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Changes in small organic N during early stages of soil development

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ABSTRACT

During the early stages of ecosystem development there are increases in plant and soil microbial biomass, nutrient availability and rates of nutrient cycling; but little is known about how pools of small organic N vary during the initial stages of soil development. The aim of this study was to examine how the pool of small organic N compounds varies during the initial stages of soil development, and if age differentially affects D- and L-enantiomers of protein amino acids. Measurements were made at a soil chronosequence on the east coast of Tasmania that comprised a series of sub-parallel beach dunes and ridges varying in age from <100 years to 5500 years. 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. Small organic N was dominated by protein amino acids, while there were also large amounts of quaternary ammonium compounds and alkylamines. There were differences among chronosequence sites in the profile of small organic N, but these differences were not monotonically related to age and there was no evidence for a build-up of recalcitrant compounds over time. Differences were instead site-specific and related to presence/absence of particular non-protein amino acids which probably related to the presence/absence of specific plants and/or microbes that produce and/or can metabolise different non-protein amino acids.

In free solution and microbial biomass D enantiomers of many amino acids were below detection limits (i.e. < 0.125 nmol g^{-1}) and D-enantiomers were at low concentrations relative to L enantiomers such that across all ages and replicates the summed concentration of D-amino acids was 0-3-0.6% of L amino acids. There was no evidence that absolute or relative concentrations of D-enantiomers in free solution, microbial biomass or hydrolysates were larger at the older chronosequence sites. The consistent lack of an effect of soil age 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 soil age is vastly different.

1. Introduction

Chronosequences are a series of soils of different ages that formed on the same parent material in the same locality. They allow us to study soils of different age at the same time (i.e. space-for-time substitution), and have advanced our understanding of process of soil formation and development occurring at timescales that are too long to be directly observed within a lifetime (Walker and Syers, 1976; Pearson and Vitousek, 2002; Walker et al., 2010). Studies of chronosequence have typically observed that in the early stages of succession there are increases in plant and soil microbial biomass, nutrient availability and rates of nutrient cycling (Chapin et al., 2002). This so-called progressive phase can continue for thousands of years before ecosystems may enter a so-called retrogressive phase in which there are decreases in nutrient availability and plant biomass (Walker et al., 2001; Vitousek, 2004; Peltzer et al., 2010a, 2010b; Walker et al., 2010). In terms of nitrogen (N) availability studies of chronosequences have focused on inorganic N and mineralization, but chronosequences could equally be used to test ideas about organic forms of N. Shifting focus to organic N is timely because we now know that plants can take up at least some low molecular weight organic N compounds (Näsholm et al., 2009), and organic N compounds play key roles in soil N cycling (Schimel and Bennett, 2004).

Chronosequences may be able to provide insight into some of

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the factors driving differences among soils in the pool of low molecular weight organic N. Recent studies that have taken a broad view of the pool of organic N established that the pool of monomeric organic N in the soil solution is chemically diverse, and 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). In addition to the 20 standard protein amino acids many of the most abundant organic N compounds in free solution and microbial biomass have been osmolytes such as betaine and ectoine (Warren, 2014c,a), while aliphatic (poly)amines (alkylamines) have accounted for a large fraction of the adsorbed fraction (i.e. the fraction that is extractable with strong salt solution) (Warren, 2014b). There are large scale differences among soils in relative proportions of compound classes and individual compounds (Warren, 2013b), yet we have little idea why the profile of small organic N differs among soils It may be the case that variation among soils could be partially due to soil age. For example, during the initial stages of soil development on new substrates (up to ~10,000 years) there are increases in biodiversity, soil C and N, plant and microbial biomass (Schlesinger, 1990; Harden et al., 1992; Chapin et al., 2002; Peltzer et al., 2010b) and perhaps also shifts in the relative abundance of organic N compounds. The effects of age on soil C, N, and microbial biomass probably reflects age-related differences in inputs, outputs and transformations by plants and microbes. For example, the effect of ecosystem age on inputs of plant litter (Jones et al., 2015), inputs of roots and root exudates (Hutsch et al., 2002), synthesis and exchange of osmolytes and other small organic compounds by soil microbes (Warren, 2014c), and incorporation of microbial residues (Roth et al., 2011).

Chronosequences may also be able to provide insights into some of the factors controlling the relative abundance of L- and D-enantiomers of amino acids. In general terms we do not know a lot about D-versus L-enantiomers of amino acids in soil, yet this information may be critical to understanding the N cycle due to vast differences between enantiomers in uptake and metabolism (Bruckner and Westhauser, 2003; Hill et al., 2011; Vranova et al., 2012). Previous studies have shown D-amino acids can account for >10% of amino acids in hydrolysed soil (Pollock et al., 1977; Amelung and Zhang, 2001; Amelung, 2003; Wichern et al., 2004), D-amino acids such as alanine and glutamine are presumed to enter the soil via D-amino acids that make up the peptidoglycan links in bacterial cell walls (Hernandez and Cava, 2016) while other D-amino acids such as lysine are not found in microbes and are instead assumed to be the product of abiotic racemisation (Amelung, 2003). Several factors may cause D/L ratios to increase with soil age. First, D-amino acids could accumulate over time because they are less prone to microbial transformation (than L enantiomers) (Amelung, 2003; Broughton et al., 2015). Second, the extent of abiotic racemisation may be larger in older soils (Mahaney and Rutter, 1989; Amelung, 2003) if proteins are sufficiently protected from microbial transformation, for example, via physical occlusion or stabilisation in organo-mineral associations with clay minerals and/or iron oxides (Eusterhues et al., 2003; Kogel-Knabner et al., 2008). Qualified support for increases in D/L with age came from a study of a soil profile where waterlogging inhibiting mineralization resulting in a ^{14}C age gradient of ~10,000 years in the top 20 cm of soil. It was shown that D/L ratios were larger in parts of the soil profile where mineralization was slower and ^{14}C ages older (Amelung, 2003). Nevertheless, there remains little evidence that D/L ratios change during soil development. In a long-term chronosequence in Hawaii D/L ratios of hydrolysed soil did not differ among sites from 300 years to 4.1 million years old (Mikutta et al., 2010), but all but one of the sites was dated at 20,000 years or older and thus the Hawaiian chronosequence provided few insights into the first 10,000 years of soil development. In any case, testing the generality of the Hawaiian findings is important because responses of D/L ratios to time may differ depending on parent material.

To date most studies of amino acid enantiomers have examined hydrolysed soi; even less is known about the occurrence of amino acid enantiomers in fractions of soil that cycle more quickly such as microbial biomass, adsorbed and free solution (Vranova et al., 2012). Knowing the abundance of amino acid enantiomers in free solution is important because it is these amino acids that are potentially available for uptake by plants and microbes. It's plausible that there are substantial amounts of D-enantiomers in free solution and/or microbial biomass given that studies using aqueous ethanol as extractant have reported soil D/L ratios of anywhere between 1 and 40% (Kunnas and Jauhiainen, 1993; Bruckner and Westhauser, 2003). Nevertheless, the exact localisation of D-enantiomers cannot be deduced for aqueous ethanol extracts because aqueous ethanol extracts some variable (and unknown) proportion of amino acids from microbial biomass (Vranova et al., 2012).

The broad aim of this study was to use a short-term chronosequence to explore how the pool of monomeric organic N varies during the initial stages of soil development. Measurements were made at a soil chronosequence on the east coast of Tasmania that comprised a series of sub-parallel beach dunes and ridges varying in age from <100 years to 5500 years (Fig. 1 and Bowden and Kirkpatrick, 1974; Bowman, 1986, 1987). Previous studies established that the chronosequence has uniform parent material of quartz sand of marine origin and soils display only slight variation in mineralogy and granulometry across the chronosequence (Bowman, 1986, 1987), and thus the chronosequence represents a simple system for studying soil development. 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 (Warren, 2013a); while chiral liquid chromatography-mass spectrometry (Claus, 2011) was used to quantify amino acid enantiomers in hydrolysed soil and the free, adsorbed and microbial fractions of soil. These measurements allowed us to document how small organic N changes during the initial stages of soil development and test specific hypotheses.

- Previous studies have established that during initial stages of soil development N is in short supply and there are increases in microbial biomass (Chapin et al., 2002; Vitousek, 2004), thus we predict that older soils will contain more small organic N in microbial biomass than younger soils, while amounts of small organic N in the adsorbed and free pools will remain low and invariant throughout the chronosequence.
- Rates of input, turnover and loss differ among chemical forms of small organic N, and thus one might predict that over time we would see an accumulation of organic N compounds that are recalcitrant (e.g. those based on aromatic structures and secondary metabolites) whereas there would be no change in amounts of ubiquitous readily transformed compounds such as protein amino acids.
- Age is suggested to be one of the main drivers of D/L ratios of amino acids (Amelung, 2003) and thus we predict that D/L ratios will be larger in older stages of the chronosequence than younger stages. Following similar logic, N in soil proteins tends to be older than N in more rapidly cycling fractions of soil (e.g. free solution, adsorbed and microbial), and thus we predict that D/L ratios will be larger in soil hydrolysates than in soil extracts.