Contrasting effects of organic and mineral nitrogen challenge the N-Mining Hypothesis for soil organic matter priming

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Addition of easily available organic substances to soil often increases the CO_2 efflux from pre-existing soil carbon (C). This phenomenon is often explained in terms of the Nitrogen (N)-Mining Hypothesis. According to this proposed – but never conclusively proven – mechanism, increased C availability induces N limitation in microbes, which then access N by degrading soil organic matter (SOM) – a priming effect. This is supported by some experiments demonstrating reduced CO_2 efflux after mineral N addition. However, amino acids cause priming, despite their very low C:N ratios and rapid deamination to mineral N. To explore this contradiction, we applied ^{14}C- and ^{15}N-labelled C and N sources (glucose, alanine and ammonium sulfate) to rigorously test two key predictions of the N-Mining Hypothesis: (i) an amino acid should stimulate much less priming than glucose, and (ii) priming should be similarly suppressed for an amino acid or a stoichiometrically equivalent addition of glucose plus mineral N. Both of these key predictions of the N-Mining Hypothesis failed. Efflux of CO_2 from native C was essentially determined by the type and amount of C added, with alanine stimulating more priming than glucose (16–50% cumulative increase relative to control, versus 0–25%, respectively). Higher C additions caused more priming than low additions. Mineral N reduced native-C-derived CO_2 efflux when added alone or with organic substrates, but this effect was independent of the organic C additions and did not influence C-induced priming. These results were inconsistent with the hypothesized role of N mining in priming. We conclude that the N-Mining Hypothesis, at least in its current form, is not a universal explanation for observed priming phenomena.

Instead, we observed a strong correlation between the rates of priming and the mineralization of the added substrates, especially during the first 8 days. This indicated that priming was best explained by energy-induced synthesis of SOM-degrading exoenzymes, possibly in combination with apparent priming from accelerated turnover of microbial biomass.

1. Introduction

The processes governing soil organic matter (SOM) mineralization are not yet fully understood, despite their considerable importance to C sequestration, greenhouse gas emissions, soil fertility and groundwater protection. Mechanistic explanations often point to interactive effects of carbon (C) and other nutrients, notably nitrogen (N). The N-Mining Hypothesis is a prominent example. According to this hypothesis, N-limited microorganisms mineralize SOM to access the N contained within (Fontaine et al., 2011; Moorhead and Sinsabaugh, 2006). This can elegantly explain why an easily available C input often increases the CO_2 efflux from pre-existing soil C (Kuzyakov, 2010): when supplied with an abundant source of C and energy, microorganisms

become N-limited and actively degrade organic materials that they would not degrade to acquire C alone (Garcia-Pausas and Paterson, 2011). Therefore, according to the N-Mining Hypothesis, SOM mineralization is negatively correlated to N availability, and positively correlated to C availability (Fontaine et al., 2011).

A change in SOM mineralization rate in response to relatively moderate treatment is termed a priming effect (PE) (Kuzyakov et al., 2000). Organic substances added to soil will be mineralized and therefore contribute to the total CO_2 efflux, but isotopic labelling (^{13}C or ^{14}C) allows this to be distinguished from CO_2 derived from pre-existing soil C pools. Here we refer to these pools as "native C", which includes the living soil microbial biomass and non-living SOM at the time of exogenous C addition. Exogenous carbon could increase or

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suppress the mineralization of native C (Blogodatskaya and Kuzyakov, 2008). An increase in isotopically unlabelled (i.e. native-C-derived) CO_2 efflux could arise from two sources (in a carbonate-free soil): (i) altered SOM mineralization rate, which corresponds to the definition of priming and is termed "real priming" (Kuzyakov, 2010); or (ii) accelerated microbial turnover – "apparent priming" – in which older microbial biomass C is released as unlabelled CO_2 without necessarily affecting SOM mineralization (Blogodatskaya and Kuzyakov, 2008; Hamer and Marschner, 2005a). Although this mechanistic distinction is widely acknowledged, priming is typically measured as the overall change in CO_2 from native C (e.g., Bernal et al., 2016; Blogodatskaya and Kuzyakov, 2008; Di Lonardo et al., 2017), since apparent and real contributions to CO_2 are isotopically indistinguishable. Differences in native-C-derived CO_2 are often explained by the N-Mining Hypothesis, including in cases where evidence is not available to definitively confirm that real priming occurred (e.g., Chowdhury et al., 2014; Liu et al., 2017; Qiao et al., 2016). Hence, as it is applied in practice, the N-Mining Hypothesis explains changes in overall native-C-derived CO_2 efflux by invoking a real priming mechanism.

Isotopic labelling of the added C allows straightforward quantification of native-C-derived CO_2, but methods to directly distinguish apparent from real priming are not available (Kuzyakov, 2010). Direct quantification of SOM mineralization is hampered by the very high background SOM against which these relatively small changes occur. However, if positive priming is accompanied by a decline of similar magnitude in SOM-derived soil microbial biomass, this provides evidence of apparent priming (Blogodatskaya and Kuzyakov, 2008). Analysis of SOM-degrading potential enzyme activities has also been used to differentiate real and apparent priming (Wild et al., 2017).

The N-Mining Hypothesis is supported by evidence that mineral N fertilization reduces both native C mineralization (Zang et al., 2016), and priming by low molecular weight organic substances (Fontaine et al., 2011; Garcia-Pausas and Paterson, 2011), although this is not consistently observed (Chowdhury et al., 2014; Tian et al., 2016). However, an apparent contradiction emerges from numerous studies that have reported positive priming by amino acids (Dalenberg and Jager, 1989; Hamer and Marschner, 2005b; Wild et al., 2014). Amino acid C:N ratios are very low (from 1.3 for arginine to 7.7 for phenylalanine and tyrosine) and, like simple sugars and mineral N, they are readily available to a wide range of soil microorganisms (Barraclough, 1997; Jones, 1999). If N mining is in fact the main mechanism of observed priming phenomena, the N-Mining Hypothesis predicts that (i) amino acids should stimulate much less priming than glucose, and (ii) priming should be similarly suppressed for an amino acid or a stoichiometrically equivalent addition of glucose plus mineral N. To our knowledge, these predictions are yet to be tested under rigorously comparable conditions, with ^{14}C (or ^{13}C) labelling to distinguish between CO_2 from the added organic substrate and CO_2 from native C, and ^{15}N to trace the added N. Confirmation of these predictions would support the validity of the N-Mining Hypothesis. If these predictions fail, then priming cannot be fully explained by N limitation or the N-Mining Hypothesis in its current form.

We took the above two predictions as our hypotheses, and tested them under conditions that minimized potential confounding factors that could arise from, for example, different levels of mineral and organic N additions, or plant effects. Soil was incubated under controlled conditions and C was supplied in the form of either glucose or alanine, with simultaneous mineral N additions (ammonium sulfate) to control C and N stoichiometry (Table 1). The levels of both C and N were varied, so that N limitation was increased (by glucose addition) or decreased (by N addition). Glucose and alanine have the same formal carbon oxidation state of zero (Gunina et al., 2017), and the difference in standard free energies of formation between alanine and glucose plus ammonia is only 15 kJ mol^{-1} C (Hammes and Hammes-Schiffer, 2015), small in comparison to the total free energy change of -478 kJ mol^{-1} C for glucose oxidation (Milo et al., 2010). These physicochemical

Table 1

Schematic of the experimental design. The five types of organic addition (reference and two levels of each of glucose and alanine) are shown in the columns, and these were fully crossed with the mineral N additions shown as rows. Due to the organic N in alanine, this scheme provided several treatments with the same C:N (shown in gray shading) but different organic and mineral N contributions. Nt values are total added N. Treatment abbreviations are shown in each cell (e.g. GLN0 = glucose low, zero mineral N).

		C input (µg C g^{-1} soil)				
		0	GL (45)	GH (90)	AL (45)	AH (90)
Mineral N input (µg N g^{-1} soil)	N0	0N0 Nt = 0	GLN0 Nt = 0	GHN0 Nt = 0	ALN0 Nt = 17.5 C:N = 2.6	AHN0 Nt = 35 C:N = 2.6
	NL (17.5)	0NL Nt = 17.5 C:N = 0	GLNL Nt = 17.5 C:N = 2.6	GHNL Nt = 17.5 C:N = 5.1	ALNL Nt = 35 C:N = 1.3	AHNL Nt = 52.5 C:N = 1.7
	NH (35)	0NH Nt = 35 C:N = 0	GLNH Nt = 35 C:N = 1.3	GHNH Nt = 35 C:N = 2.6	ALNH Nt = 52.5 C:N = 0.86	AHNH Nt = 70 C:N = 1.3

similarities are also reflected in their bioenergetics: conversion of glucose and ammonia to alanine via the glycolysis pathway is energetically neutral in terms of ATP and reducing equivalents, if the prerequisite assimilation of ammonium into glutamate (as N source for transamination of pyruvate) occurs via glutamine synthetase (Voet and Voet, 2004). Corresponding glucose and alanine additions therefore represented similar amounts of metabolic energy and carbon (subject to uptake and catabolism), but different C:N ratios.

2. Materials and methods

2.1. Soil

Ap horizon (0–30 cm) of a silty loam Haplic Luvisol, with a pH of 6.2 and water holding capacity (WHC) of 34% of dry weight, was sampled from the Reinshof experimental farm near Göttingen, Germany, in late October 2016. The site conditions and soil properties have been previously reported in detail (Ehlers et al., 2000). Soil was sampled from three randomly selected locations on the same plot and combined into a single composite sample. The soil was slightly air dried overnight at room temperature to facilitate sieving, reaching approximately 60% of WHC, then sieved to 2 mm and stored at 4 °C for two days. Soil samples (35 g dry weight) were weighed into 125 mL sealable glass jars and pre-incubated for 12 days under experimental conditions to achieve stable CO_2 efflux before substrate additions.

2.2. Experimental design

The experiment included five levels of organic substrate: reference (no C addition), D-glucose or L-alanine, with these organic substances both at two concentrations (45 and 90 µg C g^{-1} soil). These C additions corresponded respectively to 45 and 90% of extractable microbial biomass C (by chloroform fumigation-extraction, see Supplementary Material). The five organic additions were fully crossed with three levels of mineral N (ammonium sulfate at 0, 17.5 and 35 µg N g^{-1} soil), selected to provide the same increments of N as the alanine additions. Hence, for example, low glucose + low mineral N (GLNL) had the same C and N stoichiometry as low alanine + zero mineral N (ALN0). The resulting 15 treatments (Table 1) provided a range of C and N addition levels and C:N ratios, reflecting C and N limitation as well as C and N excess. Total N addition ranged from zero, for 0N0, GLN0 and GHN0, to 70 µg N g^{-1} soil for AHNH. For each treatment, a total of 12 replicates were prepared from the composite field sample, providing four