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Significant release and microbial utilization of amino sugars and D-amino acid enantiomers from microbial cell wall decomposition in soils

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ABSTRACT

Amino sugars and D-amino acid enantiomers are major components of bacterial and fungal cell walls (i.e. peptidoglycan and chitin) and are often used as biomarkers of microbial residue turnover in soils. However, little is known about the *in situ* decomposition rates of microbial cell wall residues and how soil physicochemical properties affect this process. In this study, we investigated the *in situ* gross production and consumption rates of free amino sugars (glucosamine and muramic acid) and amino acids (meso-diaminopimelic acid, L-alanine, and D-alanine) by a novel isotope pool dilution assay using ^{15}N-labeled amino compounds. Soils were obtained from six sites differing in land management (cropland, pasture, and forest) and bedrock (silicate and limestone) and incubated at three temperatures (5, 15, and 25 °C). Free glucosamine released during the decomposition of peptidoglycan and chitin contributed significantly to the extractable soil organic nitrogen pool. Gross production and consumption rates of glucosamine were higher than those of individual amino acids, i.e. L- and D-alanine. Muramic acid had a longer mean residence time (68 h compared to 2.7 h for glucosamine, L- and D-alanine) and made a negligible contribution to soil organic nitrogen fluxes, indicating that free muramic acid was not a major decomposition product of peptidoglycan in soils. Meso-diaminopimelic acid and D-alanine exhibited comparable gross production and consumption rates with L-alanine. These amino acids can be used as indicators to estimate the decomposition of peptidoglycan from bacterial cell wall residues. We found that chitin decomposition was greater in silicate soils, while peptidoglycan decomposition dominated in limestone soils. Glucosamine production rates were not correlated with soil total amino sugars, microbial community structure, or hydrolytic enzyme activities, but were highest in soils with low pH and high sand content, indicating that soil texture and soil pH may strongly influence the decomposition of amino sugar polymers. In contrast, mDAP, L- and D-alanine gross production and consumption rates were positively correlated with soil pH and clay content, due to greater depolymerization of peptidoglycan stem peptides in limestone soils. This isotope pool dilution approach strongly improves our understanding of the mechanisms and environmental controls on microbial cell wall decomposition in soils.

1. Introduction

Soil organic matter (SOM) decomposition is an essential process controlling carbon (C) and nitrogen (N) cycling in terrestrial ecosystems and the feedback from short-term/long-term climate change legacies (Schmidt et al., 2011; Lehmann and Kleber, 2015). Soil microbes are believed to be the major decomposers, including fungi and bacteria, and are responsible for the decomposition of SOM. Moreover, non-living biomass (necromass) of the soil microbial community is a component of SOM, contributing to > 50% of extractable SOM (Kögel-Knabner, 2002; Simpson et al., 2007; Liang and Balser, 2011; Miltner et al., 2011). Soil microorganisms preferentially decompose and metabolize microbial residues (cell wall materials) compared to other SOM fractions in N-limited soils, leading to a rapid turnover of microbial residues (Zeglin and Myrold, 2013). Acid hydrolysis of soil total nitrogen indicates that it is composed of 30-60% polymeric amino acids and 5–8% amino sugar polymers, which are key components of microbial cell walls (Amelung et al., 1996; Schulten and Schnitzer, 1997). A major component of fungal cell walls is chitin, a homopolymer of 1,4-linked N-acetylglucosamine (Rinaudo, 2006) (Fig. S1). Bacterial cell walls are constructed of peptidoglycan (PGN) consisting of glycan strands, repeating units of N-acetylglucosamine and N-acetylmuramic acid, crosslinked by short peptide stems (Steen et al., 2003) (Fig. S1). The peptide stems generally consist of four to five amino acids, with the first two being L-alanine and D-glutamine and the last one or two being D-alanine (Vollmer et al., 2008). The third one is lysine in Gram-positive

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bacteria, and meso-diaminopimelic acid (mDAP) in Gram-negative bacteria (also in some Gram-positive bacteria such as *Bacillus* spp.) (Vollmer et al., 2008). These cell wall-derived amino compounds, i.e. amino sugars, mDAP, and D-amino acids, contribute to the soil organic N pool together with proteinogenic L-amino acids, and are often used as indicators of soil microbial necromass composition (Zhang and Amelung, 1996; Glaser et al., 2004; Veuger et al., 2005). A major research gap is understanding how the decomposition of high-molecular weight microbial cell wall materials contributes to the soil organic N pool and the subsequent conversion to biologically available N.

Depolymerization of microbial cell wall residues by extracellular hydrolytic enzymes yields low-molecular weight organic compounds (i.e. oligomers, free amino acids, free amino sugars), which is the rate limiting step for microbial-mediated recycling of organic N in the soil N cycle (Schimel and Bennett, 2004; Wanek et al., 2010). Free amino acid and amino sugar pools are available for microbial uptake, thus they are very small (less than 1% of the total pool) (Warren, 2014; Hu et al., 2017) and highly dynamic, e.g. half-life times of amino acids ranging from only minutes to a few hours (Jones, 2002; Roberts et al., 2007; Wanek et al., 2010; Hu et al., 2017). Current methods for estimating the dynamics of soil microbial cell wall residues are based on acid hydrolysis of SOM at high temperature to yield total amino sugars, which is the sum of free amino sugars and those bound in microbial cell wall residues (e.g. Zhang and Amelung, 1996; Pronk et al., 2015). Alternative methods to quantify and measure the dynamics of amino sugars were tracing the changes in $\delta^{13}C$ and $\delta^{15}N$ of soil total amino sugars by GC/MS, GC/C/IRMS, or LC/IRMS (He et al., 2006; Bode et al., 2009; Decock et al., 2009). Rapid microbial utilization of free glucosamine was demonstrated by measuring the respiration of added ^{14}C-labeled glucosamine to soil (Roberts et al., 2007; Roberts and Jones, 2012), and free glucosamine concentration was $< 0.02\,\mu M$, which is low relative to free amino acid concentrations of 0.1–1 μM in soil water and 6 nmol/(g dry soil) in K_2SO_4 extracts (Warren, 2013, 2014). In this work, mDAP concentrations were below the detection limit ($< 0.06\,\mu M$) in soil extracts (Warren, 2014). Moreover, D-amino acids were reported to exhibit comparable or slightly slower rates of microbial utilization and/or respiration compared to L-amino acids in soils (Vranova et al., 2012; Hu et al., 2017).

An alternative way to investigate the decomposition processes of microbial residues is to measure the activities of microbial cell wall hydrolytic enzymes, e.g. soil exochitinases, endochitinases, and N-acetyl-beta-glucosaminidases (Howard et al., 2003). However, all present approaches only measure potential enzyme activities by adding synthetic substrates at saturating concentrations and optimal pH, and subsequently measuring the release of the dye or the fluorescent adduct, but this approach does not provide adequate information on the actual decomposition rates of chitin and peptidoglycan (Wallenstein and Weintraub, 2008; Jackson et al., 2013). The *in situ* decomposition process is likely governed by complex interactions between substrate availability, microbial biomass, microbial community structure, enzyme concentration, soil pH, temperature, and necromass stabilization (Sinsabaugh et al., 2008, 2009; Wallenstein et al., 2009). For instance, *in situ* decomposition rates of proteins in litter were shown to be constrained by the available or accessible protein pool and not by the protease activity (Wanek et al., 2010).

However, none of the existing approaches allowed to measure *in situ* gross production rates of amino sugars and D-amino acids deriving from the depolymerization of microbial cell wall residues. Isotope pool dilution (IPD) is the only approach for measuring *in situ* gross production and consumption rates, in which by tracing the dilution of the isotope-labeled tracers by unlabeled compounds over time, production and consumption rates of the target compounds can be quantified (Kirkham and Bartholomew, 1955; Di et al., 2000). The IPD technique has been applied in various SOM decomposition assays, such as for studying soil protein and glucan decomposition (Wanek et al., 2010; Leitner et al., 2012; Mooshammer et al., 2014; Wild et al., 2015). Recently, we pioneered a methodology for the quantification of *in situ* gross production and consumption rates of soil amino compounds, including free amino sugars and D- and L-amino acid enantiomers based on the ^{15}N-IPD technique and a novel liquid chromatography-high resolution mass spectrometry platform (Hu et al., 2017). In this assay, ^{15}N-labeled monomeric amino sugars and amino acids were used to label the free amino compound pool, and the ^{15}N enrichment is subsequently diluted by the decomposition of unlabeled native microbial cell walls and uptake of monomers by soil microbes. It is therefore for the first time possible to quantify controls and contributions of the decomposition of amino sugar polymers to soil organic N cycling and microbial N use.

The objective of this study was to estimate the production and consumption of microbial cell wall-derived free amino sugars and amino acid enantiomers in soils, as well as to assess how soil characteristics and microbial community structure affect these transformation. Therefore, we applied the novel IPD assay to soils from six sites differing in land management (cropland, pasture, and forest) at two bedrock types (silicate and limestone) to determine the *in situ* gross production and consumption rates of amino sugars and amino acid enantiomers. Soils were characterized for physico-chemical properties and microbial community structure was profiled by phospholipid fatty acid (PLFA) analysis. Experiments were conducted at three temperatures, i.e. 5, 15, and 25 °C to determine the temperature sensitivity of *in situ* gross production and consumption rates.

2. Materials and methods

2.1. Site description and soil sampling

Soil samples were taken from six sites, from three land management types (cropland, pasture, and forest) replicated on two bedrocks (silicate and limestone) in the upper Enns valley, Styria, Austria. Silicate soils (Spodo-dystric Cambisols) were sampled from Gumpenstein (47.49 °N, 14.10 °E, 690 m a.s.l.) and limestone soils (Leptosols) from Moarhof, Pürgg-Trautenfels (47.51 °N, 14.07 °E, 700 m a.s.l.). The sites are close-by but on opposing faces of the Enns valley differing in geology but not in climate, with mean annual precipitation of 980 mm and mean annual temperature of 7.2 °C. Sites in Gumpenstein included a cropland grown with cabbage, beans, potatoes, and onions, a sheep-grazed pasture, and a forest composed of *Picea abies* and *Vaccinium myrtillus*. At Moarhof the arable site was grown with barley, oat, and wheat, the pasture was grazed by cattle, and the forest was dominated by *Picea abies* and *Fraxinus excelsior*. Further details on site location, land management, and vegetation can be found in Table S1. At each site, mineral topsoil was sampled in four replicates to 15 cm depth using a root corer with 8 cm diameter (Eijkelkamp, Netherlands), after the removal of the organic soil layer.

2.2. Soil characterization

After being brought back to the laboratory on the same day, fresh soil samples were immediately sieved through a 2-mm sieve and incubated at field moisture at 15 °C in sealed polyethylene bags prior to the experiments. Bags were opened shortly every three days to allow for gas exchange and water contents were adjusted when necessary. Soil pH was determined in 10 mM $CaCl_2$ (1:2.5 w/v soil: $CaCl_2$) by an ISFET electrode (Sentron, Austria). Soil samples were dried at 80 °C for 48 h and ground to a fine powder using a ball mill (MM2000, Retsch, Germany) for elemental analysis. Total soil organic C (C_{org}) and total N (TN) content were determined using Element Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS; EA 1110 elemental analyzer coupled to a Finnigan MAT Delta^{Plus} IRMS, Thermo Fisher Scientific, USA). Soil dissolved organic C (DOC) and total dissolved N (TDN) were measured in 1 M KCl extracts (1:5 w/v fresh soil, 30 min extraction time) by a DOC/TN analyzer (TOC-VCPH/CPN/TNM-1, Shimadzu, Austria). Ammonium and nitrate concentrations were determined