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Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories

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Abstract

The increasing input of anthropogenically derived nitrogen (N) to ecosystems raises a crucial question: how does available N modify the decomposer community and thus affects the mineralization of soil organic matter (SOM). Moreover, N input modifies the priming effect (PE), that is, the effect of fresh organics on the microbial decomposition of SOM. We studied the interactive effects of C and N on SOM mineralization (by natural ^{13}C labelling adding C_4-sucrose or C_4-maize straw to C_3-soil) in relation to microbial growth kinetics and to the activities of five hydrolytic enzymes. This encompasses the groups of parameters governing two mechanisms of priming effects – microbial N mining and stoichiometric decomposition theories. In sole C treatments, positive PE was accompanied by a decrease in specific microbial growth rates, confirming a greater contribution of K-strategists to the decomposition of native SOM. Sucrose addition with N significantly accelerated mineralization of native SOM, whereas mineral N added with plant residues accelerated decomposition of plant residues. This supports the microbial mining theory in terms of N limitation. Sucrose addition with N was accompanied by accelerated microbial growth, increased activities of β-glucosidase and cellobiohydrolase, and decreased activities of xylanase and leucine amino peptidase. This indicated an increased contribution of r-strategists to the PE and to decomposition of cellulose but the decreased hemicellulolytic and proteolytic activities. Thus, the acceleration of the C cycle was primed by exogenous organic C and was controlled by N. This confirms the stoichiometric decomposition theory. Both K- and r-strategists were beneficial for priming effects, with an increasing contribution of K-selected species under N limitation. Thus, the priming phenomenon described in 'microbial N mining' theory can be ascribed to K-strategists. In contrast, 'stoichiometric decomposition' theory, that is, accelerated OM mineralization due to balanced microbial growth, is explained by domination of r-strategists.

Keywords: C cycle, extracellular enzyme activity, microbial growth kinetics, priming mechanisms, r and K strategy, soil microbial biomass, soil organic matter turnover

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Introduction

Soil organic matter (SOM) is a heterogeneous mixture of organic substances with different forms and degradability (Lal, 2009). The input of exogenous organic matter in soil may intensify or retard SOM decomposition, causing positive or negative priming effects (Kuzyakov *et al.*, 2000; Paterson *et al.*, 2008; Guenet *et al.*, 2010). The positive 'priming effect' is proportional to the amount of added substrate-C (Mary *et al.*, 1993; Paterson & Sim, 2013). Besides the amount, the quality of organic substances and their availability to decomposers affect the decomposition of SOM pools (Six & Jastrow, 2002). Availability here is defined as the biochemical recalcitrance of organic compounds, that is, their susceptibility to enzymatic degradation with further uptake of reaction products by soil microorganisms. Complex organic

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substrates, for example green manure, wheat straw, and ryegrass, showed stronger priming effects than direct energy substrates such as glucose or fructose (Fontaine *et al.*, 2003). While priming effects have been extensively reported after organic C incorporation, few studies have successfully linked priming effect with substrate availability depending on nutrient supply (e.g., Blagodatskaya *et al.*, 2009).

Globally, many ecosystems are experiencing increased inputs of anthropogenically derived N (Galloway *et al.*, 2008), originating mainly from increased N deposition in natural ecosystems and application of mineral N fertilizer in agricultural ecosystems. This reactive nitrogen caused globale ecological changes in terrestrial ecosystems, such as significant soil acidification, nitrate leaching, and loss of biodiversity. Furthermore, nitrogen strongly influences the below-ground carbon cycling due to their close interaction (Janssens *et al.*, 2010). Even minor changes of soil organic carbon storage cause a profound impact on atmospheric CO_2 concentration (Manlay *et al.*, 2007). This calls for thorough studies on the effects of increased N input on litter and SOM decomposition, as well as on soil C stocks. Divergent results have been reported, with positive (Conde *et al.*, 2005; de Graaff *et al.*, 2006), negative (Janssens *et al.*, 2010) or nil (Liljeroth *et al.*, 1994) effects of N on SOC decomposition. That is why several theories explaining the different aspects of SOM decomposition during priming were currently suggested, for example, cometabolism (Kuzyakov *et al.*, 2000), shift in microbial communities composition (Fontaine *et al.*, 2003), microbial activation (Drake *et al.*, 2012), as well as preferential substrate utilization (Cheng, 1999) and competition for mineral nutrients (Cheng & Kuzyakov, 2005) in the rhizosphere. Among them there are two competing hypotheses – 'stoichiometric decomposition' and 'microbial nitrogen mining' – regarding the impacts of nutrient availability on organic matter decomposition (Craine *et al.*, 2007). According to 'stoichiometric decomposition' theory, the microbial activity is highest, and decomposition rates are maximal, if C and N input with substrate matches microbial demands, that is, when this input corresponds to stoichiometric C and N ratios (Hessen *et al.*, 2004). The 'microbial nitrogen mining' hypothesis assumes that the N-acquiring microbes use labile C as an energy source to decompose recalcitrant organic matter, which contains N (Moorhead & Sinsabaugh, 2006). This means that low-N availability in pools accessible for microorganisms facilitates the decomposition of recalcitrant SOM to acquire N. Thus, the two hypotheses assume an opposite effect of N on SOM stability, that is, according to 'stoichiometric decomposition' high-N availability (nutrient-rich condition) is likely beneficial for SOM decomposition, while according to 'microbial N mining' low-N availability (nutrient-poor condition) is likely facilitating SOM decomposition. However, both above-mentioned mechanisms are associated with the availability of organic C, which represents the clear linkage between two hypotheses. Therefore, to better explain the coupling mechanisms between carbon and nitrogen cycles in soils a pot experiment with sole mineral N, with sole organic C, and their combined addition was set up in the present study to understand the single and interactive effects of C and N on SOM mineralization.

Mineralization of SOM is essentially the result of activity of microorganisms growing on the decomposed substrate. So, growth parameters of microorganisms metabolizing soil organics determine which kind of functional groups of heterotrophic decomposers benefit in the competition for substrates in soil. The metabolic activity and functional structure of microbial communities have been widely discussed in attempts to explain the mechanisms of priming effects (Brant *et al.*, 2006; Schmidt *et al.*, 2011). A conceptual model of the priming effect (Fontaine *et al.*, 2003) is based on competition for energy and nutrient acquisition and on community shifts between microbial communities specialized for utilizing various sources: fast-growing r-strategists benefited by utilizing easily available substrates and slow-growing K-strategists having an advantage in utilizing recalcitrant organics. Nonetheless, clear experimental support for the above- mentioned mechanisms by determination of microbial growth parameters *in situ* in soil is missing. Most studies fail to demonstrate the mechanisms because the experimental designs aim to identify the priming effect, not the underlying mechanisms (Blagodatskaya & Kuzyakov, 2008).

Under natural conditions, soil microorganisms are able to degrade large organic molecules of cellulose, hemicelluloses, lignin and proteins via the activity of excreted hydrolytic and oxidative enzymes (Pérez *et al.*, 2002). β-glucosidase and cellobiohydrolase are commonly measured enzymes (Sinsabaugh & Shah, 2011) responsible for degrading cellulose. Xylanase can degrade xylan to xylose, thus being responsible for breaking down hemicelluloses (Li *et al.*, 2009), a major component of plant cell walls. Chitinase and leucine aminopeptidase are two common enzymes involved in degrading organic N compounds – chitin and proteins, respectively (Sanaullah *et al.*, 2011). Extracellular enzyme activities reflect well the functions of decomposer communities, depending on metabolic requirements and on nutrient availability (Caldwell, 2005). Thus, even a small shift in the activity of one or two critical enzymes can significantly alter decomposition rates of organic matter at a global, long-term scale

(Sinsabaugh *et al*., 2002). The linkage between extracellular enzyme activities and the succession of microbial communities, in particular from r-strategists to K-strategists, is one possible explanation for the observed dynamics of the priming effect during litter decomposition (Hamer & Marschner, 2005). This assumption needs to be proven, considering the interactive effect of C and N availability on priming, to recognize the applicability of 'microbial mining' and 'stoichiometric decomposition' theories, both of which are linked to such successions and such enzyme activities.

The present study examines the mechanisms of priming effects as a response to exogenous organic C, added mineral N and their interactions. The study is designed to (i) link the growth parameters of soil microbial communities and extracellular enzyme activities with the observed priming effect; (ii) test two associated but competing hypotheses of N effect on priming: either N limitation increase SOM decomposition ('microbial N mining') or sufficient N supply stimulate microbial growth and SOM decomposition ('stoichiometric decomposition').

Materials and methods

Soil

The soil used in these experiments was collected from the upper 20 cm of the Hohenschulen experimental farm of Kiel University (10.0°E, 54.3°N), 15 km west of Kiel, northern Germany. The soil is classified as Stagnic Luvisol, with a sandy loam texture, pH 6.5 (0.01 M $CaCl_2$ 1:4), and a water-holding capacity (WHC) of 31% (w/w). The chemical properties of the soil, and the applied organic substrates are presented in Table 1. Before use, the soil was air-dried, homogenized and sieved <2 mm. Roots and other plant residues were carefully removed.

Experimental design

The pot experiment included six treatments (three replicates): nonamended C_3-soil (control); soil with mineral N (min-N); soil with C_4-sucrose (Suc-C); soil with sucrose+mineral N (Suc-C+min-N); soil with C_4-maize straw (MS-C); soil with maize straw+mineral N (MS-C+min-N). These treatments were chosen to prove whether different availability of C (sugar or straw) will affect the C/N interactions and PE mechanism. Mineral N was applied to the soil at an N rate of 110 mg NH_4 + -N kg^{-1} dry soil equivalent to150 kg NH_4 + -N ha^{-1}, which was the conventional amount of mineral N fertilizer application in northern Germany. Considering the optimized C/N of added substrates as ca. 23, maize straw was applied to yield a total organic C of 2.5 g C kg^{-1} dry soil. Sugar was applied at only 1/5 of the total C of maize straw (0.5 g C kg^{-1} soil), as this amount can stimulate sufficient PE according to published data (Conde *et al*., 2005) and larger dose of sugar will cause very intensive microbial growth masking thus N interaction effects under study. Detailed description of experimental treatments and the amount of N and C applied are given in Table 2.

About 150 g air-dried soil was homogeneously mixed with the above-mentioned organic substrates and immediately filled into glass jars (250 ml, 12 cm in height). The same physical mixing of the soil was performed with the unamended control soil. Soil moisture was adjusted with distilled water to 75% of WHC. Jars were incubated in a dark chamber at 19 °C and a relative air humidity of 65% and remained open during the incubation. The CO_2 efflux was monitored during 500 h with the goal to distinguish the intensive and slow phases of decomposition. As after 210 h, the decomposition rates strongly slowed down, and to minimize possible ^{13}C-discrimination (Werth & Kuzyakov, 2010; Blagodatskaya *et al*., 2011), the PE was determined at this time and then followed by destructive sampling and measurements of microbial activity.

Analysis of CO_2 fluxes

Gas probes were sampled using an adapted closed chamber method. Before sampling, the incubation chamber was ventilated for 15 min, and then, air-tight lids were fitted onto each jar. Zero, 20 and 40 min after sealing, the gas was collected using a gas-tight syringe and stored in pre-evacuated Exetainer glass bottles (Labco, High Wycombe, UK). CO_2 concentrations were analyzed by ECD gas chromatography (Varian Star 3400 CX; Varian, Palo Alto, CA, USA). Hourly

Table 1 Chemical properties of soil and organic substrates used in the experiment

	TOC (%)	$\delta^{13}C_{TOC}$ (‰)	TN (%)	C/N
Soil	1.47	−28.8	0.098	15
Sucrose	40.61	−13.9	n.a	n.a
Maize straw	39.6	−14.4	0.94	42

TOC, content of total organic C; $\delta^{13}C_{TOC}$, $\delta^{13}C$ of total organic C; TN, content of total N; C/N, ratio of total organic C and total N.

Table 2 Experimental design and applied substrates

	Applied C		
Treatment	Substrate	Amount (g C kg^{-1} dry soil)	Applied N as $(NH_4)_2SO_4$ (mg N kg^{-1} dry soil)
Control	No	0	0
min-N	No	0	110
Suc-C	Sucrose	0.5	0
Suc-C + min-N	Sucrose	0.5	110
MS-C	Maize straw	2.5	0
MS-C + min-N	Maize straw	2.5	110

CO_2 emission rates were calculated from the linear regression of CO_2 concentration vs. time. Cumulative CO_2 emissions were estimated by linear interpolation of hourly CO_2 emission rates. $\delta^{13}C$ of CO_2 was analyzed by GC-IRMS using a preconcentration unit (Thermo 19 Finnigan Delta C+ and Precon, Thermo Finnigan, Bremen, Germany).

Kinetics of substrate-induced respiration and use of derived growth parameters

The kinetics of substrate-induced growth response (SIGR) in the soil was analyzed at the end of the incubation period according to Blagodatsky *et al*. (2000). We used SIGR approach as it is based on microbial physiology and enables distinguishing total and active biomass fractions along with parameters of microbial growth (Panikov, 1995). In terms of determination of total biomass, the SIGR approach is very similar to the basic and classic physiological SIR-method (Anderson & Domsch, 1978). It has to be noted that despite substrate addition is required by SIGR approach all kinetic parameters (specific growth rate, active and total microbial biomass and their turnover times, see below) analyzed by SIGR represent the characteristics of the soil microbial community at the sampling instant, that is before substrate addition. Ten grams of fresh soil was amended with a mixture containing 10 mg g^{-1} glucose, 20 mg g^{-1} talcum, 1.9 mg g^{-1} $(NH_4)_2SO_4$, 2.25 mg g^{-1} K_2HPO_4, and 3.8 mg g^{-1} $MgSO_4{\cdot}7H_2O$. Soil samples were placed (in triplicate) in an ADC2250 24-Multichannel Soil Respiration System (ADC BioScientific Limited, Hoddesdon, UK) at 19 °C. Each sample was continuously aerated (300 ml min^{-1}), and the rate of CO_2 production from each sample was measured every hour using an infrared detector and mass-flow meter.

The kinetics of microbial growth was estimated by fitting the parameters of Eqn (1) to the measured CO_2 evolution rate (Panikov & Sizova, 1996):

$$CO_2(t) = A + B\exp(\mu \times t) \quad (1)$$

where A is the initial respiration rate uncoupled from ATP generation, B is the initial rate of the growing fraction of total respiration coupled with ATP generation and cell growth, μ is the maximal specific growth rate of soil microorganisms, and t is time. The parameters of Eqn (1) were optimized by minimizing the least-square sum using Model Maker-3 software (Cherwell Scientific Publishing Ltd., Oxford, UK). Three replicates of respiration curves were used for each treatment. Fitting was restricted to the initial phase of the curve, which corresponded to unlimited exponential growth (Wutzler *et al*., 2012).

Other parameters of microbial growth kinetics were calculated from the optimized parameters of the fitted respiration curve Eqn (1). The duration of the lag period (Tlag) was calculated using the equation:

$$T_{lag} = \frac{\ln(A/B)}{\mu} \quad (2)$$

· The total microbial biomass (TMB) and growing microbial biomass (GMB) before substrate addition were calculated using Eqns (3) and (4), respectively.

$$TMB = \frac{B}{r_0 Q} \quad (3)$$

$$GMB = TMB \cdot r_0 \quad (4)$$

In Eqns (3) and (4), r0 is the so-called physiological state index of the microbial biomass (MB) before substrate addition and was calculated from Eqn (5).

$$r_0 = \frac{B(1-\lambda)}{A + B(1-\lambda)} \quad (5)$$

λ is a basic stoichiometric constant, which has an accepted value of 0.9 (Panikov & Sizova, 1996). Q is the total specific respiration activity and was calculated from Eqn (6).

$$Q = \frac{\mu}{\lambda Y_{CO_2}} \quad (6)$$

The theory of microbial growth kinetics has been presented in detail earlier (Panikov, 1995). Note that in Eqn (6), Y_{CO_2} is the MB yield per unit of glucose-C and was assumed to be constant throughout the monitoring period with a mean value of 0.6 (Petersen *et al*., 2005).

Microbial maximal specific growth rate (μ), derived from Eqn (1) was used as an intrinsic property of microorganisms for the estimation of relative abundance of fast-growing (r-strategists) or slow-growing (K-strategists) populations in soil microbial community. According to the definitions (Pianka, 1970; Andrews & Harris, 1986) higher μ reflects relative domination of r-strategists, while lower μ shows relative domination of K-strategists. This approach is based on the common view linking microbial community structure and substrate availability (e.g., Fierer *et al*., 2007; Panikov, 2010) and was validated against other physiological parameters of total microbial community, such as affinity to substrate (Ks) and/or substrate use efficiency (Blagodatskaya *et al*., 2007, 2009).

Measurement of soil enzyme activity

Potential extracellular enzyme activities in the soil were measured at the end of the incubation period under optimal conditions using fluorogenically labelled substrates (Pritsch *et al*., 2004). Five types of artificial fluorogenic substrates were used: 4-Methylumbelliferyl-β-D-glucopyranoside (MUF-G, EC 3.2.1.21) for the detection of β-glucosidase activity; 4-Methylumbelliferyl-β-D-cellobioside (MUF-C, EC 3.2.1) for the detection of cellobiohydrolase activity; 4-Methylumbelliferyl-β-D-xylopyranoside (MUF-X, EC 3.2.1) for the detection of xylanase activity; and 4-Methylumbelliferyl-N-acetyl-β-D-glucosaminide dehydrate (MUF-NAG, EC 3.2.1.14) for the detection of chitinase activity. L-Leucine-7-amino-4-methyl coumarin was used to analyze leucine amino peptidase (LAP) activity, which is involved in the hydrolysis of L-peptide bonds. All substrates were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Calculations and statistical analysis

With the difference in $\delta^{13}C$ signatures between C4-substrates and C3-soil, total CO_2 emissions from the soil (CT) can be

separated into SOM-derived CO_2 emissions (CSOM) and substrate-derived CO_2 emissions (CSUB) using the following equations (Cheng, 1996):

$$C_{SOM} = C_T \times (\delta_T - \delta_4)/(\delta_3 - \delta_4) \quad (7)$$

$$C_{SUB} = C_T - C_3 \quad (8)$$

where CT and δT was obtained using Keeling plot (Keeling, 1961; Pataki *et al.*, 2003). δ3 in the equations was obtained from control jars/plots and already included fractionation in the system. The δ4 in equations was assumed to be equal to the $\delta^{13}C$ of maize straw in the experiment. In this, we followed Mary *et al.* (1992) who showed that fractionation during the biodegradation processes was very small or negligible, although $\delta^{13}C$ of CO2 evolving from degrading maize roots was slightly lower than $\delta^{13}C$ of total root C.

Priming effects (PE) were calculated with Eqn (9)

$$PE = C_{SOM\_AME} - C_{SOM\_CON} \quad (9)$$

where C_{SOM_AME} is the SOM-derived CO_2 emissions in soils with different amendments, and C_{SOM_CON} is the SOM-derived CO_2 emissions in the control treatment.

Data are presented as the means of three replicates with standard errors. Tukey's HSD post hoc test was used to identify statistically significant differences among the treatments at $P < 0.05$. Correlation between CO_2 emissions and extracellular enzyme activity was measured by the Pearson correlation method. Statistical analyses were conducted using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA).

Results

Cumulative CO_2 emissions and priming effects

A total of 54.5 μg C g^{-1} CO_2 was released from the control soil during the 8-day period. CO_2 emissions from soils amended with mineral N (min-N) showed a similar pattern as the control (Fig. 1). Added mineral N alone had no significant effect on soil CO_2 emission (i.e., SOM-derived CO_2) compared with the control. There was no statistically significant PE in min-N treatment (Fig. 2). The other four treatments with organic C substrates (Suc-C, Suc-C+min-N, MS-C, MS-C+min-N) produced a clear increase of CO_2 emissions, starting from the initial amendment (Fig. 1). Added sugar and maize straw significantly increased SOM-derived CO_2 accumulated during the incubation period, causing positive priming effects of 38.0 and 60.3 μg C g^{-1}, respectively (Fig. 2).

There was a strong interaction of added organics with mineral N. The respiratory response was stronger and quicker in Suc-C+min-N than in Suc-C, with an earlier and higher flush of CO_2 (Fig. 1). SOM-derived CO_2 in Suc-C+min-N was significantly higher than in Suc-C (Fig. 2), causing a positive PE of 92.3 μg C g^{-1} (Fig. 2), whereas sucrose-derived CO_2 did not differ significantly between the two treatments. Unlike

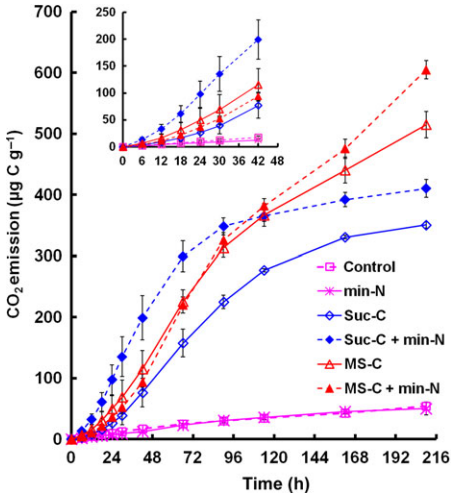

Fig. 1 Cumulative CO_2 released from soils amended with mineral N (min-N), sucrose (Suc-C), sucrose+mineral N (Suc-C + min-N), and water (Control) (top), while maize straw (MS-C) and maize straw + mineral N (MS-C + min-N) (bottom). Data are means of 3 replicates ± standard errors. An inset: the CO_2 emissions over the first 42 h.

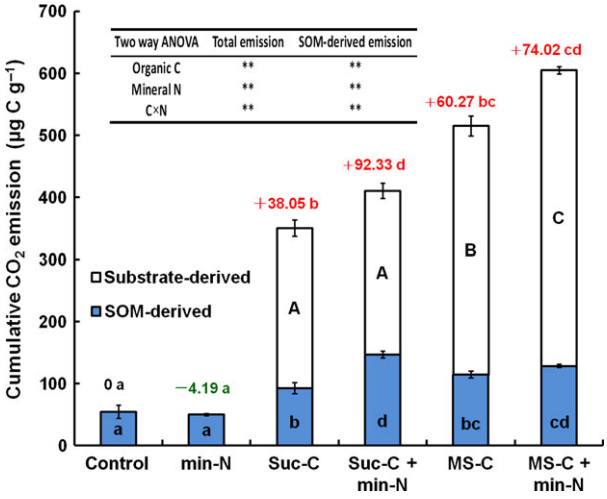

Fig. 2 Cumulative SOM-derived CO_2 (gray bar) and CO_2 derived from added organic substrates (blank) and priming effects (data above the bars) in soils amended with mineral N (min-N), sucrose (Suc-C), sucrose + mineral N (Suc-C + min-N), maize straw (MS-C), maize straw + mineral N (MS-C + min-N), and water (control) at the end of incubation. Data are means of 3 replicates ± 1 SE. Bars contained the same letter are not significantly different ($P < 0.05$); capital letters for substrate-derived CO_2 and small letters for SOM-derived CO_2 and priming effects.

treatments with sugar, SOM-derived CO_2 was not significantly different in MS-C+min-N and MS-C treatments (Fig. 2). Furthermore, MS-C-derived CO_2 emission in MS-C+min-N was 19% higher than in MS-C.

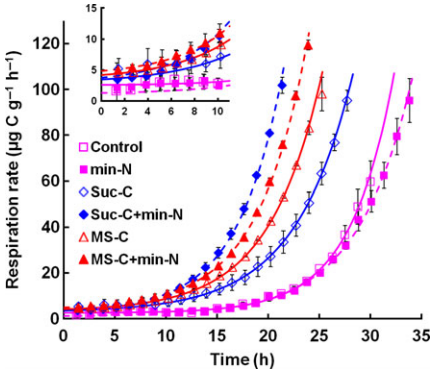

Fig. 3 Substrate-induced respiration of microorganisms in soils amended with mineral N (min-N), sucrose (Suc-C), sucrose+mineral N (Suc-C+min-N), maize straw (MS-C), maize straw+mineral N (MS-C+min-N) and water (control). Experimental data are shown as symbols and model fittings as curves. An inset: the CO_2 emissions over the first 10 h.

This clearly indicates enhanced mineralization of plant residues caused by improved availability of mineral N.

Kinetics of substrate-induced respiration

The application of glucose with nutrients to the soil (to analyze microbial growth kinetics) induced an exponential increase in the CO_2 evolution rate within a few hours (Fig. 3). This indicates quick microbial growth in all treatments. The glucose-induced respiration rates increased significantly earlier in treatments Suc-C, Suc-C+min-N, MS-C, and MS-C+min-N as compared to the control; the rates were slightly delayed in treatment min-N vs. Suc-C+min-N and MS-C+min-N (Fig. 3). The lag-time was shortest in Suc-C+min-N and longest in the control treatment (Table 3). The lag-time differed more between the two treatments with sucrose (2.4 h) than between the two treatments with maize straw (0.2 h, Table 3).

Compared with the control, the min-N treatment decreased the respiratory response to glucose addition, shortened the lag-period, increased the active microbial biomass but decreased the total microbial biomass (Table 3). The four treatments with organic C amendments (Suc-C, MS-C, Suc-C+min-N, MS-C+min-N) stimulated microbial growth in soil, with a 16–18 times increase in GMB and a 1.2–1.8 times increase in TMB compared with the control. TMB in the treatments with sucrose was lower than in treatments with maize straw: a 12% difference was observed between Suc-C and MS-C, a 32% difference between Suc-C+min-N and MS-C+min-N (Table 3).

As an inherent property of microorganisms, the specific microbial growth rate (μ) can reflect the functional structure of soil microbial community as a whole. The μ value in control soil was the highest among all the treatments (Table 3). Organic C amendments clearly decreased the μ value according to the ranking: Suc-C< MS-C<MS-C+min-N<Suc-C+min-N<control (Table 3). This indicates a shift of dominant microbial strategies in soils with different amendments. When applied in combination with organic C, mineral N boosted the μ value, with Suc-C+min-N being 27% higher than Suc-C, and MS-C+min-N being 7.2% higher than MS-C. This points to the response of fast-growing microbial strategists to the combined addition of mineral N and organic C substrates. The trend of μ values was similar to the trend in the amount of priming effects, which was Suc-C<MS-C<MS-C+min-N<Suc-C+min-N (Fig. 3).

Extracellular enzyme activities

Compared with the control, mineral N alone slightly increased the activity of *β*-glucosidase, cellobiohydrolase and chitinase, slightly decreased the activity of LAP but significantly decreased the activity of xylanase

Table 3 Microbial characteristics of growth response, lag-time, actively growing biomass, total microbial biomass and their generation times, calculated by the glucose-induced microbial growth approach

Treatments	Specific growth rate, μ (h^{-1})	Initial nongrowth respiration, A (μg C g^{-1})	Initial growth respiration, B (μg C g^{-1})	Lag-time (h)	Microbial biomass		
					Total (μg C g^{-1})	Growing (μg C g^{-1})	Growing (% of total)
Control	0.235 ± 0.013	2.53 ± 0.49	0.053 ± 0.031	16.50	107.1	0.22	0.21
min-N	0.206 ± 0.015	1.82 ± 0.54	0.093 ± 0.04	14.47	88.13	0.44	0.5
Suc-C	0.178 ± 0.015	2.80 ± 1.49	0.666 ± 0.265	8.04	159.0	3.70	2.32
Suc-C+min-N	0.226 ± 0.011	2.94 ± 0.63	0.820 ± 0.165	5.64	132.3	3.59	2.71
MS-C	0.194 ± 0.001	3.46 ± 0.14	0.762 ± 0.027	7.79	180.4	3.88	2.15
MS-C+min-N	0.208 ± 0.003	4.00 ± 0.41	0.821 ± 0.059	7.62	194.8	3.91	2.01

μ, A, and B are best-fitted model parameter values ± SE, which were given by Model Maker software. Lag-time, total and active microbial biomass are calculated according to Eqns (2)–(4), respectively. Treatments: soils amended with mineral N (min-N), sucrose (Suc-C), sucrose+mineral N (Suc-C+min-N), maize straw (MS-C), maize straw+mineral N (MS-C+min-N), and water (Control).

(Fig. 4). There was an overall significant effect of Suc-C and MS-C on increasing the activities of all measured enzymes (except that the effect was not significant with chitinase in Suc-C due to a high scattering of data). Finally, enzyme activity was typically stimulated more by MS-C than by Suc-C (Fig. 4).

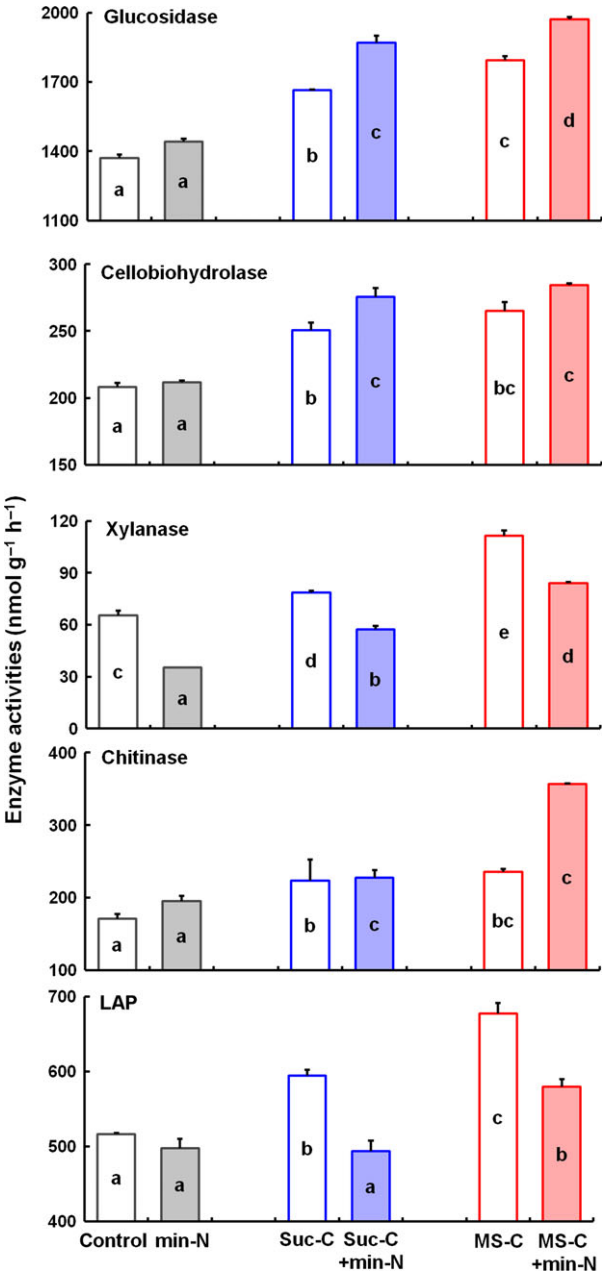

Fig. 4 Activity of extracellular enzymes β-glucosidase, cellobiohydrolase, xylanase, chitinase, and leucine amino peptidase (LAP) in soils amended with mineral N (min-N), sucrose (Suc-C), sucrose+mineral N (Suc-C+min-N), maize straw (MS-C), maize straw+mineral N (MS-C+min-N), and water (control). Data are means of 3 replicates ± 1 SE. Bars not contained the same letter differ significantly at $p < 0.05$.

There was also a strong interaction of added organics with mineral N, which influenced the activities of extracellular enzymes. The activities of β-glucosidase and cellobiohydrolase, which are involved in cellulose decomposition, were significantly higher in combined application treatments than in sole organic C treatments (Fig. 4, Suc-C+min-N compared with Suc-C; MS-C+min-N compared with MS-C). However, mineral N in combined amendment with organic C significantly decreased the xylanase activity (involved in hemicellulose decomposition) and the LAP activity (involved in protein decomposition).

Discussion

Priming effects in response to organic C, mineral N and their interactions

Sole amendment of mineral N did not cause a positive PE. This agrees with the previous laboratory (Köster *et al.*, 2011) and field monitoring of CO_2 emissions (Ginting *et al.*, 2003). It also corresponds well to DNDC-model simulation (Li, 2000), which predicts only small changes in CO_2 emissions by 50% variation in N fertilizer application rates (Grant *et al.*, 2004). As mineral N alone had a minimal impact on SOM-C mineralization, the effect of anthropogenic N deposition or mineral N fertilizer application on extra CO_2 emission via SOM mineralization must always be considered in conjunction with soil C availability.

Adding organic C with and without mineral N both accelerated native SOM mineralization, causing a positive priming effect (Fig. 2). The experimental design does not allow us direct distinguishing real and apparent priming by differences in ^{13}C signatures, taking that real PE is acceleration of nonliving SOM mineralization and apparent PE is additional CO_2 efflux due to accelerated microbial biomass turnover (see also Blagodatskaya *et al.*, 2007 and Blagodatsky *et al.*, 2010). An amount of primed C in our study accounted up to 92 μg C g^{-1}, that is it was comparable with initial size of microbial biomass (107 μg C). The microbial turnover time usually ranged between 21–75 days (Blagodatskaya *et al.*, 2009) and as determined by natural abundance approach was about 30 days (Blagodatskaya *et al.*, 2011). Considering a much shorter monitoring period of PE in this study (about 9 days) as compared with microbial turnover, it is likely that only a small fraction of primed C comes from microbial biomass. The stimulation of SOM mineralization caused by combined addition of mineral N and organic C was stronger than that caused by sole addition of organic C (Fig. 2). The same result was obtained in a 90-day incubation experiment by Moran *et al.* (2005); however, no

increase in SOM-derived CO_2 was observed after concurrent addition of mineral nutrients (N, P) and glucose, as compared with the PE effect induced by sole addition of glucose (Hartley *et al*., 2010). In the latter case added glucose still remained at 4.2 mg C g^{-1} in soil after incubation. This indicates that soil microorganisms still focused on the added sugar. In contrast to Hartley *et al*. (2010), substrate-C was limited considering that two orders of magnitude less substrate C (0.06 mg C g^{-1}) remained in our experiment. So, the N effect on priming depends on time course of incubation experiment and 'real' N limitation which can occur or not due to the other factors such as decomposition rates and possible limitation by other nutrients.

High mineral N amount in the applied substrate did not increase PE induced by maize straw in our experiment but did enhance the mineralization of plant residues (Fig. 2). Therefore, steady existence of available C combined with mineral N (e.g., in maize straw + mineral N amendment) may delay the switch of microbial utilization from the added organics to native SOM. Although the sources of extra-respired CO_2 were different in the sucrose and maize residue treatments, large amount of mineral N did enhance the cumulative mineralization of organic substances in soil. This echoes experiments with organic fertilizers containing both mineral N and available C, for example, animal slurries (Bol *et al*., 2003) and biogas residues (Chen *et al*., 2012).

Microbial mechanisms of priming effects

PE mechanism based on microbial succession is currently under discussion (Fontaine *et al*., 2011). It was assumed that domination strategy of soil microbial community is changing after the exhaustion of labile C in soils, that is, K-strategists outcompeter-strategists by producing extracellular enzymes that decompose recalcitrant SOM (Fontaine *et al*., 2003). On the other hand, an increased activity of r-strategists, as well as extracellular enzymes produced by the activated r-strategists are efficient and sufficient for decomposing recalcitrant SOM, followed by the subsequent use of released SOM-compounds by various nonspecific microbial groups (Blagodatskaya & Kuzyakov, 2008). A special finding of our study is that both high- and low-N availabilities could induce the PE. Thus, we hypothesize that both r-and K-strategists can induce the priming effect, possibly at subsequent stages of substrate and SOM decomposition.

To distinguish relative domination of r- and K-strategy in the community, we used the basic physiological.differences between fast-growing r- vs. slow-growing K-strategists (specific growth rate, μ). Although genetic analysis is powerful in estimating structure of the microbial community, the SIGR approach is a most relevant approach to determine the shift in growth strategy in soil *in situ*. Such physiological response enables interpretations on relative domination/shift in functional structure of the whole microbial community but tells few about its' species composition. It represents the characteristic of total microorganisms that are mainly active and functioning *in situ*.

In treatments with only C addition (Suc-C or MS-C), the PE was accompanied by significant decrease in specific growth rates (μ) and by ca. 15 times increase in growing microbial biomass as compared with control at the end of incubation (Table 3). This clearly demonstrated the physiological shift of microbial communities after C amendments. Lower μ values indicate relative dominance of slow-growing K-strategists, which were more competitive after exhaustion of sucrose (Blagodatskaya *et al*., 2009). Thus, our study experimentally demonstrated the increased dominance of K-strategists coupled with positive PE. The addition of sole sucrose led to the lowest μ value and to N immobilization because no mineral N was added to the soil and microbial biomass increased (Table 3). This indicated that K-strategists are more competitive in decomposing substrates with low N availability (Blagodatskaya *et al*., 2010). N limitation was at least one reason behind this microbial shift from r- to K-strategists (Fig. 5). Such a shift can occur when active r-selected microbial groups are unable to uptake sufficient N to meet their growth requirements. Consequently, the K-strategists have preference under N limitation, as this group is able to decompose recalcitrant SOM for mineral N acquisition (Fontaine *et al*., 2011). The r-strategists could still commensally use SOM-derived compounds solubilized by the K-strategists.

In the presence of available C, higher N availability stimulates the growth of r-strategists (Blagodatskaya *et al*., 2007). As the concurrent availability of labile C and mineral N significantly promoted the mineralization of organics (Fig. 2) and increased the μ values, the r-strategists contributed to priming in Suc-C+min-N and MS-C+min-N larger as compared with Suc-C and MS-C treatments, respectively (Fig. 3 and Table 3). A similar significant shift in microbial community structure from fungi (especially white rot basidiomycetes) to bacteria was associated with increased availability of N and C, which was demonstrated by PCR-DGGE (Milcu *et al*., 2011). Thus, PE intensity is a consequence of interactions of factors affecting the competitive ability of fast-growing microorganisms. These factors include the presence of energy-rich substrates and nutrient availability.

Two hypotheses and microbial strategies. The soils amended with sucrose or straw had a positive PE,

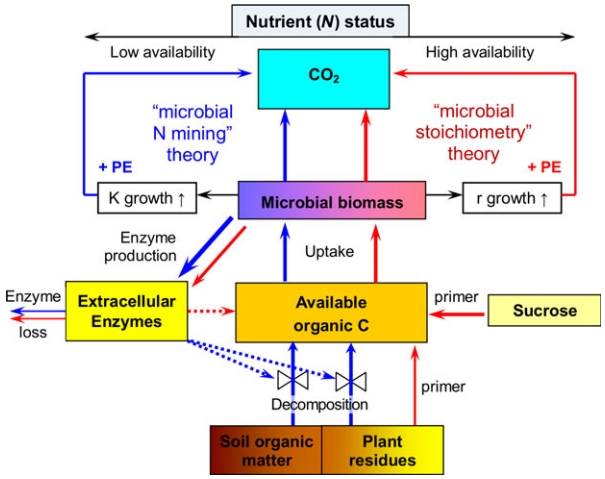

Fig. 5 Concept linking 'microbial N-mining' and 'microbial stoichiometry' theories by interactive effects of C and N availability on priming of soil organic matter decomposition in relation to microbial growth strategies and enzyme activity. The blue lines show the fluxes controlled by K strategists; the red lines show the fluxes controlled by r strategists. The dotted lines show the actions of extracellular enzymes. The darkness of colour of organic matter pools represents schematically their availability. See further explanations in text.

mainly due to 'microbial N mining' mediated by K-strategists (Fig. 2 and Table 3). However, in parallel, we observed larger PE in treatments with concurrent addition of C and N which corresponded to larger contribution of r-strategists in decomposition (Table 3), and such phenomenon contradicts both the 'microbial nitrogen mining' hypothesis (Moorhead & Sinsabaugh, 2006) and the bank hypothesis (Fontaine *et al.*, 2011), both predicting higher PE at low-nutrient availability. Thus, another mechanism, for example, 'stoichiometric decomposition' (Craine *et al.*, 2007), seems to be responsible for the PE under high-C and N availability in the substrate. This theory serves as an explanation for PE: namely increase in biomass and general enzymatic activity (Fig. 2 and Table 3) was accompanied by domination of r-strategists and increase in SOM decomposition.

Despite that the two mechanisms 'microbial N mining' and 'stoichiometric decomposition' look conflicting at the first glance, in reality both may operate individually or in combination depending on the availability of soil C and N at different spatial and temporal scales (Cheng & Kuzyakov, 2005). Our results demonstrated that 'microbial N mining' and 'stoichiometric decomposition' can co-exist in the same system and co-influence the strength and size of PE (Fig. 5). In addition, as a new finding we related it with the dominating contribution of r-strategists and K-strategists, respectively. K-strategists contribute more to PE in soils with low-nutrient availability, while r-strategists contribute more in soils with abundant nutrients (Fig. 5). Accordingly, the phenomenon described in 'microbial N-mining' theory (Moorhead & Sinsabaugh, 2006) can be ascribed to the contribution of K-strategists, whereas 'stoichiometric decomposition' theory (Hessen *et al.*, 2004), that is, accelerated OM mineralization due to balanced microbial growth, is explained by the domination of r-strategists.

Status switch between dormancy and activity. Our study experimentally revealed for the first time the domination of either r- or K-strategists during the priming effect in soil. In the control without additions, the lag-time was the longest as most microorganisms were dormant (Table 3). Faster microbial specific growth rates in control vs. amended soil (Table 3) revealed that r-strategists in the nutrient-poor soil responded faster to the glucose addition and won in a short-term the competition with K-strategists for occasional substrate input. However, in community growing on plant residues with low availability, or in active but starving community, the K-strategists also contribute to microbial growth – as a result the μ values decrease as compared with unamended control.

The addition of labile C and mineral N to soils drastically (by a factor of 16–18) increased the fraction of active biomass (Table 3). This explains why despite varying μ values the respiratory response of microorganisms in substrate treated soils was 2–3 times faster than in the long-term starving community (control). The duration of the lag-period, therefore mainly depends on the fraction of active microorganisms, while microbial growth rate is a consequence of the strategy currently dominating in the microbial community (Chen *et al.*, 2012).

We also demonstrated that the microbial switch from dormancy to activity is fast with extra energy input. In our study, this switch occurred within several hours so that respiratory maximum was reached in 17 h in the control (Fig. 3). In contrast, the reverse switch from activity to dormancy takes much longer (Nannipieri *et al.*, 1979; Blagodatsky *et al.*, 2000). In our experiment, it required longer than 10 days after substrate exhaustion and was reflected by a high level of active biomass (Table 3) even after the added sucrose had been utilized.

Priming effects and extracellular enzyme activities

Overall, adding sucrose and maize straw stimulated extracellular enzyme activities. In our study, this included *β*-glucosidase, cellobiohydrolase, chitinase, LAP, and xylanase (Fig. 4); in other studies, it included

protease and phosphatase (Cayuela *et al*., 2009). The enzymes produced after C amendment efficiently degraded C polymers (Dilly *et al*., 2007) and thus caused positive PE (Fig. 2). Total CO_2 emissions significantly correlated with all the tested extracellular enzymes (Table 4), indicating a good correspondence of microbial growth and extracellular enzyme production (Dorodnikov *et al*., 2009).

In contrast, the effects of N availability on extracellular enzymes were enzyme specific. High-N availability stimulated cellulose-degrading enzymes (Fig. 4), supporting many previous reports (Sinsabaugh *et al*., 2002; Frey *et al*., 2004). Other studies reported that high-N availability reduced the activity of ligninolytic enzymes, for example, phenol oxidase (Carreiro *et al*., 2000; Frey *et al*., 2004). In the present study, we determined that high-N availability significantly inhibited the activity of xylanase (Fig. 4), a hemicellose-degrading enzymes. A recent study (Tischer *et al*., 2014) reported that various drivers are responsible for hydrolytic enzyme activity in soil: *β*-glucosidase and cellobiohydrolase are related to abiotic factors controlling microbial biomass (e.g., pH, N, and P availability), while xylanase is mainly governed by the quality of plant residues. Supporting these findings, high-N availability stimulated cellulose decomposition and retarded lignin decomposition (Fog, 1988; Berg & Matzner, 1997). Our results suggest that N amendment tends to retard hemicellulose decomposition in unamended soils. As mineral N had various impacts on the decomposition of cellulose, hemicelluloses, and lignin, the resulting PE might reflect the integrated impacts on various organic C compounds. Therefore, the size of PE depends in some extent on the proportions of different organic components in soil, for example, on the cellulose/hemicellulose ratio. This understanding is helpful to explain the contrasting PE observed after N addition.

SOM mineralization and CO_2 emission in response to high-N availability tended to increase in soils of high-quality (with low-lignin and high-nitrogen content), but decline in soils of low-quality (with high-lignin and low-nitrogen content that decompose very slowly), especially forest soils (Janssens *et al*., 2010). Increased decomposition rate were often observed in forest soils with a high-lignin content (Janssens *et al*., 2010). The declines may attribute to the inhibitory effects of high-N availability on lignolytic enzyme production by white-rot fungi (Prescott, 2005), or to the increase in carbon use efficiency after N application (Fog, 1988). It indicated that high-N availability positively affected the transformation of exogenous C into more stable SOM (Moran *et al*., 2005). This call for studies on priming effects in forest soils with low pH, containing more fungi and with high-lignin and low-nitrogen litter.

Greater LAP activity under N limitation (control) compared with N-treated soil demonstrated that low mineral N availability generally induced protease activity (Sinsabaugh & Moorhead, 1994). The latter activity mitigates N limitation by mobilizing organic N compounds in soil (Lindén *et al*., 2012). Increasing utilization of N-containing compounds was also observed during the decomposition of maize litter, which has a high C/N ratio (Sharma *et al*., 1998). In contrast, high-N availability significantly reduced LAP activity (Fig. 4). This illustrates well the microbial shift from usage of soil organic N compounds toward usage of the soil mineral N pool at high mineral N availability.

Chitinase activity significantly correlated with total CO_2, SOM-derived CO_2, and substrate-derived CO_2 (Table 4). Chitinases generally reflect the dissolution and digestion of chitin (Sámi *et al*., 2001), which is mainly derived from the cell walls of fungi and the exoskeletons of soil animals. A direct link between fungal growth and PE was confirmed by PLFA (Nottingham *et al*., 2009) and by molecular fingerprinting techniques (Fontaine *et al*., 2011). Note, however, that not only fungal growth, but also the decomposition of dead fungal mycelium by bacteria was indicated by recycling of ^{13}C-substrates in the microbial PLFA. This reflects C redistribution in the soil during the microbial succession accompanying the priming effect (Rinnan & Bääth, 2009). The correlation between chitinase activity and priming effects was significant but slightly weaker than that between chitinase and total CO_2 efflux (Table 4).

Table 4 The Pearson correlations between CO_2 emissions and extracellular enzyme activity in soils amended with mineral N (min-N), sucrose (Suc-C), sucrose+mineral N (Suc-C+min-N), maize straw (MS-C), maize straw+mineral N (MS-C+min-N), and water (Control)

	β-glucosidase	Cellobiohydrolase	Xylanase	Chitinase	LAP
$TOTAL_{CO2}$	0.884†	0.865†	0.714†	0.774†	0.592*
SOM_{CO2}	0.832†	0.876†	0.448	0.610†	0.213
$Substrate_{CO2}$	0.559	0.347	0.577†	0.726†	0.435

*Correlation is significant at 0.05.

†0.01 levels (two-tailed). $TOTAL_{CO2}$, total CO_2 emissions; SOM_{CO2}, SOM-derived CO_2 emissions; $Substrate_{CO2}$, added substrate-derived CO_2 emissions; LAP, leucine amino peptidase.

This points nontargeted SOM decomposition by enzymes produced to degrade added substrate which is followed by commensal use of SOM-compounds during normal food web processes.

In conclusion, addition of mineral N alone had minimal impact on SOM mineralization. Exogenous organic C acted as a primer of SOM decomposition, switching microorganisms from a dormant to an active status and producing extracellular enzymes. In their active state, the contribution of microorganisms to SOM decomposition was controlled by nutrient (mineral N) availability. Interactions between C and N availability, however, drive the strength and extent of the priming effects (Fig. 5). Microbial K- and r- growth strategists promoted PE, depending on N availability: K-strategists contributed to PE more under low-N availability, whereas r-strategists contributed more under high-N availability. Although microbial growth and extracellular enzyme production were correlated, the effects of N availability depended on enzyme type. N addition stimulated the activities of β-glucosidase and cellobiohydrolase but decreased the activities of xylanase and LAP. We proposed that N amendment tends to retard the decomposition of hemicellulose. This calls for factoring in C availability when considering the effect of anthropogenic N deposition or mineral N fertilizer application on extra CO_2 released from soil. We coupled the $\delta^{13}C$ of CO_2 released by SOM decomposition with microbial growth kinetics and enzyme activities. This indicated that the priming phenomenon described in 'microbial N-mining' theory can be ascribed to the contribution of K-strategists, while 'microbial stoichiometry' theory, that is, accelerated OM mineralization due to balanced microbial growth, is explained by domination of r-strategists. Further studies on the effects of C and N availability on soil C cycling should go beyond merely evaluating the flow rate of C or C pool size. They should also pay close attention to SOM composition and structure as well as to changes in enzyme and microbial activities, as our work uncovers the more complex dynamics driving whether the priming effect does or does not occur with C and N amendments.

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