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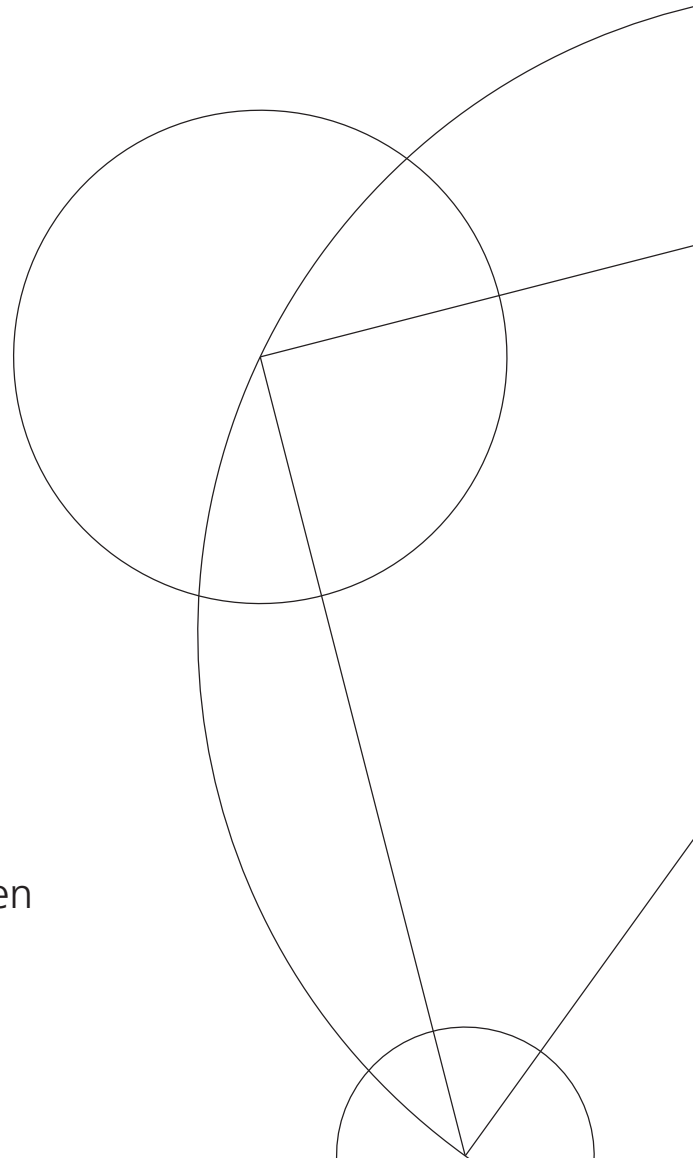
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Carbon and Nitrogen Dynamics of Temperate and Subarctic Heath Ecosystems with Emphasis on Cold-Season Processes

Ph. D. Thesis By
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2006



Preface

This Ph.D. thesis was written at the Dept. of Terrestrial Ecology, Institute of Biology, University of Copenhagen, and gathers the results of three field experiments focusing on the cold-season cycling of carbon and nitrogen in temperate and subarctic heath ecosystems. Over the last three years, I have been surrounded by helpful people who have made the project period an interesting and inspirational time. My supervisors Sven Jonasson, Anders Michelsen and Claus Beier were always helpful with ideas and feedback whenever needed. I am also very grateful to Heidi Sjursen Konestabo who initiated the freeze-thaw experiment in Abisko providing several years of treatments to be studied.

Niels Bruun, Karna Heinsen and Gosha Sylvester had the pleasure of the numerous soil extracts brought back from field trips and I am very thankful for their great effort with the laboratory analyses. A special thanks also to all my colleagues and fellow Ph.D. students at the Dept. of Terrestrial Ecology for great company and not least for interesting coffee break discussions. During the project period, about 3½ months were spent with field work at Abisko Scientific Research Station in Northern Sweden where the staff was always very helpful and, together with other visiting researchers, provided tundra bars and camp fires at the lake as nice social breaks during field trips. Mols Bjerge and the Mols Laboratory were visited on many shorter trips. Thank you to Anni Kjeldsen for always taking care of logistics on short notice and to Lars Hougaard for his help in the field. I also spent one month at the Dept. of Biosystems at the Risø National Laboratory during the work with photosynthesis and respiration models, where especially Andreas Ibrom has spend many hours introducing me to modeling carbon exchange, thank you. Also thanks to Karina Clemmensen, who gave valuable comments on the thesis.

My wife, Nina, has supported me always and has given many helpful comments on the manuscripts and the thesis, for which I am very grateful.

Klaus Steenberg Larsen
September 2006

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Papers I-IV

List of papers

Paper I: Larsen KS, Grogan P, Jonasson S and Michelsen A. Respiration and microbial dynamics in two sub-arctic ecosystems during winter and spring-thaw: Effects of increased snow depth. *Arctic, Antarctic, and Alpine Research* (accepted).

Paper II: Larsen KS, Ibrom A, Jonasson S, Michelsen A and Beier C. Significance of cold-season respiration and photosynthesis in a subarctic heath ecosystem in Northern Sweden. Submitted to *Global Change Biology*.

Paper III: Larsen KS, Ibrom A, Beier C, Jonasson S and Michelsen A. Ecosystem respiration depends strongly on photosynthesis in a temperate heath. Submitted to *Biogeochemistry*.

Paper IV: Larsen KS, Michelsen A, Jonasson S and Beier C (*in prep.*). Significant plant nitrogen uptake during the cold season in a temperate and a subarctic heath ecosystem.

Summary

Large amounts of carbon are stored in terrestrial ecosystems and the annual carbon exchange with the atmosphere due to photosynthesis and respiration is high. Terrestrial ecosystems may therefore represent major positive or negative feedbacks to the carbon dioxide concentration of the atmosphere and thus to future climate change. In order to assess the impacts of global changes we need to understand the controls of important ecosystem processes under the current climate. However, recent research has made it clear that our knowledge of some processes, including the cold season carbon and nitrogen dynamics, is still limited.

In this thesis, I investigated the ecosystem respiration and photosynthesis in a temperate heath ecosystem at Mols Bjerge, Denmark, and in subarctic heath and birch understory ecosystems at Abisko, Northern Sweden. I focused on the cold season fluxes in order to estimate the contribution of cold season respiration and photosynthesis to the annual carbon budget. At the sites in Abisko, possible future changes in snow depth and in the freeze-thaw regime were simulated *in situ*, to investigate the ecosystem responsiveness to such changes. Isotopic tracer studies were also performed at both the temperate and the subarctic sites in order to investigate plant nitrogen uptake during the cold season.

The main findings include: 1) Cold-season ecosystem respiration and, more surprisingly, also photosynthesis were considerable and important in the annual carbon budget in both the temperate and subarctic ecosystems (Papers II and III). 2) Increased freeze-thaw frequency at the subarctic heath site had little effect on ecosystem carbon exchange and no effect on ecosystem nitrogen exchange (Papers II and IV, respectively) suggesting that the ecosystem will respond slowly to future changes in the freeze-thaw regime. 3) All investigated plant groups at the temperate heath had significant nitrogen uptake throughout the winter, while evergreen dwarf shrubs as the only plant group showed a considerable nitrogen uptake immediately after snowmelt at the subarctic heath site (Paper IV). 4) For the snowmelt period at a subarctic heath and birch understory, a classic temperature-dependent ecosystem respiration model was improved when incorporating a measure of substrate supply, in the form of dissolved organic carbon or nitrogen into the model (Paper I). 5) At the temperate heath, a better model fit, as well as a lower and more realistic temperature sensitivity, was achieved when photosynthetic rates were incorporated into the temperature-dependent model (Paper III). The results from these model approaches support the recent critique of the wide-spread use of respiration models, which only depend on temperature, and highlight the need for incorporating other potentially important factors into the models.

Sammenfatning (Danish summary)

Terrestriske økosystemer indeholder store mængder kulstof og den årlige kulstofudveksling mellem økosystem og atmosfære som følge af fotosyntese og respiration er stor. Positiv eller negativ feedback fra terrestriske økosystemer på atmosfærens kuldioxidkoncentration kan derfor have afgørende betydning for det fremtidige klima. For at kunne forudsige mulige effekter af klimaforandringer er det nødvendigt at vi har en indgående viden om vigtige økosystemprocesser under nuværende klimaforhold. Nyere forskning har imidlertid gjort det klart at vores viden om nogle af disse processer, herunder kulstof- og kvælstofomsætningen i vinterhalvåret, stadig er begrænset.

I dette ph.d.-projekt har jeg undersøgt økosystemrespiration og -fotosyntese i et tempereret hedeøkosystem i Mols Bjerge, Danmark, og i subarktiske hede- og birkeunderskovsøkosystemer i Abisko, Nordsverige. Jeg fokuserede på vinterhalvåret for at estimere bidraget for denne periode i det årlige kulstofbudget. I områderne i Abisko undersøgte jeg desuden mulige effekter på kulstof- og kvælstofomsætningen af *in situ* forøget snemængde samt forøget antal fryse-tø-cykler. Isotop-indmærkningsforsøg blev ligeledes foretaget på både det tempererede og det subarktiske hedeøkosystem for at undersøge planternes kvælstofoptag i vinterhalvåret.

Hovedresultaterne inkluderer: 1) Økosystemrespirationen i vinterhalvåret, og mere overraskende også økosystemfotosyntesen, udgjorde en betydelig del af den årlige kulstofudveksling med atmosfæren (Manuskript II og III). 2) Et øget antal af fryse-tø-cykler i det subarktiske hedeøkosystem havde kun en beskedent effekt på såvel respiration som fotosyntese og ingen effekt på kvælstofomsætningen (hhv. Manuskript II og IV), hvilket tyder på at økosystemrespons på fremtidige ændringer i antallet af fryse-tø-cykler vil være begrænset. 3) Alle undersøgte plantegrupper på den tempererede hede viste signifikant optag af kvælstof gennem hele året, mens de stedsegrønne dværgbuske var den eneste gruppe som viste et signifikant optag af kvælstof umiddelbart efter sneafsmeltningens afslutning på den subarktiske hede (Manuskript IV). 4) Økosystemrespiration under sneafsmeltningsperioden i en subarktisk hede og en birkeunderskov kunne modelleres bedre end med en klassisk temperaturafhængig respirationsmodel når et mål for tilgængeligt substrat for jordens mikroorganismer, i form af opløst organisk kulstof eller kvælstof, blev indarbejdet i modellen (Manuskript I). 5) For den tempererede hede kunne en bedre økosystemrespirationsmodel opnås ved at indarbejde fotosynteseraten i den klassiske respirationsmodel (Manuskript III). Modellerne støtter derfor den nyligt rejste kritik af den udbredte brug af respirationsmodeller der kun er baseret på temperatur og viser vigtigheden af at andre vigtige faktorer indarbejdes i økosystemrespirationsmodeller.

1. Introduction

The plants of terrestrial ecosystems globally hold approximately 560 Gt of carbon (C) and sequester about 120 Gt C – or 1/6 of the atmospheric carbon pool – every year through photosynthesis (Figure 1; (Schlesinger and Andrews 2000)). An almost similar amount is returned to the atmosphere due to respiratory processes. In comparison to these flux rates, the release of carbon into the atmosphere caused by human activities is small (about 7 Gt C), but very important because of its unidirectional nature. Since the beginning of the industrialization the burning of fossil fuels and land-use changes has therefore increased the atmospheric concentrations of greenhouse gasses, e.g. the atmospheric concentration of CO₂ has increased 31 % from 1750 until present (IPCC 2001).

The changes in the composition of the atmosphere induced by human activities and the physical properties of the greenhouse gases as insulators of long-wave radiation are the hard facts in the global climate debate. What puzzles scientists around the world today is: How dramatically will the global climate be affected and how will the worlds ecosystems respond and adapt to the new conditions for living.

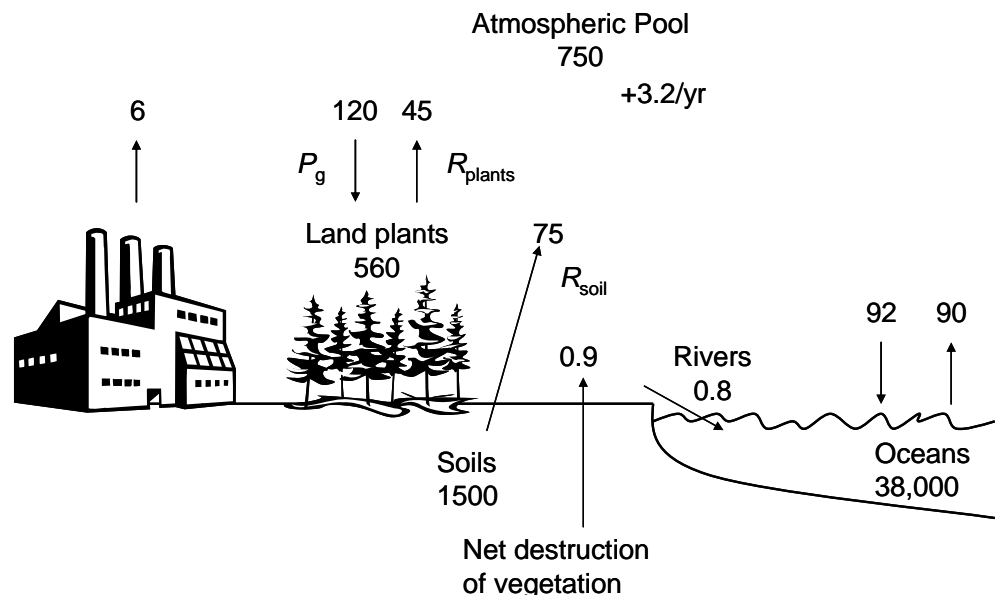


Figure 1. The global carbon cycle. All pools are expressed in units of 10¹⁵ gC and all fluxes in units of 10¹⁵ gC/yr, averaged for the 1980s. P_g is gross photosynthesis, R_{plants} is plant respiration and R_{soil} is soil respiration. Modified from Schlesinger and Andrews (2000).

*Climate
projections*

During the last decade, it has become increasingly evident that global climate changes are already taking place (IPCC 2001; ACIA 2005; Kohler et al. 2006). Simultaneously, models of the global climate have improved. The latest report from the international panel of climate change (IPCC 2001) estimates that global temperatures have increased by 0.6 °C over the last century and that temperatures will increase by 1.4 to 5.8 °C over the next century depending on the future development in atmospheric concentrations of greenhouse gasses. Because of the large annual fluxes of carbon between the atmosphere and terrestrial ecosystems, and because soil globally contain more than 1500 Gt C (Schlesinger and Andrews 2000), terrestrial ecosystems holds the potential to be major positive or negative feedbacks to global climate change. Consequently, the impact of possible climate changes is today an essential aspect of most investigations of the function of terrestrial ecosystems.

*Importance of
the cold season*

The prerequisite for unraveling possible ecosystem feedbacks to environmental changes is that we understand well the processes that govern the biogeochemical cycling of key elements on all spatial and temporal scales. Recent research has made it clear that our knowledge in certain areas and aspects of ecosystem processes is still limited. For instance, in ecosystems with high seasonality like arctic ecosystems, research has traditionally focused on the growing season, when plant activity is high and the turnover of key elements is presumed to be rapid. During the last decade, however, evidence of considerable cold-season biological activity in arctic ecosystems and significant fluxes of carbon dioxide from the ecosystems to the atmosphere has grown (e.g., Sommerfeld et al. 1993; Zimov et al. 1996; Sommerfeld et al. 1996; Oechel et al. 1997; Fahnestock et al. 1999; Grogan et al. 2001). This activity has most often been accredited to the soil microbial community, which furthermore seems to be structurally different in winter than during summer (Schadt et al. 2003; Lipson and Schmidt 2004). Also, the net mineralization rate of nitrogen, the element that limits plant growth in many terrestrial ecosystems (Killham 1994) and practically all arctic ecosystems (Nadelhoffer et al. 1992; Jonasson et al. 1999b), has been shown to be considerable or even higher during the cold season from late fall to early spring, than during the short, intense growing season (Hobbie and Chapin 1996; Grogan and Jonasson 2003; Schimel et al. 2004). Consequently, although processes may be much slower during the cold season, they may be fundamental for ecosystem functioning and in part set the boundaries for plant growth during the growing season. Although the research record of cold-season processes has increased during the last decade, our knowledge is still limited. Furthermore, almost all studies have been done in arctic-alpine areas (e.g. Brooks et al. 1996; Mast et al. 1998; Grogan et al. 2001) and very little is known about the processes in the temperate areas.

1.1 Aims and objectives

This thesis focuses on investigating the importance of cold-season processes in the annual cycle of carbon and nitrogen in temperate and subarctic heath ecosystems. The aim was also to determine the drivers of ecosystem respiration and incorporate these drivers into models in order to make carbon budgets. At the subarctic site the impacts of possible future climate changes were also studied, i.e. the effects of increased snow cover and increased freeze-thaw cycles. Major questions that were addressed include:

- What is the contribution of cold-season ecosystem respiration to the annual carbon budget? (Papers I, II and III)
- What is the contribution of cold-season photosynthesis to the annual carbon budget? (Papers II and III)
- What are the primary drivers of ecosystem respiration during early spring (Paper I) and annually (Paper III)
- How do changes in snow depths and in the freeze-thaw regime affect the microbial community and nutrient turnover? (Papers I and IV)
- Do plants have active uptake of nitrogen during the cold-season (Paper IV)

1.2 Study sites

The field investigations included in this thesis were all carried out in temperate and subarctic heath vegetation and in one experiment also in the understory vegetation of a subarctic birch forest (Paper I). The low-statured vegetation allowed for ecosystem level measurements of the carbon exchange with the chamber method. Despite great latitudinal differences the similarities in plant morphological types at all sites (dwarf shrubs, mosses and graminoids) implied a similar, major control on community composition, thus also allowing for sensible comparisons over a broad climatic gradient to be made.

The temperate site (Figure 2) is situated in Mols Bjerge on the east coast of Jutland in Denmark (56°23'N, 10°57'E) and is part of a 3000 ha preserved area. The experimental area is close to the CLIMOOR site (Beier et al. 2004) and the nearby Mols Laboratory (University of Aarhus) provides logistic support. It is a semi-natural ecosystem which was cultivated until the middle of the 19th century (Pedersen et al. 2001). Currently the low-statured vegetation, which is dominated mainly by the evergreen dwarf shrub *Calluna vulgaris* (L.), the grass *Deschampsia flexuosa* (L.) and various mosses, is preserved by extensive cattle grazing. The annual mean air temperature is 9.4°C (1.6°C in January and 18.1°C in July) and mean annual precipitation (1998-2000) is 758 mm (Beier et al. 2004). Further details are given in Paper III.



Figure 2. The temperate heath site studied in Paper III and IV at Mols Bjerge, Denmark.



Figure 3. The subarctic heath site studied in Paper II and IV near Abisko, Northern Sweden.

The subarctic birch understory and heath sites (Figure 3) are situated near Abisko in Northern Sweden (68° 20' N, 18°47' E). Abisko is an area with intensive research activity facilitated by the Abisko Scientific Research Station, which provides housing as well as extensive laboratory facilities. Birch forest and heath ecosystems are both dominant ecosystem types in Northern Scandinavia (Sjörs 1971) and in contrast to the semi-natural temperate heath site, both subarctic sites are natural ecosystems with minimal human impact.

The climate in the region is sub-arctic/alpine and records (1970–2000) from a nearby climate station show a mean annual temperature of -0.5°C and permafrost is patchy in the region although not present at the study sites. The mean air temperatures in winter (December – February) and spring (March – May) are -9.9°C and -2.3°C , respectively. Annual precipitation is 315 mm, with 75 mm falling in the winter and 41 mm in the spring. Compared to the temperate heath site, the vegetation is co-dominated by several species. Various species of mosses comprise a large fraction of the above-ground biomass together with the ericoid dwarf shrubs *Empetrum nigrum* ssp. *hermaphroditum* (Hagerup), *Vaccinium uliginosum* (L.), *Vaccinium vitis-idaea* (L.), *Andromeda polifolia* (L.) and *Rhododendron lapponicum* (L.). Other common species are *Arctostaphylos alpinus* (L.), *Cassiope tetragona* (L.), *Tofieldia pusilla* (Michx.), *Carex vaginata* (Tausch), and *Betula nana* (L.). The species composition at the birch (*Betula pubescens* ssp. *czerepanovii*) site differs mainly by *Vaccinium myrtillus* (L.) as an additional dominant species. More details are given in Paper I.

2. The carbon cycle

Photosynthesis

Terrestrial ecosystems sequester nearly all their carbon from atmospheric CO₂ through the process of photosynthesis. The largest fraction of photosynthesis in terrestrial ecosystems usually takes place in specialized, above-ground organs, such as the leaves of higher plants, while algae and autotrophic bacteria usually contribute only little to the ecosystem carbon fixation. An even smaller fraction of the ecosystem carbon sequestration may be accredited to other processes than photosynthesis, such as the bacterial oxidation of methane (Killham 1994). Photosynthesis depends strongly on environmental factors such as solar radiation, temperature, humidity and atmospheric concentrations of CO₂ (Lambers et al. 1998) and the theoretical foundation of photosynthesis is well-established (Trumbore 2006). This means that we are capable of modeling photosynthesis based on mechanistic processes, like enzyme kinetics (see Papers II and III), enabling us also to a certain degree to predict possible impacts of a changed, future climate.

The uptake of carbon by terrestrial ecosystems through photosynthesis has the potential to reduce the increasing atmospheric concentrations of CO₂ (Van Minnen and Voigt 2004). For example, European terrestrial ecosystems have been estimated to assimilate between 7 % and 12 % of the anthropogenic emissions over the last decade (Janssens et al. 2003). The correct estimation of ecosystem photosynthesis, as well as a credible assessment of the impacts of climate change on photosynthesis, are therefore key stones in the prediction of the future climate development.

Respiration

On the ecosystem level, respiration is a much more complex process than photosynthesis. Where photosynthesis is practically the single process of ecosystem carbon sequestration, a variety of different pathways, collectively referred to as respiration, are involved with the return of carbon to the atmosphere (Trumbore 2006). Functionally, carbon may be respired by all parts of the autotrophic plants, above-ground as well as belowground (autotrophic respiration, R_a), and by a wide range of different heterotrophic organisms in the litter and soil layers involved in the decomposition of dead organic matter (heterotrophic respiration, R_h) including symbiotic mycorrhizal fungi and saprotrophic fungi and bacteria. Consequently, R_a depends directly on the carbon input from the canopy and may be expected to more or less mimic the responses of photosynthesis to major ecosystem drivers like temperature and humidity. In contrast, the resulting carbon storage and nutrient turnover of ecosystems depend on the activity of a diverse group of heterotrophic soil organisms (Binkley et al. 2006) each depending on the physical conditions as well as on their specific substrate supply. Below-ground food webs with several trophic levels in the decomposer community add further complexity to the soil matrix and the resulting soil respiration.

2.1 Measuring and modeling ecosystem carbon cycling

Eddy co-variance

No current method for measuring the ecosystem exchange of CO₂ can produce carbon budgets over longer time intervals without the use of models to fill the gaps between measurements. Even the most continuous method, the eddy co-variance technique (used e.g. by the EUROFLUX project, see Janssens et al. 2001), depends on models for gap filling. The concept of the eddy co-variance technique is to measure the gas concentration above the vegetation and quantify the mass flow by measuring also the vertical fluctuations in wind speed (for a detailed theoretical description, see Aubinet et al. 2000). Consequently, the method requires adequate turbulent mixing and data must be modeled, or at least corrected, during periods with calm weather and boundary layer formation in the air. Second, the eddy co-variance technique only yields the net ecosystem carbon exchange and infers respiration from night time measurements, leaving photosynthesis as well as daytime respiration to be estimated by modeling.

Chamber technique

The most widely used method for measurement of the CO₂ exchange in terrestrial ecosystems is probably the chamber technique (see e.g., Zimov et al. 1993; Alm et al. 1999; Christensen et al. 2000; Grogan et al. 2001). As described by Vourlitis et al. (1993), a cuvette (chamber) attached to a portable infrared gas analyzer (IRGA) is placed on top of a plot, and due to the higher concentrations of CO₂ in the soil caused by respiration processes, CO₂ diffuses from the soil into the chamber atmosphere. If the chamber is transparent for incoming photosynthetic active radiation (PAR) and the plot contains plants, there may be active photosynthesis as well as respiration. The measured flux thus resembles the net flux of CO₂ (F_n). Respiration (R) may be measured with a darkened chamber and gross photosynthesis (P_g) estimated as $F_n - R$. The advantage is therefore, that both respiration and gross photosynthesis can be estimated. However, measurements are usually performed over short time intervals of, e.g. two minutes, since longer time spans will ultimately affect the gradient between the soil atmosphere and the chamber atmosphere thus affecting the measured gas flux. Being non-continuous per se, the method depends on modeled data for the periods between measurements. Because the necessary equipment is portable the method is more flexible than the eddy co-variance method, which relies on stationary flux towers. However, compared to the eddy co-variance technique, which usually integrates measured fluxes over areas of more than 1,000 m², the plot scale is much smaller with the chamber technique (usually below 1 m²) and the time gaps between measurements often much higher. Obviously, the method also can not be applied on the ecosystem level in tall vegetation like in forests. Using the two methods in combination may thus provide important knowledge of the carbon cycling on different spatial scales (e.g., Pilegaard et al. 2001) and provide the opportunity for data rectification and model improvement. Still, in order to estimate the carbon balance over longer time spans, e.g. months or years, models of respiration and photosynthesis are needed.

Models

Because the single process of photosynthesis dominates the carbon sequestration of ecosystems, both empirical and mechanistic models, usually

can simulate ecosystem photosynthesis with high accuracy. Especially the mechanistic models are important, because they may be able to assess possible effects of a changed climate with much higher confidence than an empirical model.

Somewhat surprising, the much more complex ecosystem respiration may often be modeled with high accuracy by simple first-order exponential equations related only to temperature. However, such equations often produce temperature dependencies (i.e., Q_{10}) that are much higher than can be explained by mechanistic processes such as enzyme kinetics. It has therefore recently been suggested that if Q_{10} exceeds 2.5 it is likely that factors other than temperature, e.g. humidity and substrate supply, are affecting the respiration rate (Davidson et al. 2006). Since these other factors are excluded from such models but potentially may change in a warmer future climate, these models are poorly suited for making predictions of effects of climate changes on ecosystem respiration. Much research attention has therefore recently been directed towards unmasking the true drivers of respiration and improving our mechanistic understanding of ecosystem respiration (Subke et al. 2006).

2.2 Results of the carbon flux studies

In Papers I and III, the controlling factors of ecosystem respiration are investigated by model approaches. In Paper I, we show that ecosystem respiration during spring-thaw in subarctic heath and birch understory ecosystems varies greatly in spite of almost constant soil temperatures around 0°C and that incorporation of dissolved organic carbon (DOC) or dissolved organic nitrogen (DON) into the respiration model greatly improved the model fit. It is therefore evident that substrate supply restricts soil respiration during snowmelt and needs to be measured with high frequency in order to model ecosystem respiration rates at this time of year. In Paper III, a better model fit of the ecosystem respiration model at a temperate heath was achieved when photosynthesis rates were incorporated into the model (Figure 4), suggesting that daytime respiration increased with increasing photosynthesis. Also, the Q_{10} of the modified model was 2.5 as opposed to 3.3 to 3.9 of a classic model depending only on temperature, showing that the modified model is mechanistically more sound than the classic model. Furthermore, Papers I, II and III also highlight the importance of cold-season fluxes of ecosystem respiration, as well as photosynthesis, in all the investigated ecosystems.

$$R_E = (R_{0m} + \lambda P_g) e^{b_m T}$$

Figure 4. The modified ecosystem respiration (R_E) model from Paper III. A linear relationship between respiration and photosynthesis was added to a classic first-order exponential equation related to temperature (van 't Hoff 1898). R_{0m} is the basal ecosystem respiration at 0 °C in the absence of photosynthesis, λ is the respiratory costs of gross photosynthesis (P_g) as a fraction of P_g , b_m is the temperature sensitivity of respiration and T is temperature (°C).

This thesis reveals the importance of photosynthesis outside what is generally believed to be the growing season in both the temperate (Paper III) and subarctic (Paper II) heaths. Interestingly, despite the differences in the length of the cold season, as well as different patterns within the cold-season, the contribution of cold-season photosynthesis in the annual budgets was almost similar for the temperate heath (22 %) and the subarctic heath (19 %, Figure 5). At the temperate site, where considerable mid day photosynthesis rates could be measured even at winter solstice, the results indicate that photosynthesis is an year-round process and that the dominant species *Calluna vulgaris* seems active throughout the year and not seasonally dormant as previously suggested (Miller 1979; Kwolek and Woolhouse 1981). At the subarctic site, the results indicate that especially the late fall and early spring are important periods with considerable photosynthetic activity and that these periods should be included in our definition of the growing season.

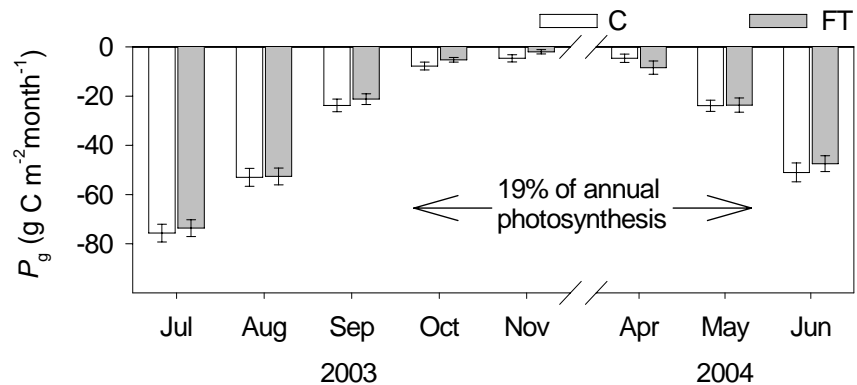


Figure 5. Modeled monthly gross ecosystem photosynthesis (P_g) in a subarctic heath ecosystem. Photosynthesis during October-November and April-May, which are usually not considered part of the growing season, constituted 19% of annual P_g in both controls (C) and plots with increased freeze-thaw frequency (FT). Modified from Paper II.

3. The nitrogen cycle

The carbon cycle interacts with the cycling of other elements associated with the organic molecules in the soil, of which some are important plant nutrients (Killham 1994). Proteins, peptides and amino sugars are examples of compounds containing nitrogen, which limits plant growth and biomass production in many terrestrial ecosystems and especially in arctic regions (Nadelhoffer et al. 1997; Jonasson et al. 1999b).

Paradigm change

Until the 1990s it was generally assumed that plants take up nitrogen in inorganic form (Figure 6A). However, more recent studies with isotopic tracers (see below) have shown that some plants are able to take up nitrogen in organic form as well (Chapin and Matthews 1993; Lipson and Näsholm 2001; Schimel and Bennett 2004) thus short circuiting the mineralization step of the classical N cycle model and leading to a new paradigm of the soil N cycling (Figure 6B). Furthermore, many plants may acquire organic nitrogen indirectly through the uptake by their mycorrhizal symbionts. For example, both ecto- and ericoid mycorrhizae can use complex organic nitrogen compounds (Lambers et al. 1998), which may be transferred to the plant in return for carbohydrates. The importance of each of

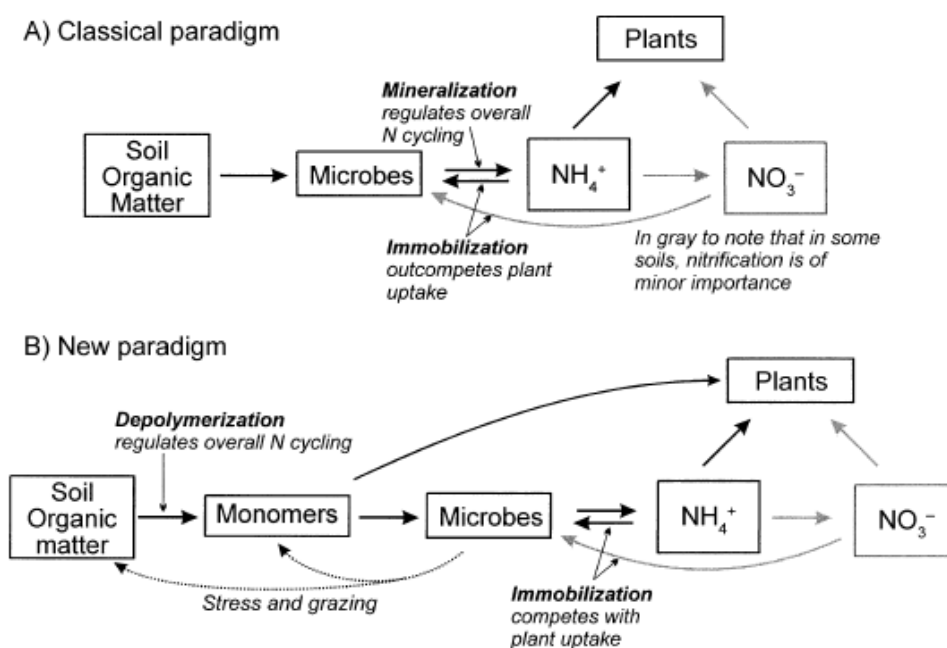


Figure 6. The changing paradigm of the soil N cycle. (A) The dominant paradigm of N cycling up through the middle of the 1990s. (B) The paradigm as it developed in the late 1990s. From Schimel and Bennett (2004).

these processes is still subject for discussion and is therefore currently being intensively investigated.

Timing of nitrogen cycling

Another important aspect of plant nitrogen uptake is the timing of microbial mineralization and plant nutrient acquisition, which was addressed specifically in Paper IV. Because of the link between the cycling of carbon and other elements associated with the SOM pool of the soil the observations of considerable carbon release from arctic ecosystems during the cold season (e.g., Sommerfeld et al. 1993; Zimov et al. 1996; Sommerfeld et al. 1996; Oechel et al. 1997; Fahnestock et al. 1999; Grogan et al. 2001) indicate that the cold-season nitrogen cycling may be important as well. The observations of considerable cold-season net mineralization rates (Hobbie and Chapin 1996; Grogan and Jonasson 2003; Schimel et al. 2004) suggest that this is in fact the case, although the importance of cold-season nutrient uptake as well as possible differences between plant species are still uncertain. The observed differences between winter and summer soil microbial communities (Schadt et al. 2003; Lipson and Schmidt 2004) furthermore suggest that the mineralization processes may differ with season, which possibly affects seasonal patterns of plant nutrient uptake as well.

3.1 Isotopic tracer studies

Isotopes

The study of ecosystem nutrient cycling has developed fast in recent years with the extensive application of stable and radioactive isotopic tracers, mainly ^{15}N and ^{13}C , but also ^{14}C and ^{32}P . Because these isotopes are rare in nature compared to the dominant isotopes, e.g., ^{14}N and ^{12}C , the addition of only small amounts of the heavier isotopes will lead to considerable changes in the isotopic ratios (e.g. the $^{15}\text{N}/^{14}\text{N}$ ratio) when incorporated into residues. After the addition and a certain incubation time the harvest of specific ecosystem pools (e.g. roots, shoots and soil microbes) and subsequent determination of ^{15}N or ^{13}C abundance by mass spectrometry can reveal the fate of the added nitrogen. The addition of different substances labeled with ^{15}N , e.g. $^{15}\text{NH}_4^+$, $^{15}\text{NO}_3^-$ or various amino acids, has provided important knowledge of the preferential N-form taken up by various plant species (Kielland 1994). The seasonality of nitrogen uptake has also been studied by ^{15}N addition and harvest at various times of the year revealing that temperate heath plants are capable of nitrogen uptake throughout the winter (Andresen and Michelsen 2005; Paper IV) and that subarctic heath plants have considerable uptake during late fall in a Northern Swedish birch understory (Grogan and Jonasson 2003).

3.2 Seasonal patterns in N cycling

Snowmelt and freeze-thaw

Especially in the Arctic, the decomposition of dead organic material is slow because of low temperatures and/or water saturation (Post et al. 1982). Plant photosynthesis is usually less restricted than microbial decomposition (Post et al. 1982) leading to a high content of organic matter in arctic soils and a high

biomass of soil microbes. Large amounts of nitrogen is therefore often tied up in the soil microbial biomass (Jonasson et al. 1999a) and periods with large changes in the microbial community could represent events of significant immobilization or mineralization in the ecosystem.

Previous studies have shown that especially the snowmelt period may be a very dynamic period for the soil microbial community (Lipson and Schmidt 2004) with decrease in total microbial biomass (Brooks et al. 1998; Paper IV) and possibly high nutrient turnover. The changes in microbial biomass and composition have been suggested to be caused by freeze-thaw cycles. A single freeze-thaw cycle may kill up to 50 % of the soil microbes (Soulides and Allison 1961) causing high increases in the concentrations of simple sugars and amino acids in the soil (Ivarson and Sowden 1966; Ivarson and Sowden 1970). However, our knowledge of effects of freeze-thaw cycles are primarily based on laboratory studies, where the severity of the applied freeze-thaw events may not always be realistic. With less severe freeze-thaw cycles Lipson et al. (2000) found no effects of freeze-thaw cycles and suggested that substrate supply and temperature were more important factors controlling the microbial community changes during spring in an alpine dry meadow. Using a similar temperature regime, however, Larsen et al. (2002) found decreased microbial biomass carbon but unchanged microbial biomass nitrogen as response to freeze-thaw cycles in subarctic/alpine fellfield and heath mesocosms. The diverging results and the dominance of laboratory studies highlight the need for *in situ* experiments focusing on the effects of soil freeze-thaw cycles on the microbial community and nutrient cycling.

3.3 Results of the nitrogen cycle studies

At the subarctic heath, the possible effects of a changed freeze-thaw regime were addressed by experimentally increasing the frequency of soil freeze-thaw cycles by erecting greenhouses and partly removing the snow during fall and spring for three consecutive years (Papers II and IV).

The effects of increased freeze-thaw frequency on the ecosystem carbon exchange were minimal (Paper II and Figure 5) and there were no effects on the cycling of nitrogen (Paper IV). These results indicate that the ecosystem will react more slowly to future changes in freeze-thaw regime than expected based on earlier laboratory studies and therefore provide support for the results obtained by Lipson et al. (2000).

Paper IV also addressed the general importance of the cold-season nitrogen cycling in the investigated temperate and subarctic heath ecosystems as well as the timing of plant nitrogen uptake throughout the cold season. At the subarctic site, significantly lower amounts of labeled nitrogen was recovered in the soil microbes during spring than during fall indicating considerable net mineralization over winter. The different plant morphological groups showed different temporal patterns in their nitrogen uptake (Figure 7), with significant uptake by evergreen dwarf shrubs and transfer to above-ground tissue already during the first ten days after snowmelt completion. Significant uptake by deciduous dwarf shrubs followed with a time delay of approximately one month, and at the final harvest in the end of June, the graminoids still had not increased their concentration or total amount of labeled nitrogen. The deciduous shrubs and the graminoids therefore seem to depend more on the nitrogen becoming available during the growing season, whereas the evergreens during early spring are capable of exploiting the pool of nutrients released by the soil microbes over winter.

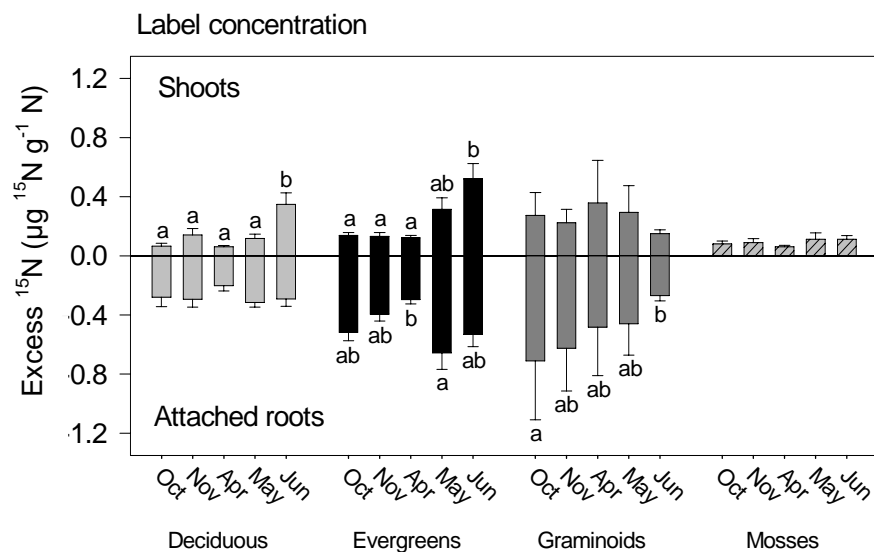


Figure 7. Partitioning among plant functional groups of added ¹⁵N-label at the subarctic heath shown as fraction of total N mass in each pool. Different letters above bars indicate significant differences in recovered label between different times of harvest (P ≤ 0.05; Tukey's test). From Paper IV.

At the temperate heath, all investigated plant groups had a significant uptake of nitrogen throughout the winter although at lower rates than during summer (Figure 8). These results provide further evidence of winter activity by the dominant plant species, *Calluna vulgaris*, as also implied by the measurements of considerable photosynthetic activity during this period.

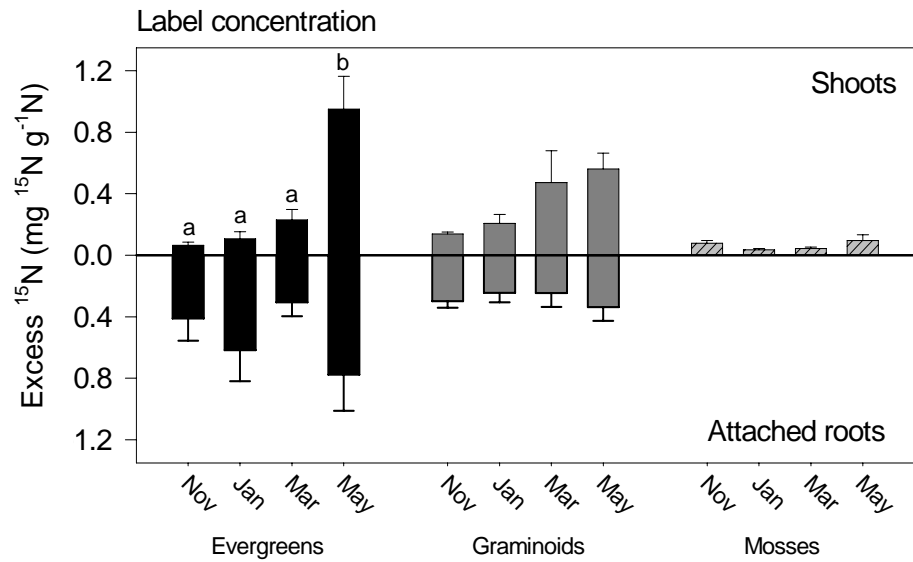


Figure 8. Partitioning among plant functional groups of added ^{15}N -label at the temperate heath shown as fraction of total N mass in each pool. Different letters above bars indicate significant differences in recovered label between different times of harvest ($P \leq 0.05$; Tukey's test). From Paper IV.

4. Conclusions and perspectives

The results from the four papers that comprise this thesis show that cold-season processes are important for the cycling of both carbon and nitrogen in temperate and subarctic heath ecosystems. The findings also imply a close link between the cycling of the two elements during the cold season. Results based on measurements during the growing season alone, may therefore overlook important processes that influence the overall ecosystem function and productivity. While cold-season respiratory activity in arctic ecosystems has been acknowledged through the last decade, it is more surprising that also cold-season photosynthesis may be considerable in both temperate (Paper III) and in subarctic heath ecosystems (Paper II). Also, at the temperate heath all plant groups had significant nitrogen uptake year-round and the evergreen dwarf shrubs at the subarctic site acquired considerable amounts on nitrogen early in the season immediately following snowmelt (Paper IV). The effects of three years of increased freeze-thaw cycles in the subarctic heath were small and suggest that future changes in the freeze-thaw regime may be of minor importance (Papers II and IV). However, it still remains to be clarified whether freeze-thaw cycles are generally less important than usually believed in controlling the springtime transition from a winter to a summer microbial community.

The ecosystem respiration model approaches in Paper I and III provides support for the recent critique of classic first-order exponential equations based on temperature alone by showing that models may be improved by incorporating substrate supply during early spring in a subarctic heath (Paper I) and photosynthesis rates in a temperate heath (Paper III). The model approach may help determine other important drivers of respiration than temperature and also provides an easily applied, non-intrusive tool for investigating seasonal patterns in the respiratory costs of photosynthesis. The lower, and mechanistically more sound, temperature sensitivity of the modified ecosystem respiration model presented in Paper III also implies that the expected positive feedback of ecosystem carbon loss to a warmer future climate may be lower than projected based on temperature-based models alone.

Future studies should aim to increase the temporal resolution, e.g. of indicators of soil microbial substrate supply, in order to capture important events throughout the cold season and depict plant species differences in photosynthetic activity as well as in nutrient uptake. Especially in the Arctic, a high temporal resolution is necessary during the dynamic snowmelt period, not least for improving the modeling of ecosystem respiration, as shown in Paper I. The relationship between ecosystem photosynthesis and respiration also needs further investigation and the model presented in Paper III should be tested for other ecosystems.

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Paper I

Respiration and microbial dynamics in two sub-arctic ecosystems during winter and spring-thaw: Effects of increased snow depth

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Respiration and microbial dynamics in two sub-arctic ecosystems during winter and spring-thaw: Effects of increased snow depth

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Abstract

Recent evidence suggests that biogeochemical processes in the Arctic during late winter and spring-thaw strongly affect the annual cycling of carbon and nutrients, indicating high susceptibility to climate change. We therefore examined the carbon and nutrient dynamics in a sub-arctic heath and a birch forest with high temporal resolution from March until snow-melt at both ambient and experimentally increased snow depths.

Ecosystem respiration (ER) from mid March to snow-melt at ambient snow was high, reaching 99 ± 19 (birch) and 67 ± 1.4 g C m⁻² (heath). Enhanced snow depth by about 20–30 cm increased ER by 77–157% during late winter but had no effects during spring-thaw. ER rates at the birch site were poorly described by classic first-order exponential models ($R^2=0.06-0.10$) with temperature as a single variable, but model fit improved considerably by including the supply of dissolved organic carbon (DOC) or nitrogen (DON) in the model ($R^2=0.40-0.47$). At the heath, model fit with temperature as the single variable was better ($R^2=0.38-0.52$), yet it improved when the supply of DOC or DON was included ($R^2=0.65-0.72$).

Microbial carbon decreased by 43% within a few days after the first soil freeze-thaw event, while microbial nitrogen and phosphorus decreased more slowly. Because soil inorganic nitrogen and phosphorus concentrations were low, nutrients released from lysed microbial cells may have been sequestered by surviving microbes or by plants resuming growth. The fast change in microbial biomass and the dependence of ER on substrate availability stress the need for high temporal resolution in future research on ecosystem carbon and nutrient dynamics at snow-melt in order to make robust models of their turnover.

Introduction

Biological activity in plants and soil microbes of the Arctic was traditionally assumed to be very low during the cold season due to long periods with subzero air and soil temperatures. Appreciable CO₂ emissions have, however, been observed in field studies at soil temperatures at least down to -5°C (Brooks et al., 1997) because the thermal insulation of snow cover restricts winter soil temperature minima despite low air temperatures. Consequently, wintertime respiration constitutes 10–50% of the total annual respiration in a variety of arctic/alpine ecosystems (Sommerfeld et al., 1993; Clein and Schimel, 1995; Zimov et al., 1996; Oechel et al., 1997; Alm et al., 1999; Elberling and Brandt, 2003; Grogan and Jonasson, 2005). At the same time, net mineralization of nutrients in arctic ecosystems often appears to be higher during the cold season than during summer (Hobbie and Chapin, 1996; Schimel et al., 2004), which evidently is related to major structural and functional changes in the microbial community (Schadt et al., 2003; Schimel et al., 2004; Lipson and Schmidt, 2004). These recent findings suggest that ecosystem functioning is fundamentally different during the winter and summer seasons, yet we are still far from understanding the dynamics of winter soil processes, such as organic matter turnover and nutrient cycling (Brooks et al., 1997; Schimel et al., 2004).

Except for low air temperatures, the distribution of snow may be the single most important factor controlling the cold season respiration in these ecosystems (Walker et al., 1999). Snow insulates the soil, reduces temperature fluctuations and constrains minimum temperatures (Brooks et al., 1997; Groffman et al., 2001). General circulation models predict increasing precipitation at high latitudes during winter (Maxwell 1992; Kattenberg et al. 1996; Giorgi and Francisco, 2000; Hulme et al., 2000), which will lead to increased snow cover in some regions, and decreased cover in others if precipitation falls as rain. In currently low-statured vegetation like arctic tundra, increased shrub growth due to climate warming (Chapin et al., 1996) may also act to increase snow depths by as much as 10–25% due to increased wind shelter (Sturm et al., 2001). Manipulating natural snow depths with snow fences, as has been done at Toolik Lake, Alaska, and Niwot Ridge, Colorado (Walker et al., 1999) may therefore provide important new insight in snow-ecosystem dynamics. In the Toolik Lake experiment, the fences typically create a drift with a depth of up to 3 m, compared to ambient snow depths of 20–40 cm, resulting in dramatically increased soil temperatures during winter, higher winter respiration, prolonged period of snow cover and changes in nutrient cycling (Schimel et al., 2004; Wahren et al., 2005). Similar strong effects have been observed at Niwot Ridge where the fences typically enhance snow depths by about 80 cm from ambient levels of 30–80 cm (Williams et al., 1998).

In this study, we established a much more moderate increase in snow accumulation, comparable to current inter-annual variability, in order to mimic a realistic scenario of

changed future snow depth in a sub-arctic dwarf shrub heath and a birch forest, the two dominant vegetation types of Northern Scandinavia (Sjörs 1971). We measured the late winter and spring-thaw ecosystem CO₂ efflux and the soil and microbial pools of carbon, nitrogen and phosphorus with high temporal resolution. The purpose was to: A) quantify the biogeochemical transformations at short time-steps during the transition from winter to spring, which has been suggested to be a very dynamic period for soil microbial processes (Lipson and Schmidt, 2004), and B) quantify the effects of moderately increased snow depth. We expected that relatively moderate increases in the snow depths would increase soil temperatures, CO₂ flux rates, soil and microbial nutrient pools and enhance nutrient transformation rates, with most pronounced effects at the heath, where ambient snow cover normally is lowest.

Methods

SITE DESCRIPTION AND EXPERIMENTAL DESIGN

The experiment took place near Abisko in Northern Swedish Lapland at 68° 20' N, 18° 47' E (heath site) and 68° 20' N, 18° 50' E (birch site). The elevation is approximately 430 m a.s.l. Climate is sub-arctic/alpine and records (1970–2000) from a nearby climate station show a mean annual temperature of –0.5°C. The mean air temperatures in winter (December – February) and spring (March – May) are –9.9 °C and –2.3°C, respectively. Annual precipitation is 315 mm, with 75 mm falling in the winter and 41 mm in the spring. The two sites have a non-significant difference in depth of the organic soil layer with means ± standard errors of 12.1±0.7 cm and 10.7±0.4 cm at the heath and birch site, respectively, (t-test; $n = 72/140$, $P = 0.08$). The organic soil layer overlays stones and bedrock, and a mineral soil layer is often absent. Soil organic matter (SOM) content ranged from 69 to 95% and 88 to 95% of dry soil mass at the birch and heath sites, respectively, while soil water content of dry soil mass was 472–829% at the birch site and 324–721% at the heath.

Dominant species at the heath are the ericoid dwarf shrubs *Empetrum nigrum* ssp. *hermaphroditum*, *Vaccinium uliginosum*, *Vaccinium vitis-idaea*, *Andromeda polifolia* and *Rhododendron lapponicum*. Other common species are *Arctostaphylos alpinus*, *Cassiope tetragona*, *Tofieldia pusilla*, *Carex vaginata*, *Betula nana* and various mosses. The species composition at the birch (*Betula pubescens* ssp. *czerepanovii*) site differs mainly by *Vaccinium myrtillus* as an additional dominant species. Plant biomasses were not determined, but in similar vegetation types within 100–1000 m from the sites in this study, plant biomasses were 1426±96 g m⁻² and 1792±92 g m⁻² in the birch understory and the heath vegetation, respectively, with approximately 30% above-ground and 70% below-ground in both ecosystems (Grogan and Jonasson, 2005).

On 11 January 2000, when the snow depths still were <5 cm, 12 plots of about 150 m² were selected at each site. At six of the plots per site, we installed 1.2 m high snow fences made by semi-permeable plastic, while the other six plots served as controls. At the heath site, each fence was 6 m long and was erected perpendicular to the expected prevailing winds. A prevailing wind direction could not be predicted at the more sheltered birch site. We therefore erected 12 m long fences with a 90° bend at the middle. One fence in the heath fell down after the first measurement, and this plot was hereafter excluded from the experiment. On 9 and 10 March, 12 temperature loggers (Gemini Tinytags) were placed in three fenced and three control plots at each site at 5 cm soil depth, and temperature was logged every 10 minutes from 10 March to 18 May 2000.

CHAMBER CO₂ FLUX MEASUREMENTS

Closed chamber CO₂ flux measurements were done once during late winter (medio March) and six and seven times during spring-thaw (April-May) in the birch and heath ecosystems, respectively. We used a LICOR 6200 Infrared Gas Analyzer attached to a 35.5 L Perspex chamber with a basal area of 1076 cm² and equipped with a fan to insure proper air mixing. During measurements, the rate of evapotranspiration usually was below 0.01 μmol H₂O m⁻²s⁻¹. We therefore set the air flow through the desiccant to zero and calculated the CO₂ fluxes using the original LI-COR equations (LI-COR 1990; Hooper et al., 2002).

At each date of measurements, the gas fluxes were measured at new, random positions within the plots, to make sure that they were always done in places where the snow had been undisturbed. Rather than measuring fluxes on the snow surface, which may be influenced by snow physical structure (e.g. ice layers) and CO₂ storage, we removed the snow prior to measurements in order to measure instant respiration instead of instant release. Previous test studies have shown a pulse of CO₂ from the soil after snow removal due to changes in the CO₂ level and diffusion rates at the soil-atmosphere interface, but with reduced and stable flux rates after a maximum time of 25 minutes (Grogan et al., 2001; Grogan and Jonasson, 2005). The snow was therefore always removed 45–90 min before measurements were done. We monitored the soil temperatures from the time of snow removal until measurements and did not observe soil temperature changes at any time.

Snow was packed tightly to the sides of the chambers to seal the chamber air from the atmosphere. Sunlight was excluded by covering the chambers with a double layer of black plastic, and the ecosystem respiration (ER) was measured as the CO₂ flux over six successive 20 s intervals and averaged (Alm et al., 1999; Grogan et al., 2001; Grogan and Jonasson, 2005). Further details on the methodology of the flux measurement are given by Grogan and Jonasson (2005).

SOIL COLLECTION AND ANALYSES

In four plots at each site on every measurement day, a 10 x 10 cm soil sample was sawed out of the frozen soil to a depth of 5 cm after the CO₂ measurements. The soil samples were enclosed in plastic bags, kept cool (2–5°C) and sorted within 48 hours.

As many roots as possible were removed by hand sorting during a standardized time of 30 minutes, and the soil was divided into three sub-samples. One sub-sample of 30 g was used for determination of water content by drying for 24 hours at 90°C. A second sub-sample of 10 g was immediately extracted in 50 ml of 0.5 M K₂SO₄ to recover soil inorganic N and P and dissolved organic C (DOC) and N (DON). After the K₂SO₄ addition, the samples were shaken, filtered through a Whatman GF-D filter, and kept frozen at –18 °C until analysis. The third sub-sample of 10 g was fumigated for 24 h in chloroform (CH₃Cl) vapour to release nutrients from the microbial biomass (Jenkinson and Powlson, 1976), after which the samples were extracted and handled in the same way as the unfumigated samples.

The extracts were analysed on a Shimadzu TOC-5000A total organic C analyzer for DOC and extractable microbial C, estimated as the difference between the DOC content in fumigated and unfumigated samples.

The NH₄⁺-N content was determined by the indophenol method, inorganic P by the molybdenum blue method and NO₃⁻-N with the cadmium reduction method (Allen 1989). Sample values for NO₃⁻-N were mostly below the detection limit of about 0.05 µg g⁻¹ SOM, and the soil NO₃⁻-N content was therefore considered to be negligible.

Ten ml sub-samples of all extracts were used for estimates of extractable microbial N content. The extracts were digested for four hours in 10 ml concentrated H₂SO₄ and selenous acid mixture with 1 ml of H₂O₂ added to reduce all N to NH₄⁺-N. After digestion, the sample tubes were filled with distilled water to 100 ml, and the diluted extracts were analysed using the indophenol method. Extractable microbial N was determined by subtracting the concentration in the non-fumigated, digested sample from the concentration in the fumigated, digested sample (Brookes et al., 1982, 1985a, b; Vance et al., 1987). The extractable microbial P was determined by subtracting the P content of the unfumigated, undigested sample from the P content of the fumigated, undigested sample (Brookes et al., 1982; Jonasson and Michelsen, 1996).

STATISTICAL ANALYSES AND DATA PROCESSING

Statistical analyses were conducted using the GLM procedure (SAS Institute ver. 8.0). Since all CO₂ flux measurements and the soil samplings were done at new positions within plots at each date, repeated measurement Anova (Analysis of variance) was not used. Instead, an overall three-way Anova was used with site (birch vs. heath), treatment (control

vs. snow-fenced), and time as main effects and their interactions. Effect of site was always significant and two-way Anova was therefore used to test the effects of time and treatment and their interactions at the individual sites. Since the study included one measurement during winter and the remaining measurements during spring-thaw, two-way Anova also was conducted to test for effects of site, treatment, and their interactions at individual measurement dates.

Some data were transformed to pass Brown and Forsythe's test for homogeneity of variance. When transformation failed to homogenize variances between groups, nonparametric one-way Anova (NOA) was used to test for differences on individual measurement dates. Data from the two sites were tested together to examine differences between sites, and separately to test for differences between fenced and control plots within each site. T-test was used to test for selected differences when appropriate.

In order to investigate the major controls of ecosystem respiration, we related it to soil temperature and chemistry by fitting a classic first-order exponential equation (van't Hoff 1898) in the form:

$$ER = A \exp^{(BT)}$$

to the data, where A ($\text{g C m}^{-2} \text{ d}^{-1}$) is the respiration rate at 0 °C representing an index of substrate availability, B ($^{\circ}\text{C}^{-1}$) is a constant representing the temperature sensitivity of respiration and T is temperature in °C (Grogan and Jonasson, 2005).

However, it has recently been suggested that robust models of ecosystem respiration need to incorporate also the effects of substrate supply and desiccation stress (Davidson et al., 2006). We therefore also tested models that incorporated simple, linear relationships between respiration and dissolved organic carbon (DOC, mg g^{-1} SOM) and nitrogen (DON, mg g^{-1} SOM), as indicators of substrate availability, and soil water content (SW, % of wet soil) in the form:

$$\text{Respiration rate} = (A + c \times \text{DOC} + d \times \text{DON} + e \times \text{SW}) \exp^{(BT)}$$

where c, d and e are constants in flux units times the inverse of the units of the variable they are associated with. In this model, A integrates the respiration at 0 °C with any offset (y-axis intercept different from zero) of the inferred linear relationships between respiration and DOC, DON and SW. We ran the model with various combinations after removal of one or several of the variables in order to find the models with best fit. Similar models with microbial biomass C, N or P were also tested, but did not produce good model fits and are therefore not presented.

Results

SNOW DEPTH AND SOIL TEMPERATURE

The snow fences caused a moderate increase of the snow cover by about 20–30 cm added to the depth of 74 ± 3 and 27 ± 3 cm at the time of maximum snow depth at the birch and heath site, respectively. The increased depth was within the range of current inter-annual variation and, hence, simulated a realistic future snow depth within the limits of climate change projections (Giorgi and Francisco, 2000). However, the temporal patterns in the distribution of snow and the effects on soil temperatures varied between the two sites. On 9 March, both snow depths and soil temperatures were similar in controls and snow-fenced plots at the birch site (Fig. 1a, b). Until 24 April there was an increase in snow depth

FIGURE 1

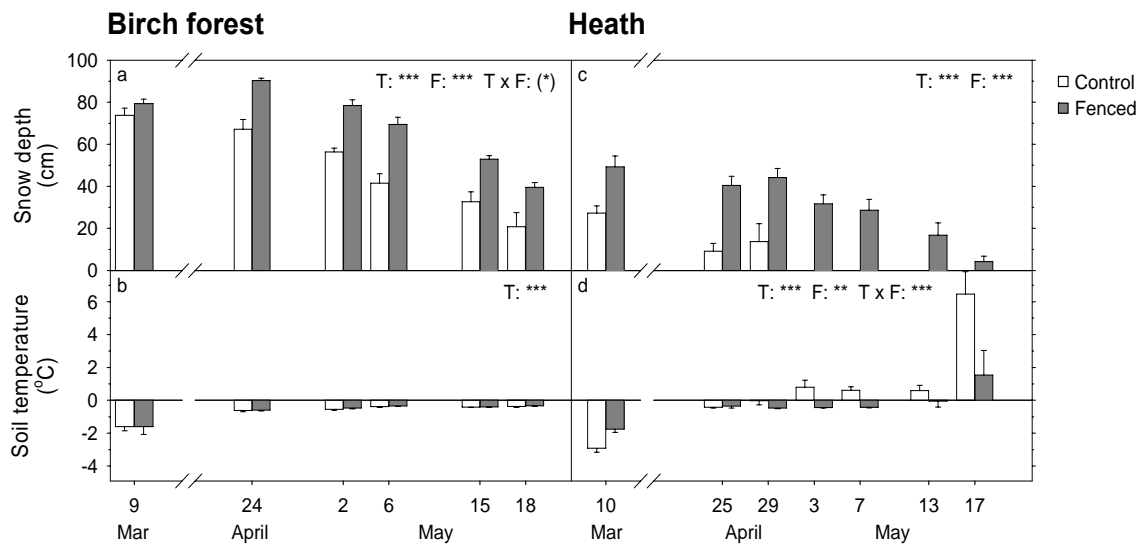


FIGURE 1. Snow depths (a, c) and soil temperature at 5 cm soil depth (b, d), in the birch and heath ecosystems (means + 1 SE of 6 replicated samples except for the heath in April/May with 5 samples). Differences at $P \leq 0.1$ from two-way Anova of effects of time (T) and snow fence (F) and their interactions (T x F) are shown in the upper right corner of each panel. Significance levels are: (*), $P \leq 0.1$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

in the fenced plots resulting in 18–27 cm deeper snow than in the controls with the difference lasting throughout the remaining study period (Fig. 1a, Treatment_{1,60}, $F = 88.1$, $P < 0.0001$). At no time at the birch site did the differences in snow depth lead to differences in soil temperatures, which were just below 0°C and only increased slowly throughout the

period (Fig. 1b). The temperature loggers recorded neither thaw nor freeze-thaw cycles during the study period (Table 1).

At the more wind-exposed heath site, the snow depth was significantly lower than at the birch site through the entire period (Fig. 1a, c; NOA, $P < 0.0001$ at all dates). Until snow-melt was completed, there was significantly deeper snow in fenced plots than in controls on all measurement days, ranging from a difference of 22 cm on 10 March to 31 cm on 25 April (Fig. 1c, Treatment_{1,58}, $F = 98.2$, $P < 0.0001$). In contrast to the birch site, where snow still remained on 18 May, snow-melt in the heath controls was completed already on 3 May. In the fenced plots, however, some snow remained on the last measurement day on 17 May, and completion of snow-melt was therefore delayed by more than 14 days.

TABLE 1

Mean soil temperatures (°C) at 5 cm soil depth and recorded freeze-thaw cycles from 10 March to 19 May 2000. Temperature was logged with 10-minute-intervals, and a freeze-thaw cycle was defined as thaw ≥ 3 hours followed by freezing ≥ 3 hours. SE are shown in brackets, $n = 3$. Significant effects of snow fences on temperature tested by t-test are shown with the P -levels: (*), $P \leq 0.1$; *, $P \leq 0.05$; **, $P \leq 0.01$.

	Birch forest (control)	Birch forest (fenced)	Heath (control)	Heath (fenced)
Mean March	-1.15 (0.12)	-1.31 (0.20)	-2.11 (0.20)	-0.99 (0.06) **
Mean April	-0.64 (0.03)	-0.84 (0.16)	-1.24 (0.19)	-0.71 (0.06) (*)
Mean May	-0.60 (0.00)	-0.60 (0.00)	0.67 (0.68)	0.05 (0.29)
Max March	-0.60 (0.00)	-0.60 (0.00)	-0.60 (0.00)	-0.60 (0.00)
Max April	-0.60 (0.00)	-0.60 (0.00)	-0.60 (0.00)	-0.60 (0.00)
Max May	-0.60 (0.00)	-0.60 (0.00)	6.20 (2.96)	7.27 (1.97)
Min March	-1.93 (0.27)	-2.80 (0.60)	-4.00 (0.52)	-1.93 (0.27) *
Min April	-1.13 (0.27)	-1.13 (0.27)	-4.30 (1.31)	-1.40 (0.46)
Min May	-0.60 (0.00)	-0.60 (0.00)	-0.60 (0.00)	-0.60 (0.00)
First freeze-thaw	none	none	1 May	5 May
No. of freeze-thaw cycles	0.0 (0.0)	0.0 (0.0)	7.3 (4.1)	5.7 (2.7)

On 10 March, the soil temperatures in the fenced plots at the heath were significantly higher than in controls (Fig. 1d; NOA, $P = 0.0042$). The temperature loggers in the fenced plots never recorded soil temperatures lower than -2°C , while soil temperatures sometimes decreased to below -4°C in the controls in both March and April (Table 1). The differences were significant in March (Table 1, t-test, $P = 0.0055$) and near significant ($P = 0.0585$) in April. The temperature loggers recorded the first freeze-thaw cycle on 1 May and 5 May in control and fenced plots, respectively. In total, the 7.3 ± 4.1 and 5.7 ± 2.7 freeze-thaw cycles during the period from 1 to 19 May in the controls and fenced plots, respectively, were not significantly different (Table 1).

CO₂ FLUXES

The ecosystem respiration (ER) rates (Fig. 2) were generally significantly higher at the birch site than at the heath (Vegetation_{1,118}, $F = 23.6$, $P < 0.0001$), and the temporal patterns were different at the two sites (Vegetation \times time_{5,118}, $F = 8.2$, $P < 0.0001$). On 9-10 March, there was a significant effect of the snow fences on ER rates (Treatment_{1,20}, $F = 7.4$, $P = 0.013$), which were 77% and 157% higher in fenced plots than in the controls of the birch and heath sites, respectively. In the April-May period, there was no significant effect of different snow depths on ER in either vegetation type (Treatment_{1,20}, $F < 2.6$, and $P > 0.12$ on all dates).

FIGURE 2

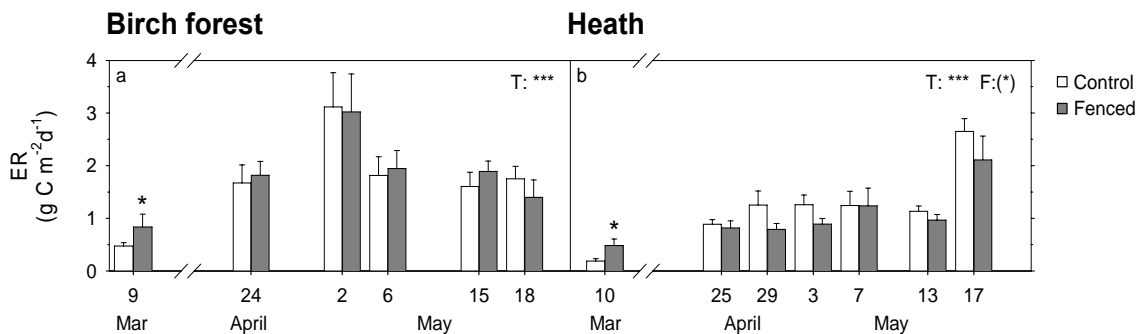


FIGURE 2. Ecosystem respiration (ER), in the birch (a) and heath (b) ecosystem (means + 1 SE). Sample sizes, effect abbreviations and significance levels are denoted as in Fig. 1. The asterisks on 9-10 March denotes significant effect of snow fence tested by two-way Anova of effects of vegetation and snow fence on individual measurement dates.

The ER rates at the birch site was poorly described by the simple temperature-dependent exponential equation in both control and fenced plots (Table 2, model 1, $R^2 = 0.10$ and 0.06 , respectively), as opposed to a better model fit at the heath site (Table 2, model 1, $R^2 = 0.52$ and 0.38 , respectively). However, the model fit was significantly improved, especially at the birch site, when either DOC or DON were added to the equations as indicators of substrate availability (Table 2, models 4 and 5, $R^2 = 0.40$ and 0.47 with DOC included and 0.65 and 0.72 with DON included at the birch and heath, respectively). The more complex models, which included more variables (Table 2, models 2 and 3), did not significantly improve the model fits further compared to models 4 and 5.

We were unable to estimate the respiratory carbon loss during the full study period at the birch site by using the various exponential equations, because of the poor model fit of the simple temperature-dependent exponential equation (model 1), and because temperature

was the only parameter that was logged continuously. However, linear extrapolation over the measurements at the different dates estimated the respiration of the birch ecosystem to 99 ± 19 g C m⁻² in the controls and to 112 ± 21 g C m⁻² in plots with increased snow depths.

At the heath site, total respiratory carbon loss using model 1 was 67 ± 1.1 and 68 ± 1.3 g C m⁻² in controls and fenced plots, respectively, indicating no overall significant effect of increased snow depth during the study period. However, during the 20 days in March, when soil temperatures were recorded, modelled respiratory losses were significantly different (*t*-test, $P = 0.02$) reaching 17.6 ± 0.4 g C m⁻² in controls and 19.2 ± 0.2 g C m⁻² in plots with increased snow depths.

TABLE 2

Mean coefficients (SE in brackets), explained model variances (R^2) and sample sizes (n) for first-order exponential relationships between ecosystem respiration and soil temperature, dissolved organic carbon and nitrogen (DOC and DON, respectively) and soil water (SW) in various combinations. See text for explanations and units of model parameters.

Model		Birch forest (control)	Birch forest (fenced)	Heath (control)	Heath (fenced)
(1)	A	2.40 (0.49)	2.22 (0.36)	1.05 (0.10)	1.05 (0.09)
	B	0.53 (0.35)	0.35 (0.28)	0.12 (0.02)	0.16 (0.02)
	R^2	0.10	0.06	0.52	0.38
	n	36	36	36	36
ER = A exp ^(BT)					
(2)	A	-1.33 (2.39)	-3.42 (3.31)	3.80 (2.37)	11.11 (4.97)
	B	0.86 (0.43)	0.12 (0.25)	0.07 (0.03)	1.10 (0.24)
	c	0.59 (0.47)	0.64 (0.25)	-0.09 (0.09)	0.17 (0.16)
	d	2.20 (1.20)	1.07 (1.06)	0.87 (1.10)	0.34 (0.74)
	e	3.13 (2.84)	4.74 (3.83)	-3.23 (2.92)	-11.46 (5.92)
	R^2	0.48	0.51	0.72	0.68
	n	23	24	20	21
ER = (A + c × DOC + d × DON + e × SW) exp ^(BT)					
(3)	A	1.16 (0.67)	0.69 (0.48)	1.19 (0.17)	1.52 (0.34)
	B	0.73 (0.38)	0.17 (0.25)	0.09 (0.03)	1.36 (0.26)
	c	0.55 (0.43)	0.56 (0.25)	-0.09 (0.08)	0.06 (0.02)
	d	2.28 (1.13)	1.56 (1.04)	0.46 (0.80)	0.34 (0.86)
	R^2	0.47	0.5	0.71	0.64
ER = (A + c × DOC + d × DON) exp ^(BT)					
(4)	A	0.87 (0.70)	0.54 (0.47)	0.98 (0.12)	1.50 (0.24)
	B	0.87 (0.41)	0.18 (0.25)	0.13 (0.02)	1.47 (0.17)
	c	1.03 (0.47)	0.82 (0.20)	-0.02 (0.05)	0.11 (0.14)
	R^2	0.40	0.47	0.72	0.65
	n	24	24	28	28
ER = (A + c × DOC) exp ^(BT)					
(5)	A	1.93 (0.43)	1.56 (0.38)	1.04 (0.11)	1.61 (0.23)
	B	0.59 (0.34)	0.26 (0.27)	0.12 (0.02)	1.33 (0.24)
	d	2.88 (1.05)	3.17 (0.91)	-0.01 (0.4)	0.37 (0.82)
	R^2	0.44	0.41	0.71	0.66
	n	23	24	20	21
ER = (A + d × DON) exp ^(BT)					

SOIL AND MICROBIAL POOLS OF C, N AND P

The microbial biomass carbon (MBC; Fig. 3) was significantly lower at the heath than at the birch site (Vegetation_{1,78}, $F = 28.5$, $P < 0.0001$) and the temporal patterns were different in the two vegetation types (Vegetation \times Time_{5,78}, $F = 8.8$, $P < 0.0001$). This was mainly due to a 43% reduction between 25 April and 3 May (t-test, $P = 0.03$) in the control plots at the heath as they became snow-free, while MBC was constant or even tended to increase in both controls and fenced plots at the birch site. MBC stayed on the significantly lower level at the heath controls throughout the study period (Treatment_{1,42}, $F = 40.4$, $P < 0.0001$).

FIGURE 3

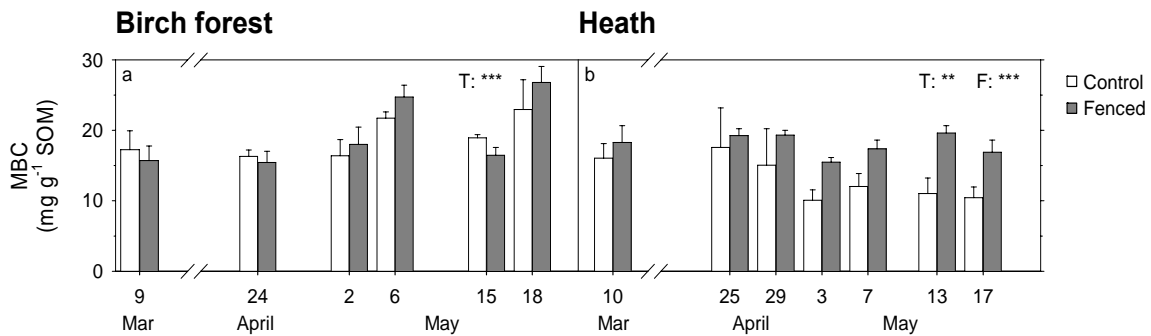


FIGURE 3. Extractable microbial biomass carbon (MBC) in the birch (a) and heath (b) ecosystems (means + 1 SE; $n = 4$). Effect abbreviations and significance levels are denoted as in Fig. 1.

The contents of nitrogen and phosphorus in the microbial biomass (MBN and MBP; Table 3) also were significantly higher at the birch site than at the heath (Vegetation_{1,78}, $F > 25.7$, $P < 0.0001$ for both), and both decreased significantly with time at the heath (Time_{1,42}, $F = 3.77$, $P = 0.0043$ and $F = 6.45$, $P < 0.0001$, respectively). However, the decrease was much less pronounced than the decline of MBC in the controls, and there was no significant treatment effect.

The concentrations of soil NH_4^+ -N (Table 3) were very low and at similar levels in both ecosystems, except for significantly higher NH_4^+ -N content at the birch than at the heath site on 9-10 March (NOA, $P = 0.0109$). The inorganic P showed more pronounced differences (Table 3), with higher concentrations in the birch plots on all measurement days (NOA, $0.0001 < P < 0.0013$) except 13-15 May (NOA, $P = 0.1239$). No treatment effect was observed for any of the nutrients, but P concentrations decreased significantly with time at the birch site (Time_{5,36}, $F = 4.45$, $P = 0.003$).

TABLE 3

Mean concentrations of soil microbial biomass N (MBN) and P (MBP), dissolved organic C (DOC) and N (DON) and of soil NH_4^+ and inorganic P in control and fenced plots at all measurement days. Data are means with SE in brackets, $n = 6$ except for at the heath in April/May with $n = 5$. b.d. = below detection limit.

	MBN (mg g^{-1} SOM)		MBP (mg g^{-1} SOM)		$\text{NH}_4^+\text{-N}$ ($\mu\text{g g}^{-1}$ SOM)	
	control	fenced	control	fenced	control	fenced
Birch forest						
9 Mar	0.85 (0.10)	0.75 (0.09)	0.44 (0.08)	0.36 (0.06)	3.6 (0.3)	4.6 (2.5)
24 Apr	0.75 (0.06)	0.71 (0.03)	0.37 (0.02)	0.27 (0.05)	5.3 (1.7)	2.0 (0.7)
2 May	0.48 (0.06)	0.68 (0.19)	0.38 (0.04)	0.31 (0.04)	10.0 (4.4)	11.6 (6.8)
6 May	0.46 (0.07)	0.91 (0.12)	0.29 (0.03)	0.39 (0.01)	b.d.	9.9 (6.3)
15 May	0.68 (0.06)	0.55 (0.09)	0.37 (0.03)	0.35 (0.07)	b.d.	b.d.
18 May	0.67 (0.24)	0.56 (0.13)	0.28 (0.05)	0.38 (0.09)	10.3 (5.4)	3.8 (2.8)
Heath						
10 Mar	0.51 (0.10)	0.53 (0.05)	0.25 (0.02)	0.27 (0.04)	b.d.	b.d.
25 Apr	0.67 (0.12)	0.51 (0.06)	0.36 (0.04)	0.34 (0.05)	12.2 (7.2)	3.2 (1.1)
29 Apr	0.41 (0.06)	0.53 (0.03)	0.26 (0.02)	0.29 (0.02)	2.5 (1.6)	4.3 (2.8)
3 May	0.50 (0.09)	0.64 (0.05)	0.28 (0.02)	0.31 (0.01)	14.5 (7.8)	10.0 (6.6)
7 May	0.37 (0.03)	0.30 (0.10)	0.23 (0.02)	0.21 (0.03)	b.d.	b.d.
13 May	0.39 (0.04)	0.51 (0.05)	0.23 (0.01)	0.27 (0.02)	3.9 (1.6)	b.d.
17 May	0.27 (0.07)	0.45 (0.08)	0.18 (0.02)	0.21 (0.03)	b.d.	5.3 (5.6)
	DOC (mg g^{-1} SOM)		DON ($\mu\text{g g}^{-1}$ SOM)		Inorganic P ($\mu\text{g g}^{-1}$ SOM)	
	control	fenced	control	fenced	control	fenced
Birch forest						
9 Mar	1.953 (0.37)	1.435 (0.27)	78.5 (16.9)	69.9 (23.6)	53.5 (13.1)	51.0 (9.9)
24 Apr	2.658 (0.50)	1.946 (0.29)	146.7 (26.3)	96.0 (8.7)	66.8 (7.7)	41.8 (12.3)
2 May	2.101 (0.30)	2.692 (0.90)	604.9 (143)	472.9 (229)	67.5 (15.6)	55.8 (8.2)
6 May	1.484 (0.39)	2.502 (0.46)	40.8 (21.2)	175.0 (67.2)	39.3 (9.2)	40.2 (8.5)
15 May	1.528 (0.34)	1.147 (0.23)	73.0 (34.7)	49.4 (28.6)	16.9 (10.0)	20.2 (9.4)
18 May	2.021 (0.34)	2.004 (0.44)	188.5 (91.1)	415.3 (61.9)	21.3 (13.5)	33.1 (3.4)
Heath						
10 Mar	1.832 (0.58)	1.431 (0.32)	22.5 (22.5)	9.4 (6.0)	17.5 (9.6)	9.1 (2.4)
25 Apr	3.341 (1.62)	2.306 (0.37)	179.1 (86.7)	88.9 (24.3)	18.0 (5.5)	10.5 (1.9)
29 Apr	1.203 (0.25)	1.959 (0.40)	29.2 (20.1)	44 (34.4)	7.3 (1.1)	10.4 (1.5)
3 May	1.341 (0.32)	1.548 (0.21)	40.2 (28.7)	28.6 (17.5)	10.3 (2.5)	10.9 (0.7)
7 May	1.176 (0.26)	1.552 (0.19)	67.6 (22.2)	207.1 (148.6)	7.6 (1.1)	9.3 (0.7)
13 May	1.626 (0.63)	1.837 (0.48)	43.3 (26.6)	26.7 (15.4)	7.5 (1.5)	8.6 (0.8)
17 May	0.866 (0.15)	1.396 (0.30)	224.5 (92.6)	275.6 (47.3)	b.d.	b.d.

Discussion

ECOSYSTEM RESPIRATION DURING WINTER AND SPRING-THAW

It is well-documented that soil respiration rates in late winter depend on the timing of snow accumulation in late fall and early winter, because the snow constraints both temperature fluctuations and soil cooling, providing improved conditions for microbial decomposition (Brooks et al., 1998; Brooks and Williams, 1999; Olsson et al., 2003; Schimel et al., 2004). Snow build-up later in the season is usually thought to be less important. In our study, however, despite a long and cold period of bare ground in early and mid winter, lasting until mid January, the increased snow depth in fenced plots yet increased the respiration rate in March by more than 70% in the birch forest and by more than 150% in the heath. In contrast, there were no effects of increased snow depths on respiration during snow-melt in April/May.

Soil temperatures in March were significantly increased by increased snow depths from 30 to 50 cm at the heath, while there was no temperature difference between heath soil with 50 cm snow depth and the birch soil with about 80 cm deep snow. Our results, therefore, suggest that 40–50 cm of snow resulted in uncoupling of soil and air temperatures, which is slightly higher than the approximately 30 cm inferred from other studies (Brooks et al., 1997; Monson et al., 2006). The lack of effect of increased snow on soil temperatures during spring-thaw probably was due to higher air temperature during this period, which reduces the importance of snow as insulator and protector against extremely low temperatures. Indeed, the soil temperatures were just below 0°C in both the birch and heath sites despite a wide range of different snow depths. In contrast to the minimal effect of increased snow on soil temperatures and ER during spring-thaw, an important side-effect of the increased snow cover at the heath site was the delayed completion of snow-melt, because the length of the snow-free period strongly affects the ecosystem C balance during the growing season (Tieszen 1978; Soegaard and Nordstroem, 1999; Soegaard et al., 2000; Christensen et al. 2001; Monson et al., 2006).

The soil temperatures were above the empirical threshold of about –5°C for appreciable microbial activity (Brooks et al., 1997) throughout the entire study period in both controls and plots with increased snow. Indeed, the total respiration during the 66 days from 10 March to 17 May in the heath controls of $67 \pm 1.4 \text{ g C m}^{-2}$ is high compared with estimated growing season C sequestration in the net primary production of 125 g C m^{-2} at a similar heath nearby (Jonasson et al., 1999a). This demonstrates the importance of ecosystem respiration in late winter and during spring-thaw for the annual carbon budget.

Grogan and Jonasson (2005) estimated winter-time (late October to late May) respiration to $128 \pm 25 \text{ g C m}^{-2}$ in a birch understory and $65 \pm 12 \text{ g C m}^{-2}$ in a nearby heath ecosystem similar to ours by using first-order exponential models of annual ecosystem

respiration and soil temperature. The explained variances in the models were high ranging from 0.76 to 0.92. If applying their equations to our data, the estimated carbon loss at our heath would be only $29 \pm 1 \text{ g C m}^{-2}$ (57% reduction compared to model 1 estimate). The annual model, therefore, may underestimate ecosystem respiration during late winter and spring-thaw, in spite of high R^2 -values. Higher than expected ecosystem respiration during spring-thaw may be partly due to the thawing of ice layers in the soil and release of trapped CO_2 produced earlier in the winter. However, classic exponential equations, which only include temperature as a variable and substrate availability as a constant are unfit for modelling respiration when substrate availability and free soil water are likely to fluctuate strongly because of freezing and thawing of soil water (Davidson et al. 2006). Indeed, at soil temperatures around $0 \text{ }^\circ\text{C}$, we observed a striking variability in respiration rates in both ecosystems. The equations, which included a measure of substrate availability greatly improved model fits particularly at the birch site and, therefore, indicate that ecosystem respiration was substrate-limited.

The higher substrate-limitation at the birch site than at the heath was unexpected, however, because the substrate quality of birch litter apparently is higher than of the litter from mainly evergreen shrubs (Grogan and Jonasson, 2005). It could be that the deeper snow at the birch site, creating higher and more stable soil temperatures, led to higher microbial activity and growth, as also indicated by the higher microbial nitrogen and phosphorus concentrations there, which depleted the available substrate. Substrate limited respiration during late winter may therefore in itself be an indication of high microbial activity during the winter.

MICROBIAL BIOMASS DYNAMICS

The contents of microbial carbon and phosphorus, but not the nitrogen content, were appreciably higher in both ecosystems during the snow-covered period than summer estimates in other similar heaths nearby (Jonasson et al., 1999b). However, contrary to our expectation, we found no effects of increased snow depth on the microbial and soil nutrient pools. Nor did we find any correlation between the size of the microbial biomass pools and ER, demonstrating that the microbial biomass is a poor indicator of microbial activity (Wardle, 1992; Michelsen et al., 2004).

The high microbial C in the control plots of the heath measured from mid March to late April rapidly decreased towards the earlier reported levels once the soil became snow-free. At the same time also microbial N and P decreased, although at a less pronounced rate. This pattern of changes corresponds well with observations in alpine ecosystems of the Rocky Mountains (Lipson et al., 1999) and suggests that microbial populations may

increase through the winter, if an insulating snow pack ensures stable temperature and if free water is available (Brooks et al., 1998).

Later, during spring-thaw, when the sampling was done with higher temporal resolution, we found pronounced variability in microbial biomass over short time intervals in both ecosystems. It appears, therefore, that the size of the microbial biomass, being high in winter, can vary strongly and decline significantly within a few days at the time of snow-melt. The fluctuations suggest large and rapid cycles of mobilization and immobilization of microbial nutrients, which are overlooked if sampling is done with larger time intervals. Indeed, it is possible that earlier reported high net nutrient mineralization rates in arctic soils during winter, measured as differences between autumn and spring content of soil inorganic N and P (Giblin et al., 1991; Nadelhoffer et al. 1992; Jonasson et al., 1993; Hobbie and Chapin, 1996; Schmidt et al., 1999) may reflect a high rate of mineralization in early spring rather than throughout the winter.

Brooks et al. (1998) suggested that freeze-thaw cycles were the main cause of decreased microbial biomass during snow-melt, and other studies have shown microbial diebacks when soils are exposed to freeze-thaw cycles (Schimel and Clein, 1996; Larsen et al., 2002) but initiated grazing by the soil fauna may also affect the microbial community (Ruess et al., 1999). The decline of C in the microbial biomass in the controls at the heath coincided with the first freeze-thaw cycle (Fig. 3b, Table 1), and the microbial C stayed low thereafter. It appears, therefore, that the first thaw had the most important effect on the microbial C content. Microbial N and P, however, decreased less and more slowly, indicating uptake of released nutrients by surviving microbes and causing decreased C-to-N and C-to-P ratios of the microbial biomass. The latter suggests structural changes in the microbial community when the food sources, such as plant labile carbon, increased as the vegetation resumed root growth. Indeed, substantial differences between winter and summer microbial communities have been shown for both fungi (Schadt et al., 2003) and bacteria (Lipson and Schmidt, 2004) in alpine soils of the Rocky Mountains with fungi being most dominant during winter. If structural changes in the microbial community were the main reason for the changes, they apparently take place over a relatively short interval of time.

The decline in content of microbial nutrients was not accompanied by corresponding increases in soil concentrations of DON or inorganic N and P. Although some nitrogen can be lost by leaching and denitrification (Grogan et al., 2004), it is possible that plants, when they started nutrient uptake in spring, were strong sinks for the released nutrients (Brooks et al., 1998). Recent observations of subnivean photosynthesis (Starr and Oberbauer, 2003) indeed give support for possible plant sequestration of N and P earlier in the season than usually believed. Although further research is needed to establish the significance of sub-

nivean photosynthesis it may, therefore, partly cancel out the significant respiratory carbon losses during late winter and spring-thaw.

From our study, it seems most likely that most nutrients released from the microbial biomass at snow-melt are retained in the ecosystem by surviving microbes and by plants resuming growth and nutrient uptake at this period, when the availability of free water and light levels increase. High plant demand for nutrients would, indeed, minimize nutrient losses from the ecosystems, and the timing of the release of microbial nutrients may contribute to optimize plant growth and ecosystem production.

CONCLUSION

Our study provides several important insights. First, although the snow build-up in late fall and early winter is a key determinant of late winter soil temperatures and respiration rates in arctic ecosystems, delayed snow accumulation until mid-late winter may still affect late winter soil respiration and the timing of completion of snow-melt. Second, adding a simple, linear relationship between respiration and the supply of DOC or DON to classic first-order exponential equations with only temperature as a variable significantly improved ecosystem respiration models. Third, the microbial community may change rapidly within few days indicating high nutrient turnover and high net mineralization rates just around completion of snow-melt. The high microbial turnover, contrasting with much smaller changes in organic and inorganic soil nutrient concentrations, indicate that released nutrients are immobilized rapidly and may even provide an important nutrient source for plants resuming their growth earlier than previously thought.

Our observations of fast changes in microbial biomass and the dependence of ER on substrate availability stress the need for high temporal resolution in future research on ecosystem carbon and nutrient dynamics at snow-melt in order to make robust models of their turnover.

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Paper II

Significance of cold-season respiration and photosynthesis in a subarctic heath ecosystem in Northern Sweden

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Significance of cold-season respiration and photosynthesis in a subarctic heath ecosystem in Northern Sweden

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Abstract

While substantial cold-season respiration has been documented in most arctic and alpine ecosystem in recent years, the significance of cold-season photosynthesis in these biomes is still believed to be small. In a mesic, subarctic heath during both the cold and warm season, we measured *in-situ* ecosystem respiration and photosynthesis with a chamber technique at ambient conditions and at artificially increased frequency of freeze-thaw cycles during fall and spring. We fitted the measured ecosystem exchange rates to respiration and photosynthesis models with R^2 -values ranging from 0.81 to 0.85. As expected, estimated cold-season (October, November, April, and May) respiration was significant and accounted for at least 22 % of the annual respiratory CO_2 flux. More surprisingly, estimated photosynthesis during this period accounted for up to 19 % of the annual gross CO_2 uptake, suggesting that cold-season photosynthesis partly balanced the cold-season respiratory carbon losses and can be significant for the annual cycle of carbon. Still, during the full year the ecosystem was a significant net source of $120 \pm 12 \text{ g C m}^{-2}$ to the atmosphere.

Neither respiration nor photosynthetic rates were much affected by the extra freeze-thaw cycles, although the mean rate of net ecosystem loss decreased slightly, but significantly, in May. The results suggest only a small response of net carbon fluxes to increased frequency of freeze-thaw cycles in this ecosystem.

Introduction

Traditionally, the annual carbon exchange between the atmosphere and ecosystems in the Arctic was assumed to take place primarily during a short growing season of typically two to four months, on the northern hemisphere often beginning in early to mid June and ending in late August to mid September. However, the evidence of considerable biological activity outside this period has grown rapidly within the last decade. The estimates of ecosystem respiration during the cold season from a variety of studies are, e.g., 25 % of the annual respiration in a sub-alpine wetland (Sommerfeld *et al.*, 1993), 21 to 23 % in a boreal peatland (Alm *et al.*, 1999), 10 to 30 % in various tundra and taiga soils (Clein *et al.*, 1995), 32 to 51% in tussock tundra (Grogan *et al.*, 1999) and 30 to 60 % in forest tundra (Zimov *et al.*, 1996). None of these studies included measurements of plant photosynthesis during the cold season, although photosynthesis in both bryophytes and lichens has long been known to continue at temperatures below 0 °C and under snow (Lange, 1965; Tieszen, 1974; Oechel *et al.*, 1978; Kappen *et al.*, 1996). In contrast, different studies have shown no or very low photosynthesis by higher plants as long as they are snow-covered (Tieszen, 1974; Hamerlynck *et al.*, 1994). However, a more recent study has documented significant photosynthesis by various evergreen species under snow during spring in an Alaskan tussock-dwarf shrub tundra ecosystem (Starr *et al.*, 2003). In their study, temperatures in the snow had risen to about 0 °C, light levels were high enough to allow substantial amounts of photosynthetic active radiation (PAR) to penetrate through the snow, and CO₂ concentrations in the snow were beneficial for photosynthesis. Similar conditions may not have been present in the previous studies showing no photosynthesis by higher plants, although they are likely to occur during spring in many ecosystems in the Arctic. However, it remains unclear whether photosynthesis at this time of the year, in general, is of significance for the annual carbon budget.

It is also uncertain how future climate change may affect the seasonal patterns of ecosystem photosynthesis, as well as respiration. Climate models predict increased temperatures in the Arctic especially during the cold season (Watson *et al.*, 2000; Hassol *et al.*, 2004), which are likely to increase the number of thaw events during winter and induce earlier completion of snowmelt during spring. Earlier exposure of bare ground than at present will make the soil more susceptible to day-time thaw and night-time frost. Such freeze-thaw cycles have been shown to cause microbial dieback affecting the turnover of carbon and nutrients (Schimel *et al.*, 1996; Brooks *et al.*, 1998; Larsen *et al.*, 2002), and may play a critical role in ecosystem functioning. Temporarily increased respiration rates have been observed concurrent with freeze-thaw induced microbial diebacks (Ivarson *et al.*, 1970). The increased rates (Skogland *et al.*, 1988; Schimel *et al.*, 1996) have been attributed to enhanced activity by surviving microbes as they decompose easily degradable compounds released by freeze-thaw intolerant microbes (Soulides *et al.*, 1961; Morley *et al.*, 1983).

However, our knowledge of the effect of freeze-thaw cycles and changes in their frequency is almost entirely based on laboratory studies. Hence, there is a need for *in-situ* experimental manipulation of the freeze-thaw frequency in order to confirm the findings from the *in-vivo* studies. In this study, we investigated the significance of photosynthesis and respiration for the ecosystem carbon balance during both the cold and the warm season in a subarctic heath

ecosystem. We focused on the fall (October – November) and spring (April – May) periods, which are thought to be the periods outside the growing season when biological activity is likely to be most affected by future climate changes (Olsson *et al.*, 2003). We measured the carbon dioxide flux rates of respiration and photosynthesis both at ambient conditions and in plots that had been covered by open-top plastic greenhouses during fall and snowmelt in three years. The treatment significantly increased the frequency of freeze-thaw cycles, and we hypothesized that (A) respiration rates would increase in treated plots in response to the increased release of easily decomposable substrates from lysed microbial cells during the extra freeze-thaw cycles. In contrast, we hypothesized that (B) photosynthesis would be unaffected by the applied increase in freeze-thaw frequency, but that the plants would respond positively to the lengthening of the snow-free season and increased daytime temperatures.

Materials and methods

Experimental site

The experiment was carried out in a mesic, subarctic/alpine heath ecosystem near Abisko Scientific Research Station, Northern Sweden (68° 21' N, 18° 49' E). Climate records (1970–2000) show an annual mean temperature of -0.5 °C, and annual precipitation of 315 mm. Permafrost is patchy in the region but not present at the study site. Because of wind redistribution, winter snow depth rarely exceeds 30–40 cm and usually peaks in March, and melting occurs during May. The highly organic soil (87.4±6.8 % SOM) overlays bedrock and has a depth of 5–20 cm. The soil has a mean dry mass of 0.113±0.004 g cm⁻³, corresponding to 5–20 kg dry soil m⁻². The vegetation is dominated by mosses (433±33 g above-ground dry biomass m⁻²), evergreen and deciduous dwarf shrubs (151±12 and 97±7 g m⁻², respectively), and graminoids (42±3 g m⁻²). The total below-ground plant biomass (1717±75 g m⁻²) is approximately twice the size of the above-ground biomass (724±33 g m⁻²).

Treatments

For three years from August 2001 to June 2004, six dome-shaped 1 m × 1 m open-top plastic greenhouses were erected for about four weeks during fall and four weeks during spring, aiming to increase the number of freeze-thaw cycles (FT treatment). The 0.05 mm polyethylene plastic covering the domes transmitted about 90 % of the solar radiation for wavelengths between 300 nm and 1100 nm (Havström *et al.*, 1993) but prevented heat conductance between the atmosphere and the ground. As a consequence, the diurnal surface temperature fluctuations were expected to increase. The six increased-freeze-thaw plots (FT plots) and a similar number of control plots were randomly located within a homogeneous area of approximately 600 m². During fall, the greenhouses were in place from mid October to mid November, when the natural soil freeze-thaw period was ending, and during spring from early-mid April to mid May, starting a few weeks before the soil thaw was anticipated to begin. When setting up the greenhouses, any loose snow within each treated plot was carefully removed, thereby reducing snow depths by approximately 5 cm. The 2003 to 2004 season experienced lower than average snowfall with depths on 3 April ranging from 4 cm to 14 cm. Snowmelt was completed by 1 May.

Temperatures were logged continuously every 60 minutes in 2-5 plots per treatment at the soil surface and at 3 cm soil depth from August 2001 until June 2004 using Tinytag loggers (Gemini Data Loggers, Chichester, UK). Air temperature (2 m) and global radiation were logged with intervals of 10 minutes at a nearby weather station. A freeze-thaw cycle was defined as ≥ 3 hours of temperatures above 0 °C followed by ≥ 3 hours below 0 °C.

CO₂ flux measurements

CO₂ fluxes were measured with a transparent (PAR transmission: 90 %) static chamber technique. A metal collar was installed into the soil at each plot on 24 June 2003. A water-filled channel along the edge of the collars ensured a tight fit with the 13.3 L Perspex chamber, which was attached to a LICOR 6200 or 6400 IRGA.

Measurements of CO₂ fluxes were performed in July, August, October, and November 2003 and in April, May, and June 2004, i.e. the last of the three years of the FT treatment. Only during the April field campaign, the measurements were performed when the greenhouses were in place. Night-time measurements were performed during all campaigns except in October due to technical problems, and in November when low temperatures made the chamber freeze to the collars. In April and May, diurnal measurements were performed with four repetitions per plot within 24 hours. Fluxes from all plots were measured 38-40 times from July 2003 to June 2004 ($n = 234$ and 240 in controls and FT plots, respectively). In November 2003 and April 2004, snow depths were low enough to measure the CO₂ fluxes without removing snow from the collars. During all other periods of measurements, there was no snow.

The detection limit of the fluxes is approximately $\pm 0.025 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ (Grogan *et al.*, 2001). When using large chambers, the water vapour concentration during measurements often increases beyond the scrub capability of the desiccant because of evapotranspiration. To prevent problems with inadequate internal equations of the LICOR 6200, the desiccant was always switched off (Hooper *et al.*, 2002). Also, to prevent overestimation of respiration, the chamber fan was switched off if measurements were performed in calm weather (Hooper *et al.*, 2002).

Net Ecosystem CO₂ exchange (F_n) was estimated from the mean rate of change of the CO₂ concentration in the chamber over a two-minute interval. Ecosystem Respiration (R_E) was estimated in a similar way from measurements done in darkness by covering the chamber with a double-layer of black plastic. Gross Ecosystem Photosynthesis (P_g) was estimated as F_n minus R_E . Thus, CO₂ uptake has a negative sign and release is positive.

Statistical analysis and data processing

F_n , R_E , and P_g flux rates were tested with two-way mixed model Anova with time as repeated effect, treatment as regular effect (control vs. greenhouses), and their interactions (SAS Enterprise Guide 3.0). Due to seasonal differences, data from each field campaign were tested individually in order to achieve homogeneity of variance (Brown and Forsythe's test). In some cases, data were log-transformed in order to pass the test. Due to the dependence of

incident light on flux rates, PAR was used as a covariate in all statistical tests of CO₂ flux data.

A similar mixed model Anova was used to test for effect of the greenhouses on the number of freeze-thaw cycles (FT) at the soil surface and at 3 cm soil depth for fall and spring separately with year as repeated effect and treatment as regular effect, and their interactions. Treatment effects on mean soil temperatures during fall 2003 and spring 2004 were tested with two-way Anovas with depth (soil surface vs. 3 cm soil depth) and treatment as regular effects, and their interactions. In all Anovas, the interaction term was removed from the model if P exceeded 0.15.

Ecosystem respiration R_E was modelled (SAS System 8.02) using a classic, first-order exponential equation (van't Hoff, 1898):

$$R_E = R_0 e^{bT} \quad (1)$$

where R_0 represents the ecosystem respiration at 0 °C depending on substrate availability ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), b is the temperature sensitivity ($^{\circ}\text{C}^{-1}$) of respiration and T is the temperature ($^{\circ}\text{C}$). We fitted models with various combinations of temperature data from soil (-3 cm), soil surface (0 cm) and air (2 m) and found the best model fit when using the mean of the soil and soil surface temperature, which was subsequently used in the models.

The ecosystem gross photosynthetic rate (P_g) was modelled using the non-rectangular hyperbola (Thornley, 1976):

$$P_g = \frac{(\alpha \cdot I + P_m) - \sqrt{(\alpha \cdot I + P_m)^2 - 4\alpha \cdot I \cdot P_m \cdot \theta}}{2\theta} \quad (2)$$

where I is the incident global radiation ($\text{J m}^{-2}\text{s}^{-1}$), α is the radiation use efficiency ($\mu\text{mol CO}_2 \text{ J}^{-1}$), θ is a dimensionless curvature parameter, and P_g is the gross ecosystem photosynthesis rate ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$). P_m is the temperature dependency of photosynthesis per unit area and was estimated using a temperature response equation similar to the equation used by dePury *et al.* (1997) for photosynthesis enzyme kinetics:

$$P_m = P_{mr} \cdot e^{\frac{E_a(T-T_r)}{R T T_r}} \cdot \frac{1 + e^{\frac{\Delta S T_r - E_d}{R T_r}}}{1 + e^{\frac{\Delta S T - E_d}{R T}}} \quad (3)$$

where P_{mr} is the photosynthesis per unit area at reference temperature (T_r) of 298 K, T is temperature in K, E_a is the activation energy (kJ mol^{-1}), R is the universal gas constant ($8.341 \text{ J mol}^{-1} \text{ K}^{-1}$), ΔS is an entropy term ($\text{J mol}^{-1} \text{ K}^{-1}$), and E_d is the energy of deactivation (kJ mol^{-1}).

As for the respiration models, we tested various combinations of temperature data from soil (-3 cm), soil surface (0 cm) and air temperature (2 m). For the photosynthesis models, we

found the best model fit with the mean of soil surface temperature and air temperature probably because it represents best the temperature around the plants.

Separate models of respiration and photosynthesis were fitted to the control and FT plots. We used the model parameters to extrapolate the fluxes from 234 plot measurements in control plots and 240 measurements in FT plots to mean monthly fluxes, excepting the period December-March, when no flux measurements were done. In addition, we used the residuals (observed minus modelled fluxes) to test the seasonal robustness of the models and then corrected the model outcome for seasonal patterns in the residuals. The resulting variability in the monthly CO₂ fluxes therefore incorporates the variation in temperature data as well as the variation in the seasonal residual patterns.

Results

Soil temperatures and freeze-thaw cycles

Mean daily soil temperatures from July 2003 to June 2004 were similar in controls and treated plots (Fig. 1). Even during the periods when the greenhouses were in place, the daily temperatures at both depths were not significantly higher in FT plots than in the controls in neither fall (Anova_{1,11}, $F = 0.02$, $P = 0.89$) nor spring (Anova_{1,11}, $F = 1.41$, $P = 0.27$).

Figure 1

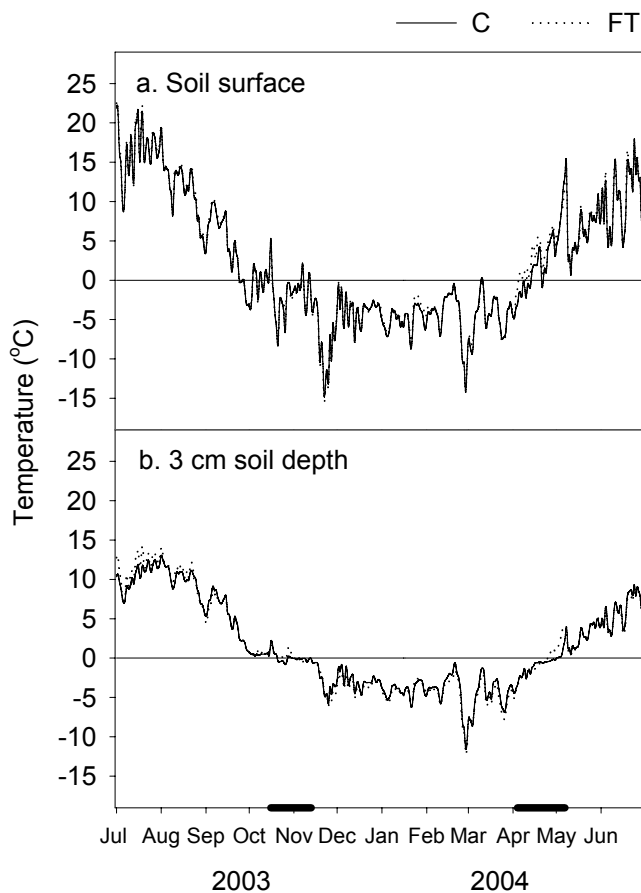


Figure 1. Mean daily temperatures from July 2003 to June 2004 at the soil surface (a) and at 3 cm soil depth (b) in controls (C, solid lines) and in plots with extra freeze-thaw cycles (FT, dotted lines). $n = 3$. Bold parts of the x-axes represent the greenhouse periods.

Soil temperatures showed a seasonal pattern with highest means in July reaching an average of 15 °C at the surface (Fig. 1a) and 10 °C at 3 cm soil depth (Fig. 1b). The temperatures then steadily decreased, with daily means passing below 0 °C for the first time in late September at the soil surface, and in October at 3 cm soil depth. The lowest daily mean soil temperature at 3 cm depth was recorded in late February (-12 °C) while March had the lowest monthly mean temperature of -5 °C. The daily mean temperature at 3 cm depth stayed below 0 °C until late April/early May.

Table 1. Number of freeze-thaw cycles recorded by temperature loggers ($n = 3$) during fall and spring 2001-2004 at the soil surface (0 cm) and in the soil (-3 cm). A freeze-thaw cycle was defined as at least 3 hours of frost followed by at least 3 hours of thaw. Data from 2001 to spring 2003 are from (Sjursen, 2004). SE are shown in brackets.

Season	Year	Control (0cm)	Treatment (0cm)	Control (-3cm)	Treatment (-3cm)
Fall	2001	26.5 (0.5)	30.5 (0.5)	2 (0)	1.8 (0.3)
Fall	2002	21 (1.0)	25.5 (0.9)	1.5 (0.5)	0.5 (0.3)
Fall	2003	16 (1.0)	21 (1.9)	1 (0.6)	3.3 (0.9)
Spring	2002	18.5 (0.5)	21.8 (1.0)	2.5 (0.5)	10.4 (0.2)
Spring	2003	31.5 (0.5)	36.3 (0.6)	0.5 (0.5)	12.8 (1.1)
Spring	2004	37.3 (2.9)	42.5 (1.3)	9.7 (4.1)	16 (4.9)

In the period from 2001 to 2004, the greenhouses had a profound effect on the number of surface freeze-thaw cycles (Table 1), which significantly increased on average by 21 % during the fall seasons (Treatment_{1,19}, $F = 117.9$, $P = 0.0001$) and by 15 % during the spring seasons (Treatment_{1,20}, $F = 35.6$, $P = 0.001$). At 3 cm soil depth, no significant increase was found during the three fall seasons, while during spring the number of freeze-thaw cycles was significantly increased by a mean of 209 % (Treatment_{1,21}, $F = 82.7$, $P < 0.0001$). However, the effect differed between years (Treatment \times year_{2,21}, $F = 11.0$, $P = 0.01$) mainly because of a higher number of naturally occurring freeze-thaw cycles during spring 2004.

Ecosystem CO₂ fluxes

Mid day flux rates of CO₂ peaked in July and August as expected, but significant fluxes of both ecosystem respiration and gross ecosystem photosynthesis were measured during all field campaigns (Fig. 2a, b). Even in November, when mean solar radiation during measurements was only $17 \pm 2 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$, the vegetation maintained mid day P_g flux rates of -0.28 ± 0.09 and $-0.21 \pm 0.04 \mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ in controls and FT plots, respectively (Fig. 2b). In May, mid day flux rates of R_E reached about half of peak season rates, while P_g rates were about 1/3 of the highest annual rates. During mid day, F_n rates were negative only in August 2003 and June 2004, i.e. these were the only periods when the ecosystem had a mid day net uptake of CO₂ from the atmosphere (Fig. 2c).

In May, following the most intense annual period of soil freeze-thaw frequency, F_n rates (Fig. 2c) were significantly lower (Treatment_{1,60}, $F = 8.22$, $P = 0.017$) in FT plots

($0.19 \pm 0.2 \text{ g C m}^{-2} \text{ d}^{-1}$) than in controls ($0.48 \pm 0.09 \text{ g C m}^{-2} \text{ d}^{-1}$), P_g tended to be higher (Treatment_{1,60}, $F = 3.71$, $P = 0.083$) while R_E rates were not significantly different (Fig. 2). At the other times of the year, the observed R_E , P_g or F_n flux rates were not significantly different between FT and control plots, except for the tendency of higher net carbon loss in controls than in FT plots in November.

Figure 2

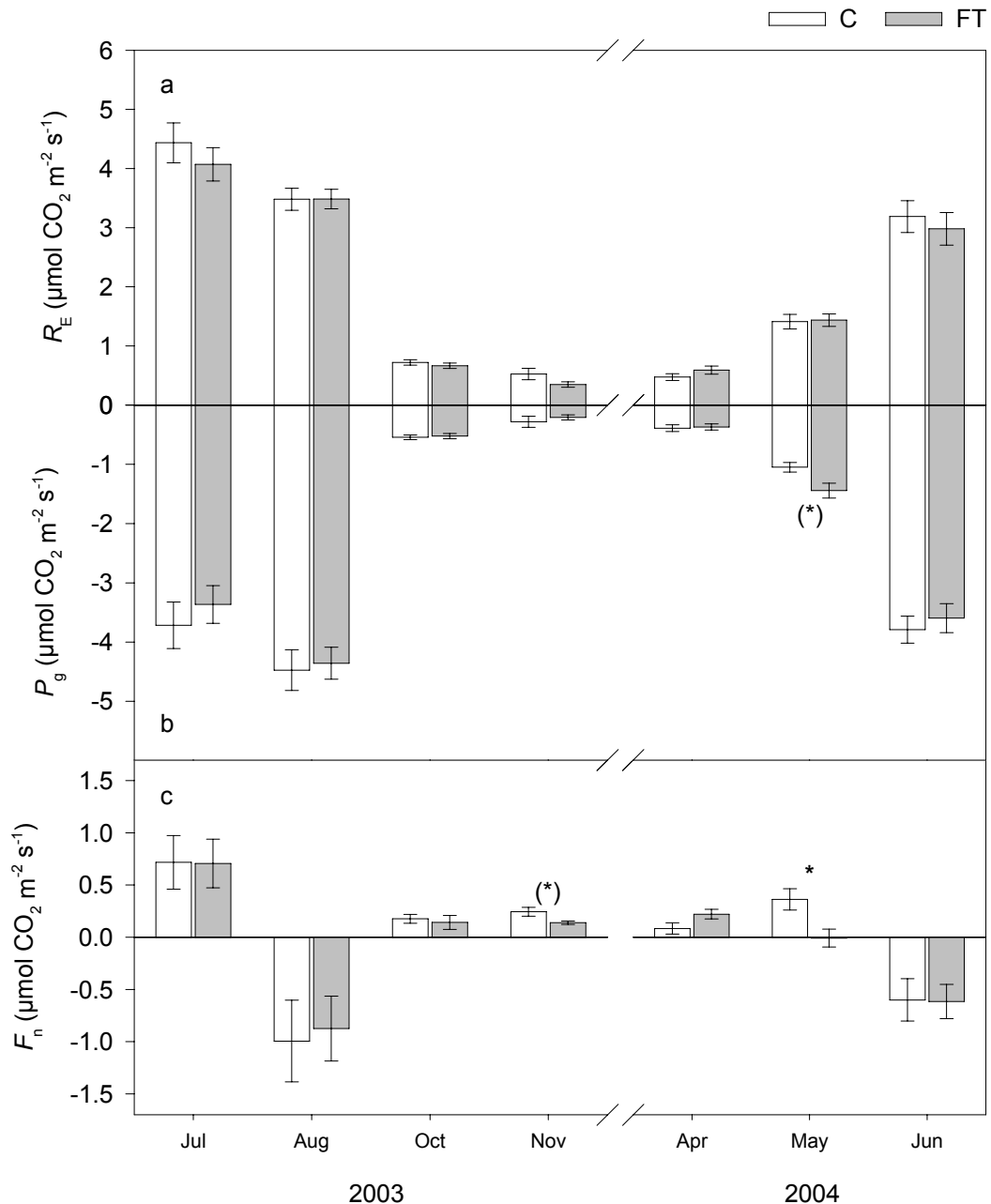


Figure 2. Mean mid day (10:00 h – 14:00 h) flux rates of (a) ecosystem respiration (R_E), (b) gross ecosystem photosynthesis (P_g) and (c) net ecosystem exchange (F_n) during field campaigns from July 2003 to June 2004. Data are means with SE, $n = 24\text{--}72$ (six replicates repeated 4 to 12 times per campaign). C = controls, FT = plots with extra freeze-thaw cycles. Significant treatment effects from two-way mixed model Anovas on repeated time and treatment for each month are shown. P -levels: (*), $P < 0.10$; *, $P < 0.05$.

Figure 3

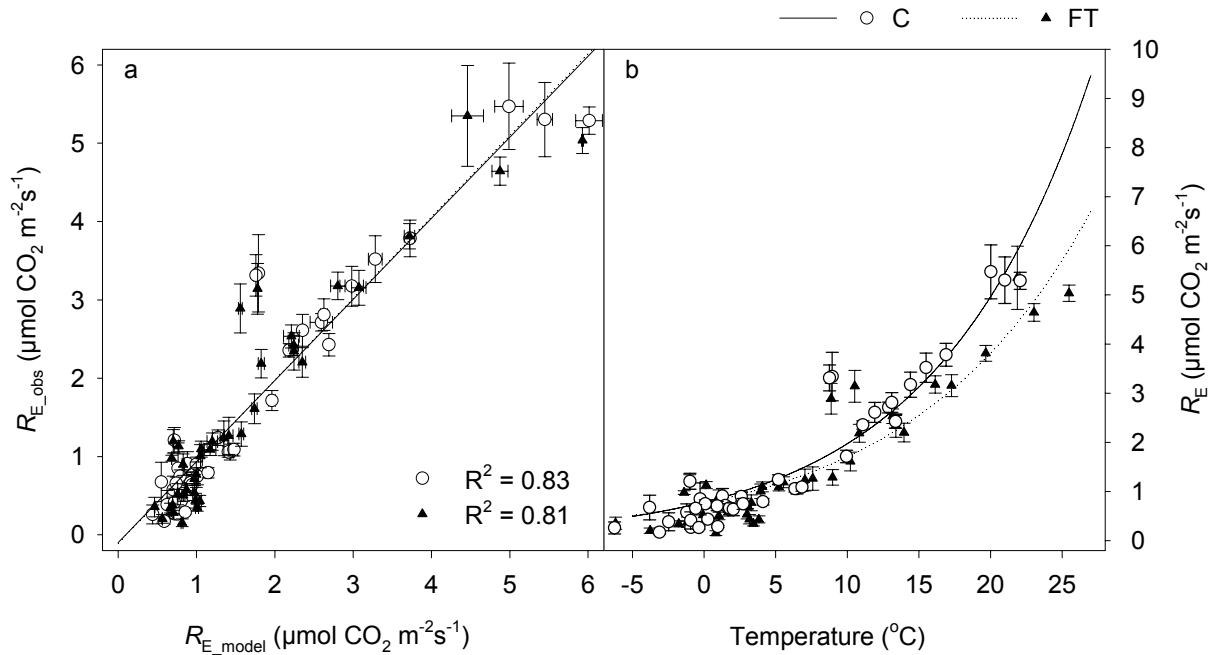


Figure 3. Relationship between modelled (R_{E_model}) and observed (R_{E_obs}) rates of ecosystem respiration (R_E) (a) and temperature response curves of the R_E models plotted together with observed rates of R_E (b). Data are means with SE, $n = 234$ (control) and 240 (treatment). C = controls, FT = plots with extra freeze-thaw cycles. Lines represent the linear regressions of observed vs. modelled R_E rates in (a) and modelled R_E rates in (b).

R_E was modelled with a high fit to the observed values with $R^2 = 0.83$ for controls and 0.81 for treated plots (Fig. 3a, Table 2). The high model fit is also reflected in the regression lines of the observed vs. modelled R_E rates with slopes of 1.035 ± 0.030 and 1.041 ± 0.032 for the control and FT model, respectively, and intercepts with the Y-axis of -0.098 ± 0.066 for the controls and -0.107 ± 0.065 for the FT plots. The slopes were not significantly different from 1 and the intercepts were not significantly different from 0. The substrate availability terms (R_0)

Table 2. Parameter estimates for ecosystem respiration (R_E) (see eq. 1) and gross ecosystem photosynthesis (P_g) models (see eq. 1-3). Different superscript letters indicate significant differences ($P < 0.05$) from Student's t-test. All parameters were significant in the models at $P < 0.01$. See text for parameter abbreviations and explanations.

R_E						
	n	R_0	b			
Control	234	0.78 ± 0.04	0.092 ± 0.003^a			
FT	240	0.76 ± 0.04	0.080 ± 0.003^b			
P_g						
	n	α	θ	P_{mr}	E_a	E_d
Control	234	0.022 ± 0.007	0.92 ± 0.14	3.73 ± 0.33	135044 ± 16474	202414 ± 1082
FT	240	0.018 ± 0.003	1.00 ± 0.01	3.57 ± 0.15	113876 ± 9147	204201 ± 659

of the control and FT treatment were similar (Table 2) while the temperature sensitivity (*b*) of the FT model was slightly, but significantly, lower than for the controls (Table 2), resulting in lower modelled respiration in FT plots than in controls at high temperatures (Fig. 3b). The model fit to equation (3) of P_g (Fig. 4a, Table 2) was also high for both controls ($R^2 = 0.84$) and treated plots ($R^2 = 0.85$) and, as for the R_E models, the regression lines of observed vs. modelled P_g rates were not significantly different from a slope of 1 (0.991 ± 0.028 and 0.999 ± 0.027 for the control and FT model, respectively) and an intercept of 0 (-0.030 ± 0.058 and $-8.41 \cdot 10^{-5} \pm 0.0053$, respectively). Nor were there any significant treatment differences between the estimate of model parameters of the controls and FT plots (Fig. 4b, Table 2). Consequently, the differences between the temperature response curves of the FT- and the control models are not significant (Fig. 3d).

Figure 4

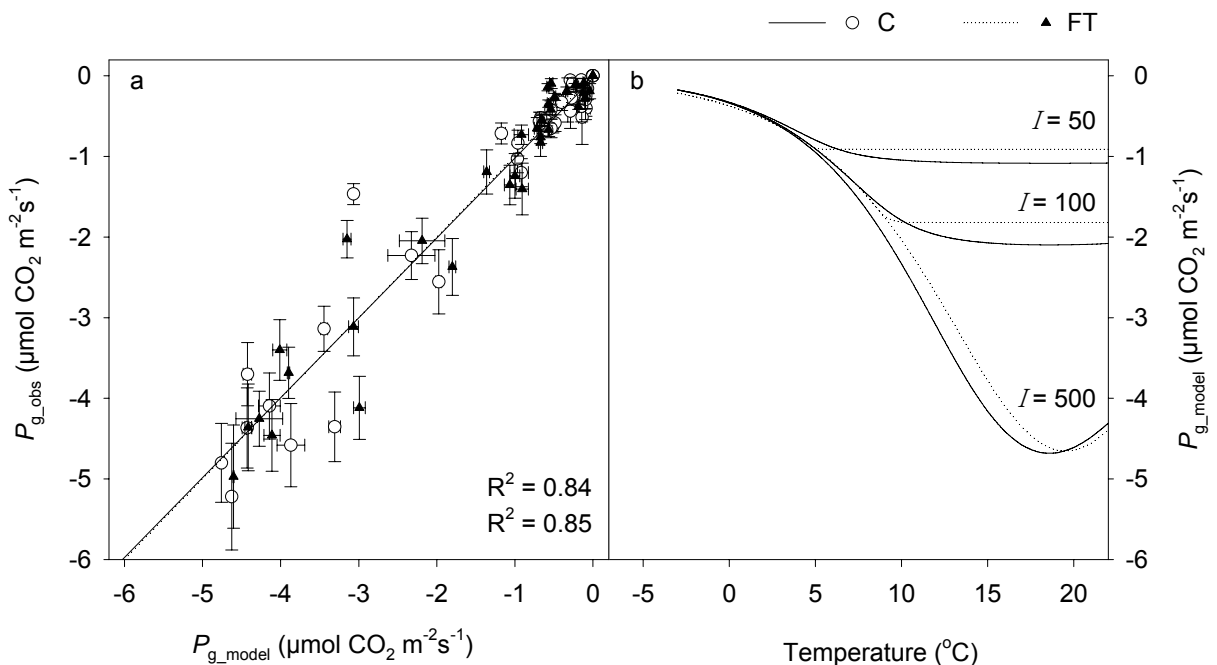


Figure 4. Relationship between modelled ($_{model}$) and observed ($_{obs}$) rates of gross ecosystem photosynthesis (P_g) (a) as well as temperature response curves of the control and FT models at global radiation (I) rates of 50, 100 and 500 Wm^{-2} (b). Data are means with SE, $n = 234$ (control) and 240 (treatment). C = controls, FT = plots with extra freeze-thaw cycles. Lines represent the linear regressions of observed vs. modelled P_g rates in (a) and modelled P_g rates in (b).

The residuals of observed and modelled R_E rates were small in comparison to the measured rates but did show a seasonal pattern of slight model overestimation during spring and fall and underestimation during summer (Fig. 5a). The residuals of P_g were even smaller than for R_E and were not significantly different from 0 during summer for any of the models and during fall for the FT model while the control model slightly underestimated P_g during fall. During spring, both models showed a small overestimation of P_g .

Figure 5

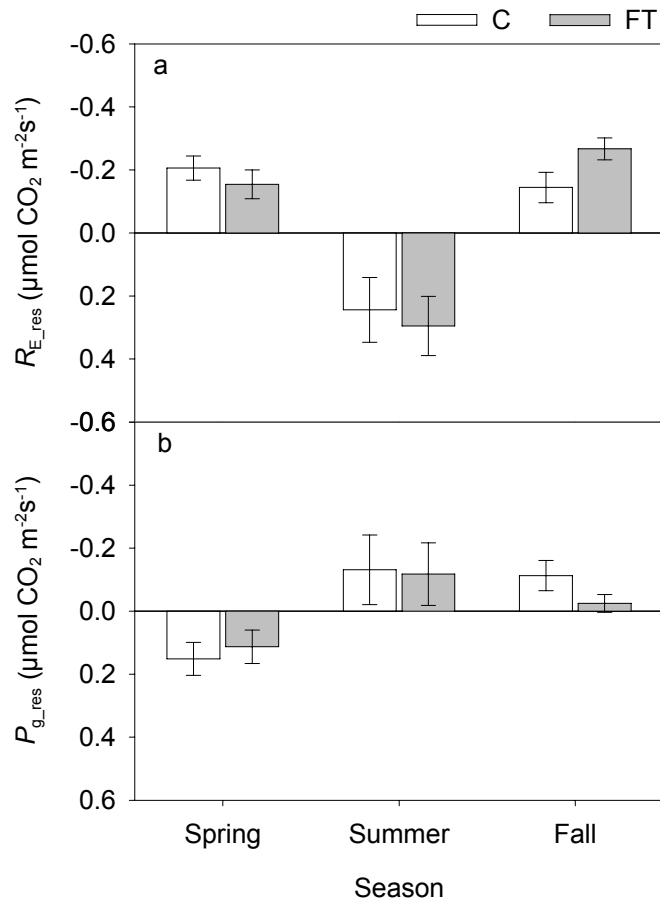


Figure 5. Residual (observed minus modelled) flux rates of ecosystem respiration (R_E) (a) and gross ecosystem photosynthesis (P_g) (b) during spring (April and May), summer (June, July and August) and fall (October and November). Data are means with SE, $n = 66-96$. C = control plots, FT = plots with extra freeze-thaw cycles.

The small differences of R_E between the FT- and control model outputs resulted in very similar seasonal patterns of respiration rates, indicating that the significant differences in temperature sensitivities of the two models was of minor importance. Both models showed the highest ecosystem respiratory carbon loss during the warmest months from June to August, when 67 % of the annual respiration took place (Fig. 6a). The periods from October to November and from April to May together account for about 22 % of the annual respiration while the respiration in September and May was similar each with about 11 % of the annual flux.

The gross ecosystem photosynthesis showed a seasonal trend surprisingly similar to the respiration (Fig. 6b). Even during October and November when the light level was low, modelled monthly P_g fluxes still reached 5–8 $\text{g CO}_2\text{-C m}^{-2}$ and 2–5 $\text{g CO}_2\text{-C m}^{-2}$, respectively, accounting together for 3–5 % of the annual photosynthesis. During spring, the photosynthetic rate was 5–8 $\text{g CO}_2\text{-C m}^{-2}$ in April, i.e. similar to the October rate, and about 24 $\text{g CO}_2\text{-C m}^{-2}$ in May. Together, 12–14 % of annual photosynthesis took place during the April plus May period. Consequently, 15–19 % of annual photosynthesis took place during periods, which traditionally has not been considered as part of the growing season.

As a consequence of the temporal patterns of R_E and P_g , F_n showed much smaller seasonal variability (Fig. 6c). F_n was never negative, i.e. the photosynthesis never balanced the

Figure 6

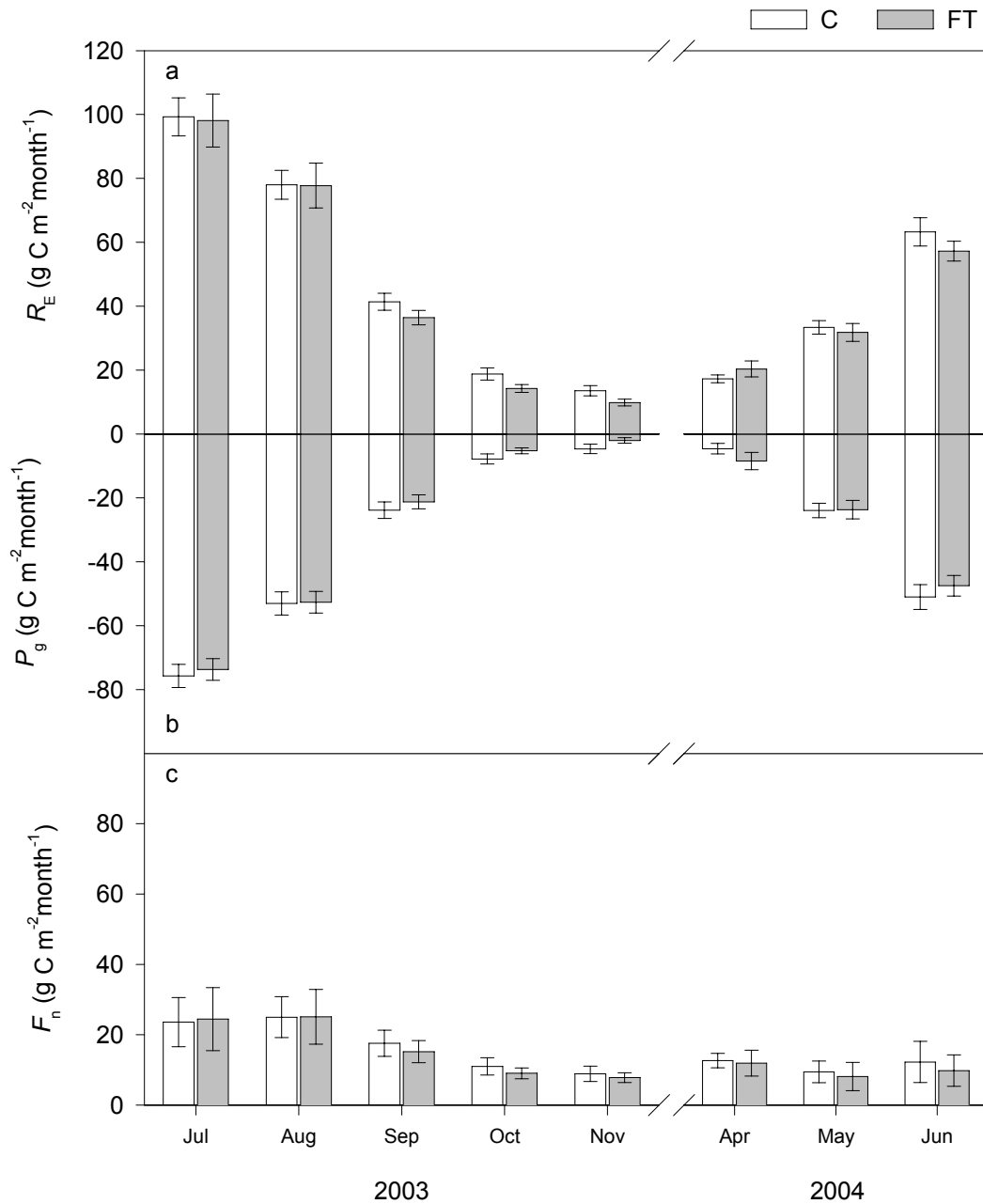


Figure 6. Modelled fluxes of (a) ecosystem respiration (R_E), (b) gross ecosystem photosynthesis (P_g) and (c) net ecosystem exchange (F_n) on a monthly basis. Models were run on 10 min-interval weather data from temperature loggers placed inside three control and three treated plots, respectively, combined with data of global irradiance and air temperatures from the nearby weather station and then corrected for the seasonal residuals (observed vs. modelled) presented in Figure 5. C = control plots, FT = plots with extra freeze-thaw cycles. See text for model equations.

respiratory carbon loss, not even during the peak of the warm season. In fact, seasonal net CO₂ loss was highest during the warmest part of the year. The modelled fluxes result in a high net annual loss (excl. December-March) of 120±12 and 111±14 g C m⁻² y⁻¹ in controls and FT plots, respectively, which was not significantly different.

Discussion

Cold-season CO₂ fluxes

Recently, the use of classic exponential equations to describe respiration at the ecosystem level has been debated (Davidson *et al.*, 2006a; Davidson *et al.*, 2006b) not least because the models have been considered inadequate when soil temperatures are close to or below 0 °C, and because potentially important factors, e.g. water availability, are not included in simple Q₁₀-models. In spite of these potential shortcomings, we found high fits between modelled and observed data with R² of 0.81 to 0.83, resulting in small residuals with moderate seasonal patterns. Therefore, it appears that soil temperature was by far the most dominating factor controlling R_E in this ecosystem, while substrate availability, plant phenology, water availability, or other factors not included in the models, were of minor importance or co-varied with temperature. Considering the high R²-values and low seasonal changes in residuals of both our respiration and photosynthesis models, we believe that the seasonal fluxes in the present study were estimated with high confidence.

Most of the studies of cold-season respiration in arctic ecosystems have focused on the total cold-season contribution to the annual carbon budget (Sommerfeld *et al.*, 1993; Clein *et al.*, 1995; Zimov *et al.*, 1996; Alm *et al.*, 1999; Grogan *et al.*, 1999), while the intra-seasonal patterns of gas exchange within the cold period of the year has received less attention. Olsson *et al.* (2003) suggested a division of the cold season of the Alaskan arctic into five stages with different characteristics of driving ecosystem processes based on intra-seasonal climate patterns, snowpack development and distinct transition periods of the soil active layer. They hypothesized that climate variability during the mid winter stages have the least impact on ecosystem processes. In contrast, conditions during the first stages in fall, when the soil still is warm, together with the thaw period in spring were considered to have much greater implications for variations in biological activity, including effects also of the length of the snow-free season and effects of climate change.

Indeed, at our study site, 22% of the annual respiration took place during the first and last stages of the cold season from October to May (33% from September to May), showing the importance of cold-season respiration also in this ecosystem. Ecosystem respiration was lower in November (3–4 % of annual flux) and October (4–5%) than in April (5–6 %) and May (about 9 %), indicating an intra-seasonal trend opposite to earlier reported higher fluxes during the early cold season than at the thaw period at various sites of the Alaskan Arctic (Oechel *et al.*, 1997; Jones *et al.*, 1999). Hence, the magnitude of the gas exchange may differ among the climatic stages, as well as between arctic regions in response to regional and local climate variability.

Our estimate of cold-season ecosystem respiration is conservative, because the mid winter fluxes during December-March, which we did not measure, should be added to our data.

However, the mid winter fluxes probably were low (Olsson *et al.*, 2003) because of a mean soil temperature of -4.2 °C at 3 cm depth, compared to -2.3 °C in November and -1.4 °C in April. The empirical limit for *in situ* measurable fluxes is at about -5 °C (Brooks *et al.*, 1997) and at temperatures below zero, respiration rates decrease rapidly with decreasing temperature (Mikan *et al.*, 2002; Elberling *et al.*, 2003) most likely because of fast decreasing free water and substrate availability (Davidson *et al.*, 2006b).

While cold-season ecosystem respiration in high-latitude ecosystems are known to constitute an important part of the annual carbon budget, cold-season photosynthesis has been far less investigated and has been assumed to be negligible. The soil microbial community has generally been accredited as the dominating source of wintertime CO₂ production. However, vascular plants absorb nitrogen as late as at the onset of winter in arctic ecosystems (Grogan *et al.*, 2003) and throughout the winter in temperate ecosystems (Andresen *et al.*, 2005), indicating activity also during the cold season. Furthermore, the long-known photosynthetic capacities at low temperatures in mosses and lichens (Lange, 1965; Tieszen, 1974; Oechel *et al.*, 1978; Kappen *et al.*, 1996) and the recent finding of photosynthesis also by higher plants before completion of snowmelt during spring (Starr *et al.*, 2003) indicate that cold-season photosynthesis may also be an important process in arctic ecosystems. In our study, the plants were photosynthetically active even in October and November when both soil and air temperatures were often below zero and light levels were low. In spite of the unfavourable conditions, photosynthesis during these two months still accounted for 3 to 5 % of the annual photosynthesis. The photosynthetic activity was even higher during the spring months in April and May, making up about 12 to 14 % of annual photosynthesis. Our study therefore shows that measurements restricted to the growing season from June to September, which is the common period of reported photosynthetic rates, may underestimate the annual carbon sequestration, in our study by up to 19%. Our results, therefore, stress the need for including cold-season and especially spring-thaw measurements of photosynthesis as well as respiration, to attain a more precise estimate of the annual ecosystem carbon balance. Furthermore, in many studies of carbon exchange in mesic, heath ecosystems, the focus has often been entirely on the higher plants, i.e. the evergreen and deciduous dwarf shrubs and graminoids, although mosses often contribute significantly to the above-ground biomass (60 % in our study). Bryophytes generally have lower light compensation points and can maintain positive net assimilation at lower temperatures than most higher plants (Oechel *et al.*, 1978; Tenhunen *et al.*, 1992; Longton, 1997; Sveinbjörnsson *et al.*, 1997; O'Neill, 2000). In addition, the mosses may also take advantage of higher light levels penetrating through the vascular plant cover in periods prior to bud break and after leaf abscission of the deciduous dwarf scrubs. It is therefore reasonable to assume that the mosses were significant contributors to the cold-season photosynthesis we measured.

The large discrepancy between annual respiration and gross photosynthesis, resulting in a modelled net loss of carbon of 120±12 and 111±14 g C m⁻² y⁻¹ in control and FT plots, respectively, shows that the ecosystem was a large source of carbon to the atmosphere. The high annual C loss was highlighted by the net loss of carbon even during the peak growing season. High inter-annual variability in the carbon balance of arctic ecosystems has been

reported (Christensen *et al.*, 2001; Lafleur *et al.*, 2003) and we may have done our study in a year with particularly high respiration. However, the early completion of snowmelt during spring 2004 should rather give the opposite trend, because of higher light availability and lengthening of the growing season. Additionally, the modelled mid day photosynthesis rates during the peak growing season in July and August were approximately 5 g C d^{-1} and very close to reported peak values from a similar ecosystem in the same area of $5.2\text{--}6.5 \text{ g C d}^{-1}$ (Olsrud *et al.*, 2004). Net growing season ecosystem carbon loss has also been reported for other nearby heath areas (Illeris *et al.*, 2004). Based also on the high R^2 -values of our R_E and P_g models, it therefore seems likely, that the ecosystem was in fact a substantial source of carbon to the atmosphere during the experimental year. The evidence of climate warming already taking place in the Arctic has grown rapidly in recent years (Hassol *et al.*, 2004). For instance, air temperatures have risen by $2.8 \text{ }^\circ\text{C}$ during winter and by $1.5 \text{ }^\circ\text{C}$ during spring over the last 50 years in the area of the present study concurrent with a significant increase in winter snow depths (Kohler *et al.*, 2006). Together, increased air temperatures and snow depths are likely to have increased soil temperatures during the cold season as well and the observed carbon loss could therefore be a response to soil warming and increased microbial decomposition rates. Our results therefore suggest that the ecosystem type we worked in may represent a potentially major source for positive feedbacks to ongoing and anticipated climate warming.

Effects of increased freeze-thaw

The expected higher respiration rates in the treated plots, due to the respiratory pulses as microbes are lysed during freeze-thaw and quickly decomposed by surviving microbes, were not observed and we therefore did not confirm our hypothesis (A). However, the high number of freeze-thaw cycles naturally occurring in the control plots during spring 2004, compared to the previous years, may have increased respiration rates in control plots as well as in FT plots, thus hiding any extra effect. With our hypothesis (A) of higher respiration and (B) unchanged or increased photosynthesis, the resulting net ecosystem CO_2 loss of the ecosystem should have been most likely to increase. In contrast, we observed significantly lower rates of F_n compared to the controls during the May field campaign, which was most likely because of increased photosynthesis and unchanged respiration although the difference in P_g rates between controls and FT-treated plots only tended to be significant. Snowmelt was just completed prior to the May field campaign and we expect that snowmelt completed earlier in FT plots due to the increased daytime warming and removal of snow. As expected, rather than being an effect of increased freeze-thaw frequency, the tendency to higher P_g was most likely caused by the earlier exposure of the plants, speeding up above-ground plant development and photosynthetic capacity, while the mean soil temperature was unaffected during the greenhouse period, causing no change in soil respiration. In spite of these observed differences, the overall pattern was a general lack of treatment effect on R_E and P_g rates during the observed period, which was also highlighted by the very similar model output for both controls and FT plots. The ecosystem carbon exchange was therefore rather unresponsive to the applied increase in freeze-thaw frequency.

Conclusion

During the traditional non-growing season from October to May, we found significant respiration rates accounting for at least 22 % of annual respiration. More surprisingly, plants were photosynthetically active during the cold season as well, with rates accounting for up to 19 % of annual photosynthesis, suggesting that photosynthesis during the cold season is an important component of the annual carbon budget in this subarctic heath ecosystem, at least in years with low snowfall and early completion of snowmelt. Mosses are often overlooked in mesic heath ecosystems but they are likely to be major contributors to the observed cold-season photosynthesis. The effects on ecosystem fluxes after three years of increased frequency of freeze-thaw during fall and spring were either low or absent suggesting a slower ecosystem response to a possible future climate scenario than was expected. Our study stresses that future research on the carbon balance of high-latitude ecosystems needs to include measurements of cold-season photosynthesis as well as respiration, in order to improve models of annual carbon exchange. More research is also needed on *in-situ* photosynthesis of both vascular plants and bryophytes at low temperatures and low light levels, as well as under the snow, in order to clarify their photosynthetic activity during the cold season.

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Paper III

Ecosystem respiration depends strongly on photosynthesis in a temperate heath

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Ecosystem respiration depends strongly on photosynthesis in a temperate heath

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Abstract

We measured net ecosystem CO₂ flux (F_n) and ecosystem respiration (R_E), and estimated gross ecosystem photosynthesis (P_g) by difference, for two years in a temperate heath ecosystem using a chamber method. Model fit of R_E of a classic, first-order exponential equation related to temperature (second year; $R^2 = 0.65$) was improved when incorporating a simple, linear relationship between R_E and P_g (second year; $R^2 = 0.79$), suggesting that daytime R_E increased with increasing photosynthesis. The model introduces R_{photo} as a measure of the instant respiratory costs of photosynthesis. It increases the reference value of R_E by 5% per unit assimilated carbon dioxide flux at 0°C and by 35% at 20 °C implying a high sensitivity of respiration to photosynthesis during summer.

The simple model provides an easily applied, non-intrusive tool for investigating seasonal trends in the relationship between ecosystem carbon sequestration and respiration. R_{photo} showed a seasonal pattern with very low rates from November to February (5 % of R_E). It increased strongly from March to September, peaking with up to 38 % of R_E in June. Annually, it accounted for 24 % of R_E . Furthermore, the temperature sensitivity of R_E decreased from apparent Q_{10} values of 3.3 to 3.9 by the classic equation to a more realistic Q_{10} of 2.5 by the modified model.

Considerable rates of photosynthesis and respiration were observed throughout the year with mid day net carbon sequestration even at winter solstice. Estimated total gross photosynthesis during the cold season (October to April) was 22 % of annual photosynthesis while cold-season respiration accounted for 30 % of annual respiration. The ecosystem was a large net sink of 293 ± 12 g C m⁻² y⁻¹ during a year of high heather productivity and cessation of earlier cattle grazing.

Introduction

The carbon (C) exchange of terrestrial ecosystems is a key factor in the prediction of the future global climate because these ecosystems contain significantly more carbon than is present in the atmosphere (Davidson and Janssens 2006) and sequester 1/6 of the global atmospheric CO₂ annually (IPCC 2001). Consequently, they have a high potential for creating negative or positive feedbacks to climate change. The net ecosystem exchange of carbon is the relatively small difference between much larger fluxes of C sequestration by plants and release of C through respiration by plants, soil microbes and animals. Small errors in the estimation of photosynthesis and respiration may therefore lead to relatively large errors in the estimate of net ecosystem exchange.

All current methods for estimating ecosystem carbon balance, including the two most frequently applied techniques, eddy co-variance and the chamber method, depend on models of photosynthesis and respiration or gap filling methods, because measurements are not continuous. Furthermore, the eddy covariance technique only provides the net ecosystem carbon exchange, and other methods are required to estimate the components of the net balance, i.e. photosynthesis and respiration. Models of photosynthesis and respiration are therefore important tools in studies of carbon cycling and play a fundamental role for the estimation of ecosystem carbon exchange.

The enzymatic processes involved in photosynthesis have evolved conservatively across taxa (Davidson et al. 2006), and the theoretical understanding of photosynthesis is well-established (Trumbore 2006). Unless plants are drought stressed, temperature and solar radiation are by far the most important drivers of instant ecosystem photosynthetic rates. If the number of photosynthesis measurements and their distribution in space and time are sufficient, ecosystem photosynthesis can be modelled with relatively high confidence using well-known empirical temperature and light response equations.

In contrast, respiration processes span over many different enzymatic systems across taxa (Davidson et al. 2006). Still, ecosystem respiration (R_E) has traditionally been modelled based on thermodynamic principles by using simple, first-order exponential equations with temperature as the only determinant (Craine et al. 1999). Their use and appropriateness are currently being questioned, because the simple temperature-dependency of these models only poorly reflects the complex nature of the different components of ecosystem respiration and the drivers that control them (Craine et al. 1999; Davidson et al. 2006). For instance, the evidence that the photosynthetic rate influences R_E is currently growing (Hogberg et al. 2001; Tang et al. 2005; Irvine et al. 2005; Knohl et al. 2005), and the interaction between photosynthesis and respiration needs to be incorporated into the models (Craine et al. 1999). This has not generally been done, possibly because the empirical modelling of respiration by temperature-dependent exponential equations often fit well with observed respiration rates. The good fit of the model may, however, be misleading, because several factors as e.g. phenology, photosynthesis, substrate supply or soil water content often co-vary with temperature (Trumbore 2006; Davidson et al. 2006) and mask other important drivers of the

respiration. However, despite the often high model fit of the classic Q_{10} equation, the resulting temperature sensitivities often become unrealistically high with Q_{10} values exceeding 2.5, which suggests that other factors than temperature are affecting the respiration (Davidson et al. 2006).

In most ecosystems, soils contain high amounts of carbon, and soil respiration may account for as much as 60-90% of total R_E (Goulden et al. 1996; Longdoz et al. 2000). Consequently, soil respiration plays an essential role in the carbon balance and the partitioning of soil respiration has been intensely studied (Subke et al. 2006). Soil respiration is the sum of respiration from roots and their mycorrhizal symbionts, as well as from the rhizosphere, the bulk soil microbes and the soil fauna. Especially the separation of soil respiration into autotrophic (R_a) and heterotrophic (R_h) respiration is important, because R_a reflects the carbon input from the canopy while the resulting carbon storage and nutrient turnover in the soil depend on the activity of the heterotrophic soil community (Binkley et al. 2006). R_a and R_h are therefore likely to respond differently to the major ecosystem conditions of, e.g., temperature, water availability, and anticipated future climate changes.

Because of the complex structure of the soil environment, the separation of the various sources of R_E is difficult, and many different approaches have been applied in the attempt to partition the respiration components. Destructive methods include different forms of root exclusion (e.g. clipping, trenching, and girdling) as well as physical separation of components, while isotope techniques and modelling represents the non-destructive methods; see reviews of e.g., Hanson et al. (2000), Subke et al. (2006) or Kuzyakov (2006).

In grasslands and heathlands dominated by low-statured plants, the gas exchange can be estimated on ecosystem level by chamber techniques. This technique has the advantage that both respiratory carbon loss and photosynthetic uptake are estimated independently and at the same scale. Although the method does not allow for a separation of R_E into its above-ground and soil component, we wanted to investigate if models of R_E could be improved by incorporation of photosynthetic activity as a biological driver for R_E . If so, this could provide important information on the interaction between autotrophic and heterotrophic respiration on the ecosystem level.

In this study, we estimated the net ecosystem CO_2 flux (F_n), the R_E , and their difference (gross ecosystem photosynthesis; P_g) for two years in a semi-natural, temperate heath ecosystem. In order to investigate seasonal patterns of photosynthesis and respiration, we performed measurements through all seasons of the years. Flux rates were modelled separately for each of the two years, which differed in grazing intensity by cattle.

Materials and methods

Study site and experimental setup

The experimental area is a heath close to the CLIMOOR site (Beier et al. 2004) at Mols Bjerger, Eastern Jutland, Denmark (56°23' N, 10°57' E), 58 m above sea level. Annual mean air temperature is 9.4°C (1.6°C in January and 18.1°C in July) and mean annual precipitation

(1998-2000) is 758 mm. The soil is a sandy podzol with a pH of 4.6 (Jensen et al. 2003). The heath has an approximately 3 cm thick organic top layer with a mean soil organic matter (SOM) content of $19.3 \pm 1.7\%$, and the upper 7 cm of the mineral soil has a SOM content of $3.2 \pm 0.3\%$. Together, the two soil layers have 5.6 ± 0.1 kg SOM m^{-2} in the top 10 cm. The vegetation is dominated by the evergreen dwarf shrub *Calluna vulgaris* (L.), various mosses and the grass *Deschampsia flexuosa* (L.). The area is grazed extensively and mostly during winter by Galloway cattle.

In fall 2003, 6 replicate plots of 2×2 m^2 were randomly selected within the experimental site, and a soil collar of 30×30 cm^2 was installed within each plot reaching 2 to 10 cm into the soil. Rather than placing the soil collars randomly, we made sure that all of them contained a patch of *C. vulgaris* small enough to fit into the collar, as well as a substantial cover of mosses and *D. flexuosa*. Tinytags (Gemini Data Loggers, Chichester, England) were installed ca. 30 cm outside each soil collar, logging the temperature at the soil surface (T_{surf}) and at 3 cm soil depth (T_{soil}). From 27 September 2004, a Campbell CR10X datalogger (Campbell Scientific Inc., Logan, USA) also recorded the soil volumetric water content (SW) at two points within the experimental area using Campbell TDR probes, as well as the incident photosynthetic active radiation (PAR) at one point in the middle of the experimental site (Campbell quantum sensor). PAR was recorded once per minute, temperatures every 10 minutes, and SW every hour. All data was stored by the loggers as one-hour averages. From 24 February 2004 to 26 September 2004, PAR flux densities (I) were extrapolated from measurements of global radiation (2 h intervals) from the weather station at the Mols Laboratory approximately 200 m from the site. Because of animal disruption of equipment, the experimental area had to be fenced and was therefore not grazed after 1 October 2004 until the end of the observation period.

Vegetation biomass and height

Four times during the cold season of 2004 and 2005, we collected 20×20 cm^2 cores of soil with vegetation to a depth of 10 cm within each plot. The cores were taken at a minimum distance of 30 cm from the soil collars and brought to the laboratory, where the plants were carefully separated from the soil with as many roots as possible attached. The plants were subsequently sorted into five fractions: Evergreens (shoots and roots), graminoids and herbs (shoots and roots) and mosses. Dead plants were discarded except for partly senesced grass leaves. *C. vulgaris* was the sole evergreen, and the grass *D. flexuosa* accounted for more than 90% of the graminoid/herb fraction of each sample. A sub-sample of about 100 cm^3 of the soil was sorted for a maximum of 30 minutes in order to extract remaining roots for determination of total root biomass. The mean vegetation height within each soil collar was measured during spring (March) and fall (September) each year as an average of six point measurements.

CO₂ flux measurements

From 30 September 2003 to 20 September 2005, CO₂ fluxes were measured 58 times at each soil collar, summing up to a total of 348 measurements. The flux observations include nine diurnal measurement campaigns, usually with four measurements per plot within 24 hours and 25 additional measurement dates with fluxes measured between 10 a.m. and 2 p.m. CO₂ fluxes were measured with a LICOR-6400 infrared gas analyzer (IRGA, LI-COR, Lincoln, NE, USA) attached to a 33.5 L Perspex chamber. A water-filled channel on the sides of the soil collars ensured a tight seal of the chamber atmosphere during measurements.

F_n was measured during three consecutive one-minute intervals after allowing the chamber to equilibrate for about 20 s from the time when the chamber was placed on the soil collars. In most cases, the photosynthetic rate decreased from the first to the third minute of measurement, indicating an effect of the chamber by the decreasing CO₂ concentration as photosynthesis progressed. We therefore use the fluxes during the first minute of measurement as an estimate of F_n .

After the F_n measurements, the chamber was vented and repositioned, followed by measurements of R_E with the chamber covered by a thick layer of black plastic. As for the F_n measurements, we observed a chamber effect of decreasing rate of CO₂ flux into the chamber with time, presumably because of reduced diffusion from the soil as the chamber CO₂ concentration increased. In order to avoid bias due to disturbance when the chamber was repositioned and the effect of reduced diffusion with time, we use the flux during the second of three consecutive one-minute-measurements for the estimate of R_E . P_g was estimated as $F_n - R_E$ and has a negative sign, while R_E is positive.

Statistical analysis and data processing

Changes over time in plant biomasses of the plant fractions and their sum (*C. vulgaris*, mosses and *D. flexuosa* and their sum) and in mean height of the vegetation were tested using one-way ANOVA followed by Tukey's test (SAS Enterprise Guide 3.0).

To investigate the controls of ecosystem CO₂ fluxes, we first modelled R_E using a classic, first-order exponential equation by (van't Hoff 1898):

$$R_E = R_0 e^{bT} \quad (1)$$

where R_0 ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) represents the ecosystem respiration at 0 °C depending on substrate availability, b is the temperature sensitivity ($^{\circ}\text{C}^{-1}$) of respiration, and T is the temperature ($^{\circ}\text{C}$). We fitted models with T_{soil} (-3 cm), T_{surf} (0 cm) and their mean (T_{mean}), and found the closest model fit when using the T_{soil} , which therefore is reported here. During the diurnal field measurements, we noticed that respiration during the nights usually was lower than respiration during mid day, presumably because the respiration rate was affected by the rate of photosynthesis. To include this effect, we therefore modified the classic exponential equation to:

$$R_E = (R_{0m} + \lambda P_g) e^{b_m T} \quad (2)$$

λ is a unitless measure of the respiratory costs of photosynthesis as a fraction, comprising both above-ground effects of enhanced assimilate metabolism and transport, and/or enhanced soil respiration, e.g. through increased autotrophic respiration, mycorrhizal and rhizosphere respiration. R_{0m} is the basal ecosystem respiration at 0 °C in the absence of photosynthesis, and b_m is the temperature sensitivity as in equation (1). In this way the assumptions of the model are:

$$R_E = R_{\text{base}} + R_{\text{photo}} \quad (2b)$$

$$R_{\text{base}} = R_{0m} e^{b_m T} \quad (2c)$$

$$R_{\text{photo}} = \lambda P_g e^{b_m T} \quad (2d)$$

R_{base} is the basal respiration rate of the ecosystem unaffected by photosynthesis, and R_{photo} is the fraction of R_E directly linked to the instant photosynthetic rate. The temperature sensitivity (b_m) is assumed to be identical for R_{base} and R_{photo}

P_g was modelled using the non-rectangular hyperbola (Thornley 1976):

$$P_g = \frac{(\alpha \cdot I + P_m) - \sqrt{(\alpha \cdot I + P_m)^2 - 4\alpha \cdot I \cdot P_m \cdot \theta}}{2\theta} \quad (3)$$

where I is measured in $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, α is the PAR use efficiency ($\text{mol CO}_2 \text{ mol photon}^{-1}$), θ is a dimensionless curvature parameter, and P_g is measured in $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$. P_m is maximum P_g at a certain leaf temperature. Temperature dependency of P_g was described using an equation similar to the equation used by (dePury and Farquhar 1997) for electron transport enzyme kinetics:

$$P_m = P_{m_r} \cdot e^{\frac{E_a(T-T_r)}{R T T_r}} \cdot \frac{1 + e^{\frac{\Delta S T_r - E_d}{R T_r}}}{1 + e^{\frac{\Delta S T - E_d}{R T}}} \quad (4)$$

where P_{m_r} is P_m at the reference temperature (T_r) of 298 K, T is temperature in K, E_a is the activation energy (kJ mol^{-1}), R is the universal gas constant ($8.341 \text{ J mol}^{-1} \text{ K}^{-1}$), ΔS is an entropy term ($\text{J mol}^{-1} \text{ K}^{-1}$), and E_d is the energy of deactivation (kJ mol^{-1}). As for the respiration models, we fitted the model based on temperatures of the soil, the soil surface and their mean and found the closest fit using the T_{mean} , which is reported here.

Because the grazing stopped in October 2004, we evaluated the model parameters for ecosystem CO₂ fluxes separately for the first and second year of the study. Hence, measurements from 30 September 2003 to 13 September 2004 were used to model the fluxes the first year, while measurements from 27 September 2004 to 20 September 2005 were used to model the fluxes the second year. All six replicate plots were included in the same model. The models were used to extrapolate from the measurements to the whole period. When applying the models to the climate data, the models were run on the temperature recorded by each temperature logger individually and then corrected for seasonal residuals to include potential seasonal differences in the physiological response of the ecosystem. Standard errors of the reported monthly estimates therefore represent the variability in temperatures as well as the residuals caused by variations over the seasons.

Results

Biometrical measurements of vegetation

The vegetation was dominated by *C. vulgaris*, which constituted on average 52±4 % of the above-ground dry biomass, followed by mosses (29±3 %) and *D. flexuosa* (18±2 %). The mean total above-ground biomass over the four samplings during the cold season 2004/2005 was 980±43 g m⁻², and did not change significantly with time (ANOVA_{3,20}, $F = 0.85$, $P = 0.48$). However, the biomass of *D. flexuosa* was low from November to March and then increased significantly from March to May (One-way ANOVA_{3,20}, $F = 17.3$, $P < 0.0001$). Total below-ground dry biomass was on average 768±64 g m⁻², resulting in a mean ratio of 1.3 between above- and below-ground biomass.

Figure 1

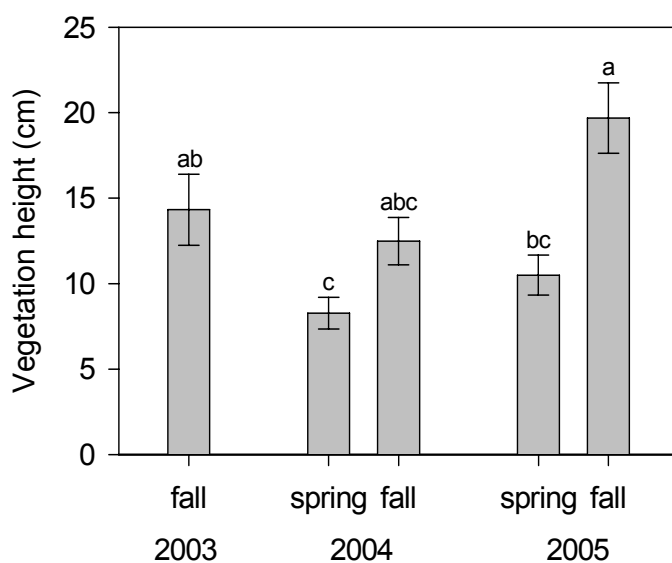


Figure 1. Mean vegetation height in fall (September) and spring (March) during the experiment. Data are means ± SE, $n = 6$. Results of Tukey's test following a one-way Anova are shown. Bars, which do not share letters are significantly different ($P < 0.05$).

Figure 2

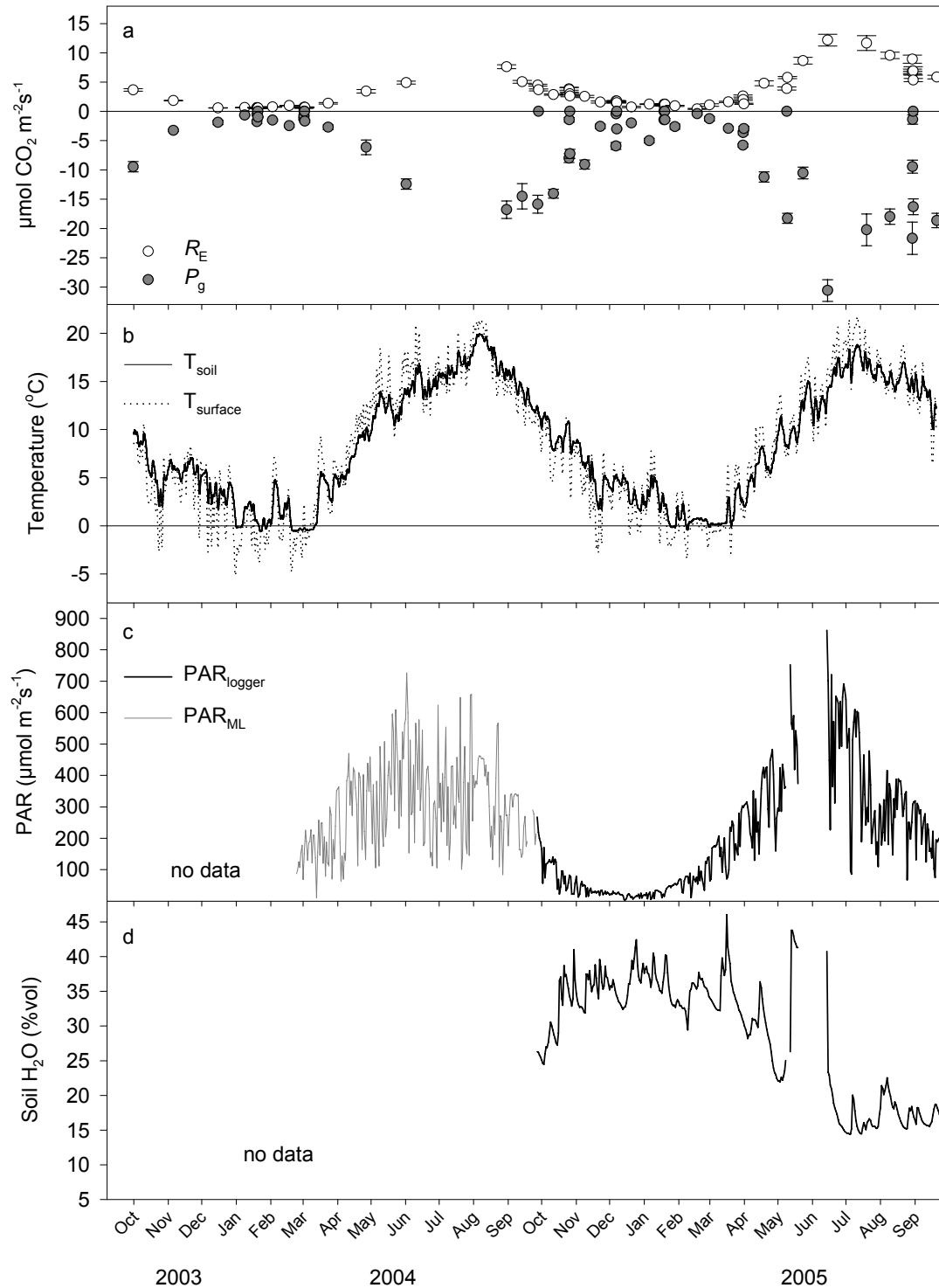


Figure 2. Observed flux rates of ecosystem respiration (R_E) and photosynthesis (P_g) from 30 September 2003 to 20 September 2005 (a). Data are means \pm SE, $n = 6$ and the number of measurements per plot is 58. Several circles at the same point in time represent the diurnal variation in flux rates. When only one measurement was done, it was performed at mid day during 10 a.m. and 2 p.m. Also shown are (b) temperatures at 3 cm soil depth (T_{soil}) and at the soil surface (T_{surface}), (c) photosynthetically active radiation (PAR) and (d) soil water content (SW). No data on PAR was available prior to 24 February 2004 and SW was logged with start on 27 September 2004. From 24 February to 26 September 2004, PAR data are extrapolated from global radiation data measured at the Mols

Laboratory weather station situated about 200 m from the site (PAR_{ML}). From 27 September 2004, the data are from a data logger placed within the site (PAR_{logger}).

The mean vegetation height (Fig. 1) was 14 ± 2 cm in fall 2003 and decreased significantly (Tukey's test, $P < 0.05$) over the cold season due to cattle grazing, reaching only 8 ± 1 cm in spring 2004. As expected, the vegetation height tended to increase over the following growing season, although this was not statistically significant. In the second winter, when the area was un-grazed, the vegetation height was constant, but doubled during the subsequent growing season.

Observed and modelled CO_2 fluxes

The observed R_E rates (Fig. 2a) showed the expected seasonal pattern decreasing from late September until the winter months (December-February), followed by low and relatively constant rates between 0.59 and $0.99 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ during the first winter. The R_E rates fluctuated more during the second winter ranging between 0.43 and $1.77 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. From the beginning of April, the rates increased rapidly with time, especially during the second year when there was no cattle grazing, reaching peak rates of 12.2 ± 1.0 and $11.7 \pm 1.3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in June and July, respectively. A similar seasonal trend was observed for P_g

Figure 3

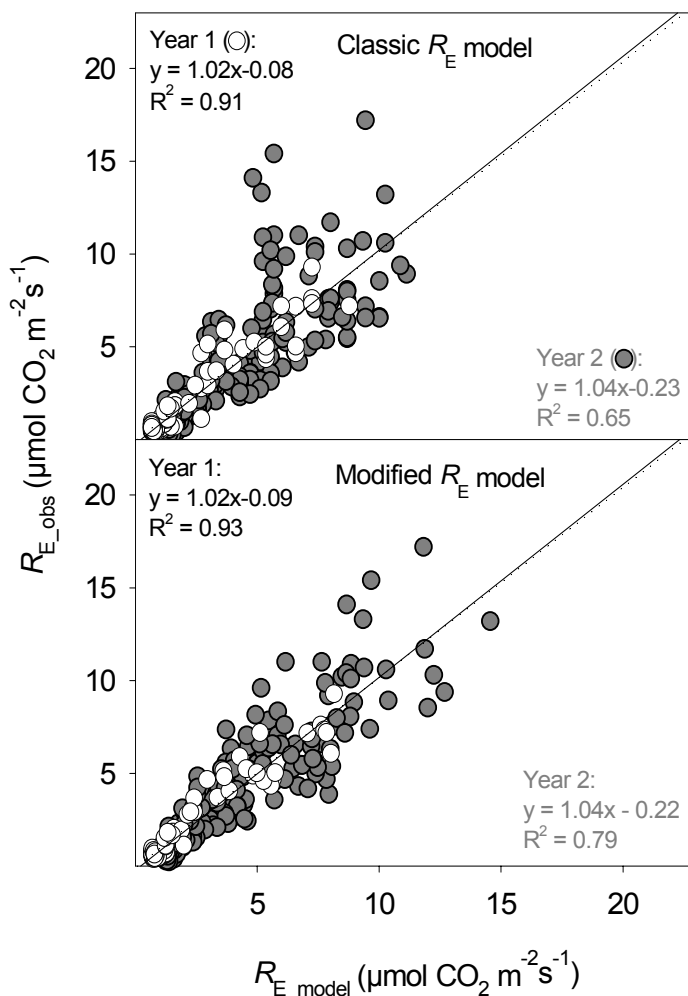


Figure 3. Modelled (R_{E_model}) and observed (R_{E_obs}) flux rates of ecosystem respiration (R_E), using classic (upper panel) and P_g -modified (lower panel) first-order exponential equations with regression line equations for each year and explained variances (R^2).

See text for model equations and Table 1 for the parameter estimates.

(Fig. 2a) but with highest values more than twice the R_E rates peaking at $-30.6 \pm 1.9 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ in June 2005. Considerable P_g rates were measured even at mid day during mid winter with e.g., $-5.0 \pm 0.4 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ in January 2005, leading to a negative mid day net carbon exchange (i.e. ecosystem sequestration of C) at all occasions of measurement. The seasonal pattern of the observed R_E and P_g rates followed closely the seasonal development in temperatures and solar radiation (Fig. 2b, c). For instance, during the second year, R_E correlated significantly with T_{soil} ($R^2 = 0.65$), and moderately with the soil water content (Fig. 2d, $R^2 = 0.39$). In comparison, P_g was more related to PAR ($R^2 = 0.62$) than to T_{mean} ($R^2 = 0.39$) and less to soil water content ($R^2 = 0.20$).

Table 1. Parameters and standard errors (in brackets) from model estimation of R_E using first-order exponential equations (classic R_E model, eq. 1) and a similar model but with addition of a linear relationship of R_E with P_g (modified R_E model, eq. 2). See text for equations.

Year	R_E model	R_0 / R_{0m}	b / b_m	Q_{10}	λ	R^2	n
2003-2004	Classic	0.738 (0.05)	0.137 (0.005)	3.9	-	0.91	114
	Modified	0.738 (0.05)	0.093 (0.009)	2.5	-0.048 (0.013)	0.93	114
2004-2005	Classic	1.291 (0.12)	0.118 (0.007)	3.3	-	0.65	234
	Modified	1.217 (0.09)	0.090 (0.006)	2.5	-0.058 (0.007)	0.79	234

Using the classic, first-order exponential equation, the best model fit for R_E was found using T_{soil} , which resulted in $R^2 = 0.91$ during the first year and $R^2 = 0.65$ during the second year (Fig. 3a, Table 1). Interestingly, the model fit was improved to $R^2 = 0.93$ and $R^2 = 0.79$, respectively, when the R_{photo} was included in the modified R_E model (Fig. 3b, Table 1).

The cost term of respiration during photosynthesis in the modified R_E model, λ , was significant in both years ($P < 0.0001$), showing that ecosystem respiration increased considerably concomitant with increasing photosynthetic rates. λ reached about -0.05 in both years, i.e. the reference respiration at 0 °C increased by 5% of the instant P_g rates. At higher temperatures, however, the proportion of the assimilated carbon that is instantly respired through enhanced R_E , may be calculated as $\lambda e^{b_m T}$. According to the modified R_E model, therefore, the proportion increased with temperature to 14 % of the instant P_g rates at 10°C and 35 % at 20°C.

Due to the pronounced sensitivity of R_E to P_g rates, the modified R_E model estimated significantly lower temperature sensitivities (b_m) than the classic model (Student's t-test, $P < 0.01$ in both years), resulting in annual Q_{10} values of 2.5 in both years as compared to 3.3 or 3.9 by the classic model. Consequently, the slopes of temperature response curves generally were lower than for the classic R_E model, except at high P_g rates (Fig. 4).

The model fit improvement by the modified R_E model compared to the classic model, and the resulting high R^2 values indicates that temperature and the photosynthetic rates were the major drivers of the instant respiration. In a similar way, we also investigated the effect of

Figure 4

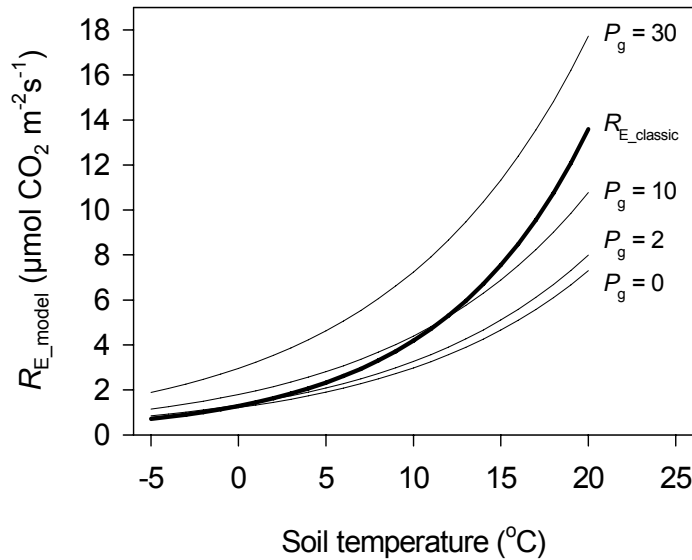


Figure 4. Temperature response curves for the ecosystem respiration (R_E) models from the second year of the study. The solid line represents the classic R_E model ($R_{E_classic}$) and thin lines represent the response to temperature by the modified R_E model at different rates of instant photosynthesis. See table 1 for parameter estimates.

Figure 5

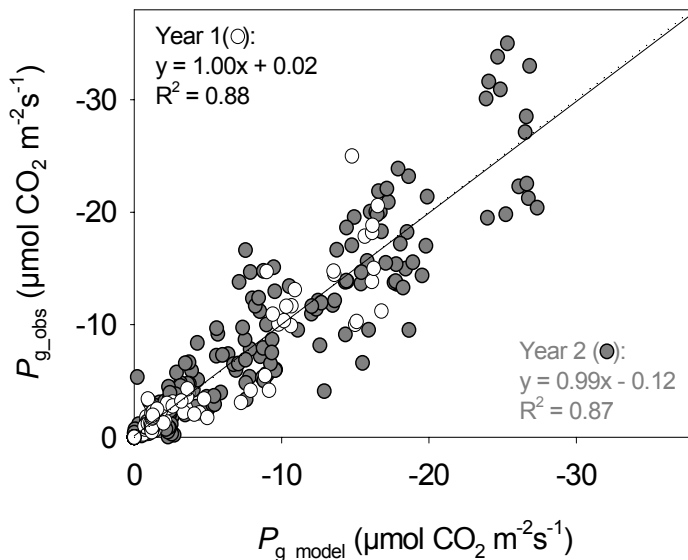


Figure 5. Modelled (P_{g_model}) and observed (P_{g_obs}) flux rates of ecosystem photosynthesis (P_g). Regression line equations for each year and explained variance (R^2) are shown. See text for model equations and table 2 for the parameter estimates.

the soil water content on R_E during the second year, using a modification of the classic model with a linear relationship between soil water content and R_E included. This improved the

model fit only marginally ($R^2 = 0.68$, data not shown) compared with the classic model ($R^2 = 0.65$), and the constant related to the water content in this model (similar to λ in equation 2) only tended to be significant ($P = 0.08$).

Table 2. Parameters and standard errors (in brackets) from model estimation of P_g . See text for equations.

Year	α	θ	P_{mr}	E_a	E_d	R^2	n
2003-2004	0.030 (0.003)	0.996 (0.02)	14.2 (1.2)	143929 (21329)	202826 (1543)	0.87	114
2004-2005	0.055 (0.015)	-0.795 (1.70)	46.3 (18.1)	153940 (25177)	201615 (1555)	0.87	234

The P_g models yielded high R^2 -values of 0.87 during both years (Fig. 5, Table 2), clearly indicating that temperature and solar radiation were the major determinants of the photosynthetic rates, as also shown by the linear regression analyses. The close model fit for both the R_E and the P_g rates is reflected by small residuals between observed and modelled fluxes (Fig. 6) relative to the level of the measured flux rates. Often, the residuals were not significantly different from zero. Still, both models showed a seasonal trend of slightly overestimated model fluxes during fall and winter and underestimation during spring. This trend tended to be decreased, although not significantly, by the modified temperature response model. Similarly, the residuals of P_g generally were not significantly different from zero, except during spring both years, when the model slightly overestimated the photosynthetic rates.

Figure 6

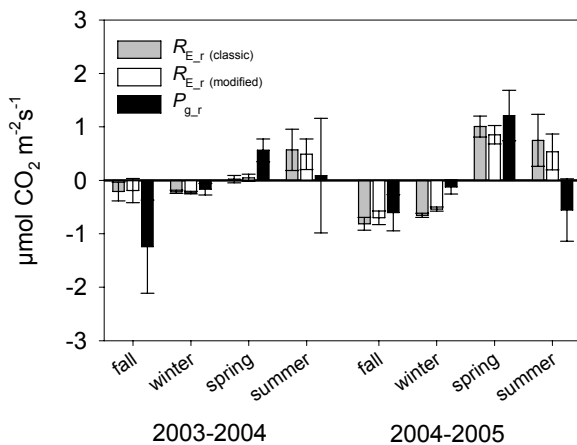


Figure 6. Seasonal residuals (r) over the season of ecosystem respiration (R_E , classic and modified models) and photosynthesis (P_g). Residuals were calculated as observed minus modelled fluxes; i.e. positive residuals means model overestimation of P_g and underestimation of R_E . The seasons were defined as fall (Sep-Nov), winter (Dec-Feb), spring (Mar-May) and summer (Jun-Aug).

In general, the low residuals and high R^2 -values give considerable confidence in the estimates of the gas fluxes. We therefore ran the modified R_E model (equation 2) and the P_g model (equations 3 and 4) on the available temperature and radiation data (Fig. 7a, b) in order to extrapolate from our measurements to the entire time period. We estimated R_{photo} using equation (2d) with the parameter estimates of λ and b_m from the model fit of equation (2). We

modelled R_E by the classic model (equation 1) during the first six months of the study period, when P_g could not be modelled due to the lack of radiation data.

Figure 7

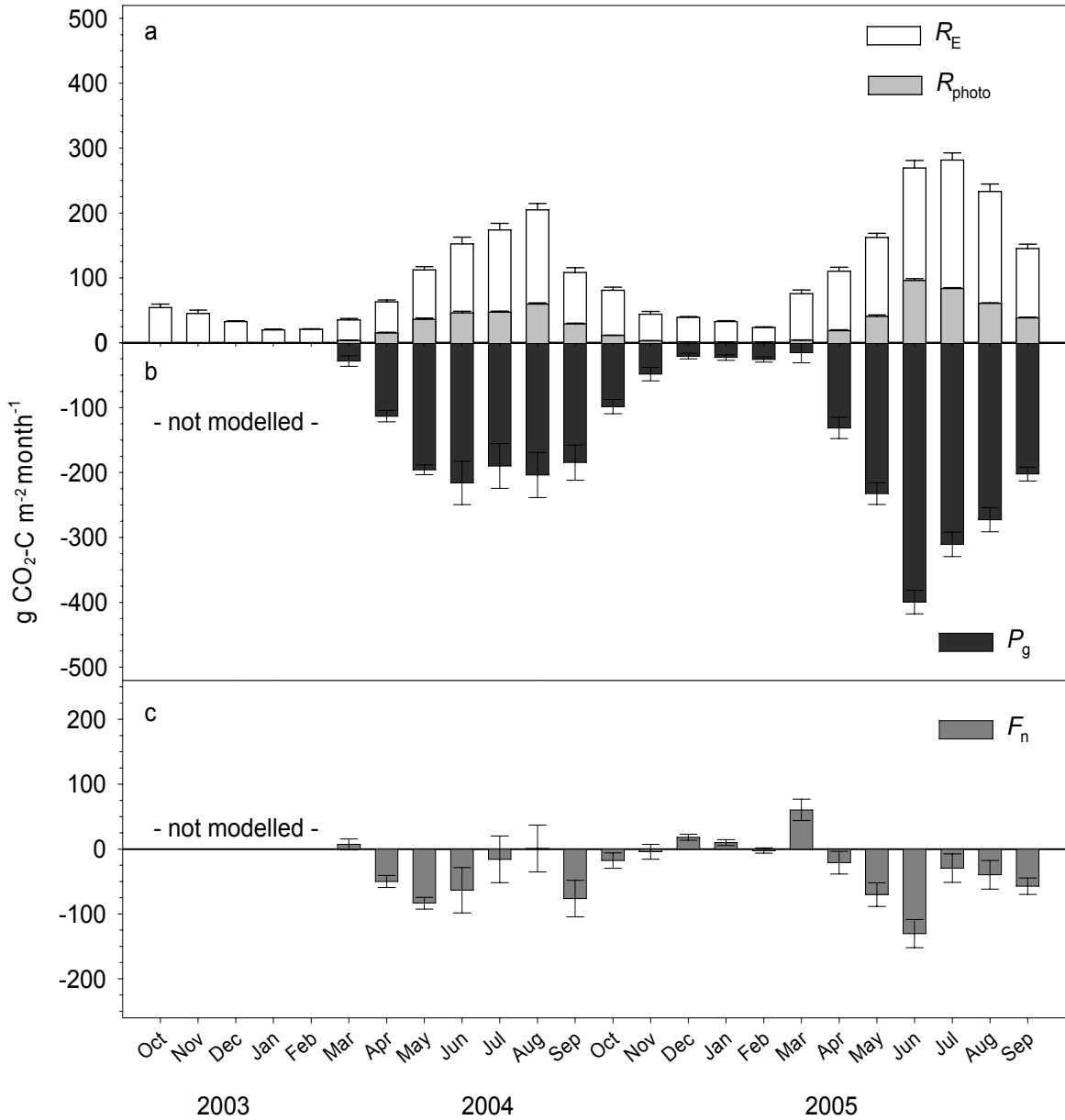


Figure 7. Estimated monthly ecosystem respiration (a) as total respiration (R_E) and the proportion that is related to the instant photosynthesis rate (R_{photo}), (b) estimated ecosystem gross photosynthesis (P_g), and (c) monthly net exchange of carbon (F_n). Because of lack of data prior to 24 February 2004, monthly rates of P_g , R_{photo} and F_n could only be modelled from March 2004. For the same reason R_E until March 2004 was modelled using the classic first-order exponential equation (see text, equation 1).

The modelled monthly estimates of ecosystem respiration and photosynthesis revealed a seasonal pattern of 70% of annual respiration taking place during April to September, while the remaining 30% occurred during the cold season from October to March (Fig. 7a). The lowest rates were in January and February during both years, which together accounted for 4 to 6% of the annual respiration, while monthly respiration peaked in August during the first year and in June / July during the second year. However, the respiratory carbon loss was almost entirely cancelled out by similar or higher photosynthesis rates at all times, except in March 2005 (Fig. 7b, c). As a result, cold-season photosynthesis accounted for 22% of the annual C uptake. Total annual respiration of the second year was estimated to 1481 ± 11 g C m⁻²y⁻¹ when using the modified R_E model (equation 2) compared to 1662 ± 16 g C m⁻²y⁻¹ if using the classic model (equation 1). R_{photo} was close to zero from November to March in both years, i.e. the plants had a constant respiration over the day in spite of considerable observed and modelled P_g -rates during mid day (Fig. 2b). From March to June, P_g increased strongly and so did the R_{photo} , thereby accounting for 34 % and 38 % of R_E in June 2004 and June 2005, respectively. During the second year, when all fluxes were modelled through the entire year, R_{photo} accounted for 24 % of the annual R_E and 20 % of annual P_g . With annual P_g during the second year of 1774 ± 11 g C m⁻²y⁻¹, the ecosystem was an estimated sink of 112 ± 19 g C m⁻²y⁻¹ using the classic R_E model (data not shown) compared to 293 ± 12 g C m⁻²y⁻¹ if using the modified R_E model.

Discussion

Application and effects of the modified R_E model

Our modified model of ecosystem respiration introduces R_{photo} as an estimate of the fraction of the ecosystem respiration, which is linked to the instant rate of photosynthesis. R_{photo} includes the direct effect of the instant rate of photosynthesis on plant respiration as well as the indirect effects of photosynthetically supplied C to the mycorrhizal fungi and rhizosphere organisms. Consequently, our method does not separate the autotrophic and heterotrophic respiration, which is attempted in some studies on ecosystem respiration partitioning. However, our non-intrusive modelling approach indicates several important traits of the ecosystem carbon cycling, partitioning and seasonality.

First, our results indicate that the ecosystem respiration was greatly affected by the instant carbon sequestration of the plants. This interpretation is supported by recent reports of links between photosynthesis and respiration from a variety of forest (Hogberg et al. 2001; Irvine et al. 2005; Knohl et al. 2005), an oak-grass savanna (Tang et al. 2005), and grassland ecosystems (Craine et al. 1999). Second, we could only find marginal effects of drought on respiration, although drought effects have been reported to modify respiration, e.g. in Mediterranean ecosystems (Reichstein et al. 2002). Third, the modified R_E model provided a direct measure of the instant respiratory costs of carbon assimilation, which was unexpectedly large at about 5% of the instant P_g rate at 0 °C and increasing to ca. 35 % of the instant P_g rate at 20 °C during both years.

Due to inclusion of the photosynthetic rate in the temperature response of R_E , the temperature sensitivity of ecosystem respiration was considerably decreased from an annual Q_{10} of up to 3.9 by the classic model to only 2.5 using the modified model, corresponding to about the upper limit for physiologically realistic Q_{10} values proposed by (Davidson et al. 2006). They argue that if observed Q_{10} values exceed 2.5, processes other than temperature fluctuations are likely to influence the respiration rates. This important effect of our modified R_E model exemplifies how a high model fit of a classic model can be misleading (Trumbore 2006). For instance, during the first year of our study, the classic model yielded a high R^2 of 0.91, which was only slightly improved by the modified model ($R^2 = 0.93$). However, the significant decrease of the temperature sensitivity by the modified model strongly suggests that the photosynthesis was an important driver of respiration, but that the effect was hidden in the classic model because the P_g rates co-varied with temperature. Similarly, other potentially important factors, e.g. phenology, substrate supply or soil water content may co-vary with temperature (Trumbore 2006; Davidson et al. 2006) and mask the true drivers of the respiration. Our approach indicates that the possible hidden effects may be revealed by our simple modification of the model. For instance, a similar approach revealed that the supply of dissolved organic carbon and nitrogen to the soil microbes played a dominant role controlling the ecosystem respiration in a subarctic heath during spring-thaw (Larsen et al. 2006).

Unravelling of the seasonal patterns and controls of autotrophic and heterotrophic respiration is important for understanding ecosystem functioning as well as for assessing possible impacts of future climate changes. Still, studies focusing on the seasonal relationship between these two components are lacking. Our modified R_E model provided the opportunity to estimate ecosystem respiration without photosynthesis, because the model can easily distinguish between R_{base} and R_{photo} . The model indicated a strong seasonal pattern of the photosynthesis-related respiration being of minor importance from October to February while contributing considerably with of up to 38% of total respiration during the growing season.

The model fit improvement of the modified R_E model compared to the classic model was higher during the second year in the absence of grazing ($R^2 = 0.79$ and 0.65, respectively) than during the first year ($R^2 = 0.93$ and 0.91, respectively) when the area was still grazed by cattle. The instant respiratory costs of photosynthesis (λ), however, were similar in both years. Still, the doubling of plant height over the second growing season suggests that photosynthesis became a more important factor for ecosystem respiration relative to temperature during the second year. As a consequence, the peak season respiration related to the photosynthesis was slightly higher during the second (38% of R_E) than during the first year (34%).

Seasonality of carbon fluxes

The flux rates of ecosystem respiration were high, usually reaching between 5 and 10 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ during the growing season, peaking at $12.2 \pm 1 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ in June 2005. In comparison, peak rates from plots at the nearby CLIMOOR site without *C. vulgaris*, and

with less than half of standing plant biomass of that in our plots, were 6.3 ± 0.3 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ (Tesgaard 2006). Peak season R_E rates of 9 to 10 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ have been reported from North-American grassland ecosystems (Xu and Baldocchi 2004; Flanagan and Johnson 2005) with annual ecosystem respiratory carbon loss of e.g. 735 to 758 $\text{g C m}^{-2}\text{y}^{-1}$ (Xu and Baldocchi 2004). Our annual estimate of $1481 \pm 11 \text{ g C m}^{-2}\text{y}^{-1}$ is about twice as high and even slightly higher than the annual ecosystem respiration of various temperate European forest ecosystems, estimated on average to $1100 \pm 260 \text{ g C m}^{-2}\text{y}^{-1}$ (Janssens et al. 2001). However, several factors are likely to explain the high rates in our study. First, the *C. vulgaris* was most probably re-established after an outbreak of the heather beetle (*Lochmaea suturalis*) in the area in 1998. Being about six years old, the shrubs were entering the building phase of their life cycle (Gimingham 1960), and the rapidly increased plant biomass in the absence of grazing during the second year indicates that growth respiration was considerable. Second, we did not find any indication that respiration rates were suppressed by water stress at any time.

These factors may also explain why the annual P_g was high and comparable to the highest estimates from temperate forest ecosystems. Another indication for high plant productivity is the relatively high leaf area index (LAI) of *C. vulgaris* estimated to $5.3 \pm 0.4 \text{ m}^2 \text{ m}^{-2}$ at another Danish heath site (Sørensen, unpublished data), which is only slightly less than reported LAI of, e.g., evergreen forests (Ibrom et al. 2006), but higher than peak values in a broadleaved forest ecosystem (Pilegaard et al. 2001).

An alternative reason for the relatively large gross CO_2 fluxes of R_E , and P_g , if compared to, e.g., the estimates for forests (Janssens et al. 2001), may be methodological. The estimates for forest are all based on the eddy correlation technique where daytime R_E have been extrapolated from night time fluxes, overlooking any particular enhancement of daytime R_E that is not related to temperature. If a similar relationship between photosynthesis and respiration exists for forest ecosystems as seen in the current study, the respiration based on night time measurements only would correspond to R_{base} in our study, indicating that R_E and P_g are underestimated with eddy correlation technique by about 20 % annually judging from our results. However, unlike in our study, a time lag between carbon sequestration and increased ecosystem respiration rates on a diurnal (Tang et al. 2005) as well as seasonal (Hogberg et al. 2001) basis has been reported for forest ecosystems, indicating a different pattern of the photosynthesis-respiration interaction. Clearly, more research on this interaction is urgent in order to increase the confidence of carbon flux estimates.

The high net fluxes coincide with the observed increase in plant height. The net carbon gain of $293 \pm 11 \text{ g C m}^{-2}\text{y}^{-1}$ during the second year represents a biomass gain of approximately $580 \pm 22 \text{ g dry mass m}^{-2}\text{y}^{-1}$ assuming a carbon content of dry biomass of 50 %. This corresponds to a net increase of about 34% of the total above- and below-ground biomass. The large P_g during all seasons of the year and the high net carbon gain during the second year of the study demonstrate that heath ecosystems may act as large carbon sinks when *C. vulgaris* is in its peak growth phase. Second, although the photosynthetic rates during winter were low compared to peak growing season rates, they had a considerable effect on the net

carbon exchange by practically always cancelling out the respiratory carbon loss. The carbon exchange during the cold season of the year is therefore at least as important for the resulting carbon balance as during the growing season, when both ecosystem respiration and photosynthesis are much higher. *C. vulgaris* has previously been suggested to be dormant from October to February based on the cessation of growth (Miller 1979), irregular stomata opening and accumulation of sugar in the leaves (Miller 1979; Kwolek and Woolhouse 1981). Recently, however, both *C. vulgaris* and *D. flexuosa* have been shown to absorb considerable amounts of nitrogen during the cold season (Andresen and Michelsen 2005; Larsen et al. unpublished data). Although mosses and graminoids may have contributed to the observed photosynthesis during the cold season, the dominance of *C. vulgaris*, the fact that is evergreen, and that mid day flux rates were considerable, indicate that it was photosynthetically active throughout the year.

Conclusion

The exchange rates of carbon in the investigated heath ecosystem were high and of similar magnitude as for productive forest ecosystems. Our results showed that the carbon sink strength of heather-dominated ecosystems may be considerable when *C. vulgaris* is in the building phase of its life cycle. By incorporating a simple, linear relationship between instant photosynthesis rates and ecosystem respiration into a classic, first-order exponential equation related to temperature, we showed that instant ecosystem respiration was significantly affected by the carbon uptake of the vegetation. We estimated the annual instant respiratory costs of photosynthesis to 20 % of the assimilated carbon. In addition, the modified model resulted in lower and more realistic temperature sensitivity of respiration and provided an opportunity to explore seasonal patterns in the relationship between ecosystem respiration and photosynthesis. Our study indicates that the traditional method of extrapolating daytime respiration from night time flux measurements will underestimate daytime ecosystem respiration for ecosystems, where respiration is related to instant photosynthesis. Where photosynthesis is extrapolated as the difference between daytime net carbon flux and respiration rates based on night time measurements, i.e. the eddy co-variance method, photosynthesis may also be underestimated. We suggest that our easily applied and non-intrusive approach is tested in future modelling of ecosystem respiration in other ecosystems to test its general applicability.

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Paper IV

Significant plant nitrogen uptake during the cold season in a temperate and a subarctic heath ecosystem

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Manuscript ready for submission

Significant plant nitrogen uptake during the cold season in a temperate and a subarctic heath ecosystem

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Abstract

In two experiments with *in situ* addition of ¹⁵N-labelled glycine, we found significant plant nitrogen uptake during the cold season in a temperate, as well as in a sub-arctic heath ecosystem, both dominated by dwarf shrubs, mosses and graminoids. At both sites, the soil microbes always acquired most of the added ¹⁵N with especially high uptake rates during fall. Uptake rates were lower over the winter than in late spring at the temperate heath, and the microbial pool size of ¹⁵N label decreased significantly over the winter at the subarctic heath. At the temperate heath, all functional plant groups absorbed N throughout the cold season, showing that the plants were active year round. This speaks against the general assumption that at least the dominant evergreen dwarf shrub, *Calluna vulgaris*, is periodically dormant during the cold season. At the subarctic site, the plants usually lost N during winter. The evergreen dwarf shrubs resumed N uptake by April and the deciduous shrubs by May, while the graminoids and mosses had not resumed net N accumulation by June. Hence, the results suggest different temporal patterns in nitrogen acquisition of the different subarctic plant functional groups, with evergreen dwarf shrubs starting to exploit the nutrients that have been mineralized over winter by the soil microbes, whereas the faster-growing graminoids and deciduous dwarf shrubs seem to depend mostly on nitrogen made available during the growing season.

Introduction

The most extreme future temperature increases in response to the increasing atmospheric concentrations of greenhouse gasses are expected to take place at high latitudes and especially during the cold season (IPCC 2001). Still, the ecosystem processes controlling cold-season biological activity are far less understood than processes during the growing season (Brooks et al. 1997; Hobbie et al. 2000), which hampers our ability to predict ecosystem responses to a changing climate. In temperate and arctic ecosystems, which seasonally experience subzero temperatures, the cold season has traditionally been believed to be a dormant period for plants and a period with low microbial activity. However, the evidence of considerable biological activity even at temperatures well below zero has grown rapidly within the last decade (e.g., Sommerfeld et al. 1993; Zimov et al. 1996; Sommerfeld et al. 1996; Oechel et al. 1997; Fahnestock et al. 1999; Grogan et al. 2001) and, consequently, so has the need for understanding cold-season ecosystem functioning and the possible effects on it by climate changes.

Because previous investigations of higher plants under snow indicated no photosynthetic activity (Tieszen 1974; Hamerlynck and Smith 1994), a consensus developed that plants are dormant until snowmelt is completed in spring. Hence, the majority of cold-season investigations have focused on the microbial community, and wintertime CO₂ emissions are usually accredited to the activity of soil microbes slowly decomposing soil organic matter. However, there are several indications of plant activity also outside the growing season. For instance, Grogan et al. (2001) showed that plant removal significantly decreased winter CO₂ efflux, indicating that maintenance and/or growth respiration by plants may contribute to the wintertime CO₂ loss. Also, Starr and Oberbauer (2003) recently found evidence of significant photosynthesis by higher plants while they were still snow-covered during early spring. Furthermore, plant growth in most temperate and high-latitude ecosystems is limited by nitrogen availability (Shaver and Chapin 1980; Vitousek and Howarth 1991), and numerous studies have shown that growing season net nitrogen mineralization rates often do not meet the demand of the plants (Hart and Gunther 1989; Giblin et al. 1991; Jonasson et al. 1993; Fisk and Schmidt 1995; Schmidt et al. 1999, 2002; Kaiser et al. 2005). In contrast, considerable net mineralization has been shown to take place within the winter and spring thaw periods (Hobbie and Chapin 1996; Grogan and Jonasson 2003; Schimel et al. 2004). These observations may partly be due to a bias in the methodology, because the most widely used method for measuring mineralization, the buried bag technique, excludes the plant roots and may lead to increased microbial immobilization in the absence of plant nutrient uptake (Jonasson et al. 2004). Furthermore, plants may partially short-circuit the microbial mineralization by utilizing nitrogen in organic forms, such as amino acids (Chapin and Matthews 1993; Lipson and Näsholm 2001; Schimel and Bennett 2004). In spite of these uncertainties, the observed high cold-season mineralization rates, together with the numerous observations of microbial release of nutrients at spring thaw (Brooks et al. 1998) and the shown plant activity outside the growing season (Lipson et al.

1999; Grogan and Jonasson 2003; Larsen et al. 2006) suggest a significant release of nutrients accessible for plants during these periods. However, only few field investigations of plant nutrient uptake have focused on the cold season uptake. The few studies performed provide contrasting evidence of, e.g., high cold-season nitrogen uptake only during fall in a subarctic birch understory (Grogan and Jonasson 2003), very high plant uptake during spring snowmelt in an alpine moist meadow (Jaeger et al. 1999), very low plant uptake during snowmelt in an arctic tussock tundra ecosystem (Bilbrough and Welker 2000), and considerable root uptake by all investigated plant groups in a temperate heath throughout the cold-season (Andresen and Michelsen 2005). The scarcity of cold-season investigations of plant nitrogen uptake and the lack of general trends in these studies imply that there is still only limited knowledge of the magnitude of the nitrogen sink capacity of plants outside the traditional growing season (Grogan and Jonasson 2003).

In the present study, we aimed to investigate the significance as well as the timing of the cold-season plant nitrogen uptake by adding ^{15}N -labelled glycine as a stable isotopic tracer. We performed the experiment in two heath ecosystems, a temperate and a subarctic heath, both dominated by dwarf shrubs, mosses and graminoids, but situated in different climatic zones. The temperate heath site usually experiences night-time frost during most of the winter with sporadic cold periods when the soil freezes, whereas the subarctic site usually has a long period of continuous soil frost. The possible similarities and differences of cold-season plant nutrient uptake at the extremes of this broad climatic gradient could therefore be examined.

Materials and methods

Site descriptions

The temperate heath site is close to the CLIMOOR site (Beier et al. 2004) at Mols Bjerger, Eastern Jutland, Denmark (56°23' N, 10°57' E), 58 m above sea level. Annual mean air temperature (1961-1990, Danish Meteorological Institute) is 7.7°C ranging from a monthly mean of 0.2 °C in January and 16.2 °C in July. Mean annual precipitation is 758 mm (1998-2000). The soil is a sandy podzol with a pH of 4.6 (Jensen et al. 2003). It has an approximately 3 cm deep organic horizon with a mean SOM content of 19.3±1.7%. The mineral soil beneath has an organic matter content of 3.2±0.3%. The vegetation is dominated by the evergreen dwarf shrub *Calluna vulgaris* (L.), various mosses and the grass *Deschampsia flexuosa* (L.).

The subarctic site is situated near Abisko Scientific Research Station, Northern Sweden (68° 21' N, 18° 49' E), 380 m above sea level. Climate records (1970–2000) show an annual mean temperature of -0.5 °C (-10.2 °C in January and 12.6 °C in July), and annual precipitation of 315 mm. According to long-term records at the Abisko Scientific Research Station, the snow-covered period usually lasts from early October to late May. The highly organic soil horizon containing an average of 87.4±6.8% soil organic matter (SOM) overlays bedrock and has a depth of 5-20 cm and a pH of 5.8 (H.J. Konestabo, unpubl. data). Permafrost is patchy in the region and not present at the site. The vegetation is dominated by

mosses, evergreen and deciduous dwarf shrubs, and graminoids. Dominant higher plants are *Empetrum nigrum* ssp. *hermaphroditum* Hagerup, *Vaccinium uliginosum* ssp. *alpinum* (L.), *Andromeda polifolia* ssp. *polifolia* (L.), *Arctostaphylos alpina* (L.) *Betula nana* (L.), *Equisetum arvense* (L.) and *Carex vaginata* (L.).

Experimental design

Because we wanted to investigate the possible cold-season transfer of N from soil and soil microbes to the plants we wanted as much of the added ^{15}N -residue to go to the soil microbes after addition, since it would make it easier at later harvests to measure increases in plant ^{15}N concentrations. Although some plants are capable of taking up various organic N-compounds (Chapin et al. 1993; Lipson and Näsholm 2001; Schimel and Bennett 2004), we assumed that plants still compete better for N in inorganic forms than in organic forms compared to microbes. Therefore, we added the ^{15}N as the single-labelled amino acid glycine.

At the temperate heath, one randomly chosen subplot of $20 \times 20 \text{ cm}^2$ within each of six plots was labelled with $0.129 \text{ g glycine-}^{15}\text{N m}^{-2}$ (99 % ^{15}N) 14 days prior to each harvest. Labelling was done by gently pressing down a $20 \times 20 \text{ cm}^2$ perspex plate with metal pegs in each corner down on top of each plot until the plate was 2-3 cm above the soil surface. With a needle attached to a dispenser, 10 ml of label was added through each of 25 holes evenly distributed on the plate, thus adding a total of 250 ml per subplot. The needle was pushed down about 10 cm into the soil, and the label was evenly dispensed as the needle was pulled up. The labelled subplots were excavated to a soil depth of 10 cm as intact plant/soil cores on each of four harvest campaigns that took place on 22 November 2004, 19 January 2005, 31 March 2005 and 24 May 2005 (24 subplots in total). In March, six none-labelled plant/soil cores were also extracted, for determination of background levels of ^{15}N .

At the subarctic heath, the soil is frozen for a long period of the winter. Because it is impossible to apply a homogeneous addition of the ^{15}N labelled glycine to frozen soils, all subplots used in the experiment were labelled on 22 August 2003. The ^{15}N -labelled glycine was added using the same procedure and subplot size as at the temperate heath. One subplot within each of twelve plots was then excavated to a depth of 10 cm on each of five harvest campaigns, which took place on 4 October 2003, 12 November 2003, 9 April 2004, 11 May 2004 and 21 June 2004 (60 subplots in total). In May, six additional none-labelled cores were excavated for determination of background levels of ^{15}N . Half of the plots at the subarctic site had been exposed to increased soil freeze-thaw frequency during spring for three years by using passive greenhouse warming for about three weeks during fall and spring (HS Konestabo, unpubl. data). Effects of the freeze-thaw treatment on the ^{15}N -content of all plant, soil and microbial fractions were tested by two-way ANOVA with sampling month and treatment as main effects and their interaction. Since there were no significant differences, we assumed that there was no effect of the treatment and therefore pooled the data from the control and treated plots.

At both sites, the excavated cores were brought to the laboratory and stored at 5 °C. Within 48 hours after collection, the plants were gently pulled out of the soil cores with as many roots attached as possible. At the temperate site, plants were sorted into the following five pools: Mosses/lichens, and shoots and roots of evergreen shrubs and graminoid/herbaceous plants, respectively. At the subarctic heath, plants were sorted into seven fractions because of the presence here also of deciduous dwarf shrubs. *C. vulgaris* was the only evergreen shrub at the temperate heath while *D. flexuosa* estimatedly constituted more than 90% of the graminoid/herbaceous fraction here. At the subarctic heath *Carex* and grass species constituted more than 90% of the graminoid/herbaceous fraction in all cores. We therefore hereafter refer to this fraction as the graminoid fraction for both sites. The roots were washed in 0.5 mM CaCl₂ to remove loosely adhered label (Persson et al. 2003), and all plant parts were dried at 90 °C for 24 hours. The dried samples were crushed with a mill or a mortar and kept in darkness at room temperature until further analysis.

At both sites, soil temperatures at 3 cm depth were logged continuously with 15-minute intervals throughout the study period at six plots (Gemini Data Loggers, Chichester, England). Air temperatures for most of the experimental period at the temperate heath were logged at all plots 10 cm above the soil surface and with 1-hour intervals (Campbell Scientific Inc., Logan, USA). At the subarctic site, air temperatures were recorded at a nearby weather station at Abisko Scientific Research Station, measured at 2 m height and with 10-minute intervals.

Below-ground fractionation

At the subarctic heath, a soil subsample of about 100 cm³ was taken from the excavated plant/soil cores after the removal of shoots and adhering roots. The exact volume of the subsample was measured and used for calculation of soil density. As many remaining roots as possible were then hand-picked from the soil subsample within a standardized time of 20 minutes per sample. At the temperate heath, the organic and mineral soil layers were treated individually, using the same methodology for each soil layer as for the organic soil at the subarctic site.

After the removal of remaining roots, about 30 gram of soil per sample was freeze-dried at -8 °C for determination of soil water content, while 10 gram was immediately extracted in 50 ml of demineralised H₂O, followed by filtration through a Whatman GF-D filter. Another 10 gram was chloroform-fumigated for 24 hours (Jenkinson and Powlson 1976) before extraction in demineralised H₂O to release microbial nutrients. The extraction with pure H₂O, rather than e.g. 0.5M K₂SO₄ or other salt solutions, was chosen to avoid the large amounts of crystallized salts when extracts were freeze-dried prior to mass spectrometry (see below). The extracts were stored at -25 °C until analysis.

For determination of total root biomass at the temperate site, the attached roots from the evergreen plants were pooled with the remaining roots from the organic soil subsample, whereas the roots of graminoids were pooled with the remaining roots from the mineral soil

layer, based on the general pattern in the spatial root distribution of the two groups observed in the field. Total root biomass at the subarctic site was determined as the sum of attached roots from all plant groups plus the remaining roots picked out from the soil subsamples.

Mass Spectrometry

Total carbon (C), total nitrogen (N) and the $^{15}\text{N}/^{14}\text{N}$ ratio in the plant and soil fractions from each harvest were analyzed on 5 to 10 μg of sample with a CN elemental analyser (Eurovector, Milan, Italy) coupled to an Isoprime isotope ratio mass spectrometer (IRMS, Micromass-GV Instruments, Manchester, UK) using continuous flow. Unlabelled samples from the March (temperate site) and May (subarctic site) harvests were used to correct for background isotope ratios. Total C and N in the soil microbial biomass and the microbial $^{15}\text{N}/^{14}\text{N}$ isotope ratio were determined from the soil extracts. A fraction of each extract was oxidized with the persulphate method and analysed for total dissolved nitrogen with the cadmium reduction method (Allen 1989). Knowing the N concentration, 3-20 ml of un-oxidized samples, containing about 100 μg N, was freeze-dried in 20 ml plastic vials together with a 12.5 mm² quartz fibre filter disc (Millipore, Billerica, USA). After drying, the filters were used to wipe any adhered dry matter off the sides and bottom of the vials. An additional quartz filter was wetted with 10 μl demineralised H₂O, and the vial was wiped again in order to recover as much dried material as possible. The two filters were subsequently dried in a desiccator at room temperature and analysed as described above. The microbial N contents (MBN) are based on the N masses determined by IRMS and calculated as the difference between N content of fumigated and non-fumigated samples, using an extractability factor of 0.4 (Jonasson et al. 1996; Schmidt et al. 2002). The non-fumigated sample resembled the total dissolved nitrogen fraction (TDN). Extracted amounts of N from the mineral soil extracts were often below 10 μg N per sample, which was too low for accurate determination of $^{15}\text{N}/^{14}\text{N}$ ratios by IRMS. Therefore, microbial ^{15}N at the temperate heath could only be determined in the organic soil layer. Bulk soil ^{15}N content was calculated as the ^{15}N content in dried soil samples minus the ^{15}N content of MBN and TDN. Total amounts of recovered label in the different soil layer pools were recalculated from dry mass basis to area basis, using the soil density estimates down to a depth of 10 cm; at the temperate heath by taking into account the distribution of depth and the volumetric densities of the organic and mineral horizons of each sampled core.

All samples were analysed with reference gas calibrated against international standards IAEA C5, CH6, CH7, N1, N2 and USGS 25, 26, 32, and corrected for drift using internal standards of calibrated leaf material. The standard deviation of the $\delta^{15}\text{N}$ measurements was ± 0.2 ‰.

Statistical analysis

The plant biomass and ^{15}N pool data were tested for effects of sampling month at each site for each individual fraction, by one-way ANOVA followed by Tukey's studentized test to

localise the differences using $\alpha = 0.05$. Wilcoxon's non-parametric test was used to test if $\delta^{15}\text{N}$ -values in samples from labelled plots were significantly higher than the background level in un-labelled samples.

Results

Seasonal environmental conditions

The temperate heath experienced a warmer winter than the long-term average with mean diel air temperature in January of 2.9 °C as compared to a long-term average of 0.2 °C and with only short periods of a mean temperature below 0 °C for the first part of winter (Fig. 1, upper panel). However, from the beginning of February, a cold period with a consistent snow cover decoupled air and soil temperatures, stabilizing the latter near 0 °C. Snow melt was completed by mid March, concurrent with the start of the March labelling and harvest. Sub-zero soil temperatures were only recorded before the March harvest. The March post-labelling period also had the lowest number of degree days reaching only 35, while the 14 day pre-harvest periods in November, January, and May had 70, 50 and 138 degree days, respectively (Table 1).

At the subarctic site, the mean diel soil temperature (Fig. 1, lower panel) was close to 0 °C from the beginning of October to mid November. The soil temperature then dropped and stayed stable around -5 °C from mid December to mid February and was only slightly affected by large fluctuations in air temperatures, indicating strong thermal insulation by a persistent snow cover. A warm period with occasional above-zero air temperatures in mid to late February apparently reduced the depth of the insulating snow cover, judged from the considerable soil cooling during the following cold period and higher variability in soil temperatures thereafter. On 1 May the soil temperature reached 0 °C and then increased during the following days, indicating that snow melt was completed.

The seasonal pattern in soil temperatures resulted in large differences in the number of hours with temperatures above 0 °C, as well as the number of degree days (>0 °C) during the inter-harvest periods (Table 2). From the October to the November and April harvests, inter-harvest degree days (with the number of inter-harvest days in brackets) decreased from 241 ± 16 (43) to 22 ± 11 (39) and 1 ± 1 (149), respectively. During the 32 days between the harvests in April and May, mean soil temperatures were still below 0 °C but increased at the end of the period resulting in 20 ± 7 degree days. Between the May and June harvests, mean soil temperatures increased and the soil temperatures were above 0 °C for most of the time, resulting in a much higher number of degree days of 169 ± 16 (41) compared to the previous periods.

Figure 1

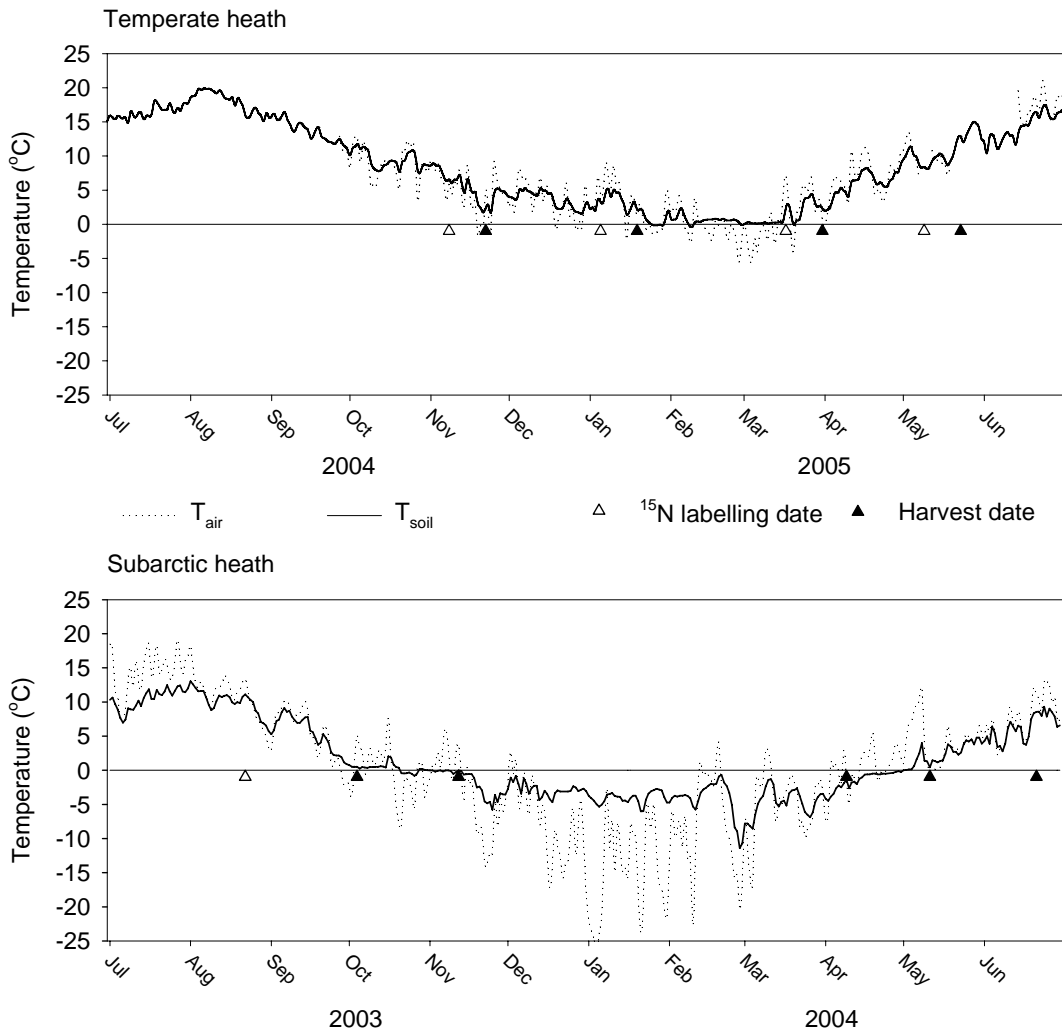


Figure 1. Mean diel temperatures at the temperate (upper panel) and subarctic heath (lower panel). T_{air} is the temperature 10 cm above the ground at the temperate heath and 2 m above the ground at the subarctic site. T_{soil} is the temperature 3 cm below the soil surface. Times of addition of ^{15}N -labelled glycine and harvests are indicated. Note that T_{air} data is discontinuous at the temperate heath.

Table 1. Soil temperatures (3 cm depth) at the temperate heath ecosystem during the 14-day periods between the time of ^{15}N labelling and harvest. Data are means with standard errors (in brackets) from six temperature loggers. Time resolution and calculation of degree days as in Table 1.

	Pre-harvest period			
	8 Nov - 22 Nov	5 Jan - 19 Jan	17 Mar - 31 Mar	10 May - 24 May
mean	5.0 (0.3)	3.6 (0.1)	2.4 (0.2)	9.9 (0.1)
min.	1.3 (0.3)	1.2 (0.1)	-0.4 (0.2)	6.6 (0.3)
max.	7.9 (0.3)	5.7 (0.2)	5.7 (0.4)	15.4 (0.6)
hours > 0 °C	336 (0)	336 (0)	298 (7)	336 (0)
hours < 0 °C	0 (0)	0 (0)	38 (7)	0 (0)
degree days > 0 °C	70 (4)	50 (2)	35 (2)	138 (2)
No. of days	14	14	14	14

Effects of season on plant biomasses

At both sites, only the above-ground graminoid biomass changed significantly over time (Fig. 2), being significantly higher in May than in November at the temperate heath (Fig. 2, upper panel), while it was higher in October than in May at the subarctic heath (Fig. 2, lower panel). At the temperate heath, the attached root biomasses did not change significantly with time, although there was a tendency to a lower root mass in evergreens in March than in November (Anova_{3,20}, $F = 2.34$, $P = 0.10$). At the April harvest at the subarctic heath, i.e. at the end of winter, the amount of attached roots was significantly lower than in October for both deciduous and evergreen shrubs.

Table 2. Soil temperatures (3 cm depth) at the subarctic heath ecosystem from 22 August 2003 to 21 June 2004 separated into the five inter-harvest periods. Data are means with standard errors (in brackets) from six temperature loggers. Temperatures were recorded continuously every 15 minutes. Degree days were calculated for each 15-minute interval individually and positive values were summed for each inter-harvest period.

	Inter-harvest period				
	22 Aug - 4 Oct	5 Oct - 12 Nov	13 Nov - 9 Apr	10 Apr - 11 May	12 May - 21 Jun
mean (°C)	5.6 (0.4)	0.2 (0.4)	-3.8 (0.3)	-0.5 (0.3)	4.1 (0.4)
min. (°C)	0.1 (0.5)	-1.0 (0.4)	-12.6 (0.5)	-12.6 (0.5)	-0.2 (0.3)
max. (°C)	16.8 (1.8)	3.1 (0.5)	-0.1 (0.3)	7.1 (1.4)	11.7 (1.1)
hours > 0 °C	988 (28)	417 (160)	42 (35)	243 (52)	832 (117)
hours < 0 °C	44 (28)	519 (160)	3534 (35)	525 (52)	152 (117)
degree days > 0 °C	241 (16)	22 (11)	1 (1)	20 (7)	169 (16)
No. of days	43	39	149	32	41

Figure 2

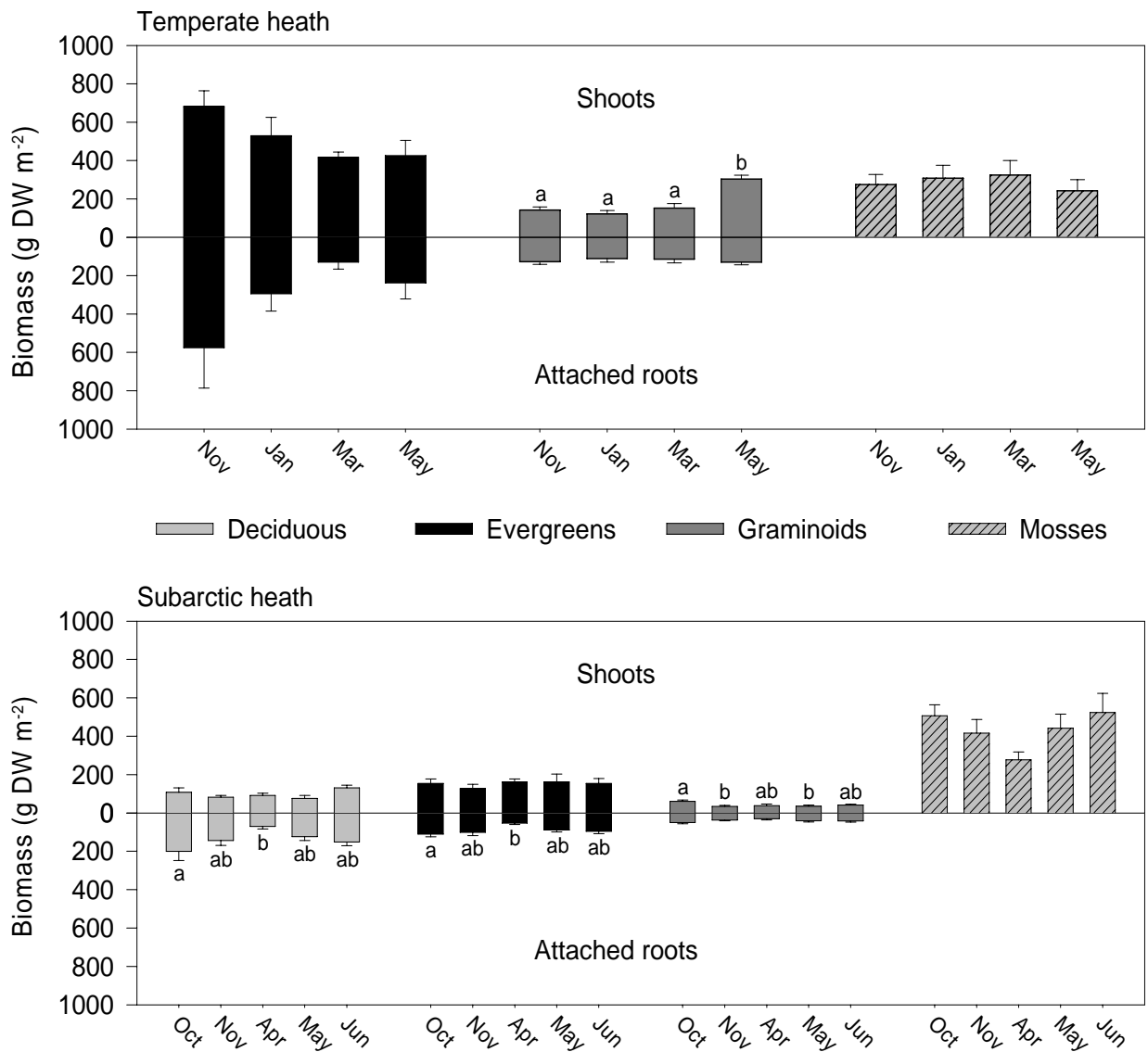


Figure 2. Biomasses of different plant functional groups at four times at the temperate heath (upper panel) and five times of harvest at the subarctic heath (lower panel). Different letters above bars indicate the location of significant differences ($P \leq 0.05$; Tukey's test) between biomasses at the different times of harvest.

Seasonal nitrogen turnover at the temperate heath

The mean recovery of added ¹⁵N label at the temperate heath of 85±4 % indicates that some of the label entered deeper, unsampled soil layers or was lost by leaching or denitrification. Total recovery was lowest in November (74±9 %) and highest in March (105±11 %). The largest proportion of the recovered label was found below-ground (97±1 % on average) with 28±8 %

recovered in the organic horizon and 69 ± 15 % in the mineral soil layer, while 3.3 ± 1.6 % of the recovered label was found in above-ground plant parts. These fractions varied considerable with time with recovery in the mineral soil layer peaking in March at 91 ± 13 % and recovery in above-ground plant part increasing from about 1.3 ± 0.4 % in November and January to 2.3 ± 0.9 % in March and 8.3 ± 1.2 % in May.

Figure 3

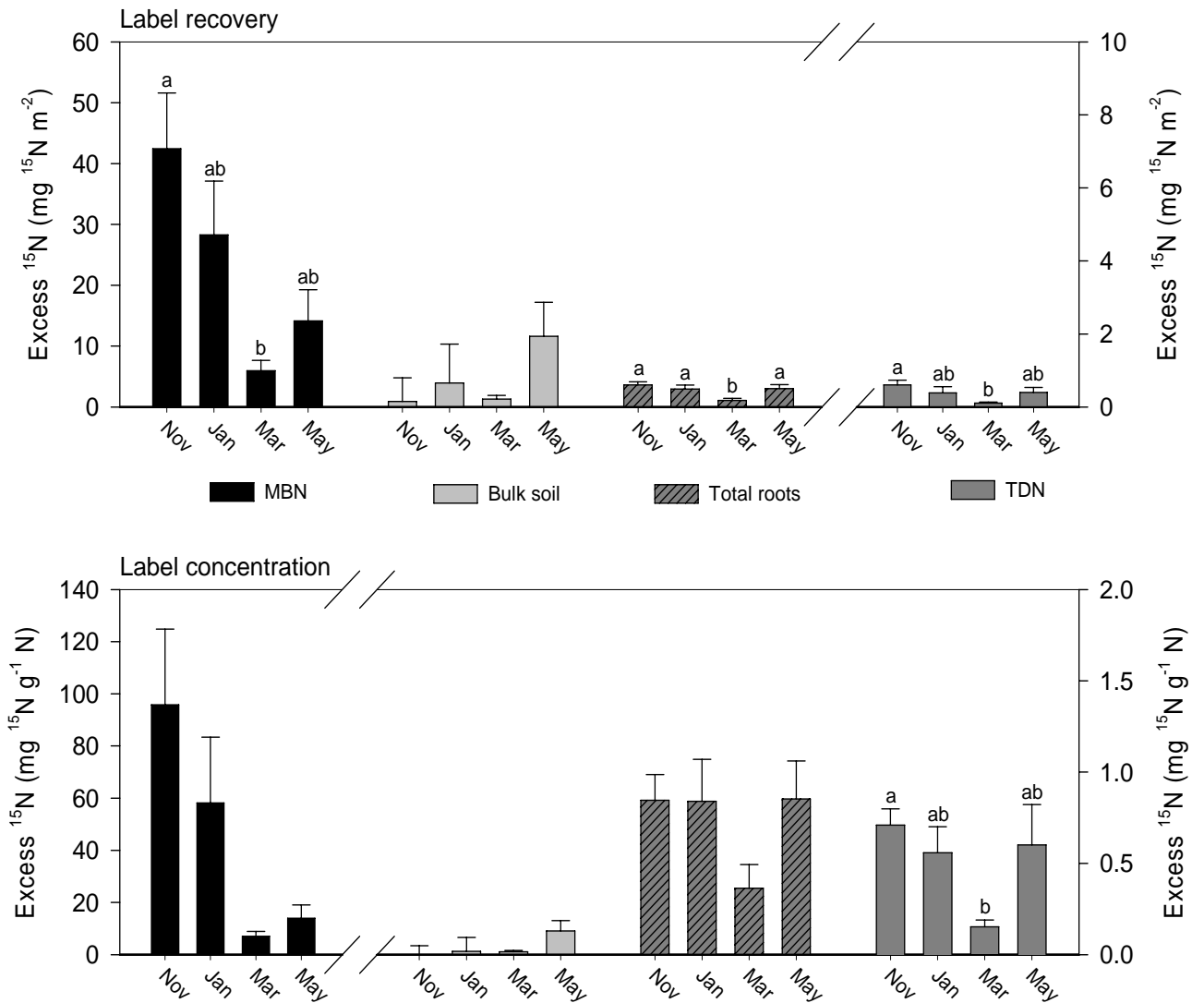


Figure 3. Partitioning of added ¹⁵N-label in the organic soil layer at the temperate heath shown as mass recovery per area (upper panel) and as concentration of the total N mass in each pool (lower panel). MBN is microbial biomass and TDN is total dissolved nitrogen. Bulk soil ¹⁵N is the added label recovered in the soil minus the ¹⁵N content of MBN and TDN. Different letters above bars indicate significant differences ($P \leq 0.05$; Tukey's test) between biomasses at the different times of harvest. Note the different scale on the Y-axis for the TDN fraction in the upper panel and the MBN fraction in the lower panel.

Of the ^{15}N recovered in the organic soil, more than 90% was immobilized in the soil microbes in November, decreasing to 48% in May (Fig. 3, upper panel), when a larger fraction was recovered in the remaining bulk soil. The lowest amount recovered in the microbes was in March. The concentration of label recovered in the microbial biomass showed a similar, although not significant, trend (Fig. 3, lower panel). The recovery of label in the total root fraction, as well as in TDN in the organic soil layer was also significantly lower in March than at the other times of harvest. The overall pattern of low recovery in the organic soil layer in March was balanced by a the high recovery in the mineral soil layer at this time when the recovery was significantly higher than at all other harvest dates (Fig. 4, upper panel). The higher recovery in the mineral soil resulted from similar or lower concentrations of added label in both the total soil and root fractions (Fig. 4, lower panel) than in the organic soil layer (Fig. 3, lower panel), but a much higher soil mass and moderately higher root biomass on an area basis (data not shown).

Figure 4

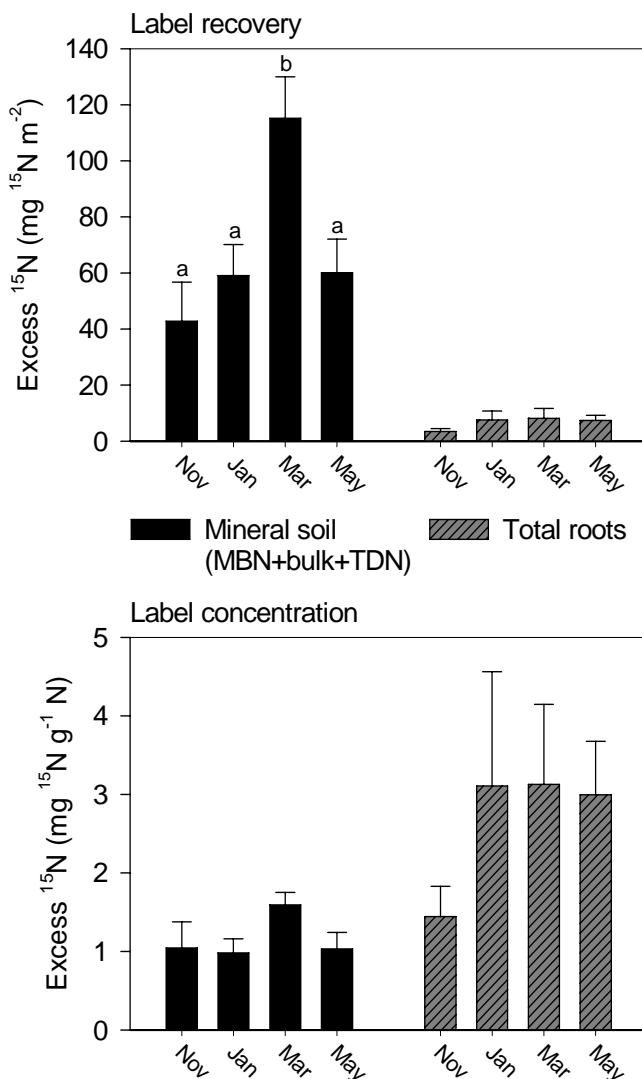


Figure 4. Partitioning of added ^{15}N -label in the mineral soil layer at the temperate heath shown as mass recovery per area (upper panel) and concentration of the total N mass in each pool (lower panel). Because concentrations in soil extracts were too low, the ^{15}N content of MBN and TDN could not be estimated for the mineral soil layer. Different letters above bars indicate significant differences ($P \leq 0.05$; Tukey's test) between biomasses at the different times of harvest.

The concentration of label in the plant roots and shoots was significantly higher than the background levels in all plant groups at all harvests (Fig. 5, Wilcoxon's test, $P < 0.0001$ at all times). The recovered amounts of label in shoots increased with time from March to May in the evergreens and from January to May in the graminoids, while both recovered amounts and the concentrations in the roots did not change significantly during the experimental period. Mosses showed no seasonal differences in either total amount recovered or in the tissue concentration of label.

Figure 5

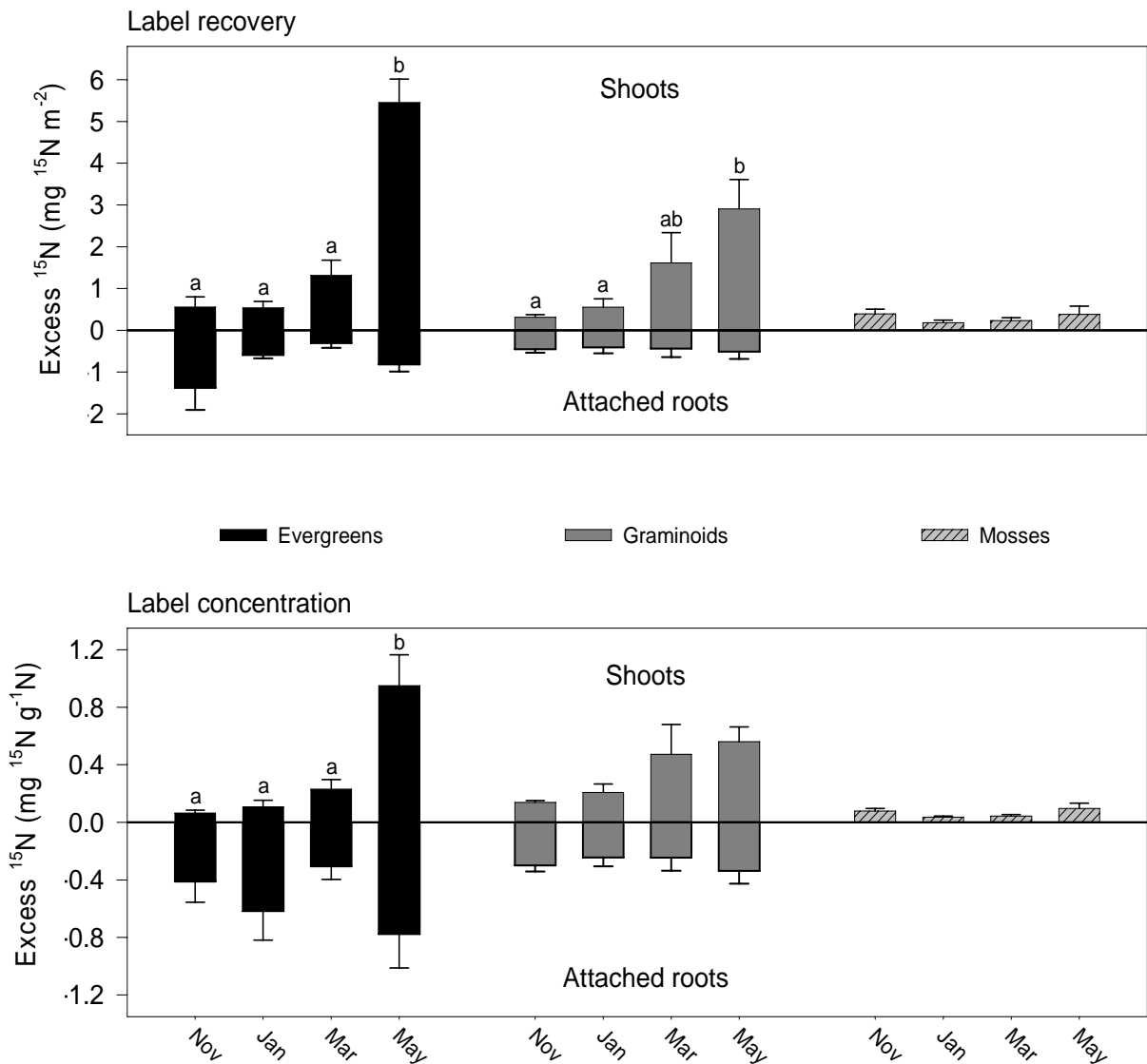


Figure 5. Partitioning among extracted plants of added ¹⁵N-label at the temperate heath shown as mass recovery per area (upper panel) and as concentration of the total N mass in each pool (lower panel). Different letters above bars indicate significant differences ($P \leq 0.05$; Tukey's test) between biomasses at the different times of harvest.

Seasonal nitrogen turnover at the subarctic heath

The mean recovery of ^{15}N label in the soil plus above-ground plant biomass was $104.1 \pm 5.8\%$ of the added amount over the five harvests at the subarctic heath, ranging from $92.8 \pm 11.3\%$ in November to $122.0 \pm 11.1\%$ in October. As for the temperate heath, the largest fraction was recovered below-ground ($99.4 \pm 0.11\%$ on average) with about equal proportions of $42.6 \pm 3.7\%$ in soil microbes and $48.1 \pm 3.3\%$ in the bulk soil, and a smaller proportion of $7.8 \pm 0.8\%$ in roots and $0.9 \pm 0.1\%$ in the TDN fraction. Only $0.6 \pm 0.1\%$ was found in above-ground plant biomass.

Figure 6

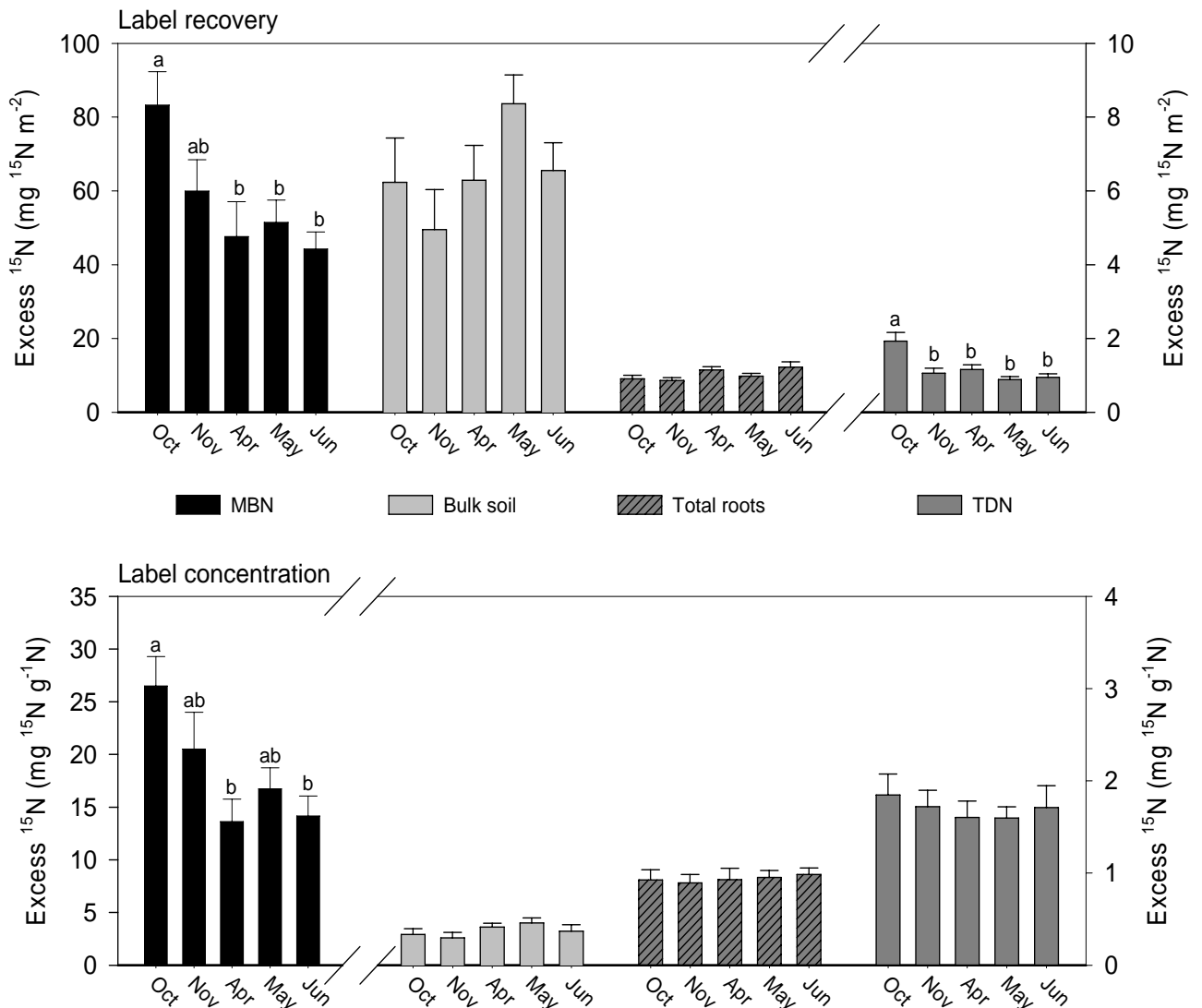


Figure 6. Partitioning of added ^{15}N -label in the soil at the subarctic heath shown as recovery per area (upper panel) and as concentration of the total N mass in each pool (lower panel). MBN is microbial biomass and TDN is total dissolved nitrogen. Bulk soil ^{15}N is the added label recovered in the soil minus the ^{15}N content of MBN and TDN. Different letters above bars indicate significant differences ($P \leq 0.05$; Tukey's test) between biomasses at the different times of harvest. Note the different scale on the Y-axis for the TDN fraction in the upper panel and the MBN fraction in the lower panel.

While the content in the microbial biomass and the bulk soil were comparable on an area basis (Fig. 6, upper panel), the microbes had a much higher, although variable, concentration of label per g of nitrogen than the bulk soil (Fig. 6, lower panel). While having immobilized about 65 % of the added label by the first harvest in October, the microbial biomass lost about 43 % of the acquired label from October to April, primarily as a consequence of reduced label concentrations rather than a decrease of microbial biomass.

Figure 7

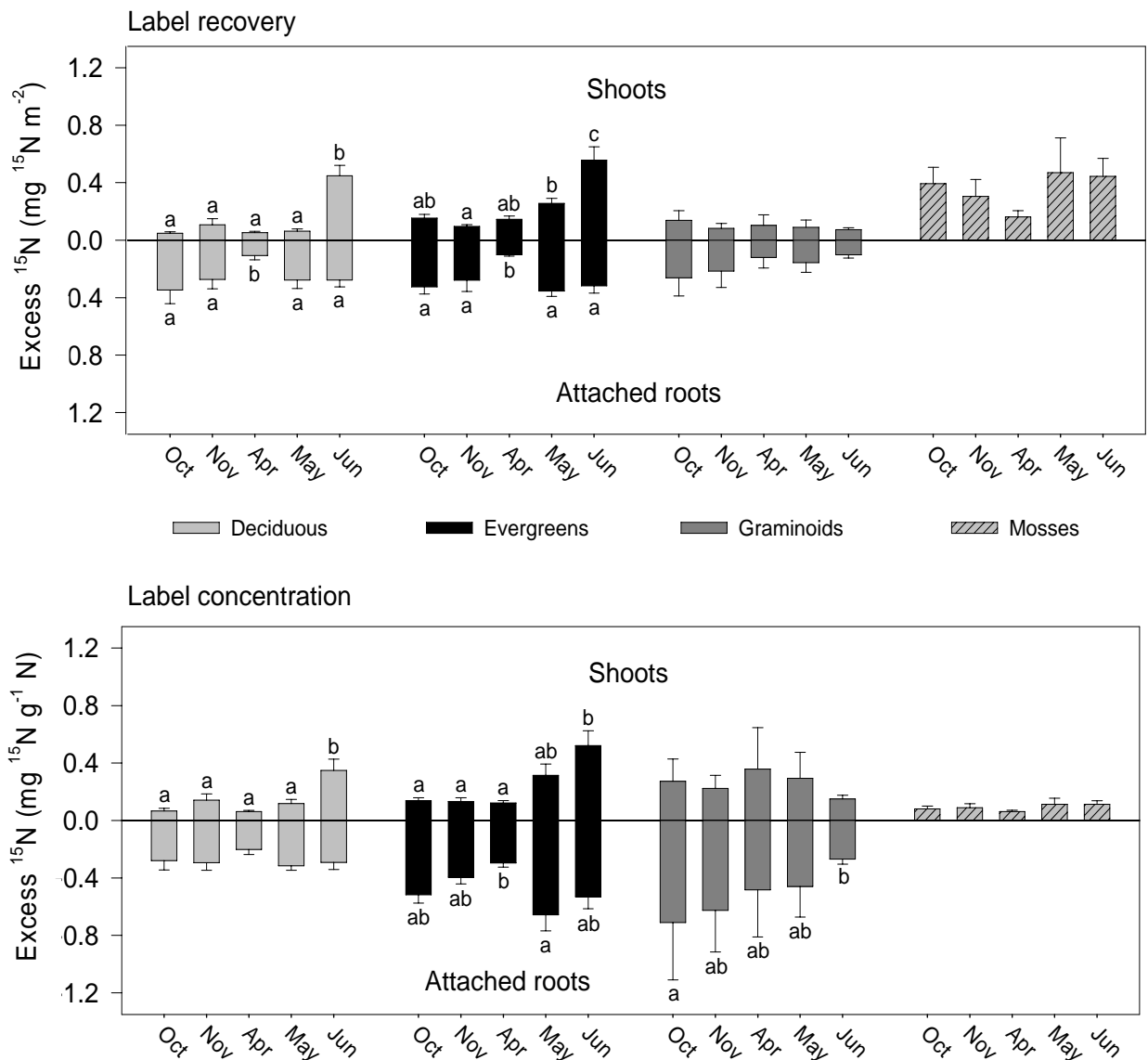


Figure 7. Partitioning among plant functional groups of added ^{15}N -label at the subarctic heath shown as mass recovery per area (upper panel) and as concentration of the total N mass in each pool (lower panel). Different letters above bars indicate significant differences ($P \leq 0.05$; Tukey's test) between biomasses at the different times of harvest.

However, the recovery in the root biomass, both by area and concentration did not change significantly after the first harvest, whereas label recovery in the TDN was significantly higher in October than at the other harvest dates when compared on an area basis.

The ^{15}N recovery in roots of both deciduous and evergreen shrubs was lowest in the early April harvest and increased significantly until the next harvest in early May (Fig. 7, upper panel). The low April recovery in roots of deciduous shrubs was because of a significantly lower biomass at this time compared the remaining harvests, while the concentration of label did not change significantly (Fig. 7, lower panel). The concentration of label in the roots of evergreen shrubs also increased significantly from early April to early May concomitantly with increased shoot concentration, indicating a considerable nitrogen uptake. The increase resulted in significantly higher uptake of label in the evergreen shoots by May than by November, with a further increase from May to June when also the concentration of label in shoots increased significantly. The deciduous shrubs showed a similar pattern, but the increase of concentrations and amounts of added label in the shoots did not appear until the May – June period. The temporal pattern of the label recovery in herbs and mosses was more stable over the year and did not change (mosses) or even tended to decrease slowly and almost linearly with time (graminoids), particularly in the roots (Fig. 7 lower panel).

Discussion

Annual N cycling in a heath with a long period of frozen soil

Our knowledge of soil microbial activity during the cold season and the seasonal community dynamics of microbes in the Arctic have increased considerably over the last decade, while much less focus has been directed to the significance of plant activity outside the growing season. A few generalizations may be drawn from previous studies of cold-season microbial dynamics in arctic and alpine ecosystems: 1) The soil microbial community is dominated by other functional groups than during summer (e.g., Schadt et al. 2003; Lipson and Schmidt 2004), 2) under a permanent snow-cover, the microbial biomass usually increases and peaks before or during snowmelt (e.g., Brooks et al. 1998; Larsen et al. 2006), and 3) large changes in community structure coupled with a decrease in total microbial biomass and potentially high nutrient turnover takes place rapidly at the time of snowmelt (e.g., Brooks et al. 1998; Larsen et al. 2006). It would therefore be advantageous to plants and also for the retention of ecosystem nutrients, if plants were able to mobilize nutrients when the microbial release is high.

Our results support the hypothesis that some arctic plants are capable of nitrogen uptake early in the season, but we found no evidence of significant nitrogen uptake during the mid winter period with continuously subzero temperatures. However, the large change in the label recovered in the microbial biomass from November to April indicates a major cold-season loss of microbial nitrogen during the winter, whereas no further reduction in of microbial ^{15}N

was seen during snowmelt. Many studies have shown that repeated freezing and thawing of the soil reduces the microbial biomass (e.g. Ross 1972; Schimel and Clein 1996; Larsen et al. 2002) and observed changes in microbial biomass during snowmelt has often been accredited directly to freeze-thaw events (Brooks et al. 1998; Jaeger et al. 1999). However, it has also been suggested that the changes in carbon availability and temperature, and not freeze-thaw cycles, may be the main factors controlling the changes in the microbial community and the size of the biomass in the winter-summer transition (Lipson et al. 2000). In our study, it is possible that the relatively warm period in late February followed by a cold period with soil temperatures rapidly cooling down to -12 °C led to a crash in the winter microbial populations, which had built up during the previous months when soil temperatures were much more stable. Although this remains unclear, our study shows that large cold-season mineralization may take place prior to the springtime periods of soil freeze-thaw.

High N uptake by the evergreen dwarf shrubs took place between early April and May, when mean soil temperatures were still below 0 °C and the number of degree days above zero were only about 20. In comparison, Bilbrough and Welker (2000) found significant nitrogen uptake by alpine plants during the spring snowmelt, whereas arctic plants showed a much lower uptake of less than 1% of annual nitrogen uptake under the snow. They suggested that the low uptake in arctic plants could have been due to much lower mid winter soil temperatures of down to -14.5 °C at the site compared to -8 °C at the alpine site, where they found plant uptake. In our study, soil temperature in late February was, however, -12 °C, which is comparable to the arctic site in the study by Bilbrough and Welker (2000). Still, only ten days after snowmelt was completed, the evergreen shrubs had acquired considerable amounts of nitrogen. If uptake under the snow is assumed low based on the results by Bilbrough and Welker (2000), our results indicate that the uptake rates by the evergreen dwarf shrubs after snowmelt were several times higher than later during the growing season. It has often been suggested that the post-snowmelt period is of high importance to plant nutrient uptake but the present study is to our knowledge the first *in situ* experiment that has actually shown this for an arctic heath ecosystem. Second, our results indicate that it is primarily the evergreen dwarf shrubs rather than all plants in general that are capable of considerable nutrient uptake at this time of the year.

Considerable plant uptake of nitrogen during fall, as well as high microbial release of nitrogen over the following winter-summer transition concurrent with increased plant uptake, has previously been shown in a subarctic birch understory (Grogan and Jonasson 2003). In their study, no plant nitrogen uptake was observed from October to May, whereas significant ¹⁵N uptake by all functional plant groups took place between a harvest in May and July. The higher temporal resolution of the late winter measurements in our study shows a more detailed pattern of nitrogen uptake by the plant functional groups during the period when the snow is melting. The evergreen dwarf shrubs showed a substantial nitrogen uptake immediately at the onset of soil thaw in April - May, and they rapidly allocated nitrogen to above-ground tissues. In contrast, the uptake and allocation to above-ground tissue by

deciduous dwarf shrubs was delayed to the May – June period, while the graminoids had not increased their ^{15}N pool by June in comparison to the October pool.

Our results therefore indicate a temporal sequence of N uptake and allocation among the functional plant groups with the evergreen shrubs being active as the soil thaws, followed by an onset of N uptake by the deciduous and later by the graminoids. This coincides with a report of earlier onset of fine root production and earlier re-greening of leaves in Alaskan evergreens shrubs than root and new leaf development in deciduous dwarf shrubs (Kummerow et al. 1983). As the summer proceeds, it appears, however, that the faster growing deciduous shrubs and herbs increase the rate of N uptake and reach the same level of N acquisition as the evergreens, judged from July data by Grogan and Jonasson (2003). This could also explain the lack of differences in the N acquisition pattern in leaves of different longevity in their study. The uptake in early spring may give the evergreen species an advantage that compensates for their generally slower growth rates during summer compared to the deciduous shrubs and graminoid/herbaceous species (Lambers et al. 1998). The early-season nutrient acquisition by the evergreens may also act to maximize ecosystem nitrogen retention in highly nitrogen-limited ecosystems.

Annual N cycling in soils with sporadic soil frost during winter

In spite of soil temperatures often above zero through most of the cold-season in many temperate ecosystems, plants are usually assumed to have a low potential for nutrient uptake at this time of year in addition to low photosynthetic activity. However, measurements of considerable photosynthetic rates during winter in the investigated temperate heath (KS Larsen, unpubl. data) suggest that the plants may be much more active than usually assumed. Indeed, there was a considerable N uptake at all times over the winter in both the evergreen shrubs and the graminoids with levels of ^{15}N concentrations in roots at all harvests that were not significantly different from the concentration at the early growing-season in May. To our knowledge, only one previous study has focused on the cold-season nitrogen uptake of temperate heath plants (Andresen and Michelsen 2005) with reported N uptake throughout the cold season by all investigated species. Compared to the uptake during the growing season in tussock tundra (Schimel and Chapin 1996), grassland (Näsholm et al. 2000; Bardgett et al. 2003) and forests (Näsholm et al. 1998; Persson et al. 2003), the cold-season nitrogen uptake we and Andresen and Michelsen (2005) found in the temperate heaths is only slightly lower than growing season uptake in these ecosystems. The similar results in both studies therefore strongly suggest that cold-season nitrogen uptake contributes significantly to the annual nitrogen economy of temperate heath plants. Similar studies for a wider range of temperate ecosystems are necessary, however, to generalize about the importance of cold-season nitrogen acquisition.

Our results with similar seasonal recovery in roots but increased recovery and concentrations of ^{15}N in shoots of both *C. vulgaris* and *D. flexuosa* in May as compared to at the earlier harvests suggest a seasonal reduction of the uptake potential as well a down-

regulation of allocation to above-ground tissue in both species during the cold-season. In contrast to the subarctic site, we found no indications of different temporal patterns between the plant groups.

The significant uptake potentials observed throughout the cold-season combined with evidence of considerable *in situ* mid day photosynthetic rates even at winter solstice (KS Larsen, unpubl. data) speaks against suggested winter dormancy of *C. vulgaris* inferred from cessation of growth, irregular stomata opening and accumulation of sugar (Miller 1979; Kwolek and Woolhouse 1981). It seems more likely that the nitrogen uptake and photosynthetic potential, as well as the accumulation of sugar, is a result of seasonal changes in the translocation pattern of photosynthates. During the growing season, a large fraction of the assimilated carbon supports new shoot growth, whereas during the cold season it is used for storage and maintenance and, inferred from our study, facilitating nutrient uptake.

The microbial biomass showed a different seasonal nitrogen uptake pattern than the plants with highest uptake in late fall (November), lowest in March and intermediate in January and May. Furthermore, the highest proportion of label was recovered in the deeper mineral soil layer in March, when microbial and plant root uptake in the organic soil layer was lowest. It therefore appears that both microbes and the plants in the organic soil layer were negatively affected by the cold, snow-covered period at the time of labelling and harvest in March. This indicates differences in adaptations between the plants at the temperate and the subarctic heath, where the evergreens had a considerable uptake at lower soil temperatures. However, the lower uptake by microbes and plants in the organic layer at the temperate heath was compensated for by a higher retention in the mineral soil layer, probably keeping nitrogen loss by leaching relatively low. Still, the lack of any plant group being able to acquire more nitrogen in a period where microbial immobilization is low, together with the lack of differentiation in temporal patterns of N acquisition among the species, suggest that the retention of N at the temperate heath is lower than the subarctic ecosystem possibly reflecting less serious nitrogen limitation.

Conclusions

The significant uptake potentials of N throughout the winter at the temperate heath and during spring at the subarctic heath coupled with observations of significant cold-season photosynthesis in both heath ecosystems (Larsen et al. 2006; KS Larsen unpubl. data) indicate considerable cold-season plant activity at both sites although we found no evidence of plant N-uptake during the coldest part of winter at the subarctic site (November – April). However, our results from the two different climatic zones indicate that plants need only a few degree days above 0°C in order to absorb considerable amounts of nutrients. It also appears that the first, few days after snowmelt are critical for the annual nitrogen uptake by evergreens at the subarctic site, but apparently not for deciduous dwarf shrubs and graminoids. In a warmer future climate, when especially winter temperatures are expected to rise and the growing season is expected to be longer (ACIA 2005), a general shift towards a higher cold-season

nitrogen uptake is probable, and may lead to changes in inter-species competition for nitrogen on an annual basis and possibly to changes in plant community composition.

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