Organic Matter Dynamics in a Forest Soil as affected by Climate Change

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STELLINGEN

1. Er is geen bewijs om aan te nemen dat een verhoging van de atmospherische CO_2 concentratie resulteert in een tragere afbraak van bladstrooisel.

2. De temperatuursgevoeligheid van decompositie van organische stof neemt af met de diepte in het bodemprofiel.
Dit proefschrift

3. Het effect van temperatuur op de mineralizatiesnelheid van C zijn afhankelijk van de incubatietijd en de manier waarop dit wordt uitgedrukt.
Dit proefschrift en Kirschbaum (1995)

4. Behandelingseffecten met betrekking tot netto C of N uitwisseling zoals gemeten in ecosysteem-manipulatie experimenten kunnen het gevolg zijn van het feit dat deze behandelingen vaak schoksgewijs worden opgelegd.
Dit proefschrift

5. De bosbranden in Zuidoost Azië resulteren in een netto C vastlegging in de biosfeer.

6. Broeikasvraagstukken spelen zich in Nederland vooral in het Westland af.

7. Om het gevaar van zwaar metaal voor de volksgezondheid aan te geven is de decibel een meer relevante maat dan het totaalgehalte in de bodem.

8. Het fietsvriendelijk maken van het centrum van Houten leidt tot een toename van het aantal gereden kilometers met zowel de fiets als de auto.

9. De toenemende nadruk op de opleiding van AIO's zal de concurrentiepositie van Nederlandse ten opzichte van buitenlandse promovendi verslechteren.

10. Instituten die frequent reorganiseren moeten e-mailadressen hebben die onafhankelijk zijn van de organisatiestructuur.

11. Omdat de stemming van Pythagoras niet zuiver is, is deze minder algemeen aanvaard dan zijn stelling.

12. 'Foute' noten bestaan niet zolang ze in de juiste context gespeeld worden.

13. De kans dat de langhalsluiten (waartoe de banjo behoort) uitsterven is groter dan de kans dat de korthalsluiten (waartoe de gitaar behoort) uitsterven gelet op de diversiteit binnen de families.

14. Vanwege het vermeende gebrek aan veiligheid op de luchthaven Schiphol kan uitbreiding het best op de Langebaan geschoven worden.

15. De ware eco-toerist blijft thuis.

Stellingen, behorend bij het proefschrift 'Organic matter dynamics in a forest soil as affected by climate change'. Paul Verburg, Wageningen, 22 juni 1998

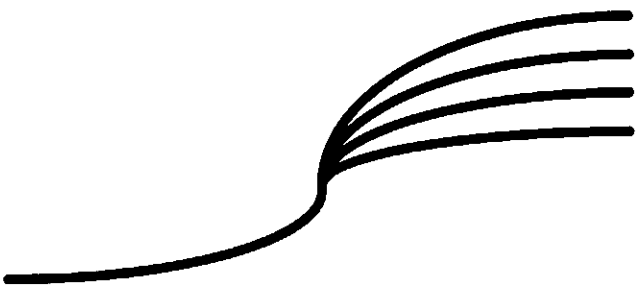
CONTENTS

Als ik maar raak kakel kakel ik altijd wel één keer raak, moet hij gedacht hebben.

G. Komrij (1983) Dit Helse Moeras

Chapter 1

GENERAL INTRODUCTION



GENERAL INTRODUCTION

During the past decades, concentrations of CO_2, CH_4 and N_2O in the atmosphere have significantly increased compared to pre-industrial levels. This rapid increase is mainly caused by combustion of fossil fuels and changes in land use such as deforestation. Although, on geological time scales, concentrations of these gasses have changed as well (e.g. Barnola et al., 1987), it is the current rapid rate of change which causes a major concern. The concern stems from the fact that these gasses absorb longwave radiation reflected by the earth surface which is called the 'greenhouse effect'. A natural greenhouse effect has always existed; without the natural greenhouse effect, temperature at the earth surface would be around -18°C (Ramanathan, 1988). However, the anthropogenic greenhouse effect may cause a significant increase in temperature on top of the natural greenhouse warming (Houghton et al., 1995). Even though its radiative effect is less than that of CH_4 and N_2O, CO_2 is by far the most important greenhouse gas and contributes for about 50% to the greenhouse effect due to its abundance and relatively long residence time (IPCC, 1990).

Atmospheric carbon dioxide is continuously produced and consumed both in biotic and abiotic transformations. In the abiotic cycle, CO_2 is produced through metamorphic and magmatic breakdown of carbonates and carbonate formation in the ocean. Atmospheric CO_2 is mainly consumed by weathering of rocks (Berner et al., 1983). This abiotic cycle is very slow and theoretically it would take millions of years for a CO_2 molecule to be completely recycled. The biotic C cycle is much faster and time scales involved are in the order of days to millennia (Table 1.1). Carbon dioxide is taken up by green plants to produce carbohydrates through photosynthesis. Part of these carbohydrates are used to build plant biomass whereas another part is used for metabolism and respired as CO_2 (autotrophic respiration). In terrestrial ecosystems, when plants die or shed their leaves at the end of the growing season, plant litter is deposited on the soil. In the soil, the litter is consumed by microorganisms to build microbial biomass for metabolic energy use which produces CO_2. This last process is called heterotrophic respiration since most microorganisms do not produce their own metabolic C compounds in contrast to green plants. A small part of the litter is transformed into stable organic components both through biotic and abiotic processes. These stable compounds (humus) decompose much more slowly than fresh litter. Theoretically, during soil development, humus accumulates until total humus decomposition equals production. However, it may take thousands of years to reach equilibrium.

Table 1.1. Estimated turnover times of various C reservoirs

Reservoir	Turnover time (yr)	Source[1]
Atmosphere	4.8	1
Vegetation	10-17	1, 2
Soils	26	2
Ocean	433	1
Lithosphere	110,000-170,000	2

[1] 1 Schimel (1995), 2 cited by Schlesinger (1991)

Since climatic conditions and vegetation cover may change on shorter timescales, soil organic C content may rarely be in equilibrium with the vegetation. The global amount of C stored in soils is approximately 1500 Gt or about twice the amount present in the atmosphere (Schimel, 1995). The living biomass contains approximately 600 Gt of C. The annual C uptake by the biomass from the atmosphere through photosynthesis is estimated at 61.4 Gt whereas the annual release through respiration is 60 Gt (Schimel, 1995). Therefore, the terrestrial biosphere is considered to be a sink for C. It is, however, not clear whether terrestrial ecosystems will continue to act as a sink for C when climate changes.

Increased atmospheric CO_2 concentrations and temperature will affect both C assimilation as well as respiration and thus affect C storage in atmosphere, vegetation and soils. Atmospheric CO_2 affects C pools mainly via the vegetation whereas temperature primarily affects soil processes (Fig. 1.1). Elevated CO_2 will stimulate growth of C_3 plants. This process is known as CO_2 fertilization (Cure and Acock, 1986; Poorter, 1993; Fig. 1.1A). This increased biomass production is often thought to be a potential sink for atmospheric C because at elevated CO_2 plants attain a higher standing and have a higher litter production (Fig. 1.1B). If partitioning of decomposed litter C into microbial C, humus, and CO_2 remains unchanged, both vegetation C, soil C and atmospheric C increase proportionally. However, elevated CO_2 may also affect chemical composition decreasing the decomposability of the litter (Cotrufo et al., 1994; Coûteaux et al., 1991). If litter material becomes more recalcitrant, relatively more C is sequestered in the soil since relatively more humus may be formed (Fig. 1.1C). Direct effects of elevated atmospheric CO_2 concentrations on decomposition are generally considered to be negligible since concentrations of CO_2 in soil air tend to be orders of a magnitude higher than in the atmosphere (Van de Geijn and Van Veen, 1993).

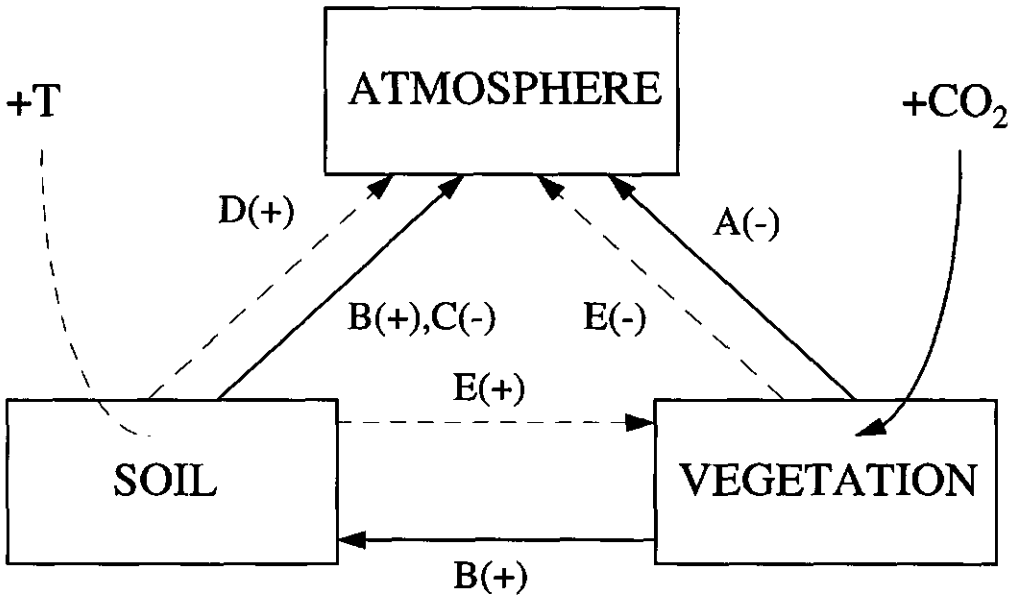


Figure 1.1. Summary of the main effects of elevated temperature and CO_2 on C storage in the soil, vegetation and atmosphere. Temperature effects (dashed lines) are assumed to primarily affect soil processes whereas effects of elevated CO_2 (filled lines) will be mediated through the vegetation. A '+' sign represents an increase in C storage in the pool to which the arrow points and a '-' sign a negative effect. The letters near the arrows refer to the following processes which are explained in more detail in the text.
A. Increased Net Primary Production (NPP)
B. Increased litter production resulting in increased CO_2 emissions
C. Decreased decomposability of 'high-CO_2' litter
D. Increased decomposition
E. Increased nutrient mineralization resulting in increased NPP

As the kinetics of most biological processes, decomposition rates are temperature dependent (Swift et al., 1979). If temperatures increase, decomposition of both litter and humified organic matter will be stimulated and may cause increased emissions of greenhouse gasses CO_2, CH_4 and N_2O from the soil to the atmosphere (Kirschbaum, 1993; Fig. 1.1D). Upon decomposition, microbes use nutrients present in the substrate for synthesis of biomass. If the plant material contains more nutrients than is needed for synthesis of microbial biomass, the excess is released into the soil which is called mineralization. These nutrients can be taken up by plants again. If decomposition rates of

organic matter increase as a result of elevated temperature, more nutrients may be mineralized. In many natural ecosystems, availability of N limits plant growth, so increased N availability may stimulate plant growth, thereby enhancing CO_2 fertilization (Rastetter et al., 1991; Fig. 1.1E). Any nutrients mineralized in excess of plant uptake may leach to the ground- and surface waters, and affect C and N cycling in downstream aquatic ecosystems (Wright and Schindler, 1995). A possible direct effect of elevated temperature is increased plant biomass production by increasing the length of the growing season.

Although the most important effects of climate change have been summarized above, some effects which are hard to predict may have a large impact on C cycling in terrestrial ecosystems too. First, below-ground rhizosphere processes and root turnover which play an impotant role in soil organic matter dynamics (Merckx et al., 1986; Van Veen et al., 1989) can be affected by elevated CO_2 in that more C is allocated to roots. In addition, root litter may become more difficult to decompose due to a decrease in substrate quality. Plants release low-N organic compounds in the rhizosphere which are used as C source by microorganisms. Changes in amount and composition of these root exudates will undoubtedly affect soil C budgets. Second, elevated CO_2 and temperature may indirectly affect organic matter decomposition by changing soil moisture conditions. Under elevated CO_2, plants partially close their stomata which decreases exchange of water vapor between leaf and ambient air (Raschke, 1975). This mechanism causes a reduction in transpiration so less water is needed to produce the same amount of biomass (Mooney et al., 1991). If the increase in biomass is less than the increase in this water use efficiency, soils become wetter. This effect of elevated CO_2 on soil water availability is counteracted if the the biomass is increased more strongly. Also, elevated temperature will increase evaporation which causes the surface soil to dry out. Finally, increased CO_2 concentrations and changes in nutrient availability may shift plant species composition which will affect several processes influencing C dynamics, a.o. quantity and quality of litter produced.

OBJECTIVE

From the previous paragraph it is clear that the net response of terrestrial ecosystems to climate change is difficult to predict due to a multitude of interactions between plant, soil and atmosphere. Production and decomposition of soil organic matter is one of the key processes in C cycling in terrestrial ecosystems. The objective of this thesis is to assess the effects of elevated CO_2 and temperature on soil organic matter dynamics in a boreal forest. The boreal and tundra zone cover 14% of total land surface but contain 25% of the global

amount of soil C (Schlesinger, 1991). Therefore, changes in decomposition induced by climate change may significantly affect the net C exchange between terrestrial biosphere and atmosphere.

METHODOLOGY

The research presented in this thesis was carried out within the framework of the Climate Change Experiment (CLIMEX). The main goal of this experiment was to assess the effects of climate change on an entire headwater catchment with special emphasis on the soil-plant-water linkages. In this project, two small forested catchments in southern Norway were experimentally manipulated by increasing atmospheric CO_2 concentration and/or (soil) temperature. The site is located on a large granite plain near the town of Grimstad (58°23' N, 8°19' E). Mean annual precipitation is 1400 mm and mean annual temperature is 5°C (-3°C in January and +16°C in July). Elevation of the site is approximately 300 m. Small, patchy, depressions in the granite surface are filled with post-glacial soil material, leaving 30-50% of the bedrock uncovered. Soil depth varied between 0 and 70 cm. The vegetation is dominated by dwarf shrubs (*Calluna vulgaris* (L.) Hull, *Vaccinium myrtillus* L., *Vaccinium vitis-ideaea* L. *Vaccinium uliginosum* L.). The main tree species are Scots pine (*Pinus sylvestris* L.), birch (*Betula pubescens* Ehrh.) and Norway spruce (*Picea abies* (L.) Karsten) and are confined to areas with deeper soils. Although the soils are shallow and vegetation relatively sparse, the site is representative for most boreal ecosystems in terms of climate, dominance of coniferous trees and presence of acid podsolic soils. In 1983, two catchments were covered by a transparent roof as part of the RAIN (Reversing Acidification In Norway) project (Wright et al., 1993). Precipitation was collected from the roof and recycled under the roof using a sprinkler system. In the largest catchment (KIM; 800 m^2) prior to sprinkling, water was cleaned and natural amounts of sea salt were added. In the second catchment (EGIL; 400 m^2) ambient, acid, rain was sprinkled under the roof. In 1994, the clean-rain catchment (KIM) was completely sealed by transparent walls and was split in two parts. In June 1994, about 80% of the catchment was manipulated by increasing the CO_2 concentration by 200 ppmv during the growing season and increasing temperature by 5°C in winter and 3°C in summer with intermediate temperature increases in the intervening months. This CO_2 and temperature increase agrees with predictions made by Global Circulation Models for the middle of the next century at the latitude of southern Norway (Houghton et al., 1995; Sellers et al., 1996). In the acid-rain catchment (EGIL), in spring 1994, heating cables were put on top of the litter layer in 80% of the catchment. These cables caused

soil temperatures to increase by the same amount as the air temperature in KIM. In both catchments, the remaining 20% acted as untreated controls. Three, uncovered, catchments (METTE, ROLF, CECILIE) were used as outside controls.

Within the CLIMEX project, scientists from different disciplines cooperate by carrying out measurements on nutrition, phenology and gas-exchange of the vegetation, catchment hydrology, soil chemistry, soil fauna and runoff chemistry. Integration of these measurements will allow for quantification of whole ecosystem response to climate change as well as rates of individual processes. These data can be used to test models predicting future effects of climate change on ecosystems. In field experiments, environmental conditions often show temporal variation whereas soil characteristics exhibit spatial variation. In addition, variables may interact which sometimes hampers interpretation of field data. Therefore, complementary to the field study, laboratory experiments were carried out to look at the effect of specific factors or interactions under more controlled conditions.

OUTLINE OF THE THESIS

The second and third chapter describe the effects of elevated temperature and CO_2 on litter decomposition and N mineralization under field conditions. Litter was produced at ambient and elevated CO_2 concentrations to assess the effect of CO_2 on litter quality and subsequent decomposition. The litter was incubated in both enclosures to determine the direct effects of elevated temperature and CO_2 on litter decomposition. In addition, net N mineralization was measured both in- and outside the enclosures to determine effects of climate change on N availability.

Chapter 4 describes the effects of elevated temperature on decomposition by incubating soil cores under constant temperature and moisture conditions. We used large undisturbed cores to bridge the gap between poorly controlled large-scale field and highly controlled small-scale laboratory experiments often using pre-treated soil samples. Emissions of CO_2 as well as chemical composition of the water leaching from the columns was monitored during a 4 month incubation at different temperatures. Since many studies have shown that decomposition rates are depth dependent, soil material from different soil layers was incubated separately to determine temperature sensitivity of decomposition as a function of depth.

In Chapter 5, the effects of elevated temperature on microbial C and N transformations in organic surface horizons was measured. Although these layers contain only a few percent of the total amount of C present in the soil, C and N transformations in

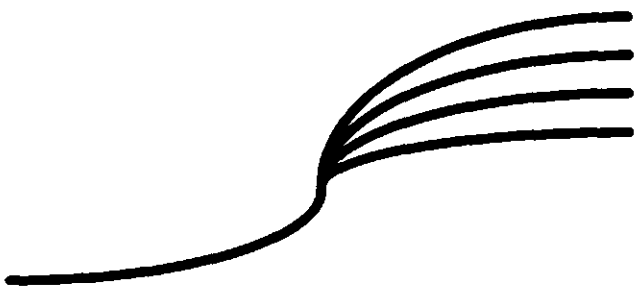
these layers are very rapid due to the presence of fresh, labile, organic matter. Therefore, short-term response to changes in environmental conditions are likely to be most pronounced in these layers. The effects of elevated temperature on gross N fluxes were determined using the ^{15}N enrichment technique. Using a simulation model, gross C and N fluxes in these horizons were calculated.

In Chapter 6, a greenhouse experiment was conducted to measure allocation of C in different plant-soil compartments as a function of CO_2 concentration and nutrient level. Heather plants were pulse-labeled with ^{14}C-CO_2 to determine whether elevated CO_2 causes increased below-ground allocation of C. The labeled soil was subsequently incubated to follow the fate of the root-derived organic matter after harvest of the plants.

Finally, Chapter 7 attempts to synthesize the main results. By integrating soil and plant measurements using the NICCCE model, some predictions on overall ecosystem response to global climate change are made. In addition, some of the gaps in our knowledge are pointed out which need to be filled in to decrease the uncertainty in predictions on ecosystem behavior in a high CO_2 and temperature world.

Chapter 2

MANIPULATION OF A FORESTED CATCHMENT: LITTER DECOMPOSITION AND N MINERALIZATION



P.S.J. Verburg and N. van Breemen

MANIPULATION OF A FORESTED CATCHMENT: LITTER DECOMPOSITION AND N MINERALIZATION

ABSTRACT

Model predictions on the response of soil processes to global warming are mostly inferred from small-scale laboratory studies. We experimentally treated a forested catchment in southern Norway by increasing CO_2 (to 560 ppm) and temperature (+3-5°C) and studied treatment effects on litter decomposition and N mineralization. *Betula* and *Calluna* litter produced under ambient and elevated CO_2 in greenhouses was incubated for 1 year in the manipulated catchment. After 1 year exposure to elevated CO_2, *Betula* produced litter with a higher C/N ratio but a similar decomposition rate as litter produced under ambient CO_2. Two years of exposure to elevated CO_2 did not affect the C/N ratio of both *Betula* and *Calluna* litter. However, this high-CO_2 *Betula* litter decomposed faster than the low-CO_2 litter, whereas elevated CO_2 had no effect on decomposition of *Calluna* litter. We expected the higher temperature in the climate manipulation treatment to increase decomposition, but no such effect was found probably due to drier conditions of the litter at elevated temperature. Net N mineralization from the 0-10 cm soil layer significantly increased, presumably as a result of increased temperature. The effect was largest under *Calluna*.

INTRODUCTION

Increased emissions of CO_2 and other greenhouse gasses due to combustion of fossil fuels and land use change may lead to a significant increase in global temperature over the next decades (Houghton et al., 1995). An increase in temperature would stimulate decomposition of litter and humified soil organic matter which, in turn, would cause increased soil emissions of greenhouse gasses such as CO_2, CH_4 and N_2O. Increased N mineralization plus CO_2 fertilization are likely to favor Net Primary Production, resulting in increased C fixation in the living biomass. If N mineralization exceeds uptake by vegetation, N may leach to ground- or streamwater causing acidification and eutrophication of aquatic ecosystems (Hessen and Wright, 1993; Wright and Schindler, 1995). However, emissions of C and N to atmosphere and hydrosphere through enhanced decomposition may be buffered if litter produced under elevated CO_2 is more recalcitrant

to decomposition (Norby et al., 1986b; Lambers, 1993; Cotrufo et al., 1994). So far, predictions regarding whole ecosystem responses have largely been based on modeling in which results from small-scale greenhouse studies and laboratory incubations have been extrapolated to the ecosystem level (Overpeck et al., 1990; Rastetter et al., 1992; Schimel et al., 1997). In addition, to date, most large-scale experiments manipulate either CO_2 (e.g. Hendrey et al., 1993; Miglietta and Raschi, 1993) or temperature (e.g. Peterjohn et al., 1993; Mitchell et al., 1994; Rustad et al., 1995). Combined temperature and CO_2 enrichment experiments are scarce even though interactions between CO_2 and temperature are crucial in evaluating whole ecosystem response to climate change. Oechel et al. (1994) found that in an arctic tundra ecosystem, elevated CO_2 and temperature resulted in a persistent net ecosystem carbon sequestration due to increased nutrient availability caused by increased mineralization. However, elevated CO_2 alone resulted in a transient response and after 3 years of treatment, net ecosystem C exchange returned to pre-treatment levels (Oechel et al., 1994)

CLIMEX (Climate Change Experiment) is an international multidisciplinary project in which temperature and CO_2 are manipulated in a forest catchment ecosystem (Jenkins and Wright, 1995). This facility provides an opportunity to integrate measurements on vegetation and soil response and to assess future response of a forest ecosystem to climate change. Current studies include: phenology, photosynthesis and biomass of tree and shrub vegetation; soil fauna and decomposition; soil and soil solution chemistry; soil and catchment hydrology; trace gas fluxes, and runoff chemistry. In this paper, we report the effects of elevated CO_2 and temperature on litter decomposition and N mineralization.

MATERIAL AND METHODS

Site description

The CLIMEX site is located at Risdalsheia (58°23' N, 8°19' E) near Grimstad, southernmost Norway. The site is 300 m above sea level on a large biotite granite plateau, and is representative for large areas of upland southern Norway. Mean annual precipitation is 1400 mm and mean annual temperature is 5°C (-3°C in January and +16°C in July). Depressions in the granite surface are filled with post-glacial soil material in which acid, peaty podsolic soils have developed. Maximum soil depth is 70 cm. About 30-50% of the bedrock is exposed. The vegetation is dominated by dwarfshrubs (*Calluna vulgaris* (L.) Hull, *Vaccinium myrtillus* L., *Vaccinium uliginosum* L. and *Vaccinium vitis-idaea* L.) and scattered trees (*Pinus sylvestris* L., and *Betula pubescens* Ehrh.).

Experimental design

The KIM catchment (860 m^2) was covered by transparent roofs in 1983 as part of the RAIN (Reversing Acidification In Norway) project (Wright et al., 1993). Precipitation was collected from the roof, filtered, and ion-exchanged. Natural levels of sea salt were added before the water was distributed under the roofs at a fixed rate of 2 mm h^{-1} using a sprinkler system. In 1993, this catchment was completely enclosed with air-tight, transparent walls. The greenhouse was separated in two parts by a transparent wall such that 20% of the catchment acts as an untreated control (KIM-c). From June 1994, in the treated part (KIM-t), during the growing season CO_2 was increased up to 560 ppm and the temperature was increased 5°C compared to KIM-c in January and 3°C in July with intermediate temperature increases in the intervening months. The difference in air temperature during the treatment between KIM-c and KIM-t closely followed the targets (Fig. 2.1). Prior to the treatment, air temperature throughout the KIM catchment was homogeneous due to the absence of side walls. Two uncovered catchments (METTE, ROLF) served as outside controls.

Litter decomposition

One-year old birch (*Betula pubescens* Ehrh.) and heather (*Calluna vulgaris* (L.) Hull) were grown for 2 years in 10l pots containing sandy soil (C=2.16%, N=0.10%, pH-H_2O=6.59) at either 365 or 700 ppm CO_2 in greenhouses in The Netherlands. Temperature, light and humidity followed ambient outside conditions. *Betula* litter was collected each year whereas *Calluna* litter was collected after 2 years. Since *Calluna* generally produces little litter, we reduced water supply to increase litter production. Total C and N of the litter was measured using an EA 1108 CHN element analyzer. For Klason-lignin determinations, ethanol-soluble components were removed by extraction with 80% ethanol (3 x 30 min). The residual material was treated with 10 ml 72% H_2SO_4 at 30°C for 1 h. This mixture was diluted to 3% H_2SO_4 and refluxed for 2 h. The residual solid material was washed, filtered and dried overnight at 105°C. Lignin content was determined as the mass loss upon ignition (650°C; 2 h) of this residue.

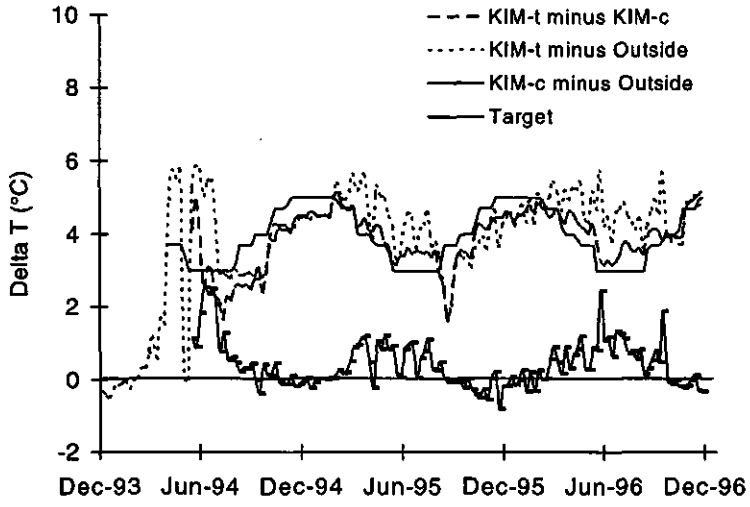


Figure 2.1. Differences in air temperature between KIM-t and KIM-c, KIM-t and outside, KIM-c and outside.

Litterbags with *Betula* litter collected after 1 year were incubated in KIM-c and KIM-t in April 1994. *Betula* and *Calluna* litter collected after 2 years was incubated in April 1995 in KIM-c, KIM-t and METTE. We incubated both low- and high-CO_2 litter in each section to allow for separation of litter quality effects from climate treatment/site effects. All litterbags were incubated for 1 year. Prior to the incubations, the *Betula* litter was frozen for use in soil fauna studies (Vreeken-Buijs and Brussaard, 1995) whereas *Calluna* litter was not. All litterbags contained 4 g of litter. Mesh size of the bags was 1.5 mm for *Betula* litter and 1 mm for *Calluna* litter. Every 6 months, 10 litterbags of each litter type were collected. After each sampling, litterbags were cleaned, dried at 70°C and weighed. Prior to the climate manipulation, we carried out litterbag experiments with standard pine litter (Berg et al., 1982) to assess the effect of environmental site differences. In October 1991, we incubated air-dried pine litter for 1 year under *Vaccinium* or *Calluna* in KIM and ROLF using 25 replicates for each vegetation-catchment combination. In October 1992, we incubated pine litter for 1 year in KIM-c, KIM-t and ROLF using 25 replicates per site. Litterbags had a mesh of 1 mm and contained between 0.5 and 1 g of pine needles. The exact weight was written on a tag present in the litterbag.

At the start of the incubations, ten litterbags of both high- and low-CO_2 *Calluna* litter were sampled randomly to determine the flower-to-leaf ratio, C, N, and lignin contents of flowers and leaves. To estimate the relative contributions of *Calluna* flowers and leaves to the total decomposition, we measured respiration by incubating 1 g of litter from flowers or leaves (n=3) at 20°C. The flower and leaf material were mixed with 10 g A-horizon material from a sandy forest soil (C=1.29%; N=0.06%; pH-H_2O=3.81) having a moisture content of 40%. Respiration was measured as the change in conductivity of 10 ml 0.6M KOH upon CO_2 absorption present in a vial in the headspace of the 250 ml sample containers (Nordgren, 1988). Empty sample containers and containers with soil only were used as blanks. CO_2 production was measured hourly and data were stored automatically on a personal computer.

Soil nitrogen mineralization

Net soil N mineralization was measured in plots dominated by either *Calluna* or *Vaccinium* in the CLIMEX roof before the high CO_2 and temperature treatment started (June 1993-June 1994) and during the first 2 treatment years until August 1996 using the sequential core incubation method (Berendse et al., 1987; Raison et al., 1987; Berendse et al., 1989; Berendse, 1990). In each vegetation type in KIM-c, KIM-t and METTE, we measured N mineralization in ten plots of 20 by 50 cm. Per year we had four incubation periods: April-June, June-August, August-October, and October-April. At the start of each incubation period, two samples were taken 5-10 cm apart using pre-weighed PVC tubes (length 15 cm, diameter 2.8 cm, wall thickness 2 mm). Soil was sampled to a depth of 10 cm unless bedrock was shallower. One sample was taken to the lab whereas the second sample was covered on both sides with soft polyethylene caps and put back into the soil. The incubated cores had four 4 mm holes to allow for gas diffusion. At the end of the incubation period, the samples were collected from the field and new incubations were started. The retrieved tubes were weighed and stored overnight at 4°C. Soil material including the organic surface horizons and litter layer were mixed after measuring length of the soil cores. Subsequently, 20 g of field moist soil was extracted with 50 ml 1 M KCl by shaking for 1 h. The KCl extracts were immediately filtered (Schleicher and Schuell 595.5) and analyzed for NH_4-N and NO_3-N by colorimetry on an auto analyzer. Net N mineralization was calculated as the difference in NO_3-N + NH_4-N between the incubated and reference samples.

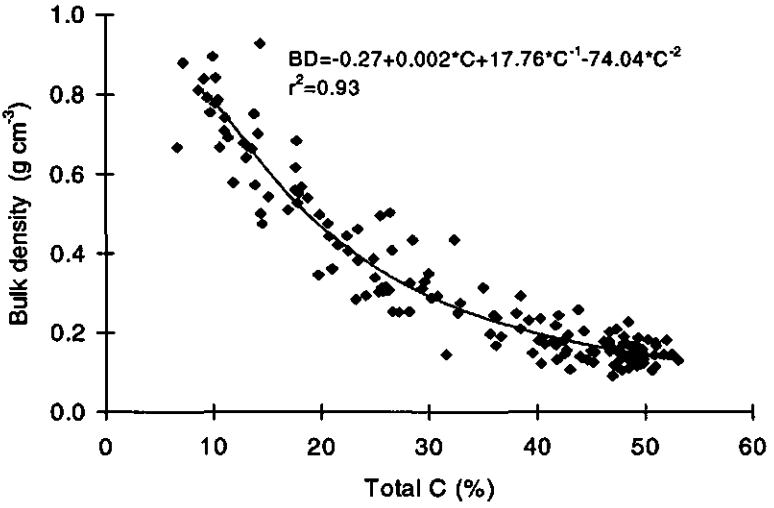


Figure 2.2. Relation between dry bulk density (BD) and C content as measured in the mineralization tubes.

Soil moisture content was measured after drying the samples at 105°C for 24 h. Gravimetric water content (g H_2O g dry $soil^{-1}$) in samples collected at any one time showed a large variation reflecting differences in soil organic C content (6.7 to 53.0%). We normalized water content to total pore space rather than to dry soil, and expressed moisture as relative water content (RWC; = volume fraction water/total pore space; Skopp et al., 1990). Total pore space was calculated from volume fractions of organic (ϕ_{om}) and mineral (ϕ_m) material as 1- ϕ_{om}- ϕ_m. In the period August-October 1994, we measured total C and N on 80 reference and 80 incubated samples used in the mineralization measurements. Organic C content correlated well (r^2 =0.93) with bulk density which justified conversion of mass fraction to volume fraction (Marnette and Stein, 1993; Fig. 2.2). We calculated organic matter content by multiplying C content by 1.7 (Schachtschabel et al., 1984). ϕ_{om} and ϕ_m were calculated by assuming a bulk density of 1.4 g cm^{-3} for organic matter and 2.65 g cm^{-3} for mineral matter (Koorevaar et al., 1983). Volumetric water content of the soil in the mineralization tubes was calculated by multiplying gravimetric water content with bulk density.

Statistical methods

Data on residual mass of *Betula* and *Calluna* litter were analyzed by multiple analysis of variance (MANOVA) using 'catchment'(METTE, KIM), 'field treatment', and 'litter quality' as factors. 'Field treatment' was nested in the factor 'catchment'. N mineralization and RWC data were analyzed by MANOVA with 'treatment', 'vegetation' and 'location'(METTE, KIM-c, KIM-t) as main factors. The factors 'treatment' and 'vegetation' were nested within the factor 'location'. We tested for interannual variation by including the factor 'year' instead of 'treatment'. METTE data were analyzed separately as well using factors 'vegetation' and 'year'. For MANOVA on RWC data we added the factors 'RWC prior and after incubation' and 'season' Statistical analysis was carried out using SPSS version 6.1. Effects were considered to be significant if $p<0.05$.

RESULTS AND DISCUSSION

Litter decomposition

One year of exposure to elevated CO_2 caused a significant increase in C/N ratio of the *Betula* litter. However, 2 years of exposure to elevated CO_2 had no significant effect on C/N ratio of *Betula* leaves while C/N of *Calluna* leaves and flowers decreased (Table 2.1). Most studies report an increase in C/N ratio at elevated CO_2 (Luxmoore et al., 1986; Norby et al., 1986b; Coûteaux et al., 1991; Cotrufo et al., 1994) but Franck et al. (1997) found that for some grass species C/N ratio increased whereas for others it decreased upon elevated CO_2. Ball (1997) found that C/N ratio decreased for several C_4 species, but increased for C_3 species at elevated CO_2. Our data on *Betula* suggest that the effects of CO_2 on C/N ratio may vary with exposure time or plant age. Few data are available on the effects of elevated CO_2 on secondary metabolites such as lignin. In our study, after 2 years exposure lignin content significantly decreased for *Betula* but slightly increased for *Calluna* leaves (Table 2.1). Lignin content of *Calluna* flowers was not affected by CO_2. Norby et al. (1986b) found a decrease in lignin content for white oak which contrasts results from Cotrufo et al. (1994) who found an increase in lignin content for four tree species under elevated CO_2. Ball and Drake (1997) found no significant effect of elevated CO_2 on lignin content of a C_3 sedge and a C_4 grass. Lambers (1993) suggested that the

Table 2.1. Chemical characteristics of Betula and Calluna litter and respiration of Calluna litter

Species	Treatment (ppm)	Exposure time (years)	C/N[1]	Lignin[1] (%)	Respiration (mg C g^{-1})
Betula	365	1	62	16.7	nd[2]
	700	1	76**[3]	16.0	nd
Betula	365	2	94	17.8	nd
	700	2	87	16.1***	nd
Calluna leaves	365	2	65	23.0	39.4[4]
	700	2	49***	24.1*	34.7
Calluna shoots	365	2	119	35.5	22.2
	700	2	86***	34.5	23.6

[1] n=4 for *Betula* and 10 for *Calluna*

[2] nd = not determined

[3] * $p<0.05$;** $p<0.01$;*** $p<0.001$

[4] n=3

concentration of secondary metabolites may increase due to nutrient limitations induced by elevated CO_2, not as a direct effect of elevated CO_2 itself. Consequently, the opposing trends in lignin content between *Betula* and *Calluna* leaves may reflect differences in nutrient requirements between the two species.

Although 1 year of exposure to elevated CO_2 resulted in an increase in C/N ratio of *Betula* litter, this change did not affect decomposition (Table 2.2). However, after 2 years of exposure, high-CO_2 litter decomposed significantly faster although C/N ratio was not affected by CO_2. For *Calluna*, exposure to elevated CO_2 did not affect decomposition. Results were not caused by differences in percentage of flowers which was 26.1±2.8% at ambient CO_2 and 24.9±5.9% at elevated CO_2. However, in KIM-c and KIM-t, the effect of *Calluna* litter quality was reversed which caused a significant interaction between litter quality and treatment (Table 2.2). Low- and high-CO_2 grown litter incubated in METTE did not show a difference in mass loss. The opposite effect of elevated CO_2 in KIM-c and KIM-t appeared to be caused by an anomalously high residual mass value of the high-CO_2 litter in KIM-c. Residual mass after 1 year was higher than after 6 months (not shown) and

Table 2.2. Residual mass (%) of Calluna and Betula litter after 1 year of incubation. Values are means and standard deviations (in parentheses) for n=10, except if noted otherwise

		Calluna		*Betula*		*Betula*	
Exposure time	(years)	2		2		1	
Treatment	(ppm)	365	700	365	700	365	700
KIM-c		63 (4)	79 (10)	55 (7)	51 (5)	48 (8)[1]	50 (5)
KIM-t		68 (4)	65 (13)	60 (9)	54 (10)	nd[2,3]	51 (9)[1]
METTE		76 (6)	77 (9)	61 (5)	53 (2)	nd	nd
Catchment[4] (C)			*		*		nd
Treatment (T)			ns		ns		ns
Litter quality (L)			ns		***		ns
C x L			*		ns		nd
T x L			**		ns		nd

[1] n=6

[2] not enough low-CO_2 litter was produced to allow for incubation in KIM-t as well

[3] * p<0.05; ** p<0.01; *** p<0.001; ns = not significant; nd = not determined

[4] 'Catchment'= KIM versus METTE; 'Treatment'= Field treatment; 'Litter quality'= low versus high-CO_2 litter

was also higher than in METTE. Two out of ten litterbags showed no mass loss which may have been caused by incomplete removal of mineral material that sometimes entered the bags. Cumulative CO_2 production in lab incubations after 80 days was slightly higher for high-CO_2 leaves (p=0.12) (Table 2.1). Cotrufo et al. (1994) found a significantly slower decomposition of high-CO_2 litter for several tree species, in agreement with higher C/N ratios. Franck et al. (1997) found no consistent effect of elevated CO_2 and nutrient conditions on mass loss among four grass species, and found no correlation between mass loss and C/N ratio across species and treatments. Neither our *Betula* nor *Calluna* incubations support presence of a correlation between C/N ratio and decomposition rates as found by Cotrufo et al. (1994).

Comparing studies remains difficult due to differences in incubation conditions and measurement period. Cotrufo et al. (1994) and Franck et al. (1997) incubated litter in meso- or microcosms at constant temperature and moisture whereas we incubated litter

under field conditions. Under field conditions the decomposer community may be very different from laboratory conditions, but is probably more natural. Coûteaux et al. (1991) showed that respiration of high-CO_2 litter was higher than low-CO_2 litter when a complex food web was present, whereas the reverse held when the food web was simple. In addition, substrate effects may be time-dependent: Cotrufo et al. (1995) observed that mass loss after incubating litter for 1 year was the same for low- and high-CO_2 litter whereas at intervening samplings, significant litter quality effects were observed. Berg and Ekbohm (1991) showed that nutrient-rich litters initially decompose fastest but that accumulated mass loss at later stages tends to be lower than for nutrient-poor litters. So, even under similar incubation conditions, substrate quality effects are time-dependent and can be opposite depending on the time measured.

During pre-treatment years, decomposition of pine litter was lowest in the outside control (ROLF), similar in KIM-c and KIM-t, and independent of the vegetation under which litterbags were placed (Table 2.3). The higher decomposition in KIM may have been caused by a combination of higher temperatures and more favorable moisture conditions. In winter, average soil temperature was about 2°C higher in KIM than in ROLF whereas in summer, soil temperatures were similar. In addition, the roof substantially reduced radiation which may have depressed evaporation, resulting in wetter soils. In general, soils were deeper in KIM, so drought stress may have occurred less

Table 2.3. Residual mass (%) of pine litter after 1 year of incubation in KIM and ROLF.

Site	Vegetation	Residual mass	n
October 1991			
KIM	*Calluna*	69.3 (5.3)a[1]	25
	Vaccinium	71.5 (5.0)a	24
ROLF	*Calluna*	77.8 (4.3)b	25
	Vaccinium	78.0 (6.1)b	25
October 1992			
KIM-c		66.6 (11.9)a,b	22
KIM-t		62.2 (11.9)a	26
ROLF		71.7 (6.5)b	18

[1] different letters indicate significant differences within each year of incubation at $p<0.01$

frequently than outside. During the treatment period too, decomposition rates were higher in KIM than in the outside control (METTE) but were generally not affected by the climate treatment in KIM-t. In KIM-t, moisture content of the litter layer may have decreased due to the elevated temperatures. Therefore, a positive temperature effect may have been (partly) offset by a negative moisture effect leaving the overall treatment effect to be negligible. Direct effects of elevated CO_2 on decomposition were not likely. Ball and Drake (1997) found that litter decomposition was similar at CO_2 concentrations of 350 and 700 ppm. Koizumi et al. (1991) showed that between 0 and 300 ppm, decomposition decreased with increasing CO_2 concentration. When the CO_2 concentration was increased above 300 ppm, decomposition did not change.

Soil nitrogen mineralization

In KIM-t, both in *Calluna* and *Vaccinium* plots, N mineralization increased during the first treatment year (Fig. 2.3). In the second treatment year, mineralization in *Calluna* plots continued to increase. When including data for KIM-c and METTE, significant treatment effects became apparent (Table 2.4). No significant year effects were found, suggesting that the increase in mineralization in KIM-t was indeed caused by the climate treatment. In METTE, both under *Vaccinium* and *Calluna* and in KIM-c under *Calluna*, mineralization did not vary between years. In KIM-c under *Vaccinium*, mineralization was significantly higher in the first treatment year than in the pre-treatment year. This increase was most likely due to sampling problems. A loose litter layer covered shallow pine roots leaving an air layer between the litter layer and the rest of the soil. Using the 15 cm sampling tubes, we could not sample 10 cm soil. After the first incubation period in the pre-treatment year, we switched to 20 cm sampling tubes to sample a larger amount of soil. This resulted in higher mineralization rates expressed per meter squared. In KIM-t and METTE, *Vaccinium* plots were not in the vicinity of large tree roots so in these plots we did not have sampling problems. The low mineralization under *Vaccinium* in KIM-c may have contributed to a significant overall 'location' effect on N mineralization (Table 2.4). However, pre-treatment N mineralization in *Calluna* plots was similar in KIM-c, KIM-t and METTE suggesting that no chamber- or site-effects were present. In KIM-t and METTE, mineralization was similar under *Vaccinium* as well and consistently higher than under *Calluna*.

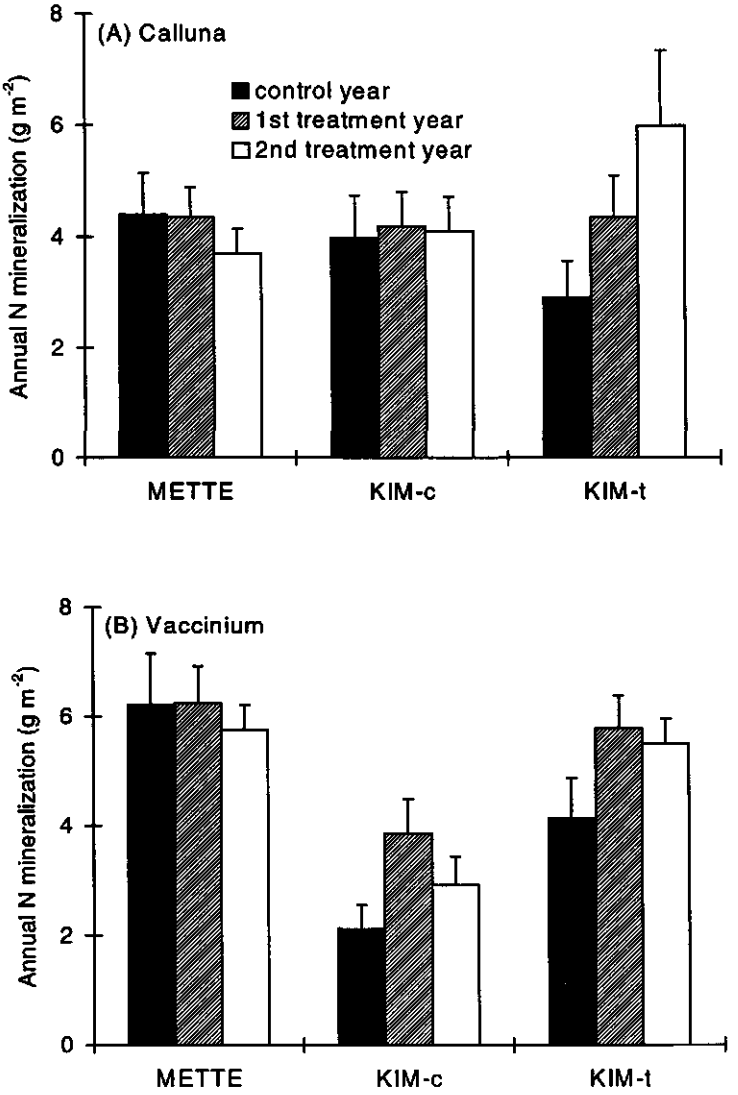


Figure 2.3. Annual N mineralization in METTE, KIM-c and KIM-t during control and treatment years under Calluna (A) and Vaccinium (B). Error bars represent standard errors of the mean.

Table 2.4. MANOVA results for N mineralization in METTE and KIM

Factor	KIM+METTE	METTE
Treatment (T)	**[1]	nd
Vegetation (V)	ns	**
Location (L)	***	nd
Year (Y)	(ns)[2]	ns
T x V	ns	nd
V x Y	nd	ns

[1] * p<0.05; ** p<0.01; *** p<0.001; ns = not significant; nd = not determined

[2] Factor 'year' was not included when factor 'treatment' was used.

Table 2.5. MANOVA results on relative water content in mineralization tubes in METTE and KIM.

Factor	KIM+METTE	METTE
Treatment (T)	**[1]	nd
Vegetation (V)	***	***
Location (L)	***	nd
Season (S)	***	***
Year (Y)	(**)[2]	**
Incubation (I)	***	***
T x S[3]	***	nd
T x I	*	nd
T x V	ns	nd
V x S	*	ns
S x Y	nd	***
S x I	**	*
S x L	***	nd
Y x I	nd	**

[1] * p<0.05; ** p<0.01; *** p<0.001; ns = not significant; nd = not determined

[2] Factor 'year' was not included when factor 'treatment' was used.

[3] higher order interactions were calculated but not included in the table. Most higher order interactions were not significant.

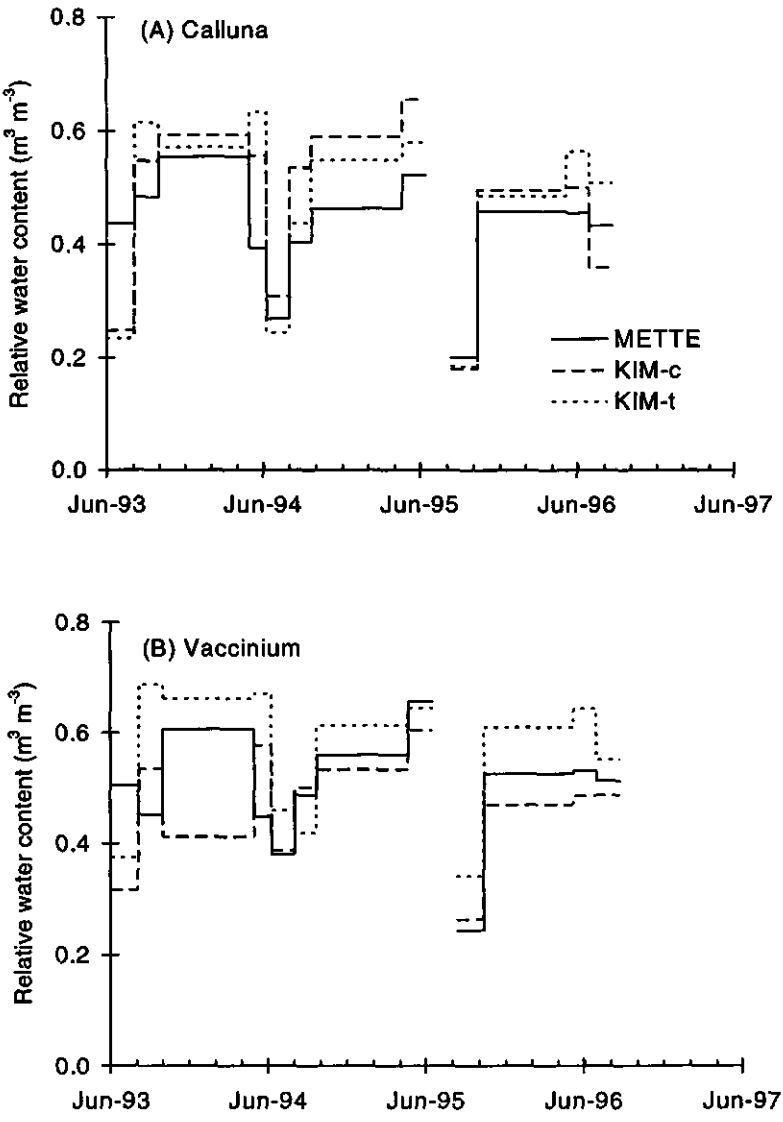


Figure 2.4. Relative water content (= volume water divided by total pore space) of soil inside mineralization tubes under Calluna (A) and Vaccinium (B) during pre-treatment and treatment period. Values are averages of reference and incubated cores for each incubation period. The climate manipulation started in June 1994.

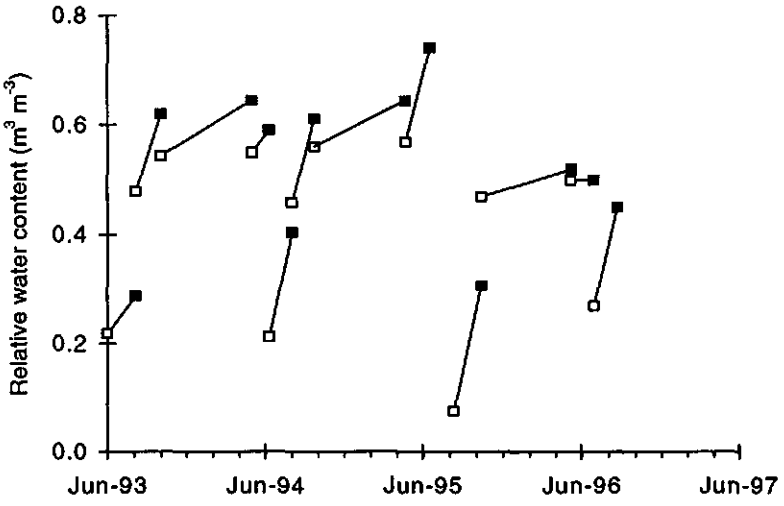


Figure 2.5. Relative water content under Calluna in KIM-c in reference samples (open squares) and incubated samples (closed symbols). The lines connect reference and incubated samples for each incubation period. The climate manipulation started in June 1994.

The climate treatment appeared to lower the relative water content (RWC) of the soils in KIM (Table 2.5). However, in METTE, soil moisture was significantly lower in the first and second treatment year compared to the pre-treatment year as well. When the factor 'year' was included in the MANOVA instead of 'treatment', significant year effects were found. For all sites, especially during summer, moisture content was lower in *Calluna* than *Vaccinium* plots (Fig. 2.4). We ascribe this difference in soil moisture to *Calluna* being largely confined to fringes of soil pockets or other shallow areas, whereas *Vaccinium* grows in areas with deeper soils. Especially under *Vaccinium*, moisture levels were lower in the shallow soils of KIM-c than in the deeper soils of KIM-t. Consequently, in KIM-c, soils are more susceptible to dry out due to a limited moisture supply from the subsoil.

Although mineralization tubes were capped at both ends, soil moisture increased during the incubations even when the surrounding soil dried out (Fig. 2.5), especially at low moisture content, as observed also by Van Vuuren et al. (1992). While differences in temporal patterns in moisture between the tubes and the surrounding soil may have resulted in a different mineralization in- and outside the tubes, increased net N

mineralization must be ascribed to the treatment. This is corroborated by the observations that growth and total N uptake of *Calluna* increased (Arp, pers. comm.) and total inorganic N in runoff from KIM increased as a result of elevated CO_2 and temperature (Wright, 1998). Both observations suggest a higher N availability, in agreement with our measurements on N mineralization.

CONCLUSIONS

Our results show that the climate change treatments increased soil N availability but not litter decomposition. The absence of a clear temperature effect on litter decomposition may be due to a desiccation of the litter induced by higher air temperatures during the treatment years. We found no evidence for reduced decomposition rates by elevated CO_2 via production of more recalcitrant litter. In fact, in our experiment elevated CO_2 caused an increase in decomposition of high-CO_2 *Betula* litter. In the literature, both increased and decreased decomposition of high-CO_2 litter have been found which illustrates that the effects of elevated CO_2 on litter quality and subsequent decomposition are not well understood. Consequently, predictions concerning the fate of C and N in terrestrial ecosystems upon climate change are still constrained by the lack of understanding of mechanisms controlling decomposition. Whole-ecosystem manipulations such as CLIMEX may help to test current hypotheses concerning the response of ecosystems to climate change (Carpenter et al., 1995).

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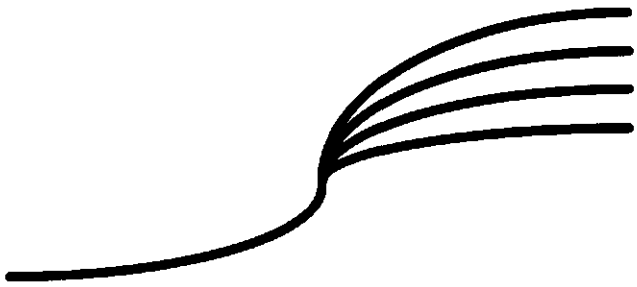
Chapter 3

THE CLIMEX SOIL-HEATING EXPERIMENT: SOIL RESPONSE AFTER 2 YEARS OF TREATMENT



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submitted to Biology and Fertility of Soils

THE CLIMEX SOIL-HEATING EXPERIMENT: SOIL RESPONSE AFTER 2 YEARS OF TREATMENT

ABSTRACT

Most of the model predictions concerning the response of boreal forest ecosystems to climate change are inferred from small-scale experiments on artificial, simplified, systems. Whole-ecosystem experiments to validate these models are scarce. We experimentally manipulated a small forested catchment in southern Norway by increasing soil temperature (+3°C in summer to +5°C in winter) using heating cables installed at 1 cm depth in the litter layer. *Betula* litter, produced after exposing trees for 2 years to ambient and elevated CO_2 in greenhouses, was incubated for 1 year in the manipulated catchment. Exposure to elevated CO_2 did not affect the C/N ratio or decomposition of *Betula* litter. We expected the higher temperature in the climate manipulation treatment to increase decomposition, but no such effect was found probably due to desiccation of the litter. Both net N mineralization and nitrification in the 0-10 cm soil layer increased as a result of the climate manipulation. The heating cables caused a permanent increase in soil temperature in this soil layer, but when soils were dry, the temperature difference between control and heated plots decreased with increasing distance from the cables. When soils were wet, no gradients in temperature increase occurred.

INTRODUCTION

Elevated concentrations of greenhouse gasses due to combustion of fossil fuels and changes in land use may cause an increase in global temperatures (Houghton et al., 1995). Estimates on temperature increases at northern latitudes during the last decades range from 0.7°C (Möberg and Alexandersson, 1997) to 2-4°C (Lachenbruch and Marshall, 1986). Elevated temperature may stimulate decomposition of both litter and humified soil organic matter. In the boreal and tundra zone, soil C stocks are large and net primary production (NPP) is low. Therefore, C losses due to increased decomposition of humus may exceed allocation of C to pools in plant and soil organic matter due to CO_2 fertilization. However, higher N availability due to increased N mineralization may favour NPP on top of CO_2 fertilization. Excess N that is not taken up by plants and micro-organisms can leach to groundwater and surface waters which will affect nutrient cycling

in downstream aquatic ecosystems (Wright and Schindler, 1995). Emissions of C and N to atmosphere and hydrosphere through enhanced decomposition may be buffered if litter produced under elevated CO_2 is more recalcitrant to decomposition (Norby et al., 1986b; Lambers, 1993; Cotrufo et al., 1994). So far, predictions regarding whole ecosystem responses have largely been based on modeling in which results from small-scale greenhouse studies and laboratory incubations have been extrapolated to the ecosystem level (Overpeck et al., 1990; Rastetter et al., 1992; Schimel et al., 1997). To date, few large-scale experiments exist providing data that can be used to validate these models.

CLIMEX (Climate Change Experiment) is an international multidisciplinary project in which forested catchments are subjected to increased air temperature and CO_2 or to increased soil temperature alone (Jenkins and Wright, 1995). The CLIMEX facility provides an opportunity to integrate measurements on vegetation and soil response in order to assess future response of a boreal forest ecosystem to climate change. Current studies include: phenology, photosynthesis and biomass of tree and shrub vegetation; soil fauna and decomposition; soil and soil solution chemistry; soil and catchment hydrology; trace gas fluxes, and runoff chemistry.

In this paper we concentrate on the large-scale soil heating experiment and report on the effects of soil heating on in-situ N mineralization and litter decomposition. In addition, we studied effects of elevated CO_2 on litter quality and decomposition by incubating birch litter produced under ambient and elevated CO_2. We hypothesized that elevated temperature will increase N mineralization as well as litter decomposition whereas elevated CO_2 may reduce litter decomposition due to a decrease in litter quality. We will present tests of these hypotheses and discuss the use of soil-heating as a tool to study effects of elevated temperature on soil processes.

MATERIALS AND METHODS

Site description

The CLIMEX site is located at Risdalsheia (58°23' N, 8°19' E) near Grimstad, southernmost Norway. The site is located at 300 m above sea level on a large biotite granite plateau, and is representative for large areas of upland southern Norway. Mean annual precipitation is 1400 mm and mean annual temperature is 5°C (-3°C in January and +16°C in July). Depressions in the granite surface are filled with post-glacial soil material in which acid, peaty podsolic soils have developed. In the studied EGIL catchment (400 m^2), maximum soil depth is 40 cm. Vegetation is dominated by heather (*Calluna vulgaris*

(L.) Hull) with few scattered Scots pines (*Pinus sylvestris* L.) and birches (*Betula pubescens* Ehrh.). In 1983, the catchment was covered by a transparent roof as part of the RAIN (Reversing Acidification In Norway) project (Wright et al., 1993). During rain events, precipitation was collected from the roof and distributed under the roofs at a fixed rate of 2 mm h^{-1} using a sprinkler system. In spring 1994, in the lower 80% of the catchment (EGIL-t), heating cables were put at a depth of 1 cm in the litter layer with a spacing of 10-15 cm. The upper 20% of the catchment (EGIL-c) act as an untreated control. Temperatures are monitored hourly using a network of 120 thermistors. The thermistors are placed throughout the control and treatment areas at 6 levels (25 and 10 cm above the soil, 0, 5, 15, and 30 cm in the soil). From June 1994, the temperature in the treated part (EGIL-t) was increased by 5°C in January and 3°C in July with intermediate temperature increases in the intervening months (Fig. 3.1). Two uncovered catchments (METTE, ROLF) serve as outside controls.

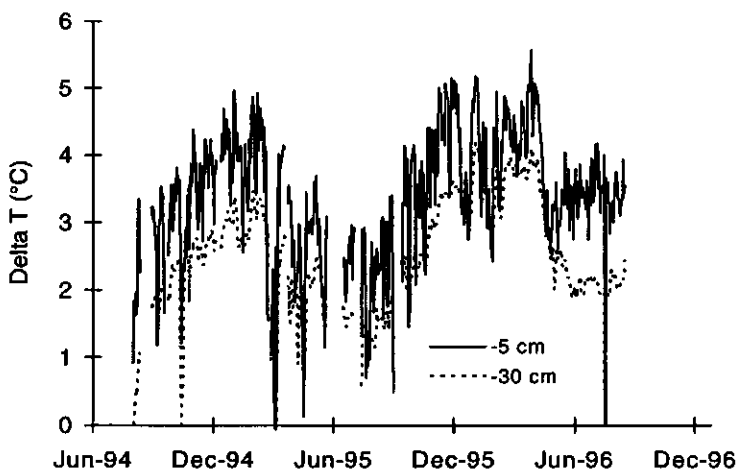

Figure 3.1. Average temperature difference between soil-heated and control plots at 5 and 30 cm depth.

Litter decomposition

To study the effect of elevated CO_2 and temperature on decomposition, we incubated litter produced under ambient and elevated CO_2 in EGIL (soil heating) and METTE (control). We grew 1-year old birches (*Betula pubescens* Ehrh.) in 10 l pots in a sandy soil (C=2.16%, N=0.10%, pH-H_20=6.59) at either 365 or 700 ppm CO_2 in greenhouses in The Netherlands. Temperature, light and humidity followed ambient outside conditions. The litter was collected after 2 years exposure to elevated CO_2. Total C and N of the litter was measured using an EA 1108 CHN element analyzer. For Klason-lignin determinations, ethanol-soluble components were removed by extraction with 80% ethanol (3 x 30 min). The residual material was treated with 10 ml 72% H_2SO_4 at 30°C for 1 h. This mixture was diluted to 3% H_2SO_4 and refluxed for 2 h. The residual solid material was washed, filtered and dried overnight at 105°C. Lignin content was determined as the mass loss upon ignition (650°C; 2 h) of this residue.

In April 1995, thirty litterbags with either low- or high-CO_2 litter were incubated in EGIL-c, EGIL-t and METTE. Each litterbag (15 x 15 cm; mesh size 1.5 mm) contained 4 g of litter. At the start of the incubations and after 6 and 12 months, ten litterbags of each littertype were collected from the three locations. After sampling, litterbags were cleaned, dried at 70°C and weighed. In October 1992, prior to the climate manipulation, we incubated standard pine litter (Berg et al., 1982) in EGIL and ROLF for 1 year to detect catchment differences. We used the ROLF catchment as control since monitoring of runoff and precipitation in the METTE catchment did not start before 1993. We used 25 replicates per catchment. Litterbags had a mesh of 1 mm and contained between 0.5 and 1 g of pine needles. The exact weight was printed on a tag present in the litterbag.

Soil nitrogen mineralization and nitrification

Net N mineralization was measured in EGIL-c, EGIL-t and METTE during pre-treatment (June 1993-June 1994) and treatment period until August 1996 using the sequential core incubation method (Berendse et al., 1987; Raison et al., 1987; Berendse et al., 1989; Berendse, 1990). We measured N mineralization in ten plots of 20 x 50 cm. Each year, was split into four incubation periods: April-June, June-August, August-October, and October-April. At the start of each incubation period, two samples were taken 5-10 cm apart using pre-weighed PVC tubes (length 15 cm, diameter 2.8 cm, wall thickness 2 mm). Soil was sampled to a depth of 10 cm unless soils were shallower. One sample was taken to the lab whereas the second sample was covered on both sides with soft

polyethylene caps and put back into the soil. The incubated cores had four 4 mm holes to allow for gas exchange. At the end of the incubation period, the samples were collected from the field and new incubations were started. The retrieved tubes were weighed and stored overnight at 4°C. Soil material including litter were mixed after measuring length of the soil cores. A subsample of 20 g field moist soil was extracted with 50 ml 1 M KCl. The KCl extracts were analyzed for NH_4^+-N and NO_3^--N by colorimetry on an auto analyzer. Net N mineralization was calculated as the difference in NO_3^--N + NH_4^+-N between the incubated and reference samples. Net nitrification was the difference in NO_3^--N between reference and incubated samples.

Soil moisture content was measured after drying the samples at 105°C for 24 h. Gravimetric water content (g H_2O g dry $soil^{-1}$) in samples collected at any one time showed a large variation reflecting differences in soil organic C content (6.7 to 53.0%). We normalized water content to total pore space rather than to dry soil, and expressed moisture as relative water content (RWC; = volume fraction water/total pore space; Skopp et al., 1990) according to Chapter 2 of this thesis.

Heat distribution

At selected locations, additional thermistors were installed at 1 cm depth at 1, 3 and 7.5 cm from the cable and at 5 cm depth, at 1 and 7.5 cm from the cable. We measured thermal conductivity (λ) of the organic surface soil and mineral subsoil at different moisture levels according to Van Loon et al. (1989). After each conductivity measurement, gravimetric moisture content was determined by drying a subsample for 24 h at 105°C. Dry bulk density was calculated by weighing wet soil in the measurement cylinders and correcting for moisture content. Volumetric heat capacity (C_h) of the soil was calculated as:

$$C_h = \Sigma \theta_i C_i$$

With θ_i being the volume fraction of soil constituent *i* and C_i is the volumetric heat capacity of material *i* assuming a volumetric heat capacity of 2.0 MJ m^{-3} K^{-1} for minerals, 2.5 MJ m^{-3} K^{-1} for organic matter and 4.2 MJ m^{-3} K^{-1} for water (Koorevaar et al., 1983). Organic matter content of the soil material was measured as Loss On Ignition at 600 °C after 4 h of heating. Thermal diffusivity (D_h) was calculated as λ/C_h.

Statistical methods

Residual mass data of the birch litter incubations were analyzed by multiple analysis of variance (MANOVA) using 'catchment' (EGIL, METTE), 'field treatment', and 'litter quality' as factors. The factor 'field treatment' was nested in the factor 'catchment'. N mineralization, nitrification and RWC data were analyzed by MANOVA with 'treatment', and 'location' (METTE, EGIL-c, EGIL-t) as main factors. The factor 'treatment' was nested within the factor 'location'. For MANOVA on RWC we added 'RWC prior and after incubation' and 'season' as additional factors. Tests for significance were carried out using 2-tailed t-tests. Effects or differences were considered significant when $p<0.05$. For the statistical analyzes we used SPSS version 6.1.

RESULTS AND DISCUSSION

Litter decomposition

To test if elevated CO_2 causes a decrease in decomposability of litter, we grew birch trees at 350 and 700 ppm CO_2 in greenhouses and incubated leaf litter in the CLIMEX catchments. Two years of exposure to elevated CO_2 did not significantly alter the C/N ratio of the birch litter. Most studies report an increase in C/N ratio of tree leaves at elevated CO_2 (e.g. Norby et al., 1986b; Coûteaux et al., 1991; Cotrufo et al., 1994). On the other hand, Franck et al. (1997) found that for some grass species C/N ratio increased whereas for others it decreased at elevated CO_2. Ball (1997) reported that C/N ratio at elevated CO_2 decreased for several C_4 species, but increased for C_3 species. Less data are available on the effects of elevated CO_2 on secondary metabolites such as lignin. In our study, lignin content was significantly lower in the high-CO_2 litter (16.1%) than in the low-CO_2 litter (17.8%). Norby et al. (1986b) found a decrease in lignin content for white oak which contrasts results from Cotrufo et al. (1994) who observed an increase in lignin content for four tree species under elevated CO_2. Lambers (1993) suggested that the concentration of secondary metabolites may increase due to nutrient limitations induced by elevated CO_2.

Table 3.1. Residual mass (%) of Betula litter after 1 year of incubation. ANOVA results are given for residual mass data after 6 and 12 months of incubation.

	6 months		12 months	
	365	700	365	700
EGIL-c	65 (2)	65 (8)	57 (4)	61 (9)
EGIL-t	70 (7)	73 (11)	62 (8)	65 (8)
METTE	71 (4)	64 (4)	61 (6)	53 (2)
Catchment (C)[2]	ns		ns	
Treatment (T)	**		ns	
Litter quality (L)	ns		ns	
T x L	*		ns	

[1] * $p<0.05$; ** $p<0.01$; ns = not significant

2 'Catchment' = EGIL versus METTE; 'Treatment' = field treatment; 'Litter quality' = low- versus high-CO_2 litter

Although lignin content of the high-CO_2 litter was significantly lower, this did not affect decomposition at the control and heated areas of the CLIMEX soil-heating site (Table 3.1). However, in the control catchment METTE, the high-CO_2 litter decomposed faster. In EGIL, the residual mass data showed much variation which may have obscured any effects of litter quality. Cotrufo et al. (1994) found a significantly slower decomposition of high-CO_2 litter for several tree species, in agreement with higher C/N ratios. Franck et al. (1997) found no consistent effect of elevated CO_2 and nutrient conditions on mass loss among four grass species, and found no correlation between mass loss and C/N ratio across species and treatments. In our experiment, C/N ratio was not affected by elevated CO_2 but the measurements from the control catchment indicated a more rapid decomposition of high-CO_2 litter.

In EGIL-t, soil temperatures increased due to the heating cables but litter decomposition did not. In fact, after 6 months, decomposition of birch litter was significantly lower in EGIL-t than in EGIL-c (Table 3.1). After 1 year, residual mass was similar in EGIL-c and EGIL-t. Both after 6 months and 1 year of incubation, litter from EGIL-t was drier than in EGIL-c but this difference was only significant after 6 months. The heating treatment may have caused the soils to become drier which may have depressed decomposition rates. Moore (1986) showed that effects of moisture and temperature on litter decomposition strongly interact. When moisture conditions are

unfavorable for decomposition, effects of temperature on decomposition rates are reduced as well. Residual mass of pine litter incubated for 1 year prior to the start of the heating treatment was similar in EGIL (70.8±11.6) and ROLF (71.7±6.5) suggesting that the presence of a roof did not affect decomposition. In addition, residual mass data of birch litter showed no overall catchment effects.

Soil nitrogen mineralization and nitrification

In METTE, EGIL-c and EGIL-t, N mineralization and nitrification in the 0-10 cm soil layer did not significantly change in treatment years compared to the pre-treatment period (Fig. 3.2 and 3.3). During the pre-treatment year, mineralization was significantly higher in EGIL-t than in EGIL-c. However, the level of significance increased from $p=0.018$ in the pre-treatment year to $p<0.001$ in the second treatment year. In the pre-treatment year, nitrification was similar in EGIL-t and EGIL-c but during the second treatment year, nitrification was significantly higher in EGIL-t than in EGIL-c. Since standard errors did not change between the years, we conclude that in the second treatment year, both mineralization and nitrification must have increased in EGIL-t relative to EGIL-c. Mineralization and nitrification in EGIL-t also significantly increased compared to METTE between the pre-treatment and the second treatment year.

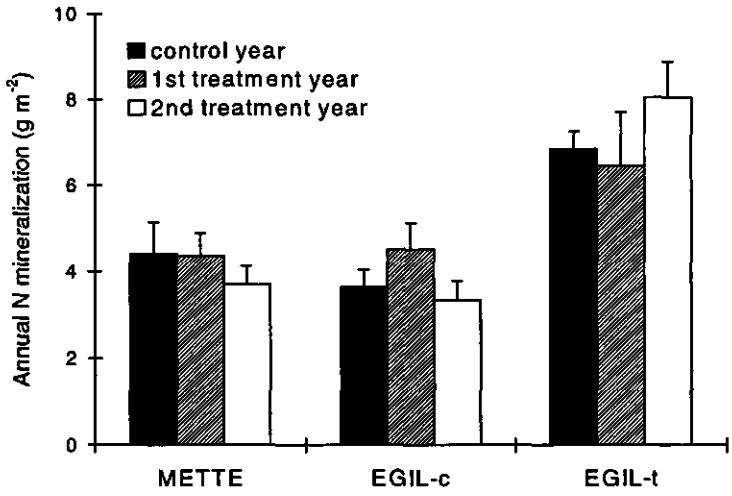

Figure 3.2. Annual net N mineralization in METTE, EGIL-c and EGIL-t during pre-treatment and treatment years. Error bars represent standard errors of the mean (n=10)

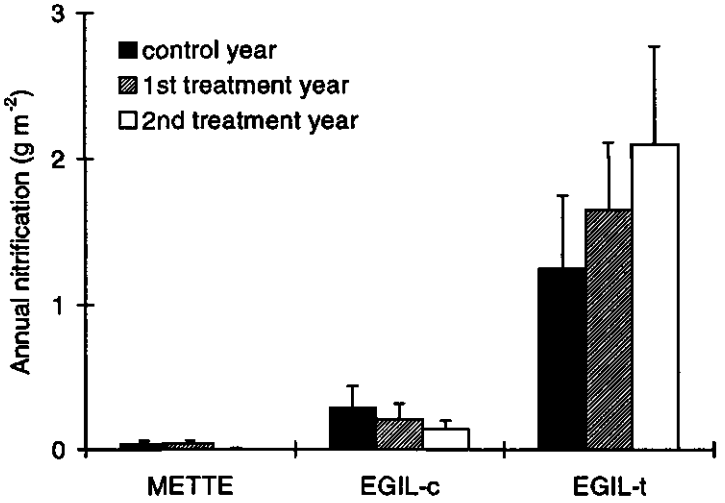

Figure 3.3. Annual nitrification in METTE, EGIL-c and EGIL-t during pre-treatment and treatment years. Error bars represent standard errors of the mean (n=10)

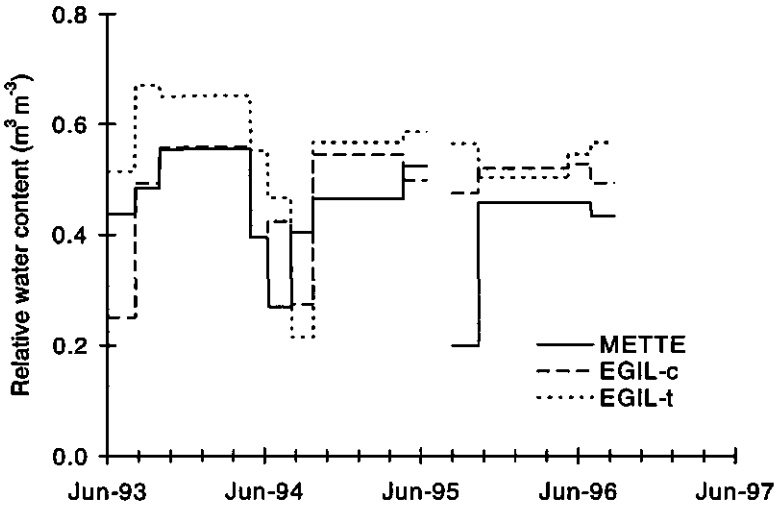

Figure 3.4. Relative water content (=volume water divided by total pore space) of soil inside mineralization tubes during treatment and pre-treatment period. The soil-heating started in June 1994.

The lower mineralization in EGIL-c compared to EGIL-t prior to the start of the treatment was most likely caused by a lower moisture content of soil inside the mineralization tubes in EGIL-c (Fig. 3.4). Soils in EGIL-c are shallower than in EGIL-t so, in EGIL-c, soils are more susceptible to dry out due to a limited moisture supply from the subsoil. During the two treatment years, moisture content in the tubes was similar in EGIL-c and EGIL-t. Still, during the treatment period the higher soil temperatures in EGIL-t may have caused mineralization and nitrification to increase even though moisture levels decreased compared to EGIL-c. In the control catchment METTE, pre-treatment mineralization and nitrification were similar to EGIL-c. Both mineralization and nitrification were lower than in EGIL-t but only the difference in nitrification was significant. Whether or not the presence of the roof affected N mineralization and nitrification remains difficult to assess. Organic matter quality, and thus N dynamics (Van Vuuren et al., 1992), may vary between (or even within) catchments due to differences in contribution of litter produced by overstorey vegetation to the soil organic matter pool. Some plots may receive significant amounts of pine litter whereas other areas receive additional birch litter or mixtures of both. Although tubes were capped at both sides, soil moisture content was generally higher after than before the incubations even when the moisture content of the soil surrounding the tubes decreased. This difference between reference and incubated samples was significant during summer when moisture levels of the reference samples were low. It is not clear whether the effects of the core incubation method on soil moisture favored or hampered N mineralization. Since moisture in the tubes increased during incubations, mineralization may have been favored (Tietema et al., 1992) whereas periodic drought may have occurred in the surrounding soil. However, repeated wetting and drying of soil is known to stimulate N mineralization (Nordmeyer and Richter, 1985) which is more likely to have occurred outside than inside the tubes.

The increases in mineralization and nitrification at elevated temperature appeared to have exceeded plant uptake since both NH_4^+ and NO_3^- efflux in runoff increased during the first 2 years of treatment (Lükewille and Wright, 1997). By contrast, Van Cleve et al. (1990) found that after 2 years of soil heating, NO_3^- concentrations in the soil solution were lower in soil heated plots which these authors ascribed to an increase in denitrification potential. These same authors observed, however, increased NH_4^+ and total N concentrations in heated plots. Peterjohn et al. (1993) found increased CO_2 emissions and lower C and N concentrations in surface horizons in soil-heated plots which suggested faster decomposition.

Heat distribution

Under dry conditions, temperature difference between treatment and control (ΔT) decreased with increasing distance from the cable (Fig. 3.5). Under these conditions, thermal conductivity (λ) was very low but increased with depth from 0.025 W m^{-1} K^{-1} in the LF layer to 0.116 W m^{-1} K^{-1} in the mineral soil (Bw horizon; Fig. 3.6) in agreement with a higher conductivity of minerals than of organic matter. Diffusivity (D_h), however, was highest in the organic surface layers (Fig. 3.7). When soils were wet, ΔT was similar at all points. In all layers, λ increased with increasing moisture content. In the organic layers (LF, H and Ah), D_h tended to decrease with increasing moisture content but increased in the mineral soil . The diurnal pattern in ΔT under dry conditions may be caused by rewetting of the soils at night due to condensation. Water films around soil particles will increase λ so heat from the surface is redistributed. Although the field observations can be explained qualitatively using the laboratory measurements, differences

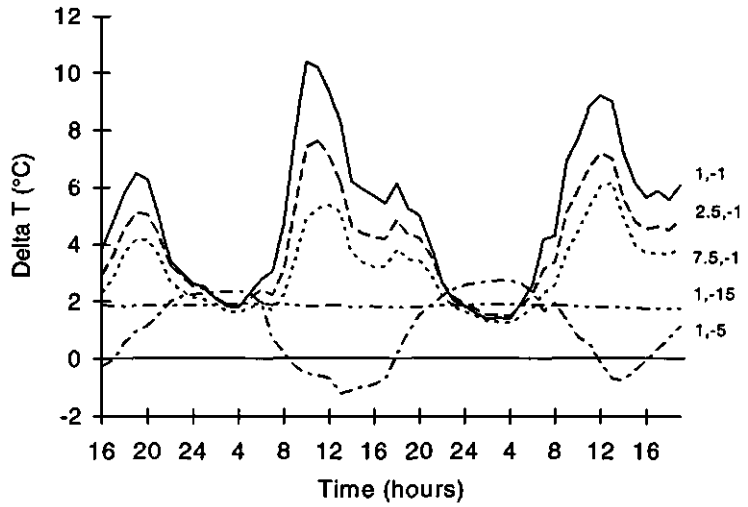

Figure 3.5. Temperature difference between soil-heated and control plots under dry conditions, 2 weeks after a rain event in July, as a function of vertical and horizontal distance from the cable. The first number of the line label gives horizontal distance (cm) from the cable, the second gives the depth (cm) in the soil.

in λ and C_h between field and lab may have occurred due to differences in arrangement of soil particles. Bulk density measured in the laboratory under dry conditions was generally lower than measured in the field (Chapter 4 of this thesis). In the laboratory, contact between individual soil particles was likely to be less than in the field where soil is more compacted. As a result, λ in the field will be higher than measured under laboratory conditions. We could calculate D_h using field data and thus check if this value was consistent with calculations based on laboratory measurements. Diffusivity in the field was calculated using the following relation (Van Wijk, 1966):

$$\varphi = d\sqrt{(\omega/2D_h)}$$

φ is the phase shift in the diurnal heat cycle between two depths (-), d is the difference between depths (mm), ω is the angular frequency ($=2\pi/(24*3600)$ radians per second) and D_h is diffusivity ($mm^2\ s^{-1}$). To determine φ, we fitted a sinus function through the temperatures measured at 1, 5 and 15 cm depth. Under dry conditions, φ was 3 hours between 1 and 5 cm. The resulting D_h was 0.15 $mm^2\ s^{-1}$ which agrees well with the value

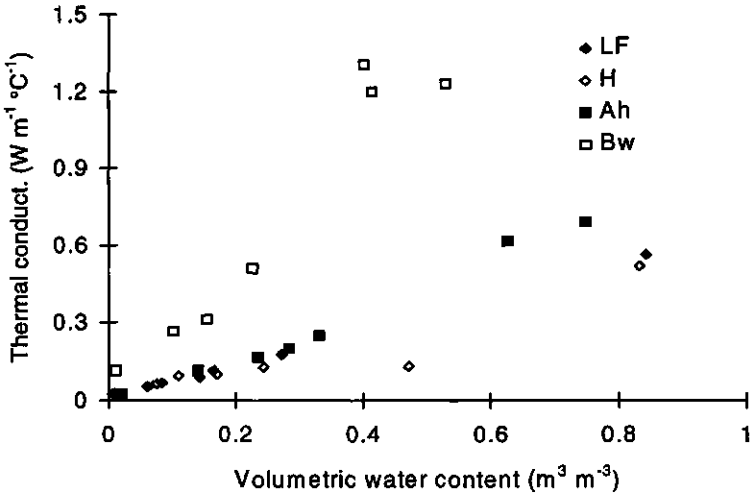

Figure 3.6. Thermal conductivity of organic surface soil (LF, H and Ah) and mineral subsoil (Bw) as a function of moisture.

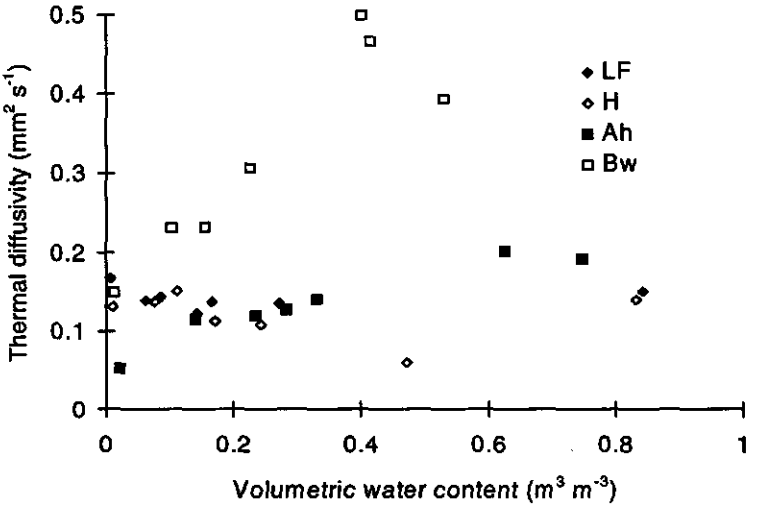

Figure 3.7. Thermal diffusivity of organic surface soil (LF, H and Ah) and mineral subsoil (Bw) as a function of moisture.

for the LF and H layer measured in the lab (Fig. 3.7). Between 5 an 15 cm depth φ was 12 hours giving a D_h of 0.23 $mm^2 s^{-1}$. This higher value reflects presence of mineral material at greater depth. Under wet conditions, φ between 1 and 5 cm remained 3 hours whereas between 5 and 15 cm it was 7 hours. In the organic soil D_h remained 0.15 $mm^2 s^{-1}$ whereas in the subsoil D_h increased to 0.4 $mm^2 s^{-1}$. The calculations do not resolve whether λ was measured correctly because when the soil is more compacted, both C_h as well as λ will increase which could leave D_h ($=\lambda/C_h$) unchanged. However, under dry and wet conditions, laboratory estimates of D_h were consistent with field data.

Peterjohn et al. (1993) showed that when heating cables are buried at 10 cm depth, at 5 cm depth heat is distributed more evenly than when cables are buried at 5 cm depth. It is, however, very likely that when cables are buried at 10 cm depth, at this depth still strong heating gradients occurred which may have affected decomposition of organic matter deeper in the soil. In addition, burying the cables may cause disruption of fine roots, leading to increased N concentrations in soil solution which may last for up to 1 year after installation (McHale and Mitchell, 1996). Our measurements suggest that gradients in heating will be lower in mineral and continuously moist or wet soils which have a high thermal conductivity. In permanently wet systems, heating cables will work well even when put on top of the soil. Heating patterns will also depend on the location of

the control sensors. If control sensors are located far from the cables, especially under dry conditions the majority of the soil will be heated above the target temperature causing large risks of desiccation. Installing control sensors close to the cables will reduce this risk of desiccation, but will cause the bulk of the soil to be heated below the targets when soils are dry. However, at low soil moisture, effects of elevated temperature are likely to be small (Moore, 1986). Under wet conditions, most of the soil will be heated to the target temperatures due to the high conductivity of the soil. Therefore, in wet soils, location of control sensors is less critical.

CONCLUSIONS

After 2 years of elevated soil temperature, we measured an increase in net N mineralization and nitrification in the 0-10 cm soil layer. This suggests that N availability will increase when atmospheric temperatures rise. In our experiment, the increased N availability apparently exceeded plant demand as evidenced by increased N export from the catchment in runoff. Under elevated CO_2 concentrations, N demand by plants will probably increase. At least during the growing season most of the extra N will be sequestered by the vegetation instead of leaving the ecosystem (Van Breemen et al., 1998). The effect of elevated temperature on decomposition of litter at the soil surface appeared to be dominated by a desiccation of the litter which may have been enhanced by the heating cables. However, if atmospheric temperatures increase, evaporation from the soil may be stimulated which could result in drier surface soil layers. The often predicted increase in decomposition at elevated temperature may thus be reduced due to lower soil moisture levels. We found no evidence for a decrease in decomposability of litter produced under elevated CO_2. In fact, the incubations in the control catchment METTE suggested the opposite. Since direct effects of CO_2 on decomposition are thought to be negligible (Koizumi et al., 1991; Ball and Drake, 1997), elevated CO_2 may affect above-ground litter decomposition only by increasing litter production. Although heating cables caused a permanent increase in soil temperature spatial heating patterns depend on moisture status of the soil. When soils are dry, heating patterns are not uniform which may affect measurements carried out on small sub-plots within soil-heated areas. Burying heating cables will reduce desiccation of the litter layer but will cause variable heating patterns at larger depth which will affect decomposition of subsoil organic matter. However, disturbance effects after burying the heating cables may exceed soil warming effects at least during the first year of treatment. Provided these limitations and problems are recognized, soil heating can be a suitable approach. It is a robust and relatively cheap

method for long-term and large-scale manipulations of soil temperature which may help to reduce uncertainty in predictions concerning soil response to elevated temperatures.

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Chapter 4

THE INFLUENCE OF TEMPERATURE ON C MINERALIZATION IS DEPTH DEPENDENT: EVIDENCE FROM A BOREAL FOREST SOIL

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THE INFLUENCE OF TEMPERATURE ON C MINERALIZATION IS DEPTH DEPENDENT: EVIDENCE FROM A BOREAL FOREST SOIL

ABSTRACT

To study the effects of temperature on C mineralization, we incubated undisturbed soil cores from a boreal forest at 5, 10 and 17°C. We measured CO_2 evolution as well as chemistry of the drainage water and estimated the contribution of different soil layers to the total CO_2 production by incubating samples of separate layers. With increasing temperature, C mineralization from the columns increased and leachate showed increased acidification due to NO_3 production. Stimulation of respiration at high temperatures was larger near the surface and decreased with depth. Respiration per unit C invariably decreased with depth indicating a decrease in substrate quality. Both microbial biomass and microbial activity decreased with depth. The Carbon Availability Index for all layers was well below 1 suggesting that decomposition was C-limited in all layers. In addition, glucose decomposition proceeded much slower in the subsoil than in the surface soil which could be partly due to nutrient limitation. Our data indicate that the temperature sensitivity of the microbial population decreased with decreasing substrate quality. By assuming organic matter to consist of a labile and stable fraction having different decomposition rate constants with a different temperature dependence, it was shown that effects on decomposition become time-dependent. This complicates establishment of general relationships between temperature and decomposition using a wide variety of studies differing in measurement times.

INTRODUCTION

Elevated concentrations of greenhouse gasses due to combustion of fossil fuels and changes in land use may cause an increase in global temperatures (Houghton et al., 1995). Indeed, during the last decades, temperature has increased by 0.7°C (Möberg and Alexandersson, 1997) to 2-4°C (Lachenbruch and Marshall, 1986) for the boreal and tundra zone. These areas represent approximately 14% of the total land area but contain 25% of the global soil C pool (Schlesinger, 1991). Hungate et al. (1997) argued that an increase in CO_2 concentration may result in increased net primary production (NPP) but

that most of the C is allocated to pools having fast turnover rates. Consequently, the vegetation may have limited capacity to sequester C. However, elevated temperature is likely to favour decomposition of both fresh and old, humified, soil organic matter (SOM) which may result in a significant C loss from the soil in a warmer world. If this C loss from the soil exceeds allocation of C to stable plant and ultimately soil pools due to CO_2 fertilization, these areas may become a significant source for CO_2. Oechel et al. (1993) and Peterjohn et al. (1993) showed that soil respiration increased upon heating which they partly be ascribed to increased SOM decomposition. Increased SOM decomposition is likely to favour N mineralization. In N-limited ecosystems, increased N availability may facilitate increased NPP in addition to CO_2 fertilization. Nadelhoffer et al. (1991) demonstrated that in tundra soils both C and N mineralization increased when temperature increased from 9 to 15°C. Below 9°C, they found no temperature response, an observation confirmed by Pöhhacker and Zech (1995). However, Ross and Cairns (1978), Howard and Howard (1993), and MacDonald et al. (1995) showed that CO_2 evolution increased with increasing temperature for several soils even at low temperatures, and Kirschbaum (1995) found that the relative increase in C mineralization was generally largest at low temperature.

Several studies show that C mineralization decrease with soil depth (Federer, 1983; Nadelhoffer et al., 1991 and Van Dam et al., 1997). Although C mineralization rates per unit substrate are highest in the surface horizons, the slowly decomposing humified material in the subsoil may contribute significantly to the total efflux of CO_2-C because of the large amounts present. We do not know if the sensitivity of C mineralization to a change in temperature varies with depth. Johansson (1986) hypothesized that decomposition rates of fresh litter are controlled by temperature and moisture whereas for more humified material, substrate quality determines decomposition rates and soil physical parameters are less important. In addition, Liski et al. (submitted) could only simulate observed soil C stocks for coniferous forest soils in Finland by assuming a lower temperature sensitivity of humified SOM. Still, most models simulating organic matter dynamics (e.g. Parton et al., 1987; Johnsson et al., 1987; Jenkinson, 1990; Van Dam and Van Breemen, 1995), use the same temperature responses for labile and stable C pools.

In this study, we determined the temperature response of C mineralization and N leaching of a boreal forest soil by incubating soil cores at 5, 10 and 17°C for 4 months. The contribution of different soil layers to the total CO_2 production was assessed by measuring respiration of the individual soil layers.

MATERIALS AND METHODS

Study site

We studied soils in an open forested ecosystem on a large granite intrusion at Risdalsheia, Southern Norway at the location of the CLIMEX project (Jenkins and Wright, 1993). Small, patchy, depressions in the granite surface are filled with post-glacial soil material, leaving 30-50% of the bedrock uncovered. Soil depth varied between 0 and 70 cm. On the shallow soils (< 30 cm), vegetation is dominated by dwarf shrubs (*Calluna vulgaris* L., *Vaccinium myrtillus* L., *Vaccinium vitis-ideaea* L.) whereas in areas with deeper soils, also scattered trees (*Pinus sylvestris* L. and *Betula pubescens* L.) are present. The sampling site is a plot with approximately equal soil depth of 25 cm dominated by *Calluna vulgaris*. The soil (Dystric Leptosol; FAO, 1988) is acid, has a high organic matter content throughout the profile (Table 4.1), and showed evidence of podsolization. No saprolite occurred at the soil-rock interface. The mineral fraction consisted mainly of coarse quartz and feldspar grains.

Sample collection and preparation

Soil cores were collected in June 1994 using polyethylene tubes (length 50 cm, diameter 16 cm). After removal of the above-ground vegetation, the columns were gently cut down to the rock surface. A root mat of a few mm thickness present at the soil-rock interface was removed to improve drainage of the columns. A 2 cm layer of non-calcareous sand was added to the bottom before closing the soil core with an air- and watertight cap. Prior to the experiment, the columns were stored for 2 weeks at approximately 15°C. At the same site, we took samples from different depths for C mineralization measurements and chemical analyses. Soil samples for C mineralization measurements were air-dried at room temperature until they reached constant weight. Field moist samples for chemical analyses were stored at 2°C. Both dry and moist mineral soil samples were sieved (mesh 2 mm) and roots were removed. The air-dried organic horizons were sieved with a 1 cm mesh size. Organic matter content of the samples was determined as Loss On Ignition (LOI) at 600°C after 4 h of heating. Total C and N were determined by dry combustion using an EA 1108 CHN element analyzer. Cation Exchange Capacity (CEC) and exchangeable base cations were measured following the method of Gilman (1979). NH_4 and NO_3 were extracted with 1 M KCl using a 1:30 (w/w) soil to extractant ratio.

Table 4.1. Selected physical and chemical properties of the studied soil

Depth (cm)	BD[1] ($g\ cm^{-3}$)	LOI[2]	C	N	pH-H_2O	pH-$CaCl_2$	BS[3]	NH_4-N	NO_3-N
		----------(%)----------			--------(-)--------		(%)	------($mg\ kg^{-1}$)------	
Litter	nd[4]	97.8	49.7	1.3	4.2	3.0	nd	582	7
0-5	0.13 (0.01)	95.9	46.2	1.8	4.7	3.1	32.8	329	2
5-10	0.35 (0.07)	74.8	39.4	1.9	4.4	3.2	20.1	213	4
10-14	nd	23.2	11.1	0.6	4.3	3.2	11.2	26	0.4
14-19	1.12 (0.14)	12.1	6.4	0.4	4.6	3.6	9.4	23	0.4
19+	1.07 (0.18)	10.0	4.9	0.4	4.5	3.8	9.4	10	0.4

[1] BD = bulk density (n=10)

[2] LOI = Loss On Ignition

[3] BS = base saturation (= Σ base cations/ Cation Exchange Capacity *100)

[4] nd = not determined

NH_4-N and NO_3-N were measured colorimetrically on a Technicon autoanalyzer. Soil pH was measured in a 1:5 water and a 1:5 0.01 M $CaCl_2$ solution. All chemical analyses were done on duplicate samples. Bulk density was measured at 0-5, 5-10, 14-19 and 19-24 cm using ten replicates.

Column incubations

We incubated five soil columns for 4 months in dark climate chambers at target temperatures of 5, 10 or 17°C. CO_2 emitted from the columns was adsorbed by 10 g oven dry (105°C) sodalime in a petri dish (diameter 10 cm) present in the headspace of the columns. The sodalime was replaced weekly to maximize CO_2 absorption (Edwards, 1982). When sodalime reacts with CO_2, water is formed which is lost upon oven-drying. Therefore, the increase in oven dry weight of the sodalime has to be multiplied by 1.69 (= molar $weight_{CO2}$ / (molar $weight_{CO2}$ - molar $weight_{H2O}$)) to account for this formation and subsequent loss of water. A second CO_2 trap, connected to a removable airtight lid, allowed for free exchange of O_2 but prevented absorption of CO_2 from the surrounding air. Three Rhizon lysimeters were placed in the sand layer at 1 cm above the bottom of each column and were connected to a 500 ml glass bottle. The bottles were connected to a hanging water column to create a suction of -50 cm. The suction ensured continuous water percolation and collection of runoff without the soils being saturated. In two columns per temperature treatment, tensiometers were installed at 5, 15 and 25 cm depth below the soil surface. Once a week, 300 ml demineralized water was added to each column using a plate with fifty evenly distributed needles (diameter 0.4 mm) to ensure homogeneous water distribution and to prevent ponding. The water was acidified by adding H_2SO_4 to pH 4.6 which equals the pH of rain water in Norway. Runoff samples were analysed for pH, total Al, NH_4, and NO_3 and Total Organic Carbon (TOC). The pH was measured using a glass electrode. Total Al was measured colorimetrically after complexing Al with pyrocatechol violet. NH_4-N and NO_3-N were measured colorimetrically on a Technicon autoanalyzer. TOC was measured as CO_2 evolved after combustion at 950°C using a Shimadzu TOC-5000 analyzer.

Carbon mineralization of separate layers

Triplicate samples of each layer were incubated for soil respiration measurements at 5, 10 and 17°C at moisture levels corresponding to field capacity as determined on disturbed samples (Table 4.2). CO_2 was absorbed by 10 ml 0.6 M KOH present in a vial in the

headspace of 250 ml air-tight sample containers. CO_2 production was calculated after measuring the change in electrical conductivity of the KOH solution upon absorption of CO_2 (Nordgren, 1988). The conductometer was attached to a personal computer for continuous measurements and hourly data storage. Empty sample containers were used as blanks. The KOH was replaced when the total amount of CO_2 absorbed was around 50% of the calculated maximum absorption capacity of 132 mg. The amount of sample incubated varied depending on the organic matter content (Table 4.2). All samples were preincubated at the final moisture level and temperature for 7 days (Jenkinson, 1988). Initial microbial biomass was determined using the fumigation-extraction method (Jenkinson and Powlson, 1976; Brookes et al., 1985; Voroney et al., 1993). The soil was fumigated for 24 h. Before and after fumigation, the soil was extracted by 1 M KCl. We used an extraction efficiency factor (K_{ec}) of 0.43 (Martens, 1995) to convert the difference in KCl-extractable C prior to and after fumigation to microbial C. TOC in the KCl extracts was measured as CO_2 after combustion at 950°C using a Shimadzu TOC-5000 analyzer. Microbial activity was measured at 20°C as substrate-induced respiration (SIR) after addition of glucose (Anderson and Domsch, 1978). SIR was measured the same way as the basal respiration measurements. Tests showed that for the organic soil layers a maximum glucose response was found at an addition of 10 mg glucose-C per g sample whereas in the mineral layers 1.6 mg glucose-C per g soil was sufficient. Glucose was added in 3 ml water using a syringe.

Table 4.2. Amounts and moisture content of incubated soil samples

Depth	Amount incubated		Grav. moisture
	5°C	10°C, 17°C	
(cm)	(g)		(%)[1]
Litter	2.5	1	300
0-5	5	2.5	300
5-10	5	5	200
10-14	10	5	200
14-19	20	10	40
19+	20	10	40

[1] Gravimetric moisture content in g H_2O g dry soil^{-1} * 100%

RESULTS

Column incubations

Respiration from soil columns increased with temperature (Fig. 4.1). At 17°C, respiration showed considerable temporal fluctuation. The 17°C climate chamber did not function properly causing temperatures to vary between 15 and 19°C. At 5 and 10°C temperature varied less than 0.2°C. NO_3 concentrations in the leachate significantly increased with temperature (Fig. 4.2). Increased NO_3 leaching was accompanied by a decrease in pH and an increase in total Al. TOC concentrations only increased at 17°C. NH_4 concentration was similar at all temperatures. Analysis of variance (ANOVA) showed significant ($p<0.001$) overall temperature effects on TOC, Al, and NO_3 even when time trends were ignored. ANOVA on pH values showed a significant ($p=0.015$) interaction between temperature and time; pH decreased more rapidly with time at 17°C.

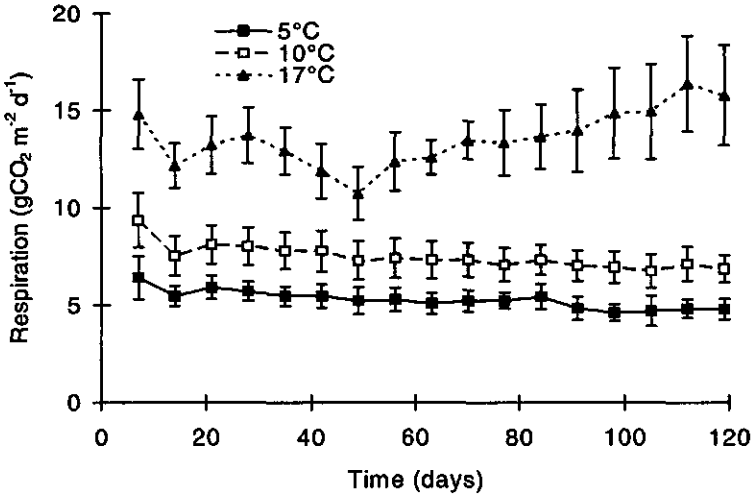

Figure 4.1. CO_2 emissions from soil columns at 5, 10 and 17°C. Error bars represent standard deviations.

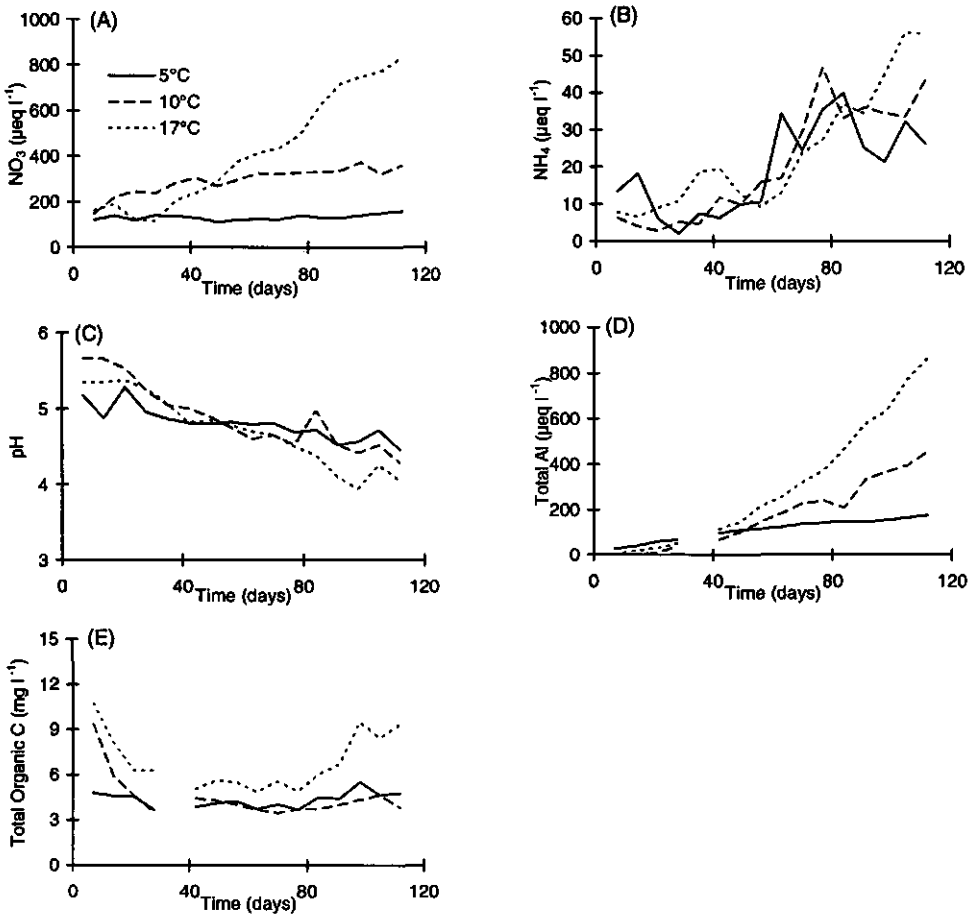

Figure 4.2. NO_3 (A) and NH_4 (B) concentrations , pH (C), total Al (D) and total organic C (E) concentrations in column leachate at 5, 10 and 17°C. Lines represent average values of 5 columns. See text for statistics.

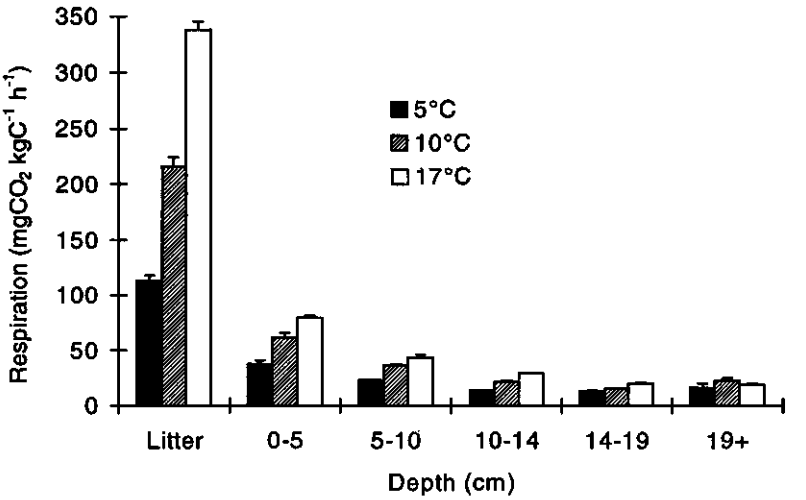

Figure 4.3. Respiration rates per gram C in different soil layers measured at 5, 10 and 17°C after 100 h of incubation. Error bars represent standard deviations.

Carbon mineralization of separate layers

Respiration rates decreased from 250 in the litter layer to 30 mg CO_2 kg C^{-1} h^{-1} in the subsoil averaged among temperatures. In most soil layers, respiration rates increased with temperature between 5 and 17°C (Fig. 4.3). The relative increase in respiration rate at elevated temperature decreased with depth from 70% in the litter layer to -4% in the subsoil averaged over all measurement times and temperature intervals ($p<0.01$; Fig. 4.4). This trend was confirmed by ANOVA using layer, temperature interval and time as main effects. The relative increase in rates showed significant layer effect ($p<0.001$) and a significant interaction between layer and temperature interval ($p=0.026$). All other main effects and interactions were insignificant. Microbial C, SIR and CAI decreased with depth except for microbial C between 5 and 10 cm, which more than doubled with increasing depth (Table 4.3). In the deepest soil layers, SIR was less than 0.5% of that in the litter layer. Both microbial C and SIR showed an abrupt decrease going from the organic to the mineral soil. The Carbon Availability Index (CAI; basal respiration/SIR) of all layers was well below 1.

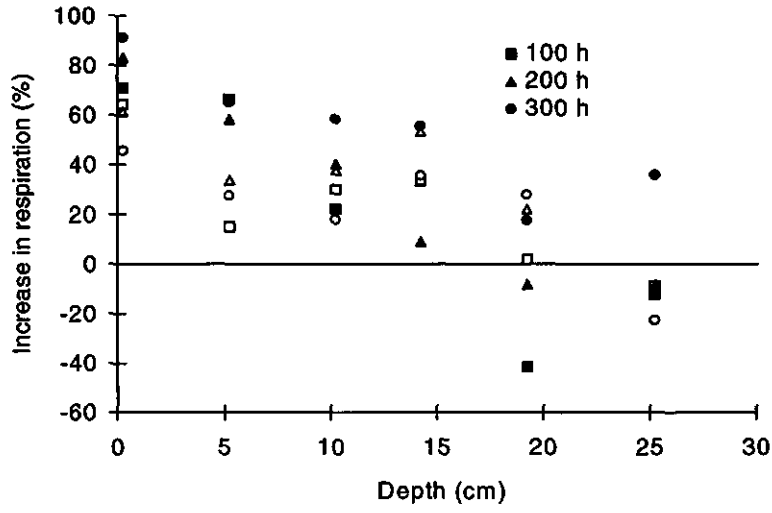

*Figure 4.4. Increase in respiration (R) measured after 100, 200 and 300 h. Values represented by closed symbols are calculated as ($R_{10°C}/R_{5°C}$)*100%; values represented by open symbols are calculated as ($R_{17°C}/R_{10°C}$)*100%.*

Table 4.3. Microbial parameters

Depth (cm)	Microbial C (mg g^{-1})	SIR[1] (mg CO_2 g^{-1} h^{-1})	CAI[2] (-)
Litter	3.84 (0.24)	0.53 (0.01)	0.37 (0.02)
0-5	1.00 (0.03)	0.21 (0.03)	0.13 (0.03)
5-10	2.58 (0.06)	0.13 (0.01)	0.17 (0.02)
10-14	0.35 (0.04)	0.018 (0.001)	0.19 (0.004)
14-19	0.19 (0.01)	0.011 (0.001)	0.11 (0.02)
19+	0.05 (-)	0.002 (0.001)	0.45 (0.23)[3]

[1] Substrate Induced Respiration

[2] Carbon Availability Index

[3] CAI = 0.29 (0.03) excluding one sample with a value of 0.77

DISCUSSION

The increased CO_2 emissions and leaching of C and N indicate that decomposition in the soil columns was stimulated by higher temperatures. By removing the above-ground vegetation and keeping the soil columns in the dark to prevent regrowth, we tried to limit interference from root respiration. By cutting the above-ground vegetation, we inadvertently added fresh root material to the SOM pool which may have caused an overestimation of C mineralization. Silvola et al. (1996) found that root respiration in a similar soil and sparse vegetation, was less than 10% of the total respiration during the growing season with living plants present. Thus, the interference from root respiration if roots stayed alive after cutting was likely to be less than 10%. Lükewille and Wright (1997) and Wright (1998) measured increased NO_3 and TOC leaching in two experimentally heated catchments close to the sampling location which agrees with our results. However, in the soil columns, plant uptake of NH_4 was absent which may have favoured nitrification and subsequent acidification compared to field conditions where at least part of the NH_4 is immobilized by plants.

The increase in respiration per degree temperature increase in the different soil layers tended to be higher between 5 and 10°C than between 10 and 17°C ($p=0.09$) which agrees with conclusions by Kirschbaum (1995) who found larger Q_{10} values at low temperatures. Nadelhoffer et al. (1991) and Pöhhacker and Zech (1995) ascribed the absence of a temperature response below 10°C for surface and subsurface soil horizons of selected tundra and forest soils to the lack of temperature sensitivity of specific enzymes at low temperatures. In our experiment, both temperature sensitivity as well as respiration per kg C decreased with depth. If the decrease in respiration with depth in our experiment is due to a decrease in substrate quality, our data would support the hypothesis by Johansson (1986). Several mechanisms have been proposed to explain the often observed decrease in respiration per unit substrate with depth rate such as O_2 deficiency (Hunt, 1977), protection of C in microaggregates (Detwiler, 1986) or slower substrate diffusion to microbes due to differences in soil structure between surface and subsoils as well as lower substrate quality (Van Dam et al., 1997). While O_2 decreased below atmospheric levels by decomposition in closed vessels, availability of O_2 never limited decomposition. Even in the surface soil, respiration aggregates was not very likely since the soils were coarse textured without clear structure elements rates did not increase after opening the vessels when replacing KOH. Protection of SOM in and organic matter contents remained high in the subsoil.

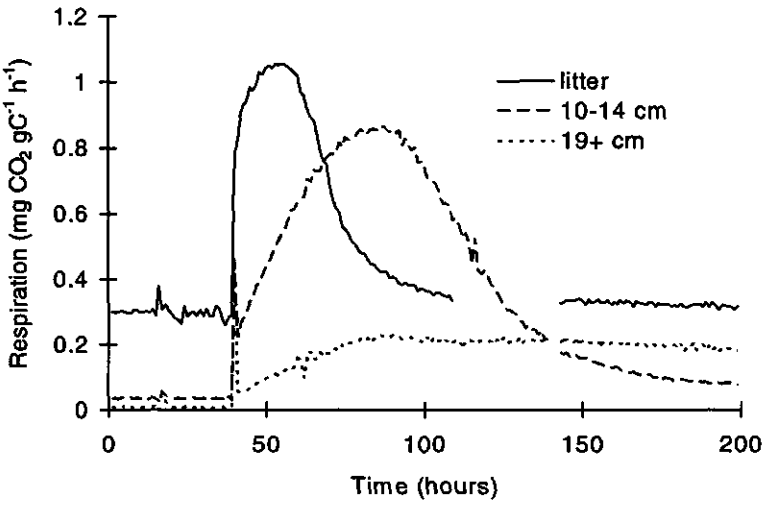

Figure 4.5. Glucose respiration rates in different soil layers. Rates are normalized to initial amount of C present in the soil layer to emphasize dynamic behaviour of glucose respiration.

Zech and Guggenberger (1996) showed that in forest soils the amount of aromatic structures increases with depth. In addition, polysaccharide concentrations decrease due to increased cellulose decomposition. These observations suggest a vertical differentiation in chemical composition and decrease in decomposability of the organic matter with depth in the soil profile. In our study, we also found increased stability with depth under field conditions since ^{14}C age of the organic matter increased with depth as well with the deepest material being approximately 2000 years old (Verburg, unpublished data).

Both total microbial C as well as activity of the microbial population was much higher in the topsoil than in the deeper soil layers. SIR decreased with depth whereas the time needed to decompose glucose increased (Fig. 4.5). Van Dam et al. (1997) showed that in an Andosol from Costa Rica, in the subsoil glucose decomposition rates increased upon additions of nutrients, but that even under optimal nutrient conditions, glucose decomposition proceeded slower in the subsoil than in the surface soil. In the soil used in this study, inorganic N levels were low in the subsoil which may have partly limited glucose decomposition. However, the study presented in Chapter 5 of this thesis showed that when N was abundant, the microbial population present in the L layer consumed

glucose more rapidly than in the FH layer. The CAI of all layers was well below 1 indicating that decomposition was C limited in all soil horizons (Parkinson and Coleman, 1991). In addition, CAI generally decreased with depth suggesting that deeper in the soil this C limitation was more severe. The high CAI in the deepest soil layer may indicate additions of fresh C due to root turnover although the glucose decomposition itself was slower than in overlaying horizons.

Our data indicate that the decreasing microbial activity with increasing depth, is most likely caused by a decrease in substrate quality. Our results are consistent with the hypothesis that, when labile C is absent, breakdown of specific, more recalcitrant, components limits decomposition rates as was suggested by Nadelhoffer et al. (1991) and Pöhhacker and Zech (1995). The overall temperature response will then be determined by the specific temperature sensitivity of the enzyme involved (McClaugherty and Linkins, 1990). Linkins et al. (1984) showed that especially endocellulase shows a very low temperature sensitivity at temperatures below 10°C. Differences in temperature response between soils may thus be directly related to organic matter quality; soils having relatively small amounts of labile C may be less responsive to changes in temperature. If decomposition of stable C is less stimulated by higher temperatures than easily decomposable C, effects of temperature on decomposition of SOM are time dependent. As an example, we assumed organic matter to consist of a labile and stable fraction and decomposition of the organic matter was described by a double exponential function according to Deans et al. (1986):

$$C(t) = C_0 \cdot S \cdot (1 - e^{-k_1 \cdot t}) + C_0 \cdot (1 - S) \cdot (1 - e^{-k_2 \cdot t})$$

C is the amount of decomposed organic carbon at time t, C_0 is the potentially decomposable C, S represents the labile fraction having a decomposition rate constant k_1 and (1-S) represents the stable fraction having a decomposition rate constant k_2. We assumed that C_0 is 100 g, S is 5% of C_0, k_1 is 0.035 d^{-1}, k_2 is 0.001 d^{-1}, and that k_1 doubles at a certain temperature increase where k_2 increases by only 50%. The increase in daily respiration due to increased temperature indeed varies with time (Fig. 4.6). When comparing cumulative decomposition, the temperature effect shows a different pattern and becomes constant with time. Although our example is a gross simplification, it illustrates a potential problem when comparing different studies. Indeed, Kirschbaum (1995) suggested that his conclusions may have been biased by using data from studies using different incubation times. For instance, respiration rates measured immediately after the start of the incubation as done by Howard and Howard (1993) may be influenced by a

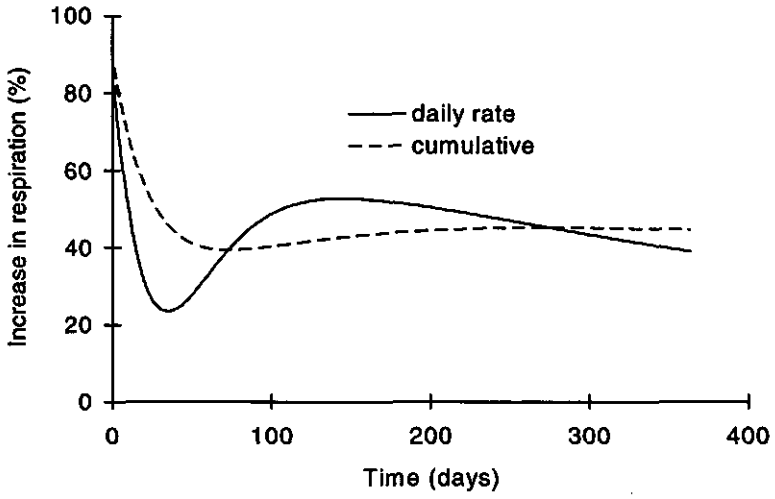

*Figure 4.6. Effects of increased temperature effect on decomposition assuming a two-pool model where rate constants for the stable pool has a lower temperature dependence. The increase is calculated as ($R_{Televated}/R_{Tambient}$)*100%. The solid line represent increase in daily respiration rates. The dotted line represents the increase in cumulative respiration.*

dominance of decomposition of microbial metabolic products. Howard and Howard (1993) also determined temperature effects on the same samples by subsequently increasing temperature, so their results may have been influenced by effects of decreased substrate quality during the course of the experiment, resulting in an underestimation of temperature effects with time. Nadelhoffer et al. (1991) compared cumulative decomposition at different temperatures after 13 weeks of incubation, which may have emphasized decomposition of more stable material. In our study we compared rates measured at different times. Although the magnitude of the temperature effects varied with time, the patterns with depth remained the same. Under natural conditions, C mineralization is likely to be dominated by decomposition of labile C supplied by roots (Hungate et al., 1997) which would argue for short-term incubation experiments. However, effects of disturbance during pre-treatment of samples are also most important in short-term experiments.

Our study shows that decomposition in the studied soil was stimulated by elevated temperature which resulted in increased emissions of CO_2 from the soil and acidification of drainage water. Temperature sensitivity decreased with depth most likely due to a decrease in the amount of labile C. Consequently, temperature sensitivity of enzymes, involved in breakdown of specific recalcitrant compounds, may determine the overall temperature response. If this depth-dependent temperature sensitivity is common in forest soils, global predictions of C losses at elevated temperature are likely to be overestimated when using a uniform temperature dependence for both litter and humified organic matter.

Acknowledgments-- We thank K. de Valk, N. Nakken and L. Rou for assistance in the laboratory.

Chapter 5

MICROBIAL TRANSFORMATIONS OF C AND N IN A BOREAL FOREST FLOOR AS AFFECTED BY TEMPERATURE

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submitted to Soil Biology and Biochemistry

MICROBIAL TRANSFORMATIONS OF C AND N IN A BOREAL FOREST FLOOR AS AFFECTED BY TEMPERATURE

ABSTRACT

The effects of temperature on N mineralization were studied in two organic surface horizons (LF and H) of a boreal forest soil incubated at 5°C and 15°C after adding ^{15}N. Gross N fluxes were calculated using a numerical simulation model. The model was calibrated on microbial C and N, basal respiration, and KCl-extractable NH_4^+, NO_3^-, $^{15}NH_4^+$ and $^{15}NO_3^-$. In the LF layer, increased temperature resulted in a faster turnover of all N pools. Net N mineralization did not increase during the 15 days of incubation because both gross NH_4^+ mineralization and NH_4^+ immobilization increased. In the H layer, however, both gross NH_4^+ mineralization and NH_4^+ immobilization were lower at 15°C than at 5°C and the model predicted a decrease in microbial turnover rate at higher temperature although measured microbial activity was higher. Decreased gross N fluxes in spite of increased microbial activity in the H layer at elevated temperature could have been caused by uptake of organic N. Disparities between the layers were ascribed to differences in physiology of microbial population. Microbial C/N was around 13 in the LF layer pointing at a fungi-dominated decomposer community whereas it was close to 6 in the H layer, probably due to predominance of bacteria. Respiration and microbial C were difficult to fit by the model if the microbial C/N ratio was kept constant with time. The lack of response of net N mineralization to elevated temperature is probably transient. When the microbial N level has become stable, it is expected that N mineralization will increase. A separate ^{15}N enrichment study with the addition of glucose showed that glucose was metabolized faster in the LF than in the H layer. Because no evidence for N limitation for decomposers was found, decomposition appears to be limited by C availability in LF and H layers.

INTRODUCTION

An increase in the concentration of greenhouse gasses in the atmosphere is expected to result in global warming. General Circulation Models predict an increase in temperature of about 4.5°C in the Northern latitudes for the next 50 years (Parton et al., 1995). Soils in

these areas are usually rich in organic matter and could become an important source of CO_2 if decomposition would increase at elevated temperature (Oechel et al., 1993). This could at least partly offset the predicted increase in C fixation by the living biomass at higher atmospheric CO_2 concentration (Schimel, 1995). In addition to increased C loss, higher decomposition rates at elevated soil temperatures may favor N mineralization. Especially in ecosystems where plant growth is limited by N availability, the effect of CO_2 fertilization on Net Primary Production (NPP) may be enhanced by higher N levels (Arp et al., 1997). Microbial activity is a major control on C mineralization and N availability. Consequently, insight in the effects of temperature on microbial C and N transformations may provide valuable information on how soils will respond to climate change.

At elevated temperature we may expect microbial activity to increase, resulting in increased substrate decomposition. As a result, on the short term, N may be immobilized which will lead to production of either more microbial biomass and/or biomass with a lower C/N ratio. Kuikman et al. (1991) showed that upon addition of N, the microbial population decomposes more C rich substrates than under low N conditions, suggesting that decomposition of C rich substrates can be limited by N availability. Jonasson et al. (1996) demonstrated that in arctic soils, microbial activity is stimulated upon addition of inorganic N. These authors also showed that addition of labile C, such as glucose, resulted in an increase in microbial biomass suggesting a C limitation. Pöhhacker and Zech (1995) speculated that microbial stress due to substrate limitations may cause the amount of microbial biomass to be more sensitive to temperature changes. Consequently, presence of C and/or N limitations is likely to influence effects of temperature on gross C and N fluxes.

The aim of this study was to assess the short-term effects of temperature on microbial gross C and N fluxes by combining a ^{15}N addition experiment with numerical simulations (Tietema and Van Dam, 1996). We used soil material from 2 organic surface horizons of a forest soil and measured C and N mineralization, and microbial characteristics at 5 and 15°C. In addition, we assessed whether decomposition is limited by C or N availability by combining an addition of glucose and ^{15}N.

MATERIALS AND METHODS

Study site and sampling

Soil samples were collected in a boreal forest near Risdalsheia, Southern Norway, at the location of the CLIMEX project (Jenkins and Wright, 1993). The site is located on a large

granite plateau. Small, patchy, depressions in the granite surface are filled with post-glacial soil material, leaving 30-50% of the bedrock uncovered. Soil depth in the sampling area varies between 0 and 70 cm. In the shallow parts, the ground vegetation is dominated by heather (*Calluna vulgaris* (L.) Hull) whereas different blueberry species (*Vaccinium myrtillus* L., *V. vitis-idaea* L., *and V. uliginosum* L.) are dominant in deeper soils. Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karsten) and birch (*Betula pubescens* L.), the main tree species, are confined to the deeper soils. The soils are classified as Dystric Leptosol (FAO, 1988) but show evidence of podzolisation. The mineral fraction consists of coarse grains of partially weathered, quartz and K-feldspar. We took soil samples from a plot of approximately 2 m^2 dominated by *Calluna vulgaris*. The upper 2 cm-thick layer contained easily recognizable plant remains which showed some discoloration, the LF layer (Klinka et al., 1993). The next 3 cm-thick layer consisted of humified material without recognisable plant structures except for stems and coarse root remains, and is referred to as the H layer. Samples of the LF and H layer were air-dried at room temperature until constant weight. After drying, large roots and stems were removed and the remaining material was coarsely ground (1 cm). The samples were stored in the dark at 2°C for about 3 months until use.

Litter characterization

We determined organic matter contents of the samples as Loss On Ignition (LOI) at 600°C after 4 h, and total C and N using a EA 1108 CHN element analyzer. For lignin determinations, ethanol-soluble components were removed by extraction with 80% ethanol (3 x 30 min). The residual material was treated with 10 ml 72% H_2SO_4 at 30°C for 1 h. This mixture was diluted to 3% H_2SO_4 and refluxed for 2 h. The residual solid material was washed, filtered and dried overnight at 105°C. Lignin content was determined as LOI (650°C; 2 h) of this residue. The pH was measured in a 1:5 (w/w) KCl extract. All analyses were carried out on duplicate samples.

C and N mineralization

We conditioned soil samples for 1 week at the target incubation temperature of 5 and 15°C at a gravimetric moisture content of 300% corresponding to field capacity. After the preincubation, at each temperature, 12 samples of 6 g (air-dry weight) of each layer were put in a respirometer (Nordgren, 1988). Respiration was continuously measured as the hourly change in electrical conductivity of 10 ml 0.6 M KOH in a vial in the headspace of the 250 ml sample jars. Nitrogen 15 was added as $(NH_4)_2SO_4$ to each sample in 6 ml

water. The solutions containing 99% ^{15}N were added dropwise using a syringe. The added ^{15}N did not exceed 10% of the resulting total amount of KCl-extractable NH_4^+-N. The increase in moisture due to addition of the isotope solution did not affect respiration. Initially, the soil samples contained very little NO_3^-. To test whether the low NO_3^- level was due to low nitrification rates or to rapid NO_3^- turnover, we carried out a separate NO_3^- labeling. Six samples of 10 g of each layer were incubated in the respirometer simultaneously with the NH_4^+ labeling experiment. Since initial NO_3 levels were too low for accurate isotope measurements, we added a mixture of ^{15}N- and ^{14}N-KNO_3 to reach a final NO_3^- concentration of 100 µg per g sample with a maximum ^{15}N atom percentage of 10%. At t=0, 3, 9 and 15 d, three ^{15}N-NH_4^+ labeled samples of each layer were analyzed for N and microbial biomass. Three ^{15}N-NO_3^- labeled samples of each layer were analyzed only at t=0 and 15 d. Approximately half of each sample was extracted with 1 M KCl using a 1:30 (w/w) soil to extractant ratio. The remainder was used for measurement of microbial C and N. NH_4^+-N and NO_3^--N in the extracts were measured colorimetrically on a Technicon autoanalyzer. Total N was measured colorimetrically after digestion by $K_2S_2O_8$ and subsequent conversion to NO_2^-. Total Organic Nitrogen (TON) was calculated as the difference between total N and $NH_4^+ + NO_3^-$. Total Organic Carbon (TOC) was analyzed with a Shimadzu TOC-5000 analyzer. ^{14}N and ^{15}N were recovered from the extracts by microdiffusion (Sørensen and Jensen, 1991). NH_4^+ was trapped on an acidified glass filter after conversion of NH_4^+ to NH_3 by adding MgO to the KCl extracts. In the NO_3^- enriched samples, NO_3^- was reduced to NH_4^+ by Devarda's alloy after all NH_4^+ had diffused to the glass filter. The $^{15}N/^{14}N$ ratio of the N present on the glass fiber filters was measured using an on-line combustion gas isotope-ratio mass spectrometer.

Microbial biomass

We determined microbial C and N by fumigation-extraction (Jenkinson and Powlson, 1976; Brookes et al., 1985; Voroney et al., 1993). Microbial C and N were calculated as the difference in KCl-extractable TOC and TON prior to and after 24 h of fumigation with chloroform, divided by the extraction efficiency factor (K_{ec} and K_{en}). We used a K_{ec} of 0.43 (Martens, 1995) and a K_{en} of 0.31 (Voroney et al., 1993). After the preincubation, at each temperature, 12 samples of 6 g (air-dry weight) of each layer were put in a respirometer simultaneously with the labeling experiment for determination of Substrate Induced Respiration (SIR) (Anderson and Domsch, 1978). Samples were conditioned at a moisture level of 350%. At each sampling date, glucose was added in 3 ml water to three untreated samples per layer. Tests revealed that a maximum response was obtained when

10 mg glucose-C per g sample was added. The SIR was taken to be the average respiration during the 6 h lag phase before the start of microbial growth.

At 15°C, SIR measurements were combined with isotope measurements. Samples were conditioned at a moisture level of 300%. At day 0, ^{15}N was added as $(NH_4)_2SO_4$ to 15 samples of each layer in the same amounts as described above in 3 ml water. At day 3, 10 mg glucose-C per g sample was added in 3 ml water. Five sampling dates were selected depending on the respiration response. Measurements were the same as for the non-amended ^{15}N enrichment study.

Statistics

To test whether net N mineralization or immobilization occurred, we performed a linear regression on the KCl-extractable NH_4^+ and NO_3^- data. We tested whether the slopes of these lines significantly differed from 0. Data on microbial C, N, C/N ratio, respiration and SIR were analyzed by 3-way Analysis of Variance (ANOVA) with temperature, soil layer and sampling day as main factors. Interactions between all factors were calculated. Main effects and interactions were considered significant if $p<0.05$.

Simulation model

We calculated gross N fluxes using the numerical simulation model as described by Tietema and Van Dam (1996). In addition to NH_4^+ and NO_3^-, the model considers three organic C and N pools; labile organic matter (LOM), refractory organic matter (ROM) and microbial biomass (Fig. 5.1). Decomposition of ROM (1C, Fig. 5.1) is a first order process with the rate constant being corrected by the ratio of actual and initial microbial biomass. Decomposition of LOM (2C) is assumed to be equal to the input of microbial necromass and organic metabolites. The C/N ratio of the LOM is assumed to be constant, implying steady state composition of labile C and N. N mineralized from ROM (1N) and LOM (2N) is divided in a fraction (ß) NH_4^+ (arrow 3) and (1-ß) NO_3^- (arrow 4) where the amount of NO_3^- equals heterotrophic nitrification (arrow 4). Autotrophic nitrification (arrow 5) is first order with respect to total NH_4^+. Dissimilatory NO_3^- reduction (arrow 6) is first order with respect to the active microbial biomass. Gross immobilization of both NH_4^+ (arrow 7) and NO_3^- (arrow 8) is independent of the concentration but first order with respect to the amount of microbial biomass. We assumed that the C/N ratio of the

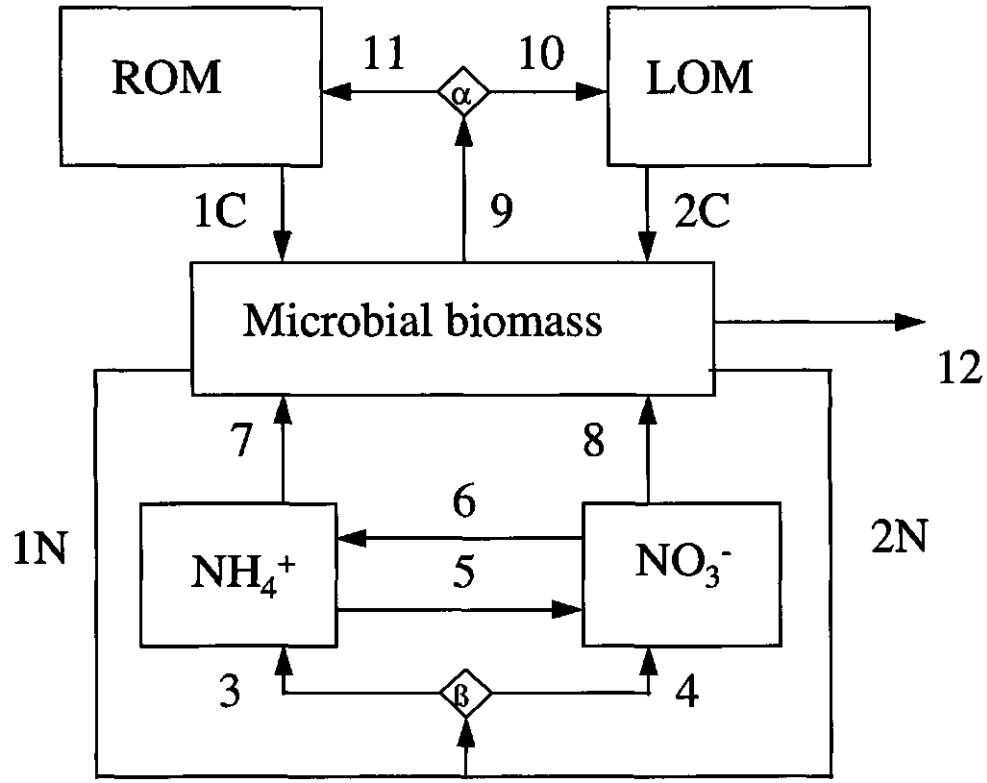

Figure 5.1. Carbon and nitrogen pools and fluxes as used in the simulation model. The numbers of the fluxes and symbols correspond with numbers of the processes and symbols as mentioned in the text. LOM = Labile Organic Matter; ROM = Refractory Organic Matter (adapted from Tietema and Van Dam (1996)).

microbes is constant so that gross growth of the microbial biomass follows from gross N mineralization (Schimel, 1988). The production rate of microbial necromass plus dissimilation of organic metabolic compounds (arrow 9) is first order with respect to the microbial biomass. This necromass is distributed over LOM (fraction α, arrow 10) and ROM (fraction 1-α, arrow 11). A fraction of the total C is respired (arrow 12), the remainder being used for growth. The initial ^{15}N concentrations of all compartments are assumed equal to the natural abundance of ^{15}N with an isotope proportion of 0.0366%. Isotope discrimination during the various transformations was assumed negligible.

By means of a Simplex procedure (Caceci and Cacheris, 1984) we optimized parameters for fitting of the non-linear functions using data on respiration, NH_4^+ and NO_3^- concentrations, isotope ratios, and microbial C for each sampling date. Ten parameters were optimized; the six rate constants, the C use efficiency, the parameters regulating the partitioning of microbial necromass into LOM and ROM and the mineralization of organic N into NH_4^+ and NO_3^- and the amount of labile N. Since C use efficiency and the rate constant for microbial turnover both depended on microbial C/N ratio, we treated microbial C/N as a fixed parameter. Using a lower C/N ratio in the simulations would result in a lower C use efficiency and a lower turnover rate of the microbial population (cf Tietema and Van Dam, 1996). We simulated the glucose decomposition by adding a separate glucose pool since the kinetics of glucose consumption are different from other components (Coody et al., 1986). Also, the microbial C/N ratio could vary since we expected a more dynamic behavior of the microbial population due to the rapid kinetics of glucose decomposition, while it was kept constant in the simulations without glucose addition. In all simulations, the time step was halved if the calculated fluxes would change state variables or isotope percentages by more than 1%. The maximum time step used was 8 h.

RESULTS AND DISCUSSION

The organic matter, %C, %N, C/N ratio, lignin content and pH of the morphologically distinct LF and H layer were similar (Table 5.1). The NH_4^+ and NO_3^- concentration were lower in the H layer. At 5°C, in both layers, NH_4^+ did not significantly increase during the incubation whereas NO_3^- increased significantly (LF layer: $p<0.001$, H layer: $p=0.014$). At 15°C, NH_4^+ increased significantly ($p=0.014$) in the H layer and tended to increase in the LF layer ($p=0.079$) but NO_3^- remained constant in both layers (Fig. 5.2). At day 0, the percentage of $^{15}NH_4^+$ showed little variation suggesting that distribution of the label was homogeneous (Fig. 5.3). However, in the H layer at 5°C the atom % ^{15}N at day 0 seemed anomalously low suggesting incomplete recovery of the ^{15}N on the glass fibers filters. At both temperatures, the ^{15}N percentage decreased more strongly with time in the LF layer than in the H layer indicating a faster turnover of inorganic NH_4^+. The atom % ^{15}N of the nitrate labelings remained unchanged with time in all layers/temperatures (data not shown) suggesting that NO_3^- turnover was slow compared to turnover of NH_4^+. The relatively small net mineralization may have been due to the high initial NH_4^+ concentration. Air-drying and storage of samples could have caused accumulation of NH_4^+. However, Tietema and Van Dam (1996) found a net mineralization of around 200 mg N kg^{-1} during

a 2-week incubation of LF material of a coniferous forest soil with inorganic N concentrations comparable to our samples. We do not know whether the observed trends will be sustained over longer time periods. During incubations generally exceeding 10 weeks, annual net N mineralization under field conditions at the sampling site increased after increasing soil temperature by 2-3°C (Chapter 2, this thesis). Nadelhoffer et al. (1991) found increased net N mineralization at 15°C compared to 3°C but no response between 3°C and 9°C in an arctic tundra soil after a 13-week incubation. Emmer and Tietema (1990) found a linear increase in net N mineralization between 0 and 30°C during a 4-week incubation of LF material from a deciduous forest soil.

Table 5.1. Chemical characteristics of the forest floor material

	LF	H	
Total analysis			
LOI[1]	96	94	ns[2]
C[1]	48.8	46.8	ns
N[1]	1.7	1.8	ns
C/N[3]	29.3	25.9	ns
Lignin[1]	44.7	46.4	ns
KCl-extractions			
NH_4-N[4]	522	450	*
NO_3-N[4]	8.4	2.6	*
pH	3.6	3.4	ns

[1] LOI (Loss On Ignition), C, N and lignin in % of total.

[2] * = significant differences between LF and H at $p<0.05$; ns = not significant ($p>0.05$)

[3] Mass ratio

[4] Nutrient concentrations in mg kg^{-1}

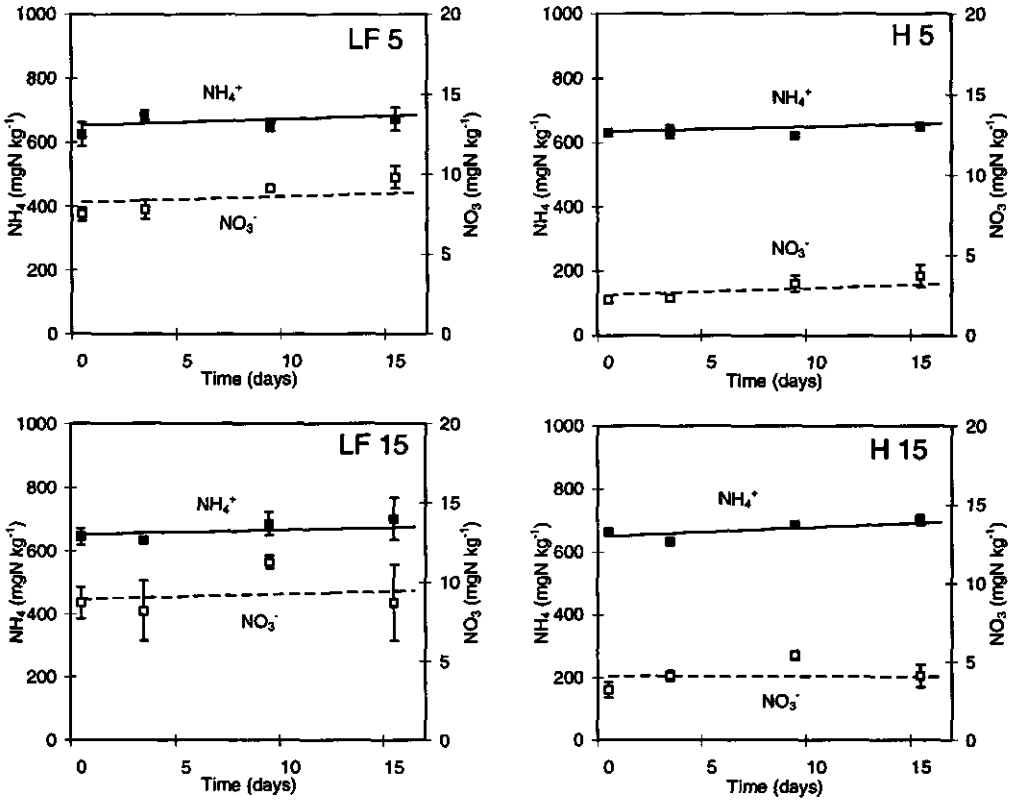

Figure 5.2. Observed (mean and standard deviation; n=3) and simulated NH_4^+ and NO_3^- concentrations in LF (A) and H layer (B) at 5 and 15°C. Lines represent simulations.

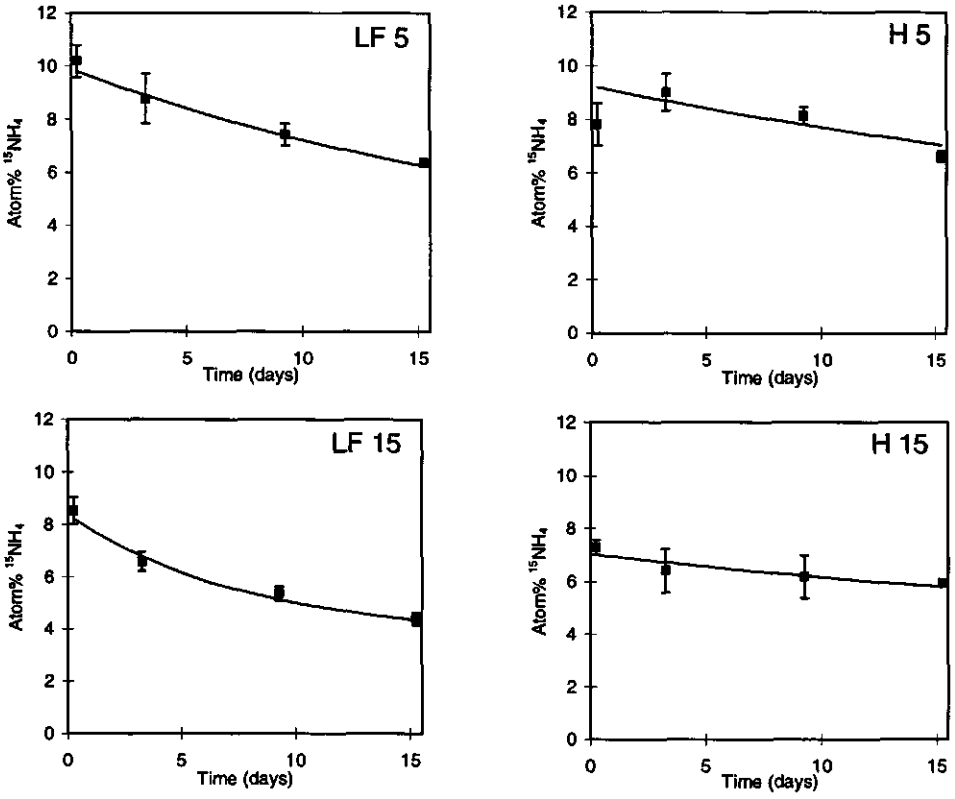

Figure 5.3. Observed (mean and standard deviation; n=3) and simulated isotope percentages of the NH_4^+ enriched samples of LF and H layer at 5 and 15°C. Lines represent simulations.

Table 5.2. Analysis of variance results for microbial parameters.

	MB-C[1]	MB-N[1]	MB-C/N[1]	Respiration	SIR[1]
Day (D)	***[2]	ns	ns	ns	**
Layer (L)	ns	***	***	***	***
Temperature (T)	***	ns	ns	***	***
D*L	ns	ns	ns	ns	**
D*T	ns	ns	ns	ns	*
L*T	*	ns	ns	***	***
D*L*T	ns	ns	ns	ns	ns

[1] MB-C = microbial C; MB-N = microbial N; MB-C/N = microbial C/N ratio; SIR = Substrate Induced Respiration.

[2] * $p<0.05$; ** $p<0.01$; *** $p<0.001$; ns = not significant ($p>0.05$).

Despite the absence of clear differences in net N mineralization between both soil layers, microbial respiration was higher in the LF than in the H layer (Fig. 5.4A). Since microbial C was similar in both layers (Fig. 5.4B), microbial activity appeared to be higher in the LF layer. Especially in the LF layer, respiration increased with temperature whereas microbial C did not change (LF layer) or decreased (H layer; Table 5.2). Our data agree with results from Pöhhacker and Zech (1995) who observed that, in the L layer of a beech forest soil, the amount of microbial C was less sensitive to temperature changes than in the humic horizon whereas respiration more strongly increased in the L layer. These authors hypothesized that their observations reflected microbial stress in the humic layer due to substrate limitations. In our study, microbial C significantly varied with time whereas respiration did not. Microbial N was significantly lower in the LF layer resulting in a higher C/N ratio in the LF layer. The average C/N ratio was 12.6 ± 5.0 (n=20) in the LF layer and 6.4 ± 1.8 (n=20) in the H layer. Although microbial C showed significant day and temperature effects and microbial N did not, we found no such effects on microbial C/N ratio presumably due to the large variation in microbial C and N (Table 5.2). The SIR was highest in the LF layer (Fig. 5.5). In both layers, SIR increased with temperature but the increase was strongest in the LF layer (Table 5.2). Especially in the LF layer, SIR was lowest at day 3 which resulted in significant day effects and a significant interaction between day and layer. At 15°C, temporal variation was stronger than at 5°C.

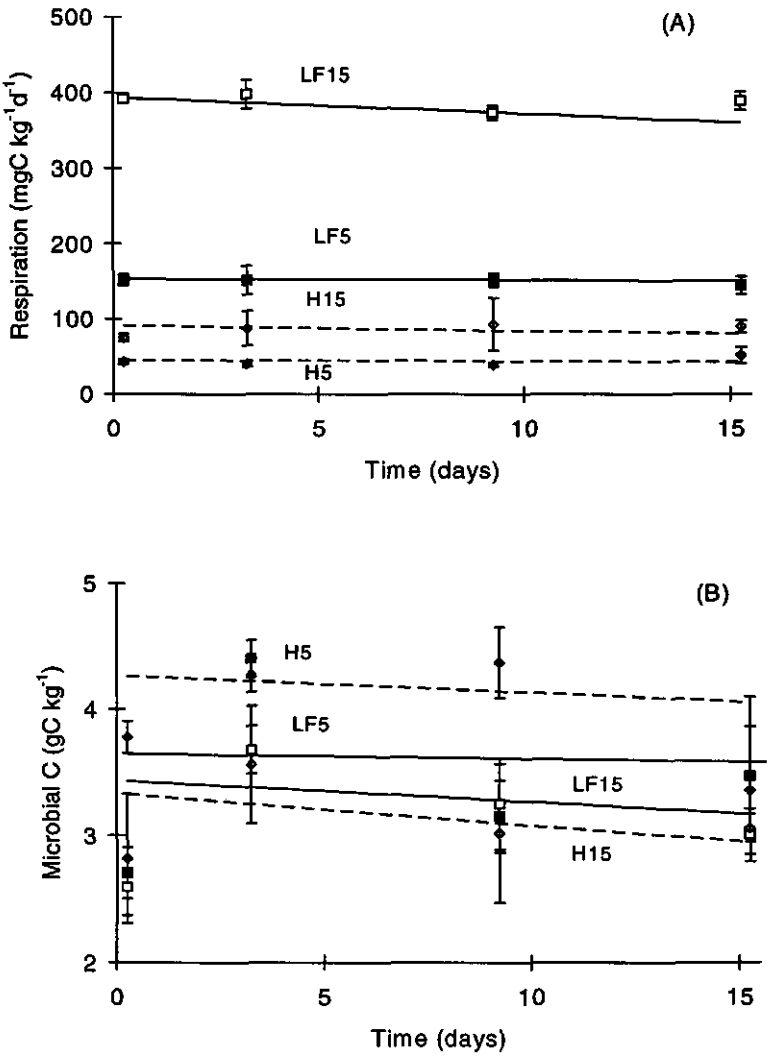

Figure 5.4. Observed (mean and standard deviation; n=3) and simulated microbial respiration (A) and microbial C (B) in LF and H layer at 5 and 15°C. Squares represent LF samples. Closed symbols represent 5°C measurements. Lines represent simulations.

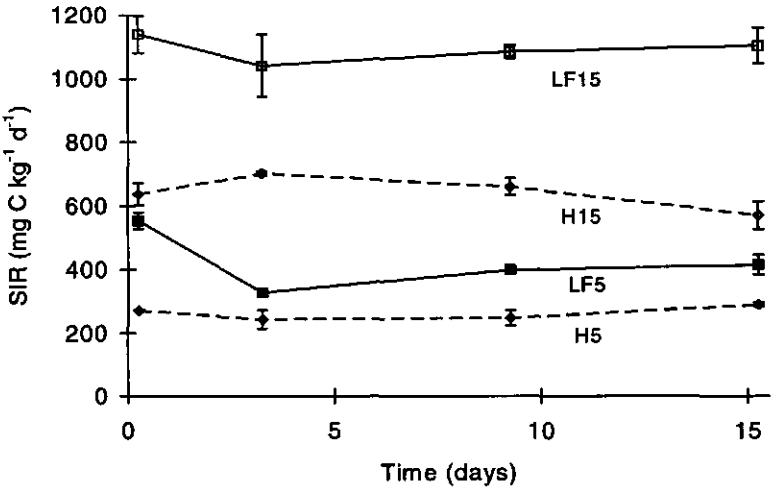

Figure 5.5. Substrate Induced Respiration (mean and standard deviation; n=3) of LF and H layer at 5 and 15°C. Squares represent LF samples. Closed symbols represent 5°C measurements.

Although the main trends in NH_4^+ and NO_3^- and microbial C were simulated, the temporal variation was generally not captured by the model (Fig. 5.2 and 5.4). In general, the model fitted isotope percentages within the standard deviations of the measurements except in the H layer at 5°C, where the isotope percentage on day 0 was lower than on day 3 (Fig. 5.3). Microbial C and respiration could not be simulated simultaneously using fixed values of the microbial C/N ratio and the LOM pool size (Fig. 5.4). In the simulations, we used a microbial C/N ratio of 13 for the LF and 6 for the H layer. Especially in the LF layer, measured microbial C fluctuated with time so the assumption that both microbial biomass and LOM pool are in steady state may not be valid. Apparently, 1 week of preincubation was not enough for the microbial population to stabilize. Still, even though Tietema and Van Dam (1996) found less temporal variation in microbial C than we did, they were also unable to simulate both respiration and microbial C assuming a fixed C/N ratio of the microbial biomass. In the LF layer, both gross mineralization and NH_4^+ immobilization more than doubled with increasing temperature (Table 5.3). By contrast, in the H layer, gross NH_4^+ mineralization decreased by 22% and immobilization by 40% with increasing temperature. In all simulations, nitrate immobilization and nitrification rates were negligible compared to the gross NH_4^+ fluxes

indicating that the low NO_3^- concentrations were not due to rapid NO_3^- turnover. The turnover rates of the LOM, ROM and, except in the H layer, microbial C increased with temperature. Turnover rates of these organic pools were higher in the LF than in the H layer. Estimating the pool size of the LOM, and consequently turnover rate, in the both layers was very difficult. Near the end of the optimization process, an increase of the LOM pool size in the H layer from 36 to 94 mg kg^{-1} resulted in a decrease in residuals of only 5%. Measurement of isotopic ratios of NH_4^+ in the NO_3^- labeling experiment would have contributed to a better estimate of the LOM pool size. Turnover of LOM in the H

Table 5.3. Modeling results for LF and H layer at 5 and 15°C

	LF		H	
	5	15	5	15
LOM-N (mg N kg OM^{-1})	488	81	506	46
ROM-N (g N kg OM^{-1})	15.3	15.7	17.1	17.4
Turnover rate LOM (fraction d^{-1})[1]	0.044	0.510	0.042	0.212
Turnover rate ROM (fraction y^{-1})	0.220	0.483	0.025	0.055
Turnover rate MB-C (fraction d^{-1})	0.084	0.176	0.033	0.024
NH_4^+ immobilization (g N kg^{-1} MB-C d^{-1})	6.40	13.10	3.04	2.05
Carbon use efficiency	0.66	0.60	0.75	0.37
Δ ROM (mg N kg^{-1} OM)[2]	-28.0	-0.8	-14.6	+21.8
Δ NH_4^+ (mg N kg^{-1} OM)	+32.3	+21.8	+24.8	+45.1
Δ MB-N (mg N kg^{-1} OM)	-5.0	-21.6	-11.5	-66.8
Fluxes (mg N kg^{-1} OM d^{-1})				
NH_4^+ mineralization	22.1	44.5	12.1	9.4
NH_4^+ immobilization	20.1	43.2	10.6	6.4
NO_3^- immobilization	<0.01	<0.01	<0.01	<0.01
Heterotrophic nitrification	0.05	0.04	0.04	<0.01
Autotrophic nitrification	<0.01	<0.01	<0.01	<0.01
Dissimilatory NO_3^- reduction	<0.01	<0.01	<0.01	<0.01

[1] LOM = labile organic matter; ROM = refractory organic matter; MB-C = microbial C; MB-N = microbial N.

[2] Δ .. = change in pool size between day 15 and day 0 in simulation

layer at 15°C was faster than at 5°C even though in- and output fluxes decreased, which was caused by a stronger decrease of the pool size compared to the fluxes. The simulations suggest that at 5°C, net N mineralization is caused by decomposition of ROM whereas at 15°C most N is mineralized at the expense of microbial N. ROM may even act as a sink for N as is the case in the H layer (Table 5.3). Although the model calculated a decrease in microbial turnover and gross N transformations in the H layer, the experimental data suggested a higher microbial activity at elevated temperature. This apparent contradiction can be explained by increased utilization of organic N at higher temperature. Direct uptake of organic N from the LOM and ROM implies that N bypasses the labeled inorganic NH_4^+ pool, leaving the isotopic ratio of the NH_4^+ pool unaffected. Hadas et al. (1992) and Barraclough (1997) concluded from ^{15}N enrichment experiments that organic and inorganic N may be taken up simultaneously by microbes. Direct assimilation of organic N, necessarily accompanied by consumption of C, was not accounted for in our simulations. If we had accounted for this extra C consumption, simulated C use efficiency would have increased instead of decreased (Table 5.3) with increasing temperature.

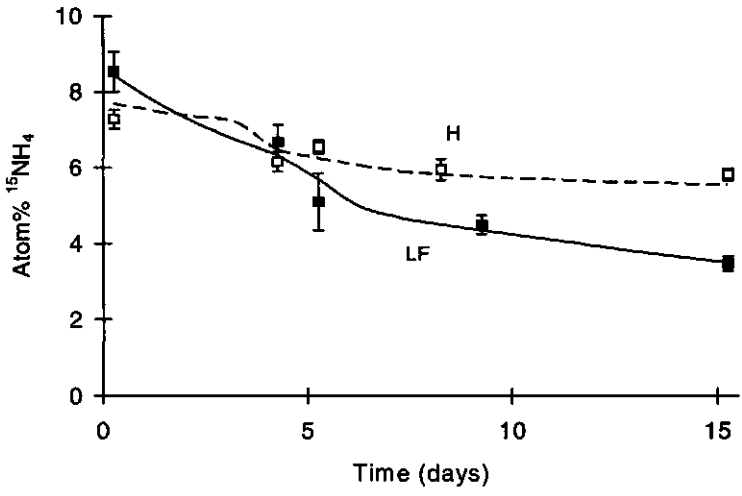

Figure 5.6. Observed (mean and standard deviation; n=3) and simulated NH_4^+ concentration in LF and H layer at 15°C in glucose amended samples. Glucose was added at day 3. Lines represent simulations.

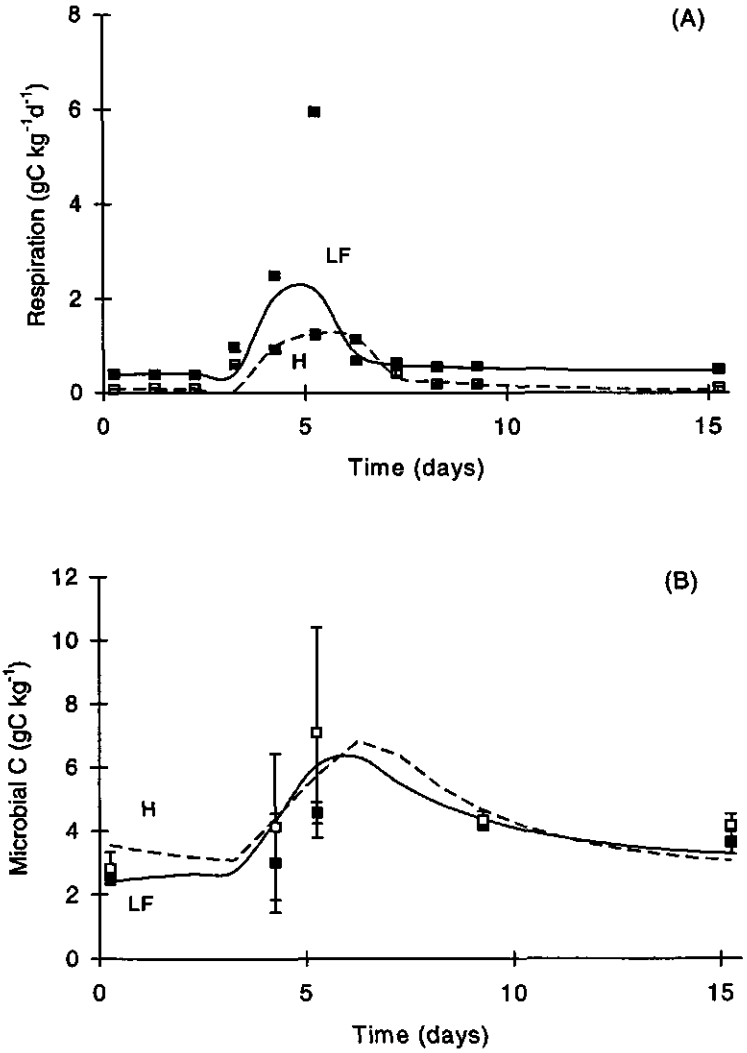

Figure 5.7. Observed (mean and standard deviation; n=3) and simulated microbial respiration (A) and microbial C (B) in LF and H layer at 15°C in glucose amended samples. Glucose was added at day 3. Closed symbols represent LF layer. Lines represent simulations.

After addition of glucose, NH_4^+ was immobilized, and most so in the LF layer (Fig. 5.6) which agrees well with results found by Jonasson et al. (1996) in a field study in arctic soils. Apparently, in the LF layer most of the KCl-extractable NH_4^+ in that layer was available for microbes suggesting that decomposition is not limited by N. The same was probably true for the H layer. The time-averaged Carbon Availability Index (CAI) defined as basal respiration/SIR was 0.37 ± 0.05 (n=8) in the LF layer and 0.15 ± 0.02 (n=8) in the H layer. These CAI values are well below 1, indicating that in both layers decomposition is limited by C (Parkinson and Coleman, 1991). The calculated glucose-C use efficiency was similar for the LF and H layer. Still, glucose was respired faster in the LF layer than in the H layer (Fig. 5.7). Isotope percentages were generally fitted within the standard deviations of the measurements (Fig. 5.8). By adding a separate glucose pool, the model captured the dynamic behavior of the glucose consumption even though a perfect fit of all data remained difficult.

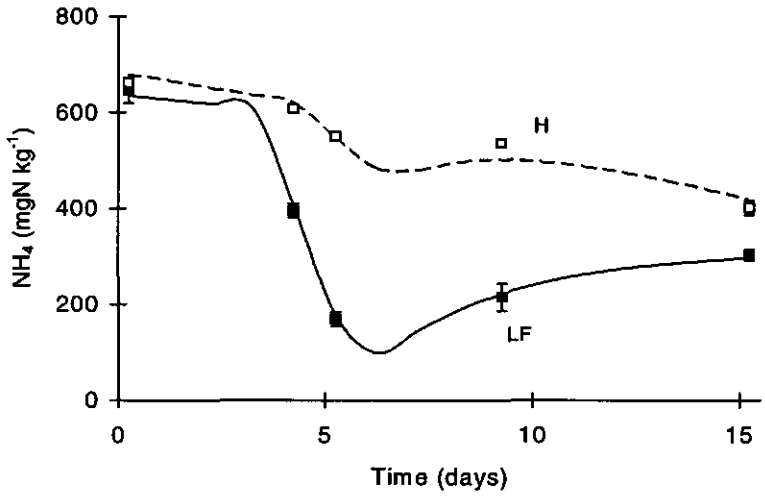

Figure 5.8. Observed (mean and standard deviation; n=3) and simulated isotope percentages of the NH_4^+ enriched and glucose amended samples of the LF and H layer at 15°C. Lines represent simulations.

In general, turnover of C and N pools and glucose decomposition were more rapid in the LF than in the H layer. Carbon mineralization and SIR in the LF layer were higher than in the H layer. Differences in net N mineralization with depth were not evident. Microbial C/N ratios suggest that in the LF layer the decomposer community was dominated by fungi whereas in the H layer it was dominated by bacteria. Our results are consistent with results from Berg et al. (submitted) who observed a shift from a fungi dominated decomposer community in the recent LF layer to a bacteria dominated community in the older H layer of a coniferous forest soil. In our study, the temperature effect on microbial respiration and SIR was larger in the LF than in the H layer. The lower CAI in the H layer indicates a lower substrate quality in this layer. Our data support the hypothesis by Johansson (1986) that sensitivity of decomposition rates to changes in physical conditions decreases with decreasing substrate quality. However, the lower temperature effect on respiration in the H layer was partly due to a decrease in microbial biomass. So, even though the overall effect of temperature on C loss decreased, the effects on the microbial population itself appeared to be stronger when substrate quality was lower. Whether the difference in sensitivity to short-term changes has implications for long-term effects of climate change on microbial C and N dynamics is difficult to assess from our experiment. Overall, our data showed that at elevated temperature more C is lost from the forest floor through respiration. If both mineralization and immobilization continue to increase at the same rate at elevated temperature, the C/N ratio of the organic matter would decrease. Ultimately, gross mineralization would exceed immobilization resulting in increased net mineralization. However, this possible long-term response does not take into account any changes in quantity and quality of litter produced at elevated CO_2 and temperature. Under field conditions, microbial C and N dynamics may be affected by presence of a more complex food web (e.g. Coûteaux et al., 1991 and De Ruiter et al., 1994). In addition, N immobilization by microbes may be reduced due to competition for N between plants and microbes. Van Veen et al. (1989) observed that decomposition of high C/N compounds was slower in the presence of plants. Kaye and Hart (1997), however, suggested that microbes are stronger competitors for inorganic N than plants. Occurrence of competition may vary with depth in the soil depending on whether microbes use inorganic or organic N as main N source. Although direct extrapolation of small-scale, short-term, experiments both in space and time remains difficult, the integration of simulation modeling with ^{15}N enrichment studies revealed some basic responses of microbial C and N transformations to a changing environment.

Chapter 6

CARBON ALLOCATION AND DECOMPOSITION OF ROOT-DERIVED ORGANIC MATTER IN A PLANT-SOIL SYSTEM OF *CALLUNA VULGARIS* AS AFFECTED BY ELEVATED CO_2

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Soil Biology and Biochemistry (in press)

CARBON ALLOCATION AND DECOMPOSITION OF ROOT-DERIVED ORGANIC MATTER IN A PLANT-SOIL SYSTEM OF *CALLUNA VULGARIS* AS AFFECTED BY ELEVATED CO_2

ABSTRACT

The effect of elevated CO_2 on C allocation in plant and soil was assessed using soil columns planted with 1-year-old heather (*Calluna vulgaris* (L.) Hull). Plants were pulse-labeled with $^{14}CO_2$ at ambient and elevated CO_2 and two nutrient levels. After harvesting the plants, the soil was incubated to monitor total respiration and decomposition of ^{14}C-labeled rhizodeposits. Total and shoot biomass increased at high N but were not affected by CO_2. Root biomass was not affected by either N or CO_2 treatments. Total ^{14}C uptake and shoot-^{14}C increased upon adding N and elevating CO_2 but the N effect was strongest. Total ^{14}C uptake per unit shoot mass decreased with N, but increased with CO_2. Root-^{14}C content was not significantly affected by the N or CO_2 treatment. Total soil-^{14}C slightly increased at elevated CO_2 whereas microbial ^{14}C increased due to high N. C allocation to shoots increased at the expense of roots, soil and respiration at high N but was not affected by the CO_2 treatment. Variation in ^{14}C-distribution within each treatment was small compared to variation in total ^{14}C amounts in each plant/soil compartment. Initially, ^{14}C respiration from rhizodeposits correlated well with root-^{14}C, total soil-^{14}C, soil solution-^{14}C and microbial ^{14}C, at harvest time and was increased by elevated CO_2. At the end of the incubation, decomposition of labeled organic matter was not affected by the treatments whereas total ($=^{12}C+^{14}C$) respiration was lowest for the elevated-CO_2 soils. We speculate that initially, respiration is dominated by decomposition of fresh root exudates whereas on the longer term, respiration originates from decomposition of more recalcitrant root material formed during the entire experiment. The increased net ^{14}C uptake and unchanged distribution pattern, combined with an increased decomposition of easily decomposable compounds and a decreased decomposition of more recalcitrant root-derived material indicated a small sink function of a *Calluna* plant-soil system under elevated CO_2.

INTRODUCTION

Increased combustion of fossil fuels and changes in land use have caused an indisposed increase in atmospheric CO_2 concentration during the last decades. Current business-as-usual scenario's predict concentrations of approximately 600 ppm for the middle of the next century (Houghton et al., 1995). Increased biomass production is considered to be a potential, although temporary, sink for C (Schimel, 1995). Indeed, many experiments show that total plant biomass production, especially of C_3 plants increases under elevated CO_2 (Poorter et al., 1996). However, the response to elevated CO_2 may be lower under water- or nutrient limited conditions (Oechel et al., 1994; Arp et al., 1997). Several studies show that at elevated CO_2, production of root biomass is more stimulated than that of shoot biomass (Norby et al., 1986a; Körner and Arnone, 1992; Newton et al., 1994; Van Ginkel et al., 1996). However, in a literature review, Norby (1994) showed that a wide range in response of shoot/root ratios to elevated CO_2 may occur depending on species, nutrient and water conditions. Van Ginkel et al. (1997) found an absolute increase in root C as well as soil C at elevated CO_2 in a ^{14}C labeling experiment with *Lolium perenne*. Increased root production could enhance rhizodeposition which, in turn, could affect rhizosphere processes (Rogers et al., 1994). Consequently, increased belowground C allocation could cause soils to act as a sink for C at elevated CO_2 in as far as soil C is limited by inputs rather than by its C-protection capacity (Hassink, 1996). In this paper, 'rhizodeposits' include (1) root exudates, (2) secretions upon metabolic processes and (3) lysates including cell walls, sloughed cells and decaying roots (Whipps, 1990; Swinnen, 1994).

Not only the amount of C deposited in the soil by roots, but also the output through decomposition may be affected by CO_2 due to changes in substrate quality and/or microbial activity. Very few studies dealt with decomposition rates of roots. Cotrufo and Ineson (1995), Gorissen et al. (1995b) and Van Ginkel et al. (1996) found evidence for a decreased decomposition for roots grown at elevated CO_2. Several studies show the importance of decomposition processes in the rhizosphere for soil C dynamics (e.g. Cheng et al., 1993) but decomposition of rhizodeposits as such under elevated CO_2 has not been measured yet. These deposits likely to be easily decomposable, and will probably be preferentially used by soil microorganisms (Lekkerkerk et al., 1990; Cheng et al., 1996), provided that sufficient N is available (Van Veen et al., 1993). At low N supply, microorganisms would have to utilize soil organic matter as main substrate.

The objective of this experiment was to quantify the effects of both CO_2 and N level on C allocation in plant and soil as well as decomposition of fresh rhizodeposits

using ^{14}C as a tracer. Most labeling experiments dealt with fast growing agricultural plant species. To our knowledge very few labeling experiments have been carried out with slow growing, natural species. In our experiment we used heather (*Calluna vulgaris*) being a common species growing under nutrient poor conditions. One-year-old *Calluna* plants were grown at two CO_2 and two nutrient levels for 2 months and subsequently pulse-labeled with $^{14}CO_2$ for 1 day. Three weeks after labeling, ^{14}C allocation was measured in the plants and soil. The soil was subsequently incubated to follow the decomposition of rhizodeposits.

MATERIALS AND METHODS

Plant and soil

One-year-old heather plants (*Calluna vulgaris* (L.) Hull) of 5 cm height, were collected in May 1996 near Risdalsheia, southern Norway at the location of the CLIMEX project (Jenkins and Wright, 1993). Twenty four seedlings were transplanted into PVC columns (diameter 100 mm; height 190 mm) containing 2 kg of moistened soil. The soil consisted of a mixture of 2/3 pure sand and 1/3 topsoil of a loamy sand. The mixture contained 0.46% organic C, 0.06% total N, 15 mg kg^{-1} mineral N, and had a pH-KCl of 4.6. The particle size distribution was 1% < 2 µm, 4% 2-50 µm and 95% > 50 µm. Twelve columns received 6 mg P (as $Ca(H_2PO_4)_2$), 30 mg of K (as K_2SO_4) but no N. These columns were considered as a low-N treatment. Twelve remaining columns for the high-N treatment received 120 mg N (as urea), 30 mg P, and 150 mg K. All nutrients were added as slow release fertilizer (Grace/sierra, The Netherlands). Gravimetric moisture content was kept at 15% (w/w) throughout the experiment with tap water by weighing the columns twice a week. At the end of May 1996, the high-N and low-N columns were put in greenhouses either at 380 or 580 ppm CO_2. Average relative humidity was 72% and the average daily temperature in both greenhouses varied between 19 to 22°C with an amplitude of 5°C. The light regime followed outside ambient conditions.

Pulse-labeling

In early August 1996, 11 weeks after the start of the N and CO_2 treatments, all columns were covered with a PVC lid fitted with a PVC tube (diameter 2 cm, length 10 cm) containing two layers of sodalime separated by a cotton plug allowing for free exchange of oxygen and for trapping root and microbial respiration (referred to as 'soil respiration').

Approximately 8 g of sodalime was used in the lower layer to capture soil respiration. The upper layer, containing 6 g sodalime, prevented entrance of CO_2 from the surrounding air. A second hole, sealable with a rubber plug, allowed for watering the plants. The columns were air-tight sealed with silicone rubber (Q3-3481; Dow Chemical) at the base of the plants to separate the shoot from the root and soil compartment. After sealing, the plants were put in two separate Experimental Soil Plant Atmosphere System (ESPAS) chambers at either 380 or 580 ppm CO_2. In the ESPAS systems, temperature, light, humidity and CO_2 concentration were controlled as described by Gorissen et al. (1996). Relative humidity and Photosynthetically Active Radiation were kept at 75% and 300 μmol m^{-2} s^{-1} respectively. The day/night rhythm was 16/8 h. Prior to the experiment, environmental parameters were checked with independent equipment to assure that conditions were identical in both chambers. Day/night temperature varied according to a sinus function with an average of 20°C and an amplitude of 5°C. After 3 days acclimation, plants were pulse-labeled by exposing them to $^{14}CO_2$ during one whole photoperiod to avoid physiological artifacts that may occur when shorter labeling periods are used. Both CO_2 and $^{14}CO_2$ were supplied from gas cylinders (100% CO_2) and the inflows were controlled automatically. Specific activities of the CO_2 entering the chambers were 11.38 and 11.97 kBq mg C^{-1} for the 380 and 580 ppm CO_2 chamber, respectively. In calculating the total ^{14}C uptake by the different plant-soil compartments, the elevated-CO_2 values have been corrected for the higher specific activity of the CO_2 by multiplying ^{14}C contents by 0.95. During the 3 weeks after labeling, the plants were continuously exposed to 380 or 580 ppm CO_2.

Decomposition of rhizodeposits

Three weeks after the pulse-labeling, the plants were harvested. The soil material was separated from the roots by gently shaking. Any remaining soil was removed by washing roots over a sieve (250 μm mesh). We subsequently incubated the fresh, ^{14}C-labeled, soil to determine decomposition of labeled and total soil C. We incubated an equivalent of 50 g of dry soil in 250 ml sample jars and added water to a moisture content of 15% (w/w). Evolving CO_2 was captured in 0.1 M KOH present in a vial in the headspace of the sample containers. The CO_2 production was calculated after measuring the change in electrical conductivity of the KOH solution upon absorption of CO_2 (Nordgren, 1988). The soils were incubated in a closed chamber for 45 days at 20°C. Total ^{14}C content of the KOH solution was measured by liquid scintillation counting after 2, 5, 8, 19, 29, 37, and 46 days. KOH removed for ^{14}C analyses (0.5 ml) was replaced by the same amount of

fresh KOH. Empty containers with KOH were used as blanks. In all vials, KOH was replaced when the amount of CO_2 adsorbed exceeded 50% of the calculated maximum CO_2 adsorption capacity to avoid diffusion limited CO_2 adsorption.

Analyses

Shoots, roots and soil were dried at 70°C for 48 h and analyzed for dry weight, total C, and ^{14}C. Subsamples of plant and soil material were destructed using a modified wet combustion method (Dalal, 1979). Dried, ground plant material (30 mg) and soil (1 g) were digested in 5 ml of a 10% (w/v) solution of $K_2Cr_2O_7$ in a mixture of concentrated H_2SO_4 and H_3PO_4 (3:2 v/v) at 160 °C for 2 h. The evolving CO_2 was trapped in 10 ml of 0.5 M NaOH. Total CO_2 was determined after 18 h by titration of 2 ml NaOH with 0.1 M HCl after precipitating dissolved carbonate by excess $BaCl_2$. ^{14}C was determined in 0.5 ml of the NaOH solution mixed with 3 ml Ultima Gold (Packard) by liquid scintillation counting (Tri Carb 2100 TR; Packard) with a counting efficiency of 91%. The sodalime from the respiration traps was transferred into bottles and dissolved by injecting excess 6 M HCl through a septum in the lid. The evolving CO_2 was trapped in 10 ml 5 M NaOH. Total C and ^{14}C content were determined after 18 h by titration and liquid scintillation counting, respectively. The ^{14}C content of the soil microbial biomass was determined using the fumigation-centrifugation method (Van Ginkel et al., 1994). The difference in ^{14}C in the soil solution before and after fumigation is referred to as Soil Microbial Flush (SMF). The 'soil solution-^{14}C' refers to the amount of soluble ^{14}C in the soil solution prior to fumigation.

Statistics

During the experiment, one out of six plants in both high-N treatments died. The results on the remaining twenty two plant-soil columns were analyzed by 2-way ANOVA (Genstat 5; release 3.1) with CO_2 treatment and N level as main factors. In the incubation experiment, ANOVA was carried out on the respiration rates at each measurement date with CO_2 and N as main factors. Interactions between CO_2 and N were calculated in all ANOVA's. Correlation between selected parameters was calculated by linear regression.

RESULTS

Carbon allocation

Plant dry weight did not increase at elevated CO_2 (Table 6.1). However, at high N, total plant dry weight increased by more than 100% which was mainly caused by increased shoot growth. Shoot/root ratios remained unaffected by CO_2 but increased at high N by 217% at ambient CO_2 and by 157% at elevated CO_2. At elevated CO_2, soil respiration increased by 50% at low N and by 22% at high N. No significant interactions between CO_2 and N were observed for any of the measured parameters.

Total net ^{14}C uptake (defined as the sum of the ^{14}C recovered in shoot, root, soil and soil respiration) increased by 43% with increasing CO_2 and by more than 100% at high N (Table 6.2). Total ^{14}C uptake per gram shoot mass increased with CO_2 by 30% and decreased with N by 25%. Shoot-^{14}C increased by 50% at elevated CO_2 and by almost 200% at high N. Mean ^{14}C contents of roots increased at elevated CO_2 and high N, but not significantly. Soil-^{14}C increased due to elevated CO_2 at both N levels. Soil Microbial Flush-^{14}C (SMF-^{14}C) was about 100% higher at high N and increased non-significantly with CO_2. Soil ^{14}C respiration increased both with CO_2 (39% at low N, 25% at high N) and N (42% at ambient CO_2, 27% at elevated CO_2). Respiration of ^{14}C per gram root biomass was not affected by CO_2 but increased at high N.

Table 6.1. Plant biomass (g) and total soil respiration (mg C) of Calluna vulgaris at harvest (3 weeks after pulse-labeling).

	L-N[1]	L+N	H-N	H+N	CO_2	N	CO_2*N
Total	2.36	4.98	2.59	5.55	ns[2]	***	ns
Shoot	1.50	4.22	1.67	4.47	ns	***	ns
Root	0.87	0.77	0.92	1.08	ns	ns	ns
Shoot/Root	1.97	6.24	2.14	5.51	ns	***	ns
Respiration[3]	316	474	352	431	ns	***	ns

[1] L-N = ambient CO_2, low N; L+N = ambient CO_2, high N; H-N = elevated CO_2, low N; H+N = elevated CO_2, high N.

[2] ***$p<0.001$; ns = not significant ($p>0.1$).

[3] Respiration represents root and microbial respiration during the three weeks after pulse-labeling.

Table 6.2. Total net ^{14}C uptake (kBq), shoot/root ratio, uptake per unit shoot mass (kBq g^{-1}), soil respiration per unit root mass (kBq g^{-1}) and distribution (%) of ^{14}C among the plant and soil compartments.

	L-N[1]	L+N	H-N	H+N	CO_2	N	CO_2*N
Total net uptake[2]	428	897	622	1273	*[3]	***	ns
Shoot	200	598	308	882	*	***	ns
Root	73	94	110	134	ns	ns	ns
Soil respiration	106	150	147	187	**	**	ns
Soil	48	55	56	69	**	*	ns
Soil solution	0.2	0.9	0.3	0.6	ns	***	ns
SMF[4]	1.5	3.3	2.2	4.0	ns	***	ns
Shoot/root ratio	2.93	7.44	3.13	8.27	ns	***	ns
Uptake/shoot[5]	289	212	370	277	***	***	ns
Resp./root[6]	135	217	188	251	ns	*	ns
%-distribution							
Shoot	46.1	66.6	49.3	68.0	ns	***	ns
Root	17.3	10.2	17.3	9.3	ns	***	ns
Soil respiration	25.2	16.8	24.2	16.2	ns	***	ns
Soil	11.4	6.4	9.2	6.6	ns	***	ns
Soil solution	0.05	0.09	0.05	0.06	ns	*	ns
SMF[4]	0.4	0.4	0.4	0.4	ns	ns	ns

[1] L-N = ambient CO_2, low N; L+N = ambient CO_2, high N; H-N = elevated CO_2, low N; H+N = elevated CO_2, high N.

[2] Total net uptake = shoot-^{14}C + root-^{14}C + soil-^{14}C + soil respiration-^{14}C

[3] * $p<0.1$; ** $p<0.05$; ***$p<0.001$; ns = not significant ($p>0.1$).

[4] SMF = Soil microbial flush

[5] Total ^{14}C uptake per gram dry shoot

[6] Total ^{14}C respiration per gram dry root

The distribution of ^{14}C over the different plant-soil compartments, calculated as the percentage ^{14}C present in each compartment of total net ^{14}C uptake, was only affected by N but not by CO_2 (Table 6.2). At high N, the percentage ^{14}C retained in the shoots

increased by approximately 40% at both CO_2 levels at the expense of ^{14}C in roots, soil and soil respiration.

Decomposition of rhizodeposits

Respiration rates from ^{14}C rhizodeposits from the incubated soil were initially higher both at elevated CO_2 and high N (Fig. 6.1). After 3 weeks, ^{14}C respiration from the elevated-CO_2 soil remained higher while the effect of N had disappeared. After approximately 5 weeks, neither CO_2 nor N effects were present. During the first 2 days, total ($=^{12}C+^{14}C$) respiration was highest at high N without effects of CO_2 (Fig. 6.2). From day 2 until day 14, no significant CO_2 or N effects were found. After day 14, respiration rates were significantly higher for the ambient-CO_2 soil irrespective of the N treatment.

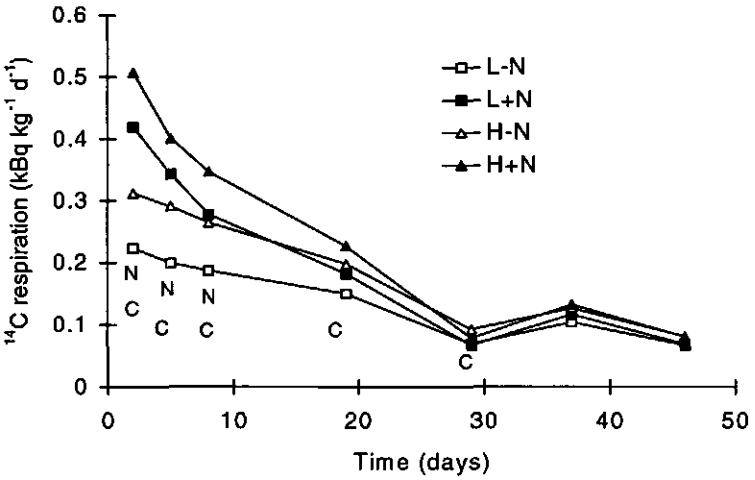

Figure 6.1. ^{14}C soil respiration in kBq kg soil^{-1} d^{-1}. "N" and "C" indicate significant N or CO_2 effects at $p<0.05$ after carrying out ANOVA at each measuring date. L-N = ambient CO_2, low N; L+N = ambient CO_2, high N; H-N = elevated CO_2, low N; H+N = elevated CO_2, high N. The low rate at day 29 was caused by improper calibration of the pipette used to take ^{14}C samples. The error was systematic and did not affect conclusions concerning treatment effects.

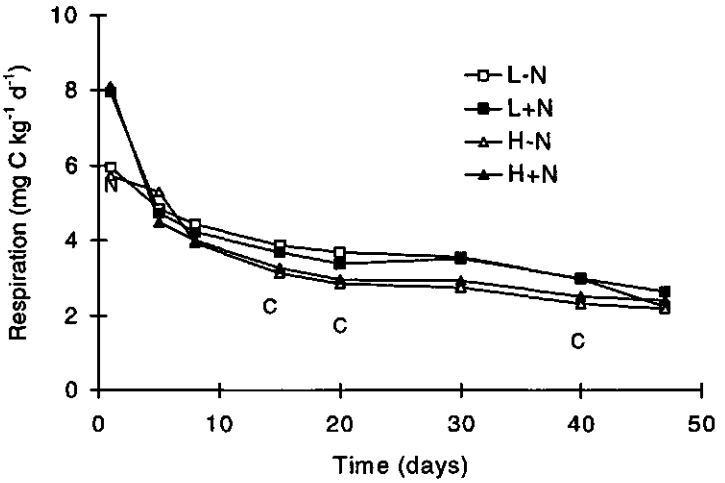

Figure 6.2. Total soil respiration in mg C kg soil^{-1} d^{-1}. "N" and "C" indicate significant N or CO_2 effects at p<0.05 after carrying out ANOVA at each measuring date. L-N = ambient CO_2, low N; L+N = ambient CO_2, high N; H-N = elevated CO_2, low N; H+N = elevated CO_2, high N.

DISCUSSION

In contrast to many studies dealing with other plant species (e.g. Norby et al., 1986a; Newton et al., 1994; Rogers et al., 1994; Schenk et al., 1995; Van Ginkel et al., 1996), we found no significant effect of elevated CO_2 on total biomass or dry weight shoot/root ratios at both nutrient levels. We found no evidence for a growth stimulation by elevated CO_2 at low N due to increased nitrogen use efficiency as suggested by Goudriaan and De Ruiter (1983). We also found no support for the suggestion by Bazzaz (1990) that elevated CO_2 may cause a strong decrease in shoot/root ratio at low nutrient levels. At the time of labeling though, more C was fixed at elevated CO_2, which was preferentially allocated to shoots. The amount of ^{14}C uptake per unit shoot mass increased at elevated CO_2 which agrees well with a general observation that photosynthetic rates of C_3 plants increase at elevated CO_2 (Farquhar and Von Caemmerer, 1982). In a greenhouse experiment with *Calluna* under the same CO_2 and N conditions, elevated CO_2 caused the percentage leaves of the total shoot mass to decrease from 71% to 64% at high N and from 73% to 67% at low N (Arp, unpubl.data). Consequently, the effect of elevated CO_2

on C uptake is even more apparent when total ^{14}C uptake is expressed per amount of photosynthesizing leaf instead of total shoot mass.

The ^{14}C shoot/root ratio was higher than the dry weight shoot/root ratio in all treatments. First, the dry weight numbers represent an integrated response over a longer period whereas the ^{14}C numbers are representative only for the time of labeling. Keith et al. (1986), Gorissen et al. (1995a), and Swinnen et al. (1995) showed that for several species the largest ^{14}C allocation to roots after pulse-labeling took place in the earlier growing stages. However, in a pulse-labeling study with sweet chestnut trees, Rouhier et al. (1994) observed that towards the end of the growing season of the first year of treatment, more ^{14}C was allocated to roots irrespective of the CO_2 treatment. At the end of the second growing season, at ambient CO_2, relatively more ^{14}C was allocated to roots whereas at elevated CO_2, more ^{14}C was allocated to shoots. In both years, both at ambient and elevated CO_2, the amount of root derived C in the soil increased at the end of the growing season. Second, pulse-labeling only affects labile C pools. Swinnen et al. (1995) showed that with increasing time little re-allocation from shoot to root occurs suggesting that the difference between dry weight and ^{14}C shoot/root ratio is not an artifact of the method.

Elevated CO_2 did not cause a change in C distribution in *Calluna* which does not support the hypothesis by Gorissen (1996) who suggested that a shift in C distribution may be restricted to perennial species. At low N availability, relatively more C is invested in roots than at high N availability confirming a general observation that plants invest more in their root systems when nutrient availability is limited (Brouwer, 1962). Within each treatment, the variation in ^{14}C allocation was much smaller than in the total amount of ^{14}C in each soil-plant compartment suggesting that the treatment effects we found are independent of plant size and result from a general mechanism.

Although root-^{14}C and SMF-^{14}C were not affected, soil ^{14}C respiration increased at elevated CO_2 indicating a higher activity of roots, soil microbial biomass or both. Still, ^{14}C respiration per gram root mass was not affected by CO_2 even though root biomass did not significantly increase at elevated CO_2. However, variation in root mass was large which may have caused the increase in respiration per gram root mass to be non-significant. Our results are consistent with results found by Billès et al. (1993) using 4-weeks-old wheat plants. By contrast, Van Ginkel et al. (1997) found an increase of 50% in ^{14}C-respiration per unit root biomass due to elevated CO_2 but no change due to high N with *Lolium perenne*.

Both high N and elevated CO_2 caused increased C deposition in the soil even though root mass did not significantly increase. In our study, the amount of C deposited

did not depend on root biomass alone as was suggested by Rogers et al. (1994) but on total plant biomass. Initially, decomposition of the labeled rhizodeposits was higher both at high N and elevated CO_2. Van Veen et al. (1993) and Van de Geijn and Van Veen (1993) suggested that decomposition of rhizodeposits is stimulated by N. However, even in our low-N treatments, ^{14}C rhizodeposits were used as substrate. With increasing time, both effects of CO_2 and N on ^{14}C respiration disappeared. Upon decomposition of labeled rhizodeposits, ^{14}C will be incorporated in the microbial biomass. Consequently, with time, ^{14}C respiration will reflect turnover of microbial ^{14}C in addition to decomposition of rhizodeposits. Respiration rate of ^{14}C in the first 2 days after incubation correlated well with the amount of ^{14}C in roots, soil, SMF, and soil solution measured directly after the harvest irrespective of the treatments (Table 6.3). The size of the SMF positively correlated with the amount of ^{14}C in the roots and with dissolved ^{14}C. The initial ^{14}C respiration rates were high enough to consume all ^{14}C in the soil solution in only a few days. Therefore, not the soluble ^{14}C alone but a labile fraction associated with this soluble fraction was used as substrate. Van Ginkel and Gorissen (1998) showed that soluble organic C was strongly correlated with both root weight and soil microbial biomass during growth of *Lolium perenne*. We only found a correlation between soil solution ^{14}C and ^{14}C-SMF which, together with the lack of correlation between the ^{14}C in roots and soil solution ($p=0.62$), suggests that in our experiment the amount of soluble material was the result of microbial activity itself. The CO_2 treatments did not affect microbial respiration rates expressed per amount of soil-^{14}C (data not shown) suggesting that substrate quality of the labeled rhizodeposits was not affected by the CO_2 treatments.

Table 6.3. Correlations between ^{14}C in various soil compartments, initial ^{14}C respiration and soil microbial flush (SMF).

	Root	Resp.[1]	Soil	Solution	SMF
Root	nd[2]	**	ns	ns	**
Resp.[1]		nd	***	***	***
Soil			nd	nd[3]	nd[3]
Solution				nd	**
SMF					nd

[1] Resp. = ^{14}C respiration rate during first 2 days after incubation of rhizodeposits.

[2] * $p<0.1$; ** $p<0.05$; *** $p<0.001$; ns = not significant ($p>0.1$); nd = not determined

[3] No correlations were calculated since 'Soil' includes soil solution and SMF.

From the specific activity of the CO_2, we calculated that the amount of ^{14}C in the soil varied between 2.1 (ambient CO_2, low N) and 3.0 (elevated CO_2, high N) mg C kg^{-1} soil. Before the start of the experiment, the total biomass of the seedlings was about 0.4 g indicating that 84-93% of the total biomass was formed during the 105 days of the experiment. At unchanged allocation, the amount of ^{14}C-labeled organic matter, originating from the 1-day ^{14}C-pulse, would be less than 1% of the total amount of rhizodeposits formed during the 105 days of the experiment. However, this percentage may be higher since the plants were larger at the time of labeling than at the start of the experiment. Still, decomposition of this small fraction may not be representative for that of all root derived organic matter formed throughout the experiment. We could not discriminate between decomposition of unlabeled organic matter formed during the experiment and native soil organic matter (SOM) so we cannot quantify possible effects of CO_2 and N on native SOM decomposition. Still, our results are consistent with results reported by Lekkerkerk et al. (1990) who found an increased $^{14}CO_2$ respiration in a soil planted with wheat, and a decreased decomposition of native SOM. Although these authors could not distinguish between ^{14}C respiration of roots and soil microorganisms, they hypothesized that microorganisms preferred easily decomposable root-derived material over native SOM which agrees with results from Cheng et al. (1996). Our results do not indicate that elevated CO_2 causes SOM content to decrease as suggested by Körner and Arnone (1992), because total respiration after 2 weeks was lowest at elevated CO_2 whereas inputs of rhizodeposits were highest. It appears that differences in total respiration rate between ambient-CO_2 and elevated-CO_2 rhizodeposits could be caused by substrate differences. Cotrufo and Ineson (1995), Gorissen et al. (1995b) and Van Ginkel et al. (1996) reported that roots produced under elevated CO_2 decomposed slower than those produced at ambient CO_2, in agreement with our longer term respiration measurements. We, therefore, speculate that the initial respiration is dominated by decomposition of labeled easily decomposable compounds such as fresh root exudates, whereas decomposition of more recalcitrant, unlabeled, root material formed during the entire experiment dominates respiration on the longer term. Both in our experiment as well as in root incubation experiments, supply of fresh rhizodeposits was stopped as soon as the samples were incubated. Consequently, in incubation studies without plants, the respiration flux on the longer term tends to represent the decomposition of the more stable organic matter pools. Even though labile material may be decomposed in a few days and comprises a small fraction of the total amount of soil organic matter, under continuous deposition more than 50% of the respired CO_2 may originate from this material (Fig. 6.2).

Our pulse-labeling experiment shows that C storage in a soil with *Calluna vulgaris*, a native, slow growing, woody species, was stimulated by elevated CO_2. The ^{14}C data suggested that increased C storage in the soil was primarily caused by an increase in net photosynthesis and not by increased relative allocation to below-ground compartments, as was observed for grasses by Newton et al. (1994, 1995) and Van Ginkel et al. (1996). We found, however, no significant effects of elevated CO_2 on plant dry weights but the trends in biomass pointed into the same direction as the ^{14}C data. High N availability had relatively little effect on C storage in *Calluna* soils, because most extra-formed C was allocated above-ground. None of the measured parameters showed interactions between CO_2 and N although the plants' response to CO_2 may be stronger at high nutrient levels (Goudriaan and De Ruiter, 1983). Often, average values of plant dry weight and ^{14}C content suggested presence of interactions but variation caused these interactions to be insignificant. Decomposition of soluble C appeared to be related to the amount and activity of microbial biomass present. Fresh substrate seemed to be readily used by the soil microbial biomass. The decomposability of this labile material was not affected by CO_2 whereas the more stable material formed at elevated CO_2 appeared to decompose slowest whereas inputs were highest. Consequently, not the inputs of readily decomposable rhizodeposits but continuous inputs of more recalcitrant structural root material at elevated CO_2 may cause the soil to become a sink for C. Microbial activity may be stimulated depending on the size of the root system which is consistent with the hypothesis of Zak et al. (1993). However, in our experiment this did not appear to increase decomposition of native SOM as was suggested by Van de Geijn and Van Veen (1993). The results from the pulse-labeling experiment suggested that the total CO_2 uptake in *Calluna* is stimulated under elevated CO_2. Although the C allocation appeared to be unchanged, the increased absolute amounts will possibly cause the soil to become a sink for C, when other factors such as temperature and moisture remain constant.

Acknowledgments--We thank J.H. van Ginkel for helpful discussion.

Chapter 7

DISCUSSION AND CONCLUSIONS

DISCUSSION AND CONCLUSIONS

The objective of this thesis was to quantify the effects of climate change on soil organic matter decomposition in a boreal forest soil. This was done by employing a combination of field and laboratory studies. The field experiments were carried out in two experimentally manipulated catchments. In one catchment, both air temperature and CO_2 concentration were increased whereas in a second catchment only soil temperature was increased. The research addressed (i) the impacts of climate change on litter decomposition and N mineralization under field conditions (Chapter 2 and 3), (ii) the impacts of elevated temperature on gross and net C and N fluxes in micro- and mesocosms (Chapter 4 and 5) and (iii) effects of elevated CO_2 and nutrient conditions on C allocation in a soil-plant system and decomposition of below-ground organic matter (Chapter 6). In this last chapter, the main conclusions are summarized and evaluated in the light of model predictions about the net response of a boreal forest to climate change.

ELEVATED TEMPERATURE

The micro- and mesocosm studies presented in Chapter 4 and 5 showed that C mineralization increased with increasing temperature. The results from Chapter 4, however, suggested that, with increasing soil depth, the relative effect of temperature on decomposition decreased, most likely due to increased substrate limitations at greater depth. If temperature sensitivity depends on substrate quality, the effect of elevated temperature on decomposition rates of 'old' organic matter may be smaller than predicted by most models, because they use a similar temperature response for decomposition rates of both labile and stable C pools. In the field, elevated soil temperatures did not increase decomposition of plant litter, a relatively labile pool of soil C (Chapter 2 and 3). Especially upon soil-heating, increased evaporation may have caused desiccation of the litter which hampered (micro)biological activity. Indirect temperature effects on decomposition through reduced soil moisture will reduce decomposition in those soils where moisture levels are already sub-optimal. In permanently wet soils, where decomposition is depressed by anoxic conditions, increased evaporation may improve aeration which will stimulate decomposition. This effect was shown in arctic tundra systems (Oechel et al., 1993).

Both field and laboratory experiments suggested that N availability increased at elevated temperatures. In the air- and soil-heated parts of the CLIMEX catchments, net N

mineralization increased (Chapter 2 and 3). With increasing temperature, both C mineralization and NO_3 leaching from isolated, devegetated, soil columns increased (Chapter 4). In the soil columns, nitrification may have been stimulated due to higher NH_4 availability compared to field conditions, where part of the mineralized N will be taken up by plants. Indeed, in the air-heated KIM catchment, increased soil N availability caused a transfer of N from the soil to the vegetation (Van Breemen et al., 1998). Biomass of especially *Calluna* increased while C/N ratio remained constant (Arp and Berendse, 1997). The N content in *Pinus* needles increased during the treatment period. Although tree biomass did not appear to have increased as a result of the treatment, more N may be present in the overstory vegetation (Beier and Rasmussen, 1997). In both treated catchments, the increase in mineralization exceeded plant demand since effluxes of inorganic N in runoff increased compared to pre-treatment levels (Wright, 1998). These data suggest that the ecosystem may, at least, initially loose N. Whether increased N leaching will be sustained is not clear. The initial observed soil response may be due to mineralization of labile N. If this pool of labile N becomes depleted, then soil response is expected decrease (Nadelhoffer et al., 1991).

Both field (Chapter 2 and 3) and soil column (Chapter 4) experiments showed that soil N availability increased at higher temperature. The temperature response on gross N fluxes differed between soil layers (Chapter 5). In the litter and fermentation layer, at 15°C both gross mineralization and immobilization increased compared to 5°C whereas net mineralization remained unchanged. Initially microbes, immobilize N resulting in either an increase in microbial biomass and/or a decrease in microbial C/N ratio. However, on the longer term, net mineralization is likely to increase at elevated temperature. Eventually, microbial growth will be limited either by C availability or predation by other soil animals. Indeed, the field and column measurements showed increased net mineralization at higher temperatures. In the humic layer, neither gross mineralization nor immobilization increased although measured microbial activity was higher. I could only explain this apparent contradiction if microbes in the humic layer are able to use organic N as substrate. In the studied forest ecosystem, plant growth appears to be limited by inorganic N availability so plants may compete for NH_4 or NO_3 with microorganisms (Kaye and Hart, 1997). Some plant species present in boreal/tundra ecosystems use organic N as their primary N source which is either taken up directly or through mycorrhizal fungi (e.g. Chapin et al., 1993 and Michelsen et al., 1996). Consequently, not only competition for inorganic N but also for organic N may occur, depending on N source used by plants and microorganisms in each soil layer.

ELEVATED CO_2

Elevated CO_2 may affect soil organic matter dynamics by 1) increasing litter inputs into the soil through increased Net Primary Production (NPP; Poorter, 1993), and by 2) reducing litter decomposition due to production of more recalcitrant litter (Cotrufo et al., 1994). Direct effects of elevated CO_2 on decomposition were not studied in this thesis, these effects are found to be negligible in the range of 350-700 ppm (Ball and Drake, 1997; Koizumi et al., 1991). In a pot experiment, biomass of *Calluna* did not significantly increase when plants were exposed to elevated CO_2 (Chapter 6). Plant biomass only increased when nutrient availability was higher. In the field, *Calluna* biomass and the number of leaves on *Vaccinium* branches increased during the second treatment year (Arp and Berendse, 1997). Whether increased biomass production is due to CO_2 fertilization only remains difficult to assess. In the soil-heated catchment (EGIL), biomass did not increase but mineralization did which may indicate that indeed elevated CO_2 caused biomass to increase in the catchment where both air temperature and CO_2 were manipulated (KIM). However, prior to the start of the treatment, N availability was likely to be higher in EGIL since in the KIM catchment during the last 12 years NH_4 and NO_3 were removed from the precipitation (Wright et al., 1993). Consequently, increased biomass production in KIM was most likely at least partly due to a higher N availability caused by increased mineralization during the CLIMEX treatment.

The results presented in Chapter 2 and 3 of this thesis do not provide conclusive evidence that elevated CO_2 will alter litter chemistry and subsequent decomposition rates of this litter in the studied ecosystem. Effects of elevated CO_2 on litter chemistry varied among species and duration of exposure. For *Betula*, C/N ratio only increased after 1 year of exposure to elevated CO_2 whereas lignin content remained unaffected. After 2 years of exposure C/N ratio was not affected whereas lignin content decreased. For *Calluna*, C/N ratios of both leaves and flowers decreased after 2 years exposure to elevated CO_2 whereas lignin content of leaves increased. Changes in litter chemistry did not always affect decomposition rates. After 1 year of incubation in the field, mass loss of the *Betula* litter produced after 2 years of exposure to elevated CO_2 tended to higher than that of the control litter. An increasing number of studies (e.g. Franck et al., 1997; Raiesi, in press.) including the one presented in this thesis, show that effects of CO_2 on litter chemistry and subsequent decomposition are still not well understood.

Many studies suggested that at elevated CO_2 relatively more C would be allocated to roots and soil which could cause the soils to become a significant sink for C (e.g. Zak et al., 1993; Newton et al., 1994). In a pot experiment where *Calluna* plants were pulse-

labeled with ^{14}C, C allocation did not change at elevated CO_2 compared to ambient conditions (Chapter 6), except at high nutrient availability, where relatively more C was allocated to shoots than at low nutrient levels. Consequently, when both CO_2 concentration and N mineralization increase, shoot/root ratio is more likely to increase rather than to decrease. The amount of root-derived C deposited in the soil appeared to depend only on total plant biomass rather than on changes in allocation induced by elevated CO_2. Decomposition of the most labile root-derived organic matter was more rapid at high-CO_2 and contributed for almost 50% to the total microbial respiration. These labile deposits were about 1-2% of the total amount of root derived organic matter produced during the experiment. When this labile material was decomposed, decomposition of low-CO_2 root-derived organic matter was higher. Although, on the short term (days), decomposition of labile C appears to be stimulated by elevated CO_2, on the longer term (weeks), more C may be fixed in the soil. Still, most of the root-derived organic matter appears to be very labile and decomposes rapidly which supports the conclusions from Hungate et al. (1997) that the potential of the soil to act as a sink for C in a high CO_2 world may be limited.

METHODOLOGICAL ASPECTS

Field experiments

Large-scale ecosystem manipulation experiments provide the most direct way to improve predictions of the response of ecosystems to environmental changes (Carpenter et al., 1995). Large-scale ecosystem manipulations, however, have restrictions. First, enclosing a forest by a greenhouse caused alterations in other environmental conditions besides CO_2 and temperature; notably photosynthetically active radiation and windspeed reduces which will strongly affect ecosystem behavior. Second, both CO_2 and temperature were increased rapidly whereas in real life temperature and CO_2 increase more gradually. Feedback or adaptation mechanisms operating on longer time-scales than the measurement period may not be accounted for in the interpretation of the results from the manipulation. In addition, the response of the ecosystem to the treatment may be more pronounced than under gradual increases in temperature and CO_2. Third, in many cases, available finances limit the number of replicates, presence of proper controls and duration of the experiment (Carpenter et al., 1995). In the experimental setup of the CLIMEX project, only a combined CO_2-temperature and a soil-temperature manipulation were employed. It was therefore not possible to completely separate CO_2 from temperature

effects. Still, the combined CO_2 and temperature manipulation was most realistic for assessing response of ecosystems to future climatic conditions as predicted by General Circulation Models. The measurements on vegetation, soil and water showed that interactions between CO_2 and temperature effects largely determine total catchment response. These interactions would not have been detected when carrying out either a CO_2 or temperature manipulation only.

The soil-heating technique proved to be a useful tool for assessing effects of elevated soil temperature on soil processes on a catchment scale. However, one has to be aware of the limitations of this technique when studying processes on a subplot scale. Cable installation may cause disturbance of the soil which can affect measurements for up to a year after installation depending on the time of installation (McHale and Mitchell, 1996). I found that the heating cables caused increased desiccation of the litter layer, which may have affected litter decomposition and N mineralization as discussed in Chapter 3. Especially when soils are dry and have a high organic matter content, heat from the cables will not be distributed evenly throughout the soil.

Laboratory experiments

Many predictions on the long-term response of ecosystems to global climate change are derived from small-scale, short-term experiments. Results of these experiments can not be extrapolated directly to real life due to the artificial conditions in these experiments and partial or complete lack of interactions with other organisms or ecosystem compartments (Van Breemen et al., 1998). Artifacts may also result from sample pretreatment (e.g. Taylor and Parkinson, 1988). Moreover, small-scale studies typically emphasize short-term responses to changes in environmental conditions as was discussed in Chapter 3. However, because separation of factors affecting decomposition such as temperature and moisture was not possible under field conditions in the experimental setup used, small-scale studies had to be carried out to assess the effect of specific parameters while keeping other parameters constant. I used results from laboratory incubation experiments only in a qualitative sense although Teuben and Verhoef (1994) showed that especially results from mesocosm studies are often also quantitatively comparable to field measurements. Most results obtained from micro- and mesocosm studies presented in this thesis were consistent with the field measurements.

BOREAL FORESTS IN A WARMER WORLD: A SOURCE OR A SINK FOR C?

Much of the current debate concerning global change and terrestrial ecosystems revolves around the question whether these ecosystems will act as a source or a sink for C when climate changes. CLIMEX results on soils and vegetation were integrated for making long-term predictions on net ecosystem C and N fluxes using the Nitrogen Isotope and Carbon Cycling in Coniferous Ecosystem (NICCCE) model (Van Dam and Van Breemen, 1995). This model includes transport of heat and water, primary production, decomposition and cycling of C and N isotopes in a mature forest. Plant litter is divided into four components (polysaccharides, proteins, hemicellulose and lignin). Microbial litter is divided into metabolic (low C/N) and structural litter (high C/N) having a longer turnover time. Soil organic matter is divided into humified (turnover time 10-20 yrs), stable (turnover time 50-100 yrs) and resistant (turnover time 500-2000 yrs) organic matter. Microbial C and N transformations are explicitly simulated. Both C and N mineralization depend on C and N use efficiencies. Both the C use efficiency and N mineralization decrease with increasing C/N ratio of the microbial biomass; at a high C/N ratio, an increasing amount of C is transformed into less oxidized C products than CO_2 such as low-molecular-weight acids. The model was calibrated using estimates on C and N pools in soil and vegetation for the outside control catchment METTE. Parameters were optimized until the labile C and N pools in soil and plant were in steady state. In the first scenario, CO_2 concentration and atmospheric temperature were increased stepwise to levels as employed by the CLIMEX treatments.

The model predicted that a stepwise increase in both CO_2 concentration and temperature caused a rapid sequestration of C and N in vegetation and litter (Fig. 7.1A). Initially, litter C decreased since increased decomposition at elevated temperature was not entirely compensated for by litter production. About 50 years after the start of the "treatment", total ecosystem C had increased by approximately 25%. On the long term, C fixation due to CO_2 fertilization is larger than C losses from increased decomposition of soil organic matter. The percentual increase in N storage in the vegetation is smaller than the increase in C fixation, increasing the C/N ratio of plant material (Fig. 7.1B). However, net ecosystem N sequestration was zero, suggesting that N is transferred from the soil to the vegetation.

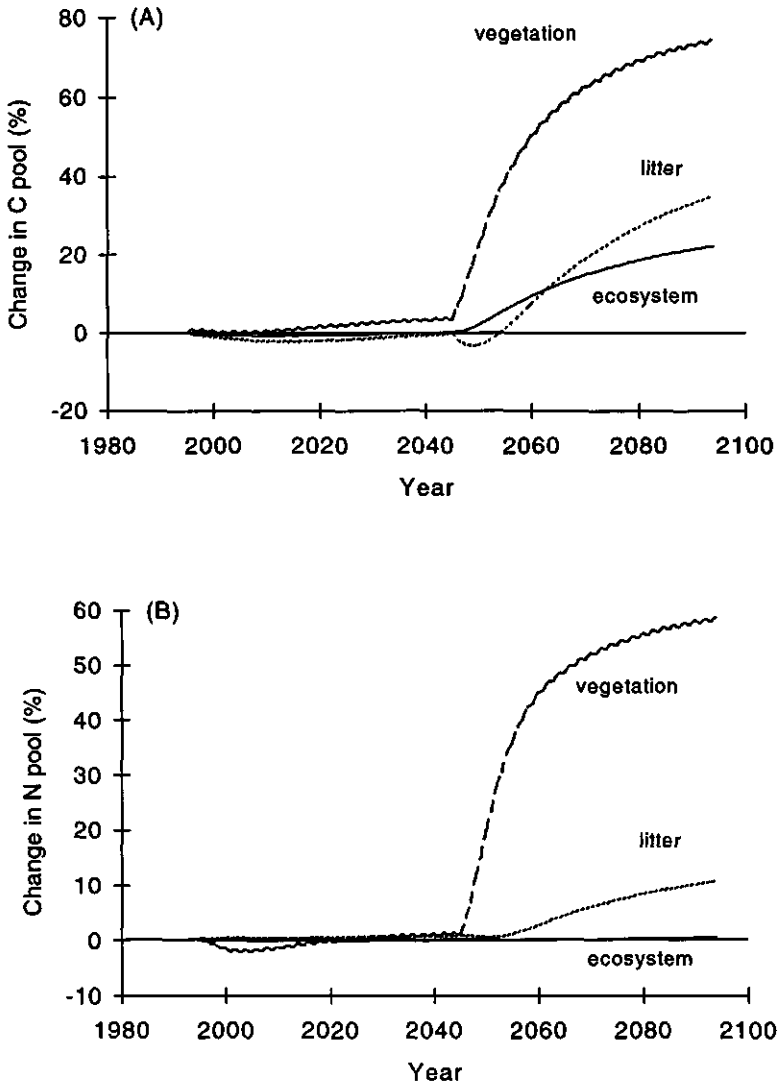

Figure 7.1. Simulated changes in C (A) and N (B) stocks in vegetation, litter and the whole ecosystem after a step-increase in both atmospheric CO_2 concentration and temperature.

To evaluate the effect of the experimental stepwise increase in temperature and CO_2 against the expected gradual changes in climate, we also ran the model assuming a gradual increase in CO_2 and temperature during a period of 50 yrs to the same levels as used for the stepwise increase. When both temperature and CO_2 concentrations increase gradually, the ecosystem acts as a sink for C and N (Fig. 7.2). Both stepwise as well as gradual increase in temperature and CO_2 yield a similar long-term (>50 yrs) response in terms of net ecosystem source-sink behavior for C and N although the absolute magnitude and temporal pattern differed.

To evaluate the effect of the presence of a roof over the canopy, we assumed a reduction in incoming radiation by 45% corresponding to conditions in the CLIMEX enclosure. When reducing incoming radiation, the increase in NPP as a result of elevated CO_2 and temperature is smaller than under normal light conditions. Under reduced light, C losses due to increased decomposition are larger than the C accumulation in the living biomass causing the ecosystem to become a source for C compared to normal light conditions (Fig. 7.3A). This stepwise, reduced light, scenario reflects the treatment as employed in the CLIMEX project and illustrates an important potential artifact on net ecosystem source-sink behavior. When CO_2 and temperature gradually increase, initially the ecosystem loses C (Fig. 7.3B). Fifty years after the start of the treatment, the amount of C present in the ecosystem is the same as in the pre-treatment period.

The vegetation response to elevated CO_2 concentrations may become smaller with time if down-regulation of photosynthesis occurs (Sellers et al., 1996). After 3 years of treatment, the experimental data from the CLIMEX sites do not indicate occurrence of down-regulation (Beerling and Wills, 1997). If, on the longer term, down regulation occurs, less C and N will be sequestered in the biomass. In addition, if decomposition of litter is not affected by changes in litter quality, heterotrophic respiration may be larger than suggested by the model. This may be partly compensated by a lower temperature sensitivity of stable organic matter decomposition. With a stepwise increase, especially under normal light conditions, the amount of C sequestered in the ecosystem almost doubles when temperature sensitivity of the stable pool is reduced by 30% and that of the passive pool by 50% (Fig. 7.4A). In the gradual scenario, the effect of a reduced temperature sensitivity of stable soil organic matter is much smaller (Fig. 7.4B); both under normal and reduced light, net ecosystem C sequestration slightly increases.

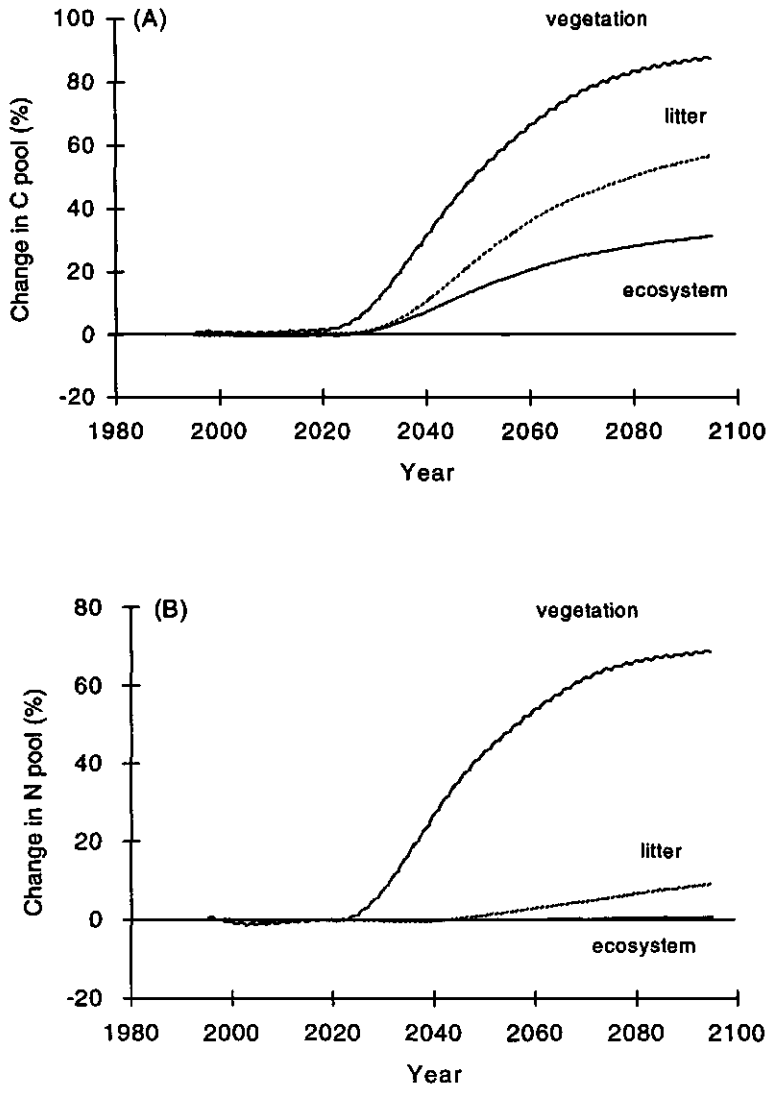

Figure 7.2. Simulated changes in C (A) and N (B) stocks in vegetation, litter and the whole ecosystem after a gradual increase in both atmospheric CO_2 concentration and temperature during 50 years.
the soil to the vegetation.

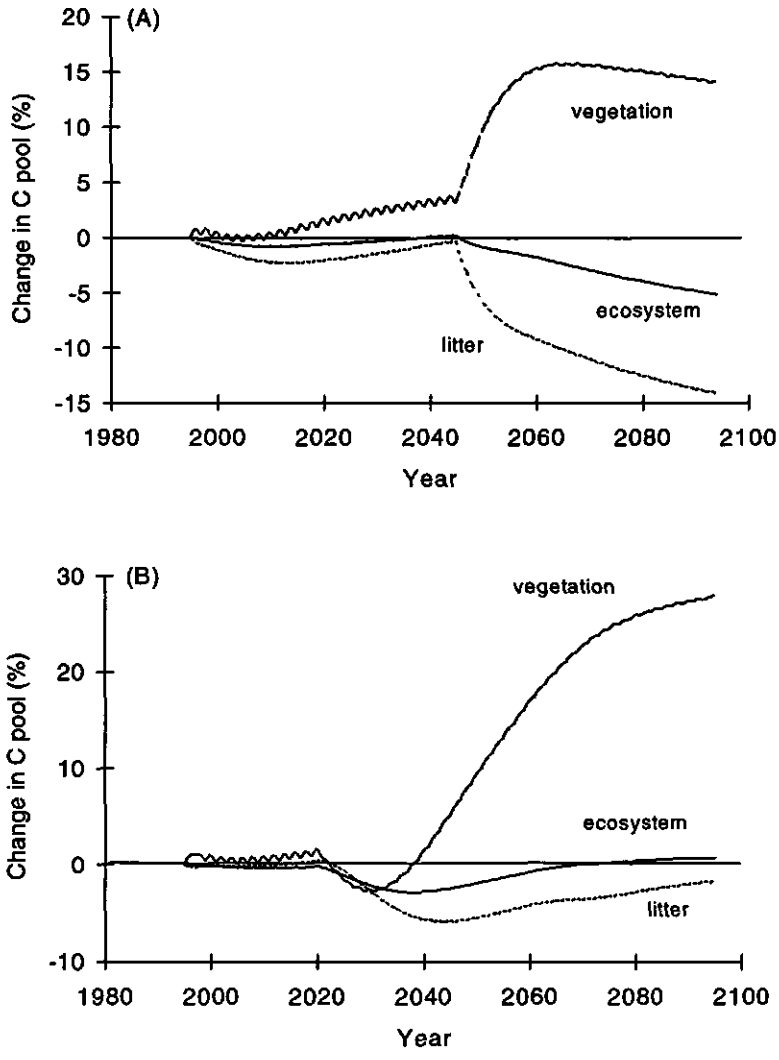

Figure 7.3. Simulated changes in vegetation, litter and ecosystem C after a step-increase (A) or a gradual increase (B) in atmospheric CO_2 concentration and temperature when incoming radiation is reduced by 45%.

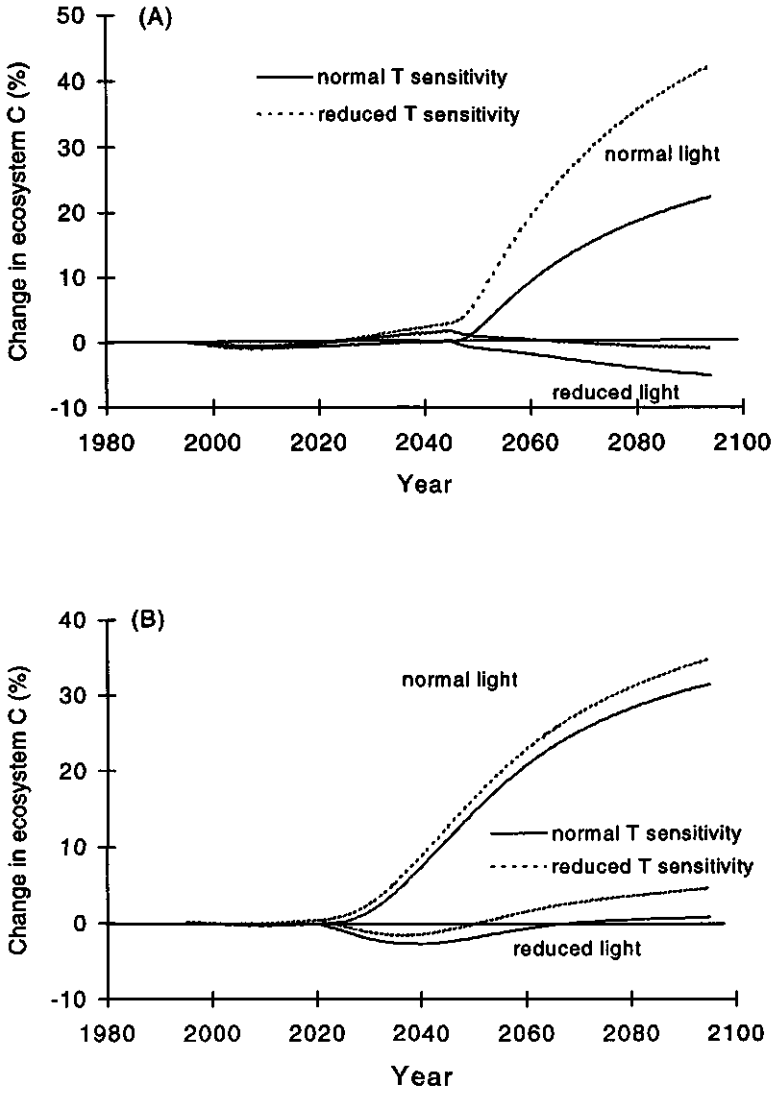

Figure 7.4. Simulated changes in ecosystem C after a step-increase (A) or gradual increase (B) in atmospheric CO_2 concentration and temperature with normal and low temperature sensitivity of the stable and passive organic C pool. The temperature sensitivity of stable organic matter is reduced by 30% and that of passive organic matter by 50%.

FINAL REMARKS

It is beyond doubt that the human population has put its signature on the composition of the atmosphere and the global climate. In order to allow political institutions to enforce measurements for the reduction of greenhouse gas emissions, it is critical to make an assessment of the impacts of global change on natural ecosystems since these systems cover about 90% of the land surface and contain about 99% of the total amount of terrestrial organic C. Some ecosystems may have a potential to sequester C whereas other systems may act as a source for C in a warmer world. Whether natural ecosystems can and should be managed in a way that these systems may act as a sink for C is an important scientific as well as ethical issue. The study presented in this thesis has shown some of the potential effects of climate change on soil processes. It did not produce an unequivocal answer to the question whether soils will loose or sequester C upon climate change. It showed, however, the complexity of the processes involved. In addition, it emphasized the need for answering the question how to improve the accuracy of long-term predictions based on short-term experiments.

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SUMMARY

During the past decades, concentrations of the greenhouse gasses CO_2, CH_4 and N_2O in the atmosphere have significantly increased compared to pre-industrial levels. The main causes for this increase are the combustion of fossil fuels and land use changes such as deforestation. The gasses are referred to as 'greenhouse gasses' since they absorb longwave radiation reflected by the earth surface and cause global temperatures to increase. Carbon dioxide is by far the most important greenhouse gas and contributes for about 50% to the greenhouse effect due to its abundance and relatively long residence time. Increased atmospheric CO_2 concentrations may cause an increase C fixation in the living biomass through increased Net Primary Production (NPP) which is referred to as CO_2 fertilization. However, litter composition may change at elevated CO_2 which could affect decomposition rates. An increase in temperature may cause increased C losses through enhanced decomposition of soil organic matter. Increased decomposition could favor N availability which may enhance NPP on top of CO_2 fertilization. The difference between C fixation by the vegetation and C losses from decomposition will determine whether terrestrial ecosystems become a net source or sink for C when climate changes. The study presented in this thesis assessed the effects of elevated temperature and CO_2 concentration on soil organic matter dynamics in a natural forest ecosystem using a combination of field and laboratory experiments.

The field experiments were carried out within the framework of the Climate Change Experiment (CLIMEX). CLIMEX is an international multidisciplinary project in which temperature and CO_2 are manipulated in an entire forest ecosystem. The CLIMEX site is located at Risdalsheia (58°23' N, 8°19' E), southernmost Norway. The site is 300 m above sea level on a large biotite granite plateau, and is representative for large areas of upland southern Norway. Mean annual precipitation is 1400 mm and mean annual temperature is 5°C (-3°C in January and +16°C in July). Depressions in the granite surface are filled with post-glacial soil material in which acid, peaty podsolic soils have developed. Maximum soil depth is 70 cm. About 30-50% of the bedrock is exposed. The vegetation is dominated by heather (*Calluna vulgaris* (L.) Hull) and various blueberry species (*Vaccinium myrtillus* L., *V. uliginosum* L. and *V. vitis-idaea* L.). The main tree species are Scots pine (*Pinus sylvestris* L.) and birch (*Betula pubescens* Ehrh.).

The KIM catchment (860 m^2) and EGIL catchment (400 m^2) were covered by transparent roofs in 1983 as part of the RAIN (Reversing Acidification In Norway) project. In both catchments, precipitation was collected from the roof and distributed

under the roofs. In the KIM catchment, acidifying components were removed and natural levels of sea salt were added before the water was sprinkled under the roof. In 1993, the KIM catchment was completely enclosed by air-tight, transparent walls. The greenhouse was separated in two parts by a transparent wall. From June 1994, in the lower 80%, CO_2 concentration was increased up to 560 ppm during the growing season and the temperature was increased by 5°C in January and 3°C in July compared to ambient conditions with intermediate temperature increases in the intervening months. In spring 1994, heating cables were put at a depth of 1 cm in the litter layer in the lower 80% of the EGIL catchment. From June 1994, the soil temperature in the treated part was increased to the same levels as the air temperature in KIM. In both the KIM and EGIL catchment, the upper 20% acted as untreated control areas. Three uncovered catchments served as outside control areas to detect possible 'roof effects'.

To assess the effect of elevated CO_2 on litter chemistry and decomposability, *Betula* and *Calluna* were grown at 350 and 700 ppm CO_2 in greenhouses and foliar litter was collected (Chapter 2 and 3). The litter was subsequently incubated for 1 year in the control and treated parts of the CLIMEX catchments. After 1 year exposure to elevated CO_2, *Betula* produced litter with a higher C/N ratio. Mass loss after incubation was the same for the elevated- and ambient-CO_2 litter. Two years of exposure to elevated CO_2 did not affect the C/N ratio of *Betula* but the elevated-CO_2 litter decomposed faster than the low-CO_2 litter. Two years of exposure of *Calluna* to elevated CO_2 caused a significant decrease in C/N ratio of leaves and flowers. Decomposition was similar for both ambient- and elevated-CO_2 litter. The higher temperature in the manipulated parts of the CLIMEX catchments did not cause an increase in litter decomposition rates. It was hypothesized that elevated temperatures caused increased desiccation of the litter especially in the soil-heating site. Although the heating cables caused a permanent increase in soil temperature, under dry conditions, the temperature difference between control and heated plots decreased with increasing distance from the cables. When soils were wet, no gradients in temperature increase occurred. Although litter decomposition did not increase, net N mineralization in the 0-10 cm soil layer increased in both catchments as a result of the climate treatments.

Undisturbed, devegetated, soil cores (diameter 16 cm; length 25 cm) were incubated for 16 weeks in climate chambers at 5, 10 and 17°C to determine the effect of temperature on decomposition under controlled conditions (Chapter 4). The CO_2 emissions from the soil columns and the NO_3 concentrations in the drainage water increased with temperature. Incubation of soil samples from different depths showed that temperature effects on respiration decreased with depth. At all temperatures, respiration

per unit C invariably decreased with depth indicating a decrease in substrate quality. In all layers, decomposition appeared to be limited by availability of C. Both microbial biomass and microbial activity decreased with depth. Glucose was decomposed much slower in the subsoil than in the surface soil which could be partly due to nutrient limitation. The data indicate that the temperature sensitivity of the microbial population decreased with decreasing substrate quality. By assuming organic matter to consist of a labile and stable fraction with decomposition rate constants having a different temperature dependence, it was shown that temperature effects on decomposition may be time-dependent. This complicates establishment of general relationships between temperature and decomposition using studies where different measurement times have been employed.

Effects of temperature on gross and net microbial C and N transformations in two organic surface soil horizons (LF and H) were studied using the ^{15}N-enrichment technique (Chapter 5). The soil material was incubated in microcosms at 5°C and 15°C. Gross N fluxes were calculated using a numerical simulation model using data on microbial C and N, basal respiration, and KCl-extractable NH_4^+, NO_3^-, $^{15}NH_4^+$ and $^{15}NO_3^-$. In the LF layer, increased temperature resulted in a faster turnover of all N pools. While both gross NH_4^+ mineralization and NH_4^+ immobilization increased, net N mineralization did not increase during the 15 days of incubation. By contrast, in the H layer both gross NH_4^+ mineralization and NH_4^+ immobilization were lower at 15°C than at 5°C. For this layer, the model calculated a decrease in microbial turnover rate at higher temperature although measured microbial activity was higher. Decreased gross N fluxes in spite of increased microbial activity in the H layer at elevated temperature could have been caused by increased uptake of organic N at elevated temperature. The differences found between the layers may have been caused by differences in physiology of microbial population. Microbial C/N was around 13 in the LF layer pointing at a fungi-dominated decomposer community whereas it was close to 6 in the H layer, probably due to predominance by bacteria. Decomposition in the LF and H layer did not appear to be limited by availability of N but by availability of C especially in the H layer.

In a pot experiment, 1-year-old *Calluna* plants were pulse-labeled for 1 day with ^{14}C-CO_2 after plants had been exposed to 350 or 560 ppm CO_2 for 3 months (Chapter 6). Plants were grown at either high or low nutrient availability. After harvesting the plants, the soil was incubated to monitor total ($=^{12}C+^{14}C$) respiration and decomposition of ^{14}C-labeled rhizodeposits. Total and shoot biomass increased at high N but were not affected by CO_2. Root biomass was not affected by either N or CO_2 treatments. Total ^{14}C uptake and shoot-^{14}C increased upon adding N and elevating CO_2 but the N effect was strongest. Total ^{14}C uptake per unit shoot mass decreased with N, but increased with CO_2. Root-^{14}C

content was not affected by the N or CO_2 treatment. Total soil-^{14}C increased at elevated CO_2 whereas microbial ^{14}C increased due to high N. C allocation to shoots increased at the expense of roots, soil and respiration at high N but was not affected by the CO_2 treatment. Variation in ^{14}C-distribution within each treatment was small compared to variation in total ^{14}C amounts in each plant/soil compartment. Initially, ^{14}C respiration from the incubated soil was higher for the elevated-CO_2 soil. ^{14}C respiration rates measured during the first 2 days correlated well with root-^{14}C, total soil-^{14}C, soil solution-^{14}C and microbial ^{14}C measured directly after the harvest. After 4 weeks, decomposition of labeled organic matter was not affected by the treatments but total respiration was lower for the elevated-CO_2 soils. Initially, respiration may have been dominated by decomposition of fresh root exudates whereas on the longer term, respiration originates from decomposition of more recalcitrant root material formed during the entire experiment. The increased net ^{14}C uptake and unchanged distribution pattern, combined with a decreased decomposition of more recalcitrant root-derived material indicate that the *Calluna* plant-soil system is a small sink for C under elevated CO_2.

Data on soil and vegetation were integrated to make predictions on the net source-sink behavior of the studied ecosystem using the NICCCE model (Chapter 7). The simulations suggested that on the long term (> 50 yr) the studied ecosystem acts as a sink for C. Sink strength is about the same when CO_2 and temperature are increased gradually or stepwise. Under reduced light conditions as employed in the CLIMEX treatments, the increase in Net Primary Production at elevated CO_2 is smaller than C losses from the soil due to increased decomposition at elevated temperature causing the ecosystem to become a source for C. When temperature and CO_2 concentrations suddenly change, the ecosystem continues to loose C whereas when both CO_2 and temperature increase gradually, the amount of ecosystem C remains unchanged. The simulations did not account for occurrence of down-regulation of photosynthesis so the C fixation in the living biomass may have been overestimated. However, if temperature sensitivity of decomposition decreases with decreasing substrate quality, less C will be lost from the soil at elevated temperatures.

SAMENVATTING

Gedurende de afgelopen decennia zijn de concentraties van de broeikasgassen CO_2, CH_4 en N_2O in de atmosfeer sterk toegenomen ten opzichte van pre-industriële niveaus. De belangrijkste oorzaken voor deze toename zijn de verbranding van fossiele brandstoffen en veranderingen in landgebruik zoals ontbossing. Eerdergenoemde gassen worden 'broeikasgassen' genoemd omdat zij lang-golvige straling absorberen die door de aarde wordt gereflecteerd wat resulteert in een opwarming van de atmosfeer. Kooldioxyde is veruit het belangrijkste broeikasgas en draagt voor ongeveer 50% bij aan het broeikaseffect vanwege de hoge concentratie en lange verblijftijd in de atmosfeer. Een toename in atmosferische CO_2 concentratie kan een verhoogde C vastlegging in de levende biomassa veroorzaken omdat plantegroei van met name C_3 gewassen wordt gestimuleerd door hogere CO_2 concentraties. Dit effect wordt 'CO_2 bemesting' genoemd. Echter, plantenmateriaal geproduceerd onder verhoogd CO_2 heeft vaak een andere samenstelling dan materiaal geproduceerd bij laag CO_2. Deze veranderingen in samenstelling kunnen gevolgen hebben voor de afbreekbaarheid van dood plantenmateriaal dat in de bodem terechtkomt. Een verhoging van de temperatuur kan de afbraak van bodemorganische stof versnellen waardoor het ecosysteem meer C verliest. Een verhoogde afbraaksnelheid van bodemorganische stof kan de N beschikbaarheid verhogen. Hierdoor kan plantengroei extra worden gestimuleerd in combinatie met CO_2 bemesting. Het verschil tussen C fixatie door de biomassa en C verliezen door afbraak van bodemorganische stof zal bepalen of terrestrische ecosystemen netto C zullen vastleggen of verliezen wanneer het klimaat verandert. Het onderzoek weergegeven in dit proefschrift had als doel om de effecten van verhoogde CO_2 concentraties en temperatuur op de afbraaksnelheid van bodemorganische stof te kwantificeren in een natuurlijk bosecosysteem. In dit onderzoek zijn veldexperimenten met laboratoriumexperimenten gecombineerd.

De veldexperimenten zijn uitgevoerd in het kader van het Climate Change Experiment (CLIMEX). CLIMEX is een internationaal multidisciplinair project waarin zowel CO_2 concentratie als temperatuur worden gemanipuleerd in een bebost stroomgebied. Het CLIMEX proefgebied ligt in Risdalsheia (58° 23' N, 8° 19' O) in zuid Noorwegen. Het studiegebied ligt op 300 m boven zeeniveau op een granietplateau en is representatief voor grote delen van zuid Noorwegen. De gemiddelde jaarlijkse neerslag is 1400 mm en de gemiddelde jaarlijkse temperatuur is 5°C (-3°C in januari en +16°C in juli). Depressies in het granietoppervlak zijn opgevuld met post-glaciaal bodemmateriaal

waarin zich venige podzolen hebben ontwikkeld. De maximale bodemdiepte is 70 cm. Ongeveer 30-50% van het gesteente is onbedekt. De vegetatie wordt gedomineerd door struikheide (*Calluna vulgaris* (L.) Hull) en diverse bosbessoorten (*Vaccinium myrtillus* L., *V. uliginosum* L. en *V. vitis-idaea* L.) De belangrijkste boomsoorten zijn grove den (*Pinus sylvestris* L.) en zachte berk (*Betula pubescens* Ehrh.).

Het KIM stroomgebied (860 m^2) en EGIL stroomgebied (400 m^2) zijn in 1983 afgeschermd door transparante daken in het kader van het RAIN (Reversing Acidification In Norway) project. In beide stroomgebieden wordt de neerslag opgevangen en uitgesproeid onder de daken. In het KIM stroomgebied worden verzurende componenten uit de neerslag verwijderd en een natuurlijk gehalte aan zeezout toegevoegd voordat de regen wordt uitgesproeid. In 1993 werd het KIM stroomgebied volledig afgesloten door middel van transparante muren. De 'broeikas' is opgesplitst in twee delen door middel van een transparant tussenschot. Vanaf juni 1994 werd in 80% van de kas de atmosferische CO_2 concentratie verhoogd tot 560 ppm. De luchttemperatuur werd met 5°C verhoogd in januari en met 3°C in juli. De temperatuursstijging varieerde tussen 3 en 5°C in de tussenliggende maanden. In het voorjaar van 1994 werden in 80% van het EGIL stroomgebied verwarmingskabels geplaatst op 1 cm diepte in de strooisellaag. Vanaf juni 1994 werd de bodemtemperatuur in dezelfde mate verhoogd als de luchttemperatuur in het KIM stroomgebied. In beide kassen diende de resterende 20% van het stroomgebied als onbehandeld controlegebied. Drie onoverdekte stroomgebieden dienden als referentiegebied om eventuele 'dak-effecten' te bepalen.

Om het effect van een verhoogde CO_2 concentratie op de samenstelling en afbreekbaarheid van strooisel te onderzoeken werden *Calluna* en *Betula* blootgesteld aan 360 en 700 ppm CO_2 in klimaatskassen (Hoofdstuk 2 en 3). Het strooisel werd opgevangen en vervolgens geïncubeerd gedurende 1 jaar in de behandelde en onbehandelde delen van de CLIMEX stroomgebieden. *Betula* strooisel gevormd na 1 jaar blootstelling aan verhoogd CO_2 had een hogere C/N verhouding. Gewichtsverlies na incubatie was hetzelfde voor laag- en hoog-CO_2 strooisel. Na twee jaar CO_2 behandeling was de C/N verhouding van laag- en hoog-CO_2 strooisel gelijk maar het hoog-CO_2 strooisel brak sneller af. Twee jaar blootstelling van *Calluna* aan verhoogd CO_2 resulteerde in een verlaging van de C/N verhouding van scheuten en bloemen. De afbraaksnelheid was gelijk voor hoog- en laag-CO_2 strooisel. De verhoogde temperatuur in de stroomgebieden had geen snellere afbraak van het geïncubeerde strooisel tot gevolg. Mogelijk leidde vooral in het bodemverwarmingsexperiment een verhoging van de temperatuur tot uitdroging van de strooisellaag. Hoewel de verwarmingskabels een continue verhoging van de bodemtemperatuur veroorzaakten, was de opwarming tussen

de kabels zeer gering als de bodem droog was. Onder natte omstandigheden werd de bodem homogeen verwarmd. Hoewel afbraak van strooisel niet werd gestimuleerd door een verhoging van de temperatuur nam de netto mineralisatie van stikstof toe in de bovenste 10 cm van de bodem in de behandelde delen van het KIM en EGIL stroomgebied als gevolg van de klimaatsmanipulatie.

Om de effecten van temperatuur op decompositie te bepalen onder gecontroleerde omstandigheden werden bodemkolommen zonder vegetatie (diameter 16 cm; lengte 25 cm) 16 weken geïncubeerd in klimaatskamers bij 5, 10 en 17°C (Hoofdstuk 4). De CO_2 emissie vanuit de kolommen en de NO_3 concentratie in het drainagewater namen toe bij een verhoging van de temperatuur. Incubaties van bodemmateriaal afkomstig van verschillende dieptes in het profiel lieten zien dat het temperatuureffect op respiratie afnam met de diepte. De respiratie per eenheid C nam sterk af met de diepte wat een aanwijzing kan zijn voor een afname van de substraatkwaliteit. In alle lagen leek de decompositie van organische stof beperkt te zijn door een lage beschikbaarheid van C. Zowel microbiële biomassa als activiteit namen sterk af met de diepte. Bovendien werd glucose sneller afgebroken in de bovengrond dan in de ondergrond. De tragere glucose afbraak in de ondergrond werd mogelijk deels veroorzaakt door een nutrienten deficiëntie. De data suggereerden dat temperatuursgevoeligheid van de microbiële populatie afnam met een dalende substraatkwaliteit. Als aangenomen wordt dat de decompositiesnelheid van labiele en stabiele C een verschillende temperatuursgevoeligheid hebben, heeft dit tot gevolg dat het effect van temperatuur op de decompositiesnelheid afhangt van de tijd waarop dit wordt gemeten. Deze tijdsafhankelijkheid bemoeilijkt het vaststellen van algemene verbanden tussen temperatuur en decompositiesnelheid aan de hand van datasets waarin verschillende meettijden zijn gebruikt.

De effecten van een verhoogde temperatuur op bruto en netto C en N omzettingen in twee organische horizonten (LF en H) werden bestudeerd met gebruik van ^{15}N als tracer (Hoofdstuk 5). Het bodemmateriaal werd 15 dagen geïncubeerd bij 5 en 15°C. Bruto N fluxen werden berekend met een numeriek simulatiemodel. Het model werd gecalibreerd op gemeten microbiële C en N, respiratie, en KCl-extraheerbaar NH_4^+, NO_3^-, $^{15}NH_4^+$ en $^{15}NO_3^-$. In de LF laag nam de turnover van alle N fracties toe bij een verhoging van de temperatuur. Zowel bruto NH_4^+ mineralizatie als immobilizatie namen toe maar netto N mineralizatie bleef gelijk gedurende de incubatie. Echter, in de H laag namen bruto NH_4^+ mineralizatie en immobilizatie af bij een verhoging van de temperatuur. Het model berekende een afname van de microbiële turnover bij 15°C hoewel de metingen een toename in activiteit lieten zien. De berekende afname in bruto N omzettingen kon verklaard worden wanneer werd aangenomen dat de microben in de H laag stikstof

opnemen in organische in plaats van anorganische vorm. De verschillen in temperatuurrespons tussen beide lagen hingen zeer waarschijnlijk samen met verschillen in fysiologie van de microbiële populatie. De C/N verhouding van de microbiële biomassa was 13 in de LF laag. Dit kan een aanwijzing zijn voor een dominantie door schimmels. In de H laag was de microbiële C/N verhouding 6 wat kan duiden op dominantie door bacteriën. In beide lagen leek decompositie niet gelimiteerd te worden door N maar door C.

In een potproef werden 1 jaar oude heideplanten voor een dag blootgesteld aan ^{14}C-CO_2 nadat deze planten gedurende 3 maanden waren opgekweekt bij 350 of 560 ppm CO_2 (Hoofdstuk 6). De planten werden opgekweekt bij een lage en hoge N beschikbaarheid. Na de oogst werd het met ^{14}C verrijkte bodemmateriaal geïncubeerd. Tijdens de incubatie werden zowel totale (=^{12}C+^{14}C) als ^{14}C respiratie gemeten. De totale biomassa en scheut biomassa namen toe bij een hoger N niveau. Een verhoging van de CO_2 concentratie had geen effect. Wortelbiomassa veranderde niet ten gevolge van de N en CO_2 behandelingen. Totale ^{14}C opname en ^{14}C gehalte van de scheuten was hoger bij een verhoogd CO_2 en N niveau. Het N effect was het sterkst. Totale ^{14}C opname per eenheid scheutmassa was lager bij hoog N maar hoger bij verhoogd CO_2. Het ^{14}C gehalte van de wortels werd niet beinvloed door N en CO_2. De totale hoeveelheid ^{14}C in de bodem was hoger bij verhoogd CO_2 maar de hoeveelheid ^{14}C in de microbiële biomassa nam alleen toe bij een hoger N niveau. Bij hoog N werd relatief meer C gealloceerd naar bovengrondse plantendelen. Allocatie was hetzelfde bij hoog en normaal CO_2. De variatie in allocatie was klein ten opzichte van variatie in ^{14}C gehalte in elk afzonderlijk plant/bodem compartiment. ^{14}C respiratie van de geïncubeerde bodem was hoger voor de hoog-CO_2 bodem. Gedurende de eerste 2 dagen was ^{14}C respiratie gecorreleerd met wortel-^{14}C, bodem-^{14}C, ^{14}C in bodemoplossing en ^{14}C in de microbiële biomassa als bepaald net na de oogst. Na 4 weken was ^{14}C respiratie voor alle behandelingen gelijk maar totale respiratie was het laagst voor de hoog-CO_2 bodems. In het begin werd respiratie waarschijnlijk gedomineerd door decompositie van verse organische stof afkomstig van wortels. Op de lange termijn werd respiratie gedomineerd door afbraak van meer recalcitrante organische stof gevormd gedurende het opkweken van de planten. Omdat bij verhoogd CO_2 de totale ^{14}C opname toenam bij een gelijkblijvende allocatie en op de lange termijn de afbraaksnelheid van hoog-CO_2 organische stof afkomstig van wortels lager was, werd geconcludeerd dat het plant-bodem systeem met *Calluna* een kleine netto put voor C wordt onder verhoogd CO_2.

Gegevens met betrekking tot bodem en vegetatie werden geïntegreerd om te kunnen voorspellen of het bestudeerde ecosysteem een bron of een put voor C wordt als

het klimaat verandert (Hoofdstuk 7). De modelberekeningen werden uitgevoerd met het NICCCE model. De simulaties lieten zien dat het bestudeerde ecosysteem op de lange termijn (>50 jaar) netto C vastlegt wanneer temperatuur en CO_2 concentratie worden verhoogd. De hoeveelheid vastgelegde C was gelijk bij zowel een geleidelijke als plotselinge verhoging van de temperatuur en CO_2 concentratie. Wanneer inkomende straling werd gereduceerd, zoals het geval is in de CLIMEX stroomgebieden, was de voorspelde toename in netto primaire productie minder groot dan de toename in afbraaksnelheid van bodemorganische stof bij een verhoging van de temperatuur zodat het ecosysteem C verliest. Wanneer het klimaat plotseling werd veranderd, bleef het ecosysteem C verliezen terwijl bij een geleidelijke klimaatsverandering er uiteindelijk netto noch C werd vastgelegd noch verloren. In de simulaties is geen rekening gehouden met adaptatie van de vegetatie aan verhoogd CO_2 ('down-regulation'). Als deze adaptatie optreedt, zal er minder C worden vastgelegd in de levende biomassa dan werd berekend in de simulaties. Echter, wanneer de temperatuursgevoeligheid van afbraak van stabiele C minder is dan die van labiele C, zal er ook minder C verloren gaan.

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My (ex)room- and housemates had to deal with my high's and low's most often accompanied with music played on a very high volume no matter whether it was a 'high' or a 'low'.

The BenG-boys, squash partners, concert visitors, musicians, bar visitors, Van Rossums and other friends provided the necessary distraction and at times put my attention at more immediate issues in life such as soccer tactics and whether or not to play a C# demolished scale over a G#maj7#4 chord.

CURRICULUM VITAE

Paulus Stephanus Jacobus Verburg werd geboren op 18 juli, 1968 te Houten. In september 1987 startte hij zijn studie Regionale Bodemkunde aan de Landbouwuniversiteit. In zijn eerste doctoraalonderzoek bestudeerde hij bodemvorming op andesitische lavas in Costa Rica. In zijn tweede doctoraalonderzoek bestudeerde hij de invloed van kristaldefecten op de verweringssnelheid van mineralen. Zijn praktijktijd bracht hij door bij de vakgroepen Scheikunde en Bosbouw op Oregon State University waar hij zich bezig hield met het modelleren van de effecten van zure depositie op bosecosystemen. In september 1992 studeerde hij af met als hoofdvakken Bodemvorming en Bodemmineralogie. Van 1992 tot en met 1997 had hij een aanstelling bij de vakgroep Bodemkunde en Geologie om het onderzoek uit te voeren dat beschreven is in dit proefschrift. Het onderzoek werd uitgevoerd in het kader van het Climate Change Experiment (CLIMEX). In dit projekt werden de gevolgen van klimaatsverandering op een boreaal bosecosysteem bestudeerd.

Paulus Stephanus Jacobus Verburg was born on July 18, 1968 in Houten, The Netherlands. He started his study Soil Science at Wageningen Agricultural University in September 1987. His first M.Sc. thesis described soil formation on andesitic lavas in Costa Rica. His second thesis described the influence of crystal defects on weathering rates of minerals. During his study, he worked at the departments of Chemistry and Forest Science at Oregon State University modeling the impacts of acid deposition on forest ecosystems. He graduated in September 1992 specializing in soil formation and soil mineralogy. From 1992 to 1997, he was employed by the department of Soil Science and Geology of the Wageningen Agricultural University to carry out the research presented in this Ph.D. thesis. The research was part of the Climate Change Experiment (CLIMEX). In this project, the effects of climate change on a boreal ecosystem were studied.