The vegetation systems of East Kent colliery waste and a study of the factors limiting their development.

by

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Abstract

The ecological restoration of derelict and contaminated land necessarily focuses on the establishment of self-sustaining vegetation to provide; stability, pollution control and improved visual appearance to the land. The colliery waste, on the three sites investigated in this research, is deficient in plant resources. However, plants do colonise it. The species richness and functional composition of the vegetation, and associated mycorrhizal symbionts, have been investigated on these resource-limited substrata. The effects of environmental gradients, especially of nitrogen, on vegetation systems were used to gain an understanding of the ecological processes which operate in successional vegetation systems. The findings enabled the better understanding of functional components of the developing vegetation systems and what major parameters limit them. The findings have also been used in developing ecological restoration strategies.

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1 Prologue

Derelict and contaminated land is a potential valuable resource for development. There are real pragmatic reasons for improving methods for converting land not in use into valuable useable land e.g. improved methods may make more land available for reuse and improved methods may be cheaper than conventional techniques (King 1991). Land reclamation and restoration increasingly use ecological principles as engineering solutions become prohibitively expensive. A reduction in the cost of reclaiming land not only reduces the price for a development, but also reclamation projects which are too expensive to undertake will become affordable (Cairns & Pratt 1995).

There is growing environmental and political pressure to redevelop brown field sites, rather than increasing green field development (Geist & Galatowitsch 1999). Vegetation systems which naturally develop on derelict and contaminated sites can indicate possible approaches to novel reclamation methods; these natural systems are sustainable. Understanding how natural systems function will enable innovative reclamation schemes to be prepared (Prach *et al.* 2001).

This research primarily focuses on the ecology of vegetation systems on East Kent colliery waste. The structure and functioning of the vegetation systems in relation to age of spoil tipping, including the chemical and biological components of the systems, are investigated. The findings are tested in the field for their potential suitable inclusion into reclamation strategies.

The overall aims of this thesis are therefore to:

1. review the history and development of land reclamation especially for colliery waste sites in the UK.
2. review the ecological processes especially of succession and plant community structure which occur naturally on derelict sites, and which can be emulated in reclamation.
3. carry out empirical investigations into the development of plant systems on colliery waste. This will inform 2 above.
4. interpret the findings from practical investigations in terms of community ecology and further, to make recommendations for practical application.

1.1 Land, a finite resource.

Land is a finite resource and the demand for all types of land use is on the increase. Perhaps the only exception is agricultural land in Europe which, due to intensification of farming methods, is being taken out of production (Robinson & Sutherland 2002). However, this set-aside agricultural land does not increase the useable land stock, as it has restrictive legislation preventing its development. Land for housing or industrial development is becoming increasingly scarce. Land surrounding existing conurbation in Britain has been designated green belt (new build free zones) to prevent urban sprawl. This has led to acute shortages of land available for development in some areas (O'Riordan 1995).

The demand for new sources of land suitable for development is leading developers to look at Brown field sites (areas of derelict or contaminated land which have had a previous industrial or commercial development upon them) as potential development sites. There is economic advantage for developers who use vegetative cover for erosion and pollution control, independent of the advantages gained in addressing environmental or regulatory concerns. Homebuyers and realtors perceive vegetated lots to be worth more than un-vegetated lots and this increased value exceeds the cost of seeding. Developers, therefore, have an economic incentive in vegetative cover because of the potentially high return on the investment (Herzog *et al.* 2000).

Derelict land is considered to be land which has become so damaged by industrial or other developments that it is incapable of beneficial use without treatment. Such land includes closed and disused waste tips; worked-out mineral excavations which are not subject to enforceable planning conditions or other arrangements providing for restoration; abandoned military or service installations; abandoned industrial installations; and areas of land which are affected by actual surface collapse resulting from disused underground mining operations (DETR 1995).

Contaminated land is any land which appears to a local authority to be in such a condition, because of the substances it contains, that water pollution or significant harm is being, or is likely to be, caused (Novotny & Olem 1994; DETR 1995). The

Environment Agency has specific duties under the Environment Act 1995 with respect to contaminated and derelict land. Broadly the duties are to ensure no health hazard is represented by the waste land.

The previous industrial and commercial use of brown field sites usually necessitates some form of their remediation before they can be developed. There are different sources of grant available to enable derelict land to be brought back into beneficial use. These include the Land Reclamation Programme, which is administered by English Partnerships which also has specific money available for colliery land reclamation through their Coalfield Programme and the Coalfield Regeneration Trust. Under the Local Government and Housing Act 1989, local authorities may also apply to the Department of the Environment for supplementary credit approvals in respect of capital expenditure on contaminated land (DETR 1998).

The stock of derelict land is continually changing as brown field sites are developed and land is taken out of use. However as demand for land increases, the price of land also increases. Land which has been uneconomic to reclaim in the past is now becoming viable to restore, because of the increase in the land's value for development. The increased viability of restoration is also due, in part, to new approaches and techniques which are making land reclamation more cost effective (King 1991). Adhering to Agenda 21 objectives, for sustainable development, and ensuring long term economic, social or environmental improvement are also dimensions of land reclamation in the Twenty First Century (Spangenberg 2002).

1.2 Degraded and contaminated land in Britain - the quantity and location.

Changes in the estimated stock for England made in 1974, 1982, 1988 and 1993 are shown in Figure 1.2.1; they are divided into four principal named categories, plus the remainder. It can be seen that the largest single category of derelict land (excluding *Others*) is covered by spoil heaps, but at the same time the total amount has remained remarkably constant.

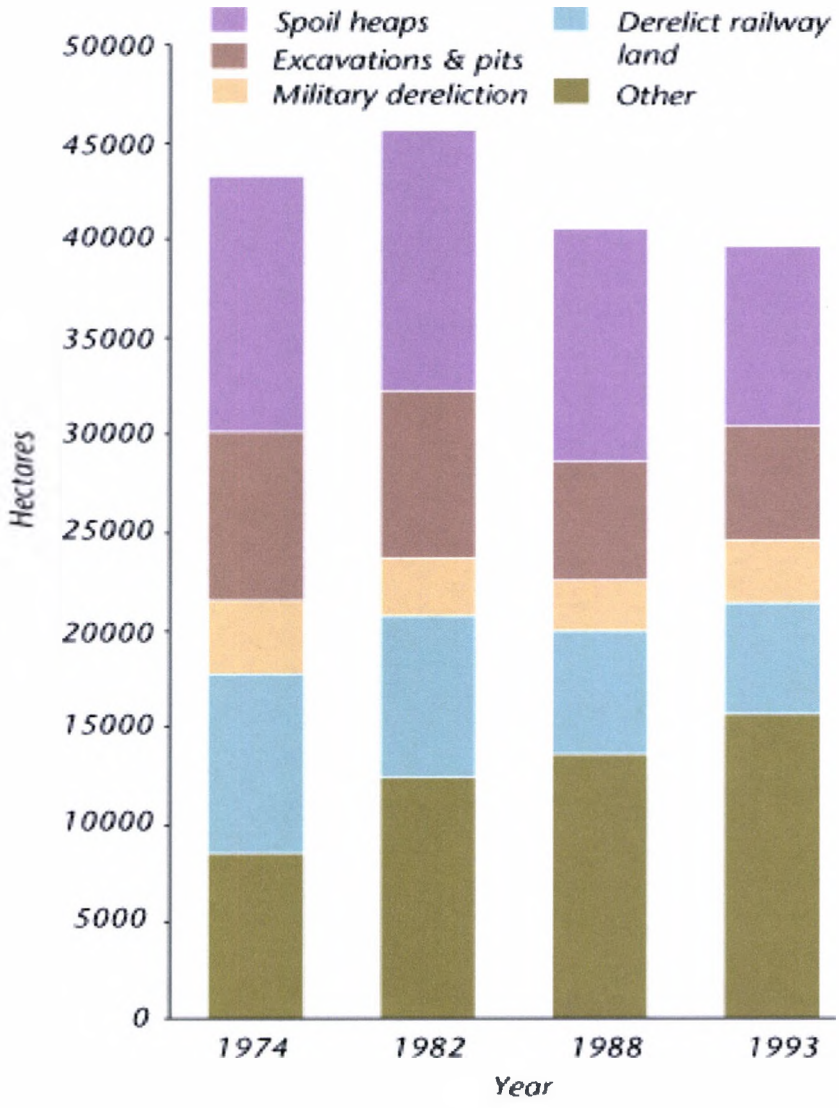

Figure 1.2.1 Changes in derelict land (adapted from the derelict land survey DETR 1995).

The amounts of derelict land in different regions of England are shown in Figure 1.2.2. These are categorised into inner-city areas, other urban areas, and rural areas. Of the whole total in 1991 it was estimated that 22000 hectares of land in England and Wales was covered by 3600 million tonnes of colliery waste. Although most of British coalfields have stopped production, the legacy of the deep coal mining industry persists (Glyn & Machin 1997). The land covered by colliery spoil is a particular significant resource which, if restored, would add a significant contribution to the UK land stock.

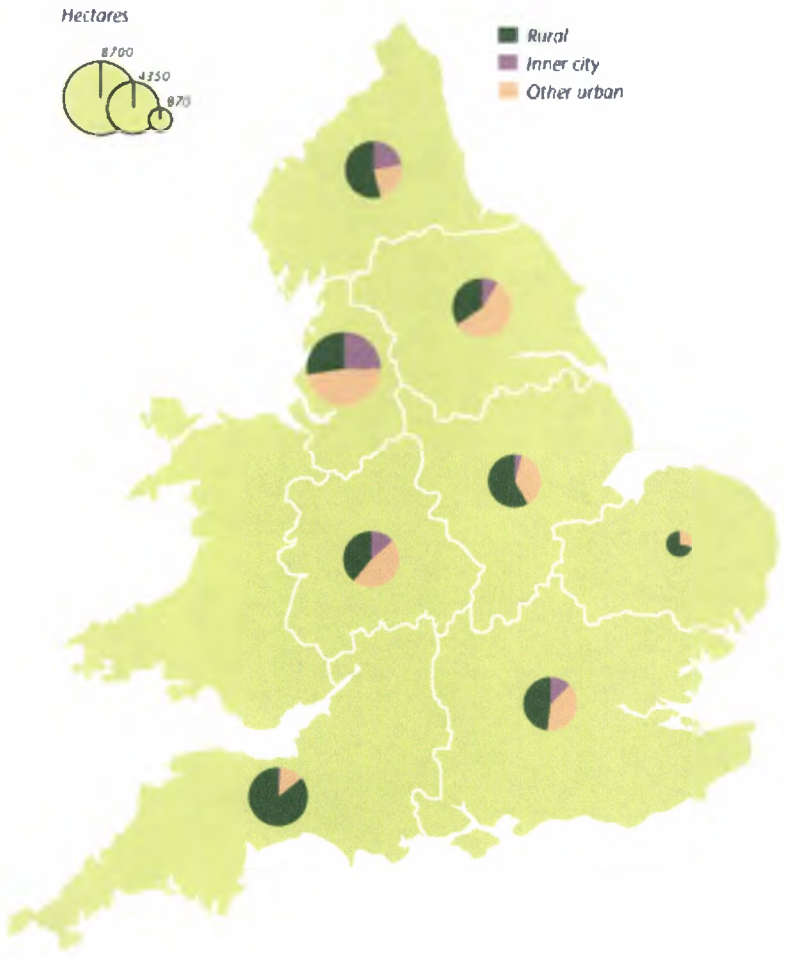

Figure 1.2.2 Derelict land by region in England (adapted from the derelict land survey DETR 1995).

Although significant amounts of derelict land are being redeveloped, the most common beneficial use made of reclaimed derelict land is that of creating open spaces for public use. Some 98% of land reclaimed in inner-city areas over the last decade was put to this beneficial use, as was over 84% of land reclaimed in other urban areas; over 90% of rural reclaimed land was similarly used (Richards, Moorhead and Laing Ltd. (DOE) 1996). Although open spaces for public use may indeed enhance the aesthetic quality of an area they do not, necessarily, generate a tangible economic gain (Dutton & Bradshaw 1982). Therefore there are more constraints on the monies and resources made available for improving contaminated and derelict land for this end use. Other end uses, which have a direct economic or commercial gain, can offset their costs of restoration against this gain (Schulz & Wiegleb 2000).

1.3 Approaches to land reclamation.

The strategy used for reclaiming derelict land depends on the intended end-use of the land. The end uses of derelict land are as numerous as there are land-use types. The constraint on the end use is determined by the funds available for the restoration and to a lesser extent the value of the land once reclaimed (Bradshaw 1984).

Agriculture and pasture end uses were prioritised in European land reclamation strategies until European farming overproduction led to the need for agricultural land to be taken out of production (Broughton 1985). Today there is a lack of land as a resource for domestic housing and commercial development so land reclamation is tending towards these end uses. However the majority of reclamation is to *other* end uses such as public open spaces (Figure 1.2.1).

Conventional reclamation techniques involving regrading, deep cultivation and the application of inorganic fertilisers are often effective but the results can be very short lived (Gemmell 1973; Chadwick *et al.* 1978). The regression of a restored vegetation to a simpler community structure than intended is a serious problem in land restoration (Richardson & Evans 1986). Regression has implications for the sustainability of the restored vegetation and the intended end use. Bulky organic material such as farmyard manure, sewage sludge, river, canal and estuarine dredges, spent mushroom compost and municipal waste have all been assessed as techniques of preventing

regression (Bradshaw & Chadwick 1980; Atkinson 1991). The addition of organic material helps to overcome many of the physical problems associated with restoring colliery spoil. Soil porosity, aggregate stability, and water availability are increased with the addition, improving the conditions for root penetration and plant growth (Roberts & Simpson 1987). Long term management strategies are often still necessary to avoid the regression of restoration. Emulation of a succession as a restoration technique inherently prevents the regression of restored vegetation by facilitating natural ecological processes thus enabling a sustainable vegetation to develop (Richards, Moorhead and Laing Ltd. (DOE)1996; Prach *et al.* 2001).

1.4 Colliery waste: the importance it has played in developing land reclamation strategy.

Small scale land restoration projects have been undertaken in Britain by local authorities, notably in the Lancashire coalfield, since the 1950's. However, the history of land reclamation in Britain dates back to 1966 and the Aberfan disaster. A colliery spoil heap on the side of a Welsh valley destabilised and slipped onto the mining town of Aberfan burying the town's school. 116 children and 28 adults died in the tragedy (Bradshaw 1984). This incident galvanised public and government opinion to look at means of improving Britain's degraded land to prevent another such disaster. Erosion is a problem on colliery spoil (Plate 1.4.1).

Plate 1.4.1 Erosion of colliery spoil at a Kent pit heap in 2000.

Historically, after the Aberfan disaster, the reclamation of derelict and degraded land has focused on four major aims:

1. gross stability,
2. surface stability,
3. pollution control,
4. visual appearance (Bradshaw 1998).

To achieve these aims, there is effectively only one choice: the establishment of a vegetation cover as a means to achieve physical stability and/or simple landscape improvement at a minimum cost (Bradshaw 1998).

After over three decades of land reclamation, the commonest physical and chemical problems associated with establishing vegetation on degraded land are well understood, as are their treatments (Burt & Bradshaw 1986). However, the present position on the reclamation of derelict land is moving on from the traditional reclamation methods. One of the commonest traditional methods of land reclamation involves burying the problem substrata with topsoil, usually taken from road improvement schemes, and then planting directly into this imported topsoil (Gildon, Stanton & Daglish 1982). The use of a topsoil covering over colliery waste provides a seed bed which promotes seedling establishment and protects against toxicities and low fertility inherent within colliery spoil (Gildon & Rimmer 1993). A topsoil layer increases water availability and improves other plant resource availability for developing vegetation (McKell 1978, Power, Sandovel & Reis 1978).

Topsoil can be an important source of indigenous plant propagules in restoration strategies and can also provide important inoculums of micro-organisms such as rhizobia and mycorrhiza (McKell 1978, Hodder 1978). However, imported soils can also import problems of weed species into reclamation sites. Pioneer species included in the topsoil's seed bank can dominate the community on restoration sites (D'Antonio & Meyerson 2002). This is because pioneer species often do very well in disturbed areas which have been top soiled, in comparison to species associated with older more stable ecosystems which are often the desired end point of a restoration (Chapman & Younger 1995).

Plate 1.4.2 Planting directly in to topsoil does not always work: at Tilmanstone Colliery, colliery spoil was covered with soil but the trees planted into it had a very low survival rate. Every empty tree guard represents a dead tree.

Planting directly in to topsoil does not always work (Plate 1.4.2), also the changing economic climate and changes in road building policy has led to traditional land reclamation strategies being reviewed in Britain. As road building schemes declined in the 1990s, the availability of topsoil decreased and the cost of topsoil use in restoration projects greatly increased. Land reclamations relying largely on engineering solutions are subsequently declining in favour of new strategies for solving the problems associated with establishing a vegetation cover over waste land. Traditional methods have been superseded by a shift towards ecological solutions to create and maintain reclamation strategies for derelict land (Ewel 1987, Grace 2000). Although the four aims of land reclamation, developed after the Aberfan disaster, still underpin land reclamation strategies today, the shift towards ecological solutions is in response to a number of factors:

1. an escalating cost of land reclamation, to spend ten times the value of the land on restoration is not justifiable,
2. surplus agricultural production in Europe means that there is no precedent for bringing degraded land into production,
3. there is increased concern that the biodiversity created on reclaimed land should be maintained by natural solutions rather than intensive management aftercare (Bradshaw 1984).

Incorporating ecological principles into restoration strategies is deemed sympathetic to trends in public opinion. More pragmatically, understanding how natural systems establish on problem substrata and then using nature's solutions to the problems are often cost effective, both in the short term in respect to an initial restoration and in the long term management of the restored land (Bradshaw 1984). The new strategies rely on ecological principles and theory to provide practical solutions to the problems of land reclamation (Parker 1997; Haigh & Gentcheva-Kostadinova 2002).

1.5 The ecology of colliery waste: the importance to land reclamation.

The ecosystems developing on old colliery pit heaps provide a unique opportunity to investigate natural vegetation systems developing on stressed sites (Kirmer & Mahn 2001). Understanding the natural systems, occurring on these derelict areas, will provide information on how the ecosystem structure, physiology and processes enable vegetation to establish and to survive in harsh environmental conditions (Ewel 1987; Wiegleb & Felinks 2001). This resource of information can provide useful insights into the important components of vegetation systems required for improving sustainability in restoration or reclamation strategies.

Ecological restoration strategies often attempt to exactly imitate natural systems to provide recognisable and persistent vegetation systems (Jackson, Lopoukhine & Hillyard 1995). Only by understanding what is happening in natural systems can this be achieved.

The usefulness, as an information resource, of vegetation systems occurring on stressed sites cannot be overstated. Although the vegetation is typically species impoverished, it is this lack of species which enables the crucial components of a vegetation system to be firstly recognised, and then understood (Zobel, van der Maarel & Dupré 1998). Just as physical scientists simplify systems to understand them, the simplicity of vegetation systems occurring on stressed sites promotes understanding for the ecologist. The very nature of colliery sites enables the most important aspects of ecosystem functioning to be observed. The confusion of a multi-species ecosystem can mask the important functioning components of the system which can be hidden by

the *white noise* of the many taxonomic levels. Observing successional vegetation sequences on colliery sites, in which the complexity of the ecosystem increases with time, the components of the complexity of the systems development can be observed (Schuster & Hutnik 1987; Prach 2001). The ecological concepts investigated and predictions made in this research are expanded in subsequent chapters. The unique situation of colliery sites enables a fuller understanding of the processes involved in the vegetation dynamics at the ecophysiology and eco-functioning level, as well as at the species and community level.

1.6 The National Coalfield Regeneration Programme.

In 1998, the National Coalfield Regeneration Programme was launched to provide a centralised governing and funding body for the regeneration of the coalfield areas in Britain. One of the main outcomes of the National Coalfield Regeneration Programme has been the national management structure of all Britain's colliery sites. English Partnerships was the organisation given the task, by the government, of developing the national strategy for the collieries' regeneration programme. Through English Partnerships, the regional development agencies (RDAs) were given specific targets and funding to regenerate the economies of the coalfield areas. Part of the RDAs remit is to restore the environment blighted by colliery waste tips (DETR 1998). It was due partly to the National Coalfield Regeneration Programme and the resurgence in the interest and requirement of the RDAs to restore colliery waste that led to this research.

1.7 Research Design.

The main hypothesis tested is that developing ecosystems are limited by environmental, physical and chemical characteristics of the locale on which they are developing (Marrs & Bradshaw 1993). Developing ecosystems are also limited by the biologically functioning groups within the ecosystem and the whole developing system is limited by the effectiveness of nutrient cycling systems, especially nitrogen (Dancer, Handley & Bradshaw 1977a; Skeffington & Bradshaw 1980; Marrs 1989; Jochimsen 2001). The investigation of key parameters relating to vegetation systems and

exploring the relationships between the factors controlling their growth, will give a holistic picture of ecosystem development on colliery waste in East Kent.

The focus of this study is on chronosequenced colliery spoil at two sites in East Kent and the associated vegetation systems developing in relation to the characteristics of different ages of colliery spoil. A third colliery site is used to test the application of some of the research findings to developing novel land reclamation strategies. Site descriptions are given in Chapter 3.

The main underlying premise of the research was that productivity in plants is dependent on whichever resource or condition is most limiting to growth in their environment: thus an increase in productivity would be associated with an increase in resource supply rates. Species richness can also increase with productivity as a function of resource availability and is linked to niche availability (Tilman, Lehman & Thomson 1997). Importantly, species richness is also related to the stability of community structure (Tilman & Downing 1994). The relationship between developing vegetation systems and nutrient status within succession were investigated in both natural and experimental situations. This was to promote the understanding of ecological processes which control ecosystem development on colliery waste.

1.8 Aims.

The overall aims of this thesis are therefore to:

1. review the history and development of land reclamation especially for colliery waste sites in the UK,
2. review the ecological processes especially of succession and plant community structure which occur naturally on derelict sites, and which can be emulated in reclamation,
3. carry out empirical investigations into the development of plant systems on colliery waste. This will inform 2 above,
4. interpret the findings from practical investigations in terms of community ecology and further to make recommendations for practical application.

2 Ecological concepts in land reclamation.

Since Aberfan, the methods of land reclamation and restoration have changed greatly, reflecting the huge technical and theoretical advances of the past decades. In this time, social and political opinion has shifted to include ecocentric ideals which have contributed to the move towards ecological approaches to land reclamation (Pepper 1984). However, the greatest influence on land reclamation strategy has been the increasing cost of reclamation. Bradshaw (1989) estimated that top soiling a site could cost £20K ha^{-1}: by 1996 Richards, Moorhead and Laing Ltd. suggests this figure had risen to £30-40K ha^{-1}. The escalating costs have directed research into strategies which are increasingly reliant on ecological principles for solutions to land restoration and reclamation. The inclusion of ecological principles can reduce the engineering intensity as well as reducing the aftercare and management of restoration (Haigh & Gentcheva-Kostadinova 2002). This trend to include ecological solutions will necessarily continue into the future, ensuring the future increased use of ecological principles in land reclamation.

Ecological restoration relies on the understanding of natural ecosystems and being able to utilise that understanding. Ecological restoration demands the creation of communities which resemble natural communities they emulate (Jackson, Lopoukhine & Hillyard 1995). The restored communities must display the complex functional relationships typified by high level of species diversity found in natural communities (Gross 1987). The success of such revegetation can be judged by five criteria.

1. Sustainability. The restored ecosystem must be able to perpetuate itself.
2. Invasibility. The reconstructed ecosystem should be resistant to invasion by new species.
3. Productivity. A restored community should be as productive as the original.
4. Nutrient retention. A restored community that loses more nutrients than the original will be limited with respect to productivity.
5. Biotic interactions. Spontaneous colonisation by animals and micro-organisms, following the restoration of formerly associated plant populations, often leads to the reconstruction of an entire community. However, community functionality can be severely restricted by the absence of a key species. A key pollinator, micro-

organism essential for N or P uptake, or an essential link in the food web missing in a community can have severe implications for the integrity of that community (Ewel 1987).

One approach to ecological restoration is the emulation of a vegetation succession. This is considered an efficient and cost effective method of restoring land (Pietsch 1996). Ecological restoration aims to develop a desired plant community as practically feasible in both time and cost. Understanding the dynamics of vegetation in succession makes restoration strategies possible which rely on these vegetation dynamics (Kirmer & Mahn 2001). This enables not only the desired vegetation to develop (Holl 2002), but can also facilitate colonisation by the faunal components of the ecosystem (Brady *et al.* 2002).

2.1 Succession.

Succession provides a useful model to examine the major changes in a vegetation system as it develops towards stability. The changes in species structure and the sequence of communities are not as predetermined as the first exponents of the concept suggested. Stochastic factors render the essentially predictive and deterministic successional models of Clements (1916, 1936), with regards to community development, over simplified (Connell & Slatyer 1977). However, there are recognisable trends within developing vegetation systems which the succession concept helps to explain.

The species forming a community at any specific place and time will be a selection from those which can tolerate the prevailing environmental conditions. The nutrient cycling and energy flows within ecosystems ultimately rely on the development of the system and the species present (Montoya, Rodríguez, & Hawkins 2003). Organisms influence and modify their environment by influencing soil formation and microclimates so that even without climatic change or disruption, the physical conditions in any location will not be constant (Clements 1916). The transition stages within a succession inherently possess a greater number of species than the original community and the community to which it is evolving. This is because the transitional stages are composed of a mixture

of species from successive communities (Tilman 1986). Consequently, the species structure of any developing ecosystem will not be constant. This is particularly evident in ecosystems developing on bare land such as sand dunes, volcanic deposits or colliery waste.

A succession on colliery waste, as it is newly exposed, can be classified as an autogenic succession (Begon, Harper and Townsend 1996). As the waste has not been influenced previously, by biotic factors, the succession can also be described as a primary succession. Investigating the processes and environmental factors, which are controlling the vegetation patterns on colliery waste, enables the formulation of revegetation plans for colliery waste and other brown field sites (Jochimsen 2001).

Connell and Slatyer (1977) proposed three models, of succession, to explain the different mechanisms and processes resulting in different community organisation as a result of succession. The three models bring together the observations and arguments which have surrounded the succession concept since its inception by Clements (1916). The climax vegetation is the result of processes acting within a succession and is not always as predictable as Clement's succession concept suggests (1916). The species reservoir, from which vegetation in the succession can be recruited, the biotic influences exerted on the environment and on other plant species by the developing plant communities, and the abiotic factors (including climatic conditions) all influence the vegetation dynamics and climax community end point to a succession. The three models proposed by Connell and Slatyer (1977), to explain the differing types of succession and their end points, are:

- facilitation model,
- inhibition model,
- tolerance model.

2.1.1 The facilitation model of succession.

Clements (1916, 1936) first proposed succession as an ecological concept, developed to explain the vegetation patterns that are found in areas with strong environmental gradients. Clements suggested that a community developed through a series of predictable and directed stages to an end point; the climax community. A climax community is stable over time, with little further change in community composition. Tansley's (1935) description of the successional end point: *"vegetation climax represented the nearest approach to dynamic equilibrium that could be attained under the system developed in the given conditions and with the available components"* succinctly expresses the climax vegetation concept.

The distinct vegetation patterns which occur along environmental gradients are the result of different responses of vegetation to different environmental conditions e.g. the vegetation transitions from seashore to sand dune to scrub to mature woodland. Succession as a model of the vegetation dynamics occurring along environmental gradients is a useful tool for interpreting the processes that are involved (Alvarez, Alcaraz & Ortiz 2000; Callaway *et al.* 2002).

Watt's (1970) work on hummock and hollow cycles of heather in the Breckland supported Clements' (1916) succession concept, but added an extra dimension to the model of the dominant taxa. Watt's concluded that the environment decided spatial patterns in vegetation systems but species composition of communities determined the temporal pattern. Different environmental conditions enable competitive advantages of certain functional types of vegetation over other vegetation functional types. Therefore, individuals with different heritable life histories were found on areas with different environmental conditions. Importantly, this model of succession relies on a feedback system: the environmental parameters are changed over time due to the influence of organisms and the changes in the environmental parameters then influence the species composition able to inhabit that locale (Watt 1970; Figueroa *et al.* 2003).

The typical model of succession suggests there is a directed progression from lower plants through to higher plant communities over time (Clements 1936; Crocker & Major 1955). The direction and rate of a succession is controlled by the availability of resources at the locale. In the facilitation model, the typical vegetation dynamics as described from Glacier Bay Alaska are observed, communities composed of lichens and bryophytes give way to grasses and herbs which in turn are replaced by shrubs and scrub woodland (Cooper 1923; Crocker & Major 1955). The culmination of the sequence of vegetation communities is the climax vegetation which in southern Britain is deciduous woodland Oak or Beech dominated (Kuiters & Slim 2003). The different communities within the succession are called seral stages and the sequence from bare substrate to climax vegetation a sere. The seral stages are not really discrete from each other as there is a gradual and continuous change from one community to another. Each new community changes the local environment. Pedogenesis, and the development of associated nutrient cycles within the succession, controls the rate and type of plant community able to inhabit a locale at any particular time (Roberts & Simpson 1987).

The climax vegetation is that plant community which has the maximum possible resource use and resource turnover. It is determined by the geology, aspect topography and climate at the locale. The environmental conditions are modified, by the developing communities, so that the environment becomes more favourable for *K*-selected species and *r*-selected species form a progressively less important role in the community (MacArthur and Wilson 1967; Boyce 1984). Just as large scale vegetation patterns in biogeography can be described in terms of its physiognomy, rather than the species composing the community, so the vegetation composing the climax vegetation, can also be described in physiognomy and functional terms (Watt 1970; Chapman & Reiss 1992; Lavorel & Garnier 2002).

The facilitation model of succession advocates a definitive and directional development of community structure within the succession. The climax vegetation is essentially mono-climactic i.e. there is only one possible vegetation type which the succession will tend towards. The successive communities change the environmental conditions at a locale and facilitate the establishment of new species to that locale. Regression of the

succession sequence, in this model, is not possible unless some environmental disturbance occurs. The succession is therefore essentially deterministic with a distinct and characteristic end point (Clements 1916; Clements 1936; Figueroa *et al.* 2003).

2.1.2 The inhibition model of succession.

The facilitation model prescribes a definable sequence of plant communities. However, in natural plant communities deviations from the predicted sequence of communities within a succession sequence have been observed. The climax community is not realised, and the plant community that becomes stable at a locale differs from the expected climax. This is believed to be the result of a plant community dominated by species that can resist the progression of the succession. The resistant community alters the environmental conditions which would enable future communities in the sequence from establishing. This resistance by a plant community to a subsequent replacement caused by its modification of the environment is described as the inhibition model of succession. Olson (1958) described the first vegetation pattern which fitted the inhibition model. Olson working on the raised beaches formed by the retreat of glaciers around the Great Lakes in the USA described the stable community as Black-oak dominated woodland. The climax community predicted for the area was Beech and Maple forest. The Black-oaks had reduced the soil pH from 7.6 at the beginning of the sere to 4. The low pH inhibited the establishment of the Beech and Maple forest which develop optimally in a neutral pH.

The inhibition of the predictable climax vegetation type by a community has connotations not only for naturally occurring communities but also for those in restoration/reclamation projects (Vitousek *et al.* 1987; D'Antonio & Meyerson 2002). If an inhibitory seral stage arises within a restoration strategy relying on successional community development, then the desired end vegetation type will never come into being.

2.1.3 The tolerance model of succession.

Gleason (1926) suggested that a plant community is made up of whatever species happened to be at a locale, and could withstand the prevailing environmental conditions. Unlike the facilitation model, the tolerance model of succession proposes that there is no definable starting community. The succession can be started with any species but it does follow a predictable sequence. The first community in a tolerance model sere is composed of species from a previous community. This *initial floristic* composition is provided by a seed bank or the vegetative parts of a previous community which has been destroyed by some environmental catastrophe. All the species which are to be found in all the seral stages of the succession can be present at the outset of the succession, but it is the species that establishes first which starts the succession sequence. The successional vegetation system developing after the Mount St. Helens eruption follows this model (Wood & del Moral 1987, Turner *et al.* 1998).

Colliery waste which has not been previously colonised has no vegetation residue to provide a species reservoir to initiate a succession sequence. In the vegetation patterns observed on colliery waste, the fact that there has been no previous plant community to provide a species reservoir, would suggest the elimination of the tolerance model as an explanation of the vegetation patterns within colliery waste. However, the surrounding plant communities are so spatially close to the colliery waste that the tolerance model cannot wholly be ruled out. The influence on the successional sequence on the sites, from immigrating species from the surrounding communities, must play a part in the successional vegetation patterns.

2.2 Biodiversity and its relationship to ecosystem stability.

Stability is defined as the ability of a system to return to an equilibrium state after a temporary disturbance, whilst resilience is the ability of a system to absorb changes in all its inputs and still to function (Walker 1989). Both seem to be linked, to some extent, to the diversity of a community.

Alpha diversity or species richness, the number of species in a system, can be used as an effective state variable for conservation and management purposes. Changes in species richness over time can provide a basis for predicting and evaluating community responses to environmental change both, natural and managed.

The effect of biodiversity on ecosystem functioning has become a major focus in ecology. Biodiversity and its significance in a fluctuating environment is still poorly understood. The insurance hypothesis proposes that high biodiversity insures ecosystems against declines in their functioning; this is because many species provide greater guarantees that some species will maintain functioning even if others fail (Yachi & Loreau 1999). However, species richness can also respond disproportionably to changes to factors such as community composition e.g., the removal of a keystone species (Nichols *et al.* 1998; Ryerson & Parmenter 2001). To a certain degree, species composition in a multi-species ecosystem is as important as the total number of species (Lavorel & Garnier 2002). However, the more species within a system, the more possible interactive associations are also present (Gaston 1996). The number of species within the system is therefore important in relation to resource utilisation and energy dynamics in a system. The proportion of total energy flow allocated to ecosystem organisation can be considered proportional to the species number (Odum 1970).

Beta-diversity, or biodiversity, is the number and variety of taxa per unit area in an ecological system. Stability of an ecosystem can be interpreted in different ways:

- Resistance to change i.e. community interactions prevent changes in the community structure therefore the more components in the system the more likely it is to be unchanged by a disturbance.
- Resilience to change-refers to the ability of a community to return to equilibrium after perturbation:
    - Elasticity = the speed at which a community can return to normal.
    - Amplitude = the distance of disturbance from which a community can return to normal (Stiling 1996).

Whilst species composition may vary substantially in response to disturbances, ecosystem variables, including species richness, productivity and energy use, may

remain relatively constant (Chesson & Case 1986; Wardle *et al.* 1999; Brown *et al.* 2001a and Brown *et al.* 2001b). It is also possible that species fluctuations may represent a compensatory mechanism that contributes to ecosystem stability in some ecosystems (Morgan-Ernest & Brown 2001). However, there seems to be good reasons why stable ecosystems would have a diverse species composition or high biodiversity. The effects of a catastrophe can be limited by large numbers of interacting species (Naeem *et al.* 1994, Yachi & Loreau 1999). However, this is not always the case. If one considers systems in which:

- Only one higher plant species is present, for instance in an artificial agro-system.
- Every single individual of a community is of a different species.

The monoculture has to be artificially protected from pathogens and the depletion of key nutrients as intraspecific competition is intense. The species rich community rather than being stable is also compromised. One cannot imagine how the population dynamics necessary for sustainability, in relation to reproduction, could be achieved. The community could not be self sustaining unless there were complex mechanisms for immigration and dispersal from outside the community. Therefore, there is possibly some middle ground in which, the integrity of a system is maximised by the diversity of species composing it, and also the characteristics of the system must be maintained by an optimum diversity of species.

Community stability, and its resistance to environmental stresses, is in part the result of the adaptations of member species to environmental stresses. Each species member of the community has to be the best competitor in its particular niche to prevent displacement by species better suited for that environment (Pontin 1982).

In experimental communities, such as *Ecotron*, enhanced ecosystem functioning is attributed to increased biodiversity (Naeem *et al.*1994). Tilman *et al.* (2001) also found a positive relationship between community diversity and ecosystem functioning. However, there is conflicting evidence, from natural ecosystems, which suggests that the functional characteristics of the species that compose the community are more important than the number of species in respect to ecosystem processes (Archer, Boutton & Hibbard 2001, Ryerson & Parmenter 2001), evidence suggests that loss of a

few predator species often has impacts comparable in magnitude to those stemming from a large reduction in plant diversity (Duffy 2002, Duffy *et al.* 2003). The notion that high biodiversity promotes ecosystem sustainability can be questioned on this basis. Although the experimental mathematical models, investigated by May (1972 & 1973), could not support diversity causing stability; the models of Yachi & Loreau (1999) do. Naeem (2002) also supports the concept that the greater the biodiversity of a system the more stable is that system. Whether there is a point at which the loss of a key functional species hinders ecosystem processes cannot be resolved, but the concept has important implications for both ecological restoration and successional vegetation systems.

2.3 The importance of species number in community development.

May (1972) explores the relationships between species diversity and species interactions which are necessary for stability within models of systems. Those models which have high species diversity with weak interactions are less stable than models of systems which have fewer species but stronger interactions. The model, put forward by Norberg *et al.* (2001) on the contrary, suggests that phenotypic variance, within functional groups, is linearly related to their ability to respond to environmental changes. The model suggests that the long-term productivity, for a group of species with high phenotypic variance, may be higher than for the best single species. This is so even though high phenotypic variance decreases productivity, in the short term, because sub-optimal species are present. High productivity, as already discussed, is an important attribute of communities in later seral stages of successional vegetation systems. This phenomenon is important to ecosystem functioning and hence also to the development of restoration strategies which need to simulate natural systems.

The greater the species richness the more parts to the succession system there are likely to be. This enhances the plasticity of the system, improving both the stability and resilience of the developing succession. As long as no critical overload occurs to the succession system, the more species there are, the greater the capacity of the system to maintain itself (Waide *et al.* 1999).

In natural ecosystems the optimum number of species and type of species are influenced by the environmental characteristics of the locale. The characteristics of the community are, also, directed by the species available to colonise that locale. Models of competition within communities predict, and some field experiments confirm, that greater plant diversity leads to greater primary productivity (Odum 1970, Brown *et al.* 2001a). This diversity-productivity relationship results both from the greater chance that a more productive species would be present at higher diversity, the sampling effect (Norberg *et al.* 2001), and from the better "coverage" of habitat heterogeneity caused by the broader range of species traits in a more diverse community, the niche differentiation effect (Hubbell 2001). Both effects cause more complete utilisation of limiting resources at higher diversity, which increases resource retention, further increasing productivity. This retention of limiting resources within the community, and higher levels of diversity, are predicted to decrease the susceptibility of an ecosystem to invasion, supporting the diversity-invasibility hypothesis (Tilman 1999a).

Increased species richness, arguably therefore, enhances the flexibility of a community improving both the stability and resilience of a developing succession (Grime 1998). Identifying the plant nutrient supply rate, and form of plant nutrient, which promotes species rich plant communities, could have significant connotations for the success of restoration projects. Greater species richness enables greater resource use and environmental modification, thus increasing the rate of succession (Marrs & Bradshaw 1993). As long as no critical overload occurs to the succession system, the more species there are, the greater the capacity of the system to maintain itself (Walker 1989). Therefore the importance of establishing species rich vegetation in reclamation projects is three fold.

1. Species richness gives stability and therefore sustainability to a plant community, reducing the need for intensive management aftercare.
2. The natural process of succession is augmented by greater species richness, so a sustainable climax vegetation end point can be reached in less time than land restorations relying on species poor vegetation (Chapman & Younger 1995).
3. An increase in plant species also increases the likelihood of higher species diversity in other taxonomic groups, such as microarthropods or

bryophytes, developing within the community (Hansen 2000; Pharo, Beattie & Binns 1999): this is an important concept with respect to the potential sustainability of restoration strategies.

The interaction of groups of species should not be underestimated, especially when successional development is investigated (Jonsson & Malmqvist 2000). Herbivory can play an important role in plant community structure. The development of a soil flora and fauna must also be deemed as important as the community development of the higher plants especially in relation to nutrient cycling and pedogenesis (Tilman 1986). However: "*It is difficult exploring relationships of the total species richness within any study*" (Walker 1989 p93). This is because the study of even modest species assemblages can only deal with one or two major groups of organisms at a time. The specialist taxonomic and enumerating requirements, for dealing with each major group of organisms, discourages studies of total diversity in favour of those of one or a few major groups (Walker 1989). My research focuses on the higher plants and mycorrhizae symbionts within the developing vegetation systems studied, i.e. animals and other micro-organisms are necessarily neglected.

Although predictions about diversity development within a succession will be made from the study, the true question of the holistic species diversity relies on inferences made from diversity within the functional groups studied. The functional relationships within the study are valid in relation to forming ecological restoration strategies for the East Kent colliery waste and gaining an understanding of the ecological process controlling the vegetation patterns on the project sites. However, there are fundamental constraints to predicting vegetation responses to environmental changes, due to the complexity of interactions between plants and their biotic and abiotic environment (Connell and Slatyer 1977; Tilman 1994; Montoya, Rodríguez, & Hawkins, 2003). A change in the environmental resources leads to a change in a community. Plants competing for limiting resources change the resource availability and therefore the criteria for competitive success (Gersani, Abramsky & Falik 1998). An understanding and ability to confidently predict vegetation responses to changing environmental conditions would greatly enhance the success of ecological restoration strategies.

Ecosystem viability and sustainability may well depend on some level of biodiversity, but the connection between ecosystem processes and biodiversity has not been demonstrated to be consistent. The process of succession is directed towards maximising resource utilisation within a system. The examination of the functional consequence of biodiversity, and mechanisms controlling biodiversity within succession, could therefore be crucial in determining the rate and direction of the vegetation dynamics (Cottingham, Brown & Lennon 2001). The vegetation developing on colliery waste gives a unique opportunity to explore the role of biodiversity with respect to ecosystem functioning. By manipulating resources and the functional groups, within vegetation systems on colliery waste, the connection between biodiversity and ecosystem function can be investigated within this system.

2.4 Community Structure and composition; functional species and their importance in developing ecosystems.

Population biologists suggest that on islands, the number of species in a plant community simply depends on the rate at which species become extinct from a community and the rate at which species establish within a community (MacArthur & Wilson 1967). However, this view takes no account of the mechanisms controlling species number at the starting point of the early seral stages of a succession. Disregarding the tolerance model for succession, the rates of extinction from and immigration to a community are closely associated with the nutrient availability and cycling within a developing succession (Mitchell *et al.* 2000). Studies have focused on the effects of nutrient supply on the species diversity within established plant communities but few have attempted to analyse experimentally how nutrient supply affects the establishment of plant species (Bengtsson 1998).

Hubbell (2001) interprets that there are two possible explanations for the community structures in ecosystems.

1. The **niche-assembly explanation** for community structure suggests that the species observable in a community are dependent on the functional roles and ecological niches of individual species. The interacting groups of species in communities can be extrapolated from species assembly rules. A species

persistence in a community is dependent on some spatial, temporal or niche separation from other species within that community.

2. The **dispersal-assembly explanation** for community structure suggests, that, community structure is the result of species brought together by chance, site history and random dispersal. Communities are essentially open and not in equilibrium, species coming and going. The presence or absence of any species in a community, at a locale, being dictated by random dispersal and stochastic extinction. The species within a community are essentially neutral with respect to excluding other species from that community. Macarthur and Wilson's (1967) Island biogeography theory is the basis for this explanation for community structure.

Neutrality of species, with respect to competitive exclusions, can operate at levels within systems where niches are not filled and potential resource utilisation is low. However, within systems which have maximum resource utilisation and filled niches, the niche-assembly rules play an important part in determining community structure. Therefore, in any of the three successional models proposed by Connell and Slatyer (1977), the importance of dispersal-assembly rules will influence early seral stages and in later seral stages, niche-assembly influences will play increasingly important roles in the community structure.

In the developing community of a succession a species may well have an exaggerated role, in the functioning of the system, due to the lack of the potential number of species within the community. Grime (1998) also has developed community structure explanations which have in some part aspects of both niche-assembly and dispersal-assembly rules. The functional importance of a species depends both on the specific composition of a community and the variation in environmental resources in both space and time (Grime 1998). As a generalisation, the loss of a species has a more pronounced effect on the functioning of a species poor community than a diverse community. This is because the loss of a species function, in a species rich community, has a greater probability of being compensated for by the remaining species (Ekschmitt & Griffiths 1998; Ruesink & Srivastava 2001).

2.5 Niche concept and resource acquisition: their role in limiting species diversity and type.

Hutchinson (1957) first introduced the niche concept defined as: the sum of environmental factors acting on a species. The niche, so defined, is a region of an *n*-dimensional hyper-space in which the *n*-dimensions represent all the environmental factors including the physical, chemical and biological resources and interactions which limit the range of a species within time and space (Schoener 1989; Tokeshi 1999).

Niche theory as a concept, enables an understanding of the abundance and organisation of the number of species within a community. It can also be used to explain the extent of species distribution and the variability from community to community in species composition based on resource utilisation (Tilman 1986). The more diverse a community the more multi-dimensional niche space is available for exploitation within the system (Martinez 1986). The number of species within a system influences the community on more than one level (Gilbert 1989; Schmitz, Beckerman & Litman 1997) e.g. consider a single higher plant species and only its affect on the physical environment. It not only provides substratum stability with its root system but it also influences the above-ground micro climate with its leaves and its litter provides substrates for decomposers. The more species of plant, the more levels of influence are experienced by the community. This is simply due to the morphological differences of the different plant species. Therefore, species contribute to a community's overall function in several dimensions (Ekschmitt & Griffiths1998; Díaz *et al.* 2003).

There is a general increase in primary productivity from the poles to the tropics. This is correlated with an increase in resource availability (light levels, temperature, the length of the growing season etc.) (Gaston 2000). This productivity gradient is also mirrored by species diversity gradients; there are more species in the tropics than at the poles (Cardillo 2002). The relationship between resource availability, productivity and species diversity could be crucial for formulating an understanding of successional vegetation dynamics. Productivity is an important ecological measure of an ecosystem with respect to successional development. There is an increase in productivity associated with an increase of resource supply rates of nitrogen, phosphorus and potassium.

However, the species present, at any point in a succession, create environmental modifications which control the rate and direction of the succession. There is, therefore, an expectation that species richness and biodiversity will increase with productivity as a function of resource availability. This is because species diversity can reflect resource availability (Currie & Paquin 1987; Tilman & Downing 1994; Waide *et al.* 1999). For a given range of resources, more species are able to occupy the range if:

a) the species are more specialised in their use of resources (resource partitioning),
b) species overlap in their use of resources so that more species can coexist along a resource continuum,
c) the community is fully saturated; there will be fewer species if the resource continuum is not fully exploited (Brodie 1985).

Therefore the number of species able to occupy a locale will increase if the given ranges of resources also increase (Currie & Paquin 1987). However, a decline in species diversity with increase in plant nutrients has been observed in some plant communities. For example, there is evidence for the decrease in phytoplankton species richness, but with a corresponding increase in primary productivity due to the eutrophication of water systems (O'Riordan 1995). The 'Parkgrass' experiment, running from 1856 at Rothamsted in England, also questions the universality of increase diversity with increase resource availability. A $3.2 \times 10^4 m^2$ pasture was divided into 20 plots two serving as controls the rest receiving a fertiliser treatment once a year. The diversity of the two controls remained unchanged whilst the fertilised plots showed a progressive decline in diversity (Rosenzweig 1971).

2.6 The importance of ecological concepts for land restoration.

The restoration and management of ecological systems has generally been accepted to involve intervention, even on sites being colonised by vegetation (Niering 1987; Luken 1990). A degraded site often requires considerable initial rehabilitation: restoration ecology in this context requires specific objectives (Cairns 1990). In some cases these objectives may include a particular composition of species (Ewel 1987), some functional process re-established such as nutrient cycling in severely degraded sites such as colliery waste heaps (Marrs 1989), or comparison against a reference site

(Bradshaw 1983). Intervention might require the reduction or removal of species or circumstances that might prevent site restoration, such as invasive species populations (D'Antonio & Meyerson 2002) or toxic soil conditions such as acidity in colliery waste (Costigan, Bradshaw & Gemmell 1982). The principal objective should be the creation of a species mix and environmental conditions that permit the site to become self-sustaining. The understanding of ecological concepts is therefore crucial to developing sustainable restoration strategies (Bradshaw 1987).

The successional development of a particular climax vegetation is dependent on climatic conditions and can be explained in terms of different resource utilisation and the modification of environmental conditions by successive communities at the same locale (Chadwick 1987). The modification of environmental conditions by a community enables the colonisation by species new to the locale and the subsequent competitive exclusion of the existing species assemblage (Grime 1973a). Intermediate stages within succession are composed of a mixture of species from successive communities; therefore, they are inherently species rich (Tilman 1986).

In Southern Britain the climax vegetation, predicted by the facilitation model of succession, is deciduous woodland. Deciduous woodland represents the system of species which can maximise the resource utilisation within this geographical locale (Watt 1970; Chapman & Reiss 1992). The natural vegetation succession on derelict land in Southern Britain, if following the facilitation model of succession, will typically progress towards this climax vegetation and given time deciduous woodland will develop (Smith & Olff 1998).

"A basic observation about natural populations is that they fluctuate with changing environmental pressures (DeWitt & Yoshimura 1998 p.616,).

Plant species richness is important to the vegetation development on derelict land. The more species which exist at a locale the more they influence the environment and therefore the rate of succession is increased (Gray, Crawley & Edwards1987; McGrady-Steed & Morin 2000). Prach, Pysek & Smilauer (1999) also suggest that if it was possible to maintain the species assemblage at a non-equilibrium transitional stage, in a restoration, the successional dynamics would be faster than a system where

community equilibrium was achieved. Greater species richness also increases the potential ability of a developing community to withstand environmental extremes (Walker 1989; Cottingham, Brown & Lennon 2001). The insurance hypothesis also supports this: the more species there are, the more likely there will be a species adapted for the changing environmental conditions caused by the changing plant communities (Yachi & Loreau 1999). It therefore follows that the more plant species present in the initial seral stages of succession, the higher the resource utilisation in the succession. The increased resource utilisation would maximise the environmental modification; until the resource utilisation was at its maximum which would maintain the environmental conditions at equilibrium and the climax vegetation would develop (Niering 1987). Therefore, one would expect developing vegetation with naturally high biodiversity to flourish and it also follows those restoration strategies which incorporate high biodiversity as an aim will be more successful than those which do not. High biodiversity as a restoration target may also gain financially. A restoration project targeted at a rare or endangered habitat can gain funding where restoration to some other use may not (Mountford & Keppler 1999). An understanding of the successional dynamics and the ecological controls and influences on biodiversity are, therefore, crucial for designing successful and self-sustaining restoration strategies.

3 The East Kent Coalfield.

In 1990 the last deep coal mine of Kent ceased production. Unlike other Coalfields, which have to be limed to reduce acidity before vegetation will establish on them, the Kent colliery waste has developed extensive natural vegetation (Atkinson 1991). The Kent colliery waste therefore presents a unique opportunity to examine natural plant colonisation and succession and to incorporate the observations into derelict land restoration strategies.

The Kent colliery waste is the rock excavated to enable the extraction of coal. It is composed of a mixture of crushed and broken sedimentary rocks which are associated with the coal deposits. The coal measures of the Kent coalfield are Carboniferous of age, the same as most other coalfields in the UK, (Wood, Shephard-Thorn & Harris 2000) and are divided into two parts:

1. The Lower Westphalian Shale Division (213m) at the base comprises mudstones with subordinate sandstones and includes eight main coal seams and at least four marine horizons.
2. The Upper Westphalian Sandstone Division (670m), which comprises a lower coal-bearing succession with sandstones, thick mudstones and six main coal seams, of which the lowest (Kent No. 6) was formerly of considerable economic importance; and an upper succession with few workable coals in which thick sandstones form over 70 per cent of the lithologies (Wood, Shephard-Thorn & Harris 2000).

These Kent coal measures include sandstone, limestone, mudstone and, also in larger proportions, shale (Tucker 2001). The rocks were formed from the deltaic sedimentary deposits laid down at the same time as the swamp forests which were eventually lithofied to produce the coal (Thomas & Thomas 1995). Many of the deep coal fields such as the Lancashire and Durham fields have waste which is very acidic, pH as low as 3.2 has been recorded in the Lancashire waste. This low pH is due to iron pyrites and other sulphur rich minerals in their shale, weathering to produce sulphuric acid (Costigan, Bradshaw & Gemmell 1981; Chadwick 1987). In contrast the colliery spoil from the Kent coalfield does not have this acidity problem; its surface pH ranges from 4.5-7.2. Part of the reason for the relative high pH is that the surface sulphur minerals

have already weathered, releasing their acidic products which have been subsequently leached out of the colliery waste system. It is thought the benign pH is also due to the environment in which the rocks, especially the shale, were deposited. The environmental conditions during the formation of the Kent coal measures did not promote the development of sulphur minerals (produced in anaerobic conditions). The Kent coalfield is also overlain by chalk. During the coal extraction process, the chalk has become mixed with the shale waste. The chalk is composed of calcium carbonate which acts as a natural buffer, preventing the low pH found in other colliery spoil. The Kent colliery waste is a relatively benign (in comparison with other colliery waste) homogeneous mix of broken rock with a particle size ranging from a clay matrix to large boulder clasts. It does not have associated toxicity problems of other Coalfields' waste, but like other colliery waste is deficient in all plant nutrients (NPK) (Palmer *et al.* 1979; Atkinson 1991).

Compaction is probably the most common form of substratum damage associated with reclamation and restoration projects on colliery waste (Doubleday 1974), and is also a problem for strategies on the East Kent colliery sites. Compaction is caused by compressive forces which the spoil does not have the strength to resist. Spoil loosened for the restoration procedure is at its structurally weakest, and is therefore most susceptible to compaction (Rimmer 1979; McRae 1989). The replacement and modification of the upper layers of spoil for the restoration by mechanical plant can cause a serious compaction problem. Compaction in the substratum has a number of serious deleterious effects on establishing vegetation as a result of a decrease in:

- root penetration,
- water availability,
- drainage (Rimmer 1982).

Therefore any reclamation strategy should endeavour to cause minimum compaction to the substratum, ensuring the substratum:

- is not compacted, as it would inhibit root penetration,
- has an adequate root-penetrable depth to provide a water reserve in dry conditions,
- is structured so that it is porous and drains to maintain aeration (Richards, Moorhead & Laing Ltd. 1996).

3.1 Project sites

This research investigates vegetation systems on three former colliery sites in East Kent: Tilmanstone, Betteshanger and the Chislet and Hersden mine waste at Stodmarsh (Figure 3.1.1).

Three project sites were chosen, as they presented a unique possibility to study chrono-sequenced vegetation on a relatively biologically sterile substratum. The three sites chosen also enabled the investigation of some real problems associated with vegetation establishment on the colliery waste of the East Kent coalfield. Hanson Brick Ltd (UK), the owner of the site, has had significant tree planting failures in the past (Gilchrist 1998).

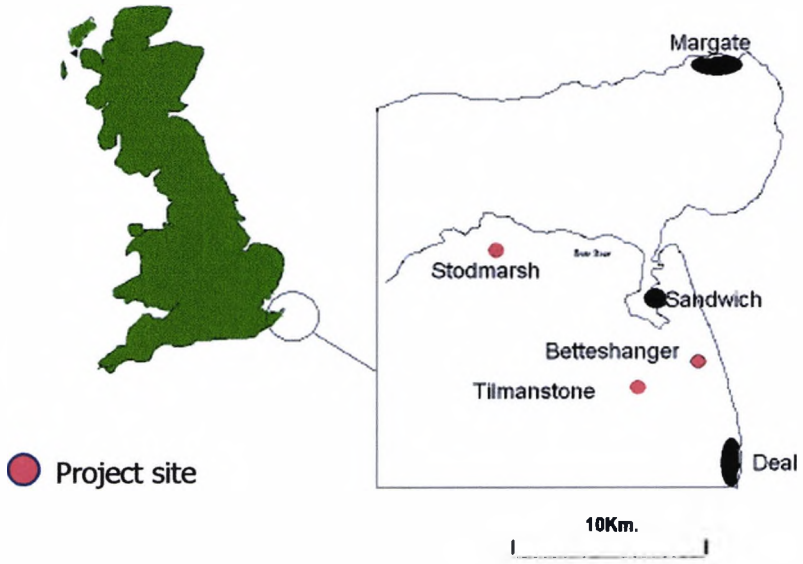

Figure 3.1.1 Location of the project sites.

3.2.1 Tilmanstone.

The Tilmanstone research site is situated at the former Tilmanstone colliery (now owned by Hanson Brick Limited UK), Tilmanstone Works, Pike Road, Eythorne, Kent Longitude 1^0 183'E Latitude 51^0 125'N. The factory is adjacent to the waste tailings from the Tilmanstone Coal Mine decommissioned in 1989. The shale from the old mine workings is added to clay and is used to manufacture house bricks.

In 1994, Dover District Council granted planning permission to the Tilmanstone Brick Company for the development of the site, conditional on landscaping the area and returning the worked shale to indigenous woodland (D0/88/1679 August 1994).

The boundary of the works has some scrub vegetation, comprising mostly of colonising tree species; *Betula pendula* (Birch), *Viburnum lantana* (Wayfarer Tree) *Quercus ilex* (Holm Oak) and *Quercus robur* (Pedunculate Oak) is establishing. Although there is a noticeable absence of extensive herb cover on the shale, some annual pioneer species are present. The tree planting schemes on the site have focused on augmenting this established vegetation and the provision of perimeter screen plantations to conceal the factory. Most of the vegetation located on the colliery shale originates from this planting scheme which has had limited success, as the planted tree survival rate is low.

3.2.2 Stodmarsh.

The Stodmarsh project site is composed of 22 ha of colliery mine spoil from the Chislet and Hersden mine (Longitude 1^0 113'E Latitude 51^0 185'N). The mine head from which the spoil originated is on the north side of the river Stour. The spoil was transported by way of a conveyor belt to the spoil tip on the south side of the river. The first spoil deposited on the site was used to reinforce the river bank. Successive tipping worked away from this initial stabilised bank. The Mine ceased production in 1972 and the final spoil movement occurred in 1973. Coal Authority plans and aerial photographic records of the development of the spoil tip are kept at KCC Maidstone. Figure 3.2.1 illustrates the interpretation of these records into an aged sequence (chronosequence) of the spoil tipping at the site. The records have enabled five distinct areas within the

chronosequence of spoil tipping to be identified. All the colliery spoil, the natural vegetation and mycorrhizal investigations have been carried out along the five transects illustrated in Figure 3.2.1.

The colliery spoil is adjacent to a national nature reserve and SSSI wetland and has had no remediation. All the vegetation on site is natural colonisation. The site is jointly owned by a local land owner, English Nature and the Environment Agency. The multi-ownership of the site has meant a lack of consensus to remediate it. This has lead to the fortuitous situation of a unique vegetation system developing without interference.

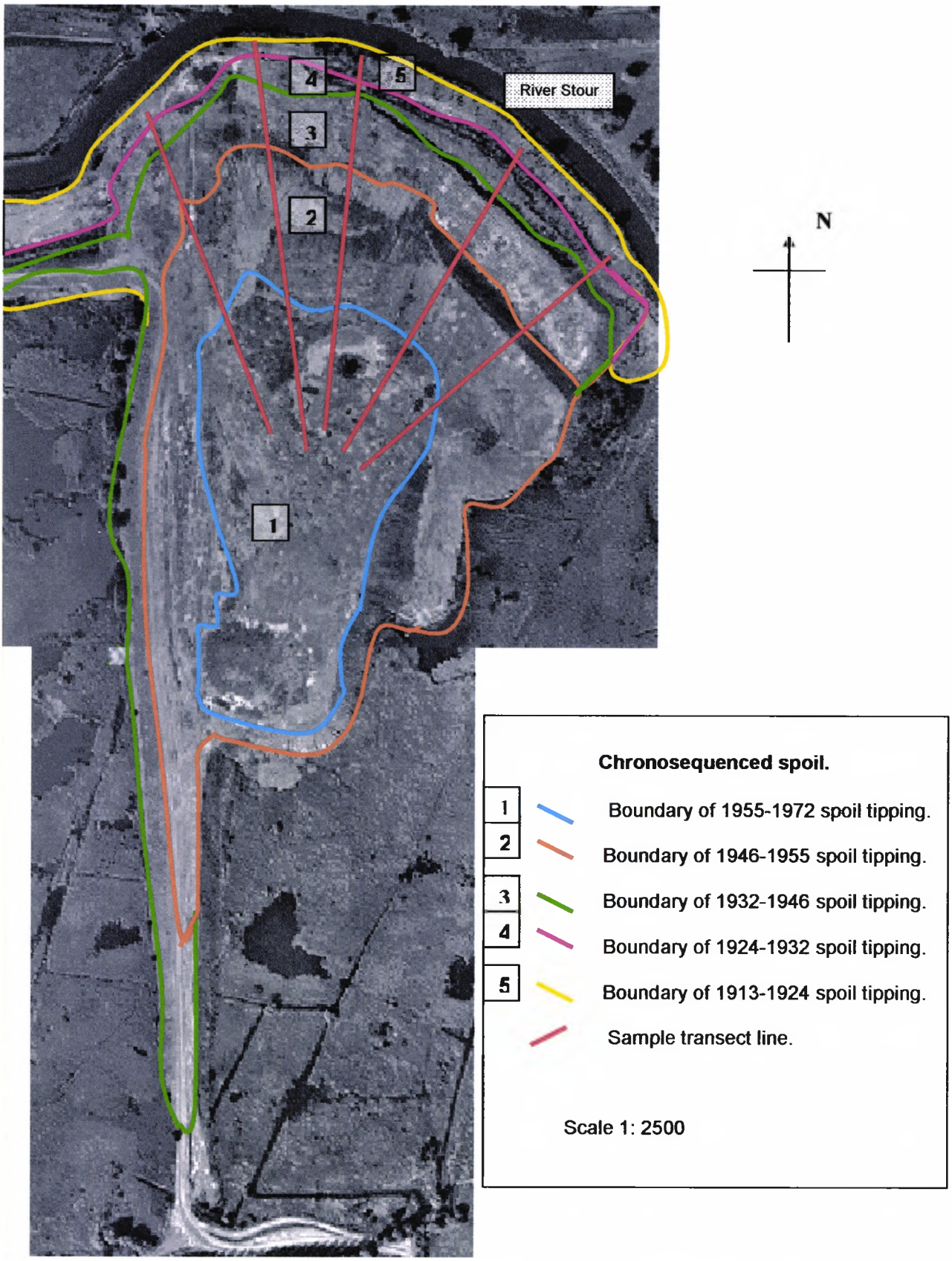

Figure 3.2.1 Aerial photograph of the Chislet Colliery waste at Stodmarsh. The 5 tipping ages of the spoil and 5 sampling transects are shown.

3.2.3 Betteshanger.

The Betteshanger project site is situated on the colliery waste from the Betteshanger colliery (Longitude 1^0 233'E Latitude 51^0 140'N). Deep coal mining at Betteshanger ceased in 1990 but part of the tip was restructured in 1991. The colliery waste has been undisturbed since. There have been two main tipping regimes at Betteshanger. The first tipping regime involved loose tipping of colliery waste from a conveyor belt. The second stage of tipping was from railway wagons. The waste was transported from the pit head *via* a specially constructed rail line. The waste was then spread by earth-moving vehicles on the pit heap. Aerial photographs and Coal Authority plans have been used to identify differently aged spoil on site. The chronosequence at Betteshanger is identified in Figure 3.2.2.

There has been a very small amount of screen planting including *Pinus nigra var. maritima* (Corsican pine) and X *Cupressocyparis leylandii* (Leyland cypress) on the margins of the pit heap. A soil material, from a local development, has also been spread over part of the site which covered the old lagoons (Figure 3.2.2). This research however has concentrated on the naturally occurring vegetation systems which have developed on the Betteshanger colliery waste. As the result of the tipping episodes at the site, there is a distinct chronosequence within the colliery waste. The chronolosequence represents a unique opportunity to investigate the vegetation patterns with respect to both community structure and functionality that develop at the site over time.

Betteshanger is naturally colonised in places by scrub woodland. The scrub woodland is dominated by two tree species Birch (*Betula pendula*) and Holm Oak (*Quercus ilex*). *B. pendula* has very light winged seeds which are wind dispersed. However, the acorns of *Q. ilex* are thought to have been transported by birds, probably Jays, from local parkland some 3km away (Atkinson 1991).

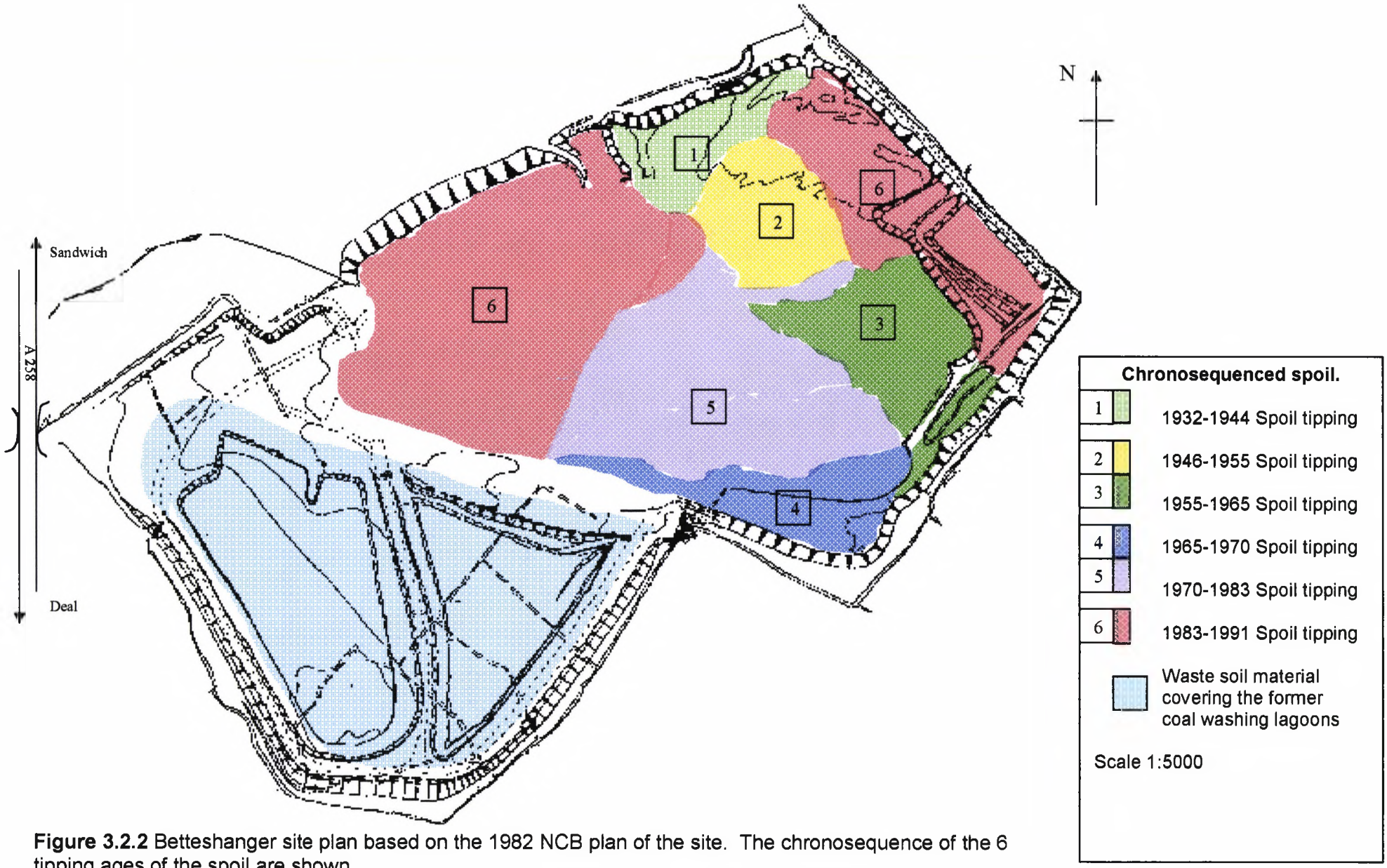

Figure 3.2.2 Betteshanger site plan based on the 1982 NCB plan of the site. The chronosequence of the 6 tipping ages of the spoil are shown.

4 Ecological processes: succession and the initial physico-chemical characteristics controlling vegetation structure.

Vegetation has a number of barriers to overcome before it is able to establish on a site. One barrier is simply the ability for a plant species to migrate onto a site. Another is the ability to exploit what few resources are available when it gets there.

The vegetation on any substratum is controlled by a number of limiting environmental factors such as nutrient availability, water availability etc. (Leibig's Law of the minimum). Perhaps the most important factor to the community development in terrestrial ecosystems is the nitrogen status and re-cycling ability of the establishing community (Bradshaw 1983).

The vegetation patterns on colliery sites, at Betteshanger and Stodmarsh, have been influenced by the environmental conditions of the colliery waste: whether there has been a positive interaction between colonisation and subsequent plant growth is one of the keys to identifying:

- successional development of the vegetation,
- whether the environmental conditions of the waste are fixed in time.

The chemical composition and the vegetation systems, developing on the Betteshanger and Stodmarsh sites, were investigated. The investigations were designed to ascertain if there was any relationship between the age of the colliery spoil, chemical composition and the vegetation systems which had established at the sites.

4.1.1 Method

The age of different tipping episodes of colliery spoil, at Stodmarsh and Betteshanger, were identified from aerial photographs of the sites dating from 1946 and prior to this from NCB plans of the sites (see Figures 3.2.1 and 3.2.2).

Five colliery waste samples were taken from each of the different aged sequences of tipping at the two sites; Stodmarsh and Betteshanger. The spoil was collected by auger and in pairs; one sample from 0.1m and one 1m below the surface at each

sample point. The samples were placed in polythene bags which were sealed for transportation and stored at 0-5°C until analysed.

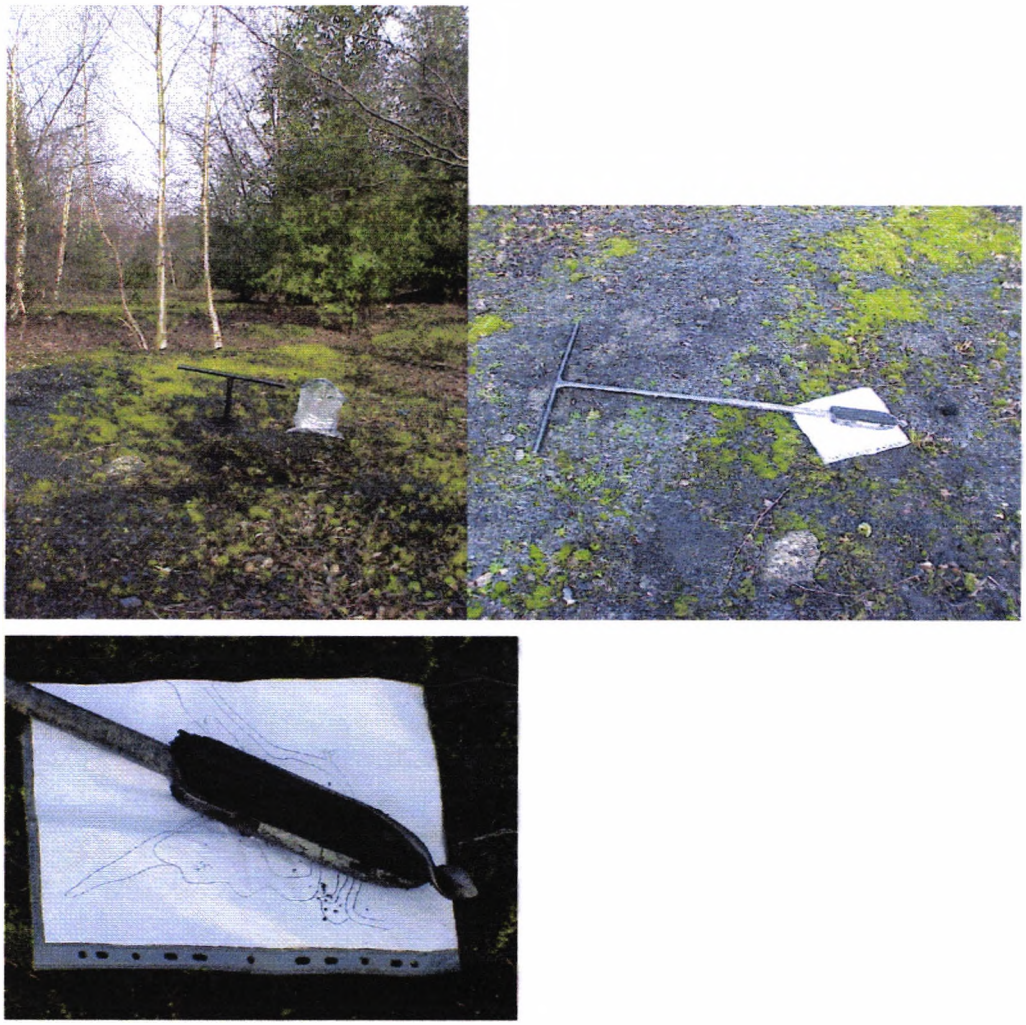

Plate 4.1 Spoil sampling with an auger.

The colliery waste samples collected from the chronosequences at Stodmarsh and Betteshanger were analysed for: cadmium, chromium, copper, lead, nickel, zinc, mercury, arsenic, molybdenum, tin, fluorine, pH and soil moisture content. Standard methods were used for the determination of the analyses of the colliery waste (ISO/FDIS 11047; Allen 1989; Radojevic & Bashkin 1999). The elements analysed were chosen as they can be toxic to plant growth at high concentrations (Kimber, Purford & Duncan 1978, Radojevic & Bashkin 1999). The samples from 1m below ground were analysed to give a baseline composition for the sites. The colliery waste 1m below the surface is beneath the major rhizosphere and biologically active part of the spoil. The surface 0.1m, of a soil profile, is the most biologically active part of the spoil (Allen 1989). It was therefore predicted that the most change, due to

biological modification, would occur at the 0.1m depth (Schottelndreier & Falkengren-Grerup 1999). The spoil tipping age and regime had been identified, for both Betteshanger and Stodmarsh; however, there may well have been stochastic reasons for differences in the spoil composition across the sites. Examination of the colliery spoil at a 1m depth gave a baseline for the spoil composition across the sites. This baseline tested the validity of the assumption that the spoil tipped was equitable in composition across the different ages of the tips.

The following method, adapted from Allen (1989) was used to determine the pH of each fresh spoil sample. The pH, of the spoil samples, was measured using a buffer calibrated, glass bulb electrode digital pH probe, with a compensatory temperature probe attached. Field sampled spoil was placed into a 100ml beaker to the 50ml mark. Deionised distilled water was then added to the 100ml mark to give an approximate 1:2 ratio by volume. The spoil and water was then thoroughly stirred with a glass rod and allowed to stand for 10 minutes. The pH and temperature probes were then immersed into the supernatant and swirled. Once the pH reading had stabilised, and the temperature had been compensated for, the pH reading was recorded. The probes were rinsed thoroughly in deionised distilled water and touch-dried with a tissue between measurements. The probe's calibration was checked every five samples with buffer solutions of pH: 3.5, 7 and 10. The pH measurements were replicated three times for each spoil sample.

The moisture content, of fresh spoil samples, was calculated using the following method adapted from Allen (1989). 10-20g of fresh sample was weighed into an evaporating basin and the weight of the spoil recorded. The spoil was not sieved but large stones or plant roots were removed. The evaporating basin and weighed spoil was placed in an air-circulating oven set at 105°C and dried to a constant weight. The spoil was cooled in a dessicator and weighed. The percentage fresh moisture content of each sample was calculated from the loss in weight. The moisture content analysis was replicated three times for each spoil sample.

To analyse for the elements in the colliery spoil, each spoil sample was prepared and analysed by the following method adapted from ISO/FDIS 11047 and Radojevic and Bashkin (1999): the colliery spoil was air-dried and sieved to remove fractions above 1mm. 2g of the air-dried colliery spoil was placed in a 150ml beaker and 10ml of 1:1 HNO_3 was added. The sample was then covered with a watch glass and refluxed on a hot plate at 95°C for fifteen minutes. The digestate was allowed to cool and 5ml of

concentrated HNO_3 was added. The beaker was re-covered and refluxed for a further 30 minutes at 95°C. The watch cover was partly removed and the solution reduced to 5ml without boiling. The sample was allowed to cool and filtered through a Whatman® No. 42 filter paper using a vacuum pump and Buchner flask. The filtrate was diluted to 50 ml with deionised water and analysed using atomic absorption spectrophotometry (AAS) see Table 4.1.1. Calibration standards (See Appendix 4.1.1) for each metal were analysed; this enabled the construction of calibration curves from which the concentration of metals, extracted from the spoil samples, were determined.

Chromium was extracted as above, but before the sample was filtered it was allowed to cool and 2ml of deionised water and 3ml of 30% H_2O_2 were added to it. The beaker was gently heated to initiate the peroxide reaction. If the effervescence became excessively vigorous, the sample was removed from the hot plate. 30% H_2O_2, in 1ml increments, was added followed by gentle heating until the effervescence subsided. The sample was allowed to cool, and then 5ml of concentrated HCl and 10ml of deionised water were added to the beaker. The beaker was re-covered and the sample refluxed again for fifteen minutes without boiling. The sample was then uncovered and reduced to 5ml without boiling, allowed to cool, and then filtered and tested as above (Radojevic & Bashkin 1999).

Table 4.1.1 AAS Wavelengths used for analysis (adapted from: ISO/FDIS 11047, and Radojevic & Bashkin 1999).

Metal	Wavelength (nm)
Cd	228.8
Cr	357.9
Cu	324.7
Pb	217.0
Ni	232.0
Hg	240.0
Mo	313.3
Sn	224.6
Zn	213.9

The relationships between the chemical characteristics and the area, within the chronosequence from which the spoil was sampled, were tested with both linear and polynomial regression and analysis of variance using the statistical package Minitab (release 13). The tests which gave the highest regression coefficient between data points, either linear or polynomial regression, are displayed as trend lines in the results' figures. Polynomial regression was used to fit a regression line to curved sets of data as a quadratic term ($Y = a + bX + cX^2$). The quadratic term was used as it employs a linear model to fit a curved line to data points. The linear term in a quadratic polynomial regression represents the overall effect on the dependent variable of low to high values of the independent variable. The quadratic term, therefore, does not impact the overall effect of the relationship between the variables. Including the quadratic term when there is curvature in the data trend makes the estimate of the linear term more precise (Townend 2002). The analysis of variance tested the significance of the relationship between the chemical characteristics and the area, within the chronosequence from which the spoil was sampled.

4.1.2 Results

Figures 4.1.1-4.1.12 represent the chemical analyses results from the Stodmarsh site. Figures 4.1.13-4.1.24 represent the chemical analyses results from the Betteshanger site. Figure 4.1.25 illustrates the soil water content measured from both sites. The Figures summarise the data from both sample depths collected from the sites. They include the regression equation and R^2 values which illustrate the relationship between the chemical compositions of the spoil with the tipping ages of the spoil. Table 4.1.2 summarises the statistical significance of these relationships at P=0.05, for full statistical analyses see Appendix 4.1.2.

In the samples from Stodmarsh, none of the chemical characteristics significantly vary with time, P>0.05 (Table 4.1.2). The exception was soil moisture content in the 0.1m samples which significantly increased with age (P=0.035).

At Stodmarsh, copper, chromium, lead, nickel, zinc, mercury, arsenic and tin (Figures 4.1.1, 4.1.2, 4.1.3, 4.1.5, 4.1.6, 4.1.7, 4.1.8 and 4.1.10) all have a similar concentration against time relationship. In general at 1m deep the newer spoil had less metal than older spoil. However, the lowest concentration of arsenic is in the oldest spoil deposited 1913-1924, and arsenic exhibits a humped relationship with time (Figure 4.1.8). The metal extracted from the 0.1m samples was less than the 1m samples, except in the newest spoil in which the metal concentrations were similar between the two depths. The concentration of cadmium and fluorine in 1m samples from Stodmarsh increased as the younger spoil, in contrast the concentration in the 0.1m samples decreased as the spoil became younger (Figure 4.1.4 and 4.1.11). There is wide variation in the concentrations of molybdenum at spoil samples from both depths at Stodmarsh (Figure 4.1.9).

In the samples from 0.1m at Stodmarsh, pH ranged from 5.8 in the youngest spoil to 6.8 in the oldest spoil R^2=0.86 (Figure 4.1.12). At 1m deep, the pH relationship decreased with an increase in age from 8.1 to 7 (R^2=0.77), but was not significant (P=0.09). There was little variation in pH at Stodmarsh in comparison to Betteshanger, and the pH at Stodmarsh was close to neutral.

At Betteshanger the chemical characteristics of the spoil do not illustrate a clear trend with age. Some characteristics increase with age, and some decrease with the age of the spoil from which they were sampled (Figures 4.1.13-4.1.25). Table 4.1.2

summarises the chemical characteristics which significantly varied with the chronosequence age from which they were sampled. In the samples from 1m deep, copper, chromium, lead, zinc and molybdenum, as well as pH, change significantly with the age of the spoil sampled (Cu P=0.037, Cr P=0.049, Pb P=0.043, Zn P=0.044, Mo P=0.037 and pH P=0.049). Copper and zinc decrease as the spoil gets younger (Figures 4.1.13, 4.1.8 and 4.1.24), chromium, lead and molybdenum increase as the spoil gets younger (Figures 4.1.14, 4.1.15, 4.1.21)

In the 0.1m samples, from Betteshanger, mercury (P=0.041), soil moisture content (P=0.001), and pH (P=0.017) had a significant variation with age (Table 4.1.2). Mercury concentration, in the spoil, decreases as the spoil gets older, whereas water content increases as the age of the spoil increases.

At Betteshanger, pH varied linearly from 4.5 in the youngest spoil to 6 in the oldest spoil in the 0.1m samples (R^2=0.92), and between 5 and 6.5 in the 1m samples. However, the relationship in pH with age at the 1m depth was not as strong R^2=0.2 (Figure 4.1.24).

Table 4.1.2 Summary of the significance of regression analyses for the chemical investigation versus the age of the sampled area.

Analysis	Betteshanger 1m samples	Betteshanger 0.1m samples	Stodmarsh 1m samples	Stodmarsh 0.1m samples
Cadmium	ns	ns	ns	ns
Chromium	*	ns	ns	ns
Copper	*	ns	ns	ns
Lead	*	ns	ns	ns
Nickel	ns	ns	ns	ns
Zinc	*	ns	ns	ns
Mercury	ns	*	ns	ns
Arsenic	ns	ns	ns	ns
Molybdenum	*	ns	ns	ns
Tin	ns	ns	ns	ns
Fluorine	ns	ns	ns	ns
Soil moisture content	ns	***	ns	*
pH	*	**	ns	ns

Key: *** = P<0.001, ** = P<0.01, * = P<0.05, ns = non significant difference.

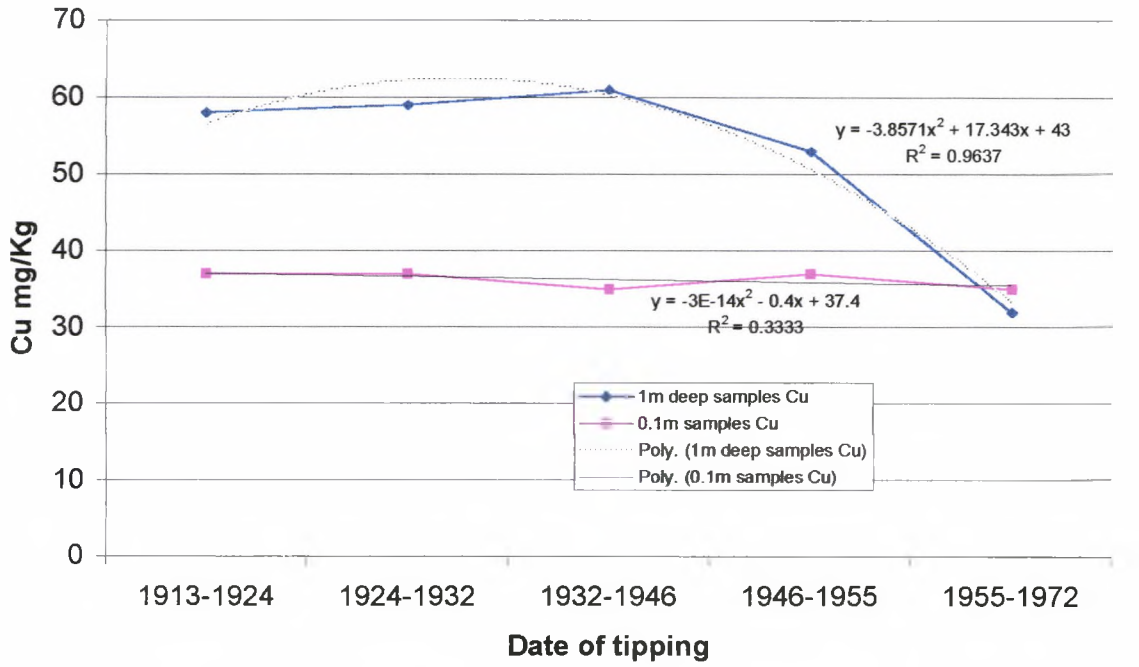

Figure 4.1.1 Copper concentration in the chronosequence at Stodmarsh

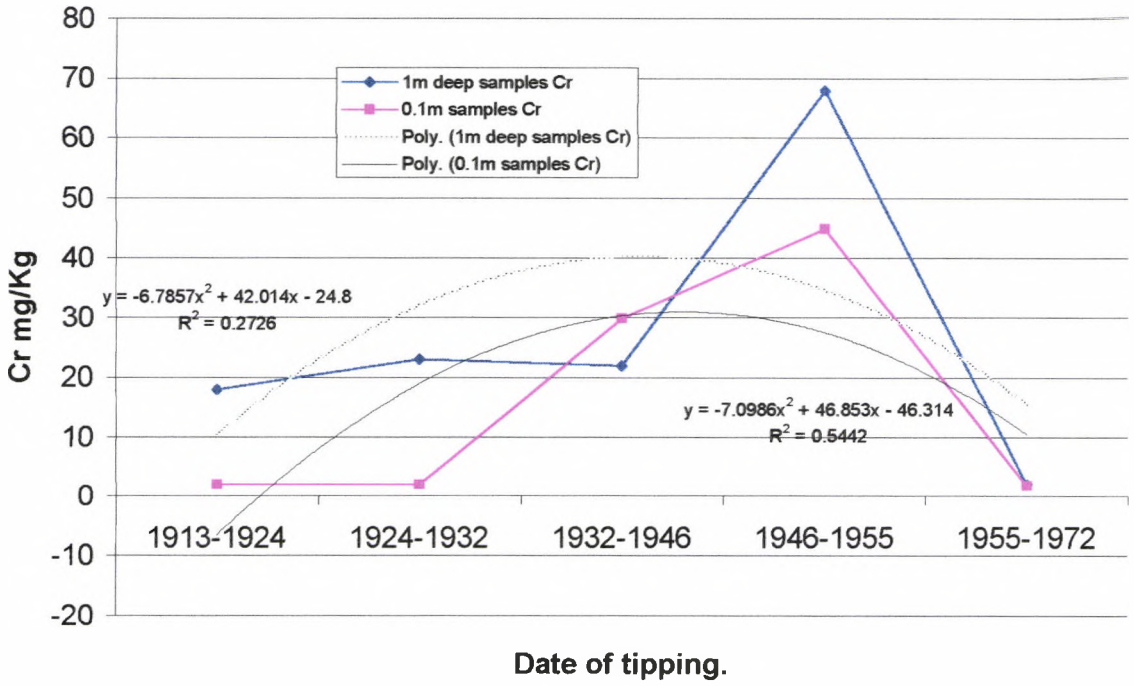

Figure 4.1.2 Chromium concentration in the chronosequence at Stodmarsh

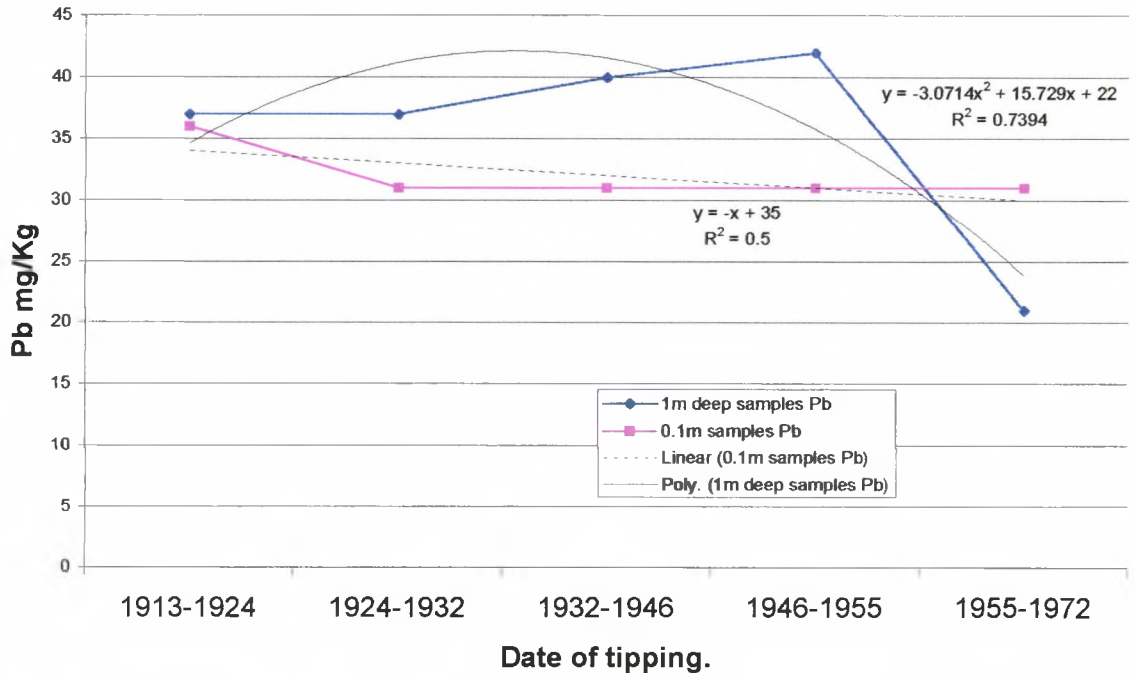

Figure 4.1.3 Lead concentration in the chronosequence at Stodmarsh

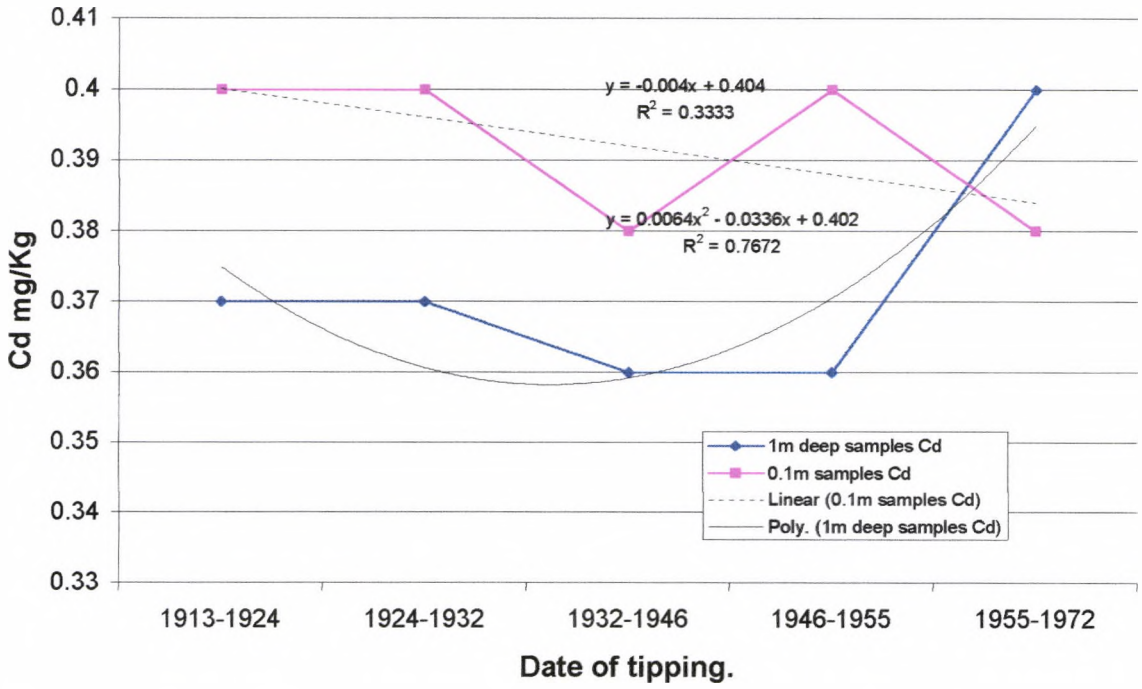

Figure 4.1.4 Cadmium concentration in the chronosequence at Stodmarsh

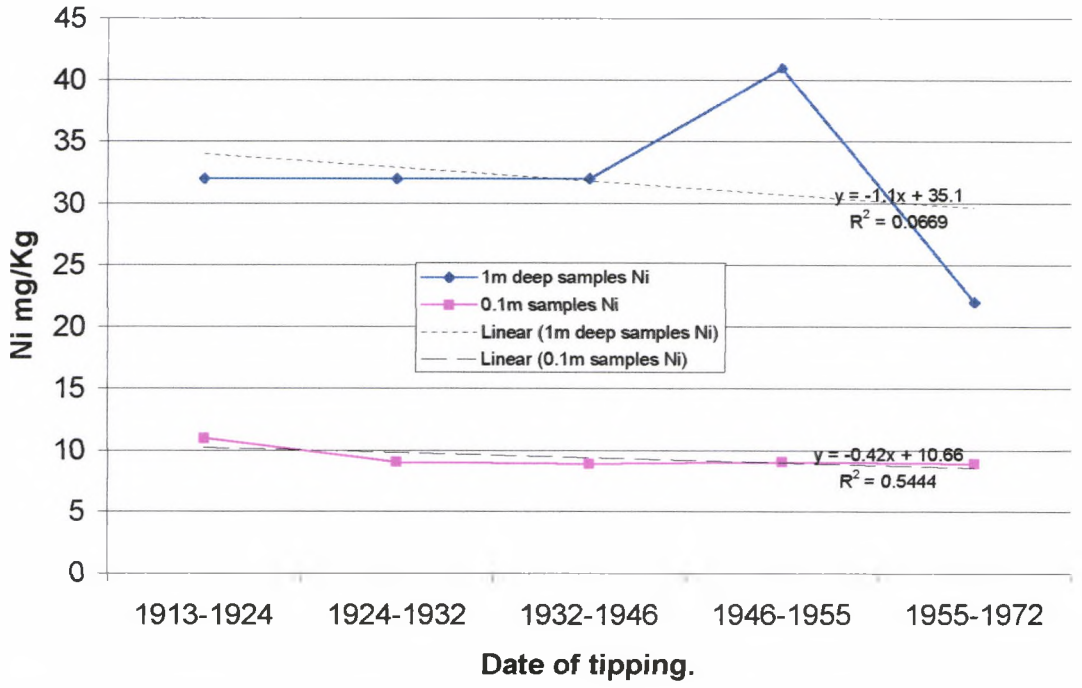

Figure 4.1.5 Nickel concentration in the chronosequence at Stodmarsh

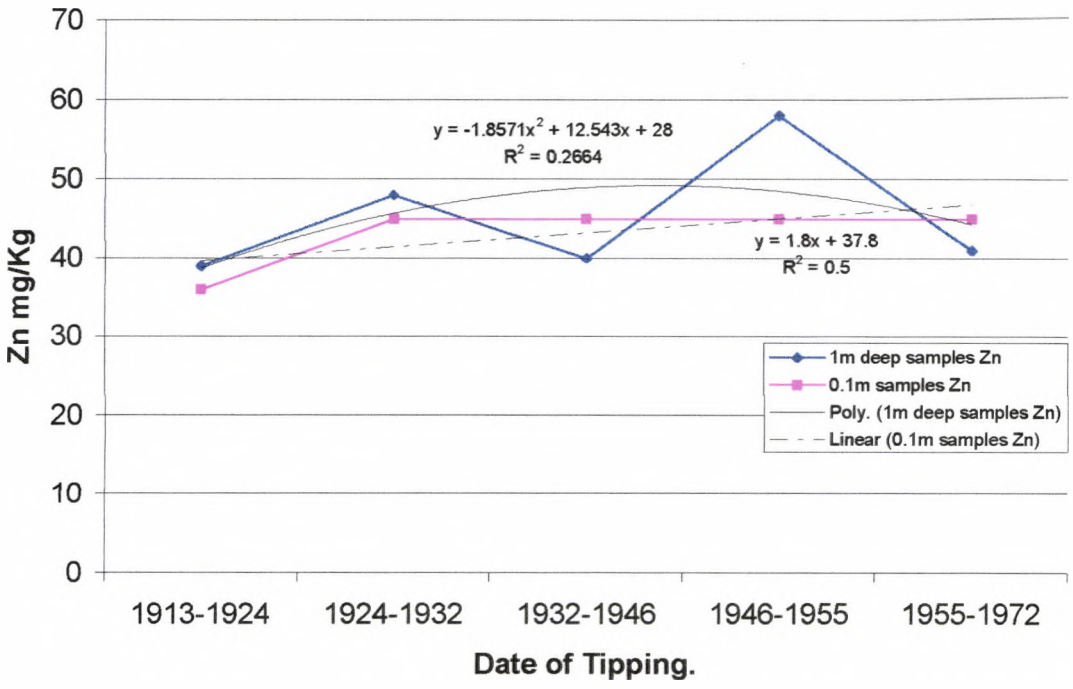

Figure 4.1.6 Zinc concentration in the chronosequence at Stodmarsh

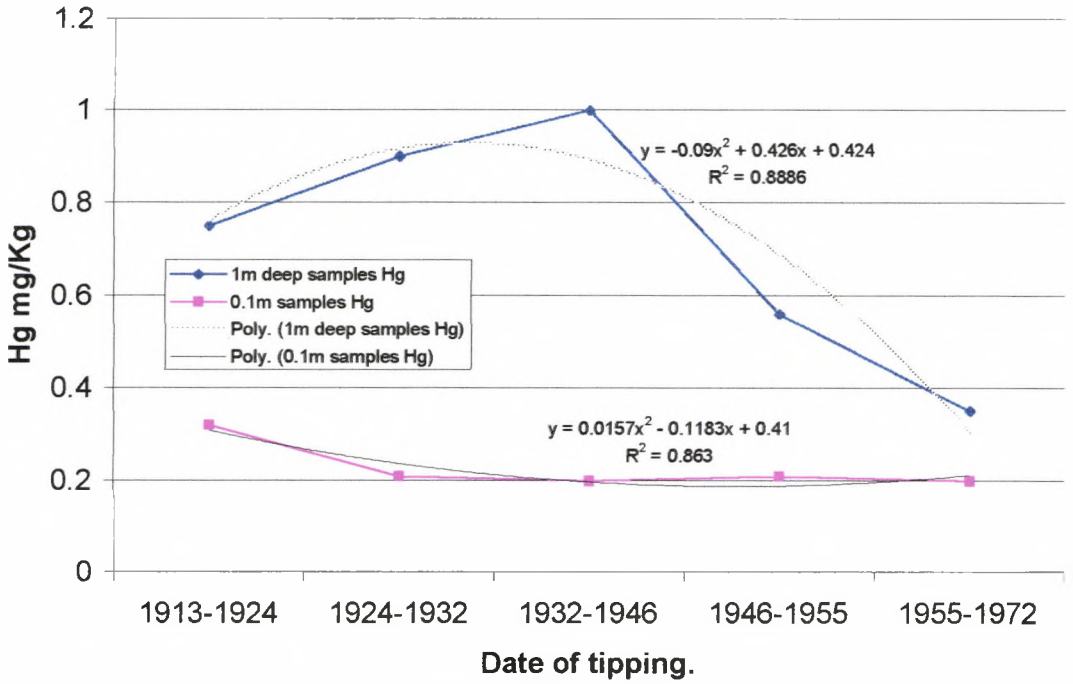

Figure 4.1.7 Mercury concentration in the chronosequence at Stodmarsh

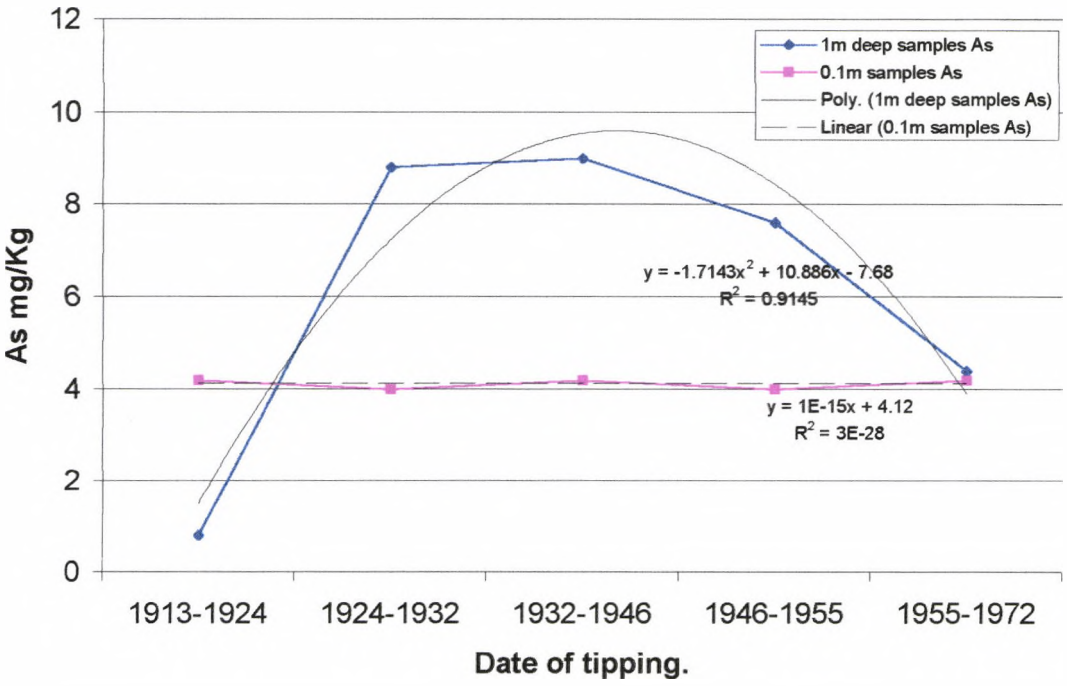

Figure 4.1.8 Arsenic concentration in the chronosequence at Stodmarsh

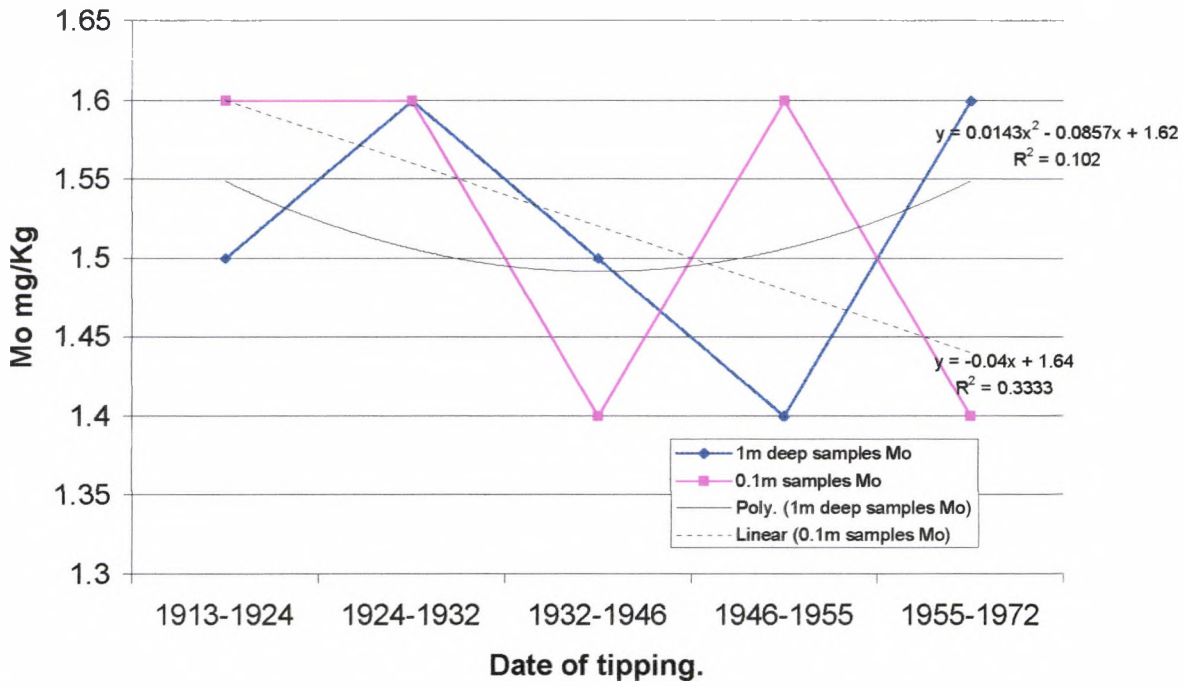

Figure 4.1.9 Molybdenum concentration in the chronosequence at Stodmarsh

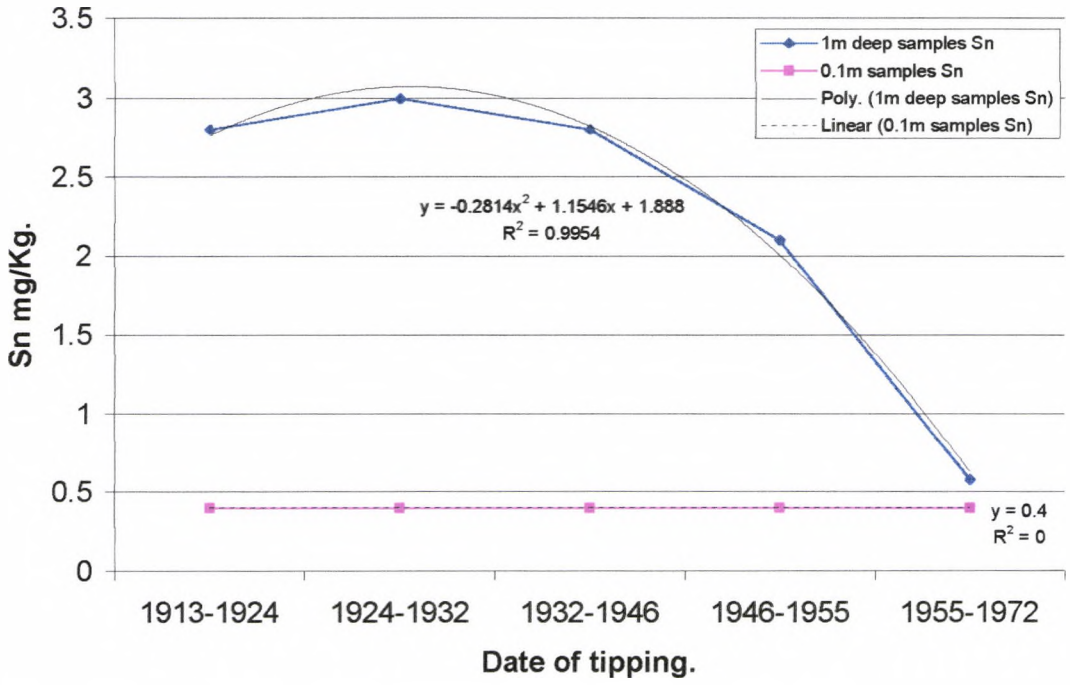

Figure 4.1.10 Tin concentration in the chronosequence at Stodmarsh

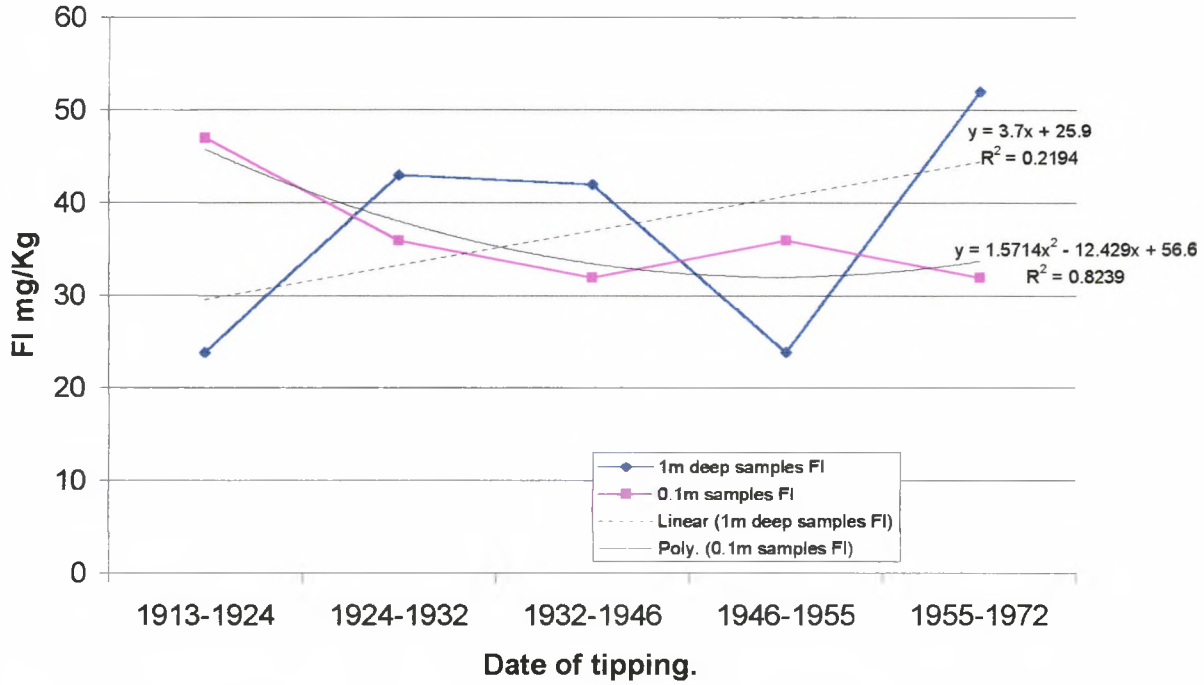

Figure 4.1.11 Fluorine concentration in the chronosequence at Stodmarsh

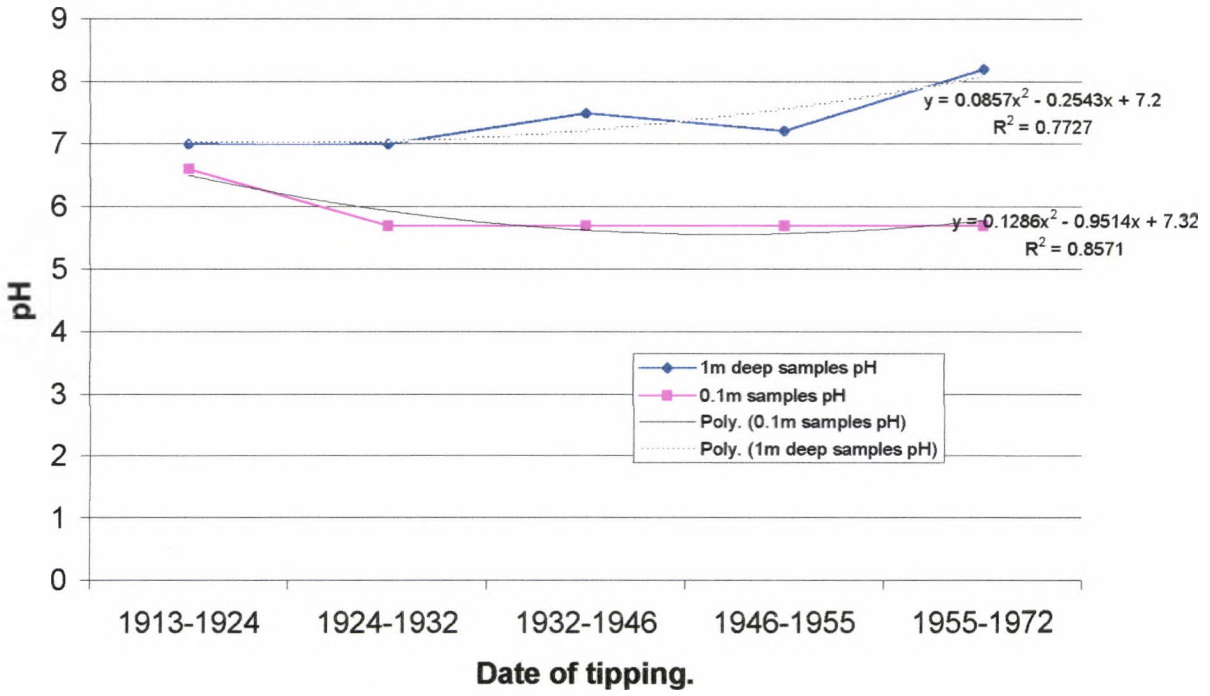

Figure 4.1.12 pH across the chronosequence at Stodmarsh

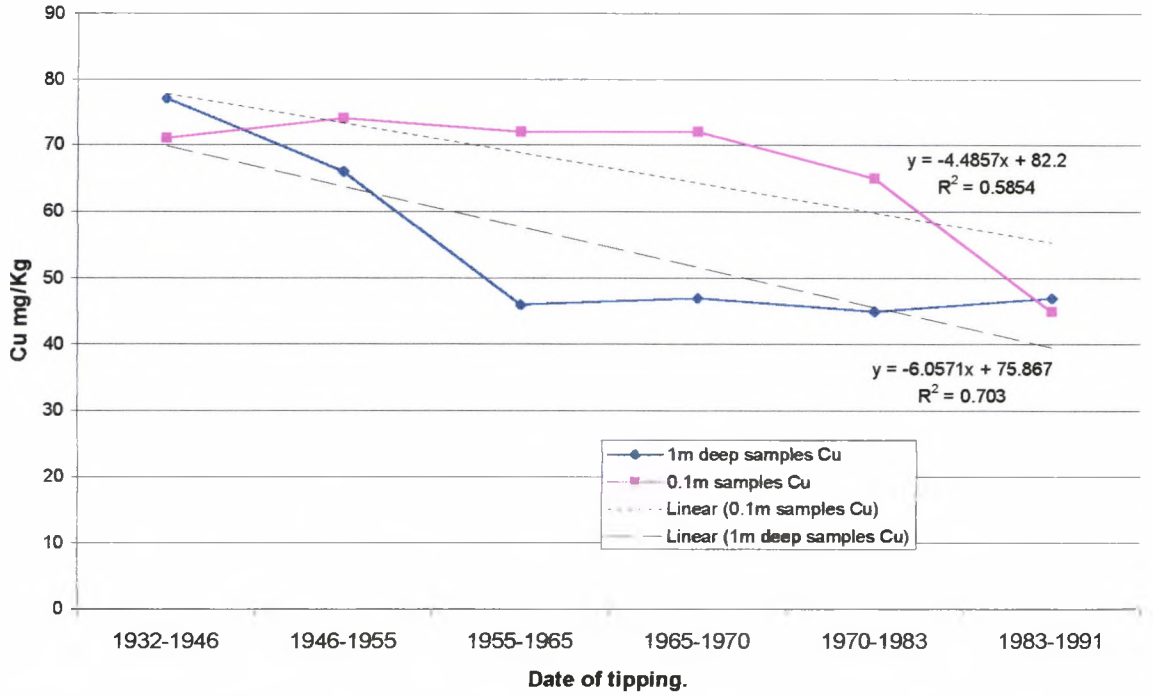

Figure 4.1.13 Copper concentration in the chronosequence at Betteshanger

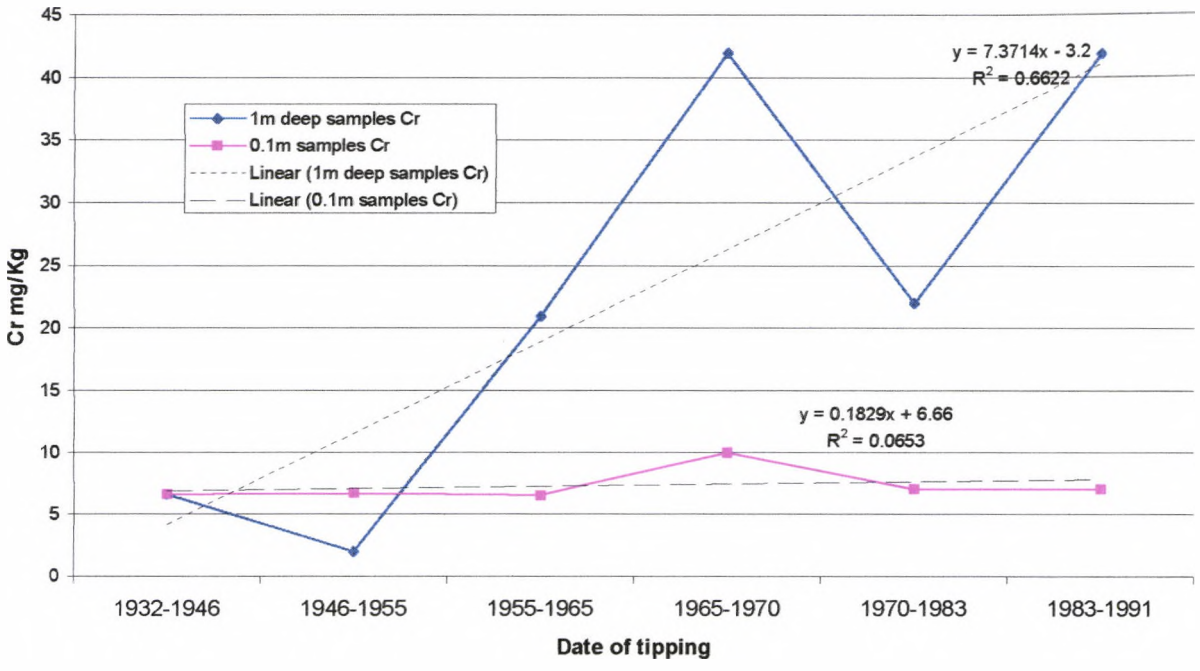

Figure 4.1.14 Chromium concentration in the chronosequence at Betteshanger

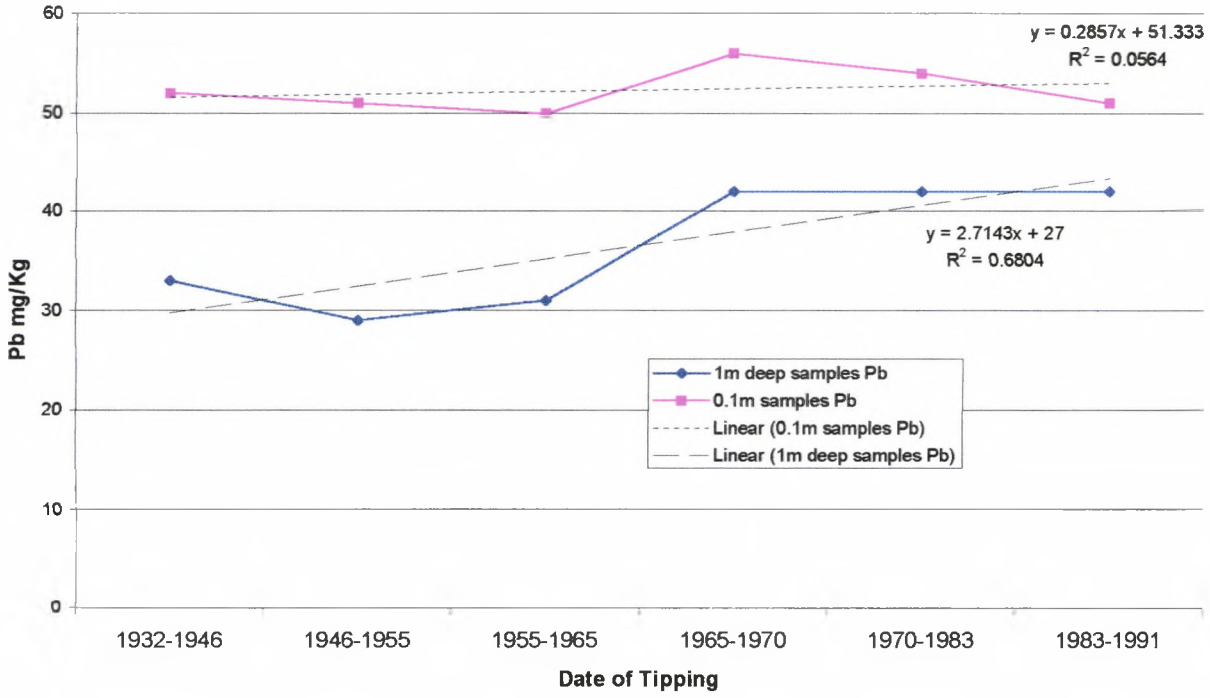

Figure 4.1.15 Lead concentration in the chronosequence at Betteshanger

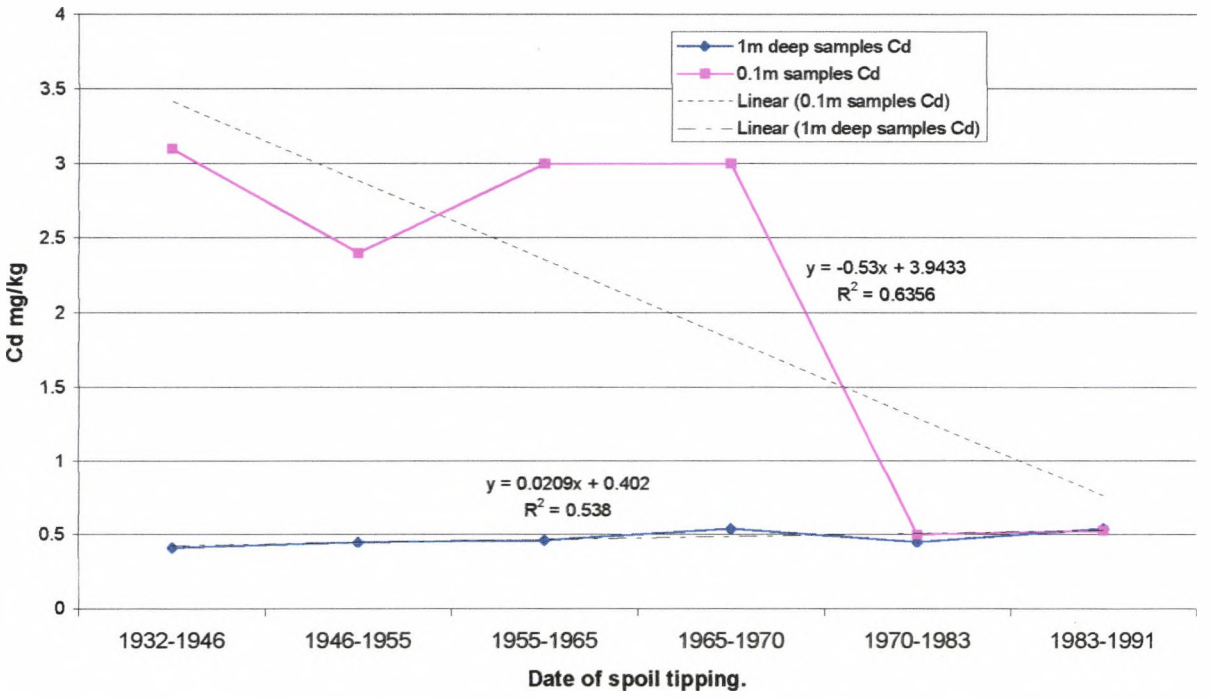

Figure 4.1.16 Cadmium concentration in the chronosequence at Betteshanger

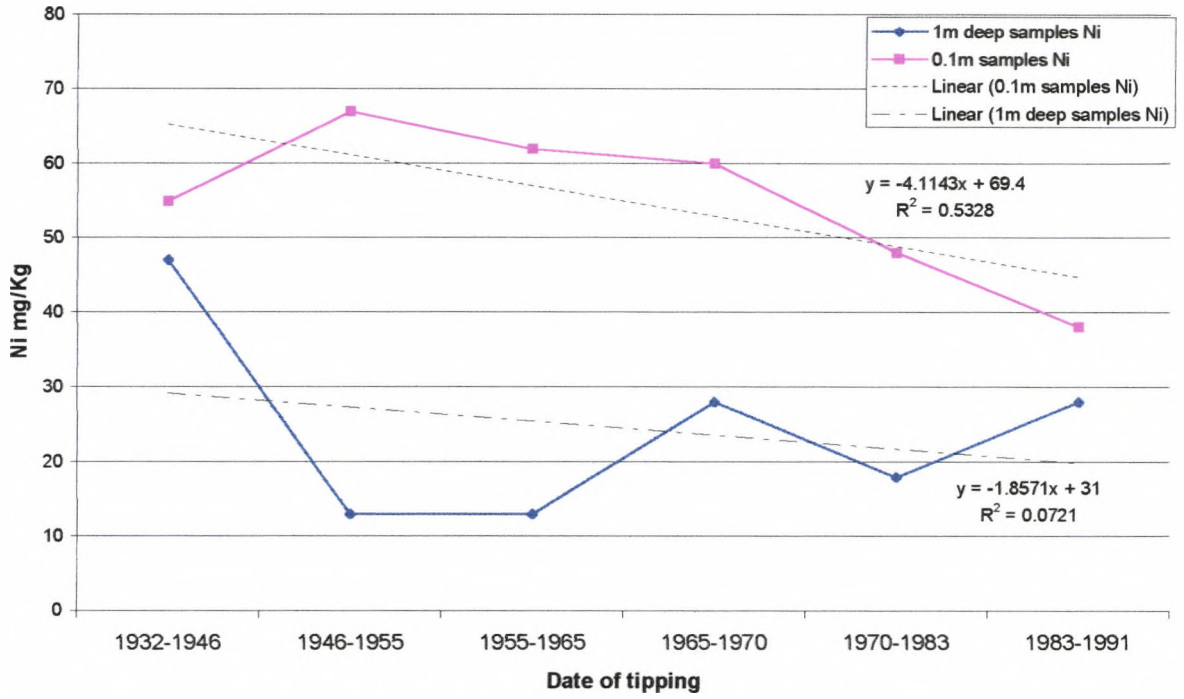

Figure 4.1.17 Nickel concentration in the chronosequence at Betteshanger

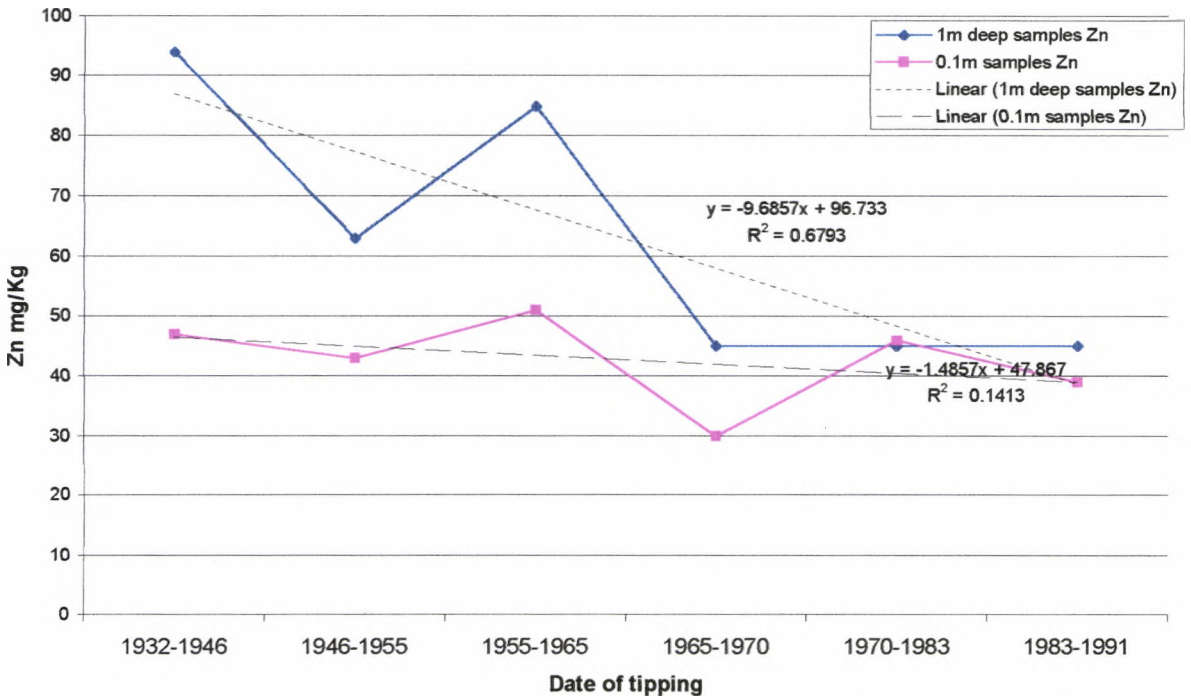

Figure 4.1.18 Zinc concentration in the chronosequence at Betteshanger

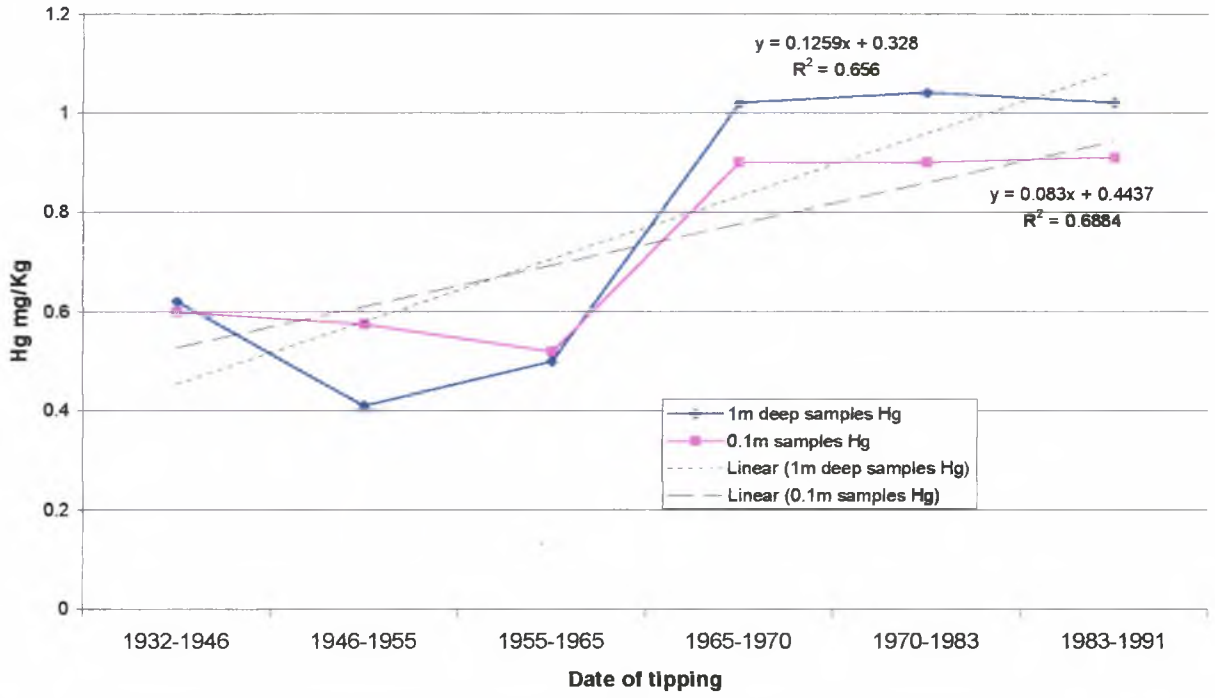

Figure 4.1.19 Mercury concentration in the chronosequence at Betteshanger

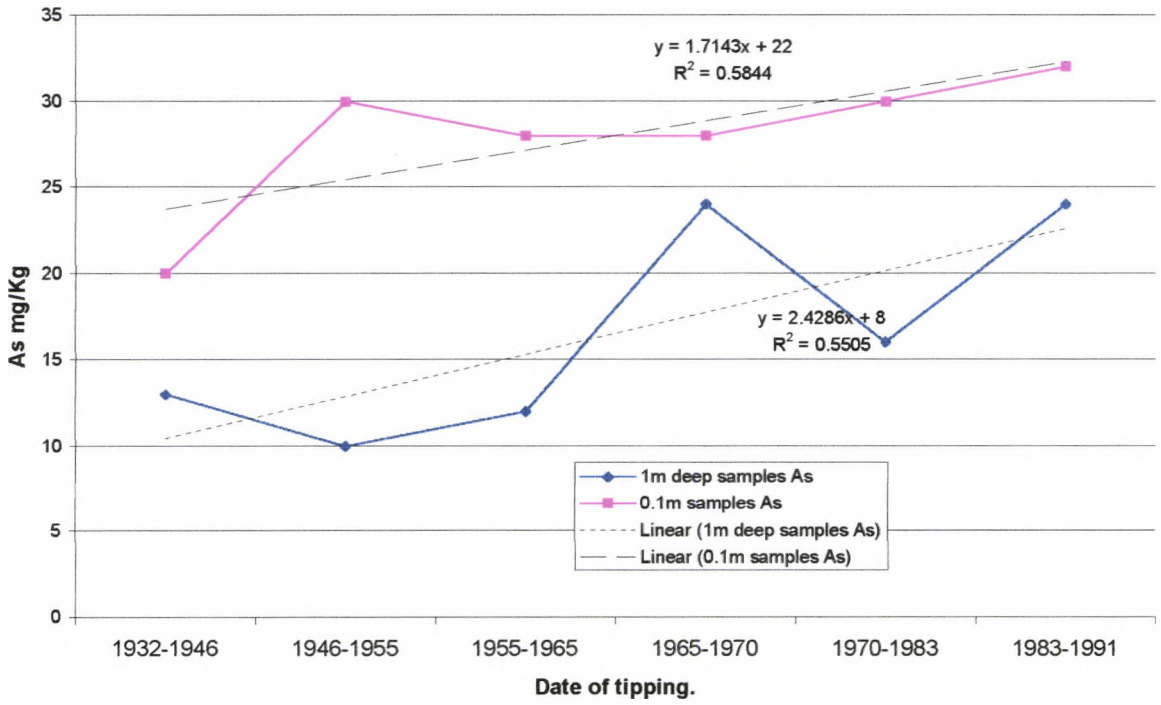

Figure 4.1.20 Arsenic concentration in the chronosequence at Betteshanger

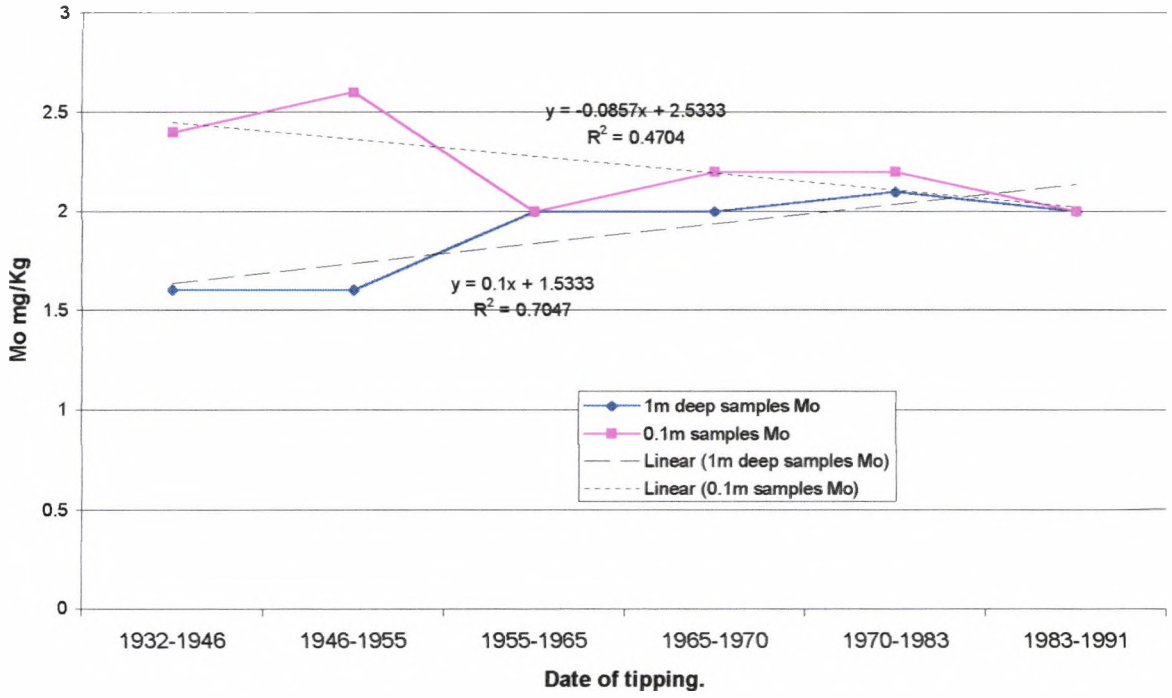

Figure 4.1.21 Molybdenum concentration in the chronosequence at Betteshanger

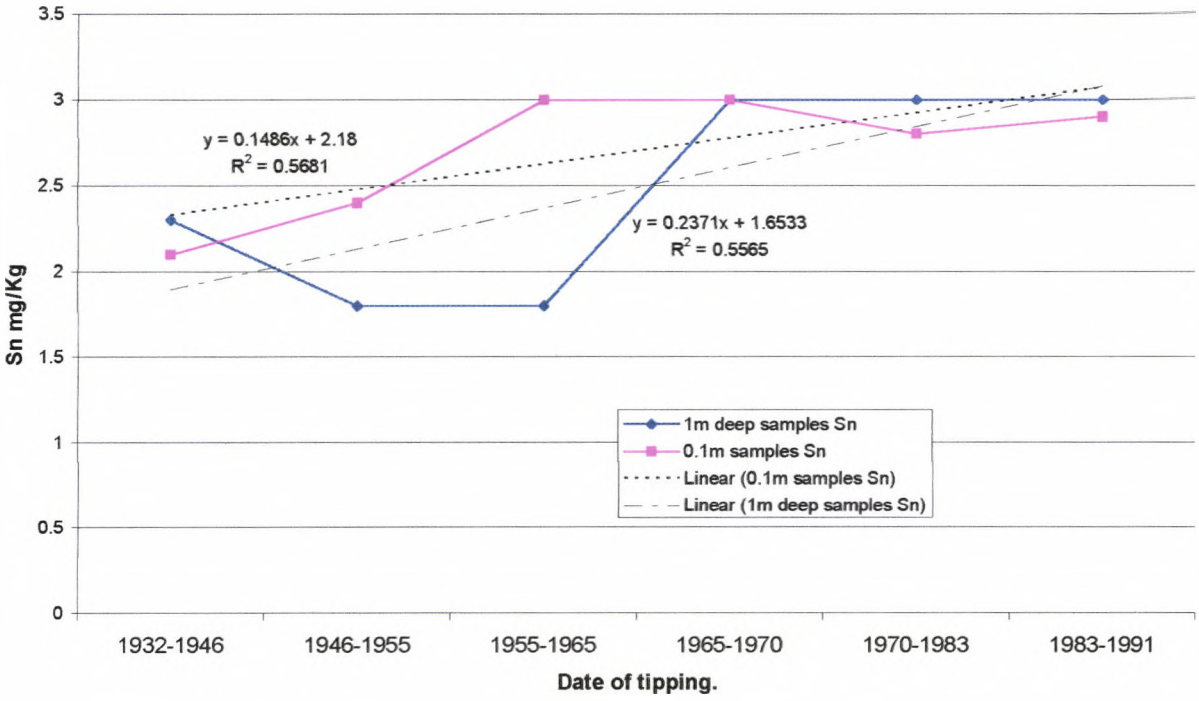

Figure 4.1.22 Tin concentration in the chronosequence at Betteshanger

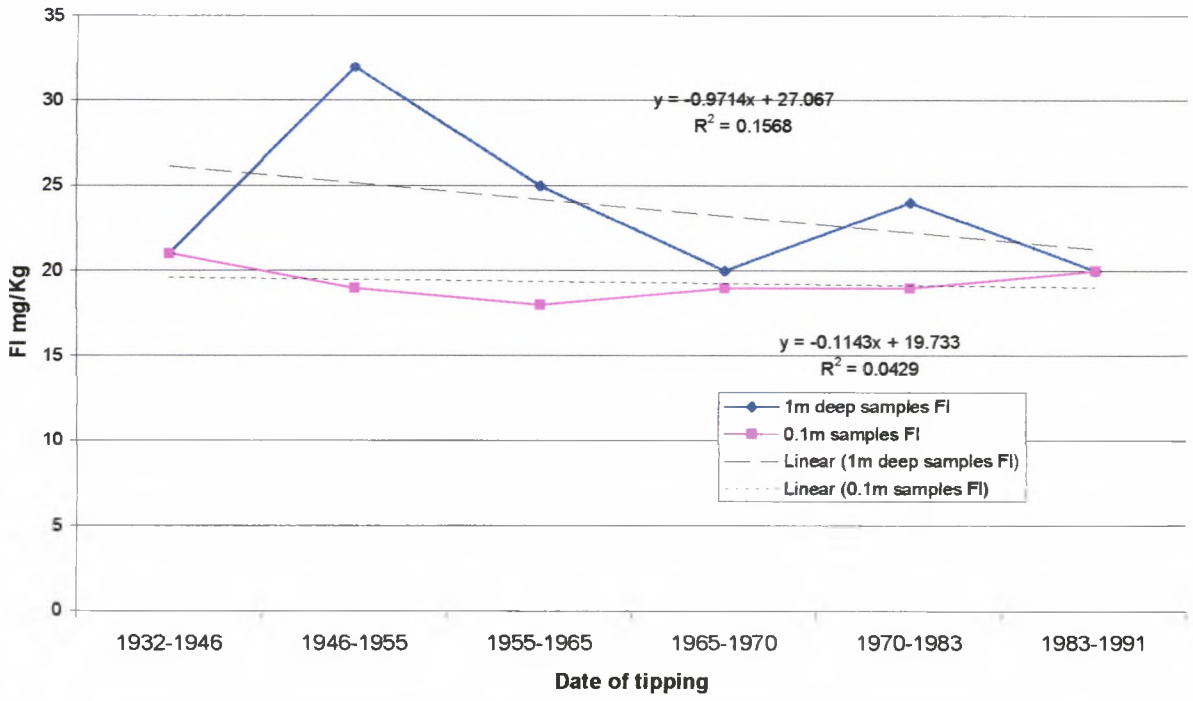

Figure 4.1.23 Fluorine concentration in the chronosequence at Betteshanger

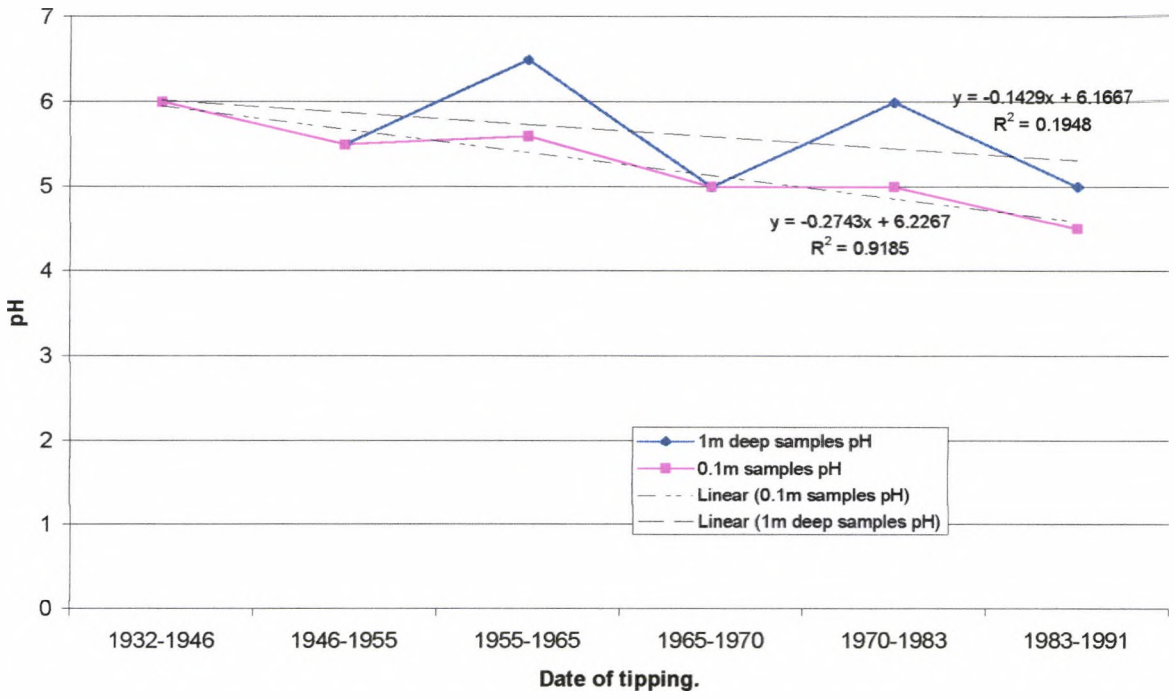

Figure 4.1.24 pH across the chronosequence at Betteshanger

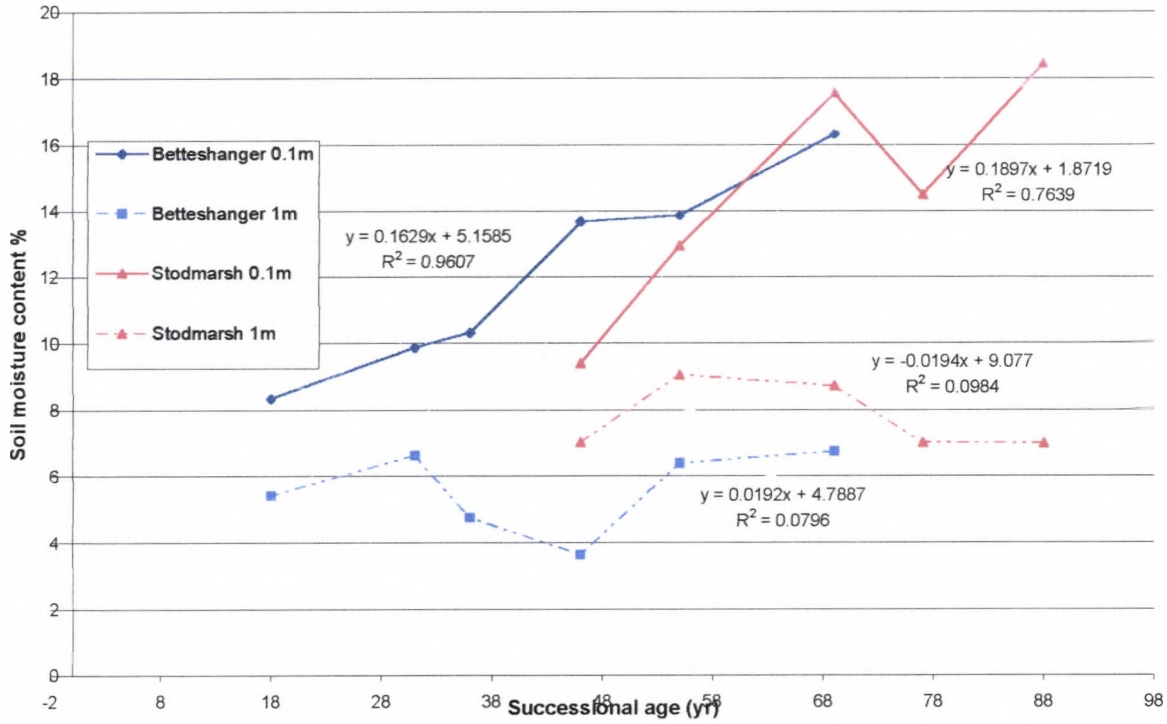

Figure 4.1.25 Soil moisture content in the upper 10 cm and 1 m deep spoil from the chronosequences at Stodmarsh and Betteshanger

4.1 .3 Discussion.

The relationship between age and chemical concentration was not the same for all elements (Figures 4.1.1.to 4.1.25). There was also in-site and between-site variation in composition of the colliery spoil. This suggests that the chemical composition was not equitable across the age range and sites. However, the concentrations of chemicals which could have toxic effects on the vegetation system are well below the critical toxicity levels and those elements which are micro nutrients are, also, within tolerable levels (Doubleday 1974: Bentham *et al.* 1992; Radojevic & Bashkin 1999; Perfus-Barbeoch *et al.* 2002). For these elements, there would not be a critical effect on the establishing vegetation within the concentration variations analysed see Table 4.1.3.

The changing concentrations for elements, at the two different depths sampled, indicate how the elements move through the developing spoil system with time (Figures 4.1.1-4.1.11 and Figures 4.1.13-4.1.23). In the 0.1m samples it was possible that the surface environment had led to conditions which promoted

weathering and then there was leaching of these elements out of the surface 0.1m of the spoil. The subsequent increased concentration with age of these minerals, sampled at 1m, could have been the result of their progress through the spoil profile. The decline in the metal concentration, in the oldest spoil, could have been due to their loss from the spoil profile, probably via ground water transport or less likely from plant uptake (Levine *et al.* 1989). The metal concentrations within the spoil may well influence other characteristics of the spoil such as pH (Machin & Navas 2000). Some traces of the elements were found in the spoil profile at all ages. These traces probably represented mineral complexes and residues which were not soluble, so would not be leached from the profile and also not available for plants (Singh & Narwal 1984). However, the metal concentrations measured were such that they would not influence the vegetation developing on Stodmarsh (*Ibid.*) as they were below critical levels (Table 4.1.3).

Table 4.1.3 Normal and critical ranges of heavy metals in soils (adapted from Radojevic & Bashkin 1999; Perfus-Barbeoch *et al.* 2002).

Metal	**Normal range in soils** (mg kg-1)	**Critical concentration in soils which affect plant growth** (mg kg-1)
Cd	0.01-2.0	3-8
Cr	5-1500	75-100
Cu	2-250	60-125
Hg	0.05	0.3-5
Mo	0.1-4	2-10
Ni	2-750	100
Pb	2-300	100-400
Sn	1-200	50
Zn	1-900	70-400

The significant changes in pH at Betteshanger, and soil moisture content at both sites, in relation to the age of the spoil (Figures 4.1.24 & 4.1.25 respectively and Table 4.1.2), will potentially have influenced the vegetation at the two sites. Both characteristics increase with spoil age. The relationship between pH and substratum age supports the findings of Costigan, Bradshaw, & Gemmell (1981). They found that pH increases in older colliery spoil as a result of the oxidation of sulphur minerals and the acidic products then leaching from the spoil profile. Low pH does have an

influence on the species able to establish and tolerate acid conditions (Bell 2001). The pH scale is logarithmic and therefore the difference in pH of 1.5 will have a large effect on the vegetation pattern establishing, especially as the variation takes the pH to very acidic conditions (Kimber, Purford, & Duncan 1978). However, the changing pH at Stodmarsh (Figure 4.1.1)2, therefore, would not have the same magnitude of influence on the vegetation as the variation in pH at Betteshanger (Figure 4.1.24).

The low pH at Betteshanger will also affect the developing nitrogen cycle at the site. Nitrogen fixation and denitrification are inhibited by acid conditions (Imeka & Cooperb 2002), and the overall nutrient budget at Betteshanger could be limited by the low pH. A major source of nitrogen fixation in colliery spoil can be from Leguminosae species (Chapman, Collins, & Younger 1996; Bradshaw 1997), but the formation of root nodules is hindered by the acid sensitivity of *Rhizobium* bacteria. Tate (1985) found that a pH of 5 and below prevents the root nodulation process. Nitrogen studies for Stodmarsh and Betteshanger are described in Chapter 5.

Soil moisture content (Figure 4.1.25) in 0.1m samples significantly increased with the age of the spoil across both Stodmarsh (R^2=0.76) and Betteshanger (R^2=0.96) (Table 4.1.2). The soil moisture content analysed from 1m deep spoil did not significantly change with age at either Stodmarsh (R^2=0.1) or Betteshanger (R^2=0.08) (Table 4.1.2). The change in soil moisture content at the biologically active surface illustrates an important change in the property of the spoil. Since the soil moisture is linked to the increase in the vegetation component of the spoil (Down 1975). The different soil water availability can influence plant community structure (Kadmon 1995). The soil moisture content of the 1m deep samples did not significantly change with age, probably due to the lack of a biologically active component in the spoil at this depth. As the vegetation system developed on the two sites, it increased the organic component in the 0.1m of the soil which was forming. The soil moisture content was therefore a function of the modification of the spoil by the vegetation systems which were developing. The increase in the soil moisture content has implications for the vegetation system developing on the colliery waste, as water availability is a limiting environmental component on colliery waste (Down 1975, Chadwick 1987, Holl 2002, Ludwig, Hindley, and Barnett 2003).

4.2 Natural vegetation colonisation of chronosequenced tipping episodes.

The chemical characteristics have been shown to change with the age of the spoil at the Stodmarsh and Betteshanger sites (4.1). The natural vegetation developing at Betteshanger and Stodmarsh was investigated to identify if there was a corresponding change in vegetation structure and composition, with age of the colliery waste, at the two sites.

4.2.1 Method

The vegetation at the Stodmarsh site was sampled along 5 transects (Figure 3.1), 17/7/2000-27/7/2000. Three $1m^2$ quadrats, were randomly placed along each of the five transects in each of the 5 chronsequenced areas, to give 15 X$1m^2$ quadrats per aged area of the site. In each $1m^2$ quadrat, data from 30 randomised point samples were recorded. Each time a species intersected with a randomised point within the quadrat, it was scored.

The vegetation at the Betteshanger site was hierarchically sampled using 10 randomised $1m^2$ quadrats per aged area (Figure 3.2.2), 1/8/2000-18/8/2000. In every $1m^2$ quadrat, data from 30 randomised point samples were recorded. Each time a species intersected with a randomised point within the quadrat, it was scored.

A point quadrat sampling method, for measuring the vegetation at the sites, was decided upon because point sampling is one of the most objective ways to sample vegetation cover (Bonham 1989, Silvertown *et al.*1992), and can be an indirect method of assessing yield or biomass without destructive sampling (Greig–Smith 1983). Morrison and Yarranton (1970) used cross wires as approximation to points with zero diameters. However, Goodall's (1952) work indicated that a point, with a diameter of 0.5mm, would be an appropriate size to score the vegetation at the Betteshanger and Stodmarsh colliery sites. A pin of 0.5mm was therefore randomly positioned 30 times within the $1m^2$ quadrats. The position of the pin was determined by dividing the $1m^2$ quadrat into a 100 square grid using lines 10cm apart. A random number table was then used to select the position of each pin which was placed into the centre of the corresponding grid square. The vegetation in contact with each pin was scored so that each part of a plant touching the pin, of each species, was recorded. In areas with tree cover, pins 3m long were used to determine canopy cover. The pins were used to sight along with X15 magnification binoculars, an

adaptation of Reynolds and Edwards (1977) method, to determine the number of hits/counts for each tree species.

The vegetation data was categorised into four functional groups: Gramineae, Leguminosae, woody species and others to enable the functional comparison of the vegetation at the sites. Point quadrat score and species data for each area within the chronosequences at Betteshanger and Stodmarsh were subjected to principal components analyses and hierarchical cluster analyses using the statistical package Minitab (release 13). The analyses were used to determine if there were any distinguishable patterns within the plant communities developing at Betteshanger and Stodmarsh.

4.2.2 Results.

Figures 4.2.1 and 4.2.2 illustrate the mean point quadrat scores for the functional vegetation types sampled from the chronosequences at Betteshanger and Stodmarsh. Error bars represent standard deviation.

At Betteshanger woody species are found on all the different aged spoil, although they tend to become increasingly dominant in the older spoil (Figure 4.2.1). The exception is in the very youngest spoil at Betteshanger, where woody species are the only plants to be found. Leguminosae are only found in the plant community from the 36 year old spoil at Betteshanger. This contrasts to the presence of Leguminosae in every plant community found at Stodmarsh. Leguminosae are found dominating the plant communities in the youngest spoil at Stodmarsh, and decreasing with importance as the spoil ages (Figure 4.2.2). Woody species only appear in spoil 69 years old and older at Stodmarsh, but become increasingly important within the plant communities as the spoil ages. Grasses and other species do not show any definitive trend with the age of the spoil.

Figures 4.2.3 and 4.2.4 illustrate the first two principal components (eigenvalues) plotted against each other. The eigenvalues were calculated from the principal component analyses for the species point quadrat scores at Betteshanger and Stodmarsh respectively (Appendices 4.2.1 and 4.2.3). At Betteshanger, only two discernable communities were differentiated, one being the plants located on the spoil 36 years old, and the other comprising the plants from all the other aged

communities sampled. At Stodmarsh five different communities were differentiated which corresponded to the age of the spoil from which they were sampled.

Figure 4.2.5 and 4.2.6 summarise the similarity matrix dendrograms generated by the hierarchical cluster analyses, which illustrate the relationship of each species to every other species within the plant communities at the two sites. Table 4.2.1 and Table 4.2.2 are the final partition of the hierarchical cluster analyses. These tables illustrate the species composition which differentiates the communities found within the chronosequences at Betteshanger and Stodmarsh (for full Hierarchical Cluster Analyses see appendices 4.2.2 and 4.2.4).

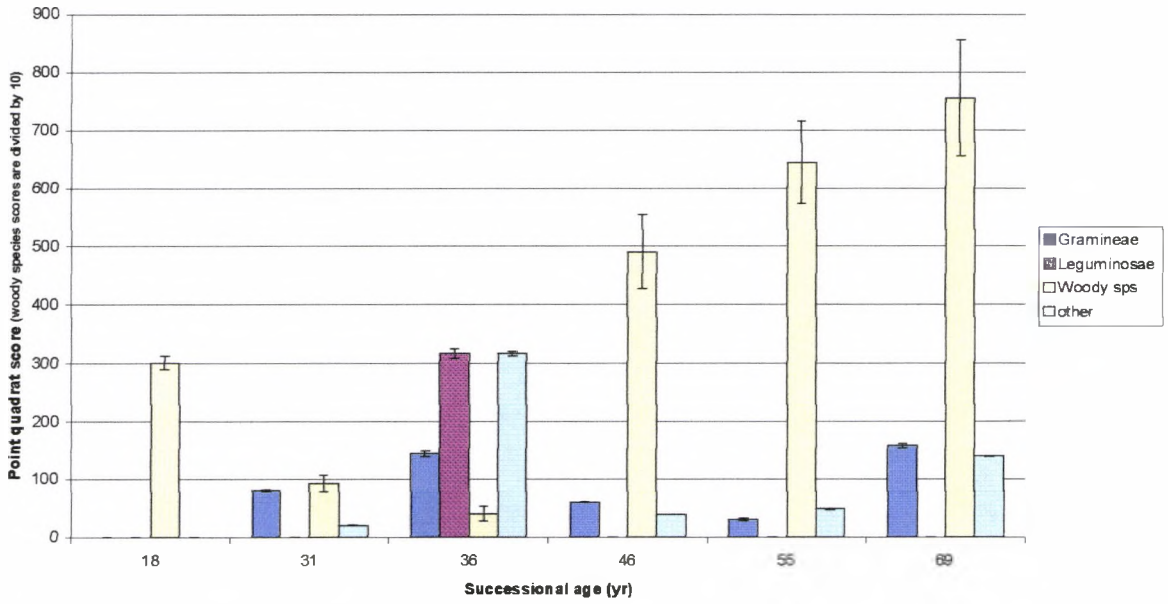

Figure 4.2.1 Functional vegetation types plotted against chronosequenced areas. Data collected 1st-18th August 2000 from randomised quadrats on Betteshanger.

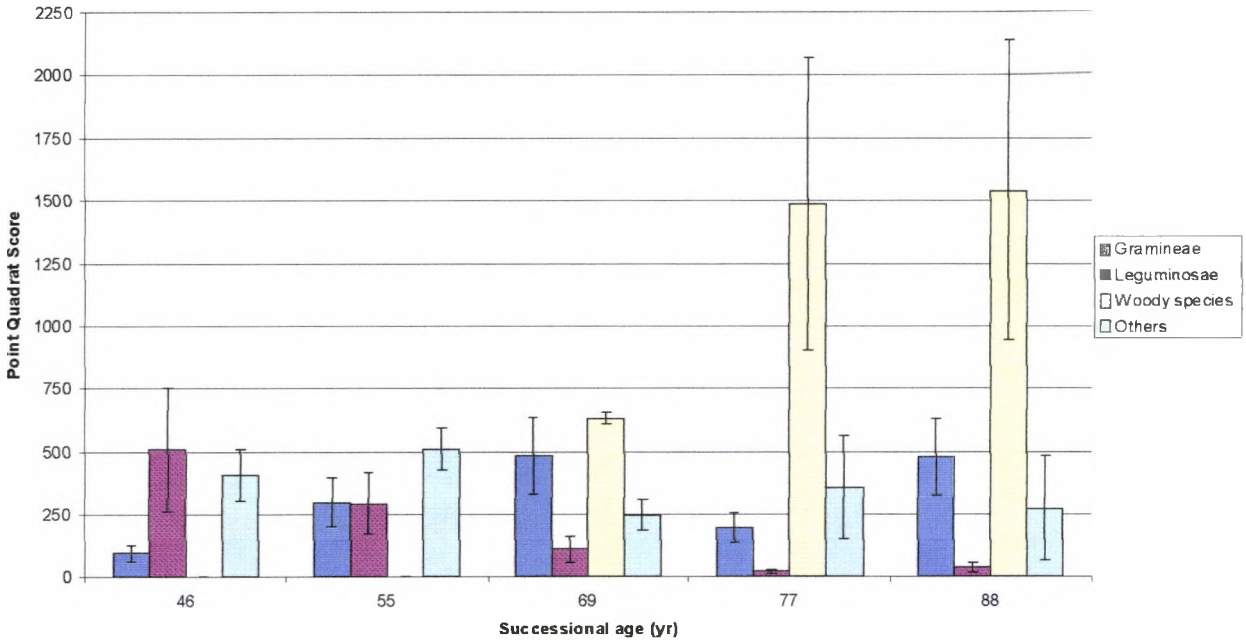

Figure 4.2.2 Functional vegetation types plotted against chronosequenced areas. Data collected 17th-27th July 2000 from five vegetation transects on Stodmarsh.

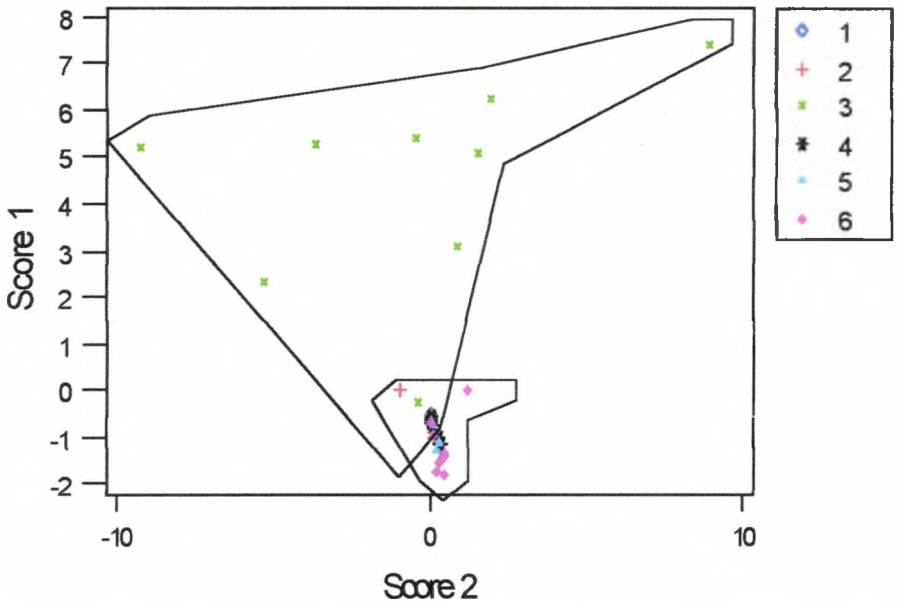

Figure 4.2.3 The first two principal components (eigenvalues) plotted against each other from the principal component analysis of the species point quadrat scores from the chronosequence at Betteshanger.

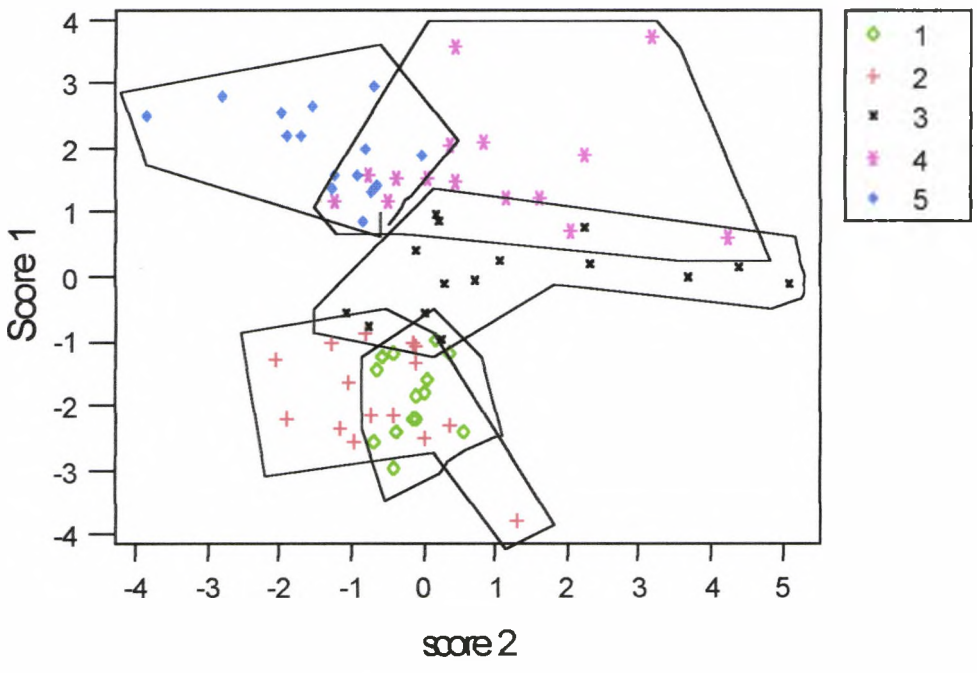

Figure 4.2.4 The first two principal components (eigenvalues) plotted against each other from the principal component analysis of the species point quadrat scores from the chronosequence at Stodmarsh.

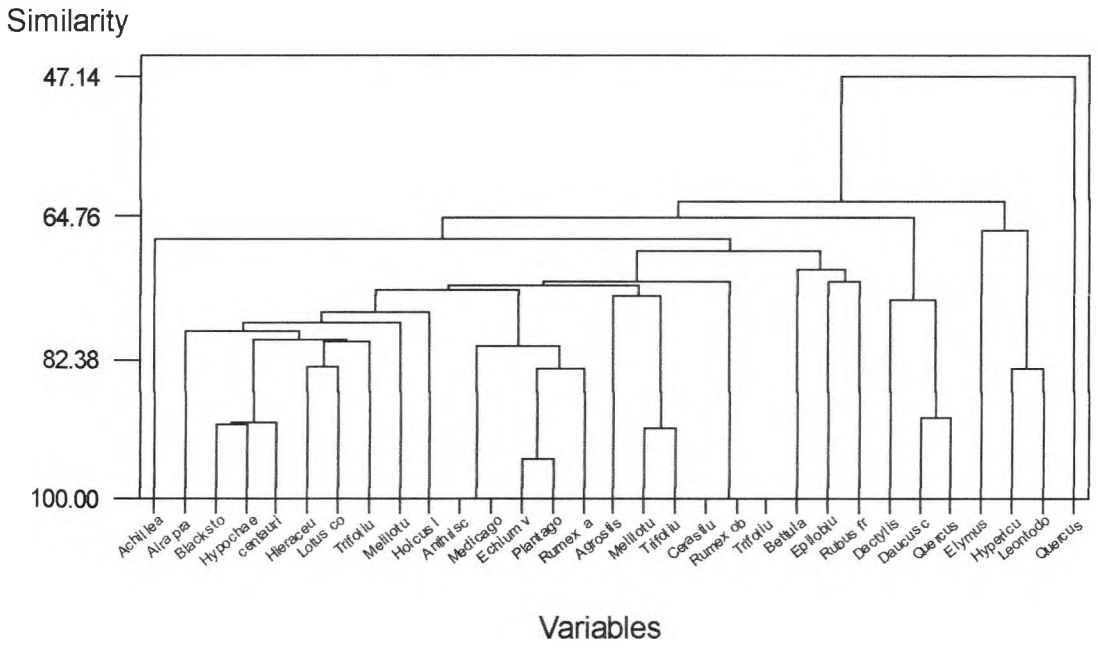

Figure 4.2.5 Similarity matrix dendrogram summarising the hierarchical cluster analysis of the Betteshanger vegetation data from the chronosequenced areas.

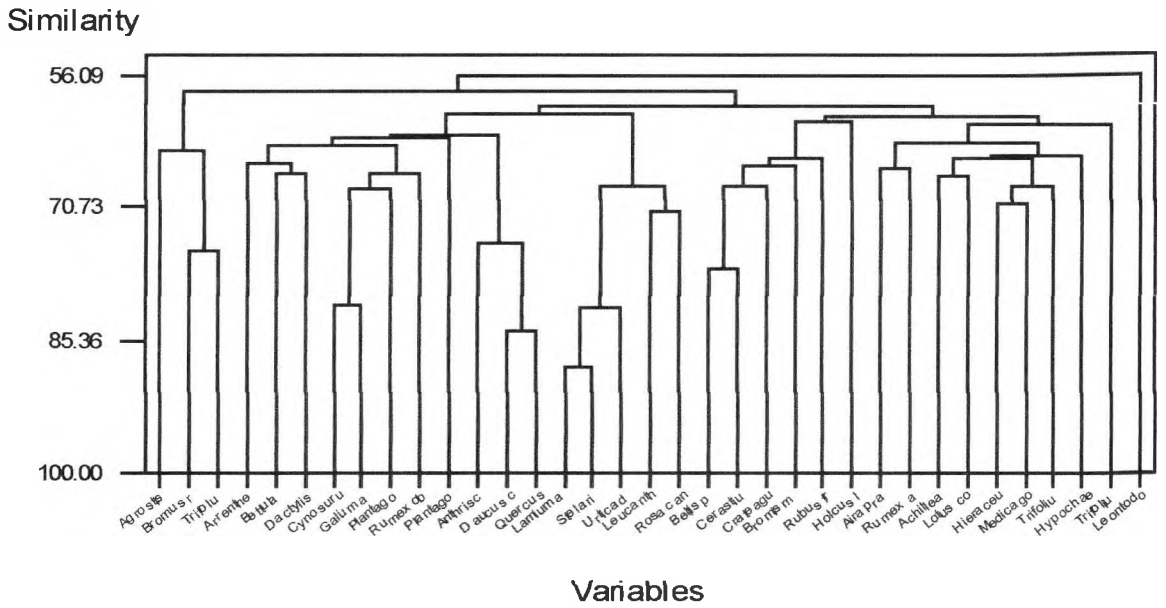

Figure 4.2.6 Similarity matrix dendrogram summarising hierarchical cluster analysis of the Stodmarsh vegetation data from the chronosequenced areas.

Table 4.2.1 Final partition from the Hierarchical Cluster Analysis of Variables of the species clusters from the chronosequenced vegetation transects at the Stodmarsh site.

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Achillea millefolium, Aira praecox, Hieracium pilosella, Hypochoeris radicata, Lotus corniculatus, Medicago lupulina, Rumex acetosella, Trifolium repens, Trifolium pratense.	*Agrostis capillaris Bromus ramosus, Trifolium arvense.*	*Anthriscus sylvestris, Arrenatherum elatius, Betula pendula, Cynosurus cristatus, Dactylis glomerata, Daucus carrota, Galium aparine, Plantago lanceolata, Plantago media, Quercus robur, Rumex obtusifolius*	*Bellis perennis, Bromis mollis, Cerastium arvense, Crataegus monogyna, Holcus lanatus, Rubus fruticosus.*	*Lamium album, Leucanthemum vulgare, Rosa canina, Stellaria media, Urtica dioica.*	*Leontodon autumnalis.*

Table 4.2.2 Final partition from the Hierarchical Cluster Analysis of Variables of the species clusters from the chronosequenced vegetation transects at the Betteshanger site.

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Achillea millefolium, Agrostis capillaris, Aira praecox, Anthriscus sylvestris, Betula pendula, Blackstonia perfoliata, Centaurium erythaea, Cerastium arvense, Echium vulgare, Epilobium angustifolium, Holcus lanatus, Hieracium pilosella, Hypochoeris radicata, Lotus corniculatus, Medicago lupulina, Melilotus alba, Melilotus altissima, Plantago lanceolata, Rubus fruticosus, Rumex acetosella, Rumex obtusifolius, Trifolium arvense, Trifolium repens, Trifolium pratense.	*Dactylis glomerata, Daucus carrota, Quercus robur.*	*Elymus caninus.*	*Hypericum perforatum, Leontodon autumnalis.*	*Quercus ilex.*

4.2.3 Discussion

In the oldest aged spoil, at both sites, woody species dominate the community (Figures 4.2.1 & 4.2.2). At Betteshanger the under-storey was quite sparse and this was probably partly due to the presence of *Quercus ilex*. *Q. ilex*, an evergreen, substantially shades the spoil all year round, preventing the establishment of an extensive ground cover (Martens, Breshears and Meyer 2000). This assumption was reinforced by *Q. ilex* not being associated with any other species in the hierarchical cluster analysis of variables from the chronosequenced vegetation sampling at the Betteshanger site (Cluster 6 Table 4.2.2). The presence of *Q. ilex* will influence the plant communities at Betteshanger very like its native Mediterranean garrigue woodland. Garrigue is composed of evergreen trees which out-shade the under-storey thus preventing competition for water and other resources (Bragg & Westoby 2002). This strategy obviously works well on the colliery spoil at Betteshanger which has similar environmental stresses to *Q. ilex's* natural habitat. *Q. ilex* does not occur on Stodmarsh; there are presumably no trees which occur locally and could act as a seed source (Clark *et al.* 1999). This lack of intense competition maybe why the under storey is better established at Stodmarsh. *Betula pendula* is a dominant woody species found on both sites. The survey at Stodmarsh indicates that woody species do not appear until the spoil is at least 69 years old. The woody species at Stodmarsh appear to follow the expected facilitation model of succession (Connell & Slatyer 1977), in which a build up of a soil and nutrient cycling is required before a new substratum can sustain the requirements of tree species (Li & Daniels 1994). This pattern of successional development has been described in functional terms from other mine sites (Down 1975; Brenner, Werner & Pike 1984; Holl & Cairns 1994; Holl 2002).

The Betteshanger vegetation survey shows that the woody species appear in the youngest spoil (Figure 4.2.1). *B. pendula* was the first plant species to colonise the colliery waste at Betteshanger. One would not expect a tree species to be a primary coloniser as the resource requirements for trees are substantial (Torbert *et al.* 1985; Torbert *et al.* 2000). The colliery spoil was assumed to be deficient in plant resources (Chapter 5) so the ability of *B. pendula* to colonise is an ecological paradox. *B. pendula*'s role as a pioneer species at this site, suggests a traditional succession model is not being followed at Betteshanger. However, woody species tend to become more dominant in the plant communities as the spoil gets older (Figure 4.2.1).

The young successional plant communities at Stodmarsh were dominated by legume species, the most common of which was *Lotus corniculatus*. Leguminosae progressively become less dominant in the plant communities at Stodmarsh as the age of the spoil increases (Figure 4.2.2 and Table 4.2.2). The ability for Leguminosae to fix nitrogen, could explain their success in the youngest spoil. The ability to fix nitrogen would have promoted a competitive advantage in the Leguminosae over those groups of plants that do not fix nitrogen (Knops, Bradley and Wedin 2002). This advantage could be crucial in the mine spoil where nitrogen availability maybe limiting (Schuster and Hutnik 1987). As the spoil gets older it is probable that nutrient cycling systems develop (Marrs 1989): this is explored further in Chapter 5. As a nitrogen cycle develops, the benefit of fixing nitrogen would diminish and so the advantage the Leguminosae have over other plant groups would also diminish (Maron and Connors 1996, Knops, Bradley and Wedin 2002). This is a possible explanation of why the Leguminosae have become less important in the plant communities on the older spoil. Unlike Stodmarsh, legumes were only found in the 36 year old spoil at Betteshanger; however, the dominant legume species is *L. corniculatus* as at Stodmarsh. As *L. corniculatus* was present on the Betteshanger site, the lack of their dominance in the vegetation system can not be due to their lack of dispersion to the site. It must be the result of their competitive advantage for fixing nitrogen being less effective on Betteshanger than Stodmarsh. This could be linked to the acidity of the spoil which inhibits *Rhizobium* bacteria (Tate 1985).

The 36yr old plant community at Betteshanger had similar structure and species composition to the 46yr old spoil at Stodmarsh, except at Stodmarsh there is a noticeable absence of woody species on younger spoil (Figures 4.2.1, 4.2.2 and Tables 4.2.1 and 4.2.2). The community composition of the 31 and 36 year old spoil at Betteshanger shows quite a different functional group composition. The differences in these two plant communities cannot be easily explained in terms of spoil age as there are only five years between their tipping. The differences are possibly due to changing tipping regimes of the spoil rather than the influence of successional plant communities (Skousen, Johnson & Garbutt 1994).

The principal component analysis of the Stodmarsh site illustrates that there are very different communities separated by parameters most closely related with community age (Figure 4.2.4). However, the principal components analyses of the Betteshanger communities do not illustrate this pattern of differentiation in relation to age (Figure 4.2.3). The presence of *B. pendula* in most areas of the site probably restricts the

differentiation of the communities' components by age. The most notable exception is the community sampled from area 3 which has a similar functional structure to areas in the early successional stages at Stodmarsh. The community has no woody species and a high Leguminosae component. The principal component analyses (Figure 4.2.4) show strong evidence that the vegetation pattern on Stodmarsh does illustrate a typical successional development. However, the communities sampled from Betteshanger do not illustrate the typical predicted vegetation pattern associated with successional development (Figure 4.2.3).

Figures 4.2.5 & 4.2.6 and Tables 4.2.1. & 4.2.2 summarise the hierarchical cluster analysis of variables from the chronosequenced vegetation samples at Betteshanger and Stodmarsh. The summaries depict the different clusters of species representing novel community associations which characterise different aged spoil at the two sites. Stodmarsh has distinct groups of species associated with different ages of the colliery spoil; woodland flora dominate the older spoil indicating the good rehabilitation of the site (Cluster 3 Table 4.2.1) (Ludwig, Hindley and Barnett 2003). However, at Betteshanger a large number of species cluster together in the first division of the analysis, suggesting that the communities on the different aged spoil at Betteshanger do not show a great differentiation in species composition. This is supported by the summary of the principal components analysis (Figure 4.2.3), in which communities from areas 2-6 do not differentiate. The lack of community differentiation at Betteshanger indicates the uniqueness of the site and its deviation from the facilitation successional model.

The difference in the plant communities at the two sites maybe related to the differences in the physico-chemical characteristics of their spoils (Brenner, Werner & Pike 1984), or the ability of species to migrate to the sites (Tilman 1994). The disparity between the plant communities at the two sites suggests that, although facilitation processes are important for community development, the species pool available for recruitment is also important. This fits the tolerance model of succession as proposed by Connell and Slatyer (1977).

5 Nitrogen and its availability to plants in colliery spoil.

Nitrogen in soils is found as insoluble organic matter, soluble nitrogen and as gaseous nitrogen in soil air. Soluble nitrogen is available for use by plants and therefore is critical for ecosystem functioning. Soluble nitrogen is released from organic complexes by decomposition processes and is also directly fixed from the atmosphere. However, the major source of soluble soil nitrogen is biologically fixed from soil air (Marrs 1989; Postgate 1998).

Nitrogen is a metabolically important molecule and is crucial in all aspects of ecosystem functioning. It is found in many organic compounds: proteins, peptides, amino acids, nucleic acids, chitin, mucopeptides, amino sugars etc. Plants, bacteria and fungi primarily use inorganic nitrogen: NH_3 (ammonia) and NO_3^- (nitrate) as nitrogen sources (Li & Daniels 1994). Other organisms must acquire their nitrogen in organic form as: amino acids, proteins, nucleic acids etc (Bray 1983). Nitrogen does not occur in primary soil-forming materials (Bradshaw *et al.* 1982). Therefore, an organic derived nitrogen store must be accessible to organisms in the soil before an ecosystem functions at its maximum potential (Dancer, Handley & Bradshaw 1977a; Bradshaw 1997).

There is a perceived critical nitrogen requirement for a community, below which a succession is slow and above which a succession develops relatively quickly towards climax (Bradshaw *et al.* 1982; Bradshaw 1983; Marrs 1989). Although the concentration of potassium in plants is often equal to that of nitrogen, the difference in atomic weight means that plant tissue contains three to four times as many nitrogen atoms as potassium and between eight and ten times the number of atoms of any other nutrient (Marrs and Bradshaw 1993). As an ecological and niche dimension, nitrogen availability is therefore often limiting to vegetation systems (Bray 1983; Marrs 1989; Killham 1994).

All organisms require nitrogen to produce biomass and in some forms it is extremely mobile within ecosystems. Nitrogen has been shown, repeatedly, to be a limiting resource on colliery wastes; controlling the type and number of species which are able

to colonise the colliery waste substratum (Kimber, Purford & Duncan 1978, Chadwick 1987; Atkinson 1991; Chapman, Collins & Younger 1996).

The movement and storage of nitrogen within a system, to a degree, is controlled by the types of species present, especially with regards to functionality (Foster & Gross 1998). In a multi-species ecosystem, community composition can be as important as the total number of species with respect to how an ecosystem functions (Tilman, Lehman & Thomson 1997). However, the more species within a system the more possible interactive associations are also present. The number of species, within a system, is therefore important in relation to niche availability and resource utilisation; exploitable nitrogen is an important dimension within a system which is influenced, not only by the species present, but also by how many there are (Bengtsson 1998; Foster & Gross 1998).

5.1 Mineralizable Nitrogen.

Nitrogen when bound in organic molecules is normally unavailable for uptake by plants. Only by the decomposition process does organic nitrogen normally become available as inorganic nitrogen. This process is called mineralization. The major proportions of nutrients in the soil are locked in organic forms and are unavailable to plants; therefore, mineralization rates and reactions are critical to the functioning of an ecosystem (Voos & Sabey 1987). Mineralization of soil organic nitrogen produces three soluble inorganic forms of nitrogen: ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) (Allen 1989). The ionic form of the mineralized nitrogen has an influence on the redox potential of the soil (Reeder & Berg 1977). The differences in charge, carried by the different inorganic nitrogen forms, mean that all the reactions within the nitrogen cycle can be associated with pH changes to soil. Plants taking up nitrate release bicarbonate ions to maintain an internal charge balance. This has the effect of raising the pH, whereas those plants which absorb mainly ammonium release protons thereby lowering pH. The pH of the rhizosphere is therefore, fundamentally linked with nitrogen uptake by plants (Stanford & Smith 1972). The modification of soil pH, as the nitrogen cycle develops with pedogenesis, can play an important role in natural succession (Olson 1958) and can be crucial in maintaining a desired vegetation type in land restoration (Killham 1994).

Vegetation development increases the immobilised nitrogen within a system; as the vegetation and soil develop, nitrogen is progressively incorporated into the living tissues within that system and forms complex organic molecules (Knops, Bradley & Wedin 2002). The rate at which nitrogen is mineralized will also increase as the soil biota develops and becomes more diverse and abundant. The mineralization and immobilisation of nitrogen occur simultaneously and are dependent on each other. These two processes tend towards equilibrium and form a balanced feedback system in the climax community (Knops, Bradley & Wedin 2002).

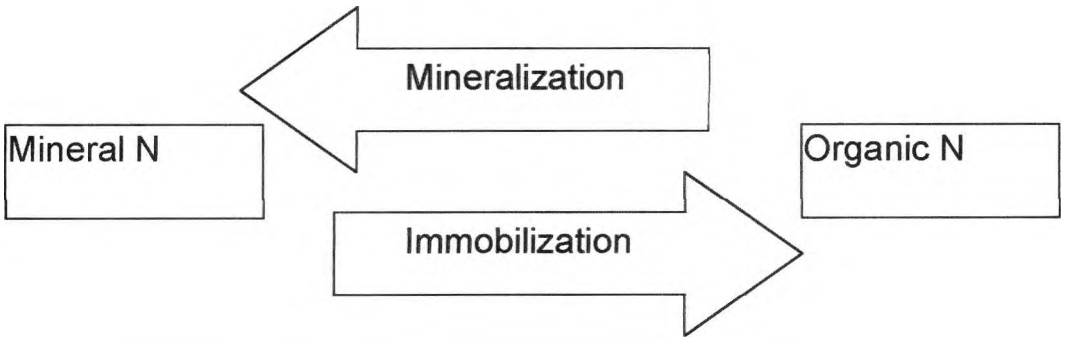

Figure 5.1.1 Balanced feed back between mineral nitrogen and organic nitrogen in the soil system.

The amount of nitrogen immobilised by ecosystems varies. Table 5.1.1 indicates the levels of nitrogen uptake in different terrestrial ecosystems.

Table 5.1.1 The approximate percentage of the total soil nitrogen pool taken up each year by different ecosystems (adapted from Killham 1994).

Ecosystem	Approx. % total soil N removed by vegetation annually[a].	Approx. total soil N (Kg N ha^{-1})
Tundra	0.4%	10 000
Temperate, upland moorland	0.5%	10 000
Temperate, coniferous forest	0.75%	20 000
Temperate, deciduous forest	0.7%	7500
Tropical rain forest	1-2%	9000
Temperate (high-yield cereal)	5%	1000

[a] Total off take is greater because off take is from fertiliser N + soil + litter.

Natural succession studies, such as chronosequences of glacial moraines (Crocker and Major 1955), have documented rates of nitrogen accumulation. These studies have found total soil N increased by 1000Kg N ha^{-1} over a 100yr period from a starting nitrogen budget of 200Kg N ha^{-1}. Nitrogen fixing groups, such as Rhizobia and *Frankia*, can be important contributors to the nitrogen accumulation of a system and can accumulate between 50-150Kg N ha^{-1} yr^{-1} (Walker 1993; Bradshaw 1997). 1600Kg N ha^{-1} is an estimated minimum amount of nitrogen in the nitrogen pool, in developed temperate grasslands, below which the grassland will not develop non-nitrogen fixing based vegetation (Bradshaw 1983). Marrs (1989) estimated minimum values for the amounts of nitrogen required in the soil for natural ecosystems, developed on raw substrata, before they could be self sustaining (Table 5.1.2).

Table 5.1.2 Estimates of target nitrogen contents and the time taken to reach these targets on four raw substrata (adapted from Marrs 1989).

Substrate	Time to develop non-nitrogen fixing vegetation (yr)	Nitrogen content in soil (Kg N ha^{-1})
Glacial moraines	100	1200
Sand dunes	21	400
Ironstone	100	600
China clay waste	>70	700
China clay waste	>120	1200

Table 5.1.2 illustrates the nitrogen range for self sustaining vegetation to develop. This is estimated to be 600-1200Kg N ha^{-1} and is less than 1600Kg N ha^{-1} which is the figure estimated for temperate grasslands by Bradshaw (1983). The figure of 1200Kg N ha^{-1} in the china clay waste is the amount of nitrogen in the soil required for a *Betula/Quercus* woodland to be sustained. Values calculated by Marrs (1989) can be used to compare the amount of nitrogen accumulated in any naturally colonised vegetation systems such as those found on colliery waste.

Total nitrogen, available nitrogen exploitable by plants and mineralizable nitrogen of different aged colliery waste, were investigated at Betteshanger and Stodmarsh. The total nitrogen and mineralizable nitrogen were examined to explore the role of fertility in the chronosequence of spoil tipping at the project sites.

5.2 Experiments to compare nitrogen levels in material from different stages in succession.

Five colliery waste samples were taken from each of the different aged sequences of tipping at the two sites; Stodmarsh and Betteshanger. The method of sample collection is described in 4.1.1. Five soil samples were also taken from woodland: *Chequers Wood, The Old Park*, a SSSI in Canterbury, Kent, UK, Longitude 1°080 Latitude 51°170. Chequers Wood's soil has developed on the Green Sand geological sequence and is acidic (pH 5.5). In East Kent this particular soil pH is rare but comparable with the pH found on the colliery spoil (Chapter 4). The woodland soil was selected to act as a control for comparing how nitrogen behaved in a mature soil of the region with that of colliery spoil.

5.3 Available nitrogen and Mineralizable nitrogen determination.

The accumulation of organic nitrogen occurs when inputs into the soil exceed the rate at which soil micro-organisms can mineralize the organic nitrogen input. The build up of an organic nitrogen pool is essential for the development of ecosystem function in successional systems (Marrs 1989; Ryel & Caldwell 1998). However, the rate at which nutrients are made available for plant uptake, by mineralization processes, is also essential for ecosystem functioning (Stanford & Smith 1972). If the available mineral nitrogen released by mineralization does not supply the demands of the developing vegetation system, then development will be restricted and regression and die back can occur (Reeder & Berg 1977; Palmer *et al.* 1979; Chapman & Younger 1995; Chapman, Collins & Younger 1996).

Available nitrogen is influenced by a range of edaphic parameters such as leaching rate, plant uptake, microbial activity and pH, so concentrations can vary considerably temporally. The measure of available nitrogen, therefore, only gives a picture of the

available nitrogen in the spoil at the given time the spoil was sampled (Palmer, Morgan & Williams 1985).

Mineralizable nitrogen is the difference between the initial inorganic nitrogen in a soil and inorganic nitrogen released from it or absorbed (immobilized) by microbial activity after a period of time (Palmer, Morgan & Williams 1985). The mineralizable nitrogen is a measure of nitrogen availability on a temporal scale and relates to the fauna and flora activity within a soil. It is a useful measure of the nitrogen cycling rate within an ecosystem and enables empirical comparisons between the functioning of different systems (Dancer, Handley & Bradshaw 1977b).

Mineralizable nitrogen is not as simple to measure as total organic nitrogen of a sample. Organic nitrogen composes up to 98% of all soil nitrogen but is unavailable to plants. The problem with only analysing organic nitrogen is that it does not indicate how well an ecosystem is functioning. Organic nitrogen must be broken down to ammonium and nitrate before it can be used by plants. The rate at which this occurs is not related to the total amount of nitrogen in the soil at any one time (Williams & Cooper 1976). It is, never the less, a better measure of ecosystem functioning than available nitrogen, which is merely a snapshot of the nitrogen available for uptake by plants in a moment in time (Stanford & Smith 1972; Palmer, Morgan & Williams 1985; Voos & Sabey 1987).

Ecological functional efficiency is a measure of the effectiveness of ecological processes or mechanisms and how proficiently they operate (Lavorel & Garnier 2002). In terms of nutrient cycling the more nutrient a system can recycle, in a given period of time, the better the ecological functional efficiency of that system (Craine *et al.* 2002). This concept is particularly important in the context of successional plant communities and restoration ecology, as the ecological functional efficiency can determine plant community structure and its sustainability (Gray, Crawley & Edwards 1987; Marrs *et al.* 2000).

Available nitrogen and mineralizable nitrogen were used as a comparative measure of the nitrogen cycling capacity, and therefore the ecological functional efficiency, between different ages of a succession system, on the colliery waste at the two sites,

and with those found in woodland. Incubation techniques were employed to calculate the mineralizable rates of nitrogen.

5.3.1 Method.

To determine the mineralizable nitrogen content of a spoil sample, firstly its available nitrogen at time zero was analysed. The sample was then incubated for a fourteen day period and the available nitrogen re-analysed. The difference between the two available nitrogen concentrations represented the amount of new nitrogen which was mineralised (or immobilized) by the spoil biota.

The procedure for calculating the available and mineralizable nitrogen (adapted from Allen 1989 and ISO 14238) was as follows: the spoil and soil samples were dried in circulating air at a low temperature (40°C). The dried samples were then passed through a 2mm sieve to remove coarse fragments: soil passing this mesh contains almost the whole of the nutritionally important fraction (Allen 1989).

Five grams of the air-dried sieved samples were placed into 250ml soil sample tubes (A) and 5g into beakers (B). Fifteen grams of acid-rinsed sand was added to both A and B and swirled to mix. Deionised water (6ml) was added to each vessel and mixed. Samples (B) were immediately extracted in 125ml 6% KCl by shaking for 1 hour on a rotary shaker. The extractions were filtered through Whatman ® No.44 filter paper using a vacuum pump and Buchner flask.

Ammonium, nitrate and nitrite concentrations were determined for the extractions using the Aquatec auto-analyser and represented inorganic nitrogen (N) available to plants in the spoil.

Samples (A) were incubated for 14 days at 24 ^{0}C keeping the soil sample tubes sealed with porous film. At the end of the 14 days the samples were extracted and analysed as previously described for samples (B). Sand and water blanks were run with samples (A) and (B) to enable determination of any background nitrogen contamination.

Inorganic mineralizable nitrogen (N-mineralization gN/Kg) was calculated for each sample by summation of NH_4N, NO_2N and NO_3N (µg N/g, dry weight) and then subtracting this figure from the available-nitrogen concentrations calculated for samples (B) at time zero (ISO 14238).

The nitrogen data versus the age of the spoil, from which they were sampled, were analysed with a Kruskal-Wallis test (a nonparametric equivalent of a one-way analysis of variance) using the statistical package Minitab (release 13). There is no *post hoc* statistical test available for determining which group of nitrogen samples were different from another as the data were not normally distributed; the differences between the groups of nitrogen samples were determined by inspection of the summary statistics (Zar 1996; Dytham 1999).

5.3.2 Results: available nitrogen.

All results have been analysed using Kruskal-Wallis non parametric one-way analysis of variance as the data were not normally distributed (Zar 1996, Dytham 1999) (for full analyses see Appendices 5.3.1 & 5.3.2). Table 5.3.1 summarises the results from the Kruskal-Wallis tests of the available nitrogen analyses versus the age of the spoil from which the nitrogen was extracted. All forms of nitrogen varied significantly with the age of the spoil from which they were sampled at the 0.1m sample depth at Betteshanger. However, the 1m deep samples did not show significant variation in concentration of extracted nitrogen forms with age across the chronosequences, except nitrate at Betteshanger (Table 5.3.1). Ammonium and nitrate varied significantly with the age of the spoil from which they were sampled at the 0.1m sample depth from Stodmarsh, but nitrite did not. All nitrogen forms did not vary significantly with age in the 1m deep samples at Stodmarsh (Table 5.3.1).

Table 5.3.1 Summary of the results from the Kruskal-Wallis tests: inorganic available nitrogen concentrations (at time zero) versus the age of the spoil.

	Sample location			
Available inorganic nitrogen species	Betteshanger 1m	Betteshanger 0.1m	Stodmarsh 1m	Stodmarsh 0.1m
Ammonium	ns	***	ns	*
Nitrate	**	***	ns	*
Nitrite	ns	**	ns	ns

Key: *** = $P<0.001$, ** = $P<0.01$, * = $P<0.05$, ns = non significant difference.

Figures 5.3.1-5.3.4 *illustrate the means of inorganic available*, the nitrogen available immediately for plant uptake from the spoil, which were extracted from the spoil samples; error bars represent standard deviations. The concentration of inorganic available nitrogen increases with the age of the spoil from which it was sampled in 0.1m samples at Stodmarsh and Betteshanger (Figures 5.3.1 and 5.3.3 respectively). The greatest available nitrogen was extracted from the woodland control soil. In the 1m samples there was no discernable relationship between the concentration of nitrogen extracted from the samples and the age of the spoil (Figures 5.3.2 and 5.3.4). The woodland control soil yielded high rates of ammonium and nitrate in comparison to the colliery spoil in the 1m samples.

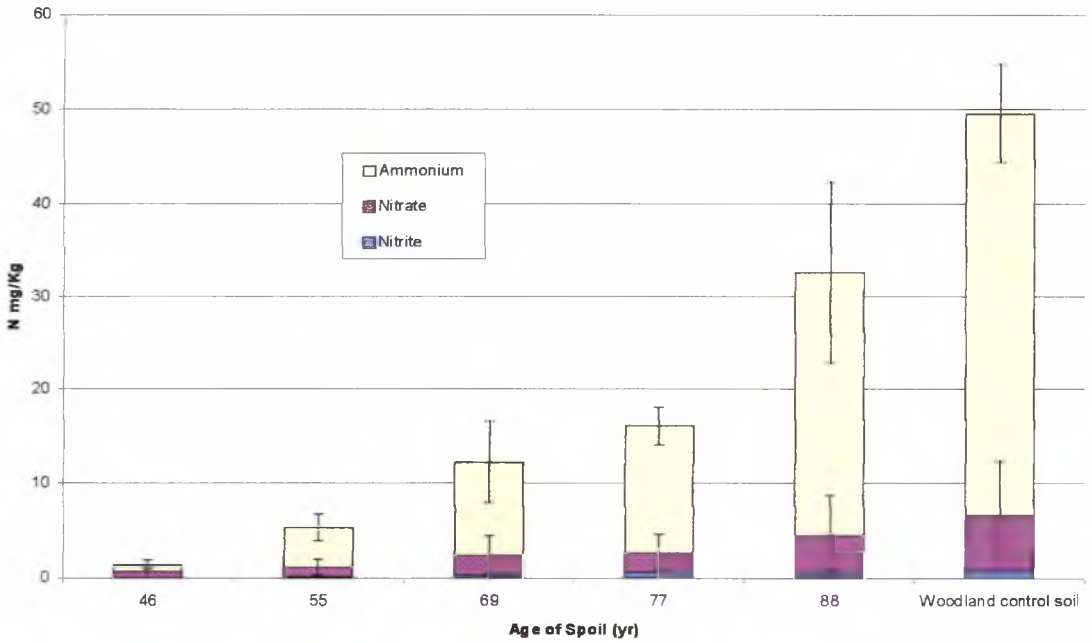

Figure 5.3.1 Available inorganic N from the chronosequence at Stodmarsh 0.1m below spoil surface.

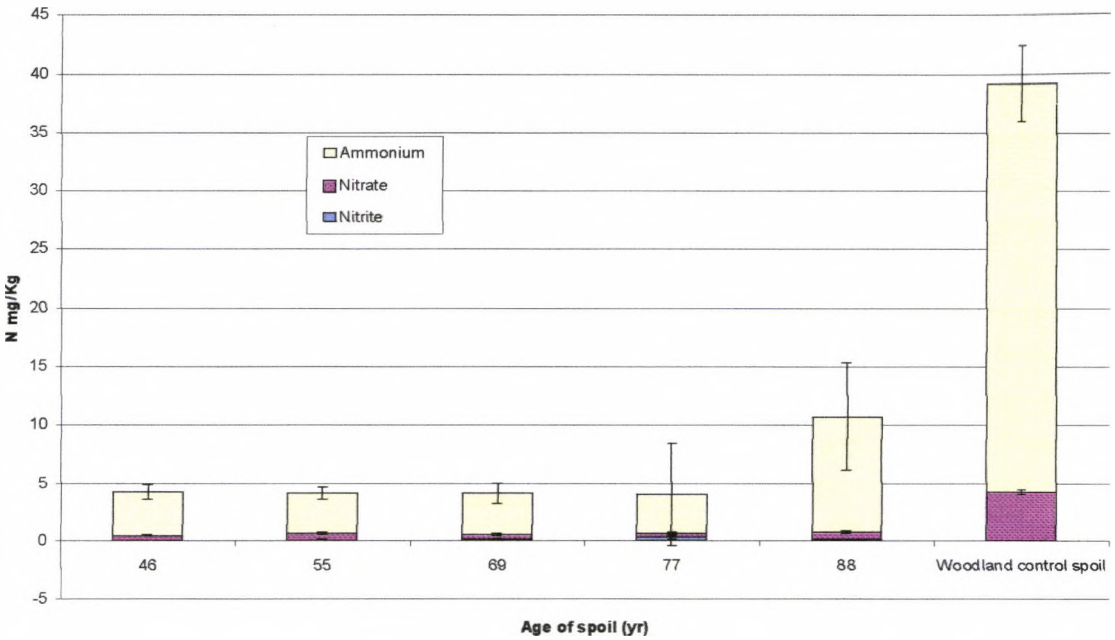

Figure 5.3.2 Available inorganic N from the chronosequence at Stodmarsh 1m below spoil surface.

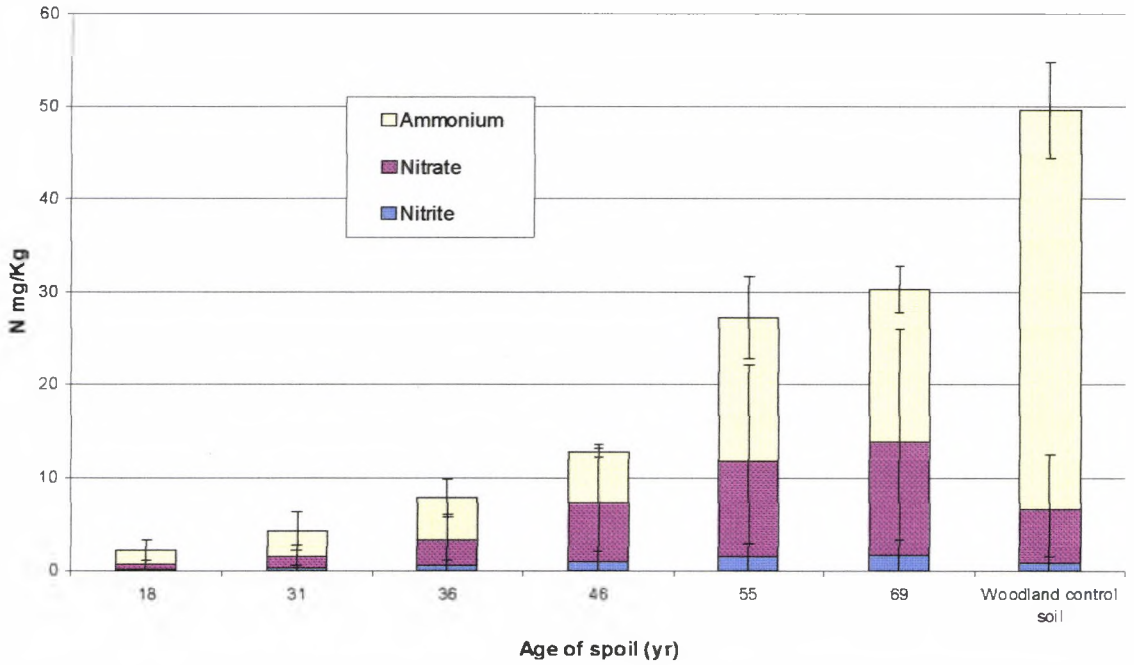

Figure 5.3.3 Available inorganic N from the chronosequence at Betteshanger 0.1m below spoil surface.

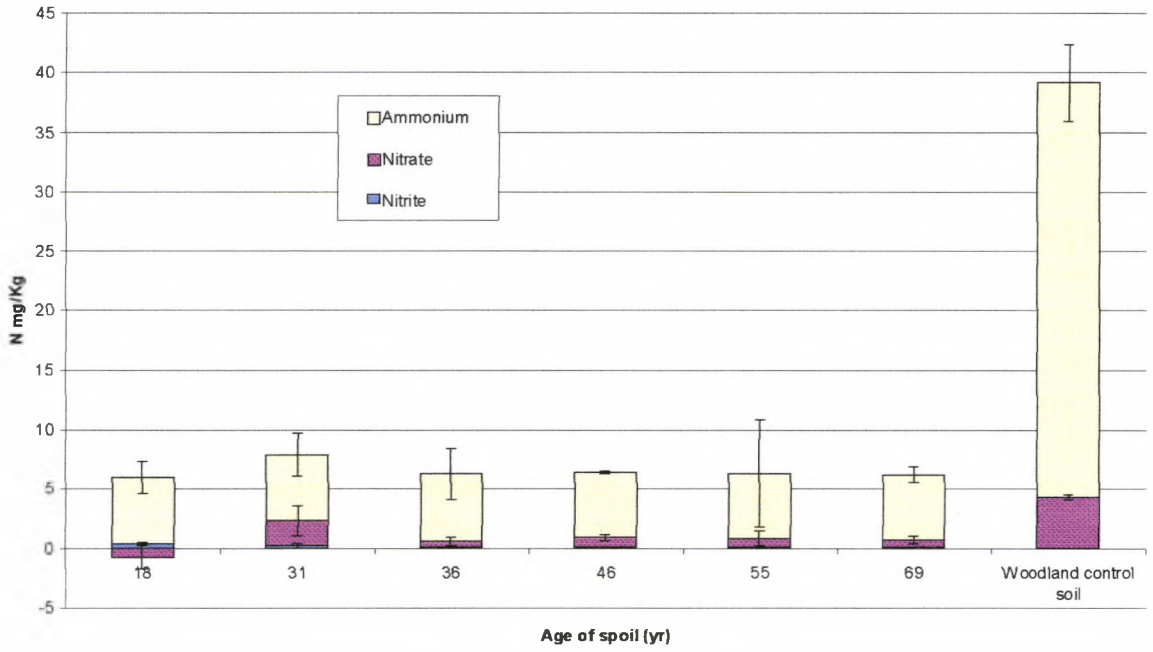

Figure 5.3.4 Available inorganic N from the chronosequence at Betteshanger 1m below spoil surface.

5.3.3 Discussion: available nitrogen.

The available nitrogen increases from the youngest to oldest spoil in the 0.1m samples, and emulates the nitrogen availability patterns found in other primary materials (Dancer, Handley & Bradshaw 1977a). However, in the 1m deep samples only nitrate varied significantly with age at Betteshanger (Table 5.3.1). This reinforces the view that the 1m deep samples were below the major biological portion of the spoil horizon (Figures 5.3.2 & 5.3.4). In comparison with the woodland soil, the nitrogen available was low in the colliery spoil, only ~25% of that found in the woodland soil (Figures 5.3.2 & 5.3.4) and is indicative of the poor nitrogen status of the colliery spoil at both sites. The high concentration of nitrogen in the deep woodland samples probably indicates the depth of biological activity within a soil formed under woodland.

The summary statistics from the 0.1m samples suggest that the oldest spoil at both sites has significantly greater available nitrogen than the younger spoil (Figures 5.3.1 and 5.3.3). The summary statistics also indicate that the relationship between age of the spoil and ammonium concentration was greater than that between the age of the spoil and nitrate concentration. At Stodmarsh, the nitrate concentration did not vary significantly from that sampled in the woodland soil (Figure 5.3.1). This suggests the nitrate dynamics within the spoil was mirroring the nitrate dynamics within woodland soil. However, the ammonium concentration was significantly less in the Stodmarsh spoil than the woodland soil. This suggests, that this component of the nitrogen cycling system, as described by Craine *et al.* (2002) and Lavorel and Garnier (2002), was not functioning as efficiently as that of the woodland soil. This observation has implications for the successional vegetation patterns described in Chapter 4.

At Betteshanger nitrate concentration increased from the youngest to oldest spoil in the 0.1m samples. However, the standard deviations are so large that the significance of this relationship is questionable (Figure 5.3.3). The nitrate available in the Betteshanger samples, in the surface samples at the oldest spoil, was larger than that found in the woodland soil. The large standard deviations again call into question the significance of the observations. Ammonium availability varied significantly with the age of the spoil. The available ammonium in the oldest spoil was significantly less than the available ammonium in the woodland spoil, suggesting, just as with Stodmarsh, that

this component of the nitrogen cycling system was not functioning as efficiently as that of the woodland soil and develops with age of the spoil (Craine *et al.* 2002; Lavorel and Garnier 2002).

Nitrite was rare in all the spoil sampled. Nitrite is the easiest form of nitrogen to be taken up by plants and is extremely transient in soil systems. It is also notoriously difficult to analyse from soil samples as it degrades rapidly and is lost as N_2 gas (Allen 1989). However, it was significant that no nitrite was analysed from the samples collected from 1m deep yet there was nitrate in the 0.1m samples at both sites and the woodland soil; this is undoubtedly related to the transience of this nitrogen form in soils.

Nitrate and ammonia, at Betteshanger and Stodmarsh, were the largest proportion of available nitrogen and both compounds significantly increased in the spoil with age at 0.1m deep. In general, samples from 1m deep at Betteshanger had more available nitrogen than the Stodmarsh samples although there was no significant variation with age at this depth. The amounts of available nitrogen found at 1m, at both sites, were probably a response to the type and quantity of vegetation. Stodmarsh had a good ground cover over all its chronosequence, therefore available mineral nitrogen would be readily taken out of the developing system by the cover vegetation and immobilized (Foster & Gross1998). Betteshanger had less vegetation cover than Stodmarsh (Figures 4.2.1 and 4.2.2); any nitrogen that was available could therefore persist longer in the spoil horizon at Betteshanger as the vegetation was not present to use it. Available nitrogen is mobile in soil systems; therefore, the greater quantity found in 1m deep samples at Betteshanger was probably the result of differential movement of available nitrogen through the spoil horizon, controlled by the vegetation structure (Li & Daniels 1994). It was expected that available nitrogen at Stodmarsh would have been greater than that at Betteshanger. This was because of the legume-dominated vegetation surveyed at the site (Chapter 4); legume-dominated vegetation has been reported to increase the available nitrogen in vegetation systems (Dancer, Handley & Bradshaw 1977b; Jeffries, Bradshaw & Putwain 1981), but this was not the case at Stodmarsh.

5.3.4 Results: mineralizable nitrogen.

Table 5.3.2 summarises the results from the Kruskal-Wallis tests of the mineralizable nitrogen analyses, versus the age of the spoil from which the nitrogen was extracted (see Appendices 5.3.3 & 5.3.4 for full statistical tests). Figures 5.3.5-5.3.8 illustrate the means of mineralizable nitrogen which were extracted from samples; error bars represent standard deviations.

Mineralizable ammonium varied significantly with age at Betteshanger and Stodmarsh in the 0.1m samples ($P<0.000$ & $P=0.13$ respectively). In the 0.1m samples, nitrate varied significantly with spoil age at Stodmarsh ($P=0.043$), but not at Betteshanger ($P=0.058$). Nitrite varied significantly with age in the 0.1m samples at Betteshanger ($P=0.020$), but not at Stodmarsh ($P= 0.156$). In the 1m deep spoil, nitrate varied significantly with the age of the spoil at both sites and at Stodmarsh ammonium significantly varied with the age of the spoil (Table 5.3.2). There are, therefore, differences between the two sites in the way the different mineralizable nitrogen forms behave.

In general, however, the trend for mineralizable nitrogen is to increase from younger to older spoils at 0.1m deep, although there were some negative mineralizable nitrogen rates (Figures 5.3.5 & 5.3.7). The greatest mineralizable nitrogen was extracted from the woodland control soil (Figures 5.3.5-5.3.8).

In 1m deep samples at Stodmarsh, total mineralizable nitrogen and mineralizable nitrate samples increase with spoil age. There was no nitrite extracted from the 1m deep samples at Stodmarsh, and the mineralizable ammonium did not increase with the age of the spoil (Figure 5.3.6).

In 1m deep samples at Betteshanger, the mineralizable nitrogen did not have a consistent relationship with the age of the spoil. However, in general, mineralizable nitrate decreases as the spoil gets older at Betteshanger (Figure 5.3.8).

Table 5.3.2 Summary of the results from the Kruskal-Wallis tests: inorganic mineralizable nitrogen concentrations versus age of the spoil.

	Sample location			
Mineralizable nitrogen species	Betteshanger 1m	Betteshanger 0.1m	Stodmarsh 1m	Stodmarsh 0.1m
Ammonium	ns	***	*	*
Nitrate	**	ns	*	*
Nitrite	ns	*	ns	ns

Key: *** = $P<0.001$, ** = $P<0.01$, * = $P<0.05$, ns = non significant difference.

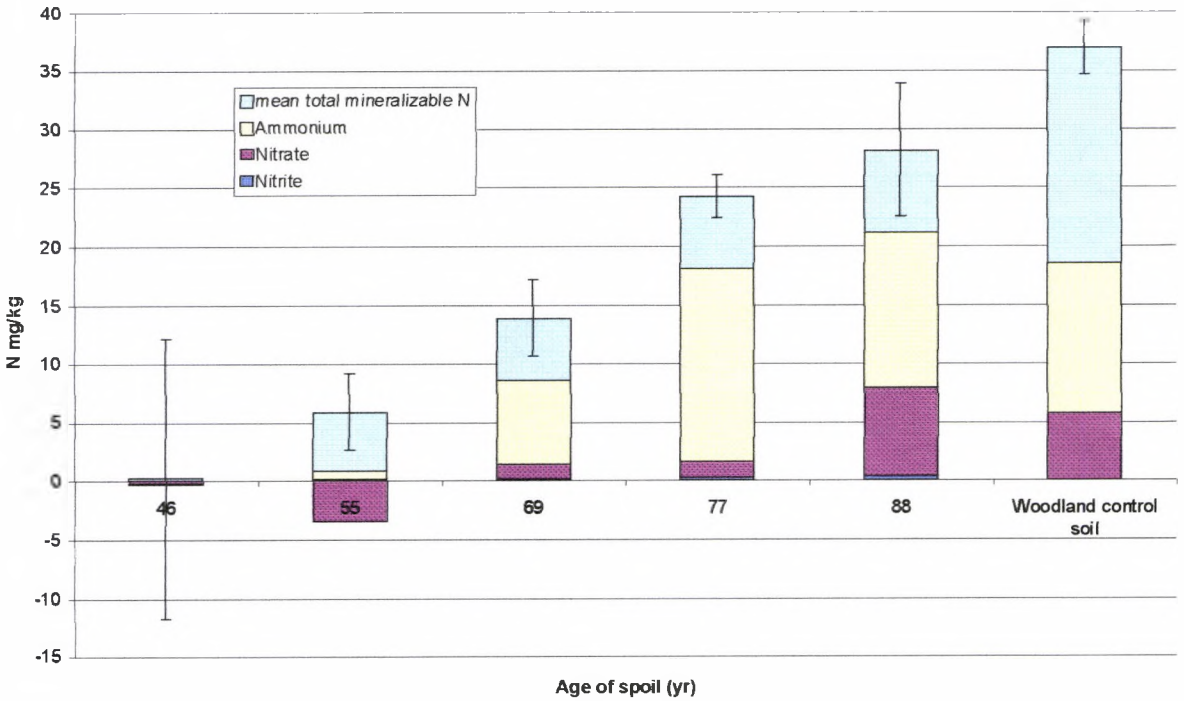

Figure 5.3.5 Mineralizable N from the chronosequence at Stodmarsh 0.1m below spoil surface.

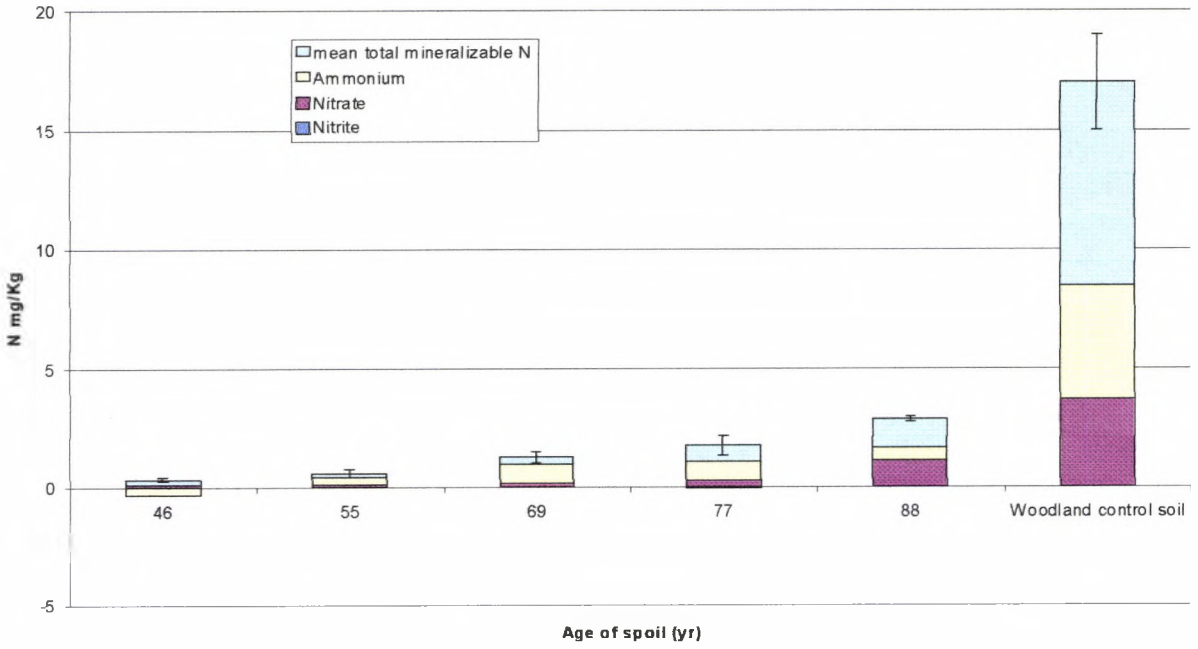

Figure 5.3.6 Mineralizable N from the chronosequence at Stodmarsh 1m below spoil surface.

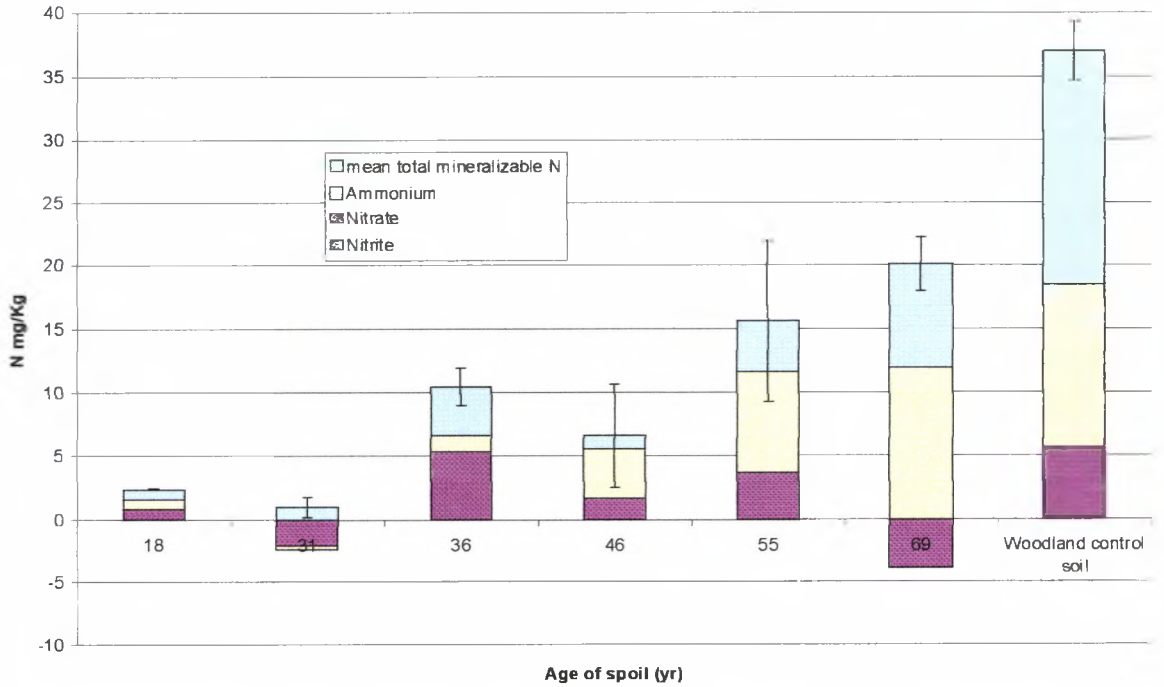

Figure 5.3.7 Mineralizable N from the chronosequence at Betteshanger 0.1m below spoil surface.

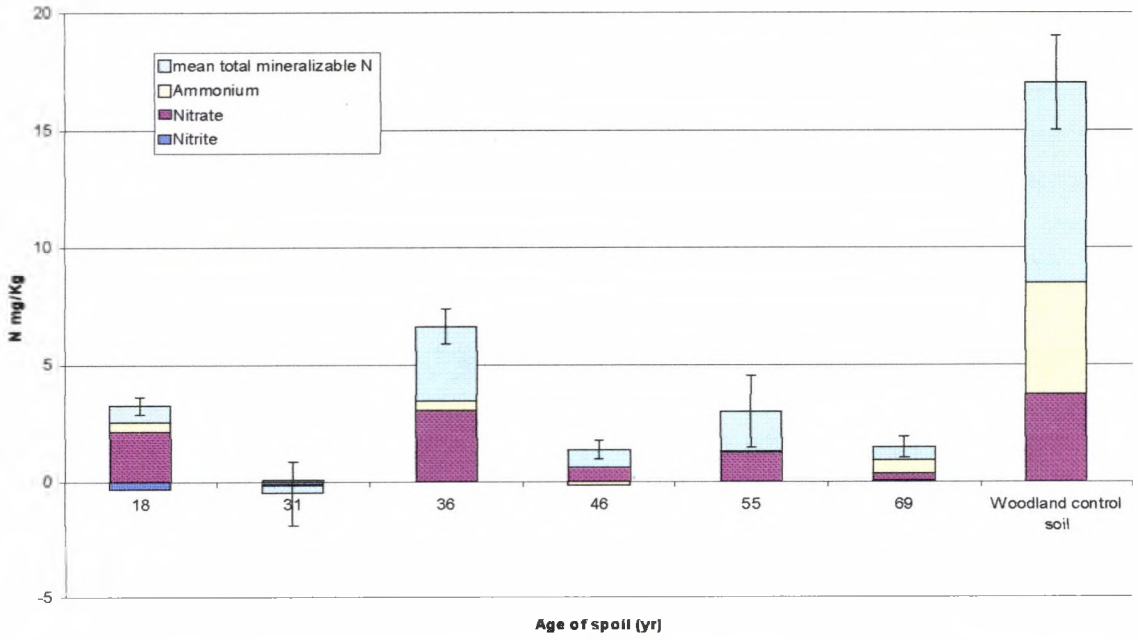

Figure 5.3.8 Mineralizable N from the chronosequence at Betteshanger 1m below spoil surface.

5.3.5 Discussion: mineralizable nitrogen.

The increase in mineralization of nitrogen, with age (Figures 5.3.5 & 5.3.7), confirms that the older vegetations systems had better functioning nitrogen cycling in place than the younger vegetation systems. This supports similar findings by Reeder and Berg (1977) and Craine *et al.* (2002). The availability of nitrogen is crucial for vegetation systems to be sustainable in the long term. The implication for the older vegetation at the sites, with better developed nitrogen cycling, will be that they are more stable than the younger vegetation (Gray, Crawley & Edwards 1987). Therefore, the community dynamics and species replacements will be less than in younger spoil. The community in the older spoil will therefore be controlled by niche assembly rules, and the communities in the younger spoil controlled by dispersal assembly rules (Hubbell 2001)

There were negative mineralizable rates for nitrate in two age categories of 0.1m samples at Betteshanger (Figure 5.3.7). The negative mineralizable rates observed in the colliery spoil (Figures 5.3.5, 5.3.57 & 5.3.8) are due to nitrogen being removed from the spoil and being locked into biomass as organic nitrogen in the incubation process. Bacterial or fungal components, of the spoil biota, are most likely to be responsible for this removal from the available inorganic nitrogen released in the incubation process. This locking away of nitrogen, which is unavailable to plants, has implications for the vegetation systems where this occurs. It represents a potential limitation to the system, as nitrogen is crucial to the development of vegetation systems on new substrata (Chadwick *et al.* 1978; Bloomfield, Handley & Bradshaw1982; Bradshaw 1997).

1m spoil samples have much less mineralizable nitrogen, even in the woodland soil, in comparison to the 0.1m samples (Figures 5.3.5-5.3.8). This indicates that the 1m samples are indeed below the major biological active area. The older spoil vegetation systems, although they support woody species (Figures 4.2.1 and 4.2.2), do not have mineralizable nitrogen rates comparable with the woodland control soil. This suggests that time is an important factor to the development of a fully functional ecosystem with maximised nitrogen use and recycling. The low levels of nitrogen mineralisation support findings by Williams and Cooper (1976). At Betteshanger, the mineralizable rates in the 1m samples do not increase with the age of the spoil. The highest mineralizable rates of nitrogen were in the spoil from the 36 year old category. It is not

fully understood why this area has a large mineralizable nitrogen rate. The plant community associated with this spoil category was composed of legumes and herbs (Figure 4.2.1); legume dominated communities have been associated with higher mineralization rates in primary materials (Dancer, Handley & Bradshaw 1977b, Jeffries, Bradshaw & Putwain, 1981). However, a similar community structure was also found on the 46 year old spoil category at Stodmarsh (Figure 4.2.2), but without a corresponding large mineralizable nitrogen rate at 1m deep.

5.4 Total organic (Kjedahl) nitrogen.

Nitrogen is incorporated into ecosystems *via* atmospheric fixation and most importantly from biological fixation by soil micro-organisms. Ultimately, all biologically available nitrogen comes from nitrogen gas which comprises 80% of Earth's atmosphere. N_2 gas is not directly available to plants or most other organisms. It must be fixed into a useful form by a limited number of nitrogen fixing bacteria such as *Rhizobium* spp. which can convert nitrogen to ammonia, which is then assimilated into biomass. Nitrogen is immobilized in ecosystems by being incorporated into organic molecules i.e. biomass. The amount of organic N in the soil is an indication of the productivity and fertility of an ecosystem (Williams & Cooper 1976; Bradshaw *et al.* 1982).

Total organic nitrogen of soil is the sum of organic nitrogen compounds that are found in that soil. These nitrogen compounds can be extracted by Kjeldahl digestion. Organic nitrogen compounds are digested with sulphuric acid to form ammonia which can then be analysed (Allen 1989). However, previous investigations into organic nitrogen on colliery spoil suggest organic nitrogen determination is complicated by the properties of colliery spoil (Chadwick *et al.* 1978; Jeffries, Bradshaw, & Putwain 1981). A large fraction of the nitrogen in colliery spoil is in the form of fossilised organic nitrogen, thought to originate from lithified plant and detritus that formed the coal measures. This fossilised nitrogen can be extracted along with the organic nitrogen in the Kjeldahl method, and thus give exaggerated readings for the nitrogen which has accumulated in the developing ecosystems on colliery spoil (Palmer, Morgan & Williams1985). The spoil and sites investigated, were all from the same coalfield. The environment in which the coal measures were formed, was therefore presumed to have been the same. The amounts of fossilised nitrogen within the spoil sampled were

therefore also assumed to be the same across the sites. The extracted nitrogen may have included fossilised nitrogen, but the extractions were believed to be comparable across the Kent coalfield because of these assumptions (Palmer *et al.* 1986).

5 .4.1 Method.

The spoil and soil samples were dried in circulating air at a low temperature (40°C). The dried samples were then passed through a 2mm sieve to remove coarse fragments. The soil and spoils samples were then analysed for total nitrogen at Southern Laboratories, Ashford, Kent using the following method: 5g of sample was placed in a Kjeldahl flask containing 2 glass beads. AR grade concentrated sulphuric acid (25ml) and 2 Kjeldahl tablets (containing 5g potassium sulphate, 0.15g copper (II) sulphate, and 0.15g titanium oxide) were added and swirled to mix.

The Kjeldahl flask was then placed in a Nitro-Foss 435 digestion unit and heated at setting 8 until the solution turned green and white fumes had ceased to be evolved. The flask was allowed to cool. Sodium hydroxide solution (32% M/V) was added to the sample until it turned dark brown (178 -185ml were used for the spoil samples). It was then distilled into a receiving vessel containing 25ml boric acid solution (4% M/V) and 2-3 drops of screened methyl red indicator.

The distillate was then titrated with 0.1N hydrochloric acid until the pink/red end point was reached. The volume of HCl titre used was recorded. The procedure was repeated for each sample (Allen 1989).
The Kjeldahl nitrogen in the sample was calculated as follows:

- The relative atomic mass of nitrogen = 14.∴;
- 1 litre of 1N HCl titre is equivalent to 14g of N,
- 1ml of 1N HCl is equivalent to 0.14g of N,
- 1ml of 0.1N HCl titre is equivalent to 0.0014g N,
- so HCl titre X 0.0014 = g of N / 5g of sample,
- So HCl titre X (0.0014/5) X 1000 = g of N per Kg of sample.

The data were analysed using linear regression in the statistical package Minitab (release 13). The analyses included one-way analyses of variance to determine the

significance of the relationship between the age of the spoil and the total nitrogen extracted from the samples (Minitab 2000). The results were then adjusted to Kg N ha^{-1} using the calculated bulk density for colliery spoil from Bradshaw and Chadwick (1980). The results were then compared to total nitrogen targets for developing systems calculated by Marrs (1989).

5.4.2 Results

Table 5.4.1 summarises the significance of the relationship between the total organic nitrogen and the age of the spoil from which it was extracted (see Appendix 5.4.1 for full statistical analyses). The means and standard deviations for total organic nitrogen Kg N ha^{-1} from the different aged spoils sampled at Stodmarsh and Betteshanger are displayed in Tables 5.4.2 and 5.4.3 respectively. Figures 5.4.1-5.4.4 illustrates the total nitrogen Kg N ha^{-1} extracted from the chronosequences at Stodmarsh and Betteshanger at the two different sample depths; trend lines represent fitted regression lines.

The total organic nitrogen extracted from the chronosequences, at the Betteshanger and Stodmarsh sites are all lower than the control soil from the woodland at Canterbury (Table 5.4.2 & 5.4.3).

Total organic nitrogen sampled from 0.1m in the chronosequence, at Stodmarsh, did not show a significant relationship with the age of the spoil from which it was sampled (P=0.065). However, all other samples did have a significant relationship with the age of the spoil from which they were sampled (P<0.0001 in all cases: Table 5.4.1). The regression analyses indicated positive relationships between the age of the spoil and total organic nitrogen in both chronosequences (Figures 5.4.1-5.4.4). The lowest total organic nitrogen was found in the youngest spoil and the oldest spoils had the highest total organic nitrogen (Table 5.4.2 & 5.4.3).

Table 5.4.1 Summary of the analyses of variance: testing the significance of the relationship between the total organic nitrogen and the age of the spoil from which it was extracted.

Site	0.1m samples	1m samples
Betteshanger	***	***
Stodmarsh	n/s	***

Key: *** = $P<0.001$, ** = $P<0.01$, * = $P<0.05$, ns = non significant difference.

Table 5.4.2 Total organic nitrogen (Kg N ha^{-1}) from the Stodmarsh samples.

	Successional age (yr)	Mean	S.D.
0.1m samples	46	752	65.1
	55	720.7	47.3
	69	764.5	162.1
	77	1008	116
	88	1101	221.7
	woodland control soil	1440	68.4

	Successional age (yr)	Mean	S.D.
1m samples	46	229.4	3.76
	55	225.6	3.76
	69	312.1	66.8
	77	323.4	41.4
	88	438.7	25
	woodland control soil	537.7	16.9

Table 5.4.3 Total organic nitrogen (Kg N ha^{-1}) from the Betteshanger samples.

	Successional age (yr)	Mean	S.D.
0.1m samples	18	245.2	38.3
	31	315.1	32
	36	365.5	27.6
	46	307.6	55.6
	55	700.9	96.2
	69	780.6	68.7
	woodland control soil	1440	68.4

	Successional age (yr)	Mean	S.D.
1m samples	18	225.6	2.7
	31	230.9	13.7
	36	228.6	38.3
	46	270	13.2
	55	318.5	53.4
	69	358.6	51.5
	woodland control soil	537.7	16.9

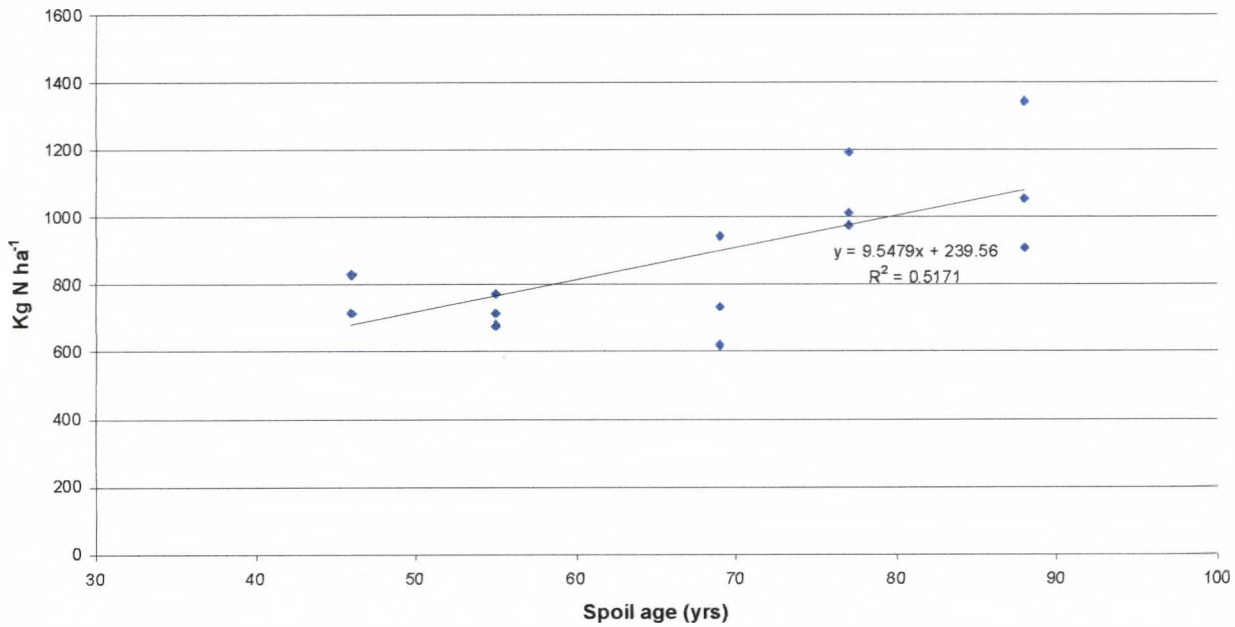

Figure 5.4.1 Total accumulated organic nitrogen (Kg N ha^{-1}) versus age of spoil for 0.1m spoil samples from Stodmarsh.

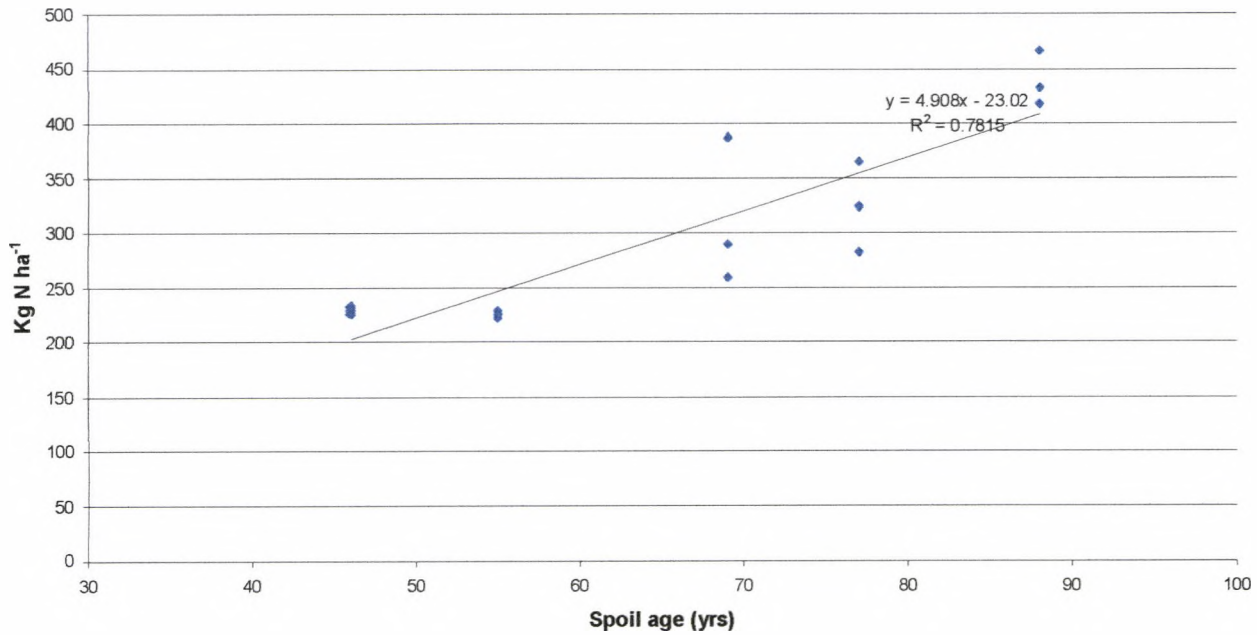

Figure 5.4.2 Total accumulated organic nitrogen (Kg N ha^{-1}) versus age of spoil for 1m spoil samples from Stodmarsh.

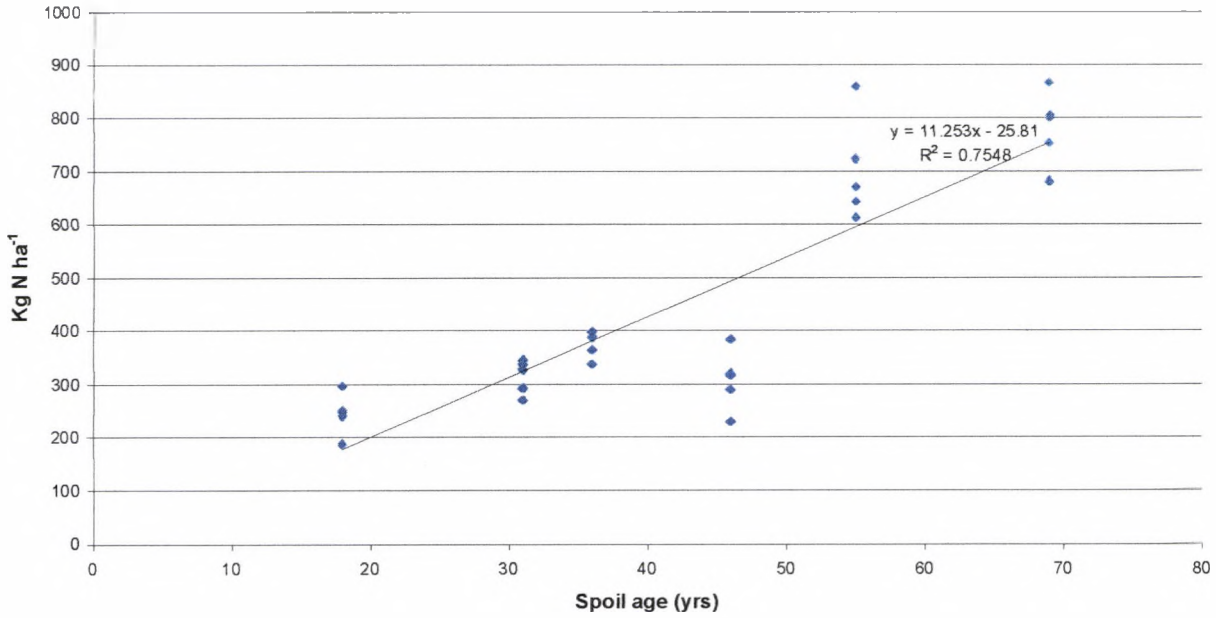

Figure 5.4.3 Total accumulated organic nitrogen (Kg N ha^{-1}) versus age of spoil for 0.1m spoil samples from Betteshanger.

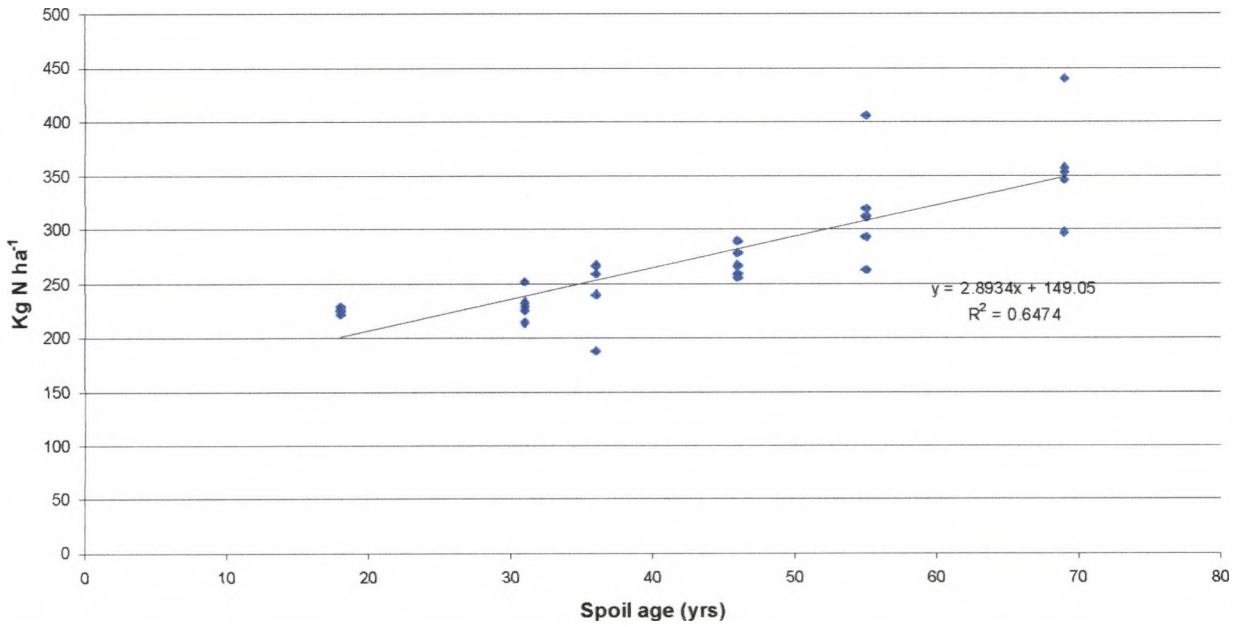

Figure 5.4.4 Total accumulated organic nitrogen (Kg N ha^{-1}) versus age of spoil for 1m spoil samples from Betteshanger.

5.4.3 Discussion.

The woodland control soil has more total organic nitrogen than any of the colliery spoil sampled from the chronosequences, at the Betteshanger and Stodmarsh. This true even where recognisable woodland communities have established on the older spoil at both Stodmarsh and Betteshanger (Chapter 4). One would expect the low levels of nitrogen accumulating in young successional seral stages, such as the communities developing on colliery waste (Dancer, Handley & Bradshaw 1977a; Marrs 1989; Li & Daniels 1994; Foster & Gross 1998; Knops, Bradley & Wedin 2002) and this is supported by the results from this investigation (Tables 5.4.2 & 5.4.3). Nitrogen studies on other colliery sites suggest these total organic nitrogen results could include a significant quantity of fossilised nitrogen, up to 70% (Palmer, Morgan and Williams1985). If this 70% figure is taken into account then the nitrogen accumulation was low on both sites. It is perhaps therefore more feasible that the Kent colliery waste has less fossilised nitrogen than colliery waste from other coalfields.

The amount of accumulating organic nitrogen was predicted to increase with the age of the spoil. This is because, as a succession progresses, above ground plant biomass increases and the biomass in the rhizosphere also increases (Marrs & Bradshaw 1993). The increase in total organic nitrogen with age confirmed findings that young plant communities will have small amounts of accumulated nitrogen in comparison to older plant communities (Li & Daniels 1994).

The samples from the surface 0.1m of the chronosequences had more total nitrogen than the 1m deep samples and indicate the differences in biological composition between the two sample depths. The deeper samples are below the major biologically active part of the rhizosphere and so do not have as high an organic component as the samples from 0.1m deep (Allen 1989). The total nitrogen extracted from the 1m deep samples, at Stodmarsh and Betteshanger, have mean values of 229.4 Kg N ha^{-1} (S.D. 3.76) and 225.6 Kg N ha^{-1} (S.D. 2.7) per kilogram of spoil, respectively. As these samples were below the major biologically active area of the spoil these figures may represent the fossilised nitrogen component of the Kent colliery spoil; they are significantly lower than those which are found in other colliery spoils where figures of 440 Kg N ha^{-1}, of fossilised N, have been reported (Palmer, Morgan and Williams1985).

The highest of these figures (229.4 Kg N ha^{-1}) is the possible amount of fossilised nitrogen in the Kent colliery spoil and the N Kg ha^{-1} figures (Tables 5.4.2 & 5.4.3) can be adjusted by subtracting 229.4 Kg N ha^{-1} from them: the results represent the true organic nitrogen accumulated in the colliery spoil, and can be compared with published data for nitrogen accumulation in raw substrata from Marrs (1989).

The rates of accumulation were calculated for the 0.1m samples as yearly figures by dividing the adjusted figures by the their successional age i.e. length of time they have had to develop; which gives the average nitrogen accumulation Kg N ha^{-1} yr^{-1}. The time to develop non-nitrogen fixing vegetation on each of the aged areas was then calculated by subtracting the amount of accumulated nitrogen from the target nitrogen figure; the highest figure of 1200 Kg N ha^{-1} (Table 5.1.2; Marrs 1989) has been used in the calculations, this was then divided by the yearly rate of accumulation; these calculated results are displayed in Table 5.4.4. Table 5.4.4 illustrates the different nitrogen concentration and the time to develop non-nitrogen fixing dependent vegetation. The woodland control soil has a nitrogen pool of 1483 Kg N ha^{-1} and therefore, has a nitrogen pool above the target level. This suggests that this woodland was not limited by its nitrogen pool. Table 5.4.4 illustrates, that in general, the Stodmarsh samples contained more nitrogen than Betteshanger samples and therefore the Stodmarsh ecosystems were calculated to take less time to develop the target nitrogen level than Betteshanger's.

Table 5.4.4 Estimates of target nitrogen contents and the time taken to reach these targets on four raw substrata, and calculated nitrogen contents for colliery spoil and the time for each stage of the chronosequences to reach a target nitrogen content of 1200 Kg N ha^{-1} (adapted from Marrs 1989).

Substrate	Time to develop non-nitrogen fixing vegetation (yr)	Nitrogen content in soil (Kg N ha^{-1})
Glacial moraines	100	1200
Sand dunes	21	400
Ironstone	100	600
China clay waste	>70	700
China clay waste	>120	1200
Age of spoil in yrs		
46	58	522.6
55	83	491.3
Stodmarsh 0.1m samples 69	85	535.1
77	42	778.6
88	33	871.6
18	>1000	15.8
31	412	85.7
Betteshanger 0.1m samples 36	288	136.1
46	303	78.2
55	85	471.5
69	74	551.2
Woodland control soil	-	1483

At Stodmarsh, the greatest quantities of total organic nitrogen are found in the top 0.1m of the oldest spoils of 77 and 88 years old (Table 5.4.2). The large amount of nitrogen recovered in comparison with other aged spoils may reflect the maturity of the plant communities on these spoils. This is supported by results from Betteshanger where the largest quantity of nitrogen was extracted from the top 0.1m of the 55 & 69 year old spoils (Table 5.4.3).

Determining the effects of differing nitrogen budgets on plant species diversity, within developing vegetation, is important to the understanding of community successional development. In this study it was found as the community structure changed with age (4.2) there was a corresponding change in the nitrogen budget.

The Leguminosae composition of the plant communities at Stodmarsh and Betteshanger did not seem to have a strong influence on the total organic nitrogen extracted from these sites. At Stodmarsh, Leguminosae dominate in the 46-69 year old plant communities, and at Betteshanger in the 36 year old plant community (Chapter 4). The increase in nitrogen by Leguminosae and other nitrogen fixing species in reclamation sites are well documented (Dancer, Handley & Bradshaw 1977b; Skeffington and Bradshaw 1980; Jeffries, Bradshaw & Putwain 1981; Walker 1993; Chapman, Collins & Younger 1996). At Betteshanger and Stodmarsh the expected increase in total organic nitrogen associated with Leguminosae was not observed. The exception maybe the 36 year old community at Betteshanger where the time to develop the target nitrogen content is less than would be predicted from the age time sequence illustrated in Table 5.4.4, and the community is dominated by legumes (4.2).

The time to reach the target nitrogen of 1200 Kg N ha^{-1} is less for Stodmarsh samples than for Betteshanger samples (Table 5.4.4). In general this is due to the higher nitrogen levels in Stodmarsh samples than in Betteshanger and is related to the age of the spoil samples, but the relationship between the age of the spoil and accumulation time differs between the two sites. At Stodmarsh, there is a linear decrease in the time to reach the target nitrogen level related to the age of the spoil, where as at Betteshanger, there is an exponential decrease in the time to reach the target nitrogen level and the age of the spoil (Figures 5.4.5 & 5.4.6).

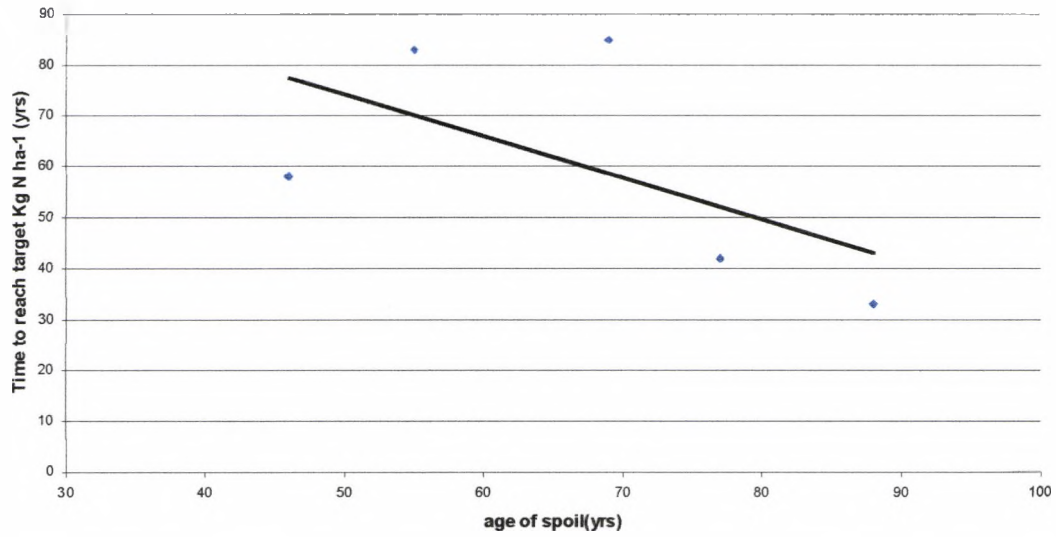

Figure 5.4.5 Time to reach the target nitrogen level of 1200kg N ha^{-1} versus the age of the spoil at Stodmarsh.

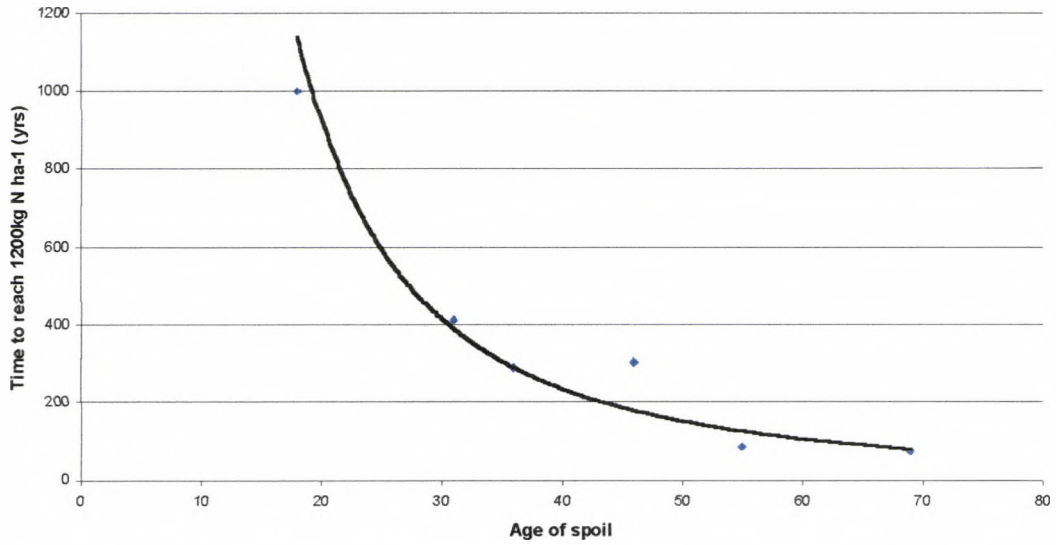

Figure 5.4.6 Time to reach the target nitrogen level of 1200 kg N ha^{-1} versus the age of the spoil at Betteshanger.

The difference in nitrogen accumulation at the two sites is probably controlled by the different vegetation structure at the two sites (4.2), but also could be the function of the age range of the chronosequence from which the nitrogen was sampled (Li & Daniels1994); as very young spoil was not available to analyse at Stodmarsh this

probably influenced the lack of significance for the relationship between total nitrogen levels and the age of spoil. The spoil age categories of 55 and 69 years at both sites have similar nitrogen levels and their times to reach the target nitrogen level are also similar, although the vegetation structure in these age categories are dissimilar (4.2). The exponential relationship observed at Betteshanger supports models of nitrogen and successional development in ecosystems. Models predict a nitrogen limiting threshold, below which ecosystem development is slow and which once reached the ecosystem develops rapidly with an associated increase in nitrogen storage in the system (Bradshaw *et al.* 1982; Bradshaw 1983; Marrs 1989). The vegetation system at Betteshanger does not follow a typical successional model (4.2). However, the total nitrogen does. Conversely the Stodmarsh vegetation system follows a typical facilitation successional model, but the total nitrogen dynamics do not follow a predicted pattern (Li & Daniels 1994). This suggests that the species composition of a community must be intimately linked with the ecological functionality of an ecosystem, with regards nitrogen, and supports experimental findings by Tilman *et al.* (2002).

6 Plant resources and mycorrhizal fungi in stressed vegetation systems.

Mycorrhizal fungi (*myco*=fungus *rhiza*=root), in general, form symbiotic (mutualistic) relationships with plants. The mycorrhizae obtain some of the sugars they require from plants (Gavito and Olsson 2003), while the higher plant can benefit from the increased efficiency in the uptake of mineral nutrients (Smith and Read 1997), increased drought resistance (Ruiz-Lazano and Azcon 1995), and increased resistance to insect herbivory (Gange and Bower 1997). The interactions between mycorrhizal fungi and higher plant hosts are not always beneficial though. At certain levels of colonisation or where resources, especially phosphorus, are not limiting then the relationship between plant benefit and mycorrhizal fungi can be negative (Son and Smith 1988; Peng, Eissenstat & Graham 1993). A curvilinear relationship between mycorrhizal colonisation and plant 'benefit' is proposed by Gange and Ayres (1999), as a more realistic model of plant/mycorrhizal fungal interaction.

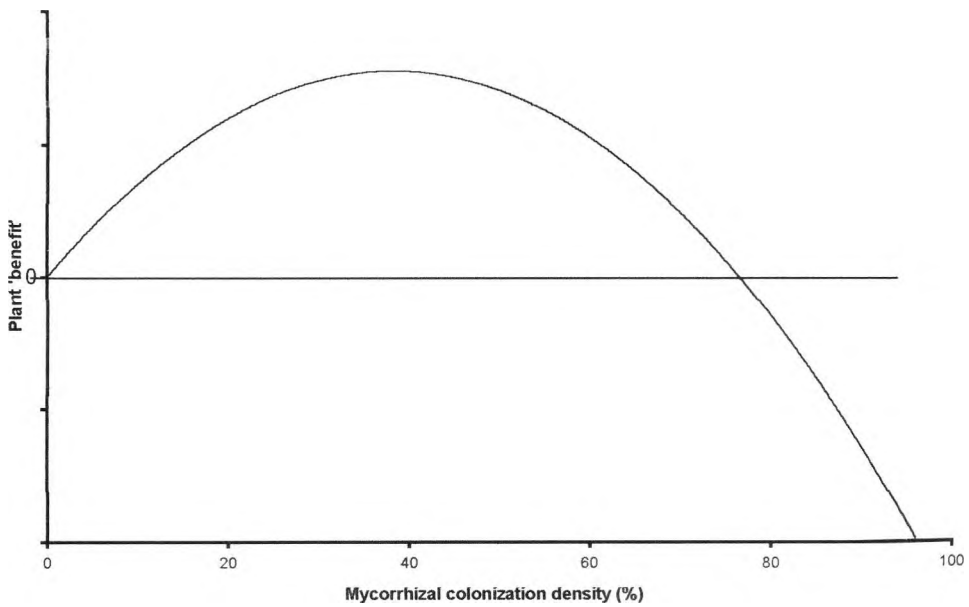

Figure 6.1 "*The proposed curvilinear relationship between mycorrhizal colonization density and plant 'benefit'. The model predicts that over a range of colonization densities, there will be a positive effect of the mycorrhiza on plant performance, but only up to a point; after this 'benefit' declines and can become negative if colonization is too high*" (Gange and Ayres1999 p. 617).

It is estimated that more than 70% of the species of higher plants, including crop plants, form relationships with mycorrhizal fungi, as do many pteridophytes and some bryophytes (Brundrett 1991). Mycorrhizae, both in wild and cultivated plant communities, have been shown to be important or even essential for plant

performance. The association between plants and mycorrhizae is the norm rather than the exception (Allen 1992). The importance of mycorrhizal fungi within plant communities, and their effect on the structure and composition, is becoming recognised as crucial (Smith and Read 1997). Importantly, with respect to land reclamation and ecological restoration, plant species coexistence and community structure are influenced by their associated mycorrhizal fungi. Mycorrhizal fungi affect plant communities indirectly by influencing the pattern and strength of plant competitive interactions (van der Heijden *et al.* 1998a; van der Heijden *et al.* 1998b; Smith, Hartnett and Wilson 1999; Hart, Reader and Klironomos 2003). Mycorrhizae have also been thought to influence community structure in successional plant systems (Gange, Brown and Farmer 1990; Boerner, De Mars and Leicht 1996; Smith 2000).

There are four common types of mycorrhizal fungi; orchid mycorrhizae, ericaceous mycorrhizae, ectomycorrhizae and arbuscular mycorrhizae (Clapp *et al.* 2002). Each of them represents a distinctive association. Orchid mycorrhizae and ericaceous mycorrhizae, as their names suggests, form symbiotic relationships with members of the Orchidaceae family and members in the *Erica* genus respectively. Orchid mycorrhizae have been linked with seed germination as well as nutrient uptake and pathogen protection within the family Orchidaceae (Hadley1982). However, orchid mycorrhizae and ericaceous mycorrhizae are of limited interest within the scope of this study.

6.1 Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizae are named after the arbuscule (*tree like*) structures which they develop within plant root cells. The fungi involved are members of the Zygomycota (related to *Mucor*). AMF are currently classified into approximately 150 species (Clapp *et al.* 2002). The low global diversity of AMF is not mirrored by the diversity which can be found at a single site; routinely 10-30 spore types (Eom, Hartnett and Wilson 2000). Arbuscular mycorrhizae are axenic, i.e. they cannot be cultured without a (Dodd 2000).

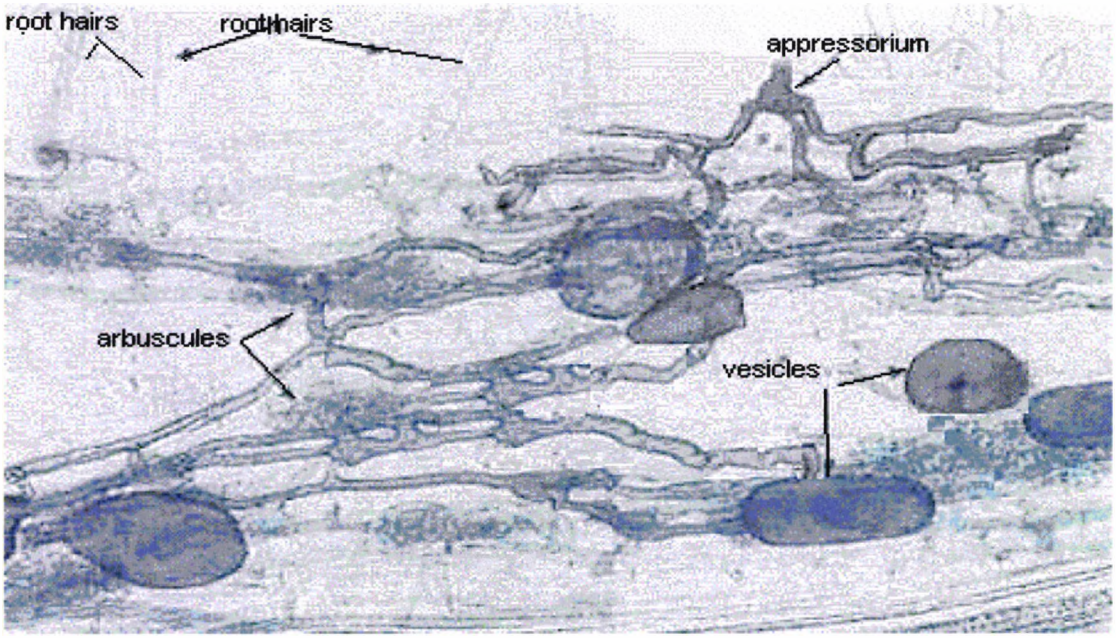

Plate 6.1.1 Stained arbuscular mycorrhizal fungal structures in a clover root.

In Plate 6.6.1 mycorrhizal fungal colonisation has been exposed by clearing the root tissues with strong alkali and then staining with trypan blue to reveal the mycorrhizal fungal structures. The appressorium was the site of root penetration where the fungus produced a pre-penetration swelling. It grew between the root cells and formed finely branched arbuscules and swollen vesicles.

The arbuscule is a repeatedly dichotomous branching structure which forms inside a root cell. The branching nature of the arbuscule gives a large surface area over which exchange of minerals/sugars can occur. The plant cell remains alive, because the cell's membrane extends to encase all the branches of the arbuscule. Strictly speaking, therefore, the fungus is always outside of the cell, surrounded by the cell membrane. The vesicles are thought to be used for storage of nutrients (Allen 1992).

Arbuscular mycorrhizae are found on the vast majority of wild and crop plants, with an important role in mineral nutrient uptake and sometimes in protecting against drought or pathogenic attack. Structures resembling those of the present-day AMF have been found in fossils of primitive pteridophytes of the Devonian period. It is thought that these fungi colonised the earliest land plants and that mycorrhizal associations could have been essential for the evolution of terrestrial plants (Taylor, Remy & Kerp 1995).

AMF play an important role in the absorption of mineral nutrients; their hyphae extend into the soil system giving a large surface area over which absorption can

occur (Robinson and Fitter 1999; Dodd 2000). If a soil system is deficient in minerals, or the minerals are immobile, then AMF can be crucial in the success of vegetation (Van Aarle, Rouhier and Saito 2002), this has important connotations for land restoration projects where the substrate is deficient in nutrients. Although AMF are thought to play a particularly important role in the absorption of phosphate, a poorly mobile plant nutrient within ecosystems, they also promote the decomposition and absorption of nitrogen (Hodge, Campbell and Fitter 2001) and other plant nutrients (Olsson, Jakobsen and Wallander 2002).

6.2 Ectomycorrhizal fungi.

Ectomycorrhizal fungi (sometimes termed ectotrophic mycorrhizae) are normally associated with trees and do not produce arbuscules (Plate 6.1.2). Their hyphae grow to form a sheath surrounding a plants roots (a Hartig net) (Bending & Read 1995). Ectomycorrihzae share the same properties as AMF in that they increase the symbiotically associated plants mineral nutrient foraging capabilities in exchange for sugars (Bowen & Theodorou 1973). Ectomycorrhizae are characteristic of many tree species in the cooler parts of the world, for example the genera: *Pinus*, *Picea*, *Abies*, *Quercus*, *Betula* etc. in the Northern Hemisphere and *Eucalyptus* in Australia. However, some trees (e.g. species of *Salix* and *Betula*) can have both ectomycorrhizae and arbuscular mycorrhizae (Chilvers, Lapeyrie & Horan 1987). Most tropical trees have only arbuscular mycorrhizae (Alexander & Hogberg 1986).

Plate 6.2.1 Fruiting body of *Scleroderma citrina* an ectomycorrhizal fungus associated with *Betula pendula* on Betteshanger and Stodmarsh sites.

Ectomycorrhizae are mainly from the two fungus classes, Ascomycota and Basidiomycota, including many that produce characteristic toadstools. Most of these fungi can be grown in laboratory culture but, unlike the wood-rotting fungi, they are poor degraders of cellulose and other plant wall materials. They therefore gain most of their sugars from the living plant roots in natural conditions (Dighton, Thomas and Latter 1987).

Ectomycorrhizal fungi can degrade proteins (they release protease enzymes) and thus can obtain nitrogen from decomposing leaf litter (Pérez–Moreno and Read 2000). It is hypothesised that they might play a crucial role in the nitrogen nutrition of trees (Abuzinadah and Read 1988; Smith and Read 1997). This could be highly significant for land reclamation strategies in substrata such as colliery waste, where the rates of mineral nutrient recycling are low.

Experiments with radioactive tracers have shown that when labelled CO_2 is supplied to leaves of tree seedlings, the labelled carbon is found in plant sugars (sucrose, etc.) which move to the roots and eventually the label enters the fungal sheath. Here the labelled carbon occurs in the form of typical 'fungal carbohydrates' such as mannitol and trehalose (Högberg *et al.* 1999). Most plants and plant tissues cannot metabolise these compounds, so there is, in effect, a one-way flow of carbohydrate to the fungus (Graham, Duncan and Eissenstat 1997). The cost of this to the plant may be considerable; however, the plant can also benefit from the association, because the fungal hyphae that ramify into soil are efficient in capturing mineral nutrients. These mineral nutrients accumulate in the sheath, but at least some minerals are transferred to the plant, presumably, from the Hartig net (Rayner 1995).

Young tree seedlings growing in the shade of 'mother' trees can be attached to the 'parent' by a common network of mycorrhizal hyphae in the soil. In these conditions, at least some movement of labelled elements has been shown to occur from the roots of the parent to the younger trees, perhaps helping to nurture the seedlings (Finlay and Read 1986; Finlay 1989; Pérez–Moreno and Read 2000).

6.3 The influence of mycorrhizal fungi and nutrient additions on the establishment and survivorship of *Betula pendula* and *Lotus corniculatus* grown at different population densities.

At Betteshanger former colliery, trees act as primary colonisers on the colliery waste. This appears to be a paradox with respect to successional theory (Chapter 4). A more typical succession system is seen at Stodmarsh where nitrogen fixing species, such as *Lotus corniculatus,* colonise the nutrient poor substrata before tree species. However, *B. pendula* colonise the colliery waste before herb cover at Betteshanger and seem to provide the first organic component for pedogenisis. In conjunction with the tree colonisation there is also ectomycorrhizal colonisation. The presence of mycorrhizal fungi may be crucial to the ability of the trees to colonise the waste. *B. pendula* can be colonised by both ecto- and endo- mycorrhizal fungi (Harley and Harley 1987). Ectomycorrhizal fungi can infect *B. pendula* very rapidly in laboratory conditions; mature mycorrhizae have been recorded to develop between 2 to 8 days after inoculation (Feugey *et al.* 1999). This rapid colonisation could give an advantage to the trees when colonising a nutrient poor substrate such as colliery waste.

To investigate the influence of mycorrhizal fungi on the development and competition of *B. pendula* and *L. corniculatus* on colliery waste an experiment was initiated in April 2000. Different densities of mixtures and monocultures of *B. pendula* and *L. corniculatus* were planted with different inputs of nitrate, phosphate, and mycorrhizal fungal inoculate.

The association of mycorrhizal fungi with *B. pendula,* as observed in the field, could be crucial in enabling the tree to access, more effectively, resources. Thus *B. pendula* can perhaps colonise colliery waste, which is deficient in plant nutrients, more effectively than would be otherwise without its mycorrhizal association. The foraging advantage mycorrhizal fungi give to *B. pendula,* is predicted to manifest in the difference in production in biomass between treatments. Biomass will be greatest in those treatments with mycorrhizae compared with those without. However, where there are added nutrients, the advantage to the trees with a mycorrhizal infection will be lessened. Therefore, it was aimed to measure the difference in biomass production between treatments.

6.3.1 Method.

An area of colliery waste at Betteshanger was double tilled with a sub-soiler to a depth of 1m.

Plate 6.3.1 Site preparation to negate compaction problems associated with the site.

The experimental area was enclosed with a rabbit proof fence and subdivided into 480 experimental units 1.2m X 1.2m. The factorised treatments for all the planting densities were: +/- mycorrhizal fungal inoculate, +/- nitrogen at 75g/m^2, +/- phosphorus at 75g/m^2. Experimental units were randomly ascribed planting treatments using a random number generator. Five replicates of each treatment were established.

Table 6.3.1 Planting densities of *L. corniculatus and B. pendula*.

	8 *L. corniculatus*	*16 L. corniculatus*	*24 L. corniculatus*
8 B. pendula	8 *L. corniculatus* 8 *B. pendula*	16 *L. corniculatus* 8 *B. pendula*	
16 *B. pendula*	8 *L. corniculatus* 16 *B. pendula*		
24 *B. pendula*			

Yearling *B. pendula* were planted as 30 cm whips in April 2000. The trees were all root dipped (in 1:3 water storing gel: deionised distilled water) immediately prior to planting (see Plates 6.3.2 and 6.3.3).

Plate 6.3.2 Root dipping birch prior to planting.

Plate 6.3.3 *Betula pendula* were planted immediately after root dipping.

The mycorrhizal treatment was made with AMF isolated from the site. Arbuscular mycorrhizal fungal spores were extracted from spoil collected from the rhizosphere at Betteshanger in June 1999, by wet sieving and sucrose density gradient centrifugation. The spoil was washed and material flushed through a 500µm sieve and collected in a 45µm sieve. The contents of the fine sieve were back-washed into centrifuge tubes and a 60% (w/v) commercial sucrose solution added to the backwashed material using a syringe. After centrifugation at 3000 rpm for 2 minutes, the supernatant was washed into a 45µm sieve and the contents back-washed into a Petri dish (Brundrett, Melville and Peterson 1994). AMF spore identification was based on spore morphology under a dissecting microscope and individual AMF spores were collected and rinsed. Plantworks Ltd, of Sittingbourne Research Centre, Sittingbourne, Kent, UK, then grew the isolated AMF to sufficient quantities using *Trifolium pratense* as a host plant. The mycorrhizal material was added to the root dipping medium and mixed well before use. The mycorrhizal fungi treatment was made up of 180g/l of axenically cultured AMF spores and infected root of *T. pratense,* and 20g/l of *Scleroderma citrina* spore material collected directly from the site. The

AMF species were: *Glomus geosporum* (45g), *Glomus intraradices* (45g), *Glomus claroideum* (45g), *Acaulospora morrowiae* (45g).

L. corniculatus was planted in small depressions, approximately 2cm deep, which were scooped out of the spoil. Five seeds per depression were sown; the seeds were lightly covered with spoil. In the experimental units with a mycorrhizal treatment, 20g of mycorrhizal inoculate was placed in the depression prior to sowing. Five *L. corniculatus* seeds were then placed on top of the inoculate and then lightly covered with spoil. The fertiliser treatments were added as a top dressing after planting. Both plant species were bought from suppliers guaranteeing British provenance.

The stem diameter 1cm above ground and the first internodal length, of the yearling *B. pendula*, were measured at the start of the experiment. This was to negate any initial tree size bias, in the tree stock, and to enable these measurements to be removed as covariates in the analyses and interpretation of the data. The trees were checked in June 2000 and dead trees were replaced. In June 2001 stem diameter was measured 1cm above ground using callipers with a vernier scale; the first internodal length of *B. pendula* was also measured. The *L. corniculatus* plants grew with a prostrate form producing a circular, flattened disc of stems and leaves in the experiment. This disc form is referred to in the rest of this study as leaf rosettes. The leaf rosette diameter and the number of *L. corniculatus* flower heads per plant were measured. Root samples were also collected from specimens in each treatment to enable the presence of mycorrhizae to be assessed.

Mycorrhizal presence was assessed using the following method. The root samples were cleared in 2% (w/v) KOH, for 1h at 90°C in a water bath, and then rinsed with water three times using a mesh and forceps. The roots were subsequently covered with 2% (v/v) HCl for 45 minutes. The HCl was decanted and the roots covered with 0.05% (w/v) trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) and placed for 15minutes to 1hour at 90°C in a water bath, until the roots were strongly stained. The roots were then placed into a Petri dish with 50% (v/v) glycerol for de-staining, so that only the fungal structures kept their blue colour, and observed with a compound microscope.

The data were confirmed to be normally distributed by plotting and comparing: residual histograms, normal probability plots of residuals, residuals versus fitted residuals and residuals versus order using Minitab (release 13) statistical package

(Appendix 6.3.1). The data were analysed by the General Linear Model analysis of variance using the statistical software Minitab (release 13). The initial measurements of the trees, from 2000, were used as covariates in the analysis. Tukey's simultaneous pairwise comparison tests were include in the analyses. These enabled the identification of significant differences between each treatment and level of treatment (Minitab 2000). See Appendix 6.3.1 for full statistical analyses.

6.3.2 Results.

The full analyses of variance, and Tukey's simultaneous pairwise comparison tests, are displayed in Appendix 6.3.1. The responses of tree growth to the treatments are summarised in Tables 6.3.2 and 6.3.3. Tables 6.3.4 and 6.3.5 summarise the measured responses, of *L. comiculatus,* to the treatments. Figures 6.3.1, 6.3.2 and 6.3.3 illustrate the mean growth response, of the first internodal lengths, of *B. pendula* to the treatments. Figures 6.3.4, 6.3.5 and 6.3.6 illustrate the mean growth response, of the stem diameter, of *B. pendula* to the treatments. Figures 6.3.7 and 6.3.8 illustrate the mean response of tree growth to treatments and different planting densities. In all figures error bars represent standard deviation. Columns labelled with the same lower case letter were not significantly different from each other following analysis of variance and Tukey's simultaneous pairwise comparison tests.

Only the root samples taken from the treatments with mycorrhizal inoculate showed mycorrhizal structures to be present. By contrast, root samples from experimental units without mycorrhizal inoculate had no evidence mycorrhizal fungi. This result indicated that the mycorrhizal inoculate had infected the treated plants and surprisingly there had been no background infection in the trial.

Table 6.3.2 displays the significance of treatments and planting density on the stem diameter growth of *B. pendula*. Additions of mycorrhizal fungi ($P<0.001$), nitrogen ($P<0.001$) and phosphorus ($P<0.001$) significantly increased the stem diameter of *B. pendula*. Stem diameter was significantly increased by the synergistic affects of combined treatments of mycorrhizal inoculate and nitrogen additions ($P=0.045$) and mycorrhizal inoculate and phosphorus additions ($P=0.002$). Increasing the birch planting density significantly decreased stem diameter ($P<0.001$).

Table 6.3.3 displays the significance of treatments and planting density on the growth of the internodal length of *B. pendula*. Additions of mycorrhizal fungi ($P<0.001$), nitrogen ($P<0.001$) and phosphorus ($P<0.001$) significantly increased the internodal

extension of *B. pendula*. Internodal length was significantly increased by the synergistic affects of the combined mycorrhizal inoculate and phosphorus treatments (P=0.001). Increasing the birch planting density significantly decreased internodal length (P<0.001).

Table 6.3.4 illustrates the significance of treatments, and planting density, on leaf rosette diameter growth of *L. corniculatus*. Addition of nitrogen (P<0.001), phosphorus (P=0.012) significantly increased the diameter of the leaf rosette diameter of *L. corniculatus*. Increasing Birch planting density (P<0.001) and *L. corniculatus* planting density (P<0.001) significantly reduced the leaf rosette diameter of *L. corniculatus*.

Table 6.3.5 summarises the significance of treatments and planting density on flower head production. Without phosphorus and nitrogen the mean flower head per experimental unit was 8.75 $\pm$3.42. Adding nitrogen significantly increased flower head production to 16.25$\pm$5.59 (P=0.025), when phosphorus was added 17.5$\pm$5.23 (P<0.001) and when phosphorus and nitrogen were added together the mean was 21.25$\pm$3.42, but there was no significant synergy between the two treatments (P=0.111).

The data summarised in Figures: 6.3.1, 6.3.2, 6.3.3, 6.3.4, 6.3.5 and 6.3.6 illustrate that all treatments, at all planting densities, increased the stem diameter and internodal length of *B. pendula*, in comparison with the control units with no treatments. Experimental units with a planting density of twenty four trees, per experimental unit, suppressed growth in comparison to all other planting densities. The experimental control units had significantly less growth than those experimental units with a treatment. The experimental units which had the mycorrhizal treatment generally had greater growth than the experimental units which had just mineral fertiliser treatments. However, the units with just the mycorrhizal treatment, had no significant difference in growth of the internodal length from those units which had both, nitrate and phosphate additions in planting densities of sixteen trees per unit (Figure 6.3.2). There was a similar pattern for stem diameter in all planting densities (Figures 6.3.4, 6.3.5 and 6.3.6).

Figure 6.3.7 summarises the effect of planting density and treatments on the mean growth of the first internodal length of *B. pendula*. The experimental control units, which had no treatments, had significantly less growth than experimental units which had treatments. In general, the experimental units with sixteen *B. pendula* had less

growth than experimental units with eight trees. The exceptions to this were experimental units with just the mycorrhizal treatment, in these there were no significant differences in growth between planting densities. The experimental units with eight trees and which included the mycorrhizal treatment and an addition of inorganic nitrogen, phosphorus or both fertilisers did not have significantly different growth from each other. In the experimental units with sixteen trees there was a similar pattern observed, except where additions of both nitrogen and phosphorus together. In these experimental units there was a significant increase in internodal length.

Figure 6.3.8 summarises the effect of planting density and treatments on the mean increase in stem diameter of *B. pendula*. The experimental control units, which had no treatments, had significantly less growth than experimental units which had treatments. However, there was no significant difference between the different planting densities and combinations of treatments on stem diameter growth.

Figure 6.3.9 illustrates the response of the mean leaf rosette diameter of *L. corniculatus* to treatments. Increasing planting density of *B. pendula* had a significant limiting effect on the above ground growth of *L. corniculatus* ($P<0.001$). In experimental units with 16 trees, the addition of extra nitrogen or phosphorus had no effect on the rosette diameter of the *L. corniculatus* plants.

The planting density of *L. corniculatus,* in monoculture experimental units affected its ability to produce flower heads. Generally, in all treatments, eight plants per experimental unit had the highest mean flower heads decreasing as the planting density increased (Figure 6.3.10). Mixed planting experimental units reduced flower head production in comparison to monoculture plantings of *L. corniculatus*, even in the highest density monoculture planting of 24 *L. corniculatus* plants per unit (Figure 6.3.10). In experimental units where *L. corniculatus* were planted with eight *B. pendula,* flower head numbers were severely reduced and *L. corniculatus* planted with sixteen *B. pendula,* per experimental unit, did not produce any flower heads. Table 6.3.5 summarises the significance of treatments and planting density on flower head production. Without phosphorus and nitrogen the mean flower head per experimental unit was 8.75 ± 3.42. Adding nitrogen significantly increased flower head production to 16.25 ± 5.59 ($P=0.025$), when phosphorus was added 17.5 ± 5.23 ($P<0.001$) and when phosphorus and nitrogen were added together the mean was 21.25 ± 3.42, but there was no significant synergy between the two treatments ($P=0.111$).

Table 6.3.2 Summary of the analysis of variance for the stem diameter response to treatments.

Treatment additions	Significance	Response
Mycorrhizal inoculate	***	+ve
Nitrogen	***	+ve
Phosphorus	***	+ve
Nitrogen X phosphorus	ns	=
Mycorrhizal inoculate X nitrogen	*	+ve
Mycorrhizal inoculate X phosphorus	**	+ve
Mycorrhizal inoculate X nitrogen X phosphorus	ns	=
Birch Planting density	***	-ve
L. corniculatus planting density	ns	=

Key: *** = $P<0.001$, ** = $P<0.01$, * = $P<0.05$, ns = non significant difference.

Table 6.3.3 Summary of the analysis of variance for the internodal length response to treatments.

Treatment additions	Significance	Response
Mycorrhizal inoculate	***	+ve
Nitrogen	***	+ve
Phosphorus	***	+ve
Nitrogen X phosphorus	ns	=
Mycorrhizal inoculate X nitrogen	ns	=
Mycorrhizal inoculate X phosphorus	**	+ve
Mycorrhizal inoculate X nitrogen X phosphorus	ns	=
Birch Planting density	***	-ve
L. corniculatus planting density	ns	=

Key: *** = $P<0.001$, ** = $P<0.01$, * = $P<0.05$, ns = non significant difference.

Table 6.3.4 Summary of the analysis of variance for the leaf rosette diameter response to treatments in experimental units with the mycorrhizal treatment (experimental units without a mycorrhizal treatment were omitted from the analysis, summarised below, as no *L. corniculatus* germinated in these units).

Treatment additions	Significance	Response
Nitrogen	***	+ve
Phosphorus	*	+ve
Nitrogen X phosphorus	ns	=
Birch Planting density	***	-ve
L. corniculatus planting density	***	-ve

Key: *** = $P<0.001$, ** = $P<0.01$, * = $P<0.05$, ns = non significant difference.

Table 6.3.5 Summary of the analysis of variance for the number of *L. corniculatus* flower heads per experimental unit in response to treatment (experimental units without a mycorrhizal treatment were omitted from the analysis summarised below as no *L. corniculatus* germinated in these units).

Treatment additions	Significance	Response
Nitrogen	*	+ve
Phosphorus	***	+ve
Nitrogen X phosphorus	ns	=
Birch Planting density	***	-ve
L. corniculatus planting density	***	-ve

Key: *** = $P<0.001$, ** = $P<0.01$, * = $P<0.05$, ns = non significant difference.

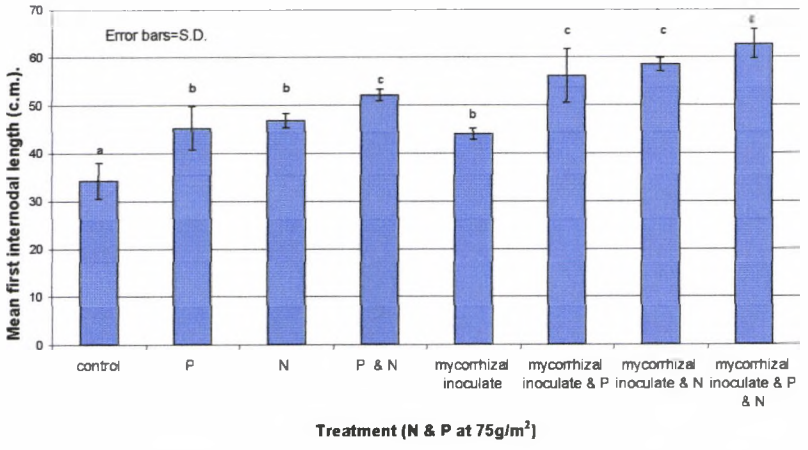

Figure 6.3.1 The mean growth response to treatments, of the first internodal length, of *Betula pendula* planted at eight trees per experimental unit.

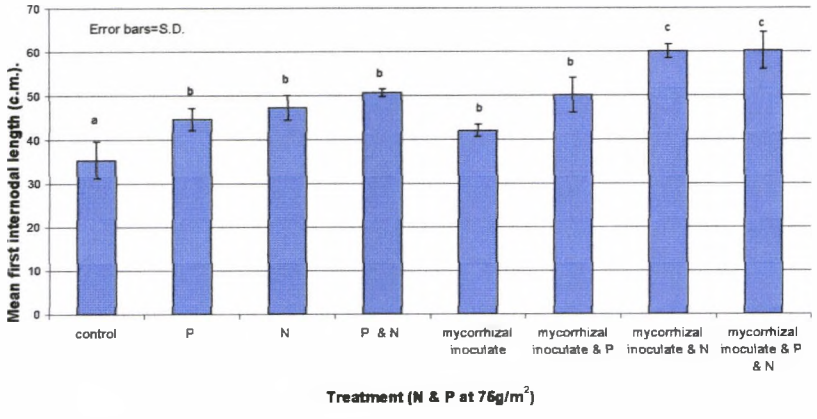

Figure 6.3.2 The mean growth response to treatments, of the first internodal length, of *Betula pendula* planted at sixteen trees per experimental unit.

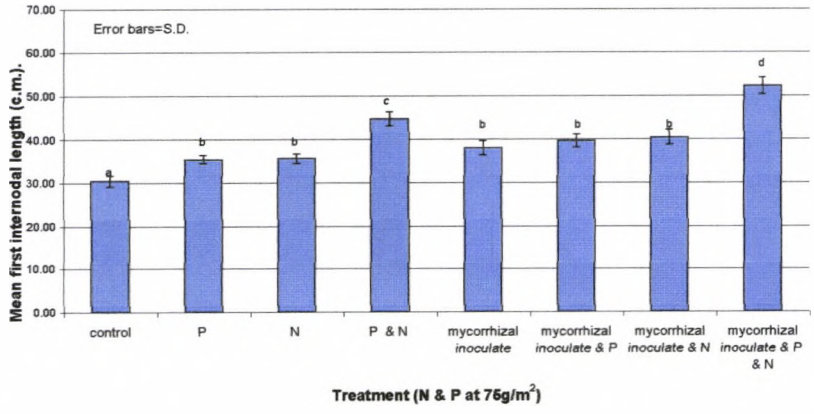

Figure 6.3.3 The mean growth response to treatments, of the first internodal length, of *Betula pendula* planted at twenty four trees per experimental unit.

Columns labelled with the same lower case letter, in figures: 6.3.1, 6.3.2 and 6.3.3, were not significantly different at $P<0.05$ following analysis of variance and Tukey's simultaneous pairwise comparison tests.

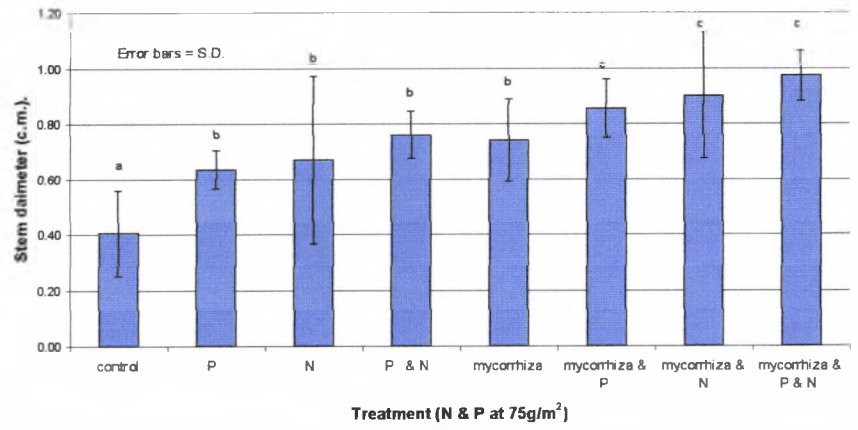

Figure 6.3.4 The mean growth response to treatments, of the stem diameter, of *Betula pendula* planted at eight trees per experimental unit.

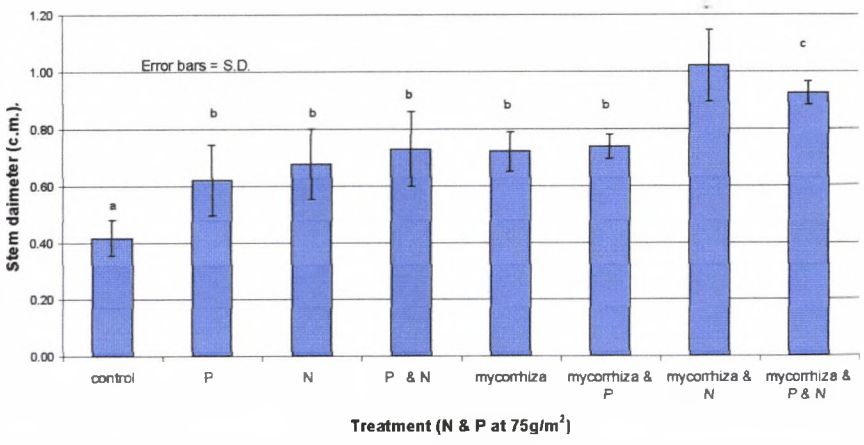

Figure 6.3.5 The mean growth response to treatments, of the stem diameter, of *Betula pendula* planted at sixteen trees per experimental unit.

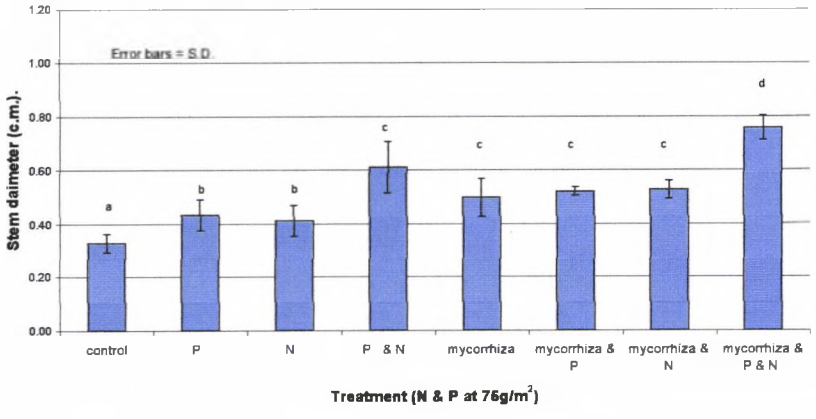

Figure 6.3.6 The mean growth response to treatments, of the stem diameter, of *Betula pendula* planted at twenty four trees per experimental unit.

Columns labelled with the same lower case letter, in figures: 6.3.4, 6.3.5 and 6.3.6, were not significantly different at $P<0.05$ following analysis of variance and Tukey's simultaneous pairwise comparison tests.

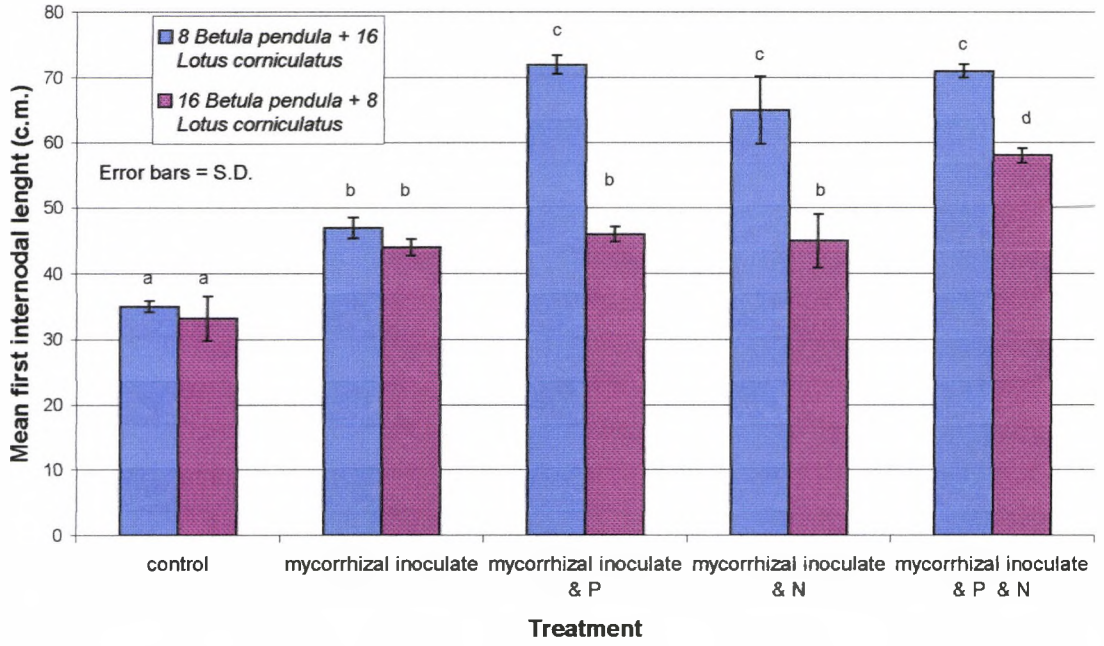

Figure 6.3.7 The effect of planting density and treatments on the mean growth of the first internodal length of *Betula pendula* from May 2000 – August 2001.

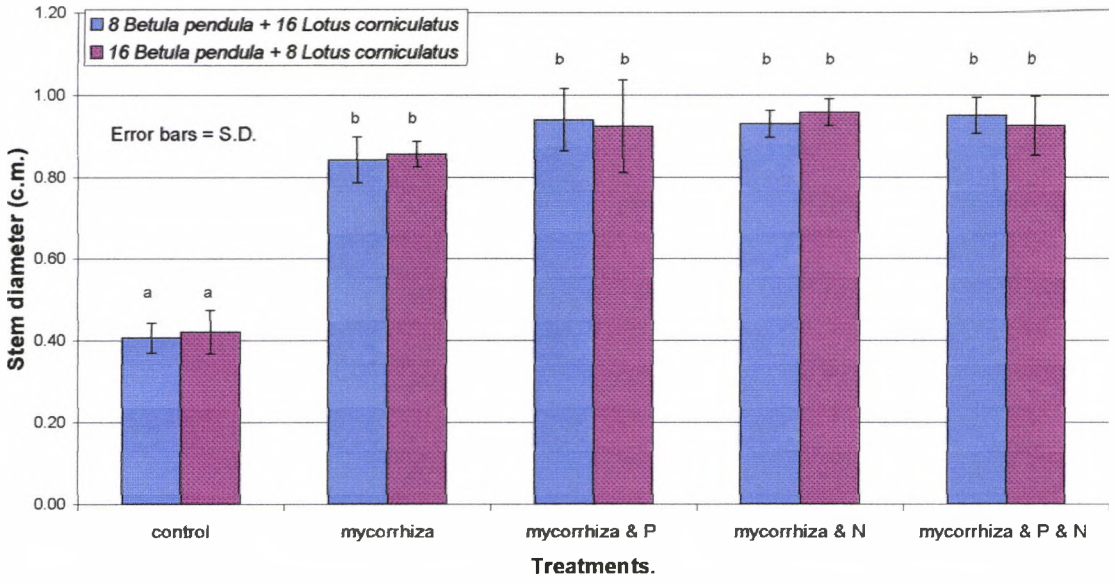

Figure 6.3.8 The effect of planting density and treatments on the mean increase in stem diameter of *Betula pendula* from May 2000 – August 2001.

Columns labelled with the same lower case letter were not significantly different at $P<0.05$, following analysis of variance and Tukey's simultaneous pairwise comparison tests.

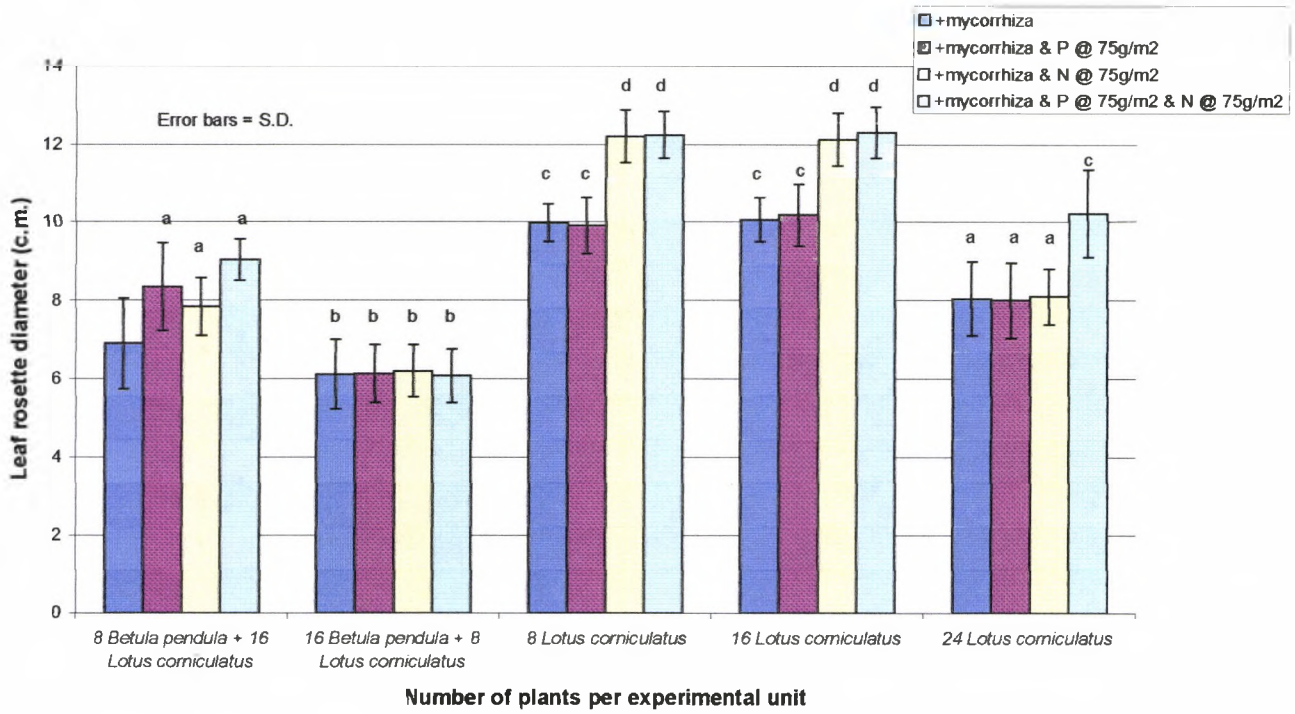

Figure 6.3.9 Mean leaf rosette diameter of *Lotus corniculatus.*

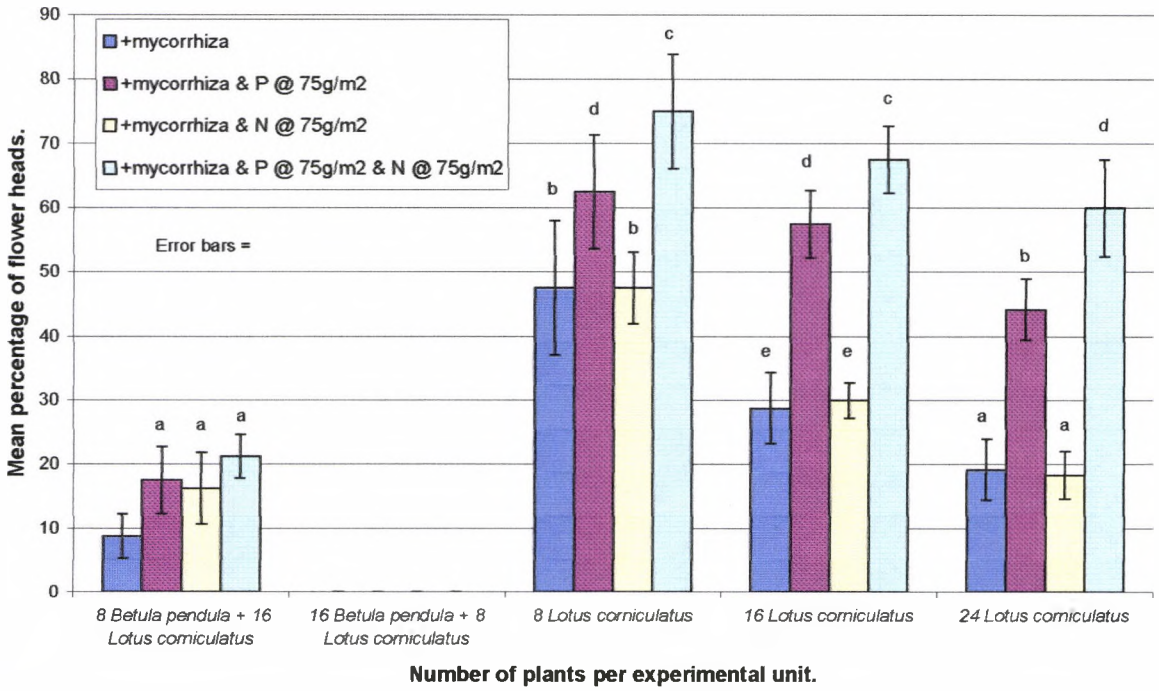

Figure 6.3.10 Mean percentage of *Lotus corniculatus* flower heads per experimental unit.

Columns labelled with the same lower case letter were not significantly different at $P<0.05$, following analysis of variance and Tukey's simultaneous pairwise comparison tests.

6.3.3 Discussion.

The results indicated that interspecific competition for resources, between the two plant species *B. pendula* and *L . corniculatus,* does occur. The results also revealed intraspecific competition influences growth in both species, at certain planting densities (Tables 6.3.2, 6.3.3, 6.3.4 and 6.3.5). Phosphorus and nitrogen are both limiting in the colliery spoil, but the growth response of the two species depends on the planting density and the mix of planting (Figures 6.3.1-6.3.10).

The most startling result from the experiment was the complete absence of *L. corniculatus* germination when sown without mycorrhizal fungal inoculate. There was good germination of *L. corniculatus* when planted with the mycorrhizal fungal inoculate. Possible reasons could be:

i) although every care was taken to guard against it, some preparation of the ground affected the germination of the *L. corniculatus,*

ii) the treatment without mycorrhizal inoculate prevented the *L. corniculatus* from germinating,

iii) some crucial preparation of the ground with the mycorrhiza (other than the mycorrhiza themselves) enabled the *L. corniculatus* to establish,

iv) a crucial relationship between *L. corniculatus* and mycorrhizae in their germination and establishment requirements at the site. This type of crucial dependence is well-documented for the Orchids (Hadley 1982) but not for Legumes which have well-reported associations with Rhizobia (Jeffries, Bradshaw & Putwain 1981).

AMF cannot be cultured axenically (Dodd 2000) and *Trifolium pratense* were used as the host plants to bulk the AMF inoculate up for the field trial. The *T. pratense* seeds were surface sterilised, in 3% (v/v) NaOCl for 5 minutes, before sowing and there was no obvious nodulation of the *T. pratense* root systems. However, Rhizobia were possibly present in the AMF inoculate and could explain the drastic difference in germination between the two treatments. Nodulation, in the root systems of *L. corniculatus,* in the trial was observed when roots were sampled for the presence of mycorrhizal fungi. There are Legumes including *L. corniculatus* at the Betteshanger site and one would expect some natural background Rhizobia in the spoil, which would have been present in the experimental units without the mycorrhizal inoculate. Further work is required to answer the questions raised by these observations.

In Figures: 6.3.1, 6.3.2, 6.3.3, 6.3.4, 6.3.5 and 6.3.6 the increase in tree growth with addition of treatments indicates that intraspecific competition for resources was limiting at this planting density (Berntson & Wayne 2000). This has implications for the community development on colliery spoil where resources are limited, and suggests a potential mechanism for poor vegetation cover observed in the youngest spoils at Stodmarsh and Betteshanger, described in Chapter 4.

The addition of mycorrhizal inoculate increased the internodal length of the trees, indicating that they in some way improved resource availability. Comparisons of trees in the treatments with mycorrhiza and mycorrhiza with phosphorus, in experimental units with a planting density above eight trees per unit, showed no significant difference between their internodal growths. Addition of nitrogen to the mycorrhiza treatment, however, significantly increased the growth of the trees in units planted at sixteen trees per unit. Nitrogen must therefore limit tree growth in the colliery shale, at this planting density, and mycorrhiza enabled the trees to access more phosphorus. In experimental units without mycorrhiza, but with additions of nitrogen, phosphorus and phosphorus plus nitrogen, the observed increase in internodal length was, generally, not as great as in comparative treatments which also include mycorrhiza. Mycorrhizal fungi appear to have facilitated resource acquisition, which was reflected in increased tree growth.

The growth response of *B. pendula* summarised in Figures 6.3.7 and 6.3.8 was probably the result of intraspecific competition between the trees for resources, rather than the interspecific competition between *L. corniculatus* and *B. pendula*. *L. corniculatus* may well have provided a benefit to the trees in the form of increased nitrogen availability which was suggested by the results. Stem diameter measurements displayed no significant differences between planting densities in any of the treatments (Figure 6.3.8). However, the stem diameter was reduced in the control units, in comparison to those units with treatments. This indicates that nitrogen and phosphorus were limiting stem growth and mycorrhiza negated the effects of this limitation. This was deduced as there was no difference in tree growth between those units with just mycorrhiza, and those with fertiliser additions. However, the measurements of the stem diameter were perhaps not sensitive enough to pick up possible between treatment differences.

Table 6.3.4 shows the significance of treatments, and planting density, on leaf rosette diameter growth of *L. corniculatus*. Figure 6.3.9 illustrates the response of the mean leaf rosette diameter of *L. corniculatus* to the treatments. Increasing planting density

of *B. pendula* had a significant limiting effect on the above ground growth of *L. corniculatus* ($P<0.001$). In experimental units with 16 trees, the addition of extra nitrogen or phosphorus had no effect on the rosette diameter of the *L. corniculatus* plants. This indicates that nitrogen and phosphorus were not limiting, at this planting density, but other resources such as water and sunlight were (Martens, Breshears, & Meyer 2000). The trees must be better competitions for these resources, thus limiting the growth of the leaf rosette of the *L. corniculatus* plants. The ability for plants to compete effectively for resources, in crowded populations, is thought to be intimately linked with their physiognomy rather than their functional roles within ecosystems (Berntson & Wayne 2000). This is another example of a possible mechanism for the control of community structure at Betteshanger and Stodmarsh.

At eight trees per experimental unit, there was an increase in *L. corniculatus* rosette diameter from the units with sixteen trees per unit. In the *L. corniculatus* monoculture experimental units, the rosette diameter was significantly bigger in all treatments than the mixed planting units with trees (Figure 6.3.9). However, there was no significant difference between the rosette diameters in the monoculture planted experimental units with eight and sixteen *L. corniculatus* plants per unit area. At a density of twenty four plants, per unit area, the rosette diameter was significantly reduced in comparison with the eight and sixteen plants densities. Intraspecific competition for both nitrogen and phosphorus limited leaf production, in *L. corniculatus*, at the planting density of twenty four plants per experimental unit. In the twenty four plants per unit area there was a synergy between the addition of nitrogen and phosphorus, suggesting that both elements limited leaf production on colliery waste at this planting density. Addition of phosphorus only to the experimental units, with eight and sixteen *L. corniculatus,* did not significantly increase the rosette diameter above that measured in plants from the mycorrhizal control units. Therefore, phosphorus was not limiting in the colliery waste with regard to leaf production at these planting densities. Potentially this could be because mycorrhizae are thought to aid phosphorus acquisition in plants more than any other resource (Finlay & Read 1986; Van Aarle *et al.* 2002). These results suggest that this may have been occurring. However, the addition of nitrogen did significantly increase the mean rosette diameter in experimental units with eight and sixteen plants per unit, indicating that nitrogen was limiting at these planting densities. This was confirmed in units with both nitrogen and phosphorus added, as the growths in these units were not significantly different from those units with only nitrogen additions. This was surprising as the presence of root nodules indicates that *L. corniculatus* was able to fix nitrogen.

Flower head production of *L. corniculatus* is an indication of the plant's ability to produce offspring. Fecundity of a species is important for ecological success in stressful environments (Stanton, Roy & Thiede 2000). The ability to produce offspring is crucial to long term establishment and success of vegetation (Tilman 1994 b; Grime 1998; Robinson & Handel 2000). Figure 6.3.10 illustrates the results of how planting density and treatments affected flower head production in *L. corniculatus*. *L. corniculatus* did not germinate in experimental units without mycorrhizal inoculate. Therefore, the mycorrhiza fungal treatment was used as a control, in the analyses, to compare the planting density and additions of nutrients on the flower head production.

The lowest density of *L. corniculatus* planting had the highest number of flower heads; this suggests intraspecific competition for resources also influenced flower head production in individual *L. corniculatus* plants. From the results summarised in Figure 6.3.10, phosphorus had the largest effect on flower head production, signifying it was more limiting in the colliery waste than nitrogen with regard to flower head production. The presence of root nodules, and therefore the presence of Rhizobia in association with *L. corniculatus,* potentially provided a source of nitrogen for the plants.

Figure 6.3.10 illustrates that nitrogen and phosphorus limited flower head production. However, it was likely that competition for light and water, by *B. pendula*, also lead to the suppression of flower head production by *L. corniculatus* (Martens, Breshears, & Meyer 2000). The influence of *B. pendula* on flower head production, in this experiment, can help explain the natural vegetation patterns occurring on Betteshanger and Stodmarsh. At Betteshanger, *B. pendula* is the primary coloniser, its competitive ability to prevent *L. corniculatus* from producing flower heads, and therefore reproducing, would effectively exclude the legume from the vegetation system (Stanton, Roy & Thiede 2000). This also supports the hypothesis that physiognomy can be as important as functionality with respect to resource acquisition (Berntson & Wayne 2000). This is strengthened by the field evidence, since *L. corniculatus* is found in only one area of Betteshanger where few trees have colonised (Chapter 4). This lack of trees, in the community, could have enabled *L. corniculatus* to establish. At Stodmarsh, the newer spoil is not dominated by *B. pendula*. *L. corniculatus* is therefore not precluded, and is able to establish and dominate, no doubt aided by its symbiotic relationship with Rhizobia (Jeffries, Bradshaw & Putwain 1981).

6.4 Field survey of the arbuscular mycorrhizal status of plants in the colliery spoil tipping chronosequence at Stodmarsh.

Mycorrhizal fungi are integral to the structure of most terrestrial vegetation communities (Brundrett 1991; Smith & Read 1997; van der Heijden *et al.* 1998a). By examining the arbuscular mycorrhizal status of plants, in the chronosequenced areas of Stodmarsh, the relationship between the age of the site and the arbuscular mycorrhizal fungi can be explored.

6.4.1 Method.

Plants and their root systems were sampled from the five distinctly aged tipping sequences at Stodmarsh (Figure 3.2.1), for the presence of AMF in their root systems. Species chosen were selected from those species found on each chronosequenced area of the site and also known to be mycorrhizal (Harley & Harley 1987). Five individuals of each species of plant (*Hieraceum pilosella, Lotus corniculatus* and *Agrostis capillaris*) were chosen at random from each of the five distinctly aged areas of the site. They were removed from the spoil with a hand trowel 26th July 2000. The main root systems, together with attached spoil, were sealed in polythene bags and taken to the laboratory.

Several pieces of young, living white lateral roots were cut from each root ball and washed thoroughly in tap water. The roots were cleared in 2% (w/v) KOH for 1h at 90°C in a water bath. The roots were rinsed with water three times using a mesh and forceps. The roots were then covered with 2% (v/v) HCl for 45 minutes. The HCl was decanted and the roots covered with 0.05% (w/v) trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) and placed for 15minutes to 1hour at 90°C in a water bath, until the roots were strongly stained. The roots were then placed into a Petri dish with 50% (v/v) glycerol for de-staining, so that only the fungal structures kept their blue colour.

The roots were then assessed, for percentage root length colonised by AMF, using Phillips and Hayman's (1970) protocol. The percentage root length colonised was evaluated by mounting a sub-sample, of approximately 10cm of stained root, using the mountant PVLG (Polyvinyllactoglycerol) onto a slide. The presence or absence of typical mycorrhizal structures (arbuscules, vesicles and hyphae), in a gridline intercept system, were counted in each field of view at x100 magnification, using a compound microscope (Axioskop, Zeiss, Germany). This technique was used to

compare the relative degree of AMF infection across the chronosequence at Stodmarsh rather than direct comparisons between plant species.

Infection rate versus the age of the colliery spoil was analysed by product moment correlation in Minitab statistical software (release 13).

6.4.2 Results

The relationship between infection rate of AMF within the three plant species sampled and the age of the spoil from the chronosequence at Stodmarsh, are summarised in Figure 6.4.1. Table 6.4.1 summarises the significance of the relationship.

In *A. capillaris* there is a significant relationship between the age of spoil and the percentage of AMF colonisation ($P<0.001$); older areas from which the grass was sampled had higher percentage colonisation. This mycorrhization pattern is not mirrored in *H. pilosella* or *L. corniculatus*. *H. pilosella* shows no significant relationship ($P=0.165$) between the age of the area, from which the plants were sampled, and the mycorrhization of the plants, infection rates are stable at between 71% and 76% for all sample areas. By contrast, mycorrhization of *L. corniculatus* has a significant relationship with the age of the site ($P<0.001$), such that older the site the less AMF colonised *Lotus corniculatus*.

Table 6.4.1 Summary of the product moment correlation coefficient analysis between age of the spoil and percentage of AMF infection.

Species	*P*-value
H. pilosella	0.165
L. corniculatus	<0.001
A. capillaris	<0.001

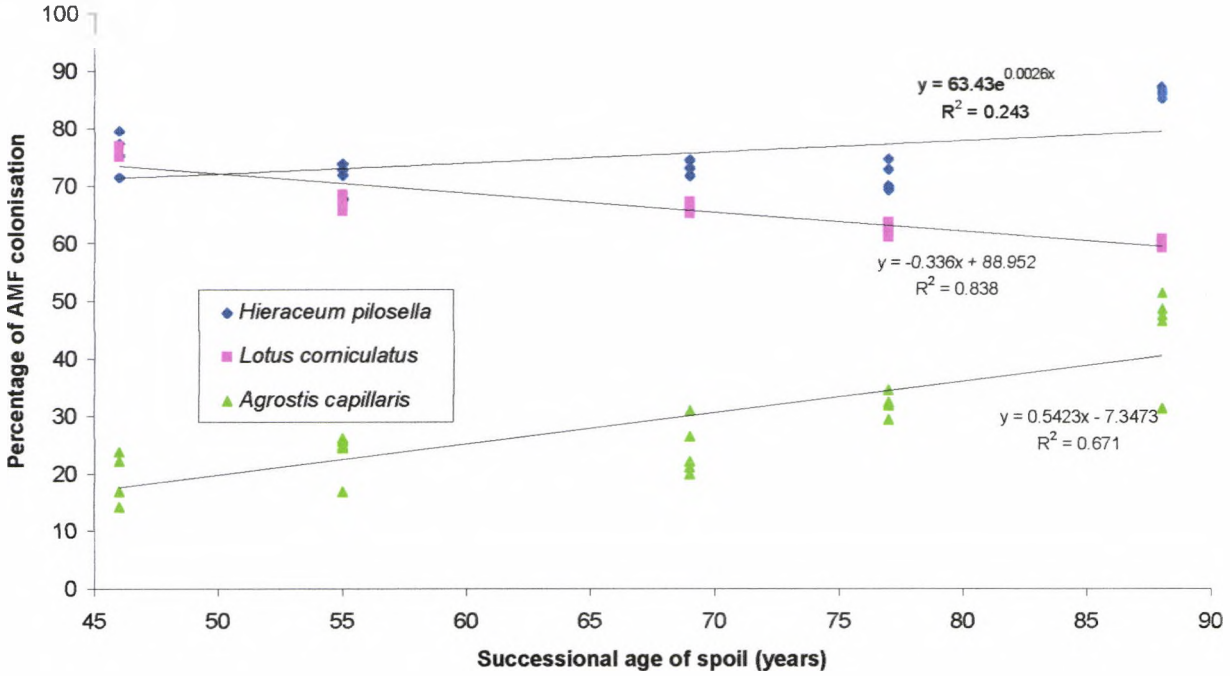

Figure 6.4.1 AMF infection rates of *Agrostis capillaris, Hieraceum pilosella* and *Lotus corniculatus* roots. Collected 26/07/00 from the chronosequence at Stodmarsh.

6.4.3 Discussion.

The different patterns of AMF colonisation, exhibited by the three plant species studied across the site, may have been in part due to the tolerance of environmental conditions by the host plants and or their associated mycorrhizal symbionts (Moora & Zobel 1998). The pattern of AMF infection observed in *H. pilosella* may be due to only one, or a few species of AMF infecting the roots of *H. pilosella.* These AMF species may be very good colonisers of the site, and therefore do not exhibit any temporal variation in their infection rates (Smith 2000). Another explanation of the difference in AMF colonisation, between the host plants, could be due to the root architecture of the host plant (Dodd 2000); A. *capillaris* has shallow fibrous roots, however, both *L. corniculatus* and *H. pilosella* have very deep and extensive tap roots with fine lateral roots branching from them. They therefore provide ideal hosts for AMF in stressed environments (Fitter 1987), such as the youngest spoil at Stodmarsh. Both plant and AMF therefore derive benefit from their association in the early successional spoil at Stodmarsh, this supports findings by Gange, Brown, and Farmer (1990).

The AMF species which associate with *A. capillaris,* by comparison, may well not colonise the site very well, and therefore they manifest a temporal variation or *succession* of AMF species: they are more infective with successional age. *L. corniculatus* exhibits less mycorrhization as the age of the area in which it is growing increases. This may be due to the *L. corniculatus* relying on AMF more to forage for, and provide resources in the youngest deposited spoil than in the older spoil (Robinson & Fitter 1999).

All three species of plants sampled had high rates of AMF infection across the different aged spoil areas at the Stodmarsh site (Figure 6.4.1). It is possible that the AMF at these densities may have a negative influence on the plant species (Gange and Ayres 1999). Although this cannot be determined form this study plant, it is unlikely because of the poor resource status of the colliery waste (Smith and Read 1997).

A. capillaris in general had lower mycorrhization rates than *H. pilosella* or *L. corniculatus*. However, direct comparisons of mycorrhization between plant species is not reliable because this can be dependent on the age, health, stress etc. of the plant population sampled. Equivalent individuals of each species in different plant populations would be needed to meaningfully compare mycorrhization between plant species (Sanders & Fitter 1992), despite this patterns of mycorrhization within a species are comparable.

As plant resources are limiting on colliery waste, the importance of mycorrhization for the plant communities at Stodmarsh are that they may benefit from an:

- increased ability to acquire nutrients (Smith & Read 1997),
- increased ability to survive drought conditions (Ruiz-Lazano and Azcon 1995),
- increased resistance to herbivory (Gange & Bower 1997).

Although the plant species may have some negative impacts, due to their association with mycorrhizal fungi, such as a carbon cost (Fitter *et al.* 1998; Gavito and Olsson 2003). The increased ecological functional efficiency of plants, imparted to them by mycorrhizal fungi, will give advantages for the colonisation and establishment on colliery waste over those plants which do not have mycorrhizal associations. It is therefore possible that the mycorrhizal fungi will be influencing the plant community structure because of their influence on the competitive abilities of plants (van der Heijden *et al.* 1998b).

6.5 Examining AMF communities in the colliery spoil tipping chronosequence at Stodmarsh.

Higher plant communities change in the chronosequence of colliery spoil at Stodmarsh (Chapter 4). It is anticipated that the endo-mycorrhizal fungi communities also change with the age of spoil. Mycorrhizal fungi within plant communities are thought to crucially affect the structure and composition of those plant communities they associate with (Brundrett 1991; Smith and Read 1997; van der Heijden *et al.* 1998a). By investigating the AMF communities, within the Stodmarsh chronosequence, this relationship between community structure and chronosequence age can be examined.

6.5.1 Method.

Arbuscular mycorrhiza fungi trap cultures were set up 26 July 2000 using the plant samples collected for 6.4.1. The root ball (4-6cm diameter) of each sample was placed into a 14cm diameter plastic pot. The aerial parts of the plants were cut back and the remaining volume in the pots was filled with a 2:1 mix of an attapulgite clay (Agsorb 8/16, from Oil-Dri Ltd, Wisbech, UK.) and a durite sand (a particulate by-product of calcined flint pebbles heated in a furnace which consists of 97% silica with small percentages of iron oxides, calcium oxides and alumina and a mean pH of 8.3). Seeds of *Trifolium pratense* were surface sterilised in 3% (v/v) NaOCl for 5 minutes and sown around the plant to trap AMF present in the sample. Trap cultures were transferred to a greenhouse (maintained between 8°C-34°C). Plants were watered with de-ionised water as required and supplied with nutrients (NPK 18:0:18) once per week using 1.4g l-1 Vitafeed 102 (Vitax Ltd., Leicester, UK.) with trace elements [www.bio.ukc.ac.uk/beg/protocols/extraction.htm].

Arbuscular mycorrhizal fungal spores were extracted from the trap cultures by wet sieving and sucrose density gradient centrifugation. Two 50cm^3 cores of the attapulgite clay/durite sand mixture were removed from each trap. The material was flushed through a 500µm sieve and collected in a 45µm sieve. The contents of the fine sieve were back-washed into centrifuge tubes and a 60% (w/v) commercial sucrose solution added to the pellet using a syringe. After centrifugation at 3000 rpm for 2 minutes, the supernatant was washed into a 45µm sieve and the contents back-washed into a Petri dish (Brundrett, Melville and Peterson 1994). Taxonomic identification was based on spore morphology under a dissecting microscope and on diagnostic slides examined under a compound microscope at x100-400. Samples of

substrate were removed after 3, 6 and 9 months. Several control 'trap cultures' containing only the attapulgite clay/durite sand substrate were also incorporated amongst the pots in the greenhouse to check whether airborne or splash contamination of pots was occurring (www.bio.ukc.ac.uk/beg/Protocols/slide.htm).

AMF species richness versus the age of the colliery spoil was analysed by product moment correlation in Minitab statistical software (release 13). The data was also subjected to Principal Components Analysis (PCA) and Hierarchical Cluster Analysis using the statistical software Minitab (release13).

6.5.2 Results

Tables 6.5.1, 6.5.2 and 6.5.3 summarise the AMF species, extracted from the AMF traps, from the difference aged areas on the chronosequence at Stodmarsh. Table 6.5.4 illustrates the AMF extracted at different harvests from the AMF traps. Table 6.5.5 illustrates the product moment correlation coefficient analysis between the AMF species colonising the host plants and age of the colliery spoil. Figure 6.5.1, displays the trends in AMF species colonisation, extracted from the three host plant species, in the chronosequence at Stodmarsh. Figures 6.5.2, 6.5.5 and 6.5.8 display the first 2 principal components (eigen values) plotted against each other from the PCA. Figures 6.5.3, 6.5.6 and 6.5.9 are similarity matrices from the Hierarchical Cluster Analyses. Figures 6.5.4, 6.5.7 and 6.5.10 illustrate the final partition from the Hierarchical Cluster Analyses (see appendix 6.5.1 for full statistical analyses).

Successful sporulation by AMF species occurred in the traps which enabled identification.

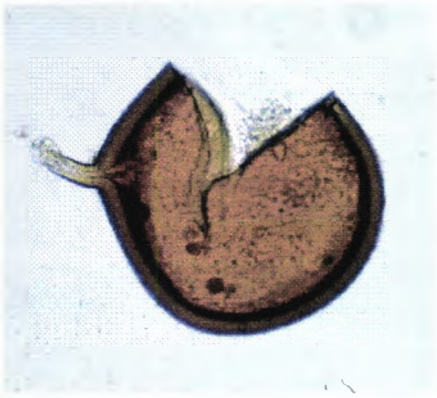

Plate 6.5.1 AMF spore preparation enabling identification: in this example *Glomus geosporum* (X 100)

In total 11 species of AMF were identified from the traps, of the three plant species, monitored over a 9 month period (Tables 6.5.1-6.5.4). No AMF spores were isolated from the control pots sampled over the same period indicating that there had been no accidental contamination of the traps.

Table 6.5.4 indicates that the number of AMF isolated at each harvest increased until the final harvest May 2001. Time constraints meant that further harvests were not possible. *G. geosporum* and *G. intraradices* were the only two species found at each harvest; *G. coronatum* was only found in the first harvest from *H. pilosella* traps.

All species of host plant had a significant correlation between AMF species colonisation and the age of spoil (Table 6.5.5); in both *H. pilosella* and *A. capillaris* AMF species richness increased with the age of the spoil. In *L. corniculatus* no discernable trend was evident (Figure 6.5.1)

The species richness of AMF was greatest in samples from *H. pilosella* (mean=8.6, S.D.=0.9) followed by *A. capillaris* (mean=4.8, S.D=2.6) then *L. corniculatus* (mean 4.6, S.D.=0.55).

AMF species richness changes across the chronosequence (Figure 6.5.1). However, whether the species assemblages of AMF change, in the different aged spoil, is not discernable from this analysis. PCA, and Hierarchical Cluster Analyses, enabled different communities of AMF, isolated from the host plants, in the chronosequence to be identified.

Figures 6.5.2-6.5.10 summarise the AMF communities isolated from the different host plants on the different aged spoil at Stodmarsh. Figures 6.5.2, 6.5.5 and 6.5.8 illustrate that each distinct aged area of the chronosequence has a distinct AMF community associated with it. The precise make up of these communities are dependent on the species of host plant (Figures 6.5.3, 6.5.4, 6.5.6, 6.5.7, 6.5.9, and 6.5.10). The AMF communities identified from *H. pilosella* and *L. corniculatus*, host plants, indicated very distinct AMF community assemblages associated with the three youngest spoil divisions of the chronosequence. However, the two oldest spoil samples have the same final partition for their similarity matrices, indicating similar community structure in these aged spoils. The AMF communities isolated from host plants of *A. capillaris* were distinct. However, in the third youngest area there was an exception and the AMF communities shared a final partition for their similarity

matrices with AMF communities isolated from the third youngest spoil from *H. Pilosella*.

Table 6.5.1 AMF species extracted from the trap cultures of the host plant: *Hieraceum pilosella*.

		Sampling sites				
AMF		Area 5	Area 4	Area 3	Area 2	Area 1
	Date of spoil deposition	1913-1924	1924-1932	1932-1946	1946-1955	1955-1972
	Mean age of spoil (yr)	88	77	69	55	46
Acaulospora morrowiae		X	X	X	X	X
Entophopora infrequens		X	X	X	X	
Glomus claroideum		X	X	X	X	X
Glomus coronatum		X	X	X		
Glomus etunicatum		X	X	X		
Glomus fasciculatum		X	X	X	X	X
Glomus geosporum		X	X	X	X	X
Glomus intraradices		X	X	X	X	X
Glomus microaggregatum						X
Glomus mosseae		X	X		X	X
Glomus occultum			X		X	X
Total		9	10	8	8	8

Table 6.5.2 AMF species extracted from the trap cultures of the host plant: *Lotus corniculatus*

		Sampling sites				
AMF		Area 5	Area 4	Area 3	Area 2	Area 1
	Date of spoil deposition	1913-1924	1924-1932	1932-1946	1946-1955	1955-1972
	Mean age of spoil (yr)	88	77	69	55	46
Glomus coronatum						
Glomus etunicatum						
Glomus fasciculatum						X
Glomus geosporum		X	X	X	X	X
Glomus intraradices		X	X	X	X	X
Glomus microaggregatum		X	X	X	X	
Glomus mosseae		X	X	X	X	X
Glomus occultum			X	X	X	
Total		4	5	5	5	4

Table 6.5.3 AMF species extracted from the trap cultures of the host plant: *Agrostis capillaris*

		Sampling sites				
AMF		Area 5	Area 4	Area 3	Area 2	Area 1
	Date of spoil deposition	1913-1924	1924-1932	1932-1946	1946-1955	1955-1972
	Mean age of spoil (yr)	88	77	69	55	46
Acaulospora morrowiae						
Entophopora infrequens		X	X			
Glomus claroideum		X	X	X		
Glomus coronatum						
Glomus etunicatum		X	X			
Glomus fasciculatum						X
Glomus geosporum		X	X	X	X	X
Glomus intraradices		X	X	X	X	X
Glomus microaggregatum		X	X	X		
Glomus mosseae		X	X			
Glomus occultum			X			
Total		7	8	4	2	3
Total number AMF Species collected		**10**	**11**	**11**	**9**	**9**

Table 6.5.4 The species of AMF isolated at each harvest from plants sampled from Stodmarsh and trapped over a 9 month period.

Plant host	Arbuscular Mycorrhizal fungi	Harvest		
		November 2000.	February 2000.	May 2001.
Hieraceum pilosella	*Acaulospora morrowiae*		X	
	Entophopora infrequens		X	X
	Glomus claroideum		X	X
	Glomus coronatum	X		
	Glomus etunicatum			X
	Glomus fasciculatum			X
	Glomus geosporum	X	X	X
	Glomus intraradices	X	X	X
	Glomus microaggregatum			X
	Glomus mosseae			X
	Glomus occultum			X
	Total	**3**	**5**	**9**
Lotus corniculatus	*Acaulospora morrowiae*			X
	Entophopora infrequens		X	X
	Glomus claroideum		X	X
	Glomus coronatum			
	Glomus etunicatum			X
	Glomus fasciculatum		X	X
	Glomus geosporum	X		X
	Glomus intraradices	X	X	X
	Glomus microaggregatum			X
	Glomus mosseae			X
	Glomus occultum			X
	Total	**2**	**4**	**10**
Agrostis capillaris	*Acaulospora morrowiae*			
	Entophopora infrequens		X	X
	Glomus claroideum		X	X
	Glomus coronatum			
	Glomus etunicatum			X
	Glomus fasciculatum			X
	Glomus geosporum	X	X	X
	Glomus intraradices	X	X	X
	Glomus microaggregatum			X
	Glomus mosseae			X
	Glomus occultum			X
	Total	**2**	**4**	**9**

Table 6.5.5 Summary of the product moment correlation coefficient analyses between age of the spoil and AMF species colonisation.

Species	*P*-value
H. pilosella	0.000
L. corniculatus	0.016
A. capillaris	0.000

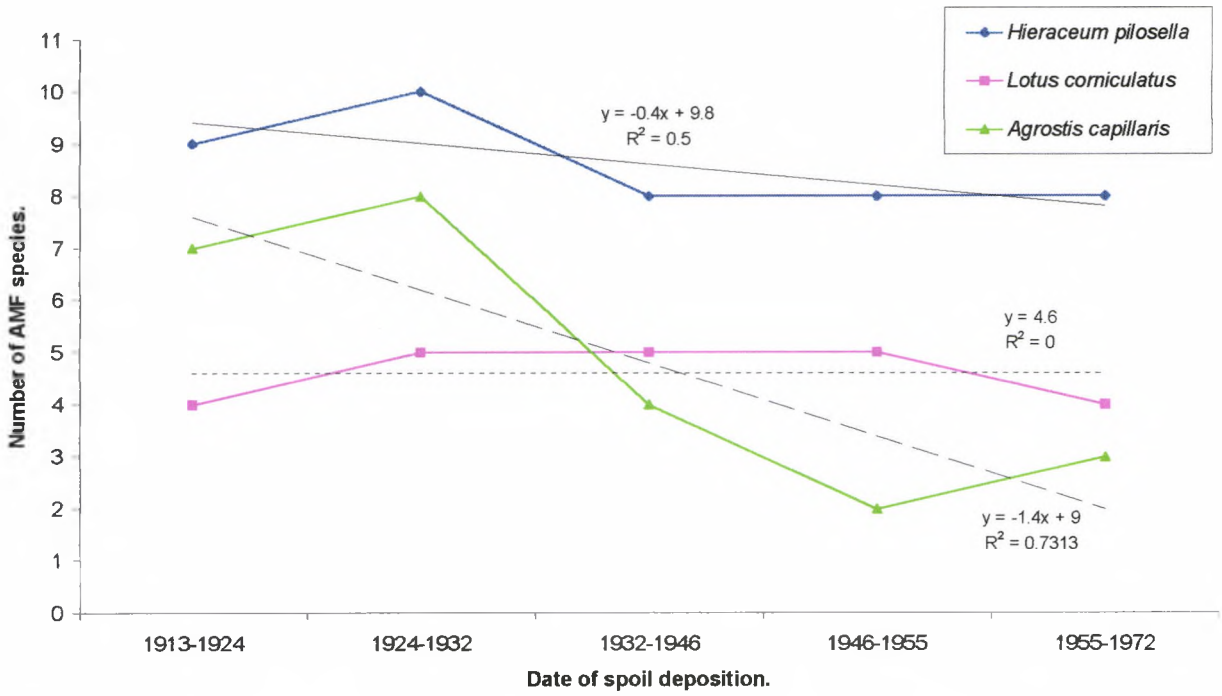

Figure 6.5.1 Species richness of AMF from the five chronosequenced areas at Stodmarsh.

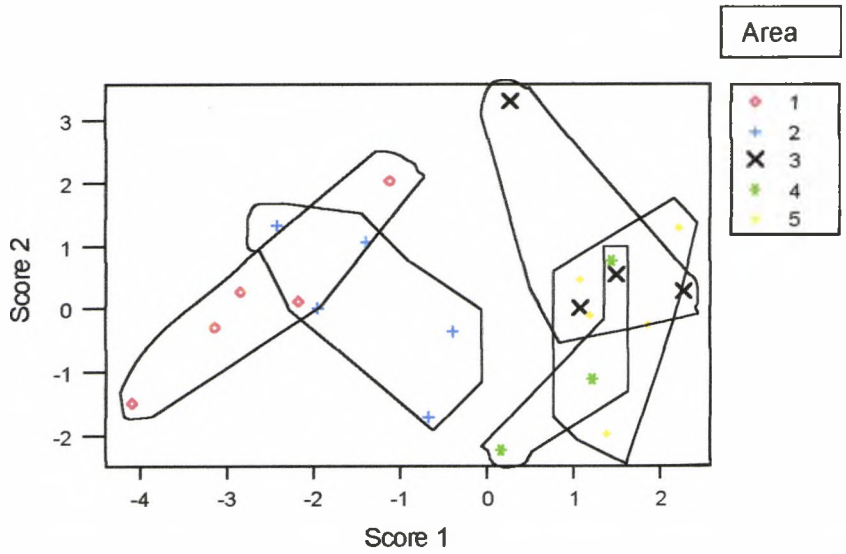

Figure 6.5.2 The first 2 principal components (eigen values) plotted from the principal components analysis for AMF species isolated from culture traps with *Hieraceum pilosella* as the host plant.

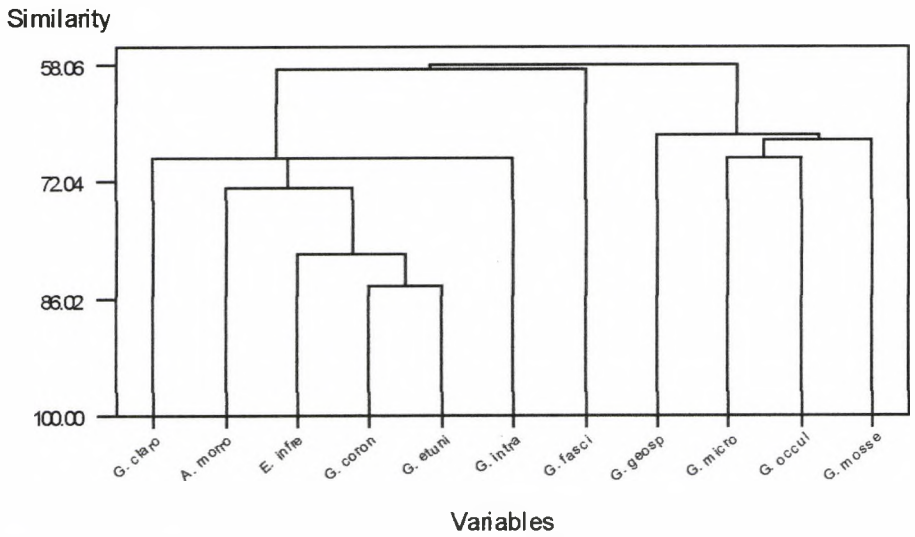

Figure 6.5.3 Similarity matrix from Hierarchical Cluster Analysis of Variables from AMF species isolated from culture traps with *Hieraceum pilosella* as the host plant.

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Acaulospora morrowiae *Entophopora infrequens* *Glomus claroideum* *G. coronatum* *G. etunicatum* *G. intraradices*	*G. fasciculatum*	*G. geosporum*	*G.microaggregatum* *G. occultum*	*G. mosseae*

Figure 6.5.4 Final Partition from Hierarchical Cluster Analysis of Variables from AMF species isolated from culture traps with *Hieraceum pilosella* as the host plant.

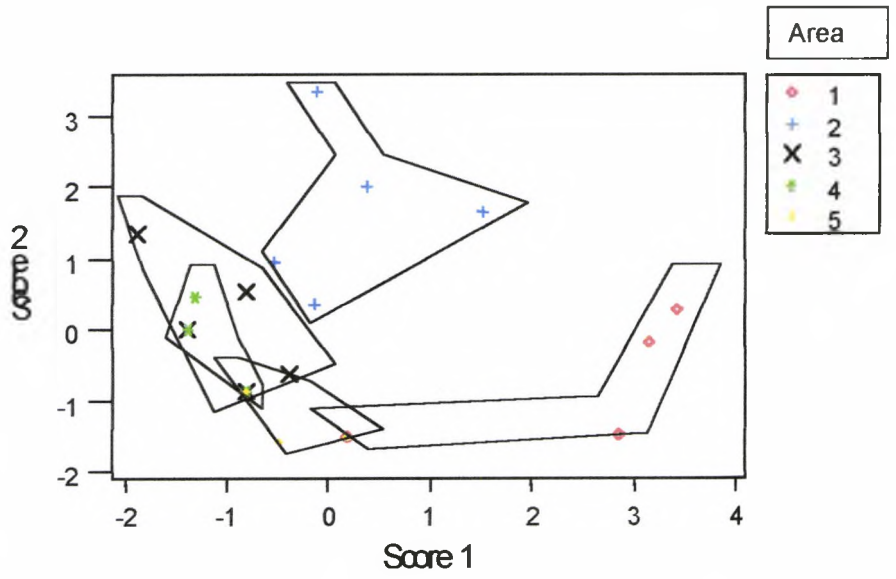

Figure 6.5.5 The first 2 principal components (eigenvalues) plotted from the principal components analysis for AMF species isolated from culture traps with *Lotus corniculatus* as the host plant.

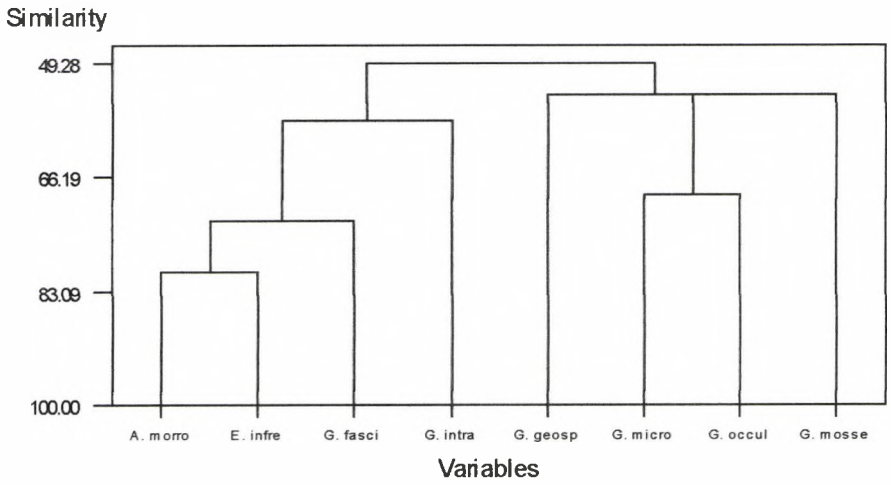

Figure 6.5.6 Similarity matrix from Hierarchical Cluster Analysis of Variables from AMF species isolated from culture traps with *Lotus corniculatus* as the host plant.

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Acaulospora morrowiae *Entophopora infrequens* *Glomus fasciculatum*	*G. geosporum*	*G. intraradices*	*G. microaggregatum* *G. occultum*	*G. mosseae*

Figure 6.5.7 Final Partition from a Hierarchical Cluster Analysis of Variables from AMF species isolated from culture traps with *Lotus corniculatus* as the host plant.

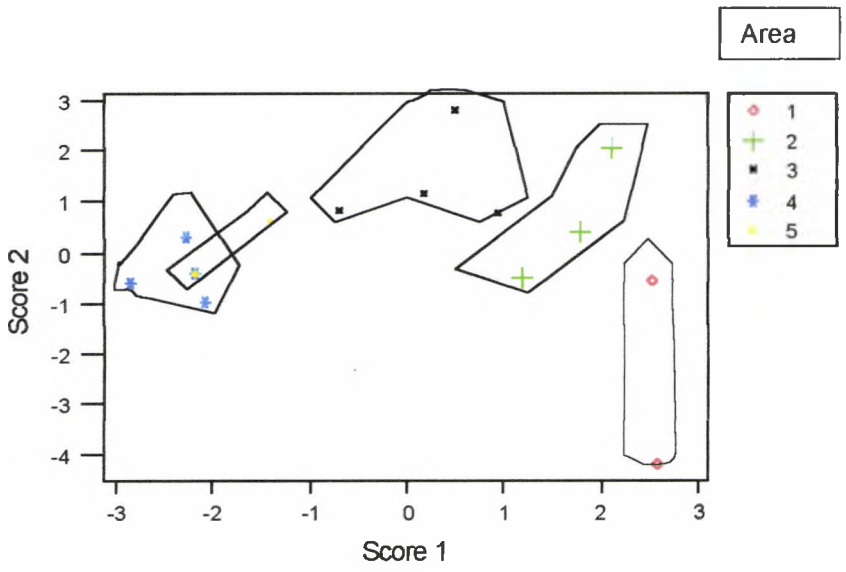

Figure 6.5.8 The first 2 principal components (eigenvalues) plotted from the principal components analysis for AMF species isolated from culture traps with *Agrostis capillaris* as the host plant.

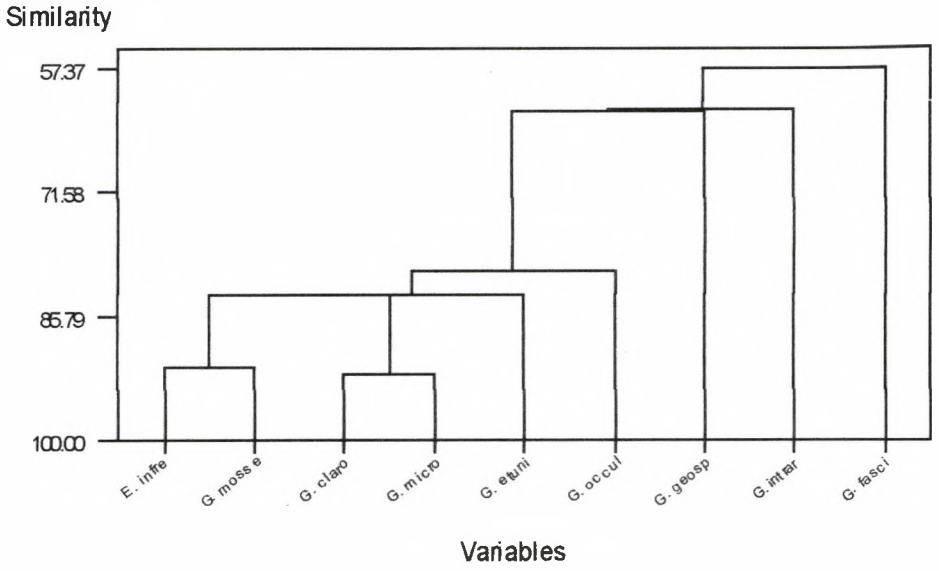

Figure 6.5.9 Similarity matrix from Hierarchical Cluster Analysis of Variables from AMF species isolated from culture traps with *Agrostis capillaris* as the host plant.

Cluster 1	Cluster 2	cluster 3	Cluster 4	Cluster 5
Entophopora infrequens *G. claroideum* *G. etunicatum* *G. microaggregatum* *G. mosseae*	*G. fasciculatum*	*G. geosporum*	*G. intraradices*	*G. occultum*

Figure 6.5.10 Final Partition from a Hierarchical Cluster Analysis of Variables from AMF species isolated from culture traps with *Agrostis capillaris* as the host plant.

6.5.3 Discussion.

The difference in AMF species richness patterns (Figure 6.5.1), between the host plants, could be due to the root architecture of the host plant (Dodd 2000; Fitter 1987). This is especially true for patterns of species richness within *L. comiculatus* which displays no relationship between AMF species richness and the spoil age (R^2=0). However, as there is a significant relationship between AMF species richness and spoil age for *H. pilosella* and *A. capillaris,* this suggests that the colliery waste environment may influence the AMF community structure (Gardner & Malajczuk 1988).

Tables 6.5.1-6.5.3 illustrate that single species are not isolated from only one aged area; it is the assemblage of species which change with the age of the spoil. However, there are exceptions to this rule: *G. fasciculatum* tends to be found only in the youngest spoil, and *G. etunicatum* is only found in the oldest spoils. This indicates that these species could, perhaps, be used as indicators of the age of the succession on Stodmarsh. Whether they could be used as indicators for other sites would require further work (Husband *et al.* 2002). However, the different AMF species assemblages found in different aged spoil have implications for the use of AMF in restoration strategies. The use of soil biota associated with different successional ages in restoration strategies has merit for increasing the effectiveness of restoration (Biondini, Bonham & Redente 1985). Identifying those AMF which are associated with young successional vegetation systems could, possibly, enable them to be used to aid vegetation establishment (Danielson 1985; Brantlee & Stutz 2002).

Table 6.5.4 indicates that the longer the trapping process was continued, the more species of AMF were isolated from the traps. It is therefore very likely that other AMF species could have been isolated in further harvests. This obviously has implications for the confidence of the results, but it is assumed that the isolated AMF are representative of the AMF community structure.

The identification of distinct AMF communities associated with different aged areas at Stodmarsh (Figures 6.5.2-6.5.10) indicate, just like the higher plant communities (Chapter 4), AMF communities change with the successional age of the colliery spoil. The distinctions between the community AMF structures in the three oldest spoil areas are less well defined than in the younger spoil. This suggests that niche-assembly and dispersal-assembly controls maybe influencing the AMF community

structure (Hubbell 2001). However, the exact mechanisms cannot be identified from this work.

The overlap of AMF communities, observed in the PCA, supports the idea that successional developments of AMF communities mimic the successional structural dynamics of plant communities (Boerner, De Mars & Leicht 1996), where the successional community stages merge in the seral sequence (Janos 1980; Niering 1987)

In successional systems where vegetation is resource limited, the mycorrhizal community play a crucial role in the plant community structure (Boerner, De Mars & Leicht 1996; Hart, Reader & Klironomos 2003). It is therefore likely that AMF do mediate plant communities at Stodmarsh. However, Hart, Reader, and Klironomos (2001) put forward a theoretical context for AMF community dynamics on a successional time scale. It was based on differences in AMF life-history strategies and modelled two possible hypotheses:

- the *Driver hypothesis*, interactions within AMF communities are responsible for changes in the plant community over time.
- the *Passenger hypothesis,* AMF community dynamics are a by-product of changes within the plant community.

The AMF communities at Stodmarsh could be following either of these models. The implied competitive advantages given to plants with mycorrhizal associations (van der Heijden *et al.* 1998a), and the observed influences of mycorrhizal inoculants, used in experimentation (section 6.3), suggest that the AMF will be influencing plant community structure within the chronosequence at Stodmarsh. The work by Stampe & Daehler, (2003) also suggests this is the case. Therefore, the identification of those AMF species that colonise and facilitate the plant communities in the youngest spoil could be crucial for the success of restoration strategies which utilise AMF. This is because AMF could be essential in directing community composition in successional vegetation systems. However, to determine the exact role of mycorrhizal fungi in the successional vegetation dynamics at the site still requires further work.

7 Plant resources and the development of vegetation systems.

Functional species within developing communities can have a profound influence on their structure (Semenova & van der Maarel 2000). It has been demonstrated that nitrogen fixing species can increase the availability of nitrogen in plant communities (Dancer, Handley & Bradshaw 1977b; Jeffries, Bradshaw & Putwain 1981).

Community structure varies with fluctuations in abiotic factors within the environment (DeWitt & Yoshimura 1998), and vegetation structure is closely linked with resource gradients (Theodose & Bowmana 1997; Rydgren, Økland, & Økland 2003). Although, a general understanding of the relative importance of factors governing the distribution of species response curve shapes along environmental gradients, has not been reached (Austin 1976, 1987; Austin & Gaywood 1994; Lawesson & Oksanen 2002). The successional sequence and development of communities is intimately linked with resource gradients (Clements 1916, 1936; Connell & Slatyer 1977; Tilman 1985). Considering the response of vegetation to different levels of resources is fundamental for understanding successional sequences and how vegetation responds in natural systems (Davis, Grime, & Thompson 2000), as well as restoration schemes (Bradshaw 1987).

As there are fundamental constraints to predicting vegetation responses to environmental changes, due to the complexity of interactions between plants and their biotic and abiotic environment (Connell and Slatyer 1977; Tilman 1994; Montoya, Rodríguez, & Hawkins, 2003). This chapter investigates experimental manipulation of vegetation *ex-* and *in- situ*, on colliery waste. In an attempt to control some of the complexity of interactions and to further establish the relationship between resource availability and community structure with respect to vegetation development.

7.1 The experimental manipulation of nitrogen, carbon and a functional group in developing *ex-situ* vegetation.

Nitrogen deficiency is a feature common to most derelict land and must be overcome if vegetation is to be sustainable (Bradshaw & Chadwick 1980; Broughton 1985). Natural plant communities developing on the East Kent colliery sites are associated with differing resource availabilities and vegetation structure has been demonstrated to be closely linked with the nitrogen resource available on the Betteshanger and Stodmarsh sites (Chapters 4 and 5). As a control to community structure, nitrogen availability is often limiting to species diversity in developing vegetation systems (Bray 1983; Marrs 1989; Killham 1994), and especially on colliery waste (Palmer, Morgan, & Williams 1985; Bradshaw 1997). Levels of nitrogen are thought to increase with the successional age of plant communities (Tilman 1985; Olff, Huisman & van Tooren 1993)

The importance of the role of nitrogen in plant metabolism and its availability crucially controls the functioning and structure of ecosystems (Li & Daniels 1994). The availability of carbon in the form of plant litter has also been demonstrated to influence species diversity (Tilman 1994: Tilman *et al.* 2001). This experiment investigates the influence of different applications of nitrogen and a source of carbon i.e. a peat based compost, to the establishment of a species rich community from a species rich seed mix. The addition of nitrogen-fixing species was included as a treatment to investigate whether this functional group would influence the community which developed in the experiment.

7.1.1 Method.

A balanced factorial experimental design was used for the investigation. This method of experimental design enables, not only the exploration of the effects of individual treatments, but also the detection of treatment interactions (Watt 1997). Cricklade Meadow mix (Appendix 7.1.1), a species rich seed mixture with some 90+ species, was chosen to provide a species pool. The seed mix was chosen as it was readily

commercially available and has a proven record of establishment on colliery waste (Gilchrist 1998).

Eighty woven planter bags, each with 100l capacity, were filled with colliery spoil taken from Tilmanstone Colliery. Tilmanstone Colliery waste was used as this was logistically and economically the easiest to transport to the experimental site. Large clasts of greater than 5cm diameter were removed. The equivalent of 100g/m^2 of water storing granules was added to each planting bag (Plate 7.1.1) and mixed in to the spoil to a depth of 10cm.

Treatments were applied to provide 4 randomised replicates.

Treatments application rates:

i) +/- peat (organic matter at 200 g/m^2),

ii) 4 levels of nitrogen (in the form of ammonium nitrate) plus a control, were applied in 3 doses per year to give equivalent covering of 0 g/m^2, 25 g/m^2, 50 g/m^2, 100 g/m^2, 200 g/m^2 of nitrogen.

iii) +/- 1g legume mix composed of: *Trifolium pratense* (45%), *Trifolium repens* (15%), *Trifolium hybridum* (30%) and *Lotus corniculatus* (10%).

The experiment was sown (14/4/1999) with Cricklade Meadow mix, at the Mount Ecology Unit, Stodmarsh Road, Kent, and irrigated Monday, Wednesday and Friday.

Plate 7.1.1 Ex-situ factorial experiment: woven planter bags containing colliery waste and sown with seed mixture.

The numbers of individuals of each plant species were recorded from the experiment by using a point quadrat method. A pin of 0.5mm was randomly positioned 20 times within each planting bag. The position of the pin was determined by dividing the top of the bag into a 100 squares using lines 5cm apart. A random number table was used to select the position of a grid square, within the planting bag, and then the pin was placed into the centre of that grid square. The vegetation in contact with each pin was scored so that each part of a plant touching the pin, of each plant species, was recorded. The experiment was scored on a monthly basis (Plate 7.1.2).

Plate 7.1.2 Plant establishment in a planter bag containing colliery spoil.

The data were analysed using an analysis of covariance (orthogonal design) in the statistical package Minitab (release 13). The legume treatment was used as the covariate to distinguish between those seed treatments which had a higher species reservoir due to their inclusion. All treatments and treatment interactions were analysed.

7.1.2 Results

See appendices 7.1.2 and 7.1.3 for full analyses of covariance (orthogonal design) for the experimental data. Figures 7.1.1 and 7.1.2 summarise the point quadrat scores, from the experiment, sampled in August 1999 and August 2000 respectively. Figures 7.1.3 and 7.1.4 summarise the species richness sampled in August 1999 and August 2000 respectively. These four figures illustrate the vegetation response to all the

treatments. Figures 7.1.5 and 7.1.6 illustrate the vegetation response to the different applications of nitrogen as measured in 1999 and 2000 respectively. All figures have polynomial fitted regression lines to illustrate the relationship of the vegetation response to treatment, as they have curved data sets. The exception to this is mean point quadrat score data in Figure 7.1.6 which has a linear regression fitted. The choice of fitted regression was based on the type which gave the largest regression coefficient therefore describing the best data relationships (Townend 2002).

In 1999 all treatments significantly affected the point quadrat scores, as a measure of productivity, recorded for the experimental vegetation: legume (as a covariate) $P<0.001$, nitrogen $P<0.001$, peat $P=0.001$. However, there was no significant treatment interaction between peat and nitrogen $P=0.403$. The totals of species recorded from the experiment were also significantly affected by the treatments in 1999: legumes (as a covariate) $P<0.001$, nitrogen $P<0.001$, however the peat treatment did not significantly influence the number of species within the experiment $P=0.824$. There was also no significant interaction between nitrate and peat $P=0.160$.

The only treatment which significantly affected the point quadrat scores by 2000 was nitrogen ($P<0.001$). Peat, $P=0.757$, and legumes, (as a covariate) $P=0.281$, no longer had a significant influence on the point quadrat score. The interaction between peat and nitrogen, ($P=0.422$), had no significant influence on the vegetation. The totals of species recorded from the experiment were significantly affected by the nitrogen treatment in 2000 ($P<0.001$). The peat treatment did not significantly influence the number of species within the experiment ($P= 0.059$). Legumes (as a covariate) did not significantly influence the number of species in the experimental vegetation ($P=0.260$). There was also no significant interaction between nitrate and peat ($P=0.958$).

Point quadrat score (a function of productivity) and species richness were significantly influenced by the availability of nitrogen in the experiment. The addition of peat significantly affected the productivity but not species richness in 1999; by 2000 the addition of peat no longer had a significant influence on the developing community in the experiment. No significant synergism between nitrate and peat applications was found. Nevertheless application of peat equivalent to an annual carbon deposition of

200g/m^2 increased percentage cover and species number in comparison with plots without added nitrogen. Addition of legumes had a significant influence on the developing plant communities increasing both the recorded number of species and the point quadrat score in the 1999 sampling. The legume treatment, by 2000, was not significantly affecting the species number or point quadrat score sampled.

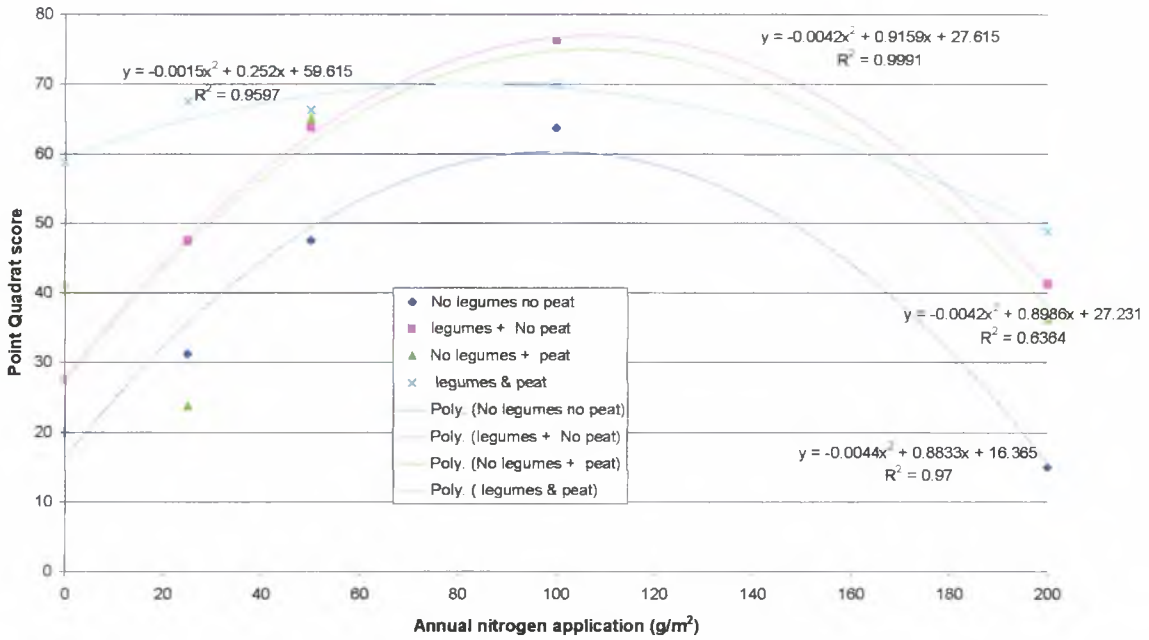

Figure 7.1.1 Mean point quadrat score data collected 23/8/1999.

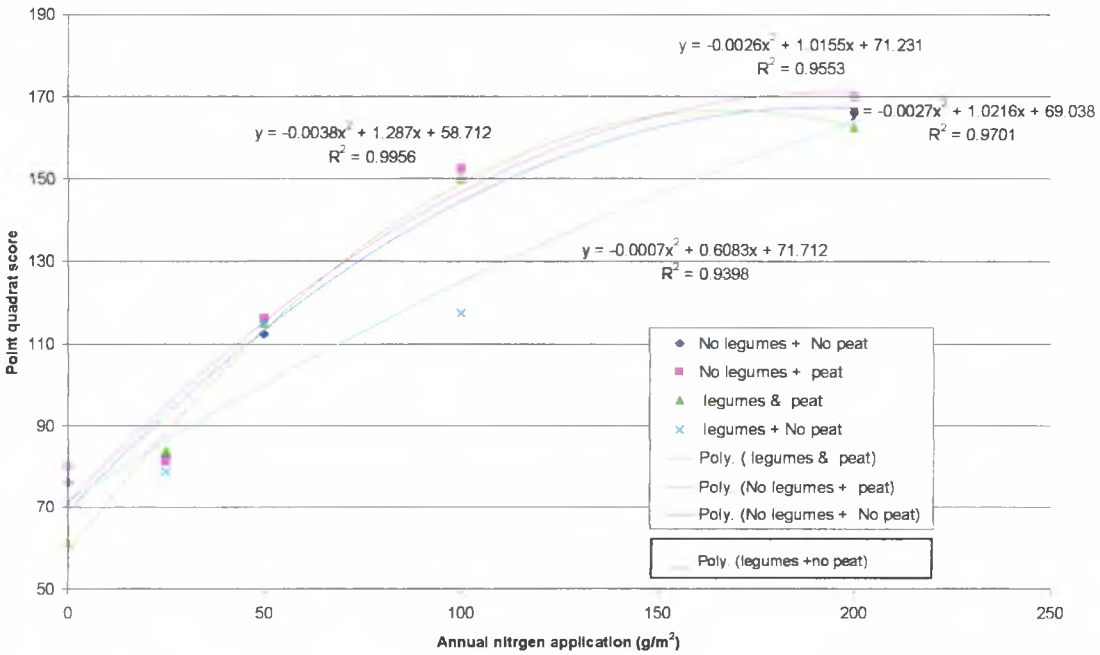

Figure 7.1.2 Mean point quadrat score data collected 25/8/2000.

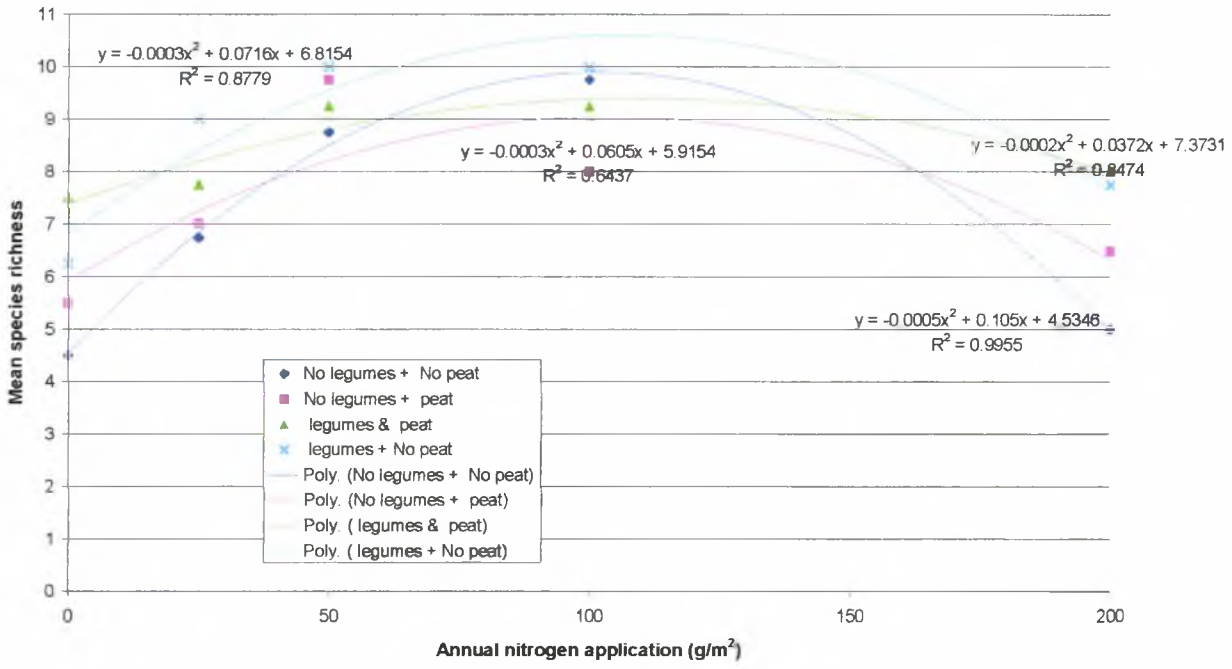

Figure 7.1.3 Mean species richness data collected 23/8/1999.

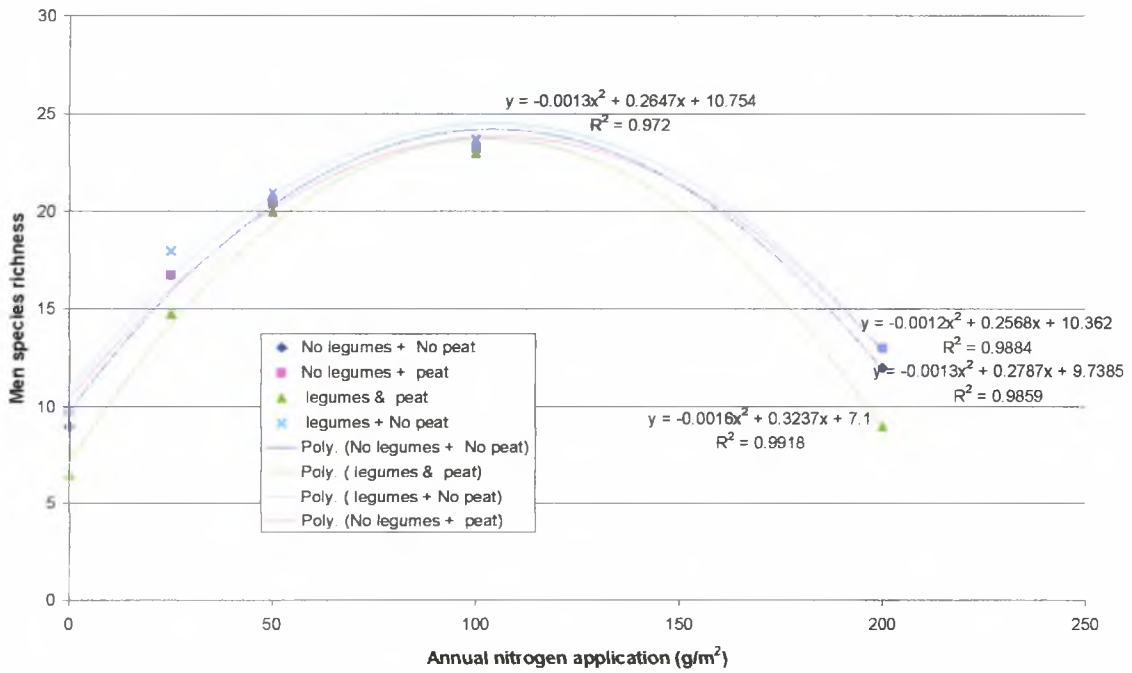

Figure 7.1.4 Mean species richness data collected 24/8/2000.

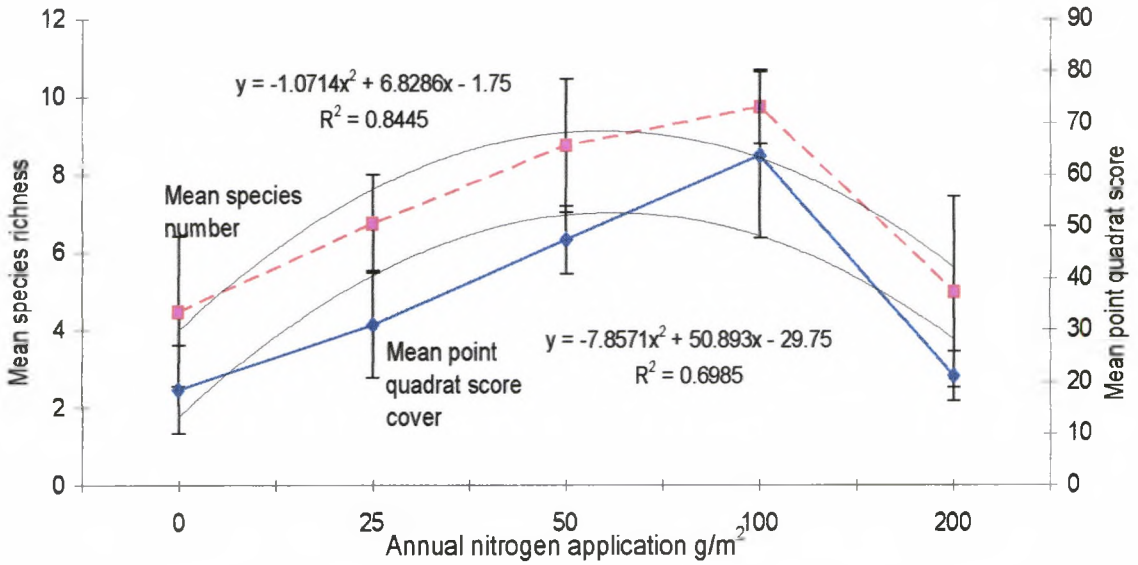

Figure 7.1.5 Mean species richness and mean point quadrat score against annual nitrogen application (data collected 23/8/99).

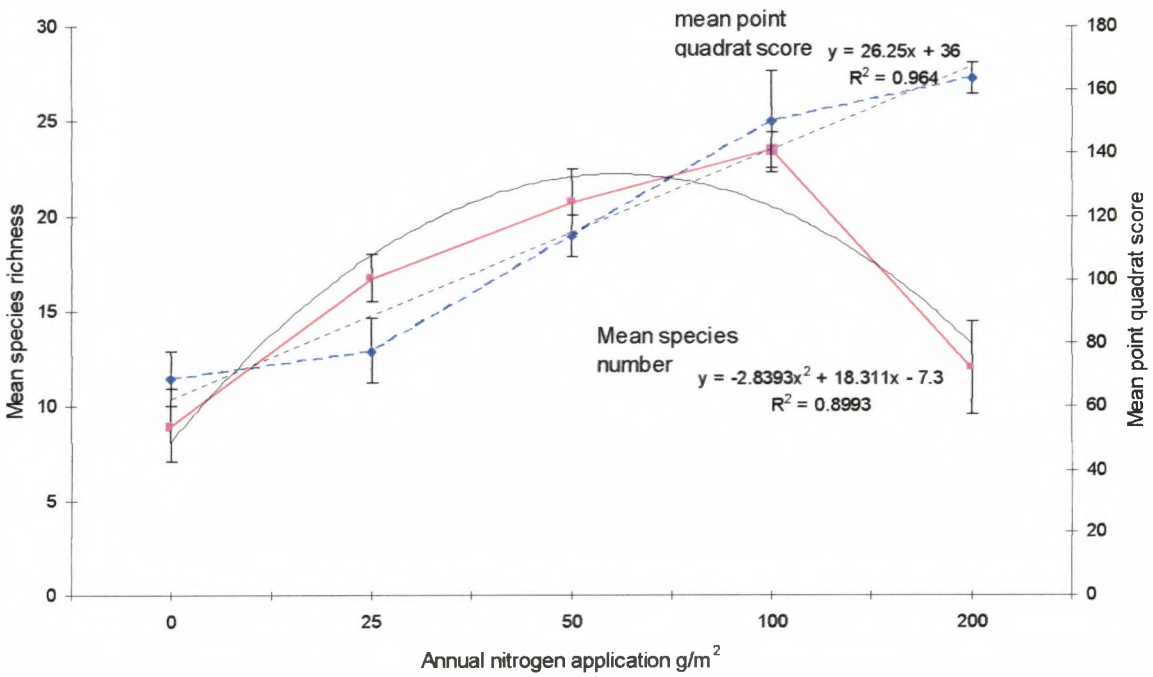

Figure 7.1.6 Mean species richness and mean point quadrat score against annual nitrogen application (data collected 25/8/00).

7.1.3 Discussion

It had been predicted that the inclusion of a nitrogen fixing group. would have influenced the developing community in the long term (Jeffries, Bradshaw, & Putwain 1981; Mulder *et al.* 2002). The results indicate the significant influence of the legume species in 1999 had dissipated by 2000 (Figures7.1.1-7.1.4). The fact that the inclusion of a functional group had a significant affect on increasing both species richness and productivity, in the short term, has implications for developing vegetation systems. Increase in species diversity and productivity have been reported by Mulder *et al.* (2002) although their findings suggested that long term influences of legume species to be more influential on both productivity and species diversity. The legumes were noted to have root nodules by the end of the experiment, so they would have been contributing to the nitrogen budget of the experimental units. The importance of legumes in the nitrogen budget of vegetation and soils developing on new substrates especially colliery waste is well documented (Dancer, Handley & Bradshaw 1977a & 1977b; Chadwick *et al.* 1978; Skeffington & Bradshaw 1980; Jeffries, Bradshaw & Putwain 1981; Marrs 1989; Bradshaw 1997). Chapman, Collins & Younger (1996) also recognised the importance of legume contributions to the nitrogen budget of communities developing on colliery spoil. However, they found the need to control legumes in communities developing on opencast spoil, as they could become dominant and reduce diversity.

In 1999 the $100g/m^2$ annual nitrogen application, equivalent to the annual nitrogen uptake level for temperate high-yield cereal crops (Killham 1994), yielded maximum point quadrat score and species richness; these yields being less at higher and lower nitrogen levels (see Figures 7.1.1, 7.1.3 and 7.1.5). In the year 2000, 100 g/m^2yr^{-1} of nitrogen gave the highest species richness, but the $200g/m^2yr^{-1}$ application gave the highest productivity. There was a depression in species number with the higher nitrogen application (Figures 7.1.2, 7.1.4 and 7.1.6). Depression of species richness has been reported in natural systems with nitrogen inputs in excess of $50g/m^2yr^{-1}$ (Kirkham 2001), and in agricultural systems additions of 40-120 g/m^2yr^{-1} of nitrogen is usually not exceeded to promote yield, the addition depending on the crop and soil type (Clarkson & Benson 1980; Nasholm, HussDanell, & Hogberg, 2000; Singh & Arora 2001). The low levels of nitrogen within the spoil (Chapter 5) may explain why such

high additions influence the experimental vegetation. Nitrogen additions have the greatest impacts on ecosystems in which it is the limiting factor (Marrs *et al.* 1983; Kirkham 2001). As vegetation on colliery waste has been demonstrated to be limited by nitrogen, then it is perhaps not surprising that the nitrogen additions have such a dramatic impact on the experimental vegetation communities.

Nitrogen is important for developing ecosystems and is though to control vegetation structure (Marrs *et al.* 1983). The results, from this experiment, have significant implications for developing vegetation systems. Nitrogen availability is demonstrated as crucial for controlling not only productivity but also species richness and therefore community structure in the establishing experimental vegetation. The depression of productivity observed in1999 (with respect to the highest nitrogen application) had been reversed by 2000, and the $200g/m^2yr^{-1}$ nitrogen application had the highest yields (measured as point quadrat scores). However, at this highest nitrogen application species number was depressed. The reduction in species richness was the result of one or two species making up most of the yield in the highest nitrogen units and depressing other species (Berntson & Wayne 2000). . The two species noted as being most dominant in the high nitrogen application units were the tall growing plants: *Rumex acetosella* and *Leucanthemum vulgare* with mean point quadrat scores of 120 and 150 respectively.

The determination of the effects of differing nitrogen budgets on plant species diversity within developing vegetation can help our understand community successional development. Even the application of $25g/m^2yr^{-1}$ nitrogen had a significant positive affect on species richness and productivity in the developing vegetation, indicating nitrogen was limiting in the colliery waste. This implies that it is necessary for developing vegetation systems on colliery waste to gain, and maintain, nitrogen cycling capabilities.

Tilman's (1985) resource-ratio hypothesis, suggests that competition for nutrients changes to competition for light at late successional stages. These results indicate that at least competition for nutrients is present at both successional stages, because the productivity of the tall growing plants (*R. acetosella* and *L. vulgare*) increased

when nitrogen was added. This implies that the smaller plants may have been limited by another factor such as light. The increase in biomass of tall growing plants irrevocably results in a further decrease in the amount of available light for other species. Competition for light therefore may become more important at late successional stages. The dramatic effect of the $200g/m^2yr^{-1}$ nitrogen application on species diversity, as a result of a few tall growing species growing at the expense of small plants, is unlikely to be encountered in natural state systems. Although there is an increasing trend in nitrogen deposition in the UK (RGAR 1997), the maximum natural nitrogen inputs from atmospheric deposition have been recorded as $33.2g/m^2yr^{-1}$ in the UK (Kirkham 2001). However, the depression of species richness found in this experiment has important connotations for land restoration strategies which utilise artificial nitrogen sources, and natural systems which have large anthropogenic inputs of nutrients.

7.2 The effect of an NPK gradient on natural vegetation with respect to species richness and productivity.

Resource manipulation in natural systems has been utilised to examine the relationship between resource availability and community structure and diversity (DiTommaso & Aarssen 1989). The influence of changing biodiversity on ecosystem functioning has been a central and rapidly growing theme in ecology (Loreau *et al.* 2001). Research in this area is motivated in significant part by the practical issue of understanding how declining diversity influences ecosystem services on which ecological integrity depends. Empirical research on biodiversity-ecosystem functioning linkages has included manipulating diversity in grasslands, and testing how ecosystem-wide biomass accumulation is affected by species diversity associated along gradients of environmental resources (Tilman *et al.* 2002). Experimentally this has been achieved by randomly assembling test communities from a pool of species (Rajaniemi, 2002). This investigation examines the influence on productivity and species diversity, by manipulating plant nutrient gradients in a natural community.

The landscaping strategy at the Tilmanstone site has included the provision of a vegetation screen to conceal a brick manufacturing factory. For this end, a shale bank was formed at the northern boundary of the site. The bank was covered in topsoil to a

depth of 1m in 1995 with the intention of sowing grasses and planting trees to form a vegetation screen. The topography of the bank shale (angle >45^0) was such that the topsoil covering was eroded in less than twelve months leaving an amalgamation of mixed sediment (soil, shale and chalk) covering the shale bank to a depth of only a few centimetres in places. Although there was very little soil on the bank, and the sediment was unstable, there was some natural colonisation by perennial and annual plant species. This investigation examined the effect of additions of a 20:10:10 NPK inorganic fertiliser gradient to the naturally colonising vegetation.

7.2.1 Method.

The vegetation on the bank was surveyed in March 1997, at the start of the experiment, to ensure that there was no bias in the selection of the area which was to be treated. The bank vegetation was surveyed using 20 X 0.25m^2 randomised quadrats. A pin of 0.5mm was randomly positioned 20 times within the 0.25m^2 quadrats. The position of the pin was determined by dividing the 0.25m^2 quadrats into a 100 squares using lines 5cm apart. A random number table was used to select the position of a grid square, within the quadrat, and then the pin was placed into the centre of that grid square. The vegetation in contact with each pin was scored so that each part of a plant touching the pin, of each plant species, was recorded. The survey data was analysed by one-way analysis of variance using the statistical package Minitab (release 11). The pattern of vegetation was shown to be random in terms of species, P=0.981, and point quadrat score P=0.392 (Appendix 7.2.1).

An area of the bank which had been surveyed was then divided into a grid of 20 X 1m^2 experimental units with 0.5m isolation areas around each unit. Individual units were ameliorated with one of five concentrations of 20:10:10 NPK slow release *Osmocote* fertiliser. Application rates were equivalent to an annual nitrogen application rate of: 0g/m^2, 25g/m^2, 50g/m^2, 100g/m^2 and 200g/m^2. Experimental units were randomly selected to give four replicates of the five treatments.

The vegetation on the bank was monitored from in March 1997 and September 1997. The number of plant species and the point quadrat score of each experimental unit was

measured. A pin of 0.5mm was randomly positioned 20 times within the $1m^2$ quadrats. The position of the pin was determined by dividing the $1m^2$ quadrats into a 100 squares using lines 10cm apart. A random number table was used to select the position of a grid square, within the quadrat, and then the pin was placed into the centre of that grid square. The vegetation in contact with each pin was scored so that each part of a plant touching the pin, of each plant species, was recorded. Data was collected in March 1997 and September 1997.

The data collected in September 1997 was analysed using analysis of covariance (orthogonal design) in Minitab (release 13). The data collected in March 1997 was used as covariates in the analyses.

7.2.2 Results

Data from this experiment was first presented in an undergraduate dissertation (Gilchrist 1998). The data has been reworked with analysis of covariance (orthogonal design). This enabled the vegetation measurements before treatment to be used as covariates when analysing the experimental data (Appendices 7.2.2 and 7.2.3). The adjusted means are presented in Figure 7.2.1. Plates 7.2.1 and 7.2.2 illustrate the vegetation development within the experimental area.

Point quadrat score and species number are significantly affected by the application concentrations of NPK (P=0.006 and P<0.001 respectively from the analyses of covariance, Appendices 7.2.2 and 7.2.3). Figure 7.2.1 illustrates the vegetation response to different 20:10:10 NPK concentrations at the northern boundary bank. The data pattern is very similar to the pattern illustrated in Figure 7.1.6 which is for nitrogen only. Point quadrat score (a function of productivity) increases linearly with the increase in NPK dosage, whereas, species number is limited at higher concentration of fertiliser. Point quadrat score and species number have correlation coefficients R^2=0.85 (Pearson correlation P=0.002) and R^2=0.98 (Pearson correlation P<0.001) respectively (Figure 7.2.1).

Plate 7.2.1 Tilmanstone bank experiment marked out March 1997

Plate 7.2.2 Experimental area September 1997

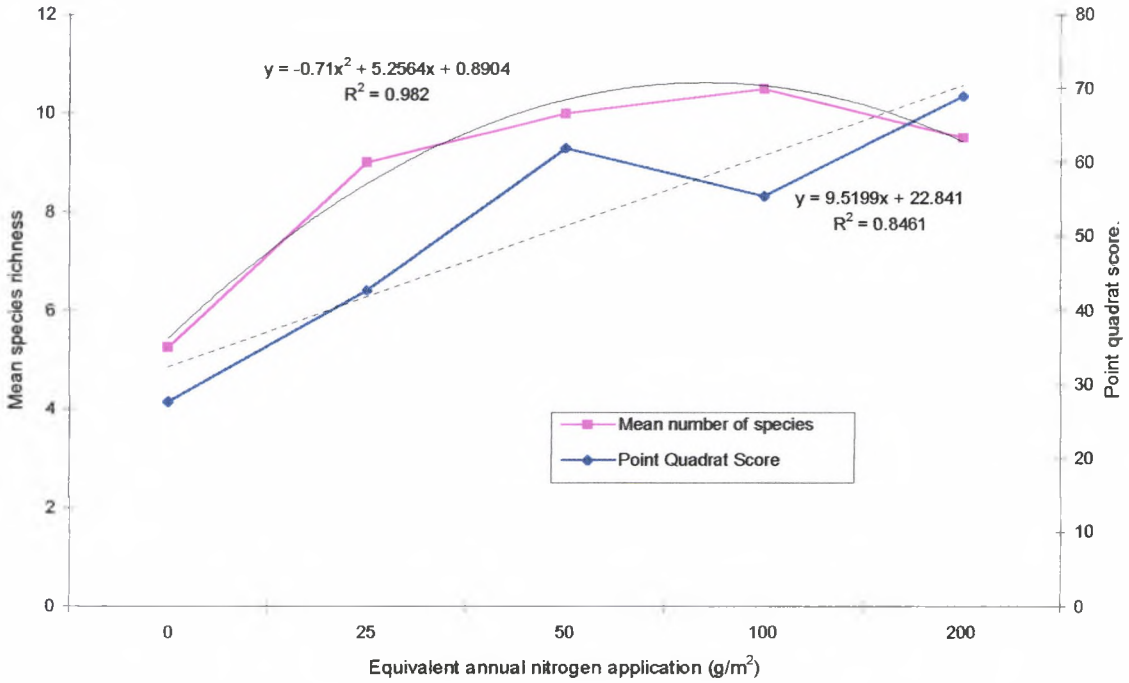

Figure 7.2.1 Adjusted mean species richness and point quadrat score against annual nitrogen application.

7.2.3 Discussion.

The majority of variation within the two measured variables can be explained by the concentration of NPK applied to the vegetation (Figure 7.2.1). Augmentation of productivity and species richness with some NPK addition and the suppression of species number in this natural vegetation at the equivalent of 200g/m^2 of nitrogen, have implications for restoration strategies which include the addition of NPK, as well as for community ecology.

Tilman's (1985) resource-ratio hypothesis assumes that each plant species is a superior competitor for a particular proportion of the limiting resources and predicts that community composition should change whenever the relative availability of two or more limiting resources changes. This can be seen in the change in species diversity within

the natural plant community as a response to the artificial NPK gradient imposed at Tilmanstone, and supports some of Tilman's proposals that:

- the major limiting resources for terrestrial habitats are often a soil resource, often nitrogen, and light;
- these resources are naturally inversely related, the habitats with poor soils having high-light availability and the habitats with rich soils having low light availability;
- the life history of a plant species will depend on the point along the soil-resource to light gradient at which it is a superior competitor to other plant species;
- primary succession and secondary succession on poor soils result from a temporal gradient in the relative availabilities of a limiting soil resource and light.

The plant community had higher productivity, as measured by point quadrat scores, at higher resource rates. This implied that light at the substratum surface would be reduced due to interception by above ground growth, in comparison with those plants with low productivity in low NPK experimental units. The NPK gradient and light intensity at the substratum surface was therefore inversely related and would influence the plant community development (Siemann & Rogers 2003).

In this experiment, it is not fully understood why species number should be influenced by NPK addition in natural vegetation. Although the niche dimension of plant resources had been altered in some experimental units, there was no artificial input of extra species to the area by sowing. Plants already germinated and establishing could exploit the increase in plant nutrients (Chapin, Vitousek & van Cleve (1986). It is presumed they changed the micro-environmental conditions on the experimental site and emulated succession (Olff, Huisman, & van Tooren 1993). These new conditions could have promoted recruitment to the system either from the seed bank or from seed rain. The increase in height of the vegetation could have disrupted air flow over the experiment and wind dispersed seeds may have been preferentially deposited on the site (Plates 7.2.1 & 7.2.2). Harvey (2000) reported this phenomenon in agricultural systems. However, the productivity of the vegetation was not limited at the highest NPK application but species richness was. Possibly the reduction in species may have been caused by competitive exclusion the mechanism of which was observed in

experiment 7.1 (Berntson & Wayne 2000). The plants that could exploit the high application of NPK grew vigorously influencing the developing community composition (Figure 7.2.1) by competing effectively for other resources such as light (Martens, Breshears, & Meyer 2000) and supporting Tilman's (1985) resource-ratio hypothesis.

7.3 Vegetation manipulation.

Successional vegetation systems can exhibit a gradual and progressive change of community structure towards an end point (Clements 1916 & 1936). This progressive change is in response to the modification of the environmental conditions by species present at that locale over time (Olff, Huisman & van Tooren 1993; Tilman1994).

The pattern of successional dynamics is also controlled by the limiting environment constraints operating at a locale. To understand the successional patterns of vegetation a degree of simplification of the complex system is required (Pakes & Maller 1990). This can be achieved by experimental manipulation to enable meaningful interpretation of the functional response of vegetation to changing conditions.

Experimental manipulation of the natural vegetation systems on Betteshanger and Stodmarsh were performed to examine the process of colonisation at the two sites. Hils and Vankat (1982) experimented on old-field successions in Ohio by removing functional vegetation types and then measuring the response of the remaining vegetation. They did not find conclusive evidence in their experiment for any one successional model. However, in sand dune successions natural vegetation removal occurs. The vegetation that grows back is indicative of the successional processes occurring within the dune system (Olson 1958). Marram grass is a primary coloniser in UK sand dunes and dominates the early dune vegetation community. Marram grass is replaced as the dominant species, when the sand substratum is sufficiently modified to enable other species to colonise it. If late seral vegetation is stripped from an area by a storm leaving bare sand (a blow out), the whole successional process starts again. The vegetation reverts to an early seral succession stage dominated by Marram grass (Pemadasa, Greig-Smith & Lovell 1974).

The Hils and Vankat (1982) approach was adapted by removing all vegetation rather than just functional types and the inclusion of different resource applications to the de-vegetated areas to simulate different ecological inputs and to mimic different seral stages of succession (Olff, Huisman, & van Tooren 1993)

The removal of the vegetation from colliery waste and filling the space with material taken from an un-vegetated part of the site, simulated the natural process of vegetation removal similar to that observed in dune systems. The vegetation that re-established on the de-vegetated colliery waste, the colonising patterns and essential components of the vegetation system were examined to assess the successional processes occurring.

7.3.1 Method

Sixteen squares of vegetation ($1m^2$) were removed from the surface of the spoil deposited between 1932 and 1946 (area 3 Figure 3.2) on the Stodmarsh site and $16X1m^2$ of vegetation were removed from spoil deposited between 1965 and 1970 on Betteshanger site (Figure 3.3). The vegetation including the root system was removed as a turf. Species composition and percentage cover were recorded prior to removal of the turfs.

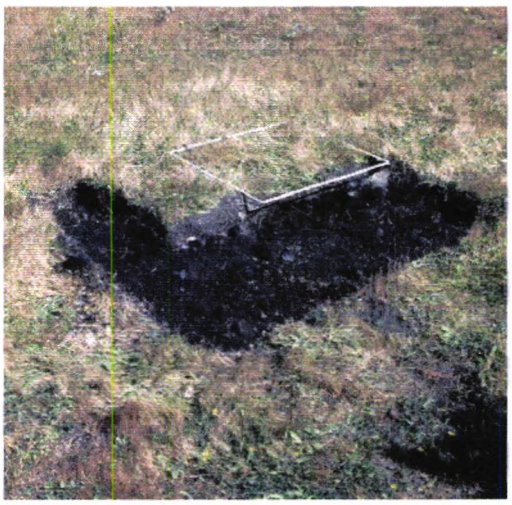

Plate 7.3.1 Removal of a $1m^2$ turf.

Plate 7.3.2 1m^2 areas were filled with spoil taken from un-vegetated areas.

The 32 X 1m^2 de-vegetated areas were treated in a randomised factorial design. The treatments were: a once only treatment of ± 200g/m^2 peat, equivalent annual application rates of: ± 50g/m^2 nitrogen, ± 25g/m^2 of phosphorus and potassium added quarterly (P & K as one treatment). Two replicates of each possible combination were set up in April 1999 at Stodmarsh and Betteshanger. The species number and point quadrat score of the vegetation developing in the de-vegetated areas were sampled in April 2001. A pin of 0.5mm was randomly positioned 20 times within the 1m^2 experimental units. The position of the pin was determined by dividing the 1m^2 units into a 100 squares using lines 5cm apart. A random number table was used to select the position of a grid square, within the quadrat, and then the pin was placed into the centre of that grid square. The vegetation in contact with each pin was scored so that each part of a plant touching the pin, of each plant species, was recorded. The data were subjected to principal components analysis using the statistical package Minitab (release 13).

7.3.2 Results

Plate 7.3.4 illustrates the plants colonising the experimental areas in 2001. Figure 7.3.1 represents the first 2 principal components (eigenvalues) from the data pre- and post- treatment. See Appendix 7.3.1 for full analyses.

Figure 7.3.1 illustrates the correlation coefficients for each species in the first 2 principal components (eigen values) for the plant communities in 1999 before treatment and after treatment in 2001. The 1999 communities at both Stodmarsh and Betteshanger are very different. However, all the communities of plants are very similar in 2001 regardless of treatment.

Plate 7.3.4 Plants colonising one of the de-vegetated areas in 2001.

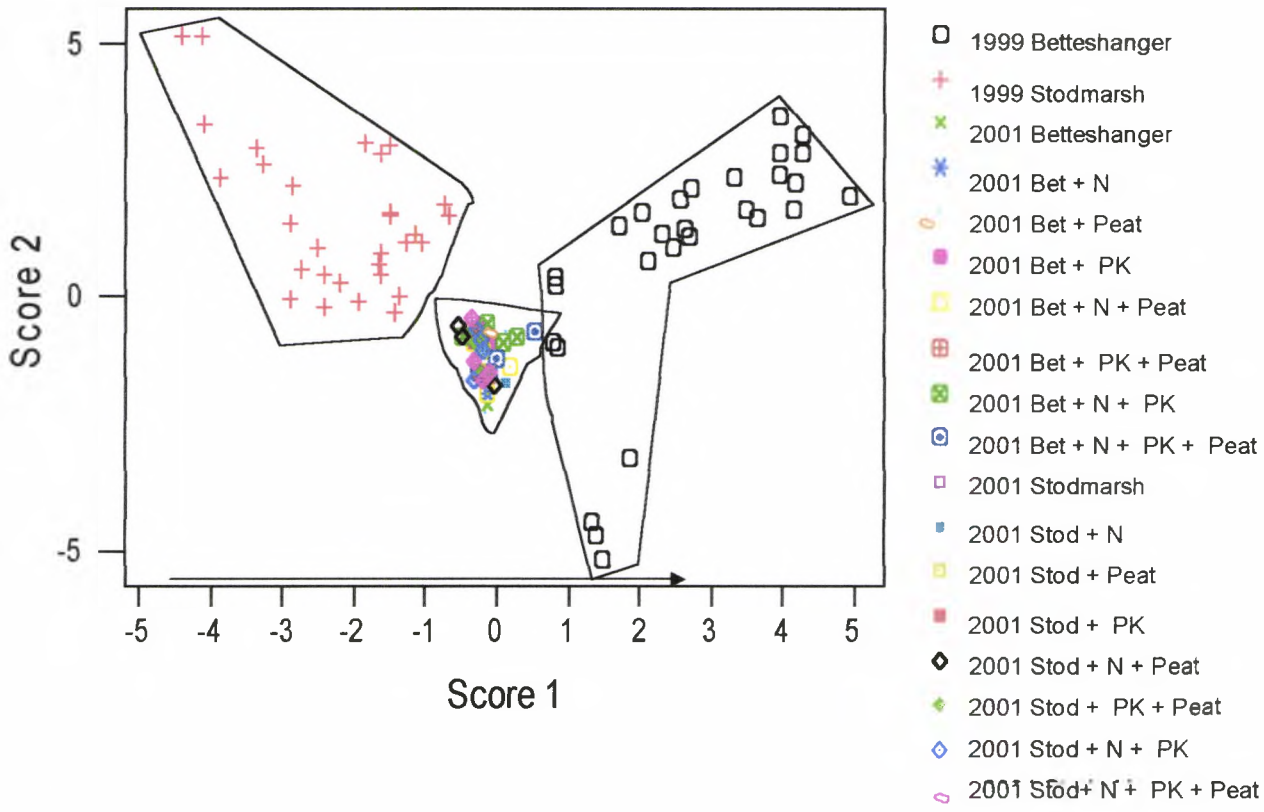

Figure 7.3.1 The first 2 principal components (eigenvalues) plotted from the principal components analysis of plant communities at the Betteshanger and Stodmarsh sites before and after treatment.

7.3.3 Discussion

It was predicted that if the vegetation structure which re-established on the bare colliery spoil was similar to the vegetation that was removed, then there was strong evidence that the controlling processes were a result of the physico-chemical environment of the shale and not related to age of the substratum (Olson 1958; Pemadasa, Greig-Smith & Lovell 1974). However, after two years the plant communities were significantly different from the original 1999 communities which were removed (Figure 7.3.3). This would suggest that successional processes were controlling community development and that the age of the spoil was important to the community able to colonise it (Brenner, Werner & Pike 1984; Schuster & Hutnik 1987).

It was anticipated that resource availability provided by the treatments would have had an influence on the developing plant communities in the de-vegetated plots (Biondini, Bonham & Redente 1985; Olff, Huisman & van Tooren1993; Brown *et al.* 2001a). This was also because treatments related to resource availability associated with older spoil had already had a demonstrable affect on plant community development (Chapter 5). However, this was not evident except in the Betteshanger community which had NPK and peat added. This community was very similar to a community found in the 1999 community analysis for Betteshanger (Figure 7.3.1). The relatively small influence of resource availability on the developing communities suggests that other parameters were controlling the community structure developing within the de-vegetated plots. An important factor may be the ability of plant species to migrate to the experimental areas (Collingham & Huntley 2000). However, the experimental plots were surrounded by a plant community which could have provided species for recruitment. *Cirsium vulgare* and *Senecio vulgaris* were noted as common in the 2001 communities on both Stodmarsh and Betteshanger. It was perhaps because these species are wind dispersed ruderals that enabled them to exploit the de-vegetated areas in preference to other less mobile species (Soons & Heil 2002). Competitive exclusion is thought not to be an issue on the de-vegetated plots, as percentage cover was low (less than 40%) in all the experimental units at the sampling time.

7.4 Reciprocal vegetation experiment.

One of the limitations to the development of a plant community is the availability of species which can migrate into an area and survive the local conditions once they get there (MacArthur & Wilson 1967; Collingham & Huntley 2000). Studies of biological invasions indicate that natural recruitment of new species can occur as a "nucleation" phenomenon, in which scattered colonisation, focus, spread and coalesce (Levine 2000; Symstad 2000). Ecological reclamation might make use of this potential for enhanced natural dispersal and establishment, by inoculating sites with multiple small plantings (Robinson & Handel 2000). The colliery waste sites are surrounded by plant communities which have developed on a very different substrate: rendzina soil covering chalk country rock. The potential species for recruitment, to the plant communities developing on the colliery waste, are therefore limited (Brenner, Werner & Pike 1984).

Transplanting plant communities, from vegetated areas to non-vegetated areas is a method of establishing vegetation in restoration schemes (van Keulen, Paling & Walker 2003; Bull, Reed & Holbrook 2004). To assess whether this methodology was appropriate for colliery waste restoration, vegetated plugs in the form of $1m^2$ turfs were transplanted into un-vegetated areas on colliery waste. The vegetation that established and the rate at which this occurred was monitored. It was intended to demonstrate the species dispersal capabilities of those species which have already formed communities on the colliery spoil, and the potential for this type of approach for establishing vegetation on colliery waste.

7.4.1 Method

On Betteshanger ten of the turfs removed in method 7.3.1 were transplanted into an area of shale that was not vegetated (Plate 7.4.1). The orientation of the turfs was randomised to prevent the possibility of confounding the results of dispersal between treatments with prevailing wind direction.

Colliery waste was tilled to provide $1m^2$ wide boundaries of un-compacted material around the individual turf transplants. $1m^2$ areas within the boundary and adjacent to

the transplants were treated with ammonium nitrate (applied quarterly) equivalent to an annual nitrogen application rate of 50 g/m^2 and a once only application of 200g/m^2 of peat (Figure 7.4.1).

Plate 7.4.1 *One of the ten 1m^2 turfs from 7.3.1 in the process of transplanting into an* un-vegetated area on Betteshanger.

-N +Peat	-N +Peat	-N +Peat
+N -Peat	Transplanted vegetation	-N -Peat
-N +Peat	+N +Peat	-N +Peat

Figure 7.4.1 Plan of one experimental unit.

7.4.2 Results

Transplanted turfs survived and were monitored for two years. *L. corniculatus, Centaurium erythraea, A. capillaris* and *H. pilosella*, which were present in the original turfs, also flowered and produced seed in this time. However, there was no spread of individuals into the prepared tilled colliery spoil. Therefore, there was no community development to monitor and none of the treatments instigated the spread of species outside the turfs (Plate 7.4.2).

Plate 7.4.2 A turf in 2001 illustrating that although the turfs themselves survived, there was no spread of plants from the turf.

7.4.3 Discussion

It is not fully understood why there was no spread of plant species outside the ten transplanted turfs as the individuals of the turf communities were already adapted to the site conditions (D'Antonio, Levine& Thomsen 2001). There may have been some toxicity in the spoil, in which the turfs were planted, but this was not found in the analyses performed in Chapter 4.1. Also, the colliery waste removed to enable the turfs to be transplanted was used in 7.3, and plant communities did establish on it.

The lack of dispersal of plants from the transplants could be the result of the time that this type of colonisation can take (Collingham & Huntley 2000), and the lack of

vegetation is simply a function of the experiment having not been monitored for long enough. However, as a restoration technique, with the time scale greater than two years before any vegetation colonises, it is unviable.

It has been suggested that the size of the vegetation transplant can influence the success of species to colonise substratum outside the transplant (Soons, & Heil 2002; van Keulen, Paling, & Walker 2003), and maybe $1m^2$ turfs were not a viable size to facilitate colonisation from the transplant.

The use of reservoirs of endemic species, in the form of turfs already adapted to the conditions on site, was a potential land restoration strategy for establishing vegetation at the site (Bull, Reed & Holbrook 2004). The lack of dispersal of species from the transplanted turfs makes this impractical technique in this instance.

The invasion from introduced species into the un-vegetated spoil was anticipated to be facilitated in those areas adjacent to the transplants which had a treatment (Huenneke *et al.* 1990). The treatments related to resources which would be available in older successional substratum and which related to potential limiting resources for the colonisation of the colliery spoil (Brenner, Werner & Pike 1984). The absence of facilitated colonisation of the amended colliery spoil suggests that there are other barriers to the colonisation other than the availability of nitrogen, phosphorus and organic carbon. Further work to understand the processes of species dispersal to the site is therefore required, to try and explain the lack of dispersal into the prepared boundaries surrounding the turfs.

8 Novel restoration strategies developed from the research.

The introduction of a self-sustaining ecosystem is the primary objective of most reclamation strategies (Allen, Covington & Falk 1997; Bradshaw 1998). Vegetation establishment, development of nutrient cycling and the restoration of beneficial soil micro-organisms are all necessary considerations in this process (Marrs 1989; Bradshaw 1997). The establishment of a sustainable vegetation cover is also a useful tool for stabilising unconsolidated materials (Haigh & Gentcheva-Kostadinova 2002), such as colliery waste (Plate 8.1). However, the majority of restoration schemes employ engineering solutions which concentrate on substratum remediation and only consider establishing vegetation in this context (Bradshaw 1987). They often do not consider the functional components of the vegetation system that, may, ensure the long term sustainability and success of the scheme. Ecological findings, from research reported in this thesis, were incorporated into novel restoration techniques which were evaluated at Tilmanstone former colliery.

Plate 8.1 Tilmanstone colliery waste has a significant erosion problem which a sustainable vegetation system may remedy.

The inclination of colliery waste tip slope sides at the Tilmanstone site are $>45^0$ in places. It is unfeasible to use traditional tilling machinery at this angle (Merlin, DiGioia, & Goddon 1999). Some natural vegetation colonisation has, however, occurred. *Betula pendula* and other tree species have established at the site. If traditional sowing techniques were employed these trees would have to be removed (Roberts & Bradshaw 1985). Establishing vegetation on the site is difficult (Plate 1.4.2); therefore, any natural vegetation is important to maintain. Plants which naturally colonise colliery waste are innately sustainable and persistent; they also improve the stability of the waste (Prach *et al.* 2001). Gross and surface stability provided by the colonising trees, and other natural alterations to the shale environment which the trees promote, facilitate further vegetation to establish (Rimmer 1982). The leaf litter from the trees provides an organic component for soil formation and the canopy cover provides shade. The shade drastically reduces the surface temperatures of the banks in the summer months. A successful hydroseeding technique would augment the naturally occurring vegetation, and the vegetation system which develops would be both self sustaining and robust (Roberts & Bradshaw 1985).

8.1 Hydro-seeding.

Establishing vegetation on the steep banks at Tilmanstone is an ultimate test for a restoration technique. The colliery waste is difficult to vegetate because of its physical and chemical characteristics; there is also the added problem of its topography.

Hydroseeding is a method which has been used in other restoration projects with similar problems of topography as found at Tilmanstone former colliery (Roberts & Bradshaw 1985). Hydroseeding is a process by which seed, soil conditioners and growth promoters are mixed in water and sprayed onto substrata which are not suitable for traditional tilling machinery (Merlin, DiGioia, & Goddon 1999). The potential for using a hydro-seeding technique, to develop a sustainable vegetation system on steep colliery spoil slopes, was evaluated in this experiment.

Nutrient availability was shown to be crucial for influencing not only productivity, but also species richness and, therefore, community structure on colliery waste (Chapters

4, 5 and 7). A new slow release nutrient was evaluated as a restoration tool in this experiment. The novel slow release product, a bacterially digested and pelleted biosolid, was developed by Southern Water Plc and trades as: *Biotech-granules*. *Biotech-granules* have not been used in hydroseeding previously, and if they proved successful could be an important resource for future restoration schemes. Three rates of application were selected for evaluation.

Plant community composition and structure were also observed to be crucial for vegetation establishment on colliery waste (Chapter 5). Drought tolerant seed mixes, as well as an autumn sowing, were incorporated into the experimental design. These measures were taken to promote establishment before potential summer drought conditions could prevent establishment. Seed mixes, with differing species compositions and different functional types, were chosen to be evaluated. They included a nurse grass species to aid initial establishment and to promote structure in the colliery waste (Carrillo-Garcia *et al.* 1999; Pinaya *et al.* 2000), and legumes to improve the nitrogen fixing potential of the developing vegetation (Dancer, Handley & Bradshaw 1977b; Jeffries, Bradshaw & Putwain 1981). A high and low density sowing rate was also selected for investigation. A high rate, of 15g/m^2, was chosen to increase the possibility of survivorship after germination. A low rate, 5g/m^2, was selected so that if there was good germination and establishment, the competition for resources would be lower than the demand in the high density vegetation. A low resource demand could be crucial for the long term sustainability of the vegetation on the resource poor colliery waste (Zobel, van der Maarel & Dupré 1998).

8.1 .1 Method.

A steep, south facing colliery bank (the slope ranging from 40°-49° measured with a Silverman clinometer) was selected at the Tilmanstone site. The bank was divided into 10 equal areas (16m X 12m) with 2m gaps between each area. The 2m wide isolation areas, between each block, were not sprayed to prevent cross contamination between treatments. See Figures 8.1.1a and 8.1.1b for the experimental plan. Five seed mixes (Tables 8.1.1-8.1.5) were applied by hydroseeding on the 7/5/99 (Plate 8.1.1). The treatments were borne in mains water which included a hydroseeding carrier. The

hydroseeding carrier mix (per *English Landscapes* Industrial Standard©) was composed of: *Bentonite* clay to give 120g/m^2 coverage, wood pulp cellulose to give 100g/m^2 coverage, sphagnum peat moss to give 100g/m^2 coverage, *Seanure Seaweed* to give 70 g/m^2 coverage, *Terrabind* soil stabilising fluid to give 50 g/m^2 coverage and *Biotech granules* to give 200 g/m^2, 400 g/m^2 and 800 g/m^2 coverage.

Due to the constraints and logistics of the hydroseeding process, the different factorial treatment applications could not be replicated in separate blocks. Instead the large area that contained each combination of treatments was randomly sub-sampled to give data which fitted a replicated statistical model. Three 1m^2 quadrats were randomly selected, in each treatment area. A pin of 0.5mm was randomly positioned 20 times within the 1m^2 quadrats. The position of the pin was determined by dividing the 1m^2 quadrats into a 100 squares using lines 10cm apart. A random number table was used to select the position of a grid square, within the quadrat, and then the pin was placed into the centre of that grid square. The vegetation in contact with each pin was scored so that each part of a plant touching the pin, of each plant species, was recorded. The vegetation was scored in June 1999. Analysis of variance was performed on the data using Minitab (release 13) statistical package.

Seed mix A 5g/m^2 *Biotech-granules* @ 200g/m^2	Isolation area	Seed mix B 5g/m^2 *Biotech-granules* @ 200g/m^2	Isolation area	Seed mix C 5g/m^2 *Biotech-granules* @ 200g/m^2	Isolation area	Seed mix D 5g/m^2 *Biotech-granules* @ 200g/m^2

Figure 8.1.1a Plan of the hydroseeding experiment (not to scale).

Seed mix A 15g/m^2 *Biotech-granules* @ 200g/m^2	Isolation area	Seed mix B 15g/m^2 *Biotech-granules* @ 200g/m^2	Isolation area	Seed mix C 15g/m^2 *Biotech-granules* @ 200g/m^2	Isolation area	Seed mix D 15g/m^2 *Biotech-granules* @ 200g/m^2	Isolation area	Seed mix A 15g/m^2 *Biotech-granules* @ 400g/m^2	Isolation area	Seed mix A 15g/m^2 *Biotech-granules* @ 800g/m^2

Figure 8.1.1b Plan of the hydroseeding experiment continued (not to scale).

Plate 8.1.1 The treatments were applied by spraying the experimental area.

The *Biotech-granules* (NPK fertiliser) were applied at a rate of 200g/m^2 (equivalent to 50 g/m^2 of N) on 9 of the 11 blocks and at 400 and 800g/m^2 on two areas with seed mix A sown at 15g/m^2 (Figures 8.1.1a and 8.1.1b).

Table 8.1.1 Seed mix A

Species		**% of mix by weight.**
Lolium perenne	Perennial Rye grass (Candisa)	12
Lolium multiflorum	Westerwold Rye grass (Mowester)	40
Festuca rubra	Strong Creeping Fescue (Boreal).	35
Agrostis capillaris	Brown Bent (Highland).	5
	Legume mix (see Table 8.1.5)	8

Table 8.1.2 Seed mix B

Species		**% of mix by weight.**
Lolium perenne	Perennial Rye grass (Candisa)	21
Festuca rubra	Strong Creeping Fescue (Boreal).	62
Agrostis capillaris	Brown Bent (Highland).	9
	Legume mix (see Table 8.1.5)	8

Table 8.1.3 Seed mix C

Species		**% of mix by weight.**
Lolium perenne	Perennial Rye grass (Candisa)	14
Lolium multiflorum	Westerwold Rye grass (Mowester)	40
Festuca rubra	Strong Creeping Fescue (Boreal).	38
Agrostis capillaris	Brown Bent (Highland).	8

Table 8.1.4 Seed mix D

Species		**% of mix by weight.**
Lolium perenne	Perennial Rye grass (Candisa)	21
Festuca rubra	Strong Creeping Fescue (Boreal).	70
Agrostis capillaris	Brown Bent (Highland).	9

Table 8.1.5 Legume mix

Species		**% of mix by weight.**
Trifolium pratense	Red Clover	45
Trifolium repens	White Clover	15
Trifolium hybridium	Alsike Clover	30
L. corniculatus	Bird's-foot trefoil	10

8.1.2 Results.

Plate 8.1.2, illustrates the germination of grass and *Trifolium* species established in June 1999 after the May 1999 hydroseeding. Plate 8.1.3 indicates the state of the vegetation on the hydroseeded area in November 1999. The full analyses of variance using Minitab (release 13) statistical package can be seen in Appendices 8.1.1 and 8.1.2. Figures 8.1.2, 8.1.3 and 8.1.4 summarise the response of the mean point quadrat score, of the sown vegetation, to the different treatments incorporated in the experiment. In all Figures error bars represent standard deviation.

The vegetation monitored in June 1999 indicated that there were significant differences, in point quadrat score, between treatments: for seed mix type (P=0.046), for sowing rate (P=0.011) and for *Biotech granule* application rate (P=0.036). No

species information was collected, as the grass and legume species were immature most species could not be reliably identified. However, *Lolium multiflorum* (annual westerwold ryegrass) was identifiable and appeared to have germinated relatively better than other grass species in the seed mixes. There was also germination of the legumes, notably the *Trifolium* species (plate 8.1.2).

In August 1999 there was a significant failure of all the vegetation due to a period of hot dry weather. Temperatures of 42^{0}C were recorded at the spoil surface and the vegetation was badly scorched: the vegetation monitoring was abandoned.

Plate 8.1.2 Germination of all the seed mixtures was observed by June 1999.

Plate 8.1.3 The hydroseeded bank with some vegetation recovery (30/11/99).

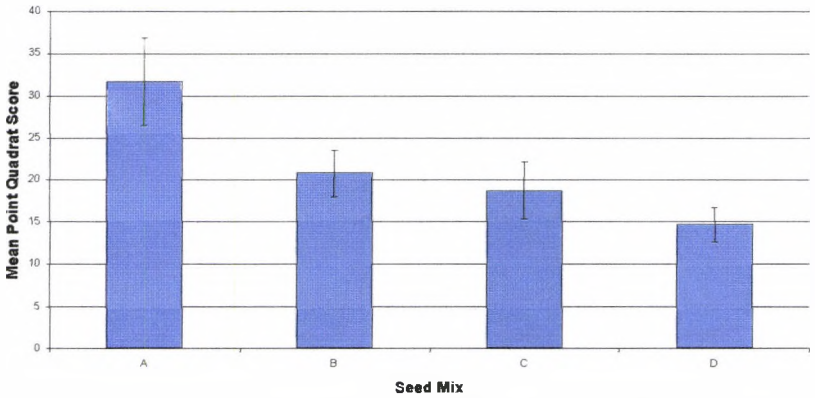

Figure 8.1.2 Mean point quadrat score from the different seed mix applications: data collected June 1999.

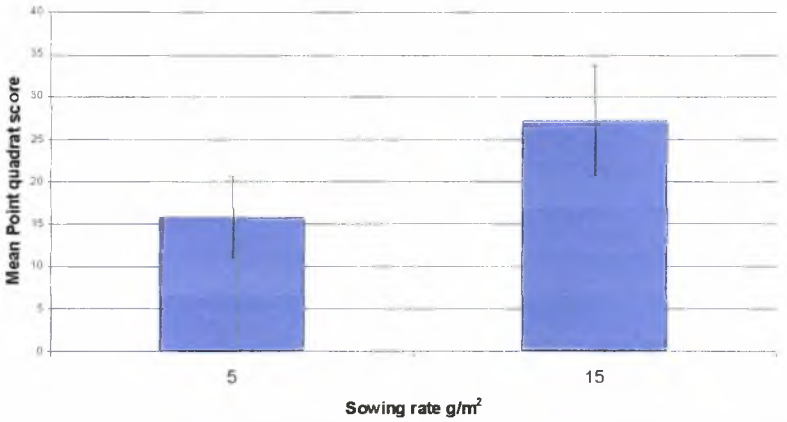

Figure 8.1.3 Mean point quadrat score against sowing rate: data collected June 1999.

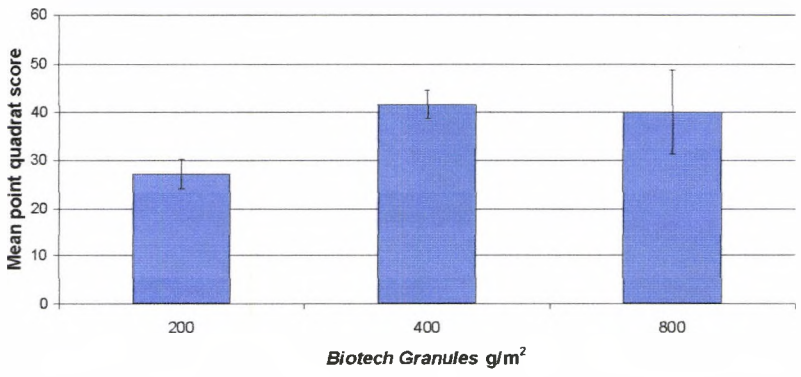

Figure 8.1.4 Mean point quadrat score versus *Biotech Granule* application: data collected June 1999.

8.1.3 Discussion

Although the hydroseeded vegetation failed, the June sample provided important information for future restoration trials. Figure 8.1.2 illustrates the cover achieved by the different seed mixes. Seed mix A germinated and had a larger point quadrat score in comparison with the other seed mixes and could be recommended for future hydroseeding at the site. Figure 8.1.3 summarises the difference in point quadrat score between the $5g/m^2$ and $15g/m^2$ sowing rates. The $5g/m^2$ sowing rate was chosen to minimise competition for scarce plant resources in the developing vegetation (Peltzer Wilson & Gerry 1998). However, because the establishment of vegetation is so difficult on the site, the higher application rate of $15g/m^2$ maybe more applicable in future trials, as over twice the cover was achieved with this higher sowing rate. Although there are significantly higher point quadrat scores for the $15g/m^2$ rate (P=0.011), the scores are not three times as high as the $5g/m^2$ rate. This may be due to initial competition between the young plants (Goldberg & Barton 1992; Silvertown *et al.* 1992), and has a cost implication for future seeding strategies (King 1991); a sowing rate between the two rates chosen may well give comparable coverage, as the $15g/m^2$, and would be less expensive. However, further seeding rates would need to be investigated to establish if the relationship between seeding rate and cover is linear. The vegetation would also need to be monitored over a number of seasons to determine whether competition, within the higher sowing rate, had any long term implications for the sustainability of the developing plant community (Marrs *et al.* 2000).

Plant nutrients are limiting on the colliery waste (Chapter 7), and this limitation has to be overcome for sustainable vegetation to establish (Bradshaw & Chadwick 1980; Broughton 1985). The different application rates, of *Biotech-granules,* significantly influenced the percentage cover of the vegetation (P=0.036). An increase in percentage cover was recorded for the $400g/m^2$ application rate in comparison with the $200g/m^2$ application rate (equivalent of $100g/m^2$ and $50g/m^2$ of nitrogen respectively). At the $800g/m^2$ application rate (equivalent to 200 g/m^2 of nitrogen) there was a slight reduction in the percentage cover in comparison with the $400g/m^2$ application rate. Agricultural systems do not exceed additions of 40-120 g/m^2yr^{-1} of nitrogen to promote yield, the soil quality and the type of crop influencing the amount of nitrogen added

(Clarkson & Benson 1980; Nasholm, HussDanell, & Hogberg, 2000; Singh & Arora 2001). The vegetation response, to nutrient availability, was similar to that observed in experiments described in Chapter 7 i.e. at high rates of nitrogen addition, in the first growing season, depresses productivity. This could be due to some plants utilising the excess nitrogen more effectively than other species, and then out-competing species for resources other than nitrogen (Tilman 1985), or the nitrogen levels being toxic for some of the species in the seed mix (Kirkham 2001). The results give important information on application rates for *Biotech-granules* in hydroseeding strategies on colliery waste, and suggest an application of 400g/m^2 should, probably, not be exceeded. The two functional groups, the nurse grass and legumes, both germinated well, which have implications for future restoration strategies (Dancer, Handley; & Bradshaw 1977b; Jeffries, Bradshaw & Putwain 1981; Ashton *et al.* 1997; Carrillo-Garcia *et al.* 1999). However, as the vegetation failed, due to an extended hot dry period, there was no observable influence on the establishing vegetation by the functional species. There was, however, some recovery of the vegetation when growing conditions improved which can be seen in Plate 8.1.3.

8.2 Hydroseeding application of mycorrhizal fungi.

Mycorrhizal fungi were demonstrated to be integral to the vegetation systems developing on colliery waste, in East Kent (Chapter 6). Hydroseeding is a potential technique which could introduce mycorrhizal fungi into vegetation systems and improve the success and therefore the cost effectiveness of restoration schemes on colliery waste (King 1991). Mycorrhizal fungi could present a solution to some of the limiting effects of scarce plant resources in colliery waste (Danielson1985).

The inclusion of mycorrhizae, in a hydroseeding mixture, may promote the developing vegetation to become infected with mycorrhizal fungi (Biermann & Linderman 1983). The vegetation could then benefit from any symbiosis which may develop. The vegetation, and mycorrhizal system, would essentially be able to forage for plant resources more effectively than plants without mycorrhizal symbionts (Olsson, Jakobsen & Wallander 2002). Therefore, the developing vegetation could survive harsher conditions, and be more sustainable, than vegetation sown without the

inclusion of mycorrhizal fungi on colliery waste (Jasper *et al.* 1994). To test this, a number of arbuscular mycorrhizal fungi species, isolated from stressed sites, and ecto-mycorrhizal fruiting bodies from *Scleroderma citrina*, found at Tilmanstone colliery, were collected. The collected mycorrhizal fungi were then evaluated in a hydroseeding trial to gain an understanding of their influence on hydroseeded vegetation.

Plant nutrients were shown to limit plant growth in colliery waste (Chapter 7). *Biotech-granules*, a slow release organic fertiliser developed by Southern Water and already tested in a previous hydroseeding (8.1), were included in the trial design to provide a slow release source of NPK. The seed mix selected included four legume species to provide a potential ecological solution to nitrogen limitation within the experimental vegetation system (Dancer, Handley, & Bradshaw, 1977b; Jeffries, Bradshaw & Putwain 1981).

8.2.1 Method

AMF species were isolated from plants and spoil, sampled from the Tilmanstone and Betteshanger sites (see 6.3 for method), and then grown to sufficient quantities by Plantworks Ltd on *Trifolium pratense* host plants. The mycorrhizal fungi treatment, for the hydroseeding, was made up of 100g/l of axenically cultured AMF spores and infected root of *Trifolium pratense* and 2g/l of *Scleroderma citrina* spore material collected directly from the Tilmanstone site. The AMF species were: *Glomus geosporum* (24.5g/l), *Glomus intraradices* (24.5g/l), *Glomus claroideum* (24.5g/l) and *Acaulospora morrowiae* (24.5g/l).

A steep, south facing colliery bank (the slope ranging from 40°-45° measured with a Silverman clinometer) was selected at the Tilmanstone site. The experimental area was marked out into 12 equal experimental units each measuring 13m X 25m = 325m^2. The total trial area to be sprayed = 12 X 325 = 3900m^2. 2m wide isolation areas, between each block, were not sprayed to prevent cross contamination between treatments. The treatments were borne in mains water which included a hydroseeding carrier. The hydroseeding carrier mix (per *English Landscapes* Industrial Standard©) was composed of: *Bentonite* clay to give 120g/m^2 coverage, wood pulp cellulose to give 100g/m^2 coverage, sphagnum peat moss to give 100g/m^2 coverage, *Seanure*

Seaweed to give 70 g/m^2 coverage and *Terrabind* soil stabilising fluid to give 50 g/m^2 coverage.

Table 8.2.1 illustrates the seed mix chosen for this experiment. The species chosen were mycorrhizal (Harley & Harley1987). Drought resistant varieties were selected to mitigate summer conditions experienced on the colliery waste banks. Seeds of *B. pendula* were collected from the site and included in the seed mix. *B. pendula* was included because it naturally colonised the colliery waste, is both ecto- and endo-mycorrhizal, and the seed was readily available on site.

Table 8.2.1 Seed mix for hydroseeding.

		% of mix
Lolium perenne	Perennial Rye grass (Candisa)	11
Lolium multiflorum	Westerwold Rye grass (Mowester)	20
Festuca rubra	Strong creeping fescue (Pernille).	35
Agrostis capillaris	Brown Bent (Highland).	5
Festuca trivialis	Hard fescue (Triana)	6
Festuca ovina	Sheeps fescue (Quatro)	6
Poa compressa	Flattened meadow grass (Cannon)	2
Holcus lanatus	Yorkshire fog	2
Betula pendula	Silver birch (collected from site)	1
	Legume mix (see Table 8.1.5)	8

The Hydroseeding experiment consisted of 3 replicates of 2 factorial treatments:

- +/- biotech granules at 200g/m^2,
- +/- mycorrhizal inoculants.

Three blocks had the mycorrhizal treatment added: two had their mycorrhizal inoculants added to the hydroseeding carrier and a third experimental unit had the mycorrhizal inoculants broadcast by hand (11/10/00) prior to hydroseeding. The trial was hydroseeded 12/10/00.

To ensure that the selected experimental areas were equitable, in respect to their initial vegetation cover, point quadrat scores of the experimental areas were taken 4/10/00 (pre-trial). The Hydroseeding was also monitored by the same point quadrat method on 12/12/00 & 08/05/01: three 1m^2 quadrats were randomly selected, in each treatment area. A pin of 0.5mm was randomly positioned 20 times within the 1m^2 quadrats. The

position of the pin was determined by dividing the $1m^2$ quadrats into a 100 squares using lines 10cm apart. A random number table was used to select the position of a grid square, within the quadrat, and then the pin was placed into the centre of that grid square. The vegetation in contact with each pin was scored so that each part of a plant touching the pin, of each plant species, was recorded. Analysis of variance was performed on the data using Minitab (release 13) statistical package.

Randomly selected individual plants of *Holcus lanatus, Agrostis capillaris and Trifolium pratense* were collected in December 2000 from each of the 12 experimental blocks and tested for mycorrhization. The plants were then transplanted into sterile pots and grown on in a glass house. Plants from the 12 experimental blocks were sampled 08/05/01 and tested for mycorrhization. The potted plants were again tested for mycorrhization 11/05/01.

The plants were tested for mycorrhization by the following procedure: several pieces of young, living white lateral roots were cut from each root ball and washed thoroughly in tap water. The roots were cleared in 2% (w/v) KOH for 1h at 90°C in a water bath. The roots were rinsed with water three times using a mesh and forceps. The roots were then covered with 2% (v/v) HCl for 45 minutes. The HCl was decanted and the roots covered with 0.05% (w/v) trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) and placed for 15minutes to 1hour at 90°C in a water bath, until the roots were strongly stained. The roots were then placed into a Petri dish with 50% (v/v) glycerol for destaining, so that only the fungal structures kept their blue colour (Brundrett, Melville & Peterson 1994). The roots were then assessed, for percentage root length colonised by AMF, using Phillips and Hayman's (1970) protocol. The percentage root length colonised was evaluated by mounting a sub-sample, of approximately 10cm of stained root, using the mountant PVLG (Polyvinyllactoglycerol) onto a slide. The presence or absence of typical mycorrhizal structures (arbuscules, vesicles and hyphae), in a gridline intercept system, were counted in each field of view at x100 magnification, using a compound microscope (Axioskop, Zeiss, Germany).

8.2.2 Results.

For full statistical analyses see Appendices 8.2.1, 8.2.2, 8.2.3 and 8.2.4. The analysis of variance of the point quadrat data for the vegetation cover pre-trial, in the different trial areas to be seeded, had a probability value P=0.979 (Appendix 8.2.1). This indicated that the vegetation at the start of the trial was not significantly different within the 12 experimental blocks i.e. the starting vegetation cover was equitable across the trial area. Plates 8.2.1 and 8.2.2 illustrate the good germination across the hydroseeded experiment although no *Betula pendula* seedlings were observed.

Plate 8.2.1 Root development in the vegetation on the hydroseeding was tested for mycorrhizal infection.

Plate 8.2.2 Grass species and legume species were evident in the hydroseeded vegetation.

Figure 8.2.1 illustrates the mycorrhizal status of plant species sampled from the experimental areas 08/05/01 and Figure 8.2.2 illustrates the mycorrhizal status of plants sampled 12/12/00 and grown under glass which were tested for mycorrhization 11/05/01. Figure 8.2.3 summarises the point quadrat score data for the different treatments from the experimental area. In all Figures error bars represent standard deviations.

The mycorrhizal status of the plants, sampled from the site, in general was low, less than 10% and only the mycorrhization of *A. capillaris* was significantly (P=0.049) affected by the mycorrhizal treatment broadcast by hand. There was a marked difference between the mycorrhizal status of plants in the different treatments (Figure 8.2.1). However, direct comparisons of mycorrhization between plant species is not reliable because this can be dependent on the age, health, stress etc. of the plant population sampled. Figure 8.2.2 illustrates the mycorrhizal status of plants on 11/5/01 which had been collected from the hydroseeded blocks 12/12/00 and then grown under glass. The mycorrhizal status of *T. pratense* and *A. capillaris* was significantly increased by the hydroseeded mycorrhizal treatment (P<0.001 and P=0.017 respectively), but *H. lanatus* was not (P=0.071). None of the plants tested from the hand broadcast treatment were mycorrhizal.

Germination and establishment of the sown vegetation on the hydroseeded trial was initially good; all treatments had a mean point quadrat score of 35 or above. However, there was little evidence, above ground, that the different treatments i.e. +/- mycorrhiza, broadcast by hand or included in the hydroseeding mix, had influenced the germination and establishment of the vegetation (Figure 8.1.3).

Vegetation cover (measured by point quadrat score) had increased across the hydroseeded bank (Figure 8.2.3) between October 2000 and May 2001. Figure 8.2.3 illustrates that the treatments had not significantly affected the vegetation when the data were collected 12/12/00 (P=0.974). Data collected in May 2001 also did not indicate the treatments had influenced the vegetation developing on the hydroseeded areas: P=0.517 for *Biotech Granules*, P=0.406 for mycorrhizal inoculants and P=0.926 for the interaction of the two treatments.

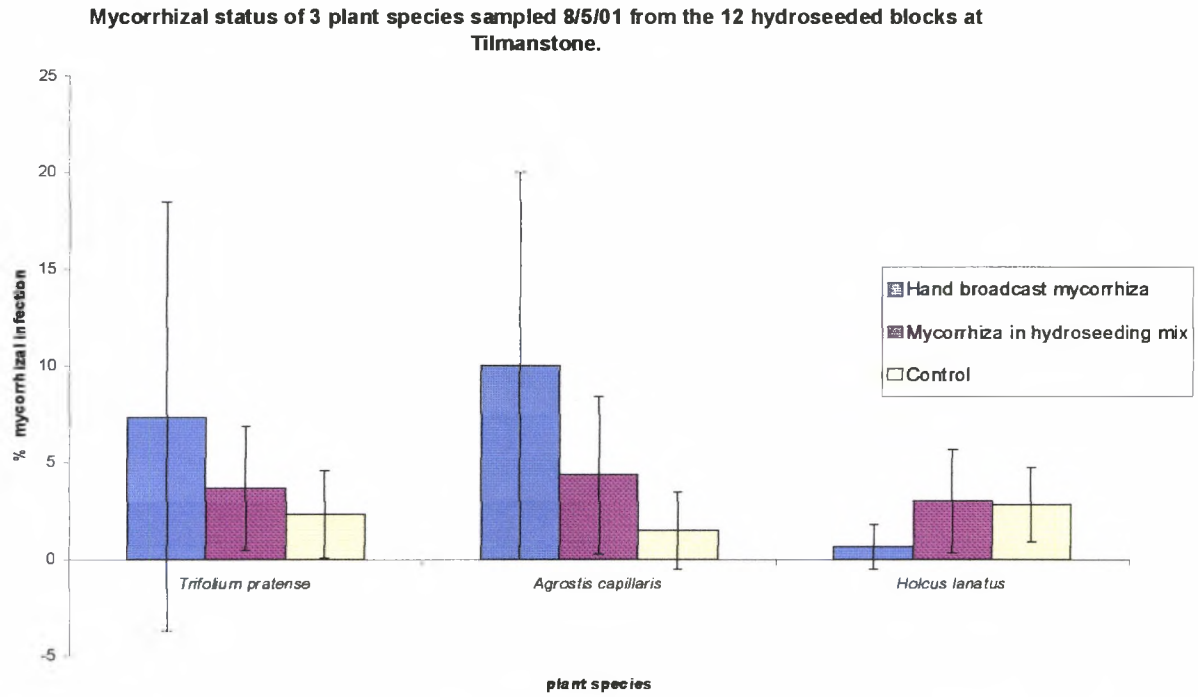

Figure 8.2.1 Mycorrhizal status of 3 plant species sampled 8/5/01 from the 12 hydroseeded blocks at Tilmanstone.

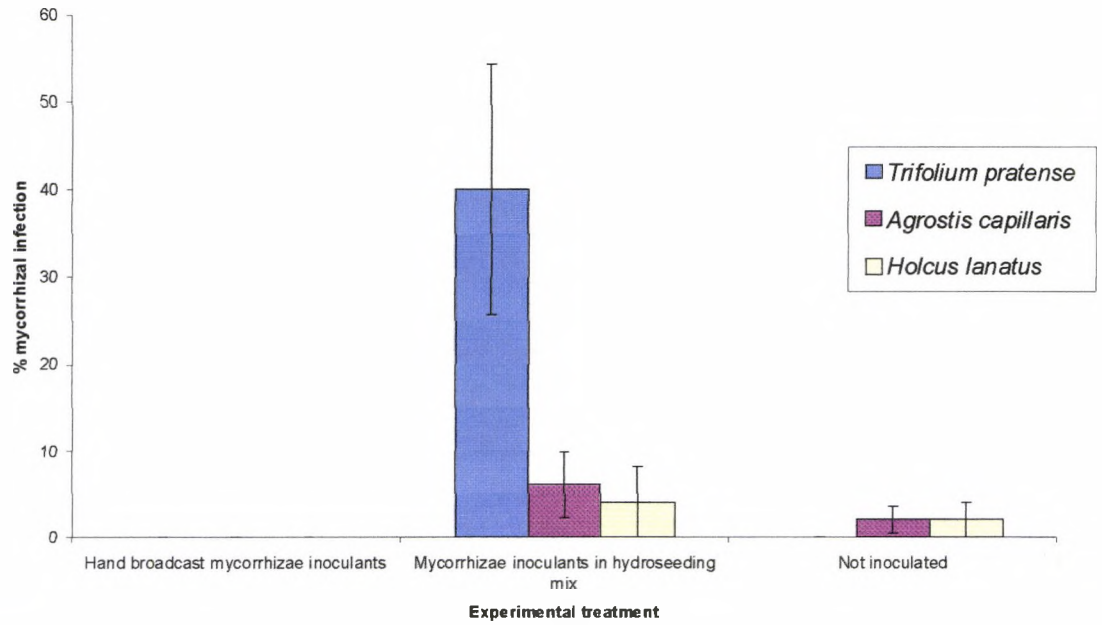

Figure 8.2.2 Mycorrhizal status of plants (on 11/5/01) collected from the hydroseeded blocks (12/12/00) at Tilmanstone, and grown under glass.

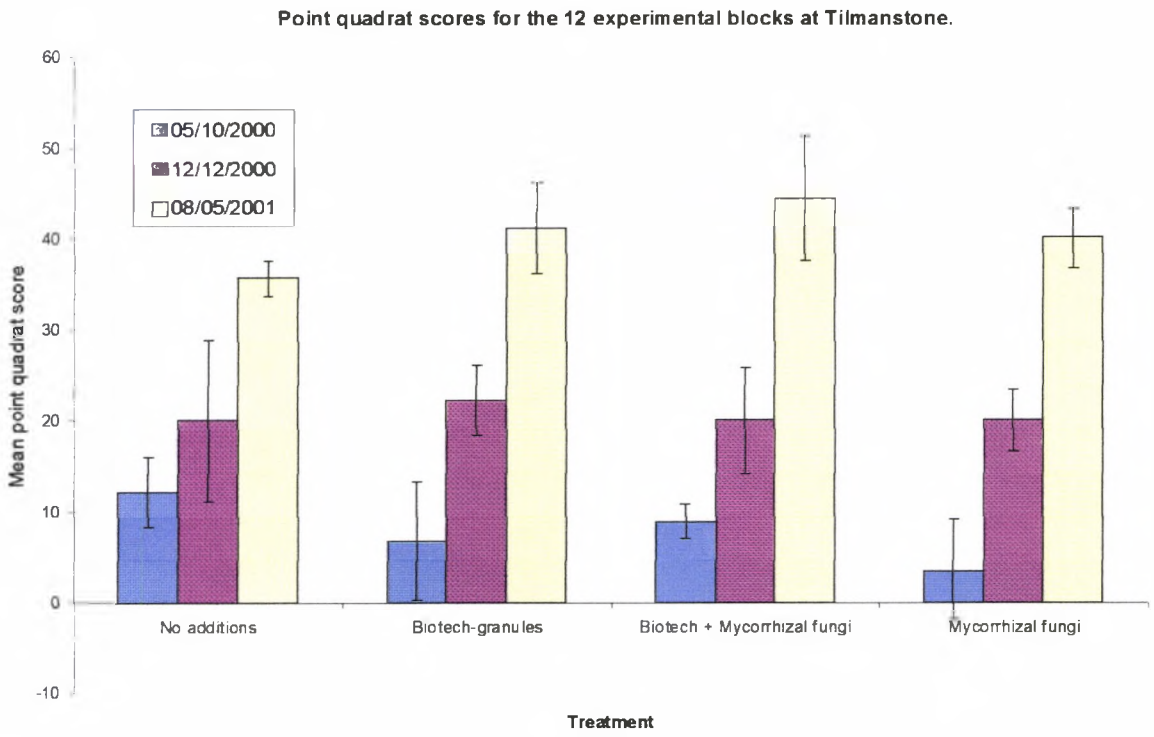

Figure 8.2.3 Point quadrat scores for the 12 experimental blocks at Tilmanstone.

8.2.3 Discussion

The increase in vegetation cover in the hydroseeded blocks (Figure 8.2.3) suggests the hydroseeding technique, *per-se*, was a successful tool for initiating vegetation establishment on the boundary banks at Tilmanstone. As a method for enabling steep colliery banks to be, seeded without the destruction of the naturally colonising vegetation, it was also successful.

The mycorrhizal status of the plants sampled 8/5/01 in general was very low (<10%). There was also a marked difference between the mycorrhizal status of plants in the different treatments (Figure 8.1.1). In general plants in the mycorrhizae inoculated plots had a higher percentage of infestation than those blocks with no mycorrhizae added. The exception was the mycorrhizal status of *H. lanatus* which had a lower infection rate to the control. The low infection rate might have been due to the timing of the grass's germination (West 1996). *H. lanatus* did not germinate until the spring, and therefore would not have provided a protective host in which mycorrhizal fungi could

over winter. In future hydroseedings, this should be taken into account and the vernalisation of the seeds broken by treatment prior to sowing.

In plants sampled direct from the field and tested for mycorrhization, the hand broadcast mycorrhizal treatment had the highest infection rates for both *T. pratense* and *A. capillaris*, but the lowest infection rate for *H. lanatus* (Figure 8.2.1). There was wide variation between the infection rates within the hand broadcast treatment in comparison to the hydroseeded application of the mycorrhizal treatment. The variation was probably due to the innately patchy nature of the coverage produced by applying the mycorrhiza by hand. The infection rate of the mycorrhiza included in the hydroseeding mix did not show such a wide variation, but the infection rate is generally lower than that broadcast by hand (Figures 8.2.1 and 8.2.2). The density of mycorrhizal in a system can control the infection rate of plants which is supported by these results (Abbott & Robson 1991).

The mycorrhization of plants grown under glass showed a marked difference between treatments. The hand broadcast treatment had no discernible infection and again this may be the result of the uneven distribution of the inoculants; the plants sampled simply did not come into contact with mycorrhizae. Areas without any additions showed a background level of mycorrhization which was expected in a natural plant community. The experimental blocks which were inoculated with mycorrhizal fungi carried in the hydroseeding mix had good infection. *T. pratense* had an average of 40% infection. *A. capillaris* and *H. lanatus* had much lower infection rates, but significantly higher than the control. The presence of mycorrhizal fungi in the spoil could in themselves help to promote the bank stabilisation, as reported by Habte *et al.* (1988).

The area was hydroseeded in October to negate the problems associated with the harsh summer conditions which were observed in the trial described in 8.1. The autumn sowing enabled germinated seedlings to over winter, so that the growing period in the spring was maximised. Ground cover was well established before the summer. The proof of the technique would have been the survival of the vegetation over the summer. Under the shade of existing Birch trees, the hydroseeded vegetation endured, but unfortunately by June 2001, after a prolonged dry period of 16 days

without rain, the vegetation failed over large areas of the banks and monitoring the vegetation was stopped.

The hydroseeding technique could be a successful tool for initiating vegetation establishment on the boundary banks at Tilmanstone (Figure 8.2.3). The problem with maintaining the ground cover through dry hot periods is, however, not resolved. The colliery waste banks are dark coloured and south facing. There have been surface temperatures of 42^{0}C recorded on the trial site. High temperatures will kill most UK indigenous plant species. The vegetation that survived under the Birch trees probably did so due to the shade and the water relationships that maintained lower temperatures improving ground cover survivorship. There was a loose soil structure developed by the root systems of the vegetation, before it failed. The ground cover, which survived under the trees, may supply an important seed reservoir which could promote the spread of vegetation on the banks (Zobel, van der Maarel & Dupré 1998). This vegetation will also promote improvement of the shale environment for plants. These observations led to the site owners, Hanson Brick Ltd. (UK), planting trees in 2002 to augment the canopy formed by the naturally colonising trees. The planting mimics natural regeneration at the site, and is expected to promote the sustainability of future ground cover establishment. Future strategies, for establishing vegetation on colliery waste banks, may require hydroseeding which is repeated a number of times. The hydroseeding could be repeated until the soil profile is developed and the vegetation becomes self sustaining.

The hydroseeding trial did not conclusively identify the advantage of including mycorrhizal fungi in a hydroseeding scheme, although there was an increase in the mycorrhizal status of plants which had been treated with mycorrhizal inoculates. The plants sampled from the field, and grown under glass, showed the potential success of the mycorrhizal treatment as they had high infection rates. The positive influence of mycorrhiza on the trial vegetation was not apparent in the first growing season. The positive influence on vegetation by mycorrhiza is sometimes not apparent until the second or third growing season; this is because there is often an initial carbon cost to the plants which are infected with mycorrhizal fungi (Dodd 2000; Gavito & Olsson 2003). The vegetation was scorched on most of the trial site. It was, therefore, impossible to assess the influence of the mycorrhizal treatment on the developing

vegetation in subsequent growing seasons in the field. From the mycorrhizal investigations, in natural vegetation systems developing on colliery waste (Chapter 6), the role of mycorrhizal fungi in the success of restoration strategies could be crucial. If mycorrhizal fungi are to be used in future hydroseedings, at the site, then this research suggests that the mycorrhyzae should be included in the hydroseeding mix to provide an even coverage. The hydroseeding should also be applied in early autumn, which would enable plants to become infected before winter frosts and would promote the survival of the mycorrhizal fungi (Sanders & Fitter 1992).

9 General discussion and conclusions.

Reclamation of derelict land is of increasing concern for industrial countries such as Great Britain. Technically produced and biologically inactive soils can be hostile to plants. Therefore, great effort is applied, in order to reintegrate them into the landscape and to remediate them for re-development (Schulz & Wiegleb 2000). Ordinarily, plant colonisation starts with pioneer plants (Prach, Pysek Smilauer 1999; Wiegleb & Felinks 2001), although the species and vegetation structure can differ widely, as seen on Kent colliery waste (Chapter 4.2).

Technical and engineering land reclamation can be important, for example, in creating gentle slopes at sites exposed to accelerated erosion, in the vicinity of settlements and at margins of colliery waste heaps (Schulz & Wiegleb 2000). However, it is not necessary to use this approach on all sites. In Germany, 85% of land disturbed by mining is technically reclaimed and 15% left to spontaneous succession (Schulz & Wiegleb 2000; Wiegleb & Felinks 2001). Reclamation or restoration of disturbed sites is often in the hands of technically oriented people who usually have little ecological background (Pietsch 1996). This social condition is changing as restoration ecologists become more involved in various restoration projects, and as economically driven considerations require ecological solutions to traditional restoration problems (Allen, Covington & Falk 1997). In this context the aims of this thesis were to examine some of the factors which limit natural vegetation establishment, examine the primary succession occurring on East Kent colliery waste, and relate these to potential restoration strategies. Particular emphasis has been placed on the role of nitrogen within developing vegetation systems (Chapter 5), the changing plant communities with respect to age of spoil tipping and associated mycorrhizal communities and their role in vegetation development on colliery waste. The findings from the natural vegetation investigations (Chapter 4), experimental manipulation (Chapters 6 & 7) and their testing in restoration trials (Chapter 8), illustrated the potential for incorporating ecological understanding in approaches to ecological restoration.

For a plant community to establish and survive in an area it must overcome environmental conditions which would prevent it from doing so. Environmental

conditions therefore control the spatial patterns of vegetation systems. The chemicals most likely to cause toxicity problems in the spoil and therefore preclude vegetation were analysed in Chapter 4. Samples were taken at two depths to attempt to give a baseline measurement at 1m deep of the spoil before biological modification. The top 10cm of the spoil was also analysed and shown to widely vary in composition from the 1m deep samples. The variation was mainly attributed to biological modification of the spoil although proximity to the surface and the increased exposure to rain and oxygen will also have affected the spoil. Analysis revealed no toxicity in the spoil, but at the Betteshanger site, pH was related to spoil age. This relationship confounded results with respect to vegetation development and age at the site, as pH can control vegetation structure on colliery waste. The pH was not as acidic as measured in other coalfields where pH has been shown to control vegetation patterns (Down 1973; Kimber, Purford & Duncan 1978; Chadwick 1987; Richards, Moorhead & Laing 1996); this pH pattern was not seen at the Stodmarsh site. Vegetation development could, therefore, be directly correlated to age of the spoil and the differences in vegetation patterns, observable on the colliery spoil, could be attributed to biological activity and modification.

A method of mimicking natural succession has ecological and economic advantages (Prach *et al.* 2001). Imitating natural succession and developing vegetation close-to-naturalness (allowing natural dynamics to take place without human interference) is a sustainable alternative to intensive engineering or technical restoration (Kirmer, & Mahn 2001; Prach *et al.* 2001). Understanding the vegetation dynamics and community composition of naturally occurring vegetation systems developing on degraded land, enables species selection for ecological restoration strategies which maximises restoration success and habitat protection (Baig 1992). Vegetation investigations and mycorrhizal investigations, Chapters 4 & 6 respectively, provided information which could be utilised for species selection with regards ecological restoration strategies for Kent colliery waste and beyond. The findings were investigated further in experimental and restoration field trials in Chapters 7 and 8. One of the significant findings from the research was the importance of mycorrhizal fungi in the successional stages of vegetation development on colliery waste.

The plant communities that were developing on Stodmarsh and Betteshanger were very different from each other. The Stodmarsh communities followed a typical facilitation successional model. However, the plant communities on Betteshanger did not follow the predicted development pattern suggested by successional models (Connell & Slatyer 1977). The vegetation patterns at Betteshanger may have been the result of pH gradients, but the vegetation at both sites were also controlled by the patterns of resource availability and in both cases the relationship between vegetation and nitrogen appeared to be crucial (Chapters 4 and 5).

At Stodmarsh, ecophysiology was observed to change with age as did the higher plant community structure which was mirrored by changes in the mycorrhizal community. Dispersal-assembly influences on community structure did seem to dominate early seral stages and in later seral stages of the successional development, niche-assembly influences played increasingly important roles in the community structure. This was observed at Betteshanger as well. *B. pendula*, which has wind dispersed seed, dominated the early seral stage communities. This observation was also supported by the results from the experiments reported in 7.3. These observations support the models proposed by Macarthur & Wilson's (1967) *theory* of *island biogeography* for community development on new substrata, and Hubbell's (2001) *unified neutral theory of biodiversity and biogeography* explanations for community structure controls in older communities. The communities which developed on fresh spoil differed widely from the later seral stage community which had been removed (7.3). Wind dispersed ruderals were present which were absent in the removed vegetation turfs. The new communities which developed in two years at both Stodmarsh and Betteshanger showed surprising similarities and suggested that the divergence of the communities at the two sites observed (Chapter 4.2), maybe related to stochastic events regarding species migration to the sites. At Betteshanger, *Q. ilex* dominates the oldest plant communities but is absent from Stodmarsh. Species composition of a seral community can determine the direction and speed of a succession (Kuiters & Slim 2003). The ability of species to migrate to an area can critically control community development (Soons & Heil 2002), which is supported by this study.

The leaf litter produced by the colonising birch forms the organic matter crucial for soil development in the colliery waste. The litter can have a positive effect on plant biodiversity by improving the physical and chemical characteristics of the colliery spoil. It could also have a negative effect by preventing germination of ruderal species which can require a certain light incidence to stimulate germination (Berends 1999).

The youngest plant communities, at Stodmarsh, were dominated by species of legumes and *H. pilosella* which, on analysis, turned out to be highly mycorrhizal. Legumes have a symbiotic relationship with Rhizobia which fix nitrogen which is available for exploitation by the legumes. Legumes tend to dominate communities where nitrogen is limiting (Jeffries, Bradshaw & Putwain 1981). This was supported by the subsequent analysis of nitrogen in different ages of spoil; nitrogen increased with age of the spoil (Chapter 5). The symbiotic relationship of the mycorrhiza and *H. pilosella* also indicates a competitive advantage for acquisition of nutrients again suggesting the spoil is nutrient deficient. The community development at Betteshanger could not be confidently related to age, due to the significance of the pH relationship with the age of the spoil confounding the vegetation associations. However, another symbiotic relationship was observed in the earliest plant community at Betteshanger, between *B. pendula* and the ectomycorrhiza *S. citrina*. Understanding the system that enables establishment of a tree species in a poor environment has implications for the increased success of restoration strategies (Aber 1990), but moreover the importance of symbiotic relationships within early seral stages is a crucial observation from this research. It points to a mechanism, outside traditional ecological understanding of successional dynamics, for the direction and rate of community development within succession.

In evaluating reclamation success many aspects must be considered. The success will depend on the aims and objectives of the restoration process, the character of the site and the ecological characteristics of involved species and sometimes a broader (e.g., geographical) context must be considered (Chambers & Wade 1990; Palik *et al.* 2000).

There are a number of approaches to reclamation or restoration of a degraded site:

(1) using technical reclamation with sowing or planting target species, accompanied by restoration or improvement of site conditions (Schulz & Wiegleb 2000),

(2) doing nothing allowing spontaneous succession (Schuster & Hutnik 1987; Skousen, Johnson, & Garbutt 1994),

(3) directing spontaneous (natural) succession with the aim to reach a target community (Luken 1990; Parker 1997; Prach *et al.* 2001),

(4) improvement of site conditions and then directing revegetation using ecological concepts to deliver an end point functioning vegetation (Bradshaw 1987).

The results from this research can inform any of the restoration approaches listed above and in 1,2 and 4, improve their likely success on colliery waste.

The experimental approaches used in Chapter 7, i.e. manipulating immature plant assemblages, could be argued not to mimic either natural or human-caused processes of species extinction, accumulation, or combination in communities (Huston *et al.* 2000). However, as an understanding of initial successional development and community sequences of ecological restoration were being sought, the approach of using immature communities was valid in trying to emulate both natural and anthropogenic influences on vegetation systems (Hector 1999; Loreau *et al.* 2001). As these small scale experiments were also then related to natural vegetation systems, occurring on colliery waste, this increased their validity (Waide *et al.* 1999).

Biodiversity possibly plays a vital role for ecosystem functioning in a changing environment such as successional systems and ecological restoration projects. Benefits of biodiversity arise if interspecific competition is less intense on average than intraspecific competition; this is generally the case. Yet theoretical approaches that incorporate diversity into classical ecosystem theory do not provide a general dynamic theory based on mechanistic principles (Norberg *et al.* 2001). In Chapter 7, the influence on the experimental community development by a functional group, legumes, had only a transient effect on the community development. The increase in species richness and productivity in the experimental community, due to the presence of the functional group, had dissipated by the second growing season. This was possibly due to their inability to respond to environmental changes brought about by the

developing community i.e. as the soil conditions improved, the net positive influence of the legumes was lessened to such an extent that none of the measured system parameters were eventually significantly influenced by their presence (Walker 1993). As a result, the long-term productivity for the experimental communities was as high in less diverse communities, as for high diversity communities regardless of their functional component. This result does not support the modelled predictions by Norberg *et al.* (2001). They predict long-term productivity for a group of species with high phenotypic variability may be higher than for the best single species; however, the results from the first growing season do support their model. Thus, importantly, the time a system has been developing is as crucial as its functional composition in respect to its productivity, and therefore has implications for successional vegetation; this was also supported by the natural vegetation community investigations reported in Chapter 4.2.

Ecosystem productivity is determined by individual species responses to environmental fluctuations; the availability of nitrogen seems crucial to the response of species at a community level and to productivity in plant communities developing on colliery waste (Chapters 4, 5, 7 & 8). The legume dominated communities of Stodmarsh were superseded by more productive communities in the older colliery spoil. This suggests that in dynamic successional systems functional traits which no longer provide an advantage are lost. The relationship between soil biological activity, including nitrogen mineralization rates, and vegetation structure related to successional age are intimately linked (Biondini, Bonham & Redente 1985).

BIODEPTH, a major international experiment on the response of plant productivity to variation in the number of plant species, has found a reduction of average aboveground biomass with loss of species. In the experiment a halving of diversity leads to a 10 to 20% reduction in above ground plant productivity (Loreau *et al.* 2001). However, in Chapter 7, the increase in productivity measured by point quadrat score was greatest where species richness was lowest in the second growing season (Figure 7.1.6). This was due in part to a few very successful species being able to exploit the higher levels of nitrogen available in the experiment. The availability of resources such as nitrogen is therefore important in influencing the diversity of the community and its productivity. These findings support Tilman's (1985) *resource-ratio hypothesis* which

suggests that differing resources become important at different stages of succession. The importance of the resource, to the community, is determined by the life strategies of the species composing the community.

This research found unimodal relationships between diversity and nitrogen availability, but a corresponding positive linear relationship between productivity and nitrogen availability. Although this relationship has been reported in other systems (Gough & Grace 1998; Davis, Grime & Thompson 2000), and Grime (1977a &1977b) reported systems in which low diversity is associated with high fertility (nitrogen) systems. These results do seem to contradict the generally accepted ecological principle of increased productivity with increased diversity (Tilman, Lehman, & Thomson 1997; Loreau *et al.* 2001; Norberg *et al.* 2001; Tilman *et al.* 2001; Naeem 2002). This finding also has implications for successional systems, in which it is generally accepted that diversity and productivity both increase with resource availability which is linked to the age of the developing system (Brenner, Werner & Pike 1984; Gray, Crawley & Edwards, 1987; Foster & Gross 1998).

The proposed mechanisms underlying the effects of diversity depend on the functional traits of individual species and groups. Huston *et al.* (2000), suggest that although the contributions of most individual species to the effects of biodiversity are small, those of a functional group are large. It was anticipated from the experiments with functional groups (Chapter 7) to find that community development would have complementary and positive effects (Jeffries, Bradshaw, & Putwain, 1981). It was also expected that nitrogen-fixers would play a large role in the species richness (Walker 1993); hence, legumes were a *priori* functional group in the design. Although the legumes did have an initial significant effect on both community composition and productivity these effects were short lived. This community response was undoubtedly the result of experimentally manipulated resource availability, but it is mirrored in field experiments Chapter 8 and the natural successional systems on colliery waste described in Chapter 4.2. Although these findings question that an ecosystem that contains many different plant species is more productive and successful than one containing only a few, the niche complementarity model in which certain combinations of species are able to use resources in the ecosystem more efficiently than if each species grew on its own

concentrations of nitrogen lead to investigating how it was able to survive where other less nutrient demanding plants could not. It was these investigations which lead to the discovering the crucial relationships between the vegetation developing on the colliery waste and their mycorrhizal symbionts reported in full in Chapter 6.

Plate 9.1 *Betula pendula* is often the primary coloniser of the Kent colliery waste.

Plate 9.2 The leaf litter produced by the tree accumulates over time and is the primary component of soil formation which enables grasses and herbs to establish.

The leaf litter produced by the colonising birch forms the organic matter crucial for soil development in the colliery waste. The litter can have a positive effect on plant biodiversity by improving the physical and chemical characteristics of the colliery spoil.

(Tilman 199b), may be crucial in understanding successional dynamics. The roles of symbionts within a successional system have been recognised within this research.

Coexistence is the term that is used to describe a balanced mixture of species in a biotic community. Communities should be the exception rather than the norm, in ecology, because the tendency towards competitive exclusion should favour a monoculture. Theories attempting to explain plant coexistence have focused on either interactions among species, such as competitive balance (Grime 1973b; Aarssen 1983) or temporal or spatial mechanisms such as resource partitioning to promote the avoidance of interaction among species (Ricklefs 1977; Bliss *et al.* 2002).

External interactions, such as the action of herbivores and pathogens from outside the plant populations themselves, may promote coexistence within plant communities (Gough & Grace 1998). External interactions promote non-interaction between plant populations and encourage competition avoidance; it is therefore a mechanism for maintaining multi-species assemblages in plant communities.

Arbuscular mycorrhizal fungi interact externally to plant populations and are thought to promote plant coexistence (Hetrick *et al.* 1994). In Chapter 6, the different AMF communities sampled from the different aged spoil but from the same plant species, suggest that some form of interaction between the plant community and AMF community is occurring related to the age of the spoil. As the chronosequence ages and succession proceeds on the colliery spoil, the distribution and abundance of AMF also changes (Chapter 6). Traditionally, AMF have been considered to be generalists with regard to the hosts that they infect. They have also been considered to be functionally equivalent in their effects on a host. AMF can successfully infect a wide range of plant species when grown experimentally in monocultures. However, when different plants and fungi are grown together, AMF growth and species composition is often found to be host specific (Douds & Millner 1999). Consequently, some AMF species are more beneficial to a host plant than are others, because of incompatibilities between an AMF and its host (van der Heijden, *et al.*1998a). The shared mycelial networks, AMF species richness and plant–fungal feedbacks, therefore, might be important determinants of community structure on colliery waste. If an otherwise less competitive plant species is infected by more AMF than is a highly

competitive plant species, then AMF should promote coexistence by increasing the ability of less competitive species to access nutrients (Moora & Zobel 1996). Alternatively, if a highly competitive plant species is also more infected by AMF, then AMF would simply reinforce competitive dominance by that species (West 1996). The different plant communities and AMF communities sampled from the chronosequence at Stodmarsh, suggest these competitive interactions are taking place within the vegetation system. The competitive advantages imparted by AMF to plant species, are a possible mechanism that could account for the changing plant communities within the colliery waste succession.

Hartnett & Wilson (1999) found that diversity (both richness and evenness) increased when mycorrhizal fungi were suppressed over several growing seasons in a tallgrass prairie. However, Grime *et al.* (1987) showed that mycorrhizal association increased plant species diversity (owing to increased evenness). In field experiments it has also been demonstrated that a reduction in mycorrhizal activity was correlated to a decrease in plant species richness (Gange, Brown & Farmer 1990). These reports suggest that AMF, have differential feedback effects on plants which possibly depend on the plant species mixtures (Bever 1999). In the colliery waste successional systems, this could be crucial for determining the plant community dynamics. Janos (1980) found in older more fertile soils of a succession mycorrhizal-dependent plants would be selected against, whereas infertile soils would support a highly mycorrhizal-dependent plant community. Although there is a relationship between increasing nitrogen and increased age of the colliery spoil, more species of AMF were isolated from the older spoil in the chronosequence (Figures 6.5.1-6.5.3). It is possible that these results reflect the nitrogen concentrations in the spoil which were significantly less than the nitrogen concentrations found in mature woodland soil (Chapter 5). Therefore, it could be that the colliery spoil fertility is below that which would initiate a selection pressure against mycorrhizal-dependent plants even in the older soil. It is not known though, whether changes in soil nutrient availability alter the ability of AMF to mediate plant coexistence (Hart, Reader & Klironomos 2003), although different AMF communities have been reported along artificially created nitrogen gradients (Egerton-Warburton & Allen 2000).

In the *Betula pendula* and *Lotus corniculatus* experiments at Betteshanger (Chapter 6), the influence of mycorrhizae on individual plant growth was also affected by interactions between the individual plants. Once established the mycorrhizal mycelia were a potential resource (Newman *et al.* 1992) and results showed that co-occurring plant species differed in their ability to compete for this resource despite showing individual responsiveness to mycorrhizal association when grown in separate plots. The extent of host-plant benefit from mycorrhizas is density dependent (Koide & Li 1991; Facelli *et al.* 1999) and may be influenced by neighbour competition (Hartnett *et al.* 1993); this was also supported by the experimental results from Betteshanger, in particular where there was lack of flower production in *L. corniculatus* in plots with sixteen *B. pendula* (Chapter 6, Figure 6.3.10). This has significant implications for the developing vegetation systems on the Kent colliery waste, and suggests mechanisms for the observed structure of the vegetation. In an Oak seedling establishment experiment on colliery waste, Lunt & Hedger (2003) reported that seedling growth response was dependent on the availability of nutrients and importantly the species of ectomycorrhizal fungi used as an inoculate. This differential growth observation may in part explain the difference in ecological success observed between *L. corniculatus* and *B. pendula.* The response of both plant species might be crucially mediated by the species of mycorrhizal fungi selected for inclusion in the experimental inoculate. The mycorrhizal inoculate contained a spore extract from *S. citrina,* an ectomycorrhizal fungi, which could not have formed an association with *L. corniculatus.* Therefore *B. pendula* had an extra mycorrhizal resource to that of *L. corniculatus*

The recurring theme of plant communities dominated by symbiotic partnerships associated with young systems and poorly developed nutrient cycling on the colliery waste is important. Understanding the successional processes which occur on new substrates can help to explain the future community development (Hodder 1978; Prach, Pysek, & Smilauer 1999; Jochimsen 2001). At Betteshanger many of the plant communities have poor ground cover and this is undoubtedly linked to the tree dominated communities and mycorrhizal associations. The symbiotic associations can also point to possible reclamations strategies: by emulating early communities and including symbiotic partnerships in their design, then possible alternatives to traditional reclamation schemes can be developed. An understanding of these symbiotic

relationships was tested by the use of mycorrhizal inoculants in restoration trials Chapter 8. Unfortunately other factors led to the failure of these trials, but using mycorrhizal fungi to establish trees at Betteshanger has proved successful in increasing survivorship and first year growth (Gilchrist and Dodd 2000), and mycorrhizal inoculation can benefit restoration efforts in semiarid regions (Richter & Stutz 2002).

Reclamation strategies tend to advocate planting schemes which specify seeds or plants that have a local (to the restoration) provenance for sound ecological reasons (D'Antonio & Meyerson 2002). However, as climate change continues to influence ecosystems it may be prudent to advocate plantings which utilise species which are better adapted to the changing climate. The East Kent colliery waste certainly exhibits extreme conditions and utilising species better adapted to these conditions, for example using species from a Mediterranean climate, might promote revegetation success. There is evidence from the natural plant communities at Betteshanger, where *Q. ilex* is a dominant in the oldest spoil (Chapter 4), that this strategy might indeed prove successful.

Vegetation is limited by the availability of resources. This research has identified nitrogen as one of the crucial plant resources which controls not only productivity but also the species richness in plant communities developing on East Kent colliery waste. Biological and abiological parameters all influence mineral nitrogen concentrations in soils (Reeder & Berg 1977; Voos & Sabey 1987). Mineral nitrogen concentrations therefore change within ecosystems. However, the observed trends of increasing mineralizable nitrogen across the Betteshanger and Stodmarsh chronosequences suggest that time is required for nitrogen cycles to become developed in vegetation systems.

The total organic nitrogen pools analysed within the colliery waste systems were found to have a significant relationship with the age of the colliery spoil and the developing plant communities (Chapters 4 & 5). These results supported Gray, Crawley & Edwards' (1987) findings that there is a relationship of increased organic nitrogen pools to increased stability in successional systems. This is important for understanding the

community dynamics and therefore the direction towards targeted species assemblages within restoration strategies.

Artificially augmenting available and organic nitrogen, or introducing systems which increase nitrogen mineralization, are possible ways of emulating natural older vegetation systems and therefore improving the likely success of restoration strategies.

9.1 Further work

Important insights into ecosystem functioning have been reported in this study. Aspects that would be worthy of further work include the roles other plant resources, apart from nitrogen, such as phosphate and water etc have on the vegetation patterns on the East Kent colliery waste.

Mycorrhizal fungi have been demonstrated to be important components of the communities on colliery waste, but further work on understanding how they influence the community structure and resource acquisition is needed. The use of mycorrhizal fungi in restoration strategies is also an area requiring further investigation to develop successful and sustainable reclamation strategies.

Soil micro- and macro-faunal activity influences ecosystem functioning and is undoubtedly linked to the nitrogen budget within a developing ecosystem (Postgate 1998). Soil faunal community structure is also thought to exhibit a similar community development pattern to the higher plants with respect to the number of species in a succession i.e. they both increase with the age of the succession (Hutson 1980, Boschker *et al.* 1998). Soil faunal community development and functionality should be considered in relation to ecological processes within a succession, but this is an area which was not evaluated in this thesis and needs further exploration.

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Appendices.

4.1.1 Preparation of 100mgl-1 stock calibration solutions.

The calibration solutions for use with the AAS were prepared by dissolving each metal in the primary solvent and diluting to 1l with deionised distilled water in a volumetric flask. The amount of metal, metal form and the primary solvent are stated in the table below adapted from Radojevic and Bashkin (1999). All salts were air dried at 110°C for 2hrs before being weighed. Calibration standards were then prepared from the stock solutions. Aliquots of the standard solutions were pipetted into volumetric flasks and 1ml of concentrated nitric acid added to each flask. The acid and aliquot mix was then diluted to the mark to make a standard range of known metal concentrations for use in calibrating the AAS.

Metal	*Reagent*	*Weight (g)*	*Primary solvent*
Cd	Cd metal	1.00	minimum volume of 1:1 HCl
Cr	$K_2Cr_2O_7$	2.828	200ml water + 1.5ml conc. HNO_3
Cu	Cu metal	1.00	15ml 1:1 HNO_3
Pb	$Pb(NO_3)_2$	1.598	200ml water + 1.5ml conc. HNO_3
Ni	NiO	1.273	minimum volume of 10% (v/v) HCl
Mo	MoO_3	1.5	10% HCl
Sn	Sn metal	1.00	100ml conc. HCL
Zn	Zn metal	1.00	20ml 1:1 HCl

4.1.2 Regression analyses and analyses of variance for chemical characteristics versus the age of the colliery spoil from Betteshanger and Stodmarsh.

Betteshanger spoil analysis results from 1m samples.

Regression Analysis: Cd versus Area

```
The regression equation is
Cd = 0.402 + 0.0209 Area
Predictor        Coef     SE Coef          T        P
Constant      0.40200     0.03763      10.68    0.000
Area         0.020857    0.009664       2.16    0.097

S = 0.04043     R-Sq = 53.8%     R-Sq(adj) = 42.3%

Analysis of Variance
Source            DF          SS          MS         F        P
Regression        29    0.007613    0.007613      4.66    0.097
Residual Error    23    0.006537    0.001634
Total             24    0.014150
```

Regression Analysis: Cr versus Area

```
The regression equation is
Cr = - 3.2 + 7.37 Area

Predictor        Coef     SE Coef          T        P
Constant        -3.20       10.25      -0.31    0.771
Area            7.371       2.632       2.80    0.049

S = 11.01       R-Sq = 66.2%     R-Sq(adj) = 57.8%

Analysis of Variance
Source            DF          SS          MS         F        P
Regression        29       950.9       950.9      7.84    0.049
Residual Error    23       485.1       121.3
Total             24      1436.0
```

Regression Analysis: Cu versus Area

```
The regression equation is
Cu = 75.9 - 6.06 Area

Predictor        Coef     SE Coef          T        P
Constant       75.867       7.667       9.90    0.001
Area           -6.057       1.969      -3.08    0.037

S = 8.235       R-Sq = 70.3%     R-Sq(adj) = 62.9%

Analysis of Variance
Source            DF          SS          MS         F        P
Regression        29       642.06      642.06      9.47    0.037
Residual Error    23       271.28       67.82
Total             24       913.33
```

Regression Analysis: Pb versus Area

```
The regression equation is
Pb = 27.0 + 2.71 Area

Predictor        Coef     SE Coef          T        P
Constant       27.000       3.623       7.45    0.002
Area           2.7143       0.9302      2.92    0.043
S = 3.891       R-Sq = 68.0%     R-Sq(adj) = 60.0%
```

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	128.93	128.93	8.51	0.043
Residual Error	23	60.57	15.14		
Total	24	189.50			

Regression Analysis: Ni versus Area

The regression equation is
Ni = 31.0 - 1.86 Area

Predictor	Coef	SE Coef	T	P
Constant	31.00	12.98	2.39	0.075
Area	-1.857	3.332	-0.56	0.607

S = 13.94 R-Sq = 7.2% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	60.4	60.4	0.31	0.607
Residual Error	23	777.1	194.3		
Total	24	837.5			

Regression Analysis: Zn versus Area

The regression equation is
Zn = 96.7 - 9.69 Area

Predictor	Coef	SE Coef	T	P
Constant	96.73	12.96	7.46	0.002
Area	-9.686	3.328	-2.91	0.044

S = 13.92 R-Sq = 67.9% R-Sq(adj) = 59.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	1641.7	1641.7	8.47	0.044
Residual Error	23	775.1	193.8		
Total	24	2416.8			

Regression Analysis: Hg versus Area

The regression equation is
Hg = 0.328 + 0.126 Area

Predictor	Coef	SE Coef	T	P
Constant	0.3280	0.1775	1.85	0.138
Area	0.12586	0.04557	2.76	0.051

S = 0.1906 R-Sq = 65.6% R-Sq(adj) = 57.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	0.27720	0.27720	7.63	0.051
Residual Error	23	0.14539	0.03635		
Total	24	0.42259			

Regression Analysis: As versus Area

The regression equation is
As = 8.00 + 2.43 Area

Predictor	Coef	SE Coef	T	P
Constant	8.000	4.273	1.87	0.135
Area	2.429	1.097	2.21	0.091

S = 4.590 R-Sq = 55.0% R-Sq(adj) = 43.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	103.21	103.21	4.90	0.091
Residual Error	4	84.29	21.07		
Total	5	187.50			

Regression Analysis: Mo versus Area

The regression equation is
Mo = 1.53 + 0.100 Area

Predictor	Coef	SE Coef	T	P
Constant	1.5333	0.1261	12.16	0.000
Area	0.10000	0.03237	3.09	0.037

S = 0.1354 R-Sq = 70.5% R-Sq(adj) = 63.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	0.17500	0.17500	9.55	0.037
Residual Error	4	0.07333	0.01833		
Total	5	0.24833			

Regression Analysis: Sn versus Area

The regression equation is
Sn = 1.65 + 0.237 Area

Predictor	Coef	SE Coef	T	P
Constant	1.6533	0.4122	4.01	0.016
Area	0.2371	0.1058	2.24	0.089

S = 0.4428 R-Sq = 55.7% R-Sq(adj) = 44.6%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	0.9841	0.9841	5.02	0.089
Residual Error	4	0.7842	0.1960		
Total	5	1.7683			

Regression Analysis: Fl versus Area

The regression equation is
Fl = 27.1 - 0.97 Area

Predictor	Coef	SE Coef	T	P
Constant	27.067	4.387	6.17	0.004
Area	-0.971	1.126	-0.86	0.437

S = 4.712 R-Sq = 15.7% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	16.51	16.51	0.74	0.437
Residual Error	4	88.82	22.20		
Total	5	105.33			

Regression Analysis: % soil moisture content versus area

The regression equation is
Betteshanger 1m = 4.79 + 0.0192 Area

Predictor	Coef	SE Coef	T	P
Constant	4.789	1.492	3.21	0.033
Area	0.01923	0.03270	0.59	0.588

S = 1.327 R-Sq = 8.0% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	0.609	0.609	0.35	0.588
Residual Error	4	7.039	1.760		
Total	5	7.648			

Regression Analysis: pH versus Area

The regression equation is
pH = 9.12 - 0.849 Area

Predictor	Coef	SE Coef	T	P
Constant	9.120	1.181	7.72	0.002
Area	-0.8486	0.3032	-2.80	0.049

S = 1.268 R-Sq = 66.2% R-Sq(adj) = 57.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	12.601	12.601	7.83	0.049
Residual Error	4	6.434	1.608		
Total	5	19.035			

Betteshanger spoil analysis results from 0.1m samples.

Regression Analysis: Cd versus Area

The regression equation is
Cd = 3.94 - 0.530 Area

Predictor	Coef	SE Coef	T	P
Constant	3.9433	0.7814	5.05	0.007
Area	-0.5300	0.2007	-2.64	0.057

S = 0.8394 R-Sq = 63.6% R-Sq(adj) = 54.4%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	4.9158	4.9158	6.98	0.057
Residual Error	23	2.8183	0.7046		
Total	24	7.7341			

Regression Analysis: Cr versus Area

The regression equation is
Cr = 6.66 + 0.183 Area

Predictor	Coef	SE Coef	T	P
Constant	6.660	1.347	4.94	0.008
Area	0.1829	0.3459	0.53	0.625

S = 1.447 R-Sq = 6.5% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	0.585	0.585	0.28	0.625
Residual Error	23	8.375	2.094		
Total	24	8.960			

Regression Analysis: Cu versus Area

The regression equation is
Cu = 82.2 - 4.49 Area

Predictor	Coef	SE Coef	T	P
Constant	82.200	7.351	11.18	0.000
Area	-4.486	1.887	-2.38	0.076

S = 7.896 R-Sq = 58.5% R-Sq(adj) = 48.2%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	352.13	352.13	5.65	0.076
Residual Error	23	249.37	62.34		
Total	24	601.50			

Regression Analysis: Pb versus Area

The regression equation is
Pb = 51.3 + 0.286 Area

Predictor	Coef	SE Coef	T	P
Constant	51.333	2.276	22.56	0.000
Area	0.2857	0.5844	0.49	0.650

S = 2.445 R-Sq = 5.6% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	1.429	1.429	0.24	0.650
Residual Error	23	23.905	5.976		
Total	24	25.333			

Regression Analysis: Ni versus Area

The regression equation is
Ni = 69.4 - 4.11 Area

Predictor	Coef	SE Coef	T	P
Constant	69.400	7.502	9.25	0.001
Area	-4.114	1.926	-2.14	0.100

S = 8.059 R-Sq = 53.3% R-Sq(adj) = 41.6%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	296.23	296.23	4.56	0.100
Residual Error	23	259.77	64.94		
Total	24	556.00			

Regression Analysis: Zn versus Area

The regression equation is
Zn = 47.9 - 1.49 Area

Predictor	Coef	SE Coef	T	P
Constant	47.867	7.131	6.71	0.003
Area	-1.486	1.831	-0.81	0.463

S = 7.660 R-Sq = 14.1% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	38.63	38.63	0.66	0.463
Residual Error	23	234.70	58.68		
Total	24	273.33			

Regression Analysis: Hg versus Area

The regression equation is
Hg = 0.444 + 0.0830 Area

Predictor	Coef	SE Coef	T	P
Constant	0.4437	0.1087	4.08	0.015
Area	0.08300	0.02792	2.97	0.041

S = 0.1168 R-Sq = 68.8% R-Sq(adj) = 61.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	0.12056	0.12056	8.84	0.041
Residual Error	23	0.05456	0.01364		
Total	24	0.17512			

Regression Analysis: As versus Area

The regression equation is
As = 22.0 + 1.71 Area

Predictor	Coef	SE Coef	T	P
Constant	22.000	2.815	7.82	0.001
Area	1.7143	0.7228	2.37	0.077

S = 3.024 R-Sq = 58.4% R-Sq(adj) = 48.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	51.429	51.429	5.63	0.077
Residual Error	23	36.571	9.143		
Total	24	88.000			

Regression Analysis: Mo versus Area

The regression equation is
Mo = 2.53 - 0.0857 Area

Predictor	Coef	SE Coef	T	P
Constant	2.5333	0.1771	14.30	0.000
Area	-0.08571	0.04548	-1.88	0.133

S = 0.1902 R-Sq = 47.0% R-Sq(adj) = 33.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	0.12857	0.12857	3.55	0.133
Residual Error	4	0.14476	0.03619		
Total	5	0.27333			

Regression Analysis: Sn versus Area

The regression equation is
Sn = 2.18 + 0.149 Area

Predictor	Coef	SE Coef	T	P
Constant	2.1800	0.2523	8.64	0.001
Area	0.14857	0.06478	2.29	0.084

S = 0.2710 R-Sq = 56.8% R-Sq(adj) = 46.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	0.38629	0.38629	5.26	0.084
Residual Error	23	0.29371	0.07343		
Total	24	0.68000			

Regression Analysis: Fl versus Area

The regression equation is
Fl = 19.7 - 0.114 Area

Predictor	Coef	SE Coef	T	P
Constant	19.733	1.052	18.76	0.000
Area	-0.1143	0.2700	-0.42	0.694

S = 1.130 R-Sq = 4.3% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	0.229	0.229	0.18	0.694
Residual Error	23	5.105	1.276		
Total	24	5.333			

Regression Analysis: % soil moisture content versus Area

The regression equation is
Betteshanger 0.1m = 5.16 + 0.163 Area

Predictor	Coef	SE Coef	T	P
Constant	5.1585	0.7515	6.86	0.002
Area	0.16287	0.01648	9.89	0.001

S = 0.6683 R-Sq = 96.1% R-Sq(adj) = 95.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	43.652	43.652	97.72	0.001
Residual Error	4	1.787	0.447		
Total	5	45.439			

Regression Analysis: pH versus Area

The regression equation is
pH = 8.92 - 0.720 Area

Predictor	Coef	SE Coef	T	P
Constant	8.9200	0.7102	12.56	0.000
Area	-0.7200	0.1824	-3.95	0.017

S = 0.7629 R-Sq = 79.6% R-Sq(adj) = 74.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	29	9.0720	9.0720	15.59	0.017
Residual Error	23	2.3280	0.5820		
Total	24	11.4000			

Stodmarsh spoil analysis results from 1m samples.

Regression Analysis: Cd versus Area

The regression equation is
Cd = 0.357 + 0.00500 Area

Predictor	Coef	SE Coef	T	P
Constant	0.35700	0.01745	20.46	0.000
Area	0.005000	0.005260	0.95	0.412

S = 0.01663 R-Sq = 23.1% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	0.0002500	0.0002500	0.90	0.412
Residual Error	18	0.0008300	0.0002767		
Total	19	0.0010800			

Regression Analysis: Cr versus Area

The regression equation is
Cr = 22.7 + 1.30 Area

Predictor	Coef	SE Coef	T	P
Constant	22.70	29.73	0.76	0.501
Area	1.300	8.963	0.15	0.894

S = 28.34 R-Sq = 0.7% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	16.9	16.9	0.02	0.894
Residual Error	18	2410.3	803.4		
Total	19	2427.2			

Regression Analysis: Cu versus Area

The regression equation is
Cu = 70.0 - 5.80 Area

Predictor	Coef	SE Coef	T	P
Constant	70.000	9.159	7.64	0.005
Area	-5.800	2.762	-2.10	0.127

S = 8.733 R-Sq = 59.5% R-Sq(adj) = 46.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	336.40	336.40	4.41	0.127
Residual Error	18	228.80	76.27		
Total	19	565.20			

Regression Analysis: Pb versus Area

The regression equation is
Pb = 43.5 - 2.70 Area

Predictor	Coef	SE Coef	T	P
Constant	43.500	8.655	5.03	0.015
Area	-2.700	2.610	-1.03	0.377

S = 8.252 R-Sq = 26.3% R-Sq(adj) = 1.7%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	72.90	72.90	1.07	0.377
Residual Error	18	204.30	68.10		
Total	19	277.20			

Regression Analysis: Ni versus Area

The regression equation is
Ni = 35.1 - 1.10 Area

Predictor	Coef	SE Coef	T	P
Constant	35.100	7.865	4.46	0.021
Area	-1.100	2.371	-0.46	0.674

S = 7.499 R-Sq = 6.7% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	12.10	12.10	0.22	0.674
Residual Error	18	168.70	56.23		
Total	19	180.80			

Regression Analysis: Zn versus Area

The regression equation is
Zn = 41.0 + 1.40 Area

Predictor	Coef	SE Coef	T	P
Constant	41.000	9.287	4.41	0.022
Area	1.400	2.800	0.50	0.651

S = 8.854 R-Sq = 7.7% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	19.60	19.60	0.25	0.651
Residual Error	18	235.20	78.40		
Total	19	254.80			

Regression Analysis: Hg versus Area

The regression equation is
Hg = 1.05 - 0.114 Area

Predictor	Coef	SE Coef	T	P
Constant	1.0540	0.2297	4.59	0.019
Area	-0.11400	0.06926	-1.65	0.198

S = 0.2190 R-Sq = 47.5% R-Sq(adj) = 29.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	0.12996	0.12996	2.71	0.198
Residual Error	18	0.14392	0.04797		
Total	19	0.27388			

Regression Analysis: As versus Area

The regression equation is
As = 4.32 + 0.60 Area

Predictor	Coef	SE Coef	T	P
Constant	4.320	4.077	1.06	0.367
Area	0.600	1.229	0.49	0.659

S = 3.887 R-Sq = 7.4% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	3.60	3.60	0.24	0.659
Residual Error	18	45.33	15.11		
Total	19	48.93			

Regression Analysis: Mo versus Area

The regression equation is
Mo = 1.52 + 0.0000 Area

Predictor	Coef	SE Coef	T	P
Constant	1.5200	0.1013	15.00	0.001
Area	0.00000	0.03055	0.00	1.000

S = 0.09661 R-Sq = 0.0% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	0.000000	0.000000	0.00	1.000
Residual Error	18	0.028000	0.009333		
Total	19	0.028000			

Regression Analysis: Sn versus Area

The regression equation is
Sn = 3.86 - 0.534 Area

Predictor	Coef	SE Coef	T	P
Constant	3.8580	0.6429	6.00	0.009
Area	-0.5340	0.1938	-2.75	0.070

S = 0.6130 R-Sq = 71.7% R-Sq(adj) = 62.2%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	2.8516	2.8516	7.59	0.070
Residual Error	18	1.1272	0.3757		
Total	19	3.9787			

Regression Analysis: Fl versus Area

The regression equation is
Fl = 25.9 + 3.70 Area

Predictor	Coef	SE Coef	T	P
Constant	25.90	13.36	1.94	0.148
Area	3.700	4.029	0.92	0.426

S = 12.74 R-Sq = 21.9% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	136.9	136.9	0.84	0.426
Residual Error	18	487.1	162.4		
Total	19	624.0			

Regression Analysis: %soil moisture content versus Area

The regression equation is
Stodmarsh 1m = 9.08 - 0.0194 Area

Predictor	Coef	SE Coef	T	P
Constant	9.077	2.324	3.91	0.030
Area	-0.01936	0.03385	-0.57	0.607

S = 1.138 R-Sq = 9.8% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	0.424	0.424	0.33	0.607
Residual Error	3	3.883	1.294		
Total	4	4.307			

Regression Analysis: pH versus Area

The regression equation is
pH = 6.60 + 0.260 Area

Predictor	Coef	SE Coef	T	P
Constant	6.6000	0.3489	18.92	0.000
Area	0.2600	0.1052	2.47	0.090

S = 0.3327 R-Sq = 67.1% R-Sq(adj) = 56.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	0.6760	0.6760	6.11	0.090
Residual Error	18	0.3320	0.1107		
Total	19	1.0080			

Stodmarsh spoil analysis results from 0.1m samples.

Regression Analysis: Cd versus Area

The regression equation is
Cd = 0.404 - 0.00400 Area

Predictor	Coef	SE Coef	T	P
Constant	0.40400	0.01083	37.30	0.000
Area	-0.004000	0.003266	-1.22	0.308

S = 0.01033 R-Sq = 33.3% R-Sq(adj) = 11.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	0.0001600	0.0001600	1.50	0.308
Residual Error	18	0.0003200	0.0001067		
Total	19	0.0004800			

Regression Analysis: Cr versus Area

The regression equation is
Cr = 3.4 + 4.26 Area

Predictor	Coef	SE Coef	T	P
Constant	3.38	23.05	0.15	0.893
Area	4.262	6.949	0.61	0.583

S = 21.97 R-Sq = 11.1% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	181.6	181.6	0.38	0.583
Residual Error	18	1448.6	482.9		
Total	19	1630.2			

Regression Analysis: Cu versus Area

The regression equation is
Cu = 37.4 - 0.400 Area

Predictor	Coef	SE Coef	T	P
Constant	37.400	1.083	34.53	0.000
Area	-0.4000	0.3266	-1.22	0.308

S = 1.033 R-Sq = 33.3% R-Sq(adj) = 11.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	1.600	1.600	1.50	0.308
Residual Error	18	3.200	1.067		
Total	19	4.800			

Regression Analysis: Pb versus Area

The regression equation is
Pb = 35.0 - 1.00 Area

Predictor	Coef	SE Coef	T	P
Constant	35.000	1.915	18.28	0.000
Area	-1.0000	0.5774	-1.73	0.182

S = 1.826 R-Sq = 50.0% R-Sq(adj) = 33.3%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	10.000	10.000	3.00	0.182
Residual Error	18	10.000	3.333		
Total	19	20.000			

Regression Analysis: Ni versus Area

The regression equation is
Ni = 10.7 - 0.420 Area

Predictor	Coef	SE Coef	T	P
Constant	10.6600	0.7357	14.49	0.001
Area	-0.4200	0.2218	-1.89	0.155

S = 0.7014 R-Sq = 54.4% R-Sq(adj) = 39.3%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	1.7640	1.7640	3.59	0.155
Residual Error	18	1.4760	0.4920		
Total	19	3.2400			

Regression Analysis: Zn versus Area

The regression equation is
Zn = 37.8 + 1.80 Area

Predictor	Coef	SE Coef	T	P
Constant	37.800	3.447	10.97	0.002
Area	1.800	1.039	1.73	0.182

S = 3.286 R-Sq = 50.0% R-Sq(adj) = 33.3%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	32.40	32.40	3.00	0.182
Residual Error	18	32.40	10.80		
Total	19	64.80			

Regression Analysis: Hg versus Area

The regression equation is
Hg = 0.300 - 0.0240 Area

Predictor	Coef	SE Coef	T	P
Constant	0.30000	0.04247	7.06	0.006
Area	-0.02400	0.01281	-1.87	0.158

S = 0.04050 R-Sq = 53.9% R-Sq(adj) = 38.6%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	0.005760	0.005760	3.51	0.158
Residual Error	18	0.004920	0.001640		
Total	19	0.010680			

Regression Analysis: As versus Area

The regression equation is
As = 4.12 + 0.0000 Area

Predictor	Coef	SE Coef	T	P
Constant	4.1200	0.1327	31.06	0.000
Area	0.00000	0.04000	0.00	1.000

S = 0.1265 R-Sq = 0.0% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	0.00000	0.00000	0.00	1.000
Residual Error	18	0.04800	0.01600		
Total	19	0.04800			

Regression Analysis: Mo versus Area

The regression equation is
Mo = 1.64 - 0.0400 Area

Predictor	Coef	SE Coef	T	P
Constant	1.6400	0.1083	15.14	0.001
Area	-0.04000	0.03266	-1.22	0.308

S = 0.1033 R-Sq = 33.3% R-Sq(adj) = 11.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	0.01600	0.01600	1.50	0.308
Residual Error	18	0.03200	0.01067		
Total	19	0.04800			

Regression Analysis: Sn versus Area

The regression equation is
Sn = 0.400 +0.000000 Area

Predictor	Coef	SE Coef	T	P
Constant	0.400000	0.000000	*	*
Area	0.00000000	0.00000000	*	*

S = 0 R-Sq = *% R-Sq(adj) = *%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	0.000000000	0.000000000	*	*
Residual Error	18	0.000000000	0.000000000		
Total	19	0.000000000			

Regression Analysis: Fl versus Area

The regression equation is
Fl = 45.6 - 3.00 Area

Predictor	Coef	SE Coef	T	P
Constant	45.600	4.737	9.63	0.002
Area	-3.000	1.428	-2.10	0.127

S = 4.517 R-Sq = 59.5% R-Sq(adj) = 46.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	90.00	90.00	4.41	0.127
Residual Error	18	61.20	20.40		
Total	19	151.20			

Regression Analysis: % soil moisture content versus Area

The regression equation is
Stodmarsh 0.1m = 1.87 + 0.190 Area

Predictor	Coef	SE Coef	T	P
Constant	1.872	4.182	0.45	0.685
Area	0.18973	0.06090	3.12	0.035

S = 2.047 R-Sq = 76.4% R-Sq(adj) = 68.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	40.676	40.676	9.70	0.035
Residual Error	18	12.574	4.191		
Total	19	53.250			

Regression Analysis: pH versus Area

The regression equation is
pH = 6.42 - 0.180 Area

Predictor	Coef	SE Coef	T	P
Constant	6.4200	0.3447	18.63	0.000
Area	-0.1800	0.1039	-1.73	0.182

S = 0.3286 R-Sq = 50.0% R-Sq(adj) = 33.3%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	24	0.3240	0.3240	3.00	0.182
Residual Error	18	0.3240	0.1080		
Total	19	0.6480			

4.2.1 Principal Component Analysis for species data from the Betteshanger site.

Eigen analysis: Correlation Matrix

Eigenvalue	4.3810	3.6894	2.8453	2.5618	2.4395	2.2559
Proportion	0.141	0.119	0.092	0.083	0.079	0.073
Cumulative	0.141	0.260	0.352	0.435	0.513	0.586
Eigenvalue	2.0748	1.9022	1.5608	1.2813	1.1932	0.9388
Proportion	0.067	0.061	0.050	0.041	0.038	0.030
Cumulative	0.653	0.715	0.765	0.806	0.845	0.875
Eigenvalue	0.7779	0.7358	0.6318	0.6028	0.4296	0.2552
Proportion	0.025	0.024	0.020	0.019	0.014	0.008
Cumulative	0.900	0.924	0.944	0.964	0.977	0.986
Eigenvalue	0.1895	0.1734	0.0692	0.0108	0.0000	0.0000
Proportion	0.006	0.006	0.002	0.000	0.000	0.000
Cumulative	0.992	0.997	1.000	1.000	1.000	1.000
Eigenvalue	0.0000	0.0000	-0.0000	-0.0000	-0.0000	-0.0000
Proportion	0.000	0.000	-0.000	-0.000	-0.000	-0.000
Cumulative	1.000	1.000	1.000	1.000	1.000	1.000
Eigenvalue	-0.0000					
Proportion	-0.000					
Cumulative	1.000					

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Achillea	-0.069	0.023	0.020	0.146	-0.083	0.170
Agrostis	-0.026	0.040	0.061	0.403	-0.232	-0.012
Aira pra	0.289	0.211	-0.008	0.267	0.235	0.038
Anthrisc	0.156	-0.328	0.273	0.067	-0.027	-0.011
Bettula	-0.172	0.046	0.003	0.205	-0.049	0.144
Blacksto	0.286	0.201	-0.012	-0.009	-0.430	-0.042
Centauri	0.247	-0.020	-0.274	-0.203	-0.345	0.061
Cerastiu	0.223	0.318	0.335	-0.118	0.097	0.034
Dactylis	-0.042	0.045	0.013	0.099	0.028	0.429
Daucus c	-0.061	0.019	0.018	0.077	-0.031	0.500
Echium v	0.133	-0.319	0.212	-0.066	0.012	0.022
Elymus	-0.081	0.020	-0.024	0.010	0.053	-0.162
Epilobiu	-0.061	-0.006	0.003	0.089	-0.045	0.213
Holcus l	0.232	-0.236	-0.087	-0.016	0.148	0.125
Hypericu	-0.021	0.038	-0.108	0.099	0.184	-0.202
Hieraceu	0.277	-0.056	-0.328	0.096	0.295	0.064
Hypochae	0.187	0.069	-0.214	-0.165	-0.456	0.007
Leontodo	-0.055	0.019	-0.044	0.032	0.101	-0.163
Lotus co	0.399	0.046	-0.248	-0.018	-0.010	0.058
Medicago	0.156	-0.328	0.273	0.067	-0.027	-0.011
Melilotu alt	0.144	0.049	-0.013	0.486	-0.118	-0.161
Melilotu alb.	0.162	-0.017	-0.199	0.041	0.223	0.085
Plantago	0.205	-0.390	0.185	-0.074	0.019	0.047
Quercus ro.	-0.041	0.016	0.013	0.032	-0.002	0.481
Quercus il.	-0.075	0.005	0.009	-0.142	0.002	-0.145
Rubus fr	-0.084	0.018	0.017	0.138	-0.093	0.151
Rumex a	0.171	-0.218	-0.080	-0.157	0.056	0.094
Rumex ob	0.223	0.318	0.335	-0.118	0.097	0.034
Trif.arv	0.223	0.318	0.335	-0.118	0.097	0.034
Trif. prat	0.153	-0.121	0.181	0.413	-0.213	-0.139
Trif. rep	0.153	0.055	-0.197	0.237	0.243	-0.060

4.2.2 Hierarchical Cluster Analysis of Variables from the Betteshanger

Correlation Coefficient Distance, Single Linkage

Amalgamation Steps

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of Obs in new cluster
1	30	100.00	0.000	28	29	28	2
2	29	100.00	0.000	8	28	8	3
3	28	100.00	0.000	4	20	4	2
4	27	95.04	0.099	11	23	11	2
5	26	91.27	0.175	21	30	21	2
6	25	90.71	0.186	6	17	6	2
7	24	90.52	0.190	6	7	6	3
8	23	89.82	0.204	10	24	10	2
9	22	83.82	0.324	11	27	11	3
10	21	83.80	0.324	15	18	15	2
11	20	83.51	0.330	16	19	16	2
12	19	80.94	0.381	4	11	4	5
13	18	80.34	0.393	16	31	16	3
14	17	80.21	0.396	6	16	6	6
15	16	79.00	0.420	3	6	3	7
16	15	78.04	0.439	3	22	3	8
17	14	76.67	0.467	3	14	3	9
18	13	75.15	0.497	9	10	9	3
19	12	74.57	0.509	2	21	2	3
20	11	74.02	0.520	3	4	3	14
21	10	73.49	0.530	2	3	2	17
22	9	72.82	0.544	2	8	2	20
23	8	72.77	0.545	13	26	13	2
24	7	71.38	0.572	5	13	5	3
25	6	68.93	0.621	2	5	2	23
26	5	67.42	0.652	1	2	1	24
27	4	66.45	0.671	12	15	12	3
28	3	65.00	0.700	1	9	1	27
29	2	62.93	0.741	1	12	1	30
30	1	47.14	1.057	1	25	1	31

Final Partition

Cluster 1

Achillea	Agrostis	Aira pra	Anthrisc	Bettula	Blacksto	centauri
Cerastiu	Echium v	Epilobiu	Holcus l	Hieraceu	Hypochae	Lotus co
Medicago	Melilotu	Melilotu	Plantago	Rubus fr	Rumex a	Rumex ob
Trifoliu	Trifoliu	Trifoliu				

Cluster 2

Dactylis	Daucus c	Quercus robur

Cluster 3

Elymus

Cluster 4

Hypericu	Leontodo

Cluster 5

Quercus illex

4.2.3 Principal Component Analysis for species data from the Stodmarsh site.

Eigenanalysis of the Correlation Matrix

Eigenvalue	3.3186	2.3986	2.2543	2.0275	1.8923	1.7655
Proportion	0.095	0.069	0.064	0.058	0.054	0.050
Cumulative	0.095	0.163	0.228	0.286	0.340	0.390
Eigenvalue	1.6887	1.5464	1.4483	1.3979	1.3213	1.2444
Proportion	0.048	0.044	0.041	0.040	0.038	0.036
Cumulative	0.438	0.483	0.524	0.564	0.602	0.637
Eigenvalue	1.1482	1.0873	1.0402	1.0291	0.9363	0.8333
Proportion	0.033	0.031	0.030	0.029	0.027	0.024
Cumulative	0.670	0.701	0.731	0.760	0.787	0.811
Eigenvalue	0.7483	0.6954	0.6668	0.6486	0.6265	0.5832
Proportion	0.021	0.020	0.019	0.019	0.018	0.017
Cumulative	0.832	0.852	0.871	0.890	0.908	0.924
Eigenvalue	0.4904	0.4084	0.3931	0.3498	0.2759	0.2401
Proportion	0.014	0.012	0.011	0.010	0.008	0.007
Cumulative	0.938	0.950	0.961	0.971	0.979	0.986
Eigenvalue	0.2170	0.1178	0.0938	0.0668	-0.0000	
Proportion	0.006	0.003	0.003	0.002	-0.000	
Cumulative	0.992	0.995	0.998	1.000	1.000	

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Achillea	-0.158	-0.040	-0.033	0.036	0.041	0.033
Agrostis	0.040	-0.251	0.133	0.118	-0.267	-0.203
Aira pra	-0.158	-0.004	0.049	0.043	-0.008	0.114
Anthrisc	0.242	0.041	-0.195	-0.301	-0.160	0.018
Arrenthe	0.140	-0.068	0.177	0.153	-0.116	0.165
Bellis p	-0.063	0.376	0.127	-0.124	0.133	0.180
Bettula	0.423	-0.130	0.082	0.146	0.028	0.142
Bromus r	-0.099	-0.110	0.082	0.103	-0.392	-0.258
Bromis m	0.036	0.139	0.050	-0.124	-0.157	-0.349
Cerastiu	0.072	0.425	0.153	-0.168	0.056	0.103
Crataegu	0.052	0.346	0.056	-0.174	-0.028	-0.156
Cynosuru	0.109	-0.278	0.067	-0.033	0.503	-0.180
Dactylis	0.152	-0.053	0.296	0.095	-0.231	0.330
Daucus c	0.242	-0.129	-0.293	-0.367	-0.046	-0.023
Galium a	0.142	-0.146	0.052	-0.023	0.411	-0.250
Holcus l	0.071	0.136	0.352	0.058	0.055	-0.035
Hieraceu	-0.421	-0.105	-0.177	0.006	-0.007	0.018
Hypochae	-0.082	0.017	0.053	0.028	0.079	0.102
Lamium a	0.127	0.021	-0.317	0.263	-0.012	0.042
Leontodo	-0.059	-0.051	-0.028	0.010	0.049	-0.041
Leucanth	0.121	0.313	-0.051	0.226	0.067	-0.140
Lotus co	-0.368	-0.047	-0.053	0.019	0.082	0.049
Medicago	-0.202	0.025	-0.124	-0.043	0.076	0.114
Plantago	0.078	-0.256	0.075	-0.043	-0.025	0.350
Plantago	0.135	-0.200	0.101	0.011	0.134	0.083
Quercus	0.138	-0.084	-0.274	-0.455	-0.148	0.067
Rosa can	0.028	0.110	0.032	0.123	0.010	-0.148
Rubus fr	0.077	-0.017	0.094	-0.047	0.042	-0.357
Rumex a	-0.091	-0.046	-0.045	0.004	-0.007	0.121
Rumex ob	-0.045	-0.100	-0.015	-0.002	0.205	-0.115
Stellari	0.184	0.117	-0.431	0.393	0.008	0.035
Trifoliu	-0.116	-0.033	-0.080	0.005	0.010	-0.012
Trifoliu	-0.154	0.040	-0.103	-0.044	0.078	0.105
Trifoliu	-0.078	-0.093	0.009	0.078	-0.299	-0.252
Urtica d	0.132	0.155	-0.289	0.294	0.027	0.004

4.2.4 Hierarchical Cluster Analysis of Variables from the Stodmarsh site.

Correlation Coefficient Distance, Single Linkage

Amalgamation Steps

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of Obs in new cluster
1	34	88.23	0.235	19	31	19	2
2	33	84.38	0.312	14	26	14	2
3	32	81.70	0.366	19	35	19	3
4	31	81.48	0.370	12	15	12	2
5	30	77.54	0.449	6	10	6	2
6	29	75.44	0.491	8	34	8	2
7	28	74.65	0.507	4	14	4	3
8	27	71.18	0.576	21	27	21	2
9	26	70.31	0.594	17	23	17	2
10	25	68.72	0.626	12	25	12	3
11	24	68.46	0.631	17	33	17	3
12	23	68.30	0.634	6	11	6	3
13	22	68.28	0.634	19	21	19	5
14	21	67.26	0.655	1	22	1	2
15	20	66.99	0.660	12	30	12	4
16	19	66.85	0.663	7	13	7	2
17	18	66.37	0.673	3	29	3	2
18	17	66.09	0.678	6	9	6	4
19	16	65.95	0.681	5	7	5	3
20	15	65.22	0.696	6	28	6	5
21	14	65.22	0.696	1	17	1	5
22	13	65.01	0.700	1	18	1	6
23	12	64.54	0.709	2	8	2	3
24	11	63.89	0.722	5	12	5	7
25	10	63.67	0.727	1	3	1	8
26	9	62.93	0.741	5	24	5	8
27	8	62.60	0.748	4	5	4	11
28	7	61.62	0.768	1	32	1	9
29	6	61.35	0.773	6	16	6	6
30	5	60.83	0.783	1	6	1	15
31	4	60.57	0.789	4	19	4	16
32	3	59.52	0.810	1	4	1	31
33	2	57.97	0.841	1	2	1	34
34	1	56.09	0.878	1	20	1	35

Final Partition

Cluster 1
Achillea Aira pra Hieraceu Hypochae Lotus co Medicago Rumex a
Trifoliu Trifoliu

Cluster 2
Agrostis Bromus r Trifoliu

Cluster 3
Anthrisc Arrenthe Bettula Cynosuru Dactylis Daucus c Galium a
Plantago Plantago Quercus Rumex ob

Cluster 4
Bellis p Bromis m Cerastiu Crataegu Holcus l Rubus fr

Cluster 5
Lamium a Leucanth Rosa can Stellari Urtica d

Cluster 6
Leontodo

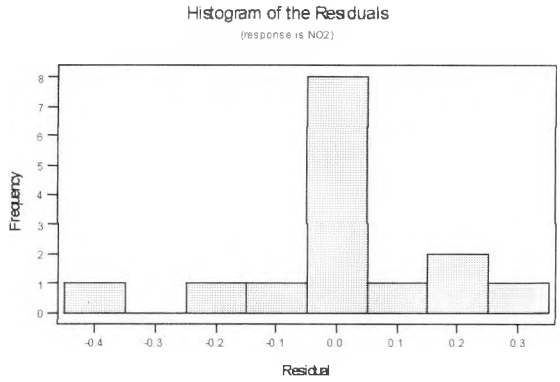
5.3.1 Kruskal-Wallis tests on the available nitrogen versus age of spoil data for spoil samples collected from Stodmarsh.

Kruskal-Wallis Test for available NO_2 extracted from 0.1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	0.00E+00	3.3	-2.02
2	3	1.26E-01	7.3	-0.29
3	3	1.54E-01	6.8	-0.51
4	3	4.45E-01	12.7	2.02
5	3	4.38E-01	9.8	0.79
Overall	15		8.0	

H = 7.31 DF = 4 P = 0.120
H = 7.44 DF = 4 P = 0.114 (adjusted for ties)

Poor normality plot for NO_2 extracted from 0.1m samples across the chronosequence at Stodmarsh data are not normally distributed.

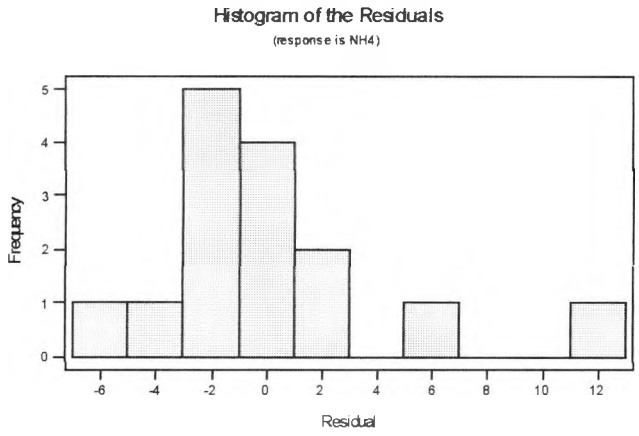


Kruskal-Wallis Test for available ammonia extracted from 0.1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	0.8064	2.0	-2.60
2	3	3.8871	5.0	-1.30
3	3	7.4588	8.7	0.29
4	3	12.4683	10.3	1.01
5	3	23.4398	14.0	2.60
Overall	15		8.0	

H = 13.03 DF = 4 P = 0.011

Poor normality plot for ammonia extracted from 0.1m samples across the chronosequence at Stodmarsh data.

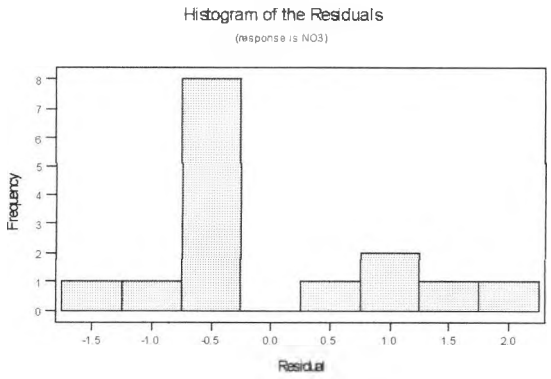


Kruskal-Wallis Test for available NO_3 extracted from 0.1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	0.2275	3.0	-2.17
2	3	0.6606	5.0	-1.30
3	3	1.5777	8.7	0.29
4	3	1.7917	10.0	0.87
5	3	3.5832	13.3	2.31
Overall	15		8.0	

H = 10.03 DF = 4 P = 0.040

Poor normality plot for NO_3 extracted from 0.1m samples across the chronosequence at Stodmarsh data are not normally distributed.

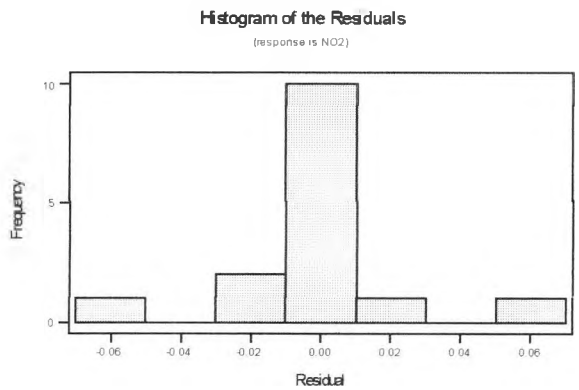


Kruskal-Wallis Test for available NO_2 extracted from 1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	0.00E+00	4.0	-1.73
2	3	0.00E+00	5.3	-1.15
3	3	2.91E-02	9.7	0.72
4	3	6.01E-02	12.7	2.02
5	3	1.71E-02	8.3	0.14
Overall	15		8.0	

H = 7.17 DF = 4 P = 0.127
H = 7.96 DF = 4 P = 0.093 (adjusted for ties)

Poor normality plot for NO_2 extracted from 1m samples across the chronosequence at Stodmarsh data are not normally distributed.

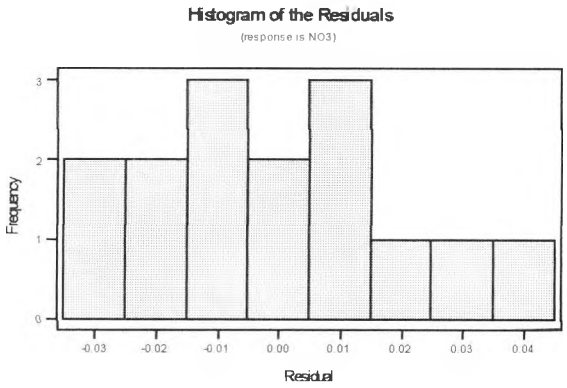


Kruskal-Wallis Test for available NO_3 extracted from 1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	0.09760	6.3	-0.72
2	3	0.13410	11.3	1.44
3	3	0.10110	5.3	-1.15
4	3	0.06316	4.0	-1.73
5	3	0.15050	13.0	2.17
Overall	15		8.0	

H = 9.30 DF = 4 P = 0.054

Poor normality plot for NO_3 extracted from 1m samples across the chronosequence at Stodmarsh data are not normally distributed.

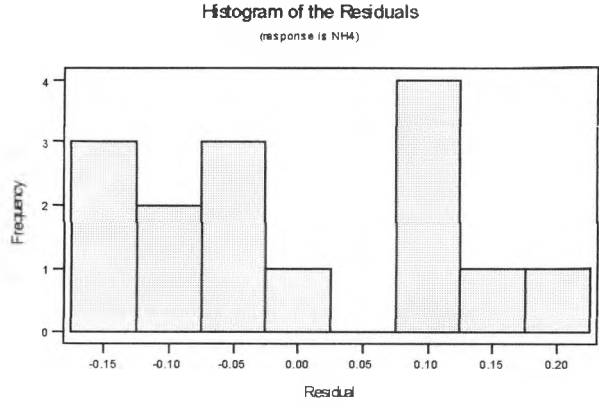


Kruskal-Wallis Test for available ammonia extracted from 1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	0.9139	7.7	-0.14
2	3	0.7157	6.0	-0.87
3	3	0.7357	7.0	-0.43
4	3	0.7253	5.7	-1.01
5	3	1.1320	13.7	2.45
Overall	15		8.0	

H = 6.40 DF = 4 P = 0.171

Poor normality plot for ammonia extracted from 1m samples across the chronosequence at Stodmarsh data are not normally distributed.



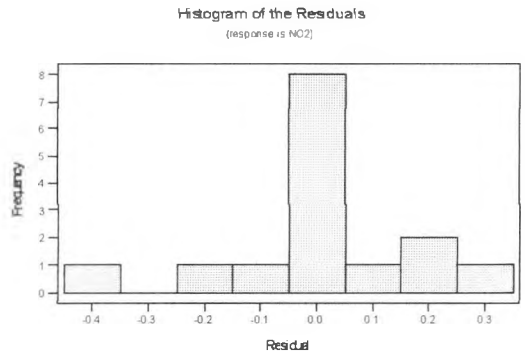
5.3.2 Kruskal-Wallis tests on the available nitrogen versus age of spoil data for spoil samples collected from Betteshanger.

Kruskal-Wallis Test for available NH_4 extracted from 0.1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	0.8034	3.2	-3.42
2	5	1.4977	7.8	-2.14
3	5	2.4120	14.0	-0.42
4	5	2.9886	17.0	0.42
5	5	4.2361	24.8	2.59
6	5	4.4829	26.2	2.98
Overall	30		15.5	

H = 26.84 DF = 5 P = 0.000

Poor normality plot for NH_4 extracted from 0.1m samples across the chronosequence at Betteshanger data are not normally distributed.

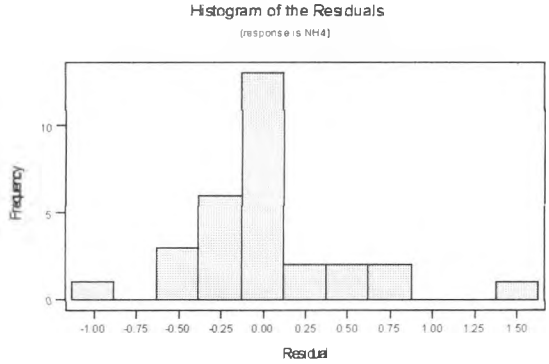


Kruskal-Wallis Test for available NO_3 extracted from 0.1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	0.2566	3.0	-3.48
2	5	0.6056	8.2	-2.03
3	5	1.1692	13.4	-0.58
4	5	1.7320	19.4	1.09
5	5	4.6169	23.8	2.31
6	5	6.0057	25.2	2.70
Overall	30		15.5	

H = 25.30 DF = 5 P = 0.000

Poor normality plot for NO_3 extracted from 0.1m samples across the chronosequence at Betteshanger data are not normally distributed.

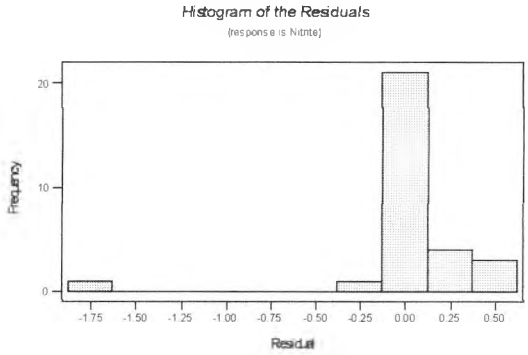


Kruskal-Wallis Test for available NO_2 extracted from 0.1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	0.05361	4.0	-3.20
2	5	0.22827	9.0	-1.81
3	5	0.51642	14.0	-0.42
4	5	0.90854	19.0	0.97
5	5	1.40805	24.0	2.37
6	5	2.05334	23.0	2.09
Overall	30		15.5	

H = 20.48 DF = 5 P = 0.001

Poor normality plot for NO_2 extracted from 0.1m samples across the chronosequence at Betteshanger data are not normally distributed.

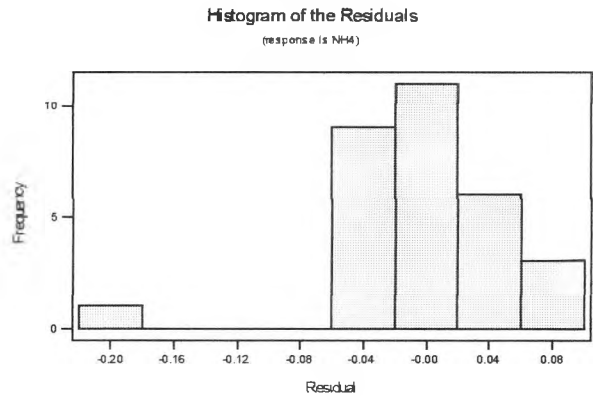


Kruskal-Wallis Test for *available* NH_4extracted from 1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	1.440	19.2	1.03
2	5	1.382	16.5	0.28
3	5	1.382	16.9	0.39
4	5	1.382	13.3	-0.61
5	5	1.382	13.1	-0.67
6	5	1.383	14.0	-0.42
Overall	30		15.5	

H = 1.90 DF = 5 P = 0.862
H = 1.93 DF = 5 P = 0.858 (adjusted for ties)

Poor normality plot for NH_4 extracted from 1m samples across the chronosequence at Betteshanger data are not normally distributed.

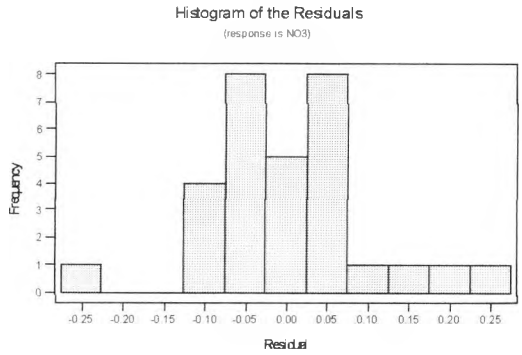


Kruskal-Wallis Test for available $N0_3$ extracted from 1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	-0.2174	3.0	-3.48
2	5	0.5961	27.8	3.42
3	5	0.1535	11.6	-1.09
4	5	0.2074	19.0	0.97
5	5	0.1704	17.0	0.42
6	5	0.1615	14.6	-0.25
Overall	30		15.5	

H = 21.81 DF = 5 P = 0.001

Poor normality plot for $N0_3$ extracted from 1m samples across the chronosequence at Betteshanger data are not normally distributed.

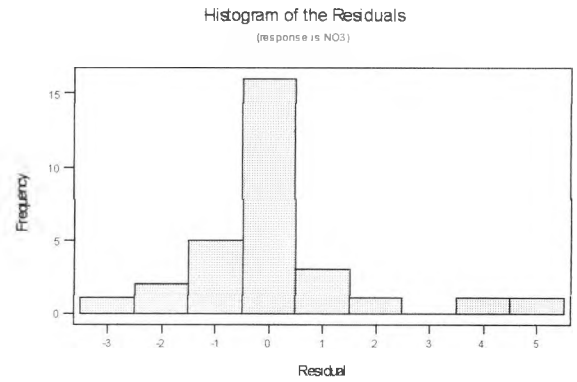


Kruskal-Wallis Test for available nitrite NO_2 extracted from 1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	0.40657	22.6	1.98
2	5	0.04189	15.2	-0.08
3	5	0.04084	10.0	-1.53
4	5	0.04177	14.8	-0.19
5	5	0.04796	16.8	0.36
6	5	0.04165	13.6	-0.53
Overall	30		15.5	

H = 5.58 DF = 5 P = 0.349

Poor normality plot for $N0_3$ extracted from 1m samples across the chronosequence at Betteshanger data are not normally distributed.



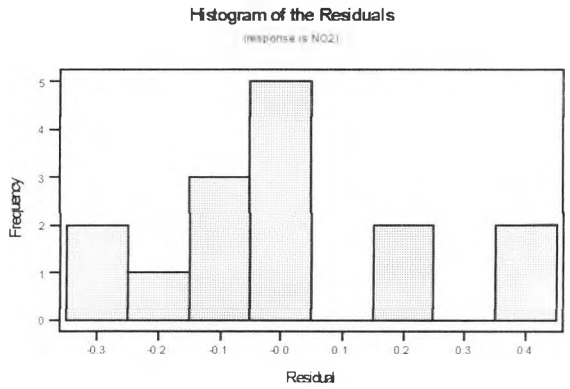
5.3.3 Kruskal-Wallis tests on the mineralizable nitrogen versus age of spoil data for spoil samples collected from Stodmarsh.

Kruskal-Wallis Test for mineralizable NO_2 extracted from 0.1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	0.00E+00	8.7	0.29
2	3	1.87E-02	13.3	2.31
3	3	-1.5E-01	6.8	-0.51
4	3	-3.3E-01	6.7	-0.58
5	3	-4.4E-01	4.5	-1.52
Overall	15		8.0	

H = 6.64 DF = 4 P = 0.156
H = 6.76 DF = 4 P = 0.149 (adjusted for ties)

Poor normality plot for mineralizable NO_2 extracted from 0.1m samples taken across the chronosequence at Stodmarsh data are not normally distributed.

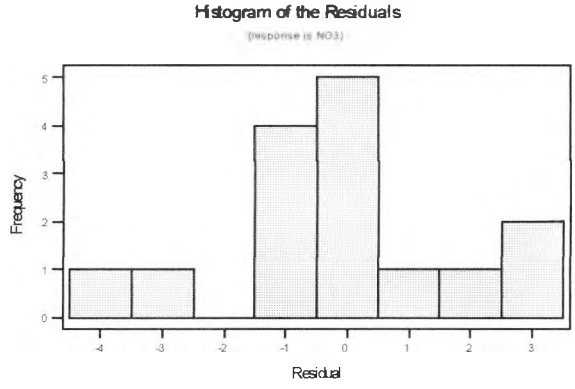


Kruskal-Wallis Test for mineralizable NO_3 extracted from 0.1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	0.03964	3.0	-2.17
2	3	3.00548	10.7	1.15
3	3	0.54678	6.0	-0.87
4	3	1.30778	7.0	-0.43
5	3	9.04887	13.3	2.31
Overall	15		8.0	

H = 9.83 DF = 4 P = 0.043

Poor normality plot for mineralizable NO_3 extracted from 0.1m samples taken across the chronosequence at Stodmarsh data are not normally distributed.

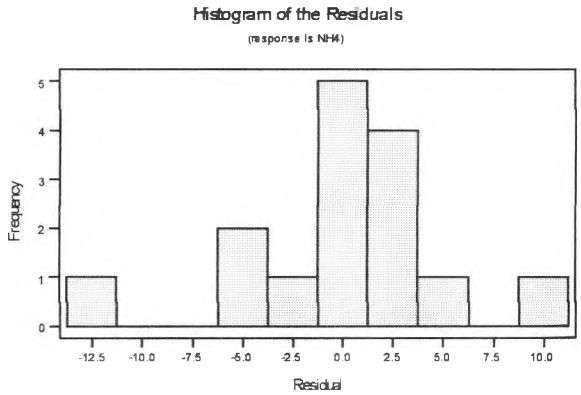


Kruskal-Wallis Test for mineralizable NH_4 extracted from 0.1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	-0.2720	3.0	-2.17
2	3	1.1022	4.0	-1.73
3	3	9.3558	8.0	0.00
4	3	15.9256	11.3	1.44
5	3	36.8174	13.7	2.45
Overall	15		8.0	

H = 12.63 DF = 4 P = 0.013

Poor normality plot for mineralizable NH_4 extracted from 0.1m samples taken across the chronosequence at Stodmarsh data are not normally distributed.

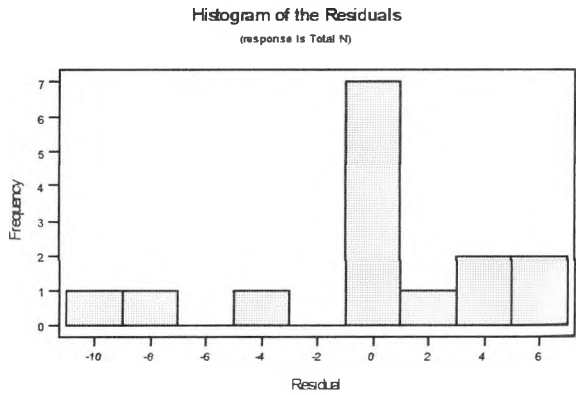


Kruskal-Wallis test for total mineralizable nitrogen extracted from 0.1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	-0.3198	2.3	-2.45
2	3	4.1079	6.0	-0.87
3	3	9.9026	7.0	-0.43
4	3	17.0264	10.7	1.15
5	3	44.9735	14.0	2.60
Overall	15		8.0	

H = 12.03 DF = 4 P = 0.017

Poor normality plot for total mineralizable nitrogen extracted from 0.1m samples taken across the chronosequence at Stodmarsh data are not normally distributed.

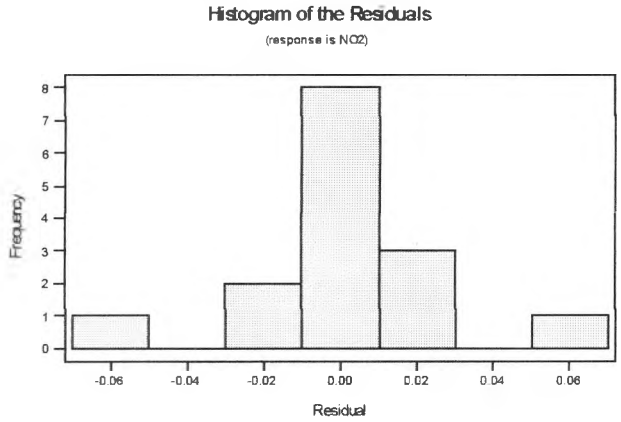


Kruskal-Wallis test for mineralizable NO_2 extracted from 1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	0.00E+00	11.5	1.52
2	3	0.00E+00	11.5	1.52
3	3	-2.9E-02	6.2	-0.79
4	3	-6.0E-02	3.3	-2.02
5	3	-1.7E-02	7.5	-0.22
Overall	15		8.0	

H = 7.48 DF = 4 P = 0.112
H = 7.98 DF = 4 P = 0.092 (adjusted for ties)

Poor normality plot for mineralizable NO_2 extracted from 1m samples taken across the chronosequence at Stodmarsh data are not normally distributed.

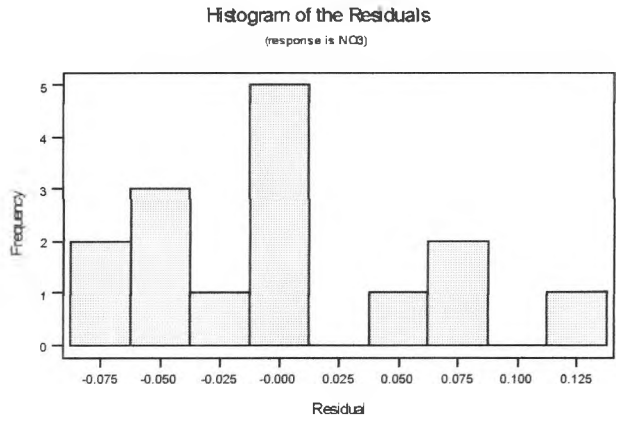


Kruskal-Wallis test for mineralizable NO_3 extracted from 1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	0.08530	4.7	-1.44
2	3	0.04110	4.3	-1.59
3	3	0.12476	6.0	-0.87
4	3	0.26634	11.0	1.30
5	3	1.11648	14.0	2.60
Overall	15		8.0	

H = 11.03 DF = 4 P = 0.026

Poor normality plot for mineralizable NO_3 extracted from 1m samples taken across the chronosequence at Stodmarsh data are not normally distributed.

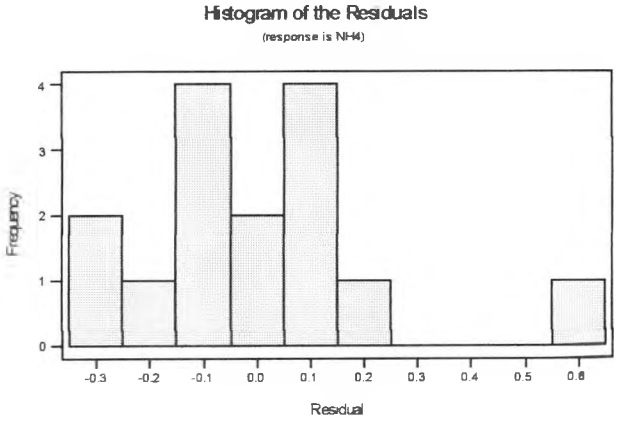


Kruskal-Wallis test for mineralizable NH_4 extracted from 1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	-0.3743	2.0	-2.60
2	3	0.3339	5.0	-1.30
3	3	0.9141	13.0	2.17
4	3	0.5879	11.3	1.44
5	3	0.5381	8.7	0.29
Overall	15		8.0	

H = 12.23 DF = 4 P = 0.016

Poor normality plot for mineralizable NH_4extracted from 1m samples taken across the chronosequence at Stodmarsh data are not normally distributed.

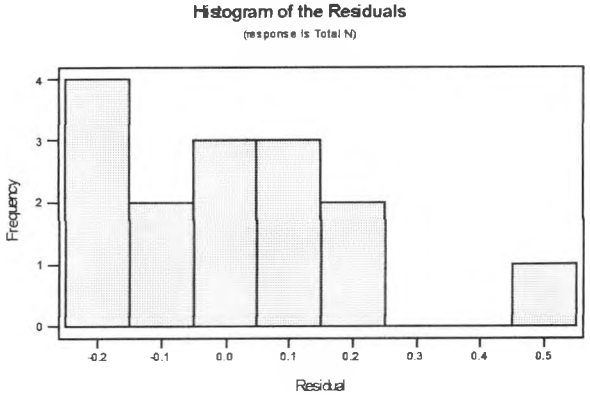


Kruskal-Wallis test for total mineralizable nitrogen extracted from 1m samples across the chronosequence at Stodmarsh.

Area	N	Median	Ave Rank	Z
1	3	-0.2129	2.0	-2.60
2	3	0.3536	5.0	-1.30
3	3	0.9889	9.3	0.58
4	3	0.8043	9.7	0.72
5	3	1.7223	14.0	2.60
Overall	15		8.0	

H = 12.83 DF = 4 P = 0.012

Poor normality plot for total mineralizable nitrogen extracted from 1m samples taken across the chronosequence at Stodmarsh data are not normally distributed.



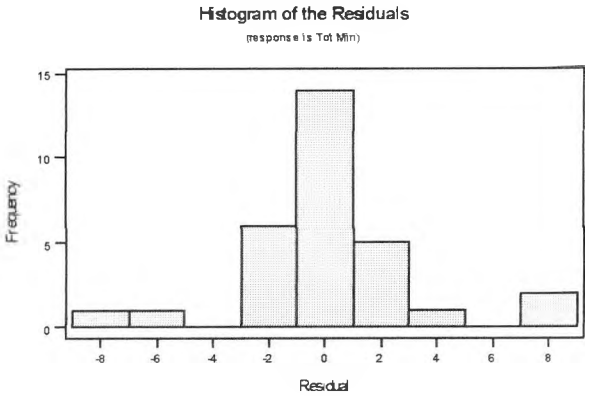
5.3.4 Kruskal-Wallis tests on the mineralizable nitrogen versus age of spoil data for spoil samples collected from Betteshanger.

Kruskal-Wallis Test for total mineralizable nitrogen extracted from 0.1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	-0.03968	5.4	-2.81
2	5	0.27462	9.0	-1.81
3	5	4.21560	21.2	1.59
4	5	1.77148	16.4	0.25
5	5	8.74613	20.4	1.36
6	5	4.68868	20.6	1.42
Overall	30		15.5	

H = 14.68 DF = 5 P = 0.012

Poor normality plot: data are not normally distributed.

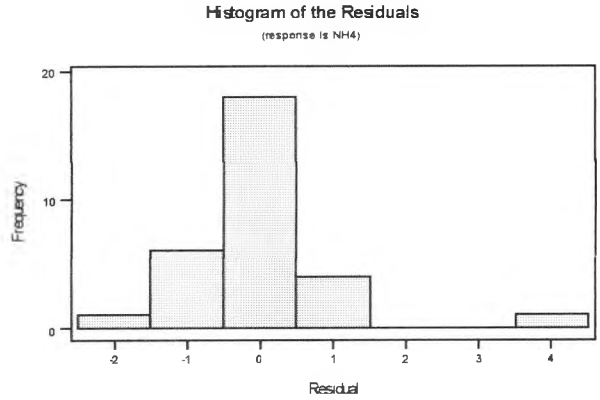


Kruskal-Wallis Test for total NH_4 extracted from 0.1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	-0.4699	3.8	-3.26
2	5	-0.1796	7.2	-2.31
3	5	1.0236	13.0	-0.70
4	5	2.3931	18.0	0.70
5	5	4.3997	24.0	2.37
6	5	7.9565	27.0	3.20
Overall	30		15.5	

H = 27.28 DF = 5 P = 0.000

Poor normality plot data are not normally distributed.

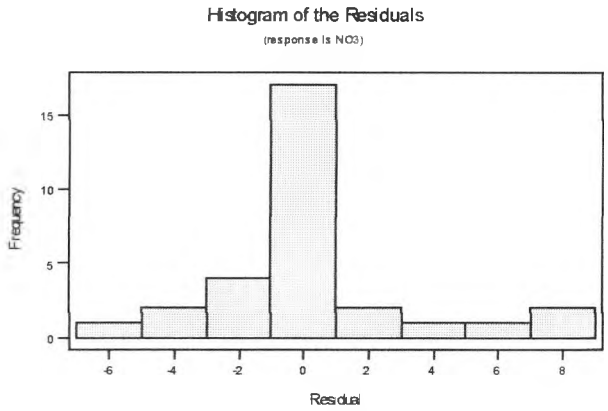


Kruskal-Wallis Test for total NO_3 extracted from 0.1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	0.5278	16.0	0.14
2	5	0.4614	16.2	0.19
3	5	4.0285	24.8	2.59
4	5	0.2504	14.0	-0.42
5	5	-0.4302	15.2	-0.08
6	5	-3.4552	6.8	-2.42
Overall	30		15.5	

H = 10.66 DF = 5 P = 0.058

Poor normality plot data are not normally distributed.

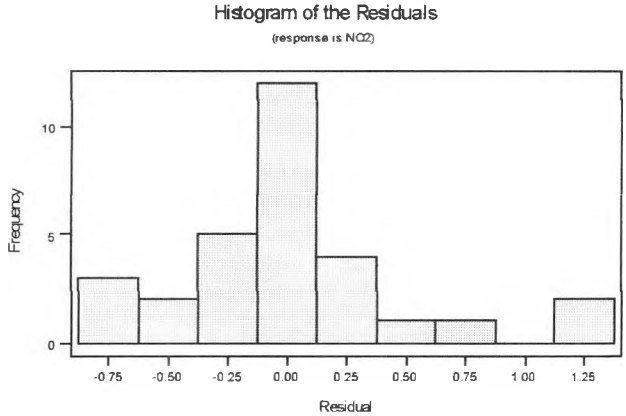


Kruskal-Wallis Test for total NO_2 extracted from 0.1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	-0.05152	26.0	2.92
2	5	-0.22827	20.2	1.31
3	5	-0.45969	14.8	-0.19
4	5	-0.66269	9.6	-1.64
5	5	-1.26389	10.0	-1.53
6	5	-2.05334	12.4	-0.86
Overall	30		15.5	

H = 13.39 DF = 5 P = 0.020

Poor normality plot data are not normally distributed.

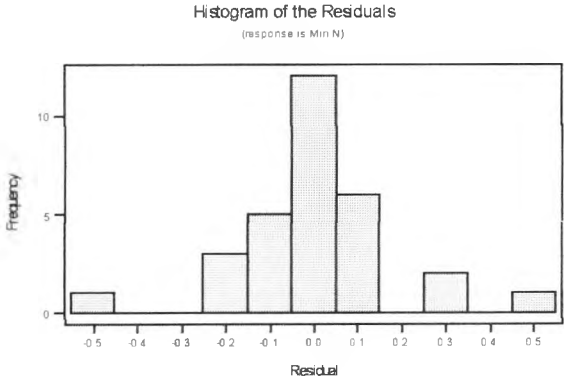


Kruskal-Wallis Test for total mineralizable nitrogen extracted from 1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	0.18774	17.0	0.42
2	5	-0.08877	6.4	-2.53
3	5	0.65740	27.2	3.26
4	5	0.07369	9.6	-1.64
5	5	0.05429	14.8	-0.19
6	5	0.19225	18.0	0.70
Overall	30		15.5	

H = 17.00 DF = 5 P = 0.004

Poor normality plot: data are not normally distributed.

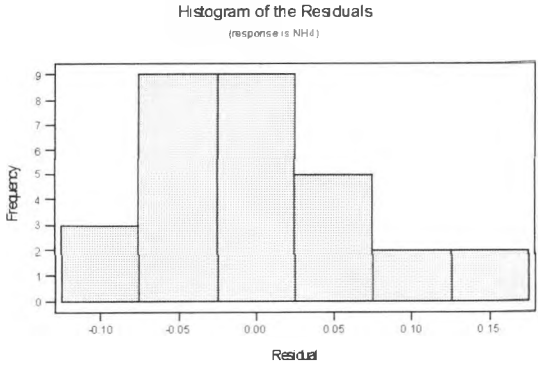


Kruskal-Wallis Test for NH_4 nitrogen extracted from 1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	0.06008	19.0	0.97
2	5	0.03380	11.2	-1.20
3	5	0.11749	19.6	1.14
4	5	-0.06490	8.0	-2.09
5	5	-0.04696	12.2	-0.92
6	5	0.11097	23.0	2.09
Overall	30		15.5	

H = 11.03 DF = 5 P = 0.051

Poor normality plot: data are not normally distributed.

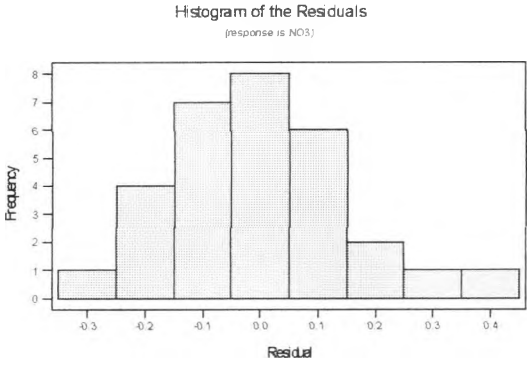


Kruskal-Wallis Test for nitrate (NO_3) extracted from 1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	0.47376	22.0	1.81
2	5	-0.07971	7.2	-2.31
3	5	0.62213	26.8	3.14
4	5	0.13655	10.6	-1.36
5	5	0.19158	16.6	0.31
6	5	0.13975	9.8	-1.59
Overall	30		15.5	

H = 19.13 DF = 5 P = 0.002

Poor normality plot: data are not normally distributed.

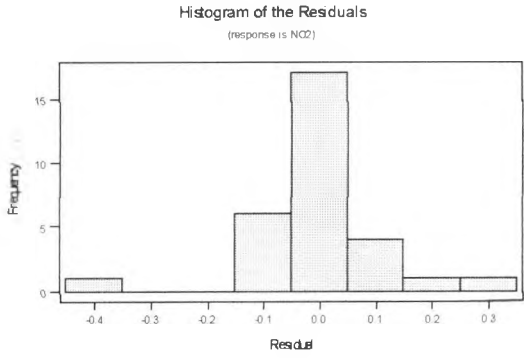


Kruskal-Wallis Test for total NO_2 extracted from 1m samples across the chronosequence at Betteshanger.

Area	N	Median	Ave Rank	Z
1	5	-0.40657	7.8	-2.14
2	5	-0.04189	15.0	-0.14
3	5	-0.04084	19.2	1.03
4	5	-0.04177	14.8	-0.19
5	5	-0.04796	13.4	-0.58
6	5	-0.01629	22.8	2.03
Overall	30		15.5	

H = 8.48 DF = 5 P = 0.132

Poor normality plot: data are not normally distributed.



5.4.1 Regression analyses for total organic nitrogen against sample age.

Regression Analysis: Ng/Kg versus Successional age (yrs) Stodmarsh 0.1m samples.

Key

Successi = successional age of the spoil.

The regression equation is
Ng/Kg = 5.76 + 0.241 Successional age (yrs)

Predictor	Coef	SE Coef	T	P
Constant	5.761	8.185	0.70	0.494
Successi	0.2407	0.1192	2.02	0.065

S = 6.941 R-Sq = 23.9% R-Sq(adj) = 18.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	196.34	196.34	4.08	0.065
Residual Error	13	626.24	48.17		
Total	14	822.58			

Unusual Observations

Obs	Successi	Ng/Kg	Fit	SE Fit	Residual	St Resid
8	69.0	2.50	22.37	1.81	-19.87	-2.96R

R denotes an observation with a large standardized residual

Regression Analysis: Ng/Kg versus Successional age (yrs) Stodmarsh 1m deep samples.

The regression equation is
Ng/Kg = - 0.61 + 0.131 Successional age (yrs)

Predictor	Coef	SE Coef	T	P
Constant	-0.612	1.315	-0.47	0.649
Successi	0.13053	0.01915	6.82	0.000

S = 1.115 R-Sq = 78.1% R-Sq(adj) = 76.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	57.760	57.760	46.48	0.000
Residual Error	13	16.153	1.243		
Total	14	73.913			

Regression Analysis: Ng/Kg versus Successional age (yr) Betteshanger 0.1m samples.

The regression equation is
N/Kg = - 0.69 + 0.299 Successional age (yr)

Predictor	Coef	SE Coef	T	P
Constant	-0.686	1.470	-0.47	0.644
Successi	0.29929	0.03224	9.28	0.000

S = 2.924 R-Sq = 75.5% R-Sq(adj) = 74.6%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	736.97	736.97	86.20	0.000
Residual Error	28	239.40	8.55		
Total	29	976.37			

Unusual Observations

Obs	Successi	N/Kg	Fit	SE Fit	Residual	St Resid
17	46.0	6.100	13.081	0.546	-6.981	-2.43R
23	55.0	22.800	15.774	0.669	7.026	2.47R

R denotes an observation with a large standardized residual

Regression Analysis: Ng/Kg versus Successional age (yr) Betteshanger 1m deep samples.

The regression equation is
N/Kg = 3.96 + 0.0770 Successional age (yr)

Predictor	Coef	SE Coef	T	P
Constant	3.9642	0.4895	8.10	0.000
Successi	0.07695	0.01073	7.17	0.000

S = 0.9735 R-Sq = 64.7% R-Sq(adj) = 63.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	48.719	48.719	51.41	0.000
Residual Error	28	26.535	0.948		
Total	29	75.254			

Unusual Observations

Obs	Successi	N/Kg	Fit	SE Fit	Residual	St Resid
23	55.0	10.790	8.197	0.223	2.593	2.74R
26	69.0	11.700	9.274	0.335	2.426	2.65R

R denotes an observation with a large standardized residual

6.3.1 General Linear Model analyses of variance for the Betteshanger mycorrhizal and *B. pendula* and *L. corniculatus* planting density experiments.

General Linear Model (with stem diameter on planting (2000) used as a covariate) analysis of variance for stem diameter of *B. pendula* measured 2001

Abbreviations: Myc = Mycorrhizal inoculate
P = Phosphorus @ 75g/m^2
N = Nitrogen @ 75g/m^2

Factor	Type	Levels	Values
Myc	fixed	2	0 1
N	fixed	2	0 1
P	fixed	2	0 1
Birch planting density	fixed	3	8 16 24
Lotus planting density	fixed	3	0 8 16

Analysis of Variance for Stem diameter, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Stem diameter	1	11.2982	11.1234	11.1234	1062.57	0.000
Myc	1	3.1448	3.1448	3.1448	300.41	0.000
N	1	1.1123	1.1116	1.1116	106.19	0.000
P	1	0.4883	0.4878	0.4878	46.60	0.000
Myc*N	1	0.0314	0.0314	0.0314	3.00	0.045
Myc*P	1	0.1087	0.1085	0.1085	10.36	0.002
N *P	1	0.0309	0.0312	0.0312	2.98	0.086
Myc *N *P	1	0.0094	0.0093	0.0093	0.89	0.346
Birch Planting density	2	1.7709	1.3524	0.6762	64.60	0.000
Lotus Planting density	2	0.0185	0.0185	0.0093	0.89	0.414
Error	477	1.9576	1.9576	0.0105		
Total	479	19.9710				

Expected Mean Squares, using Adjusted *SS*

Source	Expected Mean Square for Each Term
1 Stem diameter	(11) + Q[1]
2 Myc	(11) + Q[2, 5, 6, 8]
3 N	(11) + Q[3, 5, 7, 8]
4 P	(11) + Q[4, 6, 7, 8]
5 Myc *N	(11) + Q[5, 8]
6 Myc *P	(11) + Q[6, 8]
7 N *P	(11) + Q[7, 8]
8 Myc*N *P	(11) + Q[8]
9 Birch planting density	(11) + Q[9]
10 *Lotus* planting density	(11) + Q[10]
11 Error	(11)

Error Terms for Tests, using Adjusted *SS*

Source	Error DF	Error MS	Synthesis of Error MS
1 Stem diameter	467.00	0.0105	(11)
2 Myc	467.00	0.0105	(11)
3 N	467.00	0.0105	(11)
4 P	467.00	0.0105	(11)
5 Myc *N	467.00	0.0105	(11)
6 Myc *P	467.00	0.0105	(11)
7 N *P	467.00	0.0105	(11)
8 Myc*N *P	467.00	0.0105	(11)
9 Birch planting density	467.00	0.0105	(11)
10 *Lotus* planting density	467.00	0.0105	(11)

Variance Components, using Adjusted SS

Source	Estimated Value
Error	0.01047

Means for Covariates

Covariate	Mean	StDev
Stem diameter	0.3025	0.08301

Least Squares Means for Stem diameter

Myc	*N	*P	Mean	SE Mean
0			1.496	0.011815
1			1.746	0.011814
N				
0			1.546	0.011816
1			1.696	0.011814
P				
0			1.572	0.011815
1			1.670	0.011814
Myc	*N			
0	0		1.408	0.015630
0	1		1.583	0.015629
1	0		1.684	0.015630
1	1		1.808	0.015630
Myc	*P			
0	0		1.423	0.015632
0	1		1.568	0.015629
1	0		1.720	0.015629
1	1		1.772	0.015629
N	*P			
0	0		1.485	0.015629
0	1		1.608	0.015632
1	0		1.659	0.015631
1	1		1.732	0.015633
Myc	*N	*P		
0	0	0	1.316	0.021300
0	0	1	1.500	0.021300
0	1	0	1.529	0.021303
0	1	1	1.636	0.021300
1	0	0	1.653	0.021299
1	0	1	1.716	0.021303
1	1	0	1.788	0.021299
1	1	1	1.829	0.021304
Birch planting density				
8			1.701	0.014268
16			1.691	0.014267
24			1.471	0.019443
Lotus planting density				
0			1.607	0.009341
8			1.635	0.020887
16			1.621	0.020885

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Myc

Myc = 0 subtracted from:

Myc	Lower	Center	Upper	--+---------+---------+---------+----
1	0.2223	0.2508	0.2794	(----------------*----------------)
				--+---------+---------+---------+----
				0.224 0.240 0.256 0.272

Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Myc

Myc = 0 subtracted from:

Level Myc	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0.2508	0.01447	17.33	0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of N

N = 0 subtracted from:

N	Lower	Center	Upper	-----+---------+---------+---------+-
1	0.1206	0.1491	0.1777	(----------------*----------------)
				-----+---------+---------+---------+-
				0.128 0.144 0.160 0.176

Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of N

N = 0 subtracted from:

Level N	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0.1491	0.01447	10.30	0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter.
All Pairwise Comparisons among Levels of P

P = 0 subtracted from:

P	Lower	Center	Upper	----------+---------+---------+---------
1	0.07024	0.09878	0.1273	(----------*----------)
				----------+---------+---------+---------
				0.080 0.096 0.112

Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of P

P = 0 subtracted from:

Level P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0.09878	0.01447	6.826	0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Myc*N

Myc = 0
N = 0 subtracted from:

Myc	*N	Lower	Center	Upper	------+---------+---------+---------
0	1	0.1211	0.1742	0.2273	(----*---)
1	0	0.2228	0.2759	0.3290	(---*---)
1	1	0.3468	0.3999	0.4531	(---*----)
					------+---------+---------+---------
					0.12 0.24 0.36 0.48

Myc = 0
N = 1 subtracted from:

Myc	*N	Lower	Center	Upper	------+---------+---------+---------
1	0	0.04858	0.1017	0.1548	(---*----)
1	1	0.17262	0.2257	0.2788	(----*---)
					------+---------+---------+---------
					0.12 0.24 0.36 0.48

Myc = 1
N = 0 subtracted from:

Myc	*N	Lower	Center	Upper	------+---------+---------+---------
1	1	0.07094	0.1241	0.1772	(---*----)
					------+---------+---------+---------
					0.12 0.24 0.36 0.48

Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Myc*N

Myc = 0
N = 0 subtracted from:

Level Myc	*N	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0.1742	0.02046	8.513	0.0000
1	0	0.2759	0.02046	13.482	0.0000
1	1	0.3999	0.02047	19.540	0.0000

Myc = 0
N = 1 subtracted from:

Level Myc	*N	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0.1017	0.02046	4.969	0.0000
1	1	0.2257	0.02047	11.030	0.0000

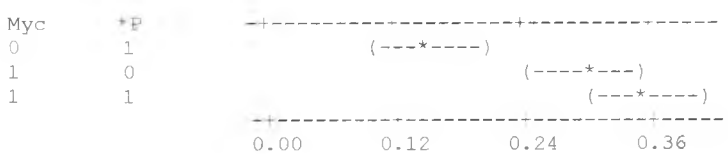
Myc = 1
N = 0 subtracted from:

Level Myc	*N	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	0.1241	0.02047	6.061	0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Myc *P

Myc = 0
P = 0 subtracted from:

Myc	*P	Lower	Center	Upper
0	1	0.09225	0.1454	0.1985
1	0	0.24428	0.2974	0.3505
1	1	0.29648	0.3496	0.4027

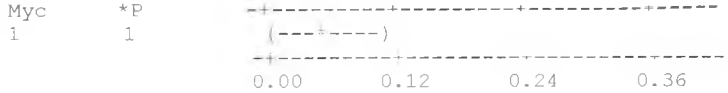


Myc = 0
P = 1 subtracted from:

Myc	*P	Lower	Center	Upper
1	0	0.09893	0.1520	0.2051
1	1	0.15113	0.2042	0.2573

Myc = 1
P = 0 subtracted from:

Myc	*P	Lower	Center	Upper
1	1	-0.000903	0.05220	0.1053



Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Myc*P

Myc = 0
P = 0 subtracted from:

Level Myc	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0.1454	0.02047	7.103	0.0000
1	0	0.2974	0.02047	14.531	0.0000
1	1	0.3496	0.02047	17.082	0.0000

Myc = 0
P = 1 subtracted from:

Level Myc	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0.1520	0.02046	7.429	0.0000
1	1	0.2042	0.02046	9.980	0.0000

Myc = 1
P = 0 subtracted from:

Level Myc	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	0.05220	0.02046	2.551	0.0556

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of N @ 75g/*P @ 75g/

N = 0
P = 0 subtracted from:

N	*P	Lower	Center	Upper
0	1	0.07065	0.1238	0.1769
1	0	0.12100	0.1741	0.2272
1	1	0.19480	0.2479	0.3010

```
N   *P  -+---------+---------+---------+-----
0   1         (----*-----)
1   0              (----*-----)
1   1                   (-----*----)
        -+---------+---------+---------+-----
        0.00      0.10      0.20      0.30
```

N = 0
P = 1 subtracted from:

N	*P	Lower	Center	Upper
1	0	-0.002755	0.05035	0.1035
1	1	0.071024	0.12416	0.1773

```
N   *P  -+---------+---------+---------+-----
1   0    (----*----)
1   1          (----*-----)
        -+---------+---------+---------+-----
        0.00      0.10      0.20      0.30
```

N = 1
P = 0 subtracted from:

N	*P	Lower	Center	Upper
1	1	0.02068	0.07381	0.1269

```
N   *P  -+---------+---------+---------+-----
1   1     (----*-----)
        -+---------+---------+---------+-----
        0.00      0.10      0.20      0.30
```

Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of N *P

N = 0
P = 0 subtracted from:

Level N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0.1238	0.02046	6.047	0.0000
1	0	0.1741	0.02046	8.508	0.0000
1	1	0.2479	0.02047	12.113	0.0000

N = 0
P = 1 subtracted from:

Level N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0.05035	0.02046	2.460	0.0696
1	1	0.12416	0.02047	6.064	0.0000

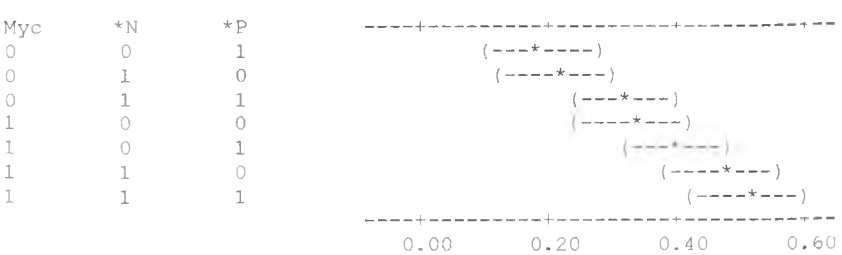
N = 1
P = 0 subtracted from:

Level N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	0.07381	0.02047	3.605	0.0023

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Myc *N *P

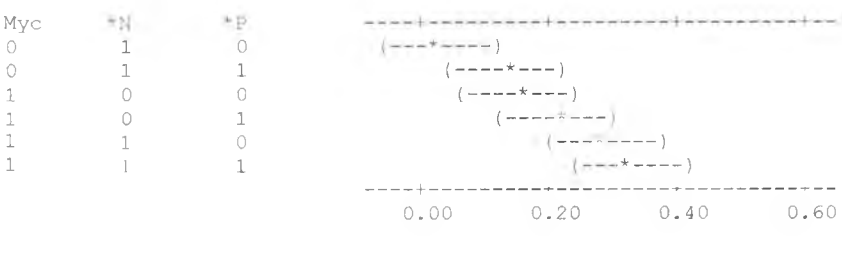
Myc = 0
N = 0
P = 0 subtracted from:

Myc	*N	*P	Lower	Center	Upper
0	0	1	0.09519	0.1840	0.2728
0	1	0	0.12403	0.2128	0.3017
0	1	1	0.23075	0.3196	0.4084
1	0	0	0.24732	0.3361	0.4249
1	0	1	0.31083	0.3996	0.4885
1	1	0	0.38269	0.4715	0.5603
1	1	1	0.42355	0.5124	0.6012



Myc = 0
N = 0
P = 1 subtracted from:

Myc	*N	*P	Lower	Center	Upper
0	1	0	-0.05997	0.02884	0.1177
0	1	1	0.04675	0.13557	0.2244
1	0	0	0.06332	0.15213	0.2409
1	0	1	0.12683	0.21564	0.3045
1	1	0	0.19869	0.28750	0.3763
1	1	1	0.23955	0.32839	0.4172



Myc = 0
N = 1
P = 0 subtracted from:

Myc	*N	*P	Lower	Center	Upper
0	1	1	0.01789	0.1067	0.1956
1	0	0	0.03447	0.1233	0.2121
1	0	1	0.09799	0.1868	0.2756
1	1	0	0.16984	0.2587	0.3475
1	1	1	0.21069	0.2995	0.3884

```
Myc     *N      *P          ----+---------+---------+---------+--
0        1       1               (---*----)
1        0       0                (---*----)
1        0       1                   (---*----)
1        1       0                      (----*---)
1        1       1                         (---*---)
                             ----+---------+---------+---------+--
                                0.00      0.20      0.40      0.60

Myc  = 0
N    = 1
P    = 1 subtracted from:

Myc     *N      *P            Lower     Center     Upper
1        0       0         -0.07225    0.01656    0.1054
1        0       1         -0.00876    0.08007    0.1689
1        1       0          0.06311    0.15193    0.2407
1        1       1          0.10400    0.19282    0.2816

Myc     *N      *P          ----+---------+---------+---------+--
1        0       0          (----*---)
1        0       1               (---*---)
1        1       0                  (----*---)
1        1       1                     (----*---)
                             ----+---------+---------+---------+--
                                0.00      0.20      0.40      0.60

Myc  = 1
N    = 0
P    = 0 subtracted from:

Myc     *N      *P            Lower     Center     Upper
1        0       1         -0.02531    0.06351    0.1523
1        1       0          0.04655    0.13536    0.2242
1        1       1          0.08744    0.17625    0.2651

Myc     *N      *P          ----+---------+---------+---------+--
1        0       1             (---*----)
1        1       0                (----*---)
1        1       1                   (----*---)
                             ----+---------+---------+---------+--
                                0.00      0.20      0.40      0.60

Myc  = 1
N    = 0
P    = 1 subtracted from:

Myc     *N      *P            Lower     Center     Upper
1        1       0         -0.01696    0.07185    0.1607
1        1       1          0.02389    0.11274    0.2016

Myc     *N      *P          ----+---------+---------+---------+--
1        1       0             (----*---)
1        1       1               (----*---)
                             ----+---------+---------+---------+--
                                0.00      0.20      0.40      0.60

Myc = 1
N   = 1
P   = 0 subtracted from:

Myc     *N      *P            Lower     Center     Upper
1        1       1         -0.04793    0.04089    0.1297

Myc     *N      *P          ----+---------+---------+---------+--
1        1       1            (---*---)
                             ----+---------+---------+---------+--
                                0.00      0.20      0.40      0.60

Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Myc*N /*P

Myc = 0
N = 0
P = 0 subtracted from:
```

Level Myc	*N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	0	1	0.1840	0.02894	6.358	0.0000
0	1	0	0.2128	0.02894	7.355	0.0000
0	1	1	0.3196	0.02894	11.042	0.0000
1	0	0	0.3361	0.02894	11.615	0.0000
1	0	1	0.3996	0.02894	13.810	0.0000
1	1	0	0.4715	0.02894	16.292	0.0000
1	1	1	0.5124	0.02895	17.701	0.0000

Myc = 0
N = 0
P = 1 subtracted from:

Level Myc	*N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0	0.02884	0.02894	0.9967	0.9744
0	1	1	0.13557	0.02894	4.6841	0.0002
1	0	0	0.15213	0.02894	5.2567	0.0000
1	0	1	0.21564	0.02894	7.4515	0.0000
1	1	0	0.28750	0.02894	9.9344	0.0000
1	1	1	0.32839	0.02895	11.3442	0.0000

Myc = 0
N = 1
P = 0 subtracted from:

Level Myc	*N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	1	0.1067	0.02895	3.687	0.0071
1	0	0	0.1233	0.02894	4.260	0.0009
1	0	1	0.1868	0.02894	6.455	0.0000
1	1	0	0.2587	0.02894	8.937	0.0000
1	1	1	0.2995	0.02895	10.346	0.0000

Myc = 0
N = 1
P = 1 subtracted from:

Level Myc	*N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0	0.01656	0.02894	0.5723	0.9992
1	0	1	0.08007	0.02895	2.7662	0.1101
1	1	0	0.15193	0.02894	5.2496	0.0000
1	1	1	0.19282	0.02894	6.6626	0.0000

Myc = 1
N = 0
P = 0 subtracted from:

Level Myc	*N	P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	1	0.06351	0.02894	2.194	0.3600
1	1	0	0.13536	0.02894	4.677	0.0002
1	1	1	0.17625	0.02894	6.090	0.0000

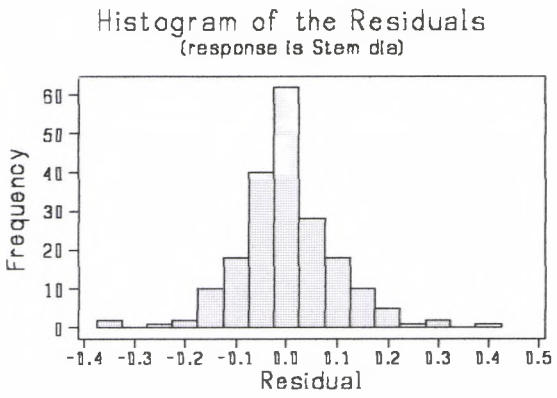
Myc = 1
N = 0
P = 1 subtracted from:

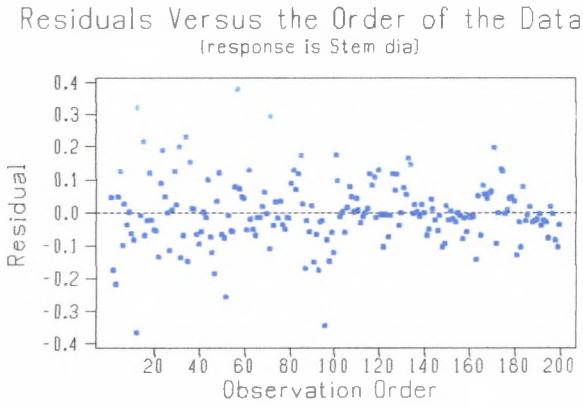
Level Myc	*N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	0	0.07185	0.02894	2.483	0.2094
1	1	1	0.11274	0.02895	3.894	0.0034

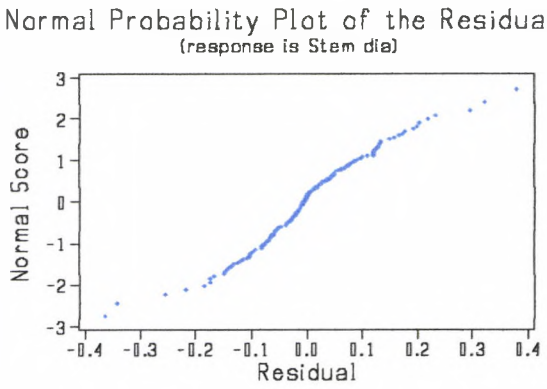
Myc = 1
N = 1
P = 0 subtracted from:

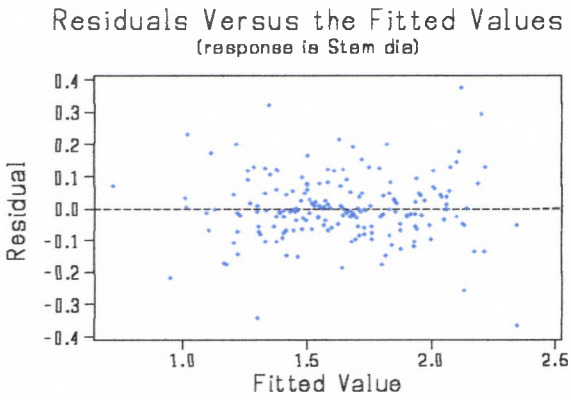
Level Myc	*N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	1	0.04089	0.02894	1.413	0.8505

Good normal distribution plots for the stem diameter data from the Betteshanger *B. pendula* and *L. corniculatus* multi-factor experiment.









General Linear Model analysis of variance for internodal data from the Betteshanger *B. pendula* and *L. corniculatus* experiment.

Abbreviations: Mycorrhi = Mycorrhizal inoculate
P = Phosphorus @ 75g/m^2
N = Nitrogen @ 75g/m^2
Internod = internodal length

Factor	Type	Levels	Values
Mycorrhi	fixed	2	0 1
N	fixed	2	0 1
P	fixed	2	0 1
Birch planting density	fixed	3	8 16 24
Lotus planting density	fixed	3	0 8 16

Analysis of Variance for Internodal length, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Internodal length	1	260.290	256.255	256.255	1062.84	0.000
Mycorrhi	1	72.621	72.620	72.620	301.20	0.000
N	1	25.807	25.790	25.790	106.97	0.000
P	1	11.053	11.043	11.043	45.80	0.000
Mycorrhi*N	1	0.724	0.726	0.726	3.01	0.084
Mycorrhi*P	1	2.513	2.508	2.508	10.40	0.001
N @ 75g/*P	1	0.726	0.732	0.732	3.04	0.083
Mycorrhi*N *P	1	0.238	0.238	0.238	0.99	0.322
Birch Planting density	2	40.763	31.181	15.590	64.66	0.000
Lotus Planting density	2	0.415	0.415	0.207	0.86	0.425
Error	467	45.086	45.086	0.241		
Total	479	460.237				

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Internodal length	(11) + Q[1]
2 Mycorrhi	(11) + Q[2, 5, 6, 8]
3 N	(11) + Q[3, 5, 7, 8]
4 P	(11) + Q[4, 6, 7, 8]
5 Mycorrhi*N	(11) + Q[5, 8]
6 Mycorrhi*P	(11) + Q[6, 8]
7 N *P	(11) + Q[7, 8]
8 Mycorrhi*N*P	(11) + Q[8]
9 Birch Planting density	(11) + Q[9]
10 Lotus Planting density	(11) + Q[10]
11 Error	(11)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Internod	467.00	0.241	(11)
2 Mycorrhi	467.00	0.241	(11)
3 N	467.00	0.241	(11)
4 P	467.00	0.241	(11)
5 Mycorrhi*N	467.00	0.241	(11)
6 Mycorrhi*P	467.00	0.241	(11)
7 N *P	467.00	0.241	(11)
8 Mycorrhi*N * P	467.00	0.241	(11)
9 Birch tr	467.00	0.241	(11)
10 Lotus pl	467.00	0.241	(11)

Variance Components, using Adjusted SS

Source	Estimated Value
Error	0.2411

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Internodal length
All Pairwise Comparisons among Levels of Mycorrhi

Mycorrhi = 0 subtracted from:

Mycorrhi	Lower	Center	Upper	-------+---------+---------+---------
1	1.068	1.205	1.342	(----------*----------)
				-------+---------+---------+---------
				1.120 1.200 1.280

Tukey Simultaneous Tests
Response Variable Internodal length
All Pairwise Comparisons among Levels of Mycorrhi

Mycorrhi = 0 subtracted from:

Level Mycorrhi	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1.205	0.06945	17.36	0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Internodal length
All Pairwise Comparisons among Levels of N

N = 0 subtracted from:

N	Lower	Center	Upper	--------+---------+---------+---------
1	0.5813	0.7183	0.8553	(----------*----------)
				--------+---------+---------+---------
				0.640 0.720 0.800

Tukey Simultaneous Tests
Response Variable Internodal length
All Pairwise Comparisons among Levels of

N = 0 subtracted from:

Level N	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0.7183	0.06945	10.34	0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Internodal length
All Pairwise Comparisons among Levels of P

P = 0 subtracted from:

P	Lower	Center	Upper	---------+---------+---------+-------
1	0.3330	0.4700	0.6070	(----------*----------)
				---------+---------+---------+-------
				0.400 0.480 0.560

Tukey Simultaneous Tests
Response Variable Internod
All Pairwise Comparisons among Levels of P @ 75g/

P = 0 subtracted from:

Level P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0.4700	0.06945	6.768	0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Internodal length
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/

Mycorrhi = 0
N = 0 subtracted from:

Mycorrhi*N		Lower	Center	Upper
0	1	0.5840	0.8388	1.094
1	0	1.0709	1.3258	1.581
1	1	1.6687	1.9236	2.178

Mycorrhi*N		-------+---------+---------+---------
0	1	(---*---)
1	0	(---*---)
1	1	(---*---)
		-------+---------+---------+---------
		0.60 1.20 1.80

Mycorrhi = 0
N = 1 subtracted from:

Mycorrhi*N		Lower	Center	Upper
1	0	0.2321	0.4870	0.7418
1	1	0.8298	1.0847	1.3396

```
Mycorrhi*N          -------+---------+---------+---------
1        0           (---*---)
1        1                     (---*---)
                    -------+---------+---------+---------
                         0.60      1.20      1.80
```

```
Mycorrhi = 1
N    = 0 subtracted from:

Mycorrhi*N            Lower    Center     Upper
1        1           0.3429    0.5978    0.8527

Mycorrhi*N          -------+---------+---------+---------
1        1            (---*---)
                    -------+---------+---------+---------
                         0.60      1.20      1.80
```

```
Tukey Simultaneous Tests
Response Variable Internodal length
All Pairwise Comparisons among Levels of Mycorrhi*N

Mycorrhi = 0
N        = 0 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*N               of Means  Difference   T-Value     P-Value
0        1                 0.8388     0.09821     8.542      0.0000
1        0                 1.3258     0.09821    13.500      0.0000
1        1                 1.9236     0.09823    19.583      0.0000

Mycorrhi = 0
N        = 1 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*N               of Means  Difference   T-Value     P-Value
1        0                 0.4870     0.09821     4.958      0.0000
1        1                 1.0847     0.09822    11.044      0.0000

Mycorrhi = 1
N        = 0 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*N               of Means  Difference   T-Value     P-Value
1        1                 0.5978     0.09822     6.086      0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Internodal length
All Pairwise Comparisons among Levels of Mycorrhi*P

Mycorrhi = 0
P        = 0 subtracted from:

Mycorrhi*P            Lower    Center     Upper
0        1           0.4391    0.6940    0.9489
1        0           1.1744    1.4292    1.6841
1        1           1.4204    1.6752    1.9301

Mycorrhi*P          -+---------+---------+---------+-----
0        1                (----*---)
1        0                          (---*---)
1        1                             (---*---)
                    -+---------+---------+---------+-----
                    0.00      0.60      1.20      1.80

Mycorrhi = 0
P        = 1 subtracted from:

Mycorrhi*P            Lower    Center     Upper
1        0           0.4804    0.7353    0.9901
1        1           0.7264    0.9813    1.2361

Mycorrhi*P          -+---------+---------+---------+-----
1        0                  (---*----)
1        1                     (---*----)
                    -+---------+---------+---------+-----
                    0.00      0.60      1.20      1.80
```

```
Mycorrhi = 1
P        = 0 subtracted from:

Mycorrhi*P            Lower    Center     Upper
1        1        -0.008849    0.2460    0.5008

Mycorrhi*P          -+---------+---------+---------+-----
1        1           (---*---)
                    -+---------+---------+---------+-----
                    0.00      0.60      1.20      1.80

Tukey Simultaneous Tests
Response Variable Internodal length
All Pairwise Comparisons among Levels of Mycorrhi*P

Mycorrhi = 0
P        = 0 subtracted from:

Level                 Difference       SE of             Adjusted
Mycorrhi*P              of Means  Difference   T-Value    P-Value
0        1                0.6940     0.09822     7.066     0.0000
1        0                1.4292     0.09822    14.552     0.0000
1        1                1.6752     0.09822    17.056     0.0000

Mycorrhi = 0
P        = 1 subtracted from:

Level                 Difference       SE of             Adjusted
Mycorrhi*P              of Means  Difference   T-Value    P-Value
1        0                0.7353     0.09820     7.487     0.0000
1        1                0.9813     0.09820     9.992     0.0000

Mycorrhi = 1
P        = 0 subtracted from:

Level                 Difference       SE of             Adjusted
Mycorrhi*P              of Means  Difference   T-Value    P-Value
1        1                0.2460     0.09820     2.505     0.0624

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Internodal length
All Pairwise Comparisons among Levels of N*P

N= 0
P= 0 subtracted from:

N       *P            Lower    Center     Upper
0        1           0.3362    0.5911    0.8459
1        0           0.5845    0.8394    1.0942
1        1           0.9334    1.1883    1.4432

N       *P          -+---------+---------+---------+-----
0        1                (------*-----)
1        0                      (-----*-----)
1        1                            (------*-----)
                    -+---------+---------+---------+-----
                    0.00      0.40      0.80      1.20

N  = 0
P  = 1 subtracted from:

N       *P            Lower    Center     Upper
1        0        -0.006539    0.2483    0.5032
1        1         0.342246    0.5972    0.8522

N       *P          -+---------+---------+---------+-----
1        0           (-----*------)
1        1                 (-----*-----)
                    -+---------+---------+---------+-----
                    0.00      0.40      0.80      1.20

N   = 1
P   = 0 subtracted from:

N       *P            Lower    Center     Upper
1        1          0.09397    0.3489    0.6039
```

```
N       *P          -+---------+---------+---------+-----
1        1             (------*-----)
                    -+---------+---------+---------+-----
                    0.00      0.40      0.80      1.20
```

Tukey Simultaneous Tests
Response Variable Internodal length
All Pairwise Comparisons among Levels of N*P

N = 0
P = 0 subtracted from:

Level N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0.5911	0.09821	6.018	0.0000
1	0	0.8394	0.09821	8.547	0.0000
1	1	1.1883	0.09823	12.098	0.0000

N = 0
P = 1 subtracted from:

Level N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0.2483	0.09821	2.528	0.0588
1	1	0.5972	0.09826	6.078	0.0000

N = 1
P = 0 subtracted from:

Level N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	0.3489	0.09825	3.552	0.0027

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Internodal length.
All Pairwise Comparisons among Levels of Mycorrhi*N *P

Mycorrhi = 0
N = 0
P = 0 subtracted from:

Mycorrhi*N	*P	Lower	Center	Upper
0 0	1	0.4578	0.8840	1.310
0 1	0	0.6026	1.0289	1.455
0 1	1	1.1066	1.5328	1.959
1 0	0	1.1925	1.6187	2.045
1 0	1	1.4906	1.9169	2.343
1 1	0	1.8424	2.2686	2.695
1 1	1	2.0362	2.4625	2.889

```
Mycorrhi*N  *P     ----+---------+---------+---------+--
0 0          1          (---*---)
0 1          0           (---*----)
0 1          1               (---*----)
1 0          0                (---*---)
1 0          1                   (---*---)
1 1          0                      (----*---)
1 1          1                        (----*---)
                      ----+---------+---------+---------+--
                        0.0       1.0       2.0       3.0
```

Mycorrhi = 0
N = 0
P = 1 subtracted from:

Mycorrhi*N	*P	Lower	Center	Upper
0 1	0	-0.2814	0.1449	0.5711
0 1	1	0.2226	0.6488	1.0751
1 0	0	0.3085	0.7347	1.1610
1 0	1	0.6066	1.0329	1.4591
1 1	0	0.9584	1.3846	1.8108
1 1	1	1.1522	1.5785	2.0048

```
Mycorrhi*N       *P          ----+---------+---------+---------+---
0        1        0           (---*----)
0        1        1                (---*----)
1        0        0                 (---*----)
1        0        1                    (---*----)
1        1        0                         (---*---)
1        1        1                           (---*---)
                              ----+---------+---------+---------+---
                                 0.0       1.0       2.0       3.0
```

```
Mycorrhi = 0
N        = 1
P        = 0 subtracted from:

Mycorrhi*N       *P            Lower    Center     Upper
0        1        1          0.07765    0.5040    0.9303
1        0        0          0.16360    0.5899    1.0162
1        0        1          0.46179    0.8880    1.3142
1        1        0          0.81353    1.2398    1.6660
1        1        1          1.00723    1.4336    1.8601

Mycorrhi*N       *P          ----+---------+---------+---------+---
0        1        1               (---*---)
1        0        0                (---*---)
1        0        1                   (---*---)
1        1        0                      (---*----)
1        1        1                        (---*----)
                              ----+---------+---------+---------+---
                                 0.0       1.0       2.0       3.0
```

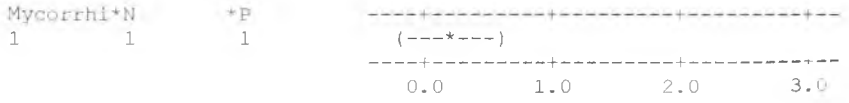
```
Mycorrhi = 0
N        = 1
P        = 1 subtracted from:

Mycorrhi*N       *P            Lower    Center     Upper
1        0        0          -0.3403   0.08590    0.5121
1        0        1          -0.0423   0.38403    0.8103
1        1        0           0.3096   0.73580    1.1620
1        1        1           0.5035   0.92967    1.3559

Mycorrhi*N @     *P          ----+---------+---------+---------+---
1        0        0           (---*---)
1        0        1              (---*---)
1        1        0                 (---*----)
1        1        1                   (---*----)
                              ----+---------+---------+---------+---
                                 0.0       1.0       2.0       3.0
```

```
Mycorrhi = 1
N        = 0
P        = 0 subtracted from:

Mycorrhi*N       *P            Lower    Center     Upper
1        0        1          -0.1282   0.2981    0.7244
1        1        0           0.2237   0.6499    1.0761
1        1        1           0.4175   0.8438    1.2700

Mycorrhi*N       *P          ----+---------+---------+---------+---
1        0        1              (---*---)
1        1        0                 (---*----)
1        1        1                   (---*----)
                              ----+---------+---------+---------+---
                                 0.0       1.0       2.0       3.0
```

```
Mycorrhi = 1
N        = 0
P        = 1 subtracted from:

Mycorrhi*N       *P            Lower    Center     Upper
1        1        0          -0.07447   0.3518    0.7780
1        1        1           0.11923   0.5456    0.9721

Mycorrhi*N       *P         ----+---------+---------+---------+---
1        1        0             (----*---)
1        1        1               (---*----)
                              ----+---------+---------+---------+---
                                 0.0       1.0       2.0       3.0
```

Mycorrhi = 1
N = 1
P = 0 subtracted from:

Mycorrhi*N	*P	Lower	Center	Upper
1	1	-0.2324	0.1939	0.6202



Tukey Simultaneous Tests
Response Variable Internodal length
All Pairwise Comparisons among Levels of Mycorrhi*N*P

Mycorrhi = 0
N = 0
P = 0 subtracted from:

Level Mycorrhi*N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	0	1	0.8840	0.1389	6.365	0.0000
0	1	0	1.0289	0.1389	7.408	0.0000
0	1	1	1.5328	0.1389	11.035	0.0000
1	0	0	1.6187	0.1389	11.655	0.0000
1	0	1	1.9169	0.1389	13.802	0.0000
1	1	0	2.2686	0.1389	16.335	0.0000
1	1	1	2.4625	0.1389	17.726	0.0000

Mycorrhi = 0
N = 0
P = 1 subtracted from:

Level Mycorrhi*N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0	0.1449	0.1389	1.043	0.9671
0	1	1	0.6488	0.1389	4.671	0.0002
1	0	0	0.7347	0.1389	5.290	0.0000
1	0	1	1.0329	0.1389	7.437	0.0000
1	1	0	1.3846	0.1389	9.970	0.0000
1	1	1	1.5785	0.1389	11.362	0.0000

Mycorrhi = 0
N = 1
P = 0 subtracted from:

Level Mycorrhi*N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	1	0.5040	0.1389	3.628	0.0087
1	0	0	0.5899	0.1389	4.247	0.0009
1	0	1	0.8880	0.1389	6.394	0.0000
1	1	0	1.2398	0.1389	8.926	0.0000
1	1	1	1.4336	0.1390	10.318	0.0000

Mycorrhi = 0
N = 1
P = 1 subtracted from:

Level Mycorrhi*N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0	0.08590	0.1389	0.6185	0.9986
1	0	1	0.38403	0.1389	2.7644	0.1106
1	1	0	0.73580	0.1389	5.2978	0.0000
1	1	1	0.92967	0.1389	6.6937	0.0000

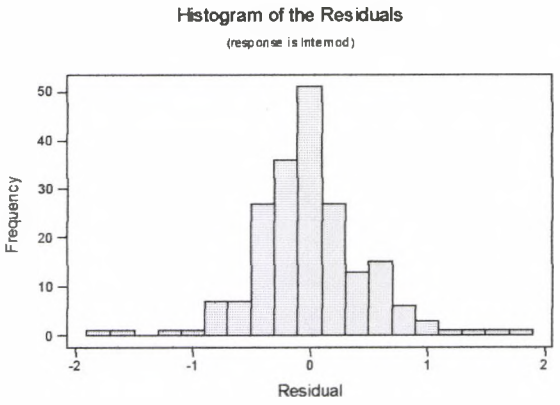
Mycorrhi = 1
N = 0
P = 0 subtracted from:

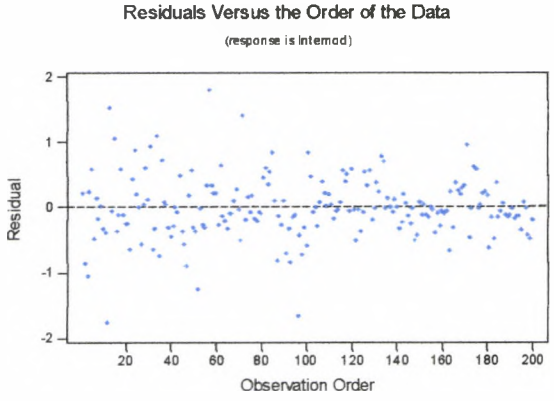
Level Mycorrhi*N	*P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	1	0.2981	0.1389	2.146	0.3895
1	1	0	0.6499	0.1389	4.679	0.0002
1	1	1	0.8438	0.1389	6.075	0.0000

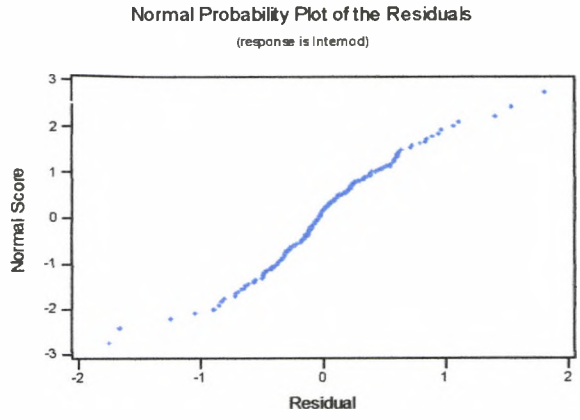
Mycorrhi = 1

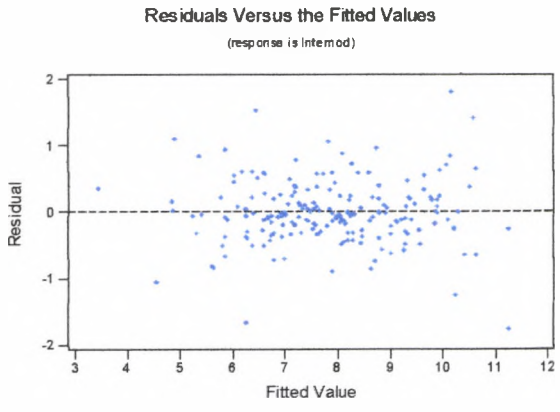
```
N        = 0
P      = 1 subtracted from:

Level                          Difference       SE of              Adjusted
Mycorrhi*N       *P              of Means  Difference   T-Value     P-Value
1        1        0                0.3518      0.1389     2.533      0.1884
1        1        1                0.5456      0.1390     3.927      0.0030

Mycorrhi = 1
N        = 1
P        = 0 subtracted from:

Level                          Difference       SE of              Adjusted
Mycorrhi*N       *P              of Means  Difference   T-Value     P-Value
1        1        1                0.1939      0.1389     1.396      0.8583
```

Good normal distribution plots for the internodal length data from the Betteshanger *B. pendula* and *L. corniculatus* multi-factor experiment.









General Linear Model: 8 Birch trees stem diameter versus treatments.

Key: mycorrhi = mycorrhizal treatment, stem dia = stem diameter, N @ 75g/ = N @ 75g/m^2, P @ 75g/ = P @ 75g/m^2.

```
Factor     Type Levels Values
Mycorrhi   fixed      2 0 1
N @ 75g/   fixed      2 0 1
P @ 75g/   fixed      2 0 1

Analysis of Variance for stem diameter, using Adjusted SS for Tests

Source                    DF     Seq SS     Adj SS     Adj MS       F      P
Stem diameter              1    1.79153    1.61921    1.61921  119.08  0.000
Mycorrhi                   1    0.66668    0.66859    0.66859   49.17  0.000
N @ 75g/                   1    0.30804    0.30846    0.30846   22.68  0.000
P @ 75g/                   1    0.17891    0.17880    0.17880   13.15  0.001
Mycorrhi*N @ 75g/          1    0.00730    0.00730    0.00730    0.54  0.469
Mycorrhi*P @ 75g/          1    0.01221    0.01219    0.01219    0.90  0.351
N @ 75g/*P @ 75g/          1    0.01175    0.01174    0.01174    0.86  0.360
Mycorrhi*N*P               1    0.00624    0.00624    0.00624    0.46  0.503
Error                     31    0.42155    0.42155    0.01360
Total                     39    3.40419

Term                          Coef   SE Coef        T      P
Constant                   1.07314   0.05942    18.06  0.000
Stem diameter               2.0325    0.1863    10.91  0.000

Unusual Observations for Stem diameter

Obs  Stem diameter  Fit      SE Fit  Residual   St Resid
  3     0.73000   1.03325  0.05881  -0.30325     -3.01R
 12     1.98000   2.19850  0.06409  -0.21850     -2.24R

R denotes an observation with a large standardized residual.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Mycorrhi

Mycorrhi = 0 subtracted from:

Mycorrhi     Lower    Center     Upper  ----+---------+---------+---------+---
1           0.1835    0.2588    0.3340   (--------------*--------------)
                                        ----+---------+---------+---------+---
                                          0.200     0.250     0.300     0.350

Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Mycorrhizal treatments

Mycorrhi = 0 subtracted from:

Level        Difference       SE of              Adjusted
Mycorrhi       of Means  Difference   T-Value    P-Value
1                0.2588     0.03690     7.012     0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of N @ 75g/

N @ 75g/ = 0 subtracted from:

N @ 75g/     Lower    Center     Upper  ----------+---------+---------+------
1           0.1005    0.1758    0.2510  (--------------*--------------)
                                        ----------+---------+---------+------
                                                0.150     0.200     0.250

Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of N @ 75g/

N @ 75g/ = 0 subtracted from:

Level        Difference       SE of              Adjusted
N @ 75g/       of Means  Difference   T-Value    P-Value
1                0.1758     0.03690     4.763     0.0001
```

```
Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of P @ 75g/

P @ 75g/ = 0 subtracted from:

P @ 75g/     Lower    Center     Upper  ---------+---------+---------+-------
1          0.05854    0.1338    0.2090   (--------------*--------------)
                                         ---------+---------+---------+-------
                                                0.100     0.150     0.200


Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of P @ 75g/

P @ 75g/ = 0 subtracted from:

Level          Difference       SE of              Adjusted
P @ 75g/       of Means  Difference   T-Value    P-Value
1                0.1338     0.03690     3.626     0.0010

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/

Mycorrhi = 0
N @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/     Lower    Center     Upper
0        1          0.06113    0.2028    0.3444
1        0          0.14413    0.2858    0.4274
1        1          0.29271    0.4345    0.5763

Mycorrhi*N @ 75g/  ---+---------+---------+---------+---
0        1               (------*------)
1        0                   (------*------)
1        1                         (------*------)
                   ---+---------+---------+---------+---
                     0.00      0.20      0.40      0.60

Mycorrhi = 0
N @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/     Lower    Center     Upper
1        0         -0.05860   0.08300    0.2246
1        1          0.09008   0.23174    0.3734

Mycorrhi*N @ 75g/  ---+---------+---------+---------+---
1        0         (------*------)
1        1                 (------*------)
                   ---+---------+---------+---------+---
                     0.00      0.20      0.40      0.60

Mycorrhi = 1
N @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/     Lower    Center     Upper
1        1          0.007079   0.1487    0.2904

Mycorrhi*N @ 75g/  ---+---------+---------+---------+---
1        1            (------*-------)
                   ---+---------+---------+---------+---
                     0.00      0.20      0.40      0.60

Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/

Mycorrhi = 0
N @ 75g/ = 0 subtracted from:

Level                Difference       SE of              Adjusted
Mycorrhi*N @ 75g/      of Means  Difference   T-Value    P-Value
0        1               0.2028     0.05217     3.887     0.0027
1        0               0.2858     0.05217     5.478     0.0000
1        1               0.4345     0.05223     8.320     0.0000
```

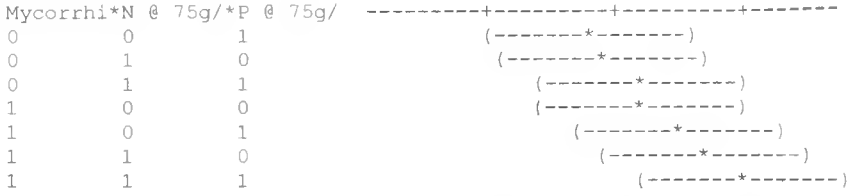
```
Mycorrhi = 0
N @ 75g/ = 1 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*N @ 75g/        of Means  Difference   T-Value     P-Value
1        0               0.08300     0.05215     1.592      0.3980
1        1               0.23174     0.05217     4.442      0.0006

Mycorrhi = 1
N @ 75g/ = 0 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*N @ 75g/        of Means  Difference   T-Value     P-Value
1        1                0.1487     0.05217     2.851      0.0366

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Mycorrhi*P @ 75g/

Mycorrhi = 0
P @ 75g/ = 0 subtracted from:

Mycorrhi*P @ 75g/     Lower    Center     Upper
0        1          0.02703    0.1687    0.3104
1        0          0.15198    0.2937    0.4354
1        1          0.25076    0.3925    0.5343

Mycorrhi*P @ 75g/  ---+---------+---------+---------+---
0        1              (--------*--------)
1        0                       (--------*--------)
1        1                                (--------*--------)
                   ---+---------+---------+---------+---
                     0.00      0.16      0.32      0.48
Mycorrhi = 0
P @ 75g/ = 1 subtracted from:

Mycorrhi*P @ 75g/     Lower    Center     Upper
1        0         -0.01664    0.1250    0.2666
1        1          0.08221    0.2238    0.3655

Mycorrhi*P @ 75g/  ---+---------+---------+---------+---
1        0            (--------*--------)
1        1                     (--------*--------)
                   ---+---------+---------+---------+---
                     0.00      0.16      0.32      0.48
Mycorrhi = 1
P @ 75g/ = 0 subtracted from:

Mycorrhi*P @ 75g/     Lower    Center     Upper
1        1         -0.04275   0.09887    0.2405

Mycorrhi*P @ 75g/  ---+---------+---------+---------+---
1        1          (--------*--------)
                   ---+---------+---------+---------+---
                     0.00      0.16      0.32      0.48

Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Mycorrhi*P @ 75g/

Mycorrhi = 0
P @ 75g/ = 0 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*P @ 75g/        of Means  Difference   T-Value     P-Value
0        1               0.1687     0.05218     3.233      0.0146
1        0               0.2937     0.05218     5.628      0.0000
1        1               0.3925     0.05222     7.518      0.0000

Mycorrhi = 0
P @ 75g/ = 1 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*P @ 75g/        of Means  Difference   T-Value     P-Value
1        0               0.1250     0.05215     2.396      0.0988
1        1               0.2238     0.05216     4.291      0.0009
```

```
Mycorrhi = 1
P @ 75g/ = 0 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*P @ 75g/       of Means  Difference   T-Value     P-Value
1        1               0.09887     0.05216     1.896      0.2506

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of N @ 75g/*P @ 75g/

N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

N @ 75g/*P @ 75g/     Lower    Center     Upper
0        1          0.02649    0.1681    0.3097
1        0          0.06846    0.2101    0.3517
1        1          0.16776    0.3095    0.4513

N @ 75g/*P @ 75g/  -------+---------+---------+---------
0        1                    (--------*---------)
1        0                       (--------*--------)
1        1                            (---------*--------)
                   -------+---------+---------+---------
                        0.00      0.15      0.30

N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

N @ 75g/*P @ 75g/     Lower    Center     Upper
1        0         -0.09964   0.04197    0.1836
1        1         -0.00042   0.14145    0.2833

N @ 75g/*P @ 75g/  -------+---------+---------+---------
1        0         (---------*---------)
1        1                   (--------*---------)
                   -------+---------+---------+---------
                        0.00      0.15      0.30

N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

N @ 75g/*P @ 75g/     Lower    Center     Upper
1        1         -0.04235   0.09948    0.2413

N @ 75g/*P @ 75g/  -------+---------+---------+---------
1        1              (---------*--------)
                   -------+---------+---------+---------
                        0.00      0.15      0.30
Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of N @ 75g/*P @ 75g/

N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Level                  Difference       SE of              Adjusted
N @ 75g/*P @ 75g/       of Means  Difference   T-Value     P-Value
0        1               0.1681     0.05215     3.223      0.0150
1        0               0.2101     0.05215     4.028      0.0018
1        1               0.3095     0.05222     5.928      0.0000

N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Level                  Difference       SE of              Adjusted
N @ 75g/*P @ 75g/       of Means  Difference   T-Value     P-Value
1        0              0.04197     0.05215     0.8047     0.8517
1        1              0.14145     0.05225     2.7073     0.0508

N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Level                  Difference       SE of              Adjusted
N @ 75g/*P @ 75g/       of Means  Difference   T-Value     P-Value
1        1              0.09948     0.05224     1.904      0.2470
```

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/*P @ 75g/

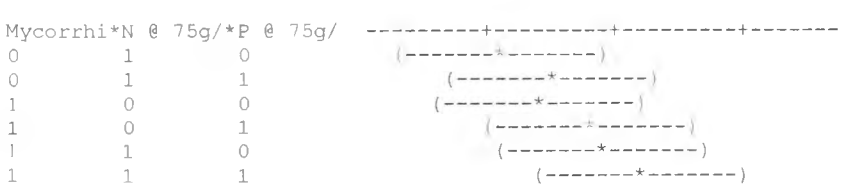
Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
0	0	1	-0.01137	0.2280	0.4674
0	1	0	0.02269	0.2621	0.5014
0	1	1	0.13191	0.3715	0.6110
1	0	0	0.10623	0.3457	0.5851
1	0	1	0.21449	0.4539	0.6933
1	1	0	0.26432	0.5037	0.7432
1	1	1	0.35355	0.5933	0.8330



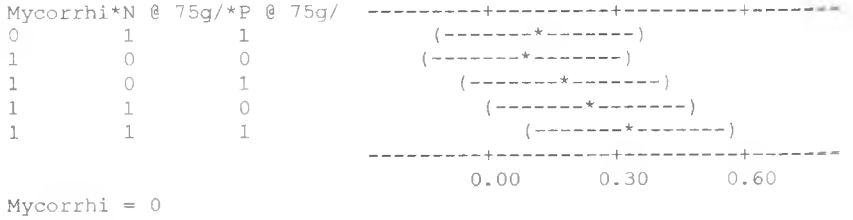
Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
0	1	0	-0.2053	0.03407	0.2734
0	1	1	-0.0961	0.14348	0.3830
1	0	0	-0.1218	0.11767	0.3571
1	0	1	-0.0135	0.22587	0.4653
1	1	0	0.0363	0.27574	0.5152
1	1	1	0.1255	0.36528	0.6050



Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
0	1	1	-0.1302	0.10941	0.3490
1	0	0	-0.1559	0.08361	0.3231
1	0	1	-0.0476	0.19180	0.4312
1	1	0	0.0022	0.24167	0.4811
1	1	1	0.0914	0.33122	0.5710



Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
1	0	0	-0.2652	-0.02580	0.2136
1	0	1	-0.1571	0.08239	0.3219
1	1	0	-0.1072	0.13226	0.3717
1	1	1	-0.0176	0.22180	0.4612

```
Mycorrhi*N @ 75g/*P @ 75g/  ---------+---------+---------+-------
1        0        0         (-------*-------)
1        0        1             (-------*-------)
1        1        0              (-------*-------)
1        1        1                  (-------*-------)
                             ---------+---------+---------+-------
                                    0.00      0.30      0.60
```

Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
1	0	1	-0.1312	0.1082	0.3476
1	1	0	-0.0813	0.1581	0.3974
1	1	1	0.0081	0.2476	0.4871

```
Mycorrhi*N @ 75g/*P @ 75g/  ---------+---------+---------+-------
1        0        1              (-------*-------)
1        1        0               (-------*-------)
1        1        1                  (-------*-------)
                             ---------+---------+---------+-------
                                    0.00      0.30      0.60
```

Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
1	1	0	-0.1895	0.04987	0.2893
1	1	1	-0.1002	0.13941	0.3790

```
Mycorrhi*N @ 75g/*P @ 75g/  ---------+---------+---------+-------
1        1        0            (-------*-------)
1        1        1               (-------*-------)
                             ---------+---------+---------+-------
                                    0.00      0.30      0.60
```

Mycorrhi = 1
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
1	1	1	-0.1500	0.08954	0.3291

```
Mycorrhi*N @ 75g/*P @ 75g/  ---------+---------+---------+-------
1        1        1            (-------*-------)
                             ---------+---------+---------+-------
                                    0.00      0.30      0.60
```

Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/*P @ 75g/

Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Level Mycorrhi*N @ 75g/*P @ 75g/			Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	0	1	0.2280	0.07375	3.091	0.0710
0	1	0	0.2621	0.07375	3.553	0.0241
0	1	1	0.3715	0.07381	5.033	0.0005
1	0	0	0.3457	0.07378	4.686	0.0012
1	0	1	0.4539	0.07376	6.154	0.0000
1	1	0	0.5037	0.07377	6.829	0.0000
1	1	1	0.5933	0.07387	8.032	0.0000

Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Level Mycorrhi*N @ 75g/*P @ 75g/			Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0	0.03407	0.07375	0.4619	0.9998
0	1	1	0.14348	0.07381	1.9439	0.5332
1	0	0	0.11767	0.07378	1.5950	0.7496
1	0	1	0.22587	0.07376	3.0624	0.0757
1	1	0	0.27574	0.07377	3.7380	0.0152
1	1	1	0.36528	0.07387	4.9453	0.0006

Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Level Mycorrhi	*N @ 75g/	*P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	1	0.10941	0.07383	1.482	0.8108
1	0	0	0.08361	0.07379	1.133	0.9442
1	0	1	0.19180	0.07376	2.600	0.1940
1	1	0	0.24167	0.07378	3.276	0.0467
1	1	1	0.33122	0.07389	4.483	0.0021

Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 1 subtracted from:

Level Mycorrhi	*N @ 75g/	*P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0	-0.02580	0.07376	-0.3499	1.0000
1	0	1	0.08239	0.07379	1.1166	0.9482
1	1	0	0.13226	0.07377	1.7930	0.6292
1	1	1	0.22180	0.07376	3.0071	0.0854

Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Level Mycorrhi	*N @ 75g/	*P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	1	0.1082	0.07376	1.467	0.8185
1	1	0	0.1581	0.07375	2.143	0.4114
1	1	1	0.2476	0.07379	3.356	0.0387

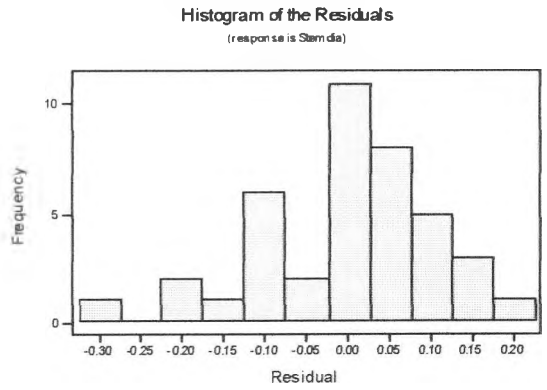
Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

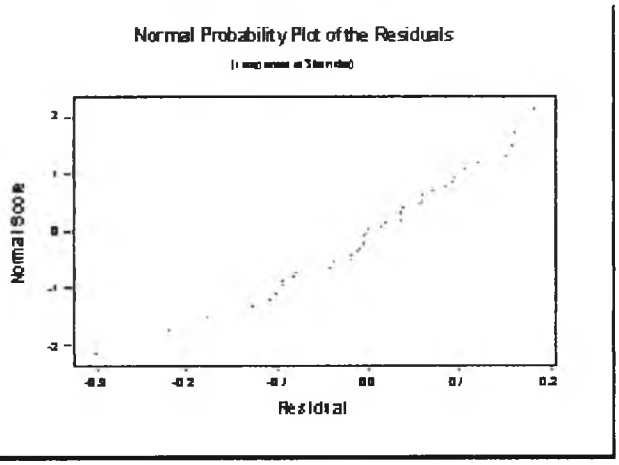
Level Mycorrhi	*N @ 75g/	*P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	0	0.04987	0.07376	0.6762	0.9971
1	1	1	0.13941	0.07383	1.8884	0.5685

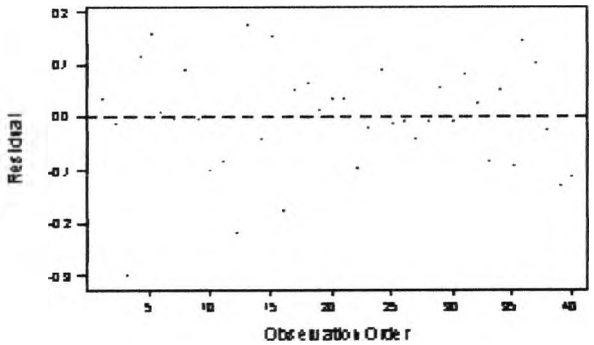
Mycorrhi = 1
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

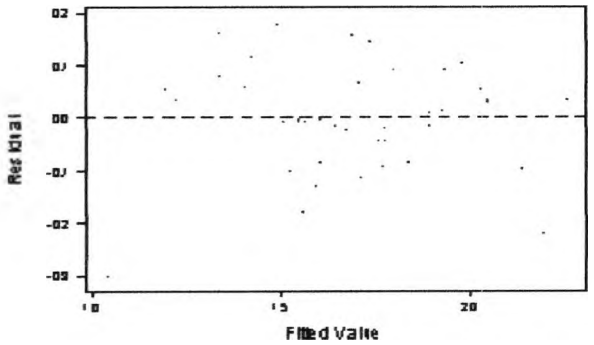
Level Mycorrhi	*N @ 75g/	*P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	1	0.08954	0.07380	1.213	0.9218

Reasonable normal distribution plots for the eight Birch trees per plot stem diameter measurements versus treatments data.









General Linear Model: 16 trees stem diameter versus treatments

Key: mycorrhi = mycorrhizal treatment, stem dia = stem diameter, N @ 75g/ = N @ 75g/m^2, P @ 75g/ = P @ 75g/m^2.

```
Factor      Type Levels Values
Mycorrhi    fixed      2 0 1
N @ 75g/    fixed      2 0 1
P @ 75g/    fixed      2 0 1

Analysis of Variance for Stem diameter, using Adjusted SS for Tests

Source                      DF     Seq SS     Adj SS     Adj MS       F       P
Stem dia                     1    4.43882    4.25067    4.25067  294.31   0.000
Mycorrhi                     1    1.18218    1.23324    1.23324   85.39   0.000
N @ 75g/                     1    0.73538    0.75578    0.75578   52.33   0.000
P @ 75g/                     1    0.15190    0.15755    0.15755   10.91   0.002
Mycorrhi*N @ 75g/            1    0.00029    0.00008    0.00008    0.01   0.940
Mycorrhi*P @ 75g/            1    0.07219    0.07430    0.07430    5.14   0.026
N @ 75g/*P @ 75g/            1    0.04918    0.04968    0.04968    3.44   0.068
Mycorrhi*N  *P               1    0.00616    0.00616    0.00616    0.43   0.516
Error                       70    1.01098    1.01098    0.01444
Total                       78    7.64707

Term                            Coef   SE Coef        T      P
Constant                     0.92169   0.04663    19.77  0.000
Stem diameter                 2.5131    0.1465    17.16  0.000

Unusual Observations for Stem diameter

Obs  Stem dia       Fit      SE Fit  Residual   St Resid
 17   2.50000   2.15323     0.04042   0.34677      3.06R
 32   2.50000   2.15896     0.05040   0.34104      3.13R
 42   0.73000   0.96736     0.04633  -0.23736     -2.14R
 51   1.98000   2.23467     0.04835  -0.25467     -2.31R
 52   1.67000   1.35509     0.04361   0.31491      2.81R
 54   1.85000   1.60640     0.03860   0.24360      2.14R

R denotes an observation with a large standardized residual.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi

Mycorrhiza = 0 subtracted from:

Mycorrhiza   Lower    Center     Upper  -----+---------+---------+---------+-
1          0.1961    0.2501    0.3041  (-----------------*-----------------)
                                       -----+---------+---------+---------+-
                                          0.210     0.240     0.270     0.300

Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi

Mycorrhi = 0 subtracted from:

Level          Difference       SE of              Adjusted
Mycorrhi         of Means  Difference   T-Value     P-Value
1                  0.2501     0.02706     9.241      0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of N @ 75g/

N @ 75g/ = 0 subtracted from:

N @ 75g/     Lower    Center     Upper  ---+---------+---------+---------+---
1          0.1418    0.1958    0.2498  (-----------------*-----------------)
                                       ---+---------+---------+---------+---
                                        0.150     0.180     0.210     0.240


Tukey Simultaneous Tests
Response Variable Stem diameter
All Pairwise Comparisons among Levels of N @ 75g/
```

```
N @ 75g/ = 0 subtracted from:

Level        Difference       SE of              Adjusted
N @ 75g/       of Means  Difference   T-Value    P-Value
1                0.1958     0.02707     7.234     0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of P @ 75g/

P @ 75g/ = 0 subtracted from:

P @ 75g/     Lower    Center     Upper  ---------+---------+---------+-------
1          0.03540   0.08937    0.1433   (------------------*-----------------)
                                        ---------+---------+---------+-------
                                               0.060     0.090     0.120

Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of P @ 75g/

P @ 75g/ = 0 subtracted from:

Level        Difference       SE of              Adjusted
P @ 75g/       of Means  Difference   T-Value    P-Value
1              0.08937     0.02706     3.303     0.0015

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/

Mycorrhi = 0
N @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/     Lower    Center     Upper
0        1          0.09243    0.1938    0.2951
1        0          0.14670    0.2480    0.3494
1        1          0.34450    0.4459    0.5473

Mycorrhi*N @ 75g/  ---+---------+---------+---------+---
0        1                 (----*----)
1        0                   (----*----)
1        1                             (----*----)
                   ---+---------+---------+---------+---
                     0.00      0.20      0.40      0.60

Mycorrhi = 0
N @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/     Lower    Center     Upper
1        0         -0.04570   0.05427    0.1542
1        1          0.15212   0.25214    0.3522

Mycorrhi*N @ 75g/  ---+---------+---------+---------+---
1        0           (----*----)
1        1                     (----*----)
                   ---+---------+---------+---------+---
                     0.00      0.20      0.40      0.60

Mycorrhi = 1
N @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/     Lower    Center     Upper
1        1          0.09783    0.1979    0.2979

Mycorrhi*N @ 75g/  ---+---------+---------+---------+---
1        1                 (----*----)
                   ---+---------+---------+---------+---
                     0.00      0.20      0.40      0.60

Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/


Mycorrhi = 0
```

N @ 75g/ = 0 subtracted from:

Level Mycorrhi*N	@ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0.1938	0.03853	5.029	0.0000
1	0	0.2480	0.03853	6.438	0.0000
1	1	0.4459	0.03855	11.567	0.0000

Mycorrhi = 0
N @ 75g/ = 1 subtracted from:

Level Mycorrhi*N	@ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0.05427	0.03800	1.428	0.4864
1	1	0.25214	0.03802	6.631	0.0000

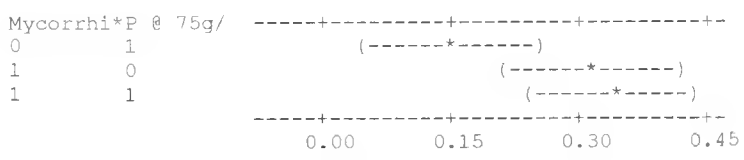
Mycorrhi = 1
N @ 75g/ = 0 subtracted from:

Level Mycorrhi*N	@ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	0.1979	0.03803	5.203	0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*P @ 75g/

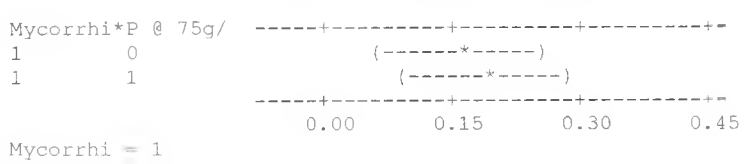
Mycorrhi = 0
P @ 75g/ = 0 subtracted from:

Mycorrhi*P	@ 75g/	Lower	Center	Upper
0	1	0.04939	0.1507	0.2521
1	0	0.21010	0.3115	0.4128
1	1	0.23810	0.3395	0.4408



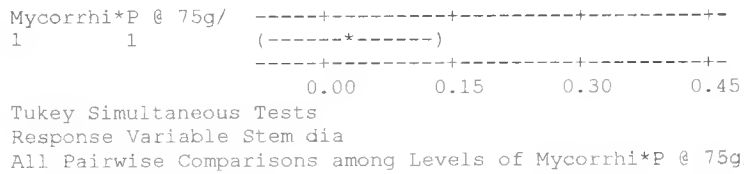
Mycorrhi = 0
P @ 75g/ = 1 subtracted from:

Mycorrhi*P	@ 75g/	Lower	Center	Upper
1	0	0.06075	0.1607	0.2607
1	1	0.08875	0.1887	0.2887



Mycorrhi = 1
P @ 75g/ = 0 subtracted from:

Mycorrhi*P	@ 75g/	Lower	Center	Upper
1	1	-0.07197	0.02800	0.1280



Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*P @ 75g/

Mycorrhi = 0
P @ 75g/ = 0 subtracted from:

Level Mycorrhi*P	@ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0.1507	0.03853	3.912	0.0012
1	0	0.3115	0.03853	8.083	0.0000
1	1	0.3395	0.03853	8.809	0.0000

Mycorrhi = 0

```
P @ 75g/ = 1 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*P @ 75g/        of Means  Difference   T-Value    P-Value
1        0                0.1607     0.03801     4.229     0.0004
1        1                0.1887     0.03801     4.966     0.0000

Mycorrhi = 1
P @ 75g/ = 0 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*P @ 75g/        of Means  Difference   T-Value    P-Value
1        1               0.02800     0.03800    0.7368     0.8819

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of N @ 75g/*P @ 75g/

N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

N @ 75g/*P @ 75g/     Lower    Center     Upper
0        1          0.03824    0.1397    0.2411
1        0          0.14474    0.2461    0.3475
1        1          0.18382    0.2852    0.3866

N @ 75g/*P @ 75g/  -----+---------+---------+---------+-
0        1                 (-----*------)
1        0                       (-----*------)
1        1                         (------*------)
                   -----+---------+---------+---------+-
                      0.00      0.15      0.30      0.45

N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

N @ 75g/*P @ 75g/     Lower    Center     Upper
1        0          0.006470    0.1064    0.2064
1        1          0.045288    0.1455    0.2457

N @ 75g/*P @ 75g/  -----+---------+---------+---------+-
1        0               (------*------)
1        1                 (------*-----)
                   -----+---------+---------+---------+-
                      0.00      0.15      0.30      0.45

N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

N @ 75g/*P @ 75g/     Lower    Center     Upper
1        1          -0.06106   0.03906    0.1392

N @ 75g/*P @ 75g/  -----+---------+---------+---------+-
1        1           (------*-----)
                   -----+---------+---------+---------+-
                      0.00      0.15      0.30      0.45

Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of N @ 75g/*P @ 75g/

N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Level                  Difference       SE of              Adjusted
N @ 75g/*P @ 75g/        of Means  Difference   T-Value    P-Value
0        1                0.1397     0.03857     3.622     0.0030
1        0                0.2461     0.03855     6.386     0.0000
1        1                0.2852     0.03854     7.400     0.0000

N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Level                  Difference       SE of              Adjusted
N @ 75g/*P @ 75g/        of Means  Difference   T-Value    P-Value
1        0                0.1064     0.03801     2.801     0.0326
1        1                0.1455     0.03810     3.819     0.0016
```

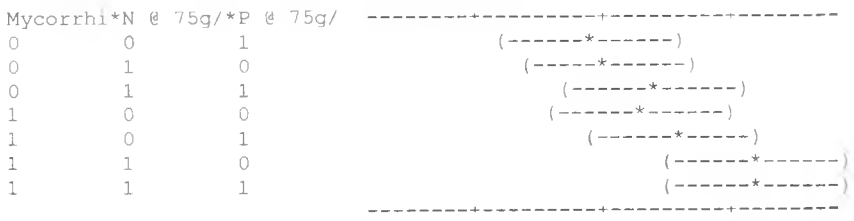
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Level N @ 75g/*P @ 75g/		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	0.03906	0.03806	1.026	0.7347

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/*P @ 75g/

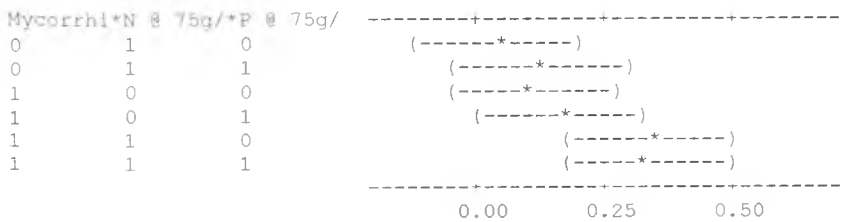
Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
0	0	1	0.04605	0.2187	0.3914
0	1	0	0.08904	0.2618	0.4345
0	1	1	0.17193	0.3445	0.5171
1	0	0	0.15450	0.3271	0.4997
1	0	1	0.21505	0.3877	0.5604
1	1	0	0.38501	0.5576	0.7302
1	1	1	0.38033	0.5529	0.7256



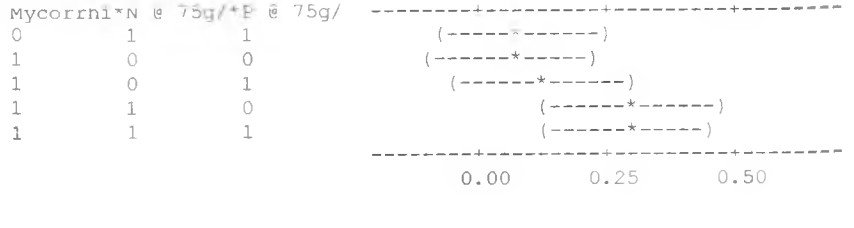
Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
0	1	0	-0.1250	0.04303	0.2110
0	1	1	-0.0423	0.12579	0.2939
1	0	0	-0.0597	0.10836	0.2764
1	0	1	0.0010	0.16900	0.3370
1	1	0	0.1708	0.33887	0.5069
1	1	1	0.1660	0.33422	0.5025



Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
0	1	1	-0.0854	0.08276	0.2509
1	0	0	-0.1028	0.06533	0.2334
1	0	1	-0.0420	0.12597	0.2940
1	1	0	0.1278	0.29584	0.4639
1	1	1	0.1229	0.29120	0.4595



```
Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/     Lower    Center     Upper
1        0        0          -0.1854  -0.01743    0.1506
1        0        1          -0.1249   0.04321    0.2113
1        1        0           0.0451   0.21308    0.3811
1        1        1           0.0404   0.20843    0.3764

Mycorrhi*N @ 75g/*P @ 75g/  --------+---------+---------+--------
1        0        0            (-----*------)
1        0        1              (------*-----)
1        1        0                     (------*-----)
1        1        1                     (-----*------)
                             --------+---------+---------+--------
                                    0.00      0.25      0.50

Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/     Lower    Center     Upper
1        0        1          -0.1074   0.06064    0.2287
1        1        0           0.0625   0.23051    0.3985
1        1        1           0.0578   0.22587    0.3939

Mycorrhi*N @ 75g/*P @ 75g/  --------+---------+---------+--------
1        0        1             (-----*------)
1        1        0                    (------*-----)
1        1        1                    (------*-----)
                             --------+---------+---------+--------
                                    0.00      0.25      0.50

Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/     Lower    Center     Upper
1        1        0          0.001831   0.1699    0.3379
1        1        1         -0.003025   0.1652    0.3335

Mycorrhi*N @ 75g/*P @ 75g/  --------+---------+---------+--------
1        1        0                   (------*------)
1        1        1                   (------*------)
                             --------+---------+---------+--------
                                    0.00      0.25      0.50

Mycorrhi = 1
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/     Lower    Center     Upper
1        1        1          -0.1727 -0.004644    0.1634

Mycorrhi*N @ 75g/*P @ 75g/  --------+---------+---------+--------
1        1        1           (------*------)
                             --------+---------+---------+--------
                                    0.00      0.25      0.50

Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/*P @ 75g/

Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Level                          Difference       SE of              Adjusted
Mycorrhi*N @ 75g/*P @ 75g/       of Means  Difference   T-Value    P-Value
0        0        1               0.2187     0.05525     3.959     0.0042
0        1        0               0.2618     0.05526     4.737     0.0003
0        1        1               0.3445     0.05522     6.239     0.0000
1        0        0               0.3271     0.05522     5.923     0.0000
1        0        1               0.3877     0.05525     7.018     0.0000
1        1        0               0.5576     0.05522    10.098     0.0000
1        1        1               0.5529     0.05523    10.012     0.0000
```

Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Level Mycorrhi	N @ 75g/	P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0	0.04303	0.05375	0.8006	0.9926
0	1	1	0.12579	0.05380	2.3383	0.2883
1	0	0	0.10836	0.05377	2.0152	0.4794
1	0	1	0.16900	0.05374	3.1445	0.0474
1	1	0	0.33887	0.05376	6.3028	0.0000
1	1	1	0.33422	0.05383	6.2086	0.0000

Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Level Mycorrhi	N @ 75g/	P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	1	0.08276	0.05381	1.538	0.7842
1	0	0	0.06533	0.05378	1.215	0.9249
1	0	1	0.12597	0.05375	2.344	0.2855
1	1	0	0.29584	0.05377	5.502	0.0000
1	1	1	0.29120	0.05385	5.408	0.0000

Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 1 subtracted from:

Level Mycorrhi	N @ 75g/	P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0	-0.01743	0.05375	-0.3244	1.0000
1	0	1	0.04321	0.05380	0.8032	0.9924
1	1	0	0.21308	0.05375	3.9641	0.0042
1	1	1	0.20843	0.05375	3.8779	0.0055

Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Level Mycorrhi	N @ 75g/	P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	1	0.06064	0.05377	1.128	0.9484
1	1	0	0.23051	0.05375	4.289	0.0014
1	1	1	0.22587	0.05376	4.201	0.0019

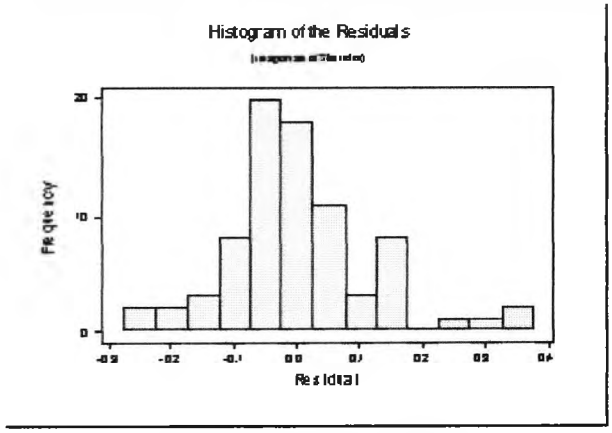
Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

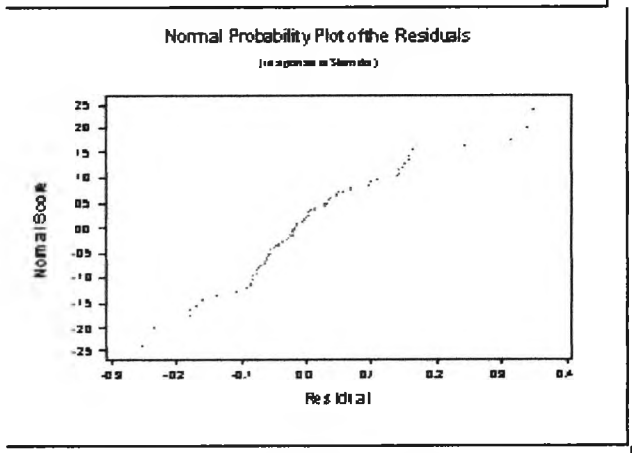
Level Mycorrhi	N @ 75g/	P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	0	0.1699	0.05376	3.159	0.0456
1	1	1	0.1652	0.05383	3.069	0.0578

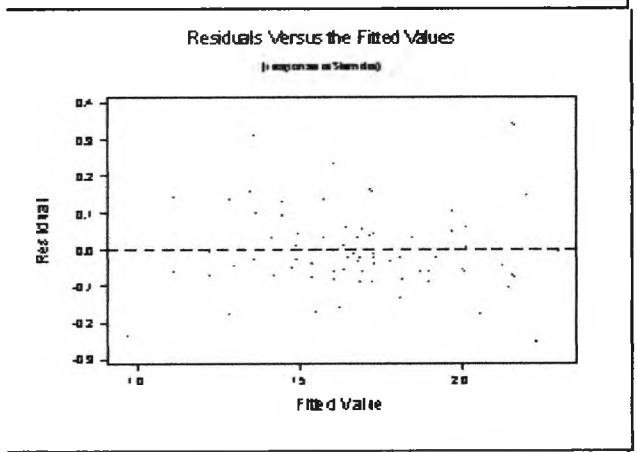
Mycorrhi = 1
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

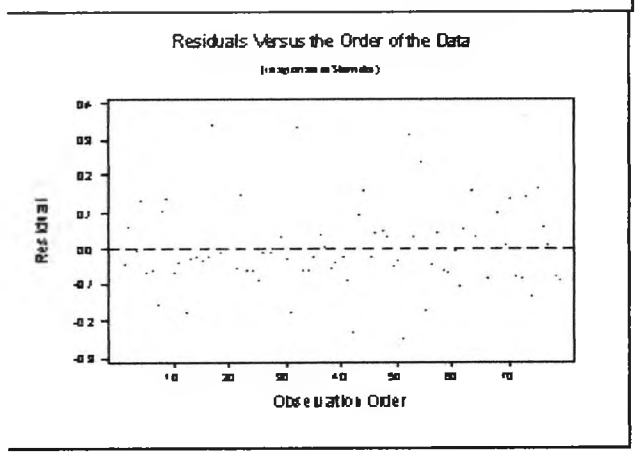
Level Mycorrhi	N @ 75g/	P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	1	-0.004644	0.05377	-0.08637	1.000

Reasonable normal distribution plots for the sixteen Birch trees per plot stem diameter measurements versus treatments data.









General Linear Model analysis of variance: 24 trees stem diameter versus treatments

```
Factor     Type Levels Values
Mycorrhi   fixed      2 0 1
N @ 75g/   fixed      2 0 1
P @ 75g/   fixed      2 0 1

Analysis of Variance for Stem dia, using Adjusted SS for Tests

Source                       DF     Seq SS     Adj SS     Adj MS       F      P
Stem dia                      1     7.4923     7.1293     7.1293  274.38  0.000
Mycorrhi                      1     1.2287     1.3037     1.3037   50.18  0.000
N @ 75g/                      1     0.8736     0.9049     0.9049   34.83  0.000
P @ 75g/                      1     0.3180     0.3263     0.3263   12.56  0.001
Mycorrhi*N @ 75g/             1     0.0000     0.0000     0.0000    0.00  0.987
Mycorrhi*P @ 75g/             1     0.0595     0.0611     0.0611    2.35  0.128
N @ 75g/*P @ 75g/             1     0.0026     0.0028     0.0028    0.11  0.741
Mycorrhi*N @ 75g/*P           1     0.0161     0.0161     0.0161    0.62  0.432
Error                       109     2.8322     2.8322     0.0260
Total                       117    12.8231

Term                          Coef   SE Coef        T      P
Constant                   0.77774   0.05260    14.79  0.000
Stem dia                    2.7401    0.1654    16.56  0.000

Unusual Observations for Stem dia

Obs  Stem dia       Fit      SE Fit  Residual   St Resid
 16    0.96000   1.45447     0.04574  -0.49447     -3.20R
 56    2.50000   2.02989     0.04451   0.47011      3.03R
 71    2.50000   2.12650     0.05514   0.37350      2.47R
 91    1.67000   1.22960     0.04801   0.44040      2.86R
 93    1.85000   1.50361     0.04227   0.34639      2.23R

R denotes an observation with a large standardized residual.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi

Mycorrhi = 0 subtracted from:

Mycorrhi     Lower    Center     Upper  -------+---------+---------+---------
1           0.1516    0.2105    0.2694  (----------------*----------------)
                                       -------+---------+---------+---------
                                            0.175     0.210     0.245


Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi

Mycorrhi = 0 subtracted from:

Level        Difference       SE of              Adjusted
Mycorrhi       of Means  Difference   T-Value    P-Value
1                0.2105     0.02972     7.083     0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of N @ 75g/

N @ 75g/ = 0 subtracted from:

N @ 75g/     Lower    Center     Upper  -------+---------+---------+---------
1           0.1165    0.1755    0.2344  (----------------*----------------)
                                       -------+---------+---------+---------
                                            0.140     0.175     0.210


Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of N @ 75g/
```

```
N @ 75g/ = 0 subtracted from:

Level        Difference       SE of              Adjusted
N @ 75g/       of Means  Difference   T-Value    P-Value
1                0.1755     0.02973     5.901     0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of P @ 75g/

P @ 75g/ = 0 subtracted from:

P @ 75g/     Lower    Center     Upper  -------+---------+---------+---------
1          0.04640    0.1053    0.1642  (-----------------*----------------)
                                        -------+---------+---------+---------
                                             0.070     0.105     0.140


Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of P @ 75g/

P @ 75g/ = 0 subtracted from:

Level        Difference       SE of              Adjusted
P @ 75g/       of Means  Difference   T-Value    P-Value
1                0.1053     0.02971     3.544     0.0006

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/

Mycorrhi = 0
N @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/     Lower    Center     Upper
0        1          0.06524    0.1759    0.2866
1        0          0.10031    0.2110    0.3217
1        1          0.27521    0.3860    0.4967

Mycorrhi*N @ 75g/  -----+---------+---------+---------+-
0        1                   (------*------)
1        0                     (------*------)
1        1                               (------*------)
                   -----+---------+---------+---------+-
                      0.00      0.16      0.32      0.48

Mycorrhi = 0
N @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/     Lower    Center     Upper
1        0         -0.07354   0.03506    0.1437
1        1          0.10143   0.21005    0.3187

Mycorrhi*N @ 75g/  -----+---------+---------+---------+-
1        0         (------*------)
1        1                   (------*------)
                   -----+---------+---------+---------+-
                      0.00      0.16      0.32      0.48

Mycorrhi = 1
N @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/     Lower    Center     Upper
1        1          0.06635    0.1750    0.2836

Mycorrhi*N @ 75g/  -----+---------+---------+---------+-
1        1                 (------*------)
                   -----+---------+---------+---------+-
                      0.00      0.16      0.32      0.48

Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/

Mycorrhi = 0
N @ 75g/ = 0 subtracted from:
```

Level Mycorrhi*N @ 75g/		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0.1759	0.04242	4.147	0.0004
1	0	0.2110	0.04242	4.974	0.0000
1	1	0.3860	0.04245	9.092	0.0000

Mycorrhi = 0
N @ 75g/ = 1 subtracted from:

Level Mycorrhi*N @ 75g/		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0.03506	0.04162	0.8424	0.8341
1	1	0.21005	0.04163	5.0458	0.0000

Mycorrhi = 1
N @ 75g/ = 0 subtracted from:

Level Mycorrhi*N @ 75g/		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	0.1750	0.04164	4.203	0.0003

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*P @ 75g/

Mycorrhi = 0
P @ 75g/ = 0 subtracted from:

Mycorrhi*P @ 75g/		Lower	Center	Upper
0	1	0.04020	0.1509	0.2615
1	0	0.14540	0.2561	0.3668
1	1	0.20514	0.3158	0.4265

```
Mycorrhi*P @ 75g/  ----+---------+---------+---------+--
0        1                (------*------)
1        0                       (------*------)
1        1                              (------*------)
                  ----+---------+---------+---------+--
                    0.00      0.15      0.30      0.45
```

Mycorrhi = 0
P @ 75g/ = 1 subtracted from:

Mycorrhi*P @ 75g/		Lower	Center	Upper
1	0	-0.003401	0.1052	0.2139
1	1	0.056345	0.1650	0.2736

```
Mycorrhi*P @ 75g/  ----+---------+---------+---------+--
1        0             (------*------)
1        1                    (------*------)
                  ----+---------+---------+---------+--
                    0.00      0.15      0.30      0.45
```

Mycorrhi = 1
P @ 75g/ = 0 subtracted from:

Mycorrhi*P @ 75g/		Lower	Center	Upper
1	1	-0.04887	0.05973	0.1683

```
Mycorrhi*P @ 75g/  ----+---------+---------+---------+--
1        1          (------*------)
                  ----+---------+---------+---------+--
                    0.00      0.15      0.30      0.45
```

Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*P @ 75g/

Mycorrhi = 0
P @ 75g/ = 0 subtracted from:

Level Mycorrhi*P @ 75g/		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0.1509	0.04241	3.557	0.0031
1	0	0.2561	0.04242	6.037	0.0000
1	1	0.3158	0.04242	7.446	0.0000

```
Mycorrhi = 0
P @ 75g/ = 1 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*P @ 75g/       of Means  Difference   T-Value     P-Value
1        0                0.1052     0.04163     2.528      0.0613
1        1                0.1650     0.04162     3.963      0.0008

Mycorrhi = 1
P @ 75g/ = 0 subtracted from:

Level                  Difference       SE of              Adjusted
Mycorrhi*P @ 75g/       of Means  Difference   T-Value     P-Value
1        1               0.05973     0.04162     1.435      0.4805

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of N @ 75g/*P @ 75g/

N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

N @ 75g/*P @ 75g/     Lower    Center     Upper
0        1         0.004319    0.1151    0.2260
1        0         0.074623    0.1853    0.2960
1        1         0.170069    0.2808    0.3914

N @ 75g/*P @ 75g/  ----+---------+---------+---------+--
0        1             (---------*---------)
1        0                       (---------*---------)
1        1                                 (---------*---------)
                   ----+---------+---------+---------+--
                     0.00      0.12      0.24      0.36

N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

N @ 75g/*P @ 75g/     Lower    Center     Upper
1        0         -0.03849   0.07017    0.1788
1        1          0.05670   0.16561    0.2745

N @ 75g/*P @ 75g/  ----+---------+---------+---------+--
1        0           (---------*---------)
1        1                     (---------*---------)
                   ----+---------+---------+---------+--
                     0.00      0.12      0.24      0.36

N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

N @ 75g/*P @ 75g/     Lower    Center     Upper
1        1         -0.01325   0.09544    0.2041

N @ 75g/*P @ 75g/  ----+---------+---------+---------+--
1        1             (---------*---------)
                   ----+---------+---------+---------+--
                     0.00      0.12      0.24      0.36
Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of N @ 75g/*P @ 75g/

N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Level                  Difference       SE of              Adjusted
N @ 75g/*P @ 75g/       of Means  Difference   T-Value     P-Value
0        1                0.1151     0.04248     2.711      0.0385
1        0                0.1853     0.04242     4.368      0.0002
1        1                0.2808     0.04242     6.618      0.0000

N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Level                  Difference       SE of              Adjusted
N @ 75g/*P @ 75g/       of Means  Difference   T-Value     P-Value
1        0               0.07017     0.04164     1.685      0.3366
1        1               0.16561     0.04174     3.968      0.0008
```

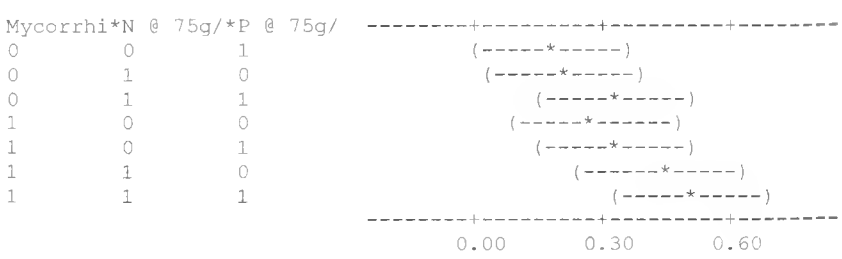
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Level		Difference	SE of		Adjusted
N @ 75g/*P @ 75g/		of Means	Difference	T-Value	P-Value
1	1	0.09544	0.04166	2.291	0.1064

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/*P @ 75g/

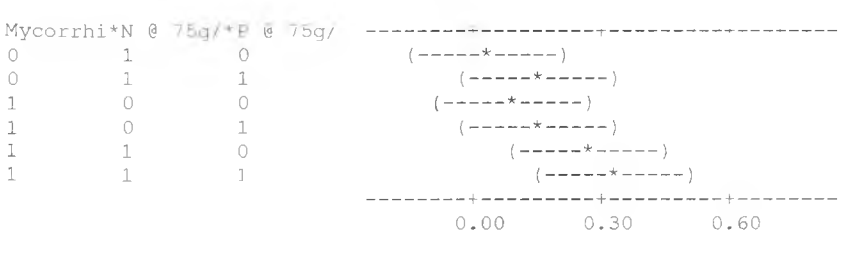
Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
0	0	1	-0.004688	0.1841	0.3730
0	1	0	0.020447	0.2092	0.3980
0	1	1	0.138029	0.3268	0.5155
1	0	0	0.091200	0.2800	0.4688
1	0	1	0.137312	0.3261	0.5150
1	1	0	0.252657	0.4414	0.6301
1	1	1	0.325851	0.5147	0.7035



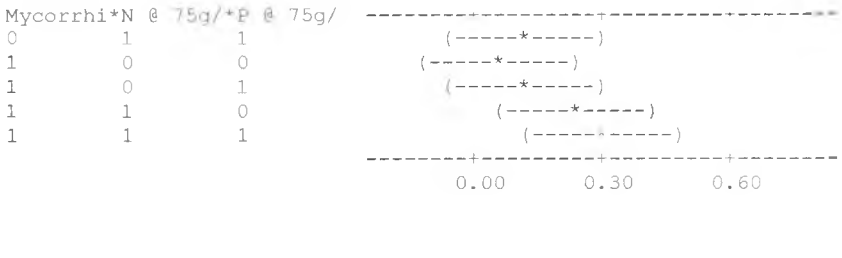
Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
0	1	0	-0.1568	0.02507	0.2070
0	1	1	-0.0394	0.14265	0.3247
1	0	0	-0.0863	0.09584	0.2779
1	0	1	-0.0399	0.14200	0.3239
1	1	0	0.0753	0.25727	0.4392
1	1	1	0.1483	0.33056	0.5128



Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/			Lower	Center	Upper
0	1	1	-0.0643	0.11759	0.2995
1	0	0	-0.1112	0.07077	0.2527
1	0	1	-0.0650	0.11693	0.2988
1	1	0	0.0503	0.23220	0.4141
1	1	1	0.1234	0.30549	0.4876



```
Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/     Lower    Center     Upper
1        0        0          -0.2287  -0.04681    0.1351
1        0        1          -0.1827  -0.00065    0.1814
1        1        0          -0.0673   0.11461    0.2965
1        1        1           0.0060   0.18791    0.3698

Mycorrhi*N @ 75g/*P @ 75g/  --------+---------+---------+--------
1        0        0         (-----*------)
1        0        1           (-----*-----)
1        1        0               (-----*-----)
1        1        1                 (-----*-----)
                            --------+---------+---------+--------
                                   0.00      0.30      0.60


Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/     Lower    Center     Upper
1        0        1          -0.1359   0.04616    0.2283
1        1        0          -0.0205   0.16143    0.3433
1        1        1           0.0528   0.23472    0.4166

Mycorrhi*N @ 75g/*P @ 75g/  --------+---------+---------+--------
1        0        1            (------*-----)
1        1        0                (-----*-----)
1        1        1                  (-----*-----)
                            --------+---------+---------+--------
                                   0.00      0.30      0.60


Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/     Lower    Center     Upper
1        1        0         -0.06669    0.1153    0.2972
1        1        1          0.00631    0.1886    0.3708

Mycorrhi*N @ 75g/*P @ 75g/  --------+---------+---------+--------
1        1        0              (-----*-----)
1        1        1                (-----*-----)
                            --------+---------+---------+--------
                                   0.00      0.30      0.60


Mycorrhi = 1
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Mycorrhi*N @ 75g/*P @ 75g/     Lower    Center     Upper
1        1        1          -0.1087   0.07329    0.2553

Mycorrhi*N @ 75g/*P @ 75g/  --------+---------+---------+--------
1        1        1             (-----*------)
                            --------+---------+---------+--------
                                   0.00      0.30      0.60


Tukey Simultaneous Tests
Response Variable Stem dia
All Pairwise Comparisons among Levels of Mycorrhi*N @ 75g/*P @ 75g/

Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Level                          Difference       SE of              Adjusted
Mycorrhi*N @ 75g/*P @ 75g/       of Means  Difference   T-Value     P-Value
0        0        1               0.1841     0.06111     3.013      0.0616
0        1        0               0.2092     0.06109     3.425      0.0190
0        1        1               0.3268     0.06109     5.350      0.0000
1        0        0               0.2800     0.06109     4.583      0.0003
1        0        1               0.3261     0.06111     5.337      0.0000
1        1        0               0.4414     0.06108     7.226      0.0000
1        1        1               0.5147     0.06111     8.422      0.0000
```

Mycorrhi = 0
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

Level Mycorrhi*N @ 75g/	*P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	0	0.02507	0.05887	0.4258	0.9999
0	1	0.14265	0.05891	2.4213	0.2418
1	0	0.09584	0.05893	1.6263	0.7335
1	0	0.14200	0.05886	2.4125	0.2460
1	1	0.25727	0.05888	4.3691	0.0007
1	1	0.33056	0.05898	5.6047	0.0000

Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

Level Mycorrhi*N @ 75g/	*P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
0	1	0.11759	0.05888	1.997	0.4886
1	0	0.07077	0.05889	1.202	0.9298
1	0	0.11693	0.05887	1.986	0.4958
1	1	0.23220	0.05886	3.945	0.0035
1	1	0.30549	0.05892	5.185	0.0000

Mycorrhi = 0
N @ 75g/ = 1
P @ 75g/ = 1 subtracted from:

Level Mycorrhi*N @ 75g/	*P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	-0.04681	0.05886	-0.7953	0.9930
1	0	-0.00065	0.05891	-0.0111	1.0000
1	1	0.11461	0.05887	1.9470	0.5222
1	1	0.18791	0.05887	3.1918	0.0378

Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 0 subtracted from:

Level Mycorrhi*N @ 75g/	*P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0.04616	0.05893	0.7833	0.9937
1	1	0.16143	0.05887	2.7420	0.1214
1	1	0.23472	0.05887	3.9873	0.0030

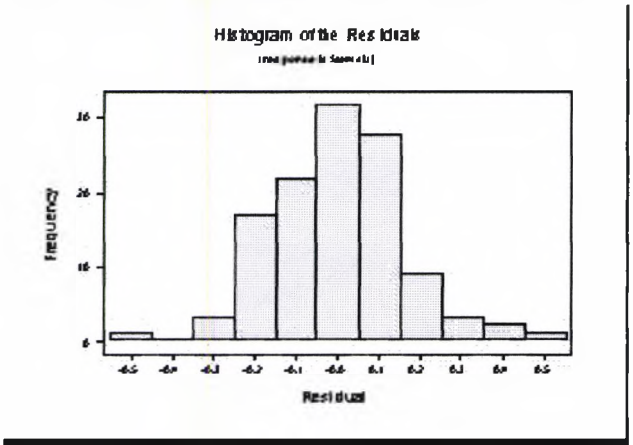
Mycorrhi = 1
N @ 75g/ = 0
P @ 75g/ = 1 subtracted from:

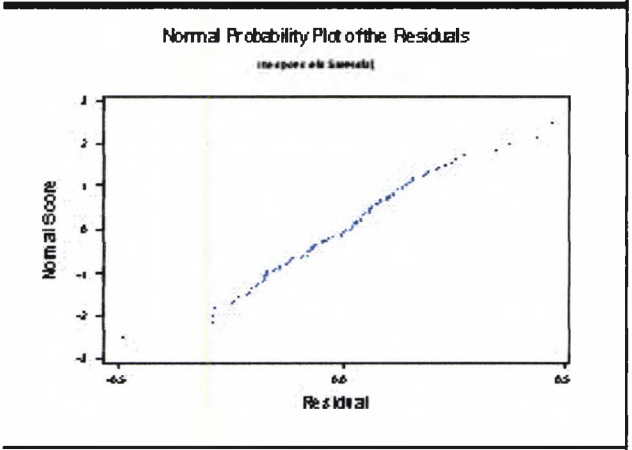
Level Mycorrhi*N @ 75g/	*P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0	0.1153	0.05888	1.958	0.5151
1	1	0.1886	0.05898	3.197	0.0372

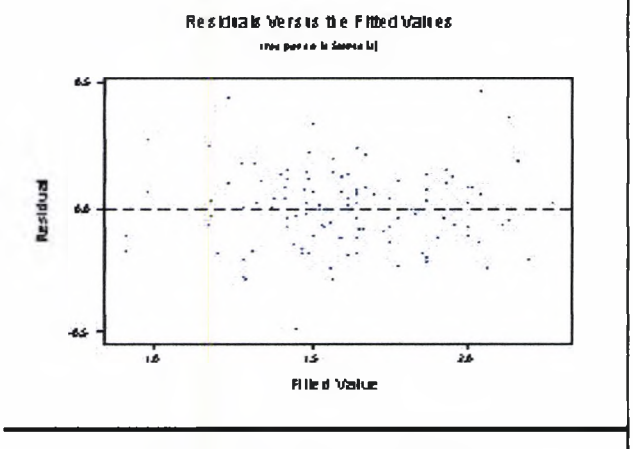
Mycorrhi = 1
N @ 75g/ = 1
P @ 75g/ = 0 subtracted from:

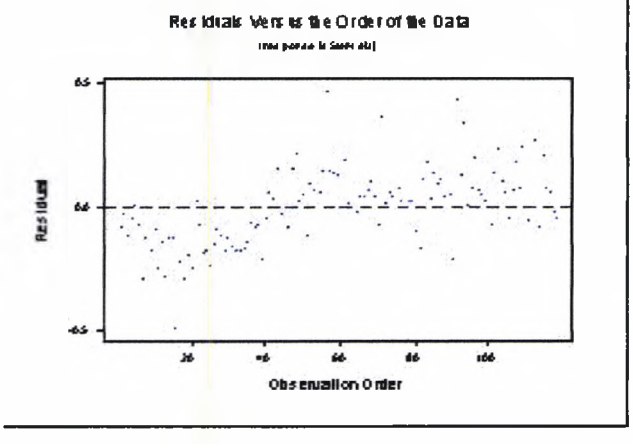
Level Mycorrhi*N @ 75g/	*P @ 75g/	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	1	0.07329	0.05890	1.244	0.9166

Reasonable normal distribution plots for the twenty four Birch trees per plot stem diameter measurements versus treatments data.









General Linear Model analysis of variance for the leaf rosette diameter data from the Betteshanger *B. pendula* and *L. corniculatus* experiment. Experimental units without a mycorrhizal treatment have been omitted from this analysis as no *L. corniculatus* germinated in these units.

Abbreviations: P = Phosphorus @ 75g/m^2
N = Nitrogen @ 75g/m^2
B. pendu = *B. pendula*
L. corni = *L. corniculatus*

Factor	Type	Levels	Values
B. pendu	fixed	3	0 8 16
L. corni	fixed	3	8 16 24
P	fixed	2	0 1
N	fixed	2	0 1

Analysis of Variance for Rosette, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
B. pendu	2	281.031	342.813	171.406	190.42	0.000
L. corni	2	85.586	85.586	42.793	47.54	0.000
P	1	5.954	5.954	5.954	6.61	0.012
N	1	40.196	40.196	40.196	44.65	0.000
P*N	1	0.960	0.960	0.960	1.07	0.304
Error	92	82.813	82.813	0.900		
Total	99	496.540				

Unusual Observations for Rosette

Obs	Rosette	Fit	SE Fit	Residual	St Resid
1	5.1000	7.2500	0.2683	-2.1500	-2.36R
7	7.2000	5.3550	0.2683	1.8450	2.03R
28	9.5000	7.5420	0.2683	1.9580	2.15R
64	13.2000	11.3720	0.2683	1.8280	2.01R
66	13.4000	11.4570	0.2683	1.9430	2.14R

R denotes an observation with a large standardized residual.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Rosette
All Pairwise Comparisons among Levels of P

P = 0 subtracted from:

P	Lower	Center	Upper	------+---------+---------+---------+
1	0.1111	0.4880	0.8649	(---------------*--------------)
				------+---------+---------+---------+
				0.25 0.50 0.75 1.00

Tukey Simultaneous Tests
Response Variable Rosette
All Pairwise Comparisons among Levels of P

P = 0 subtracted from:

Level P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0.4880	0.1898	2.572	0.0117

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Rosette
All Pairwise Comparisons among Levels of N

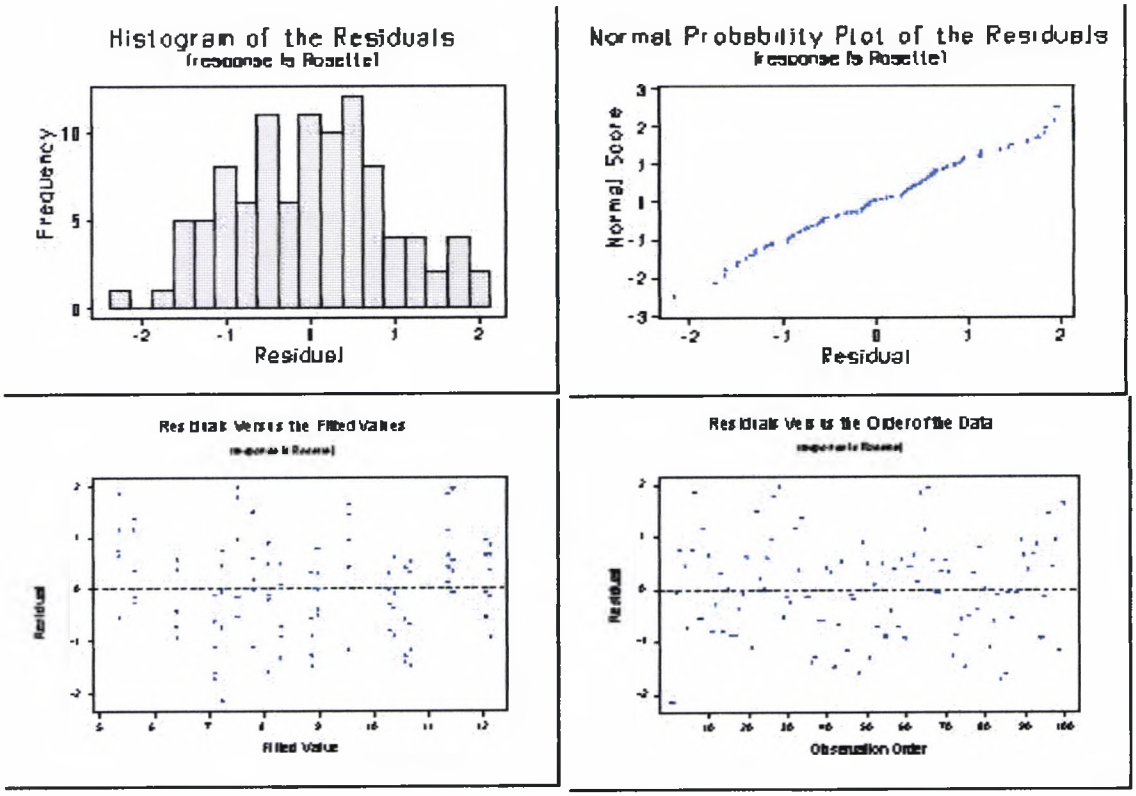
N = 0 subtracted from:

N	Lower	Center	Upper	-----+---------+---------+---------+-
1	0.8911	1.268	1.645	(--------------*--------------)
				-----+---------+---------+---------+-
				1.00 1.25 1.50 1.75

Tukey Simultaneous Tests
Response Variable Rosette
All Pairwise Comparisons among Levels of N
N = 0 subtracted from:

```
Level Difference       SE of              Adjusted
N       of Means  Difference   T-Value     P-Value
1          1.268      0.1898     6.682      0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Rosette
All Pairwise Comparisons among Levels of P*N

P = 0
N = 0 subtracted from:

P*N     Lower    Center     Upper  ---+---------+---------+---------+---
0 1    0.3699    1.0720    1.7741                  (-----*-----)
1 0   -0.4101    0.2920    0.9941            (----*-----)
1 1    1.0539    1.7560    2.4581                       (-----*----)
                                  ---+---------+---------+---------+---
                                   -1.2       0.0       1.2       2.4


P = 0
N = 1 subtracted from:

P*N     Lower    Center     Upper  ---+---------+---------+---------+---
1 0   -1.482   -0.7800  -0.07792   (----*-----)
1 1   -0.018    0.6840   1.38608             (-----*-----)
                                  ---+---------+---------+---------+---
                                   -1.2       0.0       1.2       2.4


P = 1
N = 0 subtracted from:

P*N     Lower    Center     Upper  ---+---------+---------+---------+---
1 1    0.7619     1.464     2.166                     (-----*-----)
                                  ---+---------+---------+---------+---
                                   -1.2       0.0       1.2       2.4


Tukey Simultaneous Tests
Response Variable Rosette
All Pairwise Comparisons among Levels of P*N

P = 0
N = 0 subtracted from:

Level   Difference       SE of              Adjusted
P*N       of Means  Difference   T-Value     P-Value
0 1         1.0720      0.2683     3.995      0.0008
1 0         0.2920      0.2683     1.088      0.6977
1 1         1.7560      0.2683     6.544      0.0000

P = 0
N = 1 subtracted from:

Level   Difference       SE of              Adjusted
P*N       of Means  Difference   T-Value     P-Value
1 0        -0.7800      0.2683    -2.907      0.0233
1 1         0.6840      0.2683     2.549      0.0591

P = 1
N = 0 subtracted from:

Level   Difference       SE of              Adjusted
P*N       of Means  Difference   T-Value     P-Value
1 1           1.464      0.2683     5.456      0.0000
```

Reasonable normal distribution plots for the leaf rosette data from the Betteshanger *B. pendula* and *L. corniculatus* multi-factor experiment. Experimental units without a mycorrhizal treatment have been omitted from this analysis.



Analysis of variance (General Linear Model) of the number of *L. corniculatus* flower heads per experimental unit (from the Betteshanger *B. pendula* and *L. corniculatus* experiment). Experimental units without a mycorrhizal treatment have been omitted from this analysis as no *L. corniculatus* germinated in these units.

Abbreviations: P = Phosphorus @ 75g/m^2
N = Nitrogen @ 75g/m^2

Factor	Type	Levels	Values
Birch density	fixed	3	0 8 16
Lotus density	fixed	3	8 16 24
P	fixed	2	0 1
N	fixed	2	0 1

Analysis of Variance for Flower h, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Birch	2	806.51	446.62	223.31	68.80	0.000
Lotus	2	156.23	156.23	78.12	24.07	0.000
P	1	259.21	259.21	259.21	79.86	0.000
N	1	16.81	16.81	16.81	5.18	0.025
P*N	1	8.41	8.41	8.41	2.59	0.111
Error	92	298.62	298.62	3.25		
Total	99	1545.79				

Expected Mean Squares, using Adjusted SS

Source	Expected Mean Square for Each Term
1 Birch	(6) + Q[1]
2 Lotus	(6) + Q[2]
3 P	(6) + Q[3, 5]
4 N	(6) + Q[4, 5]
5 P*N	(6) + Q[5]
6 Error	(6)

Error Terms for Tests, using Adjusted SS

Source	Error DF	Error MS	Synthesis of Error MS
1 Birch	92.00	3.25	(6)
2 Lotus	92.00	3.25	(6)
3 P	92.00	3.25	(6)
4 N	92.00	3.25	(6)
5 P*N	92.00	3.25	(6)

Variance Components, using Adjusted SS

Source	Estimated Value
Error	3.246

Least Squares Means for Flower h

	Mean	SE Mean
Birch		
0	6.833	0.2326
8	2.033	0.5201
16	2.183	0.5201
Lotus		
8	1.500	0.3553
16	4.200	0.3553
24	5.350	0.4842
P		
0	2.073	0.2942
1	5.293	0.2942
N		
0	3.273	0.2942
1	4.093	0.2942
P*N		
0 0	1.953	0.3892
0 1	2.193	0.3892
1 0	4.593	0.3892
1 1	5.993	0.3892

Tukey 95.0% Simultaneous Confidence Intervals. Response Variable Flower heads per experimental unit. All Pairwise Comparisons among Levels of Birch density
Birch = 0 subtracted from:

Birch	Lower	Center	Upper	-----+---------+---------+---------+-
8	-6.158	-4.800	-3.442	(-----*----)
16	-6.008	-4.650	-3.292	(----*-----)
				-----+---------+---------+---------+-
				-5.0 -2.5 0.0 2.5

Birch = 8 subtracted from:

Birch	Lower	Center	Upper	-----+---------+---------+---------+-
16	-1.770	0.1500	2.070	(-------*------)
				-----+---------+---------+---------+-
				-5.0 -2.5 0.0 2.5

Tukey Simultaneous Tests
Response Variable Flower heads per experimental unit
All Pairwise Comparisons among Levels of Birch de

Birch = 0 subtracted from:

Level Birch	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
8	-4.800	0.5697	-8.425	0.0000
16	-4.650	0.5697	-8.162	0.0000

Birch = 8 subtracted from:

Level Birch	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
16	0.1500	0.8057	0.1862	0.9811

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Flower heads per experimental unit. All Pairwise Comparisons among Levels of Lotus/pl

Lotus/pl = 8 subtracted from:

Lotus/pl	Lower	Center	Upper	--+---------+---------+---------+----
16	1.342	2.700	4.058	(--------*--------)
24	2.492	3.850	5.208	(--------*--------)
				--+---------+---------+---------+----
				0.0 1.5 3.0 4.5

Lotus/pl = 16 subtracted from:

Lotus/pl	Lower	Center	Upper	--+---------+---------+---------+----
24	-0.2076	1.150	2.508	(--------*--------)
				--+---------+---------+---------+----
				0.0 1.5 3.0 4.5

Tukey Simultaneous Tests
Response Variable Flower heads per experimental unit. All Pairwise Comparisons among Levels of Lotus density per experimental unit.

Lotus/pl = 8 subtracted from:

Level Lotus/pl	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
16	2.700	0.5697	4.739	0.0000
24	3.850	0.5697	6.758	0.0000

Lotus/pl = 16 subtracted from:

Level Lotus/pl	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
24	1.150	0.5697	2.019	0.1135

Tukey 95.0% Simultaneous Confidence Intervals Response Variable Flower heads per experimental unit. All Pairwise Comparisons among Levels of Phosphorus

P = 0 subtracted from:

```
P     Lower    Center     Upper  --------+---------+---------+--------
1     2.504     3.220     3.936   (-----------------*-----------------
                                 --------+---------+---------+--------
                                       2.80      3.20      3.60
```

Tukey Simultaneous Tests
Response Variable Flower heads per experimental unit.
All Pairwise Comparisons among Levels of P

P = 0 subtracted from:

Level P	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	3.220	0.3603	8.936	0.0000

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Flower h
All Pairwise Comparisons among Levels of N

N = 0 subtracted from:

```
N     Lower    Center     Upper  --------+---------+---------+--------
1    0.1044    0.8200     1.536   (-----------------*-----------------)
                                 --------+---------+---------+--------
                                       0.40      0.80      1.20
```

Tukey Simultaneous Tests
Response Variable Flower h
All Pairwise Comparisons among Levels of N

N = 0 subtracted from:

Level N	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	0.8200	0.3603	2.276	0.0252

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable Flower h
All Pairwise Comparisons among Levels of P*N

P = 0
N = 0 subtracted from:

```
P*N     Lower    Center     Upper  ------+---------+---------+---------+
0 1    -1.093    0.2400     1.573   (-----*------)
1 0     1.307    2.6400     3.973               (-----*------)
1 1     2.707    4.0400     5.373                     (-----*------)
                                 ------+---------+---------+---------+
                                      0.0       2.0       4.0       6.0
```

P = 0
N = 1 subtracted from:

```
P*N     Lower    Center     Upper  ------+---------+---------+---------+
1 0     1.067     2.400     3.733            (------*------)
1 1     2.467     3.800     5.133                  (------*------)
                                 ------+---------+---------+---------+
                                      0.0       2.0       4.0       6.0
```

P = 1
N = 0 subtracted from:

```
P*N     Lower    Center     Upper  ------+---------+---------+---------+
1 1   0.06679     1.400     2.733      (------*------)
                                 ------+---------+---------+---------+
                                      0.0       2.0       4.0       6.0
```

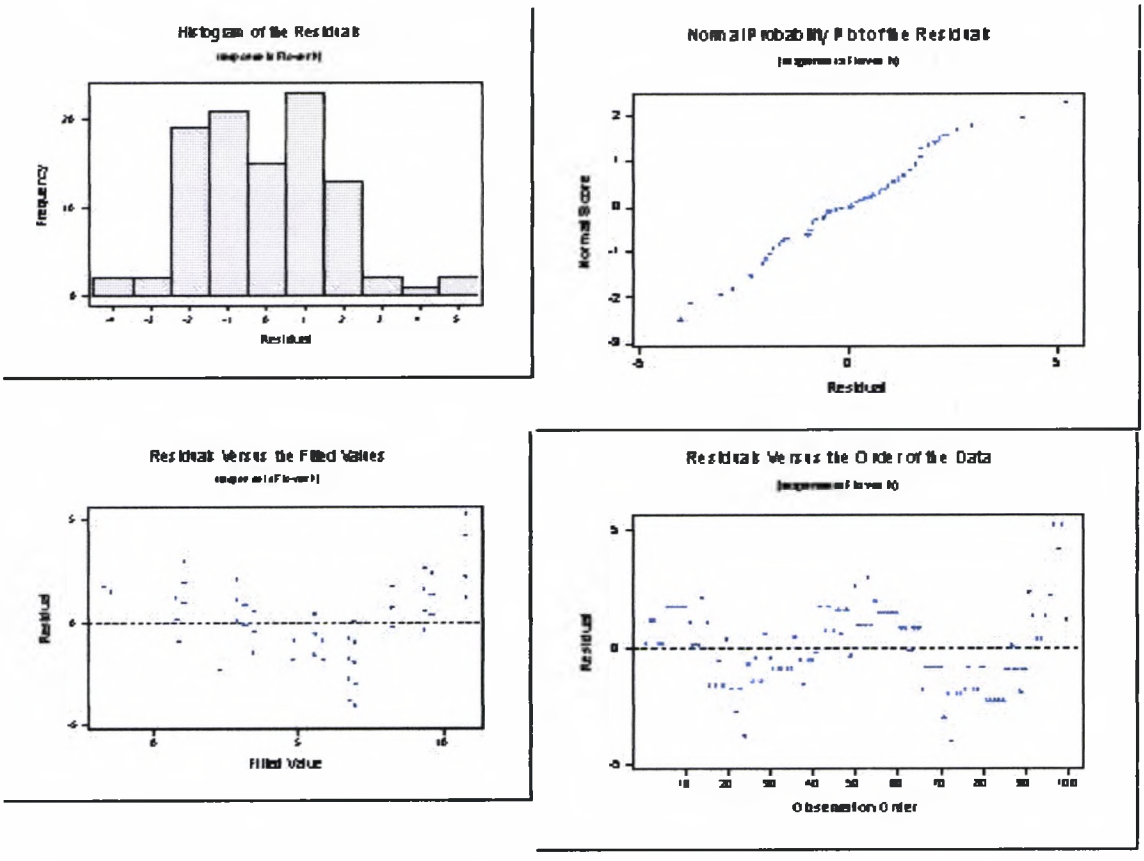
Tukey Simultaneous Tests
Response Variable Flower heads per experimental unit.
All Pairwise Comparisons among Levels of P*N

P = 0
N = 0 subtracted from:

Level	Difference	SE of	Adjusted

```
P*N       of Means  Difference   T-Value     P-Value
0 1         0.2400      0.5096    0.4710      0.9653
1 0         2.6400      0.5096    5.1808      0.0000
1 1         4.0400      0.5096    7.9281      0.0000

P = 0
N = 1 subtracted from:

Level    Difference       SE of              Adjusted
P*N        of Means  Difference   T-Value     P-Value
1 0           2.400      0.5096     4.710      0.0001
1 1           3.800      0.5096     7.457      0.0000

P = 1
N = 0 subtracted from:

Level    Difference       SE of              Adjusted
P*N        of Means  Difference   T-Value     P-Value
1 1           1.400      0.5096     2.747      0.0358
```

Reasonable normal distribution plots for the number of flower heads per experimental unit from the Betteshanger *B. pendula* and *L. corniculatus* multi-factor experiment.



6.5.1 Principal Component Analyses and Hierarchical Cluster Analyses for AMF species extracted from the chronosequence at Stodmarsh.

Key

	AMF
A. morro	*Acaulospora morrowiae*
E. infre	*Entophopora infrequens*
G. claro	*Glomus claroideum*
G. coron	*Glomus coronatum*
G. etuni	*Glomus etunicatum*
G. fasci	*Glomus fasciculatum*
G. geosp	*Glomus geosporum*
G. intra	*Glomus intraradices*
G. micro	*Glomus microaggregatum*
G. mosse	*Glomus mosseae*
G. occul	*Glomus occultum*

Principal Component Analysis AMF species extracted from AMF culture traps with *L. corniculatus* as the host plant.

Eigenanalysis of the Correlation Matrix

Eigenvalue	2.5644	1.4645	1.1383	0.9654	0.7484	0.5753
Proportion	0.321	0.183	0.142	0.121	0.094	0.072
Cumulative	0.321	0.504	0.646	0.767	0.860	0.932

Eigenvalue	0.3289	0.2148
Proportion	0.041	0.027
Cumulative	0.973	1.000

Variable	PC1	PC2	PC3	PC4	PC5	PC6
A. morro	0.414	0.485	-0.009	0.170	-0.067	-0.087
E. infre	0.390	0.440	0.257	0.008	0.219	-0.430
G. fasci	0.560	-0.082	0.079	-0.116	0.002	0.248
G. geosp	-0.189	-0.176	0.719	-0.334	0.367	-0.223
G. intra	0.159	-0.441	-0.248	0.544	0.565	-0.305
G. micro	-0.457	0.296	-0.008	0.305	-0.248	-0.479
G. mosse	-0.110	0.229	-0.581	-0.606	0.415	-0.181
G. occul	-0.287	0.447	0.113	0.299	0.510	0.584

Hierarchical Cluster Analysis of Variables AMF species extracted from AMF culture traps with *L. corniculatus* as the host plant.

Correlation Coefficient Distance, Single Linkage

Amalgamation Steps

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of Obs in new cluster
1	7	80.16	0.397	1	2	1	2
2	6	72.84	0.543	1	3	1	3
3	5	68.66	0.627	6	8	6	2
4	4	58.06	0.839	1	5	1	4
5	3	53.97	0.921	6	7	6	3
6	2	53.97	0.921	4	6	4	4
7	1	49.28	1.014	1	4	1	8

Final Partition

Cluster 1
A. morro E. infre G. fasci

Cluster 2
G. geosp

Cluster 3
G. intra

Cluster 4
G. micro G. occul

Cluster 5
G. mosse

Principal Component Analysis of AMF species extracted from AMF culture traps with *Hieraceum pilosella* as the host plant.

Eigenanalysis of the Correlation Matrix

Eigenvalue	3.5898	1.6156	1.2576	1.0114	0.9677	0.7524
Proportion	0.326	0.147	0.114	0.092	0.088	0.068
Cumulative	0.326	0.473	0.588	0.679	0.767	0.836
Eigenvalue	0.6297	0.4529	0.3542	0.2275	0.1411	
Proportion	0.057	0.041	0.032	0.021	0.013	
Cumulative	0.893	0.934	0.966	0.987	1.000	

Variable	PC1	PC2	PC3	PC4	PC5	PC6
A. morro	0.273	-0.070	-0.163	-0.491	-0.435	-0.235
E. infre	0.377	-0.358	-0.216	-0.179	-0.014	-0.070
G. claro	0.192	-0.262	0.702	0.115	-0.141	0.013
G. coron	0.396	-0.131	0.047	0.339	-0.136	-0.111
G. etuni	0.422	-0.254	0.004	0.227	-0.131	0.072
G. fasci	0.100	0.509	-0.209	0.471	-0.262	-0.249
G. geosp	-0.104	-0.427	-0.291	0.394	0.364	-0.492
G. intra	0.322	0.139	0.063	0.204	0.395	0.514
G. micro	-0.382	-0.096	0.392	0.220	-0.338	-0.188
G. mosse	-0.195	-0.237	-0.377	0.278	-0.523	0.534
G. occul	-0.320	-0.437	-0.055	-0.049	0.105	0.183

Hierarchical Cluster Analysis of Variables from AMF species extracted from AMF culture traps with *Hieraceum pilosella* as the host plant.

Correlation Coefficient Distance, Single Linkage
Amalgamation Steps

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of Obs in new cluster
1	10	84.52	0.310	4	5	4	2
2	9	80.69	0.386	2	4	2	3
3	8	72.92	0.542	1	2	1	4
4	7	69.34	0.613	9	11	9	2
5	6	69.22	0.616	1	8	1	5
6	5	69.22	0.616	1	3	1	6
7	4	67.23	0.655	9	10	9	3
8	3	66.63	0.667	7	9	7	4
9	2	58.73	0.825	1	6	1	7
10	1	58.06	0.839	1	7	1	11

Final Partition

Cluster 1
A. morro E. infre G. claro G. coron G. etuni G. intra

Cluster 2
G. fasci

Cluster 3
G. geosp

Cluster 4
G. micro G. occul

Cluster 5
G. mosse

Principal Component Analysis from AMF species extracted from AMF culture traps with *Agrostis capillaris* as the host plant.

Eigenanalysis of the Correlation Matrix

Eigenvalue	4.2035	1.6124	0.9526	0.7707	0.6131	0.4521
Proportion	0.467	0.179	0.106	0.086	0.068	0.050
Cumulative	0.467	0.646	0.752	0.838	0.906	0.956

Eigenvalue	0.1688	0.1335	0.0934
Proportion	0.019	0.015	0.010
Cumulative	0.975	0.990	1.000

Variable	PC1	PC2	PC3	PC4	PC5	PC6
E. infre	-0.430	-0.158	-0.032	-0.134	0.197	-0.287
G. claro	-0.392	0.186	-0.199	-0.164	-0.518	-0.205
G. etuni	-0.296	-0.459	-0.332	-0.108	0.453	0.066
G. fasci	0.308	-0.405	0.127	0.065	-0.240	-0.777
G. geosp	-0.128	0.539	0.514	-0.251	0.413	-0.319
G.intrar	-0.094	-0.457	0.620	-0.476	-0.244	0.331
G. micro	-0.423	0.181	-0.100	-0.070	-0.408	0.029
G. mosse	-0.444	-0.169	0.075	0.134	0.164	-0.204
G. occul	-0.279	-0.077	0.411	0.792	-0.072	0.116

Hierarchical Cluster Analysis of Variables from AMF species extracted from AMF culture traps with *Agrostis capillaris* as the host plant.

Correlation Coefficient Distance, Single Linkage

Amalgamation Steps

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of Obs in new cluster
1	8	92.49	0.150	2	7	2	2
2	7	91.67	0.167	1	8	1	2
3	6	83.33	0.333	1	2	1	4
4	5	83.33	0.333	1	3	1	5
5	4	80.62	0.388	1	9	1	6
6	3	62.50	0.750	1	5	1	7
7	2	62.04	0.759	1	6	1	8
8	1	57.37	0.853	1	4	1	9

Final Partition

Cluster 1
E. infre G. claro G. etuni G. micro G. mosse

Cluster 2
G. fasci

Cluster 3
G. geosp

Cluster 4
G.intrar

Cluster 5
G. occul

7.1.1 Cricklade Meadow mix species list

Achillia millefolium	Yarrow
Agrostis tenuis	Common Bent Grass
Ajuga reptans	Bugle
Allium vineale	Crow Garlic
Alopecurus pratensis	Meadow Foxtail
Anthoxanthemum odoratum	Sweet Vernal Grass
Anthriscus sylvestris	Cow Parsley
Arctium minus	Lesser Burdock
Arrhenatherum elatius	False Oat Grass
Bellis perennis	Common Daisey
Briza media	Quaking Grass
Bromus commtatus	Meadow Brome
Bromus erectus	Upright Brome
Bromus mollis	Soft Brome
Bromus racemosus	Smooth Brome
Cardamine pratensis	Lady's Smock
Carex flacca	Glaucous Sedge
Carex hirta	Hairy Sedge
Carex nigra	Common Sedge
Carex panicea	Carnation Grass
Centaurea nigra	Lesser Knapweed
Cerastium fontanum	Common Mouse-ear
Chrysanthemum leucanthemum	Ox-eye Daisy
Cirsium arvense	Creeping Thistle
Cirsium vulgare	Spear Thistle
Cornopus squamatus	Swinecress
Crataegus monogyna	Hawthorn
Cynosurus cristatus	Crested Dog's Tail
Dactylis glomerata	Cocksfoot
Dactylorhiza incarnata	Early Marsh Orchid
Deschampsia cespitosa	Tufted Hair Grass
Equisetum arvensis	Field Horsetail
Festuca pratensis	Meadow Fescue
Festuca rubra	Red Fescue
Filipendula ulmaria	Meadowsweet
Fritillaria meleagris	Snake's Head Fritllary
Galium mollugo	Hedge Bedstraw
Galium verum	Marsh Bedstraw

7.1.2 Analysis of Covariance (Orthogonal Designs) for the ex-situ factorial experiment data 1999.

Factor	Levels	Values				
Nitrate	5	0	25	50	100	200
peat	2	0	200			

Analysis of Covariance for 1999 point quadrat score

Source	DF	Adj SS	MS	F	P
Covariates	1	3920.0	3920.0	13.59	0.000
Nitrate	4	17591.9	4398.0	15.25	0.000
peat	1	3251.3	3251.3	11.27	0.001
Nitrate*peat	4	1176.9	294.2	1.02	0.403
Error	69	19898.7	288.4		
Total	79	45838.8			

Covariate	Coef	StDev	T	P
No. of 1	3.500	0.949	3.687	0.000

F-test with denominator: Error
Denominator MS = 288.39 with 69 degrees of freedom

Numerator	DF	MS	F	P
Nitrate	4	4398.0	15.25	0.000
peat	1	3251.3	11.27	0.001
Nitrate*peat	4	294.2	1.02	0.403

Analysis of Covariance for 1999 species richness

Source	DF	Adj SS	MS	F	P
Covariates	1	35.113	35.113	15.58	0.000
Nitrate	4	148.125	37.031	16.43	0.000
peat	1	0.113	0.113	0.05	0.824
Nitrate*peat	4	15.325	3.831	1.70	0.160
Error	69	155.512	2.254		
Total	79	354.187			

Covariate	Coef	StDev	T	P
No. of 1	0.3312	0.0839	3.947	0.000

F-test with denominator: Error
Denominator MS = 2.2538 with 69 degrees of freedom

Numerator	DF	MS	F	P
Nitrate	4	37.0312	16.43	0.000
peat	1	0.1125	0.05	0.824
Nitrate*peat	4	3.8312	1.70	0.160

Adjusted Means

Nitrate	N	1999 score	1999 species richness
0	16	36.562	5.9375
25	16	42.500	7.6250
50	16	60.625	9.4375
100	16	73.125	9.2500
200	16	35.313	6.8125

peat	N	1999 score	1999 species richness
0	40	43.250	7.7750
200	40	56.000	7.8500

Nitrate	peat	N	1999 score	1999 species richness
0	0	8	23.125	5.3750
0	200	8	50.000	6.5000
25	0	8	39.375	7.8750
25	200	8	45.625	7.3750
50	0	8	55.625	9.3750
50	200	8	65.625	9.5000
100	0	8	70.000	9.8750
100	200	8	76.250	8.6250
200	0	8	28.125	6.3750
200	200	8	42.500	7.2500

7.1.3 Analysis of Covariance (Orthogonal Designs) for the ex-situ factorial experiment data 2000.

```
Factor   Levels Values
Nitrate       5     0    25    50   100   200
peat          2     0   200
```

Analysis of Covariance for 2000 point quadrat score

Source	DF	Adj SS	MS	F	P
Covariates	1	61	61	1.18	0.281
Nitrate	4	109372	27343	526.63	0.000
peat	1	5	5	0.10	0.757
Nitrate*peat	4	204	51	0.98	0.422
Error	69	3583	52		
Total	79	113225			

Covariate	Coef	StDev	T	P
No. of l	-0.4375	0.403	-1.086	0.281

F-test with denominator: Error
Denominator MS = 51.920 with 69 degrees of freedom

Numerator	DF	MS	F	P
Nitrate	4	27343.0	526.63	0.000
peat	1	5.0	0.10	0.757
Nitrate*peat	4	51.1	0.98	0.422

Analysis of Covariance for 2000 species richness

Source	DF	Adj SS	MS	F	P
Covariates	1	8.45	8.45	1.29	0.260
Nitrate	4	2335.18	583.79	88.96	0.000
peat	1	24.20	24.20	3.69	0.059
Nitrate*peat	4	4.18	1.04	0.16	0.958
Error	69	452.80	6.56		
Total	79	2824.80			

Covariate	Coef	StDev	T	P
No. of l	-0.1625	0.143	-1.135	0.260

F-test with denominator: Error
Denominator MS = 6.5623 with 69 degrees of freedom

Numerator	DF	MS	F	P
Nitrate	4	583.794	88.96	0.000
peat	1	24.200	3.69	0.059
Nitrate*peat	4	1.044	0.16	0.958

Adjusted Means

Nitrate	N	2000 score	2000 species richness
0	16	71.25	8.750
25	16	80.63	16.562
50	16	114.06	20.563
100	16	150.31	23.375
200	16	165.00	11.750

peat	N	2000 score	2000 species richness
0	40	116.00	16.750
200	40	116.50	15.650

Nitrate	peat	N	2000 score	2000 species richness
0	0	8	68.75	9.375
0	200	8	73.75	8.125
25	0	8	83.12	17.375
25	200	8	78.13	15.750
50	0	8	113.75	20.875
50	200	8	114.38	20.250
100	0	8	150.00	23.625
100	200	8	150.63	23.125
200	0	8	164.38	12.500
200	200	8	165.62	11.000

7.2.1 One-way analysis of Variance for the point quadrat score March 1997 Tilmanstone bank data.

```
Source      DF        SS        MS        F        P
NPK          4      60.3      15.1     1.10    0.392
Error       15     205.5      13.7
Total       19     265.8

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level     N      Mean     StDev  ------+---------+---------+---------+
    0     4    23.000     4.163                (--------*---------)
   25     4    17.750     3.096   (--------*---------)
   50     4    19.000     4.163      (--------*---------)
  100     4    19.750     3.304        (--------*---------)
  200     4    20.000     3.651        (---------*---------)
                                   ------+---------+---------+---------+
Pooled StDev =    3.701             16.0      20.0      24.0      28.0
```

One-way analysis of Variance for the number of species March 1997:

```
Source      DF        SS        MS        F        P
NPK          4      0.70      0.18     0.10    0.981
Error       15     26.25      1.75
Total       19     26.95

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level   N      Mean     StDev    --+---------+---------+---------+----
  0     4     5.750     1.708       (-------------*--------------)
 25     4     5.500     1.291     (-------------*-------------)
 50     4     5.250     1.258   (-------------*--------------)
100     4     5.250     0.957   (-------------*--------------)
200     4     5.500     1.291     (-------------*-------------)
                                  --+---------+---------+---------+----
Pooled StDev =    1.323           4.0       5.0       6.0       7.0
```

7.2.2 Analysis of Covariance (Orthogonal Designs) for species number on the bank at Tilmanstone data collected September 1997 (covariate data collected March 1997)

```
Factor   Levels Values
NPK           5     0    25    50   100   200

Analysis of Covariance for the number of species per treatment: data from
September 1997 (covariate March 1997 data).

Source       DF     Adj SS         MS       F      P
Covariates    1      0.060      0.060    0.04  0.844
NPK           4     91.109     22.777   15.41  0.000
Error        14     20.690      1.478
Total        19    113.750

Covariate       Coef     StDev         T         P
March sps    -0.04762     0.237   -0.2007     0.844

F-test with denominator: Error
Denominator MS = 1.4779 with  14 degrees of freedom

Numerator  DF       MS      F       P
NPK         4    22.78  15.41   0.000

Adjusted Means

 NPK     N  Sept sps
  0      4     5.264
 25      4     9.002
 50      4     9.990
100      4    10.490
200      4     9.502
```

7.2.3 Analysis of Covariance (Orthogonal Designs) point quadrat score from the Tilmanstone bank data collected September 1997 (covariate data collected March 1997).

```
Factor   Levels Values
NPK           5     0    25    50   100   200

Analysis of Covariance for point quadrat score: data from September 1997
(covariate March 1997 data).

Source       DF     Adj SS         MS       F      P
Covariates    1        1.2        1.2    0.01  0.936
NPK           4     4043.2     1010.8    5.85  0.006
Error        14     2420.8      172.9
Total        19     6744.8

Covariate       Coef     StDev         T         P
March Score  -0.07543     0.917  -0.08223     0.936

F-test with denominator: Error
Denominator MS = 172.92 with  14 degrees of freedom

Numerator  DF       MS      F       P
NPK         4     1011   5.85   0.006

Adjusted Means

NPK     N   Sept Score
  0     4     27.734
 25     4     42.838
 50     4     61.932
100     4     55.489
200     4     69.008
```

7.3.1 Principal Component Analysis for plant community data from the Vegetation manipulation experiments.

The output below shows the proportion of variation accounted for by each species in each of the first six principal components (eigen values).

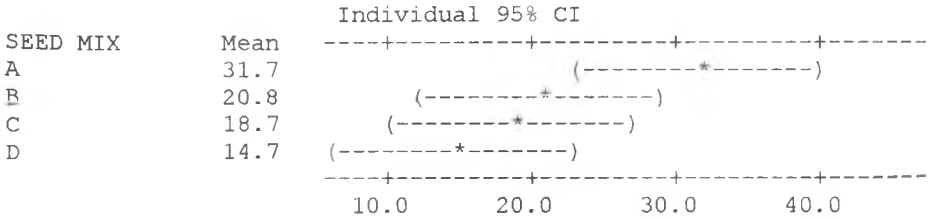
Eigenanalysis of the Correlation Matrix

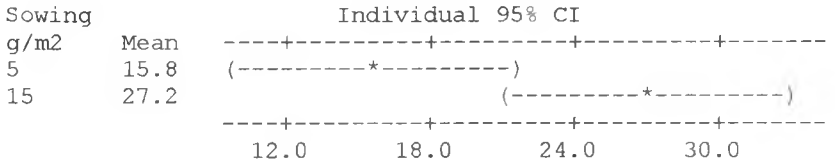
Eigenvalue	3.5313	3.1884	2.9829	2.5753	2.3084	2.2082
Proportion	0.104	0.094	0.088	0.076	0.068	0.065
Cumulative	0.104	0.198	0.285	0.361	0.429	0.494
Eigenvalue	1.9694	1.5680	1.3479	1.2334	1.1071	1.0577
Proportion	0.058	0.046	0.040	0.036	0.033	0.031
Cumulative	0.552	0.598	0.638	0.674	0.706	0.738
Eigenvalue	1.0397	1.0152	0.9324	0.8389	0.7782	0.6956
Proportion	0.031	0.030	0.027	0.025	0.023	0.020
Cumulative	0.768	0.798	0.825	0.850	0.873	0.893
Eigenvalue	0.6657	0.5269	0.4744	0.3667	0.3087	0.2743
Proportion	0.020	0.015	0.014	0.011	0.009	0.008
Cumulative	0.913	0.929	0.943	0.953	0.962	0.970
Eigenvalue	0.2457	0.2053	0.1340	0.0947	0.0885	0.0705
Proportion	0.007	0.006	0.004	0.003	0.003	0.002
Cumulative	0.978	0.984	0.988	0.990	0.993	0.995
Eigenvalue	0.0669	0.0432	0.0353	0.0211		
Proportion	0.002	0.001	0.001	0.001		
Cumulative	0.997	0.998	0.999	1.000		

Variable	PC1	PC2	PC3	PC4	PC5	PC6
A. milli	0.109	0.065	-0.094	0.459	-0.101	0.245
A. cappi	-0.046	0.300	-0.417	0.002	0.005	0.084
A. praec	0.160	0.212	0.205	-0.189	-0.189	0.232
A. elati	-0.212	0.224	-0.173	-0.050	-0.092	0.006
B. peren	-0.159	0.024	0.164	-0.046	0.417	0.361
A. sylve	0.029	-0.037	0.036	0.002	0.026	-0.087
B. pendu	-0.087	0.043	-0.137	0.006	-0.033	0.062
B. racem	-0.205	0.268	-0.182	-0.084	-0.140	-0.040
B. molli	-0.109	0.148	-0.017	-0.093	-0.167	-0.103
C. fonta	-0.164	0.026	0.159	-0.043	0.416	0.359
C. vulga	-0.012	-0.134	0.050	0.043	-0.039	-0.084
C. monog	-0.109	-0.006	0.090	0.000	0.243	0.189
D. glome	-0.021	0.158	0.061	-0.158	0.068	0.055
C. eryth	0.058	-0.272	-0.302	-0.216	-0.089	0.236
D. carot	0.197	0.139	0.225	-0.170	-0.260	0.211
E. angus	0.363	0.185	-0.017	-0.183	0.170	-0.088
H. lanat	-0.258	0.282	-0.139	-0.051	0.116	0.107
H. tetra	0.302	0.186	-0.138	-0.049	0.309	-0.186
H. pilos	-0.131	0.123	0.002	-0.036	-0.146	-0.080
L. hispi	0.330	0.117	-0.220	-0.150	0.217	-0.047
L. vulga	-0.203	0.101	-0.047	0.004	0.106	0.088
L. corni	-0.069	0.283	0.095	-0.184	-0.116	0.157
M. lupul	0.207	0.145	-0.183	0.448	0.036	0.158
M. altis	0.218	0.118	-0.116	-0.050	0.268	-0.181
M. alba	0.272	0.186	0.230	-0.216	-0.208	0.183
P. lance	-0.013	-0.082	0.066	0.057	-0.030	-0.085
P. prate	-0.134	0.128	-0.153	0.000	-0.066	-0.080
R. canin	-0.181	0.144	-0.166	0.009	-0.038	-0.065
R. fruti	0.000	0.136	0.019	-0.106	-0.077	-0.124
R. acet	0.069	-0.300	-0.323	-0.223	-0.083	0.234
R. obtus	0.077	-0.247	-0.351	-0.248	-0.069	0.272
S. vulga	-0.003	-0.060	0.006	0.006	-0.014	-0.023
T. arven	0.207	0.137	-0.029	0.394	-0.176	0.312
T. Prate	0.125	0.062	-0.043	0.040	0.079	-0.063

8.1.1 Two-way Analysis of Variance percentage cover data from seed mix treatments collected June 1999.

Source	DF	SS	MS	F	P
SEED MIX	3	951.1	317.0	3.34	0.046
g/m2	1	782.0	782.0	8.23	0.011
Interaction	3	1084.1	361.4	3.80	0.031
Error	16	1520.7	95.0		
Total	23	4338.0			





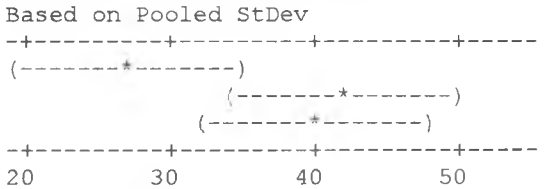
8.1.2 One-way Analysis of Variance: % cover versus *Biotech-granules* g/m^2 data collected June 1999.

Analysis of Variance for % cover

Source	DF	SS	MS	F	P
Biotech	2	375.9	188.0	6.11	0.036
Error	6	184.7	30.8		
Total	8	560.6			

Level	N	Mean	StDev
200	3	27.200	3.000
400	3	41.667	2.887
800	3	40.000	8.660

Pooled StDev = 5.548



8.2.1 One-Way Analysis of Variance for the vegetation on the experimental area at Tilmanstone prior to hydroseeding

```
Analysis of Variance for Quadrat score for data collected 5/10/00.
Source      DF        SS        MS         F         P
Block       11       564        51      0.30     0.979
Error       24      4067       169
Total       35      4631
                          Individual 95% CIs For Mean Based on Pooled StDev
Expt. Area   N      Mean     StDev  -+---------+---------+---------+-----
 1           3     10.00     10.00         (-----------*-----------)
 2           3     10.00     17.32         (-----------*-----------)
 3           3      3.33      5.77   (-----------*-----------)
 4           3     13.33     23.09           (-----------*-----------)
 5           3     10.00     10.00         (-----------*-----------)
 6           3      3.33      5.77   (-----------*-----------)
 7           3      3.33      5.77   (-----------*-----------)
 8           3     10.00     10.00         (-----------*-----------)
 9           3     10.00     10.00         (-----------*-----------)
10           3      6.67     11.55      (-----------*-----------)
11           3     16.67     20.82              (-----------*-----------
12           3      6.67     11.55      (-----------*-----------)
                             -+---------+---------+---------+-----
Pooled StDev =     13.02          -12         0        12        24
```

8.2.2 Analyses of variance on the data from the hydroseeding trials at Tilmanstone.

Analysis of Variance: point quadrat score for data collected 12/10/00

Key:
Broadcast= Mycorrhizal treatment broadcast by hand
Hydro= Mycorrhizal treatment carried in hydroseeding mix.

```
Source      DF        SS        MS         F         P
treatment    2        11         6      0.03     0.974
Error       33      6978       211
Total       35      6989
                          Individual 95% CIs For MeanBased on Pooled StDev
Level        N      Mean     StDev  ----+---------+---------+---------+--
control     18     21.11     15.68           (----------*----------)
Broadcast    9     20.00     14.14   (----------------*----------------)
Hydro        9     20.00     12.25   (----------------*----------------)
                             ----+---------+---------+---------+--
Pooled StDev =     14.54          12.0      18.0      24.0      30.0
```

Analysis of Variance: point quadrat score for data collected 8/5/01.

```
Source      DF        SS        MS         F         P
treatment    2       225       113      0.36     0.699
Error       33     10272       311
Total       35     10497

                          Individual 95% CIs For MeanBased on Pooled StDev
Level        N      Mean     StDev  -----+---------+---------+---------+-
control     18     38.33     15.43    (----------*---------)
Broadcast    9     40.00     21.21  (--------------*--------------)
Hydro        9     44.44     18.10         (--------------*--------------)
                             -----+---------+---------+---------+
Pooled StDev =     17.64          32.0      40.0      48.0      56.0
```

ANOVA: point quadrat score 8/5/01 versus biotech-granules & mycorrhizal inoculants

Key:
Biotech= Biotech Granules
Mychoriz= Mycorrhizal treatment

```
Factor     Type Levels Values
biotech-   fixed      2      0      1
Mychoriz   fixed      2      0      1

Analysis of Variance for Quad sco

Source                DF         SS         MS       F       P
biotech-               1      225.0      225.0    0.71   0.406
Mychoriz               1      136.1      136.1    0.43   0.517
biotech-*Mychoriz      1        2.8        2.8    0.01   0.926
Error                 32    10133.3      316.7
Total                 35    10497.2

Means

biotech-      N  Quad sco
0            18    37.778
1            18    42.778

Mychoriz      N  Quad sco
0            18    38.333
1            18    42.222

biotech- Mychoriz     N  Quad sco
0        0            9    35.556
0        1            9    40.000
1        0            9    41.111
1        1            9    44.444
```

8.2.3 General Linear Model analysis of variance: mycorrhizal infection of *Trifolium pratense, Agrostis capillaris, Holcus lanatus* versus hydroseeded and hand broadcast mycorrhizal inoculate, data from field sampled plants.

```
Factor          Type  Levels Values
Hydroseeded     fixed      2 0 1
Hand broadcast  fixed      2 0 1

Analysis of Variance for Trifoliu, using Adjusted SS for Tests

Source      DF     Seq SS     Adj SS     Adj MS       F      P
Hydrosee     1       0.25       3.56       3.56    0.11  0.747
Hand bro     1      50.00      50.00      50.00    1.56  0.243
Error        9     288.67     288.67      32.07
Total       11     338.92

Unusual Observations for T. pratense.

Obs  Trifoliu       Fit      SE Fit  Residual   St Resid
  3   20.0000    7.3333      3.2698   12.6667      2.74R

R denotes an observation with a large standardized residual.

Analysis of Variance for Agrostis, using Adjusted SS for Tests

Source      DF     Seq SS     Adj SS     Adj MS       F      P
Hydrosee     1       0.00      16.06      16.06    0.57  0.468
Hand bro     1     144.50     144.50     144.50    5.16  0.049
Error        9     252.17     252.17      28.02
Total       11     396.67

Unusual Observations for A. capillaris

Obs  Agrostis       Fit      SE Fit  Residual   St Resid
  1    0.0000   10.0000      3.0561  -10.0000     -2.31R
  2   20.0000   10.0000      3.0561   10.0000      2.31R

R denotes an observation with a large standardized residual.

Analysis of Variance for H. lanatus, using Adjusted SS for Tests

Source      DF     Seq SS     Adj SS     Adj MS       F      P
Hydrosee     1      1.778      0.056      0.056    0.01  0.908
Hand bro     1      9.389      9.389      9.389    2.38  0.157
Error        9     35.500     35.500      3.944
Total       11     46.667
```

8.2.4 General Linear Model analysis of variance: mycorrhizal infection of *Trifolium pratense, Agrostis capillaris, Holcus lanatus* versus hydroseeded and hand broadcast mycorrhizal inoculate. Data from plants collected 12/12/00 and grown under glass, mycorrhization tested 11/5/01.

Factor	Type	Levels	Values
Hand bro	fixed	2	0 1
hydrosee	fixed	2	0 1

Analysis of Variance for Trifoliu, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Hand bro	1	1848.1	0.0	0.0	0.00	1.000
hydrosee	1	4363.6	4363.6	4363.6	122.18	0.000
Error	14	500.0	500.0	35.7		
Total	16	6711.8				

Analysis of Variance for Agrostis, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Hand bro	1	73.925	15.709	15.709	3.27	0.092
hydrosee	1	35.345	35.345	35.345	7.36	0.017
Error	14	67.200	67.200	4.800		
Total	16	176.471				

Analysis of Variance for Holcus l, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Hand bro	1	37.091	10.909	10.909	3.82	0.071
hydrosee	1	10.909	10.909	10.909	3.82	0.071
Error	14	40.000	40.000	2.857		
Total	16	88.000