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RESEARCH ARTICLE

The dependence of soil microbial activity on recent photosynthate from trees

S. G. Göttlicher · K. Steinmann · N. R. Betson · P. Högberg

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Abstract It remains a challenge to quantify and assess the importance of the direct plant belowground flux of photosynthate carbon (C) to soil microorganisms, especially in forests because of the size of the plants and the great spatial heterogeneity of soils. We studied the importance of labile C inputs from trees on the respiratory activity of soil microorganisms by comparing the response of plots with and without girdled pine trees (*Pinus sylvestris* L.) to additions of C_4-sucrose, thus enabling us to differentiate between utilization of endogenous C_3-soil C sources and exogenous C_4-sucrose. In both girdled and non-girdled plots the respiration rate after sucrose application, i.e. substrate induced respiration measured in the field, was on average ca. double that of basal respiration rate measured in the field. However, the C_4-sucrose-induced increase in respiration of endogenous C_3-C was significantly higher in non-girdled plots. Expression of C_3-respiration as a percentage of induced respiration in the field showed that in girdled plots, C_3-respiration decreased after sucrose addition and, consequently, the induced respiration in the field was totally C_4-C based. A previous laboratory experiment found no increase in total respiration rate when C_4-sucrose was added to the soil substrate of non-mycorrhizal and ectomycorrhizal pine plants. Hence, we see no reason to attribute the increased respiration to (mycorrhizal) roots. Thus, our results indicate that despite the alleged C limitation of the soil microorganisms there is a fraction of SOM, or C within the microbial biomass that is available to microbial metabolism if their C limitation is relieved by the supply of labile C. This fraction corresponds to roughly 10–20% of biomass C of the heterotrophic organisms and seems to become exhausted in the long-term absence of supply of photosynthate to roots.

Keywords Carbon isotope · Carbon limitation · Forest soil · Priming · Soil microorganisms · Tree-girdling

Abbreviations

BR_{field}	The basal respiration of the untreated undisturbed soil measured directly in the field
IR_{field}	Induced respiration in the field is $SIR_{field}-BR_{field}$
SIR_{field}	Substrate induced respiration in the field is the total respiration rate after addition of sucrose measured in the field

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S. G. Göttlicher (✉) · N. R. Betson · P. Högberg
Section of Soil Science, Department of Forest Ecology, SLU, SE-901 83 Umeå, Sweden
e-mail: sabine.goettlicher@sek.slu.se

K. Steinmann
Dept. of Landscape Dynamics, Swiss Federal Research Institute WSL, 8903 Birmensdorf, Switzerland

Introduction

The metabolism of a few cultured microorganisms is comparatively well known at the cellular and molecular levels, but factors limiting the activity and growth of microbial populations in native soils are poorly understood. The classical view is that soil microorganisms are carbon (C)-limited, while in the same systems plants are nutrient-, notably nitrogen (N)-limited (Alexander 1977; Paul and Clark 1996; Tate 1995). However, the term ''microorganisms'' combines organisms that metabolise different fractions of soil organic matter (SOM) and more recent litter with symbiotic mycorrhizal fungi and other organisms in the rhizosphere that receive recent plant photosynthate. As a result, the term ''microorganisms'' encompasses organisms likely to be very limited by C, with those that are directly supplied by a significant quantity of highly labile C (Chapin et al. 2002; Cheng et al. 1996; Jones et al. 2004).

The fundamental difference in the degree of dependence on recent photosynthates between mycorrhizal and saprotrophic fungi was demonstrated in a boreal forest by a large-scale tree girdling experiment, in which the flux of photosynthate to roots and hence, associated organisms was terminated. Two months after girdling the number and biomass of fruiting bodies of ectomycorrhizal fungi was reduced almost to zero, whereas there was no effect on the number or biomass of saprotrophic fungi (Högberg et al. 2001). Thus, in this paper we will make a functional distinction between heterotrophic microorganisms decomposing younger and older plant remains and microorganisms directly supplied with C from recent photosynthates.

It remains a challenge to characterize and quantify the importance of the photosynthate-based C-flux to these latter organisms, especially in forests with large trees and considerable soil heterogeneity. In the mentioned girdling experiment, soil respiration was reduced by 50% within weeks (Högberg et al. 2001) and the soil microbial biomass C was reduced by ca. 30% within a few months (Högberg and Högberg 2002). These effects and the disappearance of ectomycorrhizal fruiting bodies indicate the potential importance of the current photosynthate C-supply on soil microbial activity.

Soil microbial biomass and activity can be assessed by a variety of methods. For example, substrate induced respiration (SIR) is frequently used to estimate the amount of C in living, non-resting biomass in soils. The method is based on the initial respiratory response of microbial biomass to a supply of a C-source, and is performed under standard laboratory conditions (Anderson and Domsch 1978). Högberg and Ekblad (1996) applied this approach in the field, taking advantage of the difference in stable C isotope composition ($\delta^{13}C$) between C_3- and C_4-plants. The $\delta^{13}C$ in C_3-plants ranges from −35 to −21‰, relative to the Vienna Pee Dee Belemnite, and in C_4-plants from −20 to −9‰ (O'Leary 1988). In this paper we refer to the field version of the SIR approach as SIR_{field}. In contrast to the initial SIR study by Anderson and Domsch (1978) SIR_{field} does not aim to calibrate the respiratory response with the chloroform fumigation method in order to calculate microbial biomass, as the requirement of constant temperature for this calibration is violated in the field. The observation of the respiratory response is extended over the period of linear increase of the respiration rate. The rationale behind combining the SIR approach with ^{13}C isotope studies in a boreal forest was that the induced respiration carries the isotopic signature of the added C_4-substrate, while the basal respiration carries the isotopic signature of the C_3-system.

However, the relationship between C_3- and C_4-derived CO_2 fluxes is made more complicated by ''priming effects'', i.e. ''strong, short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil'' (Kuzyakov et al. 2000). Application of C_4-sucrose to boreal forest soils induced a short-term increase in C_3-respiration (C_{3resp}) (Ekblad and Högberg 2000; Ekblad et al. 2002; Högberg and Ekblad 1996). This increase can be attributed to microbial metabolism, excluding that of mycorrhizal fungi, because there is experimental evidence that sucrose addition does not induce an increase in respiration rate in non-mycorrhizal roots and ectomycorrhizal roots (Ekblad and

Högberg 2000). Furthermore, the C_4-sucrose-induced increase in C_3-respiration is similar in sieved (root-free) mor-layers and in the field (with roots) (Ekblad and Högberg 2000; Kelliher et al. 2005). Two of the explanations given by Ekblad and Högberg (2000) for the additional C_3-C metabolised after addition of C_4-sucrose (in this article referred to as ''primable'' C) were increased use of C already present in the microbial biomass (a phenomenon also suggested by Dalenberg and Jager 1981, 1989), or accelerated decomposition of SOM. Both mechanisms are listed as mechanisms of real priming (Kuzyakov et al. 2000). A further possibility suggested by Högberg and Ekblad (1996) is discrimination against ^{13}C in newly added substrate during respiration leading to an overestimation of the proportion of C_{3resp}. The possibility of an isotope effect during microbial metabolism has been tested thoroughly (Ekblad and Högberg 2000; Ekblad et al. 2002), and it seems extremely unlikely that C isotope fractionation occurs in the forest type studied. Ekblad and Högberg (2000) found no shift in the $\delta^{13}C$ of evolved CO_2 after adding C_3-sucrose to sieved mor-layer material and Ekblad et al. (2002) found similar values for calculated contributions from added C and endogenous C_3-C to SIR_{field} after addition of C_4-sucrose (−10.8‰) or ^{13}C labelled glucose (103.7‰). These values should clearly differ, if discrimination is significant. Both tests confirmed that C isotope discrimination during microbial respiration is minor in this system.

Thus, the most likely explanations for additional C_3-C metabolised after addition of C_4-sucrose are increased use of C already present in the microbial biomass or accelerated decomposition of SOM. However, the existence of an unexploited endogenous C-pool in a system in which microorganisms are thought to be C-limited seems to be a paradox. Are these reserves of ''primable'' C exhausted or does ''primable'' C accumulate in the prolonged absence of constant priming by root exudates? We investigated this question by comparing the response of endogenous C_3-soil respiration to additions of C_4-sucrose in tree-girdled plots (lacking input of recent photosynthate to their roots and below-ground microbial communities) with non-girdled control plots. We hypothesized that a C_4-sucrose-induced increase in C_{3resp} would be significantly less in the girdled plots, reflecting a lack of labile C inputs from recent photosynthesis.

Material and methods

Forest site

The experiments were conducted in a 48–59-year-old Scots pine (*Pinus sylvestris* L.) forest at Åheden in northern Sweden (64°14′N, 19°46′E, 175 m above sea level). The soil is a weakly podzolized sediment of sandy silt (Högberg et al. 2001). The organic mor-layer was 2 cm thick and had a bulk density of 0.16 ± 0.05 g cm^{-3} (Högberg and Högberg 2002). The understorey vegetation is very sparse and consists mainly of the dwarf shrubs *Vaccinium vitis-idea* L. and *Calluna vulgaris* L. In August 2000, trees in three 900 m^2 plots were girdled, i.e. the bark was removed over 0.3 m long circular sections at breast height, terminating the supply of current photosynthate to roots. Three non-girdled plots served as controls. Each plot contained about 120 trees. Girdled trees lost their foliage in the third summer after the treatment. This added additional needle litter onto the forest floor, but this was removed temporarily, together with the forest floor lichen and moss layer, during measurements of soil respiration, as described below. C and nutrient input to the soil through leachates of the litter and decomposing roots cannot be excluded, but Bhupinderpal-Singh et al. (2003) calculated that decomposition of the standing root biomass would yield a small amount of C in comparison with the total soil respiratory activity for this study site. However, if the experimental manipulation associated with girdling provides an additional source of C then the difference between girdled and non-girdled plots should diminish.

Girdling led to a reduction of 32% of the microbial biomass within the first year, which was calculated to correspond to 58 kg C ha^{-1} (Högberg and Högberg 2002). The remaining heterotrophic microbial biomass was thus ca. 120 kg C ha^{-1}. For more details on the site see Bhupinderpal-Singh et al. (2003), Högberg and Högberg (2002) and Högberg et al. (2001).

Substrate addition treatments

In each of the three girdled (G) and three non-girdled (NG) plots, two plastic cylinders were installed. The positions of the plastic cylinders were chosen randomly within the plot centres (to avoid an edge effect) and we did not aim for a fixed distance to the neighbouring trees. Shoots of the understorey vegetation—if present—were cut back with scissors. The moss and lichen layer, and fresh litter found on top of the mor-layer were removed. One cylinder (control) served to measure basal respiration (BR_{field}) and the other to measure SIR_{field}. The surface area enclosed by the cylinders received ten 10 ml injections (amounting to 100 ml) of either water or C_4-sucrose solution (100 g sucrose l^{-1}, from sugar cane, *Saccharum officinarum* L.) for the control or the SIR_{field} cylinders, respectively. The sucrose solution and water were injected with a syringe fitted with a 4-sideport needle. Injections started at the interface between the mineral soil and the mor-layer, with the needle slowly lifted through the mor-layer during injection. The C isotope composition of the sucrose solution was $-11.8 \pm 0.0‰$ in 2003 and $-11.6 \pm 0.1‰$ in 2004.

Gas sampling

The gas sampling method basically followed that of Högberg and Ekblad (1996). Briefly, the moss and lichen layer, and fresh litter found on top of the mor-layer were removed during measurements. Cylinders of opaque PVC-plastic (diameter 243 mm, height 135 mm) were put onto the soil and covered by a removable PVC-lid, thus creating a headspace of ca. 6 l. In order to provide a tight seal between the soil surface and the cylinder a 2 kg stone was placed on top of each lid. Gas samples of 12 ml were withdrawn with a syringe through a rubber membrane stopper in the lid and immediately transferred to pre-evacuated 12 ml glass vials. The first gas sample was taken directly after closure of the lid, and then every 2 (non-girdled plots) or 3 (girdled plots) -min. A pilot study showed that due to lower respiration rates, sampling intervals of 3 min were more suitable for girdled plots. At each sampling, and for each cylinder, five gas samples were withdrawn in total, resulting in a sampling time of 8 and 12 min in non-girdled and girdled plots, respectively. Gas sampling was performed between 12:00 and 14:00. However, we did not expect different respiration rates at different hours of the day as a detailed study of soil respiration in a nearby boreal forest found no diurnal variation in soil respiration rate or in the $\delta^{13}C$ of the CO_2 efflux (Betson, N.R. unpublished). After sampling the plastic cylinders stayed in place, but the lids were removed and the moss and lichen layer was replaced.

The sampling periods were between 29th August and 4th September 2003 and between 3rd and 6th September 2004. The positions of the plastic cylinders were not identical in 2003 and 2004. Gas samples were taken four times during each sampling period. In 2003, the sampling interval was 48 h resulting in sampling times 0 (before addition), 48, 96, 144 h after sucrose addition. In 2004 the sampling interval was 24 h and the sampling times 0, 24, 48, 72 h after sucrose addition.

Measurements of CO_2 concentration and C isotope composition of CO_2

An autosampler transferred the gas samples from the glass vials to a Model 20-20 stable isotope analyser (Europa Scientific Ltd, Crewe, UK), via an ANCA-NT gas purification module, using He as a carrier gas. For further information on the measurements see Högberg and Ekblad (1996).

Results of the C isotope composition are reported in $\delta^{13}C$ (‰):

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

where $R = {}^{13}C/{}^{12}C$. The standard used was 5% CO_2 in N_2 with a $\delta^{13}C$ of 5‰ relative to the international standard Vienna-Pee Dee Belemnite. The analytical precision was $\leq \pm 0.06\%$ for the CO_2 concentration measurements and $\leq \pm 0.15‰$ for the $\delta^{13}C$ measurements ($\pm$ 95% C.I. n = 14–32).

Calculations

The respiration rate was determined as the rate of CO_2 increase with time. Respiration rates are

given in mg C m^{-2} h^{-1}. To assess the isotopic composition of soil respired CO_2, the Keeling plot approach was used (Keeling 1958). This is based on a two-component isotope mixing model, consisting of the $\delta^{13}C$ of both the atmospheric and soil-respired CO_2. After closure of the cylinder, the CO_2 concentration increases linearly, but the $\delta^{13}C$ of CO_2 decreases non-linearly. The intercept of a linear regression of $\delta^{13}C$ of sampled CO_2 versus 1/[CO_2] provides an estimate of $\delta^{13}C$ of soil-respired CO_2 (where [CO_2] is the CO_2 concentration in the sample in %). Some samples had to be removed due to sampling problems. If r^2 of the regression line was <0.9, the Keeling plot was excluded from further analysis. As the r^2 of the Keeling plots used were high (0.98 ± 0.00 for 2003 and 0.97 ± 0.00 for 2004) a simple linear regression of $\delta^{13}C$ of sampled CO_2 versus 1/[CO_2] was used, since it has been shown that for Keeling plots with high r^2 values (>0.95), the results of this approach are reliable (Pataki et al. 2003). Examples of Keeling plots for BR_{field} and SIR_{field} on a non-girdled and girdled plots are given in Fig. 1.

The fraction (f) of C_4-C-derived CO_2 in total SIR_{field} was calculated as:

$$fC_{4resp} = (\delta^{13}C_{SIRfield} - \delta^{13}C_{BRfield}) / (\delta^{13}C_{4suc} - \delta^{13}C_{BRfield}) \quad (2)$$

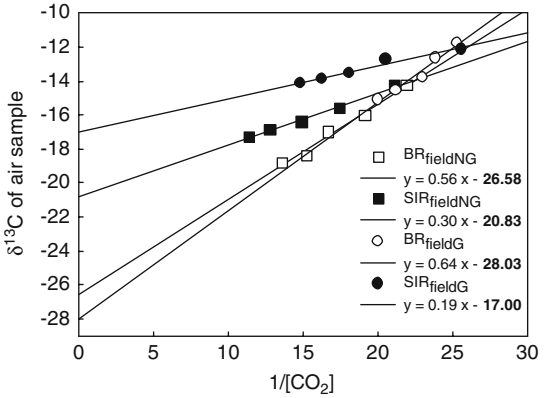


Fig. 1 Example Keeling plots in a non-girdled plot and in a girdled plot. □ BR_{field} in non-girdled plots ■ SIR_{field} in non-girdled plots ○ BR_{field} in girdled plots ● SIR_{field} in girdled plots. The regression equation with the intercept is given in the figure

where fC_{4resp} is the proportion that can be attributed to the respiration of C_4-substrate, $\delta^{13}C_{SIRfield}$ is the C isotope composition (‰) of soil respired CO_2 in sucrose-amended plots, $\delta^{13}C_{BRfield}$ is the C isotope composition in control plots and $\delta^{13}C_{4suc}$ is the C isotope composition of the C_4 sucrose solution applied.

The fraction of C_3-C-derived CO_2, fC_{3resp}, in total SIR_{field} is:

$$fC_{3resp} = 1 - fC_{4resp} \quad (3)$$

We did not use a correction factor for possible fractionation during microbial respiration, as C isotope fractionation during microbial respiration should be minor in the studied system (Ekblad and Högberg 2000; Ekblad et al. 2002). The standard errors of fC_{4resp} and fC_{3resp} were calculated using the Excel Microsoft Corporation Spreadsheet provided by Phillips and Gregg (2001).

The contribution of C_4-C-derived and C_3-C-derived CO_2 to total SIR_{field}, C_{4resp} and C_{3resp} (mg C m^{-2} h^{-1}), as a respiration rate, was calculated by multiplying fC_{4resp} and fC_{3resp} by SIR_{field}, the respiration rate in sucrose-amended plots (mg C m^{-2} h^{-1}).

$$C_{4resp} = fC_{4resp} * SIR_{field} \quad (4)$$

For each measurement day, all of the above parameters were calculated using the mean values for girdled and non-girdled plots.

The contribution of C_3-C-derived CO_2 and C_4-C-derived CO_2 to the induced respiration rate in the field, IR_{field}, was calculated as a percentage of IR_{field} using the mean values of the replicate plots for the calculations:

$$IR_{field} = SIR_{field} - BR_{field} \quad (5)$$

$$\%C_{4IR} = (C_{4resp}/IR_{field}) * 100 \quad (6)$$

$$\%C_{3IR} = (C_{3resp}/IR_{field}) * 100 \quad (7)$$

where IR_{field} is the induced respiration rate in the field, BR_{field} the respiration rate in the control plots, $\%C_{4IR}$ and $\%C_{3IR}$ are the percentages of C_4-C- and C_3-C-derived CO_2 of IR_{field}, respectively. $\%C_{4IR}$ and $\%C_{3IR}$ were calculated for

girdled and non-girdled plots using the mean values of replicate plots.

The time taken to cycle C through the heterotrophic fraction of the microbial biomass, T_{mic} (d) was calculated by dividing the microbial biomass C (C_{micG}) (g C m^{-2}) in the girdled plots (values for C_{micG} taken from Högberg and Högberg, 2002) by the basal respiration rate of the girdled plots averaged over the sampling period BR_{fieldG} (g C m^{-2} d^{-1}):

$$T_{mic} = (C_{micG}/BR_{fieldG}) \quad (8)$$

Further, additional C_3-C, mobilized after sucrose addition, was expressed as a fraction of the heterotrophic microbial biomass ($C_{3IR/mic}$):

$$C_{3IR/mic} = (C_{3IRNG24}/C_{micG}) \quad (9)$$

where $C_{3IRNG24}$ is the amount of C_3-C liberated additionally after C_4-sucrose addition during a 24 h period in the non-girdled plots (g C m^{-2} d^{-1}), taking the average value for the period during which a positive priming effect was observed.

Statistics

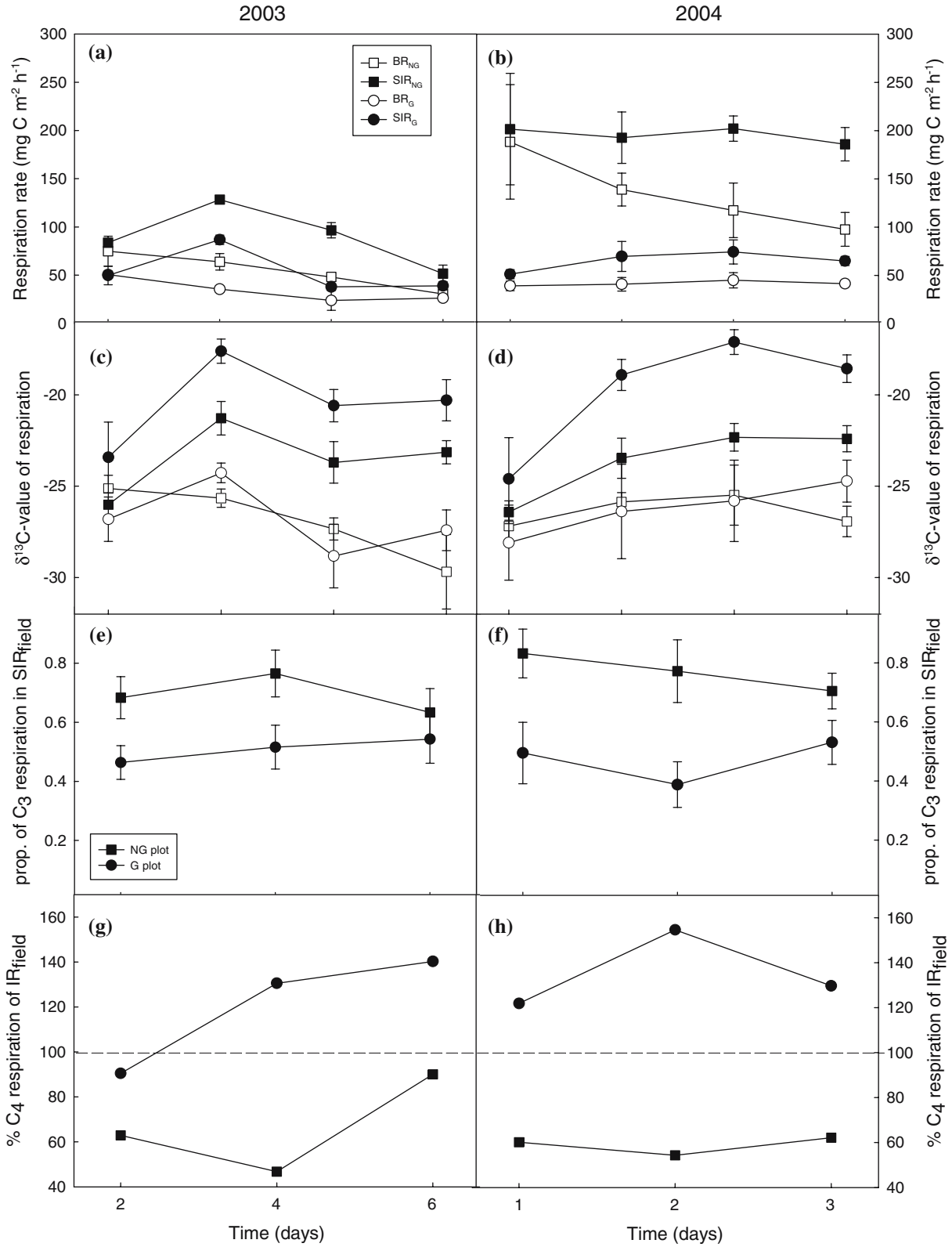
The statistical package MINITAB 14 (Minitab Inc., State College, PA, USA) was used for data analysis. Effects of girdling and sucrose addition were tested by application of a mixed general linear model ANOVA for split-plot designs and subsequent manual recalculation of the significance of the main plot error (girdling) (Mead et al. 1993). For comparisons between years or between treatments (girdled and non-girdled) one-way ANOVA was performed.

Results

The basal respiration rate in non-girdled plots, averaged over the sampling period, was higher in 2004 than in 2003 (one-way ANOVA, $P < 0.001$), 137 ± 19 and 54 ± 7 mg C m^{-2} h^{-1}, respectively, but in girdled plots there was no difference between the years: 41 ± 3 and 34 ± 4 mg C m^{-2} h^{-1} in 2004 and 2003, respectively (Fig. 2a, b). In both years and treatments (girdled and non-girdled), respiration rates increased significantly after sucrose additions in comparison to the respiration rate of the control cylinder, except on day 3 in 2004, where there was no significant effect (Fig. 2a, b). Based on ANOVA for split-plot experimental designs girdling had a significant impact on the respiration rate ($P < 0.05$). The relative increase after sucrose addition (as a percentage of the BR_{field}), averaged over the 3- or 6-day sampling period was similar between treatments (199 ± 18 and 213 ± 35% in 2003 and 169 ± 13 and 173 ± 21% in 2004 for non-girdled and girdled plots, respectively, Table 1). There was no significant difference in these increases calculated as a percentage of BR_{field} between treatments within years. In 2003, 183 and 184 mg C_4-C were respired on average per cylinder during the 6 day sampling period and in 2004, 148 and 124 mg C_4-C were respired on average per cylinder during the 3-day sampling period, in non-girdled and girdled plots, respectively. In both years and for both treatments less than 5% of the C_4-sucrose applied was respired, which is comparable with values published by Ekblad and Nordgren (2002). These calculations were based on the assumption that there were no differences in respiration rate between night and day.

The absolute increase in respiration rate (after addition of sucrose), averaged over the sampling period, was higher in non-girdled plots than in girdled plots in 2004 (one-way

Fig. 2 (**a**–**h**) Respiration rate (mg C m^{-2} h^{-1}) in girdled and non-girdled plots in (**a**) 2003 and (**b**) 2004 and stable C isotope composition (‰) of the respired CO_2 in (**c**) 2003 and (**d**) 2004 □ BR_{field} in non-girdled plots ■ SIR_{field} in non-girdled plots ○ BR_{field} in girdled plots ● SIR_{field} in girdled plots. Error bars represent standard error (n = 2–3). Proportion of C_3-C derived CO_2 in SIR_{field} in (**e**) 2003 and (**f**) 2004 ■ proportion of C_3-C-derived CO_2 in SIR_{field} in non-girdled plots, ● proportion of C_3-C-derived CO_2 in SIR_{field} in girdled plots. Percent of C_4-C-derived CO_2 of the IR_{field} in (**g**) 2003 and (**h**) 2004 ■ percentage of C_4-C-derived CO_2 of the IR_{field} in non-girdled plots, ● percentage of C_4-C-derived CO_2 of the IR_{field} in girdled plots. The percentages were calculated using the mean values of the respiration rate and the mean $\delta^{13}C$ values of the respiration of the three non-girdled and the three girdled plots


2003
2004
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
BR_NG
SIR_NG
BR_G
SIR_G
Respiration rate (mg C m-2 h-1)
δ13C-value of respiration
prop. of C3 respiration in SIRfield
% C4 respiration of IRfield
NG plot
G plot
Time (days)

ANOVA, $P < 0.01$); $IR_{fieldNG}$ were 45 ± 8 and 73 ± 8 mg C m^{-2} h^{-1}, as compared to IR_{fieldG} 26 ± 7 and 29 ± 8 mg C m^{-2} h^{-1} in 2003 and 2004, respectively. The difference in the $\delta^{13}C$ between sucrose-amended and control cylinders over the course of the sampling period was larger in girdled plots (7.3 ± 0.7‰ in 2003 and 7.1 ± 1.3‰ in 2004 compared with 4.9 ± 1.0‰ in 2003 and 3.9 ± 0.7‰ in 2004 in non-girdled plots; Fig. 2c, d). The differences between girdled and non-girdled plots were statistically significant in 2004 (one-way ANOVA, $P < 0.05$).

The fraction of C_3-C derived respiration in SIR_{field} was higher in non-girdled plots than in girdled plots (Fig. 2e, f). In 2003 it ranged from 0.63 ± 0.08 to 0.77 ± 0.08 in non-girdled plots and from 0.46 ± 0.06 to 0.54 ± 0.08 in girdled plots. In 2004 it ranged from 0.7 ± 0.06 to 0.83 ± 0.08 in non-girdled plots and from 0.39 ± 0.08 to 0.53 ± 0.07 in girdled plots. The C_3-C-derived respiration in SIR_{field} consists of basal respiration in the field and C_4-sucrose induced C_3-respiration. Calculation of $\%C_{4IR}$ illustrates the importance of $\%C_{3IR}$ to IR_{field} (Fig. 2g, h). The $\%C_{3IR}$ averaged over the sampling period was 33 ± 13% in 2003 and 41 ± 2% in 2004 in non-girdled plots, but −20 ± 15% in 2003 and −35 ± 10% in 2004 in girdled plots. Averaged over the sampling period, these values for girdled and non-girdled plots were significantly different in 2004 (one-way ANOVA, $P < 0.01$). During the days in which a positive priming effect (i.e. increased respiration of endogenous C_3-C), was observed, an amount of C corresponding to between 10 and 20% of the heterotrophic microbial biomass-C was liberated as C_{3IR}-C in the non-girdled plots (equivalent of ca. 1/20 of the microbial biomass each day).

Table 1 SIR_{field} as percentages of BR_{field} in non-girdled (NG) and girdled (G) plots. Calculations were performed with the single values of the three non-girdled and three girdled plots. Values are means ± 1 SE. The mean value ± 1 SE of all sampling days is given in bold

Time	Percent of $BR_{fieldNG}$	Percent of BR_{fieldG}
2003	(%)	(%)
Day 2	207 ± 14	249 ± 8
Day 4	205 ± 18	242 ± 6
Day 6	186 ± 28	147 ± 6
Average	**199 ± 18**	**213 ± 35**
2004		
Day 1	139 ± 10	170 ± 29
Day 2	173 ± 25	184 ± 80
Day 3	198 ± 22	167 ± 10
Average	**169 ± 13**	**173 ± 21**

Discussion

In both years, C_{4IR} in non-girdled plots accounted for less than 100 percent of IR_{field}, indicating that a fraction of IR_{field} was C_{3IR}. A short-term increase in C_3-respiration after application of C_4-sucrose to boreal forest soils has been observed before (Ekblad and Högberg 2000; Ekblad et al. 2002; Högberg and Ekblad 1996). In contrast, in girdled plots C_{4IR} accounted for more than 100 percent of IR_{field}, indicating that C_{3resp} decreased after C_4-sucrose addition (Fig. 2g, h). Thus, our hypothesis that the $\%C_{3IR}$ (C_4-sucrose-induced increase in C_{3resp}) should be less in girdled plots than in non-girdled plots was confirmed. In 2004 this difference was significant when averaged over the sampling period. Our data suggest that the reserves of ''primable'' C become exhausted in the absence of a continuous plant phloem-C flux to the soil, thus, amplifying the C-limiting conditions experienced by the microorganisms. The decrease in C_{3resp} after sucrose addition indicates that the microorganisms switch from decomposing endogenous SOM to the more labile sucrose added, a mechanism previously suggested by Kuzyakov et al. (2000). The most obvious difference between girdled and non-girdled plots is that girdled plots lack active (mycorrhizal) roots. Still, we consider it unlikely that the observed $\%C_{3IR}$ in non-girdled plots is a response of (mycorrhizal) roots as (1), a pot experiment on pine seedlings showed that sucrose addition does not induce an increase in respiration rate in non-mycorrhizal roots and ectomycorrhizal roots alone (Ekblad and Högberg 2000); and as (2), the C_4-sucrose-induced increase in C_3-respiration was similar in sieved (root free) mor-layer and in the field (with roots) (Ekblad and Högberg 2000; Kelliher et al. 2005).

The observed differences in the respiratory response to substrate addition (i.e. lower absolute increase in IR_{field} and no priming effect in girdled plots), in the long-term absence of living plant

roots and root exudates can accordingly be attributed to heterotrophic microorganisms and may be partly linked to differences in the microbial species composition. A change in microbial species composition after girdling was found in a proteomic study of dissolved organic matter. In undisturbed forest soils, proteins from nine distinct taxonomic groups were found in contrast to soils of a forest that was girdled 1 year before, where proteins of only four taxonomic groups were found. In the non-girdled forest soil, 30% of the proteins were of bacterial origin, whereas in girdled forest soil, 60% were of bacterial origin (Schulze et al. 2005). Moreover, results from a study of phospholipid fatty acids conducted at the site of the present study showed that the abundance of the fungal phospholipid fatty acid biomarker 18:2ω6,9 was reduced by 50% in girdled plots (Mona N. Högberg, pers. comm.).

It is obvious that microorganisms have a key role in priming; Kuzyakov et al. (2000) state it is an ''undisputed fact'' that real priming effects have never been observed under sterile conditions, i.e. in the absence of microorganisms. The additional endogenous C that is liberated during priming could be derived from the endocellular reserves of microorganisms or from SOM. The fact that considerable positive priming is only found in non-girdled plots with an intact, active rhizosphere indicates that the C-status of the microbial biomass in the girdled plots was inferior to that of the non-girdled plots. This could be due to decreased endocellular reserves of individual microorganisms or due to shifts in the microbial community composition towards species with less endocellular reserves. If the C_{3IR}-C is only due to mobilization of endocellular reserves, then a considerable fraction (approximately 1/20) of the microbial C-biomass would be turned over during one day of priming. Alternatively, the absence of a positive priming effect in the girdled plots might indicate that the fraction of SOM that can be mobilized if the C limitation is relieved, had already been exhausted during the period after girdling. Differences in the magnitude of the priming effect between girdled and non-girdled plots have been observed before. The presence of fresh litter caused a stronger and more persistent priming effect in non-girdled than in girdled plots (Subke et al. 2004), indicating a positive role for active mycorrhizal roots in the decomposition of SOM.

BR_{field} in the girdled plots, where only respiration by heterotrophic organisms occurs, suggests that it takes a few weeks to turn over the microbial biomass C of the heterotrophs. The results of the calculations based on C_{mic} are only rough estimates, indicative of an order of magnitude rather than exact values, as C_{mic} was not measured in the same years as our experiments were performed. However, it was measured at the same time of the year (at the beginning of September) and thus, seasonal variation in C_{mic} should not confound our calculations. Similarly, it is unlikely that inter-annual variation in the respiration rate is another confounding factor since we also obtained similar results when using the respiration rates which were obtained in the same year as the microbial biomass measurements (Högberg et al. 2001).

The different patterns of soil respiration after sucrose addition in girdled and non-girdled plots showed that terminating the C-flux from the canopy to the root–soil system not only reduces root and mycorrhizal respiration immediately (Högberg et al. 2001), but also influences the soil microorganisms on a long-term basis. In the absence of a direct supply of photosynthate to the soil, the amount of ''primable'' C was reduced to zero. This raises the question of why microorganisms in a soil with an intact rhizosphere, where ''primable'' C is present, do not exhaust all their C sources, i.e. why do allegedly C-limited organisms have C reserves? A hypothesis that explains the observed presence of ''primable'' C in a system where soil microorganisms are thought to be C-limited has been put forward by De Nobili et al. (2001), according to which the soil microbial survival strategy is based on a population of ''resting cells'' maintaining a state of ''metabolic alertness'' and being prepared to invest more energy (endocellular reserves) if a ''food event'' is sensed. If this is true, then the lack of a priming effect and the smaller absolute respiratory response to sucrose addition in girdled plots suggests that this ''metabolic alertness'' is reduced after a prolonged absence of supply with photosynthate C.

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