THE UNIVERSITY OF EDINBURGH

INSTITUTE OF ECOLOGY AND RESOURCE MANAGEMENT

EFFECTS OF ATMOSPHERIC NITROGEN DEPOSITION ON HEATHLAND ECOSYSTEMS

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A Thesis submitted for the Degree of Doctor of Philosophy

August 2001

DECLARATION

This thesis has been composed by myself from results of my own work, except where stated otherwise, and has not been submitted in any other application for a degree.

Rashneh N. Pardiwala

August, 2001.

To my parents, Roda and Noshir,
And brothers, Cyrus and Dinshaw.

Where the mind is without fear and the head is held high,
Where knowledge is free,
Where the world has not been broken up into fragments
By narrow domestic walls,
Where words come out from the depth of truth,
Where tireless striving stretches its arms towards perfection,
Where the clear stream of reason has not lost its way
Into the dreary desert sand of dead habit,
Where the mind is led forward by thee,
Into ever-widening thought and action
Into that heaven of freedom, my Father, let my country awake.

from ***'Gitanjali'***,
Rabindranath Tagore, 1861-1941

ABSTRACT

The heathlands of north-west Europe, dominated by the species *Calluna vulgaris* (L.) Hull, have for centuries determined the physiognomy of the landscape. In recent times, however, many countries have recorded an alarming decline in the area of lowland dry heaths. The mechanisms that underlay the impoverishment process have still not been fully understood but the deterioration has been linked to high loads of atmospheric nitrogen and sulphur pollutants. Nitrogen pollutants may also disrupt the terrestrial carbon cycle, which modern studies suggest is vulnerable to climate warming. *Calluna*-dominated heathlands are an important carbon store that cover about 15 % of the land area in the UK but contain nearly 75 % of the soil organic carbon. Therefore, understanding and predicting the response of soil carbon in heathland ecosystems to changes in global temperature and nitrogen pollution is critical, particularly since increased release of respired carbon dioxide to the atmosphere has the potential to exacerbate global warming.

This study examined the degree to which enhanced nitrogen inputs in a *Calluna*-dominated ecosystem can alter plant physiological responses, affect the response of soil respiration to environmental parameters by disturbing acclimatised soil microbial populations, influence the relationship between soil carbon fluxes and soil microbial populations, and change soil mineral nitrogen availability to the plants.

A pilot study investigated the response of nitrogen deposition on *Calluna vulgaris* plants maintained in open-top chambers. Heathland monoliths were exposed to acid mist treatments of ammonium nitrate spanning across extreme values. Growth response to increasing fertiliser additions was detectable and high nitrogen fertiliser inputs significantly stimulated shoot growth. Fertiliser inputs were reflected in soil and tissue nitrogen concentrations with an increase in total nitrogen content within actively growing tissues while shoot phenolic concentration decreased in response to nitrogen additions in agreement with the carbon-nutrient hypothesis.

A field study was conducted in experimental plots set up in a dense stand of mature heather at Castlelaw Hill, near to Edinburgh. A new, simple methodology is developed and operated to accurately measure soil respiration under controlled laboratory conditions using small soil microcosms with a gas analysis unit. Annual seasonal pattern of soil carbon dioxide effluxes and environmental parameters of soil temperature, moisture, pH, organic matter, microbial biomass and plant growth were measured. Soil temperature, pH, organic matter and microbial biomass were found to be important determinants of carbon dioxide fluxes from soil. In all the soil horizons, carbon dioxide efflux in response to temperature followed the exponential first order equation with an increase with increasing temperature but soil carbon dioxide fluxes decreased with depth. Nitrogen inputs significantly increased soil respiration and the results suggest that long-term effects of atmospheric N deposition, with accelerated mineralisation at higher temperatures, could disrupt the carbon balance of nutrient-poor ecosystems, as noted for heathlands.

ACKNOWLEDGEMENTS

The following people have contributed, either their time, energy, effort, knowledge, expertise, advice, or friendship, faith, love and encouragement in an important and appreciated manner:

- Professor John Grace, supervisor, mentor and guide in the truest of sense
- Dr. Andrew Vinten, motivating tutor ever since my days as a masters student
- Derek Scott, laboratories manager and most helpful fieldtrip companion
- Andy Gray, Rab Howard, Lesley Swan and Dave Mackenzie, technical staff
- Sheila Wilson and Connie Fox, secretarial staff
- Piedad Martin-Olmedo, Changming Fang, Mark Rayment, Patrick Meir, Lisa Wingate and Sandra Patiño, colleagues in the department
- Katy Rustom, spirited friend and confidant during good and bad times
- Peter and Gemma Rebello, guardian angels in Edinburgh
- Gorwala family, providers of a warm, caring home away from home in London
- Barbara Panvel, benevolent supporter in every hour of need
- My family and friends in India, UK and across the world

I thank the University of Edinburgh, Institute of Ecology and Resource Management, Scottish Agricultural College and the Centre for Ecology and Hydrology for generously extending research facilities for my field and laboratory experiments.

Financial support was provided through the Overseas Research Students Award Scheme and receipt of the tuition grant is gratefully acknowledged.

And finally, my infallible lifeline and pillars of strength, without whom the endeavour would not have been possible, or even worthwhile - my parents, Roda and Noshir, and brothers, Cyrus and Dinshaw.

My sincere gratitude to all.

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LIST OF SYMBOLS AND ABBREVIATIONS

Greek Alphabet

ΔC	Change in CO_2 concentration (μmol CO_2 mol^{-1})

Roman Alphabet

A	Surface area (m^2)
ANI	Added nitrogen interaction
C	Carbon
^{14}C	Carbon isotope
C:N	Carbon : nitrogen ratio
E	Arrhenius activation energy (kJ mol^{-1})
E_{Nin}	Difference between extracted ninhydrin-reactive-nitrogen from fumigated and unfumigated soil (μg-N g^{-1} dry soil)
F	Flow rate (mol s^{-1})
FE	Fumigation-extraction
IRGA	Infra-red gas analyser
k	Coefficient for the exponential response of CO_2 efflux to temperature ($°C^{-1}$)
k_{EC}	Extractable component of microbial biomass-carbon
MB_C	Microbial biomass-carbon (mg-C g^{-1} dry soil)
MB_N	Microbial biomass-nitrogen (mg-N g^{-1} dry soil)
MB_C:MB_N	Microbial biomass-carbon : microbial biomass-nitrogen ratio
MB_{Nin}	Microbial biomass ninhydrin-reactive-nitrogen (mg-N g^{-1} dry soil)
MB_T	Microbial biomass-total (mg-MB g^{-1} dry soil)
M_A	Mass of air-dry soil (g)
M_I	Mass of ignited soil (g)

M_O	Mass of oven-dry soil (g)
N	Nitrogen
^{15}N	Nitrogen isotope
NH_4- N	Ammonium derived nitrogen
NH_4^+	Ammonium ion
NH_4NO_3	Ammonium nitrate
NO_3- N	Nitrate derived nitrogen
NO_3^-	Nitrate ion
OTC	Open-top chamber
PO_3^-	Phosphate ion
Q_{10}	Relative increase in the rate of a chemical reaction in response to an increase of temperature by 10 °C (dimensionless)
R	Efflux rate of soil CO_2 (μmol m^{-2} s^{-1})
$\Re$	Universal gas constant (8.314 mol^{-1} K^{-1})
SOM	Soil organic matter (%)
T	Temperature (°C)
T	Temperature (K)

1. INTRODUCTION

1.1 HEATHLANDS AND THEIR CHANGING ENVIRONMENT

The term heathland describes a landscape covered largely by semi-natural, evergreen, dwarf-shrub communities of ericoid plant species, such as *Calluna vulgaris, Erica tetralix* and *Vaccinium myrtillus*, which form a closed canopy with few or no trees. This vegetation type, extending over the geographical range of north-west Europe, generally occurs under humid, oceanic climatic conditions in a nutrient-poor environment (Gimingham, 1972).

Since the early nineteenth century there has been a steady reduction in the heathland regions of Europe, primarily due to changing land-use practices and large-scale human impacts (Aerts, 1993 b). The substantial losses have been exacerbated over recent decades by the successional conversion of heathlands to grasslands, brought about by an increase in the deposition of atmospheric pollutants. The widespread eutrophication of nutrient-poor ecosystems, as a result of nitrogen fertilisation, poses a constant threat to the remaining *Calluna*-dominated heaths. The change from ericaceous towards gramineous dominance has been found to progress sequentially, wherein the unnatural opening of the vegetation canopy via stress and disturbance factors increases susceptibility to the invasion of competitive grass species. Management techniques adopting an integrated approach need to be urgently implemented if the declining trend is to be halted.

Heathland ecology has been the subject of numerous research projects through the years. Studies have focused on ecosystem dynamics with respect to nutrient cycling (Helsper, Glenn-Lewin and Werger, 1983; Brunsting and Heil, 1985; Heil and Bruggink, 1987; Berdowski and Zeilinga, 1987; Berendse *et al.*, 1987), and nutrient impoverishment of heathlands (Diemont and Heil, 1984; Aerts, 1989), while some

have explored the conversion of heathlands to grasslands as an example of natural selection on different adaptive strategies with respect to varying levels of nutrient availability (Aerts, 1990). The mechanisms which underlay the deterioration process are still not fully understood but they are widely believed to include the deposition of oxides of nitrogen and ammonium from anthropogenic sources. This chapter summarises our state of knowledge of heathlands and examines the complex interaction between heathlands and their changing environment.

1.1.1 The Atmosphere

Over the past 30 years heather moorlands in upland regions of the UK have been under threat from increased atmospheric N deposition, changes in the land use and management, and climate warming (Thompson, McDonald and Marsden, 1995; Werkman, Callaghan and Welker, 1996). Emissions of atmospheric sulphur dioxide, nitrogen oxides and ammonia are elevated across Western Europe (Rodhe and Rood, 1986; Fowler *et al.*, 1989). The main source of sulphur compounds is industry, while oxides of nitrogen primarily originate from the combustion of fossil fuels and biomass, lightning, ammonia oxidation, microbial soil processes, stratospheric inputs and marine biological processes, and ammonia is volatilized in great quantities from intensive agricultural and livestock production systems (ApSimon, Kruse and Bell, 1987).

Drastic changes in the nitrogen cycle have occurred with time and currently, approximately 140 Tg of reactive nitrogen per year are being released into the environment (Lee, 1998). Galloway *et al.* (1995) estimate that fertiliser production, fossil fuel burning and agriculture are now releasing more combined nitrogen into the environment than that due to nitrogen fixation by micro-organisms in semi-natural ecosystems and lightening. Estimates include an increase of atmospheric nitrogen deposition rates by more than ten-fold over the last 40 years to current values of 0.5 – 2.5 g N m^{-2} yr^{-1} in eastern North America and 0.5 – 0.6 g N m^{-2} yr^{-1} in

northern Europe (Wedin and Tilman, 1996). This trend is likely to continue as a result of fossil fuel consumption and fertiliser use and to lead to a projected 60 % increase by 2020 in combined annual nitrogen release (Matthews, 1994).

Measurements made across Europe have indicated a distinct upward trend in the nitrogen component of rainfall since 1950 (Grennfelt and Hultberg, 1986; Pitcairn, Fowler and Grace, 1995). The inputs of nitrogen, in the form of nitrates and ammonium in the rain, range from 5 - 20 kg N ha^{-1} annually, of which atmospheric ammonia contributes 50 - 80 %. The average deposition of ammonia in the UK is approximately 15 - 20 kg ha^{-1} yr^{-1} while in the Netherlands, which records the highest rates of deposition, the value fluctuates between 40 and 50 kg ha^{-1} yr^{-1}. Within the past decade, the concentrations of ammonia in rural air, as recorded in Wales, has risen from 0.1 parts in 10^9 to 5 parts in 10^9 by volume (Ashenden and Edge, 1995), with the result that in many regions of Europe nitrogen inputs are believed to be dominated by reduced nitrogen species, collectively known as NH*y* (Sutton, Fowler and Moncrieff, 1993).

Forests are aerodynamically rough, and the surface structure promotes dry deposition of sulphur and nitrogen (Mayer and Ulrich, 1974; Van Breemen *et al.*, 1982; Johnson and Siccama, 1983; Nihlgard, 1985; Fowler, Cape and Unsworth, 1989). Similarly, modern research has shown that in contrast to the conventional views, the leaf canopy of heathlands is also quite rough and can capture gaseous ammonia (Miranda, Jarvis and Grace, 1984; Lindberg *et al.*, 1986; Sutton, Moncrieff and Fowler, 1992, Sutton, Pitcairn and Fowler, 1993). The importance of the nitrogen biogeochemical cycle has long been recognised but until recently much less emphasis has been placed on the ecological consequences of the perturbations of these cycles by human activities. The best documented evidence of vegetation change caused by atmospheric nitrogen deposition is provided by the acidic heathland ecosystems in the Netherlands where the decline of many plant species has been caused by the acidification of the environment by nitrogen (Van der Eeden *et al.*, 1991).

1.1.2 The Vegetation

Heaths in Europe, traditionally represented by woody shrubs, form characteristic heathlands on acid soils and moorlands on peaty soils (Gimingham, 1972). However with the passage of time, heathland ecosystems have undergone a dramatic change in terms of the floristic composition (Gimingham, Chapman and Webb, 1979; Bunce 1989). Since the end of the 1970's, a serious deterioration in species diversity has been apparent with the disappearance of *Calluna*-dependent flora and fauna (Usher, 1992), both in terms of species number and in cover percentage as documented by De Smidt and Van Ree (1991) for ten heathland areas in The Netherlands. The alarming loss of lowland dry heath has been characterised by the invasion of either monospecific stands of perennial grass species such as *Deschampsia flexuosa* (L.) Trin. and *Molinia caerulea* (L.) Moench. (Marrs, 1993; Pitcairn, Fowler and Grace, 1995) or scrub and bracken (Marrs, 1987 a, b).

The change in species composition is thought to have commenced during a period in which nitrogen and phosphorus nutrient availability in the originally nutrient-poor ecosystems steadily rose. Much of the published literature documenting the effects of increased atmospheric nitrogen deposition on heathlands has originated from the Netherlands, where the change from ericaceous towards gramineous dominance has been widespread (Heil and Diemont, 1983; Aerts, 1990). The main attributing causes for this increase have been stated as, a) the high loads of atmospheric nitrogen, b) the continuous accumulation of litter and humus which leads to the formation of a thick organic layer, with a high mineralisation rate acting as a catalyst and c) attacks of *Calluna*-dominated heathlands by the heather beetle, *Lochmaea suturalis* (Brunsting and Heil, 1985; Berdowski and Zeilinga, 1987).

Nitrogen eutrophication has been established to be the most significant factor in determining the species composition of heathland vegetation (Buijsman, Maas and Asman, 1987; Hargreaves *et al.*, 1992). Nitrogen availability has essentially risen

simultaneously with the atmospheric depositions of pollutants, which amounts on average to 3 - 4 kg N ha^{-1} yr^{-1}. Raised nutrient availability levels in turn drastically alter competitive relationships between dominant plant species thereby encouraging the replacement of heather by grasses (Heil and Diemont, 1983; Bobbink, 1991). This hypothesis has been supported by the field and laboratory experiments of Heil and Diemont (1983), Berendse and Aerts (1984), Heil and Bruggink (1987) and Aerts *et al.* (1990) which confirm the importance of nutrient inputs.

It was hypothesised by Loach (1968) that the high productivity of *Molinia* is a crucial feature responsible for the exclusion of *Calluna* and *Erica* from high-nutrient sites. Enhanced productivity due to fertilisation leads to a higher litter production with a higher organic nutrient input into the soil. Elevated rates of accumulation of litter and humus thereby promote higher rates of nitrogen mineralisation. In many heathlands in the Netherlands, this accumulation has led to rates of nitrogen mineralisation as high as 10 - 13 g N m^{-2} yr^{-1} (Berendse, 1990).

Increased nitrogen availability alone cannot explain the replacement of *Calluna* by grasses, as a number of field experiments suggest that *Calluna* is capable of remaining dominant even with the high rates of nitrogen input. Intact *Calluna* plants appear to be competitively superior to *Molinia* and *Deschampsia* plants, even at a nitrogen availability of 20 - 25 kg N ha^{-1} yr^{-1} (Aerts, *et al.*, 1990). Heathlands have a strong filtering effect on ammonia/ammonium and the canopy structure of the vegetation has been proven to play a pivotal role (Heil and Bruggink, 1987; Heil *et al.*, 1988). It appears that *Calluna* is a superior competitor for light, due to its evergreen nature, which permits canopy closure early in the growing season, thus suppressing potential competitors. The rapid conversion to grassland seen in the field is therefore thought to be triggered by gap formation in the *Calluna* canopy, allowing understorey grasses access to light, which in combination with greater nitrogen availability, permits them to become dominant and prevent regeneration of *Calluna*. Increased nitrogen deposition could accelerate natural canopy breakdown by causing

high shoot nitrogen contents, which may be associated with an increase in plant susceptibility to insect attack, frost and drought (Brunsting and Heil, 1985; Marrs, 1986; Van der Eerden *et al.*, 1991; Caporn, Risager and Lee, 1994). During an outbreak of heather beetle pest, Heil and Diemont (1983) observed that *Calluna vulgaris* was more severely affected by heather beetles in the more heavily nitrogen fertilised vegetation. This observation indicated that the replacement of *Calluna vulgaris* was not due to the higher potential growth rate of the grasses at a higher level of nitrogen availability, but because more *Calluna* plants died in the heavily fertilised zones and as a result grasses invaded the barren places. Therefore, Lee (1998) concluded that heather can remain dominant in moorland under high nutrient conditions provided that the integrity of the canopy is maintained to suppress faster growing plant species. However, overgrazing has also resulted in extensive canopy losses of heather moorland and stands are also being competitively displaced by bracken, *Pteridium aquilinum* (L.) Kuhn, which has more than doubled its area over this period (Armstrong, 1991; Marrs *et al.*, 1992). As the bracken stand moves forward discrete surface layers of organic matter develop overlying material derived from heather (Anderson and Hetherington, 1999). Such changes in physical and chemical properties of the litter layers can have important feedback effects on the growth and species balance of moorland vegetation (Van Vuuren and Berendse, 1993; Snow and Marrs, 1997). These interactions between plants and soils can be further complicated by mixtures of plant litters showing enhanced rates of decomposition and soil organic matter turnover (Chapman, Whittaker and Heal, 1988; Williams and Alexander, 1991; McTiernan, Ineson and Coward, 1997). There is no consensus on the mechanisms involved and so the magnitude and importance of organic matter and nutrient dynamics, and plant species interactions, are unpredictable (Facelli and Pickett, 1991).

Nutrient economy and secondary metabolite production are the two main plant physiological processes found to govern the changing patterns in semi-natural vegetation associated with heathlands.

1.1.2.1 *Nutrient Economy*

In many terrestrial ecosystems plant productivity is limited by the availability of nutrients as is shown by many fertilisation experiments in the field (Vermeer, 1986; Bobbink, Bik and Willems, 1988; Aerts, 1989; Aerts and De Caluwe, 1989). In all plants, there are losses and gains of nutrients to and from the shoot environment (Clarkson, Kuiper and Luttge, 1986), so that net accumulation within plant tissues is the resultant of these two processes, upon which the success of perennial plant species is largely dependent. Increased nutrient availability not only leads to an increased production, but very often causes a dramatic shift in the species composition of plant communities. This has been shown in heathlands (Heil and Bruggink, 1987; Aerts and Berendse, 1988), grasslands (Williams, 1978; Berendse, 1983; Elberse, Van den Bergh and Dirven, 1983; Bobbink, Bik and Willems, 1988) and fens (Vermeer and Berendse, 1983).

Exogenous dissolved nitrogen is available to the shoots of terrestrial plants mainly from three sources. One is the inorganic nitrogen in rainwater and occult precipitation derived from ammonia, nitrogen, or nitrogen oxides in the gas phase (Nihlgard, 1985; Duyzer, 1994); another is the organic solutes leached from the shoots of taller plants, by rainwater and dew and the third being in the form of solution sprays employed in agriculture and horticulture (Mengel and Kirkby, 1978). The relationship between atmospheric nitrogen deposition and tissue nitrogen content of vegetation, is therefore based on the degree of reliance of that vegetation on atmospheric inputs for nutrient supply.

Leaf nitrogen concentrations are generally higher in comparison to other plant tissues, because of the high concentration of nitrogen-containing enzymes, which are involved in photosynthesis (Mooney *et al.*, 1981; Hunt, Weber and Gates, 1985; Evans, 1989). In the dry heathland different seasonal patterns were observed, with nitrogen concentrations being largest in young leaves particularly in early summer and at a minimum in winter, although the difference is negligible in mature plants

(Brunsting and Heil, 1985). In *Calluna*, leaf nitrogen concentrations showed high values shortly after leaf emergence and lower values during the growing season, declining to a steady low level after about six years. Most nutrients, especially nitrogen are known to attain their greatest concentration in the current year's shoot tips in June, when growth is most active (Robertson and Davies, 1965; Grant and Hunter, 1966).

In a field experiment by Lee, Caporn and Read (1992) involving the addition of ammonium nitrate at rates of 40 - 200 kg ha^{-1} yr^{-1} of nitrogen to an upland *Calluna* monoculture in the UK, shoot nitrogen levels increased from 1.1 % in the control treatment to 1.4 % in the 40 kg ha^{-1} treatment and to 2.3 % in the 200 kg ha^{-1} treatment following only two years of application. Increases in nitrogen content resulting from nitrogen applications in the range 60 - 200 kg ha^{-1} yr^{-1} of nitrogen have also been quoted in the Netherlands (Brunsting and Heil, 1985; Aerts, 1989; Van der Eerden *et al.*, 1991). In a study conducted by Dueck *et al.* (1991), shoots exposed to a range of ammonia treatments revealed varying nitrogen contents. Shoot concentration in plants grown without added nitrogen and exposed to ammonia at 11 μg m^{-3} was 1.4 %, which increased to 2.6 % with the highest rate of added nitrogen but surprisingly, percent nitrogen escalated to 3.3 % upon exposure to ammonia at 550 μg m^{-3}. The results suggest that there is a significant positive correlation between tissue nitrogen concentrations in this investigation and the atmospheric inputs of nitrogen.

The most recently published figures set a mean foliar nitrogen content of 1.5 % for a number of European sites studied in the 1970s (Heil and Diemont, 1983) and 1.75 % to 1.9 % in the Netherlands in the 1980s and 1990s respectively (Brunsting and Heil, 1985; Pitcairn, Fowler and Grace, 1995). The foliar nitrogen content of *Calluna* can effectively range from 0.8 % to 2.6 %. These figures reflect the increasing deposition of nitrogen in Europe over the past 30 years.

In yet another study, Helsper, Glenn-Lewin and Werger (1983), clearly showed germination and growth inhibition of *Calluna* by repeated applications of nitrogen as either NPK, ammonium nitrate or even as sheep manure. In the manner in which nitrogen fertilisation negatively affects the germination and growth of *Calluna vulgaris* seedlings it is possible that nitrogenous air pollutants may act in accordance. To date, the application of nitrogen expressed in terms of total deposition rates slightly in excess of what is generally regarded as the 'critical load' has exhibited no deleterious effects on *Calluna*. However, initial application of nitrogen even at moderate doses, stimulate *Calluna* and bolster regeneration. Large positive effects on growth have been shown, which may actually result in a denser canopy, thereby reducing the likelihood of grass invasion. In a thorough review, Kinzel (1982) concluded that nitrogen fertiliser concentrations of 100 ppm or more in soil retard the growth of members of the *Ericaceae*, but growth was usually stimulated by nitrogen in lesser amounts. At low concentrations, ammonium is reported to be a stronger growth stimulator than nitrate. While some experiments suggest that nitrate may indeed stimulate growth when the initial content is low, Kinzel (1982) concluded that it is uncertain if the *Ericaceae* can use nitrate as a nitrogen source. Fertilisation except for an initial pulse of nitrogen, is not advantageous for hastening *Calluna* regeneration and the effects of the fertiliser will disappear after a few years, probably due to leaching of the soil.

Elevated foliar nitrogen levels are so strongly associated with the decline of *Calluna* in Europe that current concentrations in important *Calluna* heaths and moors in the UK must be viewed with grave concern (Armstrong, 1991).

1.1.2.2 *Secondary Metabolites*

A major goal of ecologists has been to explain herbivore feeding habits since increased foliar nitrogen levels have been shown to increase the likelihood of heather beetle outbreaks in the Netherlands, which can open up the *Calluna* canopy and promote the invasion of grasses (Brunsting and Heil, 1985; Berdowski and Zeilinga,

1987; Uren, 1992). Food selectivity is thought to be a response to plant metabolite composition, with preferred plant species having the highest concentrations of nutrients or the lowest levels of secondary compounds (Freeland and Janzen, 1974). Orkney voles, *Microtus arvalis orcadensis*, have shown marked selectivity in their choice of food plants, especially utilising herbaceous food low in fibre, which is difficult to digest, and low in total phenolics which exhibit detrimental effects (Hartley, Nelson and Gorman, 1995).

A fundamental problem facing herbivores is the low nitrogen content of plants. Nitrogen is limited in time and space; for example it may be in a form that herbivores cannot digest. Therefore maximising nitrogen assimilation is an important goal for herbivores, and for most insect herbivores increased availability of nitrogen is associated with an increase in the growth rate, survival, or population density (Mattson, 1980). Consequently plant nutritional quality is a significant factor in determining patterns of abundance of sap-feeding insect herbivores, although how this interacts with other host plant characteristics remains to be determined.

The chemical response of different plant species to variations in the availability of nutrients has long been studied (Chapin, 1980). Species associated with nutrient-rich environments tend to be fast growing, allocating resources to growth and reproduction rather than to defensive compounds, whilst species associated with nutrient-poor habitats are slow growing and usually have a higher allocation to secondary metabolites (Coley, Bryant and Chapin, 1985). Compounds such as phenolics and tannins, which have a slow turnover constant and which are also the end products of pathways, are suggested to be the most sensitive to nitrogen availability (Bryant *et al.* 1992), particularly since protein and phenolic biosynthesis share a common precursor (Margna, Margna and Vainjärv, 1989). Furthermore, plants adapted to low nutrient environments have been shown to exhibit smaller chemical responses to changes in soil nutrient levels than plants from richer habitats (Chapin and Shaver, 1985; Bryant *et al.*, 1987).

Given the current concern over the loss of *Calluna*-dominated moorlands to grassland in Europe (Thompson, McDonald and Marden, 1995), the fluctuations in plant chemical composition in response to nutrient inputs has warranted further investigation. The carbon/nutrient balance hypothesis (Bryant, Chapin and Klein, 1983) predicts a decrease in the level of carbon-based secondary compounds in plants in response to fertiliser. This has been shown for many plants (Bryant *et al.*, 1987), but Iason and Hester (1993) found that phenolic and tannin levels in *Calluna* did not decrease in response to increased nutrient supply. In a subsequent study, in a series of fertilised plots, conducted by Hartley and Gardner (1995), *Calluna* shoots had a greater nitrogen content and lower fibre and lignin content with respect to the controls, and the authors concluded that in general, *Calluna* plants exposed to high levels of fertiliser tend to exhibit reduced levels of shoot secondary metabolites, total phenolics and condensed tannins.

The resource quality of litter is an expression of the degree to which its chemical constituents meet the nutritional requirements of soil microorganisms and is therefore a key factor determining the rates of litter and soil organic matter decomposition (Anderson, 1991). Hence the concentration of secondary metabolites and modifiers such as tannins, which can inhibit enzyme or organism activities (Swift, Heal and Anderson, 1979), can significantly influence soil respiration. Heal, Latter and Howson (1978) studied the decomposition rates of organic matter and concluded that within groups of similar resource types decomposing in similar environments, lignin, nitrogen and tannins appear to act as a hierarchical series of controls. In high quality resources, the effect of tannins on the microbial community acts as a rate determinant (Palm and Sanchez, 1991) but as resource quality declines organic matter decomposition depends largely on the initial lignin concentration (Fogel and Cromack, 1977; Berendse, Berg and Bosatta, 1987) or the lignin:N ratio (Aber and Melillo, 1980; Melillo, Aber and Muratore, 1982; Harmon *et al.*, 1986). Therefore, the concentration of secondary metabolites in the litter of *Calluna* plants

exposed to elevated nitrogen inputs may play a significant role in determining carbon dioxide effluxes from heathland soils.

1.1.3 The Soil

Soil profile studies are capable of accurately reflecting the history of the resident vegetation, as there exists a clear relationship between soil properties and vegetation type. The first heathlands were probably composed of grasses and herbs preferring nutrient-rich sandy soils which gradually developed into moder podsols. Later with the expansion of heathland areas, nutrient-poor sandy soils, which progress to form humus podsols, were also incorporated. With the abandonment of ancient management regimes, as part of the old agricultural system, the soil of heathlands became poorer in nutrients until the turn of the century.

In general heathlands have since been restricted to oligotrophic, nutrient-poor, weakly buffered, sandy soils consisting of an acidic substrate, with pH ranging from 3.8 - 4.8 (Gimingham,1989). They tend to be freely drained and podsolic with a layer of raw humus, of approximately 5 centimetres in thickness, at the surface, overlying the mineral material.

Gimingham (1972), pointing out the low nutrient status and high carbon-nutrient ratios of heathland soils, stated that nitrogen fertilisation produces a rapid response in *Calluna*, and that there is a considerable demand for nitrogen in heathlands. Moreover, in many nutrient-poor ecosystems, most of the nutrients that are absorbed by the vegetation originate from the decomposition of the plant litter and humus (Staaf and Berg, 1977; Rosswall and Granhall, 1980). Although heathland soils are mainly acidic by nature, there are often zones where, due to natural causes such as upwellings, or due to disruptive human activities the soil becomes slightly buffered thus less acidic. It is well known that nitrifying bacteria are scarce or absent in moorland soils and most inorganic nitrogen comes from rainfall or from the decomposition of plant and animal residues in the soil. Consequently, ammonium

ions exhibit a definite impact on heathland vegetation by causing soil acidification and nitrogen enrichment since if the soil on which ammonium is deposited is acidic, a strong accumulation of nitrogen occurs in the soil layer, because ammonium is bound much more strongly to the soil absorption complex than nitrate. Ammonia uptake and assimilation from the soil is found in plants that tend to be perennial, and the *Ericaceae* seems to be a family where ammonia is preferentially favoured (Smirnoff, Todd and Stewart, 1984).

Early findings of consistently higher productivity, over a period of seven years, at a moor site in north-east Scotland overlying a relatively base-rich rock in comparison to an adjoining moor over granite (Moss and Miller, 1976) revealed the importance of soil nutrient composition in plant growth. Originally the plant species were restricted to the slightly buffered, less acidic sediments but in the course of time a heterogeneous environment with a rich plant community subsequently developed. The atmospheric deposition of ammonium at these slightly buffered locations was quickly transformed into nitrate by the process of nitrification, causing soil acidification and nutrient enrichment. Subsequent fertilisation experiments *Calluna*-dominated moorlands by Miller (1979) confirmed that soil type and nutrient availability greatly influenced productivity at different sites, since apart from calcium, the availability of nitrogen and phosphorus is a major restraint on herbage production. Similarly, in the case of heathlands, when there is competition between heather species such as *Calluna vulgaris* and grasses such as *Molinia caerulea*, the grasses profit from higher nitrogen levels (Sheikh, 1969). Field experiments have shown that nitrogen enrichment indeed stimulates the development of grasses in heathlands and the biomass of the grasses is not negatively influenced by the acidity of the precipitation (Heil and Bruggink, 1987; Berendse and Elberse, 1989; Aerts *et al.*, 1990; De Smidt and Van Ree, 1991). However, the problem with many field fertilisation experiments is that the high input of atmospheric nitrogen deposition are not taken into account (Roelofs, 1986). Natural levels of ammonium deposition in 'cleaner areas' of the Netherlands have already caused a marked increase in biomass

of grass species (Aerts, 1993). Therefore, it can be concluded that the high atmospheric nitrogen enrichment is a main cause for changes from heather dominated into grass dominated heathlands.

Another ecological effect of nitrogen pollutants involves a disruption of the carbon cycle (Rastetter *et al.*, 1991). Moorlands, typically dominated by heather, *Calluna vulgaris* (L.) Hull, cover about 15 % of the land area in the UK but contain nearly 75 % of the soil organic carbon (Howard *et al.*, 1995). Turnover times for decomposition and mineralisation of organic matter have been found to be relatively slow with high rates of litter accumulation (Swift, Heal and Anderson, 1979). The primary production of upland vegetation is fairly low and these large accumulations of organic matter are a consequence of poor quality litters decomposing slowly in predominantly cold, wet and nutrient-limited environments (Heal, Latter and Howson, 1978). Organic matter generally accumulates on the soil surface under these conditions and little is chemically or physically stabilised in the mineral soil horizons. Consequently, rates of carbon mineralisation and soil respiration can show rapid responses to changes in the climate or resource quality (Anderson, 1991). The Q_{10} temperature responses of soil microbial activity in cool temperate systems are generally higher than those for plant production (Lloyd and Taylor, 1994) and small increases in temperatures can result in significant net losses of unstabilised soil organic matter over one or two growing seasons (Ineson, Coward and Hartwig, 1998). Kirschbaum (1995) has suggested that the low quality of soil organic matter can limit the magnitude of these short-term temperature responses but this can change as a consequence of higher quality litter inputs caused by increased nutrient availability, changes in plant species cover, or a combination of these factors (Hobbie, 1996).

Nitrogen fertilisation experiments are providing the evidence for a strong interaction between atmospheric nitrogen deposition and nitrogen mineralisation processes resulting in increased nitrogen availability (Morecroft, Sellers and Lee, 1994; Fisk

and Schmidt, 1996; Wedin and Tilman, 1996). Vinton and Burke (1995) examined mineralisation processes in plots of undisturbed shortgrass steppe from a 1971 nitrogen addition experiment, wherein 5 g N m^{-2} yr^{-1} ammonium nitrate had been added for three years to treatment plots which subsequently produced a 5 to 10 fold increase in net primary productivity and large changes in species composition (Lauenroth, Dodd and Sims, 1978), to show that 20 years after the experiment had ended, nitrogen mineralisation was still simulated in the treated plots and plants from these plots still had lower C:N tissue ratios. Thus nitrogen enhancement in semi-natural ecosystems potentially has large and long-lasting effects on plant community composition and soil processes, making a prolonged contribution to the total nitrogen economy. These changes in mineralisation suggest that major changes in microbial communities and activities may be occurring in soils subject to high atmospheric nitrogen inputs, but there are few studies that have investigated its importance in terms of interactions with the carbon cycle. Caporn *et al.* (1995 b) demonstrated little effect of nitrogen deposition on mycorrhizal infection in an upland *Calluna* heathland soil but a later study showed large increases in utilisation of substrates by bacteria at the same site, suggesting changes in microbial activity and composition to long-term nitrogen addition. Other workers have shown a decrease in mycorrhizal infection in response to atmospheric nitrogen deposition (Arnolds, 1991). There is considerable need to extend the studies and examine the underlying dynamics of soil microbial processes in response to elevated nitrogen inputs because although vegetation and soil accumulate anthropogenic nitrogen, the soil is the major nitrogen and carbon sink (Nadelhoffer *et al.*, 1994) susceptible to environmental fluctuations. Thus, atmospheric nitrogen deposition through the partial removal of nitrogen limitation on the growth of plants in nutrient-deficient ecosystems may have an appreciable influence on atmospheric carbon dioxide concentrations.

1.1.3.1 *Respiration*

Soils and vegetation of tropical forests represent the largest terrestrial carbon pools in the biosphere and their destruction is a significant contribution to non-industrial

sources of increased CO_2 concentrations in the atmosphere. Soil organic matter contains approximately 1,500 Pg C in the top meter of the soil, which is about 2.5 times more than is contained in terrestrial vegetation (Schlesinger, 1977). An additional 840 Pg C resides between 1 and 3 m depth (Jobbágy and Jackson, 2000). Plant production creates approximately 60 Pg C of organic C each year, while approximately 77 Pg C returns to the atmosphere as soil respiration reflecting the sum of root respiration and decomposition of organic matter (Raich and Potter, 1995). Even a small net change in the flux of carbon from soils could dramatically affect the accumulation of atmospheric CO_2.

Soil respiration is a major flux in the global carbon cycle and is greatly influenced by climatic change. Rates of microbial and root respiration are highly sensitive to soil properties of temperature, moisture and nutrient availability. Any changes in temperature, moisture or nutrient availability due to climate change or the addition of fertilisers could have an important effect on carbon fluxes and rates of carbon sequestration in an ecosystem. Current climate models predict that global mean temperatures are likely to increase by 3 $\pm$ 1.5 °C in this century (Liss and Crane, 1983; Dickson, 1986; Bouwman, 1990). But above 50 degree latitude in the Northern Hemisphere temperatures would increase by a factor of two in summer and three in winter (Manabe and Stouffer, 1980; Wigley, Jones and Kelly, 1980; Anderson, 1991). There is considerable debate over predictions based on global climate models but if current climate-warming trends continue, it will affect species composition of plant and soil communities, the mass chemical composition of above- and belowground litter inputs, litter composition, soil organic matter dynamics and nutrient cycling. The long-term status of the biomes as net carbon sources or sinks under changing climate conditions will thus depend upon the interactions of all these factors determining the balances between production and decomposition.

Two critical questions limit our ability to accurately predict feedbacks between the biosphere and atmosphere CO_2 namely, a) to what extent does carbon storage in

vegetation and soils change with elevated atmospheric nitrogen deposition, and b) to what extent does soil respiration respond to increased temperature.

Lundegårdh (1927) first stated that fertilisation of agricultural crops generally increases soil respiration rates, but few direct comparisons of annual CO_2 efflux from fertilised and unfertilised crops have been investigated. In forests too the impacts of fertilisation on soil respiration rates are ambiguous, though fertilisation with a broad array of mineral nutrients increased soil respiration rates in pine forests studied by Repnevskaya (1967). Soil respiration can increase after the addition of N fertiliser for a range of reasons such as high root respiration rates since energy is required for the reduction of nitrate, incorporation of ammonium into amino acids, and maintenance of high N content in tissue (Johnson, 1983; Morris and Dacey, 1984; Margolis and Waring, 1986; Lambers, 1987; Vessey and Layzell, 1987; Goodwin and Mercer, 1990; Ryan, 1991), elevated organic matter decomposition due to increased microbial biomass (Anderson, 1991) and raised plant productivity resulting in increased root exudates (Waring and Schlesinger, 1985). The net result of these different effects may vary among sites, soils and vegetation types, and no clear patterns are apparent in the available data. Short- and long-term effects of fertilisation may also differ as vegetation adapts to the new nutrient regime (Chapin, Shaver and Kedrowski, 1986). Therefore, the potential impacts of fertilisation on soil respiration rates are poorly documented and few studies have investigated heathlands.

Understanding and predicting the response of soil carbon in specific ecosystems to changes in global temperature and pollution is critical, particularly since increased release of respired carbon dioxide to the atmosphere has the potential to exacerbate global warming (Woodwell *et al.*, 1978; Jenkinson, Adams and Wild, 1991; Schimel *et al.*, 1994; Kirschbaum, 1995). The present study addresses this question in Chapter 5.

1.1.4 Conservation and Management

Awareness regarding the conservation of *Calluna*-dominated heathlands in lowland Europe has steadily grown with time. The two main objectives for the conservation of lowland heaths are a) to prevent succession, in order to maintain the heathland free of invasion species such as birch and bracken, and b) to encourage regeneration of *Calluna* so that all phases of the *Calluna* cycle are present as a mosaic in the same area (Marrs, 1987 a).

Various management measures, in conjunction with associated land uses, are aimed to control the species composition of heathlands as part of conservation and restoration programs (De Smidt, 1983). Regulated species performance can be achieved by decreasing nitrogen availability and enhancing the establishment of specific plants. The practices of mowing, burning and sod cutting ensure a periodical removal of nutrients from the ecosystem, besides creating a diversity of habitats ranging from bare ground to sheltered heather niches. Data over a period of 25 years also showed the effects of abiotic and biotic stress and disturbance factors such as summer drought, frost, heather beetle infestations and sheep grazing (De Smidt, 1977; Berendse, 1985).

Nutrient balances of the most important types of heathland farming systems were reconstructed and summarised by Gimingham and De Smidt (1983) in order to reveal that during the past few decades, the equilibrium between the input and output of nutrients has been lost by increased atmospheric nitrogen deposition and a change in management priorities. Increasingly, ideas on the management of heathlands have changed since the establishment of heathland reserves began (Gimingham, 1992). Currently pollution control policy on a European scale is being focused around the concept of the critical load. This approach can support an effects-based policy for emission control and is considered by many to be a scientific method of linking emission reductions on both national and international levels to environmental benefits. Critical loads set on the basis of dynamic, simulation models which

incorporates information from field observations and experimental studies have proposed a critical load of 15 - 20 kg N ha^{-1} yr^{-1} for dry heathland (Van Breemen *et al.*, 1982; Bobbink, Heil and Raessen, 1992; Heil and Bobbink, 1993).

It must be emphasised, however, that the long term conservation of heathlands require a substantial reduction of the current, unacceptably high atmospheric nitrogen deposition levels in addition to the short term corrective measures. The success of a management strategy strongly depends on setting management goals, which in order to achieve, ought to adopt a technically feasible, economically viable and environmentally sustainable integrated approach.

1.2 AIMS OF THE STUDY

This study aimed to determine the degree to which enhanced nitrogen deposition in *Calluna*-dominated heathland ecosystems can

i) alter plant physiological responses in terms of a) shoot growth, b) tissue nitrogen, c) tissue carbon and d) secondary phenolic metabolites, which serve as defence compounds,

ii) affect the response of soil respiration to environmental parameters by disturbing acclimatised soil microbial populations,

iii) influence the relationship between soil carbon fluxes and soil microbial populations, and

iv) change soil mineral nitrogen availability to the plants.

1.3 STRUCTURE OF THE THESIS

This thesis investigates the effects of atmospheric nitrogen deposition on heathland ecosystems. There are seven chapters.

Chapter 1: Introduces the subject of heathland ecology and provides a review of literature in order to summarise our knowledge of heathlands in relation to their changing environment. A database of previous work in the field was created with an aim to compare and contrast the results obtained by different studies, compile the conclusions into a coherent structure and finally, map changing trends with the passage of time, so as to gain a better understanding of nutrient cycling between atmospheric processes, heathland communities and soil properties in order to identify areas of weakness, which would benefit from further research.

Chapter 2: This chapter describes an experimental study investigating the effects of nitrogen deposition on *Calluna vulgaris* (L.) Hull grown in open-top chambers providing a range of fertiliser input rates, spanning across extreme values. Plant physiological responses, within a controlled system simulating field conditions, were carefully monitored to determine the degree to which environmental changes are reflected in heather, confirm reported trends and validate proposed critical loads with regards dry heathlands.

Chapter 3: The field site and experimental design is described. A new, simple methodology is developed and operated to accurately measure soil respiration under controlled laboratory conditions using small soil microcosms with an 18 channel, continuous flow, multipoint, gas analysing unit that was specially built for the study. Data from four supporting experiments are presented to establish the best working technique to measure soil respiration.

Chapter 4: Soil microbial biomass was monitored during the period of study to gain an insight into the soil microbial community governing soil carbon fluxes. The fumigation-extraction technique was employed to determine the relationships between biomass-C, biomass-N, and microbial C:N ratio for heathland soils. The respiration profile in soils following the addition of a specific C or N nutrient can demonstrate if that factor increases respiration with or without simulating microbial growth. This approach was developed in an attempt is made to link the compartments of soil carbon, namely microbial biomass and respiration, in conjunction with the climatic conditions and elevated nitrogen inputs.

Chapter 5: The annual seasonal patterns of soil carbon dioxide effluxes and environmental parameters were measured to determine the effect of elevated nitrogen inputs on the response of soil respiration to soil temperature, moisture, pH, organic matter and microbial biomass. The modelling of respiration in soil is critically discussed and the ability of the three most commonly used models to accurately describe the temperature dependence of soil respiration was tested.

Chapter 6: An unconventional, though simple, growth measurement technique is described whereby growth rings of mature *Calluna vulagris* (L.) Hull can be studied by staining shoot cross-sections with blue ink. The effect of ammonium nitrate fertiliser on the carbon:nitrogen ratio of shoot tissues is also examined.

Chapter 7: A summary of the main conclusions drawn from all the experiments so as to bring together the entire body of work and highlight the main findings of the study.

2. PILOT STUDY – RESPONSES OF *CALLUNA VULGARIS* TO NITROGEN DEPOSITION

2.1 INTRODUCTION

The heathlands of north-west Europe, dominated by the species *Calluna vulgaris* (L.) Hull, have for centuries determined the physiognomy of the landscape (Gimingham, 1972). In recent times however, many countries have recorded an alarming decline in the area of both lowland dry heath and upland heather moorland (Farrell, 1989; Webb 1990; Aerts, 1993 b). The loss has been characterised by a transition from heathlands to grasslands with the invasion of non-native grass species, such as *Deschampsia flexuosa* (L.) Trin. and *Molinia caerulea* (L.) Moench and this observation has been investigated in field and laboratory experiments by several authors (Heil and Diemont, 1983; Berendse and Aerts, 1984; Heil and Bruggink, 1987; Aerts, 1990; Aerts *et al.*, 1990; Bobbink, 1991).

Modern research has linked the impoverishment of heathlands to large-scale environmental disturbances; the key threatening factors being nutrient enrichment due to high loads of atmospheric pollutants and the discontinuation of traditional heather management practices (Gimingham, 1992). The three environmentally most damaging air pollutants across Europe are sulphur dioxide, nitrogen oxides and ammonia. The main source of sulphur dioxide is industry while nitrogen oxides originate mainly from the burning of fossil fuels and ammonia emissions are elevated in areas with high densities of animal husbandry (ApSimon, Kruse and Bell, 1987; Sutton, Fowler and Moncrieff, 1993; Ashenden and Edge, 1995).

Studies have shown that environmental acidification and eutrophication as a result of sulphur and nitrogen deposition have led to a decline in species abundance in

nutrient-poor aquatic and terrestrial ecosystems (Buijsman, Maas and Asman, 1987; Hargreaves *et al.*, 1992). Documented evidence has proved that elevated tissue nitrogen levels in heathland plant species can dramatically change plant physiological and soil chemical processes that lead to greater stress sensitivity, alter growth rates, suppress regenerative capacities, promote gap formations within the closed vegetation canopy and increase the probability of devastating pest outbreaks (Brunsting and Heil, 1985; Marrs, 1986; Berdowski and Zelinga, 1987; Van der Eerden *et al.*, 1991; Caporn, Risager and Lee, 1994). Increases in *Calluna* foliar nitrogen content resulting from experimental nitrogen fertiliser applications in the range of 40 - 200 kg ha^{-1} yr^{-1} have been periodically reported by workers in the UK (Lee, Caporn and Read, 1992; Caporn *et al.*, 1995 a, b) and the Netherlands (Dueck *et al.*, 1991; Van der Eerden *et al.*, 1990; 1991).

Species associated with nitrogen-rich habitats tend to allocate vital resources to growth and reproduction rather than to defensive compounds, whilst in species residing in nutrient-poor niches, carbon-based secondary metabolites accumulate as growth is limited by nutrient deficiency (Coley, Bryant and Chapin, 1985). In 1983, Bryant, Chapin and Klein put forth the carbon/nutrient balance hypothesis which predicts a decrease in the levels of carbon-based secondary compounds in plants upon fertiliser treatment, which would cause a direct effect on pest resistivity. The theory was shown to apply to many plant species by Bryant *et al.* (1987); however, Iason and Hester (1993) found that phenolic and tannin concentrations did not decrease in *Calluna* in response to elevated nutrient supply. Similar results were found in a study conducted by Hartley and Gardner (1995), lending support to the idea that though inputs of nitrogen greatly influence the nutritional status of plants, species surviving in nutrient-low environments, such as *Calluna vulgaris* may exhibit smaller fluctuations in chemical composition when subject to raised nutrient inputs, as opposed to species adapted to nutrient-rich environments. In 1994 the United Nations Economic Commission for Europe (UN-ECE, 1994) proposed a critical load of 15 - 20 kg N ha^{-1} yr^{-1} based on a computer simulation model for dry heaths in the

Netherlands which incorporated only limited data from field and experimental observations.

The impact of large-scale atmospheric pollution on the complex biotic and abiotic interactions of heathlands is an intriguing field of research providing an insight into species dynamics. This study aimed to investigate some aspects of the response of *Calluna vulgaris* (L.) Hull, maintained in open-top chambers, to nitrogen deposition. Much of the published experimental work undertaken to propose critical values at which a conversion from ericaceous to gramineous dominance would be imminent, relates to experimental additions at rates much higher than the proposed critical load. This experiment involved the regular application of six acid mist treatments of 2, 20, 40, 60, 80, 100 kg N ha^{-1} yr^{-1} to lowland dry heath monoliths in a controlled environment. Ammonium nitrate was employed as the fertiliser in view of the co-deposition of nitrogen oxides and ammonia frequently observed in the field. By investigating soil and plant physiological responses in a controlled experimental system simulating field conditions, it was hoped to determine the degree to which present day environmental changes impact upon *Calluna*-dominated ecosystems, and validate proposed critical loads of N with regards to heathlands.

2.2 STUDY SITE AND METHODS

To investigate the effect of increased atmospheric pollutants on vegetation dynamics an experiment was conducted in which the supply of nitrogen was varied to heathland monoliths maintained in a series of open-top chambers (Fowler *et al.*, 1989; Leith *et al.*, 1989) at the Centre for Ecology and Hydrology (CEH), Bush Estate, Edinburgh (Figure 2.1). The work described in this chapter was done in 1997, and formed part of an experiment which had been designed and established in 1995 by colleagues at CEH (formerly the Institute of Terrestrial Ecology, ITE).

Figure 2.1 Open-Top Chamber site, Centre for Ecology and Hydrology, Edinburgh.

2.2.1 Treatment

2.2.1.1 *Chamber Design*

Six octagonal open-top chambers, glazed with 3 mm horticultural glass set in aluminium frames, with a side length 3.0 m, floor area 7.0 m^2 and height 2.3 m to the base of a frustum, were used. Air was supplied to each chamber by a pump unit at a rate of 40 m^3 min^{-1} to ensure two air changes per minute. The air was filtered through activated charcoal plates, to remove ozone, nitrogen dioxide, sulphur dioxide, and subsequently injected from a polyethylene manifold 1.5 m above the plants to provide uniform air distribution at the chamber floor. Rainfall was excluded from the system by fixing a polyethylene ceiling inside the chamber below the frustum, with a central outlet drain to divert the rainwater out of the chamber. The environment within the open-top chamber was inevitably modified relative to ambient conditions by the enclosure and air delivery system. The framework intercepted approximately 15 % of the short-wave solar radiation, and modified the net long-wave energy exchange above the plant canopies. The air blower altered the microenvironment by

raising the air temperature inside the chambers by 0.5 – 2.0 ºC above the ambient temperature and reducing the relative humidity by 5 - 13 %.

2.2.1.2 *Acid mist application*

Six simulated acid mist treatments, at a rate of nitrogen supply of 2, 20, 40, 60, 80 and 100 kg N ha^{-1} yr^{-1}, were applied using serial dilutions of a stock solution of ammonium nitrate. The treatment commenced in July 1995. NH_4^+ and NO_3^- were applied in a 1:1 ratio, which is consistent with the average ratio of these ions in UK precipitation (RGAR, 1997). The solutions were simultaneously pumped to the chambers from 25 litre polypropylene bottles, via 6 mm diameter horticultural polyethylene tubing, using compressed air, at an application rate of 3 mm h^{-1}. Droplets with a mean mass diameter of 90 μm and a mean diameter of 40 μm were generated using a spinning disc, rotating at 5000 rpm (Micromax 84, CDA-Micron Sprayers Ltd., UK) mounted centrally 1.5 m above the plants. The treatments were applied twice weekly to give 2 l m^{-2} ground per treatment which is equivalent to 2 mm precipitation. This was just sufficient to exceed the water-holding capacity of the young plants, so that water began to drip from foliage after misting. The distribution and deposition of droplets within the chamber was uniform over the area occupied by the plants and the material used for the spray delivery systems did not modify the composition of the spray droplets. To avoid excessive rates of evaporation from foliage and scorch, the treatments were applied early in the morning.

2.2.2 Sampling Procedure

The monoliths were collected on 31 May 1995 from an upland *Calluna*-dominated moorland site at an altitude of 450 m above sea level near Glossop in Derbyshire (53° 26′ N, 1° 56′ W). The site receives a wet N deposition of approximately 40 kg N ha^{-1} yr^{-1}. The soil profile revealed a peat depth of 46 cm with a pH of 4.04. Eighteen monoliths were cut with a spade and carefully transferred to 16 litre buckets with dimensions 30 cm diameter × 45 cm depth. All the monoliths were transported back

to CEH, Edinburgh and randomly established in six open-top chambers (three per chamber) by the end of July 1995 and subsequently fertiliser treatment commenced.

In 1997, 30 young shoots for each treatment, 10 per monolith, were randomly selected and marked with white latex paint at the beginning of the growing season and growth was periodically measured via length increments. Shoot samples for chemical analysis were harvested in three replicates on a fortnightly basis from May to July 1997. The top 2.5 cm of growing shoot tips were excised and transported to the laboratory in dark, sealed polythene bags. At the same time, soils were collected at a depth of 5 cm from the soil surface and sampled at the commencement of the study period.

2.2.3 Chemical Analysis

Shoot and soil samples were oven dried at 60 °C for 16 hours and then the replicates of each treatment were individually ground in a ball-mill. A further three sub-samples for each replicate were taken for chemical analysis. Total tissue nitrogen and carbon were estimated by mass spectrometry (Carlo Erba 1400 Automatic Nitrogen Analyser interfaced to a VG Isogas Micromass 622 Triple Collector Mass Spectrometer, Milan, Italy). Total phenolics were determined by the Prussian Blue Method (Price and Butler, 1977; Waterman and Mole, 1994). Results were expressed as percent dry matter. Analyses were validated by inclusion of a standard reference material and concentrations obtained for the replicates were always within 5 % of the certified value.

2.2.4 Statistical Analysis

The effects of fertiliser on growth, the concentration of nutrients and secondary compounds in *Calluna vulgaris* were assessed using regression analysis and one-way factorial analysis of variance (Fowler and Cohen, 1990). Where significant treatment effects were found on shoot growth ($P < 0.001$) histograms were plotted for the maximum lengths of seedlings (Sokal and Rohlf, 1981).

2.3 RESULTS

2.3.1 Shoot Growth

Growth of new *Calluna* shoots increased with higher nitrogen inputs and time. Differences in shoot extension between the higher nitrogen applications and the lowest control treatment were statistically significant (Figures 2.2 and 2.3).

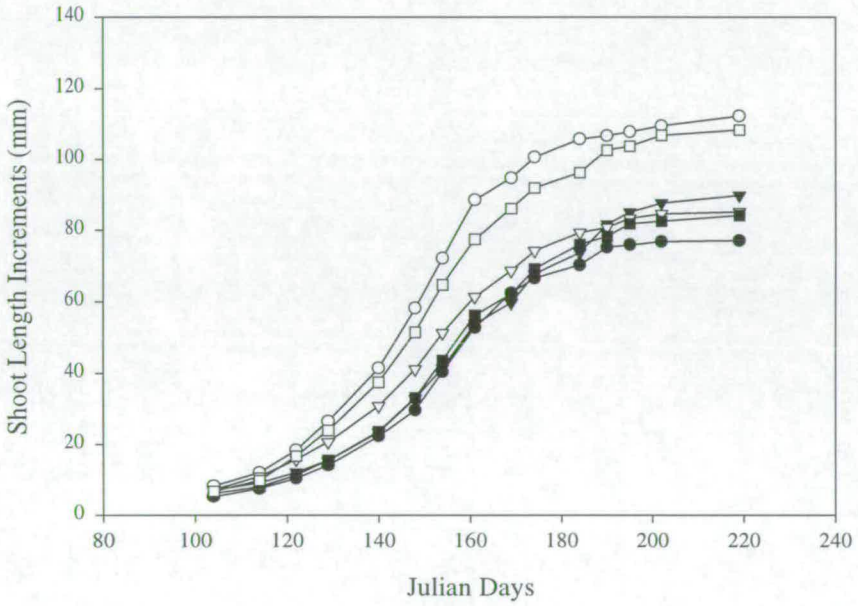


Figure 2.2 Mean growth curves plotted for *Calluna* shoot exposed to fertiliser treatments equivalent to 2 (•), 20 (o), 40 (▾), 60 (▽), 80 (■) and 100 ( ) kg N ha^{-1} yr^{-1} within open-top chambers.

The growth curves showed that the shoots exposed to a nitrogen input of 20 kg N ha^{-1} yr^{-1} grew most rapidly, attaining maximum annual growth (Figure 2.3). Shoots increased by more than 100 mm in length over a period of three months for 20 kg N ha^{-1} yr^{-1} and 100 kg N ha^{-1} yr^{-1} while for the remaining treatments the asymptotic growth ranged between 76.7 and 87.6 mm.

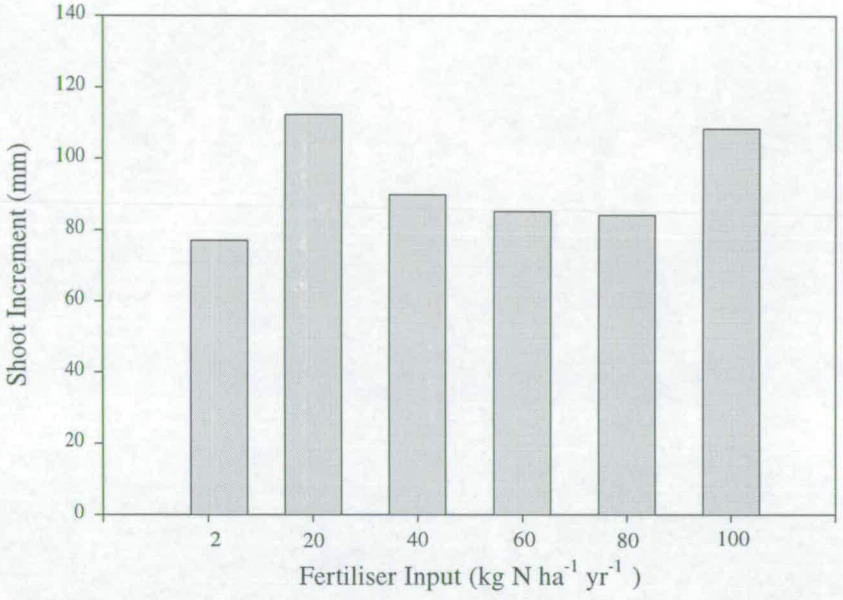


Figure 2.3 Maximum *Calluna* shoot length increments (mean ± S.E., n = 18) for each fertiliser treatment. The differences between the mean values were statistically significant ($P < 0.05$). Tukey's critical difference, $T = 4.11$, for comparing the means, based on ANOVA.

2.3.2 Plant and Soil Nitrogen

Results of one-way analysis of variance to test the effect of treatment on total tissue nitrogen concentration showed that there was a statistically significant ($r^2 = 0.73$, $P < 0.001$) positive correlation between percent nitrogen expressed as a dry weight and fertilisation (Figure 2.4). In May the percent tissue nitrogen concentration increased from a mean value of 1.37 % to 2.08 % for 2 kg N ha^{-1} yr^{-1} and 100 kg N ha^{-1} yr^{-1} input levels respectively. During the growing season the mean tissue nitrogen concentration was 1.53 %. The upward trend was sustained for the subsequent two months of the study period through June ($r^2 = 0.89$, $P < 0.001$) and July ($r^2 = 0.95$, $P < 0.001$). However, with the commencement of the *Calluna* flowering season, the relationship was no longer significant and the average dropped to 1.25 %.

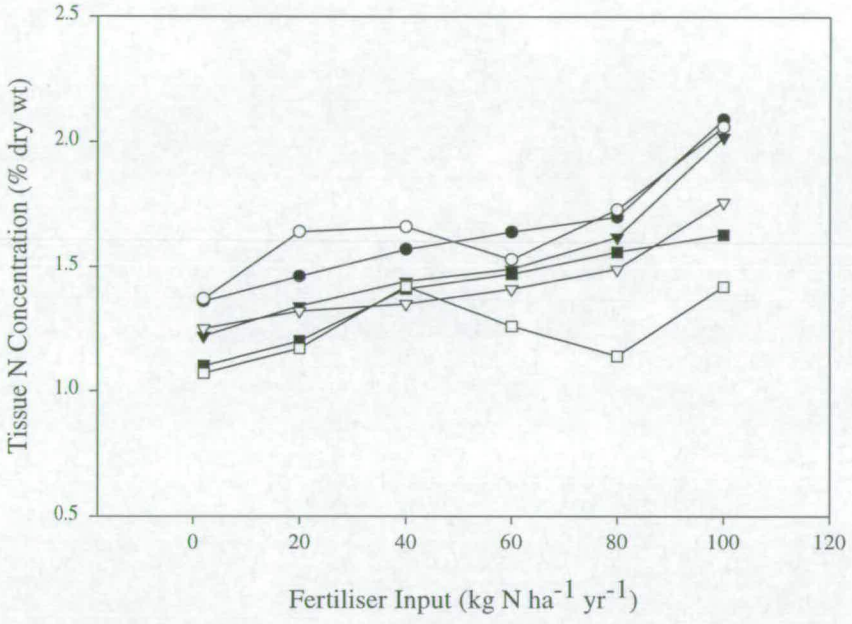


Figure 2.4 Relationship between tissue nitrogen concentration (% dry weight) of *Calluna vulgaris* (L.) Hull terminal shoots and fertiliser input levels for samples collected on 9th May 1997 (•), 28th May 1997 (o), 10th June 1997 (▾), 23rd June 1997 (▿), 8th July 1997 (■), and 21 July 1997 ( ). Points represent mean tissue N concentration per treatment and statistical significance of this relationship is $r^2 = 0.73$, $P < 0.001$.

Soil analysis revealed that there was a positive relationship between total soil nitrogen concentration and ammonium nitrate fertiliser treatment levels. A steady statistically significant increase ($r^2 = 0.98$, $P < 0.001$) from a mean value of 1.63 % for 2 kg N ha^{-1} yr^{-1} to 1.72 % for 100 kg N ha^{-1} yr^{-1} was observed during the period of study, as shown in Figure 2.5.

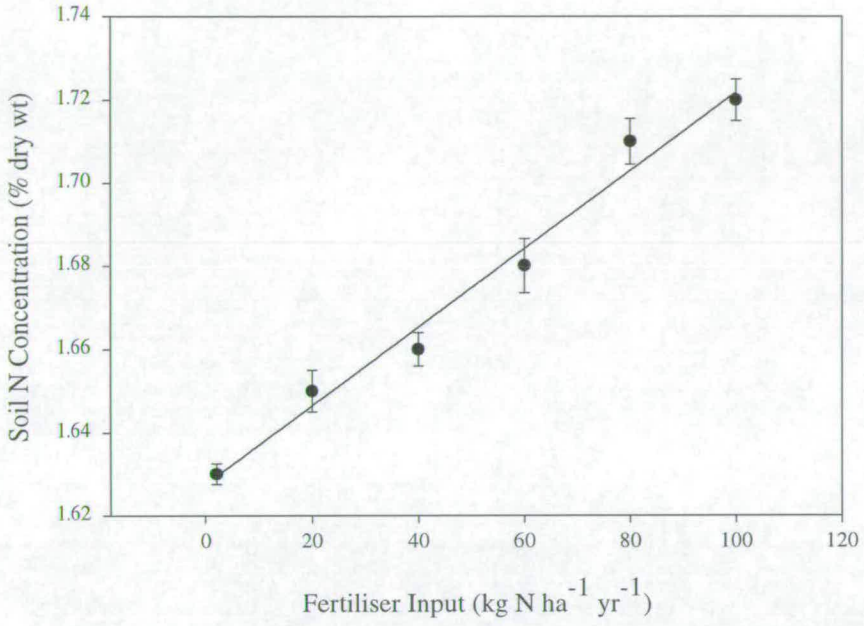


Figure 2.5 Relationship between soil nitrogen concentration (% dry weight) and ammonium nitrate fertiliser input levels equivalent to 2, 20, 40, 60, 80 and 100 kg N ha^{-1} yr^{-1}. Points represent tissue N concentration (mean ± S.E., $n = 18$) during the study period per treatment and the fitted line shows the least squares regression line. The significance of this relationship is $r^2 = 0.98$, $P < 0.001$.

2.3.3 Tissue and Soil Carbon

Results of the carbon estimation study reveal that the total tissue carbon concentration for *Calluna* shoots values were not significantly ($r^2 = 0.28$, $P < 0.05$) affected by elevated nutrient supply during the growing season (Figure 2.6).

Soil studies for total carbon concentration also recorded no rise or fall in mean values during the period of study ($r^2 = 0.18$, $P < 0.05$; figure not shown).

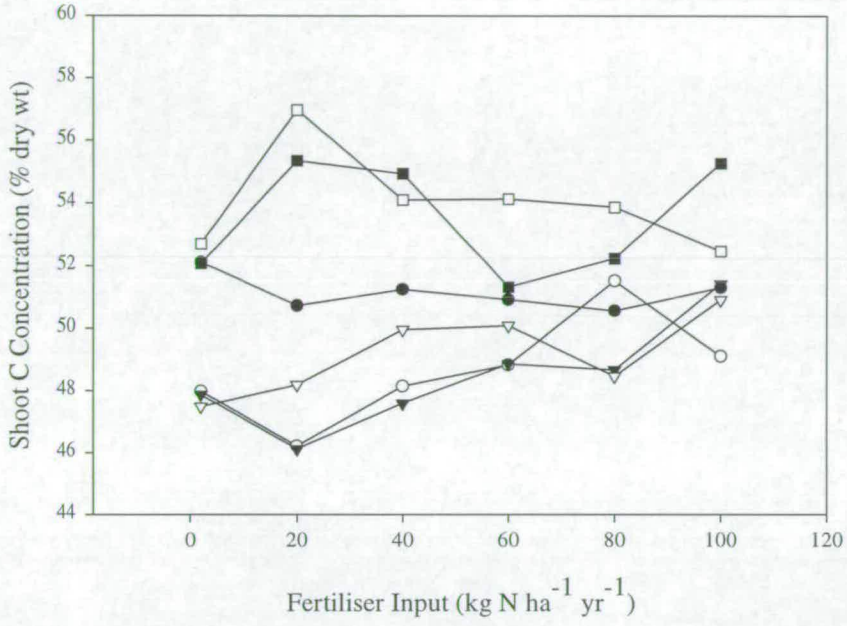


Figure 2.6 Relationship between tissue carbon concentration (% dry weight) of *Calluna vulgaris* (L.) Hull terminal shoots and fertiliser input levels for samples collected on 9th May 1997 (•), 28th May 1997 (o), 10th June 1997 (▾), 23rd June 1997 (▽), 8th July 1997 (■), and 21 July 1997 ( ). Points represent mean tissue C concentration per treatment. The statistical significance of this relationship is $r^2 = 0.28$, $P < 0.05$.

Ammonium nitrate application had a strong effect on the phenolic secondary compounds of *Calluna* shoots (Figure 2.7). The fertilised monoliths revealed a significant (r^2 = 0.91, P < 0.001) decrease in the levels of total phenolic concentration with increasing nitrogen inputs during active growth. Prior to the growing season there were no statistically significant interaction effects between the factors, though a trend is observed. During the growing season a sharp decline is observed from a peak value of 14.9 % in May for an input of 2 kg N ha^{-1} yr^{-1} to 12.2 % in July for 100 kg N ha^{-1} yr^{-1}. At the end of the growing season, the phenolic levels once again rise correspondingly through the fertiliser gradient.

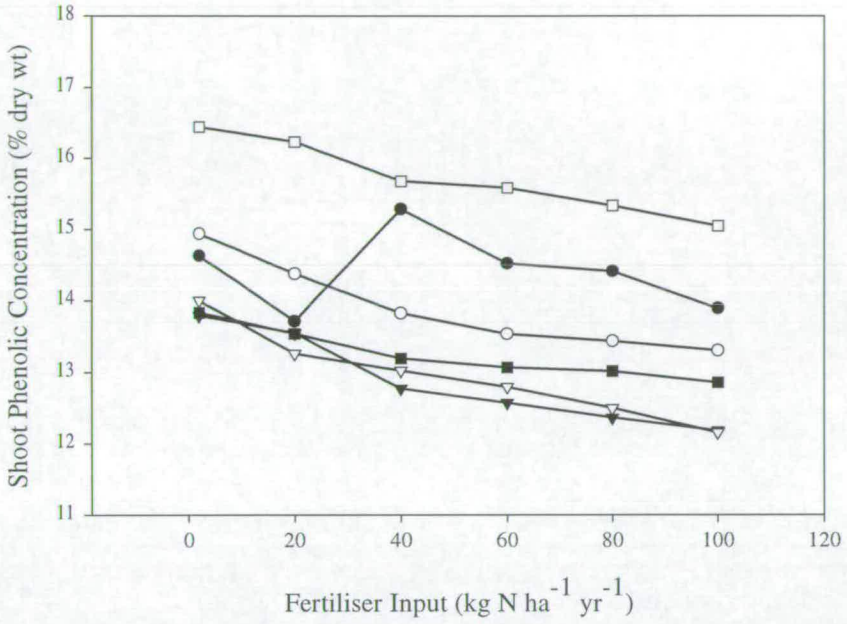


Figure 2.7 Relationship between tissue phenolic concentration (% dry weight) of *Calluna* terminal shoots and fertiliser input levels for samples collected on 9th May 1997 (•), 28th May 1997 (o), 10th June 1997 (▾), 23rd June 1997 (▽), 8th July 1997 (■), and 21 July 1997 ( ). Points represent mean tissue phenols concentration per treatment. The significance of this relationship is $r^2 = 0.91$, $P < 0.001$.

2.4 DISCUSSION

The experimental design allowed for a study of the response of *Calluna vulgaris* (L.) Hull to fertilisation treatments in a controlled environment. Field conditions were simulated within the constraints of the chamber construction with the possible rise in air temperature and fall in relative humidity not being viewed as major contributory factors when considered in relation to the annual seasonal variations. Moreover, there was no evidence of any visible injury to *Calluna* plants, such as leaf browning, in response to the high concentrations of ammonium nitrate during the period of study as reported by other researchers (Van der Eerden *et al.*, 1990).

2.4.1 Growth

Under natural conditions, the growth rate of plants is largely regulated by the nutrient flux density from the soil to the roots, that is, the amounts of nutrients made available for plant uptake through mineralisation per unit time and unit area (Ingestad, Aronsson and Ågren, 1981). Therefore, growth is markedly suppressed within an ecosystem where the natural fertility is too low to support the maximum biomass potential, as observed within nutrient-poor heathlands. However, plants readily respond to increased nutrient availability with rapid increases in growth rate, as first documented by long-term forest fertilisation experiments with Scots pine and Norway spruce in Sweden (Tamm, 1961; Nohrstedt *et al.*, 1989; Linder, 1990) and Ecalyptus stands in Portugal (Pereira *et al.*, 1989). Similar positive effects on growth of *Calluna* by the application of ammonium sulphate in the field have also been periodically reported (Heil and Bruggink, 1987; Aerts, 1989; Caporn *et al.*, 1995 a, b; Uren *et al.*, 1997). The importance of nutrient availability during active growth is reflected in the response of *Calluna vulgaris* (L.) Hull to elevated nitrogen levels with experiments undertaken by other authors suggesting that ammonium uptake leading to a stimulation in growth is predominantly via the shoots (Raven, 1988). Isotopic studies conducted by Van der Eerden *et al.* (1990) with ^{15}N-labelled ammonium sulphate have illustrated shoot dominance over root uptake and canopy adsorption studies in the field revealed that 45 – 90 % net throughflow of wet deposited ammonium was directly assimilated upon uptake by *Calluna* shoots.

In this experiment, the growth response of *Calluna vulgaris* (L.) Hull shoots with increasing fertiliser inputs was weak and not easily detectable between low and high treatment levels. The length increment histogram revealed a statistically significant increase in length increments for shoots exposed to a fertilisation rate of 20 kg N ha^{-1} yr^{-1}, which is the United Nations Economic Commission for Europe proposed critical load, in comparison to 2 kg N ha^{-1} yr^{-1} treatment level. Thereafter the nitrogen effect appears to decline with higher ammonium nitrate inputs of 40, 60 and 80 kg N ha^{-1} yr^{-1} but then unexpectedly the shoots exposed 100 kg N ha^{-1} yr^{-1} exhibit a sharp

increase in length so that no clear trend emerges. A number of possible explanations may exist as to why neither a linear trend nor an 'optimum' response curve was noted.

Firstly, ammonia-nitrogen, which can be taken up by shoots, is also made available to the plants by the root system upon deposition on the soil surface through nitrification or ammonium accumulation (Cowling and Lockyer, 1981; Dueck, Dil and Pasman, 1987). The mineralisation-nitrification process is driven by the soil microbial biomass and activity, which in turn are affected, either positively or negatively, by the fertiliser treatment levels (Anderson, 1991; Arnold, 1991; Nadelhoffer *et al.*, 1994; Caporn *et al.*, 1995 b). The results suggest that high levels of nitrogen inputs may have decreased microbial activity and had deleterious effect on microbial biomass as a result of which nitrogen availability was reduced above the critical level and growth did not reach the maximum attainable rate. A number of field studies have recorded a significant decline in microbial activity and biomass after high additions of urea and ammonium nitrate to experimental plots (Kowalenko, Ivarson and Cameron, 1978). In a fertilisation experiment, Bååth, Lundgren and Söderström (1981) found that microbial biomass and soil respiration rate decreased by 81 – 91 % at all sites within three months after application of 150 kg NH_4NO_3 - N ha^{-1} to different coniferous forest podsols. High concentrations of nitrogenous compounds to a small volume of soil can have a direct inhibiting effect on microbial processes as demonstrated for ligninolytic enzyme production (Keyser, Kirk and Zeikus, 1978).

Secondly, soil carbon may have also become less available owing to the condensation of nutrient-rich compounds (Haider, Martin and Filip, 1975; Witter, Mårtensson and Garcia, 1993); there might have been a partial sterilisation effect owing to a toxic potential in the soil solution (Leuken, Hutchinson and Paul, 1965) or an increase in soil acidity could impose a stress factor on microbial biomass thereby reducing yield and efficiency of the biomass (Killham, 1985). Other nutrient

limitations within the closed microcosm system may have set in as well. Optimal biomass production in a given climate is achieved when all essential mineral elements are available at a time and rate suitably adjusted to soil mineralisation rates and nutrient demand of the crop (Ingestad, 1987). Therefore, plant growth could be inhibited by limiting factors such as phosphorus, even in the presence of excess nitrogen, as emphasised by the results of a series of long-term experiments conducted by Van der Eerden *et al.* (1990; 1991). Furthermore, prolonged inputs of high nitrogen can adversely affect root growth (Vessey and Layzell 1987; Wallander and Nylund, 1990; Lu *et al.*, 1998) and that too can stunt plant growth.

Another possibility is that the 100 kg N ha^{-1} yr^{-1} treatment chamber was a 'rogue' chamber in the series of six OTC's. It was economically not feasible to replicate all the chambers hence the experimental set-up could not accommodate the existence of an erroneous chamber. The monoliths within the chamber could have received comparatively more sunlight, which could have increased photosynthesis and consequently shoot length.

This study highlights the need to fine-tune the critical load concept for nitrogen inputs to heathlands, which must be set on a site-specific basis because a number of inter-related environmental factors come into play that may determine the effects of excessive nitrogen availability to *Calluna*-dominated heathlands. Site-specific critical loads for specific species would prove to be more accurate and effective in preventing the nutrient enrichment of terrestrial ecosystems that alter the balance of species composition as observed in the conversion of heathlands to grasslands.

2.4.2 Tissue and Soil Nitrogen

The relationship between atmospheric nitrogen deposition and tissue nitrogen concentration of vegetation depends on the degree of reliance of that species on atmospheric inputs In the case of *Calluna*, which grows in acidic upland soils, rainfall is an important source of nutrients, especially nitrogen, and during the last

two decades the atmospheric deposition of fixed nitrogen has increased throughout Europe from 2 - 6 kg N ha^{-1} yr^{-1} to 15 - 60 kg N ha^{-1} yr^{-1} (Pitcairn, Fowler and Grace, 1995). Field experiments have been conducted across the continent to demonstrate the effects of such a rising trend and have recorded significant increases in the foliar nitrogen concentrations resulting from fertiliser applications, ranging from 40 kg N ha^{-1} yr^{-1} in the UK to 200 kg N ha^{-1} yr^{-1} in the Netherlands (Brunsting and Heil, 1985; Aerts, 1989, Van der Eerden *et al.*, 1990; Lee, Caporn and Read, 1992; Pitcairn, Fowler and Grace, 1995).

In this experiment ammonium nitrate has a significant effect on the tissue nitrogen percentage which rises in accordance with increasing fertiliser inputs. A clear upward trend in nitrogen levels is seen during the growing season with the mean tissue nitrogen value rising by 0.61 % in a period of three months, indicating a considerable seasonal variation with time. Recently published figures for tissue N concentrations of *Calluna* range from a mean value of 1.75 % and 1.90 % in The Netherlands in the 1980's and 1990's respectively (Pitcairn, Fowler and Grace, 1995). The figures indicate a rise in atmospheric pollution in Europe with time, and if foliar nitrogen concentrations reflect atmospheric nitrogen levels as suggested by Pitcairn, Fowler and Grace (1995), then the overall mean value of 1.53 % recorded in this study indicate that nitrogen deposition is still lower in UK in comparison to The Netherlands, which records the highest rates of nitrogen depositions (Heil and Aerts, 1993). Therefore, atmospheric nitrogen inputs are capable of stimulating plant growth within nutrient-deficient ecosystems and the degree of pollution is successfully reflected in plant tissue nutrient levels.

The significant increases in soil nitrogen with increasing fertiliser inputs suggest that a fraction of the applied N was taken up directly by the shoots while the balance remained in the soil. Calculations revealed that approximately half of the fertiliser N was found in the soil, and with no concomitant increase in soil carbon being detected, the results support the theory that microbial biomass did not increase with

increasing ammonium nitrate additions. Hence, soil nitrogen availability to the plant via microbial activity did not increase with an increase of N additions as a result of which the growth response was weak. The microbial population too may not have been able to utilise the applied N due to a number limitations within the closed system, as also noted by Söderström, Bååth and Lundgren (1983).

2.4.3 Tissue and Soil Carbon

The carbon-nutrient balance hypothesis predicts that in nutrient-poor environments, when plant growth is limited by nutrient deficiency, carbon-based secondary compounds accumulate within tissues (Bryant, Chapin and Klein, 1983). Defence compounds, determining susceptibility to pests and insect attacks, such as phenolics and tannins, which have a slow turnover and are also the end-products of pathways, are most sensitive to nitrogen availability (Bryant *et al.*, 1992), especially since protein and phenolic biosynthesis share a common precursor (Margna, Margna and Vainjarv, 1989). A reduction in the concentration of carbon-based secondary metabolites in response to fertiliser treatment has been observed experimentally for many plants (Bryant *et al.*, 1987) but poorly reported for *Calluna*.

The levels of total secondary metabolites in *Calluna vulgaris* (L.) Hull shoot tissue responded sharply to the treatments in this study as stated by the carbon-nutrient balance theory. Prior to the growing season the level of phenolics does not decline with rising fertiliser inputs. Previous studies by Iason and Hester (1993) and Hartley and Gardner (1995) during the non-growing months also failed to demonstrate a decline in phenolics when treated with fertilisers. The observed statistically significant decreases in the concentration of phenolics, during the growing season, are in agreement with previous laboratory experiments. The total carbon concentration of heather shoots was less drastically affected by the additions of ammonium nitrate and no fertiliser effects were observed. The problem with detecting carbon differences in plant material with time is that tissue carbon concentrations are sensitive to changes in the ratio of leaf:wood with tissue

maturation (Poorter and Bergkotte, 1992), and in *Calluna* shoots this is naturally quite variable. However, this response may suggest that higher nutrient availability alters the allocation of carbon to defence compounds, while maintaining the crucial level of total carbon percent within tissues to promote the active growth of new shoots. Chapin and Shaver (1985) found that the plants of low nutrient habitats, as *Calluna*, revealed smaller changes in chemical composition in response to fertiliser addition in comparison to plants which are dependent on frequent nutrient inputs. Therefore it may be stated that the carbon-nutrient hypothesis holds true during the growing season for the ericaceous shrubs. Consequently, new growing shoots would be highly susceptible to devastating pest outbreaks since insects show feeding preferences for young succulent plant tissues with lower defence compounds (Hartley, Nelson and Gorman, 1995). Therefore, *Calluna* growing in nitrogen-rich conditions would exhibit diminished pest resistivity due to a higher carbon:phenol ratio. Researchers have successfully linked the frequency of heather beetle attacks to the high nitrogen deposition (Heil and Diemont, 1983) and attribute 'gap formation' with a closed *Calluna* canopy to be the factor that allows understorey grasses to gradually dominate over heather (Brunsting and Heil, 1985; Bobbink, Heil and Raessen, 1992). Increased atmospheric nitrogen deposition is thus the trigger that aids the conversion of heathlands to grasslands by altering the carbon:nutrient ratio in plant tissues. No changes in soil carbon levels were detected which revealed that microbial biomass did not utilise or benefit from increasing fertiliser inputs, hence suggesting the possible limitation of other important nutrients such as phosphorus.

2.5 CONCLUSIONS

Growth was remarkably unresponsive to the applied N, much less so than one expects from previous work on forest stands, and may be attributed to the possible inhibitory effects of excessive nitrogen on soil microbial biomass and activity, and other nutrient limitations, such as phosphorus, within the closed environment of a

microcosm. Shoot tissue-N responded significantly over a period of three months with increasing fertiliser inputs. The results suggest that a fraction of the applied N was directly taken up by the shoots while approximately half was found remaining in the soil. No changes were observed in the soil carbon concentration, which suggests that microbial biomass did not increase with increasing nitrogen additions. However, phenolics in the shoots declined significantly with increasing N. Since secondary metabolites govern chemical defence systems, the plants may be more susceptible to pest attack by herbivores. There was not a threshold response but a gradual decline in phenolics with increasing nitrogen application. The study reveals the need for the 'critical load concept' for atmospheric nitrogen depositions to be more site and species specific in order to be truly effective.

3. A LABORATORY TECHNIQUE TO MEASURE CO_2 FLUXES FROM SOIL

3.1 INTRODUCTION

The storage of carbon as soil organic matter plays a vital role in the global carbon cycle. Approximately $1{,}500 \times 10^{15}$ g of carbon is stored in the upper meter of soil (Jobbágy and Jackson, 2000), which constitutes the second largest pool of carbon in the biosphere after oceans (Schlesinger, 1991). The reservoir of carbon stored in the soil has the potential to greatly influence atmospheric CO_2 concentrations because the process of decomposition returns carbon sequestered by photosynthesis to the atmosphere. CO_2 flux from the soil to the atmosphere is estimated to be $50 - 70 \times 10^{15}$ g of carbon per year and makes up 20 - 38 % of annual inputs of carbon in the form of CO_2 to the atmosphere from terrestrial and marine sources (Raich and Potter, 1995). Thus, the efflux of CO_2 from the soil is an important component of the global carbon balance (Baldocchi *et al.*, 1986). Currently, the global terrestrial biosphere is a carbon sink of about 2×10^{15} g because the net primary productivity is enhanced by elevated CO_2 and nitrogen deposition (IPCC, 2000). However, climatic warming ranging from 1.5 – 5.0 °C is predicted in 100 years that may effectively change the sink into a source because of the impact of temperature on decomposition of soil organic matter (IACGEC, 1996). Many previous studies have measured CO_2 evolution and demonstrated the relationship between soil respiration and environmental factors (Anderson, 1973; Weber, 1985; Gordon, Schlenter and Van Cleve, 1987, Lloyd and Taylor, 1994). However, it is not entirely known how climate change would affect the storage of organic carbon in soils (Van de Geijn and Van Veen, 1993; Post *et al.*, 1990). In some models, the entire global stock of carbon is deemed to be vulnerable to climatic warming and is considered to be uniformly sensitive to temperature (Cox *et al.*, 2000), but in practice this sensitivity is not well

established (Liski *et al.*, 1999; Valentini *et al.*, 2000). Hence, the ability to accurately quantify carbon dioxide flux from soil is of paramount importance to improve the understanding of soil organic matter turnover and incorporate this knowledge into simulation models (Hansen *et al.*, 1991). Moreover, the rate of soil respiration is governed directly or indirectly not only by a major environmental factor such as temperature but also affected by secondary factors of soil depth, nutrient status of the soil, moisture, oxygen status and the availability of the substrate. All these factors vary with depth, hence, the need arises to determine the region within a soil profile that contributes most to soil respiration and the response of that layer to changes in temperature and nutrient inputs.

Soil respiration has previously been determined in the field as well as in the laboratory, as summarised by Schinner *et al.* (1996). Field investigations are commonly done with closed measuring devices (Fang and Moncrieff, 1996; Rayment, 2000). However, the measurements are subject to uncontrolled variations in the environment. Laboratory methods on the other hand enable single variables to be controlled at will whilst others are held constant. The three main laboratory techniques involve a) carbon dioxide measurements in a closed system, b) carbon dioxide measurements with continuous measurements, and c) continuous measurements of oxygen uptake. In this study an 18 channel, continuous flow, multi-point gas analysing unit was constructed wherein soil microcosms were continuously aerated with ambient air and the CO_2 flux was derived from the difference in carbon dioxide concentration between the inflowing and outgoing air. In laboratory experiments, sample preparation also plays an important role in determining the accuracy of measurements. A majority of the previous studies have used restructured soil. However, altering the inherent structure of the soil profile may change the response of soil respiration to environmental variables such as temperature (Fang and Moncrieff, 2001). In this project intact soil microcosms have been studied under laboratory conditions with the aim of introducing minimum disturbance to the soil structure and composition so as to increase the accuracy and reliability of

measurements. The phase of initial CO_2 flush was also closely monitored to determine the time period taken by small soil samples to attain a state of equilibrium.

While a significant correlation between soil temperature and carbon mineralisation is well established, there is no agreement about which function best describes the relationship between soil carbon mineralisation and temperature (Lloyd and Taylor, 1994). From laboratory experiments, two main approaches can be distinguished to obtain data for fitting functions: first, comparing instantaneous CO_2 efflux rates at different soil temperatures and second, an analysis of the time series of CO_2 efflux using a decomposition model. Each approach has certain disadvantages: when measuring instantaneous CO_2 efflux one has to cope with the problem that the apparent temperature sensitivity of CO_2 efflux may be dependent on the point in time when the flux is measured, implying that studies are not comparable when measuring respiration at different pre-incubation times. On the other hand, when measuring longer time series CO_2 efflux, artificial conditions may arise due to the formation of toxic by-products. These problems introduce uncertainty into the estimation of parameters for temperature response functions of decomposition used in ecosystem and soil organic matter models. In this study the response of soil respiration to temperature was analysed during short- and long-term laboratory incubations using instantaneous CO_2 efflux rates and monitoring decomposition to determine the temperature sensitivity of the process.

The aim of this thesis is to use the laboratory technique to explore the effect of temperature and N-deposition on CO_2 fluxes. In this chapter the basic methodology of flux measurement is presented and some of the critical aspects of the technique are demonstrated with a series of experiments to investigate i) the equilibration time of small soil samples, ii) the influence of sampling depth in the soil, iii) the effect of incubating at a given temperature prior to measurement, iv) the occurrence of hysteresis, and v) differences in field and laboratory measurements.

3.2 MATERIALS AND METHODS

3.2.1 Site

Field experiments were carried out in six plots located at Castlelaw Hill (55° 52′ 22″ N, 3° 14′ 3″ W) in the Pentland Hills near to Edinburgh, at an altitude of 435 meters above mean sea level. The area receives an annual rainfall of 1000 - 1200 mm; however, 1999 - 2000 was wetter than average. The moderately steep hill slope was covered with a dense stand of mature heather, *Calluna vulgaris* (L.) Hull (Figure 3.1). Based on a study by Fowler *et al.* (1989), wherein atmospheric inputs were measured for an area of moorland along the Scottish borders in Northern Britain, the background levels of nitrogen inputs were estimated to be 12.4 kg N ha^{-1} yr^{-1} made up from 8.0 kg N ha^{-1} yr^{-1} of wet deposition, 4.0 kg N ha^{-1} yr^{-1} of dry deposition and 0.4 kg N ha^{-1} yr^{-1} by cloud interception.

Figure 3.1 Site location of the experimental plots at Castlelaw Hill, in the Pentland Hills near Edinburgh, Scotland. A dense stand of mature heather *Calluna vulgaris* (L.) Hull covers the moderately steep hill sides.

Soil at the site is classified as a 'Dirrington', a freely drained humus iron podzol of the Bemersyde association, which averages 25 cm deep (Ragg and Futty, 1967; MISR, 1969). The parent material is stony drifts derived from rhyolites and trachytes (Trudgill, 1989). The heathland floor is mainly composed of a thin 0.5 - 1 cm litter layer, also known as the O horizon, followed by a 3 - 5 cm raw humus H horizon that exhibits a strongly acid reaction. The A_1 horizon measures 1 cm, is dark in colour and incorporates humic acids. The 7 - 10 cm thick, grey bleached A_2 horizon dark with a low organic content, is pale in colour. The underlying 5 - 7 cm B_2 horizon is well developed and bright in colour with strong humus/iron staining at the top edges. There is a sharp change into a paler, indurated, intermittent B_3 and C horizon of parent bed rock. Most heather roots were concentrated in the upper H and A horizons of the soil profile.

3.2.2 Experimental Design

Three treatment and three control plots, each measuring a 3 m × 3 m square, were laid out with a separation gap of 1m between adjoining plots. Nitrogen was applied to three randomly selected treatment plots as an ammonium nitrate, NH_4NO_3, solution (BDH Laboratory Supplies, Poole, England) in six, 10 kg N ha^{-1} yr^{-1} doses over a period of one year; thus input being equivalent to a total of 60 kg N ha^{-1} yr^{-1}. Every N aliquot was applied uniformly over each plot in 2 litres of distilled water as a fine mist using a controlled pressure knapsack sprayer (KS - 15L: P/N 100221, Oregon, Blount, UK). The treatment plots were fertilised in August, October, December 1999 and February, April, June August 2000.

A wooden temperature probe, was inserted into the soil profile with type-T thermocouple wires measuring soil temperature at depths of 1, 3, 5, 10 and 20 cm from the surface. The output data were collected by a data logger (DL-5864, Delta-T Devices, Cambridge, England) during different seasons of the year.

3.2.3 Soil Collection

A sampling corer was designed to collect blocks of soil with minimum disturbance to the structural integrity. The galvanised steel corer was rectangular in shape with a movable head plate, which was internally connected to a piston enclosed within a short central shaft (Figure 3.2). When the corer was pushed into the soil, the piston rose out of the central shaft to provide a handle to remove the corer from the soil and subsequently the sample could easily be ejected from the corer by pushing down the piston. The dimensions of the corer were 1.0 cm smaller than the microcosm chamber so that the sample could be directly and swiftly transferred to the chamber, allowing an aeration space around the sample. The lids of all the chambers were immediately sealed with non-corrosive silicon rubber (Vallance, Leeds, UK) in the field and transported to the laboratory in a cold box at approximately 5 °C. Soil respiration was measured in the laboratory with an 18 channel, continuous flow, multipoint gas analysing unit.

Figure 3.2 A sampling corer designed to collect small, intact soil samples with minimum disturbance to the structural integrity.

To explore the effect of sample size, samples of two sizes were collected. The larger samples had a volume of 21 × 14 × 4 cm while the smaller samples measured 9 × 7 × 4 cm. All microcosm samples for CO_2 flux studies were collected from the H horizon of the soil profile in autumn after the growing season in October 2000. Samples were incubated at the field temperature of 5 °C and soil respiration was monitored on an hourly basis using the laboratory gas analysis system. After all the samples had achieved a steady state of respiration, the incubation temperature was increased to 15 °C and CO_2 fluxes were measured once again.

To investigate the effect of depth in the soil, additional soil samples were collected from the experimental site in the summer month of July 2000 at the peak of the heather growing season and once again in October 2000 at the end of the growing season in late autumn. A soil corer of length 65 cm, with an inner diameter of 5.8 cm was used to collect an undisturbed, intact core from the soil profile. The core was subsequently sliced into 4.0 cm thick sections at mean depths of 2 cm in the H horizon, 7 cm in the A_2 horizon and 12 cm in the B_2 horizon from the top surface, and the sections were carefully transferred to soil chambers. All chambers were kept in a cooled incubator (Gallenkamp, Loughborough, England) at 6 °C for an initial period of 18 hours for the soils to stabilise after the initial flush of CO_2. Carbon dioxide efflux from the different depths was determined at 6 °, 12 °, 20 ° and 25 °C using the gas analysis system.

To study the effect of incubating at a fixed temperature prior to measurement, two batches of soil samples were collected from the H horizon of all plots in the month of December 1999 for the incubation experiment. The first batch of soil samples was further sub-divided into two sets, where one was incubated at 5 °C and the other at 15 °C, for a period of seven weeks in cooled incubators (Gallenkamp, Loughborough, England). Carbon dioxide evolution was measured by the gas analysis unit on days 1, 6, 15, 29 and 49 at the respective incubation temperatures. The second batch was incubated at 5 °C for a period of 44 days and respiration readings were periodically

determined on days 1, 5, 13, 25, and 44 across a temperature gradient from 5 °C to 25 °C in 5 degree intervals. During the period of incubation, the inlet and outlet tube openings of the soil chambers were left open so as to allow diffusion of gases to and from the soils. The samples were frequently weighed and moistened with distilled water, if necessary, to replace water lost by evaporation.

To examine the response of soil respiration across an increasing and thereafter decreasing temperature gradient, the hysteresis effect, soil samples were collected from the H horizon in July 2000 from the experimental field with the help of the sampling corer. Samples were stored in a cooled incubator (Gallenkamp, Loughborough, England) set at 5 °C for 18 hours to allow for the initial flush of CO_2 before measuring the response of soil respiration to temperature using the gas analysis system. Soil respiration was measured across a rising temperature gradient of 5° – 25 °C with 10 °C increments and thereafter a falling temperature gradient from 25 °C to 5 °C with a step-wise 10 °C interval.

To compare the laboratory reading of soil respiration with field measurements, a portable CO_2 analyser with an environmental gas monitor (WMA3 - EGM2, PP Systems, Hertfordshire, England) was used to take soil surface carbon dioxide effluxes from the experimental field site at a mean depth of 3 cm from the surface. Thereafter, the upper H horizon of soil was removed and soil respiration was measured at a mean depth of 7 cm. Simultaneously, soil samples were collected in June 2001 from the H horizon of all plots, immediately transported to the laboratory in cold boxes, and CO_2 fluxes were measured at field temperature in the laboratory using the gas analysis system.

3.2.4 Gas Analysis System

An analytical unit was designed to measure CO_2 fluxes at a range of temperatures in the laboratory from a series of soil samples collected from the field. The 18 channel, continuous flow, multi-point, 'open chamber system' is shown in Figure 3.3.

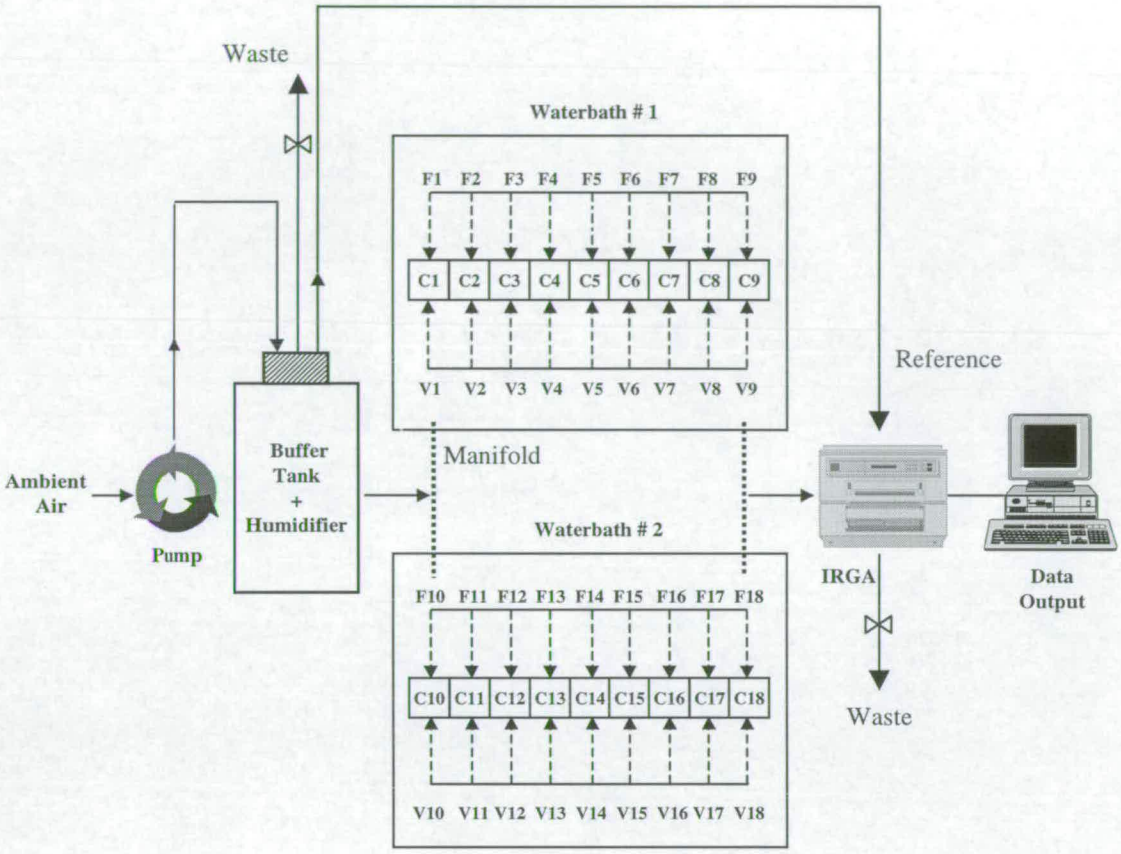


Figure 3.3 Gas analysis system for measuring carbon dioxide fluxes from small, intact soil cores in the laboratory. Key : C = soil chamber, F = flow meter, V = 3-way solenoid valve

A diaphragm pump (B100DE-Duplex 2F, Charles Austen, Weybridge, UK) drew ambient air at a rate of approximately 20 litres per minute from an external pipe into a 10 litre humidifying 'buffer' tank and pumped the moist air into all the microcosm chambers. The humidifier provided water saturated air, and prevented desiccation of the samples, while the volume of the tank reduced fluctuations in atmospheric CO_2 concentrations in the gas handling unit. The chambers were constructed from standard sealed diecast boxes (224 - 802, RS Components, Corby, England) with inlet and outlet connectors fitted on to the lids. The temperature of each soil sample was recorded by a type-T thermocouple, inserted into the soil and connected to a

datalogger (21X, Campbell Scientific, Shepshed, UK). The chambers stood immersed in a controlled temperature water bath (W46 DC10 - EK20, Haake, Karlsruhe, Germany). Each sample line was fitted with a polycarbonate air flow meter (Key Instruments, USA) to enable the flow rates to be adjusted and balanced, and a 3-way solenoid valve, which when activated, diverted the outlet airstream to the Infra Red Gas Analyser (225 MK3, Analytical Development Co. Ltd., Hoddesdon, England), through which air was circulated by the IRGA's internal pump and further monitored by the in-built flow meter. The solenoid valves were manually controlled by illuminated neon switches mounted on a control panel. A heating tape was fitted around both the reference and analysis air lines to prevent condensation of water vapour in the lines and minimise errors resulting from cross-sensitivity to water vapour in the gas analyser. Sequential measurements of soil respiration were made for all the samples at either a set temperature or across a range of temperatures. Every chamber was sampled for 5 minutes at every set temperature, with the mean value being recorded. After every reading the sensor cell of the gas analyser was flushed for a minute. For each temperature change, the soil sample was allowed to slowly attain the temperature over a period of 30 minutes and thereafter stabilise for an additional four hours with continuous aeration before readings were taken. The entire system was monitored on a personal computer with a graphical display of the CO_2 concentrations and corresponding chamber temperature readings.

3.3 RESULTS

3.3.1 Sample Size and Time Course

Figure 3.4 shows the time course of respiration when large and small soil samples were incubated initially at 5 °C and then exposed to a stepwise increase of temperature to 15 °C. A marked difference was noted for the time taken by the smaller soil cores to achieve a steady state of respiration as compared to the larger samples. Constant soil respiration readings were recorded within 16 hours for the

smaller samples but required an additional 3 hours in the larger samples. Thereafter, rates were constant and independent of the size of the soil sample.

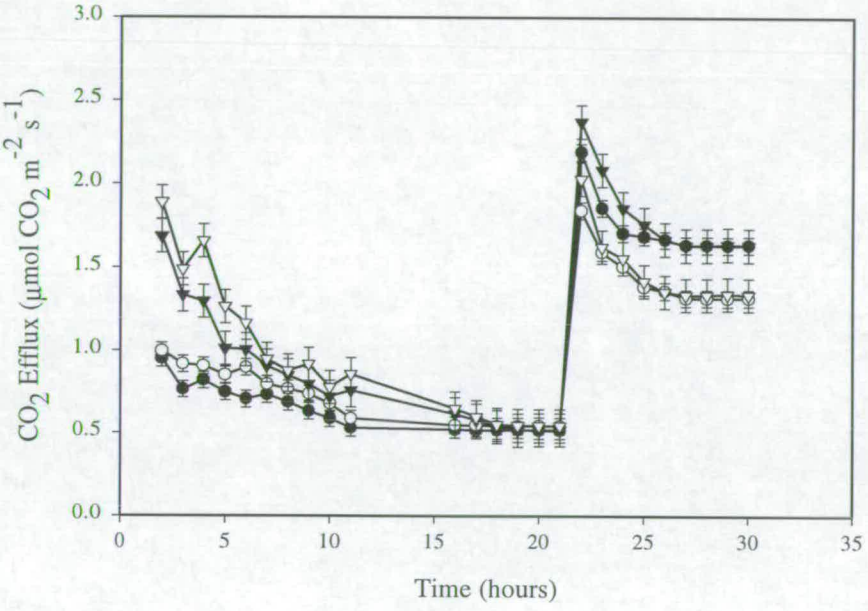


Figure 3.4 The time course of respiration when small [control (o), treatment (•)] and large [control (▽), treatment (▼)] soil samples were incubated and monitored, immediately upon collection, at initially 5 °C and then at 15 °C after 21 hours (mean ± S.E., $n = 9$).

Following the stepwise change in incubation temperature, after 21 hours of incubation, the smaller microcosms reached a state of thermal equilibrium in 35 minutes while the larger microcosms took an average of 50 minutes. All the samples recorded a sharp increase in soil respiration at 21 hours followed by a gradual decline to a constant. The period of initial CO_2 flush after a 10 degrees rise in temperature for the small and large samples was 4 and 6 hours respectively.

Both small and large cores reached the same state of equilibrium at 5 and 15 °C and soil respiration readings did not differ significantly between the large and small samples.

Treatment had no significant effect on the either the length of time taken to attain thermal equilibrium or the period of CO_2 flush in both the small and large microcosms. However, all cores collected from treatment plots showed higher rates of respiration than the controls at 15 °C.

3.3.2 Sample Depth

Soil respiration declined significantly ($P < 0.005$) with sample depth (Figure 3.5) and at all incubation temperatures, the H horizon at a mean depth of 2 cm had the highest rate of respiration in both control and treatment plots, followed by A_2 (mean depth = 7 cm) and B_2 (mean depth = 12 cm) horizons. The rates of respiration from all sample depths increased with temperature in accordance with the exponential first-order equation. The estimated Q_{10} decreased with depth over the temperature range of 6 °C to 25 °C (Figure 3.6).

In summer, for control plots, the Q_{10} decreased from 2.47 in the H horizon to 2.27 in the A_2 horizon and then to 2.00 in the B_2 horizon. A similar trend was observed in the treatment plots with the Q_{10} value dropping with each depth level; from 2.53 in the H horizon to 2.37 in the A_2 to 2.01 in the B_2. The same declining trend was recorded in autumn for all the plots, however, in comparison to summer values, Q_{10} was slightly lower in the H and A_2 horizons while remaining constant in the B_2 horizon over the two seasons.

Although treatment had no statistically significant effect on the response of soil respiration to temperature, during both sampling times, respiration in all horizons is slightly higher in the treatment plots as compared to control plots.

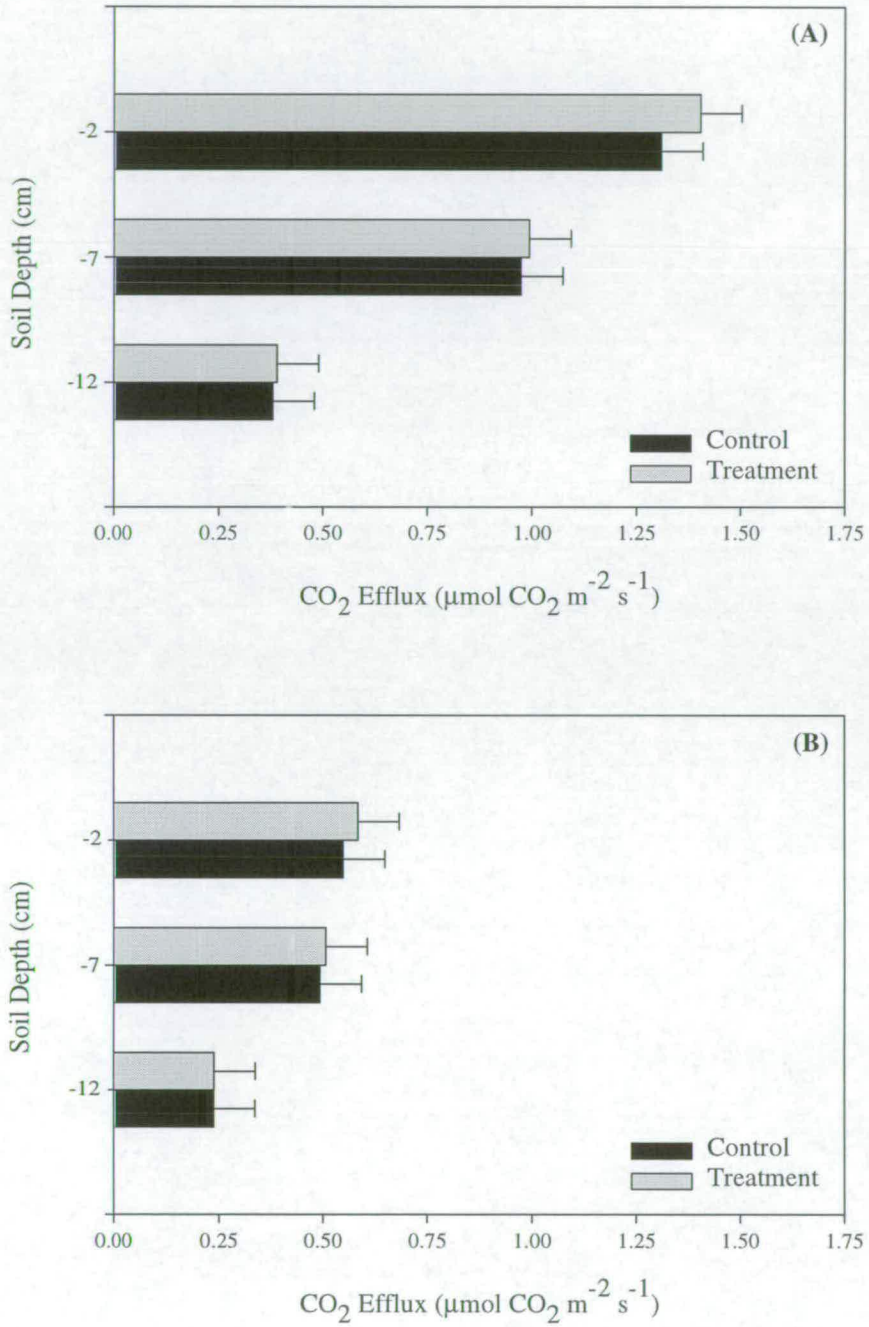
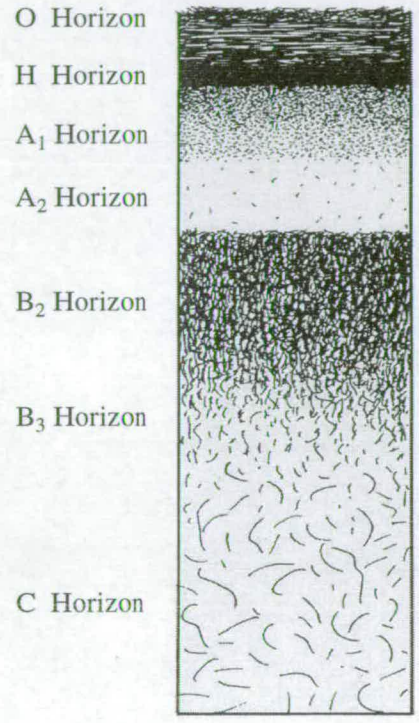


Figure 3.5 Soil CO_2 flux rates at depths of 2, 7 and 12 cm from the surface during (A) summer – July 2000 and (B) autumn – October 2000 (mean ± S.E., $n = 9$).



SOIL PROFILE	DEPTH	CONTROL		TREATMENT	
	(cm)	July	October	July	October
O Horizon					
H Horizon	2	2.47	2.42	2.53	2.46
A_1 Horizon					
A_2 Horizon	7	2.27	2.17	2.37	2.27
B_2 Horizon	12	2.00	2.01	2.01	2.01
B_3 Horizon					
C Horizon					

Figure 3.6 The decreasing response of Q_{10} with depth across the soil profile, as noted for the H, A and B horizons during summer – July 2000 and autumn – October 2000.

The daily temperature of the different soil horizons was logged during the study period in 2000 and the temperature probe interfaced with a datalogger recorded a gradual decrease in temperature with an increase in soil depth as shown in Figure 3.7.

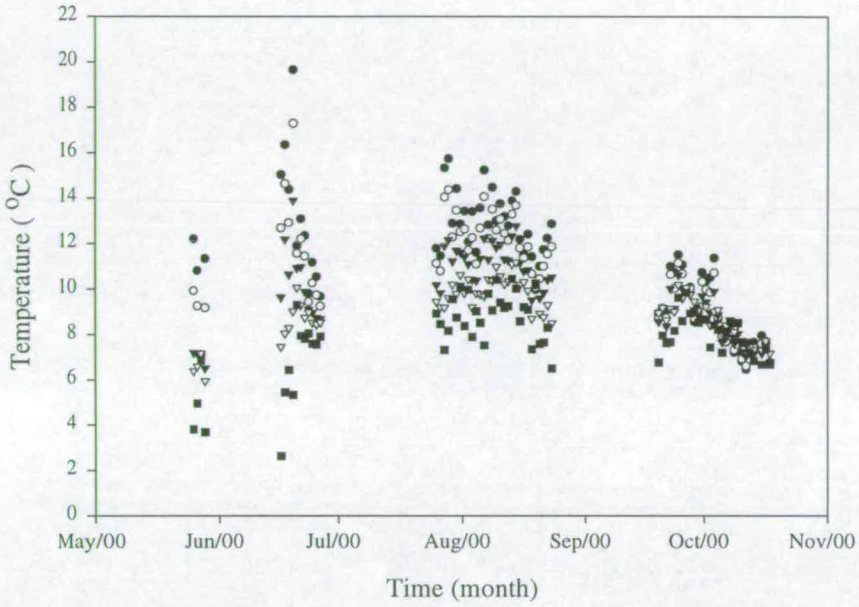


Figure 3.7 Diurnal soil temperatures during summer and autumn at 1, (•), 3 (o), 5 (▾), 10 (▽), and 20 (■) cm depths as recorded by a temperature probe interfaced with a datalogger at the experimental field site at Castlelaw Hill. Data points are the mean temperatures recorded at 1200 hours.

3.3.3 Incubation Time

In the incubation experiments respiration rates for both control and treatment samples remained constant during the first 15 days of incubation at 5 °C and 15 °C with variation less than 0.005 μmol CO_2 m^{-2} s^{-1} in absolute respiration rates (Figure 3.8). Thereafter, a constant decline in soil respiration was noted in all samples incubated at both temperatures.

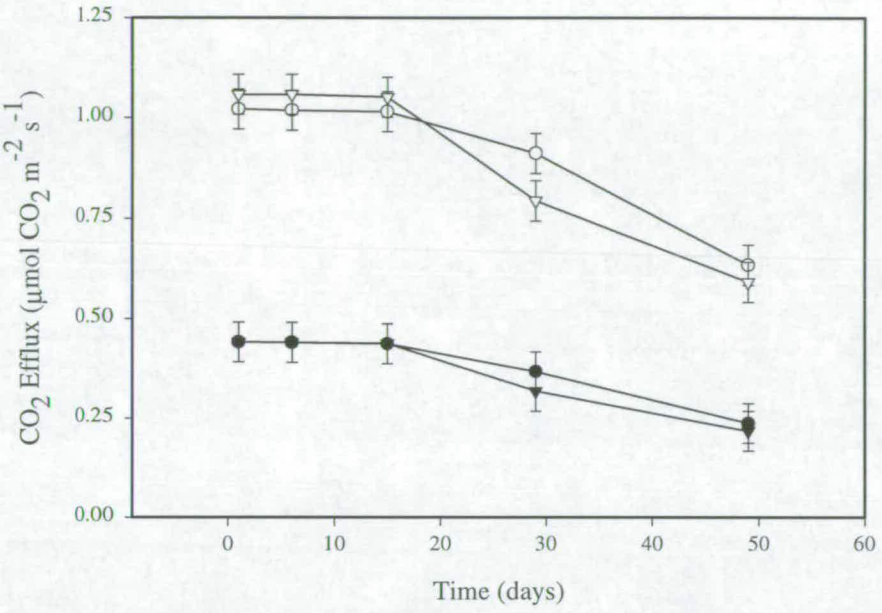


Figure 3.8 Soil respiration rates for small, intact cores, from control [5 °C (•), 15 °C (o)] and treatment [5 °C (▾), 15 °C (▿)] plots (mean ± S.E., n = 9), incubated and measured at the same temperature of 5 °C or 15 °C.

A similar pattern was observed for the Q_{10} values, which fluctuated no more than 0.01 during the first 15 days of incubation and then sharply increased (Figure 3.9) from 2.31 to 2.67 and from 2.39 to 2.72 for control and treatment samples respectively.

Nitrogen fertiliser inputs had no significant effect on the rates of soil respiration during incubation but treated samples recorded slightly higher readings at 15 °C.

During the last 34 days of incubation, soil respiration values recorded a greater absolute decline at 15 °C than at 5 °C, for both control and treatment samples.

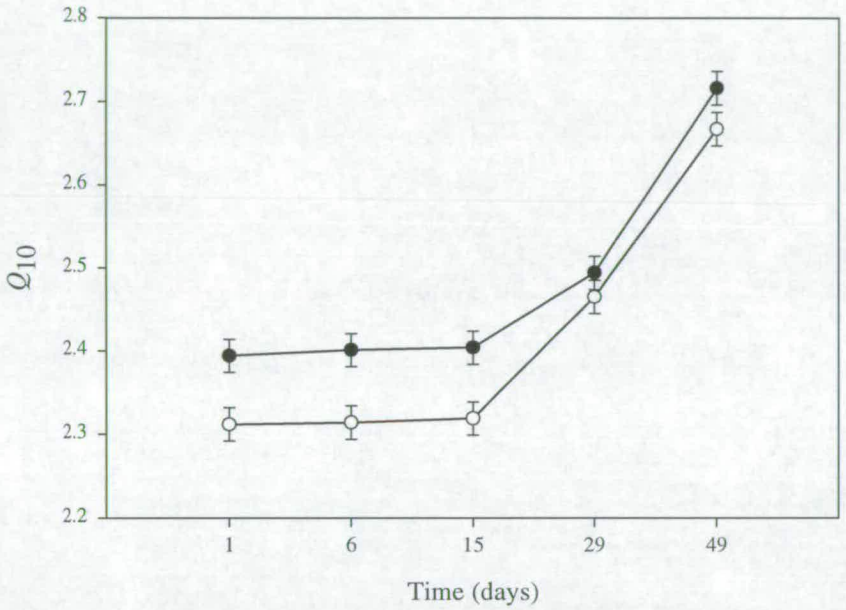


Figure 3.9 Response of Q_{10} with time over a period of 46 days for control (o) and treatment (•) samples (mean ± S.E., $n = 9$) incubated and measured at 5 °C or 15 °C.

When control and treatment samples, maintained for 44 days at a constant temperature of 5 °C were subjected to an increasing range of temperatures, they showed an exponential increase in soil respiration with respect to temperature (Figure 3.10). The respiration rate at 5 °C was fairly constant till Day 5 and thereafter rapidly dropped to almost half the initial respiration rate by the end of the incubation period.

The Q_{10} values were constant till Day 5 but then steadily increased (Figure 3.11). After 2 weeks of incubation the Q_{10} increased from 2.5 to 2.9. There was no detectable effect of treatment on this pattern.

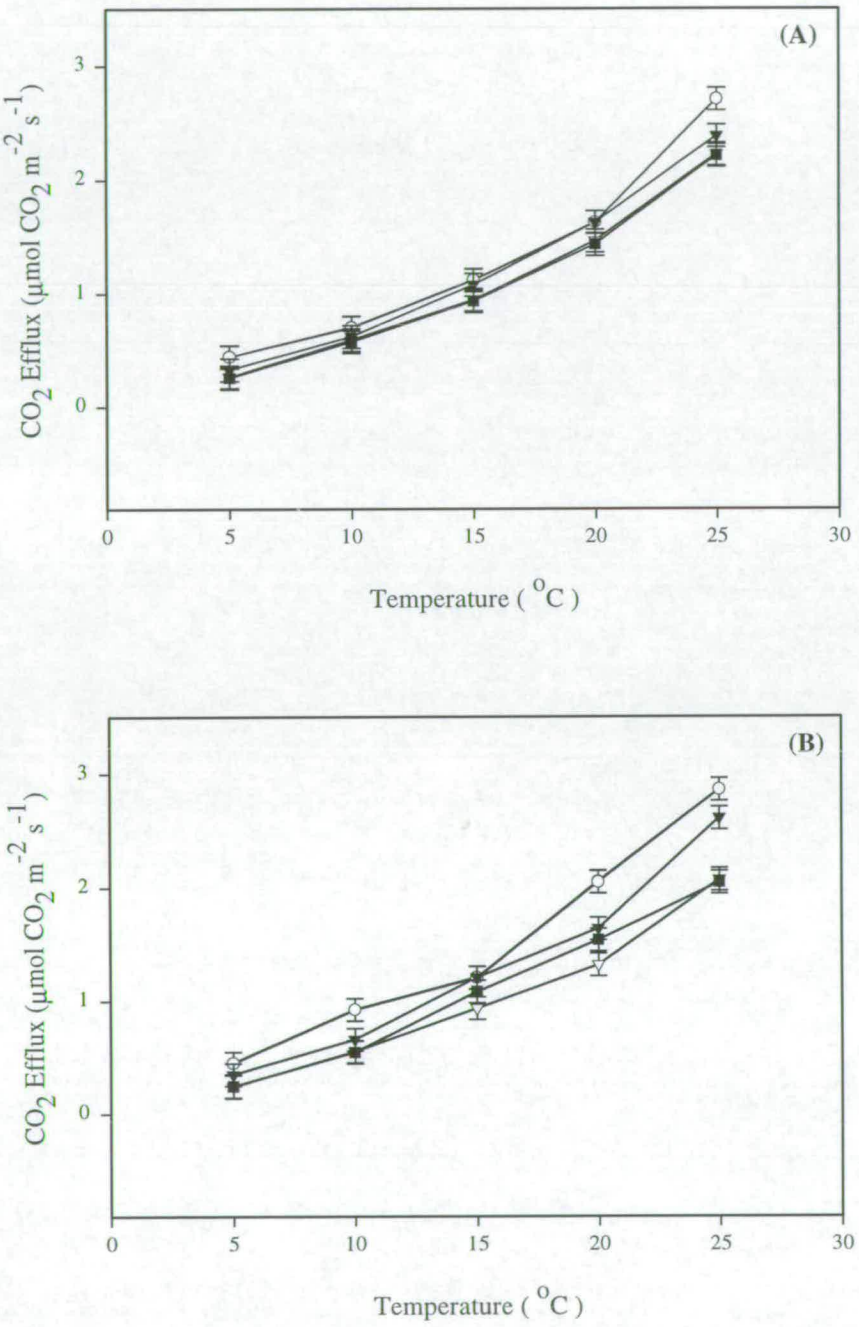


Figure 3.10 The time response of soil respiration rates for (A) control and (B) treatment samples (mean ± S.E., $n = 9$) incubated at 5 °C but measured across a rising temperature gradient of 5 – 25 °C on Day 1 (•), Day 5 (o), Day 13 (▾), Day 25 (▿), and Day 46 (■).

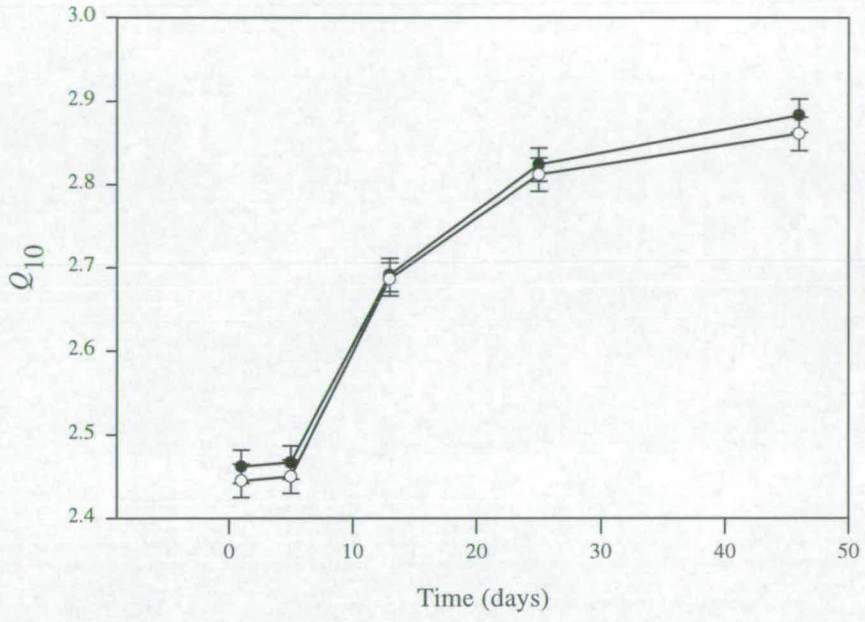


Figure 3.11 Response of Q_{10} with time over a period of 49 days for control (o) and treatment (•) samples (mean ± S.E., n = 9) incubated at 5 °C but measured across an increasing temperature gradient of 5 - 25 °C.

3.3.4 Hysteresis

The effect of increasing and decreasing temperatures on carbon mineralisation of heathland soils is shown in Figure 3.12. In this experiment the warming and cooling cycles took 11 hours each. In all samples, both control and treatment, soil respiration increased exponentially with an increase in soil temperature and thereafter decreased exponentially in the same way. The trend was reflected in the Q_{10} values with no significant difference between the Q_{10} values across the temperature gradients, either while increasing or decreasing.

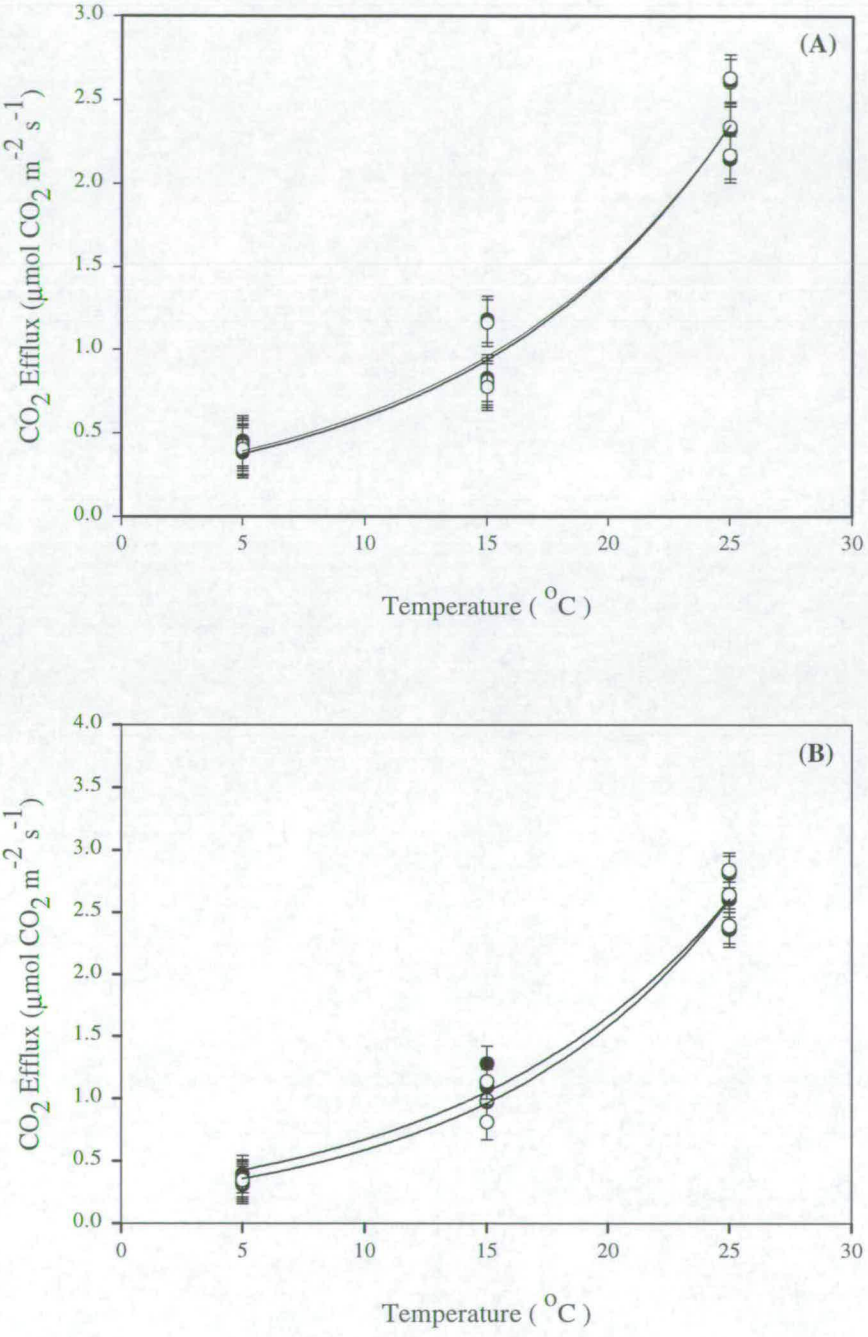


Figure 3.12 The response of soil CO_2 fluxes with temperature for (A) control and (B) treatment samples (mean ± S.E., $n = 9$) across an increasing (•) and decreasing (o) temperature gradient.

Treatment had no significant effect on the response of soil respiration and did not change the magnitude of the response at any given specific temperature over a period of 22 hours, irrespective of the previous soil temperature. However, higher Q_{10} values were noted for treatment samples as compared to control. Treatment plots recorded Q_{10} values as 2.70 and 2.75 while control plots revealed slightly lower Q_{10} values at 2.36 and 2.40 for increasing and decreasing temperature intervals respectively.

3.3.5 Field versus Laboratory Measurements

Carbon dioxide fluxes from the soil profile as measured in the field ranged from a mean value of 1.25 to 1.45 μmol CO_2 m^{-2} s^{-1} for control and treatment plots respectively.

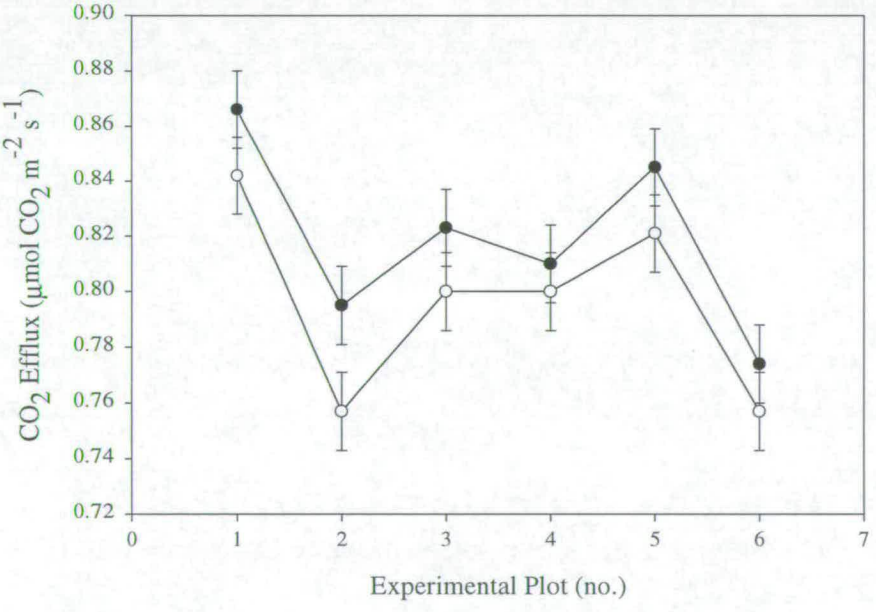


Figure 3.13 Comparison of soil carbon dioxide fluxes from the H horizon as measured in the laboratory (•) and in the field (o) for control (no. 2, 4, 6) and treatment (no. 1, 3, 5) plots (mean ± S.E., $n = 9$).

Upon removing 4 cm of H horizon and determining soil respiration from the subsequent reduced profile, the carbon dioxide fluxes contributed by the H horizon could be calculated. The field values compared well with the laboratory measurements of soil respiration from the H horizon and there was no statistically significant difference between the means for each plot (Figure 3.13).

3.4 DISCUSSION

3.4.1 Sample Size and Time Course

A general problem faced with laboratory incubated soil samples is that the respiration rate is initially very high due to the disturbance of the soil structure during sample preparation but then gradually declines to a lower level (Winkler, Cherry and Schlesinger, 1996). Even after the initial flush, CO_2 efflux may be several times higher during the initial stages of decomposition than that observed at the end of the incubation period under constant conditions (Liebeg *et al.*, 1995; Torbert, Prior and Rogers, 1995). This flush is not well-understood. Disturbance may provoke a 'wound' response in broken roots and hypae. Another possibility is that we are dealing with the release of carbon dioxide trapped in gas-filled pores, and the larger samples take longer because the diffusion path is longer. Irrespective of the cause, determining the time period of flush and stabilisation for a specific sample size is vital to accurately determine soil respiration rates in the laboratory. Moreover, incubating soil samples to study the response of soil respiration to temperature under laboratory conditions may lead to anomalies in CO_2 efflux rates due to the altered structure and size of the soil sample (Lomander, Kätterer and Andrén, 1998). Lomander, Kätterer and Andrén (1998) modelled the effects of temperature on carbon dioxide evolution from soil and concluded that flux measurements performed on intact soil cores yield far more accurate results than disturbed samples.

Several authors have argued that soil samples should be large in order to adequately represent field conditions (Thomson *et al.*, 1997; Fang and Moncrieff, 1998 b). However, harvesting large samples from field sites may not prove to be ideal or convenient if the samples need to be frequently collected, with replication, especially during long-term, intensive studies monitoring seasonal trends. The removal of large volumes of soil from experimental plots would affect the plant-soil interactive balance and introduce a further element of error. Moreover, small soil samples would ensure a rapid equilibration time. Therefore, the smaller the sample, the more accurate will be the laboratory representation of the response of soil respiration to temperature under natural field conditions. Small re-constructed soil samples have been previously studied but some authors have shown a continual decrease in respiration rates after an initial CO_2 flush (Pohhacker and Zech, 1995; Winkler, Cherry and Schlesinger, 1996). This has been attributed to the removal of roots, which may deactivate microbes during incubation, and the constant depletion of soil organic matter with time. The use of small intact soil cores has not yet been thoroughly explored. Hence, one objective of this study was to determine if smaller samples of intact soil could provide accurate estimates of soil respiration during laboratory incubations at different temperatures.

The results in the present experiments do prove that small samples of intact soil can provide accurate measures of soil respiration by the laboratory incubation technique. The steady state of respiration reached by the small and large samples within a short period of 16 and 21 hours respectively may be attributed to the fact that the samples were not tightly fitted into the chambers but had a circulation space all around. Thus CO_2 molecules within the sample could more rapidly diffuse into the airstream, thereby also preventing the build-up of a CO_2 gradient in the soil profile. Thus the equilibration time for temperature was much shorter than the 18 hours reported by Fang and Moncrieff (2001) for a 4500 cm^3 chamber. After reaching a steady state in the small chambers, soil respiration was recorded for a further 5 hours and no significant decrease with time was noted. This suggests that the soil organic matter

does not rapidly deplete in agreement with the findings of Winkler, Cherry and Schlesinger (1996) who found organic matter to be considerably stable even after a 120 day incubation. Recently, a similar result was also recorded by Grisi *et al.* (1998) for temperate and tropical soils. Hence, it may be concluded that the soil organic matter of small samples can support the resident microbial population just as well as larger samples because the proportion of microbial load will correspond with soil volume.

The flush of CO_2 from all samples after the temperature change, when the soil temperature was raised to 15 °C, might be accounted for by the reduction of carbon dioxide solubility in the soil solution upon warming and the consequent release of CO_2 from CO_2-saturated solution. Thereafter, the smaller chambers attain a steady state of respiration within 4 hours while the larger chambers take an additional two hours. However, all cores reach the same state of equilibrium at both incubation temperatures proving that small samples do not reduce the accuracy of response of soil respiration to temperature. On the contrary, small samples provide a rapid and convenient measure of CO_2 efflux from soil.

3.4.2 Sample Depth

Previous studies have shown contrary trends in soil respiration rates with an increase in soil depth. Early investigations (Lundegårdh, 1924; Smith and Brown, 1932; Makarov, 1958) revealed a decrease in CO_2 concentration down the soil profile and De Boois (1974) found that Q_{10} between 5 °C and 20 °C in a woodland soil profile averaged 3 for the upper litter layers and 2 for humus layers, thereby concluding that the magnitude of microbial temperature responses may decrease down the soil profile as the residual organic matter becomes depleted in available carbon. Similar patterns of decreasing Q_{10} values with increasing depth in soil profiles have been described by De Jong and Schappert (1972); Bunnell *et al.* (1977); Ross and Tate (1993); Winkler, Cherry and Schlesinger (1996); Lomander, Kätterer and Andrén (1998). However, contrary patterns with an increase in soil CO_2 concentration with

increasing soil depth have also been periodically documented (Cosby *et al.*, 1985; Castelle and Galloway, 1990; Crill, 1991; Hersterberg and Seigenthaler, 1991; Fernandez *et al.*, 1993; Yavitt, Fahey and Simmons, 1995; Fang and Moncrieff, 1998 b). Despite the widespread belief that higher global temperatures will increase the rates of microbial decomposition in soils, there is little information on the magnitude of this effect in different soil horizons of specific ecosystems (Kirschbaum, 1995), and especially in conjunction with elevated deposition of atmospheric pollutants.

The surface horizons contain 'fresh' organic material with constituents such as protein and cellulose, which are known to decompose rather easily (Kladivko and Keeney, 1987). The older deeper horizons are likely to be depleted in these labile constituents and enriched in recalcitrant compounds such as lignin and chitin. A range of techniques have been adopted through the years to measure soil respiration down the soil profile. In laboratory investigations, to determine the soil respiration of a specific layer, De Jong and Schappert (1972) took undisturbed core samples and measured CO_2 evolution in a controlled environment while Witkamp and Frank (1969) calculated the respiration of individual layers of the soil profile by progressively removing layers from the top of the profile and measuring the changes in CO_2 evolution. In later ex-situ studies (Ross and Tate, 1993; Winkler, Cherry and Schlesinger, 1996) samples from different horizons were collected from the wall of a large single pit, excavated to expose the entire soil profile. In this project, soil corers were utilised due to the shallow depth of the soil profile.

The results observed in the present study suggest that in spring, with the commencement of the growing season after a low period of metabolic activity in winter, decomposition rapidly increases in the top of the soil profile with soil respiration rates being highest in the uppermost horizon and gradually decreasing with depth. Soil organisms are capable of long period of minimal activity or dormancy and becoming highly active when environmental conditions are most favourable (Paul and Clark, 1989). Winkler, Cherry and Schlesinger (1996) studied

CO_2 effluxes in the soil profile of a temperate forest and also found that the soil respiration varied with soil horizon, wherein the A-horizon had the highest rates of respiration, followed by B- and E- horizon soils. Lomander, Kätterer and Andrén (1998) modelled soil C evolution rates under laboratory conditions to conclude that the CO_2 evolution rate per unit of soil carbon is about twice as high for topsoil than for subsoil. This trend can be explained by higher soil temperatures and microbial-plant activity in the surface layers of soils. The H horizon contains the bulk of heather plant roots, and probably also the highest microbial density. Microbial population, root growth and plant exudates would thus be maximum in the H horizon, which consists of raw humus while the underlying grey-coloured bleached A_2 horizon has little organic matter, which is the key substrate of soil respiration. Respiration in the mineral B_2 layer is constant with no fluctuations between all the plots because it is a root-free zone and the soil material would not be able to support a proliferating microbial population. Ross and Tate (1993) documented microbial C and N, and respiratory activity in a Beech forest soil and reported that microbial C and N, and total soil C and N percentages decrease with depth. The lower CO_2 fluxes measured in late October 2001, at the end of the growing season in autumn, can thus be attributed to reduced activity with decreasing carbon inputs, as a result of which the CO_2 profile in the soil strata drops to a slightly lower level.

Soil respiration for each horizon increased across the incubation temperature gradient. The rates of respiration from all the horizons increased with temperature in accordance with the exponential first order equation. The Q_{10} for the horizons decreased with depth and varied between 2.53 – 2.00 over the temperature range of 6 °C to 25 °C. This result compares well with the findings of Ross and Tate (1993) and Winkler, Cherry and Schlesinger (1996) where metabolic quotients in all samples, and under all incubation conditions were higher in organic than in mineral horizons in a vertical soil profile. Under field conditions, soil temperature decreased with depth, as recorded by the temperature probe during the summer and early spring of 2000. Hence, the results suggest that *in situ* soil carbon dioxide fluxes would also

decrease down the soil profile since in the laboratory soil respiration increases with temperature.

No significant difference was noticed between the control and treatment plots during both sampling times but respiration and Q_{10} values were consistently higher in the treatment plots for the upper two horizons. This pattern suggests that the nitrogen inputs do support higher rates of respiration in the H and A_2 horizon but the fertiliser does not reach the lower B_2 horizon.

3.4.3 Incubation Time

Winkler, Cherry and Schlesinger (1996) incubated soil samples to find soil respiration rates increased exponentially in accordance with the Arrhenius equation for temperatures between 4 – 38 °C, however, CO_2 evolution varied significantly with time of incubation. In a similar incubation study, Holland, Townsend and Vitousek (1995) found that increasing the temperature from 5 - 15 °C caused large increases in CO_2 evolution whereas increasing the temperature beyond 25 °C had a lesser effect on respiration, which may be attributed to substrate limitation. In the incubation studies of this project it was demonstrated that respiration remains stable for two weeks after the samples had been collected and a high correlation between soil respiration and soil temperature, that is in general agreement with many previous reports (Oberbauer *et al.*, 1992; Bridgham and Richardson, 1992; Fang *et al.*, 1998). The main reason for the high correlation between CO_2 efflux and soil temperature in the experiment could be that soil moisture was never a limiting factor during the period of incubation. Thereafter a steady decline in soil respiration was observed till the end of the incubation period suggesting that the labile substrate concentration has decreased to a value lower in relation to the rates of respiration because the pool of labile carbon was not replenished by new inputs of organic C from roots and above ground litter. This trend, presented in Figure 3.8, contrasts with previous investigations (Pohhacker and Zech, 1995; Holland, Townsend and Vitousek, 1995; Winkler, Cherry and Schlesinger, 1996), wherein respiration rates decreased during

incubation without an initial steady state. However, the constant decline recorded in the studies may have been due to the pre-treatment, where homogenisation of the soil samples could have made larger surfaces available to microbial attack (Caberra and Kissel, 1988), thereby disrupting the natural decomposition regime. In the present study, the sharper decline in soil respiration after 15 days for all samples incubated at 15 °C, in comparison to samples at 5 °C, may be attributed to the fact that at higher temperatures, the easily decomposable fraction will be mineralised more quickly than at lower temperatures (Pal, Broadbent and Mikkelsen, 1975; Kralova, Kubat and Novak, 1980; Donnelly *et al.*, 1990; Trumbore, Bonani and Wolfi, 1990; Townsend, 1993; Schimel *et al.*, 1994). Hunt (1977) pointed out the risk of substrate depletion with exhaustion of readily available substrates under conditions favourable for microbial activity, but not under less favourable conditions. Thus soil respiration determined within the first two weeks of incubation would best represent natural field conditions where the microbial physiological response to temperature is not confounded with substrate limitation.

The soil respiration pattern over time is supported by the dynamics of Q_{10} for instantaneous C-mineralisation rates. The carbon mineralisation rates were clearly dependent on soil temperature between 5 and 15 °C. The temperature dependence was best described by the exponential Q_{10} model with higher Q_{10} values with increasing time intervals. The absolute values of Q_{10} during the period of incubation range between 2.38 and 2.85 for treatment and control samples, which fall within the reported range of 1.9 and 3.7 (Anderson and Domsch, 1986; Carlyle and Than, 1988; Crill, 1991; Kim and Verma, 1992; Hanson *et al.*, 1993; Howard and Howard, 1993; Peterjohn *et al.*, 1994; Fang *et al.*, 1998). Initially the Q_{10} values for both control and treatment plots are fairly constant, with a variation of no more than 0.002 for all the plots, and consistent with the mean value of 2.4 given by literature reviews (Raich and Schlesinger, 1992; Kätterer *et al.*, 1998), but when the easily decomposable fraction is almost completely decayed the Q_{10} values start rising sharply. This suggests that other interactions may influence the mineralisation response to

temperature, such as substrate quality (Novak, 1974; Bunnell *et al.*, 1977; Anderson, 1991) and incubation time (Lomander, Kätterer and Andrén, 1998). Therefore, it may not be advisable to derive the Q_{10} of C-mineralisation from the respiration rates at any one arbitrary incubation time, because the temperature effect is affected by the incubation-time effect. Nicolardot, Fauvet and Cheneby (1994) conclusively proved that mineralisation rates are influenced by substrate-temperature interactions. Subsequently, temperature responses will depend on the time selected for the analysis. One way to solve the problem faced by the incubation technique to determine the temperature response of soil carbon mineralisation under laboratory conditions is to calculate the Q_{10} values from only the respiration rates at the beginning of the incubation, soon after the initial flush, introduced by sample preparation because the samples are still unaltered. Soil respiration readings within the first week of incubation would best represent field measurements.

Nitrogen addition had no statistically significant effects on the response of soil CO_2 fluxes over time and increased temperature rates but ammonium nitrate treated samples incubated at 15 °C had higher respiration rates during the first 15 days and a sharper decline thereafter in comparison to the control samples. An identical trend was also observed by Söderström, Bååth and Lundgren (1993) on coniferous forest podzolic soils treated with ammonium nitrate fertiliser and incubated for 45 days, wherein supplemented soils had higher respiration rates during the first 5 - 12 days and subsequently lower CO_2 production rates as compared to control soils. The initial increase could be due to increased activity of microorganisms that were nitrogen limited and the decrease may be attributed to the more rapid depletion of carbon by the microbial population.

Control and treatment samples incubated for a period of 44 days at a constant temperature of 4 °C showed a clear dependence of carbon mineralisation rates on soil temperatures. Soil respiration was periodically measured and at every sampling time CO_2 efflux increased with increasing temperatures. The trend was reflected in the Q_{10}

values calculated by the exponential first-order equation. The initial Q_{10} values for all samples fall within the range of Q_{10} calculated by previous investigators (Schleser, 1982; Kirschbaum, 1995) and are similar to the average Q_{10} of 2.4 calculated by Raich and Schlesinger (1992). This result corresponds well with the findings of previous soil warming studies (Pohhacker and Zech, 1995). Peterjohn *et al.* (1993) found exponential increases in respiration rates with increasing temperature from measurements of a forest floor. It is interesting to note that even during the period of incubation, the response of soil respiration to temperature follows an exponential trend with an increase in incubation temperature. This suggests a significant robustness of the response of soil respiration to temperature even during a long period of incubation under fluctuating temperatures. However, a decline in the rate of soil respiration is seen earlier, after 5 days, in samples incubated at a constant temperature of 5 °C but measured across an increasing temperature gradient, as compared to after 15 days in samples incubated and measured at a constant temperature. This could be due to the rise in incubation temperature from 5 - 25 °C while taking measurements as a result of which more soil organic matter must have been consumed by the activated microbial population. In samples where a constant incubation and measurement temperature was maintained the microbial communities may have got acclimatised with a steady state of respiration.

3.4.4 Hysteresis

Soil respiration may vary significantly with fluctuating soil temperature and the response may be influenced by the temperature regime. The magnitude of the hysteresis shift in response to temperature cycles could provide a useful measure of labile carbon pools in litter and soil organic matter in a soil sample and hence the potential response of upland soils to rapid environmental change. Little is known about the uniformity of the response of soil respiration to temperature.

Few researchers have studied the influence of fluctuating temperatures on soil respiration during laboratory incubations of soil microcosms, especially when using

small intact cores. Chapman and Thurlow (1998) conducted peat incubation experiments to find differing results where the majority of samples showed no change in soil respiration rates whether the temperature was rising or falling but others showed a hysteresis effect with higher respiration rates for rising than falling temperatures. Anderson and Hetherington (1999) studied heather and bracken litter samples, and concluded that litter decomposition also shows a similar hysteresis pattern across a temperature gradient. However, Fang and Moncrieff (2001) subjected different continuously and staggered temperature changes on large intact soil cores from a sitka spruce site and could detect no significant change in soil respiration to varying temperature regimes.

The results suggest that soil respiration rates respond in an exponential manner to temperature variations of a small intact soil core over a range of 5 - 25 °C. However, soil respiration at a specific soil temperature behaves conservatively and is not significantly influenced by the temperature in preceding hours. The lack of hysteresis may be partially attributed to the stable chemical composition of peats and soil organic matter during the initial phases of decomposition (Anderson and Hetherington, 1999). The Q_{10} values fall within the generally accepted range of 1.9 – 3.7 (Anderson and Domsch, 1986; Carlyle and Than, 1988; Crill, 1991; Kim and Verma, 1992; Hanson *et al.*, 1993; Howard and Howard, 1993; Peterjohn *et al.*, 1994; Fang *et al.*, 1998) and do not deviate much from the mean value of 2.4 as suggested by literature reviews (Raich and Schlesinger, 1992; Kätterer *et al.*, 1998). The results agree well with the recent findings on large intact soil samples by Fang and Moncrieff (2001) wherein the different procedures for changing the incubation temperature, such as continuously increasing/decreasing or increasing and decreasing alternately, did not affect soil respiration rates. A similar result was also documented by Chapman and Thurlow (1998) investigating temperature responses of microbial respiration in 15 Scottish peats, with the exception of samples from two locations that showed higher respiration rates for rising than falling temperatures. The inconsistent data in their study may possibly have resulted from a high load of raw

litter in the soil samples because the chemical composition of litter is far less stable than that of soils and changes rapidly during the early stages of decomposition. Anderson and Hetherington (1999) studied heather and bracken litters incubated in a laboratory experiment under fluctuating temperatures to find microbial respiration of the litter samples showed a similar hysteresis pattern with higher rates of CO_2 production for rising temperatures than at the same temperature when temperatures were falling. Hence microbial respiration rates for a soil, as opposed to litter, are the same for a particular temperature whether the temperatures are rising or falling.

3.4.5 Field versus Laboratory Measurements

Laboratory measurements of soil respiration using the gas analysis system compared well with field measurements and there were no statistically significant differences between the comparative mean values for control and treatment plots. However, laboratory measurements were slightly higher than the field values. Hokkanen and Silvola (1993) also found that dependency of respiration measurements on temperature in intact soil corers differed slightly from those measured in the field and concluded that the enhanced mineralisation in the laboratory was due to pre-treatment, wherein the digging out of the sample would make larger surfaces available to microbial attack (Cabrera and Kissel, 1988) thereby increasing fluxes; a response that seems to increase with the clay content of soils (Craswell and Waring, 1972). Lomander, Kätterer and Andrén (1998) attributed the relatively higher CO_2 fluxes observed in their comparative study to the disintegration of carbonates upon homogenisation of the soil samples, which Coleman *et al.* (1980) showed may contribute 30 - 60 % of the carbon dioxide released from carbonate-rich soils. Field techniques using static, closed or dynamic chambers, are not entirely error free (Ewel, Cropper and Gholz, 1987; Raich and Nadelhoffer, 1989; Nakayama, 1990; Rochette, Gregorich and Desjardins, 1992; Hutchinson and Livingston, 1993; Dugas, 1993; Rayment, 2000) however, the gross over-estimation of C mineralisation by the laboratory technique is almost certainly due to the pre-treatment. In the present study where intact soil corers were collected with minimum disturbance to the structural

integrity of the soil, the laboratory measurements compared well with the field measurements. The insignificant differences between the field and laboratory measurements could also be attributed to the fact that removing the H horizon in the field may have caused an initial flush of CO_2 from the disturbed pore spaces within the soil matrix thereby over-estimating soil respiration for the remaining profile. Nevertheless, the differences were negligible thus suggesting that the laboratory incubation technique using intact soil corers as described in this chapter is an accurate, reliable and rapid technique.

3.5 CONCLUSIONS

This experiment showed that CO_2 fluxes from soil can be successfully measured in the laboratory using small intact soil cores. Microcosms provide a rapid and convenient way to measure soil respiration and allow considerable replication. There was no evidence to suggest a depletion of substrate in the small samples over a periods of several days. Ideally soil respiration must be estimated within the first two weeks of a laboratory incubation experiment using small intact soil core samples because decomposers may become substrate-limited after a period of time. The initial period of CO_2 flush is considerably reduced by employing a small volume of soil, surrounded by an aeration space, which also allows thermal equilibrium to be quickly achieved within the soil chamber. The laboratory incubation technique can accurately estimate the response of soil respiration to temperature under controlled conditions. The results suggest that in all the horizons, soil respiration in response to temperature follows the exponential first order equation with an increase with increasing temperature. However, soil carbon dioxide fluxes decrease with depth, and the decline down the soil profile may be caused by decreasing temperatures and lower microbial activity. Ammonium nitrate fertiliser did not show a statistically significant effect on heathland soils but respiration in plots that received inputs of nitrogen inputs was higher in both the H and A_2 horizons as compared to the control

plots. Heathland soils rich in organic matter did not show a hysteresis effect. Laboratory measurements with the gas analysis system compare well with field measurements proving that the gas analysis system designed to measure carbon dioxide fluxes from intact soil corers provides an accurate and reliable laboratory technique for determining soil respiration.

4. ESTIMATING SOIL MICROBIAL BIOMASS

4.1 INTRODUCTION

Soil microorganisms play a critical role in the sustainability of ecosystems by being both a source and sink for plant nutrients. On one hand, the microbial biomass in soil is a relatively large and labile pool of organic matter containing important plant nutrients, such as nitrogen and phosphorus, and on the other hand, microorganisms are also the main mediators of carbon turnover in soil. During soil organic matter decomposition, nutrients pass from the organic matter, through the microbial population and are released to become available for plant use. Thus, soil microbial biomass performs an important function in nutrient cycling and the capacity of microoganisms to serve as a relatively labile source of nutrient elements in soils, is well recognised (Bonde, Schnürer and Rosswall, 1988; Duxbury, Lauren and Fruci, 1991; Franzluebbers, Hons and Zuberer, 1994).

A measure of the size of soil microbial biomass is of importance in studies of nutrient cycling in soils and has been used as an ecological marker (Smith and Paul, 1990). It has been found that within certain limits there often is a close relationship between the soil microbial biomass and the soil's organic carbon content (Jenkinson and Ladd, 1981; Smith and Paul, 1986), although the underlying mechanisms for this relationship are less well understood. The relationship between the size of the biomass and the soil organic carbon content has been shown to be modified by factors such as the macroclimate and the presence of pollutants (Chander and Brookes, 1991; Christie and Beattie, 1989). Microbial life in the soil is largely determined by environmental factors. Seasonal changes in soil moisture, soil temperature and carbon input from plant roots, rhizosphere products (such as, root exudates, mucilage and sloughed cells) and plant residues can have a great effect on soil microbial biomass and its activity (Ross, 1987), which in turn, affect the ability

of soil to supply nutrients to plants through soil organic matter turnover (Bonde and Rosswall, 1987). Nitrogen fertilisation has been documented to enhance the growth of forest trees in boreal and temperate podzolic soils (Söderström, Bååth and Lundgren, 1983; Tamm, 1991) but apart from increasing plant growth, the fertiliser treatment may also affect the soil animals (Lohm *et al.*, 1977) and microorganisms. A number of studies dating back to the 1960's have shown that after nitrogen inputs, an increase in microbial activity occurs (Roberge and Knowles, 1967; Salonius and Mahendrappa, 1975; Roberge, 1976; Kelly and Henderson, 1978; Van Cleve and Moore, 1978). However, these results were in contrast to other studies, in which after urea or ammonium nitrate additions, soil microbial activity and biomass were significantly lowered in fertilised plots (Leuken, Hutchinson and Paul, 1962; Viro, 1963; Nömmik and Popovic, 1971; De Jong, Schappert and MacDonald, 1974; Kowalenko, Ivarson and Cameron, 1978; Bååth, Lundgren and Söderström, 1981; Söderström, Bååth and Lundgren, 1983). The inconsistency in the cited results suggests the subject clearly merits further studies on the effect of nitrogen fertilisation on soil microorganisms.

Microbial biomass is an active participant in nutrient cycling that mediates residue decomposition, which results in the efflux of carbon dioxide from the soil surface that represents a major flux of carbon to the atmosphere. Estimating the pool sizes of microbial carbon and nitrogen is therefore required for understanding one of the important driving forces behind soil respiration. The ability to accurately quantify soil surface carbon dioxide flux *in situ* is of importance if we are to improve our understanding of the soil carbon budget. One of the key questions in climate change research relates to the future dynamics of the large amount of carbon that is currently stored in the soil organic matter, and specifically, whether the amount of carbon in this pool increases or decreases with global warming (Grace and Rayment, 2000). The future trend in amounts of soil organic carbon will depend on the relative climatic sensitivities of net primary productivity and soil organic matter decomposition rate. Literature on the climatic effects on soil microbial biomass is

however limited and hence, there is a need to link the seasonal patterns of soil carbon and nitrogen with climatic conditions and elevated nitrogen inputs. Nitrogen fertilisation enhances the growth of *Calluna vulgaris* (L.) Hull in temperate podzolic soils as documented in the pilot study. Apart from increasing plant growth, the fertiliser treatment may also affect microorganisms. Indeed, the stimulation of plant growth may have been caused partly by enhanced mineralisation of the organic matter. In this study, biomass-C and biomass-N for the control and treatment plots was measured to study the effects of nitrogen fertilisation on the microbial biomass. The seasonal distribution of carbon and nitrogen, and fertiliser-induced changes in C:N ratios is also investigated. Measurements of ninhydrin-reactive nitrogen released and extracted from fumigated soils are known to provide a useful sensitive assay of biomass-C and biomass-N, however the relationship varies for different soil types (Carter, 1991; Rowell, 1994). The experiment also aims to determine the relationship between biomass-C, biomass-N and ninhydrin-N for heathland soils.

4.2 MATERIALS AND METHODS

4.2.1 Soil Collection

Soil samples were regularly collected, for a period of one year from September 1999 to September 2000, from the control and treatment plots established at the experimental field site located at Castlelaw Hill, in the Pentland Hills of Penicuik near Edinburgh. Soil samples for microbial studies were collected from the H horizon of the soil profile at the same instance when microcosms were collected for laboratory soil respiration measurements and the field conditions of soil temperature, pH, moisture and organic matter content were simultaneously monitored. In autumn and winter, samples were collected every alternate month viz. September, November 1999, February 2000 while during spring and summer the frequency of collection was increased to once-a-month from March through till September 2000, with the exception of June. Samples were transported to the laboratory in a cold box and

thereafter, stored overnight in a cold room maintained at 5 °C before microbial biomass analysis on the following day.

4.2.2 Microbial Biomass Analysis by Fumigation-Extraction Method

The estimation of soil microbial biomass by the fumigation-extraction (FE) method works on the basic principle that soil microorganisms die after their cell membranes are attacked by a strong fumigant like chloroform, and part of the microbial constituents, especially in the cytoplasm, is degraded by enzymatic autolysis and transformed into extractable components (Öhlinger 1996 a, b). Microbial biomass-C and biomass-N may be estimated by the FE method using chloroform as the fumigant and potassium sulphate as the extractant.

a) Biomass-C

The fumigation-extraction method was performed according to Vance, Brookes and Jenkinson (1987). Each moist soil sample was split into two portions of 25 g (on an oven dry basis), one for fumigated and the other for the unfumigated treatment. One set of samples was fumigated with ethanol-free chloroform for 24 hours at 25 °C in a vacuum oven (7627F, Gallenkamp, Loughborough, England) containing a vial of soda lime. After fumigant removal all the samples were extracted with 100 ml of 0.5 M potassium sulphate on an orbital shaker set at 80 rpm (5B-6736B, Gallenkamp, Loughborough, England) for 1 hour. The samples were filtered through filter paper (Ashless Paper 42, Whatman Ltd., Maidstone, England) for 3 hours and the clear filtrate diluted in the ratio 1:1 with 5 % sodium hexametaphosphate solution, pH 2.1. Organic C in the extracts was measured using a total organic carbon analyser (Dohrman 80, Sartec Ltd., Kent, England) with UV-persulphate oxidation and IR detection (Wu *et al.*, 1990). Microbial biomass-C was calculated as follows:

$$MB_C = E_C / k_{EC}$$

where, E_C is (organic C extracted from fumigated soil) – (organic C extracted from unfumigated soil) and k_{EC} is the extractable component of microbial biomass-C, which was estimated to be 0.45 for C analysis after UV-persulphate oxidation (Wu *et al.*, 1990; Joergensen, 1996).

b) Ninhydrin-Reactive Biomass-N

The soil filtrate generated from the fumigation-K_2SO_4 extraction technique was utilised to determine biomass ninhydrin-reactive N as describes by Joergensen and Brookes (1990). The clear filtrates were treated with 0.2 M citric acid buffer and ninhydrin reagent in thick-walled glass test tubes, mixed thoroughly and heated for 25 minutes in a boiling water bath (CC20-CC25, Grant Instruments, Cambridge, England). The solutions were then cooled to room temperature, a 1:1 ethanol-water solution added to each tube and the absorbance read at 570 nm in a spectrophotometer against a water blank. Microbial biomass ninhydrin-reactive-N was calculated as follows:

$$MB_{Nin} = E_{Nin}$$

where, E_{Nin} is (ninhydrin-N extracted from fumigated soil) – (ninhydrin-N extracted from unfumigated soil)

Ninhydrin-N released by fumigation is significantly correlated to microbial biomass-N, biomass-C and total microbial biomass and the relationships can be defined by following the Rothamsted conversions (Ocio and Brookes, 1990), wherein the factors used were:

$$MB_N = 4.6 \times MB_{Nin}$$

$$MB_C = 31 \times MB_{Nin}$$

$$MB_T = 62 \times MB_{Nin}$$

where, MB_N is microbial biomass-N, MB_C is microbial biomass-C, MB_T is microbial biomass-total and MB_{Nin} is ninhydrin-reactive microbial biomass-N.

The conversion factors stated are an average for all soil types and hence may vary slightly for specific soil types under varying environmental conditions.

4.3 RESULTS

An important observation for microbial biomass was that biomass-C and biomass-N for both treatment and control followed a similar annual pattern of increasing at the height of winter, decreasing slightly over spring and then reaching a steady state during summer and autumn (Figure 4.1 and Figure 4.2). However, treatment charted the same trend as control but at an elevated level, and the difference between treatment and control increased progressively with cumulative fertiliser input.

4.3.1 Biomass-C

A statistically significant difference ($P < 0.001$) in biomass-C between treatment and control samples was visible but not great for the first six months of the experiment (Figure 4.1). Subsequently, with an increase in cumulative N inputs, treatment samples exhibited a gradual increase evident from May 2000, corresponding to the onset of growth. During the study, the difference between the control and treatment plots rose from 0.12 mg MB_C g^{-1} dry soil to 0.80 mg MB_C g^{-1} dry soil. Biomass-C measured for the treatment plots in September 1999 and 2000 was the same at 3.37 mg MB_C g^{-1} dry soil with seasonal fluctuations through the year, but control values steadily fell from 3.26 mg MB_C g^{-1} dry soil to 2.57 mg MB_C g^{-1} dry soil over the period of one year. After the one-year fertilisation period, the treatment recorded an average annual mean value of 3.30 mg MB_C g^{-1} dry as opposed to 2.28 mg C g^{-1} for control.

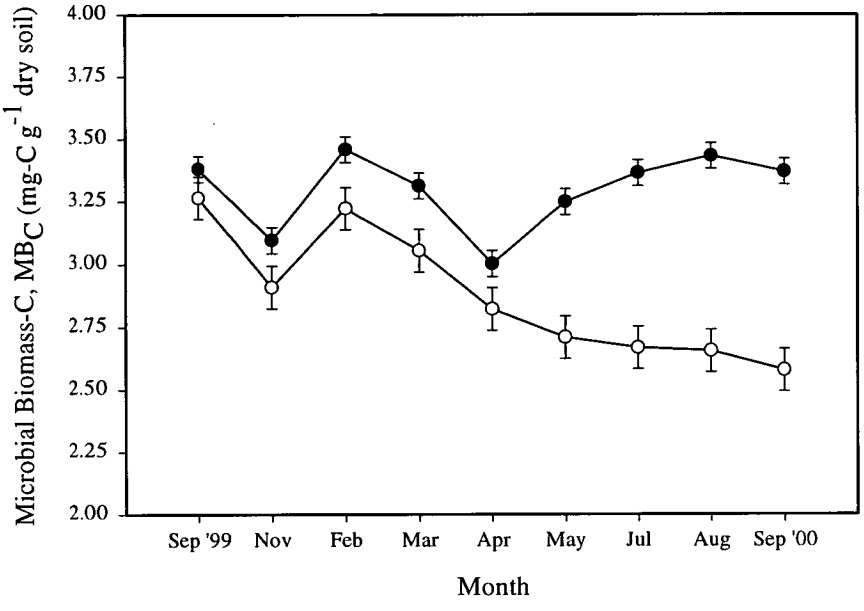


Figure 4.1 The effect of ammonium nitrate fertiliser treatment on soil microbial biomass-C, MB_C, for control (o) and treatment (•) samples (mean ± S.E., n = 9) as measured by the fumigation-extraction method from September 1999 to September 2000.

4.3.2 Biomass-N

Fertiliser inputs had a significant effect ($P < 0.001$) on microbial biomass-N, with treatment and control plots exhibiting annual trends that were similar to biomass-C (Figure 4.2). After April 2000 the concentration of microbial biomass-N (MB_N) per gram of dry soil with the treatment plots started increasing while control showed a gradual decline. After one year's treatment, plots showed an increase of 0.002 mg MB_N g^{-1} dry soil from 0.108 mg MB_N g^{-1} dry soil in September 1999 to 0.110 in September 2000 while the control recorded a drop of 0.022 mg MB_N g^{-1} dry soil from 0.104 mg MB_N g^{-1} dry soil to 0.082 in the same period of time. At the end of the study, samples that received 60 kg N ha^{-1} yr^{-1} recorded an annual average of 0.106

mg MB_N g^{-1} dry soil in comparison 0.091 mg MB_N g^{-1} dry soil measured in the control plots.

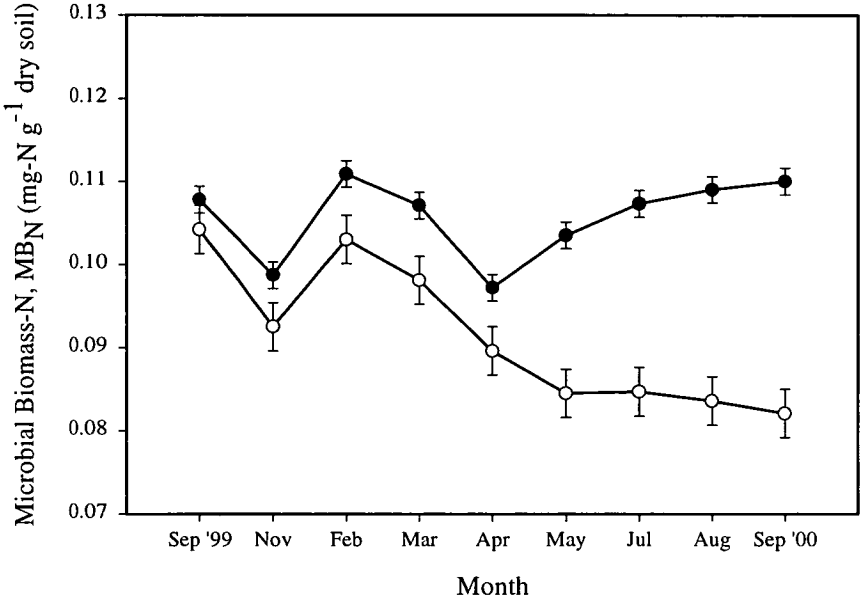


Figure 4.2 The effect of nitrogen inputs on ninhydrin-reactive soil microbial biomass-N, MB_N, for control (o) and treatment (•) samples (mean ± S.E., n = 9) as determined by the fumigation-extraction method from September 1999 to September 2000.

Biomass-C calculated indirectly from ninhydrin-reactive N agreed very closely with the observed values for biomass-C during the entire period of study. There was no significant difference between the observed and calculated values of biomass-C with the average deviation in mean readings being 0.043 and 0.003 for control and treatment samples respectively.

4.3.3 Microbial C:N Ratio

The microbial C:N ratio as derived indirectly from biomass-C and biomass-N, both calculated as functions of ninhydrin-reactive N, agree closely to the observed values. The observed mean value 6.81:1 for the C:N ratio of the soil microbial biomass compares well with the calculated C:N ratio of 6.74:1. Both the observed and calculated mean C:N ratios of soil microbial biomass closely agree to the mean $MB_C:MB_N$ value of 6.7 as reported by Anderson and Domsch (1980) and Shen, Pruden and Jenkinson (1984) for a heterogeneous population of soil microorganisms. Fertiliser input did not have a statistically significant effect on the observed microbial C:N ratio as shown in Figure 4.3.

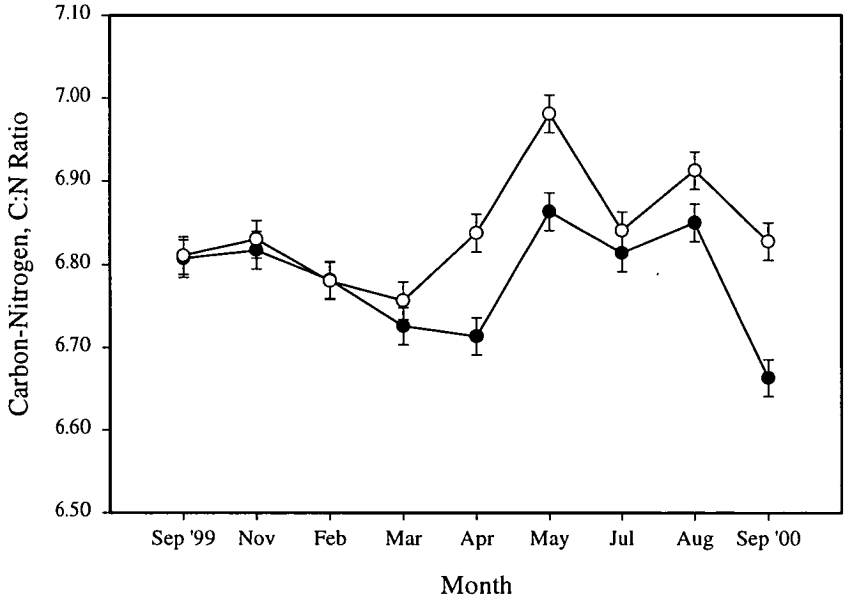


Figure 4.3 Profile of the time course of the effect of fertiliser treatment on the soil microbial C:N ratio for control (o) and treatment (•) samples (mean ± S.E., $n = 9$) over a period of one year.

However, after four doses of fertiliser application during the first 7 months of the study, treatment samples consistently showed a lower microbial C:N ratio as compared to the control plots. Hence confirming that microbial-N within the treatment samples increased with time while control samples had decreasing N values as a result of which the C:N ratio steadily decreased.

4.4 DISCUSSION

Nutrient additions to an ecosystem can have a marked influence at the species level, especially in a system that is nutrient limited (Macura and Kunc, 1961). Plant responses to environmental change are underpinned by mechanisms that control microbial community processes and by potential nutrient feedback loops that govern net primary production in terrestrial ecosystems (Cardon, 1996; Berntson and Bazzaz, 1997; Robinson and Conroy, 1999). Several authors have shown that in nutrient-poor ecosystems, the nitrogen concentration is an important factor which largely regulates the organic matter decomposition process (Berg and Staaf, 1980 b; Melillo, Aber and Muratore, 1982) and past studies on soil transformation processes have documented significant increases in nitrogen mineralisation with nitrogen salt additions (Johnson and Guenzi, 1963; Broadbent and Nakashima, 1971; Heilman, 1975; Bååth *et al.*, 1978). In this study, inorganic N application resulted in significantly higher microbial biomass within plots that were treated with ammonium nitrate as compared to plots that did not receive nitrogen inputs during the period of study. Insam, Mitchell and Dormaar (1991) also found the effect of inorganic fertilisation on microbial biomass was more pronounced in soils with low nutrient status, such as those of heathlands and moorlands.

Cheng (1999) put forth a 'preferential substrate utilisation' model to explain how the nutritional status of a soil determines the process of mineralisation. The hypothesis states that that in fertile soils, micro-organisms preferentially exploit labile root-

derived carbon to soil-derived carbon resulting in decreased SOM decomposition but if mineral nutrients are in short supply then soil micro-organisms must resort to utilising nutrient-rich soil organic matter, resulting in increased SOM decomposition. Therefore, microbial activity is essentially governed by the surplus of an easily metabolised energy source. Liljeroth, Kuikman and Van Veen (1994) suggested that negative effects on SOM decomposition in high N availability could be due to a disruption of the competition between potent and less potent decomposers or a decrease in the production of specific enzymes by fungi.

The seasonal trend observed for microbial biomass-C and biomass-N in this study suggests that during the winter period ecosystem activity is at a minimum and the nutritional requirements of both plant and microbial communities can be met by the background nutrient levels of soil (Insam, Parkinson and Domsch, 1989). In late spring, as the soil temperatures begin to rise, the heather growing season commences and plant nutrient uptake rapidly increases, thereby decreasing the source of freely available nitrogen for the microbial population to maintain itself (Drury, Voroney and Beauchamp, 1991). Hence, the control plots record a decline during summer but in the treatment plots, receiving regular inputs of nitrogen, the microbial population is sustained at a steady state. Berendse, Berg and Bosatta (1987) studied the effect of nitrogen on the decomposition of litter in nutrient-poor ecosystems and also concluded that nitrogen limits microbial growth, if the soil nitrogen concentration is below a critical level and the external supply of inorganic nitrogen is not sufficiently great to satisfy the nutritional needs of the growing biomass. The annual pattern of change for microbial biomass in the control plots was high in autumn, low in winter, fluctuating in spring due to fluctuating spring temperatures (Martin and Kemp, 1980) and low in summer, which agrees with studies conducted on pastoral soils (Lynch and Panting, 1982; Sarathchandra *et al.*, 1988; Sarathchandra, Perrott and Littler, 1989). A similar trend was observed for treatment plots though with an increase through summer.

Seasonal changes in microbial biomass associated with the low concentrations of total inorganic-N measured in the rhizosphere soil for both control and treatment plots, shown in Chapter 5, suggest that there is a strong competition for mineral nutrients between root and soil micro-organisms. Under conditions of nutrient stress, the nutrient cycling process becomes stringent with lower inputs being counterbalanced by lower losses resulting in a decrease of plant nutrients released (Cheng, 1999). Hence, with the commencement of the plant growing season, the microbial community would solely rely on SOM decomposition to meet all nutritional requirements, which is supported by the annual trend observed for soil organic matter content, shown in Chapter 5, wherein a sharp decline in total organic matter was recorded in May 2000. During summer, through till autumn, MB_C and MB_N were noted to gradually decrease in the control plots with the depletion of nutrients and the effectiveness of the internal circulation of nitrogen between the soil organisms was probably less effective than the root uptake of the system. However, the treatment plots showed a gradual increase because the N inputs would provide additional available soil nutrients, aid plant growth and also increase fine root growth, thereby increasing organic C inputs via the roots in the soil, which would further support microbial activities. Anderson and Domsch (1986) proposed that the stabilised fraction of the C input to the soils is the basis for the size of the soil microbial biomass under steady-state conditions and the labile fraction would then be responsible for the short-term fluctuations of biomass content during the year. The theory is supported by studies of seasonal changes in microbial biomass in wheat management systems which showed soil microbial biomass-C to increase during the flowering season in early spring due to increased C inputs from rhizosphere products to the soil (Xu and Juma, 1993). Therefore, the effect of fertiliser inputs may also be indirect, wherein inorganic N stimulates plant growth with increased C inputs by enhancing production of root and shoot biomass (Fauci and Dick, 1994), and promoting the release of C exudates (Coleman, 1976; Prikryl and Vancura, 1980). The trend observed for treatment plots supports the 'priming effect' theory (Jenkinson, Fox and Rayner, 1985) that suggests that the extra input of labile root-

derived C initially decreases SOM decomposition as a result of the increase in immobilisation of mineral nutrients, but subsequently stimulates SOM decomposition and nutrient release with the turnover of this newly grown microbial biomass. The rate at which these reactions take place would determine the overall N availability, with important consequences on long-term nutrient dynamics.

The priming effect is supported by the findings of this study. In a uniform substrate with known chemical components, the rate of mineralisation or immobilisation is a positive linear function of the C:N ratio of the substrate (Rowell, 1994). If the C:N ratio is higher that the critical C:N ratio, a net release of nitrogen is expected and a net immobilisation of nitrogen occurs if the C:N ratio is below the critical threshold value. Moreover, high C:N ratios are prevalent in soils where conditions are less favourable for decomposition and the C:N ratio becomes narrower as soil organic matter is rapidly broken down by microbes (Bosatta and Staaf, 1982). The soil C:N ratio, as determined for the experimental field site at the end of the study (Chapter 5), showed a high value in the control plots and a comparatively lower value in the treatment plots suggesting that fertiliser inputs may increase microbial populations in soil with a shift to net immobilisation, thereby increase SOM decomposition and control the release of N in soils.

Thus the results suggest that the long-term effect of inorganic N additions on MB_C and MB_N under field conditions may be a combination of direct and indirect nutrient availability to the plant and microbial populations during changing seasons of plant growth.

The continuous supply of energy and nitrogen to the soil mixture would also strongly influence the microbial population. Ingestad (1962) showed that in nitrogen deficient conditions of a pine forest soil, additions of N had a positive effect on the fungal mycelium biomass. Moreover, very few algae were found in the upper horizons in the forest soils, all arthropods were fungivorous and the species composition of

nematodes shifted to the fungivorous species. The microbial C:N ratio provides an insight into the relative biomass of fungal and bacterial populations within a soil microbial community. Hence, the results of this study indicate that increased ammonium nitrate inputs to the treatment plots, not only supported plant and microbial growth but also increased bacterial populations in relation to the fungal population, and in turn could have influenced the microbial consumers. Anderson and Hetherington (1999) also suggest that N treatments affect the composition and activities of fungal communities in heather moorland and Park (1976) found that the cellulolytic ability of a wide range of fungi showed positive responses to increasing concentrations of mineral N in cultures. Hence the structure of fungal communities that decompose plant materials, and their net functional characteristics are affected by application of mineral-N and the extent to which is determined by the total N inputs. Build up of microbial biomass as evidenced by accumulation of MB_N during winter and early spring in all plots may be a result of the dominance of fungal biomass decomposing dead roots because bacteria in the rhizosphere mainly depend on soluble root exudates for energy but fungi on the other hand are able to digest cellulose and pectin and obtain energy from dead roots and organic matter (Newman, 1985). Onset of higher temperatures in late spring may induce considerable release of N from soil microbial biomass, which would supplement the nutritional needs of the plants, increase plant activity, and thereby cause a decline of microbial nutrients with a decrease of fungal biomass in the control plots. However, the same effect was not observed in the treatment plots, which continue to receive N inputs, and these nutrients being taken up by growing plants and microbial communities. Nicolardot, Fauvet and Cheneby (1994) concluded that variations in nutrient releases from the biomass might be attributed to changes in atmospheric temperature. A controlled climate experiment using soil cores revealed that microbial N, and anaerobic mineralisation increased during winter-early spring and then declined with increasing temperatures (Sarathchandra, Perrott and Littler, 1989) and in a study by Nicolardot, Fauvet and Cheneby (1994), more carbon was incorporated in the microbial biomass at low temperatures of 4 °C. Okano, Nishio and Sawada (1987) calculated that 21 kg

ha^{-1} yr^{-1} of N was released through the soil biomass in the root mat layer of a pastoral soil, and concluded that soil biomass plays an important role as a source of available N. Therefore, the seasonal trend observed for the microbial biomass was likely to be driven by the environmental parameter of temperature and suggests that the fluctuations portray changes in the composition of microbial population.

The relative amounts of fungal and bacterial biomass are reflected in the microbial C:N ratios. The observed and calculated C:N ratios of microbial biomass, namely 6.81 and 6.71, compared well with data in the literature and helped give an insight into the species composition of the microbial population. Anderson and Domsch (1980) found a mean C:N ratio of 5.61 for 10 species of soil bacteria; Marumoto, Anderson and Domsch (1982) a mean of 4.59 for an actinomycete and a bacterium and Jenkinson (1976) a mean of 3.83 for 7 bacteria and actinomycete, giving an overall mean of 4.85 for all 19 organisms of bacteria and actinomycetes. However corresponding values for 14 species of soil fungi were much higher at 8.26 (Anderson and Domsch, 1980); for 2 species of fungi and 2 yeasts 8.08 (Jenkinson, 1976) and for 3 species of fungi 10.26 (Marumoto, Anderson and Domsch, 1982), giving a weighted mean for all 21 organisms of 8.51. Anderson and Domsch (1980) proposed a mean value of 6.7 for the C:N ratio of soil microbial biomass based on a study of 3 fungi and 1 bacterium. Shen, Pruden and Jenkinson, (1984) also supported a common value of 6.7 for all microbial biomass, assuming that the C:N ratio of the larger spherical organisms, like protozoa and fungal spores, are similar to those of bacteria and that the ratio of hyphal to spherical biovolume is 1 (Jenkinson, Powlson and Wedderburn, 1976, mean value for 8 soils). However, it must be stressed that most C:N values were derived for microflora and the value of 6.7 for the C:N ratio of soil biomass was computed from measurements made on organisms grown *in vitro* and the relevance to soil organisms *in situ* is not confirmed by models computing the dynamics of carbon and nitrogen in soils (McGill *et al.*, 1981). In this study, microbial biomass-C and biomass-N were determined for soil samples collected from

the field and hence proves that the value of 6.7 is an accurate estimate of the soil microbial C:N ratio under natural *in situ* conditions.

Anderson and Domsch (1980) concluded that higher microbial C:N ratios could be indicative of a comparatively high fungal component growing in an environment with appreciable available C, as seen in the control plots wherein the soil microbes were relatively starved of nitrogen. Hence, the narrower MB_C:MB_N ratio, as observed in the treatment plots, would indicate a shift from fungal to bacterial populations. No qualitative data were available for the experimental site but the occurrences of large and varied fungal populations in the soil profile of heathlands has been reported (Gimingham, 1992).

In summary, the changes in microbial C and N pools would have resulted from the seasonal differences in C and N inputs from litter and roots in conjunction with rates of N immobilisation in the present study, as hypothesised by the 'added nitrogen interaction' (ANI) or 'priming effect'. The increase or decrease of biomass-C and biomass-N would also be influenced by climatic conditions, especially by temperature and soil organic matter content. The C:N ratio in both control and treatment plots recorded a low in winter and early spring, but a sharp increase is observed in summer, followed by a drop in autumn. The decrease in biomass corresponds to the decline in available substrates with the commencement of the plant growing season (Robertson *et al.*, 1988; Sarathchandra, Perrott and Littler, 1989), and the simultaneous release of nutrients from biomass which is, in turn related to the temperature (Anderson and Domsch, 1986; Insam 1990). Ross and Tate (1993) also found that microbial N differed significantly between the late-spring and autumn samples as a result of which MB_C:MB_N were significantly higher and lower respectively for the two seasons, and hence stated that climatic factors have a major impact on the overall levels of microbial biomass in the uppermost litter layers. Therefore, short-term fluctuations in soil microbial biomass may increase microbial

activity and subsequent turnover rates are important factors in the nutrient transfer from soil to plants (Bottner, Sallih and Billés, 1988).

4.5 CONCLUSIONS

This study indicated that fertiliser treatment sustained and increased microbial biomass. Nitrogen inputs were rapidly utilised by the microbial population which caused a decline in the C:N ratio of microbial biomass. The change in C:N ratio also indicated a possible shift in microbial species composition from fungi to bacteria. It may therefore be concluded that ammonium nitrate fertiliser treatment may act in conjunction with climatic conditions, like temperature, to set annual patterns of nitrogen utilisation by plants and microbes within a soil profile and is capable of altering the storage and release of nutrients by causing a shift in species dominance.

5. MEASURING CO_2 FLUXES FROM HEATHLAND SOILS

5.1 INTRODUCTION

Soils are a major reservoir of carbon in terrestrial ecosystems, containing more than two-thirds of total carbon in the terrestrial fraction of the biosphere (Lin *et al.*, 1999), and the evolution of carbon dioxide from soils represents a major component in the global carbon cycle (Baldocchi *et al.*, 1986). Estimates of carbon recycled to the atmosphere from belowground sources range from 70 (Raich and Schlesinger, 1992) to greater than 100 Pg per year globally (Musselman and Fox, 1991) and therefore, the response of soil carbon stores to anticipated climate changes is of vital importance. Even small percentage changes in the average soil carbon pool could result in relatively large changes in fluxes of CO_2 to the atmosphere and measurement of climate-driven changes in fluxes can be useful indicators of changes in soil organic matter turnover, and rates of energy and nutrient cycling (Prentice and Fung, 1990; Trumbore, Chadwick and Amundson, 1996). Many studies have measured soil CO_2 fluxes and demonstrated the relationship between soil respiration and environmental factors, especially temperature, but we have limited knowledge of the magnitude of fluxes, the interrelationships between factors that regulate these fluxes, and potential responses of factors to climatic change. Moreover, there is rather little work on the effect of nitrogen deposition on the temperature sensitivity of the process, and in particular from heathland soils.

Early studies of soil respiration were summarised by Romell (1932) and since then estimates of soil respiration have been made in a range of ecosystems, which have been in reviewed by Schlesinger (1977), Singh and Gupta (1977), Raich and Nadelhoffer (1989) and Raich and Schlesinger (1992). Factors known to influence the rate of CO_2 evolution include soil temperature and moisture (Wiant, 1967;

Garrett and Cox, 1973; Edwards, 1975; Schlenter and Van Cleve, 1985; Weber, 1985; Fung, Tucker and Prentice, 1987; Naganawa *et al.*, 1989; Hanson *et al.*, 1993; Peterjohn *et al.*, 1993, 1994; Holland, Townsend and Vitousek, 1995; Fang *et al.*, 1998; Fang and Moncrieff, 2001), soil pH (Kowalenko, Ivarson and Cameron, 1978), soil nitrogen content (Söderström, Bååth and Lundgren, 1983), litter quality (Rout and Gupta, 1989), and content (Van Cleve and Sprague, 1971), soil organic content (Chapman, 1979; Ewel, Cropper and Gholz, 1987; Gordon, Schlenter and Van Cleve, 1987; Rout and Gupta, 1989), and management practices (De Jong, Schappert and MacDonald, 1974; Weber, 1985, 1990; Gordon, Schlenter and Van Cleve, 1987; Vose *et al.*, 1995). Since many of these factors vary temporally, either diurnally or seasonally, considerable variation in soil CO_2 evolution has been recorded (Garrett and Cox, 1973; Edwards and Sollins, 1973; Schlenter and Van Cleve, 1985; Hanson *et al.*, 1993; Lloyd and Taylor, 1994; Vose, Elliot and Johnson, 1994; Fang and Moncrieff, 2001).

Inherent soil properties have both direct and indirect effects on soil efflux, and interactions of these limiting properties can be complex (Keith, Jacobsen and Raison, 1997) and appear to vary greatly depending on environmental conditions at a site (Ellis, 1969; Holt, Hodgen and Lamb, 1990; O'Connell, 1987; Richards, 1981).

There have been insufficient studies to assess these relationships for heathlands and any changes in temperature, moisture, pH, and nutrient availability due to climate change or land use management such as addition of fertilisers could have important effects on carbon fluxes and rates of sequestration of carbon in heathland ecosystems. To predict such potential impacts on ecosystem carbon dynamics it is necessary to define the responses of both above-ground and below-ground components to changes in each of these environmental variables (Anderson, 1991). Moreover, few studies have examined the direct effects of nitrogen availability, in a nutrient-poor ecosystem, on soil CO_2 efflux (Keith, Jacobsen and Raison, 1997), although nutrient availability influences soil respiration via total productivity and

allocation of assimilate belowground, quality and quantity of the substrate entering the soil organic matter and biomass of the microbial community.

The specific objectives of the study were a) to quantify seasonal patterns of soil CO_2 evolution, b) to determine the impact of elevated nitrogen deposition on soil carbon fluxes by applying ammonium nitrate to experimental plots, c) to examine the temperature dependence of soil respiration under laboratory conditions with an intact soil incubation technique and d) to evaluate the capacity and accuracy of the exponential, quadratic and Arrhenius equations to define the response of soil respiration rates to temperature, in the absence of soil moisture limitations.

5.2 MATERIALS AND METHODS

5.2.1 Field Site and Treatment

Field experiments were carried out in experimental plots located at Castlelaw Hill (55° 52′ 22′′N, 3° 14′ 3′′W) in the Pentland Hills of Penicuik near Edinburgh (Ordinance Survey Map - Penicuik & Dalkeith Pathfinder 420 NT 26/36, Great Britain), at an altitude of 435 meters above mean sea level. The moderately steep hill slope was covered with a dense stand of heather, *Calluna vulgaris* (L.) Hull, and the site received an annual rainfall of 1200 – 1400 mm during 1999 - 2000. Soil at the site is a freely drained humus iron podzol averaging a depth of 25 cm. Total background levels of nitrogen deposition in the region is approximately 12 kg N ha^{-1} yr^{-1} (Fowler *et al.*, 1989). Six 3 m × 3 m plots were laid out, of which three were demarcated as control and another three as treatment plots. Nitrogen was applied to three randomly selected treatment plots as an ammonium nitrate, NH_4NO_3, solution in six, 10 kg N ha^{-1} doses over a period of one year; thus at a rate equivalent to a total of 60 kg N ha^{-1} yr^{-1}. Every N aliquot was uniformly applied over each treatment plot in 2 litres of distilled water as a fine mist using a Controlled Pressure Knapsack Sprayer (Oregon – KS. 15L P/N 100221, Blount, UK). The treatment plots were

fertilised every alternate month in August, October, December 1999 and February, April, June August 2000.

5.2.2 Sample Collection

A soil corer was designed to collect small, intact soil microcosms from the experimental plots with minimum disturbance to the soil structure. Microcosm samples for CO_2 flux studies were collected from the upper 5 cm of the soil surface in the H horizon of the soil profile, on a regular basis for a period of one year, from September 1999 to September 2000. In autumn and winter, samples were collected every alternate month viz. September, November 1999, February 2000 while during the growing season, samples were collected every month viz. March, April, May, July, August September 2000, with the exception of June.

5.2.3 Environmental Monitoring

Environmental variables of soil temperature, pH, organic matter and moisture were recorded at every sample time during the entire period of study from September 1999 to September 2000. Mineral-N, namely ammonium-N and nitrate-N, and phosphate ion concentrations in the plots was determined at the end of the study period in September 2000 after the treatment plots had received a total dose of 60 kg N $ha^{-1}yr^{-1}$ of ammonium nitrate fertiliser.

5.2.3.1 *Temperature*

Field temperature readings at sample collection times were recorded using a hand-held digital thermometer with a stainless steel sensor probe (AT1, Airflow Developments Ltd., High Wycombe, UK). A multi-channel, data logger (DL-5864, Delta-T Devices, Cambridge, England) with copper-constantan (type-T) thermocouples was used to log soil temperatures at 1, 3, 5, 10 and 20 cm depths for every half an hour interval during different seasons of the year.

5.2.3.2 *pH*

25 ml of distilled water was added to 10 g of moist soil and stirred thoroughly. pH was measured after 20 minutes using a pH meter (CD-620, Russell Scientific Instruments, Norfolk, England), calibrated using two buffer solutions with pH values of 4 and 7.

5.2.3.3 *Moisture*

10 grams of each soil sample were weighed in aluminium foil dishes and oven dried at 105°C for 18 hours for water content determination. The samples were cooled in a dessicator to room temperature and weighed again.

$$\text{Water content (\%)} = [(M_A - M_O) / M_O] \times 100 = \text{g } H_2O \text{ per 100 g air-dry soil}$$

where, M_A is mass of air-dry soil (g) and M_O is mass of oven-dry soil (g).

5.2.3.4 *Organic Matter as Loss on Ignition*

10 grams of soil was weighed into a porcelain crucible and oven dried at 105 °C for 18 hours and re-weighed. Each sample was ashed in a muffle furnace (FR-520, Gallenkamp, Loughborough, England) at 450 °C for 4 hours. The samples were cooled in a dessicator to room temperature and weighed again.

$$\text{Loss on ignition (\%)} = [(M_O - M_I)/ M_O] \times 100 = \text{g per 100g oven-dry soil}$$

where, M_O is mass of oven-dry soil (g) and M_I is mass of ignited soil (g).

5.2.3.5 *Soil C:N Ratio*

Organic carbon and nitrogen contents in soil samples were determined by the 'flash combustion' technique described by Verardo *et al.*, (1990) using a Carla Erba NA-1500 Analyzer and AS200 Autosampler interfaced with a Hewlett-Packard 3390A Integrator. The soil samples were tested for the presence of calcium carbonate by

treating a group of randomly selected sub-samples with 8 % sulphurous acid. No traces of calcium carbonate were observed in the samples viewed under a compound microscope, so the acidification step was omitted. The soil samples were oven dried at 60 °C for 36 hours. The samples were ground in a clean agate mortar and pestle, sieved using a 0.353 mm aperture sieve (Endecotts Ltd., London, England) and stored in high density polyethylene scintillation glass vials with linerless screw caps (Wheaton Science Products, New Jersey, USA). Approximately 5 mg of sample was weighed into tin foil capsules (Elemental Microanalysis Ltd., UK) using a microbalance (AT261 Delta Range, Mettler-Toledo Ltd., Leicester, UK) and the sample cups were carefully moulded into small round pellets measuring less than 5 mm in diameter with the help of fine forceps. The sample balls are placed into the autosampler tray and loaded into the autosampler of the NA-1500 Analyzer for direct analysis.

5.2.3.6 *Mineral N*

The chemical extraction techniques for determining soil ammonium derived nitrogen (NH_4-N) and nitrate derived nitrogen (NO_3-N), are important for the measurement of available nutrients in soil. Fresh soil was sieved through a 1.5 mm aperture sieve (Endecotts Ltd., London, England) and 10 g weighed into a glass bottle. 100 ml 1M KCl extractant was added to each sample and a set of blanks, then the bottles were well sealed and the solution thoroughly mixed on an orbital shaker (Gallenkamp, Loughborough, UK) for 2 hours. The solution was transferred to 15 ml centrifuge tubes and centrifuged (T52 Centrifuge, Clandon Scientific Ltd., Farnborough, England) at 4500 rpm for 20 minutes. NH_4-N was determined by the salicylate method using an autoanalyser system (Autoanalyser III, Bran and Luebbe, Norderstedt, Germany) as described in the Bran & Luebbe Application Sheet G-102-93. NO_3-N was determined using a flow-injection analyser (Flow Solution 3000 Analyser, Perstorp Analytical, USA), wherein nitrate is reduced to nitrite by passing through a cadmium column. The nitrite is colorimetrically determined as an azo dye

at 540 nm following a reaction with sulfanilamide and naphthylethylenediamine as described in the Perstorp Application Sheet 001975 Rev. B, 11/94.

5.2.3.7 *Phosphate*

The soil extraction procedure for soil phosphorus analysis is similar to the technique for soil mineral nitrogen determination. Fresh soil was sieved through a 1.5 mm aperture sieve (Endecotts Ltd., London, England) and 10 g weighed into a glass bottle. 100 ml of 2 % acetic acid extractant was added to each sample and a set of blanks. The bottles were well sealed and the solution thoroughly mixed on an orbital shaker (Gallenkamp, Loughborough, England) for 2 hours. The solution was transferred to 15 ml centrifuge tubes and centrifuged (T52 Centrifuge, Clandon Scientific Ltd., Farnborough, England) at 4500 rpm for 20 minutes. Phosphorus was determined as phosphate by the molybdate-ascorbic acid method using an autoanalyser system (Autoanalyser III, Bran and Luebbe, Norderstedt, Germany) as described in the Bran & Luebbe Application Sheet G-103-93.

5.2.4 Laboratory Measurements of Soil Carbon Dioxide Fluxes

Soil respiration in the laboratory was measured by an Infra Red Gas Analyser, IRGA, (ADC 225 MK3, Analytical Development Co. Ltd., Hoddesdon, England) with an 18 channel continuous flow multi-point gas analysing unit specially constructed for the study, as described in Chapter 3. A humidifier was placed in line to prevent drying of the samples in the soil chambers, which were maintained in a temperature-controlled water bath (W46-DC10-EK20, Haake, Germany). Carbon dioxide concentrations were recorded by a data-logger (21X, Campbell Scientific, Shepshed, UK) and the results displayed on a personal computer. The concentrations were converted to fluxes as follows:

$$R = \Delta C \times F / A$$

where, R is efflux rate of soil carbon dioxide, ΔC is the change in CO_2 concentration, F is air flow rate and A is surface area.

5.2.4.1 *Rate of Respiration*

The seasonal pattern of the response of soil respiration to temperature was defined on the basis of area, mass, microbial-C and microbial-N, so as to facilitate easy comparison with previously published data and account for the seasonal fluctuations in microbial biomass that naturally occur through the year. The three most commonly used models to describe the relationship between soil respiration and temperature, namely the first-order exponential, Arrhenius and quadratic equations, were fitted to the response of soil respiration across the temperature gradient ranging from 5 °C to 25 °C to determine the model of best fit.

5.2.4.2 Q_{10}

Q_{10} was computed according to the first-order exponential, Arrhenius and quadratic equations and the seasonal values compared to determine the model that best describes the response of soil respiration to temperature.

5.2.5 Data Analysis

A statistical approach was taken to parameterising the relationship in a multiple regression model and quantifying the interaction between all the variables and soil respiration. Regression analyses were used to explore relationships between carbon dioxide fluxes with environmental variables, and to examine the possible effects of fertiliser treatment. The statistical computer packages used were MINITAB (version 12.1 for Windows), SIGMA PLOT (Version 4.00 for Windows) and EXCEL (Office 97).

5.3 RESULTS

5.3.1 Environmental Monitoring

5.3.1.1 *Temperature*

Soil temperature of the top layer of the soil profile showed a strong seasonal pattern, increasing from April to August and then decreasing after September ($P < 0.001$), with no significant difference between the control and treatment plots (Figure 5.1 A). All plots followed the identical seasonal pattern through the year with the lowest temperature being recorded in the month of February 2000 and the highest in August 2000.

There was also a marked seasonal pattern in soil temperature at 1, 3, 5, 10, and 20 cm depth ($P < 0.001$) as documented earlier in Chapter 3 in Figure 3.7. The amplitude of diurnal soil temperature fluctuations was greater in the upper 5 cm of the soil, especially during the summer season. During late autumn and winter, the soil temperature range decreased across the entire soil profile and did not exhibit sharp diurnal changes. Nitrogen addition did not affect the soil temperature.

5.3.1.2 *pH*

The control plots followed an annual pattern wherein the pH gradually decreased from pH 3.93 in September 1999 to pH 3.40 in spring of 2000, and then increased again in summer to reach a pH of 3.91 (Figure 5.1 B). In contrast, the addition of ammonium nitrate solution affected the soil pH of the treatment plots, with a pH drop of 0.75 in the year from 3.90 in September 1999 to 3.15 in September 2000. Treatment plots also showed a drop in pH in autumn but the steady decline continued through winter, spring and the summer with the acidifying effect of the fertiliser becoming clearly visible in March 2000 after the first four doses of ammonium nitrate.

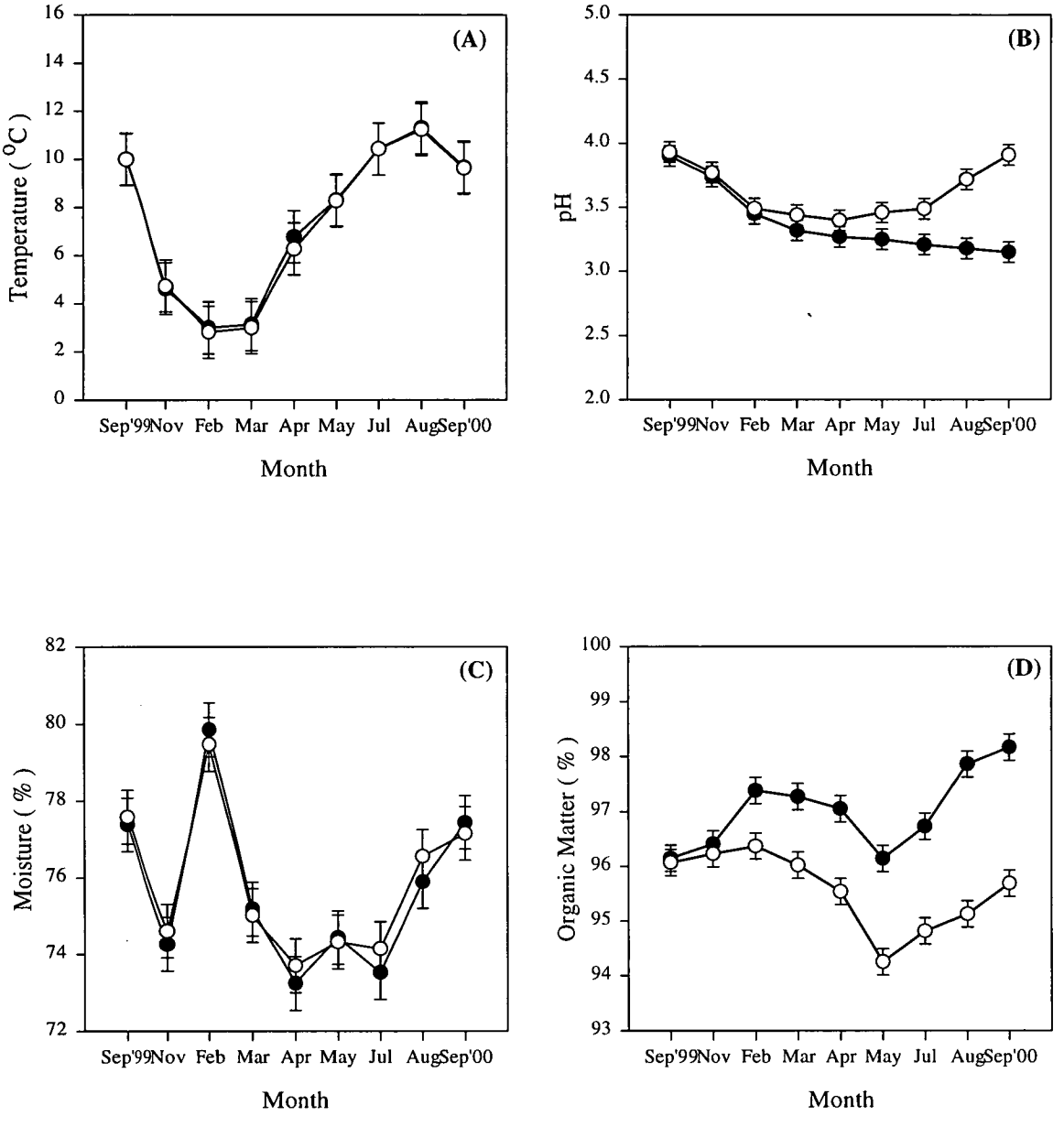


Figure 5.1 The seasonal pattern plotted by the environmental variables of (A) temperature, (B) pH, (C) moisture and (D) organic matter (mean ± S.E., $n = 9$) for control (o) and treatment (•) samples collected at a mean depth of 5 cm during the study period from September 1999 to September 2000.

5.3.1.3 *Moisture*

There was no significant difference in total moisture content among the six plots (Figure 5.1 C). The control and treatment plots both recorded an annual average of 75.7 %. The highest moisture was 79.0 % at the height of winter in February 2000 when the field site was covered with approximately 4 cm of snow.

5.3.1.4 *Organic Matter as Loss on Ignition*

The organic matter of the control plots varied in an annual cycle with a decrease during spring and summer, reaching the lowest value of 94.3 % in May 2001, and subsequently increased with a decrease in soil temperature (Figure 5.1 D). The treatment plots followed a similar trend, albeit at a slightly elevated level. Ammonium nitrate additions had a positive affect on the soil organic matter content, visible in March 2000 and thereafter. At the end of the study in September 2000 the treatment plots recorded mean organic content at 98.2 % as compared to 95.7 % in control plots.

5.3.1.5 *Soil C:N ratio*

Soils in the control and treatment plots showed significant differences ($P < 0.001$) in the total organic carbon and nitrogen percentages, determined at the end of the study period in September 2000. In the control plots the mean carbon and nitrogen values were 29.0 % and 0.8 % respectively, while treatment recorded a far higher average of 44.3 % average for carbon and 1.4 % for nitrogen. The soil C:N ratio for control plots was 37.8 while treatment plots had only 30.9 (Table 5.1).

5.3.1.6 *Mineral-N*

Mineral-N was determined for all plots at the end of the experiment as shown in Table 5.1. None of the six plots showed nitrate-N with all the readings being well below the 0.005 mg 100 g^{-1} detectable limit of the instrument. However, there was a significant difference in ammonium-N between the treatment and control plots.

Control samples exhibited a mean concentration of 0.08 mg per 100 g fresh soil as against a high 1.32 mg per 100 g fresh soil for the treatment samples.

5.3.1.7 *Phosphate*

There was a negligible difference in the concentration of phosphate ions between the control and treatment plots. At the end of the study period in September 2000, control and treatment plots showed similar concentrations of phosphate ions in the soil with a mean value of 4.29 and 4.24 mg per 100 g fresh soil respectively (Table 5.1).

ELEMENT	CONTROL *(S.E.)*	TREATMENT *(S.E.)*
Carbon (%)	29.02 *(0.54)*	44.24 *(0.37)*
Nitrogen (%)	0.77 *(0.03)*	1.43 *(0.03)*
C:N	37.69 *(0.78)*	30.94 *(0.56)*
NH_4 - N (mg 100 g^{-1} dry soil)	0.07 *(0.01)*	1.32 *(0.09)*
NO_3 - N (mg 100 g^{-1} dry soil)	< 0.005 *(N.A.)*	< 0.005 *(N.A.)*
PO_4^{3-} (mg 100 g^{-1} dry soil)	4.29 *(0.11)*	4.24 *(0.08)*

Table 5.1 Soil chemical analysis conducted at the end of the study period in September 2000 (n = 9). Soil samples were collected from the H horizon at a mean depth of 5 cm from the surface. (*N.A.* = Not Applicable).

5.3.2 Laboratory Measurements of Soil Carbon Dioxide Fluxes

5.3.2.1 *Rate of Respiration*

The response of soil respiration to an increasing temperature from 5 °C to 25 °C was computed for every soil microcosm collected from September 1999 to September 2000. Mean CO_2 effluxes showed a highly significant temperature effect ($P < 0.001$) and responded exponentially to increasing temperatures with a minimum mean efflux of 0.41 and 0.41 μmol CO_2 m^{-2} s^{-1} at 5 °C in the winter month of February and a maximum mean efflux of 2.96 and 3.65 μmol CO_2 m^{-2} s^{-1} at 25 °C in May for control and treatment plots respectively (Figure 5.2).

Treatment had a statistically significant effect on soil respiration across the range of temperatures (2-way ANOVA). At higher temperatures, ≥ 15 °C, the CO_2 efflux from treated soils was markedly higher at all sampling times during the entire period of study from September 1999 to September 2000.

The response of soil respiration to temperature calculated on the basis of : area of soil (μmol CO_2 m^{-2} s^{-1}), microbial-C content (μmol CO_2 g-micro-C^{-1} s^{-1}), and microbial-N content (μmol CO_2 g-micro-N^{-1} s^{-1}), showed a similar seasonal trend at every temperature (Figure 5.3). The rate of respiration was seen to fall to a minimum during winter in February 2000 and thereafter increase to a peak value during the summer.

The statistically significant effect of ammonium nitrate additions on the response of soil respiration to temperature was also observed when computed on a different basis. The effect is clearly visible at higher temperatures as seen at 25 °C in comparison to 5 °C as shown in Figure 5.3.

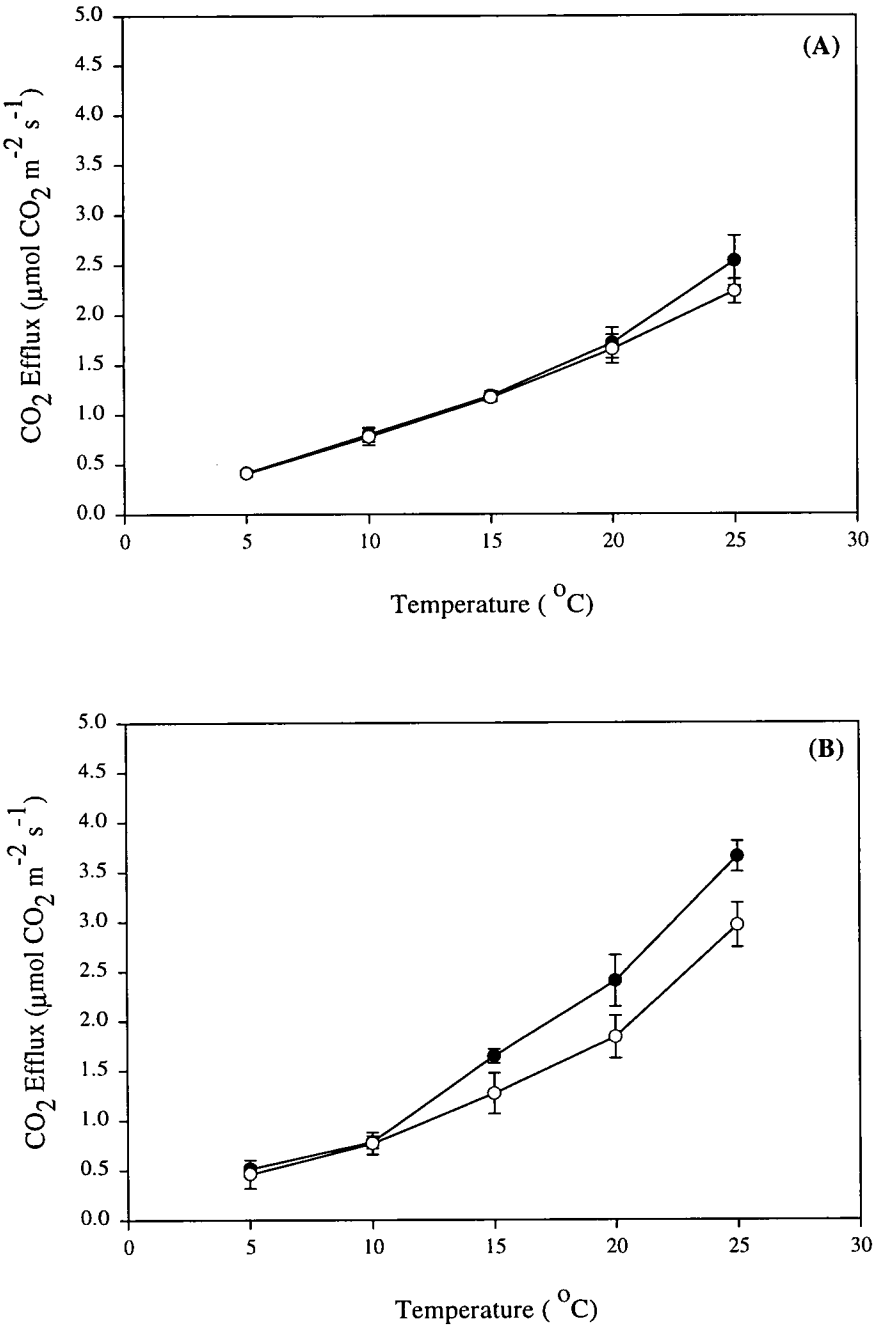


Figure 5.2 Examples of the response of soil respiration (mean ± S.E., n = 9) across a temperature range of 5 °C to 25 °C during (A) winter – February 2000 and (B) spring – May 2000 for control (o) and treatment (•) samples.

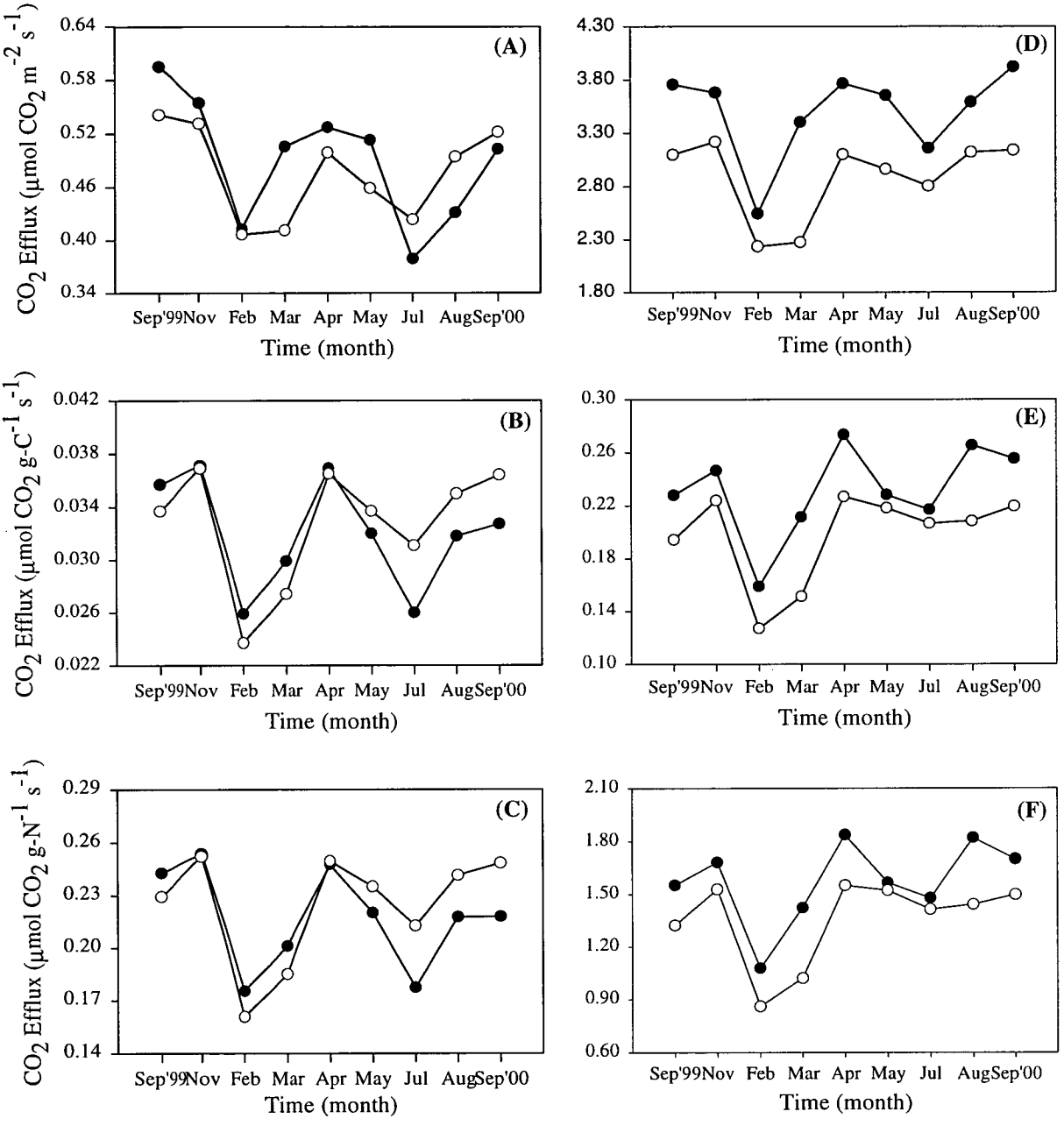


Figure 5.3 Soil respiration calculated at 5 °C on the basis of (A) area, (B) microbial-C, (C) microbial-N and at 25 °C on the basis of (D) area, (E) microbial-C and (F) microbial-N (n = 9) for control (o) and treatment (•) samples from September 1999 to September 2000.

The relationship between soil respiration and temperature was described by first-order exponential, quadratic and Arrhenius types of equations (Table 5.2). All three functions could be fitted to the data and there is no significant difference between the values calculated from the different models for any sampling time through the year.

EQUATION	FITTED PARAMETERS	r^2
EXPONENTIAL	Control: a = 0.36 *(0.03)*, b = 0.08 *(0.003)*	0.99
$k = ae^{bT}$	Treatment: a = 0.38 *(0.04)*, b = 0.09 *(0.005)*	0.98
ARRHENIUS	Control: a = 0.83 *(0.03)*, E = 5.85×10^4 *(2.19×10^3)*	0.99
$k = ae^{(\frac{E}{\Re(T+273.2)} \times \frac{T-10}{283.2})}$	Treatment: a = 0.91 *(0.05)*, E = 6.30×10^4 *(5.85×10^4)*	0.98
QUADRATIC	Control: a = 0.41 *(0.04)*, b = 0.004 *(0.0002)*	0.99
$k = a + bT^2$	Treatment: a = 0.38 *(0.07)*, b = 0.005 *(0.0002)*	0.98

Table 5.2 Fitted relationships between soil respiration and temperature. The fitted parameters were calculated as an annual average of all the sampled months (± S.E.) for control and treatment plots.

Figure 5.4 shows an example of the fitted curves using the first-order exponential equation between simulated and measured data for both control and treatment plots through the period of study. Residual analysis did not show a systematic variation in differences between the observed and calculated data across the temperature range, although the residual is more variable at 15 °C for all equations and for both control and treatment plots.

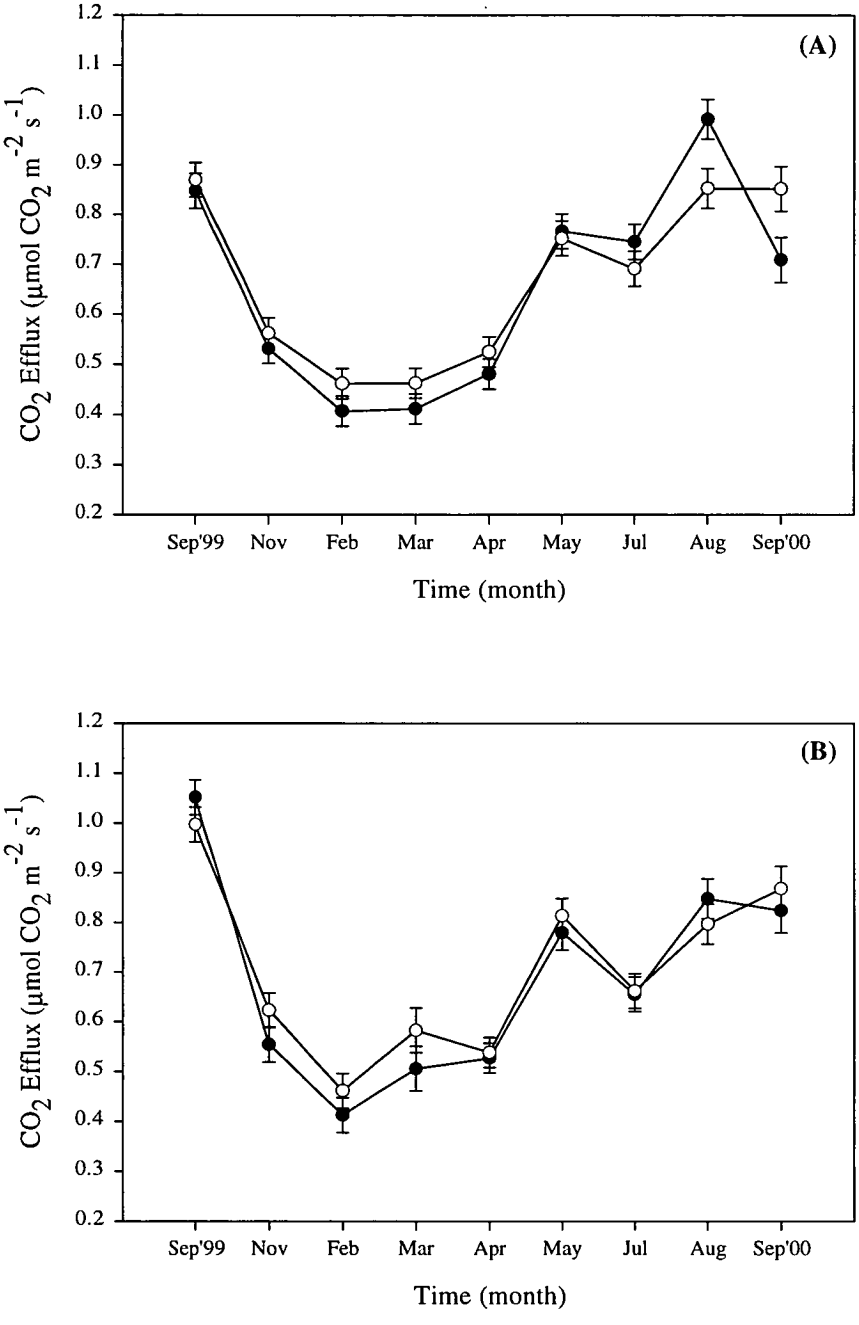


Figure 5.4 The observed (•) and calculated (o) soil respiration (mean ± S.E., n = 9) for (A) control and (B) treatment samples during the entire study period.

5.3.2.2 Q_{10}

Q_{10} values were calculated for each sampled month with the three fitted relationships between soil respiration and temperature. Despite the fact that all three equations fitted the observed data well and provided similar estimates of soil respiration at different temperatures, derived Q_{10} values from the first-order exponential and Arrhenius equations compared very closely while the quadratic equation gave slightly higher values (Table 5.3).

MONTH	EXPONENTIAL $k = ae^{bT}$		ARRHENIUS $k = ae^{(\frac{E}{\Re(T+273.2)} \times \frac{T-10}{283.2})}$		QUADRATIC $k = a + bT^2$	
	Control	**Treatment**	**Control**	**Treatment**	**Control**	**Treatment**
Sep. '99	2.26 *(0.99)*	2.35 *(0.98)*	2.29 *(0.99)*	2.31 *(0.98)*	2.44 *(0.99)*	2.52 *(0.98)*
Nov. '99	2.28 *(0.98)*	2.31 *(0.98)*	2.31 *(0.98)*	2.35 *(0.98)*	2.47 *(0.98)*	2.49 *(0.98)*
Feb. '00	2.07 *(0.98)*	2.24 *(0.98)*	2.10 *(0.99)*	2.28 *(0.98)*	2.23 *(0.99)*	2.40 *(0.98)*
Mar. '00	2.08 *(0.98)*	2.29 *(0.98)*	2.11 *(0.98)*	2.33 *(0.98)*	2.23 *(0.98)*	2.46 *(0.98)*
Apr. '00	2.34 *(0.99)*	2.48 *(0.93)*	2.38 *(0.99)*	2.53 *(0.93)*	2.54 *(0.99)*	2.72 *(0.93)*
May '00	2.45 *(0.99)*	2.50 *(0.99)*	2.50 *(0.99)*	2.55 *(0.99)*	2.65 *(0.99)*	2.81 *(0.99)*
Jul. '00	2.57 *(0.99)*	2.76 *(0.97)*	2.62 *(0.99)*	2.83 *(0.97)*	2.71 *(0.97)*	3.06 *(0.97)*
Aug. '00	2.32 *(0.99)*	2.58 *(0.99)*	2.36 *(0.99)*	2.64 *(0.99)*	2.50 *(0.99)*	2.91 *(0.99)*
Sep. '00	2.34 *(0.99)*	2.52 *(0.98)*	2.38 *(0.99)*	2.58 *(0.99)*	2.49 *(0.98)*	2.88 *(0.99)*

Table 5.3 Monthly Q_{10} values (r^2) derived from different equation models to describe the relationship between soil respiration and temperature. The Q_{10} for the Arrhenius and Quadratic models, was estimated from the calculated respiration rates at 10 °C and 20 °C.

A seasonal pattern was observed for Q_{10} derived from the first-order exponential equation during the one-year study period from September 1999 to September 2000 with the lowest value being recorded in winter and the highest in summer (Figure 5.5). Treatment did not have a significant effect on the Q_{10} value during the initial stages of the study in the winter season. However, after spring the Q_{10} value of the treatment plots increased with time, while the control plots exhibited a relatively constant value.

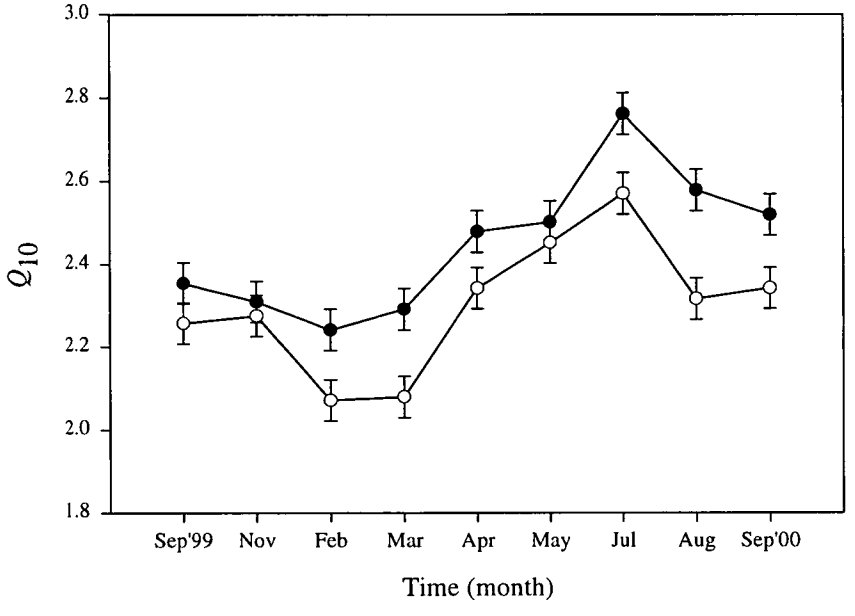


Figure 5.5 The seasonal trend of Q_{10} (mean ± S.E., n = 9) for control (o) and treatment (•) plots during the study period from September 1999 to September 2000.

Q_{10} was plotted across different temperature ranges (Figure 5.6) and the seasonal pattern charted by both control and treatment plots was observed to be similar for each temperature interval with insignificant shifts in magnitude.

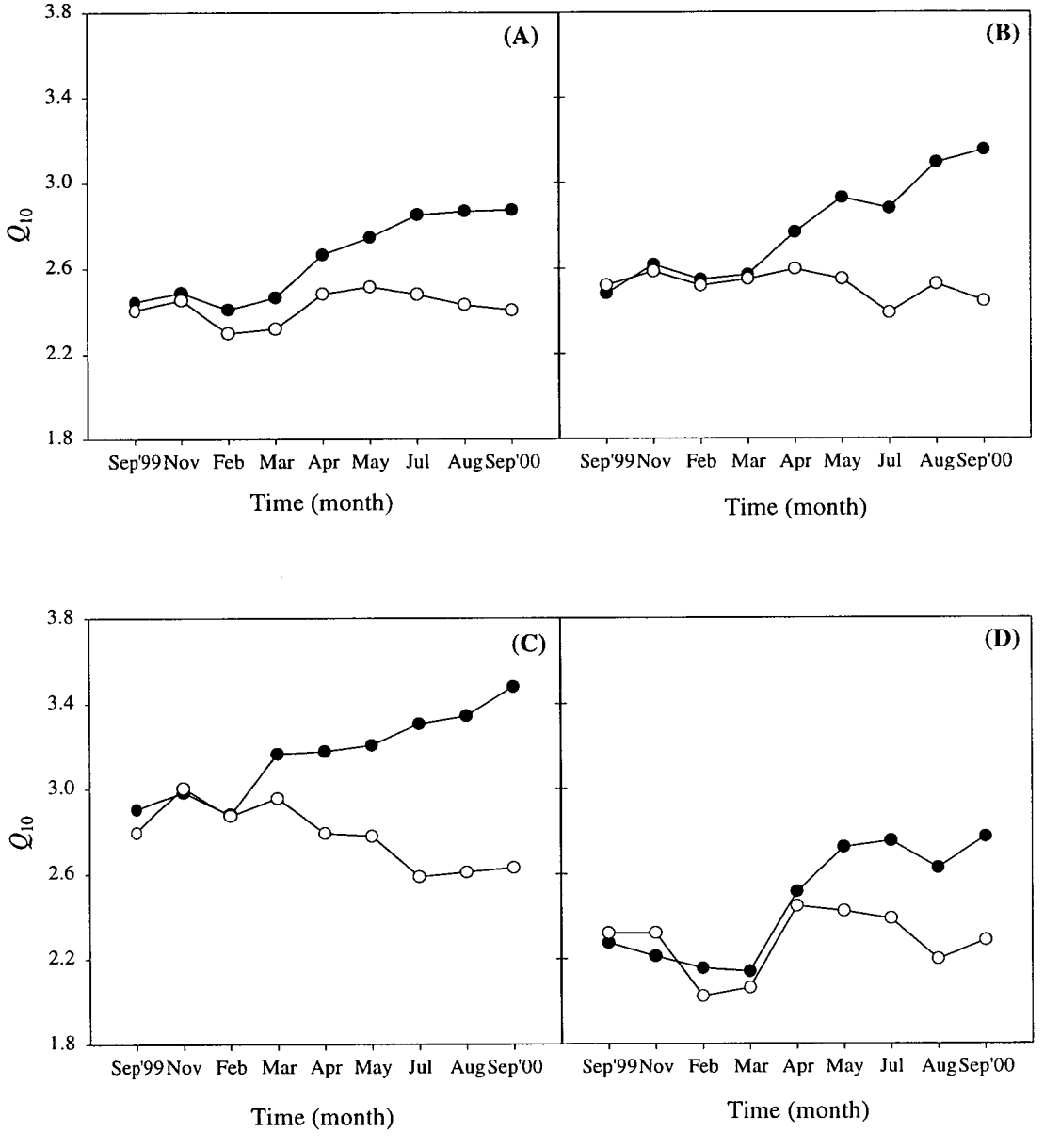


Figure 5.6 The seasonal trend of Q_{10} (n = 9) for control (o) and treatment (•) plots during the study period from September 1999 to September 2000 across the temperature intervals of (A) 5 – 25 °C, (B) 5 – 20 °C, (C) 5 – 15 °C and (D) 10 – 25 °C.

5.4 DISCUSSION

The seasonal changes in CO_2 fluxes from the soil were comparable with other published studies on the rates of soil respiration from heathland ecosystems (Singh and Gupta, 1977; Raich and Schlesinger, 1992), ranging from 0.407 – 3.92 μmol CO_2 m^{-2} s^{-1} over a temperature range of 5 – 25 °C. Chapman (1979) working at a lowland *Calluna* heath in southern England, found that soil respiration increased exponentially with temperature from 0.69 – 2.84 μmol CO_2 m^{-2} s^{-1} over the temperature range 2 – 18 °C. Brown and Macfadyen (1969) studied a *Calluna* heath in Denmark, where respiration of the soil varied according to the age of a heather stand: pioneer stage, 0.79 – 2.29 μmol CO_2 m^{-2} s^{-1}; mature stage, 0.92 – 2.20 μmol CO_2 m^{-2} s^{-1}; building stage, 0.81 – 2.03 μmol CO_2 m^{-2} s^{-1}; and degenerate stage, 0.71 – 1.26 μmol CO_2 m^{-2} s^{-1}. The clear decline in the degenerate phase suggests a strong link between soil respiration and primary productivity.

In the present study soil respiration was positively related to soil temperature, especially under non-limiting moisture conditions, as shown in numerous other ecosystems (Reiners, 1968; Garrett and Cox, 1973; Edwards, 1975; Schlentner and Van Cleve, 1985; Weber, 1985; Ewel, Cropper and Gholz, 1987; Peterjohn *et al.*, 1993; Hanson *et al.*, 1993). Soil respiration closely followed changes in soil temperature in both control and treatment plots with values being high during the summer, reaching a maximum in August and thereafter rates gradually declining during late autumn. The seasonal pattern in soil temperature was observed down the entire soil profile suggesting that heat penetration occurred through the successive soil strata to a depth of 20 cm, though the amplitude of seasonal soil temperature change decreased exponentially with depth, as also noted by Anderson (1991), with the uppermost layers showing maximum diurnal and seasonal fluctuations. The top 5 cm of the soil profile were constantly subjected to wide fluctuations in temperature and consequently moisture due to evapotranpiration, especially during the summer

season, and hence would have had the greatest influence on soil respiration. However, as a general approximation, the annual wave is known to be virtually extinguished at only 19 times the depth penetration of the diurnal wave (Rosenberg, 1974), therefore, in areas with shallow soil profiles as observed in the present study, lower mineral soil horizons are also exposed to wide temperature fluctuations and hence, also contribute to the variation in soil carbon dioxide fluxes at the experimental site. The seasonal pattern of soil respiration agrees closely with previously published data which describes a similar trend for a range of ecosystems and soil types with a seasonal maximum in middle to late summer, followed by a rapid decrease during winter (Anderson, 1973; Edwards and Sollins, 1973; Singh and Gupta, 1977; Edwards and Harris, 1977; Lawrence and Oechel, 1983; Sowell and Spomer, 1986; Raich and Schlesinger, 1992; Bowden *et al.*, 1993; Lloyd and Taylor, 1994; Toland and Zak, 1994; Jensen *et al.*, 1996; Silvola *et al.* 1996; Zogg *et al.*, 1996; Boone *et al.*, 1998; Lin *et al.*, 1999). For example, Edwards and Sollins (1973) also found that the mean CO_2 release from a deciduous forest ecosystem was low in spring from March to July, peaked in August and thereafter steadily decreased while a similar trend was also observed by Jensen *et al.* (1996) for an arable soil, wherein the soil surface CO_2 flux was maximum in August followed by a gradual decline.

Though moisture is not a limiting factor at this site, which in most years receives moderate rainfall throughout the year and is well drained, it is important to note that the relationship between CO_2 efflux and soil temperature usually involves complex interactions with the soil moisture content, depending on the relative limitation of each variable to both microbial and root activity under given conditions. Howard and Howard (1993) and Schlentner and Van Cleve (1985) found a strong interaction between temperature and moisture content in determining the soil respiration. However, in the present work, the rainfall was high enough to maintain moisture close to field capacity. It is quite unlikely that the experimental addition of water with ammonium nitrate additions, equivalent to merely 0.22 mm per month, would have influenced the result.

Carbon dioxide fluxes from the soil have both an autotrophic and heterotrophic component. These heterotrophic fluxes arise from the metabolic activity of a wide range of soil bacteria, fungi and micro and macro fauna which are responsible for organic matter decomposition (Christensen *et al.*, 1996). This in turn depends on favourable environmental conditions, especially temperature, because microorganisms are capable of long periods of minimal activity or dormancy to be followed by a highly active phase when the natural conditions are more conducive and populations flare up (Holland, Townsend and Vitousek, 1995). Hence, the rate of soil respiration may also be affected by the microbial population and organic content of soil, which is an indicator of the quantity and quality of litter. Litterfall can influence the seasonal pattern of CO_2 efflux from the soil surface by adding both soluble carbon and fresh organic material (Schlesinger, 1977). The effects of litterfall on patterns of soil CO_2 efflux have been shown in a study by Weber (1985), wherein patterns of above-ground litter would also be accompanied by seasonal patterns of high carbon loss from the soil, especially roots due to mortality and exudation that would also stimulate CO_2 efflux. The timing of litterfall inputs to the soil surface is generally high during the summer and autumn, after the flowering season. In the present study, soil respiration was highest during late summer and autumn with the beginning of summer litterfall in September 1999 and 2000 probably contributing to the markedly high values in CO_2 efflux at those sampling times. Moreover, organic matter increased with an increase in nitrogen inputs because fertiliser addition would aid plant productivity, which in turn would lead to higher levels of organic matter in the soil by enhancing litter quality and quantity (Jager, 1971; Fauci and Dick, 1994) and promoting the release of highly decomposable root exudates (Coleman, 1976; Prikryl and Vancura, 1980). Therefore, as Swift, Heal and Anderson (1979) proposed, the rate of decomposition of organic matter is regulated by four groups of variables namely, a) the soil organisms, both vertebrates and microorganisms, b) physical rate determinants, primarily temperature, c) mineralogical constituents of soil, and d) the chemical composition of litter, which is a function of carbon

availability, mineral nutrient content and the presence of modifiers, such as lignin, tannins and nitrogen. Heal, Latter and Howson (1978) investigated the decomposition rates in a peat bog of 14 litter types, including *Calluna vulgaris*, to conclude that in N-limited sites, decomposition rates are highly sensitive to variations in nitrogen concentrations. This was also shown in fertiliser trials on Scots pine stands in Sweden (Berg and Staaf, 1980 a, b), where increased concentration of nitrogen in needles were reflected by increased decomposition rates of up to 5 % over 2 years (Berg and Ekbohm, 1993). In this study, nitrogen inputs clearly had a strong effect on the organic composition of soil with the ammonium nitrate fertiliser increasing both organic carbon and nitrogen levels in the soil and typically, a high rate of respiration is found in soils with a recent input of easily degradable substrates. The low soil C:N ratio in the treatment plots, as compared to the control plots, may be attributed to an increase in the microbial population, organic root exudates and organic matter. The functional relationship between microbial biomass and soil respiration is not yet fully understood. Sparling (1981) considered soil respiration to be representative of the active part of microbial biomass while Anderson and Domsch (1986), viewed soil respiration as representing the activity of the whole microbial community, both dormant and active stages. However, Insam (1990) and Rodrigo *et al.* (1997) demonstrated an intimate relationship between climatic conditions, respiratory C flux and the entire microbial soil C pool by explaining part of the climatic effect to be responsible for an altered quantity of metabolizable substrates due to an influence on primary production or substrate allocation to the roots, in addition to decomposition responding to climatic conditions as well. Both factors result in distinct effects upon the mediator of decomposition, the microbial biomass and in turn, soil respiration. Hence, in the present study the rate of soil respiration in the treatment plots would have been greatly influenced by the increased microbial activity due to increased soil organic matter content with added fertiliser treatment.

Another factor influencing rates of CO_2 evolution is soil pH, which determines the types of microorganisms active in soil organic matter decomposition. When organic tissue or exudates enter the soil, the bulk of the material, readily-degradable compounds mainly composed of recently-incorporated plant remains plus the components of the growing and dying microbial population, undergoes enzymatic oxidation with carbon dioxide, heat and water as the major products, while the essential elements, nitrogen, phosphorus and sulphur are released and/or immobilised by a series of secondary reactions specific for each element (Vanhala, Fritze and Neuvonen, 1996). The more degradation-resistant components, chiefly humic acid, that are the major components of soil organic matter (Tate, 1980) and have been shown to vary with soil type and vegetation cover (Stevenson, 1982), may be decomposed in alkaline or neutral soils by bacteria (Kontchou and Blondeau, 1992). However, at low pH ($\leq$ 4.0) such as those observed in heathland ecosystems, fungi become more important (Haider and Martin, 1988; Blondeau, 1989). Therefore, a fall in soil pH would strongly influence the soil microbial biomass and community dynamics, which in turn would affect the rate of soil respiration, as noted in the present study. The significant drop in soil pH observed in the treatment plots shows that the addition of ammonium nitrate fertiliser would affect soil respiration by increasing the acidity of the soil. In most soils previously studied, nitrification of ammonium salts occurs with the production of nitric acid, which lowers pH, whereas nitrate salts have no effect on the pH (Fog, 1988). Foster, Beachamp and Corke (1980) found that ammonium sulphate lowered the pH of a pine forest soil by 1 unit, while ammonium nitrate reduced the pH by 0.75 unit over one year. Hence, the higher rates of soil respiration observed in the treatment plots may possibly be attributed to a drop in soil pH caused by fertiliser additions, which triggers an expansion of fungal hyphae.

Treatment plots exhibited a build up of ammonium-N while nitrate-N was not detected in any of the experimental plots. This may be attributed to the fact that treatment plots with high organic matter content, would also have high

mineralisation rates compared to the control plots, which would lead to higher ammonium-N concentrations upon fertilisation (Stanford, Frere and Schwaninger, 1973; Schenk and Wehrmann, 1979). Simultaneously, a high denitrification rate of nitrate by the plant root system and microbial population, and the immobilisation and leaching of nitrates through the soil pores would result in negligible nitrate-N values (Stanford, 1982). Although it is generally assumed that plants are an important sink for the atmospheric deposition of NO_3^- (Aber *et al.*, 1989; 1991), it appears that NO_3^- is of less importance than NH_4^+ in the nitrogen metabolism in N-deficient soils, as also postulated by Rothstein, Zak and Pregitzer (1996). However, the added NO_3^- may cycle through microbial biomass and ultimately re-enter soil solution as NH_4^+ thereby indirectly influencing respiratory rates over the long term by increasing total N availability (Aber *et al.*, 1991). Changes in soil N availability could influence soil respiration by several mechanisms. For example, an increase in the quantity of available N in soil will likely affect root respiration by increasing tissue N concentration because NH_4^+ can be assimilated directly into biologically active plant compounds (Veen, 1980; Johnson, 1983), and the associated protein maintenance and construction costs (Merino, Field and Mooney, 1982; Waring *et al.*, 1985; Irving and Silsbury, 1987; Ryan 1991) with increasing protein concentration (Ryan, 1991). If an increase in N availability results in higher N concentrations in root tissue then root respiration is likely to increase, thereby increasing total soil respiration. Zogg *et al.* (1996) studied the effect of nitrogen availability on root respiration in a hardwood forest to also conclude that differences in root respiration rates among stands resulted from differences in soil N availability.

Differences in soil CO_2 efflux in response to nutrient availability, especially soil nitrogen, represent the net result of many processes that involve changes in root and microbial activity with biomass production (Söderström, Bååth and Lundgren, 1983). In a nutrient-poor ecosystem, the processes that could potentially be affected by an increase in soil N availability include first, stimulation of microbial activity and second, increase in allocation of assimilate belowground by plants, thus increasing

root and microbial respiration. The net change in total CO_2 efflux as soil respiration therefore depends on the balance between initially, microbial and root respiration, and subsequently, total productivity and allocation above and belowground. The immediate response after fertiliser application as observed in the treatment plots, before significant root growth could have occurred, indicated differences in CO_2 efflux due to microbial activity. This suggests a small short-term stimulation of microbial activity due to N addition followed by the response of the plant root system in later months. In late summer the treatment plots showed a marked increased in soil respiration rates. This may be an indication of the greater effect of high temperatures on stimulating microbial activity, with the gradual build-up of nutrient availability in soil and increase in nutritional quality of litter, all of which would influence the microbial utilisation efficiency of organic compounds. Van Veen *et al.* (1991) found lower bacterial biomass in nutrient-poor as compared with nutrient-rich soils having a higher proportion of soil organic carbon in an available form, but decomposition was instantly stimulated when soil nutrient levels were increased. Changes in nutrient availability can cause changes in the composition of microbial populations, as noted in Chapter 4 while estimating the soil microbial biomass, and thus alter soil CO_2 efflux. Tewary, Pandey and Singh (1982) also found a positive correlation between soil CO_2 efflux and percent nitrogen in soil and litter. As well as the direct effect of N addition to the soil on microbial activity, it is likely that the composition of root exudates and dying fine roots was enhanced by increased N availability. Orchard, Cook and Corderoy (1992) also found that a higher soil nutrient availability increased the capacity of microorganisms to utilise higher carbon inputs from litterfall resulting in increased rates of respiration, and suggested that this was due to a change in microbial population. These direct and indirect effects would both tend to stimulate microbial activity and therefore, soil respiration.

A number of factors influence the fluxes of carbon dioxide from soils but with growing concerns regarding global warming, the temperature sensitivity of soil respiration will largely determine the effects of a warmer world on net carbon flux

from soils to the atmosphere. Hence, temperature is the single best predictor of the annual soil respiration rate of a specific location (Raich and Schlesinger, 1992).

The temperature dependence of all biochemical processes is widely recognised, and soil respiration in particular is known to vary with temperature (Amthor, 1984; Johnson, 1990; Reichstein *et al.*, 2000). The response of soil respiration and other mineralisation processes in the soil to temperature are commonly described using exponential, Arrhenius and quadratic equations. However, uncertainties remain in understanding and describing the relationships and in recent times, a range of variations have been introduced by researchers to produce an accurate universal model to best describe the response of soil respiration to temperature, for example, exponential or Arrhenius based equations (Lloyd and Taylor, 1994; MacDonald, Zak and Pregitzer, 1995; Thierron and Laudelout, 1996); linear models (Rochette, Desjardins and Pattey, 1991); quadratic models (Holthausen and Caldwell, 1980); logistic models (Schlentner and Van Cleve, 1985; Jenkinson, 1990). Although these models may fit well with sets of experimental data under specific conditions, they suggest different explanations for the response of soil respiration to temperature. The Q_{10} value, which defines the temperature dependence or sensitivity of soil respiration to temperature variation, when derived from different models is also different, whether by magnitude or with respect to temperature (Howard and Howard, 1993; Lloyd and Taylor, 1994; Kirschbaum, 1995; Lomander, Kätterer and Andrén, 1998; Winkler, Cherry and Schlesinger, 1996). Nevertheless, an exponential increase in soil respiration with respect to temperature is still commonly accepted and observed for biological systems over a limited range of temperatures (O'Connell, 1990; Thierron and Laudelout, 1996; Winkler, Cherry and Schlesinger, 1996). Hence, the relationship between soil respiration and temperature in the present study has been described by the three most commonly used models are viz. first-order exponential, Arrhenius and quadratic equations.

In 1898, Van't Hoff described the following exponential relationship to examine the effect of temperature on different reactions, over a limited temperature range:

$$\log_{10} k = a + bT$$

where, k is the rate constant, a and b are constants, and T is the temperature. Using natural logarithms, the equation can be expressed for the rate of respiration (R) as:

$$R = ae^{bT}$$

The above-mentioned first-order exponential is widely used by researchers to determine the relationship between soil respiration and temperature.

Another widely used equation is the following Arrhenius rate equation derived in 1889, which takes into consideration the energy distribution of reacting molecules:

$$k = ae^{-E/(\Re T)}$$

where, k is the rate constant (respiration rate), a is a constant, E is the activation energy, $\Re$ is the universal gas constant, and T is the absolute temperature (K).

The third equation commonly applied is a simple quadratic equation that was originally developed by Ratkowsky *et al.* (1982) to explain temperature responses of microbial growth for pure bacterial cultures, namely

$$k = a + bT^2$$

where, k is the rate constant (respiration rate), a and b are constants, and T is the temperature.

Días-Raviña, Frostegård and Bååth (1994) showed that the quadratic function can also be applied to predict microbial temperature responses in soil and since microbial activity is the basis of much of soil respiration, the model has been used to describe the relationship between soil carbon fluxes and temperature.

The temperature dependence of respiration is often expressed as the Q_{10} value. When the relationship between soil respiration and temperature is exponential, Q_{10} is related to the slope of log R versus temperature and can be calculated by the following linear relationship.

$$\ln R = \ln a + k T$$
$$\ln Q_{10} = 10 k$$
$$Q_{10} = e^{10k}$$

where, R is rate of respiration, a is a constant, k is the rate constant, T is temperature.

Q_{10} is the factor by which the respiration rate (R) differs for a temperature (T) interval of 10 °C, as defined below, and has been the focus of many studies.

$$Q_{10} = R_{(T+10)} / R_T$$

In this study, soil respiration is positively correlated with soil temperature and the Q_{10} derived from the exponential, quadratic and Arrhenius equations range from 2.11 to 2.91 across a temperature gradient from 5 to 25 °C. Chapman (1979) had also recorded values between 2.09 – 2.68 across a temperature range from 2 – 18 °C. The values compare well with published Q_{10} values for soil CO_2 efflux given by literature reviews, which range from 1.3 to 3.3 with a mean value of 2.4 (Raich and Schlesinger, 1992; Kätterer *et al.*, 1998). Schleser (1982) noted that the wide variation in Q_{10} values for soils is dependent on natural conditions such as nutrient supply because the metabolism of soil organic matter by microorganisms is

influenced by the physico-chemical conditions and the quality of organic matter. Howard and Howard (1993) also recorded an increase in Q_{10} values with increasing temperatures for podzols and high peat soils with pH 3.0 - 3.7 with *Calluna*-dominated vegetation and concluded that in acidic soils decomposition of plant detritus is naturally slow and resistant organic matter tends to accumulate and hence a lower rate of organic matter decomposition. The first-order exponential and Arrhenius equations, both gave similar Q_{10} values suggesting that soil respiration does increase exponentially with temperature. The first-order exponential equation was chosen to describe the response of soil respiration to temperature, which also helped facilitate the comparison of this project to the results of previous studies.

In the present study, it can be stated that factors affecting the rate of soil respiration were ammonium nitrate fertiliser treatment, temperature, pH and organic matter content. In order to express the magnitude of influence of each environmental factor (± S.E.), multiple regression was applied on Q_{10} to generate the following equation (r^2 = 61.4 %):

$$Q_{10} = 4.929\ (\pm 0.85) + 0.206\ (\pm 0.04)\ N + 0.029\ (\pm 0.01)\ T - 0.150\ (\pm 0.08)\ pH - 0.025\ (\pm 0.01)\ S$$

where, T is field temperature (°C), N is nitrogen treatment (1 or 2) and S is soil organic matter (%).

Residuals showed slight seasonal behaviour but it proved impossible to improve the fit by using an index of seasonality. The use of non-linear terms was tried but they did not prove to be significant.

It is noteworthy that the Q_{10} increases significantly with temperature. Thus with climate warming, temperature may exert a stronger influence than would be predicted by a simple Q_{10} model, at least until substrate exhaustion becomes important. Simulation of the annual soil carbon efflux under conditions of increased and decreased temperature and organic matter by using the above regression model illustrated that substantial changes in soil changes in soil carbon fluxes could occur (Figure 5.7).

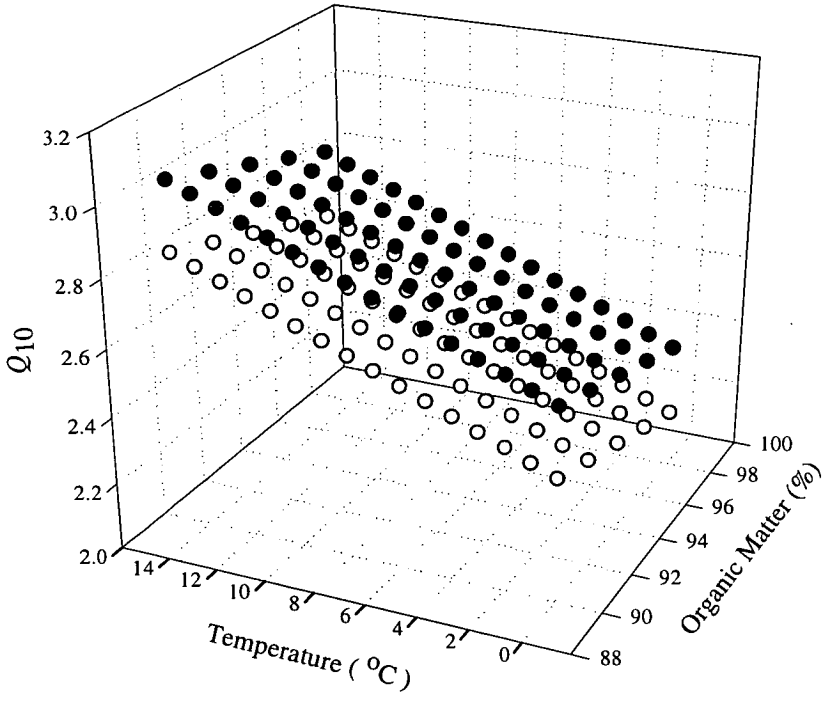


Figure 5.7 Model results of the effects of temperature and organic matter on carbon dioxide fluxes from the soil using the multiple regression equation for control (o) and treatment (•) samples.

The dynamics between soil properties and belowground carbon fluxes provides an essential baseline for understanding and predicting potential changes in terrestrial ecosystems and their capacity to sequester carbon. The close relationship of belowground biological activity with temperature, moisture and soil nutrient availability indicates that potential impacts of disturbance to these soil properties can greatly influence CO_2 efflux from the soil. The high correlation of soil CO_2 efflux with temperature and nutrient status of the soil means that the carbon flux can be predicted at any given time with accuracy, if these variable and the relationships between them are known for a specific site, moisture not being a limiting factor, and substrate quality and quantity does not change as a consequence of depletion of carbon pools that constitute the substrate. These caveats are in practice very important, and long term soil warming experiments suggest that the decomposition process does in fact show an acclimation process, probably associated with the depletion of labile carbon pools (Jarvis and Linder, 2000). This acclimation process tends to undermine the use of fixed Q_{10} in global circulation models (eg. Cox *et al.*, 2000), and may explain why the temperature sensitivity of soil respiration is markedly less in long-term experiments (Grace and Rayment, 2000). Nevertheless, predicted changes in global climates include significant increases in temperature, and this could result in higher rates of soil CO_2 efflux (Raich and Schlesinger, 1992). This effect is likely to provide a positive feedback to the greenhouse effect, particularly as CO_2 efflux, as respiration is an exponential function of temperature.

The results in this study represent short-term responses in CO_2 efflux from soils that are related to root and microbial activity. However, even small changes in soil carbon fluxes, if sustained, will have major impacts on carbon cycling; for example, using the Rothamsted model of soil organic matter turnover, Jenkinson, Adams and Wild (1991) predicted that the increase in CO_2 release from soil organic matter due to a temperature rise of 0.03 °C per year will be 6×10^{15} g C, over the next 60 years.

5.5 CONCLUSIONS

In the present study, environmental variables of soil temperature, pH, organic matter and microbial biomass were found to be important determinants of carbon dioxide fluxes from soil. Nitrogen application as ammonium nitrate significantly increased soil respiration and the results suggest that long-term effects of atmospheric N deposition, with accelerated mineralisation at higher temperatures, could disrupt the carbon balance of nutrient-poor ecosystems, as noted for heathlands. Therefore, soil environmental parameters and nitrogen availability are important factors that influence the carbon dioxide effluxes from soil and should be considered in terrestrial C budget models describing the response of ecosystems to global change.

6. EFFECTS OF N DEPOSITION ON PLANT GROWTH AND TISSUE C:N RATIO

6.1 INTRODUCTION

The physiological effects of excessive nitrogen consumption by plants is not always easy to predict, although, the consequences at the ecosystem level can be profound (Lee, 1998). Amongst, the ecological repercussions, increases in the concentration of organic nitrogen compounds in leaves may have significant influence on herbivory (McNeill and Southwood, 1978; Mattson, 1980; Bryant, *et al.*, 1987, 1992, Hartley, Nelson and Gorman, 1995). Secondly, a decrease in the C:N ratio in litter may have marked stimulatory effects on decomposition processes (Fog, 1988; Anderson, 1991; Van Vuuren and Berendse, 1993). Carbon dioxide is an end product of microbial respiration during the process of organic matter decomposition, and respiration by live roots, and so N deposition can be expected to increase the carbon dioxide fluxes to the atmosphere. Microbial respiration is related to the quantity and quality of substrate and root respiration is related to the rate of assimilate transfer belowground; hence both processes are dependent on total productivity, allocation of assimilate and nutritional status of the plant.

Vegetation change, as result of excessive N inputs, may also alter the distribution of soil nutrients because as a soil-forming factor, plants affect the pattern and rate of rock weathering, the rate of organic inputs to the soil, and the distribution of soil nutrients spatially and temporally, all of which influence root and microbial growth and respiration. Therefore, changes in plant life forms that alter root profiles, maximum rooting depths and microbial dynamics may consequently alter vertical nutrient distributions (Jama *et al.*, 1998), including the soil C pool, which is the largest terrestrial pool of organic C (Trumbore, 2000). Thus, soil dynamics, including

carbon dioxide fluxes, bears the imprint of plant activity through time. When these community responses are considered together with the potential effects of nitrogen deposition on individual species, far reaching effects on ecosystems can be envisaged. Hence the need in this present study to estimate the effects of ammonium nitrate fertiliser inputs on plant growth and tissue C:N ratio of *Calluna vulgaris* (L.) Hull, especially since plants confined to low nutrient status soils are susceptible to enhanced nitrogen deposition (Ellenberg *et al.*, 1991).

In early investigations, the age of *Calluna* plants was estimated by counting growth rings on a sample of stems (Watt, 1955; Gimingham, 1960; Kayll and Gimingham, 1965; Miller and Miles, 1970). Beijernck (1940) in a detailed study entitled, 'Calluna: A Monograph on the Scotch Heather', wrote "the annual rings are pretty distinctly delineated and can be clearly recognised with low magnifications by the differences in size of the vessels in the winter- and summer-wood, especially when stained they show well" (Figure 6.1).

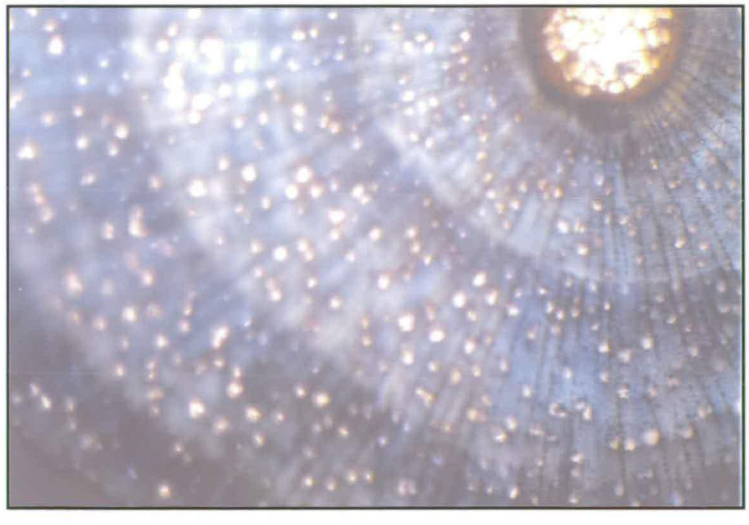


Figure 6.1 Annual growth rings of a *Calluna vulgaris* (L.) Hull stem sample as observed under low magnification with a compound microscope after staining with blue ink.

By extension, the last season's incremental growth may be determined by the width of the last annual ring. A number of studies have shown that nitrogen inputs can promote shoot growth and alter tissue C:N ratio as discussed in Chapter 2 and supported by the pilot study findings.

This chapter presents a technique to accurately determine shoot diameter increments of *Calluna* plants as an indicator of the effect of fertiliser treatment on plant growth. Tissue C:N ratio was also estimated to study the degree to which applied nitrogen inputs are reflected in tissue nitrogen concentrations, which can directly influence soil processes like soil respiration and microbial communities via root exudates.

6.2 MATERIALS AND METHODS

Terminal shoots from all six plots were studied for shoot increments, in order to determine the growth pattern during the last growing season with higher nitrogen inputs, and total organic carbon and nitrogen content to determine the effects of ammonium nitrate fertiliser additions. Samples were taken at the end of the study period in October 2000.

6.2.1 Growth

Growth during the last growing season was estimated by measuring the width of the last growth ring as observed in a cross-section of apical shoot samples collected from all the experimental plots. The terminal shoots were excised at a point corresponding to three years old, that is, there were three radial growth increments. The thin cross-sections were stained with blue writing ink (Helix, Austria) for 10 seconds and viewed under a binocular microscope with a calibrated eyepiece. The average width of the last growth ring was measured in millimetres to determine incremental increases in shoot diameter.

6.2.2 Total Carbon and Nitrogen

Total carbon and nitrogen contents in plant samples were determined by the 'flash combustion' technique described by Verardo *et al.*, 1990 using a Carla Erba NA-1500 Analyzer and AS200 Autosampler interfaced with a Hewlett-Packard 3390A Integrator. The samples, consisting of the young apical shoots of individual heather plants, were oven dried at 60 °C for 36 hours. Each sample was ground in a clean agate mortar and pestle, sieved using a 0.353 mm aperture sieve (Endecotts Ltd., London, England) and stored in high density polyethylene scintillation glass vials with linerless screw caps (Wheaton Science Products, Millville, USA). Approximately 5 mg of sample was weighed into tin foil capsules (Elemental Microanalysis Ltd., Devon, UK) using a microbalance (Delta Range AT261, Mettler-Toledo, Leicester, UK) and the sample cups were carefully moulded into small round pellets measuring less than 5 mm in diameter with the help of fine forceps. The sample balls are placed into the autosampler tray and loaded into the autosampler of the NA-1500 Analyzer for direct analysis.

6.3 RESULTS

6.3.1 Growth

The growth increments were readily measured on the ink-stained cross-sections (Figure 6.2).

Nitrogen fertiliser had a significant effect ($P < 0.01$) on the shoot growth. The width of the last growth ring within the treatment plots recorded a mean value of 0.708 mm (S.E. ± 0.007) in comparison to a mean of 0.503 mm (S.E. ± 0.008) in control plots as shown in Figure 6.3.

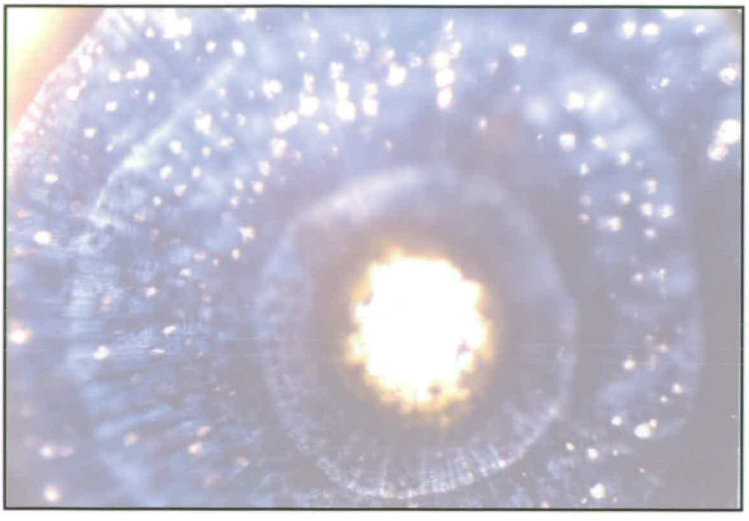


Figure 6.2 A stained cross-section of a terminal shoot of *Calluna vulgaris* (L.) Hull excised at a point corresponding to three years old so that three radial growth increments are visible for measurement.

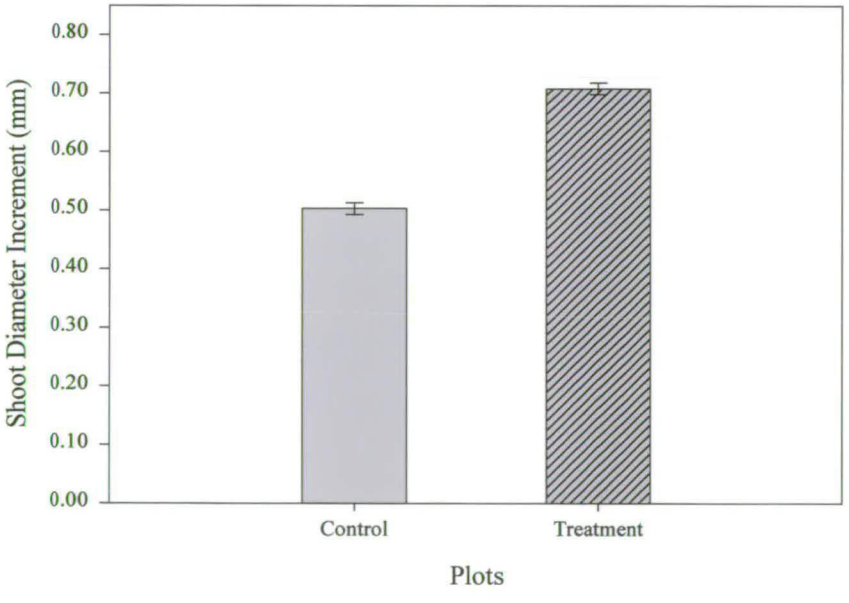


Figure 6.3 Annual shoot diameter increments (mean ± S.E., n = 18) during the last growing season as measured in October 2000 after fertiliser treatment.

6.3.2 Plant C:N Mass Ratio

Figure 6.4 shows that nitrogen inputs do not significantly affect the carbon concentration in foliar tissue as observed from the tissue composition of shoots from both control and treatment plots. The average carbon percentage in control and treatment plots was 50.41 % and 50.43 % respectively. However, the nitrogen concentration in young shoots was significantly higher ($P < 0.01$) in plants treated with ammonium nitrate. The mean percentage was 1.15 % (S.E. ± 0.007) in control plots while treatment showed a higher concentration, 1.34 % (S.E. ± 0.008), after one year of fertiliser application. The C:N ratio was therefore observed to be higher in control at 43.7 (S.E. ± 0.26) as compared to 37.7 (S.E. ± 0.25) in treatment plots (Figure 6.5).

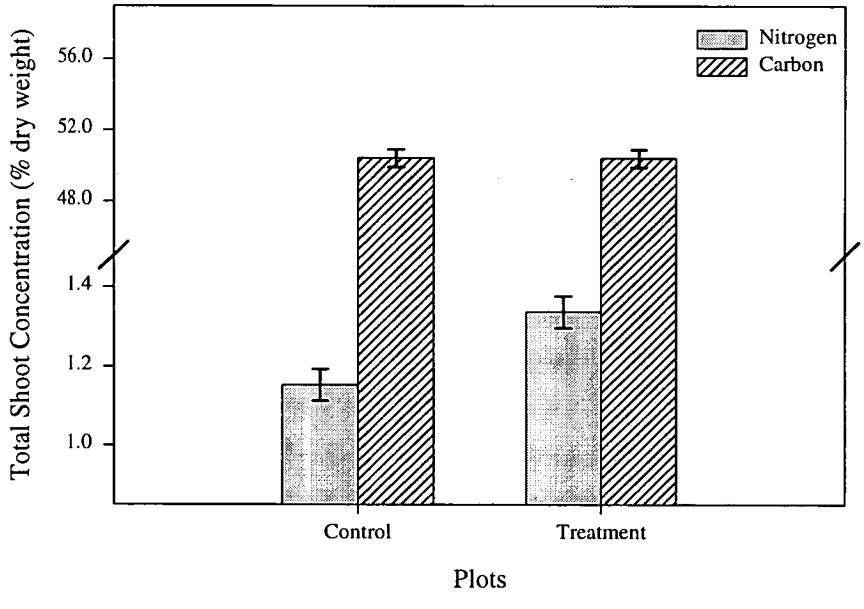


Figure 6.4 Total shoot carbon and nitrogen concentration expressed as a percentage of dry weight (mean ± S.E., $n = 9$) measured at the end of the fertilisation period in October 2000.

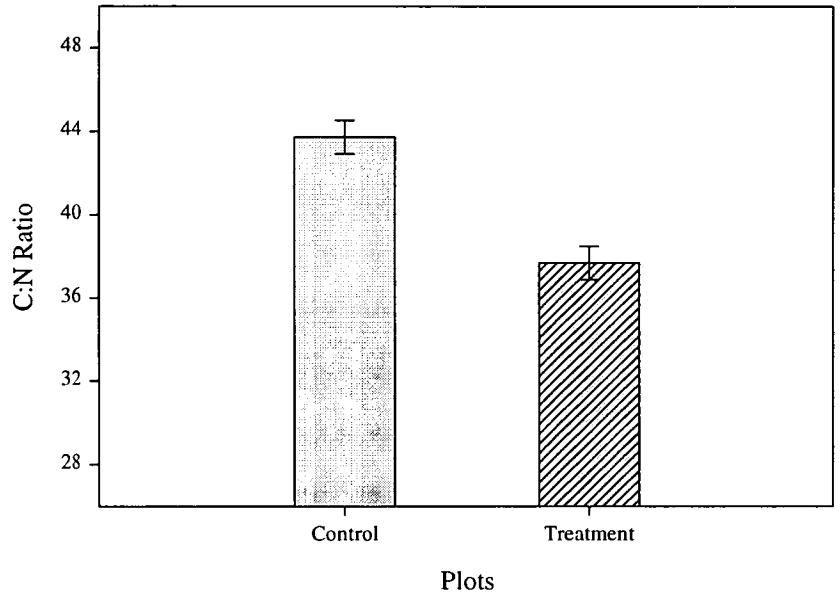


Figure 6.5 Shoot C:N ratio (mean ± S.E., n = 9) determined at the end of the treatment regime in October 2000.

6.4 DISCUSSION

The effect of nutrition on the growth and yield of plants have long been studied in trees. More than a hundred years ago, the German scientist Ebermayer (1876) could demonstrate the deleterious effects of forest yield on repeated litter removal. Thereafter, Swedish scientists took an early interest in the nutritional requirements of trees and clearly proved that nitrogen was the most limiting element in old conifer forests in Sweden (Tamm, 1968), a fact that may also hold true for most heathlands that grow in similar organic soils and under similar climatic conditions. In long-term fertiliser experiments in forest stands of Scots pine in Central Sweden which included repeated additions of nitrogen, a significant increase in the concentration of nitrogen in the foliage was noted with a pronounced growth response, and stem

volume increment doubled on all plots receiving nitrogen (Linder and Axelsson, 1982). The results of this present study also show that nitrogen inputs have a marked effect on plant growth and tissue C:N mass ratio.

The observations recorded in this chapter were in agreement with the trends observed in the pilot study. Enhanced shoot growth of *Calluna vulgaris* plants was observed in all treatment plots in comparison to the control plots, in a manner similar to the positive effects elicited by ammonium nitrate fertiliser inputs on the shoot extension of plants maintained within the open-top chambers. The stimulation of growth may be attributed to the direct removal of nitrogen-limitation in the nutrient-poor ecosystem by regular fertiliser additions. Similar results have been reported for fertilisation studies conducted on *Calluna* wherein nitrogen additions significantly enhanced growth (Heil and Bruggink, 1987; Aerts, 1989; Caporn *et al.*, 1995 a, b; Uren *et al.*, 1997). Importantly, the observation suggest that the staining technique employed can accurately represent the growth pattern of a *Calluna* plant, thereby providing a powerful tool for determining the effects of fertilisers on plant growth and productivity.

The high percentage of tissue nitrogen in treated plants suggests that added fertiliser could significantly disrupt the tissue C:N ratio within plants. The high concentrations of foliar N suggest a foliar uptake of nitrogen in conjunction with the absorption of nutrients via the root system. In isotopic studies conducted by Van der Eerden *et al.* (1990), on heathland vegetation, using ^{15}N-labelled ammonium sulphate, the authors revealed that the uptake of ammonium ions from atmospheric sources is predominantly via the shoots. Moreover, field experiments have shown that 45 - 90 % net throughflow of wet deposited ammonium can be directly assimilated upon uptake by *Calluna* shoots (Fowler *et al.*, 1989). The foliar N concentrations observed for treated and control plants compare well with the values observed during the pilot study, as described earlier in Chapter 3, and increases in foliar nitrogen concentration upon fertiliser application have also been observed by other researchers (Brunsting

and Heil, 1985; Van der Eerden *et al.*, 1990; Lee, Caporn and Read, 1992). In a fertilisation experiment, Aerts (1993 b) showed that foliar N concentration in dwarf shrub species of *Calluna vulgaris* and *Erica cinerea* correlated with N deposition. Pitcairn, Fowler and Grace (1995) reported increases in foliar N content in *Calluna vulgaris* over the last 30 years in the British Isles, reflecting N deposition levels. The foliar carbon concentrations are less drastically affected by nitrogen inputs as also seen in the pilot study. There it was concluded that although the total carbon percentage may not vary considerably, the carbon allocation pattern changes wherein less carbon is utilised for the production of secondary metabolites, which serve as vital defence compounds against pest attacks, while more energy is channelled towards growth. Therefore, foliar C:N ratios provide a good indicator of the effects of atmospheric fertilisers on plant physiological processes and stress susceptibility.

N enrichment promotes plant growth, with a differential uptake of NH_4^+ and NO_3^- ions, and thereby increases productivity (Jefferies and Maron, 1997). An increase in the fine root structure and production of root exudates would in turn influence root and microbial respiration within the soil and thereby increase carbon dioxide effluxes from the soil. The plant-soil interactions, including the carbon balance, can thus be easily disrupted by elevated nitrogen inputs.

6.5 CONCLUSIONS

The study showed that ammonium nitrate fertiliser treatment significantly increased plant growth and altered tissue C:N mass ratio, which may consequently result in more litter and root exudates to the soil and exert an influence on the soil microbial profile. Therefore, elevated nitrogen inputs can indirectly cause an increase of carbon dioxide effluxes from soils, especially in nitrogen-limiting ecosystems where plant growth responds rapidly to elevated nutrient levels. It may hence be concluded that

atmospheric deposition of nitrogen directly affect the growth and yield of plants and subsequently affect soil processes, including root and microbial respiration.

7. CONCLUSIONS AND SUMMARY

The heathlands of north-west Europe, dominated by the plant species *Calluna vulgaris* (L.) Hull, have for centuries characterised the physiognomy of the landscape (Beijernck, 1940; Watt, 1955; Kayll and Gimingham, 1965; Miller and Miles, 1970). Distinctive assemblages of plants and animals have, therefore, had time to develop in the tree-less habitats, created years ago by forest clearance, and these ecological niches have with time been adopted by species which are now rare or of scientific interest (Gimingham, 1972; Uren, 1992). In recent times a steady decline in the area of dry heaths has been the cause of much concern, especially in the Netherlands, Denmark and the south of England, and today heathlands serve as important indicators of nutrient-poor terrestrial ecosystems which are constantly threatened by large-scale human activities. The substantial loses have been exacerbated over the past years by a dramatic change in the floristic composition with a successional conversion of heathlands to grasslands (Heil and Diemont, 1983; Aerts, 1990; Marrs, 1993). *Calluna vulgaris* (L.) Hull is thus a typical example of a high conservation value plant species, playing a pivotal role in the maintenance of plant-animal species diversity and of considerable economic importance for sheep grazing and for sporting purposes, however presently succumbing to a suite of anthropogenic stress factors. In the future, heathlands may suffer even more as a result of climate warming, and further anthropogenic nitrogen deposition.

Since the end of the 1970's, a serious deterioration in species diversity has been apparent with the disappearance of *Calluna*-dependent flora and fauna (Bunce, 1989; Usher, 1992). The impoverishment process has been the subject of many research studies, which suggest that increased atmospheric nitrogen depositions cause the eutrophication of heathland ecosystems and nitrogen appears to be the crucial trigger controlling the conversion of heathlands to grasslands (Helsper, Glenn-Lewin and Werger, 1983; Brunsting and Heil, 1985; Berdowski and Zeilinga, 1987; Berendse *et*

al., 1987; Van der Eerden *et al*, 1991; Anderson and Hetherington, 1999). However the mechanisms which underlie the complex deterioration process are rather complicated and still poorly understood. Moorlands dominated by the heather species *Calluna vulgaris* (L.) Hull, cover 15 % of the land area in the UK but contain nearly 75 % of the soil organic carbon (Howard *et al.*, 1995). However, few investigations have linked the ecological effects of nitrogen pollutants to the disruption of the carbon cycle. Soils are a major terrestrial sink of carbon and the response of carbon dioxide effluxes to environmental change is of vital importance in the global carbon cycle. In the boreal region, the organic soils contain vast deposits of carbon in the form of undecomposed and partially decomposed phytomass (Rapalee *et al.*, 1998). In the UK, such soils are found mainly as bogs, moors and heaths (Grace and Marks, 1978). Therefore, an important goal is to characterise factors such as soil temperature, moisture, pH, organic matter, carbon:nitrogen ratio and microbial composition that determine soil respiration rates, with respect to elevated nitrogen inputs in a heathland ecosystem. There is often a lack of integrated studies of the biogeochemical cycling of carbon and nitrogen in terrestrial ecosystems, in which inputs from atmospheric depositions are related to outputs, in terms of respiration and net mineralisation of soil elements. This has caused major gaps in knowledge regarding the rates of transfer of anthropogenic pollutants within specific ecosystems and not enough is known about how these rates affect population dynamics of individual species and trophic relationships.

The research project aimed at investigating the response of *Calluna*-dominated heathlands to nitrogen deposition as large-scale environmental disturbances, notably nutrient enrichment due to high loads of atmospheric pollutants, have been identified as the key factors threatening the existence of heathlands. The experimental design of the research project allowed for the study of plant, soil and microbial community interactions in a heathland ecosystem exposed to a fertiliser treatment that simulated atmospheric inputs, within both a controlled and field environment. In the pilot study, field conditions were carefully simulated in open-top chambers within the

physical constraints of the chamber construction. The environment within the OTC was inevitably modified relative to ambient conditions by the enclosure and air delivery system. The exposure system was noted to alter the microenvironment, by raising the air temperature inside the chambers by 0.5 - 2.0 °C above the ambient temperature and reducing the relative humidity by 5 - 13 % (Leith *et al.*, 1998). However, the alterations are not viewed as major contributory factors when considered in relation to the annual seasonal variations. In the field experiment, the investigation was taken a step further, shifting the emphasis to the response of soil to atmospheric pollutants. The annual pattern of soil respiration was monitored under elevated and ambient nitrogen conditions with the simultaneous determination of important soil properties.

The research project revealed that increased nitrogen availability in a heathland ecosystem can drastically change plant physiological processes which increase growth rates, and raise pest susceptibility levels by decreasing secondary defence metabolite production, while influencing the carbon storage potential of soils by increasing soil respiration and microbial biomass. The general conclusions may briefly be enumerated as follows.

✓ Growth of new *Calluna* shoots increased with nitrogen inputs of greater than 20 kg N ha^{-1} yr^{-1} and differences in length and diameter increments between the control and treatment samples were clearly significant.

✓ Dilute solutions of ammonium nitrate, applied to the plants in order to simulate present-day and possible future inputs, had a significant effect on tissue nitrogen content as observed during the pilot and field study periods.

✓ Higher nutrient availability reduced the plant allocation of carbon to herbivore-defence compounds, measured as phenolic secondary metabolites.

✓ Nitrogen additions significantly altered soil C:N ratio from control levels.

✓ Soil respiration increased with temperature. The Q_{10} was in the range 2.07 - 2.57.

✓ Inputs of nitrogen fertiliser magnified the response of soil respiration to temperature; carbon dioxide effluxes in a nutrient-poor ecosystem were observed to increase across a rising temperature gradient.

✓ Soil respiration was noted to decrease with soil depth and across long periods of incubation due to substrate depletion.

✓ Microbial biomass and species composition are sensitive to fluctuations in soil properties, such as temperature, pH, organic matter content and nitrogen concentration as reflected in the microbial C:N ratios and biomass.

The results indicate that biogeochemical processes in a heathland ecosystem are sensitive not only to the effects of changes in climatic variables but to N availability. Nitrogen enrichment may initiate changes in plant tissue chemistry and microbial decomposition processes, which could have important indirect effects on species interactions in plant and microbial communities that may enhance the rates at which the system responds to environmental changes, as well as affecting rates of herbivory, all of which may be expected to result in changes in plant species assemblages. Changes in plant species assemblages associated with N additions may result in feedbacks that reflect interactions between plant tissue chemistry, litter accumulation and N mineralisation. Enrichment can change competitive interactions between species, which may favour introduced species, and also increase palatability of forage plants to herbivores. Nitrogen deposition may not necessarily lead to increased biomass, particularly where phosphorus is limiting at a specific site, and a series of studies in the UK has shown that plant growth appears to be co-limited by the availability of both N and P (Wilson, Wells and Sparks, 1995). However,

elevated levels of N in plant tissues can increase herbivory. The most dramatic example is the increased mortality of *Calluna vulgaris* plants brought about by the heather beetle, *Lochmaea suturalis*, which feeds on the N-rich shoots of heather and densities of beetles may be as high as 2000 m^{-2} (Jefferies and Maron, 1997). The death of *Calluna* plants and their replacement by *Deschampsia flexuosa* can create a vegetation mosaic as seedlings of *Calluna* are unable to establish in the dense litter mats of the grass (Prins, Berdowski and Latuhihin, 1991). Hence, the interaction results in the changes in plant assemblages and in soil structure. Autotroph-herbivore relations, once modified, become increasingly destabilised as predicted by the paradox of enrichment. Over-consumption of N-rich plant tissue set up positive feedbacks between sustained nitrogen deposition, herbivory and species composition, leading to instability and destruction of systems. Once soil-N is raised to the level where plant biomass is increased, and rates of nitrogen cycling are altered, it may take many years for the pool of soil-N to diminish, even in the absence of new inputs. Thus anthropogenic inputs of nitrogen may ultimately produce long-term changes in ecosystem function. Therefore, individual concepts such as 'annual nitrogen saturation' (Ågren, 1983) and 'critical load' (Nilsson, 1986), used to describe the effects of enrichment on soil, community, ecosystem processes and species assemblages, cannot easily accommodate the range of interactions between nitrogen and different environmental processes. The critical load depends on availability of inorganic nutrients at a site, nutrient-use efficiency by different species are different stages in their life history, and land-use practices. Hence, the critical load in reality is a 'moving target' that depends on nitrogen inputs and their chemical form, the responses of individual species, and the ecosystem attribute under consideration. The critical loads set by the United Nations Economic Commission for Europe (UN-ECE, 1994) for heathlands need to be viewed with due caution.

It is clearly difficult to fully examine heathland ecosystem dynamics in response to experimental N-additions but the integrated responses to high inputs of nitrogen may entail a cascade effect as shown in Figure 7.1.

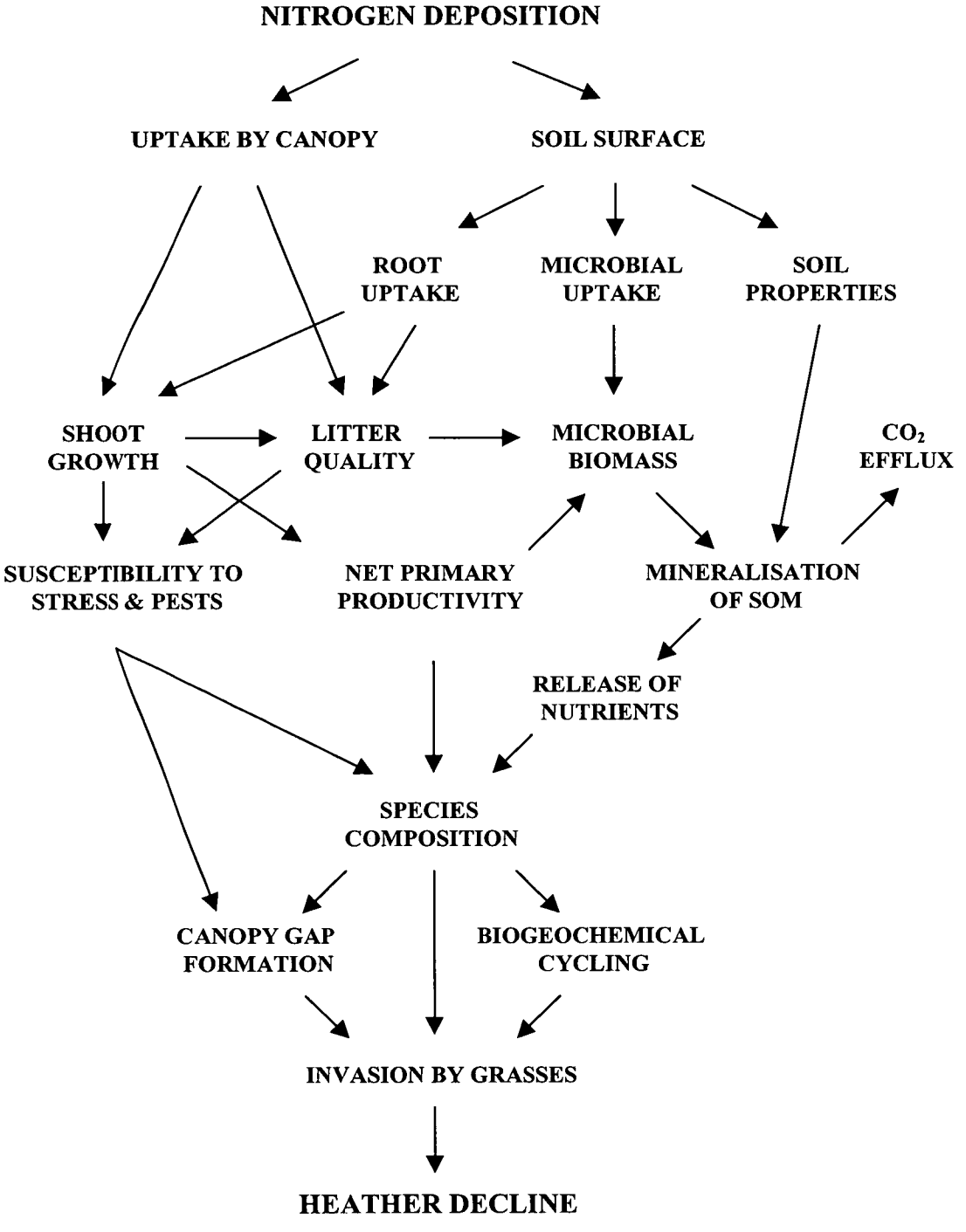


Figure 7.1 A schematic and hypothetical influence diagram showing the sequential manner in which atmospheric nitrogen deposition promotes the conversion of heathlands to grasslands.

In heathlands dominated by *Calluna vulgaris*, the change from ericaceous towards gramineous dominance proceeds in a sequential manner wherein the opening of the vegetation canopy by senescence or by stress and disturbance factors such as drought, frost and heather beetle infestation promotes the invasion of competitive grass species. However, far more work is needed to turn this flow chart into a quantitative tool for management purposes. In order to gain a complete understanding of heathland ecology, long-term experiments simulating field conditions within a controlled environment are vital to investigate how large-scale atmospheric pollutions can modify *Calluna* response patterns to natural elements of stress. Impact assessment studies on heathlands can help evaluate how increasing nitrogen inputs dramatically change plant physiological and soil chemical processes that lead to greater stress sensitivity, alter growth rates, disrupt the tissue carbon/nutrient balance, suppress vegetative regeneration, promote pest outbreaks and subsequently govern species assemblage. Moreover, in soils, although organisms are the proximate determinants of biological transformations, process measurements are closely related to a range of distal variables like organic matter quality and physical environmental variables. Results from the soil respiration–microbial biomass experiments, effects of nitrogen fertilisation and the temperature responses of microbial respiration revealed that the composition of the microbial community to be a neglected factor in understanding the effects of environmental change on upland systems. The laboratory experiments described have largely concerned the initial stages of decomposition, which are dominated by the utilisation of carbohydrates, which are readily accessible to the fungal community. However, under field conditions, over a period of years, the depletion of these pools is likely to affect the successional development, species dominance and activity of fungi decomposing cellulose and ligno-cellulose complexes which form the bulk of peaty organic soils. Hence, a number of approaches need to be used in tandem and far greater emphasis is required on these longer term processes which determine the turnover of organic matter at the system level.

Long-term multidisciplinary research, exploiting new technology, to advance the science of terrestrial ecology is crucial to explore the physical, chemical and biological processes of nature. Stable isotope analysis of organic and inorganic compounds at natural abundance levels is a powerful tool in the resolution of the sources, history and pathways that an element can have as it enters an environment. Studies of ^{15}N natural abundance in ecosystems at various stages of development and across wide ranges of environmental conditions should be pursued to thoroughly explore how distributions of nitrogen isotopes in ecosystems may be linked to nitrogen cycling and other biochemical processes. Utilisation of the stable isotope of carbon, and the ^{14}C 'bomb carbon' signature can shed further light on the carbon dynamics within heathland ecosystems (Harrison, Harkness and Bacon, 1990; Trumbore, 2000).

Impact assessment studies incorporating satellite imagery can help provide data for computer modelling programs to accurately evaluate the vitality of heathland vegetation and set site-specific critical loads. Priority ought to be placed on developing and advancing knowledge regarding the factors which determine the composition, structure and reactions of terrestrial ecosystems, taking into consideration the characteristics of individual plant and animal species. For example, the high capacity of both *Calluna* and *Erica* to produce a long-lived seed bank (Heil and Aerts, 1993 b) can be successfully exploited for the regeneration of heathlands with ericaceous dominance. Furthermore, a decrease in carbon-based secondary defence metabolites to the changing nutritional status of *Calluna* raises wider concerns about the potential long-term effects of additional nutrient inputs from atmospheric deposition on the balance of insect-plant interactions as elevated foliar nitrogen levels have been shown to increase the frequency of pest attacks and alter the feeding behaviour of invertebrates. Well-monitored field and laboratory investigations help provide data for computer modelling programs in order to present an accurate experimental appraisal of the validity of proposed critical loads for dry heathlands and ultimately help draft a technically feasible, economically viable and

socially acceptable integrated management plan for the conservation of heathlands so as to conserve and restore the characteristic landscape of dry heathlands. Long-term conservation of heathlands would require a substantial reduction of the current, unacceptably high atmospheric deposition levels

Ecologists ought to focus on the securing, expansion and dissemination of ecological data so as to further scientific research and provide the basis for management schemes on environmental protection, conservation and the sustainable use of natural resources. The case of *Calluna* is only one example where the ecosystem is threatened, directly or indirectly, by human activities. In a broader context, this project and many other projects are beginning to contribute to the development of a sound scientific database for the monitoring, modelling and predicting of environmental trends in order to define the past, present and future effects of natural and anthropogenic pressures.

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APPENDIX A

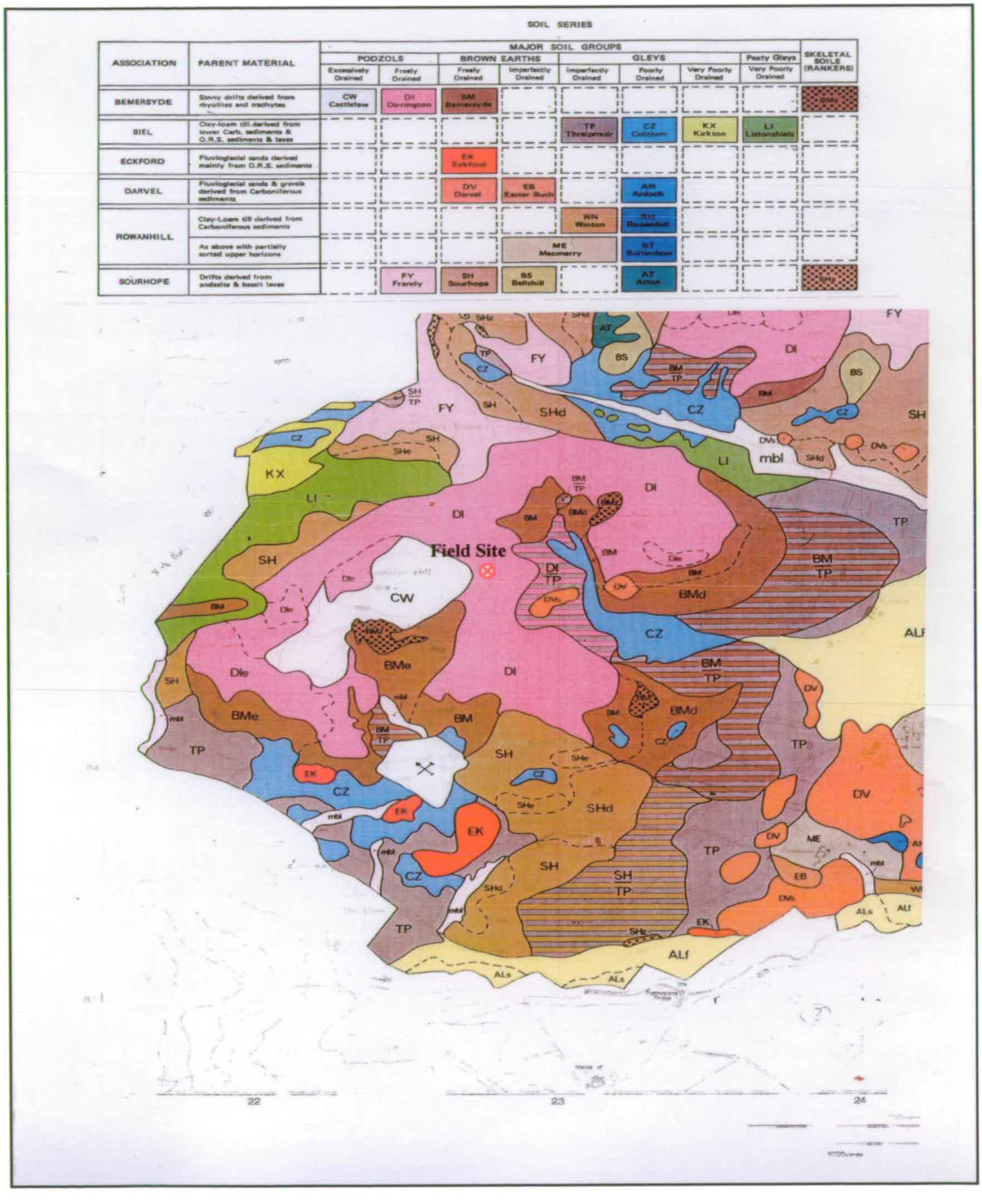
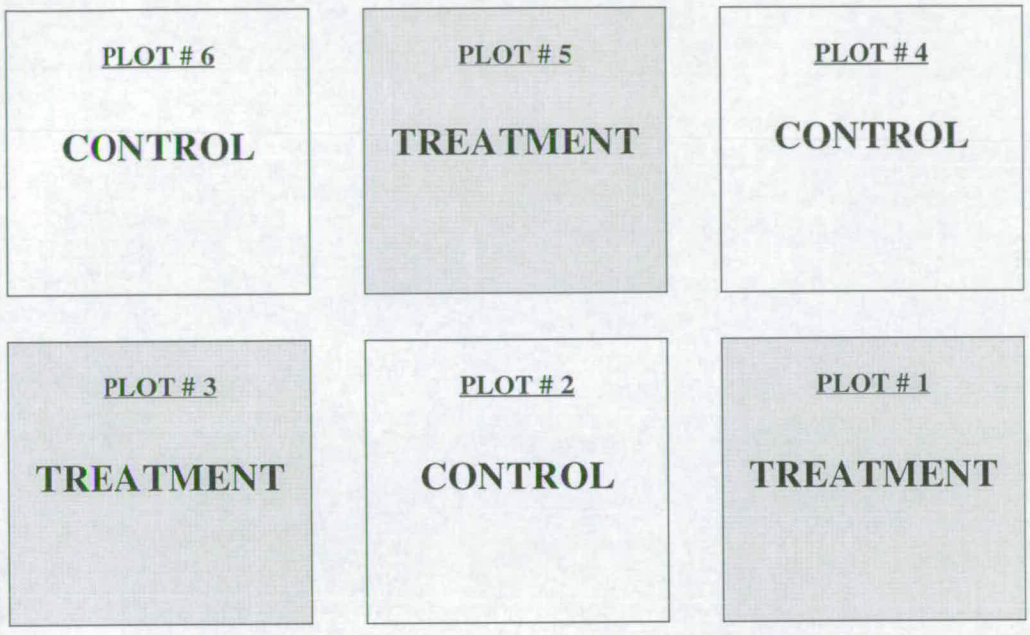


Figure A The soils of the Bush Estates, Pentland Hills, Edinburgh, UK.

APPENDIX B



EACH PLOT = 3m × 3 m = 9 m^2

TREATMENT = 60 kg N ha^{-1} yr^{-1}

Figure B Experimental plots at Castlelaw Hill, in the Pentland Hills near to Edinburgh, UK.