MICROBIAL RESPONSE TO SIMULATED CLIMATE CHANGE IN ANTARCTIC FELLFIELD SOIL

A thesis submitted to the University of Kent for the degree of Doctor of Philosophy in the Faculty of Natural Sciences

by

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ABSTRACT

A chronosequence of soil samples, taken over the 1992-3 austral summer, was used to evaluate the bacterial response to simulated climate change brought about by passive greenhouses (cloches) placed on fellfield soil at Signy Island, Maritime Antarctic. Bacterial numbers, electron transfer chain (ETS), glucosidase and galactosidase activity were significantly elevated by the simulation, with a greater increase in numbers and activities beneath a warming and UV-blocking cloche in place for eight years prior to sampling than beneath a cloche that warmed soil under natural UV-B flux in place for five years. The simulation had no effect on soil saccharide at either site, but soil carbon was significantly greater ($P < 0.05$) in the cloche-cover soil beneath the warming/UV-screening cloche. In addition, moss shoots were evident beneath this cloche, leading to the conclusion that climate change will increase the rate at which microbially-dominated polygon mineral soil accumulates carbon and succeeds to moss-dominated peat soil.

The yield coefficient of a dominant bacterial isolate was calculated to determine the seasonal bacterial energy expenditure on biomass production. Cloche-cover increased bacterial energy expenditure in both sites (e.g. from 4.95 to 54.28 μg glucose g^{-1} soil 53 d^{-1} at polygon WW2). Microbial ATP production, calculated from measurements of ETS activity, was also significantly greater beneath warmed soil (e.g. from 9.76 to 27.71 μmol ATP g^{-1} soil 53 d^{-1} in polygon WW2). The potential release of hexose by polysacchridase activity during the course of the season was also greater beneath the cloches, compared to exposed soil, at both sites (e.g. from 58.57 to 189.5 μmol hexose g^{-1} soil 53 d^{-1} in polygon WW2).

A second field study was conducted to assess the response of the bacterial and microalgal communities to separate warming and UV-B protection. A cloche that screened UV-B under ambient temperature resulted in a significantly greater microalgal colonisation ($P < 0.001$), than in exposed soil, and a reduction in the biomass ratio between microalgae and bacteria. A cloche simulating global warming under a natural UV-B flux caused a significantly greater cyanobacterial colonisation, and a succession from domination by the filamentous *Phormidium autumale* to the aseriate *Nostoc commune*.

Twenty five bacterial isolates from fellfield soil at Signy Island, maritime Antarctic, were investigated to determine their ability to metabolise the two key carbon sources (polysaccharide material and low molecular weight carbohydrates) reported in polygon soil. The majority of isolates (88%) produced at least one exopolysaccharidase enzyme (of four screened: α-D-glucosidase, β-D-glucosidase, α-D-galactosidase and β-D-galactosidase). Fifteen of the isolates required yeast extract for growth: the remaining ten were screened for abilit y to grow on 11 sugars and polyols recorded in polygon soil. A 'nutritional flexibility index' was used to compare the ability of isolates to metabolise these carbohydrates. There was no evidence of a distinct metabolic strategy for bacterial use of soluble carbohydrates against polysaccharide. All the isolates were pyschrotrophic (maximum growth rate above 20°C with growth at 0°C), rods with 21 Gram-negative and 19 pigmented.

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1. INTRODUCTION

1.1 Terrestrial Antarctic Microbiology

The first microbiological studies to be carried out in the Antarctic were performed by Ekelöf in 1908 who isolated and identified bacteria, yeasts and fungi from a variety of environments (Wynn-Williams, 1990a). Further studies took place up until the outbreak of the First World War. Co-ordinated microbial investigations began in the late 1950s initially concentrating on taxonomic studies then developing, during the 1970s, to include microbial ecology, ecophysiology, biochemistry and biophysics. Long term ecology based research projects were initiated, such as the Antarctic Cryptoendolithic Microbial Ecosystems (ACME), in the Ross Desert of Antarctica and the British Antarctic Survey's (BAS) Fellfield Ecology Research Program (FERP) on Signy Island, in the maritime Antarctic.

Antarctic microbiology has developed under several influences. They include: the assessment of life detection systems for use on the USA Mars probe (Horowitz, *et al.,* 1972); the use of the pristine, isolated habitats as a natural cold laboratory to study ecological hypotheses (Upton, 1988); the study of freeze-tolerance mechanisms (Melick and Seppelt, 1992; Arnold, 1995); the assessment of the environmental impact of drilling and human habitation on Antarctic ecology (Cameron, 1972; Roser *et al.,* 1992a; Kerry, 1993) and investigations into the biological effects of ozone depletion (Karentz, 1991) and global warming (Wynn-Williams, 1996).

1.1.1 Antarctic Habitats

The Antarctic is divided into three zones (sub-Antarctic, maritime Antarctic and continental Antarctica) based upon location, climate and biology (Holdgate, 1977).

Sub-Antarctic
The sub-Antarctic includes islands north of 60°S latitude, excluding the South Sandwich Islands (Figure 1.1). The climate is cool oceanic, defined as having temperature above freezing for more than six months of the year and precipitation of more than 900 mm. The macrobiota includes vascular and nonvascular plants as well as molluscs, spiders, insects and land birds. Microbiological studies have focused on bacterial populations (French and

Smith, 1986), specific bacterial activity (Grobler *et al.*, 1987), cellulose decomposition (Walton, 1985) and nitrogen turnover (Smith and Steenkamp, 1992).

Maritime Antarctic

The maritime Antarctic zone extends south between 66 and 70°S on the Antarctic peninsula and its offshore islands and on the north-east coast of the Peninsula to 63°S (Figure 1.1). It is characterised by cold maritime climate - mean air temperatures above freezing for between one to four months and a mean monthly winter temperature that rarely falls below -15°C and precipitation of between 350-500 mm per annum. The vegetation is mainly composed of nonvascular plants (cyanobacteria, chlorophytes, lichens, liverworts and mosses), with two species of angiosperm: the grass *Deschampsia antarctica* Desv. and the pearlwort *Colobanthus quitensis* Bartl. This vegetation supports nematodes, enchytraeids, springtails, mites and microorganisms. Microbiological investigations have included: community structure, seasonal population changes and response to climate change of the cyanobacterial and chlorophyte colonists (Davey, 1991a; Wynn-Williams, 1996); protozoan and fungal abundance (Baker and Smith, 1972; Pugh and Allsopp, 1982; Smith and Tearle, 1985); and bacterial activities in peat and fellfield soil (Wynn-Williams, 1984; Foister *et al.*, 1993).

Continental Antarctica

The continental Antarctic zone includes all of mainland Antarctica, excluding the Peninsula north of 63°S (Figure 1.1). Exposed terrestrial habitats include coastal yregions, interior slope and plateau and isolated nunataks. The climate is cold and dry becoming more severe towards plateau areas (e.g., McMurdo Dry Valleys). In coastal areas, winter temperatures are between -5 and -25°C with precipitation between 100-150 mm with temperatures rising above 0°C for less than one month in the summer. Interior slope and plateau areas are classified as cold deserts with mean monthly temperatures between -5 and -15°C and very little precipitation. Colonists are restricted to endolithic habitats in the interior cold deserts and consist of lichen dominated microbial communities. Studies have focused on the productivity, rates of growth and diversity of these communities (Hirsch *et al.*, 1988; Johnston and Vestal, 1991; Freidmann *et al.*, 1993).
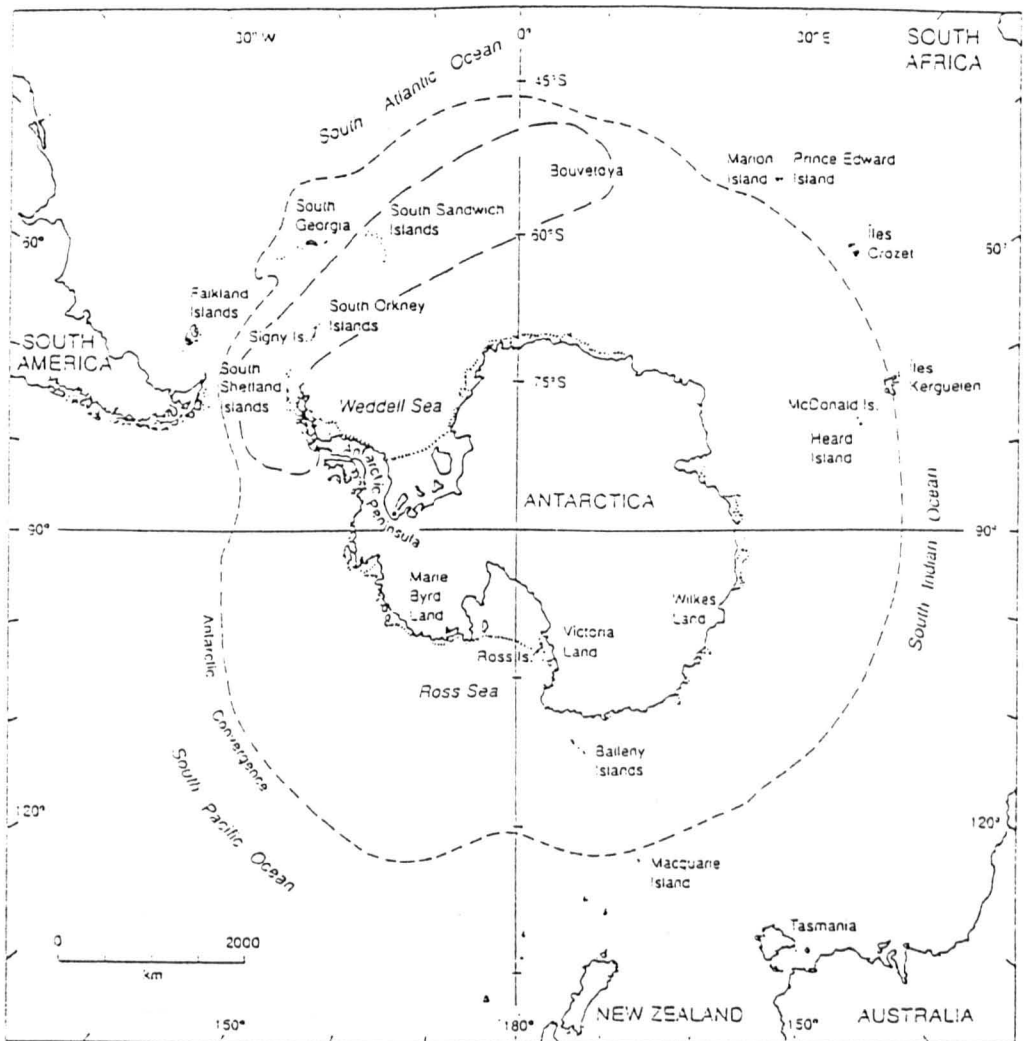
Figure 1.1 Map of Antarctica showing the Antarctic Convergence (---), maritime Antarctic (— — —), and Signy Island (from Block, 1994).

1.1.2 Signy Island

Signy Island is one of the South Orkney Islands, an Archipelago which forms part of the Scotia Ridge, an arc extending from the Antarctic Peninsula (Plate 1.1, Figure 1.1). Sea-ice encases the Island during the winter (June-July) causing a fall in winter temperatures to -10 to -20°C. Conversely, when the ice-sheet retreats the resulting drop in albedo causes a rapid transition from winter to spring. As the snow melts, a greenhouse effect causes ground thaw which is accelerated by melt water. The transition from frozen, inactive soil to thawed active soil can occur within one day and can give rise to a microbial bloom as water stimulates soil microflora to metabolise available substrate (Wynn-Williams, 1980). During summer (December to March) temperatures average around 3-4° C, with a diurnal range from upwards of 20°C during the day to around 0°C at night (Walton, 1982; Davey, 1991a). As winter approaches (April-May) temperatures drop to around freezing and snow begins to accumulate which acts as a blanket of insulation during the winter, buffering the ground from extremes of cold.

Terrestrial habitats include fellfield mineral soils (Plate 1.2), peat banks and guano-enriched ornithogenic soils. Fellfields are formed by the grinding action of glaciers on bedrock and the subsequent shattering of rocks by freeze-thaw action. Smaller rock fragments or fines, collect together to form characteristic polygons on flat areas and stone stripes on sloped areas (Chambers, 1966). Polygons are unstable as ice needles form around the fines and push up the centre of the polygon. This frost-heave sorts the polygon particles by size, with the centre consisting of fines of 12 to 132 μm, with larger stones and rocks rolling to the periphery (Plate 1.3 and Holdgate *et al.*, 1967). Disturbed polygons return to a sorted state after a period of three years (Chambers, 1967). The instability of polygon fines precludes their colonisation by moss and lichen with immature polygons being dominated by cyanobacteria (Wynn-Williams, 1993). Moss carpets are also established on the island and can lead to the formation of peat soil. Such soil decomposes at a slow rate (Wynn-Williams, 1980) such that peat from two metres depth from Signy Island has been calculated to be approximately 5500 years old (Smith, 1990). The input of guano around bird colonies provides nutrient enrichment and such ornithogenic soil can support significantly elevated microbial activities compared with non-ornithogenic soils (Spier and Ross, 1984).

Plate 1.1   Signy Island during the summer. The Jane Col ice-free plateau is visible in the foreground, surrounded by permanent glacier (reproduced with permission of D.D. Wynn-Williams).
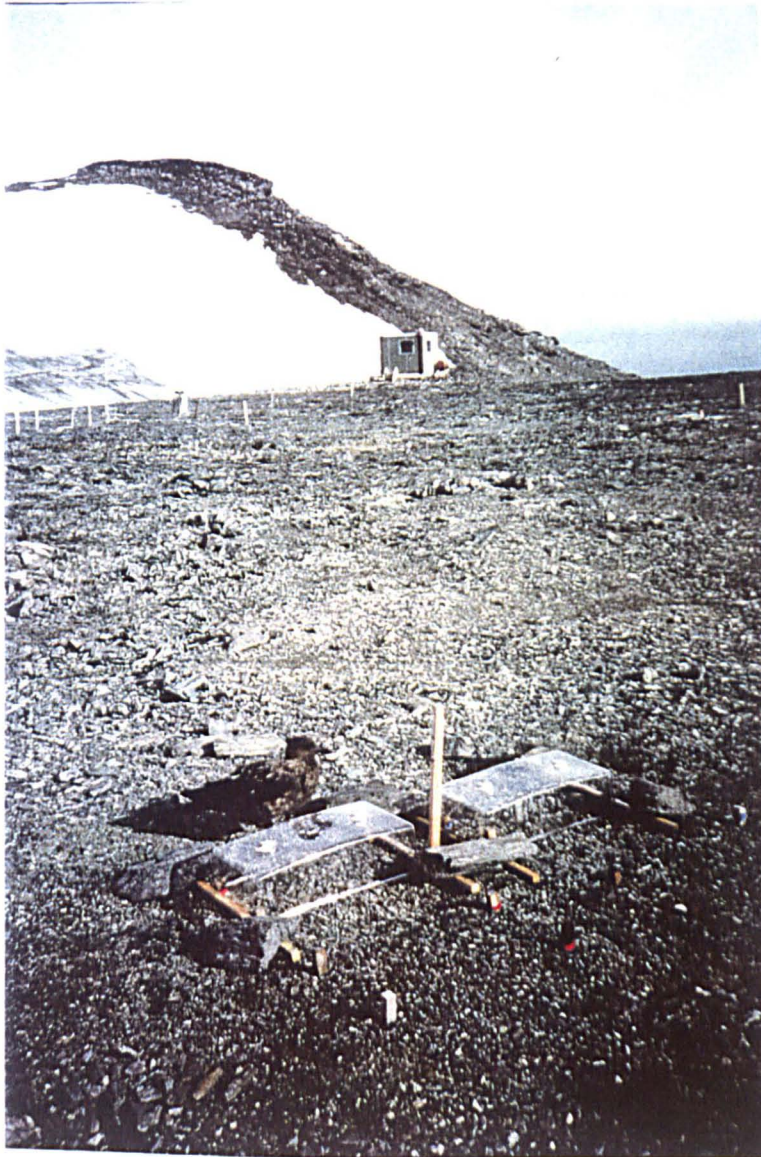
Plate 1.2 The Jane Col plateau. A typical fellfield with plastic cloches visible on a polygon (reproduced with permission of D.D. Wynn-Williams).
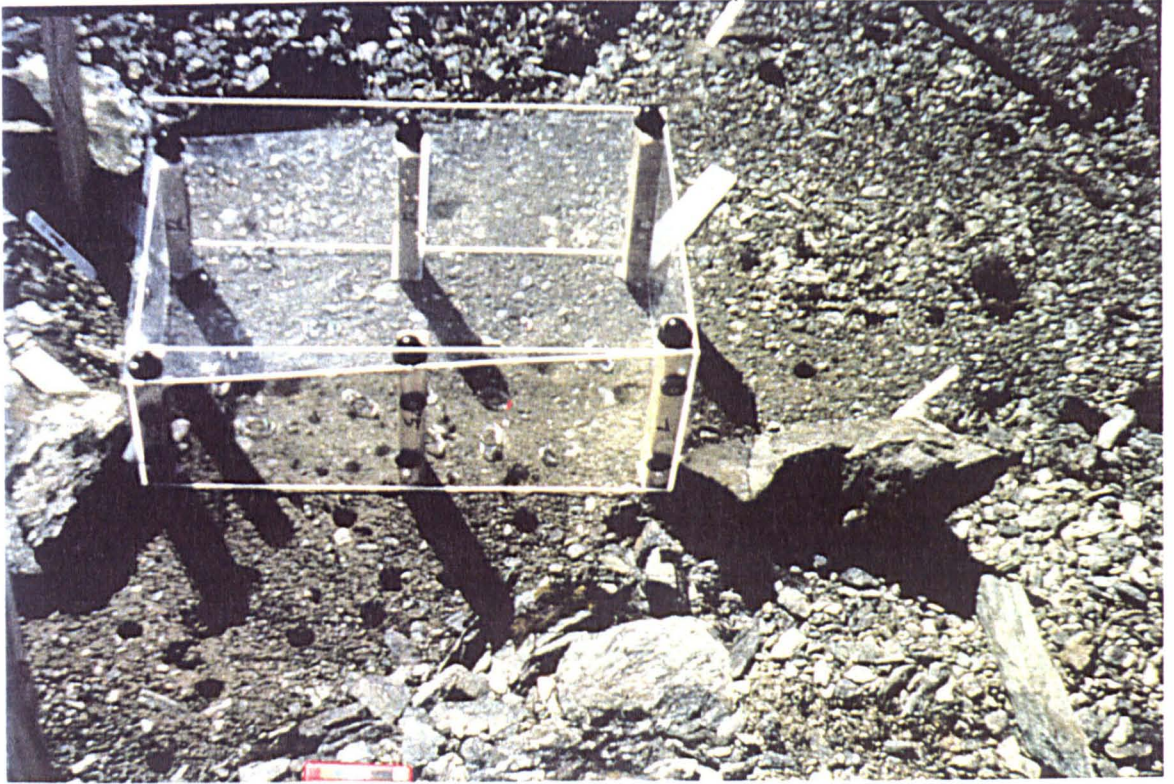
Plate 1.3 A fellfield polygon with a cloche in place. Holes left from soil sampling are visible both inside and adjacent to the cloche (reproduced with permission of D.D. Wynn-Williams).

Water Availability

Several factors, acting singly and in concert, limit the colonists of Signy Island. The principal limiting factor for Antarctic colonists has been considered to be the availability of water (Kennedy, 1993). The addition of water alone caused bacterial growth in continental Antarctic soil samples (Bölter, 1993; Roser *et al.*, 1993). However, amendment studies during the summer growing season at Jane Col provided no evidence that the addition of water could stimulate the growth of the indigenous cyanobacteria and chlorophyte populations (Wynn-Williams, 1990a). This is a reflection of the high summer rainfall at Signy Island.

Diurnal Freeze-Thaw Cycles

Diurnal freeze-thaw cycles during the spring and summer are also an obstacle to colonisation of Signy Island polygons: the process of freeze-thaw can cause cell membrane damage and result in leakage of cellular material (Tearle, 1987; Melick and Seppelt, 1992). However, such leachate can act as a nutrient source for the heterotrophic colonists (Tearle, 1987; Bölter, 1993; Melick *et al.*, 1994) and can increase the microbial productivity of polygons by bringing compatible solutes from macroscopic colonists (moss and lichen), frequently at the periphery of polygons, to the microbial colonists at the centre of polygons. Despite the low average temperature during the growing season (3-4°C), the mechanism that causes the freeze-thaw cycles (i.e., solar radiation warming exposed rocks in the day and cold permafrost cooling the surface at night), results in wide temperature variations (from 0 to 35°C; Walton, 1982). Such diurnal temperature change can explain the predominance of psychrotrophic colonists at Signy Island polygons: they are able to out-compete psychrophiles during brief warm periods. The domination of psychrotrophs is a common situation in environments that undergo temperature fluctuations (Russell, 1990). The relevance of this to global warming is discussed in section 1.2.2.

Nutrients

Inorganic nutrients have been shown to be limiting to fellfield algae (Davey and Rothery, 1992) and fellfield soils have low concentrations of mineral nitrogen and phosphate (Allen and Northover, 1967). Indeed the dominant cyanobacterial species, *Phormidium autumnale,* grows significantly better at low levels of inorganic phosphate which is consistent with its ability to compete well in fellfield soils (Gapp, 1996). A further adaptation of this species to low nutrient levels is its ability to mobilise resources from photosynthetic pigments to maintain cellular C:N ratios (Gapp, 1996).

Visible Light and UVR

Kappen *et al.* (1991) observed photoinhibition of lichens in continental Antarctica during periods of high summer irradiance. Conversely, the high albedo (reflectance) of snow, reduces incident radiation to the soil surface by up to 98% (Walton, 1984), although growth of cyanobacteria has been observed beneath snow cover prior to the spring thaw (Davey, 1991a).

Normal incident levels of UV-B radiation at the Antarctic causes inhibition of photosynthesis (Helbling *et al.*, 1992). However, the advent of spring time depletion of ozone in the Antarctic (Farman *et al.*, 1985) has resulted in elevated spring levels of UV-B and this may have significant effects on springtime populations as they recover from winter dormancy (Karentz, 1991). The effect of UVR and the steps taken to avoid UV-damage by polygon colonists is discussed in section 1.2.4.

1.1.4   Primary Colonists of Fellfield Polygons

According to Wynn-Williams (1993) the first colonisers of bare polygon fines at Signy Island are microscopic eukaryotic algae (chlorophytes) and cyanobacteria. These primary producers are restricted to the phototrophic zone comprising the top 2 cm of the fines. Cyanobacterial and chlorophyte populations increase up the soil profile but no colonisation of the polygon surface is evident (Davey and Clarke, 1991) and this is thought to be a either a desiccation or UV-avoidance strategy (Davey, 1991a). The latter explanation is supported by Wynn-Williams (1993) who observed growth of cyanobacteria on the surface of fines beneath cloches and this is attributed to the reduction of UVR beneath the cloche. Davey (1991a) recorded seasonal periodicity in the colonisation of fellfield fines. Populations of eukaryotic filamentous algae increased beneath spring snow and ice cover, whilst cyanobacterial populations dominated the latter part of the growing season. This succession was attributed to the ability of the cyanobacterial species to survive periodic freezing and desiccation and to grow rapidly under summer field temperatures.

A survey of microagal colonists revealed an average of 24 taxa in fellfield soils at Signy Island (Broady, 1979). However, at the Jane Col plateau, on fellfield recently exposed by glacial retreat, fewer taxa (approximately seven) are evident (Davey, 1991a; Davey and Rothery, 1993). *Phormidium autumnale* was frequently found to be the dominant cyanobacterial colonist immature fellfield polygons (Davey, 1988). Suggested reasons for

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its predominance include: motility which enables colonisation of the entire polygon (the non-motile *Nostoc* is found with greater frequency towards the polygon periphery); the ability to survive repeated freeze-thaw cycles; the ability to fix nitrogen and greater desiccation resistance relative to other fellfield cyanobacteria and chlororphytes (Davey and Clarke, 1991). Interestingly, under experimental cloches, an aseriate form of *Nostoc commune* became dominant over *Phormidium* suggesting the selective advantages enjoyed by *Phormidium* were reduced under such experimental conditions (Wynn-Williams, 1993, 1996). In a comprehensive study of 65 polygons at Jane Col, Signy Island, Davey and Rothery (1993) reported that *Phormidium* spp. comprised the largest biovolume of the cyanobacteria and chlorophytes in the majority of polygons, with just one polygon dominated by *Nostoc* spp. They found little variation in environmental and chemical factors between polygons and attributed differences in the size and composition of colonists to the "vagaries of colonisation" and likened the process of successful polygon colonisation to that of small ponds where Talling (1951) suggested an "element of chance" played a role in successful colonisation. Although close together, polygons are physically isolated by areas of larger rocks and stones and there is no evidence that meltwater runs across the Jane Col plateau to provide a route for cross-polygon transfer of colonists (Davey and Rothery, 1993). The major source of algal colonists reach polygons by an airborne route and potential colonists, including *Phormidium* spp., have been collected from the air around Signy Island (Wynn-Williams, 1991).

The phototrophic community is associated with bacteria, fungi, flagellates and testate amoeba (Smith and Tearle, 1985). Microbial activities in soil usually decrease with depth (Eivazi and Tabatabai, 1990) and this is apparent in the heterotrophic activities in polygon soil with neutral phosphatase activity in the top 4 cm of soil twice that in soil from 4-8 cm depth (Foister *et al.,* 1993). There is evidence of seasonal periodicity within the bacterial community analogous to that observed by Davey (1991a) for cyanobacterial and chlorophyte populations: bacterial enzyme activities show temporal peaks during the growing season indicative of functional successions (e.g., from trehalase-positive species dominating periods of high trehalase activity to α- and β-glucosidase positive species during periods of high glucosidase activity, Foister *et al.,* 1993).

1.1.5   Microbial Stabilisation of Polygon Soil

Many of the primary polygon colonists produce a peripheral polysaccharide sheath (Wynn-Williams, 1993; Foister *et al.,* 1993) which cements fines and microorganisms together and improves the soil structure (Martin, 1971; Roberson *et al.,* 1995). In addition, the filamentous microorganisms (e.g., *Phormidium autumnale*) interweave fines to provide a mucilagenous framework of microorganisms and soil particles (Wynn-Williams, 1993). Soil-microorganism "rafts" form near the surface of the soil, providing structural stability for further community development (Nedwell and Gray, 1987). As these two processes of soil organic matter increase and aggregation of fines develop, the soil structure improves and becomes more stable, enabling colonisation by moss and lichen. This is the beginning of the secondary stage of colonisation and ultimately leads to the formation of moss-covered peat soil (Wynn-Williams, 1993). The pattern of initial colonisation and stabilisation of pristine substratum by microorganisms leading to a succession to moss and lichen is considered to be the process which ultimately results in an explosion of taxa including herbs, shrubs and trees for any given favourable habitat (Smith, 1993). The colonisation process at Signy Island is restricted by climate to the secondary stage of colonisation, with moss peat banks forming the climax community. Exceptions to this sequence do occur and include the moss and lichen colonists of the stony fellfield between polygons.

1.1.6   Compatible Solutes

The lichen and moss colonisers of fellfields are able to survive the Antarctic winter using physiological adaptations similar to those reported by Block (1982) allowing the successful overwintering of Antarctic arthropods. The arthropods withstand the Antarctic winter by supercooling - maintaining their body fluids in the liquid phase below the freezing point of water. This is achieved by increasing internal cellular osmotic potential by the accumulation compatible solutes (sugars and polyhydric alcohols - polyols). Tearle (1987) reported seasonal variations in the polyol concentration of the fellfield lichens *Usenea* and *Himantormia* and in the moss *Andrea*. The levels of polyols in the spring and summer were markedly higher in than those reported in species isolated from temperate environments (Hale, 1983) indicating an accumulation in response to cold. In addition, these cryptogamic species contained winter levels of polyols sufficient to confer frost tolerance (according to calculations done by Sakai (1961) working on woody plants in the winter). More recently, Montiel and Cowan (1993) have shown increases in the levels of polyols in a maritime Antarctic lichen (*Umbilicaria antarctica*), a moss (*Drepanocladus*

uncinatus) and an alga (*Prasiola crispa*) when incubated at -3.6°C. The levels of accumulated polyols (100-150 millimolar) were again calculated to be sufficient to confer osmoregulation and cryoprotection. The concentration of glycerol and erythritol in cyanobacteria and chlorophytes has been shown to increase at the end of the summer in Signy Island polygons and also to increase in response to controlled freeze-thaw cycles suggesting their use as cryoprotectants by fellfield cynaobacteria and chlorophytes (Arnold, 1995).

Roser *et al.* (1992a) reported lower concentrations of polyol and soluble sugars in continental Antarctic mosses, lichens and algae than found by Tearle (1987) in maritime Antarctic counterparts. The difference has been suggested by Roser *et al.* (1992a) to be related to the more extreme environment on the Antarctic continent with the continental cryptograms having lower water concentrations compared to maritime counterparts and thus require less polyol to confer cryoprotection. Alternatively, it is not known how much of the lichen material studied is viable cell mass, and this could change the water-to-polyol ratio of the biomass. A third explanation is that it is not energetically possible for the cryptogams to divert material to cryopreservation, given their slow growth and the low net photosynthetic gain reported in continental cryptoendolithic communities (Friedmann *et al.*, 1993).

Tearle (1987) monitored sugars and polyols leached into fellfield fines from the surrounding lichens and mosses and found large increases immediately after the spring thaw. At this time, the concentration of these carbohydrates reached 1 % (w/w) fresh weight of fellfield soil. Leached carbohydrates from frost damaged mosses have been suggested to provide the substrates used by heterotrophic bacteria during the significant population increase observed in the spring in Signy Island peat soil (Wynn-Williams, 1980). The fellfield heterotrophs can survive such a nutritional shock and grow on these nutrients (Tearle, 1987). Indeed, in soil of such low organic matter, this spring input of nutrients may cause the most significant growth of the heterotrophic community for the entire season. Melick *et al.* (1994) have shown that bacteria in soil samples from continental Antarctica can also respond rapidly to supplemented carbohydrates. Rates of polyol leakage from maritime Antarctic mosses (Melick and Seppelt, 1992; Roser, 1992a) were used to calculate appropriate levels for carbohydrate addition and glucose, sucrose and arabitol (common components of leachate) were significantly reduced after a seven day incubation of supplemented soil at 15°C. The bacterial community of ornithogenic soil on continental Antarctica also respond to high levels of supplemented sugars (Roser *et al.* 1993). Bölter (1993) found the carbohydrates that promoted growth of bacteria from continental Antarctica included the major components of cryptogamic leachate with

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polyols normally found in lower concentrations in the soil causing little or no growth. Polyol leakage under stress has led to the use of lichens as indicators of pollution near an Antarctic base. Lichens near a concrete mixing site showed significant reductions in polyols and oligosaccharides compared to controls (Roser *et al.*, 1992b).

1.2.1   *Global Warming*

Palaeoclimatic evidence from ice cores drilled at the Vostock Antarctic station demonstrate that for the last 160 000 years there has been a close correlation between near-surface air temperature and CO_2 concentrations (Lorius *et al.,* 1985). It is also now established that atmospheric CO_2 levels have risen, from a pre-industrial concentration of c280 ppm to the 1994 concentration of 358 ppm. This rise is largely due to human activities, in particular fossil fuel combustion, but also land-use conversion and cement production (IPPC, 1996). This has lead to concern that anthropogenic CO_2 emissions will result in increased global temperatures - the so called greenhouse effect. Other gases implicated in the greenhouse effect include methane, nitrous oxide, ozone (increased concentrations in the troposphere, which contrasts with ozone depletion in the stratosphere), halocarbons and other halogenated compounds. Although these gases are at lower atmospheric concentrations in comparison with CO_2 (e.g. 1994 level of methane is 1.72 ppm; IPCC, 1996) their radiative forcing capacity (ability to perturb the energy balance of the Earth-atmosphere system) is greater per molecule. As a result, although CO_2 is considered to be the primary atmospheric forcing agent, contributing a projected warming of 1.56 Wm^{-2}, methane contributes 0.47 and nitrous oxide 0.14 Wm^{-2}.

Global surface air temperatures have risen between 0.3 and 0.6 C° since the late 19th century and recent years have been the warmest since 1860, providing evidence of the action of such forcing agents on global temperatures (IPCC, 1996). In addition, sea levels have risen between 10 and 25 cm over the past 100 years and much of this rise may be the result of thermal expansion of water. The melting of polar ice is not yet considered to be having a significant effect on sea level. However, the latest report of the Intergovernmental Panel on Climate Change (IPPC, 1996) concluded that "the balance of evidence suggests a discernible human influence on global climate".

The most recent IPCC projection for global warming is a 2 C° rise by 2100, relative to 1990 temperatures (IPCC, 1996). This projection is based on the mid-range greenhouse-gas emission scenario, the "best estimate" value of climate sensitivity and takes into account the latest information on the negative influence of anthropogenic aerosols. Using "low" and "high" values for climate sensitivity gives

projected temperature increases of 1 and 3.5 C°, respectively. These projections are lower than reported by the IPCC in 1990, as new knowledge has enabled calculations to include the cooling effect of aerosols, lower emission scenarios (CO_2 and CFCs) and improved treatment of the carbon cycle. Nevertheless, there have been recent reports voicing concern over this latest model by the IPCC: Hansen *et al.* (1997a and b) argue that observed global warming since the industrial revolution is only about half that expected from the current models and suggest that further consideration should be taken of the aerosol effect and stratospheric ozone depletion.

1.2.2   *Antarctic Terrestrial Microorganisms and Global Warming*

It is unclear how global warming will affect Antarctica. It has been suggested that the high albedo of ice and snow combined with Antarctic weather systems will result in temperatures 2 - 2.4 times greater than the global mean in the winter and 0.5 - 0.7 times the global mean in the summer (Maxwell and Barrie, 1989). However, the recent recognition of the aerosol effect, which predominates in northern industrialised zones, may partially offset this imbalance (Kennedy, 1995a). There have been reports of warming in the Antarctic: the Southern Hemisphere warmed by 0.3 C° between 1955 and 1985 (Houghton *et al.,* 1992); a sub-Antarctic island warmed by 1 C° since 1949 (Adamson *et al.,* 1988); temperatures have risen at Signy Island, in the maritime Antarctic (Smith, 1990) and at sites on the Antarctic continent (Adamson and Adamson, 1990; Stark, 1994). However, it is still unclear whether such temperature increases are due to the greenhouse effect or natural climatic variability (King, 1994).

The short term effect of a warmer Antarctic may be increased snow fall leading to ice-sheet growth (Morgan *et al.,* 1991). However, in the longer term, it is considered that warming will to lead to melting of Antarctic ice-sheets (Hall and Walton, 1992). There is some dispute over the effect of ice recession on the progress of global warming. Ramanathan (1988) argued that the exposed land or sea, being darker than snow and ice, will absorb more solar energy and amplify global warming by 10-20%. However, Bretherton *et al.* (1990) used a dynamic-ocean model to conclude such a positive "ice-albedo" effect is precluded and the progress of global warming will actually be slowed. However, regardless of the model used, global warming is expected to lead to the exposure of more pristine terrestrial substratum (Hall and
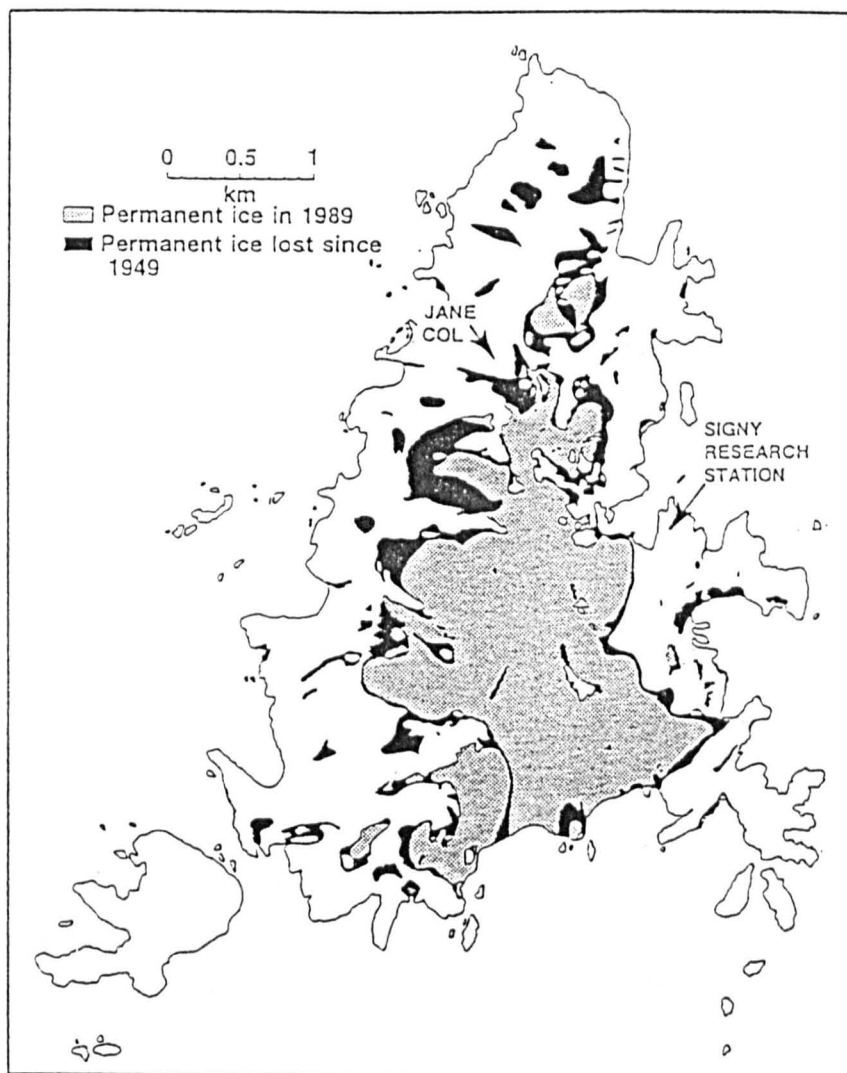
Figure 1.2   The loss of permanent ice from Signy Island between 1949 and 1989 (see Smith, 1990).

Global warming will have an influence on the fate of such exposed substratum. Two factors have been suggested to play an important role under a warmer climate: increased formation of rock microfractures which allow ingress of water (which can subsequently expand on freezing and shatter the bedrock) and thawing of permafrost which will increase soil active depth (Hall and Walton, 1992). Both of these factors can be expected to increase the rate of bedrock weathering and soil formation. The resulting fellfield soil is the most common substratum at exposed Antarctic sites. Fellfield soil can, under favourable conditions, become colonised by microbes (section 1.1.4), and such colonists can mature the soil (section 1.1.5) enabling colonisation by moss and ultimately to the formation of peat soil (Wynn-Williams, 1993). Simulated climate change experiments have shown that the pioneer colonists at Signy Island polygons are able to respond to global warming conditions with dramatic increases in cyanobacterial and chlororphyte biomass after three years of the simulation (Wynn-Williams, 1996). Moss shoots were apparent after a further period of three years indicating that global warming will increase the rate at which the cyanobacteria and chlorophytes proliferate and stabilise the soil to a degree sufficient for moss colonisation. That investigation illustrated the potential of using indigenous species as biological indicators for the progression of global warming, and Wynn-Williams (1996) suggested the fellfield cyanobacterium *Phormidium autumale* as a suitable indicator organism for use in immature polygon soil.

There is evidence that moss and lichens have already started to colonise previously bare glacial moraines in response to warmer summer temperatures at Signy Island (Smith, 1990). Simulated global warming has also revealed the potential of substantial moss-propagule banks to germinate during warmer periods (Smith, 1993). Populations of the two native Antarctic vascular plants, *Deschampsia antarctica* and *Colobanthus quitensis,* have increased their population size by 25 and 5 times respectively between 1964 and 1990 at the Argentine Islands, Antarctic Peninsula (Fowbert and Smith, 1994). An increase in the range of colonisation of Antarctic plants has been recorded at a sub-Antarctic island with three species recorded for the first time and three other species expanding their range of colonisation (Selkirk, 1992). Kennedy (1995a) suggests that community structure will diversify with global change, with dwarf, cushion and prostrate plant forms giving way to taller canopy and hummock forming species. Interestingly, the opposite has been observed under experimental temperature elevation in Arctic tundra with simulated warming causing a decrease in species richness of the indigenous plant community (Chapin *et al.*, 1995). There is evidence that warmer temperatures will also change reproductive behaviour of native Antarctic plants, with experimental warming,

particularly increased degree-days above 0°C, increasing production and germination of spores and seeds in mosses and phanerogams (Longton, 1990; Smith, 1994).

Information derived from studies on the optimum temperatures for growth and photosynthesis of Antarctic colonists suggests that global warming will also change the rate of production of indigenous colonists. For example, the maximum net photosynthesis of the two native vascular plants and of two mosses occurs above 12°C (Collins, 1977; Edwards and Smith, 1988; Harrison *et al.,* 1989), which is above the average soil temperature (Kennedy, 1995a). Upton and Nedwell (1989) have also found similar growth optima for fellfield bacteria isolated from the maritime Antarctic. This suggests that there is selection for psychrotrophic organisms in the type of Antarctic environments where summer daytime temperatures fluctuate and may reach 20°C and exceptionally 30°C (Walton, 1984). In the polar seas, where temperatures fluctuate within a much smaller range of -1.5 to 3.5°C, a psychrophilic strategy would appear to be more competitive. However, low ratios of psychrophilic to psychrotrophic bacteria have been isolated from Antarctic freshwater lakes and sea water (Upton and Nedwell, 1989). Such a low proportion of psychrophiles in constant low temperature environments was unexpected and the authors proposed that the psychrotrophs entered these environments from other, more temperature-variable environments (e.g. being washed down from fellfields into freshwater lakes or by being moved by sea currents from warmer waters into Antarctic waters).

Roser *et al.* (1993) showed that continental Antarctic ornithogenic soils also have a predominance of psychrotrophic bacteria. It was found that substrate induced-respiration rates increased linearly with temperature from 3 to 20°C suggesting significant numbers of bacteria present have growth optima approaching 20°C. Similar increases have also been found in soil samples from fellfield-like habitats at a continental Antarctic site (Bölter, 1990). Additionally, out of fifteen bacterial isolates from a continental cold desert site only one was an obligate psychrophile (Siebert and Hirsch, 1988). At such sites soil is below freezing for most of the year, however, when temperatures do rise above freezing, rock temperatures have been recorded at 15°C providing a selective advantage to the psychrotrophic colonists (McKay and Friedmann, 1985).

The result of the psychrotropic nature of many Antarctic colonists indicates that they will respond to elevated temperatures with increased rates of net photosynthesis (Edwards and Smith, 1988), decomposition (Upton and Nedwell, 1989) and

predation (Block, 1990). The consequence for carbon flow into such systems has yet to be firmly established. Experiments with climate-changing cloches at Signy Island have shown that although there is increased bacterial decomposition activity in warmed soil (Foister *et al.,* 1993), an increase in moss colonisation (Wynn-Williams, 1996) suggests that such sites are accumulating carbon and becoming more stable.

1.2.3   *Antarctic Ozone Depletion*

Ozone depletion occurs above the Antarctic because the polar vortex occurring in this region isolates a large mass of cold air and stratospheric cloud that act as a substrate for ozone-destroying chemicals (Hoffmann, 1989). The chemicals are chlorinated radicals that have derived from anthropogenic chlorofluorocarbons (CFCs) and hydrochlorofluorocarbons (HCFCs, Anderson *et al.,* 1991). The ozone-destroying reaction is catalysed by early springtime sunshine and results in a predictable seasonal decrease in ozone at this time (Farman *et al.,* 1985; Solomon, 1990). Satellite measurements have revealed that the total springtime ozone column above Antarctica has decreased by over two thirds between the late 1950s and 1993 (Kerr, 1994). However, the growth in the concentration of CFCs, but not HCFCs, has slowed to about zero because of the implementation of the Montreal Protocol (an international agreement on the reduction of CFC and HCFC production, IPCC, 1996). As a result, the concentrations of CFCs and HCFCs, and consequent ozone depletion, are projected to decrease substantially by 2050 (IPCC, 1996).

Stratospheric ozone is the only major atmospheric gas to absorb radiation below 300 nm and so has a vital role in regulating the amount of UVR (100 - 400 nm) that reaches the Earth's surface. Springtime ozone depletion has resulted in levels of incident UVR being as high in the spring as that which occurs naturally in the summer, when the increased solar zenith angle allows greater incident radiation (Jones and Shanklin, 1995). Indeed a 65% decrease in ozone column, similar to the observed difference between the 1950s and 1993, has been calculated to cause a 14-fold increase in UV-B at 305 nm (Frederick and Snell, 1988).

The primary biological effect of UV-B (280 - 315 nm) and UV-C (100 - 280 nm) is to cause lesions in DNA, which can lead to mutation (Mitchell and Karentz, 1992). Secondary effects include absorption by RNA, proteins and other biological

molecules and the production of radicals by catalysis of photochemical cell reactions (Caldwell, 1981). Radicals have toxic effects and cause oxidative stress in the organism (Karentz, 1991). The result of one or a combination of these effects is mutagenesis, changes in membrane structure and impairment of metabolic activities. Studies on the long term consequences of UVR exposure have been conducted on organisms from temperate climates and include changes in morphology and growth, restricted mobility, a decrease in chlorophyll, lipid, protein and mRNA of leaves and disturbances in stomatal physiology (Jordan *et al.*, 1991; Strid and Porra, 1992).

1.2.4   Terrestrial Antarctic Microorganisms and UVR

The ecological consequence of springtime ozone depletion depends upon the tolerance of the indigenous species to UVR. Tolerance depends upon the effectiveness of UVR screening molecules and of DNA repair processes. Organisms can also reduce UV-induced damage by reducing exposure to incident UVR. These three strategies are discussed below.

Protection from UVR
(i)   Physical barriers
Antarctic microorganisms are faced with a paradoxical situation concerning UVR avoidance. In order for microbial primary producers to function, sufficient photosynthetically active radiation (PAR) must be absorbed to allow photosynthesis to occur. Exposure to PAR inevitably leads to UVR exposure. Cyanobacteria in polygons at Signy Island show behavioural activity which allows them to avoid direct exposure to solar radiation. The cyanobacteria and chlorophytes do not grow on the surface of the soil, but have been observed in a layer beneath the upper layer of fine particles (Davey and Clarke, 1991, 1992). The cyanobacteria in such a community are able to respond to UV-B radiation and change their orientation and position in response to changing light conditions using gliding motility (Häder, 1987). The effectiveness of this avoidance strategy is demonstrated by the fact that the dominant cyanobacterial colonist of Signy Island fellfield polygons, *Phormidium autumale* (Davey and Rothery, 1993), is highly sensitive to UV-B, even at ambient levels (Häder, 1984). The McMurdo Dry Valleys, cold deserts of continental Antarctica, feature ice-free areas with no surface colonists. There are lichen-dominated microbial communities below the rock surface, however, this appears to be primarily to avoid desiccation, rather than UVR (Nienow and Meyer, 1986). Nevertheless, the resulting UV-protection gained in this manner may be a significant

factor in long-term survival given the exceptionally low growth rates of such communities (Vestal, 1988).

Protection from UVR

(ii)   UVR-absorbing compounds

There is a range of screening and quenching molecules which are used by microorganisms to combat the effect of UVR. For example, scytonemin is a lipid-soluble extracellular pigment that has been found in the mucilagenous sheath of an Antarctic *Nostoc commune* isolate (Garcia-Pichel and Castenholz, 1991). This pigment acts as a sunscreen, by absorbing UV-A whilst allowing PAR to enter the cell. Removal of this pigment from the sheath of *Chlorogloeopsis* induced bleaching of chlorophyll during periods of metabolic inactivity, such as desiccation (Garcia-Pichel and Castenholz, 1993). Scytonemin has been found in every major taxonomic cyanobacterial group leading to the suggestion that it was a constituent of cyanobacteria prior to the diversification of the group in the Precambium (Garcia-Pichel and Castenholz, 1991). A comparable water-soluble UV-A/UV-B absorbing pigment has been recorded in the capsule of a strain of *Nostoc commune* isolated in China (Scherer *et al.*, 1988) which is related to species found in Antarctica (Broady, 1979).

In a survey of 57 species of Antarctic marine organisms (fish, invertebrates and algae) showed that nearly 90% contained mycosporine-like amino acids (MAAs), intracellular UVR-absorbing compounds (Karentz *et al.*, 1991). MAAs absorb wavelengths of UV-A and UV-B from 310 to 360 nm, depending upon their chemical structure (presence of side groups and attached amino acids; Figure 1.3). Cyanobacteria isolated from UVR-exposed habitats also contain MAA, in addition to scytonemin (Garcia-Pichel and Castenholz, 1993). Organisms often contain several MAAs and, if the *in vitro* absorbencies are assumed to be the same when located intracellularly, such combinations of MAAs could protect against large regions of the UV spectrum (Karentz *et al.*, 1991).

Photosynthetic pigments are particularly vulnerable to UVR-damage. However, the photosynthetic pigments found in fellfield cyanobacteria appear to be differentially sensitive to UVR. The accessory pigment phycoerythrin bleaches first, followed by carotenoids and then chlorophyll (Rodriguez *et al.*, 1989; Häder and Worrest, 1991). This sequential loss of pigments may provide a level of defence by allowing sacrificial loss of the more sensitive pigments allowing the remaining pigments to remain functional during periods of UV-exposure.

Pigments associated with photosynthesis also play a role in UV-defence. There is evidence that phycoerythrin provides UV-protection in addition to protection from intense visible light (Tyagi et al., 1992). It is also known that carotenoids protect cyanobacteria by quenching excess solar energy (especially UV-B) not used for photosynthesis and which can otherwise lead to the formation of lethal singlet oxygen (Buckley and Houghton, 1976; Paerl, 1984). Interestingly, several Antarctic marine, freshwater and terrestrial bacterial isolates have been shown to contain carotenoids (Shiba, 1991; Dobson et al., 1991; Chauhan and Shivaji, 1994), presumably for the same protection from high incident radiation.

Protection from UVR

(iii)   DNA repair

Although the absorption peak of DNA is 260 nm, UV-B (280 to 315 nm) can cause significant and lethal DNA damage (Karentz, 1991). There are three known enzymic mechanisms of DNA repair: photoreactivation; excision (dark) repair and postreplication (recombination) repair. There is evidence that Antarctic marine bacteria and diatoms repair UV-induced DNA damage using photoreactivation (Karentz, 1994), with diatoms also able to use excision repair (Karentz et al., 1991). Repair mechanisms have also been reported in cyanobacterial species related to those at terrestrial Antarctic sites (Broady, 1979; O'Brien and Houghton, 1982; Levine and Thiel, 1987), leading Wynn-Williams (1994) to suggest a similar UVR-repair capability may exist in Antarctic species.

Effect of UVR on Antarctic Microbial Colonists

One of the biggest obstacles in assessing the impact of increased UV-B on Antarctic macro and microorganisms is the lack of data for sites prior to ozone depletion. However, Markham et al. (1990) showed a relationship between decreasing levels of ozone with increasing levels of flavonoids in dried Antarctic moss samples collected between 1957, prior to the ozone hole, and 1989. Exposure of Antarctic phytoplankton to UV-B radiation caused inhibition of photosynthesis and reduction of primary production (Helbling et al., 1992). Blocking of UV-B under a normal ozone column has yielded higher production rates, indicating that UV-B is limiting photosynthesis under "normal" ozone protection (Behrenfeld et al., 1993). The cold polar seas contain a greater amount of dissolved atmospheric gases than warmer temperate waters, enabling them to be more productive. Exposure of polar primary

producers, such as the phytoplankton, to increased UV-B levels could have global implications in terms of the amount of carbon cycled in these waters.

Very little is known about the response of terrestrial Antarctic microbiota to the increasing levels of UVR caused by the ozone hole. However, Kennedy (1995a) argues that increased UVR is likely to change the taxonomic composition of terrestrial communities as UVR susceptibility is species-specific (Vincent and Quesada, 1994) and that reduced ecosystem productivity will result from photoinhibition of cyanobacteria and chlorophytes.

mycosporine - Gly
λ_{max} = 310nm

palythine
λ_{max} = 320nm

asterina - 330
λ_{max} = 330nm

palythinol
λ_{max} = 332nm

palythene
λ_{max} = 360nm

porphyra - 334
λ_{max} = 334nm

mycosporine -
glycine:valine
λ_{max} = 335nm

shinorine
λ_{max} = 334nm
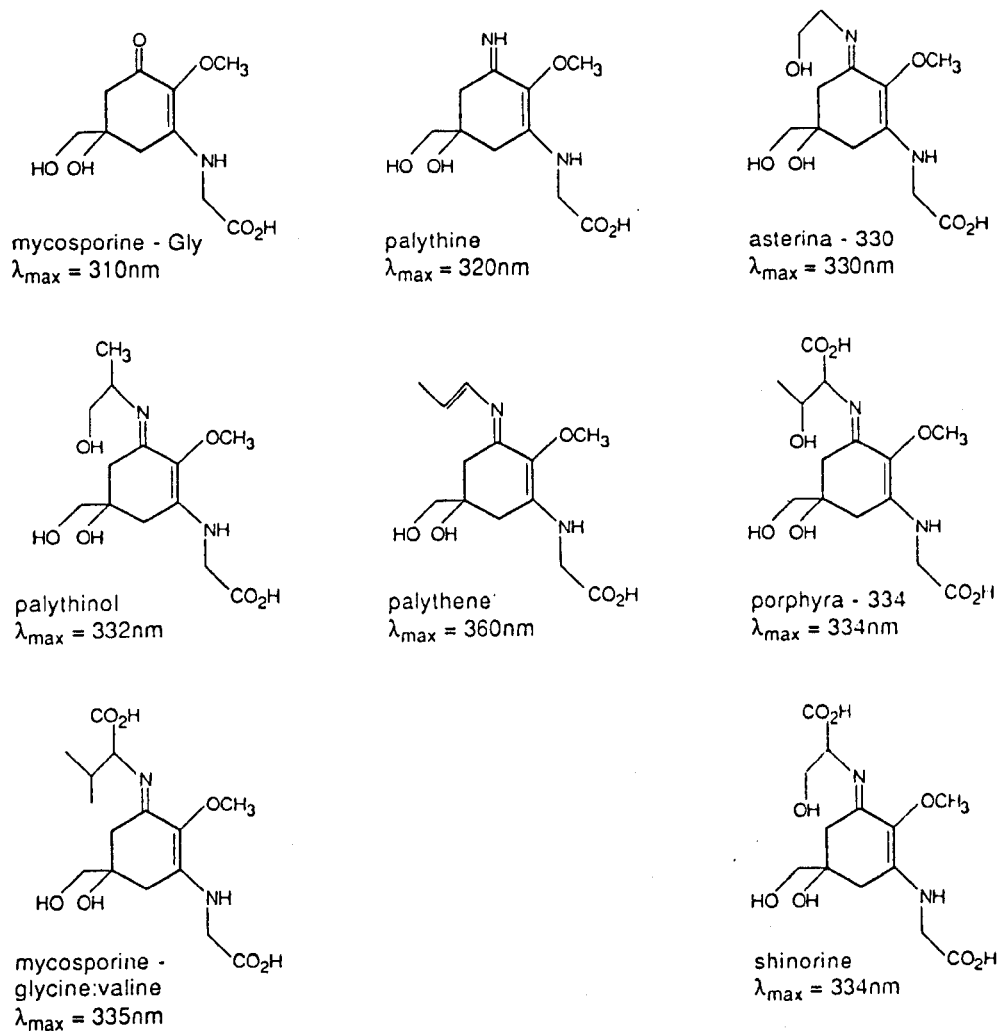
Figure 1.3   Molecular stuctures and wavelength of maximum absorbance (λ_{max}) of eight mycosporine-like amino acids identified in Antarctic species (from Karentz *et al.*, 1991).

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1.3. Aims and Objectives

1.   To carry out an ecophysiological survey of fellfield bacterial isolates. This will allow the morphological and functional diversity of the bacteria in fellfield polygons to be assessed. This includes the ability to metabolise the two main carbohydrate nutrient sources in polygon soil: compatible solutes leached into the soil from cyanobacteria and chlorophytes and from mosses and lichens colonising the periphery of polygons (e.g. glucose, glycerol, erythritol; Arnold, 1995); and EPS material of the polygon photo and heterotrophs (Wynn-Williams, 1993) via the production of extracellular polysaccharidases.

2.   To monitor the bacteriological response to simulated global warming, with and without incident UV-B radiation at two Antarctic polygons. Chronosequences of soil samples will be used to determine the effect of climate change throughout the growing season. The study will focus on the effect of the simulation on bacterial numbers and metabolic activity (dehydrogenase and phosphatase activity), and on microbial carbon turnover (bacterial polysaccharidase activity and soil carbon and saccharide content).

3.   To use field measurements taken from two Antarctic polygons to evaluate changes in microbial energy use caused by the simulated climate change. Seasonal bacterial energy expenditure (SBEE) will be assessed by considering the energetic cost of bacterial biomass production and by using microbial dehydrogenase activity to determine microbial electron transfer system activity and hence microbial ATP production. The potential hydrolysis of EPS will be determined by conversion of bacterial polysaccharidase activity into total available hexose.

4.   To measure the separate effects of simulated global warming conditions and UV-B exposure on the cyanobacterial and bacterial communities at two polygons during two consecutive Antarctic summers. The biomass relationship between cyanobacteria and bacteria will be investigated to determine how climate change affects the bacterial carrying capacity of the phototrophic community.

2. MATERIALS AND METHODS

2.1 Antarctic Field Site

2.1.1 Location

The fellfield site is an area of pattern ground on Jane Col, Signy Island, South Orkney Islands (60°43'S, 45°35'W) in the maritime Antarctic. This site was gradually exposed from permanent ice cover from approximately 1950 onwards as part of a recession of the ice cap on this island (Smith, 1990). The fieldfield site is characterised by polygons: pools of rock fragments (up to 4 m diameter) formed by frost and ice formation collecting small fines (12 to 132 μm) at the centre, with larger rocks at the periphery (Chambers, 1967). Polygon soil is typically poor in both organic and iorganic nutrients (Allen and Northover, 1967; Allen et al., 1967) with a water holding capacity of around 20% (w/w; Davey and Rothery, 1993).

2.1.2 Climate Change Simulation

Climate change simulation was brought about with the use of plastic cloches placed on fellfield soil. The cloches used were of three designs (Table 1.1) to provide simulated global warming conditions and to block the transmission of incident UV-B, either together or in isolation. The cloches (570 × 290 × 150 mm high) were placed on a polygon with an adjacent area of equal size used as a control plot (Plate 1.3).

2.1.3 Soil Sampling (Wynn-Williams, 1992a)

Soils were sampled using a sterilised (microwave, 2 min) syringe as a corer. Five cores (25 mm depth and 16 mm diam) were taken from beneath the cloche and from the adjacent control area at each sampling point. The piston and plunger were used to draw up th moist cores as the barrel was screwed into the soil. The cores were transferred to the individual compartments of a sterile repli dish and the dishes sealed in sterile polythene bags to minimize water loss and transported to the research station immediately. The cores were then placed in a refridgerator (-8°C) overnight before storage at -20°C during transport to the UK. In the UK the samples were stored at -70°C.

Prior to analysis, soil cores were stored at -20°C for 24 h and then defrosted at 4°C for 24 h. Preliminary studies by Foister *et al.* (1993) established that the soil must be homogenised in order to reduce the variation between cores taken from within the same polygon area. Further cores could have been taken to account for the intra-polygon variation but this would have depleted the soil of the polygon field sites. The five cores were mixed together using a flame-sterilised, cooled (4°C) pestle and mortar.

Table 1.1   Stucture and function of cloches installed on fellfield polygons.

Cloche structure	Primary cloche effects (1)	Location (polygon)	Year of installation	Year(s) of sampling
Polystyrene box	temperature (2) elevation (+3.43 C°) UV-blocking	WW 2	1985	1992
Acrylic box	temperature elevation (+2.22 C°) UV-transparent	WW 5 WW 2	1988 1988	1992 1993 and 1994
Acrylic shield -no side walls	UV-blocking (3)	WW 4	1988	1993 and 1994

(1)   Cloches also have secondary effects as reviewed by Kennedy (1995b) and discussed in Chapter 7.
(2)   Temperature effects re averages from data taken between 2-1-94 and 17-3-94 (Wynn-Williams, 1996).
(3)   Temperature was elevated by 1.11 C° between 2-1-94 and 17-3-94 (Wynn-Williams, 1996).

2.2   Soil Analysis

2.2.1   Gravimetric Moisture Content (Forster, 1995)

To determine the moisture content of soil, sub-samples (1g) were weighed, placed at 105°C (Hotbox oven size 2, Gallenkamp, UK) for 24 h, cooled in a desiccator and reweighed in order to calculate moisture loss.

2.2.2   Water Holding Capacity (Forster, 1995)

Oven dry soil (105°C, 24 h, 10g) was placed on a filter paper (No 1, Whatman, UK) in Hilgard cups which were immersed in water to below their rim and left for 24 h. Cups were then removed and excess water allowed to drain (6 h). The moisture content was determined (section 2.2.1) and results expressed as g water g^{-1} soil.

2.2.3   Soil Saccharide Measurement (Dubois et al., 1956)

Soil (1 g) was placed in a 250 ml quick fit round bottom flask and 20 ml (0.25 M) sulphuric acid added. The suspension was refluxed for 16 h to hydrolyse soil polysaccharides. The cooled hydrolysate was neutralised with 5 M sodium hydroxide to pH 6.0 and filtered to remove the dark green precipitate. The filtrate was then passed through ion exchange columns to remove Fe^{3+}, NO_3^- and Cl^- ions which are reported to interfere with saccharide determination (Martens and Frankenburger, 1990). Cation exchange columns (SCX, Altech, UK) were conditioned with 3 ml methanol followed by 10 ml 10 mM hydrochloric acid and 400 μl of distilled water. The filtrate was then passed through the column at a flow rate of 0.5 ml min^{-1}. Eluates were then passed through anion exchange columns (SAX, Altech, UK) with the same flow rate after the columns were conditioned with 3 ml methanol followed by 20 ml 100 mM sodium acetate and 400 μl of distilled water. The sample (200 μl) was added to 200 μl 5 % (v/v) aqueous phenol and 1 ml of concentrated sulphuric acid added. After 10 min, the mixture was vortexed and the absorbance read at 490 nm after a further 30 min. Absorbances were related to saccharide concentration by comparison to a calibration curve of glucose standards (0-20 μg glucose ml^{-1}).

2.2.4 Elemental Analysis

Soil carbon, nitrogen and hydrogen was measured using a 110C Carlo-Erba simultaneous C H N analyser.

2.3 Bacterial Isolation and Characterisation

2.3.1 Bacterial Isolation and Viable Counts

Ten-fold serial dilutions were prepared from 1g soil samples using 50 mM Tris pH 7.0 containing 0.1% Tween 80. Aliquots (100 µl) were spread, in triplicate, on full strength and 1/10 strength casein peptone starch (CPS) agar plates (Collins and Willoughby, 1962) supplemented with cycloheximide (Sigma, UK) at a final concentration of 50µg l^{-1}. Plates were incubated at 10°C for 28 d prior to counting. Three 1g samples were spread plated in this way from each combination of five soil cores. Bacterial isolates for use in characterisation studies were picked off isolation plates, sub-cultured at 10°C and stored in CPS sloppy agar (0.4% agar) at 4°C.

2.3.2 Colony Form

The colony form and pigmentation of isolated bacterial colonies on CPS agar plates were examined after a 7 d incubation at 25°C. Isolates were subsequently grouped into nine broad categories according to pigmentation: white, translucent, yellow, orange, brown, red, bright pink, pale pink and purple. This provided a scheme for the initial grouping of large numbers of isolates grown on agar plates.

2.3.3 Morphological Characterisation

Isolates were incubated in 10 ml CPS broth in glass universals at 25°C for 24 to 72 h until visible growth had occurred. Microscopic observations of bacterial shape and size was then assessed using phase contrast microscopy (Zeiss A10 microscope). Grams stains were then performed according to Davey and Thompson (1971).

2.3.3   Enzyme Activities (adapted from Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1988)

Bacterial neutral phosphatase, α-D-glucosidase (EC 3.2.1.2.0), β-D-glucosidase (EC3.2.1.2.1), α-D-galactosidase (EC3.2.1.22) and β-D-galactosidase (EC3.2.1.23) enzymes was assessed. This gave an indication of the proportion of the culturable bacterial community able to produce the enzymes whose activity were subsequently assayed for in soil samples. Isolates were grown on CPS agar supplemented with a *p*-nitrophenyl (*p*NP) analogue (phosphate or α-, β-glucose or galactose, Sigma, UK) for 72-144 h at 25°C. Production of the enzyme by the bacterial colony would cause cleavage of the bond between the *p*NP and substrate giving a yellow halo of *p*NP the colony.

2.3.4   Carbohydrate Use

The loss of sugars and polyols from mosses under freeze-thaw stress in Antarctic environments has been widely reported (Melick and Seppelt, 1992; Roser *et al.,* 1993). Tearle (1987) reported that the bacterial community in Signy Island fellfield soil responded to polyol and glucose supplements. Bacterial isolates were screened for ability to grow on a range of carbohydrates recorded in fellfield polygons. The growth of bacterial isolates was compared on a minimal salts medium with and without a supplement of carbohydrate (1% w/v) after incubation at 25°C for 144 h.

Minimal Medium

Na_2HPO_4	2 g	
KH_2PO_4	1.4 g	
$(NH_4)_2SO_4$	1.1 g	
$MgSO_4$	0.2 g	
yeast extract	10 mg	
trace elements sol^n	5 ml	
agar	6 g	Made up to 1 litre with distilled water

<u>Trace element solution</u>   (grams per litre)

EDTA	12.0	$Na_2MoO_4. 2H_2O$	0.1
NaOH	2.0	$FeSO_4. 7H_2O$	2.0
$ZnSO_4. 7H_2O$	0.15	H_3BO_3	0.01
$MnSO_4. 4H_2O$	0.15	$CaCl_2$	0.2
$CuSO_4. 5H_2O$	0.05	$NiCl_2. 6H_2O$	0.002
H_2SO_4 (conc.)	0.5	$CoCl_2. 6H_2O$	0.01
Na_2SO_4	10.0		

2.3.5   Determination of Isolate Optimum Growth Temperature

Bacterial isolates were replica plated onto CPS agar plates (section 2.3.1) and the plates incubated at 0, 4, 10, 20, 25 and 37°C. Growth was visually assessed by awarding arbitary units in relation to the size of colony after four weeks.

2.4   Soil Enzyme Activities

2.4.1   Electron Transfer System (ETS) Activity (adapted from Trevors 1984; von Mersi and Schinner, 1991)

INT (2(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride, Sigma, UK) reductase activity was determined for 0.5 g (dry weight equivalent) subsamples in sterile bijou (7 ml) bottles. Sterile controls were prepared by autoclaving soil (121°C, 15psi) for 30 min on three consectutive days. An aqueous solution of INT (0.5%) was prepared by dissolving 0.5 g of INT in N.N. dimethylformamide (2 ml), making the volume up to 100 ml with distilled water and filter sterilising. A constitutive and substrate-induced activity was assayed. The former involved adding 1 ml of INT solution (0.5 %) and 1 ml of sterile distilled water to each 0.5 g soil sample. The latter assay involved replacing the distilled water with 200 μl yeast extract solution (1 % w/v) and 800 μl Tris buffer (0.5 M, pH 7.0). Samples were incubated at 10 °C for 48 h. After the incubation period, the INTF (iodonitrotetrazolium formazan) produced was extracted by adding 5 ml of ethanol/N.N. dimethylformamide (1:1) and samples were stored in the dark for 60 min and vortexed every 20 min. Aliquots (1 ml) were centrifuged (9464 x g, 20 min, MSE Microcentaur) to remove soil particles and the absorbance at 464 nm recorded using soil extracts (controls

with INT solution replaced with distilled water) as a blank. A standard curve (0-100 nmoles ml^{-1} INTF) was prepared in ethanol/N.N. dimethylformamide (1:1) and the sterile control reading subtracted from the sample reading and compared with INTF standards. Activities were expressed as nmoles INTF 48 h^{-1} g soil^{-1} (dwt).

2.4.2 *Neutral Phosphatase Activity* (Tabatabai and Bremner, 1969)

Soil (0.5 g dry weight equivalent) was placed in a sterile bijou with 2 ml modified universal buffer (MUB) pH 7.0 and 0.5 ml 50 mM *p*-nitrophenyl phosphate added. Soil solutions were vortexed and incubated at 30°C for 4 h. CaCl2 (0.5 M, 0.5 ml) was added to flocculate humic substances and 2 ml Tris buffer (0.1 M, pH 12.0) added to stop the reaction and increase the solution pH to intensify the colouration of *p*-nitrophenyl. Aliquots (1 ml) were centrifuged at (9464 x *g*, 20 min, MSE Microcentaur) to remove soil particles and the absorbance at 410 nm recorded with soil extracts (controls with *p*NP phosphate replaced with distilled water) as a blank. A standard curve of *p*-nitrophenyl (10 - 100 nmol ml^{-1}) was prepared and the sterile control reading subtracted from the sample reading and compared with *p*NP standards. Activities were expressed as nmoles *p*NP h^{-1} g soil^{-1} (dwt).

2.4.3 *α-D- and β-D-glucosidase, α-D- and β-D-galactosidase Activity* (Eivazi and Tabatabai, 1988)

Soil (0.5 g dry weight equivalent) was placed in a sterile bijou with 2 ml modified universal buffer (MUB) pH 6.0 and 0.5 ml 50 mM of the appropriate *p*-nitrophenyl substrate added. Soil solutions were vortexed and incubated at 30°C for 4 h. CaCl2 (0.5 M, 0.5 ml) was added to flocculate humic substances and 2 ml Tris buffer (0.1 M, pH 12.0) added to stop the reaction and increase the solution pH to intensify the colouration of *p*-nitrophenyl. Aliquots (1 ml) was centrifuged at (9464 x *g*, 20 min, MSE Microcentaur) to remove soil particles and the absorbance at 410 nm recorded with soil extracts (controls with *p*NP-substrate replaced with distilled water) as a blank. A standard curve of *p*-nitrophenyl (1 - 10 nmol ml^{-1}) was prepared and the sterile control reading subtracted from the sample reading and compared with *p*NP standards. Activities were expressed as nmoles *p*NP h^{-1} g soil^{-1} (dwt).

2.5   *Growth of* Arthrobacter oxydans *in Sterilised Antarctic Soil*

2.5.1   *Soil Preparation, Inoculation and Enumeration of* A. oxydans

Surface slices of soil from polygons (WW3 and 7, sampled 1988/9) at Jane Col, Signy Island were homogenised, dried at 105°C for 24 h and sieved (500 μm mesh). Soil (1 g) was placed into a glass universal and autoclaved (30 min, 121°C, 15 psi). An aliquot (200 μl) of *A. oxydans* suspension was then inoculated into the cooled soil. This volume of suspension brought the soil to 80 % water holding capacity, typical for this soil in the field (Davey and Rothery, 1992). The *A. oxydans* suspension was obtained from a 24 h culture in CPS broth, incubated at 25°C, which was then centrifuged (9464 x g, 15 min) and the cell pellet resuspended in sterile distilled water. The volume of the cell suspension was subsequently diluted to give the desired cell density for inoculation (typically 1 × 10^7 per microcosm) by relating the absorbance of the suspension at 600 nm cell numbers.

Filter sterilised (0.22 μm pore size) glucose supplements were added in 25 μl aliquots to give final concentrations up to 5 mg g^{-1} soil. An equal volume of sterile distilled water was added to control samples.

In order to enumerate the *A. oxydans* population, 10 ml Tris buffer (0.5 M, pH 7.0, 0.1 % Tween 80) was added to each microcosm and a ten-fold serial dilution prepared. Three replicates of 10 μl from successive dilutions were inoculated onto CPS agar plates and incubated at 25°C for 7 d prior to counting colony forming units.

2.5.2   *Rate of Evolution of* $^{14}CO_2$ *from Radiolabelled Glucose by* A. oxydans

A. oxydans was inoculated into sterile Antarctic soil in sterile glass universals with glucose supplements added which incorporated 1 kBq of ^{14}C radiolabelled glucose (Sigma). Plastic scintillation vials (L.I.P. Ltd, UK) containing 1 ml NaOH (0.5 M) were placed on the surface of the soil in each universal. The scintillation vials were periodically replaced with vials containing fresh NaOH solution. Scintillation fluid (Optiphase, Wallac, UK; 4 ml) was added to the removed vials which were left at 4°C for 24 hours to allow chemiluminescence to subside prior to measuring the radioactivity using a scintillation counter (Rackbeta, Wallac, UK). Control vials containing 1 kBq of ^{14}C radiolabelled glucose were counted alongside samples to determine the proportion of the glucose

sample mineralised. This negated the need to determine the absolute counting efficiency of the scintillation counter.

2.6   Conversion of Field Data into Microbial Energy Use

2.6.1   Calculation of Glucose Consumption Equivalent (GCE) of Fellfield Bacterial Communities

The yield coefficient of Arthrobacter oxydans (2.42×10^8 cells mg^{-1} glucose) was used to calculate the Glucose Consumption Equivalent (GCE) of fellfield bacterial communities. The increase in bacterial number between successive samples was calculated and was then divided by the above yield coefficient number to give the GCE required to give the increase in bacterial numbers. The seasonal GCE of the bacterial community was calculated by the adding the GCEs calculated for each successive sampling time.

2.6.2   Conversion of ETS Activity into Seasonal ATP and Oxygen Consumption

Total seasonal constitutive ETS activity was calculated by multiplying the activity per hour by the time in hours between samples to give the total activity between sampling times. The activity between all sampling times was calculated as the total seasonal activity. In order to take into account the relationship between INT-based ETS activity and the actual microbial ETS activity of the microbiota, a conversion based on the work of Benefield et al. (1977) was used. These authors report that INT-based activities represent 5.41% of total microbial ETS activity measured by oxygen consumption. Thus the ability of INT to compete with oxygen during the assay is accounted for. The total seasonal microbial ETS was then converted into ATP produciton on the bases of a 1:1 ratio between moles of INT reduced and moles of ATP formed by oxidative phosphorylation (Neidhardt et al., 1990). The moles of oxygen consumed was then calculated by assuming a ratio of 4 ATP formed for every one oxygen molecule reduced (Neidhardt et al., 1990).

2.6.3   Conversion of Polysaccharidase Activity into ATP Production

Total seasonal polysaccharidase activity of all four enzymes was calculated by multiplying the activity per hour by the time in hours between samples to give the total activity between sampling times. The activity between all sampling times was calculated as the

total seasonal activity. The total seasonal activity of all four enzymes was calculated and converted to moles of hexose hydrolysed, assuming that one mole of pNP cleaved from pNP-hexose, is equivalent to one mole of hexose released from polysaccharide. The total ATP available from the hydrolysed hexose was calculated by assuming that 60 % of the hexose would be used for fuelling (Cheshire et al., 1960; Payne, 1970; Shields et al., 1974) and that 1 mole of hexose yielded 1.076 moles of ATP (section 5.2.1.2).

2.7   Image Analysis of Fellfield Cyanobacteria   (Wynn-Williams, 1988)

2.7.1   Sample Preparation and Epifluoresence Microscopy

Circular coverslips (19 mm diam) were lightly pressed onto the surface of a soil core to give a flat surface and sterile distilled water was added where needed to moisten the soil to aid microscopy. Observations were made with an epifluorescence microscope (Leitz Laborlux 12, Ploemopak epifluorescence illuminator, HB-200 50 W mercury arc lamp, N2.1 filter block). This allowed the visualisation of autofluorescing algae and cyanobacteria containing the photosynthetic pigment chlorophyll a, phycocyanin or phycoerthyrin.

2.7.2   Image Capture and Analysis

A charge-coupled device (CCD) video camera captured the microscopic images and transmitted them to a Seescan I3000 image analyser (Seescan PLC, Cambridge, UK). The analyser digitised the images by converting them to a monochromatic 256×256 map of pixels, each of a 0-128 shade of grey. A subroutine to eliminate electronic "noise" generated by the camera was incorporated into the image capture process. Ten fields of view were randomly selected (by lightly flicking the microscope's stage positioning dial) for each core sampled and the ten captured images were stored together on an optical disk for subsequent analysis.

Autofluorescing organisms were taken as being the pixels of above a grey shade threshold of 74 (out of 128 shades). This allowed discrimination against background. The total number of such pixels was calculated and used to calculate the percentage colonisation of soil on the soil surface. The area, volume and breadth of individual organisms was also calculated. Three expressions for the calculation of length were used,

each appropriate for different types of filament observed. One measure was based on area and perimeter and was useful for rods and short filaments. Longer filaments were measured on the basis of the total number of x- and y-intercepts. Entwined filaments were deconvoluted and measured with a Seescan software routine (Wynn-Williams 1990).

2.7.3 Calculation of the Biovolume Ratio of Cyanobacteria to Bacteria

The cyanobacterial biovolume per microscopic field of view ($1.8 \times 10^5 \ \mu m^2$) was calculated from the average of ten fields of view analysed by computer-aided image-analysis (section 2.7.2). This value was multiplied by 1117.22 (the number of fields of view of each soil core). This give the total cyanobacterial biovolume in each syringe-core soil sample.

The number of viable bacteria in the one core was then calculated. The sampling syringe has an internal volume of 5.02 cm³ (0.8 cm dia, 2.5 cm depth) and was found to contain on average 5.18 g of field moist soil. The soil moisture content was then used to calculate the amount of dry soil. The number of viable bacteria per gram of dry soil (section 2.3.1) was then multiplied by the mass of dry soil per syringe to calculate the total number of viable bacteria in one soil core. This was multiplied by 1.178 (the average volume in μm^3 of a fellfield bacterial isolate; Chapter 3) to give the total biovolume of bacteria per core. The biovolume ratio of cyanobacteria to bacteria was calculated by dividing the total cyanobacterial biovolume by the total bacterial biovolume.

3. ECOPHYSIOLOGICAL SURVEY OF FELLFIELD BACTERIAL ISOLATES

3.1 Introduction

The impact of climate on a microbial ecosystem can be assessed by monitoring changes in the central activities of that system such as carbon turnover. As a result, many studies on Antarctic habitats have focused on carbon fixation and phototrophic communities, from microscopic algae and cyanobacteria to cryptogamic lichen and moss colonists (Friedmann and Ocampo, 1976; Smith, 1985; Wynn-Williams, 1996). Tearle (1987) reported a flush of compatible solutes from such fellfield colonists into soil in a Signy Island polygon during spring thaw and noted subsequent utilisation of these compounds by the heterotrophic community. Melick and Seppelt (1992) reported the loss of compatible solutes from Antarctic mosses following freeze-thaw cycles. Wynn-Williams (1980) also observed exponential increases in the bacterial microflora at a moss peat site on Signy Island as a result of the postulated release of sugars after freeze-thaw action. Given that microalgae are the primary colonists of fellfield polygons, which consist of mineral soil of low organic matter (Allen and Northover, 1967), compatible solutes released from freeze-thaw damaged phototrophs may act as a significant carbon source for the fellfield bacterial community. The microalgae also produce polysaccharide sheath material, visualised by direct microscopic observation (Wynn-Williams, 1993). Such material may also provide an important carbon source for the heterotrophic microbial community. Thus, it can be concluded that compatible solutes and extracellular polysaccharides (EPS) are both important carbon sources for the bacterial community.

This chapter reports an investigation of 25 bacterial isolates and their metabolic capabilities in relation to the two potential carbon and energy substrates. The experiments determined whether the two carbon sources are the basis of two bacterial metabolic strategies: (a) the ability to utilise the wide range of the compatible solutes recorded in polygons; and (b) the ability to produce enzymes that hydrolyse bonds within the EPS. The relationship between isolate growth rate and temperature is also reported in order to establish the proportion of psychrophiles, psychrotrophs and mesophiles. Gram stain and cell morphology are also reported to facilitate comparisons with bacterial surveys from other habitats.

3.2 Results and Discussion

3.2.1 Selection of Bacterial Isolates

Twenty five bacterial isolates were chosen from bacteria isolated from exposed soil from the centre of polygon WW5 (sampled on 28-12-92). A large proportion (70%) of the bacterial colonies isolated were pigmented and isolates were selected reflect the relative abundance of each pigment type. Isolates were randomly chosen from each of nine pigment categories (Table 3.1; six white, one translucent, four yellow, two orange, one brown, four pink, two pale pink, four red and one purple).

Pigmented bacteria have been isolated from a variety of Antarctic habitats: maritime and continental terrestrial soil (Wynn-Williams, 1983; Shivaji *et al.,* 1989, 1991, 1992); continental cold desert soil (Siebert and Hirsch, 1988) and hypersaline lake water (Dobson *et al.,* 1991). However, little is known about the relative abundance of pigmented strains at these different habitats. Instead, studies have focused on the function of pigments (Pemberton *et al.,* 1993; Chauhand and Shivaji, 1994) or on the use of pigmented bacteria as marker species (Wynn-Williams, 1983).

Bacterial pigments may protect cells from incident solar radiation and this is of particular importance to microorganisms living near the surface of bare soil. Chauhan and Shivaji (1994) recorded that the yellow pigment of the terrestrial Antarctic bacterium, *Sphingobacterium antarcticus,* was due to carotenoid pigments - UV-protection molecules previously reported in phylloplane (leaf surface) bacteria such as *Erwinia herbicola* (Sandmann *et al.,* 1990). Carotenoids quench excess solar energy which could otherwise lead to the formation of lethal singlet oxygen (Buckley and Houghton, 1976; Paerl, 1984). Shiba (1991) reported a carotenoid-containing pink-pigmented bacterium for which the new genus *Roseobacter* has been proposed. Dobson *et al.* (1991) isolated orange and yellow bacteria from an Antarctic hypersaline lake and identified them to be carotenoid containing members of the *Flavobacterium-Cytophaga* group. The cyanobacterial colonists of fellfield polygons also contain UV-protection pigments such as phycoerythrin (Wynn-Williams, 1988) and all major cyanobacterial groups contain scytonemin, a UV-absorbing compound found in the mucilagenous sheath where it acts as a biological sunscreen (Garcia-Pichel and Castenholz, 1991). Thus it appears that, for surface dwelling Antarctic phototrophic organisms, including the microalgae of the fellfield polygon, protection from solar radiation is an important feature. The presence of a large proportion of pigmented bacteria suggests that such bacteria are located near the soil surface, alongside the microalgae and within the zone of penetration of incident solar

radiation, where pigmentation would be beneficial to bacterial survival. Indeed, Tong and Lighthart (1997) reported that solar radiation selected for pigmented bacteria in an atmospheric bacterial population.

Pigmentation alone may not, however, be sufficient to confer protection from UV radiation. Siebert and Hirsch (1988) isolated 14 bacteria from the McMurdo Dry Valleys, Antarctica and, although six had red pigments, only two of them (both belonging to the genus *Deinococcus*) were UV resistant. Other factors such as presence and efficiency of DNA repair enzymes may also be key elements contributing to UV resistance (Karentz, 1991). Pigmentation may also have other biological functions. For example, a definite function has not been assigned to violacein, the purple pigment found in *Janthinobacterium lividium*, a bacterium frequently isolated from terrestrial Antarctic environments (Wynn-Williams, 1983) and one of the 25 isolates used in our studies. Although crystalline violacein has antibiotic activity against some Gram-positive bacteria other suggested functions include: protection against UV and visible radiation; the inhibition of predation by protozoa; a respiratory intermediate and a sink for excess tryptophan or indole compounds (Pemberton *et al.*, 1991).

3.2.2 Isolate Physiology and Morphology

All isolates were rod-shaped, with 21 Gram-negative, two Gram-positive and two isolates showing a variable Gram response (Table 3.1). Bölter (1995) carried out direct microscopic observations of bacteria from soils from a comparable habitat, King George Island, and found rod-shaped bacteria comprised 70 to 90% of the total. Rod-shaped bacteria also showed the greatest difference in abundance between samples (e.g. large rods decline from 20 to 5% of the community with depth). The domination of rods amongst the isolates in our study and in the direct observations of the former study, contrast with bacterial isolations from Antarctic cold deserts. Siebert and Hirsch (1988) have established a culture collection of 1500 isolates from the Dry Valleys cold desert with the majority being coccal forms with all of the 14 isolates selected for further study being Gram-positive. A change in the abundance of cocci to rod shaped bacteria may indicate a reduction in habitat severity, and Bölter (1995) suggests such changes may prove useful in monitoring the progress of climate change at Antarctic sites.

3.2.3 Influence of Temperature on Isolate Growth Rate

Isolates were replica plated onto CPS agar plates (section 2.3.3) and incubated at 0, 4, 10, 20, 25 and 37°C to determine optimum growth rate. All isolates showed growth at 0°C after 28 days of incubation, with the greatest growth of all isolates (measured by speed of appearance of colonies) occurring at 25°C. No isolate grew at 37°C. All isolates can therefore be defined as being psychrotrophic according to the widely accepted definition of Morita (1975): "an organism, irrespective of its optimum temperature for growth, whose maximum exceeds 20°C but which is still capable of growth at 0°C". This is in agreement with previous studies on temperature optimum of bacteria isolated from maritime Antarctic soil (Upton and Nedwell, 1989) and freshwater and marine Antarctic waters (Herbert and Bhakoo, 1979; Ellis-Evans, 1981). In addition, other organisms indigenous to Signy Island fellfields have been reported to have optimum growth temperatures above the average soil temperature during the summer. For example, the maximum net photosynthesis of the two vascular plants and of two mosses occurs above 12°C (Collins, 1977; Edwards and Smith, 1988; Harrisson *et al.*, 1989) with the optimum growth temperature for microalgae reported to be between 15-20°C (Davey *et al.*, 1992). Interestingly, even in some Antarctic freshwater lakes psychrotrophs dominate even though temperature is lower and more constant than adjacent terrestrial sites. The reason for the success of psychrotrophs at such sites is thought to be because it is advantageous for the indigenous bacteria to be metabolising sub-maximally, with long generation times, to avoid exhausting the food supply (Ellis-Evans, 1985).

The predominance of macro and microorganisms with optimum growth temperatures above the average fellfield temperature is probably due to temperature fluctuations at this field site. Although the average summer temperature of Signy Island fellfield soil has been recorded as 1.10°C (mean ground temperature at a Signy Island fellfield polygon, 2-1-94 to 17-3-94; Wynn-Williams, 1996), there is a wide diurnal fluctuation from below 0°C to above 20°C (Chambers, 1966). Such fluctuations may lead to the success of psychrotrophy over psychrophily: organisms which are able to increase growth rate as temperatures increase above 10-15°C will outcompete bacteria which grow optimally at low temperatures (<10°C). This supports an accepted view that psychrotrophs dominate low temperature environments that undergo thermal fluctuations (Russell, 1990). Indeed, the psychrotrophic nature of the indigenous bacterial community makes it well placed to respond to future global warming.

Table 3.1   Bacterial isolates from fellfield polygon WW5: physiological traits.

Isolate	Pigment (1)	Gram stain (2)	Shape (3)	Motility
A1	W	v	SR	-
A2	W	v	SR	-
A3	W	+	P	+
B1	W	-	SR	-
B2	W	-	R	-
M2	W	-	R	+
D1	T	-	R	+
E1	Y	-	R	+
F1	Y	-	SR	-
F2	Y	-	SR	-
G3	Y	-	SR	-
G1	O	-	R	+
G2	O	-	R	+
G4	B	-	R	+
C1	P P	-	R	+
C2	P P	-	SR	-
K1	P	+	R	-
L1	P	-	R	-
L2	P	-	SR	-
N1	P	-	SR	-
J1	R	-	R	-
H2	R	-	R	-
I1	R	-	R	-
I2	R	-	R	-
M1	Pu	-	R	+

(1)   W = white, T = translucent, Y = yellow, O = orange, B = Brown,
       PP = pale pink, P= pink, R= red, Pu = purple.
(2)   v = variable
(3)   SR = short rod (< 1.5 μm long x 1μm wide), R = rod (≥ 1.5 μm long), P = pleomorphic

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3.2.4 Production of Glucosidase, Galactosidase and Phosphatase Enzymes

A screen of glucosidase, galactosidase and neutral phosphatase production by the 25 isolates revealed that 13 are α-glucosidase positive, 11 are β-glucosidase positive, 10 are α-galactosidase positive, 21 are β-galactosidase positive and 16 are phosphatase positive (Table 3.2). The four glucosidase and galactosidase enzymes were chosen because glucose and galactose have been reported to be the two most abundant components of polygon soil polysaccharide (Foister *et al.*, 1993). The enzyme assayed for are exopolysaccharidases and are thus capable of releasing monosaccharide residues for microbial metabolism. Neutral phosphatase was used to measure general bacterial metabolic activity in whole soil samples (Chapter 4 and 5) and was included in the screen to determine the proportion of isolates able to contribute to this activity. Siebert and Hirsch (1988) characterised 14 isolates from the extreme Dry Valley habitats of continental Antarctica: 71% of isolates had phosphatase activity with 50 % having β-galactosidase activity. This compares with 64% and 84% in the present study.

3.2.5 Carbohydrate Utilisation

Isolates were screened for ability to utilise a range of single sugars and polyols, previously recorded in Signy Island fellfield polygons (Arnold, 1995), by comparison of growth on a minimal salts medium to that on minimal medium supplemented with 0.5 g l^{-1} carbohydrate (section 2.3.4). Fifteen of the isolates showed no growth on any of the carbon compounds provided. This was subsequently overcome by adding yeast extract to the medium. A modified minimal salts medium including yeast extract was then used and isolate growth was found to be directly related to yeast extract concentration in the range 0 - 1.0 g l^{-1}. Supplementary carbon compounds had little effect on growth of these isolates and these 15 isolates were excluded from further screening. The remaining 10 isolates were screened for utilisation of 11 carbon compounds (Table 3.3).

Bölter (1993) determined the extent to which supplemented carbohydrates stimulated the growth of bacteria from a continental Antarctic site and found that glucose, sucrose, maltose, sorbitol and mannitol stimulated the most growth. Such carbohydrates are consistent with the available carbohydrate both in the habitat studied in his study and at in our study (i.e. polyols and sugars leached from moss, lichen and algae). His study confirms that the carbohydrates screened in our study also stimulate the growth of bacteria in a more extreme Antarctic habitat.

Upton (1988) carried out a complementary screen of bacterial isolates from Heywood Lake, a freshwater lake on Signy Island, and proposed the use of the Nutritional Flexibility Index (NFI) to compare the range of carbon compounds used by different isolates. The NFI is defined as:

Nutritional Flexibility   =   number of carbon compounds utilised by an isolate
　　　Index　　　　　　　　　　　total number of carbon compounds tested

Upton (1988) screened copiotrophic bacteria isolated on CPS agar plates and oligotrophic bacteria, isolated on the low nutrient enrichment medium of Griffiths and Morita (1973). The mean NFI were calculated as being 0.22 for the copiotrophs and 0.89 for the oligotrophs. The mean NFI for the present screen was 0.58. As CPS was used to originally isolate the bacteria screened in the present study the screen can be compared with that carried out on the 'copiotrophs' of the former study, also isolated on CPS. The lower mean NFI value in the former study suggests that given the same isolation medium, freshwater isolates from Signy Island can utilise a narrower range of carbon compounds than terrestrial isolates. However, 15 of the 31 copiotrophs screened in the former study had a nutritional index of 0. Such isolates may be limited by yeast extract, in the same manner as the 15 isolates excluded from the present screen. This is likely given that CPS isolation medium contains $0.5 \ g \ l^{-1}$ yeast extract and would support the growth of obligate yeast extract requiring isolates. If such isolates are excluded from Upton's study (as they have been in the present study), the mean NFI becomes 0.56, remarkably close to the mean NFI of 0.58 obtained in the present study. This indicates that bacteria, isolated on the same medium, from terrestrial and freshwater habitats at Signy Island can utilise a similar range of carbohydrates. Indeed, Ellis-Evans and Wynn-Williams (1985) proposed that the dissolved organic carbon released from terrestrial cryptogamic cells damaged by freeze-thaw cycles may ultimately become washed into the freshwater lakes of Signy Island. Given that five out of seven carbon sources used by Upton are included in the present screen and the similarity of the mean NFIs it can be postulated that the two communities may well have similar capacities to utilise the polyols and sugars which become available to them from frost-damaged phototrophic organisms.

3.2.6 Evidence for Distinct Metabolic Strategies

The ability for isolates to utilise two sources of carbohydrates has been assessed: the production of glucosidase and galactosidase enzymes indicates a capacity to hydrolyse bonds between the most abundant components of the fellfield polysaccharide whilst

growth on polyols and sugars (used as compatible solutes by phototrophic organisms) indicates an ability to grow on leachate material. There is no clear evidence of a distinct strategy favouring one type of activity over the other, albeit given the small number (10) of isolates screen for carbohydrate use. For example, isolate E1 with the lowest NFI (0.09) produces all four glucosidase, galactosidase enzymes along with isolate A1 which has the highest NFI (1.00). If such distinct, polarised, strategies existed then it may be postulated that bacteria utilising a range of compatible solutes would be favoured following a flush of such compounds (e.g., after a freeze-thaw event). Polysaccharidase producing bacteria may subsequently succeed during periods when free polyols and sugars are depleted.

Bacterial population dynamics are still likely to be driven by the prevailing nutrient status of the soil, but success may depend on other factors, such as substrate affinity rather than breadth of substrate range. It is becoming accepted that substrate affinity decreases with temperature (Herbert and Bell, 1977; Wiebe et al., 1992, 1993) and that the more psychrophilic a bacterium (i.e., the lower the optimum growth temperature), the greater affinity at low temperatures (Fukunaga and Russell, 1990; Nedwell and Rutter, 1994). Indeed, bacterial isolates from freshwater lakes on Signy Island (which feature smaller temperature fluctuation than terrestrial sites), have a greater substrate affinity at low temperatures than terrestrial bacterial isolates. Conversely the terrestrial isolates have a greater substrate affinity at higher temperatures and outcompete the lake isolates (Ellis-Evans and Wynn-Williams, 1985).

Furthermore, Nedwell and Rutter (1994) report that change in substrate affinity with temperature is itself substrate-dependent. They argued that, if substrate affinity varies with substrate-type and temperature, this would increase the number of metabolic niches available to bacteria within a community and could help to maintain diversity during periods of low temperature and low substrate availability. Such a situation can be envisaged within the fellfield polygon during a growing season. The nutrient content of fellfield polygons exhibits periodical changes in content and concentration during a growing season characterised by a wide diurnal temperature range (Allen and Northover, 1967; Walton, 1982). For example, during the spring thaw, concentrations of polyols and sugars leached from frost-damaged organisms can reach 1 mg ml^{-1} in the soil (Tearle, 1987) and can subsequently be utilised rapidly by the bacterial community (up to four d in the former study). Such a situation would allow bacteria with a similar substrate range (e.g. isolate D1 and M1) to occupy distinct niches, with a difference in their substrate affinity-profiles allowing each being competitive under periods of different temperatures and substrate concentrations. Such a heterogeniety in bacterial substrate affinities would

be expected to result in a greater diversity of bacterial strains than would be expected from considering substrate input in the system alone.

Table 3.2   Bacterial Isolates from fellfield polygon WW5: glucosidase, galactosidase and phosphatase enzyme production.

Isolate	Pigment (1)	α-D-gluco-sidase	β-D-gluco-sidase	α-D-galacto-sidase	β-D-galacto-sidase	Total polysacc-haridases	Neutral phos-phatase
A1	W	+	+	+	+	4	+
A2	W	+	+	+	+	4	+
A3	W	-	-	-	-	0	+
B1	W	-	-	-	-	0	+
B2	W	+	+	+	+	4	+
M2	W	-	-	-	-	0	+
D1	T	-	-	-	+	1	-
E1	Y	+	+	+	+	4	+
F1	Y	-	-	-	+	1	+
F2	Y	-	-	-	+	1	-
G3	Y	-	-	-	+	1	-
G1	O	+	+	+	+	4	+
G2	O	+	+	+	+	4	+
G4	B	+	+	+	+	4	+
C1	P P	-	+	-	+	2	-
C2	P P	-	+	-	+	2	-
K1	P	-	-	-	+	1	-
L1	P	-	-	-	+	1	-
L2	P	-	-	-	+	1	-
N1	P	+	-	+	+	3	-
J1	R	+	+	+	+	4	+
H2	R	+	+	+	+	4	+
I1	R	+	-	-	+	2	+
I2	R	+	-	-	+	2	+
M1	Pu	+	-	-	-	1	+
percentage of enzyme positive isolates		52%	44%	40%	84%	88% (2)	64%

(1)   W = white, T = translucent, Y = yellow, O = orange, B = Brown, PP = pale pink, P= pink, R= red, Pu = purple
(2)   Perecentage of isolates with one or more glucosidase or galactosidase enzyme.

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Table 3.3.   Representative isolates from fellfield polygon WW5: utilisation of selected polyols and sugars recorded in Antartic fellfield polygons (Arnold, 1995).

Isolate	Pigment (1)	glucose	fructose	sucrose	glycerol	arabitol	mannitol	adonitol	sorbitol	trehalose (2)	erythritol	myo inositol	Nutrional Flexibilty Index
A1	W	+	+	+	+	+	+	+	+	+	+	+	1.00
A2	W	+	+	+	+	+	+	+	+	+	-	+	0.91
B2	W	+	+	+	+	-	-	-	-	+	-	-	0.45
M2	W	+	+	-	+	+	+	+	+	+	+	+	0.91
D1	T	+	+	+	+	-	+	-	+	+	-	-	0.64
E1	Y	+	-	-	-	-	-	-	-	-	-	-	0.09
G1	O	+	+	+	-	-	-	+	-	-	-	-	0.36
G2	O	+	+	+	-	-	-	+	-	-	-	-	0.36
G4	B	+	+	+	-	-	-	+	-	-	-	-	0.36
M1	Pu	+	+	+	+	+	+	-	+	+	-	-	0.73

(1)   W = white, T = translucent, Y = yellow, O = orange, B = Brown, Pu = purple.
(2)   Trehalose was detected in Jane Col polygon soil by gas chromatography and the enantiomer was not determined (Arnold, 1995).
D(+) α-D-glucopyranosyl α-D- glucopyranoside trehalose isomer (Sigma, UK) was used on the basis of commercial availablity.

4. SIMULATED CLIMATE CHANGE AT TWO ANTARCTIC FIELD SITES: MICROBIOLOGICAL OBSERVATIONS

4.1 Introduction

The need for information on the biological response to climate change is becoming of increasing importance with the accumulating body of evidence for global warming and the continued occurrence of springtime ozone depletion above Antarctica (Jones and Shanklin, 1995; Jiang et al., 1996). Antarctic ecosystems are particularly sensitive to both of these aspects of climate change: there is evidence of a threat of retreat of the West Antarctic Ice Sheet due to temperature increases (Alley and MacAyeal, 1993) and the elevated UV-B dose beneath the ozone hole is thought to be affecting the productivity of the Southern Ocean (El Sayed et al., 1990; Behrenfeld et al., 1993). Terrestrial Antarctic habitats are also vulnerable to climate change as soil and rock surfaces respond rapidly to temperature changes (Walton, 1982). This may become particularly evident at habitats with temperatures around 0°C during the growing season when small temperature increases could significantly alter water availability, and consequently, biological activity (Kennedy, 1995a). In addition, the phototrophic producers (mosses, lichens and microscopic algae) are, by necessity, restricted to zones of photosynthetically available radiation (PAR) and are therefore exposed to UV-B radiation (Vincent and Quesada, 1994).

Recently-deglaciated fellfields at Signy Island are suitable for the study of the response of terrestrial Antarctic colonists to climate change: pioneer colonists are microbial and are capable of rapid response to environmental change (Wynn-Williams, 1996). The simulation of the two aspects of climate change has been achieved by the use of cloches placed on the surface of fellfield soils (Wynn-Williams, 1992b; Smith, 1993). Such cloches are designed to either simulate global warming by increasing soil temperatures by approximately 3°C (in line with the most recent estimates of the medium-term progression of global warming, Intergovernmental Panel on Climate Change, 1996), or to block the transmission of UV-B radiation, or to combine both effects (Wynn-Williams, 1992b). Wynn-Williams (1996) observed substantial increases in the dominant phototrophic colonists, cyanobacteria and microscopic eukaryotic algae (collectively referred to as microalgae) in soil beneath such cloches. This study indicated the potential response of the primary producers in the fellfield ecosystem to projected warming events and their use as bio-indicators of the progression of global warming.

The study of microbial communities also indicates changes in carbon flux and the extent to which Antarctic soils will act as carbon sinks or sources under future warming. Changes in bacterially-mediated carbon flux are of particular significance as carbon dioxide is considered to be the primary atmospheric forcing agent responsible for global warming (Intergovernmental Panel on Climate Change, 1996). Indeed, studies on a climosequence of New Zealand soils indicated that soil organic matter may act as a significant source of atmospheric carbon dioxide as the soils warm and microbial activity increases (Jenkinson et al., 1991; Tate, 1992).

The present study uses the cloche-based experimental system to study the bacterial response to simulated climate change at two polygons (WW2 and WW5) at the Jane Col Plateau, Signy Island (60°43'), maritime Antarctic.

Microbiological study of polygons WW2 and WW5

Polygon WW5, dominated by the cyanobacterium *Nostoc commune,* was covered with a UV-B transparent cloche (that warmed the soil by an average of 2.22 C°; Wynn-Williams, 1996) for five years prior to sampling. Foister et al. (1993) carried out microbiological analysis on exposed soil sampled from this polygon during the austral summer of 1991, the summer before samples were taken for this study. Their results are discussed below, alongside those from the present study.

Polygon WW2 had a polystyrene cloche (that warms the soil by an average of 3.43 C° and blocks the transmission of incident UV-B radiation; Wynn-Williams 1996) in place for eight years prior to sampling. Wynn-Williams (1996) investigated the response of the microalgal community to the enhanced conditions provided by the cloche at this polygon. After three years of the simulation, microalgae had colonised 73.4% of the soil surface beneath the cloche compared with 4.4% of the exposed soil. After a further three years (the year before samples were taken for the present study) the values were 23.2 and 8.3%, respectively. A change of dominant colonists from filamentous cyanobacteria (*Phormidium autumale* and *Pseudanabaena* spp.) to aseriate *Nostoc* spp. was also recorded between year three and six of the simulation. Foister et al. (1993) carried out a preliminary investigation of the response of the bacterial community at this fellfield polygon after six years of simulated climate change. Significant increases in bacterial (x 24), fungal (x 116) and yeast (x 19) numbers and microbial activities including acid phosphatase (x 3.7), trehalase (x 8.0) , α- and β- glucosidase (x 4.5 and x 2.0) and β-

galactosidase (x 100) were recorded. However, these results were from a single field sample taken during the 1991-2 Antarctic summer and may not be representative of the response of this community over a full growing season. Indeed, considerable variation in the size and composition of a fellfield microbial phototrophic community (Davey, 1991a) and in microbial counts and respiration in Signy Island peat soil (Wynn-Williams, 1980) have been recorded during Antarctic summers. For these reasons field samples were taken at approximately seven day intervals during an austral summer in the present study. This sampling regime also allowed total seasonal activity (e.g. microbial respiration) to be estimated (Chapter 5).

The aim of the experiment reported in this chapter is to assess the response of the fellfield bacterial community to prolonged simulated climate change (of five and eight years). The size and activity of the bacterial community is investigated with special regard to microbial carbon turnover and the implications of this on ecosystem stability during periods of climate change.

4.2   Results and Discussion

4.2.1   Field Temperature

Microclimate facilities installed at the field site on Signy Island measured the soil temperature inside and adjacent to cloches on fellfield polygons. However, for the period of this experiment, the climate loggers proved unreliable and did not produce usable data. Soil temperatures for a previous period (11-1-91 to 13-3-91, Figure 4.2) illustrate the range of expected temperatures in exposed soil at this field site. Although the average daily temperature beneath a cloche may only be 1 - 3 C° higher than exposed soil (Kennedy 1995c), this can mask large temporal increases. Smith (1993) recorded the temperature at 2 cm above moss surface inside a cloche and above bare soil at five minute intervals over three days (Figure 4.2). Comparison of the data sets illustrates the diurnal temperature effects of cloches: temperatures can be greatly elevated around midday beneath a cloche (from approximately 1°C on bare ground to 20°C beneath a cloche) and then fall to a temperature close to that of the bare soil at night (0°C, Figures 4.1 and 4.2).
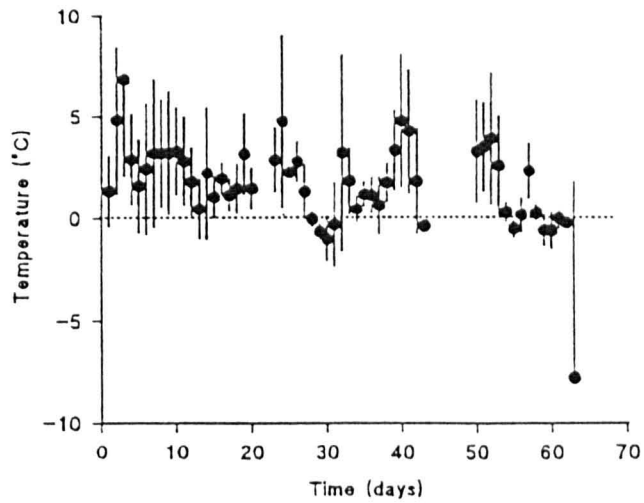
Figure 4.1. Mean daily soil temperatures, c. 5 mm depth, in a polygon at Jane Col, Signy Island. Measurements taken over a 63 day period (11 January to 13 March 1991). Error bars represent standard deviations about the mean.
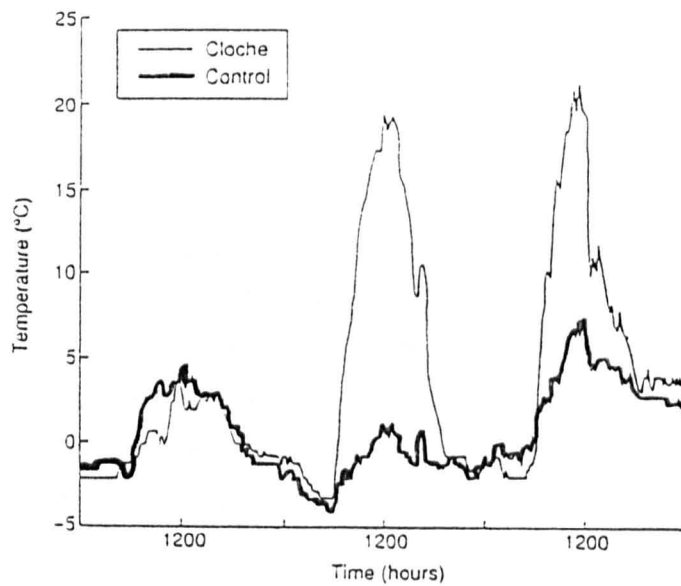
Figure 4.2. Temperature at 2 cm above moss surface inside cloche and above exposed soil over 3 days (3-5 March 1989) from a site at Jane Col, Signy Island. Data recorded at 5 min intervals. (From Smith, 1993).

4.2.2   Field Observations from Polygon WW5

A chronosequence of soil samples was taken from exposed and cloche-covered soil at polygon WW5 at approximately seven-day intervals. The first sample was taken just prior to soil thaw on 10 December 1992 with a second sample taken on 15 December 1992 immediately after the thaw. The 15 December sampling is designated day 0, i.e. the first day of the spring thaw. The final sample in the sequence was taken on day 51 (6 February 1993).

4.2.2.1   Soil Water Content

The water content of the soil cores from exposed soil was between 66 - 82% of total WHC, with greater variability at the start of the season (77% of total WHC at day 0, 66% at day 6, 82% at day 13, Figure 4.3). The water content of the cloche-covered soil also fluctuated in the first 13 days but at a lower percentage of water holding capacity (from 60 to 69% WHC).

The water contents of both plots were sufficient to allow microbial activity to occur, i.e., the soils were never water logged nor desiccated (Tu, 1982; West *et al.*, 1987; van Gestal *et al.*, 1992). However, a more important consideration in a polar ecosystem is not the quantity of water but its availability. This is been considered to have a direct limiting effect on terrestrial biota (Kennedy, 1993) and forms the experimental basis of the Fellfield Ecology Research Programme being undertaken by BAS. In the present experiment, as there was always water present in the soil cores (> 50% WHC), temperature would have played a more important role than water content in influencing microbial activity by making the water biologically available (i.e. liquid).

4.2.2.2   Bacterial Numbers

Viable bacterial numbers were significantly greater in cloche-covered soil (Students t-test, $p < 0.005$, Figure 4.4), indicating a greater utilisation of existing carbon and energy reserves. Therefore, it is possible that the indigenous microbial community is responding directly and solely to the enhanced conditions provided by cloche cover. This assumes that the community in the exposed soil are unable to utilise all available nutrients because of sub-optimal environmental conditions (e.g. low temperature) of which there is evidence from the ETS activities (section 4.2.2.3). However, the bacterial heterotrophs
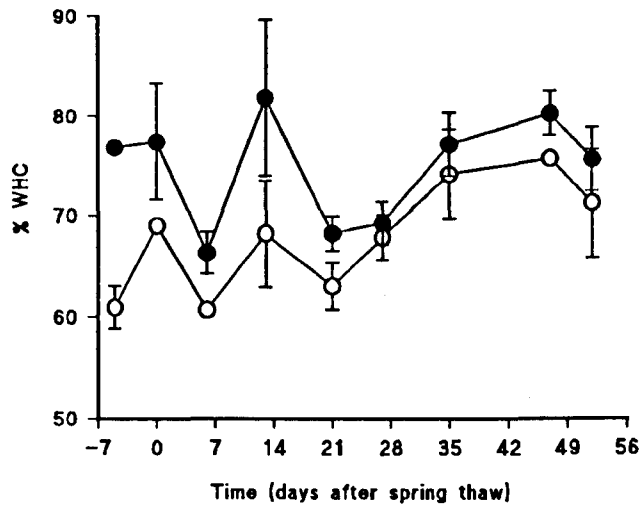
Figure 4.3. Water content of fellfield soil at polygon WW5, exposed (•) and inside cloche (o). Values are expressed as a percentage of water holding capacity (WHC) of the soil. Standard error bars are plotted within the symbols where not visible.
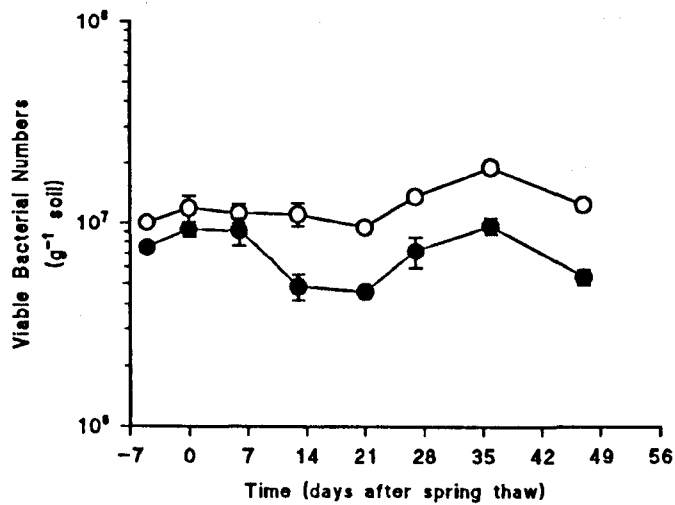
Figure 4.4. Total bacterial viable counts during the austral summer of 1992-3 in polygon WW5, in exposed (•) and cloche-covered (o) soil. Standard error bars are plotted within the symbols where not visible.

are surviving at the expense of nutrient leachates and from primary production by fellfield microalgae. Wynn-Williams (1996) showed that microalgal communities were also able to increase in size under cloche-cover. The response of the bacterial community is thus likely to be a combination of the enhanced environmental conditions beneath the cloche (allowing increased metabolism of pre-existing substrates) plus metabolism of new substrates produced by the increased microalgal community.

Bacterial numbers followed a similar pattern throughout the season both in and outside the cloche. An initial increase in numbers at the start of the season preceded a fall at day seven followed by a rise in numbers from day 21 (Figure 4.4). This rise was coincident with a small increase in water content of both soils. However, as this was only about 5% of total water holding capacity, it is unlikely to account for the increase in bacterial numbers. Indeed, bacterial numbers do not correlate well with soil water content (Table 4.1), although it is the best correlation of any of the measured parameters in the cloche-covered soil ($r^2 = 0.44$).

Isolation plates of lower nutrient content (1/10 strength CPS) were also used to enumerate bacteria (as recommended for Antarctic microbiology by Wynn-Williams, 1992a). This isolation medium gave consistantly lower viable counts than did full strength CPS plates: in exposed soil the seasonal average was 7.3×10^6 g^{-1} soil from full strength CPS and 4.8×10^6 g^{-1} from 1/10 strength CPS. The corresponding cloche-covered values were 1.2×10^7 and 8.2×10^6 g^{-1} soil. Full strength CPS was used for all enumerations in order to represent as fully as possible the actual numbers of viable bacteria in each soil sample.

Foister *et al.* (1993) monitored viable bacterial numbers in exposed soil from polygon WW5 during the austral summer of 1991. The numbers reported were at least ten-fold lower than those in the present study. This may be accounted for by less favourable environmental conditions (e.g. temperature) or reduced nutrient availability. However, the higher numbers may be due to methodological differences. Although the soil cores were sampled and stored in the same manner, in the former study, they were air dried prior to microbiological analysis (Foister *et al.*, 1993) whereas in the present study field-wet soil was homogenised with a cooled pestle and mortar. The latter procedure is likely to give greater viable bacterial counts given the decreased bacterial viability associated with soil drying (Tu, 1982; West *et al.*, 1987; van Gestal *et al.*, 1992). A second difference was that the isolation plates (full strength CPS in both studies) were incubated at 15°C for 21 days by Foister *et al.* (1993), but at 10°C for 28 days in the present study. A preliminary experiment showed that up to 50% more bacteria grew on plates incubated at 10°C

compared with 25°C. At 10°C there was also less overgrowth of slower growing isolates by faster growing ones, enabling a more complete count of total isolates.

Table 4.1 Relationship between bacterial numbers and other microbial and soil properties in polygon WW5 during the 1992-3 austral summer. Correlation values, r^2, given. Values close to 1.0 are considered as significant positive correlations.

	Exposed soil	Cloche-covered soil
water content	0.02	0.44
constitutive ETS	0.14	0.06
substrate-induced ETS	0.14	0.16
α-D-1,4 glucosidase	0.26	0.02
β-D-1,4 glucosidase	0.13	0.02
α-D-1,4 galactosidase	0.08	0.01
β-D-1,4 galactosidase	0.29	0.05
saccharide	0.32	0.10
carbon	0.29	0.02
nitrogen	0.24	0.04

A simple method for measuring bacterial population changes during a season was evaluated. This involved grouping isolates according to colony pigmentation into the nine bacterial pigment groups used in Chapter 3. Changes in the numbers of each group could then be used to follow seasonal dynamics of the different pigment types and would allow the effect of climate change on population structure to be recorded (e.g., proportion of red strains in exposed soil and soil under a UV-B blocking cloche). However, wide variability in pigmented isolates within replicate isolation plates taken from the same sample prevented this procedure yielding statistically valid results.

Indicator (or biomarker) species can also be used to follow the impact of climate change on Antarctic communities. Plants (Smith, 1994), microalgae (Wynn-Williams, 1996) and a soil ciliate (Smith, 1996) have been suggested as appropriate indicators for such a study. Ellis-Evans and Wynn-Williams (1985) used *Chromobacterium* spp. (now *Janthinobacterium*) as a biomarker in a freshwater lake at Signy Island and it was hoped that the distinctive purple isolates of this genus could be use as an indicator of climate

change in the present study. However, the bacterium was rarely isolated and cloche cover gave no discernible change in the frequency of *Janthinobacterium* isolation.

4.2.2.3 Electron Transfer Chain (ETS) Activities

Microbial metabolic activity was measured using the ETS assay. This was chosen because it gives a relative measure of the activity of the respiratory chain (Trevors *et al.*, 1982) - a direct link to microbial energy production via the phosphorylation of ADP to ATP (Neidhardt *et al.*, 1990). The assay of ETS activity without the addition of exogenous nutrients is a measure of the basal level of respiratory activity carried out using indigenous nutrients. The addition of yeast extract gave a greater stimulation of ETS activity than with glucose as reported by Trevors *et al.* (1982), and was used to give a maximal microbial activity which is in relation to the amount of active respiratory chains (i.e. viable microorganisms) present (section 2.4.1).

The pattern of ETS activities in the exposed and cloche-covered soil (Figure 4.5) follows the trend observed for bacterial numbers: a spring peak (day 0 to 14) followed by a dip (day 21) and then an increase (day 28 to day 49). However, neither ETS activity correlated well with bacterial numbers (Table 4.1, r^2 < 0.20). The initial peak of activity may be the result of metabolism of nutrients held in frozen soil water until the spring thaw. Such nutrients may be the cellular contents of frost-damaged microbes or material from mosses and lichens at the polygon periphery, trapped during the winter freeze and subsequently released during the spring thaw: Roser *et al.* (1992a) showed that fellfield moss and lichen accumulate sugars and polyols to concentrations that endow a cryoprotectant function (Sakai, 1961); loss of such soluble carbohydrates from mosses under freeze-thaw stress has been reported by Melick and Seppelt (1992); Tearle (1987) showed the indigenous microbial community was able to utilise the relatively high concentrations of leachate entering the soil (1% w/w of fresh soil) within four days. Additionally, a spring activity flush has been recorded in the microbial community of moss peat banks on Signy Island (Baker, 1970; Wynn-Williams, 1980) and from a continental Antarctic site (Boyd and Boyd, 1963).
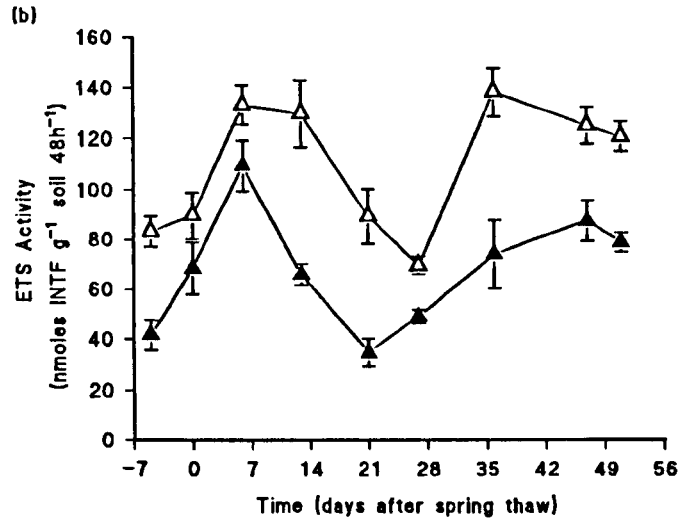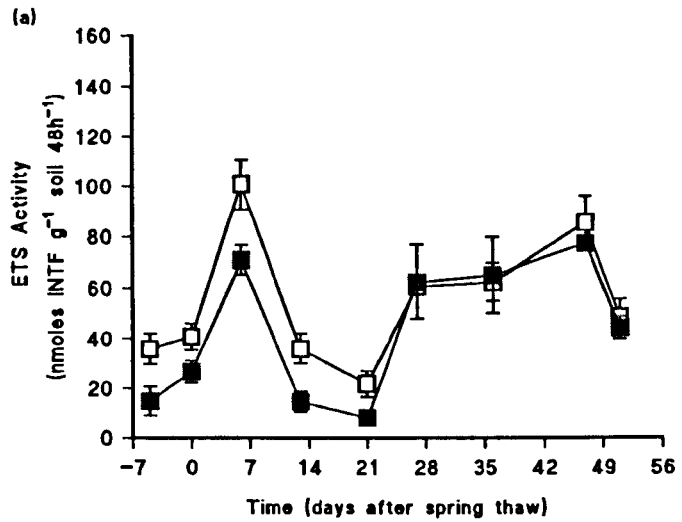
The substrate-induced ETS activity in the cloche-covered soil was always greater than the constitutive activity. This was also the case up to day 21 in the exposed soil after which the addition of nutrients did not result in higher activity. As the addition of nutrients failed to stimulate greater activity, the levels of nutrients present in the soil at the time of

sampling must have been sufficient to give the maximum ETS activity (under the optimised assay conditions). It can be concluded that the bacterial community had excess nutrients in the soil at the time of sampling and were limited by environmental conditions (e.g. low temperature). The existence of a difference between the constitutive and substrate-induced activities in the cloche-covered soil indicates that the microbial community were always able to utilise increased substrate. This in turn suggests that the bacterial community would have been limited more by substrate availability than environmental conditions in the field. Thus, the warmth provided by cloche-cover appears to have reduced a constraint present in the exposed soil and enabled the bacterial community to be more active and utilise more substrate. As the water content of the exposed and cloche-covered soil were similar (Figure 4.3) it is proposed that the increased temperature provided by the cloche allowed the increased bacterial activity.

Nutrients not used by the bacterial community (e.g., due to environmental constraints, such as postulated to occur in exposed soil from day 21 onward) can leach from the polygon and ultimately reach the freshwater lakes of Signy Island (Ellis-Evans and Wynn-Williams, 1985). The bacterial community of the freshwater lakes are predominately oligotrophic and can utilise a wide range of carbohydrates, including the common fellfield sugars and polyols (e.g. glucose, glycerol, trehalose; Arnold, 1995) and utilise such leachate (Upton, 1988). The effect of warming may be to enable the fellfield bacteria to use more of the soil nutrients which would result in a reduced nutrient input into the freshwater lakes with a resulting impact on the lake bacteria.

Expressing ETS activities per bacterium (Figure 4.6) also provides an insight into the activity of the bacterial community. The activity per bacterium in warmed soil is a constant value, 4×10^{-6} nmoles INTF $48h^{-1}$, with a temporal increase to 1×10^{-4} nmoles INTF $48h^{-1}$ bacterium^{-1} at day seven. This suggests that environmental conditions (e.g., temperature) allowed the bacterial community to maintain a constant activity during most of the season (except for the greater activity at day 7). This is unlike the situation in the exposed soil where activity per bacterium is lower and shows grater variability (2.1×10^{-6} at day -5 to 1.0×10^{-5} at day 7).

Figure 4.5. Microbial ETS activity during the austral summer of 1992-3 in soil polygon WW5. Exposed soil (a), constitutive (■) and substrate-induced activity (□). Cloche covered soil (b), constitutive (▲) and substrate-induced activity(△). Standard error bars are plotted within the symbols where not visible.

Figure 4.6. Microbial ETS activity per bacterium during the austral summer of 1992-3 in soil polygon WW5. Exposed soil (a), constitutive (■) and substrate-induced activity ( ). Cloche covered soil (b), constitutive (▲) and substrate-induced activity(Δ). Standard error bars are plotted within the symbols where not visible.

The activities of four exopolysaccharidase enzymes (α-D-1,4 and β-D-1,4 glucosidase, α-D-1,4 and β-D-1,4 galactosidase) in soil samples were significantly greater (Students t-test; $p < 0.05$) in the cloche-covered soil throughout the austral summer (Figure 4.7 and 4.8). This indicates that global warming could result in greater turnover of polysaccharide, a key carbon source in this low organic matter soil (Chambers, 1967). However, the lack of a significant difference in soil saccharide between exposed and cloche-covered soil (Figure 4.9) indicates that such elevated glucosidase and galactosidase activity is being offset by a corresponding increase in polysaccharide production.

The greater enzyme activities in the cloche-covered soil may be the result of a more active microbiota producing a greater number of enzymes. Such a situation may be envisaged beneath the cloche as the ETS activities indicate that the microbial community is more active than in exposed soil. Greater enzyme activities may also be the result of enzymes with increased substrate affinities. Fukunaga and Russell (1990) have demonstrated that a psychrotrophic bacterium produced enzymes with greater substrate affinities (for glucose uptake at 20°C) when grown at 20 rather than 5°C. Thus, the elevated temperature provided by cloche-cover may have allowed the microbiota to produce enzymes with substrate affinities which are greater at higher temperatures. This would have resulted in a greater activity per enzyme under the relatively high temperature (30°C) of the assay compared with enzymes synthesised in exposed soil.

The enzyme activities did not follow the trend of bacterial numbers in either exposed or cloche-covered plots (Table 4.1). The activity of β-D-1,4 glucosidase in the exposed soil did show a better correlation with water content ($r^2 = 0.71$) but the correlations of the other three enzymes with water content were poor ($r^2 < 0.40$). The lack of a correlation between the glucosidase and galactosidase activities and bacterial numbers (Table 4.1) is unsurprising given that not all of the representative isolates of the bacterial community surveyed in Chapter 3 showed polysaccharidase activity (i.e., 52% of isolates had α-D-1,4 glucosidase activity, 44% β-D-1,4 glucosidase, 40% α-D-1,4 galactosidase and 84% β-D-1,4 galactosidase activity). Also numbers of cells and extracellular enzymes do not necessarily relate well to each other because extracellular enzymes can remain active in the soil for prolonged periods (Burns, 1977) and cells may grow without synthesising such enzymes if, for example, other easily assimilated substrates are available (e.g. sugars and polyols) which will be used preferentially.

Foister *et al.,* (1993) speculated that peaks of glucosidase, galactosidase and trehalase activity during an austral summer were the result of a succession of bacteria capable of producing such enzymes. There were no clear peaks of glucosidase or galactosidase activity in the present study and the survey of glucosidase and galactosidase production and sugar and polyol use found no evidence of distinct nutritional strategies (i.e., polyol and sugar use versus polysaccharides, Chapter 3). However, successions within the bacterial community are possible as other factors will play a role in determining bacterial success (e.g., substrate affinity and growth rate under field temperatures, Upton and Nedwell, 1989; Nedwell and Rutter, 1994). Indeed, significant changes in the composition of fellfield microalgal communities have been reported due to postulated UV-B radiation, desiccation or nitrogen limitation (Davey, 1991a; Davey and Rothery, 1992).

The relatively high value for the final saccharide measurement (day 52) in the exposed soil may reflect the accumulation of compatible solutes, such as glucose and sucrose, prior to winter in the fellfield soil (Arnold, 1995). This build up may be because the saccharides are intracellular and not available for metabolism by the heterotrophic community. Alternatively, the lower autumn temperatures (Davey, 1991b) may have inhibited the *in situ* activity of the glucosidase and galactosidase enzymes. Note that the glucosidase and galactosidase assay measures activity under optimised conditions and reflects the potential activity in the field. Thus, actual *in situ* activity can be much lower, depending on such environmental conditions as temperature. Indeed, it has already been argued that the ETS activities point to environmental conditions limiting the microflora in the exposed soil during the latter part of the season (section 4.2.2.3).

Foister *et al.* (1993) recorded the activities of α- and β-glucosidase, and β- galactosidase in polygon WW5 during 1992 using the same enzyme assay. The activities of all three enzymes are from two-fold (α-glucosidase) to five-fold (β-glucosidase) greater in the present study. The reasons for higher activities in this study are the same as discussed above for the viable bacterial numbers (i.e., more favourable field conditions such as temperature allowing greater microbial synthesis of glucosidase and galactosidases and field-moist, not ground, air-dried soil used in the present assay). The lower saccharide measurements in the former study may also reflect the sensitivity of the assay used. Saccharides were assayed by Foister *et al.* (1993) using the anthrone method (Cheshire and Mundie, 1966). This method has been criticised for being less sensitive than the phenol-sulphuric acid method (Dubois *et al.,* 1956) used in the present study and to overestimate the contribution of glucose to total saccharide levels, to produce variable results and to be subject to interference by Fe^{++}, NO_3^- and Cl^- ions (Martens and Frankenburger, 1990).
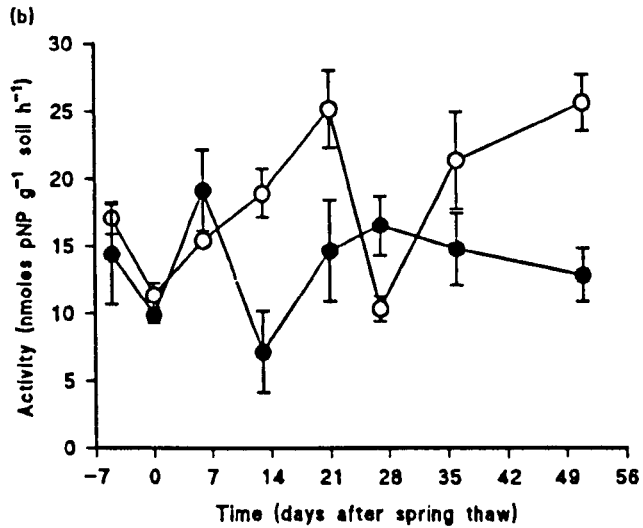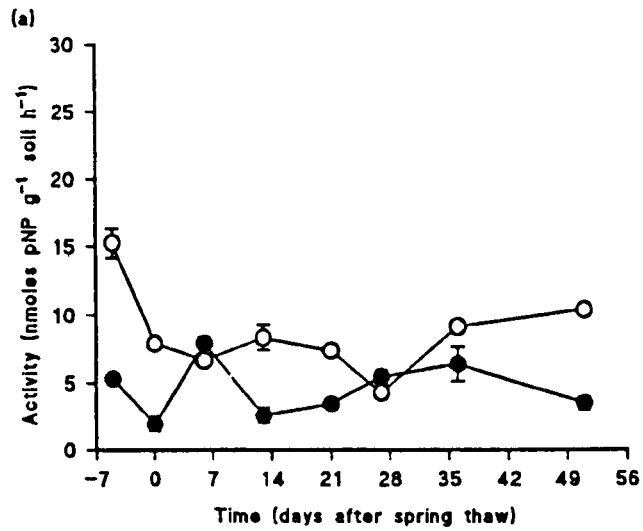
Figure 4.7   α-D-1,4 Glucosidase activity (a) and β-D-1,4 glucosidase activity (b) during the austral summer of 1992-3 in polygon WW5, in exposed (•) and cloche-covered (o) soil. Standard error bars are plotted within the symbols where not visible.
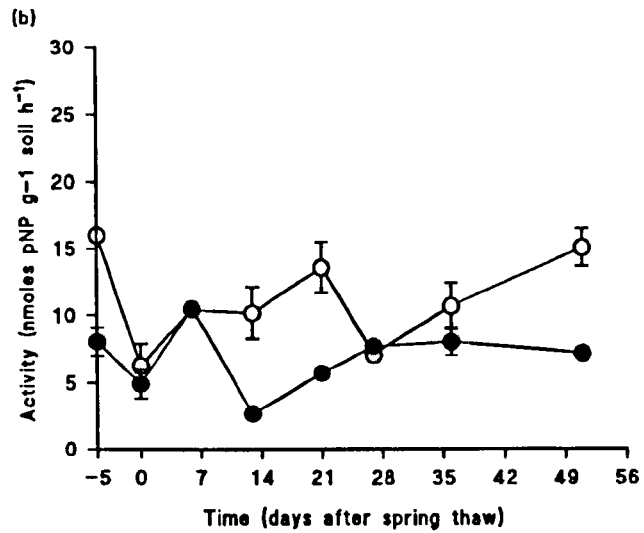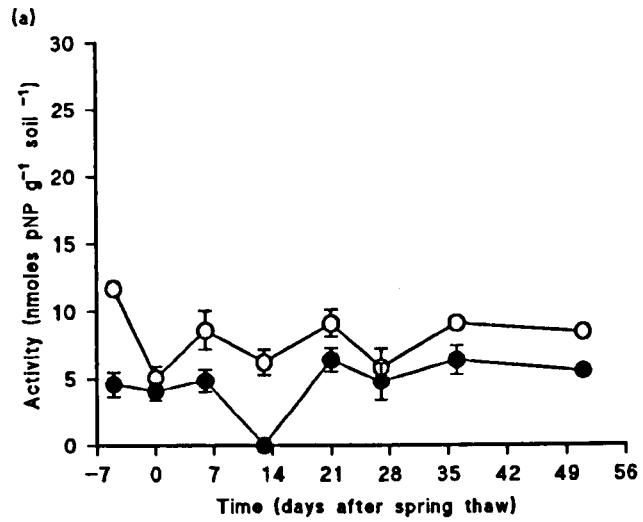
Figure 4.8.   α-D-1,4 Galactosidase activity (a) and β-D-1,4 galactosidase activity (b) during the austral summer of 1992-3 in polygon WW5, in exposed (•) and cloche-covered (o) soil. Standard error bars are plotted within the symbols where not visible.

4.2.2.5   Soil Carbon and Nitrogen

There were no significant differences in soil carbon and nitrogen contents of exposed and cloche-covered soil (Figure 4.10a and b, Students t-test on paired seasonal counts). In the control soil the seasonal trend recorded for bacterial numbers was repeated with soil carbon, however the two measures did not correlate well (Table 4.1, r^2.= 0.29). Nitrogen fluctuated at 0.2 mg g^{-1} soil and temporarily fell below detectable limits at day 21.

A comparison of the saccharide and carbon measurements (taken as averaged seasonal values) showed that 15.82 and 13.85% of the total carbon was in the form of saccharide in the exposed and cloche-covered soil respectively. The fact that neither carbon nor saccharide increased in the cloche-covered soil suggests that the soil did not act as a carbon source or sink for the duration of this simulation.

Figure 4.9. Total soil saccharide during the austral summer of 1992-3 in polygon WW5, in exposed (•) and cloche-covered (o) soil. Standard error bars are plotted within the symbols where not visible.
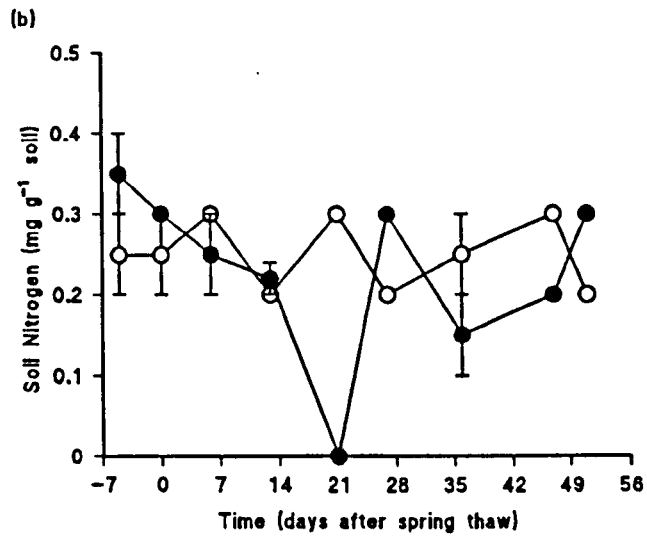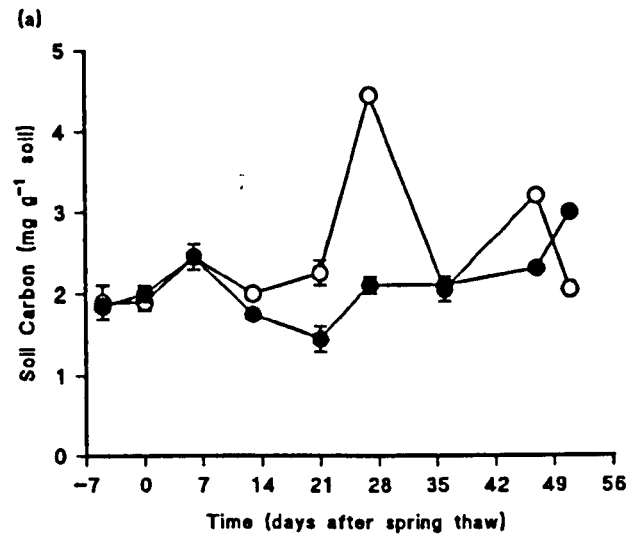
Figure 4.10. Total soil carbon (a) and nitrogen (b) during the austral summer of 1992-3 in polygon WW5, in exposed (•) and cloche-covered (o) soil. Standard error bars are plotted within the symbols where not visible.
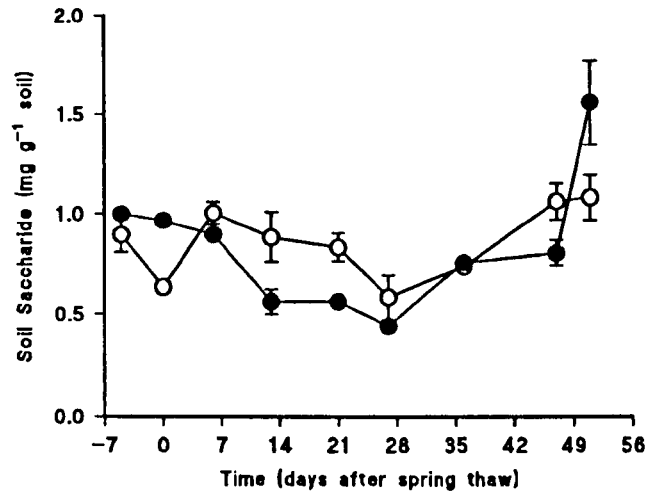
4.2.3   Field Observations from Polygon WW2

A second chronosequence of soil samples was taken from exposed and cloche-covered soil at polygon WW2 at approximate seven-day intervals. The first sample was taken after soil thaw on 15 December 1992 (day 0). The final sample was taken on day 51 (6 February 1993).

4.2.3.1   Soil Water Content

The water content in WW2, like WW5, never fluctuated enough to cause detrimental effect on the microbial community (exposed and cloche-covered soil with 50 -62% WHC). Indeed, the soil water measurements do not regress well with the majority of measurements taken on the sample (r^2 values below 0.25) with stronger correlations with soil carbon ($r^2 = 0.68$ in exposed soil and 0.37 in cloche-covered soil). This independence of microbial numbers, enzyme action and soil composition suggests that water content did not play a significant role in determining the activity of the microbial community.

4.2.3.2   Bacterial Numbers

Bacterial numbers in WW2 were significantly higher in the cloche-covered soil than in exposed soil (Students t-test, p<0.005) and follow a similar seasonal trend: high spring numbers precede a fall at day 21 followed by a rise from day 28 continuing to the end of the summer (Figure 4.12). This reflects the spring peak observed for activities and numbers in WW5. However, the lower numbers preceding the spring peak observed in WW5 were not recorded in WW2. This was probably due to the initial sample being taken five days later than the first sample from WW5, and thereby missing the low numbers that preceded the spring thaw.

The numbers in the exposed soil in polygon WW2 were around ten-fold lower than in WW5: a seasonal average of 0.82×10^6 g^{-1} soil in WW2 compared with 7.3×10^6 in WW5. The difference in the size of these communities may reflect differences in primary production in the two polygons. Indeed, the microalgal communities in Signy Island show marked variation between polygons which is thought to be due to the vagaries of the colonisation process and to reflect the probability of successful colonisation by the microalgae (Davey and Rothery, 1993)
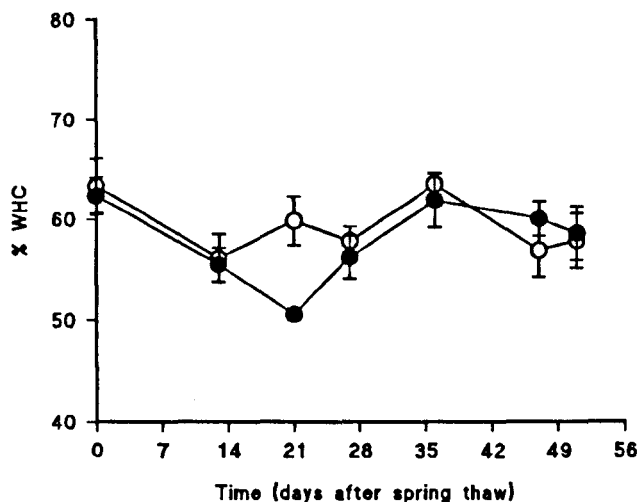
Figure 4.11. Water content of fellfield soil at polygon WW2, exposed (•) and inside cloche (o). Values are expressed as a percentage of water holding capacity (WHC) of the soil. Standard error bars are plotted within the symbols where not visible.
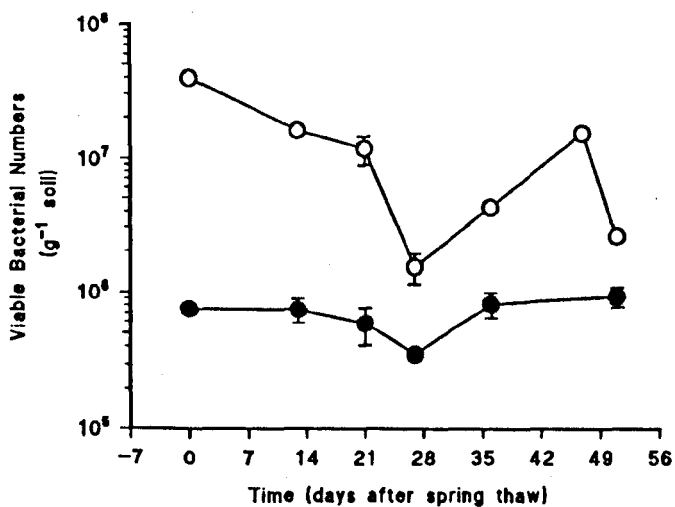
Figure 4.12. Total bacterial viable counts during the austral summer of 1992-3 in polygon WW2, in exposed (•) and cloche-covered (o) soil. Standard error bars are plotted within the symbols where not visible.

Table 4.2   Relationship between bacterial numbers and other microbial and soil properties in polygon WW2 during the 1992-3 austral summer. Correlation values, r^2, given. Values close to 1.0 are considered as significant positive correlations.

	Exposed soil	Cloche-covered soil
water content	0.18	0.14
constitutive ETS	0.08	0.30
substrate-induced ETS	0.14	0.68
phosphatase	0.61	0.43
α-glucosidase	0.40	0.80
β-glucosidase	0.27	0.51
α-galactosidase	0.68	0.64
β-D-1,4 galactosidase	0.75	0.32
saccharide	0.13	0.03
carbon	0.04	0.02
nitrogen	0.40	0.15

4.2.3.3.   ETS Activities

ETS activities in WW2 follow the same trend as the bacterial numbers in both exposed and cloche-covered soil (Figure 4.13a and b), although the two measurements do not correlate well (Table 4.2). The activities in the covered soil are significantly greater than in WW5, even though bacterial numbers are similar. Expressing ETS activity per bacterium (Figure 4.14a and b) allows a direct comparison between activity of the bacterial community at different soil sites. This shows a higher seasonal average activity in polygon WW2 (1.09×10^{-5} nmol INTF 48h^{-1} g^{-1} soil) than in WW5 (1.65×10^{-5} nmol INTF 48h^{-1} g^{-1} soil) in the cloche-covered soil. This, in turn indicates that there was a higher proportion of actively respiring bacteria beneath the cloche at WW2.

(a)

(b)
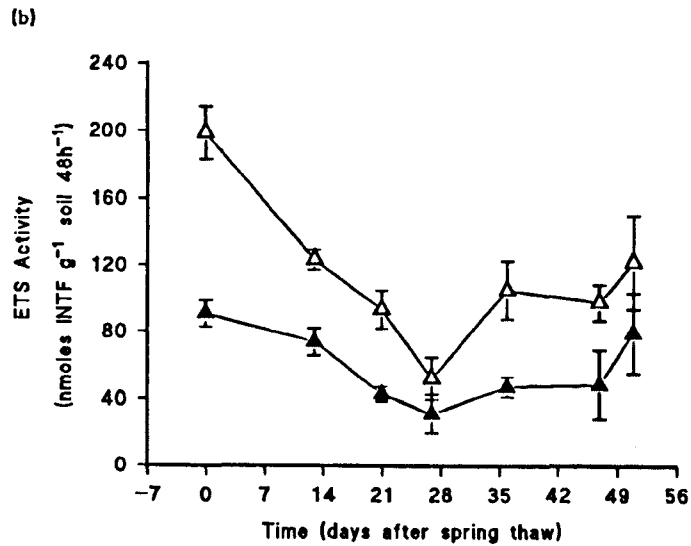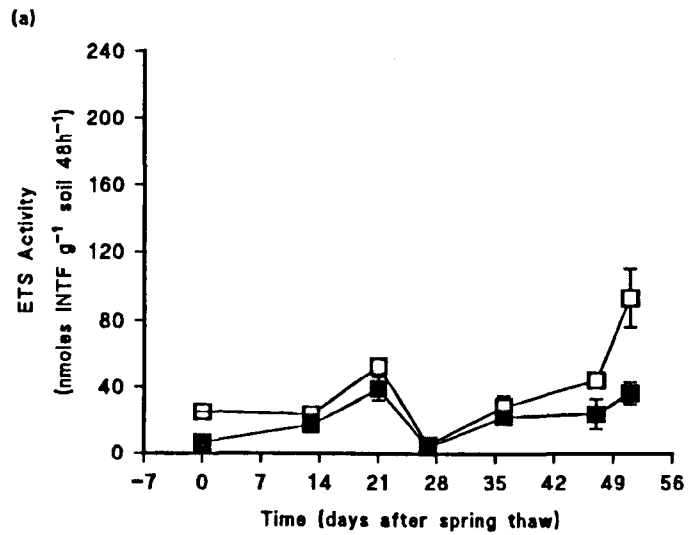
Figure 4.13. Microbial ETS activity during the austral summer of 1992-3 in soil polygon WW2. Exposed soil (a), constitutive (■) and substrate-induced activity ( ). Cloche covered soil (b), constitutive (▲) and substrate-induced activity(△). Standard error bars are plotted within the symbols where not visible.
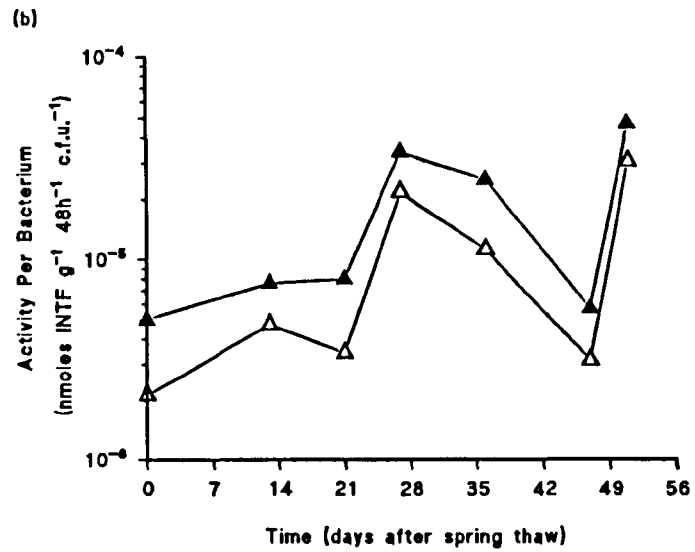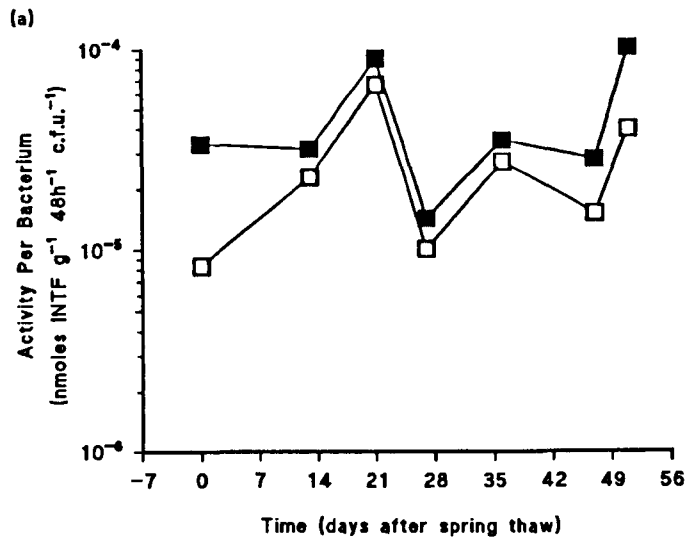
Figure 4.14. Microbial ETS activity per bacterium during the austral summer of 1992-3 in soil polygon WW2. Exposed soil (a) constitutive (■) and substrate-induced activity ( ). Cloche covered soil (b), constitutive (▲) and substrate-induced activity(△). Standard error bars are plotted within the symbols where not visible.

The difference between constitutive and substrate-induced activity shows a similar pattern to that in WW5; a large difference between the two measures of activity in cloche-covered soil, and no difference between the two in exposed soil. The conclusion reached in section 4.2.2.2 are relevant to this site: environmental conditions limit bacterial nutrient use in exposed soil, whereas nutrients provide greater limitation in cloche-covered soil.

4.2.3.4 Phosphatase Activities

As with the ETS activities, the activity of phosphatase follows the same seasonal trends as total bacterial number (Figure 4.15). Phosphatase activity was 10 x greater in cloche-covered soil compared with exposed soil, as were bacterial numbers. ETS activities were only two to three-fold greater. This suggests that the phosphatase activity may correlate more precisely with the number of viable bacteria, whereas the ETS assay measures metabolic activity. However, phosphatase activity did not correlate well with bacterial numbers (r^2 = 0.61 in exposed and 0.43 in cloche-covered soil) and this may be because not all bacteria are phosphatase positive. Indeed, only 64% of 25 bacterial isolates screened (Chapter 3) were phosphatase-positive. Although the composition of the bacterial community is unlikely to remain constant during the growing season and the proportion of phosphatase-positive bacteria could change, the screen does show that phosphatase activity in this soil does not directly relate to the size of the whole bacterial community.

4.2.3.5 Glucosidase and Galactosidase Activities

Activities of four exopolysaccharidase enzymes (α-D-1,4 and β-D-1,4 glucosidase, α-D-1,4 and β-D-1,4 galactosidase) were significantly greater in the cloche-covered soil than in exposed soil (Figures 4.16 and 4.17). Unlike polygon WW5, the fluctuations in the activity of all the enzymes follow those recorded with the bacterial numbers. There does appear to be a relationship between the polysaccharide degrading enzymes and bacterial numbers (Table 4.2), with the highest correlations of any activity with bacterial numbers (e.g., r^2 = 0.80 for α-glucosidase in cloche-covered soil). Thus, it appears that warming could elicit an increase in decomposition activity in this soil and the link of such activity to bacterial numbers suggests it is central to the response of the bacterial community . Indeed, it has been argued that increases in the carbon content of Arctic soil under global warming depend on the response of the decomposer community to increased primary product input (Callaghan and Jonasson, 1995). The relationship between phototrophic

and heterotrophic fellfield communities under global warming conditions is studied in chapter six.

4.2.3.6   Soil Carbon, Nitrogen and Saccharide

Although there are clear patterns of glucosidase and galactosidase activity in WW2, there is no corresponding pattern of changes in carbon or saccharide levels (Figures 4.18 and 4.19a). The absence of a significant decline in saccharide during periods of potentially high glucosidase and galactosidase activity suggests that the phototrophic organisms produce sufficient saccharide to offset any loses to the heterotrophic community. Total soil nitrogen levels in both plots show very little fluctuation from 0.30 mg g^{-1} soil (Figure 4.19b).

The seasonal average saccharide level was greater in the cloche-covered soil (0.410 mg g^{-1} soil) compared to the exposed soil (0.332 mg g^{-1}, Figure 4.18) although there was no significant difference (Students t-test) between saccharide in either plot. Soil carbon was, however, significantly greater in the cloche-covered soil (Students t-test $p<0.05$). This suggests that the greater amount of carbon in the warmed soil was not due to saccharide and may be in the form of biomass or other forms of soil organic matter such as humus.

Moss shoots were observed on the surface of the soil from the cloche-covered plot. Such colonists are rarely observed on immature fellfield polygons (Smith, 1990), as the relative instability of the substratum precludes their survival. In order to tolerate the polygon environment, the mosses would require a soil of higher organic matter content, acting to stabilise the mineral soil (Wynn-Williams, 1993; Smith, 1993). Soil maturation is the result of successful colonisation of bare fellfield and is hypothesised to ultimately lead to succession to secondary, moss-dominated, community - the climax community for such habitats on Signy Island (Smith, 1990). The observation of moss colonists and the increased soil carbon in cloche-covered soil, suggests that global warming conditions will increase the rate at which this succession will occur.
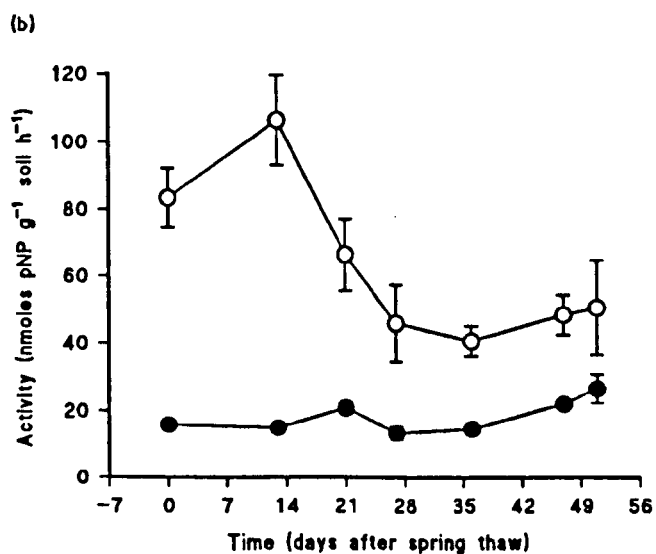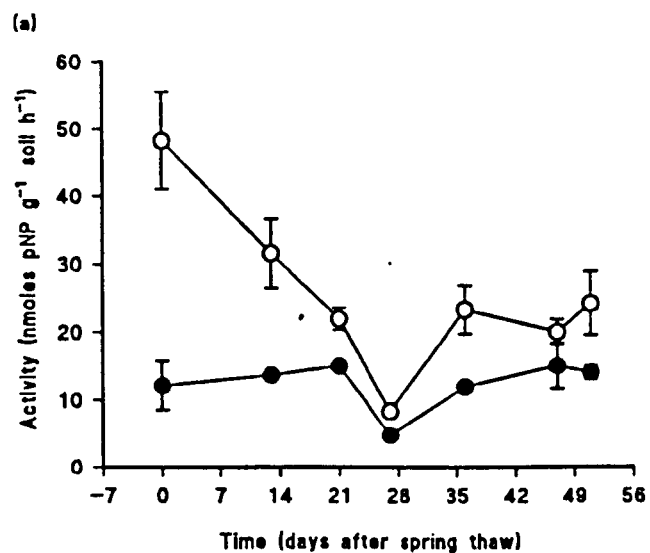
Figure 4.15 α-D-1,4 Glucosidase activity (a) and β-D-1,4 glucosidase activity (b) during the austral summer of 1992-3 in polygon WW2, in exposed (•) and cloche-covered (o) soil. Standard error bars are plotted within the symbols where not visible.

(a)

(b)
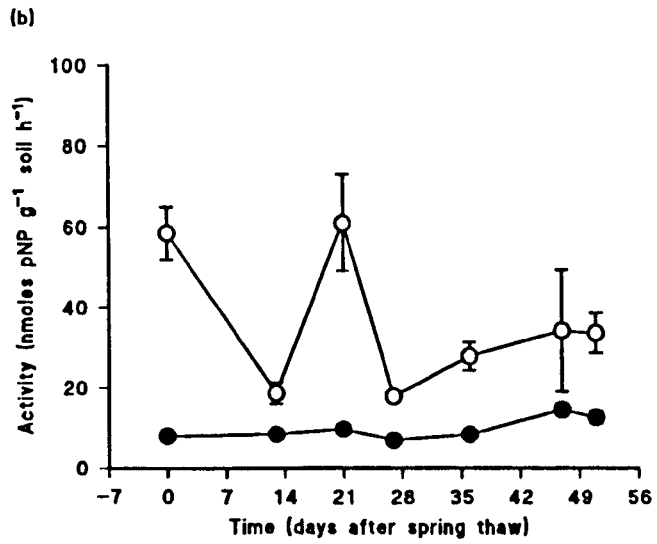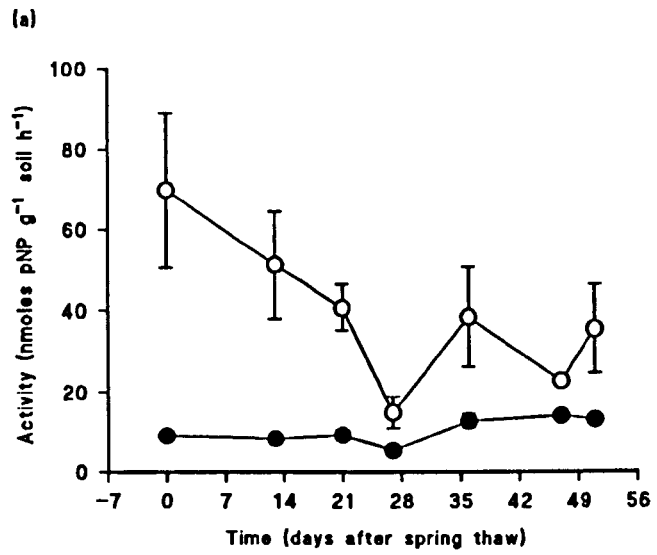
Figure 4.16   α-D-1,4 Galactosidase activity (a) and β-D-1,4 galactosidase activity (b) during the austral summer of 1992-3 in polygon WW2, in exposed (•) and cloche-covered (o) soil. Standard error bars are plotted within the symbols where not visible.
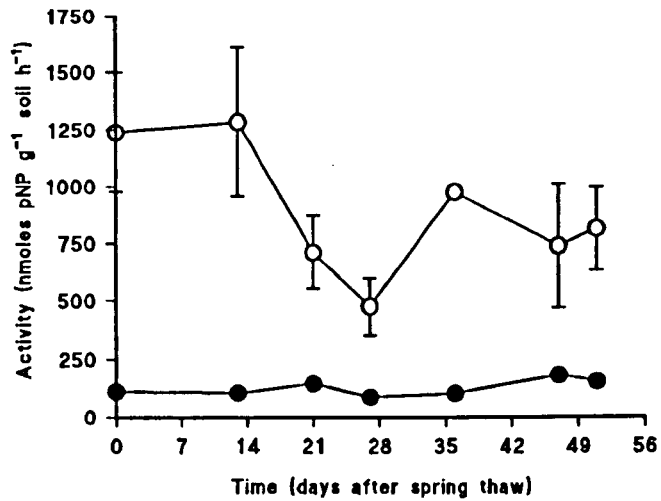
Figure 4.17   Phosphatase activity during the austral summer of 1992-3 in polygon WW2, in exposed (•) and cloche-covered (o)soil. Standard error bars are plotted within the symbols where not visible.
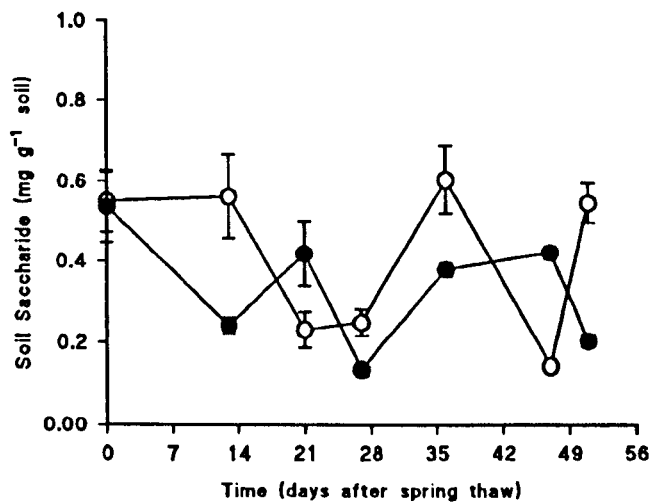
Figure 4.18.   Total soil saccharide during the austral summer of 1992-3 in polygon WW2, in exposed (•) and cloche-covered (o) soil. Standard error bars are plotted within the symbols where not visible.

Smith (1994) provided evidence that climate warming has been occurring at Signy Island and at Faraday Station, Argentine Island, mid-west Antarctic Peninsula (65°15'S, 64° 17'W) during a 45-year period up to 1990. Given this, it is likely that the permanent ice cover at Signy Island will recede, exposing pristine fellfield habitats (Hall and Walton, 1992). The observations from WW2 suggest that, once primary phototrophic colonists are established, they will increase the carbon content and stabilise the soil allowing secondary colonisation to succeed at an increased rate under global warming. The net result may mean faster accumulation of carbon by such freshly exposed substratum. Callagan and Johasson (1995) raise the question as to the fate of primary production in Arctic terrestrial environments under global warming. They argue that increases in primary productivity may be offset by increased decomposer activity. From the results of this study, it appears that at polygon WW2, the net carbon accumulation indicates that increased decomposer activity is not fully offsetting increases in primary production. Given the importance of this observation and the implication that such Antarctic sites may act as carbon sinks under global warming, further work should be carried out to assess annual carbon build up beneath climate-changing cloches.

4.2.4 Comparison of Measurements from Polygons WW2 and WW5

It is interesting to note that even though bacterial numbers in exposed soil at WW5 were ten-fold higher than in WW2 (4.2.3.2), numbers in the cloche-covered soils were comparable: a seasonal average of 1.32×10^7 g^{-1} in WW2 compared with 1.20×10^7 in WW5. This may reflect the greater duration of cloche-cover at site WW2. After eight years of cloche-cover the smaller initial microbial community of WW2 may have developed into a comparable size to that achieved by the larger community of WW5 after five years of cover. This assumes that the size of the exposed community does not undergo dramatic change on an annual basis, and that the relatively small size of the WW2 community is not a reflection of poor growing season.

Alternatively, the differences may be due to the cloches installed on the two polygons. The cloche on polygon WW5 acts to simulate global warming, whereas the cloche on WW2 also blocks the transmission of incident UV-B radiation (Wynn-Williams, 1992b). Although the spring time ozone hole does not reach as far north as Signy Island, constitutive summer levels of incident UV-B radiation are high enough for terrestrial colonists to have UV-B avoidance and protection mechanisms (e.g., production of UV absorbing compounds such as mycosporin-like amino acids and scytonemin, Karentz et al., 1991). The majority of bacteria isolated in the present study were pigmented and

many pigmented Antarctic bacterial isolates contain carotenoids (Shiba, 1991; Dobson *et al.,* 1991; Chauhan and Shivaji, 1994), pigments that quench photochemical energy and protect from UV and bright PAR (Vincent and Quesada, 1994).

Thus the relatively large difference in bacterial numbers from exposed to cloche-covered soil in WW2 may be attributed to a response to UV-B protection. Such a response may be led by increased production by phototrophs supporting a greater bacterial community, or may reflect enhanced survival of bacteria near the soil surface. Indeed, the bacterial numbers were similar beneath both cloches but the ETS and glucosidase and galactosidase activities were greater beneath the warmed and UV-B screened cloche on WW2. This suggests a greater activity per bacterium, as shown by the higher specific ETS activities in the cloche-covered WW2 compared to WW5. This interesting observation indicates that the constitutive levels of UV-B radiation may reduce microbial activity but not microbial numbers. This effect is studied in more detail in Chapter 6, from field observations from cloches that provide only UV-B screening or global warming simulation.
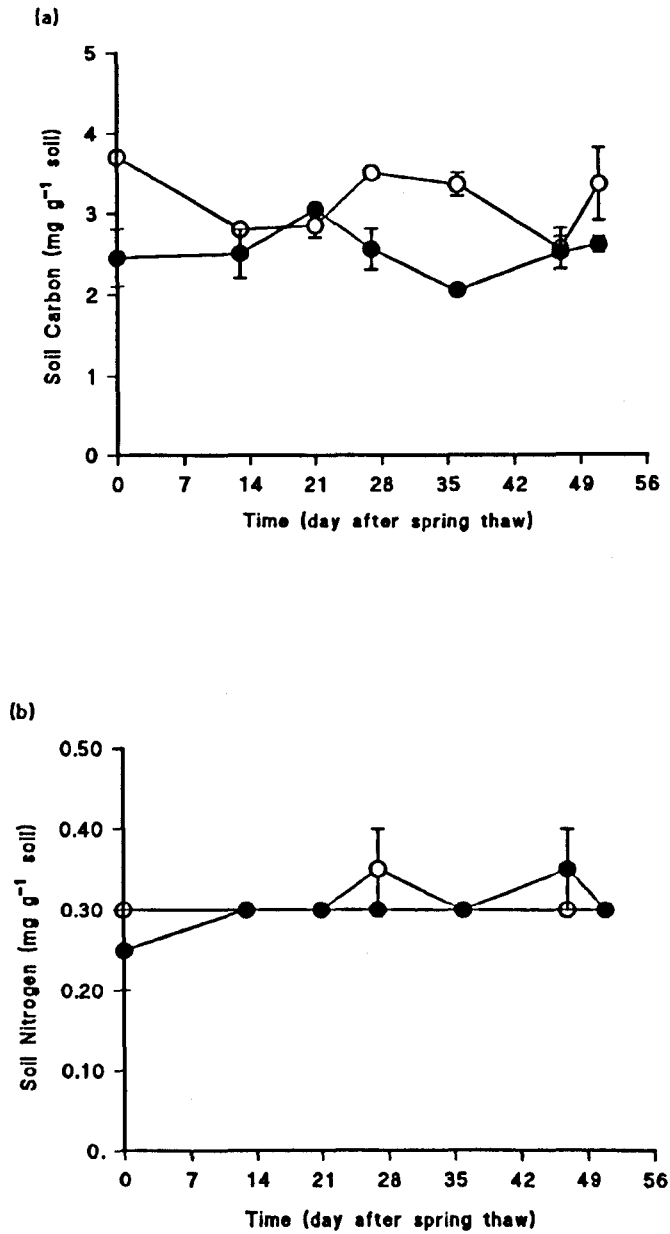
Figure 4.19. Total soil carbon (a) and nitrogen (b) during the austral summer of 1992-3 in polygon WW2, in exposed (•) and cloche-covered (o) soil. Standard error bars are plotted within the symbols where not visible.

5. SIMULATED CLIMATE CHANGE AT TWO ANTARCTIC FIELD SITES: ENERGETIC CONSIDERATIONS

5.1 Introduction

This chapter explores methods of calculating the effect of climate change on seasonal microbial energy use at two Signy Island field sites (WW2 and WW5). This information is then used to put the changes caused by climate change into context with microbial activity at moss-dominated peat soils at Signy Island. Microbial energy use has been estimated by ATP measurements (Nannipieri *et al.*, 1978), incorporation of radiolabelled carbon into lipids (Vestal, 1988) and rate of thymine incorporation into DNA and leucine into biomass (Bölter, 1993; Berger *et al.*, 1995). Oxygen uptake has been used to monitor aerobic decomposition at moss-dominated sites at Signy Island and other maritime Antarctic sites (Wynn-Williams, 1984). Microbial photosynthesis and respiration has also been monitored using carbon dioxide flux in one of the most extreme Antarctic ecosystems, that of the Ross Desert (Friedmann *et al.*, 1993). These authors also calibrated microbial activity with temperature and irradiance to estimated annual respiration and net photosynthesis. In this manner the annual net ecosystem productivity of the cryptogamic microflora of this cold desert was calculated to be the lowest recorded (3 g C m^{-2} y^{-1}).

Three methods of calculating energy use are reported, all involving extrapolations from the microbiological data presented in Chapter 4. The first method involves quantifying the total energy required to give increases in bacterial number recorded from field samples. The second approach involves the conversion of microbial dehydrogenase activities into electron transfer system (ETS) activity and hence ATP production. This information is then used to calculate total microbial oxygen uptake during the duration of the field study and is compared with previous work on the oxygen uptake of the microbiota in Signy Island peat soils. Finally, the potential hydrolysis of hexose from soil polysaccharide is calculated in order to estimate the effect of climate change on the release of this important carbon source.

5.2   Results and Discussion

5.2.1   Energy Expenditure Calculated from Observed Biomass Production

The first method for calculating seasonal bacterial energy expenditure involved assessing the energy required to give the increases in bacterial biomass recorded in field samples (Chapter 4). This involved using the yield coefficient (quantitative nutrient requirement of an organism; Neidhardt *et al.*, 1990) of an individual fellfield bacterial isolate to calculate the total nutrient requirement of the bacterial community during the growing season. There is no information on the yield coefficient of Antarctic bacteria and in order to determine an ecologically relevant yield coefficient, to reflect the growth conditions of the bacterial community in the field, the study was carried out on a fellfield isolate growing in Antarctic fellfield soil under simulated field conditions (i.e. temperature and soil water content).

Isolate A2 was chosen as the model fellfield bacterium as it represented a morphology and colony form (short rod, 1.5. x 1 μm, entire, raised, white colony, diameter >2 mm) consistently isolated from cloche-covered and exposed soil from polygons WW2 and WW5. The isolate was identified by FAME analysis to be closest to *Arthrobacter oxydans* with 71% homology to the database reference strain (Thompson *et al.*, 1993). All Antarctic bacterial isolates identified with 16S RNA analysis have proven to be new species (Franzmann and Dobson, 1993; Franzmann, 1996) and so the relatively poor match of isolate A2 to the reference strain is not unexpected.

The growth of A2 on glucose as the sole carbon source was studied. Glucose was chosen as the substrate because it has been recorded in fellfield soil, both as a free sugar (Arnold, 1995) and as a major component of fellfield polysaccharide (Foister *et al.*, 1993). Glucose was also used by all 10 isolates screened for sugar and polyol use (section 3.2.5) and, after carrying out growth experiments with Antarctic bacterial isolates, Bölter (1993) concluded that glucose can serve as a model substrate to evaluate bacterial metabolism in Antarctica.

5.2.1.1 Calculation of Yield Coefficient for Isolate A2

Fellfield soil from two Signy Island polygons (WW 3 and WW 7) sampled during the 1988-9 austral summer and stored at -70°C was pooled, homogenised and heat-sterilised (section 2.5) to provide a uniform, ecologically appropriate substratum onto which isolate A2 was inoculated. Inoculations of between 1×10^6 and 1×10^7 g^{-1} soil resulted in $4 \pm 0.5 \times 10^7$ g^{-1} soil after 48 h of incubation at 25°C (Figures 5.1 and 5.2). An increase of a population of an *Arthrobacter* sp. has been observed during the first 48 h after inoculation into sterilised and non-sterilised soil in laboratory microcosms (Thompson *et al.*, 1990) but not when the inoculation was repeated into field soil (Thompson *et al.*, 1992). The response was observed whether the cells were harvested during exponential growth and inoculated immediately (as in the present study) or following starvation - suggesting the bacteria were not simply carrying on the process of cell division carried out during the exponential phase of growth. The increase may be due to microbial growth on nutrients in the soil as Thompson *et al.* (1990) found the bacterial number increase corresponded with a peak of microbial respiration. Powlson and Jenkinson (1976) reported that soil homogenisation, with or without sterilisation, solubilised nutrients and thereby made them more available for microbial metabolism.

Prior to substrate amendment studies, the initial increase was investigated to confirm if it was due to growth on nutrients in the sterilised soil. Cells were inoculated into hot washed (4 h, 60°C - to remove soluble nutrients; Martens and Frankenburger, 1990), burnt (12 h, 550°C - to oxidise organic carbon; Forster, 1995) and heat sterilised soil. The populations in the washed and burnt soils did show increases ($4.0 \pm 1.6 \times 10^6$ g^{-1} soil) relative to the inoculum (1×10^6) after 24 h. The population in the heat sterilised soil increased to a greater extent ($2.8 \pm 0.6 \times 10^7$ g^{-1} soil; Figure 5.1). The growth of A2 was probably due to nutrients present in the heat-sterilised soil which were unavailable (burnt or washout out) in the two treated soils. The smaller increase in the washed and burnt soils can be attributed to nutrient carry-over despite the washing of cells prior to inoculation (section 2.5.1.). Nutrients in the heat-sterilised soil become reduced to levels unable to promote bacterial growth after 48 h and glucose amendments in subsequent experiments were given after this period.

The length of time between inoculation and glucose amendment (1 mg g^{-1} soil) had no significant effect on the increase in cell number: the response to all amendments (after 24, 48, 72 and 144 h) being $2.64 \pm 0.29 \times 10^8$ g^{-1} soil (Figure 5.2). It was assumed from this that the period of time elapsed between nutrient input into the fellfield would not have significant effect on the efficiency of subsequent biomass production of the community.

However, such extrapolations may not hold for a population under field conditions, especially in an Antarctic environment with stresses due to lack of water availability and frequent freeze-thaw cycles (Davey, 1991a).

The effect of concentration of glucose on biomass production was studied with amendments of 1.0, 0.5 and 0.1 mg glucose g^{-1} soil (Figure 5.3). ^{14}C radiolabelled glucose was used to assess the efficiency of glucose utilisation by allowing the rate and yield of conversion of glucose to carbon dioxide to be monitored. The increase in cell number per mg glucose did not differ significantly with the concentration of the amendment. The 0.1 mg amendment did, however, result in a large standard error about the mean, attributable to the multiplication required to give the cell number increase per mg glucose. Approximately 60% of the amended glucose was converted to carbon dioxide in 144 h with all three amendments (Figure 5.3 b). This is in agreement with other studies on the efficiency of utilisation of substrates during aerobic growth (Janson, 1960; Cheshire *et al.*, 1969; Shields and Paul, 1973). Thus, it can be assumed that the increase in bacterial numbers is a result of complete aerobic metabolism of glucose and that the cell growth is due to metabolism of all the amended glucose.

It would have been advantageous ensure all CO_2 dissolved in the soil water was volatilised prior to the estimation of total CO_2 evolved by microbial respiration. However, acidification of the soil to flush out dissolved CO_2 was not performed as the relatively small quantities of soil available necessitated the use of continuos rather than destructive sampling. However, Martens (1986) reported no loss of supplemented CO_2 to soil water in sealed flasks of soil below pH 6.0 (the soil used in our study was pH 5.7).

The initial amendment studies involved incubations at 25°C, the optimum growth temperature of *A. oxydans* (section 3.2.3). Subsequent experiments were carried out at 4, 10 and 25°C to reflect the range of field temperatures during the summer at Signy Island (Walton, 1982). Interestingly, increases in cell number were not significantly influenced by temperature. This suggests no change in yield coefficient with temperature and contrasts with reports of microbial metabolic efficiency increasing with decreasing temperature (Herbert and Bell, 1977; Wiebe *et al.*, 1992, 1993). Given that the mean temperature of the fellfield during the austral summer is 1.10°C in exposed soil and 4.88° C in cloche-covered soil, the response at 4°C was used for extrapolations to fellfield samples. At 4°C, the cellular yield coefficient was calculated as 2.42 x 10^8 cells mg^{-1} glucose. Note that yield coefficient (Y_{ATP}) is defined as the yield in grams dry matter per mole of ATP obtained in catabolism of substrate (Pirt, 1975). However, in our study, a

modified coefficient was used to give the cellular yield. This cellular yield coefficient is defined as increase in biomass (g) per substrate (g).

Table 5.1 Increase in numbers of *Isolate A2* inoculated into heat sterilised Antarctic fellfield soil to glucose amendments.

Temperature (°C)	Concentration of glucose amendment (mg glucose g^{-1} soil)	Increase in bacterial number (mg glucose^{-1}) [1]
25	1.0	$2.64 \pm 0.29 \times 10^8$
25	0.5	$2.82 \pm 0.53 \times 10^8$
25	0.1	$5.30 \pm 2.41 \times 10^8$
10	1.0	$2.81 \pm 0.35 \times 10^8$
4	1.0	$2.42 \pm 0.33 \times 10^8$ [2]

[1] Bacterial increase from an initial inoculum of 1×10^7 cells g^{-1} soil after 96 h at 25°C, 144 h at 10°C and 336 h at 4°C.

[2] Increase in bacterial number used for extrapolations of energy use by bacterial communities in field samples.
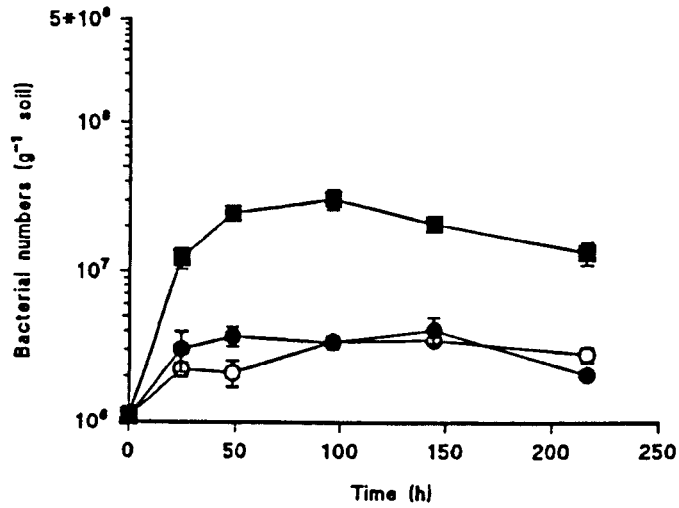
Figure 5.1   Growth of *Arthrobacter oxydans* after inoculation into heat sterilised (■), burnt (●) and hot washed (O) Antarctic fellfield soil. Standard error bars are plotted within the symbol where not visible.
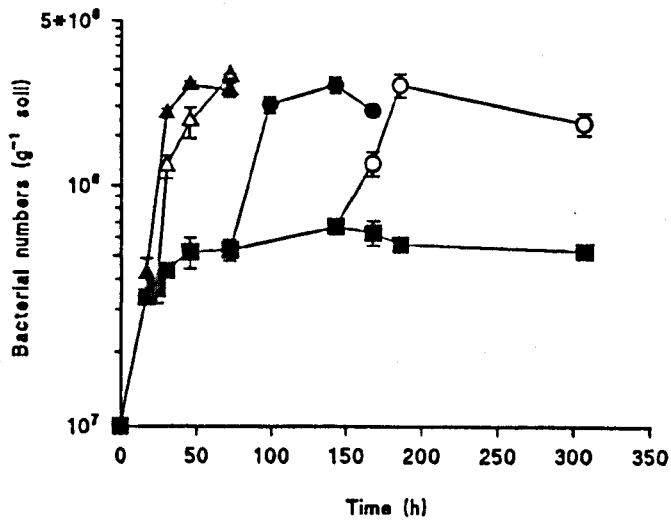
Figure 5.2   Growth of *Arthrobacter oxydans* after inoculation into heat sterilised Antarctic fellfield soil. Non-amended soil (■), soil amended with glucose (1 mg g^{-1}) at 24 h (▲), 48 h (△), 72 h (●) and 144 h (O) after inoculation. Standard error bars are plotted within the symbol where not visible.

(a)

(b)
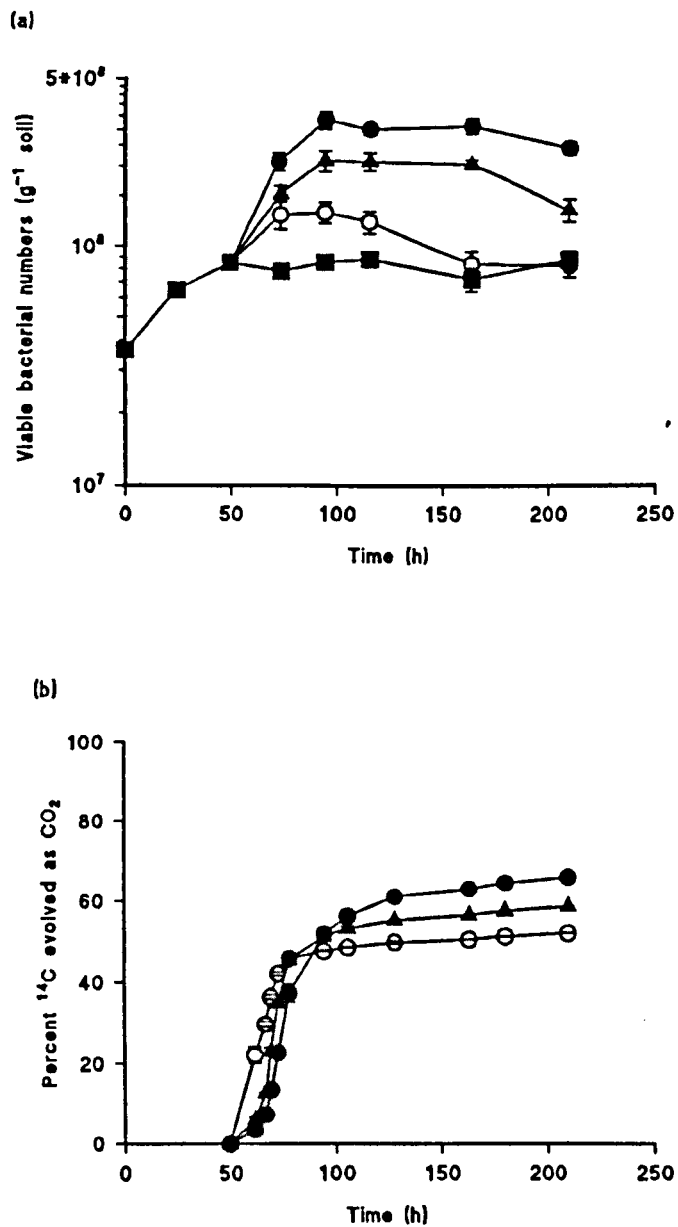
Figure 5.3 Response of *Arthrobacter oxydans* in heat sterilised Antarctic soil amended with radiolabelled glucose. (a) Population numbers and (b) evolution of $^{14}CO_2$ with 0 (■), 1 (●), 0.5 (▲) and 0.1 (O) mg glucose per gram of sterilised Antarctic fellfield soil. Standard error bars are plotted within the symbol where not visible.

5.2.1.2 Seasonal Bacterial Energy Expenditure Calculated from Biomass Increase

The yield of Isolate A2 with glucose amendments was used to convert bacterial increases observed between successive field samples into Glucose-Consumption-Equivalent (GCE; section 2.6.1). The calculated energy use reveals that the climate change simulation allowed a significantly greater substrate use in both polygons (Table 5.2). This is unsurprising given that the information is derived from field measurements which clearly show greater bacterial numbers beneath the climate changing cloches compared to exposed soil (Figures 4.4 and 4.12). However, whereas the counts of bacterial number are useful to show seasonal trends, only when the increases in cell number are converted into GCE can information about the effect of climate change on seasonal bacterial energy use be obtained.

A direct comparison with the values for seasonal ATP production calculated from dehydrogenase activities (section 5.2.2) can be made by converting the GCE into ATP use. This was done by converting the cellular yield coefficient of A2 into molar yield coefficient $Y_{substrate}$ by transforming the number of cells per mg glucose, into mass of cells per mole of glucose (Neidhardt *et al.*, 1990). Assuming the average size of an *A. oxydans* cell to be 1.5 x 1 μm (Chapter 3) and a specific gravity of 1.1 and that 80 % of a cell mass is water (Neidhardt *et al.*, 1990), each cell has a dry mass of 2.592×10^{-13} g. Therefore, 1 mg glucose (5.55×10^{-6} moles) gave 6.272×10^{-5} g of biomass. Thus, molar yield coefficient ($Y_{substrate}$):

$$Y_{substrate} = biomass\ (g)\ /\ substrate\ (moles)$$
$$= 6.272 \times 10^{-5}\ /\ 5.55 \times 10^{-6}$$
$$= 11.301$$

This compares well with other studies on bacteria and yeast grown in laboratory culture ($Y_{substrate}$ for bacteria growing on glucose, e.g. 8.3 for *Pseudomonas lindneri*, 21.0 for *Streptococcus faecalis* and 33.6 for *Escherichia coli* ; Pirt, 1975). Given the relationship between $Y_{substrate}$ and Y_{ATP} (biomass increase mol^{-1} ATP):

$$Y_{ATP} = Y_{substrate}\ /\ n$$

where n = moles of ATP per mole of substrate.

The moles of ATP per mole of glucose used by *A. oxydans* can be calculated given the standard value of Y_{ATP}, Bauchop's constant = 10.5 g mol^{-1}:

$$n = Y_{substrate} / Y_{ATP}$$
$$= 11.301 / 10.5$$
$$= 1.076$$

The seasonal ATP use for biomass increase is given in Table 5.2.

This calculation of energy use cannot, however, take into consideration additional energy required for growth under field conditions. This includes the synthesis and secretion of enzymes to metabolise complex polysaccharide or the cost of survival in under fluctuating diurnal temperatures. Fellfield mosses and lichens, for example, have the cost of production of compatible solutes, which protect from frost damage (Tearle, 1987). Although there are no reports of fellfield bacteria accumulating compatible solutes, purple and green sulphur bacteria and a halophilic sulphate-reducing bacterium have been reported to accumulate trehalose during osmotic stress (Welsh and Herbert, 1993; Welsh *et al.*, 1996). Fellfield cyanobacteria also produce UV-protection compounds, such as scytonemin and mycosporin-like amino acids (Karentz, 1991). Indeed, in the most extreme Antarctic ecosystem, the Ross Desert, only around 0.025% of gross primary productivity has been calculated to be converted into ecosystem productivity (Friedmann *et al.*, 1993). No account can also be made of maintenance energy of the bacterial standing stock. Interestingly, maintenance energy has been recorded at 2% of carbon input at 2°C and 10% at 20°C in a psychrophilic *Vibrio* sp. (Herbert and Bell, 1977). Thus, the maintenance energy may be relatively small given the low temperatures of fellfield soil, but may become increasingly significant in the warmed soil beneath the cloches. Individual bacterial populations may also fluctuate without giving a change in the size of the community. Indeed, it has been calculated that all the microbial heterotrophic production in a marine ecosystem was consumed by predators and so no increase in bacterial biomass was observed (Solic and Krstulovic, 1995).

Finally, these calculations are based on microbial counts from soil samples taken at approximately seven day intervals. These are intervals chosen for convenience and do not take account of any significant changes between sampling dates.

Nevertheless, given that this information is based on recorded bacterial increase, it is the only method of the three described in this chapter which is based on an event that has happened (i.e. energy has been used to give the bacterial number increases). Other approaches depend upon enzyme assays which give activity under idealised conditions (e.g. temperature and nutrients). Such assays give a measure of the potential maximum activity (in the field) at the time of sampling but will, in all probability, overestimate the actual microbial activity.

Table 5.2   The effect of climate change on seasonal energy expenditure on observed bacterial biomass increases in polygons WW2 and WW5. Equivalent glucose use is based on calibration of the response of fellfield isolate *A. oxydans* to glucose amendments.

Polygon	Treatment	Seasonal Bacterial Energy Expenditure	
		Equivalent glucose use	ATP use [3]
		(μg glucose g^{-1} soil 53 d^{-1})	(nmoles ATP g^{-1} soil 53 d^{-1})
WW 2	exposed	4.95	29.59
WW 2	cloche [1]	54.28	324.47
		(μg glucose g^{-1} soil 58 d^{-1})	(nmoles ATP g^{-1} soil 58 d^{-1})
WW 5	exposed	27.38	163.67
WW 5	cloche [2]	42.27	252.68

[1]   Cloche on polygon WW2 provided an average increase in summer temperature of +3.78 C° and blocked the transmission of UV-B radiation (Wynn-Williams, 1996).

[2]   Cloche on polygon WW5 provided an average increase in summer temperature of +2.22 C° and was transparent to UV-B radiation (Wynn-Williams, 1996).

[3]   Conversion of GCE to ATP production uses a conversion of 1 mole of glucose yielding 1.076 moles of ATP.

This assay measures the activity of microbial electron transfer system (ETS, Trevors *et al.*, 1982): a supplemented tetrazolium salt (INT) is reduced to a coloured formazan by ETS activity. The tetrazolium salt thus competes with oxygen during aerobic metabolism to be the terminal electron acceptor. Although INT has been shown to be a more effective competitor than the formerly widely used 2,3,5-triphenyltetrazolium chloride (TTC, Trevors, 1984a; Friedel *et al.*, 1994), a negative relationship has been shown between oxygen concentration and INT activity (Trevors, 1984). This suggests that INT is not able to fully out-compete oxygen in the role of terminal electron acceptor. However, in order for INT to be as competitive as possible the assay was performed with soil suspensions, incubated stationary for 48h (section 2.4.1), to restrict oxygen diffusion.

A link between ETS activity, as measured by the INT assay, with oxygen consumption has been shown in soil (Trevors *et al.*, 1982). Dehydrogenase activity and oxygen use followed a similar sequence of events following nutrient supplements to a soil microbial community (Casida, 1977). This indicates that although INT may not be accepting 100% of the electrons from the ETS, there is a relationship between ETS (as measured by oxygen consumption), and INT reduction. Furthermore, in woodland soils, the amount of INT reduction correlated well with oxygen use but accounted for less than 6% of total ETS activity when measured separately by oxygen consumption (Benefield *et al.*, 1977). In the absence of further studies on the direct relationship between oxygen uptake and reduction of INT, it is proposed to use a conversion factor based on the latter study to account for the ability of to INT compete for reduction. A conversion based on the reduced INT representing 5.41% of oxygen use, and therefore actual ETS activity, has been used to calculate a "modified" dehydrogenase activity. This conversion is based on data from experiments carried out by Benefield *et al.* (1977) which were the closest to match the experimental conditions of the present study.

The conversion of INT reduction into ETS activity allows calculation of ETS-based ATP production. In the ETS of *Escherichia coli*, a pair of hydrogen atoms (which subsequently split into two protons and electrons) have sufficient energy (protonmotive force) to phosphorylate two molecules of ADP to form ATP (Neidhardt *et al.*, 1990). This gives a hydrogen to ATP ratio (H:P, Neidhardt *et al.*, 1990) of 1:1. Given that one molecule of INT is reduced by one hydrogen atom, the ratio between reduced INT and ATP production (INT:P) is also 1:1. In order to estimate the total "modified" dehydrogenase activity during the sampling period (10-12-92 to 6-2-93), account is taken of the activity and time between successive samples (section 2.6.2).

Total ATP production values for the duration of the field study are given in Table 5.3. Two measures of dehydrogenase activity were measured: constitutive (no supplemented nutrient) and substrate-induced (section 4.2.2.3). Both are maximal activities, i.e. the substrate-induced activity is a measure of maximal activity with excess nutrients (provided by yeast extract) whilst the natural activity is a measure of the maximal activity on nutrients in the soil at the time of sampling. The latter may still be greater than the expressed activity *in situ* as the soil was homogenised prior to the assay (which may release nutrients available for metabolism).

ATP production calculated from constitutive dehydrogenase activity and probably the more realistic measure of *in situ* activity (activity with no nutrient supplements), is greater than the energy required to give biomass increase (Table 5.2). For example, ATP production from exposed soil from WW2, was 330 x that calculated from the energy expenditure-microbial numbers relationship, and 85 x that for cloche-covered soil. As discussed above, the energy calculated to give observed biomass increase is expected to underestimate actual energy use. The dehydrogenase assay measures the activity of the community at all sampling times, not just when there are periods of observable biomass increase. This assay, therefore, takes into account many of the energy requirements of the microbial community not measured from biomass increase alone (e.g. survival of freeze-thaw events, production of polysaccharidases and UV-defence pigments).

Table 5.3 Effect of climate change on maximal energy production by microbial communities in polygons WW2 and WW5. Calculation based on dehydrogenase activity.

Polygon	Treatment	Seasonal Microbial Energy Expenditure	
		Natural dehydrogenase (3)	Substrate-induced dehydrogenase (4)
		(μmoles ATP g^{-1} soil 53 d^{-1})	(μmoles ATP g^{-1} soil 53 d^{-1})
WW 2	exposed	9.759	16.239
WW 2	cloche (1)	27.711	53.393
		(μmoles ATP g^{-1} soil 58 d^{-1})	(μmoles ATP g^{-1} soil 58 d^{-1})
WW 5	exposed	25.065	30.800
WW 5	cloche (2)	37.058	59.968

(1)   Cloche on polygon WW2 provided an average increase in summer temperature of +3.78 C° and blocked the transmission of UV-B radiation (Wynn-Williams, 1996).

(2)   Cloche on polygon WW5 provided an average increase in summer temperature of +2.22 C° and was transparent to UV-B radiation (Wynn-Williams, 1996).

(3)   Dehydrogenase activity with no nutrient supplement.

(4)   Dehydrogenase activity with yeast extract (0.1% w/v final concentration) supplement.

Given the relationship between INT reduction and ETS activity, it is also possible to calculate microbial respiration in terms of moles of oxygen consumed (section 2.6.2). During respiratory chain activity four hydrogen atoms reduce one molecule of oxygen. Thus, one mole of oxygen is consumed to generate four moles of ATP. The seasonal microbial oxygen consumption at the two polygons is compared with the oxygen uptake of the heterotrophic microflora from peat soil at Signy Island in Table 5.4. The oxygen consumption values during the observed season are around one order of magnitude lower than those recorded at two moss-covered soil sites on Signy Island. However, it must be noted that in our study the "annual" respiration was calculated from microbial dehydrogenase activity during a 58 day period (10-12-92 to 6-2-93), and not on based extrapolations for the entire year as with the peat soil study (Wynn-Williams, 1984). This period can be considered to be the spring and summer season (December-March, Davey *et al.*, 1992) and features the highest annual temperatures (large diurnal fluctuations,

average 1.1°C, Wynn-Williams, 1996) and would be expected to include the period of most of the annual microbial activity (Davey, 1991a). The autumn season (March-May) features stable temperatures around 0°C and is considered to be of ecological importance in promoting cold-hardiness prior to winter (Davey et al., 1992; Arnold, 1995). However, significant microbial activity may also occur during this autumn period, not included in our study, as microbial respiration and photosynthesis have been recorded below 0°C (Wiencke et al., 1992; Clein and Schimel, 1995) and significant winter and spring growth of chlorophytes has been recorded beneath snow and ice at Signy Island polygons (Davey, 1991a). This may lead to a greater true annual respiratory activity.

Nevertheless, the large difference between the aerobic respiration at the two peat sites at Signy Island (Wynn-Williams, 1984) and the bare polygons of the present study is probably due to the different nutrient status of the two soil types. The moss peat soil has a high organic matter content (40-46% w/w) and has a concomitantly greater input of primary product. The increased productivity of soil beneath the cloches illustrates the beginnings of a successional change caused by climate change observed in polygon WW 2 and discussed in Chapter 4. However, the comparable data for microbial respiration at the moss peat site (the secondary stage of colonisation), illustrates that the increased productivity caused by the cloches is some way from attaining that expressed by the peat soil.

The values for annual microbial respiration at the Signy Island peat site were calculated from just one sample per field site. The relationship between respiration and temperature was studied in field samples and was used, along with field microclimate temperature data, to calculate the annual respiration of the field site (Wynn-Williams, 1984). This approach does yield valuable information on annual respiration, however, like the dehydrogenase-based respiration data of our study, it is based on a number of assumptions. The first of these is that the relationship between respiratory activity and temperature holds for the entire year. This requires the nutritional status of the field soil to be the same throughout the year as the soil sampled for use in the laboratory study. This was not the case in our study. The difference between consitutive and substrate-induced dehydrogenase activity shows that nutrients are more limiting in exposed fellfield soil during the spring (day -5 to day 21 onwards) to than during summer (day 21; Figure 4.5 and 4.13). It is likely that the nutritional status of moss peat soil will also change during the growing season, as nutrient flushes have been recorded which give rise to periods of high respiratory activity (Wynn-Williams, 1980). In addition, the former study assumes the microflora has the same respiratory ability and a constant active biomass throughout the year. The present study has shown this not to be the case: bacterial numbers underwent

significant changes during the experimental period (Figures 4.4 and 4.12). Decreases in the microscopic phototrophs at Signy Island polygons have also been recorded during the spring melt and their populations fluctuate during the austral summer and autumn (Davey, 1991a). The whole-seasonal sampling approach of the present study does take into account changes in respiratory activity due to nutrient availability and biomass size by measuring dehydrogenase activities in field samples throughout the period of study.

Table 5.4   Comparison of microbial oxygen consumption in Signy Island peat and fellfield soil.

Soil type	Year	Treatment	Estimate annual oxygen consumption $(l\ m^{-2})$
fellfield	1992-3	exposed	19.14 (3)
polygon WW 2		cloche (1)	54.34 (3)
fellfield	1992-3	exposed	49.15 (3)
polygon WW 5		cloche (2)	72.67 (3)
peat dominated by the	1972-3	exposed	555.5 (4)
moss *Polytrichum*	1973-4		569.7 (4)
peat dominated by the	1972-3	exposed	320.9 (4)
moss *Drepanocladus*	1973-4		382.9 (4)

(1)   Cloche on polygon WW2 provided an average increase in summer temperature of +3.78 C° and blocked the transmission of UV-B radiation (Wynn-Williams, 1996).

(2)   Cloche on polygon WW5 provided an average increase in summer temperature of +2.22 C° and was transparent to UV-B radiation (Wynn-Williams, 1996).

(3)   Based on dehydrogenase activities taken during 53 d (WW 2) and 58 d (WW 5) sampling period during the 1992-3 austral summer (section 2.6.3).

(4)   Based on laboratory measurements of O_2 and CO_2 flux and one year of field microclimate data (Wynn-Williams, 1984).

5.2.3   *Potential Release of Saccharides by Microbial Polysaccharidases*

The potential amount of hexose released by exopolysaccharidases has been calculated from assays performed on field samples (section 4.2.2.4 and 4.2.3.4). Four exopolysaccharidase enzymes were assayed, chosen because they cleave the most abundant components of fellfield soil polysaccharide (Foister *et al.*, 1993). Seasonal activities were derived in the same way the seasonal dehydrogenase activity was calculated (i.e. by taking into account the activity and duration between samples throughout the period of the field study, section 2.6.3). The activity can be considered to be near to the maximum, as the assay was carried out under conditions of excess substrate with agitation at 30°C. Thus, this activity is likely to considerably overestimate the actual field activity. Furthermore, to extrapolate the enzyme activities during the assay to field activity requires the assumption that the enzymes function extracellularly to cleave polysaccharide to their component sugars. There is evidence that β-glucosidase enzymes are located extracellularly in soil, having cellulase activity and that they can be detectable using pNP analogues, the assay used in our study (Hope and Burns, 1987).

The mean annual temperature of exposed fellfield soil is 1.1°C (Wynn-Williams, 1996) and a temperature-activity relationship was established in order to convert laboratory data to the field.

5.2.3.1   *Relationship Between Temperature and Polysaccharidase Activity*

Whole soil activities of four enzymes were assayed at 5 C° intervals between 0 and 37°C (Figure 5.4a and b). There was a peak of activity at around 5-10°C (α-D- and β-D-glucosidase) or 5-15°C (α-D- and β-D- galactosidase) with an increase from 20 to 37°C for all four enzymes. These temperature-activity profiles are similar to that found for photosynthesis and heterotrophic incorporation of bicarbonate in the cryptoendolithic microbiota from an Antarctic cold desert (Vestal, 1988). Interestingly the two peaks of photosynthetic activity were 5 and 15°C which may reflect the lower basal temperature of the continental site of that study, with the normal temperature range of the rock surface of -22 to +5°C (December to January; Friedmann *et al.*, 1987).

The temperature response of polysaccharidase enzymes in fellfield soil does not follow directly the growth of the 25 bacterial isolates surveyed in Chapter 3. The growth rates of the heterotrophic community respond in a similar psychrotrophic manner to the

phototrophic community, suggesting a close tie between the two communities. There is broad agreement that the fellfield bacterial community comprises mostly psychotrophic members (Ellis-Evans and Wynn-Williams, 1985; Upton and Newell, 1989), supported by this study (section 3.2.3) and that the photosynthetic rates of members of the microscopic phototrophic communities have similar growth rate patterns, with optima around 20°C (Davey, 1991b).

The results suggest that psychrophilic (peak at 5-15°C) and mesophilic (activity peak greater than 35°C) forms of the four enzymes exist and may be a result of selection of a bacterial community capable of responding to the diurnal temperature fluctuations at Signy Island. However, given the paucity of psychrophilic isolates both in this study and that of others (Upton and Nedwell, 1989), the two temperature-related peaks may reflect two forms of an enzyme being synthesised at different temperatures.

Interestingly, the Antarctic lichens, Lecoanora tephroeceta and Lecidea sp. have also been recorded to have two optima for photosynthesis at 7°C and 15°C, and 3°C and 19°C respectively (Schofield and Ahmadjian, 1972). These authors speculated that activity at the higher temperature is a relic from a more mesophilic past, either when the Antarctic was warmer or from the lichen's habitat before it colonised Antarctica. However, the rates of photosynthesis and respiration in maritime Antarctic mosses have revealed no specialisation to the Antarctic environment (Convey, 1994).

The results from the laboratory assay conducted at 30°C have been transformed to their corresponding activity at a mid-summer temperature at Signy Island of 5°C. This relationship has also been used for extrapolations of microbial respiration at a Signy Island site (Wynn-Williams, 1984). However, the activity of the enzymes, with the exception of β-D-galactosidase, were not significantly different between 5 and 30°C (Figure 5.4a and b) and so the activity of the laboratory assay was used to calculated seasonal activity in the field. The activity of β-D-galactosidase in the field was taken as being 67.8% of the assay activity (Figure 5.4b). The reason for the similar activities of the enzymes at 5 and 30°C is probably because of the presence of a community of enzymes with a range of activity optima which combine to give the overall recorded activity. For example, soil with two enzymes with optima at 5°C and 15°C would give a high activity at 5°C (from the more psychrophilic enzyme) and at 15°C (from the psychrotrophic enzyme) with the overall activity at 10°C being the same (both enzymes being half as active at this temperature).

5.2.3.2   Potential Release of Saccharides in Polygons WW2 and WW5

The seasonal enzyme activity (Table 5.5) serves to illustrate the potential release of low molecular mass saccharides during the duration of the study. The effect of climate change, as based on total potential ATP production, is to increase soluble carbon available for metabolism from 1.65 x (WW 5) to 3.2 x (WW2) in comparison with the non-warmed soil (Table 5.5).

The figures for release of saccharide have been converted into ATP, based on a 60 % conversion of hexose use for fuelling pathways (Cheshire et al., 1969; Figure 5.3b) and of one mole of hexose into 1.076 moles of ATP (section 5.2.1.2). As the polysaccharidase enzymes would be hydrolysing their high molecular weight insoluble substrates extracellularly, the activity of these enzymes can function independently of microbial metabolism. Hence the figures for ATP produced from the released hexose greatly exceeds that measured by the dehydrogenase assay (e.g., 100 x greater in cloche-covered WW2 soil). It is possible, however, that actual hydrolysis of polysaccharide in the field may exceed the rate of hexose uptake by the microbiota resulting in residual hexose being washed out of the fellfield environment and contributing to the nutrient flow into freshwater lakes of Signy Island hypothesised by Ellis-Evans and Wynn-Williams (1985).

Table 5.5   Effect of climate change on potential seasonal release of hexose from soil polysaccharide and on subsequent potential energy made available for microbial respiration.

Polygon	Treatment	Potential Hexose Release by Polysaccharidase Enzymes					Total potential ATP production[3]
		α-D-gluco-sidase	β-D-gluco-sidase	α-D-galacto-sidase	β-D-galacto-sidase	Total	
		(μmol hexose g^{-1} soil 53 days^{-1})					(μmol ATP g^{-1} soil 53 days^{-1})
WW 2	exposed	15.534	21.901	8.815	12.321	58.571	37.81
WW 2	cloche [1]	33.296	78.514	33.069	44.673	189.552	122.37
		(μmol hexose g^{-1} soil 58 days^{-1})					(μmol ATP g^{-1} soil 58 days^{-1})
WW 5	exposed	6.576	19.105	4.491	9.528	39.700	25.63
WW 5	cloche [2]	11.484	31.713	7.436	15.173	65.806	42.48

[1]   Cloche on polygon WW2 provided an average increase in summer temperature of +3.78 C° and blocked the transmission of UV-B radiation (Wynn-Williams, 1996).

[2]   Cloche on polygon WW5 provided an average increase in summer temperature of +2.22 C° and was transparent to UV-B radiation (Wynn-Williams, 1996).

[3]   Based on a conversion of 60% of hexose used for fuelling (respiration) with the remainder for biomass production.
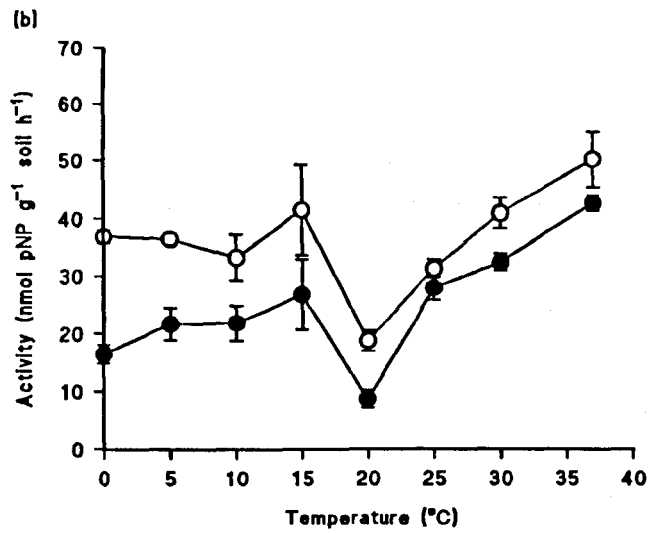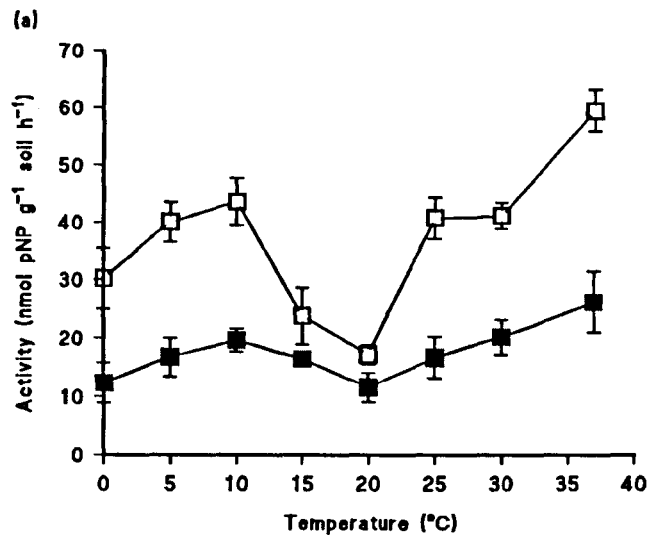
Figure 5.4 Effect of temperature on activity of (a) α-D-glucosidase (■), β-D-glucosidase (□) and (b) α-D-galactosidase (O), β-D-galactosidase (●) in Antarctic fellfield soil. Standard error bars are plotted within the symbol where not visible.

6. MICROBIAL RESPONSE TO SEPARATE GLOBAL WARMING SIMULATION OR UV-B PROTECTION AT TWO ANTARCTIC FIELD SITES

6.1 Introduction

There is little experimental evidence to indicate how Antarctic terrestrial microbial communities will respond to global warming and the effect of elevated incident UV-B beneath the ozone hole. Studies on Arctic habitats indicate that global warming will change the composition of the plant community (Chapin *et al*, 1995) and will increase bacterial decomposition which may release carbon from high-latitude tundra sites (Callagan and Jonasson, 1995). In the Antarctic, experimental manipulation of climate (to simulate global warming and protect from UV-B) has given rise to large increases in fellfield cyanobacteria and revealed a considerable bank of moss propagules at Jane Col, Signy Island (Smith, 1993; Wynn-Williams, 1996). Foister *et al.* (1993) recorded significantly increased bacterial numbers and enzyme activities beneath such cloches. Chapter 4 of this thesis reports increased size, metabolic activity and polysaccharidase activity of the bacterial community beneath cloches at two such sites throughout the summer growing season.

The cloches used in all previous studies (except at one site, WW5; Chapter 4) combine two aspects of climate change: they elevate soil temperature by about 3 C° and they block the transmission of incident UV-B radiation (Wynn-Williams, 1992b). The use of such cloches only provides information on the microbial response to global warming in the absence of UV-B radiation. However, Kennedy (1995a) argued that both global warming and UV-B could change the species composition of Antarctic terrestrial communities. Thus, the change in cyanobacterial dominance from *Phormidium* spp. to *Nostoc* spp. beneath cloches (Wynn-Williams, 1996) can be attributed to either warming, protection from UV-B radiation or a combination of both. In addition, the two aspects of climate change may have a contrasting influence on ecosystem productivity (i.e. elevated temperatures may increase productivity whilst increased incident UV-B may decrease productivity; Kennedy, 1995a), which would be masked in such combined-cloches.

New cloches were installed at Jane Col polygons (1988) and were designed to simulate single aspects of climate change by either warming soil under constitutive UV-B flux or to block UV-B and maintain ambient temperature. This chapter reports an investigation into the microbial response of communities beneath both kinds of cloche. Computer-aided

image analysis was used to observe the size and structure of the cyanobacterial community, and bacterial enumerations and enzyme assays were performed to determine the relationship between microbial phototrophic and heterotrophic activity under the two, separate, aspects of climate change.

6.2   Results and Discussion

Soil samples were taken from two polygons (WW 4 and WW10), at the Jane Col plateau, from inside and adjacent to climate-changing cloches. The cloche on polygon WW4 consisted of a perspex sheet on stilts which blocked the transmission of UV-B radiation, whilst the cloche on polygon WW10 consisted of a perspex box which increased soil temperature by 2.22 C° (without altering incident solar radiation). In addition to the primary effect of the cloches, a number of secondary effects has been predicted by Wynn-Williams (1992b). They include a reduction in severity of freeze-thaw cycles, reduction of propagule input, an extension of the growing season and a reduction of rain splash and snow accumulation relative to exposed soil under the cloche type on polygon WW 10. Soil beneath the cloche on WW 2 has decreased rain splash. Such secondary effects are discussed in Chapter 7. A UV-screening cloche was reported to increase soil temperature by 1.1 C° during a 73 day period in the summer (Wynn-Williams, 1996). It would have been advantageous to place both cloches on the same polygon to allow direct comparisons of the microbial response, however, the size of the polygons (dia c1 m) prevented this. Samples were taken twice during each of two successive growing seasons. The 1993-4 growing season is henceforth referred to as Season 1 and the 3-1-94 sampling time is referred to as Spring 1 and the 4-2-94 sampling time as Summer 1. Correspondingly, the 1994-5 season is referred to as Season 2 and sampling time 10-12-94 is referred to as Spring 2 and the 13-2-95 sample as Summer 2.

6.2.1 Field Observations from Polygon WW4: Effect of Screening of Constitutive Incident UV-B Radiation Under Ambient Temperature

6.2.1.1 Increased Cyanobacterial Colonisation

There was a significantly (Students t-test, $P < 0.01$) larger cyanobacterial community beneath the UV-screening cloche, compared to exposed soil, at both times during Season 1 (e.g. 8.38% cover in UV-screened soil compared to 5.00% in exposed soil at Spring 1 and 7.78% and 3.98% at Summer 1, respectively; Table 6.1), with no significant difference between exposed and covered soil in the sample during Season 2. This indicates that UV-B radiation limits the growth of the cyanobacteria. UV-B radiation is known to cause damage to DNA, proteins and other biological molecules and can lead to the formation of toxic radicals (Caldwell, 1981; Karentz, 1991). Photosynthesis is also particularly sensitive to UV-inhibition and the pigments of the fellfield cyanobacteria become bleached with prolonged exposure (Häder and Worrest, 1991; Garcia-Pichel *et al.*, 1992, 1993). Removal of UV-B is likely to have increased the photosynthetic rate of the cyanobacteria through decreased photoinhibition. Thus, the protection from UV-B provided by cloche-cover would stop the direct damage caused by UV and would result in increased primary production, total biomass and area colonised by the cyanobacteria. Comparable UV-screening experiments have been carried out on the marine ecosystem of the Southern Ocean and have demonstrated that constitutive levels of incident UV-B radiation can reduce primary production by phytoplankton by 10 - 20% (Helbling *et al.*, 1992; Holm-Hansen *et al.*, 1993).

UV-B exposure may also induce the production of UV-defence pigments (section 1.2.4) which are likely to cause a significant decrease in productivity. For example, in photoinhibited cells of the algae *Anacystis nidulans,* up to 10% of cellular protein synthesis was required to repair the D1 protein of photosystem II (Raven and Samuelsson, 1986). Antarctic mosses also respond to increased levels of UV-B by diverting primary production to the synthesis of flavenoids (Markham *et al.,* 1990). It is probable that the indigenous cyanobacteria of the WW4 polygon are also diverting resources to the production of UV-defence compounds: the dominant cyanobacterium *Phormidium autumnale* contains phycoerythrin, a UV and visible light protection pigment and UV-induced damage repair systems are known to exist in cyanobacteria related to those at fellfield polygons (O'Brien and Houghton, 1982; Levine and Thiel, 1987).

It is interesting to note that the dominant cyanobacteria, *Phormidium autumnale*, has been reported to be highly sensitive to UV-B (Häder, 1984). This cyanobacterium appears to avoid UV-B radiation by moving down the soil profile, rather than by screening from, or repairing UV-damage (Häder, 1987). Thus, the increased size of the phototrophic community beneath the cloche may also have resulted from the established cyanobacteria responding to the absence of UV-B by moving closer to the surface of the soil. The greater amount of photosynthetically active radiation (PAR) nearer the soil surface would then increase photosynthetic rates and, in turn cyanobacterial productivity and community size. Microscopic examinations did not, however, provide evidence of any change in the position of the cyanobacteria relative to the soil surface.

6.2.1.2   *Compositional Changes within the Cyanobacterial Community*

UV-screening had no effect on the composition of the cyanobacterial community (Table 6.1). Mean breadth of filaments, total numbers per area colonised and volume per area colonised were similar in exposed and covered soil (Table 6.1). This assumes that changes in the cyanobacterial population structure would be observable, i.e. morphological, as was the case for the change in dominance from filamentous *Phormidium* (Plates 6.1 and 6.2) to aseriate *Nostoc* spp. in a previous cloche experiment (Wynn-Williams, 1996). The similarity between the breadth data (section 2.7.2) does suggest that if species composition had changed, the emergent species have a similar morphology to that of *Phormidium*.

The reason for a lack of observable change may be because the dominant cyanobacteria survive sub-optimally under the constitutive levels of incident UV-B and are able to remain competitive when it is removed. Indeed, the cyanobacterial communities have relatively low species diversity (Davey and Rothery, 1993) and Wynn-Williams (1996) observed increases in *Phormidium* spp. (up to 17 x) after three years beneath a combined warming and UV-screening cloche suggesting that this species can take advantage of UV-screening. Thus, UV-B resistance may have a minor role in selection of cyanobacterial species, as *P. autumnale* is sensitive to UV-B, and behavioural avoidance (section 1.2.4) may play an important part in dominance. It is conceivable that even without UV-B, such a strategy would be competitive by enabling survival of high visible irradiance.

6.2.1.3 Seasonal Differences in the Cyanobacterial Community

A reduction in the size of the cyanobacterial community was evident between the two seasons (Figure 6.1). The decline was particularly noticeable in the UV-screened site, with 4.45% of the soil surface colonised in Summer 2, compared with 8.39 and 7.78% in the two samples from Season 1. Both communities, however, have the same composition: mean breadth and numbers of colonists per area colonised are similar. Davey (1991a) recorded significant changes in the total size of the cyanobacterial community at a Jane Col polygon between January 1987 and March 1988. The largest disturbance to the community occurred during the spring thaw when a flush of melt water is thought to have caused the dramatic decline in all members of the algal community. Such seasonal washout led Davey (1991a) to suggest that the remaining cyanobacteria acts as an inoculum which rapidly increases in size, following the thaw, to recolonise the polygon. This hypothesis can explain the difference in size of the cyanobacterial community between growing seasons: a smaller degree of colonisation during one season may be the result of reduced regrowth from the spring washout.

Spring washout of cyanobacterial colonists may limit the accumulation of organic matter in polygon mineral soils. The maturation of the soil is dependent on successful colonisation by cyanobacterial and bacteria which stabilise the soil and ultimately lead to moss colonisation (Wynn-Williams, 1993). Given that these primary colonists suffer a significant annual biomass removal, polygon maturation would be greatly slowed. It is also interesting to note that constitutive levels of UV-B are greater during the summer than during the spring (increased solar zenith angle results in greater solar radiation; Jones and Shanklin, 1995) and so the spring recovery period occurs under lower incident UV-B. However, screening the soil from even the low spring levels of UV-B had a significant effect on the percentage of the soil colonised by the cyanobacteria (Spring 1, Table 6.1). Thus, as Vincent and Quesada (1994) argue, the advent of the springtime ozone hole may prove to be of particular detriment to Antarctic habitats as colonists will receive summer doses of UV-B, under the springtime ozone hole, during the so called spring 'recovery period'.

6.2.1.4   Biomass Ratio of Cyanobacteria to Bacteria

The ratio of cyanobacterial biomass to bacterial biomass was calculated from total volume of cyanobacteria (direct observations using epifluoresence microscopy; section 2.8.1) and from viable counts of bacteria (spread plate counts; section 2.3.1). Bacterial counts were converted into volume by assuming that the average dimensions of bacterial cells in the soil were the same as the average of the 25 bacterial isolates characterised in Chapter 3. It is known that bacterial volume is not a constant morphological property and that cell size is dependent on growth conditions: Van Veen and Paul (1979) reported a five-fold variation in the volume of *Arthrobacter globiformis* depending upon the nutrient status of the growing environment. However, in the present study bacterial volume was used to show relative changes in the biomass ratio of cyanobacteria and bacteria between UV-exposed and UV-screened soil and so accurate measurements of *in situ* bacterial volume were not needed. Instead, it was assumed that the average *in situ* bacterial volume was not significantly different between exposed and UV-screened soil samples. Bölter (1995) analysed direct microscopic observations of bacteria in Antarctic soil and found the most important parameters affecting bacterial size to be soil water and organic matter content. However, when taking such parameters into account the contribution of short bacterial rods (the dominant morphology of bacterial isolates from polygon WW5; Chapter 3) to the overall bacterial community only varied between 40 and 55% in the former study. Thus, it may be expected that the distribution of bacterial size classes may be similar between UV-exposed and UV-screened soil, given the small difference in soil water, saccharide and carbon contents (soil saccharide was only significantly greater in UV-screened soil at Spring 2, and soil carbon only significantly greater in Spring 1 in UV-screened soil compared to UV-exposed soil; Table 6.2).

The volume of algae in a unit surface area was compared to the volume of the bacteria beneath the same surface area to a depth of 25 mm, the depth of the soil sample cores (section 2.8.3). Van Veen and Paul (1979) demonstrated bacterial and fungal specific gravity changed in relation to moisture stress, but given that both cyanobacteria and bacteria would be expected to be under the same moisture stress (in the same upper 25 mm of polygon soil), the specific gravity was taken to be the same. Thus, the ratio of volume was directly converted into a ratio of biomass.

UV-screening had an effect on the biomass ratio in two out of the three samples analysed. The ratio between cyanobacteria and bacteria was 30:1 in UV-exposed soil and 20:1 in UV-screened soil at Spring 1 and 32:1 in UV-exposed and 9:1 in UV-screened soil at Summer 2 (Table 6.1). Because of a lack of quantitative studies on the effect of

constitutive levels of UV-B on microbes it is difficult to conclude why the bacterial community increased, relative to the cyanobacterial community, under the UV-screen. It is possible that the constitutive UV-B flux is to some extent inhibitory to the bacterial population and the greater size of the community beneath the screen reflects a greater survival of the bacteria in the absence of UV radiation. However, if UV-B was inhibiting the bacterial community a change in the frequency of pigmented isolates may have be expected under the UV-B screen. Pigmented bacteria contain carotenoid pigments, which quench excess solar and UV energy (Buckley and Houghton, 1976; Pearl, 1984). Thus, pigmentation can be expected to confer a selective advantage if UV-B was a significant limiting factor to the bacteria. The lack of a change in the frequency of isolation of pigmented bacteria (Table 6.2) suggests that the selective advantage of pigmentation is not influenced by the removal of UV-B. This, in turn, suggests that UV-B did not have a significant role in selecting pigmented bacteria and may therefore not have a significantly inhibitory effect on the bacterial populations.

It is possible that the increase in bacteria, relative to cyanobacteria, occurred because cyanobacterial productivity increased under the UV-B screen. The cyanobacteria had an increased community size beneath the UV-B screen (Table 6.1) because of the absence of inhibitory UV effects, as discussed above. It is also likely that the cyanobacteria increased productivity beneath the screen given that photosynthesis has been demonstrated to be inhibited by the constitutive levels of incident UV-B at the Southern Ocean at a latitude close to that of Signy Island (Holm-Hansen et al., 1993). Thus, a greater amount of product, per unit biomass of cyanobacteria, would enter the microbial domain. This would result in the increased bacterial community, relative to the cyanobacteria, observed under the UV screen.

The direct comparison can be criticised because the cyanobacterial community was measured by direct microscopic observation, whereas the bacterial community was measured by spread plate counts. It is accepted that spread plate counts underestimate total bacterial numbers in comparison to direct counts (Atlas and Bartha, 1987). However, the direct observations of the cyanobacterial community were made by epifluoresence microscopy with the photosynthetic pigments of the cyanobacteria (i.e., chlorophyll, phycocyanin, phycoerythrin) being visualised by their own autofluoresence (Wynn-Williams, 1988). Soil chlorophyll has been used to measure the viable algal biomass in soil, as chlorophyll relates well to viable phototrophic biomass (Davey, 1991a). Thus, such direct observations of the cyanobacterial community measure viable phototrophs. Spread plate counts, however, also underestimate bacterial numbers because clumps of viable bacteria will form one colony forming unit and isolation media and incubation

temperatures will not favour the growth of all bacterial types (Atlas and Bartha, 1987). However, this inherent limitation of spread plate counts can be reconciled by the fact that surface observations of the cyanobacterial community also underestimates the total community size (although not necessarily to the same extent): a small proportion of cyanobacteria have been reported to be deeper in the soil profile, presumably moved there by movement of soil fines during freeze-thaw heave (Davey and Clarke, 1991, 1992).

6.2.1.5   Size and Activity of the Bacterial Community

As argued above, the lower ratio between cyanobacteria and bacteria caused by UV-screening suggests a greater flow of energy from cyanobacteria to bacteria. In turn, greater bacterial enzyme activities would be expected. However, although bacterial numbers are consistently higher beneath the UV-screening cloche, the majority of enzyme activities were not significantly greater (Figures 6.1 and 6.2). There are three exceptions: β-galactosidase activities were greater in UV-screened soil at Spring 1; and ETS and β-glucosidase activities were greater at Spring 2 (Figures 6.1d and 6.2d). The general lack of elevated enzyme activities may have been because the increased photosynthate may have entered the bacterial domain at a slow rate. Such a small increase in nutrients may not have stimulated increased enzyme synthesis but would have resulted in an elevated levels in situ activity of the existing enzymes. This would have lead to increased bacterial growth leading to higher bacterial numbers. Thus, without an increase in enzyme numbers, the enzyme assay would not reveal a greater in vitro enzyme activity. This illustrates a limitation of the enzyme assays performed in this study - the enzyme activity measured is a reflection of the number and substrate affinity of the enzymes present in the soil at the time of sampling, and is not a direct measure of in situ enzyme activity. There have been attempts made to estimate in situ microbial activities in the Antarctic by measuring the decomposition of cellulose strips placed in soil (Wynn-Williams, 1980; Walton, 1985) and by monitoring the disappearance of sugars added to soil (Melick et al., 1994). However, such approaches are not possible when working on Antarctic samples removed from the field, frozen and defrosted prior to analysis in the laboratory.

An alternative explanation is that as only two samples were taken during each year, periods of increased enzyme activities in UV-screened soil may have occurred between sampling times. The nine samples taken during the 1992-3 season from polygon WW5 illustrate this point (Chapter 4). Although bacterial numbers were consistently greater

beneath the cloche on this polygon, enzyme activities were not greater in every sample and activities in exposed soil were occasionally higher (e.g., β-glucosidase activities were greater in exposed soil at day 28; Figure 4.7b).

The lack of a seasonal increase in soil carbon or saccharide between Season 1 and Season 2 supports the hypothesis that the spring washout has a significant effect on the long-term maturation and carbon-accumulation of the soil. The lower saccharide level in Season 2 may be the result of a reduced regrowth by the cyanobacteria after the washout, compared to Season 1. Cloche-cover also appeared to have a minimal impact on soil water content (Table 6.2). This was expected given that horizontal water movement through the soil polygon would quickly cancel any protection from precipitation.

Table 6.1 Effect of screening of constitutive UV-B radiation on the size and morphological characteristics of cyanobacteria in soil polygon WW4. Samples were taken six (Season 1) and seven years (Season 2) after the cloche was installed.

| Parameters | Treatment | Season 1 (1993-4) | | Season 2 (1994-5) | |
		Spring 1	Summer 1	Spring 2	Summer 2
Colonisation (% area)	exposed	5.00 α (2)	3.98 αβ	ND	3.26 β
	cloche (1)	8.39 χ	7.78 χ	ND	4.45 αβ
Numbers (3)	exposed	69.98 α	92.78 β	ND	84.60 αβ
	cloche	156.52 χ	170.76 χ	ND	121.98 χ
Numbers per % area colonised	exposed	14.0 α	23.3 χ	ND	25.9 χ
	cloche	18.7 β	21.9 χ	ND	27.4 χ
Breadth (mean - μm)	exposed	3.92 α	3.38 β	ND	3.16 β
	cloche	3.50 β	3.18 β	ND	3.11 β
Total volume (3) (μm^3)	exposed	133,002 α	87,614 β	ND	82,572 β
	cloche	231,944 χ	198,884 χ	ND	108,128 β
Volume per % area colonisation	exposed	26,600 α	22,180 α	ND	25,330 α
	cloche	27,650 α	25,560 α	ND	24,300 α
Biomass ratio(4) (cyanobacteria: bacteria)	exposed	30 : 1	15 : 1	ND	32 : 1
	cloche	20 : 1	18 : 1	ND	9 : 1

(1)   Cloche on polygon WW4 blocked the transmission of UV-B radiation at ambient temperature (Wynn-Williams, 1992b).

(2)   Data for each parameter grouped according to significance (Students t-test, $P < 0.01$).

(3)   Per field of view of $1.8 \times 10^5 \ \mu m^2$.

(4)   Biomass ratio based on cyanobacterial volume (calculated from epifluoresence microscopy) and bacterial volume (calculated from viable bacterial counts; section 2.8.3).

ND   Not determined.

Table 6.2 Soil properties and frequency of isolation of pigmented bacteria from polygon WW4.

Parameters	Treatment	Season 1 (1993-4)		Season 2 (1994-5)	
		Spring 1	Summer 1	Spring 2	Summer 2
Water content (% WHC)	exposed	51.4 α (2)	51.5 α	51.5 α	50.8 α
	cloche (1)	44.3 α	55.0 α	55.1 α	55.3 α
Saccharide (mg g^{-1} soil)	exposed	0.451 α	0.270 α	0.119 β	0.174 α
	cloche	0.487 α	0.340 α	0.266 α	0.371 α
Carbon (mg g^{-1} soil)	exposed	2.35 α	2.75 β	2.70 β	3.05 βχ
	cloche	3.15 χ	3.05 βχ	3.05 βχ	3.35 χ
Nitrogen (3)	exposed	BDL	BDL	BDL	BDL
	cloche	BDL	BDL	BDL	BDL
Pigmented bacteria (frequency)	exposed	63.8 α	61.2 α	64.9 α	63.1 α
	cloche	65.6 α	64.3 α	62.8 α	64.1 α

(1)   Cloche on polygon WW4 blocked the transmission of UV-B radiation at ambient temperature (Wynn-Williams, 1992b).

(2)   Data for each parameter group according to significance (Students t-test, $P < 0.01$).

(3)   Limit of detection = 0.2 mg g^{-1} soil.

BDL   Below detectable limits.
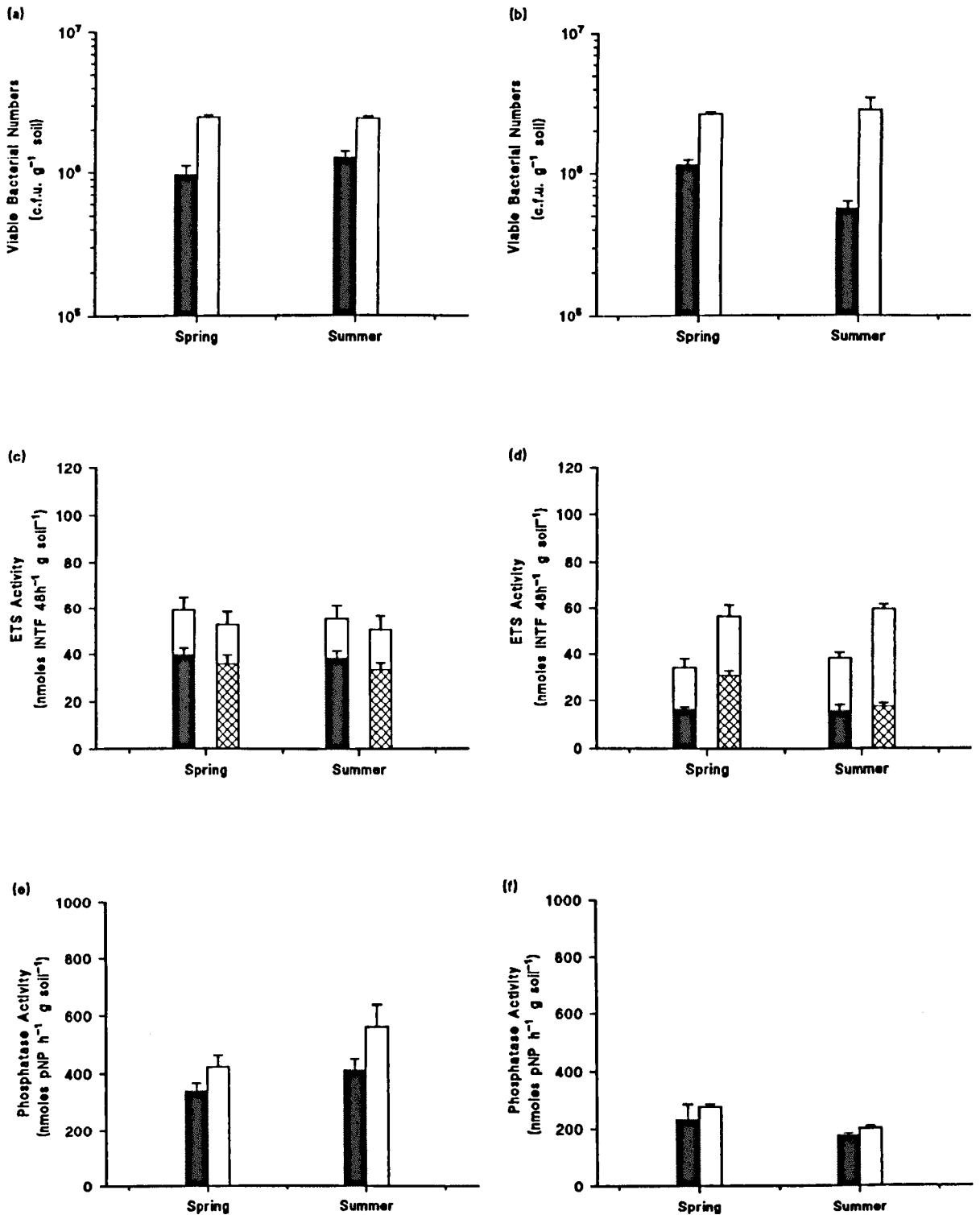
Figure 6.1   Bacterial viable counts at (a) Season 1 (1993-4) and (b) Season 2 (1994-5) from UV-exposed (■) and UV-screened soil (□) at polygon **WW4**. ETS activity at (c) Season 1 and (d) Season 2. Constitutive activity (▨) and substrate-induced activity (□) from UV-exposed soil. Constitutive activity (▨) and substrate-induced activity (□) from UV-screened soil. Phosphatase activity at (e) Season 1 and (f) Season 2 from UV-exposed (■) and UV-screened soil (□). Standard error bars are plotted within the symbol where not visible.
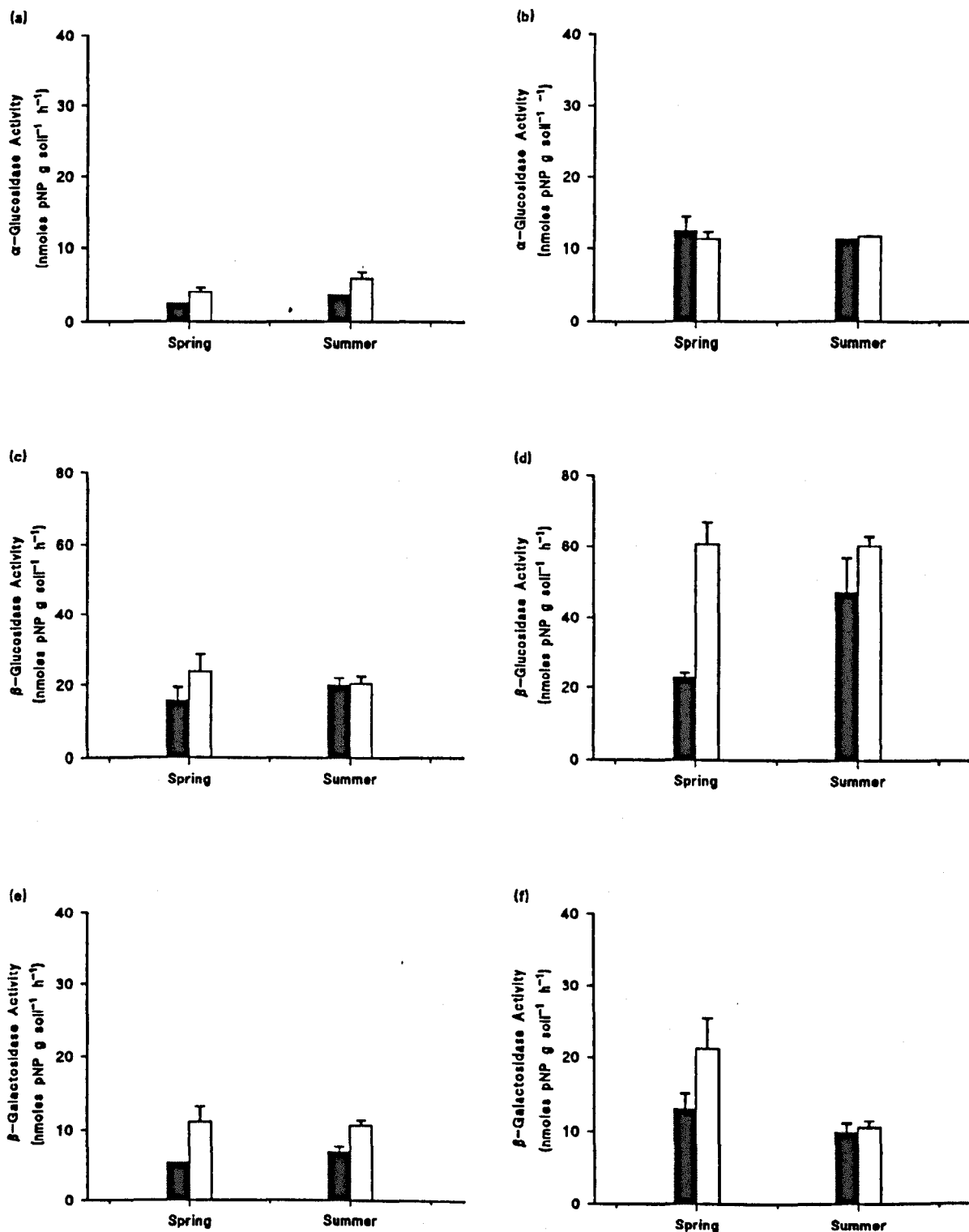
Figure 6.2   α-D-1,4 Glucosidase activity at (a) Season 1 (1993-4) and (b) Season 2 (1994-5) from UV-exposed (■) and UV-screened soil (□) at polygon **WW4**. β-D-1,4 glucosidase activity at (c) Season 1 and (d) Season 2 from UV-exposed (■) and UV-screened soil (□). β-D-1,4 galactosidase activity at, (e) Season 1 and (f) Season 2 from UV-exposed (■) and UV-screened soil (□). Standard error bars are plotted within the symbol where not visible.

6.2.2 Field Observations from Polygon WW10: Effect of Global Warming Simulation

6.2.2.1 Increased Algal Colonisation

The cyanobacterial community at polygon WW10 was analysed at two sampling times (Summer 1 and Summer 2). The sample collected at Summer 1 showed a significantly greater colonisation of the soil surface (18.83%) beneath the global warming cloche compared to exposed soil (3.99%). The colonisation was comparable at Summer 2: 6.56% in exposed soil and 5.54% in cloche-covered soil (Table 6.3). This illustrates the interseasonal differences in cyanobacterial colonisation observed in polygon WW4 (7.78% colonisation in UV-screened soil at Summer 1 and 4.45% at Summer 2; Table 6.1) and supports the hypothesis that the spring washout of algae reduces the cyanobacterial community at the beginning of each season. It would appear from the much lower colonisation in the cloche-covered soil at Summer 2 that the cyanobacterial community did not regrow to the same extent in the two seasons. It is possible that the *Nostoc* spp. which dominated the soil beneath the cloche (see below), are more prone to being washed out than the dominant cyanobacteria of exposed soil. Indeed, given the dramatic impact of the washout, the ability to withstand it may prove to be a highly important selection factor. For example, the intermeshing of cyanobacterial filaments with soil fines may be enable such species to resist the flood of melt water better than the non-filamentous, spherical *Nostoc* spp.

The larger extent of the colonisation beneath the cloche in Summer 1 indicates that temperature may have a significant limiting effect on the cyanobacteria. However, a change in the composition of the cyanobacterial population was also evident under the cloche at both sampling times, with the community being dominated by aseriate *Nostoc* spp, compared with the filamentous species (*Phormidium autumnale* and *Pseudanabaena* spp.; Wynn-Williams, 1996) that dominate exposed soil. This is the same effect as observed by Wynn-Williams (1996) beneath cloches that combined UV-screening and temperature elevation. This change was recorded visually (Plate 6.3), and was evident by larger breadth values (e.g. mean breadth of 3.63 in exposed and $7.79\mu m$ in cloche-covered soil in summer 1) and fewer cyanobacteria per percent area colonised (e.g. 22.5 in exposed and 13.0 in cloche-covered soil in Summer 1; Table 6.3), consistent with a large proportion of larger, spherical cells compared with thinner filaments. Thus, although the percentage colonisation of the soil surface was comparable at Summer 2, the

morphology of the dominant colonists resulted in a larger cyanobacterial volume than in the exposed soil (Table 6.3).

A change in the composition of Antarctic communities under global warming was predicted by Kennedy (1995a). It appears that the fellfield polygon undergoes such a compositional change under simulated global warming, with the same change evident in polygon WW10 in the present study as recorded at two other Jane Col polygons under similar cloche treatment (Wynn-Williams, 1996). It is unclear whether the change in dominance is due to the direct effect of the warming caused by the cloche which would imply that *Nostoc* was more competitive under the cloche conditions (i.e. elevated temperature, reduced diurnal freeze-thaw severity) than *Phormidium*. However, Wynn-Williams (1996) suggested that the presence of moss shoots beneath the cloches indicates that nitrogen limitation may be a possible cause of the success of *Nostoc*. Mosses are efficient at scavenging nitrogen from soil and this would give *Nostoc*, a nitrogen fixer, an advantage under nitrogen limiting conditions. There is however, no direct proof that nitrogen is more limiting beneath global warming cloches as soil nitrogen levels were below detectable limits (0.2 mg g^{-1} soil) in both polygons WW4 and WW10 in the present study, both inside and out of cloches (Tables 6.2 and 6.4). Davey and Rothery (1992) also suggested that cyanobacteria are limited by mineral nitrogen, although this has not been directly demonstrated. However, the use of molecular probes with *Phormidium* has indicated that the N:P ratio may be more important and on occasions limiting (Gapp, 1996). In addition, Arnold (1995) demonstrated sporadic availability of ammonium, the major N source used by *Phormidium*, in fellfield polygons, increasing the possibility of limiting N:P ratios.

6.2.2.2   *Biomass Ratio of Cyanobacteria to Bacteria*

The large difference in the ratio between cyanobacteria and bacteria in the UV-screened polygon WW4 was not observed in polygon WW10 (Table 6.3). Although conclusions must be drawn with care, given two sampling times, the results suggests that the global warming cloche has not affected the productivity of the cyanobacteria. Thus, both bacterial and cyanobacterial communities have increased under the cloche with the resulting larger communities being in similar proportions as in exposed soil. The ratios in cloche-covered and exposed soil in the Summer 1 sample are far greater than recorded for the other samples in either polygon. This again illustrates the variation in the size of the microbial communities. Such constitutive variation in the microbial standing stock was also observed between the bacterial communities in exposed soil from polygons WW2

and WW5, with the latter having a seasonal average ten times greater than the former (Chapter 4).

6.2.2.3   Size and Activity of the Bacterial Community

Bacterial numbers were significantly greater beneath the global warming cloche in all four samples analysed (Figures 6.3a and b). Phosphatase activities were also significantly greater in all samples and polysaccharidase activities were greater in eight out of 12 samples (Figures 6.3e and f; Figure 6.4). These results indicate greater bacterial activity under global warming conditions, confirming those presented in Chapter 4 on bacterial changes beneath a cloche of the same design (on polygon WW5).

Interestingly, neither the constitutive nor substrate-induced ETS activities were elevated in any sample (Figure 6.3c and d). Given that this assay measures bacterial metabolic activity, it can be concluded that the number of active bacteria were similar in cloche-covered and exposed soil. This contradicts the results from the other enzyme assays and the bacterial counts. However, extracellular phosphatase and polysaccharidase enzymes and some bacteria may persist in the soil for a period after the time when microbial activity caused their production. For example, extracellular enzymes can associate with soil clays and humics and retain their activity after being synthesised and secreted from bacterial cells (Burns, 1977). Bacteria can also persist in soil in a dormant state for considerable periods (Chen and Alexander, 1973; Thompson et al, 1992). ETS activities, however, are only associated with the extant microbial population, as respiratory chain activity is central to oxidative phosphorylation and fuelling pathways (Neidhardt et al., 1990). Thus, the absence of an increase in ETS activities beneath the cloche may mean that any increase in bacterial numbers and enzyme activities had occurred prior to the time of sampling. This again illustrates the limitation of only having two samples per year for this study. However, the small number of samples were taken so as not to deplete the polygon soil reserves and to allow sufficient soil to remain for samples to be taken in future years (section 2.1.3).

As in polygon WW4, the soil water content in WW10 was not significantly different within the cloche compared to outside. However, unlike the UV-screened soil beneath the cloche on polygon WW4, soil carbon and saccharide were not significantly elevated in the cloche covered soil compared to exposed soil at polygon WW10. A similar observation was made on cloche-covered soil at polygon WW5 (Chapter 4). Given the increased

microbial community beneath the cloches at both of these sites, the soil carbon and saccharide might be expected to be elevated compared with exposed soil, especially as the microbiota of fellfield polygons are known to produce extracellular polysaccharide sheath material (Wynn-Williams, 1993; Foister et al., 1993). It would appear that this contribution to soil carbon or saccharide content is too low to be measurable. It is also evident that there is no apparent annual accumulation of carbon beneath the cloche. It can be concluded that, although there was an increase in the size and activity of the microbiota beneath the cloche, there were no long term changes in soil carbon content after six and seven years. In addition, at polygon WW5, after five years of cloche cover, no accumulation of soil carbon, relative to exposed soil, was evident (Chapter 4). Microbial increase in the absence of a significant long-term change in soil carbon content can be explained by the seasonal washout of the cyanobacteria observed by Davey (1991a) and of which there is evidence in the present study.

It is notable that the most dramatic changes under any of the climate-change simulations carried out at this field site have occurred beneath cloches that combine the effects of temperature elevation and UV-screening (polygon WW2, Chapter 4; Wynn-Williams, 1996a). In fact, it was only at polygon WW2, beneath a combined cloche, that there was a significant elevation in soil carbon relative to exposed soil (Students t-test). At polygons WW1 and WW2 Wynn-Williams (1996) reported that the cyanobacteria beneath combined cloches showed a greater increase in percentage colonisation of the soil, relative to exposed soil, after three and six years than reported for either polygons in this chapter. Thus, the combination of UV-screening and elevated temperature seem to have an additive effect at such sites. Indeed, Kennedy (1995a) argues that the stimulatory effect of future global warming may be counteracted by elevated UV-B levels beneath the ozone hole. In the present study, it appears that constitutive UV-B does inhibit the cyanobacteria and that it does offset the microbial response to simulated global warming. This suggests that, in habitats beneath the ozone hole, the inhibitory effect of elevated UV-B may well play an increased role in offsetting any increased ecosystem productivity brought about by future warming.

Table 6.3   Effect of global warming simulation on the size and morphological characteristics of cyanobacteria in soil polygon WW10. Samples were taken six (Season 1) and seven years (Season 2) after the cloche was installed.

Parameters	Treatment	Season 1	(1993-4)	Season 2	(1994-5)
		Spring 1	Summer 1	Spring 2	Summer 2
Colonisation (% area)	exposed	ND	3.99 α (2)	ND	6.56 β
	cloche (1)	ND	18.63 χ	ND	5.54 β
Numbers (3)	exposed	ND	89.66 α	ND	149.40 β
	cloche	ND	27.24 χ	ND	49.64 δ
Numbers per % area colonised	exposed	ND	22.5 α	ND	22.8 α
	cloche	ND	13.0 β	ND	8.9 χ
Breadth (mean - μm)	exposed	ND	3.63 α	ND	3.17 β
	cloche	ND	7.79 χ	ND	5.24 δ
Total volume (3) (μm^3)	exposed	ND	165,474 α	ND	173,910 α
	cloche	ND	6,925,260 β	ND	350,732 χ
Volume per % area colonisation	exposed	ND	41,470 α	ND	26,510 β
	cloche	ND	371,720 χ	ND	63,310 δ
Biomass ratio(4) (cyanobacteria: bacteria)	exposed	ND	49 : 1	ND	20 : 1
	cloche	ND	60 : 1	ND	17 : 1

(1)   Cloche on polygon WW10 provided an average increase in summer temperature of +2.22 C° and was transparent to UV-B radiation (Wynn-Williams, 1996a).

(2)   Data for each parameter group according to significance (Students t-test, $P < 0.01$).

(3)   Per field of view of 1.8×10^5 μm^2.

(4)   Biomass ratio based on cyanobacterial volume (calculated from epifluoresence microscopy) and bacterial volume (calculated from viable bacterial counts; section 2.8.3).

ND   Not determined.

Table 6.4   Soil properties from polygon WW10.

Parameters	Treatment	Season 1 (1993-4)		Season 2 (1994-5)	
		Spring 1	Summer 1	Spring 2	Summer 2
Water content (% WHC)	exposed	61.9 α (2)	51.5 β	51.4 β	49.1 β
	cloche (1)	52.2 β	55.0 β	51.4 β	51.3 β
Saccharide (mg g^{-1} soil)	exposed	0.096 α	0.156 α	0.237 α	0.298 α
	cloche	0.195 α	0.197 α	0.163 α	0.213 α
Carbon (mg g^{-1} soil)	exposed	2.20 α	2.05 α	2.35 α	2.40 α
	cloche	2.40 α	2.20 α	2.75 β	2.65 αβ
Nitrogen (3)	exposed	BDL	BDL	BDL	BDL
	cloche	BDL	BDL	BDL	BDL

(1)   Cloche on polygon WW10 provided an average increase in summer temperature of +2.22 C° and was transparent to UV-B radiation (Wynn-Williams, 1996a).

(2)   Data for each parameter group according to significance (Students t-test, P < 0.01).

(3)   Limit of detection = 0.2 mg g^{-1} soil.

BDL   Below detectable limits.
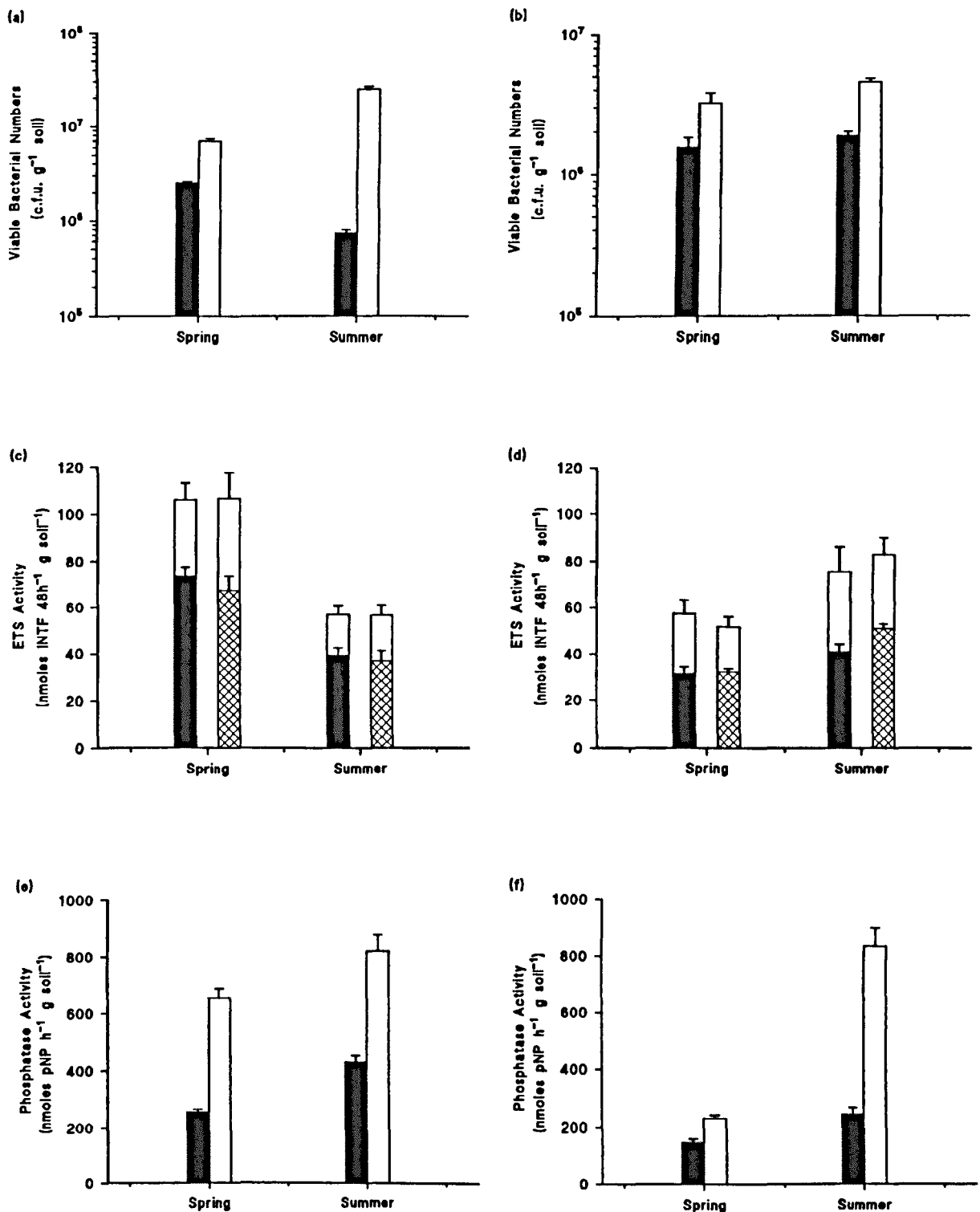
Figure 6.3 Bacterial viable counts at (a) Season 1 (1993-4) and (b) Season 2 (1994-5) from exposed (■) and cloche-covered (warmed) soil (□) at polygon **WW10**. ETS activity at (c) Season 1 and (d) Season 2. Constitutive activity (▨) and substrate-induced activity (□) from exposed soil. Constitutive activity (▨) and substrate-induced activity (□) from cloche-covered soil. Phosphatase activity at (e) Season 1 and (f) Season 2 from exposed (■) and cloche-covered soil (□). Standard error bars are plotted within the symbol where not visible.
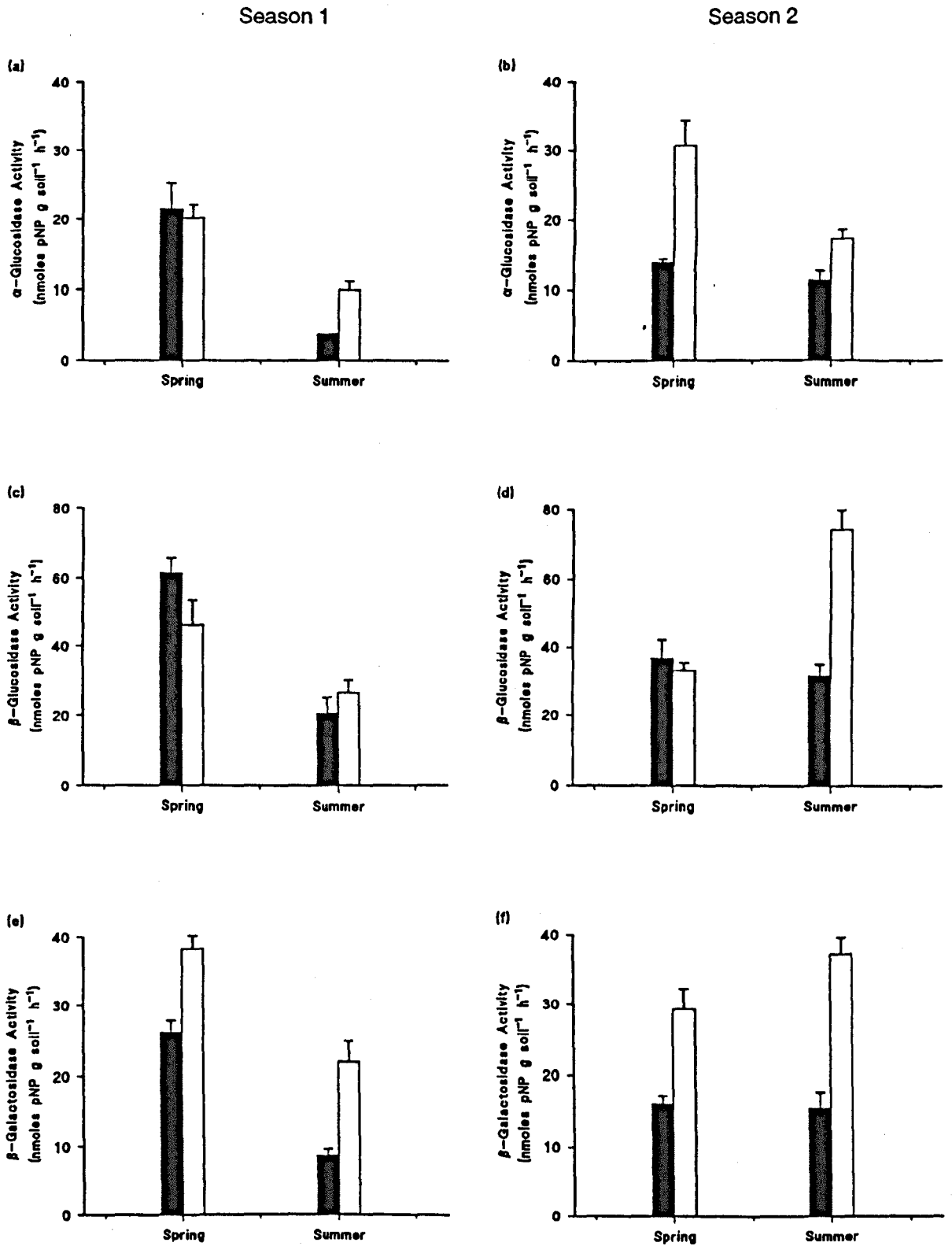
Figure 6.4 α-D-1,4 Glucosidase activity at, (a) Season 1 (1993-4) and (b) Season 2 (1994-5) from exposed (■) and cloche-covered (warmed) soil (□) at polygon **WW10**. β-D-1,4 glucosidase activity at, (c) Season 1 and (d) Season 2 from exposed (■) and cloche-covered soil (□). β-D-1,4 galactosidase activity at, (e) Season 1 and (f) Season 2 from exposed (■) and cloche-covered soil (□). Standard error bars are plotted within the symbol where not visible.

25 μm

Plate 6.1   Filaments of *Phormidium autumnale* on fines from exposed soil at polygon WW4 as observed by epifluoresence microscopy.
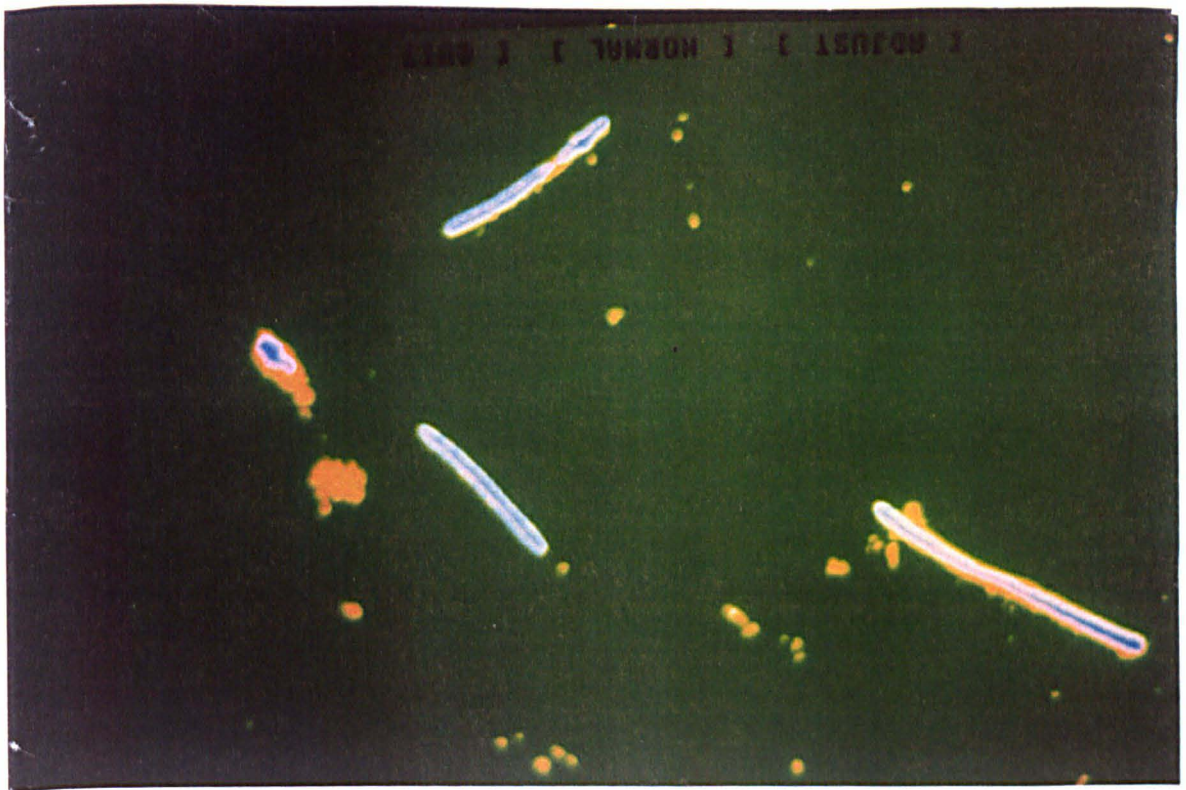
25 µm

Plate 6.2   Computer-generated pseudocolour display of the filaments in Plate 6.1. This display was subsequently used for image analysis (section 2.8.2).

25 µm

Plate 6.3   Aseriate *Nostoc commune* cells present on fines from polygon WW10 after six years of global warming simulation.

7. GENERAL DISCUSSION

The accumulation of a body of evidence supporting anthropogenically-mediated climate change has lead to concern over the response of biological systems. Areas of specific interest include: the response of plants to elevated temperature and the fertilisation effect of increased CO_2 levels (Gifford, 1992); the effect of increased temperatures on the microbial release of soil carbon (Callaghan and Jonasson, 1995); and the reduction of productivity, particularly of the Antarctic marine ecosystem, beneath the ozone hole (Behrenfeld et al., 1993). This thesis reports a study of the response of microbial communities in Antarctic fellfield soil to simulation of global warming and to the blocking of incident UV-B radiation.

Antarctic ecosystems are particularly sensitive to climate change. Although Morgan et al. (1991) proposed that a warmer Antarctic may result in ice-sheet growth due to increased snow-fall, longer-term forecasts predict that warming will cause increased melting of ice-sheets (Hall and Walton, 1992). Indeed, there is evidence of a threat of retreat of the West Antarctic Ice Sheet (Alley and MacAyeal, 1993) and the permanent ice-cover at Signy Island has already been reduced by 35% between 1949 and 1989, under an increase of approximately 1 C° (Smith, 1990). There is also a consensus of opinion that increased global temperatures will result in net loss of carbon from soils due to increased microbial decomposition rates. Such an effect is of importance to polar habitats given the potential of a small temperature increase to elongate growing seasons, to increase water availability and to directly increase microbial activity. In addition, the annual springtime ozone depletion above the continent is likely to be having an effect on the productivity of the Southern Ocean (Helbling et al., 1992; Holm-Hansen et al., 1993).

Smith (1990) considered the islands of maritime Antarctic (including Signy Island) to warrant particular attention from an ecological viewpoint: the ice-cover is relatively thin and, as mentioned above, is already retreating. Glacial retreat has lead to the exposure of pristine terrestrial substratum, typified by the fellfield of the Jane Col plateau at Signy Island (Hall and Walton, 1992). The effect of global warming on the colonists of this plateau has been subject to attention of the British Antarctic Survey and has lead to investigations using plastic cloches placed on polygons of this field site in an effort to mimic the effect of climate change. These studies have demonstrated that the primary colonists of such fellfields, microscopic algae and cyanobacteria (cyanobacteria), are capable of significant increases in colonisation after a three year period (Wynn-Williams, 1996). In addition, the secondary (and climax), colonists of such sites, mosses such as

Andreaea spp., were conspicuous beneath the cloches, in one case bare mineral fines showed a 95% covering after four years (Smith, 1993).

This project has increased the scope of these first Antarctic climate change experiments by assessing the response of heterotrophic bacterial community and its relationship with the cyanobacterial community. Bacterial numbers and enzyme activities were elevated beneath all cloches that caused soil warming, whilst the results from a UV-screening cloche demonstrated that the incident UV-B radiation at this site (which is not beneath the ozone hole), inhibit the phototrophic community.

7.1   *Experimental Design*

Like the earlier climate change studies at Signy Island, the project was based on analysis of field samples adjacent to and beneath climate-changing cloches within Antarctic fellfield polygons. The cloches used consisted of one of the same design as these previous experiments (polygon WW2) and three others of a modified design (section 2.1.2). The modified cloches were installed in an effort to separate the effects of the earlier cloche design by causing soil warming under incident UV-B (polygons WW5 and WW10) and by screening UV-B under ambient temperature (polygon WW4).

However, cloches (or passive greenhouses - requiring no artificial power to produce the treatment effect) have been criticised for producing artefacts (Kennedy, 1995b). The criticisms include concerns over the ability of cloches to mimic changes to the global circulation model (GCM) predicted under global warming and the presence of secondary effects, for example, changes in humidity and soil water content.

Kennedy (1995c) reported a detailed analysis of the temperatures changes inside a 'combined' cloche (as used on polygon WW2) installed at a polygon on the Jane Col plateau. He found that, although temperature elevation was within the projections of the IPPC when considered as monthly averages during the summer (e.g. +1.5 C° in January, +2.5 C° in February), closer inspection of the diurnal and annual changes revealed four areas where the temperature changes did not mimic projected changes in the GCM. They include (i) GCMs predict that global warming will either be of a similar magnitude throughout the diel cycle, or will result in larger increases at night, thereby reducing diel temperature variation. However, the cloche caused greater temperature elevation during the day (up to +10 C°) with either no elevation or depression of temperature relative to

exposed soil at night, (ii) the rate of change of temperature was greater in the cloche than in exposed soil, (iii) the tops of cloches remain snow-free for longer than exposed soil causing greater thermal fluctuations during the winter, (iv) cloud cover had a significant effect on the extent of warming in the cloche, adding further variation not predicted by GCMs.

Concern has also be made over changes in soil water contents beneath cloches (Wynn-Williams, 1992b; Kennedy 1995). However, in the present study the water content beneath cloches did not significantly vary in comparison to the adjacent exposed plot (Figures 4.3 and 4.11, Tables 6.3 and 6.5). Davey and Rothery (1992) also showed that fellfield fines were unlikely to provide drying conditions lethal to cyanobacteria and that the soil beneath minicloches were moister than exposed soil. This led Wynn-Williams (1996) to conclude that lateral and vertical diffusion of water would ensure that moisture would be unlikely to be a limiting factor for cyanobacteria either inside or outside cloches. This illustrates the fact that predicted cloche secondary effects, such as given by Wynn-Williams (1992b) must be investigated *in situ* to determine the significance of any biological effect. For example, Smith (1993) reported a decreased humidity inside cloches during the daytime, although he concluded that this change was not important in the response of the colonists beneath the cloche. Reduced propagule input into cloche covered soil may also be of minor importance given that an abundant *in situ* moss-propagule bank germinated beneath a cloche and gave a fully integrated moss community associated with a well-established community of invertebrates after four years (Smith, 1993).

Nevertheless, given the poor fit of cloche temperature changes to GCMs (Kennedy, 1995c), alternatives to passive greenhouses have be sought - such as the use of infra red heating elements suspended above treatment plots (Harte and Shaw, 1995; Harte *et al.*, 1995). However, such an approach is not practical for polar field experiments where maintenance, portability, energy use and climate must be considered. Such considerations were used to design the modified, second-generation cloches that have been installed at various Antarctic sites (Wynn-Williams, 1996). They include those installed on polygons WW4, 5 and 10, which are small, robust, portable and capable of being set up in a variety of configurations to suit the experimental objectives. For example, a UV-opaque cloche can be installed with or without side walls to block incident UV-B with or without warming the soil. Strathdee and Bale (1993) reported a new design of cloche, installed at an Arctic field site, which better mimics changes predicted by GCMs for global warming. The cloche is circular, to minimise edge effects, with plexiglass walls and a water- and air-permeable fabric top which transmits 80% of incident radiation. The

cloche is also has a height of only 15 cm which would reduced the duration that the cloche would be snow-free during winter months - one of the criticisms of the cloches investigated by Kennedy (1995c). This cloche design may point the way for future field manipulations in Antarctica, although it must be noted that the more extreme Antarctic climate, particularly the high wind speeds (in excess of 100 mph on the continent; Wynn-Williams, 1990a) and greater snowfall may require further design modifications.

Thus, given the constraints of Antarctic field manipulations the use of passive greenhouses remains the most realistic option for *in situ* climate change. However, among the shortfalls of the cloches used in our experiments, changes in soil water content, humidity and propagule immigration can be excluded from having a significant effect on the microbiota of interest in the present study. This leaves the discrepancies from the GCM forecast of global warming and of these little can be done practically. However, it is felt that given the paucity of field data on the microbial response to climate change the field manipulations of the present study have validity in that they show the potential of the microbiota to respond to warming, even though the actual pattern of warming *in situ* can not yet accurately reflect current predictions.

7.2 Response of the Polygon Microbiota to Simulated Global Warming

7.2.1 Response of Fellfield Microbiota to Cloche Cover

The simulation of global warming brought about by cloches installed on fellfield polygons caused a significant bacterial response in all three polygons studied. Bacterial numbers were up to ten fold greater in warmed soil compared to exposed (polygon WW10, average for Season 2). Bacterial metabolic activity was significantly greater in warmed soil, as measured by phosphatase and ETS activity (e.g. x 2.8 and 7.0 in polygon WW2). Exopolysaccharidase activity was also greater in warmed soil (e.g. x 3.7 in polygon WW2). Increased enzyme activity is indicative of an increase in enzyme synthesis or an improvement in substrate affinity, suggesting either greater bacterial action to produce the extra enzymes, or a change in the nutrient status of the soil to select fior enzymes of increased affinity.

ETS activity provided additional information on the nutrient status of the soil at the time of sampling (section 4.2.2.3) and provided evidence that the bacterial community in the warmed soil was more nutrient limited than that of exposed soil, particularly towards the

latter half of the season (from day 21 onward Figure 4.5 and 4.13). Thus, cloche cover reduced the environmental limits that prevent the bacterial community in the exposed soil from metabolising available nutrient. It would therefore appear that the bacterial community responded to the direct effect of the enhanced conditions beneath the cloche. More extensive field microclimate data could have aided in the analysis of the environmental changes beneath the cloches that caused the bacterial changes. However, in their absence, information on factors determining bacterial activity must be inferred from enzyme assays. Thus, it was concluded that the cloches, although not significantly altering soil water content, increased soil temperature and enabled the fundamental change from temperature-limited bacterial community, to one limited more by nutrients.

Image analysis of the cyanobacteria near the soil surface were studied in one polygon (WW10) that had a global warming cloche. In Season 1 (1993-4) the cloche cover showed a dramatic increase in cyanobacterial cover and a succession from domination by filamentous *Phormidium* and *Pseudanabaena* spp. to aseriate *Nostoc* spp., as previously recorded beneath such cloches (Wynn-Williams, 1996). Thus, increased primary product from the cyanobacteria is suggested to play a part in the increased bacterial community. However, the ETS assay revealed that the community was also more nutrient limited (and was always able to utilise supplemented nutrients, in contrast to exposed soil). Thus, it would appear that the bacterial community in warmed soil would be able to respond to further primary product from the cyanobacteria. The question therefore arises as to what then limits the cyanobacterial population in warmed soil. Wynn-Williams (1996) observed much larger increases in cyanobacterial community beneath cloche (on polygon WW2) after three and six years than was observed in WW10, after six and seven years of cloche-cover. However, the increase after three years (73.4% colonisation, compared with 4.4% in exposed soil) was due to increased colonisation by filamentous species, notably *Phormidium autumnale* and *Pseudanabaena* spp. After six years, however, the succession to the nitrogen fixing *Nostoc commune* was recorded, leading to the suggestion that nitrogen limitation drove this successional change. Indeed, there is evidence that ammonium, the major N source for *Phormidium* falls to limiting levels in fellfield polygons during the summer (Arnold, 1995) and Davey and Rothery (1992) suggest that polygon cyanobacterial communities are N limited. Gapp (1996) provided evidence that the N:P ratio may also be important in the growth of *Phormidium autumnale*. Thus, if the succesional change was driven by limiting soil N, it would follow that nitrogen-fixation by *Nostoc* spp., although giving selective advantage, would also reduce the productivity of the community (microbial nitrogen fixation requires significant diversion of metabolic resources). However, the cyanobacteria would also be able to remain productive during periods of low soil N. Indeed, nitrogen fixation may only be a

more successful strategy in such soils under warmer conditions, to enable the increased metabolic cost to be met. It may then be that availability of P limits the nitrogen fixing cyanobacteria in warmed soil.

7.2.2 Ability of Indigenous Fellfield Colonists to Respond to Elevated Temperature

It is becoming clear that in cold, fluctuating habitats (e.g. Signy Island), a pyschrotrophic strategy is more common than a psychrophilic one (Russell, 1990). This was also found to be the case in the present study (section 3.2.3) and points to the bacteria being well suited to responding to elevated temperatures. This is particularly evident when the pattern of temperature change caused by cloches is taken into account. As discussed above, the cloches exaggerate the daytime temperature elevation during cloud-free days, with temperatures recorded up to 20°C inside a cloche compared with 1°C in exposed soil (Plate 4.2; Smith, 1993). Thus, the psychrotrophic colonists are able to take advantage of such large temporal increases which occur, rather than an averaged temperate increase of around 3 C°. It is interesting to speculate on the effect of a more realistic simulation of global warming; i.e. which better mimicked the diel changes predicted by the GCMs. As discussed above the models predict a more even temperature elevation - this would not be so advantageous to a psychrotrophic microbial community as constant temperature elevation of 3 C° would not allow burst of microbial activity which could occur during such cloud-free days beneath cloches. Indeed, Panikov (1994) used a computer model to demonstrate that a constant 5 C° temperature increase in tundra soil would not change the population dynamics of bacteria with r, K and L strategies (exemplified by *Pseudomonas*, *Arthrobacter* and *Bacillus* spp. respectively) or alter the rate of balance between dead organic matter production and decomposition.

7.3   Effect of Climate Change on Carbon Turnover

7.3.1 Evidence for Long-Term Changes in Soil Carbon Content

Although temporal increases in soil carbon and saccharide (which play a major role in soil stabilisation; Burns and Davies, 1986) were observed in the warmed soil of polygon WW5, only soil beneath the cloche on polygon WW2 showed a significant increase in soil carbon, compared with exposed soil. There was also no evidence from polygon WW10 of

an annual accumulation of carbon or saccharide in exposed or cloche-covered soil. However, the increased polysaccharidase activity in all warmed soils indicates that warming increases the rate of organic carbon decomposition and formation, resulting in no net change in the soil carbon or saccharide pools. Interestingly this was the conclusion drawn by Panikov (1994) from a model of the response of tundra bacteria to a 5 C° temperature elevation. Fellfield soil is, however, of a lower carbon content than tundra soil and as such soils develop into peat soil at Signy Island, carbon accumulation, with time, would be expected. However, the formation of peat soil at Signy Island has taken place over a considerably larger time scale than that of our experiment: peat at depth of 2 m has been estimated to have been formed 5500 years ago (Smith, 1990), whereas the cloche at polygon WW2 was installed for eight years prior to the present study. It is suggested, therefore, that as the microbial response to the simulation has been established over a relatively short time period (five years in polygon WW5) the soil carbon content in warmed and exposed soil be periodically monitored over a longer time period.

7.3.2   *The Significance of Springtime Washout*

Given the observed increase in size and activity of the polygon microbiota to our simulation the reason for the lack of soil carbon increase in the 2 of the 3 sites studied should be considered. As discussed in Chapter 6 (section 6.2.1.1) the annual spring thaw may play a significant role in checking the advance of polygon colonisation: considerable interseasonal variation in the extent of cyanobacterial colonisation was recorded in polygons WW4 and WW10, in exposed and cloche-covered soil. Davey (1991a) also recorded a considerable decline in cyanobacterial community size during the spring and concluded that the flow of melt water had washed out the vast majority of the cyanobacterial biomass.

The primary reason for fellfield polygons remaining at the pioneer (microbial) stage of colonisation may well be the inability to prevent such considerable, annual, biomass removal. It is also conceivable that the onset of moss colonisation may stabilise the fine materials and reduce the impact of this annual event. Once such a situation existed, the microbiota would be able to further stabilise the fines and a process of positive feedback may then lead to the secondary, moss dominated, stage of fellfield colonisation.

This leads to a paradoxical situation for succession of fellfield soils: elevated soil carbon (giving increased soil stability) is required before mosses can successfully colonise polygon fines, whereas the absence of soil stability results in poor resistance of the

microbiota to melt water washout. Thus, it may take considerable time for the microbiota to cross the hurdle of carbon accumulation and explains, even with elevated microbial activity, why carbon accumulation in warmed soil was only recorded in 1 out of 3 polygons investigated.

Smith (1993) reported that temperature played a significant role in limiting moss colonisation of bare polygons, although the study was performed on fines cultured at constant temperature under laboratory conditions. However, the lack of moss shoots on the surface of samples (beneath cloche on polygons WW5 and WW10) studied in our experiments suggest that, even with the temperature elevation provided by cloche-cover, mosses were unable to colonise the fines. This may reflect the freeze-thaw cycles occurring *in situ* not accounted for in the former study. The only site with moss shoots in evidence was beneath the cloche on polygon WW2, which is also the only site with elevated C, thereby supporting the hypothesis that carbon accumulation is required for moss colonisation.

7.3.3   *Global Warming and Carbon Flux: Do Current Models Fit With Observations from Signy Island Fellfield Soil?*

Models of the effect of global warming on microbial decomposition of soil organic matter are of great importance in predicting whether a biologically-mediated positive feedback mechanism will occur. Jenkinson *et al.* (1991) have modified the Rothamstead model for soil organic matter turnover to predict that increased microbial activity under global warming will release 61×10^{15} g carbon into the atmosphere in the next 60 years (~19% of carbon released by combustion of fossil fuels, under present consumption). This study did not, however, take into account increased net primary production under future warming, which could offset such carbon release. Studies that have taken net primary production into account have concluded that the higher Q_{10}s for microbial decomposition, compared with primary production, indicate that warming will result in a net loss of carbon from soils (Raich and Schlesinger, 1992; Kirschbaum, 1995). Panikov (1994) modelled the response of tundra microbiota to increased temperature and reported a net release of soil carbon at 10°C, but not at 5°C.

Zogg *et al.* (1997) have shown that such estimates, from models based on carbon accumulation versus decomposition may have to be modified to take into account functional changes in microbial communities with increased temperature. They found that elevated temperature (from 5°C to 25°C) increased the pool of decomposable substrate

and hence resulted in a larger than expected release of carbon with soil warming. they concluded that such changes arise from functional successions within the microbial communities enabling a wider range of substrate to be metabolised.

In contrast to these studies, it is concluded that global warming has the potential to increase the soil carbon of Antarctic fellfield soils. However, as disused above, such increases rely on warming tilting the balance between microbial colonisation and annual biomass washout, which, during the duration of our field experiments, was observed only in 1 out of 3 warmed soil. However, given that global warming will speed up the rate of fellfield soil carbon accumulation and succession to moss dominated soil, the impact of warming on this such secondary stage of colonisation should also be investigated. Smith (1993) has shown that moss colonisation is limited by temperature (in laboratory incubated soils under constant temperature, with no freeze-thaw cycles). As discussed above, it may then be expected that, as mosses colonise bare fellfield soils, the microbiota would suffer reduced biomass washout and this may enable carbon to accumulate at a faster rate. However, such soils would start to resemble the moss-dominated peat soils of Signy Island, whose fate under global warming has yet to be established.

7.4.   Effect of UV-Protection on Polygon Microbiota

The UV-screening cloche on polygon WW4 was used to determine the effect of normal levels of incident UV-B on the indigenous colonists in the exposed soil. The size of the cyanobacterial and bacterial communities were larger beneath the screen with β-galactosidase activity greater at Spring 1 (x 2.0) and ETS and β-glucosidase activities were greater at Spring 2 (x 1.8, x 2.5). It was concluded from these results that polygon microbiota are inhibited by the natural UV-B radiation at such sites. These findings are in agreement with comparable UV-screening experiments carried out on the marine ecosystem of the Southern Ocean, at a similar latitude, where it was concluded that the natural UV-B flux inhibited the phytoplankton production by 10 - 20% (Helbling et al., 1992; Holm-Hansen et al., 1993).

It is interesting to note that the springtime ozone hole does not normally extend to Signy Island and that such results can be considered as useful baseline studies into the effects of incident UV-B under a 'normal' ozone column of approximately 300 Dobson Units (DU; World Meteorological Organisation (WMO), 1992). However, in October 1992, ozone

mapping showed that the most northerly tip of the elliptical ozone hole (170 DU) extended as far as Tierra del Fuego, Southern Argentina, and would have included Signy Island (WMO, 1992). Thus, the polygon colonists of exposed soil would have been temporally exposed to significantly elevated UV-B levels during spring 1992 (one year before Season 1). It is possible that the smaller cyanobacterial community in exposed soil during Season 1 may have been due to the higher UV-B levels of the previous year. However, the spring washout is likely to have a greater effect in determining the predicament of the microbiota at the start of each season, given that Davey (1991a) reported a significant decline in the cyanobacterial community with the size of four out of five individual species (*Phormidium, Pseudanabaena, Pinnularia, Zygnema*) dropping below detectable limits at this time. Nevertheless, unpredictable movement of the lobes of the ozone hole mean that associated elevated UV-B levels can be considered to be a threat to all Antarctic ecosystems (Karentz, 1991).

Suggested reasons for the inhibition of the polygon colonists beneath normal incident UV-B have been given in Chapter 6 and include: the direct inhibition of cyanobacterial photosynthesis; the synthesis of UV-defence pigments and DNA repair enzymes which led to reduced productivity; and restriction of cyanobacterial to a zone below the soil surface which reduced the PAR available for photosynthesis (section 6.2.1.1). However, the reason for the smaller bacterial community in exposed soil is more difficult to determine: the bacterial community under the UV-screen may be metabolising increased substrate levels from a larger and more productive cyanobacterial community; or the UV-exposed bacterial community may be suffering from UV-inhibition. The lack of difference in the proportion of pigmented bacteria does suggest that UV-screening has not altered the selective pressure for pigmentation. As pigments in other Antarctic bacteria have been shown to be carotenoids, which provide protection from UV and visible radiation, it was concluded that either the bacteria were not normally exposed to levels of UV-radiation which would make pigmentation and advantage, or the pigments were predominantly for protection from visible radiation. This does illustrate the lack of UV photo biology data for Antarctic colonists. If such data were available for bacteria it may be possible to determine whether the natural levels of UV-B (approximately 500 μW cm^{-2}) are inhibitory to bacteria at or near the soil surface.

The cloche on polygon WW2 blocked incident UV-B in addition to warming soil and it is likely that the two effects are additive: separate UV-screening (WW4) and warming (WW5 and WW10) cloches both elevate the activity of polygon microbiota. However, given the greater bacterial activity, and cyanobacterial colonisation beneath the warming cloches compared to the UV-screen it is suggested that soil warming has a greater effect on the

colonists. Nevertheless, in the absence of detailed microclimate data and lack of quantitative information on the effects of natural UV-B radiation, it is not possible to determine to what extent the two factors where responsible for the microbiological response under the combined cloche on WW2. It is interesting to note that the bacterial response was the greatest beneath the cloche on this polygon, but again, it is not possible from our data to determine the extent to which warming, UV-B, the duration of cloche cover or simply natural variation gave the greater microbial response in comparison to the three other cloche sites. The greater response beneath the combined cloche may also act to illustrate the point made by Kennedy (1995a) who suggested that the effects of global warming at polar sites may be offset by increased UV-B flux caused by ozone depletion. This was shown by the response to simulated global warming being less in the presence of natural UV-B (WW4 and WW10) than in the absence of UV-B (WW2).

7.5   Suggested Future Work

7.5.1   Further Field Studies

Further field samples taken in conjunction with detailed microclimate data (including temperature, PAR, UV-B) would allow the microbiological changes beneath cloches to be analysed in more detail. For example, the conclusion that the bacterial communities in exposed soil in polygons WW2 and WW5 were limited by temperature during the latter half of the season (from day 21 onward; 4.2.2.3) could be have been tested by comparisons of temperature in and outside cloches. Samples taken a intervals prior to the spring thaw could be used to determine if the bacterial community suffers a washout or a significant viability decrease (e.g. by comparison of the ratio of viable plate counts and direct microscopic observations).

Cloches placed on moss-dominated peat soil would also be invaluable in determining the response of Antarctic soil of higher carbon content to climate change. This would enable a more complete picture of the effect of global warming on the fate of bare polygon soil. For example, if warming leads to succession of mineral soil to peat soil, would such peat soil be vulnerable to carbon loss, as predicted for Arctic tundra soil, and thereby remain at an equilibrium between the low carbon mineral soil and higher carbon peat soil? Such a study would also determine whether Signy Island will be a net source or sink of carbon by comparing the potential for carbon flow into mineral soils and out of peat soils.

Cloches have been placed at fellfield sites south of Signy Island at Alexander Island and will provide information on the response of the microbiota at this more extreme habitat. This site is beneath the ozone hole and should provide useful data on the effect of the elevated springtime UV-B levels which can then be compared with data from this study (WW4).

7.5.2 Growth Cabinet Studies

Growth cabinets allowing precise control of temperature, PAR, humidity, UV-B etc. (as exist at the Rothera Research Station, on the Antarctic Peninsula) can be used to deduce the important environmental features that limit the microbiota of fellfield soil. Whole Antarctic microbial ecosystems, such as surface slices of fellfield polygons, could be transferred intact into such cabinets where a variety of experiments, complementary to our field study, can be conducted. Experiments could include:

(i)   Soil samples incubated under a simulated Antarctic growing season both at field temperature and at a temperature that mimics the diel changes predicted under global warming by GCM (a constant temperature increase of 2-3 C°). This approach may reveal that cloches (criticised by Kennedy (1995b) for producing warming that does not match GCM predicted diel changes) produce a greater microbial response than to be expected under a more realistic simulation of global warming (e.g. cloche produce unavoidable secondary effects and soil beneath cloches can be up to 20 C° greater than exposed soil resulting in large, temporal microbial activity). Such a study could also determine the effect of a simulated spring thaw and whether, in the absence of melt water, the microbiota suffer the same loss of viability as recorded in the field.

(ii)   The factors enabling moss colonisation of bare polygon soil can be investigating by seeding soil with moss propagules and monitoring germination and growth under controlled temperature, freeze-thaw cycles and soil organic matter content. Arnold (1995) has successfully mimicked freeze-thaw cycles in a laboratory study and revealed that cyanobacteria respond by the accumulation of compatible solutes. Such a study would be useful in determining the fate of bare polygon soil to global warming by ascertaining to what extent microbial stabilisation of fines is required before moss are able to survive in situ freeze-thaw cycles.

7.5.3 Modelling of Carbon Flux

An alternative method of estimating the effect of global warming on carbon flux in fellfield soils is to model microbial production and decomposition with temperature and then use field temperature data and elevated (+3 C°) field temperature to extrapolate *in situ* annual carbon flux. This approach has the advantage of also enabling further global warming (e.g. +6 C°) to be modelled. Wynn-Williams (1980) has extrapolated annual microbial carbon dioxide and oxygen flux at Signy Island peat soil by extrapolating from experimentally derived microbial activities and Friedmann *et al.* (1993) have calculated annual gross and net photosynthesis at a continental cold desert site. The data from these two studies could also be used to study the potential effects of warming by repeating the calculations at higher temperatures than for the field temperatures reported.

Unfortunately, models cannot take into account available substrate. Furthermore, freeze-thaw cycles have an important role in bringing substrates into the bacterial domain (e.g. from mosses at polygon periphery). The modelling of polysaccharidase activity (section 5.2.3) illustrates that such an approach gives maximal substrate turnover possible. Nevertheless, the modelling of bacterial decomposition and phototrophic productivity with temperature combined with an analysis of the quantitative effect of freeze-thaw cycles would allow estimates of ecosystem productivity under present and future field temperatures. It must be noted that some of this information is currently available: Convey (1994) has reported photousynthesis rates of Signy Island mosses with temperature; Arnold (1995) has studied the release of compatible solutes from fellfield cyanobacteria under freeze-thaw cycles; Wynn-Williams (1980) has modelled the effect of temperature on microbial oxygen and carbon dioxide flux for the microbiota of Signy Island peat soil.

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