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Contrasting effects of glucose, living roots and maize straw on microbial growth kinetics and substrate availability in soil

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Summary

The most modern molecular approaches fail to link structure of soil microbial community with its functions. We used classical physiological approaches based on (i) microbial growth kinetics and (ii) microbial affinity to the substrate to show the shift in functional properties of the soil microbial community after amendments with substrates of contrasting availability. Kinetic parameters of substrate-induced respiration and substrate availability in soil were monitored during the growth of maize, and decomposition of glucose or maize straw. Input of small amounts of easily available substrates (i.e. glucose or root exudates) significantly increased the specific growth rates of soil microorganisms by up to 13 and 20%, respectively. This increase, showing the shift from K to r strategies, was confirmed by a 50% decrease in the affinity of microorganisms to the easily available substrates. In contrast, maize straw lowered specific growth rates by 16–30% and increased the affinity of microorganisms to the substrate by 23–131% compared with untreated soil. After maize straw addition, the shift of the microbial community to K strategy was accompanied by an increase in the fraction of microbial biomass responding to glucose addition by immediate growth. The generation time of this fraction was 1.8 to 2.8 hours, which was 100 to 1000 times faster than that of the whole microbial community. Easily available carbon (C) in soil amended with maize straw comprised only half of that extracted by 0.05 M K_2SO_4. Therefore, we conclude that C extracted by 0.05 M K_2SO_4 from the soil with maize straw residues had a lower availability for microorganisms as compared with glucose. Adding N to the soil prolonged the intensive mineralization period of plant residues, decreased specific microbial growth rates and increased the amount of easily available C in the soil. This indicates a more efficient use of maize residues by K-selected microorganisms after the removal of N limitation. Combination of two complementary physiological approaches based on microbial growth kinetics and substrate affinity showed contrasting effects of easily and less available substrates on the shift of growth strategies (r vs. K) of the whole microbial community. These approaches are also suitable for estimation of microbial availability of indigenous C in the soil.

Introduction

Increased interest in carbon (C) sequestration in soil has prompted the investigation of the mechanisms, rates and triggers of soil organic matter (SOM) decomposition *in situ*. Apart from environmental conditions (temperature, moisture, oxygen availability, pH, etc.), the main factor limiting decomposing activity of soil heterotrophic microorganisms is energy supply with fresh organic matter (i.e. plant residues and root exudates). Thus, increase in the SOM decomposition rate stimulated by supply of energy-rich substrates (i.e. a positive priming effect) (Kuzyakov *et al*., 2000), may affect long-term C sequestration. However, the priming effect caused by addition of soluble energy-rich substances such as glucose has not been accompanied by increased mineralization of recalcitrant SOM (Dalenberg & Jager, 1989). This phenomenon was designated as an 'apparent' priming effect (Blagodatskaya *et al*., 2007).

Fontaine *et al*. (2003) and Fontaine & Barot (2005) put forward a theory linking the 'real' priming effect (i.e. acceleration of recalcitrant SOM turnover) with development of

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K-strategists in the competition between microbial functional types. This approach is based on the common view linking microbial community structure and substrate availability (Winogradsky, 1952; Panikov, 1995; Morris & Blackwood, 2007). It is believed that ample quantities of easily available (mainly water-soluble) substrates are quickly consumed by fast-growing microorganisms with enzymes of low substrate affinity (i.e. r-strategists). The slow-growing K-strategists with enzymes of high substrate affinity are better adapted for growth on substrates of low availability. Thus, the most pronounced real positive priming action (i.e. acceleration of recalcitrant SOM decomposition) should occur at a later stage of decomposition of fresh plant residues when species with K-strategy features dominate in the population.

There is a lack of experimental studies confirming or rejecting the hypothesis that relates functional properties of soil microbial community (specific growth rate (μ) or substrate affinity of microbial enzymes (K_s)) with substrate quality or decomposition rate of SOM. This is because (i) direct measurements of μ_m and K_s in pure cultures cannot be transferred to real process rates and microbial interactions in soil, and (ii) there is an absence of direct methods for satisfactorily estimating microbial growth *in situ*.

There are at least two indirect approaches suitable for (i) estimation of microbial specific growth rates (μ) based on kinetic analysis of substrate-induced respiration (Blagodatsky *et al*., 2000; Stenström *et al*., 2001), and (ii) determination of substrate affinity of microbial enzymes (K_s) and maximal mineralization rate (V_{max}) based on a modified Wright & Hobbie (1966) equation relating respiratory response rates to increasing substrate concentration (Sikora & McCoy, 1990; Panikov *et al*., 1992). These approaches provide information concerning the overall microbial community.

Kinetic parameters of microbial growth 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 community (Blagodatsky *et al*., 1994; Blagodatskaya *et al*., 2007). Comparative analyses of the amounts of available substrate in soil (S_n) and apparent substrate affinities (K_s) have been completed for rhizosphere and bulk soils (Bradley & Fyles, 1995b) as well as for soils under contrasting management and fertilizer systems (Anderson & Gray, 1990; Sikora & McCoy, 1990; Blagodatsky *et al*., 1994; Hopkins & Shiel, 1996; Blagodatskaya *et al*., 2001). It is unclear, however, how these microbial characteristics change during mineralization of substrates with different composition and availability in relation to the nutrient status of the soil and the C/N ratio of the applied substrate. Assessment of functional characteristics of soil microbial communities developing after application of substrates with different availability may support (or reject) theories linking the decomposition rate of SOM with features of microbial biomass.

The aim of the present study is to compare the specific growth rate and substrate affinity constant of soil microorganisms during mineralization of simple and complex organic compounds at different levels of nitrogen (N) amendment. Glucose and root exudates of *Zea mays* L. permanently exuded by growing roots into the rhizosphere were studied as easily available substrates. Plant residues of *Zea mays* L. simulated the input of substrate with lower availability. We also analysed the effect of applied substrates on other microbial characteristics derived as parameters of a modified Monod equation (i.e. heterotrophic potential (V_{max}) and the amount of available for mineralization substrate S_n).

Materials and methods

Soil sampling and preparation

The Ap horizon of a loamy Luvic Chernozem (C_{org} = 5.0%; N_{total} = 0.346%, pH (H_2O) 6.1) was sampled from 0–10 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-gram (dry weight) sub-samples were weighed and put into 250-ml Schott-jars or 50 ml plastic vessels designed for growing plants. The moisture content was adjusted to 50% of the water holding capacity (WHC), and then the soil was pre-incubated at 22°C for 24 hours.

Experiment design and substrates application

Three substrates of decreasing microbial availability were added to the soil: (i) glucose, (ii) exudates of living roots, and (iii) plant residues. Glucose was applied to the soil either with or without N (G and GN treatments) at 50 μg C g^{-1} soil and comprised about 0.1% of soil organic C content. This is *c*. 5 to 10 times larger than the daily C input into soil through root exudation (Kuzyakov & Domanski, 2000). Seeds of *Zea mays* L. were placed into plastic vessels with soil and incubated for 14 days until plants were *c*. 30 cm tall (R treatment) when the dry weight of the roots was 0.2 g. Plant residues are much less easily decomposed than glucose. Therefore, the residues (ground leaves) of *Zea mays* L. (MR and MRN treatments) were added at a greater rate (8 mg g^{-1}) and were thoroughly mixed with the soil. Both glucose and residue amendments were with (GN and MRN, 5 and 400 μg N g^{-1} soil, respectively) or without (G and MR) N application. The latter treatments were included to check how N limitation of microbial growth could modify the kinetic characteristics and thus functional properties of microbial community. Distilled water or N as aqueous KNO_3 solution were added to the incubation vessels just after glucose or maize residues were added. The volume of water added (2 ml) was calculated to reach 60% of water holding capacity (WHC).

Incubation and sampling

After adding glucose and plant residues to the soil, 3 ml of 1 M NaOH in small vials was placed in the incubation vessels to trap CO_2. The vessels were then closed air-tight and incubated for 14 days at 22 °C at 60% of WHC. At 4, 10, 14, 21, 30 and 38 hours after glucose addition and thereafter daily, the vials were removed and replaced by vials with another 3 ml aliquot of 1 M NaOH. After 16 hours and 4 days of treatment with glucose, and after 4, 6, 14 and 20 days of treatment with plant residue addition, three replicate incubation vessels were used to estimate the kinetics of substrate-induced respiration (see below). At the same time, the soil from another three replicate incubation vessels was used for chemical analyses and for estimation of mineralization activity by the Wright & Hobbie (1966) method.

Microbial biomass and the kinetics of substrate-induced respiration

The kinetics of substrate-induced respiration (KSIR) has been described according to the model proposed by Panikov & Sizova (1996). The model simulates the transition of soil microorganisms from maintenance to an active state, including both the lag and exponential growth phases. The KSIR approach helps to estimate the specific growth rate (μ) as well as the sustaining and growing fractions of microbial biomass (Panikov, 1995; Blagodatsky *et al*., 2000).

Ten grams (dry weight) of soil were amended with a mixture containing glucose (10 mg g^{-1}), talcum (20 mg g^{-1}) and mineral salts (i.e. $(NH_4)_2SO_4$, 1.9 mg g^{-1}; K_2HPO_4, 2.25 mg g^{-1}; $MgSO_4{\cdot}7H_2O$, 3.8 mg g^{-1}). After substrate addition, the soil samples were placed in triplicate into an ADC2250 24-Multichannel Soil Respiration System (ADC Bioscientific Ltd, Great Amwell, UK), which consisted of 24 plastic tubes, to measure CO_2 production rate 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 microorganism growth) 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

$$CO_2(t) = A + Bexp(\mu t), \quad (1)$$

to the measured CO_2 evolution rate ($CO_2(t)$) after glucose addition, and where A is the initial respiration rate uncoupled from ATP production, B is the initial rate of the growing fraction of total respiration coupled with ATP generation and cell growth and t is time (Panikov & Sizova, 1996; Blagodatsky *et al*., 2000).

r_0, the physiological state index of microbial biomass at time zero (before substrate addition), was calculated from the ratio between A and B (Panikov & Sizova, 1996). The total glucose-metabolizing microbial biomass (sustaining + growing) is:

$$x_0 = \frac{B}{r_0 Q_r}, \quad (2)$$

where the total specific respiration activity Q_r is:

$$Q_r = \frac{\mu}{\lambda\, Y_{CO_2}}. \quad (3)$$

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

$$x'_0 = x_0 r_0. \quad (4)$$

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

$$t_{lag} = \ln(A/B)/\mu. \quad (5)$$

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

Measuring mineralization activity by the Wright & Hobbie method

Additional characteristics reflecting mineralization activity of microbial biomass can be obtained by measuring initial rates of soil respiration after applying increasing amounts of easily available substrate such as glucose. The maximum mineralization rate V_{max}, the substrate affinity constant K_s, the amount of endogenous soil substrates with availability similar that of the added substrate (S_n) and turnover time (T_t) are derived from the model based on Monod kinetics. This approach is known in aquatic microbiology as the Wright & Hobbie method (1966) and has been modified for application for soil conditions (Ferroni *et al*., 1985; Panikov *et al*., 1992).

Subsamples of soil from incubation jars (1 g) were placed in 15 ml glass vials. Then 200 µl of ^{14}C(U)D-glucose solutions was added at a rate of 15–600 µg C g^{-1}, corresponding to an activity of 5.8×10^3 Bq. The appropriate concentration interval was determined for each treatment in preliminary experiments (data not shown). Immediately after adding labelled glucose, the glass vials were tightly sealed with plastic stoppers provided with folded strips of filter paper saturated

with 200 µl of 1 M NaOH. The soil was then incubated at 22°C for 20 minutes before $^{14}CO_2$ measurements.

The $^{14}CO_2$ production rate (v) depends on concentration of applied labelled glucose (S) as described by the model based on Monod kinetics (Panikov *et al.*, 1992):

$$v = V_{max}S/(K_s + S_n + S), \quad (6)$$

where V_{max} is the maximum mineralization rate or heterotrophic potential, $K_s + S_n$ is the sum of the enzyme saturation constant and concentration of soil indigenous C equivalent to glucose.

The turnover time T_t of soil substrate equivalent to glucose is the total period needed by the microbial population to metabolize S_n and was estimated by the following equation:

$$T_t = (K_s + S_n)/V_{max}. \quad (7)$$

The quantity of S_n was then calculated using the basal respiration rate v_0 (i.e. respiration rate of soil not supplied with labelled substrate) as:

$$S_n = T_t v_0. \quad (8)$$

The saturation constant values (K_s) were obtained after subtraction of S_n estimated by Equation (8) from the sum ($K_s + S_n$) estimated by Equation (6).

Analyses

After destructive sampling or at the end of the experiment, the soil was carefully mixed and 10 g soil were extracted with 0.05 M K_2SO_4 in a 1:4 ratio for exchangeable NH_4^+, NO_3^- and organic C and N. Total C and N content of soil samples was determined using a CN auto-analyser (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. The excess NaOH was then titrated with 0.2 M HCl using the phenolphthalein indicator (Zibilske, 1994).

The ^{14}C activity collected as $^{14}CO_2$ and adsorbed by paper strips impregnated with NaOH was measured in 4 ml of the scintillation cocktail Rotiszint Eco Plus (Carl Roth, Karlsruhe, Germany) after decay of the chemiluminescence. ^{14}C was measured using a Wallac 1411 Liquid Scintillation Counter (Wallac Oy, Turku, Finland). The ^{14}C counting efficiency was *c.* 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).

Statistics

The experiment was conducted with three replicates for every treatment. Standard errors (SE) for CO_2 dynamics, and standard deviations (SD) for microbial biomass and growth characteristics, were calculated as a variability parameter. The significant effects of soil treatments were assessed by one-way ANOVA at $P < 0.05$.

The parameters of Equation (1) or (6) were fitted by minimizing the least-square sum using ModelMaker© Version 3.0.3 software (ModelMaker, 1997). Three replicate respiration curves were used for each sampling date and treatment when parameter optimization was made for Equation (1). Fitting was restricted to the part of the curve that corresponded better to applied model Equation (1), as indicated by maximum values of statistic criteria: r^2, the fraction of total variation explained by the model defined as ratio of model weighted sum of squares to total weighted sum of squares. Further goodness of fit estimations were made and based on Q value derived from χ^2 (ModelMaker, 1997) to show that the probability that the difference between the model and the data is pure chance and not a fault in the model. Small values of Q indicate that the model fails to describe the real process.

Results

CO_2 efflux as affected by C source and N addition

Microbial respiratory response to substrate addition strongly depended on the substrate type and N addition (Figure 1). Glucose application (with and without N) increased the microbial respiration rate only for the first 12 to 20 hours (Figure 1a). The maximum CO_2 evolution rate in the G treatment was *c.* twice as great as in the GN treatment. Glucose was exhausted in both treatments after 1 day and had no further direct effect on CO_2 efflux. Addition of maize residues (MR) also increased the CO_2 efflux from soil, but this increase lasted much longer compared with glucose addition. The N addition to the soil with maize residues (MRN) shifted the time of maximum CO_2 evolution from day 3 to day 6 (Figure 1b). However, the maximum CO_2 evolution rates did not depend on N availability in this case.

For both substrates added as a pulse (i.e. glucose and maize residues) there was two mineralization periods: (i) intensive mineralization in the first 20 hours for glucose and 3 to 9 days for MR, and thereafter (ii) slow mineralization after exhaustion of the initial glucose or available compounds of maize residues.

The cumulative CO_2 released after glucose addition (i.e. CO_2 evolved from amended soil minus CO_2 evolved from control treatments) during 14 days reached 122 and 226% (± 10%) of C input for G and GN treatments, respectively. Thus, more CO_2-C was evolved than glucose-C added (i.e. a positive priming effect was induced by glucose). The cumulative CO_2 production induced by maize residues over 14 days was 13 and 25% of maize-C input for MR and for MRN treatments, respectively.

Changes in soluble C and N content in soil

Twenty-four hours after glucose addition, the content of K_2SO_4-soluble C in glucose-amended soil exceeded soluble C in the

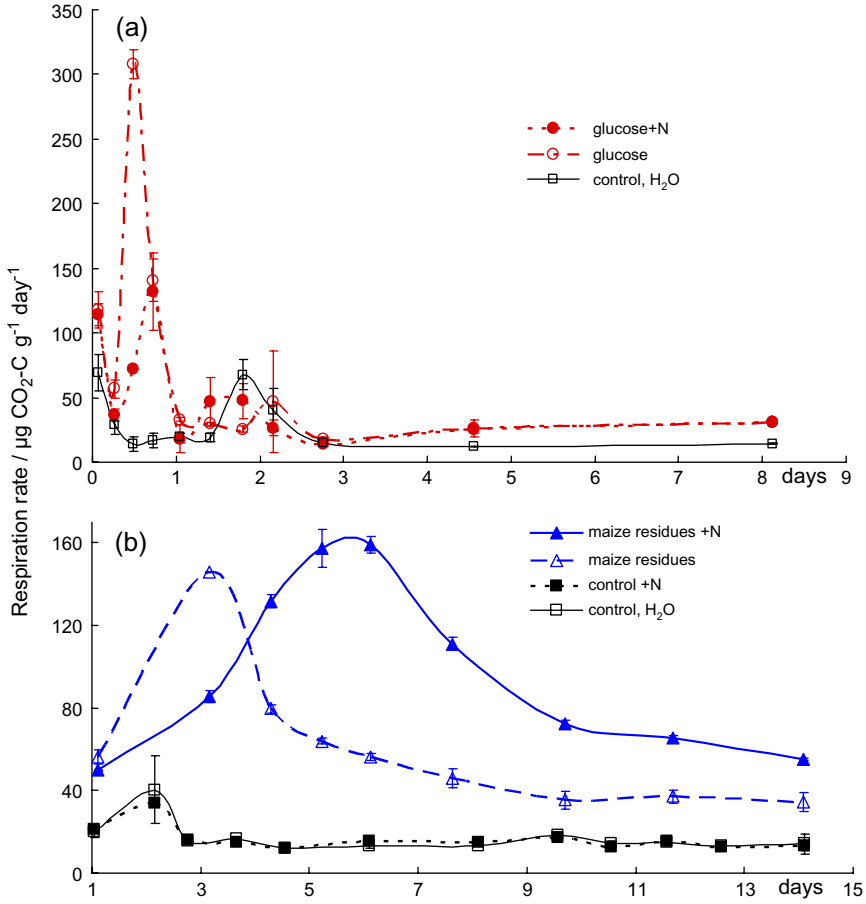

Figure 1 CO_2 efflux rate after soil amendment with glucose and N (a) and with maize residues (b). Control treatments: water only (H_2O), water and KNO_3 (N). Bars show standard errors of the means ($n = 3$). Standard errors are not shown when covered by the symbol.

soil without glucose by 2 µg C g^{-1} (Table 1), indicating that 96% of applied glucose was consumed by microorganisms during the period of intensive mineralization. No differences between soluble C content in control soil and in soil amended with glucose and N were observed (i.e. N application led to a more complete C consumption). Growing plants enriched the soil with K_2SO_4-soluble C by a factor of 1.8. In contrast, the extractable N content in soil with maize plants was smaller by a factor of seven than that in control soil (Table 1). This indicates intensive N uptake by the roots and strong N limitation. During the period of intensive mineralization of maize residues (3–5 days after addition), K_2SO_4-soluble C increased by seven to eightfold and soluble N increased by 4.4 and 1.6 times for treatments without and with N, respectively (Table 1).

During the slow mineralization period (i.e. after 14 days of incubation) the amount of soluble C in soils amended with maize

Table 1 C and N extractable with K_2SO_4 during incubation of soils with glucose (G), living roots exudates (R) and maize residues (MR) and with (+) or without (−) N; ± denotes standard error. The amount of nitrogen added to 'control treatment with N' corresponds to the N amount applied in MRN treatment

		0.05M K_2SO_4 extract			
		C /µg (g soil)$^{-1}$		N /µg (g soil)$^{-1}$	
Treatment	Period of substrate mineralization	−N	+N	−N	+N
Control		6.6 ± 0.7	6.6 ± 1.1	7.6 ± 0.7	141 ± 21
G	Intensive	8.6 ± 1.2	6.4 ± 1.0	8.0 ± 0.9	7.5 ± 0.9
G	Slow	8.3 ± 1.2	7.7 ± 1.3	7.9 ± 2.3	9.6 ± 0.7
R	Intensive	11.4 ± 1.5	ND	1.1 ± 0.6	ND
MR	Intensive	47.5 ± 4.6	51.3 ± 5.3	33.6 ± 4.9	222 ± 21
MR	Slow	11.4 ± 1	15.9 ± 0.9	0.6 ± 0.2	166 ± 8

residues decreased significantly, but still exceeded the control treatment by a factor of 1.8–2.5. The soluble N content in the MR treatment was 10 times smaller than in controls, showing extreme N limitation (Table 1), while no N limitation occurred in the MRN treatment during the whole incubation period.

Analysis of kinetic respiration curves

Nine to 14 hours after addition, the added glucose with nutrients caused an exponential increase in the CO_2 evolution rate (Figure 2), indicating microbial growth after the lag-phase. The microbial growth kinetics depended on substrate availability and the period after the substrate addition.

Respiration rates increased more steeply in both glucose and root exudate treatments than in the controls (Figure 2a). Parameterization of Equation (1) revealed the response of fast-growing microorganisms to available substrate addition: significantly greater specific growth rates (μ) were obtained for soil amended with available substrates than in soil without any additions (Figure 3a). Permanent substrate input from growing maize roots yielded significantly greater μ-values than did glucose-treated soils. Specific growth rates increased significantly during the period of slow mineralization compared with the period of intensive glucose mineralization (Figure 3a). The percentage of growing biomass, however, was smaller during slow compared with intensive mineralization (Table 2). No significant changes in the duration of the lag-period and in total glucose consuming biomass were found between control and treatments with addition of glucose or root exudates (Table 2).

The initial CO_2 evolution rates after adding maize residues were greater, while respiration curves sloped more gently compared with unamended soil (Figure 2b,c). Specific growth rates

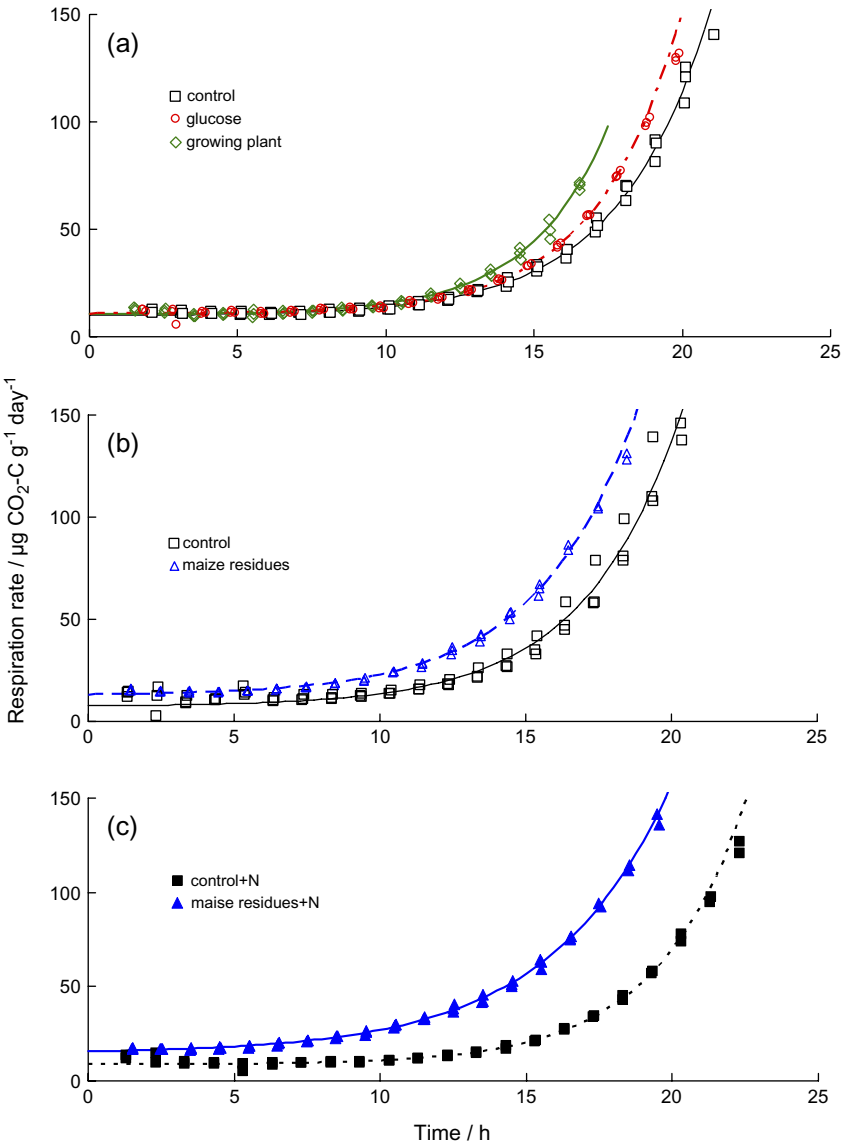

Figure 2 Glucose-induced respiratory response of microbial community during period of slow mineralization of substrate added to soil: glucose and root exudates (a); maize residues (b); maize residues and N (c). Experimental data are shown as symbols and model simulation (Equation 1) as curves.

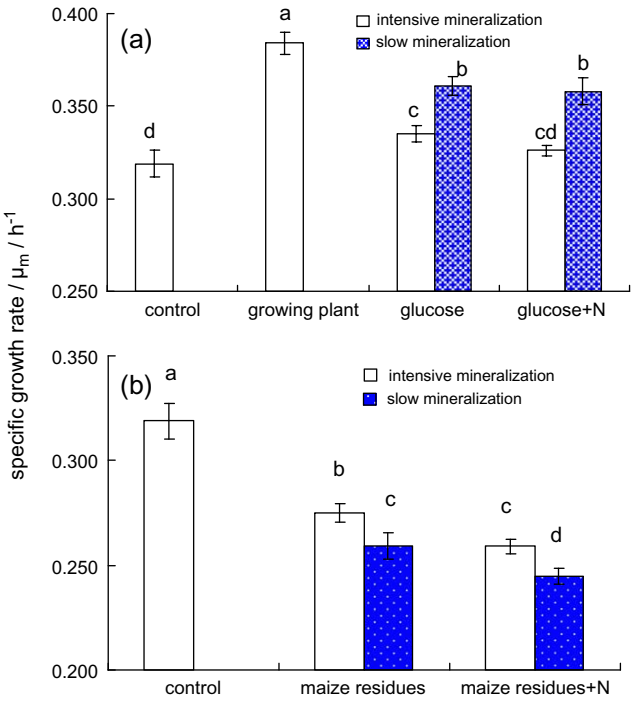

Figure 3 Specific growth rates (μ) of soil microorganisms during intensive and slow mineralization of readily (a) and poorly (b) available substrates. Bars show standard deviations of the means ($n = 3$). Values with the same letter are not significantly ($P < 0.05$) different from each other.

in soil amended with maize residues were significantly smaller than in control soil, especially after N addition (Figure 3b), which indicated the response of slow-growing microorganisms to maize residue application. In contrast to glucose-treated soils, μ-values (Figure 3b) were significantly smaller during the period of slow than during intensive residue mineralization. The percentage of growing biomass, however, was larger during slow mineralization (Table 2).

The lag-time was *c*. 2 hours shorter, total microbial biomass 1.5–2 times greater and the percentage of growing microorganisms three to six times greater in soils amended with plant residues compared with the control soil (Table 2).

Activity of soil microorganisms estimated by the Wright and Hobbie method

Characteristics of microbial mineralization activity (substrate affinity, turnover time and heterotrophic potential V_{max}) are shown in Table 3 and mineralization activity during intensive mineralization period in Figure 4. The most pronounced differences were between the control and the planted soil (Figure 4a), as well as between control soil and soil with maize residue addition (Figure 4b).

K_s values were significantly greater for soils treated with available substrates compared with the control (Figure 5a). This indicated lower substrate affinity of microorganisms in soil amended with glucose or root exudates compared with soil without amendments. The differences between treatments with glucose and with root exudates were insignificant.

The K_s values significantly decreased after 3–6 days from maize residues addition (Figure 5b). This effect indicated activation of microorganisms with high substrate affinity, and remained stable after 14 days. K_s decreased during the incubation, with maize residues having smaller values during the slow versus intensive mineralization period. This trend was significant, however, only in the MRN treatment and coincides with a similar decrease in μ-values (Figure 3b).

Addition of glucose and maize residues slightly increased the maximum mineralization rate (V_{max}) (Table 3). However, the V_{max} increased very strongly in soil with root exudates and exceeded values in the control soil by a factor of 3.

During the period of intensive mineralization of glucose or root exudates, the amount of available substrate (S_n) estimated by Equation (8) increased by a factor of 2.6–3.9 compared with untreated soil (Figure 6). Adding maize residues

Table 2 Microbial biomass (KSIR), lag-period and generation times (T_g) of actively growing and total microbial biomass consuming glucose during incubation of soils with glucose (G), living roots (R) and maize residues (MR) and with (+) or without (−) N; ± denotes standard deviation. Control values are given for the first day of incubation because no significant differences were observed between control values during the experiment. The amount of nitrogen added to 'control treatment with N' corresponds to the N amount applied in MRN treatment. ND = not determined

				Microbial biomass				Generation time			
	Period of substrate	lag-time /hour		Growing /% of total		total /μg C g^{-1}		growing biomass / hours		total biomass / days	
Treatment	mineralization	−N	+N	−N	+N	−N	+N	No N	+N	−N	+N
Control		12.7 ± 0.2	14.1 ± 0.1	0.17	0.09	260 ± 21	262 ± 45	2.2	2.1	53.3	97.2
G	Intensive	12.6 ± 0.5	12.9 ± 0.2	0.16	0.15	278 ± 39	319 ± 27	2.2	2.1	53.3	97.2
G	Slow	12.8 ± 0.2	12.6 ± 0.3	0.10	0.11	295 ± 34	282 ± 53	1.9	1.9	80.0	73.3
R	Intensive	11.5 ± 0.5	ND	0.11	ND	266 ± 38	ND	1.8	ND	68.4	ND
MR	Intensive	9.9 ± 0.5	11.3 ± 0.3	0.65	0.53	484 ± 10	479 ± 25	2.50	2.7	16.2	21.0
MR	Slow	9.0 ± 0.3	10.7 ± 0.4	0.97	0.73	390 ± 25	595 ± 36	2.70	2.8	11.5	16.1

Table 3 Mineralization activity characteristics according to the Wright & Hobbie model (Equation 6) during incubation of soils with glucose (G), living roots (R) and maize residues (MR) and with (+) or without (−) N; ± denotes standard error. Control values are given for the first day of incubation because no significant differences were observed between control values during the experiment. The amount of nitrogen added to 'control treatment with N' corresponds to the N amount applied in MRN treatment

Treatment	Period of substrate mineralization	Saturation constant and concentration of soil indigenous C ($K_s + S_n$) /μg C g^{-1}		Maximal mineralization rate V_{max}/μg C g^{-1} hour^{-1}		Turnover time of indigenous substrate equivalent to glucose T_t /hour		Competitive ability of microorganisms at low substrate concentrations μ-to-K_s /g hour^{-1} μg C^{-1}	
		no N	+N	no N	+N	no N	+N	no N	+N
Control		43.3 ± 3.1	33.9 ± 7	7.7 ± 0.2	8.9 ± 0.6	4.6 ± 0.7	3.8 ± 0.8	0.008	0.010
G	Intensive	69.6 ± 5.3	61.3 ± 3.0	10.6 ± 0.7	10.3 ± 0.4	6.5 ± 0.9	6.0 ± 0.5	0.005	0.006
R	Intensive	77 ± 5.2	ND	23 ± 0.4	ND	3.3 ± 0.2	ND	0.006	ND
MR	Intensive	68.6 ± 8.3	62.9 ± 11.8	9.1 ± 0.6	11.7 ± 1.1	7.5 ± 1	5.4 ± 1.1	0.008	0.008
MR	Slow	36.7 ± 2.6	27.1 ± 0.3	9.8 ± 0.5	6.7 ± 0.5	3.7 ± 0.3	4.0 ± 0.7	0.008	0.014

increased the content of available substrate by a factor of 12–15.

The turnover time of available substrate (T_t, Equation 7) during the active mineralization period was significantly longer in both G and MR treatments than in the control (Table 3). T_t significantly decreased during the second week of soil incubation with maize residues.

Effect of nitrogen application

In the glucose treatments the effect of N was not significant for any of the investigated microbial growth characteristics. In treatments with maize residues, adding N prolonged the lag phase and reduced the portion of growing microorganisms during the intensive period of maize residue mineralization. During the period of slow mineralization, adding N also resulted in more total microbial biomass (Table 2). The N effect on the amount of easily available C in the soil went in the opposite direction for periods of intensive and slow mineralization. S_n was less in MRN as compared with MR treatment at the beginning of the incubation and greater in MR in the same soil during the second week of incubation (Figure 6b).

Discussion

Parameters of microbial mineralization activity and competitive ability of soil microorganisms

The substrate affinity (K_s values) ranged from 17.9 (plant residues) to 67.8 μg C g^{-1} (glucose amendment). These values agree with the range of K_s of 21.1–79.1 μg C g^{-1} reported for soils under various tree species (Bradley & Fyles, 1995a). The turnover times T_t (3.3–7.5 hours) were comparable with T_t (2.8 hours) for forest silty loamy Haplic Luvisol (Panikov *et al.*, 1992) but were considerably shorter than those (19.3–20.4 hours) reported by Ferroni *et al.* (1985) for soil slurry. The main difference between the latter experiment and ours lies in oxygen availability.

K_s values are known to vary with V_{max} (i.e. the two parameters are not completely independent) and affect each other by the fitting procedure (Kovarova-Kovar and Egli, 1998).

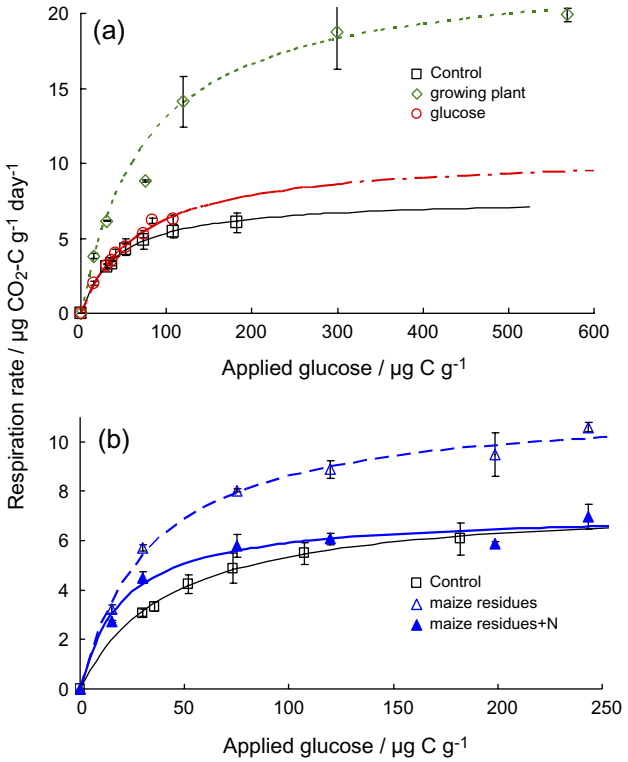

Figure 4 Glucose-induced respiratory response of microbial community during intensive mineralization of readily (a) and poorly (b) available substrates as a function of glucose concentration. Experimental data are shown as symbols and model simulation based on Monod kinetics (Equation 6) as curves. Bars show standard deviations of the means ($n = 3$).

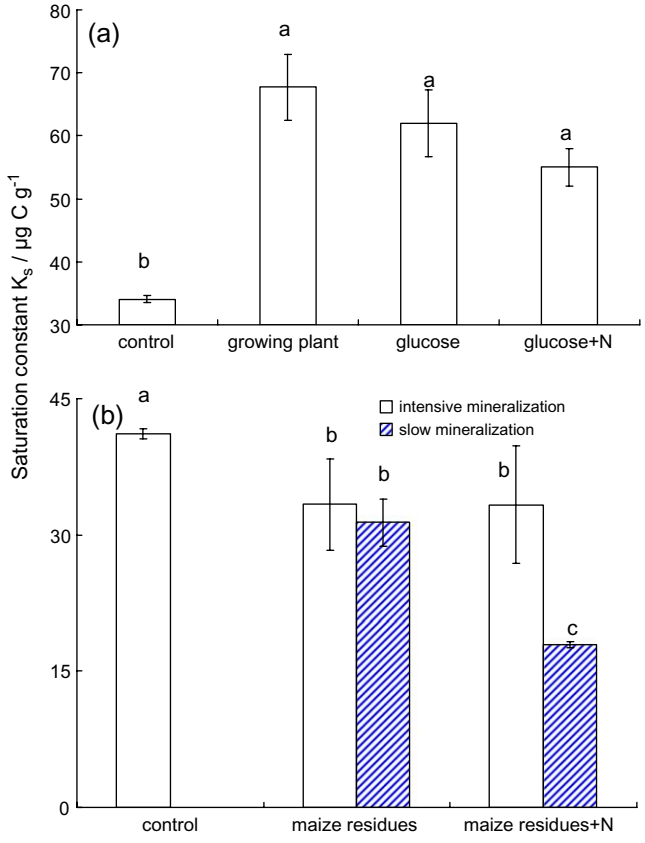

Figure 5 Saturation constant (K_s) values during mineralization of readily (a) and poorly (b) available substrates. Bars show standard errors of the means ($n = 3$). Values with the same letter are not significantly ($P < 0.05$) different from each other.

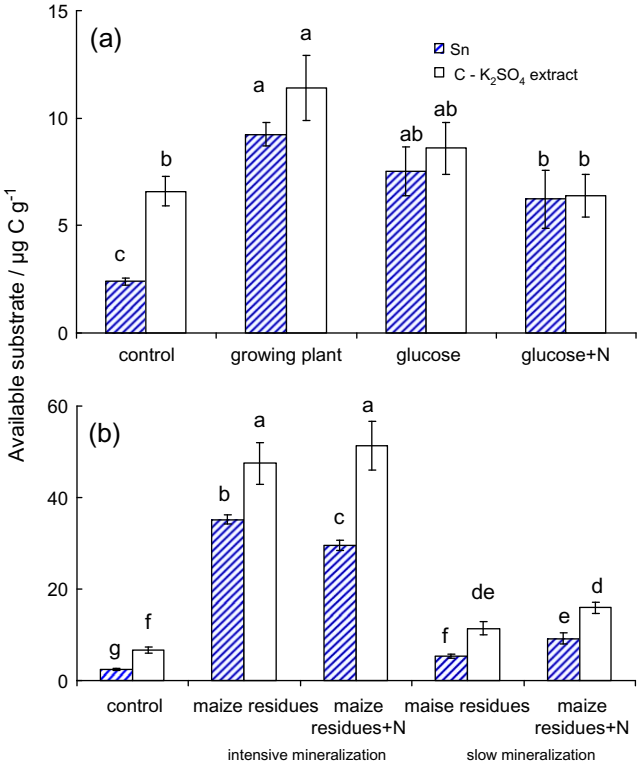

Figure 6 Carbon available to microbial mineralization (S_n) and carbon extractable with K_2SO_4 during mineralization of readily (a) and poorly (b) available substrates. Bars show standard errors of the means ($n = 3$). Values with the same letter are not significantly ($P < 0.05$) different from each other.

Therefore, Healey (1980) proposed that the V_{max}/K_s (or μ/K_s) ratio is a better factor to assess the advantage in competition for nutrients at low concentrations. To overcome the problem of co-correlation of V_{max} and K_s, we used the ratio μ/K_s, in which both characteristics are obtained independently in separate experiments. The lowest μ/K_s ratios were obtained in our study for treatments with readily available substrates: glucose and root exudates. This value was greatest during slow mineralization of maize residues with N. These data demonstrate that during decomposition of maize residues amended with N, a microbial community developed that was more competitive at higher population density (K-strategists). The decrease in concentration of substrate available for microorganisms (S_n) at the slow phase of decomposition (Figure 6) supports this conclusion. In contrast, the competitive ability of K-strategists decreased in treatments with available substrates.

Assessing generation time of soil microorganisms in situ

The kinetic approach allowed the assessment of generation time (T_g) of both actively growing and total microbial biomass consuming glucose. The estimation of T_g for actively growing biomass is based on specific growth rates, i.e:

$$T_g = \ln(2)/\mu. \quad (9)$$

T_g values for growing biomass (1.8 and 2.8 hours) were of the same order as generation times for continuous pure cultures at room temperatures. If generation time is attributed to all living biomass responding to glucose addition by respiration flush, then the ratio between growing and total biomass (physiological state index r_0) has to be considered, i.e.:

$$T_g' = T_g/r_0. \quad (10)$$

T_g' values for total biomass (between 11 and 97 days) were 100–1000 times longer than generation times for actively growing microorganisms. The generation times for total biomass were several times greater than those (3.5–5.9 days) found for bacteria extracted from soil by Bååth (1992, 1994) using ^{3}H-labelling by thymidine and ^{14}C-labelling by leucine. Similar turnover rates were obtained by decomposition and incorporation into microbial biomass of ^{14}C-glycine and ^{14}C-glucose (Kuzyakov & Demin, 1998). However, if fungal contribution to the overall generation time of 130–150 days (Rousk & Bååth,

2007) is considered, our estimation corresponds very well to figures obtained by alternative methods. These include kinetics of substrate-induced respiration and thymidine and leucine incorporation into DNA and protein of extracted bacteria, or acetate incorporation into ergosterol. This correspondence supports the validity of the theoretical approach (Panikov, 1995) used in this study. The relative simplicity of the KSIR technique (Blagodatsky *et al*., 2000) for estimating microbial generation time underlines its usefulness for further application in evaluating C turnover in soil.

Available carbon estimation: methodological issues

Reliable estimation of soil C available for microorganisms is an important problem, because the size of this small pool with high turnover rate determines heterotrophic microbial activity and is a key component in many SOM models. The quantity of available C in soil could stimulate or limit the development of microbial functional groups with specific growth strategy (r or K). We compared direct extraction of soluble C with the Wright & Hobbie (1966) approach. The amount of available substrate estimated by the latter in treatments with both easily available substrates was nearly identical to the C content estimated by K_2SO_4 extraction (Figure 6). However, in control soil and in soil with maize residues, the S_n values were 1.3–2.7 times smaller than K_2SO_4-extractable C during the active mineralization period. This indicates that K_2SO_4-extractable C consists of various substances with various availability to soil microorganisms. The higher content of K_2SO_4-extractable C compared with that estimated by the Wright & Hobbie (1966) approach remained stable during the slow mineralization phase of maize residues.

The amount of available C estimated in our study for untreated soil (2.4 μg C g^{-1}) was at the upper range of those (0.4–2 μg C g^{-1}) estimated by Sikora & McCoy (1990), while it was several times smaller than the S_n values of 4.2–4.9 μg C g^{-1} found earlier by Blagodatsky *et al*. (1994) and 16 μg C g^{-1} by Bradley & Fyles (1995b) for untreated soil. Sikora & McCoy (1990), Blagodatsky *et al*. (1994) and Bradley & Fyles (1995b) used the same Wright & Hobbie approach with an important modification. In contrast to the original suggestion (Wright & Hobbie, 1966; Panikov *et al*., 1992), unlabelled glucose was used as the substrate. When applying unlabelled substrate, the S_n value can be estimated by the combination of two equations (Bradley & Fyles, 1995a):

$$v = (V_{max}S)/(K_{m1} + S) + V_{basal}, \quad (11)$$

$$v = V_{max}(S + S_n)/(K_{m2} + S_n + S), \quad (12)$$

where V_{max} is the maximum respiratory response at glucose-C saturation levels; K_{m1} and K_{m2}, the Michaelis constants; S_n, the glucose equivalent of indigenous available C in the soil; and V_{basal} the basal respiration rate when S = 0 (Bradley & Fyles, 1995a). The sensitivity of S_n estimation when applying unlabelled substrate is, however, much smaller compared with ^{14}C-glucose application. The detectable level of $^{14}CO_2$ is achieved within several minutes after adding ^{14}C-glucose to soil, while a much longer incubation period is necessary to accurately determine unlabelled CO_2. The method requires that the incubation times chosen for the kinetic studies are in the range where mineralization rates for each substrate concentration are a linear function of time (Ferroni *et al*., 1985). In our study, a 30-minute threshold was a suitable incubation time for glucose concentrations <50 μg C g^{-1}. Furthermore, regarding methodological restrictions (Wright, 1973), the amount of respired $^{14}CO_2$ in our study never exceeded 5% of added C. The long incubation time exceeding this threshold can distort both S_n and K_s values, causing inconsistency between different studies.

Microbial growth strategies in soil amended with substrates of contrasting availability

The common ecological concept of r- and K-selection classifies organisms according to their competitive abilities. The theory of microbial ecology requires that both K- and r-strategists consume glucose, but that the specific growth rates and substrate affinity to glucose differ. Compared with K-strategists, r-strategists are characterized by higher μ-values under unlimited nutrient conditions, but lower substrate affinity (corresponding to higher K_s values) (Andrews & Harris, 1986). Because both types of microorganisms are abundant in soil, changes in growth rates and substrate affinity of the whole population after substrate addition reflect a dominance shift.

Easily available substrates (glucose, glucose with N, root exudates) added to the soil increased the specific growth rates and decreased the substrate affinity of microorganisms. Thus, a strong increase in r-strategy features compared with the control was observed and reflects the advantage of fast-growing microorganisms in competing for easily available substrate. The significantly greater μ values were observed during the slow versus the intensive mineralization of available substrates. r-strategists therefore remained dominant for at least 4 days after substrate application.

The total input of C in the G and GN treatments was comparable with C input to soil by rhizodeposition during 14 days of plant growth (Kuzyakov & Domanski, 2000). Small amounts of applied available substrates were quickly consumed and did not significantly affect the measured content of K_2SO_4-extractable C, but strongly increased C availability (S_n). This may reflect the activation of glucose transport systems with lower substrate affinity in cell membranes. Accordingly, the sum of ($K_s + S_n$) increased by a factor of 1.6–1.8 (Table 3), v_0 increased by 15–30 times, while V_{max} increased only minimally (1.2–1.4 times); thus, the S_n values increased (Equations 7, 8). A similar difference in substrate availability between unplanted and maize-planted soil was shown by

Nguyen & Guckert (2001) using the Wright & Hobbie approach.

The amount of added C in substrates of low availability was enough to induce microbial growth. The most available components of maize residues were primarily consumed during the intensive mineralization period and as a result the microbial biomass was almost doubled. Treatments with maize residues (MR and MRN) resulted in a decrease of both μ and K_s values. Thus, slow-growing but more substrate-specific K-strategists were more competitive for substrates of low availability and r-strategists failed in competition under such conditions.

The amount of soluble C produced during maize residues decomposition decreased several-fold at the slow mineralization phase of microbial succession, when less available compounds of maize residues were exposed to microbial degradation. Nutrient limitation reduced the participation of r-strategists in substrate uptake, causing μ and K_s values to drop more during the slow than during the intensive stage of mineralization, again indicating the increased domination of K-strategists over r-strategists during plant residue mineralization.

The contrasting tendency of changing μ-values clearly showed that the small amount of added glucose in G and GN treatments was enough to activate the metabolism (De Nobili *et al*., 2001) of r-strategists (higher μ-values) but insufficient for microbial growth. Thus, exhaustion of available substrate over 4 days after glucose addition caused the decrease in actively growing biomass in both G and GN treatments whereas the applied amount of maize residues was sufficient to activate the K-strategists with lower μ- and K_s values and to support their growth. The consistent increase in growing biomass in MR and MRN treatments demonstrated this for intensive and slow decomposition periods of maize residues.

Effect of nitrogen on respiration and specific growth rates

There were variable effects of the N on microbial activity. Thus, the relatively low N application with the GN treatment decreased significantly the initial CO_2 efflux rates, but no effect of N application on specific growth rate or substrate affinity was observed in the glucose treatments. Nitrogen application to maize residues (C:N 10:1) unexpectedly caused μ-values to drop significantly (but insignificantly in K_s values) compared with residue treatment without N. Numerous studies have reported the inconsistent effect of N availability on microbial respiration (Waldrop & Zak, 2006). Not only the C/N ratio and soil properties, but also physiological attributes of the decomposer community such as substrate utilization efficiency (Hobbie, 2005; Magid *et al*., 2006), apparently determine the N effect on soil respiration. A reduced utilization efficiency of the microbial biomass was observed under N-limiting conditions (Korsaeth *et al*., 2001). Accordingly, the efficiency of microbial decomposers increased after N enrichment (Ågren *et al*., 2001). In our study, the increase of initial CO_2 efflux in the G as compared with the GN treatment can be explained by the lower substrate use efficiency under N limitation. The lower μ values in MRN as compared with MR treatment can be explained by development of microbial community with greater population density when K-strategists with smaller growth rates but more efficient enzyme systems overcame the r-strategists in the course of plant residue decomposition.

Conclusions

Two different approaches, microbial growth kinetics and the Wright & Hobbie (1966) approach, showed the same tendency and revealed the clear shift in the growth strategy of soil microorganisms after substrate addition. Input of easily available substrates such as glucose or root exudates stimulated fast-growing r-strategists. Adding substrate of low availability (plant residues) shifted the microbial community to slow-growing K-strategists. The Wright & Hobbie (1966) approach was suitable for estimation of the amount of indigenous soil substrate easily available to microorganisms and corresponded well to C-content in K_2SO_4 extracts. However, for plant residue decomposition, the Wright & Hobbie (1966) approach predicted lower availability of soluble substrates in contrast to chemical extraction method.

Applications of substrates with different availability (i.e. sugars or plant residues) change functional properties of soil microbial community. These changes control the direction and size of apparent or real priming effects. Soil microorganisms have a great potential for adaptive variation of their growth traits in dependence on energy source and its availability. This should be considered by studies on C sequestration and stability of soil organic matter as well as for future model development.

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