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Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies

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ABSTRACT

Input of easily available C and N sources increases microbial activity in soil and may induce priming effects (PE)—short-term changes in SOM decomposition after substrate addition. The relationship between the origin of priming and growth characteristics of the microbial community is still unclear. We related real and apparent PEs induced by glucose and N addition with growth strategies of soil microorganisms. Two concentrations of uniformly labeled ^{14}C glucose with and without N were added to Chernozem, and the released $^{14}CO_2$ and CO_2 efflux were monitored over a 300 h period. The shift in strategies after glucose addition was monitored by microbial growth kinetics based on the estimation of maximal specific growth rate. The production of unlabelled extra CO_2 induced by glucose was completed after 3 days and amounted to about 15–19% of the microbial biomass-C. The presence of real or apparent PE depended on the level of added C and N. An apparent positive PE was observed when the amount of applied glucose-C was 13 times lower than the amount of microbial biomass-C, i.e. under C-limiting conditions. Apparent PE was accompanied by a higher maximal microbial specific growth rate, i.e. by a shift towards r-strategy features. The absence of a priming effect was observed under N-limiting conditions at an eightfold excess of glucose-C versus microbial biomass-C. A large excess of glucose and N lowered maximal specific growth rates of soil microorganisms and had a negative priming effect. Accordingly, slow-growing microorganisms (K-strategists) switched from SOM mineralization to glucose uptake, probably due to preferential substrate utilization.

Analysis of microbial growth kinetics was an efficient approach for evaluating short-term changes in the response of microorganisms to substrate addition; this approach is therefore suitable for assessing transitions between K and r strategies.

1. Introduction

The input of easily available organic substances in soil may strongly change the turnover of native soil organic matter (SOM), i.e. cause priming effects (PE). As PE can be real (connected with altered SOM mineralization) or apparent (extra CO_2 due to microbial endogenous respiration), it is crucial to distinguish CO_2 produced from individual C pools when investigating PE mechanisms. Two mechanisms for the extra CO_2 production released during apparent priming effects have been suggested: the increased turnover of microbial biomass (Dalenberg and Jager, 1989; Wu et al., 1993; Degens

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and Sparling, 1995; Chander and Joergensen, 2001; Luna-Guido et al., 2001) and triggered activation of microbial metabolism (De Nobili et al., 2001). Apparent PE induced by glucose addition to soil has been demonstrated by relatively low (34 μg C g^{-1}, De Nobili et al., 2001) and relatively high (800 μg C g^{-1}, Dalenberg and Jager, 1989) rates of glucose application. Soil amendments with glucose caused a positive (Mary et al., 1993; Asmar et al., 1994; Dalenberg and Jager, 1989) or negative (Chapman, 1997; Kuzyakov and Bol, 2006) priming effect or no effect at all (Wu et al., 1993). One suggestion is that the acceleration or retardation of SOM decomposition depends mainly on the nutrient status of the soil and the C-to-N ratio of the active SOM pool (Kuzyakov et al., 2000; Kuzyakov, 2002). However, clear proof of this hypothesis has been missing until now.

One recent hypothesis regarding the mechanisms of the priming effect is based on the succession of microorganisms from r- to K-strategy after the exhaustion of the easily available components of fresh organic substances added to the soil (Fontaine et al., 2003). The common ecological concept of r- and K-selection classifies organisms according their competitive abilities (Pianka, 1970). One of the main fitness-determining phenotypic traits differentiating the K- and r-strategists is the maximum specific growth rate under uncrowded conditions with excess food (Andrews and Harris, 1986). Applying ecosystem ecology approaches to the microbial community level allows the dominating strategy to be estimated by using growth characteristics of soil microorganisms. r-strategists show quick growth on easily available substrates, whereas K-strategists use the resources more efficiently and grow slowly even in the absence of limitation. K-strategists have an advantage over r-strategists when the population density is close to the carrying capacity of the environment and when the increase in the population's specific growth rate is close to 0. K-strategists are able to degrade complex insoluble organic substances due to diversity of metabolic pathways, providing the minimal substrate flux necessary for slow growth. Since both types of microorganisms – r- and K-strategists – are abundant in soil, changes in growth rates of the whole population after adding easily available substrate can reflect the shift of domination of the two types. Fontaine et al. (2003) hypothesized that K-strategists outcompete r-strategists in the late stage of plant residue decomposition (when energy-rich compounds have been exhausted) and then start to decompose SOM more strongly than before plant residue addition. According to this hypothesis, adding easily available carbon (e.g. sugars) can not cause the real PE right after substrate addition by activating K-strategists decomposing SOM because r-strategists (which better take up available substrates) will dominate in the first hours and days. This microbial-strategy-based hypothesis remains to be proven experimentally. Hamer and Marschner (2005), who criticized this succession mechanism, also failed to check it experimentally. In our opinion, PE induced by a low amount of easily available substrates, such as glucose, is due to accelerated microbial biomass turnover, i.e. such PE is apparent. In this case the microbial community's higher specific growth rate is supposed to reflect the dominance of r-strategy features. Application of easily available substrates in high amounts, which activate and increase microbial biomass, should cause a more complicated effect. Furthermore, the rate and intensity of these dynamic changes clearly depend on the availability of nitrogen and other essential mineral nutrients.

Estimating kinetic parameters of microbial communities, such as apparent substrate affinity (K_s) and specific growth rate (μ) on added substrate are suitable tools to trace the shift and succession in the microbial growth strategy (Anderson and Gray, 1990; Blagodatsky et al., 1994; Bradley and Fyles, 1995; Panikov, 1995; Hopkins and Shiel, 1996). The kinetic approach developed to measure the substrate-induced growth response of soil microorganisms also allows the total and growing biomass of the glucose-consuming part of the microbial community to be determined (Panikov and Sizova, 1996).

We hypothesized that the observed PE were connected with changes in microbial growth kinetics and our goal was to relate the microbial growth strategy with the extra (primed) CO_2 evolved at high and low levels of available C and N application.

2. Materials and methods

2.1. Field site, soil sampling, and preparation

The Ap horizon of loamy Luvic Chernozem (C_{org} = 5.0%; N_{total} = 0.346%, pH_{H_2O} = 7.4) was sampled from 0 to 10 cm depth in an unfertilized plot of a long-term field experiment at the Institute of Sugar Beet (Ramon, Voronezh region, Russia). 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, samples were sieved (<5 mm) and fine roots and other plant debris were carefully removed. Twenty grams (dry weight) sub-samples were weighed out and put into 250-ml Schott-jars. The moisture was adjusted to 50% of the WHC, and then the soil was preincubated at 22 °C for 24 h.

2.2. Experiment design and glucose application

A 2 × 2 factorial experiment was established. The first factor was the amount of glucose added to the soil: 48.7 μg and 4.87 mg C glucose g^{-1} soil as aqueous solution for low (GL) and high glucose (GH) treatments, respectively. The high glucose concentration was approximately eight times higher than the microbial C content in the soil and close to the amounts applied in the SIR method (Anderson and Domsch, 1978), allowing the growth of microorganisms in all soil microsites during the first 1–2 days. The treatment was aimed at tracing possible changes in SOM decomposition after peak of microbial growth and substrate exhaustion. The low glucose concentration was two orders less than that in the high treatment and was sufficient only to activate microbial biomass, but not for its growth. Uniformly labeled ^{14}C glucose was added to the unlabeled glucose (7.0×10^6 DPM per jar) before being added to the soil for three of six replicate sub-samples.

The second factor was the N application. N as aqueous KNO_3 solution (GLN or GHN treatment) was added to the incubation vessels. The C-to-N ratio in added substrate was equal to 10. The volume of water added (2 ml) was calculated to reach 60% of WHC (water holding capacity).

Based on these treatments (GL, GLN, GH, GHN) we monitored: (1) CO_2 originating from the added glucose (as

$^{14}CO_2$), (2) unlabelled CO_2 originating from all other sources: microbial biomass and all pools of SOM, 3) the amounts of available C and N in the soil solution, and (4) the parameters of microbial growth kinetics.

2.3. Incubation and sampling

After adding glucose and N to the soil, 3 ml of 1 M NaOH in small vials were placed in the incubation jars to trap CO_2. The jars were then closed air tight and incubated for 14 days at 22 °C at 60% of WHC. Periodically (at 4, 10, 14, 21, 30, 38 h after glucose addition and thereafter daily), the vials with NaOH solution with absorbed CO_2 evolved from the soil were sampled and substituted by fresh vials with 3 ml portions of 1 M NaOH. Aliquots of sampled NaOH were used for measurement of ^{14}C activity and amount of trapped CO_2. After 14 h and 4 days of treatment with low glucose, and after 4, 6, and 14 days of treatment with high glucose addition, three replicates of incubation jars (with addition of the unlabelled glucose only) were used to estimate the microbial biomass and the kinetics of substrate-induced respiration (see Section 2.5). At the same time soil from another three replicates of incubation jars were used for chemical analyses.

2.4. Chemical analyses

After destructive sampling or at the end of the experiment, the soil was carefully mixed and the 10 g soil samples were extracted with 0.05 M K_2SO_4 in a 1:4 ratio. These extracts included exchangeable NH_4^+, NO_3^- and organic C and N (Hart and Nason, 1994). Application of 10-fold diluted K_2SO_4 solution (50 mM instead of 500 mM) does not significantly affect the amount of N measured (Appel, 1998), but allows the use of a Dimatoc-100 TOC/TIC analyzer (Dimatec, Germany) without dilution for C and N determination. Additionally, lower K content strongly reduce background of scintillation counting caused by natural ^{40}K. The second part of the soil sample was dried at 60 °C and ground in a ball mill (MM2, Fa Retsch) for ^{14}C residue analysis. The total C and N content of soil samples was determined using a CN auto-analyzer (RC 412, LECO, St Joseph, MI, USA).

CO_2 trapped in NaOH solution during the sampling was precipitated with 0.5 M $BaCl_2$ solution and then the excess of NaOH was titrated with 0.2 M HCl using the phenolphthalein indicator (Zibilske, 1994).

The ^{14}C activity collected as $^{14}CO_2$ in NaOH solution was measured in 2 ml aliquots added to 4 ml of the scintillation cocktail Rotiszint Eco Plus (Carl Roth, Germany) after the decay of the chemiluminescence. ^{14}C was measured using a Wallac 1411 Liquid Scintillation Counter (Wallac Oy, Finland). The ^{14}C counting efficiency was about 87% and the ^{14}C activity measurement error did not exceed 2%. The absolute ^{14}C activity was standardized by adding NaOH solution as a quencher to the scintillation cocktail and using the spectrum of an external standard (SQP(E) method). ^{14}C in solid soil samples was measured after combusting 1 g of sample within an oxidizer unit (Model 307, Canberra Packard Ltd., USA), the absorption of the $^{14}CO_2$ in the Carbo-Sorb E (Perkin Elmer, Inc., USA), and adding the scintillation cocktail Permafluor E$^+$ (Perkin Elmer, Inc.).

2.5. Microbial biomass and the kinetics of substrate-induced respiration

Soil microbial biomass-C in control unamended soil was determined using the initial rate of substrate-induced respiration (SIR) (Anderson and Joergensen, 1997) and recalculated according to the equation by Anderson and Domsch (1978):

$$\text{biomass-C}\ (\mu g\, g^{-1}\, \text{soil}) = (\mu l\, CO_2\, g^{-1}\, \text{soil}\, h^{-1}) \times 40.04 \quad (1)$$

The kinetic parameters of microbial growth response were estimated by the dynamics of the CO_2 emission from soil amended with glucose (Blagodatsky et al., 2000). Ten grams (dry weight) of soil was amended with a powder-mixture containing glucose (10 mg g^{-1}), talcum (20 mg g^{-1}), and mineral salts: $(NH_4)_2SO_4$–1.9 mg g^{-1}, K_2HPO_4–2.25 mg g^{-1}, and $MgSO_4{\cdot}7H_2O$–3.8 mg g^{-1}. After substrate addition, the soil samples were placed in triplicate into the respiration-apparatus, which consisted of 24 plastic tubes, to measure the rate of CO_2 production at 22 °C. Each sample was continuously aerated (300 ml min^{-1}), and the evolved CO_2 was measured every hour using an infrared detector and a mass-flow meter (Heinemeyer et al., 1989). Air-flow rate, CO_2 concentration, and standard error of CO_2 measurements were continuously monitored. Optimal glucose concentrations added to the soil (sufficient for unlimited exponential growth of microorganisms) were estimated in preliminary experiments with the same soil. The amount of mineral salts selected was based on the pH-values and buffer capacity of the soil, so that the pH changes were less than 0.1 units after addition.

Specific growth rate (μ) of soil microorganisms was estimated by fitting the parameters of the equation:

$$CO_2(t) = A + B\exp(\mu \times t), \quad (2)$$

to the measured CO_2 evolution rate ($CO_2(t)$) after glucose addition, where A is the initial respiration rate uncoupled from ATP generation, B the initial rate of the productive fraction of total respiration coupled with ATP generation and cell growth, and t is the time (Blagodatsky et al., 2000; Panikov and Sizova, 1996). The parameters of Eq. (2) were fitted by minimizing the least-square sum using Model Maker-3 software (SB Technology Ltd.). Three replicates of respiration curves were used for each sampling date and treatment. The fitting was restricted to the part of the curve that corresponded to unlimited exponential growth, as indicated by maximal values of r, F, and Q statistic criteria.

$r_{0,}$ the so-called physiological state index of microbial biomass at time zero (before substrate addition), was calculated from the ratio between A and B (Panikov and Sizova, 1996). The total glucose-metabolizing microbial biomass (sustaining + growing) is

$$x_0 = \frac{B}{r_0 Q_r}, \quad (3)$$

where the total specific respiration activity Q_r is

$$Q_r = \frac{\mu}{\lambda Y_{CO_2}}. \quad (4)$$

Biomass yield per unit of C–CO_2 (Y_{CO_2}) was assumed to be constant during the experiment and equals 1.5, corresponding to a mean value of 0.6 for the microbial yield per unit of glucose-C consumed. $\lambda = 0.9$ may be accepted as a basic stoichiometric constant (Panikov and Sizova, 1996). The growing (active) microbial biomass at time zero (less than the total biomass) is given by

$$x_0' = x_0 \cdot r_0. \quad (5)$$

The duration of the lag period (t_{lag}) was determined as the time interval between the moment of glucose addition and the moment when the increasing rate of growth-related respiration $B\exp(\mu \times t)$ becomes as high as the rate of respiration uncoupled from ATP generation A; it was calculated using the parameters of the approximated curve of the respiration rate of microorganisms by the equation:

$$t_{lag} = \frac{\ln(A/B)}{\mu} \quad (6)$$

The theory of the microbial growth kinetics was presented in detail earlier (Panikov, 1995).

2.6. Calculations and statistical analysis

Priming effects were calculated as the difference of the non-glucose-CO_2 from soil with glucose addition and CO_2 evolved from soil without glucose.

$$PE = \underbrace{(\text{total } CO_2 - \text{glucose-derived } CO_2)}_{\text{soil amended with glucose}} - \underbrace{\text{total } CO_2}_{\text{unamended soil}} \quad (7)$$

The glucose-derived CO_2 was calculated according to the specific ^{14}C activity of the added glucose. The priming effects are presented in two ways: (1) as mg C of extra CO_2 per g soil; (2) cumulative extra CO_2 expressed as a percentage of the cumulative CO_2 evolution from soil without glucose addition.

The experiment was conducted with three replicates for every treatment. ^{14}C data are presented as a percentage of ^{14}C input activity. Standard errors (S.E.) for CO_2 dynamic and PE effects, and standard deviations (S.D.) for microbial biomass and growth parameters, were calculated as a variability parameter.

The effect of glucose and nitrogen was assessed by two-way ANOVA with glucose treatment and nitrogen treatment as independent factors. When significant treatment effects were observed ($P < 0.05$), least significant differences ($LSD_{0.05}$) were used to separate means.

3. Results

3.1. Total and labelled CO_2 efflux as affected by glucose and N addition

The microbial respiratory response to substrate addition strongly depended on the glucose amount and N addition (Fig. 1, top). Low glucose amounts (with and without N) increased the microbial respiration rate only for the first 12–20 h (Fig. 1, top). The maximal CO_2 evolution rate was about two times higher in the GL than the GLN treatment. After adding glucose without N, the CO_2 evolution rates for both the GH and GL treatments peaked at nearly the same time and intensity. The decrease in CO_2 production, however, took much longer for the GH treatment (Fig. 1, top).

The rate of CO_2 efflux from soil amended with high amounts of glucose and N increased for more than 40 h and was 2.3 times more intensive than CO_2 efflux from soil treated with the same glucose amount but without N addition (Fig. 1, top). Hence, N was the limiting factor for the CO_2 evolution rate, when a high glucose amount was used.

Despite the different dynamics of CO_2 efflux rates from soil with and without N addition, the total amounts of CO_2 evolved during 13 days after the treatments with the equal glucose amounts were nearly the same (Fig. 1, bottom). Total CO_2 evolution from soil amended with a high glucose amount (after subtracting CO_2 from the control) was approximately 25 times greater than that from soil after GL treatment. The initial difference between high and low glucose inputs to the soil, however, was 100-fold. The cumulative total CO_2–C evolution after 13 days approached 50% of C at high glucose input, whereas at low glucose level the cumulated CO_2 efflux from glucose reached 90 and 208% of C input for treatments with and without N, respectively.

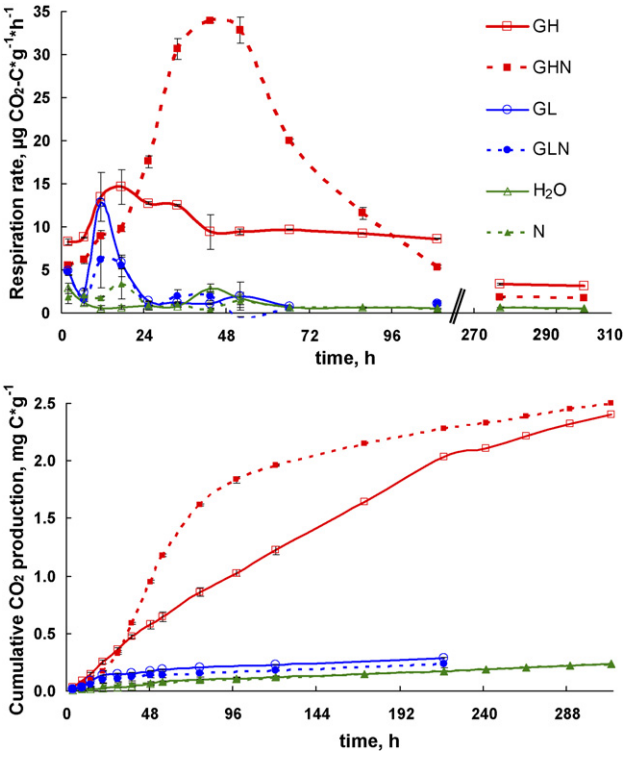

Fig. 1 – Total CO_2 efflux rate (top) and cumulative CO_2 production (bottom) after addition of low (GL) or high (GH) amounts of glucose or glucose and N (GLN, GHN) to the soil. Control treatments: water only (H_2O), water and KNO_3 (N). Bars show standard errors of the means ($n = 3$). Standard errors are not shown when less then the symbol size.

$^{14}CO_2$ efflux showed glucose decomposition, depending on the amount added and N availability. $^{14}CO_2$ efflux was maximal during the first 2–3 h after addition for treatments with low C input and after about 40 h for addition of high glucose amounts and N (Fig. 2, top). During a 9-day period, 25% of glucose added at low level was decomposed to CO_2, whereas 45 and 50% were respired in treatments with high glucose addition level without and with N, respectively (Fig. 2, bottom).

3.2. *Priming effects induced by glucose or/and N addition*

The production of unlabeled CO_2 originating from sources other than glucose was highest in soil with low glucose addition (Fig. 3, top and middle). The cumulative unlabeled CO_2 made up about 26–32% and 5–26% of C in microbial biomass for the treatments with low and high glucose, respectively (Fig. 3, middle). Starting from 14 h after glucose addition, cumulative production of unlabelled CO_2 induced by glucose was higher without than with nitrogen treatments (Fig. 3, middle). In GL and GLN treatments the unlabeled CO_2 efflux was significantly higher than that in the control treatment without glucose (Fig. 3, bottom). PE in GL and GLN treatments was positive and was most pronounced between 13 and 38 h after glucose addition (Fig. 3 bottom, Fig. 4, top). PE was insignificant during 2 (GHN) and 4 (GH) days after treatment, while further incubation even

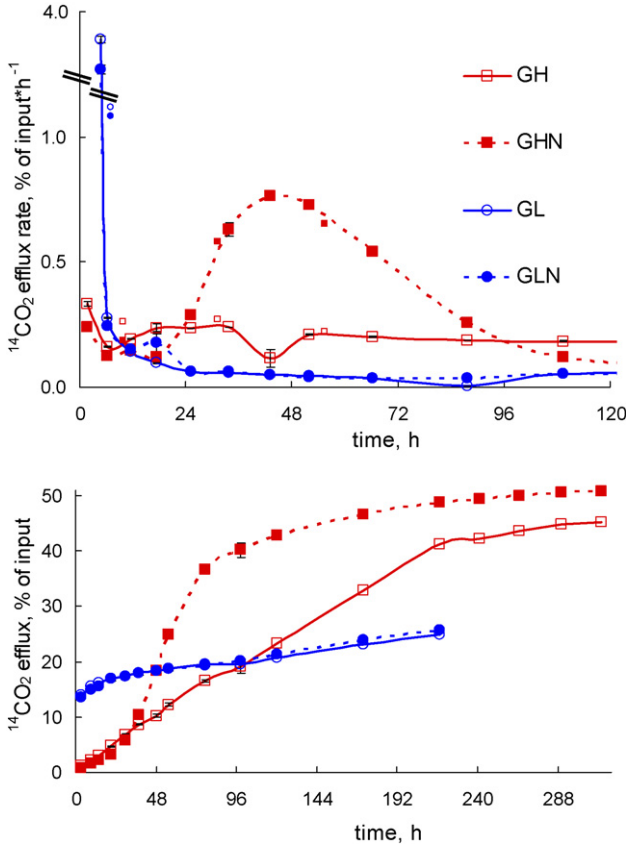

Fig. 2 – $^{14}CO_2$ efflux rate (top) and cumulative $^{14}CO_2$ production (bottom) after addition of low (GL) or high (GH) glucose amounts or glucose and N (GLN, GHN) to the soil. Bars show standard errors of the means (n = 3). Standard errors are not shown when less then the symbol size.

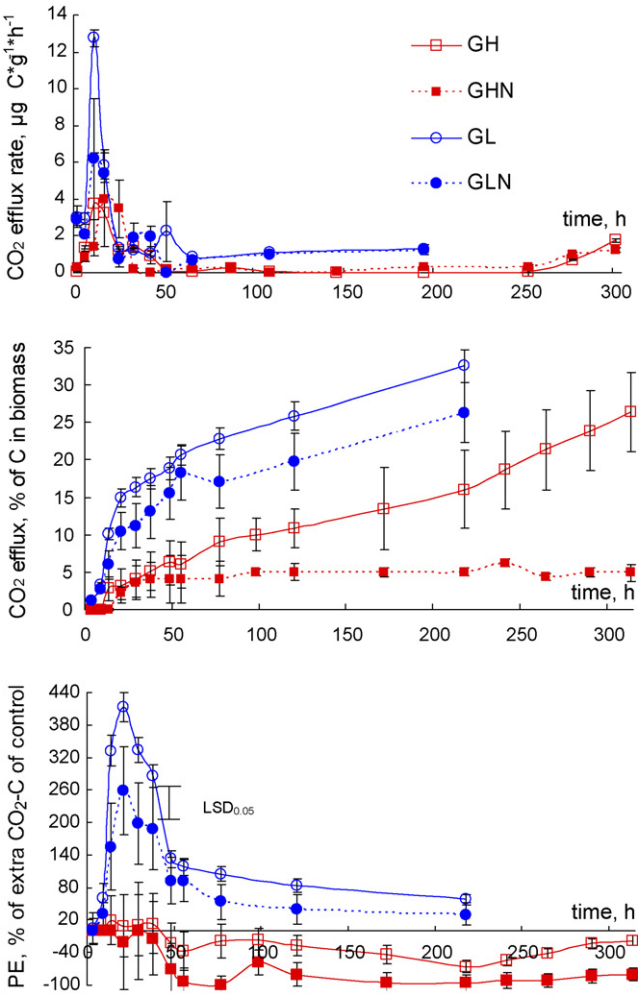

Fig. 3 – Unlabeled CO_2 efflux expressed as CO_2 evolution rate (top); as percentage of C in microbial biomass (middle); as priming effect in percent of cumulative extra CO_2–C of control (bottom). Low (GL) or high (GH) glucose amounts or glucose and N (GLN, GHN) were added to the soil. Bars show standard errors of the means (n = 3). $LSD_{0.05}$, the least significant differences between treatments.

yielded a negative PE in both GH and GHN treatments (Figs. 3 and 4, bottom).

3.3. *Changes in soluble C and N content in soil*

The addition of glucose and N at low rates did not change contents of C and N extracted from soils with 0.05 M K_2SO_4 compared to the control (Table 1). Most of the glucose (92.5%) added at a high rate (4872 µg C g^{-1}) without N was taken up by soil microorganisms before the 4th day after amendment (Table 1). This treatment resulted in a 52-fold excess of available C, accompanied by a 7-fold decrease in available N content, compared to the control. Soil amendment with glucose and N at high rates caused both K_2SO_4-extractable C and N to significantly increase during the 14 days after treatment (Table 1).

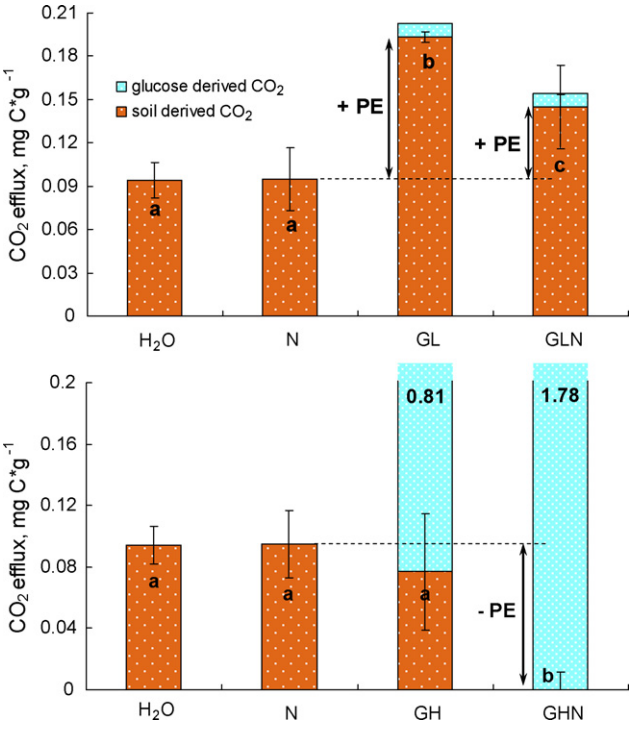

Fig. 4 – Partitioning of cumulative total CO_2 efflux (77 h) after addition of glucose at low rate (top, GL) and at high rate (bottom, GH) for CO_2 derived from glucose (based on ^{14}C) and from other sources. Control treatments: water only (H_2O), water and KNO_3 (N). Bars show standard errors of the means ($n = 3$). Values with the same letter are not significantly ($P < 0.05$) different from each other.

3.4. *Changes in microbial biomass and growth kinetics during the utilization of glucose*

Microbial biomass was determined by the SIR method only in control samples because the SIR technique is only applicable for soils not amended with available C (Anderson and Domsch, 1978; Sparling et al., 1981). Growing cells release more CO_2 per mass unit than non-growing cells, so the standard factor 40.0 recommended for mostly non-growing soil populations is not applicable for soil recently amended with available C. The approach based on microbial growth kinetics allows microbial biomass to be estimated in soils amended with available substrate (Blagodatsky et al., 2000). The values for total microbial biomass-SIR in control samples were not included in Table 1 since they did not changed during the experiment and amounted to 636 ± 39 and 635 ± 22 μg C/g at the first and 14th days of incubation, respectively. The biomass of the glucose-consuming part of the soil microbial community (kinetic approach) in control samples was approximately two times lower than total biomass determined by the SIR technique (Table 1).

To estimate microbial growth kinetics, glucose and nutrients were added to the sub-samples of incubated soils. This glucose addition exponentially increased the CO_2 evolution rate (Fig. 5), indicating microorganism growth after the lag-phase. Patterns of the exponential CO_2 evolution rate were

Table 1 – Microbial biomass, calculated by kinetic approach, growth characteristics and content of K_2SO_4-extractable C and N during incubation of soils treated with high and low rate of glucose

Treatment	Incubation time (days)	0.05 M K_2SO_4 extract				Lag-time (h)		μ_m (h^{-1})		Microbial biomass			
		C (μg C/g)		N, μg N/g						growing, % of total		total, (μg C/g)	
		No N	+N	No N	+N	No N	+N	No N	+N	No N	+N	No N	+N
Control		6.5 ± 0.7	6.6 ± 1.1	7.6 ± 0.7	141 ± 21	12.7 ± 0.2	14.1 ± 0.1	0.323 ± 0.007	0.330 ± 0.006	0.17	0.09	302 ± 65	262 ± 45
Glucose 48.7 (μg C/g)	1	8.6 ± 1.2	6.4 ± 1.0	8.0 ± 0.9	7.5 ± 0.9	12.6 ± 0.5	12.9 ± 0.2	0.335 ± 0.005	0.326 ± 0.003	0.16	0.15	278 ± 39	319 ± 27
	4	8.3 ± 1.2	7.7 ± 1.3	7.9 ± 2.3	9.6 ± 0.7	12.8 ± 0.2	12.6 ± 0.3	0.361 ± 0.005	0.358 ± 0.007	0.10	0.11	295 ± 34	282 ± 53
Glucose 4872 μg C/g	4	336 ± 60	20 ± 2	1.0 ± 0.3	98 ± 9	No growth	7.3 ± 0.6	No growth	0.226 ± 0.012	No growth	1.90	No growth	613 ± 69
	6	41 ± 5	28 ± 5	1.9 ± 0.5	119 ± 8	7.0 ± 0.3	4.2 ± 0.6	0.146 ± 0.007	0.246 ± 0.031	3.49	3.45	439 ± 85	502 ± 33
	14	17 ± 1	16 ± 1	1.0 ± 0.2	121 ± 6	5.9 ± 0.8	4.7 ± 0.9	0.262 ± 0.007	0.226 ± 0.006	2.07	3.30	321 ± 51	689 ± 7

Variants with no N (no N) and with 487 μg N (+N) are presented; ±standard deviation. Control values are given for the first day of incubation since no significant differences were observed between control values during the experiment.

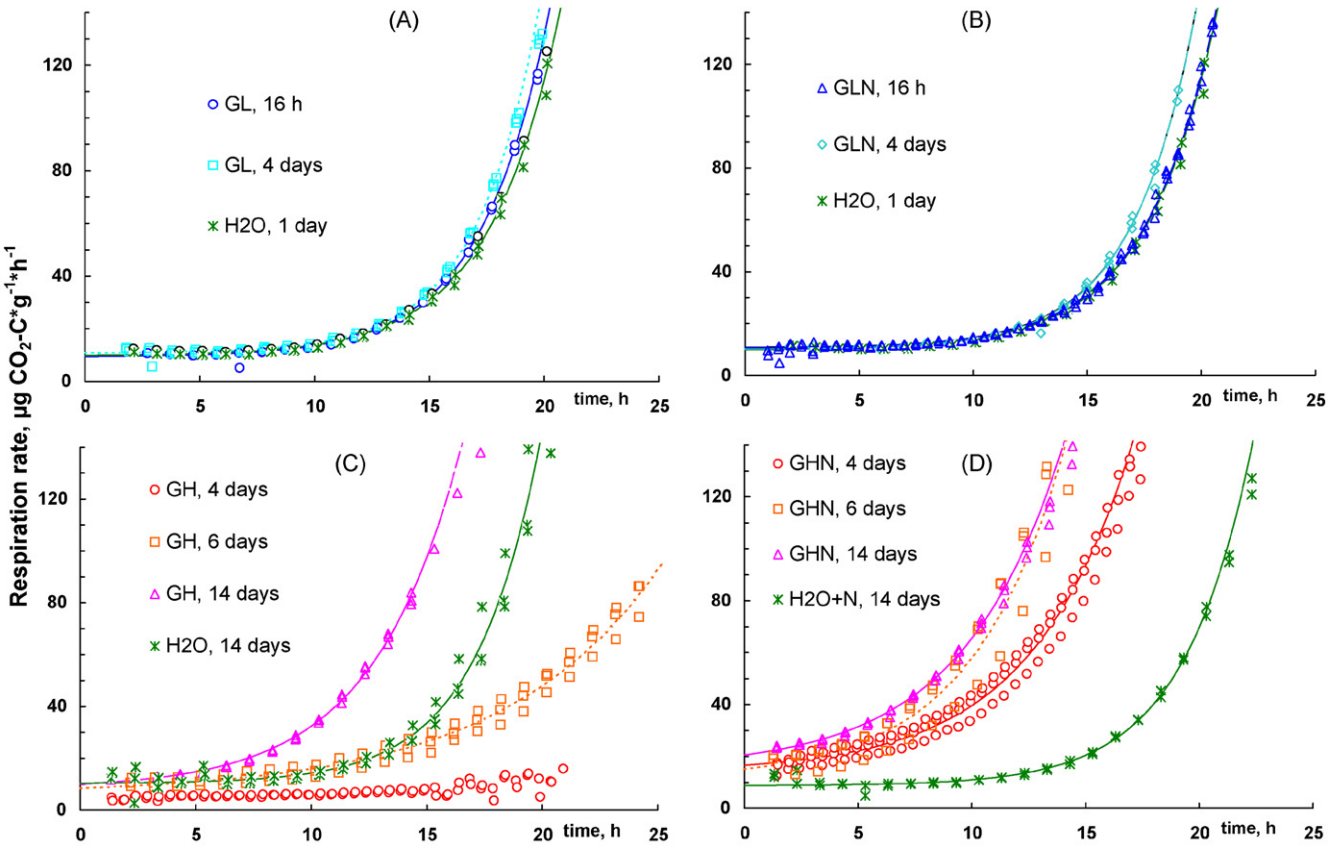

Fig. 5 – Substrate-induced respiratory response of the microbial community during incubation of soils treated with glucose at low rate (A); with glucose and N at low rate (B); with glucose at high rate (C); with glucose and N at high rate (D). The incubated soil was sampled for kinetic studies 16 h, 4 days, 6 days and 14 days after the glucose addition.

similar in soils with a low rate of glucose addition and in the control soil with only H_2O addition (Fig. 5A and B). Specific growth rates (μ) of microorganisms in soil with low glucose addition were slightly but significantly ($P = 0.05$) higher after 4 days of incubation compared to the control soil and to the soil after 16 h of incubation (Table 1). The effect of adding nitrogen on specific growth rate was not pronounced in GLN-treated soils (Table 1). Little glucose amount did not significantly changed the amount of microbial biomass or the duration of the lag-period compared with the control (Table 1).

At least for the first 4 days, the GH treatment suppressed the respiratory response to the subsequent addition of glucose and nutrients (applied to estimate microbial growth parameters) (Fig. 5C). The respiratory response pattern did not show pronounced exponential growth, indicating nutrient limitation (Nordgren, 1992). Indeed, the application of large glucose amounts (GH) at the beginning of the experiment activated soil microorganisms and led to rapid consumption of available N (Table 1) and other nutrients from the soil. Consequently, due to strong nutrient limitation, the glucose surplus added the second time (to estimate microbial growth kinetics) resulted in a poorly developed or virtually no exponential phase followed by a period of constant respiration until the glucose was consumed (Stotzky and Norman, 1961; Joergensen and Scheu, 1999; Ilstedt and Singh, 2005). Therefore, the growth parameters cannot be adequately estimated for high glucose treatment at that moment and are not presented in Table 1.

On the 6th day after GH treatment, the respiratory response to glucose added to estimate microbial growth kinetic parameters was well pronounced (Fig. 5C). A 10-fold difference between the contents of microbial and available C indicated the exhaustion of the initially added glucose (Table 1). The specific growth rate was lower by a factor of 2.2 compared to the control soil (Table 1).

Fourteen days after glucose addition, the μ values of the microbial community in GH-treated soil were 1.2 times lower than the initial μ values. The duration of the lag-period was gradually shortened during the incubation and, on the 14th day after glucose addition (GH), the lag time was twice as short as in the control (Table 1). The increase in the percentage of active (growing) microbial biomass after amending the soil with abundant glucose was approximately 20 times higher than both the control soil and the corresponding soil amended with little glucose (Table 1).

The initial respiratory response after applying the glucose-mineral nutrients mixture increased in the GHN treatment compared with the control soil or GH-treated soil (Fig. 5D). The

N addition with high glucose amounts resulted in a 2.3–2.6 increase of glucose-consuming microbial biomass (Table 1), as calculated by the kinetic approach. The GHN treatment was accompanied by a 2–3-fold decrease of the lag-period, a 20–38 fold increase of growing microbial biomass, and a 1.5-fold decrease of the specific growth rate compared to the control (Table 1).

4. Discussion

4.1. *Glucose applied at low rate: r-strategists activation and apparent positive PE*

The significant increase in microbial specific growth rates (μ) in soil amended with a low glucose amount (GL) as compared to the control soil (Table 1) is evidence of the dominance of r-strategy features in the microbial community of glucose amended soil. The shift to an r-strategy reflects the advantage of fast-growing microorganisms in competing for small amounts of easily available substrate added to the soil. Positive PE, i.e. stimulation of non-labeled CO_2 production from sources other than added ^{14}C-glucose occurred in this case.

Extra CO_2 production was found after addition of a low amount of available carbon (8% of microbial C) and comprised less than 20% of the C content in the microbial biomass. No increase in microbial biomass was observed after available C addition at a low rate. The extra CO_2 production coincided with the activation of the fast-growing part of the microbial community. As microbial biomass did not increase, we assume that the short-term extra CO_2 production was caused by acceleration of internal microbial metabolism. According to Dalenberg and Jager (1981), the accelerated decomposition of SOM is a real priming action, while the accelerated turnover of microbial C is an apparent priming effect. The latter can reflect the triggering of microorganisms by low amounts of glucose (Dalenberg and Jager, 1981, 1989; De Nobili et al., 2001). Adding glucose at low rates may initiate the switch from dormant to active state in soil microorganisms, increasing the maintenance energy requirements (Chapman and Gray, 1986; Anderson and Domsch, 1985), but without additional SOM decomposition. We compared two experiments with soil amendment with glucose at low rates (our one and the experiment by De Nobili et al., 2001) which differed in soil type (Chernozem and Chromic Luvisol), microbial biomass (636 and 330 μg C g^{-1}), added glucose amounts (48.7 and 34 μg C g^{-1}), and in extra CO_2 evolved (48.5 and 13 μg extra CO_2–C g^{-1}). In spite of such differences the amount of primed CO_2 per unit of microbial C was similar in both soils and was equivalent to 0.15 and 0.12% of C added per μg C_{mic} for Chernozem and Luvisol (De Nobili et al., 2001), respectively. These findings provide additional evidence for the apparent nature of PE in both experiments. So, at a low level of added available substrate, our results support the hypothesis that the supply of soluble and quickly utilizable carbon has no effect on actual SOM mineralization (Fontaine et al., 2003), and contradict the opinion of Hamer and Marschner (2005). However, longer monitoring of CO_2 and $^{14}CO_2$ efflux, and comparing these with the C in the microbial biomass, would be necessary to obtain a definitive answer.

4.2. *Glucose applied at high rate: K-strategists contribution and negative PE*

Glucose oversaturation of microorganisms in the GH treatment resulted in no growth on the 4th day. We assume that the growth response to additional glucose input was retarded at day 4 due to the strong N limitation, along with the excess of available C, as the C-to-N ratio in the K_2SO_4 extract on day 4 was 336 (Table 1). Therefore, the kinetic approach failed to estimate the real changes in specific growth rates μ, and was not suitable to evaluate growth strategies at that moment. Later, on 6th and 14th days microorganisms with r-strategy clearly failed to compete under strong mineral nutrient limitations, while K-strategists participated more in the glucose uptake, thus decreasing the measured specific growth rate of the whole soil microbial community (both K- and r-strategists). This assumption is also supported by the mechanism of glucose uptake by K-strategists (i.e. *Arthrobacter globiformis* – typical soil K-bacteria, Panikov, 1995): the glucose taken up is first stored as intracellular reserves without observable growth (absence of growth on the 4th day after treatment corresponded with this phase in our experiment, Fig. 5 C). Thereafter, the K-strategists would start to grow slowly, using the intracellular reserves, independent of the amount of available substrate in the soil.

The priming effect in soil after high glucose and N addition was close to zero or even negative (Fig. 3). Thus, microorganisms switched mainly from SOM decomposition to glucose utilization and did not utilize recalcitrant SOM in Chernozem soil, at least not during the 14 days after treatment. This mechanism has frequently been termed preferential substrate utilization (Cheng, 1999; Cheng and Kuzyakov, 2005). High N fertilization of arable loamy sandy soil caused a negative priming effect, i.e. lower soil organic carbon mineralization in a ^{14}C-labeling experiment (Liljeroth et al., 1990). Wu et al. (1993), however, found a positive priming effect in grassland soil with pH 5.2 after applying glucose at a rate similar to ours. This discrepancy can be explained by the lower C:N ratio in the applied substrate of the Wu et al. (1993) experiment. Clearly, further studies comparing priming effects and shifts in microbial strategies in soils with contrasting properties and SOM availability are needed.

The switch of microorganisms from SOM decomposition to glucose uptake, and the preferred uptake of the added C- and N-rich substrates are assumed to be mechanisms of real negative priming effects (Kuzyakov, 2002; Kuzyakov and Bol, 2006). Our assumption that the observed negative priming effect was real – and caused when microorganisms switch to growth on glucose in the GHN treatment – was supported by the respiratory growth kinetic. The microbial specific growth rate (μ) decreased after GHN treatment and did not increase again until day 14. The r–K competition was negligible under conditions of large C and N excess, and SOM-decomposing K-strategists switched to utilizing added glucose leading to a real negative PE. The involvement of K strategists to glucose decomposition decreased the average μ values of the whole microbial community as estimated by the kinetic approach. This is partly in agreement with Fontaine et al. (2003), who suggested that available C may be metabolized by K-strategists, but that r-strategists have an advantage in the r–K

competition immediately after adding available substrates to the soil. However, Fontaine et al. (2003) suggested that even if large amounts of energy and nutrients are supplied, K-strategists may not have enough time to assimilate them because they grow too slowly compared to r-strategists. Our results revealed that no r–K competition occurred because of the large excess (compared with microbial biomass-C) of glucose and N in the soil. Under such conditions, not only r-strategists but also K-strategists participate in glucose uptake and utilization, causing decrease in the specific growth rates of the whole microbial community. Therefore, the specific growth rates were lower compared to μ after a GL treatment, where mainly r-strategists consumed the glucose.

We are aware that applying glucose or other easily available substrates (CLPP approach) to estimate microbial growth parameters does not assess responses by all soil microorganisms. On the other hand, molecular approaches estimating the diversity of soil microbial communities (16S rDNA-DGGE, PLFA) are inadequate for quantification of microbial growth rates. Therefore, molecular approaches can be used together with the quantitative analysis of microbial growth kinetics, which is a suitable tool for explaining the growth strategies of microorganisms. Other physiological approaches are also available to estimate microbial growth strategies, i.e. estimation of the saturation constant (K_s) for the microbial community (Blagodatsky et al., 1994; Bradley and Fyles, 1995). When the assumed differences in the substrate use efficiency of r and K strategists are considered, the approaches based on respiratory quotient may be useful as well (Randerson et al., 2006). Thus, the combination of isotopic approaches (for functional aspects) with molecular methods (for community composition) (Liebich et al., 2006), along with the tracing of respiratory quotient changes, are very promising strategies to elucidate the connection between microbial growth strategies and induced priming effects.

4.3. *Substrate use efficiency and priming mechanisms*

As estimated by ^{14}C in trapped CO_2, 25% of glucose added at a low rate and 45–50% of glucose added at high rate were mineralized at the termination of our experiment (218 and 314 h, respectively) (Table 2). Our results *prima facie* support lower substrate use efficiency with higher glucose application (Witter and Kanal, 1998), when calculated based on ^{14}C. The comparison with total respiration, however, showed that estimation of substrate use efficiency based on

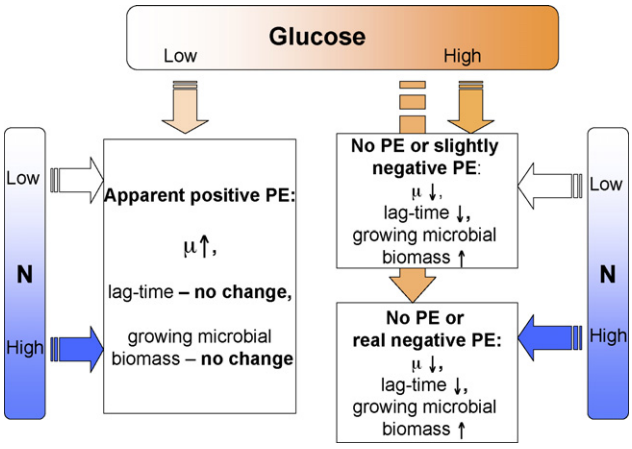

Fig. 6 – Priming effects (PE) and microbial growth parameters in dependence on the availability of glucose and N.

percentage of ^{14}C-glucose mineralized to $^{14}CO_2$ is misleading for GL treatments, since CO_2 emission from endogenous sources exceeded glucose C input by 25 to 136% (Table 2). The apparent PE observed under C-limiting conditions after the addition of little glucose can be explained by the ''signal'' or ''triggering'' effect. This activated more microorganisms than expected after consumption of such an amount of glucose (Stenström et al., 2001; De Nobili et al., 2001).

Our estimation of the proportion of ^{14}C–CO_2 evolved from glucose was very close to that by Šantruckova et al. (2004). They found 27% of ^{14}C evolved as CO_2 after 72 h of aerobic incubation of soil with 315 μg glucose-C g^{-1}. Our values, estimated based on ^{14}C (25 and 47%), also agreed with those reported by Bremer and Kuikman (1994) for sandy loam soil: 30 and 42% for low (36 μg C g^{-1}) and high (2304 μg C g^{-1}) glucose amendments, respectively.

Depending on application level and N availability, glucose addition to soil has three potential consequences: positive priming effect (GL, GLN), absence of, and negative priming effects (GH, GHN) (Figure 6). The ''direction'' of PE depended on the level of added C and N in relation to microbial biomass-C. Positive apparent PE was observed when the amount of glucose-C was 13 times lower than the C content of the microbial biomass and was insufficient for microbial growth. Under such limiting conditions the glucose would only activate microbial biomass, inducing greater respiratory

Table 2 – Cumulative CO_2, primed CO_2 and fraction of C mineralized to CO_2 as assessed by labeled and unlabeled CO_2 efflux at termination time of the experiment; ±standard error

Treatment	Cumulative CO_2			Extra (primed), CO_2	Fraction of glucose mineralized	
	^{14}C-labeled (μg CO_2–C g^{-1})	Total (μg CO_2–C g^{-1})	From control (μg CO_2–C g^{-1})		$^{14}CO_2$–C (%)	$^{12}CO_2$–C (%)
GL	12.2 ± 0.1	288 ± 9	173 ± 12	103	25	236
GLN	12.5 ± 0.1	235 ± 30	174 ± 16	48.5	25.6	125
GH	2207 ± 58	2400 ± 28	234 ± 12	−41	45.3	44.5
GHN	2475 ± 78	2500 ± 33	232 ± 19	−201	50.8	46.6

losses from the C reserves (125–236% of added glucose-C) which may be termed as triggering and were associated with the reaction of r-strategists within the microbial community. The absence of a priming effect was observed at an 8-fold excess of glucose-C compared to microbial biomass-C under N-limiting conditions. If the amount of easily available C and mineral nutrients was sufficient for microbial growth, then the growth was not associated with SOM decomposition (negative PE), at least during the first 2 weeks after substrate addition. Large excess of glucose with N addition caused r–K competition to cease and the K-strategists to switch their metabolism to glucose uptake. Partial contribution of K-strategists to glucose utilization decreased the weighted specific growth rate of microbial community.

4.4. *Conclusions*

Priming effects observed in our study were related with changes in microbial growth kinetics as induced by different levels of glucose and N addition. Acceleration of SOM decomposition (real positive PE) was not observed in our experiments both for low and high levels of glucose application. If the amount of glucose was insufficient for microbial biomass increase then microorganisms with r-strategy within the microbial community were activated by the glucose and extra CO_2 was produced by accelerated microbial metabolism (apparent positive PE). If the amount of glucose C and mineral N was in excess, then both K and r-strategists started to grow. This switch of K strategists from SOM decomposition on glucose utilization led to negative PE (preferential substrate utilization).

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