Root trenching: a useful tool to estimate autotrophic soil respiration? A case study in an Austrian mountain forest

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Abstract We conducted a trenching experiment in a mountain forest in order to assess the contribution of the autotrophic respiration to total soil respiration and evaluate trenching as a technique to achieve it. We hypothesised that the trenching experiment would alter both microbial biomass and microbial community structure and that fine roots (less than 2 mm diameter) would be decomposed within one growing season. Soil CO_2 efflux was measured roughly biweekly over two growing seasons. Root presence and morphology parameters, as well as the soil microbial community were measured prior to trenching, 5 and 15 months after trenching. The trenched plots emitted about 20 and 30% less CO_2 than the control plots in the first and second growing season, respectively. Roots died in trenched plots, but root decay was slow. After 5 and 15 months, fine root biomass was decreased by 9% (not statistically different) and 30%, (statistically different) respectively. When we corrected for the additional trenched-plot CO_2 efflux due to fine root decomposition, the autotrophic soil respiration rose to ~26% of the total soil respiration for the first growing season, and to ~44% for the second growing season. Soil microbial biomass and community structure was not altered by the end of the second growing season. We conclude that trenching can give accurate estimates of the autotrophic and heterotrophic components of soil respiration, if methodological side effects are accounted for, only.

Keywords Trenching · Soil CO_2 efflux · Rhizosphere · Autotrophic respiration · Heterotrophic respiration · Win-Rhizo · PLFAs · Fine root decomposition

Introduction

Soil respiration represents a major carbon (C) flux from ecosystems to the atmosphere (Raich and Schlesinger 1992). The distinction between its two components, i.e. heterotrophic respiration and autotrophic respiration, is important because the activity of soil heterotrophic organisms is proportional to the decomposition of soil organic matter and the autotrophic fraction (respiration of roots, mycorrhiza and associated bacteria) is dependent on the supply of recent photosynthates (Högberg et al. 2001; Högberg and Read 2006). Different responses of autotrophic and heterotrophic soil respiration have been found in relation to temperature (Boone et al. 1998; Epron et al. 2001; Lee et al. 2003), seasonal drought (Scott-Denton et al. 2006) and atmospheric CO_2 concentration (Janssens et al. 1998). Contribution of heterotrophs and autotrophs to total soil respiration is not assessed easily (Bowden et al. 1993; Ekblad and Högberg 2001; Lee et al. 2003) and a quantification of the response to changing environmental conditions is even more difficult (Trumbore 2006). Across 37 experiments in forest soils autotrophic soil respiration contributed between 10 and 90% of total soil respiration (Hanson et al. 2000). In forests roughly half of total soil respiration is autotrophic.

"Root trenching" is a root exclusion method in which roots are physically isolated by digging a trench around the

edges of a plot (Ewel et al. 1987). The assumption is that the cut roots die off within a short time and that afterwards the measured soil respiration can solely be attributed to heterotrophic soil microorganisms. The autotrophic fraction is calculated as difference between trenched plots and control plots. Trenching is relatively simple and it is thought to give reasonable estimates of annual root respiration in forest ecosystems (Epron et al. 2001; Ewel et al. 1987; Hanson et al. 2000; Kuzyakov 2006; Lee et al. 2003). However, it is a relatively severe interference in the plant–soil system, and might cause side effects that question the technique as a useful tool to separate autotrophic from heterotrophic soil respiration. Methodological problems of root trenching include (1) water regimen disturbance; (2) continued remaining respiration of dying roots; (3) a pulse of CO_2 release from decomposing severed roots; (4) major increases in understory plants within trenched plots, as well as (5) soil microbial community changes (Epron et al. 1999a; Ewel et al. 1987; Hanson et al. 2000; Kuzyakov 2006; Ross et al. 2001; Siira-Pietikäinen et al. 2001, 2003). In contrast to soil moisture, root parameters and soil microbial community structure have not been regularly assessed (Bowden et al. 1993; Buchmann 2000; Epron et al. 1999b; Lee et al. 2003; Rey et al. 2002; Sulzman et al. 2005). Fine roots can survive several months (Ewel et al. 1987; Uchida et al. 1998), and the remaining root respiration can influence CO_2 fluxes from trenched plots. It is, therefore, important to know when the desired experimental conditions (no root activity) are reached. After fine root death, decomposition of fine roots will enhance the CO_2 efflux from the trenched plots. The magnitude of this additional CO_2 source is important to know and has to be subtracted from the trenched plot CO_2 efflux (Epron et al. 1999b). Trenching immediately disrupts the supply of recent photosynthates to the roots and mycorrhiza. Especially mycorrhizal fungi and associated bacteria will suffer from the lack of labile C. Soil microbial community composition may change and thereby alter the decomposition rates on trenched plots (Brant et al. 2006; Hanson et al. 2000; Kuzyakov 2006). Since autotrophic soil respiration includes mycorrhizal respiration, the breakdown of mycorrhizal respiration is anticipated in order to separate the autotrophic fraction. However, if dramatic changes in soil microbial community occurred, heterotrophic soil respiration could be affected as well and estimates of the autotrophic fraction would be biased. Further interactions between rhizosphere and heterotrophic decomposers (e.g. priming effect) will be enabled (Kuzyakov 2006; Subke et al. 2004).

In this study, we used root trenching to assess the autotrophic contribution to total soil respiration in an Austrian mountain forest. We quantified the seasonal CO_2 loss by fine root decomposition in order to accurately estimate the autotrophic soil respiration. It is frequently assumed that fine roots decay within some months after trenching, and that afterwards no correction for extra CO_2 from decaying fine roots is necessary (Boone et al. 1998; Bowden et al. 1993; Kelting et al. 1998; Rey et al. 2002). We tested whether this assumption holds true or whether fine root decay is a longer lasting process. Further, we used phospholipid fatty acid (PLFA) analysis to figure out if trenching lead to considerable changes in the microbial community structure. Since roots were cut we hypothesised that decaying mycorrhizal fungi led to a reduction in fungal biomass.

Material and methods

Site description

The field site is located in the Northern Limestone Alps (11°38′21″ E; 47°34′50″ N), about 12 km north-west of the village Achenkirch (Austria). The climate is cool humid, with maximum precipitation in summer (mean temperatures and precipitation for the period 1991–2003 were 6.8°C and 1563 mm, respectively). The forest was characterised as a warm, central montane spruce-fir-beech forest (Englisch and Starlinger 1996). The stand is about 130 years old and dominated by Norway spruce. Approximately, 20% of the soils belong to Chromic Cambisols and are 30–60 cm deep. The other 80% are Rendzic Leptosols that are merely 15–30 cm deep. The bedrock is formed of dolomite. The soil pH is approximately 7 at the upper 10 cm (Mutsch 2001) and a C/N ratio of 15–18 indicates a high biological activity. For a detailed site description see (Herman et al. 2002).

Experimental design

The treatments 'Control' and 'Trenched' were established in June 2005 with three replicates on square plots of 4 m^2 each. At trenched plots a narrow ditch was dug down to the solid bedrock. To prevent the in-growth of new roots during the experiment, a plastic lining was placed in the trench, and it was refilled with soil. The scarce herbaceous vegetation was periodically removed.

Gas measurements

CO_2 effluxes from the soil surface were periodically measured during the snow-free season, with an average interval of 15 days. Three collars per plot were established, to assure the measure is made exactly in the same point over time. For the measurements, the collars (round plastic cylinders, 10 cm height and 20 cm diameter) were closed with a stainless-steel lid. The lid was equipped with a rubber fitting, a vent, and outlet and inlet tubes, connected to a WMA-4 Infrared Gas Analyser (PP-Systems, Hitchin, UK). The soil CO_2 efflux rates were calculated from the linear

increases of the CO_2 concentration in the chamber headspace, from 1 min after closing the chamber until 4 min after (30 s measurement interval). The analyser was calibrated every month using zero gas (pure N_2) and a CO_2 standard (500 ppm, Linde Gas, Vienna, Austria). The total CO_2 efflux of each growing season was estimated through linear interpolation between each sampling date CO_2 efflux.

Soil sampling

Soil samples were collected in June 2005 (one day before trenching), in November 2005 and September 2006, to assess the effect of trenching over time. Three cores (7 cm diameter, 15 cm long) per plot were randomly taken at each date. A 15 cm soil depth was chosen because most of the fine roots were found in the upper soil layers (Englisch 2001). The cores were placed in a portable cooler, and then stored at −18°C until root analyses were made. From each soil core, a small amount of root free soil (ca. 5 g) was separately stored at −18°C for further microbiological analysis.

Soil parameters

During CO_2 measurements, soil temperature from every plot at 5 cm depth was measured with a hand-held thermistor (±0.1°C). Soil moisture data from 5 and 15 cm soil depth were obtained from adjacent plots of a soil warming study (Schindlbacher et al. 2008). Data were taken with ECH_2O EA-10 probes (Decagon, Washington, USA) in half hourly resolution.

Total soil C content was measured by dry combustion with a Carlo Erba NA 1500 CHN Analyser (Carlo Erba Instruments, Milan, Italy). Water extractable organic carbon from the upper 15 cm of the mineral soil was measured by a TOC 5050 Analyser (Shimadzu Scientific Instruments Inc., Columbia, USA).

Root analysis

Soil cores were deposited on a tray, the soil was crumbled by hand, and the roots were extracted and deposited in warm water. Careful washing separated soil aggregates still adhered to the roots. During the cleaning and scanning procedure, a visual assessment was made whether fine roots were still alive or dead. The decision was made upon colour and flexibility of roots (Persson 1983). All the roots (live and dead) were scanned with a WinRhizo LA 1600 scanner with a resolution of 300 dots per inch (Figure 1), and analysed with the WinRhizo software (Régent Instruments, Quebec, Canada). The evaluated parameters were total root length, mean root diameter and root volume. The algorithms of WinRhizo are described in Bauhaus and Messier (1999). Each root parameter was measured for seven root-diameter classes. A 0.25 mm interval was used for root diameter from 0 to 1 mm, a 0.5 mm interval for diameters between 1 and 2 mm, and roots larger than 2 mm. Fine roots are here defined as those with a diameter of 0–2 mm; coarse roots have a diameter >2 mm (Persson 1983). Roots biomass was obtained after drying at 65°C. In September 2006, we measured the C and N content of roots with A CN 2000 automatic analyser (LECO Corp., St. Joseph, USA).

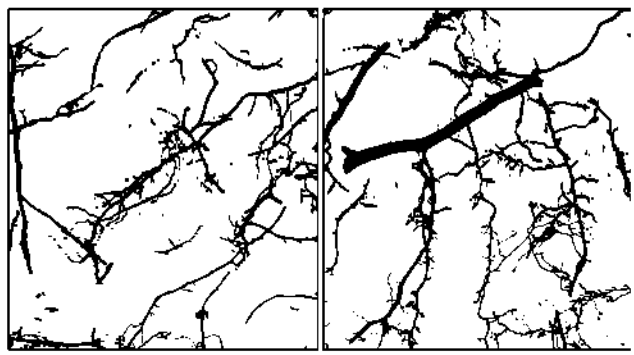

Fig. 1 Examples of images taken with WinRhizo LA 1600 scanner. The *left image* corresponds to a plot before trenching. The *right image* corresponds to the same plot 15 months after trenching. At this sampling date, all roots from trenched plots (*right*) were dead but have maintained the morphology prior to trenching (*left*). *Scale* 1:2.5

Soil microbial community analysis

The soil microbial community in the upper 15 cm of mineral soil was characterized by the pattern of PLFAs (modified PLFA method, Frostegård et al. 1991; Hackl et al. 2005): 1.5 g fresh soil was extracted with a chloroform:methanol:citrate buffer mixture (1:2:0.8). The lipids were separated into neutral lipids, glycolipids and phospholipids on a silicic acid column. The phospholipids were subjected to a mild alkaline methanolysis. Fatty acid methyl esters were separated with an Agilent 6890 gas chromatograph equipped with a split splitless inlet on a HP5 capillary column (50 m × 0.20 mm × 0.33 µm) and detected with a flame ionisation detector. Helium was used as the carrier gas. Temperatures of the injection and the detection were 280 and 300°C, respectively. The injected volume was 1 µl. The initial oven temperature of 70°C was maintained for 1.5 min, then raised to 160 at 30°C min^{-1} and increased to the final temperature of 260 at 3°C min^{-1}; the final temperature was held for 15 min. Quantification was done by the internal standard method with non-adecanoate fatty acid as an internal standard. Fatty acid methyl esters standard compound (Bacterial Acid Methyl Esters Mix from SUPELCO) was used as qualitative standard, in order to identify the different fatty acid methyl esters.

Fatty acids are designated in terms of total number of C atoms: number of double bonds, followed by the position of

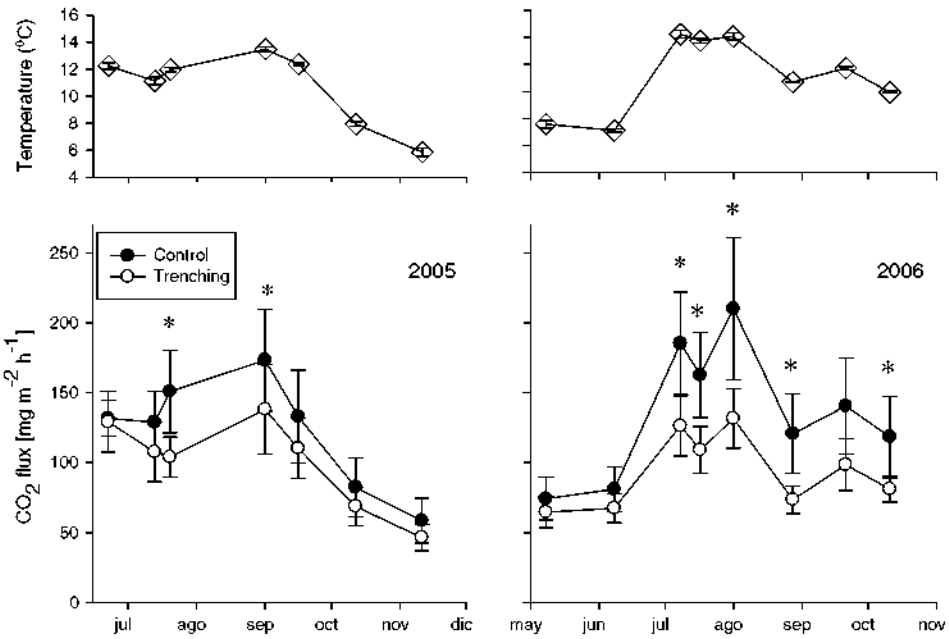

Fig. 2 Average evolution of soil CO_2 (*dots*) and soil mean temperature in 5 cm depth (*diamonds*) over time. *Vertical bars* denote the standard error; * indicates significant differences between treatments

the double bond from the carboxylic end of the molecule. Prefixes "i" and "a" denote iso- and anteiso- branching, respectively; "cy" indicates cyclopropyl fatty acid; "Me" indicates the position of methyl group from the carboxyl end of the chain.

The bacterial biomass was estimated by those PLFAs considered to be predominantly of bacterial origin (i15:0, a15:0, i16:0, i17:0, a17:0, for gram positive bacteria; 16:1(9), cy17:0, 18:1(11), cy19:0, for gram negative bacteria) (Frostegård et al. 1993). The PLFA 18:2(9,12) was used to estimate the fungal biomass (Olsson 1999). Actinomycetes biomass was estimated by 10 Me 18:0 (Kroppenstedt 1985). For arbuscular mycorrhiza biomass assessment, PLFA 16:1(11) was used (Olsson 1999). The sum of the PLFAs mentioned above was used as an estimate of the total microbial biomass ($PLFA_{mic}$). $PLFA_{mic}$ was converted to microbial C (C_{mic}) with a conversion factor of 1 nmol PLFA g^{-1} dry matter equals 5.8 μg C_{mic} g^{-1} dry matter (Jörgensen and Emmerling 2006).

Statistical analysis

Total differences in the soil CO_2 efflux from the control and trenched plots were evaluated through a repeated measures ANOVA. "Plot" and "treatment" were the factors, and soil CO_2 efflux the dependent variable. A univariate repeated measures ANOVA was chosen, to detect at which sampling dates differences between treatments were significant.

The root morphology and soil microbial community measurements of June 2005 were considered to reflect the initial situation for each plot. The change over time was calculated as difference of the values from November 2005 and September 2006, respectively, from initial values.

The statistical differences of the root parameters length, average diameter, volume, biomass and C content were analysed with a one-way ANOVA.

Treatment effects on the gram negative bacteria, actinomycetes, arbuscular mycorrhiza, the ratio of fungi to bacteria and $PLFA_{mic}$ were analysed with a one-way ANOVA. A Mann–Whitney test was used for gram positive bacteria and fungi, because the data for these parameters did not fulfil the equality of variances. All the analyses were performed with Statistica 6.0 (StatSoft Inc., Tulsa, USA) using non-transformed variables and a level of significance of 5%.

Results

Soil CO_2 efflux

Soil CO_2 efflux rates were lower from trenched plots than from control plots. The difference (± standard error, SE) in the CO_2 flux density was about 25 ± 6% (control 130.0 ± 5.5 mg CO_2 m^{-2} h^{-1}; trenching: 97.0 ± 3.5 mg CO_2 m^{-2} h^{-1}). The difference ranged from 13 to 40%. The effect of trenching was statistically significant. The differences in CO_2 efflux from control and trenched plots were on average (±SE) 20 ± 7% from June to November 2005, and 30 ± 5% from May to October 2006 (Fig. 2). Control plots emitted a total efflux (±SE) of 429 ± 90 and 502 ± 111 g C m^{-2} for the first and second season, respectively. The trenched plots emitted 344 ± 67 and 346 ± 55 g C m^{-2} in these periods. Soil CO_2 efflux followed the annual soil temperature development. Both the temporal and spatial variation in soil CO_2 efflux were high (Fig. 2).

Table 1 Soil C pools (mean ± standard error) in the upper 15 cm of the mineral soil (Tm ha^{-1}) for each treatment and sampling date divided in compartments as estimated from soil cores ($n = 9$, in three sites)

Date	Treatment	Fine roots C (Tm ha^{-1})	Coarse roots C (Tm ha^{-1})	Microbial C (Tm ha^{-1})	Total soil C (Tm ha^{-1})
June 2005	Control	2.3 ± 0.4	1.6 ± 0.4	0.26 ± 0.03	219.1 ± 44.2
	Trenching	2.9 ± 0.3	2.2 ± 0.3	0.24 ± 0.01	176.9 ± 19.7
November 2005	Control	2.3 ± 0.3	1.7 ± 0.3	0.17 ± 0.02	239.3 ± 43.1
	Trenching	2.7 ± 0.4	2.3 ± 0.5	0.24 ± 0.01	181.8 ± 33.1
September 2006	Control	2.7 ± 0.4	2.2 ± 0.4	0.27 ± 0.03	210.3 ± 4.6
	Trenching	1.9 ± 0.3	2.6 ± 0.5	0.30 ± 0.04	204.6 ± 10.2

Table 2 Mean and standard error of coarse root morphological parameters in the upper 15 cm of the mineral soil

Each value is based on $n = 9$, in three sites

Date	Treatment	Length ($m\ m^{-2}$)	Volume ($cm^3\ m^{-2}$)	Dry biomass ($g\ m^{-2}$)
June 2005	Control	204.4 ± 40.8	1521.9 ± 340.2	339.3 ± 74.1
	Trenching	266.7 ± 35.1	2092.2 ± 249.9	457.2 ± 59.4
November 2005	Control	168.0 ± 25.5	1460.1 ± 296.7	346.5 ± 65.1
	Trenching	147.6 ± 30.9	2150.4 ± 466.2	476.4 ± 104.1
September 2006	Control	147.6 ± 21.3	2175.6 ± 378.9	461.4 ± 81.3
	Trenching	189.0 ± 26.4	2397.6 ± 510.9	538.2 ± 113.1

Soil temperature, total soil carbon and water extractable carbon

Soil temperature in 5 cm depth had a mean value and standard error of 10.3 ± 0.6°C in control plots and 10.4 ± 0.6°C in trenched plots, respectively. No significant effect of root trenching was found.

The total C stock for the upper 15 cm of the mineral soil was, on average (±SE), 227.4 ± 8.3 and 192.9 ± 8.4 Tm C ha^{-1}, at control and trenched plots, respectively (Table 1).

The mean concentration (±SE) of water extractable organic C in June 2005 was 10.5 ± 2.8 mg l^{-1} and 12.2 ± 1.1 mg l^{-1} for control and trenched plots, respectively. In November 2005, water extractable organic C was 14.4 ± 5.1 and 12.7 ± 2.1 mg l^{-1} for control and trenched plots, respectively. Water extractable organic C in September 2006 was 9.4 ± 2.5 mg l^{-1} in control plots and 10.9 ± 2.3 mg l^{-1} in trenched plots. Differences due to treatment were not statistically significant at any sampling date.

Root analysis

The differences in coarse root length, coarse root diameter, coarse root volume and coarse root biomass between control and trenched plots were not significant during all sampling dates (Table 2).

Fine root length, fine root diameter and fine root volume were not affected by the treatment (Fig. 3). Differences in fine root biomass between treatments were about 9% but not significant in November 2005. However, in September 2006, the difference was approximately 30% and statistically significant. Taking into account the additional CO_2 flux from fine roots decomposition, the autotrophic respiration increased from 20 to 26% and from 30 to 44% for the first and second season, respectively.

The proportion between living and dead roots was similar during first sampling in June 2005 and second sampling in November 2005. On the contrary, all the roots collected in trenching plots in September 2006 were dead.

Roots contained (±SE) 483 ± 3 and 479 ± 3 mg C g^{-1} at control and trenched plots, respectively, and 7.6 ± 0.2 mg N g^{-1} for control plots and 7.1 ± 0.3 mg N g^{-1} for trenched plots. No statistically significant difference caused by treatment was found.

Soil microbial community

Table 3 shows the distribution of the taxonomic groups of soil microorganisms. $PLFA_{mic}$ showed temporal variation during the three sampling dates. $PLFA_{mic}$ tended to be higher in autumn than in early summer.

In November 2005, significant differences between treatments were detected in $PLFA_{mic}$, in gram positive bacteria, in gram negative bacteria and in arbuscular mycorrhizae (Table 3). Changes in gram positive bacteria were due to a significant increment on PLFAs i15:0 and i17:0 in trenched plots in comparison to control plots. PLFA 16:1(9) significant difference led the changes in gram negative bacteria. In September 2006, the differences were not significant any more.

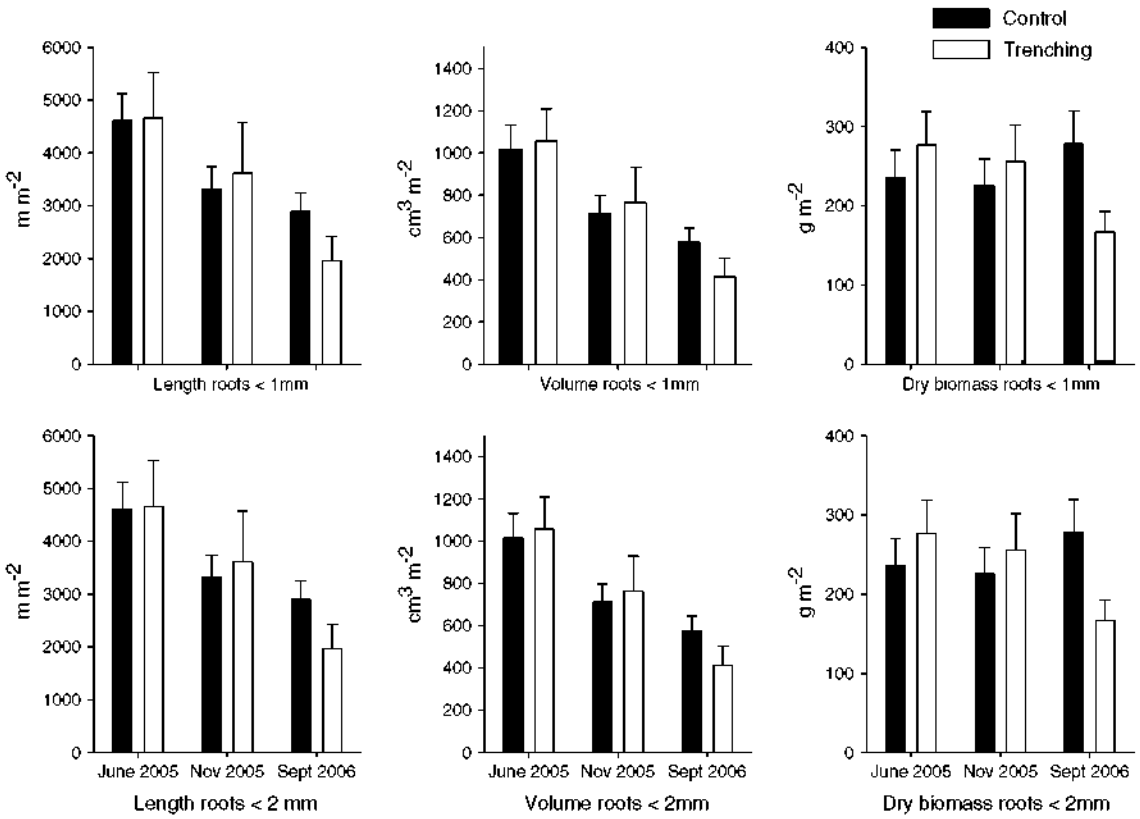

Fig. 3 Mean values (±standard error) of root <1 mm diameter length, root <1 mm diameter volume, root <1 mm diameter dry biomass, fine root length, fine root volume and fine root biomass at each sampling date in the first 15 cm of the mineral soil

Table 3 Total PLFA with microbial origin found on samples ($PLFA_{mic}$), bacterial PLFA ($PLFA_{bact}$), fungal PLFA ($PLFA_{fung}$), PLFA from arbuscular mycorrhiza ($PLFA_{am}$) and PLFA from actinomycetes ($PLFA_{act}$)

Date	Treatment	$PLFA_{mic}$	$PLFA_{bact}$	$PLFA_{fung}$	$PLFA_{am}$	$PLFA_{act}$
June 2005	Control	266.6 ± 33.1	227.2 ± 26.9	11.76 ± 3.31	21.16 ± 2.33	6.46 ± 1.13
	Trenching	260.9 ± 23.1	224.5 ± 19.8	9.75 ± 1.27	20.07 ± 1.06	6.63 ± 0.79
Nov 2005	Control	227.7 ± 24.9	196.6 ± 40.2	6.07 ± 1.20	17.95 ± 3.84	7.03 ± 1.49
	Trenching	290.8 ± 33.7*	251.3 ± 29.2*	7.31 ± 0.71	22.17 ± 2.41*	7.96 ± 1.03
Sept 2006	Control	303.4 ± 30.3	262.4 ± 25.9	9.54 ± 1.49	23.92 ± 2.37	7.51 ± 0.84
	Trenching	328.4 ± 13.9	287.0 ± 34.6	7.61 ± 1.05	24.89 ± 3.28	8.92 ± 1.19

All values are expressed in nmol PLFA g^{-1} dry matter (average ± standard error)

* Denotes a significant increase due to trenching

The populations of fungi and actinomycetes did not differ significantly between control and trenched plots at any sampling date (Table 3). In relation to $PLFA_{mic}$, fungal PLFAs tended to be decreased during the last sampling in September 2006 (Fig. 4), but the decrease was statistically not significant.

Applying the conversion factor from Jörgensen and Emmerling (2006) on $PLFA_{mic}$, we obtained an average value (±SE) of 1.59 ± 0.12 mg C_{mic} g^{-1} dry matter for control plots and 1.73 ± 0.13 mg C_{mic} g^{-1} dry matter for trenched plots, varying over time.

Discussion

Trenching reduced the soil CO_2 efflux on average by 25%. This is similar to other trenching studies in temperate forests (Buchmann 2000; Epron et al. 1999b; Ewel et al. 1987; Lee et al. 2003). Our results are also in agreement with stem-girdling experiments (Andersen et al. 2005; Scott-Denton et al. 2006). Did this response come from a reduction in autotrophic respiration alone, and were methodological artefacts important?

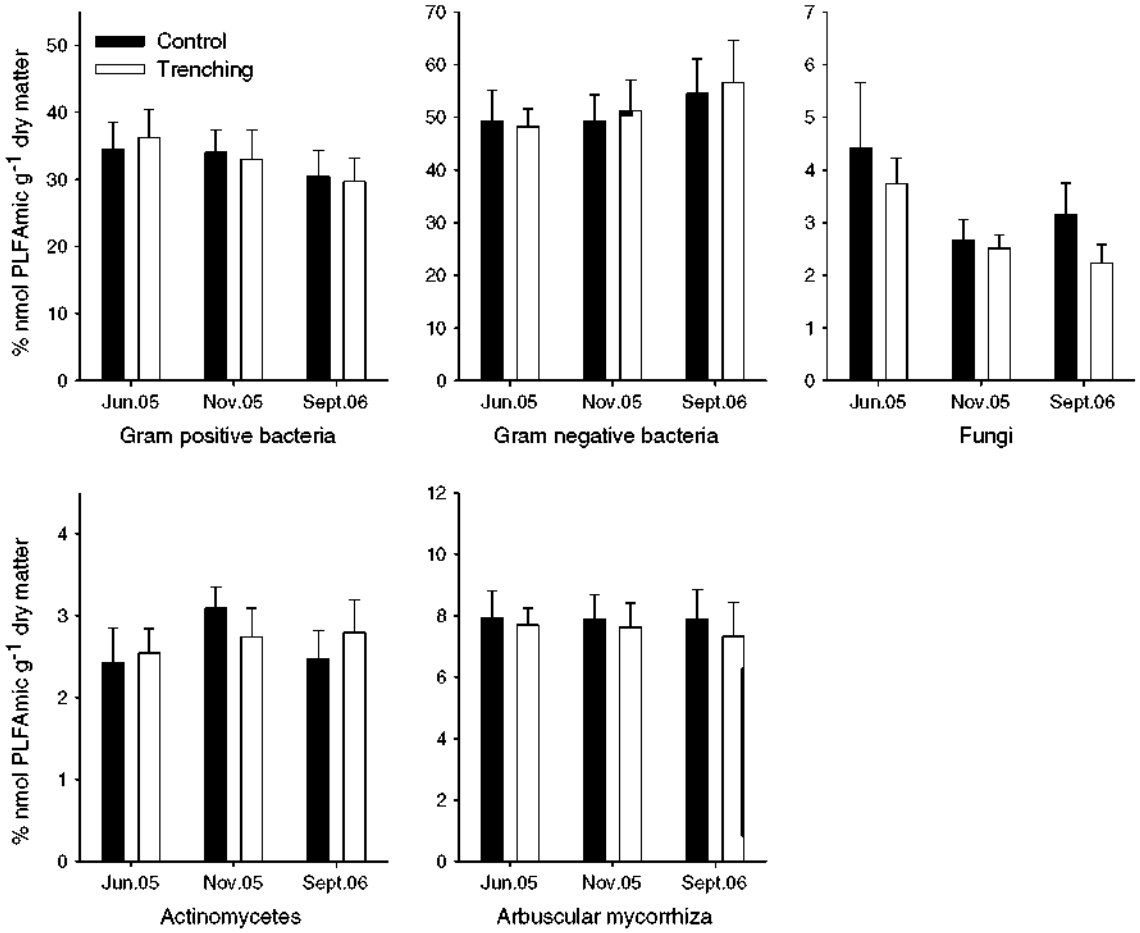

Fig. 4 Percentage contribution (±standard error) to the $PLFA_{mic}$ (g^{-1}dry matter) for gram positive bacteria, gram negative bacteria, fungi, actinomycetes and arbuscular mycorrhiza, for control (*black bars*) and trenched (*grey bars*) plots over time

A common assumption of the trenching methods is that autotrophic soil respiration is completely suppressed after trenching (Hanson et al. 2000; Lee et al. 2003). Here, we showed that the optical shape and flexibility of roots did not change much during the first five months after trenching. This suggests that fine roots used starch reserves and prolonged root respiration after trenching (Ewel et al. 1987; Lee et al. 2003). The effect of prolonged root respiration seemed to be rather insignificant because CO_2 fluxes from trenched and control plots diverged shortly after trenching (Fig. 2). Our findings show that fine root decomposition can be delayed for several months after trenching. This is in line with findings of (McClaugherty et al. 1984), who also reported a delay in root decomposition in trenched plots. The decomposition of roots that have recently died inflates the rate of heterotrophic soil respiration, and may result in an underestimation of the autotrophic fraction (Epron et al. 1999b; Hanson et al. 2000; Kuzyakov 2006). The observed fine root decay of approximately 30% during the second season after trenching showed that the autotrophic soil respiration would have been underestimated by ~14% if the additional CO_2 efflux from fine root decomposition would have been not accounted for. Our observations agree with data from Epron et al. (1999b), who measured a 53% biomass loss of roots within two years in a beech forest. About one-third of the fine root biomass decomposed within 2 years of trenching, leaving about two-thirds to continue slow decomposition in later years. We are confident that fine root decomposition lasts for several years. Hence, the frequently made assumption that fine roots are decomposed during the first season after trenching (Boone et al. 1998; Bowden et al. 1993; Kelting et al. 1998; Rey et al. 2002) did not hold true at our temperate forest.

The reactions of soil microbes to seasonal changes in environmental conditions and substrate availability likely explained the seasonal changes in microbial abundance and community structure (Monson et al., 2006). However, contrary to our expectations, microbial biomass and community structure did not vary much between trenched and control plots (Fig. 4). The slight increase in bacteria, five months after trenching, might be caused by the beginning fine root decomposition. Since easily decomposable fine

root C is decomposed first, bacteria should be primarily involved in this step (Kuzyakov et al. 2000). Beginning fine root decomposition would also explain the rather small differences between trenched and control plots CO_2 efflux during October/November 2005 (Fig. 2). Brant et al. (2006) also observed an increase in bacteria in mineral soils four months after trenching. Similar to our findings, the change was not evident any more ten months after trenching.

We expected a decrease in fungal PLFAs due to a reduction in mycorrhizal fungi, but observed no significant differences between control and trenched plots. Our results deviate from finding in other studies, where trenching has led to a decrease in fungal biomarkers. Brant et al. (2006) reported decreased fungal biomarkers in the mineral soil of three mature temperate forests, 4 and 10 months after trenching. The inhibition of fungal communities after trenching has also been reported for organic layers of boreal forests (Siira-Pietikäinen et al. 2001, 2003). Ectomycorrhizal fungi, which are associated with tree roots at our site, and heterotrophic fungi are characterised by the same PLFA. Hence, it was not possible to distinguish between the development of mycorrhizal and saprophytic fungi over time. Mycorrhizal fungi may have maintained even without fresh C supply from trees, or a shift in fungal communities from symbiotic mycorrhizae to saprophytic species had occurred (Subke et al. 2004). However, because mycorrhizal fungi have been shown to become partly heterotrophic in substrate got limited, we think that mycorrhizal PLFAs could have been present over the two seasons. The decreased (even if not statistically significant) in fungal PLFA in September 2006 (Fig. 4) could be a sign of first reductions in mycorrhizal fungi.

At our experimental site, no trees were associated with arbuscular mycorrhizae. The slight increase of arbuscular mycorrhizae in trenched plots in November 2005 might have been associated with changes in herbaceous species or abundance. However, the presence of herbaceous vegetation was scarce on control plots (only few individual of *Oxalis acetosella* L.) and herbaceous vegetation was removed from trenched plots. Hence, changes in arbuscular mycorrhizae are supposed to have had no influence on CO_2 efflux.

Conclusions

Trenching is easily accomplished but holds some drawbacks. Soil moisture is usually higher on trenched plots (not explicitly studied here) and decaying dead roots are a source of extra CO_2 from the trenched plots. If these methodological side effects are inadequately accounted for, the estimate of the autotrophic contribution to total soil respiration will be biased. A precise determination of fine root decay is tedious and correcting for differences in soil moisture is difficult. However, when methodological problems are adequately addressed, trenching yields an accurate estimate of autotrophic soil respiration. Especially for long-term studies, trenching is the method of choice, because the vitality of mature trees is hardly affected by root trenching. For short-term experiments girdling or isotope labelling may be better options.

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