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DIPLOMARBEIT

Titel der Diplomarbeit

Partitioning of Ecosystem Respiration in a Beech Forest Ecosystem

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General Introduction

Soil respiration

About 10^8 Gt carbon are stored on the earth, distributed on the following pools (Kvenvolden K A 1993; Rustad L E *et al.* 2000; Sundquist E T 1993): 1) geological formations (90,000,000 Gt), 2) dissolved oceanic carbonates (38,000 Gt), 3) gas (methane) hydrates (10,000 Gt), 4) fossil fuels (4,000 Gt), 5) atmosphere (750 Gt) 6) terrestrial biosphere excluding soils (560 Gt), and 7) soils (1,600 Gt). Soil is the largest pool for carbon in terrestrial ecosystems, containing about 1,550 Gt carbon already in the upper first meter (Eswaran H *et al.* 1993). Especially forests have a large carbon storage capacity, containing about 40 % of the soil carbon stocks (Eswaran H *et al.* 1993). Carbon exchange between terrestrial ecosystems and the atmosphere is driven by two major processes, carbon fixation by photosynthesis (or gross primary production "GPP") and carbon loss (ecosystem respiration R_{eco}).

Ecosystem respiration comprises soil respiration and aboveground respiration. Globally, soil respiration accounts for 68 - 76.5 Gt CO_2-C $year^{-1}$ and amounts for 60 to 90 % of total ecosystem respiration (Goulden M L *et al.* 1996; Longdoz B *et al.* 2000; Raich J W *et al.* 1995; Raich J W *et al.* 1992). Soil respiration differs among ecosystems and biomes, as it is controlled by abiotic (temperature, moisture, physical and chemical soil properties) and biotic factors, such as vegetation (quality and quantity of living or dead plant material, root-soil interactions). Among abiotic factors, mean annual temperature is one of the most important controls for belowground carbon processes (Litton C M *et al.* 2008). Biotic controls such as those of vegetation cover are evident, as for example soil respiration is generally higher in grasslands compared to forests under similar conditions, and lower in coniferous forests compared to broad-leaved forests on same soil types (Raich J W *et al.* 2000).

The tight connection between soil CO_2 efflux, vegetation cover and climate implies that changes in both vegetation cover and climate has the potential to influence the ecosystem CO_2 balance. Increasing anthropogenic CO_2 emissions to the atmosphere since the 19^{th} century (Chapin F S *et al.* 2009; IPCC 2007) raised concerns about potential global warming and further about possible positive feedback effects on soils, due to stimulation of enhanced release of CO_2 from terrestrial soil carbon pools. It therefore becomes more and more important to understand the controls and processes which cause soil respiration, as "even a small change in soil respiration could thus equal or exceed the annual input of CO_2 to the atmosphere via land-use changes and/or fossil fuel combustion, and could significantly exacerbate – or mitigate –

atmospheric increases of CO_2 with consequent feedbacks to climate change" (Rustad L E *et al.* 2000).

Autotrophic and heterotrophic soil respiration

Soil respiration can be paritioned into different sources, i.e. into root respiration, rhizomicrobial respiration, priming by root exudates or addition of plant residues, and respiration by microbes decomposing plant residues and soil organic matter (SOM) (Kuzyakov Y 2006). These different sources can be grouped into autotrophic respiration (root respiration, rhizomicrobial respiration) which is considered as plant derived CO_2 efflux, and heterotrophic respiration (decomposition of plant residues and SOM), which is also known as SOM- or litter-derived respiration (Högberg P *et al.* 2006). The sources for autotrophic and heterotrophic respiration differ in their mean residence times in the system (van Hees P A W *et al.* 2005). Plant assimilates and root exudates (as substrates for autotrophic respiration) exhibit short mean residence times from minutes to days, whereas plant residues and SOM have a slower turnover (Kuzyakov Y 2006; van Hees P A W *et al.* 2005). The contribution of root respiration, or in the broader sense of autotrophic respiration (including microbial respiration fuelled by recent plant assimilates) to total soil respiration can vary between 10 to 90 % of total soil respiration depending on vegetation type or season of the year (Hanson P J *et al.* 2000). High root respiration was detected during growing season, associated with high physiological activity such as root growth, and was lower during other parts of the year (Hanson P J *et al.* 2000).

Heterotrophic respiration originates from microbes or soil fauna during feeding on heterotrophic substrates, but soil fauna is thought to contribute only a few percent to total soil respiration. The bigger part of heterotrophic respiration is evolved by microorganisms such as archaea, bacteria, non-mycorrhizal fungi and actinomycetes. Therefore heterotrophic respiration is often referred as 'microbial respiration (Kuzyakov Y 2006). Heterotrophic respiration is influenced by various factors such as soil temperature, moisture, soil carbon quality, root density, microbial community structure and size, physical and chemical soil properties and vegetation type, nutrient status and growth rate (Raich J W *et al.* 2000) and shows temporal and spatial variations.
Soil microorganisms feed on a variety of different substrates, including complex substrates which can be decomposed only by specialists. Microbes are often classified into two functional groups: fast-growing microbes feeding on labile organic carbon sources (such as exudates) that are dormant when no labile carbon is present (r-strategists), and slow-growing organisms adapted to recalcitrant organic matter, such as SOM (K-strategists) (Fontaine S *et al.* 2003). Fast growing microbial communities are primarily situated in close vicinity of apical regions of fine roots (Paterson E *et al.*

2009) and are in competition with plants, which take up nutrients in this zone (Jones D L *et al.* 2004). As uptake of substrates by plants and soil microbes mainly occur in the soluble phase (Marschner B *et al.* 2003), dissolved organic carbon (DOC) links rhizosphere and soil. DOC is a small and very labile C pool, which derives from decomposition processes and, to a high proportion from root exudates (Giesler R *et al.* 2007). A small part (< 10 %; Jones D L *et al.* 2004) of DOC consists of low molecular weight compounds (LMWC) such as organic acids, amino acids and simple sugars (Jones D L *et al.* 2009; van Hees P A W *et al.* 2005). Especially LMWC can be attributed to root exudation and are considered to drive microbial activity in the mycorrhizosphere (Dakora F D *et al.* 2002). Mycorrhizae represent an important link between roots and soil. They derive assimilates directly from the plant and in return provide nutrients and water to the plant (Smith S E *et al.* 2008). They may even be able to decompose SOM (Talbot J M *et al.* 2008).

SOM stored in deeper soil layers is often considered to be recalcitrant, containing too little energy for decomposition (Fontaine S *et al.* 2007). The decomposition of dead plant material and recalcitrant SOM requires specific enzymes to break down macromolecules such lignin and humus, respectively. Decomposition of these substrates is linked to distinct microbial communities, commonly including a higher proportion of saprotrophic fungi relative to communities in the rhizosphere (Neely C L *et al.* 1991; Paterson E *et al.* 2008). The input of fresh organic carbon, however, may stimulate decomposition of SOM (Fontaine S *et al.* 2004). It is believed, that a part of fresh carbon (like root assimilates, or easily decomposable polymers, such as cellulose) is directly used by r-strategist microbes, but partly goes to the K-strategists, helping to overcome the energy-limitation usually associated with the decomposition of complex organic matter (lignin, SOM). These K-strategists are thus enabled to produce a higher amount of enzymes to break down recalcitrant molecules like i.e. humic acids (Fontaine S *et al.* 2004). The phenomenon that the input of labile carbon increases SOM decomposition is referred to as priming, or rhizosphere priming (Kuzyakov Y *et al.* 2000).

Soil respiration and stable isotopes

The application of stable isotope techniques can be used to separate autotrophic and heterotrophic respiration. Isotopic fractionation during production, transformation and break-down lead to differences in the isotopic composition of organic material, which can be used to trace carbon fluxes between atmosphere, plants and soil (Dawson T E *et al.* 2002).

Generally plants are isotopcally lighter than atmosphere (-8 ‰) which is due to different diffusion rates of $^{12}CO_2$ and $^{13}CO_2$ into leaves and discrimination during carboxylation. C_3 and C_4 plants have different isotopic signatures due to different discrimination of enzymes active in primary fixation. Rubisco (in C_3 plants) discriminates much stronger against ^{13}C than PEP carboxylase (in C_4 plants), resulting in $\delta^{13}C$ values of C_3 plants ranging between -20 to -35 ‰ and between -10 to -15 ‰ in C_4 plants (O'Leary M H 1981; Smith B N *et al.* 1971). In C_3 plants discrimination of Rubisco against ^{13}C is lower, when stomata are closed because of water stress. There is even a negative linear relation between the stomata opening and discrimination against ^{13}C during photosynthesis in C_3 plants (Farquhar G D *et al.* 1989). The $\delta^{13}C$ value of photosynthetates in the phloem also shows dynamic variation depending on aboveground weather conditions (Bowling D R *et al.* 2002; Ekblad A *et al.* 2005). Carbon compounds in the phloem sap were less depleted in ^{13}C after sunny periods with low rainfall (Pate J *et al.* 1998).

A fraction of recent photosynthetates is transported to roots, and they have shown to be tightly connected to soil respiration (Ekblad A *et al.* 2001; Göttlicher S G *et al.* 2006; Högberg P *et al.* 2001), meaning the $\delta^{13}C$ signature of the transported substances influences the isotopic signature of soil derived CO_2. The transport of assimilates to the root via phloem sap depends on plant species and tree height (Mencuccini M *et al.* 2010), including post-photosynthetic fractionation processes (starch storage and remobilisation) occurring during downward-transport (Brandes E *et al.* 2006). Phloem transport from the canopy to the roots takes between 1 to 10 days (Bowling D R *et al.* 2002; Ekblad A *et al.* 2005; Ekblad A *et al.* 2001; Högberg P *et al.* 2008) depending on the height of the treest. It has recently been shown that drought decreases transport velocity (Ruehr N K *et al.* 2009).

The $\delta^{13}C$ value of soil respiration reflects the isotopic composition of the vegetation growing on the soil, which means that the isotopic composition of soil respiration in an ecosystem dominated by C_3 plants ranges between -20 to -35 ‰ (O'Leary M H 1981) As soil respiration is a mixture of several sources (autotrophic and heterotrophic respiration) its $\delta^{13}C$ composition also consists of a mixture of several inputs (e.g. plant assimilates, SOM, leaf litter, plant or root material) with more or less different $\delta^{13}C$ signatures. Fluctuations of $\delta^{13}C$ of total soil respiration can thus be interpreted as changing contributions of autotrophic and heterotrophic respiration (Ekblad A *et al.* 2001; Kodama N *et al.* 2008; Marron N *et al.* 2009). Additionally, the control of autotrophic and heterotrophic respiration may operate at a different time-scale and may be differentially regulated. Root respiration is highly related to aboveground weather (Bowling D R *et al.* 2002; Ekblad A *et al.* 2005; Ekblad A *et al.* 2001), whereas

heterotrophic soil respiration is determined by belowground conditions like soil moisture or soil temperature, but also substrate availability and microbial community structure (Chapin F S *et al.* 2002).

Soil respiration and tree girdling

Although many techniques to measure soil respiration are available, it remains very challenging to separate heterotrophic and autotrophic respiration *in situ* as there are sensitive interactions between plants, soil and microorganisms (Baggs E M 2006). In forest ecosystems a common approach is to suppress root respiration, without physically disturbing the soil, is tree girdling. Girdling is brought about by removing the bark of the tree at breast height to terminate the transport of photosynthetates in the phloem from the canopy to roots. Water transport from roots to leaves via xylem initially remains intact, so primary production in girdled trees is continued for a certain period of time (Ekberg A *et al.* 2007). As a consequence, root respiration decreases and root exudation is reduced, affecting microorganisms tightly connected to roots, especially mycorrhiza (Högberg P *et al.* 2006; Högberg P *et al.* 2001). The difference between total soil respiration and soil respiration after girdling is considered as autotrophic respiration, and varies between ecosystems. In temperate and boreal forests autotrophic respiration makes up to about 44 % of total soil respiration (Högberg P *et al.* 2009). Girdling not only decreases root respiration, but also leads to the death of fine roots. This increases the availability of substrates, such as starch, for heterotrophic soil microbes (Högberg P *et al.* 2001). The increased supply with dead root and mycorrhizal organic matter can stimulate SOM decomposition (priming) and enhance release of heterotrophic CO_2. Furthermore the increasing number of dead roots leads to decreased water uptake by girdled trees, which in turn increases soil moisture. Higher soil moisture content may also stimulates microbial decomposition processes (Betson N R *et al.* 2007). So, both higher soil moisture and priming could lead to an underestimation of autotrophic respiration. A further effect of tree girdling is the alteration of the microbial community and processes in the soil (Högberg M N *et al.* 2007; Kaiser C unpublished data; Yarwood S A *et al.* 2009). In the absence of easily available carbohydrates and with increased root die back, ectomycorrhiza decreases (Göttlicher S G *et al.* 2006) and higher proportion of saprotrophic fungi establishes (Yarwood S A *et al.* 2009).

As girdling leads to decreased root respiration, the $\delta^{13}C$ of soil respiration should represent the $\delta^{13}C$ of heterotrophic respiration. Girdling has shown diverse effects on the $\delta^{13}C$ value of soil respiration, ranging from no change (Betson N R *et al.* 2007; Göttlicher S G *et al.* 2006) to more enriched $\delta^{13}C$ signatures of soil respiration (i.e.

Bhupinderpal S *et al.* 2003). SOM and plant material are usually enriched in lignin or humic acids, which are more depleted in $\delta^{13}C$ (Bowling D R *et al.* 2008; Ehleringer J R *et al.* 2000). The enrichment of $\delta^{13}C$ values of soil respiration after girdling might derive from increased respiration of remaining, more enriched starch and sugars stored in roots (Bhupinderpal S *et al.* 2003; Ehleringer J R *et al.* 2000). DOC represents an important interface between roots and soil. LMWC in DOC are highly related to root exudation but in case of girdling DOC is mainly fuelled by decomposition processes (Giesler R *et al.* 2007). Compound specific analyses of $\delta^{13}C$ values of DOC also indicated a shift in DOC composition after girdling, but also fluctuations on a seasonal scale (Ekberg A *et al.* 2007).

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Manuscript:

"Partitioning of soil CO_2 efflux in a girdling experiment using stable isotope techniques"

Abstract

- We characterised fluxes of soil respired CO_2 and corresponding $\delta^{13}C$ values in a temperate beech (*Fagus sylvatica*) forest on a daily and seasonal time scale to investigate autotrophic and heterotrophic contributions to total soil respiration. Partitioning of soil respiration was achieved by comparing rates of soil respiration in girdled plots with non-girdled controls. Additionally we investigated a comprehensive set of potential substrates and sources of soil respiration for their $\delta^{13}C$ values.
- In controls bulk plant litter (leaf, fine and coarse root litter) as well as soil organic matter (SOM) were more depleted in ^{13}C than plant carbohydrates (phloem sap sugars, root starch and sugars) and root respiration, indicating that a major part of soil respiration was fuelled by recently fixed carbon. We assumed that the isotopic signature of dissolved organic carbon (DOC) reflects sources contributing to DOC such as root exudates and products of decomposition. In controls, DOC was enriched in ^{13}C compared to girdled plots, and in the same range as root sugars, indicating that root exudates may contribute substantially to DOC.
- Girdling led to an average decline of total soil respiration of 36 % and to more depleted ^{13}C signatures of soil respired CO_2 by 1.5 ‰ on an annual scale, indicating reduced autotrophic respiration and a shift towards decomposition of root litter and SOM, which was corroborated by more depleted isotopic signatures of DOC in girdled plots.
- We observed daily and seasonal fluctuations of soil respiration and $\delta^{13}C$ of respired CO_2. We identified air temperature as a major predictor for soil respiration in both control and girdled plots at the various time scales. In girdled plots soil respiration was further correlated with soil temperature at an annual scale. The $\delta^{13}C$ value of phloem sap sugars, however, did not exhibit significant changes on a daily time scale and did not correlate with the $\delta^{13}C$ value of soil respiration in control plots.

Introduction

Soil decomposition processes are tightly connected to photosynthesis, either by the input of dead plant material or by belowground translocation of recently assimilated carbohydrates. The latter are the main drivers of autotrophic soil respiration (Ekblad A *et al.* 2001; Göttlicher S G *et al.* 2006; Högberg P *et al.* 2001) and are tightly connected to photosynthetic processes in the canopy reflecting environmental conditions at the time of carbon fixation. In this definition, autotrophic soil respiration comprises also microbial respiration in the close vicinity of roots, directly depending on root exudates (Fontaine S *et al.* 2003; Högberg P *et al.* 2006; Paterson E *et al.* 2009). Heterotrophic respiration mainly derives from decomposition of aboveground or belowground plant litter and of soil organic matter (SOM) (Kuzyakov Y 2006) and is thought to be controlled by physical factors such as soil temperature and soil moisture, but also by substrate availability and microbial community composition (Chapin F S *et al.* 2002; Swift M J *et al.* 1979). Microbial uptake of organic matter primarily occurs from the soluble soil phase (Marschner B *et al.* 2003). The chemical composition of dissolved organic carbon (DOC) is affected by its main components, namely root exudates, depolymerisation products of SOM or of fresh plant litter (Ekberg A *et al.* 2007; Giesler R *et al.* 2007; Jones D L *et al.* 2009). The depolymerisation of SOM is thought to be energy limited and is therefore intimately linked to a continuously active, but slow growing microbial community (Fontaine S *et al.* 2003). Nevertheless SOM depolymerisation yields nitrogen, as SOM usually has a low C/N ratio. However the break down of macromolecules into smaller molecules that can be taken up by microbes depends on the production of extracellular enzymes (Fontaine S *et al.* 2005; Paterson E *et al.* 2009). Easily assimilable compounds such as carbohydrates either originating from depolymerisation from regular polymers, like cellulose, or root exudates are considered to be the primary substrate for microbial respiration (Fontaine S *et al.* 2003). These labile substrates are consumed by fast growing r-strategists, who are thought to have only a low enzyme production capacity. In addition, they may also provide energy for slow growing K-strategists that are involved in SOM decomposition. The latter effect is referred to as priming or rhizosphere priming (Fontaine S *et al.* 2003; Kuzyakov Y *et al.* 2000).

Girdling of trees (i.e., the removal of bark) is a common method to quantitatively partition autotrophic and heterotrophic soil respiration in forests, without physically disturbing the soil (Högberg P *et al.* 2001). Girdling cuts off the translocation of assimilates in the phloem from canopy to roots. Consequently root respiration and exudation ceases, which may subsequently affect microbial community composition

and decrease priming effects (Ekblad A *et al.* 2001; Högberg P *et al.* 2001; Yarwood S A *et al.* 2009). The decline of soil respiration after girdling varies between ecosystems; in temperate forests autotrophic respiration contributes on average 44 % to total soil respiration (Högberg P *et al.* 2009). In the longer term girdling leads to decreased fine root biomass (Högberg P *et al.* 2001), which may cause a higher soil moisture content by decreased root water uptake (Bhupinderpal S *et al.* 2003; Ekberg A *et al.* 2007; Marron N *et al.* 2009) and an increased input of fresh root litter (Binkley D *et al.* 2006; Högberg P *et al.* 2001), both affecting decomposition processes. The increased availability of fresh plant root litter and the absence of root exudates alter the microbial community composition, favouring microbes that are able to decompose regular polymers, especially saprotrophic fungi (Schnecker J unpublished data; Yarwood S A *et al.* 2009). Girdling allows quantifying the contribution of autotrophic soil respiration (root respiration and respiration of recently fixed plant carbon by rhizosphere microbes and mycorrhizal fungi) to total soil respiration. A possible reduction of heterotrophic respiration by diminishing priming of soil organic matter breakdown, however, can not be assessed by this approach. Additionally, in the longer term, the amount of autotrophic respiration could be underestimated for heterotrophic respiration could increase due to priming induced by the availability of increased dead root biomass.

Thus, the application of stable isotope techniques in girdling experiments could provide further information about the origin of soil respired CO_2, given that the substrates for autotrophic and heterotrophic soil respiration exhibit different isotopic signatures (Ekblad A *et al.* 2000). The carbon isotope composition of autotrophic soil respiration is highly influenced by environmental conditions (Bowling D R *et al.* 2002; Ekblad A *et al.* 2005), as substrates for root respiration are directly derived from photosynthetic CO_2 fixation in the canopy. Temperature and air humidity control stomatal regulation and subsequently affect carbon isotope composition of assimilates (Farquhar G D *et al.* 1989). Variations in carbon isotope composition of assimilates can also be detected in autotrophic soil respiration (Bowling D R *et al.* 2002; Ekblad A *et al.* 2005). However, time delays between a few hours to 10 days can occur between carbon uptake in the canopy and respective root respiration (Bowling D R *et al.* 2002; Ekblad A *et al.* 2005; Ekblad A *et al.* 2001; Högberg P *et al.* 2008) depending on plant species and tree height (Mencuccini M *et al.* 2010), with drought effects decreasing transport velocity of sugars in the phloem (Ruehr N K *et al.* 2009). The isotopic signature of heterotrophic soil respiration is directly dependent on the isotopic signature of the substrates, with little discrimination (Ekblad A *et al.* 2002). The isotopic composition of substrates for decomposition is, however also dependent on the chemical composition of the substrate (Ehleringer J R *et al.* 2000); structural carbohydrates, such as cellulose, are

for example more enriched in ^{13}C compared to lignin (Ehleringer J R *et al.* 2000; Hobbie E A *et al.* 2004). SOM is generally more enriched compared to plant litter (Bowling D R *et al.* 2008) and becomes more enriched in ^{13}C in deeper soil layers (Boström B *et al.* 2007), i.e. with age (Fontaine S *et al.* 2007). As girdling decreases the autotrophic contribution to total soil respiration the isotopic signature of soil derived CO_2 may reflect the isotopic signatures of substrates for heterotrophic respiration and could illuminate the magnitude of priming effects with or without girdling.

Additionally fluctuations of the isotopic composition of soil respiration may be interpreted as changing contributions of its autotrophic and heterotrophic components over time (Ekblad A *et al.* 2001; Kodama N *et al.* 2008; Marron N *et al.* 2009). On a daily time scale, autotrophic soil respiration is considered to be primarily controlled by photosynthesis, which reflects recent environmental conditions (Bowling D R *et al.* 2002; Ekblad A *et al.* 2005). On an annual time scale soil respiration is highly connected to seasonal variations in temperature and water availability (Litton C M *et al.* 2008). Additionally, enhanced root growth and activity during the vegetation period lead to higher root respiration and exudation (Hanson P J *et al.* 2000). In autumn, the belowground transport of recent assimilates ceases, whereas leaf litter and fine root litter increases, providing a high input of substrate for heterotrophic soil microbes.

The overall goal of this study was to characterise variations of soil derived CO_2 and its isotopic composition *in situ* in a temperate beech forest at different time scales. On a seasonal scale we aimed at understanding the pattern of soil respiration and elucidating the environmental controls of its component fluxes. On a diurnal scale we aimed to assess major contributors to soil respiration. We approached these goals by (1) a tree girdling experiment and (2) by analysing the natural abundance of ^{13}C in a comprehensive set of possible substrates for soil respiration. We hypothesized that soil respiration would decrease in girdled plots and would be controlled by soil temperature and soil moisture, whereas in control plots air temperature and humidity would control soil respiration. Moreover, we expected that in girdled plots the isotopic signature of soil respiration would shift towards substrates for heterotrophic respiration such as SOM or root litter.

Material and Methods

Study site

The study site was situated in a mature, about 65 year old European beech (*Fagus sylvatica*) forest in Klausenleopoldsdorf, Lower Austria, about 40 km WSW of Vienna. The sampling area was at 510 m above sea level on a NNE-orientated slope. Mean annual precipitation at the site is 728 mm and mean air temperature about 8°C. The soils were cambisols over flysh with a pH about 4.8 – 5.1. The overlying organic mor-layer was about 5 – 8 cm thick and mainly consisted of beech leaf litter and woody debris. A comprehensive base characterization (soil chemistry and physics, as well as vegetation) of the research site has been published elsewhere (Hackl E *et al.* 2004; Hackl E *et al.* 2005; Hahn M *et al.* 2000; Stange F *et al.* 2000).

Experimental setup

Girdling (**G**) was performed on three 400 m² plots in April 2006. Each 400 m² plot was divided into 2 inner 10x10 m sized subplots (total n = 6), which were used for soil sampling (to avoid edge effects). Trees were girdled in breast height, which means the bark was removed over ~15 cm circular sections, to terminate the transport of photosynthetates from leaves to roots. Additional six 25 m² (5x5 m) plots were kept as controls (**K**). Understorey vegetation was removed in every single sampling plot.

We sampled monthly to determine seasonal changes in soil respiration and soil properties. Additional, an intensive sampling event took place between the 1.-3. August 2007.

Seasonal dynamic

Microclimate measurements

Soil temperature (Onset, 12 bit temperature data logger) and soil moisture (Onset ECH2o soil moisture sensor, Hobo 4-channel micro station data logger), were continuously monitored in control and girdling plots throughout the sampling period. Precipitation data, air temperature and humidity data have been provided by the *Federal Research and Training Center for Forests, Natural Hazards and Landscape* (Bundesforschungs- und Ausbildungszentrum, für Wald, Naturgefahren und Landschaft, BFW).

Gas sampling

Metal frames (1 m²) were installed in three girdled and three control plots. For gas sampling they were closed with plastic lids (creating a headspace of about 80 L), which were fixed with two rubber clamps to provide tightly sealed chambers. Sets of gas samples from each treatment (G & K; n = 3) were taken monthly from May 2007 to April 2008. Gas samples of 12 ml were taken with a syringe through a rubber membrane stopper and immediately transferred into 12 ml pre-evacuated exetainer glass-vials. The first gas sample was taken directly after closing the lid, and then after 5, 10, 15 and 30 minutes, which was identified to be the best time interval, ensuring linear respiration rates. Due to heavy snowfalls during winter 2007 (November, December and January) and snow covered sampling plots, smaller, cylindrical metal chambers (volume = 6.3 l) fitted with a rubber membrane were used instead of metal frames. Samples were, as soon as possible, analyzed for their CO_2 concentration and $\delta^{13}C$ ratio with a Finnigan GasBench coupled to a Finnigan Delta V Advantage Mass Spectrometer (both Thermo Fisher Scientific, Whaltham, MA, USA). Respiration rates were calculated as the rate of CO_2 increase in time (mg C m^{-2} h^{-1}). Isotopic composition of gas samples are reported as $\delta^{13}C$ values in ‰:

$$\delta^{13}C_{sample} = 1000 * ((R_{sample} - R_{standard}) / R_{standard})$$

with R describing the ratio of $^{13}C/^{12}C$. The $\delta^{13}C$ values were calculated relative to the Vienna-Pee-Dee-Belemnite standard. Isotopic composition of soil respired CO_2 was analysed using the Keeling plot approach (Keeling C D 1958). This method is based on a two-component isotope mixing model, where the two components are soil respired and atmospheric CO_2. Isotopic composition of soil respired CO_2 was determined by the intercept of a linear regression of $\delta^{13}C$ of sampled CO_2 versus $1/[CO_2]$. Some samples had to be removed due to sampling problems. If R^2 of the regression was <0.9, the Keeling plot was excluded from further analyses.

Daily variations

In August 2007 an intensive measuring campaign took place. Several parameters were investigated, such as fine root biomass of girdled and control trees, phloem sap sugars, soil respiration rates in the course of two days, root respiration and abiotic background.

Gas sampling

To analyze changes of soil respiration over the course of several days a measuring campaign was conducted from August 1st – August 3rd 2007. Gas samples were taken from control and girdled plots (n=3, respectively) in 3-hour-intervals starting at 10 am on August 1 and ending on August 3 at 8 am, using the same sampling procedure as mentioned above.

Root respiration

To determine root respiration soil cores from every plot were taken every 12 h starting on August 1 at 10 am. Roots were carefully picked out of the soil, washed and put into 250 ml Erlenmeyer flasks which were immediately closed with a rubber stopper. Gas samples of 12 ml were taken through the rubber stopper using a syringe and immediately transferred into 12 ml pre-evacuated glass vials and as soon as possible analysed for their CO_2 concentration and $\delta^{13}C$ ratio with a Finnigan GasBench coupled to a Finnigan Delta V Advantage Mass Spectrometer (both Thermo Fisher Scientific, Whaltham, MA, USA). The first sample was taken one minute after closing the flask and then after 10, 20 and 30 minutes. Roots were dried and weighed and stored for further use.

Isotopic composition of fine root biomass

Fine and coarse roots were grinded and weighed into smooth tin capsules for measuring organic carbon and $\delta^{13}C$ composition. Samples were measured by EA-IRMS (Carlo Erba EA 1110).

Sugar content and sugar ^{13}C ratio of fine roots

60 mg of finely ground root powder was weighed into 2 ml reaction vials and suspended using a vortex, in 1.5 ml deionised water. Samples were put in a 85 °C water bath for 30 minutes, cooled down and centrifuged at 10 000 g for 2 minutes using a Heraeus Biofuge primo centrifuge. The supernatant was frozen until further use. For ion-exchange of the samples anion exchange cartridges (Dionex OnGuard II A 1 cc cartridge) and a cation exchange cartridges (Dionex OnGuard II H 1 cc cartridge), which were connected to a 20 ml syringe, were used. First, 10 ml of deionised water

was slowly pushed through the cartridges, to let them swell for about 2 hours. Afterwards the 20 ml syringes were replaced with 1ml syringes and 1 ml of the sample was pushed through the cartridges with slight pressure into pre-weighed vials (Semadeni Cryotubes PP 5 ml). After this procedure 2 ml of deionised water was pushed slowly through the cartridges and samples and vials were weighed again. The final solutions were dried, using a concentrator (Savant Speed Vac, SC 110), redissolved with deionised water, pipetted into GC-vials and analyzed by HPLC using a Finnigan LC Isolink Interface connected to a Delta V Advantage Mass Spectrometer as described elsewhere (Wild B *et al.* 2010).

Starch content and starch $\delta^{13}C$ values of fine roots

Fine root starch content and $\delta^{13}C$ values were determined using the method described by (Göttlicher S *et al.* 2006). Finely ground root samples were cleaned of LMWC such as sugars by using a methanol-chloroform-water extract. Then samples were dried and resuspended in water, hydrolysed using a heat stable α-amylase solution, filtered, centrifuged and the supernatant pipetted into smooth tin capsules for analyses by EA/IRMS.

Phloem sap

Circles of bark with a diameter of 1 cm were cut from the stem of mature Beech trees at breast height, which was above the girdled area in girdled trees, using a corer. Two bark pieces per tree were sampled, washed with deionised water, put incubated together in 2 ml exudation solution for 4 h. 15 mM sodium hexametaphosphate (Fluka, 71600) solution has been chosen as an exudation solution as it is free of carbon or nitrogen (Gessler A *et al.* 2004). Amount and isotopic composition of the phloem sugars were analysed as described above for root sugars

DOC

Dissolved organic carbon (DOC) was analysed in soil solutions collected by tension lysimeters either through porous suction cups or suction plates installed in 5 cm soil depth connected with teflon tubes to 1 L flasks installed in control and girdled plots (n=4, respectively). DOC and $\delta^{13}C$ of DOC were measured by direct injection into a Finnigan LC Isolink Interface connected to a Delta V Advantage Mass Spectrometer.

Organic carbon in soil, rhizosphere soil and leaf litter

Organic carbon content was analysed in soils from cores taken from the upper 5 cm of mineral soil (A-layer). Four subsamples were taken from each of the six replicate plots and pooled (n=6 for G and K). Rhizosphere soil was collected directly from roots during

root biomass determination (Kaiser C unpublished data). Leaf litter was collected in litter traps (n=6 for G and K, respectively). Organic carbon was analysed by EA/IRMS (Carlo Erba EA 1110).

Calculations and statistical analyses

Calculations of soil respiration rates and $\delta^{13}C$ values were performed using Microsoft Office Excel 2003. For statistical analyses STATISTICA for Windows Version 7.1 (StatSoft, Inc; 2005) was used. Differences of soil respiration, carbon content and isotopic composition between treatments, or sampling time (seasonal or diurnal effects) were assessed by multifactor ANOVA. We used Pearson's correlations to identify relations between climate data and soil respiration and $\delta^{13}C$ values of soil respired CO_2, respectively.

Results

Daily variations

Diurnal variations of microclimate

Air temperature (T_{air}) in the forest fluctuated in a diurnal pattern (Fig. 1a) between 5.3 and 27.8 °C with lower temperatures during nights and higher temperatures during the day. Relative air humidity (RH; Fig. 1a) varied between 100 % during nights and 40.5 % during the day. Total precipitation in July 2007, the month before sampling was 48.8 mm, the last event before sampling was on July 30th with 7.5 mm. There was also a minor precipitation event with 1.2 mm at the end of the sampling period. Soil temperature (T_{soil}) at 5 cm depth (Fig. 1b) also fluctuated, although with very small magnitude with values between 13.9 and 15.1 °C with a mean value of 14.5 °C. Soil water content at 5 cm depth did not vary over the course of two days, but was significantly higher in girdled plots (36 % of dry weight) than in controls (26 % of dry weight). With regarded to seasonal scale soil moisture content in controls was lowest by the time of this experiment.

Soil CO_2 efflux

Soil respiration in control (K) plots (Fig. 1c; Tab. 1) fluctuated in a diurnal pattern, with lowest rates measured of 17.43 (± 4.91) to maximum rates of 44.53 (± 5.6) mg CO_2-C $m^{-2}h^{-1}$. On both days respiration peaked in the late afternoon. In girdled plots soil respiration was significant lower than in controls (Tab. 3) with an average decline of 19.2 % (± 3.7), but also fluctuated with a diurnal pattern over the course of two days (Fig. 1c, Tab. 3).

Pearson's correlations were calculated between soil respiration (mg CO_2-C $m^{-2}h^{-1}$) and microclimatic parameter with air temperature (T_{air}), and relative air humidity (RH) considered as aboveground parameter, and soil temperature at 5 cm depth (T_{soil}) and soil moisture content in % of dry weight (SM) as belowground parameter, for girdled and control plots, respectively (Fig. 2a-d). Additionally correlations were calculated with increasing time lags, from 0 to 48 hours, to detect if previous variations of microclimate affected recent soil respiration. T_{air} was identified as the most determinant control parameter for soil respiration in control plots (Fig. 2a). We detected highly significant ($p<0.001$) correlations outlining a 24 periodicity, phase-delayed with only 3 hours. T_{soil} significantly correlated with soil respiration in control plots, with a time lag of 18 hours. SM however correlated neither with soil respiration in control nor in girdled plots. Soil respiration in girdled plots seemed to be even

stronger related to T_{air} than soil respiration in controls. We found a highly significant correlation with 0 and 3 hour time delay, and also evidence for a 24 hour-periodicity. In contrast to control plots, soil respiration in girdled plots highly significant correlated with SM with a time lag of 18 hours. To detect time delays between photosynthesis and downward transport of assimilates as substrate for root respiration, carbon content of total sugars extracted from phloem sap, taken at breast height, was correlated with soil respiration in control plots. However, no relationship was detected.

Isotope composition of soil CO_2 efflux

Soil respiration rates significantly correlated with their corresponding carbon isotope signatures over the measuring period in both control (R=0.71; p=0.003) and girdled plots (R=0.57; p=0.027). This means that at times with higher soil respiration the respired CO_2 was less depleted in ^{13}C and vice versa. A significant diurnal variation of the $\delta^{13}C$ values of soil respiration in controls as well as in girdled plots was detectable (Tab. 2). In control plots isotopic signatures of soil respiration were most enriched in the late afternoon, with $\delta^{13}C$ values around -20 ‰ (Tab.2; Fig 1d) and nearly 6 ‰ more depleted during nights. In girdled plots $\delta^{13}C$ values of soil were significantly more depleted (Tab. 3), on average by 1.5 ‰. On a diurnal scale isotopic signatures of soil respired CO2 fluctuated even with higher magnitude than in controls (Fig. 1d) with $\delta^{13}C$ values ranging between -19.82 and -26.68 ‰.

We calculated Pearson's correlations also between $\delta^{13}C$ values of soil respiration in girdled and control plots, respectively, and microclimatic variables (T_{air}, RH, T_{soil} at 5 cm depth and SM in %). Similar as for soil respiration correlations were calculated with increasing time lags between 0 and 48 hours to determine previous effects of microclimate on recent $\delta^{13}C$ values of soil respiration (Fig. 2 e-h). For control plots additional correlations were calculated between $\delta^{13}C$ ratios of soil respiration and $\delta^{13}C$ of sucrose of phloem sap but also $\delta^{13}C$ of sucrose from phloem sap did not correlate with $\delta^{13}C$ of soil respiration.

We found correlations between T_{air} and $\delta^{13}C$ of soil respiration in controls (Fig. 2e) in a 24 h time pattern, although with an expanded time window of 3 hours. In girdled plots isotopic signatures of soil respiration exhibited a very similar pattern to control plots regarding correlations with T_{air}. T_{soil} and SM did not seem to be determinant factors for isotopic composition of soil respiration in both control and girdled plots by this time of the year.

Carbon content and $\delta^{13}C$ composition of plant and soil components contributing to soil respiration

Roots – bulk, starch and sugars, respiration

Fine and coarse roots of both controls and girdled plots did not significantly differ in bulk carbon content (Tab. 4) however $\delta^{13}C$ signature of bulk fine roots was significantly more depleted in G (Tab. 5). Starch content in fine roots although low in itself (Tab. 4) was significantly decreased in girdled plots and more depleted in ^{13}C than in control plots (Tab. 5). The amount of fine root sugars, comprising glucose, fructose and sucrose, decreased even more dramatically in girdled plots by more than 80 %. The isotopic signature of root sugar was amongst the most enriched components in control as well in girdled plots with -21.4 ‰ (± 0.2) and -22.6 ‰ (± 0.2), respectively, however significantly ($p<0.01$) more depleted in girdled plots (Tab. 5; Fig. 3). Root respiration, was highly significant ($p<0.001$) lower in girdled plots than in controls (Tab. 4), but the isotopic signature did not differ in between treatments (Tab. 5). The calculated contribution of root respiration to total soil respiration made up to about 23 % in girdled plots and 61 % on control plots.

Phloem sap

Sucrose isolated from the phloem sap was significantly lower in girdled trees (Tab. 4) and was significantly more depleted in ^{13}C (Tab. 5) than the controls, but as phloem sap samples were taken above the girdling, values should not be relevant to influence soil respiration. No diurnal pattern was found regarding sucrose concentration or its $\delta^{13}C$ values in control trees (Fig. 1f).

Dissolved Organic Carbon (DOC)

DOC content did not vary between control and girdled plots (Tab. 4) but was significant more enriched in controls ($p<0.001$), with $\delta^{13}C$ values of -21.6 ‰ (± 0.4) similar to those of root sugars (Fig. 3) in controls. In girdled plots DOC was more depleted compared to controls with a $\delta^{13}C$ of -25.7 ‰ (±0.5).

Organic matter: Soil – rhizosphere soil – Beech leaf litter

Bulk soil and rhizosphere soil did not differ concerning carbon content (Tab. 4) or $\delta^{13}C$ value (Tab. 5). The isotopic signature (around -26 ‰) was in the same range as $\delta^{13}C$ values of coarse and fine roots. No differences were detectable between soil in controls and girdled plots. Leaf litter was most depleted of all measured substrates with $\delta^{13}C$ values around -28 ‰ in both treatments.

Seasonal dynamics

Meteorological data

As typical for temperate regions T_{air} followed a seasonal pattern. T_{air} rose in spring, peaked during summer months and decreased slowly during autumn representing lowest values during winter months (Fig. 4a). At the study site highest temperatures of were measured during summer in August 2007 (34.0 °C), and lowest during winter months with in December 2007 (-5.5 °C). Unusual high temperatures of 15.0 °C were measured in January. T_{soil} (Fig. 4c) showed a similar pattern as T_{air}, but with smaller amplitude. T_{soil} ranged between 17.8 °C in July on and 2.5 °C on February, but was never lower than 0 °C. Annual precipitation in 2007 made up to 720.5 mm with peaks (Fig. 4a) in spring 2007 and was followed by a relatively dry summer. The study site was snow covered from the end of October 2007 to mid March 2008. Soil moisture (Fig. 4b) of control plots showed a slight seasonal trend, with lowest values (around 28 %) during July and August 2007 and increased during winter months. In the second year after girdling soil moisture was already severely increased especially during summer and autumn (Fig. 4b).

Soil CO_2 efflux and its isotopic composition

During the study period respiration rates in non-girdled controls (Fig. 4d) decreased from May (64.7 ± 2.9 mg CO_2-C $m^{-2}h^{-1}$) to November (2.6 ± 1.0 mg CO_2-C $m^{-2}h^{-1}$), apart from a slight peak at the end of August 2007 and increased again in spring 2008. In December and February soil respiration was not measured due to high snow cover. Girdling led to an average annual decline of soil respired CO_2 of 36 %, nevertheless a seasonal pattern, although with smaller amplitude, was detected (Fig. 4d). Especially during growing season in 2007 (May and June) soil respiration was significantly lower in girdled plots (Fig. 4d), but throughout summer and autumn no significant differences were visible. Soil respiration in girdled plots decreased to lowest values in November 2007 (1.16 ± 0.28 mg CO_2-C $m^{-2}h^{-1}$). In spring 2008 soil respiration in girdled plots constantly increased from January to April, but was again significantly lower than in control plots. Air temperature positively correlated with soil respiration in control and girdled plots, respectively (Tab. 6). Furthermore soil respiration in girdled plots positively correlated with T_{soil} in different soil depths (Tab. 6).

The trend of more depleted isotopic values of soil respiration in girdled plots was not only a diurnal phenomenon but also proceeded over the course of a year (Fig. 4e). In control plots soil respiration was most depleted in May (-24.93 ± 0.26 ‰) and June (-24.57 ± 0.76 ‰) 2007, both months with high soil respiration. In contrast $\delta^{13}C$ values

measured in August were 4.3 ‰ more enriched than in May 2007. During winter months CO_2 concentration of soil respiration was very low and $\delta^{13}C$ values of measured gas samples showed high variations. Therefore the Keeling plot approach could not be used to calculate the isotopic signature of soil respiration and values of November 2007 and January 2008 are missing. Soil respiration in control plots was most enriched in ^{13}C in March 2008 with -17.81 (± 0.49) ‰. In contrast to soil respiration rates, isotopic signatures did not correlate with any abiotic parameter (Tab. 6).

Discussion

The influence of girdling on soil decomposition processes

On average total soil respiration was about 36% lower in girdled compared to controls plots in the second year after the girdling treatment. During the relatively dry summer, however, the reduction was only 20 %, which is relatively low compared to other studies (Högberg P *et al.* 2009). A decrease in soil respiration is usually attributed to decreased root and mycorrhizal respiration, and may also originate from reduced rhizosphere priming effects. However, there are several points of evidence that suggest, that simply calculating the difference between total soil respiration in control and girdled plots may underestimates autotrophic respiration. First, in the long term girdling causes enhanced root and mycorrhiza mortality, providing additional substrates that are relatively easy to decompose (Högberg M N *et al.* 2002). Due to microbial succession the initial stages of litter decomposition are characterised by fast growing r-strategist microbes using easily assimilable low-molecular weight plant compounds (Fontaine S *et al.* 2004). A part of these easy assimilable carbon compounds, as well as the break-down products of cellulose and other regular polymers can also be used by slow growing K-strategists and can prime SOM decomposition (Fontaine S *et al.* 2003). Thus heterotrophic respiration might increase (Zeller B *et al.* 2008) and could even exceed the decline of autotrophic respiration (Ekberg A *et al.* 2007). A shift of substrates from direct autotrophic sources to heterotrophic sources (e.g., priming) may be seen in changing isotopic composition of the respired CO_2. We were indeed able to detect a shift in the carbon isotope composition of soil respiration in girdled plots compared to controls. In contrast to our expectations, however, girdling led to more depleted $\delta^{13}C$ values of soil respired CO_2. This was also a major difference to other girdling experiments, where either no change in carbon isotope composition was observed (Betson N R *et al.* 2007) or girdling led to more enriched $\delta^{13}C$ values of soil respiration (Göttlicher S G *et al.* 2006; Marron N *et al.* 2009). This observed depletion suggests either a lower contribution of root respiration, which is enriched relative to heterotrophic respiration or an increased contribution of depleted substrates for microbial respiration, such as root litter or SOM or a combination of both. In our study the $\delta^{13}C$ values of root respiration were similar in control and girdled plots but root respiration contributed much less to total respiration in girdled plots than in controls. Furthermore, not only soil respiration but also DOC was more depleted in ^{13}C in girdled plots compared to controls. In controls the $\delta^{13}C$ value of DOC was more enriched and similar to root sugars, indicating that root exudates are an important component of DOC. Plant litter and SOM, considered as

substrates for heterotrophic respiration, were more depleted in ^{13}C. Therefore a decrease of root respiration and exudation after girdling, together with the increased decomposition of plant litter due to increased input of dead roots and mycorrhiza (Ekblad A *et al.* 2001; Högberg P *et al.* 2001; Kaiser C unpublished data) could explain the shift of the isotopic signature in girdled plots. In addition microbial community composition and function was altered in girdled plots, with increased abundance of saprotrophic fungi and enhanced oxidative enzyme activity (Schnecker J unpublished data; Yarwood S A *et al.* 2009). Although soil respiration in girdled plots did not reach the level of controls as reported elsewhere (Ekberg A *et al.* 2007), our data suggest that the increased SOM decomposition in girdled plots primed by the enhanced input of root litter may have been substantial.

In control plots the average $\delta^{13}C$ value of soil respiration was relatively enriched compared to SOM, leaf litter and fine roots, all of them considered to be sources for heterotrophic respiration. This indicated a substantial contribution of other, more enriched sources, which could be related to fresh assimilates from the canopy. Phloem sap as well as root starch were more depleted in ^{13}C, whereas root sugars and DOC were more enriched than soil respiration. Based on the similarity between isotopic values of root sugar and DOC we concluded that carbohydrates exuded from roots may be an important part of DOC as it has recently been shown (Ekberg A *et al.* 2007). DOC represents a very active C pool with high turnover rates (van Hees P A W *et al.* 2008). Especially low molecular weight compounds in DOC, like organic acids, amino acids and simple sugars, attributed to root exudation, are considered to drive microbial activity in the mycorrhizosphere (Dakora F D *et al.* 2002; Fontaine S *et al.* 2003; Paterson E *et al.* 2009). We could show that the isotopic composition of DOC and microbial respiration indeed was very similar in control as well as in girdled plots, suggesting that the DOC pool is of prime importance for soil respiration.

Fluctuation of soil respiration and isotopic composition on a daily scale

Soil respiration and the correspondent $\delta^{13}C$ values varied diurnally in both control and girdled plots. Soil respiration in girdled plots fluctuated with lower magnitude but with similar periodicity as in control plots. The isotopic signature of soil respiration in girdled plots was constantly more depleted. Nevertheless the $\delta^{13}C$ signature of soil respired CO_2 was more enriched during daytime and more depleted during night in both control and girdled plots. However, similar pattern were also detected in other forests (Kodama N *et al.* 2008). In contrast another study, conducted in a temperate beech forest, found that $\delta^{13}C$ values of soil respired CO_2 were most enriched during the night (Marron N *et al.* 2009). It is believed that variations of $\delta^{13}C$ signatures of soil respired

CO_2 in the course of a day are derived by fluctuations of relative contributions of heterotrophic and autotrophic CO_2 to total soil respiration (Kodama N *et al.* 2008; Marron N *et al.* 2009). We found a clear difference between more depleted substrates for heterotrophic and more enriched for autotrophic respiration. So we considered that during the day, soil derived CO_2 consisted of a higher proportion of autotrophic respiration, which decreased during the night when $\delta^{13}C$ values of heterotrophic respiration dominated. Especially two time points, in the early morning on both sampling days, were remarkable, as soil respiration and carbon isotope composition were similar in controls and girdled plots. We concluded that these similarities indicated times where heterotrophic processes dominated also in control plots, thus indicating a small time delay between day and night rhythm and soil respiration.

The isotopic signature of root, and therefore autotrophic respiration is highly related to environmental conditions (Bowling D R *et al.* 2002; Ekblad A *et al.* 2005; Ekblad A *et al.* 2001), which affect stomatal conductance and cause fractionation of ^{13}C during CO_2 uptake (Farquhar G D *et al.* 1989). We therefore expected a tight connection between environmental conditions and $\delta^{13}C$ signatures of phloem sap sugars and subsequently with the $\delta^{13}C$ of soil respiration in control plots. Surprisingly, the $\delta^{13}C$ of sucrose from phloem sap of control trees collected at 1.5 m height did not show a diurnal pattern and did not correlate with $\delta^{13}C$ of soil respiration over the course of two days, which was also reported by others (Kodama N *et al.* 2008). Our experiment took place at a very dry summer, with low soil water content in control plots. Drought decreases transport velocity in phloem sap, so the way of assimilates from the canopy to roots could even take more than six days (Ruehr N K *et al.* 2009).

Since in girdled plots soil respiration and its $\delta^{13}C$ values are considered to be derived mainly from heterotrophic processes, we expected smaller diurnal fluctuations. In contradiction to our assumptions $\delta^{13}C$ values of soil respiration in girdled plots highly varied over the course of a day and significantly correlated with air temperature with very little time delay. These tight relations between air temperature and soil respiration probably could be attributed to decomposition processes in the organic layer (Marron N *et al.* 2009). However, the enrichment of $\delta^{13}C$ of soil derived CO_2 in girdled plots during the day which may be attributed to decomposition of more enriched compounds in litter, such as cellulose (usually 2 to 3 ‰ more enriched compared to bulk plant material; (Hobbie E A *et al.* 2004), is not able to fully explain the difference of $\delta^{13}C$ values of soil respired CO_2 between day and night by more than 6 ‰. A possible discrimination during diffusion of CO_2 in the soil, as has been described recently (Kayler Z E *et al.*), can also not be responsible for the detected pattern, as this discrimination should affect control and girdled plots similarly. Due to decoupling

of the canopy, we expected belowground temperature and moisture to highly influence soil respiration and $\delta^{13}C$ values in girdled plots (Marron N *et al.* 2009). However, soil temperature only slightly correlated with soil respiration and $\delta^{13}C$ values in both control and girdled plots, maybe due to drought effects (Andersen C P *et al.* 2005).

Fluctuations on a seasonal scale

Large variations of soil respiration and its $\delta^{13}C$ composition were detected on a seasonal time scale. In the second year after girdling, we still detected a strong decrease of soil respiration especially during spring, most likely due to high plant root activity, i.e. high root growth and exudation in spring (Hanson P J *et al.* 2000; Phillips R P *et al.* 2008). Additionally, priming effects in the rhizosphere may have been driven by higher root exudation resulting in enhanced decomposition of recalcitrant substrates and higher heterotrophic respiration in control compared to girdled plots. Soil respiration in girdled plots also followed a seasonal pattern, although with a smaller amplitude presumably because of decreasing autotrophic respiration. As predicted for temperate ecosystems (Litton C M *et al.* 2008) soil respiration in controls and also in girdled plots, strongly correlated with air temperature. Soil respiration in girdled plots significantly correlated with soil temperature, which was not evident on a daily scale. In control plots the influence of soil temperature on soil respiration was minor and probably masked as a higher autotrophic respiration, which is independent of soil temperature.

Girdling significantly affected the isotopic signature of soil respiration leading to more depleted $\delta^{13}C$ values than in control plots throughout the year. Although tight correlations between soil moisture, and $\delta^{13}C$ of soil respiration have been reported elsewhere (Marron N *et al.* 2009) we found no correlations neither for girdled nor for control plots. Soil respiration as well as the isotopic composition of soil respired CO_2 were similar in control and girdled plots during autumn (after onset of litterfall) and winter, indicating that at this time of the year similar processes dominated in both treatments - after litterfall, the supply of canopy-derived carbon to roots is also strongly decreased in control plots, while leaf litter represents a high amount of fresh substrate for decomposers.

In summary our study demonstrated, that girdling led to a decrease of soil respiration of about 20 % during the vegetation periods. The isotopic composition of soil respired CO_2, however, suggest that the reduction of the autotrophic component of soil respiration may in fact has been much larger, as enhanced input of root litter and/or increased priming of SOM have led to an increase in the heterotrophic component of soil respiration. Unfortunately, it was not possible to differentiate between priming

effects and enhanced litter decomposition, as SOM and root litter exhibited very similar carbon isotope signatures. The observed differences in the isotopic composition of respired CO_2 were also reflected in differences in the isotopic signature of DOC between control and girdled plots, indicating the predominant role of DOC as substrate for microbial respiration. In controls $\delta^{13}C$ signature of DOC was more enriched than in girdled plots, reflecting the strong contribution of root exudates to DOC, while in girdled plots the isotopic composition of DOC was similar to that of SOM or root litter. Nevertheless our data do not allow explaining the strong enrichment of $\delta^{13}C$ of soil respiration in girdled plots during the day, indicating that we may have missed a potential source for soil respiration.

Figures and Tables

Daily variations

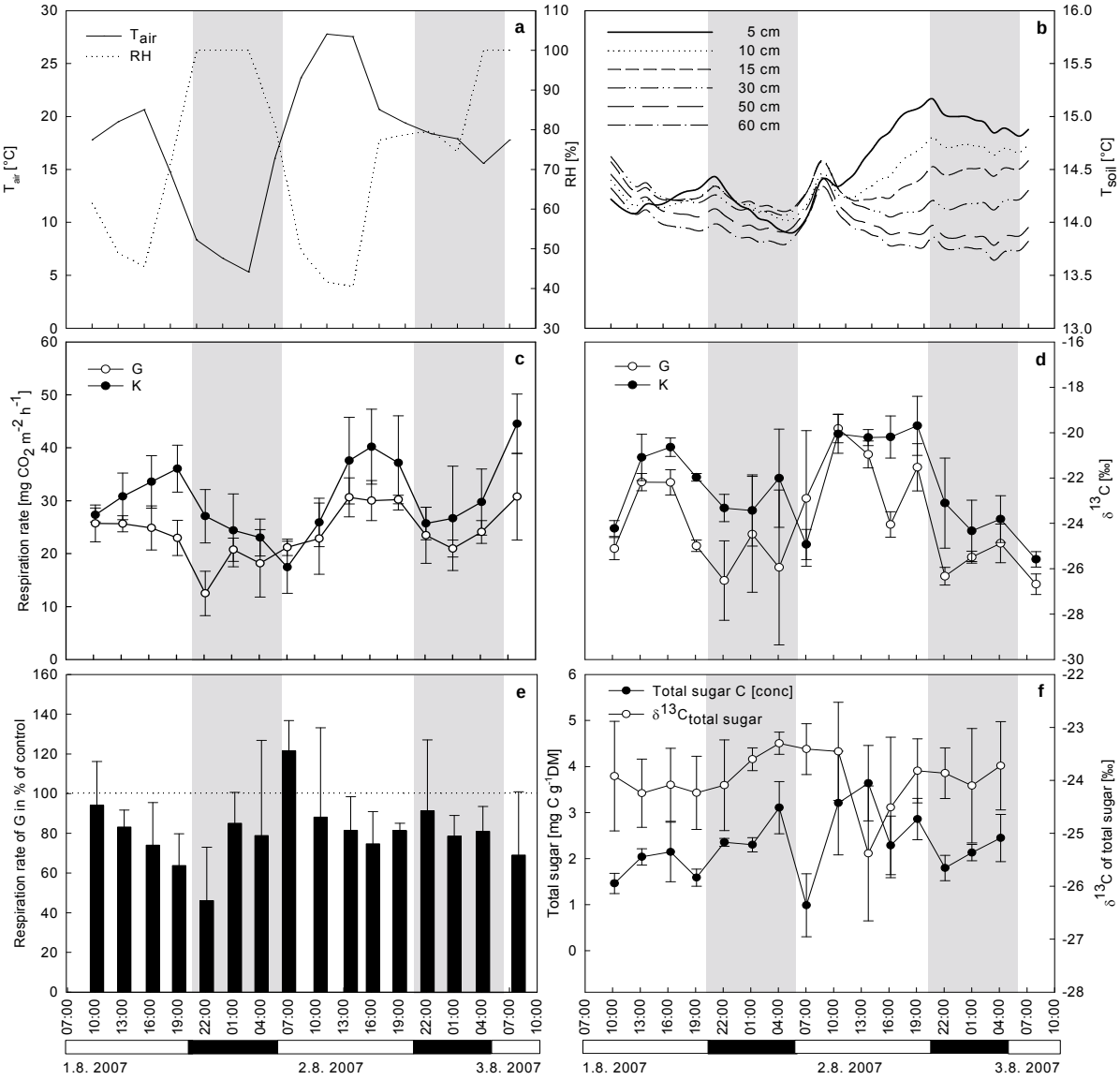

Figure 1: Data from 1.8.2007, 10 h to 3.8.2007, 8 h. **a**) Air temperature (°C) and relative air humidity (%). **b**) Soil temperature in 5, 10, 15, 30, 50 and 60 cm soil depth. **c**) Soil respiration rate of control (K) and girdled (G) plots in mg CO_2-C $m^{-2}h^{-1}$. **d**) Isotope composition ($\delta^{13}C$) of soil respiration of K and G. **e**) Respiration rate of girdled plots in % of controls. **f**) Total sugar of phloem sap exudates in mg C g^{-1} bark dry mass and $\delta^{13}C$ value of total sugar of phloem sap exudates of non girdled trees. Isotope ratios are given in ‰ relative to international V-PDB-standard. Where replicate measurements were possible values shown are means ± SE (n = 3). Open and closed bars at the bottom of the figure indicate day and night, respectively.

Table 1: Respiration rates of control (K) and girdled (G) plots (n=3) from 1.8.2007. 10 h to 3.8.2007, 8 h. Values are given in mg CO_2-$Cm^{-2}h^{-1}$. Time series differences were assessed by one-way ANOVA, homogenous groups separated by Fisher's LSD test.

	K			**G**		
Time point	Mean	± (SE)	Homogenous groups	Mean	± (SE)	Homogenous groups
01.08.2007 10:12	27.31	1.32	ab	25.70	3.48	bc
01.08.2007 13:10	30.82	4.40	abc	25.64	1.52	bc
01.08.2007 16:17	33.56	4.95	abc	24.85	4.16	bc
01.08.2007 19:07	36.05	4.42	bc	22.97	3.34	abc
01.08.2007 22:08	27.07	5.05	ab	12.48	4.20	a
02.08.2007 01:11	24.39	6.89	ab	20.74	2.20	abc
02.08.2007 04:05	23.04	3.47	ab	18.16	6.38	ab
02.08.2007 07:03	17.43	4.91	a	21.18	1.54	abc
02.08.2007 10:30	25.89	4.59	ab	22.83	6.72	abc
02.08.2007 13:46	37.58	8.19	bc	30.64	3.68	c
02.08.2007 16:11	40.20	7.08	bc	30.02	3.78	bc
02.08.2007 19:06	37.16	8.89	bc	30.21	0.82	bc
02.08.2007 22:06	25.70	3.08	ab	23.47	5.30	abc
03.08.2007 01:01	26.67	9.85	ab	20.95	1.60	abc
03.08.2007 04:08	29.75	6.25	abc	24.09	2.15	abc
03.08.2007 08:00	44.54	5.60	c	30.75	8.18	c
Total mean (n=48)	30.45	1.59		24.04	1.12	

Table 2: Isotope composition ($\delta^{13}C$) of respiration rates of control (K) and girdled (G) plots (n=3) from 1.8.2007. 10 h to 3.8.2007, 8 h. Values are given in ‰ relative to international V-PDB standard. Time series differences were assessed by one-way ANOVA, homogenous groups separated by Fisher's LSD test. (** = p<0.01).

	K			**G**		
Time point	Mean	± (SE)	Homogenous groups	Mean	± (SE)	Homogenous groups
01.08.2007 10:12	-24,22	0,35	ab	-25,11	0,49	abce
01.08.2007 13:10	-21,09	1,03	cdef	-22,18	0,38	cdef
01.08.2007 16:17	-20,64	0,41	def	-22,19	0,56	cdef
01.08.2007 19:07	-21,97	0,17	bcdef	-24,99	0,25	abcde
01.08.2007 22:08	-23,32	0,60	abcd	-26,52	1,75	ab
02.08.2007 01:11	-23,43	1,57	abcd	-24,48	2,55	bcde
02.08.2007 04:05	-22,01	2,16	bcdef	-25,94	3,42	a
02.08.2007 07:03	-24,93	0,66	ab	-22,90	2,99	bcdef
02.08.2007 10:30	-20,05	0,86	ef	-19,82	0,63	f
02.08.2007 13:46	-20,22	0,35	def	-20,96	0,59	def
02.08.2007 16:11	-20,19	0,92	ef	-24,05	0,56	bcde
02.08.2007 19:06	-19,70	1,30	f	-21,53	1,04	df
02.08.2007 22:06	-23,10	1,99	abcde	-26,33	0,39	ab
03.08.2007 01:01	-24,33	1,35	ab	-25,49	0,27	abc
03.08.2007 04:08	-23,81	1,03	abc	-24,88	0,85	abcde
03.08.2007 08:00	-25,59	0,34	a	-26,68	0,45	ab
Total mean	-22,45	0,36		-23,97	0,41	

Table 3: Significance of effects of treatment and diurnality (sampling time) on soil respiration and its isotopic values assessed by multifactor analysis of variance (ANOVA)
(Level of significance: °=p<0.1, *=p<0.05, **=p<0.01, ***=p<0.001)

	Time		Treatment		Time x Treatment	
	F	p	*F*	p	*F*	p
Soil respiration (1,80)	2.311	*	11.288	**	0.242	ns
$\delta^{13}C$ soil respiration (1, 73)	5.507	***	10.607	**	0.902	ns

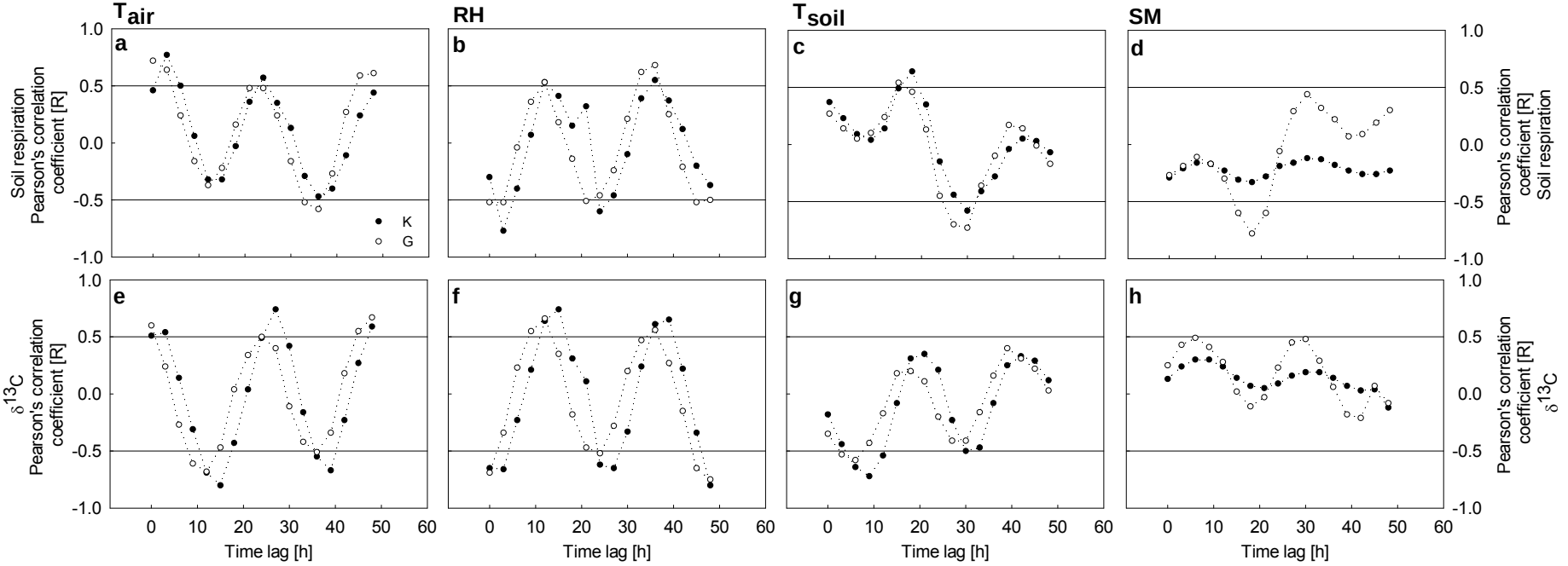

Figure 2: Pearson's correlation coefficients (R) between soil respiration (a - d) (mg CO_2-C m^{-2} h^{-1}) and $\delta^{13}C$ values of soil respiration (e - h) and T_{air} = air temperature in °C (a, e), RH = relative air humidity in % (b, f), T_{soil} = soil temperature at 5 cm depth (c, g), and SM = soil moisture in % at 15 cm (d, h), and control (K: filled dots) and girdled (G: open dots) plots, respectively, with increasing time lags (3-hour-intervals). Values for T_{air} and RH are means (n=4) of one hour before measuring soil respiration and for T_{soil} and SM means of 3 hours (n=3) before measuring soil respiration, respectively. Horizontal lines represent R > 0.5 and -0.5 reflecting level of significance: $p \leq 0.05$)

Table 4: Mean carbon concentration (±SE) of soil respiration rate [mg CO_2-$Cm^{-2}h^{-1}$] and different substrates for soil respiration [mg C g^{-1} DM]. Significant differences between control (K) and girdled plots (G) were assessed by one-way ANOVA. Levels of significance are marked by asterisks (* = p≤0.05, ** = p≤0.01, *** = p≤0.001, ns = not significant)

	n	K	±SE	n	G	±SE	
Soil respiration rate	48	30.45	1.59	48	24.04	1.12	**
Soil bulk	6	43.78	4.16	6	52.57	4.56	ns
Soil rhizosphere	6	47.89	3.47	6	54.41	3.99	ns
Leaf litter	5	515.40	22.73	6	502.43	2.76	ns
Coarse roots (>1mm) bulk	6	470.16	3.43	6	472.76	6.39	ns
Fine roots (<1mm) bulk	6	472.63	3.83	6	467.32	6.27	ns
Fine root total sugar	11	5.26	0.42	11	0.67	0.12	***
Fine root starch	11	4.36	0.28	10	3.43	0.24	*
Phloem sap sucrose	45	2.29	0.15	45	1.31	0.11	***
Root respiration	12	18.70	2.50	12	5.51	0.79	***
DOC (soil water mg L^{-1})	4	10.42	0.60	4	16.17	3.67	ns

Table 5: Mean $\delta^{13}C$ in ‰ (±SE) of soil respiration and different substrates for soil respiration. Significant differences between control (K) and girdled plots (G) were assessed by one-way ANOVA (* = p≤0.05, ** = p≤0.01, *** = p≤0.001, ns = not significant)

	n	K	±SE	n	G	±SE	
Soil respiration	43	-22.45	0.36	46	-23.97	0.42	***
Soil bulk	6	-26.06	0.15	6	-26.21	0.11	ns
Soil rhizosphere	6	-26.22	0.12	6	-26.39	0.12	ns
Leaf litter	5	-28.33	0.09	6	-28.32	0.76	ns
Coarse roots (>1mm) bulk	6	-26.79	0.14	6	-27.97	0.21	ns
Fine roots (<1mm) bulk	6	-27.28	0.08	6	-27.67	0.12	*
Fine root sugars	11	-21.37	0.23	11	-22.55	0.23	**
Fine root starch	11	-24.10	0.27	10	-25.90	0.27	***
Phloem sap sucrose	45	-23.98	0.19	45	-25.08	0.17	***
Root respiration	11	-23.09	0.40	9	-23.73	0.76	ns
DOC (soil water)	4	-21.58	0.38	4	-25.66	0.45	***

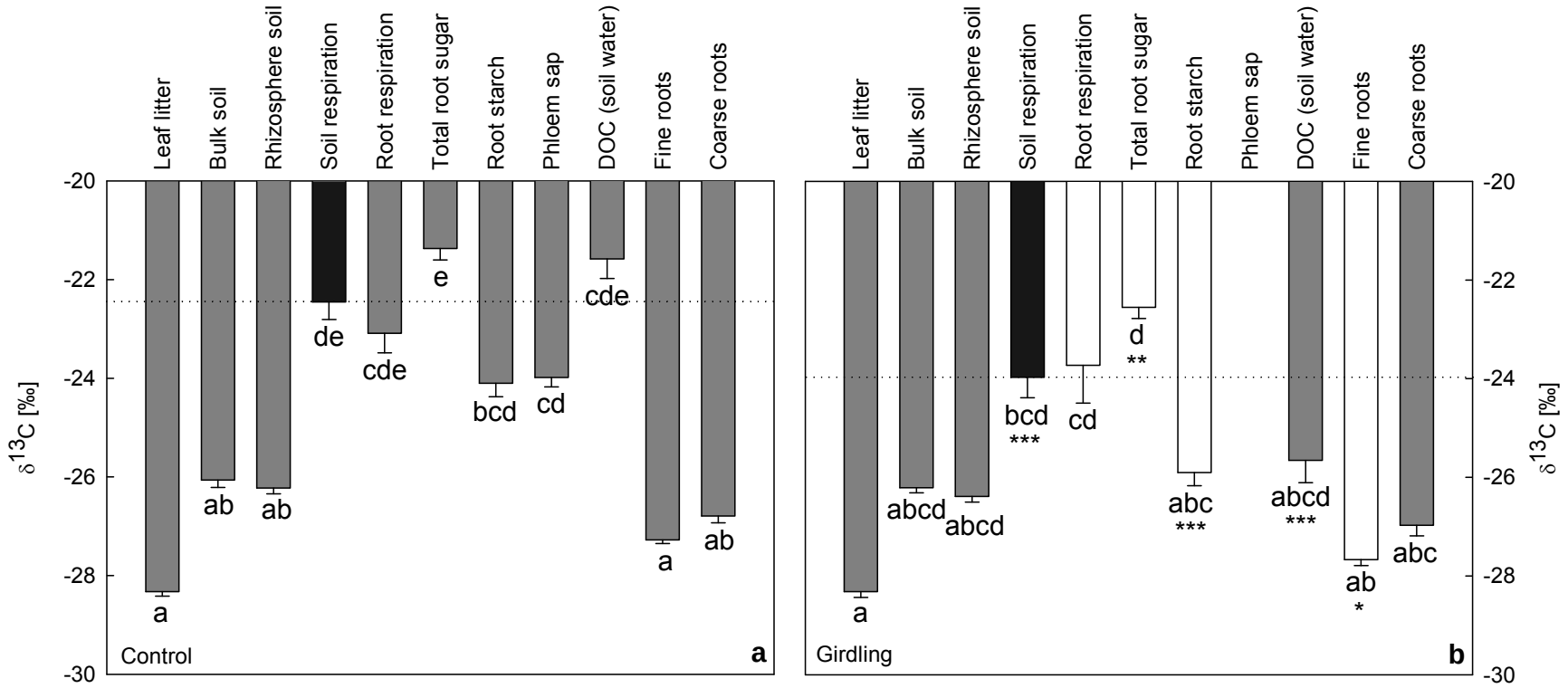

Figure 3: Mean $\delta^{13}C$ in ‰ (±SE) of soil respiration and different plant and soil compounds of **(a)** control (K) and **(b)** girdled (G) plots. Differences between measured substances were assessed by one-way ANOVA for K and G, respectively; homogenous groups among substance classes were separated by HSD-tests for non-equal n (marked by lower case letters). Differences between K and G were identified by one-way ANOVA, significant differences are marked in **(b)** by asterisks (level of significance: * = p≤0.05, ** = p≤0.01, *** = p≤0.001, ns = not significant). Black bars indicate $\delta^{13}C$ values of soil respiration, white bars indicate significant lower carbon content in G in contrast to K, and dark grey bars indicate no significant change in carbon content after girdling.

Seasonal dynamic

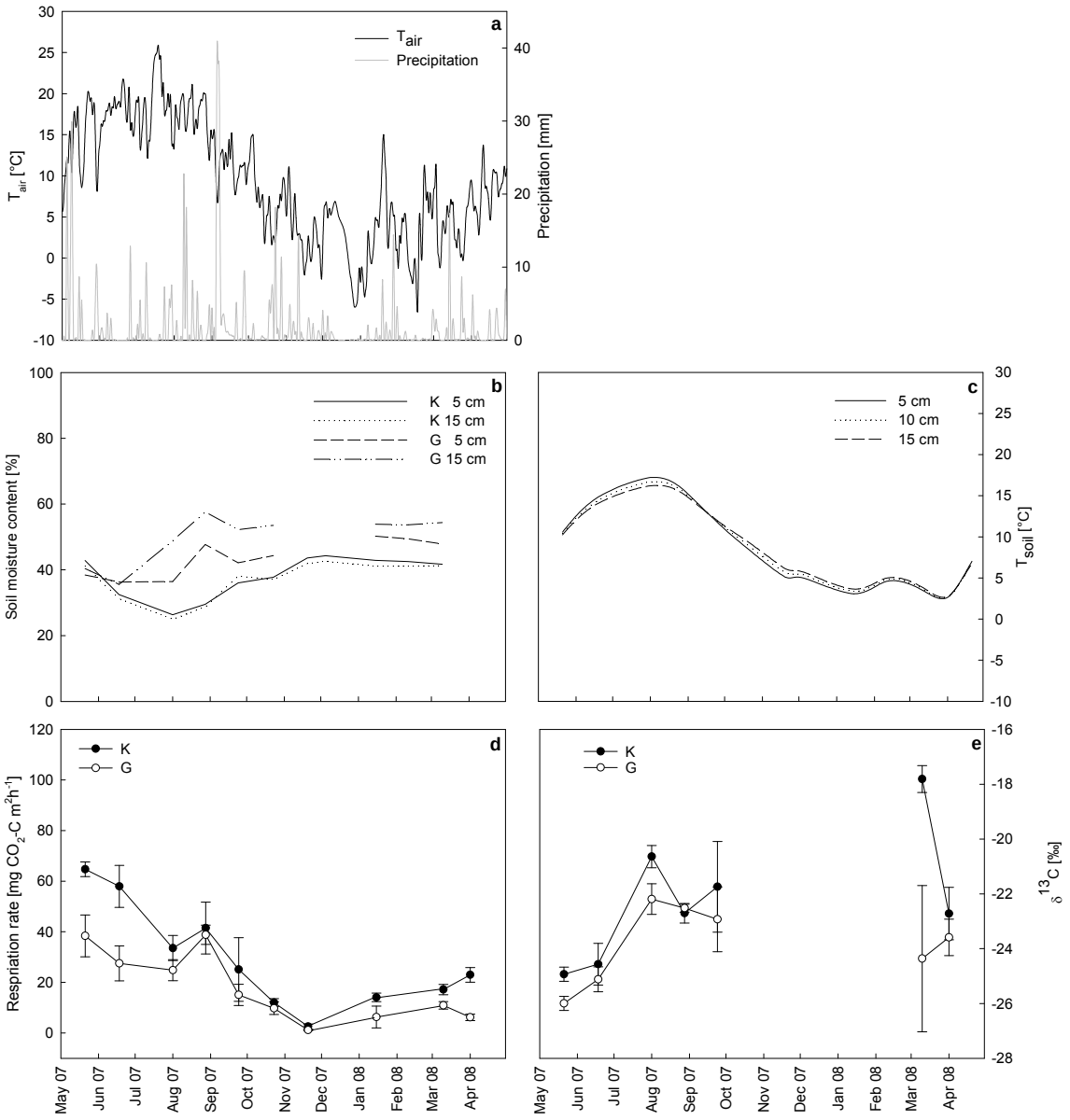

Figure 4: a) Air temperature (T_{air}) and precipitation from May 1st 2007 to April 30th 2008. Values for T_{air} are daily means; precipitation data represents the sum of precipitation of one day in mm. **b)** Soil moisture in % from control (K) and girdled (G) plots in different soil depths **c)** Soil temperature (T_{soil}) from May 2007 to April 2008 in 5, 10 and 15 cm depth. Values of soil moisture and T_{soil} represent means of one week before sampling. **d)** Soil respiration in mg CO_2-C $m^{-2}h^{-1}$ and its isotopic composition **(e)** in ‰ relative to V-PDB standard in girdled (G; white dots) and control (K; black dots) plots (*n = 3* for each treatment) from May 2007 to April 2008 (Errorbars = SE)

Table 6: Pearson's correlation coefficients calculated between soil respiration rates (resp) and their isotopic composition ($\delta^{13}C$) and T_{air} = air temperature in °C, precipitation in mm, T_{soil} = soil temperature in °C at 5, 10 and 15 cm depth, and SM = soil moisture in % at 15 cm for control (K) and girdled (G) plots, respectively, over the course of one year. Values for $T_{air,}$ T_{soil} and SM represent means of one week before gas sampling. Precipitation represents the sum of rainfall one week before gas sampling.
(Level of significance: * = p≤0.05, ** = p≤0.01, *** = p≤0.001, ns = not significant)

	Tair	Precipitation	SM	Tsoil 5 cm	Tsoil 10 cm	Tsoil 15 cm
K_{resp}	**0.71**	-0.37	-0.002	0.45	0.42	0.39
	**	ns	ns	ns	ns	ns
G_{resp}	**0.80**	-0.22	-0.31	**0.64**	**0.61**	**0.60**
	**	ns	ns	*	*	*
$K_{\delta 13C}$	-0.35	0.08	-0.05	-0.16	-0.15	-0.15
	ns	ns	ns	ns	ns	ns
$G_{\delta 13C}$	-0.25	-0.08	0.23	-0.15	-0.16	-0.16
	ns	ns	ns	ns	ns	ns

Table 7: Significance of effects of treatment and seasonality (sampling month) on soil respiration and its isotopic values assessed by multifactor analysis of variance (ANOVA)
(Level of significance: °=p<0.1, *=p<0.05, **=p<0.01, ***=p<0.001)

	Treatment		Season		Treatment x Season	
	F	p	*F*	p	*F*	p
Soil respiration (1, 39)	21.078	***	18.454	***	1.7383	ns
$\delta^{13}C$ soil respiration (1, 26)	12.255	**	6.845	***	2.446	°

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Zusammenfassung

- Wir charakterisierten die Bodenatmung (CO_2) und ihre isotopische Zusammensetzung (^{13}C) eines temperaten Buchenwaldes (Fagus sylvatica) im Tages- bzw. Jahresverlauf und untersuchten die Aufteilung der Bodenatmung in ihre autotrophen und heterotrophen Anteile. Die Aufteilung in autotrophe und heterotrophe Bodenatmung wurde durch Vergleichen von Atmungsraten in beringelten und unbehandelten Kontrollflächen erreicht. Zusätzlich untersuchten wir ein umfangreiches Set an potentiellen Substraten und Quellen der Bodenatmung auf ihre $\delta^{13}C$ Werte.
- In Kontrollflächen war Pflanzenmaterial (Laubstreu, Fein- und Grobwurzeln), sowie organisches Material im Boden (SOM) stärker an ^{13}C abgereichert, als Kohlenhydrate in Pflanzenorganen (Zucker im Phloemsaft, Stärke und Zucker in Wurzeln) und Wurzelatmung, was anzeigt, dass ein größerer Teil von Bodenatmung von kürzlich assimiliertem Kohlenstoff angetrieben wird. Wir nahmen an, dass die Isotopensignatur von gelöstem organischem Kohlenstoff (DOC) die Isotopensignatur der Quellen, wie Wurzelexudate oder Produkte von Abbauprozessen, die zum DOC beitragen widerspiegelt. In Kontrollen war DOC stärker angereichert an ^{13}C als in den geringelten Flächen, und wies ähnliche Isotopensignaturen, wie die der Wurzelzucker auf. Das könnte darauf hindeuten, dass Wurzelexudate einen wesentlichen Anteil am DOC ausmachen.
- Beringeln führte zu einem durchschnittlichen Rückgang der Gesamtbodenatmung um 36 % und zu stärker abgereicherten ^{13}C Signaturen von veratmeten CO_2 um 1.5 ‰ im Jahresverlauf, was einen Rückgang der autotrophen Atmung und eine Verschiebung Richtung Abbau von Wurzelstreu und SOM anzeigt. Dies wurde auch von stärker abgereicherten Isotopensignaturen im DOC von geringelten Flächen bestätigt.
- Wir beobachteten Schwankungen von Bodenatmung und $\delta^{13}C$ der Bodenatmung im Tages- und Jahresverlauf. Wir identifizierten Lufttemperatur als Hauptkontrollfaktor für Bodenatmung sowohl in beringelten, als auch in den Kontrollflächen auf verschieden Zeitskalen. In beringelten Flächen korrelierte im Jahresverlauf die Bodenatmung auch mit Bodentemperatur. Die $\delta^{13}C$ Werte der Zucker aus dem Phloemsaft wiesen keine signifikanten Veränderungen im Tagesgang auf und korrelierte nicht mit den $\delta^{13}C$ Werten der Bodenatmung in Kontrollflächen.

Curriculum vitae

Personal information

Name	Lucia Fuchslueger
Date of birth	20.05.1982
Place of birth	Ybbsitz, Lower Austria

Education

Since May 2007	Diplomathesis at the Department of Chemical Ecology and Ecosystem Research
October 2004	Intermediate degree in biology
November 2002	Biology at the University of Vienna
October 2001	Molecular Biology at the University of Vienna
June 2001	Matura (final exam qualifying for university admission)
1996 – 2001	Höhere Bundeslehranstalt für wirtschafliche Berufe, emphasising Environmental Economy, in Weyer, Upper Austria
1992 – 1996	Secondary School in Ybbsitz, Lower Austria
1988 – 1992	Primary School in Ybbsitz, Lower Austria

Work experience and practica

2010	Tutor for the course „Structure and function of terrestrial and aquatic ecosystems" Tutor for the course "Kenntnis mitteleuropäischer Lebensgemeinschaften"
2009	Tutor for the course „Übungen zur funktionenllen Ökologie" Tutor for the course „Kenntnis mitteleuropäischer Lebensgemeinschaften"
2007	Tutor for the lecture „Struktur und Funktion der Höheren Pflanzen"
Since 2008	Project co-worker for at the department of Chemical Ecology and Ecosystem Research including field work, laboratory analyses and sample preparation

Further work experience

2007 – 2010	La Salvia, Vienna (service and sales assistant)
2007 – 2008	Museumsquartier, Vienna (event co worker)
1998 – 2004	Welser Profile, Gresten (administration)
2001, 2002	Evangelisches Krankenhaus, Vienna (magnetic field therapist, administration)
1999	ARCOTEL Wimberger, Vienna (banquet office trainee)