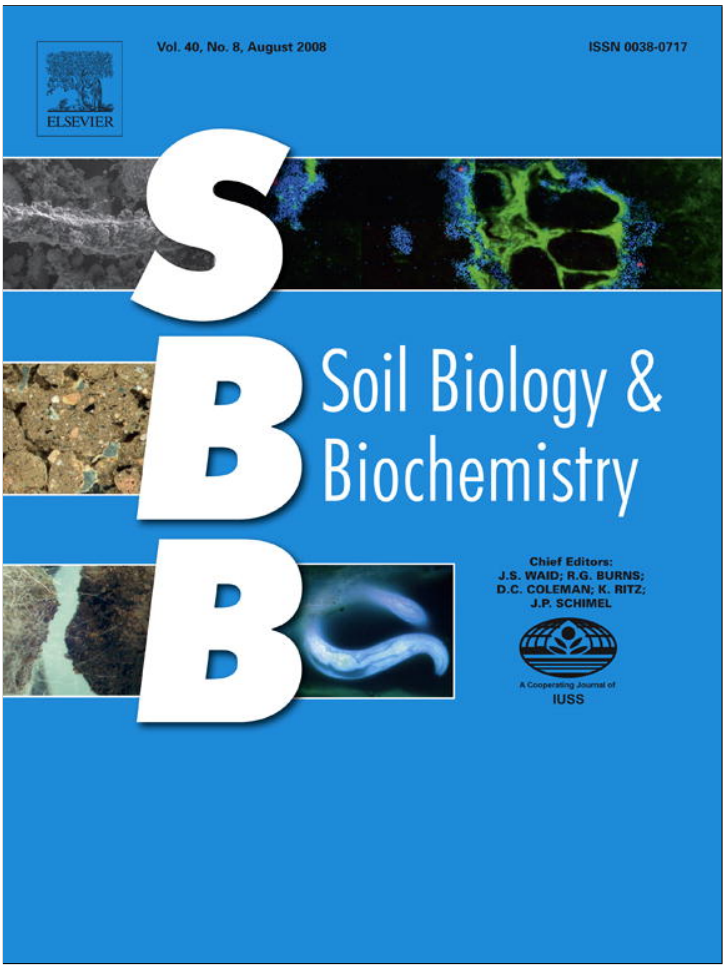

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Microbial utilization and mineralization of [^{14}C]glucose added in six orders of concentration to soil

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ABSTRACT

The substrate availability for microbial biomass (MB) in soil is crucial for microbial biomass activity. Due to the fast microbial decomposition and the permanent production of easily available substrates in the rooted top soil mainly by plants during photosynthesis, easily available substrates make a very important contribution to many soil processes including soil organic matter turnover, microbial growth and maintenance, aggregate stabilization, CO_2 efflux, etc. Naturally occurring concentrations of easily available substances are low, ranging from 0.1 μM in soils free of roots and plant residues to 80 mM in root cells. We investigated the effect of adding ^{14}C-labelled glucose at concentrations spanning the 6 orders of magnitude naturally occurring concentrations on glucose uptake and mineralization by microbial biomass. A positive correlation between the amount of added glucose and its portion mineralized to CO_2 was observed: After 22 days, from 26% to 44% of the added 0.0009 to 257 μg glucose C g^{-1} soil was mineralized. The dependence of glucose mineralization on its amount can be described with two functions. Up to 2.6 μg glucose C g^{-1} soil (corresponds to 0.78% of initial microbial biomass C), glucose mineralization increased with the slope of 1.8% more mineralized glucose C per 1 μg C added, accompanied by an increasing incorporation of glucose C into MB. An increased spatial contact between microorganisms and glucose molecules with increasing concentration may be responsible for this fast increase in mineralization rates (at glucose additions <2.6 μg C g^{-1}). At glucose additions higher than 2.6 μg C g^{-1} soil, however, the increase of the glucose mineralization per 1 μg added glucose was much smaller as at additions below 2.6 μg C g^{-1} soil and was accompanied by decreasing portions of glucose ^{14}C incorporated into microbial biomass. This supports the hypothesis of decreasing efficiency of glucose utilization by MB in response to increased substrate availability in the range 2.6–257 μg C g^{-1} (=0.78–78% of microbial biomass C). At low glucose amounts, it was mainly stored in a chloroform-labile microbial pool, but not readily mineralized to CO_2. The addition of 257 μg glucose C g^{-1} soil (0.78 μg C glucose μg^{-1} C micro-organisms) caused a lag phase in mineralization of 19 h, indicating that glucose mineralization was not limited by the substrate availability but by the amount of MB which is typical for 2nd order kinetics.

1. Introduction

Microbial biomass (MB) in soil is usually limited by available carbon (C) (Schimel and Weintraub, 2003), even though total soil organic carbon (SOC) content is high. This C limitation is caused by low availability of much of the SOC (e.g. Morita, 1988). On the other hand, easily available substrates like sugars, amino acids or organic acids with fast turnover (Jones, 1998) are continuously added to soil by root exudation and plant residue decomposition (Mary et al., 1993; Kuzyakov, 2002), by addition of plant residues (De Nobili et al., 2001) or by organic fertilizers (Leifeld et al., 2002; Bol et al., 2003). Several authors studied the turnover of these substances in soil in respect to their assimilation by micro-organisms, their incorporation into microbial biomass and their mineralization to CO_2 (Wu et al., 1993; Nguyen and Guckert, 2001). Chemically defined low molecular substances like sugar monomers or amino acids as model substances for readily available substrates are easier to apply than complex natural materials (Van Veen et al., 1985; Nguyen and Henry, 2002; Hamer and Marschner, 2005). They are commonly used to estimate the parameters that influence the substrate behaviour in soils (Falchini et al., 2003). More complex materials such as root mucilage (Mary et al., 1992, 1993) and plant residues (Cochran et al., 1988; Chotte et al., 1998) have nevertheless been used in some of these studies.

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Various soil properties affecting substrate turnover such as texture (Gregorich et al., 1991; Ladd and Amato, 1995) or CEC (Amato and Ladd, 1992) were identified in former studies. Many investigations focused on the chemical composition of the added substrates (e.g. Dalenberg and Jager, 1989; Falchini et al., 2003; Hamer and Marschner, 2005), the manner of its application (Sorensen et al., 1996) or the frequency of substrate addition or the time period between several substrate additions (Bremer and Kuikman, 1994). Most previous experiments added much higher amounts of substrates (60–700 mM) than the concentrations usually present in natural soils. Therefore, the results on assimilation by microbial biomass, decomposition to CO_2, as well as sorption by clay and organic particles may be not representative for behaviour of these substances in natural soils (Bremer and Kuikman, 1994; van Hees et al., 2005). In a root-free soil, naturally occurring concentrations of easily available substances are less than 50 μM (van Hees et al., 2005). According to Jones (1998), 0.5–10 μM of the easily available substances in a root-free soil consist of organic acids, and 0.32–4.72 μM are amino acids (Monreal and McGill, 1985). Fischer et al. (2007) found soil solution concentrations of 8.2 μmol l^{-1} for total amino acids and of 2.4 μmol l^{-1} for total carbohydrates, dominated by glucose with 0.7 μmol l^{-1}. Concentrations of low molecular substances can increase up to 100 μM in a rooted soil (van Hees et al., 2005). In the rhizosphere, concentrations of 500 μM may be attained (van Hees et al., 2005). Of this 0.4–400 nmol g^{-1} dry soil, up to 80 nmol g^{-1} dry soil is composed of organic acids. The highest values were found in root cells and ranged from 10 to 20 mM for organic acids (Jones, 1998) and from 10 to 90 mM for mono- and disaccharide (Jones and Darrah, 1996; Ryan et al., 2001).

Due to fast microbial decomposition, such substrates have short mean residence time in soils ranging from hours for carboxylic acids (van Hees et al., 2005) and amino acids (Jones et al., 2005) to 31–162 days for glucose (Saggar et al., 1999). This yields a substantial contribution of easily available substances to soil respiration, which reflects the continuous input and fast decomposition of these organic substances (van Hees et al, 2005). Therefore, knowledge about the pathway of easily available substances through microbial biomass and its mineralization to CO_2 over the whole range of concentrations in soil is crucial. Easy available substances are essential as C and energy source for microbial growth (Mary et al., 1992). For example, the activation of microorganisms from no-growth or starvation state to active state (Morita, 1988) by easily available substrates can be the main reason for accelerated SOM mineralization (Dalenberg and Jager, 1989; Kuzyakov et al., 2000; Hamer and Marschner, 2005).

Several authors showed the effect of the amount of added substrate on its microbial mineralization to CO_2 (Bremer and van Kessel, 1990; Mary et al., 1993; Bremer and Kuikman, 1994) and found positive correlations (Wu et al., 1993; Bremer and Kuikman, 1994; Marstop and Witter, 1999). However, if high amounts of easily available substrates were added, less C was incorporated into microbial biomass (Bremer and Kuikman, 1994; Marstop and Witter, 1999, Nguyen and Henry, 2002). One possible explanation for this effect is the impact on the energy state of the MB (e.g. Gottschal, 1992; Nguyen and Guckert, 2001). At low concentrations, easily available substrates are assimilated by MB and stored in a chloroform-labile pool (Hill et al., 2008), but the energy and C provided are insufficient for microbial growth (Bremer and Kuikman, 1994).

So far, studies on glucose mineralization used either only low or very high ranges of glucose concentrations to compare with naturally occurring concentrations of easily available substrates. Bremer and Kuikman (1994), for example, used glucose concentrations between 8.3 mM and 533 mM, Nguyen and Guckert (2001) concentrations between 8 μM and 13 mM. Hill et al. (2008) used glucose concentrations from 1 μM to 10 mM to reflect natural C concentrations in soil solution of root-free soil and the rhizosphere, but did not use glucose concentrations above than 10 mM although they pointed out that such concentrations can occur in soil by decomposition of plant and animal residues. Therefore, we investigated the dynamics of glucose mineralization and of its utilization by microbial biomass in relation to additions over the whole range of natural occurring substrate concentrations in the same experiment to avoid experimentally caused influences on glucose mineralization. A higher incubation temperature, for example, is known to result in an increased mineralization of the added substrate (Leifeld et al., 2002; Dioumaeva et al., 2003).

In the literature, long time intervals between samplings are often combined with long incubation periods (e.g. Bremer and Kuikman, 1994, 35 days with just three sampling times) or the incubation period is very short (Nguyen and Guckert, 2001; Hill et al., 2008). In our study, a high resolution at the beginning of the incubation when most of the glucose is mineralized was combined with a long-term monitoring of mineralization by longer time intervals. Accordingly, glucose concentrations from 0.29 μM (0.0009 μg glucose C g^{-1} soil) to 80 mM (257 μg glucose C g^{-1} soil) were added to a silt-loamy soil and traced during 22 days in increasing time intervals from 0.2 to 3 days.

We hypothesized (1) that substrate amounts ranging over 6 orders of magnitude would produce a significant, but varying effects on the assimilation and mineralization by microbial biomass and (2) that the effect of increasing glucose concentrations on its mineralization is a function of various mechanisms at different levels of glucose concentrations.

2. Material and methods

2.1. Soil

Soil samples were taken in October 2004 from the A_h horizon (0–10 cm) of a silty-loamy Gleyic Cambisol at the experimental plot "-Muttergarten" of the University of Hohenheim, Stuttgart, Baden-Württemberg, Germany (48°43′ north latitude, 9°13′ east longitude). Mean annual temperature is 8.7 °C and average rainfall 679 mm a^{-1} (1961–1990, meteorological station Stuttgart-Hohenheim). The plot was covered with the perennial energy grass *Miscanthus* × *giganteus* (Greef et Deu.) for 10 years. The soil C content was 24 g C kg^{-1}. The soil was air-dried and sieved (2 mm), after which all visible roots were carefully removed both with the electrostatic method (Kuzyakov and Siniakina, 2001) and manually by tweezers.

2.2. Experimental design

Thirty grams of the air-dried soil were adjusted to 50% of WHC and pre-incubated for 10 days at 18 °C in 250 ml incubation vessels (Schott Duran, Mainz, Germany) to exclude the influences of the rewetting on C mineralization and microbial biomass during the main experiment (Wu and Brookes, 2005). The produced CO_2 was collected in 3 ml of 1 M NaOH solution. The NaOH solution was exchanged four times during the pre-incubation.

After pre-incubation, in five different treatments, increasing D(+)-glucose amounts were added to the soil: (i) 0.00093 (0.0009) μg glucose C g^{-1}, (ii) 0.257 (0.26) μg glucose C g^{-1}, (iii) 2.57 (2.6) μg glucose C g^{-1}, (iv) 25.7 (26) μg glucose C g^{-1} and (v) 257 (260) μg glucose C g^{-1} soil. The glucose was added to the soil as an aqueous solution containing uniformly ^{14}C-labelled glucose (0.000933 μg C g^{-1} soil, activity 245 Bq g^{-1} soil) and 2.57 μg N g^{-1} soil and 0.27 μg P g^{-1} soil to avoid nutrient deficiency. Unlabelled D(+)-glucose was used to attain the different glucose amounts. For each treatment, separate solutions with ^{14}C-labelled glucose, nutrients and the respective concentration of unlabelled glucose were made. The glucose and nutrient solution was added by a fine

pipette tip to the soil as uniform as possible. In doing so the soil moisture was adjusted to 70% water hold capacity.

After the glucose addition, the vessels were incubated for 22 days at 18 °C. During the first 3 days, the NaOH solution was exchanged every 0.2–0.4 day. This period increased up to 0.6 day to 1 day for days 3–7 and to 2–3 days for days 7–22.

At days 0, 2, 6, 12, and 22, three of the replicates were removed. A part of the soil from each replicate was air-dried; the rest was kept in a refrigerator at 4 °C and, within 2 days, microbial biomass C content and ^{14}C activity were analysed.

2.3. Analyses

The amount of total evolved CO_2 was analysed by titration of an aliquot of the NaOH solution with 0.1 M HCl after precipitation of the trapped CO_2 as $BaCO_3$ using a digital burette (Brand, Wertheim, Germany). The activity of $^{14}CO_2$ trapped in the NaOH solution was measured with a Liquid Scintillation Counter (Wallac 1411, Wallac, Oy, Finland) after mixing 0.3 ml of the NaOH solution with 2 ml of the scintillation cocktail Rotiszint Eco Plus (Carl Roth, Karlsruhe, Germany).

Microbial biomass was determined with a modified chloroform-fumigation method (Vance et al., 1987). Part of the soil was fumigated with ethanol-free chloroform for 24 h. From fumigated and non-fumigated soil samples, 5 g was extracted with 20 ml 0.05 M K_2SO_4 and carbon content was determined with a Dimatoc-100 TOC/TIC analyser (Dimatec, Essen, Germany). A k_{ec}-factor of 0.45 (Wu et al., 1990) was used to calculate total microbial biomass C. The ^{14}C activity of microbial biomass was determined as described above after mixing of 2 ml K_2SO_4 extract with 4 ml scintillation cocktail. The glucose-derived microbial C was calculated based on ^{14}C activity in microbial biomass divided by ^{14}C specific activity ($^{14}C/C$) of the added glucose. The same k_{ec}-factor of 0.45 was used to determine glucose-derived C incorporated into MB. The rest to the total microbial C was defined as SOC-derived microbial C.

2.4. Calculations and statistics

Glucose mineralization was calculated as a percentage of the total added [^{14}C]glucose, assuming that labelled and unlabelled glucose was mineralized identically. The glucose C incorporated into MB was also expressed as a percentage of total added glucose.

Two-way ANOVA was used to calculate significance of two factors: (1) amounts of added glucose and (2) time. If the test of homogeneity was negative, a Tamhane-T_2 test (significance level = 0.05) was used, otherwise an LSD test was used. Standard errors of means are presented in the figures.

3. Results

3.1. Mineralization of [^{14}C]glucose

The portion of added glucose mineralized to CO_2 during the 22 days consistently increased as more glucose was added. From the added 0.0009–257 μg glucose C g^{-1} soil, 26% up to 44% were mineralized after 22 days (Fig. 1). The differences in the cumulative glucose mineralization between the increasing glucose amounts were significant ($p \leq 0.05$), except between the amendment of 0.26 and 2.6 μg glucose C g^{-1} soil ($p > 0.05$).

Most of the glucose was mineralized during the first hours after addition (Fig. 2). The maximal mineralization rate occurred in the first 0.2 day after application except after the amendment of 257 μg C g^{-1} soil. In these first 0.2 days, rates ranged between 2.3% (0.0009 μg glucose C g^{-1} soil) and 3.0% of total added ^{14}C per hour (26 μg glucose C g^{-1} soil). However, after addition of the maximal tested amount (257 μg C g^{-1}), maximum mineralization (1.3% of total added C per hour) occurred between 0.45 day and 0.8 day (Fig. 2). Therefore, adding the highest amount of glucose resulted in a short lag-phase in its mineralization as found by Wu et al. (1993). Eighteen percent (0.0009 μg glucose C) – 32% (257 μg glucose C) of total added glucose was mineralized in the first 2 days, and 22–38% in the first 6 days.

3.2. Microbial biomass

The initial content of soil microbial biomass carbon (microbial C) before glucose addition (day 0) was 331 μg C_{mic} g^{-1} soil. During incubation, microbial C increased significantly ($\alpha \leq 0.05$) to a maximum of between 445 and 1067 μg C_{mic} g^{-1} soil (Fig. 3) depending on the amount of added glucose. There were no significant differences in the level and progression of the total microbial C content for amendments

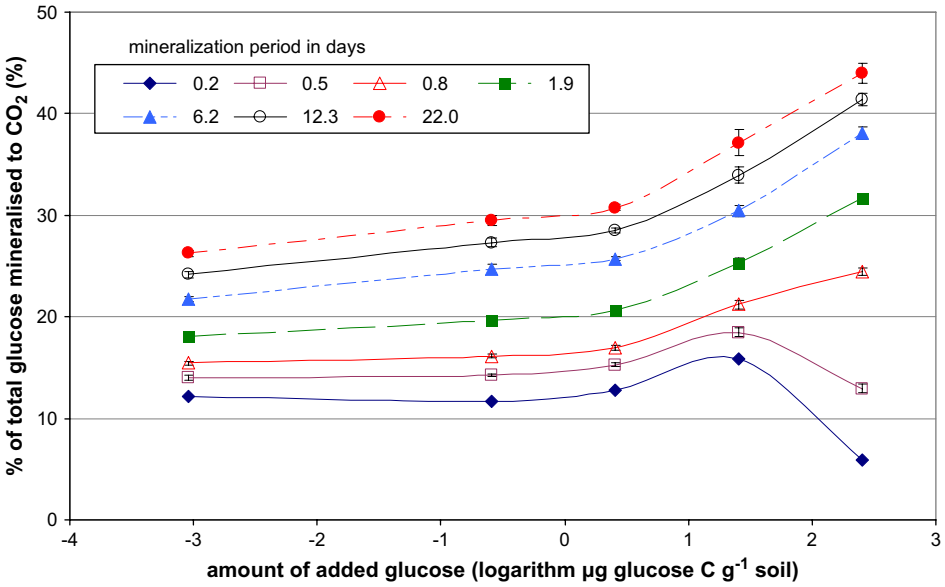

Fig. 1. Effect of the amount of added glucose on its mineralization (±SE) depending on the mineralization period. The legend shows the number of days after the start of incubation. Note logarithmic X scale of glucose concentrations.

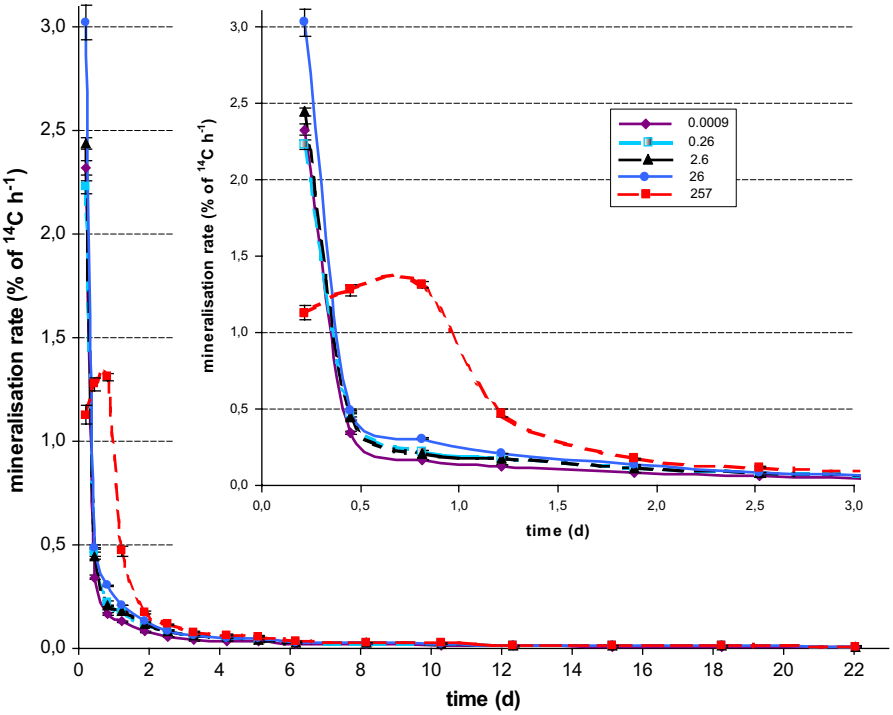

Fig. 2. Dynamics of glucose mineralization rate measured as $^{14}CO_2$ efflux (±SE) depending on the C amount added (in μg C g^{-1} soil), The inset shows magnification of the mineralization rate during the first three days.

<25.7 μg glucose C g^{-1} soil ($\alpha = 0.05$). For the 0.0009 μg amendment, the total content was barely significantly lower than for the higher amendments after 22 days. For the period before day 22, adding 257 μg soil led to a significantly stronger increase in the total microbial C content than amendments <257 μg (Fig. 3).

The total microbial C is partly derived from SOC and partly from the added glucose. Based on ^{14}C activity in the microbial biomass and ^{14}C-specific activity of the added glucose, we partitioned total microbial C into these two sources. In general, there were no differences in the total microbial C and the SOC-derived microbial C during the incubation (Fig. 3). The ratio glucose-derived microbial C to SOM-derived microbial C increased with increasing amounts of added glucose and decreased with incubation time (data not shown): With values of 1.3×10^{-6} (0.0009 μg amendment) – 0.21 (257 μg amendment), the ratio was highest on day 2.

The percentage of glucose C incorporated into MB generally decreased as added glucose increased (Fig. 4). During the first 6 days, the incorporation was also smaller after the 0.0009 and 0.26 μg amendments than after the 2.6 μg amendment. For the 0.0009 μg treatment, the incorporation remained lower than for 2.6 μg over the whole incubation period. During the whole incubation period, a significantly lower percentage of added C was incorporated for 257 μg than in the smaller amendments. Six days after glucose addition, the differences in percent incorporation into MB started to be significant ($\alpha \leq 0.05$) between the treatments. The percent incorporation decreased significantly with incubation time ($\alpha = 0.01$), except for between days 6 and 12 (Fig. 4). However, increasing glucose amounts exerted an opposite effect on incorporation into MB versus mineralization to CO_2, i.e. incorporation decreased but CO_2 fraction increased.

4. Discussion

4.1. Glucose mineralization

The cumulative glucose mineralization in our investigation was comparable to previous studies. Saggar et al. (1999) found a mineralization of 25–44% of the added 2730 μg glucose C g^{-1} soil in various textured soils within 3 days. In the same time span, 20–35% of the added 0.0009–257 μg glucose C g^{-1} soil were mineralized in our study. The 37% value in van Veen et al. (1985) (325 μg glucose C g^{-1} soil) and in Bremer and van Kessel (1990) (300 μg glucose C g^{-1} soil) also agree with our results. In contrast, the 58–92% mineralization of other easily available substrates (fructose, alanine, oxalic acid) in 26 days found by Hamer and Marschner (2005), the 37–53% mineralization of 20–500 μg glucose in 6 days in the study of Nguyen and Henry (2002) (compared to 38% of 257 μg C in 6 days in our study) or the 77% value of Sharabi and Bartha (1993) (1000 μg glucose amendment) were significantly higher than in our study. However, the latter studies were conducted at an incubation temperature 5–10 °C higher than in our experiment (18 °C). Several authors reported an exponential dependency of organic carbon mineralization on temperature (Leifeld et al., 2002; Dioumaeva et al., 2003). In Nicolardot et al. (1994), the mineralization of a 500 μg amendment increased from 41% at 4 °C to 58% at 28 °C during 140 days.

4.2. Effect of the glucose amount on its mineralization

For the whole concentration range of easily available substrate that can occur in natural soils, the addition of increasing amounts of glucose increased the portion mineralized to CO_2, as shown by Bremer and van Kessel (1990), Wu et al. (1993) and Bremer and Kuikman (1994), (Fig. 1). The hypothesis of a positive correlation was thus confirmed. In non-rhizosphere top soil, micro-organismic biosynthesis and growth are generally limited by the available C and energy (e.g. Gottschal, 1992; Cheng et al., 1996; Schimel and Weintraub, 2003), so that most of the micro-organisms are dormant, that means temporarily inactive. The switch from dormant (Morita, 1988) to active state can be triggered by release of easily available C to the soil as rhizodeposits, organic fertilizer etc. (e.g. Wu, 1993; De Nobili et al., 2001; Stenström et al., 2001). In our

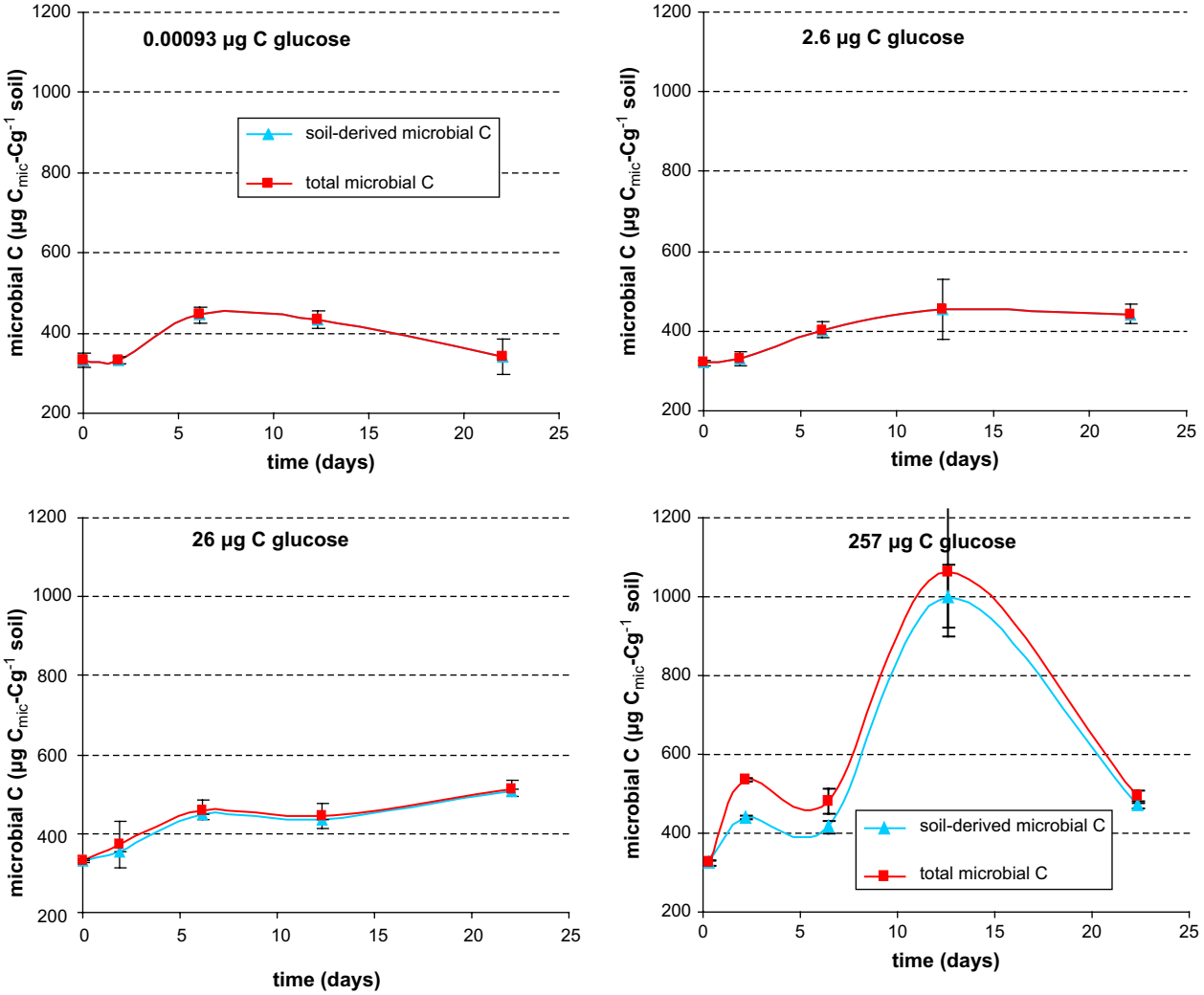

Fig. 3. Dynamics of the total, the SOC-derived and the glucose-derived microbial biomass content (as difference between total and SOC-derived microbial biomass content) in the treatments with increasing glucose amendments (treatments with 0.26 µg glucose C g^{-1} soil not presented). The difference of total microbial biomass content and the SOC-derived microbial biomass content is shown.

experiment, such a release was simulated by glucose addition. By adding increasing amounts of glucose, more microbial biomass is stimulated to grow and to mineralize the "new" added C to CO_2, yielding a positive correlation between added glucose and its mineralization. Additionally, the glucose utilization efficiency may decrease if more easily available substrate is provided. This is indicated by a decreasing relation between glucose incorporated in MB and glucose mineralized to CO_2 (Fig. 4) at glucose additions >2.6 µg C g^{-1} soil and the comparatively low increase of microbial biomass content. According to Fontaine et al. (2003), specific soil microbial populations which are unable to use the existing but hardly available soil organic C in soil switch from their starving state and begin to grow when provided with new easily available substrate. These microbial populations are normally classified as r-strategists (Blagodatskaya et al., 2007). Accordingly, changes in microbial population structure should be associated with the increased mineralization, although this was not investigated in the present study.

Because dormant MB maintains its metabolic energy state, expressed as 'adenylate energy state', even if no easily available substrate is available at that time (Brookes et al., 1987), the MB can then quickly become active when easily available substrate is added (Blagodatsky and Richter, 1998; De Nobili et al., 2001). Therefore, maximum glucose mineralization rates were measured immediately after glucose addition in all treatments (except at the 257 µg amendment, Fig. 2); and rates decreased as the available new applied C source declined with time.

4.3. Effect of added glucose amount on its incorporation into MB

The percentage of glucose incorporated into MB generally decreased with the amount of added C (Fig. 4), especially at amendments >2.6 µg. This shows that the resource use efficiency increases with rising C limitation. Nguyen and Guckert (2001) and Bremer and Kuikman (1994) proposed that increasing incorporation into MB at lower glucose levels is, among others, caused by a metabolic arrest when the energy provided with the glucose is too small for the complete activation of MB from dormant to active state. Therefore, the assimilated glucose is stored in an intermediate, chloroform-labile and "conserved" C pool and not released as CO_2 at lower glucose concentrations (Hill et al., 2008). Additionally, glucose C can be incorporated into structural compounds of the microbial biomass if enough glucose is added. These structural compounds are not chloroform-labile and therefore not detected as microbial C with the CFE method (Fig. 4). Bremer and Kuikman (1994) therefore postulated the necessity to adjust the k_{ec}-values to the glucose concentrations. Nonetheless, the sum of

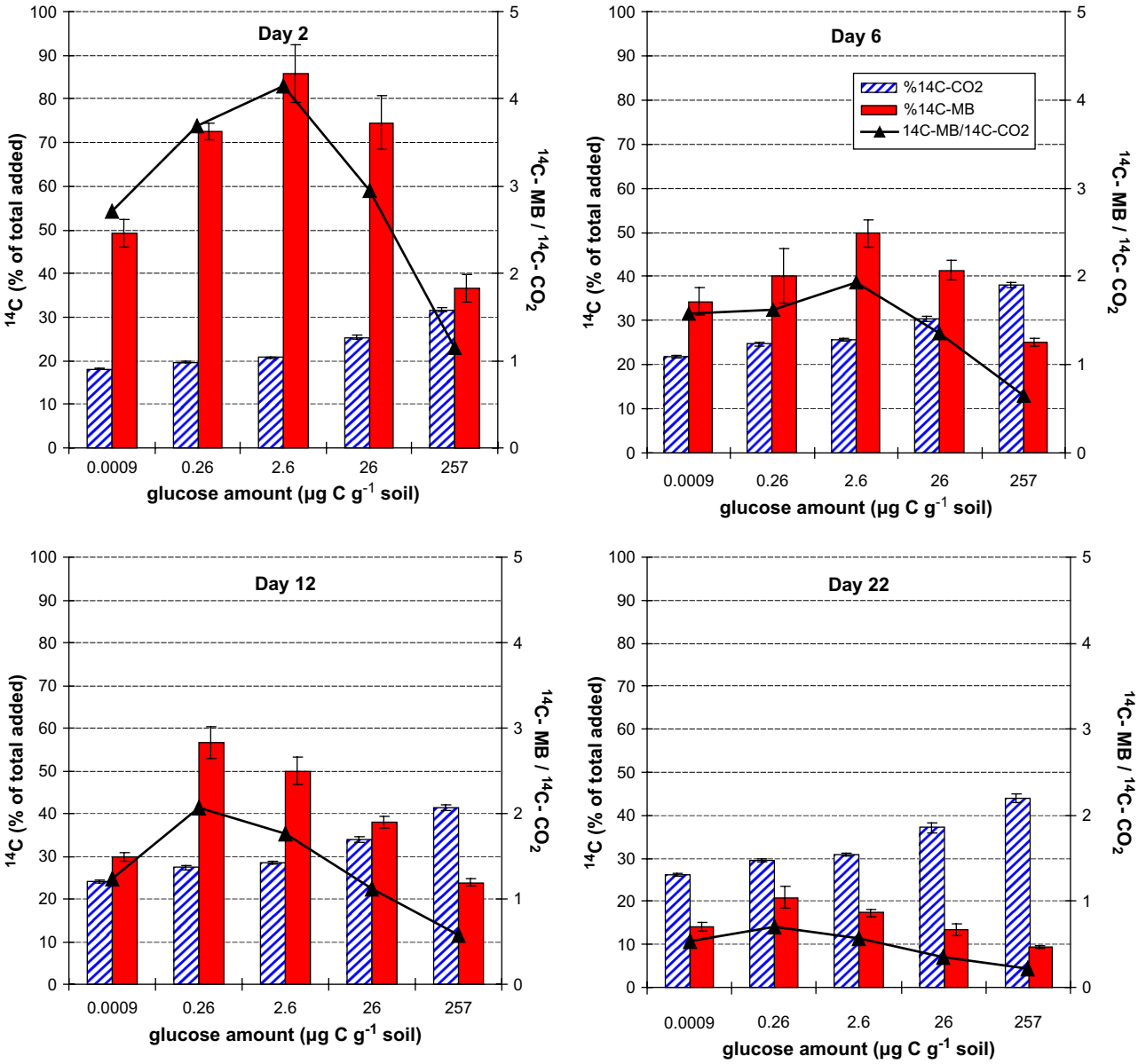

Fig. 4. ^{14}C content in microbial biomass ($^{14}C_{MB}$) as % of ^{14}C added and cumulative $^{14}CO_2$ evolved during 2, 6, 12 or 22 days (as % of ^{14}C added) depending on the amount of added glucose. The ratio between $^{14}C_{MB}$ and ^{14}C mineralized is presented as a line.

both the glucose-derived and the soil-derived MB increased slightly with the addition of increasing glucose amounts even though we used the k_{ec}-factor of 0.45 for all treatments (Fig. 3). The k_{ec}-values were not adjusted to the glucose concentrations in our study. With exception of day 2, the total recovered ^{14}C in MB and evolved CO_2 was between 40% and 84%. In general, the total recovered glucose decreased with incubation time or remained constant. Of the glucose C not recovered in MB and CO_2, 3–42% of the added glucose was found in the soil matrix (data not shown). If this ^{14}C remaining in the soil is considered, 89–99% of the total added glucose C was recovered on day 2 and 82–88% at the end of the incubation. These values are nearly the same as reported by Bremer and Kuikman (1994). They also found no relationship between the amount of added C and total glucose recovery, as we did. They explained the remaining difference to 100% by the insufficient oxidation of the soil when preparing the soil samples for measuring. Additionally, $^{14}CO_2$ might escape by opening the glass bottles to exchange the NaOH solution, as described by Chotte et al. (1998).

4.4. Effect of glucose amount on glucose mineralization

In Sections 4.2 and 4.3, the general mechanism responsible for glucose utilization by MB and its mineralization is described. But the shift in the portions of both mineralized and incorporated (in MB) glucose with increasing amendments favours the hypothesis that various mechanisms are responsible for the decreasing substrate utilization at increasing levels of easily available substrates in soil.

The logarithmic scale of the cumulative glucose mineralization on the glucose amount added (Fig. 1) shows two linear parts for amendments <2.6 µg and >2.6 µg glucose C g^{-1}. Both parts of the curve are characterized by different slopes. Although the overall differences between the former treatments were only partly significant, the increase in the glucose mineralization per mass unit of glucose added was 20–36 times greater at amounts <2.6 µg than at amounts >2.6 µg glucose C g^{-1}. After 22 days, the mineralization increased by 1.8% per µg glucose C for amendments <2.6 µg and

just by 0.05% per μg glucose C added for amendments between 2.6 and 257 μg C g^{-1}. At the same time, the percentage of glucose incorporated in the chloroform-labile pool of the MB increased up to the addition of 2.6 μg C g^{-1} during the first 6 days of incubation when 4/5 of CO_2 was evolved. In contrast, this value decreased at additions >2.6 μg C g^{-1} for the whole incubation time, in accordance to Nguyen and Henry (2002) who also found decreasing ratios in the MB-incorporated to CO_2-evolved percentage when amendments increased between 20 and 500 μg glucose C g^{-1} soil.

At the very low glucose concentrations (<2.6 μg C g^{-1}) in the soil solution, only part of the MB contributes to mineralization probably because of missing spatial contact between microorganisms and glucose molecules in soil solution (low concentration and non-uniform distribution in soil matrix). At higher amendments, this spatial contact increases rapidly and more MB contributes to mineralization. But this effect reflects increased spatial contact at glucose values <2.6 g C g^{-1} and not the increased energy for the MB provided by the glucose. This hypothesis is supported by the increased incorporation of glucose-derived C into MB up to 2.6 μg C g^{-1} soil, simultaneously with the increased mineralization of the glucose. The mechanisms of the increasing mineralization as more glucose is added (as described in Sections 4.2 and 4.3) are therefore only valid at concentrations >2.6 μg C g^{-1} soil. For glucose concentrations >2.6 μg C g^{-1} soil, the increasing efficiencies of the MB in the glucose mineralization caused the increased mineralization and not only the growth of the MB (see Sections 4.2 and 4.3). This is indicated by decreasing ratio between glucose C incorporated in MB and the glucose C mineralized to CO_2 (Fig. 4)

The lag phase in glucose mineralization after the 257 μg g^{-1} amendment did indicate that the dependency of mineralization on the amount of amendment switched to a dependency on the amount of the MB in the soil (e.g. Aoyama et al., 2000). The glucose respiration curves after adding <25.7 μg C g^{-1} soil belong to the zero-order type kinetics, with maximum respiration rates shortly after amendment and decreasing respiration rates when glucose disappears (Fig. 2). Starting at amounts >25.7 μg g^{-1} (=7.8% of microbial biomass C), it is not the available C but the content of the microbial biomass that limits CO_2 production. Without the growth, the existing MB was unable to mineralize the 257 μg C g^{-1} soil what correspond to 78% of the original microbial C content (331 μg C_{mic} g^{-1} soil). This respiration curve type is called the growth-associated type. Maximal substrate mineralization was reached between 11 and 19 h. The microbial biomass increased significantly stronger during the first 48 h after adding 257 μg versus <257 μg C g^{-1}.

Bremer and Kuikman (1994) also proposed the existence of an individual upper limit of glucose amendment above which further additions do not affect the initial glucose mineralization rate. They found no effect of glucose amendment on mineralization above 288 μg C g^{-1} soil. In our experiment, the upper limit was not attained with the addition of 257 μg glucose C g^{-1} soil. However, these limits should be compared as related not to the soil mass, but to microbial C.

5. Conclusion

There was a positive correlation between the portion of glucose mineralized to CO_2 and the amount of glucose added over 6 orders of magnitude representing the whole range of naturally occurring concentrations of easily available substances in soil. The dependency of glucose mineralization on its amount was described with two functions. Up to 2.6 μg glucose C g^{-1} soil, mineralization increased with the slope of 1.8% mineralized per μg C glucose. Amounts above 2.6 μg C g^{-1} soil significantly boosted glucose mineralization, as opposed to amendments below 2.6 μg glucose C g^{-1} soil. Nonetheless, the slope (0.05% more glucose mineralized per g glucose added) was smaller than at amendments below 2.6 μg C g^{-1}. Simultaneously, less glucose was incorporated into microorganisms at amendments starting at 2.6 μg glucose C g^{-1} soil. Adding 257 μg glucose C g^{-1} soil (0.78 μg C glucose μg^{-1} microbial biomass C) caused a mineralization lag phase of 19 h, indicating a switch from substrate limitation by CO_2 production to a limitation by the amount of MB.

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