On the Tree-Root-Soil-Continuum – Temporal and Spatial Coupling of the Belowground Carbon Flux

Sabine Göttlicher
Faculty of Forest Sciences
Department of Forest Ecology and Management
Umeå

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Abstract

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The direct flux of current assimilates from the tree canopy to the belowground compartment drives roughly half of the soil respiratory activity in boreal forests. This thesis focuses on temporal and spatial aspects of the carbon (C) flux within the tree-root-soil continuum in temperate and boreal forests. I used the stable isotopes ^{13}C and ^{15}N to follow C from the canopy to the belowground compartment and the flow of nitrogen in the reverse direction.

The C isotope composition of photosynthate varies diurnally, but such variations could not be observed in soil-respired CO_2. Labelling of small (up to 4.5 m) *Pinus sylvestris* trees with $^{13}CO_2$ showed that it took two days for the photosynthate to reach the soil. The velocity of the phloem flux was *c.* 0.1 m h^{-1}.

This flux of C is absolutely vital for the production of sporocarps by ectomycorrhizal fungi, as shown by their paucity in plots with girdled trees. It is also likely to be important for other soil microorganisms; addition of a labile ^{13}C labelled C source revealed a lack of labile C substrates in girdled plots. The reduction in the abundance of ectomycorrhizal sporocarps from the edges to the centre of girdled plots and a ^{15}N uptake experiment showed that lateral spread of ectomycorrhizal roots was on average 4 to 5 m from the trunks. Thus, it can be expected that an area of *c.* 60 m^2 of soil is under the influence of direct flux of current assimilates from the tree canopy of a single tree. Areas of influence of several trees overlapped.

I conclude that canopy and soil processes are coupled with time lags of a few days. The direct impact of plant photosynthate should be considered more often in studies of soil. The $^{13}CO_2$ labelling study demonstrated that it is now possible to follow at a very high resolution the fate of this C into the belowground system.

Key words: boreal forest, carbon, ^{13}C labelling, ectomycorrhiza, girdling, tree roots, phloem, stable isotopes, soil respiration, substrate induced respiration

Author´s address: Sabine G. Göttlicher. Department of Forest Ecology and Management. SLU, S - 901 83, Umeå.

Contents

Appendix

Paper I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Göttlicher, S., Knohl, A., Wanek, W., Buchmann, N. & Richter, A. 2006. Short-term changes in carbon isotope composition of soluble carbohydrates and starch: from canopy leaves to the root system. *Rapid Communications in Mass Spectrometry 20*, 653-660.

II. Betson, N.R., Göttlicher, S.G., Hall, M., Wallin, G., Richter, A. & Högberg, P. 2007. No diurnal variation in rate or carbon isotope composition of soil respiration in a boreal forest. *Tree Physiology 27*, 749-756.

III. Högberg, P., Högberg, M.N. Göttlicher, S.G., Betson, N.R., Campbell, C., Schindlbacher, A., Hurry, V., Lundmark, T., Linder, S., Näsholm, T. 2007. High-resolution tracing of photosynthate carbon from the tree canopy to the forest soil microorganisms. *Manuscript.*

IV. Göttlicher, S.G., Steinmann, K., Betson, N.R. & Högberg, P. 2006. The dependence of soil microbial activity on recent photosynthate from trees. *Plant and Soil 287*, 85-94.

V. Göttlicher, S.G., Taylor A.F.S, Grip, H., Betson, N.R., Valinger, E., Högberg M.N., Högberg P. 2007. The lateral spread of tree root systems in boreal forests: estimates based on ^{15}N uptake and distribution of sporocarps of ectomycorrhizal fungi. *Manuscript.*

Introduction

This thesis focuses on the direct belowground flux of tree-derived carbon (C) in temperate and boreal forest ecosystems, i.e. the C allocated directly from photosynthesis to roots and their associated microorganisms and its subsequent release back to the atmosphere. As forests cover an area of almost $40*10^6$ km^2 of the $132*10^6$ km^2 ice-free terrestrial area (Matthews, 1983) and their Net Primary Productivity (NPP) is $33*10^{15}$ g C yr^{-1} (Chapin, Matson & Mooney, 2002), C fluxes within forests are of interest on the global scale. Consequently, much recent research has focused on the quantification of forest C fluxes due to their impact on climate change, but many details of the C flux within forests remain poorly described. Knowledge of these details is fundamental to improving our understanding of both forest ecosystem functioning and making correct interpretations of data obtained from C flux studies. This thesis discusses approaches to follow the flux of C from fixation during photosynthesis to various destinations in the tree-soil continuum in the field, using natural variations in the stable C isotope composition and $^{13}CO_2$ labelling. This thesis also discusses the dependence of soil microbial activity on recent photosynthate and the lateral spread of mycorrhizal roots, a key parameter to understand the spatial distribution of direct belowground flux of tree-derived C.

The C flux from the atmosphere through plants, to the soil and back to the atmosphere

A simplified illustration of the C flux from the atmosphere through a tree, to the soil and back to the atmosphere is given in Fig. 1.

Short overview of photosynthetic C fixation

Carbon dioxide from the atmosphere is fixed during the process of photosynthesis. In C_3-plants this means that ribulose-1,5-bisphosphate (Rubisco) is carboxylated and the first product is a triose phosphate, a compound with three C atoms (hence C_3-photosynthesis). Some of the triose phosphate is exported to the cytosol, where it is used to produce sucrose and other metabolites that are used for respiration and growth in the leaves or that are exported via the phloem. Most of the triose phosphate remaining in the chloroplast is used to regenerate Rubisco but some is also used to produce starch, which is stored in the chloroplasts. During the night this starch may be hydrolysed and the resulting triose phosphate is then exported to the cytosol.

The C flux from leaves to roots

Carbon assimilated in leaves is either respired immediately by the leaves, stored or used as a structural component in growth. A proportion of the C is also exported

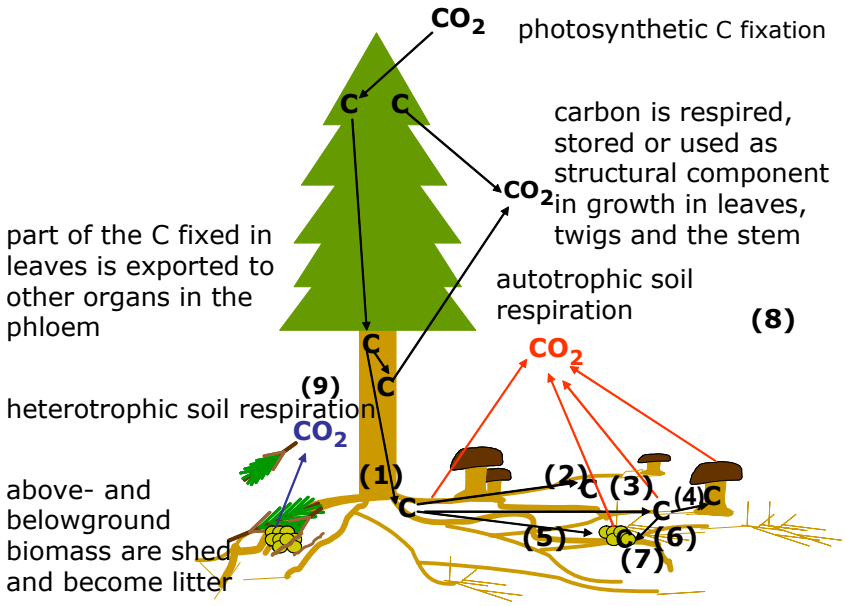

Figure 1. Simplified illustration of the carbon (C) cycle in a forest

1. Part of the C in the phloem is transported belowground.
2. Carbon is stored or used as structural component in roots.
3. Part of the C that is transported belowground is exported to ectomycorrhizal fungi.
4. Carbon is respired immediately, stored or used as structural component in growth by ectomycorrhizal fungi.
5. Roots exude C.
6. Mycorrhizal hyphae exude C.
7. Microorganisms in the rhizosphere take up this C.
8. Roots, mycorrhizal fungi and rhizosphere microorganisms respire C (red arrows). Together their respiration forms the autotrophic component of soil respiration.
9. Soil microorganisms decompose above- and belowground litter. Their respiration forms the heterotrophic component of soil respiration (blue arrow).

from the leaves to twigs, stems and roots and export of new photosynthates may start as soon as 10 min after fixation (Jahnke *et al.,* 1998). In the phloem, C is transported as sucrose (sometimes as oligosaccharides of the raffinose family or sugar alcohols (Taiz & Zeiger, 2002)) from the source (usually leaves) to the sink (e.g. stem, young leaves, seeds and roots). Phloem translocation is usually explained according to the pressure-flow model (first proposed by Münch (1930)). Briefly, the flow of solution in the sieve elements is driven by an osmotically generated pressure gradient. The pressure gradient is generated by phloem loading at the source and phloem unloading at the sink (Farrar & Jones, 2000; Taiz &

Zeiger, 2002). Reports of phloem transport velocities range from 0.2 to 2 m h^{-1} (Taiz & Zeiger, 2002; Nobel, 2005) varying with plant species and vigour of growth (Nobel, 2005). As in leaves, some C in twigs and stems is respired, stored or used for growth. Part of the C is transported further to roots.

Definitions and abbreviations:

Basal Respiration: see SIR
C_3-plant: Plant with the C_3-photosynthetic pathway. Ribulose-1,5-bisphosphate is carboxylated and the first product is phosphoglyceric acid, a compound with three C atoms (hence C_3-photosynthesis).
C_4-plant: Plant with the C_4-photosynthetic pathway. C is first assimilated in the mesophyll cells. PEP-carboxylase uses phosphoenolpyruvate and HCO_3^- as substrates. The product of this reaction is oxalacetate, a compound with four C atoms (hence C_4-photosynthesis).
EA-IRMS: Elemental Analysis-Isotope Ratio Mass Spectrometry
FACE: Free Air CO_2 Enrichment
HPAEC-PAD: High-Pressure Anion-Exchange Chromatography with Pulsed Amperometric Detection
Induced Respiration: see SIR
microsatellites: Regions within DNA sequences where short sequences of DNA are repeated one after the other. At the same location within the genomic DNA the number of times the sequence is repeated often varies between individuals, within populations, or between species and can thus be used for identification.
minirhizotrons: Minature camera inserted into the soil in a clear tube. Photographic images of root growth outside the tube can be obtained.
mycorrhizosphere: Zone of soil influenced by mycorrhizal roots and mycorrhizal hyphae
root exudates: substances released into the soil by intact plant roots
rhizoplane: surface area of a plant root
rhizosphere: soil around a plant root
SIR: Substrate Induced Respiration. Soil respiration after addition of a C source. It is the sum of Basal Respiration (respiration without substrate addition) and Induced Respiration.

Carbon flux within the belowground compartment

In the roots, C is used for growth, stored, respired, passed on to mycorrhizal fungi or released to the rhizosphere as root exudates. Root exudates include substances such as sugars, amino acids, organic acids, hormones, phenolics and enzymes (Lynch & Whipps, 1990; Jones, Hodge & Kuzyakov, 2004). The concentration of exudates decreases exponentially with distance from the roots (Kuzyakov, Raskatov & Kaupenjohann, 2003); the maximum distance reached by root exudates is thought to be *c.* 3 mm (Kuzyakov, Raskatov & Kaupenjohann, 2003). Exudates are either a result of passive diffusion, over which the plant exerts little control (basal exudation), or are released for a specific purpose, a process that is controlled by the plant and may involve opening of membrane channels (Jones, 1998). Most organic acids are almost fully dissociated in the cytosol and cell membranes are nearly impermeable to ions, thus trans-membrane diffusion is

restricted to undissociated organic acids (Ryan, Delhaize & Jones, 2001). However, the efflux of organic acids can be increased by opening of channels embedded in the lipid bilayer (Jones, 1998; Ryan, Delhaize & Jones, 2001). In the case of uncharged solutes such as sugars, it is likely that loss from roots occurs as a result of passive diffusion (Jones, 1998). Root exudates increase water soluble C concentrations in the rhizoplane and rhizosphere as compared to bulk soil (Cheng *et al.,* 1996). Thus, microbial respiration is less limited by available C in the rhizoplane and rhizosphere than in the soil further away from roots (Cheng *et al.*, 1996). The higher C availability in the rhizosphere also results in higher microbial biomass relative to in the bulk soil (Butler *et al.*, 2004; Phillips & Fahey, 2006). It has also been suggested as being a primary reason for the evolution of mycorrhizal symbiosis (Smith & Read, 1997).

Nearly all plant roots are mycorrhizal (Smith & Read, 1997) and mycorrhizal fungi are more directly supplied with C from roots than other microorgansims that take advantage of root exudates. The interface between tree roots and ectomycorrhizal fungi consists of continuous root cell walls and fungal hyphal walls with material deposited between them, creating an apoplastic compartment (Peterson & Massicotte, 2004). Carbon is transported probably mainly as sucrose from root cells to the apoplastic compartment, where acid invertase from the plant converts sucrose to glucose and fructose that can be taken up by the fungal hyphae (Peterson & Massicotte, 2004). Mycorrhizal fungi and other soil microoganisms store plant-derived C in their biomass, use it as a structural component or respire it.

In this thesis the component of soil respiration, that is directly sustained by photosynthates (i.e. respiration of roots, ectomycorrhizal fungi and rhizosphere microorganisms that utilize root exudates) is called autotrophic. The share of soil respiration coming from decomposition of above- and belowground litter (e.g. leaves, twigs and roots) is called the heterotrophic component of the soil respiration. This distinction between autotrophic and heterotrophic focuses on function. It is, however, a matter of debate if root respiration should be lumped together with the respiration of taxonomically heterotroph organisms (e.g. Kuzyakov (2006a); Kuzyakov (2006b) and Högberg, Buchmann & Read (2006)).

Carbon that is transferred belowground has different turnover times. It has been suggested that the part of the C respired or exuded by roots and mycorrhizal fungi is released within days after its fixation in the canopy (Ekblad & Högberg, 2001; McDowell *et al.*, 2004; Ekblad *et al.*, 2005). There is however, less consensus within the literature regarding the production of root litter. Studies using repeated soil coring and minirhizotrons have often suggested that fine roots of trees have short life spans with the whole fine root system turning over one or several times per year (Vogt, Grier & Vogt, 1986; Burke & Raynal, 1994; Gill & Jackson, 2000; Ostonen, Lõhmus & Pajuste, 2005). Other approaches indicate substantially longer

life spans for roots, for example data from trees grown under elevated CO_2 (^{13}C depleted CO_2) and from ^{14}C measurements indicate life spans of several years (Gaudinski *et al.*, 2001; Matamala *et al.*, 2003; Johnsen, Maier & Kress, 2005).

The importance of C that is transported belowground was demonstrated in a girdling experiment in a boreal forest, where the supply with current photosynthates was terminated by cutting through the stem bark to the depth of the current xylem (Högberg *et al.*, 2001). This experiment demonstrated that at least half of the CO_2 released from soil is root mediated (i.e. autotrophic) and that the soil system responds immediately to the withdrawal of current photosynthate supply (Högberg *et al.*, 2001; Bhupinderpal-Singh *et al.*, 2003). Within 5 days after girdling, soil respiration dropped by about 37% (Högberg *et al.*, 2001). The vital dependence of mycorrhizal fungi on current C supply from the tree was also demonstrated by the almost complete lack of production of mycorrhizal sporocarps in girdled plots 2 months after girdling (Högberg *et al.*, 2001). Disruption of the phloem also reduces soluble soil C (Högberg & Högberg, 2002; Scott-Denton, Rosenstiel & Monson, 2006; Johnsen *et al.*, 2007) and thus, it is likely that other soil organisms are affected by a disruption of the plant belowground C flux, especially in the longer term.

The area of soil that is affected by belowground transport of C is determined by the lateral root spread which is often larger than the spread of the aboveground canopy (Brunner *et al.*, 2004; Johnsen, Maier & Kress, 2005). For Scots pine and Norway spruce lateral root spread of up to 21 and 18 m, respectively have been reported (Stone & Kalisz, 1991). Lateral root spread can be determined by a range of approaches, such as excavating roots (Drexhage & Gruber, 1998), microsatellite markers (Brunner *et al.*, 2004; Saari *et al.*, 2005), by uptake of nutrient analogues, such as Rb, Cs, Li or Sr (Casper, Schenk & Jackson, 2003), by uptake of stable or radioactive isotopes (Peek & Forseth, 2005; Hartle, Fernandez & Nowak, 2006) by ground penetrating radar or by excavation using an ultrasonic air-stream (Nadezhdina & Čermák, 2003). Uptake of nutrient analogues or of stable isotopes to estimate lateral root spread has been studied for small plants (Hawkes & Casper, 2002; Casper, Schenk & Jackson, 2003; Peek & Forseth, 2005), but there are fewer studies concerning forest communities because the larger lateral root spread and the size of the trees increase the sampling effort. However, knowledge of the extent of lateral root spread in forest communities is important because within this area of influence a tree competes with other plants, causes localized increases in the C availability for microorganisms (especially within the mycorrhizosphere) and competes with micoroorganisms for nutrients (e.g. N). Furthermore, the extension of this area has implications for forest management and the design of plot based studies in forest science.

The horizontal distribution of ectomycorrhizal roots is only one of the parameters that determine the spatial distribution C that is transported belowground. Vertical root distribution is another key parameter. In boreal forests

83% of the root biomass are in the upper 30 cm (Jackson *et al.*, 1996), but roots of Norway spruce and Scots pine were found up to 6 and 8 m soil depth, respectively (Stone & Kalisz, 1991). Norway spruce was reported to have a more shallow rooting profile in mixture with beech than in pure spruce stands (Schmid & Kazda, 2001; Schmid & Kazda, 2002). Thus the rooting depth, which is also the depth to which the soil is directly supplied with C from a tree can vary substantially within a species. On a finer scale the vertical distribution of C within the soil might also depend on the ectomycorrhizal species composition. The amount of extramatrical mycelium varies between species. Ectomycorrhizal species composition itself varies between soil horizons (Rosling *et al.*, 2003). Further, the vertical distribution of extramatrical mycelium varies from that of mycorrhizas (Genny, Anderson & Alexander, 2005) adding another factor that influences vertical distribution of C within the soil.

Accounting for C that is allocated belowground

For a Swedish coniferous forest, where 60% of the NPP was allocated belowground, it was estimated that 25% of the flux into the belowground system via the stem was allocated to root growth and that 75% percent supported the respiratory activities of roots, their mycorrhizal fungi and other closely related organisms (Högberg, Nordgren & Ågren, 2002). These estimates are comparable with the results from pulse-labelling studies in a grassland, where between 12 and 22% of the C taken up by photosynthesis was allocated to root biomass (Leake *et al.*, 2006). It is estimated that between 2 and 4% of the net fixed C is used for root exudation (Jones, Hodge & Kuzyakov, 2004). However, these labile compounds are difficult to quantify (van Hees *et al.*, 2005). The cost of maintaining ectomycorrhizal associations has been estimated at between 15 and 28% of the net C fixation (Finlay, 2004). Since nearly 100% of the tree root tips in boreal forests are ectomycorrhizal (Taylor, Martin & Read, 2000) substantial amounts of fixed C are passed on to ectomycorrhizal fungi in these forests. There is, however, a considerable uncertainty about how much of the C that flows through this C sink flows further out into the soil (Godbold *et al.*, 2006).

Stable C isotopes in ecosystem research

General background

Fortunately, carbon can be traced throughout its way through ecosystems as there are several C isotopes (stable and radioactive). The abundance of these isotopes can be changed experimentally to follow and quantify C fluxes or natural variation can be exploited to trace C fluxes (Coleman and Fry, 1991). In this thesis variations in the abundances of the two stable isotopes, ^{12}C and ^{13}C have been used. About 98.9% of the atoms are ^{12}C and the remaining 1.1% ^{13}C, but precise measurements reveal that there are minute, non-random variations in the ratio

between these two isotopes. In this context the isotopic composition is usually expressed in δ values – parts per thousand differences from a standard.

$$\delta^{13}C = 1000 * \left(\frac{R_{sample} - R_{standard}}{R_{standard}} \right),$$

where R is the ratio of the heavy (^{13}C) to the light isotope (^{12}C). The international standard for $\delta^{13}C$ is Vienna Pee Dee Belemnite (V-PDB).

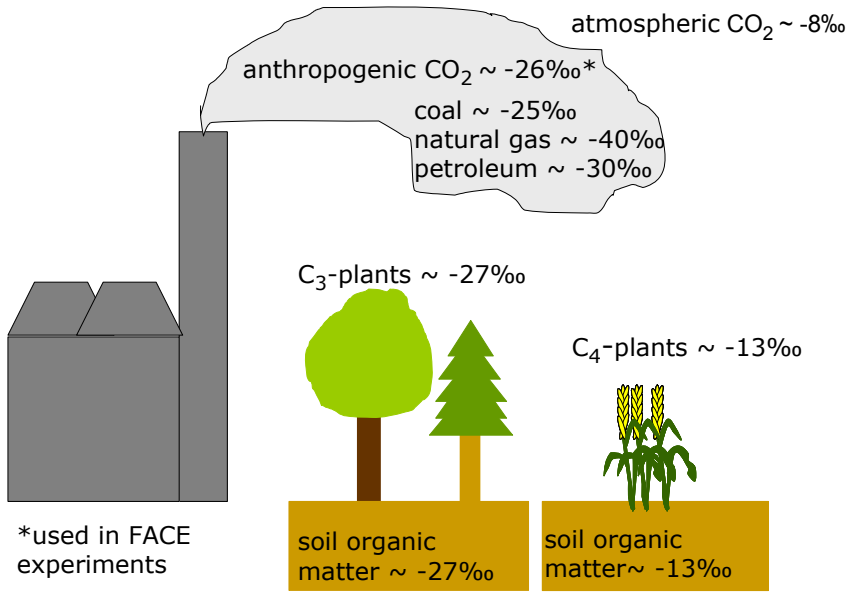

Figure 2. Variation in the carbon (C) isotope composition of important terrestrial C pools and sources.

An illustration of the variation in the C isotope composition of important terrestrial C pools and sources is given in Fig. 2. Variation in isotope composition among different compounds is due to isotope fractionation during physical, chemical and biological processes. Fractionation processes occur throughout photosynthesis in C_3-plants, namely during gaseous diffusion of CO_2 from the atmosphere, through the canopy and leaf boundary layers and through stomata to the leaf intercellular spaces, then when CO_2 is dissolved in solution (in the leaves) and finally during carboxylation (Brugnoli & Farquhar, 2000). The C isotope discrimination during C_3-photosynthesis is described in a model by Farquhar, O'Leary & Berry (1982). This model can be simplified to the following equation:

$$\Delta = a + (b - a) * \frac{c_i}{c_a},$$

where (a) is the fractionation occurring during diffusion in air (*c.* 4.4‰), (b) is the discrimination occurring during net carboxylation, (c_i) is the CO_2 concentration within the leaf intercellular spaces and (c_a) is the atmospheric CO_2 concentration. The largest of these isotope effects is the one occurring during carboxylation (b) by Rubisco, which is 28.2 to 30‰ (Brugnoli & Farquhar, 2000). The ratio between the CO_2 concentration within the leaf intercellular spaces (c_i) and the atmospheric concentration (c_a) determines if discrimination is dominated by diffusional fractionation (low c_i/c_a) or by fractionation during carboxylation (high c_i/c_a). Thus, if the c_i/c_a is low, the fractionation during photosynthesis is also low (Brugnoli & Farquhar, 2000). The c_i/c_a ratio itself is determined by the balance between stomatal conductance and photosynthetic CO_2 assimilation (Farquhar & Sharkey, 1982). Photosynthesis and stomatal conductance are influenced by a number of factors such as light conditions, water supply, temperature and air vapour pressure deficit (Chapin, Matson & Mooney, 2002). Thus, environmental factors and the C isotope composition of leaf material are linked.

C_4-plants have less negative $\delta^{13}C$ values as the C isotope discrimination by PEP-carboxylase, the enzyme responsible for primary carboxylation, is smaller (2.2‰) (Brugnoli & Farquhar, 2000).

Post photosynthesis natural variation in stable isotopes

Once C is fixed, isotope effects associated with various metabolic pathways may produce compounds which differ in their isotope composition. Starch, for example, has less negative $\delta^{13}C$ values than soluble sugars (Brugnoli *et al.*, 1988), while lipids have lower $\delta^{13}C$ values compared to the substrate they are built from (DeNiro & Epstein, 1977). Different functional components of the ecosystem also show systematic differences in their isotope composition. Host specific ectomycorrhizal fungi were found to have a $\delta^{13}C$ value 1.2 to 2.9‰ higher than foliage of their host species, but were isotopically lighter than saprotrophic fungi in the same ecosystem (Högberg *et al.*, 1999). A difference of *c.* 4‰ was observed between the C of leaves (Högberg *et al.*, 1999) or wood (Gleixner *et al.*, 1993) and the C of saprotrophic fungi.

Due to the photosynthetic pathway, the C isotope composition of C_3- and C_4-plants differs, but within C_3-plants the C isotope composition can also vary substantially. Leaf C isotope ratios of boreal plants are reported to span a range of 11‰, with a mean $\delta^{13}C$ value of -29.4‰ (Brooks *et al.*, 1997). Variation in the C isotope composition between chemical compounds, between plant organs, between the same organs depending on position within the plant and between different

plants results in a wide range of C isotope composition of substrates for respiration and decomposition (Brooks *et al.*, 1997; Damesin & Lelarge, 2003; Scartazza *et al.*, 2004). Further, temporal changes (e.g. during a growing season) in the C isotope composition of plant material add even more variation (Pate & Arthur, 1998; Damesin & Lelarge, 2003; Scartazza *et al.*, 2004). Laboratory studies have shown that leaf respired CO_2 can be enriched in comparison with carbohydrates, the most enriched metabolites (Tcherkez *et al.*, 2003), or sucrose (Duranceau *et al.*, 1999). Even measuring a range of possible respiratory substrates does not always explain the observed pattern in $\delta^{13}C$ of respired CO_2. For example, the C isotope composition of leaf respired CO_2 showed diurnal changes during a 24 h period whereas sugar, starch and lipids did not (Hymus *et al.*, 2005). As regards soil respiration the two components - the autotrophic and the heterotrophic - make it even more complex to understand the relationship between the C isotope composition of respired CO_2 and the supposed respiratory substrates.

The phloem sap usually contains a high amount of sucrose and a substantial part of the sucrose is transported belowground, where it fuels autotrophic respiration. Thus, phloem sap is of particular interest in studies of the relationship between the C isotope composition of the autotrophic component of soil-respired CO_2 and respiratory substrates. In some tree species the phloem sap bleeds out and can be collected directly, while in other species the sugars have to be extracted from phloem tissue (King & Zeevaart, 1974; Geßler, Rennenberg & Keitel, 2004).

A fundamental requirement for studying the relationship between the C isotope composition of respired CO_2 and the putative respiratory substrates is a precise method for the isolation of respiratory substrates. Very often C that is introduced during the isolation procedure (blank C) confounds results. Low and reproducible blanks are especially crucial if the samples contain very small amounts of carbohydrates, since in this case the contribution of the C isotope composition of blank C to total C is high.

Stable isotope labelling studies

If the variation in natural abundance of C isotopes is not large enough, compounds that differ substantially in their C isotope composition from the C occurring naturally can be used to study C dynamics in the system. This can be achieved by applying, for example, C_4-plant derived material in a system that is dominated by C_3-plants or *vice versa* (Robinson & Scrimgeour, 1995; Högberg & Ekblad, 1996; Ekblad & Högberg, 2000; Nyberg *et al.*, 2000), or by using CO_2 that has fossil origin and is depleted in ^{13}C (Andrews *et al.*, 1999; Keel, Siegwolf & Körner, 2006) or CO_2 or other compounds that have been technically enriched in the heavier isotope (Simard *et al.*, 1997; Ostle *et al.*, 2003). Labelling with CO_2 highly enriched in ^{13}C is usually performed as pulse labelling, because it is very expensive. While CO_2 of fossil origin is much cheaper on a unit basis, the need for long labelling periods and thereby large quantities of gas also makes this approach

expensive. The choice of method also depends on the question addressed in the study. In forest ecosystems $^{13}CO_2$ labelling is technically difficult due to the size of the trees, so this approach has primarily been attempted using small plants (Bruneau *et al.*, 2002; Johnson *et al.*, 2002; Butler *et al.*, 2003; Phillips & Fahey, 2005; Leake *et al.*, 2006). However, it has been successfully employed to trace the C flux in mature trees in a free air CO_2 enrichment experiment (Körner *et al.*, 2005; Keel, Siegwolf & Körner, 2006).

Objectives

This thesis focuses on temporal and spatial aspects of the C flux within the tree-root-soil continuum. It includes attempts to characterize the transfer times of the belowground C flux from fixation during canopy photosynthesis to soil-respired CO_2 (Paper I, II and III). The area that is influenced by a tree's root C supply, i.e. the lateral root spread, was also studied (Paper V). The ecological significance of the C that is transported belowground is addressed in a study on the role of belowground C allocation for the activity of soil microorganisms (Paper IV). Variations in the natural abundance of stable C isotopes and C and N isotope labelling experiments were employed in these studies.

Papers I and II investigate temporal variation in natural abundance of stable C isotopes in forests. It has not been investigated whether a coupling between meteorological variables and $\delta^{13}C$ of soil-respired CO_2 exists on the diurnal time scale in forest ecosystems, although the $\delta^{13}C$ of photosynthates may vary on a short time-scale, as meteorological variables such as temperature, air relative humidity and photosynthetically active radiation all vary substantially during clear days. A coupling between meteorological variables, leaf, phloem and root carbohydrates and C isotope composition of soil-respired CO_2 would be an ideal framework within which to study temporal aspects of the transfer of C from the canopy to the soil under natural conditions. We hypothesised that diurnal variation in $\delta^{13}C$ in leaf carbohydrates might result in a diurnal variation of $\delta^{13}C$ values of phloem sugars, root carbohydrates and finally of soil-respired CO_2 and that this natural variation in the C isotope composition can be used to trace C from photosynthesis to soil respiration. We tested this hypothesis in a temperate beech forest (Paper I) and a boreal Norway spruce forest (Paper II). The studies described in Paper I also encompassed the development of an improved method for the isolation of potential root respiratory substrates.

The aim of Paper III was to assess transfer times of C within trees, a feature that is poorly described. We developed a $^{13}CO_2$ labelling method where several young trees were enclosed in a chamber and exposed to ^{13}C enriched CO_2 for 90 min. We hypothesised that a short pulse of CO_2 highly enriched in ^{13}C that does not expose trees to noteworthy elevated CO_2 concentrations would make it possible to

estimate transfer times of C from leaves, to the phloem, to ectomycorrhizal root tips and to soil-respired CO_2.

The aim of Papers I, II and III was to gain insight into temporal aspects of C flux within the tree-root-soil continuum. In Paper IV we assessed differences in the metabolic activity of microorganisms in girdled and non-girdled plots 3 and 4 years after girdling. We tested whether the impact of direct belowground flux of tree-derived C is limited to roots and their mycorrhizal fungi or whether it also influences the heterotrophic microbial community. This was done by testing the ability of the heterotrophic community to respond to additions to an easily available C source. The added C was C_4-sucrose and thus it was possible to distinguish between CO_2 that is derived from the decomposition of native soil organic matter (i.e. C_3-respiration) or the added substrate. Application of C_4-sucrose to boreal forest soils induced a short-term increase in C_3-respiration (Högberg & Ekblad, 1996; Ekblad & Högberg, 2000; Ekblad, Nyberg & Högberg, 2002) which was ascribed to mobilisation of microbial reserve compounds or accelerated decomposition of soil organic matter. A difference in the response to sucrose addition could be attributed to microbial metabolism, excluding that of mycorrhizal fungi, because there is experimental evidence that sucrose addition does not induce an increase in respiration rate in ectomycorrhizal roots (Ekblad & Högberg, 2000). We hypothesised that the C_4-sucrose induced short-term increase in C_3-respiration would be significantly less in the girdled plots that lack input of recent photosynthate to the belowground system.

In Paper V we wanted to assess a spatial aspect of the tree-root-soil C flux. Based on the observation that ectomycorrhizal sporocarps disappeared within a certain distance from the plot edge on girdled plots, we hypothesised that this distance could be used to estimate the average area of soil influenced by a tree, because sporocarp production by ectomycorrhizal fungi is directly dependent on photosynthates from their host trees (e.g. Romell (1938) and Lamhamedi, Godbout & Fortin (1994). To test our hypothesis we performed a ^{15}N uptake experiment in non-girdled plots at the same two sites where girdling was also performed. We chose to perform a stable isotope uptake experiment as we wanted to assess root function rather than the physical presence of roots and to include small roots and mycorrhizas. In this study we could also address the question of whether the areas of belowground influence of trees overlap or not. Together these two approaches, observation of the spatial distribution of ectomycorrhizal sporocarps on girdled plots and assessment of ^{15}N uptake from a defined area, should give a good estimate of the area that is influenced by a tree root system.

In short I thus attempted:

- to determine if there is a diurnal pattern of soil respiration rate and of the C isotope composition of leaf and root carbohydrates, phloem sap sugars and soil-respired CO_2. Further, to test if a potential natural variation in

the C isotope composition in these C pools can be used to gain insight into the transfer times of C between ecosystem compartments and to test if foregoing weather conditions can explain possible variation in C isotope composition in these C pools. To achieve this, a method for the isolation of starch and soluble sugars had to be modified in way that is also suitable for the preparation of plant material with very low concentrations of storage carbohydrates (Papers I and II),

- to develop a ^{13}C isotope pulse-labelling method that makes it possible to follow the C flux at a high temporal resolution from the forest canopy to the belowground compartment under natural conditions in the field (Paper III),

- to test if the metabolic activity of microorganisms, other than mycorrhizal fungi, changes in the long term absence of C flux from the canopy to the belowground compartment (Paper IV), and

- to obtain an estimate of the area that is influenced by a tree root system and to describe the potential for belowground competition in two boreal forests with differences in nutrient availability (Paper V).

Material and Methods

Study sites

An overview of the most important characteristics of the five study sites is given in Table 1.

Table 1. Characteristics of the study sites.

	Hainich	**Åheden**	**Storskogberget**	**Flakaliden**	**Rosinedalheden**
Paper	I	IV, V	IV, V	II	III
Longitude	51°04´ N	64°14’ N	64°00’ N	64°07’ N	64°09’ N
Latitude	10°27´ E	19° 46’ E	20°35’ E	19°27’ E	19°05’ E
Altitude (m a.s.l.)	440	175	75	310	145
Climate zone	suboceanic	boreal	boreal	boreal	boreal
Main tree species	*Fag. syl.*	*Pin. syl.*	*Pic. ab.*	*Pic. ab.*	*Pin. syl.*
Age of the stand (yr.)	250	48-59	120	40	*c.* 15
Main understory	*All. urs.*	*Vac. vit.*	*Vac. myr.*	*Vac. myr.*	*Vac.vit.*
Mean air temp. (°C)	8	1[a]	1[a]	1[a]	1[a]
Mean prec. (mm yr^{-1})	800	600[a]	600[a]	600[a]	600[a]
Soil type	cambisol	podzol	podzol	podzol	podzol
Soil pH	5.4[b,c]	4.0[d]	4.1[d]	4.1[d]	4.5[d]
Soil C:N ratio	11.8[b]	40	32	39	33

[a] long term mean air temperature and precipitation in this region
[b] values taken from Søe & Buchmann (2005)
[c] in 1M KCl extracts
[d] in water

Abbreviations:		
	a.s.l.	above sea level
	All. urs.	*Allium ursinum* L. (wild garlic)
	Fag. syl.	*Fagus sylvatica* L. (beech)
	Pic. ab.	*Picea abies* ((L.) Karst.) (Norway spruce)
	Pin. syl.	*Pinus sylvestris* L. (Scots pine)
	prec.	precipitation
	temp.	temperature
	Vac. myr.	*Vaccinium myrtillus* L. (blueberry)
	Vac. vit.	*Vaccinium vitis-idaea* L. (lingonberry)

The study sites encompass one temperate site (Hainich, a beech dominated forest) in Germany and four boreal sites in Northern Sweden, i.e. two Scots pine (Åheden and Rosinedalheden) and two Norway spruce (Flakaliden and Storskogberget) dominated sites. The Hainich site is a “CarboEurope” study site, where we could take advantage of Net Ecosystem Exchange (NEE) measurements. Furthermore, the tower provided easy access to the canopy for sampling of leaves. At the sites Åheden and Flakaliden we took advantage of tree-girdled plots. The site Storskogberget was chosen as a contrast with the Åheden site (Norway spruce *vs*. Scots pine, older trees, lower C:N ratio, wetter) and should give our estimate of

the area that is influenced by a tree root system more general relevance. Finally, the site Rosinedalheden was chosen as the experimental setup required a short young forest.

Methodological aspects

Quantification of soluble sugars and starch content (Paper I)

Soluble sugars were identified and quantified by capillary GC (gas chromatography) as described elsewhere (Peterbauer, Puschenreiter & Richter, 1998). For determination of starch, soluble carbohydrates were first removed by repeated extraction of the plant material with ethanolic solutions. The starch was hydrolysed enzymatically with a heat-stable α-amylase from *Bacillus licheniformis*. After incubation in an amyloglucosidase solution from *Aspergillus niger* glucose released by starch hydrolysis was quantified by HPAEC-PAD on an anion-exchange column as described in Arndt *et al.* (2000).

Isolation of soluble carbohydrates for stable isotope analysis (Paper I)

Methanol/chloroform/water (MCW, 12:5:3,v/v/v) extracts from powdered plant material (beech leaves) were separated into two phases. Soluble carbohydrates were isolated by applying chloroform-free (re-dissolved in water) extracts to columns filled with a mixture of anion-exchange resin (2.6 g AG 2-X8, 20-50 mesh, BioRad, Vienna, Austria) and cation-exchange resin (1.6 g Dowex 50WX8-100, Sigma-Aldrich, Vienna, Austria). Aliquots of the dried ion-exchanged solution were analysed for $\delta^{13}C$.

Isolation of starch for stable isotope analysis (Paper I)

The method described by Wanek, Heintel & Richter (2001) for isolation of starch for stable C isotope analysis was modified to enable preparation of samples with low starch content. The starch was hydrolysed enzymatically with a heat-stable α-amylase from *Bacillus licheniformis*. To reduce the amount of C introduced to the solution by the addition of the enzyme, the solution (containing the enzyme and the hydrolysed starch) was filtered through pre-washed centrifugal ultrafiltration devices (Microcon YM-10, regenerated cellulose membrane, 10 kDa molecular weight cut-off, Millipore, Vienna, Austria). The dried filtrates were analysed for their C isotope composition.

Girdling (Papers II, IV and V)

For Papers II, IV and V, plots with girdled and untreated trees were used. To girdle a tree, the stem bark is removed to the depth of the current xylem, which terminates the supply of current photosynthate to roots. The plot area in which trees were girdled was > 700 m^2 and respiration measurements were made in the centre of the plot. The size of the area was chosen to ensure that roots from untreated trees outside do not influence the central area, where measurements of

respiration were made. For further details on the girdling see Högberg *et al.* (2001) and Olsson *et al.* (2005).

Figure 3. Opaque collars for sequential gas sampling of soil-respired CO_2 after labelling the canopy with $^{13}CO_2$.

Figure 4a and b. Application of NH_4Cl (98 atom percent ^{15}N) at Storskogberget.

Respiration rate and $\delta^{13}C$ of soil-respired CO_2 (Papers II, III and IV)

To measure respiration rates and $\delta^{13}C$ of soil-respired CO_2, cylindrical opaque collars were placed on the ground (see Fig. 3). The respiration rate was determined as the rate of CO_2 increase with time in the collar head space by sequential

sampling. To assess the isotopic composition of soil-respired CO_2, the Keeling plot approach was used (Keeling, 1958). This is based on a two-component isotope mixing model, consisting of the $\delta^{13}C$ of the atmospheric and soil-respired CO_2. The intercept of a linear regression of $\delta^{13}C$ of sampled CO_2 versus $1/[CO_2]$ provides an estimate of $\delta^{13}C$ of soil-respired CO_2 (where $[CO_2]$ is the CO_2 concentration in the sample in %). For an example of a Keeling plot see Fig. 5.

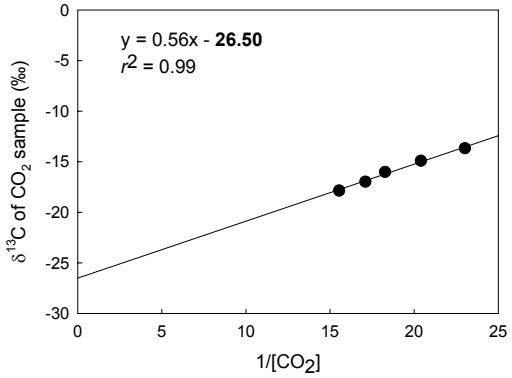

Figure 5. Example of a Keeling plot. The intercept of a linear regression of $\delta^{13}C$ of sampled CO_2 versus $1/[CO_2]$ provides an estimate of $\delta^{13}C$ of soil-respired CO_2. In this case the intercept, i.e. the $\delta^{13}C$ of soil-respired CO_2, was -26.50.

As the r^2 of the Keeling plots for Papers II, IV and III were high, a simple linear regression of $\delta^{13}C$ of sampled CO_2 versus $1/[CO_2]$ was used, since the reliability of this method for Keeling plots with high r^2 values (> 0.95) has been demonstrated by Pataki *et al.* (2003). Exceptions to the high r^2 values were some of the Keeling plots for Paper III, during the time when the $\delta^{13}C$ value of soil-respired CO_2 was close to that of atmospheric CO_2. This was due to the mixing of unlabelled components of soil respiration (that have more negative $\delta^{13}C$ values than atmospheric CO_2) with one or several labelled components of soil respiration (that had more positive $\delta^{13}C$ values than atmospheric CO_2). If the two components of the mixing model (i.e. soil-respired and atmospheric CO_2) do not differ in their isotope composition, the Keeling plot approach does not work. Where the $\delta^{13}C$ in the respiration chambers changed less than 1‰ during the 8 min the lid was closed and the r^2 of the Keeling plot was low, whereas the r^2 of the rate of CO_2 increase with time was high, we used the average of the five gas samples extracted from the chamber for the determination of $\delta^{13}C$ of soil-respired CO_2.

Measurements of CO_2 concentration and C isotope composition of CO_2

Gas samples were analysed for their C isotope composition with EA-IRMS. For further information on the measurements see Högberg & Ekblad (1996), but it should be noted that we used the Keeling plot approach (see above) rather than the

non-linear mixing model used by Högberg & Ekblad (1996). Results of the C isotope composition are reported in $\delta^{13}C$ (‰):

$$\delta^{13}C = 1000 * \left(\frac{R_{sample} - R_{standard}}{R_{standard}} \right),$$

where $R = {}^{13}C/{}^{12}C$. The standard used was 5% CO_2 in N_2 with a $\delta^{13}C$ of 5‰ relative to the international standard Vienna-Pee Dee Belemnite.

Phloem sap extraction method for $\delta^{13}C$ determination (Papers II and III)

In order to assess the $\delta^{13}C$ of the photosynthate flux to the roots, the $\delta^{13}C$ of phloem sap soluble sugars were measured. A modified version of the protocol reported by King & Zeevaart (1974) was used as described by Geßler, Rennenberg & Keitel (2004). Briefly, 2 cm^2 of bark material was excised with a scalpel, the outer bark removed and the remaining phloem material rinsed thoroughly with distilled water and immersed in 2 (or 3) mL of 10 mM EDTA solution at pH 7, to prevent chelation from occurring (Paper II) or deionized water (Paper III). After 5 h, the phloem samples were removed and discarded, with the remaining solution frozen prior to purification. After ion-exchange of the phloem sap samples (Wanek, Heintel & Richter, 2001) the solutions were dried on a rotary evaporator or freeze- dried and re-dissolved in 1 mL distilled water. An aliquot of 50 µL of this solution was dried in tin capsules and analysed for its C isotope composition.

Chamber-based $^{13}CO_2$ labelling

For the purpose of ^{13}C labelling of photosynthates a 200 m^3 transparent chamber (octagonal in shape with a diameter of 8 m and a height of 4 m) with a cooling system was installed temporarily in a young, naturally regenerated Scots pine stand end of August 2006. Carbon dioxide enriched in ^{13}C (95 atom percent) was released within the chamber, which was closed for *c.* 90 min only. This increased the CO_2 concentration within the chamber only marginally, but raised the $\delta^{13}C$ value of the chamber CO_2 to 5.74 atom percent. Both CO_2 concentration and atom percent of $^{13}CO_2$ of chamber air decreased during the 90 min of labelling. For 72 h after labelling soil-respired CO_2 was sampled every 4 h for C isotope analysis. Additionally, leaves and phloem sap and mycorrhizal roots were sampled and analysed for their C isotope composition.

Substrate addition treatments (Paper IV)

Substrate induced respiration, SIR, is frequently used to estimate the amount of C in living, non-resting microbial biomass in soils. The method is based on the initial respiratory response of microbial biomass to the supply of a C source, and is performed under standard laboratory conditions (Anderson & Domsch, 1978). Högberg & Ekblad (1996) applied this approach in the field, taking advantage of

the difference in stable C isotope composition ($\delta^{13}C$) between C_3- and C_4-plants. The rationale behind combining the SIR approach with ^{13}C isotope studies was that the induced respiration has the isotopic signature of the added C_4-substrate, while the basal respiration has the isotopic signature of the C_3-system. The difference in the C isotope composition of soil and the substrate added can be used to calculate their contributions to respired CO_2. However, in studies of the relationship between C_3- and C_4-derived CO_2 fluxes the "priming effects" ("strong, short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil" (Kuzyakov, Friedel & Stahr, 2000)) have to be considered. Application of C_4-sucrose to boreal forest soils induced such a short-term increase in C_3-respiration (Högberg & Ekblad, 1996; Ekblad & Högberg, 2000; Ekblad, Nyberg & Högberg, 2002). This increase can be attributed to microbial metabolism, excluding that of mycorrhizal fungi, because there is experimental evidence that sucrose added externally does not induce an increase in respiration rate of ectomycorrhizal roots (Ekblad & Högberg, 2000). The additional C_3-C metabolized after addition of C_4-sucrose (further referred to as "primable" C) could be caused by increased use of C already present in the microbial biomass (Dalenberg & Jager, 1981; Dalenberg & Jager, 1989; Ekblad & Högberg, 2000), or by accelerated decomposition of SOM (Ekblad & Högberg, 2000). In Paper IV, where we assessed whether restricted C flux in the tree-root-soil continuum influences the heterotrophic microbial community, we used the magnitude of the priming effect as an indicator of the C status of heterotrophic microorganisms.

Sporocarp distribution (Paper V)

The number and dry weight of sporocarps was determined in girdled and adjacent, non-girdled plots. Sporocarps were classified as ECM or saprotrophic according to Hansen & Knutsen (1992, 1997).

Tracer application (Paper V)

Tracer (98 atom percent ^{15}N labelled $(NH_4)_2SO_4$ or NH_4Cl solution) was applied in circular plots (1 m^2 each) at Åheden and Storskogberget (see Fig. 4). At 25 regularly distributed points within each circular plot, 5 mL of tracer solution was injected into the organic layer to the depth of the mineral soil layer or at least 7 cm (at Storskogberget) into the organic layer with a syringe. The concentrations of the tracer solution were chosen to give a final amount of 0.1 g added N m^{-2} in Åheden and 1 g added N m^{-2} at Storskogberget, i.e. 1 to 10 kg N ha^{-1}. In comparison in commercial forest fertilisation 150 kg ha^{-1} or more is added.

Measurement of C and N isotope composition and the C:N ratio in solid samples (Papers II, III and V)

Samples were analysed for percent N and C (%) and the N and C isotope composition with EA-IRMS, see Ohlsson & Wallmark (1999) for further details. The isotope composition was reported in $\delta^{13}C$ (‰)(see above) and $\delta^{15}N$ (‰).

$$\delta^{15}N = 1000 * \left(\frac{R_{sample} - R_{standard}}{R_{standard}} \right),$$

where $R = {}^{15}N/{}^{14}N$. The international standard for $\delta^{15}N$ is atmospheric N_2.
For the measurement of C isotope composition of starch and soluble carbohydrates, the dilution with helium by the ConFlo II interface was reduced to account for the low C content of samples, blanks and standards.

Results and Discussion

Do forest ecosystem components vary enough in their C isotope composition to study temporal aspects of the belowground C flux on a diurnal time scale?

We studied changes in the $\delta^{13}C$ of leaf and root carbohydrates, phloem sap soluble sugars and soil-respired CO_2 at a high temporal resolution to see if there was a finer scale light- or weather-driven variability than that observed previously (Ekblad & Högberg, 2001; McDowell *et al.*, 2004; Scartazza *et al.*, 2004; Ekblad *et al.*, 2005). In the study carried out in the temperate beech forest (Paper I) samples of leaves (from different canopy heights) and roots were taken six times during a day/night cycle and analysed for their concentration of starch and soluble sugars and the C isotope composition of these compounds. In the study in the boreal Norway spruce forest (Paper II) photosynthetic rate, respiration rate and the C isotope composition of soil-respired CO_2 and of phloem sap were measured *c.* every 4 h during two consecutive days. Two measurement campaigns were carried out, one in June and another in August. Air temperature, photosynthetic photon flux density, soil temperature and soil moisture were also measured. The variation in soil respiration rate and $^{13}CO_2$ of soil respiratory efflux from the control plot in the $^{13}CO_2$ labelling study (Paper III) was also examined in detail.

Methodological improvements

A method for isolating starch for stable C isotope analysis based on enzymatic hydrolysis (Wanek, Heintel & Richter, 2001) was successfully modified to allow for the low starch content of the samples. This was a pre-requisite for the study described in Paper I as the carbohydrate concentration of the samples was very low. We achieved the improvement by removing the enzyme (α-amylase) by ultrafiltration after the hydrolysis, which resulted in very low C content of blanks. Enzymatic hydrolysis of starch is an effective method as heat-stable α-amylase from *Bacillus licheniformis* cleaves exclusively α-1,4-glycosidic linkages in amylose and amylopectin. This yields the disaccharide maltose and water-soluble dextrins. Thus, the method has high specificity for starch and does not act on any of the other plant polyglucans. Further, this method yields higher starch recoveries (99 to 105%) than other methods tested (48 to 81%), i.e. (1) dissolution of starch by HCl and subsequent precipitation with ethanol or (2) solubilisation of starch by dimethyl sulphoxide followed by precipitation (Wanek, Heintel & Richter, 2001). By using ultrafiltration to remove the α-amylase we reduced the C content of blanks considerably, increasing the precision in comparison to the original protocol (Table 2).

In the case of soluble sugars, we did not try to reduce the C content of the blanks (which were already very low in the original protocol), but we did try to increase

the precision and reproducibility of the C blanks. To improve the precision we tested an anion-exchange resin in the $[HCO_3]^-$ - form, which has proven suitability for trace analysis of sugars in sea water (Skoog & Benner, 1997). However, the blanks of the ion-exchange method based on an anion-exchange resin in the $[HCO_3]^-$ - form showed a lower standard deviation for both C content and $\delta^{13}C$, but a higher C content in comparison to the other tested method based on an anion-exchange resin in the $[HCOO]^-$ - form (Table 2). Thus, the method based on the anion-exchange resin in $[HCO_3]^-$- form seemed to be useful for samples with very low C concentrations, but for samples with higher C concentrations the original method by Wanek, Heintel & Richter (2001) seemed to be more appropriate. Therefore, for the leaf extracts (Paper I) the modified protocol with the anion-exchange resin in $[HCO_3]^-$-form was chosen and for the phloem sap samples (Papers II and III) the original method was used.

Table 2. Comparison of carbon contents (µg) and $\delta^{13}C$ values (‰) of blanks assessed by different methods for (A) starch hydrolysis and (B) ion-exchange preparation of the soluble sugar fraction. Carbon blanks of starch hydrolysis were measured after ultrafiltration or precipitation with acetone or chloroform, carbon blanks of the ion-exchange procedure were determined for anion-exchange resin in the formate $[HCOO]^-$- or bicarbonate $[HCO_3]^-$ -form. Values represent means ±1SE, n = number of replicates. Methods selected for starch and sugar isolation in Paper I are in bold.

Treatment	C content (µg/Sample)	$\delta^{13}C$ (‰)	n
(A) Starch hydrolysis			
Acetone	7.1±1.3	-23.5±0.9	6
Chloroform	18.9±0.7	-23.7±0.9	6
U-Filtration	**1.8±0.5**	**-23.9±0.3**	**8**
(B) Ion-exchange			
$[HCOO]^-$	3.5±0.6	-24.7±0.6	6
$[HCO_3]^-$	**7.3±0.3**	**-25.1±0.1**	**5**

Are there diurnal changes in concentration and C isotope composition of leaf and root carbohydrates?

In the temperate beech forest (Paper I) leaf starch and soluble sugar concentrations increased during daytime and decreased during the night (for starch see Fig. 6a). For leaf starch there was a trend for ^{13}C enrichment from morning to afternoon and ^{13}C depletion during the night (Fig. 6b). The $\delta^{13}C$ values of the sugars showed a slight, but statistically insignificant increase from 08:30 to 22:30 at 5 and 10 m above the forest floor. The ^{13}C enrichment of leaf starch in the afternoon can be explained by decreased stomatal conductance that causes reduced C isotope

discrimination by Rubisco. The aldolase reaction, catalysing the condensation of triose phosphates to fructose-1,6-bisphosphate in the chloroplasts, might also contribute to this potential diurnal pattern of the C isotope composition of starch. As aldolase favours ^{13}C, the remaining triose phosphates exported from the chloroplast are depleted in ^{13}C (Tcherkez *et al.*, 2004). This leads to the production of hexoses in the cytoplasm during the light period that are isotopically lighter

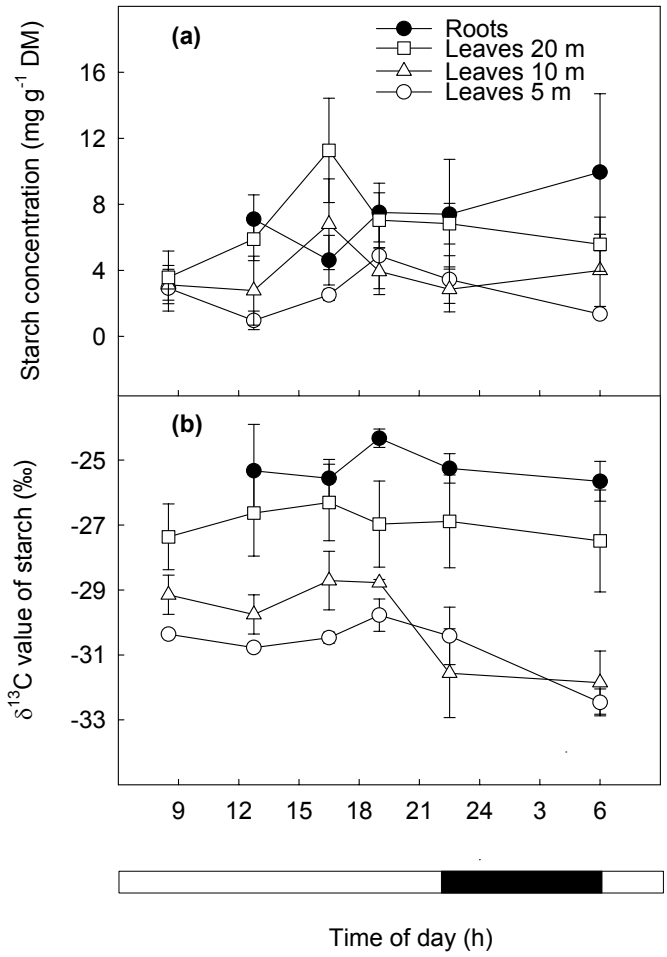

Figure 6. (a) Concentration (mg g^{-1} dry weight (DM)) and (b) C isotope signatures (‰) of foliar starch from different canopy heights (5, 10, 20 m) and in roots during a day-night cycle (28/29 July 2003) in a beech forest. Dark parts of the bar below the graph indicate night time. Symbols represent means of three to four mature trees (±1SE).

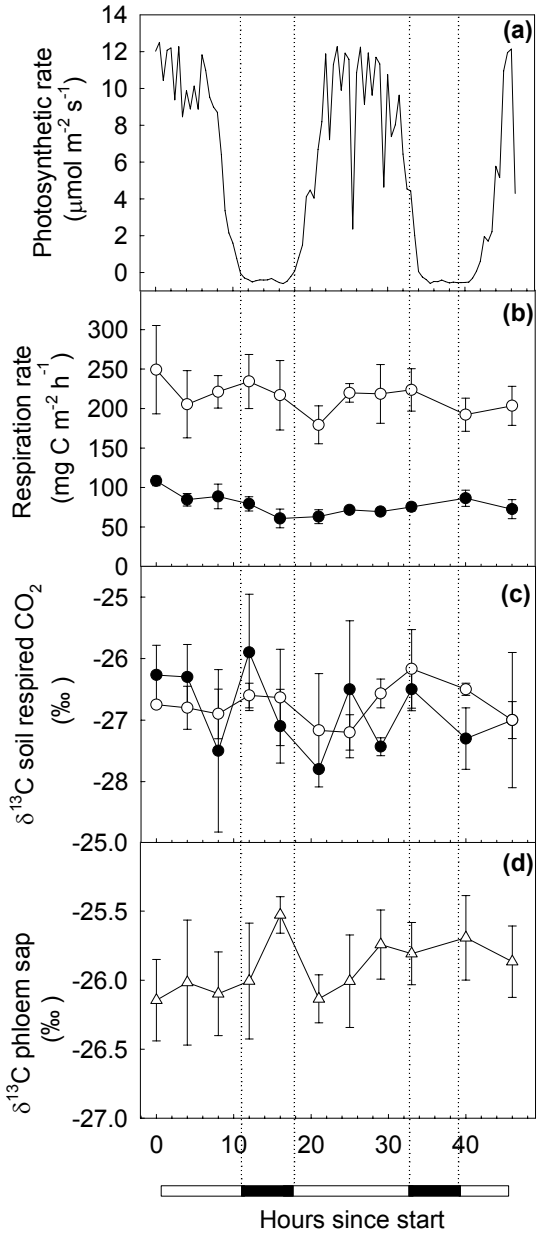

Figure 7. (a) Photosynthetic rate (µmol m^{-2} s^{-1}), (b) diurnal soil respiration rates (mg C m^{-2} h^{-1}), (c) $\delta^{13}C$ of soil-respired CO_2 (‰) and (d) $\delta^{13}C$ of phloem sap C (‰) over 48 h of experimentation in August 2004 on girdled plots (filled symbols) and non-girdled control (open symbols) plots. Values are mean ±1SE (n = 3). Dark parts of the bar below the graph indicate night time.

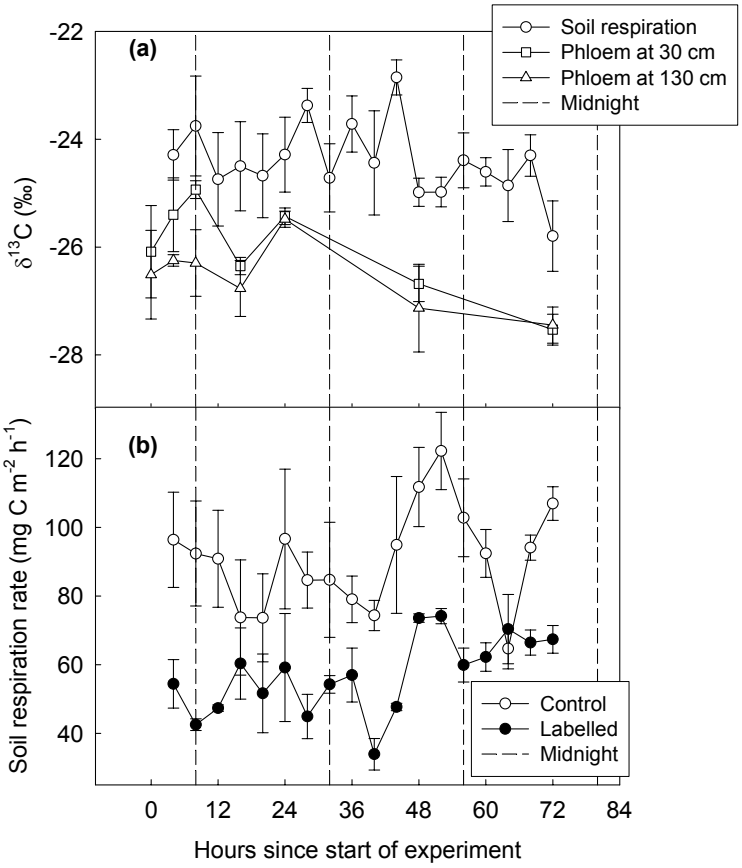

Figure 8. (a) Carbon isotope composition (‰) of soil-respired CO_2 and phloem sap at 130 and 30 cm in the control plot of the $^{13}CO_2$ labelling experiment (described in Paper III) and (b) soil respiration rates (mg C m^{-2} h^{-1}) in ^{13}C labelled and control plot during the intensive sampling campaign (72 h). Hour 0 is the end of tracer addition in the ^{13}C labelled plot. Mean ±1SE (n = 4, soil respiration and n = 3, phloem sap C). Symbols are explained in the figure.

than those in the chloroplast, where starch synthesis takes place (Tcherkez *et al.*, 2004). During the day, when isotopically lighter triose-phosphates were exported from the chloroplast to the cytosol, the newly synthesised starch was more ^{13}C enriched (Paper I). It can be assumed that the starch fraction that is mobilised during night-time has a less negative $\delta^{13}C$ value compared to the whole starch grains. If this was not the case the C isotope composition of starch would become increasingly ^{13}C enriched over time. This assumption is corroborated by the parallel trends towards ^{13}C depletion and decrease in concentration of leaf starch during the night (Fig. 6a and b). The difference between the lowest and highest $\delta^{13}C$ value of starch during one day was largest in leaves at 10 m (3.1‰, Fig. 6b).

Despite the differences observed in the C isotope composition of leaf starch and the possibilities of differences in the C isotope composition of carbohydrates mobilised and exported to roots, no diurnal variation in the $\delta^{13}C$ of starch was observed in roots during the short time frame of one day (Fig. 6b).

Are there diurnal changes in C isotope composition of phloem sap C and of soil-respired CO_2?

In the Norway spruce forest in northern Sweden (Paper II) diurnal patterns of meteorological data, net shoot photosynthesis and dark respiration (e.g. Fig. 7a) were observed during both sampling campaigns (June and August). Although there was some minor variation in both $\delta^{13}C$ of soil-respired CO_2 and in respiration rates, no systematic diurnal pattern of soil respiratory flux or C isotope composition of soil-respired CO_2 was evident in either girdled or non-girdled plots in June or August (Fig. 7b and c). No significant correlations were found between the respiration rates or the $\delta^{13}C$ of soil-respired CO_2 of both girdled and non-girdled plots and meteorological variables or photosynthetic rates averaged over various averaging periods and with different delay times in either June or August. The $\delta^{13}C$ of soil-respired CO_2 from the control plot of the $^{13}CO_2$ labelling experiment also did not show a diurnal pattern (Fig. 8a).

The C isotope composition of phloem sap and soil-respired CO_2 correlate

The $\delta^{13}C$ of phloem sap measured in August 2004 showed no variation associated with a diurnal rhythm over the 48 h of measurement (Fig. 7d). No correlations were found between the $\delta^{13}C$ of phloem sap and meteorological variables over any tested averaging period or delay time. The $\delta^{13}C$ of the phloem sap was weakly correlated with the corresponding $\delta^{13}C$ of soil-respired CO_2 measured at the same time (Fig. 7c and d, $r^2 = 0.158$, $P = 0.022$), but was generally more enriched (mean phloem sap $\delta^{13}C$ -25.9 ‰ compared with mean soil CO_2 efflux of $\delta^{13}C$ -26.7 ‰, $P < 0.001$). Correlation between $\delta^{13}C$ of phloem sap and $\delta^{13}C$ of soil-respired CO_2 measured at the same time was found again in the control plot of the $^{13}CO_2$ labelling study in 2006 (Fig. 9, $r^2 = 0.85$, $P = 0.001$). Here, the $\delta^{13}C$ of the phloem sap was generally more negative than soil-respired CO_2 (mean phloem sap $\delta^{13}C$ -26.40‰ compared with mean soil CO_2 $\delta^{13}C$ -24.80‰, $P < 0.01$; Figs 8a and 9). A period of high vapour pressure deficit (VPD) such as that occurring in August 2006 should lead to photosynthate, and thus phloem sap $\delta^{13}C$ that is less negative than that typically observed. However, this does not explain why soil-respired CO_2 had lower $\delta^{13}C$ values than phloem sap C in 2004, but higher $\delta^{13}C$ values in 2006. Possibly, as the soil was very dry in 2006 heterotrophic soil microorganisms might have used another range of substrates than those used under moister conditions.

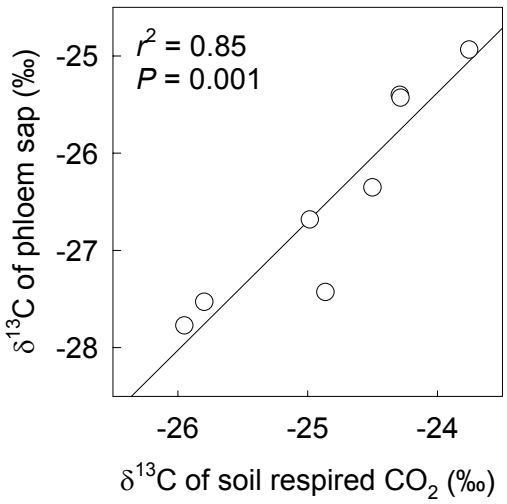

Figure 9. Correlation between $\delta^{13}C$ (‰) of soil-respired CO_2 and of phloem sap C at a height of 30 cm in the control plot of the $^{13}CO_2$ labelling experiment.

Reasons for the absence of diurnal variation in C isotope composition of phloem and root carbohydrates and of soil-respired CO_2

The absence of variation associated with a diurnal rhythm in the C isotope composition of soluble sugars in the phloem (Papers II and III), root carbohydrates (Paper I) and soil-respired CO_2 on a diurnal time scale (Papers II and III) might have several explanations. First, the C isotope composition of carbohydrates that are transported belowground is the product of several different sources, each with distinct isotopic signatures. Second, mixing in the pool of leaf carbohydrates averages the $\delta^{13}C$ values of recently fixed photosynthates and may reflect the average of sugars fixed over several consecutive days. Third, mobilised starch and sugars may differ in their C isotope composition. These three possibilities are discussed in more detail below.

(1) Variation of the C isotope composition within a tree

The $\delta^{13}C$ values of leaves increase with increasing height above the forest floor (Medina & Minchin, 1980; Ehleringer *et al.*, 1986; Schleser, 1990; Schleser, 1992; Brooks *et al.*, 1997; Buchmann, Kao & Ehleringer, 1997; Hanba *et al.*, 1997). This spatial variation in C isotope composition within trees was confirmed for bulk material, starch and soluble sugars in our study on short term changes in C isotope composition (Paper I, for starch see Fig. 6b). The $\delta^{13}C$ values of bulk material, starch and the sugars increased from the lower to the higher canopy and were highest in roots ($P < 0.05$). In this study (Paper I), mean differences between the $\delta^{13}C$ values of starch, sugars and bulk material sampled at 5 m and 20 m were around 3.8, 3.4 and 2.7‰, respectively (for starch see Fig. 6b). The C isotope

composition of the leaves from control trees in the $^{13}CO_2$ labelling experiment also differed significantly (P = 0.040) between trees of different height classes. The increase of leaf $\delta^{13}C$ with increasing height above the forest floor was attributed to changes in the $\delta^{13}C$ of canopy air (Schleser & Jayasekera, 1985; Ehleringer *et al.*, 1986; Schleser, 1990; Brooks *et al.*, 1997; Buchmann, Kao & Ehleringer, 1997) and to changing environmental conditions that may influence the c_i/c_a ratio such as, light (Ehleringer *et al.*, 1986; Schleser, 1990; Garten & Taylor, 1992; Brooks *et al.*, 1997; Buchmann, Kao & Ehleringer, 1997; Hanba *et al.*, 1997), air humidity (or VPD) and temperature (Garten & Taylor, 1992; Hanba *et al.*, 1997). However, measurements of the CO_2 concentration and its $\delta^{13}C$ values at the Hainich study site in 2002 (Paper I) showed that the CO_2 concentration was nearly constant between 5 and 20 m and that the change in the $\delta^{13}CO_2$ of canopy air between these two positions was < 0.5‰ (Knohl *et al.*, 2005), which suggests that the contribution of air source to the $\delta^{13}C$ value gradient in leaves within the canopy was minor. This increase of the $\delta^{13}C$ of foliar sugars and starch with increasing height indicates a potential problem in the study of diurnal variation in the C isotope composition of root carbohydrates and of soil-respired CO_2 (Paper II) because the signal in the soil respiratory efflux is not derived from a single, isotopically uniform source. Thus, the likelihood of seeing clear changes in the C isotope composition in phloem sap carbohydrates (Papers II and III), in respiratory substrates in the roots (Paper I) or in soil-respired CO_2 at a diurnal time scale (Paper II and III) decreases. Furthermore, clear diurnal changes in the C isotope composition in phloem sap C are unlikely, because C that was fixed at different times of the day, with different C isotope signatures mixes in the phloem depending on the distance of transport from the site of photosynthesis.

(2) Storage in the leaf carbohydrate pool averages the $\delta^{13}C$ values of recently fixed photosynthates

As mentioned above the concentrations of leaf starch and soluble sugar increased during daytime and decreased during the night (Fig. 6a) although recently assimilated C is immediately exported (Jahnke *et al.*, 1998). The increase in carbohydrate concentrations means that C fixed under varying environmental conditions is mixed in the leaf C pool. This might average the $\delta^{13}C$ values of photosynthates fixed during one day.

(3) The starch and sugars mobilised differ in their C isotope composition

The C isotope composition of the mobilised carbohydrate fraction should more closely reflect the C isotope composition of sugars that are exported to the roots than that of total leaf carbohydrates. The mobilised fraction is defined as the difference between maximum and minimum carbohydrate concentrations. The calculated C isotope composition of mobilised starch was less negative than that of mobilised leaf sugars (canopy mean pooled from all canopy levels -25.3 and -30.2‰, respectively). Part of the mobilised starch and sugars are transported to the

roots in the phloem (as sucrose). This means that C which comes from mobilised starch and mobilised sugars mix. The difference of almost 5‰ between mobilised sugars and starch indicates that already small changes in the ratio of C that comes from sugar and C that comes from starch in the phloem sap might override any diurnal changes in $\delta^{13}C$ of leaf carbohydrates. However, the results have to be considered cautiously because the calculations are representative for one day-night cycle only and because we assumed that the measured maximum and minimum values in concentration and $\delta^{13}C$ are the 'true' maximum or minimum values found *in situ*.

Concluding remarks on the studies of diurnal changes in the C isotope composition of leaf, phloem and root carbohydrates and of soil-respired CO_2

Our hypothesis that diurnal variation in $\delta^{13}C$ of leaf carbohydrates might result in a diurnal variation of $\delta^{13}C$ values of phloem sugars, root carbohydrates and finally of soil-respired CO_2 and that this natural variation in the C isotope composition can be used to trace C from photosynthesis to soil respiration was not confirmed. The presence of diurnal variation in meteorological variables, photosynthesis (Paper II) and leaf starch (Paper I) and the absence of diurnal variation in the C isotope composition of soluble sugars in the phloem (Paper II and III) and root carbohydrates (Paper I) and soil-respired CO_2 (Paper II and III) shows that relatively subtle short-term changes in meteorological variables and natural variations in the abundance of ^{13}C are very unlikely to produce traceable variations in the C flux within a forest ecosystem. This suggests it takes long term and more pronounced changes in weather to produce significant variations in $\delta^{13}CO_2$ in the soil respiratory efflux as reported by Ekblad & Högberg (2001). Therefore, under most weather conditions diurnal variation in the $\delta^{13}C$ of leaf carbohydrates can not be exploited to follow C from the tree canopy to roots and soil-respired CO_2.

Temporal aspects of the belowground C flux assessed by canopy $^{13}CO_2$ labelling

The labelling study (Paper III), gave much more insight into the C flux from the canopy to the belowground compartment than the studies on levels of variation in natural abundance (Fig. 10a, b and c). For a picture of the chamber used for labelling see Fig. 11.

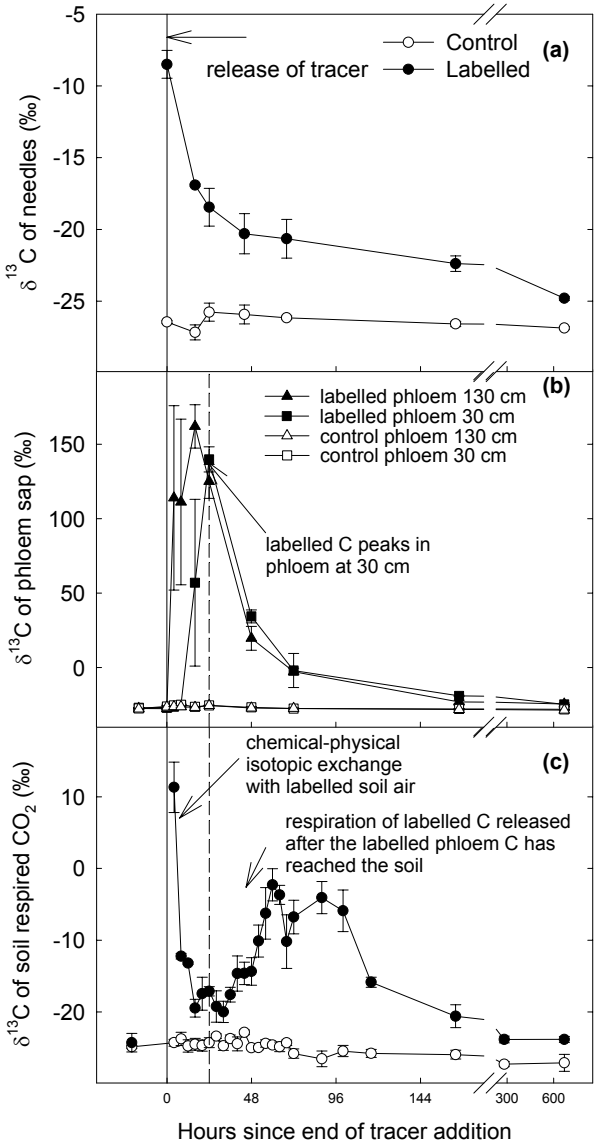

Figure 10. Carbon isotope composition (‰) of (a) needles (b) phloem sap C and (c) of soil-respired CO_2, after 90 min of $^{13}CO_2$ labelling of *c.* 100 young Scots pine trees in a chamber. Mean ±1SE (n = 4, soil respiration and n = 3, needles and phloem sap C). Solid line: end of tracer addition; dashed line: maximum ^{13}C enrichment of phloem sap C at 30 cm height. Symbols are explained in the figure.

Tracing the C from photosynthetic fixation to the phloem

Directly after labelling, leaves in the chamber plot were *c.* 18‰ less negative than in the control plot (Fig. 10a). A difference of 18‰ in total leaf material indicates

that the non-structural leaf carbohydrates were highly labelled, as they are likely to contain most of the new photosynthate. The ^{13}C enrichment in leaves declined rapidly, reaching a value of 2.5‰ ($P < 0.001$) 4 days after labelling (-22.97±0.16‰ (n = 104) compared to -25.50±0.14‰ (n = 25) in the control area). Part of the decline in enrichment can be explained by respiratory use of the new photosynthates. However, a substantial proportion was also exported as the soluble fraction of phloem sap showed high enrichment in ^{13}C 4 and 16 h after labelling at 130 and 30 cm, respectively (Fig. 10b).

The time lag between the appearance of label at 130 and 30 cm can be used to estimate the transport speed of substances in the phloem. We took samples only every 4 to 8 h, thus we can only get a rough estimate of the transport speed of between 6 and 25 cm h^{-1}, which is lower or at the low range in comparison to the usually reported phloem transport velocities (0.2 to 2 m h^{-1}) (Taiz & Zeiger, 2002; Nobel, 2005). If we use the time lags between maximum ^{13}C enrichment of the phloem at heights of 130 and 30 cm, then the estimated velocity would be 12.5 cm h^{-1}. Ekblad & Högberg (2001) found that the air relative humidity 1 to 4 days before the days of soil CO_2 sampling best explained the variation in $\delta^{13}C$ of soil-respired CO_2. The trees in their study were 20 to 25 m tall. With phloem transport velocities of 6 to 25 cm h^{-1}, as estimated in our study, the time lag for trees of that size should be 3 to 17 days. However, in the time before the labelling in our study there was unusually little precipitation, which may have slowed the transport in the phloem. When the water potential in the xylem decreases, fluid in the phloem generally moves more slowly (Nobel, 2005).

Figure 11. Photo of the 200 m^3 chamber that was used to label the canopy of young Scots pine trees with $^{13}CO_2$.

Tracing the C within the belowground compartment

During the first three samplings (≤ 12 h) after labelling, the $\delta^{13}C$ of the soil CO_2 efflux declined rapidly from an initially high value. This efflux of $^{13}CO_2$ preceded the label entering the soil via the phloem flux (Fig. 10b and c), but can be explained by an isotopic enrichment of soil CO_2 during the labelling followed by chemical-physical equilibration when the chamber was removed (Fig. 10c). The understory was very sparse and thus seems extremely unlikely to be a major contributor to the observed ^{13}C labelled CO_2 efflux at the beginning of the chase period. Between 16 and 52 h after labelling $\delta^{13}C$ of soil-respired CO_2 was not different from pre-treatment values. Between 52 and 100 h after the labelling soil-respired CO_2 was significantly less negative than pre-treatment values reaching $\delta^{13}C$ values of up to -4.18±2.37‰. This was *c.* 20‰ less negative than the pre-treatment and the corresponding control value. This efflux of labelled CO_2 followed after the maximum observed $\delta^{13}C$ value in the phloem and thus can be seen as tracing the C that was fixed in the canopy and transported belowground (Fig. 10b and c). The C in the phloem sap reached values up to 162±15‰ at 130 cm (16 h after labelling) and 140±8‰ at 30 cm above the soil (24 h after labelling), i.e. 189‰ and 165‰ above the controls, respectively. A month after labelling, phloem sap C had not returned to the natural abundance at 30 cm (Fig. 10b). On its way from the leaves, to the roots and back to atmosphere, the C that had been fixed during the ^{13}C pulse mixed with unlabelled C that had been fixed before the ^{13}C pulse. Moreover, soil-respired CO_2 consists of recently plant derived C, i.e. an autotrophic component that was partly labelled in our experiment and of soil respiration by heterotrophs that is not labelled. These two factors explain why the maximum $\delta^{13}C$ value of soil-respired CO_2 was lower than of phloem sap C.

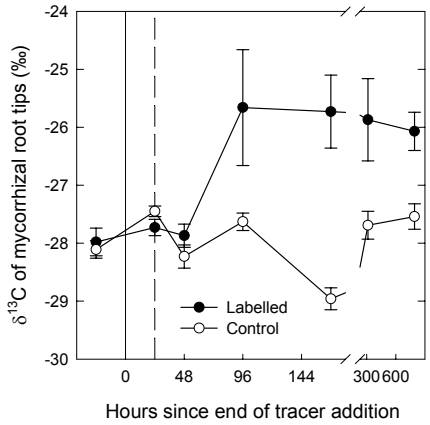

Figure 12. Carbon isotope composition (‰) of mycorrhizal root tips after 90 min of $^{13}CO_2$ labelling of *c.* 100 young Scots pine trees in a chamber. Mean ±1SE (n = 9 to 18). Solid line: end of tracer addition; dashed line: maximum ^{13}C enrichment of phloem sap C at 30 cm height. Symbols are explained in the figure.

Ectomycorrhizal root tips were *c.* 2.0 and 3.2‰ less negative on the $^{13}CO_2$ labelled plot than on the control plot, 4 and 7 days after labelling, respectively (Fig. 12). More than a month after labelling ectomycorrhizal root tips were still 1.5‰ less negative than on the control plot (Fig. 12). The slow decline of tracer levels indicates low turnover rates of ectomycorrhizal roots.

The long period during which C that has been assimilated during only 90 min was seen in the phloem (Fig. 10b) and in mycorrhizal root tips (Fig. 12) also explains why the studies (Paper I and II) that attempted to exploit natural variation to study the C flux on a diurnal time scale failed.

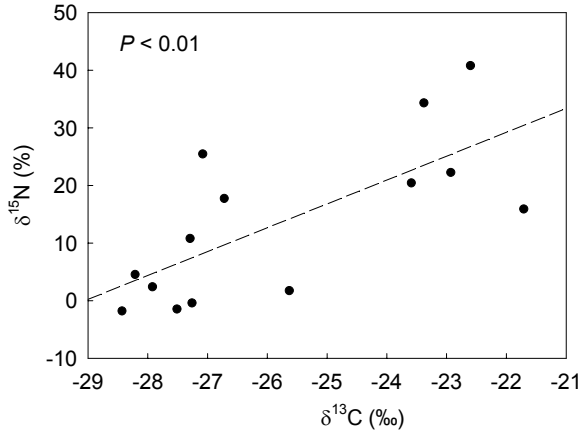

Figure 13. $\delta^{15}N$ *vs.* $\delta^{13}C$ of ectomycorrhizal root tips 7 days after labelling the soil with NH_4Cl (98 atom percent ^{15}N) and the canopy with CO_2 enriched in ^{13}C.

On the dependence of ectomycorrhizal fungi on photosynthate C

Ectomycorrhizal fungi are directly supplied with C from tree roots and it can be expected that they contain C that was fixed relatively recently. In a deciduous forest, it was calculated that 62% of the C in ectomycorrhizal sporocarps was fixed during the same growing season (Keel, Siegwolf & Körner, 2006). Moreover, tree-girdling almost eliminated the sporocarp production of ectomycorrhizal fungi; there were virtually no sporocarps in plots girdled 3 months earlier, while they were abundant in the control plots (Högberg *et al.*, 2001). To investigate which C sources were used by ectomycorrhizal fungi, we added ^{13}C labelled sucrose (*c.* 9.9 atom percent, *c.* 60 mg C m^{-2}) to small plots outside the chamber and control plots. The small amount of C added should not increase the amount of easily available C noteworthy. Two days after sucrose addition mycorrhizal root tips were not different from pre-treatment values (data not shown, $P = 0.302$). Together with the observed ^{13}C enrichment of ectomycorrhizal root tips on the $^{13}CO_2$ labelled plot this result emphasises the dependence of ectomycorrhizal fungi on direct

belowground transport of tree-derived C. Sporocarps of the ectomycorrhizal species *Suillus bovinus* collected 4 weeks after the labelling in the chamber plot (Paper III) were slightly, but not significantly (possibly due to the limited number of sporocarps that grew within the chamber plot), enriched in ^{13}C (mean on chamber plot -24.75±0.10‰ and on control plot -24.98±0.07‰, $P = 0.076$, n = 10). The same day the canopy was labelled with $^{13}CO_2$ the soil was labelled with NH_4Cl (98 atom percent ^{15}N). A correlation between $\delta^{13}C$ and $\delta^{15}N$ of ectomycorrhizal root tips showed that the root tips that were the strongest sinks for photosynthate were also the most active in taking up N from the soil (Fig. 13).

Concluding remarks on the $^{13}CO_2$ labelling experiment

Our hypothesis was confirmed: a short pulse of CO_2 highly enriched in ^{13}C that does not expose trees to noteworthy elevated CO_2 concentrations makes it possible to estimate transfer times of C from leaves, to the phloem, to ectomycorrhizal root tips and to soil-respired CO_2.

Diurnal changes in the respiration rate

There are many reports of diurnal changes in soil respiration rate (Hirano & Kim, 2003; Knohl *et al.*, 2005; Tang, Baldocchi & Xu, 2005; Liu *et al.*, 2006). Diurnal variation in the soil CO_2 efflux could be caused by the temperature dependence of respiratory processes or by differences the in supply of respiratory substrates (Davidson, Janssens & Luo, 2006; Liu *et al.*, 2006). It was reported that diurnal variation in ecosystem and soil respiration is driven by photosynthetic activity (Knohl *et al.*, 2005; Tang, Baldocchi & Xu, 2005) or soil temperature (Hirano & Kim, 2003; Tang, Baldocchi & Xu, 2005; Borken *et al.*, 2006). Nevertheless, we saw no changes, either in girdled or in non-girdled plots associated with diurnal patterns in the respiration rate in the boreal spruce forest (Fig. 7b).

The absence of diurnal variation in the respiration rate in the non-girdled plots (Fig. 7b), where the share of autotrophic respiration was high (*c.* 50% of the total respiration rate) could be due to the short nights in the boreal forests (only 2 h with no direct sunlight in June and 6 h at the beginning of August). The length of the night is in contrast with the work of Tang, Baldocchi & Xu (2005) in California during the summer (around 10 h of night). However, when the $^{13}CO_2$ labelling experiment was conducted, there where 8.5 h without sunlight, but still there was no obvious diurnal pattern in the respiration rate (Fig. 8b). As we measured soil respiration rate on girdled and non-girdled plots at Flakaliden (Fig. 7b) and did not find diurnal changes in either of them we know that the plant root activity did not uncouple relationships between heterotrophic soil respiration and temperature (Bhupinderpal-Singh *et al.*, 2003; Ekblad *et al.*, 2005).

The lack of substantial diurnal change in respiration rate and in the C isotope composition of soluble sugars in the phloem (Paper II), root carbohydrates (Paper I) and soil-respired CO_2 (Paper II) suggest that it is sufficient to measure the rate and C isotope composition of soil respiration once a day in boreal forests during the summer.

Significance of the belowground C flux

In the experiment performed 3 and 4 years after girdling of the Scots pine stand at Åheden we assessed differences in the metabolic activity of microorganisms on girdled and non-girdled plots using a field version of the SIR approach (Paper IV).

In both years and treatments (girdled and non-girdled), respiration rates increased significantly after sucrose additions in comparison to the respiration rate of the control (Fig. 14a). The relative increase after sucrose addition (as a percentage of the basal respiration (BR_{field})), averaged over the 3- or 6-day sampling period was similar between treatments (199 and 213% in 2003 and 169 and 173% in 2004 for non-girdled and girdled plots, respectively). The absolute increase in respiration rate (after addition of sucrose), averaged over the sampling period, was higher in non-girdled plots than in girdled plots in 2004 ($P < 0.01$).

The percentage of the induced respiration that can be ascribed to an increase in respiration of C_3-C, i.e. endogenous C, averaged over the sampling period was 33±13% in 2003 and 41±2% in 2004 in non-girdled plots, but -20±15% in 2003 and -35±10% in 2004 in girdled plots. This means that in girdled plots the percentage of the induced respiration that can be ascribed to an increase in respiration of added C_4-C accounted for more than 100 percent of induced respiration, indicating that respiration of C_3-C decreased after C_4-sucrose addition (Fig. 14b). Thus, our hypothesis that the C_4-sucrose induced short-term increase in C_3-respiration would be significantly less in the girdled plot was confirmed.

The observed differences in the respiratory response to sucrose addition in girdled and non-girdled plots (i.e. lower absolute increase in respiration rate and no priming effect in girdled plots) showed that terminating the C flux from the canopy to the root-soil system not only reduces root and mycorrhizal respiration immediately (Högberg *et al.*, 2001) but also influences other soil microorganisms.

We do not believe that the observed differences in the response to sucrose addition on girdled and non-girdled plots come from a reaction to C_4-sucrose addition by mycorrhizal fungi on non-girdled plots. It has been demonstrated for several ectomycorrhizal fungal species that they do not grow in cultures with sucrose as the only C source (Salzer & Hager, 1993; Kowallik *et al.*, 1998). Further, protoplasts of the ectomycorrhizal fungus *Amanita muscaria* have been shown not to take up sucrose *in vitro* (Chen & Hampp, 1993). Moreover, there is

experimental evidence that sucrose addition does not induce an increase in respiration rate in non-mycorrhizal roots and ectomycorrhizal roots (Ekblad & Högberg, 2000) and that the C_4-sucrose-induced increase in C_3-respiration is similar in sieved (root-free) mor-layers and in the field (with roots) (Ekblad & Högberg, 2000; Kelliher, Barbour & Hunt, 2005). Other conclusive evidence that ectomycorrhizal fungi do not respond to sucrose addition comes from the ^{13}C labelled sucrose (*c.* 9.9 atom percent) addition experiment in the field that complemented the $^{13}CO_2$ labelling study (Paper III), where no enrichment of ectomycorrhizal root tips was observed after two days. Further, the PLFA 18:2ω6,9, that is probably most representative for ectomycorrhizal fungi, became enriched only 4 days after sucrose addition as opposed to the fungal biomarker 18:1ω9 that became enriched 1 day after sucrose addition (personal communication M.N. Högberg). The delay in enrichment in the PLFA 18:2ω6,9 suggests that sucrose was not taken up directly.

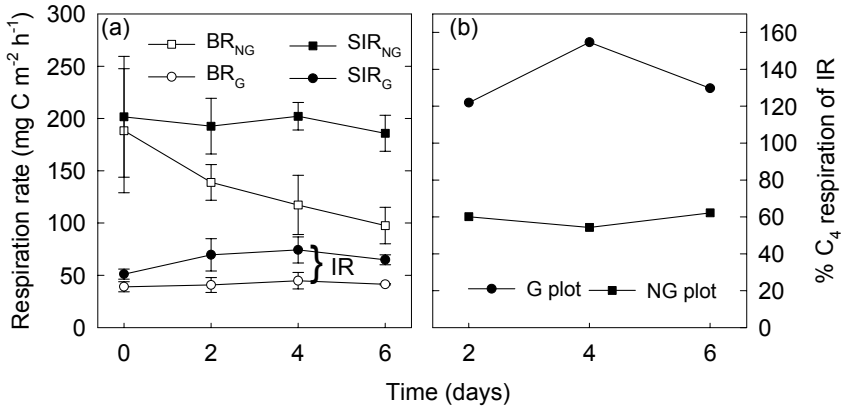

Figure 14. (a) Basal respiration rate (BR) and substrate induced respiration rate (SIR) (mg C m^{-2} h^{-1}) in girdled (G) and non-girdled (NG) plots in 2004. Error bars represent ±1SE (n = 2 to 3). (b) Percent of C_4-C derived CO_2 of the induced respiration (IR) in 2004. The percentages were calculated using the mean values of the respiration rate and the mean $\delta^{13}C$ values of the respiration of the three non-girdled and the three girdled plots.

The disappearance of the "primable" C in the long term absence of a continuous plant phloem C flux to the soil could be due to decreased endocellular reserves of individual microorganisms or due to shifts in the microbial community composition towards species with less endocellular reserves. Alternatively, the absence of a positive priming effect in the girdled plots might indicate that the fraction of SOM that can be mobilised if the C limitation is relieved had already been exhausted during the long period after girdling.

Spatial aspects on the belowground C flux

The work discussed so far has dealt with temporal aspects of the transport of C from the canopy to the root system and the dependence of soil microorganisms on C supply from tree roots. The last study focuses on spatial aspects of the belowground C flux, i.e. the horizontal distribution of tree-derived C by roots. The C flux from the canopy to the belowground compartment affects the soil only as far as tree roots and their mycorrhizal fungi reach. We assessed the lateral spread of the influence of tree root systems using two independent approaches, (1) by analysing the distribution of ectomycorrhizal sporocarps on tree-girdled and adjacent non-girdled plots and (2) by a ^{15}N uptake experiment, where the soil in circular 1m^2 plots was labelled and the uptake into the canopy of trees surrounding the labelled plot was studied.

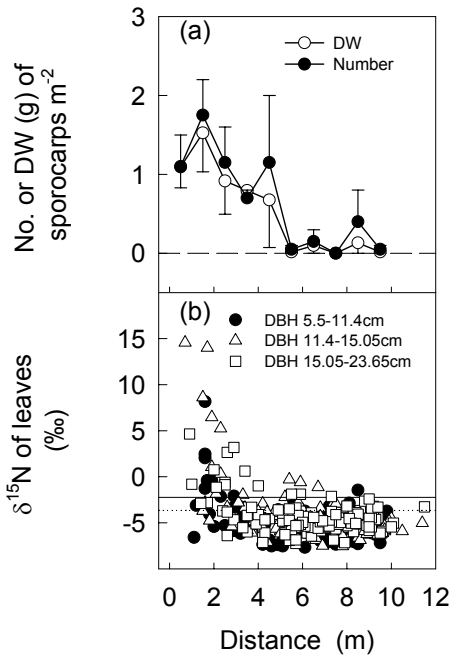

Figure 15. (a) Number and dry weight (DW, g) of ectomycorrhizal sporocarps m^{-2} on consecutive one-meter-wide strips parallel to the edge in two EG plots on 24 August 2001 at Åheden. Distance is distance from the plot edge into the girdled plots. Error bars indicate ±1SE. (b) Nitrogen isotope composition of leaves (‰) *vs.* distance from the plot centre (m), i.e. where the label was applied at Åheden. The trees were grouped into different DBH (diameter at breast height) classes. Symbols for the respective DBH classes are given in the figure. The solid line represents the limit of enrichment for the plot with the highest $\delta^{15}N$ value as limit. The dotted line represents the limit of enrichment for the plot with the lowest $\delta^{15}N$ value as limit (see Paper V for explanation). Symbols above the lines can be considered as representing enriched leaves.

Sporocarp distribution at both sites suggested that the photosynthates were distributed within 4 to 5 m from tree trunks (for Åheden see Fig. 15a). This was consistent with the estimate for the average N-foraging area by mycorrhizal tree roots (45 and 68 m^2). This estimate was based on the number of trees that took up ^{15}N and not on the assumption of a circular rooting area. Assuming a circular rooting area, the lateral mycorrhizal root spread can be converted into an area and

the average area in which N is foraged can be converted into average lateral root spread. This gave values of 50 and 78 m^2 and of 3.8 to 4.7 m, at Åheden and Storskogberget, respectively. While this does not confirm a circular shape of root systems, it does suggest that a circular shape was on average a good proxy for the form of root systems. However, the observation that not all trees close to the injection area had taken up label (Fig. 15b) indicated that either some root systems are highly asymmetric or that some root systems are very small.

Six to 11 trees took up N from a 1 m^2 area (i.e. the labelled plot) of soil. This indicated overlapping of the root systems and a high potential for belowground competition. This was confirmed by data from the sporocarp distribution study. The stand density (1300 and 1080 stems ha^{-1} at Åheden and Storskogberget, respectively) implied that the root system of a single tree would occupy only *c.* 8 and 9 m^2 if tree root systems were mutually exclusive. However, it was estimated that photosynthates were distributed to trees within an area of 50 and 78 m^2, which would only be possible if tree root systems overlapped. Overlapping of tree root systems was expected as several studies have demonstrated that fine roots of trees intermingle (Kuiper & Coutts, 1992; Brunner *et al.*, 2004; Saari *et al.*, 2005).

Our study indicated distances of 9 and 9.5 m for maximum lateral root spread of mature Scots pine and Norway spruce (for Scots pine, see Fig. 15b). These values were in the range of results from other studies that used different approaches to determine lateral root spread (Stone & Kalisz, 1991; Müller & Wagner, 2003; Johnsen, Maier & Kress, 2005; Saari *et al.*, 2005). The estimate of the average root spread, however, is more interesting in the context of ecology and forest management than observations of single roots that extend very far, but contribute little to the total root activity of a tree.

^{15}N enriched leaves of dwarf shrubs were found *c.* 2 m away from the edge of the ^{15}N labelled area. Either the roots of the dwarf shrubs explored soil within a radius of up to 2 m, or labelled N was translocated extensively within the same clone. In either case, the maximum lateral root spread of the dwarf shrubs was smaller than that of the trees.

The study clearly showed that analysing the ECM sporocarp distribution on tree-girdled plots and adjacent plots and the study of uptake of stable isotopes gave similar estimates of lateral root spread. The results emphasise the dependence of ectomycorrhizal fungi on photosynthate and indicate the high potential for belowground competition between trees in these boreal forests.

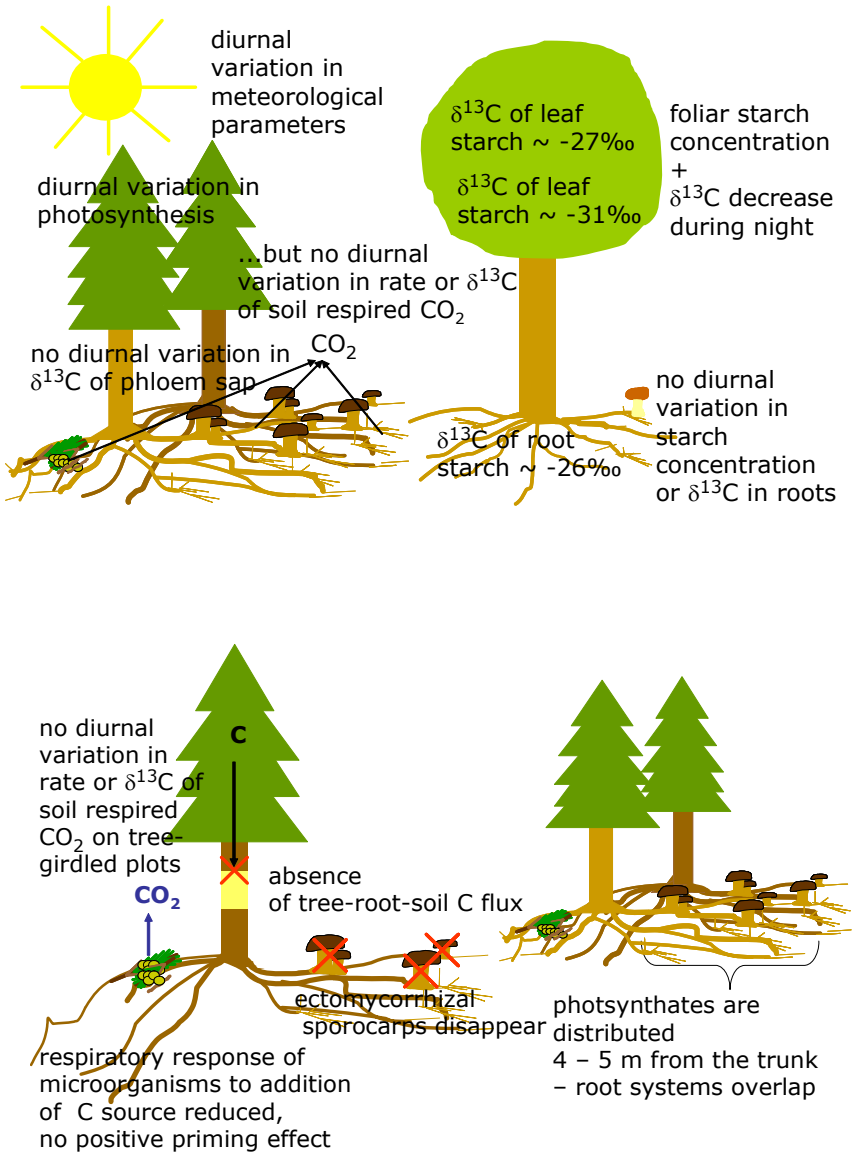

Figure16. Illustration summarising the main results of the studies on variation of natural abundance on short term scales (Papers I and II) and of the studies conducted on the girdled plots (Papers IV and V). The studies were conducted in a temperate beech forest (Hainich, Paper I) in Germany, in boreal Norway spruce forests (Flakaliden, Paper II; Storskogberget, Papers IV and V) and in a boreal Scots pine forest (Åheden, Papers IV and V) in Sweden.

Concluding remarks

The main results of this thesis are illustrated in Figs 16 and 17.

Methods for the isolation of starch and soluble carbohydrates for stable C isotope analysis were improved which enabled analysis of plant material with very low carbohydrate concentrations. Changes in the $\delta^{13}C$ associated with a diurnal rhythm were observed in the carbohydrate pool of beech leaves, but not of roots. No correlations were found between the respiration rates or the $\delta^{13}C$ of soil-respired CO_2 of both girdled and non-girdled plots and meteorological variables or photosynthetic rates in a boreal Norway spruce forest. Thus, our hypothesis that diurnal variation in $\delta^{13}C$ of leaf carbohydrates might result in diurnal variation of $\delta^{13}C$ values of phloem sugars, root carbohydrates and finally of soil-respired CO_2 was not confirmed. Consequently, it seems that variation in natural abundance of stable C isotopes can not be employed to study the temporal coupling of the C flux in the tree-root-soil continuum on the diurnal time scale.

As expected a short pulse of CO_2 highly enriched in ^{13}C made it possible to estimate transfer times of C flux from leaves, to the phloem, to ectomycorrhizal root tips and to soil-respired CO_2. The transport velocity in the phloem of young Scots pine trees was estimated to be between 6 and 25 cm h^{-1} and soil-respired CO_2 was labelled within 2 days. This emphasised that part of the C fixed during photosynthesis is rapidly respired and does not contribute to long- or medium term C sequestration. The slow decline of tracer levels in ectomycorrhizal roots, however, indicates low fine-root turnover rates. Chamber-based labelling with $^{13}CO_2$ has the potential to answer a wide range of questions concerning tree physiology and plant-soil interactions. It can also be used to assemble detailed C budgets of small forest stands.

Our hypothesis that the C_4-sucrose induced short-term increase in C_3-respiration would be significantly less in the girdled plots was confirmed. The absolute increase in induced respiration was lower than in control plots and there was no positive priming effect in girdled plots. We see this as an indication that the C flux from the canopy to the root-soil system not only influences roots and ectomycorrhizal fungi, but also other soil microorganisms.

Photosynthates were distributed within 4 to 5 m from tree trunks at two boreal forest sites with mature trees. Analysing the distribution of ectomycorrhizal sporocarps on tree-girdled and adjacent non-girdled plots and studying ^{15}N uptake into the tree biomass gave very similar results. As root systems overlapped we could demonstrate that there is a potential for belowground competition between trees. However, whether a tree supplies a certain area of soil with C or if it is in competition with neighbouring trees cannot be inferred from its position in a stand

because not all trees close to a particular area of soil necessarily have roots in that area.

The results presented in this thesis highlight the close link between plants and soil microbial activity. It is ecologically more relevant to understand the relationship and interconnectedness of these two components than to tease them apart. Variation in the stable C isotope composition of photosynthates might provide a natural label of the C assimilated under special conditions, but very often this label is not distinct enough to enable tracing of the C through the ecosystem. $^{13}CO_2$ labelling studies in the field have, as demonstrated here, the potential to give better insight into the C flux in the tree-root-soil continuum and interactions between trees and soil microorganisms.

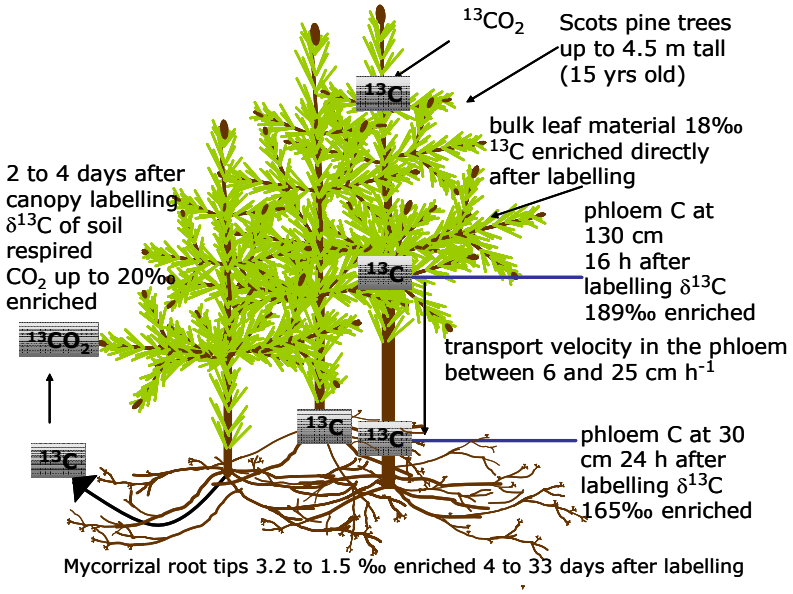

Figure 17. Illustration summarising the main results of a $^{13}CO_2$ labelling experiment in a young Scots pine forest (Rosinedalheden).

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