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

**Yuntao Hu**, **Qing Zheng**, **Shasha Zhang**, **Lisa Noll**, **Wolfgang Wanek***
Department of Microbiology and Ecosystem Science, Research Network "Chemistry Meets Microbiology", University of Vienna, Althanstraße 14, 1090, Vienna, Austria

## 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.


*Corresponding author. wolfgang.wanek@univie.ac.at (W. Wanek).

**Keywords**

Free amino sugars; Isotope pool dilution; Organic nitrogen flux; Microbial cell wall decomposition; Chitin; Peptidoglycan

## 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 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 μM, 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 μM) 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 photometrically in 1 M KCl extracts following Hood-Nowotny et al. (2010). Dissolved organic nitrogen (DON) was determined by subtracting ammonium and nitrate concentrations from TDN. Soil texture, exchangeable

base and acid cation contents, base saturation, carbonate content, and cation exchange capacity (CEC) were determined according to standard protocols (ÖNORM L 1061, L 10,694, L 1086, ISO 11,260) at the soil analysis facility of the Austrian Agency for Health and Food Safety (AGES, Vienna, Austria). A summary of selected physicochemical soil properties is presented in Table 1. All chemicals used were of the highest purity, water derived from a Milli-Q system (Millipore, USA) and LC solvents and water were of LC/MS grade (Sigma-Aldrich, Thermo Fisher, Austria).

### 2.3 Isotope pool dilution and hydrolytic enzyme activity assays at different temperatures

The novel IPD assay for amino sugars and amino acid enantiomers was described and validated in detail before (Hu et al., 2017). Briefly, uniformly $^{15}$N-labeled peptidoglycan was isolated from *Bacillus subtilis* (DMDZ 10) cultivated on uniformly $^{15}$N-labeled growth media. $^{15}$N-labeled amino sugars and amino acid enantiomers were obtained by hydrolyzing the $^{15}$N-labeled peptidoglycan in 6 M HCl (12 h, 110 °C), purified by cation exchange SPE, and freeze-dried. Prior to the IPD assay, the $^{15}$N-labeled tracer was dissolved in water and the concentration of amino compounds determined by UPLC/HRMS (Table S2). The composition of labeled compounds was as reported in Hu et al. (2017). All soils were adjusted to 60% water holding capacity seven days prior to the IPD assay and kept at 15 °C in an incubator. For the IPD assay, aliquots of 4 g fresh soil were weighed into 50 mL polypropylene tubes and preincubated at 5, 15, and 25 °C for 24 h. Based on the pool sizes of free amino sugars and amino acids in the soils three days before the start, $^{15}$N-labeled amino compounds were added at a rate comprising less than 30% of the original pool size, except for muramic acid that was increased 1.5- to 11-fold by tracer addition due to its very small pool size. The IPD assays were started by tracer addition in 0.4 mL water and terminated after 15 min, 60 min, and 240 min by adding 20 mL 1 M KCl.

Soil free amino sugars and free amino acids were extracted, purified, and analyzed as previously described in detail (Hu et al., 2017). Briefly, extraction was performed by adding 20 mL 1 M KCl to aliquots of 4 g fresh soil and shaking for 30 min at 200 rpm at room temperature. After filtration through ashfree cellulose filters, aliquots of 10 mL extract were frozen in liquid nitrogen and lyophilized for 48 h. Lyophilized extracts were re-dissolved in 10 mL dry methanol and centrifuged (10,000 g, 5 min) twice to remove insoluble salts. Samples were then dried under a gentle $N_2$ flow, re-dissolved in water and loaded onto cation exchange cartridges. Cation exchange cartridges were prepared by packing 3 g Dowex 50WX8 resin ($H^+$ form, 200 mesh particle size, Sigma-Aldrich, Austria) into 6 mL polystyrene tubes and rinsing sequentially with 12 mL $NH_4OH$, water, 1 M HCl and finally with 36 mL water. After samples were loaded, cartridges were washed with 10 mL water and amino compounds eluted with 10 mL 3 M $NH_4OH$ in 30 min. Finally, the eluted compounds were dried under a gentle $N_2$ flow and re-dissolved in 0.5 mL acetonitrile/water (80/20, v/v) for further LC/MS analysis.

Concentrations of labeled and unlabeled amino sugars and amino acids were quantified by high performance liquid chromatography coupled to a high resolution mass spectrometer (UPLC/Orbitrap-HRMS platform, Hu et al. (2017)). Separations of the amino sugars and amino acids were performed on an Accucore HILIC column (150 mm × 2.1 mm, 2.6 μm

particle size; Thermo Scientific, Germany) with a gradient starting at 95% eluent B (acetonitrile, 0.1% formic acid (v/v)) and going up to 40% eluent A (water, 0.1% formic acid (v/v)) in 24 min (Hu et al., 2017). Amino acid enantiomers were separated with an Astec chirobiotic T column (150 mm × 2.1 mm, 2.6 μm particle size; Sigma-Aldrich, Austria) by isocratic elution with methanol/water/formic acid (80/20/0.1 (v/v)). Column temperatures were set to 25 °C and the injection volume was 25 μL. The Orbitrap-MS was operated in positive mode with a scan range of 60–1000 m/z, and the mass resolution was set to 50,000. In contrast to Hu et al. (2017), no $^{13}$C-labeled internal standards were added in this study but recoveries of the target compounds were checked and accounted for by processing standards throughout the whole preparation procedure.

Soil enzyme activities were determined in separate aliquots of fresh soils without any isotope treatment in 50 mM sodium acetate buffer at pH 5, in which the protease (leucine amino-peptidase) activity was assayed by leucine-7-amino-4-methyl coumarin (AMC) addition and fluorescence measurement of the release of the coumarin adduct. Exochitinase activity was measured by following the release of 4-methylumbelliferone (MUF) from the respective MUF-N-acetyl-β-*d*-glucosaminide substrate (Kaiser et al., 2010b).

### 2.4 Soil total amino sugar and amino acid contents

Soil total amino sugars and amino acids were determined based on the methods published before with some modifications (Martens and Loeffelmann, 2003). Aliquots (40 mg dry weight) of ground soils were hydrolyzed with 10 mL 6 M HCl at 110 °C for 12 h, and norleucine was added to soils as an internal standard. Hydrolyzates were filtered through glass microfiber filters (GF/C, 2 μm pore size, Whatman) and dried under a gentle $N_2$ flow to remove HCl. Dried residues were redissolved in MilliQ water and purified by cation exchange solid phase extraction (SPE) as described in section 2.4. Samples were separated on an ion chromatography system (DX 500, Dionex, Austria) equipped with an AminoPac PA10 column (250 mm × 2 mm, Thermo Scientific, Germany) with a gradient of water, NaOH (40 mM - 200 mM), and NaOAc (0–400 mM) (Martens and Loeffelmann, 2003). An electrochemical detector in pulsed amperometric detection (PAD) mode was used to measure the amino sugar and amino acid contents. Fungal and bacterial necromass were calculated according to Appuhn and Joergensen (2006). Briefly, we assumed a 1:1 M ratio of muramic acid and glucosamine in peptidoglycan for bacterial necromass, subtracted bacterial glucosamine from total glucosamine to give fungal glucosamine, and multiplied by factors of 45 and 9 for bacterial and fungal necromass, respectively (Appuhn and Joergensen, 2006).

### 2.5 Phospholipid fatty acid analysis

Soil microbial community structure was determined by PLFA profiling. PLFAs were extracted according to Kaiser et al. (2010b) and analyzed with gas chromatography (GC) (Trace 1300, Thermo Scientific, Austria) coupled to an ISQ single quadrupole mass spectrometer (Thermo Scientific, Germany). We used branched PLFAs (a15:0, i15:0, i16:0, a17:0, and i17:0) as indicator for gram-positive bacteria, cyclopropyl and mono-unsaturated PLFAs (16:1ω7, 16:1ω9, cy17:0, and cy19:0) for gram-negative bacteria, saturated PLFAs (15:0 and 17:0) for uncategorized bacteria, poly-unsatured PLFAs (18:1ω9 and 18:2ω6,9) for fungi, 10Me-PLFAs (10Me17:0, 10Me18:0, 10Me19:0) for actinomycetes, and

unspecific PLFAs (14:0, 16:0,18:0, 20:0) for viable biomass (Hill et al., 2000; Kaiser et al., 2010a; Inglett et al., 2011). Fungi to bacteria ratios (F/B) were calculated by dividing fungal PLFAs by the sum of bacterial PLFAs. The fungal biomarker PLFA 18:1w9 however also occurs in some gram-positive bacteria, eventually causing an overestimation of fungal biomass and fungi to bacteria ratios in agricultural soils (Frostegard et al., 2011).

## 2.6 Calculations

The gross production (GP) and gross consumption (GC) rates of amino sugars and of amino acids and their enantiomers were calculated based on isotope pool dilution theory using the analytical equations developed by Kirkham and Bartholomew (1955).

$$\text{at\% excess}^{15}\text{N} = \text{at\%}^{15}\text{N}_{\text{Sample}} - \text{at\%}^{15}\text{N}_{\text{b}} \quad (1)$$

$$GP = \frac{\left(C(tot)t_2 - C(tot)t_1\right)}{t_2 - t_1} \times 60 \times 24 \times \frac{ln\left[\left(at\ \%\ \text{excess}^{15}Nt_1\right)/\left(at\ \%\ \text{excess}^{15}Nt_2\right)\right]}{ln\left(C(tot)t_2/C(tot)t_1\right)} \quad (2)$$

$$GC = \frac{\left(C(tot)t_1 - C(tot)t_2\right)}{t_2 - t_1} \times 60 \times 24 \times \left(1 + \frac{ln\left[\left(at\ \%\ \text{excess}^{15}Nt_2\right)/\left(at\ \%\ \text{excess}^{15}Nt_1\right)\right]}{ln\left(C(tot)t_2/C(tot)t_1\right)}\right) = GP - \frac{\left(C(tot)t_2 - C(tot)t_1\right)}{t_2 - t_1} \quad (3)$$

where at% excess $^{15}$N defines the $^{15}$N atom percentage excess and at %$^{15}$N$_b$ represents the natural abundance of $^{15}$N, which was set to 0.365. C(tot) is the sum of the concentrations of $^{15}$N-labeled and unlabeled amino compounds (μg N g$^{-1}$ d.w.). The termination times $t_1$ and $t_2$ were set to 15 and 60 min except for muramic acid, for which 15 and 240 min were used due to its slower turnover rate. Mean residence times (MRTs) were calculated by dividing the pool size by the mean of gross production and consumption rates. We further used the ratios of gross production rates of D-alanine/L-alanine and of D-alanine/glucosamine to assess the contributions of organic N released from peptidoglycan, chitin, and protein decomposition in soils, as D-alanine in soils solely originates from peptidoglycan decomposition and glucosamine is released from both, chitin and peptidoglycan decomposition. More specifically, the ratio of D-alanine/L-alanine is ~1 in peptidoglycan stem peptides but 0 in proteins and that of D-alanine/glucosamine is 1 in peptidoglycan but 0 in chitin (Schleifer and Kandler, 1972).

The temperature sensitivities of gross production and consumption rates of amino compounds and of hydrolytic enzyme activities were determined by the LN-transformed linear regression of temperature and process rates (Janssens and Pilegaard, 2003):

$$Ln(R) = Ln(S)/10 * T + b \quad (4)$$

where R was the measured rate, S was the temperature sensitivity (equivalent to Q10 values), and T was the temperature of incubation, which was 5, 15, and 25 °C in this study. The temperature sensitivity therefore expresses the -fold change in process rates for a 10 °C increase in temperature and was calculated across the full temperature range (5–25 °C).

### 2.7 Statistics

Data were tested for homogeneity of variance and transformed if necessary. We tested for significant effects ($p < 0.05$) of bedrock and land management by two-way analysis of variance (ANOVA) followed by the Tukey post hoc test. All F and p values of the two-way ANOVA analyses can be found in Table S3. Additionally, relationships between gross process rates and putative drivers (soil physicochemical parameters and microbial community composition) were examined by Pearson correlations. A principal component analysis (PCA) was performed separately on soil properties and on gross production and consumption rates of amino compounds to evaluate the multivariate difference between sampling sites. Furthermore, a non-metric multidimensional scaling (NMDS) analysis was performed on the PLFAs as a proxy of microbial community structure. All values in the results and discussion section are presented as means ± SE (n = 4). All statistics and calculations were conducted with R (version 3.1.3).

## 3 Results

### 3.1 Site characteristics and PLFA analysis

Physicochemical and microbial properties of the soils and effects of land use and bedrock are listed in Table 1 and Table S3. Soil pH was between 3.7 and 5.6 on silicate bedrock and between 6.0 and 7.3 on limestone. Across both bedrock types, arable soils had higher soil pH values and the lowest pH occurred in forest soils. Limestone soils had higher CEC and the lowest clay contents were found in pasture soils across the three land management types. The $C_{org}$, TN, $NO_3^-$ and free amino acid (FAA) contents were significantly higher, while DOC, DON, $NH_4^+$ and free amino sugar (FAS) contents were significantly lower on limestone soils than on silicate soils. Soil C/N ratios varied little, from 9.5 to 11.3 across all sites except forests on silicate soils, which had a soil C/N ratio of 19.6. Viable microbial biomass, as represented by bacterial and fungal PLFAs, was 2- to 3-fold greater in limestone compared to silicate soils, but the fungal and bacterial necromass and fungal/bacteria ratios were not affected by bedrock type (Table 1, Fig. S2). There was no effect of land management on microbial PLFAs (Table 1).

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### 3.2 Gross production and consumption rates, and mean residence times of amino compounds

Free glucosamine and mDAP concentrations ranged from 27.7 to 208 ng N $g^{-1}$ and from 32.5 to 211 ng N $g^{-1}$ dry soil, which were equivalent to 4%–15% of the FAA pool (Table 2). Soils on limestone had greater free glucosamine pool sizes and lower mDAP contents, while both compounds were highest in pasture and lowest in arable soils. Free muramic acid concentrations were unexpectedly low in all soils (0.2–1.5 ng N $g^{-1}$), with higher concentrations in limestone soils. L-alanine contents were 2- to 3-fold lower than glucosamine contents in most cases. D-alanine contents were equivalent to 23% of the L-alanine contents in silicate soils, but only 5% of that in soils on calcareous bedrock.

Glucosamine production and consumption rates varied widely between land management and bedrocks, ranging between 0.31 and 3.29 μg N $g^{-1}$ d.w. $d^{-1}$ (Fig. 1), with higher rates in silicate soils. It is worth noting that the other amino sugar, muramic acid, had production rates more than 2 orders of magnitude lower than glucosamine, with less than 0.01 μg N $g^{-1}$ d.w. $d^{-1}$ in all soils, showing highest gross production rates in pasture soils. Production and consumption rates of the three proxy amino acids, i.e. mDAP, L-alanine, and D-alanine, ranged between 0.02 and 1.22 μg N $g^{-1}$ d.w. $d^{-1}$ and were in almost all cases lower than those of glucosamine. But unlike glucosamine, silicate soils showed significantly lower production and consumption rates of these three amino acids compared to calcareous soils. Overall, bedrock and soil pH therefore appeared to be more important in governing the *in situ* gross production and consumption rates of these amino compounds compared to land management (Table S3).

MRT differed greatly between the amino compounds, and ranged between 0.7 and 139.5 h (Table 2). We observed comparable average MRTs (2.8 h and 1.4 h) for glucosamine and L-alanine, and both exhibited shorter MRTs in silicate soils, demonstrating faster turnover in acidic soils. Compared to L-alanine, D-alanine had longer MRTs (4.0 h) in silicate soils but shorter MRTs (0.2 h) in limestone soils. In contrast, muramic acid had much longer MRTs, which was on average 68 h. Both, the activities of exochitinase and aminopeptidase were lower in silicate soils than in limestone soils (Table S4). We also observed significant effects of land management, with highest activities of exochitinase in arable soils and highest activities of amino peptidase in pasture soils.

We observed that the ratios of gross production of D-alanine/L-alanine ranged between 0.09 and 0.71, and were 3-fold higher in calcareous soils than in silicate soils. This indicates a significantly higher proportion of peptidoglycan decomposition compared to protein decomposition in soils on limestone (42–71% of alanine production) compared to silicate soils (9–23%; Fig. 2). The ratios of gross production of D-alanine/glucosamine ranged between 0.07 and up to 1.1, indicating that in silicate soils chitin decomposition (> 93%) predominated over peptidoglycan decomposition (< 7%) while in limestone soils peptidoglycan decomposition dominated (37–100%) over chitin decomposition (0–63%).

### 3.3 Temperature sensitivity of enzyme and gross production and consumption rates

Log-linear regression models were used to estimate the temperature sensitivity (Q10) of gross production and consumption rates of amino sugars and amino acid enantiomers as well as of enzyme activities. The predictive ability ($R^2$) of the regression models for production and consumption rates ranged between 0.61 and 0.85, and for enzyme activities between 0.91 and 0.99. The temperature sensitivity of production and consumption rates of glucosamine varied between 1.01 and 2.35, except for pasture soils on silicate bedrock, which were 3.52 and 4.15 (Fig. 3). The temperature sensitivity was generally higher on silicate than on limestone soils and was lowest in arable soils and highest in pasture soils. In contrast, the temperature sensitivity of gross production and consumption rates of L-alanine were relatively stable and ranged between 0.93 and 1.55 with a mean of 1.24 ± 0.05, showing no significant differences across land management. The temperature sensitivity of D-alanine gross production and consumption rates showed relatively little variability, ranged between 1.0 and 1.2. The temperature sensitivity of exochitinase activity (1.3–3.4) differed greatly between sites and was higher in limestone than in silicate soils (Table S4), therefore positively correlating with soil pH ($p < 0.001$, $r = 0.79$). The temperature sensitivity of amino peptidase activity was relatively similar across the six sites (Table S4), varying between 1.2 and 1.7, and exhibited the same significant trends as the exochitinase activity.

### 3.4 Controls on soil amino compound gross production and consumption rates

Pearson's correlation analyses were performed to explore the relationships of soil physicochemical characteristics affecting substrate availability (total amino sugars and amino acids, soil texture), microbial community structure at biomass and necromass level, amino sugar and amino acid gross production and consumption rates, and enzyme activities. The gross production and consumption rates of glucosamine, mDAP, and L-alanine were all strongly positively correlated ($p < 0.001$), pointing to an equilibrium between decomposition and microbial utilization of each individual amino compound (Table S5). In addition, we observed positive correlations between gross production (and consumption) rates and free pool sizes of the individual amino compounds ($p < 0.01$) and across all amino compounds ($p < 0.001$), indicating that the lower pool sizes were causally linked to lower production and consumption rates. We found that gross production rates of amino compounds were not related to fungal or bacterial necromass ($p > 0.05$) and not or only weakly to extracellular hydrolytic enzyme activities ($p < 0.05$). In contrast, gross production rates of amino compounds were strongly correlated with soil physicochemical parameters such as soil texture, soil pH, and CEC. For instance, gross production rates of glucosamine exhibited significant negative correlations with soil pH, CEC, and clay and silt content ($p < 0.05$) and strong positive correlations with sand content ($p < 0.001$). In contrast, gross production rates of the other four amino compounds, i.e. muramic acid, L-alanine, D-alanine, and mDAP, were all negatively related to sand content ($p < 0.05$) and positively to pH, CEC, and silt and clay content ($p < 0.01$). The L-alanine and D-alanine gross production and consumption rates were strongly interrelated ($p < 0.001$), indicating the similarity of turnover of the alanine enantiomers. mDAP production rates exhibited a significant positive correlation with D-alanine production rates ($p < 0.05$), both originating from peptidoglycan stem peptides. Microbial PLFAs were only positively correlated to gross production and consumption rates ($p < 0.05$) of muramic acid, but not of other compounds.

### 3.5 Multivariate analyses (PCA and NMDS)

The PCA of soil physicochemical characteristics demonstrated substantial differences between soil types (Fig. 4a), and principal component 1 (PC1) explained 38.5% and principal component 2 (PC2) 27.4% of the total variance in the data. Soil pH, CEC, and soil TN were negatively correlated and sand content positively correlated with PC1, while total amino sugars and total amino acids were negatively correlated and $C_{org}$ and soil clay content were positively correlated with PC2. The silicate soils were all located on the right side of the figure and the limestone soils plotted towards the left part of the figure. Forest and pasture soils were separated along PC2.

In the PCA of gross production rates of amino compounds and enzyme activities, the six sites were clearly separated according to bedrock along PC1 (45.6% explained variance) and according to land management along PC2 (21.2% explained variance). Again, silicate soils plotted to the right side of PC1 (Fig. 4b) while forests plotted to the bottom and pastures to the top of PC2. The gross production and consumption rates and enzyme activities separated into three groups, in which glucosamine production and consumption rates positively correlated with PC1 while the other amino compound production and consumption rates were all negatively correlated to PC1. The gross production rates of the three amino acids (L-alanine, D-alanine, and mDAP) were negatively correlated and muramic acid production and consumption rates as well as enzyme activities positively correlated with PC2.

NMDS analysis of soil microbial PLFA patterns showed that soils only separated along NMDS axis 1, with limestone soils plotting to the right (high PLFA contents of actinomycetes, fungi and gram-positive bacteria) and silicate soils plotting to the left (high gram-negative PLFA content) (Fig. 4c). Soil management did not affect microbial community structure in this NMDS analysis.

## 4 Discussion

### 4.1 Glucosamine gross production rates in bacterial and fungal cell wall decomposition processes

We observed that free glucosamine accounts for a significant proportion of the DON pool, comparable to concentrations of free amino acids (L- and D-alanine in this case) in soil KCl extracts. Although only a few studies on free soil glucosamine exist due to its small pool size, Roberts et al. (2007) and Warren (2013) reported free glucosamine in soil solutions of less than 10 μg N $L^{-1}$ (below detection limit) and 0.28 μg N $L^{-1}$ in grassland soils, respectively. Glucosamine was also shown to be a major constituent of the organic N monomer fraction in 0.5 M $K_2SO_4$ extracts of a sub-alpine soil, with the concentration of 0.071 μg N $g^{-1}$ soil even exceeding those of many abundant amino acids such as Glu, Asp, Arg and Lys (range 0.011–0.027 μg N $g^{-1}$) (Warren, 2014). The concentrations of extractable glucosamine in our study (1 M KCl) were 1- to 3-fold greater than in 0.5 M $K_2SO_4$ soil extracts as reported by others (Warren, 2014) but much higher than in soil solutions, indicating that a large proportion of glucosamine is bound to the soil's solid phase and is not present freely in soil solution.

Previous research suggested that the low concentration of free glucosamine in soils is due to rapid microbial and plant uptake rather than slow production rates (Roberts et al., 2007; Roberts and Jones, 2012; Hu et al., 2017). Microbial studies also have provided numerous indications that glucosamine is a highly preferred N (and C) source for *E. coli* and *B. subtilis* and that it even shares the same membrane transporter with glucose (Imada et al., 1977; Gaugue et al., 2013). Agreed to these studies, the short MRT of glucosamine across all soil types in our study (2.78 ± 0.37 h) is consistent with that from other studies following the microbial respiration of $^{14}$C-labeled glucosamine, which was 3.0 ± 0.7 h, indicating the rapid turnover of free glucosamine in soils (Roberts et al., 2007). In contrast, the half-life times of microbial utilization of isotopically labeled chitin added to soils were 100-fold higher than for glucosamine, and ranged between 12 and 22 days (Fernandez and Koide, 2012; Zeglin and Myrold, 2013). This implies that glucosamine can be readily used but that the initial depolymerization process of microbial cell walls constrains the utilization of these polymeric amino sugars. Our results show that the mean *in situ* gross production rates of glucosamine were slightly lower than the gross consumption rates (1.15–1.51 μg N $g^{-1}$ d.w. $d^{-1}$) and gross N mineralization rates (1.79 μg N $g^{-1}$ d.w. $d^{-1}$) (Shasha Zhang, unpublished data), providing further evidence that depolymerization of the glucosamine polymer (predominant chitin/chitosan and peptidoglycan) is the rate limiting step of amino sugar decomposition and utilization, similar as reported for soil protein decomposition (Wanek et al., 2010).

In this work, we could only compare glucosamine production rates with those of a few proxy amino acids/enantiomers making up peptidoglycan, but we previously reported that glucosamine production rates accounted for 5–10% of that of total amino acids in a temperate forest and an arable soil. This indicates that amino sugars deriving from peptidoglycan and chitin decomposition contribute substantially to the bio-available soil DON pool (Hu et al., 2017). Although deacetylation of chitin was reported to be a crucial initial step in the chitin decomposition process, acetylated glucosamine, i.e. N-acetylglucosamine, may be another important product released into the bio-available DON pool through depolymerization of chitin/chitosan (Beier and Bertilsson, 2013). Microorganisms were also proven to be able to utilize N-acetylglucosamine besides glucosamine as N (and C) source and it is still unclear how much of the N-acetylglucosamine deriving from chitin and peptidoglycan decomposition becomes deacetylated to glucosamine by deacetylases *in situ* before uptake (Kadokura et al., 2007; Swiatek et al., 2012; Beier and Bertilsson, 2013). Therefore, the release of N-acetylglucosamine from chitin and peptidoglycan decomposition will most probably add to the gross production and consumption rates of free amino sugars and current measurements of amino sugar release rates therefore are likely underestimates, an issue that we are currently targeting in ongoing research. Previous research indicated that galactosamine and mannosamine (being isomers of glucosamine) represented approximately 18% and 3% of total hydrolyzable amino sugars in soils, though there was no detectable free galactosamine or mannosamine in soils (Zhang and Amelung, 1996). Concurrent release of galactosamine and mannosamine (from sources other than chitin and peptidoglycan such as from glycosylated microbial membrane proteins) with glucosamine – which we could not separate by HILIC - might have caused an overestimation of the glucosamine gross production rates

but would not affect glucosamine consumption rates that is traced by $^{15}$N-glucosamine derived from peptidoglycan that is devoid of the other hexosamine isomers (Briard et al., 2016).

### 4.2 Muramic acid, mDAP, and D- and L-alanine gross production and consumption rates in the peptidoglycan decomposition process

Surprisingly, our results together with a previous report show that free muramic acid has extremely low pool sizes (0.15–9 ng N $g^{-1}$) as well as gross production rates compared to glucosamine, although they have very similar chemical structure (only differing in a lactic acid residue) and both serve as backbones of peptidoglycan in equimolar ratios (Hu et al., 2017). This might indicate that chitin decomposition rates by far exceed peptidoglycan decomposition rates in all six soils studied here, or that free muramic acid is not a major decomposition product of peptidoglycan in soils. The relatively high microbial utilization rates of muramic acid compared to its production rates were caused by the strong increase in the concentration of $^{15}$N-muramic acid by the tracer addition to the soils (Fig. 1), but independent of this suggest that muramic acid can be efficiently used as an N source by soil microorganisms. Furthermore, we observed considerable production and consumption of D-alanine and mDAP originating mainly from depolymerization of peptidoglycan, indicating that the branched stem peptides of peptidoglycan are indeed partially hydrolyzed by extracellular enzymes in soils. These findings imply that muramic acid, mDAP, and D-alanine can be utilized by soil microorganisms but that muramic acid is rarely released during decomposition of peptidoglycan. Currently, it remains open whether free N-acetylmuramic acid or rather muropeptides are the main muramic acid-containing compounds released during peptidoglycan decomposition in soils. Culture studies have shown that the major degradation products of peptidoglycan are anhydro-muropeptides (anhyMurNAc-di-, tri- and tetrapeptides), which can be directly transported into bacterial cells by the AmpG secondary active transport system and then are further decomposed or re-utilized in the cells (Holtje, 1998; Cheng and Park, 2002; Uehara et al., 2006). The muropeptides can also be hydrolyzed extracellularly into individual amino compounds in soils, but total hydrolysis seems to be cost-ineffective as it requires more than six specific classes of enzymes including N-acetylmuramoyl-L-alanine amidases, which cleave the amide bond between muramic acid and the stem peptides (Scheurwater et al., 2007; Humann and Lenz, 2009). Therefore, it is more likely that the backbone and the stem peptide of peptidoglycan are partly hydrolyzed by extracellular enzymes releasing glucosamine, D-alanine, and mDAP while muramic acid-containing units (i.e. muropeptides such as anhyMurNAc-L-Ala-D-Glu) are not further cleaved but directly taken up by microbes in an intact form.

D-alanine was reported as one of the most abundant D-amino acids in soils (Kunnas and Jauhiainen, 1993; Warren, 2017). The D/L-alanine ratios in soil KCl extracts in our study ranged from 4% to 32% which were slightly higher than those reported for soil water extracts (6.6%) and soil hydrolyzates (3–16%) (Amelung and Zhang, 2001; Warren, 2017). In agreement with previous observations (O'Dowd and Hopkins, 1998; Farrell et al., 2012), D-alanine exhibited similar (limestone soils) or lower consumption and turnover rates (silicate soils) than L-alanine. However, differences between L- and D-alanine consumption

rates were not statistically significant when accounting for the differences in gross production rates of both enantiomers, pointing to the rapid utilization of both, D- and L-alanine in soils. We also showed that the ratio of *in situ* gross production rates of D/L-alanine was 18% in silicate soils and 57% in limestone soils (Fig. 2). Given an average ratio of D/L-alanine in peptidoglycan of ~1 (or 100%) and in protein of 0, we can apply gross production ratios of D/L-alanine to roughly estimate the contributions of peptidoglycan versus protein decomposition to alanine production in soils. In silicate soils, peptidoglycan decomposition (18%) was out-paced by protein decomposition (82%) while in limestone soils peptidoglycan decomposition (57%) predominated. The D/L-alanine ratios in peptidoglycan and in gross production rates, however, come with a large uncertainty as stem peptides (see above) are evidently not fully depolymerized, therefore releasing more D-alanine than L-alanine, the latter which might be bound in muropeptides as decomposition by-products. Values therefore are likely overestimates but were strongly positively correlated with the ratio of gross production rates of D-alanine/glucosamine, which demonstrated a similar trend. The production ratio of D-alanine/glucosamine might offer greater promise, as D-alanine and glucosamine are major decomposition products of peptidoglycan. This ratio allows the partitioning of peptidoglycan (D-alanine/glucosamine ratio ~1) relative to chitin decomposition (ratio = 0). For instance, low D-alanine/glucosamine ratios of gross production rates in silicate soils (< 0.1) indicated that peptidoglycan decomposition (< 10%) was by far exceeded by chitin decomposition (> 90%). In contrast, in limestone soils D-alanine/glucosamine ratios of production rates (0.37–1.1) indicate substantial contribution of peptidoglycan decomposition (37–100%) and smaller contributions by chitin decomposition (0–63%) at higher soil pH. We must however admit that ratios of gross production rates of D-alanine/glucosamine of > 1 in limestone forests (maximum 1.1) would lead to relative contributions of peptidoglycan decomposition > 100% which is most likely due to D-alanine/glucosamine ratios in soil bacterial cell walls higher than 1. Alternative explanations are that D-alanine is also released from the decomposition of other D-alanine containing bacterial polymers than peptidoglycan such as lipopeptides anchored in bacterial cell walls (Kaiser and Benner, 2008), or that glucosamine release is underestimated due to significant release of N-acetylglucosamine which was not assessed here. In an ongoing study, we are currently evaluating the gross production rates and the stoichiometry of glucosamine, N-acetylglucosamine, muropeptides, mDAP, D- and L-alanine, and D-glutamate release from soil peptidoglycan decomposition which will greatly advance the applicability of this approach in soil biogeochemistry.

### 4.3 Controls of the decomposition process of amino sugar polymers in soils

In our study, the six sites were situated close to each other, therefore experiencing similar precipitation and air temperature but differing in bedrock and land management. This provided us the opportunity to dissect the effects of management and bedrock on soil organic nitrogen transformation processes. Recent studies clearly showed that SOM decomposition is controlled by various factors including environmental conditions such as soil pH, soil texture, soil temperature, moisture and oxygen availability, as well as extracellular enzyme activities, resource elemental stoichiometry, and microbial community structure and activity (Wallenstein and Weintraub, 2008; Sinsabaugh et al., 2009; Kaiser et al., 2010b; Schmidt et al., 2011; Wagai et al., 2013). Soil C/N and soil pH may govern the

nutrient demand and the structure of soil microbial communities, thereby affecting the secretion of extracellular enzymes and eventually affecting the decomposition of SOM (Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015). In this study, we observed weak to non-significant relationships between extracellular enzyme activities (aminopeptidase and exochitinases) and gross production rates of amino sugars, amino acids and their enantiomers. The temperature sensitivities of the hydrolytic enzyme activities were not correlated with that of necromass decomposition, i.e. the gross production rates of glucosamine and D-alanine. Moreover, we found that the temperature sensitivity of glucosamine production was lower than that of exochitinase activity, except for forests soils on silicate bedrock. This indicates that depolymerization of glucosamine polymers was not an enzyme-limited process since the temperature sensitivity of diffusion processes is lower (close to unity) than that of extracellular enzymatic reactions (temperature sensitivity = 1.5–2.5) (Steinweg et al., 2012; Blagodatskaya et al., 2016). In addition, we measured soil total amino sugars and estimated bacterial and fungal necromass, since the production of free glucosamine from bacterial and fungal cell walls may be a substrate-limited process. However, we did not find any significant difference in total amino sugar contents as well as in microbial necromass and fungi/bacteria ratios across the six ecosystems, which agrees with previous findings, in which the amount of total amino sugars in soils did not vary strongly across sites (Stevenson, 1982; Roberts et al., 2007). Therefore, gross production rates of glucosamine, mDAP and D-alanine were not related to any of these necromass parameters indicative of the total soil pool of peptidoglycan and chitin available for decomposition. This stands in stark contrast to the large differences in peptidoglycan and chitin decomposition across soils, particularly bedrock types. Therefore our data strongly indicate that amino sugar polymer decomposition was not limited by extracellular enzyme activity or total contents of potential substrates but rather by mechanisms affecting the stabilization or accessibility of the microbial cell wall polymers in soils.

Stabilization of organic matter can be strongly increased by (i) sorption to soil minerals and can therefore be affected by soil texture, mineralogy and soil pH, or (ii) occlusion in soil aggregates which is strongly affected by texture and biological activity. We here found that glucosamine production and chitin decomposition were negatively related to soil pH, CEC and clay content which can increase sorption strength but were positively related to sand content (Table S5), suggesting that conditions of acidic soil pH and low clay content may stimulate the decomposition of amino sugar polymers, particularly chitin, through less stringent stabilization. Solomon et al. (2001) reported that the amino sugar depletion was stronger in sand-associated SOM compared to that associated with the clay fraction after land use change in tropical soils. This was also supported by artificial soil studies, which found that fungal residues tended to accumulate in high CEC montmorillonite-containing soils rather than in illite-containing soils (Pronk et al., 2015). Moreover, low soil pH favors fungal growth and therefore chitin deposition in soils, and fungi were reported to use glucosamine as a primary energy and N source (Zhang et al., 1999; Rousk et al., 2009; Moye et al., 2014). In contrast, the positive relationship between production rates of mDAP or D-alanine with soil pH suggest a faster turnover of peptidoglycan stem peptides in calcareous soils as bacteria are the major soil decomposers under neutral and alkaline soil pH conditions (Padan et al., 2005; Uehara et al., 2006), though this was not reflected in the

PLFA community structure. Mechanistic studies on sorption and aggregation processes and how these together with microbial activities affect the stabilization of bacterial and fungal necro-mass in soils therefore will greatly help advance our understanding of decomposition controls on peptidoglycan and chitin in soils relative to other polymeric organic N forms (e.g. proteins, peptides).

## 5 Conclusion

In this study, we applied a $^{15}N$ isotope pool dilution assay using $^{15}N$-labeled amino sugars and D-amino acid enantiomers in soils from six sites, across three land management types and two bedrocks, to explore the potential effects of soil characteristics on gross rates of amino compound production and consumption. Our work reveals that the production of free amino sugars, proxy amino acids, and D-enantiomers from microbial cell wall decomposition adds significantly to the bioavailable soil organic nitrogen pool. Unlike glucosamine, mDAP and D-alanine, the amino sugar muramic acid was not a major decomposition product of peptidoglycan. We found that soil pH and texture act as critical factors controlling the decomposition of microbial cell walls, in which low soil pH and clay content favored chitin decomposition while at higher pH and clay contents peptidoglycan decomposition was stimulated.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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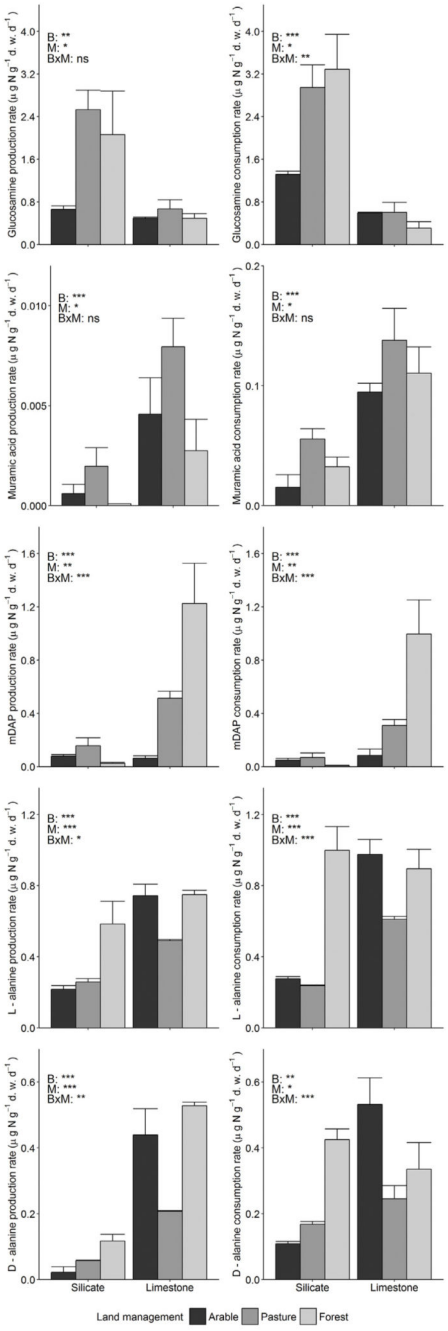


**Fig. 1.**

Gross production and consumption rates of glucosamine, muramic acid, mDAP, L-alanine, and D-alanine at 15 °C in soils from six sites differing in bedrock and land management (G, Gumpenstein; M, Moarhof; A, arable; P, pasture; F, forest). Values are means + SE (n = 4). Differences between land management and bedrock were analyzed by two-way ANOVA.

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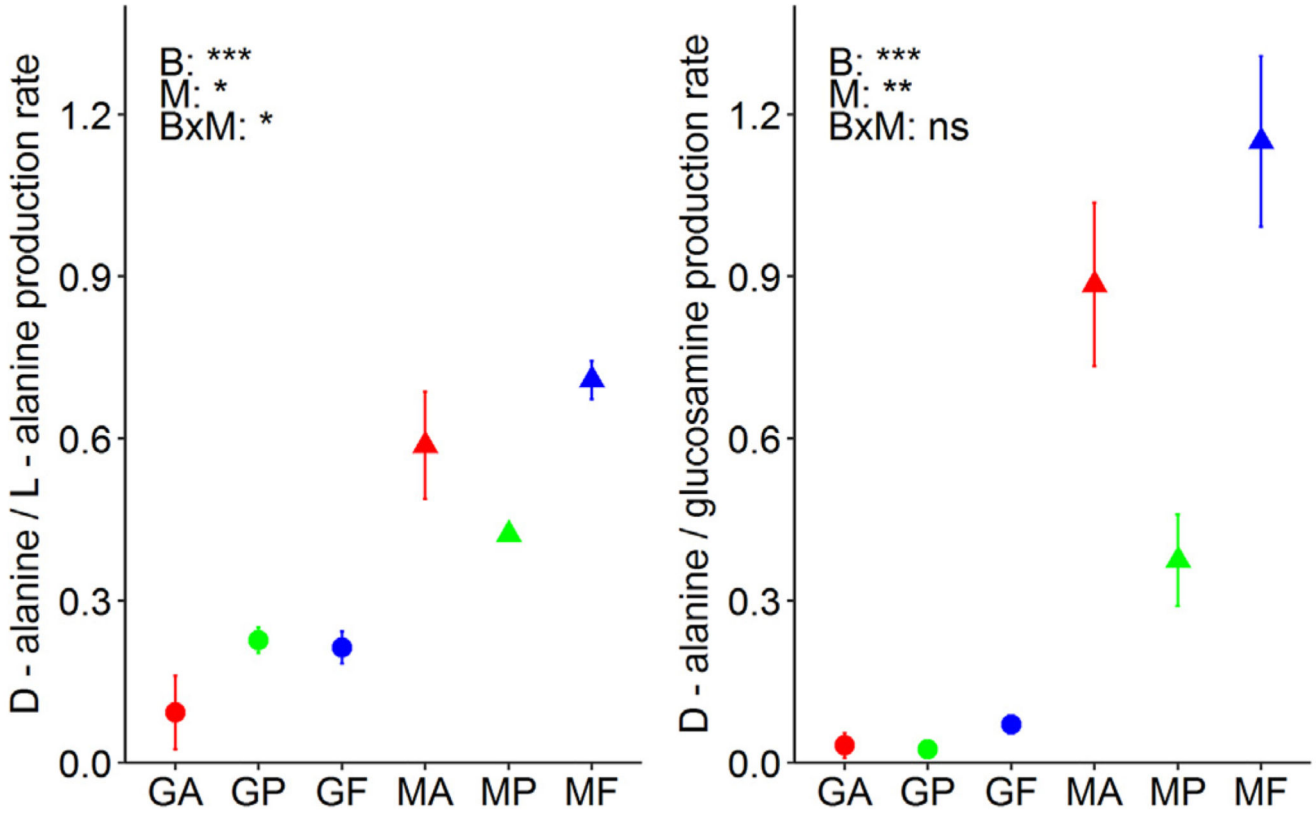


**Fig. 2.**

Ratios of gross production rates of D-alanine/L-alanine and D-alanine/glucosamine at 15 °C in soils from six sampling sites differing in bedrock and land management. Values are means ± SE (n = 4). Significant effects of land management (M) and bedrock (B) were analyzed by two-way ANOVA.

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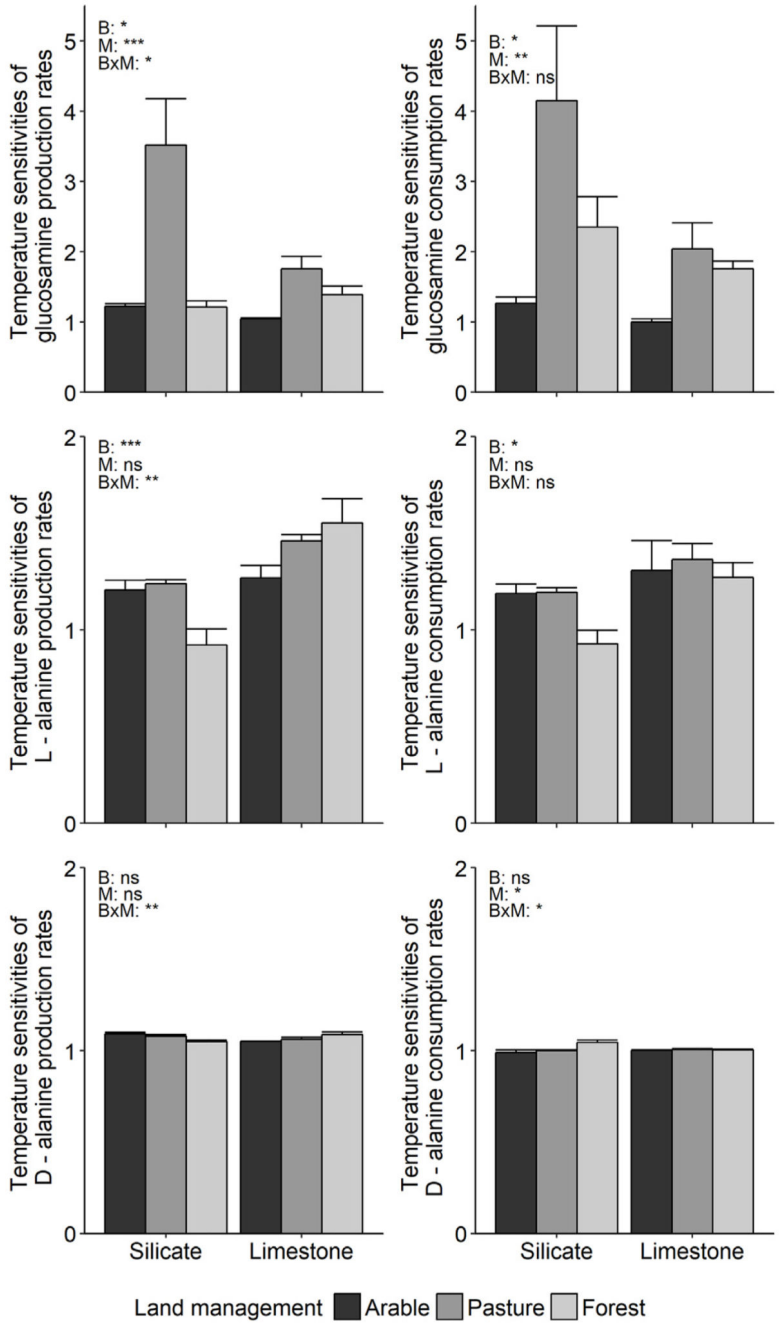


**Fig. 3.**

Temperature sensitivity of gross production and consumption rates of glucosamine, L-alanine, and D-alanine in soils from six sites differing in bedrock and land management. The temperature sensitivity was calculated for 5 °C, 15 °C, and 25 °C based on a linear regression model. Values are means ± SE (n = 4). Significant effects of land management and bedrock were analyzed by two-way ANOVA.

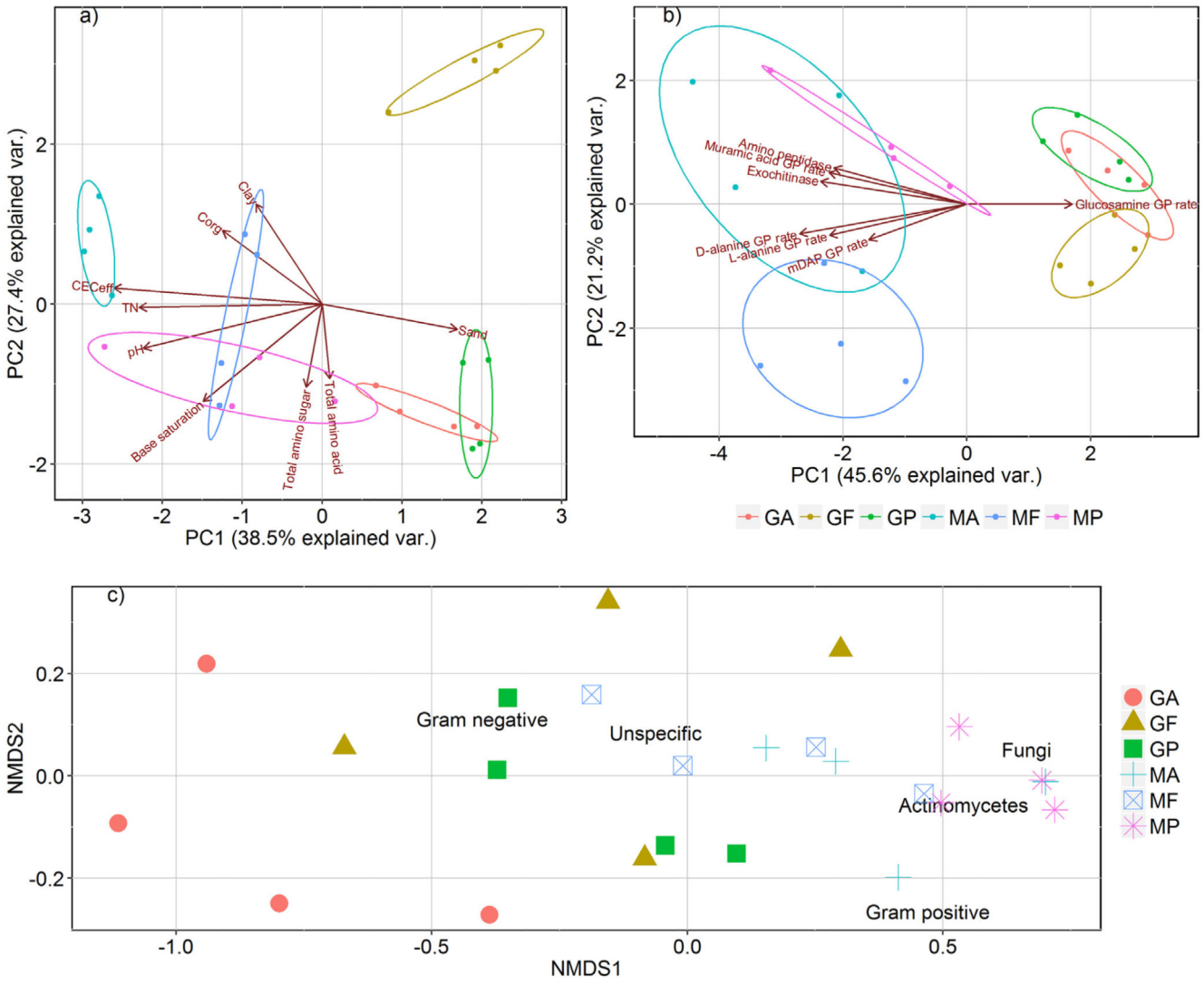


**Fig. 4.**

Score plots of two principal component analyses (PCA) of a) soil characteristics and b) gross production rates of amino sugars and amino acids and extracellular enzyme activities, and a NMDS plot of c) microbial community composition represented by PLFA biomarkers.




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## Table 1

Physicochemical properties of the soils from the six sites differing in bedrock and land management (G, Gumpenstein; M, Moarhof; A, arable; P, pasture; F, forest). Values are means ± SE (n = 4). Significance was tested by two-way ANOVA for effects of bedrock (B) and land management (M) as well as their interaction. ns: not significant.

| **Soil type** | **GA** | **GP** | **GF** | **MA** | **MP** | **MF** | ***p* value** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Bedrock (B)** | **Silicate** | | | **Limestone** | | | **B** | **M** | **BxM** |
| **Management (M)** | **Arable** | **Pasture** | **Forest** | **Arable** | **Pasture** | **Forest** | | | |
| Soil pH | 5.6 ± 0.4 | 4.8 ± 0.1 | 3.7 ± 0.1 | 7.3 ± 0.1 | 6.4 ± 0.2 | 6 ± 0.1 | *** | *** | ns |
| Sand (%) | 44.4 ± 0.5 | 61.1 ± 2.5 | 47.4 ± 1.0 | 29.1 ± 1.5 | 47.9 ± 13.2 | 22.8 ± 1.6 | ** | ** | ns |
| Silt (%) | 47.1 ± 0.6 | 33.4 ± 2.3 | 35.2 ± 0.9 | 56.2 ± 0.6 | 44 ± 11.1 | 62.5 ± 1.8 | *** | * | ns |
| Clay (%) | 8.5 ± 0.3 | 5.6 ± 0.2 | 17.4 ± 0.1 | 14.8 ± 1.0 | 8.1 ± 2.2 | 14.7 ± 1.5 | * | *** | ** |
| CEC (cmolc/kg) | 8.4 ± 2.0 | 5.4 ± 0.3 | 9.7 ± 0.7 | 33.7 ± 0.4 | 22.7 ± 3.2 | 22.8 ± 0.5 | *** | ** | * |
| Base saturation (%) | 93.4 ± 3.5 | 82.7 ± 3.0 | 5.2 ± 0.2 | 100 ± 0.1 | 99.6 ± 0.1 | 99.3 ± 0.1 | *** | *** | *** |
| $C_{org}$ (mg C $g^{-1}$) | 21.8 ± 1.1 | 26.7 ± 0.9 | 49.9 ± 7.6 | 47 ± 0.9 | 43.9 ± 5.4 | 36.8 ± 2.4 | ** | ns | *** |
| TN (mg N $g^{-1}$) | 2.2 ± 0.1 | 2.8 ± 0.1 | 2.5 ± 0.4 | 4.8 ± 0.1 | 4.6 ± 0.6 | 3.3 ± 0.1 | *** | * | * |
| Soil C:N | 10 ± 0.2 | 9.5 ± 0.1 | 19.6 ± 0.2 | 9.9 ± 0.2 | 9.7 ± 1.0 | 11.3 ± 0.4 | *** | *** | *** |
| DOC (μg C $g^{-1}$) | 64.6 ± 3.6 | 85.6 ± 4.3 | 160.9 ± 13.1 | 52.5 ± 1.8 | 53.7 ± 12.6 | 53.4 ± 6.9 | *** | *** | *** |
| DON (μg N $g^{-1}$) | 15.7 ± 1.0 | 32.4 ± 2.3 | 13.6 ± 1.9 | 16.9 ± 1.2 | 16 ± 1.4 | 15.2 ± 1.9 | ** | *** | *** |
| $NH_4^+$ (μg N $g^{-1}$) | 1.3 ± 0.7 | 0.7 ± 0.1 | 6.3 ± 0.7 | 1.8 ± 0.8 | 0.7 ± 0.2 | 1.1 ± 0.6 | ** | *** | *** |
| $NO_3^-$ (μg N $g^{-1}$) | 11 ± 1.8 | 2.1 ± 1.1 | 4.5 ± 0.9 | 20 ± 0.8 | 18.9 ± 2 | 15.7 ± 1.6 | *** | ** | * |
| Free amino sugars (ng N $g^{-1}$) | 36.6 ± 2 | 208.6 ± 31.7 | 143.4 ± 37.4 | 29.3 ± 0.9 | 128.2 ± 12.3 | 96.1 ± 12.6 | * | *** | ns |
| Free amino acids (μg N $g^{-1}$) | 0.63 ± 0.2 | 1.61 ± 0.1 | 0.97 ± 0.3 | 0.76 ± 0.1 | 2.91 ± 0.3 | 2.68 ± 0.3 | *** | *** | * |
| Fungal PLFA (μg C $g^{-1}$) | 8.8 ± 2.2 | 15.3 ± 3.8 | 24.9 ± 6.5 | 39.8 ± 8.2 | 56.4 ± 7.3 | 34.1 ± 9.9 | *** | ns | ns |
| Bacterial PLFA (μg C $g^{-1}$) | 17.3 ± 5.9 | 34.4 ± 5.3 | 33 ± 10.8 | 67.4 ± 15.7 | 85.5 ± 8.5 | 42.8 ± 8.3 | *** | ns | ns |
| Fungi:Bacteria (PLFA) | 0.65 ± 0.2 | 0.46 ± 0.1 | 0.92 ± 0.3 | 0.67 ± 0.2 | 0.67 ± 0.1 | 0.75 ± 0.1 | ns | ns | ns |
| Fungal necromass (mg C $g^{-1}$) | 2.2 ± 0.5 | 3.3 ± 0.3 | 1.6 ± 0.3 | 2.3 ± 0.4 | 2.2 ± 0.5 | 3.5 ± 1 | ns | ns | * |
| Bacterial necromass (mg C $g^{-1}$) | 5.6 ± 0.7 | 3.2 ± 0.3 | 3.9 ± 0.6 | 2.4 ± 0.2 | 5.6 ± 0.7 | 4.2 ± 0.5 | ns | ns | *** |
| Fungi:Bacteria (necromass) | 0.43 ± 0.1 | 1.06 ± 0.1 | 0.39 ± 0.1 | 0.92 ± 0.1 | 0.43 ± 0.1 | 0.83 ± 0.2 | ns | ns | ** |

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## Table 2

Free pool sizes and mean residence times of amino compounds in six soils differing in bedrock and land management (G, Gumpenstein; M, Moarhof; A, arable; P, pasture; F, forest). Values are means ± SE (n = 4). Significance was tested by two-way ANOVA for effects of bedrock (B) and land management (M) as well as their interaction. ns: not significant.

| **Soil Type** | | **GA** | **GP** | **GF** | **MA** | **MP** | **MF** | ***p* value** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Bedrock** | | **Silicate** | | | **Limestone** | | | **B** | **M** | **BxM** |
| **Management** | | **Arable** | **Pasture** | **Forest** | **Arable** | **Pasture** | **Forest** | | | |
| Free pool size (ng N $g^{-1}$ d.w.) | Glucosamine | 36.5 ± 2.0 | 208 ± 32 | 143 ± 37 | 27.7 ± 0.9 | 126.9 ± 12.3 | 94.8 ± 12.6 | ** | *** | ns |
| | Muramic acid | 0.15 ± 0.02 | 0.53 ± 0.07 | 0.45 ± 0.11 | 1.53 ± 0.14 | 1.26 ± 0.30 | 1.21 ± 0.05 | *** | ns | ns |
| | mDAP | 32.5 ± 1.4 | 89.4 ± 13.8 | 84.3 ± 22.5 | 53.3 ± 4.5 | 211 ± 14 | 175 ± 29 | *** | *** | *** |
| | L-alanine | 11.2 ± 3.3 | 8.9 ± 0.7 | 17.3 ± 6.3 | 68.8 ± 3.9 | 47.8 ± 16 | 42.4 ± 5.2 | *** | ns | ns |
| | D-alanine | 2.91 ± 0.10 | 2.83 ± 0.33 | 1.99 ± 0.32 | 2.85 ± 0.22 | 2.47 ± 0.32 | 2.91 ± 0.31 | ns | ns | ns |
| MRT (hour) | Glucosamine | 1.3 ± 0.1 | 2.0 ± 0.1 | 1.9 ± 0.3 | 1.4 ± 0.1 | 5.4 ± 1.3 | 4.7 ± 0.4 | *** | ** | * |
| | Muramic acid | 23.8 ± 10 | 41.0 ± 34.6 | 108 ± 26 | 94.9 ± 88.5 | 3.7 ± 0.5 | 140 ± 77 | ns | ns | ns |
| | mDAP | 10.6 ± 1.4 | 21.8 ± 7.4 | 76.9 ± 5.2 | 25.4 ± 6.6 | 10.0 ± 0.7 | 3.8 ± 0.7 | *** | *** | *** |
| | L-alanine | 0.8 ± 0.1 | 1.2 ± 0.4 | 0.7 ± 0.0 | 3.6 ± 0.3 | 3.3 ± 1.4 | 1.7 ± 0.3 | *** | ns | ns |
| | D-alanine | 1.5 ± 0.5 | 9.3 ± 3.7 | 0.7 ± 0.1 | 3.5 ± 1.1 | 3.2 ± 1.3 | 2.5 ± 0.8 | ** | ** | ** |