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Variation in small organic N compounds and amino acid enantiomers along an altitudinal gradient

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ABSTRACT

The absolute and relative concentration of small organic N compounds varies among soils, yet we have little idea what drives this variation among soils. Previous studies have noted differences in DON/DIN and amino acid profiles among sites differing in altitude and/or productivity, and thus it seemed plausible that similar factors would have broader effects on the molecular composition of the pool of small organic N. To test this idea we used an altitudinal transect that ranged from a low altitude forest of *Eucalyptus regnans* with a canopy height averaging 65 m through to coniferous shrubbery that was above the alpine treeline and had a canopy less than 50 cm high. From low to high altitude mean annual temperature decreased 7 °C such that turnover was likely twice as slow at the highest site than the lowest. Capillary electrophoresis-mass spectrometry was used to identify and quantify the main small organic N compounds in free, adsorbed and microbial fractions of the soil; while chiral liquid chromatography-mass spectrometry was used to quantify amino acid enantiomers in hydrolysed soil and the free, adsorbed and microbial fractions of soil.

CE-MS detected 66 small (<250 Da) organic N compounds of which 63 could be positively identified. Protein amino acids were a large fraction of the pool of small organic N, but there were also large amounts of non-protein amino acids, quaternary ammonium compounds and alkylamines. There were differences among sites in the profile of small organic N, but these differences were not monotonically related to altitude and there was no evidence pools of small organic N were larger or enriched in recalcitrant compounds at cooler high altitude sites. Among sites there was only modest variation in the molecular composition of the protein amino acid pool probably because protein amino acids are primarily derived from a common source (i.e. depolymerisation of soil proteins). In contrast, there was substantially larger variation within pools of non-protein amino acids, alkylamines and quaternary ammonium compounds; which is probably because compounds from these classes are primarily products of *de novo* synthesis by specific organisms, and thus molecular composition varies among sites depending on composition of the microbial community.

D-enantiomers of amino acids were at low concentrations relative to L enantiomers such that in soil extracts the summed concentration of D-amino acids was 0.5–0.6% of L amino acids, while in hydrolysates D-enantiomers were 0.99% of L-enantiomers. There was no evidence that absolute or relative concentrations of D-enantiomers in free solution, microbial biomass or hydrolysates were larger at high altitude sites, despite turnover likely being slower at the cooler high altitude sites. The absence of an effect of altitude on D/L probably indicates that the turnover of soil proteins is comparatively rapid and thus soil proteins are similarly young even among sites in which mean annual temperature differs by 7 °C.

1. Introduction

Management of soil nitrogen is one of the keys to supplying the world with agricultural and forest products, and is also important for conservation of natural ecosystems. Nitrogen is one of the nutrients most commonly managed because it is required in large amounts and commonly limits growth. Nitrogenous fertiliser are commonly applied to alleviate N limitation and increase productivity. In fact, 85–90 million tons of nitrogenous fertilisers are

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applied every year to soil (Vitousek et al., 1997; Tilman, 1999; Galloway et al., 2008; Gruber and Galloway, 2008). Application of nitrogenous fertilizers may increase productivity, but also contributes to a raft of problems not the last of which is eutrophication of target and non-target ecosystems. Fertiliser application coupled with other factors such as fossil fuel burning and land-use change have led to global alteration of the N cycle (Galloway et al., 2008) with many natural ecosystems receiving nitrogen as a pollutant. Nitrogen pollution is a major threat to natural ecosystems and human health (Townsend et al., 2003; Fields, 2004). One of the key ways in which nitrogen acts as a fertiliser or pollutant is by altering amounts of nitrogen available for uptake by organisms such as microbes and plants.

It is important to know which forms of N are in soil because not all chemical forms of N are equally available for uptake. Inorganic forms of N are widely bio-available and cycle rapidly in ecosystems, and the same is also true of small forms of organic N such as amino acids (Schimel and Bennett, 2004) (Hutchinson and Miller, 1912; Virtanen and Linkola, 1946; Paungfoo-Lonhienne et al., 2012). Larger forms of organic N are seemingly less available given that organic N > 1 kDa turns over slowly (~days-months) compared to the <1 kDa fraction that turns over rapidly (~hours) (Jones et al., 2004). To date we have a reasonable understanding of the availability and cycling of inorganic forms of N, but much less is known about organic forms. Understanding the availability of organic forms of N is particularly challenging because our view of organic N in the soil solution is constantly evolving (Paungfoo-Lonhienne et al., 2012). For example, recent studies that took a broad view of the pool of organic N established that the pool of small organic N in the soil solution is not necessarily dominated solely by the 20 standard amino acids that are genetically coded and can be incorporated into proteins (so-called protein amino acids, Warren, 2013a; Warren, 2013b), but also contains compounds that function as osmolytes such as betaine and ectoine (Warren, 2014a, c), and aliphatic (poly)amines (alkylamines) can be a large fraction of the adsorbed fraction (i.e. the fraction that is extractable with strong salt solution) (Warren, 2014b). The diversity of organic N in soil was in fact known for some time (Schreiner, 1912; Schreiner and Skinner, 1912; Lathrop, 1917; Dadd et al., 1953; Aseeva et al., 1977, 1978; Schlimme and Kirse, 1983) but until recently we had not recognised the quantitative significance of compounds other than protein amino acids.

The absolute and relative concentration of small organic N compounds varies among soils. Studies contrasting DON with DIN have typically shown that infertile sites have large amounts of DON and small amounts of DIN while fertile sites have large amounts of DON and large amounts of DIN (Nordin et al., 2001; Yu et al., 2003; Christou et al., 2005; Kranabetter et al., 2007; Farrell et al., 2011). These empirical observations are consistent with theory suggesting that DON ought to accumulate in low-productivity systems owing to slow turnover (Schimel and Bennett, 2004). At a finer scale there is variation among soils in relative and absolute amounts of individual compounds and compound classes. For example, studies have reported variation in amino acid profiles of soil solutions collected across natural gradients (Yu et al., 2002) or from diverse sites (Warren, 2013b). Other studies have noted differences among soils in relative proportions of compound classes (e.g. amino acids versus quaternary ammonium compounds and alkylamines) and individual compounds (Warren, 2013b), yet we have little idea why the composition of the small organic N pool differs among soils.

One specific research challenge involves determining what controls the relative and absolute abundance of amino acid enantiomers. Knowing more about amino acid enantiomers in soil is important because there are differences in uptake and metabolism between D and L enantiomers of amino acids (Bruckner and Westhauser, 2003; Hill et al., 2011b; Vranova et al., 2012; Broughton et al., 2015). In some studies D-amino acids have accounted for >10% of amino acids in hydrolysed soil (Pollock et al., 1977; Amelung and Zhang, 2001; Amelung, 2003; Wichern et al., 2004), whereas in other studies soil hydrolysates have contained much smaller amounts of D-amino acids (Mikutta et al., 2010; Warren, 2017). Less is known about amino acid enantiomers in fractions of soil that are more relevant to questions of availability such as microbial biomass and free solution (Vranova et al., 2012), but the scant data we have indicate large differences among studies. In soil from a peat bog extracted with aqueous ethanol, which presumably represents amino acids in free solution plus the microbial biomass, five D-amino acids were detected with D/L of surface soils (0–20 cm) being 1–9% in serine, aspartic acid, glutamic acid and phenylalanine and 41% in alanine (Kunnas and Jauhiainen, 1993). At depths below 40–100 cm the peat was anaerobic and D/L ratios were markedly larger (e.g. D/L from 150% in serine to 730% in glutamic acid) (Kunnas and Jauhiainen, 1993). Large concentrations of D-enantiomers were also found in soil solution of Antarctic soils with D-enantiomers of 10 amino acids being detected and D/L ratios differing among amino acids from 1% in glutamine to a remarkable 160% in phenylalanine and 190% in leucine (Broughton et al., 2015). In stark contrast a recent study examining amino acid enantiomers in free solution and microbial biomass across a 5500 year chronosequence reported that D enantiomers of many amino acids were below detection limits and the summed concentration of D-amino acids was 0-3-0.6% of L-amino acids (Warren, 2017).

There is at present no way of reconciling why there are such large differences among studies in D/L ratios of amino acids, but clues may be found in the factors controlling the rate at which D-enantiomers enter, cycle and exit soil. L-enantiomers of amino acids are used for protein synthesis and there are enzymes that recycle D-enantiomers such that D/L (of protein amino acids) in metabolically active organisms is zero or thereabouts (Brinton et al., 2002). Hence, L-enantiomers comprise the bulk of protein N added to soil, but there are two ways in which D-enantiomers can enter soil. First, when metabolism ceases amino acids undergo slow abiotic racemisation and over thousands of years D/L tends toward the equilibrium value of 100% (Brinton et al., 2002). Key to the extent of abiotic racemisation is the residence time of proteins because residence time controls the probability of racemisation and time over which racemisation products to accumulate (Amelung et al., 2006). The second way D-amino acids can enter soil is as components of bacterial peptidoglycan, which can contain D-amino acids such as alanine (Bruckner and Westhauser, 2003; Veuger et al., 2006). Irrespective of how D-amino acids enter soil, D/L ratios do not necessarily increase inexorably because soil microbes can take up and metabolise D-amino acids (Hill et al., 2011a; Broughton et al., 2015), with this decomposition and recycling by microbes moving D/L ratios towards zero (Brinton et al., 2002). Perhaps not surprisingly the soils with the largest D/L ratios have been those where rates of protein decomposition (i.e. microbial metabolism of D-enantiomers) were slow due to low temperatures and/or limited O_2, e.g. anaerobic peat (Kunnas and Jauhiainen, 1993), Antarctic soils (Broughton et al., 2015), permafrost (Brinton et al., 2002). Conversely, D/L ratios were small in studies of temperate and tropical chronosequence soils (Mikutta et al., 2010; Warren, 2017) probably because proteins turned over rapidly (e.g. on a decadal time scale: Jones et al., 2015) and residence times were too short for racemisation products to build up. Rates of protein decomposition and the likelihood of D-enantiomers accumulating could also be a function of mineralogy. For example, clays facilitate protein aging through microbial occlusion and/or stabilisation via organo-mineral associations (Eusterhues et al., 2003; von Lutzow