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Autotrophic and heterotrophic soil respiration in a Norway spruce forest: estimating the root decomposition and soil moisture effects in a trenching experiment

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Abstract The two components of soil respiration, autotrophic respiration (from roots, mycorrhizal hyphae and associated microbes) and heterotrophic respiration (from decomposers), was separated in a root trenching experiment in a Norway spruce forest. In June 2003, cylinders (29.7 cm diameter) were inserted to 50 cm soil depth and respiration was measured both outside (control) and inside the trenched areas. The potential problems associated with the trenching treatment, increased decomposition of roots and ectomycorrhizal mycelia and changed soil moisture conditions, were handled by empirical modelling. The model was calibrated with respiration, moisture and temperature data of 2004 from the trenched plots as a training set. We estimate that over the first 5 months after the trenching, 45% of respiration from the trenched plots was an artefact of the treatment. Of this, 29% was a water difference effect and 16% resulted from root and mycelia decomposition. Autotrophic and heterotrophic respiration contributed to about 50% each of total soil respiration in the control plots averaged over the two growing seasons. We show that the potential problems with the trenching, decomposing roots and mycelia and soil moisture effects, can be handled by a modelling approach, which is an alternative to the sequential root harvesting technique.

Keywords Soil moisture · *Picea abies* · PLS · Root respiration · Root decomposition · Soil temperature

Introduction

The net ecosystem carbon (C) exchange with the atmosphere is a balance between two large fluxes, the influx through photosynthesis and the outflux by ecosystem respiration. Soil respiration is the major component of forest ecosystem respiration (Lavigne et al. 1997). Today, temperate and boreal forests act as sinks for CO_2 (Dixon et al. 1994; Liski et al. 2003), though there is uncertainty whether they will persist as C sinks or become sources due to climate change (Rustad et al. 2001). A key question in this matter is whether the two components of soil respiration, autotrophic respiration (from roots, mycorrhizal fungi and free-living microorganisms in the rhizosphere) and heterotrophic respiration (from decomposition of litter and soil organic matter), will respond differently to changing environmental conditions (Boone et al. 1998; Epron et al. 2001; Högberg et al. 2001).

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Accurate estimation of the contribution of each component is thus needed to evaluate implications of environmental change on soil C cycling and sequestration.

The relative contribution of autotrophic respiration to soil respiration has been shown to vary between 1:9 and 9:1 (Hanson et al. 2000). Although some of this variability reflects differences among types of ecosystems and season of the year, a considerable proportion of it is probably due to the variety of measurement techniques, each with a unique set of limitations (Hanson et al. 2000). The methods used to estimate the contributions of root- and heterotrophic respiration to total soil respiration include; (1) *root exclusion*, (2) *component integration* of respiratory components (e.g. litter, SOM, roots, rhizosphere) and (3) *stable or radioactive isotope methods.* These methods have been reviewed by Hanson et al. (2000).

Trenching is a *root exclusion* method, commonly used in forest ecosystems (e.g. Ewel et al. 1987; Bowden et al. 1993; Epron et al. 1999; Lee et al. 2003). With the insertion of a barrier in the soil within a certain area, the connection between the roots and the tree is effectively ended and thus also the transport of photosynthetic products to the roots. A problem with the trenching method is the influence of residual decomposing roots (Hanson et al. 2000; Epron et al. 1999; Ngao et al. 2007) and of ectomycorrhizal fungi (Högberg and Högberg 2002), which can result in an overestimation of the heterotrophic respiration. In a beech forest the estimated contribution of heterotrophic respiration to total soil respiration was reduced from 73 to 48% when the decomposition of roots was taken into account (Epron et al. 1999). However, also this approach has its drawbacks and limitations. The partitioning calculation is highly sensitive to the pre-trenching root mass estimate (Ngao et al. 2007). In addition, to extract roots in a quantitative way can be difficult due to heterogeneous root-distribution, making a root harvest cumbersome, tedious and time-consuming, in particular in stony soils. Furthermore, the contribution to respiration from increased decomposition of extraradical mycorrhizal mycelium is not taken into account using the root extraction method. The mycorrhizal mycelium can have a standing biomass that is similar to that of the fine roots (Wallander et al. 2001). Increased decomposition of this mycelium after a tree-girdling treatment was indicated by increased nitrogen content and a shift upwards in the δ^{15}N (Bhupinderpal-Singh et al. 2003) in the leaves of dwarf shrubs in the understory and also by a decline in microbial C by 60 kg ha^{-1} (Högberg and Högberg 2002). The lifetime of mycorrhizal rhizomorphs, which is possibly a large part of the standing biomass, can be as long or longer than that of individual mycorrhizal root tips (Treseder et al. 2005; Pritchard et al. 2008). To reduce the impact of decomposing roots and mycorrhizal mycelium, some studies instead allowed several months to pass between the trenching and the start of soil respiration measurements (Ewel et al. 1987; Bowden et al. 1993). Another problem is that trenching terminates plant uptake of water which potentially results in higher soil water contents in trenched compared to control plots (Epron et al. 1999; Högberg et al. 2005; Kuzyakov 2006). This soil moisture effect is in well-drained soils probably most problematic during seasons with unusually dry and warm weather, when drought stress may reduce microbial respiration in the control plots. While in soils with high soil water holding capacity, long periods of wet weather may instead hamper gas diffusion in the trenched plots (Rey et al. 2002).

In this study we applied a novel empirical modelling approach to estimate the effects root and mycelia decomposition and treatment differences in soil water content have on soil respiration rates in the trenched plots. We used data from the trenched plots the second season after trenching to calibrate the model and used this model to generate heterotrophic respiration rates over two successive years. Our main aim was to estimate autotrophic and heterotrophic respiration rates corrected for the artefactual effect of the trenching treatment.

Methods

Site description

The study was performed in a 22 m tall, Norway spruce [*Picea abies* (L.) Karst] forest, planted 1934 on former farmland close to Brevens bruk, southern Sweden (59°00′N, 15°00′E, 125 m above sea level). Scattered birch (*Betula pendula* Roth) trees of approximately the same height as the spruce trees occur in the stand. No understory plants or mosses are found on the ground. The soil is a sandy, washed till with a C content, of 13.8% in the upper 2 cm of the soil profile, decreasing down to 1.6% at 20 cm depth (Boström et al. 2007). Living fine root (≤2 mm) biomass down to 20 cm depth was estimated to about 420 g dry weight m^{-2} in August 2004, with approximately two-thirds in the upper 10 cm of the soil (Boström et al. 2007). Assuming an exponential decline with depth, we estimate a root biomass of 38 g m^{-2} between 20 and 50 cm depth and 2.8 g m^{-2} between 50 and 100 cm depth. Thus, we estimate that about 0.6% of a total root biomass of 459 g m^{-2} down to 1 m depth was found between 50 and 100 cm depth.

Plot design and soil respiration measurements

Eight control plots (cross-sectional area 0.0464 m^2) where laid out in 2002, with a distance between each of 3–5 m (Ekblad et al. 2005). In the beginning of April 2003, two more plots were added, making it a total of ten control plots. In addition, ten plots that later on became trenched were placed at a distance of a minimum of 2.5 m from each of the control plots, but always with at least one tree in between the two types of plots. The trenching was carried out on June 17, 2003 by inserting PVC cylinders (29.7 cm inner diameter) to a 50-cm soil depth by pushing and rotating the cylinders.

In 2003 the soil respiration sampling covered the period April–November and occurred 19 times from the control plots and 18 times from the trenched plots, of which in both cases, 11 occurred after the trenching took place. In 2004 respiration was sampled eight times over the period June–October. Soil respiration measurements were at each occasion carried out during the middle of the day as previously described (Ekblad and Högberg 2000). Briefly, an opaque PVC cylinder (cross sectional area, 0.0464 m^2) with a removable lid was at sampling placed on the forest floor on each plot creating a 6-l headspace. Three to five gas samples (12-ml each) were then withdrawn with a syringe at intervals over a period of 8–10 min. Each sample was directly transferred to pre-evacuated (within 24 h before sampling) glass-vials (Labco Exetainer; High Wycombe, Castleford, England). Analysis of CO_2 concentrations was made within less than a week as described by Ekblad et al. (2005).

Meteorological data

Measurements of temperature and moisture in the stand were conducted at the time of gas sampling. Soil temperature at 5 cm depth and air temperature at 1.5 m height were measured using a Tinyview Plus Temp H with a 14 cm long temperature probe (Intab, Stenkullen, Sweden). In addition, soil temperature at 5 cm depth was recorded using a Tinytag Plus data logger. Soil moisture (percentage of d.w.) in the upper 8 cm of the soil was measured using a Theta probe type ML2 (Delta-T Devices, Cambridge, England).

Modelled heterotrophic and autotrophic respiration rates in control plots

Instead of simply taking the difference between the two types of plots, we estimated the autotrophic and heterotrophic respiration in the control plots with an empirical modelling approach. This was a way to handle the potential problems with, firstly, soil moisture differences between control and trenched plots and secondly, the initial flux of respiration from decomposition of roots and mycorrhizal hyphae in the trenched plots. In the modelling we used a multivariate statistical tool called partial least squares projection to latent structures analysis (PLS; Simca 8.0 program; Umetrix, Umeå, Sweden). The PLS-method is used much in the same way as multiple regression analysis but an important difference is that PLS can handle correlated *x*-variables, which preclude the use of multiple regression (James and McCulloch 1990). Since temperature and moisture variables, as used in our case, often covaries we chose the PLS-method instead of the more commonly applied multiple regression analysis (see Ekblad et al. 2005 and references therein for further information about the PLS-method). As a calibration set in the modelling we used data from the trenched plot from 2004 with soil respiration as a *y*-variable and soil moisture and soil temperature as *x*-variables to generate a PLS-model. We assumed in this modelling that the contribution from decomposition of roots and mycorrhizal mycelia was minor during this second season after trenching and that the differences in heterotrophic respiration rates between the control and trenched plots were mainly caused by moisture differences. In step two of the modelling, we used the PLS-model to predict "root-free" heterotrophic respirations rates in the control plots 2003 and 2004 and in the trenched plots 2003 by using soil moisture and soil temperature from the respective years and plots as *x*-variables. Autotrophic respiration was then calculated as the difference between the recorded soil respiration rates in the control plots and the modelled heterotrophic respiration in the same plots.

Total seasonal respiration was estimated by multiplying the average respiration rate of two adjacent measurements with the number of hours passed between the two measurements. These numbers were summed up over the whole season.

Statistical analysis

In the PLS-modelling, all the variables were scaled to unit variance before analysis and logarithmic values of soil respiration rates (in *soil respiration rate*) were used. The explained variance (Q^2) of the PLS-model was calculated by cross-validation (see Ekblad et al. 2005 and references therein for further details on PLS-modelling).

Results

Temperature and soil moisture

Air temperature measured on the day of sampling ranged between 6 and 27°C in 2003 and between 10 and 20°C in 2004, with the highest values recorded July 15 and June 2 (Fig. 1). A similar seasonal pattern was observed for soil temperature, ranging between 6 and 19°C in 2003 and between 9 and 15.5°C in 2004, with the highest values recorded July 15 and August 17. The diurnal variation in soil temperature (at 5 cm depth) was ±0.6°C and the recorded mid-day temperature was close to the diurnal mean (data not shown). Soil moisture content in control plots showed no clear seasonal trends, varying between 16 and 27% in 2003 and 17 and 27% in 2004 (Fig. 1). After trenching, soil moisture content in the trenched plots increased by 4% ($p < 0.05$) after 6 days compared to the control (June 23) and was thereafter significantly higher in the trenched plots than in the control ($p < 0.05$) by 3–7% in 2003 (except on November 6) and by 4–10% in 2004.

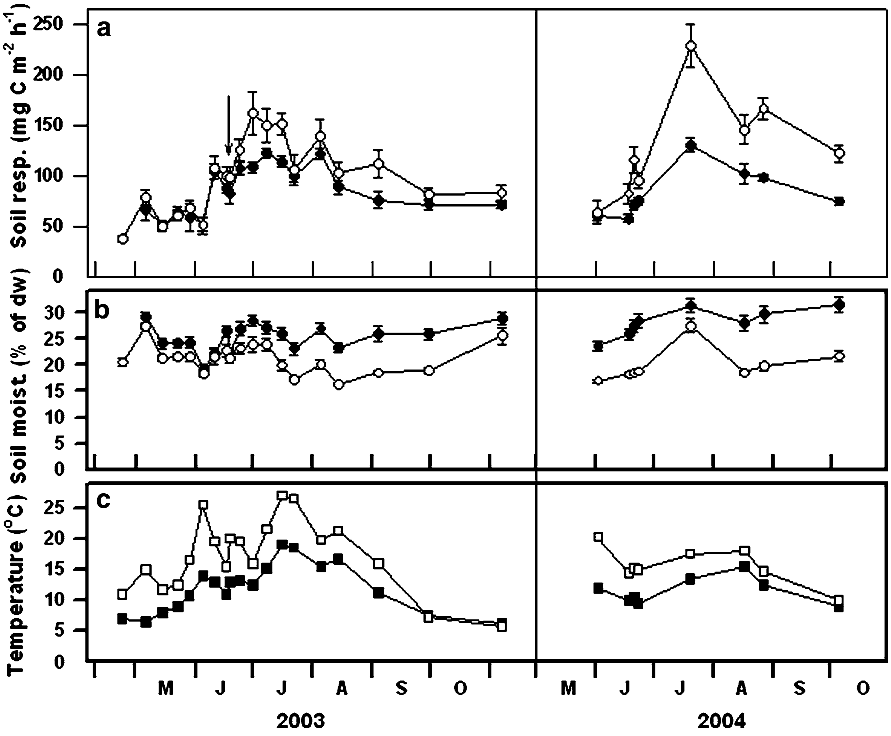

Fig. 1 Soil respiration and meteorological data from a Norway spruce forest close to Brevens bruk, Sweden, for the years 2003 and 2004. **a** Soil respiratory CO_2 efflux from control plots (*open circles*) and trenched plots (*filled circles*). **b** Soil moisture content (% of d.w.) in the control (*open circles*) and in the trenched (*filled circles*) at time of CO_2 sampling. **c** Air temperature (*open squares*) and soil temperature at 5 cm depth (*filled squares*) at time of CO_2 sampling. *Arrow* indicates the start of the trenching experiment. *Error bars* show ± SE for $n = 10$

Soil respiration in control plots

Soil respiration rates from the control plots were generally low in April to early June, ranging between 37 and 78 mg C m^{-2} h^{-1} (Fig. 1). In June 2003 and July 2004, there was a sharp increase in respiration rates. In fact, total soil respiration rates increased by more than 300% within less than 3 weeks in 2003. Respiration peaked on June 30 in 2003 (162 mg C m^{-2} h^{-1}) and on July 20, 2004 (229 mg C m^{-2} h^{-1}). The rates decreased towards the end of each season to values ranging between 72 and 122 mg C m^{-2} h^{-1}, which was slightly higher than that recorded during April to early June.

Soil respiration in trenched plots

Before the trenching on June 17, 2003, there were no significant differences ($p > 0.05$) in soil respiration rates between the control and the plots that became trenched (Fig. 1). After trenching, respiration rates in the trenched plots were significantly ($p < 0.05$) lower than the control by 53 mg C m^{-2} h^{-1} (33%) after 13 days. In the remaining part of 2003, the difference between the treatments varied from 6 to 38 mg C m^{-2} h^{-1} (6–32%), while in 2004 the difference varied from 4 to 98 mg C m^{-2} h^{-1} (7–43%). For both seasons, the greatest difference in respiration rates between trenched and control plots coincided with the seasonal peak in soil respiration rates (June 30, 2003 and July 20, 2004). Integrated for each season (June 18–November 6, 2003 and June 2–October 5, 2004), the difference in soil respiration between trenched and control plots was 19% (69 g C m^{-2}, $p \leq 0.05$, $n = 10$) in 2003, and 37% (163 g C m^{-2}, $p \leq 0.05$, $n = 7$) in 2004. Sporocarps of ectomycorrhizal fungi were found in two of the trenched plots in October 2004, while no roots were found in the soil inside the cylinders at this time.

Modelled autotrophic and heterotrophic respiration in control plots

Soil moisture or soil temperature alone explained only about 50% of the variation in soil respiration from the trenched plots in 2004 (Fig. 2a, b). Taken together in the PLS-modelling of the calibration set (data from the trenched plots 2004), soil temperature

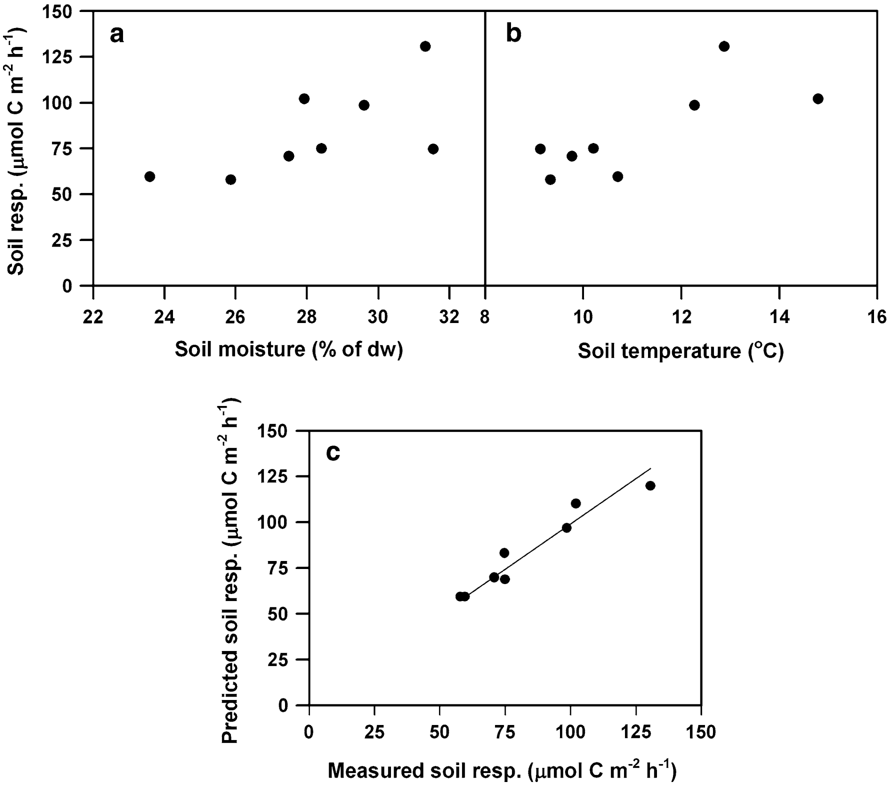

Fig. 2 **a** Measured soil respiration in the trenched plots 2004 versus soil moisture and **b** versus soil temperature. **c** Measured soil respiration in the trenched plots 2004 on the *x*-axis and predictions from the PLS-model on the *y*-axis

and soil moisture together explained as much as 90% of the temporal variation in respiration rates (Fig. 2c; cross-validated explained variance; Q^2).

This PLS-model generated heterotrophic respiration rates in the control plots that varied seasonally between 28 and 82 mg C m^{-2} h^{-1} in 2003 and between 33 and 90 mg C m^{-2} h^{-1} in 2004 (Fig. 3). The autotrophic respiration rates, calculated as the difference between the recorded soil respiration rates and modelled heterotrophic respiration rates, showed a large range between the highest and lowest value in both growing seasons. Thus, the rates varied between 6 and 99 mg C m^{-2} h^{-1} in 2003 and between 28 and 139 mg C m^{-2} h^{-1} in 2004. The autotrophic respiration rates were generally low in spring and early summer (April to early June) and peaked in late June to July. The temporal variation in autotrophic respiration rates followed that of total soil respiration closely, which was particularly obvious in 2003, when the sampling was more frequent (Fig. 3).

The relative contribution of modelled autotrophic respiration to total soil respiration was low and variable during the early part of each season (April to early June) ranging from 0% to 43% (Fig. 3). After this, the relative contribution from autotrophic respiration increased and ranged from 35 to 72% from mid June onwards. The yearly increase in relative contribution to total soil respiration coincided with the steep increase in total soil and autotrophic respiration rates observed in June, which also coincided with a period when a rapid growth of fine roots was found (D. Comstedt, personal observation). The peaks in relative proportion of autotrophic respiration occurred in late August and September at 63% in 2002, 66% in 2003 and 72% in 2004. Integrated over the seasons, the relative contribution of modelled autotrophic respiration (R_{Rp}) to control soil respiration (RC) was 55% in 2003 (calculated from the time of trenching and onwards) and 64% in 2004.

Estimating the effect of root decomposition and increased water content on respiration in trenched plots

Integrated over the period June 18 to November 6 in 2003, respiration in the trenched plots was 299 g C m^{-2} which was only 69 g C m^{-2} or 19% lower than the 368 g C m^{-2} in the control plots (Fig. 4). This is significantly less than what would be expected since the modelling estimated the heterotrophic respiration to contribute with about 165 g C m^{-2} or 45% of soil respiration in the control plots over that period (Fig. 4). This difference between the estimated heterotrophic respiration in the control and recorded respiration from the trenched plots of 134 g C m^{-2} is mainly due to root and mycelia decomposition and differences in soil moisture conditions. Thus, 45% of the respiration in the trenched plots was an unwanted artefact of the

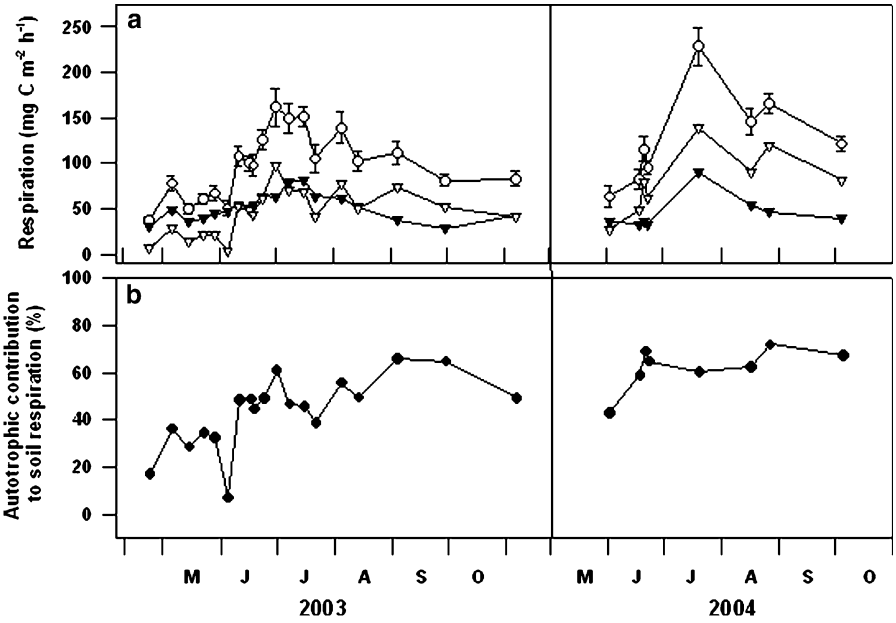

Fig. 3 **a** Measured soil respiration (*open circles*) and modelled heterotrophic respiration (*closed triangles*) and autotrophic respiration (*open triangles*) in control plots. **b** Contribution (%) of modelled autotrophic respiration to measured soil respiration in control plots. *Error bars* show ± SE for *n* = 10

trenching. By using the PLS-model and the soil moisture and temperature data of the trenched plots we estimate a "root-free" respiration in the trenched plots of 252 g C m^{-2}, which is 87 g C m^{-2} higher than the estimated heterotrophic respiration in the control. This water effect account for 29% of the recorded respiration from the trenched plots (Fig. 4). Thus, the contribution from root and mycelia decomposition is estimated to 47 g C m^{-2} or 16% of the recorded respiration in the trenched plots.

Discussion

Effect of increased C supply from decomposing roots and increased soil water content on soil respiration in trenched plots

While a post trenching increase in respiration from the flush of decomposing roots has been estimated in several studies by sequential harvesting of roots (e.g. Epron et al. 1999; Lee et al. 2003; Ngao et al. 2007), the effect of differences in soil moisture conditions is often neglected in trenching experiments. In this paper we used an empirical modelling approach to estimate both these artefacts of the trenching treatment. Without taking them into account, we would have overestimated the heterotrophic contribution to total soil respiration by 36% (81% instead of the corrected 45%) in 2003 and by 27% (63% instead of the corrected 36%) in 2004. Our results are comparable with those found in a beech forest in which the heterotrophic contribution to total soil respiration was reduced from 73 to 39% after removing the effect of root decomposition and soil water differences (Epron et al. 1999).

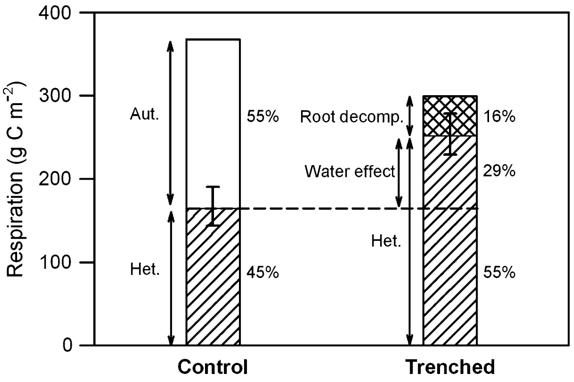

Fig. 4 The contribution of autotrophic and heterotrophic respiration to soil respiration in the control plots and from root decomposition and soil moisture changes in the trenched plots integrated over the first 5 months after root trenching in June, 2003. In the control plots, autotrophic respiration (*open bars*) was calculated as the difference between soil respiration and modelled heterotrophic respiration (*diagonal lined bars*). In the trenched plots, root decomposition (*crossed-hatched bars*) was calculated as the difference between soil respiration and modelled "root free" heterotrophic respiration in these plots. The effect of increased water content (water effect) was calculated as the difference between modelled heterotrophic respiration in the trenched plots and heterotrophic respiration in the control plots. *Error bars* show 95% CI for the predicted heterotrophic respiration using the PLS-model

In our study, the effect of differences in soil water content was about twice as large as the effect of root and mycelia decomposition in 2003 (Fig. 4). This contrasts to the results from two studies from beech forests in which the major effect was from root decomposition and only a minor part was estimated to derive from differences in soil water content (Epron et al. 1999; Ngao et al. 2007). Differences in the relative importance of soil moisture in trenching experiments may be caused by site differences in precipitation patterns, differences in soil water holding capacities as well as differences in root densities. Since the water holding capacity is relatively low in the sandy soil of our forest, water uptake by roots has caused significant differences in soil moisture contents between the control and trenched plots. The effect of treatment differences in water content on heterotrophic respiration rates is likely to be relatively more important during sunny, warm and dry years than during cloudy, cool and wet years.

A response to the trenching is not only an increased decomposition of roots, but also probably an increased decomposition of extraradical ectomycorrhizal mycelia (Högberg and Högberg 2002). The contribution to respiration from this component is probably smaller when the trenching occurs in June, as in our case, compared to if the trenching had occurred in the end of August. This is because the mycelial production is mainly in the second half of the growing season (Wallander et al. 2001; Boström et al. 2007). The respiration from this decomposition is probably passed much faster than for root decomposition. As much as 20–80% of dry matter was lost after 1 month incubation in a forest soil of mycelia enclosed in litter bags (Koide and Malcolm 2009). As a comparison, the half life of decomposing roots in a beech forest was about 2 years (Epron et al. 1999). The decomposition rates in Koide and Malcolm (2009) are based on mycelia that contained only single hyphae. However, forests in seasonally dry habitats, as in the present study, are dominated by rhizomorph forming fungi (Unestam 1991). Living rhizomorphs may have a lifetime that is similar to or even longer than that of the mycorrhizal tips (Treseder et al. 2005; Pritchard et al. 2008). Severed rhizomorphs probably decompose more slowly than single hyphae due to a smaller specific surface area, and possibly a lower nitrogen concentration. Some of them may be hydrophobic, which may retard decomposition significantly (Koide and Malcolm 2009).

Factors governing the autotrophic respiration

That the autotrophic respiration rates showed great temporal variations which were strongly correlated to total soil respiration rates in the control plots (Fig. 3), while the heterotrophic respiration rates followed instead a seasonal pattern, suggests that autotrophic respiration had a major impact on temporal variations in soil respiration rates. This is in line with results from multivariate modelling of the relation between aboveground climatic conditions and recorded soil respiration rates and its δ^{13}C signatures presented elsewhere (see Ekblad et al. 2005).

The steep increase in autotrophic respiration in June (Fig. 3) probably coincides with a flush in fine root production which is known to peak shortly after shoot growth has ceased (Majdi et al. 2001). The relative contribution of autotrophic respiration to total soil respiration peaked at 63–72% in August–September which was the period with the highest autotrophic respiration rates in two girdling experiments in northern Sweden (Högberg et al. 2001; Bhupinderpal-Singh et al. 2003; Olsson et al. 2005). This period also coincides with the time of the growing season when the production of extramatrical mycelium of mycorrhizal roots is believed to be largest (Wallander et al. 2001).

Possible uncertainties with the applied method

In our modelling we assumed the contribution of root decomposition to soil respiration in the trenched plots to be minor during the second growing season after the trenching and that the difference between control and trenched plots at that time mainly was caused by treatment differences in soil moisture conditions. It is possible that decomposition of dead roots contributed somewhat to respiration in the trenched plots also during the second season after trenching. If we assume an exponential decay with a decay constant of 0.3, which is a typical value for conifer roots (Silver and Miya 2001), and a C utilization efficiency of 0.5 (Six et al. 2006), we estimate a contribution from fine root decomposition over the period of soil respiration measurements the second season after trenching to be ~7 g C m^{-2}. This corresponds to ~2.5% of the estimated accumulated respired CO_2 from the trenched plots over that period. However, it is not obvious that our approach will result in a slightly overestimated heterotrophic respiration in the control since in this treatment, dying roots contribute with litter to the heterotrophs each year while this is not the case in the trenched plot. For example, in a cool-temperate deciduous forest the contribution of root turnover in the control was estimated to amount to 0.5–5% of total soil respiration (Lee et al. 2003).

An inherent problem with both trenching experiments and girdling experiments is that the treatment terminates all interactions between living plant roots and their mycorrhizal partners on one side and the abiotic and biotic environment around them on the other. If this has, in our case, had any effect on the respiration coming from decomposition of soil organic matter by heterotrophs is unknown. Results in the literature show increased litter decomposition (e.g. Gadgil and Gadgil 1971; Moorhead et al. 1998) as well as reduced microbial activities (e.g. Cheng et al. 2003; Subke et al. 2004; Göttlicher et al. 2006; Cheng 2008), in response to the absence of root activities. Which direction such effects take may be controlled by the availabilities of nitrogen and C (Kuzyakov 2002) but soil moisture may also play a role (see further below).

Another possible problem with root trenching possibly causing a slight overestimation of the heterotrophic component is a re-colonization of roots and mycorrhizal mycelia into the trenched area. We did not expect extensive root or mycelium colonization within the course of this study because of the very low root biomass below the depth of the root barrier. While re-colonization of roots did not take part over the time-frame of this study, the appearance of mycorrhizal sporocarps in October 2004, 16 months after the trenching, suggest that mycorrhizal mycelium has colonized the plots at a higher rate than roots. A similar colonization rate was found in a detailed study of mycelial colonization into 16 cm diameter circular plots, trenched to 25 cm depth in a Norway spruce forest (Wallander et al. 2001). Thus, despite a trenching depth of 50 cm in our case, colonization by ectomycorrhizal mycelium had obviously taken part at about the same rate as in the example mentioned. Based on the harvest of hyphal ingrowth mesh bags placed at five depths in the same forest stand (Boström et al. 2007), and in plots trenched to a depth of 20 cm (Comstedt et al., unpublished), the growth of mycorrhizal mycelium into the trenched plots in the present study was mainly in the later part of the second season after trenching.

An uncertainty in our modelling is that the range in soil moisture and temperature conditions of the calibration set did not cover the whole range of soil moisture and temperature conditions recorded over the two growing seasons (Fig. 1). In our modelling a log-linear relation between the *y* and *x*-values gave the best fit (see Methods section). Laboratory studies of decomposition (Kätterer et al. 1998) as well as studies from trenching experiments (Epron et al. 1999) show that there is an exponential relation between soil temperature and heterotrophic respiration over an even larger temperature range than was covered in the present study, which is an indirect validation of our method. However, the relation between heterotrophic respiration and soil moisture conditions is more difficult to establish with certainty due to the confounding effects of co-variations with soil temperature. Recently, this confounding effect was removed in a prolonged drought experiment in which roofs excluded the throughfall over prolonged periods in a temperate deciduous forest (Borken et al. 2006). Differences in soil respiration rates between control and drought treated plots were positively correlated to treatment differences in soil gravimetric water contents in the O horizon (Borken et al. 2006) which would give support to the use of our method. However, other studies suggest that there exist threshold soil moisture conditions below which soil moisture strongly regulates the microbial activity. This threshold was found to be 12% (v/v) in a temperate hardwood forest (Davidson et al. 1998) and 20% (v/v) in a Mediterranean Oak forest (Rey et al. 2002). In our forest the volumetric water content was never below 14% (estimated from our data and data on bulk density, Boström, pers. comm.). Thus, it is possible that heterotrophic soil respiration rates are somewhat overestimated during dry soil conditions in our study. However, this would only marginally affect our seasonal integrated estimates. This since respiration rates are low during days with dry soils and an error in the partitioning of respiratory fluxes during such days would have only minor effects on the integrated fluxes.

The use of mid-day measurements to integrate the seasonal soil respiration rates may be criticized from the view that there might be diurnal variations that are not captured in our data. Significant diurnal variations in soil respiration rates have been found in some cases (e.g. Tang et al. 2005; Heinemeyer et al. 2007) but not in others (e.g. Betson et al. 2007). Diurnal variations could potentially be caused by fluctuations in the availability of substrates used in autotrophic respiration or by fluctuations in soil temperatures or both. That the mid-day soil temperature was close to the diurnal average soil temperature, and that the diurnal variations in soil temperature were very small, suggest any error caused by diurnal temperature variations to be minor. This implies that diurnal variations in soil respiration rates in the trenched plots, which should be driven mainly by soil temperature changes, are probably minor. In the control plots, substrate availability could potentially cause diurnal variations in autotrophic respiration. In our forest, night lengths are relatively short (<8 h for most of the growing season), the trees were large and understory plants are lacking, facts that speak against large substrate availability effects on diurnal variations (Betson et al. 2007). However, there are more and more evidence that there is often a tight link between canopy photosynthesis and below ground autotrophic respiration (e.g. Ekblad and Högberg 2001; Ekblad et al. 2005; Comstedt et al. 2006; Comstedt 2008; Mencuccini and Hölttä 2009; Kuzyakov and Gavrichkova 2010). Indeed, autotrophic respiration in northern temperate forests may be more regulated by substrate availability than by soil temperature (Ekblad et al. 2005; Comstedt et al. 2006; Comstedt 2008). But, in these tall trees the lag time between changes above and below ground activities is between 1 and 4 days (Ekblad et al. 2005; Comstedt 2008), speaking against substrate driven diurnal variations in autotrophic respiration.

How problematic is the fact that our estimated cumulative respiration rates are based on relatively few data points? We tested how robust our estimated cumulative respiration rate is by repeatedly removing four of the 19 days sampled from the control plots over the 2003 growing season. When doing this analysis, we did never deviate more than 12 g C m^{-2} from the estimated total of 459 g C m^{-2}, even if we systematically removed the days with the four highest or lowest respiration rates from the data. This simple analysis suggests that the number of data points in the present study were sufficient to receive a good estimate of the accumulated respiration rate.

Applicability of the current method

The method used here is applicable in all kinds of root trenching and tree girdling experiments. It is particularly useful in situations when tree root harvesting is difficult to achieve due to e.g., deep-rooted trees, high stone content or high density of roots from plants in the understory. We recommend the use of PLS instead of multiple regression analysis to calibrate the model, because of the fact that soil temperature and soil moisture often covaries (see further in the Methods section). The robustness of the method is much determined by how good the calibration set is. Ideally this should cover all the temperature and soil moisture ranges found in the control plots. A large calibration set is of course better than a small and data collected over more than one growing season may improve the model. However, ingrowth of mycorrhizal mycelia, as in the current study, or roots may make a longer sampling period than one growing season problematic to achieve when the trenched plots are relatively small. In girdling experiments and when the trenched plots are large this is probably not a major problem.

Conclusions

In this study we have shown that the inherent root trenching artefacts, increased root and mycelia decomposition and soil moisture changes, can be compensated for by using a simple empirical PLS-modelling method. We estimate that, integrated over the first 5 months after the trenching, 45% of the recorded respiration in the trenched plots was an artefact of the treatment of which two-thirds was derived from increased soil moisture and one-third from root decomposition. Our method is an alternative to the sequential root harvesting technique (e.g. Epron et al. 1999). Both methods have similar shortcomings but our method simplifies the task, in particular at sites where quantitative root harvesting is difficult.

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References

Betson NR, Göttlicher SG, Hall M, Wallin G, Richter A, Högberg P (2007) No diurnal variation in rate or carbon isotope composition of soil respiration in a boreal forest. Tree Physiol 27:749–756

Bhupinderpal-Singh, Nordgren A, Ottosson-Löfvenius M, Högberg MN, Mellander PE, Högberg P (2003) Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. Plant Cell Environ 26:1287–1296

Boone RD, Nadelhoffer KJ, Canary JD, Kaye JP (1998) Roots exert a strong influence on the temperature sensitivity of soil respiration. Nature 396:570–572

Borken W, Savage K, Davidson EA, Trumbore SE (2006) Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. Glob Change Biol 12:177–193

Boström B, Comstedt D, Ekblad A (2007) Isotope fractionation and ^{13}C enrichment in soil profiles during the decomposition of soil organic matter. Oecologia 153:89–98

Bowden RD, Nadelhoffer KJ, Boone RD, Melillo JM, Garrison JB (1993) Contributions of above ground litter, below ground litter, and root respiration to total soil respiration in a temperate mixed hardwood forest. Can J For Res 23:1402–1407

Cheng W (2008) Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C–N budgets. Soil Biol Biochem 41:1795–1801

Cheng W, Johnson DW, Fu S (2003) Rhizosphere effects on decomposition: controls of plant species, phenology and fertilization. Soil Sci Soc Am J 67:1418–1427

Comstedt D (2008) Explaining temporal variations in soil respiration rates and $\delta^{13}C$ in coniferous forest ecosystems. Doctoral thesis, Örebro University (manuscript #3 in thesis)

Comstedt D, Boström B, Marshall JD, Holm A, Slaney M, Linder S, Ekblad A (2006) Effects of elevated [CO_2] and temperature on soil respiration in a boreal forest using $\delta^{13}C$ as a labelling tool. Ecosystems 9:1266–1277

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

Dixon RK, Brown S, Houghton RA, Solomon AM, Trexler MC, Wisniewski J (1994) Carbon pools and flux of global forest ecosystems. Science 263:185–190

Ekblad A, Högberg P (2000) Analysis of ^{13}C of CO_2 distinguishes between microbial respiration of added C_4-sucrose and other soil respiration in a C_3-ecosystem. Plant Soil 219:197–209

Ekblad A, Högberg P (2001) Natural abundance of ^{13}C in CO_2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration. Oecologia 127:305–308

Ekblad A, Boström B, Holm A, Comstedt D (2005) Forest soil respiration rate and $\delta^{13}C$ is regulated by recent above ground weather conditions. Oecologia 143:136–142

Epron D, Farque L, Lucot E, Badot P-M (1999) Soil CO_2 efflux in a beech forest: the contribution of root respiration. Ann For Sci 56:289–295

Epron D, le Dantec V, Dufrene E, Granier A (2001) Seasonal dynamics of soil carbon dioxide efflux and simulated rhizosphere respiration in a beech forest. Tree Physiol 21:145–152

Ewel KC, Cropper WP Jr, Gholz HL (1987) Soil CO_2 evolution in Florida slash pine plantations. II. Importance of root respiration. Can J For Res 17:330–333

Gadgil RL, Gadgil PD (1971) Mycorrhiza and litter decomposition. Nature 233:133

Göttlicher SG, Steinmann K, Betson NR, Högberg P (2006) The dependence of soil microbial activity on recent photosynthate from trees. Plant Soil 287:85–94

Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48:115–148

Heinemeyer A, Hartley IP, Evans SP, Carreira de la Fuente JA, Ineson P (2007) Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. Glob Change Biol 13:1786–1797

Högberg MN, Högberg P (2002) Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytol 154:791–795

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

Högberg P, Nordgren A, Högberg MN, Ottosson-Löfvenius M, Bhupinderpal-Singh, Olsson P, Linder S (2005) Fractional contributions by autotrophic and heterotrophic respiration to soil-surface CO_2 efflux in Boreal forests. In: Griffiths H, Jarvis PG (eds) The carbon balance of forest biomes. Taylor & Francis, Oxford, pp 251–267

James FC, McCulloch CE (1990) Multivariate analysis in ecology and systematics: Panacea or Pandora's box? Annu Rev Ecol Syst 21:129–166

Kätterer T, Reichstein M, Andrén O, Lomander A (1998) Temperature dependence of organic matter decomposition: a critical review using literature data analyzed with different models. Biol Fertil Soils 27:258–262

Koide RT, Malcolm GM (2009) N concentration controls decomposition rates of different strains of ectomycorrhizal fungi. Fungal Ecol 2:197–202

Kuzyakov Y (2002) Review: factors affecting rhizosphere priming effects. J Plant Nutr Soil Sci 165:382–396

Kuzyakov Y (2006) Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biol Biochem 38:425–448

Kuzyakov Y, Gavrichkova O (2010) Time lag between photosynthesis and CO_2 efflux from soil: a review. Glob Change Biol (in press)

Lavigne MB, Ryan MG, Anderson DE, Baldocchi DD, Crill PM, Fitzjarrald DR, Goulden ML, Gower ST, Massheder JMM, McCaughey JH, Rayment MB, Striegl RG (1997) Comparing nocturnal eddy covariance measurements to estimates of ecosystem respiration made by scaling chamber measurements at six coniferous boreal sites. J Geophys Res 102:28977–28985

Lee MS, Nakane K, Nakatsubo T, Koizumi H (2003) Seasonal changes in the contribution of root respiration to total soil respiration in a cool-temperate deciduous forest. Plant Soil 255:311–318

Liski J, Korotkov AV, Prins CFL, Karjalainen T, Victor DG, Kauppi PE (2003) Increased carbon sink in temperate and boreal forests. Clim Change 61:89–99

Majdi H, Damm E, Nylund J-E (2001) Longevity of mycorrhizal roots depends on branching order and nutrient availability. New Phytol 150:195–202

Mencuccini M, Hölttä T (2009) The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. New Phytol 185:189–203

Moorhead DL, Westerfield MM, Zak JC (1998) Plants retard litter decay in a nutrient-limited soil: a case study of exploitative competition? Oecologia 113:530–536

Ngao J, Longdoz B, Granier A, Epron D (2007) Estimation of autotrophic and heterotrophic components of soil respiration by trenching is sensitive to corrections for root decomposition and changes in soil water content. Plant Soil 301:99–110

Olsson P, Linder S, Giesler R, Högberg P (2005) Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. Glob Change Biol 11:1–9

Pritchard SG, Strand AE, McCormack ML, Davis MA, Oren R (2008) Mycorrhizal and rhizomorph dynamics in a loblolly pine forest during 5 years of free-air-CO_2-enrichment. Glob Change Biol 14:1–13

Rey A, Pegoraro E, Tedeschi V (2002) Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Glob Change Biol 8:851–866

Rustad LE, Campbell JL, Marion GM, Norby RJ, Mitchell MJ, Hartley AE, Cornelissen JHC, Gurevitch J, GCTE-NEWS (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126:543–562

Silver WL, Miya RK (2001) Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia 129:407–419

Six J, Frey SD, Thiet RK, Batten KM (2006) Bacterial and fungal contributions to C-sequestration in agroecosystems. Soil Sci Soc Am J 70:555–569

Subke JA, Hahn V, Battipaglia G, Linder S, Buchmann N, Cotrufo MF (2004) Feedback interactions between needle litter decomposition and rhizosphere activity. Oecologia 139:551–559

Tang J, Baldocchi DD, Xu L (2005) Tree photosynthesis modulates soil respiration on a diurnal time scale. Glob Change Biol 11:1–7

Treseder KK, Allen MF, Ruess RW, Pregitzer KS, Hendrick RL (2005) Lifespans of fungal rhizomorphs under nitrogen fertilization in a pinyon-juniper woodland. Plant Soil 270:249–255

Unestam T (1991) Water repellency, mat formation, and leaf-stimulated growth of some ectomycorrhizal fungi. Mycorrhiza 1:13–20

Wallander H, Nilsson LO, Hagerberg D, Bååth E (2001) Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytol 151:753–760