TRANSFORMATION OF FUNGAL BIOMASS IN SOIL

by

HASSAN SUBHAN NISAR HUSSAIN,
B.Sc., M.Sc., (Mysore, India) M.Sc., (Manchester)

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ABSTRACT

During this research programme the rate of mineralization of fungal residues in soil was investigated and the effects of various environmental parameters on the process analysed. An open continuous-flow soil perfusion system was designed and used for the investigation. The reactor column was packed with aggregates of a sandy clay loam which was amended in various ways with fungal material (*Aspergillus nidulans*) and perfused continuously with defined gas and liquid feeds.

This method was used to study the effects of basic soil conditions such as aeration, anaerobiosis, aggregate size, moisture fluctuations and nutritional status on the processes of mycelial degradation, mineralization and humification. Subsequently the influence of chemical treatments of the soil (glucose, potassium nitrate, ammonium sulphate and ammonium phosphate) on the rates of transformation of native organic matter and added fungal material was assessed. Further the effect of selected pesticides (Dalapon and triphenyltin acetate) on mineralization of fungal material was studied.

Carbon dioxide evolution was taken as the primary measure of carbon mineralization throughout these studies, a decision which facilitates a comparison of the present data with other studies of carbon transformation in soil. A method for determining the total activity of β-1,3 glucanase in soil has been devised and assay of this activity provided a convenient measure of the active mycolytic

microflora in the soil. Changes in the microbial population which were induced by soil amendment also were recorded.

Continuous perfusion of soil by water had an appreciable effect on the decomposition of native organic matter and the effect was enhanced under conditions of amendment with fungal mycelia. Continuous water perfusion was more effective in mineralization than cycles of soil drying and wetting. The rate of mineralization was more pronounced under aerobic than anaerobic conditions both in control and mycelium-amended soils.

Based on the cumulative production of $^{14}CO_2$ from soils amended with ^{14}C-labelled mycelia it was shown that continuous perfusion with various nutrient solution stimulated fungal mineralization in the decreasing order: potassium nitrate > glucose > ammonium sulphate > ammonium phosphate > water. Treatment with Dalapon for sixty days produced no significant difference in the overall rate of mineralization compared to the aerobic water perfused condition.

Triphenyltin acetate showed an inhibitory effect on the production of carbon dioxide. Marked inhibition was observed at the highest concentration of the fungicide used (500 ppm) in the mycelium-amended soil and also in unamended control soils at lower concentrations (100 ppm).

Half-lives of readily decomposible and humus material in soil subjected to various treatments were calculated and compared with other studies of carbon mineralization. The enhanced mineralization of the native organic matter (priming effect) following the addition of fungal material to and various treatments of soil was calculated and discussed . Some comparisons with the transformation of higher plant residues in soil have been made.

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To my wife Nazhath,

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CONTENTS

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1. Systems in the study of soil transformation:

Soil is a very complex and dynamic system and its fertility is largely dependent on the delicate balance which exists between the microflora and physico-chemical nature of the soil. The activity of the microflora in soil is determined by the presence of organic and inorganic substrates which are transformed by the microorganisms in the processes of energy and anabolic metabolism. The direction and intensity of these transformation processes does not depend only on the properties of the microflora but also to a considerable degree on the properties of the soil and prevailing climatic conditions. Thus, twenty years ago Quastel was emphasizing the necessity of studying soil as a biological whole in order to gain a better understanding of the activity of soil microbial associations under natural situations (Quastel, 1955). The introduction of soil percolation techniques (Lees & Quastel, 1944) with its application to various soil microbiological problems (Lees & Quastel, 1946a;Audus, 1951; Stevenson & Chase, 1953) was a significant development in soil microbiology.

For studies of the cycles of the biological transformation in soil selective or enrichment culture methods are usefully employed. Enrichment culture is a technique for selectively increasing the numbers of a desired microorganism or group of similar microorganisms by manipulating the growth conditions such that growth of the required population proceeds at a faster rate than the remaining microflora. The latter is accomplished by withholding, or adding, a specific nutrient or growth factor, adding a toxic material, or, in general altering the physical or chemical conditions to the advantage of desired population. The methods based on this principle facilitate the isolation of numerous characteristic microorganisms from the soil and are employed successfully in studies of the decay of organic substances.

These methods have also contributed essentially to the elucidation of several important questions concerning the ecology of the soil microflora; for example: nitrification (Alexander, 1961), Changes in specific microbial population, induction of specific enzyme systems (Stotzky, 1972). Studies of this type often utilize percolation devices in which a nutrient solution is cycled through a soil sample (Audus, 1946; Lees, 1949; Greenwood & Lees, 1959).

The Lees-Quastel soil percolation apparatus is a closed system, similar to that of conventional batch processes, i.e. there is no addition or removal of materials from the system. Substrates after addition to the soil sample are consumed and characteristic changes in the amount and composition of the soil microflora occur which, after exhaustion of the substrate, slowly return to initial states. Such methods consider soil as a closed system or environment. According to Jansson (1958) soils may be considered as closed systems only in laboratory experiments; under natural conditions, however, mineral nutrients are removed by plant roots or are leached out on the one hand and, on the other hand, enrichment with substrates originating either from plants and animals or from agricultural practices occurs such that the soil as a whole posesses the properties of an open system (Jansson, 1958). Macura & Malek (1958) developed a continuous-flow method for the study of microbiological processes in soil samples which differed from the Lees-Quastel type in treating the soil as an open system. As pointed out by Brock (1966) Macura's approach is a more realistic approximation of the situation in nature where there is an input and output of nutrients and biomass.

The applicability of Macura's method to the investigation of microbiological processes was tested in studies of various transformations in soils. Decomposition of glucose (Macura & Kunc, 1961), biological immobilization of nitrogen and phosphorus (Macura & Kunc, 1965a), transformation of glycine

(Macura & Kunc, 1965b) and nitrification (Macura & Kunc, 1965c; Bazin & Saunders, 1973) are some documented examples of the use of this experimental system. Takai, Macura & Kunc (1969) have operated the system under anaerobic conditions to study the decomposition of glucose, while Barnes, Bull & Poller (1973) adopted the approach to investigate pesticide metabolism in soil.

The continuous cultivation method applied to studies of soil in this form is, therefore, a kind of elective culture and a special case of continuous cultivation. Since the microorganisms living in the soil are adsorbed onto the surfaces of mineral and organic particles, the method possesses common features with the method of heterogenous continuous cultivation of microorganisms on solid support (Macura, 1966).

1.2. Fungal biomass and its turnover in soil

Fungi constitute an active and important component of the soil population. Although fungi are less numerous than the bacteria and actinomycetes, but are much larger in size, contributing for the greater part of the total biomass in soil. They convert a considerable percentage of the energy and carbon substrates they decompose into cell substances and other products (Waksman, 1932; Alexander, 1961). Alexander (1961) estimated that fertile agricultural soil contains 10^8 - 10^9 bacteria per gram of soil and that the average bacterial weight is 1.5×10^{-12} g. This totals 0.15 - 1.5g live weight of bacteria per kilogram soil. He estimated further that soil contains 10 - 100m of fungal mycelium per gram of soil. Assuming an average hyphal diameter of 5 µm and a specific gravity of 1.2, live weights of fungi per kilogram of soil would range from 0.24 to 2.4 g. Gray & Williams (1971) using the microbial biomass and annual litter production data for Meathop Wood soil (Satchell, 1970) reported dry weights of 7.5 kg/ha for bacteria and actinomycetes combined and 454 kg/ha for fungi. The fungal mycelia eventually die and are themselves utilized as a source of energy and carbon by new generations of soil organisms.

Fungal mycelia, therefore represent an important source of energy and carbon for the soil population. Cholodny (1930) made early observations of the bacterial colonization of fungal hyphae in soil using his buried glass slide technique. Similar associations have been revealed more recently on glass slides placed in partially sterilized soil and in soil supplemented with various kinds of organic material (Lockwood, 1967). Bacterial associations with fungi appear to predominate in most reports. Actinomycete colonization of fungal hyphae on glass slides in soil also occurs frequently and in some instances fungal mycelium seemed more attractive to actinomycetes than other types of bacteria (Thornton, 1953). Growth of bacteria and actinomycete on fungal hyphae has also been observed using other methods such as that of Waid & Woodman (1957) involving the incubation of nylon mesh in soil. The meshes became colonized first by fungi whose hyphae later became colonized by bacteria. This kind of successional development was also observed on buried cellulose filter paper (Cholodny, 1930) and with cellophane strips (Tribe, 1957).

The application of other microbiological techniques, usually based on the dilution plate method have revealed increase numbers and activity of microorganisms in soils supplemented with fungal mycelia. Heck (1929) was the first to show that total bacterial numbers increased following amendment of soil with the fungus *Aspergillus oryzae*. Subsequently Lloyd & Lockwood (1966) showed that when hyphae of *Glomerella cingulata* were added to soil the bacterial population increased approximately 65 to 70-fold in 5 days and the actinomycete population 10-fold in 9 days. The fact that mycelia and also spores of many fungi disappear rapidly when placed in contact with soil also provides evidence for their utilisation by other soil microorganisms. For example, spore germination was followed by lysis

of the germ tubes within 4 to 8 days, when glass slides, containing spores of several fungi held in thin films of agar, were placed in soil supplemented with 2% soya bean meal (Chinn, 1953).

In addition to direct observational methods, chemical and biochemical techniques have been used to assess transformation of microbial materials added to soil. In 1924 Starkey found that the decomposition of mixed spore and mycelial material in soil, as measured by carbon dioxide evolution, was as rapid as that of lucerne meal while Heck's extensive investigation showed that fungus material decomposed in soil as rapidly as other organic materials of similar C:N ratio (Heck, 1929). Jensen (1932), who in addition to reviewing the literature up to 1932, presented the results of a comprehensive study of the fate of microbial cell material added to soil. He used a number of soil fungi and actinomycete and various soil bacteria. Jensen reported that in neutral garden soil the addition of microbial substances gave rise to a more or less abundant but always temporary development of bacteria and actinomycetes, the latter group of organisms was often stimulated to a very conspicuous degree. The development of microorganisms was accompanied by the production of nitrate to varying degrees. In experiments using sand in place of soil, Jensen observed abundant ammonia production after ten days. Norman (1933) investigated various aspects of the availability of the nitrogen of fungal tissue to microorganisms during decomposition of straw. He found fungal tissue to be as suitable a source of nitrogen as ammonium salts and nitrates.

Fungal structures other than hyphae are also lysed by microorganisms in natural soil. Lysis of conidia or sporangiophores of several fungi placed in contact with soil was seen on contact slides (Park, 1955). Live or killed mycelia of numerous plant parasitic and saprophytic fungi were partially or completely lysed by soil within a few days (Lloyd &

Lockwood, 1966). There is evidence that extracellular enzymes from soil microorganisms may lyse fungal mycelia. Horikoshi & Ida (1959) showed that chitinase from streptomyces species partially lysed live fungal mycelia of *Aspergillus oryzae*. In 1965 Skujins, Potgieter & Alexander isolated from soil a streptomyces species which was active in lysing the hyphal walls of *A. oryzae* and *Fusarium solani*. The concentrated enzyme preparation completely destroyed the structural integrity of the walls of *F. solani*, subsequent work in Alexander's laboratory (Bloomfield & Alexander, 1967) demonstrated extensive digestion of the hyphal walls of *Aspergillus phoenicis* and *Sclerotium rolfsii* by the streptomyces culture filtrate and by a mixture of purified chitinase and β-1,3 glucanase preparations. However, neither the conidial walls of *A. phoenicis* nor the melanin covered sclerotia of *S. rolfsii* were attacked by the streptmyces enzymes. Recently Bull (1970a) extended these researches and showed that the hyaline and melanized walls of *Aspergillus nidulans* were degraded selectively by a crude lytic enzyme complex and highly purified β-1,3 glucanase plus chitinase mixtures produced by soil streptomycetes. It was also reported that melanin-bound chitin was extremely resistant to enzymatic degradation (Bull, 1970a).

Mayaudon & Simonart (1963) studied the humification of the mycelium of *Aspergillus niger* and the cells of the bacterium *Azotobacter vinelandii* and found that mineralization of Aspergillus was less than that of Azotobacter. Addition of the mycelium to soil lead to an enrichment of soil organic matter with complexes resistant to hydrolysis, and assumed to have an aromatic character, while addition of bacterial cells lead to an enrichment with humic materials of a proteinaceous nature that were susceptible to hydrolysis. Pinck & Allison (1944) suggested that the dark pigmented hyphae are likely to decompose slowly because they contain rather large amounts of resistant materials which they referred to as "lignin-like" substances. Martin, Ervin & Shepherd (1959) compared decomposition of

light and dark coloured fungal tissues in soil and found that more carbon dioxide was evolved from soil treated with light coloured mycelia. This type of analysis was extended by Hurst & Wagner (1969) who studied the decomposition of ^{14}C-labelled wall and cytoplasmic fractions from hyaline and melanic fungi. The rate of decomposition of wall material from both mycelial types was initially lower than that for cytoplasmic material. The hyaline wall decomposed at a relatively steady rate for a prolonged period and after six months 70% of the carbon in this fraction had been lost as CO_2 compared with 62% of the carbon in the cytoplasmic fraction. Wall and cytoplasmic fractions of the melanic species were strongly pigmented and resistant to decomposition and only 35% and 48% of the carbon in these fractions was evolved as carbon dioxide respectively.

Studies of the susceptibility and resistance of fungi to microbial lysis have shown conclusively that the presence of a dark pigment in the fungal walls confers significant protection (Potgieter & Alexander, 1966). The pigments of *Rhizoctonia solani* were reported to be melanins or melanin like and probably protect fungal structures from decomposition in natural environments (Kuo & Alexander, 1967). Bull (1970a)has provided more direct evidence that melanins are related to the resistance of fungal walls to lysis. He found that melanin-containing walls of *Aspergillus nidulans* were comparatively unaffected by a mixture of β-1,3 glucanase and chitinase to which treatment the walls of a melanin-less mutant were highly susceptible; he concluded that "the resistance of the walls to digestion was directly correlated with the melanin content of the mycelium".

The isolation and characterization of several fungal pigments has shown their similarity to humic compounds (Kang & Felbeck, 1965; Kumada & Hurst, 1967). The *Aspergillus nidulans* pigment has been analysed in detail (Bull, 1970b)and shown to be an indolic polymer related to melanins of animal origin.

1.3. Soil Enzymes

In recent years much attention has been devoted to the study of enzymes in soils (Skujins, 1967). Soil enzymes supposedly are primarily of microbiological origin but to a lesser extent are derived from plants. They are significant in regulating metabolism in soil, especially the transformation of organic substances within these processes enzymes have for instance a great influence on release and accumulation of nutrient substances. Soil enzymes are very important in the *de novo* synthesis of organic materials or complexes of the humic substance type. Thus, they participate to a high degree in the formation of organo-mineral compounds and chelates in soil, and consequently have a great influence on soil structure (Briggs & Spedding, 1963; Skujins, 1967). Enzyme activities have often been used as a measure of soil fertility (Khan, 1970).

It has been demonstrated that the soil contains a wide variety of polysaccharases: amylase (Drobnik, 1955; Hofmann & Hoffman, 1955) , cellulase (Markus, 1955) , xylanase (Sorensen, 1955) , pectinase (Hoffman, 1959), lichenase (Kiss, Bosica & Pop, 1962), levanase (Kiss, Boaru & Constantinescu, 1965) and dextranase (Dragan-Bularda & Kiss, 1972). Skujins (1967) has reviewed the techniques available for the assay of many of these enzymes including oxidoreductases, transferases, hydrolases and lyases.

Enzymes which hydrolyze β-1,3 linked glucans have been known as laminaranases because laminaran was used as a test substrate in early work. However, β-1,3 glucans have wide occurrence in nature and enzymes which depolymerize them are more suitably termed β-1,3 glucanases (β-1,3 glucan 3 - glucanohydrolase, E.C. 3.2.1.6) (Chesters & Bull, 1963; Bull & Chesters, 1966). β-1,3 Glucans are formed in microorganisms and higher plants as structural components of cell walls, as cytoplasmic and

vacuolar reserve materials and as extracellular substances of uncertain significance (Bull & Chesters, 1966). β-1,3 Glucans have been reported in a number of fungal walls (Bartnicki-Garcia, 1968), one particularly good source is the sclerotium of the fungus *Poria cocos*. This structure is the size of a large base ball (4 in diameter) and over 90% of its weight is β-1,3 glucan. While probably not uncommon, these sclerotia are found only when land is being cleared, soil overturned and a sharp-eyed collector at hand (Warsi & Whelan, 1957).

β-1,3 Glucanases are ubiquitous enzymes and appear to be implicated in the intracellular mobilization of food reserves in fungi, algae and higher plants, wall plasticity in budding and dividing fungal cells, extracellular depolymerization of plant debris by microorganisms, and in the digestive metabolism of invertebrates (Bull & Chesters, 1966). These enzymes are produced extra-cellularly in bacteria and fungi and are readily isolated from culture liquors. Their synthesis is usually constitutive or semi-constitutive. Horikoshi, Koffler & Garner (1961) reported that an enzyme preparation obtained from strains of *Bacillus circulans* which lysed *Aspergillus oryzae* walls contained chitinase and β-1,3 glucanase and other unspecified enzymes. Subsequently Horikoski & Arima (1962) prepared a chitin containing fraction from the walls of this fungus by treating them with β-1,3 glucanase. Mitchell & Alexander (1963) isolated a number of mycolytic bacteria from soil, mainly strains of Bacillus and Pseudomonas. One of the strains of *B. cereus* digested both living and dead mycelia of *Fusarium oxysporum* as well as wall preparations. Once again lysis appeared to be associated with the chitinase and β-1,3 glucanase activities.

One of the most common mechanisms of interaction between populations of microorganisms in natural environment is enzyme-induced lysis, one

species eliminating another by digesting the second species (Alexander, 1961). Moreover evidence has been obtained that a directed biological control of pathogenic fungi in nature may be achieved by modifying the indigenous microbial community in such a way as to favour destruction of the pathogen, probably through a preferential stimulation of micro-organisms capable of degrading fungal wall constituents (Mitchell, 1963; Mitchell & Alexander, 1961).

1.4. Environmental Control of biomass turnover in soil:

The soil functions as a natural waste disposal system for debris from organisms growing on and within it. The organic matter subjected to microbial decay in soil comes from several sources. Vast quantities of plant remains and forest litters decompose above the surface. Subterranean portions of plants and above-ground tissues that are mechanically incorporated into the soil become food for the microflora. Animal tissues and excretory products are also subjected to attack. A secondary addition of organic matter comprises microbial remains and animal corpses and faeces. Organic matter decomposition serves two functions for the microflora, providing energy for growth and supplying carbon for the formation of new cell material (Wagner, 1974). The efficiency of cell synthesis is governed by environmental conditions and it may vary over a considerable range. Clark (1967) reviewed growth of bacteria in soil and emphasized the specialization that microorganism have developed for utilizing energy-yielding materials. A cosmopolitan substrate is presented to the microbes and the utilization of it is governed by the ecological conditions that the substrate encounters (Clark, 1967).

The physical factors which affect the activity of soil microflora such as moisture content, degree of aeration and temperature effect have

been briefly described in the section 3.2.

Soil contains substantial quantities of a wide variety of naturally occurring chemicals at one time or another although for only a short period (Alexander, 1961). Man's activities have added many new chemicals to soil in the form of artificial fertilizers, pesticides and industrial wastes. These substances may be grouped as those with a beneficial effect on saprophytic activity and pollutants which inhibit one or more groups of organisms. Inorganic chemicals, almost all of which will be active in the liquid phase, generally favour saprophytic activity either directly in that they supply an essential element or through an increase in the productivity of the primary producers or by their action in altering the soil environment. Direct benefits may be best illustrated by the action of nitrogen which may allow active saprophytic growth on cellulose-rich debris which has a high C:N ratio (Alexander, 1961). The effect of inorganic compounds of nitrogen, phosphorous on the decomposition in soil is presented in section 3.3.

It is clear from numerous studies that the addition to the soil of any potentially toxic molecule constitutes a serious threat to the equilibrium which exists between the various types of microorganisms and hence to the future fertility of the soil. Considerable study has been made of the influence of herbicides, insecticides, fungicides and nematocides on biological processes or on the development of specific microorganisms in the soil (Alexander, 1961; Audus, 1964; Bollen, 1961; Helling, Kearney & Alexander, 1971; Martin, 1963). The action of these chemicals is not always limited to the immediate objective of killing a particular pest. Harmless or beneficial soil organisms may be killed or temporarily reduced in numbers while the qualitative nature of the soil population may be altered for varying periods of time, and abiotic

chemical changes induced.

Pesticides that become incorporated into the soil may be destroyed, inactivated or removed from the environment by a number of means, the net effect being a detoxication of the ecosystem. Such environmental detoxication may result from volatilization, leaching, chemical reactions, adsorption, photochemical destruction, removal by plants from the soil and the rhizosphere, and biological detoxication. Although in some environments, or, with certain compounds, detoxication involves one or more non-biological mechanisms, the soil microflora is frequently the major and often the sole means of freeing the treated soil of foreign chemicals. To the microflora many of the organic pesticides merely represent exotic carbonaceous substrates which are available to either a small or a large segment of the community as a source of carbon or other elements necessary to sustain growth (Alexander, 1969). Using the techniques and principles developed by workers interested in the decomposition of hydrocarbons and toxic substances in general various investigators have studied the decomposition of some of these chemicals in the soil and in pure cultures in the laboratory. A brief introduction on the effect of these chemicals are given in section 3.4.

1.5. Priming Effect

Accelerated decomposition of native soil organic matter by the microflora following addition of readily available substrates is described as a "priming action". "Priming" may also be defined as the beneficial effect of adding small amounts of fresh organic matter (mostly plant residues) on the microbial decomposition of resistant organic compounds in the soil (Bingeman, Varner & Martin, 1953; Broadbent, 1947; Broadbent & Bartholomew, 1948; Broadbent & Norman, 1946; Hallam & Bartholomew, 1953; Hiltbold, Bartholomew & Werkman,

1950; Jenkinson, 1966). Several authors have studied this effect by adding ^{14}C-labelled organic matter to the soil and subsequently measuring the $^{12}C/^{14}C$ ratio of the CO_2 evolved upon incubation. In this way it was demonstrated that in the presence of the added material more unlabelled CO_2, originating from the decomposition of the soil organic matter, was produced by microbial activity. Nearly three decades ago Broadbent & Norman (1946) showed that mineralization of soil organic matter was greatly accelerated by the addition of ^{13}C-labelled Sudan grass. Subsequent soil amendment experiments with ^{14}C-glucose (Chahal & Wagner, 1965), ^{13}C-glucose and ^{13}C-glucose plus NH_4NO_3 (Shields,Paul & Lowe, 1974) and labelled plant material or its components (Mortenson, 1963; Sorensen, 1963; Sauerbeck, 1966) have established that addition of organic materials to soil results in some degree of priming action. However, in a number of cases the primary effect was short lived and quite small in comparison to the amounts of native organic matter present in soil (Pinck & Allison, 1951; Stotzky & Mortensen, 1957; Jenkinson, 1971). The increased decomposition of native soil organic matter has also been observed after the continuous addition of ^{14}C-glucose (Macura, Szolnoki, Kunc, Vancura & Babicky, 1965).

1.6. Objectives:

This research programme was started with the aims of defining the rate of mineralization of microbial residues in soil and elucidating the effects of environmental changes on this process. In addition there was an interest in determining the fate of microbial residues in humification processes and what influence their addition to soil has on the mobilization of the native organic matter.

The experimental system of choice for these studies was considered to be the continuous-flow column reactor developed by Macura (1961) because its operation simulates the open characteristic of soil reasonably closely. Fungal material was selected for investigation because of its frequently dominating contribution to the soil biomass; in particular *Aspergillus nidulans* was used because of the previous analyses of its wall chemistry and microbial lysis *in vitro* (Bull, 1970a; 1970b).

In this study mycelium-amended and unamended control soils were subjected to a range of physico-chemical, nutritional and pesticide treatments. Mineralization of the fungal material was monitored in terms of CO_2 evolution, a parameter which facilitates the comparison of results from these experiments with other studies of carbon transformation in soils. The total β-1,3 glucanase activity in soil was taken as a convenient measure of the mycolytic activity in the soil. Changes in the microflora which were induced by soil amendments or other treatments also were recorded. In addition amendment of soil with uniformly labelled ^{14}C-mycelia studied the humification process in the soil.

The priming action of freshly added substrates also was a matter for investigation.

CHAPTER 2

METHODS AND MATERIALS.

2.1. Soil : Physicochemical Nature.

Soil was collected from an undisturbed and ungrazed grassland plot on the University of Kent at Canterbury campus. The higher plant community comprised mainly grasses with occasional Ranunculus species, some legumes (Crotalaria species) and Compositae.

The soil was sampled by digging a trench approximately 60cm long and 45cm wide and collecting soil from the various horizons and depths in the profile by means of a clean stainless steel sampling tube (2.5cm diameter, 50cm long). Soil cores were extruded with a plunger directly into sterile, wide necked bottles and, when required for microbiological analyses, were stored in the dark at 4°C. Samples for microbiological study usually were analysed within 24 h of collection. Large samples of soil intended for physicochemical studies or the preparation of soil aggregates (see Section 2.5) were collected in polyethylene bags and partially air dried on return to the laboratory. The latter samples were passed through a 10-mesh sieve, coarse stones, roots and litter fragments removed and stored in clean dry bottles in the dark at 4°C until required, usually for one to two weeks.

A soil profile description is included in the Results, Section 3.1.

2.1.1. Particle size Analysis.

A mechanical analysis based on the method of Pramer & Schmidt (1964) was used. Coarse textured, oven dry soil (100g) was weighed into the mixer cup of an M.S.E. "Atomix"

homogenizer. Sodium hexametaphosphate (50ml of a 10% w/v solution) was added to the soil and the cup then half-filled with distilled water. The mixture was stirred at maximum speed for five minutes and then washed into a sedimentation Bouyoucos cylinder. The cylinder was filled with distilled water up to the upper mark and the temperature of the suspension recorded. The cylinder mouth was covered with parafilm and the contents vigorously shaken by turning the cylinder end over end several times. After the cylinder was allowed to stand 30 s a Bouyoucos hydrometer was carefully inserted and read exactly 10s later (40s from initiation of sedimentation). The hydrometer was removed from the suspension and after two hours further thermometer and hydrometer readings were taken.

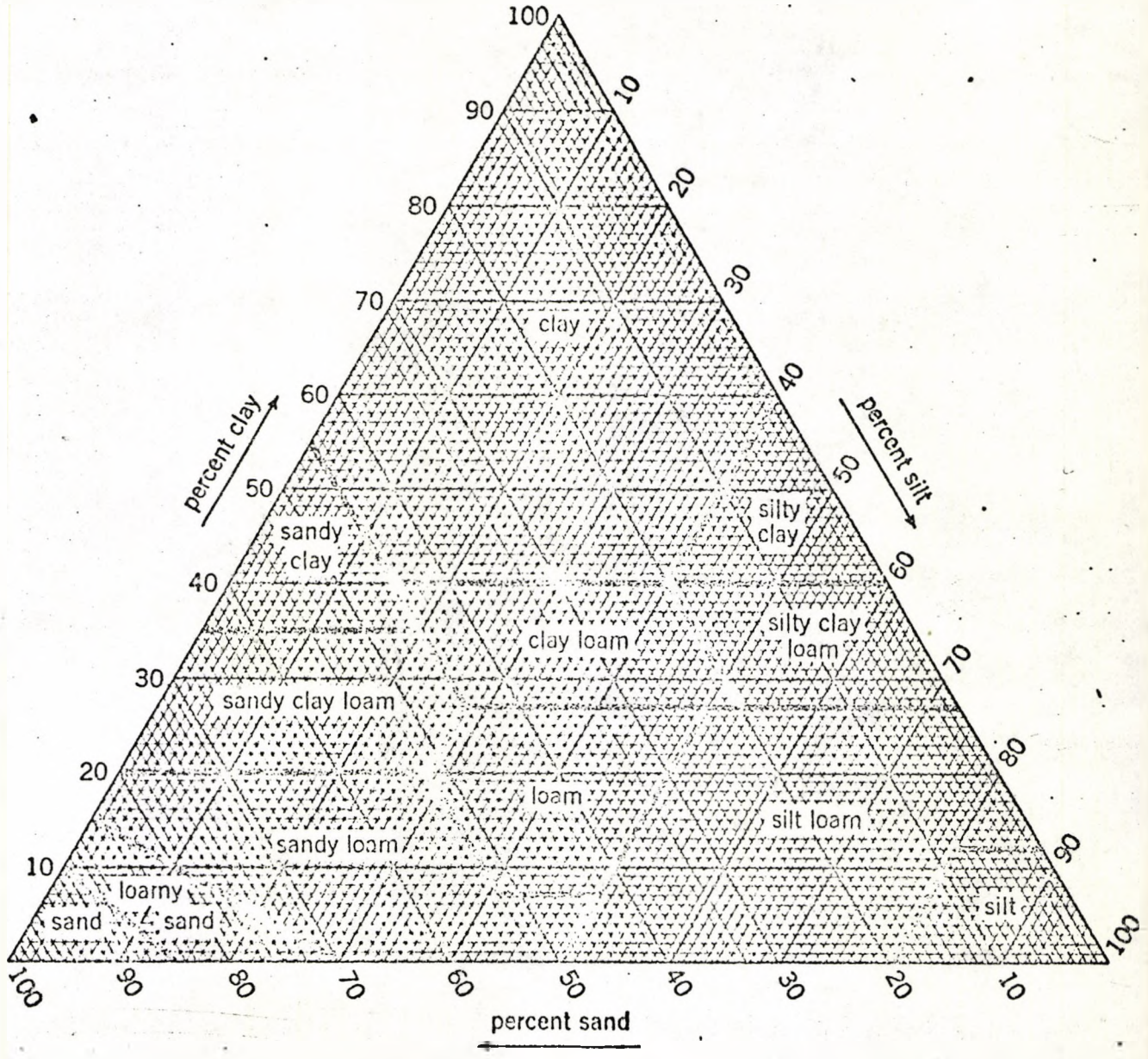
The hydrometer was calibrated so that corrected readings are referable to gram of soil material in suspension. Sand particles settle to the bottom of the cylinder within 40s and thus the first reading is of the combined silt and clay fraction remaining in suspension. The weight of sand was obtained by subtracting the hydrometer reading from the total weight of the sample. After two hours the corrected hydrometer reading represents grams of clay remaining in suspension. The quantity of silt was estimated indirectly by subtracting the sum of the clay and sand from the total soil weight. For each degree above or below 20^{o}C a factor of 0.36 was added to or subtracted from the hydrometer reading. The texture of the soil was classified by reference to a "textural triangle" (Figure 2.1).

2.1.2. Moisture Content and Water Holding Capacity.

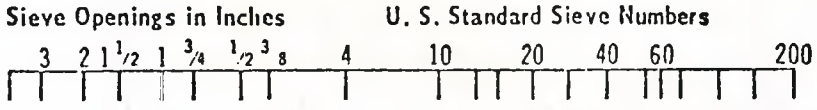
2.1.2.(a) Moisture Content.

Air dried soil (10g) was placed in a tared, dried aluminium dish. The container of soil was kept in an oven at 105^{o}C for 24h, cooled in a desiccator and re-weighed.

Figure 2.1 **GUIDE FOR TEXTURAL CLASSIFICATION** (Pramer & Schmidt,1964)



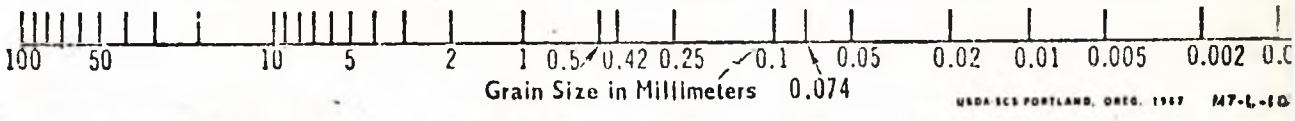
COMPARISON OF PARTICLE SIZE SCALES



USDA	GRAVEL	SAND					SILT	CLAY
		Very Coarse	Coarse	Medium	Fine	Very fine		

UNIFIED	GRAVEL		SAND			SILT OR CLAY
	Coarse	Fine	Coarse	Medium	Fine	

AASHO	GRAVEL OR STONE			SAND		SILT – CLAY	
	Coarse	Medium	Fine	Coarse	Fine	Silt	Clay



USDA SCS PORTLAND, OREG. 1947 M7-L-ED

2.1.2.(b) Water Holding Capacity.

A moist filter paper disc (Whatman No.1) was placed on the grid of Hilgard soil cup and the whole weighed. The cup was filled with oven dry soil and compacted by dropping the cup a few times through a distance of approximately 3cm. The soil surface was levelled with a spatula and the cup was re-weighed. The cup was placed in a dish containing water of sufficient depth to wet the bottom of the soil column. When the soil was saturated the cup was placed in humid enclosure until drainage was completed and then re-weighed.

The water holding capacity was calculated as the quantity of water retained per unit weight of dry soil (ml/g).

2.1.3. Soil pH.

Air dried soil (20g) was taken in a 250ml beaker and mixed with 20ml of distilled water. Twenty minutes were allowed to elapse for the paste, which was occasionally stirred, to reach equilibrium. The pH was measured with a Pye-Unicam model 292 pH meter and electrode.

2.1.4. Soil Organic Matter.

2.1.4.1. Total Organic Matter.

A porcelain crucible was heated in a Bunsen flame to red heat for 10 min, cooled in a desiccator and weighed. The crucible was half-filled with oven dry soil and re-weighed. The crucible with contents was placed on a pipestem triangle and heated with a moderate Bunsen flame; the intensity of the flame was slowly increased until a red glow was evident within the crucible. The crucible was rotated and the contents periodically stirred with a mounted needle and maintained at red heat for 30 min. The crucible was placed in a desiccator to cool and then

re-weighed.

2.1.4.2. Determination of Soil Carbon, Hydrogen and Nitrogen.

Samples of dry soil, or other materials were analysed in a Hewlett Packard Model 185 C-H-N analyser coupled to a Kent Chromalog 3 Integrator (University of Kent Chemical Laboratory, Analytical services). Results are quoted as percentage of each element in the dry material.

2.1.4.3. Determination of Soil Phosphorus.

Samples of dry soil, or other material, were analysed (University of Kent, Chemical Laboratory, Analytical services) by combustion, using the oxygen flask method (Macdonald, 1961). All forms of phosphorus present in the sample are oxidized to inorganic phosphate and this is titrated with thorium nitrate. From the titres and reference to standards the total phosphorus in soil was calculated.

2.1.4.4. Fractionation of Organic Matter.

The procedure adopted was described by Sinha (1972). Air dried soil (10g) was suspended in 100ml of acidified water pH 2.0 (1:10 w/v) in polyethylene centrifuge bottles. The bottles were shaken (flask shaker, Gallenkamp) for approximately 18h at room temperature. After 18h extraction, the soil suspension was centrifuged at 2000 rev/min for 30 min. in a Mistral 6 1. centrifuge (M.S.E.).

The supernatant was filtered through glasswool and the filtrate extracted with ether 3:1 (v/v), thrice in a separating funnel. The ether soluble extract was collected in a beaker containing dried magnesium sulphate (A.R.), stirred well and left at room temperature for the precipitate to settle. The

supernatant was decanted into a round bottomed flask and evaporated on a rotary evaporator (Gallenkamp) under reduced pressure on a water bath at 60°C.

The immiscible aqueous fraction was adsorbed on a pad of activated charcoal 1cm thick supported on a sintered glass funnel (Pyrex No.4, 5-10µm pore size). The charcoal pad was previously thoroughly washed with 0.1N hydrochloric acid. The charcoal was then eluted with 15% (v/v) ethylalcohol which was removed under reduced pressure; the residue was extracted with ethanol (100%) and the latter was evaporated to dryness on a rotary evaporator under reduced pressure at 40°C. Finally the charcoal column was eluted with 0.1N sodium hydroxide until the eluate was colourless.

The residue (soil) in the polyethylene bottles was extracted with 0.1N sodium hydroxide (1:10 w/v) on a flask shaker for 18h then centrifuged as above. The extracted soil was filtered under reduced pressure through a sintered glass funnel (Pyrex No.4, 5-10µm porosity) and further extracted (x2) with 0.1N sodium hydroxide (1:10 w/v) as above. The filtrates were pooled, acidified with concentrated sulphuric acid to pH 1.0, and allowed to stand for 24h to precipitate. After 24h the clear supernatant was siphoned off (Fulvic acid fraction). The precipitate (Humic acid fraction) was redissolved in 0.1N sodium hydroxide, twice acidified with concentrated sulphuric acid and the clear supernatant acid solution siphoned out into the same beaker. The humic acid precipitate was centrifuged at 4000 rev/min for 30 min (M.S.E. Super Minor). The centrifugate was mixed with the above supernatants and the humic precipitate was washed thrice with distilled water.

The humic acid fraction was freeze dried (Edwards-High Vacuum, Crawley, Sussex, England) and weighed.

The fulvic acid (supernatant) was fractionated by elution chromatography on a column of activated charcoal by the method of Forsyth (1947). The fulvic acid, the pH of which was adjusted to 2.5-3.0, was adsorbed onto an activated charcoal pad; all the colour was adsorbed. The charcoal was eluted with 0.1N hydrochloric acid and the elute added to the filtrate; this was designated fraction A.

The charcoal pad was then eluted with 90% (v/v) acetone in water until the eluted solvent was colourless; this was designated fraction B.

Next deionized water was sucked through the charcoal pad. The extract was a clear colourless liquid and extraction continued until it no longer produced a precipitate on addition of excess acetone. This was termed fraction C.

The organic matter remaining absorbed onto the charcoal was then completely eluted with 0.5N sodium hydroxide. The extraction was continued until the eluate was colourless. This was termed fraction D.

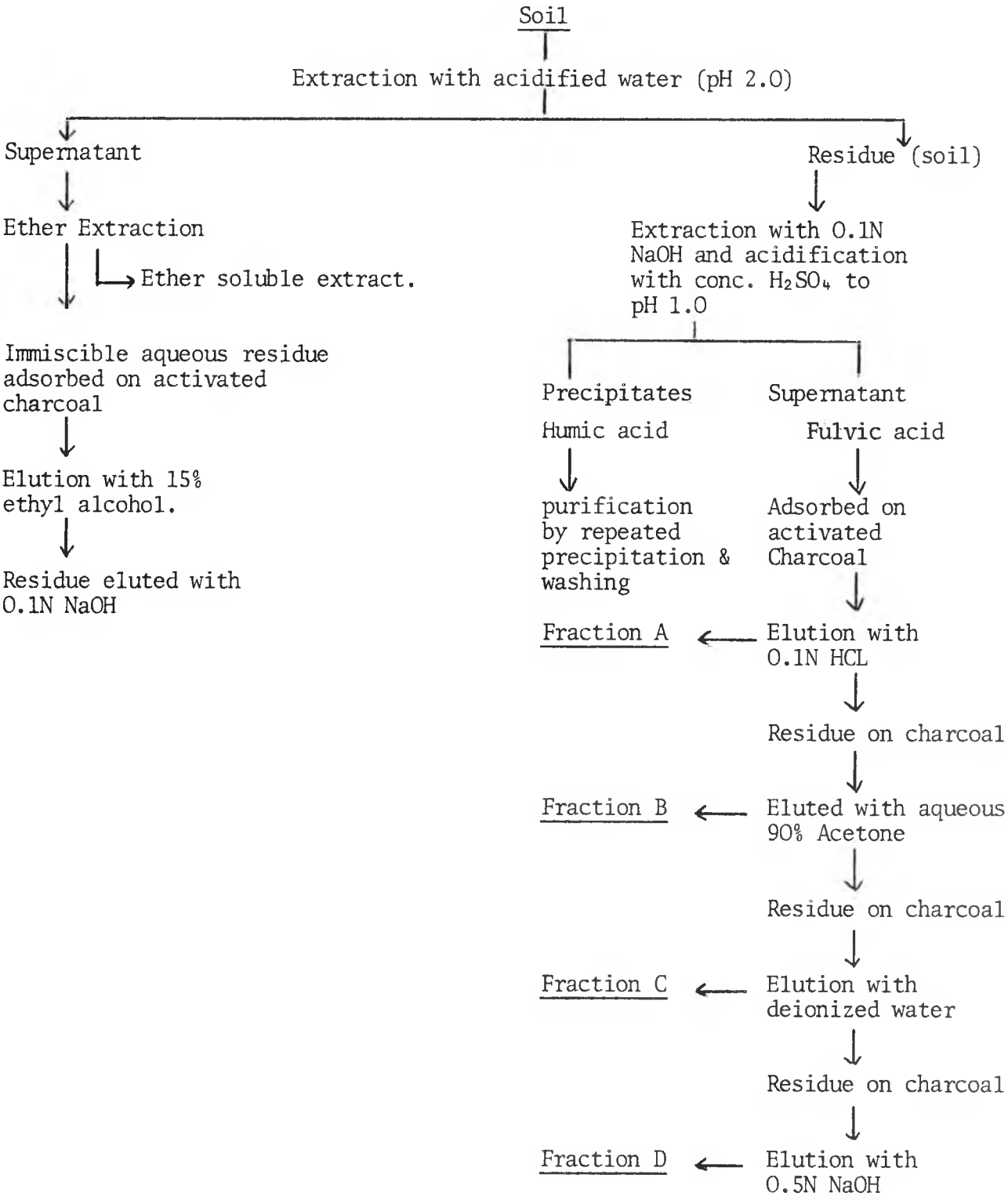
Fractions were freeze dried as above, weighed and stored in a desiccator for subsequent analysis.

The organic matter fractionation scheme is summarized in Figure 2.2.

2.1.4.5. Soil Polysaccharides.

Carbohydrates were extracted from the soil by the method described by Cheshire and Mundie (1966). Weighed quantities of air dried soil were placed in 24N sulphuric acid (5:1 v/w)

Figure 2.2. Protocol for Soil Organic Matter fractionation based on Sinha(1972)



in stoppered 250ml polypropylene centrifuge bottles. The bottles were shaken for 16h and after extraction the mixture was diluted to 1N sulphuric acid and heated at 100°C on a water bath for 8h. The cooled hydrolysate was separated from the soil residue by filtration through a sintered glass funnel (Pyrex No.3).

A small aliquot of the hydrolysate was neutralized with 6N sodium hydroxide and analysed for total reducing sugar by the Nelson Somogyi method using glucose as a standard.

(a) Deionization of the Hydrolysate.

Deionization method was described by Cheshire, Mundie & Shepherd (1969). Excess barium carbonate (A.R.) was added to the hydrolysate and the mixture stirred magnetically until the pH was about 5.0. The suspension was filtered through a sintered glass funnel (Pyrex No.3) and the precipitate thoroughly washed with water. The filtrate washings were combined and passed through an acid washed, hardened filter paper (Whatman No.542) and concentrated by rotary evaporation under reduced pressure at 40°C. The final volume was 5ml.

(b) Paper Chromatography.

About 5-10µl solutions of sugar standards and of the concentrated hydrolysate were applied with a micropipette along a line about 12cm from the end of a sheet (57 x 20cm) of Whatman No.1 chromatography paper; the opposite end of the paper was serrated to permit the solvent to drip off evenly. Descending irrigation was used for 24h with the solvent system pyridine:ethyl acetate:water, 5:12:4 (v/v). The development of separated sugars was made by drawing

the dried paper through a solution of silver nitrate in acetone (1g A.R. silver nitrate in 2ml water and diluted with 200ml acetone) (Trevelyan, Proctor and Harrison, 1950). After drying, the paper was drawn through methanolic potassium hydroxide solution (5g potassium hydroxide in 10ml water, diluted with 200ml of methanol). Spots could be made permanent by soaking the paper in a dilute solution of sodium thiosulphate followed by washing in water. After drying, the centre of each spot was marked and its mobility (distance from the origin) was compared with respect to glucose which was given an RG value of 1.00.

The amount of each sugar present was measured quantitatively by passing the Chromatograms through a Chromoscan Mark 2 (Double-Beam Recording and Integrating Densitometer, Joyce Loebel & Co., Ltd., England) and referring the peak areas to those produced by known concentrations of authentic sugars.

2.1.5. Microbial Count.

Soil samples (1g) were suspended in 100ml sterile distilled water, and dispersed by shaking for 30min. Further 10-fold serial dilutions were made as appropriate, with sterile distilled water. One ml of serially diluted soil suspension was pipetted into a sterile plastic petri dish and 15ml of the required molten agar medium, at 45°C was then added. Plates were rotated to distribute the soil suspension throughout the medium (Pramer & Schmidt, 1964). The agar was allowed to solidify and the plates were incubated in the inverted position at 25°C for a week, or longer for slow growing colonies, and the colony counts made in the standard manner.

The following media were used:

(a) Soil Extract Agar.

Glucose	1g
K_2HPO_4	0.5g
Ion agar No.2	20g
*Soil Extract	100ml
Water	900ml
pH	6.5-7.0

Sterilized by autoclaving at 15 lb/in^2 for 15 min.

(b) Chitin agar (Lingappa & Lockwood, 1962)

⁄Colloidal Chitin	2.0g
KH_2PO_4 (anhydrous)	0.4g
$MgSO_4.7H_2O$	0.05g
NaCl	0.1g
$FeCl_3.6H_2O$	5µg
$MnCl_2.4H_2O$	5µg
$CO.Cl_2.6H_2O$	5µg
Ionagar No.2	20g
Distilled water	1000ml

The medium was steamed to dissolve the agar and autoclaved at 15 lb/in^2 for 15 min.

(c) Potato Dextrose Agar.

Oxoid potatodextrose agar was used.

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* Soil extract was prepared by heating 1000g of soil with 1000ml of tap water in an autoclave for 30min. Approximately 0.5g of calcium carbonate was added and the soil suspension filtered through a double layer of filter paper. The turbid filtrate was refiltered until the extract was clear.

⁄ Colloidal chitin was prepared as follows: Crude unbleached chitin (Koch-Light) was washed alternately for 24h at a time with 1N sodium hydroxide and 1N hydrochloric acid (usually 5-6times) then with 95% ethanol (3-4 times) to remove foreign matter. This process removed about 40% of the original material and gave a white product. This clean chitin (15g) was moistened with acetone and dissolved in 100ml of cold concentrated hydrochloric acid by stirring for 20min in an ice bath. The thick syrupy solution then was filtered with suction through a thin glass wool pad in a Buchner funnel into a two litres of stirred ice cold distilled water. Chitin was precipitated as a fine colloidal suspension. The residue was redissolved in conc.HCl and refiltered usually three or four times until little or no chitin was precipitated. The colloidal chitin was alternately sedimented by allowing it to stand and washed in 5 litres of distilled water. The material was stored in a refrigerator.

2.1.6. Enzyme Activities in Soil.

2.1.6.1. Urease (EC 3.5.1.5.)

The method used was based on that described by McGarity and Myers (1967). Air-dried soil (10g) was taken in 50ml volumetric flask, 2ml toluene were added, and the mixture incubated for 15 minutes at room temperature to permit complete penetration of toluene into the soil. Following the addition of 10ml potassium citrate-citric acid buffer 0.1M, pH 6.7, and 5ml 10% (w/v) solution of urea, the flask was shaken and incubated at 37°C for six hours. Control flasks in which urea was replaced by 5ml of distilled water were set up concurrently.

After incubation the flask contents were made up to 50ml with distilled water, the toluene forming a layer above the graduation mark. The flasks were thoroughly shaken and their contents filtered through Whatman No.6 filter paper.

Urease activity was determined as ammonia released using the indophenol blue method for ammonium ions.

Procedure:

Soil filtrate (1ml) was taken in a 50ml volumetric flask and diluted with 9ml distilled water. Freshly prepared phenolate reagent (5ml) and sodium hypochlorite solution (3ml) were added. The flask contents were thoroughly mixed and left for 20 min at room temperature. The volume was then made up to 50ml with distilled water. The extinction at 630nm was measured in 1cm cuvettes in a Unicam SP 500 Spectrophotometer. The amount of ammonia -N formed was calculated by reference to a calibration curve constructed from A.R. ammonium sulphate standard solutions (0-100µg NH_3-N/ml). All samples were read against a reagent blank and heat inactivated enzyme controls were also included.

Calculations:

1ml of soil filtrate corresponds to 200mg of soil, urease activity was given per 100g soil, i.e. milligrams ammonia-N released from urea by 100 gram soil.

2.1.6.2. β-1,3 Glucanase (EC.3.2.1.6.)

The method used was based on that of Chesters and Bull (1963) and adapted by the present author for soil activities. Air dried soil (10g) was placed in a 50ml volumetric flask and incubated at room temperature for 15 min with toluene (2ml). Phosphate citrate buffer 0.05M, pH 5.8 (10ml) and 1% (w/v) Laminaran dissolved in 0.05M phosphate citrate buffer pH 5.8 (5ml) were added and the flasks shaken and incubated at 37°C for six hours. Controls in which laminaran was replaced by 5ml of distilled water were included with each series of assays.

After incubation the flask contents made up to 50ml with distilled water, the toluene forming a layer above the graduation mark. The flasks were thoroughly shaken and their contents filtered through Whatman No.6 filter paper.

Reducing sugars released as a result of β-1,3 glucanase activity were determined as glucose with low alkalinity copper reagent (Somogyi, 1952) and the arsenomolybdate chromogen of Nelson (1944).

Procedure:

Soil filtrate (1ml) was taken in test tube and 1ml of freshly prepared copper reagent added. The tubes, closed by aluminium foil, were placed in a covered, boiling water bath for 20 min. The tubes were removed, cooled and 1ml of freshly prepared arsenomolybdate reagent added, the solution was

thoroughly mixed on a rotamixer (Hook & Tucker, Ltd.). After five minutes the solution was diluted with 10ml water and again mixed thoroughly. The extinction at 520nm was read in 1cm cuvettes in a Unicam SP500 Spectrophotometer. The amount of reducing sugar released was obtained from a standard curve (0-100µg glucose/ml). All samples were read against reagent blank and heat inactivated enzyme controls also were included. β-1,3 Glucanase activities were calculated and expressed as described for urease (see 2.1.6.1. above).

2.2. Production of Fungal Mycelium.

The large scale production of fungal mycelium for amending soils was made by growing the hyaline mutant 13 mel of *Aspergillus nidulans* (Bull and Faulkner, 1964) in a glucose-limited chemostat in the chemically defined medium of Carter and Bull (1969). Cultivation conditions and fermenter specifications were those described by Rowley and Bull (1973). The chemostat was operated at a dilution rate of $0.10h^{-1}$ and steady state organism concentration of 5g dry weight per litre. The mycelium was collected in a 20 1. bottle, recovered and washed by centrifugation (M.S.E. 6L Mistral, 4000 revs/min, 30 min) and stored at $-20^{o}C$ until required.

2.3.1. Preparation of ^{14}C-Labelled Mycelium.

^{14}C-glucose (uniformly labelled with a specific activity of 281mCi/mmol) was obtained from the Radiochemical centre, Amersham, Bucks.

Sterile chemically defined medium containing 1% glucose (Carter and Bull, 1969)(400ml) was taken in a sterile 1 l. conical flask and 10µCi uniformly labelled ^{14}C-glucose was

added aseptically. The flask was inoculated with *Aspergillus nidulans* mycelium obtained from a chemostat culture (see 2.2) at 5% (v/v). Such cultures were set up in a 30°C constant temperature room and stirred continually on a magnetic stirrer (Gallenkamp). The flask was fitted with a two-holed rubber bung through which 6mm diameter bent glass tubes were passed. Through one tube was passed a slow stream of sterilized, carbondioxide free air and carbondioxide produced by the culture was absorbed in a 0.5N sodium hydroxide trap attached to the outlet tube; therefore, ^{14}C-CO_2 was not allowed to contaminate the atmosphere.

2.3.2. Measurement of ^{14}C incorporated into Mycelia.

A known quantity of washed ^{14}C-labelled mycelium was suspended in sterile distilled water and mascerated in a ground glass homogenizer. The mascerated mycelium was sonicated 3 x 1 min using a 150W M.S.E. Ultrasonic Disintegrator. A liquid scintillation counting procedure based on the methods of Daviesand Cocking (1966) and Bull (1968) was used to determine the radioactivity of sonicated mycelial suspensions. The scintillator cocktail consisted of 2,5-Diphenyloxazole (P.P.O) (3g) plus 1,4-Di{2-(5-phenyloxazolyl)} benzene (POPOP) (0.3g) in 1 l. toluene (scintillation grade). All reagents were purchased from Koch-Light Laboratories Ltd. Colnbrook, Bucks, England.

Aliquots of sonicated mycelial suspensions (0.1ml) were pipetted (Oxford Laboratories Sampler) onto 2.1cm diameter Whatman Glass fibre discs (GF/A) mounted on stainless steel pins, 6cm below a 60W lamp and dried for 45min. Dried discs were placed horizontally in the bottom of low potassium

glass 20ml counting vials and over layered with 1ml scintillator cocktail. The vials were dark adapted for 20 minutes before being counted in a model 3314 Packard "Tri Carb" - Liquid Scintillation Spectrometer (green channel, Zero amplification) at -5°C. 0.1ml water dried discs were used for background counts. All treatments were carried out in triplicate.

2.4. Production of Fungal Spores.

For certain experiments large quantities of fungal spores were required. These were prepared by growing surface cultures of *Aspergillus nidulans* 13 mel and harvesting the Conidia.

Molten malt extract agar (Oxoid Ltd.) was dispensed aseptically into large petri dishes (150mm x 20mm) to a depth of about 5mm. Each petri dish was inoculated by spraying a suspension of Conidia on to the surface of the agar. The plates were incubated at 30°C for 8 to 10 days after which the spores were harvested carefully by washing them off the mycelium with gentle scraping; subsequently they were then washed with sterile distilled water and passed through sterile cheese cloth. Mycelial debris was retained by the cloth and the spores in the filtrate were collected by centrifugation and washed twice with sterile distilled water.

2.5. Design and Operation of Continuous-flow Soil Column.

The continuous-flow column reactor used in this work was a modification of the design developed by Macura (1961). Various components of the apparatus were constructed in the Chemical Laboratory Workshops, University of Kent at Canterbury.

The arrangement of the equipment is shown in Figures 2.3 and 2.4. The apparatus consists of the following principle components:

(a) Medium reservoir and feed.

(b) Soil column.

(c) Effluent receiver.

(d) CO_2- absorption system.

(e) Soil aeration system.

(a) Medium reservoir and feed:

Solutions containing substances whose transformation or effects were to be investigated were contained in 10 l. Mariotte bottle (1). The Mariotte bottle provides an inexpensive and reliable means of regulating medium flow rates independently of the quantity of nutrient remaining in the reservoir. The neck of the bottle was tightly stoppered by a two holed rubber bung through which was passed a 6mm diameter glass tube (2), the lower end of which was widened to a diameter of 40mm, sealed off and a small hole, about 2mm in diameter, pierced near the base; this hole was aligned with the bottom outlet of the bottle. The upper end of the tube was fitted with a bacteria-proof filter (Microflow, Fleet, Hants) and a soda lime tube for absorption of CO_2. Through the other hole of the bung was passed a 6mm diameter bent glass tube fitted with rubber tubing and clamped off; this acted as a pressure release valve during autoclaving.

Through the bottom outlet of the Mariotte bottle was passed a 6mm diameter stainless steel tube, the outer end of which was connected with silicone rubber tubing (3) to a

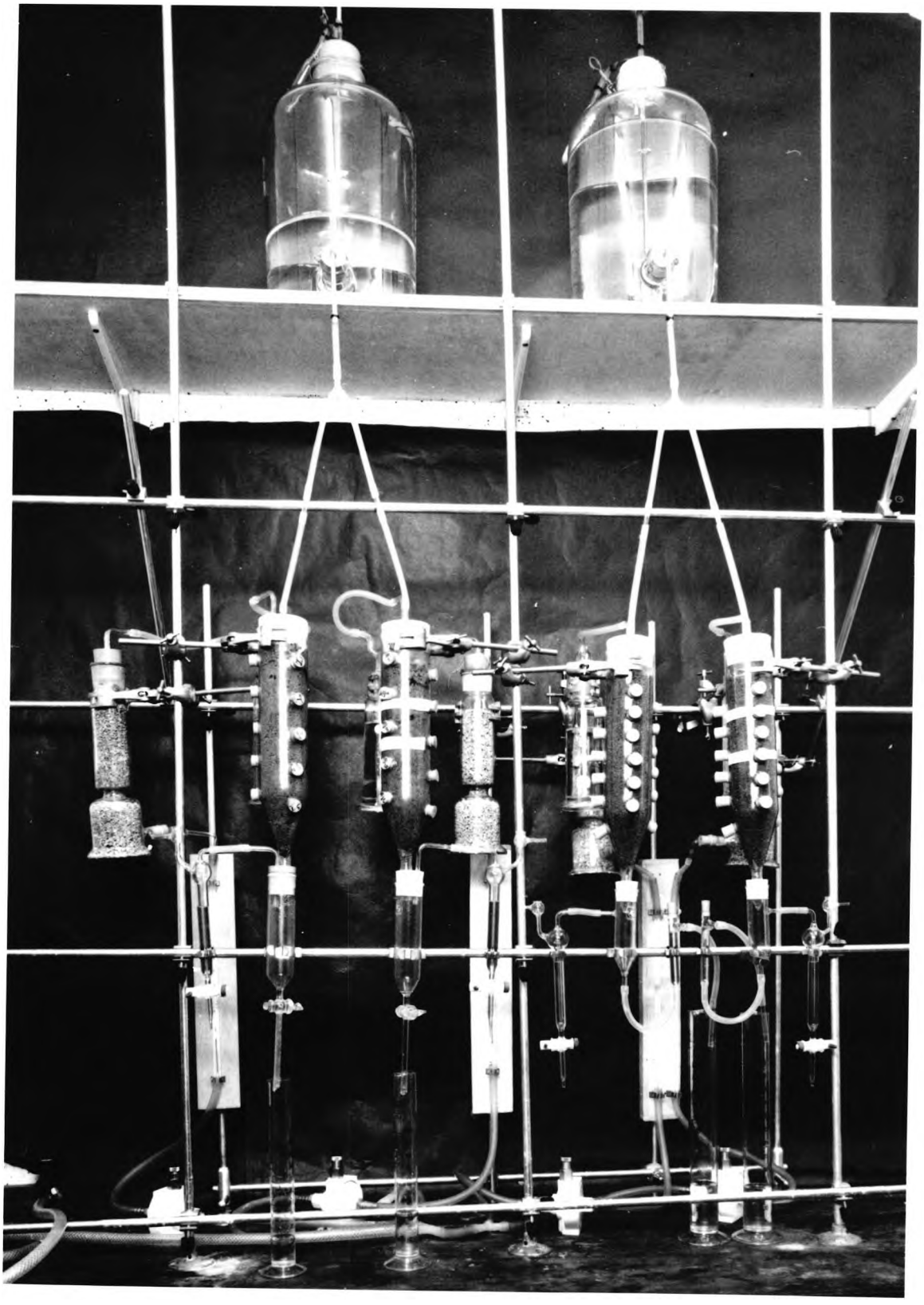


Figure 2.3. Apparatus used for studying microbiological processes in soil by means of the continuous flow method.

Figure 2.4.

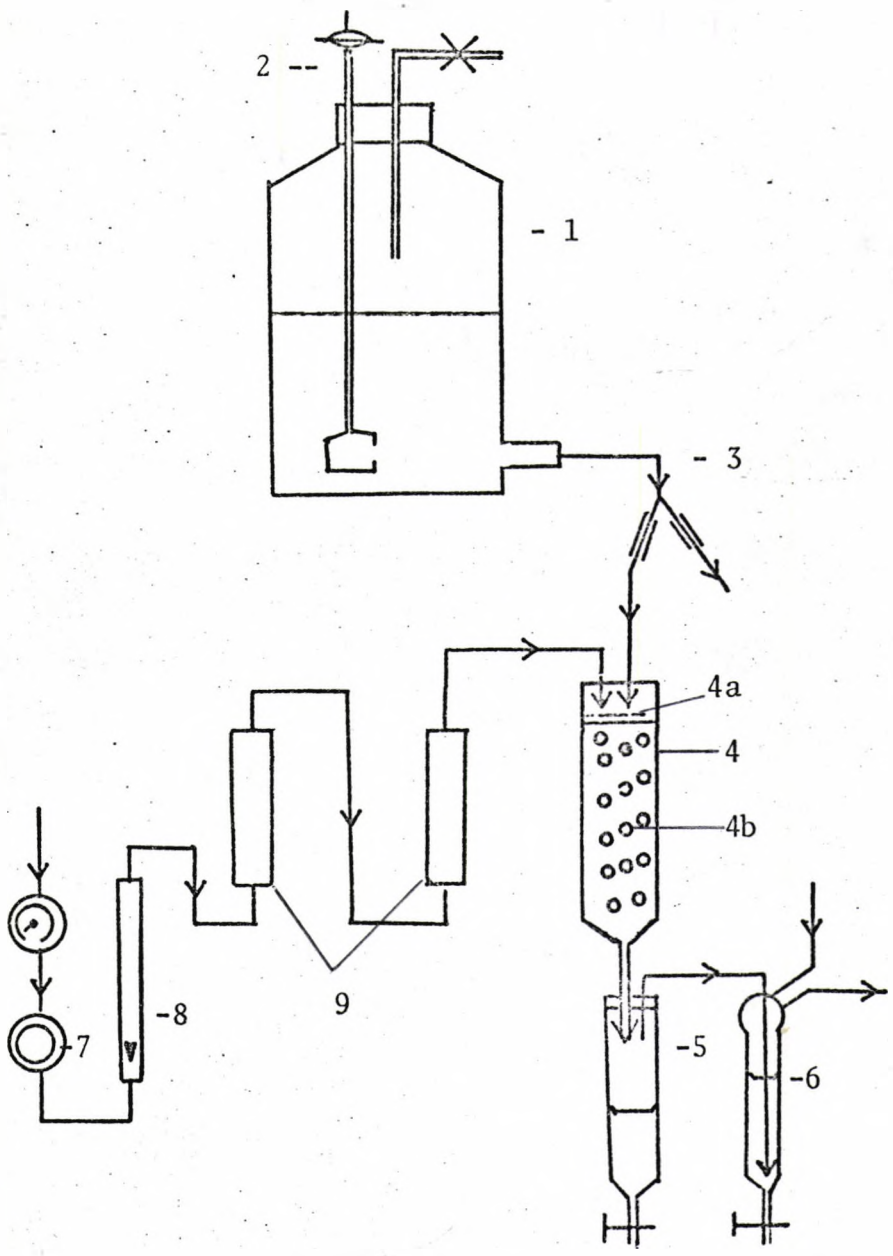


Figure 2.4. Schematic representation of the apparatus illustrated in Figure 2.3.

1. Mariotte bottle (liquid reservoir)
2. Glass tube for establishing constant hydrostatic pressure.
3. Silicone rubber tubing with resistance capillary.
4. Soil column.

4a. Nylon gauze.

4b. Sampling ports.

5. Effluent receiver.
6. CO_2-trap.
7. Flowstat for control of air flow rate.
8. Rotameter.
9. Gas Scrubbers.

calibrated resistance capillary (Jobling Laboratory Division, Stone, Staffordshire, England) for the control of medium flow rate. The other end of the capillary tube was connected via silicone rubber tubing to the soil column.

(b) Soil Column:

The reactor column (4) consists of a glass cylinder, 6cm in diameter and 35cm in length, narrowed at the lower end to about 1cm. A number of sampling ports were made in the tube and arranged in a spiral manner, approximately 4cm apart on a verticle line. The ports were closed by rubber bungs. The upper broad section of the column was fitted with a two holed rubber bung through which passed one 6mm in diamter glass tube connecting with the medium reservoir and another glass tube through which gasses were introduced into the soil column.

Partially air dried, root- and stone-free soil (Section 2.1) was sifted through a number of sieves (Gallenkamp) of mesh size ranging from 5.8mm to 1mm. Columns were packed with soil (500g), in the form of structural aggregates 2-5mm in diameter. The soil in the column was placed on a thin layer of glasswool and the upper surface of the soil was covered by a layer of nylon gauze to prevent stirring up of the soil particles by the inflowing solution.

The soil was amended with *Aspergillus nidulans* hyaline mycelium in the middle of the column in a zone approximately between 4 to 10cm region from the top, to simulate the natural condition of the soil profile. Soil sampling from the various regions of the column was made by means of clean stainless steel tubes (1cm diameter, 10cm long) and soil, approximately 2g, was extruded with a plunger directly into sterile universal

bottles. Sample removal did not have any noticeable deliterious effects on the flow characteristics of the soil column. The columns were shielded from light by covering completely with black paper to prevent the development of photosynthetic organisms.

(c) Effluent receiver:

The effluent receiver (5) consisted of a glass dropping funnel (100ml) which was fitted to the outlet of the soil column. Liquid and gaseous effluents both passed into this receiver; liquids could be removed as required from the base of the funnel and from an exhaust at its top gasses were carried to an absorption tube.

(d) CO_2-Absorption System:

Carbondioxide evolved by microbial activity in the soil was absorbed in a tube (6) containing 0.5N sodium hydroxide. The absorption tube was narrow glass section 1cm in diameter, the lower end of which was fitted with a PTFE stopcock to enable the removal of alkali. The upper end of the absorption tube was blown to form an expansion chamber fitted with two outlets. Through one of the latter passed a narrow capillary tube which reached almost to the bottom of the tube and through which effluent gas was bubbled into the alkali solution. The other outlet was used for replenishing alkali in the tube and served as a vent for unabsorbed gasses.

(e) Soil Aeration System:

Gasses of known composition were fed to the soil column at flow rates controlled and monitored by a flowstat (7) (G.A. Platon Ltd., Basingstoke, Hants, England) and a rotameter (8) (2-25 ml/min air 15°C, 760mm Hg. G.E.C. Elliott, Process Instruments Ltd., Croydon, England) system. The

gasses fed to the soil column could be freed of carbon-dioxide by passage through drying columns (9) containing soda lime and of ammonia or oxygen by passage through washing bottles containing potassium hydroxide, sulphuric acid or alkaline pyrogallol.

2.6. Analytical Methods.

2.6.1. Estimation of total CO_2 evolved.

Carbondioxide absorbed in absorption tube was determined by differential titration with a standard solution of hydrochloric acid, using phenolpthalein and screen methyl orange respectively as indicators. An aliquot of sodium hydroxide containing absorbed CO_2 was pipetted into a 50ml conical flask and a drop of phenolpthalein indicator added. The solution was titrated against 0.5N HCl until colourless when a drop of screen methyl orange was added and the titration continued until the green colour changed to grey and finally to purple. The final burette reading was recorded.

2.6.2. Estimation of $^{14}CO_2$.

Aliquots (0.1ml) of $^{14}CO_2$ absorbed sodium hydroxide were pipetted onto 2.1cm glass fibre discs and dried for about 30min under a 60Watt lamp. The dried discs were placed in the bottom of a counting vial and overlayered with one millilitre of scintillator cocktail. The vials were dark adapted for 20 min and counted in triplicate. The counting conditions were those specified in Section 2.3.2.

2.6.3. Determination of Carbohydrate.

The method used was that described by Herbert, Phipps and Strange (1971). One millilitre sample or standard

glucose solution was pipetted into thin-walled boiling tubes (6 x 1in). The tubes were cooled in ice-water. When cooled, 5.0ml of cooled anthrone reagent was added from a fast flowing pipette, swirling the tube during the addition. The tubes were allowed to stand a few minutes to equilibrate at $0^{o}C$ then transferred to a vigorously boiling water bath. After exactly 10 min the tubes were returned to the ice bath, and, after cooling, the extinction of the solutions at 625nm was measured in a Unicam SP500 Spectrophotometer. All samples were read in duplicate against a reagent blank.

The amount of carbohydrate was estimated by reference to a calibration curve (0-100µg standard glucose/ml).

2.6.4. Estimation of Ammonia.

The method used was that described by Hanna (1964). Aliquot (1ml) of sample or standard solution was pipetted into an acid washed test tube and the following additions made: 1ml 2% (w/v) Gum acacia, 1ml Nessler's reagent and 7ml distilled water. After mixing well on a rotamixer the solutions were allowed to stand for exactly 30 min for the yellow colour to develop. The extinction at 420nm was measured in a Unicam SP 500 Spectrophotometer. The amounts of ammonia-N formed were estimated by reference to a calibration curve using (0-100µg ammonia-N as A.R. $(NH_4)_2SO_4$/ml). All samples were read in duplicate against a reagent blank.

2.6.5. Estimation of Nitrate.

The method described by Bremner(1965) was used. Aliquot (1ml) of the sample were pipetted into clean 100ml beakers and 9ml distilled water and 0.75mg of A.R. calcium carbonate

added, mixed well. The solutions were evaporated to dryness on a Gallenkamp 12-place water-bath. After cooling 2ml of phenoldisulphonic acid reagent (Kodak Chemicals) were added from a rapid delivery pipette. The beakers were rotated so that phenoldisulphonic acid came into contact with the entire residue and then allowed to stand for 10min at room temperature. Distilled water (20ml) was added and further standing for 10min occurred. A 1:1 ammonium hydroxide:water solution (20ml) was added, mixed well and the solution transferred to a 100ml volumetric flask and made up to the mark with distilled water. The extinction at 420nm of the well mixed solution was measured in a Unicam SP 500 Spectrophotometer.

The amount of nitrate-N was estimated by reference to a calibration curve (0-100µg nitrate-N as A.R. KNO_3/ml). All samples were read against a reagent blank.

2.6.6. Estimation of Nitrite.

The modified Griess-Llosvay method described by Bremner (1965) was used. One ml of sample was pipetted into an acid washed test tube with 8ml distilled water and 1ml Griess-Llosvay reagent. The solution was mixed well with a clean glass rod and allowed to stand for exactly 30 min. A reddish purple colour developed and its extinction at 540nm was measured in a Unicam SP500 Spectrophotometer, against a reagent blank.

The amount of nitrite-N formed was calculated by reference to calibration curve (0-5µg nitrite-N as A.R. potassium nitrite/ml).

2.6.7. Estimation of Phosphorus.

The method of Olsen and Dean (1965) was used. Aliquots (15ml maximum) containing 2 to 20µg P were pipetted into separatory funnels with 5ml molybdate solution and the volume made up to 20ml with distilled water. Isobutyl alcohol (10ml) was added and the funnel shaken for 2 min. After standing for 5 min the aqueous layer was discarded and the isobutyl alcohol layer shaken for 1 min with 10ml 1N H_2SO_4. The aqueous layer was discarded and the organic solvent layer shaken with 15ml stannous chloride solution (2g/l) for 1 min; the aqueous layer was discarded. The blue isobutyl alcohol layer was transferred to a 25ml volumetric flask to which were added the ethanol washings from the separatory funnel; the volume was made to 25ml with ethanol. After 30 min the extinction at 720nm was measured in a Unicam SP500 Spectrophotometer against reagent blank.

The amount of phosphate -P formed was estimated by reference to a calibration curve (0-22µg phosphorus/ml).

CHAPTER 3

EXPERIMENTS AND RESULTS

3.1. SOIL DESCRIPTION.

The soil from which samples were taken for laboratory experiments was poorly stratified. Thus, examination of soil profiles showed that there was little or no trace of litter (L, Aoo) or mull (F + H, Ao) sublayers. Similarly, distinction between A and B horizons was not clearly defined and there were few signs of substantial leaching. The combined A and B horizon extended to a depth of approximately 35cm below which was revealed the heavy clay C horizon. The C horizon contained large numbers of flints and flint fragments of various sizes and shapes. The upper 10cm of the profile was extensively ramified by grass roots while the roots of other plants mostly extended to a depth of ca. 20cm. Earthworms were found throughout the profile and penetrated the C horizon to a depth of ca. 45cm.

Whole-profile soil cores were sampled from various parts of the collection site and were used for physico-chemical and microbiological studies. The total organic matter and the "bulk" pH of the soil at various depths was recorded and the results are presented in Figure 3.1.1. The soil was acidic throughout its depth but the pH varied by little more than 0.5 unit down the profile. In contrast total organic matter concentrations declined with increasing soil depth; they were high in the presumed A horizon (10-12%) and then fell by nearly 50% in the presumed B horizon. The upper region of the clay subsoil also contained organic matter and was penetrated by earthworms.

Microbial populations colonising different parts of the soil profile were estimated by dilution plate counting as

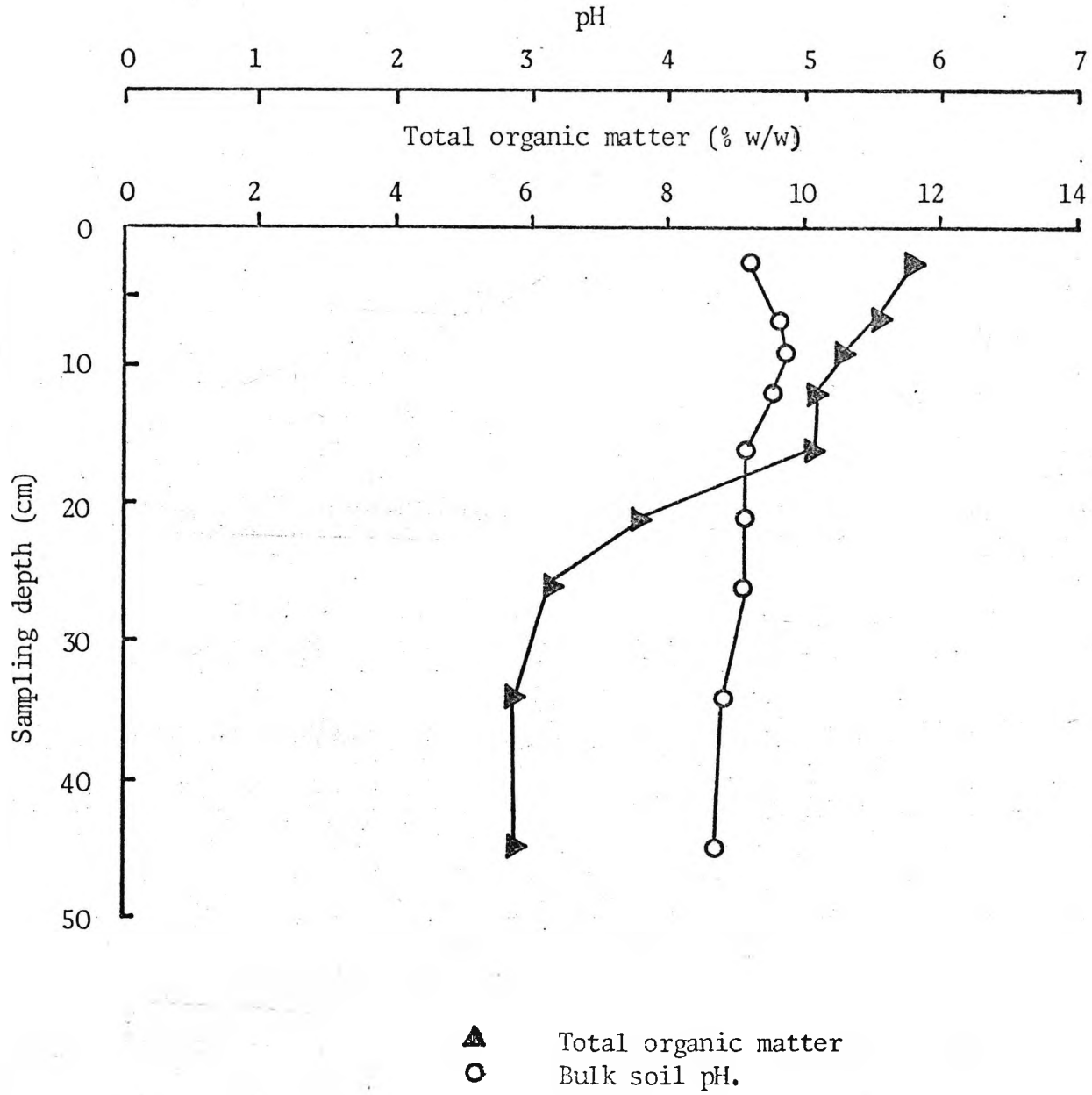


Figure 3.1.1. pH and distribution of organic matter in a soil profile constructed at the sampling site.

described in Section 2.1.5. Eubacteria were counted on soil extract agar, actinomycetes on chitin agar and microfungi on potatodextrose agar plates. Figure 3.1.2. illustrates the distribution of the microbial flora throughout the soil profile. The highest population numbers occurred in the top 10 cm of the A horizon and correlated with maximum organic matter concentration, whereas the C horizon showed the lowest number of microbes. Generally, the largest number of fungi and other microorganisms are found in the upper soil horizon and their numbers decreased with increasing depth in the soil (Alexander, 1961).

The greatest activity of marker enzymes of organic matter transformations was recorded in the upper 15cm of the soil (Figure 3.1.3). These distributions paralleled those of the organic matter concentration and microbial numbers.

Because of the markedly higher activity of soil enzymes, the size of the microbial populations and the organic matter concentration, further analyses of physicochemical properties of the soil were confined to the 0-25cm deep region and average values for the various parameters derived. The soil was sampled in bulk as described in Section 2.1 and measurements made of the mineral particle size, the total organic matter, the water-holding capacity and "the bulk" pH. Results of these analyses are presented in Table 3.1.1. Reference to a soil texture diagram and the content of organic matter identified the soil as a sandy loam. Moreover, the absence of distinct horizons strongly suggested that the soil had been under agricultural cultivation during recent times.

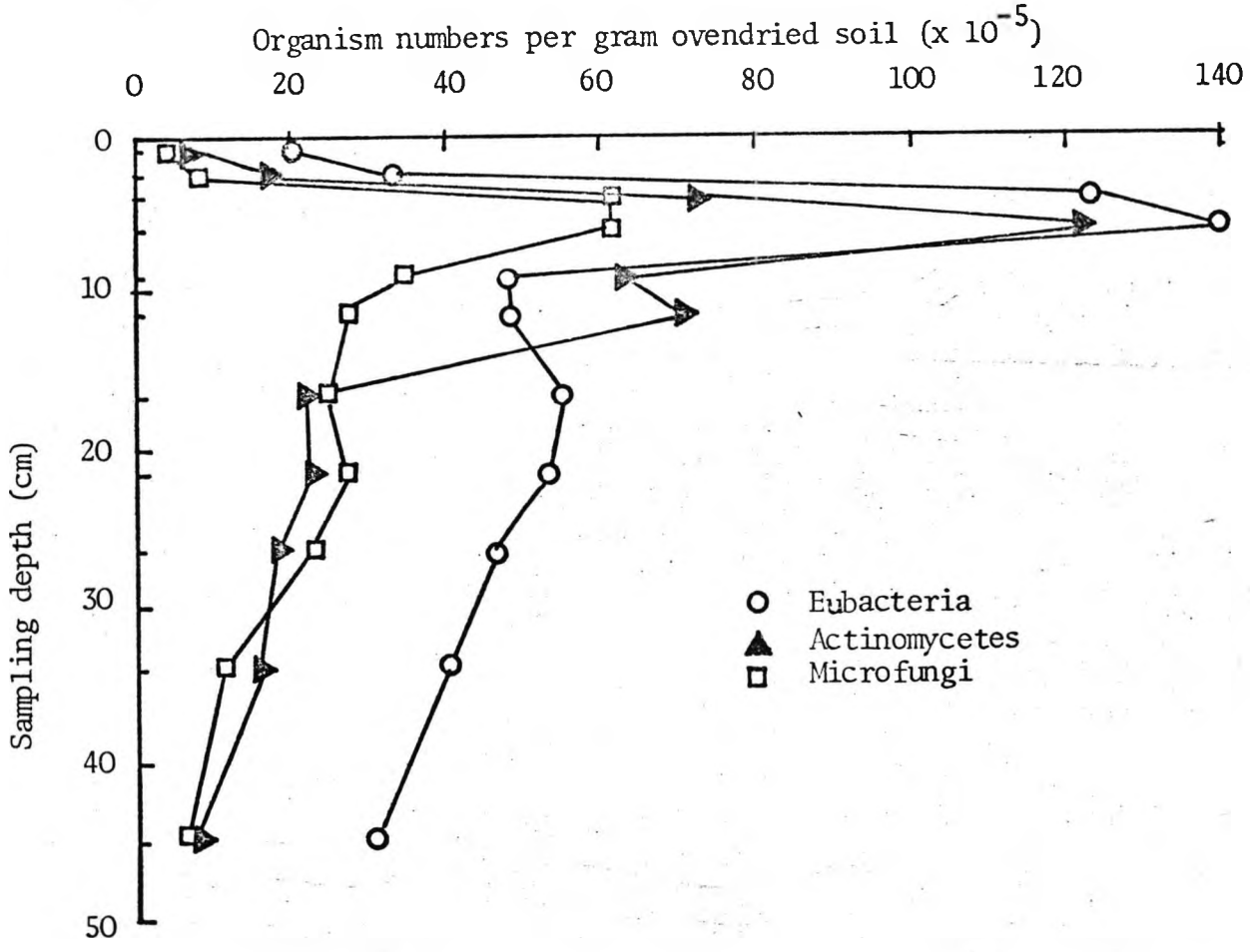


Figure 3.1.2. Distribution of Microbial populations in the soil profile constructed at the sampling site.

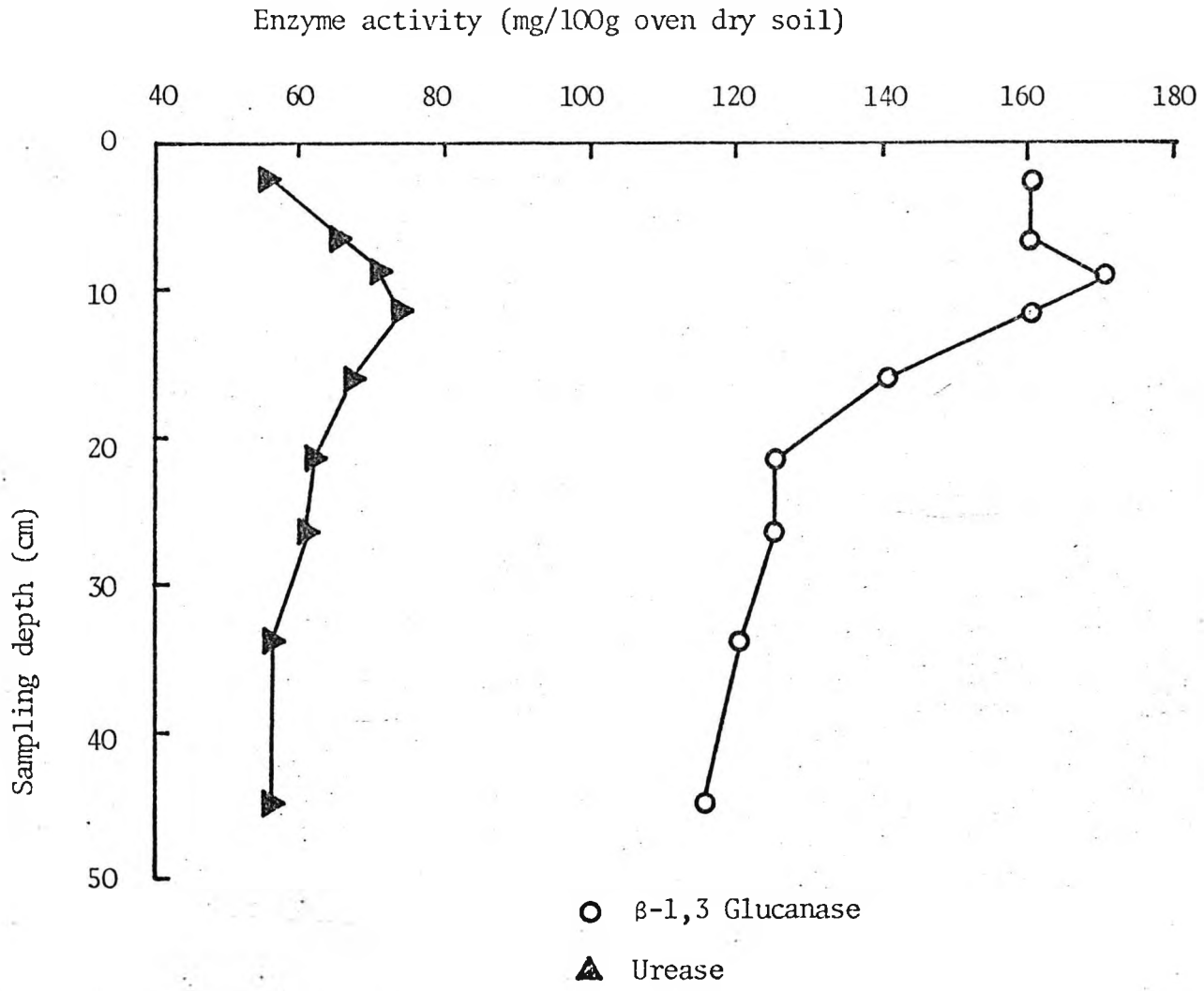


Figure 3.1.3. Distribution of enzyme activities in the soil profile constructed at the sampling site.

Enzyme activities are expressed as mg product released per 100g soil under standard conditions of assay (see Section 2.1.6).

TABLE 3.1.1.

Physicochemical Properties of the Experimental Soil

Particle Category (%, w/w)	Water holding Capacity (%, w/w)	pH	Organic matter (%, w/w)	Humic acids (%,w/w)	Fulvic acids (%,w/w)	Elemental Analysis (%, w/w)		
						Whole soil	Humic acids	Fulvic acids
Sand 55						C 4.36	5.81	10.48
Silt 26	65	4.70	11	7.92	0.25	H 1.21	0.57	0.88
Clay 19						N 0.36	0.32	0.91
						P nd	nd	1.91
± 1.25	± 0.25	± 0.10	± 0.10					
(6)	(6)	(6)	(6)					

nd : not detected

Values in parenthesis indicate number of determinations.

Note: These data are averages for the top 25 cm of soil (A + B horizons).

3.2. PHYSICAL FACTORS AFFECTING THE TURNOVER OF FUNGAL MATERIAL IN SOIL.

Introduction.

Microorganisms in soil are exposed to conditions that are variable both in space and in time. Therefore, the microbial communities of different ecosystems and of microhabitats within the same ecosystem differ from one another to greater or lesser extents. The composition and the activity of microbial communities in soil are determined by the physical and chemical characteristics of the environment. The moisture content, degree of aeration and temperature are the major physical factors in soil that determine microbial activity and several studies have been reported of their effects on the transformation of organic materials in soil (Chase & Gray, 1957; Birch, 1958; Greenwood, 1961; Sauerbeck, 1966; Sinha, 1972). All biological activities and interactions are dependent on water, the availability of which in turn is dependent, in part, on the soil structure and especially on the soil porosity. The bacterial activity in soil with respect to moisture content has been widely studied on an empirical basis, and generally, a good correlation has been found; microbial activity increases with increasing water content to about 60 to 80 percent of the water-holding capacity, conversely activity generally decreases in water-logged soils (Seifert, 1960; 1961). Changes in soil moisture status also affect other groups of microorganisms, a particularly dramatic example being revealed by Meiklejohn (1957) during the course of severe drought in Kenya. Under drought conditions the proportion of actinomycetes, that initially accounted for less than 30% of the microflora, rose

until they made up more than 90% of the viable population. It was suggested that the dominance of the actinomycetes resulted from the resistance of their conidia to desiccation.

Drying followed by remoistening of soil generally is a prelude to a transitory flush of microbial activity as measured in terms of either carbon or nitrogen mineralization. This spurt of activity has been attributed to the exposure of unavailable or inaccessible substrates to the microflora as a result of the drying and wetting sequence (Birch, 1958). Birch found that a characteristic and universal pattern of carbon mineralization occurred when dry soil was moistened; an initial, rapid decomposition stage was followed by the establishment of a slow, steady rate of breakdown. Furthermore, he observed that this pattern was repeated following successive dryings and wettings and that the magnitude of decomposition was a function of the carbon content of the soil. Birch also made the point that similar mineralization kinetics occurred under field conditions and he concluded that drying-wetting cycles were of primary importance in the run down of soil carbon.

Insight into the dynamics of soil carbon mineralization can best be obtained from a consideration of soil crumb structure. Various models of soil crumb structure have been proposed among which may be mentioned those of Emerson (1959), Greenwood (1967) and Nikitin (1973). The model of soil crumbs constructed by Greenwood is given in Figure 3.2.1. The aspects of the model that are of greatest interest are (1) the size of the aggregate, (2) the size of the pores, (3) whether the pores are gas filled or water filled, (4) the nature of mineral and organic components and (5) the ease of aggregate disruption.

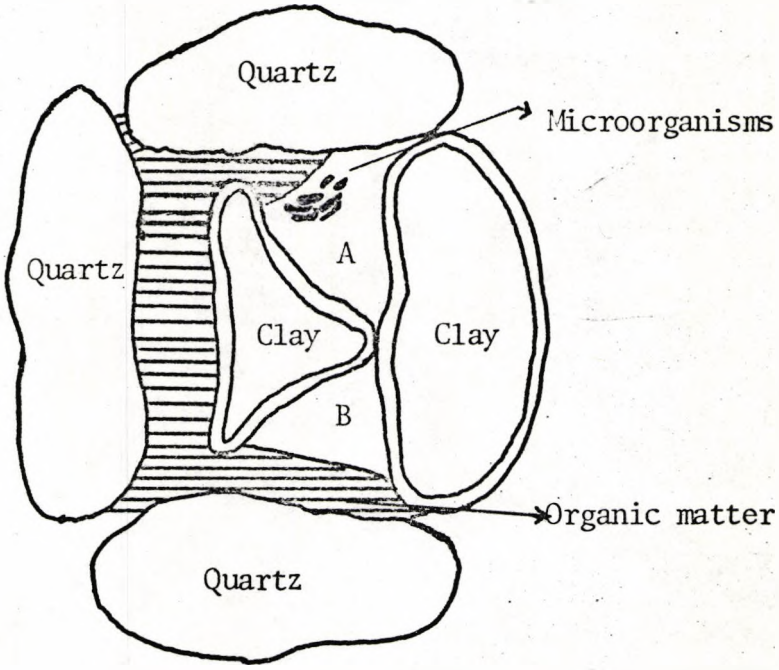


Figure 3.2.1. Model of soil aggregate.

The model depicts two water filled pores, A and B, only one of which contains organisms producing enzymes that degrade organic matter. None of the extracellular enzymes that are produced are likely to reach pore B because they would be adsorbed onto the surface of clay minerals and hence become inactivated (Skujins, 1967). Similarly, the microorganisms in pore A could not reach pore B because they are too large to traverse the connecting capillary. However, if the soil was dried and then rewetted, the resulting contraction and swelling would cause the clay domains and quartz particles to be displaced relative to one another. Such displacement would cause organic matter hitherto protected by clay or quartz to be exposed, enzymes adsorbed onto soil colloids to be brought into contact with fresh substrates, microbial surfaces hitherto in contact with other microbial surfaces to come into contact with organic matter and might even permit organisms to move between pores, i.e. from A to B. In every case these changes would create conditions leading to a greater rate of breakdown of organic matter.

Oxygen availability is particularly important to the distribution and metabolism of microorganisms in soil, the level of oxygen in the microenvironment varying considerably with the surrounding pore space, moisture content and the quantity of readily degradable organic carbon (Alexander, 1964). Greenwood (1961; 1962) examined the effect of soil oxygen levels on the aerobic and anaerobic transformation of carbon and on nitrate dissimilation. The balance between aerobic and anaerobic conditions in soils is delicately poised and Greenwood has estimated that a change from one type of metabolism to the other takes place when the oxygen concentration becomes less than ca. 3×10^{-6}M. Greenwood & Berry (1962) suggested that water saturated soil crumbs of about 3mm or more in radius would have anaerobic centres even if they were surrounded by air.

During aerobic decomposition of glucose continuously added to the soil only small amounts of volatile fatty acids, pyruvic acids, α-ketoglutaric acid and gluconic acid were produced

(Macura & Malek, 1958). On the other hand, during the breakdown of carbohydrates in the absence of oxygen, considerable quantities of organic acids accumulate in soil and the rate and extent of mineralization of the carbonaceous materials added to soils usually is decreased (Alexander, 1961). It has been suggested that the concentration of CO_2 in the soil atmosphere is significant ecologically, not only because it affects the metabolism of autotrophic organisms and modulates the pH at microsites in soil, but also because of its potential role as a differential inhibitor of heterotrophic growth (Alexander, 1964). However, Stotzky & Goos (1965) showed that soil microbes were, in general, tolerant of high CO_2 and low oxygen concentrations. A stimulatory effect of CO_2 on the rate of wheat straw decomposition was postulated by Parr & Reuszer (1962).

Temperatures below about 5°C or above 30°C will severely limit the growth of the predominantly mesophilic soil microflora (Gray & Williams, 1971). In many soils, temperatures seldom reach levels required by the majority of the soil microbes for optimum rates of growth, but it is possible that local, short-term increases of temperature occur at sites of intense microbial activity. Thus, Clark, Jackson & Gardner (1962) recorded temperature rises of 5.3°C in the vicinity of organic residues added to soil.

The aim of experiments described in this Section was the analysis of aerobiosis and anaerobiosis on transformations of the native organic matter and of fungal material added to soil and incubated under conditions of continuous liquid and gaseous flow. Additionally, the effect of repeated cycles of soil drying and wetting on the rate of mycelial mineralization was investigated. Information from these experiments was considered

to be essential for establishing an activity and kinetic baseline for the design and interpretation of experiments concerned with the effects of agricultural chemicals on fungal turnover in soil.

3.2.1. Mycelial Transformation under Aerobic Conditions.

Standard glass columns were packed with 500g of air dry soil (aggregates, 2-5mm in diameter) as described in Methods and Materials (Section 2.5). In one column the soil was amended with *Aspergillus nidulans* mycelium 16g wet weight, an amount that was equivalent to 0.5% (w/w) of the soil on a dried weight basis. The amendment was made in the middle of the column, approximately in the region of 4-10cm from the upper surface. The packed soil columns were moistened with sterile distilled water, made light proof with black paper to prevent the development of photosynthetic organisms and then clamped into position on frames in a 25°C constant temperature room as described in Section 2.5. Both control and amended soil columns were perfused continuously with CO_2-free air, usually 1200ml.h^{-1} after passing through the gas scrubbers and with sterile distilled water approximately 10ml.h^{-1}. The rate of flow was obtained from the amount of solution passed through the soil and collected in the effluent receptor. The airflow adopted for these experiments is equivalent to an aeration rate approximately twice the "normal field value" (Parr & Reuszer, 1962) and gave a rate of carbon mineralization convenient for study under laboratory conditions.

The transformation of fungal material and estimation of microbial activity in the soil columns were monitored in terms of:

(1) Carbon dioxide evolution which was measured titrimetrically at frequent intervals during the initial period of the experiment and thereafter at regular daily intervals. CO_2 evolution was indicative of the extent of carbon mineralization occurring in the soil.

(2) Population changes in the soil microflora.

(3) Levels of β-1,3 glucanase activity which gave a convenient measure of the mycolytic capacity of the soil.

(4) Humification; at the end of an experiment (usually 4 months or ca. 3000 h) the soil organic matter was fractionated and the flow of fungal residues into stable soil fractions such as humic and fulvic acids was determined.

(5) Soluble carbohydrates; the liquid effluent from the soil columns was tested regularly for the presence of carbohydrates.

RESULTS.

Figure 3.2.2. illustrates the dynamics of CO_2 evolution from mycelium-amended and control soil columns. The rate of respiration of soil amended with mycelium increased rapidly immediately flow conditions were established and reached a maximum on day 5. After this initial transitory peak at high respiration the rate of decomposition slowed down quickly and approached the rate of unamended soil respiration asymptotically. The flow conditions also promoted CO_2 evolution from the unamended soil, indicating that the rate of mineralization of the native organic matter increased in response to the continuous stimulus.

The cumulative production of CO_2 from amended and control soils is shown in Figure 3.2.3. During a period of 120 days the cumulative release of CO_2 from the control and amended soils was 4200 and 6600 mg/500g respectively. The rate of mineralization was 57% higher in the amended soil.

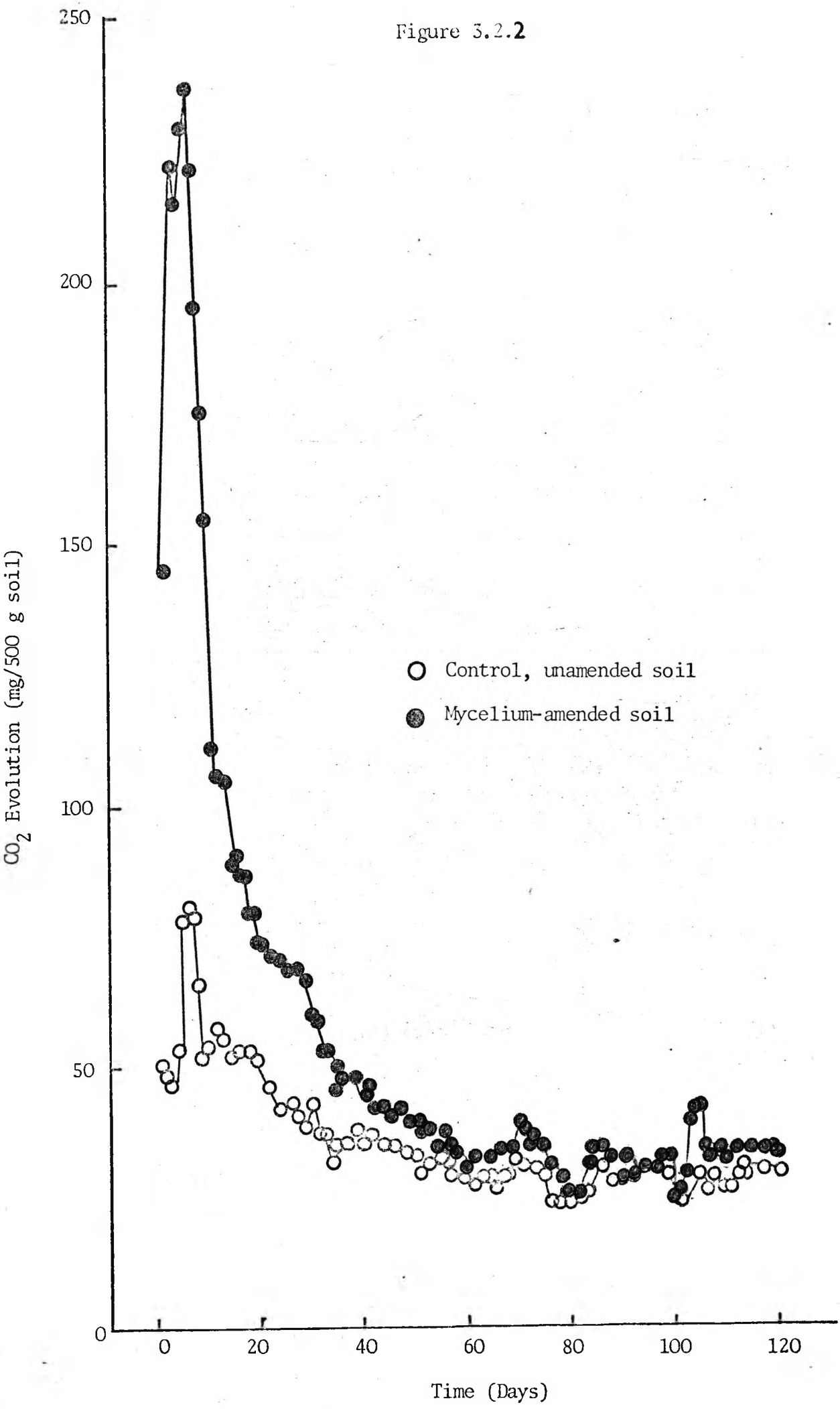
The addition of mycelium to the soil produced a steep, transistory rise in the bacterial and actinomycete numbers (Figure 3.2.4.). This increase in the size of the microflora occurred after the initial peak of mineralization and was followed by an equally sudden decline after 45 days. However, an increasing population size was observed after 80 days of incubation as shown in Figure 3.2.4. The bacterial and actinomycetes population in the control soil remained at constant level throughout the period of the experiment. Figure 3.2.5. illustrates the fact that there was a transitory increase in the fungal population after about 35 days and to a smaller extent after 100 days.

The activity of β-1,3 glucanase in amended soil was high during the initial period (Figure 3.2.6.) and it fell during the early, rapid phase of mineralization and only returned to control levels during the second slower phase of mineralization. The β-1,3 glucanase activities of unamended soil changed little with time and showed an approximate 10% decline over the period of the experiment. There was no detectable carbohydrate in the aqueous effluents from either column.

Water soluble components were not detected in either the control or amended soil; however, both humic and fulvic acid were recovered by alkali extraction of the soil at the end of the experiment. Further analysis of fulvic acid yielded only fraction D. The organic matter remaining adsorbed on to the

Figure 3.2.2. Dynamics of CO_2 production from mycelium-amended and control soils under aerobic conditions during continuous flow of water.

Figure 3.2.2


250
200
150
100
50
0
CO_2 Evolution (mg/500 g soil)
Control, unamended soil
Mycelium-amended soil
0
20
40
60
80
100
120
Time (Days)

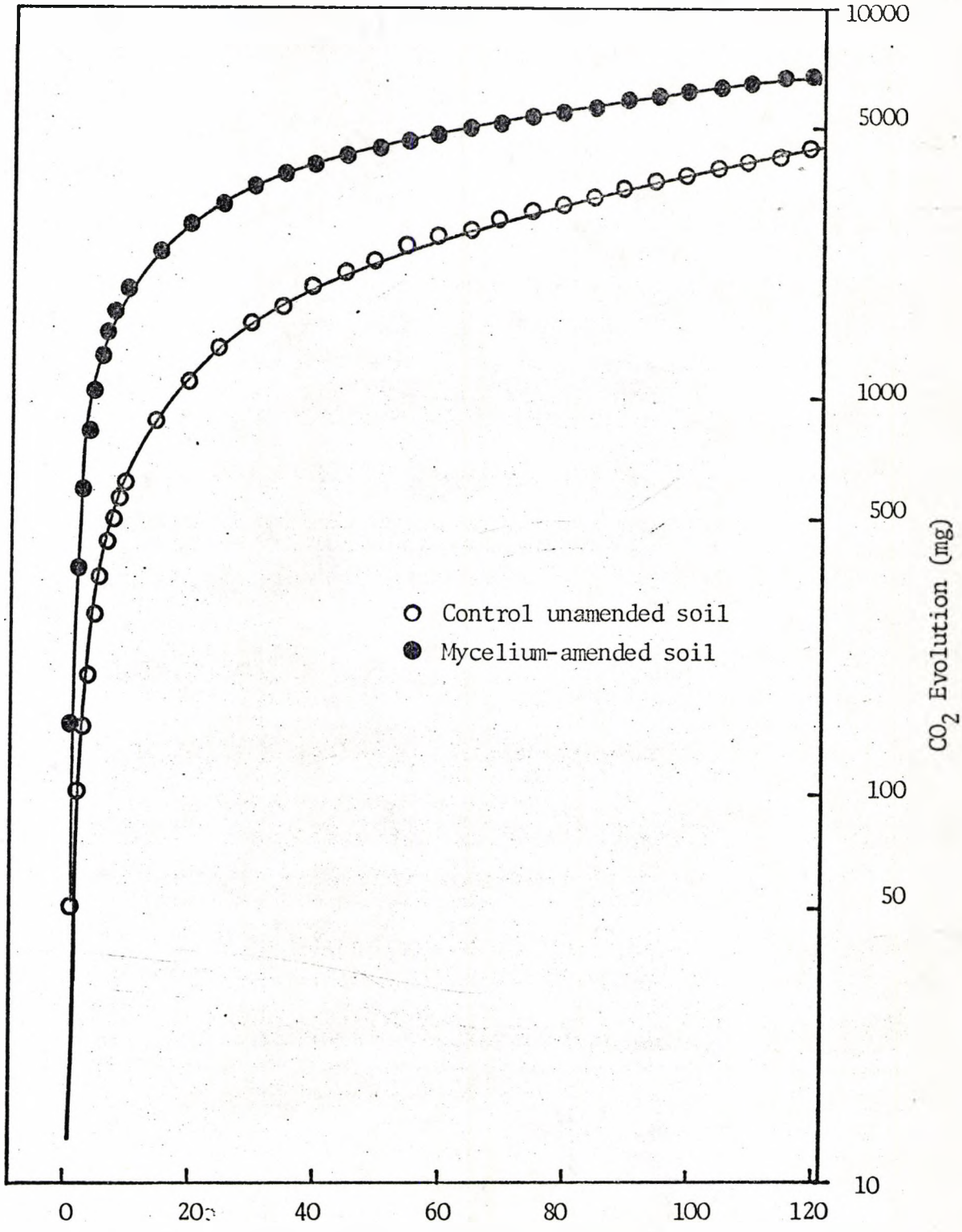


Figure 3.2.3. Cumulative CO_2 production from mycelium-amended and control soils during continuous flow of water; aerobic incubation conditions.

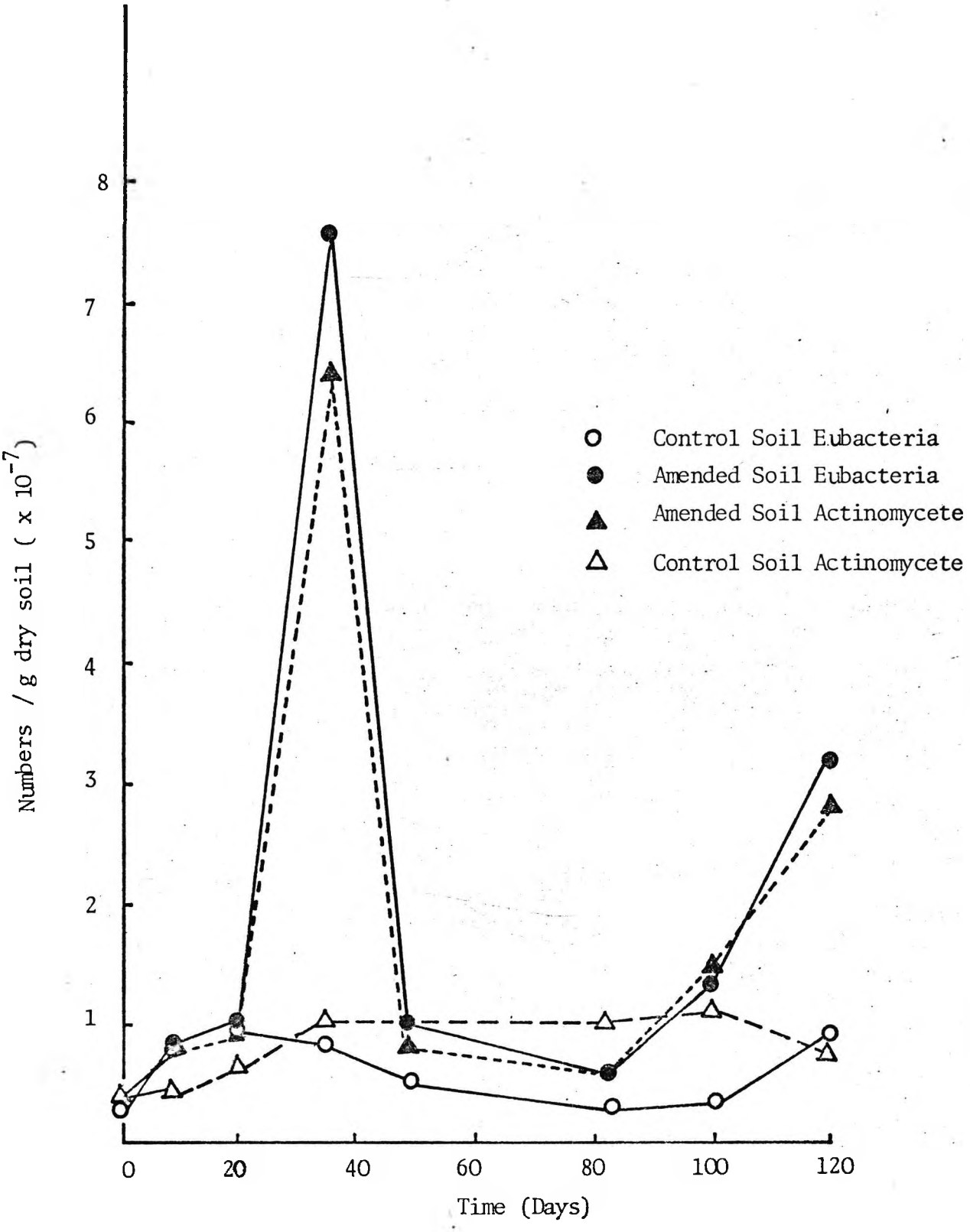


Figure 3.2.4. Changes in Bacterial and Actinomycete populations during continuous flow of water through control and mycelium-amended soil columns; aerobic incubation conditions.

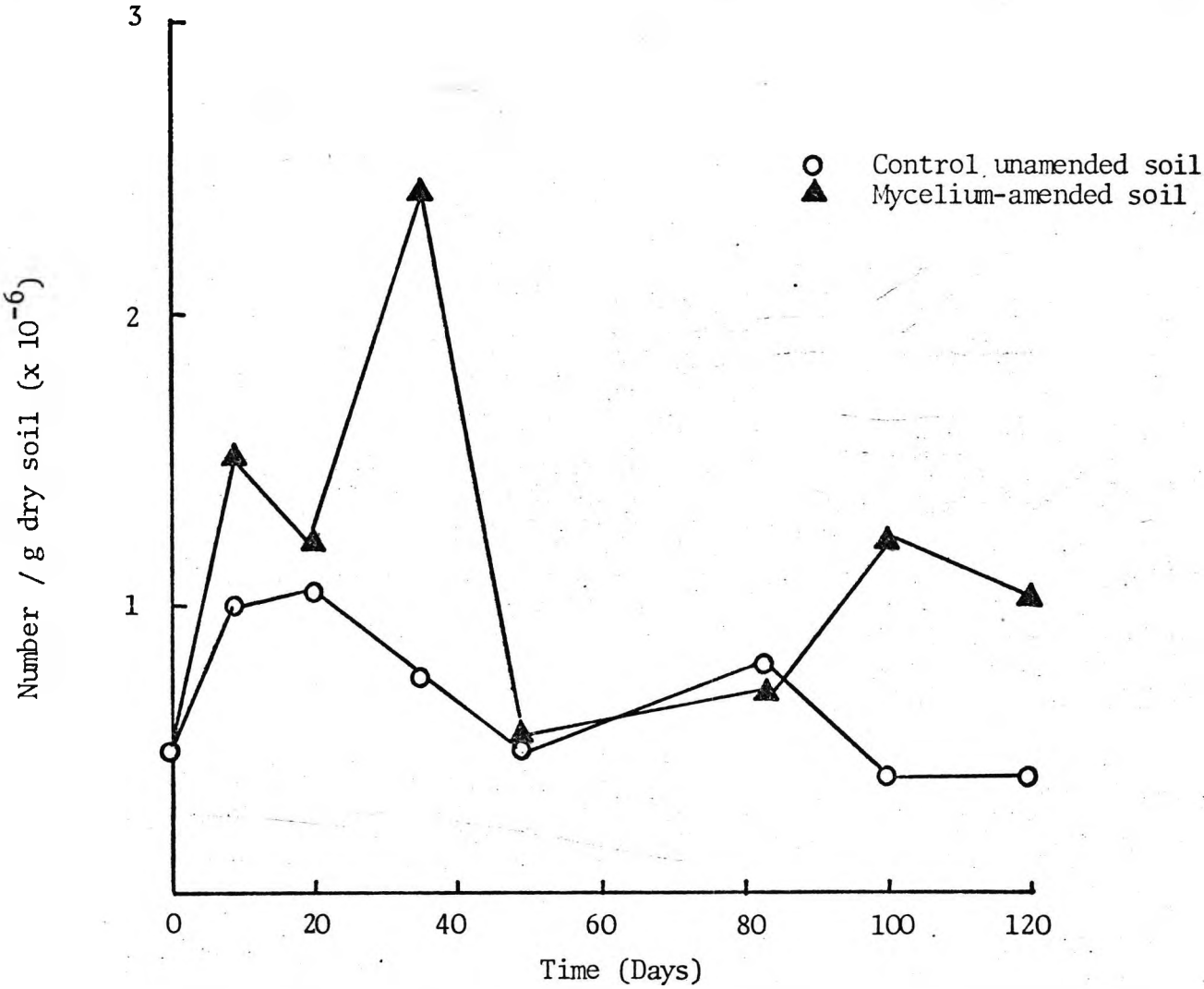


Figure 3.2.5. Changes in Fungal population during continuous flow of water through control and mycelium amended soil columns; aerobic incubation conditions.

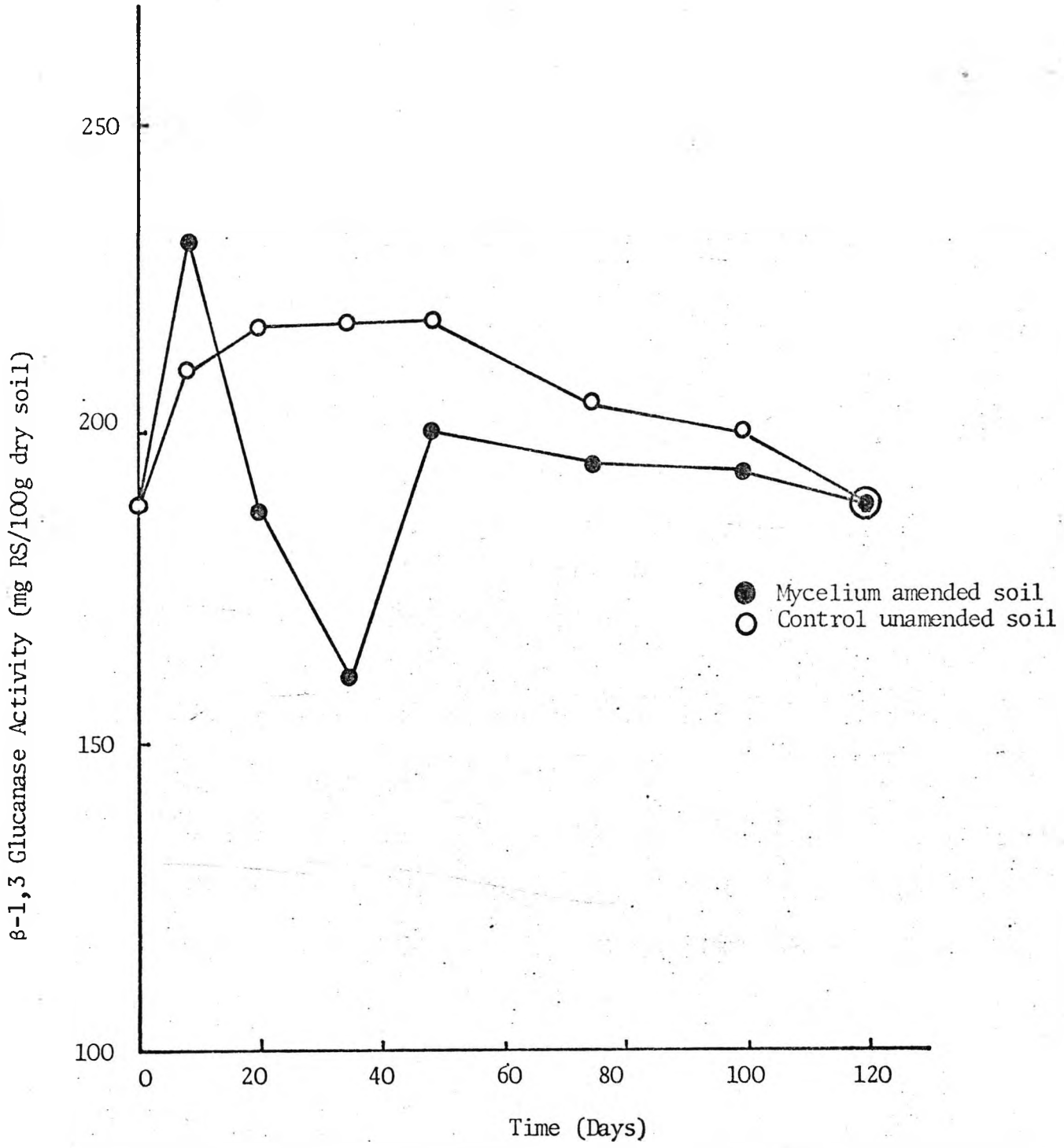


Figure 3.2.6. β-1,3 Glucanase activity in control and mycelium-amended soil columns during the continuous flow of water; aerobic incubation conditions.

TABLE 3.2.1.

Amounts of Humic and Fulvic acids extracted from the control and mycelium-amended soils at the end of incubation period.

Soil Treatment		Humic acids (%,w/w)	Fulvic acids (%,w/w)	Elemental Analysis (%,w/w)					
				Humic acids			Fulvic acids		
				C	H	N	C	H	N
Aerobic	Control [a]	4.14	0.35	9.1	2.27	0.99	12.06	1.70	0.91
	Amended [b]	3.50	0.40	9.6	2.27	1.04	12.47	1.33	0.81
Anaerobic	Control	5.56	0.40	4.15	0.32	0.74	9.85	0.65	1.19
	Amended	4.87	0.40	6.10	1.01	0.41	9.96	0.84	0.93
Drying - Wetting	Control	4.80	0.62	8.16	2.81	0.89	9.97	1.07	1.01
	Amended	4.48	0.52	8.78	2.67	0.91	10.49	0.99	0.83

a) Indicates unamended soil perfused with water under the conditions specified;

b) Indicates mycelium-amended soil perfused with water under the conditions specified.

charcoal pad after the elution with HCl, 90% acetone water and deionized water, was completely eluted with 0.5N sodium hydroxide. The amount of extractable humic and fulvic acids and their elemental analysis are shown in Table 3.2.1. The extractable humic acid fraction showed an approximate 18% increase in control compared to amended soil, whereas fulvic acid showed a decrease of about 14%. There is a slight increase in the carbon content of both humic and fulvic acid of the amended soil compared to the control soil.

3.2.2. Mycelial Transformation under Anaerobic Conditions.

Soil columns, prepared as previously described, were flushed with oxygen-free nitrogen which was scrubbed through wash bottles of alkaline pyrogallol solution. After three days of such pretreatment the soil in one column was amended with mycelium, all operations being done in a "Drypack" chamber filled with nitrogen gas. The column was returned to the incubation frame and it and control soil columns were continuously perfused with oxygen-free nitrogen gas, at a rate of 1200ml.h^{-1}, and sterile distilled water from a reservoir at a rate of 10ml.h^{-1}.

Carbon mineralization and microbial activities in the columns were monitored as described above (see Section 3.2.1).

RESULTS.

A high initial rate of CO_2 evolution was maintained for about 40 days in the amended soil (Figure 3.2.7) but the very sharp peak of mineralization characteristic of amended aerobic soil was not observed. Carbon dioxide evolution from the control soil remained at a low level throughout the experiment.

The cumulative production of CO_2 is shown in Figure 3.2.8;

although carbon mineralization was almost 100% greater in the amended soil as compared with control soil, absolute levels of mineralization were much reduced under anaerobic conditions, that of the native organic matter by one half and that of the added mycelium by one third (Cf Figure 3.2.3).

There was a marked decline with time in the numbers of both bacteria and actinomycetes in control soils under conditions of anaerobic incubation whereas somewhat smaller fluctuations in these populations were observed in the amended soils (Figure 3.2.9). In both instances, however, population sizes were reduced by an order of magnitude compared to those in aerobic soils.

The number of fungal colonies also were recorded in amended and control soils (Figure 3.2.10).

After a brief initial build up in β-1,3 glucanase level in the amended soil the level of activity then fell precipitously by 5-fold (Figure 3.2.11). This pattern of changing enzyme activity resembled that observed in aerobic mycelium-amended soils, i.e. it fell during the first phase and recovered during the second phase of mineralization. However, the fluctuations in enzyme activity were much greater in the anaerobic than in the aerobic soils. Comparable β-1,3 glucanase levels of control soils remained constant as in the aerobic control soil.

Soluble carbohydrates were not detected in the aqueous effluent from the column but considerable amounts of reducing sugars were extracted from the soil per se at various times during the experiment (Table 3.2.2).

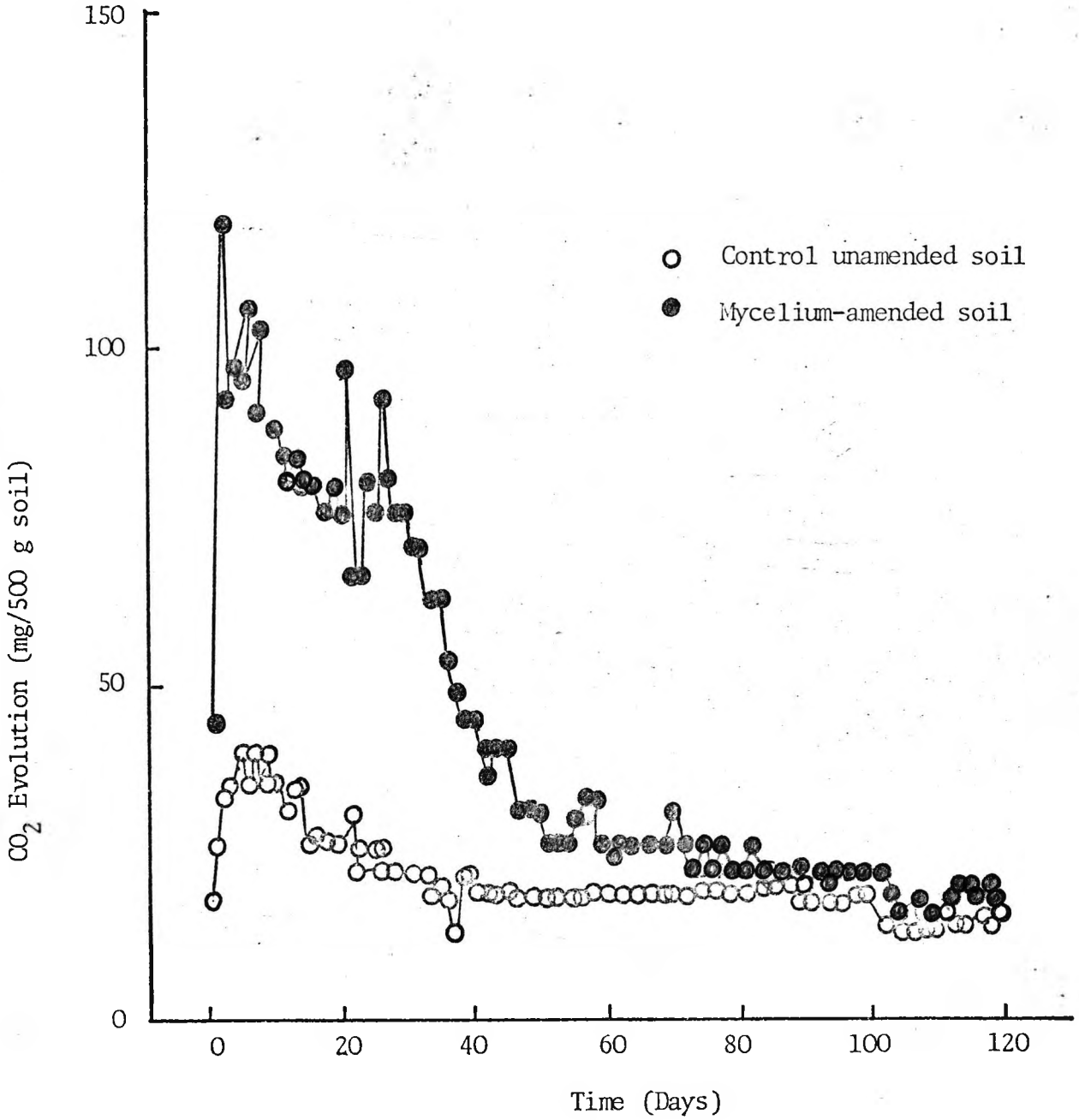


Figure 3.2.7. Dynamics of CO_2 production from mycelium-amended and control soils during the continuous flow of water; anaerobic incubation conditions.

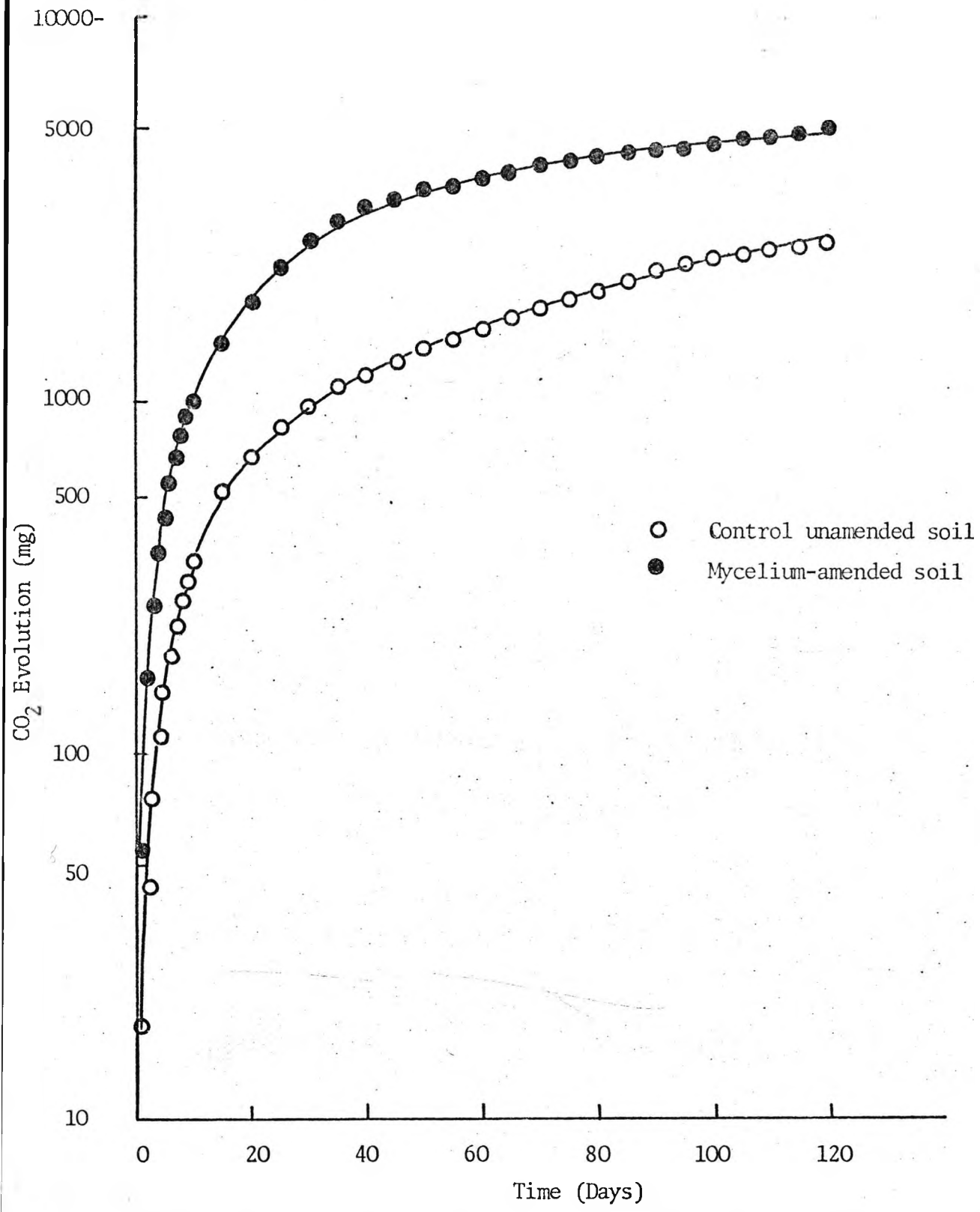


Figure 3.2.8. Cumulative CO_2 production from mycelium-amended and control soils during continuous flow of water; anaerobic incubation conditions.

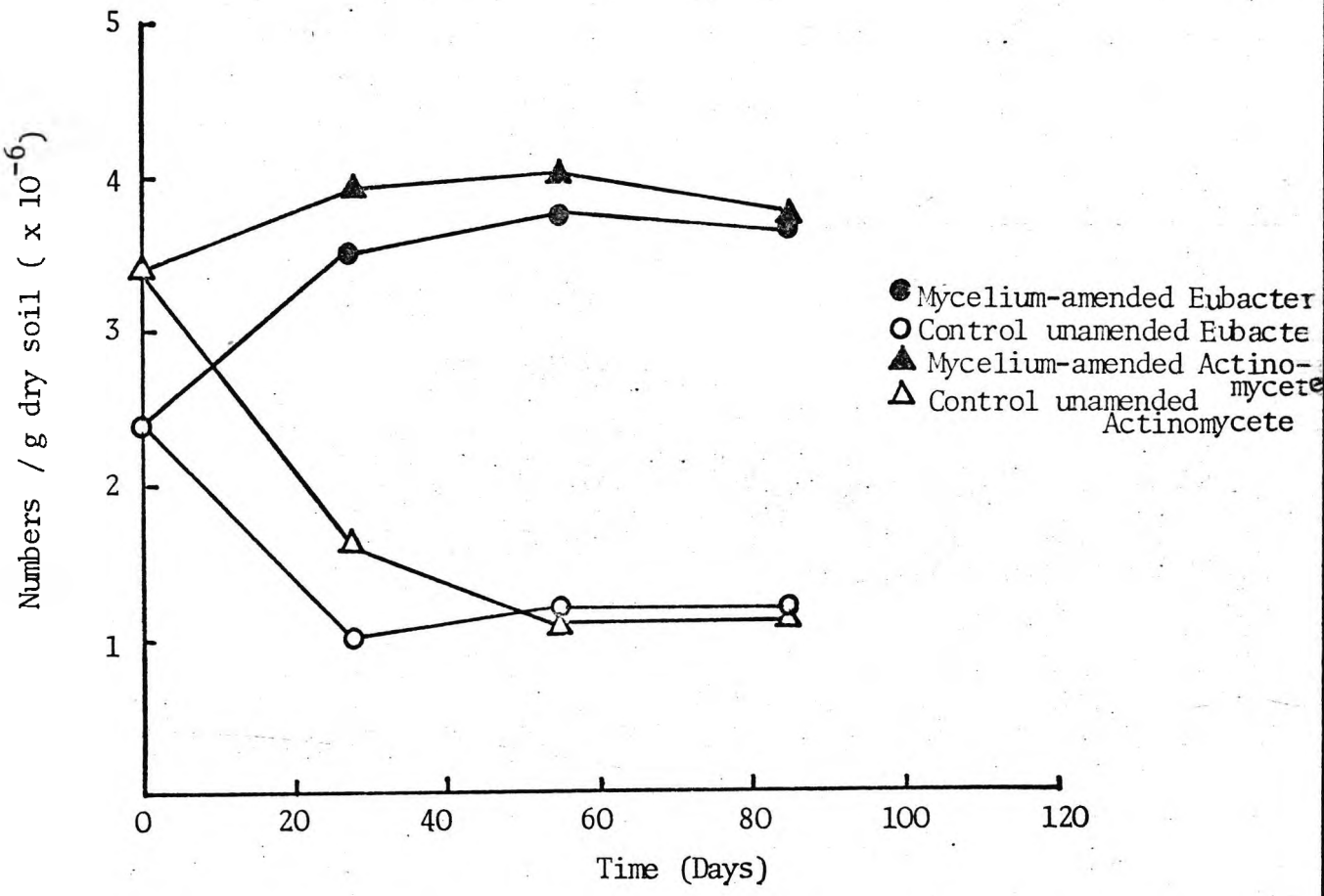


Figure 3.2.9. Changes in Bacterial and Actinomycete populations during continuous flow of water through control and mycelium-amended soil columns; anaerobic incubation conditions.

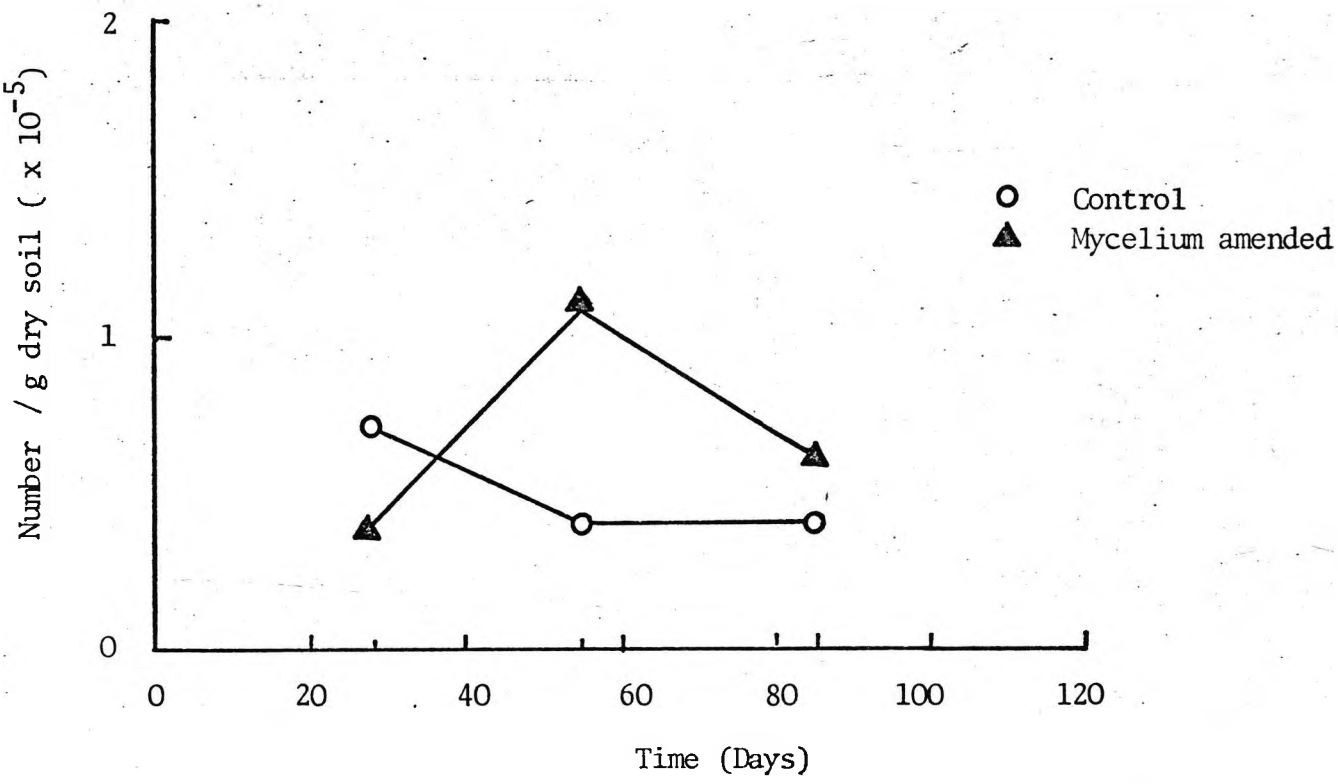


Figure 3.2.10. Changes in fungal population during continuous flow of water through control and mycelium-amended soil columns; anaerobic incubation conditions.

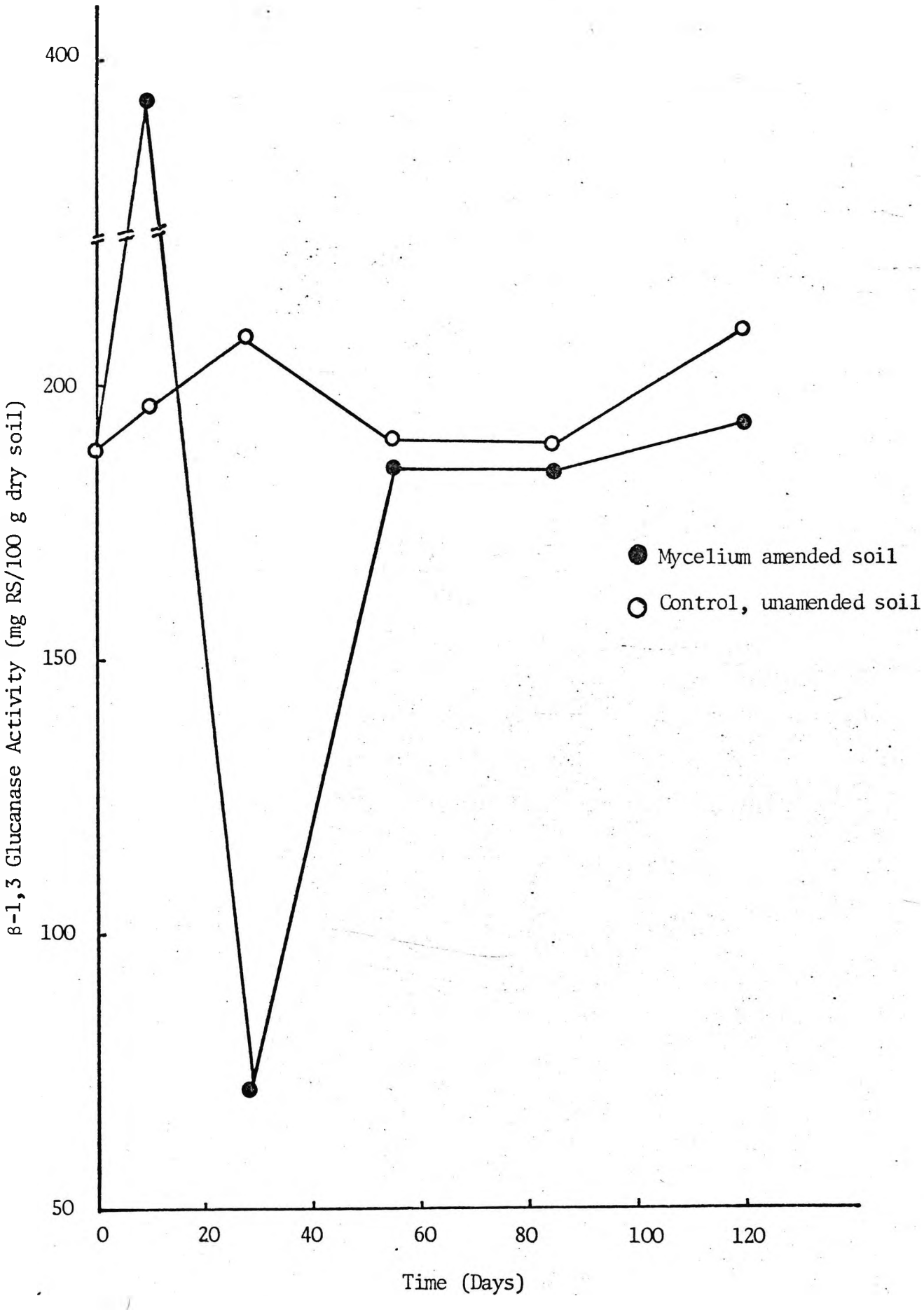


Figure 3.2.11. β-1,3 Glucanase activity in control and mycelium-amended soil columns during the continuous flow of water; anaerobic incubation conditions.

TABLE 3.2.2.

Amount of reducing sugars extracted from the control and mycelium-amended soil perfused continuously with water; anaerobic incubation condition.

Time (Days)	Reducing sugars (mg/ g soil) [a]	
	Control	Amended
0	0.28	0.28
10	0.20	0.62
28	0.14	2.48
55	0.19	0.29
85	0.20	0.29
120	0.19	0.29

a) Reducing sugars were estimated as glucose.

Fractionation of the organic matter at the end of the experiment revealed that there were no water soluble components. Humic and fulvic acids were extracted from both control and amended soils (Table 3.2.1). The extractable humic acid fraction in control soil was approximately 14% more than the amended soil. There was a significant enrichment in the carbon content of amended soil humic acid, i.e. 47% over comparable control soil fraction. There was no change in the amount and carbon content of the fulvic acid fractions of amended and control soils.

3.2.3. The Effects of Soil Drying and Wetting.

Two columns were packed with 500g of air-dried soil (2-5mm aggregates). In one column the soil was amended with mycelium (16g wet weight/500g soil); the mycelium was uniformly distributed throughout the soil. The second column was used as an unamended control. Both columns were set up at 25°C and flushed continuously with dry, CO_2-free air at the rate of 1200 $ml.h^{-1}$ for nine days. Subsequently both columns were perfused with sterile distilled water (10ml h^{-1}) for about 18 days. At the end of these first 27 days of treatment the columns were taken off stream, the soil removed and spread separately on to Alcon foil and air-dried in a dust free cabinet for 48 h. The air-dried soil samples then were returned to their respective columns which were put back on stream (gas and water). At the end of day 50 this cycle of soil drying and rewetting was repeated and the incubation continued up to 100 days.

RESULTS.

The pattern of carbon mineralization in a typical wetting and drying experiment is shown in Figure 3.2.12. Intense production of CO_2 occurred in the amended soil and it reached a peak by the third day and then began to fall in the expected manner (Cf Figure 3.2.2). There was very slight production of CO_2 from the control soil. On day 10, following the start of water perfusion the production of CO_2 again increased in control and amended soils and reached a peak after the third day on stream (≡ day 12) after which a slow fall was recorded. Similar cycles of activity followed further drying and re-wetting 28 and 50 days after the start of the experiment.

Cumulative production of CO_2 during the course of 100 days incubation is shown in Figure 3.2.13. Mineralization was 140% higher in the amended compared to the control soil (Cf Figure 3.2.3).

Changes in microbial numbers are shown in Figures 3.2.14 and 3.2.15. The bacterial and actinomycete numbers rose 4-fold by day 20, declined slowly but remaining higher than the control soil. The fungal population changes are shown in Figure 3.2.15.

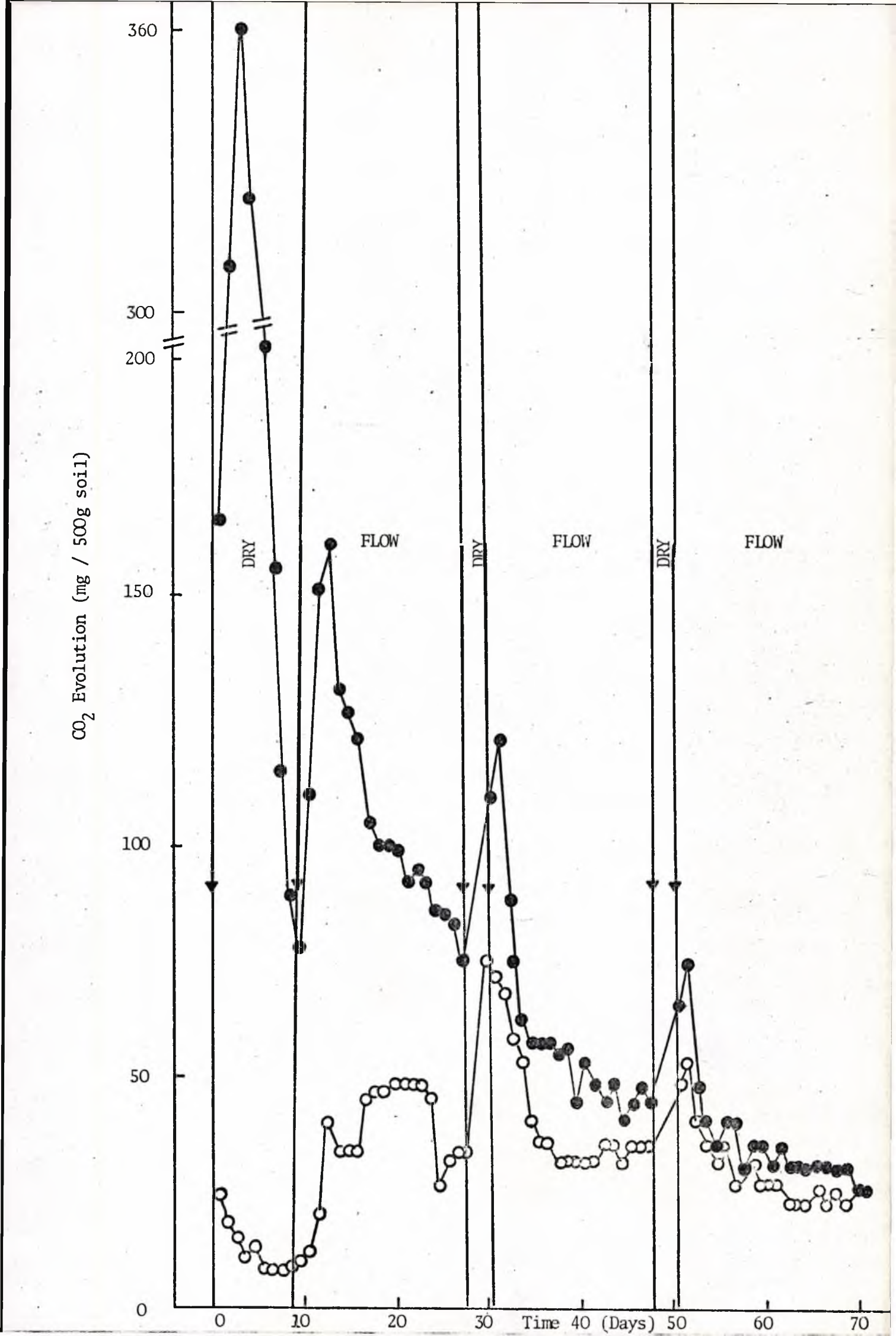
The pattern of β-1,3 glucanase activity was quite different from that observed in preceding experiments (Figure 3.2.16). Activity was substantially higher in the amended soil (45% initially and gradually falling to <10% after about 80 days) and, significantly, increased rather than decreased during the initial (wet) period (Cf. Figure 3.2.6).

Organic matter fractionation after 100 days of incubation showed no water soluble components in aqueous extracts of either control or amended soils. There was alkaline extrac-

Figure 3.2.12. Dynamics of CO_2 production from control and mycelium-amended soils during cycles of drying and wetting; aerobic incubation conditions

○ Control unamended soil

● Mycelium-amended soil


360
300
200
150
100
50
0
CO_2 Evolution (mg / 500g soil)
DRY
FLOW
DRY
FLOW
DRY
FLOW
0
10
20
30
Time 40 (Days) 50
60
70

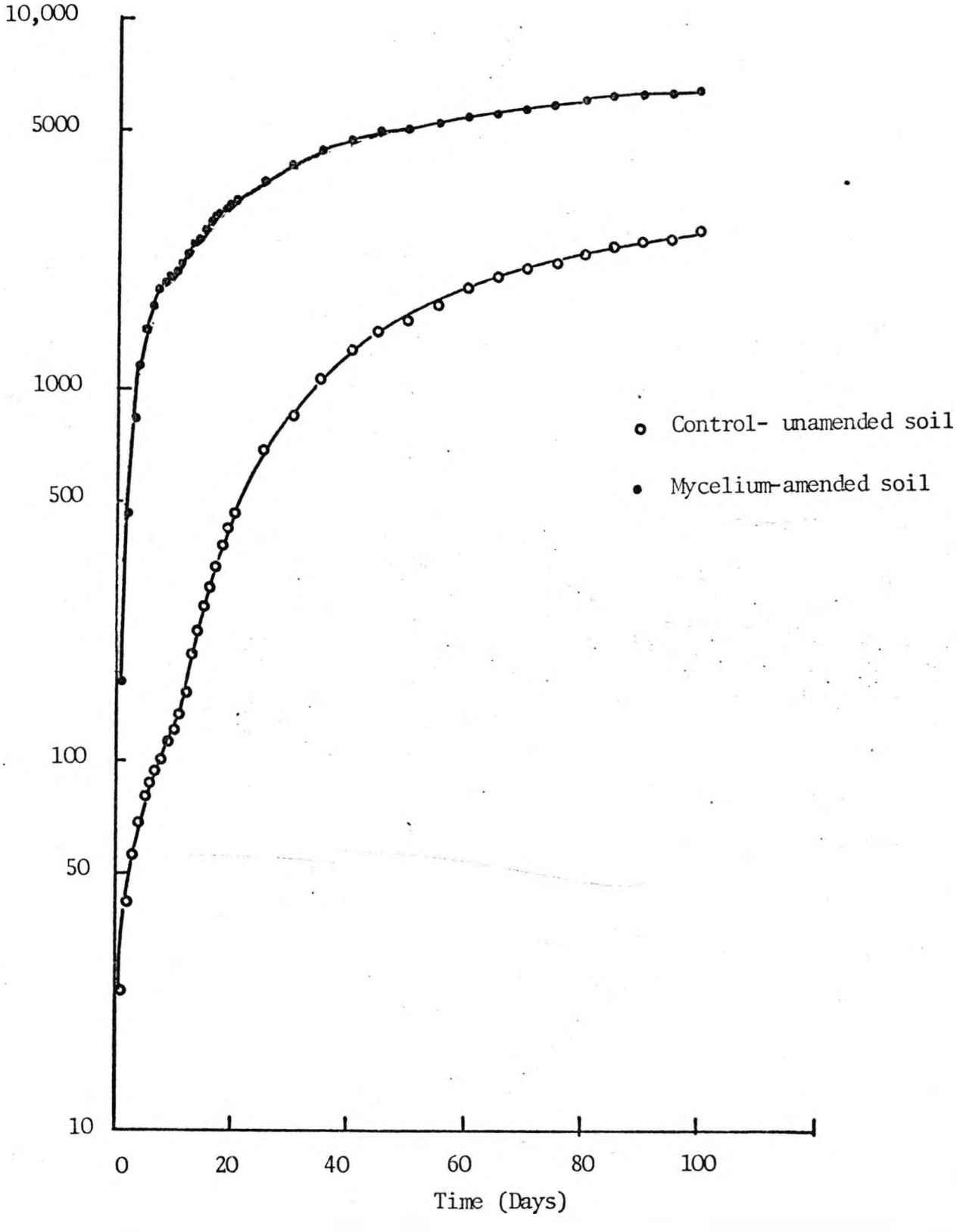


Figure 3.2.13. Cumulative CO_2 production from mycelium-amended and control soils during cycles of drying and wetting; aerobic incubation conditions.

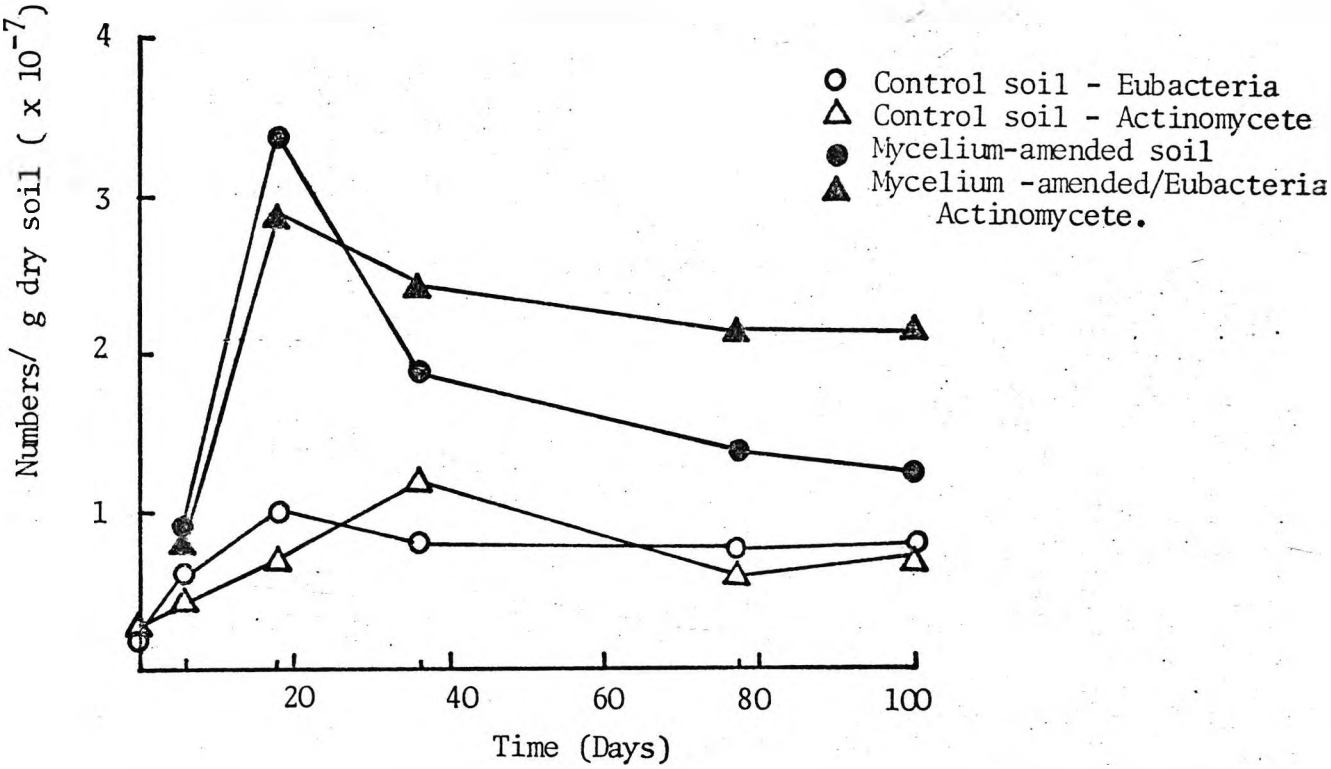


Figure 3.2.14. Changes in Bacterial and Actinomycete populations during cycles of Drying and Wetting; aerobic incubation conditions.

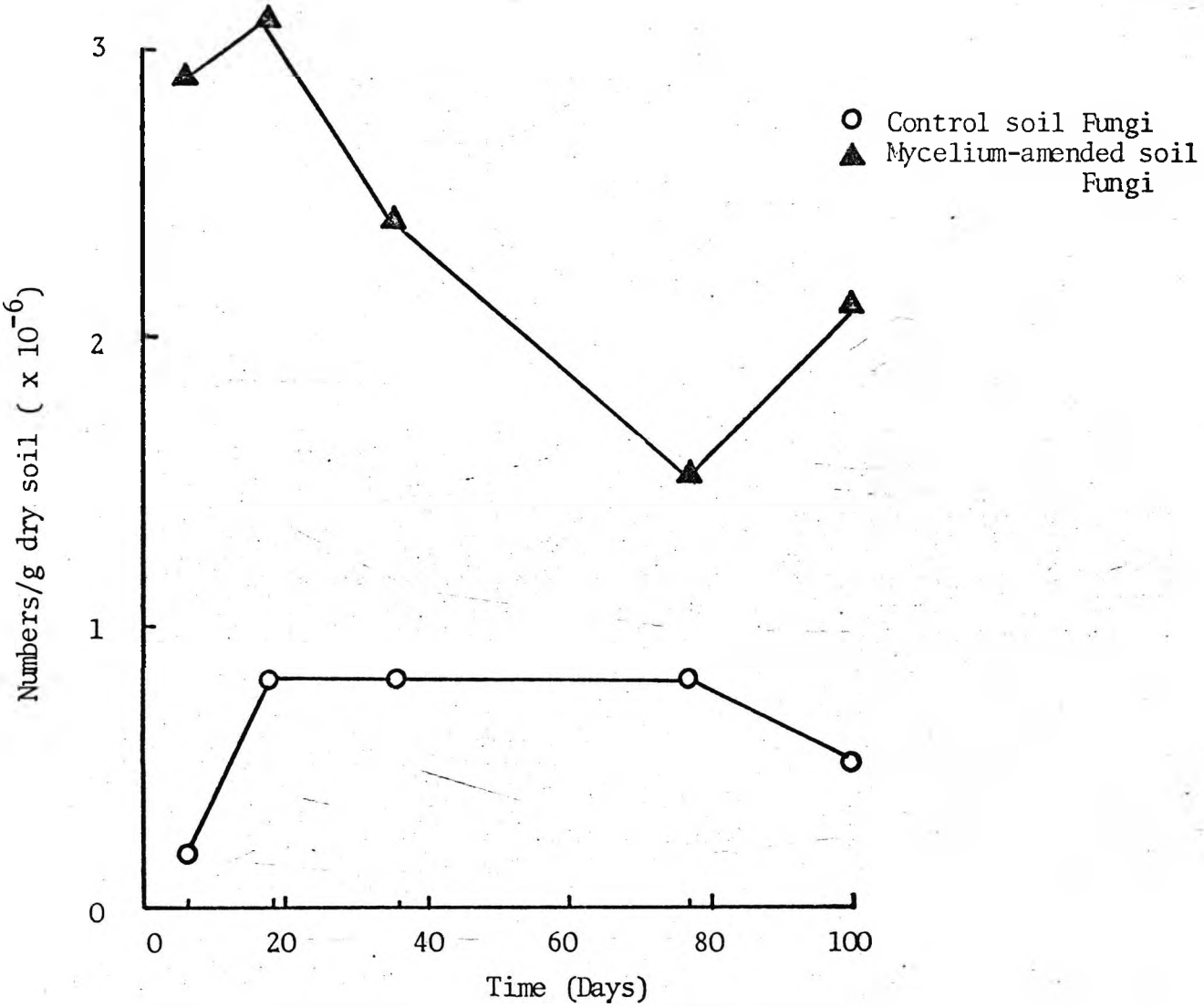


Figure 3.2.15. Changes in fungal population during cycles of drying and wetting; aerobic incubation conditions.

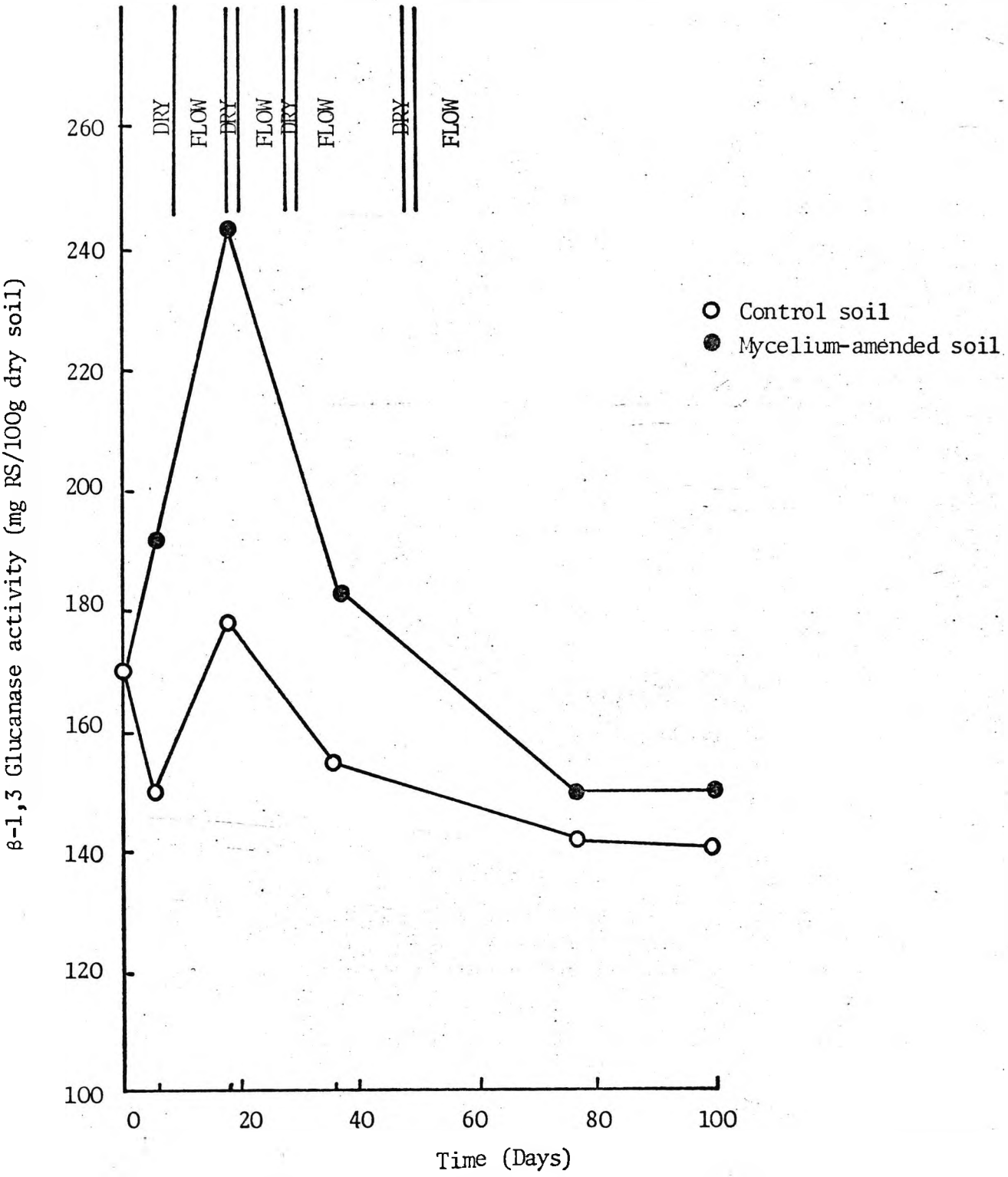


Figure 3.2.16. β-1,3 Glucanase activity in control and mycelium-amended soil during cycles of drying and wetting; aerobic incubation conditions.

tion of both humic and fulvic acid fraction D. The humic and fulvic acid components of the control soil increased by approximately 7 and 24% respectively compared to amended soil (Table 3.2.1).

3.2.4. The Effects of Soil Aggregate Size.

A column was packed with 500g soil (aggregate size <2mm) and perfused with CO_2-free air and sterile distilled water at rates of 1200ml.h^{-1} and 10ml.h^{-1} respectively. The mineralization of native organic matter was monitored as CO_2 production.

RESULTS.

The total carbon respired by the unamended soil during the incubation period is shown in Figure 3.2.17. After a small initial rise in the production of CO_2 up to day 3 a slow decline ensued and then the mineralization rate stabilized at a low level. Differences between the two sizes of soil aggregates in the cumulative production of CO_2 at the end of 120 days of incubation were negligible (Figure 3.2.18).

3.2.5. Stability of *Aspergillus nidulans* Conidia in Soil.

Soil columns were amended with *Aspergillus nidulans* conidia in the same region as described under Section 3.2.1. One column was amended with 2.5g (wet weight) of washed viable spores and the other with 2.5g (wet weight) of washed killed spores.

RESULTS.

There was a flush of CO_2 evolution from the soil on the first day of incubation with both the treatments (Figure 3.2.19) and then the rate of mineralization fell to the level

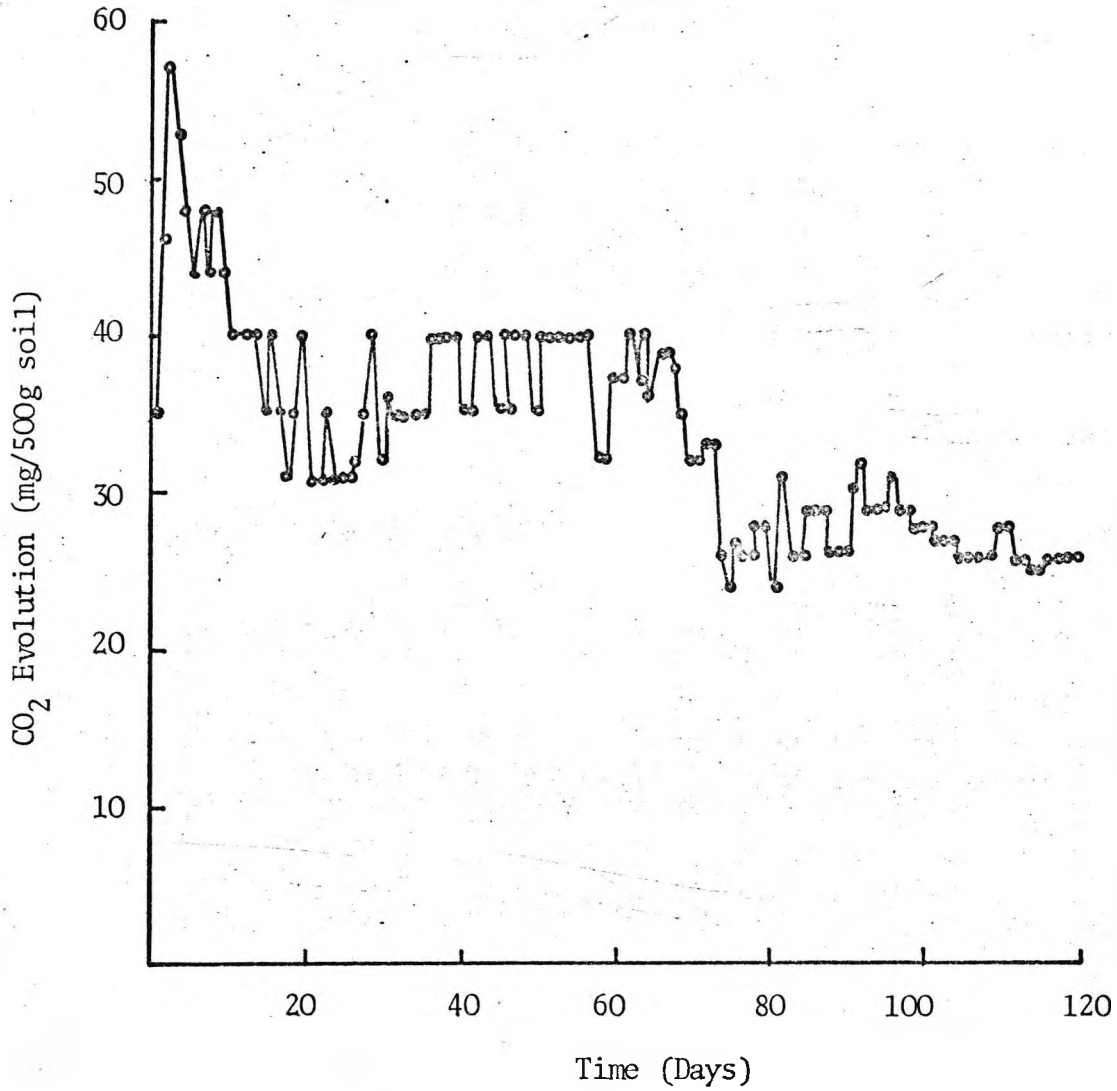


Figure 3.2.17. Dynamics of CO_2 production from unamended control soil during the continuous flow of water. Soil aggregate size < 2mm.

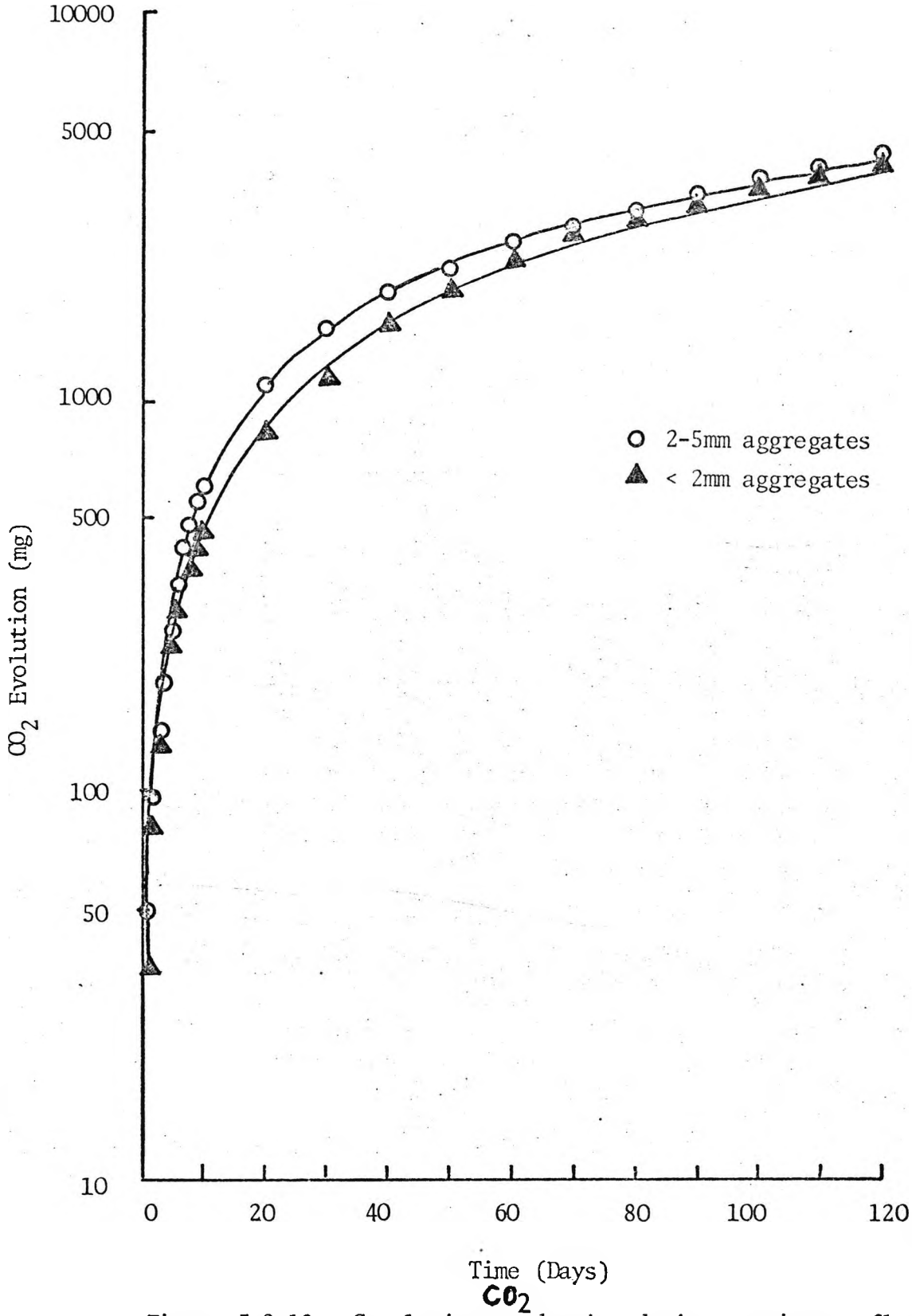


Figure 3.2.18. Cumulative CO_2 production during continuous flow of water; aerobic incubation conditions. The effect of soil aggregate size.

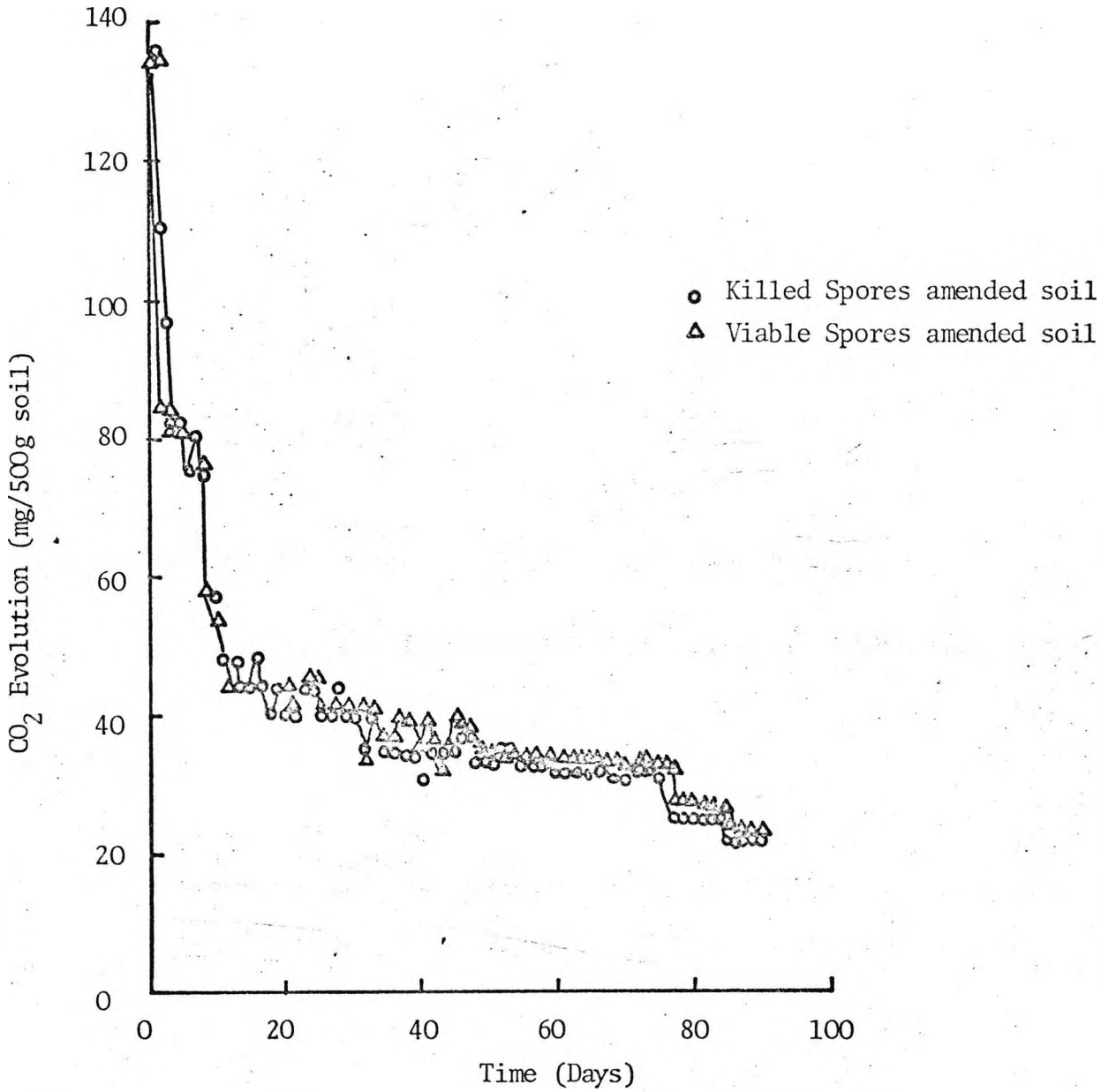


Figure 3.2.19. Dynamics of CO_2 production from soils amended with killed and viable spores during the continuous flow of water; aerobic incubation conditions.

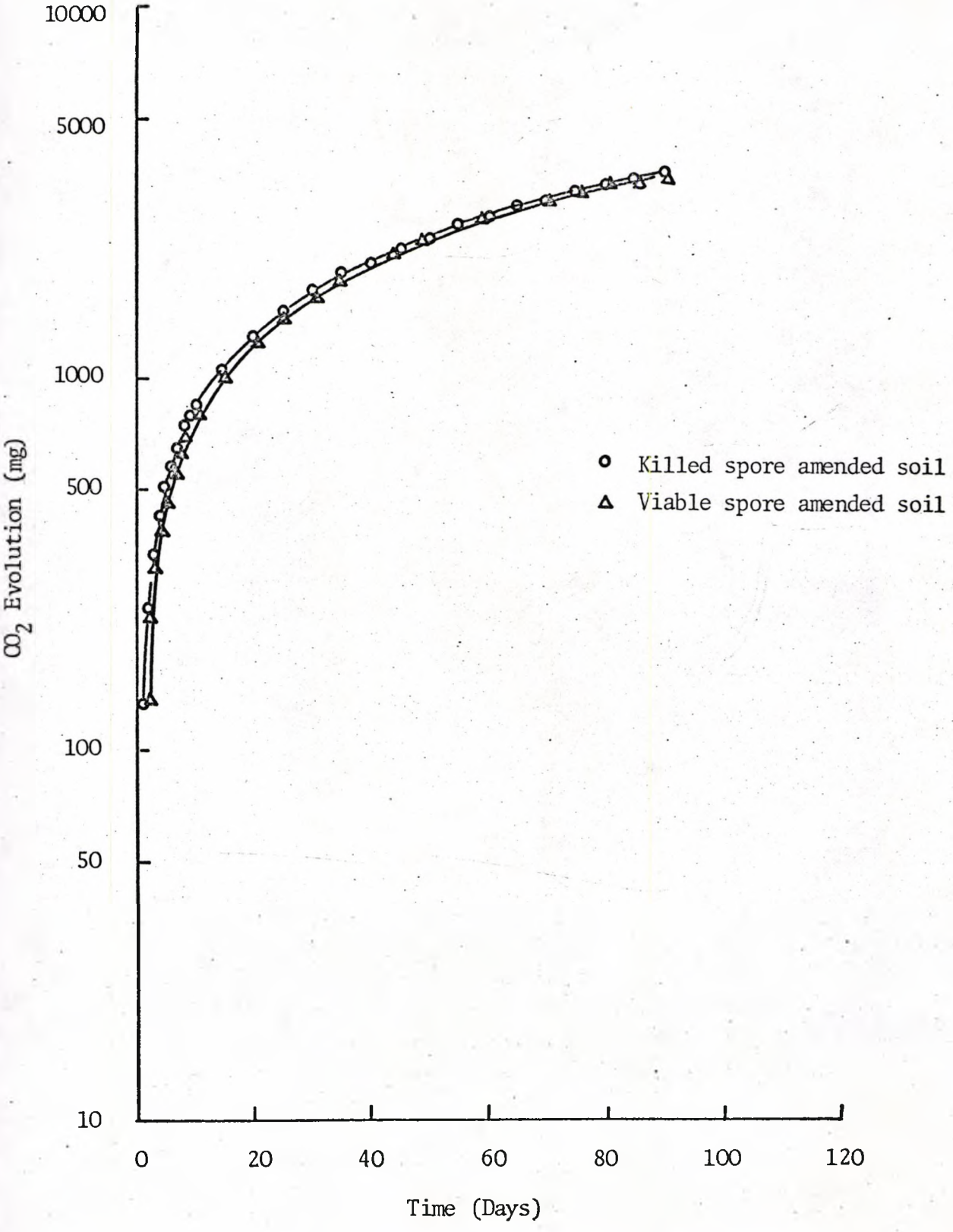


Figure 3.2.20. Cumulative CO_2 production during continuous flow of water through killed and viable spore-amended soils; aerobic incubation conditions.

Figure 3.2.21.

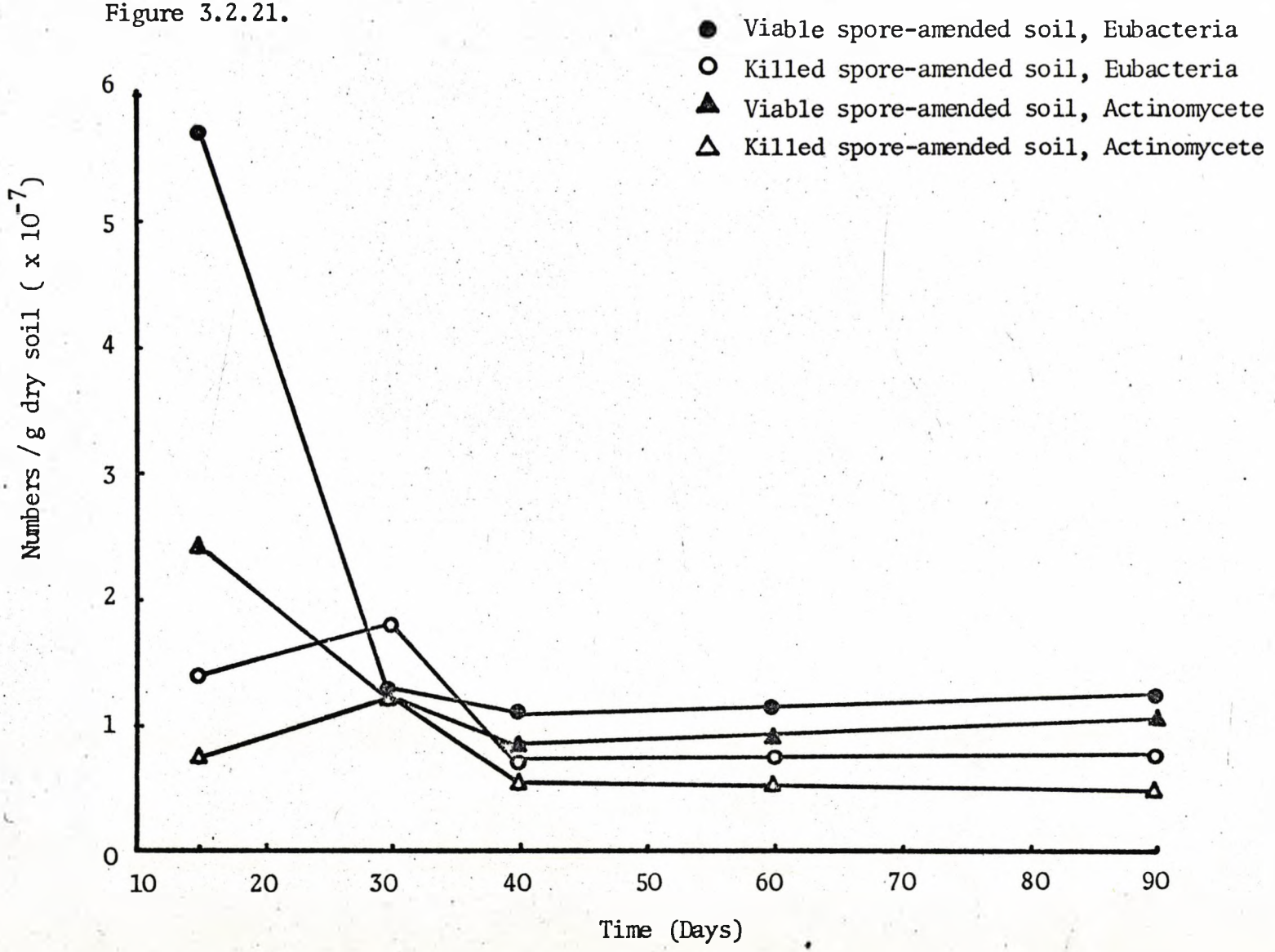

Viable spore-amended soil, Eubacteria
Killed spore-amended soil, Eubacteria
Viable spore-amended soil, Actinomycete
Killed spore-amended soil, Actinomycete
Numbers /g dry soil (x 10⁻⁷)
0
1
2
3
4
5
6
10
20
30
40
50
60
70
80
90
Time (Days)

Figure 3.2.21. Changes in Bacterial and Actinomycete populations during continuous flow of water through killed and viable spore-amended soils; aerobic incubation conditions.

Figure 3.2.22.

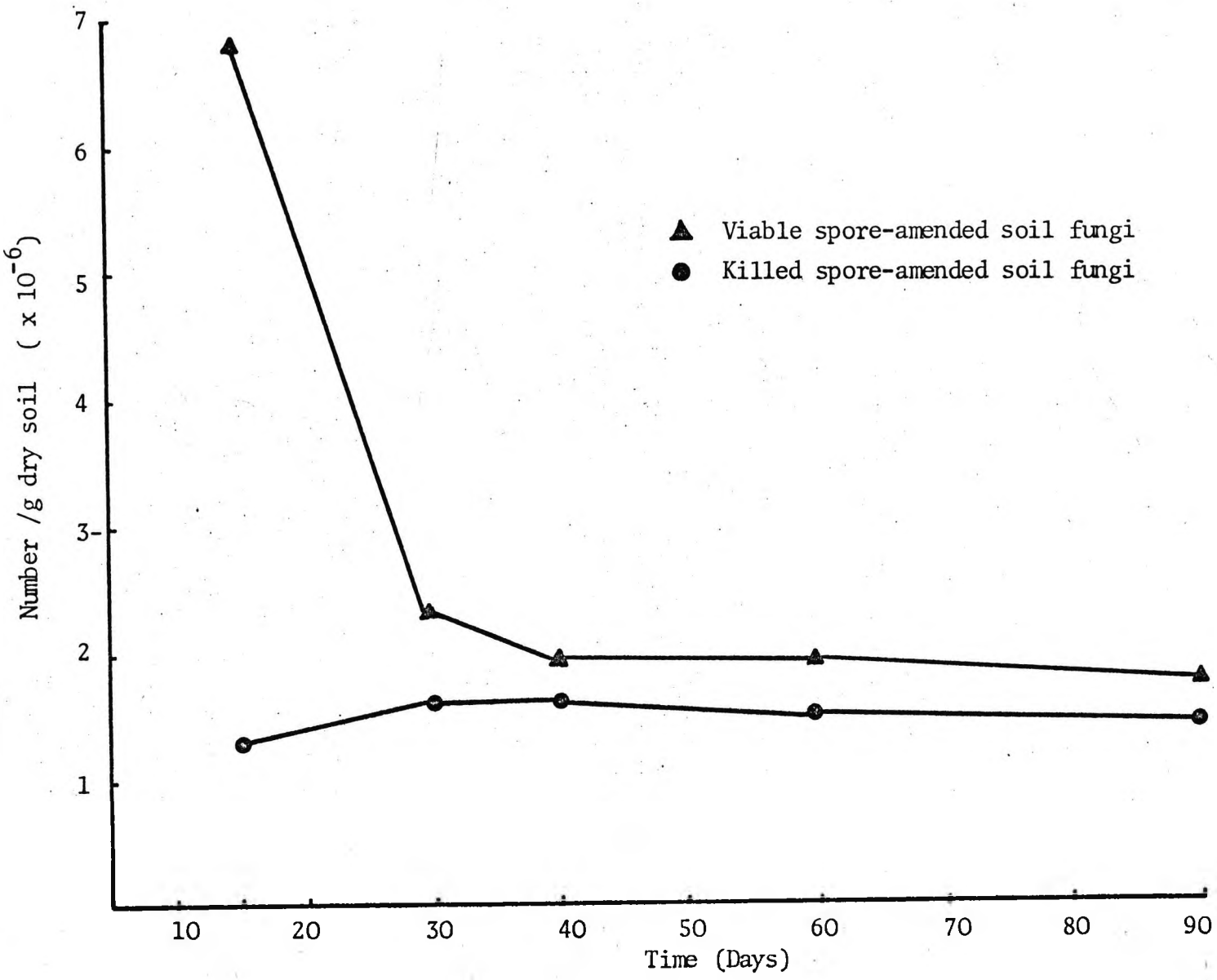

Viable spore-amended soil fungi
Killed spore-amended soil fungi
Number /g dry soil (x 10^{-6})
7
6
5
4
3
2
1
10
20
30
40
50
60
70
80
90
Time (Days)

Figure 3.2.22. Changes in fungal population during continuous flow of water through killed and viable spore-amended soils; aerobic incubation conditions.

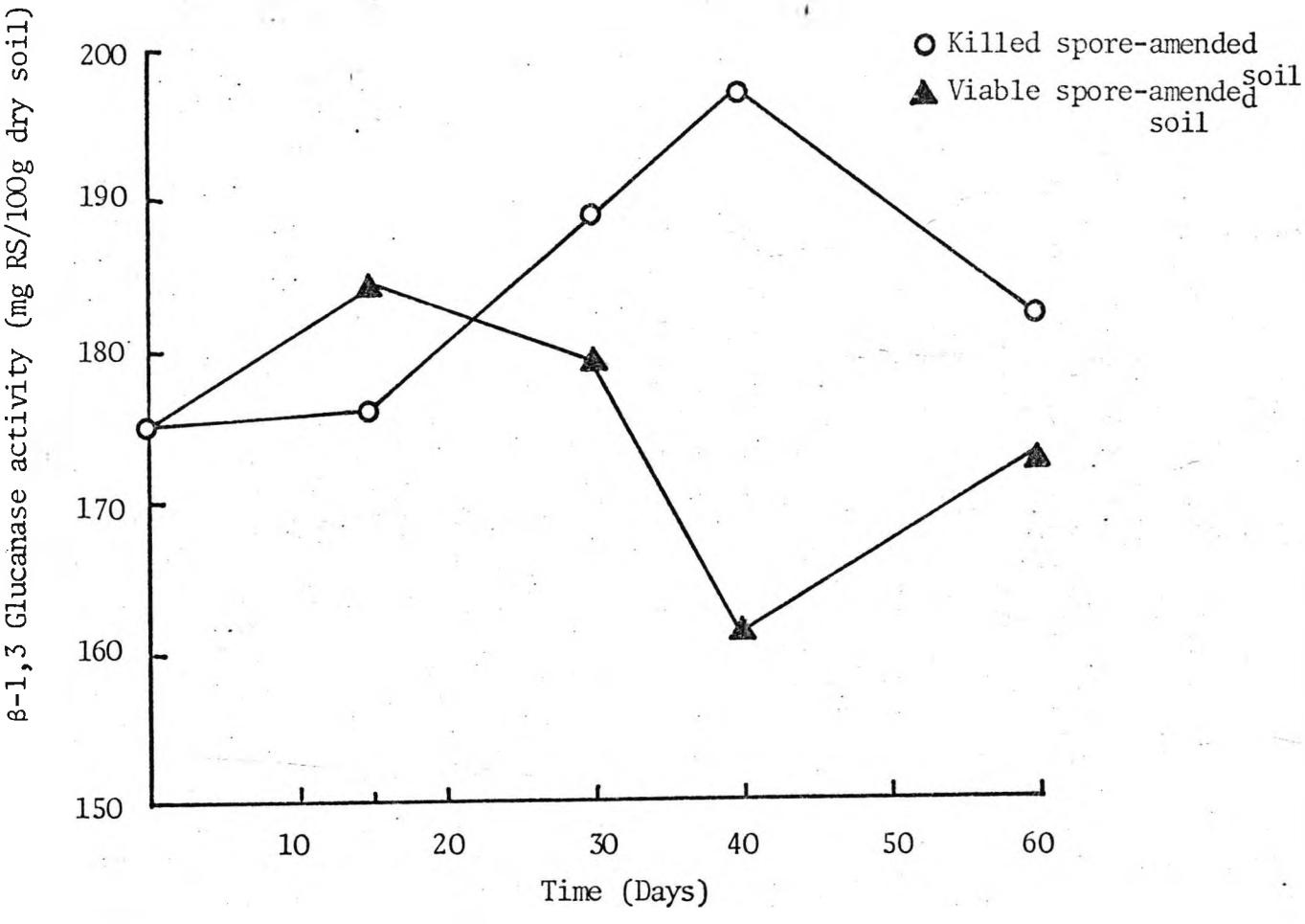


Figure 3.2.23. β-1,3 Glucanase activity in killed and viable spore-amended soils during continuous flow of water; aerobic incubation conditions.

of control soils (see Figure 3.2.2) and remained so for several months. The near identical kinetics of carbon mineralization in soils containing viable and killed conidia is evident from the cumulative CO_2 plots (Figure 3.2.20).

There was an approximately four-fold rise in the bacterial population and a two-fold increase in the actinomycetes in the soil amended with viable spores compared with the soil amended with killed spores (day 15). Subsequently the numbers fell to and remained at the level of killed spores by day 30 onwards (Figure 3.2.21). A similar fluctuation in the fungal populations was revealed (Figure 3.2.22). The fungal population that developed in the soil amended with viable spores was the largest observed in any of the present experiments.

In the soil amended with killed spores the β-1,3 glucanase activity level built up slowly by day 40 and then fell to the initial level. In the soil amended with viable spores the β-1,3 glucanase pattern resembled that found in aerobic soil amended with mycelium (Figure 3.2.23).

3.2.6. Discussion.

It was clear that fungal decomposition proceeded immediately that mycelia were added to the soil. Under aerobic conditions the cumulative production of CO_2 from amended and control soils showed that the overall rate of mineralization was 57% higher in the amended soil. The subsequent use of isotypically labelled material revealed that 42% of the mycelium carbon was converted to CO_2 in the 120 days of incubation. The data shown in Figure 3.2.24 were

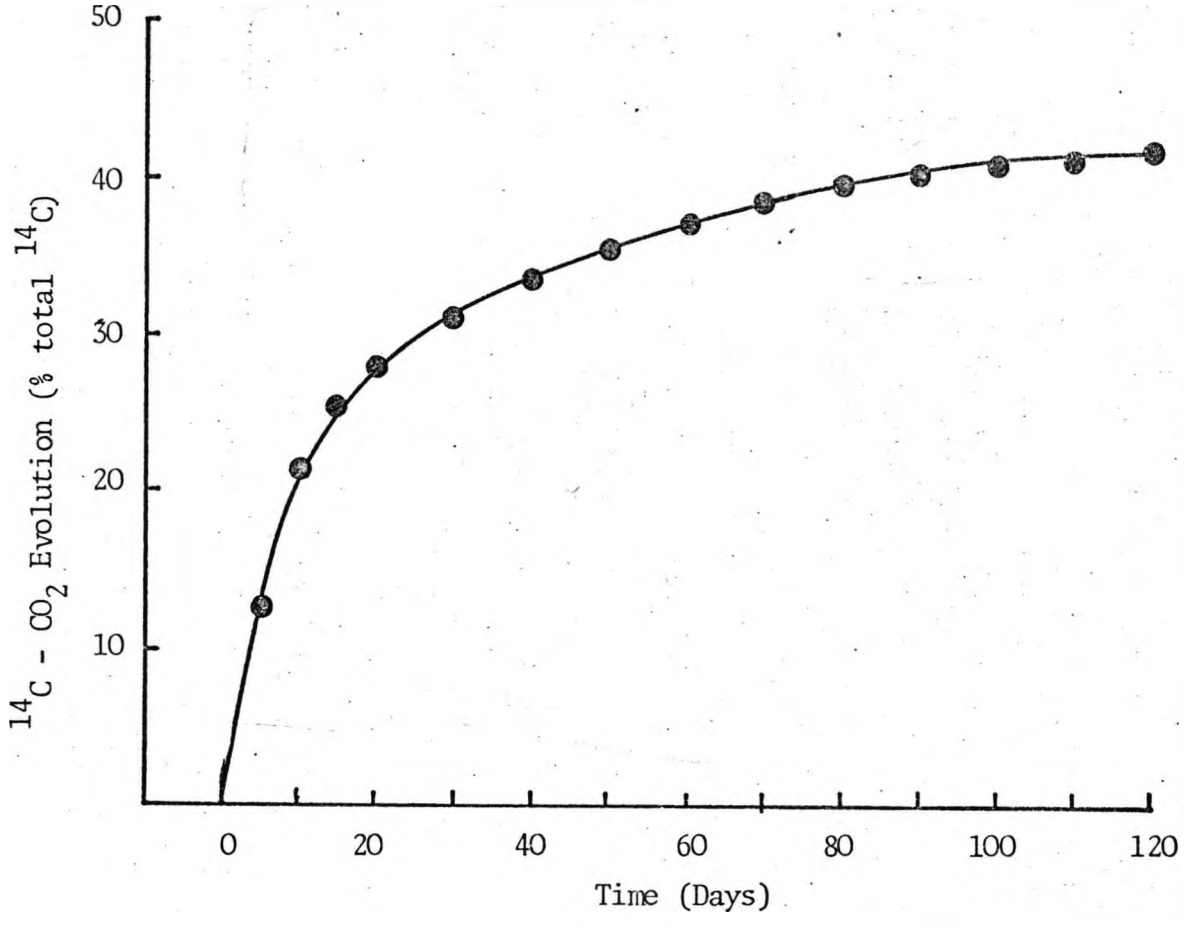


Figure 3.2.24. Release of $^{14}CO_2$ from ^{14}C-labelled mycelium during the continuous flow of water through soil; aerobic incubation conditions.

obtained from an experiment in which soil was amended with ^{14}C-labelled mycelium of *Aspergillus nidulans* and treated as described under section 3.2.1. (mycelial transformation under aerobic conditions). Based on CO_2 evolution during decomposition of a variety of plant residues and fungus cell material in sandy loam incubated aerobically at 25°C, Martin, Ervin and Shepherd (1959) found that fungal mycelium decomposed rather more quickly than did a variety of common mature plant residues.

A different pattern of carbon mineralization was observed in anaerobic soils. Anaerobiosis had a differential effect on the turnover rates of mycelial and native organic matters: thus the native organic matter was mineralized at only 50% of aerobic rate. Clearly the decomposition of relatively simple macromolecules of the type constituting exponentially grown fungal hyphae (viz. polysaccharides, proteins, nucleic acids) can proceed at high rates under severely oxygen-limited or even oxygen exhausted conditions via fermentative types of metabolism. Decomposition of native organic matter, on the other hand, necessarily must involve the cleavage of aromatic nuclei abundant in the humus fractions and this is an oxidative process. It is not unreasonable to suppose that this latter activity will be particularly susceptible to oxygen depletion. During anaerobic decomposition of glucose continuously added to the soil Takai, Macura & Kunc (1969) observed that CO_2 evolution under anaerobiosis was lower as compared to aerobic conditions but the amount of organic acids found in the effluent during continuous addition of glucose under anaerobic conditions was higher compared to aerobic conditions.

Parr, Smith & Willis (1970) studied the effect of selected environments and energy sources on respiratory activity of soil microorganisms. They reported that the respiration rates of soil amended with glucose and incubated aerobically or anaerobically (N_2, Ar or He) followed the decreasing order: CO_2-free air > N_2 > Ar = He. Further, Parr et al (1970) obtained similar results for soil amended with other saccharide substrates and natural plant products suggesting that respiration rate was largely independently of the substrate supplied. These workers also observed an incomplete recovery of added carbon from soil incubated under N_2, even after changing the gas phase to CO_2-free air: this result was attributed to a greater accumulation of certain toxic fermentative products which subsequently repressed the growth and activity of soil microorganisms.

The fact that the rate of mineralization of the native organic matter was halved under anaerobic conditions prompted us to examine the effect of soil aggregate size on its decomposition, soil particles of diameter greater than 3mm are known to be anaerobic at their centres (Greenwood, 1961). However, a comparison of the respiratory activity of 2-5mm and < 2mm diameter aggretates (Figure 3.2.18) strongly suggested that the mineralization was not oxygen limited in the larger soil aggregates. Consequently, the larger aggregates were retained for all subsequent experiments because they provided a physically stable condition in columns over long experimental periods when it was desirable to maintain constant flow rates.

Periodic wetting and drying is a significant modulator of soil metabolism under field conditions (Birch, 1958) and attempts were made to assess its importance to mycelial decomposition in laboratory experiments. Carbon mineralization was

140% greater in the amended as compared to control soil but the overall mineralization of the added mycelium compared to that in aerated, constantly wetted soil, was not significantly higher. However, when the respective control soils are compared (Figures 3.2.3. and 3.2.13) the mineralization of native organic matter in the dried and wetted soil was approximately 31% lower compared to the aerobic, constantly wetted soil, indicating that the rate of mineralization of native organic matter increased in response to the continuous stimulus.

In the present drying and wetting experiments intense CO_2 production occurred during the early dry period in the amended soil and reached a peak by the third day and then fell in the expected manner (Figure 3.2.12). Examination of soil removed from the column revealed that the added mycelium had rapidly and extensively colonized the aggregate surfaces. Thus, fungal respiration will have contributed to a significant but unquantified extent to overall CO_2 evolution during the initial dry period. Inspection of the data in Figure 3.2.25 supports the above view; it illustrates that the mineralization of killed mycelium under conditions similar to those operating in the first drying and wetting experiments (Figure 3.2.12). The production of carbon dioxide was 95% greater during the initial dry period in the soil amended with viable mycelium as compared with the killed mycelium. However, it appears that the competitive saprophytic ability of *Aspergillus nidulans* in soil declines when the water holding capacity was raised to 100%, i.e. on the return to flow conditions. Anderson and Domsch (1973), from their investigations of bacterial and fungal contributions to the soil respiration, reported that the average bacterial and fungal contribution to the soil respira-

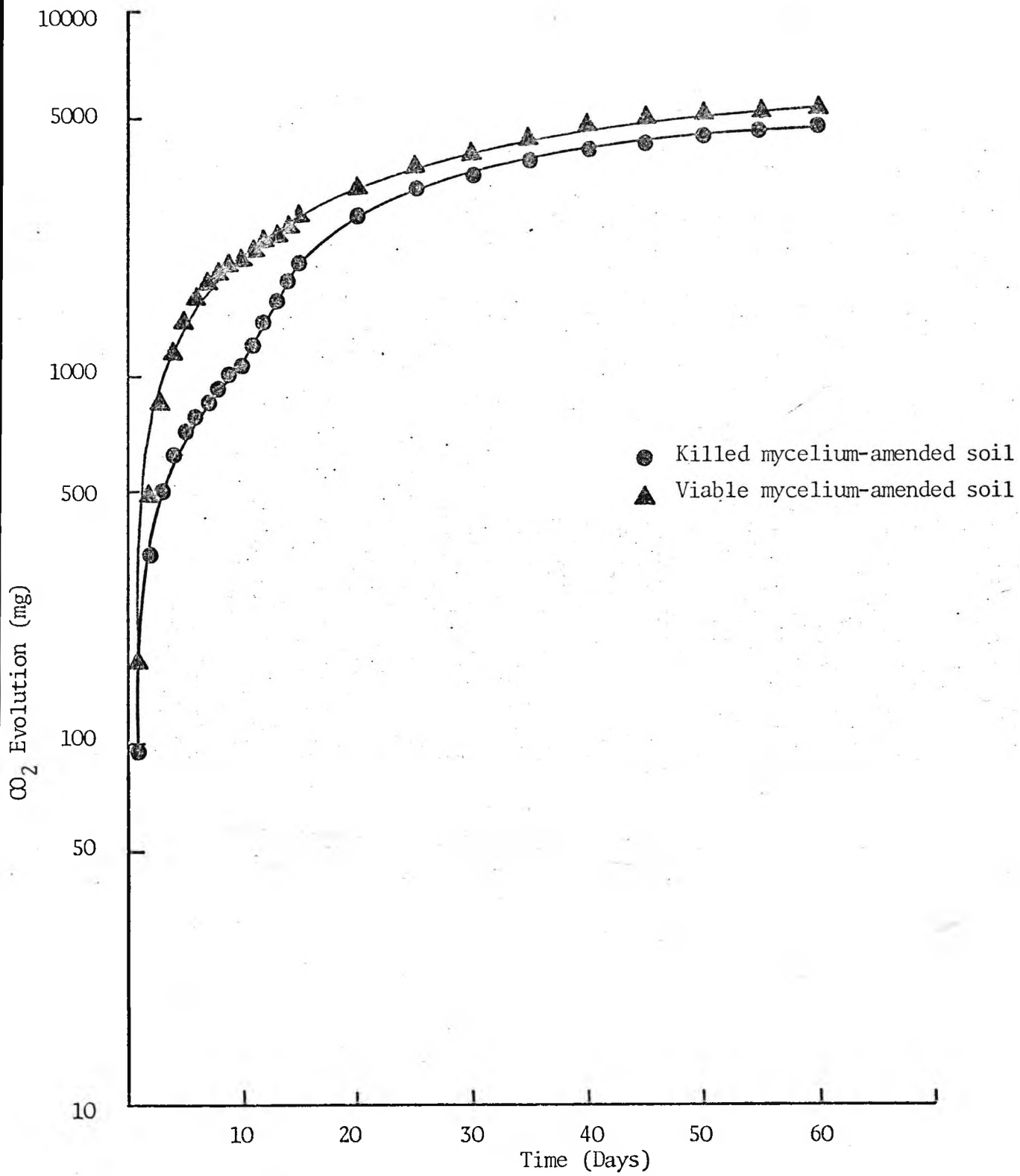


Figure 3.2.25. Cumulative CO_2 production during cycles of drying and wetting of soil; aerobic incubation conditions. The effect of dead and living mycelia.

tion were 22 and 78% respectively. Similarly, McGill, Paul, Shields and Lowe, (1973) in their laboratory studies of turn over of microbial populations and their metabolites in soil reported that the fungi are the primary organisms involved in carbon mineralization in soils.

Addition of fungal spores to soil caused a flush of CO_2 evolution on the first day followed by a stable production over a long time. Similar data were obtained when either viable or killed spores were used. The mineralization was at slightly higher rates (approximately 6 %) in spore-amended soil than in the control (unamended) aerobic soil (Figure 3.2.3.). Lingappa & Lockwood (1964) also found an initial rise in oxygen uptake of the soil supplemented with fungal spores (conidia of *Fusarium solani*, *Helminthosporium victoriae*, *Glomerella cingulata* or *Neurospora* species) and they suggested that a portion of the increased respiration may have been due to the endogenous respiration by the fungal spores. Killed conidia and cell free washings of conidia also induced similar rapid increases in oxygen uptake in soil (Lingappa & Lockwood, 1964).

The data of Figure 3.2.4 shows a steep and transistory rise in bacterial and actinomycetes numbers occurring after the initial peak of mineralization. The populations in control soils remained constant. Heck (1929) was the first to show that total bacterial numbers increased following amendment of soil with living fungi. Heck added washed, live mycelium of *Aspergillus Oryzae* to a silt-loam soil and after 30 days incubation bacterial counts in the soil supplemented with mycelium were 4 to 6 times higher than those in non-supplemented soils. Comparing the microbial population sizes and CO_2 evolutions (Figures 3.2.2

and 3.2.4) it is observed that no correlation exists between the two although Gray & Wallace (1957) observed a positive correlation between microbial numbers and CO_2 evolution in field trails. Alexander (1961) suggests that if the carbon sources were homogenous and the population composed of one species a definite relationship might be clear. However, given a diversity of the microflora presumably having differential growth rates and activities, and a variety of carbon sources, the poor correlation with CO_2 and microbial population observed here is not surprising. A contributing factor in the lack of correlation between these sets of data may be the too infrequent sampling of microbial numbers; nevertheless, the massive respiratory activity observed at the start of the experiment was not associated with increasing numbers of organisms.

Only minor fluctuations in microbial numbers were observed in anaerobic soil. In soil drying and wetting experiments the fungi proliferated rapidly during the initial dry period in the amended soil, but once the water holding capacity of the soil was raised to 100% such activity declined. Bacterial and actinomycete populations rose 4-fold in the amended soil, but the actinomycete remained at a higher level throughout the period of experiment compared to the bacterial population. There was no significant change observed in the microbial population of control soil.

There was a marked initial rise in the bacterial and actinomycete numbers in the soil amended with viable spores compared to the soil amended with killed spores. Even the fungal population of soil amended with viable spores has increased approximately six-fold and remained higher throughout the experimental period. Fungal populations in the soil amended with killed spores remained constantly low. Wong & Old (1974) reported that there was a flush of microbial

activity after conidia of *Cochliobolus sativus* were placed on to unsterile soil and a variety of bacteria and actinomycetes flourished on the spore surface. Ko and Lockwood (1967; 1970) considered that when fungal spores or mycelium were placed on or into soil there was a rapid movement of nutrients from their cell contents into the soil. It is possible, therefore, that the initial microbial activity that developed in the presence of viable spores in the present experiments was a response to such a supply of readily available substrates. In the aerobic experiment although β-1,3 glucanase activity was high initially it fell during the early rapid phase of mineralization and only returned to control levels during the second slower phase. It is postulated that these two phases of mineralization represent respectively the utilization of cytoplasmic (≡ readily available) and wall (≡ more retractable) components of the mycelium and that the initial fall in enzyme activity may be due to inhibition and/or repression following a large release of soluble materials into the soil. In contrast β-1,3 glucanase levels in amended anaerobic soil initially fell 5-fold and only eventually recovered and approached control soil values. This dramatic fall in enzyme activity was paralleled by a considerable and persistant accumulation of reducing sugars in the soil. The latter are thought to have been responsible for the repression of enzyme synthesis and or inhibition of enzyme activities. These sugars were not leached out from the soil column and only when they were eventually assimilated by the microflora did the glucanase activity again build up in the soil. Microbial β-1,3 glucanase are very susceptible to catabolite repression (see Bull, 1972; Lilley & Bull, 1974).

The data presented in Tables 3.2.1 and 3.2.3 show that perfusion of soil by water had an appreciable effect on the decomposition of native organic matter and that the effect is enhanced under conditions of amendment with fungal mycelia. Continuous water perfusion was more effective in mineralization than cycles of soil drying and wetting. The rate of mineralization was more pronounced under aerobic than anaerobic conditions of incubation both in the control and mycelium-amended soil. The extent of mineralization of the humic acids followed the decreasing order: aerobic > wetting-drying > anaerobic soil treatments. Although humic acids are mineralized, the fulvic acid content of the soil under all these conditions increases approximately 1.5 to 2.5 fold.

Recent investigations on the biological degradation of humic substances have emphasized either the organisms responsible and the effect of environmental conditions on their growth, or the chemical changes produced in the humic substances by the activities of such organisms. Burges & Latter (1960) isolated two fungal species from percolation experiments that decomposed humic acid; 33 to 43% of the humic acid was lost during a 6 to 8 week incubation period, subsequently Hurst, Burges & Latter (1962) examined 29 fungal strains for their ability to decolourize humic acid and to reduce the carboxyl group of m-hydroxybenzoic acid to the corresponding alcohol. A positive correlation was found to exist between these two activities. These workers tentatively concluded that fungal degradation of humic acid included a step involving the reduction of the carboxyl group and that the necessary reducing power was produced by aerobic growth on some substrate other than humic acid. Mishustin & Nikitin (1961) isolated a pseudomonad capable of decolourizing humic acid and they found that decomposition of humic acid was enhanced when a readily

TABLE 3.2.3.

Amounts of Humic and Fulvic acids mineralized in control and mycelium-amended soils.

Treatment	Days of Incubation	Extractable Humic & Fulvic acids (%, dry wt. of soil)	Total	Amount of Humic & Fulvic acids mineralized (%, dry wt. of soil)	Percentage of Humic & Fulvic acids mineralized
None (Control soil)	-	7.92 + 0.25	8.17	-	-
Aerobic Control [a]	120	4.14 + 0.35	4.49	3.68	45.0
Aerobic Amended [b]	120	3.50 + 0.40	3.90	4.27	52.2
Anaerobic Control	120	5.56 + 0.40	5.96	2.21	27.0
Anaerobic Amended	120	4.87 + 0.40	5.27	2.90	35.5
Drying-Wetting Control	100	4.80 + 0.62	5.42	2.75	33.6
Drying-Wetting Amended	100	4.48 + 0.52	4.98	3.19	39.0

a) Indicates unamended soil perfused with water under the conditions specified;

b) Indicates mycelium-amended soil perfused with water under the conditions specified.

decomposible carbon source, such as glucose, was added to the medium. They attributed this ability to the peroxidase activity of the organism since they demonstrated reduction of the humic acid suspension by peroxidase preparations *in vitro*.

Comparison of data in Tables 3.1.1 and 3.2.1 reveals that there was an appreciable enrichment of the carbon and nitrogen contents of both humic and to a lesser extent fulvic acids under aerobic and wetting and drying conditions of soil incubation. In the anaerobic conditions the effect was very negligible. This enrichment effect was not pronounced under aerobic conditions and it is also clear that the greatest compositional changes occurred in the humic acid fraction. As noted by other investigators the residual ^{14}C -activity from added polysaccharides e.g. ^{14}C-labelled glucose, ^{14}C-cellulose, ^{14}C-labelled straw or ^{14}C-labelled mycelium was quickly distributed into the humin, humic and fulvic acids of extracted soil fractions (Jenkinson, 1971; Sauerbeck & Fuhr, 1968; Sinha, 1972; Wagner, 1968; Zeller, Oberlander, Roth & Stadler, 1966). Moreover, the major quantity of residual ^{14}C accounted in the humin fraction (Wagner, 1968; Zeller *et al*, 1966). Sinha's (1972) study of the distribution of radioactive plant carbon into different fractions of soil fulvic acids revealed that under aerobic conditions there was a greater accumulation of fraction 'D' which consisted of high molecular weight fulvic compounds. However, under anaerobic conditions the dialysable substances of fraction 'B' were preferentially formed thus indicating that under such conditions the formation of low-molecular weight organic compounds predominated over the synthesis of specific high molecular weight fulvic acids. Results of this sort may be attributed to the assumption that under anaerobic conditions condensation of aromatic structural units of

humic and fulvic acids was restricted in the absence of free oxygen. The present concept of the biochemistry of condensation of phenolic compounds with amino-acids and protein visualizes the oxidation of aromatic compounds to quinones as the preceding step in the formation of humic substances. Wojcik - Wojtkowiak (1972) demonstrated that nitrogen of ^{15}N-tagged straw has a direct role in the synthesis of the different fractions of humic materials. Further he reported that during incubation the nitrogen content of humic acids increased significantly and independently of the fact whether it was treated with nitrogen or when straw only was introduced into the soil. In the present experiments the nitrogen-enrichment of humic acid fractions was 2.3 to 3.3.-fold; the maximum nitrogen-enrichment of the fulvic acids, in contrast, was only 30% and, under certain circumstances, the N-content actually declined (Tables 3.1.1 and 3.2.1).

3.3. CHEMICAL FACTORS AFFECTING FUNGAL TRANSFORMATION.

Introduction.

A number of factors affect the mineralization of added organic materials. The rapidity with which a given substrate is oxidized will depend upon its chemical composition and the physical and chemical status of the surrounding environment. The chemical factors which influence the decomposition of added carbonaceous material are the availability of minerals predominantly nitrogen. Phosphorus and sulphur also play an important role in the organic matter mineralization (Alexander, 1961; Stotzky & Norman, 1961a,b).

During decomposition of carbonaceous materials in soil, mineral nutrients are immobilized by the soil microorganisms thus making them temporarily unavailable for plants. Great interest has therefore been shown in the mineralization-immobilization cycle in general and in the biological immobilization of nitrogen and phosphorus in particular (Jansson, 1958; Macura & Kunc, 1965a). Very recently Shields, Paul, Lowe & Parkinson (1973) have investigated nitrogen immobilization-mineralization under field conditions using labelled $^{15}NH_4$-$^{15}NO_3$; they found that approximately 80% of the total nitrogen had been synthesized into microbial materials during the period of rapid carbon mineralization.

Ammonium and nitrate nitrogen, notwithstanding their divergent properties, generally have been considered about equally available as sources of nitrogen for microorganisms. Both ions are known to be utilized by large numbers of microorganisms and both are commonly used in microbiological experiments. That one form may be more readily absorbed by

microorganisms than the other, however, was first suggested by Richard & Shrikhande (1935). They found that ammonium was absorbed more rapidly than nitrate, particularly during the early stages of attack in the decomposition of cereal straw. Ammonium salts are the most readily assimilated nitrogen sources for most bacteria, actinomycetes and fungi. In like fashion, ammonium is preferentially utilized in the decomposition of organic matter (Alexander, 1961).

Macura & Kunc (1961; 1965a) reported that inorganic nitrogen and phosphorus levels in soil influenced the rate of glucose mineralization in the initial phases of continuous cultivation and the size and the composition of bacterial populations in the soil. Stotzky & Norman (1961a;b) studying the degradation of glucose in the usual batch culture, found that the concentration of inorganic elements like nitrogen, phosphorus and sulphur limited the rate but not the extent of glucose decomposition in soil.

In experiments concerning the mineralization of organic substances in soil, glucose is often used as a substrate (Jansson, 1960; Macura, Szolnoki, Kunc, Vancura & Babieky, 1965; Chahal & Wagner, 1965). Szolnoki, Kunc, Macura & Vancura (1963) studied the effect of glucose on the mineralization of labelled alfalfa that had already undergone decomposition for some time in soil. By incubating different fractions of alfalfa in soil it was shown that the glucose effect depended on the composition and the degree of decomposition of the added plant material. Shields, Paul & Lowe (1974) investigated in the laboratory the effect of freshly added labelled ^{13}C-glucose using soil samples which had been initially amended with ^{14}C-glucose and incubated for

60 days under field conditions. They found only a small priming action of ^{14}C-labelled materials, a result suggesting that stabilization of the relatively large amount of ^{14}C-labelled microbial material under field conditions was due to a lack of neither energy or nitrogen, nor was it susceptible to priming. However, Shields et al (1974) found that there was a marked priming of native ^{12}C during the initial incubation period effected by the addition of ^{13}C-glucose.

The purpose of the experiments described in this section was to investigate the effects of glucose, mineral nitrogen and phosphorus on mineralization of native organic matter and of fungal material added to the soil.

3.3.1. Effect of Glucose on Transformation of Mycelium in Soil.

One column was packed with 500g of air dry soil (aggregates 2-5mm in diameter) amended with a total of 16g wet weight of *Aspergillus nidulans* mycelium of which 5g were ^{14}C-labelled (275750cpm/g wet weight of mycelium). The packed soil column was moistened with sterile distilled water, made light proof and incubated at 25°C. The column was perfused continuously with CO_2 -free air, usually 1200 $ml.h^{-1}$, and with sterile glucose solution (0.1% w/v), approximately 10 $ml.h^{-1}$, for 35 days, subsequently the column was perfused with sterile distilled water at the same flow rate for 15 days and finally returned to a glucose flow until the end of the experimental period.

RESULTS.

Figure 3.3.1 shows the production of an initial large amount of CO_2 reaching a peak on day 2 and then declining. A high rate of CO_2 evolution was maintained up to day 20 but

thereafter it declined. On perfusion with water the rate fell but then remained nearly constant. Again on resumption of the glucose-flow there was a peak of carbon dioxide production which decayed gradually.

Measurements of cumulative production of carbon dioxide (Figure 3.3.2) indicated that about 8420mg carbon dioxide had been evolved during sixty days of incubation. About 71% of the label, as ^{14}C-CO_2, had been evolved during that period of incubation (Figure 3.3.3) and 62% of which had been evolved in the first ten days of the incubation.

Changes in microbial number are given in Figure 3.3.4. Numbers of bacteria rose to very high values on day 10 and declined slowly during the remainder of the experimental period. Actinomycete number increased approximately 7-fold on day 10 and showed very little change in number throughout the experimental period. The fungal population also showed a slight increase during the experimental period then declined slowly.

The level of β-1,3 glucanase activity changed only slightly during the first 35 days, but there was a significant rise following perfusion of the soil with water; the activity fell sharply after resumption of the glucose flow (Figure 3.3.5).

Less than 100µg/ml carbohydrate was detected in the effluent during the first five days of the experiment. Moreover, ^{14}C-labelled substances were not detected in the leachate throughout the experiment.

The soil was extracted for polysaccharide by treatment with 24N H_2SO_4 followed by dilution to N H_2SO_4 and heating at 100°C. The hydrolysates were neutralized with barium carbonate and the sugars separated by paper chromatography as described previously (section 2.1.4.5). The data, presented in

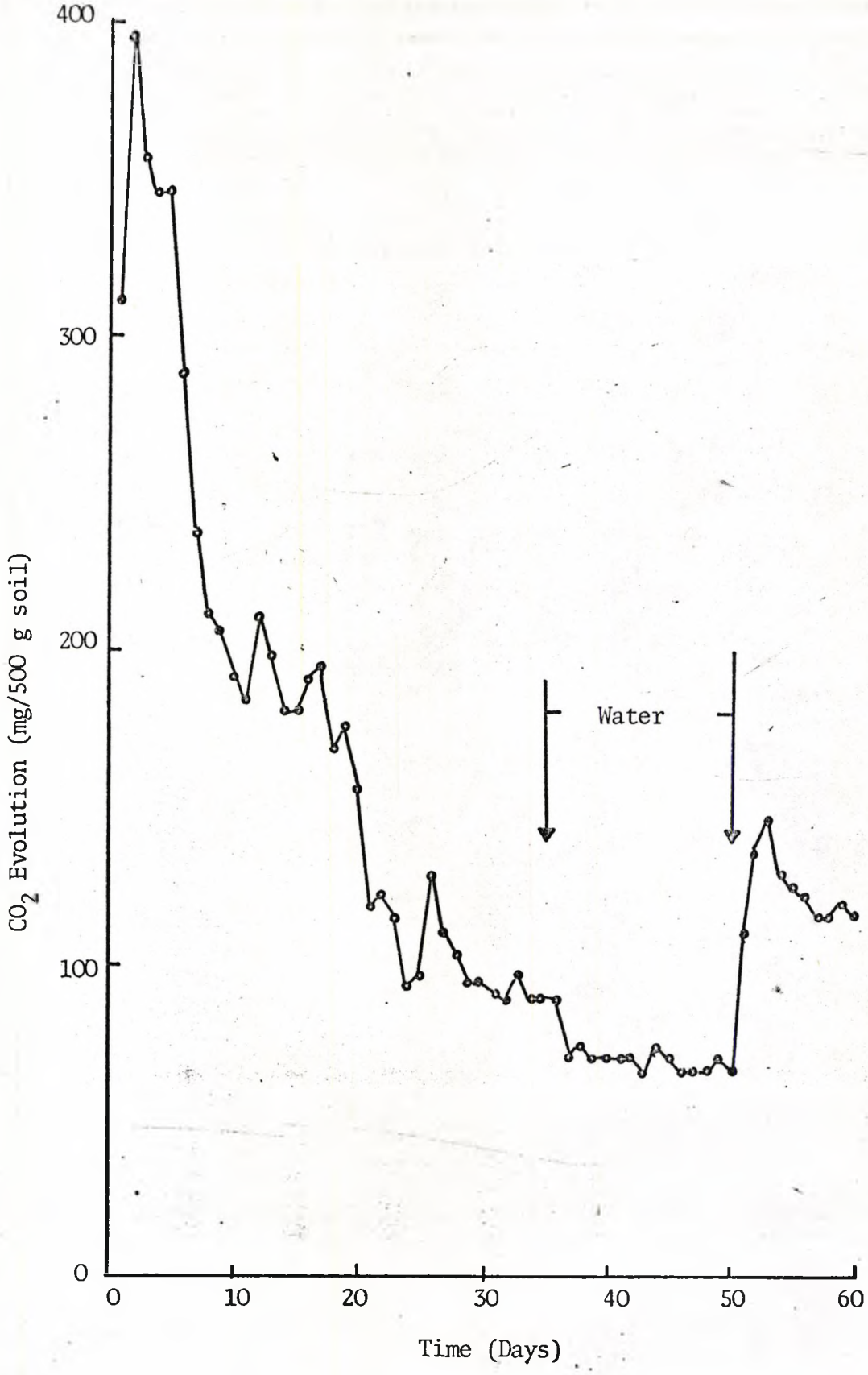


Figure 3.3.1. Dynamics of CO_2 production from mycelium-amended soil under aerobic conditions during continuous flow of Glucose solution (0.1%, w/v). At day 35 the flow was changed to one of water, and at day 50 the glucose flow was restored.

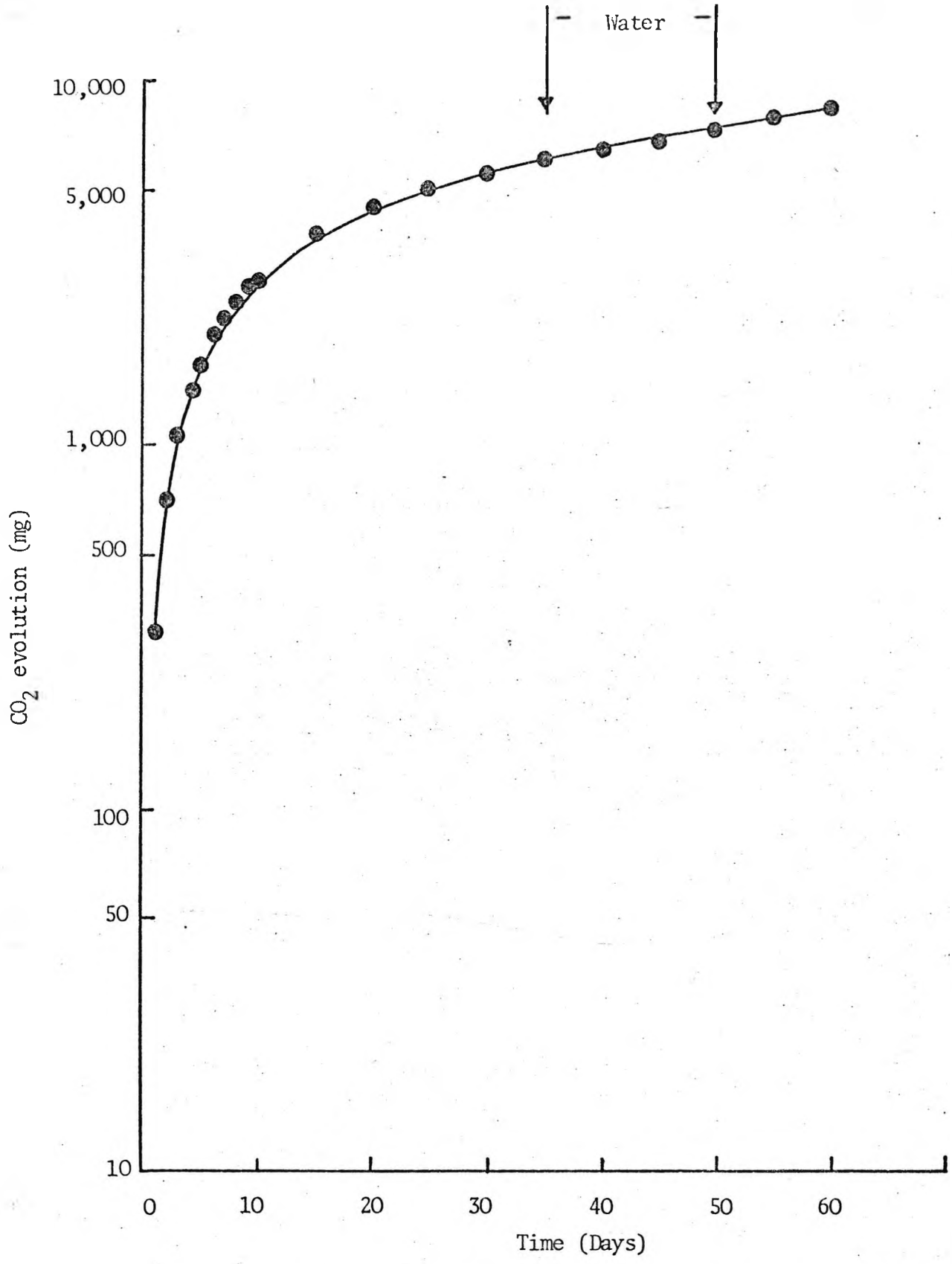


Figure 3.3.2. Cumulative CO_2 production from mycelium-amended soil under aerobic conditions during continuous flow of glucose solution (0.1%, w/v). Other conditions as in Fig. 3.3.1.

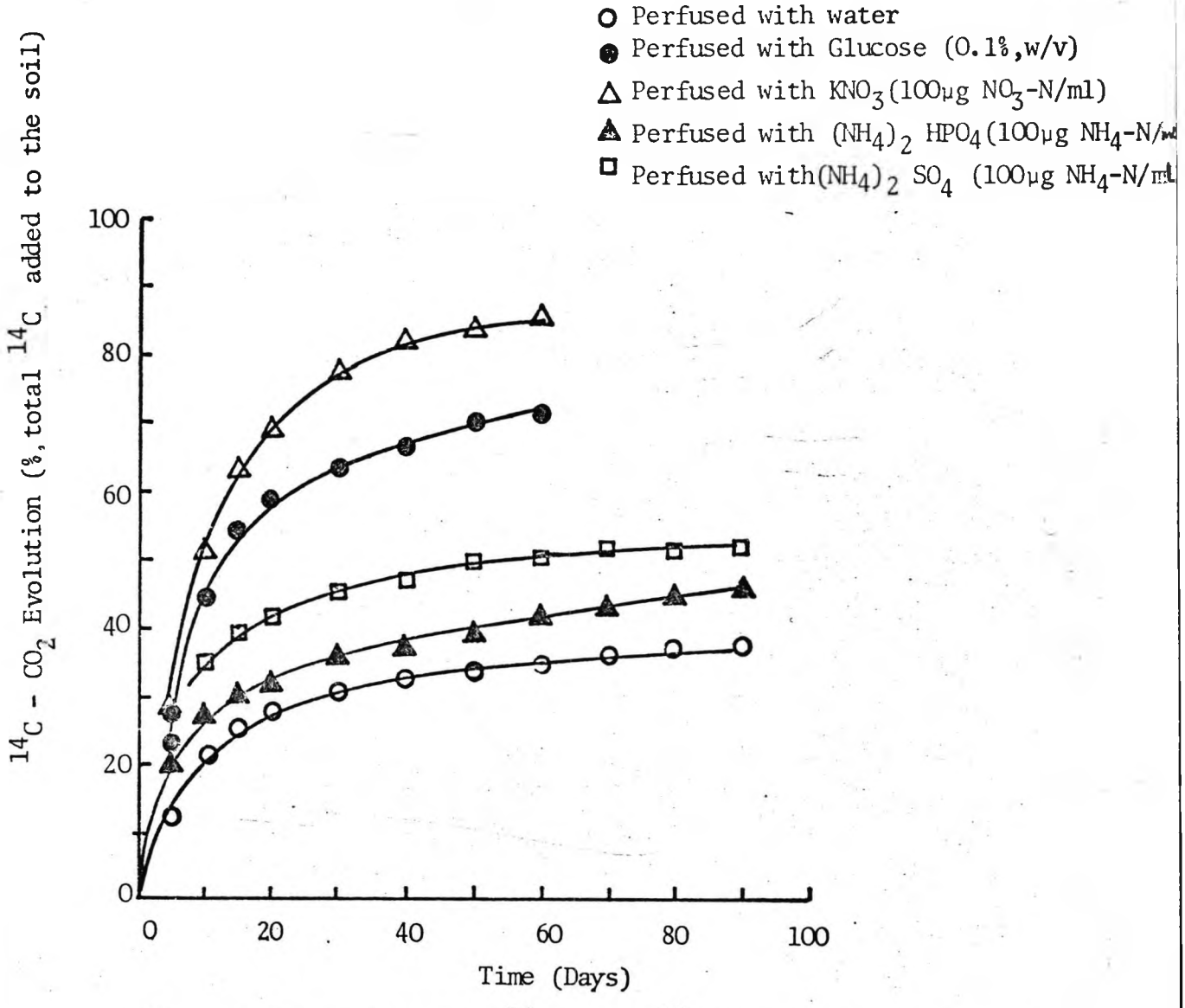


Figure 3.3.3. Release of $^{14}CO_2$ from ^{14}C-labelled mycelium under aerobic conditions during the continuous flow of various nutrient solutions (as above) through soil columns.

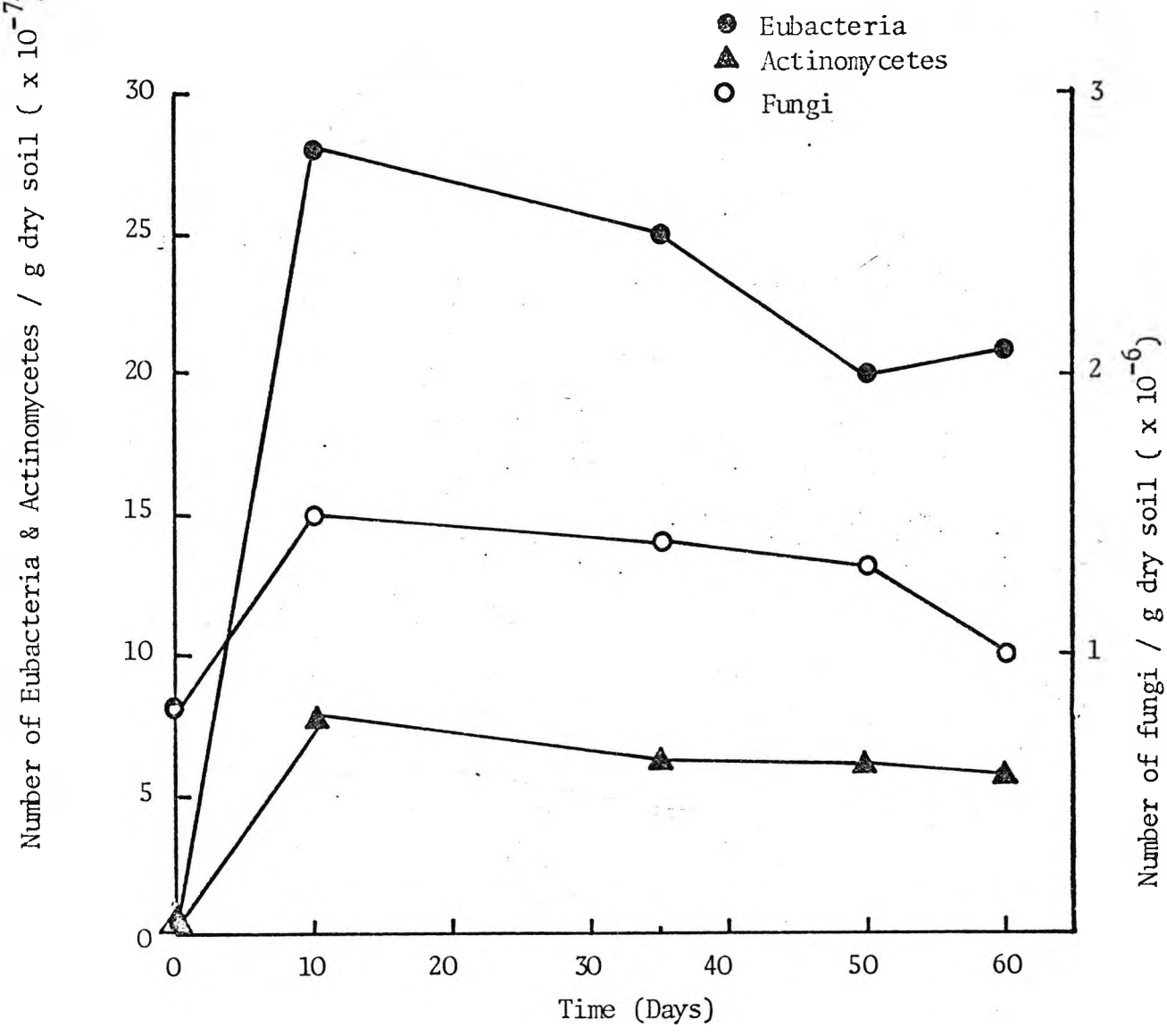


Figure 3.3.4. Changes in microbial populations during continuous flow of glucose (0.1%, w/v) through mycelium-amended soil; aerobic incubation conditions.

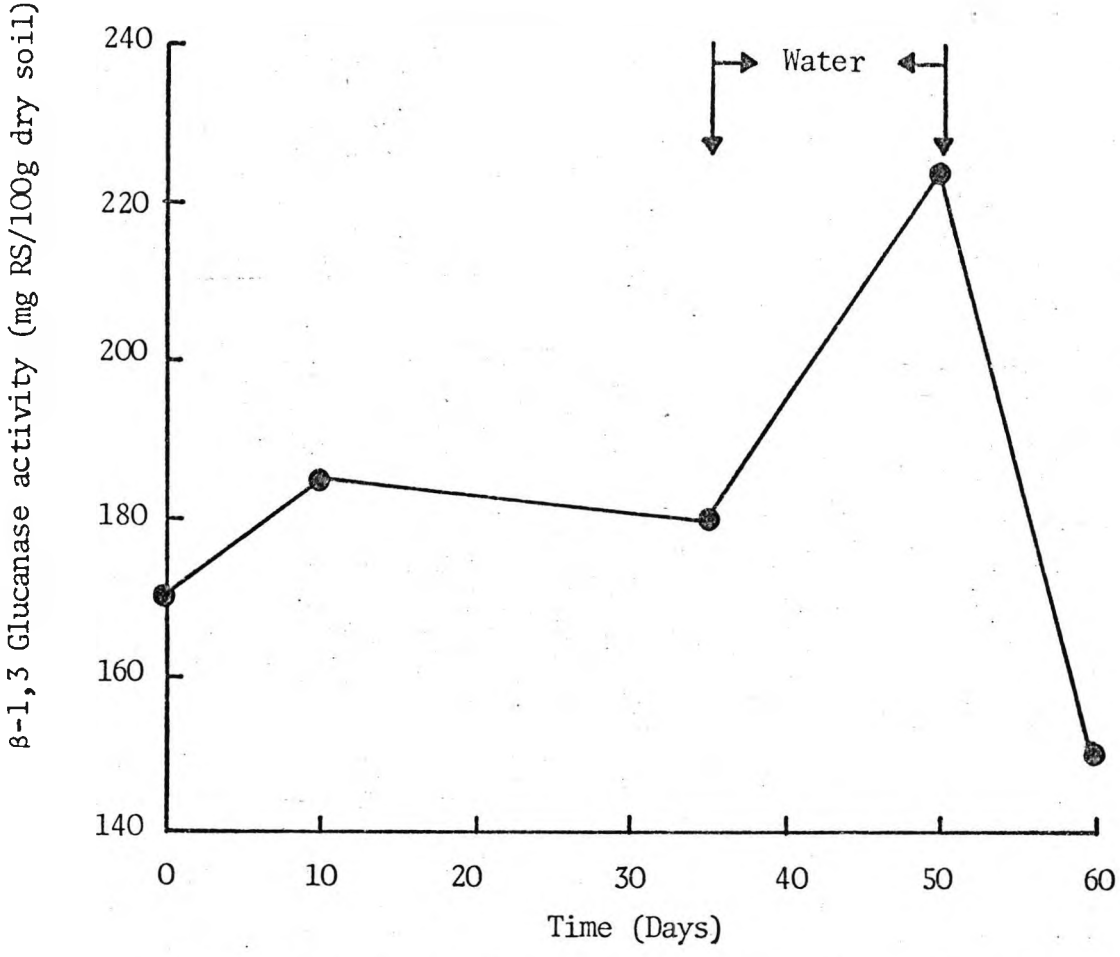


Figure 3.3.5. β-1,3 Glucanase activity in mycelium-amended soil under aerobic conditions, during the continuous flow of glucose (0.12,w/v) Other conditions as in Figure 3.3.1.

TABLE 3.3.1.

Amount of sugars in mycelium-amended soil perfused continuously with glucose.

Time (Days)	0	10	32	50	60
Sugars (mg / g soil)					
Glucose	7.5	7.3	7.2	7.2	7.3
Galactose	2.2	2.0	2.0	2.0	2.0
Mannose	2.0	2.0	1.8	1.8	1.8
Xylose	1.9	1.9	1.9	1.8	1.8
Rhamnose	1.0	0.9	0.9	0.9	0.9
Total Reducing sugar (as glucose)	20.4	18.0	18.0	17.8	18.0

TABLE 3.3.2

Amounts of Humic and Fulvic acids extracted from the control and mycelium-amended soils at the end of the incubation period.

Soil treatment		Humic acids (%, w/w)	Fulvic acids (%, w/w)	Elemental Analysis (%, w/w)					
				Humic acids			Fulvic acids		
				C	H	N	C	H	N
None (control soil) Zero time		7.92	0.25	5.81	0.57	0.32	10.48	0.88	0.91
Glucose Amended		4.32	0.75	7.09	1.99	0.85	8.97	1.11	1.23
KNO_3	Control [a]	4.62	1.10	9.54	0.71	1.16	10.00	0.69	0.91
	Amended [b]	3.96	0.70	8.15	1.10	1.08	9.65	0.91	1.24
$(NH_4)_2 SO_4$	Control	4.32	0.30	7.08	2.00	0.89	11.83	1.88	0.86
	Amended	3.70	0.80	8.39	2.14	1.05	9.30	0.80	1.01
$(NH_4)_2HPO_4$	Control	4.00	0.60	5.42	0.64	0.52	9.10	1.21	1.23
	Amended	3.00	0.60	6.81	0.32	0.46	10.05	0.57	0.97

a) Indicates unamended soil perfused with and incubated under the conditions specified.

b) Indicates mycelium-amended soil perfused with and incubated under the conditions specified.

Table 3.3.1., revealed that the pattern and quantities of sugars varied very little with time. Radioactive carbon was not detected in any of the sugars eluted.

Fractionation of organic matter at the end of the experimental period showed no water soluble components. However, both humic and fulvic acids (Fraction D) were recovered from the alkali extract of the soil. The amounts of humic and fulvic acids and their elemental analyses are presented in Table 3.3.2. No labelled carbon was detected either in humic or fulvic acid fractions.

3.3.2. Effect of Potassium nitrate on Mineralization of Fungal Mycelium.

Two columns were prepared with 500g air dry soil (2-5mm in diameter aggregate size), soil in one column was amended with mycelium as in section 3.3.1. Columns were moistened with sterile distilled water, made light proof and incubated at 25°C. Both control and amended soil columns were perfused continuously with CO_2-free air (1200 $ml.h^{-1}$) and with sterile KNO_3 solution (100µg NO_3-N/ml) approximately 10 $ml.h^{-1}$ for 35 days. Subsequently both columns were perfused with sterile distilled water till day 50 after which time perfusion with KNO_3 was restored for a further ten days.

RESULTS.

Figure 3.3.6 shows that there was a rapid and marked evolution of carbon dioxide from the soil amended with mycelium which reached a peak on day two; after this time the rate of production declined progressively, but still remained higher than the control soil. On changing to water perfusion both amended and control soil columns showed a slight increase in

the production of CO_2. On return of KNO_3 perfusion the production declined and remained constant till the end of the experimental period.

Figure 3.3.3 illustrates the evolution of $^{14}CO_2$ from the mycelium-amended soil; about 85% of ^{14}C has mineralized in sixty days.

Measurement of the cumulative production of CO_2 during 60 days of incubation revealed that mineralization was 115% higher in the amended compare to the control soil (Figure 3.3.7).

The addition of mycelium to soil perfused with KNO_3 solution enhanced the microbiological activity (Figures 3.3.8 and 3.3.9). Bacterial number rose fifty-fold by day 10, declined slightly by day 35 and then showed very little decrease till day 60. Actinomycete populations increased thirty-fold and were maintained at the high level throughout the experimental period. In the control unamended soil both bacteria and actinomycete number showed no change, remaining at a low level throughout. An increase in the fungal population also was recorded in the amended soil by day 10 which declined slowly to the level of control soil fungal population by day 50.

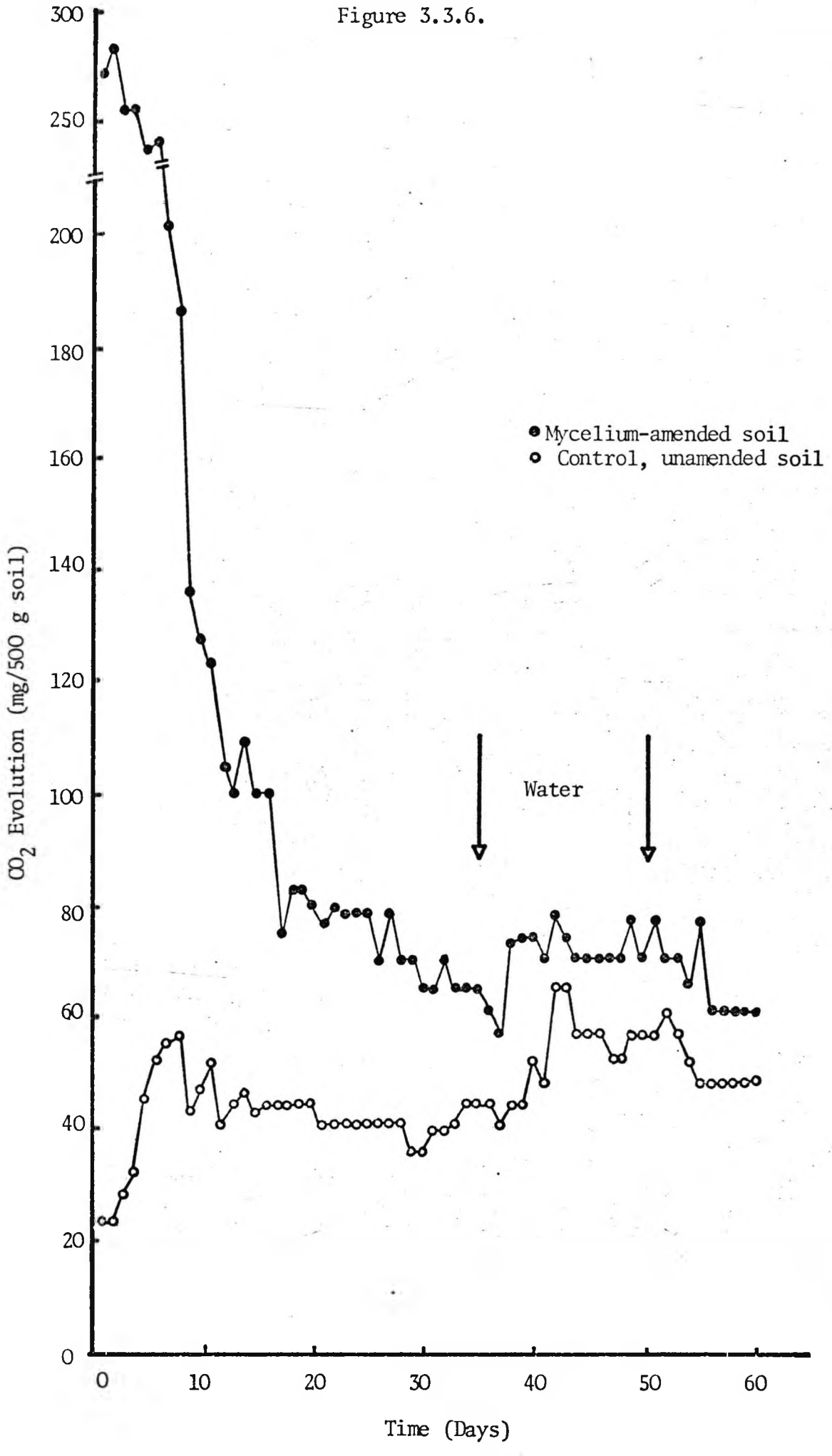
Figure 3.3.10 shows the level of β-1,3 glucanase activity in both amended and control soil. Increasing activity was observed throughout the course of the experiment; the activity only started to decline at the end of the experiment, day 60.

There was no NH_4-N or NO_2-N detectable in the effluent throughout the experiment. No marked change was observed in the concentration of NO_3-N in the effluent or in the reservoir solution throughout the period of experiment.

In the organic matter fractionation at the end of the experiment water soluble components were not detected in either

Figure 3.3.6. Dynamics of CO_2 production from mycelium-amended and control soils under aerobic conditions during continuous flow of KNO_3 solution (100µg NO_3 - N/ml).
At day 35 the flow was changed to one of water and at day 50 the KNO_3 flow was restored.

Figure 3.3.6.


300
250
200
180
160
140
120
100
80
60
40
20
0
CO_2 Evolution (mg/500 g soil)
Mycelium-amended soil
Control, unamended soil
Water
0
10
20
30
40
50
60
Time (Days)

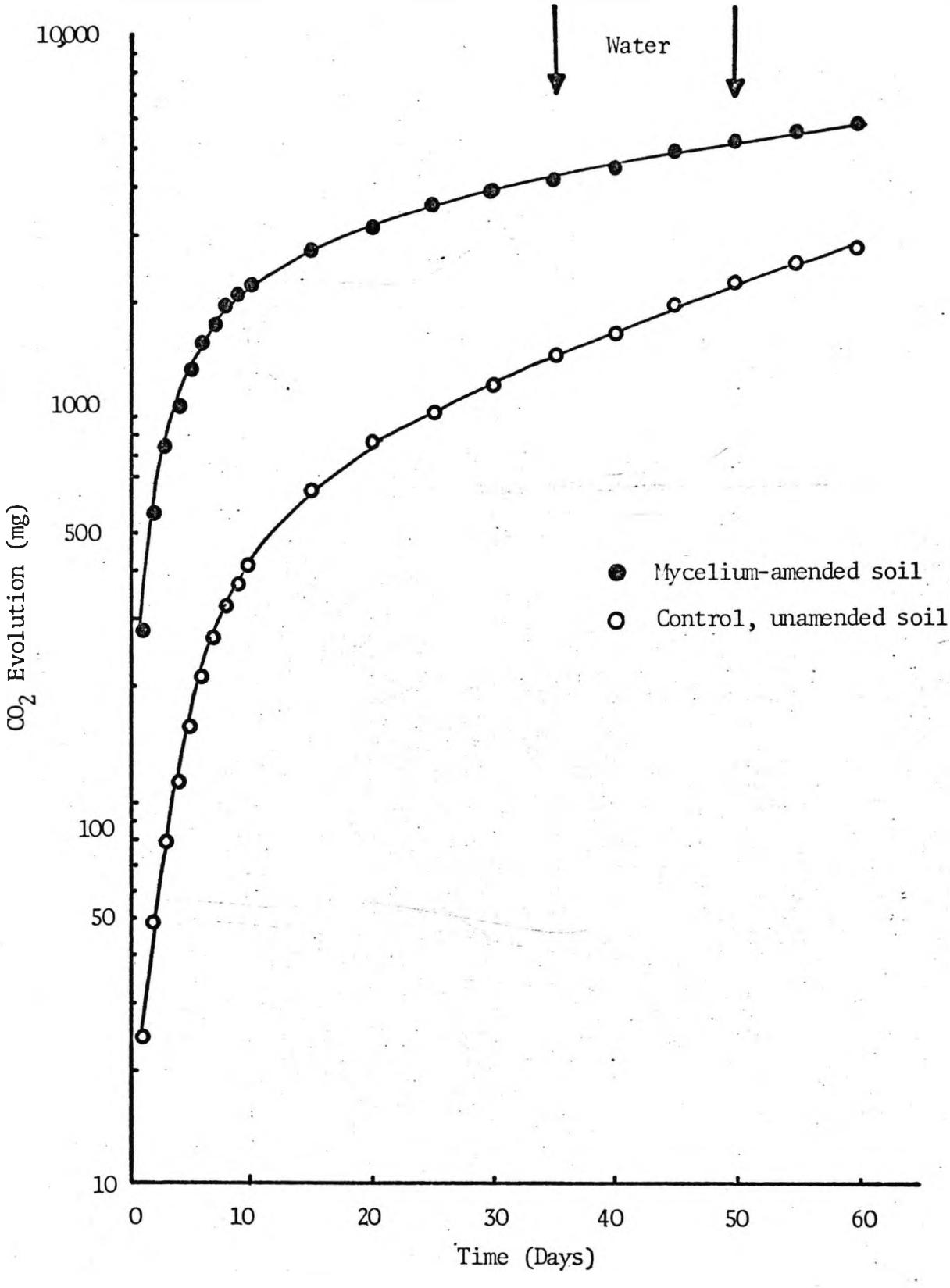


Figure 3.3.7. Cumulative CO_2 production from mycelium-amended and control soils under aerobic conditions during continuous flow of KNO_3 solution. Other conditions as in Figure 3.3.6.

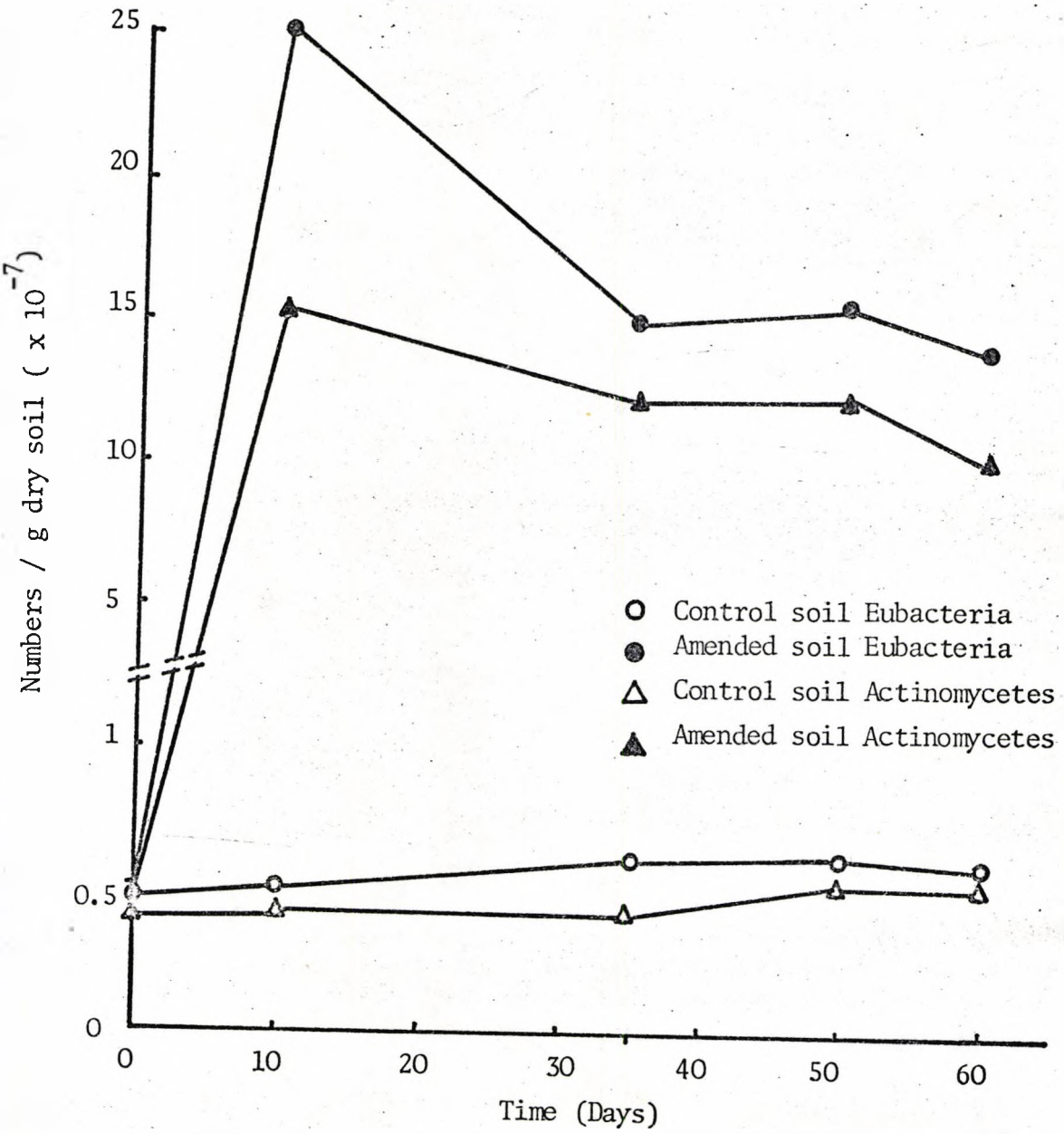


Figure 3.3.8. Changes in microbial populations during continuous flow of KNO_3 solution through control and mycelium-amended soils; aerobic incubation conditions.

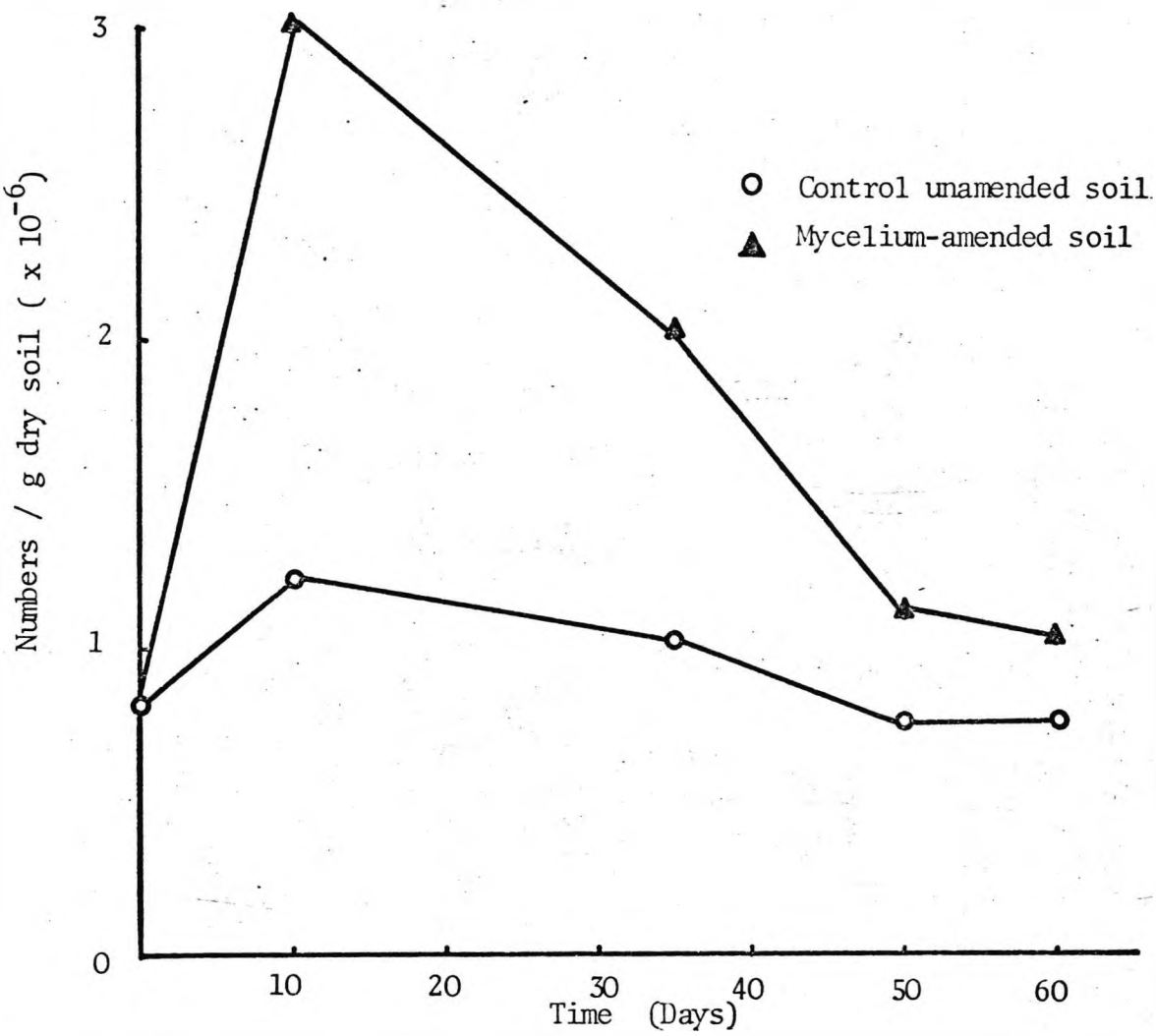


Figure 3.3.9. Changes in fungal population during continuous flow of KNO_3 solution through control and mycelium-amended soils; aerobic incubation conditions.

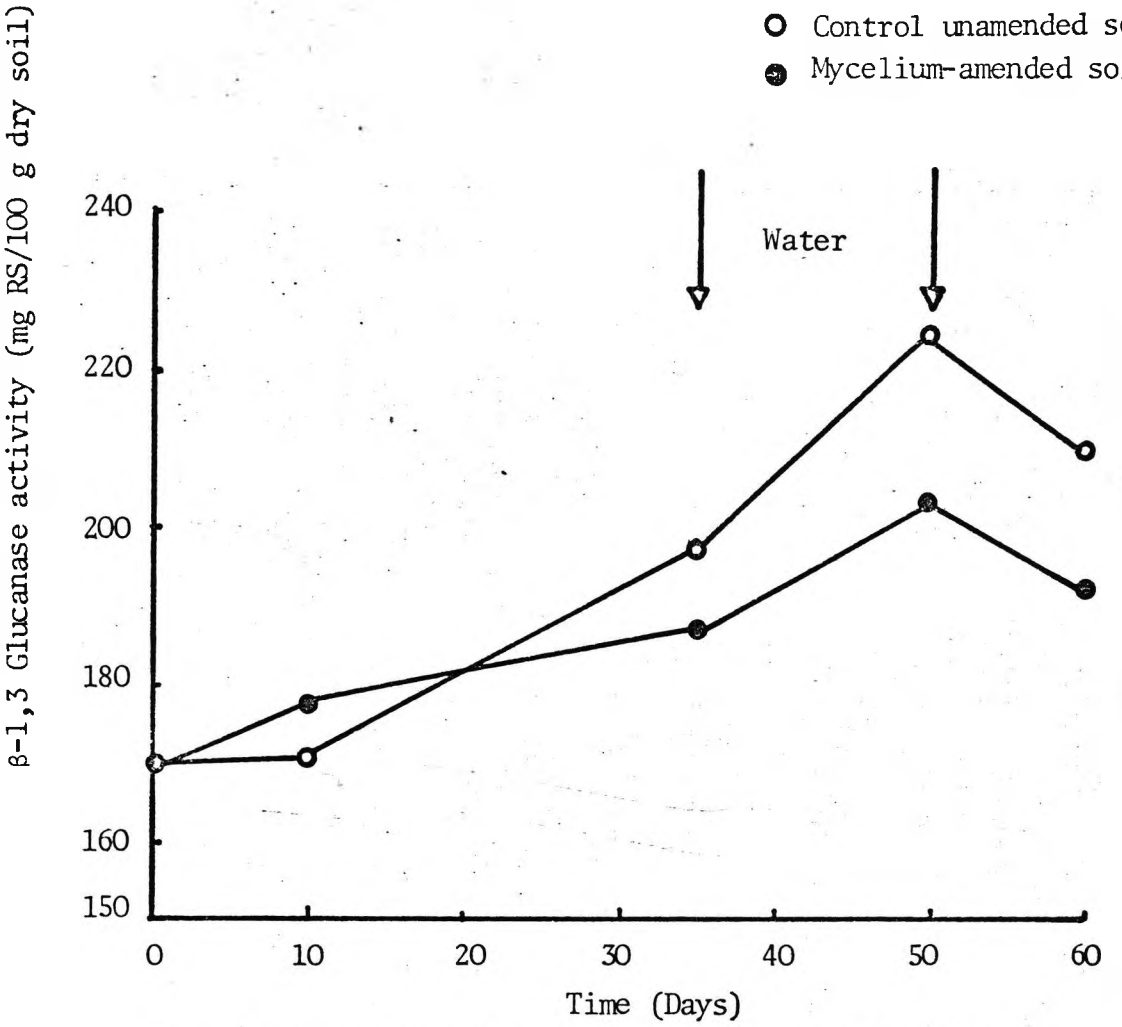


Figure 3.3.10. β-1,3 Glucanase activity in control and mycelium-amended soils during the continuous flow of KNO_3 solution; aerobic incubation conditions. Other conditions as in Figure 3.3.6.

control or amended soil; however, both humic and fulvic acids were recovered by alkali extraction of the soil as shown in Table 3.3.2. Radioactive carbon was not detected in either humic or fulvic acid fraction of the amended soil.

3.3.3. Effect of Ammonium Sulphate on the Transformation of *Aspergillus nidulans* Mycelium in Soil.

Both control and amended soil columns were perfused continuously with CO_2-free air and sterile ammonium sulphate solution (100µg NH_4-N/ml) approximately 10ml.h^{-1} for forty days. Subsequently the columns were perfused with sterile distilled water at the same rate for about twenty days then for a further period of thirty days with ammonium sulphate solution.

RESULTS.

A rapid and massive evolution of carbondioxide from the mycelium-amended soil column occurred during the first two days, after which it declined slowly (Figure 3.3.11). On the first day there was a burst of CO_2 production from the control soil column which declined and peaked again at day 6; then it declined slowly and remained constant throughout the remainder of the experiment. Change to water perfusion did not significantly alter the production rate of carbondioxide in either column. The perfusion with ammonium sulphate was restored on 61 day, but again the production of CO_2 remained at the low levels in both the columns.

Data in Figure 3.3.3. shows that only about 52% of the added ^{14}C-labelled mycelium was mineralized in 90 days and that approximately two thirds of that evolved occurred within the first 10 days.

Figure 3.3.12 illustrates the cumulative production of

carbon dioxide in control and amended soils. During 90 days of incubation 7137mg of CO_2 has evolved in amended soil column with respect to 3483 in the control. The mineralization rate is about 105% higher in the mycelium-amended than in the control.

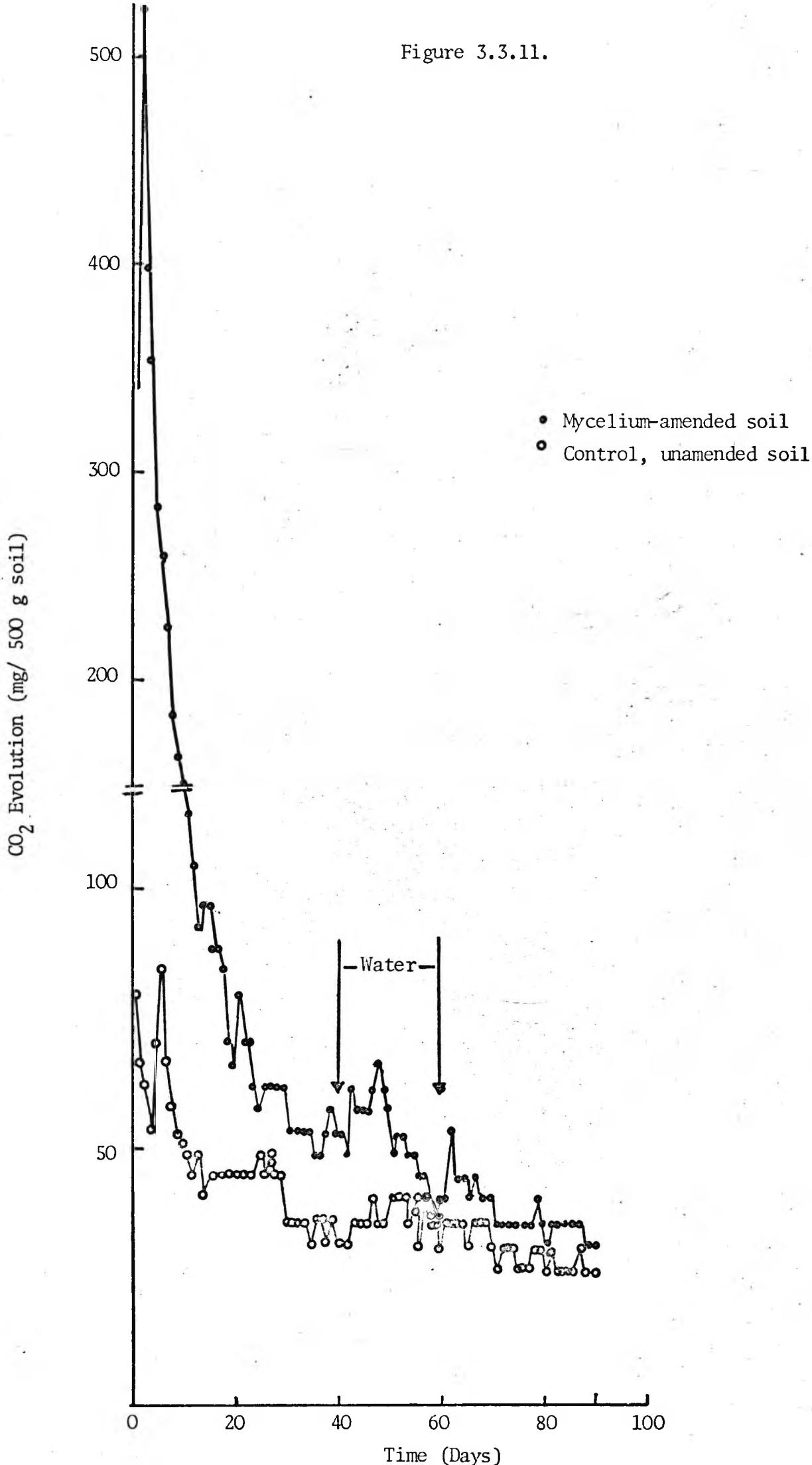
Microbiological changes during the experimental period is presented in Figures 3.3.13 and 3.3.14. There was approximately a 10-15 fold increase in the number of bacteria and actinomycete of mycelium-amended compared with control soil which was maintained throughout the experimental period. The fungal population was higher in the amended soil column up to 40 days, then it declined to the level of control soil.

The level of β-1,3 glucanase activity rose slightly in both control and amended soils. In amended soil the activity declined slightly after perfusing with water, but rose on re-establishing the flow of ammonium sulphate solution, however, the activity fell sharply in the control column after resumption of the flow of ammonium sulphate (Figure 3.3.15).

The effluent was tested for the presence of ammonium, nitrate and nitrite ions. The amount of nitrate and ammonium nitrogen determined in the outflowing fluid at daily intervals is shown in Figure 3.3.16. In the mycelium-amended soil column effluent nitrite nitrogen was detected for the first ten days (approximately 0.02µg NO_2-N/ml) but none was detected in the control column effluent. Varying amounts of ammonium nitrogen were detected in effluents from the amended soil column prior to water perfusion but after perfusion of water and re-establishment the flow of ammonium sulphate solution the amount of ammonium nitrogen increased slowly and stabilized at a higher level. In the control soil column very little ammonium nitrogen

Figure 3.3.11. Dynamics of CO_2 production from mycelium-amended and control soils under aerobic conditions during continuous flow of Ammonium Sulphate solution (100 µg NH_4-N/ml). At day 40 the flow was changed to one of water and at day 60 the flow of $(NH_4)_2\ SO_4$ was restored.

Figure 3.3.11.


500
400
300
200
100
50
CO_2 Evolution (mg/ 500 g soil)
Mycelium-amended soil
Control, unamended soil
Water
0
20
40
60
80
100
Time (Days)

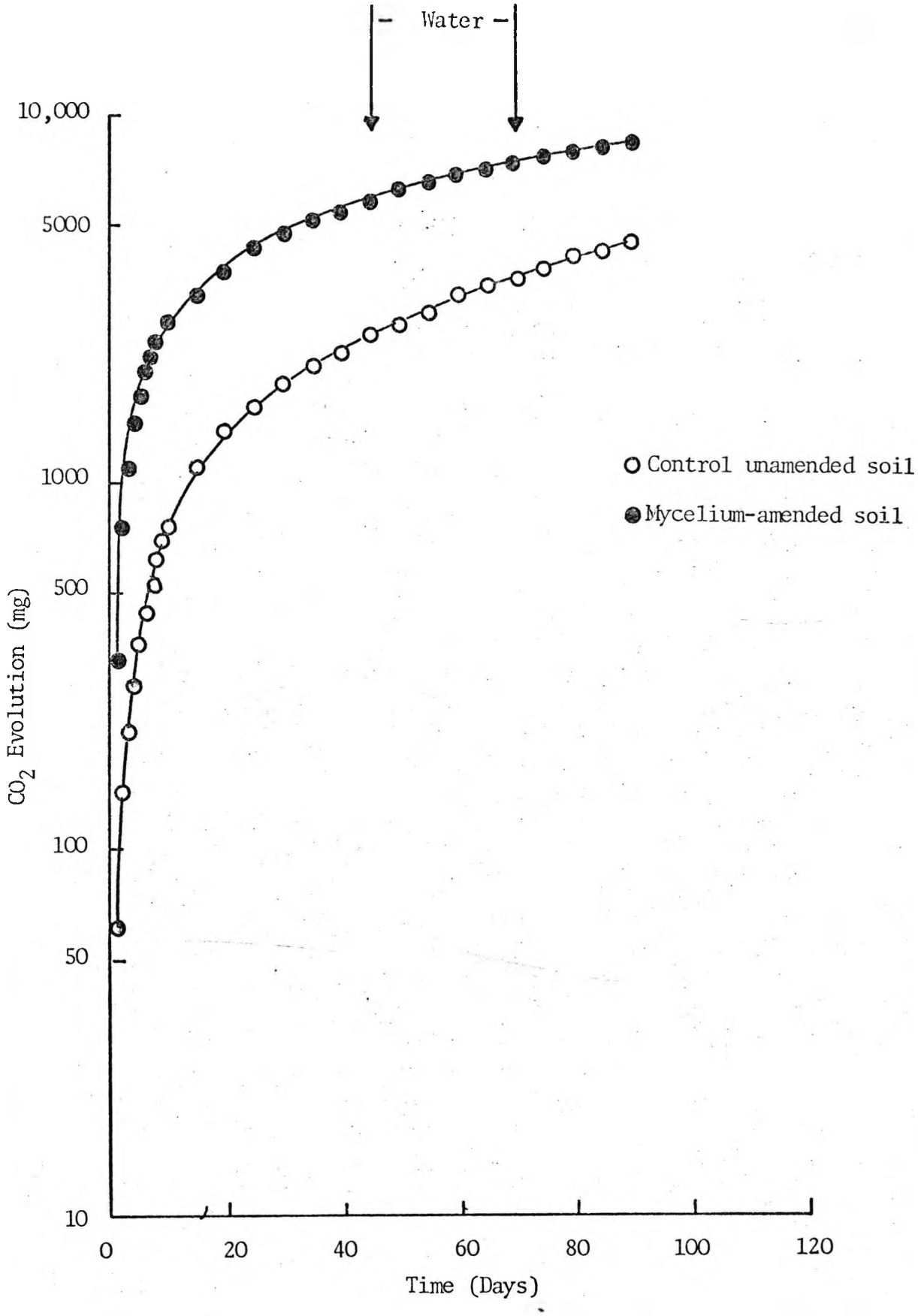


Figure 3.3.12. Cumulative CO_2 production from mycelium-amended and control soils under aerobic conditions during continuous flow of ammonium sulphate solution. Other conditions as in Figure 3.3.11.

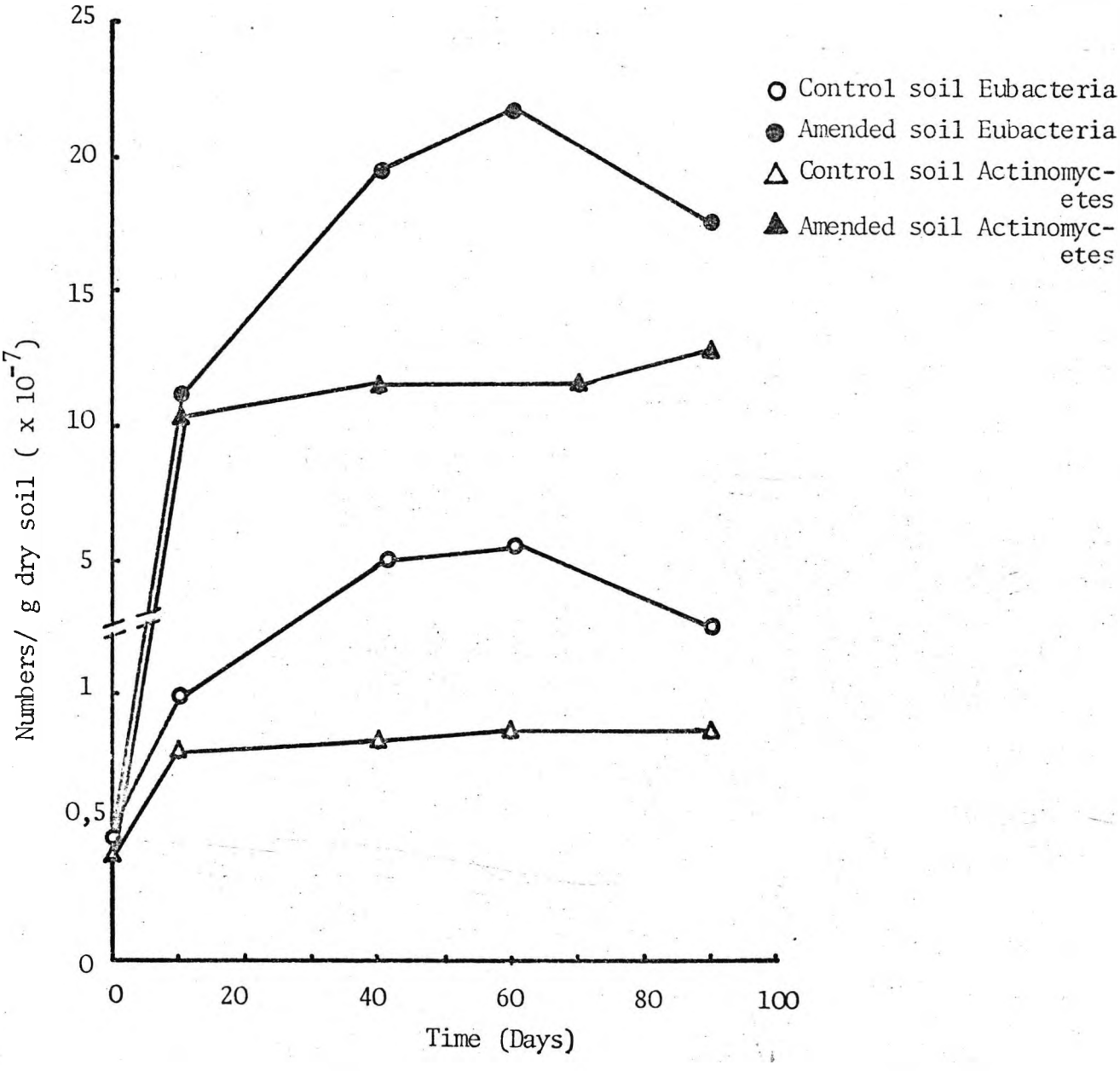


Figure 3.3.13. Changes in Bacterial and Actinomycete populations during continuous flow of ammonium sulphate solution through control and mycelium-amended soils; aerobic incubation conditions.

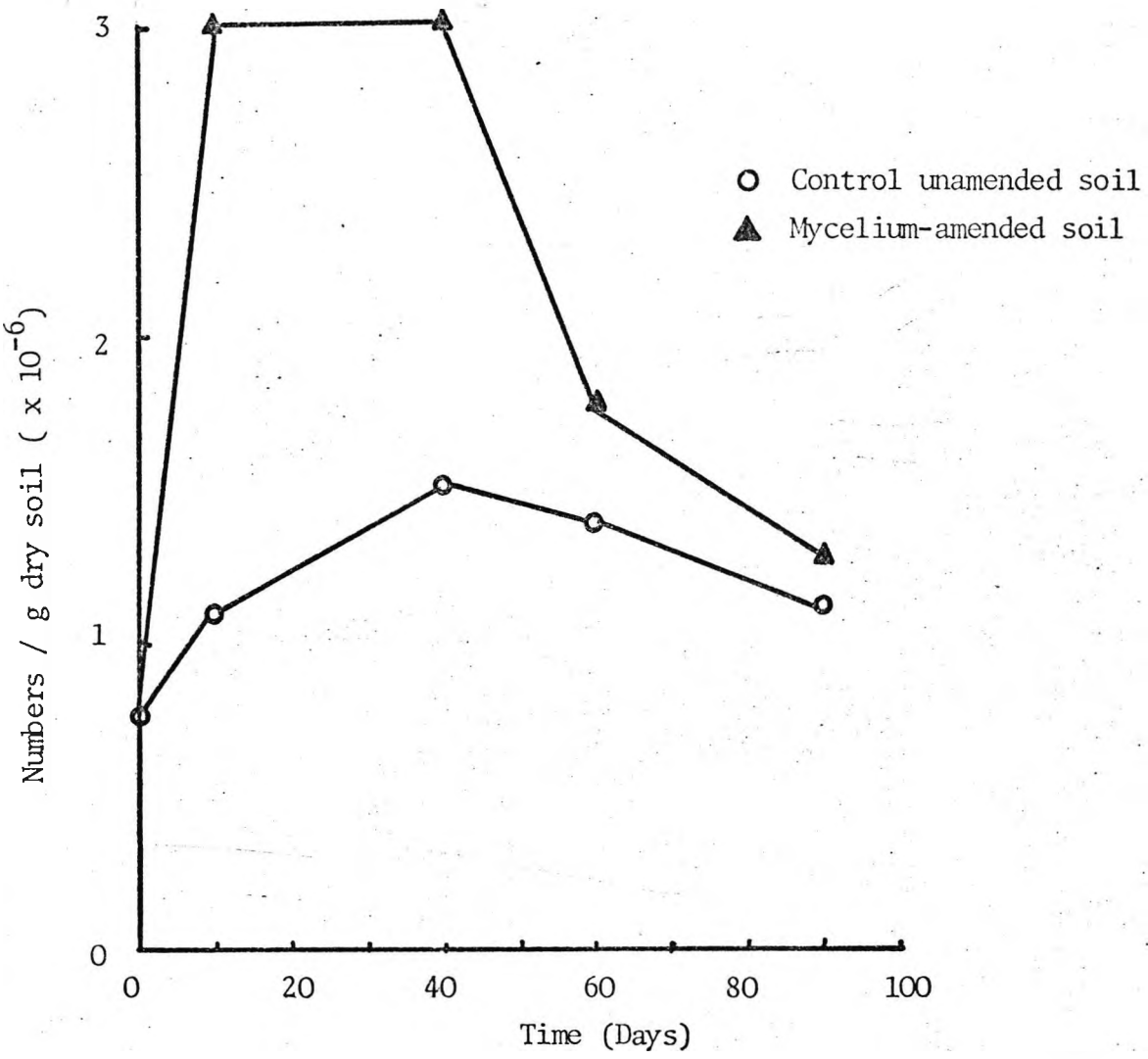


Figure 3.3.14. Changes in Fungal population under aerobic conditions during continuous flow of ammonium sulphate solution through control and mycelium amended soils.

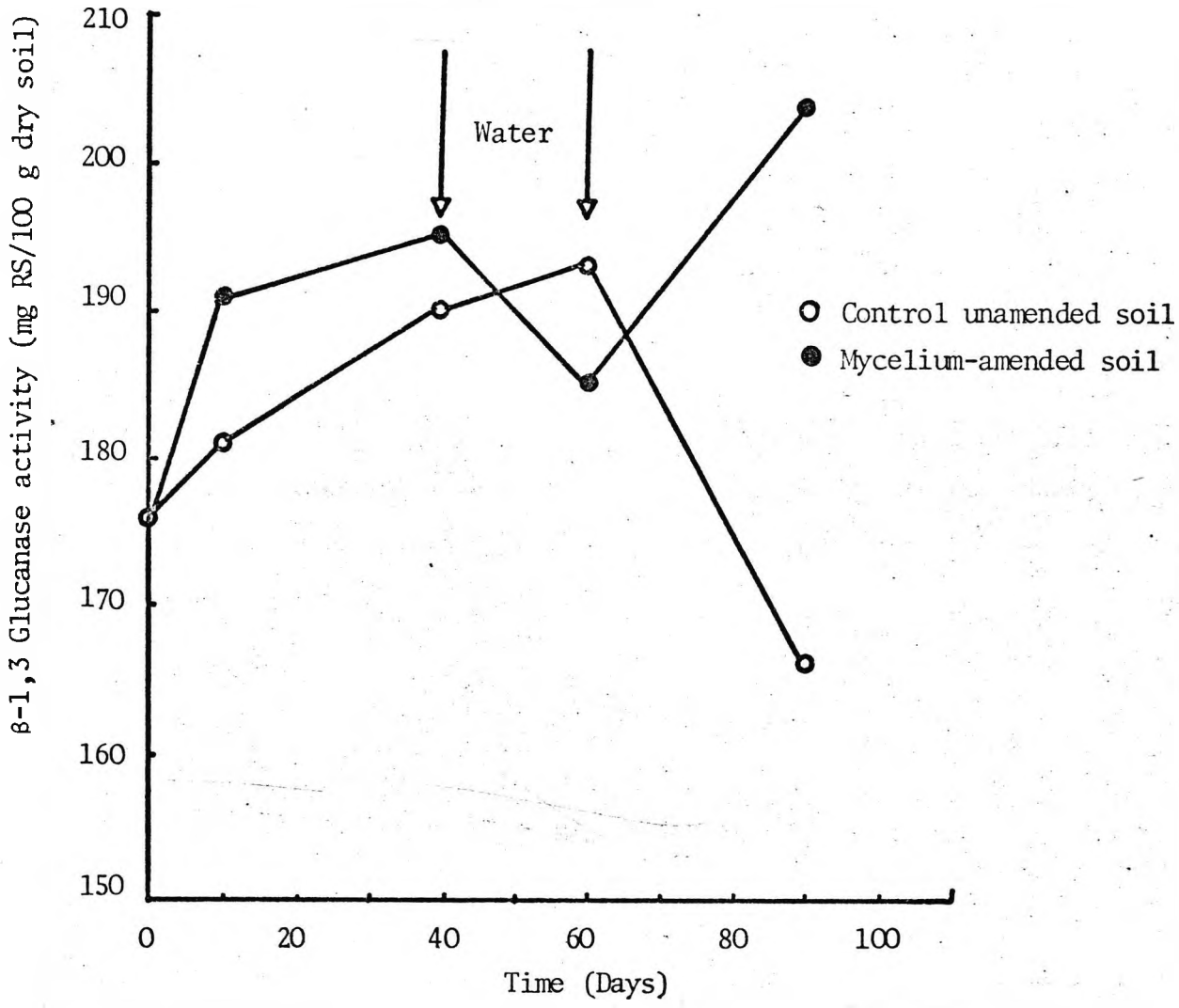


Figure 3.3.15. β-1,3 Glucanase activity in control and mycelium-amended soils during the continuous flow of ammonium sulphate solution; aerobic incubation conditions. Other conditions as in Figure 3.3.11.

Figure 3.3.16. Ammonium-N and Nitrate-N concentrations in the effluent from control and mycelium-amended soil columns during the continuous flow of ammonium sulphate solution; aerobic incubation conditions. Other conditions as in Figure 3.3.11.

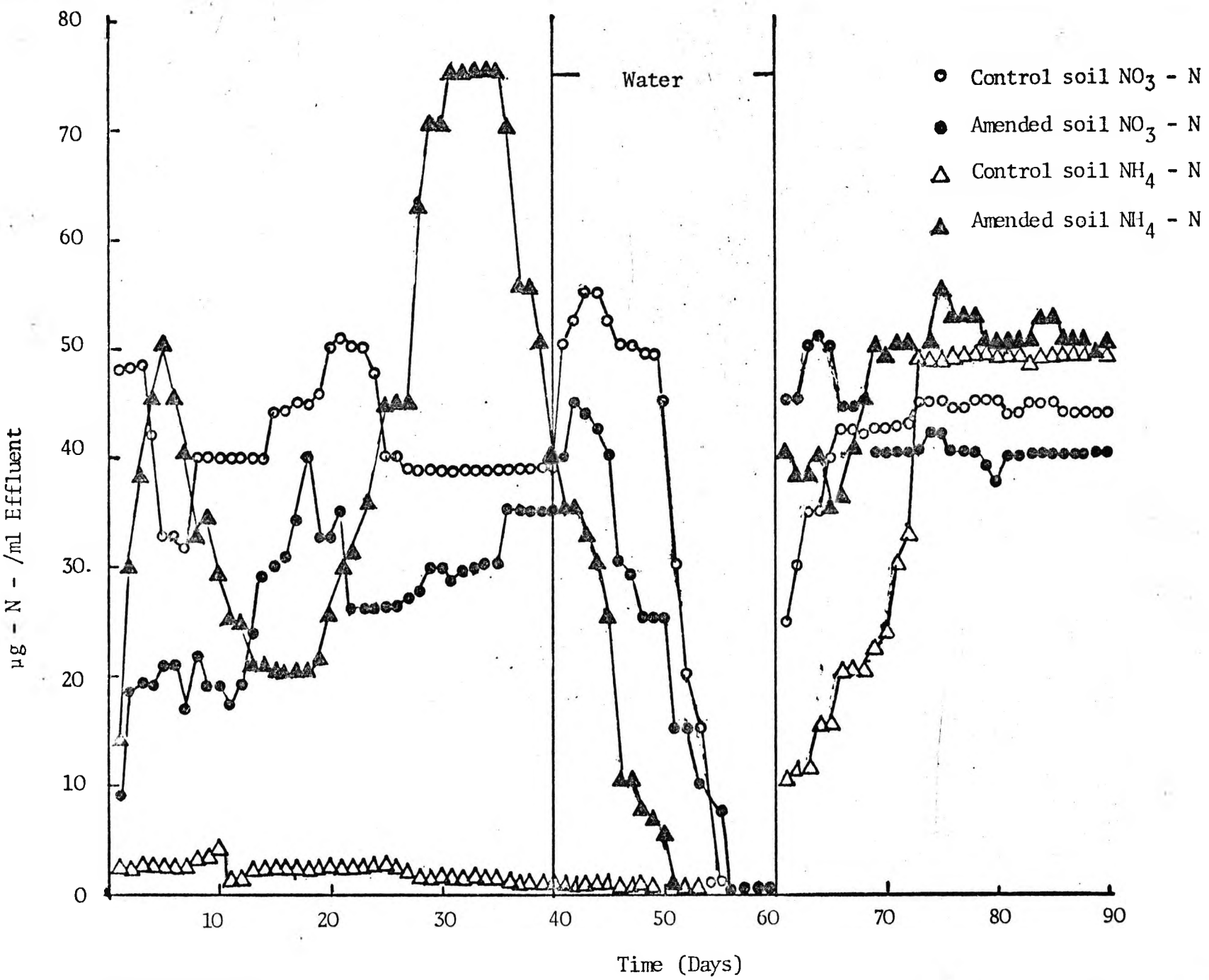

Water
Control soil NO_3 - N
Amended soil NO_3 - N
Control soil NH_4 - N
Amended soil NH_4 - N
µg - N - /ml Effluent
Time (Days)
0
10
20
30.
40
50
60
70
80
10
20
30
40
50
60
70
80
90

Figure 3.3.16.

was detected till day 40. After perfusing water and continuing the flow of ammonium sulphate solution the ammonium-nitrogen increased slowly and stabilized at a higher level as in the amended soil column effluent. Nitrate-nitrogen was at a high, more or less constant level in the effluent from the control soil column. There was a slow increase and fluctuating in concentration of nitrate-nitrogen in the effluent of amended soil column till day 40. After perfusing with water and re-establishing the flow of ammonium sulphate an increased and stable concentration of nitrate-nitrogen was recorded.

Water soluble components were not detected in the organic matter fractions of either control or amended soils at the end of 90 days of incubation. However, both humic and fulvic acids were recovered as shown in Table 3.3.2. Labelled carbon was not detected in either humic or fulvic acid fractions of the amended soil.

3.3.4. Effect of Ammonium Phosphate on the Transformation of Mycelium in Soil.

Control and amended soil columns were perfused with CO_2-free air and sterile ammonium phosphate solution (100µg NH_4-N/ml) approximately 10ml.h^{-1} for 45 days, then the columns were perfused with sterile distilled water at the same rate for 25 days and finally changed to the flow of ammonium phosphate solution for a further period of 20 days.

RESULTS.

The dynamics of carbon dioxide evolution are illustrated in Figure 3.3.17. There was an initial rapid rise in the amended soil column followed by a slow, progressive decline. There was a slow rise in the production of carbon dioxide in the control soil peaking on day 7 and then slowly declining and remaining

at a low level throughout the experiment. There was no significant change observed during the perfusion with water.

In 90 days of incubation about 45.5% of the added ^{14}C-mycelium had been mineralized, more than one half being evolved in first ten days (Figure 3.3.3).

Cumulative production of carbon dioxide evolved during 90 days of incubation of both mycelium-amended soil and control unamended soil is illustrated in Figure 3.3.18.

Microbial populations in mycelium-amended soil column showed a rapid rise on day 5 (Figures 3.3.19 and 20). The bacterial and microfungi population fell on day 35 and then onwards remained constant. Actinomycete populations remained constant throughout the experimental period in the amended soil.

β-1,3 Glucanase activity showed an initial rise in the amended soil, fell sharply by day 35 and then rose rapidly following restoration of ammonium phosphate after a period of leaching with water. The activity in the control soil increased following the second ammonium phosphate perfusion (Figure 3.3.21).

Phosphorus was not detected in the effluents from either amended or control soils throughout the experiment. Nitrite nitrogen was detected in traces (approximately 0.02µg NO_2^-N/ml) for about first 12 days in the effluent of soil amended with mycelium, but none was leached from the control soil. Nitrate nitrogen in both control and amended soil column effluents increased steadily and reached a maximum on day 21 and by day 45 it had become stabilized (Figure 3.3.22). Subsequently, a gradual fall occurred when water was perfused through the columns, while on resumption of the ammonium phosphate flow there was a rapid increase in the nitrate nitrogen which attained constant high levels till the end of the experiment. Ammonium nitrogen

Figure 3.3.17. Dynamics of CO_2 production from mycelium-amended and control soils under aerobic conditions during continuous flow of ammonium phosphate solution (100 µg NH_4-N/ml).
At day 45 flow changed to one of water and at day 70 ammonium phosphate was restored.

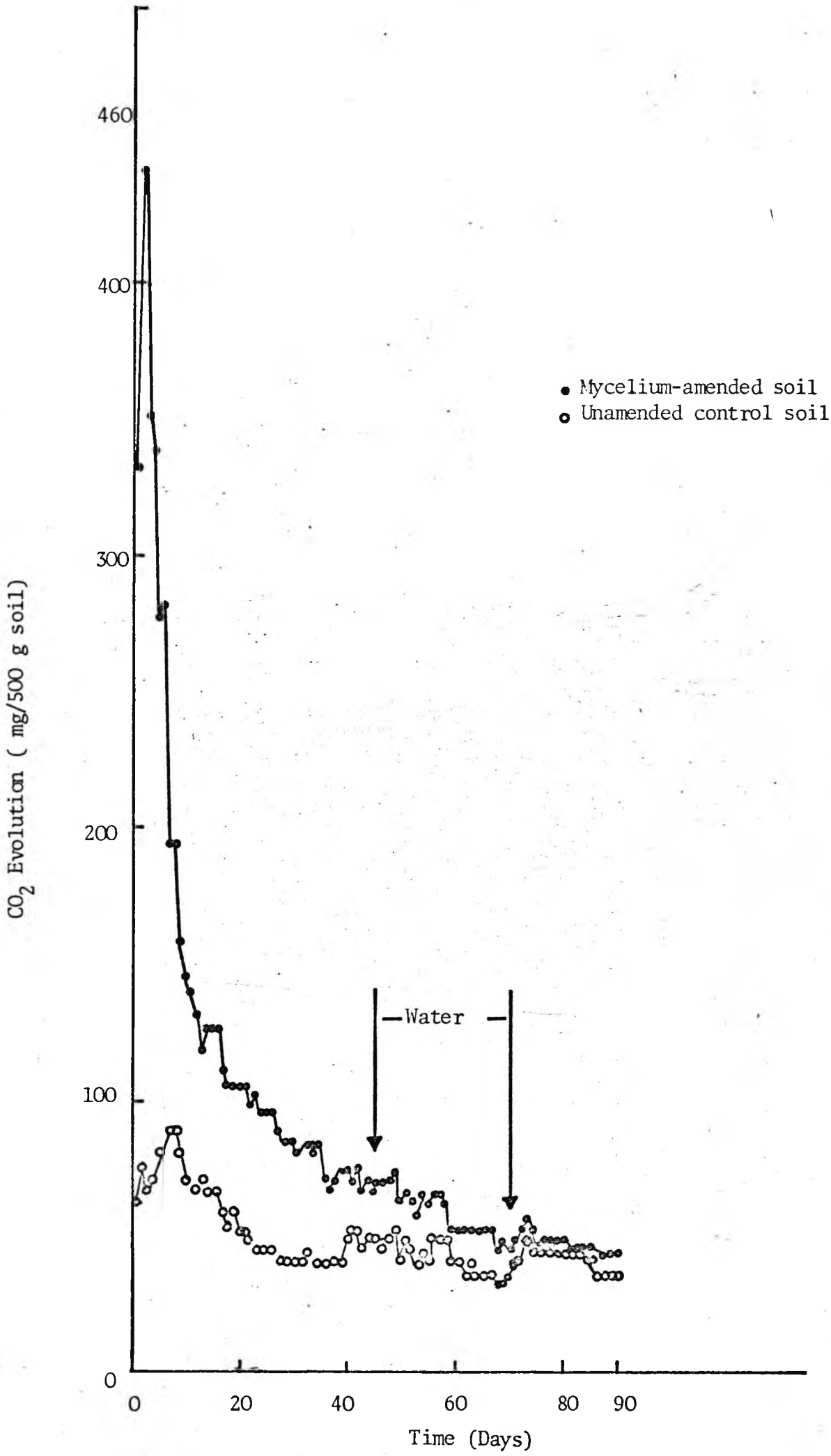

460
400
300
200
100
0
CO_2 Evolution (mg/500 g soil)
• Mycelium-amended soil
o Unamended control soil
Water
0
20
40
60
80
90
Time (Days)

Figure 3.3.17.

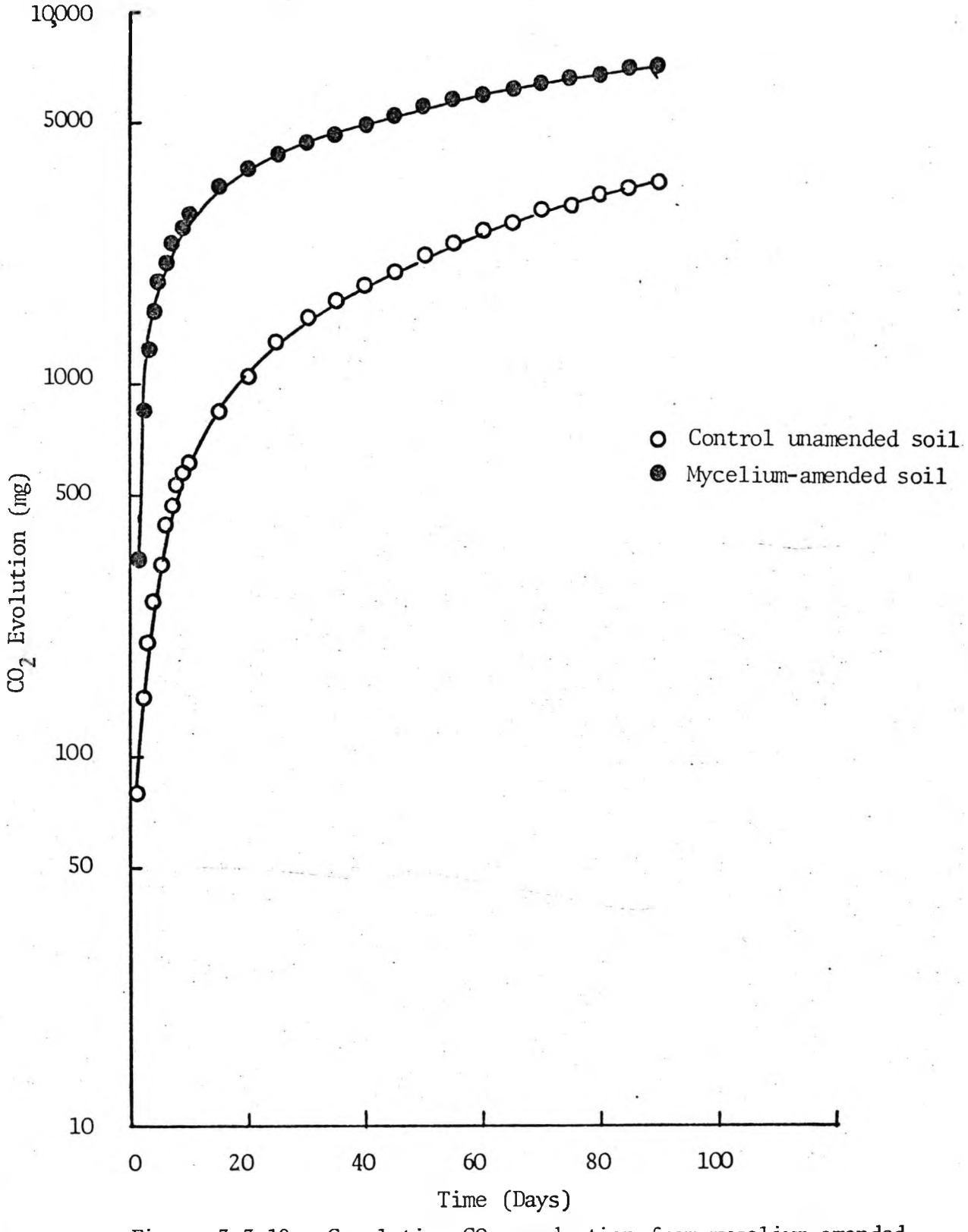


Figure 3.3.18. Cumulative CO_2 production from mycelium-amended and control soils during continuous flow of ammonium phosphate solution; aerobic incubation conditions.

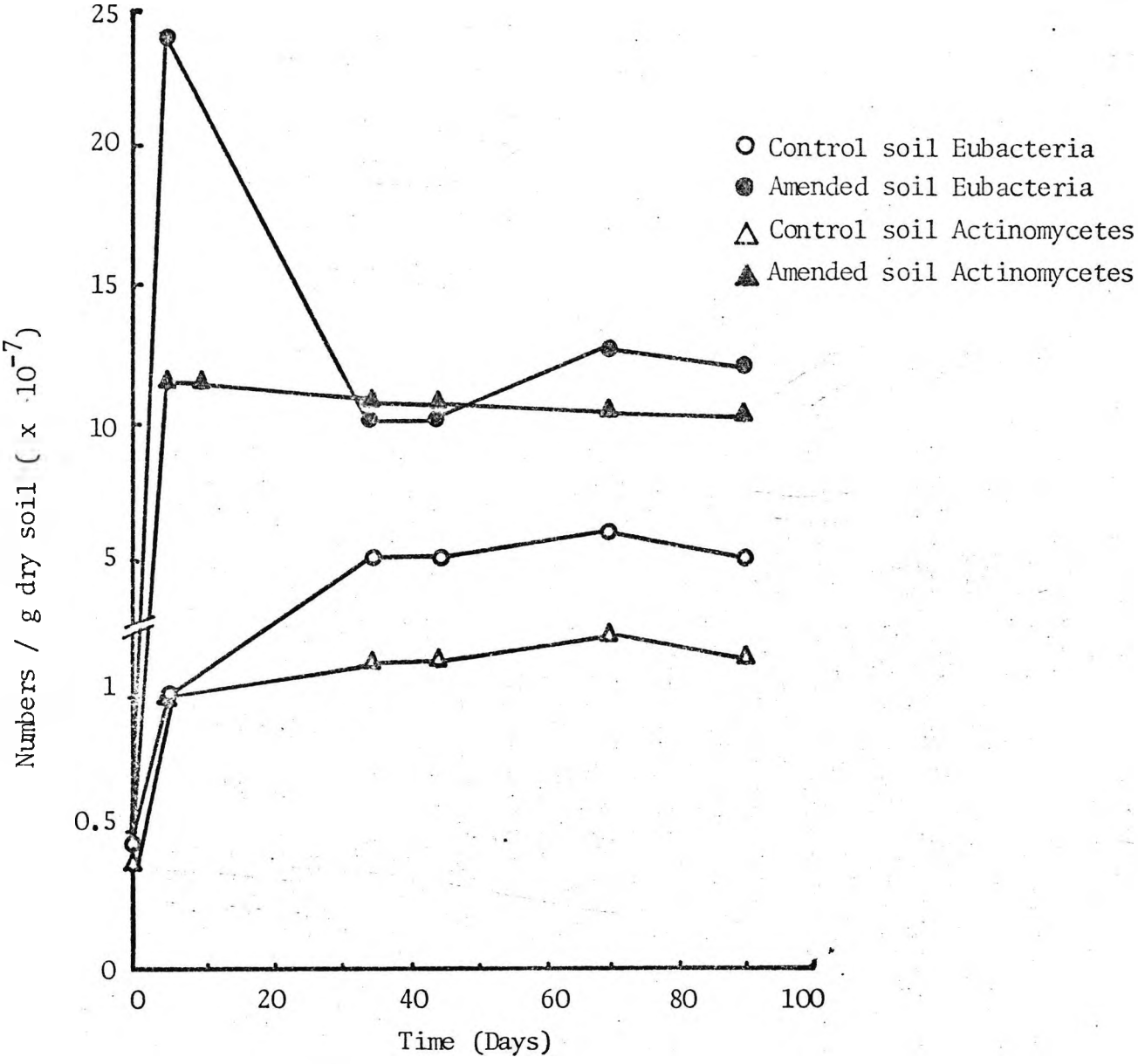


Figure 3.3.19. Changes in Bacterial and Actinomycete populations during continuous flow of ammonium phosphate solution through control and mycelium-amended soils; aerobic incubation conditions.

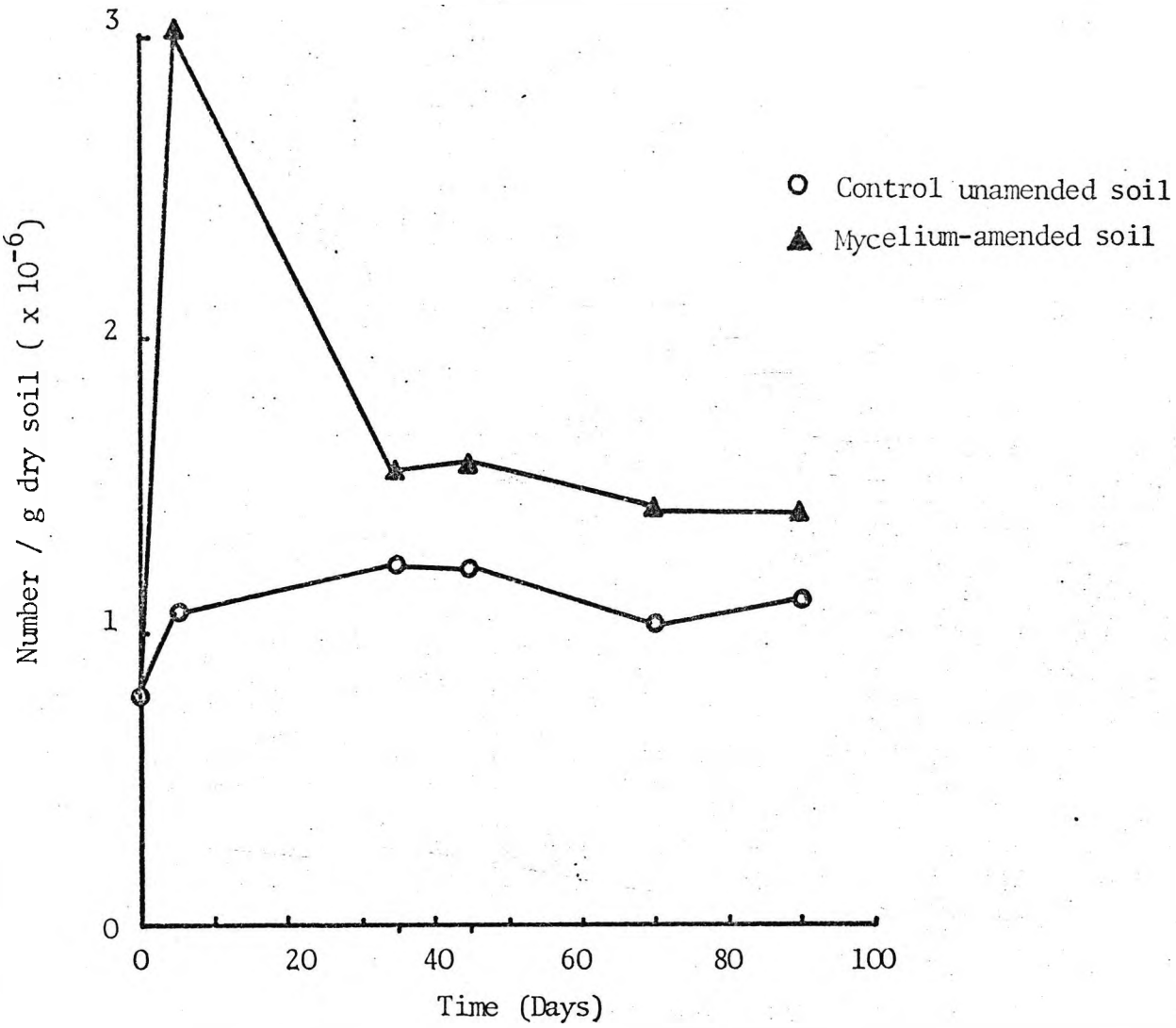


Figure 3.3.20. Changes in fungal population during continuous flow of ammonium phosphate solution through control and mycelium amended soils; aerobic incubation conditions.

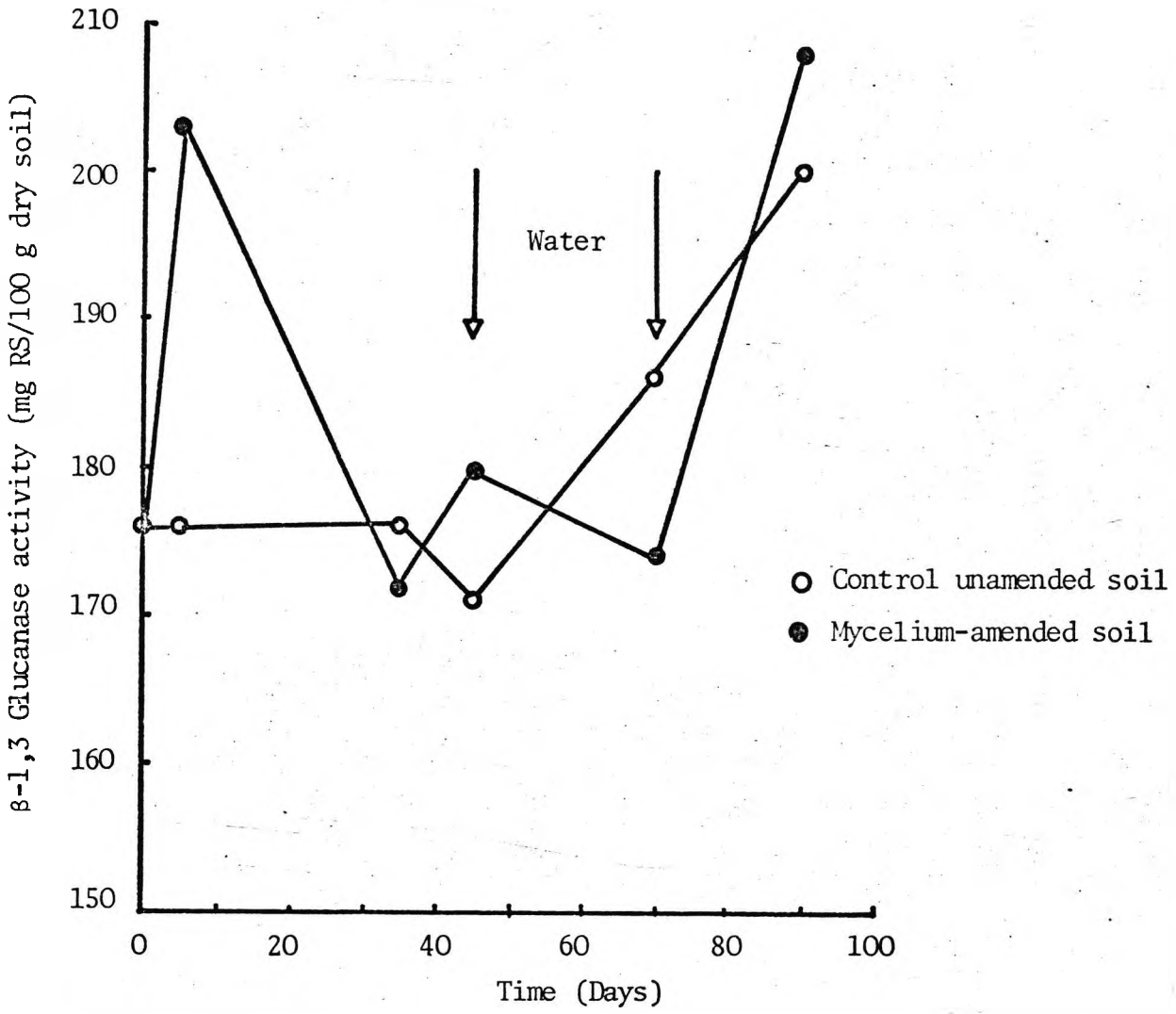


Figure 3.3.21. β-1,3 Glucanase activity in control and mycelium-amended soils during the continuous flow of ammonium phosphate solution; aerobic incubation conditions. Other conditions as in Figure 3.3.17.

Figure 3.3.22. Ammonium-N and Nitrate-N concentrations in the effluent from control and mycelium-amended soil columns during the continuous flow of ammonium phosphate solution; aerobic incubation conditions. Other conditions as in Figure 3.3.17.

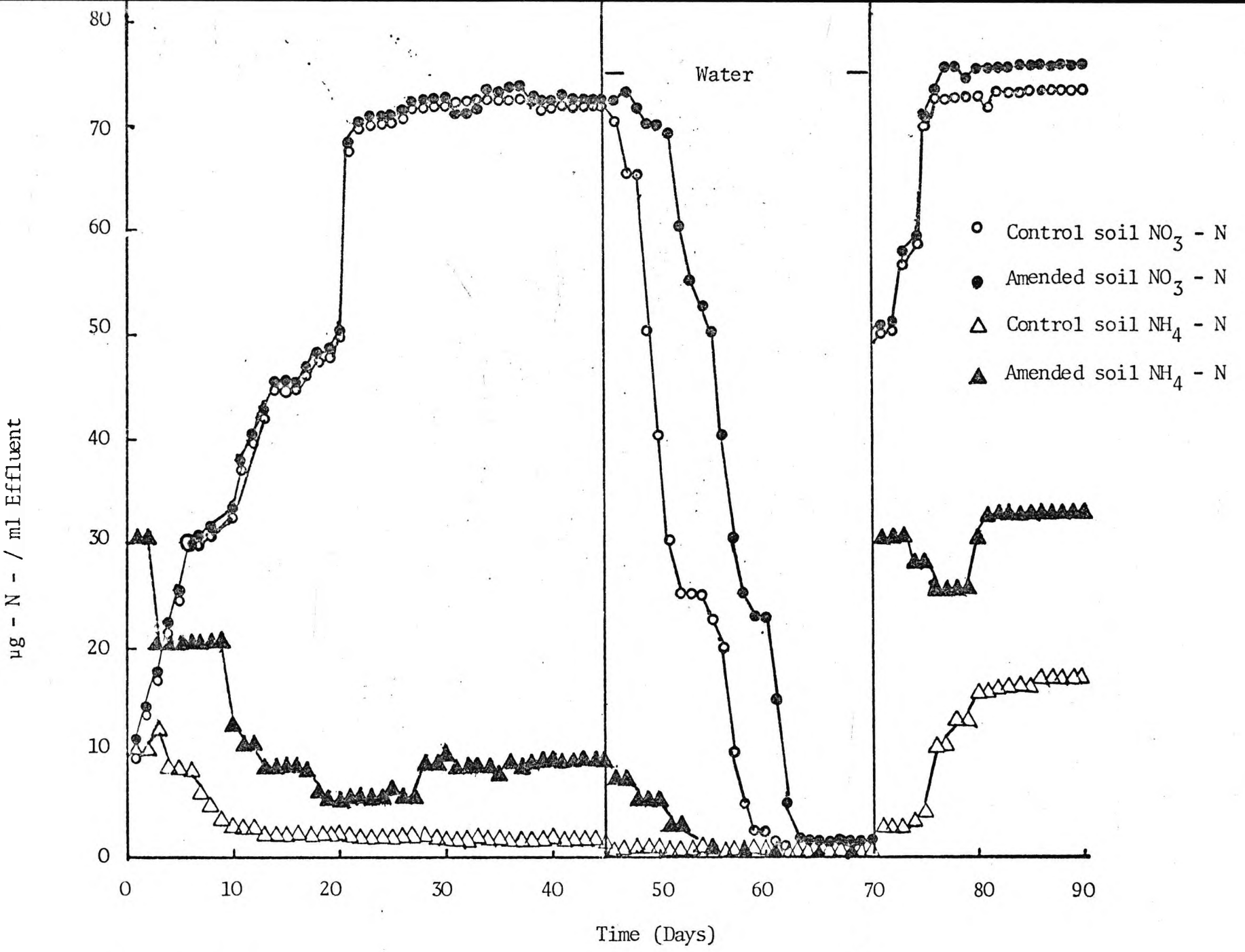

Water
Control soil NO_3 - N
Amended soil NO_3 - N
Control soil NH_4 - N
Amended soil NH_4 - N
µg - N - / ml Effluent
Time (Days)
0
10
20
30
40
50
60
70
80
90

Figure 3.3.22.

was slightly higher in the amended soil column effluent during the first perfusion sequence but gradually it decreased to very low levels during perfusion with water. Following the resumption of ammonium phosphate flow the ammonium nitrogen increased slightly and remained at this higher level. Ammonium nitrogen of the control soil column effluent remained at very low level, showed a slight increase after the water perfusion and re-establishing of ammonium phosphate solution (Figure 3.3.22).

Fractionation of the organic matter at the end of the experiment revealed that there was no water soluble components in either the control or amended soils. However, humic and fulvic acids were recovered (Table 3.3.2). Radioactive carbon was not detected in either humic or fulvic acid fractions.

3.3.5. DISCUSSION.

Based on the cumulative production of $^{14}CO_2$ during the continuous flow of glucose through amended soil, the mineralization of mycelium was increased by approximately 107% compared to that under conditions of continuous flow of water. Similarly, the overall rate of mineralization under the influence of glucose (mycelium, native organic matter and glucose) was about 79% greater than with water. Macura *et al* (1965) studied the mineralization of ^{14}C-glucose continuously added to soil and found that after 10 days, 15% of the added ^{14}C-glucose carbon had been oxidized to carbon dioxide. Further they found that some of the labelled carbon was leached from the soil, presumably in the form of metabolites because glucose was not determined in the outflow. Earlier, Vancura, Macura & Szolnoki (1964) succeeded in demonstrating

some intermediate products (small amounts of volatile fatty acids, pyruvic acid, α-ketoglutaric acid and gluconic acid) during the continuous flow of glucose solution through soil. Shields et al (1973) observed that 40 and 44% of added ^{14}C-glucose was respired by Sceptre field soil after 7 and 14 days respectively under field conditions. Subsequent experiments under laboratory conditions (Shields et al, 1974) indicated that 60 to 70% of the added carbon remained in the soil after 14 days as synthesized microbial biomass or metabolites.

In the current experiments the nitrate had a profound effect on the mineralization of mycelium in the soil. About 85% of the added labelled carbon of the mycelium was mineralized as CO_2 in sixty days. Cumulative production of CO_2 (Figure 3.3.7) revealed that the effect of nitrate was more pronounced on the mycelium amended soil than in the control soil. Nitrate induced no significant change in the rate of mineralization of native organic matter (Figures 3.2.3 and 3.3.7).

Increased mineralization (approximately 27.5%) occurred in amended soil perfused with ammonium ions, but again there was no significant effect on transformation of native organic matter (Cf Figures 3.3.12 and 3.2.3). These data agree with those of Shields et al (1974); the latter observed that the addition of NH_4NO_3 by itself had no effect on the amount of native carbon respired.

Transformation of added mycelial matter and native organic matter was stimulated approximately 47 and 29% in amended and control soils continuously perfused with $(NH_4)_2HPO_4$ compared to the soil continuously perfused with water (Cf Figures 3.3.18 and 3.2.3). The transformation of native organic matter in control soil was higher with the addition of $(NH_4)_2HPO_4$ either with KNO_3 or $(NH_4)_2SO_4$ but a reverse effect was observed on the transformation of mycelium (see Figure 3.3.3). Macura et al (1965) found that the amount of glucose carbon mineralized to CO_2 was higher if nitrogen and phosphorus were added to the soil together with glucose. They also reported that the effect of mineral nitrogen and phosphorus was most pronounced during the initial period of the experiment.

In summary, it can be concluded from the cumulative production of $^{14}CO_2$ from soils amended with ^{14}C-labelled mycelia

that continuous perfusion with a variety of nutrient solutions stimulated fungal mineralization in the decreasing order : KNO3 > glucose > $(NH_4)_2$-SO_4 > $(NH_4)_2HPO_4$ > water.

Continuous stimulation by glucose, KNO_3, $(NH_4)_2$ SO_4 and $(NH_4)_2HPO_4$ also had an especially marked effect on the microfloras of mycelium-amended soil. Thus bacterial, actinomycete and fungal populations increased several fold during these experiments and were maintained at higher levels (compared to control soils) throughout the treatment period. However, populations in control soils fed continuously with either $(NH_4)_2SO_4$ or $(NH_4)_2HPO_4$ also showed increases in number, but perfusion with KNO_3 did not produce a similar response. Macura and Kunc (1961) observed that a continuous addition of glucose alone had little effect on the number of bacteria in soil, but when glucose was supplied in the presence of ammonium and phosphate ions the number of bacteria was raised several fold. Shields et al (1974) found higher plate counts of organisms in the glucose plus NH_4NO_3 treated soil and they attributed this response to a greater efficiency of carbon utilization.

In the glucose perfusion experiments, β-1,3 glucanase activity increased slightly from the zero time level but then increased rapidly following the replacement of glucose by water; finally it fell again following the resumption of glucose perfusion. It is postulated that the presence of glucose was responsible for the repression of glucanase synthesis and/or inhibition of enzyme activities, such enzyme being particularly susceptible to catabolite repression control.

The level of β-1,3 glucanase activity in both control and amended soil perfused with KNO_3 increased with time, significantly after water perfusion, and then fell on the reintroduction of KNO_3 solution. This slight inhibitory effect of nitrate on enzyme activity was in accord with its depressive effect on the production of carbon dioxide (Figure 3.3.6).

β-1,3 Glucanase activity fluctuated in the presence of ammonium sulphate and ammonium phosphate. The level of activity fell slightly in the control soil after reintroduction of ammonium sulphate solution. The overall activity remained high in both control and amended soil perfused with ammonium phosphate. Rawald (1972) studied the effects of organic and mineral fertilizing on the activities of polyphenoloxidase, tyrosinase, catalase and dehydrogenase enzymes which are compared with soil chemical parameters and with the density of bacterial population. Rawald reported that only in the case of dehydrogenase did "complete" fertilizing (manure with NPK) stimulate enzyme activity more than did mineral fertilizing (NPK). Further, he concluded that in case of all other investigated enzymes that it was impossible to establish comparable correlations (Rawald, 1972).

There was no denitrification during the continuous flow of potassium nitrate either in the control or amended soil columns. The results agree with the findings of Macura & Kunc (1965a) from their investigation of mineralization of glucose and potassium nitrate passed continuously through the soil column.

Perusal of data in Figures 3.3.16 and 3.3.22 show the effect on addition of ammonium sulphate and ammonium phosphate on nitrification in both control and mycelium-amended soils. The nitrification effect was more pronounced and reached a steady state condition during perfusion of ammonium phosphate than ammonium sulphate. This may be due to the effect of phosphate on the increased rate of proliferation of nitrifying bacteria as noted by Chase, Corke & Robinson (1967). Further Chase *et al* (1967) reported that the effect of phosphate addition was stimulatory on nitrification. Nitrification was more rapid and in a marked steady state in both experiments after washing soil with water and on reperfusion.Lees and Quastel (1946b) had also reported that lag in nitrate accumulation apparent on first perfusing soil with a solution of ammonium did not occur if the same was washed and re-perfused with a similar solution. On reperfusion a linear rate of nitrate-N- accumulation was observed and this was interpreted to mean that during the first perfusion the nitrifying population had reached a maximum - that is the soil had become "saturated" with nitrifying bacteria (Lees & Quastel 1946b).

There was more NH_4-N in the effluent of mycelium-amended soil than in the control, and there was a lag in nitrate production compared to the control soil nitrification. Tandon (1972) has studied the effect of various organic substances on nitrification and reported that in their presence Nitrosomonas preferentially utilized the organic

substrate and derive energy. Further he reported that in the absence of organic substance (control) the bacteria derive energy for their growth and metabolism from the oxidation of ammonium salts and therefore the nitrification takes place from the very beginning.

Compared to the humic and fulvic acid extracted after 120 days of incubation of continuously water perfused aerobic soil (Cf Table 3.2.1), the amount of humic and fulvic acid extracted from the glucose and KNO_3 perfused soil after 60 days and $(NH_4)_2\ SO_4$ and $(NH_4)_2\ HPO_4$ perfused soil after 90 days of incubation is substantially higher (Table 3.3.2). Especially fulvic acid content is significantly higher in all treatments. Wojcik - Wojtkowiak (1972) studied the effect of different forms of nitrogen fertilizers on the humification of ^{15}N-tagged straw, and reported that the amount of humic and fulvic acid extracted with sodium hydroxide changed in the course of incubation. The humus fraction doubled during the first 14 days of incubation compared with organic matter contents of the original soil and subsequently decreased intensively by day 112 in all variants of the experiment. The fulvic acid content increased substantially in the first 14 days and rapidly decreased in the soil with straw and $(NH_4)_2\ CO_3$ by day 56, and soil with only straw decreased slowly by 112 days; the fulvic acid content of soil with straw and $NaNO_3$ remained at higher rate till day 112 of incubation.

Data in Table 3.3.2 reveal that there was an enrichment of carbon and nitrogen in the humic acids extracted from the soil of all the treatments studied. Compared to the humic and fulvic acids of aerobic, water-perfused soils (see Table 3.2.1), the carbon and nitrogen contents of these fractions from N-treated soil was generally lowered. The data in Table 3.3.3 demonstrates clearly the effect of addition of

TABLE 3.3.3

Amounts of Humic and Fulvic acids mineralized in control and mycelium-amended soils.

Treatment	Days of Incubation	Extractable Humic & Fulvic acids (%, dry wt. of soil) = Total	Amount of Humic & Fulvic acids mineralized	Percentage of Humic & Fulvic acids mineralized
None (control soil)	-	7.92 + 0.25 = 8.17	-	-
Glucose Amended	60	4.32 + 0.75 = 5.07	3.10	36.7
Control[a]	60	4.62 + 1.10 = 5.70	2.47	30.2
KNO_3 Amended[b]	60	3.96 + 0.70 = 4.66	3.51	42.9
Control	90	4.32 + 0.30 = 4.62	3.55	43.4
$(NH_4)_2 SO_4$ Amended	90	3.70 + 0.80 = 4.50	3.67	44.9
Control	90	4.00 + 0.60 = 4.60	3.57	43.6
$(NH_4)_2HPO_4$ Amended	90	3.00 + 0.60 = 3.60	4.57	55.9

a) Indicates unamended soil perfused with and incubated under the conditions specified.

b) Indicates mycelium-amended soil perfused with and incubated under the conditions specified.

glucose and different forms of nitrogen on the mineralization of added mycelium and native organic matter. Further calculations on the rates of organic matter and mycelium mineralization will be discussed all together in the final discussion in Section 4.

3.4. EFFECTS OF PESTICIDES ON FUNGAL TRANSFORMATION

Introduction.

An increasing number of xenobiotic compounds such as pesticides are being applied to soil in order to control plant diseases, insect pests and weeds. In addition, large amounts of chemicals which are applied to plant foliage ultimately pass into the soil where they may have secondary effects on plant growth due to their influence on soil microbial activities. Persistent pesticides may be important for the protection of certain crops but their possible phytotoxicity for succeeding ones makes their disappearance during a reasonable time period a desirable feature. Degradation by microorganisms plays an important part in the disappearance of pesticides from soil (Audus, 1960). Thus, environmental factors affecting the activity of soil microorganisms also may affect the persistance of biodegradable pesticides. Of all the pesticides that find their way into the soil, organic herbicides appear to be the most susceptible to microbial attack (Bollen, 1961).

Norman & Newman (1950) studied the persistence of some herbicides in soil and found that the addition of organic matter accelerated their disappearance. Subsequently Ogle & Warren's (1954) studies with Monuron (3-(4-Cholorophenyl)-1,1-dimethylurea), TCA (Trichloroacetate), Chloropropham (Iso propyl N-(3-Cholorophenyl) Carbamate) and Naptalam (N-1-Naphthylphythalamic acid), Rahn & Baynard's (1958) with Monuron, Kaufman's (1964) with Dalapon (2,2'-dichloropropionic acid) and McCormick & Hiltbold's (1966) with Atrazine (2-Chloro-4-ethylamino-6-isopropyl-amino-1,3,5-triazine) and Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), all indicated that herbicide breakdown rates in a range of soil types increased as the soil organic matter increased.

A number of simple chlorinated aliphatic acids are phytotoxic - but only two, Dalapon and TCA, are widely used for weed control. These compounds are commonly applied as their sodium salts at the rate of 5-50 lbs. per acre (Thomson, 1967a). Numerous studies of the persistence of these compounds in soil have been conducted (see for example Thiegs, 1962; Kearney, Kaufman &

Alexander , 1967) while several environmental factors affecting the metabolism of chlorinated aliphatic acids in soil have been studied. Thiegs (1955) reported that Dalapon disappeared most rapidly from warm, moist soils and he attributed this to decomposition by microorganisms. Holstun & Loomis (1956) described the use of a plant bioassay procedure for determining the loss of Dalapon and stated that for most herbicides detoxification by decomposition appears to be primarily a function of microbiological activity. The major factors governing microbial destruction of herbicides in soil appear to be temperature, moisture, pH, depth and organic matter level (Alexander, 1961; Kaufman, 1964).

Several investigators have demonstrated that microorganisms effectively degrade Dalapon in soil (Table 3.4.1) and the kinetics involved are consistent with the hypothesis that in the presence of Dalapon certain microorganisms undergo adaptation and became capable of utilizing it as a source of carbon. The decomposition of the chloro substituted aliphatic acids is usually accompanied by a dehalogenation.

Triphenyltin compounds are used increasingly as agricultural fungicides. Triphenyltin acetate (Fentin acetate) is used extensively in Europe to control *Phytophthora infestans* on potato (potato blight fungus), *Cercospora beticola* on sugar beet and *Septoria apii* on celeriac and other phytopathogens. Organotin compounds are comparable in their potency and specificity with the copper containing fungicides (van der Kerk, 1970). Triphenyltin acetate is applied at the rate of 0.25 to 0.5 lb. per acre (Thomson, 1967b). These compounds may be broken down by both physical and chemical actions into presumed non-toxic inorganic tin (Barnes, Bull & Poller, 1971).

Recently Barnes, Bull & Poller (1973) investigated on the persistence of fentin acetate in soil and found that it was decomposed quite rapidly by microorganisms, the half life in an agricultural loam soil being about 140 days. Further these authors accomplished the isolation of several microorganisms active in the metabolism of fentin acetate.

The object of the experiments described in this section was to investigate

TABLE 3.4.1.

Organisms which are known to attack chloro-substituted aliphatic acids.

Organism	Substrate	Reference
Pseudomonas Coryne bacterium Arthrobacter Agrobacterium	Monochloroacetate 2,2-Dichloropropionate Dalapon and Trichloro-acetate	Jensen (1957a)
Trichoderma Penicillium Clonostachys	Sodium mono Chloroacetate	Jensen (1957b)
Agrobacterium Pseudomonas	Dalapon	Magee & Colmer (1959)
Nocardia Pseudomonas	Dalapon	Hirsch & Alexander (1960)

the effects of a range of concentrations of an extensively used herbicide (Dalapon) and a fungicide currently under consideration for use in the U.K. (fentin acetate) on transformation of the native organic matter and fungal material added to soil. Preliminary experiments (Barnes et al. 1973) have shown that fentin acetate does not have a deleterious effect on nitrification in soil.

3.4.1. 2,2'-Dichloropropionic acid (Dalapon)

Two columns were packed with air dried soil (500g, 2-5 mm aggregates) amended with 2,2'-dichloropropionic acid (sodium salt, Koch light Laboratories) at the rate of 100 p.p.m. In one column the soil was amended with mycelium as described in 3.3.1. The soil columns were moistened with sterile distilled water, made lightproof to prevent photodecomposition of the herbicide and algal growth, and then incubated at 25°C. Both soil columns were perfused continuously with CO_2-free air (usually 1200ml h^{-1}) and a sterile aqueous solution of 1000 ppm Dalapon (approximately 10ml h^{-1}) for eight days. At the end of eight days the concentration of Dalapon was increased to 2500 ppm and 5000 ppm after 21 days; the incubation continued up to 60 days.

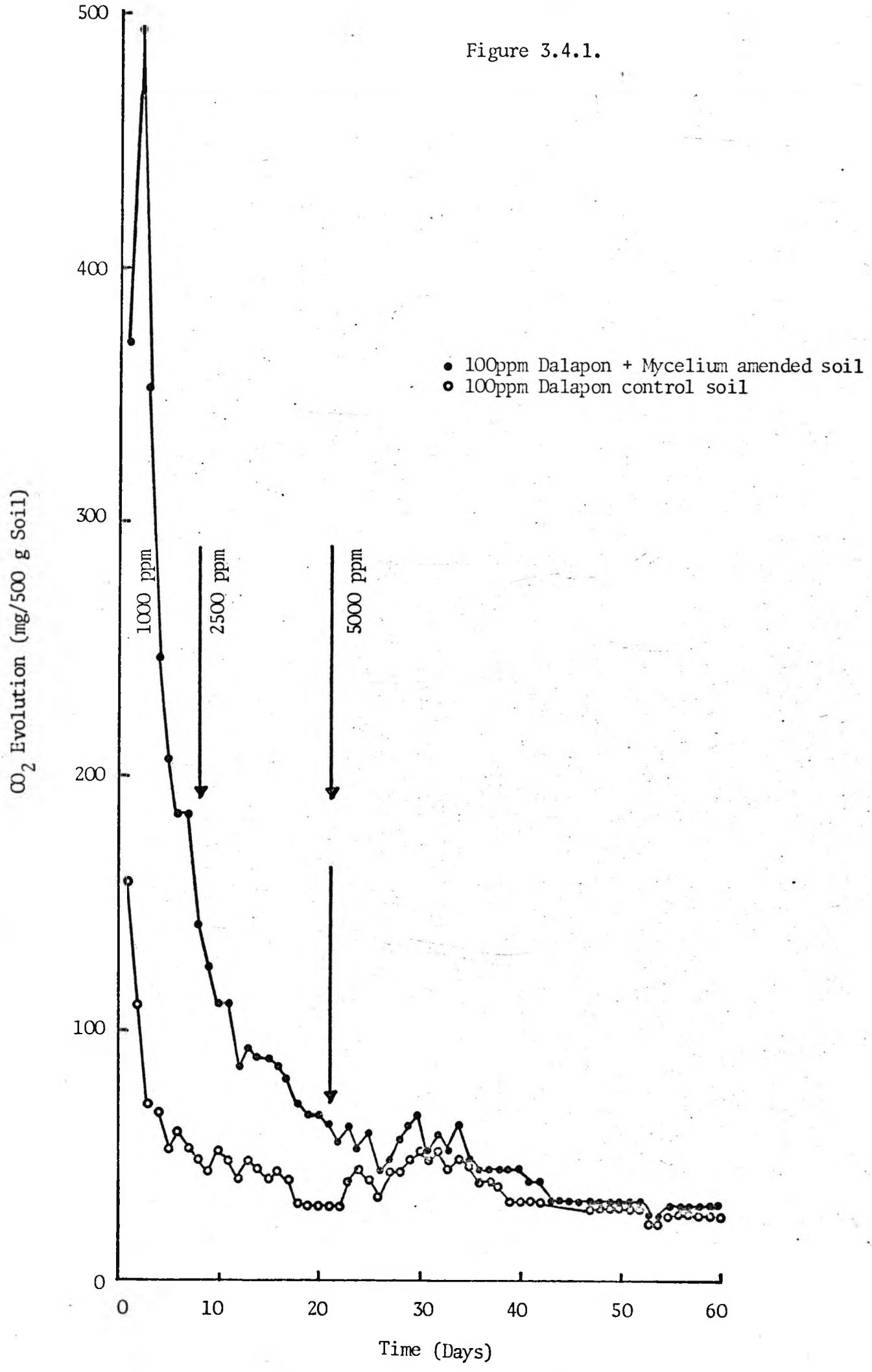
RESULTS

Evolution of CO_2 from mycelium-amended and unamended soils is shown in Figure 3.4.1. The highest rate of carbon dioxide evolution in amended soil was recorded on day two and after this initial peak the rate of decomposition fell slowly to the level of unamended soil. The cumulative production of CO_2 is illustrated in Figure 3.4.2. Carbon mineralization was 90% greater in the mycelial amended soil as compared with unamended soil in 60 days. Figure 3.4.3. shows the cumulative production of $^{14}CO_2$; about 28% of added ^{14}C-labelled mycelium was mineralized in 60 days (Cf Figure 3.3.3 "water" control).

Bacterial numbers in the mycelium-amended soil increased approximately 6-fold during perfusion with 1000 ppm Dalapon (Figure 3.4.4) but the number fell 4-fold when concentration was increased to 2500 ppm. Similarly actinomycete

Figure 3.4.1. Dynamics of CO_2 production from mycelium-amended and control soils under aerobic conditions during continuous flow of varying concentrations of Dalapon.
The arrows at days 8 and 21 indicate changes in Dalapon to higher concentrations.

Figure 3.4.1.


500
400
300
200
100
0
0
10
20
30
40
50
60
CO_2 Evolution (mg/500 g Soil)
Time (Days)
1000 ppm
2500 ppm
5000 ppm
• 100ppm Dalapon + Mycelium amended soil
o 100ppm Dalapon control soil

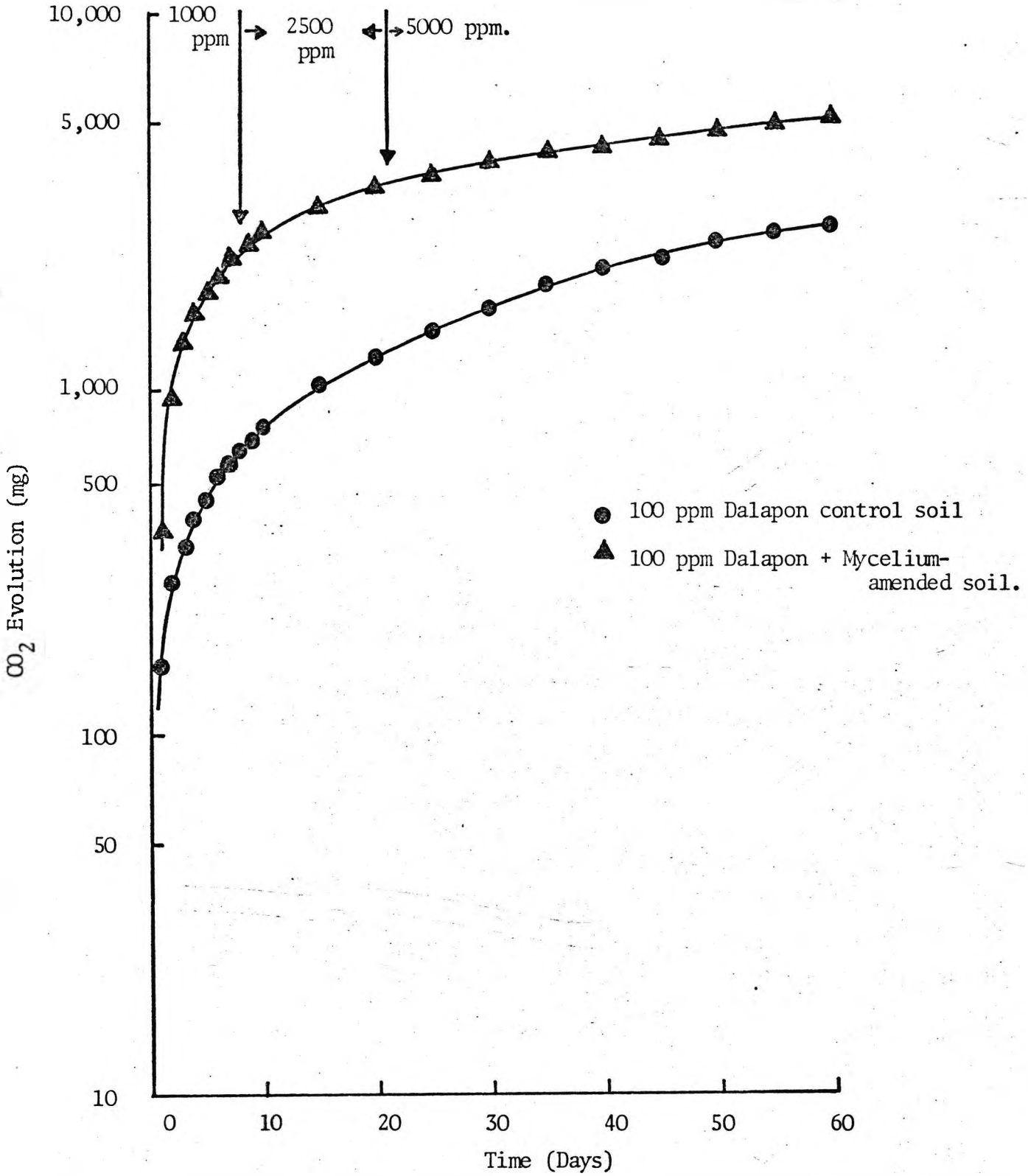


Figure 3.4.2. Cumulative CO_2 production from mycelium-amended and control soils during continuous flow of varying concentration of Dalapon.

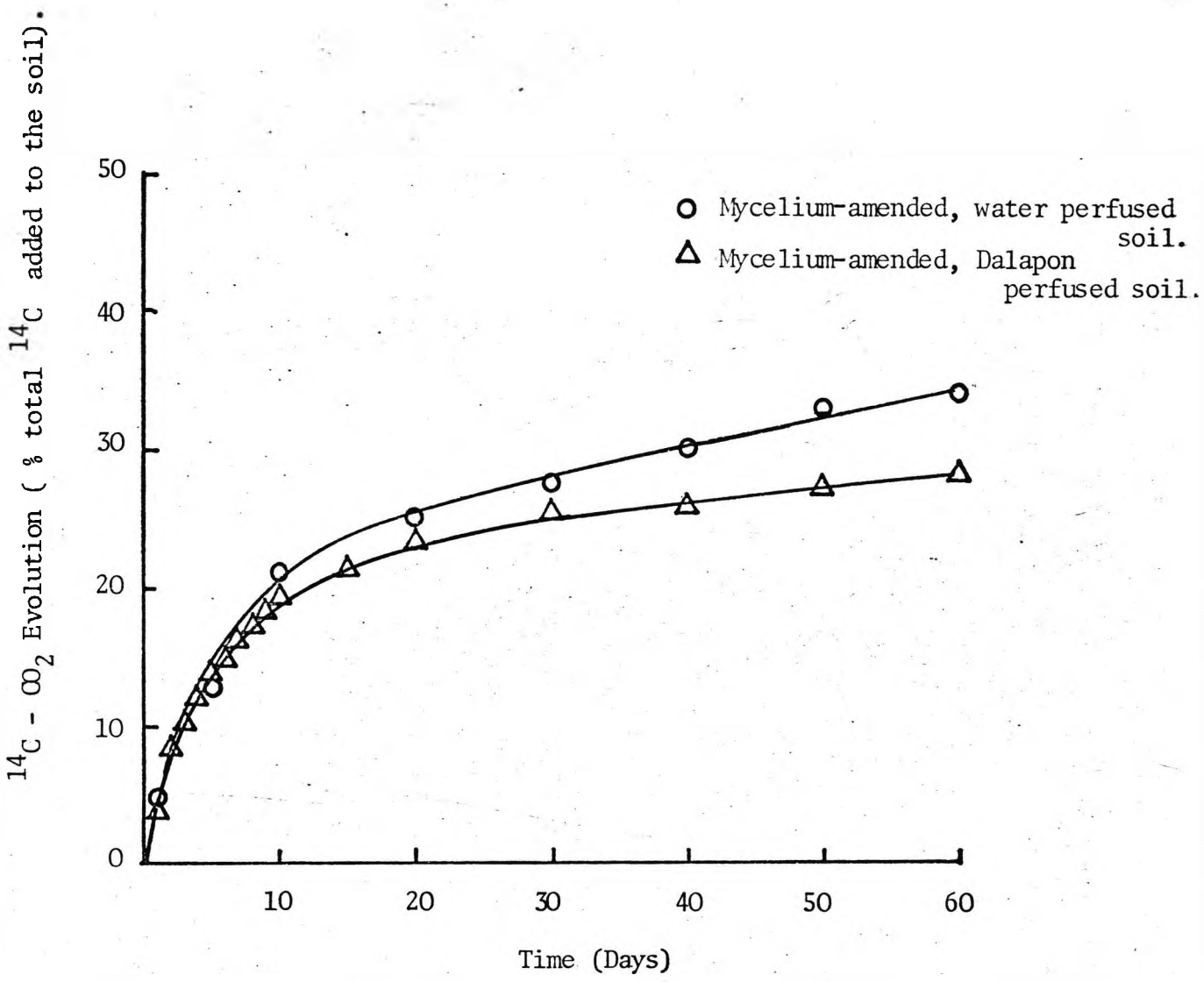


Figure 3.4.3. Release of $^{14}CO_2$ from ^{14}C-labelled mycelium during the continuous flow of water or Dalapon through soil columns ; aerobic incubation conditions.

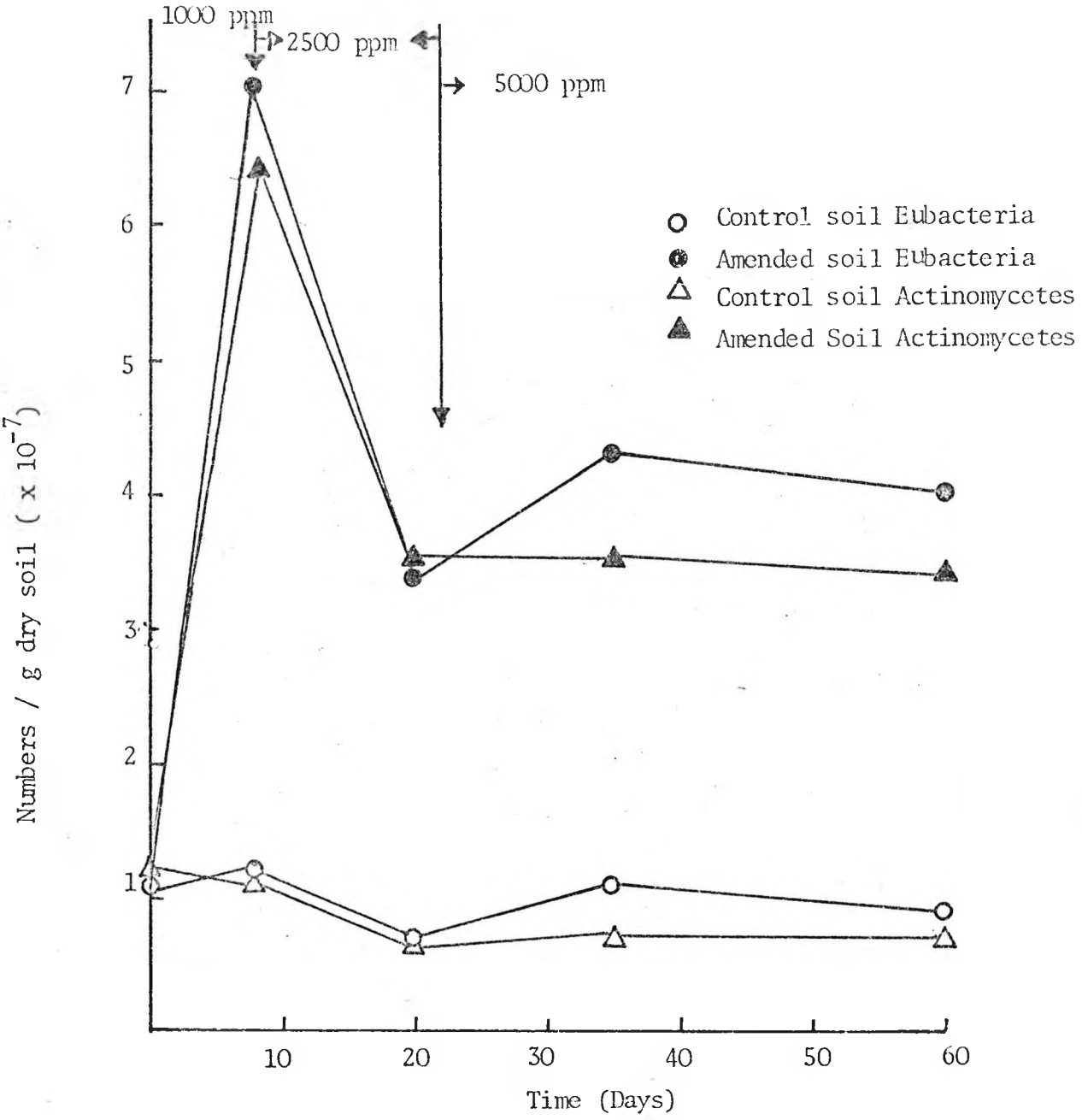


Figure 3.4.4. Changes in Bacterial and Actinomycete populations during continuous flow of Dalapon through control and mycelium-amended soils; aerobic incubation conditions. Other conditions as shown in Figure 3.4.1.

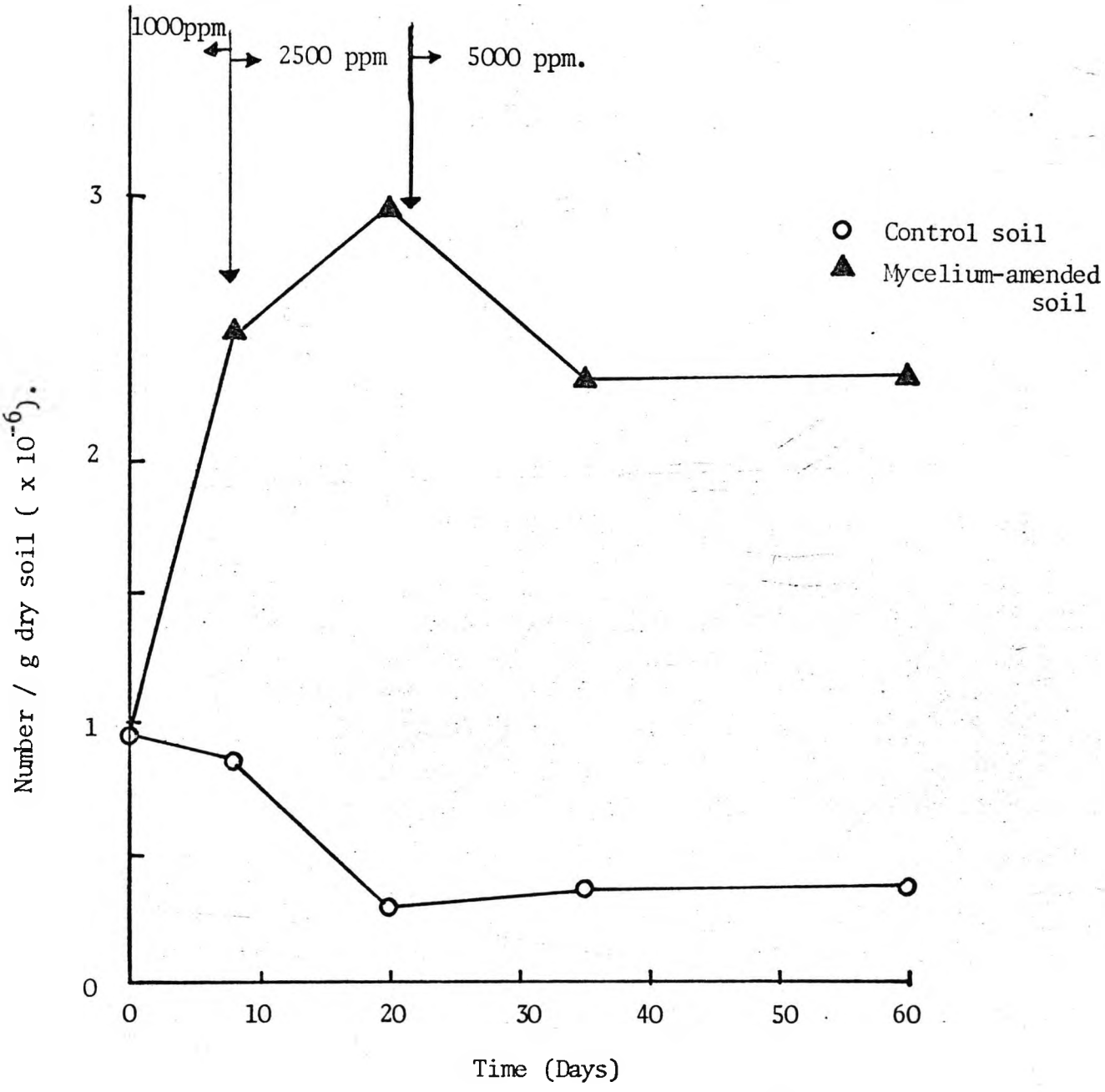


Figure 3.4.5. Changes in fungal population during continuous flow of Dalapon through control and mycelium-amended soil column ; aerobic incubation conditions. Other conditions as shown in Figure 3.4.1.

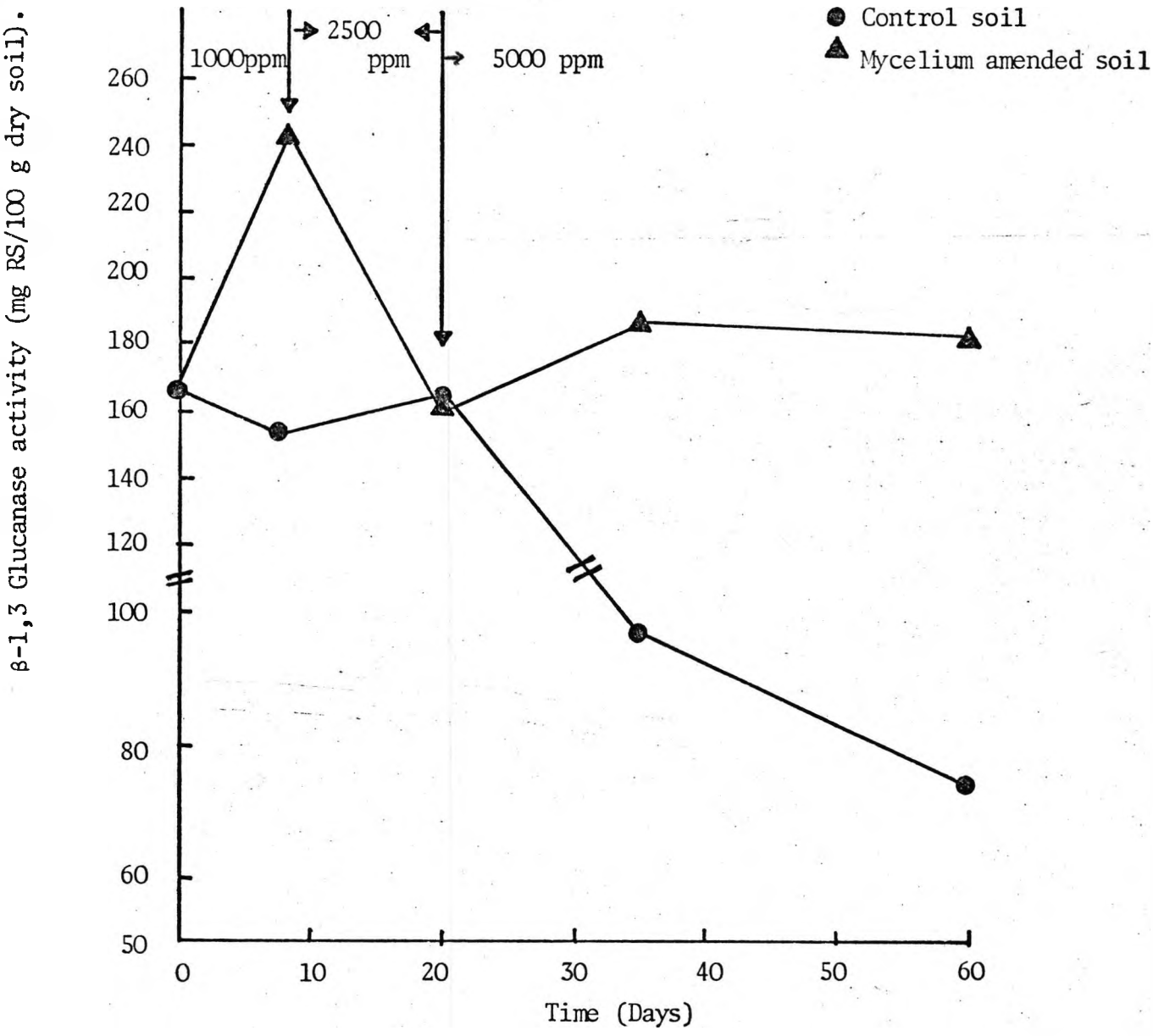


Figure 3.4.6. β-1,3 Glucanase activity in control and mycelium-amended soil columns during the continuous flow of Dalapon; aerobic incubation conditions. Other conditions as shown in Figure 3.4.1.

population increased 5-fold initially in the mycelium-amended soil, then declined as the Dalapon concentration was raised and remained constant until the end of the experiment. The bacterial and actinomycete populations in the unamended control soils remained constant throughout the period of experiment and apparently were unaffected by the presence of the herbicide. Figure 3.4.5 illustrates an increase in the fungal population in mycelium-amended soil compared to the unamended soil.

β-1,3 Glucanase activity was high in the amended soil during perfusion with 1000 ppm Dalapon and fell to the zerotime level when the concentration was raised (Figure 3.4.6). In unamended control soil the glucanase activity fell sharply when the soil was treated continuously with the highest concentration of Dalapon.

3.4.2. Triphenyltin acetate (Fentin acetate).

Four columns were packed with 500g of air dried soil amended respectively with fentin acetate (kindly supplied by Dr. R. C. Poller, Queen Elizabeth College, London) at the rate of 100 ppm, 100 ppm, 250 ppm and 500 ppm. One of the 100 ppm fentin acetate amended columns was retained as a control and the other three soil columns were amended with mycelium (for details see Section 3.3.1). The columns were moistened with sterile distilled water, made light proof (Fentin acetate is susceptible to photolytic degradation; Barnes et al, 1973) then incubated at 25°C. All four columns were perfused continuously with CO_2-free air (usually 1200 ml h^{-1}) and aqueous fentin acetate (10ml h^{-1}, 1.06 mg/litre w/v) to compensate for the predicted decay rate (Barnes et al, 1973).

RESULTS

The rate of CO_2 production was highest with 100 ppm mycelium-amended soil (Figure 3.4.7). Unamended 100 ppm Fentin acetate treated soil showed an initial burst of CO_2 production which fell rapidly to a low constant level (Figure 3.4.7). In Figure 3.4.8. is shown the cumulative production

of CO_2 from all four treatments. At the end of 60 days incubation total CO_2 evolved followed the order: 100 ppm amended > 250 ppm > 500 ppm > 100 ppm unamended soil. Carbon mineralization was almost 100% greater in the 100 ppm mycelium-amended soil as compared with 100 ppm unamended soil (control), and about 11% and 25% greater than the 250 and 500 ppm amended soils respectively. The total evolution of ^{14}C as $^{14}CO_2$ at the end of 60 days incubation (Figure 3.4.9) was 24.7%, 23.5% and 22% for 100, 250 and 500 ppm amended soils respectively (Cf Figure 3.3.3 "water" control).

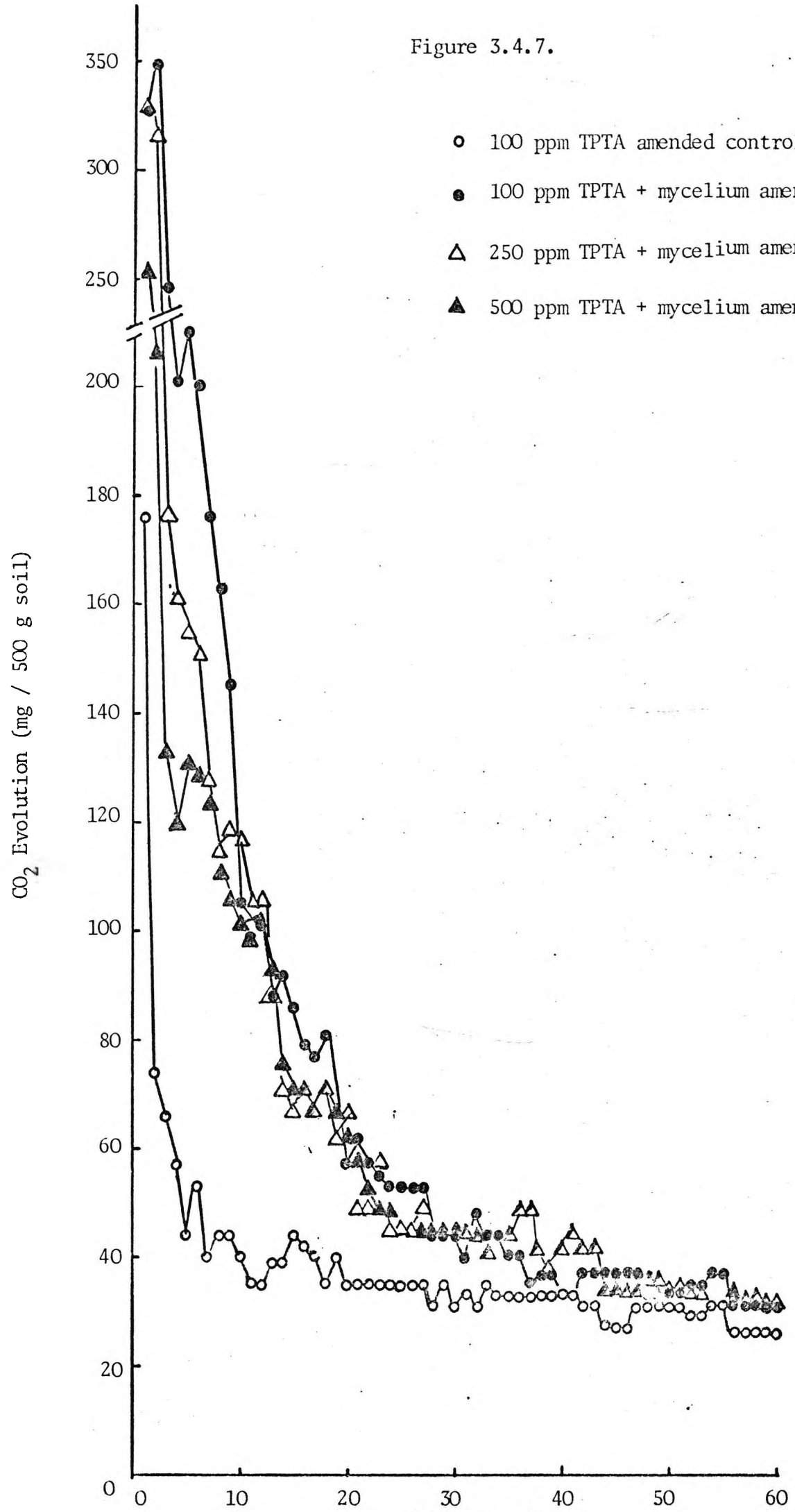
In unamended soil the bacterial population remained at a constant level throughout the experiment whereas in mycelium-amended soils the numbers of bacteria increased from zero time levels and those were maintained until the experiment was terminated (Figure 3.4.10). The actinomycete population pattern was very similar to that of the eubacteria (Figure 3.4.11).

Higher numbers of microfungi were recorded in mycelium-amended soil compare to the unamended soil (Figure 3.4.12) but a reduction in numbers was noticeable in 500 ppm amended soil.

Figure 3.4.13 shows the activity of β-1,3 glucanase. There was an initial increase in activity in the 100 ppm amended soil which fell rapidly on day 20, recovered slowly and returned to the zerotime activity by day 60. The 100 ppm control soil glucanase activity also fell to a low level on day 20 but then increased throughout the remainder of the experiment. In soil column amended with 250 and 500 ppm Fentin acetate the β-1,3 glucanase activity increased slowly all the time of the experiment.

Figure 3.4.7. Dynamics of CO_2 production from mycelium-amended and control soils under aerobic conditions during continuous flow of aqueous fentin-acetate (TPTA).

Figure 3.4.7.


o 100 ppm TPTA amended control soil.
● 100 ppm TPTA + mycelium amended soil.
△ 250 ppm TPTA + mycelium amended soil.
▲ 500 ppm TPTA + mycelium amended soil.
CO_2 Evolution (mg / 500 g soil)
0
20
40
60
80
100
120
140
160
180
200
250
300
350
0
10
20
30
40
50
60
Time (Days)

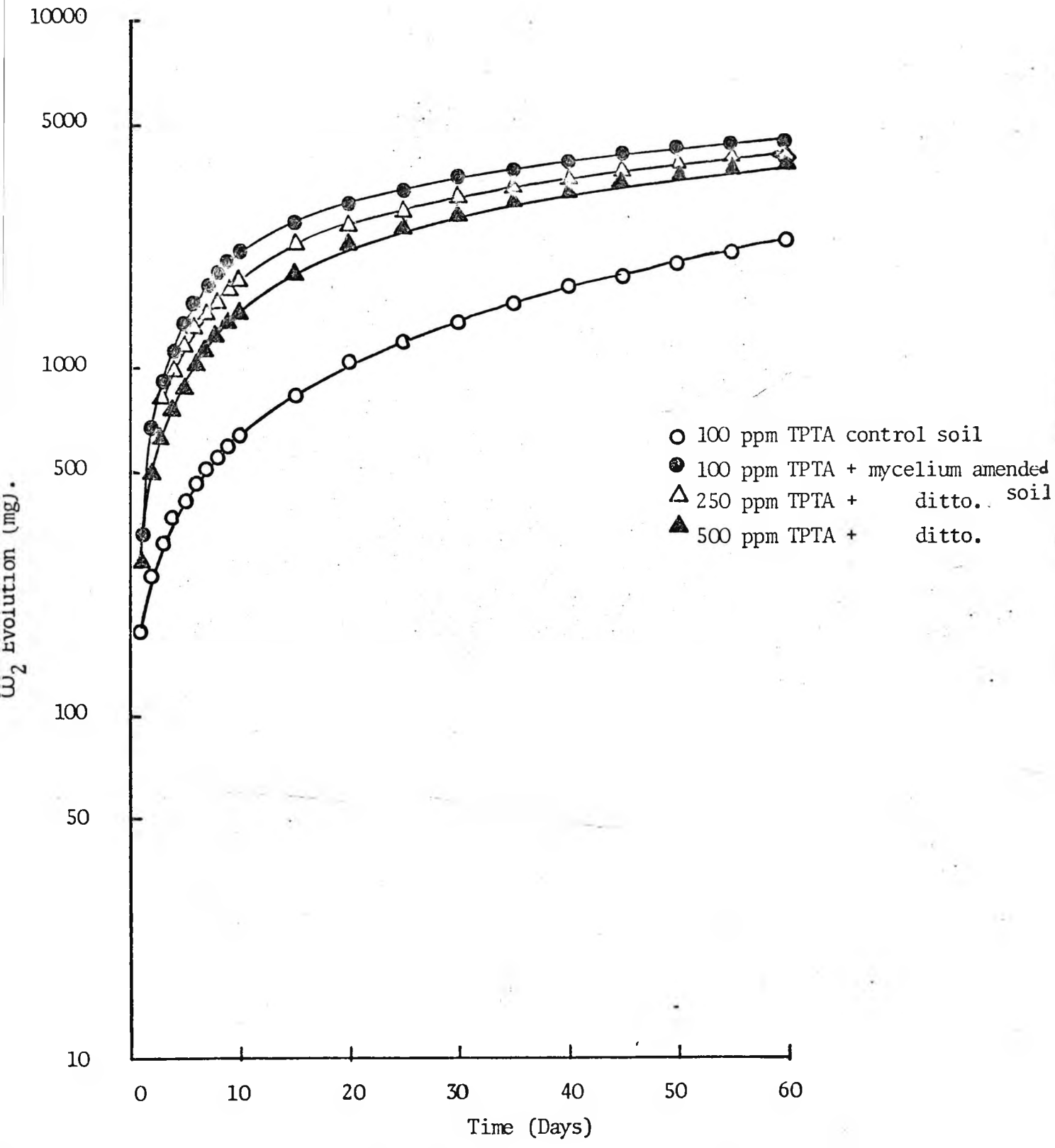


Figure 3.4.8. Cumulative CO_2 production from mycelium-amended and control soils during continuous flow of aqueous fentin acetate; aerobic incubation conditions.

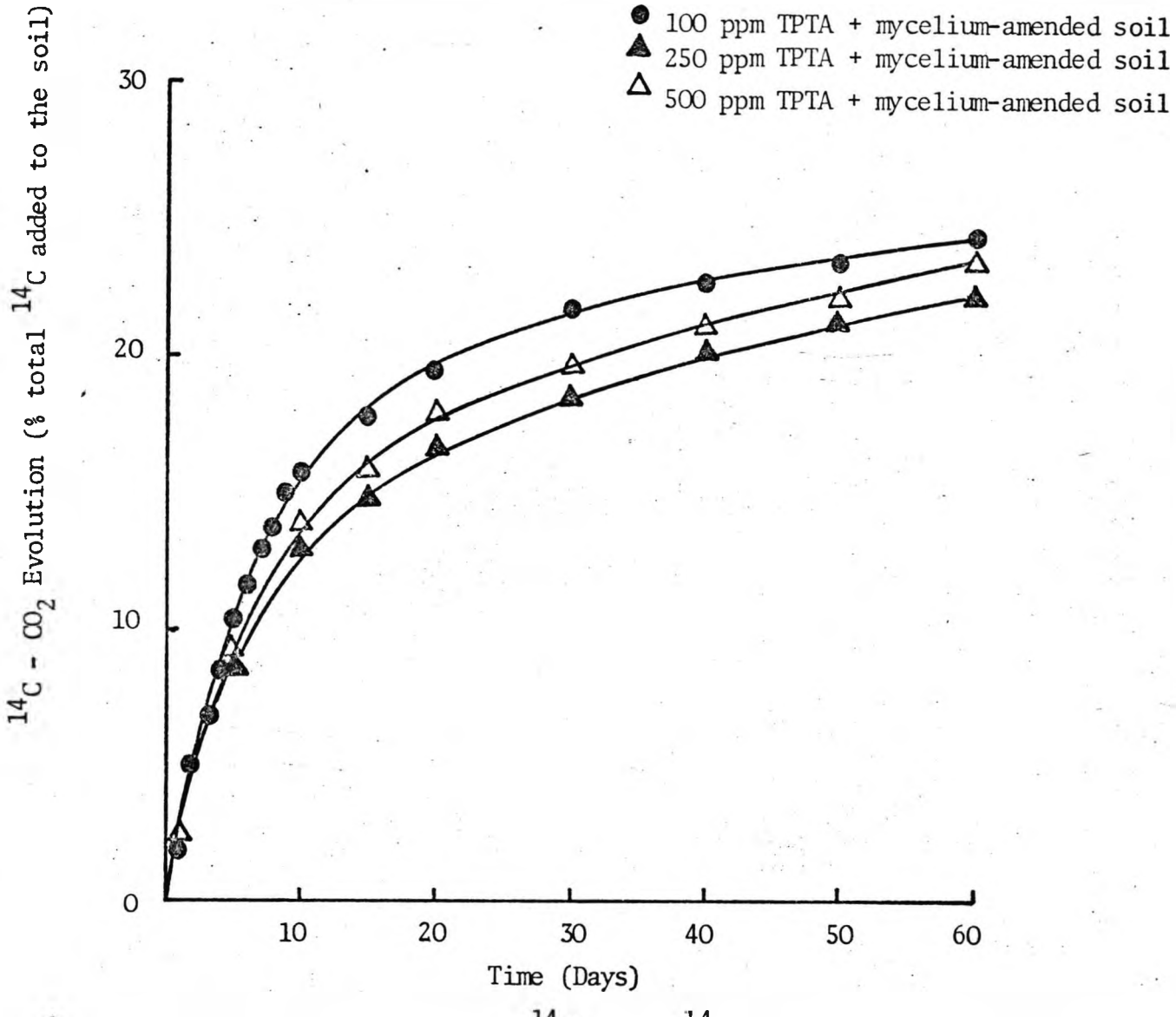


Figure 3.4.9. Release of $^{14}CO_2$ from ^{14}C-labelled mycelium during the continuous flow of aqueous fentin acetate through soil columns; aerobic incubation conditions.

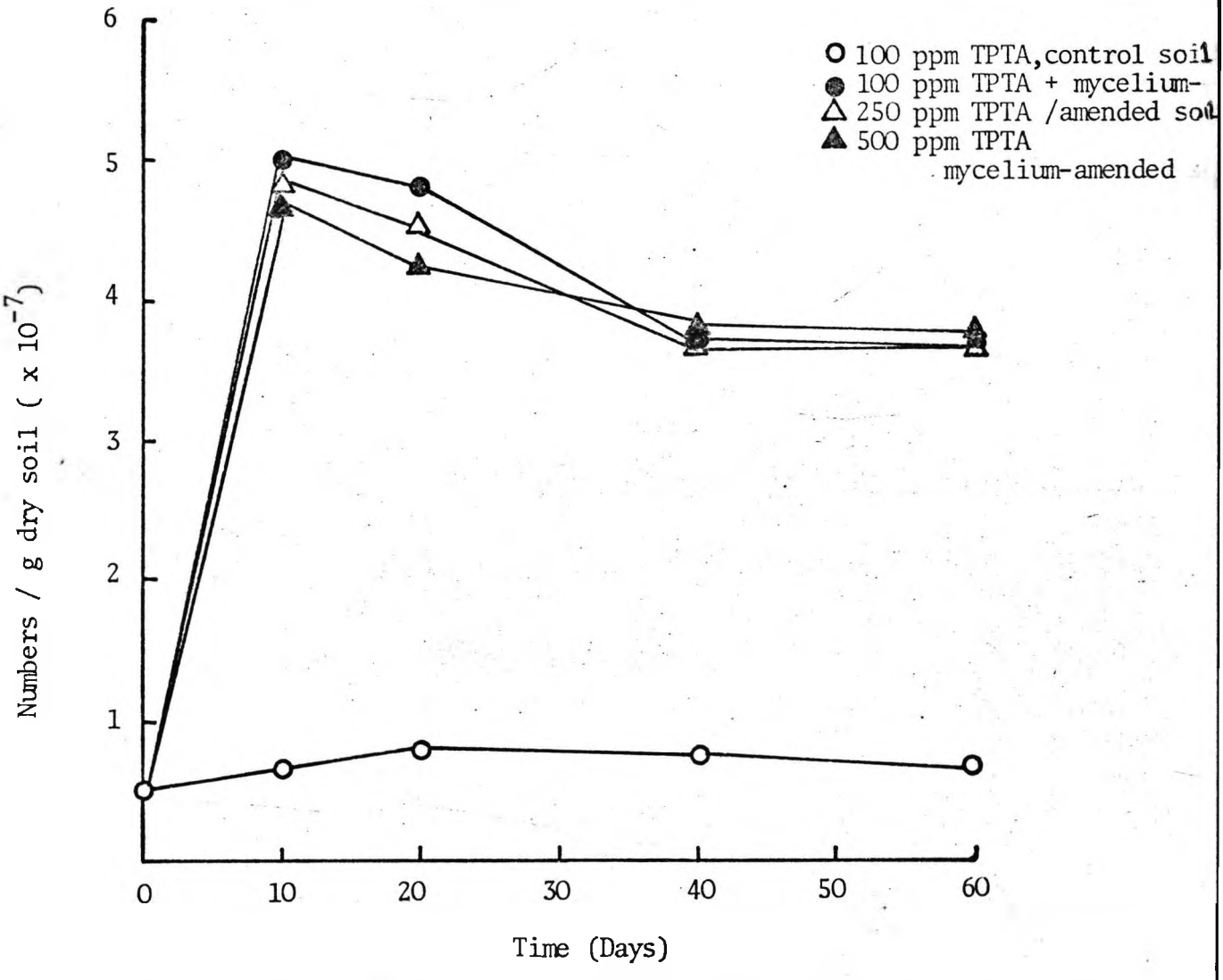


Figure 3.4.10 Changes in Eu-bacterial population during continuous flow of aqueous fentin acetate through control and mycelium-amended soils; aerobic incubation conditions.

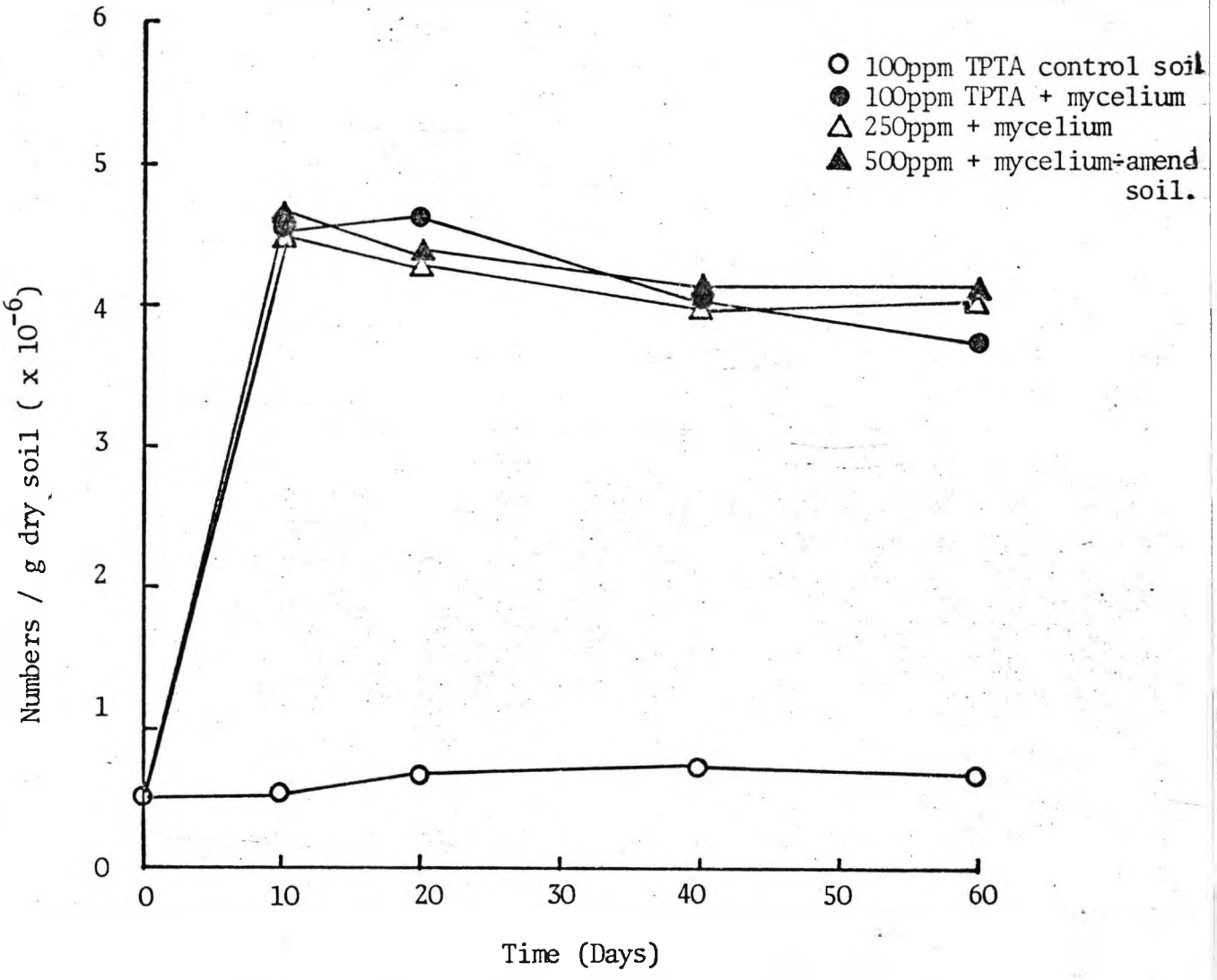


Figure 3.4.11. Changes in Actinomycete population during continuous flow of aqueous fentin acetate through control and mycelium-amended soils; aerobic incubation conditions.

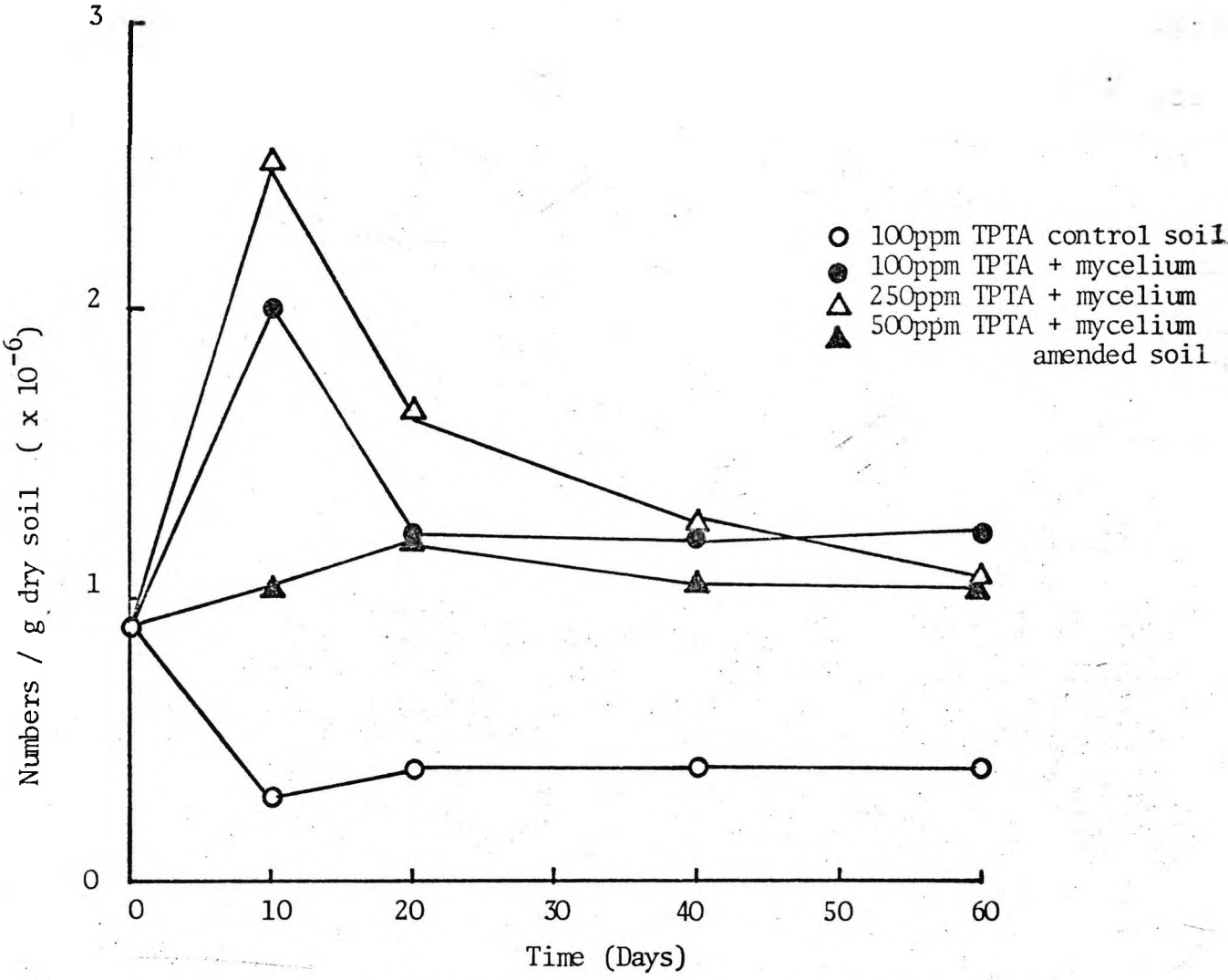


Figure 3.4.12. Changes in Fungal population during continuous flow of aqueous fentin acetate through control and mycelium-amended soils; aerobic incubation conditions.

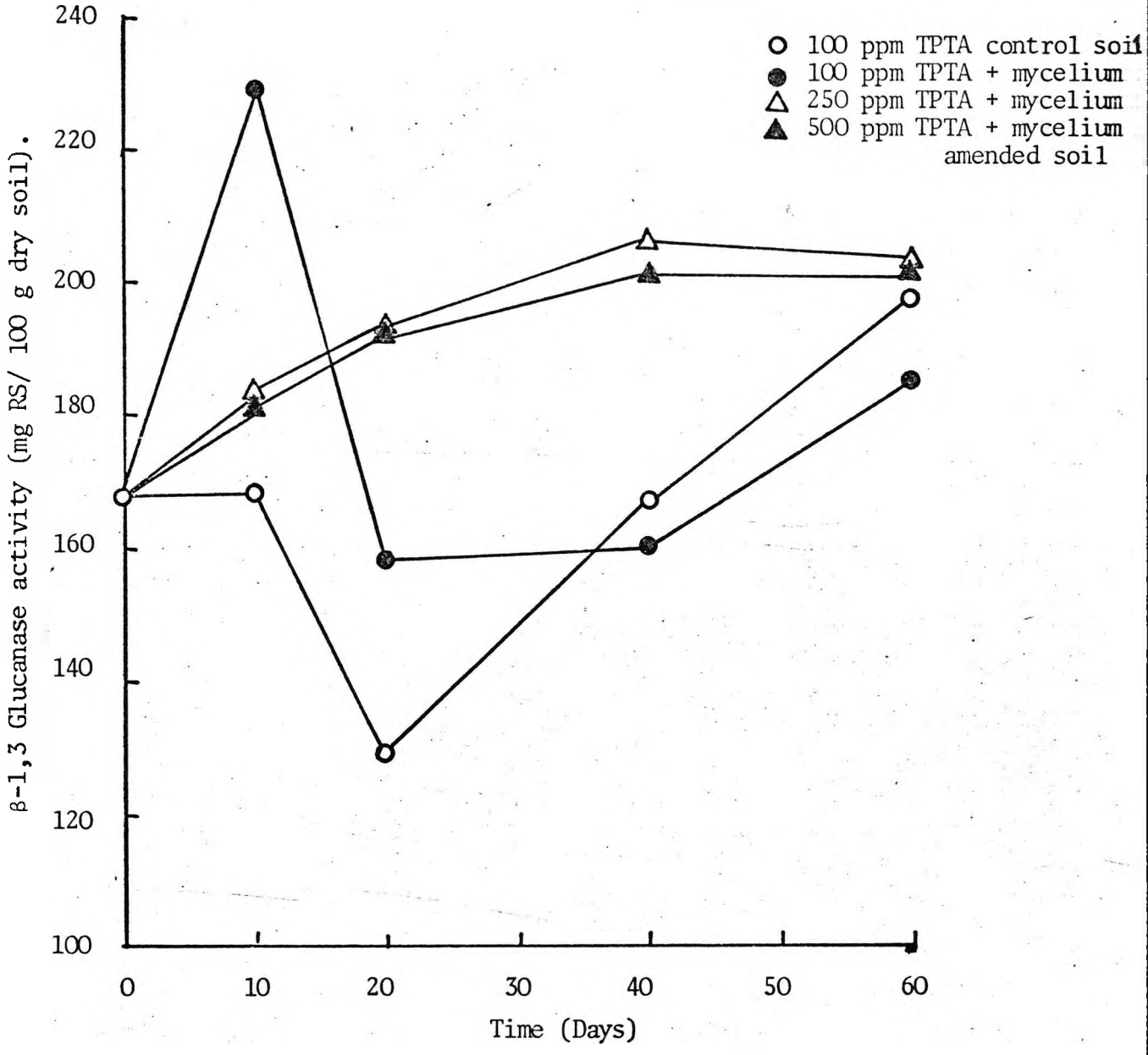


Figure 3.4.13. β-1,3 Glucanase activity in control and mycelium-amended soils during the continuous flow of aqueous fentin acetate; aerobic incubation conditions.

3.4.3. Discussion.

Cumulative production of CO_2 from both control and mycelium-amended soil perfused with varying concentration of Dalapon for sixty days showed no significant difference in the overall rate of mineralization compared to the aerobic water perfused condition (Figure 3.2.3). However, ^{14}C (CO_2) evolved from the mycelial mineralization showed about 6% less carbon was mineralized during Dalapon perfusion. Several investigators have reported the microbial degradation of Dalapon, and a number of soil microorganisms have been reported to metabolize Dalapon as shown in Table 3.4.1 (Jensen, 1957a & b., Magee & Colmer, 1959; Hirsch & Alexander, 1960). Holstun & Loomis (1956) reported that the decomposition of Dalapon in soil was primarily a function of the microbiological population and factors influencing the decomposition rate such as temperature and moisture were considered to act indirectly by affecting the activity of the micro-organisms. Thiegs (1955) found that fresh additions of Dalapon to soil were decomposed more rapidly than the initial application. He also reported that the addition of organic matter increased the rate of disappearance of Dalapon and that Dalapon did not disappear from sterilized soil. Hirsch & Alexander (1960) demonstrated the evolution of ^{14}C (CO_2) from the metabolism of ^{14}C-labelled Dalapon.

The addition of Dalapon apparently showed no inhibitory effect on the microbial population of both control and mycelium-amended soil. Newman & Downing (1958) reported that Dalapon stimulated bacterial growth when applied at 68 pounds per acre. Further they reported that the evolution of carbondioxide from soils in the presence of herbicides may be used as a criterion of influence on the microbial population as a whole.

β-1,3 Glucanase activity in the mycelium-amended soil perfused

with Dalapon showed no marked change compared to the aerobic water perfused mycelium-amended soil. However, the activity in the control soil fell sharply with increasing concentration of Dalapon. The results of many investigations have demonstrated that herbicides applied at the recommended field rates generally have no harmful effects upon the microflora or upon its biochemical activities. Only at concentrations many fold higher than those recommended are these chemicals toxic; often the first sign of inhibition does not appear until herbicide levels of one hundred times the accepted rates are added to soil (Alexander, 1961).

Data from the present study clearly demonstrate the inhibitory effect of triphenyltin acetate on the production of carbon dioxide in the soil. Marked inhibition was observed at the highest concentration of the fungicide used in the mycelium-amended soil, and also in unamended control soil with 100 ppm of fentin acetate. Compared to ^{14}C-mineralization in water-perfused soil, the evolution of $^{14}C(CO_2)$ from ^{14}C-mycelium in the presence of different concentrations of triphenyltin acetate was significantly inhibited. Recently Karnath & Vasantharajan (1973) reported the effect of fungicide Dexon (p-dimethylaminobenzendiazo sodium sulfonate) on CO_2 production from soil amended or not with organic matter. Dexon was found to retard the breakdown of glucose and paddy straw added to soil. Inhibition of CO_2 production occurred during the early stages of glucose decomposition and in the case of straw throughout the 60-day period of study. Furthermore they reported that addition of organic nutrients appeared to facilitate the degradation of Dexon, glucose being more effective than straw.

Increased total production of CO_2 in this study may be from the decomposition of fentin acetate in soil. Barnes *et al.* (1973) reported the evolution of ^{14}C (CO_2) from ^{14}C-labelled triphenyltin acetate by soil microorganisms. They reported that the fungicide decomposed in soil without any lag period. Fifty per cent of the added fungicide was

decomposed in 140 days of incubation in soil (Barnes *et al*, 1973).

Different concentrations of fentin acetate did not appear to inhibit particularly the bacterial and actinomycete population in soil. However an inhibitory effect was observed on the fungal population at the highest concentration of fentin acetate used. These results are in accord with those of Martin & Pratt (1958) who reported that both bacteria and actinomycetes multiplied rapidly after fumigation or fungicidal treatment and within a few days were usually much more numerous than in untreated soil.

In both 100 ppm fentin acetate, mycelium-amended and unamended soils the pattern of β-1,3 glucanase activity remained similar to that of the water perfused aerobic soil condition. Higher concentrations (250 & 500 ppm) of the fungicide appeared to stabilize the production and/or activity of β-1,3 glucanase ~~xxxxxxx~~ in mycelium-amended soil (Cf Figure 3.4.13).

C H A P T E R F O U R

DISCUSSION AND CONCLUSIONS

4. DISCUSSION AND CONCLUSIONS

Throughout this work the parameter of CO_2 evolution has been taken as the primary measure of carbon mineralisation. This choice was made deliberately in order to make realistic comparisons between the data obtained from this and previously published investigations. It has been advocated recently (Witkamp, 1973) that individual investigators should relate both field and laboratory observations either to rates of CO_2 evolution or rates of substrate loss. In the present studies, rates of CO_2 evolution proved to be the most easily and accurately measurable of these two parameters. This final discussion section summarises observations of the mineralization and stability of the readily decomposed and the humus components of the soil organic matter in relation to various experimental treatments (see Table 4.1); and the priming effect which such treatments induced. These data are compared with some reports of higher plant decomposition and humification.

Chase & Gray (1957) used Warburg manometry to analyse the respiratory activity of soils over periods of several days. They postulated that carbon dioxide evolution from soil resulted from (i) a slow breakdown of humus and (ii) a rapid breakdown of more readily metabolisable organic matter. Further, they argued that these two first order reactions would be superimposed and that plots of the logarithm of CO_2 evolution against linear time would produce curvi-linear graphs. By assuming that the second or linear portion of the curve would be due to the breakdown of humus, this "humus line" may be extrapolated to zero time, on the premise that humus was being degraded at a constant rate since the beginning of the experiment. Consequently it becomes possible to calculate, by difference, the rate

of oxidation of the more readily degradable organic fraction. When the data from Chase & Gray's experiments were plotted as logarithms of daily rates against time, the predicted curvi-linear result was obtained and a reasonable approximation to a second straight line was obtained from the difference calculation.

The data from the present experiments were examined by plotting the logarithms of the rates of CO_2 evolved (means of cumulative CO_2 production) against time. These plots revealed a definite tendency to give a graph with an initial curve followed by a straight line (see Figures 4.1 to 4.10). Chase & Gray (1957), by making short term experiments, ascertained the half-lives of the two substrates to be 1.4 days for "readily decomposible" organic matter and 15.6 days for "humus material". These authors suggested that the derivation of degradation rates for the more resistant forms of humus would require longer term experiments. Such prolonged incubation experiments have been made in the present study and the half-lives have been calculated for the readily decomposible organic substrates and the humus materials in the soil; these calculated values are presented in Table 4.1. Maximum time taken for decomposition of both readily decomposible substrates and humus materials occurred under anaerobic conditions of soil incubation. Both glucose and inorganic nitrogen compounds stimulated the mineralization of organic matter but the effect of Dalapon and triphenyltin acetate on these transformations was even more dramatic.

A number of studies have been made of carbon mineralization in soils using radio tracers. Thus Chahal & Wagner (1965) observed that ^{14}C-glucose decompositon was rapid in soil and after a period of several months, about 25% of the label had become incorporated into microbial tissues or into the soil organic matter; the remainder of the label was

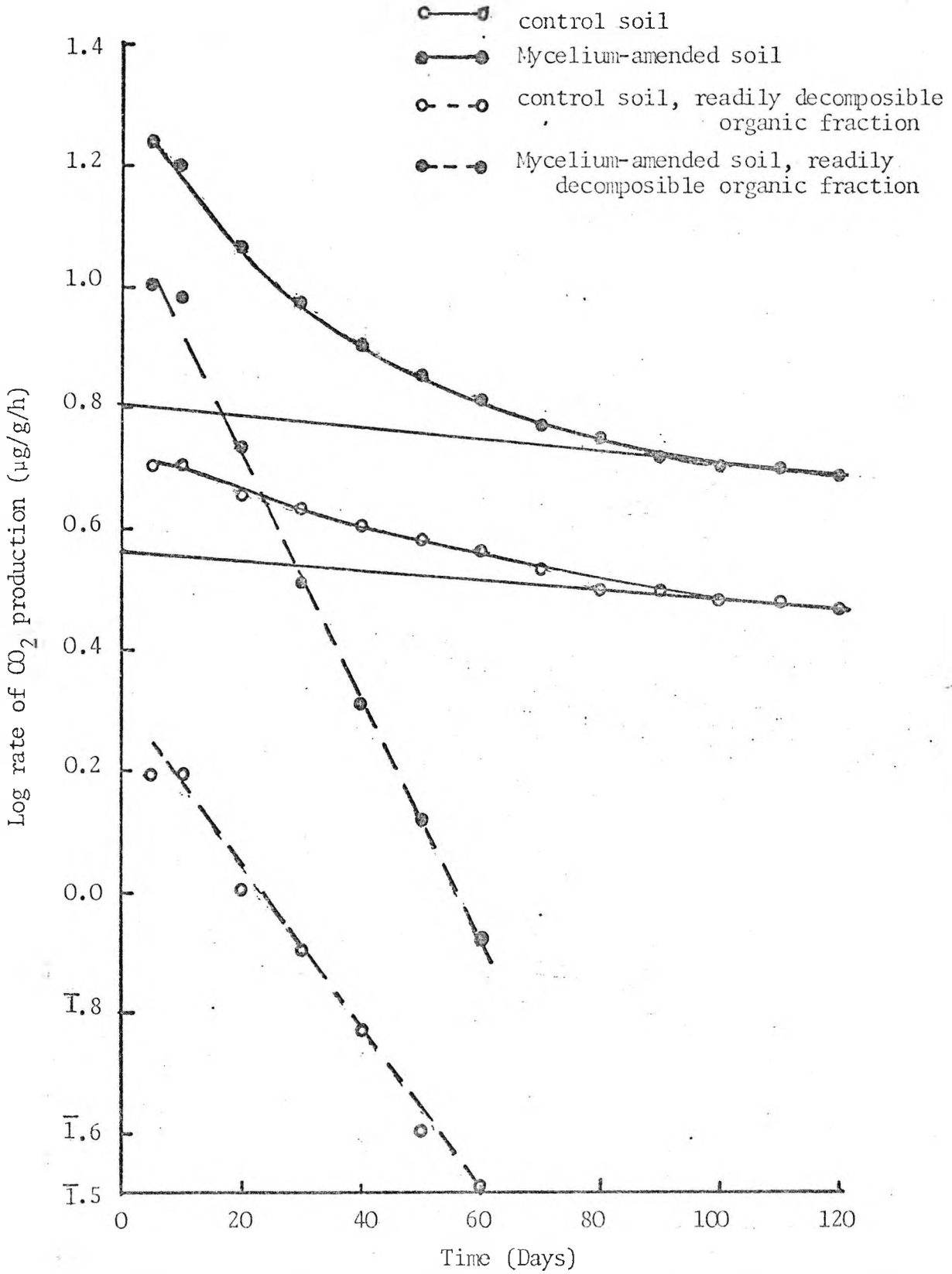


Figure 4.1. Evolution of CO_2 from mycelium-amended and control soils under aerobic conditions during continuous flow of water. The continuous straight line extrapolated to zero time from the linear position of the curve is the "humus line" (see text).

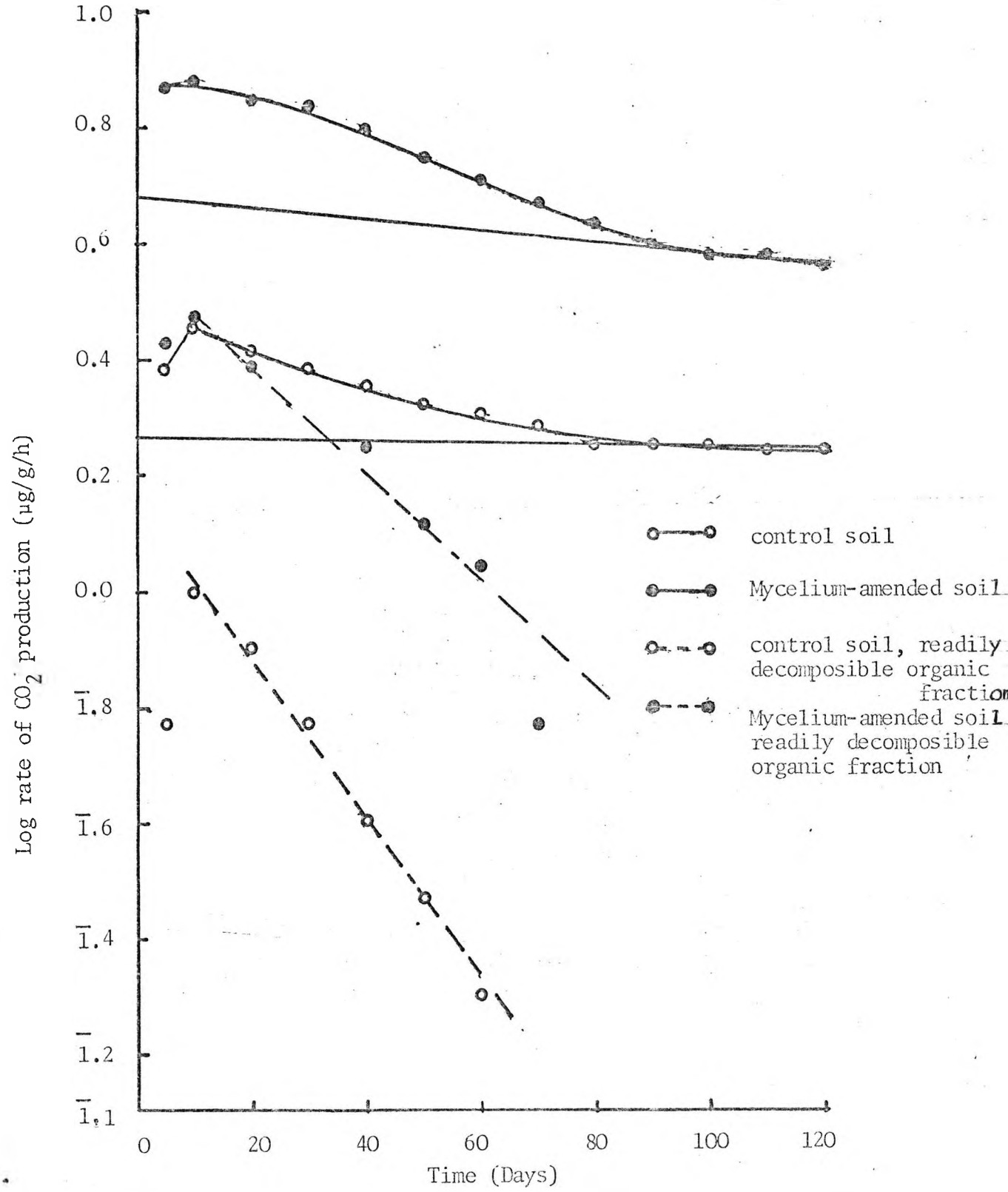


Figure 4.2. Evolution of CO_2 from mycelium-amended and control soils under anaerobic conditions during continuous flow of water. Other details as in Figure 4.1.

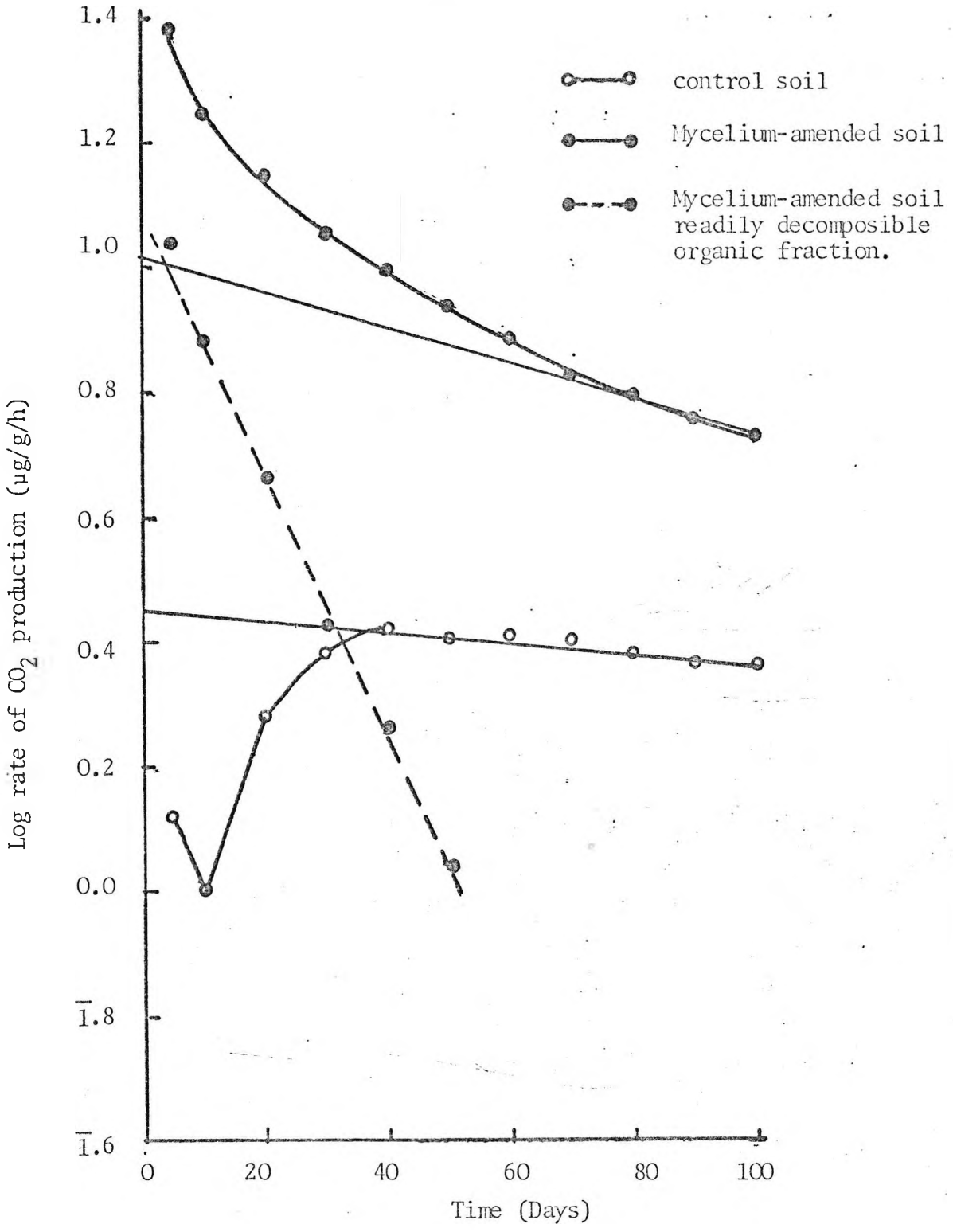


Figure 4.3. Evolution of CO_2 from mycelium-amended and control soils during cycles of drying and wetting under aerobic incubation conditions. Other details as in figure 4.1.

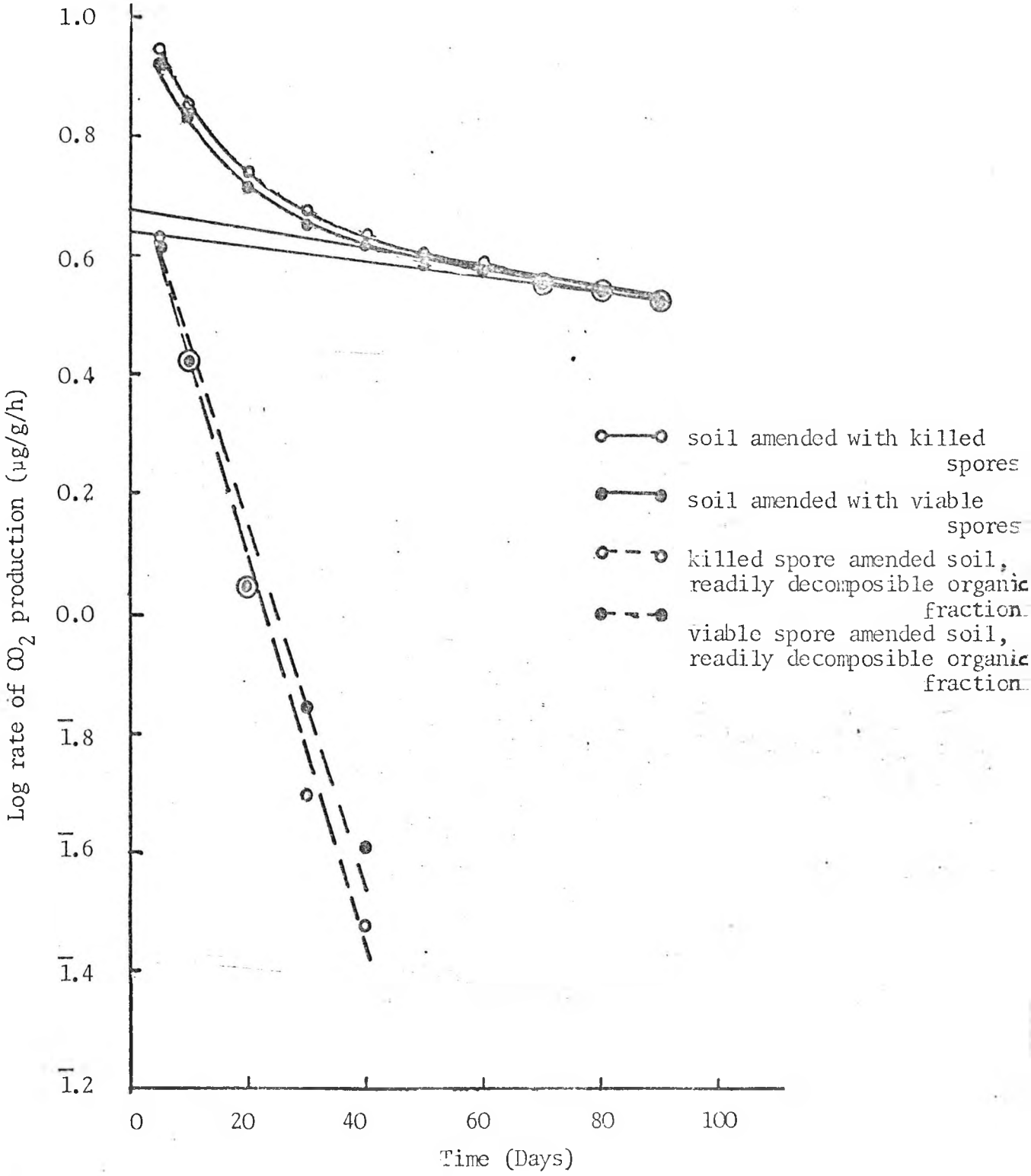


Figure 4.4. Evolution of CO_2 from soil amended with killed and viable spores during the continuous flow of water; aerobic incubation conditions. Other details as in Figure 4.1.

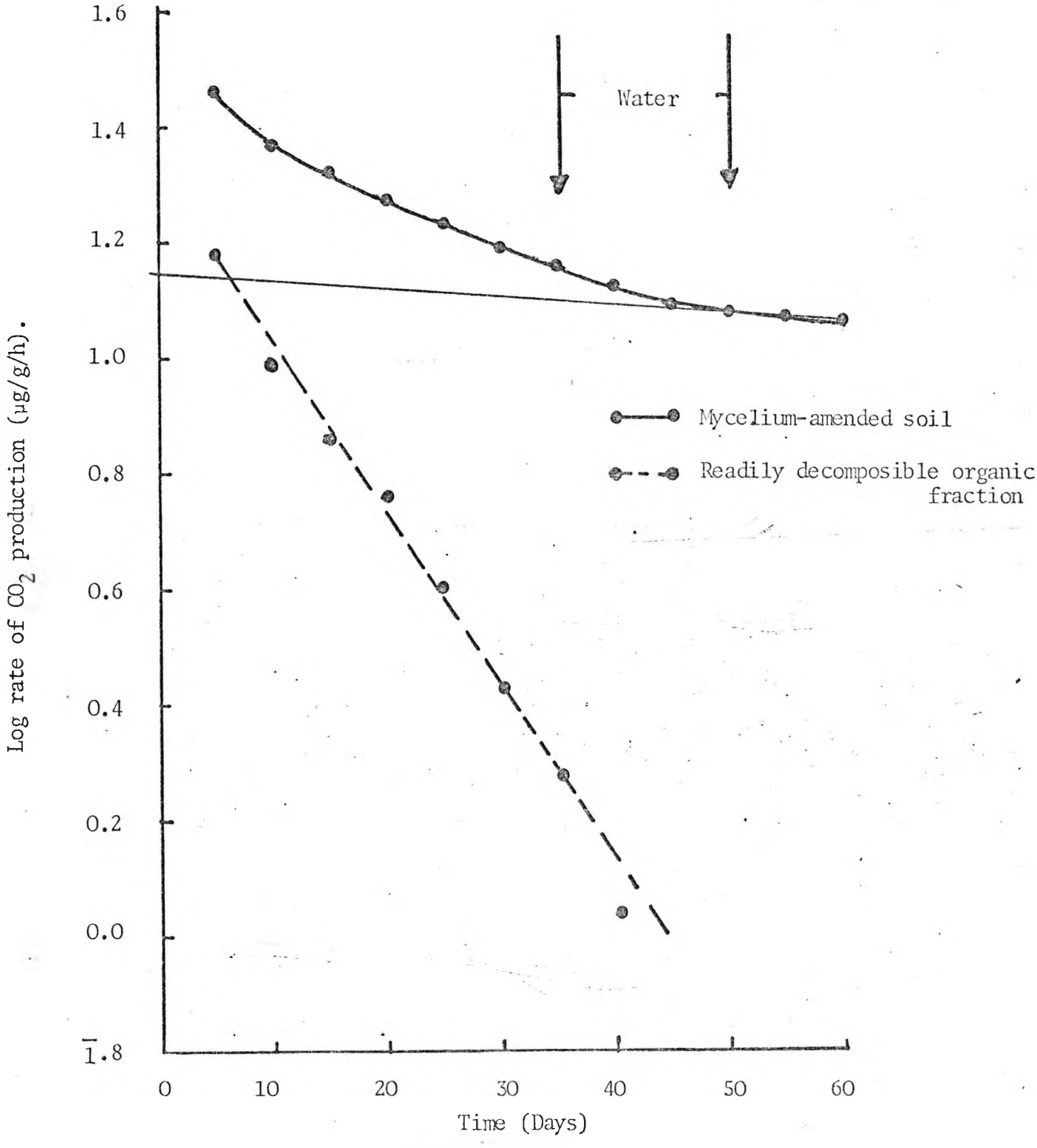


Figure 4.5. Evolution of CO_2 from mycelium-amended soil under aerobic conditions during continuous flow of glucose solution (0.1%, w/v). At day 35 the flow was changed to one of water and at day 50 the glucose flow was restored.

Other details as in Figure 4.1.

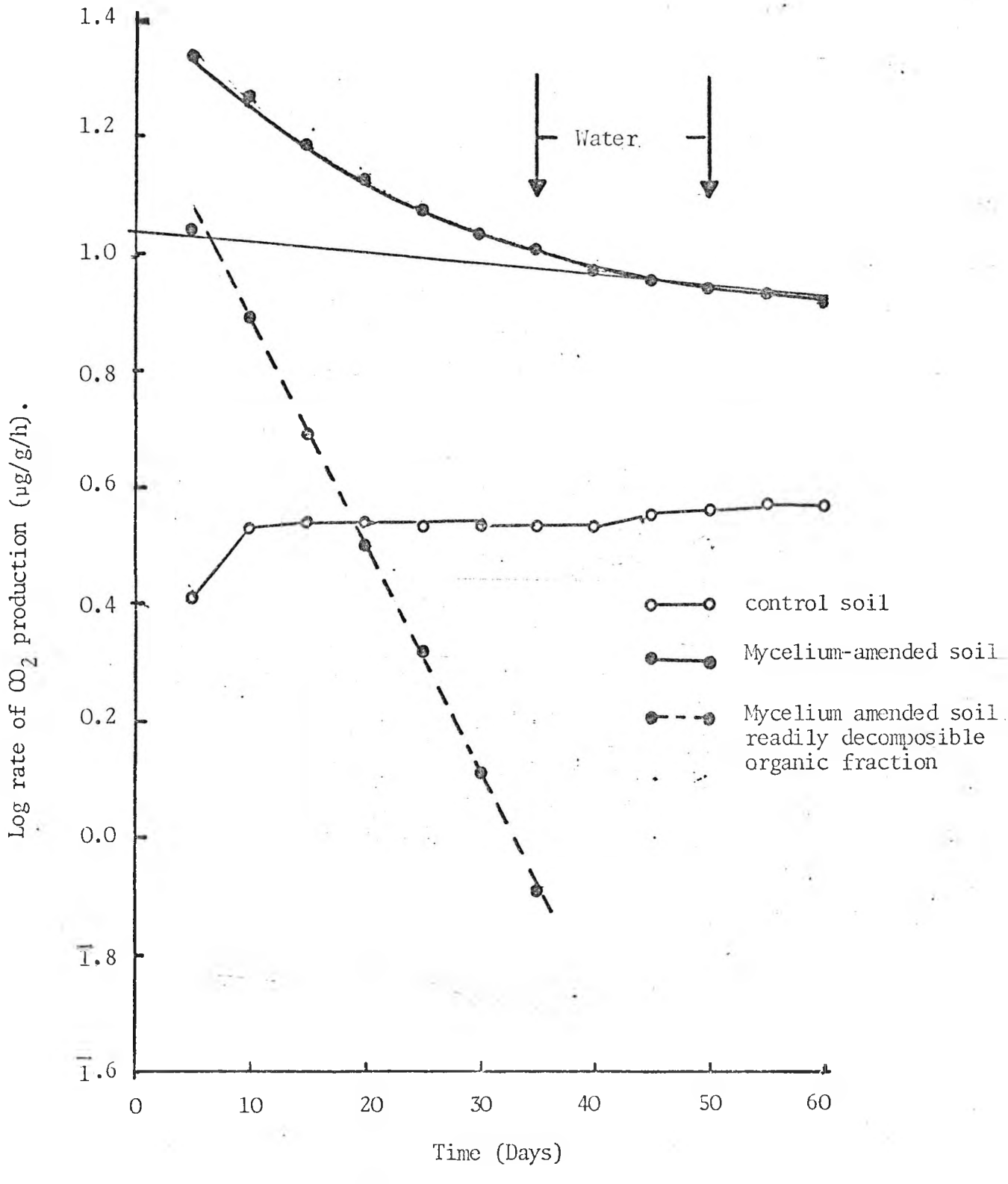


Figure 4.6. Evolution of CO_2 from mycelium-amended and control soils under aerobic conditions during continuous flow of KNO_3 solution (100µg NO_3 - N/ml). At day 35 the flow was changed to one of water and at day 50 the KNO_3 flow was restored. Other details as in Figure 4.1.

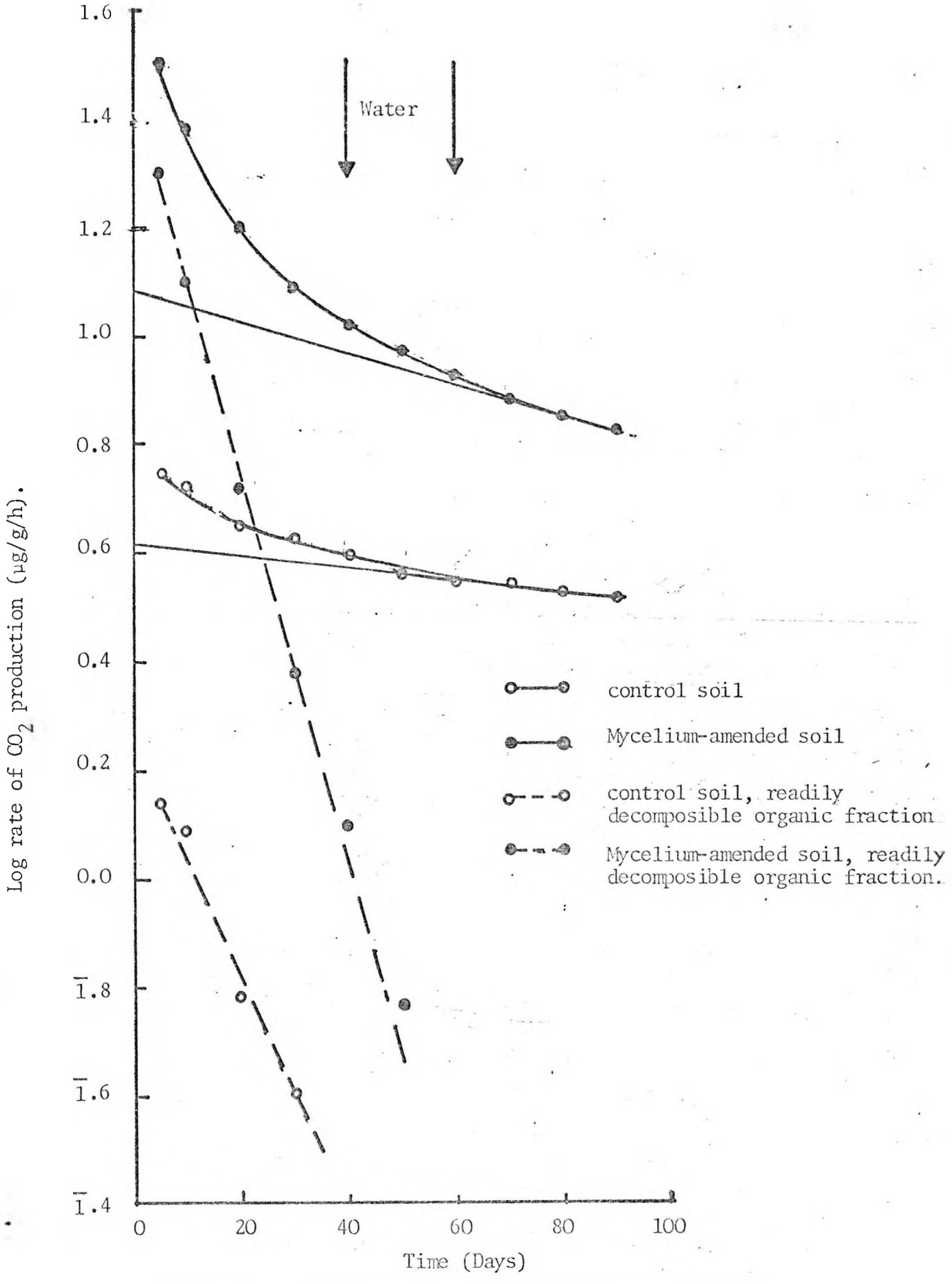


Figure 4.7. Evolution of CO_2 from mycelium-amended and control soils under aerobic conditions during continuous flow of ammonium-sulphate solution (100µg NH_4 - N/ml). At day 40 the flow was changed to one of water and at day 60 the flow of $(NH_4)SO_4$ was restored. Other details as in Figure 4.1.

Figure 4.8. Evolution of CO_2 from mycelium amended and control soils under aerobic conditions during continuous flow of ammonium phosphate solution (100µg/ NH_4 - N/ml). At day 45 flow changed to one of water and at day 70 ammonium phosphate was restored.

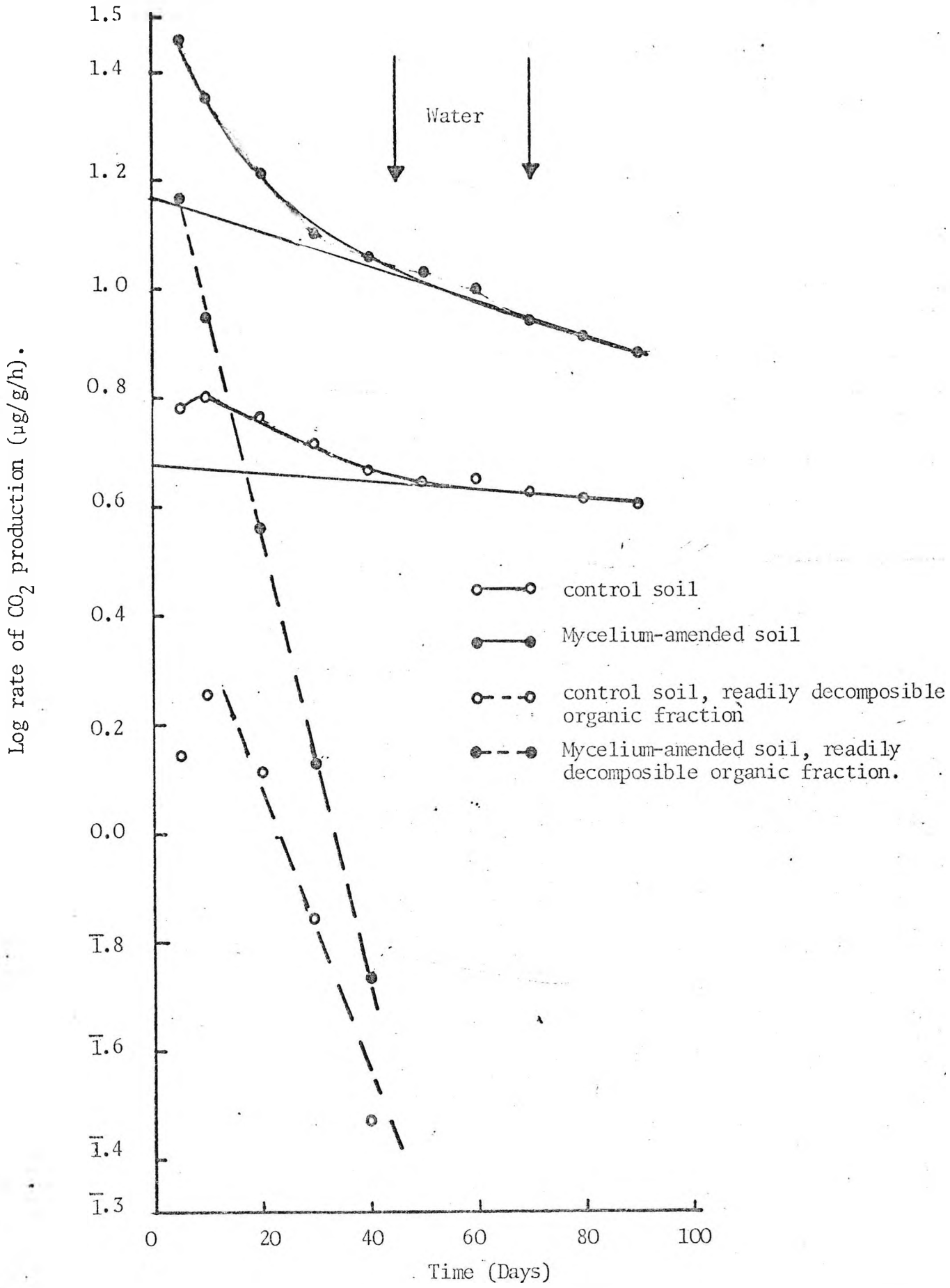

Log rate of CO_2 production (µg/g/h).
1.5
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
$\bar{1}.8$
$\bar{1}.6$
$\bar{1}.4$
$\bar{1}.3$
Water
control soil
Mycelium-amended soil
control soil, readily decomposible organic fraction
Mycelium-amended soil, readily decomposible organic fraction.
0
20
40
60
80
100
Time (Days)

Figure 4.8.

Figure 4.9. Evolution of CO_2 from mycelium amended and control soils under aerobic conditions during continuous flow of varying concentrations of Dalapon. Other details as in Figure 4.1.

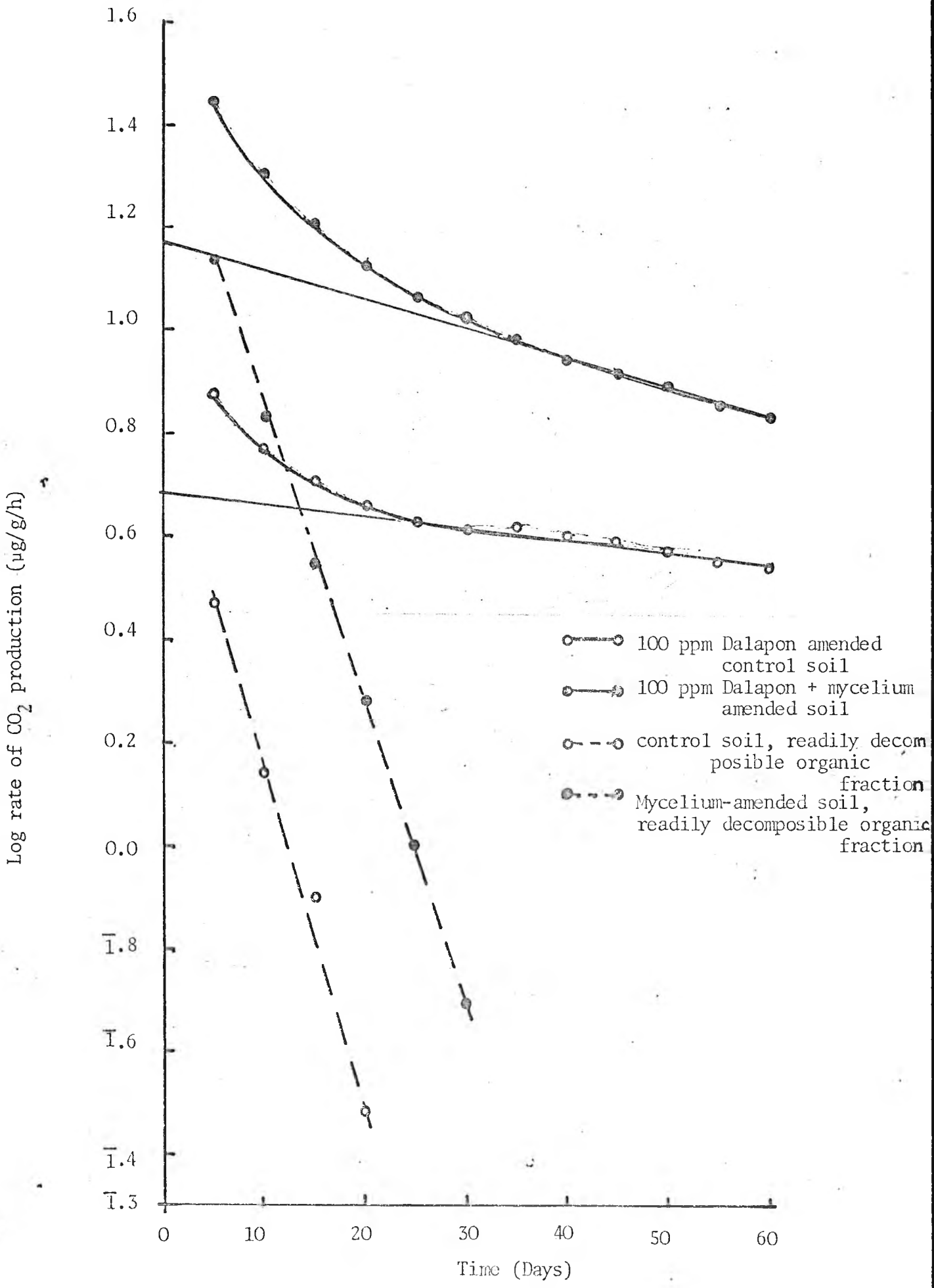

1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
$\bar{1}$.8
$\bar{1}$.6
$\bar{1}$.4
$\bar{1}$.3
Log rate of CO_2 production (µg/g/h)
0
10
20
30
40
50
60
Time (Days)
100 ppm Dalapon amended control soil
100 ppm Dalapon + mycelium amended soil
control soil, readily decomposible organic fraction
Mycelium-amended soil, readily decomposible organic fraction

Figure 4.9.

Figure 4.10. Evolution of CO_2 from mycelium amended and control soils under aerobic conditions during continuous flow of aqueous fentin acetate (TPTA). Other details as in Figure 4.1.

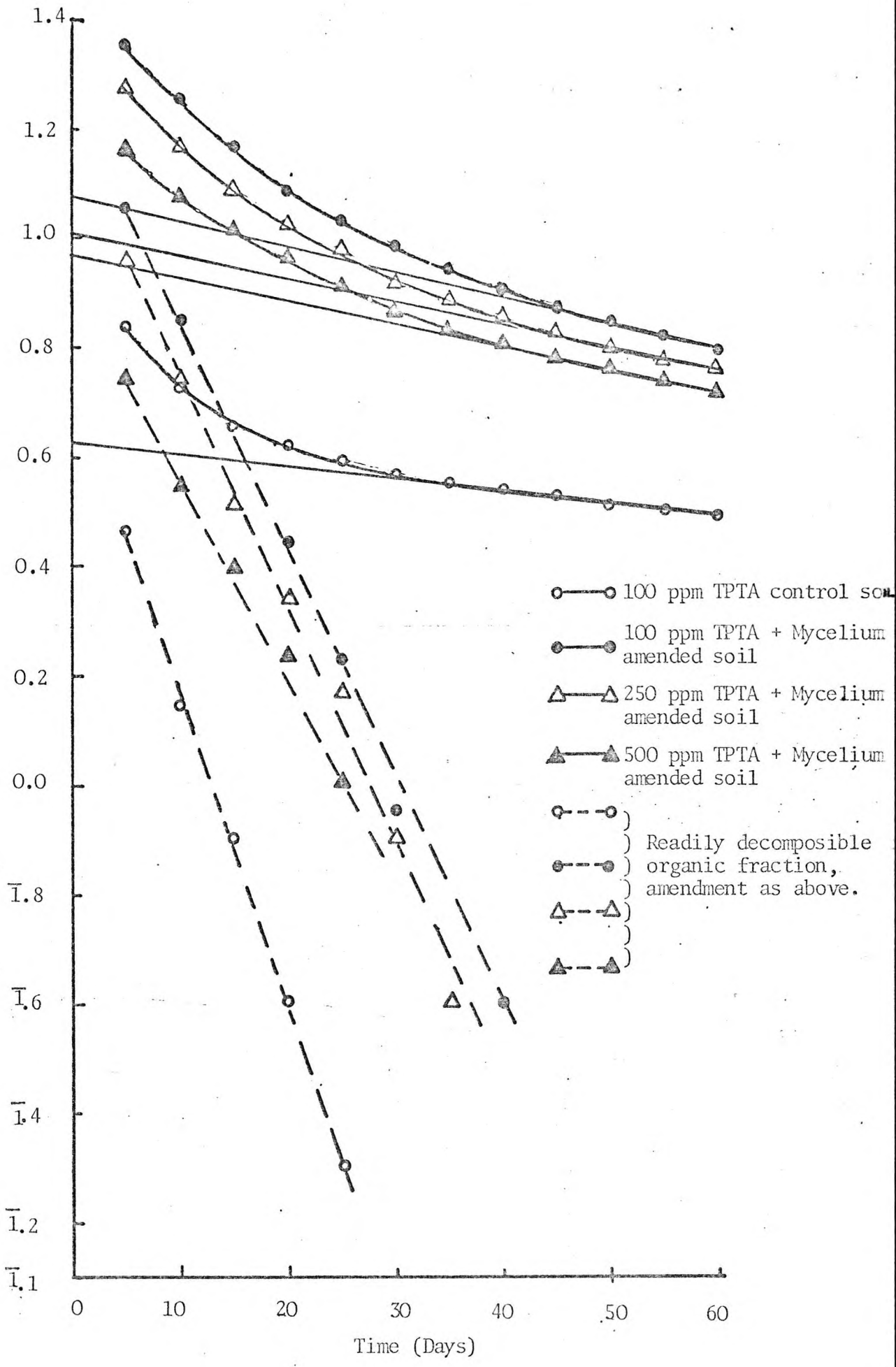

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
$\bar{1}.8$
$\bar{1}.6$
$\bar{1}.4$
$\bar{1}.2$
$\bar{1}.1$
0
10
20
30
40
50
60
Time (Days)
100 ppm TPTA control soil
100 ppm TPTA + Mycelium amended soil
250 ppm TPTA + Mycelium amended soil
500 ppm TPTA + Mycelium amended soil
Readily decomposible organic fraction, amendment as above.

Figure 4.10.

TABLE 4.1

Half-lives of readily decomposable and humus materials in soil subjected to various treatments.

Treatment	Incubation period (Days)	Readily Decomposible Material		Humus Material	
		Control Soil	Amended Soil	Control Soil	Amended Soil
Aerobic	120	33	15	430	334.5
Anaerobic	120	301	43	2006	334.5
Wetting & Drying	100	nc	14.3	376.3	141.3
Spores viable	90	nd	10.3	nd	221
killed	90	nd	10.3	nd	188
Glucose	60	nd	10	nd	125
KNO_3	60	nc	7.7	nc	150
$(NH_4)_2 SO_4$	90	14.3	6.8	273.6	103
$(NH_4)_2 HPO_4$	90	11	7.5	376.3	100
Dalapon	60	5	5	150	52.8
TPTA 100 ppm	60	5	7.5	150	75.3
250 ppm	60	nd	7.5	nd	75.3
500 ppm	60	nd	8.3	nd	75.3

nc = not possible to calculate a meaningful rate from the graph

nd = control not done.

liberated from the soil as $^{14}CO_2$ during the latter phases of experiment, a slow release of $^{14}CO_2$ suggested that microbial tissues and products resistant to rapid decay were being transformed. More recently Sorensen & Paul (1971) found that ^{14}C-labelled acetate was metabolized with a half-life of 3.4 days under laboratory conditions. This phase of metabolism was followed by a slow decomposition of ^{14}C-amino acids and ^{14}C-amino sugars formed during the initial incubation period. Sorensen (1972), from plots of decay curves, calculated that the half-lives of ^{14}C-amino acids were of the order of 6 to 7 years. Further work from Paul's laboratory (Shields *et al*, 1973) has been concerned with the transformation of ^{14}C-glucose and $^{15}NH_4$ $^{15}NO_3$ added to soil under field conditions. It was observed that the substrates added to soil were rapidly assimilated into microbial tissue or transformed into organic metabolites. Furthermore Shields *et al*reported that these immobilized radioactive metabolites had a high degree of stability throughout the period of the field study. Thus the half-life of labelled carbon remaining in the soil after 32 days was calculated to be 180 days; in comparison the half-life for the initially added glucose was only 4 days.

The fate of labelled plant residues in soil also has received considerable attention. Jenkinson (1965) investigated the loss of carbon when ^{14}C-labelled rye grass was mixed with soil and allowed to decompose in the field. He found that labelled carbon was lost rapidly during the first few months but thereafter the rate declined greatly. Jenkinson estimated that, over a six months period, the half-life of added labelled carbon was 4 years whereas the unlabelled carbon of the native organic matter had a half-life of 25 years. Shields & Paul (1973) also followed the distribution of labelled straw through the soil organic fractions and found that biological products

arising from the rapid initial attack on easily degradable plant components exhibited a high degree of stability in sceptre soil under field conditions.

Returning to the present programme it was shown that the addition of mycelium to soil generally resulted in a marked increase in the rate of decomposition of native organic matter. The magnitude of the priming action varied with the treatments. The carbon dioxide evolution data from the control and mycelium-amended soil perfused continuously with water, various nutrient and pesticide solutions during the course of 60 days incubation are presented in Figure 4.11. The addition of mycelium had a greater stimulating influence on the decomposition of native soil organic matter in the soil perfused with $(NH_4)_2\ HPO_4$, Dalapon and fentin acetate than in water and $(NH_4)_2\ SO_4$ solution. Negative priming effect in the soil perfused with KNO_3 solution was observed.

The rate of loss of native soil organic matter differed significantly between control unamended soil compared with soil perfused continuously with water and other nutrient solutions. In particular when the effects of the three nitrogen sources are compared it is clear that the mineralization of native organic matter was pronounced in the presence of $(NH_4)_2\ HPO_4$.

Shields *et al* (1974) investigated the effect of adding ^{13}C-glucose, NH_4NO_3 or ^{13}C-glucose plus NH_4NO_3 to samples of Brown chernozemic soil which had been amended initially with ^{14}C-glucose and incubated for 60 days under field conditions. At the end of 14 days incubation underlaboratory conditions they observed that 39% and 33% of ^{13}C had been evolved as CO_2 from the ^{13}C-glucose and ^{13}C-glucose plus NH_4NO_3 treated soil respectively. These two treatments resulted

in a marked priming of native ^{12}C, i.e. more than 80% mineralization. In contrast there was only a small priming action of ^{14}C-labelled materials i.e., less than 1% mineralization of ^{14}C. Furthermore Shields *et al* found that addition of NH_4NO_3 by itself had no effect on the amount of ^{12}C or ^{14}C mineralization. In the present study there was very little effect in mineralization of native organic matter of soil perfused with KNO_3 and $(NH_4)_2\ SO_4$ compared to water-perfused control soil. However, perfusing the soil with $(NH_4)_2\ HPO_4$ had a significant effect on the mineralization of native soil organic matter.

The perusal of the data in Figure 4.11 clearly shows the pronounced priming effect in mycelium-amended soils perfused with fentin acetate and with Dalapon. Karnath & Vasantharajan (1973) investigated the persistence and effect of the fungicide Dexon on soil respiration. They found that simultaneous addition of straw and Dexon to soil resulted in an increased carbon balance, but similar influence in glucose treatments was not observed. This might be due to the inhibition of straw metabolism by Dexon in soil. However, they found that the addition of fungicide alone without any supplementation resulted in a loss of organic carbon from the soil, suggesting the breakdown of native organic matter during the metabolism of Dexon.

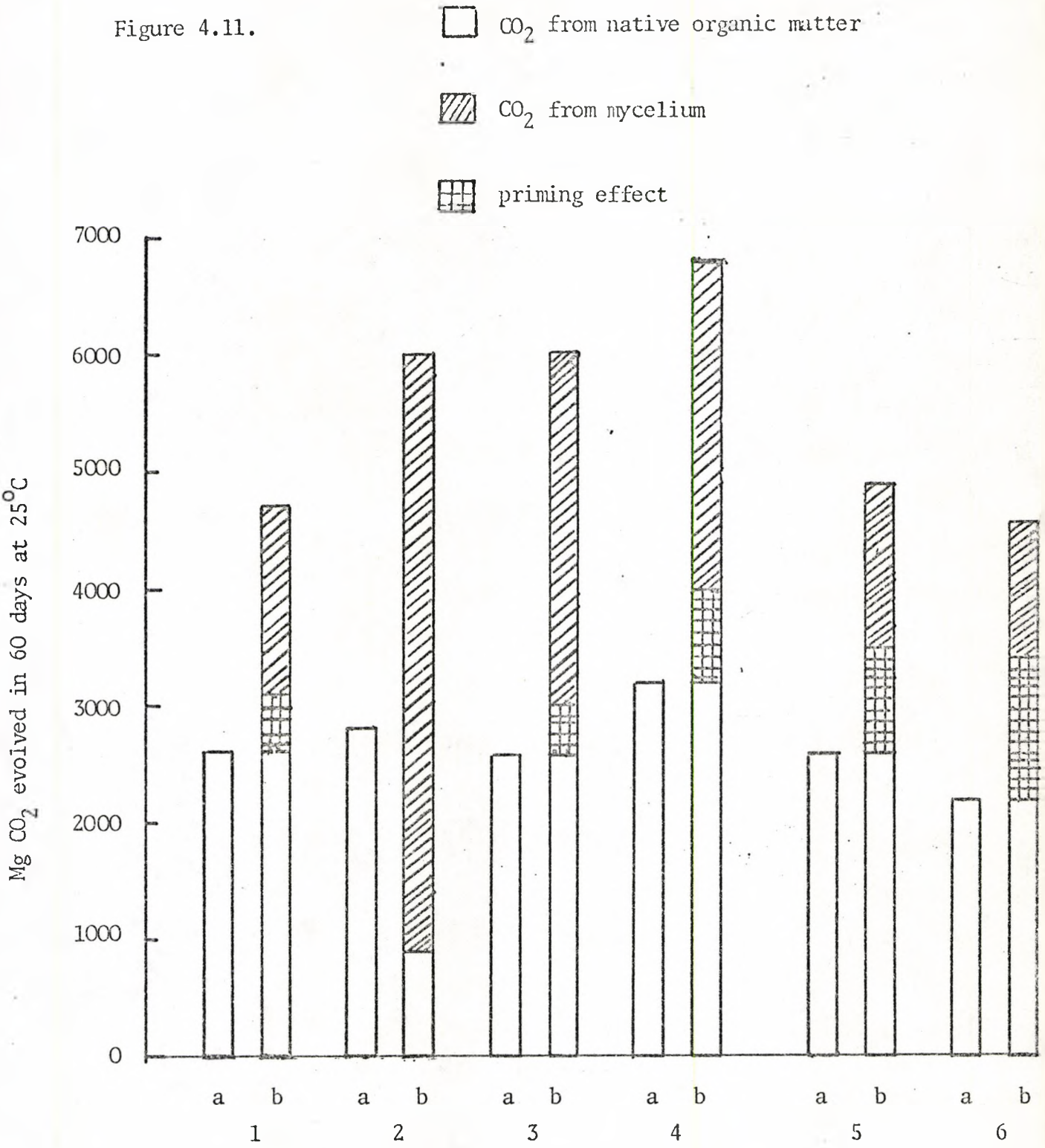
The findings of the present study suggest that the dynamics of fungal transformation in aerated soil are similar to those reported for green manure (Broadbent, 1947; Hallam & Bartholomew, 1953; Sauerbeck, 1966; Sorensen, 1963). The priming action of the fungal amendment persisted for about 45 days, i.e., for as long as it comprised the major portion of the total decomposing organic carbon. Fungal residues

persisting after this time were mineralized at the same rate as the native organic matter with which it presumably equilibrated quite rapidly. These results seem to confirm the idea suggested by Broadbent (1947) and Hallam & Bartholomew (1953) that organic amendments may exert a priming action as long as they constitute a considerable part of the total decomposing mass.

In general the continuous addition of agricultural chemicals to the soil amended with ^{14}C-labelled mycelium further enhanced the priming effect; except the addition of KNO_3 which apparently had a negative effect on the transformation of native organic matter.

Figure 4.11. Evolution of CO_2 from control and mycelium-amended soils. The priming action: The amount of organic carbon lost by the priming action in a given time was calculated as x-y-z (Jenkinson, 1966), where x is the total amount of CO_2 evolved from a soil incubated with fungal mycelium, y is that part of x which comes from the mycelium mineralization and z the background CO_2 evolved when the soil is incubated alone.

Figure 4.11.



a) control soil b) Mycelium-amended soil.

perfused with -

1. Water
2. KNO_3
3. $(NH_4)_2 SO_4$
4. $(NH_4)_2 HPO_4$
5. Dalapon
6. Triphenyltin acetate

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