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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,

microcentrifuge tube. To the surface of the soil, 191 μL ^{14}C-labelled L-trialanine (1.5 kBq ml^{-1}) was added at a concentration of 10 μM. This is equivalent to the average amount of soil moisture present (range of 18.3–20.5%) in 1 g of the fresh soil added to the tube. In order to correct for abiotic sorption of the peptide, and also to account for deviation from perfect mixing with the standing pool as a result of incomplete extraction of pore water for analysis (Hill et al., 2008, 2012), a duplicate set of samples were first sterilised by autoclaving prior to label addition. Data derived from this sterile set of replicates was used to correct microbial uptake rates in the non-sterile samples (ca. 13% ^{14}C sorbed by 1 h, with no sorption detected before 10 min). Replicate samples (sterile and non-sterile) were incubated at 15 °C for a period of 1, 5, 10, 20, 40, and 60 min before centrifugation of the tube assemblies at 4000 g for 1 min to facilitate collection of free soil solution. An aliquot of this solution was then transferred to a 6 mL scintillation vial to which 4 mL scintillation fluid (Optiphase Hisafe 3; PerkinElmer Inc.) was added before analysis by liquid scintillation counting in a Tri-Carb 3110 TR scintillation counter (PerkinElmer Inc.) to determine the amount of ^{14}C remaining in solution after incubation.

The dynamics of peptide uptake over time were described by fitting a first order single exponential decay curve of the form:

$$R = Y_0 + [a \times \exp(-kt)] \quad (1)$$

where R is the ^{14}C remaining in the soil solution, k is the exponential coefficient describing depletion by the soil microbial community, a describes the size of the depleting pool, t is time, and Y_0 is an asymptote. This equation described the uptake of the peptide well in both soils ($r^2 \geq 0.96$). The half-life ($t_{½}$) of the peptide soil solution pool (a) was calculated as:

$$t_{½} = \ln(2)/k \quad (2)$$

2.4. Effect of excess C and N on peptide uptake

To assess whether soil microbial peptide uptake is affected by C and N availability, incubations similar to those outlined above were carried out on samples from all six field treatments. Across the field treatments of mowing and fertiliser addition, an excess of C as glucose, N as NH_4Cl, or C + N was applied at a concentration of 9 mM glucose-C and 3 mM NH_4Cl–N (equivalent to 2.12 μmol C and 0.707 μmol N g^{-1} soil). This represents a 100-fold excess over the C and N applied as labelled peptide (a solution concentration of 10 μM trialanine is equivalent to an addition of 90 μM C and 30 μM N in the added solution), and these C and N excesses were applied simultaneously with the labelled trialanine. A control treatment was also used where ^{14}C-trialanine was added alone in 18.2 MΩ water. After label and excess C/N addition, average moisture content of the soils was 47% on a dry weight basis. These samples, in the same microcentrifuge assemblies described in Section 2.3, were incubated at 15 °C before centrifugation and analysis of the solution as before. An incubation time period of 3 min was selected as this is the point of maximum variance between treatments, and reflects similar earlier work (Hill et al., 2012).

2.5. Statistical analysis

Soil chemistry data from the six field treatments were analysed using a two-way general linear model (GLM) in SPSS v17.0 (IBM Corp., Armonk, NY). An independent samples t-test was used to establish differences between peptide half-life in the soil solution of the high and low fertility samples ($F_0M_1C_0$ and $F_1M_1C_1$ respectively). The effect of the field treatments and C/N additions was investigated using a four-way GLM. Tukey's HSD *post hoc* test was used to identify treatment differences.

3. Results

3.1. Soil chemistry

After 17 years of management, mowing had a greater effect on soil chemistry than fertiliser application. Six variables (electrical conductivity, available P, C:N ratio, NO_3^-, NH_4^+, and DON) were significantly affected ($P \leq 0.05$) by mowing, while only available P was significantly affected by fertiliser addition. No significant interaction between mowing and fertiliser were observed for any variable (Table 2, Fig. 1). Soil pH, total C, total N, MBC/N, microbial C:N ratio, DOC and FAA-N were unaffected by either treatment (Table 2, Fig. 1).

Mowing and subsequent removal of clippings resulted in a 43% reduction in soluble salts (measured as electrical conductivity). Despite regular removal of plant biomass, total C and N concentrations were unaffected by the mowing treatment with average concentrations of 38 g kg^{-1} and 3.1 g kg^{-1} respectively, although both the clippings retained and removed treatments resulted in a small but significant ($P < 0.001$) drop in the C:N ratio of the soil by 6% (Table 2). Curiously, available P was significantly higher in the unfertilised treatment. There were no differences in available P between the un-mowed and the clippings retained treatments, though clipping removal reduced available P by more than two thirds relative to clipping retention treatment (Table 2). Microbial biomass C, N, and C:N ratios were unaffected by the pasture management treatments (Table 2). Though not statistically significantly different ($P = 0.075$), DOC concentrations appeared lowest in the mowed, clippings removed treatment.

Across all treatments, DON (exclusive of the separately characterised FAA-N pool) made up the greatest proportion of the TDN pool, constituting two thirds of the TDN pool (Fig. 1). Mowing significantly ($P = 0.001$) reduced NO_3^- concentrations by a factor of four, regardless of whether clippings were retained or removed. Clipping retention after mowing resulted in the highest NH_4^+ concentrations of 6.1 mg kg^{-1}, while clipping removal significantly ($P = 0.029$) reduced NH_4^+ concentrations to 2.6 mg kg^{-1}. Neither mowing nor fertiliser treatment affected FAA-N concentrations, with an average of 4.0 mg kg^{-1}, and indeed, fertiliser application had no significant effect on the concentrations of all soluble N species measured.

3.2. Microbial peptide uptake rate as affected by soil fertility

Depletion of trialanine from the pore water of both soils was extremely rapid, with complete depletion of the added label remaining in the soil solution to an asymptote of ca. 10% just after 20 min (Fig. 2). The single first-order exponential decay model fitted the datasets for the high and low fertility soils well ($R^2 \geq 0.957$). The calculated half-lives of trialanine in the soil solution revealed that uptake rates were twice as fast in the low fertility soil compared to the high fertility soil ($P = 0.019$).

3.3. Effect of soil fertility and C/N excess on peptide uptake

The effect of an immediate relative excess of C and/or N overlaid upon the six field treatments on the rate of peptide uptake from the soil solution by soil microorganisms was investigated by quantifying the amount of ^{14}C remaining in solution after a 3 min incubation. This time point is within the period where most differentiation in rate of uptake occurred in the comparison between high and low fertility soils (Fig. 2), and occurs before the

Table 2
Chemical properties of the soils under the six experimental treatments (see Table 1). For the variables where significant treatment effects were observed, different letters denote significant differences at the $P < 0.05$ level between individual treatments. NS = not significant.

	F_0M_0	$F_0M_1C_1$	$F_0M_1C_0$	F_1M_0	$F_1M_1C_1$	$F_1M_1C_0$	GLM output		
							Fertiliser	Veg. Man.	Interaction
Total organic C (mg g^{-1})	38.1 ± 1.9	38.0 ± 2.5	33.9 ± 2.6	42.5 ± 3.4	42.4 ± 1.8	35.8 ± 1.8	NS	NS	NS
Total N (mg g^{-1})	2.94 ± 0.13	3.12 ± 0.23	2.75 ± 0.22	3.24 ± 0.25	3.50 ± 0.17	2.89 ± 0.12	NS	NS	NS
C:N ratio	12.9 ± 0.1a	12.2 ± 0.1b	12.4 ± 0.1b	13.1 ± 0.2a	12.1 ± 0.2b	12.4 ± 0.1b	NS	$P < 0.001$	NS
pH	5.92 ± 0.18	5.86 ± 0.09	5.64 ± 0.02	5.80 ± 0.17	5.84 ± 0.05	6.19 ± 0.33	NS	NS	NS
Electrical conductivity (μS cm^{-1})	63.2 ± 5.5a	51.6 ± 2.4 ab	36.2 ± 2.0b	62.7 ± 7.7a	52.1 ± 2.3 ab	34.4 ± 2.8b	NS	$P < 0.001$	NS
Olsen P (mg kg^{-1})	62.0 ± 5.2a	60.2 ± 6.9a	19.7 ± 3.9c	41.0 ± 0.9b	51.5 ± 4.1 ab	16.5 ± 0.6c	$P = 0.005$	$P < 0.001$	NS
Microbial biomass C (mg kg^{-1})	49.1 ± 6.1	73.8 ± 10.7	67.0 ± 5.7	55.2 ± 4.1	68.8 ± 11.2	64.9 ± 8.3	NS	NS	NS
Microbial biomass N (mg kg^{-1})	11.2 ± 4.9	15.4 ± 2.6	12.5 ± 0.8	10.4 ± 1.1	14.9 ± 2.5	11.3 ± 1.0	NS	NS	NS
Microbial biomass C:N ratio	6.13 ± 1.66	4.95 ± 0.62	5.38 ± 0.45	5.55 ± 0.78	4.69 ± 0.56	5.68 ± 0.26	NS	NS	NS
Dissolved organic C (mg kg^{-1})	142 ± 64.2	99.8 ± 34.1	41.6 ± 14.5	86.6 ± 34.1	79.2 ± 7.8	26.8 ± 2.5	NS	NS	NS

supply of peptide from the pulse becomes limiting on uptake rate as the added labelled peptide is used by the soil microbiota. A four-way factorial GLM identified that mowing ($P < 0.001$), C excess ($P < 0.001$) and the interaction between mowing and fertiliser ($P = 0.001$) were the only significant effects observed (Table 3).

In Fig. 3, we demonstrate similar trends in all but one of the six mowing × fertiliser treatments, with significant ($P \leq 0.05$) reductions in labelled peptide taken up as a result of C or C and N addition relative to the control in four out of the six vegetation management treatments. In contrast to this, on no occasion did N addition alone result in a significant reduction in labelled peptide uptake. Considering the amount of labelled peptide taken up in 3 min across all six vegetation management treatments in comparison to the measured chemical variables, several significant correlations were observed. Electrical conductivity, bulk C:N ratio, DOC and DON concentrations were all significantly negatively correlated with peptide uptake ($P \leq 0.026$), while MBC showed a weak but significant positive correlation ($r = 0.46$, $P = 0.024$).

4. Discussion

4.1. Peptide uptake rates

Recent work demonstrates that DON in the form of peptides represents a previously unrecognised, comparatively large and fast cycling source of N for microbial and plant nutrition (Farrell et al., 2011a,b, 2013; Hill et al., 2011a,b,c, 2012, 2013, Macdonald et al., 2014; Soper et al., 2011; Warren, 2013c). In this study, we demonstrated that the model peptide L-trialanine was taken up extremely quickly in these grassland soils, with a calculated half-life of 0.74 ± 0.20 to 1.41 ± 0.06 min in the low and high fertility soils respectively. These depletion rates are similar to those of L-alanine, L-dialanine and L-trialaine presented by Hill et al. (2012) in a high fertility *Lolium perenne* dominated northern hemisphere pasture soil, and also those of glucose uptake in the same soil (Hill et al., 2008).

Despite similarity in half-lives to those found in studies using similar uptake techniques (Hill et al., 2008, 2012), these half-lives are extremely rapid when compared to those estimated from the mineralisation of LMWDOC/N compounds, which are generally in the order of 1–4 h (Boddy et al., 2007; Farrell et al., 2013; Glanville et al., 2012), as opposed to timescales of minutes described by the few studies using the direct uptake technique used here. Taking the 10 μM concentration of trialanine used in this spiking study as a

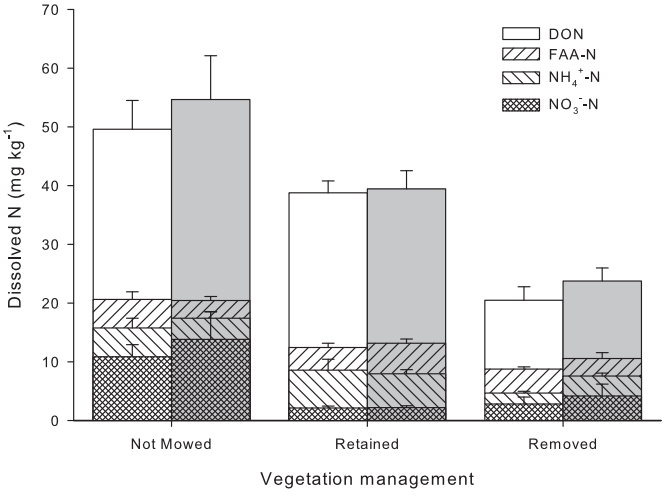

Fig. 1. Dissolved N concentrations as affected by fertiliser addition and mowing strategy. Fertiliser addition had no effect on the concentration of any of the N pools ($P > 0.05$), while mowing and removal of clippings significantly ($P \leq 0.05$) reduced NO_3^-–N, NH_4^+–N and DON concentrations. FAA-N was unaffected by mowing strategy ($P > 0.05$). White bars are unfertilised treatments, grey bars are fertilised treatments.

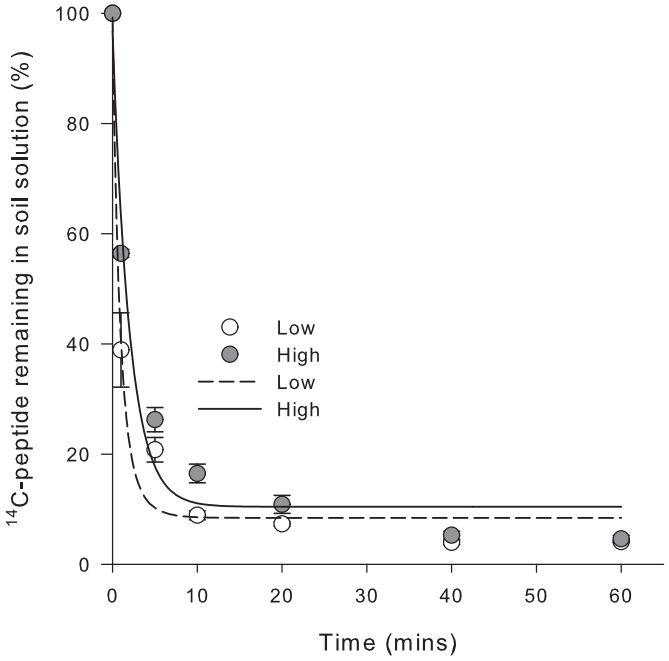

Fig. 2. Depletion of the ^{14}C-labelled peptide in the soil solution of the lowest ($F_0M_1C_0$) and highest ($F_1M_1C_1$) fertility management practices. Maximum average abiotic sorption was observed at $t = 1$ h (ca. 13%), and this is corrected for from sterile replicates as described in the materials and methods section.

Table 3
Output of the four-way GLM indicating statistical significance of treatments and interactions on peptide uptake.

Source	F	Sig.
Fertiliser	2.674	0.106
Mowed	15.553	0.000
Carbon	30.564	0.000
Nitrogen	1.765	0.188
Fertiliser × mowed	7.795	0.001
Fertiliser × carbon	0.694	0.408
Fertiliser × nitrogen	0.866	0.355
Mowed × carbon	1.795	0.174
Mowed × nitrogen	1.602	0.209
Carbon × nitrogen	0.034	0.855
Fertiliser × mowed × carbon	1.174	0.315
Fertiliser × mowed × nitrogen	0.002	0.998
Fertiliser × carbon × nitrogen	1.077	0.303
Mowed × carbon × nitrogen	0.425	0.656
Fertiliser × mowed × carbon × nitrogen	0.300	0.741

nominal concentration of peptide in the soil solution (and this is very similar to the oligopeptide-N pool measured in a UK grassland soil by Hill et al. (2011b)), we can estimate microbial C and N uptake rates on the assumption that these rates represent a continual flux:

$$\Phi = k \times Q \quad (3)$$

where Φ is the rate of uptake as a continual flux, k is the rate constant derived from Eqn. (1), and Q is the quantity of peptide C or N per m^2, assuming a soil solution concentration of 10 μM and a bulk density of 1.1 g cm^{-3}. Carbon uptake rates derived from peptide uptake in these New Zealand pasture soils to a depth of 7.5 cm as sampled would be 23.2 ± 5.3 g C m^{-2} d^{-1} in the low fertility soil, and 10.2 ± 0.4 g C m^{-2} d^{-1} in the high fertility soil. Nitrogen uptake rates would be similarly high, ranging from 9.02 ± 2.05 g N m^{-2} d^{-1} in the low fertility soil, to 3.96 ± 0.16 g N m^{-2} d^{-1} in the high fertility soil.

These flux rates are at least an order of magnitude higher than observed C and N flux through traditional respiration and mineralisation studies. Reich and Schlesinger (1992) reviewed soil respiration rates as a function of ecosystem type, finding a mean

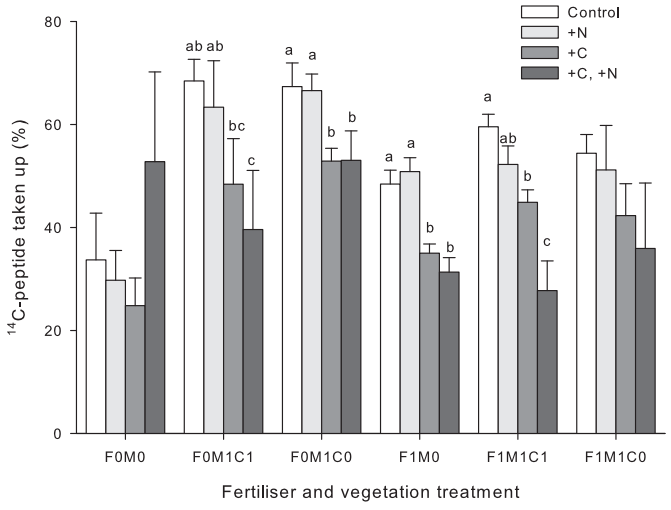

Fig. 3. Percentage of ^{14}C-labelled peptide taken up by the soil microbial community after 3 min, as affected by management practice and excess C, N or C & N addition. Different letters within each group of bars indicate a significant effect ($P \leq 0.05$) of C/N treatment in that management practice. Where no letters are present, no significant differences were observed. A lower percentage of uptake indicated reduced uptake rate assuming that 3 min is representative of peak uptake (Fig. 2).

CO_2–C flux of 1.21 ± 0.21 g C m^{-2} d^{-1} from the nine temperate grassland studies included in that analysis. Similarly, Booth et al. (2005) reported an average gross N mineralisation rate in grassland soils of 7.34 mg N kg^{-1} d^{-1} in their synthesis, which is equivalent to an average gross N mineralisation rate of 0.66 g N m^{-2} d^{-1}, assuming a bulk density of 1.1 g cm^{-3} and a soil depth of 7.5 cm as per our study. While such comparisons are crude, it is apparent that the peptidic C and N fluxes measured in the present study are far higher than gross C and N fluxes in soil, especially in the case of C when it is considered that peptides constitute only a small portion of the total LMWOC pool. Such an observation is perhaps unsurprising, given that the rate-limiting step in soil N cycling is presumed to be at the proteolysis stage (Jan et al., 2009; Weintraub and Schimel, 2005), where HMW proteins are cleaved to peptidic fragments and monomeric FAAs. Thus, our measurements quantify the peak rate of peptide uptake capacity by the soil microbial community in response to an episodic influx of a resource such as root or microbial cell lysis.

It is the microbial community's ability to respond rapidly to episodic nutrient inputs (Jenkins et al., 2010), and how this differs between soils of different nutrient statuses (Nottingham et al., 2012) that allows us to explain the significant increase in peptide uptake rate in the nutrient poor ($F_0M_1C_0$) soil over the higher fertility ($F_1M_1C_1$) treatment. There are two important concepts here: firstly, labile nutrients are a patchy resource in the soil, with large concentration gradients over the μm – mm scale (Jones et al., 2009b). Secondly, soil microorganisms, and particularly bacteria and archaea, have a limited ability to travel to capitalise on a new resource pool (Resat et al., 2012). Consequently, microorganisms must be able to react rapidly to take up available nutrients when they occur nearby. Between the nutrient rich and nutrient poor soils used in this experiment ($F_1M_1C_1$ and $F_0M_1C_0$ respectively), there was no significant difference in microbial C, N, or C:N ratio ($P > 0.05$, Table 2), indicating that the significant difference in uptake rates in these two soils may have been as a result of the specific activity of the microbial population, or differences in microbial community structure, rather than its size. However, there was a weakly significant positive correlation ($r = 0.46$, $P = 0.024$) between the amount of peptide taken up in 3 min and MBC across all six field treatments in the control of the C/N competition experiment ($n = 24$).

Though there have been no previous studies investigating peptide uptake in the soil as directly affected by soil fertility, work investigating the priming of native soil C in response to LMWOC inputs (Nottingham et al., 2012) indicates that the ability of soil microorganisms to respond within minutes to LMWOC/N inputs (Hobbie and Hobbie, 2013) may be higher in nutrient constrained soils. One hypothesis here is that in resource-poor soils, microorganisms have to be able to respond rapidly to a potentially fleeting new resource to capitalise on the energy and nutrients therein. In contrast, competition for fresh resources is likely lower in soils in which C/N are already in more plentiful supply.

4.2. What drives soil microbial uptake of peptides?

Given that oligopeptides are amongst the largest C and N bearing molecules to be taken up intact at a rapid rate by bacteria, fungi, and archaea (Farrell et al., 2013; Hill et al., 2012; Jennings, 1995; Payne, 1980), it is likely that peptides represent the point at which the terrestrial C and N cycles may become decoupled through microbial mineralisation. Using both an established fertility gradient in the form of the six pasture management treatments, and imposed C/N excess relative to peptidic C/N addition, we asked whether uptake of a peptide was driven primarily by the soil microbial requirement of the C or N contained within.

Across the six fertiliser and mowing treatments, excess of C and not N resulted in a significant reduction of peptide uptake by the soil microbial community, and there was no significant interactive effect between C and N excess across the whole dataset (Table 3). Although not all grassland management treatments behaved the same way when observed individually, uptake of the peptide relative to the control appeared to be primarily regulated by available C excess. This observation is in agreement with previous studies suggesting that uptake of LMWON compounds may be primarily for their C rather than their N (Jones and Shannon, 1999; Jones and Murphy, 2007), and observations that there is a stronger relationship between LMWON mineralisation and soil C status than the N status of a soil (Farrell et al., 2013). That being the case, uptake of LMWON for energy, either in the form of catabolism or anabolic activity likely leaves soil microorganisms with an internal excess of N to be excreted as NH_4^+. Competition between plants and microorganisms for N in both organic and inorganic forms is fierce, with wheat being outcompeted by soil microorganisms for ^{15}N-labelled glutamate in a recent study by Jones et al. (2013). However, they observed that the glutamate was quickly mineralised and excreted by the microbial cells, resulting in ca. 50% of the ^{15}N being returned rapidly to the soil as $^{15}NH_4^+$, which was subsequently taken up by the wheat roots.

We also observed weak but statistically significant ($P < 0.05$) negative correlations between the amount of peptide taken up in the control (no C/N addition) and extractable DOC ($r = -0.579$) and DON ($r = -0.453$), as well as the bulk C:N ratio ($r = -0.567$), with DOC and DON being highly co-correlated ($r = 0.620$, $P = 0.001$). Though not indicative of causation, these correlatory findings again agree with the observation that peptide uptake is lower in soils where there is a higher availability of C. This is complementary to the conclusion that LMWON is primarily assimilated for the C contained within, and explains why only C and not N excess resulted in a reduction in peptide uptake in our study.

4.3. Conclusions

Peptide uptake by soil microorganisms is extremely rapid, with $t_{½}$ in soil solution of less than 2 min measured in the present study. Fastest uptake was observed in the lower fertility soil, thus confirming our first hypothesis that peptide uptake rate would be fastest in the most nutrient depleted field trial treatment. When the model peptide trialanine was added to the soil in combination with an excess of C and/or N, it was only the addition of excess C that resulted in a significant reduction in uptake of the peptide, confirming our second hypothesis that peptide uptake would be down-regulated by the addition of an excess of labile C. By inference that peptides represent the point at which plants and microorganisms first actively compete for intact molecules (Hill et al., 2011a), we conclude that microbial uptake of LMWON appears to be mainly for the C contained within DON compounds. This observation has implications for how data on DOC/N pools and fluxes are interpreted in regard to both N availability and C loss as CO_2.

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