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Stoichiometric controls upon low molecular weight carbon decomposition

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ABSTRACT

Soil carbon (C) and nitrogen (N) cycles are inextricably linked, yet the impacts of N availability upon soil C sequestration and turnover are poorly understood. According to stoichiometric theory, in the absence of nutrient limitation substrate decomposition will reach maximum rates, with C assimilated into microbial biomass at the expense of CO_2 production. In this study, we added a ^{14}C labelled low molecular weight substrate (glucose) to a sandy soil along with eleven increasing levels of N, phosphorus (P), and sulphur (S) in relative proportions as required for microbial biomass production. Adding a simple soluble substrate allowed us to explicitly examine changes in microbial transformations of added C, rather than changes resulting from extracellular enzyme activity or the extent of substrate decomposition. We hypothesized that as nutrient addition increased, an increasing proportion of the glucose-C provided would be incorporated into microbial biomass at the expense of CO_2 production and stabilized as soil organic carbon (SOC). Instead, CO_2 production from glucose-C increased significantly with nutrient addition without measurable changes in glucose-derived microbial biomass or SOC. This suggests that if there was greater glucose-derived microbial biomass produced under higher nutrient addition it was offset by a higher rate of microbial biomass turnover. We also found greater soil-derived microbial biomass at lower nutrient addition levels, potentially supporting the concept of microbial mining of soil organic matter (SOM) for nutrients under low nutrient availability. In conclusion, our data suggest that in a sandy soil with low capacity for physical protection of SOM, nutrient addition does not immediately promote C sequestration in the soil microbial community, and that the interaction between C stabilization and nutrient addition requires further work, especially for predicting ecosystem responses.

1. Introduction

As a result of climate change and climate change policy, there is great interest in promoting terrestrial carbon (C) sequestration in plants and soils (Lal, 2004). One major uncertainty in soil organic C (SOC) stabilization lies at the nexus between C and N cycling (Gärdenäs et al., 2011). Understanding the feedbacks between C and nutrient cycling is of particular importance, due to high levels of anthropogenic nutrient deposition (in particular N) experienced worldwide (Vitousek et al., 1997; Liu et al., 2013), and potential feedbacks between soil warming, elevated CO_2, and nutrient cycling (Melillo et al., 2002; Dijkstra et al., 2012; Billings and Ballantyne, 2013).

Ecosystem and soil C stocks show variable responses to nutrient addition (Mack et al., 2004; Khan et al., 2007; Pregitzer et al., 2008). Increases in ecosystem C in response to nutrient addition have been variably attributed to increases in aboveground net primary productivity of plants (Nadelhoffer et al., 1998; Magnani et al., 2007), although changes in the rate of litter or SOM decomposition, mediated through the activity and structure of the microbial community, have also been implicated (Reay et al., 2008).

Several mechanisms have been proposed to explain microbially-mediated changes in C decomposition as a result of nutrient (in particular N) addition. Under nutrient limitation, microorganisms may decompose SOM to obtain nutrients from the soil, so that upon nutrient addition this microbial 'mining' may decrease and consequently SOC stocks should increase (Moorhead and Sinsabaugh, 2006; Craine et al., 2007; Billings and Ziegler, 2008). Conversely,

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SOC stocks may increase under nutrient limitation as microbial substrate decomposition may be highest under nutrient addition (Hessen et al., 2004). If the nutrient requirements to build microbial biomass are met (i.e. nutrient stoichiometry), this decomposing C should be used to build microbial biomass rather than respired as CO_2 (Schimel and Weintraub, 2003; Hessen et al., 2004; Sinsabaugh et al., 2009). If this microbial biomass, which can form a relatively stable SOM pool (Simpson et al., 2007; Liang and Balser, 2008, 2011; Miltner et al., 2012) is stabilized in the soil, it could potentially reduce losses of soil C (Kirkby et al., 2013, 2014). There are also impacts of nutrient addition upon C decomposition related to C chemistry, where more chemically labile or physically unprotected SOC decomposes faster with N addition, but more chemically stable or physically protected SOC decomposes more slowly (Berg and Matzner, 1997; Neff et al., 2002; Waldrop et al., 2004). This slowing of decomposition has generally been attributed to the suppression of lignin-degrading enzymes (Carreiro et al., 2000; Zak et al., 2008) although this response has been inconsistently linked to corresponding changes in C chemistry (Grandy et al., 2008; Thomas et al., 2012). Finally, there has also been recent evidence that microbial community structure, particularly the abundance of r-versus K-strategists, can alter the response of C decomposition to nutrient addition (Chen et al., 2014; Kaiser et al., 2014). Any one (or a combination) of these mechanisms may be relevant in understanding the response of soil C stocks to nutrient addition.

In this study, we set out to characterize just one of these mechanisms, specifically whether nutrient addition increased C incorporation into microbial biomass at the expense of CO_2 production, and whether this biomass was then stabilized as SOC. We chose to add a simple C substrate (glucose) rather than a complex substrate as it doesn't require extracellular enzyme production for breakdown, is not strongly sorbed to the solid phase, can be utilized almost ubiquitously by the soil microbial community, and should be completely transformed to CO_2, microbial biomass, or SOC under all nutrient treatments. In this way, we could explicitly test the concept of re-direction of C from overflow metabolism to microbial growth in response to nutrient addition. The incubation was carried out in a nutrient poor sandy soil to minimize adsorption of added nutrients to mineral surfaces. If increasing nutrients do indeed result in a greater formation of microbial biomass at the expense of CO_2 production, we should observe (a) decreased respiration of glucose derived carbon, (b) increases in glucose-derived microbial biomass carbon, and/or (c) increases in glucose-derived soil organic carbon. However, if this response is driven by the capacity of the soil to stabilize microbial biomass, the production of extracellular enzymes, C chemistry or accessibility, or microbial community structure, these trends will not be observed.

2. Materials and methods

2.1. Soil sampling

Soil (0–10 cm depth; sieved to 2 mm) was sampled in November 2012 from an agricultural aridic arenosol located in Badgingarra in Western Australia (30°20′38″S, 115°36′36″E). The soil was sandy textured, poorly structured, and was comprised of 58.0% coarse sand (200 μm–2 mm), 35.8% fine sand (20–200 μm), 1.8% silt (2–20 μm), and 1.7% clay (<2 μm) (measured as outlined in Rosewell (2002)). On a dry weight basis, the soil contained 13.0 mg g^{-1} organic C, 0.90 mg g^{-1} total N, 133.2 mg kg^{-1} total P, and 90.6 mg kg^{-1} total S. The soil had a pH of 5.91 (in water) and the absence of carbonates was confirmed upon the addition of 4 M HCl. The predominant land use and management at the site were characteristic of long term wheat (*Triticum aestivum* L.) production.

2.2. Nutrient addition

The nutrient addition levels were based upon the assumption that the glucose-C would be incorporated into microbial biomass and not respired via overflow respiration (greater respiration without associated microbial growth) if there was sufficient N, P, and S available. We framed the N, P, and S additions in the context of carbon use efficiency (CUE), which is defined as the amount of C in microbial biomass relative to the sum of C in microbial biomass and C respired as CO_2 ($\text{CUE} = \text{C}_{\text{BIOMASS}}/(\text{C}_{\text{BIOMASS}} + \text{C}_{\text{CO}_2})$). We estimated CUE under nutrient addition with the model presented in Sinsabaugh et al. (2013), which assumes C that is not used for growth as a result of nutrient limitation is re-directed to overflow respiration:

$$\frac{\text{CUE}}{\text{CUE}_{\max}} = \min\left[1, \frac{A_E \times B_{C:E}}{\text{CUE}_{\max} \times S_{C:E}}\right] \tag{1}$$

where $\text{CUE}_{\max}$ (the maximum CUE) is assumed to be 0.60 (defined here as predicted CUE) (Manzoni et al., 2010; Sinsabaugh et al., 2013), A_E is the assimilation efficiency of the nutrient E (N, P, or S), $B_{C:E}$ is microbial biomass $C:E$ ratio, and $S_{C:E}$ is the ratio of glucose-C to added nutrient E. A_E was assumed to be equal to 1, as we were adding the nutrients in inorganic forms, although it can be lower if extracellular enzymes are produced (Sinsabaugh and Follstad Shad, 2012). We assumed a microbial biomass C:N:P:S ratio of 75:8.75:1.25:1 based on values published in the literature for soil microbial communities (Banerjee and Chapman, 1996; He et al., 1997; Chowdhury et al., 1999, 2000; Cleveland and Liptzin, 2007; Kirkby et al., 2011). Based on the amount of glucose-C added and our initial soil analysis, we assumed that microbial use of soil-derived N, P, and S would be negligible. We therefore calculated $S_{C:E}$ ratios based upon the N, P, and S additions, which were added in solution as NH_4NO_3, KH_2PO_4 and Ca_2SO_4, respectively (pH 6.5) (Table 1).

2.3. Soil incubation and destructive harvesting

Oven-dried (40 °C for 48 h) soils (5 g) were placed into 50 ml polypropylene tubes, wetted to 35% water holding capacity with 18 MΩ water, capped, and pre-incubated in the dark at 22 °C for 14 d. After pre-incubation, uniformly ^{14}C labelled glucose (6.86 kBq ml^{-1}, 0.307 kBq mg^{-1} C) was added at a rate equivalent to 5% of soil-C along with one of the eleven levels of N, P, and S additions (Table 1) to bring the soil up to 65% water holding capacity. These high levels of glucose-C were provided to induce the production of microbial biomass and to minimize problems of isotopic pool dilution (Boddy et al., 2007). There were four replicates per NPS treatment.

Table 1
N, P, and S addition rates per 100 mg glucose-C added to the soil and the corresponding *C:E* ratios of the added C and nutrients, and modelled CUE values. Predicted CUE assumes a $\text{CUE}_{\max}$ of 0.60.

Nutrient addition	mg N	mg P	mg S	C:N	C:P	C:S	Predicted CUE
1	0.00	0.00	0.00	n/a	n/a	n/a	0.00
2	1.17	0.17	0.13	85.7	600.0	750.0	0.10
3	2.33	0.33	0.27	42.9	300.0	375.0	0.20
4	3.50	0.50	0.40	28.6	200.0	250.0	0.30
5	4.67	0.67	0.53	21.4	150.0	187.5	0.40
6	5.83	0.83	0.67	17.1	120.0	150.0	0.50
7	7.00	1.00	0.80	14.3	100.0	125.0	0.60
8	8.17	1.17	0.93	12.2	85.7	107.1	0.60
9	9.33	1.33	1.07	10.7	75.0	93.8	0.60
10	10.50	1.50	1.20	9.5	66.7	83.3	0.60
11	11.67	1.67	1.33	8.6	60.0	75.0	0.60