Soil Respiration Fluxes and Controlling Factors in Temperate Forest and Cropland Ecosystems

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1. Introduction and Overview

1.1. The Role of Soil Respiration in the Carbon Cycle

The biogeochemical cycle of carbon includes several reservoirs which differ in their size and turnover times (Figure 1.1). The atmosphere holds about 800 gigatons (Gt) of carbon and has the fastest turnover time with ca. 122 Gt C being taken up by the terrestrial biosphere and 90-92 Gt C being exchanged with the surface ocean every year (Sabine et al. 2003).

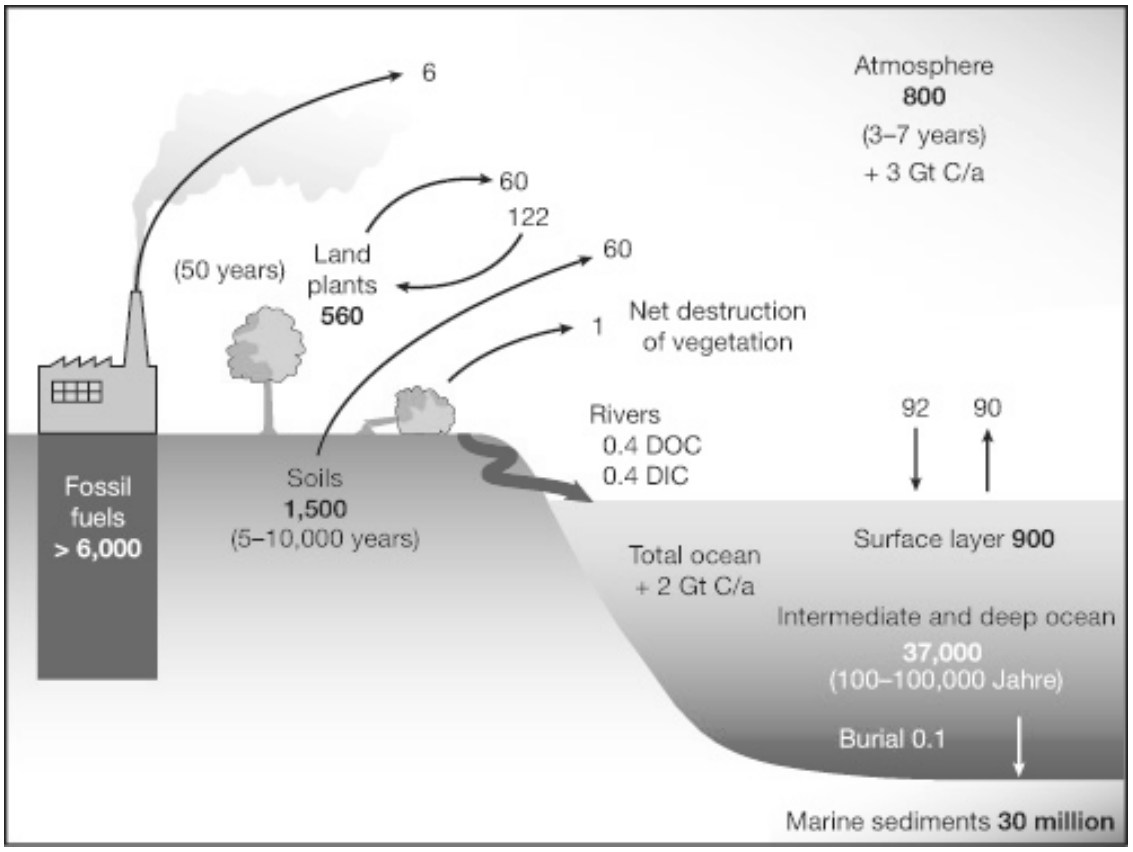

Figure 1.1: The global carbon cycle. Values are given in Gt C. Bold prints are reservoirs and normal prints are fluxes. Mean residence times are in parentheses. DOC = dissolved organic carbon, DIC = dissolved inorganic carbon. Source: WBGU (Schubert et al. 2006). Adapted after Schlesinger (1997); Sabine et al., (2003); Raven et al., (2005); NOAA-ESRL, (2006).

Carbon can remain for a period of days to several years as part of the terrestrial biomass but will eventually be respired back to the atmosphere as CO_2, or it will become litter and

form part of the pedosphere, i.e. soils. Soils, with a total global storage of approximately 1500 Gt C (Jacobson et al. 2000) hold three times as much carbon as the terrestrial biosphere and about twice as much as the atmosphere. The carbon cycle is a dynamic system sensitive to environmental changes that can influence the magnitude of the fluxes and the storage time in each reservoir. Anthropogenic emission of CO_2 from fossil fuels and land use change (e.g. deforestation, management) are currently driving this cycle away from equilibrium with largely unknown effects on the biosphere and the climate system, including positive and negative feedbacks. In order to understand and predict relations between the carbon cycle, vegetation and climate much effort is being directed towards understanding the processes involved.

Soil respiration is defined as the efflux of CO_2 from the soil surface and has been estimated at ca. 75-80 Gt of carbon per year globally (Raich and Potter 1995; Raich et al. 2002), which is nearly half of the gross primary productivity (GPP) of terrestrial ecosystems and about 10% of the total atmospheric carbon. Soil respiration is the result of the production of CO_2 in soils from a combination of several belowground processes (Ryan and Law 2005; Trumbore 2006). The most important are the biological activity of roots and their associated microorganisms and the activity of heterotrophic bacteria and fungi living on litter and soil organic matter (SOM). Non biological processes related to chemical weathering in soils are estimated to be a net carbon sink of ca. 0.3 Gt yr^{-1} (Jacobson et al. 2000), thus being of less significance.

An increase in atmospheric CO_2 concentrations has been identified as the main cause of current global warming (IPCC 2007). Given the magnitude of soil respiration fluxes, relatively small changes at the global scale can signify large changes in the amount of carbon stored in soils and in the atmosphere. A release of carbon from soils through respiration following climate change would create a positive feedback mechanism exacerbating warming effects. Conversely, increased storage of carbon in soils, as through CO_2 fertilization of plant growth leading to increased inputs into soils, would imply a negative feedback and diminished warming effects. The implications of soil carbon dynamics for climate change are therefore of great importance, not only because of changes in storage, but also in relation to ecosystem physiology, acclimation and adaptation. Climate related changes in, for example, above and belowground CO_2

concentrations, temperature changes, and water conditions will have yet largely unknown effects on respiration fluxes and carbon pools. These factors can affect carbon fluxes directly, as through temperature changes of enzymatic reaction rates (Davidson and Janssens 2006), but they may also have less direct effects through changes in vegetation, nutrient availability, etc.

The cycling of carbon through soils is determined by vegetation and soil organic matter dynamics. Plant litter is the major source of soil organic matter. Litter quality and its processing by bacteria and fungi determine the size and properties of organic pools through interactions with soil minerals, soil structure and other soil characteristics (Kogel-Knabner 2002; Lutzow et al. 2006). At the same time, organic matter affects plant growth through its role in soil development and as a source of nutrients. The flow of carbon through soils is thus not a straightforward process. Temperature and moisture are known to have a large effect on the activity of roots and microbes. For soil microbes, higher temperatures may decrease the activation energy for degrading complex molecules and it may also lead to higher mobility of cells and organic matter, thus increasing respiration rates (Davidson et al. 2006). For roots, higher temperatures lead to increased maintenance respiration for repairing living tissues (Atkin et al. 2005). However, variations in soil respiration fluxes are not explained by temperatures and moisture alone. Relations of these fluxes with plant, rhizosphere, mycorrhizal and microbial dynamics and functioning, as well as with nutrients, organic matter, and soil characteristics, are currently being explored. The relevance of each factor and the relations involved are still not understood well enough to make long-term predictions of soil respiration with accuracy at local or global scales. The importance of C input by root and mycorrhiza in determining carbon storage in soils is likewise poorly characterized. As a consequence, soils are still a source of large uncertainty in ecosystem and climate modeling.

1.2. Measuring Soil Respiration

The efflux of CO_2 from soils is the result of the respiration of different groups of organisms, a fact which has lead to the development of methods to partition and measure these fluxes separately. These include trenching and exclusion, shading and clipping,

component integration, tree girdling and isotopic techniques. Comprehensive reviews of these methods are given by Hanson (2000), Kuzyakov and Larionova (2005), Kuzyakov (2006), and Subke (2006). Most methods involve a certain degree of disturbance of the soil system that changes natural fluxes to an uncertain degree. As an example, girdling of trees is an innovative method in which the sap flow from the canopy to the roots is cut, thus stopping the transport of new photosynthates without disturbing the soil system (Högberg et al. 2001). The dependence of belowground respiration activity on the short term supply of new carbon can thus be effectively studied. However, the use of reserve carbon stored in plant tissues and the decomposition of dying roots and mycorrhiza introduce uncertainties difficult to estimate. Isotopic techniques, on the other hand, present the lowest degree of disturbance (Gaudinski et al. 2000). However, isotope fractionation by different, sometimes unknown, processes creates further uncertainties, while the work and material involved in measuring isotopes also limits their applicability.

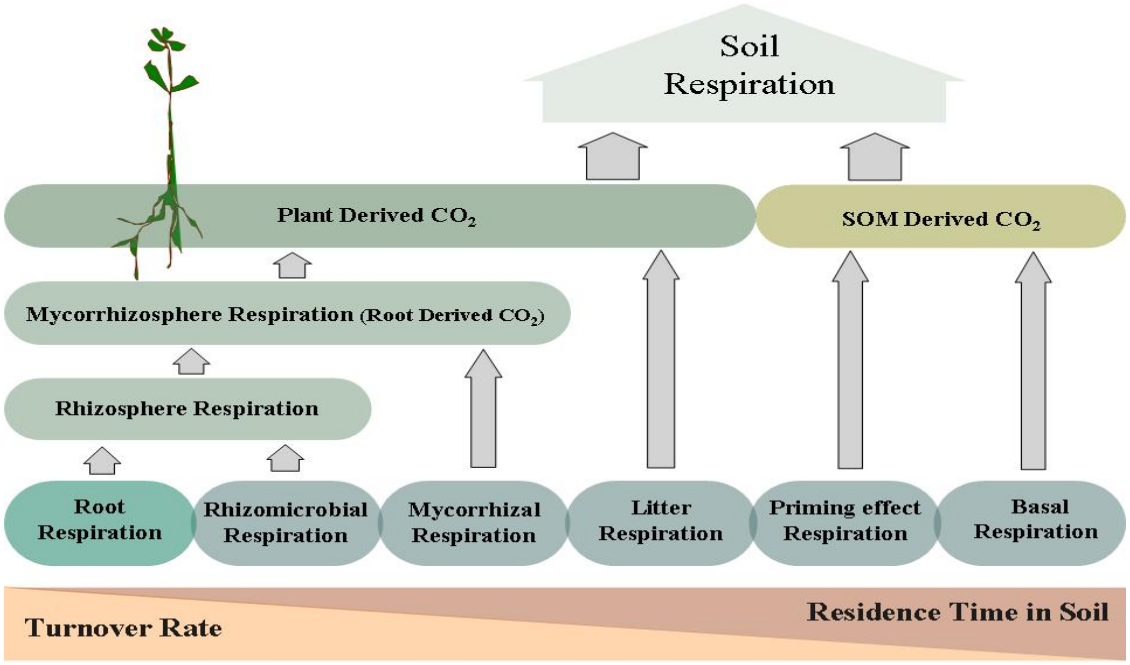

Figure 1.2: Diagram with a simplified representation of soil respiratory processes. For a more realistic view, a distinction is made between respiration by the live root tissue and respiration of rhizodeposits by microbes in the rhizosphere (rhizomicrobial respiration) and by mycorrhizal fungi (mycorrhizal respiration). Respiration of fresh plant litter is also distinguished from respiration of older, qualitatively different SOM (basal and priming respiration). Modified after Kuzyakov (2006).

Despite the complications associated with different methods, partitioning soil respiration allows researchers to measure the contribution of each respiration source to total fluxes and the individual response of each source to environmental factors. Methods for partitioning frequently allow distinguishing between fluxes derived from root-carbon and those derived from soils without roots. Thus, the terms *autotrophic soil respiration* and *heterotrophic soil respiration* are widely used to distinguish between these sources. Other equivalent terms found in literature are *root* or *rhizosphere respiration* and *microbial respiration*. Studies using different methods have shown a different response of these fluxes to temperature and moisture conditions (Lavigne et al. 2003; Scott-Denton et al. 2006). Other partitioning studies have lead to a better understanding of the regulation of root respiration by plant functioning and phenology (Bahn et al. 2006; Fahey and Yavitt 2005). However, a closer view at the soil system shows that a simple partitioning in two components is not sufficient to explain carbon fluxes adequately. Factors such as root exudations, priming effects, symbiosis with mycorrhizal fungi, as well as differences in litter and SOM pools complicate the study of these fluxes. A more precise separation of belowground carbon fluxes becomes necessary, as shown in Figure 1.2.

1.3. Study Objectives

The general aim of this study is to advance the understanding of processes controlling the activity of different belowground respiration sources at the ecosystem scale, as well as to identify relations that can serve as a basis for more realistic models and predictions of carbon fluxes. The specific objectives are:

- To partition soil respiration and to provide estimates of the relative contribution of respiration fluxes to total fluxes and their variability within and between different temperate ecosystems.
- To determine the response of individual respiration fluxes to soil temperature and soil moisture, as well as to identify associated factors influencing such relations.

- To determine the effects of plant photosynthetic activity on rhizosphere and mycorrhizal fungi respiration, together with the time relations involved for each vegetation type.
- To assess the specific spatial relation of individual respiration fluxes with relevant biological, chemical and physical soil parameters.

1.4. Study Approach and General Methodology

The objectives of this study required a partitioning of soil respiration in multiple sites and measurements of different environmental variables influencing respiration fluxes. Although many points are described with more detail in the respective chapters, this section gives an overview and a description of the core methodology.

Study Sites

The study was carried out at the three main sites of the CarboEurope Integrated Project in Thuringia, Germany. This European project aims at quantifying and predicting carbon fluxes at the continental scale and relies on a number of measurement locations equipped with eddy covariance towers for determining CO_2 exchange between the vegetation and the atmosphere (see below). The sites investigated are:

Gebesee: crop field with winter barley (*Hordeum vulgare*) during the study period

Hainich: old growth forest dominated by beech trees (*Fagus sylvatica*)

Wetzstein: 50 year old spruce (*Picea abies*) plantation forest

A more detailed description of each site can be found in the respective following chapters. Three main reasons were associated with choosing these locations. Firstly, these

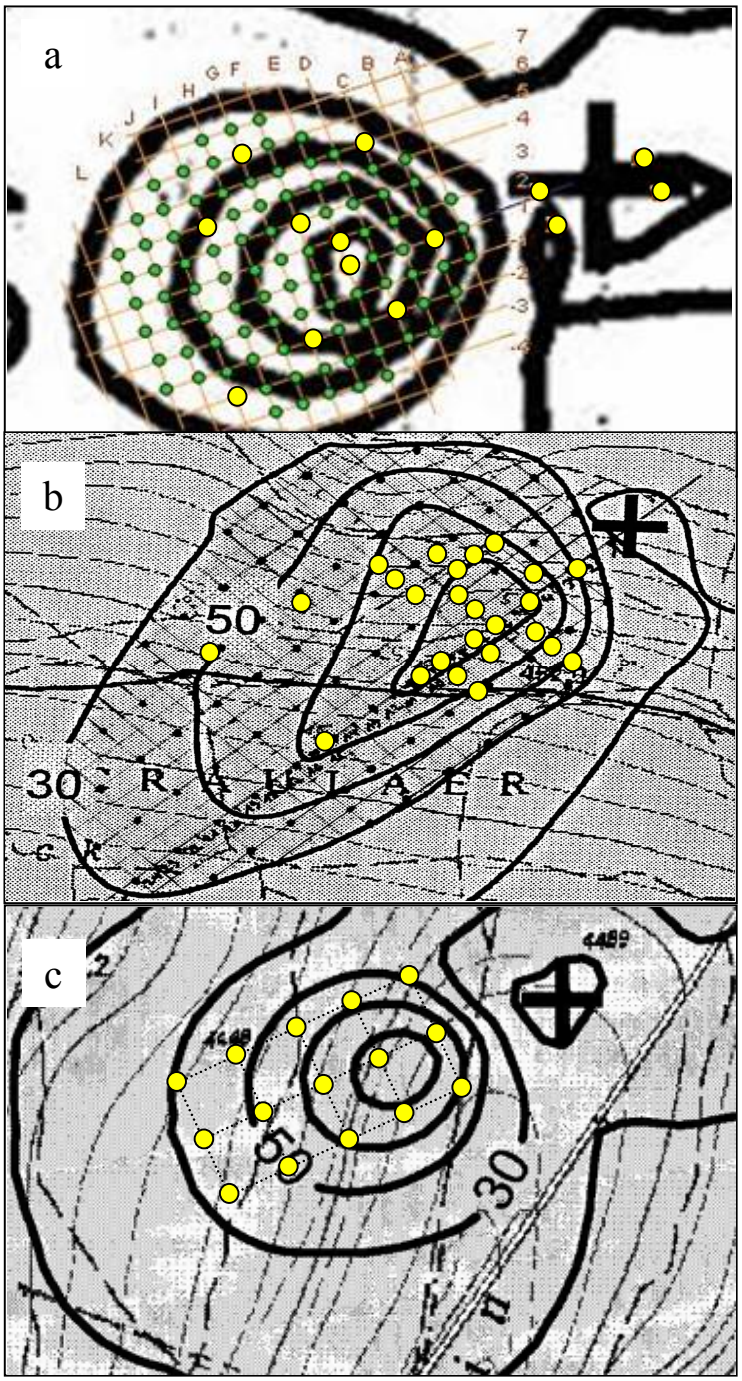

Figure 1.3: Plot location (circles) at Gebesee (a), Hainich (b) and Wetzstein (c). Thick concentric lines denote the main footprint area of the eddy covariance tower (marked with a cross). The distance between grid points is 30 m at Gebesee and Hainich, and 60 m at Wetzstein.

sites represent typical ecosystems of temperate regions across the globe which means that results obtained here can be more easily extrapolated to larger regions. Secondly, measurements of carbon exchange from eddy covariance towers as well as other measurements at the site level were available thanks to intensive monitoring and field campaigns. Finally, as part of the CarboEurope IP, results from this study can be used for integrating data in efforts to understand continental and global scale processes.

Plots for measurements of soil respiration and soil factors were installed at points falling on pre-established grids located in the main footprint area (main wind direction) of the eddy flux towers. These points were randomly distributed at Gebesee and Hainich, where the terrain is relatively homogeneous. At Wetzstein, three parallel transects were used for a better representation of the more complex terrain. The location of each plot is shown in Figure 1.3.

Soil Respiration Measurements and Micro-Mesh Partitioning Method

To measure the soil efflux of CO_2 we used a Licor 6400-09 device (Licor, Inc., Lincoln, Nebraska, USA). This system consists of a portable infrared gas analyzer (IRGA) connected to a non-steady-state through-flow chamber (Pumpanen, 2004) equipped with a pressure relief vent. When making measurements, the chamber (diameter 95 mm, volume 991 cm3) is placed on PVC collars (diameter 10 cm) inserted into the soil. Air circulates from the chamber to the infrared gas analyzer (IRGA) and back by a mixing fan. Before each cycle of flux measurement, air in the chamber headspace is scrubbed down with soda lime 3-10ppm (depending on the flux) below the ambient CO_2 concentration, and then allowed to rise as a consequence of CO_2 efflux from the soil. This procedure was usually repeated three times for each collar. The system calculates fluxes at short intervals from the increase in CO_2 concentrations in the chamber. The final flux is then calculated by regressing these flux rates versus CO_2 concentration in the chamber and computing the flux corresponding to an ambient CO_2 concentration (set by the user prior to the onset of each measurement). The instruments were calibrated yearly in 2004, 2005 and 2006.

The method used for partitioning soil respiration consists of micro-pore nylon meshes surrounding a core of soil over which CO_2 efflux is measured (Figure 1.4). The technique is described in more detail in Chapter 2 while associated advantages and disadvantages are further discussed in Chapter 5. The novelty of this method is the possibility of using different pore sizes that can selectively allow the movement of bacteria or mycorrhiza through the mesh while excluding roots. This allows separating the root derived flux into the rhizosphere component and the mycorrhizal fungal component.

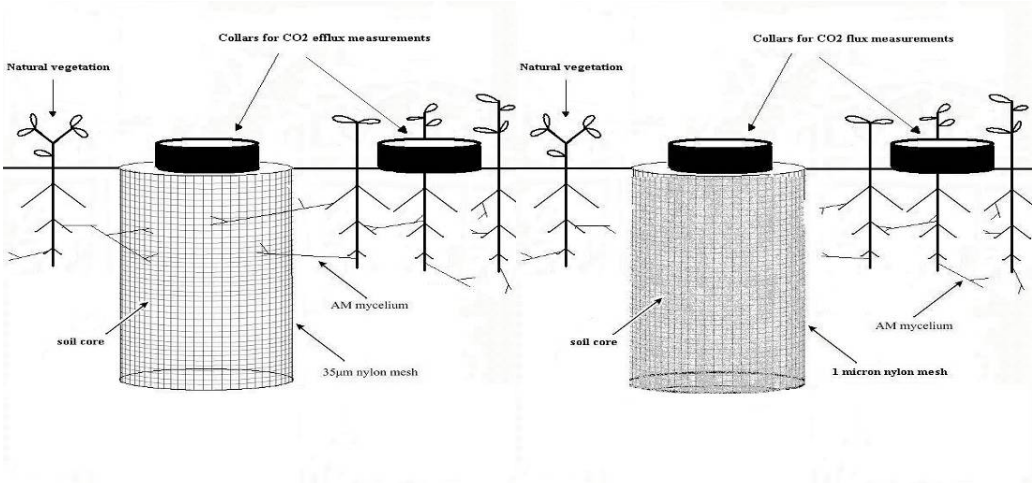

Figure 1.4: Soil cores in micro-pore nylon meshes. 35 and 1 micron mesh bags excluding the entrance of roots or roots plus mycorrhiza, respectively. PVC collars for CO_2 efflux measurements are inserted on top.

Consequently, an incentive for using this method was the possibility of investigating mycorrhizal fungal respiration, a component of soil respiration which has received little attention but which is now recognized as very significant in many ecosystems. In this study, micro-pore meshes are used to investigate the fluxes of mycorrhizal fungal respiration in forest ecosystems and, for the first time, also in croplands.

The partitioned fluxes in this study are defined as follows:

Rhizosphere respiration: includes root and rhizomicrobial respiration, as well as root litter and priming effects in the rhizosphere.

Mycorrhizal fungal respiration: respiration of the extra radical mycelia (ERM) of mycorrhizal fungi. Equivalent to the terms mycorrhizal respiration and mycorrhizal hyphae respiration.

Mycorrhizosphere respiration: equals the sum of rhizosphere and mycorrhizal fungal respiration.

Heterotrophic soil respiration: soil and litter respiration in the absence of roots and mycorrhiza.

Soil respiration measurements were carried out on undisturbed soil (control measurements) and on soil cores surrounded by micro-pore mesh bags. Meshes with pores of 1 and 35 micrometers were chosen, which do not allow the in-growth of roots and exclude or allow the in-growth of mycorrhizal hyphae, respectively (mycorrhizal hyphae have a minimum diameter of ca. 2 micrometers). Thus, PVC collars for efflux measurements were installed at each plot on one control and two different mesh treatments. At the crop field and the beech forest, soil cores were removed intact with the help of stainless steel cylinders (diameter 15 cm, depth 30 cm) and inserted back into the soil inside mesh bags. At the spruce forest, the amount of rocks made this impracticable so the soil profile was manually separated into horizons and filled in the mesh bags (Chapter 4).

Eddy Covariance Measurements

The eddy covariance technique measures the net exchange of CO_2 between the vegetation and the atmosphere. This is done by determining the simultaneous movement of turbulent air fluxes with changes in CO_2 concentrations using a sonic anemometer and a fast IRGA. Although carbon assimilation by plants, or gross primary productivity (GPP), cannot be directly measured with this technique, it can be estimated from the data. This is possible given the lack of photosynthetic activity at night so that nighttime fluxes represent only ecosystem respiration. With a temperature related function, these fluxes are extrapolated to the daytime (Aubinet 2000). Thus, daytime ecosystem respiration and GPP, by subtraction, are estimated. Technical details and references for the eddy flux system are given in Chapters 2 and 3.

Measurements obtained from eddy covariance towers offered the unique possibility of relating partitioned respiration fluxes to canopy processes. Thus, one of the main objectives of this study was to use eddy flux measurements to show the connection between above and belowground processes. Calculated values of gross primary productivity (GPP) were used for this purpose.

Soil Factors and Measurement Methods

A selection of environmental variables relevant to the objectives was measured. Temperature and moisture, usually seen as the main drivers of soil respiration, were measured at the plot and the site level. In addition, carbon content, nitrogen content, ammonium, nitrate, microbial biomass and carbon extracted with K_2SO_4 were measured on specific dates at each plot and soil treatment.

Temperature and moisture were measured manually at each plot during soil respiration measurement campaigns and continuously at the eddy covariance tower. Measurements of other factors were done in the laboratory with soil samples taken on specific dates (Chapter 4). The different methods included:

- *C and N content determination*: soil preparation, drying and grinding followed by C and N measurements with an elemental analyzer
- *Mineral nitrogen determination*: soil preparation, extraction with 1M KCl followed by ammonium and nitrate measurements with a continuous flow analyzer
- *Chloroform fumigation extraction (CFE) for microbial biomass determination*: soil preparation, fumigation with chloroform, K_2SO_4 extraction and total organic carbon determination with a “high TOC” sum parameter analyzer.

1.5. Summary of Results

Relative Contribution of Belowground Respiration Sources

Studies partitioning fluxes in different ecosystems have resulted in diverse estimates of the contribution of each component to total soil respiration. CO_2 respired by roots and associated organisms, derived largely from recently assimilated carbon, is commonly around 50% of total soil respiration. However, this relation can vary widely, depending on the vegetation type and season. The relative proportion of each respiration source resulting from this study showed significant differences between sites and significant changes during the year. The calculated contributions are discussed further in Chapters 2 and 3. In particular, the following results can be highlighted (Table 1.1):

- The respiration of mycorrhizal fungi represented a significant amount of the total in both the barley field and spruce forest. An average minimum contribution of 6±2% and 8±2% was calculated for these sites, respectively. A mean contribution of 3±1% at the beech was not significant. However, a unique relation with GPP (different from that of the rhizosphere; see below) demonstrated the importance of the mycorrhizal respiration process at this site.
- Rhizosphere respiration represented an average of 21±2, 44±2 and 45±2 percent of the total at the barley, beech and spruce site, respectively. Heterotrophic soil respiration averaged 73±3, 53±2 and 47±2 percent of the total at the same respective sites. However, results in the crop field show a large variability in these numbers depending on the crop's growth stage. Both forest sites show a higher heterotrophic respiration contribution during spring and a lower one during autumn.

Table 1.1: Contribution of heterotrophic (Rh), rhizosphere (Rr) and mycorrhizal fungal (Rm) respiration fluxes calculated from measurements in the field and shown for each ecosystem type.

Site	Rh (%)	Rr (%)	Rm (%)
Gebesee	73 (±2)	21 (±2)	6 (±2)
Hainich	53 (±2)	44 (±2)	3 (±1)
Wetzstein	47 (±2)	45 (±2)	8 (±2)

Response to Temperature and Moisture

The relation of soil respiration with temperature is typically exponential. The theoretical basis for this relation is the exponential increase of enzymatic reaction rates with temperature. Thus, current biogeochemical models predict soil respiration fluxes by relating temperature functions with a number of defined soil pools differing in their temperature sensitivity. However, it remains unclear up to what extent this observed relation, sometimes termed the 'apparent temperature sensitivity', is determined by temperature and how much other factors – such as plant and microbial growth, acclimation or substrate supply – are involved. This study presents the following main results on temperature relations (Chapters 2 and 3; Figure 1.5):

- The apparent temperature sensitivity of heterotrophic soil respiration, expressed as a Q_{10} value (the rate of change in respiration with a 10 degree increase in temperature), varied seasonally, as shown for the crop field in Chapter 3. Highest values were seen during colder periods. These time-related differences are attributed mainly to changes in the amounts of remaining litter and soil moisture. On the other hand, the apparent temperature sensitivity of total soil respiration in the crop field was highest during the period of fastest plant growth, revealing the influence of changes in plant biomass.
- Mycorrhizal fungi respiration had no significant correlation with temperature in two of the studied ecosystems, the crop field and the spruce forest. In the beech forest, this flux showed a weaker response to temperature than both heterotrophic and rhizosphere respiration. On the other hand, heterotrophic and rhizosphere

respiration correlated strongly with temperature at all sites. The correlation was similar for both fluxes at the forest sites. At the crop field (Chapter 2), however, the rhizosphere component showed a week response to temperature (R^2=0.14, P<0.01) compared to the heterotrophic component (R^2=0.81, P<0.01).

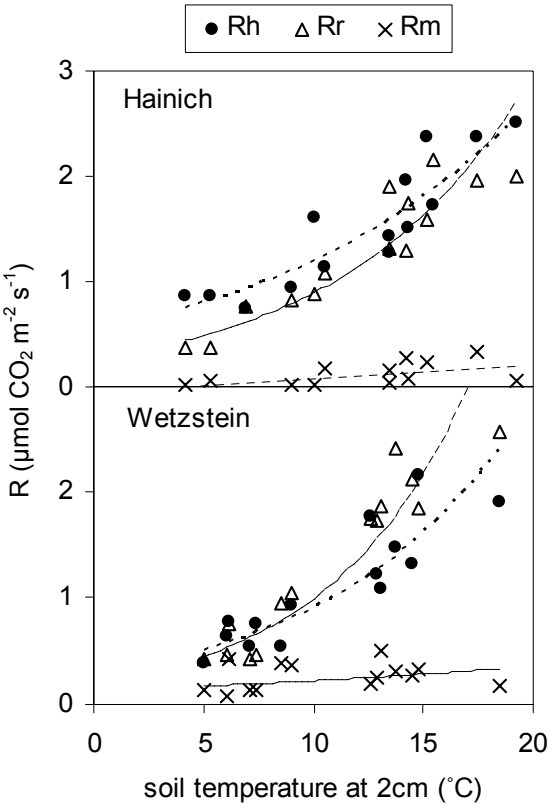

Figure 1.5: Data from Chapter 3 showing the relation of soil respiration with soil temperature at the forest sites. Fluxes in both sites showed exponential fits for hetereotrophic (closed circles) and rhizosphere (open triangles) respiration. On the other hand, mycorrhizal fungal respiration (crosses) showed a week linear relation with temperature in the beech stand and no relation in the spruce stand.

Moisture is known to affect soil respiration at low and high values, through drought stress and oxygen limitation respectively. It can also affect the ability of microbes to reach carbon sources. In this study, no significant relations with moisture, either at low or high ranges, resulted from the analysis. Although extreme values of soil moisture were not measured, the influence of soil moisture was still evident in the following results (Chapters 2 and 3):

- Soil moisture correlated negatively with temperature at all sites, thus being lowest in the warmer periods, partly explaining the decrease in the apparent temperature sensitivity from winter to summer.

- Excluding the dryer periods at the forest sites resulted in higher Q_{10} values for all the fluxes measured (Chapter 3), thus revealing a negative effect of low soil moisture on respiration.
- Negative spatial relations between soil respiration and soil moisture were observed in this as well as in previous studies (Chapter 4). Such relations are related to the activity of roots and are likely caused by the uptake of water by roots and not by oxygen limitation imposed by high levels of moisture. These results put into question previous reports of negative effects of soil moisture on soil respiration.

Response to Photosynthesis

The supply of assimilates from the canopy to the roots following photosynthesis is an important process influencing soil respiration fluxes. A few studies have already measured a response of total soil respiration as well as of the 'autotrophic' component to changes in irradiation and photosynthetic activity. However, this conection between canopy and belowground processes is not well understood, with the strength and the timing of the response being unknown for many vegetation types. In this study, photosynthetic activity was shown to be a factor with a significant influence on the activity of the rhizosphere and mycorrhiza at all the ecosystem under study. The main results include the following (Chapters 2 and 3; Figure 1.6):

- A significant relation between mycorrhizal fungal respiration and GPP was observed at all sites. At the barley field and the beech forest, mycorrhizal fungal respiration showed a strong response to GPP. The correlation between photosynthesis and the effect on respiration was strongest after a lag of 1 day at both these sites. In contrast, the spruce forest revealed a somewhat weaker relation with GPP with a delay of 5 days, similar to the rhizosphere component.
- Rhizosphere respiration was significantly related to GPP at both forest sites. The maximum correlation with rhizosphere respiration was observed 4 and 5 days after photosynthetic activity at the beech and spruce forest, respectively. A

positive but non-significant relation was observed at the crop field, with a delay of 1 day.

- These results indicate a general strong and fast link between assimilation of carbon at the leaf level and the activity of belowground plant organs and associated microorganisms. Vegetation type is shown to influence this response.

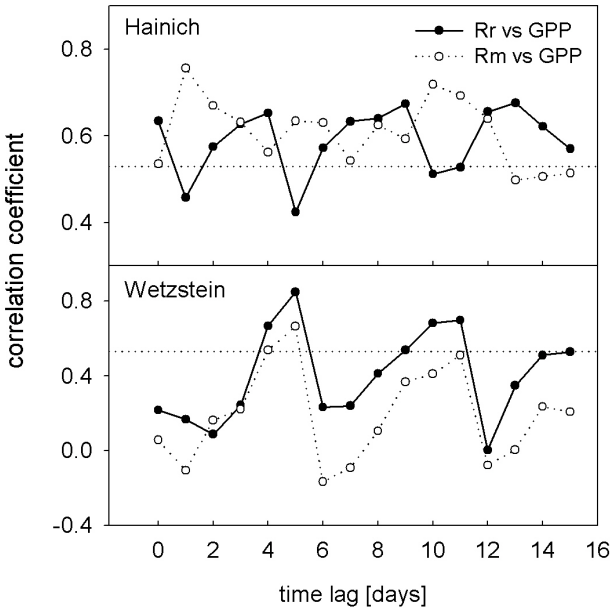

Figure 1.6: Correlation values between rhizosphere respiration (Rr) and mycorrhizal fungal respiration (Rm) with gross primary productivity (GPP). A period of 0 to 15 days previous to respiration measurements is shown for the beech forest (upper panel) and the spruce forest (lower panel). This data is discussed in Chapter 3.

Relations with Spatially Variable Soil Factors

Soil respiration is largely determined by inputs of organic litter into soils, by the amount and quality of older soil organic matter, and by the interaction of these pools with soil physical and biological factors which can vary temporally and spatially. Spatial relations of soil respiration with relevant soil factors were compared at the three study sites in order to identify general as well as ecosystem specific conditions influencing the magnitude and variability of these fluxes. The analysis resulted in the following relations (Chapter 4; Figure 1.7):

- At the forest sites, total soil respiration relations with different factors were mostly influenced by the mycorrhizosphere component, which was more spatially

variable than the heterotrophic component. The opposite relation was found at the crop field, were heterotrophic respiration showed the largest spatial variability.

- Spatial variations of rhizosphere respiration are largely determined by the availability of inorganic nitrogen. Such relations were observed with ammonium at the forest sites and with nitrate at the crop field.
- Ammonium was negatively related to heterotrophic respiration on specific dates at the forest sites. These results are in accordance with theoretical models and observations, supporting the idea of lignin decomposition inhibition at the spruce forest and of changes in carbon utilization at the beech forest.
- The input of labile carbon into the soil by mycorrhizal hyphae and roots may have a priming effect with a consequent reduction of soil organic matter amounts. This conclusion was derived from negative relations of mycorrhizal fungal and mycorrhizosphere respiration with amounts of total and extractable carbon.

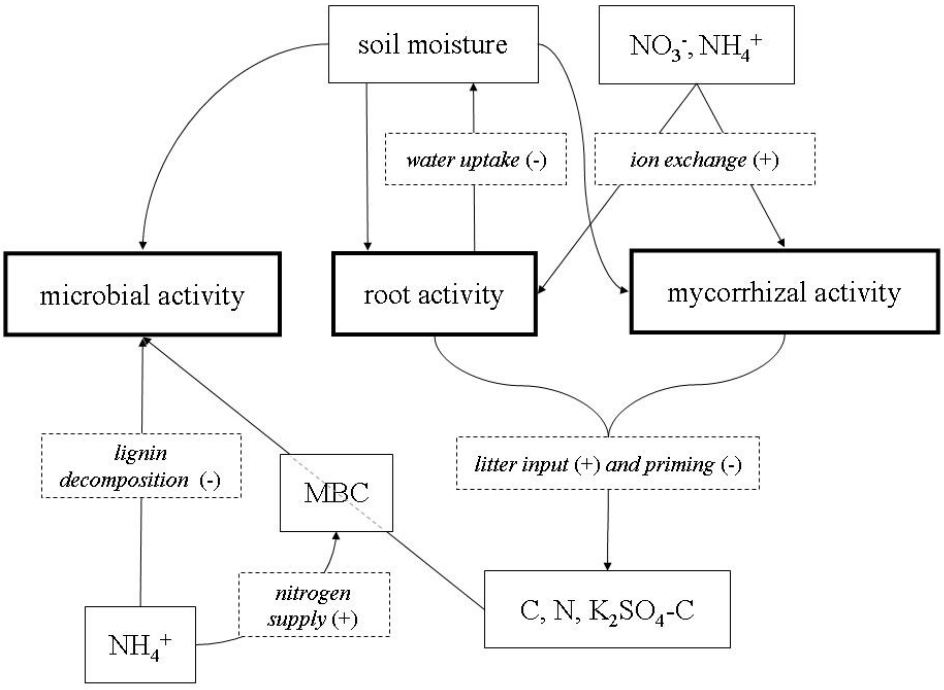

Figure 1.7: Relations between soil factors and the activity of soil microbes, roots and mycorrhizal hyphae, as discussed in Chapter 4.

1.6. Conclusions and Outlook

The flux of carbon between different global reservoirs is being actively studied in the present largely as a result of its climatic implications but also in an effort to understand the functioning of ecosystems and living organisms, two subjects which are tightly related. Soils play a central role in both respects as they contain large amounts of carbon and are key components of ecosystem functioning.

In order to investigate carbon dynamics, this study focused on the analysis of field CO_2 fluxes measured at the soil surface in combination with a method for partitioning the sources of belowground carbon. Soil respiration measurements have greatly improved with the use of IRGA sensors and soil chambers designed to reduce errors associated with pressure and concentration issues. The major challenge thus resided in obtaining reliable values of partitioned CO_2 fluxes. The use of micro-pore meshes represents an improvement over simpler root-exclusion methods in that it allows the in-growth of mycorrhizal hyphae and thus the separation of this component from plant roots. In addition, the flow of water and dissolved substances through the pores results in a better approximation of natural soil conditions.

The in-growth of mycorrhiza into root-free soil cores is the most important advantage over other partitioning methods, leading not only to an improved concept of carbon dynamics but also to a better understanding of the symbiotic relation between plants and fungi. Thus, among other insights, this method possibly gives the first measured estimates of the amount of carbon used by mycorrhiza relative to the total assimilated by the plant. This study showed that at least 5% of the total assimilated carbon was respired by mycorrhizal fungi at a barley field (Chapter 2), meaning that possibly twice as much may be exported to the fungal component (assuming 50% is used for growth). Similar estimates can be obtained for the forest ecosystems as soon as the partitioning of C within the trees itself is known.

The wider implementation of this method in the last few years, both in the field and the laboratory, is a confirmation of its value for this area of study. A large potential lies in the combination of this method with other techniques. For example, the partitioning of

carbon respired from sources with different isotopic signatures can be combined with mycorrhizal in-growth. The effect of easily degradable carbon (transported by mycorrhiza) on the use of older and more stabilized carbon compounds, i.e. the priming effect, can then be effectively studied. Another possibility with promising prospects was explored in this study with the combination of soil respiration measurements and eddy covariance data. This integration of above and belowground CO_2 fluxes is being widely used at the moment and will probably be further explored in relation to mycorrhiza and other specific soil components or processes. This will lead to a better understanding of the climatic and plant-factors controlling the flow of carbon through the plant and soil systems and back to the atmosphere.

On the other hand, as discussed in the following chapters, distinct shortcomings of the micro-pore mesh method can be signaled out. To give a conclusion on the limitations encountered during this study it is important to point out the difficulty of estimating the 'normal' activity of heterotrophic organisms after the soil system has been disturbed: by the absence of roots, the decomposition of dead roots, or the sieving of soil horizons. Allowing enough time for the system to stabilize is likely to be an effective way of avoiding some if these problems as well as having a complete in-growth of fungal hyphae. However, unwanted side effects will remain, such as changes in soil structure – influencing the physical protection of carbon or the availability of oxygen – or an increase in soil water content after roots are excluded. Results from this study indicated a moisture effect on respiration at the lower but not at the higher measured values of soil water content. The difference in water content between treated and non-treated soil would thus imply a difference in respiration activity during water-stress periods, leading to an overestimation of heterotrophic respiration. This limitation may be overcome in the future by obtaining precise moisture functions that can be applied to correct for the measured differences. It is thus important to measure water content inside the mesh-bound soil cores. The diffusion of CO_2 through the mesh pores is another problem which introduces a systematic error, again leading to an underestimation of rhizosphere respiration. This should always be considered and, when possible, avoided by using larger soil cores.

A partitioning of soil respiration from undisturbed soils may depend on the development of isotopic techniques. However, using micro-pore meshes and other methods that physically alter natural soil conditions is sometimes the best option for isolating specific respiration fluxes and studying their response to related environmental factors. More accurate estimates of carbon respired by different belowground components will likely come as a result of modeling exercises with functions derived from studies such as the present one where the relation of each respiration component with controlling factors is explored.

Results from this study showed the importance of mycorrhiza as a quick path of carbon from the canopy back to the atmosphere in croplands and temperate forests. The respiratory activity of mycorrhizal fungi was also shown to be strongly controlled by the availability of new carbon from the plant and little affected by changes in temperature in all ecosystems under study. The respiration from roots, more sensitive to changes in temperature, was also seen to be directly affected by photosynthetic activity. This already points towards limitations of current temperature driven models of soil respiration, which in the present need to be inproved by integrating biological processes reflecting basic plant and microbial functioning. To attain this, a better understanding of the drivers of belowground carbon fluxes is required, going beyond simple temperature and moisture responses. Since the influence of forest canopy processes on the activity of roots and associated organisms is particular to each vegetation type, such relations should be ideally based on physiological mechanisms inherent to different species or functional types of organisms.

Furthermore, aboveground constraints need to be coupled with belowground processes controlling the activity of roots and microorganisms. This involves nutrient and carbon supply as well as the physicochemical soil characteristics limiting the decomposition of carbon compounds. These characteristics can also be coupled with vegetation type, given the large influence of plants on soil development and characteristics. In addition, the potential for carbon storage or decomposition can be also determined by soil texture (amount of clay) and other soil parent-material related properties.

Studies in the laboratory and the field may further help understand the use of carbon by microorganisms under changing substrate quality, nutrient and moisture conditions. Careful manipulation of different soil types may help to further qualify and quantify the processes discussed in this study, such as those of inorganic nitrogen controls on decomposition and growth as well as priming effects induced by the presence of mycorrhizal hyphae. Further importance to this area of study is given by the relation of different respiration processes with temperature, which in the context of future climate changes may constitute a feedback mechanism on the soil-atmosphere system of yet unknown relevance.

1.7. Thesis Chapters

The remainder of this thesis consists of four independent manuscripts written as scientific articles or, in the case of Chapter 5, as a book chapter for publication. Chapters 2 and 3 are an analysis of the relationship between plant photosynthetic activity and temperature with soil respiration components in the cropland and forest sites, respectively. Chapter 4 deals with the relation between soil respiration components and spatially variable soil factors at all three sites. Finally, Chapter 5 is a review on root and rhizosphere respiration, influencing environmental factors, and advantages and disadvantages of related field and laboratory methods.

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2. Response of Mycorrhizal, Rhizosphere and Soil Heterotrophic Respiration to Temperature and Photosynthesis in a Barley Field

Chapter Source

Fernando Moyano, Werner Kutsch, Ernst-Detlef Schulze, 2007. Response of Mycorrhizal, Rhizosphere and Soil Basal Respiration to Temperature and Photosynthesis in a Barley Field. *Soil Biology and Biochemistry*, 39 (4), p. 843-853.

Abstract

The mycorrhizal, rhizosphere and heterotrophic components of soil respiration were partitioned in a barley field experiment with the main objective of determining the controlling effects of photosynthetic activity and temperature on soil respiration sources. Micro-pore meshes were used to create both root and mycorrhiza free soil cores over which collars for soil respiration measurements were inserted. Differences between mesh treatments were used to determine the contribution of each component. With a focus on the growing season, we analyzed the response of respiration sources to photosynthesis, temperature and moisture, as well as changes in microbial biomass, mineral nitrogen and carbon-nitrogen ratios responding to treatment and time of year. Results gave clear differences between sources in their response to both temperature and photosynthetic activity and showed that several processes are involved in determining respiration rates as well as apparent temperature relations. In particular, the respiration of arbuscular mycorrhizal hyphae was seen to be a significant amount of root derived carbon respiration (25.3%) and consequently of total assimilated carbon (4.8%). This source showed a stronger response to photosynthetic activity than the rhizosphere component (R^2=0.79, P<0.001 and R^2=0.324, P=0.53 respectively). Q_{10} values –the increase in respiration rates with a 10˚ increase in temperature– changed seasonally and showed temperature relations being dependent on the presence of mycorrhizal and rhizosphere respiration sources, as well as on plant development. Respiration from mycorrhizal

hyphae and the rhizosphere showed no response (R^2=0, P<0.99) or low response (R^2=0.14, P<0.01) to temperature, respectively. We conclude that the potential importance and controls of mycorrhizal fungi respiration in croplands are comparable to those observed in other ecosystems, and that temperature response curves should be carefully interpreted given that substrate availability and plant dynamics strongly regulate respiration rates in ecosystems.

2.1. Introduction

Interest in soil respiration (Rs) has grown in the past years as a result of the uncertainties associated with the cycling of belowground carbon, as well as the importance of soil carbon dynamics for possible feedback mechanisms in the context of climate change. Rs measurements have been used as a means to separate short term effects of environmental factors on soil CO_2 evolution and as input or validation of models that describe the long-term soil carbon dynamics. Discussions on concepts and methodology of Rs measurements have led to the following general conclusions: (i) Soil respiration cannot be analyzed as a single source of CO_2 but as a combination of different sources, each with its own seasonal behavior and response to environmental factors (Ryan and Law 2005; Trumbore 2006). A finer separation of sources is needed, as shown by Kuzyakov (2006) who divides soil respiration into soil organic matter (SOM) decomposition, additional SOM decomposition by priming, decomposition of plant litter, decomposition of rhizodeposits by root-associated microorganisms, and root respiration. (ii) The respiration of root-associated microorganisms (bacteria and mycorrhizal fungi) represents a potentially large part of the "autotrophic" component of soil respiration in many ecosystems (Högberg and Högberg 2002; Kuzyakov 2006; Moyano *et al*. in press). (iii) Temperature can be a strong controlling factor for respiration rates under certain conditions, but the limiting factors in many if not most cases are those determining substrate availability, e.g. water status and assimilate supply (Högberg et al. 2001; Davidson and Janssens 2006; Davidson et al. 2006). (iv) The development of new methods, or the improvement of currently available ones, is necessary for obtaining

reliable flux estimates from individual sources under natural and manipulated conditions (Ryan and Law 2005; Kuzyakov 2006; Trumbore 2006).

Despite these insights, it remains difficult to establish clear cause and effect relationships in belowground respiration processes given the number of factors and sources involved. This is mostly true for measurements done in field conditions where variables may covary making the effects impossible to separate or where a number of influencing factors are simply not measured. These problems gave rise to the development of a number of methods in the past years which have lead to a more precise understanding of the different processes involved in underground carbon dynamics (Hanson et al. 2000; Kuzyakov 2006).

In particular, the role of mycorrhiza is beginning to be understood with the development of compartments that exclude roots but contain the extra-radical mycelium (ERM) of the mycorrhizal fungus (Schuepp et al. 1987; Leake et al. 2004). These compartments are created by using micro-pore meshes with pore sizes smaller than the diameter of a fine root but larger than the diameter of the mycorrhizal hyphae, allowing the selective in-growth or exclusion of mycorrhizal mycelium and roots (Johnson et al. 2001; Johnson et al. 2002a; Johnson et al. 2002b). Thus, it is possible to determine the respiration rate and the response to environmental factors under near natural conditions of the ERM, a source of CO_2 that had previously been impossible to separate from rhizosphere respiration under field conditions. This method has the additional advantage with respect to other trenching/root-exclusion methods that it allows the movement of water, bacteria, organic matter and minerals through the mesh and thus reduces the disturbance of natural soil conditions that are affecting the decomposition of litter and SOM.

Mycorrhizal fungi are present in nearly every habitat (Smith and Read 1997) and a number of studies have shown that they are both an important source of CO_2 and a major pathway of carbon into the soil organic matter pool (Godbold et al. 2006). Johnson (2002b) measured a significant and fast flux of C from the plant to the atmosphere through arbuscular mycorrhizal fungi (AMF) in upland grasslands and similar conclusions have been given for ectomycorrhizal fungi (EMF) in seedling of *Pinus sylvestris* (Leake 2001). However, the mycorrhizal component of soil C fluxes has been

largely ignored, even in studies investigating the transfer of C from roots to soil microbes. For this reason, the amount of carbon allocated and respired by the ERM is very uncertain. A review by Leake et al. (2004) cites estimates of the net total C allocated from plants to AMF ranging between 2% and 20% of current assimilates.

This paper presents results of a field experiment carried out in a winter barley field where soil respiration was partitioned by using micro-pore meshes. Studies determining mycorrhizal respiration (Rm) fluxes have been few, and those done under field conditions even fewer. We give, to our knowledge, the first estimation of AMF respiration under field conditions in arable systems. We look at the contribution of heterotrophic, rhizosphere and mycorrhizal respiration sources and their response to environmental factors. Additionally, we discuss conditions that may influence substrate supplies and respiration rates, as well as calculated rate changes as a function of temperature.

2.2. Methods

Study Site

The study was carried out at the cropland research site near Gebesee. The field is 750 m x 850 m in size, situated 20 km NW of Erfurt in Thuringia, Germany (latitude 51°06'0.13''N, longitude 10°54'51.9''E, 162 m asl). The soil texture is silty clay loam (~30% clay) with granular structure, classified as Haplic Phaeozem under the World Reference Base (WRB) for Soil Resources (Schrumpf, personal communication). Crop rotation has included 50% cereals and 50% potato and sugar beet since 1970 or earlier. Soil was harrowed in August and winter barley (*Hordeum vulgare* L., cultivar Naomi) sown in September 2004. Seedlings remained mostly under snow cover from late January until early March of 2005. An eddy covariance tower for measuring energy and carbon exchange between the vegetation and the atmosphere is located at the center of the field (Anthoni et al. 2004b). Gebesee is a main site of the flux network within the integrated project CarboEurope (for more on the CarboEurope integrated project visit www.carboeurope.org).

Partitioning method

Nylon mesh bags (Plastok Associated Ltd, Birkenhead, Wirral, UK), 40 cm deep x 16 cm dia, were used to selectively exclude only roots (35 micron pores) or roots and mycorrhizal hyphae (1 micron pores; with AM-hyphae ranging between 2 and 27µm, Smith and Read 1997). Soil cores (30 cm deep, 15 cm dia) were removed by hammering a metal cylinder into the ground, and then placed into the mesh bags and back to their original position in the ground. Soil disturbance was thus kept to a minimum. By calculating differences in respiration between the 1 micron, 35 micron and control (non-treated soil) treatments, a partitioning of soil respiration into its rhizosphere, mycorrhizal, and heterotrophic components was done as follows:

- control = total soil respiration (Rt)
- control – 35 micron = *rhizosphere respiration* (Rr)
- 35 micron - 1micron = *mycorrhizal respiration* (Rm)
- 1 micron = *heterotrophic respiration* (Rh)

We prefer to define *rhizosphere respiration* in the stricter sense of *root respiration* (respiration by the living root tissue) plus *rhizomicrobial respiration* (respiration of rhizodeposits) and use the term *mycorrhizosphere respiration* (Rmr) for Rr + Rm (Moyano et al. In press). Rh is here defined as soil respiration without any influence from roots or mycorrhiza. It should be noted that any respiration of SOM by priming, if present, will be included in the respective Rm and Rr components since this effect was not partitioned.

Experimental Setup

In November 2004, a total of 14 plots were installed within the main footprint area of the eddy flux tower: 10 plots were installed at random locations, falling on grid points 30m apart, and 4 plots were installed at a few meters from the tower. At each plot 2 soil cores were installed between rows of seedlings: a 1 micron and a 35 micron mesh core. Live roots were not seen when removing the cores and any amounts within the cores were expected to be minimal. Three collars for Rs measurements (PVC, 10 cm dia) were

inserted per plot: 1 on top of each mesh core and 1 placed next to the cores, also between rows of seedlings, for measurements of Rs from non-treated (control) soil. Collars were inserted to a depth of 3-4 cm. The mesh cores remained in the field until harvest in mid-July 2005 when the collars were removed and soil cores destroyed during plowing. Collars were reinserted after harvest for measurements of total soil respiration.

Soil Respiration Measurements

We carried out soil respiration measurements with a closed soil chamber and infrared gas analyzer (Licor LI-6400-09, Licor Inc., Lincoln, NE, USA). All measurements were done in 2005, with the dates being once in mid-January, once in mid-February, and mostly weekly from March to July. Soil respiration measurements after harvest were made in 8 occasions at irregular intervals from July to November. With each soil respiration measurement we measured volumetric soil moisture in the upper 6 cm (ThetaProbe, Delta-T Devices Ltd., Cambridge, UK) and soil temperature at either 5 or 10 cm next to the collars but outside of the mesh cores to avoid disturbance. Half hour soil temperature (2, 4 and 8 cm depths) and moisture (8 and 16 cm depths) measurements were obtained next to the eddy covariance tower during the whole study period. In order to measure moisture content differences between treatments, we inserted 20 cm ECHO moisture probes (Decagon Devices, Inc. Pullman, WA, USA) vertically into each core and in non-treated soil at each of the 4 plots near the tower. Moisture was then measured continuously and stored as half hour averages from the period between the 24th of May and the 12th of July.

Eddy Covariance Measurements

Calculated gross primary productivity (GPP), used for analyzing the relationship between respiration sources and photosynthetic activity, was derived from eddy covariance measurements at the site. Ecosystem net carbon and water vapour fluxes were measured with an eddy covariance system consisting of a sonic anemometer (Solent R3, Gill Instruments, Lymington,UK) mounted variably between 3.2 and 4.5 m depending on vegetation height and a LI 7000 infrared gas analyser (LiCor, Lincoln, Nebraska, USA)

located at the base of the tower. The air was pumped through a 5 m tube (Dekabon, SERTO Jakob, Fuldabrück, Germany) and filtered behind the inlet and a second time before the gas analyser (ACRO 50 PTFE 1 µm pore-size, Gelman, Ann Arbor, MI, USA). For more details about the instrumentation we refer to Anthoni et al. (2004a).

The flux data were calculated for 30 min intervals by means of the post-processing programme 'eddyflux' (Meteotools, Jena, Germany). Raw data were converted into physical data and detrended and a 2D coordinate rotation was applied. Time lags for CO_2 and water vapour concentrations were calculated by determining the maximum correlation between the concentrations and the vertical wind component *w'*. The turbulent fluxes were calculated using conventional equations (Desjardins and Lemon 1974; Moncrieff et al. 1997; Aubinet et al. 2000; Knohl et al. 2003). CO_2 stored in the air column between the measuring system and the soil surface (storage flux) was determined as the concentration change in time within a CO_2 profile where concentrations were measured continuously at 7 heights.

We applied the stationarity test after Foken and Wichura (1996) and the integral turbulence characteristics. Thereafter, the current standard procedure for data filtering, gap-filling and flux partitioning of the CarboEurope network (Falge et al. 2001, Reichstein et al. 2005), as an online tool available at the CarboEurope database website http://gaia.agraria.unitus.it/database/eddyproc/, was used to derive the estimates of GPP that we used for the interpretation of the soil fluxes in this study. In the currently available version, this proxy contains a u*-filtering and calculates the night-time respiration by means of the Lloyd-and-Taylor (1994) regression model. GPP is then calculated by extending this model to daytime and subtracting R_{eco} from the net flux.

Soil analysis

Soil samples for determining soil characteristics were taken from selected plots at several occasions before harvest in 2005 (see below). Small corers (12 mm dia) were used to remove soil from the soil cores, taking the sample between the collars and the mesh. Larger corers were used for sampling non-treated soil.

We measured microbial biomass on 3 occasions (5th of April, 3rd of June and 12th of July, 2005) using the chloroform fumigation extraction method (Vance et al. 1987). Soil samples from 0-10 cm were kept in a cooler after removal and later stored at 4°C for a maximum of 4 days. Fumigated samples were kept under vacuum with chloroform for 2 days. TOC was determined using a high TOC analyzer (Elementar Analysensysteme Hanau, Germany). We determined mineral nitrogen contents from 0-10 cm depth soil samples on 2 occasions (9th of June and 7th of July, 2005). Samples were collected in the field and brought to the lab in a cool box. Soil was then immediately processed and extracted with 1M KCl. Amounts of NH_4 and NO_2/NO_3 where determined with a Skalar SAN Plus continuous-flow analyser. Carbon to nitrogen ratios (C:N) from 0-10 cm depths were determined the 12th of July, 2005 (vario Max Elementar Analysensysteme Hanau, Germany).

Data analysis

SPSS was used as the statistics program for data analysis. Respiration data of all treatments was log transformed to meet normal distributions assumptions. We compared treatment mean respiration values for the growing season (early April to mid-July) by analysis of variance (ANOVA) and a posteriori Tukey HSD test, with date and plot taken as random factors. Results are expressed with their associated standard deviations or standard errors. In the latter case the values for *n* are given in brackets.

Moisture relations were analyzed by linear regression. To determine temperature relationships we performed weighted least squares analysis, a procedure that accounted for the higher variance in the data at higher temperatures. We additionally calculated the increase in respiration rates with a 10° increase in temperature (Q_{10} values) by treatment for the whole pre-harvest period as well as for the January-March, April-May, and June-July periods. These were obtained by fitting non-linear regressions with the exponential function:

$$R_T = R_{0^\circ C}(e^{kT})$$

where R_T is respiration at a given temperature, $R_{0°C}$ is estimated respiration at 0°C and k is an estimated temperature coefficient. Q_{10} values were then calculated with the formula:

$Q_{10} = e^{10k}$

2.3. Results

Soil Respiration

Soil respiration mean values (Figure 2.1) were low (close to or less than 1 µmol m^{-2} s^{-1}) during winter and started increasing in early March. In general, mean values showed small differences during winter, with respiration fluxes from 1 micron and 35 micron treatments generally rising slightly over control values. Even though only young seedlings were present when the soil cores were installed, this may indicate decomposition of abscised root and fungal material. Disruption of soil aggregates and oxygen levels may also have contributed to these initial differences.

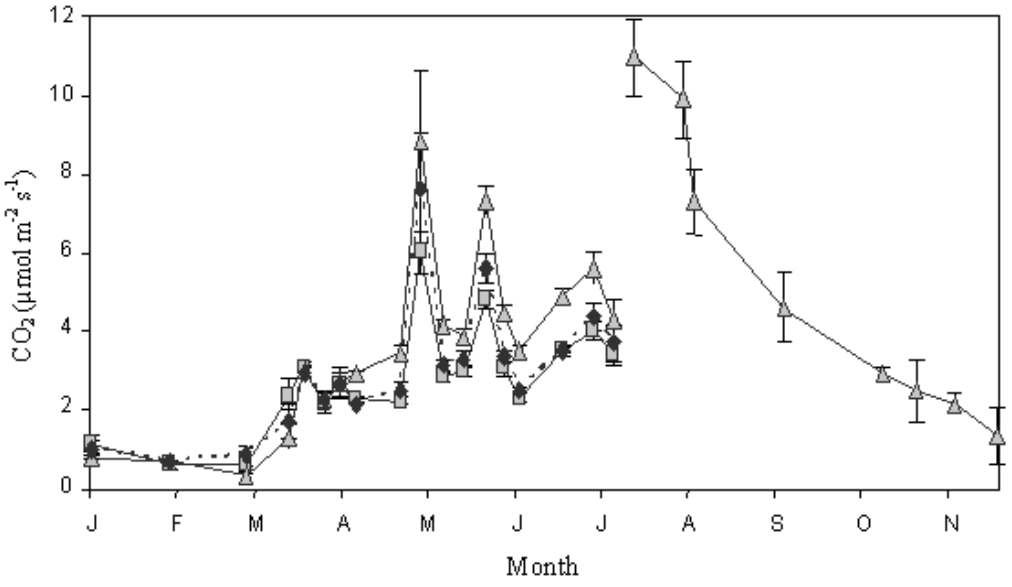

Figure 2.1: Mean soil respiration values in 2005 for control collars (triangles), 35 micron collars (diamonds, dotted line), and 1 micron collars (squares). Only total soil respiration from control collars is measured after harvest. Bars represent standard errors.

Values stabilized in late March and early April with all treatments showing similar means. Rhizosphere respiration became apparent in early April, with control mean values rising and remaining significantly higher than 1 micron and 35 micron means until harvest. The 35 micron respiration mean fell slightly under the 1 micron mean on one date in mid-April (difference not significant), but rose above the 1 micron mean in late April and remained higher until harvest. Respiration during the growing season was significantly different for all treatment comparisons ($P<0.01$, $F_{2,452}=66.2$). Mean values in μmol m^{-2} s^{-1} were 3.25±0.11 (n=159) for the 1micron, 3.59±0.14 (n=159) for the 35 micron and 4.46±0.17 (n=161) for the control treatments.

Measured total soil respiration (Rt) showed a minimum mean value of 0.35 μmol m^{-2} s^{-1} during a frost period in early March 2005 (no attempt was made to measure soil respiration under snow cover). It peaked in mid-July just after harvest, reaching a mean value of 10.97 μmol m^{-2} s^{-1}, and steadily decreased thereafter (Figure 2.1). Rr (control – 1 micron) reached a maximum of 28±6% (n=12) of Rt in early June and averaged 20.9±2.2% (n=12) during the growing season (Figure 2.2). Rm (35 micron – 1 micron) reached a maximum of 19.1±12.9% (n=12) of Rt and averaged 6.1±1.7% (n=12) during the growing season. In average, Rm represented 25.3±4.2% (n=11) of mycorrhizosphere respiration.

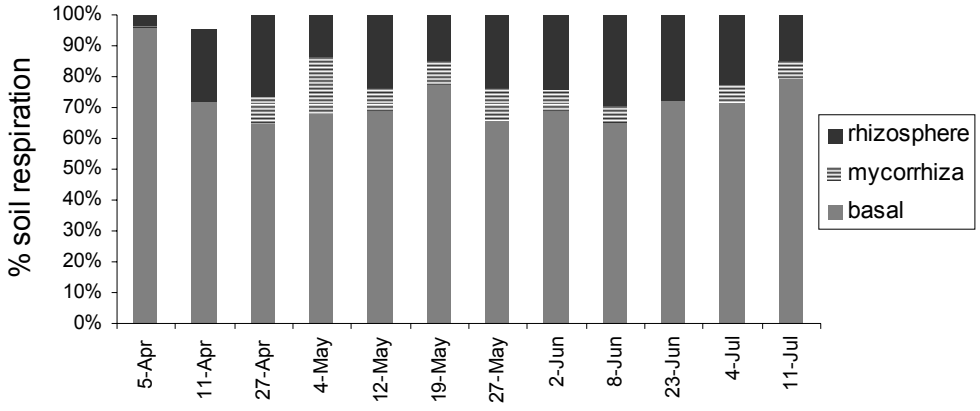

Figure 2.2: Relative contribution of rhizosphere (black bars), mycorrhizal (striped bars), and heterotrophic basal (grey bars) respiration. Bars represent percentage of total soil respiration. A negative value of mycorrhizal respiration (non significant) on April 11th is not visible.

Response to Moisture and Temperature

Volumetric soil moisture in 2005 measured next to the tower at 8 cm and 16 cm generally fluctuated between 20 and 35%. A decrease was observed in May, ending in two larger drought periods in June and July. However, soil moisture volume at these depths did not fall below 15%.

Pre-harvest measurements of soil moisture at the plot level in the upper 6 cm ranged between 37.3±2.2% in February and 9.7±0.8% June. Moisture measured at 8 cm at the site level ranged between 28.3±1.4% in May and 18.6±2.2% in July. Plot level moisture correlated negatively with both soil temperature and Rt, while 8 cm site moisture neither correlated with temperature nor respiration (data not shown). Soil moisture measured in the upper 20cm of the cores with ECHO probes was divided into weekly averages. The maximum difference between treatments, which also represented the period of lowest moisture levels, was a 4.8% difference between the control (19.9±3.2%) and

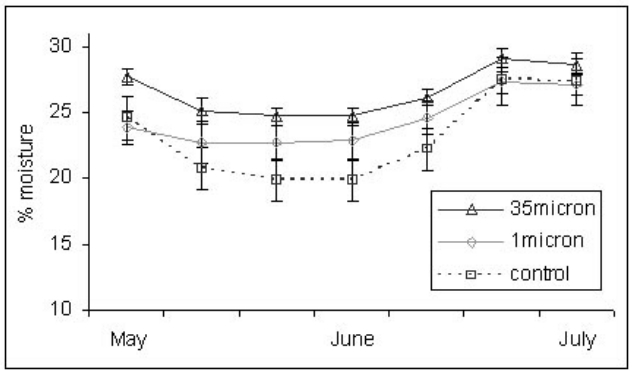

Figure 2.3: Volumetric soil moisture measured with ECHO probes in the upper 20 cm of the 35 micron (triangles) and 1micron (circles) mesh soil cores and next to control collars (squares) from mid-May to mid-July. Values shown are weekly averages. The largest differences are correlated with a period of drought in June. Error bars represent standard errors.

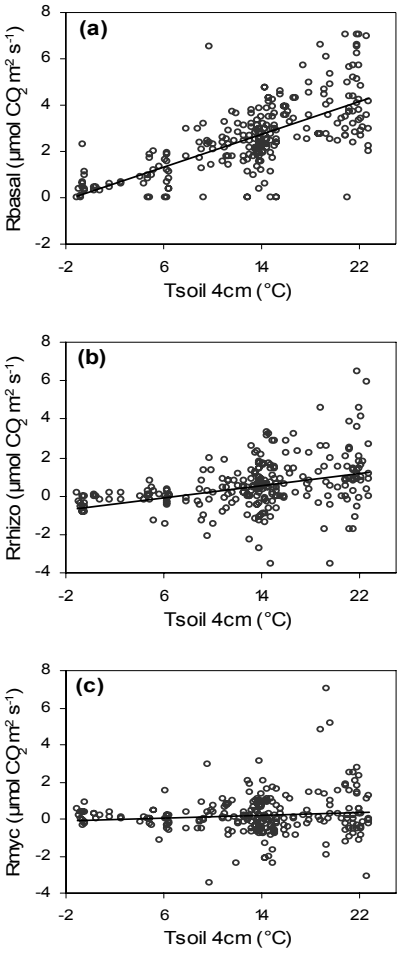

Figure 2.4: Respiration values for (a) heterotrophic, (b) rhizosphere and (c) mycorrhizal respiration components plotted against soil temperature at 4 cm depth. All measurement dates previous to harvest (January-July) were used for the analysis. Temperature response was significant only for heterotrophic (R^2=0.81, n=221, P<0.01) and rhizosphere (R^2=0.14, n=226, P<0.01) components.

the 35 micron (24.7±1.3%) treatments in mid-June (Figure 2.3).

Respiration values were found to correlate best with soil temperature (Tsoil) at 4 cm depth measured at the site level. Pre-harvest values of Tsoil at 4 cm ranged between -1.1 and 25.7°C. Temperature values showed some peaks during May and reached steady high values in late June. Temperature analyses were carried out with the calculated contribution of each soil respiration source from all plots and all measurement dates previous to harvest (January to July). Using dates only from the growing period (April-July) gave similar results (data not shown).

Response curves were different for each source (Figure 2.4). Heterotrophic and rhizosphere respiration both showed significant correlations with Tsoil. However, whereas a linear temperature function explained most of the variation in Rh (R^2=0.81, n=221, P<0.01) it explained only a small amount of the variation in Rr (R^2=0.14, n=226, P<0.01). Mycorrhizal respiration, on the other hand, presented no correlation with Tsoil (R^2=0.00, n=218, P=0.99).

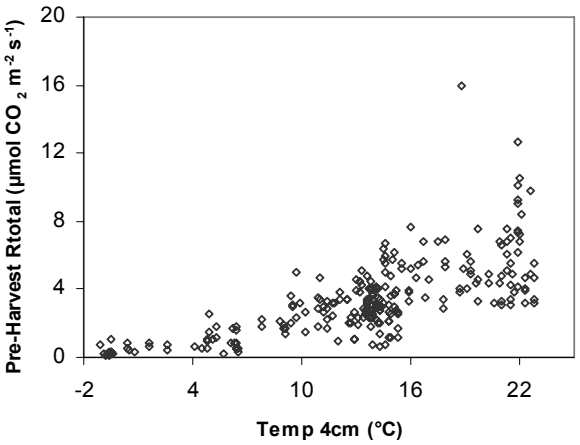

Figure 2.5: Total soil respiration for the pre-harvest period plotted against soil temperature at 4 cm depth showing an apparent exponential relationship.

Individual soil respiration components did not show any clear exponential relation with Tsoil (Figure 2.4), as most soil respiration models would predict. In all cases, curve fitting of individual sources using the exponential function $R_T = R_{0^\circ C}(e^{kT})$ resulted in lower R^2 values than those given for linear functions (data not shown). However, when plotting Rt against Tsoil an exponential relation became apparent (Figure 2.5).

Respiration Q_{10} values calculated for each treatment and for the whole pre-harvest period and January-March, April-May, and June-July periods are given in Table 2.1. The overall Q_{10} of all treatments fell close to the expected value of 2, although it was lowest for the 1micron (Rh) and highest for the control (Rt).

Table 2.1: Soil respiration Q_{10} values (R^2 values in brackets) calculated for each treatment and time period.

Time Period	1µm Q_{10} (R^2)	35µm Q_{10} (R^2)	Control Q_{10} (R^2)
Overall (Jan- July)	1.93 (0.50)	2.05 (0.52)	2.34 (0.46)
January – March	2.86 (0.57)	2.27 (0.48)	2.51 (0.43)
April- May	2.16 (0.45)	2.48 (0.52)	2.72 (0.45)
June – July	1.62 (0.21)	1.51 (0.13)	1.45 (0.16)

Values for the shorter time periods were, on the other hand, more variable. The Q_{10} of the 1 micron treatment (heterotrophic respiration) was highest in winter, intermediate in spring, and lowest in summer. Q_{10}s for control (Rt) and 35 micron (heterotrophic + mycorrhizal) treatments showed a different behavior, with values in winter and summer similar to those of the 1 micron treatment, but with the highest values present in spring.

Response to Photosynthesis

We plotted Rr and Rm mean daily values against GPP values obtained from eddy covariance data. We used growing season data, from April to June, excluding the two last measurement dates before harvest in July, which showed as outliers in the regression analysis. Growth had ceased and plants were senescent in this period, so the observed high mycorrhizosphere respiration may have been the result of the

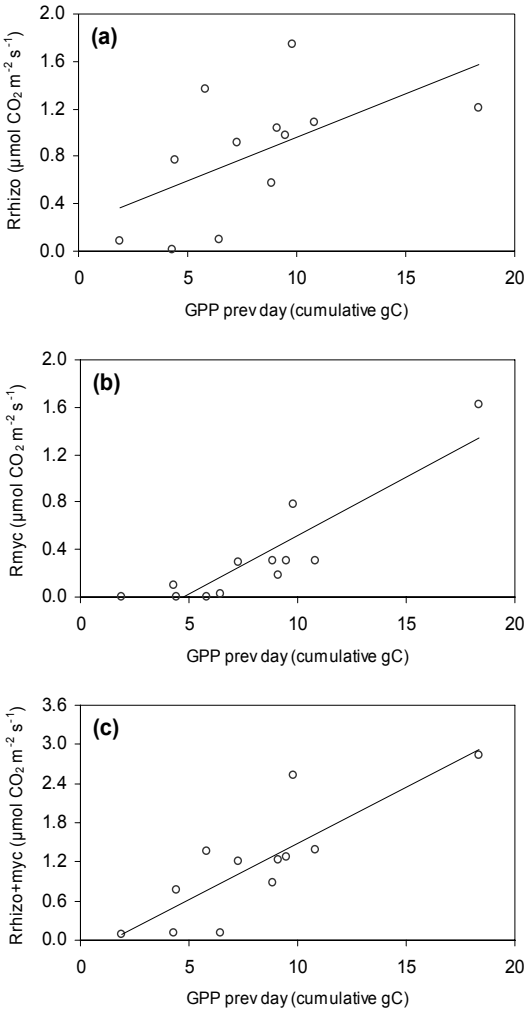

Figure 2.6: Response to the cumulative GPP of the previous day for (a) rhizosphere, (b) mycorrhizal and (c) mycorrhizosphere respiration. All daily means of measurement dates from March 24th to June 23rd were used.

decomposition of roots and fungal mycelium.

After testing for different GPP values, the cumulative GPP of the previous day, expressed as grams of carbon, resulted in the best fit for all components and was used in the analysis (Figure 2.6). Rr showed a positive but non-significant correlation with GPP (R^2=0.324, n=12, P=0.53). Rm, on the other hand, showed a strong and positive correlation (R^2=0.79, n=12, P<0.001) which was also seen for Rmr (R^2=0.69, n=12, P<0.01).

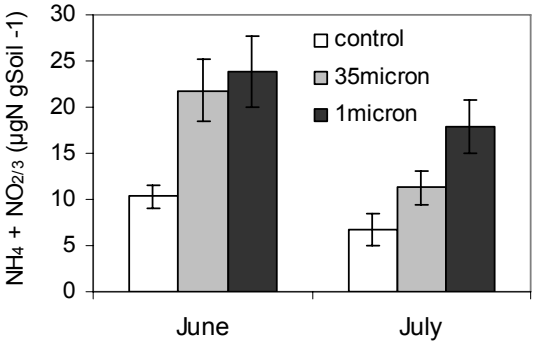

Figure 2.7: Mineral nitrogen measured in the upper 10 cm of soil for each treatment. Lowest values were observed in non treated soil, intermediate values in the 35 micron treatment, and highest values in the 1 micron treatment. Error bars represent the standard error of the mean.

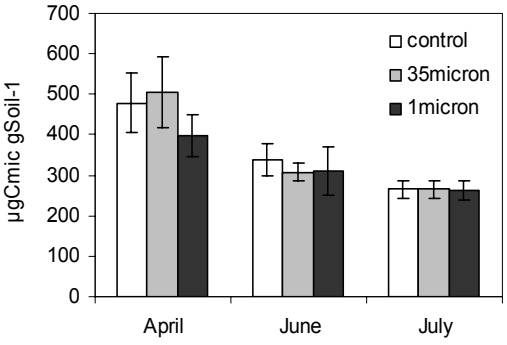

Figure 2.8: Soil microbial biomass calculated in the months of April (n=4), June and July (n=7). There were no significant differences detected between treatments. All treatment means decreased from April to July. Error bars represent the standard error of the mean.

Soil analysis

Treatment effects on mineral nitrogen values were observed on both measurement dates, with values being lowest in control soil, intermediate in the 35 micron cores, and highest in the 1 micron cores (Figure 2.7). Differences were significant between the control and other treatments in June (P<0.001, $F_{2,12}$=17.99) and between the 1micron and

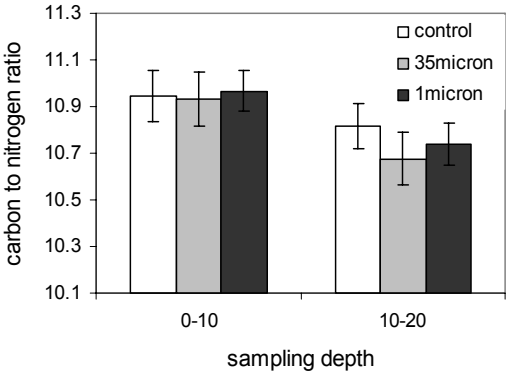

Figure 2.9: Carbon to nitrogen ratios measured outside and inside soil cores. Treatments did not show an effect at the end of the experimental period. Similar differences with depth were observed in all cases. Error bars represent the standard error of the mean.

the other treatments in July ($P<0.001$, $F_{2,10}=21.05$). Values decreased from June to July for all treatments. Measurements of soil microbial biomass in the upper 10cm resulted in no significant differences between treatments (Figure 2.8), however, a decrease similar for all treatments was observed from April to July ($P<0.01$, $F_{2,51}=14.59$). C:N values were likewise similar for all treatments and only a general difference between 0-10 and 10-20 cm depths was detected (Figure 2.9).

2.4. Discussion

Method results and respiration values

In this study we successfully applied micro-pore meshes for the selective in-growth of arbuscular mycorrhizal hyphae. This method has been confirmed as a useful tool for separating different components of the soil system in a number of other studies reviewed recently by Leake et al. (2004). The rotation of soil cores to severe mycorrhizal hyphae with the subsequent reductions in respiration rates as well as the tracing of isotopes from labeled plants to the inside of the mesh bound cores have demonstrated that these root-free compartments are effectively colonized by mycorrhiza (Johnson et al. 2001; Johnson et al. 2002a; Johnson et al. 2002b). Evidence for this can also be found in the differences in mineral nitrogen values between treatments as well as the differing response of partitioned respiration sources to environmental factors in our study.

With our experiment we have shown that mycorrhizal respiration can be an important contributor to soil respiration in arable fields. This is underlined by the fact that the Rm and Rr estimates in this study represent a minimum of mycorrhizal and rhizosphere respiration, given two reasons, both related to limitations of the method. Firstly, the mycelia of AMF generally decrease in density with the distance from the plant root. Thus, respiration from the ERM growing inside the mesh cores is likely to represent less than the average respiration of the total ERM. Secondly, CO_2 concentration differences between the outside and the inside of the mesh cores could lead to a lateral diffusion into the cores. This would then result in an underestimation of the Rm and Rr components.

Although a potential error related to lateral diffusion was not estimated, the problem can be partially overcome by taking Rm not as a fraction of total respiration but as a fraction of Rmr, i.e. root derived carbon respiration. When calculating Rm/Rmr, a theoretical proportion (1/x) of Rm or Rr diffusing into the cores can be canceled out. The relationship then remains constant even if we have lateral diffusion of CO_2 into the cores, with the simple assumption that the proportional diffusion is equal in all cases.

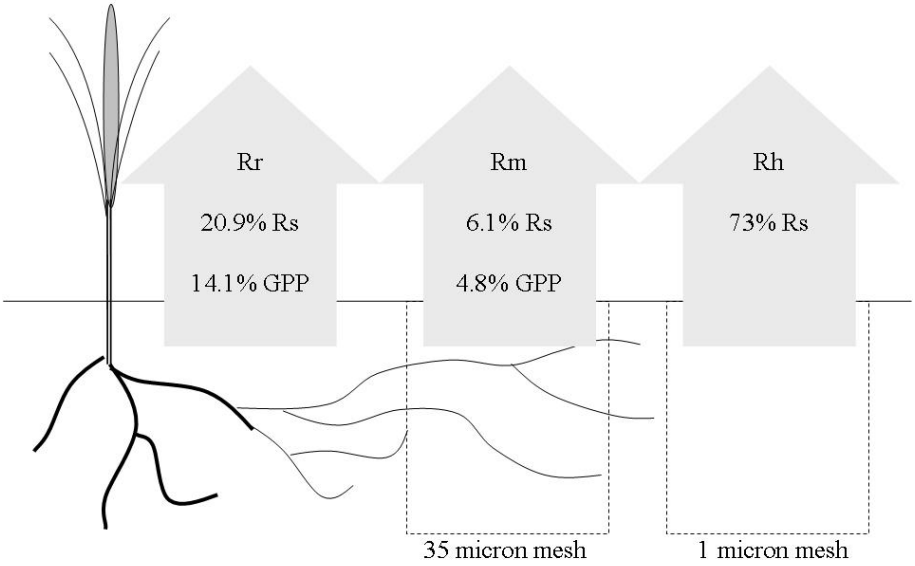

Figure 2.10: Diagram showing calculated values of rhizosphere respiration (Rr), mycorrhizal respiration (Rm) and heterotrophic respiration (Rh) as a percentage of total soil respiration (Rs) and of gross primary productivity (GPP). Rr and Rm are minimum estimates.

Our results indicate that Rm comprises 25.3±4.2% (n=11) of mycorrhizosphere respiration on average. This value suggests that even for crops that have a low mycorrhizal dependency, such as barley (Plenchette and Morel 1996), the fungal component is using a large fraction of the carbohydrates exported to the root system. A study by Whipps (1984) using barley plants grown in soil concluded that, for a day/night temperature of 18˚C/14˚C and a 16h day length, the respiration of root derived carbon accounts for 18.9±3.3% of total fixed carbon. Using this number we find that the ERM respires 4.8±2.8% of total assimilated carbon (with the remaining 14.1% as rhizosphere respiration, Figure 2.10), remarkably close to the values given by Johnson et al. (2002a)

of 3.9% - 6.2% in a grassland study. The similarity between these results reinforces such numbers, as well as those from a study estimating the total carbon allocation to the ERM at 9% (Johnson et al. 2002b).

Response of CO_2 sources to environmental factors

Results showed differences in temperature response for the different respiring components. An expected exponential relationship with different components was not obvious. Even though a large scatter at higher temperatures makes it difficult to determine the precise shape of the relationship, we do not conclude that a better fit of the linear function is a problem with the quality of the data, as done by Subke et al. (2003). Factors other than temperature effects on enzymatic activity can be limiting the increase of respiration with temperature in field conditions, namely, those determining substrate availability (Davidson and Janssens 2006). This would be the case if lower soil moisture levels dampened the increase in respiration at higher temperatures, as substrate availability can depend on water content. In the case of Rh, substrate levels presumably decreased during the growing season as litter from the previous year's harvest was being consumed. The importance of litter input as a substrate source is evident in the response of respiration after harvest and tillage in July, where values peaked at more than twice the previous numbers and slowly decrease thereafter.

The much weaker response of Rr and lack of response of Rm to temperature is also likely related to their dependence on substrate supply. Whereas a certain amount of respiration will be for maintenance and thus have a direct response to temperature, growth respiration will be controlled by the amount of photosynthates available, and only be affected by temperature when these are not limiting. Substrate dependency is most obvious for the Rm, which exhibited no response to temperature but a strong relationship with the photosynthetic activity of the previous day. Roots, as opposed to mycorrhizal hyphae, may be less dependant on new photosynthates if they have access to carbohydrate reserves, explaining their reduced response to GPP. Higher rates of respiration for tissue maintenance compared to mycorrhizal hyphae may also explain their significant response to temperature.

Our results strengthen the concept that mycorrhizosphere respiration has a short term response to photosynthetic activity (Ekblad and Hogberg 2001; Bhupinderpal et al. 2003; Kuzyakov and Cheng 2004; Tang et al. 2005). Additionally, they suggest that the main driver of this relationship is mycorrhizal respiration. This is consistent with results from Johnson et al. (2002a), which showed that C allocated to AMF hyphae peaked earlier than C allocated to roots, as well as with other studies revealing the strong dependence of AMF to current assimilates (Wright et al. 1998; Heinemeyer and Fitter 2006).

Measured soil moisture levels did not reach extreme values and presumably did not have a large effect on respiration activity. Root exclusion increased the water content in both treatments by a few percent. However, these differences are not likely to have affected respiration rates at the measured moisture levels. The negative correlation observed between moisture and respiration can be related to the positive correlation observed between moisture and temperature. Low amounts of aboveground litter, which in many ecosystems constitutes a considerable fraction of soil respiration (Ewel et al. 1987; Rey et al. 2002; Sulzman et al. 2005), may be one reason why soil respiration did not respond to the more pronounced decrease in moisture levels in the upper soil.

Implications of the calculated Q_{10} values

Kutsch and Kappen (1997) and Janssens and Pilegaard (2003) noted that Q_{10} values varied strongly throughout the year and concluded that such variations were due to temperature sensitive processes such as changes in the microbial population and diffusion of organic molecules through water. Our 1 micron treatment showed a temperature sensitivity decrease from winter to summer which reflects the pure response of the microbial community. Such seasonal Q_{10} changes have been already observed in a number of studies (Luo et al. 2001; Xu and Qi 2001; Janssens and Pilegaard 2003). Our results may be partly due to Q_{10} changes predicted by the Arrhenius equation (i.e. temperature sensitivity decreases with increasing temperatures) and by Michaelis-Menten kinetics (which can explain decreasing sensitivities at lower substrate concentrations; Davidson and Janssens 2006). However, an observed decrease of microbial biomass from April to July indicates that changes in the microbial population and activity are largely

responsible for progressively lower apparent temperature responses. A physiological acclimation of soil microorganisms connected to such temperature sensitivity changes cannot be discarded, but it seems likely that the key factor determining microbial respiration is a decrease in substrate availability, as suggested by Davidson and Janssens (2006). Substrate availability seems to depend on the large amount of input after harvest and low input during other periods, by soil water content (decreasing in summer), as well as by freezing and melting and reduced substrate usage during the colder periods.

On the other hand, the observed increase in the overall Q_{10} values when the less temperature sensitive mycorrhizal and rhizosphere components are added, as well as higher Q_{10} values for the 35 micron and control treatments when plant growth rates were high (April-May), indicate that the apparent temperature sensitivity of soil respiration will depend strongly on plant growth. This effect, observed by Yuste et al. (2004) in a mixed temperate forest, is expected to be more pronounced in crop fields where plant biomass changes are proportionally greater than in other ecosystems.

Q_{10}s are commonly used for expressing soil respiration-temperature relationships. Our results show how they are dependant on the contribution of different respiration sources and on the developmental stage of the plants. We also speculate that the observed seasonal changes in heterotrophic respiration Q_{10}s result from substrate depletion in a yearly cycle.

Soil carbon and nitrogen

Differences in soil mineral N gave a clear indication of the presence and activity of roots and mycorrhiza in the respective soil treatments. The lack of competition for N in the root/mycorrhiza free cores may have increased microbial respiration, explaining the rather high percentage of respiration from this source. However, this increase may also indicate that microbial activity is not being limited by available N. Carbon to nitrogen ratios, on the other hand, are likely determined by soil carbon corresponding to slow and passive pools. The lack of significant differences in C to N ratios measured in July may reflect the reduced effect that a season of root/mycorrhizal exclusion has on the

composition of these large fractions of soil organic matter. How these pools respond to root exclusion in the long run remains uncertain.

2.5. Conclusions

Distinguishing belowground sources of CO_2 and their individual response to different controlling factors is important for process understanding as well as for developing models that can effectively predict future changes. Mycorrhizal fungi have been shown to be a large component of soil respiration in several ecosystems, including the winter barley site under consideration in this study. Our partitioning of fluxes showed a different response to photosynthesis and temperature for different components. This together with the particular dynamics of plant growth and litter input in croplands indicate that models must be able to incorporate processes occurring in particular ecosystems.

The apparent good fit of exponential equations relating respiration with temperature may often be the result of additive factors and not a direct effect of temperature. Thus, it is not surprising that these relations frequently fail to explain a large percentage of soil respiration (Janssens and Pilegaard 2003; Lavigne et al. 2003). A simple model using temperature, soil moisture and litter input may give a yearly estimate of Rs that approximates measured values. However, a more realistic model should describe the effects of substrate limitation with relations involving temperature, water and carbon input through both litter and the translocation of photosynthates to the root system. Further, annual changes in plant growth and root biomass can affect respiration fluxes directly, but also indirectly through rhizodeposition and by priming of the microbial community. It then becomes necessary to include plant dynamics in models that predict intra-annual Rs changes.

Finally, the classification of soil respiration sources mentioned previously should be modified to include the respiration of mycorrhizal fungi as a main component. Given their particular eco-physiological characteristics, mycorrhizal fungi cannot be considered as equivalent to the root or other root associated microorganisms. A more complete division of soil respiration would then include the following processes: SOM

decomposition, SOM decomposition by priming, decomposition of plant litter, decomposition of rhizodeposits, mycorrhizal fungi respiration, and root respiration.

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3. Soil Respiration Fluxes in Relation to Photosynthetic Activity in Broad-Leaf and Needle-Leaf Forest Stands

Chapter Source

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Abstract

Soil respiration is a combination of CO_2 fluxes derived from a diversity of belowground sources, many depending directly on the input of carbon from living plants. Here we present data from two different forest ecosystems, a beech and a spruce forest, where a partitioning of soil respiration was carried out. We used soil cores inside micro-pore meshes together with periodic chamber based measurements to estimate rhizosphere, mycorrhizal fungal and microbial heterotrophic respiration. Calculated mycorrhizal mycelium respiration was 8% at the spruce forest and 3% at the beech forest. Given the nature of the partitioning method these values represent minimum estimates. The ratio of root-derived carbon respiration to heterotrophic respiration was ca. 1:1 at both forest types. The relationship of each source with temperature and photosynthesis, measured as gross primary productivity derived from eddy covariance measurements, was subsequently explored. Both factors revealed effects specific to the respiration source and the forest type. A response to temperature was evident in all cases except for mycorrhizal mycelium respiration at the spruce forest (R^2=0.06, P=0.41). Significant correlations of photosynthesis with rhizosphere and mycorrhizal fungal respiration were found in all cases. Peaks in correlation values showed time lags between photosynthetic activity and a respiration response ranging from one day for the fungal component and four days for the rhizosphere component at the beech forest (R^2=0.70, P<0.01 and R^2=0.42, P<0.05, respectively) to five days for both fluxes at the spruce forest (R^2=0.44, P<0.01 and R^2=0.72, P<0.01, respectively). Results show that respiration of the mycorrhizal

component cannot be predicted by common temperature driven models in some ecosystems. They also indicate a strong influence of forest canopy processes on the activity of roots and associated organisms. The specific response in each vegetation type should be ideally explained by physiological mechanisms inherent to different species as a next step towards understanding belowground carbon dynamics.

3.1. Introduction

Soil respiration in natural ecosystems is defined as the CO_2 efflux from the soil surface and is the outcome of a variety of biological and to a lesser extent also purely physico-chemical processes each responding individually to environmental factors determining their magnitudes and temporal variations (Schulze 2006; Trumbore 2006). In recent years, many studies have focused on partitioning total soil respiration into its components and on understanding the relationship between specific sources of CO_2 and the environmental factors controlling them, as well as the complex interactions between the different respiring organisms (Högberg and Read 2006; Kuzyakov 2006). From basic physical-chemical properties it can be deduced that all underlying biological processes will be affected to a certain extent by temperature and moisture. But even the response to these factors may vary depending on the concentration of substrates involved in enzymatic reactions (Davidson et al. 2006) or the resistance and acclimation of soil organisms to changes in their environment (Atkin and Tjoelker 2003). Other important factors, such as soil organic matter molecular complexity, biomass, nutrient availability, etc, can be related more specifically to fluxes from individual sources.

One environmental variable influencing all root-derived carbon respiration is photosynthetic activity. Although important, its effect on respiration rates is difficult to characterize given that, together with the respiration fluxes it affects, it commonly correlates strongly with temperature at a diurnal and monthly scale. The resulting confounding effect makes it difficult to distinguish the degree to which canopy processes and temperature are respectively influencing root activity, something that can easily lead to erroneous conclusions on temperature relations. The problem can be partly overcome when strong correlations with temperature are avoided, as when the lag time between

photosynthesis and its effect on respiration is large, which can be expected in the case of soil respiration of tall vegetation, or when periods of constant temperature and changing photosynthetic activity can be isolated.

Many recent studies have shown the connection between photosynthetic activity and root-derived carbon respiration, either by analyzing the isotopic signature of assimilated and respired carbon (Andrews et al. 1999; Ekblad and Hogberg 2001), or by relating changes in the amount of assimilates translocated to roots and measured respiration rates (Högberg et al. 2001). This link has been described in grass-lands (Craine et al. 1998; Johnson et al. 2002), crops (Kuzyakov and Cheng 2001; Moyano et al. 2007), boreal forests (Högberg et al. 2001) and temperate forests, both deciduous (Liu et al. 2006) and evergreen (Irvine et al. 2005). A direct connection between photosynthesis and respiration fluxes is thus expected in most, if not all, ecosystems.

The question becomes more complicated when considering the time lag and strength of the relationship in each case. Such factors are likely determined by plant physiological processes and growth patterns that depend on the vegetation type and even on the particular source of CO_2 considered (root, rhizosphere or mycorrhizal fungi). Soil respiration has been shown to respond to carbon assimilation in time periods of a few hours in grasslands, a day or two in crops and a few days in forests. Studies where the contribution of mycorrhizal fungal respiration to soil respiration was assessed (Heinemeyer et al. 2006; Moyano et al. 2007) indicate that this component is highly dependant on fresh inputs of assimilates and in many cases may be driving the short-term response of soil respiration to gross primary productivity (GPP). More indirect processes are likewise connected to root activity, as in the case of enhanced soil organic matter decomposition resulting from priming by input of root or mycorrhizal exudates (Godbold et al. 2006; Subke et al. 2004). As a result, analyzing belowground CO_2 fluxes simply as a combination of an autotrophic and a heterotrophic component ignores processes which depend on the flow of substrates from the plant, affect the whole soil system, and respond differently to environmental factors (Högberg and Read 2006).

Attempts to quantify total soil respiration with the use of empirical models have rarely included parameters other than soil temperature and soil moisture (Janssens et al. 2003).

Leaf area index as a proxy for productivity has been used as a predictive parameter (Reichstein et al. 2003). However, the relationship with daily variations in GPP has been little explored in this context.

In this study we partitioned soil respiration in a beech forest and a spruce forest with the objective of identifying general as well as site specific relations between photosynthetic activity and two root-derived carbon respiration sources: rhizosphere respiration and extra-radical mycelium respiration of mycorrhizal fungi. We hypothesize that both these fluxes will be significantly and positively related to GPP with a time lag of a few days for both sites. We additionally compare the contribution of root and soil organic matter derived respiration fluxes and discuss advantages and limitations of the employed method.

3.2. Methods

Study sites

The study was carried out at the Hainich beech forest and the Wetzstein spruce forest sites in central Germany. Both are main sites of the European CarboEurope project where continuous eddy covariance measurements are running.

The Hainich forest site, located within the Hainich National Park (51° 04' 45,36" N; 10° 27' 07,20" E; 430m a.s.l.), has mean annual values of 8°C and 750-800 mm for air temperature and precipitation. The study area is part of a large section of the forest that has been unmanaged for several decades and presents a wide range of age classes (from 1 to 250 years) and comparatively large dead wood pools. The forest is dominated by beech (*Fagus sylvatica*, 65%) and codominated by ash (*Fraxinus excelsior*, 25%) and maple (*Acer pseudoplantanus* and *A. plantanoides*, 7%). Ground vegetation mainly consists of *Allium ursinum, Mercurialis perennis* and *Anemone nemorosa*. Mean canopy height is ca. 24 m.

The Wetzstein spruce forest site is located on a low mountain range near the village of Lehesten (50° 27' 12,60" N; 11° 27' 27,12" E; 785 m a.s.l.), with mean annual air temperature and precipitation values of 5.5°C and 880-1015 mm. The forest is managed

and the main tree species is 50-year-old Norway spruce (*Picea abies*). Ground vegetation is sparse with occasional areas covered by moss. Mean canopy height is 21.6 m. See Anthoni et al. (2004) for more details.

Partitioning method and soil respiration measurements

Soil respiration was partitioned by excluding roots or roots and mycorrhizal hyphae from soil cores using nylon mesh bags of 35 and 1 µm pore size, respectively. Soil cores (30 cm deep and 15 cm diameter) were removed and restored intact inside mesh bags at the Hainich site. This was impossible at the Wetzstein site given the large amount of stones, so the soil was separated by soil horizons (O, organic A, E and B horizons) and put in the mesh bags after removing roots. At each site, plots consisted of three collars for CO_2 efflux measurements (10 cm diameter; inserted approx. 3 cm deep): one on a 35 µm mesh soil core, one on a 1 µm mesh soil core and a third control collar for measuring total soil respiration. Respiration fluxes (R) from the rhizosphere, mycorrhiza and soil organic matter were estimated from the difference in CO_2 efflux values according to the following:

- control = *total soil respiration* (Rt)
- 1 µm = *heterotrophic respiration* (Rh)
- control - 35 µm = *rhizosphere respiration* (Rr)
- 35 µm - 1 µm = *mycorrhizal respiration* (Rm)
- control - 1 µm = *mycorrhizosphere respiration* (Rmr)

We differentiate between the rhizosphere and the mycorrhizosphere. The first refers to the root itself and its zone of influence, including closely associated microorganisms living on rhizodeposits and the sheath of mycorrhizal fungi around the root. The second is the rhizosphere plus the extra radical mycelia of mycorrhizal fungi. Thus, Rr includes root and associated microbial respiration and any decomposition resulting from priming effects. Rm is respiration from the extra radical or external mycelia of mycorrhizal fungi and associated priming effects. Rmr is the sum of Rr and Rm, referred to as autotrophic

respiration in many studies. Rh includes soil organic matter and aboveground litter respiration excluding the influence of the mycorrhizosphere.

A total of 25 plots were installed at the Hainich site at random points falling on a grid of 60x60 m within the footprint area of the tower. Twelve plots were installed in January 2005 and 13 in August 2005. At the Wetzstein site, 15 plots were installed in April 2005. Plots were 30 m apart on 3 transects following the main wind direction of the tower footprint. Footprint calculations were performed according to Göckede et al. (2004) for a representative period of three months.

Soil respiration measurements were done with a soil chamber and infrared gas analyzer (LICOR LI-6400-09, Licor Inc., Lincoln, NE, USA). Measurements in 2006 started as soon as snow melted (late March in Hainich; mid-April in Wetzstein) and were carried out once a week until November, alternating between sites to obtain biweekly site measurements. Measurements started at ca. 10am and ended at 4-6pm. Depending on the temperature and amount of daylight, we were able to measure 10 to 15 plots per day. Consequently, different subsets of plots were measured on different dates at Hainich in order to obtain a higher number of plot based yearly temperature response curves.

Soil water content in the upper 6 cm (Theta Probe, Delta-T Devices Ltd., Cambridge, UK) and soil temperature at 5 cm depth were measured at the plot level along with each flux measurement. Soil temperature and moisture half hour data at several depths was obtained for each site from measurements next to the eddy covariance tower.

Eddy covariance data

Ecosystem net carbon and water vapor fluxes have been measured continuously, since 1999 in Hainich and since 2001 in Wetzstein, with an eddy covariance system consisting of a sonic anemometer (Solent R3, Gill Instruments, Lymington,UK; mounted at 43.5 m at Hainich and at 30m at Wetzstein) and a LI 6262 infrared gas analyzer (LiCor, Lincoln, Nebraska, USA). The air was pumped through a tube (Dekabon, SERTO Jakob, Fuldabrück, Germany) and filtered behind the inlet and a second time before the gas analyzer (ACRO 50 PTFE 1 µm pore-size, Gelman, Ann Arbor, MI, USA). For more details about the instrumentation we refer to Anthoni et al. (2004).

The flux data were calculated for 30 min intervals by means of the post-processing program *Eddyflux* (Kolle and Rebmann, 2007). Raw data were converted into physical data and a planar-fit rotation after Wilczak et al. (2001) was applied. Time lags for CO_2 and water vapor concentrations were calculated by determining the maximum correlation between the fluctuations of the concentrations and the vertical wind component w‘. The fluxes were calculated using conventional equations (Aubinet et al. 2000; Desjardins and Lemon 1974; Moncrieff et al. 1997) and corrections (Schotanus et al. 1983). Spectral corrections were applied on the fluxes of CO_2 and H_2O according to Eugster and Senn (1995). CO_2 stored in the air column between the measuring system and the soil surface (storage flux) was determined as the concentration change in time within a CO_2 profile where concentrations were measured continuously at 9 heights.

For Wetzstein, an additional pre-selection of the data had to be performed due to the complexity of the site. Under specific conditions (near neutral atmospheric stability, high friction velocity u* and wind direction between 200 and 280 or 30 and 40 degrees) unrealistically high CO_2-fluxes occur even if the advection terms are taken into account (Feigenwinter et al, 2007). These criteria for discarding fluxes were determined after careful analysis of the long-term data set which showed a decoupling of the flow above and below the canopy under these circumstances so that the measured turbulent flux no longer represents the net ecosystem exchange.

GPP was calculated from the difference NEE - R_{eco} (Reichstein et al. 2005), where functional dependencies of total ecosystem respiration (R_{eco}) with temperatures were determined with CO_2 fluxes (including storage) measured at night (fluxes measured during low mixed periods are discarded, see Aubinet et al., 2000). These functions were then applied to day-time data to derive GPP.

Data analysis

Significant differences between measured fluxes were determined on each date using general linear model analysis in the SPSS statistics program, for which soil treatment was taken as the fixed factor and plot as a random factor. Repeated measurement analysis was additionally used to test for significant changes over time. In addition, to check for an

increase in CO_2 efflux on the mesh cores resulting from root decomposition after installation at Hainich, we compared plots installed in January 2005 with those installed in August 2005 on several dates from September 2005 to September 2006. Differences in mean values of each date were tested with independent sample t statistics.

Temperature regressions were fitted to flux data from the entire year using the following equation:

$$R = R_{0°C} * Q_{10}^{(T/10)} \quad (1)$$

where R is the measured respiration flux, $R_{0°C}$ is the respiration flux at 0˚C, Q_{10} is the proportional change in respiration with a 10˚C change in temperature and T is the measured soil temperature in the field. At both sites, all measured soil fluxes were found to correlate best with soil temperature at a depth of 2 cm measured at the site level. These measurements were then used to normalize measured respiration data to a temperature of 15˚C using the mean annual Q_{10} calculated from Rh, as follows:

$$R_{15} = R * Q_{10h}^{((15 - T)/10)} \quad (2)$$

where R_{15} is the normalized respiration flux, R is the measured respiration flux, Q_{10h} is the Q_{10} from equation 1 using Rh values (i.e. measurements on 1 micron mesh collars), and T is soil temperature at a 2 cm depth (using temperature at 5cm did not significantly change the following analysis). Rm_{15} and Rr_{15} were then calculated from these normalized fluxes. The use of a heterotrophic Q_{10} to normalize all respiration measurements comes from assuming a similar intrinsic temperature response for all sources (a Q_{10} commonly close to 2). This in turn avoids canceling differences in respiration values that are responding to other factors which incidentally correlate with temperature (e.g. response to photosynthesis). Since both sites showed a strong negative correlation between soil moisture and soil temperature, all values where soil moisture was lower than a roughly determined critical value (lower than 25% of volume at 32 cm depth for Wetzstein and 25% of volume at 8 cm depth for Hainich) were excluded in order to estimate an additional temperature response without the influence of drought. Different normalized rhizosphere and mycorrhizal fungal respiration values (Rr_{15} and Rm_{15} respectively) were calculated using lower or higher Q_{10}s derived from including or excluding periods of low soil moisture, respectively.

After calculating Rr_{15} and Rm_{15}, Pearson correlations were performed between the mean of respiration values from each date and the mean daily GPP of 0 to 15 days prior to the respiration measurement date. Linear regressions were performed for the days where Pearson correlations showed peak values.

3.3. Results

Our mesh exclusion experiment resulted in large and significant differences between control measurements (no meshes) and both mesh treatments (Figure 1). Differences between mesh treatments were also observed but less pronounced. Respiration means from the 35 µm treatment were higher than means from the 1 µm treatment on all dates for the Wetzstein site and on most dates for the Hainich site. However, univariate and

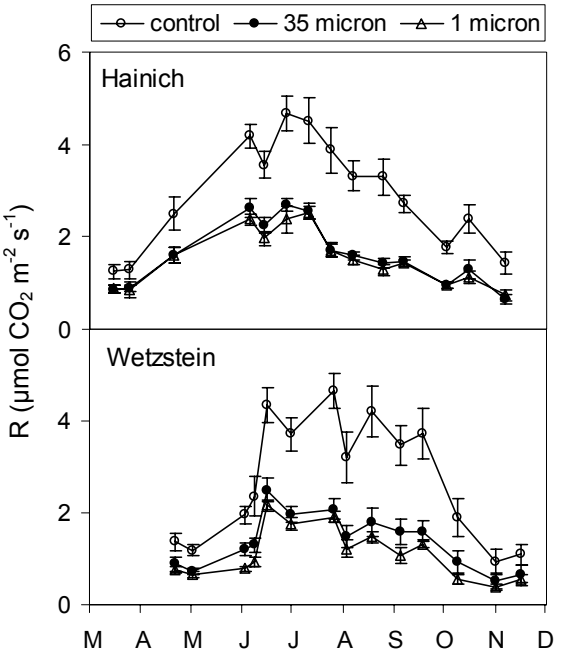

Figure 3.1: Measured soil CO_2 efflux in the year 2006. Plots show field measurements from control collars and collars on 35 µm and 1 µm mesh soil cores for the Hainich beech forest (upper panel) and the Wetzstein spruce forest (lower panel). Error bars represent standard errors.

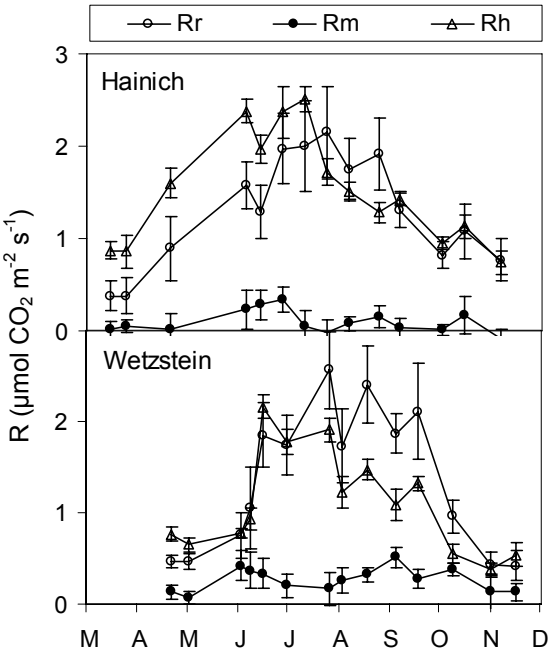

Figure 3.2: Respiration fluxes in 2006 for the rhizosphere (Rr), mycorrhizal hyphae (Rm) and heterotrophic respiration (Rh), resulting from differences in the measured fluxes. Values are shown for Hainich (upper panel) and Wetzstein (lower panel). Fluxes are significant on one or more dates ($P<0.05$) except for Hainich Rm. Error bars represent standard errors.

repeated measurement analysis showed a significant Rm flux (35 µm - 1 µm) only at the Wetzstein site.

Calculated fluxes showed a different annual course for each source (Figure 2). At Hainich, Rh peaked in late July while Rr peaked in early August, showing a steady increase in its relative contribution to total soil respiration until September. Rm values where highest in mid-July. A similar pattern was observed at Wetzstein, where Rh peaked in mid-June and later decreased while Rr peaked in late July and remained high until mid-September. Rm showed high values in early and in late summer. The relative contribution of each respiration source, as an average of all measurements, is given in Table 1.

Table 1: Contribution of heterotrophic (Rh), rhizosphere (Rr) and mycorrhizal fungal (Rm) respiration fluxes calculated from measurements in the field.

Site	Rh (%)	Rr (%)	Rm (%)
Hainich	53 (±2)	44 (±2)	3 (±1)
Wetzstein	47 (±2)	45 (±2)	8 (±2)

Figure 3 shows Rh of plots from Hainich installed in January 2005 compared to those installed in August 2005. Plots from August had significantly higher respiration levels in late 2005 and early 2006. These differences decrease and become non-significant on April 2006 and after.

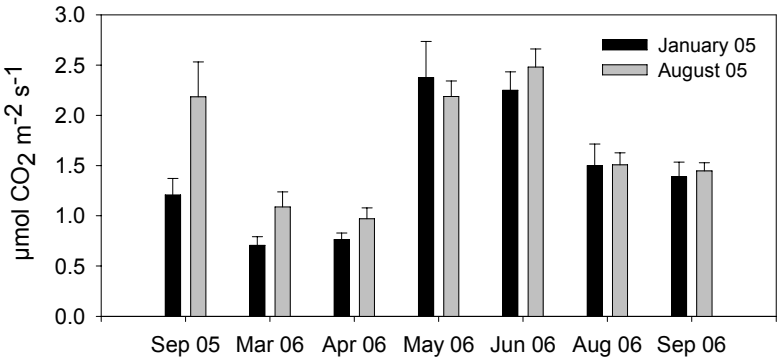

Figure 3: Mean values and standard errors of heterotrophic respiration on several dates from plots installed in January 2005 (black columns) and plots installed in August 2005 (grey columns). Values from the latter plots are significantly higher ($\alpha = 0.05$) until March 2006.

Apparent temperature sensitivity, expressed as the calculated Q_{10} values, varied between soil treatments and forest type (Table 2). For fluxes of total soil respiration as well as for fluxes from both mesh treatments, Q_{10}s in the spruce forest were higher than in the beech forest. At both sites, periods between July and October showed low levels of soil moisture, as seen in Figure 4. Excluding these periods resulted in increased temperature sensitivities for both sites (Table 2), thus suggesting a negative soil moisture effect on soil respiration. However, neither site revealed a clear relation between R_{15} values (fluxes normalized to 15˚C) and soil moisture at the plot or site level; thus, no attempt was made to correct for moisture effects.

Table 2: Q_{10} values obtained from the response of soil CO_2 efflux from control, 35µm and 1µm mesh treatments to temperature at 2cm, either including all data or excluding periods with low soil moisture. Control and 1µm treatments represent total and heterotrophic soil respiration respectively.

Rs Data	Q_{10} (control)	Q_{10} (35µm treatment)	Q_{10} (1µm treatment)
Hainich (all data)	2.3 (±0.2)	2.3 (±0.2)	1.9 (±0.1)
Hainich (SM>25%)	2.9 (±0.3)	2.8 (±0.3)	2.3 (±0.2)
Wetzstein (all data)	2.5 (±0.2)	2.2 (±0.2)	2.4 (±0.2)
Wetzstein (SM>25%)	3.8 (±0.6)	3.3 (±0.5)	3.7 (±0.4)

Temperature at 2 cm was strongly related to Rh and Rr at Hainich (R^2 = 0.83 and 0.89, respectively) and at Wetzstein (R^2 = 0.80 and 0.87 respectively). At both sites, mean values of Rh and Rr revealed significant exponential relations with the mean temperature of the corresponding day ($P < 0.01$, $n = 14$; Figure 5). Rm was positively related to temperature at Hainich (significant at $P < 0.1$) but showed no relation at Wetzstein ($P = 0.41$; Figure 5).

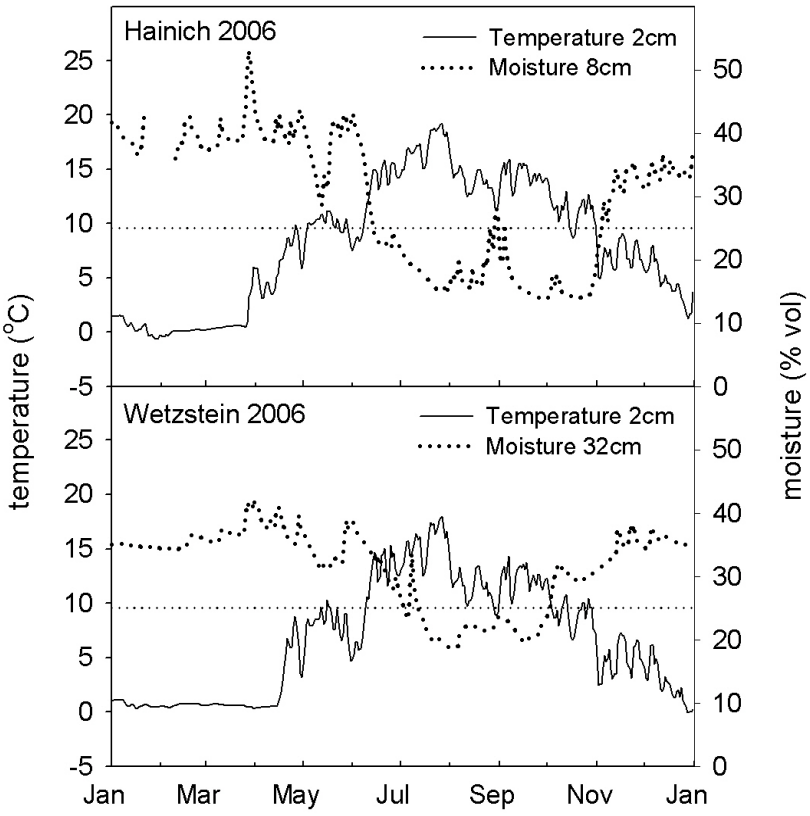

Figure 3.4. Yearly course of temperature and moisture in 2006 at Hainich (upper panel) and Wetzstein (lower panel) measured next to the eddy covariance towers. To assess moisture effects, temperature responses were calculated both including and excluding periods with soil moisture below the horizontal dotted lines.

At both sites, significant correlations between GPP and both rhizosphere and mycorrhizal fungal respiration were observed. Peaks in the correlation strength responded to time shifts between photosynthetic activity and respiration that depended on the site and the respiration source. Correlation coefficients were generally higher at Hainich and more variable at Wetzstein (Figure 6). These differences are explained by a much higher variability in day to day GPP values at Wetzstein compared to Hainich (data not shown). Excluding low soil moisture periods when calculating R_{15} improved the fit only for Rr_{15} vs. GPP at Wetzstein, reducing it in other cases.

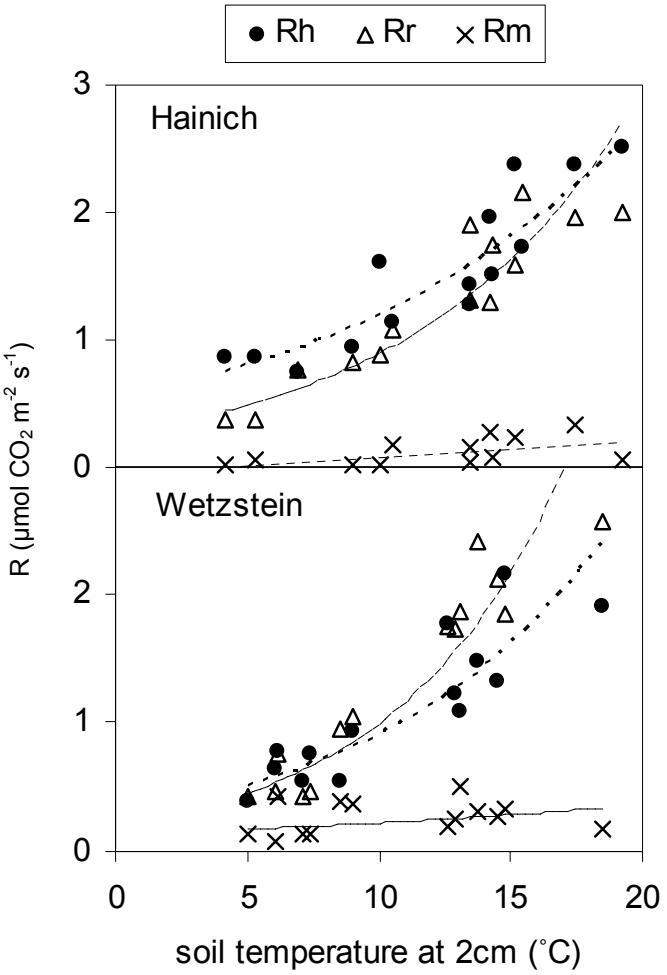

Figure 3.5. Relation of soil respiration with soil temperature at 2 cm measured at the site level. Fluxes in both Hainich (upper panel) and Wetzstein (lower panel) showed exponential fits ($P < 0.01$) for Rh (closed circles; $R^2 = 0.83$ and 0.80 respectively) and Rr (open triangles; $R^2 = 0.89$ and 0.87 respectively). Rm (crosses) showed a week linear relation with temperature at Hainich ($R^2 = 0.28$, $P = 0.07$) and no relation at Wetzstein ($R^2=0.06$, $P=0.41$).

At Wetzstein, correlations with rhizosphere respiration were especially strong after converting to Rr_{15}, showing peaks with GPP from 5, 11 and 15 days earlier. Rm showed the same behavior as Rr_{15} but with lower correlation coefficients. However, correlations were non-significant when plotting Rm_{15} against GPP. At Hainich, rhizosphere respiration correlations peaked with GPP from 4, 9 and 13 days earlier, showing a somewhat lower fit after converting to Rr_{15}. Both Rm and Rm_{15} showed the largest peak in correlation strength with the GPP from 1 day earlier, with Rm_{15} having slightly higher correlation values. Results of linear regressions for days corresponding to peaks in correlation coefficients are shown in table 3.

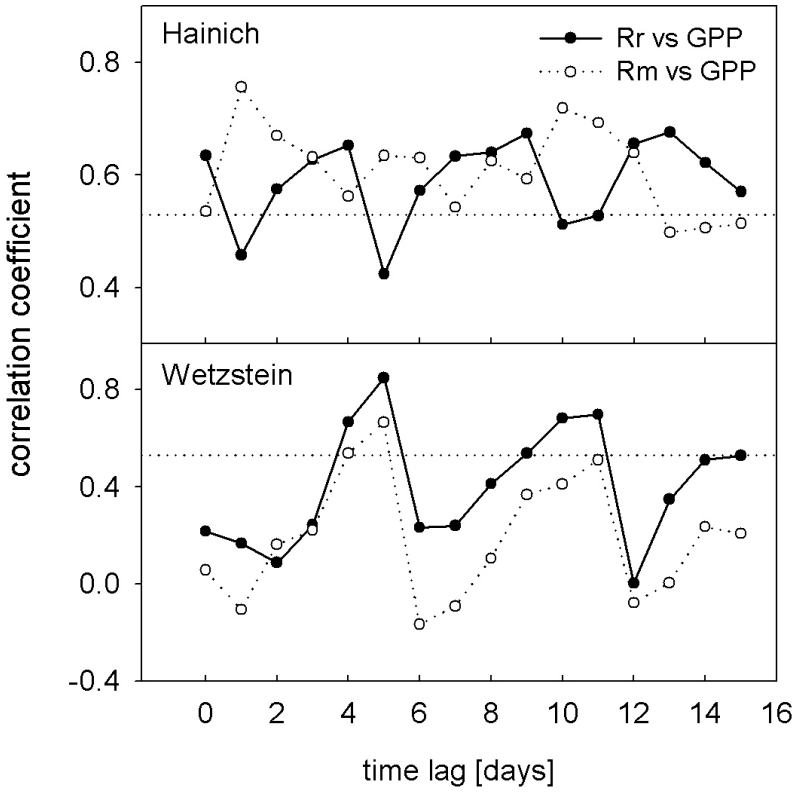

Figure 3.6: Pearson correlation coefficients of temperature normalized rhizosphere respiration (Rr) and mycorrhizal fungal respiration (Rm) against gross primary productivity (GPP) from 0 to 15 days previous to respiration measurements at Hainich (upper panel) and Wetzstein (lower panel). Values above the dotted lines are significant at $\alpha = 0.05$.

Table 3: Coefficients of determination for linear regressions between respiration fluxes and GPP values. Normalized respiration fluxes were obtained with lower or higher Q_{10} values including or excluding low soil moisture periods, respectively. GPP values correspond to peaks in correlation coefficients and are from 5 days before CO_2 efflux measurements at Wetzstein and from 4 and 1 days before measurements for Rr and Rm at Hainich, respectively.

Flux	R vs. GPP	R_{15} (lower Q_{10}) vs. GPP	R_{15} (higher Q_{10}) vs. GPP
Rr (Hainich)	$R^2 = 0.73$; $P < 0.01$	$R^2 = 0.42$; $P < 0.05$	$R^2 = 0.27$; $P > 0.05$
Rm (Hainich)	$R^2 = 0.58$; $P < 0.01$	$R^2 = 0.70$; $P < 0.01$	$R^2 = 0.67$; $P < 0.01$
Rr (Wetzstein)	$R^2 = 0.29$; $P < 0.05$	$R^2 = 0.59$; $P < 0.01$	$R^2 = 0.72$; $P < 0.01$
Rm (Wetzstein)	$R^2 = 0.44$; $P < 0.01$	$R^2 = 0.17$; $P > 0.1$	$R^2 = 0.09$; $P > 0.1$

3.4. Discussion

As with all soil respiration partitioning methods, the calculated fluxes are an approximation of real field fluxes. Limitations related to the widely used trenching-root exclusion techniques (Subke et al. 2006), and in particular the root-mycorrhiza exclusion method used in this case – namely, root decomposition, disturbance of the soil structure, lateral diffusion of CO_2 (Jassal and Black 2006), limited in-growth of mycorrhizal hyphal and differences in soil water content between treatments – may have caused the proportion of Rh to be overestimated. A side-experiment at the Hainich site where PVC tubes were inserted over mesh soil cores to estimate lateral diffusion of CO_2 showed that this effect alone can increase Rh by ca. 10% (data not shown).

The question remains whether the Rm flux actually represents respiration from mycorrhiza that have re-grown into the cores. Evidence for mycorrhizal in-growth can be found in previous studies, e.g. the movement of carbon from the plant to the inside of 35 µm mesh soil cores has been successfully traced with the use of isotope measurements (Johnson et al. 2002 and references therein). In addition, Moyano et al. (2007) showed differing levels of inorganic nitrogen between 1 and 35 µm mesh treatments, indicating an uptake of nitrogen by mycorrhiza. Average inorganic nitrogen at Hainich and Wetzstein did not differ significantly between our mesh treatments. However, mean values were still highest in the 1 µm cores, intermediate in the 35 µm cores, and lowest in control soils (5.3±1.0, 4.8±1.2, 3.1±0.5 µgN $gSoil^{-1}$ at Hainich and 20.9±3.1, 18.5±3.4, 12.7±2.0 µgN $gSoil^{-1}$ at Wetzstein for the respective treatments). This suggests mycorrhizal activity within the 35 µm cores. In addition, a strong indicator of mycorrhizal respiration is given by the unique behavior of this flux in its relation to temperature and GPP, indicating it is not simply a weaker signal of the heterotrophic or rhizosphere components.

Considering the above, and in light of evidence from other studies of its large contribution to soil respiration in forest ecosystems (Leake et al. 2004), external mycelium respiration is expected to be larger than our results have shown, especially in the beech forest. We speculate that the pore size used (35 µm) may be limiting the in-

growth of hyphae in this case. On the other hand, the 8% of external mycelium respiration relative to total soil respiration in the spruce stand is an important amount; especially considering the paucity of similar estimates to date (Högberg and Read 2006).

Soil disturbance by sieving at Wetzstein will affect fluxes in unpredictable ways. Although the measured fluxes may thus present systematic errors, making comparisons difficult, we expect them to be reduced after a year between installation and measurements. Root decomposition can also increase heterotrophic respiration. Although we were careful to avoid large roots when installing soil cores at Hainich, decomposition of fine roots can persist for several months (Chen et al. 2002). The differences seen between plots installed early and late in 2005 indicate increased decomposition is important in the first months after installation. On the other hand, differences were low or absent during most of 2006. Since root decomposition is unlikely to be stable at a high level for periods of several months (time separating the installation dates) this would indicate low root decomposition fluxes on all plots compared to the total heterotrophic flux during 2006. However, Rh estimates in March and April 2006 may be increased by some percent due to this effect.

In conclusion, results corroborate ranges of Rmr (mycorrhizosphere respiration: Rr + Rm) and soil organic matter-litter respiration (Rh) from previous studies in the same site or forest type (Andersen et al. 2005; Subke et al. 2004). Rmr in the spruce forest represented a larger amount of total soil respiration compared to the beech forest. However, considering possible systematic errors related to the issues mentioned above, we can only affirm that the relation between Rh and Rmr is similar at both forest stands and comes close to the often cited 50 to 50 percent relation. It should be noted that enhanced decomposition of soil organic matter resulting from priming of soil microbes by root or mycorrhizal exudates is included in the Rmr component. This process cannot be partitioned with most exclusion methods.

Beyond difficulties in estimating absolute magnitudes, trends in the calculated fluxes permitted an analysis of their response to environmental factors. Measurements revealed temporal changes in soil respiration which were distinct for each source, resulting in specific temperature and GPP relations. Rhizosphere respiration showed a peak response

to photosynthetic activity with a time lag of 4 days in the beech forest and 5 days in the spruce forest. These results confirm our initial hypothesis and can be compared to results from a former study at the Hainich site showing a link between photosynthesis and ecosystem respiration with a time lag of 4-5 days (Knohl et al. 2005). Given the long time lags involved and the repeating patterns observed in earlier correlation peaks (days 11 and 15 at Wetzstein; days 9 and 13 at Hainich), these are likely not related to a delayed utilization of assimilates. Such recurring peaks are better explained by autocorrelations in weather patterns. Weather fronts pass through the region in average every two weeks, creating cycles that can be observed in the temperature curves of Figure 4. The effect of these weather conditions on comparable correlation patterns was confirmed by Knohl et al. (2005) at the Hainich site. We expect a similar effect taking place at Wetzstein.

The lack of a significant relation between Rm and temperature at Wetzstein is possibly the result of Rm being largely controlled by the availability of energy substrates from roots or by changes in biomass, leaving little space for variations determined by temperature. Thus, in this case, the response to GPP should be analyzed with the measured and not the temperature normalized values. We can then conclude that Rm at Wetzstein responds to assimilate supply the same way as the rhizosphere component. This may indicate a direct relation between assimilates available to the roots and carbon exported to mycorrhizal fungi in such needle leaf forests.

This tight regulation by assimilate supply may not be the case at Hainich where Rm presented an obvious relation with temperature and the fit with GPP increased after converting to Rm_{15}. However, this flux was strongly correlated with GPP from the previous day. In contrast to the spruce forest, mycorrhizal fungi in the beech stand seemed to have a faster and more pronounced response to photosynthesis.

This faster response of Rm could explain differences between studies in the observed time between photosynthetic activity and the effect on soil respiration. While most studies report lags of a few days, some have reported lags of only a few hours (Tang et al. 2005). As noted by Högberg (2006), lag times of 1 to 5 days are consistent with rates of 0.2-2 m h^{-1} reported for phloem carbon transport (Nobel 2005). The time dependant translocation of substrates from the canopy to the roots, driven by changes in osmotic

pressure, can thus explain the lagged response of the rhizosphere and external mycelium activity. However, the decoupling of the respective responses in the Hainich beech forest suggests that the respiration of the fungal component is being regulated differently.

A possible mechanism explaining the faster response of the fungal component to photosynthesis could involve information transmission via pressure/concentration waves driven by changes in turgor, which can propagate orders of magnitude faster than the molecules themselves (Thompson and Holbrook 2004). In such a case, studies using isotopes alone would not be able to detect this connection, given that the assimilated molecules themselves are not being respired. Although tree mycorrhiza are expected to dominate in these soils, an effect of ground vegetation mycorrhiza should also be considered. A faster response associated to smaller vegetation would provide an alternative explanation.

3.5. Conclusions

Results from this study do not point towards a simple connection between photosynthetic activity and mycorrhizosphere activity that could be applied to all forest ecosystems. They indicate, on the contrary, that such relations are specific to different forest types and may respond to particular physiological mechanisms. They also provide further evidence that canopy processes should be taken into account in any theoretical explanation or modeling attempt to predict belowground CO_2 production. Identifying these relations for roots, rhizosphere and mycorrhizal fungal respiration in different ecosystems might be a necessary step before an integration of carbon fluxes is reached.

The clear importance of the mycorrhizal fungal component, also evident in this study, is an additional incentive for further research. A more physiological approach will be necessary in order to explain the mechanisms behind dissimilar responses of the fungal and root components to photosynthetic activity.

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4. Exploring the Effects of Spatially Variable Soil Factors on Mycorrhizosphere and Microbial Respiration

Chapter Source

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Abstract

We partitioned soil respiration in agricultural and forest ecosystems located in central Germany – a crop field, a beech forest and a spruce forest – with the objective of determining spatial relations between measured soil parameters and rhizosphere, mycorrhizal fungal and heterotrophic (soil organic matter) respiration fluxes. We subsequently analyze our results to identify common as well as site specific soil factors influencing mean values and spatial variability in fluxes. Site differences were large in a number of soil parameters at 0-10 cm depths: C values were ca. 2.8, 4 and 12 kg m^{-2}; the C to N ratio was ca. 11, 12.5 and 24 at the crop field, beech forest and spruce forest, respectively. Root and mycorrhizal exclusion affected soil moisture, amounts of inorganic nitrogen and carbon content, with the degree depending on the site and date, but these differences were smaller than time variations within a site. At the forest sites, the mycorrhizosphere component was more spatially variable (standard deviations of 0.57 to 1.17 and 0.43 to 1.29 μmol m^{-2} s^{-1} at the beech and spruce sites, respectively) than the heterotrophic component (standard deviations of 0.28 to 0.79 and 0.23 to 0.41 μmol m^{-2} s^{-1} for the same sites). The opposite relation was seen at the crop field (standard deviation from 0.70 to 0.86 and 0.72 to 1.30 μmol m^{-2} s^{-1} for mycorrhizosphere and heterotrophic respiration, respectively). Stepwise multiple regression resulted in significant respiration models with variables including carbon content, C to N ratios, mineral nitrogen, extractable C, microbial biomass, and soil moisture, depending on the site and date (R^2 values between 0.69 and 0.99; $P<0.05$). These results indicate important

spatial relations of nitrogen availability and water content (direct and indirect) with rhizosphere respiration, as well as of substrate supply and microbial biomass with microbial respiration. In addition, certain cases suggest a possible priming effect on decomposition by mycorrhizal fungi and negative effects of ammonium on microbial respiration. The influence of the more variable respiration component was reflected in the relations found for total soil respiration.

4.1. Introduction

The production and efflux of CO_2 from soils varies largely not only temporally but also spatially. Coefficients of variation of 50% or more are often reported for spatial variations of soil respiration in homogenous areas (Buchmann 2000; Soe & Buchmann 2005) and such differences are commonly greater in summer and lower in winter, with the variation increasing with mean temperatures. It is also typical for these spatial variations to be larger than the daily change in mean soil respiration (Matteucci et al. 2000) but lower than annual or seasonal changes.

Temperature and moisture are usually invoked as the factors driving the physiology of soil respiration rates, which is not surprising given their dominant effect on temporal changes within a site. In a global scale analysis of soil respiration data, Raich and Schlesinger (1992) presented a model where annual soil respiration rates were predicted from only two variables: mean annual temperature and mean annual precipitation. They were thus able to explain up to 50% of the variability between sites. Although this analysis serves as an approximation of global carbon fluxes from soils, it simultaneously shows that sites with similar climatic conditions can have very different soil respiration rates. By now it is clear that temperature and water relations are responsible for large seasonal variations in soil respiration; however, these two variables work in combination with other soil properties determined by site characteristics such as land use history, management, vegetation type, soil parent material, soil formation and climate. These soil properties determine the spatial variability in the response of decomposition and root activity to changes in the environment.

Soil properties that have been related with soil respiration include amounts and quality of soil organic matter (both fresh litter and older carbon pools), nutrients (N, P, K, Mn, Ca, etc), soil acidity, bulk density, site topography and below as well as above-ground vegetation characteristics (Epron et al. 2006; Fang et al. 1998; La Scala et al. 2000; Rochette et al. 1991; Xu & Qi 2001). Soil organic matter properties and interaction with soil particles have a direct influence on heterotrophic respiration while vegetation characteristics are directly determining root and mycorrhizosphere respiration. Different ecosystems can thus show differences in the magnitude and spatial variation of soil respiration fluxes even when relative temperature and moisture effects are comparable. The intra-site spatial variation of soil respiration is normally related to changes in the above mentioned soil properties, including differences in water content. Soil temperature, on the other hand, typically shows limited spatial and diurnal variation so relations with soil respiration at these scales are generally absent. Different soil factors are auto-correlated at different spatial scales (Behrens & Scholten 2006; Brocca et al. 2007; Don et al. in press; Stoyan et al. 2000). Understanding the causes of intra-site spatial heterogeneity of soil respiration can thus help understand differences among sites and vice versa.

Although several studies have explored the relations between soil factors and respiration fluxes, results have been sometimes contradictory or complicated by the strong inter-correlation of groups of factors such as amounts of C, N and other nutrients (Soe & Buchmann 2005). Thus, field studies of soil respiration are often not able to distinguish the specific response of soil respiration components to these factors. Draught, for example, may affect microbial respiration more than root respiration (Borken et al. 2006; Scott-Denton et al. 2006). As a result, many constraints affecting belowground carbon fluxes remain unresolved. One open question involves the effects of nitrogen. Nitrogen availability in soils may stimulate plant and microbial activity by removing N limitations. However, several researchers have noted that nitrogen compounds can reduce soil respiration rates, in some cases for extended periods of time (Persson 2000). The negative effect of nitrogen on soil respiration has more than one possible explanation. Several observations involve an influence of N on the degradation of lignin. On the one hand, N compounds have been seen to down regulate the production of lignin degrading enzymes

(Waldrop et al. 2004). In addition, ammonia and amino acids may react with lignin to form recalcitrant complexes (Berg 2000). Another explanation is given by Schimel and Weintraub (2003) who presented a model in which nitrogen additions reduce respiration rates as a result of available carbon being used for increasing microbial biomass instead of being respired as overflow metabolism. Finally, increased amounts of N may lead to a reduction in root or mycorrhizal biomass in the long term and therefore to lower mycorrhizosphere respiration (Coleman et al. 2004; Keyes & Grier 1981).

Dynamics in the microbial population have been proposed as yet another key element in explaining changes in the degradation and accumulation of soil organic matter. In this case, the decomposition of soil organic matter is not seen as a function of the amount of soil carbon in defined pools but of the ability of microbes to obtain energy by decomposing organic matter through the release of exoenzymes. Microbial activity would thus be controlled by the quality and spatial distribution of organic matter and by the availability of limiting nutrients such as N (Fontaine & Barot 2005; Schimel & Weintraub 2003). This idea corresponds well with observed priming effects where additions of fresh carbon promote microbial activity and enhance the decomposition of older carbon (Fontaine et al. 2003; Kuzyakov et al. 2000). It is also supported by observed effects of moisture, which in many cases may increase respiration rates by increasing substrate and microbial mobility (Davidson et al. 2006; Grant & Rochette 1994).

This study aims at identifying the response of partitioned belowground respiration fluxes to spatially variable soil factors. In this context we address the points described above involving water, nitrogen and microbial relations, among others. We obtained soil respiration measurements from three temperate ecosystems in central Germany (a cropland, a beech forest and a spruce forest) and partitioned the total flux with the use of micromeshes into three components corresponding to respiration from the rhizosphere, mycorrhizal fungi, and from litter and soil organic matter. Additionally, we measured total soil carbon (C) and nitrogen (N), carbon to nitrogen ratios (C:N), ammonium (NH_4^+), nitrate (NO_3^-), K_2SO_4-extractable carbon (K_2SO_4-C), microbial biomass carbon (MBC), and soil moisture (SM) of the upper 10 cm at individual respiration locations. We look at how these factors relate to soil treatments (root and mycorrhizal exclusion) and

respiration measurements. Compared to previous studies with a similar analysis of soil respiration spatial variability (e.g. Wang et al. 2003), we have opted for a field approach with the possibility of analyzing partitioned fluxes in near-natural conditions while contrasting different ecosystems.

4.2. Materials and Methods

Site characteristics

This study was carried out at the Gebesee crop field, Hainich beech forest and Wetzstein spruce forest sites in central Germany. All are main sites of the European CarboEurope project. Eddy covariance towers are located at the center of each site.

The Gebesee field is situated 20 km NW of Erfurt in Thuringia, Germany (latitude 51°06'0.13''N, longitude 10°54'51.9''E, 162 m asl). Soil was harrowed in August and Winter barley (*Hordeum vulgare* L.) sown in September 2004. Seedlings remained mostly under snow cover from late January until early March of 2005. Crop rotation has included 50% cereals and 50% potato and sugar beet since 1970 or earlier. Mean annual air temperature and precipitation values are 7.9°C and 500 mm. The soil is a Chernozem according to FAO classification, with depths of over 1 m. Soil texture is silty clay loam (~30% clay) with granular structure. pH values average 6.62 in the upper 10 cm of soil.

The Hainich forest site, located within the Hainich National Park (51° 04' 45.36" N; 10° 27' 07.20" E; 430m asl) is part of a large section of the forest that has been unmanaged for several decades and presents a wide range of age classes (from 1 to 250 years) and comparatively large dead wood pools. The forest is dominated by beech (*Fagus sylvatica*, 65%) and co-dominated by ash (*Fraxinus excelsior*, 25%) and maple (*Acer pseudoplantanus* and *A. plantanoides*, 7%). Mean annual values of air temperature and precipitation are 8°C and 750-800 mm. The soil is a Cambisol (FAO), with a depth of ca. 0.6 m and pH values ranging from 6 to 8.

The Wetzstein spruce forest site is located on a low mountain range near the village of Lehesten (50° 27' 12.60" N; 11° 27' 27.12" E; 785 m asl). The forest is a plantation of 50-

year-old Norway spruce (*Picea abies*). Mean annual air temperature and precipitation values are 5.5°C and 880-1015 mm. Soils are dystric Cambisols and Podsols with a mean depth of ca. 0.4 m and pH values ranging from 3.5 to 4.

Experimental Design

At Gebesee 14 plots were installed in November 2004 at random points falling on a grid of 10x10 m. At Hainich, a total of 25 plots were installed at random points falling on a grid of 60x60 m. 12 plots were installed in January 2005 and 13 in August 2005. At Wetzstein, 15 plots were installed in April 2005 on a grid of 30x30 m.

Soil respiration was partitioned by excluding roots or roots and mycorrhizal hyphae from soil cores using nylon mesh bags of 35 and 1 µm pore size, respectively. Soil cores (30 cm deep and 15 cm diameter) were removed and restored intact inside mesh bags at Gebesee and Hainich. This was impossible at Wetzstein given the large amount of stones so the soil was separated by soil horizons (O, organic A, E and B horizons) and put in the mesh bags after removing roots. Each plot consisted of both mesh bag treatments, each with a soil respiration collar (10 cm diameter; inserted approx. 3 cm deep) and an additional control collar for measuring total soil respiration. Respiration fluxes from the rhizosphere, mycorrhiza, and soil organic matter were estimated by subtracting values of soil respiration measured on the corresponding collars (Moyano et al. 2007). We use the following abbreviations:

- Rt = control (total soil respiration)
- Rh = 1 µm (heterotrophic respiration of soil organic matter including aboveground litter)
- Rr = control - 35 µm (rhizosphere respiration including seasonal root turnover and associated priming effects)
- Rm = 35 µm - 1 µm (respiration from the extra radical or external mycelia (ERM) of mycorrhizal fungi and associated priming effects)
- Rmr = control - 1 µm (mycorrhizosphere respiration, which is the sum of Rr and Rm)

Measurements

Soil respiration measurements were carried out with a closed soil chamber and infrared gas analyzer (Licor LI-6400-09, Licor Inc., Lincoln, NE, USA). Respiration was measured 0 to 2 days prior to each soil sampling date (prior to sampling if on the same date). Half hour soil temperature was obtained for the whole study period next to eddy covariance towers at the center of each site.

Soil properties were measured from selected plots on a number of dates during the growing season at each site. Table 3 shows the factors measured and included in the analysis on each date. Sampling dates at Gebesee fell between April and July 2005 with the last shortly before harvest. Sampling at Hainich occurred in autumn 2005 and for both Hainich and Wetzstein in spring, summer, and autumn 2006.

Soil samples were taken next to individual respiration locations. The upper 10 cm of soil were sampled, fresh litter not included (approximately 75% of all fine roots, as well as most soil organic matter, are at the top 10 cm in both spruce and beech forests; Stober 2000). Small corers (12 mm dia) were used to remove soil from the soil cores, taking the sample between the respiration collars and the mesh bag. Larger corers were used for sampling non-treated soil.

Roots and large litter were removed from soil samples before any analysis was carried out. Microbial biomass carbon (MBC) was measured using the chloroform fumigation extraction method (Vance et al. 1987). Soil samples were kept in a cooler after removal and later stored at 4^{o}C for a maximum of 4 days. Fumigated samples were kept under vacuum with chloroform for 2 days. Both fumigated and non-fumigated soils were extracted by shaking during one hour with a 0.5 K_2SO_4 solution. Total organic carbon was determined using a high TOC analyzer (Elementar Analysensysteme Hanau, Germany). A general conversion factor of 0.45 was used to obtain MBC (Wu et al. 1990). For determining NH_4^+ and NO_3^-, samples were collected in the field and brought to the lab in an insulated box. The soil was then immediately processed and extracted with 1M KCl. Amounts of NH_4^+ and NO_2/NO_3^- where determined with a Skalar SAN Plus continuous-flow analyzer. We determined carbon to nitrogen ratios by drying and finely

grinding the soil and subsequent analysis with a Vario Max elemental analyzer (Hanau, Germany). Soil water content was determined gravimetrically from soil sub-samples.

Statistical Analysis

Soil respiration rates were analyzed against soil factors using multiple linear regression with the SPSS statistics program. Data was first screened for outliers and data points falling over two standard deviations from the sampling mean were removed from the analysis (a total of 7 data points removed). Single dates were analyzed separately by including the soil factors measured on the same date as independent variables. Significant variables were included stepwise to obtain the best fitting model for each date.

Microbial biomass was analyzed against the remaining soil properties using the same procedure. Since the variability of microbial biomass between treatments within a plot was much lower than the variability between plots, microbial biomass values from all treatments were pooled to perform this analysis. Univariate general linear model analysis was later used to check for significant treatment interactions.

4.3. Results

Soil parameters: mean values and treatment effects

Temperature and moisture were negatively correlated at all three sites during the respective study periods (data not shown). Soil sampling dates thus captured a range of temperatures at each site but dates with relatively high soil moisture were sampled only at Hainich on cold dates (Figure 4.1). On all other occasions soil moisture was medium to low. The presence of roots affected soil moisture by significantly reducing water content in the control soil as compared to both mesh treatments (Table 4.1). The mean percent difference for each site was 14(±13), 18(±23) and 9(±19), for Gebesee, Hainich and Wetzstein, respectively. The presence or absence of mycorrhizal fungi revealed no effect on soil water content.

In order to relate soil factors with field based soil respiration measurements all values are expressed in a per area basis. This was done by using the site average bulk density of the upper 10 cm of soil. The bulk density values used were 1.26g cm^{-3}, 0.85g cm^{-3} and 0.32g cm^{-3} for Gebesee, Hainich and Wetzstein, respectively. Mean values per square meter of all soil parameters are given in Table 4.1.

Table 4.1: Mean values of soil factors at 0-10 cm depth per square meter of soil. Values are shown for Gebesee (Gs), Hainich (Ha) and Wetzstein (Ws) as well as for each mesh treatment (1 and 35 micrometers) and control soils.

Site and Date	Treatment	SM (kg m^{-2})	K_2SO_4-C (gC m^{-2})	MBC (gC m^{-2})	NH_4^+ (gN m^{-2})	NO_3^- (gN m^{-2})	C (kgC m^{-2})	N (kgN m^{-2})	C:N
Gs 05.04.05	*1 µm*	22.67	9.74	50.15	-	-	-	-	-
	35 µm	22.49	9.53	63.68	-	-	-	-	-
	control	21.19	10.97	60.22	-	-	-	-	-
Gs 02.06.05	*1 µm*	19.35	9.68	38.87	-	-	-	-	-
	35 µm	19.73	8.10	38.81	-	-	-	-	-
	control	15.51	8.90	42.65	-	-	-	-	-
Gs 08.06.05	*1 µm*	21.74	-	-	0.57	2.44	-	-	-
	35 µm	22.37	-	-	0.45	2.29	-	-	-
	control	18.19	-	-	0.62	0.68	-	-	-
Gs 11.07.05	*1 µm*	19.25	11.77	33.22	0.34	2.76	2.77	0.25	11.00
	35 µm	19.68	11.46	33.36	0.16	1.33	2.77	0.25	11.00
	control	19.62	11.86	33.36	0.13	1.58	2.80	0.25	11.10
Ha 29.09.05	*1 µm*	30.69	9.80	49.11	-	-	4.10	0.34	12.05
	35 µm	29.07	10.05	45.52	-	-	3.97	0.32	12.29
	control	24.40	12.01	64.90	-	-	4.48	0.37	12.26
Ha 29.03.06	*1 µm*	33.97	-	-	0.15	0.05	-	-	-
	35 µm	32.05	-	-	0.06	0.04	-	-	-
	control	33.89	-	-	0.19	0.02	-	-	-
Ha 07.04.06	*1 µm*	30.87	9.42	43.97	-	-	4.23	0.33	12.77
	35 µm	30.35	9.07	38.21	-	-	4.25	0.34	12.50
	control	29.39	8.70	40.72	-	-	-	-	-
Ha 11.07.06	*1 µm*	27.89	12.38	78.81	0.67	0.29	4.63	0.35	13.29
	35 µm	27.93	13.51	67.03	0.46	0.57	4.51	0.37	12.35
	control	20.14	11.13	50.07	0.31	0.06	3.86	0.31	12.27
Ha 16.10.06	*1 µm*	27.27	7.98	50.94	0.07	0.22	3.49	0.28	12.45
	35 µm	26.57	7.62	50.34	0.06	0.17	3.41	0.27	12.53
	control	20.15	9.24	63.43	0.09	0.13	4.17	0.33	12.59
Ws 21.04.06	*1 µm*	21.08	13.48	47.17	0.49	0.07	10.90	0.45	24.32
	35 µm	21.66	10.85	48.09	0.56	0.09	10.57	0.45	23.43
	control	23.33	19.30	50.43	0.50	0.09	12.95	0.58	22.24
Ws 26.07.06	*1 µm*	18.35	11.63	40.20	0.31	0.70	10.05	0.42	24.15
	35 µm	18.15	10.86	41.75	0.26	0.55	10.90	0.44	24.68
	control	15.16	12.25	41.74	0.31	0.20	12.76	0.51	25.08
Ws 09.10.06	*1 µm*	20.85	11.86	52.14	0.34	0.16	10.00	0.40	25.00
	35 µm	20.51	11.37	51.68	0.30	0.10	10.35	0.41	25.07
	control	18.67	14.43	59.60	0.19	0.01	10.61	0.42	25.32

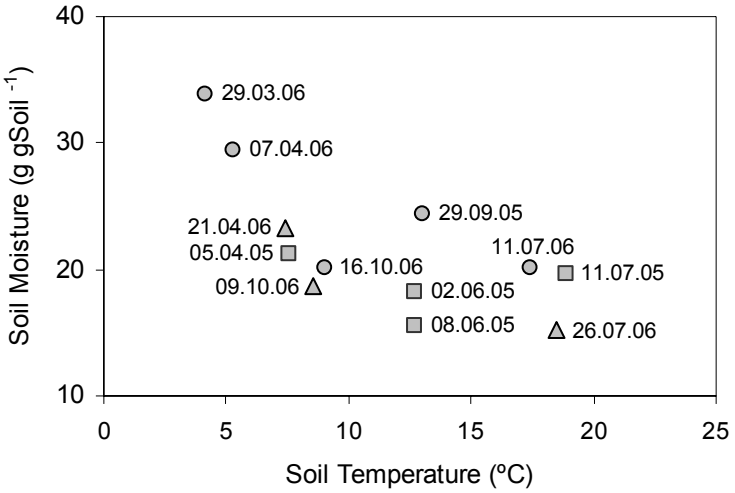

Figure 4.1: Soil temperature plotted against gravimetric moisture for the beech forest (circles), the crop field (squares), and the spruce forest (triangles) at several points in time during the study period. Each point is labeled with the date of sampling.

Carbon content was lowest at the crop field, Gebesee, with mean values of around 2.8 kg m^{-2}. Values were about twice as high in the beech forest, Hainich, with mean values ranging between 3.41 and 4.63 kg m^{-2}. The highest values were found in the spruce forest, Wetzstein, where C mean values ranged between 10 and 12.95 kg m^{-2}. Except for one date at Hainich, carbon content mean values in the control soil were higher than in both mesh treatments. These differences, however, were significant only at Wetzstein 26.07.06. Total N mean values were similarly lower at Gebesee, medium at Hainich, and highest at Wetzstein. The C:N ratio was also lowest at Gebesee, medium at Hainich and highest at Wetzstein. Soil treatments did not significantly alter N or C:N ratios.

Mineral forms of nitrogen also differed among sites and showed a large variation within sites. With values being significantly lower in the control soils compared to the mesh treatments, treatment effects were significant (P<0.05) for NH_4^+ at Hainich 11.07.06 and for NO_3^- at Gebesee 08.06.05, Hainich 11.07.05, and Wetzstein 09.10.06. Although non-significant, mean values of mineral nitrogen from all measurements showed a trend at all sites with the highest values in root and mycorrhiza free soils, intermediate in root free soils, and lowest in the control soils (Figure 4.2).

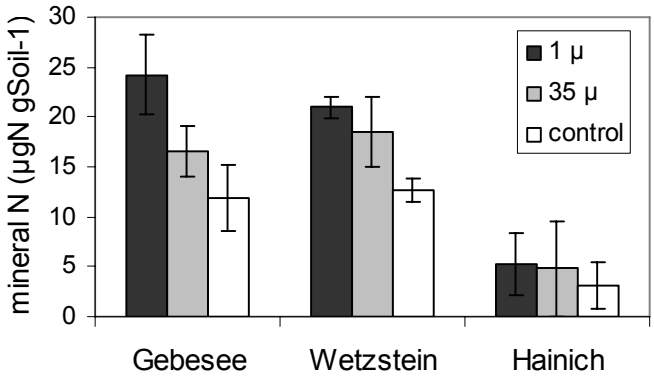

Figure 4.2: Inorganic nitrogen ($NH_4^+ + NO_3^-$) at each site and for each soil treatment. Values are means of all samples from all sampling dates.

Amounts of K_2SO_4-C per square meter of soil were similar between Gebesee and Hainich, and somewhat higher at Wetzstein. Differences were significant between sampling dates at Gebesee and Hainich (P<0.001). On the other hand, MBC showed similar values in all sites, ranging from 30 to 80 g C m^{-2}, with some higher values at Hainich. Differences in MBC were significant between dates at Gebesee and Hainich (P<0.001) but non-significant between soil treatments.

Soil respiration and microbial biomass relations

Standard deviations for each respiration source (Table 4.2) revealed a larger spatial variability of Rmr compared to Rh at the forest sites, and the opposite relation at the crop field. Rh was higher than Rmr at Gebesee and Hainich but lower than Rmr at Wetzstein (in contrast to previous studies; Buchmann 2000).

All calculated belowground respiration fluxes showed a significant relation with soil factors in at least one site and date. Conversely, most soil factors were related to respiration fluxes. Only N content was not included in any model. Wetzstein 21.04.06 was the only date lacking any significant relations. Resulting models for each source and date are shown in Table 4.3.

Table 4.2: Mean soil temperature (ºC), soil moisture (g $gSoil^{-1}$) and flux values (µmol C m^{-2} s^{-1}) with standard deviations of heterotrophic (Rh) and mycorrhizosphere (Rmr) sources.

Site	Date	ST	SM	Rh	Rmr
Gebesee	05.04.05	7.58	21.19	2.63 ± 1.30	0.14 ± 0.86
Gebesee	02.06.05	12.73	15.51	3.08 ± 0.85	1.38 ± 0.70
Gebesee	08.06.05	12.70	18.19	2.32 ± 0.72	1.22 ± 0.67
Gebesee	11.07.05	18.88	19.62	3.39 ± 1.05	0.95 ± 0.84
Hainich	29.09.05	13.00	24.40	1.61 ± 0.85	0.98 ± 1.03
Hainich	29.03.06	4.17	33.89	0.87 ± 0.41	0.40 ± 0.57
Hainich	07.04.06	5.32	29.39	0.86 ± 0.28	0.42 ± 0.99
Hainich	11.07.06	17.43	20.14	2.37 ± 0.79	2.31 ± 1.17
Hainich	16.10.06	9.00	20.15	0.95 ± 0.22	0.83 ± 0.57
Wetzstein	21.04.06	7.43	23.33	0.76 ± 0.23	0.61 ± 0.43
Wetzstein	26.07.06	18.46	15.16	1.91 ± 0.41	2.73 ± 1.29
Wetzstein	09.10.06	8.58	18.67	0.55 ± 0.23	0.83 ± 0.79

Rt was significantly related to SM, C, NH_4^+, K_2SO_4-C and MBC. Only SM at Wetzstein showed a negative relation with this flux. Rh was significantly related to C, C:N, MBC, K_2SO_4-C, and with both NH_4^+ and NO_3^-. These relationships were all positive except for NH_4^+. Rr was related to C at Hainich; no significant models for Rr were found at Gebesee or at Wetzstein. Rm was found to be negatively related to K_2SO_4-C, SM, and C, and positively related with C:N. Rmr revealed the largest number of significant models, which included K_2SO_4-C, NH_4^+, NO_3^-, C and SM. All relations were positive except for K_2SO_4-C at Gebesee and SM at Wetzstein.

The analysis of microbial carbon against the remaining soil factors revealed linear models with lower R^2 values but of higher significance compared to models of soil respiration fluxes (Table 4.4). This was at least partly due to the higher number of cases on each date available for this analysis. Positive relations were found with K_2SO_4-C, SM, N, C, and NH_4^+. Univariate general linear model tests revealed no significant effects of soil treatments in these relations.

Table 4.3: Significant models of soil respiration ($P<0.05$) for each site, date, and respiration source. Models were fitted with the soil factors available for each date using stepwise multiple regressions.

Site	Date	Variables Included	Source	Model Variables	Relation	Model R^2	Model P value
Gebesee	05.04.05	SM, K_2SO_4-C, MBC	Rm	K_2SO_4-C	-	0.99	0.002
Gebesee	02.06.05	SM, K_2SO_4-C, MBC	Rmr	K_2SO_4-C	-	0.69	0.020
Gebesee	08.06.05	SM, NH_4^+, NO_3^-	Rm	SM	-	0.74	0.027
Gebesee	11.07.05	SM, C, N, C:N, K_2SO_4-C, MBC, NH_4^+, NO_3^-	Rt	SM K_2SO_4-C	+ +	0.96	0.008
			Rh	C C:N	+ +	0.99	0.002
			Rmr	NO_3^-	+	0.99	0.001
Hainich	29.09.05	SM, C, N, C:N, K_2SO_4-C, MBC,	Rt	C	+	0.71	0.018
			Rr	C	+	0.88	0.002
			Rm	C:N C	+ -	0.80	0.039
			Rmr	C	+	0.79	0.007
Hainich	07.04.06	SM, K_2SO_4-C, MBC,	Rh	MBC	+	0.95	0.005
			Rmr	SM	+	0.71	0.035
Hainich	11.07.06	SM, K_2SO_4-C, MBC, NH_4^+, NO_3^-	Rt	NH_4^+	+	0.99	0.021
			Rh	K_2SO_4-C	+	0.99	0.015
Hainich	16.10.06	SM, C, N, C:N, K_2SO_4-C, MBC, NH_4^+, NO_3^-	Rt	NH_4^+	+	0.99	0.050
			Rmr	NH_4^+	+	0.99	0.018
Wetzstein	26.07.06	SM, C, N, C:N, K_2SO_4-C, MBC, NH_4^+, NO_3^-	Rt	SM MBC	- +	0.99	0.032
			Rh	MBC	+	0.97	0.002
			Rmr	SM NH_4^+	- +	0.99	0.001
Wetzstein	09.10.06	SM, C, N, C:N, K_2SO_4-C, MBC, NH_4^+, NO_3^-	Rh	NH_4^+ NO_3^-	- +	0.97	0.032

Table 4.4: Significant models of microbial biomass carbon ($P<0.05$) for each site and date. Models were fitted with the soil factors available for each date using stepwise multiple regressions.

Site and Date	Variables Included	Model Variables	Relation	Model R^2	Model P value
Gs 05.04.05	SM, K_2SO_4-C	K_2SO_4-C	+	0.62	0.002
Gs 02.06.05	SM, K_2SO_4-C	K_2SO_4-C	+	0.42	0.002
Gs 11.07.05	SM, C, N, C:N, K_2SO_4-C, NH_4^+, NO_3^-	-			
Ha 29.09.05	SM, C, N, C:N, K_2SO_4-C	N	+	0.53	0.000
Ha 07.04.06	SM, K_2SO_4-C	-			
Ha 11.07.06	SM, K_2SO_4-C, NH_4^+, NO_3^-	SM	+	0.52	0.002
Ha 16.10.06	SM, C, N, C:N, K_2SO_4-C, NH_4^+, NO_3^-	NH_4^+ N	+ +	0.77	0.000
Ws 21.04.06	SM, K_2SO_4-C, NH_4^+, NO_3^-	-			
Ws 26.07.06	SM, C, N, C:N, K_2SO_4-C, NH_4^+, NO_3^-	C SM	+ +	0.75	0.015
Ws 09.10.06	SM, C, N, C:N, K_2SO_4-C, NH_4^+, NO_3^-	K_2SO_4-C SM	+ +	0.85	0.000

4.4. Discussion

Site and Treatment Differences

Large differences between sites observed in several soil properties reflect the different soil types sampled. High C content at Wetzstein define the top layer as an organic horizon. At the same time, the C to N ratio at this site was about twice as high as in the cropland and beech sites. This can be seen as a sign of low soil organic matter quality as the decomposition of carbon compounds is potentially limited by the low availability of nitrogen. This is partly reflected in the MBC/C ratio of each site, which can be seen as an indicator of substrate quality. As an average of all measurements, these values were 12.0±0.5, 13.0±0.4 and 4.8±0.3 mg MBC g C^{-1} for Gebesee, Hainich and Wetzstein, respectively, again revealing poorer conditions at Wetzstein. Differences between sites were also seen in values of mineral nitrogen. Higher values of NO_3^-, as seen in Gebesee, are expected in well oxygenated and moist agricultural soils where nitrification rates are

high. At Wetzstein, low pH values (3.5-4) are expected to inhibit nitrification, explaining the comparatively high NH_4^+ values measured.

The effect of root and mycorrhizal exclusion was smaller than the differences between sites and generally less than the differences between dates. However, several factors were affected. The most obvious difference was in water content, which was lower in the control soil on most dates, clearly affected by the uptake activity of roots. For microbial biomass carbon, higher values were more common in control soils but this trend seemed to decrease or even be reverted on warm and dry dates (Hainich 11.07.06 and Wetzstein 26.07.06). This may indicate a negative effect of water uptake by roots on microbial biomass when soil moisture is limiting. Carbon and nitrogen also seemed to be somewhat affected by root exclusion with a trend towards more C and N in control soils. The exception was again on Hainich 11.07.06, a warm and dry date, although it is not clear how these conditions are lowering carbon amounts in control soils (or increasing them in the mesh cores). Finally, the significant treatment effect on NO_3^- and NH_4^+ inside mesh cores on several dates was a clear indicator of the ion exchange activity of both roots and mycorrhizal hyphae.

Regression Analysis and Variability

Most models yielded high R^2 values but relatively low levels of significance, a problem resulting from having many independent factors but few replicates on each date. Thus, existing relations between factors may not always be statistically significant, explaining the absence of models on many dates.

Partitioning total soil respiration (Rt) into heterotrophic (Rh), rhizosphere (Rr), mycorrhizal hyphae (Rm) and mycorrhizosphere (Rmr) components allowed us to detect the source of observed relations of total soil respiration (Rt) with different soil factor. Mycorrhizosphere respiration (Rmr = Rr + Rm) was used because most soil partitioning studies have used this flux, commonly referred to as the ‘autotrophic component’ and, on the other hand, because it revealed significant relationships in cases where Rr and Rm did not. Rmr was more spatially variable than the microbial flux Rh at both forest sites (Table 2). As a consequence, the total soil respiration is in these cases significantly related to the

same factors as Rmr. This can be seen for C and NH_4^+ at Hainich, and for SM at Wetzstein. At Gebesee (11.07.05), C and K_2SO_4-C were strongly inter-correlated (R^2 = 0.79, $P < 0.01$) so we can assume that Rh, which correlated with C and was more spatially variable than Rmr in this site, is determining the relation of Rt with K_2SO_4-C. These results demonstrate how the variation of the total flux is highly influenced by the larger and most variable component, thus highlighting the necessity of studying the individual processes separately.

Soil Respiration Fluxes and Soil Moisture

A further observation involves the correlation of soil moisture with Rmr. While Rh revealed no spatial relation with soil moisture, Rmr presented a negative relation at Wetzstein, resulting in a net negative relation of Rt with SM. Negative effects are expected at high levels of water content as a result of oxygen depletion. Previous studies have reported negative spatial correlations of soil respiration with soil water and concluded that this was due to lack of oxygen at higher water contents (Soe & Buchmann 2005). Our results suggest that this interpretation may be incorrect. The observed negative correlations can derive from differences in water content between soil locations due to water uptake by roots, which in turn is related to root activity and respiration rates. The idea that the highest moisture values measured did not suppress microbial activity is also supported by only positive correlations between SM and MBC (Table 4). Even at higher water contents, it is likely that enough oxygen is available from coarse primary pores and bio-pores if soils are not irreversibly compacted by heavy machinery or cemented due to soil forming processes (such as carbonate, silicate or iron crusting).

Mycorrhizosphere Respiration and Soil Carbon

As seen in other studies, mycorrhizal hyphal respiration (Rm) is highly dependant on substrate input from roots (Heinemeyer et al. 2007; Moyano et al. 2007) and may be less affected by other soil properties. However, a relation with NH_4^+ (not in results due to low significance: R^2 = 0.98; $P < 0.1$) was found at Wetzstein 21.04.06, possibly reflecting a dependency on nitrogen availability. In addition, Rm correlated negatively with SM,

K_2SO_4-C and C. Negative effects of SM are unlikely on the observed date at Gebesee (08.06.05), which was rather warm and dry, but other factors that could explain this relation were not measured on this occasion. However, correlations between SM and C (soil with higher C contents can retain more water) and between C and K_2SO_4-C are common and may partially explain an indirect relation with SM. Since Rm is not independent and is calculated by subtraction of Rh this could result in negative relations with K_2SO_4-C and C. However, corresponding positive relations with Rh were not found, even at lower significance levels. Given that negative correlations were also observed between Rmr and K_2SO_4-C (Gebesee 02.06.05), the possibility thus remains that root or mycorrhizal exudates are actually lowering the amount of soil carbon as a result of a priming effect. This phenomenon has been successfully modeled (Fontaine et al. 2003; Schimel & Weintraub 2003) and observed in the laboratory or field (Fontaine et al. 2004; Hamer & Marschner 2005; Kuzyakov & Cheng 2001). The dominant role of mycorrhizal hyphae as a source of labile carbon measured by Godbold et al. (2006) supports this hypothesis of mycorrhizal priming. Differences in the quality of dissolved organic matter measured between girdled and control plots have lead to similar conclusions at Wetzstein (Ekberg et al. 2007). This counterintuitive process is still in the area of speculation but should be carefully considered for future studies. In contrast, an increase of soil carbon as a result of root litter input would be the obvious conclusion from the positive relations between root related fluxes and C at Hainich 29.09.05. This effect, however, does not exclude priming.

Heterotrophic Respiration and Microbial Biomass

The microbial respiration flux Rh was positively related to C, C:N, K_2SO_4-C and MBC. Rodeghiero and Cescatti (2005) modeled 86% of inter-site soil respiration variability with C content as the most important factor after temperature. Wang et al. (2003) argued that K_2SO_4-C, which is an indicator of the amount of dissolved organic carbon, correlates better than C content with soil respiration when larger proportions of fresh carbon are present. This may explain the correlation of Rh with C at Gebesee, where there was no litter input since the previous year, and with K_2SO_4-C at Hainich, where the litter layer

was still present. As for C:N, positive relation with this factor may be explained by the commonly higher ratios of fresh litter compared to older soil carbon.

Most studies have found weak or no relations between MBC and soil or microbial respiration, either in the laboratory (Wang et al. 2003) or at the field (Raubuch & Joergensen 2002). Our results in this respect are varied. At Gebesee we find no relation with this parameter while at both forest sites we find a strong relation on just one date. A large proportion of the microbial population can be inactive when conditions are not favorable (Dilly & Munch 1998; Wang et al. 2003). A relation of Rh with MBC may then be expected e.g. when substrates are not limiting, or when microbial biomass size is limited by water availability. MBC correlated strongly with substrate availability indicators (K_2SO_4-C, C, and N) as well as with water availability (SM), but not on every site or date. As mentioned earlier, these factors are often inter-correlated in soils. However, relations between MBC and other soil factors in our analysis did not point towards a consistent connection between Rh, MBC and substrate supply.

Soil Respiration Fluxes and Inorganic Nitrogen

Nitrogen effects were observed on several occasions. Rmr increased with NO_3^- at the crop field and with NH_4^+ at both forest sites. An observed relation of Rmr with NH_4^+ at Hainich 11.07.06 (non-significant: $P < 0.1$) explains the significant correlation of Rt with NH_4^+. Thus, mycorrhizosphere activity was often affected by the availability of nitrogen. Such a relation can be explained by changes in the rates of ion uptake and associated respiration costs and has been observed in several studies (Lambers et al. 1981; Poorter et al. 1995). On the other hand, effects of nitrogen on Rh were observed on only one occasion at the spruce forest. The resulting model showed a negative effect of NH_4^+ and a positive effect of NO_3^-, with the negative effect being stronger (standardized coefficients = -1.37 and 0.73, respectively). As discussed previously, nitrogen may have negative effects on microbial respiration by affecting lignin decomposition (Berg 2000; Fog 1988; Thirukkumaran & Parkinson 2000; Waldrop et al. 2004) or by diverting carbon use towards biomass production (Schimel & Weintraub 2003). In particular, ammonium is known to repress the formation of lignolytic enzymes and may also accelerate the

formation of recalcitrant compounds by reacting with products of lignin degradation (Fog 1988; Keyser et al. 1978). This may be the case at Wetzstein, given that only ammonium shows a negative effect. On the other hand, MBC was not related to NH_4^+ at this site, supporting the view taken by Agren et al. (2001) who explain the negative effects of nitrogen on respiration through shifts in the microbial community towards more efficient decomposers. Considering the increase in N availability after root and mycorrhizal exclusion in our soil treatments, an effect on the microbial community is very likely. Finally, the effects of ammonium described above may differ among sites. At Hainich, negative but non-significant trends of Rh with NH_4^+ were seen on two occasions (results not shown) while at the same time we see a positive relation of MBC with NH_4^+ (Hainich 16.10.06; Table 4). These results are in good agreement with the idea of changes in C use from surplus respiration towards biomass production. At Gebesee, however, no such relations were found. It is thus difficult to determine whether a real difference with respect to nitrogen effects exists between the three study sites. However, a dependency on litter quality, described by Arnebrant et al. (1996), may be related to the significant relation found at the less fertile spruce forest.

4.5. Conclusions

The present analysis of the effects of spatially variable soil factors on partitioned soil respiration fluxes revealed a dependency of specific relations on the source of respiration, although with limited connections to site differences and climatic conditions. Field measurements in combination with root and mycorrhizal exclusion were useful for inter and intra-site comparisons in near-natural conditions. As a drawback, large variability and inter-correlation between factors has limited the interpretation of the results, with the consequent difficulty in identifying consistent relations between climate, soil characteristics and respiration fluxes. In spite of this, our analysis reinforces or reconsiders theoretical relations that have been only partially explored in natural conditions.

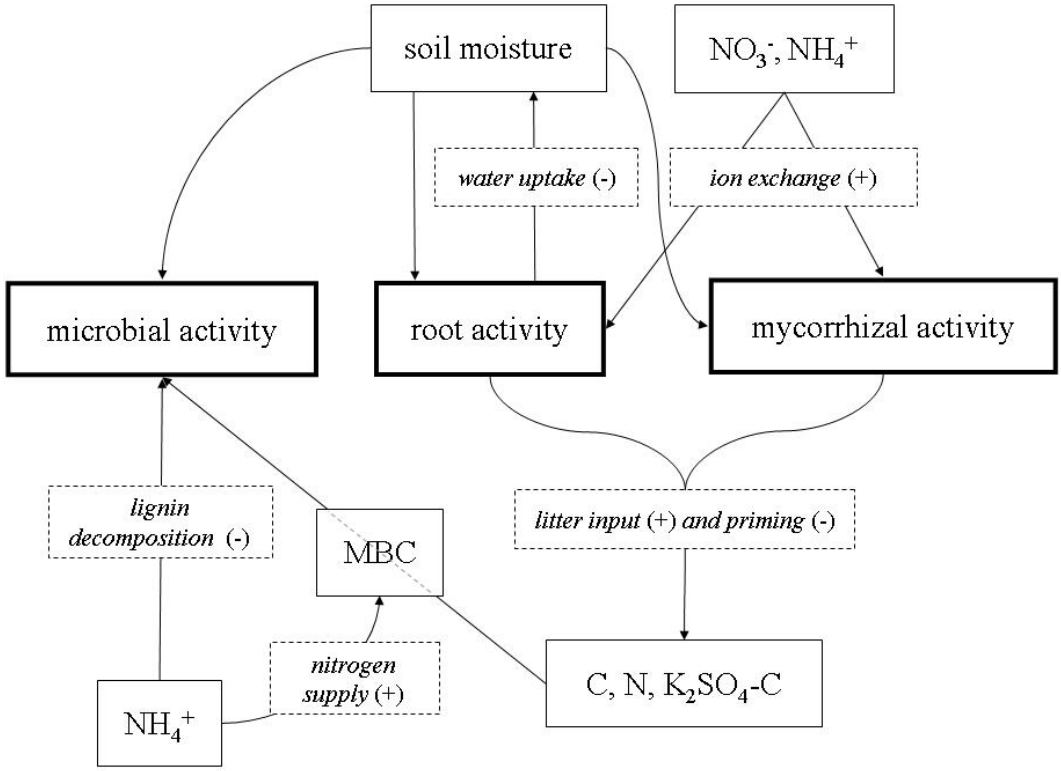

Figure 4.3: Schematic representation of potential relations involving the soil factors measured in this study with the activity of soil microbes, roots and mycorrhizal hyphae. Processes are in italic with a plus or minus sign indicating positive or negative effects.

Figure 4.3 gives a schematic representation of the processes discussed in this study. These include the positive effect of inorganic nitrogen on mycorrhizosphere respiration, the negative effects of ammonium on decomposition, relations between water content and different respiration sources, and relations between substrate availability, priming and microbial activity. Although we observed the presence of such relations on specific dates and sites, we could only hypothesize their relative importance under different ecosystem and climatic conditions. As uncertainties in climate predictions create the pressing need for more realistic models of the soil system, further studies of this kind should lead to a better understanding of the key processes in natural ecosystems.

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5. Respiration from Roots and the Mycorrhizosphere: Influencing Factors and Measurement Methods

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5.1. Introduction

The largest flux in the global carbon cycle is the uptake of CO_2 by plants as photosynthesis. Estimates of gross primary production (GPP) or total amount of CO_2 assimilated by terrestrial plants range between ca. 109 and 120 Pg C $year^{-1}$ at the global scale (Schlesinger 1997; Zhao et al. 2005). Except for carbon that remains stored in passive organic matter pools, as fossil fuel, and an estimated 0,2 Pg C $year^{-1}$ sediments in the ocean floors, assimilated carbon is eventually returned to the atmosphere by respiration, either by plants or by heterotrophic organisms. The time between the fixation of a carbon atom by the plant and its conversion back to CO_2 is extremely variable, ranging between a few hours and thousands of years. How long it remains part of organic compounds will depend on its turnover within the plant and, eventually, as part of soil organic matter.

Carbon assimilated by plants is translocated to plant organs where it can be used as building material for structural biomass, for storage, or as substrate for respiration. Carbon imported into roots can also be exudated or transferred to symbionts such as mycorrhizal fungi (Farrar 1999). The amount of assimilated carbon used for each purpose will depend on the plant's requirements, which are further determined by plant and environmental factors. Different studies on a number of plant species have shown the amount of carbon respired to be, on average, 50% of the carbon assimilated through photosynthesis per unit time, with numbers ranging between 35 and 80% (Lambers 1985;

Amthor 2000b). Other studies have shown that, on average, nearly half of the carbohydrates from photosynthesis are translocated to roots, where around 40% of this carbon is used for respiration (Lambers 1987; Farrar and Williams 1990). However, these proportions can vary. Amounts of carbon respired by roots have been seen to range between 8 and 52 percent of total fixed CO_2 (Lambers et al. 1996; Atkin et al. 2000a). Carbon exported to underground plant organs and respired in the soil environment – either by the plant itself or by other organisms – contributes to soil respiration. The factors controlling the respiration of this root derived carbon and the techniques for measuring these respiration processes are the focus of this chapter.

Part of the carbon allocated belowground is not utilized for biosynthesis or as substrate for respiration by the plant but is used by organisms associated with plant roots in the rhizosphere and the mycorrhizosphere. The *rhizosphere* is usually defined as the region of soil influenced by the root system, i.e. where the microbial population is affected by nutrient uptake and release of compounds by the root, (Russell 1982; Paul and Clark 1989). This region of influence is typically some millimetres to centimetres wide, with boundaries that can change depending on root structure, presence of root hairs and soil and plant-microbe characteristics. The release of exudates, secretions and root residues (e.g. sloughed off cells and fine roots) by the root into the rhizosphere soil is known as *rhizodeposition* (Kuzyakov and Domanski 2000; Nguyen 2003). Rhizodeposition results in a very high concentration of microorganisms in the rhizosphere as compared to the soil not directly influenced by the root (Grayston et al. 1997). The *mycorrhizosphere* extends much further in the soil than the rhizosphere, as it includes the mycorrhizal hyphal system and its zone of influence, including parts of the litter layer and very fine soil pores inaccessible to roots.

Respiration from plant roots and associated microorganisms comprise major sources to CO_2 efflux from soils. Root respiration was early recognized as an important fraction of soil CO_2 efflux that in some cases amounts to more than the fraction of CO_2 produced by decomposition of soil organic matter. Wiant (1967) defined *root respiration* as “all soil respiration derived from organic compounds originating from plants including the respiration of living root tissue, the respiration of symbiotic mycorrhizal fungi and associated microorganisms, and the decomposer organisms operating on root exudates

and recent dead root tissue in the rhizosphere". This broad definition results from the fact that the mentioned components are all linked to the direct supply of carbohydrates from the plant through its roots, as opposed to respiration coming from decomposition of soil organic matter in litter and bulk soil.

Although widely used as defined by Wiant, the term *root respiration* is now being used in a more strict sense, referring only to the respiration of the live root tissue. The terms *autotrophic soil respiration* and *assimilate fed soil respiration* have been used to include all respiration of roots and root derived compounds, although they are usually not strict and can lead to confusion. Additional terms describing processes included in Wiant's definition, among others, are *rhizomicrobial respiration*, *rhizosphere respiration*, *mycorrhizal respiration* and *mycorrhizosphere respiration*. These terms are sometimes used differently by different authors. For example, *rhizomicrobial respiration* is defined by Dilly et al. (2000) and Kutsch et al. (2001b) as respiration of both roots and associated microorganisms, while for Kuzyakov and Cheng (2001) and Nguyen (2003) the term only includes respiration of the latter. One should also be cautious with the terms *soil autotrophic* and *root respiration* which in literature have been widely used without distinguishing between respiration by roots alone and respiration by associated microorganisms. To avoid confusion and on logical grounds, *soil autotrophic respiration* should refer strictly to respiration from plants (i.e. roots) only. The following are the definitions that we think are more useful and that will be used throughout this chapter (Figure 5.1):

- **Root respiration**: respiration of the living root tissue (ie. excluding symbionts such as mycorrhizal hyphae).
- **Rhizomicrobial respiration**: respiration of rhizodeposits and plant assimilate supplies by microorganisms in the rhizosphere, not including mycorrhiza.
- **Rhizosphere respiration**: root plus rhizomicrobial respiration.
- **Mycorrhizal respiration**: respiration of mycorrhizal fungi (i.e. including carbohydrates derived from plant roots).
- **Mycorrhizosphere respiration**: rhizosphere plus mycorrhizal respiration.

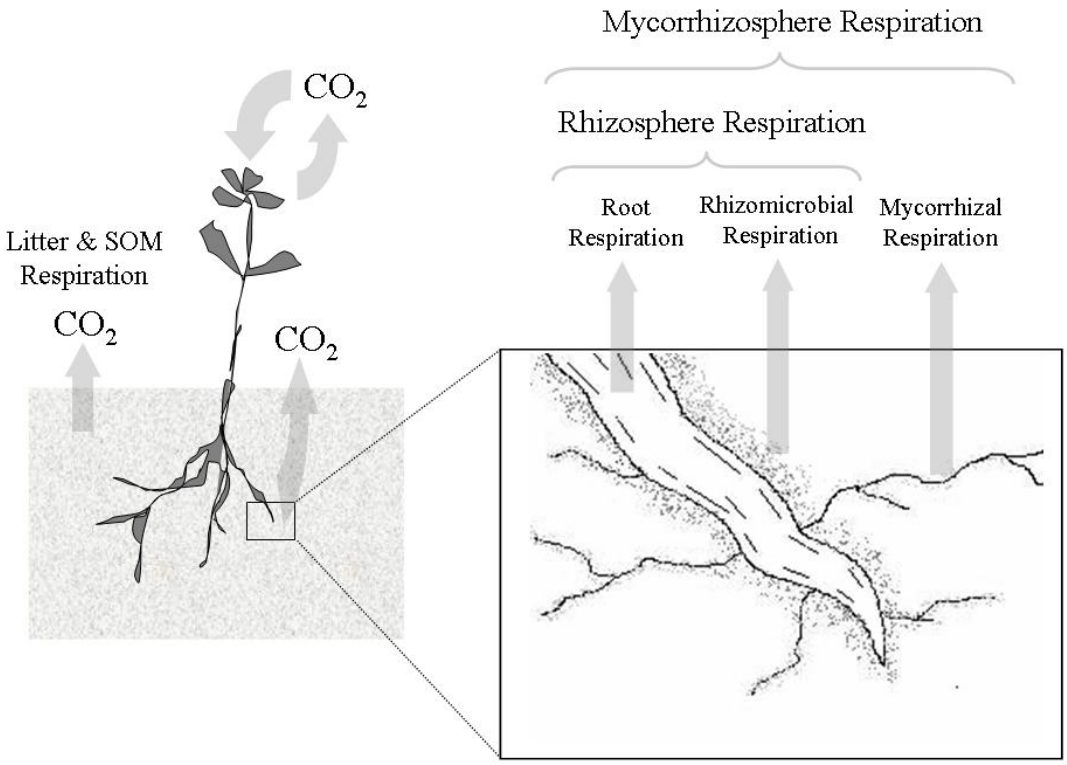

Figure 5.1: Diagram showing the sources of root derived carbon respiration - respiration from the living root tissue, respiration of rhizodeposits by microorganisms in the rhizosphere, and respiration from mycorrhizal hyphae - and their grouping into rhizosphere and mycorrhizosphere respiration. Litter and soil organic matter (SOM) respiration are the carbon fluxes not deriving from live roots.

In the following section, we will start with general aspects of root/mycorrhizosphere respiration and continue with the most important factors affecting it. In section 5.3 we will discuss methods used for measuring root/mycorrhizosphere respiration in the field and the laboratory, along with methods for determining temperature response and acclimation of root respiration. Section 5.4 presents methods for determining root biomass and turnover as well as other means for estimating root/mycorrhizosphere respiration at the ecosystem scale. Concluding remarks are given in section 5.5.

5.2. Root and Mycorrhizosphere Respiration

5.2.1. Eco-Physiology of Root Respiration

Respiration is the major loss of C in roots where it is required for a number of processes, including synthesis of biomass, translocation of photosynthates, uptake of ions from the soil, assimilation of N (including N_2-fixation) and S into organic compounds, protein turnover, and cellular ion-gradient maintenance (Amthor 2000b). Respiration can also be a result of so called wastage processes (e.g. futile cycles and mitochondrial electron transport uncoupled from oxidative phosphorylation) that only result in CO_2 and heat production. In the short term, and as a consequence of the mentioned processes, respiration is regulated by the availability of ADP and NADP, so that it responds to the demand for respiratory energy. The respiration of root derived carbon can range between 8 and 52% of the CO_2 fixed by photosynthesis, while most estimates give values of around 20% (Lambers et al. 1996; Atkin et al. 2000a). This relationship will depend on factors such as plant age, species and presence of mycorrhiza.

Theoretical models are used to explain relations between respiration and underlying processes (Amthor 2000b). A simplified approach is to divide respiration into growth and maintenance processes, a distinction which can be useful for understanding factors controlling respiration (Sprugel and Benecke 1991; Amthor 1994; Sprugel et al. 1995). *Growth respiration* is defined as the carbon costs of newly constructed tissue. The cost of producing a certain amount of new tissue (gram C respired per gram C of newly produced tissue) is unaffected by temperature (Penning de Vries et al. 1974). *Maintenance respiration* by contrast is the basal cost for maintaining established tissue (gram C respired per gram tissue C within a certain time) and is highly sensitive to temperature, as well as being positively related to plant N content (de Wit et al. 1970).

5.2.2. Regulation of Root Respiration by Plant and Environmental Factors

Many environmental, abiotic and biotic, as well as physio-morphological factors are involved in determining the short and long term rates of mycorrhizosphere respiration. The most relevant factors are listed in this section with a summary of their effects on root respiration. Both diurnal and seasonal changes may be potentially related, among others, to changes in temperature, soil moisture, nutrient demand/supply, assimilate supply and plant phenology. These factors can likewise be important for mycorrhizal and rhizomicrobial respiration. However, studies measuring the specific response of rhizomicrobial respiration are lacking, and those focusing on the mycorrhizal component are few and will be discussed separately in section 5.2.3. It should be noted, however, that although the present section deals with the response of root respiration many of the studies referred to may have included, at least partially, the rhizomicrobial and mycorrhizal components.

Temperature

Temperature is very often a limiting factor for root activity especially in the temperate and cold climates, accounting for much of the seasonal variation in root respiration (Figure 5.2). In general, CO_2 efflux is reduced to the level of maintenance respiration during winter (Linder and Troeng 1981; Benecke 1985; Wieser and Bahn 2004) and is highest during the growing season as a result of cell growth and higher temperatures increasing maintenance costs (Figure 5.2). Temperature changes result in an immediate change in respiration rates by a direct effect on enzyme activity and can also affect respiration by influencing other factors such as water availability and movement of nutrients. High temperatures – typically above 50^{o} C – can lead to disintegration of enzyme complexes, structural injury or cell death, thus lowering respiration rates (Palta and Nobel 1989b). In the longer term, plant thermal acclimation (see below) can play an important role in the temperature-respiration relationship.

The short-term temperature sensitivity (typically determined on diurnal scale) of root respiration depends on the occurring temperature range (Atkin et al. 2000a; Tjoelker et al. 2001; Atkin et al. 2005a), and often, at least partially, on the prevailing temperature of the previous days. Q_{10} values of plant respiration are often close to 2, but temperature sensitivity varies considerably between and within plant species (Atkin et al. 2000a) and Q_{10} values for fine roots tend to be slightly higher than 2 (Burton et al. 2002).

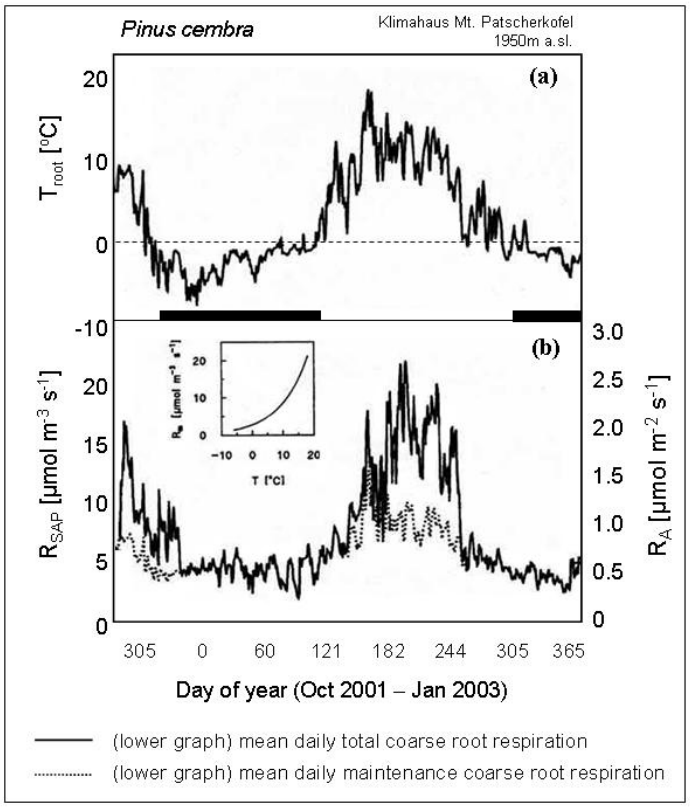

Figure 5.2: Seasonal course of mean daily root cambium temperature (T_{root}) in upper graph (a) and the corresponding mean daily total (solid line) and maintenance coarse root respiration (dotted line) per unit sapwood volume (R_{SAP}) and per unit root surface area (R_A) in the lower graph (b) of an adult Pinus cembra tree at Mt. Patscherkofel, Austria during the period 7 October 2001 to 21 January 2003. Total coarse root respiration was obtained from continuous measurements of an automated gas exchange system. Solid bars indicate the period of winter snow cover (≈ dormant period). The inner panel shows the temperature response of maintenance respiration (Rm) obtained from measurements conducted during the dormant season (temperature range -6 to 2.5°C; Rm = 2.81 exp[ln 3.10(T/19)]; R^2=0.72) extrapolated for the obtained annual temperature range (modified after Wieser and Bahn 2004).

When comparing published root Q_{10} values, it is important to consider the different growth conditions and range of measurement temperatures used in the different studies. Moreover, the nature of the tissue used in different studies must be considered. In most studies, measurements of respiration in roots are made using whole root systems (e.g., Smakman and Hofstra 1982; Bouma et al. 1997; Covey-Crump et al. 2002; Loveys et al. 2003) or root segments of differing age/function (e.g., Higgins and Spomer 1976; Crawford and Palin 1981; Sowell and Spomer 1986; Weger and Guy 1991; Zogg et al. 1996; Pregitzer et al. 1997; Pregitzer et al. 1998; Burton et al. 2002). In whole root systems, the estimates of Q_{10} will depend on the proportion of immature and mature roots and the Q_{10} of each developmental stage. Q_{10} values of 1.5 for coarse woody roots and 2.0 for fine roots (< 2 mm diameter) were reported in a *Pinus radiata* stand (Ryan et al. 1996). Comparisons of root Q_{10} values should ideally be made on tissues of defined developmental age.

The Q_{10} of plant respiration has been shown to change in response to different growth temperatures (Tjoelker et al. 1999; Atkin et al. 2000a; Loveys et al. 2003; Atkin et al. 2005a), an effect called thermal acclimation. *Thermal acclimation* of respiration is commonly defined as a change in the shape of the short-term temperature-response curve of respiratory CO_2-release and/or O_2-uptake, in response to a change in growth temperature. Acclimation can be caused by short term temperature changes, i.e. a few days, or long term temperature changes. In the latter case, thermal acclimation occurs more likely in new tissues produced following a change in the temperature regime, and thus a greater degree of acclimation might be expected in plants with rapid rates of root replacement rather than those with long-lived fine roots (Atkin and Tjoelker 2003).

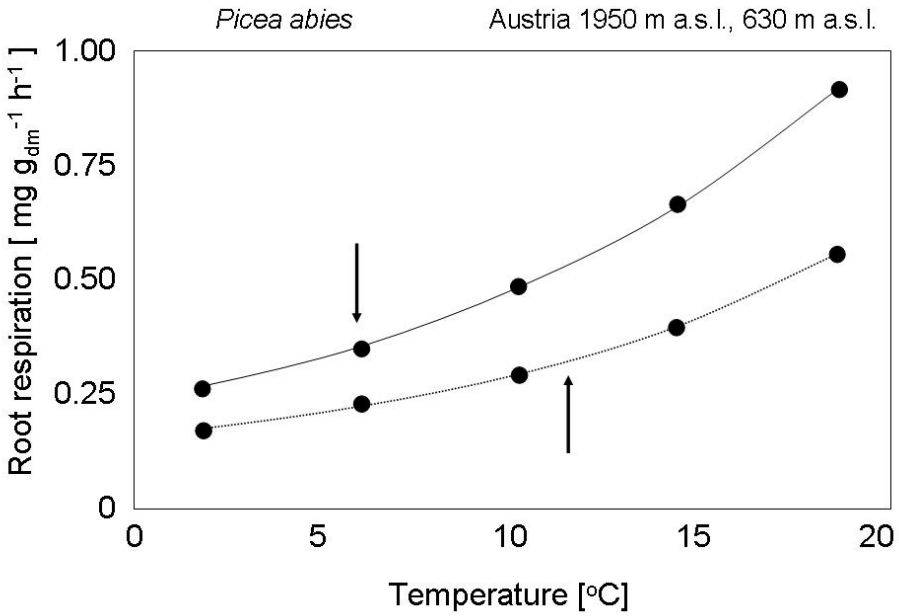

Figure 5.3: The relationship between temperature and root respiration of Norway spruce (Picea abies) seedlings grown at 1950 (dotted line) and 630 (full line) m a.s.l.. Measurements were made in laboratory conditions using excavated roots detached from the trees. Note that when measured at the prevailing temperature of each site, as indicated by arrows, no significant difference in respiration was detected. (Redrawn after data from Framba 1980).

A study on Norway spruce in a high and a low elevation site in the central Austrian Alps has shown that at the same measurement temperature respiratory CO_2 losses of roots are higher in trees from cold climates than in trees from warm climates. However, when root respiration is compared at its actual thermal site conditions (an ecological context) roots from colder high elevation sites did not exhibit a higher respiration rate when compared to the warmer low elevation sites (Figure 5.3). This would indicate the effects of acclimation, although genetic variation along elevational ecotypes may also contribute to the observed differences.

Temperature response, the use of $Q_{10,}$ and acclimation will be discussed further in sections 5.3.4 and 5.3.5.

Moisture

Soil moisture can become a limiting factor for root respiration, especially in arid environments but also in wetlands and humid temperature zones, especially in summer months in the top organic layers. Soil water potential in the rhizosphere, as determined by water content and soil physical properties, has influence on the growth and functioning of the root. Various studies relating soil respiration to soil water content show that the optimum for soil respiration is normally found at intermediate values of water content (Davidson et al. 2000). This is true for both autotrophic and heterotrophic respiration, although the response to water stress of roots may be different from that of soil microbes. The availability of soluble soil substrates as a function of water content is of less importance to roots than for soil microbes. Further, roots may have adaptations to resist water stress, such as extracting water from deeper soil profiles.

Root respiration is reduced during drought (Bryla et al. 1997; Burton et al. 1998), the effect being more pronounced in warmer than in cooler soils (Bryla et al. 2001). A result of soil drying is reduced root water content, with a subsequent reduction in root cell turgor pressure. Since turgor pressure is necessary for metabolic activity, this will lead to a reduction of growth and respiration rates. Soil drying can at some point lead to cell death and subsequent root respiration reductions. Resistance to drought varies among species and even among different roots from a same individual (Palta and Nobel 1989a). On the other hand, excess water in soils also will reduce root growth and function, mainly by decreasing the availability of oxygen.

Apparently no data are available on the effects of soil moisture on respiration of large coarse roots of field grown trees, although there is evidence that at low soil water availability coarse root respiration declines as shown for excised *Pinus teada* roots by Maier and Kress (2000).

Nutrients

Plant available mineral nutrients as well as nutrient content in plant tissues influence root respiration. Plants grown at a high supply of nutrients have higher specific root

respiration rates than plants grown at lower nutrient supplies (Lambers et al. 1981; Kuiper 1983). Additionally, root respiration at a reference temperature may be higher during periods of high nutrient demand, e.g. in late spring/early summer when the vegetation grows fast (Bahn et al. 2006). Poorter et al. (1995) report that specific root respiration decreases with decreasing nitrate supply which may vary from day to day, but especially on a seasonal scale. These relationships are presumably explained by differences in the rates of ion uptake. Higher uptake rates mean higher costs, resulting in higher respiration rates. On the other hand, increased nutrient availability can lead in the longer term to decreases in fine root biomass, with a resulting decrease in C allocation to fine root and respiration (Keyes and Grier 1981).

Nitrogen content in plant tissues also correlates with respiration rates. Ryan et al. (1996) and Burton et al. (2002) found strong correlations between fine root N content and respiration rates (Figure 5.4). Given that most nitrogen in plant cells is in protein, which needs replacement and repair and is closely linked to cell activity, the increase in respiration with higher N content is largely due to an increase in maintenance respiration.

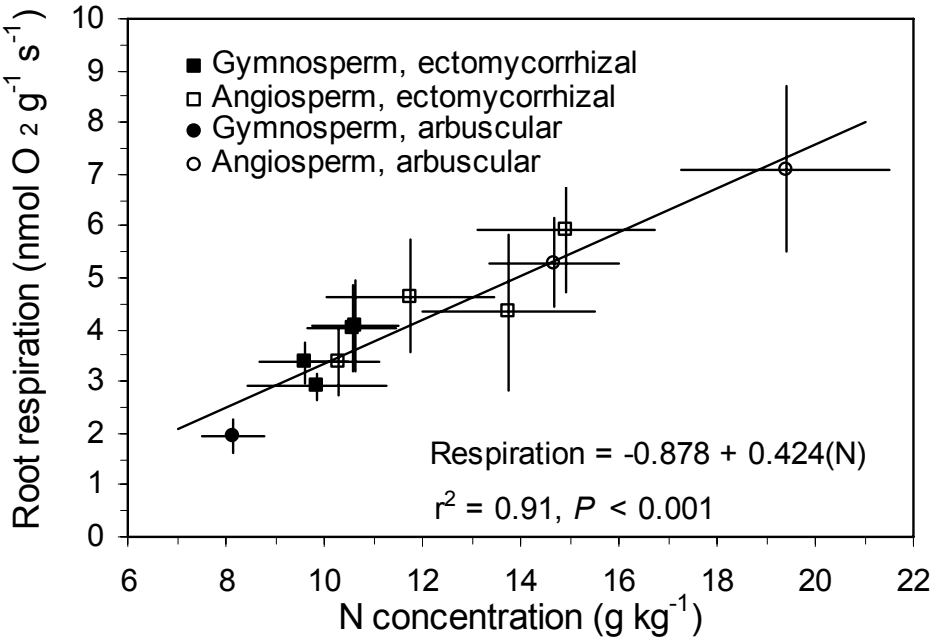

Figure 5.4: Relationship between fine root N concentration and fine root respiration at 18° C for species from ten North American forest types. Error bars indicate one standard error of the mean (Burton et al. 2002).

Insolation and Carbohydrate Supply

Carbohydrates required for root respiration are provided by the import of newly assimilated carbon, by the use of carbon stored in the plant, or even by uptake of organic molecules from the soil (Lambers 1987; Farrar and Williams 1990). Effects of changes in assimilate supply on root respiration vary with plant age and between species, with some plants being more and others less constrained (Gunn and Farrar 1999). Root metabolism can be closely related to the transport of carbohydrates from the shoots to the roots or buffered by storage, as shown in a study by Farrar (1999), where roots maintained their respiration rate for several days after plants were kept in the dark, losing their metabolic capacity only after 6 days. Respiration rates in source leaves, on the other hand, fell below normal rates within 1-2 days of darkening.

Newly assimilated carbon can reach roots and be respired in short periods of time. Even though plant respiration is well correlated with daytime photosynthesis and with tissue concentrations of soluble carbohydrates for plants in the lab (Amthor 1994; Lambers et al. 1996), this relationship is depending on the life form under field conditions (Atkin et al. 2000b; McCutchan and Monson 2001; Vose and Ryan 2002). In a field study using ^{14}C in poplar trees, Horwath et al. (1994) calculated a delay of 2 days for the respiration of newly fixed C to peak in the mycorrhizosphere. Similarly, Ekblad and Högberg (2001) calculated a delay of 1-4 days and determined a 65% contribution of new photosynthates to total soil respiration by using ^{13}C measurements in a boreal coniferous forest, whereas Kuzyakov and Cheng (2001; 2004) observed diurnal dynamics of CO_2 soil efflux responding to photosynthetic activity in wheat and maize plants.

Insolation can be an important factor explaining respiration rates in smaller plants, such as grasses and tree seedlings (Fitter et al. 1998; Lipp and Andersen 2003), whose roots cannot utilize large stored C reserves, such as those available in large woody plants (Phillips and Fahey 2005). In *Picea abies* and *Pinus cembra* (Eccher 1972; Framba 1980) seedlings root respiration was highest when shoots were illuminated, strongly suggesting that root respiration activity is directly dependent on the supply of photosynthates from the foliage (Amthor 1994). Some studies have shown cumulative radiation flux of the previous days being positively related to root respiration rates in grasslands (Fitter et al.

1998), while others observed negative (Edwards et al. 2004) or no consistent relationships (Bahn et al. 2006).

Thus, insolation, as a measure of carbohydrate supply, can be a short term factor regulating root respiration, with effects depending on the plant's carbon storage and demands.

Soil and Atmospheric CO_2 Concentrations

Belowground tissues (e.g. roots and tubers) are often exposed to [CO_2] far higher than that found in the atmosphere, with values ranging between a few hundred to more than ten thousand μmol mol^{-1} and generally increasing with soil depth (Cramer and Richards 1999; Sands et al. 2000). Direct inhibition of respiration by high [CO_2] has been reported in roots of some species (Qi et al. 1994; Burton et al. 1997; van der Westhuizen and Cramer 1998; McDowell et al. 1999), while other studies have found little or no inhibition of root respiration by elevated soil [CO_2] (Bouma et al. 1997; Burton and Pregitzer 2002). Acclimation of belowground tissue may be expected to occur to much higher [CO_2] than that of leaves; however the fluctuations of soil [CO_2] through the growing season may be high (Sands et al. 2000) as well as those within days (Bouma and Bryla 2000). Moreover, the mechanisms by which significant inhibition might occur have not been elucidated (Gonzàlez-Meler and Siedow 1999). It has been noticed that artefacts in measurements such as leakage problems and gas analyser cross-sensitivity to water vapour can lead to false results in respiration rates (Burton and Pregitzer 2002). Thus, many of the previous reports of large effects of soil [CO_2] on plant tissue respiration may be a result of such artefacts (Amthor 2000a).

Contrary to belowground [CO_2], elevated atmospheric [CO_2] can have a positive effect on root respiration by stimulating photosynthesis and reducing water requirements (Pendall et al. 2004). In addition, higher [CO_2] have been seen to increase fine root biomass and to decrease their N content (Zak et al. 2000). Atmospheric CO_2 concentrations can therefore affect root respiration through a number of factors, generally having a net positive effect which will depend on the plant species and stage of development (King et al. 2004).

Root Morphology and Plant Age

Fine roots, as opposed to *coarse roots*, are generally defined as all roots having a diameter of less than 2 mm (some definitions set limits of 5 mm or higher). Fine roots are metabolically more active and present higher specific respiration and turnover rates than coarse roots. Specific root respiration and its Q_{10} decrease with increasing root diameter (Ryan et al. 1996; Pregitzer et al. 1998; Bahn et al. 2006), decreasing specific root length (Tjoelker et al. 2005) or the ratio of long to fine roots (Kutsch et al. 2001b). Such effects may be partly related to a decrease in N concentration (Ryan et al. 1996; Pregitzer et al. 1998; Bahn et al. 2006) and an increase in tissue and plant age (Palta and Nobel 1989b; George et al. 2003; Volder et al. 2004).

Seasonal changes in ecosystem root respiration are strongly influenced by changes in root biomass (Bahn et al. 2006). Also, the proportion of roots of different diameter may vary in the course of the season and thus affect ecosystem root respiration. Within a plant or species, young fine roots have been noted to have much higher respiration rates than older roots, presumably due to higher growth respiration and their primary role for nutrient absorption (Bouma et al. 2001; Fahey and Yavitt 2005).

Plant development affects root respiratory activity and as trees become older carbon allocation patterns will change, and thus will be reflected in the respiratory demand of tissues. For example, Framba (1980) found that in both *Picea abies* and *Pinus cembra* specific root respiration rates of 4-year old seedlings was higher than in 22-year old trees.

5.2.3. Rhizomicrobial and Mycorrhizal Respiration

Rhizomicrobial Respiration

The rhizosphere is an environment that differs largely from the surrounding soil. Differences can be seen in the amounts of microbes, inorganic ions and organic material, as well as in pH values and concentrations of oxygen and CO_2 (Lambers et al. 1991). It is also a dynamic environment that is influenced by the amounts and quality of rhizodeposits and on soil characteristics. Reviews on rhizodeposition and rhizosphere

carbon dynamics are given by Grayston et al. (1997), Kuzyakov and Domanski (2000), Toal et al. (2000) and Nguyen (2003). Even though rhizodeposits represent a loss of reduced carbon, the plant benefits from higher nutrient availability resulting from the increased biological activity around the root. Uren (2000) suggests that the amount of root exudates produced varies with plant species, plant age, and substrate and stress factors. Between 5-21% of plant photosynthate was seen to be released as root exudates in studies with cereal (Haller and Stolp 1985; Flores et al. 1996).

Isotope methods have been used for estimating carbon fluxes originating from rhizomicrobial respiration. Results from a number of studies differ largely, with estimates of rhizomicrobial respiration ranging from 5% to 60% of rhizosphere respiration (Kuzyakov and Domanski 2000). Kuzyakov (2002) compared the *isotope dilution method* (Cheng et al. 1993), the *model rhizodeposition method* (Swinnen 1994), the $^{14}CO_2$ *dynamics method* (Kuzyakov et al. 1999), and the *exudates elution method* (Kuzyakov and Siniakina 2001) by applying each under the same experimental conditions, and concluded that rhizomicrobial respiration represents between 50% and 60% of rhizosphere respiration in *Lolium perenne*. These methods combine the use of isotopes with pulse labelling, sampling of respired CO_2, elusion of exudates, and modelling of carbon dynamics. Although the use of isotopes seems to be a promising path for studying microbes in the rhizosphere, uncertainties for these methods remain large (results ranging from approx. 40 - 80%) and developments in rhizosphere respiration partitioning are still needed.

Rising concentrations of atmospheric CO_2 are expected to increase rhizomicrobial respiration in response to enhanced rhizodeposition (Paterson et al. 1997; Zak et al. 2000). These changes in microbial activity in the rhizosphere could in turn affect plant growth or have a priming effect on the decomposition of soil organic matter (Pendall et al. 2004).

Mycorrhizal Respiration

Mycorrhizas are root-fungal systems where plant and fungus exchange carbon for nutrients, respectively, through extensive physical connexions. Although mycorrhizas

include roots as well as fungi, the term *mycorrhizal respiration* refers henceforth by convention only to the fungal respiration component.

Mycorrhizal fungi grow both inside roots and in the soil as a potentially far reaching extra-radical mycelium (ERM). Given the close relationship with roots and the difficulty involved in isolating the fungal mycelium, it is not surprising that the fungal component has been usually ignored or treated as an extension of the plant root when looking at sources of respired carbon. In part, this is a result of the methods used, most of which cannot distinguish between these sources. However, the importance of the mycorrhizal mycelium as a source of respiration depending on plant substrates was already observed over two decades ago (Paul and Kucey 1981; Kucey and Paul 1982; Soderstrom and Read 1987). It is thus surprising that most reviews on C flux from roots to the soil (see references in the previous section) have failed to recognize the importance of mycorrhizal fungi.

There is more than one reason to study the flow of carbon through mycorrhizal fungi, and in particular the ERM, separately from roots: (i) EM mycelia can reach 8,000 m per metre length of root (Leake et al. 2004), and make up one third of soil microbial biomass in coniferous forests (Högberg and Högberg 2002). (ii) A large fraction of the carbon trans-located belowground is allocated to ERM. Estimates range between 2% and 20% for arbuscular mycorrhizas (AM) and between 7% and 30% for ectomycorrhiza (EM) (Leake et al. 2004). Much of this is respired by the ERM itself, but another large part (e.g. cell wall components such as chitin and glomalin) might enter recalcitrant soil organic matter (Staddon 2005; Godbold et al. 2006). (iii) The mycorrhizal status can up-regulate net photosynthesis rates (Smith and Read 1997), consequently increasing the amount of carbon exported to the root system. (iv) Further, the response of mycorrhizal fungi and roots to environmental factors may differ. Although mycorrhizal C fluxes can be expected to be a major contributor to soil respiration, surprisingly little is known of how it responds to environmental changes.

Difficulties in separating root from mycorrhizal respiration have resulted in mycorrhizal fungi being included in the autotrophic flux component. Consequently, root respiration *sensu stricto* has been overestimated (Pendall et al. 2004). On the other hand using

respiration rates of excised (mycorrhizal-deprived) roots, the ignored ERM respiration component might explain why scaled-up respiration measurements show low estimates of mycorrhizosphere respiration when compared to girdling (Högberg et al. 2001), or trenching experiments which include the ERM component (Simard et al. 1997; Hogberg et al. 2002).

EM fungi seem to be a larger soil respiration component than AM fungi. Phillips and Fahey (2005) estimated around 7% and 12% mycorrhizal respiration for sugar maple (AM) and yellow birch (EM), respectively and in general it is estimated that up to 20% of net photosynthesis can be allocated to the mycorrhizal mycelium (Smith and Read 1997). For AMs so far only few studies provide fungal ERM respiration: around 1% of net-photosynthesis for *Plantago lanceolata* (Heinemeyer and Fitter 2006), similar to an estimated value of 0.8% given by Jakobsen & Rosendahl (1990), but lower than a 4.8% for winter barley from Moyano *et al.* (in press).

Until recently not much emphasis has been put on investigating the mycorrhizal response per se, i.e. on a separated mycelium, based on, for example, mesh exclusion. Heinemeyer & Fitter (2004) demonstrated with this technique for AM fungi that, even if the ERM responded positively to temperature this was due to changes in specific root length of the plant, although only the fungus experienced a temperature treatment. A recent laboratory study by Heinemeyer & Fitter (2006) combined stable isotope pulse labelling ($^{13}CO_2$) on *Plantago lanceolata* plants with warming of a separated AM mycelium. They found that mean ERM respiration was unaffected by warming the mycelium by 6°C ± 3°C but strongly covaried with PAR received during the 12 hours before gas sampling (Figure 5.5). Such fast carbon turnover was also observed by mesh separation in a grassland site (Johnson et al. 2002a; Johnson et al. 2002b). However, ERM growth responded positively to warming and increased by 75% during two weeks of warming.

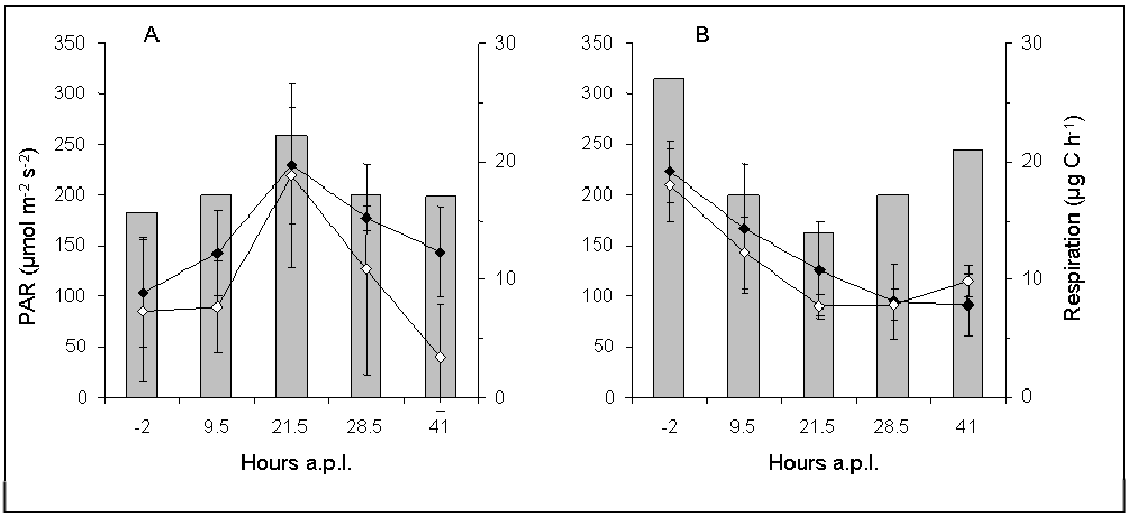

Figure 5.5: Mean total ERM respiration in ambient (open diamonds) and heated (filled diamonds) fungal compartments (as the difference of mycorrhizal and non-mycorrhizal compartments) for the first (A) and second $^{13}CO_2$ pulse label (B) during the 41 hours after pulse labelling (a.p.l.) ± SE (standard error) and corresponding mean PAR (bars) received during a 12 hours period before each measurement. There were no significant differences between temperature treatments for either pulse, however, respiration covaried significantly with PAR: (A) $F_{1,7} = 13.81$, P = 0.007; (B) $F_{1,7} = 6.71$, $P = 0.037$ (redrawn from Heinemeyer & Fitter 2006).

Recent but yet unpublished work by Heinemeyer *et al.* used continuous monitoring of soil respiration (a multiplexed 12 closed dynamic chamber system) at mycorrhizal mesh collars to explicitly determine the rate of EM respiration in the field, its environmental control and contribution to overall soil respiration. Already 38 days after collar insertion in May, the mean respiration rates from the collars with mycorrhizal in-growth had significantly increased ($P < 0.05$) relative to those without mycorrhizal in-growth (1.82 *vs.* 1.49 µmol m^{-2} s^{-1}; all unpublished data). These results indicate a rapid colonisation of the collars by EMs, supporting published EM mycelia growth rates of up to 10 mm per day (Leake et al. 2004). This experiment revealed proportional contributions of ~65% from soil, 25% from EMs and 10% from roots (calculated as a percentage of the mean rate at the shallow collars). This EM flux contribution to total soil respiration is strikingly similar to a laboratory ^{14}C based estimate of EM hyphal respiration of ~20% by Rygiewicz & Andersen (1994).

Most importantly, despite a strong exponential relationship (Q_{10}) between soil temperature (at 5 cm) and respiration in the collars excluding ($R^2 = 0.88$) or including ($R^2 = 0.68$) EM hyphae, EM respiration (the difference of the latter two) was almost

unaffected by soil temperature (Figure 5.6). This contradicts a recent suggestion that soil and rhizosphere organisms have the same Q_{10} (Baath & Wallander 2003).

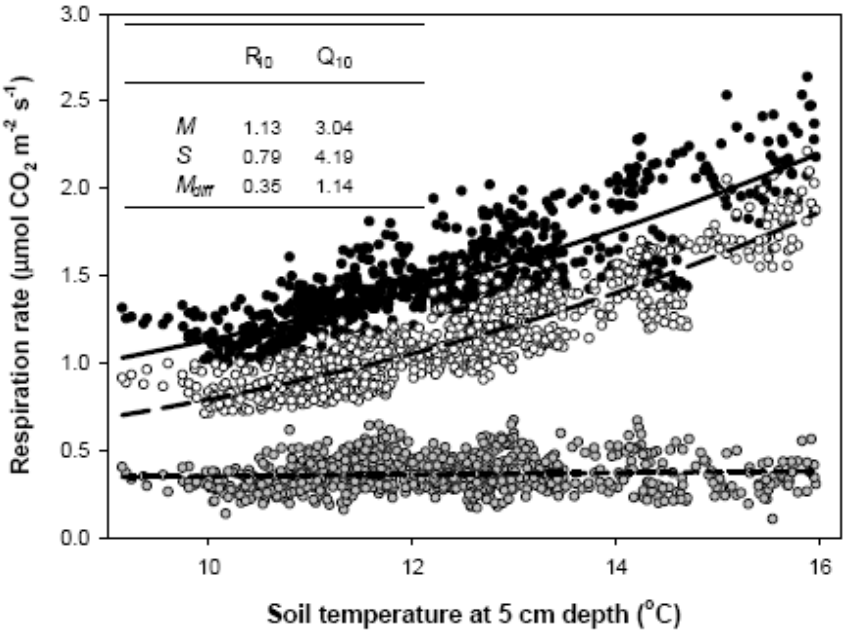

Figure 5.6: Relationship between soil temperature at 5 cm depth and the mean hourly rate of respiration at the mycorrhizal (*M*, ●) and non-mycorrhizal collars (*S*, ○) and the difference of both (M_{diff}, ●) during Campaign 2 (September 9th to October 11th 2005). Exponential relationships are plotted for each data set (*M* ——, *S* — —, M_{diff} − − −) and the Q_{10} and R^2 values displayed for each (unpublished data from A. Heinemeyer).

Diurnal cycles at non-mycorrhizal collars correlated more strongly with soil temperature than mycorrhizal collar replicates (R^2 values: 0.63 and 0.35 respectively), whereas the calculated ERM respiration (difference between treatments) was unaffected by temperature (R^2 value: 0.01). Further, clear differences in the temperature responses during daylight versus night-time hours were observed for mycorrhizal collar respiration and the calculated ERM respiration but not for non-mycorrhizal collar respiration. Consequently, factors other than temperature appear to control this diurnal cycling of ERM respiration. The most likely explanation is a time-lag in substrate supply from above-ground photosynthates to the rhizosphere as suggested previously for forest ecosystems (Tang et al. 2005).

A decline in soil moisture from 25% - 10% during a minor drought decreased the EM contribution to soil respiration considerably from ~25% to <10% ($P < 0.001$) and increased almost instantaneously with rewetting to >30% ($P < 0.001$; data not shown).

These data stress the importance of the mycorrhizal part of soil respiration and encourage to investigate the mycorrhizal component further. In particular in EM systems it may well be the ERM which contributes most of the autotrophic flux component and not roots. One key implication should be reflected in an advanced collar design for soil respiration measurements. As mycorrhizal hyphae and active fine roots are predominantly found in the organic rich top soil layers, any collar inserted only a few centimetres deep into the soil will cut through this vital assimilate supply ultimately reducing the soil CO_2 efflux. Shallow collars are a way forward and a good seal can be achieved by cutting through the top dry litter layer around the collar and then pressing it gently into the Of/Oh layer, fixing it with metal hooks down to the ground.

Finally, a recent meta-analysis demonstrated that EM growth is positively affected by elevated CO_2 (Alberton et al. 2005) and, critically, it was found that the activity of extra-radical mycelium was stimulated more than the overall plant response. The possibility of altered assimilate supply to EMs may have major effects on soil biota and belowground C cycling and storage, such as antagonistic interactions between EM and saprophytic fungi (Stark and Kytoviita 2005) or increased EM respiration (Gorissen and Kuyper 2000) and the potential for rapid turnover of mycorrhizal C (as shown for arbuscular mycorrhizal fungi, Staddon et al. 2003).

5.3. Measuring Root and Mycorrhizosphere Respiration

In this section we will discuss direct measurements of root respiration, both in the laboratory and in the field, as well as the use of Q_{10} and other approaches for quantifying acclimation. One indirect method, the use of micro meshes, will be described in this chapter. However, other exclusion (i.e. trenching) and isotope methods for determining respiration rates of mycorrhizosphere components are described in Chapters 7 and 10, respectively.

5.3.1. General Considerations

Field vs. Lab Measurements: Which Method to Use

A number of difficulties are associated with measuring root respiration and mycorrhizosphere respiration. Disturbances to the plant-soil system as a consequence of the method used will likely change natural respiration rates through changes in factors such as rhizodeposition amounts, CO_2 concentrations, moisture, disruption of fungal hyphae, etc. Further, no clear spatial limits between root derived carbon and soil organic matter respiration sources exist in natural systems. Plant rhizodeposits are respired together with older soil carbon by the same microorganisms in the mycorrhizosphere. As a consequence, some components of mycorrhizosphere respiration cannot be determined by direct measurements but need to be estimated indirectly.

The choice of method will ultimately depend on the hypotheses to be tested. Laboratory methods are carried out under conditions that differ largely from natural field conditions. It is therefore difficult to make conclusions about field fluxes only from laboratory measurements. On the other hand, one can more easily separate soil components and precisely control factors affecting respiration rates, making laboratory methods a better tool for understanding the influence of individual factors. Laboratory methods, e.g. allow for the determination of temperature response curves from a wide range of temperatures which can be applied in a relatively short time. Temperature response estimated from measurements in the field can be more problematic due to small diurnal temperature changes, and if done over longer periods, changes in other confounding factors (e.g. soil moisture, PAR etc.).

The large number of environmental factors affecting mycorrhizosphere respiration makes field methods less suitable for identifying the influence of individual factors. On the other hand, field methods have the advantage of giving values that will come closer to “real” field fluxes, and measurements will respond to the normal fluctuations of the surrounding environmental factors. However, most field methods also disturb the natural conditions of the root-soil system to some degree, resulting in changes in the fluxes measured.

Expressing Respiration Rates

Depending on the purpose of the experiment the investigator needs to consider how to express the measured rates of respiratory gas exchange as a specific root respiration. Simple standardizing and commonly used measures are: root fresh weight, dry weight, root length, or ground surface area. However, it is not straightforward to standardize at what water content the fresh weight is to be measured between species and/or experiments. Thus, the mass of dry root is often used as the base at which a specific root respiration is expressed. However, the calculated values of root respiration on a dry weight basis strongly depend on the amount of inert structural tissue in the root material. Therefore, attempts to express root respiration on a metabolically activity basis are made by relating the gas exchange measurements to for example the N content, total protein content (Lowry et al. 1951), or fumarase (mitochondrial specific enzyme) activity (van Emmerik and al. 1992).

Measuring Root Respiration Temperature Response

To generate a temperature-response curve for root respiration, it is often not practical to measure rates of root respiration using attached, intact roots. However, several investigators have developed cuvettes that enclose roots, and are placed back in the soil for measurements for extended periods of time (Rakonczay et al. 1997; Bryla et al. 2001). Typically, either detached whole roots or root pieces are used. Excision effects on root respiration when assessed within 30 mins after severing of the shoot may be minimal (Lambers et al. 1993). However, a possible increase as a response to wounding should be considered (Bloom and Caldwell 1988). Repeated measurements of respiration at different temperatures using detached roots do lead to a progressive reduction of respiration at a standard temperature (Loveys et al. 2003) due to substrate limitation. Alternatively, separate roots may be used for each measuring temperature with care taken to control for root order, size, and age.

5.3.2. Field Methods

Field methods for quantifying root respiration and mycorrhizosphere respiration typically involve either root excision or the use of intact roots that extend into chambers. In both cases, infra-red gas analyzers (IRGAs) are normally used to measure an increase in CO_2 concentrations due to respiration. Occasionally, CO_2 traps such as NaOH and KOH are used to estimate CO_2 production (Mori and Hagihara 1991; Cheng et al. 2005) or oxygen sensors are used to measure respiration as O_2 depletion (Zogg et al. 1996). CO_2 traps should be avoided as they increase the soil CO_2 gradient, leading to overestimated flux rates.

Excision Methods

Excision methods take roots that have been freshly removed from the soil and place them in a cuvette for a rapid determination of CO_2 efflux using an IRGA. In some cases, roots are washed in the field prior to measuring respiration. More commonly, soil and organic debris are physically removed prior to measurements (Burton and Pregitzer 2003), with additional adhering material washed from the roots later in a laboratory setting, in order to determine the actual dry weight of root material in the sample. The mass of material removed during this final cleaning typically is < 5 % of the total mass of the sample for which respiration was measured (Burton et al. 2002). Microbial respiration in any adhering soil and organic debris will be measured as root respiration, but its contribution will be minimal if microbial respiration per unit gram of the material is low relative to that of the root tissue. However, any mycorrhizal roots will retain much of their hyphal sheath (i.e. EM) or other mycelium parts, which will be included in the 'root respiration' measurements. Comparisons of specific respiration rates of forest soil material (Zak et al. 1999) and tree roots (Burton et al. 2002) suggest this to be the case, but actual measurements of the respiration rate of the removed rhizosphere material would have to be made to confirm this assumption.

Cuvettes used for excised roots can include standard cuvettes designed for measurement of leaf and needle photosynthesis (Ryan et al. 1996) or cuvettes designed for root respiration (Burton and Pregitzer 2003). Cuvette volumes vary widely depending in part

on the size of the sample to be analysed. Cuvettes as small as 5 cm^3 (Ryan et al. 1996) and as large as 12.7 L (Mori and Hagihara 1991) have been used. When an open system is being used for gas analysis, cuvette volume should not be greatly larger than needed to contain the sample. Excessive cuvette volume unnecessarily increases the time needed for a steady state respiration rate to be achieved after inserting a sample. It is also desirable to use cuvettes that can maintain roots at ambient temperature (or other desired temperatures). Standard photosynthesis cuvettes for IRGAs are available with temperature control devices that are adequate, but these will cause a more rapid depletion of battery power, which can be a concern in remote field locations. A simple alternative involves the use of a cuvette that contains a metal base that extends into the soil (Burton and Pregitzer 2003). The base acts as a heat sink that maintains ambient soil temperature within the cuvette chamber. In addition, drying of the roots induced by the air stream through the cuvette in open systems can be avoided by either putting the root samples on wet paper or inserting a wash bottle with water upstream of the chamber (Kutsch et al. 2001b).

There are a number of artefacts potentially related to root respiration measurements of plants grown in natural soils, especially when (1) roots are separated from the soil and (2) are excised. The removal of soil may not only affect plant-microbe interactions and potentially cause loss of highly active root hairs, but also stops the nutrient and assimilate supply. Since respiratory costs for nutrient uptake are high, the absence of nutrient supply affects root respiration. Poorter et al. (1995) showed for a slow and a fast-growing grass species that reductions in nutrient availability resulted in a decrease in root respiration. We are not aware of any study reporting how fast root respiration decreases after nutrient supply from its natural environment has been stopped by a separation of roots from the soil.

Excision of roots is unavoidable when roots are extracted from the soil in ecosystems, especially in grasslands. Excision methods allow the root-rhizosphere system to develop and function naturally, until the time roots are removed for measurement. The accuracy of excised respiration rates depends largely on the assumption that the root activity remains unaffected by excision, at least for the length of time needed to complete the measurements. Essentially, the hope is to achieve a snapshot while the roots are still

respiring at the same rate they were prior to removal from their natural environment. Effects of excision on root respiration could include increased respiration due to wounding responses (Cabrera and Saltveit 2003); decreased respiration due to the interruption of carbohydrate supply to the root, attached mycorrhiza and adhering microbes dependent on exudation; and disruption of normal physiological processes due to the removal of the physical, chemical and biological environment with which the root was interacting. Measured effects of excision on short-term root respiration rates have varied from significant decreases (Bloom and Caldwell 1988) to little or no effect (Marshall and Perry 1987; Lee et al. 2003; Lipp and Andersen 2003). Burton et al. (2002) report that respiration of fine roots from different forest sites was not affected by time since collection, which was in the range of 4 h. Bahn et al. (2006) observed for grassland roots that respiration decreased by less than 5% of the initial value within 5 h after sampling. A method to minimize possible effects of root wounding is to use single excisions and to sample intact root mats containing many fine root segments (Burton and Pregitzer 2003). Completing measurements rapidly can lessen the potential impacts of carbohydrate loss. Lipp and Andersen (2003), for example, found relatively constant rates of root respiration for six hours after excision, a time period much longer than the fifteen to thirty minutes that are required to complete usual excision measurements (Ryan et al. 1996; Burton and Pregitzer 2003). But logically this will depend on the carbohydrate status of the roots, i.e. starved roots vs. well fed roots.

It is important to consider which parts of the root-rhizosphere system are contributing to respiration rates measured by various approaches. Excision methods will exclude most of the respiration of mycorrhizal hyphae and potentially a significant portion of respiration associated with rhizosphere microbes. The respiration of internal structures of arbuscular mycorrhizas and portions of ectomycorrhizas and rhizosphere microbial communities not removed by cleaning will be included. However, since much of the soil and organic matter adhering to roots, and thus much of the rhizosphere, is removed in typical root excision procedures (Burton and Pregitzer 2003), a significant portion of the contribution of these components is missed.

In summary, the mentioned potential artefacts in studies on the respiration of roots in ecosystems can be minimized by:

- carrying out root respiration measurements in situ or, in case this is not possible, completing the measurement within 4-5 hours after sampling,
- always use the same procedure and check for time differences,
- brushing roots free of loose soil and organic matter rather than washing or rinsing them, but keeping roots moist,
- keeping the measured root system as intact as possible, i.e. few cutting effects,
- always use a fresh batch of root tissue in order to avoid substrate decline.

Measurements of root respiration at higher CO_2 concentration need to consider the problem that large concentration gradients between the chamber and its environment may result in experimental artefacts due to leakage problems (Burton and Pregitzer 2002).

Intact-Root Chamber Methods

Intact-root chamber methods are sometimes used in a similar fashion to excised-root cuvettes, with the roots inserted into the chamber just prior to measurement, and the chamber volume containing only roots and atmosphere (Cropper and Gholz 1991). In these cases, the potential problems associated with root removal apply except for an excision wounding response. More typically, intact-root chamber systems surround the roots with a soil material (Cropper and Gholz 1991; Gansert 1994; Gansert 1995; Bryla et al. 2001). The entire chamber is then buried in place, with measurements of root respiration made periodically. For these chambers, measured respiration rates will include contributions from roots, mycorrhizal fungi, including some hyphae, and rhizosphere microbes utilizing rhizodeposits. Intact-root chambers usually are sealed, but variations include open-top chambers over which soil respiration cuvettes are periodically placed to measure respiration rates (Fahey and Yavitt 2005). Another method is to insert tips of fresh fine roots into small plastic containers filled with sand or sieved mineral soil and leave them to develop a fine root system during the next months (Kutsch et al. 2001b). These containers can contain holes in the bottom, so the moisture content can be kept in the same range as the surrounding soil. For the respiration measurements the containers can be inserted into measuring chambers placed on the soil surface.

Buried chambers allow the root system to experience ambient temperatures, but if the chamber is sealed, moisture content in the system may differ substantially from that in the bulk soil. If chambers are kept in place for an extended period of time, it also becomes difficult to know the mass of the respiring material at any given point in time, other than those just prior to final destructive sampling of chamber contents.

Choice of the soil medium for intact-root chamber systems is a major factor that could influence measured respiration rates. Artificial media, such as organic-free sand (Vogt et al. 1989; Kutsch et al. 2001b; Fahey and Yavitt 2005) and sand-vermiculite mixtures (Cheng et al. 2005) have been used to minimize respiratory contributions from decomposition of bulk soil organic matter. However, mycorrhizosphere respiration measured using these media can be much lower than those for native soil (Cheng et al. 2005). It is also possible to use native soil and then determine mycorrhizosphere respiration rates by subtracting rates determined from root-free, soil only chambers. However, most intact-root chamber systems have a fairly small root mass, thus this approach is difficult to apply unless native bulk soil has low organic matter content and creates a respiration flux that is similar in magnitude to or smaller than that of mycorrhizosphere respiration. Cheng et al. (2005) used soil from C_4 ecosystems in chambers containing intact roots of C_3 plants. The difference in isotopic signature between C_4 and C_3 sources allowed separation of CO_2 efflux from bulk soil and root and rhizomicrobial components. However, they found that respiration rates of longleaf pine roots were much lower in C_4 grassland soil than in native longleaf pine-wiregrass soil.

Open and closed gas flow systems both have been used for excised root and intact-root chamber methods. In open systems, the change in CO_2 content of atmosphere entering and leaving the cuvette or chamber is used to determine respiration rate. This requires that a large enough change in concentration occur for the IRGA to accurately detect it. If changes in CO_2 concentration are too small for accurate detection, then larger samples can be used or gas flow rates can be reduced. It should be noted, however, that reducing the flow rate can increase the likelihood of significant errors if the system contains even minor leaks (Burton and Pregitzer 2003). Such effects in the past have led several investigators to falsely conclude that CO_2 concentrations used during the measurement affected plant tissue respiration rates (Amthor 2000a; Burton and Pregitzer 2003). A

potential advantage of the open system is that input CO_2 concentration can be set at levels representative of soil-atmosphere CO_2 concentrations (Burton et al. 2002; Cheng et al. 2005). This will avoid the diffusing out of CO_2 that may be stored in the roots and subsequent overestimation of respiration rates until a new steady state is reached. It is also recommended that all systems be checked using empty cuvettes and chambers at several CO_2 concentrations to assess the potential influence of gas leaks on results.

Measurement Techniques for Respiration of Large Coarse Roots

Measurements on large woody coarse roots – hereafter referred to as large coarse roots – cannot be made with the procedures described above, mainly because of the impossibility of inclosing them in regular chambers. Large coarse root respiration can either be measured using cut root sections under laboratory conditions or *in situ* by installing chambers over the root part to be sampled.

In the first case, excised root sections are removed from a tree and CO_2 evolution is measured in the laboratory under controlled conditions (Tranquillini 1959; Kimura et al. 1968). This method allows the use of a large sample size for determining within- and between tree variations in respiration rates. General complications related to excision methods will also apply, with the additional disadvantage that an adequate sampling involves considerable destruction of the tree. It is thus impossible to follow seasonal trends in respiration on the same sample (Sprugel et al. 1995) and, as a result, it may be impossible to separate maintenance from growth respiration.

Another common approach is to fix chambers permanently onto intact large coarse roots above or below the soil surface and continuously monitor the CO_2 efflux through the bark over prolonged periods of time (Linder and Troeng 1981; Benecke 1985; Wieser and Bahn 2004). This method is less disturbing than the excision method but requires complex equipment to maintain air flow through the chambers, and to monitor flow rates and changes in CO_2 concentration. Due to these hardware requirements, usually only a small sample size can be studied in parallel. Care is also needed that permanently installed respiration chambers do not significantly alter environmental conditions which may affect respiration rates.

A solution to the problem of a small sample size is the use of removable chambers that can be clamped onto a root section (Ryan et al. 1996; Desrochers et al. 2002). This allows within- and between-tree variations to be measured within a single day, and measurements on the same site over an entire growing season. Growth respiration can be easily distinguished from maintenance respiration by means of repeated measurements at the same site. However, the need to install and leak-test such clamp-on respiration cuvettes, and then to wait for an equilibrium each time a measurement will be made, reduces the number of possible measurements.

Chambers used for monitoring large coarse root respiration are often made of Perspex®, and enclose either a complete section (Figure 5.7) or only a part of the circumference of a root. Due to the absence of a large overheating, most field studies on large coarse root respiration use chambers with no environmental control.

When estimating large coarse root respiration in situ the following precautions are necessary:

- After rinsing and drying the root surface, thick or fissured bark should carefully be pared down below the point of chamber contact, stopping short before the living tissue (phloem or cambium).

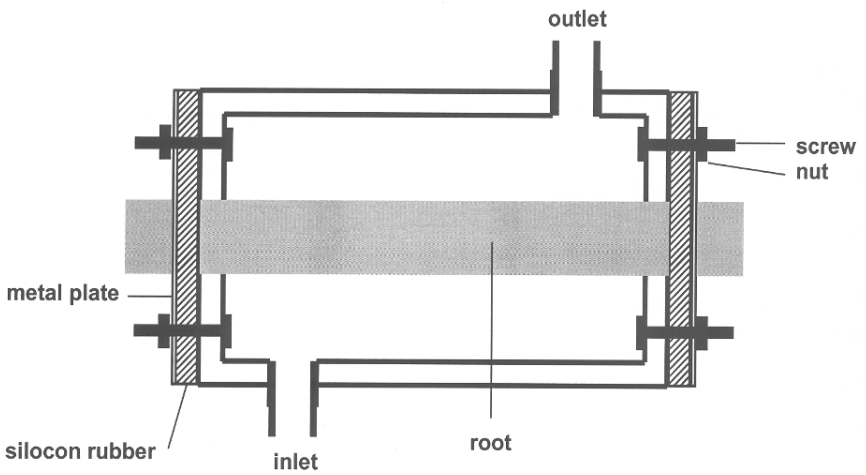

Figure 5.7: Clear Perspex chamber made of two half shells with adjacent walls for continuous in situ measurements of CO2 efflux. Half shells enclosing the root are pressed together with pile clamps and the bearing surface is sealed with a neoprene foam tape. At both ends of the chamber the root is coated with putty and a PTFE tape and the root is pressure sealed with soft silicon rubber by tightening nuts on screws which press the rubber sealing against the chamber and the root. An inlet and an outlet port allow the chamber to be connected to an infrared gas analyzer, operating in the differential mode to measure CO2 efflux, and an electronic mass flow meter for the flow rate.

- Putty and non hardening insulating foam (without toxic volatiles) should be used to ensure a gas tight seal between chambers and the bark. Chambers should be checked for gas leaks by applying an over pressure of up to 20 mbar.
- When chambers are attached to roots throughout one entire growing season or longer, allowance for expansion is necessary in order to prevent bark constriction under the seals of the chambers. It is also recommended to estimate changes in surface area and sapwood volume under the chamber covered areas.
- Simultaneously to gas exchange measurements, tissue temperature should be monitored in and outside the chambers with temperature sensors inserted into the bark down to the cambial surface.
- When working with chambers permanently sealed to a root it is important to have a continuous supply of ambient air. Condensation of moisture in tubing and chambers should be prevented by insulating and heating all the pneumatic tubing or by partial (but not complete!) drying of the inlet air.
- When measurements are not being made, glass wool can be placed into the inlet and outlet tubing in order to prevent insects from entering the chamber but allowing sufficient gas exchange (Vose and Ryan 2002).

Advantages and disadvantages of in situ large coarse root respiration measurements are similar to those for measurements in finer roots. An additional complication is that some of the CO_2 respired in the sapwood may be carried upward into stem, branches, and foliage instead of being released through the bark, so that measured root respiration may be underestimated. Teskey and McGuire (2005) observed a proportional correlation between the $[CO_2]$ in xylem sap and CO_2 efflux from the stem, which suggests that CO_2 can be easily transported within the plant. However, until now this problem has never been resolved satisfactorily (Sprugel and Benecke 1991).

Mesh Exclusion Method

Arbuscular mycorrhizal or ectomycorrhizal hyphal respiration can be separated from root and soil respiration by using nylon meshes with pore sizes that allow the ingrowth of fungal hyphae while excluding roots (Johnson et al. 2001a). Typically, cores of either undisturbed or sieved soil are established in the field and a certain time period is allowed for the ingrowth of mycorrhizal hyphae. The cores can range in diameter depending on the study (from 2 to 20 cm have been used) and can be inside deep collars which have windows covered by meshes, or directly in mesh bags. The collars can be hammered directly into the ground, thus avoiding the disturbance caused by removing soil cores. This may not be possible in some harder soils. Depending on the vegetation, one can use meshes with 41 μm pores to allow EM hyphae but no tree fine roots, or meshes with 20 μm pores to allow AM hyphae while excluding very fine (e.g. grass) roots. Additionally, 1 μm meshes can be used to exclude both hyphae and roots. The presence of pores has the additional advantage of allowing the flow of water and dissolved substances through the mesh, thus minimizing the disturbance of natural conditions. With CO_2 efflux measurements from these different treatments and of total soil respiration (using shallow collars, not cutting any roots), the contribution of mycorrhizal and root respiration can be calculated separately. The following points should be considered when using this method:

- Soil cores should be deep enough to include the main rooting zone. A problem can be deeper roots but they are unlikely to contribute much to the overall flux (because of oxygen limitation and their main functioning in passive water uptake).
- Enough time should be given for decomposition of remaining dead roots and mycorrhizal hyphae colonization of the soil core. The waiting time for the latter will depend on the soil core size and the growth activity of mycorrhizal hyphae. Decomposition and ingrowth can be considered “complete” when differences in soil respiration among treatments are stable.
- Total soil respiration shallow collars (not deep soil core collars) should be inserted to the minimum necessary, taking care not to severe fine roots and hyphae in the upper litter/soil layers which would lead to an underestimation of root and mycorrhizal respiration.

- A possible diffusion of CO_2 into the soil cores through the meshes should be estimated, e.g. by using a control collar with no windows.

In the lab, the same mesh sizes can be used and gas samples can be taken in sample vials and analysed with an IRGA to obtain fluxes. The rotation of cores in order to severe hyphal connections is sometimes performed in field or lab experiments. This technique can be used to immediately terminate the flow of carbon and nutrients through mycorrhizal hyphae and to compare fluxes before and after hyphal severance (Johnson et al. 2002a).

A limitation to the method is given by the fact that only the contribution of the mycelium inside the cores can be measured. The density of AM hyphae will decrease with the distance from the root, so that calculated mycorrhizal respiration will represent a minimum estimate; smaller cores may be desirable in this case. Hyphal density in EM systems does not necessarily decrease with root distance, but in this case, the respiration of the mycorrhizal sheath around the roots will be missed. However, such estimates are still useful to study the proportion and response of mycorrhizal respiration to environmental factors. On the other hand, massive proliferation of roots and mycorrhizal hyphae against the side of the mesh might lead to an overestimation of respiration rates. This can easily be checked for especially in EM systems by appropriate controls (e.g. deep collars without mesh).

As with other exclusion techniques, potential changes in nutrient and water status, as well as the lack of competition with roots and mycorrhiza, could alter microbial respiration of soil organic matter and litter in the mycorrhiza free soil. Possible priming effects on respiration could also change as a result of root and mycorrhizal fungi exudates being absent in treatments excluding these components.

Field Measurements to Take in Conjunction with Root Respiration

As seen in section 5.2, a variety of potential controlling factors can be measured in conjunction with field measurements of mycorrhizosphere respiration. By accounting for the effects of these factors, one can better model ecosystem level C flux from

mycorrhizosphere respiration and in some cases can utilize them as statistical covariates, allowing experimental responses to be clearly identified.

An assessment of the roots measured also should be made. Beside dry weight and length, to which mycorrhizosphere respiration is usually related, this could include the root size classes or orders measured, root N concentration, depth, root age or other demographic classifications (white, brown, woody), and presence of mycorrhizal structures. For intact-root chamber methods, such characteristics for roots in the chamber may differ significantly than those for roots in the bulk soil, leading to different respiration rates (Fahey and Yavitt 2005). For excised root methods, one has to be sure to measure roots from the wide variety of sizes, orders, ages and depths present, to truly scale results up to ecosystem level fluxes.

Researchers wishing to model seasonal or annual mycorrhizosphere respiration fluxes should take multiple measurements throughout the period of interest as well as diurnal measurements if possible. Using a single Q_{10} to adjust rates to temperatures that differ greatly from measurement days is not recommended, as Q_{10} can vary widely with species, season and temperature and will depend largely on substrate supply and status of the root (Atkin et al. 2000a; Burton et al. 2002; Atkin and Tjoelker 2003). Where possible, field-based estimates of seasonal or annual mycorrhizosphere respiration should be compared to independently derived estimates based on C budgeting, isotopic methods, or other techniques, to assess the correctness of their overall magnitudes. Careful comparisons of multiple techniques (Cheng et al. 2005; Kuzyakov and Larionova 2005) will let us further assess the accuracy and pitfalls of common techniques, such as root excision and intact-root chamber systems.

5.3.3. Laboratory Methods

O_2 Consumption and CO_2 Release Methods

In the laboratory, respiration rates in roots may be determined in enclosed cuvettes using infrared gas analyzers that measure net CO_2 efflux or using Clark-type oxygen electrodes that measure net O_2 uptake. During mitochondrial respiration, O_2 is consumed in the

electron transport chain by both the ATP producing cytochrome C oxidase and the non-ATP producing alternative oxidase. CO_2 is released by the decarboxylations of pyruvate and malate in the mitochondrial TCA cycle. Thus, root respiration measured as O_2-consumption or as CO_2-release may not be identical; the ratio of CO_2 to O_2 is termed the respiratory quotient, RQ. RQ has been found to vary between species, sections of roots and depends on the nitrogen source (Lambers et al. 1996).

Root O_2-consumption is related to the efficiency, i.e. ATP-production. "O_2-cuvettes" are typically used to determine rates of root respiration in roots bathed in a buffered hydroponic medium (e.g. 10mM HEPES and 10mM MES, pH 5.8) and temperature-controlled water bath (e.g., Burton et al. (1996)). This method has the advantage that it is easy to apply chemicals and control temperature. Further, with these types of measurements it is easier to estimate root respiration alone without any signal from microbial respiration. Prior to measuring root respiration, the O_2 electrode needs to be calibrated using air-saturated water that is in equilibrium with the temperature of measurement – large errors in calibration can occur when using air-saturated water that has not been allowed to equilibrate to the temperature of measurement. The uptake of O_2 by roots is measured polarographically after sealing roots in a closed cuvette (Walker 1985); typically, it is advisable to wait a few minutes before commencing determination of the rate of O_2 depletion. Rates are then measured over a 10-45 min period, provided the O_2 concentration in the solution remains above 10-20% of that of air saturation. A constant temperature during measurements is important, firstly because of the temperature sensitivity of the electrode and secondly because of the change in solubility of O_2 in water at different temperatures.

Root CO_2-release is related to the root's C-balance, and can be measured with IRGAs in either open or closed mode connected to an enclosing cuvette. Thus, root respiration is either measured as CO_2-differentials or as a build up of [CO_2] inside the cuvette. Currently, there are no commercially available cuvettes of this kind. Cuvettes are custom build (Qi *et al.*, 1994; Christ & Körner, 1995; Leverenz *et al.*, 1999), and usually so that it seals around the root-shoot junction. In this way, root respiration can be measured in a somewhat more natural environment, with the root still connected to the shoot and the root in a soil medium. However, this makes it difficult to add chemicals in a controlled

manner. Further, the temperature control of the root is poor and, depending on the type of medium, root respiration is measured at a background of microbial respiration. CO_2 efflux rates can also be measured using intact roots bathed in buffered hydroponic media, in an open flow-through system (Poorter et al. 1990; Atkin et al. 1996). When using an open system, enclosing more tissue within the chamber may increase the measured CO_2 differentials. This may be accomplished by measuring entire root systems in a larger cuvette. Examples of custom-built chambers for roots include a chamber using an extended heat sink for insertion into soil (Burton and Pregitzer 2003).

The potential direct inhibitory effect of high [CO_2] on root respiration is something that needs being considered for root respiration measurements in general. As pointed out by Reuveni et al. (1993), to obtain stable reliable readings of O_2-consumption by roots in closed cuvettes with O_2-electrodes, at least a 10% reduction is needed from the initial 20.8%. For simplicity, if a respiratory quotient (i.e. CO_2-release/O_2-uptake) of 1 is assumed then a build up of CO_2 in the cuvette from 350 ppm to 20,000 ppm can be expected.

Measuring Root Respiration Temperature Response in the Lab

Direct effects of temperature (T) on root respiration may be termed the T-sensitivity of root respiration. Two important methodological aspects of studying the T-sensitivity of root respiration are i) to ensure a good T-control of the root material and ii) to ensure that the response of root respiration to changing T is obtained fast and within the same root material. T-control of root material is best obtained using Clark-type electrodes coupled to a T-controlled water bath. As the root material is immersed in water medium with a stirrer the desired T-control of the root material is ensured. In cuvettes enclosing a soil-root core, in which root respiration is measured as CO_2-release, the T-control of the root tissue is usually less accurate/certain, although the cuvette can be covered by a water jacket coupled to a water bath or placed inside a T controlled growing chamber. On the other hand, root respiration measurements in Clark-type cuvettes are typically restricted to only a few measurements due to O_2-depletion. Root respiration measured as CO_2-release from soil cores do not suffer this problem. Thus, the response of root respiration

of the same material to several different Ts is better achieved by CO_2-release measurements. Therefore, the choice of how root respiration is to be measured in response to T depends on whether the investigator prioritizes accurate T-control of the tissue or that the T-response be determined within the same root material.

5.3.4. Calculating the Q_{10}

Q_{10} values are often used to describe the short-term temperature response of root respiration. Berry and Raison (1981) suggested that Q_{10} offers an important advantage over Arrhenius theory (derived from physical chemistry) when interpreting the temperature dependence of respiration; that being that Q_{10} does not imply a mechanistic explanation (whereas the use of an apparent activation energy, *E*a, does). The Q_{10} is simply the ratio of respiration at one temperature to that at 10^o C lower. The Q_{10} can be calculated according to:

$$Q_{10} = \left(\frac{R_T}{R_{T_o}} \right)^{\left[\frac{10}{(T - T_o)} \right]} \qquad \text{Eqn 1}$$

where R_T is the respiration rate measured at a a given temperature (T) and R_{To} is respiration rate measured at a reference temperature (To). In Eqn 1, T and To do not have to be 10° C apart. A rearrangement of Eqn 1 provides the following formula:

$$R_T = R_{T_o} Q_{10}^{\left[(T - T_o)/10 \right]} \qquad \text{Eqn 2}$$

in which R_T may be predicted as a function of the Q_{10} and the measurement temperature (T).

When rates of respiration have been determined over a range of measurement temperatures (but below the optimum temperature), a simple exponential function will often adequately describe the temperature response. Respiration at any given T (R_T) can be predicted using a model of the form:

$$R_T = R_{0^\circ C}(e^{kT}) \qquad \text{Eqn 3}$$

where $R_{0°C}$ is respiration at 0° C and k is a temperature coefficient. Eqn 3 may be fitted using standard non-linear regression techniques. Alternatively, k may be determined by linear regression of ln-transformed respiration plotted against measurement temperature (T) (derived by ln-transforming both sides of Eqn 3; note: this value of k may differ from that obtained from Eqn 3). Q_{10} may be then estimated from k using the following formula:

$$Q_{10} = e^{10k} \qquad \text{Eqn 4}$$

Eqns 1 through 4 do not, however, provide information on the extent to which the temperature coefficient of respiration changes with measuring temperature (rather, they provide an estimate of the average Q_{10} for the temperature range T_o to T).

The short-term response of respiration to temperature is not strictly exponential, except perhaps over a limited temperature range below the optimum temperature. In other words, the temperature sensitivity of respiration may change with measurement temperature, implying that Q_{10} is temperature-dependent. This may be revealed by a lack of fit of respiration against measurement temperature when using exponential temperature-response functions (Eqns 2 or 3). To overcome these limitations, an estimate of Q_{10} at each temperature is needed. If the regression slope of ln-transformed respiration against measurement temperature (k in Eqns 3 and 4) is linear, then a single Q_{10} value can be used across all temperatures over a defined measurement temperature interval. However, the slope may not be constant, as would be evident by the lack of linearity in the regression fit, and a significant polynomial fit to the ln-transformed respiration versus temperature data. This fitted polynomial equation can then be differentiated to get the slope (i.e. k) at each temperature; these slopes may then be used to calculate Q_{10} values at each temperature (Eqn 4). It should be noted that the use of ln-transformed respiration versus temperature plots to determine the temperature dependence of Q_{10} requires the analysis to be based on a large number of measurements conducted at several temperatures. Reliance on too few replicates and/or measurement temperatures may result in inadequate statistical power to adequately distinguish between a linear or polynomial fit to the ln-transformed respiration versus temperature plots, and perhaps erroneous conclusions being made about the temperature dependence of the Q_{10}.

When the above analysis clearly shows that Q_{10} varies with measuring temperature, the extent of that temperature dependence can be approximated via linear regression of the Q_{10} values plotted against T to yield a formula:

$$Q_{10} = c - bT \qquad \text{Eqn 5}$$

where c is the Q_{10} at 0^o C and b is the slope of the Q_{10} versus T plot. Tjoelker et al. (2001) and Atkin et al. (2005b) provide generalized equations to describe the approximate temperature dependence of root Q_{10} across biomes and contrasting plant taxa. It should be noted that if the data range includes respiration measured at values lower and higher than the optimum temperature, then Q_{10} may in fact exhibit a non-linear decline with increasing measurement temperature throughout a broad temperature range.

Fitting Curves to Measured Data

Curve fitting to measured respiration data can be accomplished in several ways. Here, we describe the use of standard non-linear regression techniques to fit curves by iteration to existing data using Eqn 3, a special case of Eqn 2, where R_{To} is $R_{0°C}$ (i.e. respiration at 0^o C) and T substitutes T-To. In cases where Q_{10} is temperature-independent, estimates of $R_{0°C}$ and the average Q_{10} over the temperature range are estimated parameters, based on non-linear regression. The resulting expression may then be used to predict respiration and plot the fitted curves.

Whenever Q_{10} is temperature-dependent (e.g., data are measured over a broad temperature range that approaches or includes the temperature optimum of respiration), curve fitting to measured data requires Eqn 2 to be modified. By substituting Eqn 5 into Eqn 2 (and T-To with T), the following expression is obtained:

$$R_T = R_{0°C}(c - bT)^{[T/10]} \qquad \text{Eqn 6}$$

where $R_{0°C}$ is respiration at 0^o C, and c and b are constants that describe the intercept and slope of Q_{10} versus temperature plots, respectively). Again, standard non-linear regression methods can be used to estimate $R_{0°C}$, c and b. These values will then be used to predict R_T and plot the fitted curves.

Predicting Respiration in the Absence of a Measured Temperature-Response

In cases where the temperature response of respiration has not been determined experimentally, respiration at different temperatures (R_T) can be modelled. Assuming that the Q_{10} value is temperature insensitive, then the rates of R_T at any given temperature (T) can be predicted using Eqn 2. However, as stated above, Q_{10} is often temperature sensitive. Then, an equation that takes into account the temperature dependence of the Q_{10} is needed to successfully predict rates of R_T at given temperatures. Intuitively, one might replace the single Q_{10} value in Eqn 2 with a term that describes the temperature dependence of the Q_{10} ($\text{Root } Q_{10} = 3.00 - 0.0450T$, where T is temperature; Tjoelker et al.(2001); Atkin et al. (2005b)). However, this approach fails to accurately predict rates of R_T, particularly at measuring temperatures that are much higher than the reference temperature, owing to the fact that the Q_{10} describes the proportional change in respiration across a 10° C interval. To predict rates of R_T when using a temperature-dependent Q_{10}, Atkin et al. (2005b) concluded that the Q_{10} at the midpoint between the reference (To) and prediction temperature (T) should be used as shown in the following equation:

$$R_T = R_{T_o}[x - y((T + T_o)/2)]^{[(T-T_o)/10]} \qquad \text{Eqn 7}$$

where x and y are constants that describe the temperature dependence of the Q_{10}. To use Eqn 7, an initial R_{To} value at To is either measured or obtained from published literature. Rates of respiration at new temperature (R_T) can then be predicted using a temperature-dependent Q_{10} equation (e.g., Eqn 5). Eqn 7 may be used in predicting temporal changes (e.g., diurnal) in respiration with changing ambient temperatures by solving for a new R_T for each successive temperature-measurement interval (Figure 5.8).

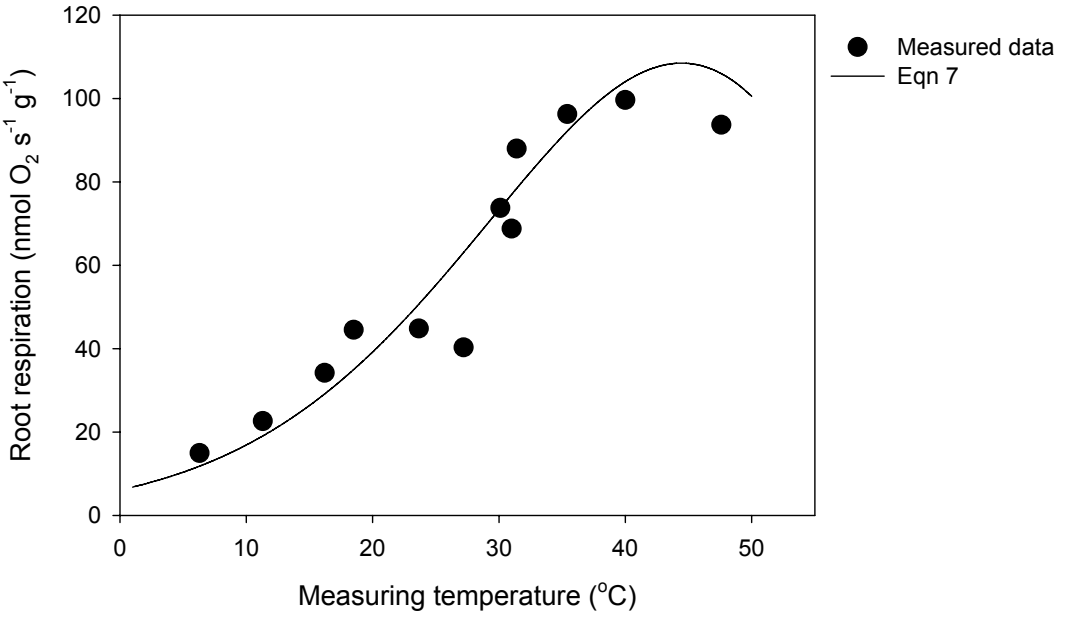

Figure 5.8: Rates of respiration predicted using Eqn 7 against observed data (Covey-Crump et al., 2002). Assumed values in the equation: R_{To} was taken as the rate of respiration at 0 °C (6.01 nmol O_2 g^{-1} s^{-1}), x = 3.00 and y = 0.045 (Tjoelker et al. 2001; Atkin et al. 2005). See text for further details.

5.3.5. Methodology for Quantifying the Degree of Acclimation

Set Temperature Method

One of the most common characteristics of acclimation is that a change in growth temperature results in a change in respiration at a set measuring temperature (Atkin et al. 2005b). Thus, comparisons of rates of respiration at a set measuring temperature can provide an indication of the degree of acclimation (*AcclimSetTemp*). Using the set temperature method, the degree of acclimation can be quantified as the rate exhibited at a particular measurement temperature by a cold-grown plant divided by the rate exhibited by a warm-grown plant. High ratios indicate high degrees of acclimation (with a ratio of 1.0 indicating no difference between cold- and warm-grown plants, i.e. no acclimation). *AcclimSetTemp* values greater than 1.0 are common, suggesting that most plant species

are capable of acclimation via up-regulation of respiration in cold compared to warm temperatures, at least when defined by the differences in respiration at a set temperature.

In cases where Q_{10} is constant after acclimation, this approach yields constant ratios of rates of respiration of cold-grown to warm-grown plants, regardless of the measuring temperature. However, changes in measuring temperature result in temperature-dependent changes in *AcclimSetTemp* if the mean Q_{10} differs between the cold- and warm-grown plants (Atkin et al. 2000a). Given that acclimation can be associated with changes in Q_{10} (Atkin et al. 2005b), this means that *AcclimSetTemp* ratios need to be treated with caution, especially when comparing results from separate studies where different measuring temperatures were used. However, within individual studies the ratio is likely to provide estimates of the extent of acclimation.

Homeostasis-Based Methods

Homeostasis is also one of the defining characteristics of acclimation; many studies have defined *full acclimation* as the situation when plants grown at different temperatures exhibit identical rates of respiration, when measured at their respective growth temperatures (Atkin et al. 2005b). Homeostasis forms the basis of several methods used to assess the degree of acclimation. In Larigauderie and Körner (1995) and Tjoelker et al.(1999), homeostasis was assessed via determining the ratio of respiration of warm-grown plants to that of respiration of cold-grown plants, each measured at their respective growth temperature. Larigauderie and Körner (1995) defined this ratio as the *Long-Term Acclimation Ratio* (LTR_{10}), the proportional change in respiration of plants grown and measured at one temperature compared with those grown and measured at a temperature 10° C lower (LTR_{10} is analogous to the Q_{10} of the short-term temperature response function). Full acclimation (i.e. perfect homeostasis) was assumed to have occurred when LTR_{10} is equal to 1.0. LTR_{10} values that are greater than 1.0, but less than the Q_{10} indicate partial acclimation. No acclimation would occur when LTR_{10} and Q_{10} values are equal. By comparing LTR_{10} values with published values of Q_{10}, Larigauderie and Körner (1995) assigned a degree of acclimation to each plant species (not a specific value, but rather whether the degree of acclimation was near-full, high, medium to low or

very low). The LTR_{10} method is useful in providing an insight into inter-specific differences in the degree of acclimation. Comparing LTR_{10} values with short-term Q_{10} values from the same species avoids potentially misleading conclusions concerning the degree of acclimation associated with low LTR_{10} values. For example, viewed in isolation, a low LTR_{10} such as 1.3 might suggest a very high degree of acclimation. However, if the Q_{10} of the same tissue were also low (e.g., 1.5), then little adjustment in rates of respiration would have occurred in response to the change in growth temperature. By comparing LTR_{10} and Q_{10} values, the magnitude of acclimation in respiration to temperature can be appropriately compared.

The method of comparing LTR_{10} with Q_{10} values does, however, have some drawbacks. Firstly, the method does not allow a definitive degree of acclimation value to be applied to any one species. Secondly, it requires LTR_{10} to be compared with Q_{10} values; however, deciding on which Q_{10} value to compare the LTR_{10} may be problematic if short-term temperature response data are missing, or if Q_{10} itself changes as a result of acclimation. If growth temperature affects Q_{10}, then the conclusions reached will depend on which Q_{10} value is used (i.e. should one use Q_{10} of the warm- or cold-grown plants?). Finally, the biological basis for stating that full acclimation is when LTR_{10} equals 1.0 has not been demonstrated; in some cases cold-grown plants exhibit faster rates of respiration than do warm-grown plants, when each is measured at their respective growth temperatures (Loveys et al. 2003).

Another limitation to the LTR_{10} method is that it does not assign a quantitative degree of acclimation value. Atkin et al. (2005b) proposed a method for assigning such a value using the range of LTR_{10} values that occur between no acclimation (i.e. $Acclim\text{LTR}_{10} = 0$, which is when the LTR_{10} and the short-term Q_{10} are equal), and full acclimation (i.e. $Acclim\text{LTR}_{10} = 1.0$). This range is equivalent to the short-term Q_{10} minus 1.0. Therefore, the degree of acclimation is:

$$Acclim_{\text{LTR}_{10}} = 1 - \left(\frac{\text{LTR}_{10} - 1}{\text{Q}_{10} - 1} \right) \qquad \text{Eqn 8}$$

Thus, knowledge of the short-term Q_{10} and LTR_{10} means that a quantitative degree of acclimation to any plant tissue can be assigned. Use of this approach requires the use of mean values of Q_{10} and LTR_{10} for different growth temperatures.

Loveys et al. (2003) proposed the *Homeostasis method* approach (a modification of the above method of comparing LTR_{10} and Q_{10} values), where the degree of acclimation (*AcclimHomeo*) is taken as the ratio of respiration exhibited by cold-grown plants divided by respiration of the warm-grown plants (each measured at their respective growth temperature). The degree of acclimation increases as *AcclimHomeo* increases. *AcclimHomeo* is the inverse of the LTR_{10} (Loveys et al. 2003); another difference is that the homeostasis method does not require that *AcclimHomeo* be compared with Q_{10}. This has advantages and disadvantages; a definitive degree of acclimation is provided without the need for comparison with variable Q_{10} values that can be growth-temperature dependent. Moreover, it does not assume full acclimation, and allows for cases where cold-grown plants exhibit faster rates of respiration than warm-grown plants, each measured at their respective growth temperature. However, the method does not take into account tissues whose Q_{10} is low (and thus already lead to a high *AcclimHomeo* value, even without large adjustments in respiration occurring). Fortunately, few species exhibit Q_{10} values that differ substantially from the common mean values of 2.0-2.5. Thus, the *AcclimHomeo* ratio probably provides a good indication of the degree of acclimation of respiration to contrasting growth temperatures. It is probably most useful for comparisons of multiple species grown under identical conditions.

Quantifying Acclimation: Which Method to Use?

If generalizations are to be made about the degree of temperature acclimation of respiration in plants, greater effort will be needed to standardize the methods by different research groups. Ideally, acclimation is best quantified using Eqn 8, as this takes into account both the short- and long-term responses of respiration to temperature. However, in some studies it may not be possible to obtain estimates of both Q_{10} and LTR_{10}; in such cases the *Set Temperature method* should be used (with the knowledge that the *AcclimSetTemp* value is measurement-temperature dependent). Alternatively,

measurements might be best made at the growth temperature (in cases where temperature control during measurements is limited) with analyses then being made using the *Homeostasis method*. Ultimately, the choice of which method to use will depend on the nature of the comparison being made (e.g., pre-existing plants that experience a change in growth temperature, versus plants that develop under contrasting temperature regimes).

5.4. Mycorrhizosphere Respiration at the Ecosystem Scale

Since direct measurements of root or mycorrhizosphere respiration are related to a certain amount of root biomass or root length, scaling-up from chamber measurements to the ecosystem scale requires additional information about the root biomass or root density and their dynamics. Since the fine root biomass is highly dynamic a singular measurement does not provide sufficient information to estimate annual sums of mycorrhizosphere respiration. In addition, a full belowground carbon balance also requires the turnover of fine root biomass. Carbon allocation to the root system and its subsequent usage for the build-up of root biomass is a highly plastic process that is driven by the phenology and the dynamic of climatic and site constraints (Hendricks et al. 1993; Middelhoff 2000; Hendricks et al. 2006).

Surprisingly, there is no standard method to derive the dynamic of the fine root biomass or fine root production. Sequential coring (Kalela 1955; Persson 1978; Vogt and Bloomfield 1991; Vogt and Persson 1991) is still the most often used approach, even if it is clear that it underestimates fine root production in most cases. Usually, this method is conducted with sharp root corers that are driven into the rooted zone of the soil. A statistically significant number of samples have to be collected at intervals of about 3 to 6 weeks. Coring locations have to be positioned randomly within a defined plot. Root production or mortality can be calculated either from the difference between minimum and maximum of fine root bio- and necromass within a measuring period of at least 12 months (‘minimum–maximum method’, Hertel and Leuschner 2002) or from the mean root mass of two consecutive measurements. The problem of the method is that one has to assume that fine root production and mortality occur asynchronously (Hendricks et al. 2006). Mortality of some roots during a net growth phase is not acquired neither root

growth during a net mortality phase. To avoid the resulting underestimation one has to quantify the changes in live and dead root mass in consecutive intervals and subtract the losses of necromass due to decomposition ('compartmental flow method'; Hertel and Leuschner 2002).

In ingrowth core experiments volumes with local soil material but free of roots are created and the amounts of roots growing into these volumes are detected (Persson 1980; Powell and Day 1991; Majdi et al. 2005). Usually soil cores are taken from the site, sieved, and relocated there after extraction of all roots. Hertel and Leuschner (2002) removed only macroscopically visible live and dead root material by hand. They assumed that smaller dead rootlets would be decomposed during the experiment. The remaining soil material can be replaced into the hole either in a mesh bag that allows fast re-sampling or directly with the edges marked at the soil surface. Care has to be taken that the structure and density of the soil samples is conserved as much as possible. Since the ingrowth cores supply a soil volume that excludes competition with other roots the method may result in an overestimation of fine root production.

Another tool to study root dynamics are minirhizotrons (Hendrick and Pregitzer 1992; Majdi 1996; Johnson et al. 2001b). They consist of clear glass or plastic tubes that are installed in the rooted soil. The roots growing along the outside walls of the tubes can be monitored by means of a specialized video camera. By taking repeated images through time, the turnover of roots can be monitored from growth to decay.

The best estimates of root dynamics and turnover are obtained by a combination of the considered methods (Lauenroth 2000; Hertel and Leuschner 2002; Hendricks et al. 2006). The total mycorrhizosphere respiration can be calculated by multiplying the biomass specific respiration rate with the root biomass representative of a certain period. However, it has to be considered that biomass specific respiration rates may vary depending on phenology, activity or structural properties of the roots (Kutsch et al. 2001b). Another error may arise if mycorrhiza-free roots are measured in the lab and ecosystem scale mycorrhizosphere respiration is calculated by multiplying the rates obtained by these measurements with root biomass. In this case the mycorrhizosphere respiration might be underestimated.

Another approach to calculate mycorrhizosphere respiration at the ecosystem scale is to subtract aboveground litter input from total soil respiration (Ewel et al. 1987; Bowden et al. 1993). However, this approach – which should not be applied at shorter time scales than annual balances – assumes that the soil carbon budget is balanced and cannot distinguish between the direct respiration of assimilates and the mineralization of dead fine roots and usually results in higher values than pure mycorrhizosphere respiration estimates.

Table 5.1: Data after Kutsch et al. (2001a) and Kutsch et al (i.p.). A model was obtained from chambers containing roots and mycorrhiza in the Bornhöved site, and later used to obtain estimates for the Hainich forest. The data for GPP given by Kutsch et al. 2001a were including night-time dark respiration of the foliage and were corrected to pure daytime photosynthesis (original data in parentheses). Aboveground respiration was therefore also corrected for foliage dark respiration.

Site	Year(s)	Vegetation	GPP g m^{-2} a^{-1}	NPP g m^{-2} a^{-1}	Aboveground Respiration g m^{-2} a^{-1}	Mycorrhizosphere Respiration g m^{-2} a^{-1}	% GPP
Bornhöved	1992-1993	Beech Forest	1324 (1047)	656	437 (160)	231	17.4
Bornhöved	1992-1993	Alder Forest	2420 (2150)	843	397 (127)	1181	48.8
Bornhöved	1992-1993	Cropland (Maize)	1682 (1425)	1059	362 (105)	261	15.5
Hainich	2004	Beech dominated mixed Forest	1518	751	364	403	26.5

Total belowground carbon usage of the vegetation (mycorrhizosphere respiration plus fine root production) can also be estimated from total ecosystem carbon or nitrogen budgets (Aber et al. 1985; Nadelhoffer et al. 1985; Kutsch et al. 2001a; Kutsch et al. 2005; Hendricks et al. 2006). In case of carbon budgeting gross primary production (GPP) has to be derived either from eddy covariance measurements or from a canopy gas exchange model. Belowground carbon usage can be calculated by subtracting aboveground plant biomass production and aboveground plant respiration from GPP. The remaining part can be split into mycorrhizosphere respiration, fine root turnover and coarse root increment. Values calculated for different ecosystems are given as an example of this method in Table 5.1.

5.5. Concluding Remarks

Mycorrhizosphere respiration combines all sources of CO_2 respiring compounds derived from live plant roots. Physiological, ecological and environmental conditions determine where this carbon is respired - either by the root itself, by bacteria and fungi in the rhizosphere, or by mycorrhizal hyphae – as well as the amounts of C respired. Although many field and laboratory methods have been developed to determine respiration rates and controls of each component, each with its own advantages and drawbacks, partitioning respired carbon in the mycorrhizosphere remains a challenge. One major problem still to be solved is the disturbance of natural soil conditions while making measurements. Linking methods described in this chapter with the use of isotopic tracers (e.g. pulse and continuous labelling) is now being explored as a way forward in this area.

Further attention should be given to studying fluxes through root associated microorganisms. The exclusion of rhizosphere and mycorrhizal hyphae components when using most methods becomes especially important when considering the high proportion of root carbon exported to these sources, as shown by isotope and micro-pore mesh techniques. These together with other innovations, such as the use of shallow collars, make it clear that method development will lead to a better understanding of mycorrhizosphere carbon fluxes and from there to an improved modelling of the soil system.

The response of mycorrhizosphere respiration to climate change posses a further challenge to this area of research. The effects of changing CO_2 concentrations and temperature on mycorrhizosphere components, as well as degrees of acclimation, can play an important role in determining soil carbon dynamics in future scenarios. The response to these factors of different vegetation types or even of particular species of mycorrhizal fungi, necessary for determining ecosystem and global scale dynamics, is only starting to be explored.

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Symbols and Abbreviations

AM: arbuscular mycorrhiza

CFE: chloroform fumigation extraction

C:N: carbon to nitrogen ratio

EM: ectomycorrhiza

ERM: extra radical mycelium

GPP: gross primary productivety

IRGA: infra-red gas analyser

DIC: dissolved inorganic carbon

DOC: dissolved organic carbon

MBC: microbial biomass carbon

NEE: net ecosystem exchange

Q_{10}: change in the rate of respiration activity with a 10^{o}C increase in tempearture

Q_{10h}: Q_{10} derived from heterotrophic respiration values

R: respiration flux

$R_{0°C}$: respiration rate at 0°C

R_{15}: respiration flux normalized to a temperature of 15 °C

Reco: total ecosystem respiration

Rh: heterotrophic soil respiration

Rm: mycorrhizal fungal respiration

Rmr: mycorrhizosphere respiration

Rr: rhizosphere respiration

Rs: soil respiration

Rt: total soil respiration

R_T: respiration at temperature T

R_{To}: respiration at reference temperature To

SM: soil moisture

SOM: soil organic matter

ST: soil temperature

TOC: total organic carbon

u*: friction velocity of eddy flux measurements

w': vertical wind component of eddy flux measurements

Summary

Soils are main components of natural ecosystems, central for the growth of plants and the recycling of organic matter through the activity of microorganisms and soil fauna. As such, they exert a large influence on the cycling of carbon between different reservoirs, storing more carbon at the global scale than either the atmosphere or the live vegetation. The flux of carbon between different global reservoirs is being actively studied in the present largely as a result of its implications for climate change but also in an effort to understand the functioning of ecosystems and living organisms. The flux of carbon dioxide from soils to the atmosphere, also termed soil respiration, is the result of belowground plant activity combined with the decomposition of soil organic matter. To understand this flux it is necessary to study the factors driving the activity of roots as well as the dynamics of soil microorganisms and their use of soil organic matter or root-derived carbon. Tightly related to biological processes are physical and chemical conditions in soils which determine the availability of carbon compounds to microbes.

This study was carried out in agricultural, broad-leaf forest and needle-leaf forest temperate ecosystems with the objective of studying the effect of vegetation and soil factors on root, mycorrhizal and microbial related respiration fluxes. To attain this, soil respiration was partitioned in the field using meshes with micrometer large pores separating soil cores from the surroundings, allowing a selective ingrowth of fungal hyphae and thus the separation of mycorrhizal fungi from roots. Soil respiration measurements on soils with different pore-sized meshes and on control soils were used to calculate values of rhizosphere, mycorrhizal fungal and heterotrophic respiration fluxes. These fluxes were then related to canopy photosynthetic activity, soil temperature, soil moisture, soil microbial biomass and a number of other relevant soil parameters. Results showed the importance of mycorrhiza as a quick path of carbon from the canopy back to the atmosphere in croplands and temperate forests. The respiratory activity of mycorrhizal fungi was also shown to be strongly controlled by the availability of new carbon from the plant and little affected by changes in temperature. The respiration from

roots, more sensitive to changes in temperature, was also shown to be directly affected by photosynthetic activity.

In particular, respiration fluxes at the crop field showed clear differences between sources in their response to both temperature and photosynthetic activity. The respiration of arbuscular mycorrhizal hyphae was seen to be a significant amount of root-derived carbon respiration (25.3%) and consequently of total assimilated carbon (4.8%), with a strong response to photosynthetic activity after a time delay of one day. Q_{10} values (the change in respiration rates with a 10˚C increase in temperature) depended on the source of respiration and on the season, and were influenced by plant growth. The importance and controls of mycorrhizal fungal respiration in croplands are shown to be comparable to those observed in other ecosystems.

At the broad-leaf and needle-leaf forests, calculated mycorrhizal mycelium respiration amounted to 3% and 8% of total soil respiration respectively, representing minimum estimates. The ratio of root-derived carbon respiration to heterotrophic respiration was nearly 1:1 at both forest types. Temperature and photosynthesis revealed effects specific to the respiration source and the forest type. Mycorrhizal respiration showed weak or insignificant temperature relations. Relations of photosynthesis with rhizosphere and mycorrhizal fungal respiration were found in all cases. Peaks in correlation values showed time lags between photosynthetic activity and a respiration response ranging from one day for the fungal component to four and five days for the rhizosphere component.

An analysis of the spatial variability of respiration fluxes at all sites showed a higher spatial variability of the root-derived flux compared to the heterotrophic component at the forest sites and the opposite relation at the crop field. Results also indicated important spatial relations of nitrogen availability and soil water content (direct and indirect) with rhizosphere respiration, as well as of substrate supply and microbial biomass with microbial respiration. In addition, results point towards possible priming effects on decomposition through the input of labile carbon by mycorrhizal fungi, and negative effects of ammonium on decomposition.

Zusammenfassung

Böden stellen einen wichtigen Teil natürlicher Ökosysteme dar und spielen eine zentrale Rolle für das Pflanzenwachstum und die Wiederverwertung organischen Materials durch Mikroorganismen und Bodenfauna. Sie haben einen großen Einfluss auf den Kohlenstoffkreislauf, indem sie auf globaler Ebene mehr Kohlenstoff speichern als die Atmosphäre oder Vegetation. Der Kohlenstofffluss zwischen verschiedenen globalen Kohlenstoffspeichern wird derzeit aufgrund seiner Bedeutung für den Klimawandel, aber auch um die Funktionsweise von Ökosystemen und lebenden Organismen zu verstehen, intensiv untersucht.

Die Bodenatmung ist das Ergebnis unterirdischer Pflanzenaktivität und der Zersetzung organischen Bodenmaterials. In enger Beziehung zu den biologischen Prozessen stehen physikalische und chemische Bedingungen im Boden, die die Verfügbarkeit von Kohlenstoffverbindungen für Mikroorganismen bestimmen. Um den Kohlenstoffkreislauf verstehen zu können, ist es erforderlich, diejenigen Faktoren zu untersuchen, die einerseits die Wurzelaktivität und andererseits die Dynamik von Bodenorganismen und deren Nutzung von organischem Bodenmaterial bzw. wurzelbürtigem Kohlenstoff steuern.

Die vorliegende Studie wurde in Agrar- sowie Laub- und Nadelwaldökosystemen der gemäßigten Breiten (Mitteleuropa) durchgeführt. Ihr Ziel ist es, die Auswirkungen von Vegetations- und Bodenfaktoren auf den Respirationsfluss von Wurzeln, Mykorrhiza und Mikroorganismen zu untersuchen. Hierfür wurde die Bodenatmung bei den Felduntersuchungen in verschiedene Komponenten aufgeteilt. Es wurden Netze mit einer Porengröße im Mikrometerbereich verwendet, die die Bodenkerne von ihrem Umfeld trennten und somit das selektive Einwachsen von Pilzhyphen ermöglichten. Um Werte für den Respirationsfluss von Rhizosphäre, Mykorrhizapilzen und heterotrophen Organismen berechnen zu können, wurden Bodenatmungsmessungen von Böden in Netzen unterschiedlicher Porengröße sowie von Kontrollböden vorgenommen. Die so ermittelten Komponenten der Bodenatmung wurden dann zu Photosyntheseaktivität, Temperatur und Wassergehalt des Bodens, der mikrobiellen Biomasse und anderen

relevanten Bodenparametern in Beziehung gesetzt. Die Ergebnisse zeigten die Bedeutung der Mykorrhiza als schneller Weg des von den Planzen assimilierten Kohlenstoffs zurück in die Atmosphäre. Die Messungen ergaben, dass die respiratorische Aktivität von Mykorrhizapilzen in hohem Maße abhängig von der Verfügbarkeit neuen Kohlenstoffs aus der Pflanze und weitgehend temperaturunabhängig war. Die Atmungsaktiviät der Wurzeln, die stärker auf Temperaturänderungen reagierten, wurde signifikant von der Photosyntheseaktivität beeinflusst.

Speziell der Respirationsfluss auf landwirtschaftlich genutzten Flächen zeigte deutliche Unterschiede zwischen den Atmungsquellen bezüglich ihrer Abhängigkeit von Temperatur und Photosyntheseaktivität. Die Ergebnisse zeigten, dass die Atmung der Hyphen arbuskulärer Mykorrhiza einen signifikanten Anteil der wurzelbürtigen Kohlenstoffrespiration (25,3 %) und demnach auch des gesamten assimilierten Kohlenstoffs (4,3 %) repräsentierte. Hierbei konnte ein deutlicher Bezug zur Photosyntheseaktivität mit eintägiger Verzögerung nachgewiesen werden. Q_{10}-Werte (Änderungen der Respirationsrate bei einer Temperaturerhöhung von 10° C) waren abhängig von der Quelle der Respiration und der Jahreszeit und wurden außerdem vom Pflanzenwachstum beeinflusst. Die Steuerung der Atmung bei Mykorrhizapilzen auf Agrarflächen war vergleichbar mit Beobachtungen in anderen Ökosystemen.

Die errechnete Respiration bei Mykorrhizapilzen in Laub- und Nadelwäldern erreichte nach minimalen Schätzungen 3 % bzw. 8 % der gesamten Bodenatmung. Das Verhältnis von wurzelbürtiger Atmung und heterotropher Atmung lag bei nahezu 1:1 in beiden Waldformen. Temperatur und Photosynthese zeigten spezifische Auswirkungen auf die jeweilige Respirationsquelle. Die Mykorrhizarespiration zeigte lediglich eine schwache oder nicht signifikante Temperaturabhängigkeit. Ein Zusammenhang zwischen Photosynthese und der Respiration bei Rhizospäre und Mykorrhizapilzen konnten in allen Fällen nachgewiesen werden. Zeitliche Verzögerungen zwischen der Photosyntheseaktivität und der darauf folgenden Respirationsreaktion betrugen von einem Tag bei der Pilzkomponente bis zu vier und fünf Tagen bei der Rhizosphärenkomponente.

Eine Analyse der räumlichen Variabilität des Respirationsflusses auf allen Versuchsflächen ergab eine höhere räumliche Variabilität des wurzelbürtigen CO_2-

Flusses im Vergleich zur heterotrophen Komponente auf den Waldflächen und eine gegensätzliche Beziehung auf landwirtschaftlich genutzten Flächen. Die Ergebnisse wiesen außerdem auf wichtige räumliche Beziehungen zwischen Stickstoffverfügbarkeit und Wassergehalt des Bodens (sowohl direkt als auch indirekt) mit der Rhizosphärenrespiration sowie zwischen Substratverfügbarkeit und mikrobieller Biomasse mit mikrobieller Respiration hin. Außerdem verwiesen die Resultate zum einen auf mögliche „priming“ Effekte bei Abbauprozessen aufgrund des Eintrags leicht verfügbaren Kohlenstoffs durch Mykorrhizapilze und zum anderen auf negative Auswirkungen von Ammonium auf Abbauprozesse.

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Fernando E. Moyano Jena, October the 24th 2007