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Three-source-partitioning of microbial biomass and of CO_2 efflux from soil to evaluate mechanisms of priming effects

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ABSTRACT

We propose and successfully applied a new approach for 3-source-partitioning based on a combination of ^{14}C labeling with ^{13}C natural abundance. By adding ^{14}C-labeled glucose to soil after $C_3 - C_4$ vegetation change, we partitioned three C sources in three compartments, namely CO_2, microbial biomass and dissolved organic C (DOC). This enabled us to estimate mechanisms and sources of priming effects (PE).

Glucose application at low and high rate (GL: 100 and GH: 1000 μg C g^{-1}, respectively) caused positive PE both short-term (during 1–3 days) and long-term (3–55 days). Despite a 10-fold difference in the amount of substrate added, the PE observed was larger by a factor of only 1.6 at the high versus low rate of glucose. The real and apparent priming effects were distinguished by partitioning of microbial C for glucose-C and SOM-derived C. As the amount of primed CO_2 respired during short-term PE was 40% lower than microbial C, and the contribution of soil C in microbial biomass did not increase, we concluded that such short-term PE was apparent and was mainly caused by accelerated microbial turnover (at GL) and by pool substitution (at GH). Both the amount of primed CO_2–C, which was 1.3–2.1 times larger than microbial C, and the increased contribution of soil C in microbial biomass allowed us to consider the long-term PE as being real. The sole source of real PE (GL treatment) was the "recent" soil organic matter, which is younger than 12-year-old C. The real PE-induced by a glucose amount exceeding microbial biomass (GH) was due to the almost equal contribution of 'recent' (<12 years) and 'old' (>12 years) C. Thus, the decomposition of old recalcitrant SOM was induced only by an amount of primer exceeding microbial C. We conclude that combining ^{14}C labeling with ^{13}C natural abundance helped disentangle three C sources in CO_2, microbial biomass and DOC and evaluate mechanisms and sources of PE.

1. Introduction

The interactions between living and dead organic matter in soil cause the phenomena of priming effects (PE)– processes changing the equilibrium between carbon (C) inputs in the form of plant residues and rhizodeposits versus C outputs as CO_2 from mineralization of various organic pools (Kuzyakov, 2010). Such equilibrium stabilizes the soil organic matter (SOM) pool (West and Six, 2007), which is the largest C stock in terrestrial ecosystems (Batjes, 1996). The input of available substrates alters microbial activity and may accelerate SOM decomposition, in turn affecting C sequestration in soil (Paterson et al., 2009). SOM is chemically not uniform and consists of relatively recent and relatively old organic compounds with different availability (von Lützow and Kögel-Knabner, 2009). This makes it important to know the extent to which the older SOM pools comprising the major C storage are involved in PEs. Plant-derived and especially root-derived 'recent' C was suggested to be a major microbial carbon source in topsoils (Rasse et al., 2005; Kramer and Gleixner, 2008). Fontaine et al. (2007), however, showed that the additional decomposition of old SOM in deep soil horizons was primed by the inputs of 'recent' plant-derived carbon. Nonetheless, these studies have not estimated the relative contribution of recent and old C to PE because no approaches to partition the three C sources were used.

The experimental design of previous studies allows distinguishing only two C sources: added substrate and SOM-originated C. Either ^{14}C (Bell et al., 2003; Hamer and Marschner, 2005; Hoyle et al., 2008) or ^{13}C (Niklaus and Falloon, 2006; Nottingham et al., 2009; Salome et al., 2010) labeling was used to trace the fate of added substrate and to evaluate PE. As only one C source is

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labeled, the PE originated from the SOM as a whole was estimated. This hindered determining the contribution of old and recent SOM to PE. Although numerous studies based on ^{14}C and ^{13}C isotopes assessed the transformation and turnover of SOM (reviewed by Paterson et al., 2009), it remains unclear which SOM pools – old and recalcitrant or recent and easily available – are involved in PEs. Depending on the ratio between added glucose and microbial biomass content, the increasing glucose amount can either increase or decrease PE (Zyakun and Dilly, 2005; Blagodatskaya and Kuzyakov, 2008). These differences in PE intensity can be explained only after clarifying the underlying PE mechanisms. Elucidating the C pathways during PEs as well as distinguishing the real (enhance the degradation of soil organic matter) and the apparent (increase of microbial C turnover) PEs (Blagodatskaya and Kuzyakov, 2008; Blagodatsky et al., 2010) requires simultaneously partitioning the SOM- and primer-derived C sources in different C pools (Kuzyakov, 2010). A few approaches (e.g. Kuzyakov and Bol, 2004) have been suggested to partition the three carbon sources in pools and fluxes, but this task remains a challenge in soil science. A successful approach would have numerous applications in basic and applied studies. The present study is the first to experimentally demonstrate whether the contribution of recent and old SOM in the PE remains unaffected by the increasing rate of primer addition.

We applied ^{14}C-glucose as a primer substance to soil originating from a $C_3 - C_4$ vegetation change in which the old and recent SOM have a different $\delta^{13}C$ signature. This allowed us to distinguish the relative and absolute contribution of three C sources (substrate added, recent and old SOM) to the three C pools: CO_2, microbial biomass and DOC.

Our study was designed to determine the extent to which 1) the older fractions of SOM comprising the major C storage pool are susceptible to PE as dependent on primer amount, and 2) the PEs estimated by CO_2 efflux correspond to PE-induced changes in DOC and in microbial C pools.

2. Materials and methods

2.1. Soil sampling and preparation

Soil (loamy Gleyic Cambisol (WRB, 1998) was sampled from the upper layer (0–10 cm) of a field under *Miscanthus giganteus* ($C_{org} = 2.4 \pm 0.1\%$; $N_{total} = 0.20 \pm 0.01\%$, pH ($CaCl_2$) 5.1) and under an adjacent grassland ($C_{org} = 2.1 \pm 0.2\%$; $N_{total} = 0.21 \pm 0.01\%$, pH ($CaCl_2$) 5.1) at the experimental station of the University of Hohenheim, Baden-Württemberg, Germany (48°43′N, 9°13′E). The C_4 plant *M. giganteus* (Greef et Deu) ($\delta^{13}C = -11.8 \pm 0.21‰$) was grown for 12 years after grassland, causing a shift in the $\delta^{13}C$ of SOM from $-27 \pm 0.29‰$ to $-19 \pm 0.28‰$, which was most pronounced in upper soil layer (Schneckenberger and Kuzyakov, 2007). This difference in $\delta^{13}C$ was used to distinguish between SOM older and younger than 12 years. We use the term 'recent' C for the C_4-C originated from *M. giganteus* because it is not older than 12 years. In contrast, for the C originated from previous C_3 vegetation, which is 12 years old or even much older, we use the term 'old' C.

The soil was stored field-fresh in aerated polyethylene bags at 4 °C for a maximum of 6 weeks after sampling. Prior to the experiment, the soil was sieved (<5 mm) and fine roots and other plant debris were carefully removed. The sieved soil was then pre-incubated for at least 10 days because sieving affects the availability of SOM for microorganisms (Hartley et al., 2007) and may cause temporal respiration flush (Blagodatskaya and Anderson, 1999). Twenty-gram (dry weight) sub-samples were weighed and put into 250-ml Schott-jars. The moisture content was adjusted to 50% of the water holding capacity (WHC), and then the soil was pre-incubated at 22 °C for 24 h.

2.2. Experiment design and substrate application

Soil from both plots (C_3–C_4 soil under *M. giganteus* and C_3 soil under grassland used as reference) was amended either with distilled water (control) or with glucose at rates of 100 (glucose low, GL) or 1000 μg C g^{-1} soil (glucose high, GH). Uniformly labeled ^{14}C-glucose (final activity of 123,600 disintegrations per minute (DPM) g^{-1} corresponding to 1950 Bq g^{-1} soil) was added to the unlabeled D(+)-glucose before being added to the soil. Glucose was applied to soil as a solution to reach final soil moisture content of 60% of WHC. The C_3 soil (with distilled water or with glucose) was used as reference to estimate the $\delta^{13}C$ shifts between the pools caused by ^{13}C isotopic fractionation. The C_3–C_4 soil under the *M. giganteus* plot treated solely with water was used as control to estimate the changes in $\delta^{13}C$ caused by preferential utilization of ^{13}C-enriched recent C. Accordingly, the experimental design included 6 treatments: 2 soils with addition of water or 2 glucose levels. Fifteen vessels were included in the incubation for each treatment of the respective soil (in total 15·6 treatments = 90 vessels).

2.3. Incubation and sampling

After adding distilled water or glucose solution to the soil, small vials with 3 mL of 1 M NaOH were placed in the incubation vessels to trap CO_2. The vessels were immediately closed air-tight and incubated for 54 days at 22 °C at 60% WHC. Periodically, the vials with NaOH were removed and replaced by vials with a new 3 mL aliquot of 1 M NaOH. Aliquots of sampled NaOH from the three randomly chosen replicate vessels for each treatment were used to measure the ^{14}C activity and total amount of trapped CO_2. These measurements were done at 2, 6, 12, 24 h, and thereafter at least every 3 days (32 samplings in total) after glucose addition. Another three vessels for each treatment were used for the sampling of $^{13}CO_2$ at 3, 7, 14 and 54 days of the incubation. It was necessary to trap CO_2 at least 3 days in order to accumulate 1–2 mg C needed for mass-spectrometry analysis. Nine incubation vessels were destructively sampled at 3, 7 and 14 days (3 replicates for each sampling date) to estimate microbial biomass and DOC and for $\delta^{13}C$ analyses. Finally, at the end of incubation (54 days), the last 6 replicates, originally used for CO_2 sampling, were sampled destructively as well.

2.4. Microbial biomass and DOC

Soil microbial biomass was determined by the chloroform fumigation extraction method (modified after Vance et al., 1987). After destructive sampling or at the end of the experiment, the soil was carefully mixed and 10 g of soil were extracted with 40 ml of 0.05 M K_2SO_4. Another 10 g of soil were firstly fumigated with chloroform for 24 h and then extracted in the same way. The extracts were frozen until analyses for total C concentrations using a TOC/TIC analyzer (Multi N/C 2100, Analytik Jena, Germany).

The total amount of extractable microbial C (C_{MB}) was determined by the difference between K_2SO_4-extractable C in fumigated and non-fumigated soil using k_{ec} factor 0.45 (Wu et al., 1990). The glucose-derived microbial C (C_{MB_G}) was calculated based on ^{14}C activity in microbial biomass divided by the ^{14}C-specific activity ($^{14}C/C$) of the added glucose.

2.5. Chemical and isotopic analyses

CO_2 trapped in NaOH solution was precipitated with 0.5 M $BaCl_2$ solution. The total amount of CO_2 trapped in the vials was measured by titration of the NaOH excess with 0.1 M HCl using the phenolphthalein indicator (Zibilske, 1994).

Since we used both ^{14}C and ^{13}C labeling in one experiment, the CO_2 samples trapped in NaOH had to be specifically prepared. For ^{13}C analyses the CO_2 trapped as Na_2CO_3 in 3 ml of NaOH was precipitated with 4 ml of 0.5 M $SrCl_2$ aqueous solution. The NaOH solutions containing the $SrCO_3$ precipitate were then centrifuged three times at 1450 g for 10 min and washed in between with deionized and degassed water to remove NaOH and to reach a pH of 7. After washing, the remaining water was removed from the vials and the $SrCO_3$ was dried at 105 °C. The $SrCO_3$ was analyzed for $\delta^{13}C$-values on the IRMS (Delta Plus XL IRMS, Thermo Finnigan MAT, Bremen, Germany). For the K_2SO_4-soluble C and for the microbial biomass C, an aliquot of the K_2SO_4 samples was pipetted directly into tin capsules and dried at 60 °C prior to IRMS analyses (according to Brant et al., 2006).

The ^{14}C activity of NaOH with trapped CO_2 was measured in the scintillation cocktail Rotiszint Eco Plus (Carl Roth, Germany) after decay of the chemiluminescence using a 1450 LSC & Luminescence Counter MicroBeta TriLux (Perkin Elmer Inc., USA). The ^{14}C counting efficiency was 87% and the ^{14}C activity measurement error did not exceed 2%.

2.6. Calculations and statistics

Because the three-source-partitioning is suggested here for the first time, we explain the calculations in detail. To partition three sources of C in CO_2, microbial biomass and DOC, we used the following steps. Initially, *the amount of glucose-originated C in each pool* ($C_{G-derived}$, μg) was calculated based on the current ^{14}C radioactivity of the corresponding pool ($^{14}C_{curr}$, DPM), the amount of added glucose (C_G, μg), and the radioactivity of the applied glucose ($^{14}C_G$, DPM):

$$C_{G-derived} = {}^{14}C_{curr} \cdot C_G / {}^{14}C_G \quad (1)$$

Then, the amount of SOM-derived C was calculated as:

$$C_{SOM-derived} = C_{total} - C_{G-derived} \quad (2)$$

where C_{total} is the total amount of C in the corresponding pool (CO_2, DOC, microbial biomass).

In the second step, we calculated *the $\delta^{13}C$ value of SOM-originated C* in each pool ($\delta^{13}C_{SOM-derived}$) based on a mass balance equation (Balesdent and Mariotti, 1996). For that purpose, the $\delta^{13}C$ signature of glucose-derived C (see below) was subtracted from the $\delta^{13}C$ signature of each pool, considering the contribution of the amount of glucose-originated C estimated in the first step based on ^{14}C:

$$\delta^{13}C_{SOM-derived} = (\delta^{13}C_{total} \cdot C_{total} - \delta^{13}C_{G-derived} \cdot C_{G-derived}) / (C_{total} - C_{G-derived}) \quad (3)$$

where $\delta^{13}C_{total}$ and $\delta^{13}C_{G-derived}$ are the $\delta^{13}C$-values of the total and glucose-originated C. The former value was measured experimentally as described in section 2.5. The shift in $\delta^{13}C$ caused by glucose addition was similar for Miscanthus and reference soils (10.2 vs 11‰) and this allowed us to assume that the $\delta^{13}C$-values of the glucose-originated C in each pool ($\delta^{13}C_{G-derived}$) are equal to $\delta^{13}C$-glucose (11.05 ± 0.05‰).

In the next step, *the contribution of recent and old C* in each pool in the C_3–C_4 soil was calculated based on the glucose-corrected $\delta^{13}C$ signature of each pool (Eq. (3)) considering isotopic fractionation, which was assumed to be equal in reference and in *Miscanthus* soil. The amount of recent C_4-originated C in each pool is:

$$C_{C4-derived} = C_{SOM-derived} \cdot (\delta^{13}C_{SOM-derived} - \delta^{13}C_{C3\_ref}) / (\delta^{13}C_{C4\_plant} - \delta^{13}C_{C3\_plant}) \quad (4)$$

where $\delta^{13}C_{C3\_ref}$ is the $\delta^{13}C$ value of the corresponding pool in the reference C_3 soil at the corresponding sampling date calculated according Eq. (3). The denominator of Eq. (4) is equal to 16.2‰, i.e. to the difference between $\delta^{13}C$ signatures of C_4 and C_3 vegetation for our experiment corrected according to Schneckenberger and Kuzyakov (2007) for isotopic fractionation during humification. C_3-originated C in each pool was then calculated by subtracting the C_4-originated C (Eq. (4)) from the total amount of C in the corresponding pool.

In the last step, *the PE and the contributions of old and recent C to the PE* were calculated based on the changes in the $\delta^{13}C$ signature and the amount of extra-C in the three pools after glucose addition (compared with the treatment without glucose).

Cumulative PE, expressed in μg CO_2–C per g soil, was calculated based on experimental data as:

$$PE = {}^{12}CO_2^{amended} - CO_2^0 - CO_2^G \quad (5)$$

where $^{12}CO_2^{amended}$, CO_2^G, and CO_2^0 is unlabeled ^{12}C–CO_2 evolved from soil amended with ^{14}C-glucose, CO_2 originated from glucose and CO_2 originated from soil without substrate addition, respectively.

The changes in $\delta^{13}C$ signature caused by preferential substrate utilization – which is not isotopic fractionation *per se* but is considered as preference for easily available and ^{13}C-enriched recent C (compared with ^{13}C depleted old C) – were considered for correct assessment of PE in all pools. The dynamic changes in $\delta^{13}C$ caused by preferential utilization were estimated in control C_3–C_4 soil treated solely with H_2O. The $\delta^{13}C$ signature was not constant and changed during incubation of control C_3–C_4 soil; these changes were most pronounced in microbial C (up to 5‰) and in the respired CO_2 (up to 7‰). Therefore, for each pool, the PE was calculated separately for C_3 and C_4 carbon sources (C_{3_PE} and C_{4_PE}, respectively) considering the changes in contribution of old and recent C in control soil for each sampling date:

$$\begin{aligned} C_{3\_PE} &= C_{3\_CO_2}^{amended} - C_{3\_CO_2}^{control} \\ C_{4\_PE} &= C_{4\_CO_2}^{amended} - C_{4\_CO_2}^{control} \end{aligned} \quad (6)$$

The following equation was used to determine *the $\delta^{13}C$ of total microbial biomass* ($\delta^{13}C_{MB}$):

$$\delta^{13}C_{MB} = \left(\delta^{13}C_f \cdot C_f - \delta^{13}C_{nf} \cdot C_{nf}\right) / \left(C_f - C_{nf}\right) \quad (7)$$

where $\delta^{13}C_f$ and $\delta^{13}C_{nf}$ are the $\delta^{13}C$-values of the fumigated and non-fumigated samples, respectively, and C_f and C_{nf} are the amounts of C in the fumigated and non-fumigated K_2SO_4 samples, respectively.

Isotopic fractionation during SOM mineralization in the course of the incubation experiment was assumed to be equal for the soil under *Miscanthus* and for the reference soil with solely C_3 vegetation (Werth and Kuzyakov, 2010). The corrections for $\delta^{13}C$ signatures of CO_2, microbial biomass, and DOC were made according to the data for isotopic fractionation in the reference soil under C_3 vegetation. The isotopic fractionation between these pools was of minor importance for the soil used in our study and did not exceed 1‰ (Blagodatskaya et al., 2011). Nonetheless, the amount of each pool derived from recent SOM (e.g. C_4–CO_2) was calculated according Eq. (4), considering thus the isotopic fractionation in the corresponding pool in reference soil.

Standard errors (SE) for CO_2, microbial biomass and $\delta^{13}C$-values were calculated to estimate the precision of the means. The

significant effects of glucose treatments (no, GL and GH) were assessed by one-way ANOVA at $p < 0.05$.

3. Results

3.1. Shift in $\delta^{13}C$ in different carbon pools

Twelve years of *M. giganteus* vegetation caused $\delta^{13}C$ shifts amounting to 8.2 ± 0.6‰ in the SOM, 4.6 ± 0.2‰ in DOC and 13.4 ± 0.7‰ in microbial biomass. The contribution of recent SOM-C to the $\delta^{13}C$ signature of all pools was always larger than the contribution of glucose-C; the latter was relatively small and did not exceed 3.2 ± 0.6‰ (Fig. 1). A larger effect of low versus high glucose amount on $\delta^{13}C$ was observed in CO_2 and in the DOC pool; in microbial biomass, only the effect of GH on the $\delta^{13}C$ value was significant.

3.2. Source partitioning for carbon pools as affected by glucose amount

3.2.1. CO_2 efflux

For both glucose treatments, two mineralization periods were observed: 1) intensive mineralization of the applied glucose in the first 1.5 d for GL and 3 d for GH, and thereafter 2) slow mineralization of glucose-derived metabolites after exhaustion of the applied glucose (Fig. 2). A larger part of the labeled CO_2, namely 65 ± 1% (GL) and 55 ± 1% (GH) of the ^{14}C–CO_2 produced during 54 days, was respired during the intensive mineralization period (Fig. 2, insert). During the slow mineralization phase, the CO_2 evolved was mainly unlabeled (Fig. 3 A and B). The CO_2–C respired during 54 days made up 47.8 ± 0.1% and 64.6 ± 2.7% of the added glucose amount for GL and GH treatments, respectively. A contribution of native, especially of recent C_4-derived CO_2–C clearly increased with time in both GL (Fig. 4A) and GH (Fig. 4B) treatments. The cumulative amount of C_4-derived CO_2 during 54 days after GL and GH addition was greater by a factor of 2.4 and 1.9, respectively, than the CO_2 efflux originated from C_3-SOM.

3.2.2. Microbial biomass

Adding glucose at the low rate resulted in slight increase in the microbial biomass (up to 26% at 54 day, Fig. 4C). After applying the high glucose amount the microbial biomass C increased by 60% in one day (data not shown). As confirmed by ^{14}C incorporation into the microbial biomass, this increase was mainly due to glucose (Fig. 4D). Even in GH treatment, 47–55% of microbial biomass still consisted of C_3- and C_4-SOM-originated C. The contribution of

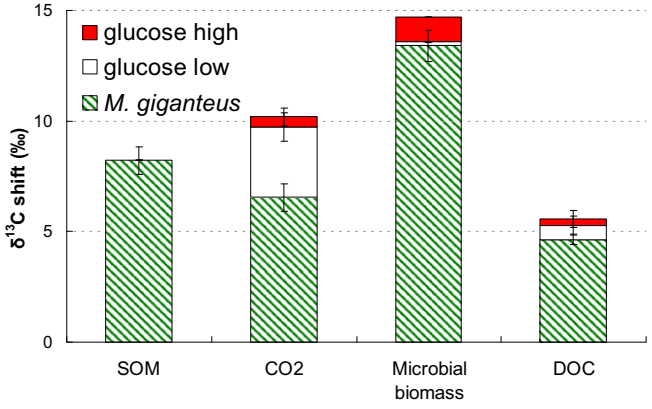

Fig. 1. Positive shift in $\delta^{13}C$-values in SOM, CO_2, microbial biomass and DOC caused by 12 years of growth of *M. giganteus* and by addition of high and low glucose amounts (in three days after glucose addition). Bars indicate standard errors of the means (n = 3).

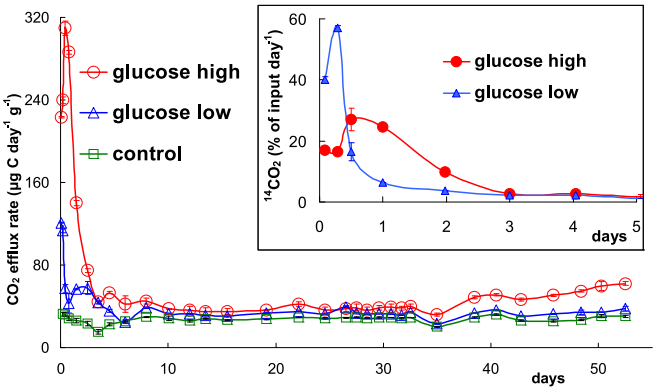

Fig. 2. Dynamics of CO_2 efflux rate from soil amended with high and low glucose amounts. Inset shows dynamics of glucose-originated ^{14}C–CO_2 (as % of input) in treatments with high and low glucose amounts. Bars indicate standard errors of the means (n = 3).

glucose-originated ^{14}C to total microbial biomass decreased during the incubation from 14 to 6% and from 52 to 19% in GL and GH treatments, respectively. As a result, at the end of the incubation, this biomass consisted of 69 and 50% of recent C_4-derived C, while old C_3-derived C contributed 25 and 31% in GL and GH treatments, respectively (Fig. 4C and D). The microbial biomass originated from recent C_4-derived soil C slightly increased up to 8% (GL treatment) or up to 23% (GH treatment) compared with soil before glucose amendment (Fig. 4). A strong increase (by a factor of 1.6 and 2.7 for GL and GH treatments, respectively) in C_3-originated microbial biomass was observed, however, at 54 days of incubation.

3.2.3. DOC

99.5% of applied glucose was taken up by microorganisms in both treatments within 3 days or earlier (Fig. 4E and F). The rest of

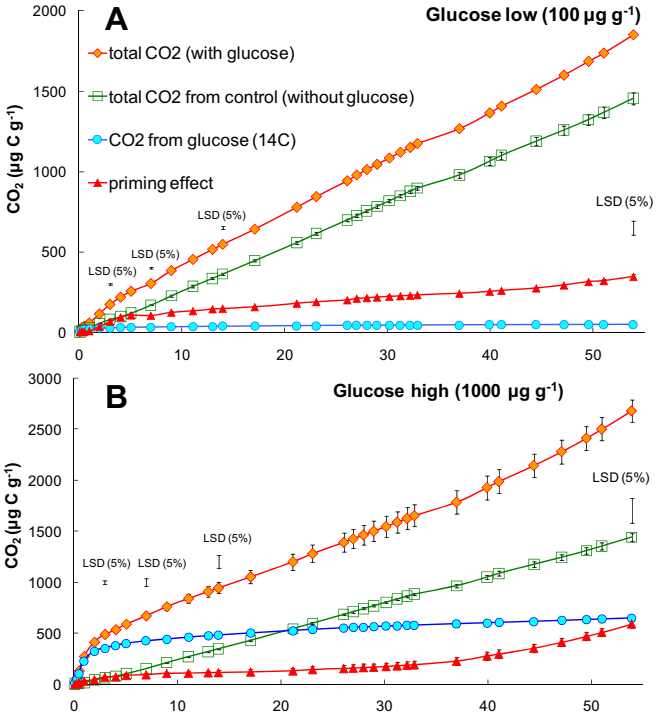

Fig. 3. Cumulative CO_2 production from soil amended with low (A) or high (B) glucose amounts. Bars indicate standard errors of the means (n = 3). The significance of differences between means for the destructive sampling dates is shown as LSD (5%).

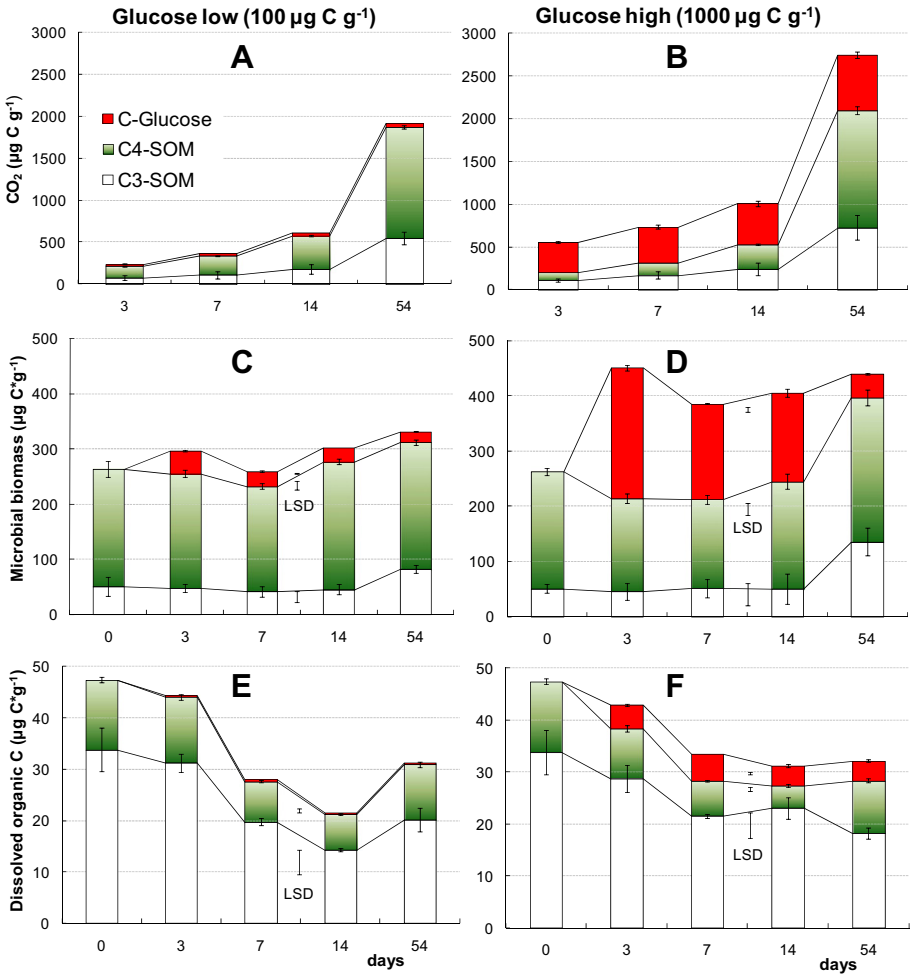

Fig. 4. Contribution of three C sources to cumulative CO_2 efflux (A, B), microbial biomass (C, D) and DOC (E, F) in soil after adding low (left panel) or high (right panel) glucose amounts. The three sources include: 1) SOM-C older than 12 years (C3-SOM), 2) SOM-C younger than 12 years (C4-SOM), and 3) C of added glucose (C-Glucose). Error bars indicate standard errors of the means (n = 3). The significance of temporal differences between means for each C source is shown as LSD (5%) on plots C – F.

the applied labeled C, i.e. 0.3–0.4% or 0.3 and 3.8 µg C g^{-1} for GL and GH treatments, respectively, was always found in the DOC pool (Fig. 4E and F). After 54 days the glucose-originated ^{14}C contributed 1 and 12% to the total (labeled + unlabeled) DOC pool in GL and GH treatments, respectively. The glucose-induced a gradual decrease in the total DOC content as compared to the untreated soil (cf. first bars with others on Fig. 4E and F). This decrease was mainly due to the gradual reduction in C_3-derived C, which at day 54 was almost twice as low as before glucose addition. Despite the gradual reduction, the contribution of 'old' C in total DOC was always higher than that of recent and of glucose-originated ^{14}C-labeled C (Fig. 4E and F). The amount of glucose-originated C in DOC was always smaller by a factor of 10–30 than the amount of ^{14}C extractable from the microbial pool. A similar ratio was observed for easily available DOC from C_4-SOM: it was smaller than the amount of microbial C by a factor of 2.5–10 (compare Fig. 4C, D and Fig. 4 E, F). In contrast, during the first two weeks after glucose addition the amount of recalcitrant C_3-derived SOM was similar or even greater in DOC than in the microbial C pool (Fig. 4C–F).

3.3. Priming effects induced by glucose and their sources

Glucose amendment induced short-term and long-term PE (calculated according Eq. (5)). These phases dominated at 1 – 9, and 16–54 days after addition. Short-term PE was most pronounced on the first day after glucose addition and was even higher in the GL versus GH treatment (125 and 110%, respectively, of the control soil). Only recent C_4-originated C was involved in short-term PE in the GL treatment, whereas in the GH treatment both recent and old C sources contributed almost equally to short-term PE (cf. first bars corresponding to day 3 on Fig. 5A and B).

Despite the 10-fold difference in glucose amendment, the cumulative PE for 54 days of incubation was smaller in the GL versus GH treatment by a factor of only 1.6, amounting to 25 and 41% of the control, respectively. CO_2 evolved during long-term PE in the GL treatment originated solely from the recent soil C (Fig. 5A). In contrast, in the GH treatment the contributions of recent C (52%) and old C (48%) to the cumulative amount of long-term PE were similar.

No significant positive PE in the microbial biomass pool was found as a short-term effect (Fig. 5C and D). Three days after both glucose additions, the contribution of recent C in the biomass even decreased compared with the control. This decrease was significant only in the GH treatment (48.8 ± 12.7 µg C g^{-1}), where it was comparable with the positive PE amount in CO_2 (72 ± 16.5 µg C g^{-1}). SOM-originated C in the microbial biomass pool increased significantly, however, as a long-term PE. This increase solely reflected the contribution of recent soil C in the GL treatment, whereas in the GH treatment 12% of primed microbial biomass consisted of the old soil C. These changes of recent and old C in

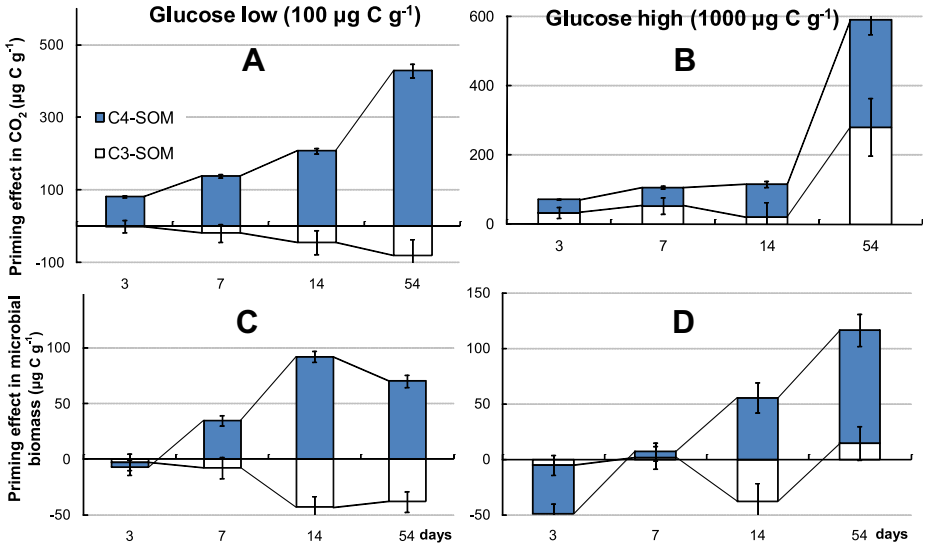

Fig. 5. Contribution of recent (C_4) and old (C_3) SOM to the priming effect in CO_2 efflux (A,B) and in microbial biomass (C, D) in soil after adding low (left panel) or high (right panel) glucose amounts.

microbial biomass corresponded well with the dynamics of both C types in the CO_2 released during PE.

In the DOC pool the PE was always negative, mainly due to the decrease of old soil C (data not shown).

4. Discussion

4.1. Three-source-partitioning approach

Until now, most studies designed to partition pools and fluxes identified only two C sources: 1) added C labeled by ^{14}C or ^{13}C, and 2) unlabeled C present in the soil before substrate addition. These two C sources were identified in released CO_2 and, in a few cases, in microbial biomass and DOC (Nottingham et al., 2009). In order to identify the SOM pools responsible for priming as well as to distinguish real and apparent PEs, more than two C sources should be detected (Kuzyakov, 2010). Only very few studies attempted this, either by using one C isotope pair and a combination of treatments (Subke et al., 2004; Kuzyakov and Bol, 2004, 2006) or by applying two C isotope pairs: labeling by ^{13}C and ^{14}C at natural abundance level (Fontaine et al., 2007). Here, we suggested and successfully tested a new approach combining ^{13}C natural abundance with ^{14}C labeling. This yielded many new advantages: 1) using highly labeled ^{14}C primer allowed exact estimation of glucose-C contributing to any pools and fluxes. Therefore, the traditional uncertainties connected with natural ^{13}C or ^{14}C variations (Bowling et al., 2008; Werth and Kuzyakov, 2010) do not affect the accuracy of partitioning. This is a clear advantage compared to approaches based solely on natural abundance. 2) Much fewer treatments are necessary to evaluate sources, and the estimation of interactions between the pools is direct, without complicated 3-way subtraction (Kuzyakov and Bol, 2004). 3) The dynamic changes in old and recent SOM induced by glucose can be traced simultaneously in various C pools and fluxes.

We used this three-source-partitioning to identify sources not only for CO_2, but also for microbial biomass and DOC. The CO_2 efflux and microbial biomass pool mainly consisted of C from current vegetation, while the DOC pool was mainly derived from old SOM (Fig. 4). This clearly illustrates the rapid uptake of easily available substrates (such as rhizodeposits of current vegetation) by microorganisms (Yevdokimov et al., 2006; Esperschütz et al., 2009; Bol et al., 2009; Fischer et al., 2010) and so, the low contribution of recent C to the DOC pool. The decrease in SOM-originated DOC content in both glucose treatments can be assigned to co-metabolism: the additional supply of energy-rich substrate (glucose) to the microbial biomass induced microbial uptake of old SOM-originated DOC (Horvath, 1972). Our approach demonstrated this phenomenon for the first time. The decline in SOM-originated DOC (Fig. 4E and F) during incubation corresponded well to the measured positive PE in CO_2 and microbial biomass (Fig. 5). The absolute increase in SOM-derived CO_2 and in primed microbial biomass, however, were much greater than the decline in SOM-derived DOC. Therefore, DOC is not the sole source of primed C, because DOC is a small intermediate pool, which is renewed at least several times during the experiment. We thus conclude that the decrease in the SOM-originated DOC pool measured as PE is mirrored in the CO_2 (at least partly) and in the microbial biomass change. Such an identification of C sources in these three pools helped further to distinguish the real and apparent PE.

4.2. Apparent and real priming as short- and long-term effects

Small differences in the intensity of short-term PE between GL and GH treatments indicated that other factors (e.g. size of microbial biomass, Blagodatskaya and Kuzyakov, 2008, or particle size, Fangueiro et al., 2007) than the primer amount were responsible for short-term PE. Disproportionally less long-term PE in the GH versus GL treatment further confirmed the observations that the PE decreases with increasing nutrient availability (Guenet et al., 2010; Fontaine et al., 2011).

We distinguished whether the observed PEs were real or apparent by comparing the CO_2 released by PE with the microbial C, as well as by their partitioning for the recent and old C. We conclude that short-term PE was apparent because no increase in microbial biomass due to SOM-originated C was observed in either treatments, and because the amount of PE was lower than microbial C. Rather, PE was caused by intensified internal microbial metabolism as a quick response to glucose addition (Blagodatsky et al., 2010). The CO_2 released by PE in the GL treatment (where added glucose did not cause the increase in microbial biomass) originated from recent soil C and was due to the accelerated microbial turnover (De Nobili et al., 2001). The apparent PE in the

GL treatment was similar to that often observed in the rhizosphere, where PE reflects enhanced microbial turnover rates instead of increased microbial biomass (Cheng, 2009).

The apparent PE in the GH treatment can be explained by pool substitution during intensive glucose decomposition (Blagodatskaya et al., 2007) because the amount of primed CO_2–C was similar to the decrease in SOM-derived C in the primed microbial biomass. At that time, microorganisms growing on the added ^{14}C-glucose respired unlabeled C from microbial cells. The short-term real PE cannot be completely excluded based only on δ^{13}C signature change, but the contribution of real PE during the first 3 days was minimal because of the absence of microbial biomass changes due to SOM-originated C and considering the CO_2 dynamics (see above).

Long-term PEs found in both treatments were greater than microbial biomass and were accompanied by a significant increase of SOM-originated C in that biomass. Accordingly, such PE can be characterized as real and caused by accelerated SOM decomposition initiated by glucose addition. Similarly, the PE determined by artificially labeling with C_4 sugar-cane sucrose showed a large initial (within 5 days) pulse of primed CO_2, followed by a smaller and more persistent flux (Nottingham et al., 2009). The significant increase of soil-derived C within PLFA biomarkers was observed only on day 14 (correspondent to the second PE-flux) after sucrose addition. Therefore, similarly to the glucose-induced PE in our study, two PEs induced by sucrose occurred (Nottingham et al., 2009): short-term apparent (or combination of apparent PE and real PE) and long-term real PE.

4.3. Contribution of recent and old C in the PE as dependent on primer amount

Partitioning of C sources revealed the increase in relatively 'recent' C in CO_2 and in microbial biomass during incubation, independently of the added glucose amount. The *source of primed* CO_2, however, strongly depended on the glucose amount.

Recent soil C was the sole source of primed CO_2 in the GL treatment (Fig. 5A and C). This agrees with the studies of Kramer and Gleixner (2006, 2008) and indicates that microorganisms preferentially use recent plant material consisting of relatively labile organic matter as a substrate (Salome et al., 2010). The preferential priming of recent SOM can be also explained by the observations of Wynn and Bird (2007) that young C_4-substances are more abundant in the labile components of SOM, while the more stable components of SOM are biased toward C_3-derived materials. As the recent (C_4-derived) organic C cycles faster than old C (originated from C_3-plants) (Wynn and Bird, 2007), we expect that the age of recent C in our study was essentially under 12 years.

Old SOM was not involved in PE in the GL treatment, whereas the high glucose amount stimulated the decomposition of old C so that it contributed 48% to the total PE over 54 days (Fig. 5B). Thus, the quality and age of the SOM mineralized by priming depends on the amount of substrate added. Following the conclusion of Paterson (2009), mineralization of old SOM is fuelled by sufficient amounts of energy-rich substrates due to stimulation of higher enzyme production (Dorodnikov et al., 2009) to cover microbial nutrient-demand.

Two situations occurred when long-term real PE was observed (Fig. 6): 1) A glucose amount smaller than microbial biomass merely activated the existing microorganisms and was insufficient to induce microbial growth. Only recent soil C was involved in PE under such conditions (Fig. 6, left panels). 2) A glucose amount much greater than microbial C induced microbial growth, which resulted in strong nutrient limitation because no other nutrients were added to soil along with the glucose. The increase in microbial biomass that consumed old SOM indicated the portion of microorganisms that was activated by glucose and switched their metabolism to SOM decomposition after glucose exhaustion (Fig. 6, right panels). We therefore assume that strong competition for nutrients in the newly formed microbial community caused the accelerated decomposition of old soil C during PE. Thus, the increase in primer amount caused the growth and then starvation

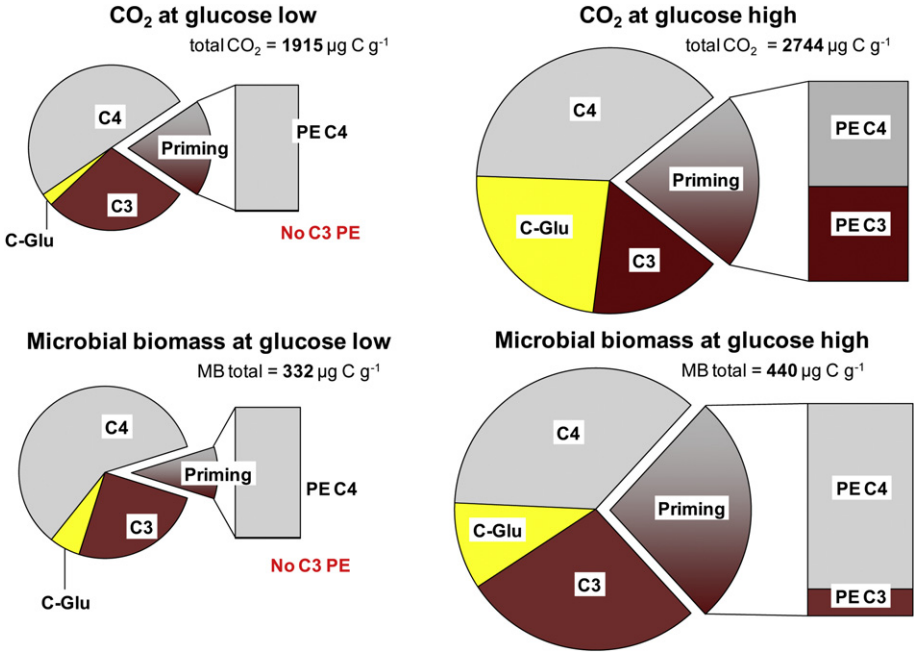

Fig. 6. Contribution of three C sources to cumulative CO_2 efflux (top) and microbial biomass (bottom) after adding low (left panel) or high (right panel) glucose amounts. The priming effect induced by glucose addition is presented as the right segment of the pie-plot and contribution of the old and recent C to the primed C is shown as stacked columns. The priming effect partitioning clearly shows that old C will be involved in priming only by high glucose addition. The three sources include: 1) SOM-C older than 12 years (C3), 2) SOM-C younger than 12 years (C4), and 3) C of added glucose (C-Glu).

of microorganisms, forcing them to decompose old recalcitrant C. The newly formed microbial biomass altered the quality of primed CO_2–C. Our study demonstrated that both the amount and quality of mobilized and decomposed SOM depend on the amount of active microbial biomass.

5. Conclusions

Using a combination of ^{14}C labeling and ^{13}C natural abundance, we successfully estimated *three* C sources in 1) CO_2 evolved from soil, 2) microbial biomass and 3) DOC. Such partitioning of pools and fluxes allowed 1) clear separation of apparent and real PEs induced by glucose and 2) evaluation of SOM pools involved in priming effects.

Apparent PE was observed as a short-term acceleration of inherent microbial metabolism within 1–3 days after glucose addition. Such PE was 40% lower than microbial C, did not depend on the amount of substrate added, and comprised 125 and 110% of CO_2 from the control, untreated soil for GL and GH treatments, respectively. Real PE was observed as a long-term response to glucose addition. The SOM origin of long-term PE was confirmed by the increased contribution of SOM-originated C in microbial biomass: at 54 days of incubation, primed (i.e. SOM-originated) C in microbial biomass comprised 20–25% of total microbial C. Long-term real PE amounted to 25 and 42% of the control for the GL and GH treatments, respectively.

Glucose addition reduced the DOC content by 30–50%, mainly due to the gradual reduction in the old C fraction of DOC as compared with untreated soil. This resulted in negative PE in DOC and confirmed that SOM was involved in microbial metabolism induced by glucose addition. We showed that low amounts of primer accelerated SOM decomposition and that the main C source originated solely from recent SOM pools characterized by relatively high availability. Adding more primer, however, stimulated microbial biomass more strongly, and older SOM pools were involved in microbial metabolism and contributed to the priming effect.

We conclude that 3-source-partitioning based on a combination of ^{14}C labeling and ^{13}C natural abundance is a very useful tool in clearly separating sources and evaluating mechanisms of priming effects.

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