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An isotope approach based on ^{13}C pulse-chase labelling *vs.* the root trenching method to separate heterotrophic and autotrophic respiration in cultivated peatlands

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We tested an isotope method based on ^{13}C pulse-chase labelling for determining the fractional contribution of soil microbial respiration to overall soil respiration in an organic soil (cutaway peatland, eastern Finland), cultivated with the bioenergy crop, reed canary grass. The plants were exposed to $^{13}CO_2$ for five hours and the label was thereafter determined in CO_2 derived from the soil–root system. A two-pool isotope mixing model was used to separate sources of respiration. The isotopic approach showed that a minimum of 50% of the total CO_2 originated from soil-microbial respiration. Even though the method uses undisturbed soil–plant systems, it has limitations concerning the experimental determination of the true isotopic signal of all components contributing to autotrophic respiration. A trenching experiment which was comparatively conducted resulted in a 71% fractional contribution of soil-microbial respiration. This value was likely overestimated. Further studies are needed to evaluate critically the output from these two partitioning approaches.

Introduction

Soils store a large amount of carbon (C) and intensive, not sustained land use induce a great loss of soil C as carbon dioxide (CO_2). In turn, CO_2 as a major greenhouse gas in the atmosphere could accelerate climate change (Houghton *et al.* 2001). Especially agricultural organic soils can release high amounts of CO_2 to the atmosphere because cultivation practices enhance decomposition processes in soil (Maljanen *et al.* 2001, Lohila *et al.* 2004). Carbon dioxide released from the soil surface results from several sources, importantly from soil organic matter decomposition by microbes (heterotrophic respiration or soil microbial respiration, SMR) and from roots (autotrophic respiration or root respiration, RR). From a methodological point of view it is difficult to partition SMR from RR, as the soil is closely inter-linked with the root system. This separation is, however, important as only SMR results in soil organic matter decomposition and thus a potential depletion of the large C reservoirs stored in soils possibly contributing to climate change. There are different approaches to separate SMR and RR, including component integration, root regression and use of C isotopes (^{13}C, ^{14}C). Each method has advantages and limi-

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tations (Hanson *et al*. 2000, Baggs 2006, Kuzyakov 2006, Subke *et al*. 2006, Trumbore 2006, Kutsch *et al*. 2009). It has been concluded that the isotopic approaches would be most reliable for estimating the amount of CO_2 released from soil organic matter decomposition (Hanson *et al*. 2000, Baggs 2006, Kuzyakov 2006) and from autotrophic sources (Kuzyakov and Gavrichkova 2010). The main advantage of isotopic methods is that there is no need to disturb the root–soil system. However, as compared with traditional techniques the isotope approaches are less frequently applied for partitioning SMR and RR (Subke *et al*. 2006). This is because these techniques have some practical restrictions including the advanced instrumentation needed. Further the natural abundance of stable isotopes of C can be successfully used for partitioning approaches only in some special soil–plant systems [transition from C_3 to C_4 ecosystems or *vice versa*; e.g. Rochette *et al*. (1999)] and there are health risks with the ^{14}C labelling techniques (Hanson *et al*. 2000).

Pulse-chase labelling is based on exposing plants for a short time to ^{13}C-enriched CO_2 (Leake *et al*. 2006, Ostle *et al*. 2000). Most frequently, the C allocation pattern through the plant–soil system is studied by using pulse-chase $^{13}CO_2$- or $^{14}CO_2$-labelling approaches (Rattray *et al*. 1995, Carbone *et al*. 2007). The labeled C compounds in plants are partly translocated into roots and root exudates (e.g. Kuzyakov 2006). As a consequence, also CO_2 derived from the roots will have a unique isotopic signature that can be used to track autotrophic respiration. In theory, SMR and RR can be separated by using an isotope mixing model (Hanson *et al*. 2000). Nevertheless, studies to distinguish SMR and RR by pulse-chase labelling are rare, and for the flow of ^{13}C in roots mainly indirect modeling approaches have been used (Kuzyakov *et al*. 1999, Sapronov and Kuzyakov 2007). To our knowledge, there are no data under field conditions.

As part of a ^{13}C pulse-chase labelling experiment to follow the flow of C in plant–soil–atmosphere continuum in a soil with high C content, a peat soil, we tested the isotope partitioning approach based on isotope labelling to separate heterotrophic respiration (SMR) and autotrophic respiration (RR). Attention was paid to the shortcomings and strengths of this method. The experiment was carried out on a cut-away peatland cultivated with reed canary grass [(RCG), *Phalaris arundinacea*] to produce biomass for bioenergy. Root respiration as determined here includes respiration from roots and root-associated microorganisms (including mycorrhiza) utilizing root exudates as their substrates, while SMR reflects the decomposition of soil organic matter by soil microbes. There has been considerable debate in the literature whether or not to collectively regard root and rhizomicrobial respiration as autotrophic when studying sources of CO_2 from soils. Kuzyakov (2006a, 2006b) considers only root respiration as truly autotrophic and strictly classifies all CO_2 from microbes which utilize organic C sources an important part of heterotrophic soil respiration. On the other hand, Högberg *et al*. (2006) regarded all respiratory components which are driven by recent photosynthesates as autotrophic (thus also rhizomicrobial respiration and respiration from mycorrhizal fungi). In our studies on soil C fluxes from plant cultivations (including the partitioning approach introduced here), the most important differentiation lays between CO_2 derived from decomposition of soil organic matter with turnover times of several years to centuries (one of the largest storehouse of C, especially large in peat soils), and CO_2 derived from ecosystem components utilizing current photosynthesates. This becomes logic especially when considering that bioenergy plants are studied here, "bioenergy" being energy which has been previously fixed. We thus describe here rhizomicrobial respiration as a part of the autotrophic respiration. However, strictly spoken, rhizomicrobes utilize a heterotrophic metabolic pathway and we would like to stress that, in more detailed investigations on C dynamics in plants and soil, the separation of root and rhizomicrobial C dynamics is very important.

We compared the results from pulse labelling with those obtained by the root-trenching technique, a common approach used in partitioning CO_2 from various soil pools (e.g., Epron 2010). Root trenching is a relatively simple technique which is based on killing roots by severing them (e.g. by trenching), and subsequently measuring SMR. After root trenching, also the supply

of current photosynthesates (and thus rhizomicrobial respiration) is ceased. The advantage of this method is that it is relatively easy to implement and that it can be applied also in remote areas. However, it has been criticized that root trenching overestimates SMR merely because of increased organic matter decomposition stemming from root residues, and also that environmental conditions (especially soil water content) are altered (Hanson *et al*. 2000, Jassal and Black 2006, Kuzyakov 2006, Subke *et al*. 2006). We thus hypothesized that the partitioning approach based on pulse-chase labeling would result in a lower, and thus more accurate, estimation of the relative proportion of SMR in soils. Accurate data on CO_2 losses from soils are important for soil C models and predictions of future atmospheric CO_2 balance. Since peat soils contain a substantial amount of the world's soil C (Gorham 1991), both traditional and modern techniques have to be explored for a more reliable estimation of the amount of CO_2 released from those soils. This will help to evaluate the impact of different land-use practices of this important soil type on the atmospheric greenhouse gas budget.

Material and methods

In July 2006, a pulse-chase labelling experiment was conducted at a cutaway peatland cultivated with a perennial grass RCG for bioenergy purpose. The peatland was used for peat extraction until 2001, when the RCG cultivation was established. The peatland complex *Linnansuo* is located in eastern Finland (62°30´N, 30°30´E), in the boreal climatic zone. The perennial RCG crop is harvested every spring. There is no tillage of the experimental site within one life cycle of this perennial plant of approximately 15 years. The study site and the cultivation practice are described in more detail in e.g., Biasi *et al*. (2008) and Shurpali *et al*. (2008). The labelling experiment was carried out with a chamber (540 l, 150 cm high) constructed from a rigid metal frame and transparent polyethylene plastic covering the frame. The chamber was installed on the soil surface gastight using a collar (60 × 60 cm) with a water groove [modified after the method of Ostle *et al*. (2000)]. Labelling with $^{13}CO_2$ (99 atom % ^{13}C) lasted for five hours. The CO_2 concentration in the labelling chamber was continuously monitored with infrared gas analyzer (IRGA). Carbon dioxide concentrations decreasing by photosynthetic uptake were kept in the range of 300–500 ppm by regular $^{13}CO_2$ additions. Four fans located in different vertical positions mixed the label with the air in the chamber. Three replicate plots, located about 50 m apart, were labelled on three consecutive sunny days. Each chamber received in total about 2.5 l of labelled CO_2. During the labelling period, chamber temperature increased by up to 10 °C above the ambient temperature. The maximum temperature during labelling corresponded to the peak temperature of the experimental season. Carbon dioxide released from the soil (SR) was measured on the 3rd and 5th days after labeling (time-shifted between the replicates) during daytime (between 10:00 and 16:00) and $\delta^{13}C$ of CO_2 was determined (*see* below). A chamber was installed on the soil surface gastight in random locations within the labeled plots, between RCG stems (60 ml volume, diameter 3 cm), and four gas samples of 1 ml were taken within the measurement period of 10 minutes. They were immediately injected into pre-evacuated, N_2-flushed vials (Valco® 12 ml) stopped with butyl rubber septa.

The area for $^{13}CO_2$ measurements was small (0.2% of total labelled area) in order to minimize disturbance caused by soil sampling. We tested the representativeness of these areas by taking initially duplicate samples for $^{13}CO_2$ analysis, and the variability of these duplicate samples was very small. After sampling of $^{13}CO_2$, a soil core (diameter 4 cm, depth 0–5 cm) with roots of RCG was taken from the same labelled plot where the gas samples were taken (n = 3). Sampling was done not earlier than 3 days after labelling, as back-diffusion of labelled CO_2 can contribute to the CO_2 release and disturbe the $\delta^{13}C$ analyses shortly after the labelling (Bahn *et al*. 2009, Subke *et al*. 2009). Soil cores were also taken from unlabelled plots within 100 m distance from the labelling experiment (n = 3). All soil cores were stored at –18 °C for three-six months until analyzed and gas samples were analyzed for $\delta^{13}C$ of CO_2 immediately as described below.

After thawing of the cores, roots and soil were separated by hand-picking and sieving. The roots were rinsed with water to remove all soil particles and dried softly with a paper towel before incubating a sub-sample of 2–3 g in a 550-ml glass flask to determine the $\delta^{13}C$ in CO_2 respired from roots (RR). Five gas samples of 1 ml were taken from the flasks within six hours of incubation time for analysis of $\delta^{13}C$ of CO_2. Simultaneously, 10 ml gas samples were taken for the analysis of CO_2 concentration. The $\delta^{13}C$ in CO_2 evolved from the unlabelled soil was determined by incubating a sieved soil sample of 10 g following the protocol applied for roots. All incubation experiments were conducted at 15 °C which is close to the mean temperature for July within the region (Shurpali *et al*. 2008).

The $\delta^{13}C$ of CO_2 and CO_2 concentration were measured within two days after sampling by a gas chromatograph coupled to an isotope-ratio mass-spectrometer (GC-IRMS; Thermo Finnigan DELTA XP^{Plus}, Bremen, Germany) equipped with a pre-concentration unit and by GC using a thermal conductivity detector (TCD) (Nykänen*et al*. 1995), respectively. The $\delta^{13}C$ values were calculated relative to the Vienna-Pee Dee belemnite (V-PDB) reference;

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and standard, respectively ($R_{standard}$ = 0.011180; Fry 2006). The external analytical precision of the $\delta^{13}C$ measurements was ±0.15‰ based on repeated measurements of a laboratory standard (n = 10) and < 2% for the CO_2 concentrations.

The $\delta^{13}C$ of respired CO_2 was calculated using the Keeling Plot approach (Pataki *et al*. 2003). The two-pool mixing model was used to calculate the fraction (f) of SMR in SR (Fry 2006). The two-pool mixing model quantifies mathematically the proportion of two different sources contributing to a mixture and is based on mass balance. This calculation is possible as the isotopic composition of the mixture is determined by the isotopic signatures and relative proportions of the two contributing sources. Many isotope applications use these two-pool mixing models:

$$\delta_{sample} = (f_{S1} \times \delta_{S1}) + (f_{S2} \times \delta_{S2}) \quad (2)$$

$$f_{S1} + f_{S2} = 1 \text{ or} \quad (3)$$

$$f_{S2} = 1 - f_{S1} \quad (4)$$

where δ_{sample} is the isotopic signature of the mixture (here: $\delta^{13}C$ value of SR including labelled RR and unlabelled SMR in pulse-labelled plots), δ_{S1} is the isotopic signature of source 1 (here: $\delta^{13}C$ value of unlabelled SMR) and δ_{S2} is the isotopic signature of source 2 (here: $\delta^{13}C$ value of labelled RR). Therefore, it is possible to calculate the fraction derived from S1 (SMR):

$$f_{S1} = (\delta_{sample} - \delta_{S2})/(\delta_{S1} - \delta_{S2}) \quad (5)$$

The $\delta^{13}C$ value of CO_2 derived from the unlabelled SMR represents CO_2 derived from decomposition of soil organic matter (SOM). Due to logistical constraints, the $\delta^{13}C$ value of RR was determined after freezing the soil cores for storage. Even though RCG is known to have excellent frost tolerance, we cannot exclude the possibility that freezing may have affected the autotrophic $\delta^{13}C$ values obtained. However, this does not hamper the main outcome of the study but is considered when interpreting the results. Also, the results (fractional contribution of SMR within realistic range; *see* Discussion) give evidence that the impact of freezing was relatively small.

Soil microbial respiration was additionally measured once from eight trenched plots in mid-July 2006 (15 July). Trenched plots were established in spring 2005 by inserting a 20-cm deep PVC collar (diameter 10 cm) into the soil and thereby severing the roots. As the maximum rooting zone of RCG is about 15 cm at this site, with the major part of the root stock found within the upper 10 cm (Shurpali *et al*. 2009), we are confident that all roots were trenched by this treatment. Plants growing in the trenched plots were regularly removed by clipping. Soil microbial respiration (CO_2 emission) was measured by a static chamber (volume 1 l, diameter 10 cm) and IRGA (Licor 6200, Lincoln, Nebraska, USA) with measurement time of 3 minutes. Respiration rates were calculated from the linear increase in CO_2 concentration in the chamber over the measurement period.

Along with the SMR measurements from the trenched plots, SR was measured as an overall

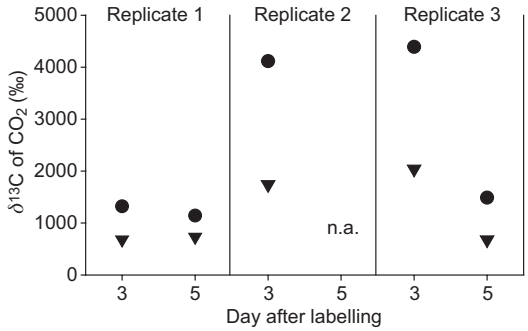

Fig. 1. The $\delta^{13}C$ values indicating the relative difference to the stable isotope composition of autotrophic root respiration (circles; RR) and soil respiration (triangles; SR) after ^{13}C pulse-chase labelling. Samples with higher label have more positive $\delta^{13}C$ values and are referred to as "isotopically enriched". In this experiment, it means that these samples carry the label derived from the $^{13}CO_2$ pulse. The three replicates are shown separately. n.a. = not analyzed.

CO_2 flux from adjacent undisturbed six random plots (control plots) located within 100 m from recently trenched plots at the RCG site. The technique applied did not disturb the roots because the collars (diameter 10 cm) between the stems of RCG were sealed just on the soil surface with wetted quartz sand. The measurements of CO_2 emissions were done as described above. The fractional contribution of SMR to overall respiration was calculated from SMR (measured in trenched plots) and SR (measured in non-trenched plots). All respiration measurements, as well as gas sampling from the labelled plots, were conducted after removing the aboveground litter layer.

If not stated differently, the results are presented as means ± standard error (SE). The statistical differences in the fractions of soil respiration obtained by the two independent methods were tested using Student's *t*-test.

Results

The $\delta^{13}C$ value of CO_2 from the unlabelled SMR was –27.3‰ (Table 1). During the post-labelling period, the $\delta^{13}C$ values of RR varied from 1140‰ to 4390‰, decreasing from day 3 to day 5 (Fig. 1 and Table 1). Due to analytical problems we have no results from day 5 for the second replicate. The $\delta^{13}C$ value of SR (heterotrophic + autotrophic) varied between 677‰ and 2040‰ (Fig. 1). In the first replicate, the $\delta^{13}C$ value of SR remained similar during the two post-labelling sampling days, and in the third replicate it decreased.

The $\delta^{13}C$ value of SR was always between the $\delta^{13}C$ value of RR and the natural abundance level of SMR. The fraction of SMR was calculated for each day using the mixing model (Eq. 5). It was 0.54 and 0.45 on day 3 and 5 after labelling, respectively (Table 1). There were no significant differences between the two post-labelling sampling days. The average fraction of soil respiration calculated from the pulse-chase labelling experiment was thus 0.50 ± 0.08), i.e. based on this method 50% of SR originated from the soil organic matter decomposition by heterotrophs (SMR).

Soil microbial respiration after root trenching varied between 304 and 419 mg CO_2 m^{-2} h^{-1}, with a mean rate of 358 ± 43 mg CO_2 m^{-2} h^{-1}. Total soil respiration was on average 504 ± 90 mg CO_2 m^{-2} h^{-1}. The mean fraction of SMR as revealed by the trenching method was thus 0.71 ± 0.07 (Fig. 2). Root trenching thus resulted in a

Table 1. The fraction ($f_{SMR/SR}$) of soil microbial respiration (SMR) in overall soil respiration (SR) as determined by the isotope partitioning approach and 2-pool mixing model; the values are mean ± SE of two (5th day) and three replicates (3rd day).

Day after labelling	δ_{sample} [a]	δ_{S2} [b]	δ_{S1} [c]	$f_{SMR/SR}$
3rd	1487 ± 412	3276 ± 980	–27.3 ± 0.8	0.54 ± 0.03
5th	705 ± 22.5	1315 ± 141	–27.3 ± 0.8	0.45 ± 0.08
Average				0.50 ± 0.08

[a] $\delta_{sample} = \delta^{13}C$ of SR.
[b] $\delta_{S2} = \delta^{13}C$ of source 2 = $\delta^{13}C$ of root respiration (RR).
[c] $\delta_{S1} = \delta^{13}C$ of source 1 = $\delta^{13}C$ of unlabelled SMR.

42% higher contribution of SMR to SR than the isotopic method as employed here (Fig. 2).

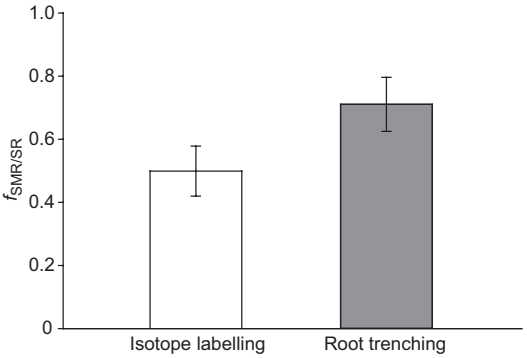

Fig. 2. Comparison of fraction ($f_{SMR/SR}$) of soil microbial respiration (SMR) in overall soil respiration (SR) as determined with ^{13}C pulse-chase labelling (isotope labelling) and root trenching. Student's *t*-test: $t_9 = -3.920$, $p < 0.01$).

Discussion

Following $^{13}CO_2$ pulse-chase labelling of RCG growing on a cut-away peatland, the $\delta^{13}C$ values of SR and RR were highly enriched, on average 1270‰ and 2760‰, respectively (equivalent to 1.37 and 2.94 at % ^{13}C excess, respectively). Organic matter decomposition stemming from SMR had, on the other hand, $\delta^{13}C$ values at the natural abundance level (–27.3‰ or –0.02 at % ^{13}C excess). Shortly after labelling, the two main sources contributing to SR (roots and microbes) were thus characterized by distinct isotope signals and large differences in $\delta^{13}C$ values. The isotopic signal of the mixture of the two sources (SR) was well between the signals of the two sources. Large differences in isotopic signals of end-members are an ideal pre-requisition for accurate source partitioning with isotope mixing model (Paterson *et al*. 2009). The method is thus, in principle, suitable for reliable separation of plant *vs*. soil respiration in undisturbed systems.

On average, a 50% contribution of SMR in SR was revealed by the isotope partitioning approach. The standard error of this mean was relatively small, just 8%. The reported portions of heterotrophic soil respiration for various ecosystems around the globe range from 10% to 90% being on average 40% for non-forested soils (Hanson *et al*. 2000). The contribution of SMR here (50%) as shown by the isotope partitioning approach was higher than this mean at least during the peak growing season. We studied organic soil, a cultivated peatland, which can explain this difference because organic soil contains a high amount of decomposable organic matter. The largest contribution of SMR to overall SR was also found in soils of boreal peatlands (Subke *et al*. 2006). Even though the proportion of SMR in SR as revealed by the isotope partitioning approach here is thus realistic, the number warrants caution as discussed below.

While the measurements of $\delta^{13}C$ of SR and unlabelled SMR posed no difficulties, the analysis of isotopic signal of RR following pulse-chase labelling was not straightforward. Contrary to continuous labelling approaches, pulse-chase labelling does not result in a homogeneous labelling of the roots, with the labile compounds being much stronger labelled than the structural compounds (Hanson *et al*. 2000). Thus, the $\delta^{13}C$ value of RR has to be either measured or indirectly computed from mass balance approaches, or modelled (Sapronov and Kuzyakov 2007). As indirect calculations and modelling tools always include assumptions, measurements would be preferred. However, roots are attached to plants and are tightly linked to the soil matrix, and analysis of RR alone in intact soil is difficult if at all possible. In theory, the roots attached to the plants could be excavated but as the soil atmosphere is different than air this might affect metabolic processes in roots and thus $\delta^{13}C$ of RR (Hanson *et al*. 2000). Root respiration and isotopic signals have to be mostly measured *in vitro* by excising the roots from the root zone. It has been shown that RR may remain stable after cutting within the first 1–3 hours (Liu *et al*. 2006), or may initially increase and later decrease (Kuzyakov *et al*. 2006). However, effects of clipping on the $\delta^{13}C$ values of RR (especially after pulse-chase labelling) remain unclear.

Working in the field in relatively remote areas often requires for the soil cores to be frozen, as here, which raises more questions regarding the viability and reliability of the roots and isotopic signals of RR. Even though RCG

is frost-tolerant, we cannot rule out such an artefact in our experiment. Another drawback of the isotope partitioning approach following pulse-chase labelling is that we do not know the isotopic signal of CO_2 respired from root-associated microorganisms including mycorrhiza, which use root exudates as their substrates, and are considered autotrophs here (Högberg and Read 2006). Since rhizosphere microorganisms are directly linked to root exudates, they are secondary consumers of plant-derived products and could thus receive already ^{13}C-depleted substrates. On the other hand, roots typically have more structural components than e.g. single-celled organisms like soil bacteria associated with roots. Those components remain unlabeled and, therefore, after labeling, CO_2 from roots could be depleted in ^{13}C as compared with CO_2 from root-associated microorganisms. This is supported by data from pulse-chase labeling experiments where soil respiratory fluxes were stronger labeled than fine roots (Högberg and Read 2006). Clipping, excavation and/or freezing most likely also causes a shift towards more storage compounds being respired, which are also less enriched in ^{13}C. Taken together, all shortcomings mentioned above may cause some uncertainties in $\delta^{13}C$ from autotrophic RR. Most likely, the $\delta^{13}C$ values are underestimated under experimental conditions. This would, consequently, result in an underestimation of the fraction of SMR. In our case, the real proportion of SMR in SR of the cultivated peat soil may be thus higher than 50%.

The trenching method resulted in a relatively higher contribution of SMR (71%). Thus, our hypothesis that the partitioning approach based on pulse-chase labeling would result in a lower estimation of the relative proportion of SMR in soils was principally confirmed. However, the results from the labelling experiment have to be considered with care, as discussed above. The major concern with the trenching method is that, in addition to the disturbance caused, dead roots contribute to CO_2 production and often environmental conditions are altered (Hanson *et al*. 2000, Jassal and Black 2006, Kuzyakov 2006, Subke *et al*. 2006). In a meta-analysis it was shown that trenching generally overestimates the contribution of heterotrophic SMR to total SR, even though corrections are frequently implemented to account for the errors (Subke *et al*. 2006). Also in this study, dead roots were found in the trenched plots after one year from establishment (data not shown). A trenching experiment by Silvola *et al*. (1996), however, revealed that 55%–65% of SR from peatlands is derived from SMR. To develop more accurate correction factors related to the contribution of root residues in trenched plots, advantage could be taken of ^{13}C pulse-chase labelling experiments: e.g., plots inside the ^{13}C-labelled area could be trenched followed by measurements of $^{13}CO_2$ emissions, which would indicate directly root-decay patterns. Such an approach should be included in future studies.

Our study showed that both methods applied here revealed approximate estimates on the proportion of SMR *vs*. RR in SR. Homogeneous labelling techniques may be more suitable for such partitioning approaches, but field applications of this method are difficult. To narrow down the proportion of SMR in SR under field conditions, more than one technique should be employed. In our case, the actual portion of SMR in cutaway peatlands cultivated with RCG is, at least during the peak growing season, most likely somewhere between the values obtained by the labelling and trenching methods (50%–71% in July). The microbial respiration component in peat soils seems to be higher than in other non-forest ecosystems. This confirms earlier reports by Shurpali *et al*. (2008) and adds to the common understanding that peat soils are prone to lose C in form of CO_2 (Maljanen *et al*. 2001, Lohila *et al*. 2004).

Summary and conclusion

We introduced here a partitioning approach based on ^{13}C pulse-chase labelling on a cut-away peatland cultivated with RCG, and compared it with one of the most commonly used approaches to separate heterotrophic and autotrophic respiration (SMR and RR), the trenching technique. Even though only preliminary data could be presented and more developmental work is needed, this technique provides important insights into heterotrophic and autotrophic respiration. The advantage of the labelling approach is clear: par-

titioning soil and root respiration can be done *in situ* in intact soil–root systems, and thus the fate of recent C compounds and interactions between plant and soil C are taken into account. However, the method is not completely non-invasive as it includes excavation and/or clipping of roots for determining the $\delta^{13}C$ values of autotrophic respiration. This problem is difficult to overcome, even though careful handling would improve the accuracy of the measurement together with indirect calculations of label distribution and modelling tools. The trenching approach is also biased by the influence of residual, decomposing roots and altered environmental conditions. Taken together, further research is needed to prove the true potential of partitioning soil and root respiration following $^{13}CO_2$ pulse-chase labelling experiments. When only approximate values can be obtained, always more than one experimental approach should be employed to determine the soil C losses from grasslands.

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