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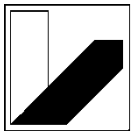

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Carbon dynamics under natural and manipulated meteorological boundary conditions in a forest and a fen ecosystem

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zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

- Dr. rer. Nat. -

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von

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CONTENTS

Summary

According to current climate models, we will face changes in the amount, intensity, frequency and type of precipitation within this century. These changes are very likely to result in an increasing frequency of severe drought periods in summer, causing irregular and extreme drought stress in well-drained soils or a lowering of the water table in water-logged soils. Additionally, rising temperatures will increase the likelihood of precipitation falling as rain rather than snow, resulting in reduced snowpacks in winter. In some regions, this can lead to an increasing frequency of soil frost. In summary, changes in the global water cycle are likely to have a significant impact on boundary conditions within soils. With soils representing important C stocks and soil respiration being the biggest flux of CO_2 from terrestrial ecosystems to the atmosphere, we have to address the question how these aspects of climate change will affect C mineralization in soils.

This thesis focused on investigating the impact of extreme meteorological boundary conditions on CO_2 fluxes in two different ecosystems in the Fichtelgebirge in South-eastern Germany. In a Norway spruce forest, the effect of prolonged periods of summer drought and of soil frost on soil C dynamics were investigated mainly by field-site manipulation experiments, but also by laboratory experiments. In a minerotrophic fen located nearby, the effect of water table lowering (as a result of summer drought) on ecosystem C dynamics was quantified. To be able to better interpret the results, soil C dynamics at both site were modeled under current meteorological conditions.

Modeling of soil C dynamics at the two sites helped to understand the site-specific preconditions under which the field-site manipulation experiments were conducted. Modeling approaches involved measurements of C stocks and the abundance of radiocarbon. For the Norway spruce forest, modeling indicated that soil C turnover predominantly occurred within the organic horizons. During the last decades, the soil has acted as a small sink. The possibility of altered C dynamics at the site due to undocumented liming has to be considered when comparing results presented here to results from other sites. For the fen, modeling also revealed that soil C turnover was clearly dominated by processes occurring within the uppermost 15 cm of the peat. Root biomass was identified to be a very important soil C stock at the site. Most important, modeling indicates that the fen is subject to marked disturbance, most likely of the hydrological conditions, turning the fen into a net C source during the last decades. Thus, results from this fen can not be regarded as representative for undisturbed peatlands.

Soil frost was induced at the forest site by removing the snowpack in the winter of 2005/2006. As the following winters were warmer than average, no repetition of this experiment was possible. Soil frost was observed down to a depth of at least 15 cm and for the duration of several weeks on the plots where snow had been removed, in contrast to naturally snow-covered plots where no soil frost occurred. Soil frost resulted in a significant reduction of soil C losses. Most likely the composition of the microbial community was markedly affected by soil frost, primarily by a reduction of fungal biomass. This would explain why the snow-removal plots featured significantly reduced soil respiration rates not only during the period of soil frost but also in the summer of 2006.

Two different approaches were used to investigate the effect of drought on soil C dynamics in the Norway spruce soil. Prolonged drought periods were experimentally induced at the field-site by excluding throughfall with a transparent roof during the summers of 2006-2008. Additionally, undisturbed soil columns from the site were subjected to drought in the laboratory. In both experiments, drought reduced total soil C losses in comparison to C losses from a control. This reduction was mainly owed to decreased soil respiration rates during the actual drought period, but water repellency also hindered rewetting of the dry soil, thus further prolonging the period of reduced soil respiration rates. In the past, mobilization of stabilized C due to drying-wetting has been repeatedly discussed as a possibility to actually enhance soil C losses. In the studies presented here, no evidence for this assumption was found. In summary, the influence of drought could be described as a temporary 'brake' slowing down soil C mineralization. Rewetting results in a switchback to pre-drought mineralization rates, possibly delayed by water repellency.

At the fen, two different approaches were used to quantify the impact of water table changes on C dynamics: (i) Experimental lowering of water tables to measure resulting C fluxes in comparison to C fluxes under natural conditions (i.e. control plots), and (ii) repeated measurements under varying natural conditions to be able to later statistically identify the main drivers of CO_2 fluxes. In contrast to the forest site, measurement techniques allowed to include C uptake and respiration by aboveground vegetation, thus being able to study ecosystem rather than soil C dynamics at the fen site. In summary, the impact of the water table on CO_2 fluxes in and out of the fen ecosystem was found to be of minor importance. The site was dominated by grass species, and assimilation of these was not affected at all by water table. In the interspersed moss species, low water tables were found to cause significant drought stress, thus decreasing the assimilation of atmospheric CO_2. However, water tables at the site are already naturally low during summer and mosses represent only a minor

proportion of the vegetation at the site, so is questionable whether lowering of the water table due to climate change can markedly affect ecosystem assimilation. Soil respiration was not affected at all by the manipulative lowering of the water table from ca. 15 cm down to more than 60 cm, most likely due to low substrate quality in deeper peat. Measurements of the natural C dynamics indicate that water table could have an impact on soil respiration within the uppermost 0-15 cm of the soil, but predominantly low water tables during summer under current boundary conditions make it unlikely that further lowered water tables due to climate change will markedly affect soil respiration rates at this site. In summary, CO_2 fluxes at the site are presumably very resilient towards an increasing frequency of summer drought resulting in lowering of the water table.

Zusammenfassung

Ausgehend von aktuellen Klimamodellen werden wir in diesem Jahrhundert mit Änderungen der Niederschlagsmenge, -intensität, -häufigkeit und -art konfrontiert sein. Es ist dabei sehr wahrscheinlich, dass diese Änderungen zu einem gehäuften Auftreten von Sommertrockenheit führen, und damit zu unregelmäßigem und schwerwiegenden Trockenstress in gut dränierten Böden bzw. zu einem Absinken des Wasserspiegels in wassergesättigten Böden. Zusätzlich werden steigende Jahresmitteltemperaturen dazu führen, dass Niederschlag zunehmend in Form von Regen anstelle von Schnee fallen wird, weshalb mit einer verringerten Mächtigkeit der Schneedecke im Winter zu rechnen ist. In einigen Gebieten kann dies zum gehäuften Auftreten von Bodenfrost führen. Zusammenfassend ist davon auszugehen, dass klimawandelbedingte Änderungen im globalen Wasserhaushalt sich signifikant auf die Randbedingungen in Böden auswirken werden. Da Böden wichtige Kohlenstoffspeicher darstellen und Bodenrespiration der größte Fluss von CO_2 zwischen terrestrischen Ökosystemen und der Atmosphäre ist, müssen wir uns mit der Frage beschäftigen, wie diese Aspekte des Klimawandels sich auf die Kohlenstoffmineralisation in Böden auswirken werden.

Die vorliegende Arbeit hat sich daher mit der Untersuchung des Einfluss von extremen meteorologischen Randbedingungen auf die CO_2 Flüsse in zwei verschiedenen Ökosystemen im Fichtelgebirge in Südostdeutschland befasst. In einem Fichtenwald wurden die Auswirkungen von Trockenheit und von Bodenfrost auf die Kohlenstoffumsätze im Boden mit Hilfe von Freiland- und Laborexperimenten untersucht. In einem nahegelegenen Niedermoor wurde die Auswirkung von Wasserspiegelabsenkungen auf die Kohlenstoffumsätze des Ökosystems untersucht. Um die Ergebnisse besser beurteilen zu können wurden an beiden Standorten die Kohlenstoffumsätze unter augenblicklichen meteorologischen Randbedingungen modelliert.

Die Modellierung der Kohlenstoffumsätze an den beiden Standorten half dabei, die Ausgangsbedingungen für die experimentelle Manipulation besser zu verstehen. Die Modellierungsansätze beinhalteten Messungen der Kohlenstoffvorräte und der Radiokarbonsignatur. Im Fichtenwald zeigten die Modellergebnisse auf, dass der größte Teil der Kohlenstoffumsätze in den organischen Horizonten stattfand. Innerhalb des Zeitraums der letzten Jahrzehnte fungierte der Boden am Waldstandort als schwache Senke für Kohlenstoff. Im Hinblick auf die Vergleichbarkeit mit Ergebnissen von anderen Standorten muss berücksichtigt werden, dass mögliche Kalkung des Standorts in der Vergangenheit zu einer geringfügigen Störung der Kohlenstoffumsätze geführt haben könnte. Für den

Niedermoorstandort ergab die Modellierung, dass die Kohlenstoffumsätze eindeutig von Umsätzen in den obersten 15 cm dominiert wurden. Wurzelbiomasse erwies sich als sehr bedeutender Kohlenstoffpool. Am bedeutendsten aber war die Tatsache, dass die Modellierungsergebnisse auf den massiven Einfluss von Störungen im Niedermoor hinwiesen, höchstwahrscheinlich Störungen der hydrologischen Randbedingungen. Diese Störungen machten das Niedermoor in den letzten Jahrzehnten zu einer Nettokohlenstoffquelle. Die Ergebnisse können daher nicht als repräsentativ für ungestörte Moorstandorte gesehen werden.

Bodenfrost wurde durch die Entfernung der Schneedecke im Winter 2005/2006 induziert. Da die folgenden Winter überdurchschnittlich warm waren, war eine Wiederholung des Experimentes nicht möglich. Bodenfrost konnte bis in eine Tiefe von wenigstens 15 cm und für die Dauer von mehreren Wochen auf den Flächen nachgewiesen werden, auf denen die Schneedecke entfernt worden war. Im Gegensatz dazu blieben die schneebedeckten Flächen frostfrei. Bodenfrost führte zu einer signifikanten Verringerung der Verluste von Bodenkohlenstoff. Höchstwahrscheinlich wurde die Zusammensetzung der mikrobiellen Zersetzergemeinschaft beträchtlich vom Bodenfrost beeinflusst, vorrangig durch eine Verringerung des Anteils pilzlicher Biomasse. Das würde erklären, weshalb es auf den Manipulationsflächen nicht nur während der Bodenfrostperiode, sondern auch im darauf folgenden Sommer 2006 zu einer erheblichen Verringerung der Bodenrespiration gekommen ist.

Zwei verschiedene Ansätze wurden gewählt um den Effekt von Trockenheit auf die Bodenkohlenstoffumsätze im Fichtenwaldstandort zu untersuchen. Im Freiland wurde Sommertrockenheit experimentell induziert bzw. verlängert, indem mit transparenten Dächern der Bestandesniederschlag ausgeschlossen wurde. Zusätzlich wurden im Labor Experimente an ungestörten Bodensäulen durchgeführt. In beiden Experimenten führte Trockenheit zu einer Verringerung der Gesamtkohlenstoffverluste aus dem Boden im Vergleich zu einer Kontrollgruppe. Diese Verringerung war in erster Linie zurückzuführen auf verringerte Bodenrespirationsraten während der eigentlichen Trockenperiode. Es kam jedoch hinzu, dass Hydrophobizität die Wiederbefeuchtung des Bodens behinderte, was dazu führte, dass die Verringerung der Bodenrespirationsraten länger anhielt als die eigentliche Trockenperiode. In der Vergangenheit wurde wiederholt diskutiert, ob es durch den Wechsel von Austrocknung und Wiederbefeuchtung zu einer Freisetzung von stabilisiertem Bodenkohlenstoff kommen kann, was letztlich zu einer Erhöhung der Bodenkohlenstoffverluste führen könnte. In der vorliegenden Arbeit wurde keinerlei Beleg für die Richtigkeit dieser Annahme gefunden. Der

Haupteffekt von Trockenheit lässt sich also beschreiben als eine vorübergehende Verlangsamung der Kohlenstoff-Mineralisation im Boden. Bei Wiederbefeuchtung kehren die Mineralisationsraten auf ihr ursprüngliches Niveau zurück, wobei Hydrophobizität diese Erholung verzögern kann.

Am Niedermoorstandort wurden zwei unterschiedliche Ansätze verwendet um den Einfluss des Wasserspiegels auf die Kohlenstoffumsätze zu beurteilen: (i) Experimentelle Absenkung des Wasserspiegels zur Messung der resultierenden Kohlenstoff-Flüsse im Vergleich zu Kohlenstoffflüssen unter natürlichen Bedingungen (d.h. auf Kontrollflächen), und (ii) wiederholte Messungen unter variierenden natürlichen Bedingungen mit dem Ziel, die Haupteinflussfaktoren für die CO_2-Flüsse bestimmen zu können. Im Gegensatz zum Waldstandort war es hier messtechnisch möglich, die Aufnahme und Abgabe von Kohlenstoff durch oberirdische Vegetation mit in die Untersuchung aufzunehmen. Zusammenfassend wurde festgestellt, dass der Wasserspiegel eine geringe Bedeutung für die Kohlenstoffumsätze im Niedermoor hatte. Die Vegetation am Standort wurde von Gräsern dominiert, und der Wasserspiegel hatte keinerlei Einfluss auf deren CO_2-Assimilation. Bei den vereinzelt vorkommenden Moosen dagegen konnte niedriger Wasserspiegel zu starkem Trockenstress führen und so die CO_2-Assimilation deutlich verringern. Da allerdings der Wasserspiegel an diesem Standort im Sommer natürlicherweise niedrig ist und Moose zudem eine untergeordnete Rolle spielen, ist es fraglich, ob eine weitere Absenkung in Folge gehäufter Sommertrockenheit sich nennenswert auf die Gesamtkohlendioxidassimilation des Ökosystems auswirken wird. Die Bodenrespiration wurde durch die manipulative Absenkung des Wasserspiegels von etwa 15 auf mehr als 60 cm nicht beeinflusst. Die Messungen der natürlichen saisonalen Dynamik der Bodenrespiration lassen vermuten, dass Wasserspiegelschwankungen innerhalb der obersten 15 cm des Bodens einen Einfluss auf die Bodenrespiration haben könnten. Alles in allem macht es der im Sommer vorherrschende niedrige Wasserspiegel an diesem Standort unwahrscheinlich, dass durch den Klimawandel bedingte weitere Absenkungen sich nennenswert auf die Bodenrespiration auswirken werden. Die CO_2-Flüsse dieses Ökosystems sind vermutlich sehr stabil gegenüber einer möglichen Zunahme der Häufigkeit von Sommertrockenheit und damit verbundener Absenkungen des Wasserspiegels.

Chapter 1

On this thesis

1 Background

1.1 Motivation

Soils contain more than twice as much carbon (C) as vegetation or the atmosphere (Batjes 1996, Schlesinger and Andrews 2000). Thus, changes in soil carbon pools can have a large effect on the global carbon budget. The possibility that climate change is being reinforced by increased carbon dioxide emissions from soils due to altered boundary conditions emphasizes the necessity to improve our understanding of climate change feedbacks on soil carbon processes. Extreme meteorological events like drought, heavy precipitation and soil frost affect many biological, chemical and physical processes in soils (Schimel *et al.* 2007), but little is known about the relevance of these events for the soil C dynamics.

1.2 Climate change as expected from climate models

Changes in the atmospheric abundance of greenhouse gases alter the energy balance of the climate system. Global atmospheric concentrations of carbon dioxide (CO_2), methane and nitrous oxide have increased significantly as a result of human activities since 1750 and now far exceed pre-industrial values (IPCC 2007). The IPCC Fourth Assessment report therefore comes to the conclusion that human activities markedly contributed to current climate change. Climate change manifests itself in a variety of phenomena, which are summarized in chapter three of the contribution of working group I to the IPCC Fourth Assessment Report (Trenberth et al. 2007). Probably the best studied aspect of climate change is the predicted increase of global mean surface temperature and its effect on ecosystem processes (Doherty *et al.* 2009). However, there are other important aspects of climate change like e.g. changes in the frequency and amplitude of extreme meteorological events, namely summer drought, heavy precipitation events, and soil frost.

Droughts are likely to occur more frequently in most land areas worldwide, especially during summer. This is rather due to changing precipitation patterns than due to changes in total precipitation amounts, so heavy precipitation events before and after drought periods are also expected to become more frequent. At the same time, as global mean surface temperature is increasing, less precipitation is expected to occur as snow. With snow being the major insulator for soils in winter, in some areas of the world this is likely to result in an increasing frequency of soil frost – an apparent paradox verbalized by Groffman et al. (2001) as the phenomenon of ‘colder soils in a warmer world’. In summary, following the projections of the

IPCC (2007), we are going to live in a world in which weather extremes will occur more frequently.

1.3 The global carbon cycle

Human activities have markedly contributed to climate change by significantly increasing the emission of greenhouse gases. Among these, CO_2 has been identified as the most important anthropogenic greenhouse gas. Its concentration has increased from a pre-industrial value in the year 1750 of ca. 280 ppm to ca. 390 ppm in 2009. The current CO_2 concentration is unparalleled in the last 650,000 years, as analyses of ice cores revealed natural fluctuations of the CO_2 concentration ranging between 180 ppm and 300 ppm (IPCC 2007). The most important anthropogenic sources of CO_2 are the combustion of fossil fuels and land use change, resulting in a combined annual flux of ca. 6.9 Pg C a^{-1} (1 Pg = 10^{15} g) to the atmosphere (**Figure 1**) (Schlesinger and Andrews 2000). However, these anthropogenic fluxes are small compared to natural fluxes within the carbon (C) cycle. Each year, around 120 Pg C are assimilated *via* photosynthesis. Roughly the same amount is returned to the atmosphere by ecosystem respiration. Ecosystem respiration consists of two major components, namely aboveground plant respiration and soil respiration. Following current C budgets, soil respiration explains up to 65% of the respiratory flux towards the atmosphere, thus representing the most important flux of CO_2 from terrestrial ecosystems. In summary, terrestrial ecosystems not only represent important stocks of C (both in the vegetation and the

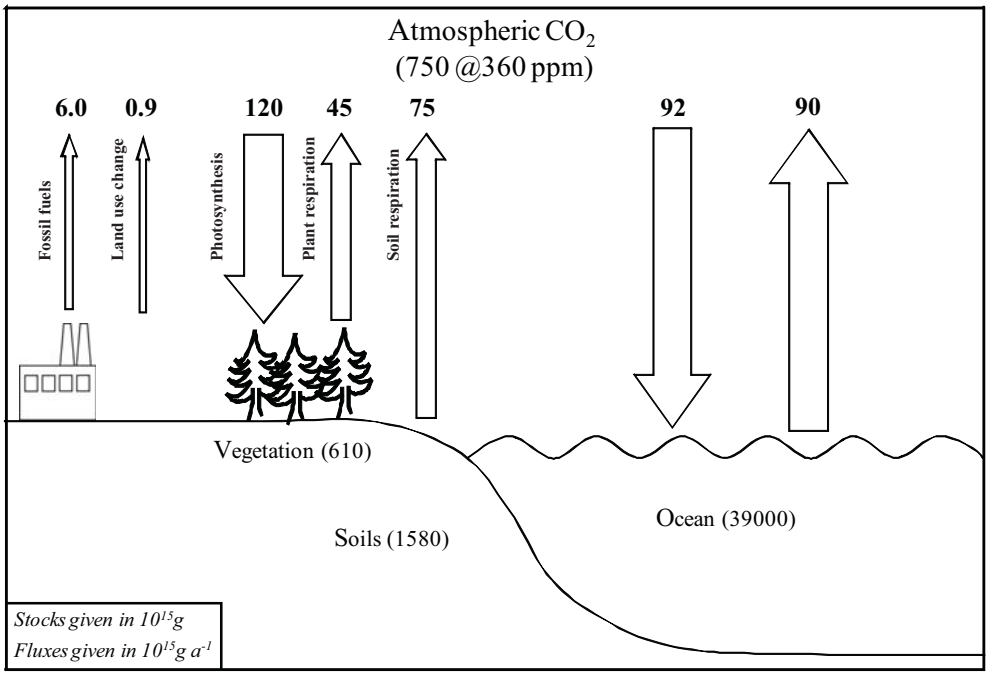

Figure 1. The global carbon cycle. Modified from Schlesinger and Andrews (2000).

soil), but also exchange C with the atmosphere at high gross rates. Thus, even small relative changes of these fluxes bear the potential to result in significant changes of net C fluxes between ecosystems and the atmosphere, thereby either further increasing or decreasing the atmospheric CO_2 concentration.

1.4 Components of soil respiration

Like mentioned before, soil respiration accounts for up to 65% of global ecosystem respiration, and therefore represents the biggest terrestrial flux of CO_2 to the atmosphere. The CO_2 emitted in soil respiration originates from various sources (for a detailed identification of these sources see Kuzyakov 2006). In a simplifying approximation, soil respiration could be partitioned into two major components: (i) roots and (ii) heterotrophic micro-organisms (comprising all kinds of decomposing bacteria, fungi, and other soil inhabitants, but also root-associated micro-organisms). Although logical, this division is not very suitable in many experiments, as root-associated micro-organisms (mycorrhizal fungi and bacteria from the rhizosphere) are inevitably linked to roots. Therefore, in this study I will repeatedly refer to a different partitioning of soil respiration and distinguish the following two major components of soil respiration: (i) Heterotrophic respiration (i.e. the respiration of all decomposing micro-organisms that are not directly associated with roots) and (ii) rhizosphere respiration (i.e. the respiration of roots, root-associated fungi, and the respiration of decomposers from the rhizosphere decomposing root exudates and young fine roots).

These two components of soil respiration are expected to react quite individually to changing environmental conditions. E. g., during drought, soil bacteria depend directly on the water content of their immediate surroundings, and normally have no mechanisms to maintain high metabolic activity when exposed to dry conditions (Schimel *et al.* 2007). Plant roots (especially deep rooting plant species), however, can access water in deeper horizons and transfer it to other regions of their root system, preferably roots in areas with high nutrient concentrations (Nadezhdina *et al.* 2006). Plant roots therefore possess a mechanism to temporarily alter the soil moisture conditions in their immediate surroundings, thereby being able to maintain high metabolic activity. Root-associated micro-organisms can also profit from this so-called hydraulic lift. It therefore is highly recommended to investigate the individual dynamics of different components of soil respiration when investigating the effects of extreme weather conditions.

1.5 Potential feedbacks of climate change on soil CO_2 emissions and vice versa

Soil respiration is governed by a variety of fundamental drivers (Ryan and Law 2005). One of the most important site-specific drivers (explaining site-to-site variability) is probably substrate supply, a factor being ultimately linked to photosynthesis and above- and belowground litter input. Other environmental factors are also important (namely soil moisture, oxygen supply, mean annual temperature, and the belowground community). Seasonal variability of soil respiration at a specific site is mainly explained by soil temperature, especially at well drained sites, but soil moisture becomes increasingly important when being below or above a broad optimum range (Bunnel and Tait 1974, Davidson 1998). In poorly or non-drained sites, water table and oxygen availability have been described as additional important factors to explain seasonal variability (Laiho 2006).

Extreme meteorological conditions normally are also reflected in changes of soil parameters like e.g. soil moisture and thus can strongly affect soil respiration and other components of the ecosystem C budget. thus bearing the possibility to either reinforce or mitigate climate change. The exceptional drought in 2003, resulting from the combination of a heat wave (high evapotranspiration) and precipitation deficit, is a good example to illustrate this climate-carbon feedback. Ciais *et al.* (2005) reported that the heat wave of 2003 significantly reduced soil respiration and, even more, also reduced plant productivity in Central Europe. Thus, it created a strong anomalous net C source to the atmosphere, reversing the effect of ca. four years of net ecosystem C sequestration. In poorly drained ecosystems, the potential for climate-carbon feedbacks has long been recognized. Here, the major concern is an increase of gross respiration fluxes due to increased oxygen availability when water table is lowered during drought periods (Alm *et al.* 1999). Thus, the ecosystem can become a net source because under dry conditions C is lost that before has been stabilized by high water tables (so-called climatic stabilization of soil organic matter, cf. Trumbore 2009).

Destabilization of C due to extreme climatic conditions has not only been discussed for poorly drained soils, but also for well drained (mineral) soils. The so-called 'Birch effect' (Jarvis *et al.* 2007) describes the rapid mobilization of C substrates during the rewetting of dry soil. This mobilization can affect previously stabilized substrates as well. Therefore, a number of studies suggested that dry/wet cycles can accelerate C losses from soil relative to what would be lost under constant 'optimum' conditions (Miller *et al.* 2005, Curiel Yuste *et al.* 2005, Schimel *et al.* 2007). Mechanistic explanations (Fierer and Schimel 2003, Xiang *et al.* 2008) of this C mobilization discuss the physical disruption of soil aggregates due to rewetting (Denef *et al.* 2001a, Denef *et al.* 2001b, Consentino *et al.* 2006), resulting in the

exposition of previously protected material to microbial attack thus resulting in its breakdown. Following this mechanistic explanation of enhanced C losses, other extreme meteorological events exerting strong physical strain on soil aggregates (like e.g. soil frost) might also result in the mobilization of previously stabilized C in soils (Schimel *et al.* 2007). In summary, it has been reported repeatedly that extreme weather conditions can have tremendous effects on soil C dynamics, but the underlying processes are complex and still poorly understood. Schulze and Freibauer (2005) subsumed the phenomenon of C mobilization due to climate or land-use change as 'carbon unlocked from soils'.

Realizing the importance of soil-atmosphere carbon fluxes, this thesis focuses on the effect of extreme meteorological boundary conditions on soil respiration in two different (semi-)natural ecosystem types that are common in Central Europe, namely a Norway spruce forest and a minerotrophic temperate fen. Norway spruce (*Picea abies* L.) currently is the most widespread tree species in Germany (Walentowski 2004). Thus, understanding the effect of projected climate change on Norway spruce soils is of high ecological relevance. Peatlands, on the other hand, cover only around 13,000 km^2 or 3.6% of the land area in Germany (Byrne et al. 2004). On a global scale, the proportion is roughly the same, with peatlands comprising 3.5% of the total land surface (Gorham 1991). Despite this relatively small area, peatlands are important C storage pools, comprising between 270-370 Pg C of the estimated 1580 Pg C stored in soils worldwide (Turunen *et al.* 2002). Under natural conditions, peatlands normally develop slowly (e.g. Hughes and Dumayne-Peaty 2002), but land-use change and/or climatic change can mediate relatively rapid changes, so peatlands have been characterized as particularly vulnerable to climate change (Alm *et al.*, 1999; Moore, 2002; Bubier *et al.*, 2003).

2 Objectives of this study

To address the uncertainties in current understanding of C dynamics under natural conditions and under the impact of extreme meteorological boundary conditions, this study has the following agenda:

(1) Quantify the soil C balance of a forest and a fen ecosystem under current (natural) climatic conditions by modeling turnover times (TT), size, input and output of soil organic carbon (SOC) pools of different horizons.

CHAPTER 2

(2) Study the effect of soil frost on the dynamics of soil respiration, total gaseous soil C losses, and the individual contribution of soil respiration components in a Norway spruce soil using a field site manipulation approach.

CHAPTER 3

(3) Investigate the importance of drought intensity for total C losses and mobilization of stabilized C in a laboratory approach with soil from a Norway spruce forest.

CHAPTER 4A

(4) Study the effect of prolonged summer drought on the dynamics of soil respiration, total gaseous soil C losses, and the individual contribution of soil respiration components in a Norway spruce soil using a field site manipulation approach.

CHAPTER 4B

(5) Study the effects of varying water table during summer (either due to natural fluctuation or due to manipulative lowering) on CO_2 emissions and CO_2 uptake in a minerotrophic fen.

CHAPTER 5

3 Materials and Methods

3.1 Study sites

This study comprises one laboratory study (**CHAPTER 4A**), three field-studies from a Norway spruce stand (**CHAPTERS 2A, 3, AND 4B**), and three field studies from a temperate fen (**CHAPTERS 2B AND 5A+B**). Soil columns for the laboratory study originate from the forest field site, thus all studies are related to two field sites in Northern Bavaria, Germany. Both sites are located in the Lehstenbach catchment, covering an area of 4.5 km^2. The mean annual air temperature (1971-2000) of the catchment is 5.3°C and the mean annual precipitation ranges around 1160 mm (Gerstberger et al. 2004). With a total of 133 frost days per year (air temperature minimum < 0°C), frost is a common event in the Fichtelgebirge (Foken 2003).

The Lehstenbach catchment area is dominated by Norway spruce (*Picea abies* L.) forest, therefore site number one (*Coulissenhieb II*) comprises a small Norway spruce stand located at 50°08'N, 11°52'E at an elevation of 770m a.s.l. The understorey vegetation at the stand is dominated by *Calamagrostis villosa* (Chaix ex Vill), *Deschampsia flexuosa* (L.), *Vaccinium myrtillus* (L.), and *Oxalis acetosella* (L.).). According to the FAO soil classification (IUSS 2006), the soil is classified as a Haplic Podzol with a sandy to loamy texture The forest floor is characterized as mor-like, exhibiting a thickness of 6–10 cm and is composed of Oi, Oe, and Oa horizons.

Site number two (*Schlöppnerbrunnen*) is a minerotrophic temperate fen located at 50°08'N, 11°51'E. It is a moderately acidic (pH 3.5-5.5) fen characterized by highly decomposed soils that are rich in sulphur and iron. The site features a slight slope (5°) from NNE to SSW, and groundwater is flowing through the site parallel to this slope. The soil is a Histosol on granite bedrock covered mainly by *Molinia caerulea* (L. Moench), *Nardus stricta* (L.), *Agrostis canina* (L.), *Carex rostrata* (Stokes) and *Eriophorum vaginatum* (L.). The site feature a ditch of unknown history and origin.

3.2 Design of the mesocosm experiment to study soil C dynamics under the effect of drought of varying intensity

A total of 20 undisturbed, vegetation-free soil columns (hereafter 'mesocosms'), comprising only organic horizons, were harvested in the *Coulissenhieb II* Norway spruce stand in the spring of 2006. The mesocosms were incubated in the laboratory at constant +15°C for a total of ca. 150 days. After an initial pre-treatment period of ca. 40 days (+15°C, 4 mm d^{-1} irrigation = equivalent to daily mean at site), the mesocosms were grouped into five

groups à four mesocosms each (one control and three manipulation groups for measuring soil C losses, and one 'batch' group for destructive sampling of soil at various stages of the experiment).

Matric potential of the control was kept constant at ca. -0.02 MPa (~pF 2) by customized irrigation to compensate for evaporation. The manipulation mesocosms were subjected to ventilation to increase evaporation and stimulate drying of the soil. After 16 days, the matric potential of the manipulation mesocosms had decreased by ca. one order of magnitude (~pF 3) compared to the control. At this time, further drying of the drying group one (D1) was prevented. After 35 days, drying of the second manipulation group (D2) was stopped at ~pF 5. The last manipulation group (D3) was dried further until ~pF 6.5 after 47 days of total drying. A detailed overview over the adjustment of individual soil moisture conditions can be found in Figure 2.

We continued to measure soil C losses while maintaining the individual matric potentials of the various groups at constant levels until day 80 after beginning of the manipulation period. At this time, irrigation (4 mm d^{-1}) of all mesocosms was started, resulting in a quick rewetting of the dry soil. We continued to measure soil CO_2 emissions and losses of dissolved organic carbon (DOC).

At various stages of the incubation (end of pre-treatment, end of drying period, a few days after rewetting) we measured the ^{14}C signature of emitted CO_2. Combining these measurements with the constant measurements of the dynamics of soil C losses allowed us to investigate whether relevant mobilization of stabilized C contributed to observed C losses from the soil.

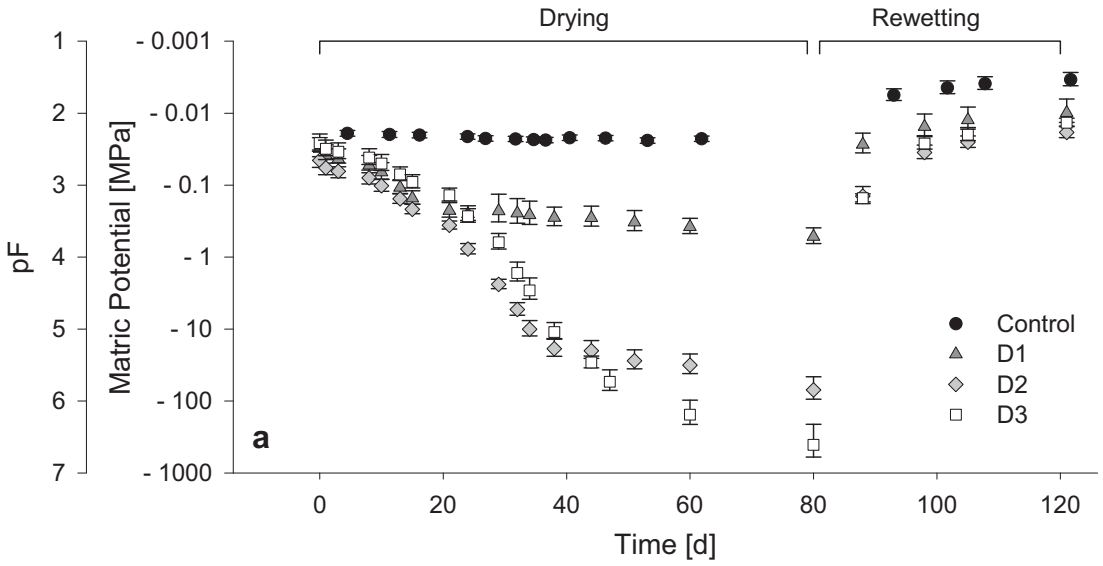

Figure 2. Simulating different soil moisture conditions in four groups of mescososms to investigate the effect of drought intensity on soil C dynamics in a Norway spruce soil.

3.3 Design of the field scale experiments to study C dynamics as affected by meteorological boundary conditions in a forest and a fen

To simulate the effects of extreme weather conditions, we manipulated the boundary conditions at the experimental sites. Although the specific design of the different manipulation experiments varied, the basic setup always comprised a set of three control plots to assess natural variability and a set of three corresponding manipulation plots. Differences between the control and the manipulation plots were used to quantify the manipulation effect.

At the forest site, we conducted two different field scale manipulation experiments. Approach number one was designed to induce soil frost by snow removal (hereafter 'SR'). Approach number two was designed to simulate prolonged summer drought by summer througfall exclusion (hereafter 'TE'). Thus, we established a total of 9 plots the forest site (3x control, 3x SR, 3x TE), each of a size of 20 x 20 m^2.

The SR manipulation was only carried out once during the winter of 2005/2006. Prevailingly warm winter temperatures made a repetition impossible. We removed snow on the SR plots from December 2005 to February 2006, thus effectively triggering soil frost from January to April 2006. To investigate the effects of soil frost on soil C dynamics, we repeatedly measured soil respiration, soil temperature, soil moisture, soil CO_2 concentration, and the ^{14}C signature of total soil respiration and components of soil respiration on all control and SR plots from September 2005 until May 2007.

The TE manipulation at the forest site was carried out during three subsequent years, in the summers of 2006 to 2008. To exclude throughfall and induce drought, the TE plots were covered with a transparent roof construction during the manipulation periods. To investigate the effects of drought on soil C dynamics, we repeatedly measured soil respiration, soil temperature, soil moisture, and soil CO_2 concentration on all control and TE plots from September 2005 until October 2008.

At the fen site, we only conducted one type of manipulation experiment. We thus established one set of control plots and one set of manipulation plots ('D' plots), each of a size of 7.2 x 5 m^2. On the manipulation we artificially lowered the water table by actively pumping the water out of the plots and by excluding precipitation using a roof construction, thus simulating the effects of prolonged dry periods on the hydrological boundary conditions of the fen. The experiment was repeated three times in the summers of 2006 to 2008. To investigate the effects of lowered water tables ecosystem C dynamics, we repeatedly measured soil respiration, soil temperature, soil moisture, water table, and the ^{14}C signature of total soil respiration on all control and D plots from June 2006 until January 2009.

3.4 Relevant analytical techniques

3.4.1 Measuring CO_2 emissions and uptake

To measure CO_2 fluxes (either soil-atmosphere or vegetation-atmosphere), we used a closed dynamic chamber approach (for definition see Pumpanen *et al.* 2004), both in the field site and in the laboratory approach. Although individual measurement systems varied between the studies, they shared the following commonalities: (1) The measurement system (chamber or mesocosm) was closed for the time of measurement, resulting in a non-steady state system with CO_2 concentrations changing over time depending on net CO_2 fluxes; (2) the measured parameter was CO_2 concentration in the headspace volume of the closed system, usually measured in intervals of 10 s over a total measurement time of 3-10 min; (3) for each individual measurement, mean change of CO_2 concentration *(dc/dt)* was calculated by performing a linear regression on the CO_2 concentration *vs.* time. (4) the *dc/dt* retrieved from the linear regression was used to calculate C fluxes ($F_{CO2\text{-}C}$, given in g m^{-2} h^{-1}) between the soil and the atmosphere by using

$$F_{CO_2-C} = \left(\frac{dc}{dt}\right) \times \left(\frac{M_M \times V_H}{M_V \times A_H}\right) \times \left(\frac{P_a}{P_N \times (1 + 0.00367 \times T_a)}\right) \qquad (1)$$

where M_M is the molar mass of C (12.01 g mol^{-1}), M_V the molar volume of CO_2 (22.26 l mol^{-1}), V_H and A_H are the individual volume and soil-atmosphere area of the chamber or mesocosm, P_N and P_a are standard (1013 hPa) and actual air pressure in [hPa], and T_a is the current air temperature in [°C]. Note that the term (1+0.00367 T_a) results from simplification of $(T_{a,[K]}/T_{N,[K]})$, where $T_{a,[K]}$ and $T_{N,[K]}$ are actual and standard (273.15 K) air temperature in [K].

3.4.2 Measuring radiocarbon signature

Measuring the relative abundance of ^{14}C isotopes in solid or gas samples requires specific facilities to process samples. These facilities were lacking at the Department of Soil Ecology in Bayreuth, so I established the modified sealed tube zinc reduction method for the preparation of AMS graphite targets like described by Xu *et al.* (2007). This method allows rapid preparation of graphite targets for measurement in an accelerator mass spectrometer with a precision of 2-3 ‰ and a relatively low background of ca. 50,000 ^{14}C years. In the studies presented within this thesis, measurements of the $^{14}C/^{12}C$ ratio were used within the context of a variety of different applications that shall be presented here in short.

Several of these applications make use of so-called 'bomb ^{14}C'. In the 1950, nuclear weapon testing nearly doubled the atmospheric concentration of ^{14}C (**Figure 3**). Subsequent burning of

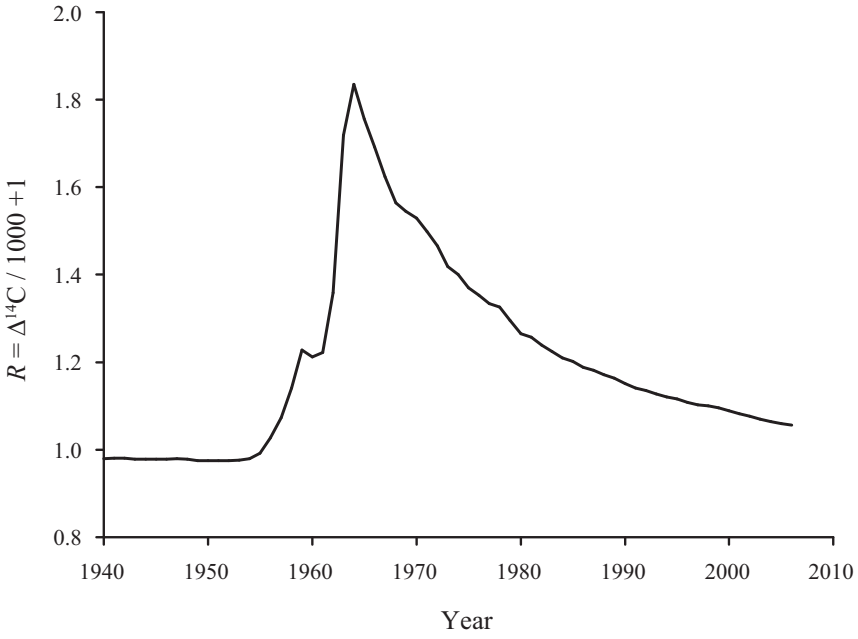

Figure 3. Atmospheric ^{14}C concentration (1940-2006). Beginning in the 1950s, nuclear weapon testing nearly doubled the concentration. After tests had been stopped in the mid 1960s, burning of ^{14}C-free fossil fuels and mixing of the atmosphere with terrestrial and marine C pools resulted in a constant decrease of the ^{14}C concentration (data from Levin *et al.* 2008).

^{14}C-free fossil fuels and mixing of the atmospheric C pool with terrestrial and marine C pools resulted in a continuous decrease of ^{14}C in the atmosphere. The annual decrease during the last decades resulted in year-to-year differences in atmospheric ^{14}C concentration that were bigger than the measurement sensitivity of AMS measurements. The year-specific atmospheric ^{14}C label is propagated to plant biomass (*via* assimilation) and to surface soils (*via* litter). Thus, bomb ^{14}C enables us to study C cycles on decadal timescales without the additional application of a tracer.

(1) Source pool identification. On short time scales, the radioactive decay of ^{14}C can be regarded as irrelevant. Making use of this simplification, ^{14}C can be used to quantify the proportion of two sources *A* and *B* in a mixture *M*, given that (i) differences in isotopic signatures of *A*, *B* and *M* are high enough with regard to the sensitivity of the measurements, (ii) *A* and *B* are the only sources contributing to *M*, and (iii) fractionation during incorporation of the sources *A*, *B* into the mixture *M* either does not occur or is corrected for during the measurement. Under these pre-conditions, a simple single isotope, two-source mixing model can be used (Philips and Gregg 2001):

$$f_A = \frac{\overline{\delta}_M - \overline{\delta}_B}{\overline{\delta}_A - \overline{\delta}_B} \qquad (2)$$

where $\overline{\delta}_M$, $\overline{\delta}_A$, and $\overline{\delta}_B$ represent the mean isotopic signatures (in this case $\Delta^{14}C$) for the mixture *M* and the sources *A* and *B*, and f_A the proportion of *A* in *M*. In the studies presented

here, this application of ^{14}C was used to quantify the proportion of rhizosphere and heterotrophic respiration in total soil respiration. Differences between these two source pools result from the incorporation of bomb ^{14}C into biomass like described above. A simplified variety of this approach has been used in the laboratory incubation experiment (**CHAPTER 4A**). In this approach, only the isotopic signature of the mixture (i.e. soil CO_2 emissions) is measured repeatedly. Significant changes in this isotopic signature are indicative of qualitative changes in the sources. This approach thus can be used to detect the mobilization of old, previously stabilized C.

(2) Radiocarbon dating. Prior to 1950, the atmospheric concentration of radiocarbon was relatively constant and is well documented from reconstructions based on tree-ring calibration techniques and radiocarbon measurements on foraminifera from marine sediments and corals (Reimer *et al.* 2004). The ^{14}C signature of a system that is (actively) exchanging carbon with its surroundings (e.g. living plants or micro-organisms) is in balance with atmospheric values (±offsets due to fractionation). In homogeneous systems (i.e. systems in which every C atom has the same chance to leave the system) without C uptake, the current ^{14}C signature of the system will be governed by radioactive decay of ^{14}C, leading to a depletion of ^{14}C over time with a half-life $\tau_{1/2}$ of 5730 years. Thus, the age T of a sample can be calculated as:

$$T = \left(-\frac{1}{\lambda_{14}} \right) \times \ln\left(\frac{N(t)}{N(0)} \right) \qquad (3)$$

where $\lambda_{14} = \ln(2)/\tau_{1/2}$, N(t) is the number of ^{14}C atoms at the time of measurement and *N(0)* at the time when C uptake into the system stopped and decay become the governing process. *N(0)* thus is identical with the ^{14}C concentration on the atmosphere at that time. The so-calculated ^{14}C age therefore has to be corrected for fluctuations in atmospheric ^{14}C concentration (using software like e.g. OxCal 4.1). In the studies presented here, this approach was used to determine the age of soil organic matter (SOM) (**CHAPTER 2**). As SOM is continuously incorporating fresh carbon, it violates the precondition of an ideally closed system. Thus, SOM ages from ^{14}C dating have to be interpreted as minimal ages for the length of soil formation (Wang *et al.* 1996).

(3) Modelling of soil C turnover. Radiocarbon data may be used in several ways to estimate input (I) and decomposition (k) rates of C stocks. In the studies presented here, three different models have been applied. They are presented in detail in the two studies comprised in **CHAPTER 2**.

4 Synthesis and discussion of the results

4.1 Quantifying soil C dynamics of a Norway spruce forest and a fen under current boundary conditions (CHAPTER 2)

Using three different models that were based on soil C stock and ^{14}C data, we quantified the mean soil C dynamics under current climatic conditions at the both field sites. The models that we applied reflect mean C dynamics on timescales from decades to millennia. These two studies complement the manipulation studies by identifying horizon specific C stocks and contribution of these stocks to total C fluxes and thus allowing to estimate the vulnerability of specific C stocks to changing boundary conditions.

The key findings for the Norway spruce soil were that (i) soil C dynamics at this site were dominated by C fluxes in and out of the organic horizons (fast turnover, high gross fluxes) (**Table 1**), thus organic horizons presumably are more vulnerable to changing boundary conditions than mineral horizons; (ii) under 'current' conditions (i.e. mean conditions of the last decade), the soil at this site was a small sink for atmospheric CO_2 in the order of 4-8 g C m^{-2} a^{-1}.

Table 1 Horizon specific gross and net C fluxes in a Norway spruce soil derived from the turnover time modeling data presented in Schulze *et al.* (2009) (cf. **CHAPTER 2**)

Horizon	C Input (*I*) (g C m^{-2} a^{-1})	C Output (*kC*) (g C m^{-2} a^{-1})	Net C accumulation (*I-kC*) (g C m^{-2} a^{-1})
Oi	153	153	< 0.1
Oe	120	120	0.3
Oa	17	11	6
Ea	9	9	0
Bsh	6	6	0
Bsh	5	5	0
Bv	3	3	0

Although a relatively high degree of spatial variation of some measured parameters complicated the interpretation of the results, we were able to establish some general characteristics of the site. A fundamental finding with regard to the manipulation experiments carried out at the site was the importance of the organic horizons for overall soil C dynamics in this soil. Between 19-35% of the total SOC stock (measurement depth 60 cm) were comprised in the organic horizons. Turnover times of this SOC were relatively fast (3-10 years), resulting in high gross C fluxes. In fact, total gross C fluxes in this soil

(including gaseous losses, DOC leaching and top-down C transfer within the profile) are clearly dominated by C turnover of the organic horizons (> 90%). Gross C fluxes are several orders of magnitude higher than calculated net C fluxes. Thus, relatively small changes in the balance of gross C fluxes of the organic horizons could result in significant changes of the net C fluxes. In comparison, relative changes in the mineral horizons would have to be much higher to result in notable changes of the net soil C balance. As organic horizons are situated directly at the interface between soil and atmosphere, weather extremes like drought or soil frost can easily have a direct impact on boundary conditions within the organic horizons, whereas the underlying mineral horizons are to some extent decoupled from changes of atmospheric boundary conditions. We thus conclude that the organic horizons at this site are more vulnerable to changing boundary conditions than mineral horizons.

Interestingly, the results of this study also raised the question whether soil C dynamics at this site reflect undisturbed conditions. The net accumulation we calculated for this site was only about half the size of net accumulation rates reported for coniferous soil in Sweden (Ågren *et al.* 2008). Given the history of the site we suspect that the site could be influenced by undocumented liming, a practice that has been common in the area in the past. Liming has repeatedly been reported to improve soil conditions, thus increasing mineralization rates of SOM and thus potentially reducing the net C balance of the soil (Persson *et al.* 1989, Fuentes *et al.* 2006).

The key findings for the fen site were that (i) under current boundary conditions the fen site is a net C source, indicating that the site is subject to disturbance, (ii) a high amount of C is stored in root biomass, (iii) fluxes in and out of SOM C stocks occur predominantly in the uppermost 15 cm, most likely due to low substrate quality in deeper peat layers.

Using a modeling approach, we quantified the soil C balance within the peat body of a minerotrophic fen. We distinguished three relevant C stock compartments within the peat body: (i) root biomass (comprising live roots and structurally intact dead roots), (ii) surface peat SOM (defined by the occurrence of bomb ^{14}C), and (iii) deep peat SOM. We used two different models to calculate the net C balances of these three compartments (Trumbore and Harden 1997, Gaudinski *et al.* 2000). Whereas peatlands in general are reported to be net C sinks with net accumulation rates between approx. 15-30 g C m^{-2} a^{-1} (Vitt *et al.* 2000, Turunen *et al.* 2001, Turunen *et al.* 2002), we calculated a slightly negative C balance for this fen under current climatic conditions, indicating disturbance of the boundary conditions at this fen site. In detail, we calculated (i) a net C loss of -24 g C m^{-2} a^{-1} from the root biomass stock, (ii) a net C loss of -5 g C m^{-2} a^{-1} from the surface peat SOM stock, and (iii) a net C accumulation

of +3 g C m^{-2} a^{-1} in the deep peat SOM stock. The net C losses from the root biomass C stock most likely reflect changing of boundary conditions on a shorter timescale, given the relatively fast turnover time of root biomass. Net C losses from the SOM stocks might also reflect disturbances on a longer timescale (up to several decades). Based on our results we are unable to identify the actual source of disturbance. The site features a ditch of unknown history, and a disturbance of the hydrological boundary conditions due to drainage by this ditch would be a very likely source of disturbance. Results from other experiments at his site have to be discussed in the context of this disturbance.

4.2 Soil carbon dynamics of a Norway spruce soil as affected by soil frost (CHAPTER 3)

The key findings of this study were that (i) C dynamics during the period of actual soil frost had a relatively small effect on total C losses from the soil, (ii) freezing-thawing does not mobilize stabilized C in this soil, (iii) soil frost alters the composition of the microbial community (preferential reduction of fungal biomass proportion), thus ultimately (iv) increasing the susceptibility of the soil microbial community towards drought stress.

Due to repeatedly warm temperatures in the winters of 2006/2007 and 2007/2008, the experimental induction of soil frost at the *Coulissenhieb II* site could only take place once in the winter of 2005/2006. In that winter, snow removal effectively induced soil frost on the manipulation plots. Following snow removal, soil frost occurred down to a depth of 15 cm and lasted ca. three months. No indication of soil frost was found on the control plots, so the snow removal successfully simulated increasing soil frost frequency.

We compared total C losses between January 2006 and January 2007 from the manipulation plots and from the control plots. Total C losses from the manipulation plots were 5.1 t C ha^{-1} a^{-1}, compared to 6.2 t C ha^{-1} a^{-1} from the control plots. Thus, soil frost resulted in a reduction of total C losses by 1.1 t C ha^{-1} a^{-1}. Surprisingly, soil respiration differences during the actual soil frost period and the subsequent thawing could only explain 14% of this reduction. The major proportion of the differences was explained by significantly reduced soil respiration fluxes from the manipulation plots during the summer of 2006. Inherent differences were excluded due to the pre-treatment period and the setup of the plots. No measurable differences were in soil temperature and soil moisture. Thus, we linked the reduction of the summer soil respiration fluxes to the stress history of the manipulation plots.

Schmitt *et al.* (2008) reported that repeated freezing-thawing of soil columns from the *Coulissenhieb II* site resulted in a reduction of the relative contribution of fungal to total microbial biomass. Similar findings have also been reported in several other studies

(Nieminen and Setala 2001, Larsen *et al.* 2002, Feng *et al.* 2007). Assuming the same phenomenon occurred under field-site conditions, we postulated that soil frost changed the composition of the microbial community on the manipulation plots, reducing fungal biomass. Fungi, in turn, have been reported to be more resistant towards drought than bacteria (Voroney 2007). Thus, an altered composition of the microbial community is likely to result in an altered susceptibility towards drought stress. The summer of 2006 was an exceptionally dry summer. We therefore conclude that soil frost indirectly reduced total soil C losses by increasing the susceptibility of the soil microbial community towards drought stress. We conclude that the exceptional combination of severe soil frost in winter and drought stress in summer were responsible for the remarkable reduction of total C losses in the manipulation plots.

Several field and laboratory studies reported a pronounced CO_2 pulse after thawing of frozen soil from agricultural, arctic or forest soils (Coxson and Parkinson 1987, Elberling and Brandt 2003, Dörsch *et al.* 2004, Goldberg *et al.* 2008). Different mechanisms have been discussed to explain this pulse. These mechanisms are very similar to the mechanisms discussed by Xiang *et al.* (2008) to explain the occurrence of such a pulse during drying-rewetting events. Thus, I will use the same terminology here, differentiating between the 'microbial stress' and the 'substrate supply' mechanism.

Following the logic of the 'microbial stress' mechanism, this pulse would originate from the release of substrates from microbial biomass. This release could be a consequence of cell death. Alternatively, it could be explained by a reversal of physiological acclimation of micro-organisms to freezing (Schimel *et al.* 2007) resulting in a release of solutes like e.g. protective molecules (Mihoub *et al.* 2003, Kandror *et al.* 2004) or antifreeze proteins (Bae *et al.* 2004). Following the logic of the 'substrate supply' mechanism, the CO_2 pulse would be due to mobilization of previously stabilized C e.g. due to physical disruption of soil aggregates. Additional mobilization of C substrates would ultimately have to result in an increase of total C losses. This second mechanism thus bears the possibility of enhanced C losses from soils due to freezing and thawing.

Based on our results, we neither observed a pulse nor did we find an increase of total C losses resulting from freezing-thawing of the soil. We therefore have to refuse the idea of mobilization of stable C due to soil frost. This result is in agreement with findings from laboratory studies on undisturbed soil columns from this site (Goldberg *et al.* 2008), but also with findings from field-site experiments by Groffman *et al.* (2006) and Coxson and

Parkinson (1987), who also reported no effect of freezing-thawing on cumulative soil C losses.

4.3 Soil C dynamics in a Norway spruce soil as affected by drying-wetting under laboratory and field-site conditions (CHAPTER 4)

Key findings of these studies: (i) The main effect of drought is a temporary reduction of decomposition, leading to (ii) a reduction of total soil C losses that can not be compensated for during subsequent wet periods, and (iii) mobilization of previously stabilized C due to drying-wetting does not occur. Thus, in summary, drought irrevocably reduces gross soil C losses in the year of drought. We did not investigate the effects of drought on the CO_2 uptake by plants and litter input. The relatively small net uptake indicates that uptake and emission fluxes are very similar in size under current climatic conditions. Hence, from the ecosystem level, we can not exclude the possibility that this forest might turn into a temporary net source of C during prolonged summer drought if CO_2 uptake is reduced stronger than soil respiration like reported by Ciais *et al.* (2005).

A laboratory study (**CHAPTER 4A**) was designed to study the effect of drought intensity on (i) dynamics of soil C losses, (ii) total quantity of soil C losses, and (iii) mobilization of stabilized C in the organic horizons in detail. As soil columns from the organic horizons were used, the study does not allow any conclusions about mineral horizons and comprises only the effects of drought on heterotrophic respiration (i.e. decomposition). The high temporal resolution of the measurements revealed that drying of the organic horizons resulted in an almost immediate reduction of decomposition, either because microorganisms became inactive or died. The more intense the drought got, the smaller were the observed CO_2 emission rates. Under very dry conditions (pF 6-7) heterotrophic respiration was close to zero. Thus, cumulative soil C losses during the drought period depended substantially from drought intensity. In contrast to this, C dynamics during rewetting of the dry soil seemed predominantly independent from precedent drought intensity: Rewetting basically restored the respiration rates back to pre-drought levels, no transient enhancement of respiration rates was observed. The effect of drought therefore can be described as a temporary reduction of decomposition that is not compensated for by enhanced decomposition during subsequent wet periods. Based on the results of the laboratory experiment, we conclude that the length and intensity of the dry conditions determine how much less C is lost from the organic horizons in comparison to what might be lost under optimum moisture conditions (cf. Borken and Matzner 2009).

A field-site manipulation experiment (**CHAPTER 4B**) was designed to study the effects of prolonged summer drought on soil respiration *in situ*. Basically, it confirmed the conclusions from the laboratory approach: Drying led to a quick reduction of soil respiration, soil respiration continued at reduced rates under dry conditions, wetting triggered an increase of soil respiration, but, consistent with the laboratory experiment, this increase was nothing more than a 'regeneration' to control level. At no time we observed enhanced soil CO_2 emissions in the manipulation plots, not even in a subsequent no-manipulation year, so we conclude that the reduction of gross soil C losses resulting from drought is preserved for at least months to years.

The field-site manipulation also yielded results beyond the findings of the laboratory approach. As the laboratory experiment was confined to the organic horizons, we were unable to assess the effect of drought on mineral horizons. As the 'substrate supply' mechanism is based on the physical disruption of soil aggregates to explain enhanced soil C losses (Xiang *et al.* 2008), organic and mineral horizons might be affected differently by drought. Due to a naturally very dry summer in 2006, we were able to observe dry conditions in the mineral horizon in that year. Based on our results, the effect of drought on organic and mineral horizons in principle was the same.

In contrast to the laboratory approach we were able to quantify the specific effects of drought on rhizosphere *vs.* heterotrophic respiration. Our results indicate that heterotrophic respiration is affected much stronger than rhizosphere respiration. We explain this phenomenon with the ability of spruce to relocate water within its root system, thereby improving soil moisture in dry regions by relocating water from deeper horizons (Nadezhdina *et al.* 2006). Thus, the observed reduction of soil respiration by drought is dominated by a reduction of decomposition.

In summary, our results contradict the idea of a possible enhancement of soil C losses like postulated by several authors (Fierer and Schimel 2002, Miller *et al.* 2005, Jarvis *et al.* 2007, Xiang *et al.* 2008). In the recent years, an increasing number of studies reported results opposing this idea (cf. Borken and Matzner 2008 and references therein). Enhanced C losses due to drying-wetting have mainly been reported for sieved mineral soil and for agricultural soils. The relevance of this mechanism for forest soil has to be readdressed.

4.4 Ecosystem C dynamics in a fen as affected by natural and manipulative water table changes (CHAPTER 5)

Key findings of these two studies: (i) Changes in water table affected respiratory C fluxes only when occurring within the uppermost ca. 0-15 cm soil depth, and (ii) photosynthetic uptake of atmospheric CO_2 was affected by water table fluctuations only in moss species, thus (iii) this fen is presumably very resilient towards an increasing frequency of summer drought. However, this resilience most likely results from the fact that the fen already is subject to a disturbance of the hydrological conditions.

A field-site manipulation experiment was designed (**CHAPTER 5A**) to quantify the effect of water table on ecosystem C dynamics by artificially lowering the water table during summer (thus simulating the effect of summer drought). In contrast to the forest site, we included CO_2 related to aboveground vegetation into our analysis. In summary, we measured (i) net ecosystem exchange (NEE), (ii) ecosystem respiration (R_{Eco}), and (iii) soil respiration (R_{Soil}) and furthermore were able to calculate (iv) gross primary production (GPP) and (v) respiration of the aboveground vegetation. In three subsequent manipulation years (2006-2008) we found no significant effect of lowered water tables on any of the measured parameters. Especially with regards to soil respiration, this was in contrast to our expectations. Generally, C in peatlands is assumed to be stabilized by high water tables, as they inhibit oxygen diffusion (Päivänen and Vasander 1994). Thus, lowering of the water table and the consequent increase of oxygen availability supposedly should increase decomposition rates.

An additional study (**CHAPTER 5B**) was designed to investigate how naturally occurring changes of the boundary conditions affected the CO_2 fluxes into and out of this ecosystem. In addition to CO_2 fluxes, the study comprised measurements of air and soil temperature, photosynthetic active radiance, changes in biomass, and water table. I will concentrate here mainly on findings that are related to changes of the water table. With respect to aboveground biomass, no evidence was found that natural fluctuations (between 0-20 cm) of the water table in any way affected gross primary production of grass species at the site. In contrast to this, biomass production of moss species was depending on water table like indicated by a significant drop of moss biomass following a periods of low water tables during early spring. Thus, it is concluded that low water tables can result in a reduction of gross primary production of mosses. This difference between grasses and roots could be explained by differences in plant anatomy, as grasses have deep rooting patterns that can guarantee sufficient water uptake even during times of low water tables (Limpens *et al.* 2008), whereas

mosses depend on water table and precipitation. However, the site is predominantly characterized by grass species; mosses represent a minor proportion of the vegetation. Furthermore, water tables at the site are already naturally low during most of summer. Thus, we expect lowering of water table due to increasing summer droughts to have only a minor impact on GPP in this ecosystem.

With regard to water table affecting R_{Eco}, the findings of this study seem to contrast the findings of the manipulation experiment: Natural lowering of the water table correlated with increasing values of R_{Eco}. However, there are two important points to notice with this correlation: (i) Data comprised in the analysis is clearly dominated by water tables between 0-10 cm below the surface (only two measurement dates with a lower water table); (ii) shifts in water table were accompanied by changes in peat temperature, making it difficult to distinguish the effects of water table from the effects of peat temperature changes. Thus, we carefully conclude that water table might effect when occurring within the uppermost peat layers (ca. 0-15 cm). This latter conclusion is based on the modeled soil C dynamics of this site (cf. **CHAPTER 2B**) and on findings reported by Reiche *et al.* (2009). As described, modeling revealed that C turnover during the last decades was clearly dominated by fluxes occurring within the uppermost 15 cm of the soil. The contribution of C turnover in deeper peat layers was almost irrelevant. As water table at least in summer (when decomposition is highest due to high soil temperatures) regularly drops deeper than 15 cm even under natural conditions, it is unlikely that the small contribution of the deeper peat can be explained only by a lack of oxygen. Indeed, Reiche *et al.* (2009) were able to demonstrate that peat in the deep peat layer (beneath 15 cm) is characterized by low substrate quality, as it has a low decomposition potential even under optimum conditions. Hence, water table fluctuations can only affect soil C losses within the uppermost 15 cm, as soil C dynamics in deeper peat are not governed by oxygen availability but by substrate quality. However, water tables are already naturally low in summer at this site. In summary, due to the preconditions at the site, we expect the effect of further lowering of the water table during summer on ecosystem C dynamics at this site to be minimal.

5 Conclusions

This thesis focused on the effect of meteorological boundary conditions on C dynamics of a forest and a fen ecosystem. Both ecosystems were located in the Fichtelgebirge in South-eastern Germany. In the forest ecosystem, the focus was on the effects of (i) drought and of (ii) soil frost on soil C dynamics. In the fen ecosystem, the focus was on the effect of (iii) water table (especially of lowered water table) on ecosystem C dynamics. Under the impressions of current climate models, all three scenarios are highly up-to-date. This thesis was especially interested in the question whether extreme meteorological boundary conditions could possibly lead to enhanced soil C losses, thus resulting in a reinforcement of climate change. This concern was based on earlier discussions of this phenomenon in the literature. A considerable number of studies points at the possibility of climate-carbon feedbacks resulting from extreme boundary conditions in soils.

However, this concern was not confirmed in the studies presented here. In the forest ecosystem, all results indicated that extreme boundary conditions like frost and drought represent significant stress for the soil organisms. As long as this stress is maintained, metabolic activity is significantly reduced. Soil organisms either die or become less active. Both types of extreme events seemed to mainly affect the heterotrophic soil organisms in the forest soil. Roots and presumably root-associated microorganisms seem to be less prone to the effects of extreme soil boundary conditions. Partially, this could be explained by the ability of roots to significantly influence soil conditions in their immediate surroundings. Heterotrophic soil microorganisms are responsible for the decomposition of soil organic matter. Thus, in the forest ecosystem extreme meteorological events lead to a transient reduction of decomposition. The more intense the stress regime and the longer it is maintained, the higher the cumulative impact on decomposition. Thus, extreme conditions like soil frost and drought primarily lead to a reduction of soil C losses as decomposition is slowed down. Whether or not such a transient reduction of soil C losses can lead to the long-term net sequestration of C depends on a variety of other factors.

First of all, net ecosystem C balance is the difference between C uptake and C losses. Thus, to determine whether reduced soil C losses are equivalent to increased sequestration of C one, the effects of extreme boundary conditions on C assimilation have to be considered. In the forest ecosystem, we have been unable to measure assimilation due to technical limitations. Thus, when observing a transient reduction of the gross C losses due to extreme meteorological conditions, it still is possible that the net ecosystem C losses are enhanced.

Furthermore, a transient reduction of soil C losses can only lead to a long-term sequestration of C as long as it is not compensated for by enhanced C losses in the aftermath of the stress period. This could happen when breakdown of complex molecules continues at high rates during the stress period but substrates are not completely mineralized (e.g. when they are instead used to produce anti-stress proteins). Furthermore, extreme boundary conditions within the soil do not only exert stress on microorganisms but also physical stress on soil aggregates. Soil aggregate breakdown might result in the mobilization of new substrates that can compensate or even over-compensate for the reduced decomposition during the stress period. However, our results in the forest disproved both these assumptions. Apart from uncertainties concerning the effect on assimilation, we therefore can conclude that soil frost and drought do both result in a clear reduction of soil C losses. Thus, based on our results, it is more likely to expect a negative climate-carbon feedback resulting from soil frost and drought than to expect a positive one.

Things were different to some extent for the fen site. Here, we did not measure the direct effects of drought, but rather the indirect effects (i.e. a possible lowering of the water table like expected as a result of increased evapotranspiration and decreased precipitation). As peatlands normally feature great amounts of C that is stabilized predominantly by high water tables and consequently low rates of decomposition, we expected a significant effect of lowered water tables on CO_2 fluxes in this ecosystem. Surprisingly, CO_2 fluxes in this fen were not affected by water table manipulations. These manipulations, however, were carried out when the water table was already low. Other data indicated that for higher water tables a change of the water table might affect the CO_2 fluxes. Still, the 'natural' water table was predominantly low under current boundary conditions, especially in summer. Modeling data indicates, however, that this condition is not actually natural, as this fen has been identified as a net C source, indicating that present boundary conditions are different compared to what they have been like during the formation of the peatland. Given the low water table and the indication of disturbance we assume a direct disturbance of hydrological conditions, probably due to draining of the site. Thus, conclusions drawn at this site are not valid for undisturbed peatland sites. The low water tables presumably result in decomposition rates that are no longer limited by oxygen availability controlled by water table but instead by peat temperature, substrate quality and the occurrence of micro-environmental structures. Based on the results here, the site might proof a suitable location for a restoration or flooding experiment. With regard to peat temperature as an important driver of decomposition, it is important to mention that manipulative lowering of the water table fails to completely

simulate the effects of summer drought. In reality, summer drought is most likely to be accompanied by high temperature and evapotranspiration. Thus, the combination of a lack of precipitation, high evapotranspiration and high temperature will not only result in lowering of the water table but will presumably also increase peat temperature. Lower water tables during summer alone were found to have no effect on CO_2 fluxes in this fen, but it has to be critically analyzed whether the manipulative approach really reflects all aspects of summer drought.

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7 Record of contributions to the included manuscripts

CHAPTER 2A - Stock, turnover time and accumulation of organic matter in bulk and density fractions of a Podzol soil (*European Journal of Soil Science* (2009), **60**, 567-577)
Kerstin Schulze, Werner Borken, Jan Muhr and Egbert Matzner

K. Schulze:	60%	(concepts, laboratory and field work, interpretation and discussion of results, manuscript preparation)
W. Borken:	10%	(concepts, discussion of results, manuscript preparation)
J. Muhr:	25%	(concepts, laboratory and field work, interpretation and discussion of results, manuscript preparation)
E. Matzner:	5%	(discussion of results)

CHAPTER 2B - Carbon dynamics in a temperate minerotrophic fen (*Biogeochemistry*, submitted July 09)
Jan Muhr, Juliane Höhle and Werner Borken

J. Muhr:	70%	(concepts, laboratory and field work, interpretation and discussion of results, manuscript preparation)
J. Höhle:	15%	(laboratory and field work, discussion of results)
W. Borken:	15%	(concepts, discussion of results, manuscript preparation)

CHAPTER 3 - Effects of soil frost on soil respiration and its radiocarbon signature in a Norway spruce forest soil (*Global Change Biology* (2009) **15**, 782-793)
Jan Muhr, Werner Borken and Egbert Matzner

J. Muhr:	85%	(concepts, laboratory and field work, interpretation and discussion of results, manuscript preparation)
W. Borken:	10%	(concepts, discussion of results, manuscript preparation)
E. Matzner:	5%	(concepts, discussion of results)

CHAPTER 4A - Drying-rewetting events reduce C and N losses from a Norway spruce forest floor (*Soil Biology & Biogeochemistry*, submitted December 08)
Jan Muhr, Janine Franke and Werner Borken

J. Muhr: 50% (concepts, laboratory work, interpretation and discussion of results, manuscript preparation)
J. Franke: 35% (concepts, laboratory work, interpretation and discussion of results)
W. Borken: 15% (concepts, discussion of results, manuscript preparation)

CHAPTER 4B - Delayed recovery of soil respiration after wetting of dry soil further reduces C losses from a Norway spruce soil (*Journal of Geophysical Research – Biogesciences*, revision submitted June 09)
Jan Muhr and Werner Borken

J. Muhr: 85% (concepts, laboratory and field work, interpretation and discussion of results, manuscript preparation)
W. Borken: 15% (concepts, discussion of results, manuscript preparation)

CHAPTER 5A - Manipulative lowering of the water table during summer does not affect CO_2 emissions and uptake in a minerotrophic fen in South-eastern Germany (*Ecological Applications*, submitted July 09)
Jan Muhr, Juliane Höhle, Dennis O. Otieno and Werner Borken

J. Muhr: 60% (concepts, laboratory and field work, interpretation and discussion of results, manuscript preparation)
J. Höhle: 15% (laboratory and field work)
D. O. Otieno: 10% (concepts, manuscript preparation)
W. Borken: 10% (concepts, discussion of results, manuscript preparation)

CHAPTER 5B - Responses of CO_2 Exchange and Primary Production of the Ecosystem Components to Environmental Changes in a Mountain Peatland (*Ecosystems* (2009) **12**, 590-603)

Dennis O. Otieno, Margarete Wartinger, A. Nishiwaki, M.Z. Hussain, Jan Muhr, Werner Borken and Gunnar Lischeid

D.O. Otieno: 50% (Concepts, field work, interpretation and discussion of results, manuscript preparation)
M. Wartinger: 15% (field work)
A. Nishiwaki: 10% (field work)
M.Z. Hussain: 10% (field work)
J. Muhr: 10% (concepts, discussion of results, manuscript preparation)
W. Borken: 10% (concepts, discussion of results, manuscript preparation)
G. Lischeid: 5% (field work)

Chapter 2

Quantifying soil C dynamics of a forest and a fen under current climatic conditions

PART A:

Stock, turnover time and accumulation of organic matter in bulk and density fractions of a Podzol soil

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Summary

Temperate forest soils store large amounts of organic matter and are considered as net sinks for atmospheric carbon dioxide. Information about the sink strength and the turnover time of soil organic carbon (SOC) are required to assess the potential response of soils to climate change. Here we report on stocks, turnover times and accumulation of SOC in bulk soil and density fractions from genetic horizons of a Podzol at the Fichtelgebirge, Germany. Stocks of SOC, total nitrogen and exchangeable cations determined in nine quantitative soil pits strongly varied with stone content and thickness of horizons in both the organic layer and mineral soil. On the basis of radiocarbon signatures, mean turnover times (TT) of 4, 9 and 133 years, respectively, were calculated for Oi, Oe and Oa horizons from three soil pits, using a non-steady state model. The Oa horizons accumulated 4-8 g C m^{-2} a^{-1} whereas the Oi and Oe horizons were close to steady-state during the past decade. Free particulate organic matter (FPOM) was the most abundant fraction in the Oa and EA horizons with TT of 70-480 years. In the B horizons, mineral associated organic matter (MAOM) dominated with over 40 % of total SOC and had TT of 390-2170 years. In contrast to other horizons, MAOM in the Bsh and Bs horizon had generally faster TT than occluded particulate organic matter (OPOM), possibly because of sorption of dissolved organic carbon by iron and aluminium oxides/hydroxides. Our results suggest that organic horizons with relatively short turnover times could be particularly vulnerable to changes in climate or other disturbances.

Introduction

The accumulation of soil organic matter (SOM) is a characteristic feature of temperate and boreal forest ecosystems. Frequent soil types of the temperate and boreal zone are Podzols covering 485 million ha throughout the world (IUSS Working Group WRB, 2006). These soils developed under heather or coniferous forests and store large amounts of SOM as a result of slow decomposition processes and accumulation of SOM in organic and spodic horizons. Referring to 1 m soil depth, Batjes (2002) estimated that Podzols had the second-largest soil organic carbon (SOC) stocks in Europe after Histosols. There is reason for concern that climate change will turn these soils from a net sink into a net source for atmospheric carbon dioxide (CO_2).

Soils contain different organic matter fractions with varying stability, turnover time (TT) and temperature sensitivity (Trumbore, 2000; Kögel-Knabner *et al*., 2008). Specific SOM fractions are more vulnerable to climate change and other disturbances than others, but the characterization of SOM fractions depends on the methodical approach. Physical fractionation techniques are less destructive than chemical fractionation procedures and relate more directly to structure and function of SOM (Christensen, 2001). Density fractionation allows the separation of free particulate (FPOM), occluded (OPOM) and mineral associated organic matter (MAOM). The FPOM fraction mainly contains recognizable plant material, fungal hyphae and responds quickly to changes in carbon (C) inputs and environmental conditions (and represent an active pool), whereas the OPOM and MAOM vary strongly with differences in soil structure and mineralogy (Baisden *et al.*, 2002; Crow *et al.*, 2007; von Lützow *et al.*, 2006). Compared with FPOM, slower turnover of OPOM is attributed to chemical recalcitrance, humification and physical stabilization by occlusion (Poirier, 2005; Kögel-Knabner *et al*., 2008). MAOM is the dominating fraction in mineral soil horizons and has very slow turnover rates because of stabilization by interaction with mineral surfaces, iron/aluminium (Fe/Al) oxides and hydroxides (Torn *et al.*, 1997; Kögel-Knaber *et al.*, 2008). However, a critical issue of the density fractionation is the removal of soluble and less degraded substrate during density fractionation (Crow *et al.*, 2007). This soluble fraction was generally discarded and therefore not characterized and considered in SOC models.

Mean TT of SOC in bulk soil or fractions can be calculated from its radiocarbon signature ($\Delta^{14}C$), SOC stock or C input, and by using a steady-state or non-steady-state model (Gaudinski *et al.,* 2000; Trumbore, 2000). The TT of SOC generally increases with increasing stability and density of the fraction. Estimates of TTs based on bulk mineral soil, however, may lead to misleading interpretations (Davidson *et al.*, 2000). The radiocarbon signature of

bulk mineral soil is governed by MAOM, but labile SOC fractions of bulk soil with short TTs could rapidly respond to global warming.

While gaseous and solute C losses from soils have been intensively investigated, little is known about the C input and net accumulation of SOC in specific soil horizons. In undisturbed coniferous forests, accumulation of SOC takes place mainly in the organic layer whereas the mineral soil seems close to steady-state and thus changes are barely detectable on decadal time scale (Ågren *et al.*, 2008; Trumbore, 2000). When tree biomass is increased or maintained at current levels, SOC stocks will also increase, but the accumulation rate is small in mature forests (Ågren *et al.*, 2008). A chronosequence study by Schlesinger (1990) suggests an average accumulation rate of 0.7-12.0 g C m^{-2} a^{-1} for boreal and temperate soils developed during the past 10,000 years. The C accumulation rate is relatively large in the initial period of soil genesis but slows down with increasing age.

The balance between litter input and losses by microbial respiration and leaching controls the build-up of organic horizons. Above-ground litterfall in coniferous forests ranges between 735-8575 kg ha^{-1} a^{-1} along a climatic gradient from north Scandinavia to Spain (Berg & Meentemeyer, 2001). Estimates for root litter input are less reliable because of methodological difficulties. Wutzler & Mund (2007) modelled root litter production for spruce and estimated fine root production of 890-1830 kg ha^{-1} $year^{-1}$.

Area-based estimates of stocks and accumulation or loss rates of SOC and nitrogen (N) in bulk soil and density fractions require representative sampling procedures in the field. The spatial variation in rock content, bulk density and thickness of soil horizons cause large uncertainties in soil surveys. Estimates of stocks of elements in soils are based on fine earth (< 2 mm), but large rock fragments make it difficult to assess the amount of fine earth in genetic horizons on larger scales (Corti *et al.*, 2002).

Only a few studies provide an area-based analysis of SOC and nutrient stocks, or of TTs of SOC in bulk or density fractions of genetic horizons. The objectives of our study were: (i) to quantify the stocks and heterogeneity of SOC and nutrients in a mountainous Podzol, (ii) to determine SOC and N stocks in different density fractions, (iii) to calculate the TT of bulk soil and SOM fractions from their $\Delta^{14}C$ signatures by a steady state or non-steady model and (iv) to assess the input and accumulation of C in the organic horizons.

Material and methods

Site description

The study was performed in a mature Norway spruce forest (*Picea abies* L.) at an elevation of 770 m above sea level in the Fichtelgebirge, SE Germany (50°08'N, 11°52'E). The mean annual air temperature is 5.3°C with warm summers and cold continental winters. Mean annual precipitation is approximately 1160 mm (Foken, 2003).

The natural vegetation consists of beech (*Fagus sylvatica* L.) and silver fir (*Abies alba* Mill.) (Gerstberger *et al.*, 2002). According to the forest administration, the area was almost completely cleared between the 16th and 18th century in order to supply the mining industry with construction wood and charcoal. The region was afforested with spruce trees during the mid 19th century. Tree rings indicate a tree age of 140 years in 2008 for our study area. The patchy ground vegetation is dominated by *Deschampsia flexuosa* (L.) and *Calamagrostis villosa* (Chaix).

The Fichtelgebirge comprises large granite formations surrounded by metamorphic rock series of gneiss, mica schists and phyllites. Erosion and solifluction formed the typical rock and boulder fields. Podzolic soils developed from deeply weathered granite and are overlain with a relatively thick humus layer (Gerstberger *et al.*, 2002). Base saturations between 52 % in the EA horizon and 40 % in the Bsh horizon indicate former application of lime to counteract soil acidification (Hentschel *et al.*, 2007). Carbonates, however, were not chemically detectable in any soil horizons.

Sampling

Within an area of one hectare, nine randomly distributed (i.e. limited to between-tree and between-boulder areas) soil pits of 0.7 m x 0.7 m were dug for area-based determination of rock volume, bulk density (BD) and element contents down to the Cv horizon. The organic (Oi, Oe, Oa) and mineral (EA, Bsh, Bs, Bv) horizons were consecutively removed and separately weighed. The volume of stones was estimated by measuring the girth of the stones at different positions and their total weight. A grid of 0.7 m x 0.7 m with 10 cm x 10 cm increments was fixed over the surface of the soil pit to measure the height of each removed soil horizon.

For each horizon, large stones, coarse roots and soil material were separately weighed in the field. Subsamples of each horizon were sieved (< 2 mm) and then dried at 105°C over 48 hours to determine the gravimetric water content and the stone fraction. The bulk density

of fine earth (< 2 mm) was calculated by dividing its mass by the averaged volume of horizon minus the volume of rocks and coarse roots. The rock weight was converted to rock volume using a density of 2.45 g cm^{-3} for parent granite at the site.

Analyses

Soil pH was determined in a 0.01 M $CaCl_2$ solution (soil:solution ratio 1:2.5). Exchangeable cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}) of the Oa and mineral soil horizons were extracted with 1 M NH_4Cl solution and then analysed by ICP-OES (Jobin-Yvon Horiba Group, JY2000, USA). The amount of exchangeable H^+ was calculated from the difference between the pH-value of NH_4Cl solution and the extracts. For C and N analysis a CNS analyser (Heraeus Elementar Vario EL, Germany) was used.

Radiocarbon signatures of above-ground litter, bulk soil (including organic and mineral soil horizons) and density fractions were determined by accelerator mass spectrometry (AMS). Subsamples of 1 mg C were combusted in 6 mm sealed quartz tubes with 60 mg CuO oxidizer and 1 cm silver wire for 2 hrs at 900°C. The resulting CO_2 was purified from water and non-condensable compounds. Afterwards, CO_2 was reduced to graphite using the zinc reduction method where TiH_2 and Zn with Fe act as catalysts at 550° C for 7.5 hrs (Xu *et al.*, 2007). All preparations took place at the Department of Soil Ecology at the University of Bayreuth. The graphite targets were analysed by the Keck-CCAMS facility of University of California, Irvine, with a precision of 2-3 ‰. Radiocarbon data are expressed as $\Delta^{14}C$ (‰ deviation was from the $^{14}C/^{12}C$ ratio of oxalic acid standard in 1950). The sample has been corrected to a $\delta^{13}C$ value of -25 ‰ to account for any mass dependent fractionation effects (Stuiver & Polach, 1977).

Density fractionation of soil

Soil samples of the Oa and all mineral horizons from three randomly chosen soil pits were fractionated by density separation. Dry soil samples were dispersed in sodium polytungstate solution (SPT, Sometu, Berlin, Germany) using a similar procedure as described in detail by John *et al.* (2005). Dry soil samples (< 2 mm, 60°C) were fractionated at densities of 1.6 g cm^{-3} and 2.0 g cm^{-3}. For the Oa and EA horizon, 10 g soil and 40 ml of SPT with a density of 1.6 g cm^{-3} were gently shaken. After sedimentation, the solution was centrifuged at 5085 g for 1 h (Varifuge 3.2RS). The supernatant was filtered through 0.45 µm pre-washed cellulose-acetate-filter (Schleicher & Schuell, Germany) and the FPOM fraction < 1.6 g cm^{-3} was washed with 200 ml de-ionized water. Then the pellet was dispersed with 2.0 g cm^{-3} SPT

and 10 glass beads and was shaken for 16 hrs at 60 rpm and centrifuged at 5085 g for 1 h. The supernatant with particles < 2.0 g cm^{-3} (OPOM) was filtered and washed (200 ml distilled water) through 0.45 µm cellulose-acetate filters. The pellet thus obtained contained the mineral associated organic matter fraction > 2.0 g cm^{-3} (MAOM). To remove the salt, the pellet was washed three times with de-ionized water. For the Bsh, Bs and Bv horizons, 20 g soil and 80 ml of SPT were used. The FPOM, OPOM, MAOM fractions and the used SPT solution were freeze-dried and then finely ground with a ball mill for analyses of SOC and N contents.

Turnover time (TT) of organic carbon

The TT for SOC in each fraction was calculated from its radiocarbon signature. Following Gaudinski *et al.* (2000), we used a non-steady state model for the Oi, Oe, and Oa horizons. We assumed that the buildup of the organic layer primarily started after reforestation in 1867, but we cannot ignore the possibility that older organic matter is included in the present Oa horizon (see below). The $\Delta^{14}C$ signature of fresh spruce litter from the year 2006 (86 ± 1 ‰) was close to the $\Delta^{14}CO_2$ signature in the atmosphere (88.5 ‰) in the year 2000 (Levin *et al.*, 2008), indicating an average age of six years. Hence, this shift in the $\Delta^{14}C$ was considered in the calculation of TT.

The C input (*I*) in kg C m^{-2} a^{-1} added by litter production in each year *t* (since 1867, time of reforestration) is represented in a sigmoid equation (e.g. Böttcher & Springob, 2001; Ågren *et al.*, 2008):

$$I_t = \frac{I_{2006}}{1 + \exp\left(-\frac{t - 1900a}{15}\right)} \quad (1)$$

The term "1900a" in Equation 1 is the year of maximal increase of litter production and the value "15" describes the slope of the graph.

The SOC stock and the $\Delta^{14}C$ measured of each organic horizon in 2006 were then calculated using Equations 2 and 3 (Gaudinski *et al.*, 2000). We assumed zero initial SOC in 1867 in the Oi and Oe horizon. For the Oa horizon the model revealed an existing SOC stock between 0.5-0.9 kg C m^{-2} in 1867 differing within the three soil pits.

$$C_{2006} = C_{1867} \times e^{-k(2006-t)} + \sum_{t=1867}^{t=2006} \left(It \times e^{-k(2006-t)} \right) \quad (2)$$

where C is the SOC stock in a specific year (kg C m^{-2}), I is the annual input rate (kg C m^{-2} a^{-1}), k is the decay constant of organic matter and reciprocally proportional to TT, and t is the year since 1867.

$$F_{m(2006)} = \frac{F_{m(1867)} \times C_{1867} \times e^{-k(2006-1867)} + \sum \left(F_{atm(t-6)} \times I_t \times e^{-k(2006-t)} \right)}{C_{2006}} \quad (3)$$

where F_m is the $^{14}C/^{12}C$ ratio in the fraction pool per year normalized to oxalic acid standard and F_{atm} is the $^{14}C/^{12}C$ ratio of the atmosphere normalized to a oxalic acid standard ($\Delta^{14}CO_2$ signature of litter input in year t is equivalent to F_{atm} six years before).

From Equations 1 and 2 and the measured C stock in each organic horizon, we estimated the turnover time ($1/k$) and C input rate (I) of the organic layer. Modern $\Delta^{14}C$ has two possible TTs for each $\Delta^{14}C$ value (Trumbore, 2000). The $\Delta^{14}C$ of the Oa horizons (107-132 ‰) corresponds to TTs of either 4-7 or to 100-160 years. Given the type and thickness of the organic layer we expect longer TTs to be more realistic.

For the mineral soil horizons, we used a time-dependent, steady state model as presented in Gaudinski *et al.* (2000):

$$F_m(t) \times C(t) = I \times F_{atm}(t) + (1 - k - \lambda) \times F_m(t-1) \times C(t-1) \quad (4)$$

where λ is the radioactive decay constant for ^{14}C, and equal to 1/8267 year.

If the pool of SOC is at steady-state, then $I=kC(t)$ and $C(t) = C(t\text{-}1)$ reduce Equation 4 to:

$$F_m(t) = k \times F_{atm}(t) + (1 - k - \lambda) \times F_m(t-1) \quad (5)$$

For the values of F_{atm}, we used radiocarbon data from dendrochronologically dated wood samples (1844 to 1958) published by Stuiver *et al.* (1998) and atmospheric $\Delta^{14}CO_2$ contents after 1959 (Levin, 2008). A steady-state model was also used for the MAOM fraction of the Oa horizon because we assumed that the MAOM fraction in the Oa horizon is partly a result of biotic or abiotic mixing processes with mineral soil.

Statistics

The soil characterization was made with nine replicates and the data are presented as mean and standard deviation (Table 1). STATISTICA 6.0 was used to display the heterogeneity of SOC and N stocks between the nine soil pits (Figure 1). The density fractionation and determination of $\Delta^{14}C$ were made on three of the nine pits (Table 2, Figure 2).

Results

Variation of physical and chemical properties in soil pits

The 7-10 cm thick organic layer consisted of litter (Oi), fermented (Oe) and humified (Oa) horizons whereas the 43-60 cm thick mineral soil comprised EA, Bsh, Bs, and Bv horizons. The mean bulk density increased from 0.07 g cm^{-3} in the Oi horizon to 1.17 g cm^{-3} in the Bv horizon (Table 1). Mean volumetric rock contents increased from 7 % in the Oa horizon to 25 % in the Bv horizon with large variations among individual soil pits.

The SOC and N contents of the soil decreased with increasing depth from 45.8 % C in the Oi horizon to 1.4 % C in the Bv horizon and from 1.7 % N to 0.2 % N, respectively (Table 1). The C/N ratio ranged between 19 and 27 in the organic layer whereas C/N ratios (21-22) were almost constant in the EA, Bsh and Bs horizons. SOC stocks increased from 2.8-4.9 kg C m^{-2} in the organic layer (sum of Oi, Oe and Oa horizons) to 2.2-7.9 kg C m^{-2} in the Bv horizon (Figure 1a). Nitrogen stocks increased from 0.05-0.16 kg N m^{-2} in the organic layer to 0.17-0.51 kg N m^{-2} in the Bv horizon (Figure 1b). The total amount of SOC and N stored in the nine soil pits were 13.1-20.3 kg C m^{-2} and 0.7-1.1 kg N m^{-2}, where the organic layer contributed 19–35 % to total SOC and 5–14 % to total N.

C and N in density fractions

The mean recovery of total soil mass after density fractionation varied between 92 % in the Oa and 98 % in the Bv horizon (data not shown). These losses coincided with the mean recovery of SOC between 90 and 96 % (Figure 2a). Some SOC was lost in the particulate fraction during density fractionation but 2–6 % of SOC was dissolved in the SPT solution. The mean recovery of N was smaller and ranged between 84 and 99 % (Figure 2b).

Consistently throughout the three soil pits, SOC contents ranged between 17-38 % C in the FPOM and OPOM fraction, whereas the MAOM fraction contained no more than 2.7 % C (data not shown). In the Oa and EA horizon, the major part of SOC was associated with FPOM (Oa = 1.6 kg C m^{-2} and EA = 1.1 kg C m^{-2}), whereas MAOM dominated the Bsh, Bs and Bv horizons with 0.8-2.2 kg C m^{-2} (Figure 2). The SOC stock of the OPOM fraction was the largest in the EA horizon (0.8 kg C m^{-2}) followed by Bv (0.6 kg C m^{-2}), Bsh (0.6 kg C m^{-2}), Oa (0.4 kg C m^{-2}) and Bs horizons (0.3 kg C m^{-2}). Overall, the soil stored 2.7-5.2 kg C m^{-2}, 2.3-3.3 kg C m^{-2} and 4.0-4.9 kg C m^{-2} in the FPOM, OPOM and MAOM fractions, respectively.

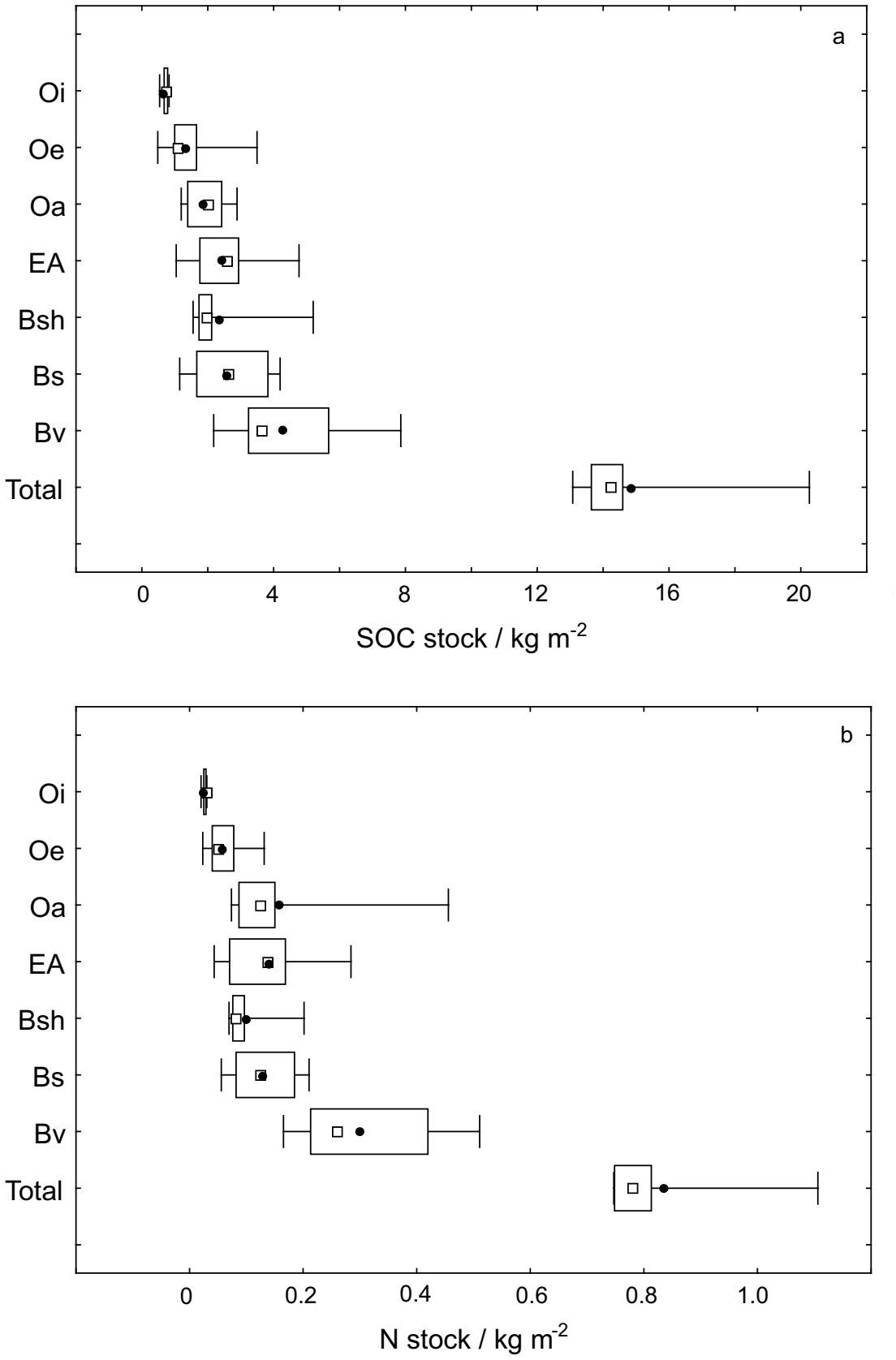

Figure 1 Box plots representing soil heterogeneity of (a) SOC and (b) N stocks in different genetic horizons of nine soil pits. The open square within the box marks the median, the black circle the mean and the boundaries of the box indicate the 25th and 75th percentile. Whiskers indicate the minimum and maximum SOC and N stocks.

Table 1 Mean thickness, bulk density of fine earth (BD), volumetric rock fraction (RF), amount of fine earth, pH ($CaCl_2$), organic C and total N contents, C/N ratio, and stocks of exchangeable cations in genetic horizons of a Norway spruce soil at the Fichtelgebirge. Numbers in parentheses are standard deviations of the means (n = 9).

	Thickness	BD	RF	Fine earth		C	N		Stock ($g\ m^{-2}$)						
Horizon	(cm)	($g\ cm^{-3}$)	(Vol.%)	($kg\ m^{-2}$)	pH	(%)	(%)	C/N	H^+	Na^+	K^+	Mg^{2+}	Ca^{2+}	Al^{3+}	Fe^{3+}
Oi	2.1 (0.3)	0.07 (0.01)				45.8 (2.7)	1.7 (0.2)	27 (2.3)							
Oe	2.2 (0.7)	0.15 (0.05)				42.1 (18.9)	1.8 (0.6)	22 (2.0)							
Oa	4.9 (1.5)	0.25 (0.09)	7 (4)	11.3 (2.9)	3.3 (0.2)	21.2 (6.9)	1.1 (0.3)	19 (1.7)	0.5 (0.7)	0.9 (1.1)	1.9 (1.1)	1.3 (0.7)	41.2 (44.8)	6.4 (4.3)	1.1 (1.0)
Ea	5.2 (2.3)	0.60 (0.05)	11 (7)	28.8 (13.1)	3.4 (0.2)	8.3 (2.6)	0.4 (0.2)	21 (3.4)	0.4 (0.3)	1.0 (0.8)	2.1 (1.3)	1.4 (1.1)	50.4 (53.1)	15.4 (9.1)	1.4 (0.7)
Bsh	5.3 (1.8)	0.75 (0.04)	15 (5)	40.1 (17.1)	3.6 (0.2)	6.0 (1.2)	0.3 (0.1)	22 (2.2)	0.4 (0.3)	1.4 (0.8)	2.0 (0.6)	1.3 (1.1)	48.1 (37.2)	32.7 (19.3)	4.6 (3.1)
Bs	11.4 (4.6)	0.79 (0.06)	18 (7)	78.5 (32.6)	3.8 (0.1)	3.6 (0.8)	0.2 (0.0)	21 (1.2)	0.2 (0.1)	1.6 (0.8)	2.8 (1.0)	1.0 (0.6)	42.8 (39.0)	59.4 (27.2)	3.0 (2.4)
Bv	30.5 (9.1)	1.17 (0.09)	25 (9)	292 (102.3)	4.2 (0.1)	1.4 (0.5)	0.2 (0.3)	8 (1.2)	0.2 (0.1)	18.1 (17.7)	10.8 (4.6)	0.8 (0.5)	21.7 (14.5)	115 (56.3)	0.7 (0.5)
Sum	59.8 (5.7)														

Table 2 Radiocarbon signature of bulk soil and density fractions of three pits expressed in ‰. The turnover times expressed in years (given in parentheses) were calculated from the radiocarbon signature with a steady-state or non-steady-state model (Gaudinski, et al. 2000). Two turnover times were calculated for bulk soil and density fractions with modern radiocarbon signatures.

	Pit 1				Pit 2				Pit 3			
Horizon	Bulk	FPOM	OPOM	MAOM	Bulk	FPOM	OPOM	MAOM	Bulk	FPOM	OPOM	MAOM
Oi	105.1 (3)*				121 (5)*				115.2 (5)*			
Oe	157.8 (9/90)*				179 (10/50)*				147.6 (8/120)*			
Oa	132 (7/100)*	150.9 (9/90)*	70.4 (120)*	38 (180)	117.8 (6/140)*	108.0 (4/160)*	28.4 (240)*	-25.9 (400)	107.1 (4/160)*	124.5 (6/140)*	48.8 (170)*	28.1 (210)
EA	91.6 (100)	119.8 (6/70)	43.7 (170)	12.1 (260)	22.5 (230)	34.7 (190)	-2.5 (320)	-13.4 (370)	-45 (570)	-31.9 (480)	-64.3 (710)	-48 (590)
Bsh	-7.9 (350)	25.3 (220)	-41 (540)	-28.4 (460)	-4.9 (330)	13.8 (250)	-27.6 (450)	-16.9 (390)	-28.7 (460)	-26.9 (450)	1.2 (300)	-20.4 (410)
Bs	-56.9 (650)	-95.2 (970)	-138.9 (1390)	-48.1 (590)	-75.3 (800)	-31 (480)	-149.3 (1500)	-63.9 (710)	-57.3 (660)	-120 (1200)	-124.4 (1250)	-43.8 (560)
Bv	-156.2 (1580)	-66.8 (730)	-182.9 (1880)	-207.2 (2170)	-155.6 (1570)	6.8 (280)	-130.7 (1310)	-180.2 (1850)	-124.4 (1250)	-30.2 (470)	-106.7 (1080)	-141.5 (1420)

* A non-steady-state model was used.

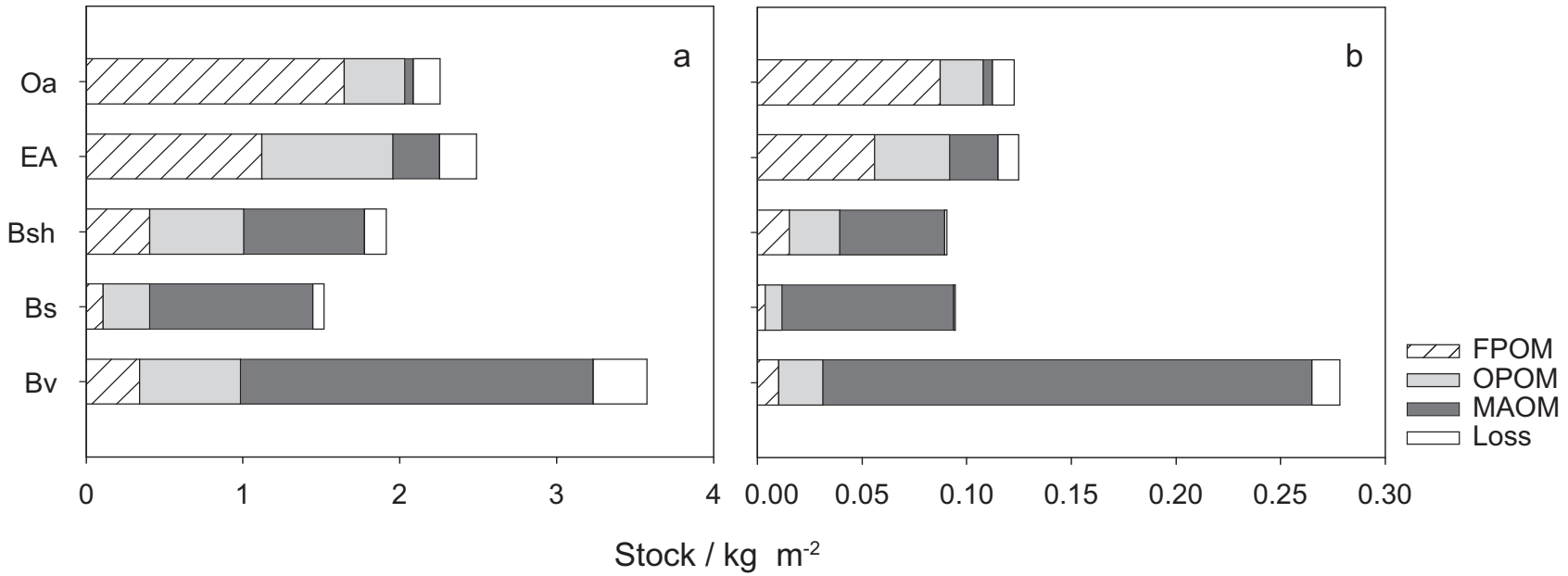

Figure 2 Stocks of SOC (a) and total N (b) in the three density fractions (FPOM, OPOM, MAOM) in genetic soil horizons from three soil pits. 'Loss' indicates the amount of organic matter lost during density fractionation.

Table 3 SOC stock, turnover time and annual C input calculated with a non-steady-state model of the organic layer of three soil pits. SOC accumulation was calculated by difference in modelled SOC stock within last 10 years (see Figure 4b). For the Oa horizon, the model revealed an existing carbon pool of 0.5-0.9 kg C m^{-2} in 1867. Turnover time and C input given in parenthesis result from either $^{14}CO_2$ of the atmosphere fixed before or after the ^{14}C-bomb peak in 1965 by the forest ecosystem (see Figure 4c).

Horizon	SOC stock /kg C m^{-2}			Turnover time /a			C input /g C m^{-2} a^{-1}			SOC accumulation /g C m^{-2} a^{-1}		
	Pit 1	Pit 2	Pit 3	Pit 1	Pit 2	Pit 3	Pit 1	Pit 2	Pit 3	Pit 1	Pit 2	Pit 3
O_i	0.68	0.77	0.78	3	5	5	180	130	150	<0.1	<0.1	<0.1
O_e	0.78	1.09	1.72	9/(90)	10/(50)	8/(120)	80/(10)	80/(30)	200/(30)	0.2	0.3	0.3
O_a	1.48	1.89	0.90	(7)/100	(5)/140	(4)/160	(210)/20	(270)/21	(220)/9	5.5	7.5	3.5
O_a (FPOM)	0.87	1.60	0.61	(9)/90	(4)/160	(6)/140	(100)/14	(380)/16	(97)/7	4.7	6.2	3

The N stock in the FPOM fraction decreased with depth from 0.09 kg N m^{-2} in the Oa horizon to 0.01 kg N m^{-2} in the Bv horizon (Figure 2b). In the B horizons, the MAOM fraction yielded the largest N stocks, contributing 74 to 83 % of total N in the respective horizon. The amount of N stored in the OPOM fraction varied between 0.01 and 0.04 kg N m^{-2} with 9-16 % of the total N in the soil.

$\Delta^{14}C$ signatures and turnover times of soil organic C

Mean radiocarbon signatures indicate modern C in litterfall (87±1 ‰), in the Oi (114±8 ‰) and Oe (161±16 ‰) horizons, but a mixture of modern and pre-bomb C in the Oa horizon (119±12 ‰) (Figure 3). The EA horizon had a large range in $\Delta^{14}C$ signature from -45 to 92 ‰, indicating that considerable amounts of modern C were incorporated in some EA horizons. Within the B horizons, the $\Delta^{14}C$ signature continuously decreased from -14 ‰ to -145 ‰. The vertical decrease in the $\Delta^{14}C$ signature below the Oi horizon coincides with the decrease in the SOC content.

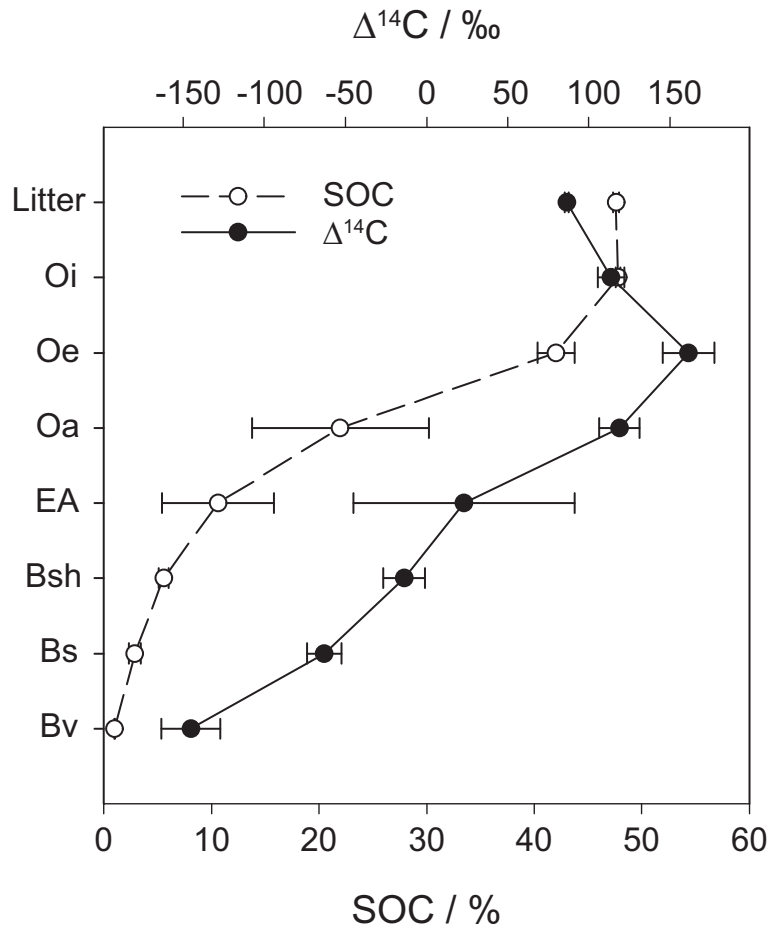

Figure 3 Vertical pattern of $\Delta^{14}C$ (‰) and total SOC (%) in litter, organic and mineral soil horizons. Error bars represent the standard deviation of the mean (n = 3).

In Pit 1 and 2, the TT of SOC in bulk soil increased consistently with soil depth from the organic horizons to the Bv horizon (Table 2). By contrast, Pit 3 had a less negative $\Delta^{14}C$ signature in Bsh (-28.7 ‰) compared with the EA horizon (-45.0 ‰), resulting in a difference of 110 years in TT. The shorter TT of bulk SOC indicates that the Bsh horizon accumulated more 'young' C than the Ea horizon.

Similar to the bulk soil, $\Delta^{14}C$ signatures of density fractions generally decreased with soil depth in the order FPOM>OPOM>MAOM (Table 2). One exception is the FPOM fraction of the Bv horizon that had consistently younger SOC (6.8 to -66.8 ‰) with faster TT than the respective FPOM fraction of the overlaying Bs horizon (-31 to -120 ‰). There was either an input of 'young' SOC in the FPOM fraction of the Bv horizon or reversely an input of 'old' SOC or stabilization mechanisms in the FPOM fraction of the Bs horizon. Another exception is the reverse order of $\Delta^{14}C$ signatures and TT in the OPOM and MAOM fractions of the Bsh (except in Pit 3) and the Bs horizon. In these horizons, MAOM had faster TT (390-710 years) compared with the OPOM fraction (450-1500 years). The shift in the TT of the MAOM fraction from the Bs horizon to the Bv horizon was relatively large in all three soil pits.

The isotopic balance approach revealed a wide spectrum for the lost fraction ranging from modern SOC with mean $\Delta^{14}C$ of 477 ‰ to relatively old SOC (-201 ‰) (data not shown). There is no systematic pattern with soil depth or for specific soil horizons.

C input and storage in organic horizon

The annual C input to organic horizons and the FPOM fractions of the Oa horizon were calculated from the non-steady-state model (Equation 2) and using the respective SOC stock and TT (Table 3). Assuming sigmoid increase of litter input (Equation 1), the estimated C input in the Oi horizon by aboveground litter varied between 130 and 180 g C m^{-2} a^{-1} in 2006. C input rates in the Oe (80 g C m^{-2} a^{-1}) and Oa (20 and 21 g C m^{-2} a^{-1}) horizons were similar in Pit 1 and 2. For the Oe horizon of Pit 3, we calculated (with a TT of 8 years) a much larger C input of 200 g C m^{-2} a^{-1}, which reflects the large C stock of 1.7 kg C m^{-2}. Here, the C input in the Oe horizon is greater than in the Oi horizon (150 g C m^{-2} a^{-1}). Again, Pit 3 differs with respect to the C input of 9 g C m^{-2} a^{-1} in the Oa horizon.

The modelled accumulation of SOC indicates that the Oi and Oe horizons are approaching a steady-state with C accumulation smaller than 0.3 g C m^{-2} a^{-1} in the last 10 years (e.g. Pit 2, Figure 4). The Oa horizon accumulated small amounts of SOC between 3.5 and 7.5 g C m^{-2} a^{-1} (Table 3), where most of that SOC was accumulated in the FPOM fraction (3.0-6.2 g C m^{-2} a^{-1}).

Discussion

Heterogeneity of soil chemical properties

The Podzol at our study site contained 13.1-20.3 SOC kg m^{-2} and 0.7-1.1 N kg m^{-2} down to a mean mineral soil depth of 60 cm (Figure 1). These values are smaller than the mean SOC (29.6 kg m^{-2}) and N stock (1.96 kg N m^{-2}) in the top 1 m of European Podzol soils (Batjes, 2002). Even small SOC and N contents in the subsoil may considerably contribute to total stocks. Several authors (e.g., Canary *et al.*, 2000; Harrison *et al.*, 2003) have emphasized that soils should be sampled to a maximum depth for accurate estimates of total SOC and N stocks. However, big rocks did not allow extending the depth in soil pits, and thus, total SOC and N stocks are relatively small at our study site.

Large amounts of SOC (19-35 % of total) and N (5-14 % of total) highlight the importance of the organic layer as SOC and N reservoirs in Podzols. Under coniferous forests, Podzols may accumulate large amounts of SOM in the organic layer within decades, making it vulnerable to climate change and other disturbances. An accurate, area-based estimate to detect changes of SOC and N stocks is hampered by the enormous spatial heterogeneity. In our study, varying thickness (7-10 cm) was the main reason for the large heterogeneity in C and N stock of the organic layer whereas bulk density, SOC and N contents were less variable.

The mineral soil down to 60 cm stores more SOC and N at our site than the organic layer. Because of slow TTs (see below), changes in the SOC stocks are generally small unless strong disturbances of the soil structure accelerate the decay of SOM. Changes in the N stock, however, could possibly be much faster in forests with large atmospheric N deposition. In view of climatic or other environmental changes, SOC and N stocks of mineral horizons are required for better understanding of C and N cycling in soils. At our site, the Bv horizon stored approximately twice as much SOC and N than the EA, Bsh and Bs horizons. The large heterogeneity of SOC and N stocks in the whole mineral soil is attributed to differences in the thickness (43-60 cm), rock fraction (12-29 % by volume) and the amount of fine earth (312-512 kg m^{-2}) among the soil pits.

SOC and N in density fractions

The portion of the FPOM fraction decreases and the portion of the MAOM fraction to total SOC stock increased with soil depth, whereas the OPOM fraction was almost evenly distributed throughout the soil pits (Figure 2). Large FPOM fractions in the Oa and EA

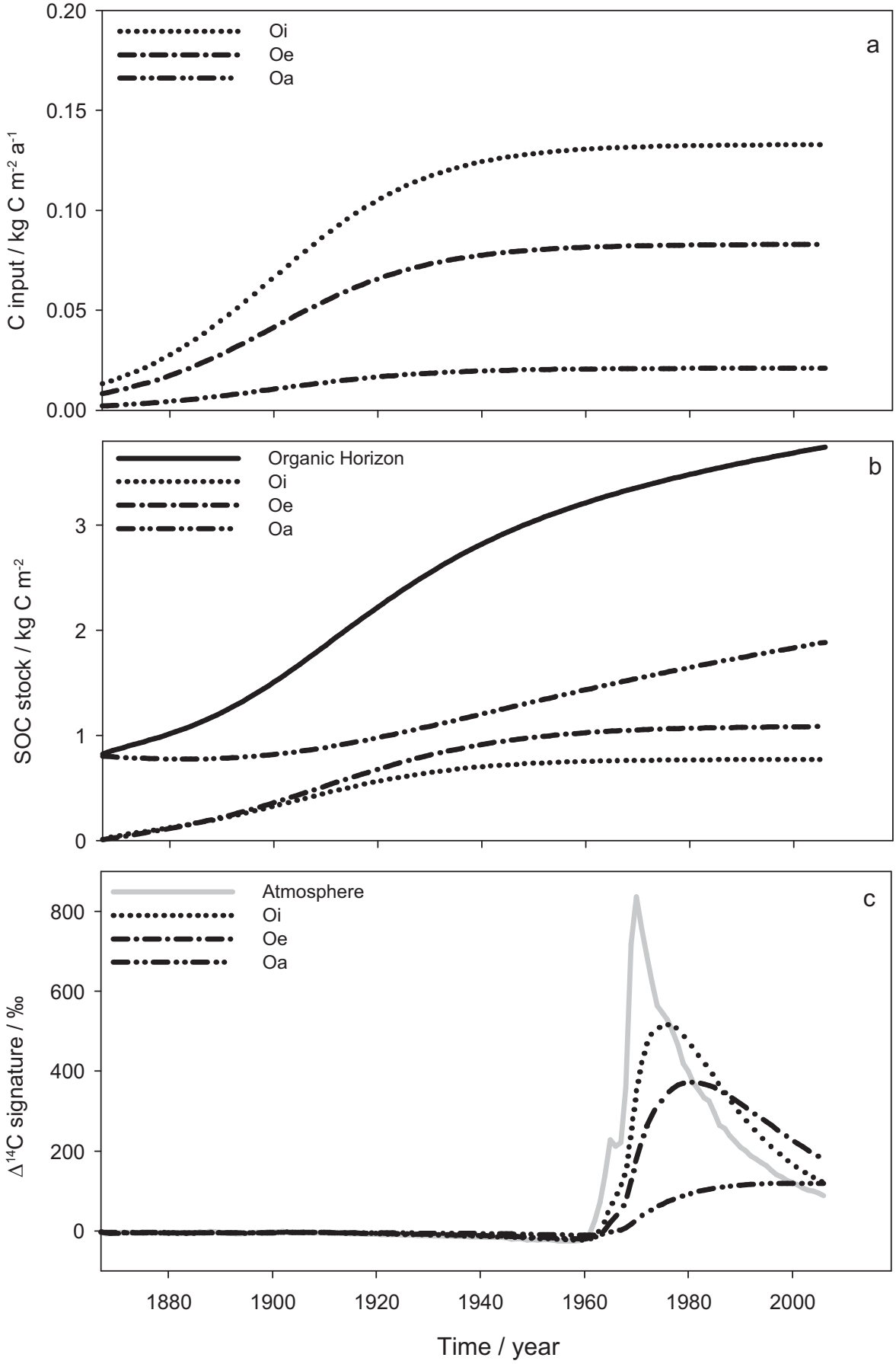

Figure 4 Carbon inputs from litter (a), SOC stocks (b), and ^{14}C signature of the atmosphere and of SOC (Pit 2 only) (c) in organic horizons from 1867 to 2006 as estimated from a non-steady state model.

horizon point to low rates of degradability of spruce litter and reduced microbial activitycompared with other litter types and less acid soils (John *et al.*, 2005). Provided that the FPOM fraction of 0.7-1.1 kg C m^{-2} (9-16 %) and 0.02-0.04 kg N m^{-2} (5-8 %) in the B horizons is accessible for microbial attack, the soil has a large potential for C and N losses. However, the slow TT of FPOM suggests that it contains not only fresh and non-decomposed material, but also charred plant debris (black carbon) as identified by Marschner *et al.* (2008). 'Recalcitrant' compounds such as lignin, lipids, and their derivates could constitute a considerable proportion of the FPOM fraction although their TTs are much faster than previously thought (Marschner *et al.* (2008). Another mechanism, the stabilization of DOC by precipitation with dissolved aluminium (Scheel *et al.*, 2007) could possibly contribute to the slow TT of the FPOM fraction from the mineral soil. Our results contradict the concept that the FPOM fraction corresponds to the active SOC pool with TT < 10 years.

The N content and C/N ratio of SOM fractions may play an important role in their stability. Decreasing C/N ratios in the order OPOM>FPOM>MAOM (not shown) are in accordance with the observation by Golchin *et al.* (1994). The large N content of MAOM is primarily related to the advanced degradation of organic C compounds. However, a N fertilization experiment suggests that 'newly' added N is partly retained in slow SOM pools, enhancing thereby its stability against microbial decay (Hagedorn *et al.*, 2003). If this stabilization mechanism is relevant, then the size and TT of SOM fractions should have been altered under the large N deposition rate at our study site during recent decades.

The Oa horizon is generally not considered for density fractionation although this horizon often contains a mineral fraction. Here, MAOM contributes 33 % to total mass in the Oa horizon, but the SOC stock of 2.3 % was relatively small. Despite the small SOC stock in the OPOM and MAOM fractions, their $\Delta^{14}C$ signatures influence the TT of the Oa horizon (Table 2). In the case of Pit 2, however, all three density fractions had smaller $\Delta^{14}C$ signatures than the bulk Oa horizon. A non-characterized fraction of SOC and N was lost by the density fractionation either as particulate or as dissolved forms in SPT solution. The mass balance approach implied that the missing fraction varied from modern SOC with mean $\Delta^{14}C$ signature of 477 ‰ to relatively old SOC (-201 ‰), representing a SOC stock of approximately 0.9-1.3 kg C m^{-2} down to 60 cm depth. Crow *et al.* (2007) found extractable young organic matter for deciduous forest soil and assumed that less degraded substrates were removed during density fractionation. Hence, the lost fraction seems to play an important role in the turnover of SOM in forest soils.

Turnover time (TT), input and accumulation of SOC in organic horizons

The estimated annual C input in the Oi horizon by aboveground litterfall varied between 130 and 180 g C m^{-2} a^{-1} in 2006. A mean annual litter input of 107 g C m^{-2} a^{-1} (assuming a C content of 50 %) was measured in an adjacent Norway spruce stand of the same age (Berg, 2004). This input rate, however, does not include branches, twigs, cones and ground vegetation which may contribute considerably to total above-ground litter input.

The C input in the Oe and Oa horizon comprises the transfer of partly decomposed and humified aboveground litter from the Oi or Oe horizon, respectively, and the input of root litter. In case of the Oe and Oa horizon, root litter input probably increased with the build-up of these horizons. The present input of 200 g C m^{-2} a^{-1} in the Oe horizon of Pit 3 can be explained by the contribution from root litter, cones or twigs to the buildup of this relatively large SOC stock. In fact, total mass of live coarse roots and cones were 2.5 to 4.5 times greater in Pit 3 than in Pit 1 and 2.

In the first decades of afforestration, SOC stocks increased rapidly in the Oi and Oe horizons and reached nearly steady-state after 80-100 years (Figure 4). In the past decade, the rate of SOC accumulation was smaller than 0.1 g C m^{-2} a^{-1} for the Oi horizon and approximately 0.3 g C m^{-2} a^{-1} for the Oe horizon. Fast TT of SOC (3-10 years) highlights the potential of these horizons to respond rapidly to an increase in temperature. It has been demonstrated that along an elevation gradient, TT of SOC in the topsoil is controlled by temperature (Trumbore *et al.*, 1996).

The radiocarbon signature indicates that the Oa horizon was partly formed from SOC before afforestration. In our model, the SOC stock did not increase during the first 30 years after afforestration which we attribute to the delay in the production and transfer of humified organic matter in the Oe horizon. In the present Oa horizon, the turnover rate (100-160 years) is slow enough to allow the annual C input to result in a SOC accumulation of 3.5-7.5 g C m^{-2} a^{-1}. Almost all of this C input is accumulating in the FPOM fraction whereas the OPOM and MAOM fractions yield SOC close to a steady-state condition.

Overall, SOC accumulation rate of the organic layer (3.8-7.8 g C m^{-2} a^{-1}) is in good agreement with estimates of 2-7 g C m^{-2} a^{-1} for a mixed deciduous forest (Gaudinski *et al.*, 2000). Slightly larger accumulation rates of 12-13 g C m^{-2} a^{-1} were estimated for coniferous soils in Sweden (Ågren *et al.*, 2008). One reason for small accumulation rates at our study site could be the application of lime to the soil surface, which is often reported to improve soil conditions and thus to increase the mineralization of SOM (e.g. Persson, 1989; Fuentes *et al.*, 2006).

Turnover time (TT) of SOC in mineral soil fractions

The TT of SOC in bulk samples increased with soil depth and exhibited little variation among the three soil pits. As with Pits 1 and 2, many studies found increasing TT with increasing soil depth and density (e.g., Bol *et al.*, 1999; Trumbore, 2000; Gaudinski *et al.*, 2000; Certini *et al.*, 2004; Eusterhues *et al.*, 2007), pointing to increasing stabilization of SOC by minerals at greater depth.

The FPOM, OPOM and MAOM fractions indicate the existence of C pools with different turnover times which are associated with the degree of degradation and humification (Baisden *et al.*, 2002: John *et al.*, 2005). For the EA and Bv horizon, TT followed a typical pattern in the order FPOM<OPOM<MAOM. In the Bsh and Bs horizon, however, the MAOM fraction had faster TT than the OPOM fraction. We attribute this finding to the sorption of DOC with a less negative $\Delta^{14}C$ signature by Fe and Al oxides/hydroxides in the MAOM fraction. Sorption of DOC in spodic horizons is a typical process of Podzols and largely contributes to the SOC stock of the Bsh and Bs horizon in the long run. The intrinsic TT of the MAOM fraction in these spodic horizons is probably much faster as predicted by our model approach. Hentschel *et al.* (2009) reported radiocarbon signatures of -65 to +38 ‰ for DOC in the soil solution below the Oa horizon at our study site. The radiocarbon signatures of DOC decreased to between -25 and -265 ‰ at 90 cm mineral soil depth and these are within the range of the FPOM fraction. The time span between the formation of DOC from 'old' particulate SOM and sorption by the soil matrix in the Bsh and Bs horizon could be relatively short.

A substantial shift in the $\Delta^{14}C$ signature and TT of the MAOM fraction was observed from the Bs to the Bv horizon in all three soil pits. Again, this shift supports the sorption of DOC in the MAOM fraction of the Bsh and Bs horizon. Turnover times of 1080-2170 years in the OPOM and MAOM fractions of the Bv horizon may be explained by small input of particulate and dissolved organic matter and strong physical and chemical stabilization.

Conclusion

- The present soil was a small sink for atmospheric CO_2 in the order of 4-8 g C m^{-2} a^{-1} during the past 10 years and might be a net CO_2 sink of similar order in the near future at similar boundary conditions. Most of SOC accumulated in the FPOM fraction of the Oa horizon whereas other organic and mineral soil horizons were in, or close to, steady-state. Sorption of DOC in the Bsh and Bs horizon seems to affect the radiocarbon signature of

the MAOM fraction, but turnover times of 390-710 years mask the true contribution to accumulation of SOC in these spodic horizons.

- The C and N stock of the organic layer is vulnerable to changes in climate conditions or other disturbances. The turnover time of non-stabilized SOM will probably decrease with increasing temperature and turn the soil from a small sink to a transient source.
- The density fractionation method revealed some uncertainties. Firstly, a portion of SOM was either lost as particulate or dissolved SOM in the order of up to 10 % (SOC) and 16 % (N). This lost fraction is generally not characterized, but might participate in the C and N cycle of soils. Secondly, the FPOM fraction, often associated with the active pool of SOC, had slow turnover times on the decadal-centennial time scale in all soil horizons. It seems that recalcitrance of SOM or stabilization processes cause a slow turnover of the FPOM fraction in this forest soil.
- The spatial variation of SOC and total N stocks in genetic horizons and density fractions was relatively large in this rocky forest soil, highlighting the importance of representative sampling approaches. The radiocarbon signatures of bulk soil and density fractions exhibited a small variation among the three pits, except the EA horizon where differences in bioturbation have apparently affected the spatial heterogeneity. Even in heterogeneous soils, measurements of the $\Delta^{14}C$ signature provide a powerful tool for the assessment of C accumulation in forest soils.

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PART B:

Carbon dynamics in a temperate minerotrophic fen

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Abstract

Globally, peatlands represent important C storage pools that are supposed to be vulnerable to climate change. Understanding of C dynamics in peatland soils is relevant for predicting the effects of changing boundary conditions on peatland C pools. Here, we present the C stocks and turnover rates of vegetation and soil organic matter (SOM) in a minerotrophic temperate fen in South-eastern Germany. In order to model soil C balance in the peat body we distinguished four C pools: (1) Aboveground biomass (relevant as input pool only), (2) root biomass (comprising live and intact dead roots), (3) surface peat soil organic matter (SOM) between 0-15 cm (defined by the occurrence of bomb ^{14}C), and (4) deeper peat SOM (defined by the predominance of pre-bomb ^{14}C). We calculated stocks of these pools and input and output fluxes. For aboveground biomass, we measured a stock of 173 g C m^{-2}. Aboveground vegetation at the site completely dies off at the end of the year, so annual input and output fluxes were equal to stock. Root biomass C stock was found to be significantly higher at this fen, comprising 1315 g C m^{-2}. We found high gross fluxes for the root biomass stock (input 242 versus output 266 g C m^{-2} a^{-1}) and calculated a negative net C balance. Gross fluxes of the surface layer SOM stock were also considerably high (total input 308 versus total output 313 g C m^{-2} a^{-1}), and net C balance was negative. Gross C fluxes of deeper layers were comparatively small (input 6.1 versus output 3.3 g C m^{-2} a^{-1}), but indicated a small net C accumulation. Total SOM C net balance for the whole peat profile was slightly negative (-2 g C m^{-2} a^{-1}), total net soil C fluxes (root biomass + SOM) were strongly negative (-27 g C m^{-2} a^{-1}). Thus, this fen site has been identified as a net source of C, indicating a disturbance of boundary conditions. Due to fast turnover of the root biomass and surface

SOM stock, this conclusion is only valid on a decadal timescale, so we can neither determine how long the fen has been a net C source nor how long it will continue to be one.

Keywords: Soil carbon balance, peat accumulation, radiocarbon, SOM turnover, root turnover

Introduction

Peatlands cover only approximately 3.5% of the earth's surface (Gorham 1991), but form an important carbon (C) store, especially in the northern hemisphere. Correcting earlier calculations made by Gorham (1991), Turunen et al. (2002) estimated global C stores in peatlands between 270-370 Pg (10^{15} g) of C, which would account for around 20-25% of the 1500 Pg C stored in soils worldwide (Schlesinger and Andrews 2000). These high amounts of C stored in peatlands result from an imbalance between input and decomposition of organic matter in peatlands. Due to high water tables, decomposition in peatland soils often is limited by oxygen availability. Since the retreat of the last ice age, this has led to the formation of thick peat bodies. The high amounts of C stored in peatlands has put them into the focus of interest under the impression of climate change scenarios predicting altered water tables and temperature increase. The question is whether peatlands, thousands of years after beginning of their formation due to the retreat of the last ice age, continue to accumulate C under current climatic conditions, and if so at which rates.

Radiocarbon (^{14}C) has proven to be a powerful tool to investigate ecosystem C dynamics that has been applied in various studies (Trumbore et al. 1995, Trumbore and Harden 1997, Gaudinski et al. 2000, Schulze et al. 2009). Natural ^{14}C can be used to determine soil C dynamics on timescales of centuries to millenia, and therefore is a suitable tool to examine the C balance of deep organic layers in peatlands. Atmospheric nuclear weapon testing in the 1950s produced high amounts of 'bomb ^{14}C', nearly doubling the atmospheric ^{14}C content (Levin et al. 2008). Due to combustion of ^{14}C-free fossil fuels and mixing of the atmospheric ^{14}C pool with terrestrial and marine C pools, the atmospheric ^{14}C content continuously decreased since the end of the nuclear weapon testing. Incorporation of atmospheric CO_2 (with the specific ^{14}C signature of the year of incorporation) into biomass results in a specific isotopic 'labeling' of annual litter input. Hence, bomb ^{14}C can be used to constrain soil C balance over the last few decades. Using these two tools, total C accumulation can be resolved into the balance of short- and long-term-averaged inputs and decomposition. Here, we present the soil C balance of a temperate fen site (*Schlöppnerbrunnen*) in southern Germany. This site has been used in a number of other studies (Knorr et al. 2009; Muhr et al. 2009; Otieno et al. 2009; Reiche et al. 2009) concerning different aspects of the C cycle.

Material and Methods

Site description

The *Schlöppnerbrunnen* fen site is located in the *Lehstenbach* catchment (*Fichtelgebirge*, Northeastern Bavaria, Germany, 50°07'54''N, 11°52'51''E). The site is characterized as a temperate minerotrophic fen covering an area of 0.8 ha at an elevation of ca. 750 m a.s.l. The soil is a Histosol on granite bedrock covered mainly by *Molinia caerulea* (L. Moench), *Nardus stricta* (L.), *Agrostis canina* (L.), *Carex rostrata* (Stokes) and *Eriophorum vaginatum* (L.). Mean annual temperature (1995-2007) is 6.3 ± 0.9 °C and mean annual precipitation (1995-2007) is 1020 ± 203 mm a^{-1} (Knorr 2009). The site features a slope from NNE to SSW and groundwater flows slowly through the site parallel to this slope (Paul et al. 2006). Since the last deglaciation, a peat body with a thickness between 40-70 cm has accumulated. The site features a water table gradient, with the north-western part being waterlogged more often than the south-eastern part. A ditch of unknown origin and history runs through the site parallel to the slope.

Definition of our system

In this study we were interested in the size and dynamics of C storage pools in the soil of a temperate fen. A major issue was the question whether the soil under current boundary conditions is a source or a sink of C. To answer this question, we defined relevant C stocks and fluxes based on the following considerations: (i) All C that is stabilized in the soil initially enters the system *via* photosynthetic assimilation; (ii) only assimilates that are used to form biomass (above- or below-ground) can result in stabilization of C in the soil, metabolic respiration of living biomass represents a direct recirculation of assimilates to the atmosphere; (iii) decomposition of aboveground litter occurs fast and the total aboveground litter pool can be assumed to be in steady state; (iv) transfer of C from root biomass (live and dead) to soil organic matter (SOM) can occur with a marked delay due to limited decomposition rates within the peat; (v) changing boundary conditions can result in non-steady state of the below-ground litter pool; (vi) input into SOM pools occurs *via* litter decomposition and due to up-down transfer between SOM pools within the peat profile. A schematic overview over the C stocks and fluxes we distinguished to calculate the soil C balance of the peat body of this fen site is presented in Figure 1.

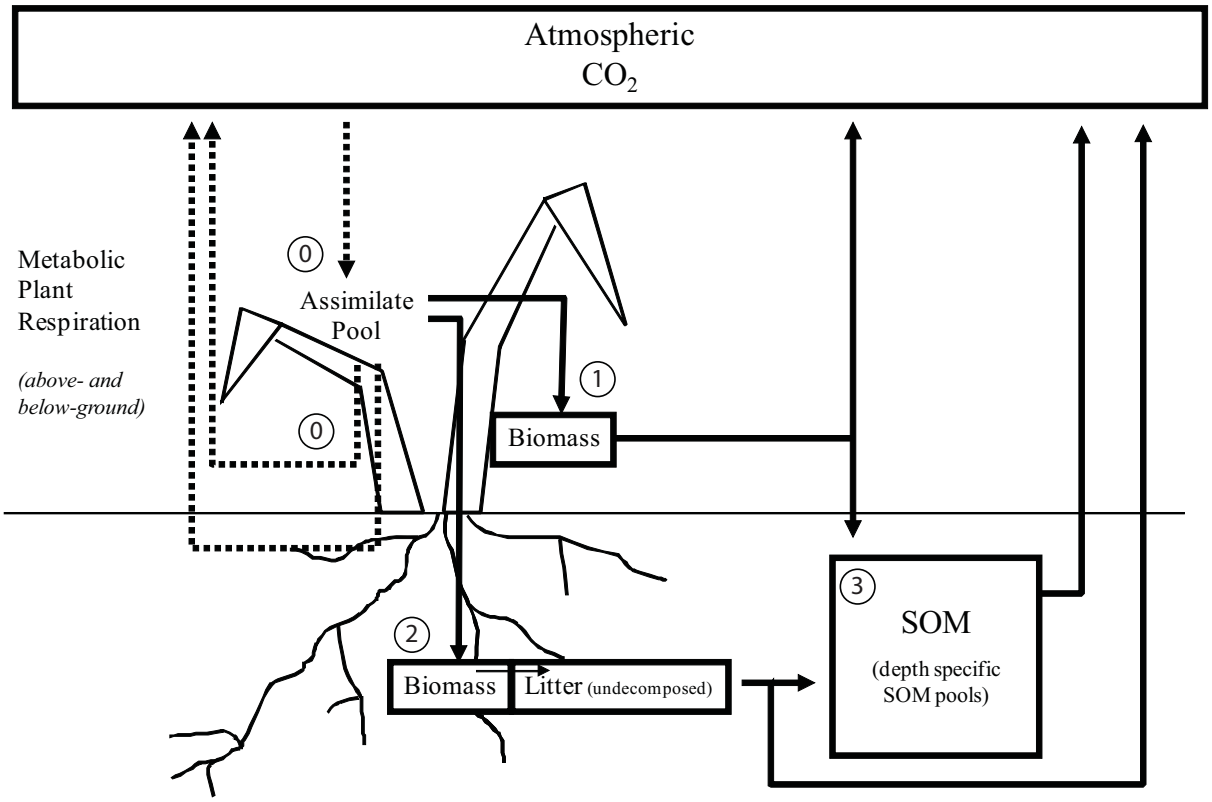

Figure 1. Schematic overview to illustrate the C pools we distinguished when modelling soil C balance in this study. We measured C stocks fluxes of (①) aboveground biomass, (②) below-ground biomass, and (③) depth-specific SOM pools. We did not quantify total C uptake by assimilation and respiration resulting from living biomass (⓪), as these were regarded irrelevant for the soil C balance. With our approaches, we can determine output fluxes from C stock pools, but we can not distinguish which proportion of these output fluxes is redirected to other soil C pools and which proportion is lost from the system.

Quantifying C stocks and fluxes

We combined a variety of different methods to quantify C stocks in aboveground and below-ground biomass and in soil organic matter (SOM), annual litter production from above- and below-ground biomass, and C input and output fluxes into SOM. Combining all results we quantified the soil C balance of the *Schlöppnerbrunnen* fen. Table 1 gives an overview over the different methods used to quantify C stocks fluxes.

Biomass

Aboveground biomass was determined by the harvesting method on a total of 12 plots, each 39 x 39 cm^2. These 12 plots were located in the immediate vicinity of the soil coring locations. Harvesting took place in September of 2007 at the end of the vegetation period. We combined this data set with biomass data for the site from Otieno et al. (2009). This second data set comprises monthly measurements from May to October of 2007. All aboveground biomass dies off at the end of the vegetation period, so annual aboveground litter input was

Table 1. Methods applied in this study to quantify C stocks and turnover.

Variable	Method
C Stock	
Aboveground biomass	Harvesting
Below-ground biomass	Harvesting (depth specific) (data from Otieno et al. 2009)
SOM	Soil coring (depth specific, using bulk density and organic C content)
Turnover	
Aboveground biomass	Complete turnover of annual production
Below-ground biomass	Non-steady state ^{14}C based model (Gaudinski et al. 2000)
SOM, surface layers	Non-steady state ^{14}C based model (Gaudinski et al. 2000)
SOM, deeper layers	Equation (2) fit to cumulative C stock versus time plot (Trumbore 1999)

assumed to be equal to annual biomass production. The C content of aboveground biomass was assumed 0.5 of total dry biomass.

Below-ground biomass data (roots) was taken from Otieno et al. (2009), who measured root biomass in 2007 down to a depth of 30 cm at the *Schlöppnerbrunnen* fen site. We measured the mean radiocarbon signature of structural C of the root biomass. As it was impossible to distinguish intact (undecomposed) dead and live roots at the site, we defined one universal root C pool comprising live roots and undecomposed root litter (decomposed root litter is comprised in the SOM pool). Root litter in peatlands can feature long turnover times due to adverse decomposition conditions. Thus, we used the same ^{14}C based non-steady accumulation model to describe this root biomass pool that was used to describe the fast-cycling surface SOM pools (see below). This results in a root biomass C stock that is allowed to increase or decrease depending on input (biomass production) and output (decomposition) fluxes. These input and output fluxes were calculated by adjusting a non-steady state accumulation model to describe best the measured root C stock and ^{14}C ratio (for details see below).

Soil organic matter (SOM)

In the summer of 2007, six soil cores (0-80 cm) were sampled by using soil corers (Ø 3cm). According to visual criteria and decomposition status, the soil cores were separated into three subsections (weakly decomposed organic horizon; intermediate horizon; mineral horizon). Each of these subsections was further divided into 5 cm segments for analysis. We measured soil pH (in water and in $CaCl_2$) and organic C content (C_{org}) on all of the resulting soil samples. Radiocarbon (^{14}C) measurements were carried out on two of the profiles. For the

modelling, we did not use mean values from all profiles to calculate C stocks, instead we used the specific values of the respective profiles where we measured ^{14}C. Dry bulk density was determined by taking additional soil samples of known volume (100 ml) and drying them at 60°C. Dry bulk density data was complemented with data for the same site from Knorr (personal communication).

We defined the peat body from the soil surface until the top of the intermediate horizon (containing increasing amounts of mineral soil and featuring reduced organic C contens). Following Trumbore and Harden (1997), we distinguished (i) a surface peat layer, which contains C fixed in the last 50 years (identified by bomb ^{14}C content), and (ii) a deeper peat layer that extends to the mineral soil. The C balance of the surface organic layer was modelled using a non-steady state accumulation model like presented in Trumbore and Harden (1997) or Gaudinski et al. (2000). As this model depends on the occurrence of bomb ^{14}C, the C dynamics of the deep organic layers were calculated based on cumulative C vs. calibrated ^{14}C age profiles.

Detailed model description

Two different models were used in this study: (i) A non-steady state accumulation model that utilizes the incorporation of bomb ^{14}C into the considered C pool and therefore is applicable on decadal timescales (cf. Gaudinski et al. 2000), and (ii) a model based on plotting cumulative C stock versus time. The first model was used for the root biomass pool and the surface peat layer (0-15 cm), the second model was used for the deeper peat layers (lack of bomb ^{14}C made it impossible to use the first model there).

Both approaches are based on one common assumption that is important for further calculation: Net change in C storage (dC/dt) represents the balance between annual C inputs (I; kg C m^{-2} a^{-1}) and decomposition (kC, where k is a first-order decomposition rate constant (a^{-1}) and $C(t)$ is the organic layer C inventory (kg C m^{-2})). The C balance for a given year then can be calculated as

$$dC/dt = I - kC(t) \quad (1)$$

Solving this equation yields:

$$C(t) = (I/k) * (1 - \exp(-kt)) \quad (2)$$

In the non-steady state accumulation model we now use ^{14}C to constrain the C dynamics of the last decades. Basically, the model makes use of the large changes in the atmospheric ^{14}C content in the atmosphere since the late 1950s and the resulting labelling of biomass when incorporating this atmospheric C. Input and decomposition rate are fitted for an optimum

agreement between modeled and observed total C content and radiocarbon signature of SOM, using the least-square method. For the non-steady state, I and k were assumed to be different from each other but constant over time. In this case, the amount of carbon that remains (C_j) in 2007 from what was added in year j can be described as:

$$C_j = I \text{ x } e^{-k(2007\text{-}j)} \quad (3)$$

The total amount of carbon in the pool in 2007 therefore can be described as the sum of all the carbon that has been added in previous years and not yet been decomposed:

$$C_{2007} = \sum_{j=j_{start}}^{j=2007} C_j \quad (4)$$

The ratio ($R = \Delta^{14}C/1000 + 1$) of ^{14}C in the atmosphere (R_{atm}) of a certain year is assumed to be identical with the ratio of ^{14}C of the C input of that year (i.e. no decoupling between assimilation and litter production). Due to the presumably fast TTs of the uppermost centimeters, radioactive decay of ^{14}C is regarded as irrelevant on this timescale, and so the ratio of ^{14}C in the SOM pool in 2007 ($R_{som(2007)}$) can be described following a simple mass balance approach:

$$R_{som(2007)} = \frac{\sum_{j=j_{start}}^{j=2007} R_{atm(j)} \times C_j}{\sum_{j=j_{start}}^{j=2007} C_j} \quad (5)$$

Below a depth of ca. 15 cm we found no bomb ^{14}C in the SOM. To determine the C balance of these layers, we used a plot of the cumulative C inventory of these layers (starting from the bottom of the surface organic layers) against time, i.e. the calibrated ^{14}C age of SOM. The calibrated age was derived from the ^{14}C age by using OxCal 4.1. The atmospheric calibration curves used for conversion was 'IntCal04' for the northern hemisphere (Reimer et al. 2004). Equation (2) was fit to the resulting plot ($C(t)$ versus t) to determine I and k.

Both ^{14}C based accumulation models are based on the following assumptions: (i) Pools are homogeneous pools (i.e. all carbon atoms have the same probability to leave the pool); (ii) There is no time lag between assimilation of carbon and transfer into the considered pool. For the input of C into the root pool and the input of C *via* aboveground litter into the SOM pool this assumption is valid, but for the input of C into the SOM pool *via* decompostition of roots this assumption can cause a bias. (iii) Mass dependent fractionation either does not occur or can be corrected for during the measurements. In our case, mass dependent fractionation is mathematically corrected for during the measurements.

C dynamics of the intermediate and the mineral horizon

As we were interested mainly in the C dynamics of the peat body, we did not explicitly model C dynamics of the intermediate and the mineral horizon for this study. Instead, we fractionated the bulk soil using density fractionation. For a detailed description of the method see Schulze et al. (2009). Density fractionation resulted in three separate fractions: Free particulate organic matter (fPOM; < 1.6 g cm^{-3}), occluded particulate organic matter (oPOM; < 2.0 g cm^{-3}), and mineral associated organic matter (MAOM; > 2.0 g cm^{-3}). The fPOM fraction is supposed to represent the active pool of the soil, whereas the other two are more recalcitrant. We measured size and ^{14}C of the different fractions. Differences between the fractions were taken as an indicator whether relevant C input does occur in the intermediate and mineral horizons.

Radiocarbon measurements

Soil and root samples were pre-treated before further processing. As non-structural C in root biomass does not reflect the atmospheric ^{14}C signature of the year the root grew, we removed it by an acid-base-acid treatment (for details of the method see Gaudinski et al. 2005). Roots were dried at 60°C and homogenized for further processing. Soil samples were manually freed from stones and roots and homogenized. Further processing followed the description of the modified sealed tube zinc reduction method for preparation of AMS graphite targets (Xu et al. 2007). Preparation completely took place in the facilities of the Department of Soil Ecology at the University of Bayreuth. Graphite targets were analyzed by the Keck Carbon Cycle AMS facility at UC Irvine, USA with a precision of 2-3 ‰. Radiocarbon data are expressed as $\Delta^{14}C$, which is the per mil deviation from the $^{14}C/^{12}C$ ratio of oxalic acid standard in 1950. Alternatively, we presented ^{14}C values as a ^{14}C ratio (R, derived from the $\Delta^{14}C$ as $R = \Delta^{14}C/1000 + 1$). The sample $^{14}C/^{12}C$ ratio has been corrected to a $\delta^{13}C$ value of -25‰ to account for any mass dependent fractionation effects (Stuiver and Polach, 1977).

Results

General description of the soil profiles

Based on visual criteria, the 6 soil profiles were very similar within the uppermost 25 cm (weakly decomposed peat). This is reflected by very similar organic C (C_{Org}) and nitrogen contents down to 25 cm depth (Figure 2). The peat dry bulk density ranged around

0.11 g cm^{-3} in the uppermost ~10 cm of the peat body and increased quickly to relatively constant 0.29 g cm^{-3} in deeper peat. Difficulties for summarizing the individual soil profiles arise mainly from differences in peat body thickness (varying between 27 and > 70 cm). This results in high variability of C_{org} beneath a depth of 25 cm, as some profiles still feature peat in this depth whereas other already feature intermediate horizons (reflected in increasing SEs in Figure 2).

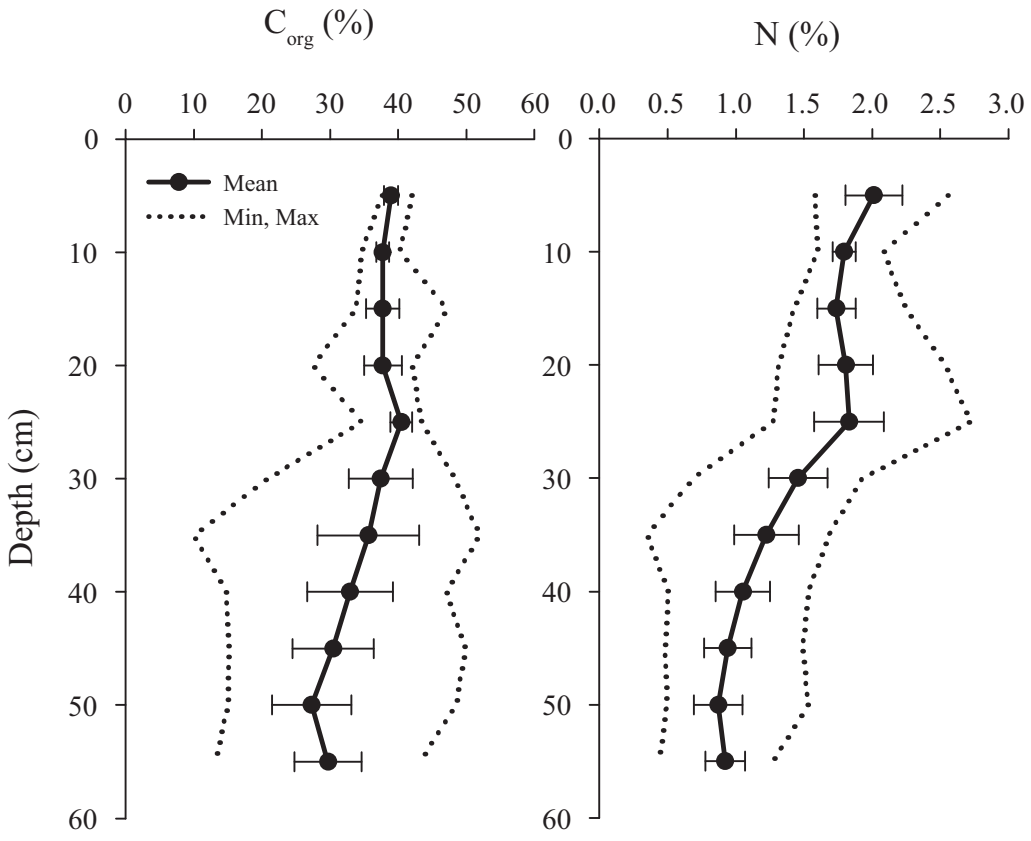

Figure 2. Mean (n = 5) organic C and nitrogen content (± SE) measured in 5 soil profiles in a temperate fen. Dotted lines represent the measured minimum and maximum values for the different depths to illustrate how differences in peat body thickness and transition to the intermediate horizon (characterized by low C_{org}) influence the C_{org} depth profile of the different soil profiles.

The two profiles chosen for additional ^{14}C analyses (hereafter profile 1 and 2) featured a peat thickness of 35 cm (profile 1; table 2) and 45 cm (profile 2; table 3). In profile 1, bomb ^{14}C dominated in the uppermost 15 cm, indicated by bulk ^{14}C values between 142 and 166 ‰, whereas C from before the 1950s dominated in deeper peat layers (bulk ^{14}C values < 0 ‰). Beneath 35 cm depth, we found an intermediate horizon where peat was intermixed with mineral soil (C contents around 15%) that continued to a depth of around 80 cm, where we found mineral soil with a C content of 1.5%. In profile 2, we found evidence for critical disturbance of the soil profile. The ^{14}C profile was untypical, with surprisingly small ^{14}C values within the uppermost 10 cm. The ^{14}C values increased significantly in a depth between

Table 2. Detailed data of profile 1 (selected for additional radiocarbon measurements). This data was used in the modelling of the C dynamics ('nd' stands for 'not determined').

	Depth (cm)	$\Delta^{14}C$ (‰)	Age (cal. Years BP)	Corg Content (%)	Dry bulk densitiy (g cm^{-3})	C Stock (kg m^{-2})	Cumulative C Stock (kg m^{-2})
Surface Peat	0 - 5	152.3	Modern	38.0	0.11	2.1	2.1
	5 - 10	166.2	Modern	34.6	0.11	1.9	4.1
	10 - 15	142.2	Modern	34.8	0.29	5.0	9.1
Deep Peat	15 - 20	-46.6	442	36.4	0.29	5.2	14.3
	20 - 26	-284.8	2816	44.8	0.29	7.7	22.0
	26 - 31	-357.5	3830	49.2	0.29	7.1	29.1
	31 - 36	-501.1	6402	52.6	0.29	7.6	36.6
Inter-mediate	36 - 41	-524.1	6788	15.1	0.36	2.7	39.3
	41 - 46	-603.1	8235	15.1	0.53	4.0	43.3
	46 - 51	-606.7	8322	15.0	0.70	5.2	48.5
Mineral	51 - 81	-594.4	8057	1.3	nd	nd	nd

Table 3. Detailed data of profile 2 (selected for additional radiocarbon measurements). Due to obvious and critical disturbance in the peat body of this soil profile, this data was not used for modelling of the ^{14}C dynamics.

	Depth (cm)	$\Delta^{14}C$ (‰)	Age (cal. yrs BP)	C_{org} Content (%)
Disturbed Peat	0 - 5	28.9	Modern	20.6
	5 - 10	-2.3	(Modern)	12.5
	10 - 15	199.5	Modern	19.7
	15 - 20	224.6	Modern	30.5
	20 - 25	-48.5	456	24.4
	25 - 30	-71.7	631	23.8
	30 - 35	-48.7	456	32.9
	35 - 40	-93.6	736	29.4
	40 - 45	-191.8	1623	22.5
Inter-mediate	45 - 50	-375.4	4128	6.9
	50 - 55	-300.1	2970	7.9
	55 - 60	-431.3	5198	8.9
Mineral	60 - 93	-566.3	7595	4.6

10-20 cm. Bomb ^{14}C was found down to a depth of 20 cm. Beneath this depth, values again were untypical, as no clear trend was recognizable until we reached a depth of ca. 50 cm. Unusually low organic C contents in the uppermost 15 cm further indicated disturbance. We thus dismissed the original plan of using the data of profile 2 for modelling C dynamics and instead had to restrict modelling to the data set of profile 1.

Fitting quality of modelled C fluxes

The non-steady state accumulation model was able to reproduce the measured C stocks and ^{14}C ratios of all considered pools (Table 4). Figure 3 summarizes the model reconstruction of past ^{14}C values of the root biomass and surface C stocks. Fitting equation (2) to the plot of cumulative C stocks versus age resulted in good fit ($r^2 = 0.94$) (Figure 4). In summary, the applied models fit well to the measured data.

Table 4. Model parameters for the non-steady state interpretation of the root biomass and the surface layers. Input (*I*) and decomposition rate (*k*) were adjusted by minimizing differences between (i) modelled C Stock (C_{Mod}) and measured C Stock (C_{Meas}) and (ii) modelled ^{14}C ratio (R_{Mod}) and measured ^{14}C ratio (R_{Meas}) following the least square method.

	k	*I*	*kC*	*I-kC*	TT	C_{meas}	C_{mod}	R_{Meas}	R_{Mod}
	($years^{-1}$)	——($kg\ C\ m^{-2}\ a^{-1}$)——			(years)	—($kg\ C\ m^{-2}$)—			
Roots	0.1972	0.2416	0.2662	-0.0246	5	1.35	1.35	1.071	1.071
SOM 0-5	0.0627	0.1297	0.1338	-0.0041	16	2.13	2.13	1.152	1.152
SOM 5-10	0.0447	0.0850	0.0869	-0.0019	22	1.94	1.94	1.166	1.166
SOM 10-15	0.0184	0.0932	0.0920	0.0012	54	5.00	5.00	1.142	1.142

Overall C stocks and fluxes

Aboveground biomass comprised a C stock of 173 g C m^{-2} at the *Schlöppnerbrunnen* site (Figure 5). Aboveground biomass at the site completely dies off each year and we assumed steady-state conditions for the above-ground litter pool, so input (annual biomass production) and output (decomposition) fluxes were assumed to be identical to the aboveground biomass stock. The root biomass C stock was significantly higher than the aboveground biomass C stock with a total of 1315 g C m^{-2} stored in root biomass down to a depth of 30 cm. Annual root biomassproduction (input) was calculated as 242 g C m^{-2} a^{-1}, compared to mean annual root biomass decomposition (output) of 266 g C m^{-2} a^{-1}. Thus, we calculated a net C loss from the root biomass C stock of 24.6 g C m^{-2} a^{-1}, that either is transferred to the SOM C stock or lost from the soil.

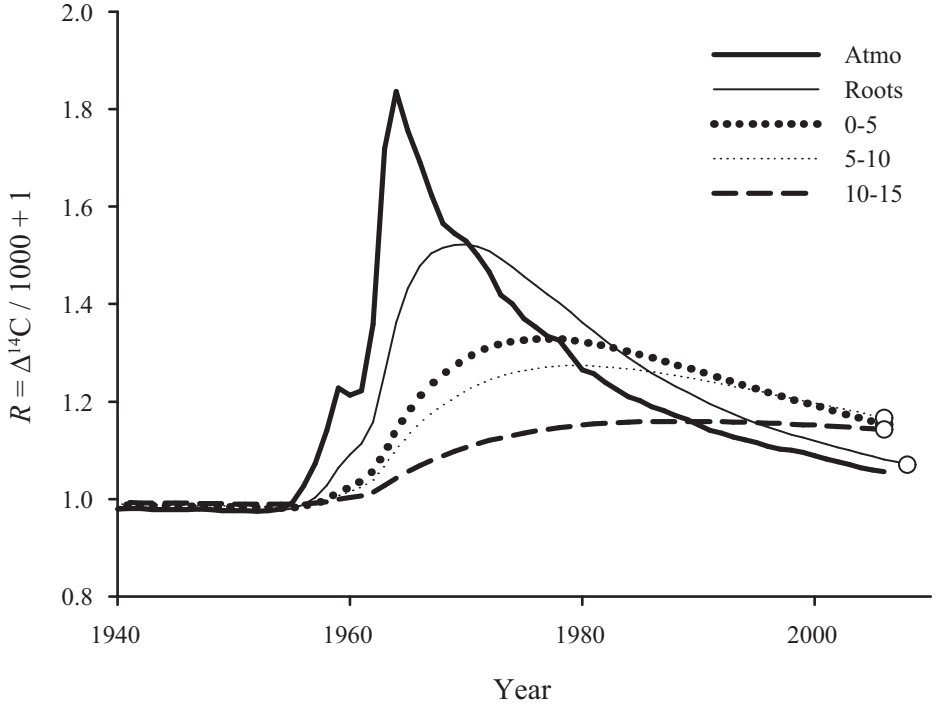

Figure 3. Atmospheric ^{14}C ratio like reported by Levin et al. (2008) and modelled ^{14}C data of the root biomass (root) and the depth specific surface SOM stocks (0-5, 5-10, 10-15). The modelled ^{14}C data is calculated using the non-steady state accumulation model. Input (*I*) and output (*kC*) were adjusted so that the modelled C stocks and ^{14}C ratios reproduce best the measured C stocks and the measured ^{14}C ratios (○).

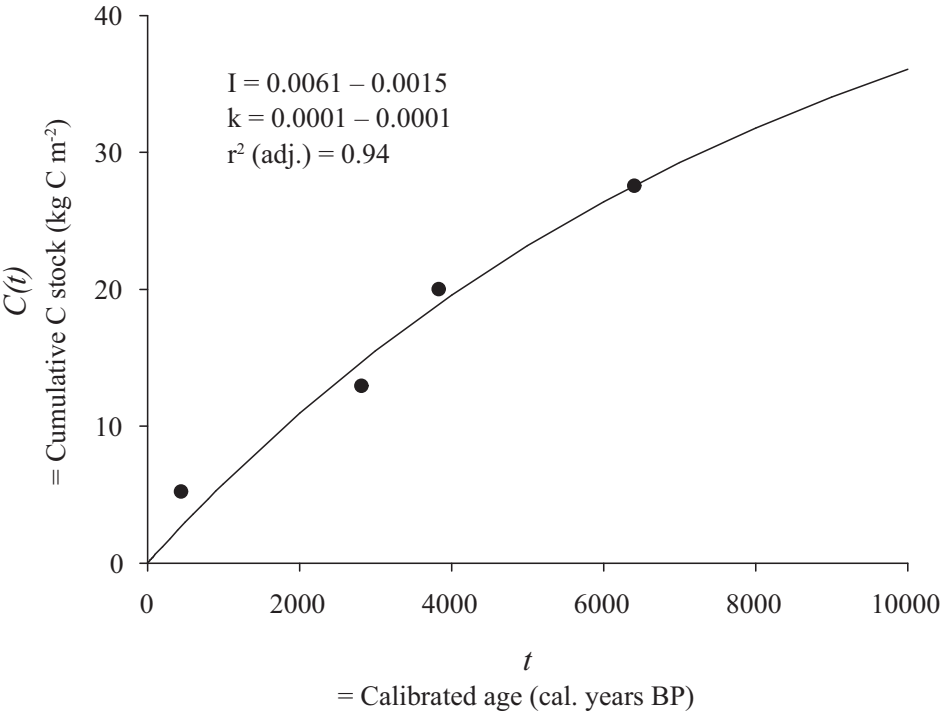

Figure 4. Derivation of long-term Input (*I*) and decomposition (*kC*) rates of the deep peat layer. I and k were determined by fitting equation (2) to a plot of cumulative C stocks (with zero at the transition of surface to deeper peat layers) versus times (=calibrated SOM age). Calibrated age was derived by converting ^{14}C ages to calendar ages using OxCal 4.1

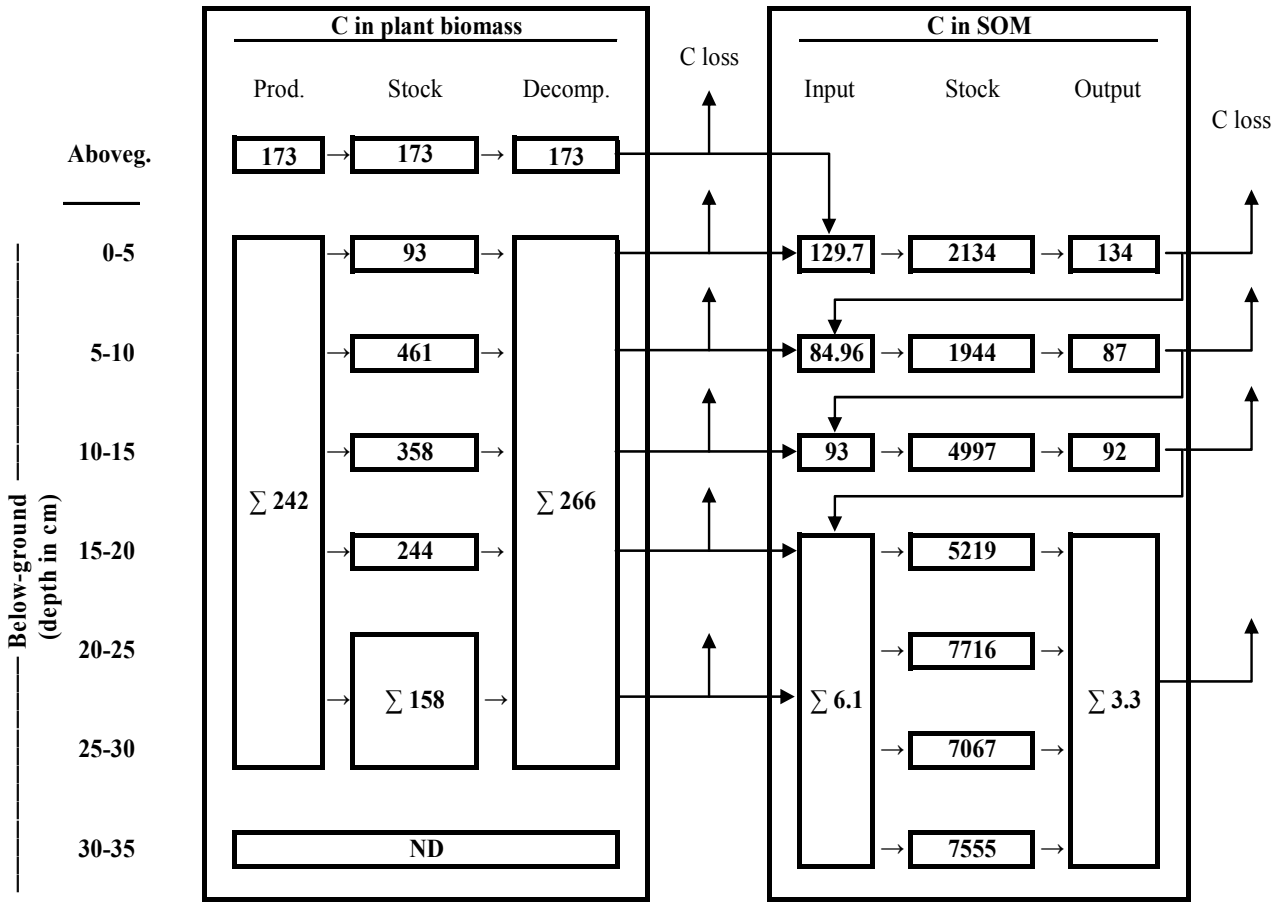

Figure 5. C stocks in biomass (aboveground and below-ground) and SOM pools, annual litter production from biomass, and annual input and output C fluxes into the SOM pools of different depths calculated on the basis of soil profile data from profile 1. Stocks are given in g C m^{-2}, fluxes (Prod., Decomp., Input, Output) are given in g C m^{-2} a^{-1}.

For the SOM C stock of the peat body, we calculated a total C stock of 36,633 g C m^{-2} down to a depth of 36 cm. Roughly 25 % of this C or 9,075 g C m^{-2} were found in the surface peat layer SOM stock compared to 27,558 g C m^{-2} in the deeper peat layer SOM stock. The mean TT of the surface peat SOM stock ranged from 16 (0-5 cm) to 54 years (10-15 cm) and thus was several orders of magnitude faster than for the deep peat layer SOM stock (4,500-23,000 years, derived from stock divided by flux). In the uppermost 5 cm, output fluxes (134 g C m^{-2} a^{-1}) were calculated to be higher than input fluxes (130 g m^{-2} a^{-1}), resulting in a small net C loss of 4.1 g C m^{-2} a^{-1}. Likewise, we calculated a net C loss from the SOM in 5-10 cm depth (output 87 g m^{-2} a^{-1}; input 85 g m^{-2} a^{-1}; net C loss 1.9 g m^{-2} a^{-1}). Between 10-15 cm, we calculated a small net uptake of ca. 1.2 g m^{-2} a^{-1}. In summary, SOM C

balance for the entire surface layers therefore was negative, with a net C loss of ca. 4.8 g m^{-2} a^{-1}.

The deep peat layer SOM stock was found to accumulate C at a net rate of 2.8 g m^{-2} a^{-1}. Gross input (6.1 g m^{-2} a^{-1}) and output (3.3 g m^{-2} a^{-1}) fluxes were between one and two orders of magnitude smaller than for the surface layer SOM stock. Combining results for the surface and deep peat SOM stocks, we calculated a total net SOM C balance for the entire peat body of -2 g m^{-2} a^{-1}, indicating a small net C loss from the SOM pool.

Intermediate and Mineral horizon

Measurements of the ^{14}C signature of the bulk soil and the three density fractions (fPOM, oPOM, MAOM) of the intermediate horizon at profile 1 and profile 2 revealed no differences between the different fractions (Table 5). In the mineral horizon, the ^{14}C signature of the fPOM fraction was increased compared to the oPOM and the MAOM fraction, indicating input of younger carbon, possibly roots. However, the fPOM fraction in the mineral horizon only accounted for 3-5 % (w/w) of the bulk soil.

Table 5. Radiocarbon signature of bulk soil and density fractions (fPOM, oPOM, MAOM) in the intermediate and the mineral horizon and weight proportion of the fractions in total bulk soil measured in profiles 1 and 2 ('nd' stand for 'not determined') .

		$\Delta^{14}C$ (‰)				Proportion of bulk soil (% w/w)		
Horizon	Depth (cm)	Bulk soil	fPOM	oPOM	MAOM	fPOM	oPOM	MAOM
Profile 1								
Interm.	36-41	-524	-542	-545	-534	20.3	26.1	51.9
Interm.	41-46	-603	-595	-603	-593	52.2	21.0	28.7
Interm.	46-51	-607	-606	-610	-608	28.6	28.3	43.4
Mineral	51-81	-594	-554	-603	-606	5.2	4.1	84.2
Profile 2								
Interm.	45-50	-375	nd	nd	nd	nd	nd	nd
Interm.	50-55	-300	-303	-315	-322	10.1	42.5	45.5
Interm.	55-60	-431	-422	-436	-410	9.8	27.5	60.2
Mineral	60-93	-566	-456	-533	-597	3.4	8.9	81.9

Discussion

Peatlands globally represent important C storage pools due to small but constant net C accumulation rates. In this study we calculated the net C balance of SOM and below-ground

biomass. We added the latter to our consideration of the soil C pool to represent the fact that adverse boundary conditions for decomposition (e.g. lack of oxygen due to water tables close to the peat surface) can lead to a significant (transient) stabilization of C in undecomposed root layer that would be ignored in root-free SOM samples. We found that SOM and root biomass pools had a negative net C balance.

Modelled input and output C fluxes at this fen site reveal net C losses of ca. 2 g m^{-2} a^{-1}. Although this net C loss appears to be small, it is already unusual for a peatland site not be a net C sink. In general, peatland sites are reported have a positive net C balance. Turunen et al. (2002) reported net C accumulation rates of 15-35 g C m^{-2} a^{-1} for Finish peatlands. Estimates for western Canada ranged around 19.4 g C m^{-2} a^{-1} (Vitt et al. 2000), and for West Siberia Turunen et al. (2001) reported a net gain of ca. 17.2 g C m^{-2} a^{-1}. A net C balance of -3 g m^{-2} a^{-1} thus indicates that boundary conditions at the fen site have been changed by disturbance, compared to the conditions during previous periods of peat accumulation.

We found significant amounts of C stored in root biomass at this site. As we were unable to precisely distinguish between live and intact dead roots, root biomass C stock comprises living roots and undecomposed root litter. Stabilization of C in the soil does not only occur in SOM C stocks. Especially under the boundary conditions found in waterlogged peatland soils (high water tables, lack of oxygen), it is possible that litter decomposes very slowly in the soil, thus root biomass litter represents another important C storage pool, though possibly on shorter timescales. Changing decomposition conditions can affect the rate of C transfer from the root biomass C stock to the SOM C stock. This might result in apparent net C losses from the SOM C stocks. We thus included the root pool into our modelling approach. As we calculated net C losses from the root biomass C stock we can exclude the possibility that reduced C transfer from the root biomass C stock to the SOM C stock resulted in apparent SOM C losses. We are aware of the fact that the fluxes calculated for the root biomass C stock are biased because we assumed a homogeneous pool. The actual fluxes therefore are uncertain. For this study it is sufficient, however, to eliminate the possibility that apparent net C losses from SOM are compensated for by transient increase of the amount of C stored root biomass. We are also aware of the fact that we lack root biomass data beneath 30 cm. Although root biomass constantly decreases beneath 10 cm we therefore underestimate the proportion of root biomass in the total C stock and gross fluxes of the peat body.

Our results reveal that turnover of the SOM C stock in this fen was mainly taking place between 0-15 cm depth, as 98 % of the gross fluxes in and out of the SOM C stock occurred there. The contribution of gross fluxes in deeper peat layers to C turnover in the entire SOM C

stock thus is relatively small. The annual mean water table for this fen site has been reported as -0.13 ± 0.19 m in 2002 (Paul et al. 2006) and -0.11 ± 0.08 and -0.10 ± 0.12 m in the years of 2007 and 2008 (Muhr et al. 2009), and, thus, is close to the transition between the surface and the deeper peat layers. Thus, the small contribution of fluxes from the deep peat SOM stocks to the entire SOM C dynamics might result from a lack of oxygen. However, the water table is subject to high seasonal fluctuations, and has been reported to drop considerably during summer: Paul et al. (2006) reported a mean water table of -0.23 ± 0.21 m between May and August of 2002. Muhr et al. (2009) found values between -0.11 and -0.19 m during the summers of 2006 to 2008. Thus, it is more likely that the small contribution of the deeper peat layers to overall SOM C fluxes is due to low substrate quality. This is also indicated by results reported by Muhr et al. (2009), who found no increase of C fluxes when they experimentally lowered the water table during summer down to values of 20 to > 60 cm beneath the soil surface. Incubation of peat samples from the deeper peat layers of this site under completely oxic conditions also revealed very little decomposition potential (Reiche et al. 2009).

The net C losses from this soil indicate disturbed boundary conditions. Based on our results, we are unable to determine the actual disturbance. There are, however, three possible explanations. (1) High nitrogen deposition into the ecosystem (Matzner et al. 2004) could explain net C losses from the root biomass C stock. Following this interpretation, production of new root biomass has decreased because nitrogen availability has increased during the last decades, whereas decomposition rates remained unaffected. (2) Reduced sulphate deposition (like observed during the last decades as a consequence of new emission laws; for data of the region cf. Matzner et al. 2004) could increase leaching of DOC from the peatland, explaining part of the net C losses from the SOM C stocks (Monteith et al. 2007). However, the difference of the net C balance of this site in comparison to other peatlands is rather big. To explain differences of that size, the disturbance has to have a critival impact on soil C dynamics. (3) Changes in hydrology could have such an impact, as stabilization by high water tables and consequent anoxia is a major reason for the net C accumulation of peatlands. As mentioned before, the site features a ditch of unknown origin and age. Profile 2 was located close to this ditch and revealed heavy disturbance within the peat body. Assuming that the ditch is of anthropogenic origin (due to undocumented peat cutting at the site), this disturbance of the ^{14}C profile could be the result of digging in that area. Thus, the ditch could significantly alter the hydrological conditions at the site, thereby possibly resulting in artificial draining of the site. Climate change could further enhance changes on the

hydrological conditions. Regardless of the cause, this site has to be regarded as a disturbed site, thereby putting results from experiments at this site in a new perspective.

Based on our model calculations, we amounted gross C fluxes from the peat body around 442 g m^{-2} a^{-1}. These C fluxes can occur as CO_2 and CH_4 emissions or as leaching of dissolved organic carbon (DOC). In 2007 and 2008, Muhr et al. (2009) measured soil respiration at the site and determined annual fluxes between 490 and 640 g C m^{-2} a^{-1}. Soil respiration includes metabolic respiration by living roots, a flux that is not considered in the model calculations of this study (cf. Figure 1). Still, these measurement data can be used to estimate a maximum loss of C from the system via CO_2 emission. Based on results by Goldberg (personal communication) and Knorr et al. (2008), CH_4 emissions do not contribute significantly to total C fluxes at this site. Carbon losses from the site *via* DOC leaching have not been quantified yet, but based on measurements of DOC concentrations in the soil solution they can be assumed to be at least one order in magnitude smaller than C losses *via* CO_2 emission (Schulze, personal communication). In summary, our modelling approach yields gross C fluxes from the soil that are similar to those estimated by measurements of soil respiration, CH_4 emissions, and DOC leaching.

As we were mainly interested in the C balance of the peat body, we did not model the C balance for the underlying intermediate and mineral horizons. Root growth, however, can easily extend down to this depth (at the two profiles chosen for radiocarbon analyses the intermediate horizon began in depths of 36 and 45 cm already), thus possibly leading to net C accumulation. However, the results from the density fractions of these horizons contradict the idea of relevant C input at least in the intermediate horizon. The different fractions analyzed here are normally considered to represent pools of different activity: The fPOM fraction mainly contains recognizable plant material and fungal hyphae and responds quickly to changes in C inputs and environmental conditions (and thus represents an active pool in contrast to the oPOM and MAOM fraction) (von Lützow et al. 2006). Based on this differentiation of the three fractions, the input of root biomass would correspond to an input of C into the fPOM fraction, thus resulting in an input of bomb ^{14}C and a consequent increase of the ^{14}C signature of this fraction compared to the other two fractions. In other words: The input of relevant amounts of root C into the intermediate horizon should result in significant differences in the ^{14}C signature of the three fractions. As we found no differences between the different fractions in the intermediate horizon, we conclude that no relevant input of young C into these horizons occurs and that C turnover in the intermediate horizon is irrelevant for the total soil C balance. In the mineral horizon, we found differences in the 14C signature of the

three fractions, but these differences were small (considering that root C has positive ^{14}C signatures) and the proportion of the fPOM fraction in bulk soil was very small (3-5 % w/w), so again we conclude that C turnover in the mineral horizon is irrelevant for the total soil C balance.

Conclusion

In contrast to most undisturbed peatlands, the total net soil C balance for the *Schlöppnerbrunnen* fen site is negative. We take this as indicative of disturbed boundary conditions. Following our model data, root biomass is a highly important C pool both in terms of storage and in terms of total soil C dynamics. Radiocarbon has proven as a tool that allows detecting even small imbalances in the investigated C pools.

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Chapter 3

Soil C dynamics in a forest as affected by soil frost

Effects of soil frost on soil respiration and its radiocarbon signature in a Norway spruce forest soil

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Abstract

Apart from a general increase of mean annual air temperature, climate models predict a regional increase of the frequency and intensity of soil frost with possibly strong effects on C cycling of soils. In this study, we induced mild soil frost (up to -5°C in a depth of 5 cm below surface) in a Norway spruce forest soil by removing the natural snow cover in the winter of 2005/2006. Soil frost lasted from January to April 2006 and was detected down to 15 cm depth. Soil frost effectively reduced soil respiration in the snow removal plots in comparison to undisturbed control plots. On an annual basis 6.2 t C ha^{-1} a^{-1} were emitted in the control plots compared with 5.1 t C ha^{-1} a^{-1} in the snow removal plots. Only 14% of this difference was attributed to reduced soil respiration during the soil frost period itself, whereas 63% of this difference originated from differences during the summer of 2006. Radiocarbon ($\Delta^{14}C$) signature of CO_2 revealed a considerable reduction of heterotrophic respiration on the snow removal plots, only partly compensated for by a slight increase of rhizosphere respiration. Similar CO_2 concentrations in the uppermost mineral horizons of both treatments indicate that differences between the treatments originated from the organic horizons. Extremely low water contents between June and October of 2006 may have inhibited the recovery of the heterotrophic organisms from the frost period, thereby enhancing the differences between the control and snow removal plots. We conclude that soil frost triggered a change in the composition of the microbial community, leading to an increased sensitivity of heterotrophic respiration to summer drought. A CO_2 pulse during thawing, such as described for arable soils several times throughout the literature, with the potential to partly compensate for reduced soil respiration during soil frost, appears to be lacking for this soil. Our results from this

experiment indicate that soil frost reduces C emission from forest soils, whereas mild winters may enhance C losses from forest soils.

Keywords: carbon dioxide, freezing, frost, partitioning, radiocarbon, soil respiration, thawing

Introduction

Soil respiration is the largest global CO_2 flux from the terrestrial biosphere to the atmosphere (Schlesinger & Andrews, 2000). Around three times more organic C is stored in soils than in terrestrial plants (Schimel, 1995). With soil temperature being the most important driver of soil respiration (Raich & Schlesinger, 1992), an increase in the mean annual temperature as predicted by the IPCC (2001) is likely to affect soil respiration rates. In addition to a mere increase of the mean annual temperature, the IPCC (2001) also predicted a changing frequency of extreme meteorological events such as the freezing and thawing of soils in mountainous regions. In some regions, increasing temperatures will lead to a reduced frequency of soil frost. In other regions, reduced snowfall together with temperatures below 0°C will lead to an increase in the frequency and intensity of soil frost.

Soil frost creates stress conditions that force organisms to acclimate or die (Schimel *et al.*, 2007). Roots, especially fine root tips, are frost sensitive (Smit-Spinks *et al.*, 1985) and normally acclimate to soil frost by accumulation of soluble carbohydrates (Tinus *et al.*, 2000). Slow growth of roots has been reported throughout the winter for *Picea abies* (Hansen *et al.*, 1996), but prolonged soil frost events can increase fine root mortality (Gaul *et al.*, 2008). Microbial cells can experience frost damage (e.g., by rupturing of cell membranes by ice crystals; Rivkina *et al.*, 2000), or may suffer from reduced solute availability due to a reduction of the amount of free water and the diffusion rate of solutes by soil frost. Ice barriers can also effectively block gas diffusion, thereby creating anaerobic conditions (Clein & Schimel, 1995). Acclimation of soil microorganisms to soil frost conditions is achieved by a number of physiological changes, including the storage of compatible solutes (e.g., carbohydrates) and the synthesis of protective molecules, including proteins and sugars. The substrates used to supply these acclimations are available neither for microbial growth nor for metabolic activity (Schimel *et al.*, 2007). In general, soil respiration is found to be reduced during periods of soil frost, but nevertheless still occurs at temperatures well below 0°C in unfrozen water films (Edwards & Cresser, 1992).

During thawing of frozen soil in laboratory experiments, a burst of CO_2 was found for both arable and forest soils and in some experiments also for arctic and tundra soils (Matzner & Borken, 2008). One explanation for this burst is dying of microorganisms during soil frost, which are then decomposed by surviving microbes during thawing. Rapid metabolization of compatible solutes or proteins stored in the cell to acclimate to soil frost is another explanation. The availability of new substrate due to aggregate disruption and the creation of new surfaces have also been discussed in this context. Field data confirm the importance of

such a CO_2 pulse during thawing for arable soils (Dörsch *et al.*, 2004), but not for alpine or arctic soils (Elberling & Brandt, 2003; Monson *et al.*, 2006). Whereas Coxson & Parkinson (1987) reported a CO_2 burst for a temperate forest soil, Groffman *et al.* (2006) found no such pulse in a northern hardwood forest soil. As data for temperate forest ecosystems are scarce and contradictory, the question remains how these soils will respond to soil freezing and subsequent thawing. To our knowledge, almost no data on the effects of soil frost on an annual scale are available.

CO_2 respired from soils normally originates from a number of different sources. CO_2 respired from live roots, mycorrhiza, or microbes in the rhizosphere that metabolize root exudates normally has a $\Delta^{14}C$ signature that is close to that of CO_2 in the atmosphere, as recent photosynthates are the major C source in this case, and therefore, will be summarized here as rhizosphere respiration (RR). CO_2 respired by heterotrophic microorganisms metabolizing soil organic matter (SOM) and plant material that has not just recently died normally has a $\Delta^{14}C$ signature that is different from that of atmospheric CO_2 in the year of the measurement and will therefore here be distinguished as heterotrophic respiration (HR). Both components of soil respiration might be affected by soil frost. Data from Herrmann & Witter (2002) for arable soils suggest that soil frost has a considerable effect on soil microbes, with mineralization of microbial biomass explaining up to 65% of the observed C flush following thawing of frozen soil. On the other hand, it has also been reported that even mild soil frost can severely damage fine roots and increase fine root mortality and turnover (Tierney *et al.*, 2001). Experiments with tundra soil (Mikan *et al.*, 2002) revealed an extremely high temperature sensitivity of microbial activity for temperatures below 0°C. To distinguish the effects of soil frost on heterotrophic vs. rhizospheric respiration, radiocarbon-based measurements have been reported to address this question with minimal disturbance (Schuur & Trumbore, 2006).

This work is testing the following hypotheses in a mountainous Norway spruce forest soil: (1) A temporary CO_2 burst can be observed during thawing of frozen soil, which can be explained by a rapid increase of microbial activity. (2) Soil frost reduces cumulative soil respiration on an annual scale. (3) Microorganisms are more severely affected by soil frost than roots, resulting in a reduction of microbial respiration during freezing. To test these hypotheses, replicated snow removal and control plots were established in a Norway spruce forest site at the Fichtelgebirge. The region experienced a significant decrease of days with snow cover over the last decades (Foken, 2004), while temperatures in winter still drop below 0°C (Foken, 2003), thereby increasing the probability of soil frost in winter and making this

region an adequate spot for our soil frost experiment. In addition to the measurements and radiocarbon analysis of soil respiration, CO_2 concentrations were measured at different soil horizons to detect potential changes in the origin of CO_2 between the control and the treatment plots.

Materials and methods

Site description

The experiment was carried out in a mature Norway spruce forest (*P. abies* L.) of an age of 145 years at the Coulissenhieb II research site, located in the Fichtelgebirge in southern Germany (50°08'N, 11°52'E) at an elevation of 770m a.s.l. *Calamagrostis villosa* (Chaix ex Vill), *Deschampsia flexuosa* (L.), *Vaccinium myrtillus* (L.), and *Oxalis acetosella* (L.) dominate the understorey vegetation. Mean annual air temperature for the site is 5.3°C and the mean annual precipitation ranges around 1160 mm (Gerstberger *et al.*, 2004). With a total of 133 frost days per year (air temperature minimum <0°C), frost is a common event at the Coulissenhieb II research site (Foken, 2003). The soil is classified as a Haplic Podzol with a sandy to loamy texture according to the FAO soil classification (IUSS, 2006). The mor-like forest floor has a thickness of 6–10 cm and is composed of Oi, Oe, and Oa horizons. The pH ($CaCl_2$) value of the soil ranges around 3.3 in the Oa horizon and increases with depth to around 4.0 in the Bw and C horizons. Carbon contents decrease with depth, ranging around 40–50% in the Oi and Oe and less than 1% in the C horizon (Table 1).

Table 1 Chemical properties (median values from nine soil profiles) in the Norway spruce stand at the Fichtelgebirge

	Depth (cm)	pH $CaCl_2$	C (%)	N ($mmol_c\ kg^{-1}$)	CEC_{eff}
Oi	10		47.8		nd
Oe	8		42.0		nd
Oa	5	3.3	18.2	1.0	206
Ea	-5	3.4	7.4	0.4	152
Bh	-12	3.4	5.5	0.3	190
Bs	-18	3.7	3.4	0.2	126
Bw	-55	4.1	1.3	0.1	48
Bw/C	< -55	4.0	0.4	0.0	43

A storm event on January 18, 2007, severely damaged the research site, considerably thinning out the forest. All results beyond this date may therefore be subject to influence by the disturbance caused by the storm.

Experimental design

For the snow removal experiment, three control and three snow removal plots, each of an area of 20 m x 20 m, were established in the summer of 2005. All plots were equipped with identical basic instrumentation for measurement of soil temperature, soil moisture, precipitation, soil solution, and litterfall. Soil moisture of the mineral horizons was measured with two custom-built, calibrated tensiometers per plot in a depth of 20 cm and automatically logged hourly.

Control plots were used to assess the natural dynamic of all measured parameters without any experimental disturbance. Snow removal plots were used to investigate the effect of soil frost on soil respiration and its components, CO_2 concentration in the soil profile, and radiocarbon signature of the emitted CO_2. Snow was removed manually from the snow removal plots in the winter of 2005/2006 between the end of December 2005 and the beginning of February 2006. Removed snow was not retransferred to the plots at the end of the snow removal manipulation period, thereby resulting in a total reduction of annual throughfall on the snow removal plots of 147 mm of water which is equivalent to 13% of the total mean annual precipitation. To avoid damage to the forest floor due to snow removal, plastic nets with a mesh width of 1 cm were used to cover the soil. The end of the snow removal period was set for February so that insulation by additional snowfall after this date would maintain the soil frost for a considerable amount of time.

Measurement of CO_2 fluxes

On each of the six plots, three plastic collars with a length of 20 cm and an inner diameter of 49.5 cm were installed permanently for soil respiration measurements. The collars were driven 5 cm into the forest floor to minimize the disturbance of near-surface roots. For gas measurements, the collars were manually closed with a plastic lid and connected to a portable infrared gas analyzer (IRGA; Li-820 from Li-Cor Biosciences GmbH, Germany). Air was circulated in this closed system by a pump at a constant flow rate of 0.5 L min^{-1} and the CO_2 concentration inside the chamber was logged every 10 s for a period of 3-5 min. A linear regression was performed on the increasing CO_2 to determine the flux rate, which was

corrected for atmospheric pressure and chamber air temperature. For more details on the method see Savage & Davidson (2003) or Borken *et al.* (2006).

Measurement of CO_2 concentrations in soil profiles

CO_2 concentrations in the soil profile were measured between April 2006 and April 2007. During winter 2005/2006, measurements were not possible due to technical problems. On each of the six plots, six gas probes were installed in the summer of 2005 to analyze the CO_2 concentrations in the soil profile. The probes were made up of plastic cylinders of a length of 50 cm and an inner diameter of 1.6 cm, resulting in a total volume of 100 mL. In the backmost 20 cm, the cylinders were perforated by drill holes, thereby allowing free gas exchange between the cylinder volume and the soil atmosphere. Cylinders were closed on both sides and two stainless steel capillaries (inner diameter 1.5 mm, capillary volume depending on length but <3 mL) were inserted into the cylinder on the side without perforating drill holes. The probes were installed horizontally in different depths at the transition among the Oa, Ea, Bh, Bs, Bw, and C horizons. An additional gas probe was installed in the middle of the Bw horizon. The protruding steel capillaries were bent in a rectangular angle and cut to a length that they poked out of the forest floor up to a height of 50 cm after the installation pit was closed again. Short PP tubes and luer lock adapters (MedNet) with a lid (MedNet) at the protruding end of the capillaries permitted the connection of a syringe (volume: 20 mL) via a three-way stopcock (MedNet) holding a cannula. For sampling, air was sucked into the syringe from the probe. After discarding 10 mL (at least three times the capillary volume), the air from the cylinder was given some time inside the syringe to adjust to air temperature, before 20 mL of air were injected into a 22 mL glass vial with a septum, which was filled with argon and depressurized before sampling. Samples were transferred to the laboratory and measured with a Shimadzu GC-14A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with an electron capture detector (ECD) and connected to a DANI HSS 1000 autosampler (DANI Strumentazione Analitica S.P.A., Monza, Italy). A calibration curve was produced by measuring six certified standards with a given CO_2 concentration of 380, 600, 1000, 3000, 10 000, and 29 495 ppm. Calibration was calculated using a sigmoid regression given by the software (DataApex Clarity by DANI). Measured concentrations were corrected for dilution due to mixing with argon in the vials.

Gas sampling for analysis of the $\Delta^{14}CO_2$ signature

Sampling of soil respiration for the determination of its $\Delta^{14}C$ signature was done on four dates (March 6, 2006; June 8, 2006; November 14, 2006; March 15, 2007) using the soil respiration chambers described earlier. For each of the sampling dates, two out of the three respiration chambers on each plot (resulting in a total of six replicates per treatment) were closed and then flushed with CO_2-free synthetic air for 90 min at a flow rate of 1.5 L min^{-1}, thereby effectively flushing the respiration chambers with an amount of gas equal to at least three times the chamber volume. Following flushing, the respiration chambers were sealed and left until CO_2 concentration inside the chambers reached at least 1500 ppm. Incubation time depended on effective CO_2 fluxes at the sampling day. Evacuated stainless steel cylinders with a volume of 2 L were connected to the respiration chambers and slowly filled with gas from inside the chambers. Gas samples were then transferred to the laboratory for further processing. The $\Delta^{14}CO_2$ signature of soil respiration ($\Delta^{14}C_{SR}$) of control or snow removal plots, respectively, was calculated as the mean value of the six replicates of each treatment for each date.

Besides monitoring changes in the $\Delta^{14}C_{SR}$, due to soil frost, it was the aim of this study to partition soil respiration into the contribution of RR and HR. The partitioning was achieved by applying the isotopic method making use of bomb-derived ^{14}C explained in greater detail by Gaudinski *et al.* (2000) or Schuur & Trumbore (2006). The basic idea is measuring the $\Delta^{14}CO_2$ signature and the flux of total soil respiration, as well as the $\Delta^{14}CO_2$ signature of the two constituents of soil respiration (RR and HR) and then applying a mass balance to calculate the fluxes of the two constituents. A major advantage of this method is that it can be carried out mostly under field site conditions and with minimal disturbances. The basic equations applied in this approach are Eqns (1) and (2):

$$F_{CO_2,SR} = F_{CO_2,Het} + F_{CO_2,Root} \quad (1)$$

$$F_{CO_2,SR} \times \Delta^{14}C_{SR} = F_{CO_2,Het} \times \Delta^{14}C_{Het} + F_{CO_2,Root} \times \Delta^{14}C_{Root} \quad (2)$$

where $F_{CO_2,SR}$ is the CO_2 flux of total soil respiration, $F_{CO_2,Het}$ is the flux of HR, and $F_{CO_2,Root}$ is the flux of RR, all in mmol m^{-2} h^{-1}. $\Delta^{14}C_{SR}$ is the radiocarbon signature of the total soil respiration, $\Delta^{14}C_{Het}$ is the radiocarbon signature of the HR, and $\Delta^{14}C_{Root}$ is the radiocarbon signature of the RR, all given in ‰.

To measure the $\Delta^{14}CO_2$ signature of RR ($\Delta^{14}C_{Root}$), two different approaches were used. As the $\Delta^{14}CO_2$ signature of CO_2 respired by roots is normally close to the $\Delta^{14}CO_2$ signature of the atmosphere, atmospheric samples were taken on three different dates (March 6, 2006;

August 16, 2006; November 14, 2006; two to three replicas for each date) by simply filling evacuated stainless steel containers with a volume of 2 L with atmospheric air in a height of 2 m above ground. As the amount of C in these containers was only around 0.3 mgC, the content of three such containers was combined to form one replicate.

As roots and microbes using root exudates may not only metabolize recent photosynthates but may use C from storage pools, the atmospheric $\Delta^{14}CO_2$ signature and the $\Delta^{14}CO_2$ signature of RR do not necessarily have to be the same. Therefore, $\Delta^{14}CO_2$ signature of RR was also measured by excavating live roots of Norway spruce from the forest floor on two different dates (November 14, 2006 and March 15, 2007, three replicas per date). Excavated roots were washed with deionized water to remove soil particles clinging to the roots and then transferred to gastight incubation containers. Before incubation, the containers were flushed with CO_2-free synthetic air to remove all atmospheric CO_2 and then incubated for 1–2 days in the laboratory at a constant temperature of +15°C. Gas samples were again taken by connecting an evacuated stainless steel cylinder with a volume of 2 L to the incubation container and sucking in gas. The value of $\Delta^{14}C_{Root}$ was calculated as the mean value of the three replicates of each sampling date.

Sampling of gas for the determination of the $\Delta^{14}C$ signature of HR ($\Delta^{14}C_{Het}$), in principle, followed the same course such as sampling of gas emitted from roots. On the sampling dates (November 14, 2006 and March 15, 2007), one soil core of a length of 25-30 cm, including the Oi, Oe, Oa, Ea, Bh, and part of the Bs horizon, was sampled at the Coulissenhieb II site on each treatment plot, resulting in three soil cores from the control plots and three soil cores from the snow removal plots for each sampling date. After soil was stored at +15°C for 4-6 weeks to reduce the effect of disturbance due to sampling, roots were manually removed and the disturbed soil was transferred to incubation containers. Organic and mineral horizons were not separated for incubation to estimate the $\Delta^{14}C$ signature of total HR from the uppermost 30 cm. Before incubation, the incubation containers were flushed with CO_2-free synthetic air to remove atmospheric CO_2 and then incubated for 1-2 days at a constant temperature of +15°C. Following previous work by Dioumaeva *et al.* (2003) and more recent experiments by Czimczik & Trumbore (2007), temperature does not affect the $\Delta^{14}C$ signature of evolving CO_2. After incubation, sampling of air from the incubation containers again took place by using the stainless steel containers described before. The value of $\Delta^{14}C_{Het}$ for the two different treatments was calculated as the mean value of the three different replicas for each treatment and sampling date.

Preparation of gas samples for $\Delta^{14}C$ signature

All gas samples sampled in stainless steel containers (originating from soil respiration, root respiration, or HR) were further processed in the laboratory for measuring the $\Delta^{14}C$ signature. The general course of action was following the zinc reduction method for preparation of AMS graphite targets described in Xu *et al.* (2007), but with some modifications. The steel containers containing the samples were connected to the extraction line via a digital mass flow controller (Type F-201C-AAA-33-V, Wagner Mess- und Regeltechnik, Germany) effectively limiting the flow rate to 20 mL min^{-1}. Gas was directed to a water trap, cooled with a 1:1 mixture of ethanol and dry ice to get rid of the water and afterwards through a CO_2 trap cooled with liquid nitrogen, thereby effectively freezing out all CO_2 from the sample. The rest of the gases, which are noncondensable at the temperatures of the two cooling traps, were discarded. The remaining procedure followed the method described in Xu *et al.* (2007). All preparation took place at the laboratories of the Department of Soil Ecology at the University of Bayreuth, Germany. Graphite targets were analyzed by the Keck Carbon Cycle AMS facility at UC Irvine, USA, with a precision of 2-3‰. Radiocarbon data are expressed as $\Delta^{14}C$, which is the per mil deviation from the $^{14}C/^{12}C$ ratio of oxalic acid standard in 1950. The sample $^{14}C/^{12}C$ ratio has been corrected to a $\delta^{13}C$ value of -25‰ to account for any mass-dependent fractionation effects (Stuiver & Polach, 1977).

Data analysis

With three soil respiration chambers per plot and three plots per treatment (control and snow removal), mean values were formed by treating the chambers on the same plot as pseudoreplicates and the different plots as true replicates. Mean values with standard error were formed for the different plots and then averaged by using error propagation.

Calculation of cumulative CO_2 emissions was achieved by linear interpolation among adjoining measurement dates. For the calculation of CO_2 concentrations in the soil profile, data from the gas probes were pooled according to the horizon. Owing to different thickness of the horizons at the different plots, depths of the probes may differ among the different plots, but a horizon specific concentration profile can be created.

Data were analyzed using STATISTICA 6.1 (Statsoft, Tulsa, OK, USA). Differences in fluxes of soil respiration and its radiocarbon signature between the treatments were tested using the nonparametric Mann–Whitney *U*-test. Comparing values of the same treatment at different dates was achieved by using the Tukey HSD test.

Results

Soil temperature and soil moisture

Before snow removal, no considerable differences in soil temperature measured in a depth of 5 cm beneath soil surface were to be found between snow removal and control plots (Fig. 1a). With the beginning of snow removal, soil temperatures of the snow removal plots became lower than on the control plots. Soil started to freeze around the mid of January 2006, when temperatures in 5 cm depth sank below 0°C on the snow removal plots, and remained frozen until April. The coldest temperature measured was around -5°C in 5 cm depth. The deepest indication of soil frost was found in 15 cm depth below surface, where temperatures reached 0°C around February 2006 (Hentschel *et al.*, 2008, this issue).

Snow removal was equivalent to a reduction of annual precipitation by 147 mm of water, so differences in soil moisture had to be considered. Matric potential measured in a depth of 20 cm revealed considerable differences in the matric potential during the freezing period (January–April), with matric potentials on the snow removal plots being more negative than on the control plots, reaching minimum values of around -20 kPa (Fig. 1b). Only minor differences were found during the rest of the year. A notable period of drought occurred during July/August of 2006, when tensiometers reported matric potentials of down to -50 kPa on both the control and the snow removal plots and then failed to operate due to the extreme drought, causing a lack of data for a few weeks.

CO_2 fluxes

In autumn 2005, before the snow removal treatment, CO_2 fluxes of the control and the snow removal plots were similar on two measurement dates and slightly different on a third one thereafter (Fig. 1c). Following the shovelling of snow and freezing of the soil, CO_2 fluxes of the snow removal plots were almost constantly smaller than CO_2 fluxes of the control plots during the winter of 2005/2006. However, although the soil was frozen down to around 15 cm depth, soil respiration fluxes from the snow removal plots still ranged around 1 mmol CO_2 m^{-2} h^{-1} compared with 1-3 mmol CO_2 m^{-2} h^{-1} on the control plots. At the beginning of the snow melt at the end of March, CO_2 fluxes increased in the snow removal plots, while CO_2 fluxes in the control plots decreased to a minimum of 0.7 mmol CO_2 m^{-2} h^{-1}. However, the differences were small and so no pronounced thawing pulse was observed in this experiment. Following snow melt at the end of March, the CO_2 fluxes of the control and

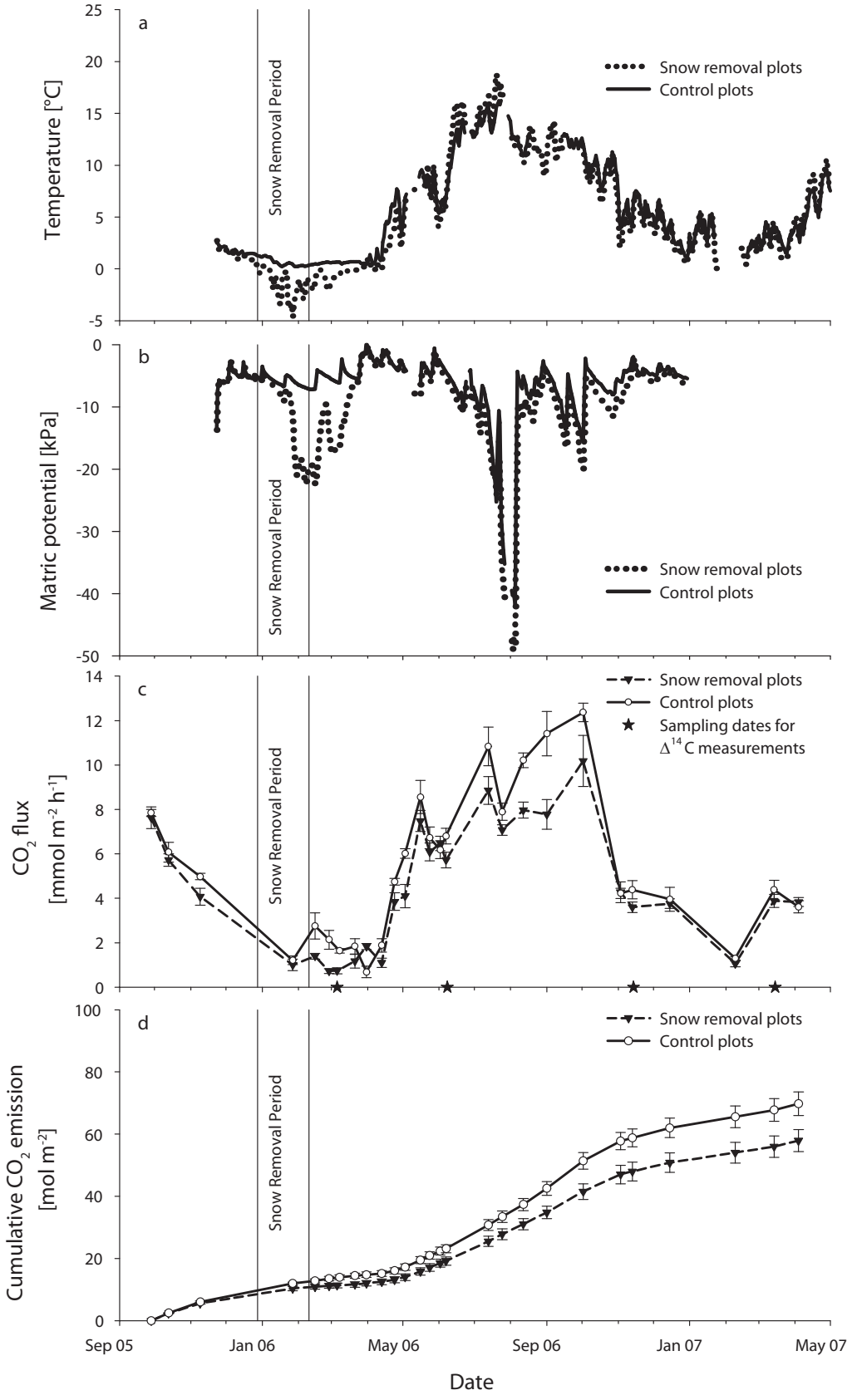

Figure 1 Measurements of (a) soil temperature in a depth of 5 cm below soil surface (mean values, $n = 3$), (b) soil matric potential in a depth of 20 cm (mean values, $n = 6$), (c) daily CO_2 fluxes (mean values, $n = 9$), and (d) cumulative CO_2 emissions calculated from daily CO_2 fluxes by linear interpolation. Stars on the x-axis of part (c) indicate sampling dates for measurement of the $\Delta^{14}C$ signature of soil respiration.

the snow removal plots were almost identical. In June 2006, fluxes of the snow removal plots started to differ again from the fluxes of the control plots, with fluxes from snow removal plots being smaller than fluxes from control plots throughout the rest of the summer. With decreasing fluxes in autumn 2006 and rewetting of dry soil, fluxes of the snow removal and control plots were about the same level and no differences were found throughout the winter of 2006/2007 and spring 2007.

In total, 70 mol CO_2 m^{-2} were emitted from the control plots throughout the whole course of the experiment (19 months), which was significantly ($P = 0.04$) > 58 mol CO_2 m^{-2} from the snow removal plots (Fig. 1d). This is equal to an annual CO_2 emission (calculated from January 2006 to January 2007) of 52 mol CO_2 m^{-2} or 6.2 t C ha^{-1} a^{-1} for the control plots and 42 mol CO_2 m^{-2} or 5.1 t C ha^{-1} a^{-1} for the snow removal plots. Thus, on an annual basis, soil respiration was 1.1 t C ha^{-1} smaller on the snow removal plots than on the control plots. The differences during frost period, lasting from beginning of January to mid of April, were responsible for 14% of this reduction or 0.16 t C ha^{-1}. Compared with this, the summer period (lasting from beginning of June to beginning of October) accounted for 63% of the difference between snow removal and control plots or 0.72 t C ha^{-1}.

CO_2 concentrations in the soil profile

Measurements of the CO_2 concentrations at the end of the frost period revealed increased CO_2 concentrations in the three lowermost horizons of the soil profiles of the snow removal plots (Fig. 2a) compared with the control plots (Fig. 2b; for differences see Fig. 2c). Following snow melt, CO_2 concentrations decreased on the snow removal plots. CO_2 concentrations were similar on manipulation and control plots during summer 2006, with only minor differences for the lowermost two horizons that were slightly more pronounced during the winter of 2006/2007 and spring 2007. Almost identical CO_2 concentrations in the uppermost mineral horizons indicate that differences in CO_2 emissions from the control and snow removal plots originate from the organic horizons.

CO_2 profiles of the control plots showed a weak seasonal pattern, with an increase in concentration during spring and winter that was interrupted during the extremely dry summer of 2006 and reached its maximum during natural rewetting of soil in September 2006. The CO_2 profiles of the snow removal plots did not show the pattern during the summer of 2006, which might be due to the lack of measurements during September and October, but showed a clear maximum at the end of the winter of 2005/2006 (Fig. 2). In general, CO_2 concentrations

in the soil profiles continuously increased with increasing depth, and no indication was found that a maximum of the CO_2 concentration was reached in 70 cm depth.

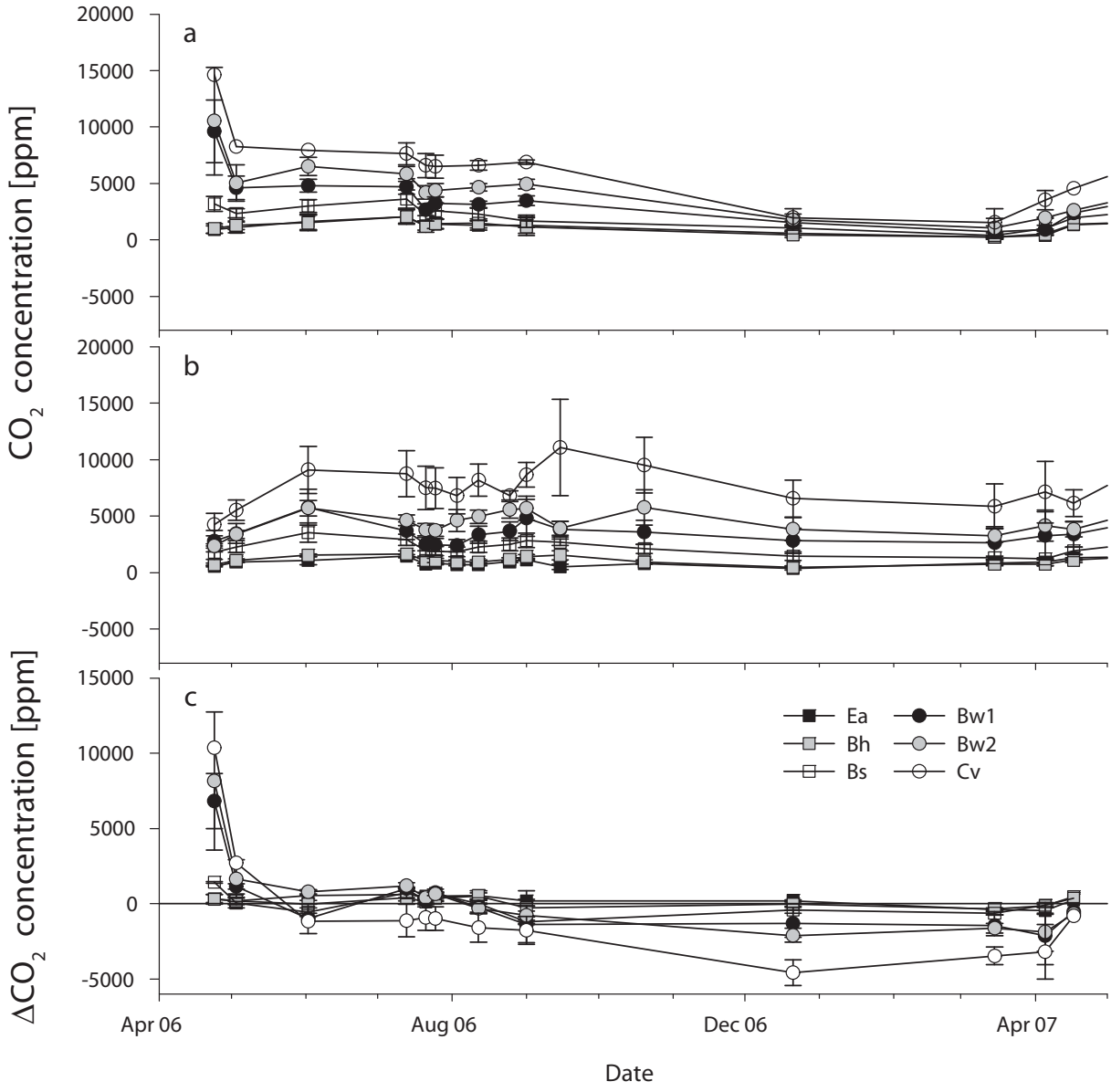

Figure 2 CO_2 concentrations in the soil profile of the (a) snow removal plots, (b) control plots, and (c) the difference in CO_2 concentrations between snow removal and control plots. All data points represent mean values (n = 3) ±SE.

Partitioning of soil respiration

Measurements of the radiocarbon signature of total soil respiration revealed significant differences (P<0.05) between the snow removal and the control plots for all measurement dates (Table 2). During and after the treatment, radiocarbon signature of total soil respiration was significantly smaller on the snow removal plots with values ranging between 74‰ and

82‰ compared with 86‰ and 91‰ on the control plots. The mean difference between control and manipulation plots was constantly around 8–12‰. Radiocarbon signature showed only a slight annual trend with slightly increased values during late spring and summer and decreased values during end of winter.

The two different approaches to estimate the $\Delta^{14}CO_2$ signature of RR yielded very similar results on all measurement dates. Atmospheric samples of the three different sampling dates yielded $\Delta^{14}CO_2$ signatures of 50.6‰, 53.0‰, and 55.9‰. Root incubation yielded a $\Delta^{14}CO_2$ signature of RR of 53.3‰ and 55.2‰. As values for both approaches were similar and no considerable annual trend was to be seen, the results of both approaches were combined to calculate one uniform $\Delta^{14}C_{RR}$ (53.6 ± 2.1‰; Table 2).

Table 2 Radiocarbon signature of total soil respiration (SR), heterotrophic respiration (HR) and rhizospheric respiration (RR) on the snow removal and control plots

	$\Delta^{14}C \pm SE$				
	Snow Removal		Control		
Date	SR (‰)	HR (‰)	SR (‰)	HR (‰)	RR (‰)
March 6, 2006	78.8 ± 3.2	na	88.8 ± 2.2	na	53.6 ± 2.1
June 8, 2006	81.7 ± 1.8	na	91.4 ± 2.2	na	53.6 ± 2.1
November 14, 2006	78.0 ± 1.6	86.7 ± 7.4	86.0 ± 2.8	91.8 ± 4.5	53.6 ± 2.1
March 15, 2007	73.6 ± 6.1	81.3 ± 1.9	85.8 ± 3.1	88.0 ± 5.8	53.6 ± 2.1

Calculating the contribution of heterotrophic and RR to total soil respiration revealed that HR was dominating total soil respiration throughout the year and for both the snow removal and the control plots, accounting for 70% to > 90% of total soil respiration fluxes (Fig. 3). This dominance of HR was more expressed on the control plots, where rhizosphere contribution ceased to play a significant role in March 2007 (Fig. 3). Converting the percentage contribution of HR and RR to actual absolute fluxes (Fig. 3) revealed that absolute HR fluxes were always considerably smaller on the snow removal plots than on the control plots. Absolute RR fluxes differed only slightly during 2006, but differences became much more pronounced in March 2007, when absolute RR fluxes on the control plots became extremely small.

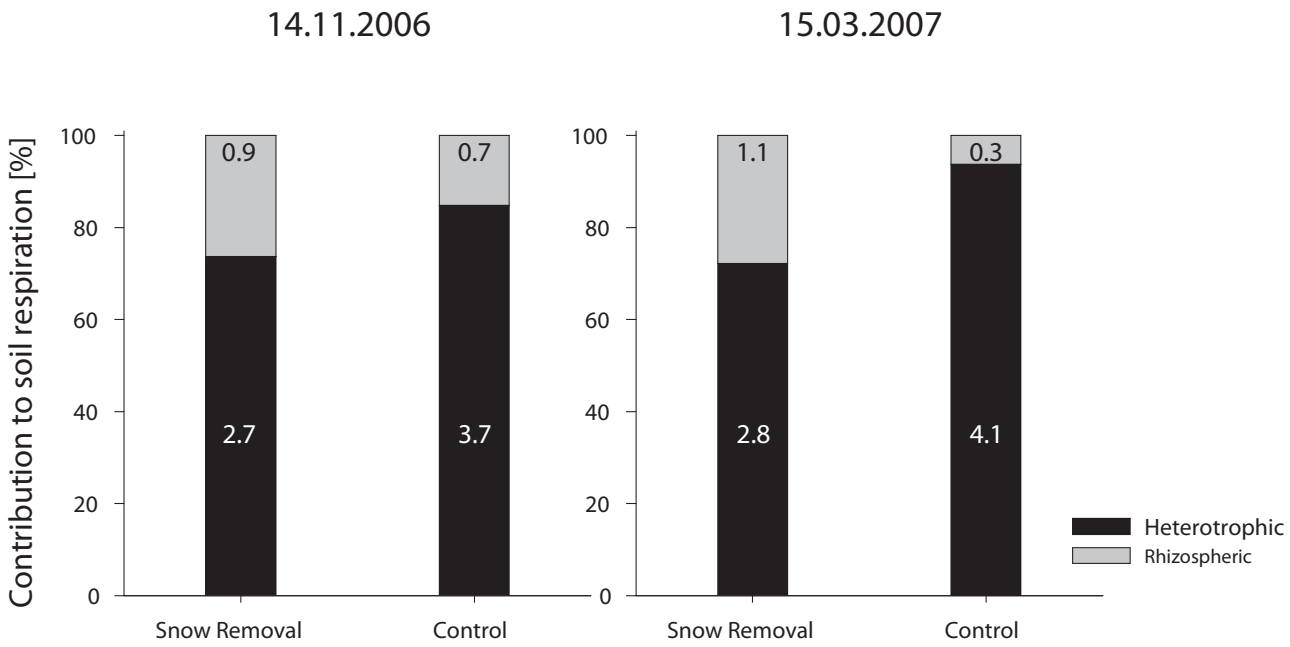

Figure 3 Relative fluxes of heterotrophic and rhizospheric respiration for two measurement dates (bars) calculated based on the radiocarbon signature of total soil respiration, heterotrophic and rhizospheric respiration. The numbers represent the absolute fluxes (mmol m^{-2} h^{-1}) for heterotrophic and rhizospheric respiration calculated based on the mean CO_2 fluxes of the snow removal plots or the control plots of the measurement day and the relative contribution.

Discussion

Soil respiration was significantly reduced in the snow removal plots during the soil frost period from January to April 2006 and during the posttreatment period from June to October 2006. No inherent differences were found during the pretreatment period that could explain these differences in soil respiration. Based on radiocarbon data, we attribute the observed decrease to damage to heterotrophic soil microbes induced by soil frost.

Differences in soil respiration observed from January to April 2006 can be directly explained by adverse living conditions for soil microbes due to frost (Schimel *et al.*, 2007) and reduced soil temperatures. Although soil temperature below 0°C can have drastic effects on microbial activity (Mikan *et al.*, 2002), the observed differences during freezing have little effect on the annual C balance, mostly owing to the fact that fluxes on both the control and the snow removal plots were small.

During thawing of soil, soil respiration for both treatments was almost identical and no pronounced pulse of CO_2 emission was found. All we found was a short-timed inversion of flux patterns, with fluxes from snow removal plots becoming higher than fluxes from control plots for a very short time around the end of March 2006 that goes along with the beginning of the snow melting period. The inversion is owed mainly to a significant reduction of fluxes

on the control plots and only partly to an increase of fluxes on the snow removal plots and insignificant on the annual scale. This lack of a pronounced CO_2 pulse during thawing is in agreement with the findings described by Groffman *et al.* (2006) for forest soil, but contradicts the findings of Coxson & Parkinson (1987) for forest soil and Elberling & Brandt (2003) for arctic soils under natural vegetation. It remains possible that due to the frequency of measurements, a short lasting pulse might have been missed in our experiment. However, results from laboratory experiments carried out with soil from our site make the occurrence of such a pulse appear unlikely (Goldberg *et al.*, 2007). In these laboratory experiments, repeated freezing of soil at three different temperatures (Group 1: -3°C, Group 2: -8°C, Group 3: -13°C) revealed that a burst of CO_2 only occurred after freezing at -8 and -13°C, but not at -3°C. Furthermore, this burst was comparatively small and did only occur during the first freezing–thawing cycle, so even if such a small pulse would have been missed it is unlikely to be relevant on an annual scale.

Finally, it is also possible that a burst occurred, but was masked by a similar burst on the control plots. When a frost induced burst of CO_2 occurs, it is normally found during the thawing of soil, which in our experiment did not start before April 2006. During that period, CO_2 fluxes on both the control and the snow removal plots start to increase significantly, likely simply due to the notable increase in soil temperature and therefore microbial activity. However, Schimel & Mikan (2005) showed for an arctic tundra soil that the acclimation shift towards frost conditions in microbial metabolism already occurs between +2 and +0.5°C. If this holds true for microorganisms of temperate forest soils, it can be assumed that the microbes on the control plots already started to acclimate to soil frost by storing sugars and proteins. Schimel *et al.* (2007) hypothesized that metabolizing of these intracellular solutes explains a large part of the flush of CO_2 during thawing. In this case, the control and snow removal plots would both react with a rapid burst of CO_2 as soon as soil temperatures become warmer again, thereby both showing what could be defined as a frost-induced CO_2 burst. This scenario remains highly speculative and for our experiment, it would not matter which scenario holds true (mere temperature increase or CO_2 burst on both plot types), but mild winters with temperatures above 2°C might be affected by this phenomenon.

Differences between the two treatments in the summer of 2006 were surprisingly high. In fact, the reduction of soil respiration in the snow removal plots between June and October 2006 is almost solely responsible for the decrease of cumulative C emissions in the snow removal plots. Soil temperature, being the most important driver of soil respiration (Raich & Schlesinger, 1992), cannot explain these differences, as soil temperature 5 cm beneath the

surface was not found to be different on the control and the snow removal plots during that period. Soil moisture, also being an important driver of soil respiration (Davidson *et al.*, 1998), was only measured in a depth of 20 cm. No differences in soil moisture between the control and the snow removal plots were found in this depth during summer. Comparative measurements of the water content of the organic horizons are lacking for the summer, but measurements of gravimetric water content made in April 2006 by Hentschel *et al.* (2008, this issue) on frozen soil cores revealed significantly higher gravimetric water contents on the snow removal plots due to frozen water in the forest floor and run-off of melting water. Two further measurements after thawing, at the beginning and the end of May 2006, revealed no differences in the water content of the organic horizons of the control and the snow removal plots (K. Hentschel, unpublished data). These data indicate that during thawing, water contents on both treatments were reset to equal levels and, therefore, the idea of a permanent reduction of water content due to snow removal on the snow removal plots can be rejected. Still, it remains possible that differences in soil moisture did occur in the organic horizons during summer. It should be mentioned in this context that no differences in root biomass were found on the two treatments, which would indicate different water uptake by vegetation (Gaul *et al.*, 2008). Furthermore, it should be noted that differences in soil respiration during the summer of 2006 coincided with a period of heavy natural drought. With the beginning of rewetting of the soil in autumn, the differences in soil respiration disappeared. Radiocarbon data reveal different $\Delta^{14}C_{SR}$ signature for the snow removal and the control plots for all four measurement dates. Data for the pretreatment period are lacking, so inherent differences between the treatments cannot be completely neglected. Considering the random distribution of the different treatment plots and the small variation in radiocarbon signature data among treatment plots of the same type, it still seems more likely that the period of soil frost induced these differences. In this context, it is interesting to mention additional radiocarbon data available for the field site (J. Muhr, unpublished data). At the same time as the control plots, we also installed three additional plots for a throughfall exclusion experiment. $\Delta^{14}C$ signature of soil respiration was measured on these plots before manipulation (i.e., they can be regarded as additional control plots) on June 8, 2006, at the same time such as $\Delta^{14}C$ signature on the snow removal and control plots. The value for $\Delta^{14}C$ signature of soil respiration on these additional plots was 88.0‰ (± 2.4, $n = 6$), which is very close to the value of the controls of 91.4‰ (± 2.2, $n = 6$) but significantly different (Student's *t*-test, $P<0.05$) from the $\Delta^{14}C$ signature on the snow removal plots, which was 81.7‰ (± 1.8, $n = 6$). These findings further contradict the idea of inherent differences.

Radiocarbon data show a reduction of HR in the snow removal plots. This reduction is still detectable in the spring of 2007, over 1 year after the manipulation period. Long-term effects of soil frost on the microbial community have been reported by some authors. Repeated freezing–thawing cycles seem to induce a shift of the microbial community towards a dominance of bacteria (Nieminen & Setala, 2001; Larsen *et al.*, 2002). Experiments by Feng *et al.* (2007) revealed that repeated lab-stimulated freezing–thawing cycles greatly reduced fungal biomass in a forest soil, whereas bacteria were unaffected. It has been hypothesized by Schimel *et al.* (2007) that this preferential effect of soil frost on fungi is due to mechanical stress resulting in damaging of fungal hyphae. In a laboratory experiment carried out with soil from the same site described here, Schmitt *et al.* (2008, in revision) investigated the effects of frost on the microbial community. Analyzing phospholipids fatty acid (PLFA) patterns in an unfrozen control (constantly kept at 15°C) and three different freeze–thaw treatments (repeatedly frozen at -3, -8, and -13°C and thawed at +5°C), they concluded that fungi were more susceptible to soil frost, as soil frost resulted in a reduction of the ratio of fungal/bacterial PLFAs. Such a reduction of fungal biomass might explain the reduction of HR in our snow removal experiment even during summer. Voroney (2007) reported that fungi are normally more tolerant towards drought than bacteria. A shift of the microbial community towards bacterial dominance (due to preferential damaging of fungi by frost), therefore, would increase the susceptibility of the surviving microbial community towards soil drought.

Whereas HR is reduced in snow removal plots, rhizospheric respiration shows a trend to increase. These results are in agreement with findings from Gaul *et al.* (2008), who reported an increase in fine root mortality and necromass, as well as fine root productivity following soil frost. Radiocarbon bulk signature of the fine roots was found to be around 85‰, so CO_2 produced from fine root decomposition should be considerably different from RR and be reflected in HR (D. Gaul, personal communication). The fact, that HR actually decreased, indicates that no significant flush of CO_2 arouse from increased decomposition of fine root necromass. In the spring of 2007, differences in RR between the control and the snow removal plots become even stronger. This phenomenon is rather unlikely to be explained by soil frost, but might be due to the mentioned storm that hit the site in January 2007 and inflicted serious and possibly spatially heterogeneous damage to the spruce stand.

Comparing partitioning data of the two treatments, it becomes evident that even in times with similar total soil respiration fluxes considerable differences concerning the origin of the emitted CO_2 do occur. The increase of rhizospheric respiration is partly compensating for the reduced HR in the snow removal plots, thereby masking the effects of soil frost. The

increased contribution of RR to total soil respiration reflects in a generally smaller radiocarbon signature of soil respiration in the snow removal plots, indicating the contribution of more recent C pools such as photosynthates respired by roots.

Considering the results of this study and of other experiments with soils under natural vegetation (Matzner & Borken, 2008), a burst of CO_2 emission during thawing of soil does not play a significant role for these soils. Instead, the more important effect of soil frost seems to be a decrease of cumulative C losses from soils such as shown in this work and others (Larsen *et al.*, 2002; Monson *et al.*, 2006). Our results for soil in the Fichtelgebirge support the idea that CO_2 emissions from soils are reduced in years with soil frosts as compared with years with mild winters. The idea of increased substrate availability due to soil frost such as postulated by Schimel *et al.* (2007) cannot be confirmed by our findings and also is not reflected by measurements of the concentration of dissolved organic carbon (DOC) carried out throughout the experiment (Hentschel *et al.*, 2008, this issue).

Considering results on fine root productivity and mortality (Gaul *et al.*, 2008), as well as the radiocarbon data presented here, C input into soils via root litter is likely to increase. However, this increase was not mirrored in an increase of total soil respiration. HR, which would be expected to increase due to increased root litter input, actually declined for the snow removal plots. Based on these results, we postulate an increased C sequestration in soils due to decreased CO_2 emissions as a result of the freezing of soils.

Conclusion

In this temperate conifer forest, soil frost reduces CO_2 emission via soil respiration by negatively affecting HR. RR is less affected and even increased probably due to increased productivity and turnover of fine roots. A pronounced CO_2 pulse during thawing of frozen soil, such as described especially for arable soils, did not occur in this experiment and can therefore not compensate for reductions of soil respiration. Long-term effects of soil frost, reducing soil respiration in the summer following the soil frost period, were more important for the annual C output than effects during soil frost itself. This effect has not been shown before, and might partly be explained by the sequence of two extreme events within 1 year (extended period of soil frost in winter and an exceptionally dry summer). Overall, CO_2 emissions from the snow removal plots were smaller by 1.1 t C ha^{-1} a^{-1} as compared with the control plots. If the observed effects of soil frost hold true, soil frost reduces the C output of soils and enhances the sink strength of the soil, considering unchanged litter input.

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Chapter 4

Soil C dynamics in a forest as affected by drought

PART A:

Drying-rewetting events reduce C and N losses from a Norway spruce forest floor

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Abstract

Periods of prolonged summer drought are likely to be expected for this century, with possibly strong effects on carbon (C) and nitrogen (N) mineralization in soils. In this experiment, undisturbed soil columns from a Norway spruce forest in Bavaria, Germany, were subjected to an 80 d drought period. Three different drought intensities were investigated in three treatment groups. During the first weeks, the soil columns were gradually dried and then kept at constant water potential. Initial water potential was between -0.01 and -0.1 MPa (pF 2-3), and reduced by drying to -0.6, -80 and -400 MPa (pF 3.5, 5.5 and 6.5) in the parallel treatment groups. Rewetting of the dry soil was achieved by irrigation (4 mm d^{-1}) for 40 d.

CO_2 fluxes from all groups were identical before drying. Over the 80 d drought period, drying treatments only emitted 72, 52 or 43 % of the amount of CO_2 emitted during the same time from a constantly moist control. Rewetting resulted in a fast increase of CO_2 fluxes to approx. the same level as in the control. No significant excess C mineralization during rewetting was observed, so adding up total CO_2 fluxes of the 80 d drought and the 40 d rewetting period, the drought treatments emitted only 88, 71 or 67 % of the CO_2 emitted by the constantly moist control. Measurements of dissolved organic carbon (DOC) did only show minor differences between control and treatment columns, indicating that no significant accumulation of DOC took place during the drought period. Radiocarbon signature of emitted CO_2 indicates that C mineralization simply was reduced with decreasing water availability and no new substrate was made bioavailable. Net N mineralization over the course of the whole experiment was reduced by drought to 77, 65 or 52 % of the control. Net nitrification

was virtually zero during drought whereas net ammonification continued at reduced levels. In total, increasing drought intensity resulted in increasingly reduced C and N mineralization. Neither C nor N mineralization were enhanced by drying-rewetting. The organic horizons seem well adapted to rapidly fluctuating water availability. The relevance of the so-called 'Birch effect' for this soil has to be challenged. Prolonged summer droughts are likely to conserve soil organic carbon (SOC) and soil organic nitrogen (SON) pools in this soil.

***Key Words*:** Summer Drought Intensity; Drying; Rewetting; Birch-Effect; CO_2; DOC; Nitrogen; Climate Change; Soil respiration; Radiocarbon

Introduction

Soils are known to play an important role as reservoirs for organic C and N. They also are important sources or sinks, respectively, for several climate relevant gases. Following the predictions of the IPCC (2007), we not only face an increase of mean annual air temperature within this century, but also a change of precipitation patterns resulting in an increasing probability of extended summer droughts followed by heavy rainfall events. This change of the global water cycle is likely to have an impact on C and N mineralization in soils.

Drying of forest floors is a phenomenon occurring regularly. It is generally understood that during drying of well-drained soils mineralization decreases, whereas rewetting leads to a rapid increase of mineralization. Several mechanisms have been proposed to explain both the reduction of mineralisation during drying and the increase during subsequent rewetting (cf. Borken and Matzner, 2009).

During drought periods, microorganisms have to respond to reduced soil water potentials. They can either dehydrate and become dormant, or they acclimate at high costs by accumulating compatible solutes (Harris, 1981). During drought, total cytoplasmic constituents can account for 30-40 % of total C and 20-60% of total N of bacteria or fungi, compared to only 3-6 % of total C and N during non-stress conditions (Schimel et al., 1989; Schimel et al., 2007). As microorganisms invest substrates into acclimatization, become inactive or even die due to severe living conditions during periods of drought (Bottner, 1985; De Nobili et al., 2006), both microbial C and N mineralization decrease. This decrease is further enhanced by diffusive limitations: Reduced water content decreases substrate diffusion and hence substrate accessibility for microorganisms (Voroney, 2007).

Drying of soil can also alter soil structure and characteristics of soil surfaces. Aggregate disruption and desorption of surfaces may occur during periods of drought, rendering new substrates available (Utomo and Dexter, 1982; Bottner, 1985; Van Gestel et al., 1993; Denef et al., 2001). At the same time drought is known to increase the hydrophobicity especially of soils rich in organic matter (Mataix-Solera et al., 2007). Although this is normally not of importance for the microbial activity during the drought itself, it becomes increasingly important during the rewetting of soil.

Wetting of the dry soil generally increases microbial activity, but the extent of this wetting pulse can vary widely depending on soil properties, intensity and length of the drying, intensity of the rewetting etc. This wetting pulse can occur very fast, even within minutes (e.g. Borken et al., 2003), and its size and duration are of major importance for the cumulative C and N net mineralization resulting from drying-rewetting events.

Two fundamentally different mechanisms are currently discussed to explain this wetting pulse. Following the nomenclature used by Xiang et al. (2008) we name them the "microbial stress" mechanism vs. the "substrate supply" mechanism.

As mentioned above, active cells have to accumulate compatible solutes to retain water and survive periods of drought. Rewetting of the soil, resulting in a sudden increase of water potential, forces cells to dispose of these compatible solutes or risk cell rupture due to massive water uptake. The no longer needed compatible solutes are set free and can now be easily mineralized by the microorganisms. The microbial stress mechanism therefore postulates that the wetting pulse during rewetting is due to the mineralization of substrates that were already present but used differently.

In contrast to this, the substrate supply mechanism assumes the creation of new substrates that were not available until the drying-rewetting event occurred. It is mainly build on the fact that physical processes during rewetting of dry soil can destabilize soil organic matter (SOM), e.g. by aggregate disruption, organic matter redistribution or desorption.

Like pointed out by Xiang et al. (2008) the effects of these two mechanisms would be dramatically different: The microbial stress mechanism bears no potential of creating new substrates, but rather might result in a loss of microbial biomass thereby reducing the metabolical capability of the microbial pool. The substrate supply mechanism on the other hand can destabilize former stable or meta-stable SOM pools and enhance C and N losses from soils (so-called "Birch effect"). In deed, several authors reported a short-term extra mineralization burst following rewetting, exceeding by far pre-drought mineralization rates or the rates of a permanently moist control (Birch, 1958; Seneviratne and Wild, 1985; Kieft et al., 1987; Fierer et al., 2003; Haney et al., 2004; Xiang et al., 2008). Such an extra boost of microbial activity indicates the creation of additional substrate due to drying-rewetting (priming effect) and is termed as the "Birch effect" (Jarvis et al., 2007). It is this Birch effect that repeatedly gave reason to discussions about the net-effect of drying-rewetting (cf. Borken and Matzner, 2009). Whereas drought generally decreases cumulative C and N fluxes out of the soil, the release of additional substrate can cause C and N fluxes during the wetting pulse which more than compensate for this reduction and result in an increase of net mineralization.

Radiocarbon (^{14}C) offers the possibility of detecting changes in the predominant sources of respired CO_2. Substrates from meta-stable or stable C pools that are made bioavailable by drying-rewetting should differ in their radiocarbon signature ($\Delta^{14}C$) from more recent C sources, thereby altering the isotopic signature of the emitted CO_2 as soon as they are

metabolized. The creation of new substrates from stable or meta-stable pools should therefore result in measurable changes of $\Delta^{14}C$ of respired CO_2.

This experiment was designed to investigate the effect of drought intensity followed by rewetting of the soil on net C and N fluxes. Undisturbed soil columns from underneath a Norway spruce stand (*Picea abies* L.) were used to test the following hypotheses: (1) increasing drought intensity reduces C and N fluxes from the soil during drought; (2) rewetting leads to a fast wetting pulse; (3) the size of the wetting pulse is depending on the intensity of the preceding drought; (4) the wetting pulse has only a minor impact on the overall C and N fluxes from the soil and cannot compensate for reduced C and N fluxes during the drought.

Material and Methods

Design of the mesocosm experiment

Undisturbed soil columns from underneath a 135 y-old Norway spruce (*Picea abies* L.) stand were sampled for this experiment. The stand is located in the "Fichtelgebirge" in NE Bavaria, Germany (50°8' N, 11°52' E) at an elevation of 775 m asl. Mean annual air temperature at the stand is 5.3°C and mean annual precipitation 1160 mm (Gerstberger et al., 2004). The forest floor at the stand is covered by ground vegetation, mainly *Calamagrostis villosa* and *Deschampsia flexuosa*, as well as *Oxalis acetosella* and *Vaccinium myrtillus*. According to the FAO soil classification (IUSS Working Group WRB, 2006), the soil at the stand is classified as a Haplic Podsol with a sandy-loamy texture, covered by a moder of 6-13 cm thickness consisting of Oi, Oe, and Oa horizons. A more detailed soil characterization can be found in Hentschel et al. (2007).

Soil sampling took place in the spring of 2006 by driving polyacrylic cylinders (ø 17.1 cm, 30 cm high) into the soil. The resulting soil columns were further prepared by removing all mineral soil and cutting off vegetation, resulting in undisturbed soil columns consisting only of the organic horizons, ranging in length from 7-13 cm. The prepared soil columns were stored at +5°C and field water conditions for several weeks before starting the experiment.

For the experiment, 20 soil columns were separated in 5 groups, each consisting of 4 replicas. Four of these groups were designed for regular measurement of CO_2 fluxes and soil solution composition. The first of these groups was designed as a control, the three other groups were designed to investigate the effects of different drought intensities and subsequent rewetting and will hereafter be called D1 to D3 (Drought treatment 1 to 3, with drought

intensity during the drying period increasing from D1 to D3). The remaining fifth group was designed for destructive soil sampling (Batch experiment) during the drying period to be able to measure important soil parameters.

With the beginning of the experiment, all soil columns were transferred to a climate chamber at constant +15°C. The cylinders were placed on ceramic base plates and covered with polyacrylic lids, resulting in gas-tight mesocosms. During a 34 d pre-run period (days -34 to -1, as day 0 is defined here as the beginning of the drying) we measured inherent differences between the treatments (for a schematic schedule see Figure 1). During this pre-run period the soil was irrigated with 4 mm artificial throughfall solution per day, representing the mean daily precipitation at the field site. The composition of the artificial throughfall solution was similar to the composition of the natural throughfall at the stand (Matzner et al., 2004) containing the following components (µmol L^{-1}): DOC 0.0, NH_4^+ 100.0, NO_3^- 100.0, Ca^{2+} 27.6, K^+ 57.5, Mg^{2+} 7.5, Na^+ 15.5, Cl^- 45.0, PO_4^{3-} 1.8, SO_4^{2-} 48.2 The solution was applied in 2 mm portions using a syringe with a fine nozzle.

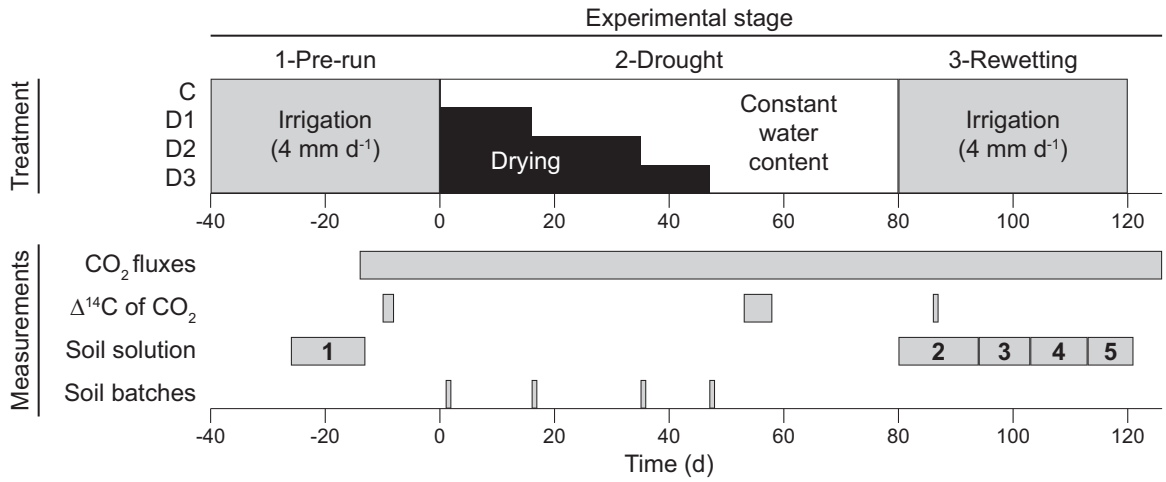

Figure 1 Schematic overview of the experimental schedule. During the pre-run period, all soil columns were irrigated with the mean daily precipitation amount at the field site. During drying, the control (C) was kept at constant water content whereas the three drought treatments (D1 to D3) where dried for different lengths of time and then kept at the new, reduced water content. At the beginning of the rewetting period, all soil columns were irrigated with 4 mm d^{-1} of artificial throughfall.

At the end of the pre-run period, the drying period began (days 0 to 80): Whereas the control was kept at constant water content throughout this period, the base plate and the lid were removed from the drought treatments for several hours a day and the soil was dried *via* ventilation with dry air. To monitor development of the drying, both gravimetric water content and remaining initial respiration were measured. Experimental design scheduled three different drought intensities for the three drought treatments, ranging from moderate to heavy drought. As CO_2 fluxes were a major focus of this experiment, the drought treatments were air

dried until measured CO_2 fluxes from the soil in D1, D2 and D3 were reduced to 65, 45 and 20% of the initial CO_2 fluxes measured during the pre-run period. As soon as the planned CO_2 flux was reached soil was kept at constant water content. This way, drying took 16 d for D1, compared to 35 and 47 d for D2 and D3, respectively. 80 d after beginning of the drying period rewetting of the dry soil was started by applying 4 mm d^{-1} of the artificial throughfall solution for each of the next 40 d (days 81 to 120). 126 d after beginning of the drying the experiment was stopped and soil columns were analyzed for gravimetric water contents, C and N content.

Gravimetric water content and matric potential

Columns were weighed regularly during the course of the drying and rewetting period. Gravimetric water contents were measured destructively after the experiment was finished and calculated back for all weighting dates. Calculation of matric potentials from specific gravimetric water contents was carried out by multiplication with mean soil bulk density to get volumetric water contents and then using the van Genuchten model (van Genuchten, 1980) to calculate matric potentials from volumetric water contents:

$$\theta(\psi) = \theta_r + \frac{\theta_s - \theta_r}{\left[1 + \left(\alpha|\psi|\right)^n\right]^m} \quad (1)$$

with: $\theta(\psi)$ = volumetric water content as a function of suction power [m^3 m^{-3}]; ψ = suction power [hPa]; θ_r = residual soil moisture [m^3 m^{-3}]; θ_s = saturation water content [m^3 m^{-3}]; α, n m = van Genuchten equation parameters with m=1-1/n.

Mean soil bulk density (0.17 g cm^{-3}) and the necessary van Genuchten parameters for this soil (θ_r = 0.005 m^3 m^{-3}, θ_s = 0.843 m^3 m^{-3}, α = 0.257, n = 1.238, m = 0.192) were taken from previous investigations with soil from the same site carried out by Zuber (unpublished data).

Measurement of CO_2 fluxes

For measuring CO_2 fluxes, the headspace volume of the mesocosms was connected to a software-controlled automated measurement system like described in Muhr et al. (2008). This system guaranteed constant CO_2 concentrations in the headspace of all 20 columns by ventilation at a mean rate (±SD) of 0.160 ± 0.015 L min^{-1} with air at ambient CO_2 concentrations. For measurements, ventilation of individual soil columns could be stopped automatically and fluxes were calculated by performing linear regression for the measured increase of CO_2 concentration over the measurement interval (precision of <1% of measured

concentration). Using the automated measurement system, up to 14 measurements of each of the soil columns could be made per day.

As mentioned above, the soil columns varied in size, and therefore in the amount of available C. To improve comparability, total weight and C content of the soil were determined at the end of the experiment to calculate the total C pool of each column. The measured CO_2 fluxes were then related to the C pool.

Radiocarbon signature

In each period (pre-run, drying, rewetting) gas samples were taken from the headspace volume above the soil to measure the $\Delta^{14}C$ signature of the emitted CO_2. Prior to sampling, ventilation was switched off and the whole system was flushed with CO_2 free synthetic air. CO_2 concentration inside the mesocosms was then allowed to increase over night. The next day, evacuated stainless steel cylinders with a volume of 2 L were connected to the mesocosms and slowly filled with gas from the headspace volume. Gas samples were then transferred to the laboratory for further processing.

Processing in general followed the zinc reduction method for preparation of AMS graphite targets described in Xu *et al.* (2007). The only notable difference was the injection of the gas samples into the extraction line: the steel containers containing the samples were connected to the extraction line *via* a digital mass flow controller (Type F-201C-AAA-33-V, Wagner Mess- und Regeltechnik) effectively limiting the flow rate to 20 ml min^{-1}. This way, extraction of CO_2 from the air inside the containers took around 3 hours per cylinder. All preparation took place at the laboratories of the Department of Soil Ecology at the University of Bayreuth. Graphite targets were analyzed by the Keck Carbon Cycle AMS facility at UC Irvine, USA with a precision of 2-3 ‰. Radiocarbon data are expressed as $\Delta^{14}C$, which is the per mil deviation from the $^{14}C/^{12}C$ ration of oxalic acid standard in 1950. The sample $^{14}C/^{12}C$ ratio has been corrected to a $\delta^{13}C$ value of -25‰ to account for any mass dependent fractionation effects (Stuiver and Polach, 1977).

Analysis of soil solution

Via the ceramic base plates (pore size 1 µm), pre-filtered soil solution could be extracted from the mesocosms. During the pre-run period, the first sampling of soil solution took place between day -26 and day -13. Soil solution was extracted by applying a negative pressure of -350 mbar. During drying soil solution sampling was not possible. Soil solution was sampled again during the rewetting period. Soil columns were freely draining during this period and

sampling took place without the application of a negative pressure. 4 subsequent samples were taken during this period, each integrating over 9-13 d of sampling (cf. Figure 1).

Soil solution was filtered using 0.45 µm polycarbonate membrane filters (Millipore), and stored at +2°C. Soil solution was then analyzed for dissolved organic carbon (DOC) and total nitrogen (N_{tot}) by high temperature analyzer (Elementar, high-TOC), for ammonium (NH_4^+-N) by flow injection analyzer (MLE, FIA-LAB) and for nitrate (NO_3^--N) by ion chromatograph (DIONEX, DX500 Chromatography system). Concentration of dissolved organic nitrogen (DON) was calculated as the difference between N_{tot} and inorganic N (the sum of NH_4^+-N and NO_3^--N). The fluxes were calculated by multiplying the concentrations in the soil solution with the measured water flux from the soil, with regard to input *via* artificial throughfall solution.

Batch experiment

In addition to the regularly measured soil columns, 4 columns for destructive soil sampling were included in the experiment (hereafter 'Batch columns', as batches of soil were removed on every sampling date). They were dried together with the drought treatments and used for analyzing concentrations of N_{tot}, NO_3^--N and NH_4^+-N in the soil during drought. Sampling took place on four different dates, and on each date, one quarter of each soil column was sampled. The first sampling took place at the beginning of the drying period (t = 0 d). The next sampling was carried out by the time when the drying of D1 was finished (t = 16 d). Sampling day 3 coincided with the end of the drying of D2 (t = 35 d) and sampling day 4 with the end of the drying of D3 (t = 47 d). Each time soil was removed from the batch columns, the sampled segment was replaced by foam to avoid lateral drying.

A subsample of each batch sample was used to determine gravimetric water content by drying the soil at 60°C in the drying oven. The rest of the samples were stored at +2°C until further analysis. For extraction, soil was mixed with Millipore water. The extract was shaken for one hour and then centrifuged for 15 min at 5250 rpm (5000 g). The supernatant was filtered using glass fiber filters (Schleicher and Schuell) and 0.45 µm polycarbonate membrane filters (Millipore). Analysis was equal to analysis of the soil solution samples.

Results

Matric potential

At the end of the pre-run period, water potential in all groups range between 0.01 and 0.1 MPa (pF 2-3, Figure 2a). During the drying period, matric potential of the control is kept constant at this level. The three drought treatments are dried out to a matric potential of -0.6, -80 and -400 MPa, respectively, and kept constantly around these values until rewetting. Irrigation during the rewetting period increases the water potential of all treatments, even of the control. This increase in the control to values between -0.001 and 0.01 MPa can be explained with differences in the soil solution sampling: Whereas soil solution during the irrigation period was sampled by free drainage, a negative pressure was applied during the pre-run period resulting in decreased water potentials. Matric potential of D1 to D3 increases to the pre-drought value of around -0.01 MPa within 8 d (D1) or 20 d (D2 and D3) of irrigation, respectively.

CO_2 fluxes

CO_2 fluxes during the pre-run period are similar for all treatments ranging between 8 and 12 mg C kg^{-1} SOC h^{-1} (Figure 2b, SOC = soil organic carbon). There is a general trend of decreasing CO_2 fluxes in all groups until day 60. Drying accelerates this decrease of the CO_2 fluxes in the drought treatments. During the first 3 d of the drying period, mean daily CO_2 fluxes decrease very fast, although mean matric potential of the soil change only slightly (Figure 3). Further reduction of the matric potential (t = 4-80d) has a smaller effect on observed CO_2 fluxes, but shows a clear logarithmic correlation ($r^2 = 0.74$). Ventilation drying of the different treatments was stopped after 16, 35 and 47 d but fluxes keep on decreasing for a few days even after ventilation was stopped. At the end of the drying period, mean CO_2 fluxes of the groups are 5.2 (control), 3.3 (D1), 1.4 (D2), and 0.5 (D3) mg C kg^{-1} SOC h^{-1}, so fluxes of the drought treatments are equivalent to 63, 27 and 10 % of the control fluxes. Differences between treatments and control are significant only for D2 and D3 ($p<0.05$, ANOVA).

The beginning of the rewetting causes a rapid increase of CO_2 fluxes. D1 and D2 regenerate back to the level of the control within around 5 d. CO_2 fluxes of D3 even exceed the level of the control fluxes for a few days. However, this excess is mainly owed to a drastic increase in only one of the replicas (replica no. 4) and therefore statistically not significant. 20 d after beginning of the rewetting, CO_2 fluxes of all treatments stabilize in the same pattern as

during the pre-run period, only at a lower level with fluxes ranging between 6 and 8 mg C kg^{-1} OC h^{-1}.

Cumulative CO_2 fluxes (Figure 4) during the pre-run period are identical for all treatments, ranging between 2.9 and 3.5 g C kg^{-1} SOC. During the drying period, 12.5 g C kg^{-1} SOC are emitted from the control, whereas total CO_2 flux from D1 was 9.0 g C kg^{-1} SOC and CO_2 fluxes from D2 and D3 are significantly ($p<0.05$) reduced with 6.5 and 5.4 g C kg^{-1} SOC. During the rewetting period, no differences are found between the treatments, with cumulative CO_2 fluxes of all groups ranging between 6.9 and 7.2 g C kg^{-1} SOC. Over the whole 126 d of the experiment, significantly less ($p<0.05$) C is emitted from the D2 and D3 treatments with 16.3 and 15.4 g C kg^{-1} SOC as compared to 22.9 g C kg^{-1} SOC that are emitted from the control. Total CO_2 flux from D1 is close to the control with 20.2 g C kg^{-1} SOC.

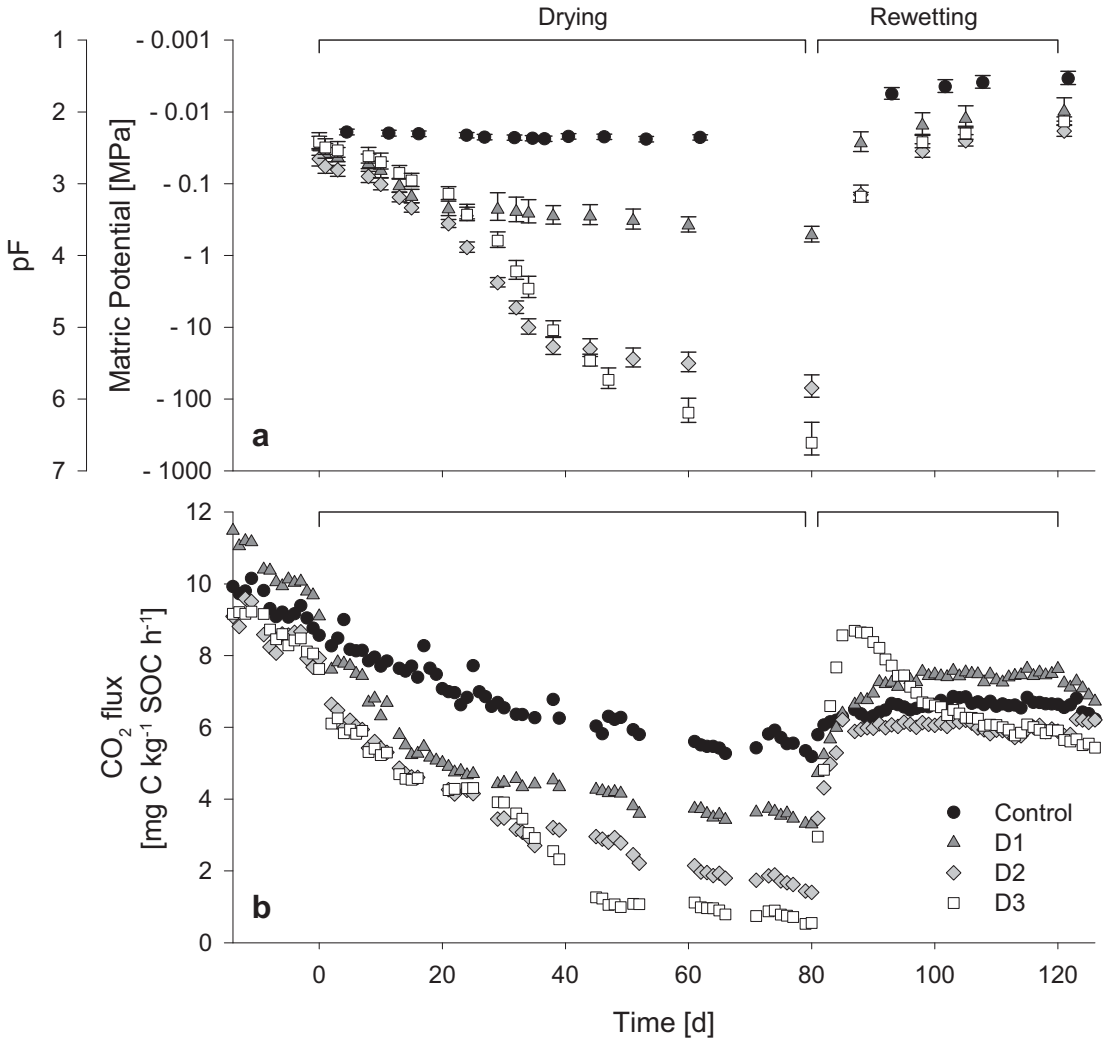

Figure 2 a) Mean (n=4) matric potential (±SE) of the control and the three different drought treatments (D1 to D3) from the beginning of the drying (t = 0 d) to the end of the experiment (t = 126 d). b) Mean daily CO_2 fluxes of the control and the three different drought treatments (D1 to D3). Mean values were from measurement data of 4 replicas per group and 2 to 14 measurements per replica and day. Error bars are omitted in favor of clarity.

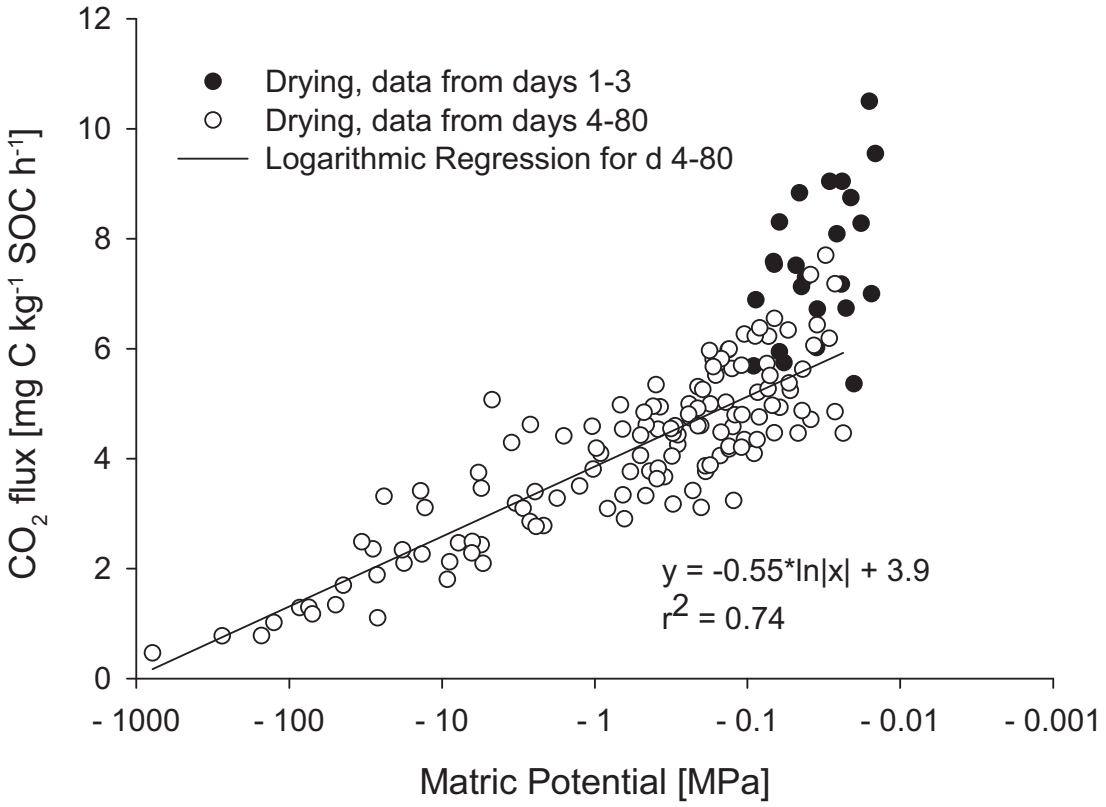

Figure 3 Correlation of observed CO_2 fluxes and matric potential in the D1 to D3 columns during the drying period. The correlation shown is calculated for data from d 4-80 of the drying period as data from d 0-3 shows a different correlation.

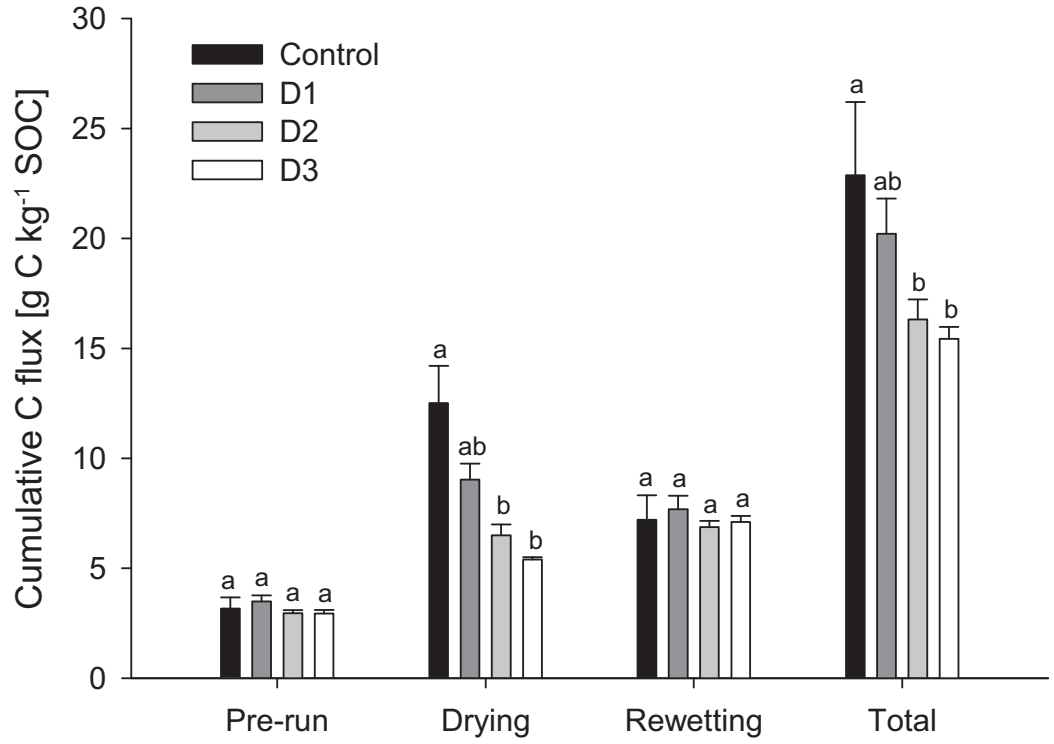

Figure 4 Mean (n=4) cumulative C fluxes (±SE) for the three separate periods and the whole experiment. Values without identical letters above the bars differ significantly ($p<0.05$).

Radiocarbon analysis

Mean radiocarbon signature of CO_2 emitted from the control shows a constant increase during the experiment, indicating mineralization of gradually older substrate (Figure 5). During the pre-run, radiocarbon signature of the control is at 106 ‰, increasing to 115 ‰ ($\Delta\Delta^{14}C$ = 9 ‰) during the drying period and 123 ‰ in the rewetting period. Radiocarbon signatures of two of the drought treatments also shows such an increase from pre-run to drying period, but at a smaller level (D1: $\Delta\Delta^{14}C$ = 6 ‰; D3: $\Delta\Delta^{14}C$ = 5 ‰). From drying to rewetting period, the radiocarbon signature in D3 remains constant. D2 differs from the other groups: No notable increase of $\Delta^{14}C$ from pre-run to drying, and in addition a drop of $\Delta^{14}C$ from drying to rewetting ($\Delta\Delta^{14}C$ = -10 ‰).

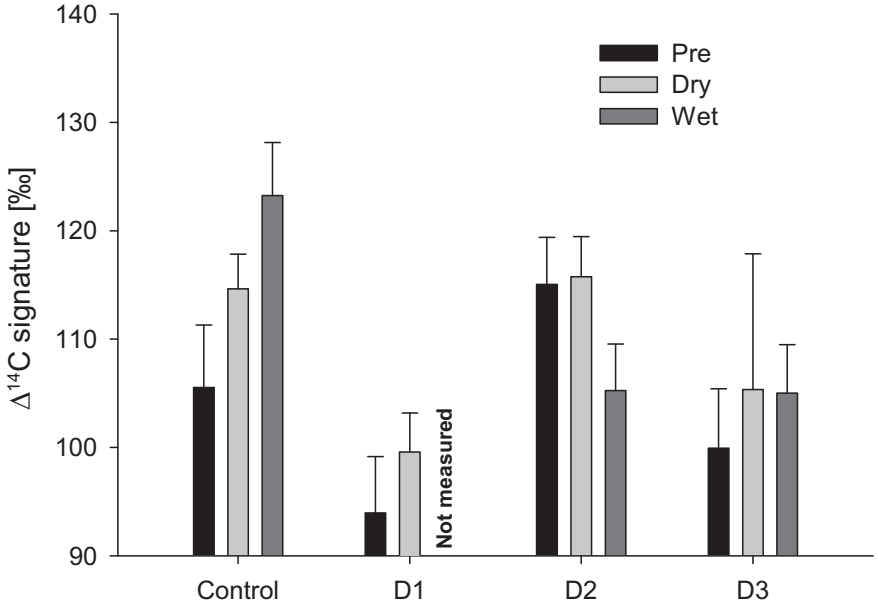

Figure 5 Mean (n=4) radiocarbon signature of emitted CO_2 in the control and of the drought treatments (D1, D2, D3) during the pre-run, drying and rewetting period.

It has to be mentioned, that the mean radiocarbon signature of D3 during the drying period excludes one of the columns (replica no. 4), as this one showed an extreme drop down to 55 ‰, possibly indicating contribution of pre-bomb C to emitted CO_2. This occurred only for one column, and only at extremely dry conditions.

Soil solution

Pre-run DOC concentrations are the same level for all treatments (around 30 mg C l^{-1}, cf. Figure 6). All treatments show only slightly increased DOC concentrations during the

rewetting period (around 50 mg C l^{-1}), except for treatment D3 with mean DOC concentrations of up to 110 mg C l^{-1}. However, this is only owed to extremely high concentrations of up to 240 mg C l^{-1} in one of the replicas (replica no. 4) and therefore statistically not significant, as the other three replicas all had DOC concentrations close to the control value. Calculated cumulative fluxes of DOC are not different for the treatments (Figure 6).

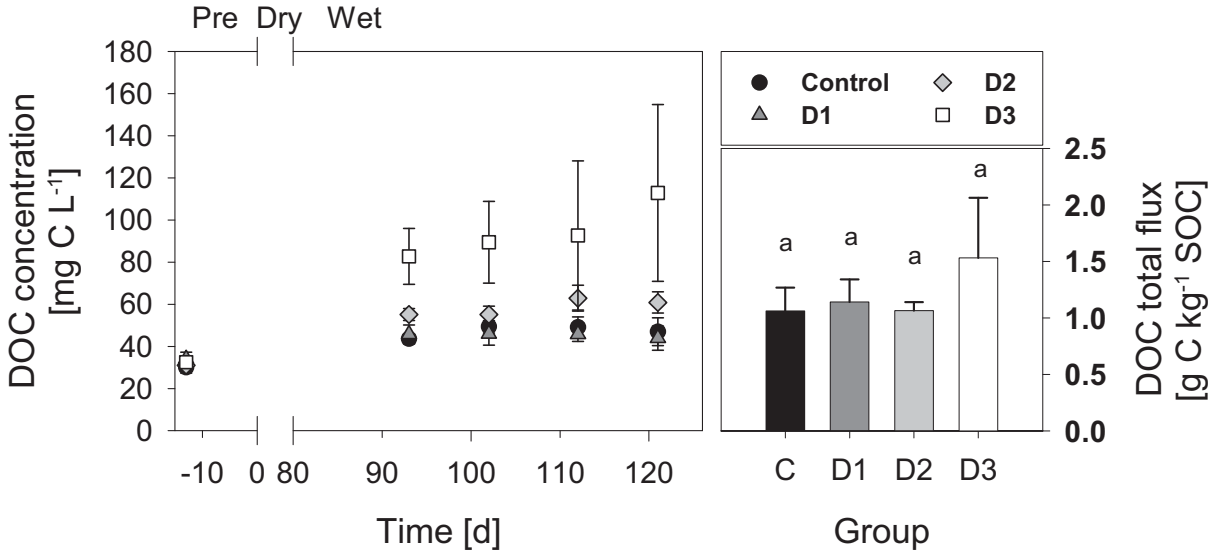

Figure 6 Mean (n=4) DOC concentration (±SE) and mean (n=4) total DOC flux (±SE) from the soil columns during pre-run, drying period and rewetting. Different letters above the total DOC fluxes indicate statistically significant differences.

The NH_4^+ concentration measured at the beginning of the rewetting period is significantly ($p<0.05$) increased for all groups as compared to the pre-run concentration of around 2 mg N l^{-1} (Figure 7). This increase is strongest for the control (ca. 28 mg N l^{-1}), whereas the NH_4 concentration in D1 to D3 increased to around 15 to 17 mg N l^{-1}. Differences wear off during rewetting, and at the end of the experiment the NH_4^+ concentration of all treatments is between 16 and 20 mg N l^{-1}. As the differences in concentration are limited to a short period of time, comparison if the total NH_4^+ fluxes reveals no significant differences between the treatments.

NO_3^- show a similar development (Figure 7). Like for NH_4^+, concentrations of the control are significantly higher at the beginning of the rewetting period (ca. 59 mg N l^{-1}) as compared to the pre-run period (ca. 23 mg N l^{-1}). Unlike NH_4^+, differences between the control and the drought treatments are far more pronounced, as the NO_3^- concentration of D1 to D3 does not increase at all from the pre-run to the rewetting. The temporary increase of the control concentrations wears off during the rewetting and in the end all treatments have NO_3^-

concentrations similar to the pre-run period again. Total NO_3^- fluxes are highest for the control (ca. 19 g N kg^{-1} N) and show a clear trend of becoming smaller with increasing drought intensity.

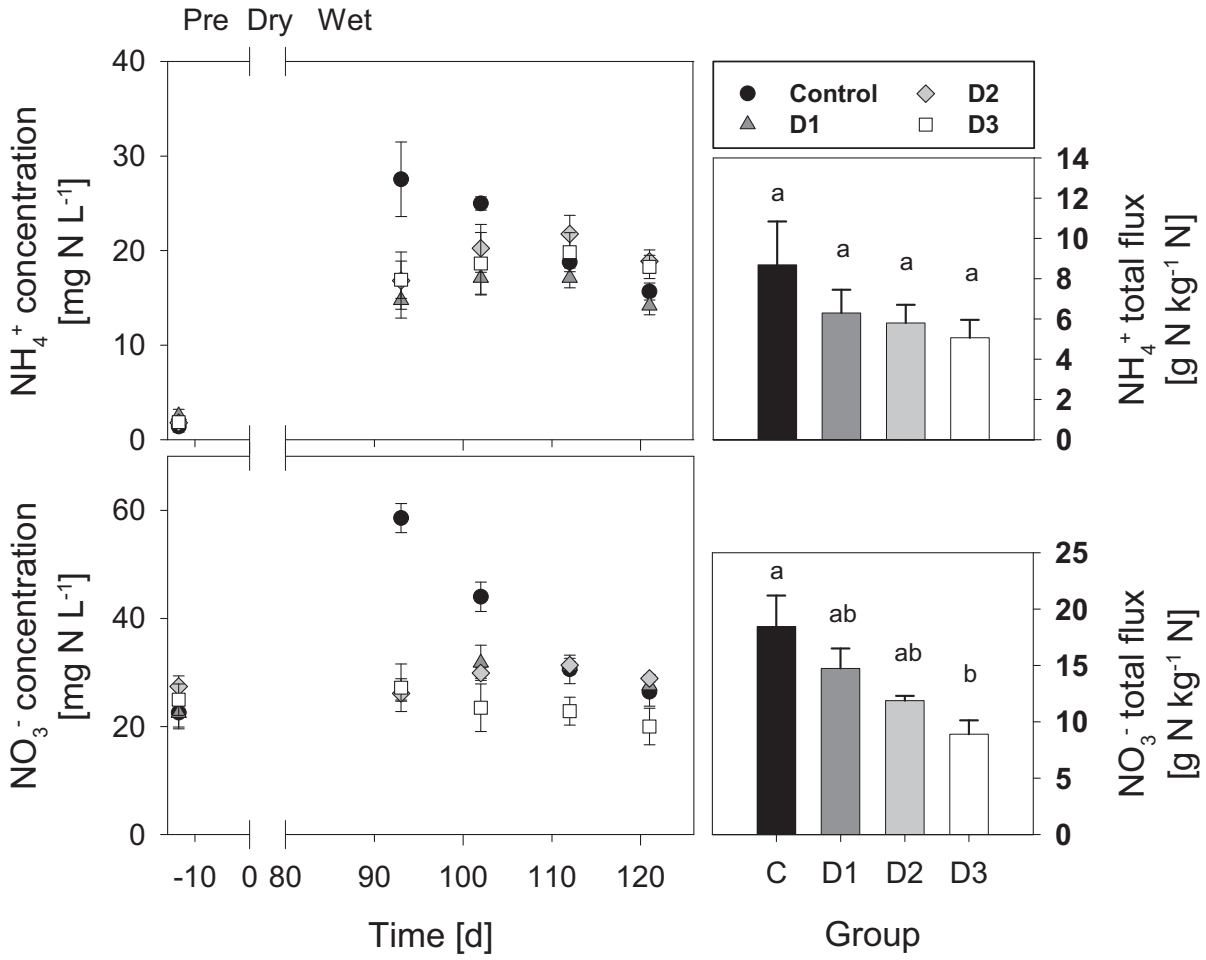

Figure 7 Mean (n=4; ±SE) concentration and total flux ammonium and nitrate measured in the soil solution samples. Samples were taken once during the pre-run period and four times during rewetting. No soil solution samples were taken during drying.

Batch experiment

The concentrations of both NH_4^+ and NO_3^- in the soil increase during the first 16 d of the drying period, indicating ongoing production of both NH_4^+ and NO_3^- down to a mean matric potential of -0.1 MPa (equal to pF 3, Figure 8). During the rest of the drying period, no significant further increase of NH_4^+ or NO_3^- is found.

The amount of DOC found in the batch columns does not vary over the whole drying and is stable at around 1.2 mg C kg^{-1} OC (data not shown).

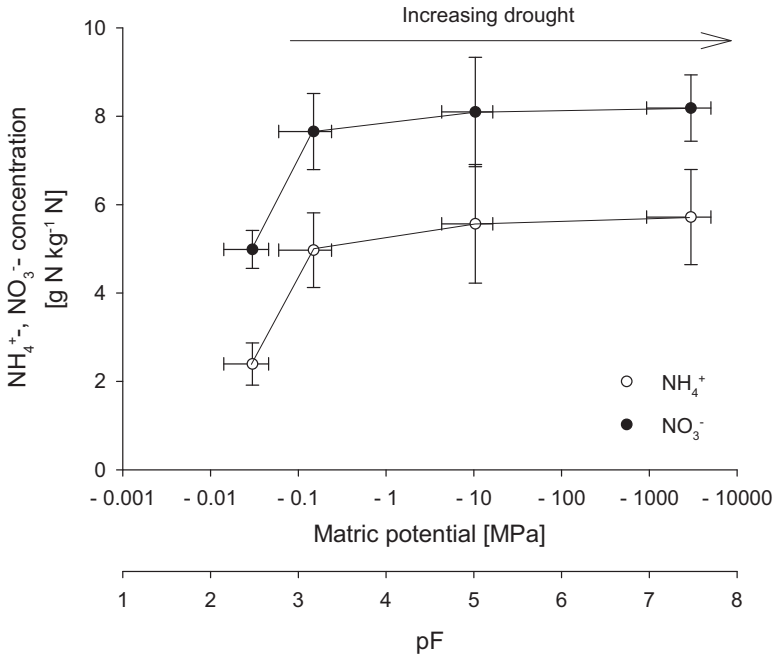

Figure 8 Mean (n=4, ±SE) NH_4^+- and NO_3^--concentration extracted from the soil of the batch columns at different matric potentials.

Discussion

C mineralization

Drying of the soil reduces the total CO_2 fluxes from the soil in all drought treatments compared to a continuously moist control. CO_2 fluxes react quickly to the beginning of the drying, decreasing from the first day on. This decrease is faster during the first 3 d than during later stages, which can be explained with the initial drying of the uppermost litter layer where a relatively high proportion of the respired CO_2 originates. Effective matric potential in these upper horizons is probably far more negative than the mean total matric potential of the soil columns, leading to an unrealistic correlation during these first 3 d. Subsequent drying in greater depth does have a smaller effect on the total CO_2 flux. The control also shows a decrease of the CO_2 fluxes over time. We explain this decrease with a reduction of the pool of bioavailable SOC sources due to ongoing mineralization. The observation during drying is in agreement with findings described by other authors (Degens and Sparling, 1995; Mikha et al., 2005; Chow et al., 2006; Xiang et al., 2008). Drying leads to a quick reduction of heterotrophic respiration, either because microorganisms become inactive or die.

Rewetting leads to an immediate increase of CO_2 fluxes in all treatments (even in the control treatments, resulting from decreased matric potentials due to soil solution sampling by the application of a negative pressure during the pre-run). This rapid increase can be

explained by fast regeneration of microbial activity. Microorganisms can survive even prolonged periods of drought (80 d in this experiment), e.g. by sporulation (Chen and Alexander, 1973) or simply by storing compatible solutes (cf. Jarvis et al., 2007; Schimel et al., 2007; Borken and Matzner, 2009). During rewetting, substrate diffusion increases; C and N rich osmolytes that are released by the microorganisms in reaction to the hypoosmotic shock can now be reassimilated by the cells (Halverson et al., 2000). Only the strongest drought treatment (D3) exceeds the CO_2 fluxes of the control for about 10 d, but this excess can not significantly increase the total CO_2 fluxes during the rewetting period and can in no way compensate for the significant reduction during the drying period, so drying-rewetting results in an overall reduction of C mineralization.

This contradicts the postulated enhancement of net C losses due to drying-rewetting events (Fierer and Schimel, 2002; Miller et al., 2005; Jarvis et al., 2007; Xiang et al., 2008). Our experiment reveals a strong reduction of CO_2 fluxes during drought compared to only a very weak burst for only the strongest drying treatment. These findings are best explained by the microbial stress mechanism, whereas the substrate supply mechanism is not supported by our results. In fact, our results indicate that mineralization is simply temporarily reduced by drought and regenerates again during rewetting. Substrates that were not used during the drought period, either because microorganisms did not have access (diffusive limitations) or because they alternatively used them as compatible solutes, can now be easily mineralized.

We measured radiocarbon signature of the emitted CO_2 to recognize shifts in the predominant substrate. The radiocarbon signature of the control steadily increases over the course of the whole experiment. This can be explained with increasing mineralization of older material with a higher radiocarbon signature, as the easily available, young substrate gradually decreases (Trumbore, 2000). D1 and D3 also show an increase of the radiocarbon signature from the pre-run to the drying period, although at a smaller level. Like in the control, we attribute this to a gradually increasing mineralization of older material: Following the $\Delta^{14}C$ sampling in the pre-run, soil is irrigated for another 8 d before the drying period starts, so mineralization of SOC continues at the level of the control for all treatments, before it decreases with decreasing matric potential. Under really dry conditions, however, when substrate mineralization is extremely small, $\Delta^{14}C$ should be conserved, unless new substrate was made available that has been physically or chemically protected before, like discussed in context of the 'Birch effect'. Such formerly stable substrate would have to be older, thereby having a significantly different radiocarbon signature and resulting in significantly different radiocarbon signature of the emitted CO_2. Conservation of the $\Delta^{14}C$ in D3 therefore supports

the idea that microbial mineralization is simply decreased during drought and increases again as living conditions improve.

The development of $\Delta^{14}C$ of D2 is different from the other groups and does not seem to fit the idea of a simple reduction of mineralization. This can be explained, however, with inhomogeneous drying of the soil during the drying period. In 3 additional soil columns we installed ECH_2O probes to measure water potential dynamics during drying in the Oe and the Oa horizon separately (data not shown). Although we dried the soil columns from the top and the bottom, drying speed of the Oe and Oa horizon is only identical for the first 20 d of drying. After this period, drying is faster in the Oe than the Oa. 35 d after beginning of the drying matric potentials in the Oe reach a constant level indicating complete drying. Drying in the Oa continues for another 10 d, so after 45 d we can assume homogeneous (dry) moisture conditions again. This means, that we had homogeneous moisture conditions in the control (no drying), D1 (16 d drying, <20d) and D3 (47 d drying, >45 d), whereas we have to expect inhomogeneous moisture patterns in D2 (35 d drying, Oe dried completely, Oa still drying). These inhomogeneous moisture patterns are likely to lead to a relatively increased contribution of CO_2 originating from the Oa horizon. Mean bulk radiocarbon signature of the Oa of this soil has been reported to be around 119 ‰, which is smaller than the bulk signature of the Oe with 161 ‰ (Schulze et al., 2009), so increasing contribution of the Oa to CO_2 fluxes is expected to reduce the resulting $\Delta^{14}C$. This reduction most likely masks the increase due to ongoing mineralization from pre-run to drying and partly explains the drop of $\Delta^{14}C$ from drying to rewetting.

Stable DOC concentrations in the batch columns during the drying period indicate that DOC production also is reduced during the drying period. Following the microbial stress mechanism, we would expect a short-timed increase of DOC concentrations due to the disposal of compatible solutes into the soil solution during rewetting. Owing to the nature of soil solution sampling we can not tell whether such an increase occurred: The first soil solution was sampled several days after rewetting, when mineralization would have decreased the easily available substrates already. Nevertheless we do see an increase of the DOC concentration in one of the replicas of D3 (replica no. 4). This soil core also shows the most pronounced CO_2 pulse during rewetting and has an extremely small $\Delta^{14}C$ of 55 ‰ during drying. This might encourage the idea that creation of new substrates indeed can take place. However, it has to be emphasized, that this one soil core also is the one with the most negative matric potential and the smallest CO_2 fluxes during the end of the drying period. In fact, CO_2 fluxes during drying are virtually zero, with respiration probably going on only at

some still moist microsites. The significant switch of $\Delta^{14}C$ is only due to a significant change of the relative contribution of substrates to the (extremely small) total CO_2 flux. Substrates at these microsites either are very young material or a mixture of young material and pre-bomb material. The pronounced CO_2 pulse during rewetting can be explained with a higher degree of conservation of easily available substrate during drying and with more water stress for the microorganisms. Therefore, we come to the conclusion that even this most extreme soil core does in no way support the idea of enhanced C mineralization due to drying-rewetting in this soil.

N mineralization

NH_4^+ concentrations during the pre-run period are surprisingly small in all treatments compared to the irrigation period. This might partly be explained by the fact that soil columns were sampled at the field site during early spring when the soil microbial community still is undergoing the change from winter to summer both in quantity and quality.

Net N losses are reduced by drought compared to a constantly moist control. Increasing NH_4^+ and NO_3^- concentrations in the control indicate accumulation of NH_4^+ and NO_3^- due to ongoing net N mineralization (defined as the sum of ammonification and nitrification) during the sampling break between pre-run and rewetting. The increase of NH_4^+ concentrations in the drought treatments during the same time is much smaller (only 60% of the control), indicating that net ammonification is going on during drought but at reduced rates. Net nitrification on the other hand is virtually zero during drought. Consequently, cumulative N losses from the drought treatments are reduced compared to the control. This reduction is independent from drought intensity for net ammonification, whereas net nitrification decreases with increasing drought intensity. This difference indicates that the reduction of net nitrification is not simply due to reduced NH_4^+ availability, but that water stress affects net nitrification rates in this soil directly.

It is unlikely that this effect can be attributed to increased immobilisation or denitrification: Previous work showed that NO and N_2O fluxes from this soil significantly decrease during drought (Muhr et al., 2008). This indicates a reduction of gross nitrification rates. As differences between treatments and control were restricted to the first two sampling dates after beginning of the rewetting of the dry soil, reduced microbial activity during drought due to reduced substrate diffusion and adverse living conditions is the most likely explanation for the reduction of net N mineralization. We therefore assume that autotrophic nitrifiers, which are known to be highly sensitive to water stress (Killham, 1990; Killham,

1994), play an important role in this soil. Results from our batch experiment reveal that this reduction net mineralization already occurs during the initial drying: NH_4^+ and NO_3^- concentrations increased only during the first 16d of drying (down to a minimum matric potential of around -0.1 MPa), almost no further increase was observed when soil was dried further.

In contrast to our results, other laboratory experiments investigating the effect of drying-rewetting repeatedly reported enhanced N losses (Marumoto et al., 1982; Seneviratne and Wild, 1985; Cabrera, 1993; Gordon et al., 2008; Xiang et al., 2008). This enhance was due to a significant increase of N mineralization during rewetting of dry soil, even exceeding the mineralization rates of a constantly moist control or during pre-drought conditions. We did not find such a wetting pulse. However, the mentioned experiments were carried out solely with sieved mineral soil from either arable land or grassland. Similar experiments carried out with intact forest soil cores (Hentschel et al., 2007) or sieved material from forest floor (Pulleman and Tietema, 1999) reported a reduction of cumulative N losses from the soil. Mikha et al. (2005), who found a reduction of cumulative N losses from arable land due to drying-rewetting attributed the occurrence of increased N mineralization in other experiments to the occurrence of soil physical disruption. This phenomenon is more likely to be of importance in mineral soil than in forest floor. However, previous experiments with mineral soil cores from this forest site (Hentschel et al., 2007; Muhr et al., 2008) also resulted in reduced C and N mineralization rates due to drying-rewetting, so we conclude that enhanced N mineralization (‘Birch’ effect) due to drying-rewetting events does not occur in this temperate forest soil. Drought does not make new substrates available but instead, like discussed for C mineralization, simply reduces N mineralization for some time until living conditions for the microorganisms improve again.

Conclusion

Drying is a process that regularly occurs in the forest floor of temperate forests and will even more so according to recent climate models. It could be shown in this work that prolonged drying (80 d) reduced net C and N losses from a Norway spruce forest soil in comparison to a constantly wet control. C losses decreased with decreasing matric potential. Wetting pulses could not compensate for the reduction during drying, and did not significantly exceed control fluxes. Rewetting seems to only reactivate mineralization that was reduced by drought. This reactivation by rewetting occurs fast, indicating that microorganisms in the forest floor are well adapted to regular drying-rewetting events. The

creation of new substrate from stable pools leading to excess C or N mineralization ('Birch effect') was not observed in this experiment. Radiocarbon signature did not indicate a change of the age of the mineralized C sources. Summarizing, it can be said that prolonged drought tends to conserve C and N pools in the forest floor. The assumption of enhanced C and N mineralization due to drying-rewetting can not be supported for this forest floor.

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PART B:

Delayed recovery of soil respiration after wetting of dry soil further reduces C losses from a Norway spruce soil

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Abstract

This experiment investigated the effects of prolonged summer drought on soil respiration (SR) in a mountainous Norway spruce forest in south Germany. On three manipulation plots we excluded summer throughfall in the years of 2006/2007 and measured SR fluxes in comparison to three control plots. Using radiocarbon measurements we quantified the contribution of rhizoshpere (RR) and heterotrophic respiration (HR) to total SR. In both manipulation years, mean CO_2 emissions (±SE) from the throughfall exclusion (TE) plots were smaller than from the control plots with 5.7 t C ha^{-1} (±0.3) compared to 6.7 t C ha^{-1} (±0.2) in 2006 and 5.9 t C ha^{-1} (±0.3) compared to 7.0 t C ha^{-1} (±0.4) in 2007. Under control conditions, CO_2 originated mainly from HR (60-95 % of SR). Prolonged drought reduced HR, whereas RR was not affected or even increased slightly. Reduction of CO_2 emissions on the TE plots was found up to 6 weeks after differences in matric potential conditions disappeared, possibly either because water repellency inhibited homogeneous rewetting of the organic horizons or because of severe damage to the microbial population. No evidence was found for the release of new, formerly protected substrates by preceding drought. Continuous measurements in 2008 (no manipulation) did not reveal increased CO_2 emissions on the TE plots that could compensate for the reduction during the years 2006/2007. Based on our results, we postulate a negative feedback between increased frequency and magnitude of summer droughts and SR in Norway spruce stands.

Keywords: Soil respiration, summer drought, radiocarbon, carbon dioxide, partitioning.

Introduction

Drought is one of the most common environmental stress that soil microorganisms experience. According to model simulations we face a globally increasing likelihood of severe drought periods in central Europe that will cause irregular and extreme water stress for soil organisms [*IPCC*, 2007]. This raises the question, how changes in the global water cycle will affect C mineralization in soils – a highly important question, regarding that soil respiration (SR) is the largest CO_2 flux from the terrestrial biosphere to the atmosphere [*Schlesinger and Andrews*, 2000].

Generally, it is understood that SR in well-drained soils is reduced during periods of drought. Liquid films become smaller and substrate accessibility for microorganisms *via* diffusion decreases [*Voroney*, 2007]. Furthermore, soil organisms have to respond to reduced soil water potentials. Strategies for surviving drought periods include the formation of dormant spores [*Chen and Alexander*, 1973] or the accumulation of compatible solutes [*Harris*, 1981, *Schimel et al.*, 2007]. Accumulation of compatible solutes demands high amounts of C and nitrogen (N) therefore further reducing SR. As soon as the dry soil is rewetted, these restrictions are lifted and SR regenerates quickly, sometimes within minutes [*Borken et al.*, 2003].

There are two fundamentally different mechanisms to explain mineralization during rewetting of dry soil: The "microbial stress" mechanism vs. the "substrate supply" mechanism [cf. *Xiang et al.*, 2008]. As mentioned above, accumulation of compatible solutes is one way for cells to survive periods of drought. Rewetting of the soil, resulting in a sudden increase of water potential, forces cells to dispose of these compatible solutes or risk cell rupture due to massive water uptake. The no longer needed compatible solutes are set free and can now be easily mineralized by microorganisms. The microbial stress mechanism postulates that the fast increase of SR during rewetting is mainly due to the mineralization of these compatible solutes, i.e. substrates that were already present but not available. The microbial stress mechanism bears no potential of creating new substrates. Instead, *Xiang et al.* [2008] postulated that it should result in a loss of microbial biomass thereby reducing the metabolic capability of the microbial pool.

However, findings by *Birch* [1958, 1959] revealed that the effect of drying-rewetting events can exceed a simple sequence of reduced and increased SR. He was the first to recognize that the cumulative CO_2 loss from soil that was subjected to drying-rewetting events can be higher than the losses from the same soil under constantly moist conditions. This indicates that - following the rewetting of dry soil - substrates were mineralized that were

not available before drought. This priming effect has recently been named the 'Birch effect' [*Jarvis et al.*, 2007]. This phenomenon is described by the substrate supply mechanism. It assumes the creation of new substrates that were not available until the drying-rewetting event occurred. Physical processes during rewetting of dry soil like e.g. aggregate disruption, organic matter redistribution or desorption result in the destabilization of formerly physically protected soil organic matter (SOM) pools and enhance labile C availability in soils. However, the occurrence of the Birch effect does not seem to be a universal phenomenon [cf. *Borken and Matzner*, 2009]. Whereas some authors report increased C losses due to drying-rewetting [*Seneviratne and Wild*, 1985, *Xiang et al.*, 2008], others did not [*Degens and Sparling*, 1995, *Muhr et al.*, 2008].

Radiocarbon (^{14}C) offers the possibility of detecting changes in the predominant sources of respired CO_2. Physically protected substrates that are made bioavailable by drying-rewetting should differ in their radiocarbon signature ($\Delta^{14}C$) from more recent C sources. The creation of new substrates from physically protected pools should therefore result in measurable changes of $\Delta^{14}C$ of respired CO_2.

The $\Delta^{14}C$ of respired CO_2 can also help breaking down SR into its components [*Dörr and Münnich*, 1986, *Gaudinski et al.*, 2000, *Schuur and Trumbore*, 2006]. CO_2 respired from soils originates from various sources. The CO_2 respired from roots, myccorrhiza or microbes in the rhizosphere that metabolize root exsudates normally has a $\Delta^{14}C$ close to that of CO_2 in the atmosphere, because recent photosynthates are the major C source. We group these sources of respiration as rhizosphere respiration (RR). The CO_2 respired by microorganisms that metabolize SOM normally has a $\Delta^{14}C$ that is different from that of atmospheric CO_2 in the year of the measurement and will here be called heterotrophic respiration (HR). During droughts source contributions to total SR may change: Whereas microorganisms are dependent on water in their immediate surroundings, trees, even shallow rooting *Picea abies* (L.) Karst. can lift up water from deeper horizons and redistribute it [*Nadezhdina et al.*, 2006]. It may be expected therefore, that RR is more resistant to drought stress than HR.

This experiment was designed to investigate the effect of prolonged summer drought followed by natural or artificial rewetting of the soil on SR underneath a Norway spruce stand (*Picea abies* (L.) Karst.). We excluded summer throughfall in two subsequent years (2006/2007) and measured one additional year without manipulation (2008) to test the following hypotheses: (1) SR is reduced during drought; (2) rewetting leads to a fast recovery of SR; (3) no additional substrates are mineralized by drying-rewetting, so that in the end (4) the cumulative CO_2 losses are reduced by drying-rewetting compared to control conditions;

(5) HR is stronger reduced by drought than RR; (6) reduced C losses in a dry year and the assumed substrate accumulation is compensated for by increased C losses in subsequent years.

Materials and Methods

Site description

The research site *Coulissenhieb II* is located in a mature Norway spruce forest (*Picea abies*, (L.) Karst., mean age 145 years) in the *Fichtelgebirge* in Southern Germany (50°08'N, 11°52'E) at an elevation of 770 m a.s.l. Mean annual air temperature is 5.3 °C and the mean annual precipitation (1971-2000) ranges around 1160 mm [*Gerstberger et al.*, 2004]. The understorey vegetation is dominated by *Calamagrostis villosa* (Chaix ex Vill), *Deschampsia flexuosa* (L.), *Vaccinium myrtillus* (L.) and *Oxalis acetosella* (L.). The soil is classified as a Haplic Podzol with a sandy to loamy texture according to the FAO soil classification [*IUSS Working Group WRB*, 2006]. The mor-like forest floor has a thickness of 6-10 cm and is composed of Oi, Oe and Oa horizons (Table 1). The pH ($CaCl_2$) value of the soil ranges around 3.3 in the Oa horizon and increases with depth to around 4.2 in the Bw and C horizon. Carbon contents decrease with depth, ranging around 40-50 % in the Oi and the Oe and less than 1 % in the C horizon [from *Schulze et al.*, 2009].

A storm event on Jan 18, 2007 severely damaged the research site, considerably thinning out the forest. All results beyond this date may therefore be subject to influence by the disturbance caused by the storm.

Experimental design

In the summer of 2005, three control and three throughfall exclusion (TE) plots were established, each covering an area of 20 m x 20 m. We chose one representative plot from each group for soil moisture measurements in the organic layer. Three ECH_2O EC-20 soil moisture probes (Decagon Devices, WA, USA) per plot were installed within the Oa horizon and logged automatically every hour. The ECH_2O probes were calibrated specifically for the Oa horizon of our site to calculate of volumetric water contents from the mV signal. To translate volumetric water contents into matric potentials, we used the van Genuchten model [*van Genuchten*, 1980]:

$$\theta(\psi) = \theta_r + (\theta_s - \theta_r)/[1 + (\alpha\,\psi)^n]^m \qquad (1)$$

with θ (ψ) = volumetric water content as a function of suction power [$m^3\ m^{-3}$]; ψ = suction power [hPa]; θ_r = residual soil moisture [$m^3\ m^{-3}$]; θ_s = saturation water content [$m^3\ m^{-3}$]; α, n, m = van Genuchten equation parameters with $m = 1 - 1/n$. The necessary parameters ($\theta_r = 0.000\ m^3\ m^{-3}$, $\theta_s = 0.860\ m^3\ m^{-3}$, $\alpha = 0.163$, $n = 1.209$, $m = 0.173$) were determined on soil from our site by Tobias Zuber (unpublished thesis).

Soil moisture in 20 cm mineral soil depth was measured on all six plots by two to four custom-built, calibrated tensiometers per study plot and automatically logged hourly. Soil temperature in the organic layer was measured on every plot by custom-built temperature data loggers. Throughfall was measured by custom-built rainwater collectors that were emptied regularly.

Control plots were used to asses the natural dynamic of all measured parameters without any experimental disturbance. TE plots were equipped with a wood-structure that was covered with transparent plastic sheets during the manipulation periods to exclude throughfall on the entire plot area (400 m^2). Roofs were built beneath the forest canopy, about 2.5 to 3 m above the forest floor. Rainwater falling on the roofs during the TE period was channelled through

Table 1 Mean thickness of the horizons, bulk density (BD), pH (CaCl2), carbon content and bulk radiocarbon signature of a Podzol soil from a Norway spruce stand in the *Fichtelgebirge*. Values represent mean values from nine soil profiles (radiocarbon signature: three soil profiles). Numbers in parentheses give the standard deviation of the mean (changed from *Schulze et al.* [2009]).

Horizon	Thickness	BD	pH	C	$\Delta^{14}C$
	[cm]	[g cm^{-3}]	$CaCl_2$	[%]	[‰]
O_i	2.1	0.07		45.8	113.8
(±SD)	(0.1)	(0.00)		(0.9)	(8.0)
O_e	2.2	0.15		42.1	161.5
(±SD)	(0.2)	(0.02)		(6.3)	(16.0)
O_a	4.9	0.25	3.3	21.2	119.0
(±SD)	(0.5)	(0.03)	(0.2)	(2.3)	(12.4)
Ea	5.2	0.60	3.4	8.3	23.0
(±SD)	(0.8)	(0.02)	(0.2)	(0.9)	(68.3)
Bsh	5.3	0.75	3.6	6.0	-13.8
(±SD)	(0.6)	(0.01)	(0.2)	(0.4)	(12.9)
Bs	11.4	0.79	3.8	3.6	-63.2
(±SD)	(1.5)	(0.02)	(0.1)	(0.3)	(10.5)
Bv	30.5	1.17	4.2	1.4	-145.4
(±SD)	(3.1)	(0.03)	(0.1)	(0.2)	(18.1)

rain gutters and water pipes over a distance of ca. 35 m before it could soak into the ground outside the plots. By trenching the TE plots down to a depth of approx. 0.4 m lateral water inflow or uptake by roots was reduced. Roofs on the TE plots were closed from 22 June to 8 August 2006 and from 2 July to 13 August 2007. In 2006, 67 mm of throughfall were excluded, compared to 121 mm in 2007. In the year 2006 we irrigated the TE plots for two days with a total of 67 mm artificial throughfall solution *via* a sprinkler system at the very beginning of the post-treatment period. By irrigating the plots, we guaranteed that the total amount of throughfall was the same on TE and control plots, so we only changed the precipitation pattern. Based on the results in 2006 and the enormous logistic effort, we decided to omit irrigation in 2007. The manipulation in 2007 therefore was a combination of prolonged summer drought and a reduction of total annual througfall.

The period before closure of the roofs, when control and TE plots both received the same amount of throughfall, will be addressed as the pre-treatment period here, the manipulation period, when roofs were closed on the TE plots will be called the TE period. The rest of the year, beginning with the re-opening of the roofs will be addressed as post-treatment period here. An overview over the exact schedule in the measurements can be found in Table 2.

Table 2 Schedule of the experiment showing starting dates and duration of the different periods in the measurement years (Pre = period before the manipulation of the respective year, TE = manipulation period, when roofs on the TE plots are closed, Post = period from the reopening of the roofs until the end of the year).

Period		**2006**	**2007**	**2008**
Pre	Start	01.01.2006	01.01.2007	**No manipulation**
	Duration (d)	172	182	
TE	Start	22.06.2006	02.07.2007	
(Manipulation)	Duration (d)	48	43	
Post	Start	09.08.2006	14.08.2007	
	Duration (d)	144	139	

Measurement of CO_2 fluxes

In each of the six plots, three plastic collars with a length of 20 cm and an inner diameter of 49.5 cm were installed permanently for SR measurements. The collars were driven 5 cm into the forest floor several months before the first measurements. Positions of the collars were chosen randomly on the plots. For gas measurements, the collars were manually closed with a plastic lid and connected to a portable infrared gas analyzer (IRGA, Li-820 from Li-Cor Biosciences GmbH). Air was circulated in this closed system by a pump at a constant

flow rate of 0.5 l min^{-1} and the CO_2 concentration inside the chamber was logged every 10 s for a period of 3-5 min. A linear regression was performed on the increasing CO_2 to determine a flux rate, which was corrected for atmospheric pressure and chamber air temperature. Measurements were conducted simultaneously on the control and the TE plots, and always between 9:00 and 12:00 am. For more details on the method see *Borken et al.* [2006].

Radiocarbon signature of CO_2 from SR, incubated roots and root-free soil

The radiocarbon signature of SR ($\Delta^{14}C_{SR}$) was measured on eight occasions during the two manipulation years 2006/2007 (Table 3). However, data from one of these dates (Jun 18, 2007) was discarded due to extreme variation. We can not eliminate the possibility that the damage from a storm in Jan 2007 partly influenced the $\Delta^{14}C$ data of 2007 and caused high variation in late spring.

For measuring $\Delta^{14}C_{SR}$, we used the same chambers as for SR measurements. Two of these chambers on each plot (resulting in a total of six replicates per treatment) were closed and then flushed with CO_2-free synthetic air for 90 min at a moderate flow rate of 1.5 l min^{-1} thereby effectively flushing the respiration chambers with an amount of gas equal to at least three times the chamber volume. Following flushing, the respiration chambers were sealed and left until the CO_2 concentration inside the chambers reached at least 1500 ppmv. Incubation time depended on CO_2 flux rates at the sampling day. Evacuated stainless steel sampling cylinders (2 l) were connected to the respiration chambers and slowly filled with gas from inside the chamber. *Via* mass-flow controllers the cylinders were connected to a high-vacuum extraction line at the University of Bayreuth. CO_2 was cryogenically purified and converted to graphite targets using the modified sealed tube zinc reduction method described by *Xu et al.* [2007]. Graphite targets were analyzed by the Keck Carbon Cycle AMS facility at UC Irvine, USA with a precision of2-3 ‰. Radiocarbon data are expressed as $\Delta^{14}C$, which is the per mil deviation from the $^{14}C/^{12}C$ ratio of oxalic acid standard in 1950. The sample $^{14}C/^{12}C$ ratio has been corrected to a $\delta^{13}C$ value of -25 ‰ to account for any mass dependent fractionation effects [*Stuiver and Polach*, 1977].

To measure the radiocarbon signature of rhizosphere respiration ($\Delta^{14}C_{RR}$), we excavated live roots of Norway spruce from the forest floor from three locations nearby our field plots and on three dates in 2007 (Table 3). Excavated roots were washed with deionised water to remove soil particles clinging to the roots and then transferred to gastight incubation containers. Prior to the incubation the containers were flushed with CO_2-free synthetic air to

Table 3 Sampling dates for the four different types of $\Delta^{14}C$ samples.

Date	Sample type			
	Soil respiration	Heterotrophic	Rhizosphere	
			Atmosphere	Roots
Mar 6 2006	-	-	x	-
Jun 8, 2006	x	-	x	-
Aug 3, 2006	x	x	x	-
Aug 16, 2006	x	x	x	-
Nov 14, 2006	-	-	x	-
Mar 15, 2007	x	x	x	-
Jun 19, 2007	x	x	x	x
Aug 9, 2007	x	x	x	x
Oct 16, 2007	x	x	x	x

remove all atmospheric CO_2 and then incubated for 1-2 days in the laboratory at a constant temperature of +15 °C. Gas samples again were taken by connecting an evacuated sampling cylinder that was then opened slowly to take in gas from the incubation container. As the $\Delta^{14}C$ of CO_2 respired by roots usually is very close to the $\Delta^{14}C$ of CO_2 in the atmosphere [*Trumbore*, 2006], we also measured the atmospheric $\Delta^{14}C$ on nine dates during 2006 and 2007 by filling evacuated sampling cylinders with gas from the atmosphere at 2 m above ground. No significant differences between the $\Delta^{14}CO_2$ of the atmosphere and RR were found and so $\Delta^{14}C_{RR}$ was calculated as the mean of all root-incubation (n=9) and atmospheric samples (n=20).

$\Delta^{14}C$ originating from the mineralization of SOM was determined by incubating root-free soil cores from the uppermost 25-30 cm of our field plots including the Oi, Oe, Oa, Ea, Bsh and part of the Bs horizon. After soil was stored at +5 °C for four to six weeks, roots were manually removed and the disturbed soil from each soil core was transferred to one incubation container. After flushing the incubation containers with CO_2-free synthetic air to remove atmospheric CO_2, the soil was incubated for 1-2 days at a constant temperature of +15 °C. Following previous work by *Dioumaeva et al.* [2003] and more recent experiments by *Czimczik and Trumbore* [2007], temperature does not affect the $\Delta^{14}C$ of evolving CO_2. Gas from the containers was sampled using stainless steel containers and processed like described before. The $\Delta^{14}C_{HR}$ values did not show a high temporal heterogeneity, therefore all results were used to calculate one uniform mean $\Delta^{14}C_{HR}$ value (±SE) of 93.4 ‰ (±1.7; n=33) for partitioning.

To partition SR into RR and HR, we used the isotopic method making use of bomb derived ^{14}C explained in greater detail by *Gaudinski et al.* [2000] or *Schuur and Trumbore* [2006]. The basic idea is measuring the $\Delta^{14}CO_2$ and the flux of total SR, as well as the $\Delta^{14}CO_2$ of the two constituents of SR (RR and HR) and then applying a mass balance to calculate the fluxes of the two constituents. A major advantage of this method is that it can be carried out mostly under field site conditions and with minimal disturbances. The basic equations applied in this approach are equations (2) and (3):

$$F_{CO_2,SR} = F_{CO_2,HR} + F_{CO_2,RR} \quad (2)$$

$$F_{CO_2,SR} \times \Delta^{14}C_{SR} = F_{CO_2,HR} \times \Delta^{14}C_{HR} + F_{CO_2,RR} \times \Delta^{14}C_{RR} \quad (3)$$

where $F_{CO2,SR}$ is the CO_2 flux of total soil respiration, $F_{CO2,HR}$ the flux of heterotrophic respiration and $F_{CO2,RR}$ the flux of rhizosphere respiration, all in mmol m^{-2} h^{-1}. $\Delta^{14}C_{SR}$ is the radiocarbon signature of the total soil respiration, $\Delta^{14}C_{HR}$ of the heterotrophic respiration and $\Delta^{14}C_{RR}$ of the rhizosphere respiration, all given in ‰.

Data analysis

Mean values for SR and $\Delta^{14}C$ measurements were formed for the nine control and the nine TE chambers. Data were analysed using STATISTICA 6.1. Differences in fluxes of SR and its $\Delta^{14}C$ between the treatments were tested using the non-parametric Mann-Whitney U-Test. Comparing values of the same treatment at different dates was achieved by using the Tukey HSD test.

Calculation of cumulative CO_2 emissions was achieved by linear interpolation between adjoining measurement dates for each individual chamber. For statistical analysis, the cumulative C emissions for the nine control chambers and the nine TE chambers were compared using the non-parametric Mann-Whitney U-Test.

Source partitioning was calculated by using formulas developed by Philips and Gregg [2001], accounting for variability in the isotopic signatures of both the sources ($\Delta^{14}C_{RR}$, $\Delta^{14}C_{HR}$) and the mixture ($\Delta^{14}C_{SR}$). When multiplying the calculated source proportions with mean SR fluxes, standard errors were calculated accounting for error propagation.

Results

Throughfall, soil moisture and soil temperature

The year 2006 was dry, with only 868 mm of throughfall, followed by a relatively wet year (2007) with 1152 mm and another dry year (2008) with 924 mm (Fig. 1a). During the manipulation period, 67 mm of throughfall were excluded in 2006, compared to 121 mm in 2007 (no manipulation in 2008). Matric potential measured beneath the Oa horizon varied between values of pF 2 (moist) and pF 5-6 (very dry) in the observed years (Fig. 1b). Driest conditions were found during the summer of 2006, when a period of natural drought hit both the control and the TE plots. A period of low matric potentials in the winter of 2008 is attributed to freezing of soil water due to deeply penetrating soil frost. Differences between control and TE plots were found only during summer time: In the summer of 2006, control plots were naturally dry with pF values around 5 compared to even drier conditions on the TE plots (pF ca. 6). In 2007, no water stress was observed on the control plots in summer (pF ca. 3), whereas the TE plots experienced a moderate drought (pF ca. 4). Although matric potentials regenerated quickly after natural rewetting of the TE plots in 2007, the volumetric water content of the organic horizon on the TE plots remained significantly reduced until winter (data not shown). It should be emphasized that the measurements represent the matric potential at the transition between organic and mineral horizon and that differences between TE and control plots within the organic horizons or even on top of the forest floor are expected to be more pronounced.

Matric potential in 20 cm mineral soil depth was slightly affected by TE in both manipulation years, resulting in more negative matric potentials on the TE plots (Fig. 1c). In the naturally dry year of 2006, TE resulted in a minimum matric potential of at least -650 hPa (tensiometers failed at this point; pF 2.8) compared to ca. -400 hPa (pF 2.6) on the control plots. In the wet summer of 2007, matric potential on the control plots ranged around -50 hPa (pF 1.7), compared to a minimum soil matric potential of -200 hPa (pF 2.3) on the TE plots. In 2008, TE and control plots showed the exact same minimum soil matric potential of ca. -300 hPa (pF 2.5). Soil temperature was not affected by the manipulation and therefore followed the same seasonal dynamic on both the control and the TE plots (Fig. 1d).

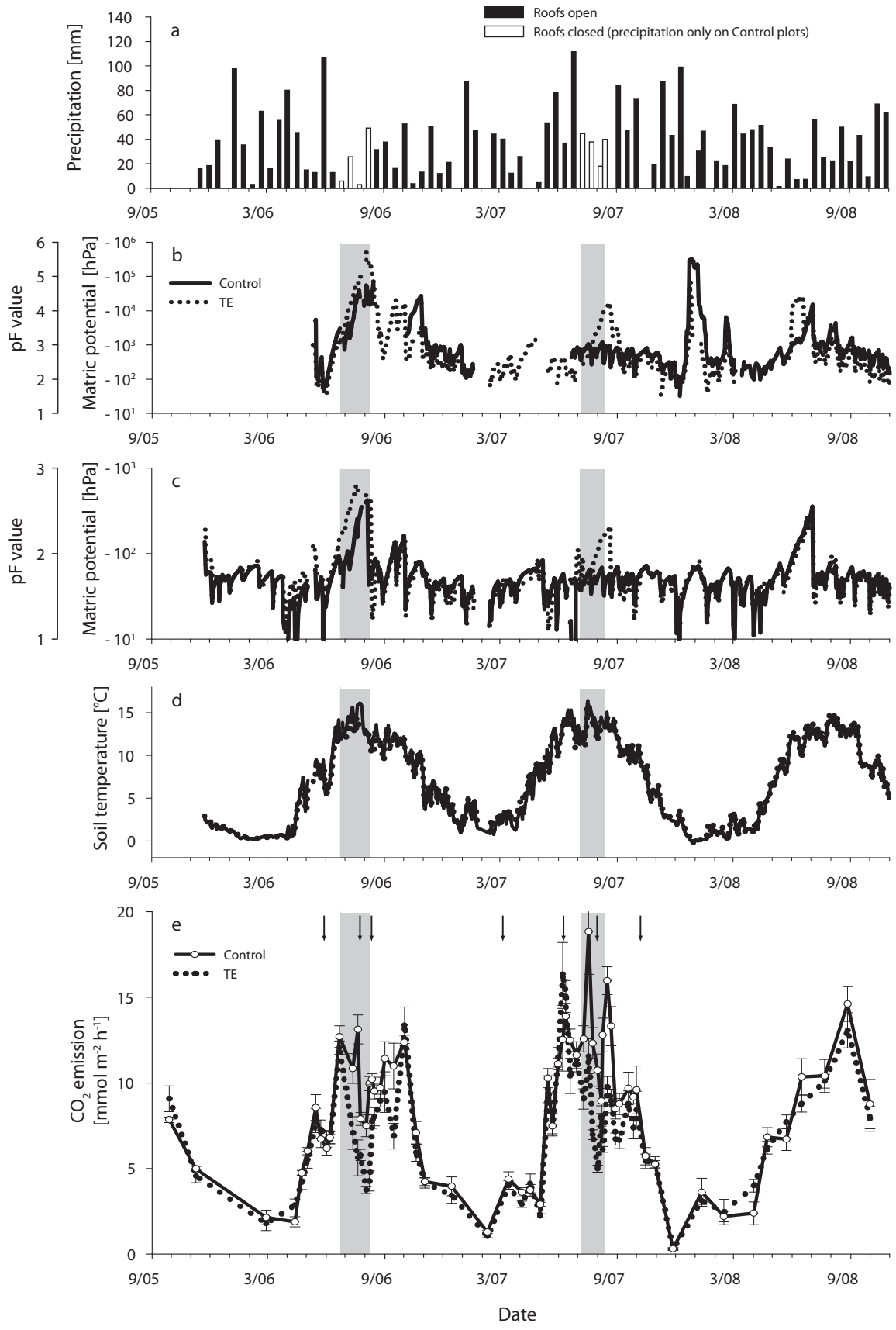

Figure 1 a) Throughfall, matric potential b) beneath the Oa horizon and c) in 20 cm mineral soil depth, d) soil temperature 10 cm below the forest floor surface and e) mean (n = 9) soil respiration (±SE) on the control and the throughfall exclusion (TE) plots from September 2005 to November 2008. Grey areas indicate periods when roofs were closed, arrows indicate $\Delta^{14}C$ sampling dates.

Soil respiration

In the two manipulation years, significantly less CO_2 was emitted from the TE plots compared to the control plots ($p < 0.05$; cf. Tab. 4). These differences on an annual basis resulted from reduced CO_2 emissions both during the TE period as well as the post-treatment period (Fig. 1e; Tab. 4). No significant differences were found during the pre-treatment period, prior to the closure of the roofs. In the year 2006, 6.7 t C ha^{-1} (±0.2) were emitted from the control plots and only 5.7 t C ha^{-1} (±0.3) from the TE plots (mean values from nine measurement chambers ±SE), resulting in a difference of ca. 0.9 t ha^{-1}. Of this total difference of 0.9 t ha^{-1}, 50 % (or approx. 0.5 t C ha^{-1}) can be explained by decreased CO_2 emissions during the TE period itself. Another 44 % of the total reduction (approx. 0.4 t ha^{-1}) can be explained by reduced CO_2 emissions during the post-treatment period.

In 2007, 7.0 t C ha^{-1} (±0.4) were emitted from the control plots, compared to only 5.9 t C ha^{-1} (±0.3) from the TE plots, so total emissions from the TE plots in 2007 were smaller by approx. 1.1 t C ha^{-1}. Again, most of this difference (54 % or approx. 0.6 t C ha^{-1}) can be explained by reduced emissions during the TE period itself. Reduced CO_2 emissions during the post treatment period explain 33 % or approx. 0.4 t C ha^{-1}.

In the year 2008, no manipulation was carried out. Mean total CO_2 emissions from the control and the TE plots were identical in this year, both ranging around 6.7 t C ha^{-1}.

Dynamics of $\Delta^{14}C$ of SR

During the pre-treatment period in the year 2006 (Jun 8, 2006), we measured little variation in the $\Delta^{14}C_{SR}$ on all plots, and we found no significant differences between TE and control plots with mean values (n = 6, ±SE) of 88 ‰ (±2.4) and 91.4 ‰ (±2.2), respectively (Fig. 2). Measurements during the first TE manipulation period (Aug 3, 2006) revealed much higher variation within both treatment groups, and $\Delta^{14}C$ values beneath contemporary atmosphere occured, indicating the influence of pre-bomb carbon. Variation of measured $\Delta^{14}C_{SR}$ decreased considerably as soon as soils were rewet again during the post-treatment period (Aug 16, 2006), and mean values on TE and control plots revealed no significant differences with 78.6 ‰ (±3.0) and 81.0 ‰ (±2.4), respectively.

Table 4: Cumulative C emissions from the control and the TE plots. Cumulative C emissions were calculated on an annual basis for the years 2006-2008, and also for the individual periods (pre-, TE-, and post-treatment period) in the two manipulation years (2006, 2007). Mean values were calculated from the data of nine individual SR chambers (n = 9) on each manipulation type (control, TE). Difference between the control and the TE plots were calculated for each period, as well as the relative contribution of differences in a certain period to the total differences of that year. Mean values of control and TE plots were statistically compared by the Mann-Whitney U-Test to detect differences in cumulative C emissions during a certain period. Values of $p < 0.05$ are considered statistically different (*).

			Cumulative C emissions [t C ha^{-1}]					
	Period		**Control**	**TE**	**Difference**		**Statistics**	
Year	Description	Duration	Mean ± SE	Mean ± SE	Absolut	[%] of annual	*p*	
2006	**Total**	**1 year**	**6.7 ± 0.2**	**5.7 ± 0.3**	0.9	100	0.031	*
	Pre	172 d	2.1 ± 0.1	2.1 ± 0.1	0.1	6	0.566	-
	TE	47 d	1.4 ± 0.1	1.0 ± 0.1	0.5	50	0.002	*
	Post	146 d	3.1 ± 0.1	2.7 ± 0.1	0.4	44	0.047	*
2007	**Total**	**1 year**	**7.0 ± 0.4**	**5.9 ± 0.3**	1.2	100	0.019	*
	Pre	182 d	3.0 ± 0.2	2.9 ± 0.1	0.2	13	0.825	-
	TE	42 d	1.6 ± 0.1	1.0 ± 0.1	0.6	54	0.001	*
	Post	141 d	2.4 ± 0.1	2.0 ± 0.1	0.4	33	0.031	*
2008	**Total**	**1 year**	**6.7 ± 0.4**	**6.7 ± 0.3**	0.0	100	0.965	-

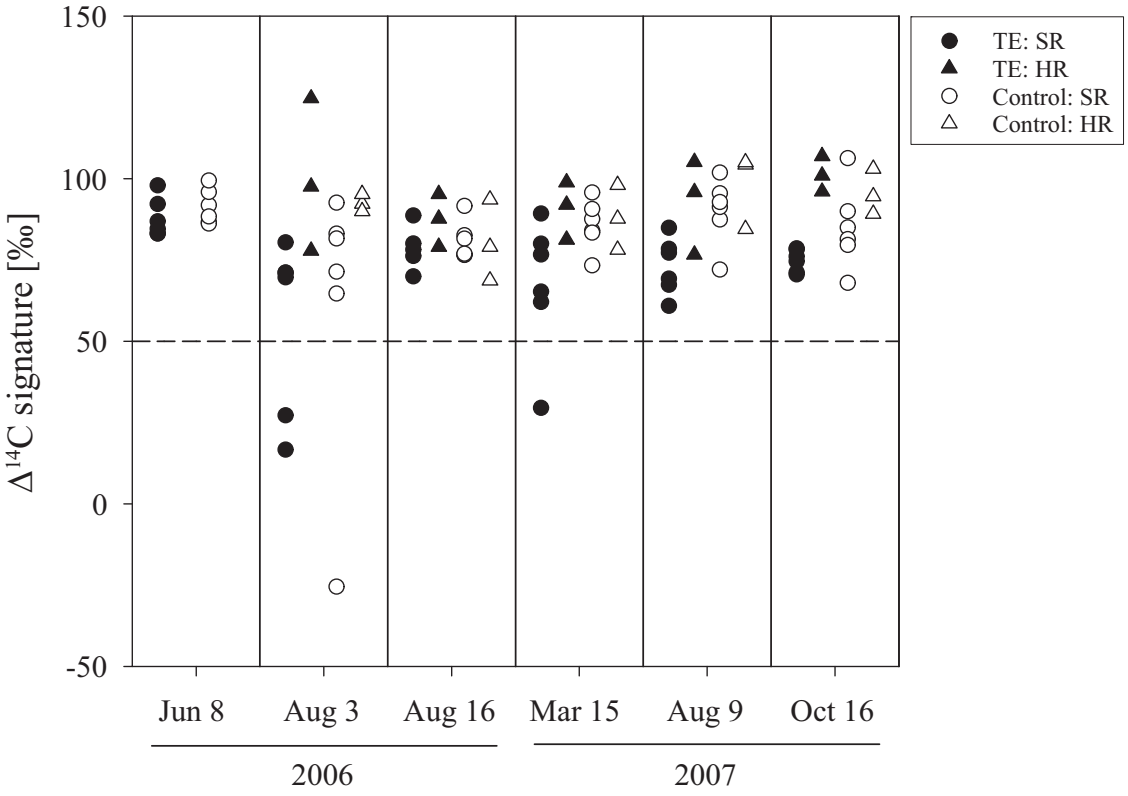

Figure 2 Radiocarbon signature of total soil respiration (SR) and of heterotrophic (root-free) respiration (HR) on the control and the throughfall exclusion (TE) plots on different sampling dates during 2006 and 2007. The dashed line at 49.2 ‰ represents the mean radiocarbon signature of atmospheric CO_2 in the measurement years. Measurements beneath this line indicate that a substantial amount of CO_2 originates from the mineralization of old substrate (pre-bomb).

In the year 2007, pre-manipulation measurements of $\Delta^{14}C_{SR}$ (Mar 15, 2007) reveal little variation for the control plots with mean $\Delta^{14}C_{SR}$ of 85.1 ‰ (±3.1), but high variation for the TE plots and the occurrence of CO_2 with pre-bomb $\Delta^{14}C$ in one of the samples. During the TE period, $\Delta^{14}C_{SR}$ measurements for both TE and control plots show a smaller variation and no samples with obvious pre-bomb influence are found. Mean values of TE and control plots during this second manipulation period differ significantly with 73.0 ‰ (±3.6) and 90.2 ‰ (±4.1), respectively. Differences decrease during rewetting with $\Delta^{14}C_{SR}$ values around 74.9 ‰ (±1.4) on TE and 85.1 ‰ (±5.2) on control plots.

Partitioning of SR

Partitioning revealed that SR on the control plots was dominated by HR on all measurement dates, with RR contribution to total SR ranging only between 5 and 40 % (Fig. 3). Absolute RR emissions were constantly small throughout both years in the control plots, except for the summer of 2006, when RR emissions ranged between 2-3 mmol CO_2 m^{-2} h^{-1}. Absolute HR emissions in the control plots followed the seasonal trend of SR.

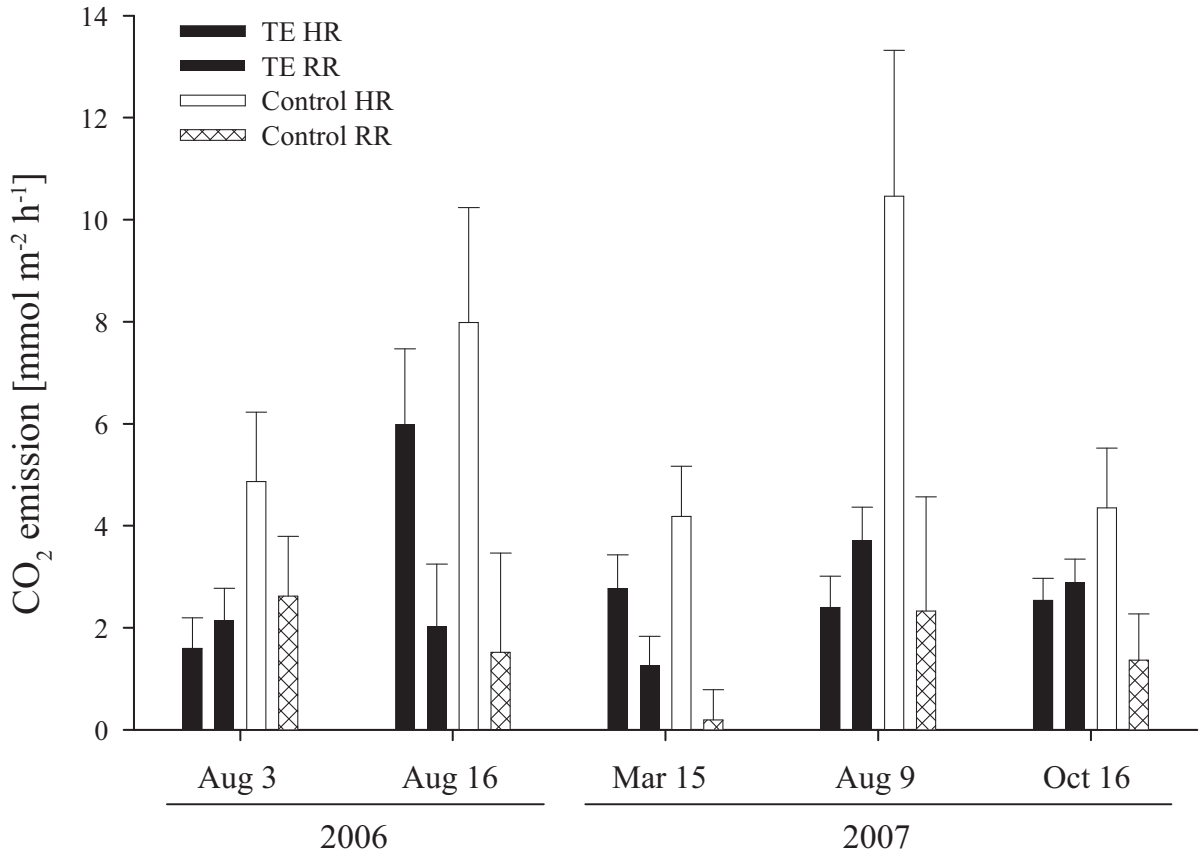

Figure 3 Absolute CO_2 emissions (±SE) calculated from partitioning of total soil respiration into heterotrophic (HR) and rhizospheric (RR) respiration on the control and throughfall exclusion (TE) plots on several dates in 2006 and 2007. Partitioning was calculated following *Philips and Gregg* [2001].

Comparing the control and TE plots (Fig. 3), we realize that natural heterogeneity makes it difficult to find differences. However, we find two measurement dates with pronounced differences: Both on Aug 3, 2006 and Aug 9, 2007, the HR on the TE plots is only about 30-40 % of the HR on the control plots, whereas RR values are similar or even slightly higher on the TE plots. It should be mentioned, that these two measurement dates are the only ones among the $\Delta^{14}C$ measurement dates on which SR on the TE plots was significantly ($p < 0.05$) smaller than on the control plots. On all other measurement dates, we neither find significant differences in SR between control and TE plots, nor in HR or RR.

Discussion

Exclusion of summer throughfall effectively reduces the total CO_2 emissions from this Norway spruce forest soil in both manipulation years. Prior to the exclusion period, CO_2 emissions on the control and TE plots did not show any inherent differences, and soil temperature (the main driver of SR) also can not explain this reduction of CO_2 emissions on the TE plots. We therefore ascribe the reduction of CO_2 emissions to reduced water

availability indicated by matric potential differences between control and TE plots. More than 50 % of the reduction are explained by reduced fluxes during the manipulation period itself. However, a major proportion of the remaining reduction is due to continuously reduced fluxes during the post-treatment period. In both years, CO_2 emissions on the TE plots remain reduced until six to seven weeks after the reopening of the roofs, although matric potential differences disappear a few weeks earlier. Based on $\Delta^{14}C$ data, we attribute the reduction of total CO_2 emissions mainly to reduced respiration by heterotrophic soil organisms. It has already been reported that for this soil C losses *via* DOC leaching are insignificant compared to CO_2 emissions [*Hentschel et al.*, 2007, *Muhr et al.*, 2008], so it is unlikely that reduced CO_2 emissions are compensated by an increase of alternate C losses in this soil.

The observed reduction of CO_2 emissions during the manipulation periods can be explained best by reduced matric potential in the organic and the uppermost mineral horizons. In the naturally dry summer of 2006, matric potentials indicate drought stress on both the control and the TE plots. Nevertheless, exclusion of summer throughfall leads to measurable differences between control and TE plots with matric potentials being one order of magnitude more negative on the TE plots than on the control plots. The soil moisture data shown here reflects the conditions 8 cm below the surface. More pronounced differences between control and TE plots can be expected in the top of the forest floor, because small precipitation events (like documented for this period) can lead to temporary increase of soil moisture and therefore SR in the control plots, whereas the TE plots are constantly dry throughout the whole manipulation period. On the other hand, when the moisture sensors indicate drought stress this close to the transition of organic and mineral horizons, we can assume that at least the uppermost centimeters of the mineral horizon will also be subjected to drought stress. This assumption is supported by the matric potential measurements in 20 cm mineral soil depth, which revealed that the manipulation produced measurable matric potential differences even in this depth.

After we removed the roofs on the TE plots, it took several weeks until the matric potentials regenerate back to the level of the control plots. In 2007, this could be due to the fact that we did not irrigate the TE plots to compensate for the excluded amount of throughfall. However, we observe the same phenomenon during the post-treatment period in 2006, despite irrigation. This indicates that the delayed regeneration of the matric potential is independent of irrigation. A reason to explain this slow regeneration of matric potential could be water repellency and preferential flow patterns in the organic layer of this soil like reported by *Bogner et al.* [2009]. It is well known that water repellency is increased under conifer

stands [*Doerr et al.,* 2000]. Drying can even further increase water repellency of soils [*Dekker and Ritsema*, 1996]. Consequently, CO_2 emissions remain smaller on the TE plots as long as matric potentials have not recovered.

However, we find that differences in SR rates persist much longer than differences in matric potential. A possible explanation for this observation that we would like to discuss here addresses the regeneration of the microbial population. Although we do not have direct measurements of microbial biomass, our $\Delta^{14}C$ data (for a detailed discussion see below) indicates significantly reduced HR rates under dry conditions. To demonstrate the severity of the drought experienced by the microorganisms, we compare our data to data from a parallel experiment at the same stand. In this experiment, the contribution of organic horizons to total SR was quantified by completely removing the organic horizons. This removal reduced SR rates in summer by maximal 40-45 % [*Froitzheim*, personal communication], indicating that CO_2 originating from the mineral horizons accounts for a high proportion of the SR emissions. This observation fits the high organic C contents of the EA, Bsh and Bh horizons in this Haplic Podzol. However, with our TE manipulation, we achieved a reduction of SR rates of up to 60 % on several measurement dates. We therefore assume that drought stress not only affected the organic horizons, but also the respiratory emissions from the uppermost mineral horizons. In previous laboratory experiments with soil columns from this stand [*Muhr et al.*, 2008, *Muhr et al.*, 2009] we tested the reaction of organic and mineral horizons to intensive drying. When intact soil cores consisting only of organic horizons were dried, mean hourly CO_2 emissions always regenerated to the same level as control CO_2 emissions after rewetting. When we subjected soil columns consisting of organic and mineral horizons to intensive drought, CO_2 emissions never regenerated back to control levels even after rewetting [*Muhr et al.*, 2008]. We attributed this difference to the fact that the microbial population inhabiting the mineral horizons is less adapted to drought. This agrees with findings by *Fritze et al.* [2000], who described that in the organic horizons of typical Podzol profiles under coniferous forest fungi are relatively abundant, whereas in the uppermost mineral horizon the relative abundance of bacteria is increasing. Fungi, in turn, are known to be more drought tolerant than bacteria [*Griffin*, 1981, *Voroney*, 2007]. To investigate the relative abundance of fungi and bacteria at the *Coulissenhieb II* site, *Schmitt et al.* [personal communication] analyzed phospholipid fatty acid (PLFA) patterns in undisturbed soil cores. Preliminary results from this investigation confirm that the ratio of fungal to bacterial PLFAs decreases from the organic to the mineral horizon at our research site. We therefore postulate that SR rates can be reduced persistently by drying because 1) water repellency and

preferential flow patterns hinder fast and complete rewetting, and 2) a part of the microbial population can be severely damaged and regenerates slowly.

Reduced CO_2 emissions during periods of drought are not surprising - it has long been described that drought reduces the activity of soil microorganisms and therefore SR [*Kieft et al.*, 1987, *Degens and Sparling*, 1995, *Borken et al.*, 2006]. However, during the last years an increasing number of experiments indicated that drought can also trigger the release of formerly protected, unavailable substrates that can result in a net increase of annual CO_2 emissions even if fluxes are significantly reduced during the actual drought period, a phenomenon recently referred to as the 'Birch effect' [*Miller et al.*, 2005, *Jarvis et al.*, 2007, *Xiang et al.*, 2008]. In this experiment, CO_2 emissions from TE plots exposed to summer drought never exceeded emissions from the control plots. One might argue that due to the nature of our measurements, we might have missed a short-lasting increase of CO_2 emissions. In the year 2006, e.g., we measured SR a few hours before we started irrigation and then again 36 h after the beginning of irrigation. We found no increase in SR between those two measurement dates. It still is possible, though, that a pulse with a duration less than 36 h occurred. However, we would like to emphasize that annual CO_2 emissions on the TE plots were reduced by 0.9 t C ha^{-1} compared to the control plots. Even when we missed a short-lasting pulse, it would have to come close to 0.9 t C ha^{-1} (or about 210 mmol m^{-2} h^{-1} on average) to compensate for this reduction. Laboratory measurements on soil from this site with high temporal resolution do not indicate that such an enormous SR pulse is likely to occur [*Muhr et al.,* 2008].

Furthermore, our findings are in agreement with nearly all other field experiments simulating prolonged drought periods we know of, all reporting reduced C losses due to drought [cf. *Borken and Matzner*, 2009]. The only field experiment that reported an increase of C losses during a drying-rewetting manipulation [*Borken et al.*, 1999] attributed this increase to artificial rewetting during an extremely warm period, when control plots received less water and probably were water limited. So far, increasing C losses from soils due to preceding drought have mainly been reported from laboratory experiments with mineral soil [cf. *Borken and Matzner*, 2009 and references therein]. In the majority of these experiments, the soil has been sieved. This represents a major disturbance, creates new surfaces and can possibly facilitate the release of formerly protected substrates. Furthermore, it changes the physical characteristic of the soil, and it has been reported that rewetting of disturbed soil occurs faster than of undisturbed soil [*Schjønning et al.*, 1999]. Delayed rewetting due to water repellency and preferential flow patterns, e.g., is not to be expected in disturbed soil.

Reduced C losses in one year do not necessarily have to result in increased C sequestration. If losses are reduced because of reduced mineralization, material that was not metabolized during the dry year might simply be mineralized in the subsequent year, resulting in increased C losses that compensate for the dry year. However, measurements of SR during 2008 do not indicate that reduced C losses in 2006 and 2007 are in any way compensated for by increased C losses in 2008. One possible explanation might be reduced metabolic capacity of the microbial community like postulated as a possible effect of drying-rewetting in the 'microbial stress' mechanism [*Xiang et al.*, 2008]. Alternatively, drying might result in stabilization and thus C sequestration of substrates. Based on our results, we so far only can show that substrates not used in dry years are not immediately metabolized in subsequent wet years.

Even though decreased SR rates on the TE plots contradict the idea of protected substrates being released, $\Delta^{14}C_{SR}$ data indicate the contribution of old pre-bomb carbon to SR mainly on the TE plots and mainly during periods of drought, thus indicating a shift in the quality of the predominant substrate. Without additional information, this might be interpreted as the release of formerly protected substrate, which would be expected to be older. However, in this experiment a second explanation is much more likely: CO_2 respired in SR originates from both organic and mineral horizons. Under normal conditions, most of the CO_2 emitted in SR is originating in the organic horizons and the uppermost mineral horizon. Bulk $\Delta^{14}C$ of these horizons is clearly dominated by post-bomb material (cf. Table 1). As soon as CO_2 emissions from organic horizons and uppermost mineral horizons decrease due to drought, the relative contribution of CO_2 originating from deeper mineral horizons increases. This will consequently lead to a change in the measured $\Delta^{14}C$ like observed in this experiment.

The calculation of the source contribution of HR and RR to SR is based on a variety of parameters (cf. Equ. 2 & 3), all of which are prone to statistical error due to natural heterogeneity. Interpretation of the results therefore has to be done very critically. However, there are two very pronounced results: (1) The contribution of HR to SR on the control plots is always bigger than the contribution of RR; (2) the CO_2 emissions from control and TE plots are always about the same size, except during the TE periods in both manipulation years: During this period, both SR and HR become considerably smaller on the TE plots than on the control plots, while RR remains unaffected.

There are two different explanations for the clear dominance of HR over RR. One explanation might be the thickness of the organic horizons and the high amount of substrate stored here. In this context, we would like to mention, that *Schindlbacher et al.* [2009] also

reported rather small contributions of RR to SR in a Norway spruce forest, ranging between 24-28 %. The other explanation might be disturbance resulting from the installation depth (5 cm) of our measurement chambers. *Wang et al.* [2005] discussed that the insertion of SR chambers into the soil cuts off superficial fine roots and thereby reduces RR. It is impossible for us to quantify this effect in our experiment, so quantitative conclusions about RR have to be considered with care. However, qualitative conclusions concerning differences in RR between control and TE plots and the reaction of RR below 5 cm depth still should be valid (see below).

During the TE periods, SR on the TE plots is significantly smaller than on the control plots. Partitioning reveals that this reduction can be explained with a corresponding reduction of HR. At the same time, RR is not affected by the TE manipulation. We therefore can conclude that drying negatively affects heterotrophic soil microorganisms, whereas roots and rhizospheric microorganisms seem to be able to withstand the drought stress caused by TE manipulation.

Summarizing, we conclude that the exclusion of summer throughfall leads to a significant reduction of SR mainly in the organic and uppermost mineral horizons during the exclusion period. HR is affected stronger than RR. Regeneration of SR takes several weeks, most likely due to a combination of water repellency and microbial casualties. The manipulation effect is strong enough to significantly reduce annual C losses. So far, no evidence has been found that metabolization of unused substrates can lead to a compensation of these reduced C losses either during rewetting of the dry soil or during the following years.

Conclusion

Prolonged summer droughts are likely to lead to a significant reduction of annual CO_2 losses in this temperate Norway spruce forest. CO_2 emissions are not only reduced during the actual drought period, due to either water repellency or serious damage in the microbial population it takes several weeks before they are restored back to control levels. Data on $\Delta^{14}C$ indicate that a reduction of SOM mineralization in the organic horizon and the uppermost mineral horizon is mainly responsible for this reduction. No evidence has been found that preceding drought can release new, formerly protected substrate and thereby result in increased carbon losses from soils, like discussed in the context of the so-called ‘substrate supply’ mechanism. So far, no evidence has been found that reduced C losses are compensated for by increased CO_2 emissions in subsequent years. Based on our results and in

face of the current climate change scenarios, we expect a negative feedback between increased frequency and magnitude of summer droughts and SR in Norway spruce stands.

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Chapter 5

Ecosystem C dynamics in a fen as affected by water table fluctuations

PART A:

Manipulative lowering of the water table during summer does not affect CO_2 emissions and uptake in a minerotrophic fen

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Abstract

We simulated the effect of prolonged summer drought by lowering the water table on three manipulation plots (D_{1-3}) in a minerotrophic fen in South-eastern Germany in three subsequent years (2006-2008). Water table was lowered below natural levels by drainage and by excluding precipitation. Soil respiration (R_{Soil}) measurements predominantly revealed no differences between the D_{1-3} plots and three control plots (C_{1-3}). Gross primary production (GPP) and aboveground respiration (R_{AG}) also were not affected by lowered water tables, indicating the lack of stress due to drought or anoxia for aboveground biomass. The water tables on the control plots were naturally low most of the time and especially during the manipulation periods. The median water table for 2006-2008 was 8 cm beneath the surface on the control plots. During summer, when respiratory activity was highest, it was even lower, with median values on the control plots between 11 and 19 cm beneath the surface during the manipulation periods. We therefore assume that oxygen availability in the uppermost at least 10 cm was not limited by water table. Thus, manipulative lowering of the water table most likely increased oxygen availability only in deeper peat layers, where we expect R_{Soil} to be limited by poor substrate quality rather than anoxia. In case that naturally low water tables on the C_{1-3} plots masked manipulation effects, we analyzed all available data in a second approach (irrespective of treatment) to estimate the influence of water table on R_{Soil}. We found a significant correlation between R_{Soil} and water table, with R_{Soil} decreasing at lower water tables rather than increasing. Summarizing, we conclude that R_{Soil} is dominated by

decomposition in the litter layer and the uppermost peat layers. Deeper peat layers bear no significant decomposition potential. We do not expect enhanced C losses from this site due to increasing frequency of summer droughts. Aboveground vegetation seems to be unaffected by water table fluctuations beneath 10 cm.

Keywords: Peatland, fen, climate change, water table, CO_2 emissions & uptake, ecosystem manipulation

Introduction

Soils store about 1500 Pg (10^{15} g) of carbon (C), roughly twice as much as the atmosphere (Schlesinger & Andrews, 2000). Around 30% (270-370 Pg) of this C is found in peatland soils (Turunen et al., 2002), although they only account for around 3.5% of the earth's surface (Gorham, 1991). This phenomenon results from the stabilization of C due to high water levels in peatlands. Consequent anoxia leads to a limitation of decomposition rates, resulting in an imbalance between biomass production and decomposition. This imbalance is rather small (Päivänen & Vasander, 1994), but has led to the formation of thick peat bodies since the last deglaciation.

As the sink function of peatlands mainly is a consequence of climatic stabilization, it has been described as labile and vulnerable to climate change (Alm et al., 1999; Moore, 2002; Bubier et al., 2003). The IPCC (2007) has predicted an increasing likelihood of summer-drought periods for this century, resulting in lowered water tables. The consequential increase of oxygen availability during a time of high temperature and, therefore, high microbial activity will presumably lead to an increase in peat decomposition. It has already been reported that peatlands can switch rapidly to a source of carbon when water table changes (Silvola, 1996). However, contrasting results on the effects of water level drawdown on decomposition have been reported (cf. Laiho, 2006 and references therein). Peatland sites may become sources of C, but also can remain sinks or even become stronger sinks when water table is lowered.

The C balance of an ecosystem does not only depend on decomposition but also on CO_2 uptake *via* photosynthetic assimilation (gross primary production or GPP). Some of the assimilated CO_2 quickly returns to the atmosphere *via* aboveground plant respiration (R_{AG}) or *via* root respiration while a fraction leaves the plant as root exudates. The rest of the assimilated C is used for the production of biomass (roots and leaves), and will finally end up as litter. Litter and root exudates serve as microbial substrates and become decomposed. All CO_2 leaving the ecosystem *via* root respiration or decomposition here will be termed soil respiration (R_{Soil}). The sum of R_{Soil} and R_{AG} is named the total ecosystem respiration (R_{Eco}) and summarizes all emissions of CO_2 from the ecosystem. The ecosystem C balance depends on the difference between uptake of C *via* GPP and emissions *via* R_{Eco}. Both parameters can be influenced by changes in the water table. To determine the effects of lowered water tables on the CO_2 source-sink function of an ecosystem one has to measure uptake and emission rates.

In this experiment, we simulated periods of prolonged summer-drought by excluding precipitation and lowering the water table by drainage in a minerotrophic fen in south-east Germany. We continuously measured soil respiration (R_{Soil}) on three manipulation plots and compared them to three control plots to assess the effect of lowered water tables on soil respiration. We also investigated effects of lowered water tables on the vegetation at our site by measuring gross primary production (GPP) and aboveground autotrophic respiration (R_{AG}) at various dates.

Material and Methods

Site description

Measurements were carried out at the *Schlöppnerbrunnen* fen site located in the *Lehstenbach* catchment (Fichtelgebirge, Northeastern Bavaria, Germany, 50°07'54''N, 11°52'51''E). The site is characterized as a temperate minerotrophic fen covering an area of 0.8 ha at an elevation of about 750 m a.s.l. The soil is a Histosol on granite bedrock covered mainly by *Molinia caerulea* (L. Moench), *Nardus stricta* (L.), *Agrostis canina* (L.), *Carex rostrata* (Stokes) and *Eriophorum vaginatum* (L.). Mean annual temperature (1995-2007) is 6.3 ± 0.9°C and mean annual precipitation (1995-2007) is 1020 ± 203 mm a^{-1} (Knorr, 2009). The site features a water table gradient, with the north-western part being waterlogged more often than the south-eastern part, and a slope from NNE to SSW. Groundwater flows slowly through the site parallel to this slope. Since the last deglaciation, a peat body with a thickness between 40-70 cm has accumulated. A schematic representation of our study site and the experimental set-up are shown in Figure 1.

Experimental design

Three control plots (named C_1, C_2, and C_3; each 7 x 5 m^2) were installed in the summer of 2005 to assess natural dynamics of measured parameters at the site. Three plots of identical size were installed a few meters downstream in terms of groundwater flow to carry out water-table drawdown experiments (named D_1, D_2, and D_3). All plots were accessible *via* wooden walkways. Each plot was equipped with soil temperature sensors in various depths (5, 10, 20, 30, 40, and 60 cm). Three collars for soil respiration (R_{Soil}) measurements (Ø 50 cm, installation depth 5 cm), and two collars (39 x 39 cm^2) for net ecosystem exchange (NEE) and ecosystem respiration (R_{Eco}) measurements were installed on each plot. Biomass was sampled on the latter at the end of the vegetation period in the year 2007, divided into species and then

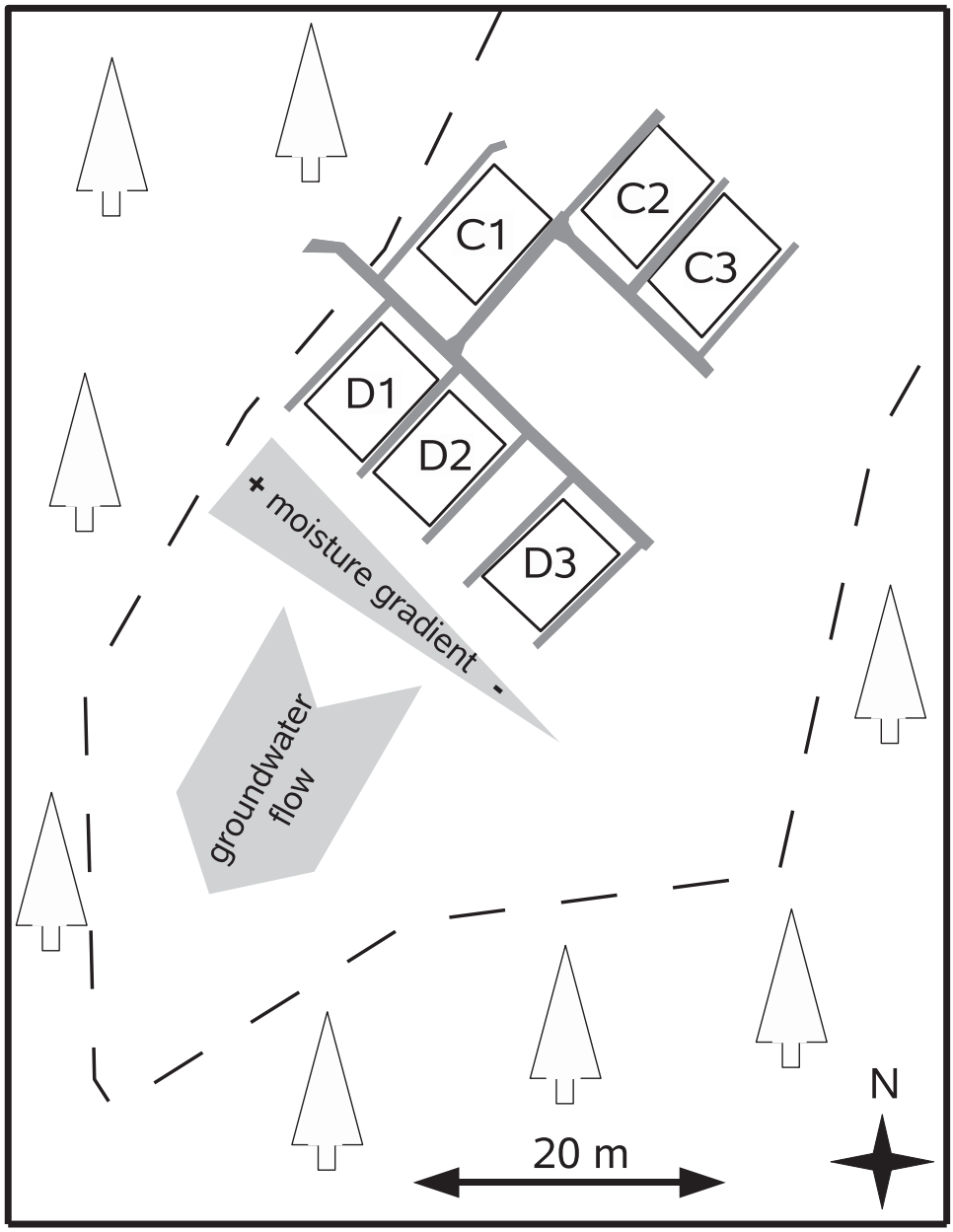

Figure 1 Schematic sketch of the study site. The non-treated control plots C_1-C_3 are located upstream of the treatments plots D_1-D_3 in terms of groundwater flow direction. Boardwalks to minimize disturbance during access are depicted as grey lines. The site is surrounded by Norway spruce forest, the open area is marked by the dashed line. At the site there is a moisture gradient from north to south. During the manipulation period, D_1-D_3 were covered with a roof construction and the drainage system was emptied constantly (Knorr et al., 2009).

oven-dried at 60°C before weighing. An existing biomass development curve for the year 2007 (Otieno et al.,2009) measured on additional plots nearby was used to calculate biomass on our plots at the specific measurement dates, by assuming the same dynamics. Piezometers (26PCBFA6D, IBA Sensorik GmbH, Seligenstadt, Germany) in the immediate vicinity of the installations allowed for continuous monitoring of the water table (for this work, the data of six piezometers per plot was used). Precipitation was measured at a climate station (*Pflanzgarten*) at a distance of ca. 1 km from the plots.

To effectively lower the water table in the D_{1-3} plots, precipitation was excluded by means of transparent roofs and ground water was actively pumped out of the plots. A drainage system was installed perpendicularly to the ground water flow both up- and downstream of the manipulation plots at a depth of 1 m. During the first manipulation period (14 August to 26 September, 2006) draining was facilitated by a pump that was operated manually every second or third day. The system was automated during the second manipulation period (10 May to 19 July 2007) to achieve better results. The automated system was also used during the third manipulation period (10 July to 7 August, 2008). A detailed overview of the dates and duration of the three manipulation periods is shown in Table 1.

Table 1 Manipulation period parameters of the three manipulation years.

	Manipulation Periods			Precipitation [mm]		
				Control	Manipulation	
Year	Start	End	Duration [d]	Total	Excluded	Irrigation
2006	Aug 14	Sep 26	43	861	109	110
2007	May 10	Jul 19	70	1265	343	182
2008	Jul 10	Aug 07	28	957	65	-

Regeneration of the water table levels on the D_{1-3} plots at the end of the manipulation period was partly achieved by natural precipitation and lateral water inflow. In the years of 2006 and 2007 we further accelerated the rising of the water table by irrigating the D_{1-3} plots with 110 and 182 mm of artificial rain water, respectively. Irrigation intensity was kept constant at 10 and 11 mm h^{-1} in these two years. The artificial rainwater consisted of deionised water to which we added (µmol l^{-1}): Na^+ 39, K^+ 30, NH_4^+ 34, NO_3^- 34, SO_4^{2-} 12, so that its chemical composition was close to natural precipitation. Irrigation raised the water table to the level of the C_{1-3} plots. In 2008, no irrigation was necessary, as water table levels quickly regenerated due to natural precipitation.

CO_2 fluxes: Measurement details (R_{Soil}, R_{Eco}, NEE)

On each plot, three non-transparent cylindrical plastic collars (length: 45 cm, Ø 50 cm) were driven approx. 5 cm into the soil for R_{Soil} measurements. Vegetation in these collars was removed regularly. For gas measurements, collars were manually closed with a non-

transparent plastic lid and connected to a portable infrared gas analyzer (Li-Cor, Li-820). Gas concentration was logged every 10 s for a period of 10 min. Measurements of R_{Soil} were carried out in rotation on the C_{1-3} and the D_{1-3} plots between 8:00 am and 12:00 am on measurement days.

Measurements of R_{Eco} and NEE were only carried out during one manipulation period in the summer of 2007, to investigate the effects of water table draw-down on the assimilation rates of the vegetation. For these measurements, we installed additional rectangular collars (39 x 39 cm^2) to a depth of 7 cm into the soil. Vegetation inside these collars was not removed during the measurement period. For NEE measurements, we used 38 x 38 x 54 cm^3 chambers built of transparent plexiglass (3 mm XT type 20070, light transmission 95 %). For R_{Eco} measurements, we used dark chambers identical in construction, but covered with an opaque insulation layer and reflective aluminium foil. R_{Eco} and NEE were always measured directly one after the other on each plot, and the C_{1-3} plots were measured in rotation with the D_{1-3} plots. Measurements were conducted between 9:00 am and 12:00 am on the measurement dates with a few exceptions due to technical problems.

During measurements, chambers were placed on the permanently installed collars and firmly secured with two elastic rubber bands fastened onto the ground on two sides of the chamber. Sealing was achieved with a flexible rubber gasket between chamber and collar. Increased air pressure inside the chamber was avoided by a 12 mm opening at the top of the chamber that was closed after placing of the chamber on the collar. Circulation of air inside the chamber was achieved by three fans creating an air-current of 1.5 m s^{-1}. The air-stream was directed over mounted ice packs inside the chamber to keep air temperature within 1°C relative to ambient.

CO_2 concentration inside the chamber was measured with a portable infrared gas analyzer (Li-Cor, Li-820) over a period of 3-5 min and logged every 10 s. Simultaneously, air temperature (20 cm above ground), peat temperature (10 cm below-ground), and photosynthetic active radiance (PAR; measured with a LI-190 quantum sensor, Li-Cor, USA) both inside and outside the chamber were measured. Detailed information on the NEE / R_{Eco} measurements can be found in Droesler et al. (2005) and Otieno et al. (2009).

Calculations of CO_2 fluxes (R_{Soil}, R_{Eco}, NEE, GPP)

CO_2 fluxes for R_{Soil}, R_{Eco} and NEE were calculated by performing a linear regression on the logged CO_2 concentration data (with a few exceptions: $r^2 > 0.95$). Data was corrected for atmospheric pressure and chamber air temperature. In the case of NEE, we limited the linear

regression to the first few measurement points, as the slope normally flattens with continuously decreasing CO_2 concentrations. For NEE and R_{Eco} measurement dates, we were able to calculate the gross primary production (GPP) as:

$$GPP = NEE - R_{Eco} \quad (1)$$

and the aboveground plant respiration (R_{AG}) as:

$$R_{AG} = R_{Eco} - R_{Soil} \quad (2)$$

By convention, negative fluxes indicate losses from the atmosphere to the ecosystem, whereas positive fluxes are directed from the ecosystem to the atmosphere.

GPP and R_{AG} were calculated to investigate whether water table drawdown influenced plant physiology on the D_{1-3} plots in comparison to the C_{1-3} plots. As biomass is not homogeneously distributed at the site, we had to normalize GPP and R_{AG} fluxes on the C_{1-3} and D_{1-3} plots for biomass. We measured biomass in each of the NEE / R_{Eco} collars in September 2007. Otieno et al. (2009) measured the seasonal biomass dynamic at the *Schlöppnerbrunnen* site in 2007. By assuming that biomass dynamic on our plots was identical to their data, we were able to calculate biomass on the measurement plots for every measurement date. We expect the biomass calculations to be more inaccurate towards spring, when heterogeneity of biomass stock is highest at the site due to initial growth. By normalizing fluxes with biomass we assume that differences in biomass between the plots are inherent and independent from the manipulation.

Application of CO_2 efflux model on R_{Soil} data

Besides the direct comparison of control and manipulation plots, we applied a simple CO_2 efflux model like presented by Mäkiranta et al. (2009) to estimate the influence of water table on CO_2 emissions. This model is based on the assumption that soil temperature and water level depth are the main abiotic drivers of R_{Soil}. It consists of an Arrhenius type of function (Lloyd and Taylor, 1994) to describe temperature dependence of peat decomposition plus a scalar dependent to test the effect of water level. Originally, it was designed to model CO_2 fluxes from root-free peat, but we use it with R_{Soil} (including root respiration):

$$f(R_{Soil})=R_{ref} \times \exp[E_0 (1/(T_{ref}-T_0) - 1/(T-T_0)] + (c \times WL) \quad (3)$$

where T is the soil temperature at 5 cm depth measured concurrently with the CO_2 efflux measurements. Other parameters were estimated by fitting the model to the dataset using non-linear regression (Sigma Plot 10.0). R_{ref} (g CO_2 m^{-2} h^{-1}) is the soil respiration rate at 10°C. E_0 (K) is an exponential parameter depicting the temperature sensitivity of soil respiration. T_{ref} is the reference temperature set at 283.15 K (i.e., 10°C), and parameter T_0 is the minimum

temperature at which respiration reaches zero, set at 227.13 K (-45.6°C) (Lloyd and Taylor, 1994). WL is the depth of the water level from the peat surface (m) measured concurrently with R_{PD}, and the parameter c describes the change in R_{PD} related to changes in WL independently of temperature.

The model developed by Mäkiranta et al. (2009) offers the calculation of other parameters, e.g. the optimum depth of the water table. However, performing these calculations did not reveal any additional interesting results and, therefore, are omitted here.

Data analysis and statistics

Due to natural gradients in water table and in peat body thickness, we formed corresponding pairs of D_{1-3} and C_{1-3} plots for analysis (D_1-C_1; D_2-C_2; D_3-C_3). To test for statistically significant differences in R_{Soil} between D_{1-3} and C_{1-3} plots we used the non-parametric Mann-Whitney U-test.

Cumulative C emissions were calculated on individual chamber basis. We interpolated linearly between adjacent R_{Soil} measurements and multiplied with time to calculate how much C was emitted in total between two measurements. By adding up the sums of the different intervals we calculated total C emissions during longer periods (e.g. years, manipulation periods). For statistical analysis, the data of the chambers from the D_{1-3} and the C_{1-3} plots was compared using a Mann-Whitney U-test. No cumulative emissions were calculated for GPP and R_{Eco} because data was insufficient.

Results

Micrometeorology

Soil temperature followed a clear seasonal trend in all measured depths down to 60 cm beneath the surface (Fig. 2a). As no differences in soil temperature were found between C_{1-3} and D_{1-3} plots (data not shown), we present a uniform soil temperature curve representative for all plots. Soil temperature in the year 2006 was slightly warmer than in 2007 and 2008, as maximum values in summer in a depth of 5 cm were about 2°C higher than in the two subsequent years, but also the measured minimum in 5 cm depth was higher in 2006 than in the other two years.

Precipitation also varied between the three years (Table 1, Figure 2b). It was lower than the long-term average of 1160 mm (1971-2000) in 2006 and 2008 with a total of 861 mm and of 1265 mm. In summary, 2006 can be described as a warm and dry year, whereas 2007 was a

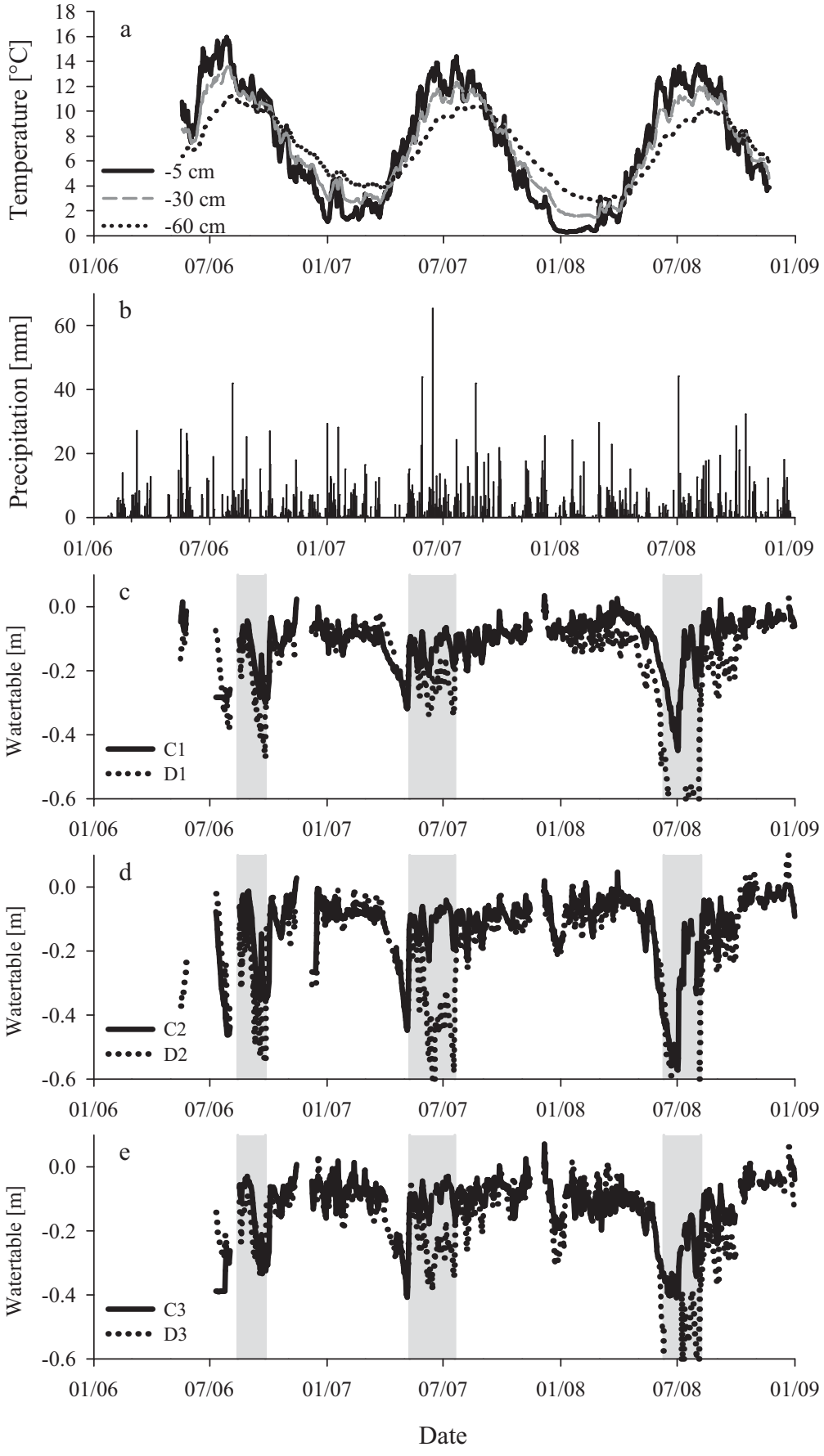

Figure 2 a) Soil temperature in various depths, b) natural precipitation, c)-e) and water table on the three pairs of control and manipulation plots in the years of 2006-2008. As soil temperature in each depth was identical on control and manipulation plots, all data is summarized in one graph per depth here.

wet year, still warm but cooler than 2006. Weather conditions in the year 2008 were intermediate with respect to the two previous years.

Water table on the C_{1-3} plots was subject to a high natural fluctuation (Figure 2c-e). The median for all three years (C_{1-3} plots only, i.e. natural fluctuation) was 0.08 m beneath the surface. During the manipulation periods, median water table of the C_{1-3} plots was even lower than this long-term average with 0.12, 0.11, and 0.19 m beneath the surface in 2006, 2007, and 2008, respectively. Further lowering of the water table was achieved by drainage and exclusion of precipitation on the D_{1-3} plots with varying success. In 2006, we failed to lower the water table on the D3 plot due to technical problems, so this plot can not be regarded as a manipulation plot for this year. On the other two plots, however, we achieved a lowering to the water table to 0.21 m beneath the surface in average. The summer of 2007 was rather wet, but we still lowered the water table to 0.25 m beneath the surface in average. The manipulation was most successful in the summer of 2008, when we lowered the water table beneath the measurement depth of our piezometers for several weeks. Raising of the water table back to the level of the C_{1-3} plots at the end of the manipulation period proceeded fast in all three years.

CO_2 emissions via R_{Soil}

CO_2 emissions on C_{1-3} and D_{1-3} plots followed a seasonal trend with highest emissions in summer and lowest emissions in winter (Figure 3). Fluxes ranged between 0 and 25 mmol m^{-2} h^{-1}. Overall, we found little differences between control and manipulation plots (Figure 3, Figure 4a and b). For the pairs C_1-D_1 and C_2-D_2 we found differences only on two measurement dates in the summer of 2007 when CO_2 emissions on D_1 and D_2 were reduced (Fig. 3). During that period, we measured extremely low water contents in the aboveground litter of the D_{1-3} plots, ranging around 22 g g^{-1}, compared to 225 g g^{-1} on the control plots (data not shown). Cumulative C emissions (annual and during the manipulation periods) were not affected by this short-term reduction (Fig. 4a and b). Differences were slightly more pronounced for the pair C_3-D_3, partly due to the fact that CO_2 emissions from C_3 were higher than from any of the other plots. The cumulative CO_2 emissions on D_3 were smaller than on C_3 during the manipulation periods of 2007 and 2008 (statistically significant with $p = 0.02$ only for the manipulation period in 2007) (Fig. 4b). This difference during the manipulation periods resulted in a visible but statistically not significant difference in the annual cumulative C emissions of 2007 and 2008 (Fig. 4a).

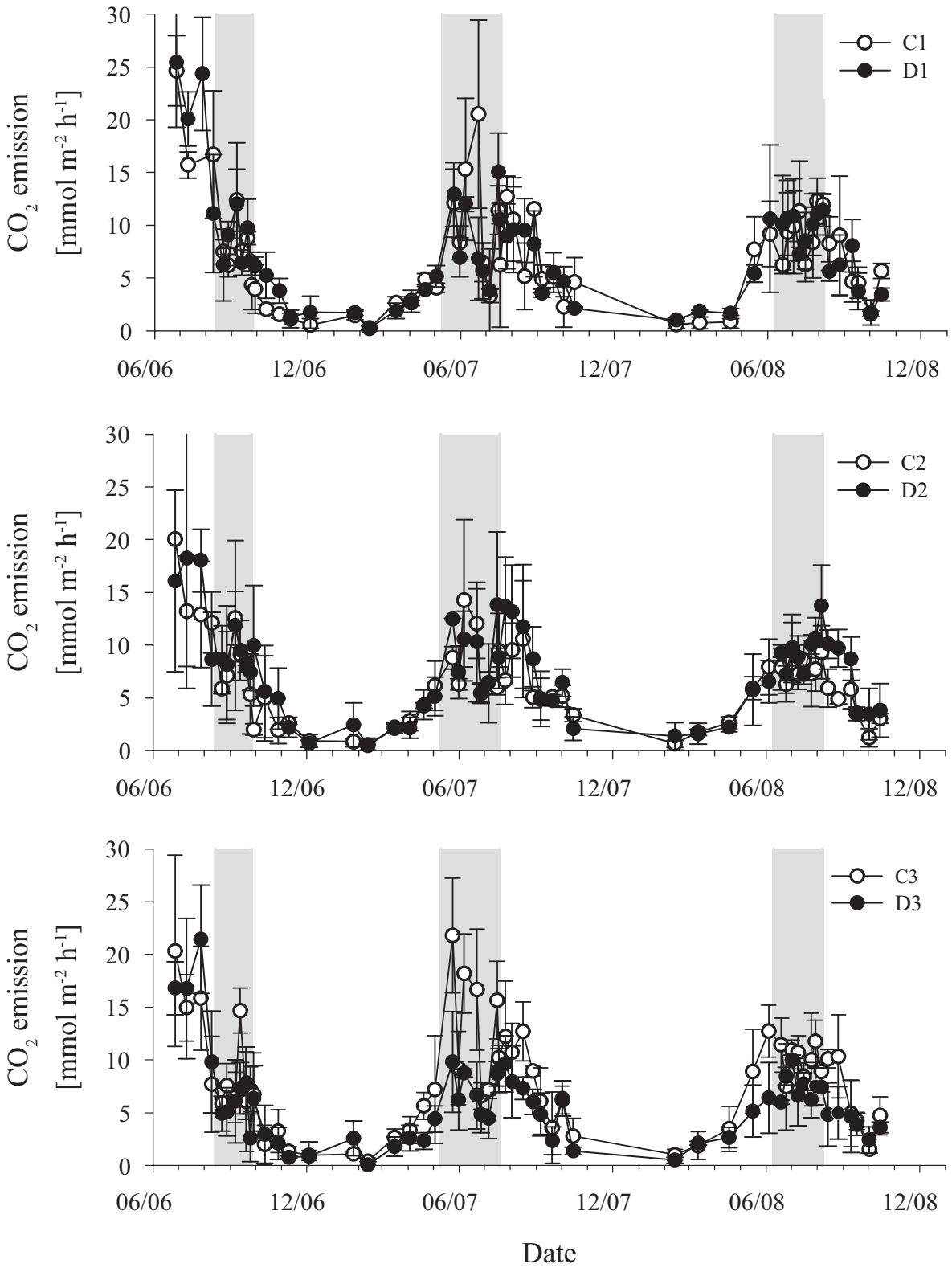

Figure 3 Mean emissions of CO_2 *via* R_{Soil} on the three pairs of control and manipulation plots (±SE; n=3). Shaded areas indicate the duration of the manipulation periods in the three years.

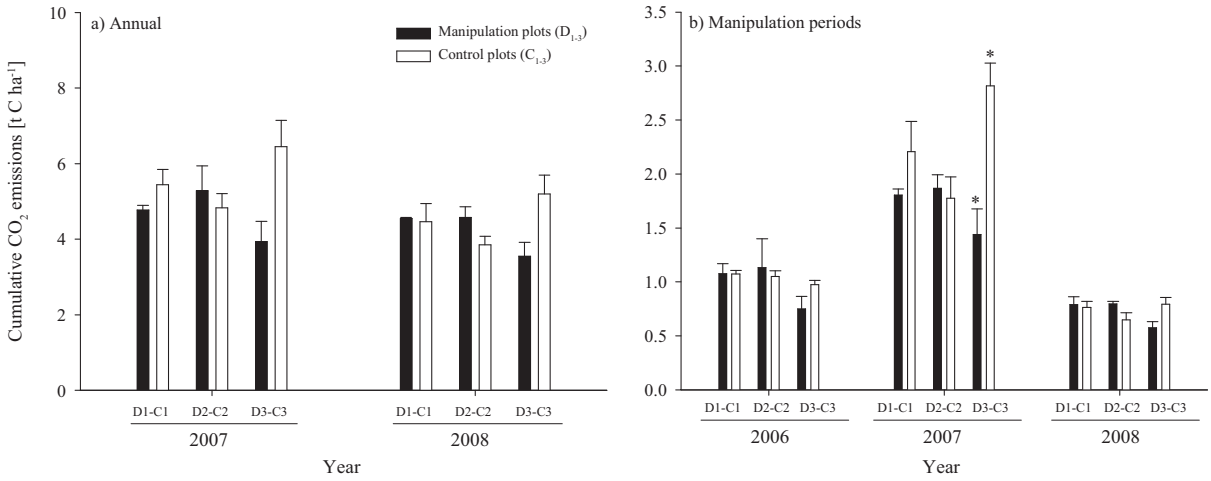

Figure 4 Cumulative CO_2 emissions a) on an annual basis and b) during the individual manipulation period of each year on the control and the manipulation plots. Cumulative annual CO_2 emissions are only shown for the years 2007 and 2008, as the experiment did not start before June 2006.

Effect of water table on GPP and R_{AG}

When harvesting the plots in the September of 2007, we found differences in species composition and biomass between the C_{1-3} and the D_{1-3} plots (Table 2). *Molinia caerulea* (L.) was the dominant species on most of the plots. On the C_{1-3} plots it was mainly accompanied by *Nardus stricta* (L.) and *Agrostis canina* (L.), on the D_{1-3} plots by *Carex rostrata* (L.). Due to differences in aboveground biomass we normalized GPP and R_{AG} data for the C_{1-3} and D_{1-3} by calculating rates per biomass unit. This way we wanted to assess the physiological effect of lowered water tables on aboveground vegetation (Figure 5). Any differences we found between C_{1-3} and D_{1-3} plots were restricted to the first three or four measurement dates and did not reveal a consistent trend. Towards the end of the manipulation period (when differences in water table were most pronounced) and during the regeneration period, we found no differences in R_{AG} or GPP between C_{1-3} and D_{1-3} plots.

CO_2 efflux model parameters

The CO_2 efflux model (Mäkiranta et al. 2009) revealed a high temperature sensitivity of R_{Soil} in our data (E_0 = 601.5 K, $p < 0.0001$) (Table 3). The model further revealed a significant ($p = 0.02$) correlation of R_{Soil} to changes in water table level independent of temperature, described by the parameter c. Interestingly, the parameter had a negative sign, indicating that R_{Soil} will decrease when water table is lowered.

Table 2 Biomass and species composition on the control and manipulation plots, as determined on two subplots per plot in September 2007.

Plot	Subplot	Biomass [g m^{-2}]	Dominant species	Other species
C1	1	216	*Molinia caerulea*	*Carex rostrata*
	2	406	*Sphagnum fallax*	*Molinia caerulea, Carex rostrata*
C2	1	311	*Molinia caerulea*	*Agrostis canina*
	2	565	*Nardus stricta*	*Agrostis canina*
C3	1	477	*Molinia caerulea, Nardus stricta*	*Agrostis canina*
	2	572	*Nardus stricta*	*Agrostis canina, Sphagnum fallax*
D1	1	238	*Molinia caerulea*	*Carex rostrata*
	2	345	*Molinia caerulea*	*Carex rostrata*
D2	1	386	*Molinia caerulea*	*Sphagnum fallax*
	2	252	*Molinia caerulea*	
D3	1	224	*Molinia caerulea*	*Carex rostrata, Agrostis canina*
	2	284	*Molinia caerulea, Nardus stricta*	

Table 3 Model parameters, their standard errors (SE), *P* values, the coefficient of determination (r^2) and the number of measurements included in the analysis (n). Parameters are: R_{ref} (g CO_2 m^{-2} h^{-1}) = basal respiration rate at 10°C; E_0 (K) = temperature sensitivity of soil respiration; c = change in soil respiration related to changes in water level independent of temperature. The model is described in detail by Mäkiranta et al. (2009).

	Value	SE	*P* value	r^2	n
R_{ref}	77.1	4.2	<0.0001	0.62	336
E_0	601.5	41.1	<0.0001		
c	-46.1	20.3	0.0238		

Discussion

The main reason for the importance of peatland soils as global C stores is a limitation of decomposition due to a lack of oxygen (climatic stabilization of C; cf. Trumbore, 2009). This lack of oxygen results from water logging, because diffusion of oxygen in water logged soils is decreased significantly. Several authors therefore discussed increasing decomposition rates in peatland sites when water tables are lowered (Hogg et al., 1992; Blodau et al., 2004; Hirano et al., 2007). Such a scenario is highly up to date, as current climate change scenarios (IPCC, 2007) predict prolonged periods of summer drought, eventually resulting in lowered water tables. This experiment was designed to assess the effect of water table lowering during summer on CO_2 emissions (R_{Soil}, R_{AG}) and uptake (GPP) in a minerotrophic fen in South-

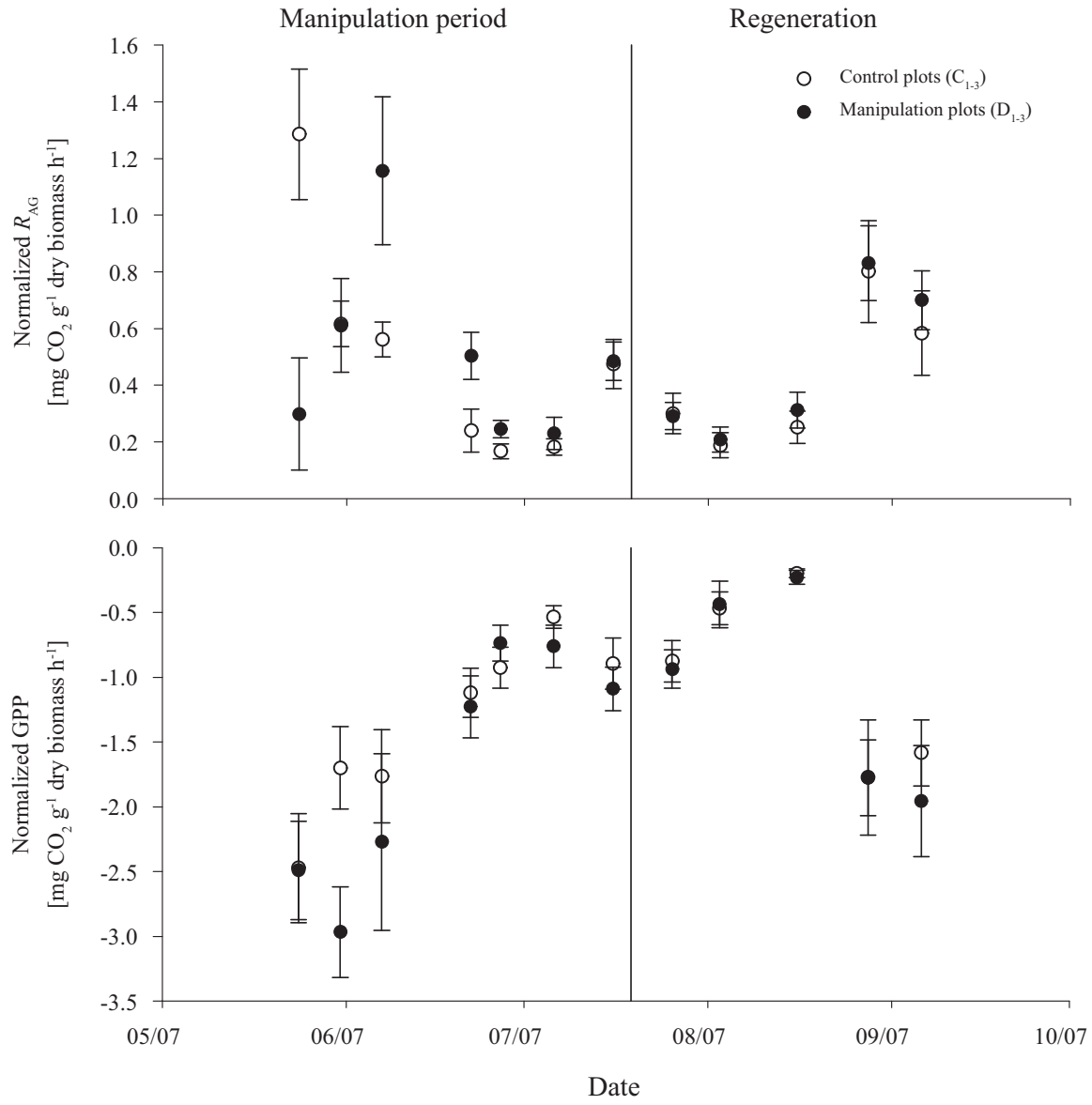

Figure 5 Mean GPP and R_{AG} on the control and manipulation plots during the summer of 2007 (±SE; n=6). Fluxes are normalized per unit biomass.

eastern Germany. Three pairs of control and manipulation plots were investigated. With one exception in the summer of 2006, we successfully manipulated the water table on all manipulation plots in three subsequent summers (2006-2008). In two of the plot combinations (D_1-C_1, and D_2-C_2), we found no significant differences in the emission of CO_2 during any of the three manipulation periods. In the third pair (D_3-C_3), R_{Soil} in the manipulation plot was smaller than in the control plot during manipulation. We found no indications that water table draw-down had any effect on plant activity in any of the three manipulation plots (GPP and R_{AG} per biomass unit).

In highly degraded and dense peat material like at our site, the water table does not have to be identical with the boundary between oxic and anoxic conditions. In a fen with similarly

degraded peat, Niedermeier and Robinson (2007) e.g. found an air entry tension of 25 cm, meaning that the zone of saturated soil would extend 25 cm above a moving water table and retard air entry to this height. As we have no direct measurements of oxygen, we refer to findings reported by Reiche et al. (2009) and Knorr et al. (2009) for this site. Reiche et al. (2009) measured maximum oxygen penetration depth with a relatively low temporal resolution (using FeS-redox probes) in 2006 and 2007. Those probes closest to our measurement chambers indicated maximum oxygen penetration of 35 cm on the control and 47 cm on the manipulation plots at the end of the manipulation period in 2006, compared to 4-13 cm on the C_{1-3} plots, and 6-20 cm on the D_{1-3} plots in 2007 (Reiche et al., personal communication). Summarizing, the year 2006 was characterized by generally deeper aeration of the peat body than the year of 2007. Furthermore, differences in the maximum penetration depth between C_{1-3} and D_{1-3} plots were more pronounced in 2006 than in 2007. Knorr et al. (2009) measured redox conditions on the plots during our experiments. They found a partial decoupling of water table and peat aeration, as they described anoxic microenvironments that were present in the uppermost horizons, regardless of water table. In summary, lowering of the water table can increase maximum oxygen penetration depth, so that deeper peat layers become aerated, but it seems not to increase oxygen availability in the uppermost horizons, where anoxic microenvironments are present regardless of water table. The water table fluctuation on the C_{1-3} plots indicates that low water tables in summer with, periodically dry conditions, seem to be a regular phenomenon at this fen site, a circumstance that is reflected in the dominant plant species, as *Molinia caerulea* (L.), *Nardus stricta* (L.) and *Eriophorum vaginatum* (L.) are all reported to be indicators of highly variable soil moisture conditions (Ellenberg et al., 1992). Paul et al. (2006) came to a similar conclusion for this site, describing the upper horizons as predominantly oxic. Lowering of the water table during summer therefore most likely has only an effect on the oxygen availability in deeper peat layers.

Radiocarbon analyses in the soil profile of the *Schlöppnerbrunnen* site revealed that the occurrence of young, easily decomposable substrates is restricted to the upper 10-15 cm of the peat body (Muhr et al., 2009). Reiche et al. (2009) incubated peat samples from the *Schlöppnerbrunnen* site in the laboratory to measure basal soil respiration, anaerobic CO_2 formation and exoenzymatic activities at different water table levels. The measured parameters were highest in the upper 0-10 cm and quickly decreased below that. Furthermore, below 10 cm, all three parameters were very constant over time and no effect of water table was found. We conclude that R_{Soil} in peat layers below ca. 10-15 cm depth is mostly not limited by oxygen availability but by poor substrate quality. This is supported by comparing

R_{Soil} data from the manipulation periods of 2006 and 2007. In 2006, we found very deep oxygen penetration on the C_{1-3} and the D_{1-3} plots. Maximum penetration depth in 2007 was considerably smaller in 2007 by up to 25 cm, but R_{Soil} rates in 2006 and 2007 were not differing at all. We conclude that aeration of deeper peat layers does not bear the potential to significantly increase R_{Soil} at the site. In summary, lowering of the water table does not result in increased CO_2 emissions because it can only increase oxygen availability in deeper peat layers where poor substrate quality rather than oxygen availability limit decomposition.

Our assumptions are supported by data from Hogg et al. (1992), who found that mass losses from peat taken from 30-40 cm depth were very low, even after 3 months of exposure to aerated conditions at 24°C. They concluded that peat from greater depths was more resistant to decomposition than surface peat. Lowered water tables therefore do not necessarily have to result in an increase of CO_2 emissions, particularly in peatlands that already have a low water table for most of the summer.

Chimner & Cooper (2003) reported similar findings for a Colorado subalpine fen. They found that CO_2 emissions strongly increased with lowered water tables, as long as water tables ranged between 10 cm above and 5 cm below the peat surface. When water tables were lowered deeper than 5 cm below the peat surface, no additional effect on the CO_2 emissions was found.

Our results also agree with findings reported by Knorr et al (2008), who investigated peat monoliths from our fen site in the laboratory. When they artificially lowered the water table from -10 cm to ca. -50 cm, CO_2 emissions at the peat surface were not affected at all by water table.

To understand future changes in the carbon balance of peatlands, it is important to not only investigate the dynamics of carbon losses *via* R_{Soil}, but also the effect of climate change on the vegetation. In this experiment we carried out very limited measurements of GPP and R_{AG} that are not sufficient to quantify annual CO_2 uptake. To normalize for heterogeneity in plant biomass on our plots, we calculated fluxes per biomass unit. By doing so, we assume that differences in biomass on the plots are inherent, and not affected by the manipulation. Instead of investigating possible changes in total biomass production, our measurements of GPP and R_{AG} were designed to estimate the effect of stress on the aboveground vegetation. Due to the predominantly identical results on the C_{1-3} and the D_{1-3} plots, we conclude that lowering of the water table did not significantly alter the stress regime on the D_{1-3} plots. Differences during the first measurement dates presumably result from inaccurate biomass data. As described above, biomass was only measured once in September 2007 and calculated

for the other dates based on data from Otieno et al. (2009). We expect increasing deviation of the biomass dynamics on our measurement plots from the dynamics on their plots towards spring, when initial growth results in high variability of biomass at the site.

Direct comparison of the C_{1-3} and the D_{1-3} plots revealed predominantly no effect of lowered water tables. We used a second approach to estimate the effect of the water table on decomposition by pooling all the data from C_{1-3} and D_{1-3} plots and applying the CO_2 efflux model by Mäkiranta et al. (2009). Using this approach, we found a significant correlation between water table and R_{Soil}. In contrast to our expectations, however, the model indicates that R_{Soil} decreases when water table is lowered, which could possibly be explained by drought stress in the litter and uppermost peat layer, as documented for the summer of 2007 on the D_{1-3} plots. This drought stress presumably results only partly from lowered water tables. More likely, what we see here is a consequence of the fact that the periods of low water tables in our data coincide with periods of warm and dry weather during summer.

Neither of the two approaches we used reveals an increase of CO_2 emissions due to lower water table. This is in contrast to additional experiments that Otieno et al. (2009) carried out in another area of the *Schlöppnerbrunnen* site. They reported increasing R_{Eco} when water table was lowered. The parameter R_{Eco} includes R_{Soil} and R_{AG} and it remains unclear how the two parameters reacted individually towards lower water tables. More important, the data they used comprise water table levels mainly between 0-10 cm below the peat surface and only some data down to 18 cm beneath the peat surface. Thus, their data differs from ours as our data includes very little water table levels above 10 cm below peat surface. Therefore, our findings are restricted to low water table levels.

Generally, findings of the various experiments at this site, as well as those reported in the literature strongly suggest that water table at the *Schlöppnerbrunnen* site is an important determinant of CO_2 emissions within the uppermost ca. 10 cm, but becomes increasingly unimportant below this depth. Therefore, a follow-up experiment was designed to examine the effect of artificially increased water table on CO_2 emissions at the *Schlöppnerbrunnen* site. Preliminary results indicate that CO_2 emissions on the flooded plots are decreased compared to control plots (Wunderlich, personal communication). So far, we found no evidence that lowering of the water table altered the activity of the aboveground vegetation. We postulate that a moderate increase of summer drought conditions, like simulated in this experiment will most likely not affect the C balance of the *Schlöppnerbrunnen* site.

Conclusion

Lowering the water table in a minerotrophic fen in South-eastern Germany during summer had predominantly no effect on gaseous C fluxes in three subsequent manipulation periods. An explanation for our findings is that gaseous C fluxes at our site during manipulation periods mostly are not oxygen limited, most likely due to already low water tables on our control plots and poor substrate quality in deeper peat layers. Instead of improving conditions for soil micro-organisms and plants, the lowering of the water table had no effect. Under most extreme drought conditions (high evapotranspiration), it might even cause drought stress in the uppermost regions of the peatland (mainly litter layer), thereby reducing decomposition rates, like observed in the summer of 2007. We anticipate that prolonged summer droughts could only affect C fluxes at our site if water table was lowered deep and long enough for drought stress to occur on the soil surface. In this case we mainly expect a reduction of CO_2 emissions *via* R_{Soil} and therefore a transient increase of the sink strength at the *Schlöppnerbrunnen* site.

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PART B:

Responses of CO_2 Exchange and Primary Production of the Ecosystem Components to Environmental Changes in a Mountain Peatland

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Summary

The complexity of natural ecological systems presents challenges for predicting the impact of global environmental changes on ecosystem structure and function. Grouping of plants into functional types, i.e. groups of species sharing traits that govern their mechanisms of response to environmental perturbations, reduce the complexity of species diversity to a few key plant types for better understanding of ecosystem responses. Chambers were used to measure CO_2 exchange in grass and moss growing together in a mountain peatland in southern Germany to assess variations in their response to environmental changes and how they influence ecosystem CO_2 exchange. Parameter fits and comparison for NEE in two ecosystem components were conducted using an empirical hyperbolic light response model.

Annual green biomass production was 320 and 210 g dwt.m^{-2}, while mean maximum Net Ecosystem Exchange (NEE) was –10.0 and –5.0 μmol m^{-2} s^{-1} for grass and moss respectively. Grass exhibited higher light use efficiency (α) and maximum gross primary production [$(\beta+\gamma)_{2000}$]. Leaf Area Index (LAI) explained 93% of light use and 83% of overall production

by the grass. Peat temperature at 10 cm depth explained more than 80% of the fluctuations in ecosystem respiration (R_{eco}). Compared to grass, moss NEE was more sensitive to ground water level (GWL) draw-down and hence could be more vulnerable to changes in precipitation that result into GWL decline and may be potentially replaced by grass and other vegetation that are less sensitive.

Key words: Carbon dioxide exchange; environmental variables; ground water level; peatlands; primary production; respiration.

Introduction

Peatlands cover about 3.5% (or $5x10^6$ km^2) of the Earth's land surface (Gorham 1991). More than 95% of total peatlands occur in cool, humid climates of the temperate belt in Northern Hemisphere. During the last millennium, northern peatlands have acted as carbon (C) sinks, with an average annual C uptake of 20-35 g C m^{-2} per year (Gorham 1991; Turunen and others 2002). This has resulted in an enormous C pool in peatland soils, amounting to approximately one-third of the world's total soil C (4.5×10^{17} g) (Turunen and others 2002). As a result of this large stock of partially decomposed plant material, peatlands are potential CO_2 sources (Chapin and others 1992; Smith and others 2004).

Despite their significant role in terrestrial C cycle, the regulation of C flow in peatlands is still poorly understood (Fenner and others 2004; Riutta and others 2007). The integrated dynamics of ground water level (GWL) may determine the long-term ecological function of temperate peatlands as C sinks as in the case of arctic tundra (cf. Ostendorf and others 1996). Lowering of the GWL could enhance decomposition over assimilation resulting in decreased net CO_2 uptake or even net CO_2 loss (Oechel and others 1995; Bubier and others 2003; Tuitilla and others 2004; Riutta and others 2007). On the other hand, several studies consider the effect of the water table as minimal and emphasise the significant influence of micro-climatic parameters such as temperature and light (see Laufleur and others 2005). Finally, rainfall and water table may indirectly influence carbon balance by modifying phenology, nutrient availability, and development of aboveground leaf area in a particular season (Shaver and others 1998; Hastings and others 1989).

Lack of consensus on how CO_2 exchange is controlled may arise from the wide range of methodologies that are employed to assess ecosystem responses (Oechel and others 1995; Ruimy and others 1995; Frolking and others 1998) and the ability to identify and describe the objects under study. Some of the methods (e.g. eddy covariance) do not take into account the inter-specific differences associated with inherent physiological adaptations that are bound to influence plant responses (Semikhatova and others 1992). Such adaptations may buffer plants from the impact of short-term habitat changes during the growing season and modify ecosystem response to environmental changes (Riutta and others 2007). Mosses for example, have shallow rhizoids and, therefore, are likely to be sensitive to shifts in GWL, with a significant influence on overall ecosystem CO_2 exchange. Moss photosynthesis and growth should, therefore, be directly influenced by the water table (Clymo and Hayward 1982; McNeil and Waddington 2003; Robroek and others 2007). This may not be the case with grass or sedge, which have relatively deep rooting systems (Bortoluzzi and others 2006;

Riutta and others 2007). Thus, characteristics of the vegetation mosaic reflect resource availability and correlate with aspects of carbon balance and net primary production (Ostendorf and others 1996; Leadley and Stocklin 1996)

Understanding of the biotic controls over functional groups and ecosystem CO_2 exchange processes and their interaction with the physical environment is crucial and provides a basis for predicting how functional groups and entire peatland ecosystems may respond to changes in the habitat. We used chamber methods to examine the variation in ecosystem CO_2 exchange response of two dominant functional groups (moss and grass) occurring in a mountain peatland of Germany. The peatland is gently sloping, homogeneous in terms of species presence, but characterised by a recurring mosaic in fine scale structure where the community is either dominated by grass and sedge tussocks with little moss, or relatively open troughs or inter-tussocks dominated by moss, with little graminoid biomass. Our objectives were; 1. To monitor annual biomass production of moss and grass in the peatland, 2. To monitor diurnal and seasonal CO_2 exchange in grass and moss dominated plots and to determine how the individual growth forms are influenced by ground water level (GWL), temperature and light and 3. To examine how grass and moss contribute to the overall net ecosystem CO_2 exchange of the plots and the peatland over the course of the season. While each plot studied is structurally unique, the exact composition of vegetation included in each measurement was determined. The average behaviour of the natural mosaic components, dominated either by moss or graminoid biomass, in gas exchange is compared, which will allow us to use abundance-weighted estimates of carbon exchange to model the mixed-functional-type community in subsequent steps.

Materials and methods

Site description

Measurements were conducted in a fen ecosystem at Schlöppner Brunnen site (50°07’54”N, 11°52’51”E) at an elevation of 700 m a.s.l., where a clearing is surrounded by spruce trees (Figure 1). The fen is dominated by two main functional groups, which include moss (*Sphagnum fallax* and *Polytrichum commune*) and grass (*Agrostis canina*, *A. stolonifera*, *Molinia caerulea*). A sedge (*Carex nigra*) is also found scattered within the vegetation, but it is overgrown quickly by the grasses and negligible in its contribution to biomass by early summer. The site structure is relatively flat but gently sloping, with a mosaic of grass-dominated tussocks and moss-dominated inter-tussock areas.

The soil at the site is classified as Fibric Histosol, moderately acidic (pH 3.5-5.5), with highly decomposed soils rich in sulphur (up to 4.6 mg kg^{-1}) and iron (up to >16 mg kg^{-1}). The fen has a slope of 3 % and water flow direction is parallel to the slope from NNE to SSW (Figure 1). The annual precipitation in the catchment varies between 900 and 1160 mm yr^{-1} and the mean annual air temperature is 5°C. The mean in-situ water table level varies annually and was 0.13 ±0.19 m (Paul and others, 2006).

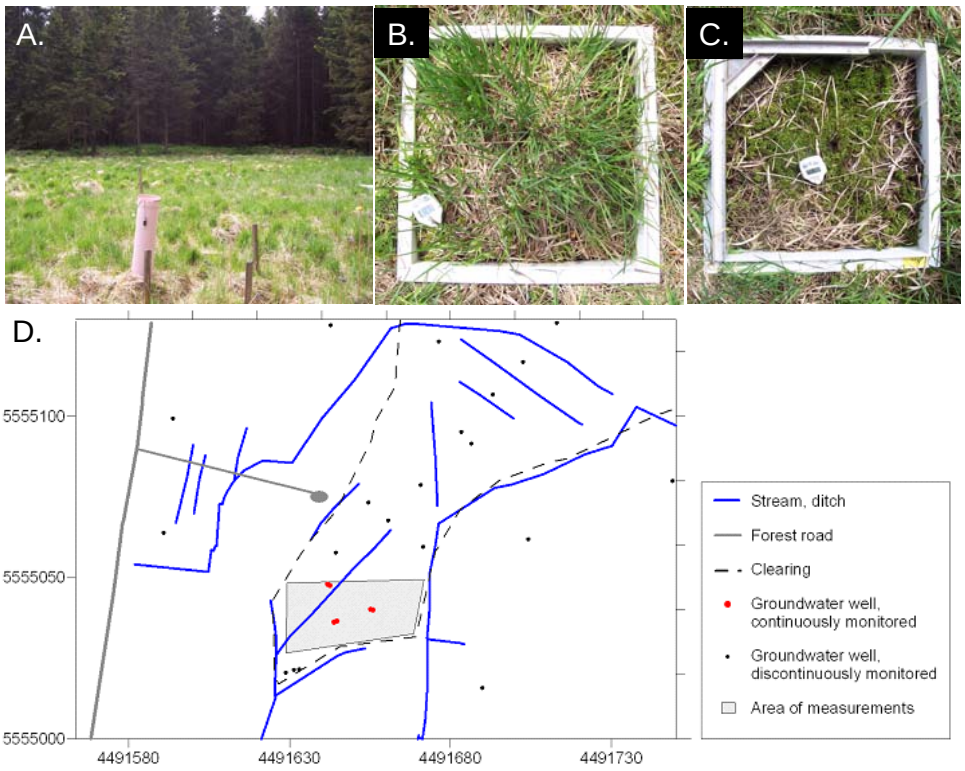

Figure 1. A. Illustration of the vegetation at the Schlöppner Brunnen wetland. B. Grass-dominated experimental plot as prepared for measurements, showing collars inserted into the organic soil. C. Moss-dominated experimental plot as in 1B. with a metal frame for mounting of cooling packs. D. Map of the Schlöppner Brunnen site indicating drainage channels and groundwater wells. The axes are Gauss-Krüger coordinates in meters.

Measurements

Microclimate

Weather conditions were continuously recorded at a meteorological station set up at the field site. Precipitation (ARG100 rain gauge, EM Ltd., Sunderland UK), global radiation, photosynthetic photon flux density (PPFD) (LI-190 Quantum sensor, Li-Cor, USA), air humidity and temperature (VAISALA HMP45A, Helsinki, Finland), and peat (0, -30, -100 cm) temperature profiles (Thermistor M841 Siemens, Germany). Data were measured every 5 min, averaged and logged every 30 min by data logger (DL2e, Delta-T Devices Ltd. Cambridge UK). Water level was measured with pressure transducers (Piezometers 26PCBFA6D, IBA Sesorik GmbH, Seligenstadt, Germany) and data recorded daily.

NEE measurements with chambers

Field measurements of ecosystem CO_2 exchange were conducted each month (1 week in a month, with 3 days of measurements) between May and October 2007, except for July when two sets of measurements were carried out. A set of 6 soil frames or collars, 3 on grass-dominated and 3 on moss-dominated vegetation were inserted into the soil a month before the measurements were conducted. Moss-dominated plots (hereafter called moss plots) were selected to have as few grass/sedges on them as possible (see Table 1 versus data in Figure 3). Also, the grass-dominated plots (hereafter called grass plots) had a combination of grass and sedge but the sedge (*Carex nigra*) and moss biomass were very low. We avoided selective vegetation removal in order to maintain the natural conditions of the plots. Each soil frame constituted a measurement plot and, hence, during each measurement week, 3 grass and 3 moss plots were monitored on day 1 and 2 to characterize CO_2 gas exchange. At the end of second measurement day (~18:00 hr), all the aboveground biomass on each of the plots was harvested. CO_2 measurements were continued on day 3, (1 day after biomass removal) to determine the peat respiration. New plots were then established for the next cycle/round of monthly measurements and the soil frames re-installed. Non-destructive determination of biomass and LAI within the studied plots was not possible hence biomass was harvested after every complete set of NEE measurements. The biomass estimates for each plot were used to normalise NEE per unit biomass; moss plots on the basis of total green moss biomass and grass plots based on green grass biomass.

Table 1. Green Biomass and LAI of grass in so-called moss plots, and green biomass of moss harvested in the 40 by 40 cm^2 grass plots (expressed to unit m^2) for each measurement campaign during the measurement period. Comparison with Figure 3 demonstrates that dominance by one or the other growth forms in the differently selected plots was almost complete.

DOY	Grass in moss plots		Moss in grass plots
	Green biomass ($g\ m^{-2}$)	LAI ($m^2 m^{-2}$)	Green biomass ($g\ m^{-2}$)
131	20.75	0.02	20.01
171	17.28	0.05	0.00
198	23.08	0.33	12.00
206	53.27	0.61	24.92
227	32.83	0.13	19.45
263	22.56	0.03	41.44
297	8.08	0.01	9.13

During each monthly measurement series (3 measurement days each month), net ecosystem exchange (NEE) and ecosystem respiration (R_{eco}) were sequentially observed with a systematic rotation over all plots using manually-operated, closed gas exchange chambers, modified from the description given by Droesler (2005), Wohlfahrt and others (2005), and Li and others (2008b) as used in central European bogs and alpine grasslands. The 38 x 38 x 54 cm^3 chambers of our system were constructed of transparent plexiglass (3 mm XT type 20070; light transmission 95%). Dark chambers, for measuring ecosystem respiration were constructed of opaque PVC, and covered with an opaque insulation layer and with reflective aluminium foil. Using extension bases, chamber height was adjusted to the canopy height. Chambers were placed on the plastic frames/collars that were inserted 7 cm into the ground. They were sealed to the chamber with a flexible rubber gasket and the chamber firmly secured using elastic bands fastened onto the ground from two sides. Tests indicated that leakage did not occur (see Droesler 2005 for details), however, this could not be examined regularly in the case of systematic field measurements and required that each set of data must be scrutinised for abnormalities.

Increased air pressure in the chamber was avoided by a 12 mm opening at the top of the chamber which was closed after the chamber had been placed onto the frame and before any records were taken. Circulation of air within the chamber was provided by three fans yielding a wind speed of 1.5 m s^{-1}. Change in chamber CO_2 concentration over time was assessed with a portable, battery operated IRGA (Li-Cor 820). Measurements were carried out in most cases within 3-5 minutes of placing the chamber on the frames. Once steady state was attained, data were logged every 15 s for 2 minutes and CO_2 fluxes were calculated from a linear regression describing the time dependent change in CO_2 concentration within the chamber. Influence of the CO_2 concentration change on plant physiological response was ignored. By mounting frozen ice packs inside and at the back of the chamber in the airflow, temperature during measurements could be maintained within 1° C relative to ambient. Air (at 20 cm above the ground surface) and peat (at –10 cm) temperatures inside and outside of the chambers were monitored during measurement and data logged at the onset and end of every round of NEE measurement on each plot. Similarly, light levels within the chamber, and above the vegetation (canopy) were monitored using a quantum sensor (LI-190, Li-Cor, USA) and data were logged every 15 seconds. Tests conducted in a controlled climate chamber showed that vapor pressure deficit (VPD) changes within our CO_2 measurement chambers were limited to 1 hPa during the period (~3 min) when the chambers were placed on the vegetation. We

therefore assumed that such small VPD changes should not affect CO_2 exchange via stomatal effects.

During each monthly measurement series, repeated light and dark chamber measurements were conducted from sunrise to sunset over single days comparing three moss and three grass plots. Eight to eleven measurement cycles were accomplished on individual days. To estimate Gross Primary Production (GPP), ecosystem respiration was estimated for each NEE observation time by linearly extrapolating between dark chamber observations (R_{eco}), and then adding it to NEE (cf. Li and others 2008a). As seen in Figure 5, the measurements of NEE and R_{eco} were closely associated in time, thus the corrections made in R_{eco} were very small. Measurements were conducted from the beginning of May until October in order to develop a picture of the seasonal dynamics of CO_2 exchange. Limitation in manpower to carry out the labour intensive chamber measurements and the nature of our experimental site prevented continuation of the observations with chambers during nighttime periods.

Estimation of model parameters describing gas exchange response

Empirical description of the measured NEE fluxes was accomplished via a non-linear least squares fit of the data to a hyperbolic light response model, also known as the Michaelis-Menten or rectangular hyperbola model (cf. Owen and others 2007):

$$NEE = -\frac{\alpha\beta Q}{\alpha Q + \beta} + \gamma \qquad (1)$$

where NEE is net ecosystem CO_2 exchange (μmol CO_2 m^{-2} s^{-1}), α is the initial slope of the light response curve and an approximation of the canopy light utilisation efficiency (μmol CO_2 m^{-2} s^{-1}/μmol photon m^{-2} s^{-1}), β is the maximum NEE of the canopy (μmol CO_2 m^{-2} s^{-1}), Q is the Photosynthetic Active Radiation, PPFD (μmol photon m^{-2} s^{-1}), γ is an estimate of the average ecosystem respiration (R_{eco}) occurring during the observation period (μmol CO_2 m^{-2} s^{-1}), (α/β) is the radiation required for half maximal uptake rate, and (β+γ) is the theoretical maximum uptake capacity. Since the rectangular hyperbola may saturate very slowly in terms of light, the term (αβQ)/(αQ+β) evaluated at a reasonable level of high light (Q = 2000 μmol photons m^{-2} s^{-1} is used in this study) approximates the potential maximum GPP and can be thought of as the average maximum canopy uptake capacity during each observation period, noted here as $(\beta+\gamma)_{2000}$. The parameters $(\beta+\gamma)_{2000}$ (e.g., NEE at PPFD(Q)=2000) and γ were estimated for each functional group using NEE data from the three measurement plots per day. Data were pooled separately for grass and moss.

Biomass and LAI measurements

After gas exchange measurements on the second day of each campaign, all the aboveground biomass within the 38 x 38 cm^2 area enclosed by the collars was harvested. The harvested moss and grass biomass was sorted into green and dead material. Leaf area (LA) of the grass was measured using leaf area meter (CI-202, CID, Camas, WA) before being oven dried at 80°C for 48 hours and weighed. The rest of the biomass was oven dried at 80°C for 48 hours and weighed to obtain the live and dead dry weight. Due to difficulties in determining the moss photosynthesising surface area, the green biomass was simply dried and weighed. Leaf area index (LAI) of the grass was calculated by dividing the LA by plot area.

During the months of July, August and September, root biomass was sampled with an 8 cm-diameter soil corer. Sampling took place in the middle of the grass measurement plots after CO_2 measurements were finished. The 30 cm soil cores were divided into sections of 5 cm each, washed under running tap water using soil sieves (mesh 2 mm) and the collected roots were oven dried at 80°C before weighing them to obtain root dry weight in each of the soil profiles. Due to difficulty in separating dead and live biomass, the reported results include both dead and live root biomass. Moss rhizoid biomass was not quantified.

Results

Meteorology

Weather conditions during the study period are shown in Figure 2. Light conditions inside and outside the chamber were not significantly different (Figure 2a). Temperature differences between the inside and outside of the chamber were maintained within ±1°C. Mean annual VPD was around 5 kPa and VPD fluctuated on a daily basis (Figure 2b). Air and peat temperatures rose to a maximum in July, with peat temperature lagging behind. Maximum air temperature (daily mean) of 15°C was recorded in July. Peat temperature varied with depth and higher fluctuations occurred near the peat surface (Figure 2c). The shallow layers warmed up faster after the snow thaw, but also cooled down more rapidly in autumn, while temperatures at 1 m depth lagged behind for several weeks. Compared to 1 m depth, peat temperatures at 30 cm depth were higher between April and August. Similarly, temperatures during the day measured at the peat surface were higher than at 30 cm, during the same period. Later in the year (autumn), the temperature profile inverted, with the warmest temperatures (~2°C warmer) at 1 m depth and the coldest near the surface, reflecting the decline in average daily air temperatures at that time.

Annual sum of precipitation was within the range of long-term average. The amount of rainfall received between April and December was 942 mm (Figure 2d). A dry spell occurred between March and May leading to a significant decrease (-0.4 m) in the ground water level (GWL), the lowest water table being recorded in May. Afterwards, GWL remained relatively high and did not decrease below -0.2 m.

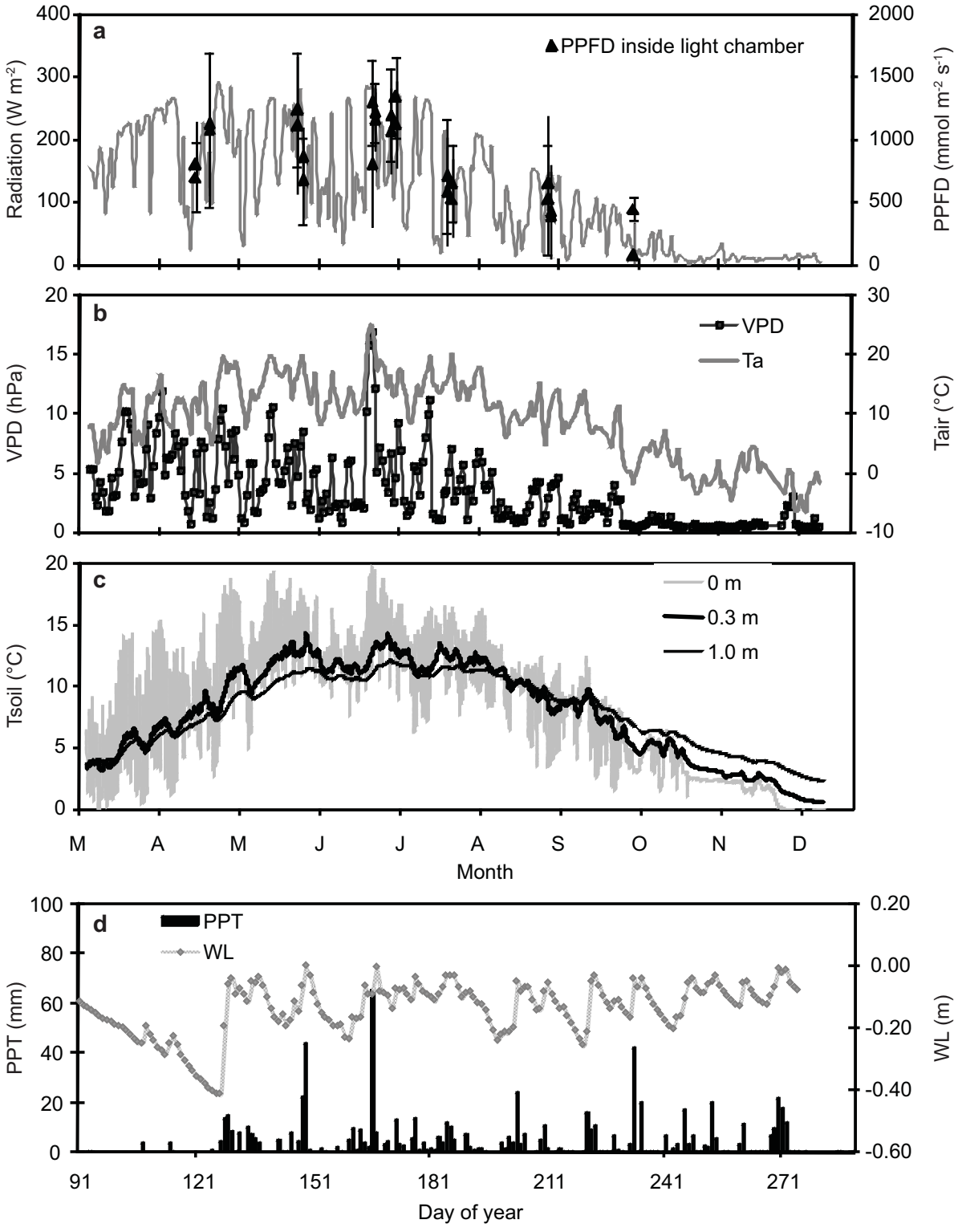

Figure 2. Prevailing weather conditions (a, b, c, d) and ground water level (d) at the study site during the measurement period.

Biomass development

Biomass did not vary greatly among the selected moss and grass plots during any single monthly measurement campaign, but changes were large between monthly measurements (Figure 3a). Green biomass development in grass was recorded from May to July, when the grasses attained maturity. Maximum green biomass (dry weight) of grass was 320 g m^{-2}. After July, grass biomass declined significantly with the declining air and peat temperatures and by the end of October, when the first snowfall occurred, there was almost no green biomass remaining (Figure 3a). The pattern of LAI development in grass was similar that of biomass development, since biomass is used as a scaling factor in the conversion from sub-samples to plot level.

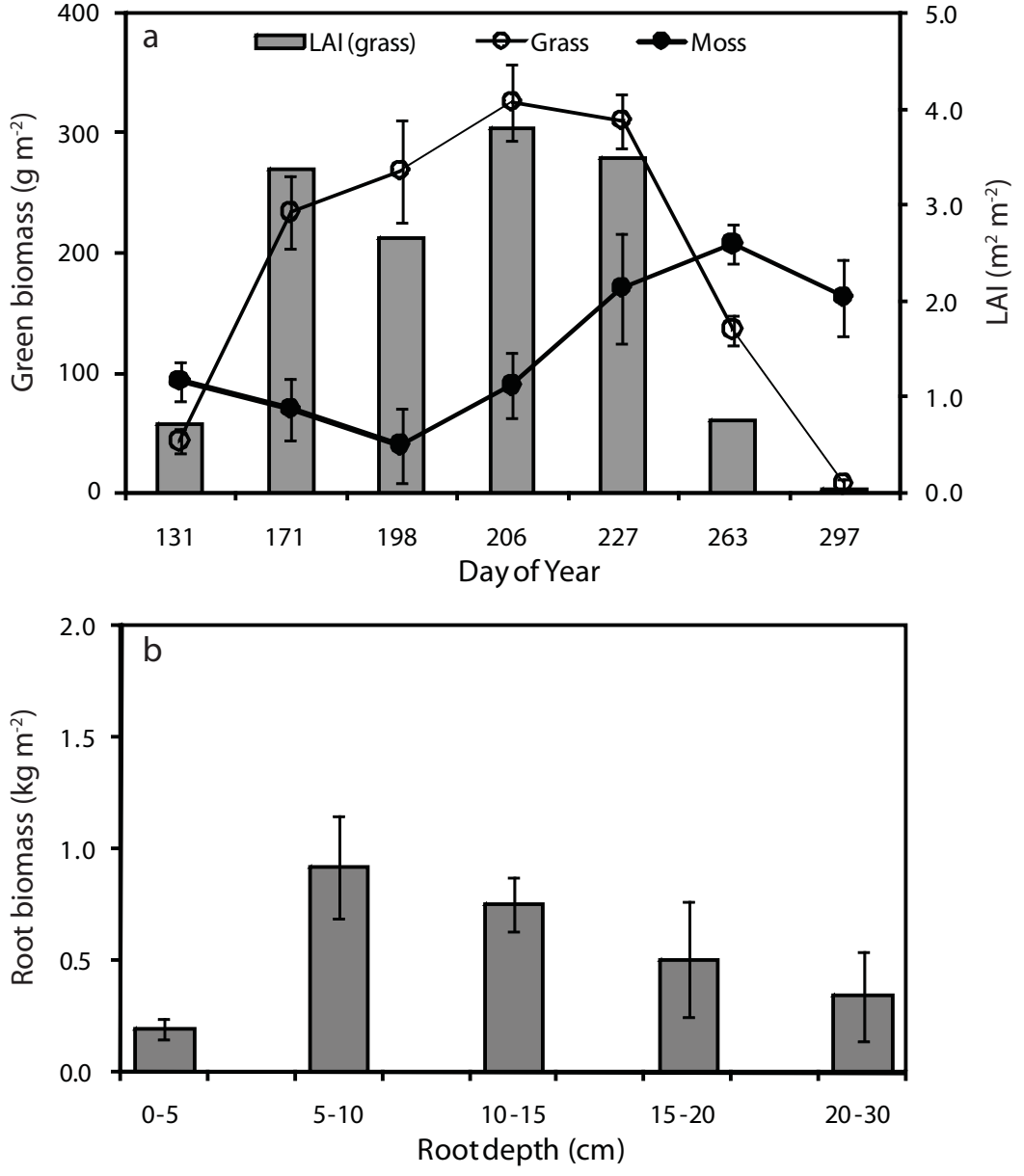

Figure 3. Seasonal variation in (a) Green biomass (moss and grass) and LAI (grass only) development. (b) Root biomass of grasses in varying soil depths determined at selected periods during the season. Bars are SE.

Highest LAI of 3.8 occurred in July (Figure 3a). Afterwards, LAI declined significantly, reaching zero values in October. Root biomass of the grass decreased with depth, but extended well below 20 cm into the soil profile (Figure 3b). Highest root mass occurred within the top 5-10 cm layer, totalling to 1.0 kg m^{-2}. The moss rhizoids were shallow and formed a thick mat within the first 1 cm top soil layer (data not shown). Moss green biomass changed in a very different manner, declining between DOY 131 and 198. Maximum biomass of 210 g m^{-2} occurred in September.

Seasonal and diurnal patterns of CO_2 fluxes

The maximum daily values for NEE, R_{eco} and GPP increased significantly between May (DOY 131) and July (DOY 206) in both moss and grass (Figure 4). Highest R_{eco} (30.0 and 22.0 μmol $m^{-2}s^{-1}$ for grass and moss respectively) occurred in mid July. R_{eco} declined to near zero at the end of the growing season. Similar patterns were observed for GPP and NEE. Compared to grass, maximum NEE attained during the day were lower in moss (close to zero) between May and June, while moss exhibited higher R_{eco} during the same period. Maximum NEE, however, occurred later in July. The highest GPP recorded during the season (37.0 μmol m^{-2} s^{-1}) occurred in the grass plots and was about 10 μmol m^{-2} s^{-1} more than in the moss plots. Parameters derived from the empirical light response model (Equation 1) are shown in Table 2. Light use efficiency (α) increased between May and June and was significantly ($p<0.05$) higher in the grass compared to the moss. The highest α recorded for grass (0.08 ±0.01) was in mid summer (DOY 198-204), while the highest for moss (0.04 ±0.01) occurred much earlier (DOY 171). These values coincide with the highest assimilation capacities (β) of -18.70 ±2.48 and -10.31 ±4.59 for grass and moss, respectively. Both α and β declined later during the growing season. Similar responses were observed for γ and $(\beta+\gamma)_{2000}$. Model results showed the maximum value of average GPP $[(\beta+\gamma)_{2000}]$ occurring in July both in moss and grass and both the model derived results and the direct GPP estimates were comparable.

Observed diurnal courses of NEE and R_{eco} on selected measurement days of the year along with the prevailing weather conditions on the respective days are shown in Figure 5. On sunny days, CO_2 assimilation increased from its lowest rates in the morning to maximum around midday, but declined to near zero values later in the day. The daily peaks of carbon assimilation changed during the season depending on the prevailing air temperature (T_{air}) and light (PPFD) conditions. CO_2 uptake in grass plots occurred only around midday in May,

while moss plots were net CO_2 sources throughout the day during May and only became CO_2 sinks from June on. July and August were characterised by CO_2 uptake by both moss and grass during most of the day. Ecosystem respiration (R_{eco}) was relatively constant during the day but changed seasonally. Very low CO_2 exchange rates occurred later in the year (see DOY 263 Figure 5).

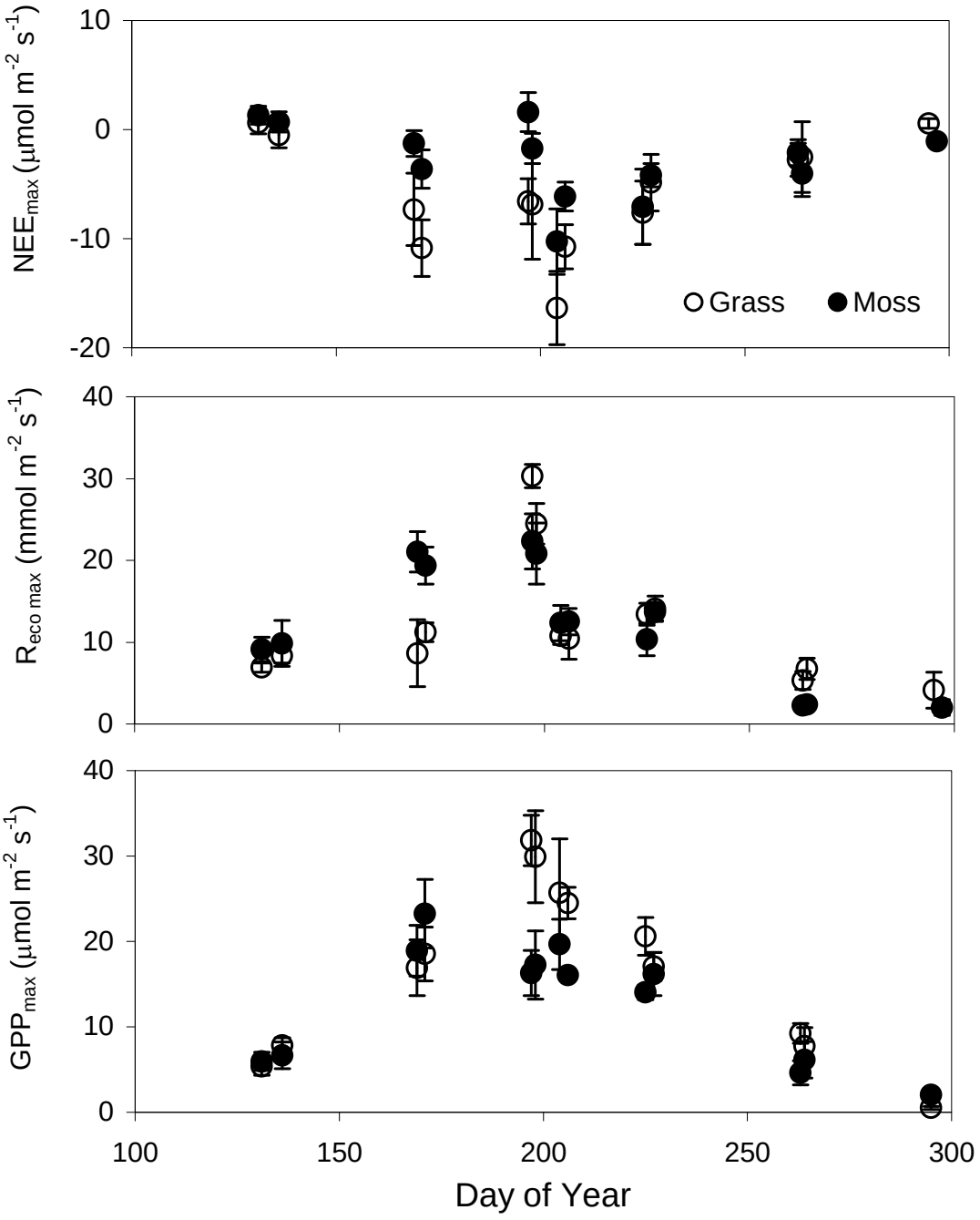

Figure 4. Seasonal variation of maximum NEE, R_{eco} and GPP determined from diurnal course flux measurements conducted on specific days during the year. Bars are SE.

Table 2. Best-Fit Parameters of the Empirical Hyperbolic Light Response Model for (A) Grass NEE and Their Statistics and (B) Moss NEE and Their Statistics

DOY	α	β	γ	$(\beta+\gamma)_{2000}$	S. E.$_{\alpha}$	S. E.$_{\beta}$	S. E.$_{\gamma}$	R^2
(a) Grass NEE								
131	0.02	-5.58	6.50	11.51	0.001	0.75	0.38	0.77
135	0.01	-11.98	5.17	10.75	0.001	2.13	1.08	0.39
169	0.07	-15.31	8.17	21.91	0.004	2.92	2.35	0.50
171	0.08	-16.70	11.94	26.98	0.002	5.40	1.84	0.79
197	0.08	-19.60	20.81	32.25	0.002	2.31	0.85	0.96
198	0.05	-20.05	16.78	34.77	0.006	2.48	1.52	0.92
204	0.08	-17.3	8.98	24.57	0.010	1.60	1.06	0.98
206	0.07	-20.92	7.48	25.67	0.002	1.63	1.08	0.85
225	0.06	-17.64	8.30	23.57	0.003	4.01	1.90	0.57
227	0.05	-16.69	7.10	25.23	0.001	8.00	1.22	0.68
263	0.02	-8.34	4.50	12.26	0.005	0.02	0.89	0.62
294	0.03	-6.26	7.26	13.32	0.007	1.55	0.88	0.61
(b) Moss NEE								
131	0.01	-8.97	7.05	14.28	0.002	1.89	2.26	0.51
135	0.01	-12.72	9.30	18.30	0.001	3.72	0.96	0.60
169	0.04	-13.83	16.04	22.04	0.003	7.34	2.99	0.43
171	0.04	-15.31	17.79	30.80	0.005	4.59	2.50	0.69
197	0.02	-14.98	17.24	31.27	0.001	4.65	1.20	0.80
198	0.03	-12.67	19.36	32.32	0.002	3.81	1.27	0.88
204	0.01	-10.49	17.71	28.96	0.001	3.98	2.47	0.46
206	0.03	-10.98	10.21	24.65	0.002	3.33	1.68	0.58
225	0.02	-8.05	6.24	17.74	0.001	2.89	0.93	0.78
227	0.02	-7.17	4.87	17.07	0.001	4.45	0.82	0.74
263	0.01	-6.60	1.43	6.85	0.005	1.07	0.34	0.79
294	0.02	-4.14	1.61	5.58	0.003	0.72	0.48	0.65

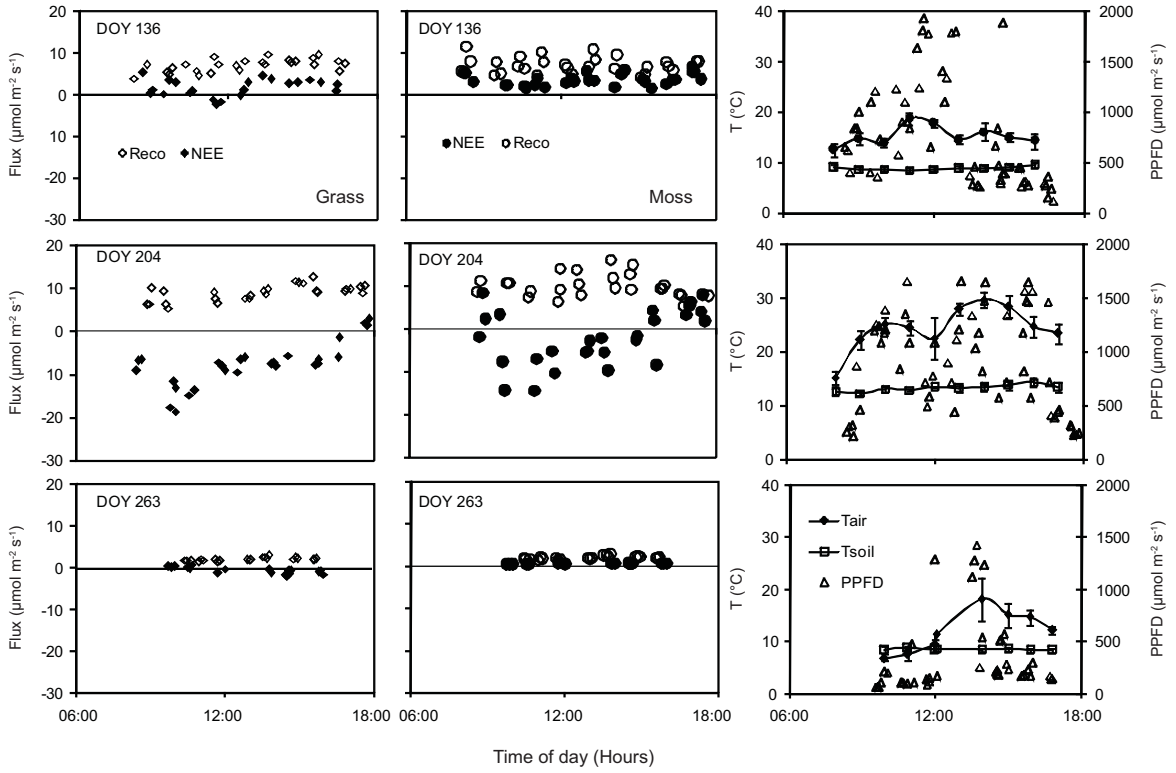

Figure 5. Diurnal variation of measured NEE and R_{eco} for selected periods during the growing season. Parallel air temperature (T_{air}) at 20 cm above the vegetation, peat temperature (T_{soil}) at –10 cm depth and Light conditions (PPFD) inside the chamber are also shown. Bars are SE.

Biotic influence on ecosystem CO_2 exchange

Both the biomass and CO_2 changed simultaneously during the season. In order to separate the influence of biomass changes on seasonal ecosystem CO_2 fluxes, NEE and R_{eco} were normalised with biomass in each of the plots (Figure 6). Until July, grass exhibited higher NEE per unit biomass than moss. After this period, both vegetation types had similar NEE per unit biomass. An abnormally high R_{eco} per unit biomass occurred in the moss on DOY 198. There was a strong correlation between LAI and model-derived physiological parameters α (R^2=0.93) and maximum GPP $(\beta+ \gamma)_{2000}$ (R^2=0.83) (Figure 7). Peat, which include soil and roots, contributed a significant proportion (~50%) of the total CO_2 output (R_{eco}). Contribution of the peat to the overall R_{eco} became dominant later in the season (Table 3).

Table 3. Mean soil and total ecosystem respiration (µmol m^{-2} s^{-1}) in grass and moss plots during respective months when measurements were conducted.

Month	Peat respiration		R_{eco}	
	Grass	Moss	Grass	Moss
June	4.9 ± 1.1	2.5 ± 5.3	9.9 ± 2.6	20.2 ± 2.4
July	13.0 ± 4.5	7.8 ± 3.3	24.5 ± 2.5	20.8 ± 3.7
August	10.5 ± 3.6	6.3 ± 2.0	14.1 ± 1.6	13.7 ± 1.1
October	3.8 ± 2.3	2.0 ± 0.9	4.2 ± 2.2	2.0 ± 0.9

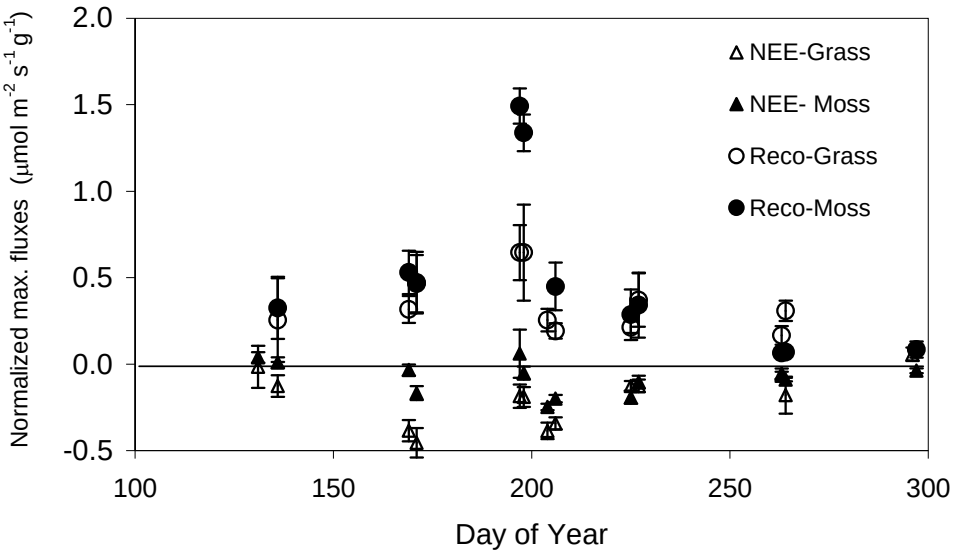

Figure 6. Seasonal courses of NEE and R_{eco} for both moss and grass/sedge normalized for green biomass. Bars are SE.

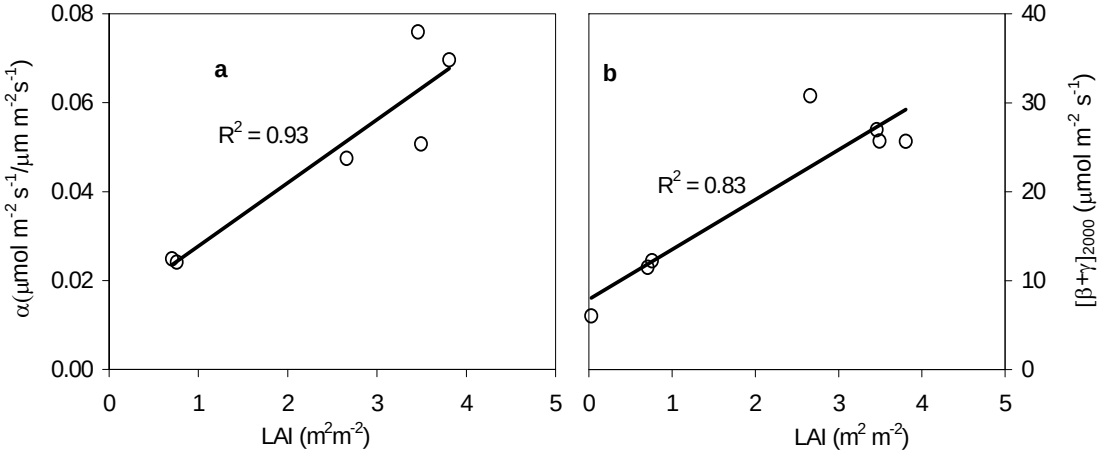

Figure 7. Relationship between LAI and (a) Light Use Efficiency (α) and (b) potential maximum GPP $(\beta+\gamma)_{2000}$ of the grass component of the ecosystem.

Abiotic influence on ecosystem CO_2 exchange

Figure 8 shows the relationship between NEE and PPFD (Q) in the grass and moss on specific periods of the year when measurements were conducted. The growing season was divided into three phases: May, July and September, which represented early, mature and late stages of growth in order to demonstrate the influence of light on NEE. During May, soon after the snow thaw, increasing light intensity was accompanied by increasing CO_2 uptake, both in grass and moss. For grass, light compensation point was reached at a proximately 1200 µmol m^{-2} s^{-1}, above which there was net ecosystem carbon gain. This was not the case with moss, where despite increased CO_2 uptake, stimulated by increasing light intensities,

there was still an overall net CO_2 production from the moss plots. In July, the vegetation showed rapid response to increasing light intensities and both grass and moss plots had relatively low light compensation points (< 500 μmol m^{-2} s^{-1}). Thus, the ecosystem was an active CO_2 sink during most part of the day. Compared to moss, higher NEE rates were observed in grass at similar light intensities during this period. Later in the season (September), although NEE rates were low, both moss and grass showed net CO_2 uptake, with low light compensation points. The results reveal that apart from light, other factors were also responsible for the regulation of CO_2 uptake in both moss and grass.

To reveal the underlying physiological mechanisms, data from the respective measurement days were fitted with a light response function (Equation 1). Physiological parameters derived from the function are summarised in Table 2. Strong positive correlation (R^2 between 0.50-0.98) between NEE and PPFD for most of the measurements and best fits occurred between June and August, particularly on days when minimum fluctuations in light intensities during the day occurred. In most cases, NEE saturated at relatively lower light intensities (900 μmol m^{-2} s^{-1}) in moss compared to grass (1200 μmol m^{-2} s^{-1}) and grass exhibited higher NEE, GPP, α and β values. Differences that occurred between moss and grass on any single measurement campaign were likely associated with differences in photosynthesising surface (LAI), α and β while seasonal differences between campaigns (Figure 7) were due to changing LAI, α, β and air temperatures. When similar fits were conducted on all the data, grouped together for the entire measurement period, the relationship was weaker, however it was better for moss compared to grass (R^2=0.68 in moss vs. R^2=0.60 in grass).

There was no correlation between air temperature and NEE. Using boundary analysis however, it was evident that net CO_2 uptake (more negative NEE) increased until an air temperature of 25°C (not shown). Further increase in air temperature above 25°C was accompanied by decline in net uptake. Although NEE declined during the time when lowest GWL (-20 cm during measurements) was experienced, the relationship between GWL and NEE was not consistent. Peat temperature at 10°C, however, explained most (>80%) of the changes in R_{eco} and similar response patterns were observed in both moss and grass (Figure 9a). There was an increase in R_{eco} with declining GWL, (R^2= 85 and R^2=39 for grass and moss respectively, Figure 9b), but shifts in GWL were also characterised by changes in peat temperature, making it difficult to discern the effects of GWL from peat temperature changes. Changes in peat temperature and GWL were however, not correlated and the influence of GWL on R_{eco} cannot be assumed.

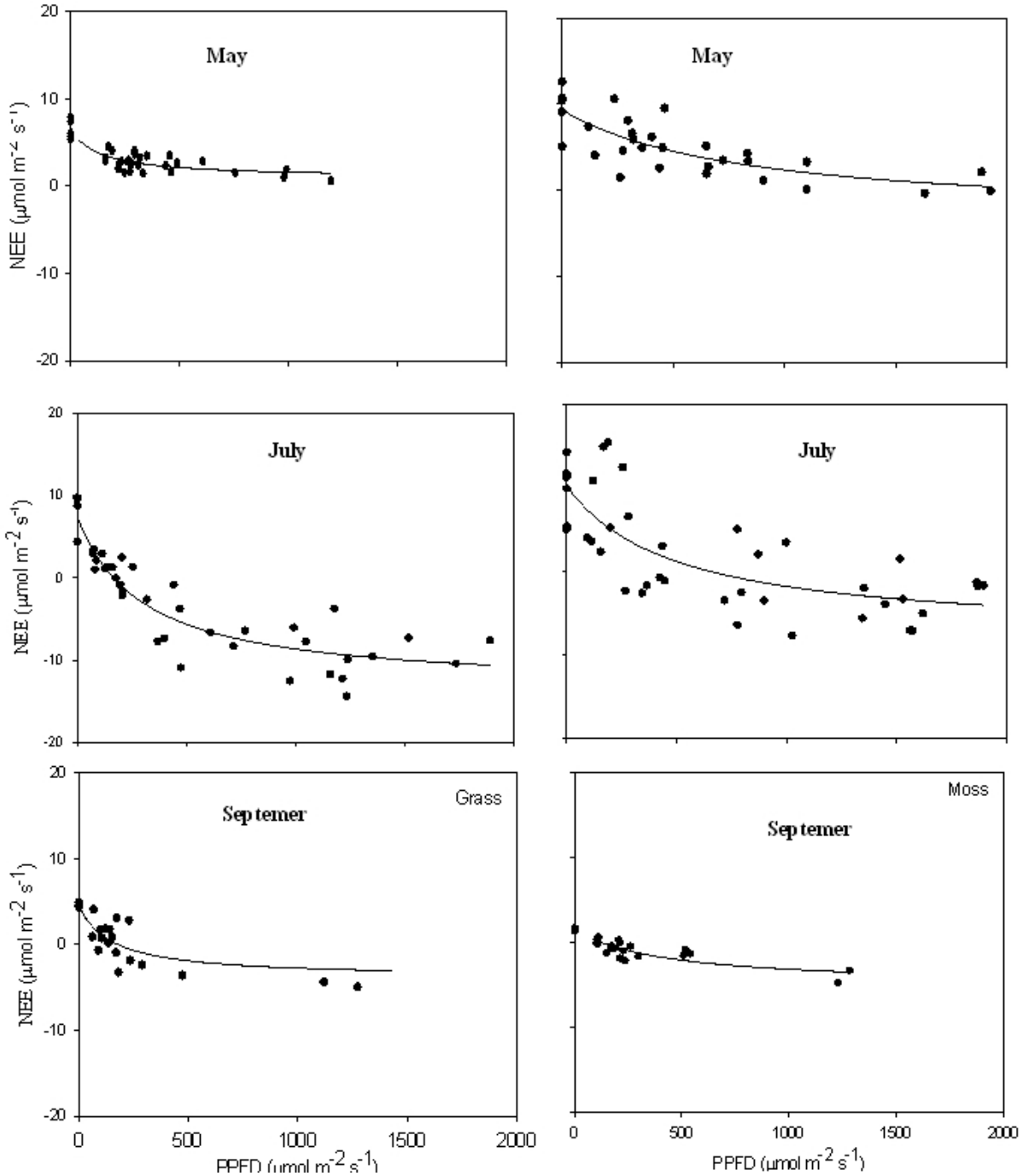

Figure 8. NEE- light response curves for grass (left panel) and moss (right panel) during early (May), mature (July) and late (September) stages of vegetation development. Regression analysis for light response curves were done with filtered data using Sigma Plot 8.0 (Residuals >5 eliminated). Results are integrated data from the three measured plots for each plant type.

Discussion

Aboveground biomass production occurred between May and October with an annual green biomass production of 320 g m^{-2} and 210 g m^{-2} for grass and moss, respectively. These values are within the range reported for most cool temperate peatlands of North America and Europe

as summarised by Moore and others (2002). Dyck and Shay (1999) reported moss capitulum biomass of 278 g m^{-2} in a peatland of central Canada based on sampling to depth of green colour. Annual biomass production of the moss capitulum in southern mires of Finland ranged between 260 and 400 g m^{-2} (Lindholm and Vasander 1990). Values obtained for eastern Canada for both vascular plant leaves and moss capitulum range between 114 and 672 g m^{-2} (Bubier and others 2006). Thus, in terms of biomass production, the studied peatland ecosystem is not very different from other peatlands occurring within the same latitudinal range. The results show that mosses are an important component of this community, representing approximately 30% of the total aboveground biomass during the growing period. Our findings are in agreement with those reported elsewhere (Shaver and Chapin 1991, Gordon and others 2001, Douma and others 2007), showing the significant contribution of moss to the overall community biomass as well as ecosystem function.

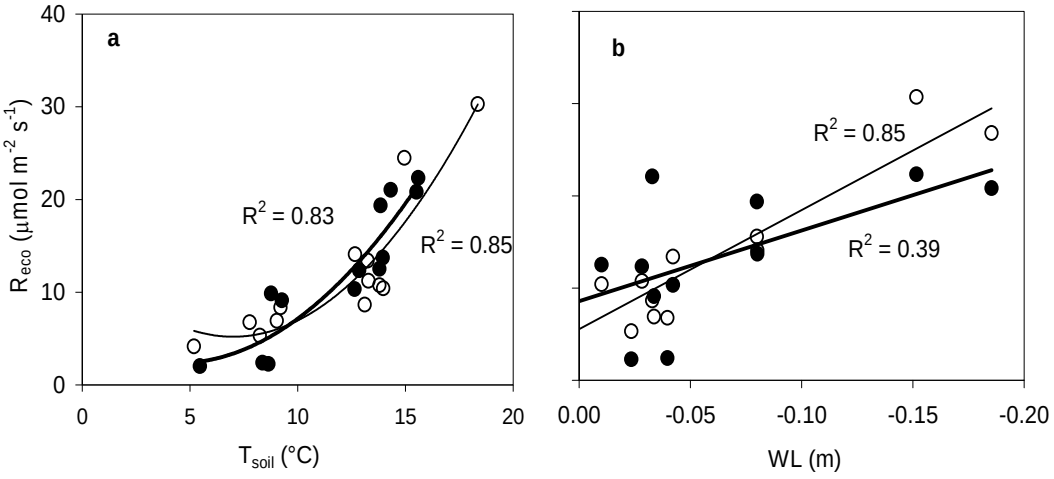

Figure 9. Relationships between daily averages of maximum R_{eco} of grass (open circles) and moss (closed circles) and (a) Peat temperature (T_{soil}) measured at 10 cm depth within the collars and (b) Mean ground water level (GWL), Thick regression lines represent moss, while the thin lines are for grass.

A combination of senescence of the grass, low radiation and rapidly dropping temperatures could be the reasons for the decline in green biomass after August. This was not the case with moss where green biomass development appeared to be more supported by low light intensities and air temperatures. The drop in moss green biomass between May (DOY 131) and June (DOY 198) may be due to be due to the early spring drought that led to the drop in GWL down to –40 cm with possible reduction in soil moisture within the top soil layers. Recovery of moss however, was a slow process, and it took almost a month before moss tissues were green and recovered. This may explain the observed 1.5 months' lag between GWL recovery and growth resumption. GWL, however, did not have any impact on

biomass development in grass. Reasons for this could be due to (1) unlike moss, green biomass development in grass started after April when there was rainfall and soil moisture conditions were already favourable. (2) Grass have deep rooting patterns, which could buffer them from low GWL compared to the moss (Limpens and others 2008). We observed grass roots growing down to -30 cm, indicating that even if the GWL drops down to this depth, grass will still have access to ground water. Moore and others (2002) reported fine root biomass accumulation of between 0.4 and 1 kg m^{-2} growing down –50 cm, making the grass vegetation less responsive to changes in GWL. Rooting depths below -50 cm have also been reported in Tundra, with mean biomass of 1.5 kg m^{-2} (Jackson et al. 1996, Canadell et al. 1996).

Green biomass influences NEE and GPP since it determines the photosynthetic surface area (Street and other 2007, Shaver and other 2007, Limpens and others 2008). Mean seasonal maximum NEE were around –10.0 and -5 μmol m^{-2} s^{-1} for grass and moss respectively, while the respective GPP were 23 and 12 μmol m^{-2} s^{-1}. These are within the range of 8-20 μmol m^{-2} s^{-1} for NEE (Tuittila and others 2004; Douma and others 2007, Riutta and others 2007; Lindroth and others 2007, Shaver and others 2007) and 10-40 μmol m^{-2} s^{-1} for GPP (Bubier and others 1998; Lindroth and others 2007) reported for most temperate peatlands of Northern Hemisphere. The moss plots, in some instances, comprised a few grass populations that could raise the CO_2 fluxes since they are more active and efficient (Douma and others 2007, Limpens and others 2008). Although NEE data of moss were corrected for contribution by the grass using aboveground biomass of the grass harvested in the moss plots, our values still remain at the extreme end of scale for moss fluxes (Tuittila and others 2004; Riutta and others 2007, Douma and others 2007). This could be explained by the high light intensity levels reaching the moss vegetation at our study site compared to those reported for the Northern Hemisphere. Our results show that moss contributes approximately 30% of the overall ecosystem CO_2 uptake in this peatland. Douma and others (2007) reported that mosses contributed between 14 – 96% of the total CO_2 assimilated in an arctic ecosystem, depending on the proportion of vascular plants (shading) growing together with the moss.

When NEE was normalised with biomass we still observed higher NEE for grass during the active growth phase. These differences result from higher CO_2 fixation capacity due to higher light use efficiency (α) and higher maximum light intensity at which saturation occurs in grass compared to moss (Street and others 2007). Our analysis showed that light alone explains more than 70% of NEE during the active phase of development in both moss and grass and that grass could increase light use efficiency by increasing LAI, with an overall

increase in potential production. At ecosystem level, increased LAI in grass may affect moss production by reducing the light levels reaching moss, since moss biomass is short and grow under the grass canopy, with a possible impact on the overall ecosystem production. Similar conclusions have been arrived at in studies conducted on moss communities in northern peatlands (Shaver and Chapin 1991, Douma and others 2007).

Ground water level of around –10 cm is regarded as the optimum water level for most physiological responses in peatland species (Tenhunen and others 1992; Semikhatova and others 1992; Tuittila and others 2004). Ground water level declined to –40 cm in April and later to –20 cm in June, with significant influence on moss NEE. Despite resumption of rainfall in May, NEE by moss remained low and moss plots were net CO_2 sources during most of the day until June. This was not the case with grass that was a CO_2 sink during this period. Differences that occur between moss and grass in response to changing GWL may be due to differences in their physiological and morphological structures (Schipperges and Rydin 1998). Mosses, unlike grasses lack roots and only possess rhizoids that don't penetrate into deeper soil layers and they show more sensitivity to tissue water changes (Schipperges and Rydin 1998), with narrow tolerance to small draw-down in GWL (Riutta and others 2007). Grass, however, are deep rooted and are less sensitive to short-term changes in GWL (Lafleur and others 2005) since they have access to water in a large soil volume. Grass also possess adaptive strategies such as leaf rolling, as shown by a declining LAI (with no change in biomass) in early June and stomatal control ability (Busch and Lösch 1999) that minimise transpiration water loss. Combined with high light use efficiency, these adaptations could enhance CO_2 assimilation during GWL decline and rapid recovery after drought in grass compared to moss.

Except for the months of June and July, R_{eco} was similar (mean = 10 μmol m^{-2} s^{-1}) in moss and grass. Our values are higher compared to those reported for peatlands of Scandinavian and North American countries (Bubier and others 1998; Tuittila and others 2004; Lafleur and others 2005; Lindroth and others 2007), but are within the range of 12-20 μmol m^{-2} s^{-1} reported by Riutta and others 2007. Higher R_{eco} from our study site could be due to (1) its southern location i.e. less continental climate with higher temperatures given that there was an exponential increase in R_{eco} with increasing temperatures. (2) Relatively longer growing season/extended decomposition period. (3) A large drop in GWL that occurred in early spring and resulting into the death of moss. Bortoluzzi and others (2006) reported R_{eco} of 8.0 μmol m^{-2} s^{-1} in a mountain bog at an altitude of 867 m.a.s.l. in France with LAI of 1.1 and mean maximum temperatures of 30°C. The exact contribution of the vegetation to the total

ecosystem respiration at this site is not known, however, measurement of peat respiration (plus roots) indicated that at least half of the R_{eco} originates from the peat. Compared to moss, a significantly large proportion of R_{eco} originated from the peat under the grass. Although no analysis of peat characteristics under the two vegetation types was conducted, we anticipated similar peat characteristics. Thus differences in R_{eco} that were observed between the two plots could only be attributed to the large root biomass in the grass plots compared to moss.

Past studies indicate that peat respiration increases with decreasing GWL (McNeil and Waddington 2003, Bortoluzzi et al. 2004, Riutta et al. 2007). We observed an increase in R_{eco} with declining GWL, and even though the effects of GWL on R_{eco} might be confounded by changes in peat temperature, the behaviour response suggested that the two vegetation types were affected differently (R^2=0.85 and 0.39 in grass and moss respectively). This was not the case with peat temperature, which had similar effects on both moss and grass. The poor correlation in moss suggests that its respiration could be influenced by GWL changes in a very narrow uppermost portion of the peat profile. This was different in grass since the extensive rooting system tracks the changing soil moisture conditions (lagging behind GWL) as aeration of the soil profiles allows for aerobic respiration of the roots and soil micro-organisms (Basiliko and others 2006), while CO_2 assimilation remained unchanged. Influence of water table fluctuation on ecosystem respiration is well documented for other ecosystems (Alm and others 1999), while Lafleur and others (2005) explained the anomalies in such relationships.

Our results emphasise the significant role of temperature in determining ecosystem CO_2 exchange processes in this peatland. At high light intensities, temperature sets the upper boundary of NEE, which increases steadily with increasing temperature to an optimum of 25°C. Since there was an exponential increase in R_{eco} with increasing temperature, rapid decline in NEE above 25°C could be the result of an increasing R_{eco} over CO_2 assimilation, an indication that at higher temperatures the peatland is likely to become a net CO_2 source.

Conclusion

Green biomass and LAI are important determinants of CO_2 assimilation by the mosses and grasses. Apart from LAI, higher NEE in grasses compared to mosses was also attributed to more efficient light use. Mosses were more sensitive to GWL draw-down, which had significant influence on their CO_2 assimilation and biomass development. Grasses however, showed less sensitivity to GWL changes, as a result of their extensive and deep rooting systems. Increasing peat temperatures at -10 cm depth resulted in exponential increase in R_{eco}.

Since future climate scenarios in Europe indicate reduced precipitation amounts and increased air temperatures, lower GWL and increased peat temperatures may turn this peatland into a net CO_2 source during most part of the year. Other possible consequences are increased death of mosses and their replacement with grasses and other vascular plant species that are more resilient. Mosses however, make significant contribution to the current total community biomass as well as ecosystem CO_2 uptake in this peatland and their decline may disrupt ecosystem CO_2 budget.

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APPENDIX

List of further publications

Ye Q, Muhr J, Steudle E (2005). A cohesion/tension model for the gating of aquaporins allows estimation of water channel pore volumes in *Chara*. *Plant, Cell & Environment*, **28**, 525-535.

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