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ORIGINAL PAPER

Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature

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Abstract The release of root exudates into the rhizosphere is known to enhance soil biological activity and alter microbial community structure. To assess whether root exudates also stimulated litter decomposition, in a rhizosphere model system we continuously injected solutions of glucose, malate or glutamate through porous Rhizon® soil solution samplers into the soil at rhizosphere concentrations. The effect of these substances on the decomposition of ^{14}C-labelled *Lolium perenne* shoot residues present in the soil was evaluated by monitoring $^{14}CO_2$ evolution at either 15°C or 25°C. The incorporation of the ^{14}C into the microbial biomass and appearance in the dissolved organic matter (DOM) pool was estimated after 32 d incubation. The presence of malate and glutamate increased the mineralization of *L. perenne* residues by approximately 20% relative to the soil without their addition at 15°C, however, no significant effects on residue decomposition were observed at 25°C. The incorporation of the ^{14}C-label into the microbial biomass and DOM pool was not affected by the addition of either glucose, malate or glutamate. Although nearly the same amount of *L. perenne* residues were mineralized at either temperature after 32 d, less ^{14}C was recovered in the microbial biomass and DOM pools at 25°C compared to 15°C. Alongside other results, this suggests that the rate of microbial turnover is greater at 25°C compared to 15°C. We conclude that the addition of labile root exudate components to the rhizosphere induced a small but significant increase on litter decomposition but that the magnitude of this effect was regulated by temperature.

Keywords Amino acids · Carbon flow · Dissolved organic carbon · Mineralization · Organic acids · Rhizosphere priming effect · Root exudation · Rhizodeposition · Sugars

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Introduction

In comparison to root-free soil, the zone of soil around roots (rhizosphere) receives greater inputs of labile C and possesses a higher microbial activity (Farrar et al. 2003). The main C input comes from root exudates, which are composed primarily of low molecular weight organic

compounds, which are readily used by rhizosphere microorganisms for growth and respiration. Due to the greater availability of C, typically both the activity and abundance of the microbial community can be an order of magnitude higher than in root-free soil (Bodelier et al. 1997). Fast turnover of exudates and microbial biomass C in the rhizosphere may lead to local changes in the rate of microbial decomposition of various C pools: dead plant residues and/or soil organic matter (SOM). Changes in the intrinsic rate of SOM decomposition in the zone of soil surrounding roots are termed rhizosphere priming (Parnas 1976; Kuzyakov et al. 2000; Kuzyakov 2002). Cheng and Kuzyakov (2005) reviewed the magnitude of these effects depending on plant species and soil conditions, and showed that SOM decomposition in the rhizosphere could decrease by 10–30% or increase by as much as 100% relative to that in non-rhizosphere soil. Many studies have suggested that climate change may induce the turnover of older reserves of SOM (Zogg et al. 1997). This may be a direct temperature effect by increasing SOM decomposition rates or an indirect effect through an increase in labile C inputs into the soil via enhanced root exudation and turnover (Van Kessel et al. 2000; Gielen et al. 2005; Xie et al. 2005). In the latter case it may be mediated by root induced changes in microbial community composition, wherein dominant populations at higher temperatures have the ability to metabolize substrates that are not used by members of the microbial community at lower temperatures (Zogg et al. 1997), or through an increase of enzyme activity (Sowerby et al. 2005). In addition, shift of microbial community structure induced by higher temperature may also change mineralization of dead plant residues or/and SOM. The mechanisms underlying these responses, however, remain unclear.

The mechanisms by which decomposition of litter and SOM can change in the presence of living roots were reviewed in detail by Dormaar (1990) and Cheng and Kuzyakov (2005). Both reviews concluded that biological rather than physical factors were responsible for this rhizosphere effect and that (1) microbial activation, (2) preferential substrate utilization, and (3) increased competition for N between plant roots and microorganisms, are the most important mechanisms for stimulating mineralization in the rhizosphere (Cheng and Kuzyakov 2005). There are too few data to conclude which mechanisms are mainly responsible for stimulated litter decomposition in the rhizosphere. Kuzyakov (2002) hypothesized that due to their labile nature, the low molecular weight components of rhizodeposits (i.e. root exudates) are the most important priming agents in the rhizosphere. This hypothesis was deduced from many studies involving the pulse addition of readily bioavailable substances to the soil (e.g. glucose, amino acids; De Nobili et al. 2001; Hamer and Marschner 2005a). However, only a few studies have investigated the capacity of repeated substrate addition to induce stimulation of decomposition (Hamer and Marschner 2005b; Zyakun and Dilly 2005). To our knowledge there have been no studies on the ability of a continuous input of low substrate concentrations, typical of root exudates, to induce priming or similar processes like stimulation of plant residue decomposition.

The three main groups of organic substances exuded by roots into the rhizosphere are sugars (50–70% of total exudate), carboxylic acids (20–30% of total exudate) and amino acids (10–20% of total exudate; Kraffczyk et al. 1984; Jones 1998; Hütsch et al. 2002). Many studies have shown that organic acids enhance plant nutrient mobilization in the rhizosphere by the formation of organometallic complexes (e.g. P, Fe, Cu, Mn; Hinsinger et al. 2003; Dakora and Phillips 2002). Directly or indirectly, organic acid release may also change the pH of the rhizosphere, which can also increase nutrient availability and affect cell membrane permeability (Jones et al. 2004a; Neumann and Römheld 1999). While sugars and proteinaceous amino acids are not directly involved in nutrient mobilization within the rhizosphere they can both act as chemoattractants to certain microorganisms and are involved in supporting microbial growth (Darrah 1991; Dakora and Phillips 2002; de Weert et al. 2002). At concentrations likely to exist in the rhizosphere (0.1–5 mM), sugars will be primarily used by soil microorganisms for anabolic processes, while organic acids will be largely used for catabolic processes and amino acids will be intermediate

between the two (Jones et al. 2003). The relative effect of individual substrates on microbial growth and activity remains unknown but might be expected to influence the stimulation response.

In previous priming or litter decomposition studies, substrates are normally added to the soil surface and the soil subsequently incubated in sealed vessels (Hamer and Marschner 2005a). The method of addition results in concentration hot spots within the vessel. Therefore, the magnitude of the stimulation effect may be significantly underestimated due to dilution by areas of soil, which receive no substrate. To simulate a growing root and rhizosphere and to achieve a uniform addition of substrate to soil, root-sized Rhizon® soil solution samplers can be used in reverse (i.e. instead of sucking solution out of the soil, root exudates can be pushed into the soil at a known rate; Dobermann et al. 1994; Bhupinderpal-Singh et al. 2005). The Rhizon samplers allow equal release of added substances across their entire surface area (ca. 6.28 cm^2) similar to the release of exudates by roots. As the rhizosphere is chemically, physically and biologically different from the bulk soil, if stimulation of litter and/or SOM decomposition is observed in the rhizosphere, it is difficult to ascribe the priming effect to individual factors. Indeed, while many factors in the rhizosphere may be collectively involved in stimulation of decomposition processes it would be useful to identify the main drivers. Here we will investigate whether specific root exudate components are more involved than others in the rhizosphere effect leading to changed mineralization of litter compared to exudate-free soil.

In most studies on SOM priming, the magnitude of the priming effect has been evaluated by measuring the difference between total CO_2 efflux and the CO_2 evolved from isotopically labelled (e.g. ^{13}C or ^{14}C) substrates. The difference, attributable to SOM-derived CO_2 efflux, is then compared by the second difference to the CO_2 evolution from the same soil without substance addition. This approach based on ''difference between differences'' can lead to an accumulation of errors when calculating priming effects (Kuzyakov 2002). To minimize these errors, we chose an alternative approach. We used unlabelled substances to induce changes in microbial activity and investigated the effect of these unlabelled substances on the decomposition of ^{14}C-labelled plant residues.

The aim of this study was to investigate whether specific components of root exudates continuously added at realistic concentrations could induce changes of litter decomposition in a simulated rhizosphere. The second aim was to investigate whether an increase in soil temperature influenced the magnitude of the decomposition changes.

Materials and methods

Soil and sampling

Soil (Eutric Cambisol) was obtained from the University of Wales—Bangor, Henfaes Agricultural Research Station located in Abergwyngregyn, Gwynedd, North Wales (53°14′ N, 4°01′ W). Soil samples were collected from the Ah horizon (5–20 cm; silty clay loam texture) of a lowland (15 m altitude) freely draining, heavily sheep-grazed grassland which receives regular fertilization (120 kg N, 60 kg K and 10 kg P y^{-1}) and supports a sward consisting predominantly of perennial ryegrass (*Lolium perenne* L.), clover (*Trifolium repens* L.) and crested dog's tail (*Cynosurus cristatus* L.). Soil was removed using a spade and stored in CO_2 permeable polypropylene bags for immediate transport back to the laboratory. In the laboratory, the soil was sieved (<5 mm) and then stored field-moist at 3°C in the same bags. Earthworms, above-ground vegetation and large roots were removed by sieving. The pH of the soil was 5.7 while the total organic C was 53 g kg^{-1} and total N was 2.6 g kg^{-1}. Further properties of the soil are presented in Jones et al. (2004b).

^{14}C-labelled plant residues

The *Lolium perenne* grown under field conditions had been previously pulse-labelled in a $^{14}CO_2$ atmosphere. The shoots were covered with a Plexiglas chamber and allowed to assimilate the $^{14}CO_2$ for 1 h. Thereafter, the chamber was removed and the plants were grown as before

the labelling. The plants were harvested 7 d after the labelling. As shown in previous study with *Lolium*, this period allowed for main ^{14}C distribution between plant parts and ^{14}C incorporation into structural cell components (Domanski et al. 2001). The specific activity of the plant residues was 0.21 kBq mg^{-1}.

The ^{14}C distribution in the plant residues were tested by sequential extraction (Poorter and Bergkotte 1992; Jones and Darrah 1994). Plant material was ground in ball mill and 50 mg put in 10 ml polypropylene centrifuge tube. Five fractions were obtained: (1) Eight millilitre of deionized water were added and heated for 30 min at 85°C. After centrifugation at 5,000*g* for 15 min, all supernatant representing hot water soluble substances was removed for analysis. (2) About 1.6 ml of 100% ethanol were added to the non-dissolved residue and heated for 30 min at 80°C. Then 6.4 ml of water (i.e. to give 20% ethanol) were added and heated for 30 min at 80°C. After centrifugation at 5,000*g* for 15 min, all supernatant was removed for analysis. (3) Five millilitre of 0.3% HCl were added to the residue, heated for 3 h at 95°C, and centrifuged at 5,000*g* for 15 min. The supernatant was collected for analysis. (4) Five millilitre of 1 M NaOH were heated for 1 h at 95°C, centrifuged at 5,000*g* for 15 min and supernatant and residue were analysed for ^{14}C activity. So, five fractions were obtained: dissolved in H_2O, C_2H_5OH, HCl, NaOH and non-dissolved residue. With exception of ethanol extract, the ^{14}C distribution was nearly homogeneous between the other fractions amounting for between 20% and 27% of total ^{14}C activity in plant residues (Fig. 1).

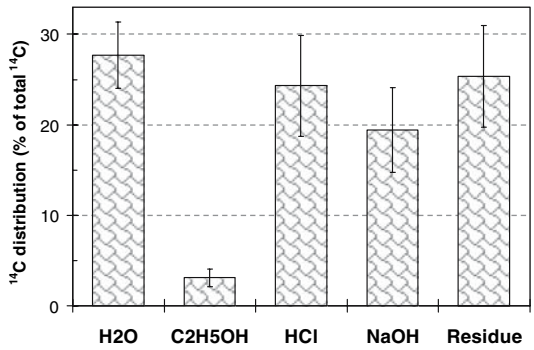

Fig. 1 Distribution of ^{14}C in fractions extracted with various solvents: distilled H_2O, C_2H_5OH, HCl, NaOH and remaining in the residue (see section "^{14}C-labelled plant residues" for description of the extraction procedure). The values represent means ± SEM (n = 4)

Simulated rhizosphere

To simulate the exudation of organic substances into the soil by roots, we used 100 mm long Rhizon® soil solution samplers (Rhizosphere Research Products, Wageningen) inserted into the centre of a soil filled 15 ml polypropylene tube (Fig. 2). The plastic tube was filled with 20 g of fresh, field-moist soil (13 g dry weight) containing 50 mg of finely ground ^{14}C-labelled *Lolium perenne* shoot residues. The activity of the plant residues added per one tube was 10.5 kBq.

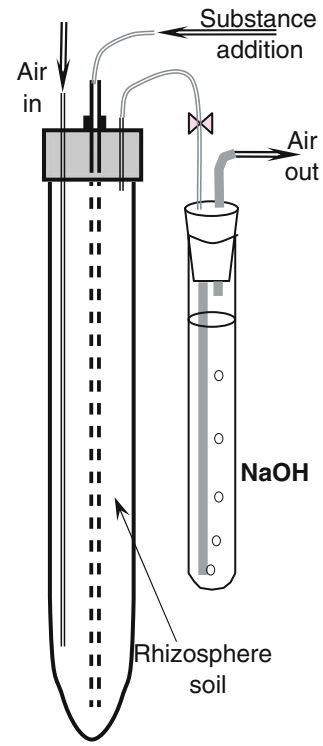

Fig. 2 Schematic representation of the microcosm used to simulate rhizosphere and to investigate the influence of root exudate compounds on plant residue decomposition. The Rhizon sampler® is located along the centre of the tube and is shown as a dotted double line. Only one of the two NaOH traps is shown for simplicity

The insertion of a thin tube to the bottom of the tube allowed air to be passed through the soil enabling the capture of CO_2 evolved from the soil

(Fig. 2). Considering the combined volumes of the Rhizon (0.31 cm^{-3}) and aeration tube (0.71 cm^{-3}), the soil density was 1.0 g cm^{-3}. The distance from the Rhizon surface to the wall of the plastic tube was 6 mm.

After constructing the simulated rhizosphere, the substrates were introduced into the soil through the Rhizon samplers. Briefly, an aqueous solution containing either glucose, malate or glutamate (100 μmol C ml^{-1}) was added to the soil at a rate of 112.5 μl d^{-1} (two injections per day). This resulted in the addition of 10 μg C g^{-1} soil d^{-1}, and corresponds to total amount of 10.8, 16.0 and 10.6 mg of glucose, malate or glutamate, respectively, added to each rhizosphere tube during 32 d of the experiment. The same volume of distilled water was added to the control treatment. During the experiment, air was pumped continuously through the rhizosphere tubes (20 ml min^{-1}) and CO_2 captured in two subsequent traps each containing 2.5 ml of 1 M NaOH (only one NaOH trap is shown in Fig. 1 for simplicity). The NaOH traps were changed at regular intervals (daily at the beginning and then every 5 d at the end) throughout the experiment. To determine the effect of temperature on residue breakdown and induced changes, the experiment was conducted in two climate-controlled chambers held at either 15°C or 25°C. The experiment was run for 32 d based on published estimates of root lifespan (Atkinson et al. 2003). Small amounts of distilled water were added to the rhizosphere tubes to compensate for soil drying caused by the continuous passage of air through the tubes.

Chemical analyses, calculation and statistics

At the end of the experiment (32 d after the start of substrate addition), the soil was removed from the tubes, mixed, and microbial biomass determined by $CHCl_3$ fumigation-extraction (Vance et al. 1987). After fumigation, 20 ml of 0.05 M K_2SO_4 solution was added to 5 g soil and the mixture shaken for 1 h at 300 rev min^{-1}. Subsequently, the mixture was centrifuged (8,000*g*, 10 min) and the supernatant filtered through a Whatman® paper No. 1. The amount of C in the K_2SO_4 extract without fumigation was used to determine dissolved organic matter (DOM; Jones and Willett 2006). The microbial biomass C, N and ^{14}C was calculated as the difference between fumigated and non-fumigated soil samples after correcting for extraction efficiency (k = 0.45; Vance et al. 1987). The 0.05 M K_2SO_4 solution was used instead of the standard 0.5 M solution to decrease the background activity caused by natural ^{40}K.

The ^{14}C content of the soil at the end of the experiment and plant residues was determined using a OX400 Biological Oxidiser (Harvey Instruments Corp., Hillsdale, NJ) and liquid scintillation counting (Wallac 1409; EG&G Ltd., Milton Keynes, UK). ^{14}C in NaOH and K_2SO_4 solutions was determined by liquid scintillation counting using Optiphase HiSafe 3 liquid scintillation cocktail (EG&G Ltd.). Total dissolved C and N in soil solution was determined with a Shimadzu TOCV-TNM1 analyzer (Shimadzu Corp., Kyoto, Japan).

A first order kinetic equation was used to calculate the half-life of the labile fraction of ^{14}C-labelled shoot residues in soil where,

$$^{14}C_{\text{remaining}}(t) = (^{14}C_i - ^{14}C_{\text{stable}}) \times \exp^{(-kt)} + ^{14}C_{\text{stable}} \quad (1)$$

and $^{14}C_{\text{remaining}}(t)$ is the amount of ^{14}C in plant residues remaining in the soil at time t, $^{14}C_i$ is the initial content of ^{14}C in plant residues (=100%) at the start (t_0), k is the rate constant, $^{14}C_{\text{stable}}$ is the percentage of ^{14}C in stable components of plant residue, which decomposition rates could not be precisely estimated within one month. The k value calculated by this approach shows only the decomposition rates of labile compounds in ^{14}C-labelled plant residue and cannot be applied for the whole residues. The half-life ($T_{½}$) of labile components of ^{14}C-labelled plant residues were calculated as follows

$$T_{\frac{1}{2}} = \ln(2)/k \quad (2)$$

The experiment was conducted with five replicates. Two-way ANOVA was applied to estimate the significance of the temperature and treatment effects on ^{14}C, C, N, C-to-N ratio and ^{14}C-to-C ratio present in the microbial biomass

and DOC. Repeated measures two-way ANOVA was applied to check the significance of differences between $^{14}CO_2$ efflux rates. Standard error of means and LSD for 5% error probability are presented on the graphs.

Results

Mineralization rate

The $^{14}CO_2$ efflux by decomposition of the ^{14}C-labelled plant residues commenced immediately (1 d) after their addition to the soil (Fig. 3). The $^{14}CO_2$ efflux rates were maximal at the first sampling event (1 d) with 2.83 ± 0.14% of the total ^{14}C added recovered as $^{14}CO_2$ at 15°C and 5.12 ± 0.14% recovered as $^{14}CO_2$ at 25°C. The $^{14}CO_2$ efflux rate at 15°C remained relatively constant during the first 5 d and thereafter decreased exponentially. In contrast, at 25°C an exponential decay pattern was observed right from the initial sampling point and after 10 d the mineralization rate was <1% of ^{14}C input d^{-1}. In addition, the $^{14}CO_2$ efflux rate was much faster at 25°C than at 15°C. In comparison to the initial incubation period, after 10 d the relative rate of residue decomposition measured as $^{14}CO_2$ efflux was very low (Fig. 3). At both temperatures, the total amount of $^{14}CO_2$ evolved over the 32 d experimental period was similar (34–40% of the total ^{14}C added; $P > 0.05$; Fig. 3).

Figure 3 also indicates that there was no strong effect of glucose, malate or glutamate addition on the $^{14}CO_2$ efflux rate by the ^{14}C-labelled residues decomposition in the simulated rhizosphere throughout the 32 d experimental period. The effects were significant only for the first 5 days at 15°C ($P < 0.05$). To evaluate the effect of the added substrates on the decomposition rate of the labile fraction of plant residues over the whole incubation period, we calculated the half-life of the plant residues by fitting a first order decay model with constant to the experimentally derived rate of $^{14}CO_2$ efflux (Fig. 3). Overall, there was a good fit of the kinetic model (Eq. 1) to the

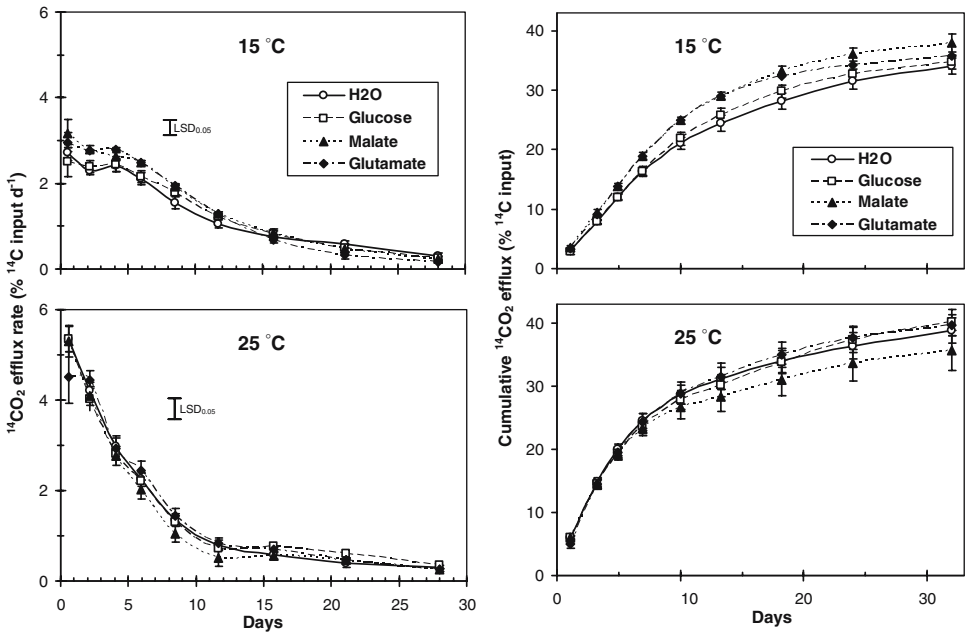

Fig. 3 $^{14}CO_2$ efflux rate (left hand panel) and cumulative $^{14}CO_2$ efflux (right hand panel) arising from the decomposition of ^{14}C-labelled *Lolium perenne* residues in a simulated rhizosphere after incubation at either 15°C (top panel) or 25°C (bottom panel). The *x*-axis units represent time in days after exudate and *Lolium* residue addition. $^{14}CO_2$ efflux rate is shown as the % of the total ^{14}C input d^{-1}; cumulative $^{14}CO_2$ efflux is shown as the % of total ^{14}C input. The values represent means ± SEM ($n = 5$). LSD value of repeated measures ANOVA for 5% error probability is presented as whisked segment

experimental data ($r^2 > 93\%$ for 15°C and $r^2 > 95\%$ for 25°C). This fit was not strongly improved by using a two-component first order kinetic model having two independent decay rate constants (data not presented). The average half-life of the labile fraction of plant residues at 15°C was 8.0 ± 0.4 d while at 25°C it was significantly lower at 4.9 ± 0.2 d ($P < 0.01$; Fig. 4). Note that because of short duration of the incubation these half-lives are responsible only for labile compounds of plant residues. Only in the glutamate treatment at 15°C was a significant decrease in the half-life of plant residues apparent ($P < 0.05$), indicating an accelerated mineralization in comparison to the distilled water control treatment (ca. 20% faster). No significant effect of substrate addition on $^{14}CO_2$ efflux rate by litter mineralization was observed in any of the other treatments ($P > 0.05$).

Compared to the CO_2 efflux rates, cumulative CO_2 efflux is less sensitive against disturbance and possible errors. To evaluate the dynamic effect of the added substrates on plant residue decomposition (measured as $^{14}CO_2$ efflux), we calculated the difference between $^{14}CO_2$ efflux in the presence and absence of substance addition (Fig. 5). While there were no significant effects of the added substrates at 25°C, the addition of malate and glutamate at 15°C increased the total $^{14}CO_2$ efflux by residue mineralization by 15–20% (Fig. 5). This effect was most evident within the first 20 d and declined thereafter.

^{14}C, C and N in microbial biomass and DOM

At the end of the experimental incubation period (32 d), between 7% and 9% of the ^{14}C initially present in the plant residues was recovered in the microbial biomass (Fig. 6). No significant differences in microbial biomass ^{14}C incorporation were observed in any of the temperature and substrate treatments ($P > 0.05$). The ^{14}C specific activity of the microbial biomass (Fig. 6, bottom right; ^{14}C-to-C ratio) was found to be negatively correlated with half-life of labile fraction of plant residues in soil ($r^2 = 0.42$, $n = 40$, $P < 0.001$). It must be noted, however, that the incorporation of ^{14}C from plant residues into the microbial biomass was measured at the end of the incubation and only partially reflects the processes occurring in the first 14 d when most of the $^{14}CO_2$ was evolved.

Total microbial biomass-C (Fig. 6) showed a strong effect of temperature ($P < 0.001$) and a small effect in response to substance addition ($P < 0.05$). The higher rate of $^{14}CO_2$ evolution and lower microbial biomass-C at 25°C in comparison to that at 15°C indicates a more active microbial biomass at higher temperatures. As no significant treatment effects were observed for microbial biomass-N ($P > 0.05$), the C-to-N ratio

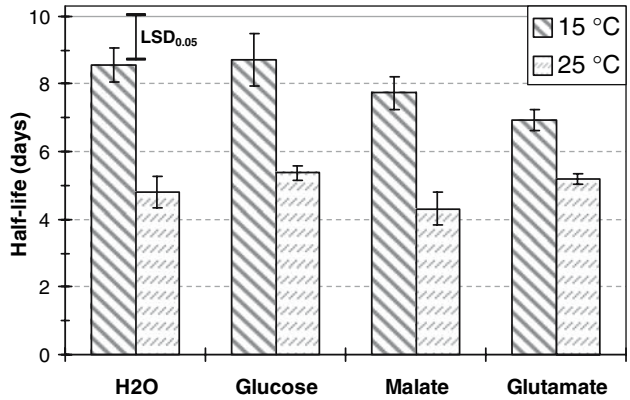

Fig. 4 Effect of the addition of glucose, malate or glutamate on the half-life (days) of ^{14}C-labelled *Lolium perenne* shoot residues in soil calculated from the $^{14}CO_2$ efflux at 15°C and 25°C. The values represent means ± SEM ($n = 5$). LSD value for 5% error probability is presented as whisked segment above the columns with H_2O addition

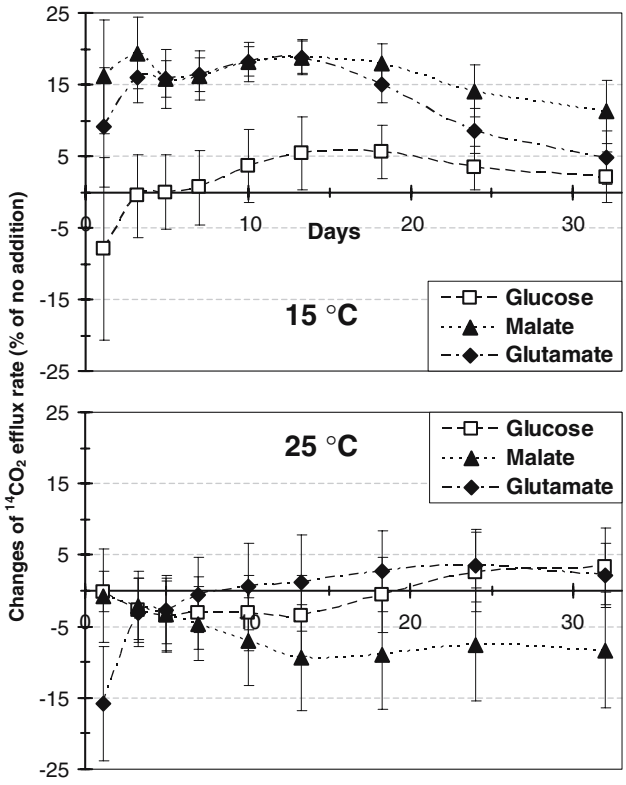

Fig. 5 Effect of the addition of glucose, malate or glutamate on changes of $^{14}CO_2$ efflux at 15°C (top panel) and 25°C (bottom panel) after the addition of ^{14}C-labelled *Lolium perenne* residues to soil. The changes of $^{14}CO_2$ efflux correspond to the difference of respective treatment to "no addition" and are presented as percentage of "no addition". The *x*-axis units represent time in days after exudate and ^{14}C-labelled *Lolium perenne* residue addition. The values represent means ± SEM (n = 5)

of the microbial biomass was significantly lower at 25°C in comparison to that at 15°C (Fig. 6).

At the end of the experiment, 0.5–0.6% of the initially added ^{14}C-label could be recovered in the soil's DOM (Fig. 7). As 27.7% of the added ^{14}C activity were present before the incubation in plant residues as water soluble substances (Fig. 1), only 2% of the added water soluble substances remains after 32 days in the soil. However, these ^{14}C in DOM originated probably not from the initial water soluble substances in plant residues, but from the transformation products of other ^{14}C-labelled litter fractions added to the soil.

Neither temperature nor substrate addition had a significant effect on the amount of ^{14}C recovered in the DOM after 32 days of incubation ($P > 0.05$; Fig. 7). In contrast, temperature did increase the total C in the native (non-labelled) DOM present in the soil ($P < 0.05$). Further, the ^{14}C specific activity of the DOM at the end of the experiment was positively correlated with half-life of the labile fraction of plant residues in soil ($r^2 = 0.31$, $n = 40$, $P < 0.001$). Both temperature and substrate addition strongly affected the N content of the DOM. Overall, the incubation of the soil at 25°C increased the N content of DOM by 2–5 times compared to the N content of DOM by incubation at 15°C (data not presented). This increased N mineralization at higher temperature led to a strong significant decrease in the C-to-N ratio of the DOM at 25°C in comparison to that at 15°C (Fig. 7). As expected, the N contained within the DOM was increased by glutamate addition at both temperatures when compared to the other treatments. In contrast, the addition of

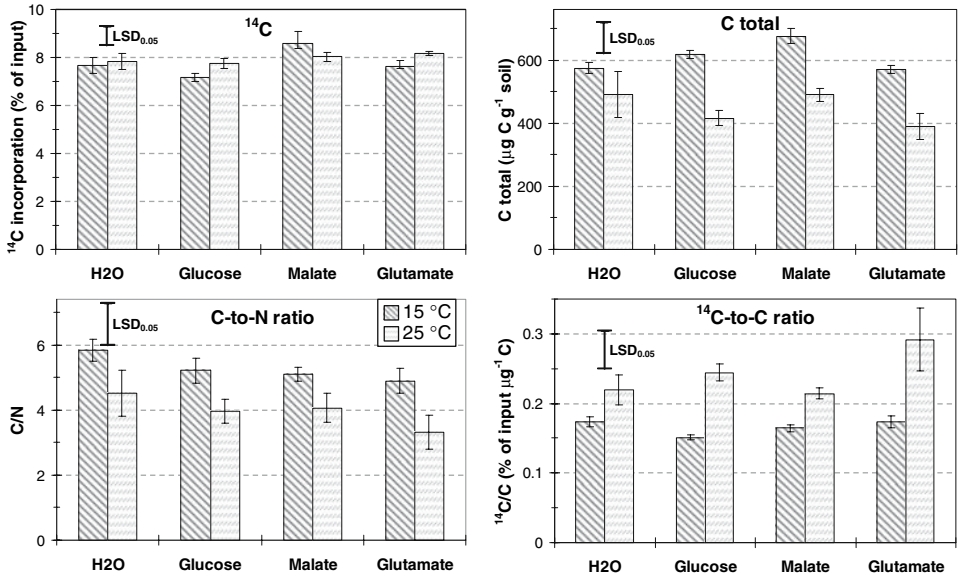

Fig. 6 Effect of glucose, malate or glutamate addition at two temperatures (15°C or 25°C) on ^{14}C incorporation into microbial biomass (top left), C content of microbial biomass (top right), C-to-N ratio of microbial biomass (bottom left) and ^{14}C incorporation per unit of microbial biomass (bottom right) after the addition of ^{14}C-labelled *Lolium perenne* residues to soil. The values represent means ± SEM (n = 5). LSD value for 5% error probability is presented as whisked segment above the columns with H_2O addition

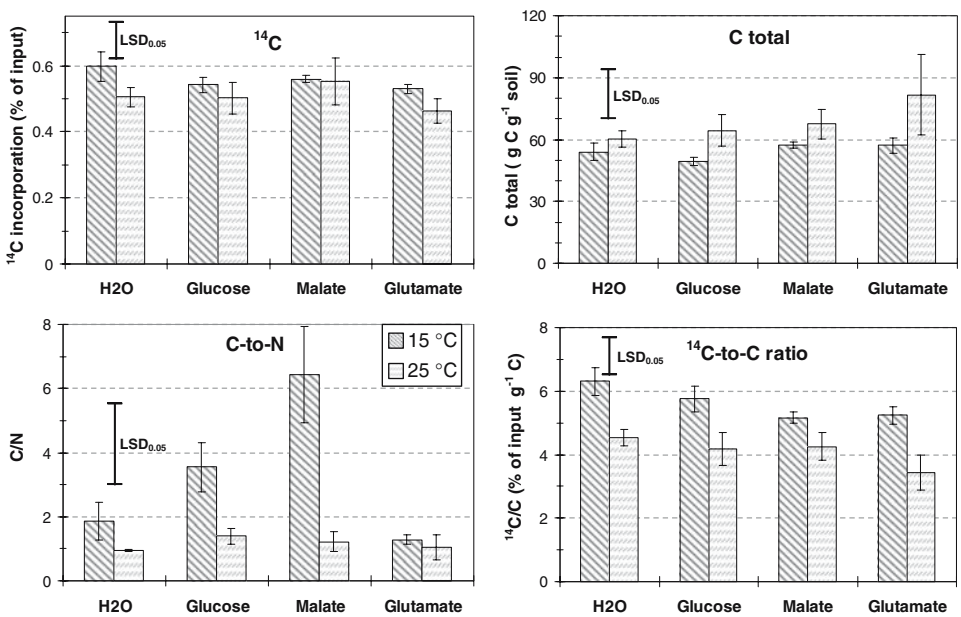

Fig. 7 Effect of glucose, malate or glutamate addition on the amount of ^{14}C recovered in the dissolved organic matter pool (DOM; top left panel), C content in DOM (top right panel), C-to-N ratio of DOM (bottom left panel), and ^{14}C incorporation per unit DOM (bottom right panel) after incubation of ^{14}C-labelled *Lolium perenne* residues in soil at either 15°C or 25°C. The values represent means ± SEM (n = 5). LSD value for 5% error probability is presented as whisked segment above the columns with H_2O addition

glucose and malate caused the immobilization of N, particularly at 15°C.

Discussion

The effect of substance addition

Exudation of organic substances by roots into the rhizosphere is a ubiquitous phenomenon in plants. It has been hypothesized that the release of this labile C into the soil will facilitate the mineralization of litter (Cheng and Kuzyakov 2005) and native soil organic matter (Kuzyakov 2002). However, it is difficult to divorce the influence of root exudation on SOM and litter mineralization over other processes operating concurrently in the rhizosphere (e.g. drying of the soil around roots, physical disturbance of the soil, extra-radical mycorrhizal hyphal growth, increased mesofaunal activity etc). Here we attempted to simulate the rhizosphere to look at the influence of root exudation on litter decomposition in isolation. We realize that the changes in plant residues decomposition induced by root exudates may not be directly transferred on SOM priming due to different C availability, chemical structure and stability, bounding on soil minerals and spatial accessibility of SOM and litter. Therefore, the interpretation of the observed results for litter and their relation to the SOM priming should be considered cautiously.

In contrast to our expectations, our results showed that the continuous addition of three common root exudate components (glucose, malate and glutamate) to a simulated rhizosphere had only a minor effect on the decomposition of ^{14}C-labelled plant residues measured as $^{14}CO_2$ efflux (Fig. 5). Further, it is clear that this response was both substrate and temperature dependent. In previous experiments using either a pulse or repeated addition of fructose, alanine and oxalic acid to soils, Hamer and Marschner (2005a, b) observed an increase in native SOM mineralization (i.e. priming effect) in the range of 10–63% at 20°C. However, the concentrations added to the soil were relatively high in comparison to that expected to occur from root exudation. Due to the uncertainties associated with quantifying rhizosphere C flow, the actual amount of root exudate-C released into the rhizosphere remains controversial. ^{14}C-labelling of plants in situ and measuring subsequent rhizodeposited-^{14}C tends to overestimate root exudation, while studies in hydroponic culture tend to underestimate root exudation (Jones and Darrah 1993). Using maize, we have estimated previously from modelling and laboratory experiments that the rate of root exudation of sugars was in the region of 3 μg C cm^{-1} d^{-1}, 0.8 μg C cm^{-1} d^{-1} for amino acids and 0.5 μg C cm^{-1} d^{-1} for organic acids (Jones et al. 2003). This is similar to that utilized in the experiments detailed here (10 μg C g^{-1} soil d^{-1}, resulting in 1.65 μg C cm^{-2} root surface d^{-1}). At 15–25°C, root growth can be expected to be rapid, at least in crop plants (1–2 cm d^{-1}; Kim and Silk 1999; Lecompte et al. 2001). Current evidence suggests that root exudation is maximal at root tips although this is clearly solute and plant species specific (Jaeger et al. 1999; Farrar et al. 2003). In such circumstances the input of large amounts of labile C into the rhizosphere can be expected to occur briefly leading to a pulse of microbial growth and an exhaustion of substrate. It could be that upon this exhaustion of substrate that SOM or litter mineralization is induced. In our experiments we added a constant amount of exudate-C, which may have reduced the need to mineralize litter. Alternatively, it could be that the amount of root exudates added is insufficient to induce sufficient microbial growth. Here we added 10 μg C g^{-1} soil d^{-1} to a total microbial biomass of 0.5 mg C g^{-1} resulting in a daily addition equivalent to 2% of the microbial C.

Glutamate was the only substrate that decreased the half-life of labile fraction of ^{14}C-labelled plant residues in the soil (Fig. 4, 15°C). This would suggest that the increased litter decomposition (measured as $^{14}CO_2$ efflux) was not a response to increased competition for N which stimulated the degradation of SOM to release greater amounts of N, but was due to a greater availability of mineral N which stimulated the degradation of the C rich plant residues (residue C-to-N ratio was 19). As we did not observe large increases in microbial-C (Fig. 6), we can conclude that the addition of small

amounts of malate and glucose did not stimulate significant microbial growth although presumably the activity of the biomass may have been enhanced by substrate addition. The lack of increase in the microbial community may have been one of the reasons for the lack of glucose and malate induced decomposition changes. This is supported to some extent by previous experiments in which a 100-fold greater C addition to soil was made and where significant SOM priming response was observed (Hamer and Marschner 2005a, b). Other studies have also shown that soil type is a major factor governing the SOM priming response (Hamer and Marschner 2005a, b). The soil type used here is a nutrient rich Eutric Cambisol. In a normal rhizosphere significant nutrient depletion may occur, however, this would not have taken place in our simulated rhizosphere. If changes in SOM or litter mineralization are induced by nutrient limitation, again our experiment would not truly reflect this attribute. Another possibility for the absence of pronounced mineralization effect in the rhizosphere may be that the labelled plant residues used here do not really represent the SOM for which the most priming effects have been reported (Fontaine et al. 2004; Kuzyakov and Bol 2006; Dijstraa et al. 2006).

The effect of temperature

Our study showed that soil temperature strongly affected a range of soil parameters in the simulated rhizosphere including (1) the intrinsic decomposition rate of labile fraction of plant residues (presented as $^{14}CO_2$ efflux rate—Fig. 3 and half-life—Fig. 4), (2) the influence of added substrates on labile fraction of plant residue decomposition (Fig. 5), and (3) the amount of microbial biomass and DOM concentration in soil at the end of incubation (Figs. 6, 7). From Fig. 3 it is apparent that the kinetics of mineralization were initially different at the two temperatures, however, after 32 d the total amount mineralized was similar in both treatments.

In our experiments it is likely that extracellular enzyme production, microbial activity and microbial community structure will be different at the two temperatures. PLFA profiles of our soil held at the two temperatures, however, revealed few short-term changes in community structure (Boddy et al., unpublished data). In contrast to our findings for litter mineralization, studies by Waldrop and Firestone (2004) and Biasi et al. (2005) have indicated that microorganisms at higher temperatures possess a greater capacity to degrade recalcitrant SOM. This may be associated with a shift towards a fungal dominated microbial community at high soil temperatures (Zhang et al. 2005; Pietikainen et al. 2005) or change of the respiratory quotient (Zyakun and Dilly 2005). In addition, a large increase (on average by 45%) in the enzyme activity of four soils was reported after a temperature increase of just 0.9°C (Sowerby et al. 2005). Clearly, further examination of the role of microbial community structural changes in the mineralization is warranted.

Despite a very similar incorporation of ^{14}C into the microbial biomass, the higher temperature decreased the amount of microbial biomass-C and so affected the parameters: C-to-N ratio and ^{14}C-to-C ratio etc. (Fig. 6). The ^{14}C-to-C ratio in the microbial biomass indicated a 15–30% faster biomass turnover rate at 25°C compared to 15°C. Although the C-to-N ratio of the DOM pool was very strongly depressed (ca. 20–400%; Fig. 7) by a temperature increase from 15°C to 25°C, in comparison, changes to the microbial C-to-N ratio were small (5.3 at 15°C and 4.0 at 25°C). However, this 15–30% faster biomass turnover induced a doubling of decomposition rate of labile litter fraction at 25°C compared to 15°C (Fig. 3, left). In contrast to Michel and Matzner (2002), we did not find any significant relationship between respiration rate and C-to-N ratio of the DOM pool (data not presented). Again further work is required to determine the chemical nature of the DOM pool and how this is affected by incubation temperature.

Conclusions

The continuous addition of representative amounts of three common root exudate components (glucose, malate or glutamate) into a simulated rhizosphere had a small, substrate-specific effect on decomposition of labile fraction of plant residues (measured as $^{14}CO_2$ efflux) and

on the microbial biomass. Glutamate and malate significantly enhanced decomposition, however, the influence of glutamate or malate addition on $^{14}CO_2$ efflux by decomposition of labile fraction of plant residues was much less than that caused by a 10°C rise in soil temperature. We conclude that the accelerated $^{14}CO_2$ efflux by litter decomposition induced by continuous input of glucose, malate or glutamate at rhizosphere concentrations was not sufficient to explain rhizosphere effects of accelerated plant residue or SOM decomposition reported in the literature. Rhizodeposition components other than root exudates may therefore be responsible for strong acceleration of SOM and/or litter decomposition (e.g. root turnover) in the rhizosphere.

The use of labelled plant residues for testing decomposition changes induced by the addition of unlabelled substances may be used as preferable alternative to addition of labelled substances. This approach contributes to error minimization, because the difference calculations are excluded.

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