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Soil microbial organic nitrogen uptake is regulated by carbon availability

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ABSTRACT

Plants and microorganisms intensely compete for nitrogen (N) at many stages of the terrestrial N cycle. In particular, the dissolved organic N (DON) pool, and competition for low molecular weight dissolved organic N (LMWDON) compounds such as amino acids and peptides (and LMW dissolved organic matter; LMWDOM as a whole) has received significant recent research interest. However, as LMWDON compounds contain both N and carbon (C), a question that remains is whether soil microorganisms are primarily taking up LMWDON mainly for the C or the N contained therein. We investigated microbial uptake rates of the model peptide L-trialanine as a rapidly cycling LMWDON compound in temperate grassland soils of differing fertility using ^{14}C labelling to assess how soil fertility status influenced microbial uptake of LMWDON. We then imposed an excess of C as glucose and/or N as NH_4Cl to ask whether the uptake of the peptide was affected by C or N excess. Our results demonstrate that L-trialanine is taken up rapidly from the soil solution ($t_{½}$ < 1.5 min), and that an excess of C, rather than N, resulted in a reduced uptake of the peptide. From this, we conclude that LMWDON is taken up primarily to fulfil the C requirement of soil microorganisms, indicating that they exist in a C-limited state, and are able to respond quickly to a transient influx of an easily metabolisable resource.

1. Introduction

Ecological stoichiometry is both an important driver of ecosystem population dynamics (Andersen et al., 2004), and litter decomposition and nutrient cycling in soils (Manzoni et al., 2008). There is strong inter- and intra-specific competition between and within plant and microbial communities for soil nutrients (Kuzyakov and Xu, 2013). In most terrestrial and maritime ecosystems, nitrogen (N) is considered to be the major limiting nutrient (Vitousek and Howarth, 1991), and this has been demonstrated to increase under elevated atmospheric CO_2 concentrations where increased root inputs of carbon (C) occur (Hu et al. 2002). However, while N and also phosphorus (P) may be the major limiting nutrients to primary production, which is a process that is not C limited due to photosynthetic C fixation, microbial heterotrophs in the soil must acquire C through the breakdown of organic inputs from primary producers.

Soil organic matter (SOM) contains a range of N compounds resulting from fertilisation by humans, animal excreta, N_2 fixation, atmospheric deposition, and the incorporation of dead and decaying plant and microbial residues, the latter of which represents the main direct input of organic N to the soil. The majority of soil N is in the organic pool, and this consists of a diverse range of polymeric molecules (Leinweber et al., 2013). Dissolved organic N (DON) in the soil solution is equally diverse, containing compounds across a mixture of molecular sizes and compound types, with high molecular weight (HMW) proteinaceous polymers dominating (Farrell et al., 2011a; Warren, 2013a, 2014).

There is an important functional distinction between HMW (>1 kDa) and low molecular weight (LMW) (<1 kDa) DON. Low molecular weight DON consists of oligomers and monomers, many of which can be taken up directly by both soil microorganisms and plants at rapid rates over a period of minutes to a few hours

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dependent upon methods used and the compounds and species in question (Farrell et al., 2011b, 2013; Hill et al., 2011a,b, 2012; Soper et al., 2011; Warren, 2013b). In comparison, HMW DON such as proteins generally require extracellular enzyme mediated degradation to oligomers and monomers (Jan et al., 2009), though slow uptake of intact proteins and even viable microorganisms has been observed in plants (Hill et al., 2013; Paungfoo-Lonhienne et al., 2008, 2010). Therefore, while plant uptake of intact proteins is a potential theoretical mechanism of N assimilation, it is the degradation of HMW proteins into LMW peptides and amino acids that appears to be at the frontier of plant-microbial competition for N in soils.

Microbial utilisation of amino acids and peptides has been observed to be universally rapid across ecosystems (Jones et al., 2009a; Farrell et al., 2013). As DON contains both C and N, one question that prevails is whether soil microorganisms take up DON compounds such as amino acids and peptides primarily for their C or their N content, and whether the soil nutrient status influences this. It has previously been speculated that the rapid utilisation of amino acids by soil microorganisms is a function of microbial C limitation, rather than N limitation (Jones and Murphy, 2007). Recently, Farrell et al. (2013) found a strong positive relationship between the C status of soils and their rate of peptide-N flux, though causality was not established. A direct indication that the soil microbial community is C, rather than N limited was also provided by Prober et al. (2005). In that study, doses of sucrose were applied to a degraded habitat to successfully reduce nutrient availability through the stimulation of microbial activity, thus encouraging native grassland regrowth through the imposition of severe N limitation. On a wider N cycling scale therefore, microbial C limitation appears to be significant, the question as to whether DON uptake by soil microorganisms is in response to C or N limitation has yet to be answered. Therefore, the aim of this study was to establish whether soil microorganisms take up DON for its C, N, or both.

As the importance of DON on an ecosystem level is presumed to be greatest under nutrient limiting systems, we sampled a long term ecological field trial in which nutrient levels had been manipulated by mowing and fertiliser treatments (Simpson et al., 2012; Adair et al., 2013). The rate of microbial L-trialanine (a model peptide) uptake (*sensu* Hill et al., 2012) was then measured in a factorial laboratory incubation experiment in which treatments of an excess of labile C (as glucose), N (as NH_4Cl) or a combination were overlaid on the soil samples from the six field fertility management regimes. We chose to analyse peptide uptake, as opposed to FAA (free amino acid) uptake, as peptides have recently been demonstrated to be the point in the protein degradation pathway in soils where plants and microorganisms can first compete for uptake of these oligomers as intact molecules (Farrell et al., 2011b, 2013; Hill et al., 2011a,b, 2012; Soper et al., 2011). We hypothesised (1) that peptide uptake rate would be fastest in the most nutrient depleted field trial treatment, and (2) that peptide uptake would be down-regulated by the addition of an excess of labile C.

2. Materials and methods

2.1. Field experiment and soil sampling

The Lincoln long-term ecology trial was established at Lincoln University, New Zealand (43°38′51″S, 172°28′05″E) in September 1994 on a silt loam soil (Udic Ustochrept [USDA]) to establish the effects of different pasture management practices on plant diversity and soil properties (Simpson et al., 2012; Adair et al., 2013). Six treatments were established in 5 × 5 m plots arranged in randomised blocks, and comprised no mowing, mowing with clippings retained, and mowing with clippings removed, with and without nitrogen fertiliser addition (50 kg N ha^{-1} applied in spring) (Table 1). Mowing was carried out when the sward reached a height of approximately 20 cm (5–6 times per annum), and the trial was not grazed and did not receive irrigation. Soils were sampled from the field plots to a depth of 7.5 cm on 3rd Nov 2011 (17 years after trial establishment), sieved to 4 mm and immediately frozen until use. Roots visible to the naked eye were removed prior to analysis.

Table 1
Grassland management treatment codes for the experimental treatments used in the study.

Code	Fertiliser	Vegetation treatment
F_0M_0	No	Not mowed
$F_0M_1C_1$	No	Mowed, clippings retained
$F_0M_1C_0$	No	Mowed, clippings removed
F_1M_0	Yes	Not mowed
$F_1M_1C_1$	Yes	Mowed, clippings retained
$F_1M_1C_0$	Yes	Mowed, clippings removed

2.2. Soil chemical characterisation

Soil pH and electrical conductivity analyses were performed on a 1:5 w/v slurry using standard electrodes. Total organic C and N was determined by automated dry combustion (Carlo Erba NA 1500 Elemental Analyser; CE Elantech, Lakewood, NJ) on soils that had been air-dried at 40 °C for 48 h. Available P was estimated by the method of Olsen et al. (1954), followed by colourimetric analysis using the malachite green method of Ohno and Zibilski (1991) on a SynergyMX microtitre plate reader (BioTek; Winooski, VT). Microbial biomass C/N (MBC/N) were determined as the difference between DOC/N concentrations in 0.5 M K_2SO_4 extracts of soil that was either extracted directly for 30 min, or fumigated in a $CHCl_3$ atmosphere for 24 h prior to the same 30 min 0.5 M K_2SO_4 extraction using K_{EC} and K_{EN} factors of 0.35 and 0.5 respectively (Voroney et al., 2008). Concentrations of C and N in the extracts were determined on a Thermalox dry combustion analyser (Analytical Sciences Ltd., Cambridge, UK).

Soil was shaken for 15 min with 18.2 MΩ water at 4 °C to avoid losses through microbial activity during extraction (Rousk and Jones, 2010). These water extracts were then analysed for NO_3^- and NH_4^+ using the methods of Miranda et al. (2001) and Mulvaney (1996), respectively, on the same SynergyMX microtitre plate reader. Free amino acid N (FAA-N) was determined by the *o*-phthaldialdehyde fluorescence method of Jones et al. (2002), again using the same SynergyMX microtitre plate reader. Dissolved organic C and total dissolved N (TDN) were determined on the same Thermalox dry combustion analyser used for MBC/N quantification, and DON was determined by subtraction of NO_3^- and NH_4^+ from the TDN value. All values are reported on a dry weight basis.

2.3. Microbial uptake of ^{14}C-labelled trialanine peptide

Treatments representing the two extremes in terms of nutrient status (low: $F_0M_1C_0$, high: $F_1M_1C_1$) were used to assess peptide uptake rates following the method of Hill et al. (2012). These treatments were selected on the basis of the total soil C and N concentrations that are a good long-term integrator of plant inputs and intrinsic fertility, and from annually collated unpublished data on the field trial (Leo Condron, pers. comm.). Briefly, 1 g fwt (equivalent to 0.81 g dwt) sieved soil was placed into a 1.5 mL microcentrifuge tube in which a hole had been pierced in the bottom. This assembly was placed into another, intact,