ECOSYSTEM CARBON BALANCE OF TEMPERATE FORESTS DIFFERING IN ELEVATION AND NITROGEN AVAILABILITY

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CHAPTER 1

GENERAL INTRODUCTION

General introduction

The central theme of this thesis is the terrestrial carbon (C) cycle in forest ecosystems, including one of its key drivers, the nitrogen (N) cycle, both in a global change context.

Forests and the global carbon cycle

Forests are covering 30% of the earth's land surface and account for half of the terrestrial net primary production (NPP; Sabine et al. 2004), thus playing a central role in the global C cycle. This paragraph summarizes the main aspects of C cycling in forests: the flow of C from the atmosphere to the vegetation, through the soil and back to the atmosphere (Figure 1).

The atmosphere currently contains about 775 Gt C in form of CO_2 (IPCC 2007). Plants fix ~120 Gt C per year through photosynthesis, the largest C flux within the global C cycle. The sum of photosynthesis by all leaves at the ecosystem scale is the gross primary production (GPP). Plants, like any organisms, acquire their energy for growth and maintenance by respiration, oxidizing C to CO_2, which is released back to atmosphere. The C costs (losses) of plant tissues are about half of the C gained through photosynthesis, and what remains is termed net primary production (**NPP)**, the net C gain by vegetation, about 60 Gt C per year globally. Total ecosystem NPP not only includes the new plant biomass produced (leafs, branches, stem, coarse- and fine roots), but also C exported by roots to rhizosphere organisms, and some volatile emissions from leaves straight to the atmosphere. A further, highly variable part of NPP is episodically removed from the ecosystem by disturbances such as fire or herbivory. For forests (in contrast to grassland), NPP can be divided into two groups, (1) components with natural (undisturbed) turnover times of more than hundred years (mainly wood), and (2) fractions with ongoing recycling and turnover times reaching from days to approximately a year (above- and belowground plant litter, root exudates and C exports to mycorrhiza). Among all vegetation types, forests represent the largest terrestrial C pool, globally accounting for > 80% of land's biomass, corresponding to ~540 Gt C. Most biomass C produced, will pass through soils sooner or later, with organic remains (humus) representing the largest terrestrial C pool. Forest soils alone account for 1100 Gt C (to a depth of 3 m; Jobbagy & Jackson 2000).

Soil organic matter (SOM) is a complex mixture of organic compounds that differ in turnover time. The majority of the soil C has a very long residence time of centuries to millennia, with residues chemically and physically protected from rapid decomposition (Anderson & Paul 1984). A minor part of the soil, in contrast, turns over within days to years (e.g. Townsend et al. 1995). In forests, the smaller, but active C pool results from the ongoing input of fresh plant litter, and, to a minor extent, from root C exports and exudates. C released from all different soil C pools, by decomposing soil microbes, accounts for about half of total soil C efflux (e.g. Högberg 2001), while the other significant part comes from autotrophic root respiration. The sum of all C fluxes from soils to the atmosphere, in form of CO_2, called **soil respiration** ($\boldsymbol{R_s}$), accounts for 75-100 Gt C a year. It is the second major terrestrial C flux after photosynthesis, more than 11-fold the current rate of fossil fuel combustion (Raich & Potter 1995).

A small but not insignificant component of the global C cycle is the flux of methane (CH_4). The dominant natural sources of atmospheric CH_4 are wetlands, where it is released as the product of microbial metabolism. The only significant sink for atmospheric CH_4 besides oxidation in the atmosphere, is its uptake by bacteria in soils (Le Mer & Roger 2001).

Although 78% of the atmosphere's volume is dinitrogen (N_2), plant available forms of N are the element most frequently limiting plant production of natural ecosystems, among them many northern and temperate forests (Vitousek & Howarth 1991). N, like C, enters ecosystems largely via the atmosphere, through atmospheric deposition or biological **N_2 fixation**. Unlike CO_2 uptake, N_2 can be assimilated by a few species of free-living or symbiotic bacteria only. These organisms convert atmospheric N_2 to organic N-compounds, by breaking the triple bond of N_2 and making the essential macronutrient available to higher organisms. The most common symbiotic N_2 fixers are *Rhizobium* species in

association with legumes, and *Frankia* species living with *Alnus*, *Ceanothus* and other woody plants. However, most plant available N within an ecosystem comes from internal, recycling, i.e. available N in soils is largely derived from decomposition of organic matter. N cycling in many forest ecosystems is of relatively closed nature, with small losses via gaseous emissions, volatilization and leaching, balanced by similar amounts via N_2-fixation or atmospheric N deposition. Since there is no plant growth without proteins, the C cycle is intimately linked to the N cycle.

Globally, emissions of the long-lived greenhouse gases CO_2, CH_4, N_2O and halocarbons through fossil fuel combustion, industrial processes, land use change and agriculture, are responsible for most of the increase in global mean temperature (IPCC 2007). Depending on the scenario, global mean air temperature is estimated to increase by 2-7 K by the end of this century (Allison et al. 2009). Currently CO_2 accounts for 77% of total greenhouse gas emissions to the atmosphere, CH_4 for 14.3%, N_2O for 7.9% when expressed in terms of CO_2 equivalents. A further component of human induced global change is the three- to fivefold rise of reactive nitrogen emissions over the last century (Denman et al. 2007) through fossil fuel and biomass combustion, industrial processes, animal husbandry and fertilizer use (IPCC 2007). The primary sink of reactive N in the atmosphere is its deposition on the land's surface. Atmospheric N deposition on land may increase by a factor of 2.5 by the end of this century (Lamarque et al. 2005).
Given the prominent role of forest ecosystems in the global C cycle, understanding their responses to the components of recent climate change is essential.

Temperature and nitrogen responses of forest carbon and nitrogen cycling

Soil respiration (R_s) is a major component of any ecosystem C balance consideration (see above). Like any metabolic process, R_s is affected by the amount and quality of substrate and environmental conditions, temperature in particular. Using established temperature responses of respiration and considering the magnitude of the soil C pool, substantial increases in soil C release could be expected as a positive feedback to climate warming (e.g. Heath et al. 2005). However, a warming-driven net C loss from soils to the atmosphere rests on the widespread assumption, that temperature is a relatively independent driver of R_s and that soil C releases would outstrip C inputs to soils and cause a net addition of CO_2 to the atmosphere. In contrast, a central hypothesis of this thesis is that R_s in the longer run (full year to multi-year scale) is driven by substrate availability, i.e. by soil C inputs from vegetation, rather than by temperature per se. Whether and how much R_s responds to climatic warming is intensively debated, and the common experimental approach is to heat soils and measure CO_2 release. This method is problematic, because it employs a step change in temperature, applies warming to soils only, and the study of responses is usually confined to a few years only. Such treatments commonly cause a rapid increase in R_s followed by a decline, with steady state effects remaining unknown. Complementary to experimental approaches such as soil heating and N fertilizer, this thesis reports on long-term established gradients of temperature and N availability.

A first objective was to assess a productivity-based explanation of annual R_s in forest ecosystems at contrasting temperatures. **Chapter 2** presents an analysis of forest productivity and concurrent soil respiratory fluxes across an elevational cline (1200 m of elevation, corresponding to 6 K), from the Swiss Central Alps to the Swiss Plateau, testing the hypothesis that cumulative annual R_s at contrasting temperatures reflects the difference in the production of short-lived biomass.

A further aim was to expand the analysis of chapter two by forest systems composed of trees living in association with N_2-fixing micro-organisms. *Alnus* stands offered systems to study long term high-N-input to forest soils. Soil N input is increasing globally, with uncertain consequences on the C cycle and storage. The basic question of **Chapter 3** is, whether or not high rates of N inputs increase the rate of C-cycling by accelerating both, NPP and R_s.

The soils of the forest sites described in chapter 3 were incubated for 600 days in order to identify soil organic pools that differ in turnover times. **Chapter 4** presents an analysis of the composition of three soil organic C pools and how these pools contribute to R_s, estimated in chapter 3.

High external N inputs have the potential to saturate the biological demand for N of forest ecosystems over time (e.g. Aber et al 1995), potentially resulting in N losses via leaching or gaseous emissions. **Chapter 5** addressed the impact of high soil N inputs under N_2-fixing trees on nitrificaiton, denitrification and subsequent N_2O emissions from soils.

Outlook

Chapter 2 is under review in *Oecologia* and **chapter 3** is ready to submit. **Chapter 4** and **5** need further development before publication. Chapter 4 and 5 are additional parts of this thesis, which offer valuable insights into mechanisms involved in forest C and N cycling. **Chapter 6** summarizes the chapters 2 to 5 and points out the main conclusions. In order to present self-containing chapters, the introduction and methodology is partly repetitive within the chapters 2 to 5, and references are given at the end of each chapter. Finally I add the abstract of a publication that is not thematically connected to this thesis, but that was prepared during this PhD.

References

Aber JD et el. (1995) Forest biogeochemistry and primary production altered by nitrogen saturation. Water air and soil pollution 85:1665-1670

Allison I, Bindoff NL, Bindoff RA et al. (2009) The Copenhagen Diagnosis 2009: Updating the World on the Latest Climate Science. The University of New South Wales Climate Change Research Centre (CCRC), Sidney, Australia

Ambus P, Zechmeister-Boltenstern S (2007) Denitrification and N-cycling in forest ecosystems. In: Bothe H, Ferguson SJ, Newton WE (eds) Biology of the nitrogen cycle. Elsevier B.V., Amsterdam, 343-358

Anderson DW, Paul EA (1984) Organo-mineral complexes and their study by radiocarbon dating. Soil science society of America Journal 48: 298-301

Binkley D, Sollins P, Bell R, Sachs D, Myrold D (1992) Biogeochemistry of adjacent conifer and alder-conifer stands. Ecology 73:2022-2033

Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. FEMS Microbiology Ecology Ca. J. Soil Sciences 61: 185-201

Chapin FS, Matson PA, Mooney HA (2002) Principles of terrestrial ecosystem ecology. Springer, New York

Denman KL et al. (2007) The Physical Science Basi – Contributing of Working Group 1 to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In: Solomon S et al. (eds) Climate Change 2007. Cambridge University Press, Cambridge, UK, pp 499-587

Heath J, Ayres E, Possell M, Bardgett RD, Blak HIJ, Grant H, Ineson P, Kerstiens G (2005) Rising atmospheric CO_2 reduces sequestration of root-derived soil carbon. Science 309:1711-1713

Högberg P et al. (2001) Large-scale forest girding shows that current photosynthesis drives soil respiration. Nature 411:789-792

IPCC (2007) Climate Change 2007: Synthesis Report. Cambridge University Press, UK

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications 10:423-436

Lamarque et al. (2005) Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: Analysis of nitrogen deposition. Journal of geophysical research 110

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: a review. Eurpean Journal of Soil Biology 37:25-50

Raich JW, Potter CS (1995) Global Patterns of Carbon-Dioxide Emissions from Soils. Global Biogeochemical Cycles 9: 23-36

Sabine CL et al. (2004) Current status and past trends of the global carbon cycle. In: Field CB, Raupach MR (eds) The global carbon cycle: Integrating humans, climate and the natural world. Island Press, Washington, pp 17-44

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea – how can it occur? Biogechemistry 13:87-115

CHAPTER 2

FOREST SOIL RESPIRATION REFLECTS PLANT PRODUCTIVITY ACROSS A TEMPERATURE GRADIENT IN THE ALPS

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ECOSYSTEM ECOLOGY - ORIGINAL RESEARCH

Forest soil respiration reflects plant productivity across a temperature gradient in the Alps

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Abstract Soil respiration (R_s) plays a key role in any consideration of ecosystem carbon (C) balance. Based on the well-known temperature response of respiration in plant tissue and microbes, R_s is often assumed to increase in a warmer climate. Yet, we assume that substrate availability (labile C input) is the dominant influence on R_s rather than temperature. We present an analysis of NPP components and concurrent R_s in temperate deciduous forests across an elevational gradient in Switzerland corresponding to a 6 K difference in mean annual temperature and a considerable difference in the length of the growing season (174 vs. 262 days). The sum of the short-lived NPP fractions ("canopy leaf litter," "understory litter," and "fine root litter") did not differ across this thermal gradient (+6 % from cold to warm sites, n.s.), irrespective of the fact that estimated annual forest wood production was more than twice as high at low compared to high elevations (largely explained by the length of the growing season). Cumulative annual R_s did not differ significantly between elevations (836 ± 5 g C m^{-2} a^{-1} and 933 ± 40 g C m^{-2} a^{-1} at cold and warm sites, +12 %). Annual soil CO_2 release thus largely reflected the input of labile C and not temperature, despite the fact that R_s showed the well-known short-term temperature response within each site. However, at any given temperature, R_s was lower at the warm sites (downregulation). These results caution against assuming strong positive effects of climatic warming on R_s, but support a close substrate relatedness of R_s.

Keywords Soil CO_2 efflux · NPP · Elevation · Temperate forest · Acclimation · Temperature sensitivity

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Introduction

Soil respiration (R_s), defined here as the the release of carbon dioxide (CO_2) from soils, is a major part of ecosystem respiration, and comprises (after photosynthesis) the second-largest terrestrial carbon (C) flux (IPCC 2001) at 75–100 Pg C a^{-1}, more than 11-fold the current rate of fossil fuel combustion (Raich and Potter 1995). Since the atmosphere contains roughly 800 Pg C, about 10 % of the atmospheric CO_2 is cycled through soils annually. Like any metabolic process, R_s is affected by the amount and quality of substrate and by soil environmental conditions such as temperature and moisture. Global mean air temperature is currently estimated to increase by 2–7 °C by the end of this century (Allison et al. 2009). Using established temperature responses of respiration in plants, soils, and whole ecosystems, substantial increases in soil C release could be expected as a positive feedback to climate warming (Trumbore et al. 1996, Heath et al. 2005, Heimann and Reichstein 2008). The rationale for a warming-driven enhancement of net C losses by soils rests on the assumptions that (1) temperature is a relatively independent driver of R_s, and (2) the sensitivity of R_s to warming will eventually cause C release to exceed C input to soils. Thus, several global biogeochemical models project soil C pools in terrestrial ecosystems to turn from a net C sink to a

net C source by around mid-century (Cox et al. 2000, Cramer et al. 2001, Friedlingstein et al. 2006). This paper aims to challenge the view that instantaneous respiratory responses to temperature are scaling to a long-term, large-area signal.

R_s is commonly separated into an autotrophic part and a heterotrophic part. Autotrophic R_s, i.e., respiration from live plant parts, includes root maintenance respiration, root growth respiration, and root nutrient uptake respiration, and is commonly assumed to contribute about half of the total soil CO_2 release (e.g., Högberg et al. 2001). The heterotrophic part of R_s is related to respiratory losses by soil microbes (including mycorrhiza), which depend on the availability of organic substrate for microorganisms and the rate of decomposition of this substrate (Trumbore et al. 1990; Schulze et al. 2000). In the short term (hours to months), rates of soil organic C decomposition are highly temperature sensitive. However, in the longer term (decades to centuries), environmental factors such as temperature and water affect CO_2 efflux only indirectly: through the rate of substrate production (Davidson and Janssens 2006, Kuzyakov and Gavrichkova 2010, Conant et al. 2011). An exception is water logging, where oxygen becomes a limiting factor (e.g., Davidson et al. 1998). Short-term substrate availability for decomposition depends on chemically and physically easily available organic compounds: plant and microbial residues and rhizodeposits of living roots. The concentrations of such substances are low (Fischer et al. 2007), and some of them have mean residence times of hours to days while others have mean residence times of a few months to a few years (litter). Hence, a sustained supply from above- and belowground litter and rhizodeposits is required to fuel these respiratory processes. Heterotrophic soil respiration can thus be assumed to strongly correlate with biomass production, as various studies have shown (Raich and Nadelhoffer 1989; Raich and Schlesinger 1992; Davidson et al. 2002b). For forests (in contrast to grassland), the heterotrophic respiratory fluxes can be divided into two parts: (1) fluxes associated with the recycling of biomass carbon (C) that has accumulated over long periods, such as wood or humus C, with natural lags in CO_2 release of more than a hundred years, and (2) fractions of NPP associated with ongoing biomass recycling and fast turnover (aboveground litter production, root and mycorrhizal turnover, and recycling of C exported by roots to rhizosphere organisms).

Accordingly, experimental approaches such as the artificial heating of forest soils without concomitant heating of the canopy produce a step change in the temperature, inducing an initial but transient net loss of C to the atmosphere, with R_s returning to rates similar to those in unwarmed soils once the fraction of labile organic material has reached a new (lower) steady-state level (Luo et al. 2001; Strömgren and Linder 2002; Melillo et al. 2002; Knorr et al. 2005; Bradford et al. 2008). This transient response of respiratory soil CO_2 release to step increases in soil temperature supports the notion that R_s is under strong substrate control in the long run. An initial but transient net loss of C to the atmosphere thus most likely overestimates the effect of long-term warming on R_s. Respiratory metabolism, including soil respiration, responds to temperature instantaneously on an hourly or daily timescale, but we hypothesize that, on a full year to multi-year timescale, soil respiration is essentially a function of substrate availability (input of plant-derived organic C to soils), which in turn depends on temperature effects on productivity.

An NPP-based explanation of annual R_s assumes that labile C input sets the limit on soil C release from forests on a long-term (several years) basis, and thus contrasts with the concept of direct temperature-driven respiration. Natural climatic gradients based on either latitude or elevation offer conditions under which C dynamics reflect long-term whole-ecosystem adjustment to contrasting temperatures, and thus permit the exploration of ecological theory related to long-term temperature effects that are not biased by imposing a step change in temperature on soils only. Only a few studies, mainly performed in the Andes, have attempted to quantify and compare forest productivity and/or respiratory fluxes along elevational gradients (e.g., Leuschner et al. 2007; Girardin et al. 2010; Zimmermann et al. 2010); to our knowledge, no such study has been conducted in the temperate zone so far.

The present study takes advantage of a mean annual temperature increase of ca. +6 K downslope of a 1200 m drop in elevation from the Central Swiss Alps to the Swiss Plateau. By restricting the comparison to alluvial sites with ample water supply, the gradient selected is not confounded by significant changes in the moisture regime. We present an analysis of the relation between NPP components in temperate deciduous forests and the concurrent soil C effluxes at two contrasting elevations along this thermal gradient. We hypothesize that the cumulative annual R_s values at contrasting temperatures reflect the difference in the production of nonrecalcitrant, short-lived biomass. An "experiment by nature," as we term it, can (1) be expected to reflect the combined effects of warming on all ecosystem processes, and (2) be expected to show signals that are well adjusted to the local temperature regime, given the rather slow rates of climatic warming (currently ca. 0.13 K per decade; IPCC 2007), thus contrasting with short-term soil-only warming trials (step changes) in forests that are otherwise exposed to the contemporary climate.

In the present study, "steady state" refers to a forest state in which annual leaf litter fall has reached a near-to-constant value (maximum LAI), and in which fine roots

have explored the available soil space so that the fine root biomass does not increase further (near-to-constant fine root turnover). At the same time, stems, branches, and coarse roots keep accumulating biomass, and the forest grows in height, so "steady state" refers to the flow of NPP fractions that are quickly turning over, including aboveground litter, root and mycorrhizal turnover, and root exudates.

Methods

Study site and experimental design

A field experiment was set up in early spring 2009, which consisted of two deciduous forest stands in the Central Swiss Alps at ca. 1500 m a.s.l., referred to as "cold" or "high-elevation" sites, and two low-elevation deciduous forest stands in the foothills of the Swiss Plateau at ca. 300 m a.s.l., referred to as "warm" or "low-elevation" sites (Table 1). The elevational cline spanned by these core sites (1200 m) corresponds to a difference in mean annual temperature of about 6 K. Each site comprised an area of 50 m × 50 m, surrounded by a buffer zone at least 10 m in width. The cold high-elevation sites were homogeneous closed-canopy stands of *Salix fragilis* L., which originate from natural regeneration and had a mean age of ca. 40 years. The warm low-elevation sites were closed-canopy stands dominated by *Acer pseudoplatanus* L. (with a few *Quercus rubra* L. individuals at one site), afforested about 40–60 years ago. Air temperature (at 2 m above the ground) and soil temperature (at 10 cm depth) were recorded throughout the field study on an hourly basis at each site (HOBO TidbiT v2, Onset Computer Corp., Bourne, MA, USA). The length of the thermal growing season was defined here as the number of days with a daily mean air temperature above 5 °C. At each site, hourly averages of soil moisture and precipitation were recorded below the canopy (EM50 data loggers connected to a ECRN-100 rain gauge and four 10HS soil moisture probes installed at 10 cm depth; Decagon Devices, Pullman, WA, USA).

Forest stand characteristics

We estimated stem basal area (*BA*, in m^2) by measuring stem circumference at breast height (1.3 m above the ground) in three plots of size 10 m × 10 m per site in early spring in three subsequent years for all trees with a diameter exceeding 10 cm (very few trees were < 10 cm in diameter). This resulted in 70 ± 8 trees and 37 ± 8 trees within the three plots at the cold and warm sites, respectively. Forest stand height (*H*, in m) was estimated as the height of five randomly selected trees per plot using an optical reading clinometer (PM-5, Suunto, Espoo, Finland). Because species-specific allocation rules for stem and branch biomass were not available, we estimated woody biomass of all tree species assuming a near-cylindrical

Table 1 Location, climatic, and stand characteristics of the two study sites at high elevation (cold sites) and the two study sites at low elevation (warm sites)

	High-elevation sites		Low-elevation sites	
Location	46°36′16″ N	46°36′21″ N	47°32′37″ N	47°32′53″ N
	8°31′01″ E	8°31′21″ E	7°46′22″ E	8°13′34″ E
Elevation (m a.s.l.)	1515	1508	296	330
Dominant species	*Salix fragilis*	*Salix fragilis*	*Acer platanoides*	*Acer platanoides*
Stand age (years)	ca. 40	ca. 40	50–60	40–50
T_{air} full year (°C)	3.8 (−23.6, 28.4)	3.6 (−25.7, 27.5)	10.6 (−15.4, 32.0)	10.4 (−14.0, 29.4)
T_{air} growing season (°C)	10.4 (5.0, 17.3)	10.7 (5.1, 16.9)	14.3 (5.1, 24.5)	14.5 (5.2, 23.3)
T_{soil} (°C)	5.3 (0.0, 15.6)	5.5 (0.1, 14.7)	10.5 (0.6, 21.0)	10.3 (1.6, 19.4)
Soil moisture (vol %)	29.8 (19.1, 39.8)	31.3 (19.9, 41.0)	36.6 (24.3, 47.7)	40.1 (29.4, 47.4)
Precipitation (mm y^{-1})	734	752	813	853
Stem basal area (m^2 ha^{-1})	37.9 ± 2.1	38.9 ± 3.2	54.9 ± 4.2	41.6 ± 1.9
Canopy height (m)	7.8 ± 0.5	6.6 ± 0.1	21.1 ± 0.1	20.5 ± 0.3
Stem and brench wood biomass (t C ha^{-1})	54 ± 3	50 ± 5	293 ± 24	128 ± 17
LAI (m^2 m^{-2})	3.7 ± 0.2	4.1 ± 0.1	5.5 ± 0.1	5.0 ± 0.2
Annual wood increment (t C ha^{-1} a^{-1})	2.29 ± 0.03	1.89 ± 0.03	5.01 ± 0.20	4.80 ± 0.34

Temperature and soil moisture are given as annual (or seasonal) means, with the minima and maxima (in parentheses) measured on an hourly basis. Precipitation was measured below the canopy of the forest stands. Basal area, canopy height, woody biomass, and LAI were recorded at the beginning of the field study (April 2009); the within-site mean ± SE is presented here

shape for the combined stem and branch xylem (sapwood area), which was estimated by stem basal area and canopy height:

$$W_C = (BA \times H) \times \rho \times 0.5, \quad (1)$$

where W_C (in kg) is the aboveground woody biomass C per ground area, ρ stands for wood density (*Salix*: 362 kg m^{-3}; *Acer*: 522 kg m^{-3}; compiled by Perruchoud et al. 1999), and dry stem biomass is assumed to contain 50 % C per unit weight.

The leaf area index (LAI) was estimated using a ceptometer (AccuPAR LP-80, Decagon Devices Inc.). The reference measurements were taken immediately after the forest measurement in an open area within a distance of 50 m under clear sky conditions. All measurements were accomplished in mid-August, when canopy foliage reached its maximum. Specific leaf area (SLA) of the litter was estimated on freshly fallen leaves that were randomly collected from the litter traps (see next section) at the end of September. Leaf area was measured using a LI-3100 area meter (LI-COR Inc., Lincoln, NE, USA) and the biomass of the same leaves was determined after drying at 80 °C.

Forest litter production

Forest NPP comprises "long-lived" components that contribute to C recycling when trees die or become harvested, such as wood and coarse-root growth, and "short-lived" components that feed more directly into respiratory belowground signals. These short-lived NPP components comprise above- and belowground litter as well as non-tissue components: root exudates and C export to mycorrhizae. In the present study, we adopted the selected short-lived NPP proxies "canopy leaf litter fall," "understory biomass," and "fine root production" in ingrowth cores (our proxy for belowground NPP). The sum of these short-lived NPP proxies is termed the "total litter production."

To estimate canopy leaf litter production, six plastic litter traps (0.25 m^2 ground area) with porous ground (sieve bottom) were installed at random locations within each study site in early autumn in two subsequent years, shortly before the leaves began to fall. The litter traps were emptied regularly, and the accumulated litterfall was dried at 80 °C. C content, quantified in a dry combustion elemental analyzer (Elementar Vario EL III, Hanau, Germany), ranged from 49 % to 54 % of dry mass at all sites. Understory vegetation biomass was determined by harvest at peak biomass at ground level in two areas of size 1 m^2 each per study site. At the beginning of the growing season, a total of 40 soil cores per study site (3.5 cm diameter × 12 cm depth) were collected. The soil cores were kept frozen until the extraction of fine and coarse roots, which were then dried at 80 °C. The holes created by root sampling were replaced by mesh cylinders (PET filament, 2 mm mesh size) and filled with sieved root-free soil taken in close proximity to the respective study site. Soil in these ingrowth cores was compacted to a similar bulk density to that found on the site. We found substantial root ingrowth after one year, and thus harvested the complete set of ingrowth cores. As a proxy of fine root turnover, we calculated the ratio of annual fine root ingrowth (NPP) to the mean fine root standing crop (Aber et al. 1985).

Soil respiration

Soil respiration (R_s) was measured using a portable custom-made static chamber system equipped with an open-path infrared gas analyzer and relative humidity/temperature sensors (GMP343 carbon dioxide probe, HMP75 rH/T probe; Vaisala, Vantaa, Finland; described in Bader and Körner 2010). This design avoids any pumps, thus preventing known problems with flow-through chambers, where minute pressure variations can alter the CO_2 flow across the delicate soil–air interface (Lund et al. 1999). Twelve polypropylene collars (20 cm diameter × 7 cm height) were installed at randomly selected positions within each study site. The collars were inserted to a depth of 3 cm into the ground 2–3 weeks prior to the first measurement. These collars permitted leakproof attachment of the soil respiration chamber, allowing the repeated measurement of the same soil area over time. The collars remained at the same location throughout the entire study. R_s was recorded biweekly throughout the growing season. From late autumn to early spring, R_s was recorded once a month at the low-elevation sites. Winter measurements at the high-elevation sites were restricted by heavy snowpack. For wintertime R_s measurement at the cold sites, snow was gently removed to reach the buried collars. Soils remained unfrozen under snow. After several attempts throughout the winter months, one reliable measurement was achieved in March 2010, when ambient air temperatures were sufficiently high for the soil surface not to freeze after excavating the collars. During these measurements, gas fluxes reached a steady state after an initial degassing period, i.e., R_s rates remained steady without a significant decrease for 0.5–2.5 h after snow removal. Soil respiration rates were calculated by linear regression of recorded headspace CO_2 concentrations against time (48 automatic readings over a period of 4 min, starting 1 min after the placement of the chamber on the collar). The data recorded during the first minute after the installation of the chamber were not used to avoid flux disturbances following chamber placement (Davidson et al. 2002a). Simultaneously with the R_s measurements, soil temperature and soil moisture at the chamber site were recorded manually (soil temperature:

GTH 175/Pt digital thermometer, Greisinger Electronic, Germany; soil moisture: ThetaProbe soil moisture sensor ML2x, Delta-T Devices Ltd., Cambridge, UK), thus complementing the data collected by the automatic data logger.

In order to ensure that results did not reflect the by-chance presence of certain tree taxa at the selected study sites, we measured R_s simultaneously at the core sites and at supplementary sites with different tree taxa in summer 2010. The supplementary sites at high elevation (cold sites) were one forest stand of *Sorbus aucuparia* L. (46°36′48″ N, 8°34′08″ E; 1635 m a.s.l.) and one forest stand of *Betula pubescens* (46°42′53″ N, 8°54′56″ E; 1325 m a.s.l.). At low elevation (warm sites), we chose five supplementary sites of mixed forest dominated by *Fagus sylvatica* L. (47°32′39″ N, 7°45′42″ E; 47°32′40′ N, 8°13′21″ E; 47°07′05″ N, 8°18′42″ E; 47°31′44″ N, 7°47′22″ E; 47°22′16″ N, 8°11′13″ E; all between 300 and 500 m a.s.l.). Daytime R_s was recorded on four occasions at the core sites and the supplementary sites (high elevation: $n = 4$; low elevation: $n = 7$) in mid-season (July and August) 2010.

Soil physicochemical analysis

Two soil pits were dug per study site and the excavated profile was sampled at depths of 5, 10, 20, 30, 40, and 50 cm using a 35 mm diameter × 50 mm length auger. The profiles included the organic topsoil layer, humic layers, and mineral layers. The soil samples were sieved (2 mm) and oven-dried at 105 °C to determine the fine earth density. Subsamples of each soil core were oven dried at 105 °C and ground to powder to quantify the total C and N concentrations in an elemental analyzer (ThermoFinnigan FlashEA 1112, Milan, Italy) after carbonates were removed from soils by acid fumigation (Harris et al. 2001). Soil pH was measured in 0.1 M KCl solution.

Data analysis

R_s rates (μmol CO_2 m^{-2} s^{-1}) were related to soil temperature (T, 10 cm depth) by fitting a nonlinear least squares model after Lloyd and Taylor (1994), which expresses R_s in terms of the respiration rate at 10 °C (R_{s10}) and a parameter E_0 that models temperature sensitivity:

$$R_s = R_{s10}\, e^{E_0\left(\frac{1}{56.02} - \frac{1}{T-227.13}\right)}. \quad (2)$$

Annual soil CO_2 efflux was estimated at each core site by predicting R_s at hourly intervals, based on the automatically logged soil temperature. The temperature sensitivity expressed by Q_{10} values within elevation were then estimated by comparing R_s rates when the temperature was increased from 5 °C to 15 °C:

$$Q_{10} = \left(\frac{R_{s\,2}}{R_{s\,1}}\right)^{\left(\frac{10\,^\circ C}{T_2 - T_1}\right)}, \quad (3)$$

where R_{s2} and R_{s1} are the R_s rates at the higher ($T_2 = 15$ °C) and lower ($T_1 = 5$ °C) soil temperatures, respectively. In order to estimate Q_{10} across elevations, annual R_s rates at the contrasting temperatures (elevations) were compared using Eq. 2.

Effects of elevation (temperature) on NPP components or the sum of NPP components, annual R_s, and pools of soil C and N were tested using a one-way analysis of variance. Normality and homoscedasticity were examined visually using diagnostic plots, and non-normally distributed rates were log-transformed (canopy leaf litter, soil C and N contents) or power-transformed ($x^{-0.4}$; NPP fine roots). Error estimates in the text and figures are standard errors of site means, and effects were considered significant at $P < 0.05$. Due to the low replication and therefore statistical power, effects with P values < 0.1 were considered marginally significant. All statistical analyses were carried out using R (R Development Core Team 2009; http://www.r-project.org).

Results

Climatic conditions

There was substantial seasonal variation in topsoil temperature and moderate changes in topsoil water content and precipitation at both the cold high-elevation and the warm low-elevation sites during the study years. Differences between sites at each elevation were almost negligible (Fig. 1c–f). The growing season (daily mean air temperature > 5 °C) accounted for 174 ± 2 days at the cold sites, and lasted 262 ± 4 at the warm sites; i.e., there was a shortening of the growing season by nearly three months with elevation. Sites at high elevation were snow-covered (to a max. of 1 m depth) from the beginning of December to mid April, whereas at low elevation the forest floor was only periodically covered with a few centimeters of snow, mainly between early December and late February. Mean annual air temperatures during the experimental period were 3.7 ± 0.1 °C and 10.5 ± 0.1 °C at the cold and warm sites, respectively (Table 1), implying a mean air temperature difference of 6–7 K for the 1200 m difference in elevation. Soil volumetric water content (VWC) was permanently higher at the warm sites, but in general all soils were moist throughout the study period (Table 1; Fig. 1e, f). By comparing tree stands on alluvial plains at both high and low elevations, we have avoided differences in the most common confounding site variable, soil moisture.

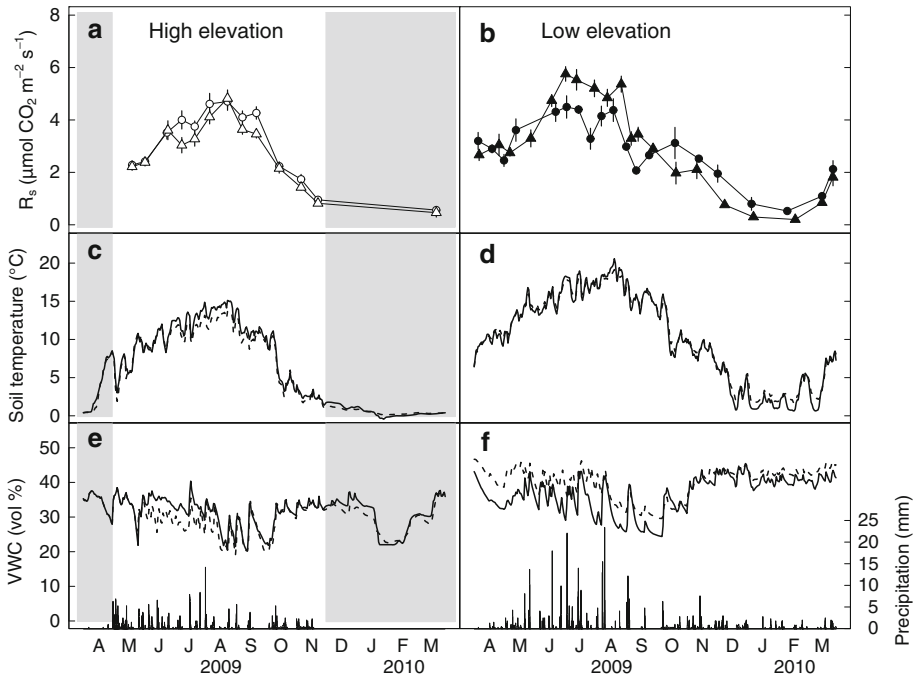

Fig. 1 Seasonal variations in **a**, **b** R_s (mean ± SE, $n = 12$), **c**, **d** soil temperature in the top 10 cm, **e**, **f** volumetric water content (VWC) in the top 10 cm and below-canopy precipitation, recorded at the individual study sites at high elevation (cold sites) and low elevation (warm sites) from April 2009 to March 2010 (full year). *Circles and solid lines* denote data from one study site, while *triangles and dashed lines* denote data from the other study site at the corresponding elevation. The *gray-shaded area* indicates the period when the soil was covered with snow

Forest stand characteristics

Stem basal area did not differ significantly between elevations, whereas tree height increased from the cold to the warm sites ($P = 0.002$), resulting in lower aboveground woody biomass at the cold high-elevation sites compared to the warm low-elevation sites, though this effect is marginally significant only due to a large variance in woody biomass at the warm sites ($P = 0.09$; Table 1). Similar to woody biomass, annual wood increment was higher at the warm compared to the cold sites (+ 140 %; $P = 0.03$; Table 1). However, accounting for the length of the growing season, wood increment was only 60 % higher at the warm low-elevation than at the cold high-elevation sites. Canopy LAI at peak season was also slightly higher at the warm sites ($P = 0.06$; Table 1), whereas SLA was similar at both elevations (160–180 cm^2 g^{-1}).

Forest litter production

Canopy leaf litter production was 117 ± 22 g C m^{-2} a^{-1} at the cold and 235 ± 27 g C m^{-2} a^{-1} at the warm sites ($P = 0.09$; Table 2), but this marginally significant difference almost disappeared when divided by the number of days of the growing season (Table 3). Production of understory vegetation (herbaceous species) was 128 ± 28 g C m^{-2} a^{-1} at the cold sites and 101 ± 44 g C m^{-2} a^{-1} at the warm sites (Table 2). When expressed per day of the growing season, this trend was amplified but remained nonsignificant (Table 3). In contrast to the aboveground trends in NPP, annual fine root ingrowth decreased considerably from 97 ± 14 g C m^{-2} a^{-1} at the cold to 28 ± 4 g C m^{-2} a^{-1} at the warm sites ($P = 0.03$; Table 2), and the effect became even stronger when expressed per day of the growing season (Table 3). Similarly, fine root biomass at the beginning of the field study declined from the cold to the warm sites (46 ± 1 g C m^{-2} a^{-1} vs. 19 ± 2 g C m^{-2} a^{-1}; $P = 0.009$). Both mean fine root turnover (cold sites: 0.37 ± 0.03 a^{-1}; warm sites: 0.31 ± 0.02 a^{-1}) and mean root duration (cold sites: 2.7 ± 0.2 years; warm sites: 3.3 ± 0.2 years) were similar at the contrasting temperature regimes. Because of the shorter season, the "functional duration" (number of days of high metabolic activity) is thus reduced at the cold sites.

Table 2 Annual NPP (in g C m^{-2} a^{-1}) of short-lived components, annual R_s, and the percentage changes in each of these annual C fluxes upon moving from cold high-elevational sites to warm low-elevational sites

	High elevation	Low elevation	In/decrease (%)	*P*
Canopy litter	117 ± 22	235 ± 27	+101	(*)
Understory vegetation	128 ± 28	101 ± 44	−21	n.s.
Fine root ingrowth	97 ± 14	28 ± 4	−71	*
Total litter	**343 ± 21**	**365 ± 13**	**+6**	**n.s.**
Annual R_s (g C m^{-2} a^{-1})	**836 ± 5**	**933 ± 40**	**+12**	**n.s.**

Total litter refers to the sum of the canopy litter, understory litter, and fine root ingrowth. The mean ± SE is presented here

(*) $0.05 < P < 0.1$; * $P < 0.05$; ** $P < 0.01$

Table 3 Productivity of short-lived components expressed per day of growing season (i.e., mean daily NPP during the growing season in g C m^{-2} d^{-1}; the number of days experiencing a 24 h temperature mean above 5 °C: high elevation 174 ± 2 days, low elevation 262 ± 4 days) and the percentage change on moving from the cold high-elevation sites to the warm low-elevation sites. The mean ± SE is presented here

	High elevation	Low elevation	In/decrease (%)	*P*
Canopy litter	0.66 ± 0.13	0.90 ± 0.09	+35	n.s.
Understory litter	0.74 ± 0.16	0.39 ± 0.17	−48	n.s.
Fine root ingrowth	0.56 ± 0.08	0.11 ± 0.01	−81	**

(*) $0.05 < P < 0.1$; * $P < 0.05$; ** $P < 0.01$

Soil respiration

R_s rates measured throughout the sampling year at the four core sites showed pronounced seasonality (Fig. 1a, b), with rates < 1 µmol CO_2 m^{-2} s^{-1} in February and March at both the cold high-elevation and the warm low-elevation sites, and peak rates of 4.8 µmol CO_2 m^{-2} s^{-1} at the cold sites in August and 5.8 µmol CO_2 m^{-2} s^{-1} at the warm sites in July. As soon as air and soil temperatures exceeded 4 °C in spring, R_s started to rise. At the cold sites, this occurred in mid-May following snowmelt, while this threshold was passed at the warm sites at the end of March. At the cold sites, R_s declined continuously during October to its lowest winter values, whereas R_s dropped more rapidly in September at the warm sites, with the temperature reducing from about 20 °C to 15 °C during a period of reduced rainfall (soil moisture: 26 % to 29 %; Fig. 1f). While R_s values at the two cold high-elevation sites ran parallel throughout the year, R_s values at the two warm low-elevation sites diverged during July and August, achieving the maximum R_s discrepancy of about 2 µmol CO_2 m^{-2} s^{-1} (Fig. 1a, b). On an annual basis (365 days), the average daytime R_s was 2.9 ± 0.1 µmol CO_2 m^{-2} s^{-1} for the cold sites and 2.8 ± 0.2 µmol CO_2 m^{-2} s^{-1} for the warm sites, so it did not vary significantly across the 6 K elevational cline in temperature.

R_s at the cold sites in mid March was 0.5 ± 0.1 µmol CO_2 m^{-2} s^{-1} at a topsoil temperature of 0.3 °C. For the annual soil CO_2 efflux, this rate is assumed to represent winter rates at the cold sites from mid-December 2009 to mid-April (the soil temperature barely changed under snow cover during that period). Had actual rates been lower (e.g., 0.1 µmol CO_2 m^{-2} s^{-1}), the elevational effect on the annual CO_2 efflux calculated by Eq. 2 would not have been significantly affected.

Soil temperature accounted for 70–80 % of the seasonal variation in R_s, and Q_{10} within elevation was similar at the two elevations (cold sites: $R^2 = 0.79$, $P < 0.001$, $Q_{10} = 2.1 \pm 0.3$; warm sites: $R^2 = 0.70$, $P < 0.001$, $Q_{10} = 2.2 \pm 0.6$; Fig. 2). Comparing annual R_s across elevation (a temperature rise of 6 K) resulted in a Q_{10} of 1.2 ± 0.1.

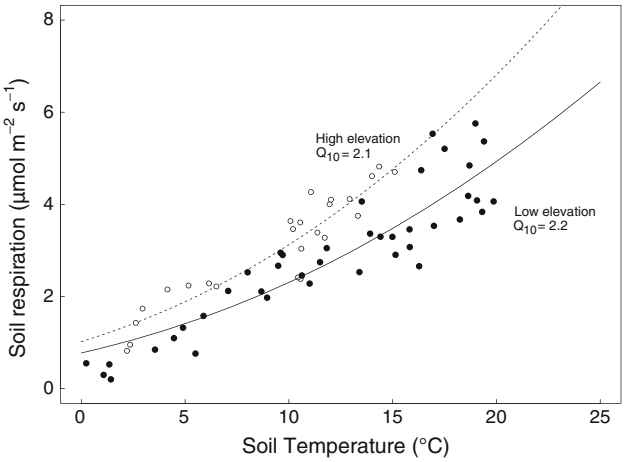

Fig. 2 Seasonal response of R_s to soil temperature (10 cm depth) at the cold high-elevational sites (*open symbols*, *dashed line*) and at the warm low-elevation (*filled symbols*, *solid line*), fitted with Lloyd and Taylor (1994) functions. Data points are campaign averages of the R_s rates at each study site ($n = 12$) throughout the sampling year. Q_{10} values are estimated by comparing the R_s rates obtained upon increasing the temperature from 5 °C to 15 °C

Total annual respired C amounts, obtained by modellng hourly values of R_s rates using Eq. 2, were only 12 % (n.s.) higher at the warm sites relative to the cold sites (836 ± 5 g C m^{-2} a^{-1} vs. 933 ± 40 g C m^{-2} a^{-1}; Table 2).

Mid-season R_s rates in summer 2010 were similar across elevations at the core sites (cold sites: 4.4 ± 0.1; warm sites: 3.4 ± 0.5), and even higher at cold high-elevation sites than at warm low-elevation sites when the supplementary sites were included (cold sites: 4.4 ± 0.1; warm sites: 3.2 ± 0.3; $P = 0.02$), and when daytime rates were averaged across measurement occasions and sites. This finding indicates that the elevational effect found for the year-round recordings at the core sites was not species specific. Mid-seasonal mean soil temperature was ca. 3 K higher at the warm sites than at the cold sites, and VWC varied between 30 % and 37 % and between 28 % and 50 % at the cold and the warm sites, respectively, during all measurements.

Soil C and N

The amount of soil organic C (0–50 cm depth) was 15.3 ± 0.3 kg C m^{-2} at the warm low-elevational sites and 4.8 ± 2.2 kg C m^{-2} at the cold high-elevational sites. These values may not represent the total soil C stocks of the study sites, since soils can be much deeper. As both soil C and soil N concentrations were higher at the warm sites,

Table 4 Soil properties along the soil profiles to a depth of 50 cm at the cold high-elevational and the warm low-elevational sites (mean ± SE; $n = 2$)

Soil depth	High elevation	Low elevation
0–5 cm		
Bulk density (g cm^{-3})	0.71 ± 0.16	0.96 ± 0.09
% C	3.27 ± 0.35	5.82 ± 0.33
C stock (kg C m^{-2})	1.18 ± 0.38	2.77 ± 0.13
C/N	11.18 ± 1.59	17.11 ± 5.77
$pH_{(KCL)}$	5.25 ± 0.01	6.26 ± 0.38
5–10 cm		
Bulk density (g cm^{-3})	1.15 ± 0.01	1.05 ± 0.06
% C	1.36 ± 0.75	3.33 ± 0.15
C stock (kg C m^{-2})	1.58 ± 0.87	3.50 ± 0.05
C/N	11.77 ± 0.56	9.86 ± 0.42
10–20 cm		
Bulk density (g cm^{-3})	1.27 ± 0.01	1.10 ± 0.01
% C	0.28 ± 0.05	2.81 ± 0.09
C stock (kg C m^{-2})	0.35 ± 0.09	3.10 ± 0.08
C/N	12.72 ± 2.45	10.70 ± 1.95
20–30 cm		
Bulk density (g cm^{-3})	1.22 ± 0.09	1.26 ± 0.02
% C	0.30 ± 0.14	1.95 ± 0.24
C stock (kg C m^{-2})	0.35 ± 0.14	2.44 ± 0.26
C/N	12.95 ± 0.68	9.03 ± 0.31
30–40 cm		
Bulk density (g cm^{-3})	1.13 ± 0.10	1.20 ± 0.06
% C	0.77 ± 0.55	1.33 ± 0.03
C stock (kg C m^{-2})	0.77 ± 0.50	1.59 ± 0.04
C/N	11.53 ± 1.23	8.37 ± 0.01
40–50 cm		
Bulk density (g cm^{-3})	1.16 ± 0.12	1.26 ± 0.06
% C	0.52 ± 0.26	1.54 ± 0.44
C stock (kg C m^{-2})	0.57 ± 0.23	1.91 ± 0.46
C/N	13.34 ± 1.62	10.46 ± 1.05
0–50 cm (total profile)		
C stock (kg C m^{-2})	4.79 ± 2.18	15.32 ± 0.27

the C/N ratios were the same for both cold and warm sites along the sampled profile (Table 4).

Discussion

The temperature difference of about 6 K upon moving from alluvial cold study sites in the Swiss Central Alps to alluvial warm study sites in the foothills of the Swiss Plateau provided us with the opportunity to test effects of temperature as a driver of both forest productivity and forest R_s. We found that neither annual R_s nor total annual litter production (i.e., the sum of the short-lived NPP components) depended significantly on elevation.

NPP components across the temperature cline

While total litter production hardly changed across elevations (+6 %, n.s.), canopy leaf litter vs. fine root litter showed contrasting components: more canopy litter (+101 %), but less fine root litter (−71 %) at the warm compared to the cold sites (Table 2).

Annual canopy leaf litter production at the cold (117 ± 22 g C m^{-2} a^{-1}) and the warm (235 ± 27 g C m^{-2} a^{-1}; Table 2) sites compare well with the Swiss Forest Inventory data on canopy leaf litter input in the respective regions (Alps: 134 g C m^{-2} a^{-1}; Jura: 229 g C m^{-2} a^{-1}; Perruchoud et al. 1999). Further, the ten-year mean for the canopy leaf litter fall in the deciduous forest at the Swiss Canopy Crane research site located in the Jura foothills is almost identical to that observed at our warm low-elevation sites (238 ± 65 g C m^{-2} a^{-1}; Körner et al. 2005).

Annual understory litter production did not differ significantly between the cold and warm sites but, remarkably, was similar to canopy litter production at the cold sites, whereas understory litter production at the warm low-elevation sites was not even half of the canopy litter production (Table 2), most likely due to the greater shade produced by a closer canopy. However, we observed the reverse trend belowground to that seen for canopy litter production, with fine root production at the warm low-elevation sites reaching only one-third of that at the cold high-elevation sites (Table 2). To the extent that new root growth into an empty soil patch can be considered proportional to overall fine root production in such a site comparison, our cold sites showed a substantially higher root activity, perhaps associated with reduced microbial activity, scarcer nutrients, or a low temperature associated reduction in specific water uptake (Persson and Ahlström 1990). A similar increase in fine root production has been reported along elevational gradients in the Ecuadorian Andes (Roderstein et al. 2005, Moser et al. 2011), whereas a recent study along an elevational gradient in the Peruvian Andes found no such trend with elevation (Girardin et al. 2010). Fine root production in ingrowth cores at the warm sites was within the ranges previously reported for deciduous temperate forests based on the ingrowth core technique (Steele et al. 1997; Bader et al. 2009), whereas no literature data for a direct comparison with our cold high-elevation sites was found. If we assume that the ratio of new roots appearing in ingrowth cores and the fine root stock is a proxy for root turnover, we arrive at rates that are slightly higher at the cold sites (0.37 a^{-1}) than at the warm sites (0.31 a^{-1}). The resultant root longevity of around three years is well within the range reported from other

temperate deciduous forests (Bader et al. 2009, Gaul et al. 2009). The ratio of fine root ingrowth to total fine root biomass may overestimate actual turnover, since root growth into an empty soil patch may be greater than new root growth in undisturbed soil, but to a first approximation we can assume that such a deviation applies to all test sites, so it does not affect the comparison.

We did not develop species- and stand-specific allometries; nevertheless, our estimates of annual wood mass increment provide a rough estimate of the increase in wood C stocks. The annual wood increment of ca. 200 g C m^{-2} a^{-1} at our cold sites is close to the 170 g C m^{-2} a^{-1} reported for a cold temperate forest in a similar climate in the northern USA (Gough et al. 2007), and the 485 g C m^{-2} a^{-1} observed at our warm sites falls in the range of 200–600 g C m^{-2} a^{-1} found under similar climatic conditions in Germany, close to our warm sites (Jacob et al. 2010).

The effects of temperature on canopy leaf litter production and wood increment largely resulted from a longer growing season; the signals almost disappear when productivity is expressed per day available for growth (Table 3). The limitation on productivity caused by a shorter season (irrespective of temperature) is a global trend across latitudinal and elevational gradients covering the major vegetation types (Schulze 1982, Körner 1998). The higher annual root production at the cold sites became further amplified when expressed per day of the growing season (Table 3).

In summary, the productivity data obtained here are representative of similar forests elsewhere, and the elevational difference in annual rates of aboveground biomass production are largely explained by season length.

Soil respiration at contrasting temperatures

As in most previous cases, our data indicate that temperature controls the short-term temporal variability of R_s (Fig. 1), but the absolute rates of R_s are such that the elevational temperature effect on R_s is greatly diminished on an annual basis (Table 2).

Annual soil C efflux was similar at both elevations (cold sites: 836 ± 5 g C m^{-2} a^{-1}; warm sites: 933 ± 40 g C m^{-2} a^{-1}), matching rates reported for other temperate deciduous forests (Malhi et al. 1999; Wang et al. 2006; Bader and Körner 2010; Ruehr et al. 2010), all of which corresponded to yearly soil C releases of between 700 and 1200 g C m^{-2} a^{-1}. R_s values for high-elevation deciduous forests that are comparable to those for our cold high-elevation sites do not exist, but cool temperate deciduous forests at a similar mean annual temperature were reported to release 700–800 g C m^{-2} a^{-1} (compiled by Chen et al. 2011). Mid-season R_s rates at the supplementary sites indicate that our results are not species specific. We have nearly exhausted the spectrum of deciduous forest types at high elevation with *Salix*, *Betula*, and *Sorbus*. Abundant *Alnus* forests, also found at this elevation, cannot readily be compared due to their symbiotic N fixation and therefore higher nutrient availability (Caprez et al., unpublished).

Estimates of annual soil C release in climates subject to seasonal snow cover are often based on recordings taken during the growing season only. However, soil C release during winter can play a significant role in the annual C budget of seasonal forest ecosystems (Sommerfeld et al. 1993, Brooks et al. 2004), with soil temperatures of −7 to −5 °C considered threshold temperatures for significant heterotrophic respiration (Brooks et al. 1997). At the cold sites of our study, below-snow soil temperatures were decoupled from air temperature by a thick snow pack, and never dropped below 0 °C (Fig. 1). Hence, our winter signals of 0.5 μmol CO_2 m^{-2} s^{-1} may be higher than what might generally apply for such elevations when the snowpack is shallower. Our estimates of R_s at high elevation in winter match those reported by Schindlbacher et al. (2007) for a forest ecosystem in the Austrian Alps (different method), while other studies that used open or closed chambers found lower (Mast et al. 1998), similar (McDowell et al. 2000), or higher (Mariko et al. 2000) below-snow R_s rates than the rates recorded in our study. The four-month contribution of below-snow soil CO_2 efflux to annual soil CO_2 efflux at high elevation was about 16 %, a contribution similar to that reported for other temperate forest ecosystems (e.g., Schindlbacher et al. 2007: 12 %; Mariko et al. 2000: < 15 %; McDowell et al. 2000: 17 %).

Temperature and substrate relatedness of R_s

The in situ relationships between R_s and soil temperature obtained here under steady state litter turnover indicate a downregulation of R_s at higher temperatures close to homeostasis; i.e., rates of R_s did not increase with increased seasonal mean temperature when moving across elevations (Fig. 2). Similar to our findings, EUROFLUX sites showed no correlation between annual R_s and mean annual temperature across a large range of European climates and tree species (Janssens et al. 2001). Further, a recent study across an elevational transect in tropical forests, spanning a larger temperature range than covered here, found no trend in R_s rates with elevation when daytime data were compared (Zimmermann et al. 2009), and only a weak positive relationship between R_s and temperature when night-time data were included (Zimmermann et al. 2010).

Responses of ecosystem respiration to temperature have often been described using the Q_{10} concept, assuming that respiration more than doubles for warming of 10 K ($Q_{10} > 2$; Lloyd and Taylor 1994, Kirschbaum 1995). On a

short-term basis (hours to days), we also found a Q_{10} of close to 2 within both cold and warm sites (Fig. 2). However, the apparent Q_{10} calculated across sites (e.g., moving from high to low elevations to simulate a 6 K warming) dropped to only $\sim$ 1.2. This estimate is compatible with an apparent Q_{10} of 1.4–1.5 across biomes differing in mean annual temperature (Mahecha et al. 2011; Bond-Lamberty and Thomson 2010). Thus, across biomes adapted to different temperatures, temperature does not exert a strong net influence on R_s. Had annual R_s in the present study responded in a similar way to the short-term Q_{10} of 2.15 (the mean of the Q_{10} values at the cold and warm sites), a 6 K higher temperature should have caused a 65 % increase in R_s relative to the substrate availability, which is not what we found. The proportionality to the short-lived NPP fraction (the amount of which also responds to temperature) when comparing cold and warm sites indicated that substrate availability rather than temperature controls annual R_s.

A full accounting of all C input components and earmarking C output according to the contributions of these components is a near-to-impossible task (see the "Introduction"). This study aimed to quantify some major input pathways, use proxy measures for others, and leave the remaining inaccessible fractions (root exudates, C export to mycorrhiza) as residuals. Further, separating out belowground C release by auto- and heterotrophic fractions in a forest is a challenging task without performing severe experimental manipulation and therefore interfering with ecosystem functioning (e.g., Kuzyakov and Larionova 2005) and introducing unknown treatment bias. Such manipulations were beyond what could be done in this study.

In contrast to the linear relationship between annual R_s and annual canopy litter production as a single C input component, as reported by Raich and Nadelhoffer 1989, this study reveals that leaf litter input is insufficient to account for annual R_s, which is not surprising. In a seasonal climate, canopy litter results from a single flush in spring, producing a site-specific LAI, with leaves shed at the same time in autumn. The seasonal C uptake by trees is then largely influenced by the leaf duration (i.e., the season length). In contrast, fine root production (and thus root turnover and associated release of organic C compounds) is likely to show a more continuous, more season length dependent C input to soils than canopy (leaf) litter production does. Since much of the belowground activity that induces respiratory C release is also related to aboveground biomass production, wood increment can be used to characterize the overall vigour of the system. However, due to the delayed respiratory recycling of wood, wood production in itself does not contribute directly to ongoing R_s on an annual basis as long as a forest grows.

We hypothesized that soil C output is largely controlled by available substrate (C input), rather than by temperature per se, and that the relevant C input fractions on an annual basis are those undergoing continuous biomass recycling. If we take the often assumed ca. 50 % autotrophic fraction of R_s (e.g., Bond-Lamberty et al. 2004; Hanson et al. 2000; Högberg et al. 2001) out of the roughly 900 g annual total respiratory C release from our soils, we arrive at ca. 400–500 g of C that should originate from the heterotrophic CO_2 source, including unknown amounts of root exudates and mycorrhizal C consumption. Our estimates of total annual litter NPP explain 343 g (41 %) and 365 g (39 %) of the respiratory C release at the high- and low-elevation sites, respectively. Hence, ca. 10 % of the assumed heterotrophic fraction of R_s remained unaccounted for, which is a reasonable estimate for root exudates and mycorrhizal consumption in temperate forests (15 % of NPP are reported by Vogt et al. 1982 for mature fir stands, 3–13 % of NPP are reported by Bekku et al. 1997 for temperate weed seedlings).

Hence, a similar quantity of C cycles through the soil within a year at both elevations, reinforcing our substrate-dependency hypothesis. Yet, in an undisturbed landscape, with all age classes of trees and forest parcels represented, including those where recalcitrant NPP (stems and branches) is at a recycling stage (decomposition of fallen logs), CO_2 will be released that is not covered by our R_s values obtained in intact forest parcels. Clearly, the 6 K warmer annual temperatures did not significantly enhance soil C release on an annual basis, and instantaneous rates of R_s were downregulated, largely reflecting the annual input of labile C. Summing up, these results caution against expectations of strong positive effects of climatic warming on R_s.

Finally, we assume that recent climatic warming was too slow to cause a significant deviation from a thermal equilibrium of metabolism (metabolism tracking temperature change) at our test sites. Thus, elevational gradients approximate the combined effects of temperature on all forest processes more closely then any experimental warming ever could. What natural thermal gradients cannot capture is long-term vegetation change, which will lag behind climatic change. The temperature dependency of C cycling across natural thermal gradients can assist in developing more realistic scenarios of C cycling in forests in a warmer climate than step changes in soil temperature only. We advocate whole-ecosystem approaches (i.e., the canopy and soils receive similar warming) and a wider appreciation and use of natural temperature contrasts in biological climate warming research.

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References

Aber JD, Melillo JM, Nadelhoffer KJ, McClaugherty CA, Pastor J (1985) Fine root turnover in forest ecosystems to quantity and form of nitrogen availability: A comparison of two methods. Oecologia 66:317–321

Allison I, Bindoff NL, Bindoff RA et al (2009) The Copenhagen Diagnosis 2009: updating the world on the latest climate science. The University of New South Wales Climate Change Research Centre (CCRC), Sydney

Bader M, Hiltbrunner E, Körner C (2009) Fine root responses of mature deciduous forest trees to free air carbon dioxide enrichment (FACE). Functional Ecology 23:913–921

Bader MKF, Körner C (2010) No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment. Global Change Biology 16:2830–2843

Bekku Y, Kimura M, Ikeda H, Koizumi H (1997) Carbon input from plant to soil through root exudation in *Digitaria adscendens* and *Ambrosia artemisiifolia*. Ecol Res 12:305–312

Bond-Lamberty B, Wang C, Gower ST (2004) A global relationship between the heterotrophic and autotrophic components of soil respiration? Global Change Biology 10:1756–1766

Bond-Lamberty B, Thomson A (2010) Temperature-associated increases in the global soil respiration record. Nature 464:579–582

Bradford MA, Davies CA, Frey SD et al (2008) Thermal adaption of soil microbial respiration to elevated temperature. Ecol Lett 11:1316–1327

Brooks PD, Schmidt SK, Williams MW (1997) Winter production of CO_2 and N_2O from alpine tundra: environmental controls and relationship to inter-system C and N fluxes. Oecologia 110:403–413

Brooks PD, McKnight D, Elder K (2004) Carbon limitation of soil respiration under winter snowpacks: potential feedbacks between growing season and winter carbon fluxes. Global Change Biology 11:231–238

Chen G, Yang Y, Guo J, Xie J, Yang Z (2011) Relationships between carbon allocation and partitioning of soil respiration across world mature forests. Plant Ecology 212:195–206

Conant RT, Ryan MG, Agren GI et al (2011) Temperature and soil organic matter decomposition rates—synthesis of current knowledge and a way forward. Global Change Biology 17:3392–3404

Cox PM, Betts RA, Jones CD, Spall SA, Totterdell IJ (2000) Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature 408:184–187

Cramer W, Bondeau A, Woodward FI et al (2001) Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. Global Change Biology 7:357–373

Davidson EA, Belk E, Boone RD (1998) Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Global Change Biology 4:217–227

Davidson EA, Savage K, Verchot LV, Navatto R (2002a) Minimizing artifacts and biases in chamber-based measurements of soil respiration. Agric For Meteorol 113:21–37

Davidson EA, Savage K, Bolstad P et al (2002b) Belowground carbon allocation in forests estimated from litterfall and IRGA-based soil respiration measurements. Agric For Meteorol 113:39–51

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–173

Fischer H, Meyer A, Fischer K, Kuzyakov Y (2007) Carbohydrate and amino acid composition of dissolved organic matter leached from soils. Soil Biol Biochem 39:2926–2935

Friedlingstein P, Cox P, Betts R et al (2006) Climate-carbon cycle feedback analysis: results from the (CMIP)-M-4 model intercomparison. J Climate 19:3337–3353

Gaul D, Hertel D, Leuschner C (2009) Estimating fine root longevity in a temperate Norway spruce forest using three independent methods. Funct Plant Biol 36:11–19

Girardin CAJ, Malhi Y, Aragão LEOC et al (2010) Net primary productivity allocation and cycling of carbon along a tropical forest elevational transect in the Peruvian Andes. Global Change Biology 16:3176–3192

Gough CM, Vogel CS, Harrold KH, George K, Curtis PS (2007) The legacy of harvest and fire on ecosystem carbon storage in a north temperate forest. Global Change Biology 13:1935–1949

Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observation. Biogeochemistry 48:115–146

Harris D, Horwath WR, Van Kessel C (2001) Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Sci Soc 65:1853–1856

Heath J, Ayres E, Possell M, Bardgett RD, Black HIJ, Grant H, Ineson P, Kerstiens G (2005) Rising atmospheric CO_2 reduces sequestration of root-derived soil carbon. Science 309:1711–1713

Heimann M, Reichstein M (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. Nature 451:289–292

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Hogberg MN, Nyberg G, Ottoson Lofvenius M, Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792

IPCC (2001) Climate Change 2001: impacts, adaptation and vulnerability. Cambridge University Press, Cambridge

IPCC (2007) Climate Change 2007: synthesis report. Cambridge University Press, Cambridge

Jacob M, Leuschner C, Thomas FM (2010) Productivity of temperate broad-leaved forest stands differing in tree species diversity. Ann For Sci 67:9

Janssens IA, Lankreijer H, Matteucci G et al (2001) Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Global Change Biology 7:269–278

Kirschbaum MUF (1995) The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biol Biochem 27:753–760

Knorr W, Prentice IC, House JI, Holland EA (2005) Long-term sensitivity of soil carbon turnover to warming. Nature 433:298–301

Körner C (1998) Alpine plants: stressed or adapted? In: Press MC, Scholes JD, Barker MG (eds) Physiological plant ecology. Blackwell, Oxford, pp 297–311

Körner C, Asshoff R, Bignucolo O et al (2005) Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2. Science 309:1360–1362

Kuzyakov Y, Larionova AA (2005) Root and microbial respiration: a review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. J Plant Nutr Soil Sci 168: 503–520

Kuzyakov Y, Gavrichkova O (2010) Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. Global Change Biology 16:3386–3406

Leuschner C, Moser G, Bertsch C, Roderstein M, Hertel D (2007) Large altitudinal Increase in tree root/shoot ratio in tropical mountain forests of Equador. Basic Appl Ecol 8:219–230

Lloyd J, Taylor JA (1994) On the temperature dependence of soil respiration. Funct Ecol 8:315–323

Lund CP, Riley WJ, Pierce LL, Field CB (1999) The effects of chamber pressurization on soil-surface CO_2 efflux and the implications for NEE measurements under elevated CO_2. Global Change Biology 5:269–281

Luo YQ, Wan SQ, Hui DF, Wallace LL (2001) Acclimatization of soil respiration to warming in a tall grass prarie. Nature 413:622–625

Mahecha MD, Reichstein M, Carvalhais N et al (2011) Global convergence in the temperature sensitivity of respiration at ecosystem level. Science 329:838–840

Malhi Y, Baldocchi DD, Jarvis PG (1999) The carbon balance of tropical, temperate and boreal forests. Plant Cell Environ 22:715–740

Mariko S, Nishimura N, Mo W, Matsui Y, Kibe T, Koizumi H (2000) Winter CO_2 flux from soil and snow surfaces in a cool-temperate deciduous forest, Japan. Ecol Res 15:363–372

Mast MA, Wickland KP, Striegl RT, Clow DW (1998) Winter fluxes of CO_2 and CH_4 from subalpine soils in Rocky Mountain National Park, Colorado. Global Biogeochem Cy 12:607–620

McDowell NG, Marshall JD, Hooker TD, Musselman R (2000) Estimating CO_2 flux from snowpacks at three sites in the Rocky Mountains. Tree Physiol 20:745–753

Melillo JM, Steudler PA, Aber JD et al (2002) Soil warming and carbon-cycle feedbacks to the climate system. Science 298:2173–2176

Moser G, Leuschner C, Hertel D, Graefe S, Soethe N, Iost S (2011) Elevation effects on the carbon budget of tropical mountain forests (S Ecuador): the role of the belowground compartment. Global Change Biology 17:2211–2226

Perruchoud D, Kienast F, Kaufmann E, Ulrich Bräker O (1999) 20th century carbon budget of forest soils in the Alps. Ecosystems 2:320–337

Persson H, Ahlström K (1990) The effects of forest liming on fertilization on fine-root growth. Water Air Soil Pollut 54:365–375

R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Raich JW, Nadelhoffer KJ (1989) Belowground carbon allocation in forest ecosystems: global trends. Ecology 70:1346–1354

Raich JW, Schlesinger WH (1992) The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B 44:81–99

Raich JW, Potter CS (1995) Global patterns of carbon-dioxide emissions from soils. Global Biogeochem Cy 9:23–36

Roderstein M, Hertel D, Leuscher C (2005) Above- and belowground litter production in three tropical montane forests in southern Ecuador. J Trop Ecol 21:483–492

Ruehr N, Knohl A, Buchmann N (2010) Environmental variables controlling soil respiration on diurnal, seasonal and annual timescales in a mixed mountain forest in Switzerland. Biogeochemistry 98:153–170

Schindlbacher A, Zechmeister-Boltenstern S, Glatzel G, Jandl R (2007) Winter soil respiration from Austrian mountain forest. Agric For Meteorol 146:205–215

Schulze ED (1982) Plant life forms and their carbon, water and nutrient relations. Encycl Plant Physiol 12B:616–676

Schulze ED, Högberg P, van Oene H, Persson T, Harrison AF, Read D, Kjöller A, Matteucci G (2000) Interactions between the carbon and nitrogen cycle and the role of biodiversity: a synopsis of a study along a north–south transect through Europe. In: Schulze ED (ed) Carbon and nitrogen cycling in European forest ecosystems (Ecological Studies). Springer, Berlin, pp 468–491

Sommerfeld RA, Mosier AR, Musselman RC (1993) CO_2, CH_4 and N_2O flux through a Wyoming snowpack and implications for global budgets. Nature 361:140–142

Steele SJ, Gower ST, Vogel JG, Norman JM (1997) Root mass, net primary production an turnover in aspen, jack pine and black spruce forests in Saskatchewan and Manitoba, Canada. Tree Physiol 17:577–587

Strömgren M, Linder S (2002) Effects of nutrition and soil warming on stemwood production in a boreal Norway spruce stand. Global Change Biology 8:1195–1204

Trumbore SE, Bonani G, Wölfi W (1990) The rates of carbon cycling in several soils from AMS ^{14}C measurements of fractionated soil organic matter. In: Bouwman AF (ed) Soils and the greenhouse effect. Wiley, New York, pp 405–414

Trumbore SE, Chadwick OA, Amundson R (1996) Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. Science 272:393–396

Vogt KA, Grier CC, Meier CE, Edmonds RL (1982) Micorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in western Washington. Ecology 63:370–380

Wang C, Yang J, Zhang Q (2006) Soil respiration in six temperate forests in China. Global Change Biology 12:2103–2114

Zimmermann M, Meir P, Bird MI, Malhi Y, Ccahuana AJQ (2009) Climate dependence of heterotrophic soil respiration from a soil-translocation experiment along a 3000 m tropical forest altitudinal gradient. Eur J Soil Sci 60:895–906

Zimmermann M, Meir P, Bird MI, Malhi Y, Ccahuana AJQ (2010) Temporal variation and climate dependence of soil respiration and its components along a 3000 m altitudinal tropical forest gradient. Global Biogeochem Cy 24:GB4012

CHAPTER 3

DOES NITROGEN INPUT ENHANCE RESPIRATORY CARBON RELEASE FROM TEMPERATE FOREST SOILS?

Does nitrogen input enhance respiratory carbon release from temperate forest soils?

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Abstract

Globally increasing soil nitrogen (N) availability implies unknown consequences on the carbon (C) cycle and storage. While there is an ongoing debate on whether atmospheric N deposition can influence the terrestrial C cycle, surprisingly little use has been made of the natural, long-term N input in forest ecosystems by trees associated with N_2-fixing bacteria. A basic question to be explored is, whether or not high rates of N input increase the rate of C cycling by accelerating both, productivity and respiratory C release. By assessing above- and belowground components of forest NPP and concurrent soil respiration (R_s) in N_2-fixing *Alnus* stands and adjacent non N_2-fixing control stands at two elevations (temperatures), we tested the hypothesis that high-N-input *Alnus* forests facilitate higher soil C release, which, however, still remains in proportion to substrate availability. We found that biological N_2 fixation enhanced total litter production (the sum the above- and belowground forest litter production) and facilitated higher R_s at low elevation, while it reduced both at high elevation. Hence R_s remained in proportion to forest litter production, irrespective of the effect of N availability or site temperature, with annual litter production explaining c. 40% of annual R_s. Assuming a c. 10% contribution of C input to soils other than by plant litter, autotrophic and heterotrophic soil respiration contribute similar fractions to total R_s.

Key words

soil CO_2 efflux, NPP, elevation, temperate forest, acclimation, temperature sensitivity

Introduction

Forests represent the largest terrestrial ecosystem carbon (C) pool, currently estimated at c. 1640 Gt C, more than two thirds of which is stored in soils (Sabine et al. 2004). Soil C pools are, in essence, balanced by the rate of soil C input via net primary production (NPP) and the rate of soil C output via soil respiration (R_s), the two major terrestrial C fluxes after photosynthesis (IPCC 2001). While there is an ongoing debate on whether atmospheric nitrogen (N) deposition can influence the terrestrial C cycle, surprisingly little use has been made of the natural, long-term N input in forest ecosystems by trees living in symbiosis with N_2-fixing bacteria, the topic of this paper.

Currently, N_2 fixation into biologically available forms through human activity exceeds natural N_2 fixation (IPCC 2007), and it is expected that the human induced N-cycle will become further enhanced two- or threefold before reaching saturation (Vitousek et al. 1997; Lamarque et al. 2005). Fertilizer use, agricultural N_2-fixation and N deposition are globally increasing soil N availability with unknown consequences on the C cycle and C storage.

In ecosystems, N is generally found in predictable proportions to C. In soil humus, this proportion is particularly high (C/N=12-20), compared to herbaceous tissue (C/N>30) or wood (C/N>200). Any soil C sequestration thus exerts a major N demand, that competes with plant N demand for growth, which rises the question, whether or not enhanced N inputs to forest ecosystems imply a net C sequestration in plants or soils (e.g. Magnani 2007).

A useful indicator of belowground C cycling is R_s (Kutsch et al. 2009), as it integrates the decomposition of organic matter at all decomposition stages and in all soil layers, as well as rhizomicrobial and root respiration, i.e. the total C flux from soils to the atmosphere. A recent meta-analysis reported enhanced N inputs (either through fertilizer or through atmospheric deposition) to negatively affect R_s in most, but not all, temperate forest ecosystems investigated, with a stronger negative effect in highly productive sites (Janssens et al. 2010). Such a slowing down of the C-cycle by N addition would increase the soil C-pool. The effect of N on C cycling though, is often described to greatly depend on the duration of N-deposition, the dose of N applied, and the decomposition stage or the quality of the substrate (e.g. Fog 1988; Makipaa et al. 1999; Knorr et al. 2005). While substrate with high N content, such as fresh litter, is rather found to be stimulated, low N content, such as in substrate in later decomposition stages, seems to be suppressed by N addition (e.g. Berg and Matzner 2005; Hagedorn, in press). This would result in either a depletion of fast turnover soil C pools or a generally faster ecosystem C turnover, and a retention or accumulation of stable soil C pools. New data also suggest, that neither N nor lignin, but often unaccounted polyphenols in litter control decomposability (Hättenschwiler et al. 2010).

Whether or not N deposition or symbiotic N_2 fixation affect litter and soil organic matter decomposition, a forest that reached steady-state nutrient cycle will commonly operate at a steady-state annual input of organic debris (plant litter production), with the decomposability only affecting the mean residence time of the litter pool, but not the input-output ratio of C.

Steady state respiratory signals from forests receiving long-term (decades or longer) high N inputs can provide useful insights for a better understanding of the C cycle under high N supply. Here we make use of naturally contrasting N availability in forest stands composed either of symbiotically N_2-fixing tree species (*Alnus*) or non N_2-fixing tree species, at two contrasting elevations but similar (high) moisture supply. Tree species living in symbiosis with N_2-fixing bacteria have been known for a long time to accumulate large amounts of N and improve N availability in soils (Hart and Gunther 1989; Binkley et al. 1992; Griffiths et al. 1998; Uri et al. 2011).

The basic question to be explored is, whether high rates of N input do increase the rate of C cycling by accelerating both NPP as well as respiratory C-release, or enhances NPP more than it enhances R_s, thus allowing C to accumulate.

By assessing above- and belowground components of forest NPP and concurrent R_s, we tested the hypothesis, that high-N-input *Alnus* forests facilitate higher soil C release, which, however, remains in proportion to NPP. In other words, we expect the high N availability in N_2-fixing forest sites to enhance R_s only to the extent it enhances labile C input in soils. The inclusion of forest sites at different temperatures (elevations) permits exploring nitrogen ×

temperature interactions, which are of interest in a global warming context.

This study represents an extension of an earlier work that focused on temperature-only effects in a global warming context (Caprez et al., under review in *Oecologia*). In order to explain the effect of natural N_2 fixation on both NPP and R_s, we are re-utilizing part of this earlier dataset for the control sites of the present study in a condensed way. In the current context we are thus refraining from a detailed analysis of temperature effects, but focus on N effects.

Methods

Study design and field sites

A field experiment was set up to study C fluxes in temperate deciduous forests differing in natural soil N input, at two contrasting elevations. Forest stands at low elevation were located at the Swiss Plateau at about 300 m a.s.l.; forest stands at high elevation were in the Swiss Central Alps at 1200 to 1500 m a.s.l., corresponding to a topography driven temperature gradient of c. 6 K (mean annual temperature). At each elevation, we compared forest stands (plots) with N_2-fixing trees with nearby plots of non N_2-fixing trees as control forest plots. The N_2-fixing plots were homogeneous stands of *Alnus glutinosa* (L.) Gaertn. and *Alnus incana* (L.) Moench, each at low and high elevation. The control plots carried either of the dominant tree species *Acer platanoides* or L., *Fagus sylvatica* L., (interspersed with *Quercus petraea* Liebl., *Carpinus betulus* L. or *Tilia platyphyllos* Scop.) at low elevation, and were homogeneous *Salix fragilis* L. *Betula pubescens* Ehrh. or *Sorbus aucuparia* L. forest stands at high elevation. In total our field study included 7 sites at low elevation, each comprising an N_2-fixing and a nearby control plot (Low1-Low7), and 4 sites at high elevation, two of which comprised an N_2-fixing and a control plot (High1 and High2), and two consisting of extra control plots only (High3 and High4; Table 1). In part of these sites, i.e. at our core sites (Low1 and Low2; High1 and High2), we monitored NPP and year-round R_s (our core sites; Table 2), whereas peak growing season R_s was recorded at all 11 sites.

We selected alluvial sites only, in order to avoid confounding effects by soil moisture. All study plots had closed canopies within an area of 50 × 50 m, surrounded by a buffer zone of at least 10 m and were older than 40 years. Larger homogeneous, monospecific N_2-fixing stands are hard to find, given most alluvial land and riverine forests were transformed into agricultural land over the past centuries.

Air temperature (2 m above ground) and soil temperature (10 cm depth) were recorded throughout the field experiment at an hourly basis (HOBO TidbiT v2, Onset Computer Corp., Bourne, MA, USA) and hourly averages of soil moisture and precipitation were recorded below the canopy (EM50 data loggers connected to a ECRN-100 rain gauge and four 10HS soil moisture probes installed at 10 cm depth, Decagon Devices, Pullman; USA) at each core site.

Leaf area index (LAI)

LAI was estimated using a ceptometer (AccuPAR LP-80, Decagon Devices Inc., Pullman, USA). Reference measurements were taken in an open area within 50 m distance under clear sky conditions immediately after below-canopy measurement. All measurements were accomplished in mid August, when canopy foliage reached its maximum.

Soil physico-chemical analysis

We excavated two soil profiles per plot at the core sites and sampled soils at 5, 10, 20 and 30 cm depth using a 35 mm diameter × 50 mm length corer (at the high-elevational N_2-fixing sites a profile depth deeper than 30 cm could not be accessed because of stony ground). All soil samples were sieved (2 mm), oven-dried at 105 °C for 24 h, and fine earth density determined. Soil pH of subsamples was measured in 0.1 M KCl solution. Carbonates were removed from subsamples from each soil core by exposure to HCl vapour (Harris et al. 2001) and C and N contents determined by dry combustion (ThermoFinnigan FlashEA 1112, Milan, Italy).

Soil microbial C and N

Soil microbial C and N were estimated by chloroform fumigation-extraction (CFE) (Brookes et al. 1985, Vance et al. 1987). In brief, the water content of sieved fresh soil samples (0-10 cm) corresponding to 10-15 g dry was adjusted to 50% water holding capacity. These subsamples were then fumigated with ethanol-free $CHCl_3$. After fumigation, $CHCl_3$ was removed by repeated evacuation and the sample extracted with 100 ml 0.5 M K_2SO_4 by shaking

Table 1 Despriction of all study sites at low elevation (Low1 to Low7) and high elevation (High1 to High 4).

Elevation (m a.s.l.)		Site	Plot	Coordinates	Dominant species
Low	296	Low1 (core sites)	Control	47°32'37" N / 7°46'22" E	*Acer platanoides*
	300		N_2-fixing	_47°32'42" N _/ 7°46'7" E	*Alnus glutinosa*
	330	Low2 (core sites)	Control	47°32'53" N / 8°13'34" E	*Acer platanoides*
	328		N_2-fixing	47°32'49" N / _8°13'38" E	*Alnus glutinosa*
	295	Low3	Control	47°32'39" N / 7°45'42" E	*Acer platanoides*
	295		N_2-fixing	47°32'39" N / 7°45'56" E	*Alnus glutinosa*
	410	Low4	Control	47°32'40" N / 8°13'21" E	*Fagus sylvatica*
	330		N_2-fixing	47°32'48" N / 8°13'27" E	*Alnus glutinosa*
	498	Low5	Control	47°07'05" N / 8°18'42" E	*Fagus sylvatica*
	485		N_2-fixing	47°07'00" N / 8°18'23" E	*Alnus glutinosa*
	449	Low6	Control	47°31'44" N / 7°47'22" E	*Fagus sylvatica*
	447		N_2-fixing	47°31'41" N / 7°47'13" E	*Alnus glutinosa*
	486	Low7	Control	47°22'16" N / 8°11'13" E	*Fagus sylvatica*
	472		N_2-fixing	47°22'10" N / 8°11'09" E	*Alnus glutinosa*
High	1515	High1 (core sites)	Control	46°36'16" N / 8°31'01" E	*Salix fragilis*
	1190		N_2-fixing	46°43'02" N / 8°54'09" E	*Alnus incana*
	1508	High2 (core sites)	Control	46°36'21" N / 8°31'21" E	*Salix fragilis*
	1390		N_2-fixing	46°32'13" N / 8°21'30" E	*Alnus incana*
	1220	High3	Control	46°42'54" N / 8°54'56" E	*Betula pubescens*
	1630	High4	Control	46°36'47" N / 8°34'11" E	*Sorbus aucuparia*

for 60 min on a table shaker. A corresponding set of unfumigated soil samples were also extracted. The extracts were then centrifuged (10 min at 1000 *g*), the supernatant filtered and frozen at -20°C until analysis for organic C and N (Dimatoc TOC/TN_b-analyser, Dimatec, Essen, Germany). Microbial biomass C and N were calculated considering the extraction efficiency for C (k_{EC}=0.45; Wu et al. 1990) and N (k_{EN}=0.54; Brookes et al. 1985).

Forest NPP

We estimated the short-lived forest NPP fractions 'canopy litter fall', 'production of understory vegetation' and 'fine root increment' in ingrowth cores (our proxy for belowground NPP), and addressed the sum of all three proxies as 'total litter production'. Stem basal area increment (BAI) was estimated as indicator of the long-lived forest NPP component 'wood'.

Canopy litter production was estimated by collecting canopy litter fall in litter traps (0.25 m^2 ground area plastic sieves); Six traps were placed randomly in each core site during two subsequent autumns. Litter traps were emptied at least every two weeks, canopy litter was dried (80 °C). For annual understory production, we harvested peak biomass at ground level (2 × 1 m^2 per core site). To assess annual fine root production, we first harvested 40 soil cores per core site at random locations (3.5cm diameter × 12 cm depth) for fine root standing biomass, which we immediately replaced the mesh cylinders (2 mm mesh size) filled with root-free soil (ingrowth cores). Ingrowth cores were harvested after one year. Both soil cores for

Table 2 Forest stand properties of the core sites. Given are the within-site means ± SE.

Site (elevation)	Low1		Low2		High1		High2	
Plot	Control	N_2-fixing	Control	N_2-fixing	Control	N_2-fixing	Control	N_2-fixing
Stand age (years)	50-60	110-150	40-50	c. 100	c. 40	40-50	c. 40	40-50
Basal area (m^2 ha^{-1})	55 ± 4.2	77 ± 4.1	42 ± 1.9	61 ± 6.3	38 ± 2.1	58 ± 9.6	39 ± 3.2	82 ± 6.6
Canopy height (m)	21.1 ± 0.1	24.6 ± 0.4	20.5 ± 0.3	26.0 ± 0.5	7.8 ± 0.5	12.3 ± 0.9	6.6 ± 0.1	15.4 ± 0.3
LAI (m^2 kg^{-1})	4.7 ± 0.1	5.5 ± 0.1	5.0 ± 0.2	5.9 ± 0.2	3.7 ± 0.2	4.4 ± 0.1	4.1 ± 0.1	4.9 ± 0.2

Canopy height was estimated using an optical reading clinometer (PM-5, Suunto, Espoo, Finland).

standing fine root biomass and ingrowth cores for fine-root production were washed carefully and dried (80 °C). To obtain a relative proxy for fine root turnover, we calculated the ratio of annual fine root ingrowth (NPP) to the mean fine root standing biomass, sampled before placing the ingrowth cores (Aber et al. 1985). The rate of fine root production was assumed to be in equilibrium with fine root decay (no net accumulation in fine root mass).

Soil respiration (R_s)

R_s was measured using a portable custom-made static chamber system (after the design of Bader et al. 2010) equipped with an open-path infrared gas analyser and relative humidity/temperature sensors (GMP343 carbon dioxide probe, HMP75 rH/T probe; Vaisala, Vantaa, Finland). In brief, twelve polypropylen collars (7 cm in height, 20 cm in diameter) were installed at randomly selected positions within each site, inserted to a depth of 3 cm into the ground, two to three weeks prior to the first measurement. The collars remained at the same location throughout the study, allowing a repeated measurement of the same soil area over time. Headspace CO_2 concentrations were automatically measured every 5 seconds during 5 minutes per measurement occasion and collar and R_s rates calculated by linear regression of recorded headspace CO_2 concentrations against time.

R_s was recorded year-round from early spring 2009 to early spring 2010 at all 8 core sites: every two weeks throughout the growing season and once a month from late autumn to early spring, whereas winter-time measurements at high elevation were restricted by heavy snowpack, resulting in one set of reliable R_s measurements between mid December and mid April. During the summer months July and August 2010, we performed weekly measurements of mid season R_s at the core and supplementary sites.

Besides data collected by the automatic data loggers, soil temperature and soil moisture at the chamber site were measured manually, simultaneously with all R_s recordings (soil temperature: Digital Thermometer GTH 175/Pt, Greisinger electronic, Germany; soil moisture: ThetaProbe Soil Moisture Sensor - ML2x, Delta-T Devices Ltd., Cambridge, UK).

Data analysis

R_s was related to soil temperature (T, 10cm depth) by fitting a non-linear least squares model following Lloyd and Taylor (1994):

$$R_s = R_{s(t)} \times e^{E_0\left(\frac{1}{56.02}-\frac{1}{T-227.13}\right)}$$

where $R_{s(t)}$ is soil respiration under standard conditions (at 10°C) and E_0 is a parameter describing temperature response. Annual R_s was estimated at the core sites by predicting R_s in hourly intervals, based on the temperature response function, using automatically logged soil temperature.

Effects of elevation ("low" vs. "high") and N availability (control vs. N_2-fixing plots) were tested using mixed-effects models fitted by restricted maximum likelihood (REML) with 'elevation' and 'N' as fixed effects and 'site' as random effect. Non-normally distributed rates were log-transformed (fine root ingrowth). Error estimates in text and figures are standard errors of 'treatment' means and effects were considered significant at $P < 0.05$. Due to the relatively low replication and therefore statistical

power, *P*-values > 0.05 but < 0.1 were considered marginally significant. All statistical analyses were carried out using R (version 2.10; mixed effects models were fit using the lme procedure from the nlme package; Pinheiro et al. 2008; R Development Core Team, 2010; www.r-project.org).

Results

Climatic conditions

Air and soil temperature showed typical seasonal variation with lowest values in January and highest values in July. Mean annual air temperature (2 m height) during the sampling period was 10.3 ± 0.1 and °C 4.3 ± 0.4 °C at low and high elevation respectively, corresponding to a topography driven temperature cline of c. 6 K, with no or minor differences between N_2-fixing and control plots. During the entire sampling time, soil temperature did not drop below the freezing point at any of the plots. Top-soil water content (VWC at 0-10 cm depth) showed moderate changes and stayed relatively high at all plots throughout the sampling period. There was a small trend within locations, with higher VWC at N_2-fixing compared to control plots at both elevations, and, as a result of higher sand content, VWC was generally lower at high elevation compared to low elevation (Figure 1). At the N_2-fixing plot Low1, the soil was flooded from early December to mid February, a period during which no R_s measurements could be accomplished at this plot. Plots at high elevation were snow covered (to a max. of 1 m thickness) from the beginning of December to mid April, whereas at low elevation the forest floor was only periodically covered with a few centimetres of snow between early December and late February.

Forest stand characteristics

The stem basal area was in the range of 38 to 82 m^2 ha^{-1} in all plots and did neither differ significantly between N_2-fixing and control plots, nor across elevations.

Trees in low elevational plots were significantly higher than trees at high elevation. LAI was slightly higher in N_2-fixing compared to control sites (P <0.1) and significantly enhanced at low relative to high elevation (P < 0.05; Table 2).

Soil C and N

Soil organic C and N differed remarkably between plots, with higher average organic C (P < 0.05) and N contents (P < 0.1) in N_2-fixing compared to control plots. Both C and N concentration decreased with soil depth at all core plots. C/N was neither affected by elevation nor N_2 fixation (Table 3).

Soil microbial C and N

Soil microbial C in the upper 10 cm was higher in N_2-fixing compared to control plots, though this signal was statistically marginally significant only (P < 0.1); a similar, also statistically marginally significant trend was found for microbial N (P < 0.1; Table 3). Accordingly, the C to N ratio of microbial biomass was similar for N_2-fixing and control plots. Elevation had no influence on microbial C, N or C/N.

Forest NPP

Annual canopy litter production was higher in N_2-fixing compared to control plots (+35%, P < 0.1), and significantly lower at high relative to low elevation (-49%, P < 0.05). Understory litter production was neither enhanced under N_2-fixing trees nor differed between elevations. Fine root ingrowth, our proxy for belowground litter production, showed reversed trends at the two elevations: at low elevation, fine root ingrowth was enhanced at N_2-fixing compared to control plots, whereas it was the other way round at high elevation, resulting in no overall N_2-fixation or elevational-effect. Fine root standing biomass was slightly reduced under N_2-fixing trees (-43%, P < 0.1), and was significantly higher at high compared to low elevation (+108%, P < 0.05). The resulting fine root turnover rates were generally between 0.28 and 0.40,
but substantially higher in low-elevational N_2-fixing plots (Low1: 1.28 a^{-1}; Low2: 0.68 a^{-1}), resulting in no overall N- and elevational-effect.

Total litter production (the sum of the forest productivity components with fast turnover: canopy litter, understory litter and fine root ingrowth) showed an slightly significant N_2 fixation effect which depended on elevation (N × elevation: P < 0.1); at low elevation total litter production was 21% higher in N_2-fixing relative to control plots, whereas at high elevation it was 23% lower in N_2-fixing compared to control plots (Table 4).

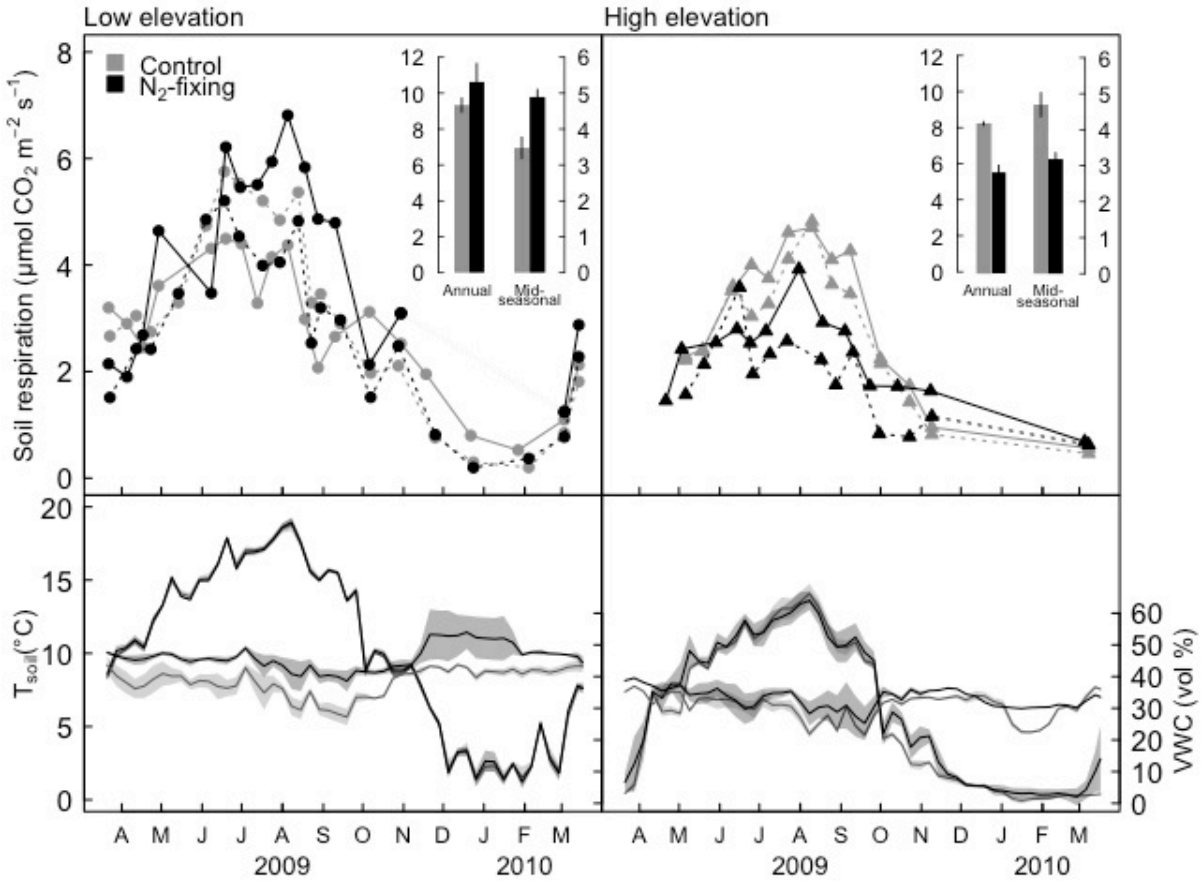

Fig. 1 Seasonal variation of R_s at the plot level of the core sites. The solid line represents the plots Low1 and High1, the dashed line denotes the plots Low2 and High2. The bar chart insets give the annual R_s (t C m^{-2} a^{-1}) of the core sites, and the mid-seasonal R_s rates (µmol CO_2 m^{-2} s^{-1}) for all sites (mean ± SE). Soil temperature (T_{soil}) and soil volumetric water content (VWC) were recorded in the top 10 cm (mean ± SE, n = 2).

Stem basal area increment was not different for N_2-fixing trees compared to controls, and neither showed an elevational effect.

Soil respiration

R_s showed pronounced seasonality, following changes in soil temperature, at the core sites. While at low elevation, R_s rates showed no clear pattern for N_2-fixing and control plots in the course of the year, at high elevation R_s rates at the control plots were higher than R_s at under N_2-fixing trees for most of the year (Figure 1).

At site Low1, cumulative annual R_s at the N_2-fixing plot (1166 g C m^{-2} a^{-1}) exceeded the control plot (894 g C m^{-2} a^{-1}) by 30%, whereas at site Low2, annual R_s was 2% lower at the N_2-fixing plot (958 g C m^{-2} a^{-1}) than at the control plot (973 g C m^{-2} a^{-1}). Mid-seasonal R_s rates (averaged across sites Low1 to Low7) were 40% higher in N_2-fixing plots than in control plots (Figure 1 a, inset), relating more to the effect of the site pair Low1 than the site pair Low2.

At high elevation, cumulative annual R_s in N_2-fixing plots (High1: 602 g C m^{-2} a^{-1}; High2: 525 g C m^{-2} a^{-1}) were on average reduced by 33% relative to control plots (High1: 841 g C m^{-2} a^{-1}; High2: 830 g C m^{-2} a^{-1}). Mid-seasonal R_s rates at high-elevational control plots High3 and High4 were similar to mid season R_s at control plots High1 and High2, thus confirming their representativeness for non N_2-fixing deciduous forest plots at this elevation (Figure 1, inset).

The contrasting signal of cumulative annual R_s at the two elevations (higher R_s in N_2-fixing plots at low elevation, but lower R_s in N_2-fixing plots at high elevation compared to control plots) resulted in a marginally significant interaction between elevation and N_2-fixation (N_2-fixation × elevation: $P < 0.1$), whereas annual R_s was higher at low compared to high elevation ($P < 0.05$; Table 4). Mid season R_s rates did not generally differ between elevations, but, similar to annual R_s, we found N_2-fixation to significantly affect R_s rates depending on

Table 3 Soil properties along the soil profiles to a depth of 30 cm at the core sites at low and high elevation. Microbial biomass (C_{mic} and N_{mic}) were measured for the upper 10 cm of the soils (mean ± SE; n = 2).

	Low elevation		High elevation	
Soil depth	Control	N_2-fixing	Control	N_2-fixing
0-5 cm				
Bulk density (g cm^{-3})	0.96 ± 0.09	0.46 ± 0.05	0.71 ± 0.16	0.85 ± 0.12
C content (kg C m^{-2})	2.77 ± 0.13	2.61 ± 0.33	1.18 ± 0.38	5.20 ± 2.03
N content (kg N m^{-2})	0.18 ± 0.05	0.23 ± 0.05	0.12 ± 0.01	0.45 ± 0.12
$pH_{(KCL)}$	6.26 ± 0.38	5.96 ± 0.72	5.25 ± 0.01	5.51 ± 0.65
5-10 cm				
Bulk density (g cm^{-3})	1.05 ± 0.06	0.59 ± 0.09	1.15 ± 0.01	1.02 ± 0.22
C content (kg C m^{-2})	3.50 ± 0.05	5.57 ± 2.26	1.58 ± 0.87	5.96 ± 1.08
N content (kg N m^{-2})	0.37 ± 0.03	0.47 ± 0.14	0.16 ± 0.05	0.38 ± 0.15
10-20 cm				
Bulk density (g cm^{-3})	1.10 ± 0.01	0.76 ± 0.20	1.27 ± 0.01	1.22 ± 0.22
C content (kg C m^{-2})	3.10 ± 0.08	4.37 ± 0.69	0.35 ± 0.09	5.87 ± 0.14
N content (kg N m^{-2})	0.30 ± 0.06	0.44 ± 0.09	0.03 ± 0.01	0.28 ± 0.22
20-30 cm				
Bulk density (g cm^{-3})	1.26 ± 0.02	0.84 ± 0.26	1.22 ± 0.09	1.07 ± 0.06
C content (kg C m^{-2})	2.44 ± 0.26	4.62 ± 2.69	0.35 ± 0.14	4.09 ± 0.75
N content (kg N m^{-2})	0.27 ± 0.04	0.39 ± 0.19	0.03 ± 0.01	0.35 ± 0.13
Microbial biomass				
C_{mic} (mg (g dry soil)$^{-1}$)	20.38 ± 1.86	67.84 ± 16.79	5.21 ± 0.65	38.17 ± 17.19
N_{mic} (mg (g dry soil)$^{-1}$)	5.05 ± 0.49	14.76 ± 4.05	1.35 ± 0.02	8.10 ± 3.21

elevation (N_2-fixation × elevation: $P < 0.05$; Table 4).

Correlation of annual plant litter production and annual R_s

Total annual litter production (the sum of the labile forest productivity components) correlated well with annual R_s ($y = 2.42x - 5.53$, $r^2 = 0.85$; Figure 2).

Discussion

We hypothesized, that high-N-input *Alnus* forests are more productive than nearby control deciduous forests, and that this higher productivity stimulates soil C release with a generally faster C cycling as the main explanation, irrespective of local temperature. What we found was, that natural N_2-fixation enhanced total litter production and facilitated higher R_s at low elevation, while it reduced both at high elevation. Hence R_s remained in proportion to forest litter production, irrespective of the effect of N-availability or site temperature.

By choosing *Alnus* stands, we intended to compare high N-input, high productive forest stands with nearby control forest stands on similar substrate and moisture. It appears however, that *Alnus* takes only advantage from high N-input in terms of litter NPP (production of plant parts with annual recycling) relative to the control forest stands at low elevation, but not at high elevation, as the incoherent picture of total fresh litter production shows (Table 4): At low elevation N_2 fixation tended to enhance canopy litter (+ 34%), to reduce understory production (- 17%), but enhance new root production (+ 57%), yielding a 21% net increase of total litter production at N_2-fixing compared to control sites. At high elevation, in contrast, the increase in canopy litter (+ 38%) of N_2-fixing trees was more than cancelled by very low production of understory (- 58%) and fine roots (- 51%), causing total litter production to be 23% lower in the N_2-fixing forest stands compared to controls.

Table 4 Annual forest C fluxes at the core sites (components forest litter production and annual R_s), and mean mid-seasonal R_s of all sites. Total litter production refers to the sum of canopy litter, understory litter and fine root production. Given is the mean ± SE (n = 2 per elevation). Results of linear mixed models are given on the right.

	Low elevation		High elevation		Effect (P-value)		
	Control	N_2-fixing	Control	N_2-fixing	Elev.	N_2-fix.	N_2-fix. × Elev.
Annual C fluxes (g C m^{-2} a^{-1})							
Canopy litter production	235 ± 27	314 ± 7	117 ± 22	161 ± 10	*	(*)	n.s.
Understory litter production	101 ± 44	84 ± 15	128 ± 28	54 ± 9	n.s.	n.s.	n.s.
Fine root ingrowth	28 ± 4	44 ± 8	97 ± 14	48 ± 23	n.s.	n.s.	n.s.
Total litter production	365 ± 13	442 ± 31	343 ± 21	263 ± 21	n.s.	n.s.	(*)
Annual R_s	933 ± 40	1062 ± 104	836 ± 5	563 ± 39	*	n.s.	(*)
Mid-seasonal R_s (μmol C m^{-2} s^{-1})	3.47 ± 0.29	4.89 ± 0.21	4.62 ± 0.01	3.18 ± 0.18	n.s.	(*)	*

(*) $0.05 < P < 0.1$; * $P < 0.05$.

Concurrently, the presence of N_2-fixing trees enhanced annual respiratory soil CO_2 release by 14% at low elevation, and reduced it by 33% at high elevation. Mid season R_s signals, including a higher number of site pairs tested, strengthened the rather weak signal of annual R_s at low elevation, with on average 41% higher R_s rates in N_2-fixing compared to control forest stands. At high elevation, mid season R_s rates supported the general high-elevational signal, with 31% lower R_s rates in N_2-fixing compared to control forest stands (Table 4).

N limitation of forest productivity

Although the productivity of temperate forests is traditionally considered N-limited (Vitousek et al. 2002), plant N demand has been observed to saturate when exposed to long-term high N input, followed by declining plant production and/or mortality, and decreased forest retention of added N (e.g. Aber et al. 1998). Increased nitrate leaching (Aber et al. 1998; Macdonald et al. 2002) or nitrous oxide emissions (Mohn et al. 2000; Jassal et al. 2011) can be the consequence of such an N saturating stage of a forest system. At high elevation, we found high stand biomass (see stem basal area and canopy height in Table 2), but low productivity (Table 4) in the N_2-fixing relative to the control plots, suggesting that our high-elevational N_2-fixing forest stands have reached N saturation (limitation by other nutrients or low temperature). At low elevation in contrast, lower forest productivity in N_2-fixing compared to control plots (Table 4) might suggest that N_2-fixing stands had not yet arrived N-saturation. Nitrous oxide emissions were particularly high in N_2-fixing stands at both elevations (see *Chapter 5* of this thesis).

R_s from soils with contrasting N-inputs

The stimulation of N_2 fixation on annual R_s we reported for the low-elevational core sites, might not be robust enough to draw conclusions. However, for mid season (when when R_s data were available for all sites), we found R_s to be significantly stimulated (directly or indirectly) by N_2-fixation (Figure 1 a, inset), with a single site (Low2) not showing an effect.

Our estimates of annual R_s of 933 ± 40 g C m^{-2} a^{-1} (control) and 1062 ± 104 g C m^{-2} a^{-1} (N_2-fixing) at low elevation, are within the range of annually respired C amounts from soils of other temperate deciduous forests (Malhi et al. 1999; Wang et al. 2006; Bader and Körner 2010; Ruehr et al. 2010) all reporting yearly soil C releases between 700 and 1200 g C m^{-2} a^{-1}. For N_2-fixing forest stands, our estimates are thus in the upper part of this range, similar to the 1234 g C m^{-2} a^{-1} reported for a black alder forest soil in northern Germany (Kutsch et al. 2001).

Mid season R_s at the low-elevational control sites was on average 3.4 ± 0.3 µmol CO_2 m^{-2} s^{-1}, and thus similar to the 3.9 µmol CO_2 m^{-2} s^{-1} mid season R_s recorded in a long-term R_s time-series in a temperate broadleaf forest located at the same elevation to our low-elevational sites (Bader and Körner 2010).

At high, in contrast to low elevation, R_s patterns between N_2-fixing and control forests were clearer, with both, annual and mid-seasonal soil CO_2 efflux rates being more than one third higher under N_2-fixing trees (Figure 1, inset). A

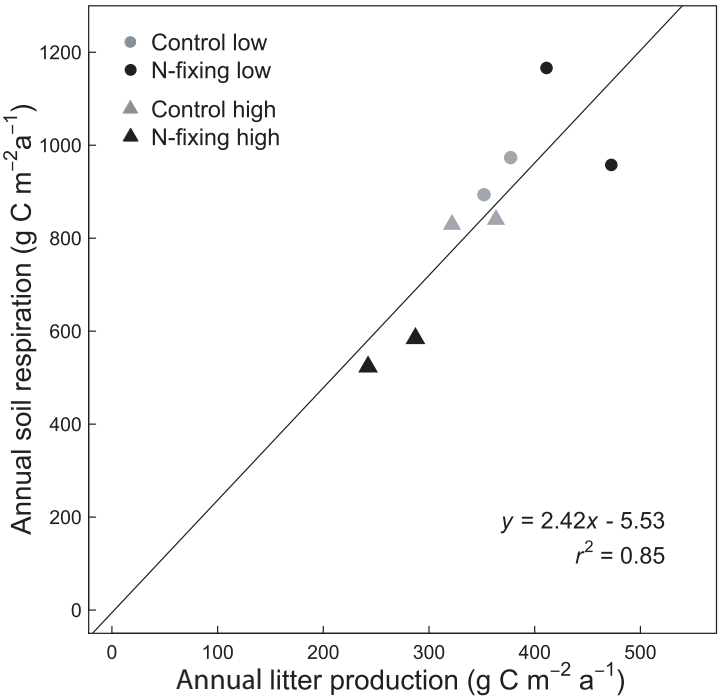

Fig. 2 Relationship (simple linear regression) between total annual litter production (the sum of canopy litter, understory litter and fine root production) and annual soil respiration (R_s) at the core sites, differing in elevation and N availability. Each data point represents a plot-mean.

phylogenetic bias, i.e. a *Salix* effect at the high-elevational control plots (High1 and High2) could be excluded by recording similar mid season R_s rates at two additional control plots dominated by either *Betula* (High3) or *Sorbus* (High4), exhausting the spectrum of deciduous forest types at this elevation. Our estimates of annual soil C efflux at high elevation of 836 ± 5 C m^{-2} a^{-1} in control and 563 ± 39 C m^{-2} a^{-1} in N_2-fixing forest stands, to our knowledge are the only R_s data of temperate high elevational deciduous forests, but annual R_s rates of 700 to 800 g C m^{-2} a^{-1} were reported in forests with similar mean annual temperature (compiled by Chen et al. 2011). We are not aware of any R_s data at *Alnus*-sites at this elevation. Studies comparing soil C effluxes in N_2-fixing and other forests are rare, but Kutsch et al. (2001 and 2005) found extraordinarily high rates of rhizomicrobial respiration in an alder/ash-dominated forest in northern Germany compared to adjacent sites composed of different tree species. By contrast, R_s in sites covered with the N_2-fixing shrub *Elaeagnus umbellate* Thunb. was reduced relative to adjacent C3 grassland sites (Baer et al. 2006), yet this comparison includes two totally different types of ecosystems.

Soils under N_2-fixing and non N_2-fixing trees

Soil N content (0-30 cm) under N_2-fixing trees, exceeded soil N content at the control forest stands by 36% at low elevation, and was four times higher than in control sites at high elevational (Table 3). The C to N ratio though, similar for all N_2-fixing and control plots, suggested that a considerable part of the abundant soil N in the N_2-fixing plots tied up with soil C to SOM. Several studies indicate that for about two-thirds of all added N, SOM is the final and long-term sink (Aber et al. 1993; Nadelhoffer et al. 1995; Magill et al. 1997; Aber et al. 1998). Another frequently reported way to incorporate mineral N into SOM is N immobilization through microbial biomass production (e.g. Hart et al. 1994; Magill and Aber 2000). In accordance with higher soil C and N contents, we found microbial C and N to be three and seven times higher in N_2-fixing than in control plots at low and high elevation respectively (Table 3).

Forest C balance

Plotting the annual C input fluxes to soils (total litter production) against the annual soil C releases via R_s across the core study sites reveals a highly significant correlation (Figure 2), suggesting a dependency of soil C release on

short-lived forest NPP fractions, irrespective of N-availability and temperature.
Here we can balance C output with C input in soils by the heterotrophic input fraction only, i.e. by proxies of plant litter input. Autotrophic (in essence root) respiration remains unaccounted by direct data, but is often assumed to account for half of total R_s, though estimates range from 10-90% (Hanson et al. 2000).
The ratio of total annual plant litter C production to annual C release from soils observed was 2.4:1 (Figure 2). In other words, annual plant litter C production is about 40% of annual C realease by R_s. Given that the labile NPP fraction of a forest ecosystem not only includes plant litter production, but, to a smaller extent, also root exudates and mycorrhizal C-consumption (esimates range from 3-15 % in the literature, e.g Vogt et al. 1982; Bekku et al. 1997), the balance of C input in soil through NPP and the C output from soils via Rs would become closer to 2:1. Hence, autotrophic and heterotrophic soil respiration, contribute similar fractions of total R_s.

We conclude that R_s exhibits a rather robust relationship to substrate availability, rather than showing direct responses to N-availability. The results of this comparison of forests with contrasting N-input rates thus lines up with the results of our previous analysis of temperature relatedness, where forest litter production offered an almost exhaustive explanation of R_s (Caprez et al., under review in Oecologia). Taken together, these studies suggest, that soil CO_2 release is tightly associated with soil C inputs, irrespective of N-availability or temperature. Our results offer no justification for modelling R_s by assuming either N or temperature to exert direct (independent) effects on R_s. R_s is largely driven by the labile fraction of NPP. In the long run, small deviations that are below the resolution of our analysis, could still affect the soil C-pool, provided element stoichiometry permits.

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References

Aber JD, Melillo JM, Nadelhoffer KJ, McClaugherty JP (1985) Fine root turnover in forest ecosystems in relation to quantity and form of nitrogen availability: A comparison of two methods. Oecologia 66: 317-321

Aber JD, Magill A, Boone R et al. (1993) Plant and soil responses to chronic nitrogen additions at the Harvard Forest, Massachusats. Ecological Applications 3:156-166

Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I (1998) Nitrogen saturation in temperate forest ecosystems. Bioscience 48:921-934

Bader MKF, Körner C (2010) No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment. Global Change Biology 16:2830-2843

Baer SG, Church JM, Williard KWJ, Groninger JW (2006) Changes in intrasystem N cycling from N2-fixing shrub encroachment in grassland: multiple positive feedbacks. Agriculture Ecosystems and Environment. 115:174-182

Bekku Y, Kimura M, Ikeda H, Koizumi H (1997) Carbon input from plant to soil through Root exudation in *Digitaria adscendens* and *Ambrosia artemisiifolia*. Ecological research 12:305-312

Berg B, Matzner E (2005) Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. Environmental Review. 5:1-25

Binkley D, Sollins P, Bell R, Sachs D, Myrold D (1992) Biogeochemistry of adjacent conifer and alder-conifer stands. Ecology 73:2022-2033

Brookes PC, Landmann A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17:837-842

Chen G, Yang Y, Gio J, Xie J, Yang Z (2011) Relationships between carbon allocation and partitioning of soil respiration across world mature forests. Plant Ecology 212:195-206

Fog K (1988) The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. 63:433-462

Griffiths RP, Homann PS, Riley R (1998) Denitrification enzyme activity of douglas-fir red alder forest soils of the Pacific Northwest. Soil Biology and Biogeochemistry 30:1147-1157

Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogechemistry 48:115-146

Harris D, Horwath WR, Van Kessel C (2001) Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Science Society 65:1853-1856

Hart SC, Gunther AT (1989) In situ estimates of annual net mineralization and nitrification in subarctic watershed. Oecologia 80:284-288

Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization, and nitrification. In: Methods of Soil Analysis, Part 2. Microbiological Biogeochemical Properties. Weaver S et al. (eds) Soil Science Society of America, Madison pp 985-1018

Hättenschwiler S, Coq S, Barantal S, Handa IT (2010) Leaf traits and decomposition in tropical rainforests: revisiting some commonly held views and towards a new hypothesis. New Phytologist 189: 950-965

IPCC (2001) Climate Change 2001: Impacts, Adaptation and Vulnerability. Cambridge University Press, UK

IPCC (2007) Climate Change 2007: Synthesis Report. Cambridge University Press, UK

Janssens IA et al. (2010) Reduction of forest soil respiration in response to nitrogen deposition. Nature Geoscience 3:315-322

Jassal RS, Black TA, Roy R, Ethier G (2011) Effect of nitrogen fertilization on soil CH_4 and N_2O fluxes, and soil and bole respiration.

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: A meta-analysis. Ecology 86:3252-3257

Kutsch WL, Staack A, Wötzel J, Middelhoff U, Kappen L (2001) Field measurements of root respiration and total soil respiration in an Alder forest. New Phytologist 150:157-168

Kutsch WL, Liu C, Hörmann G, Herbst M (2005) Spatial heterogeneity of ecosystem carbon fluxes in a broadleaved forest in Northern Germany. Global Change Biology 11:70-88

Kutsch W, Bahn M, Heinemeyer A (2009) Soil Carbon Dynamics: An Integrated Methodology. Cambridge Univ. Press

Lamarque et al. (2005) Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: Analysis of nitrogen deposition. Journal of geophysical research 110

Lloyd J, Taylor JA (1994) On the temperature dependence of soil respiration. Functional Ecology 8:315-323

Macdonald JA et al. (2002) Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. Global Change Biology 8:1028-1033

Magill AH et al. (1997) Ecosystem responses to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusats, USA. Forest Ecology and Management 196:7-28

Magill AH, Aber JD (1998) Long-term effects of experimental nitrogen additions of foliar litter decay and humus formation in fores ecosystems. Plant Soil 203:301-311

Magnani F et al. (2007) The human footprint in the carbon cycle of temperate and boreal forests. Nature 447:848-850

Mäkipää R, Karjalainen T, Pussinen A, Kellomaki S (1999) Effects of climate change and nitrogen deposition on the carbon sequestration of a fores ecosystem in the boreal zone. Canadian Journal of Forest Research 29:1490-1501

Malhi Y, Baldocchi DD, Jarvis PG (1999) The carbon balance of tropical, temperate and boreal forests. Plant, Cell and Environment 22:715-740

Mohn J, Schürmann A, Hagedorn F, Schleppi P, Bachofen R (2000) Increased rates of denitrification in nitrogen-treated forest soils. Forest Ecology and Management 137:113-119

Nadelhoffer KJ, Downs MR, Fry B, Aber JD, Magill AH, Melillo JM (1995) The fate of N-15-labeled nitrate additions to a Northern Hardwood Forest in Eastern Maine, USA. Oecologia 103:292-301

Pinheiro J, Bates D, DebRoy S, Sarkar D, The R Core Team (2009) NLME: Linear and nonlinear mixed effects models. R package version 3.1-96. Available at: http://cran.r-project.org/web/packages/nlme/index.html

R Development Core Team (2009) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Ruehr N, Knohl A, Buchmann N (2010) Environmental variables controlling soil respiration on diurnal, seasonal and annual time-scales in a mixed mountain forest in Switzerland. Biogeochemistry 98:153-170

Sabine CL et al. (2004) Current status and past trends of the global carbon cycle. In: Field CB, Raupach MR (eds) The global carbon cycle: Integrating humans, climate and the natural world. Island Press, Washington, pp 17-44

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19:703-707

Vitousek MP, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications 7:737-750

Vitousek PM et al. (2002) Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57/58:1-45

Vogt KA, Grier CC, Meier CE, Edmonds RL (1982) Micorrhizal Role in Net Primary Production and Nutrient Cycling in *Abies amabilis* Ecosystems in Western Washington. Ecology 63:370-380

Wang C, Yang J, Zhang Q (2006) Soil respiration in six temperate forests in China. Global Change Biology 12:2103-2114

CHAPTER 4

SOIL ORGANIC CARBON POOLS AND THEIR CONTRIBUTION TO SOIL RESPIRATION

Soil organic carbon pools and their contribution to soil respiration

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Abstract

Soil organic matter is a heterogeneous mixture of pools that differ in turnover time; some soil carbon (C) pools turn over within hours, others have residence times of thousands of years. Climatic warming has often been suggested to induce substantial losses of old, recalcitrant C. The temporal dynamics of C fluxes from soils though strongly depend on the composition of the different soil C pools. We incubated forest soils from different elevations for 600 days and revealed information about the contribution of three soil C pools to total R_s, and how these respiratory components relate to the size of the respective C pools. We found that more than 90% of total R_s derived from plant root respiration, rhizosphere-derived C and the contribution of soil C fractions that decompose within a growing season. The soil C fractions turning over within this relatively short time though accounted for a minor part (<5%) of total soil C content. Thus, R_s was directly linked to short-lived NPP. The contribution of recalcitrant C to total R_s in contrast, was virtually negligible (1-7%), although this fraction accounted for the major part of total soil C content (>95%). We conclude that R_s, predominantly reflecting C turning over within days to months, cannot increase under warming to any significant extent independently from NPP. However, for long-term changes in soil C stocks, small warming-induced changes of old soil C fractions, can potentially become relevant.

Key words

Long-term incubation, soil organic matter, labile carbon, recalcitrant carbon

Introduction

Soil organic matter is heterogeneous with respect to its physico-chemical structure. As a consequence, the turnover times of soil organic C vary from hours to millenia, depending on the fraction considered (van Veen and Paul 1981). Some photosynthates are exuded into the rhizosphere as easily available, low molecular-weight organic compounds, which are rapidly consumed by soil micro-organisms; part of the C contained in these compounds is released as CO_2 in the course of this process. Other plant-derived soil C inputs turn over more slowly, with mean residence times of weeks to a few months, basically dead plant tissue. Radiocarbon measurements, however, indicate that the largest part of soil organic C is characterized by mean residence times of several hundred to thousands of years (O'Brian and Stout 1978). The structure of these soil C fractions remains partly elusive, but they are generally believed to consist of chemically and physically stabilised plant and microbially-derived higher molecular weight compounds (Figure 1).

The rate of decomposition of soil organic matter is usually limited by temperature, at least when soil moisture is near to optimal. It thus has been hypothesized that global warming would result in substantial losses of "old" soil organic C and, as a consequence, further accelerate warming (Cox et al. 2000; IPPC 2007). However, net ecosystem C stock changes in an altered climate result from differential responses of primary production and associated soil C inputs on one hand, and responses of soil organic matter decomposition on the other hand. Decomposition and net primary productivity (NPP) also are tightly linked, i.e. via soil nutrient cycles (Kirschbaum 1995), and predictions of the balance of the responses of the two processes difficult.

An increasing number of soil-warming field studies are currently underway. A general pattern observed is an initial warming-induced net loss of soil C to the atmosphere, which then decreases in the further course of the experiments (e.g. Melillo 2002; Bradford et al. 2008). This soil respiratory "acclimation" has been attributed to changes in microbial community composition, physiological and ecological adjustments, and to an exhaustion of substrate supply (Luo et al. 2001). However, a main constraint of such experiments is the decoupling of effects on aboveground biomass production and soil organic matter decomposition, since soils are often warmed without a concomitant warming of the canopy. Also, warming treatments usually are implemented as a step-increase in temperature, which can result in substantial transitory imbalance between soil C pools. Comparative studies along natural temperature clines are an alternative route to investigate temperature effects of soil organic C dynamics; however, in contrast, these may underestimate transient responses as they may occur as consequence of the ongoing climatic warming.

We have established study plots in forest stands spanning an elevational cline corresponding to a difference in mean annual temperature of 6K. The forest stands were composed of plots with or without tree species symbiotically associated with N_2-fixing micro-organisms. Here we have studied the composition of soil organic C pools of these forest sites, and analyzed how these pools contribute to R_s. Using our data as an example, we discuss in more general terms which conclusions might (or might not) be drawn from our and similar data sets collected under experimental warming (i.e. from a near-steady state situation and from a simulated step-change in temperature).

Materials and Methods

Experimental design and field sites

A field experiment was set up to study C dynamics in temperate deciduous forests at low and high elevation. The low-elevation forest stands were located on the Swiss Plateau at about 300 m a.s.l.; high-elevation forest stands were situated in the Swiss Central Alps at 1200 to 1500 m a.s.l., corresponding to a topography-driven temperature gradient of c. 6 K (mean annual temperature). At each elevation, we studied two forest stands (plots) with N_2-fixing trees with two nearby plots of non N_2-fixing trees as control plots. The N_2-fixing plots were homogeneous stands of *Alnus glutinosa* (L.) Gaertn. and *Alnus incana* (L.) Moench, each at low and high elevation. The control plots were dominated by *Acer platanoides* or L.. A more detailed description of the study sites, as well as year-round temperature and soil moisture conditions are presented in *chapter 3* of this thesis.

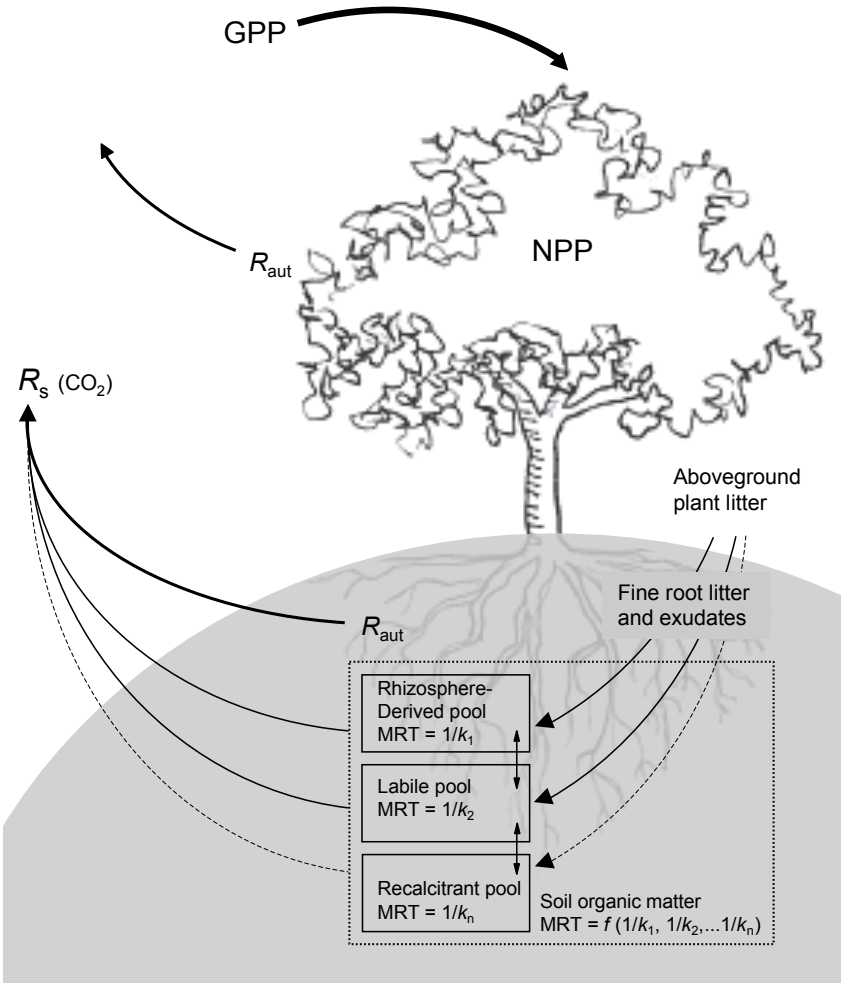

Fig. 1 The global carbon cycle and the turnover of soil organic C pools, differing in turnover time (k). The mean residence time (MRT) of total soil organic matter is a function of the turnover rates of the different pools (Six & Jastrow 2002).

Soil organic C pools

We excavated two soil profiles per plot at the core sites and sampled soils at 5, 10, 20 and 30 cm depth using a 35 mm diameter × 50 mm length corer (at the high-elevational N_2-fixing sites a profile depth deeper than 30 cm could not be accessed because of stony ground). All soil samples were sieved (2 mm), oven-dried at 105 °C for 24 h, and fine earth density determined. Soil pH of subsamples was measured in 0.1 M KCl solution. Carbonates were removed from subsamples from each soil core by exposure to HCl vapour (Harris et al. 2001) and C and N contents determined by dry combustion (ThermoFinnigan FlashEA 1112, Milan, Italy).

Soil incubation

We used long-term laboratory soil incubations to determine the decomposition dynamics of soil samples from all core sites. The aim of these incubations was not to determine *in situ* decomposition rates, but to estimate the size and decomposability of soil organic matter fractions under standardized conditions. Samples from the 0-10 cm top soil layer were sieved (2 mm mesh size), adjusted to 50% water holding capacity, and 100g dry soil equivalent incubated for 600 days at 25°C in gas-tight jars (6 replicates per plot). Soil CO_2 evolution rates were determined 16 times throughout the incubation by repeatedly sampling the jar's headspace with a gas-tight syringe. Gas samples were injected into pre-evacuated exetainers and analyzed for CO_2 by gas chromatography (Agilent 7890 GC equipped with a flame ionization detector, Agilent Technologies Inc., Santa Clara, CA, USA). Soil respiration rates were calculated by linear regression of CO_2 concentration of three subsamples against time; r^2 was generally > 0.97. The incubation jars were ventilated after every sampling to ensure sufficient oxygen supply. At the same time, the samples' soil moisture content was re-adjusted with distilled water if required.

Table 1 Soil properties along the soil profiles to a depth of 30 cm at the core sites at low and high elevation (mean ± SE; n = 2).

Soil depth	Low elevation		High elevation	
	Control	N_2-fixing	Control	N_2-fixing
0-5 cm				
Bulk density (g cm^{-3})	0.96 ± 0.09	0.46 ± 0.05	0.71 ± 0.16	0.85 ± 0.12
C content (kg C m^{-2})	2.77 ± 0.13	2.61 ± 0.33	1.18 ± 0.38	5.20 ± 2.03
N content (kg N m^{-2})	0.18 ± 0.05	0.23 ± 0.05	0.12 ± 0.01	0.45 ± 0.12
$pH_{(KCL)}$	6.26 ± 0.38	5.96 ± 0.72	5.25 ± 0.01	5.51 ± 0.65
5-10 cm				
Bulk density (g cm^{-3})	1.05 ± 0.06	0.59 ± 0.09	1.15 ± 0.01	1.02 ± 0.22
C content (kg C m^{-2})	3.50 ± 0.05	5.57 ± 2.26	1.58 ± 0.87	5.96 ± 1.08
N content (kg N m^{-2})	0.37 ± 0.03	0.47 ± 0.14	0.16 ± 0.05	0.38 ± 0.15
C_{mic} (mg (g dry soil)$^{-1}$)	20.38 ± 1.86	67.84 ± 16.79	5.21 ± 0.65	38.17 ± 17.19
N_{mic} (mg (g dry soil)$^{-1}$)	5.05 ± 0.49	14.76 ± 4.05	1.35 ± 0.02	8.10 ± 3.21
10-20 cm				
Bulk density (g cm^{-3})	1.10 ± 0.01	0.76 ± 0.20	1.27 ± 0.01	1.22 ± 0.22
C content (kg C m^{-2})	3.10 ± 0.08	4.37 ± 0.69	0.35 ± 0.09	5.87 ± 0.14
N content (kg N m^{-2})	0.30 ± 0.06	0.44 ± 0.09	0.03 ± 0.01	0.28 ± 0.22
20-30 cm				
Bulk density (g cm^{-3})	1.26 ± 0.02	0.84 ± 0.26	1.22 ± 0.09	1.07 ± 0.06
C content (kg C m^{-2})	2.44 ± 0.26	4.62 ± 2.69	0.35 ± 0.14	4.09 ± 0.75
N content (kg N m^{-2})	0.27 ± 0.04	0.39 ± 0.19	0.03 ± 0.01	0.35 ± 0.13

Soil CO_2 evolution was analysed by fitting a two-pool 1st order decomposition kinetic to the measured data:

$$R_t = r_{0,labile} \cdot e^{(-klabile\ t)} + r_{0,recalcitrant} \cdot e^{(-krecalcitrant\ t)},$$

where R_t is the soil respiration rate at time t, $r_{0,labile}$ and $r_{0,recalcitrant}$ is the respiration at t = 0 originating from the decomposition of the labile and more recalcitrant soil organic matter fraction distinguished by the double-exponential model, and k_{labile} and $k_{recalcitrant}$ are the respective first order decomposition rate constants. To discriminate against outliers, the double-exponential model was fitted using a regression procedure adopting Tukey's redescending bisquare M-estimator. The size of the labile soil pool was estimated as

$$\int_{t=0}^{\infty} R_{labile\,(t)}\, dt = \frac{r_{0,labile}}{k_{labile}}\ .$$

The rhizosphere-derived pool was calculated as 50% of mean daily R_s (measured in the field), multiplied by seven days (a rough estimate, assuming that this pool is decomposed within maximal 7 days). The recalcitrant soil C pool was estimated as the difference of total soil C content and the rhizosphere-derived plus the labile pool. The contribution of these pools to total R_s is calculated as follows: The respiration derived from the labile and the recalcitrant C pools is estimated by the initial respiration in the incubation, corrected for the mean annual temperature at the study sites (assuming a Q_{10} of 2). The rhizosphere-derived respiration is then calculated as the difference of the heterotrophic R_s (50% of R_s) and respiration derived from the labile plus the recalcitrant pools.

At the end of the incubation (when the "labile" pool was virtually completely decomposed), the temperature sensitivity of decomposition (Q_{10}) of the remaining soil fractions was determined by measuring CO_2 evolution rates at 20°C, 25°C and 30°C.

Net primary production and soil respiration

For the present study we refer to the NPP data estimated for above- and belowground

Table 2 The labile soil C pool: relative to the the incubated soil (% C_{labile}), its absolute size with regard to the C content in the upper 10 cm of the soils (C_{labile} (g m^{-2})), and its decomposition rate constant (k_{labile}) estimated by means of the two-pool model. Given is the mean ± SE (n = 2), results of linear mixed models are presented on the right.

	Low elevation		High elevation		Effect (P-value)		
	Control	N_2-fixing	Control	N_2-fixing	Elevation	N_2-fix	N_2-fix × Elev
% C_{labile}	0.94 ± 0.12	1.18 ± 0.22	4.94 ± 2.46	0.69 ± 0.24	n.s.	n.s.	n.s.
C_{labile} (g m^{-2})	59.21 ± 10.81	91.24 ± 12.86	105.24 ± 6.03	74.25 ± 20.21	n.s.	n.s.	*
k_{labile}	0.023 ± 0.005	0.017 ± 0.005	0.027 ± 0.002	0.024 ± 0.003	n.s.	*	(*)

(*) $0.05 < P < 0.1$; * $P < 0.05$

components (canopy litter, understory biomass and fine root litter production), as well as to annual R_s data that were compiled for the study presented in *chapter 3* of this thesis. A detailed description of the acquisition of these data is found in the *methods* section of *chapter 3*, and a compilation of NPP components and annual R_s is presented in *Table 4* of *chapter 3*.

Data analysis

Effects of elevation ("low" vs. "high") and N availability (control vs. N_2-fixing plots) were tested using mixed-effects models fitted by restricted maximum likelihood (REML) with 'elevation' and 'N' as fixed effects and 'site' as random effect. Error estimates in text and figures are standard errors of site means and effects were considered significant at P<0.05 whereas, due to the relatively low replication and therefore statistical power, P-values > 0.05 but < 0.1 were considered marginally significant. All statistical analyses were carried out using R (version 2.10; mixed effects models were fit using the lme procedure from the nlme package; Pinheiro et al. 2008; R Development Core Team, 2010; www.r-project.org).

Results

Soil C pools

Soil organic C and N differed remarkably between plots, with higher average organic C (P < 0.05) and N contents (P < 0.1) in N_2-fixing plots (Table 1). However, these differences might originate from variation between plots that is independent of the analyzed factors. Both C and N concentration decreased with soil depth in all plots. C/N was neither affected by elevation nor N_2 fixation.

Soil incubations

The 600-day soil incubation revealed a typical double-exponential kinetic with two clearly distinguishable soil C pools (Figure 2). Respiration rates of all soils declined sharply within the first 100—200 days, indicating the mineralisation of a labile soil pool. Following this period, respiration decreased much more slowly with time. While the size of the labile soil C pool and the associated first order decomposition rate constant could adequately be determined, the decomposition kinetics of the more recalcitrant material was less well defined; fitting a reliable decomposition rate constant would have required a much longer incubation period. The first-order decomposition rate constant of the labile soil fraction averaged ~0.023 d^{-1}, corresponding to a mean residence time of 44 days under the incubation conditions. The decomposition rate constant was 19% lower in N_2-fixing plots than in control plots (P<0.05; Table 2). Total respiration at the beginning of the incubation ($r_{0,labile}$ + $r_{0,recalcitrant}$) varied from 262 ± 40 µmol C (g dry soil)$^{-1}$ in high elevational N_2-fixing plots to 1489 ± 577 µmol C (g dry soil)$^{-1}$ in high elevational control plots. CO_2 originating from the decomposition of the labile C pool ($r_{0,labile}$) accounted for 18% -27% of total initial respiration. Both total initial respiration and $r_{0,labile}$ did not significantly depend on elevation and symbiotic N_2 fixation. The temperature sensitivity of decomposition of the slow soil fractions, determined by measuring CO_2 evolution rates at 20°C, 25°C and 30°C at 600 days, increased by a factor of 3.1-3.9 per 10°C temperature increase in all plots (Q_{10}).

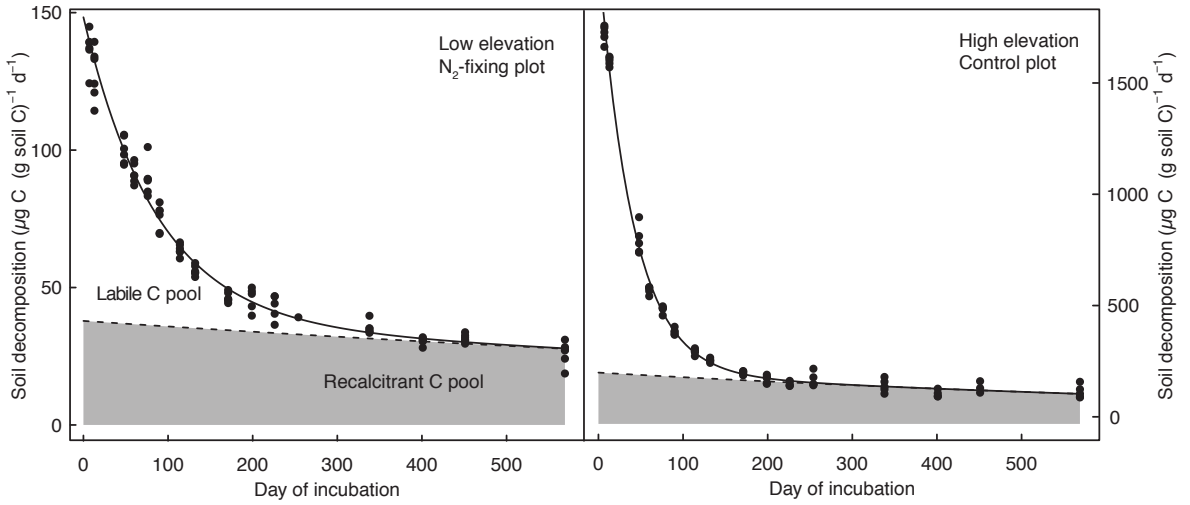

Fig. 2 Two examples of the temporal evolution of decomposition-derived soil CO_2-C fluxes of long-term incubated soils. Symbols denote the measured respiration rates (n = 6 per plot), lines are the modelled rates using a double exponential function. The white area below the solid line represents the 'labile C pool', the grey area below the dashed line represents the 'recalcitrant C pool'. The right panel is the N_2-fixing plot LOW1, the right panel is the control plot HIGH1. Please note the different scales of the y-axes.

Discussion

In the present study we present an analysis of the soil organic C pools of temperate forests and discuss how these pools contribute to total soil C release (R_s).

Root respiration generally accounts for a substantial fraction of soil CO_2 evolution. Distinguishing autotrophic from heterotrophic soil respiration is notoriously difficult, for a number of reasons (cf. Kuzyakov and Larionova 2005). Several methods have been used to answer this question, including the exclusion of plant roots by trenching, the isotopic labeling of CO_2 fixed in plants with subsequent detection in R_s, sometimes including a manipulation of rhizosphere availability of unlabelled C, and large-scale tree girdling. These approaches resulted in an estimated contribution of root respiration to R_s in the range of 10 to 90%, depending on the studied system and method applied (Hanson et al. 2000). We here assume that 50% of measured soil respiration to originate from roots by referring to several studies (e.g. Bond-Lamberty et al. 2004; Högberg et al. 2001) as well as to our findings of the study presented in *chapter 3* of this thesis. Based on this assumption, our incubation data can be used to tentatively assign the remaining heterotrophic respiration to three distinct sources. First, we subtract the temperature-adjusted initial respiration rate measured in the laboratory incubations from the estimated heterotrophic respiration component, i.e. from 50% of R_s measured in the field. The difference thereby obtained can be attributed to a soil organic C fraction that is very rapidly mineralized, i.e. that was already depleted when the laboratory incubation was started. In our study, this rapidly-cycling rhizosphere-derived C fraction contributed 20-30% to R_s, depending on plots. This rhizosphere-derived CO_2 flux did not show any statistically significant signals of elevation or N_2 fixation, but tended to exhibit the same pattern we found for total annual R_s (*chapter 3*, *Table 4*). Using the fitted double-exponential kinetics, and, assuming a similar temperature sensitivity for the labile and recalcitrant fractions separated in the incubation study, the remaining ca. 20-30% of R_s can then be assigned to these two pools. In our study, the vast majority turns out to be derived from the labile soil fraction, turning over within 34-78 days ($1/k$; Table 2), with only 1-7% of R_s originating from the decomposition of recalcitrant soil C pools. In summary, C released from soils within a year (excluding the autotrophic part) accounted for 40-50%, which is in line with results from tracer experiments (50-68%: Taneva et al. 2006; 59%: Gaudinski et al. 2000; 50-60% Trumbore 2000).

These estimates are supported by the close linear relation we found between the sum of all short-lived NPP components and annual R_s in the study presented in *chapter 3*, which hold irrespective of the presence of N_2-fixing tree species or elevation. Since R_s predominantly reflected plant respiration and the decomposition of soil organic matter fractions with turnover-times in the range up to weeks or a few months, this necessarily has to be the case, even if soil C pools would not be in equilibrium.

How are these respiratory components related to the size of the respective soil C pools? Assuming a turnover time of a maximum of a few days for the rapidly-cycling rhizosphere-derived C pool and assigning the entire heterotrophic respiration to this pool, we obtain a safe upper bound of <1% (which certainly is a gross overestimate), and was larger at low compared to high elevation ($P < 0.05$). The size of the labile soil pool was directly estimated in our incubation experiments and amounts to 1-5% of total soil C. Similar proportions for active soil C pools were found in other incubation experiments (0.7-4.3%: Townsend et al. 1997; 0.2-3.5%: Torn et al. 2005). The labile soil C pool (Table 2), similar to its contribution to total R_s, showed the same interactive effect of elevation and N_2-fixation as sum of the short-lived NPP components (*chapter 3*, *Table 4*). Finally, the recalcitrant soil C pool can be estimated as the difference between the total soil C and the rhizsphere-derived plus the labile C pool and accounts for >95% of total soil C. N_2 fixation slightly increased this recalcitrant soil C pool ($P < 0.1$), possibly due to the high N availability, which can potentially accumulate soil organic matter (e.g. Aber et al. 1998).
The conclusion emerging from this reasoning is that R_s is dominated by autotrophic respiration, and (to a similar extent each) by very quickly cycling rhizosphere C pools and the contribution from pools that turn over within a growing season; the contribution of older soil organic matter pools to R_s is virtually negligible. Therefore, under warming, R_s cannot increase to any significant extent independently from NPP. Changes in R_s are thus unsuitable to indicate changes in soil organic C stocks.

Thus, the temporal dynamic of C fluxes from soils to the atmosphere after warming, such as in soil warming experiments, strongly depends on the composition of the different soil C pools. Soil warming experiments of a few years, without concurrent warming of the canopy, inevitably overestimate potential increases of R_s with temperature, because they mainly measure the transitory responses of labile soil C pools. Labile soil C pools though lag only a couple of months to years behind NPP, and after this pool is 'depleted', a new equilibrium of NPP and R_s will be established. Respiratory fluxes from recalcitrant C pools, in contrast, rather lag hundreds of years behind NPP, thus they are the greatest unknown in terms of long-term soil C losses to the atmosphere.

Since the soil fractions of old, recalcitrant C constitute the largest part of total soil C, potentially effects of warming on these pools could become relevant for long-term changes in soil C. Several possibilities exist to detect effects on recalcitrant C dynamics in warming studies. First, if older soil C fractions possess sufficiently distinct isotopic signatures, then their contribution to R_s might be quantified. Tentative candidates for such a label are the radiocarbon bomb spike from thermonuclear bomb testing (e.g. Trumbore et al. 1993; Gaudinski et al. 2000), and isotopic changes in plant-derived soil C inputs after changes in cultivation from crops with C_3 to C_4 photosynthetic pathway or vice versa (e.g. Balesdent et al. 1988). Another possibility might be to estimate turnover rates of old soil C fractions by selectively quantifying decomposition product characteristic of these pools, e.g. phenolics originating from lignin degradation (Otto et al. 2005; Hedges and Mann 1979). However, increased decomposition rates of old soil organic matter fractions will only result in a decrease in the corresponding C stocks if there is no concomitant increase in soil C inputs from other soil C pools.

In brief, we found the smallest soil C pools, which are tightly linked to NPP and turn over within days to months, to clearly dominate R_s, whereas the contribution of the majority of soil C to R_s is virtually negligible.

References

Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I (1998) Nitrogen saturation in temperate forest ecosystems. Hypothesis revised. Bioscience 48:921-934

Balesdent J, Wagner GH, Mariotti A (1988) Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. Soil Sci Soc Am J 52:118-124

Bond-Lamberty B, Wang C, Gower ST (2004) A global relationship between the heterotrophic and autotrophic components of soil respiration? Global Change Biology 10:1756-1766

Bradford MA, Davies CA, Frey SD et al. (2008) Thermal adaption of soil microbial respiration to elevated temperature. Ecology letters 11:1316-1327

Cox PM, Betts RA, Jones CD, Spall SA, Totterdell IJ (2000) Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature 408:184-187

Gaudinski JB, Trumbore SE, Davidson EA et al. (2000) Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence time, sequestration rates and partitioning fluxes. Biogeochemistry 51:33-69

Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observation. Biogeochemistry 48:115-146

Harris D, Horwath WR, Van Kessel C (2001) Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Science Society 65:1853-1856

Hedges JI, Mann DC (1979) The characterization of plant tissues by their lignin oxidation products. Geochimica et Cosmochimica Acta 43:1803-1807

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Hogberg MN, Nyberg G, Ottoson Lofvenius M, Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789-792

IPCC (2007) Climate Change 2007: Synthesis Report. Cambridge University Press, UK

Kirschbaum MUF (1995) The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biology and Biochemistry 27:753-760

Kuzyakov Y, Larionova AA (2005) Root and rhizomicrobial respiration: A review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. Journal of Plant Nutrition and Soil Science 168:503-520

Luo Y, Wan S, Hui D, Wallace LL (2001) Acclimatization of soil respiration towarming in a tall grass prarie. Nature 413:622-625

Melillo JM, Steudler PA, Aber JD et al. (2002) Soil Warming and Carbon-Cycle Feedbacks to the Climate System. Science 298:2173-2176

O'Brian BJ, Stout JD (1978) Movement and turnover of soil organic matter as indicated by carbon isotope measurements. Soil Biology and Biogeochemistry 10:309-317

Otto A, Shunthirasingham C, Simpson MJ (2005) A comparison of plant and microbial biomarkers in grassland soils from the Prairie Ecozone of Canada. Organic Geochemistry 36:425-448

Pinheiro J, Bates D, DebRoy S, Sarkar D, The R Core Team (2009) NLME: Linear and nonlinear mixed effects models. R package version 3.1-96. Available at: http://cran.rproject.org/web/packages/nlme/index.html

R Development Core Team (2009) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Taneva L, Pippen JS, Schlesinger WH, Gonzalez-Meler MA (2006) The turnover of carbon pools contributing to soil CO2 and soil respiration in a temperate forest exposed to elevated CO2 concentration. Global Change Biology 12:983-994

Torn MS, Vitousek PM, Trumbore SE (2005) The influence of nutrient availability on soil organic matter turnover estimated by incubations and radiocarbon modelling. Ecosystems 8:352-372

Townsend AR, Vitousek PM, Desmarais DJ, Tharpe A (1997) Soil carbon pool structure and temperature sensitivity inferred using CO_2 and $^{13}CO_2$ incubation

fluxes from five Hawaiian soils. Biogeochemistry 38:1-17

Trumbore SE (1993) Comparison of carbon dynamics in tropical and temperate soils using radiocarbon measurements. Global Biogeochemical Cycles 7:275-290

Trumbore S (2000) Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. Ecological Applications 10:399-411

Van Veen JA, Paul EA (1981) Organic carbon dynamics in grassland soils. Background information and computer simulation.

CHAPTER 5

N_2 FIXATION BY *ALNUS* TREE SPECIES ENHANCES FOREST SOIL N_2O EMISSIONS

N_2 fixation by *Alnus* tree species enhances forest soil N_2O emissions

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Abstract

While nitrogen (N) cycling in most ecosystems is relatively closed, high external N inputs, such as fertilization, atmospheric N deposition or symbiotic N_2 fixation can turn the closed N cycle into a "leaky" cycle. Enhanced N losses via leaching or gaseous emissions may result. High N input and thus availability in soils further has the potential to affect soil CH_4 exchange in either direction, depending on the system. We compared fluxes of the highly potent greenhouse gases N_2O and CH_4 in N_2-fixing *Alnus* stands and adjacent control forest stands at two elevations, and found that N_2 fixation enhanced soil N_2O emissions more than threefold at lower elevations and two and a half times at the higher elevations in the Alps. Concurrently, productivity estimates of these forest sites indicated that the *Alnus* stands reached N saturation at both elevations. Soil CH_4 exchange did not show a coherent effect of N_2 fixation. We conclude that N losses from soils to the atmosphere in form of N_2O can substantially increase, once the biological demand for N reached saturation.

Key words

Greenhouse gases, N cycle, N deposition, CH_4

Introduction

Nitrogen (N) cycling in most natural ecosystems is relatively closed, with rather small N losses via soil gaseous emissions, volatilization and N-leaching. Near equilibrium, N losses are balanced by biological N_2 fixation or atmospheric N deposition. In forests subject to high external N inputs, such as through fertilization, atmospheric N deposition or symbiotic N_2 fixation, the ideally closed N cycle can turn into a "leaky" cycle (Aber et al. 1998). As a consequence, losses of N via leaching (e.g. Schulze et al. 1989) or gaseous emissions (Vitousek et al. 1997; Hall and Matson 1999) will be enhanced.

While the productivity of most temperate forests is limited by the supply of biologically available N (Vitousek et al. 2002), high rates of N supply through tree species symbiotically fixing atmospheric N_2 can saturate the biological demand for N (Johnson and Lindberg 1992). Trees of the genus *Alnus* (i.e. alder) live in symbiosis with N_2-fixing actinomycetes, which can add 100-150 kg N ha^{-1} a^{-1} to a forest ecosystem (Binkley et al. 1994; Schlesinger 1991). In comparison, recently quantified N deposition across 17 sites all over Switzerland (averaged over 7 to 10 years) reached up to 31 kg N ha^{-1} a^{-1}, with intermediate rates at the Swiss Plateau (10-20 kg N ha^{-1} a^{-1}) and lower rates in the Alps (< 5 kg N ha^{-1} a^{-1}; Thimonier et al. 2010). In response to excess N availability, nitrification and denitrification are often accelerated and result in increased N_2O emissions to the atmosphere. This effect will mainly depend on whether or not the fixed N is taken up by plants.

Although N_2-fixing woody plant species are present in many temperate forests, their provision of substantial N inputs might be limited to early successional habitats (Rastetter et al. 2001). Alder species have been reported to invade (or be afforested on) relatively large areas of former arable land and meadows across the temperate and boreal zone (e.g. Anthelem et al. 2001; Frelechoux et al. 2007; Uri et al. 2009). In the Swiss Alps for example, the N_2-fixing shrub species *Alnus viridis* (Chaix) DC. (green alder), has significantly increased within the past two decades (Frelechoux et al. 2007), expanding by more than 1000 ha per year (Brändli 2010).

Several studies found higher concentrations of most soil N forms and increased denitrification rates under *Alnus* trees relative to coniferous forests (Van Miegroet et al. 1990; Binkley et al. 1992; Griffiths et al 1998). More recent studies on a grey alder plantation (Uri et al. 2011) and a former red alder forest stand (Perakis et al. 2011), however, reported increased soil N capital with N_2-fixation, but did not assign soil N_2O emission a significant role.

In alpine ecosystems, methane (CH_4) oxidation (uptake) in soils is usually high, and soil N inputs through biological fixation or atmospheric deposition in contrast rather low compared to lowlands (Schlesinger 1997). CH_4 consumption by oxidising microorganisms and soil N availability are mechanistically linked, either positively or negatively, depending on the system (Bodelier and Laanbroek 2004). Hence, the high N availability through biological N_2 fixation could be expected to affect CH_4 oxidation. However, this interaction is complex and several other factors, soil water content in particular, play along.

Here, we report fluxes of the highly potent greenhouse gases N_2O and CH_4 in forest sites composed of either symbiotically N_2-fixing *Alnus* tree species, or non N_2-fixing broadleaf trees, at two elevations (at the Swiss Plateau and the Swiss Central Alps). *In situ* gas fluxes were recorded at four site pairs of adjacent N_2-fixing and non N_2-fixing forest stands, whereas further indicators for N-cycling (standardized enzyme activity assays) were performed with a higher number of replicates (forest sites). We hypothesized that N_2-fixation in temperate forests stimulates soils N transformation processes resulting in enhanced N_2O emissions, and that this effect is more pronounced at the warmer lowland forest sites, since both nitrification and denitrification are restricted at low temperatures (Saad and Conrad 1993). Also, warmer temperatures and longer growing seasons enhance NPP, which in turn can improve the soil environmental conditions for dentirification, by providing organic carbon to denitrifiers, and, subsequently, enhancing soil respiration and thus removing soil O_2 (denitrifiers are facultative anaerob microorganisms).

Methods

Field site and experimental design

Temperate deciduous forests sites differing in natural N availability were set up to study forest C and N fluxes. Seven 'low-elevational' sites

were located at the Swiss Plateau at elevations between 300 and 500 m a.s.l., and two 'high-elevational' sites were located in the Swiss Central Alps, at 1200 and 1500 m a.s.l.. Each site was composed of a forest stand (plot) with N_2-fixing trees and a nearby stand without N_2-fixing trees (site pairs). The control forest stands were dominated by *Acer platanoides* L. or *Fagus sylvatica* L. at low elevation and by *Salix fragilis* L. at high elevation, whereas the N_2-fixing forests were homogeneous stands composed of *Alnus glutinosa* L. at low and *Alnus incana* (L.) Moench. at high elevation. All forest sites had a size of 50 x 50 m, a closed canopy, and were surrounded by a buffer zone of at least 10 m. Two of the low elevational sites and the two high elevational sites represented the 'core sites' of this study. A more detailed description of all study sites is presented in *chapter 3*.

Mean annual air temperature (2 m height; HOBO TidbiT v2, Onset Computer Corp., Bourne, MA, USA) during the sampling period was 10.3 ± 0.1 and °C 4.3 ± 0.4 °C at low and high elevation respectively, corresponding to a topography driven temperature cline of c. 6 K, with no or minor differences between N_2-fixing and control plots. Top-soil water content (VWC at 0-10 cm depth; EM50 data loggers connected to 10HS soil moisture probes, Decagon Devices, Pullman; USA) showed moderate changes and stayed relatively high at all plots throughout the sampling period (Figure 1).

CH_4 and N_2O fluxes

We measured net CH_4 and N_2O exchange *in situ* at the core sites at nine measurement dates from March 2009 to October 2009. Six static chambers per plot (20 cm in height, 32 cm in diameter) were installed at random locations, two to three weeks prior to the first measurement. After carefully pre-trenching the soil, the chamber collars were lowered 10 cm into the ground. The headspace volume of each chamber was calculated by measuring the height of the collar (~10 cm height, corresponding to ~8.8 L). During flux measurements, the chambers were closed with detachable lids and headspace samples taken after 5, 20 and 35minutes. These samples were injected into pre-evacuated exetainers and analyzed in the laboratory for N_2O and CH_4, using a gas chromatograph (Agilent 6890 equipped with a flame ionization and an election-capture detector, Agilent Technologies Inc., Santa Clara, CA, USA). Flux rates were calculated by linear regression of CH_4 and N_2O concentrations against time; r^2 was generally > 0.98 when fluxes were different from zero. Soil temperature and soil moisture (top 10 cm) were recorded manually at the chamber location, concomitantly with the gas flux measurements (soil temperature: digital thermometer GTH 175/Pt, Greisinger electronic, Germany; soil moisture: ThetaProbe soil moisture sensor - ML2x, Delta-T Devices Ltd., Cambridge, UK).

Soil sampling for laboratory analyses

Soil samples for laboratory analyses (potential nitrification and denitrification enzyme activity) were collected from 10 randomly distributed locations within each forest plot with a steel cylinder (∅ 2.5 cm, depth 10 cm). The samples were sieved (2 mm mesh size) and kept at 4 °C until analysis (2-7 days). Soil water content was determined by drying 10 g sieved soil (105 °C, 24 hours).

Potential nitrification

Potential nitrification was estimated as NO_3^- production in a short-time laboratory incubation with excess NH_4^+ available (Schmidt and Belser 1994). In brief, fresh soil, equivalent to 10 g of dry soil, was mixed with 100 mL of buffer solution (0.5 mM KH_2PO_4/K_2HPO_4, adjusted to a pH of 7.0 by adding K_2CO_3) and incubated at 25°C on a slowly-moving table shaker. 1 mL of 0.05 M $(NH_4)_2SO_4$) solution was added to the suspension, and aliquots taken after 1, 4, 7 and 23 hours. These aliquots were then centrifuged, filtered, and analyzed for NO_3^- concentrations using a flow-injection analyser (Skalar SAN+. Skalar, Breda, The Netherlands). Potential nitrification rates were calculated by linear regression of NO_3^- concentrations against time. Potential nitrification was measured in March for the core sites and in June and October for all site pairs.

Denitrification enzyme activity (DEA)

Denitrifying enzyme activity (Tiedje et al. 1989) was estimated as short-term N_2O production under anaerobic conditions, in the presence of excess NO_3 and available organic C. DEA reflects the denitrification potential at the time of sampling; incubation times are kept short to avoid bias due to de novo synthesis of enzymes (Tiedje et al. 1989). Fresh soil, equivalent to 5 g dry mass, was placed in a 150 ml serum bottle. The headspace of each flask was evacuated and replaced by a 90:10 He-C_2H_2 mixture to provide

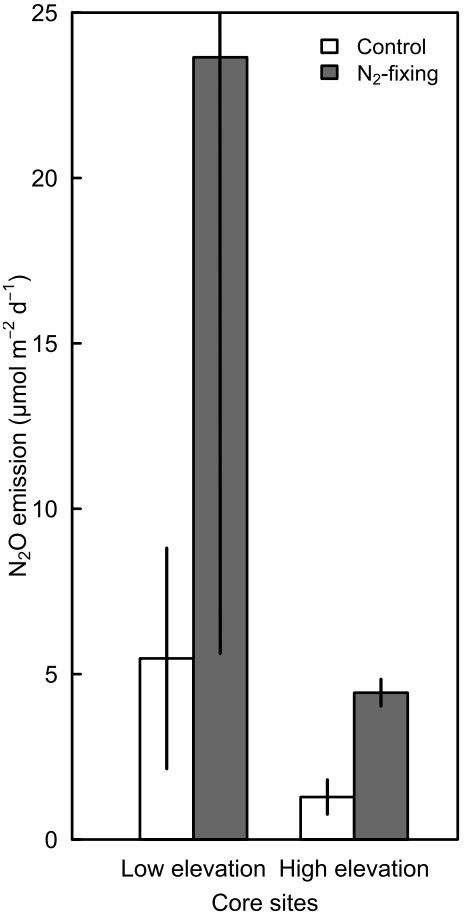

Fig. 1 N_2O emissions at the core sites in dependence of elevation and N availability (N_2-fixing vs. control sites; mean ± SE, n = 2). Shown are averages of the nine measurement dates from March 2009 to October 2009.

anaerobic conditions and inhibit N_2O-reductase activity (i.e. the conversion of N_2O to N_2). Demineralised water and a solution containing 1 mg glucose C g^{-1} dry soil, 1 mg glutamic acid C g^{-1} dry soil and 0.1 mg NO_3^--N g^{-1} dry soil were added to each sample to provide excess NO_3^- and organic C, and to raise moisture levels above water holding capacity. The soil samples were then incubated at 26 °C and headspace samples taken after 60, 90 and 120 minutes. N_2O concentrations were analysed by gas chromatography as described above. DEA was calculated as N_2O production per unit time. DEA was measured in July and September for the core sites and in June and October for all site pairs.

Forest NPP

Stem basal area was estimated by measuring stem circumference at breast height (1.3 m above ground), within 3 areas of 10 m × 10 m in each plot. At low elevation this resulted in c. 40 trees per plot at low-elevational sites and c. 70 trees per plot at high-elevational sites. Canopy litter fall was collected in litter traps (0.25 m^2 ground area plastic sieves); Six randomly placed traps per plot were emptied at least every two weeks during two subsequent autumns. Leaf litter C and N content (before and after leaf litter fall) was quantified (Elementar Vario EL III, Hanau, Germany). Peak biomass of understory vegetation was harvested at ground level (2 × 1 m^2 per core site). Fine root production was assessed using ingrowth cores: 40 mesh cyclinders (3.5cm diameter × 12 cm depth), filled with root-free soil were placed at random location in each plot. After one year, ingrowth cores were harvested and washed carefully. Biomass of all NPP components harvested was dried at 80°C. A more detailed description of forest NPP measurements at the present study sites is presented in *chapter 1* of this thesis.

Statistical analyses

Effects of elevation ("low" vs. "high") and N availability (control vs. N_2-fixing sites) were tested using mixed-effects models fitted by restricted maximum likelihood (REML) with 'elevation' and 'N' as fixed effects and 'site' as random effect. N_2O emissions, CH_4 exchange, potential nitrification and DEA were log-transformed for statistical analyses. Error estimates in text and figures are standard errors of site means and effects were considered significant at $P < 0.05$ whereas, due to the relatively low replication and therefore statistical power, P-values > 0.05 but < 0.1 were considered marginally significant. All statistical analyses were carried out using R (version 2.10; mixed effects models were fit using the lme procedure from the nlme package; Pinheiro et al. 2008; R Development Core Team, 2010; www.r-project.org).

Results

Soil N_2O emission

Measured N_2O emissions ranged from close to zero to 234 µmol m^{-2} d^{-1}, with the maximum measured in N_2-fixing plots at low elevation. However, the temporal resolution of our measurements was too low to capture the very dynamic changes typically occurring when N_2O fluxes are driven by denitrificaton; we therefore

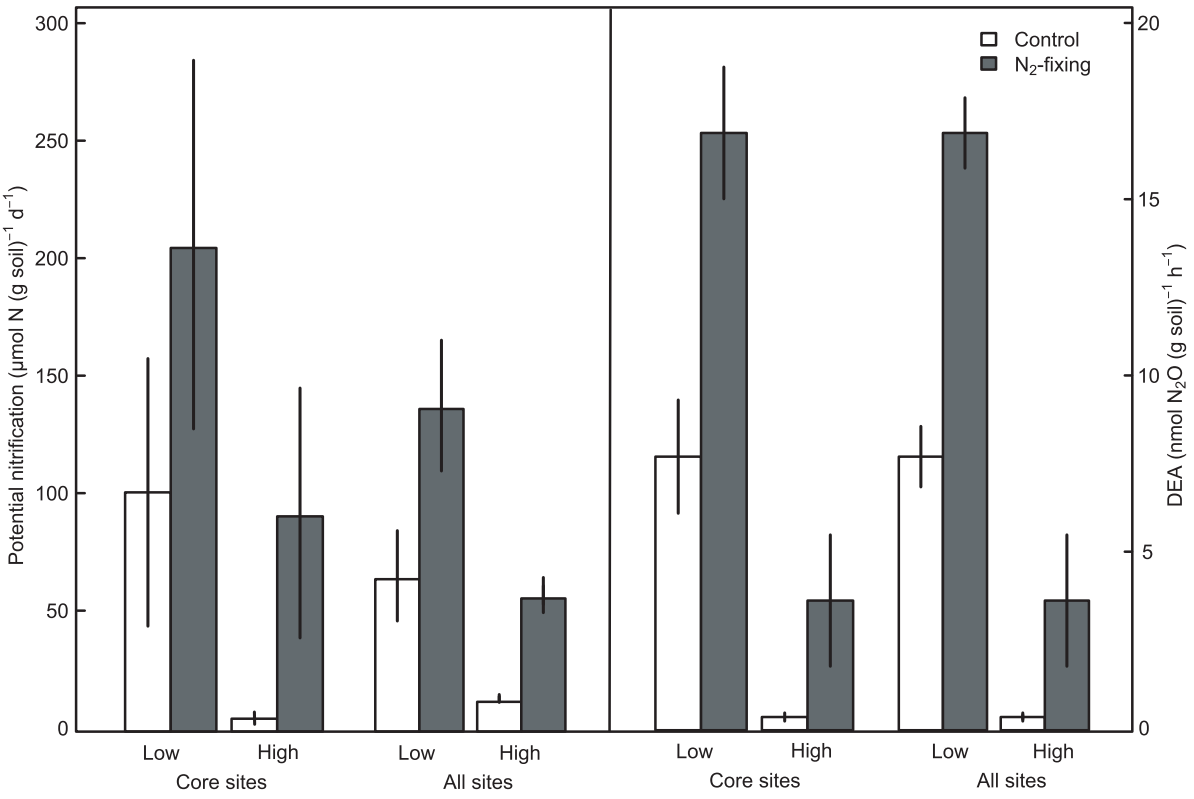

Fig. 2 Potential nitrification and denitrifying enzyme activity (DEA) for the core sites (mean ± SE, n = 2), and for all site pairs (low elevation: n = 7; high elevation: n = 2). Shown are averages across measurements.

restrict our analysis to plot-level averages of log-transformed fluxes. Our data indicate higher soil N_2O emissions from N_2-fixing plots ($P < 0.05$; Figure 1) but no significant effect of elevation.

Potential nitrification and denitrification enzyme activity

Potential nitrification was significantly higher in N_2-fixing than in control forest plots ($P < 0.05$ for core sites; $P < 0.01$ for all sites), and elevation reduced potential nitrification rates ($P < 0.1$ for core sites; $P < 0.01$ for all sites; Figure 2).

Denitrifying enzyme activity revealed the same effects than potential nitrification, with higher rates in N_2-fixing compared to control plots ($P < 0.05$ for core sites; $P < 0.01$ for all sites) and elevation generally reducing DEA, although this effect was statistically significant for the core sites only ($P < 0.05$; Figure 2).

Soil CH_4 exchange

Symbiotic N_2 fixation did neither affect CH_4 oxidation, nor CH_4 emission at any plot of the cores sites. Net soil CH_4 oxidation was observed on some dates at all low elevation sites; in contrast, high elevation plots were net methane sources on all, except for two dates in high elevational control plots (elevation: $P > 0.1$). Averaged over all sampling dates, low-elevational sites were net CH_4 sinks, since fluxes were dominated by CH_4 oxidation, whereas at high elevation all soils were net CH_4 sources.

Forest NPP

Basal area increment did neither differ between N_2-fixing and control plots, nor across elevation. Annual leaf litter was slightly higher in N_2-fixing compared to control plots (+35%, $P < 0.1$) and also enhanced at low compared to high elevation (+49%, $P < 0.05$). Understory biomass production was neither enhanced under N_2-fixing trees nor differed between elevations. Fine root ingrowth, our proxy for belowground litter production, showed reversed trends at the two elevations: at low elevation, fine root ingrowth was enhanced at N_2-fixing compared to control plots, whereas it was the other way round at

Table 1 Leaf litter and soil properties (0-30 cm depth) at the core sites (mean ± SE, $n = 2$).

	Low elevation		High elevation	
	Control	N_2-fixing	Control	N_2-fixing
Leaf litter N (%)	2.7 ± 0.2	3.6 ± 0.2	3.1 ± 0.2	3.7 ± 0.4
Leaf litter C/N	18.5 ± 1.2	14.4 ± 0.5	17.8 ± 1.7	14.3 ± 2.0
Soil C content (kg C m^{-2})	11.8 ± 0.2	17.2 ± 6.0	3.5 ± 1.5	21.2 ± 1.9
Soil N content (kg N m^{-2})	1.1 ± 0.1	1.5 ± 0.1	0.4 ± 0.1	1.7 ± 0.2
Soil C/N	10.7 ± 1.3	11.1 ± 0.4	9.8 ± 2.7	13.1 ± 2.3

high elevation, resulting in no overall N_2-fixation or elevational-effect. NPP data are compiled in *chapter 3, Table 4* of this thesis.

Litter quality

N_2 fixation increased leaf litter N content ($P < 0.01$; +143%) and, as a result, significantly decreased the C/N ($P < 0.05$; Table 1). Elevation did not affect leaf litter C and N concentrations. N resorption, calculated as difference in autumn leaf N concentration before and after litterfall was lower for N_2-fixing trees (*A. glutinosa*: 37%; *A .incana*: 19%) than for non N_2-fixing trees (*A. platanoides*: 63%; *S. fragilis*: 58%).

Discussion

Clearly, N_2O emissions were higher under N_2-fixing trees, both at the lowland sites and in the Alps (Figure 1). Denitrifying enzyme activity and potential nitrification rates validated these findings with a higher sample (Figure 2).

Productivity data indicate that *Alnus* plots already reached N saturation at both elevations. Whether wood increment, nor cumulative above- and belowground plant litter production was significantly higher with N_2 fixation (only leaf litter production was slightly enhanced in N_2-fixing plots). Obviously, the high N inputs with N_2 fixation turned N cycling in an open cycle, with higher losses of N_2O to the atmosphere.

At the lower elevation we generally recorded higher N_2O emissions than in the 6 K colder sites in the Alps. The year-round higher temperatures and the longer growing season are likely to (1) favour N_2 fixation (Roughley and Dart 1970), and thus enhancing substrate for further N transformation processes releasing N_2O (Vitousek et al. 2002), and to (2) enhance the supply of organic C as energy source for denitrification. We also found the relative N_2 fixation effect to be more pronounced at higher temperatures; N_2O emissions under N_2-fixing trees exceeded N_2O emissions from control forest stands by 332% at low elevation, and by 246% at high elevation.

Approximations of the N_2O fluxes in the *Alnus* stands for a whole here yielded 1.15 kg N_2O-N ha^{-1} a^{-1} at the lowlands (black alder) and 0.51 kg N_2O-N ha^{-1} a^{-1} in the Alps (grey alder). These values are in line with estimates from a riparian black alder forest in Germany (0.4-7.8 kg N_2O-N ha^{-1} a^{-1}, Mander et al. 2008) and a grey alder stand in Estonia (0.50 ± 0.45 kg N_2O-N ha^{-1} a-1, Uri et al. 2011). N_2O emission rates we measured in N_2-fixing plots were also within the (wide) range reported for several fertilizer experiments in temperate deciduous forests and for temperate deciduous forests receiving high N deposition (compiled by Eickenscheidt et al. 2011).

According to the net flux of CH_4 over all sampling dates, soils at the high elevational sites were CH_4 sources, whereas soils at low elevation were CH_4 sinks. This result contrasts the general assumption, that upland soils are sinks for CH_4 (Le Mer and Roger 2001). We further found no evidence of large inhibition of N on CH_4 oxidation. However, the limited spatial and temporal resolution of these soil CH_4 exchange data does not allow the drawing of general conclusions.

In summary, we found high ecosystem N inputs through symbiotic N_2-fixation to strongly stimulate nitrification and denitrification rates and subsequent soil N_2O emissions. We reason that N losses from forest soils to the atmosphere in form of N_2O can substantially increase, once the biological demand for N reached saturation.

References

Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I (1998) Nitrogen saturation in temperate forest ecosystems. Bioscience 48:921-934

Ambus P, Zechmeister-Boltenstern S (2007) Denitrification and N-cycling in forest ecosystems. In: Bothe H, Ferguson SJ, Newton WE (eds) Biology of the nitrogen cycle. Elsevier B.V., Amsterdam, 343-358

Anthelem F, Grossi JL, Brun JJ, Didier L (2001) Consequences of green alder expansion on vegetation changes and arthropod communities removal in the Northern French Alps. Forest Ecology and Management 145: 57-65

Binkley D (1981) Nitrogen fixation and net primary production in a young Sitka alder stand. Canadian Journal of Forest Research 60:281-284

Binkley D, Sollins P, Bell R, Sachs D, Myrold D (1992) Biogeochemistry of adjacent conifer and alder-conifer stands. Ecology 73:2022-2033

Binkley D, Cromack K Jr, Baker DD (1994) Nitrogen fixation by red alder: biology, rates, and controls. In: Hibbs DE, DeBell DS, Tarrant RF (eds) The biology and management of red alder. Oregon State University Press, Corvallis, pp 57-72

Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. FEMS Microbiology Ecology

Brändli UB (2010) Schweizerisches Landesforstinventar. Ergebniss der dritten Erhebung 2004-2006. Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft WSL, Birmensdorf. Bundesamt für Umwelt BAFU, Bern

Eickenscheidt N, Brumme R, Veldkamp E (2011) Direct contribution of nitrogen deposition to nitrous oxide emissions in a temperate beech and spruce forest – a ^{15}N tracer study. Biogeosciences 8:621-635

Federal office of the Environment (2011) Kenngrössen zur Entwicklung der Treibhausgasemissionen in der Schweiz 1990-2009. http://www.bafu.admin.ch/klima/09570/index.html?lang=de

Frelechoux F, Meisser M, Gillet F (2007) Secondary succession and loss in plant diversity following a grazing decrease in a wooded pasture of the central Swiss Alps. Botanica Helvetica 117:37-56

Griffiths RP, Homann PS, Riley R (1998) Denitrification enzyme activity of douglas-fir red alder forest soils of the Pacific Northwest. Soil Biology and Biogeochemistry 30:1147-1157

Hall SJ, Matson PA (1999) Nitrogen oxide emissions after nitrogen additions in tropical forests. Nature 401:152-155

Johnson DW, Lindberg SE (1992) Atmospheric deposition and forest nutrient cycling. Springer, New York

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: A review. European Journal of Soil Biology 37:25-50

Lõhmus K Kuusemets V, Ivask M, Teiter S, Augustin J, Mander Ü (2002) Budgets of nitrogen fluxes in riparian grey alder forests. Arch. Hydrobiol. 13:321-332

Mander Ü, Lõhmus K, Teiter S, Uri V, Augustin J (2008) Gaseous nitrogen and carbon fluxes in riparian alder stands. Boreal Environment Research 13:123-241

Perakis SS, Sinkhorn ER, Compton JE (2011) $\delta^{15}N$ constrainsts on long-term nitrogen balances in temperate forests. 167:793-807

Pinheiro J, Bates D, DebRoy S, Sarkar D, The R Core Team (2009) NLME: Linear and nonlinear mixed effects models. R package version 3.1-96. Available at: http://cran.rproject.org/web/packages/nlme/index.html

R Development Core Team (2009) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Rastetter EB, Vitousek PM, Field C, Shaver GR, Herbert D, Agren GA (2001) Resource optimization and symbiotic N fixation. Ecosystems 4:369-388

Roughley RJ, Dart PJ (1969) The influence of root temperature, *Rhizobium* strain and host selection on the structure and nitrogen-fixing efficiency of the root nodules of *Trifolium subterraneum*. Annals of Botany 34:631-646

Saad OALO, Conrad R (1993) Temperature dependence of nitrification, denitrification and turnover of nitric oxide in different soils. Biology and fertility of soils. 15:21-27

Schlesinger WH (1991) Biogeochemistry: an analysis of global change. Academic Press Inc., San Diego CA

Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications 7:737-750

Schmidt EL Belser LW (1994) Autotrophic nitrifying bacteria. In: Methods of soil analysis. Weaver S et al. (eds) Soil Science Society America, Madison, Wisconsin, pp 159-177

Schulze ED, Vries W, Hauhs M, Rosén K, Rasmussen L, Tamm CO, Nilsson J (1989) Critical loads for nitrogen deposition on forest ecosystems. Water, air and soil pollution 48:451-456

Thimonier A, Graf Pannatier E, Schmitt M, Waldner P, Walthert L, Schleppi P, Dobbertin M, Kräuchi N (2010) Does exceeding the critical loads for nitrogen alter nitrate leaching, the nutrient status of trees and their crown condition at Swiss Long-term Forest Ecosystem Research (LWF) sites? Eurpean Journal of Forest Research 129:443-461

Tiedje JM, Simkins S, Groffman PM (1989) Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. Plant and Soil 115:261-284

Van Miegroet H, Johnson DW, Cole DW (1990) Soil nitrification as affected by N fertility and changes in forest floor C/N ratio in four forest soils. Canadian Journal of Forest Research 20:1012-1019

Vitousek PM et al. (2002) Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57/58:1-45

Vitousek MP, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications 7:737-750

Uri V, Lõhmus K, Mander Ü, Ostonen I, Aosaar J, Maddison M, Helmisaari HS, Augustin J (2011) Long-term effects on the nitrogen budget of a short-rotation grey alder (*Alnus incana* (L.) Moench) forest on abandoned agricultural land. Ecological Engineering 37:920-93

CHAPTER 6

GENERAL SUMMARY AND CONCLUSIONS

General summary and conclusions

This PhD thesis addressed the carbon (C) balance of temperate deciduous forests across natural gradients of temperature and nitrogen (N) availability, the major drivers of net primary production (NPP) and the soil C balance. A mean annual temperature difference of 6 K across a 1200 m change in elevation from the Swiss Plateau to the Central Swiss Alps, and the presence or absence of the N_2-fixing tree species *Alnus glutinosa* or *Alnus incana* within each elevation, offered the framework (1) to test the hypothesis that cumulative annual soil respiration (R_s) at contrasting temperatures reflects the difference in the production of short-lived biomass in the longer run; (2) to test whether or not high rates of N inputs increase the rate of C-cycling by accelerating both, NPP and R_s; (3) to analyze the composition of soil organic C pools and their contribution to R_s in response to elevation and N-availability; and (4) to test whether high-N-input *Alnus* forests stimulate soil N transformations, resulting in enhanced N_2O emissions.

Chaper 2: Forest soil respiration reflects plant productivity across a temperature gradient in the Alps

In forests, the biomass components of interest for R_s are those undergoing rapid recycling, i.e. litter production by the canopy, understory and fine root system. Despite the 6 K difference in temperature and a difference in the length of the growing season of three months moving from the high to the low elevational sites, total annual litter production did not change. This is surprising, in view of the estimated doubling of annual wood increment from high to low elevation, that largely resulted from the difference in the length of the growing season. Stem growth and total NPP signals almost disappeared when expressed per day available for growth. Although following temperature variability throughout the seasonal course, cumulative annual R_s did not differ across elevations on a full-year basis. Within each elevation, the short-term temperature response of R_s (Q_{10}) was close to 2, which is in accordance to the often assumed more than doubling in respiration for a 10 K warming. However, when calculated across sites, i.e. from high to low elevation, the apparent Q_{10} dropped to ~1.2, implying a down-regulation of R_s at higher temperatures, close to homeostasis. In other words, across the sites adapted to different temperatures, temperature was not exerting a strong net influence on R_s. Adopting a simple C budget that assumes 50% of total R_s is derived from autotrophic root respiration, we arrive at c. 40% of the respiratory soil C release from concurrent litter production, both for high- and low-elevational sites. Whereas the remaining unaccounted 10% are a reasonable estimate for root exudates and mycorrhizal consumption in temperate forests. Cumulative annual soil CO_2 release thus largely reflected the input of labile C to soil, and not temperature per se. Climatic warming of the past decades most probably was slow enough, so that metabolism could track it, causing no significant deviation from a thermal equilibrium at our test sites. These results caution against expectations of strong positive effects of climatic warming on R_s.

Chapter 3: Does nitrogen input enhance respiratory carbon release from temperate forest soils?

In forests with the symbiotically N_2-fixing genus *Alnus* forest, we found that biological N_2-fixation enhanced total litter production (the sum the above- and belowground forest litter components) and facilitated higher R_s at low elevation only. At high elevation, enhanced N input was associated with lower litter production and lower Rs, compared to non N2-fixing stands. Hence, R_s remained in proportion to forest litter production, irrespective of the effect of N-availability or site temperature. Annual litter C production and annual soil C release via R_s correlated well ($r^2 = 0.85$), at a ratio of litter C to annual C release of 2.4:1. Thus, total plant litter C explained ~ 40% of R_s. Assuming a ~ 10% contribution of C input to soils by root exudates and micorrhizal C consumption, the balance of C input in soil through NPP and the C output from

soils via R_s would become closer to 2:1. Hence, autotrophic and heterotrophic soil respiration, contribute similar fractions to total R_s. In conclusion, the results of this comparison lines up with the results of chapter 2, suggesting that R_s exhibits a rather robust relationship to substrate availability, rather than showing direct responses to N availability and temperature. These findings offer no justification for modelling R_s by assuming either N or temperature to exert direct (independent) effects on R_s. In the long run though, the soil C pool could still be affected by small deviations from this relationship, provided element stoichiometry permits.

Chapter 4: Soil organic carbon pools and their contribution to soil respiration

In this study we compared C release from forest C pools by means of a 600-day incubation, that revealed information about the contribution of three soil C pools to total R_s, and how these respiratory components related to the size of the respective C pools. We identified three heterotrophic sources sources of C for R_s: rapidly cycling rhizosphre-derived C with a maximum turnover time of a few days, labile soil C turning over within a few weeks to months, and recalcitrant soil C with a residence time of several years. We found that more than 90% of total R_s is explained by the sum of autotrophic (root) respiration, rhizosphere-derived C and the contribution of soil C fractions that decompose within a growing season. The soil C fractions turning over within this relatively short time, however, accounted for a minor part (<5%) of total soil C. In other words, R_s was directly linked to short-lived NPP. The contribution of recalcitrant C to total R_s in contrast, was virtually negligible (1-7%), although this fraction accounted for the major part of total soil C (>95%). We thus conclude that R_s, in essence emerging from C pools turning over within days to months, cannot increase under warming to any significant extent independently from NPP. However, for long-term changes in soil C, small warming-induced changes of old soil C fractions can potentially become relevant.

Chapter 5: N_2 fixation by Alnus tree species enhances forest soil N_2O emissions

The comparison of fluxes of greenhouse gases from soils of N_2-fixing and adjacent control forest plots clearly revealed in enhanced soil N transformation and subsequent N_2O emissions in high N-input *Alnus* stands. Concurrently, productivity data, recorded for the study presented in chapter 3, indicated that all *Alnus* stands reached N saturation. Obviously, the high N inputs with N_2-fixation turned N-cycling into an open cycle, with higher losses of gaseous N to the atmosphere. While the N_2O emissions were generally higher at lower elevations than in the 6K colder forest stands in the Alps, the effect of N_2 fixation was also more pronounced at lower elevation. Yet, with an increase in N_2O losses of 330% and 250% in *Alnus* stands relative to controls though, the effect was considerable at both elevations. While soil CH_4 exchange did not show a conclusive effect of N_2 fixation, soils at the high elevation were CH_4 sources and soils at low elevation were CH_4 sinks over a complete season. The findings of this study suggest that N losses from soils to the atmosphere in form of N_2O can substantially increase, once the biological demand for N reached saturation.

The main conclusions

The main conclusions that can be drawn from this thesis are: (1) cumulative annual C release from forest soils largely reflects the input of labile C to soil, and not temperature per se; (2) this robust relationship of R_s to substrate availability also holds across forest sites with high N-inputs, such as under N_2-fixing trees; and (3) N_2O losses from forest soils to the atmosphere can substantially increase, once the biological demand for N reached saturation.

Drought at erosion edges selects for a 'hidden' keystone species

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Abstract

Background: The presence of plants is crucial in securing steep slopes against soil erosion. Inappropriate land use in mountains often leads to vegetation loss and thus soil degradation.
Aims: Here we ask, if the edges of large erosion gullies select for specialist plant species that reduce or prevent the progression of soil loss.
Methods: We quantified species presence and abundance across micro-transects from intact mountain pastures toward the edge of erosion gullies at ca. 1900 m elevation in the Central Caucasus, Georgia.
Results: Out of a large species pool, one particular species, *Festuca valesiaca*, was the dominant species at the very edge of erosion gullies. Increased $\delta^{13}C$ values in *Festuca valesiaca* leaves by 1.1 ‰ towards the edge confirmed that this species copes best with the dry conditions at the edge.
Conclusion: Our findings illustrate the insurance effect of a highly diverse vegetation. The importance of a single species out of this diverse species suite to sustain key ecosystem functions becomes apparent only under extreme environmental conditions, in this case at edges of erosion gullies.

Key words

Central Caucasus, pasture, drought resistance, *Festuca valesiaca*, $\delta^{13}C$, gully erosion, insurance effect, slope stability

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PUBLICATIONS

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