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Rapid microbial uptake and mineralization of amino acids and peptides along a grassland productivity gradient

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ABSTRACT

Amino acid and oligopeptide-nitrogen (N) forms only a minor component of the total dissolved N pool in grassland soils, yet the importance of these N-pools for plant productivity will ultimately depend on the rate at which these pools turnover. Fluxes of dissolved organic matter (DOM) through the soil solution are frequently estimated from measurements of respiration, but this method fails to consider any delay between microbial substrate acquisition and mineralization. Here, we added ^{14}C-labelled alanine and tri-alanine (10 μM) to 4 soils collected from a natural grassland productivity gradient and then measured substrate depletion from the soil solution and the subsequent production of $^{14}CO_2$ resulting from mineralization at 1–60 min. There was a considerable delay between microbial ^{14}C removal from the soil solution, which occurred extremely rapidly (up to 96% of added substrate depleted within a minute), and $^{14}CO_2$ evolution resulting from the fast turnover of the alanine and tri-alanine. This indicates that amino acid and peptide longevity in the soil solution of the soils in this grassland productivity gradient has been greatly overestimated from measurements of mineralization alone. Rates of substrate uptake and mineralization by microbes declined in less productive, N-limited grassland soils with lower levels of microbial biomass, suggesting that the availability of organic N for plant uptake is likely to be controlled by soil microbial activity. We estimate that amino acid and peptide pools occurring in the most productive grassland soils may turnover at a rate of up to 20 times a minute, representing a very considerable flux of N through the soil.

1. Introduction

Unravelling the processes that determine turnover rates of organic nitrogen (N) in soil and its fate within different plant and soil fractions is fundamental to improving our understanding of plant-microbial ecosystem functioning. In most agricultural soils, dissolved organic N (DON) typically represents around 30% of the total dissolved N (TDN) pool (Christou et al., 2005), whereas in lower productivity, unfertilised grasslands DON is often the dominant N pool (Farrell et al., 2011a). The fate and soil residence time of DON is therefore of particular interest in less productive systems as it has implications for the availability of N forms, such as amino acids and peptides, for microbe and plant utilisation.

Traditionally, mineralization of organic N to NH_4^+ by soil microorganisms has been regarded as the rate limiting step in releasing plant available-N; however, evidence increasingly suggests that plants from a range of ecosystems have the ability to access N directly and independently of microbial breakdown from amino acids (e.g. Owen and Jones, 2001), peptides (Hill et al., 2011a, c) and even proteins (Paungfoo-Lonhienne et al., 2008). Indeed, some studies suggest that in nutrient limited systems at least, plants are able to compete with microorganisms for significant proportions of soil organically-bound N (Bardgett et al., 2003; Hill et al., 2011a). This suggests that the depolymerisation of N compounds by extracellular enzyme activity to smaller peptides and amino acids is frequently the rate-limiting step (Chapin et al., 2002; Schimel and Bennett, 2004; Jan et al., 2009). However, the outcome of competition between plants and soil microbes is likely to depend on the ecosystem and plant species in question (e.g. Owen and Jones, 2001; Bardgett et al., 2003; Kahmen et al., 2009) and the size, activity and N-limitation of the soil microbial pool (Schimel and Chapin, 1996; Schimel and Bennett, 2004; Dunn et al., 2006).

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Whilst gross rates of N cycling in soil (N mineralisation, nitrification and inorganic N assimilation) are mainly assessed using isotope dilution studies (see review by Booth et al., 2005), rates of ON and organic carbon (OC) turnover in soil can be measured by adding isotopically labelled (^{13}C, ^{14}C) substrates to soil in the lab (although see in-situ labelling; Boddy et al., 2007) and measuring the resultant enriched CO_2 released. Such methods are useful for measuring the turnover of the specific compounds in question. These studies have quantified two main mineralization phases (Boddy et al., 2007): the first phase, rapidly respired CO_2 attributable to the immediate use of the added substrate for catabolic processes, accounts for approximately 7–16% of added amino acid-^{14}C and has a half-time ranging from 22 ± 5 min (measured in-situ in temperate grassland; Boddy et al., 2007; Table 1) to 219 ± 28 min (submontane acid grassland soil; Farrell et al., 2011b). The authors of the studies suggest that the second slower phase may be attributed to CO_2 production from the subsequent turnover of the soil microbial community, which represents 48–87% of added ^{14}C and turns over much more slowly (Boddy et al., 2007, 2008; Farrell et al., 2011b). However, it is also possible that the gradual release of abiotically retained compounds to the soil solution, followed by subsequent microbial turnover, may contribute to this secondary phase in some cases. Comparisons of turnover rates between N species suggest that peptide half-times are generally shorter, and the proportion of ^{14}C allocated to such pools is usually larger than those of amino acids (Farrell et al., 2011b; Hill et al., 2011b).

However, this approach does not provide a reliable indication of substrate longevity in the soil solution (Jones et al., 2003), and thus availability for direct uptake by plant roots, as it fails to consider any delay between microbial uptake and mineralization. Using an experimental approach designed to separate these two processes by simultaneously measuring depletion of ^{14}C-labelled glucose from the soil solution and $^{14}CO_2$ evolution from the soil itself, Hill et al. (2008) found that a 10 μM addition of glucose had a soil solution half-time of only 24 s, as opposed to ca.8 min in the rapid first phase of mineralization. Furthermore, when added to soil at a concentration of 1 μM, the microbial community removed up to 93% of added glucose within a minute. It has also recently been shown in one soil that rates of uptake of amino acids and peptides are much faster than previously estimated from rates of mineralization, with half-times in the soil solution of less than 40 s (Hill et al., 2012). However, no previous direct comparison of rates of uptake and mineralization has been made for these substrates. These findings suggest that studies relying on $^{14}CO_2$ evolution measurements vastly underestimate the residence time of compounds in soil solution.

The aim of this study was to compare the rates at which amino acids and peptides are removed from soil solution by the soil microbial community, and thus rendered unavailable for direct plant uptake, and the rate of the subsequent microbial mineralization. This was done using soil collected from 4 sites located along an elevation gradient in North Wales, that represented a gradient of grassland productivity and soil dissolved inorganic N (DIN)/DON availability (Farrell et al., 2011a). We chose to use alanine as the amino acid monomer as it is occurs commonly as free amino acids and short peptides across the 4 grasslands (Farrell et al., 2011a). Furthermore, alanine and its polymers have been previously found to undergo rapid mineralisation by microbes in grasslands (Farrell et al., 2011b) as well as taken up directly by plants in both sterile (Hill et al., 2011b) and *in-situ* conditions (Hill et al., 2011a). Specifically we addressed the following hypotheses: (1) peptide and amino acid removal from soil will be even faster than rates previously estimated using measurements of ^{14}C release from these soils (Farrell et al., 2011b; Hill et al., 2008, 2012); (2) uptake and mineralization rates at the more productive, lowland site will be faster than upland, less productive sites due to increases in microbial biomass and reductions in N-limitation (Farrell et al., 2011a; Schimel and Chapin, 1996; Schimel and Bennett, 2004); and (3) amino acid removal from soil solution will be faster than peptide removal due to the smaller size of the molecule; however, rates of peptide C and N mineralization will be faster than that of amino acids (Farrell et al., 2011b; Hill et al., 2011b) as peptides are more enriched in C and N on a molar basis.

2. Materials and methods

2.1. Soil characteristics and preparation

Soil was collected from 4 sites located along a gradient of grassland productivity, altitude and soil DIN/DON availability at Abergwyngregyn, Gwynedd, North Wales (Table 2; Farrell et al., 2011a). The 4 selected sites represent a gradient from high productivity, inorganic N (IN) dominated grassland to low productivity, high organic matter (OM) and organic N (ON) dominated grassland (Farrell et al., 2011a). Annual mean temperature and rainfall range from 9.8 °C to 800 mm at site 1 to 6.5 °C and 2300 mm at site 4. Soil classifications for the 4 sites in order of elevation (lowest first) are: Eutric Cambisol (hereafter referred to as site 1, or soil 1), Cambic Podzol (site/soil 2), Haplic Podzol (site/soil 3) and Fibric Histosol (site/soil 4). These 4 sites correspond to sites 1, 3, 4 and 5 in Farrell et al. (2011a). Soil characteristics have previously been measured for these 4 sites and summary values are presented in Table 2.

At each site, 1 kg of soil was collected from the top 10 cm of the soil profile at 3 points (representing 3 replicates) located 2 m apart, placed in gas permeable plastic bags and transported immediately to the laboratory. Soil was gently sieved to 2 mm for homogenization and to remove roots, and stones. Soil was stored at 4 °C and

Table 1
Summary of half-times and pool sizes measured in previous amino acid and peptide mineralization studies.

Soil/vegetation type	Compound	a_1 (%)	a_1 $t_{1/2}$ (min)	a_2 (%)	Reference
Temperate grassland (lab)	L-amino acids	7.0 ± 0.1	36.6 ± 6.0	47.9 ± 0.8	Boddy et al. (2007)
Temperate grassland (in-situ)	L-amino acids	9.3 ± 1.1	22.2 ± 4.8	57.4 ± 1.2	Boddy et al. (2007)
Arctic tundra (*Carex*, 10 °C): Zeppelinfjellet	L-amino acids	13.0 ± 1.3	89.4 ± 2.4	87.0 ± 1.2	Boddy et al. (2008)
Arctic tundra (*Carex*, 10 °C): Stuphallet	L-amino acids	13.0 ± 0.3	46.8 ± 3.6	86.7 ± 0.3	Boddy et al. (2008)
Global soils (mean values)	Mixed amino acids	ca. 29	108.0 ± 6.0	71.0 ± 1.0	Jones et al. (2009)
Temperate agricultural land (Eutric cambisol)	Alanine	16.0 ± 1.0	154.8 ± 10.2	83.8 ± 1.0	Farrell et al. (2011b)
Temperate agricultural land (Eutric cambisol)	Tri-alanine	29.7 ± 0.3	55.7 ± 5.2	70.7 ± 0.3	Farrell et al. (2011b)
Sub-montane acid grassland (Fibric histosol)	Alanine	15.6 ± 0.9	219.6 ± 28.2	84.8 ± 1.0	Farrell et al. (2011b)
Sub-montane acid grassland (Fibric histosol)	Tri-alanine	17.2 ± 1.7	63.0 ± 3.0	84.1 ± 1.6	Farrell et al. (2011b)
Antarctic *Deschampia antarctica* swards	Alanine	13.0 ± 2.0	36.0 ± 6.0	15.0 ± 1.0	Hill et al. (2011b)
Antarctic *Deschampia antarctica* swards	Tri-alanine	27.0 ± 0.9	30.0 ± 0.3	15.0 ± 5.0	Hill et al. (2011b)

a_1 and a_2 are estimated pool sizes for fast and slow phases of mineralization, and a_1 $t_{1/2}$ represents the half-time for pool a_1 in minutes.