

Tree species-specific effects on soil microbial residues in an upper Michigan old-growth forest system

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Summary

Microbial contribution to carbon and nitrogen cycling in forest soils is important, and may depend on tree species. The amount of amino sugars and their ratios can serve as reliable indicators for bacterial and fungal contribution. We compare forest floor microbial residues (amino sugars) beneath three canopy-tree species (Sugar Maple (SM), *Acer saccharum* Marsh; Basswood (BA), *Tilia americana* L.; Eastern Hemlock, *Tsuga canadensis* L.) replicated in five plots in an upper Michigan old-growth forest. We hypothesized that because individual tree species develop a unique microbial community over time, they will accumulate microbial residues to different degrees. In this study at three tree species sites, the absolute amount of fungal residue (glucosamine (GluN)) was relatively constant, while absolute quantities of bacterial residues (galactosamine (GalN) and muramic acid (MurA)) were least in the Hemlock site. Amino sugar ratios revealed that microbial residues were compositionally distinct in the three sites. The lower ratios of GluN to GalN and GluN to MurA in BA and SM sites relative to Hemlock site indicate the lower net accumulations of GalN and MurA in Hemlock site. In terms of microbial contribution to carbon and nitrogen cycle in forest soils, we suggest that caution may be needed when using amino sugars as a tool, especially for nitrogen pool assessment, as the amino sugars are diluted by plant-derived litter. This study provides information on the microbial residues in undisturbed forest soils which may assist interpretation of data derived from managed or damaged forests in the future.

Introduction

The influence of different plant species on soil microbial properties has been of interest for decades – initially with respect to microbial biomass and activities in stands with different dominant tree species (Turner *et al.*, 1993; Bauhus *et al.*, 1998) and more recently in identifying

changes in microbial community composition in the forest floor (Myers *et al.*, 2001; Priha *et al.*, 2001; Grayston and Prescott, 2005; Hackl *et al.*, 2005), in grasslands (Bardgett and McAlister, 1999; Grayston *et al.*, 2001) or in particular ecosystems such as deglaciated terrain and cut-away peatland (Bardgett and Walker, 2004; Potila *et al.*, 2004). However, while it is well established that

plants have species-specific effects on their associated living microbial biomass, activity and communities, little is known about the degree to which soil microbial residues persist and differ in forest floors under different tree species. Persistence of microbial residues may be an important control over carbon and nitrogen cycling or storage in forest soils (Balser, 2005).

Amino sugars are important microbially derived residues that can be quantified in forest soils to provide information about the fate of carbon and nitrogen within biomass of bacteria and fungi, in turn indicating relative microbial contribution to carbon and nitrogen cycling (Parsons, 1981; Amelung, 2001). Plants do not synthesize significant amounts of amino sugars, and amino sugars are stable against fluctuations in living microbial biomass (Nannipieri *et al.*, 1979; Chantigny *et al.*, 1997). Further, following cell death, amino sugars are significantly stabilized in soils, and accumulate over time (Guggenberger *et al.*, 1999; Glaser *et al.*, 2004).

The quantity and identity of amino sugars can indicate their origin. Glucosamine (GluN) is an important constituent of fungal cell walls, while muramic acid (MurA) uniquely originates from bacteria (Parsons, 1981; Kögel and Bochter, 1985; Chantigny *et al.*, 1997). Sowden and Ivarson (1974) demonstrated that little, if any, galactosamine (GalN) is synthesized by fungi during fungi-inoculated incubation experiments. In addition, mannosamine (ManN) is measured, but only for calculating the total amount of amino sugars, since its origin is controversial (Coelho *et al.*, 1997). The ratio of GluN to GalN is used to indicate the relative fungal contribution to the turnover and accumulation of soil organic matter (SOM) and soil organic nitrogen (SON) in organic forest soil horizons (Kögel and Bochter, 1985; Zhang *et al.*, 1998; Solomon *et al.*, 2001), while the ratio of GluN to MurA indicates the relative bacterial contribution to amino sugars in the soils (Zhang *et al.*, 1998; Solomon *et al.*, 2001). Finally, the total amount of amino sugars can serve as a useful indicator for microbial contribution to SOM and SON (Amelung *et al.*, 1999; Zhang *et al.*, 1999; Solomon *et al.*, 2001; Amelung, 2003).

Land use and agricultural management have been found to result in substantial changes in amino sugar concentrations and corresponding

pattern (Guggenberger *et al.*, 1999; Zhang *et al.*, 1999; Solomon *et al.*, 2001; Simpson *et al.*, 2004), but to date, no one has investigated how amino sugars respond to different dominant forest canopy species. Accordingly, the objectives of this study were to (1) evaluate the effect of tree species on the build-up of four amino sugars in soils and (2) assess microbial contribution to SOM and SON in an old-growth forest system.

Materials and methods

Site description and sampling

We chose a model old-growth forest site (located in the Sylvania Wilderness Recreational Area, the Ottawa National Forest in the western upper peninsula of Michigan, USA (46° 13' N, 88° 18' W; Figure 1)). The site is dominated by three tree species, Sugar Maple (SM) (*Acer saccharum* Marsh), Basswood (BA) (*Tilia americana* L.) and Eastern Hemlock (HL) (*Tsuga canadensis* L.). Understory vegetation is dominantly immature SM (personal observation by Fujinuma). The site, at 500 m elevation, has gradually undulating topography and an average growing season of ~120 days from May through October. During the growing season, air temperature averages ~18°C with 578 mm precipitation (NOAA, 2002).

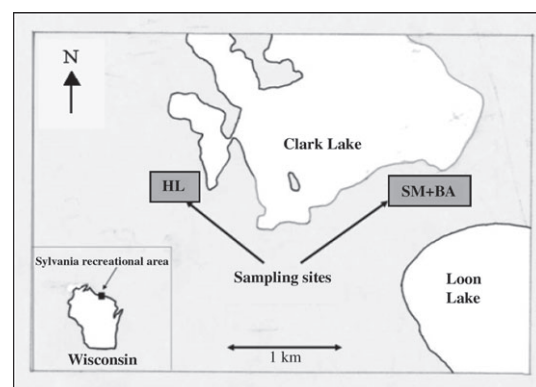


Figure 1. Locations of the three sampling sites in Sylvania Recreation Area, Michigan: SM (46° 13' N, 88° 18' W); BA (46° 13' N, 88° 18' W); HL (46° 13' N, 89° 19' W).

Additional details regarding site conditions and history are provided in Fujinuma *et al.* (2005). We chose three individual canopy-trees species (SM, BA and HL) on the same soil type (sandy, mixed, frigid Typic Haplorthod). Differences in soil properties beneath each canopy-tree species are presented in Table 1. We selected five individual canopy trees of more than 45 cm diameter at breast height in each species as replicates. In summer of 2000, soil samples (0–15 cm) were taken randomly from a point halfway between canopy drip-line and tree stem. Soil samples were frozen at -20°C within 12 h of collection and lyophilized shortly thereafter. Samples were stored at -20°C prior to analysis.

Chemical analysis

The four amino sugars were analysed by gas chromatograph (GC) after their conversion to aldonitrile acetates according to the protocol of Guerrant and Moss (1984). The method is described in detail by Zhang and Amelung (1996). Briefly, samples were hydrolyzed with 6M HCl at 105°C for 8 h, and then the solution was filtered and purified by neutralization. After drying of the supernatant, methanol was used to wash amino sugars out from the residues, and then amino sugars were transformed into aldonitrile derivatives. Excess anhydride was destroyed with water and 1M HCl, before the amino sugar derivatives were extracted from the aqueous solution with dichloromethane. GC separation of the amino sugar derivatives was carried out on an Agilent 6890A GC (Agilent Tech. Co., Wilmington, DE USA) equipped with a HP-5 (25 m \times 0.33 mm \times 0.25 μm) fused silica column and flame ionization detector. The individual amino sugars were identified by comparing their retention time with those of authentic standards. Quantification was

based on the internal standard myo-inositol, which was added to the samples prior to purification with the internal standard methyl-glucamine as recovery standard before derivatization. In addition to the individual amino sugar contents, we calculated the total amino sugar contents as the sum of four amino sugars determined, GluN, GalN, MurA and ManN. We use GalN or MurA as the biomarker for bacterial cell-wall residues, GluN for fungal cell-wall residues. ManN is not considered alone in our analysis due to its trace amounts and ambiguous origin.

Total carbon, nitrogen and sulphur concentration were analysed after dry combustion with a LECO total CNS analyzer (LECO Corporation, St Joseph, MI, USA). There was no detectable lime reaction in all samples.

Statistical analysis

Data were statistically analysed using the software package SPSS10.0 for Windows. Comparison of amino sugars and soil properties in the three forest sites was performed by one-way analysis of variance followed by a *post hoc* separation of means using the least significant difference procedure. Figures were drawn using Microsoft Excel and Sigma-Plot 8.0.

Results and discussion

In this old-growth forest soil, concentrations of the four amino sugars varied from 494 to 579 μg GluN g^{-1} soil, from 107 to 218 μg GalN g^{-1} soil, from 40 to 96 μg MurA g^{-1} soil and from 24 to 48 μg ManN g^{-1} soil (Figure 2). The contribution of each amino sugar to the total decreased ($P < 0.05$) in the order GluN > GalN > MurA > ManN.

Table 1: Soil properties beneath three tree species

Tree species	Total C (mg g^{-1})	Total N (mg g^{-1})	C/N	Total S (mg g^{-1})	Bulk density (g cm^{-3})	pH (CaCl_2)
Basswood	31.03a	2.31a	13.46b	0.298a	0.89a	4.36a
Sugar Maple	27.96a	1.89ab	14.70ab	0.222a	0.89a	4.74a
Hemlock	29.14a	1.75b	16.71a	0.271a	1.23b	3.73b

Differences from least significant difference test among mean values at the 0.05 probability level are represented by different letters.

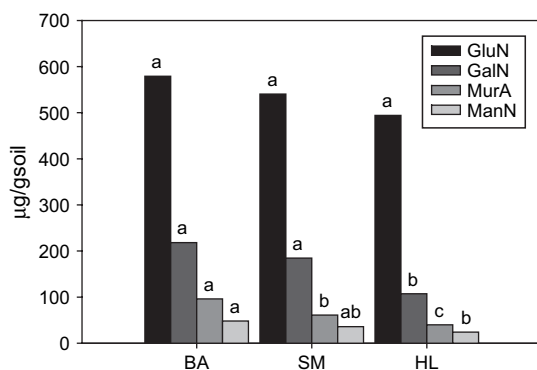


Figure 2. Amount of four amino sugars beneath three tree species sites. Bars with different letters are significantly different ($P < 0.05$) across three sites for the corresponding amino sugar.

These results are consistent with results from agricultural soils (Guggenberger *et al.*, 1999), North American grassland soils (Amelung *et al.*, 1999) and tropical soils (Solomon *et al.*, 2001). In this study, the amount of each amino sugar was influenced by canopy-tree species in different ways. Fungal-derived GluN was not significantly different ($P > 0.05$) among the three species, possibly indicating that absolute fungal contributions to microbial residues are somewhat independent of dominant tree species (Figure 2). In contrast, the bacteria-derived MurA decreased significantly in the order ($P < 0.05$) BA > SM > HL, and the bacteria-derived GalN was more enriched in BA and SM than HL ($P < 0.05$). Past research indicates MurA as a better biomarker than GalN for soil bacterial residue due to its uniqueness and ubiquitous presence in the peptidoglycan of bacterial cells (Chantigny *et al.*, 1997; Guggenberger *et al.*, 1999). In this study, MurA and GalN both showed similar trends among the three tree species, and suggest lower absolute bacterial contribution to microbial residues in HL than in SM and BA.

It has been suggested that the GluN/GalN ratio can potentially serve as a long-term indicator of amino sugars accumulation, while GluN/MurA is a short-term indicator (Zhang *et al.*, 1998). MurA uniquely originates from bacteria, and its turnover time is much shorter than the other three amino sugars due to a special carboxyl group. In its free state, MurA even decomposes

when stored in the freezer for several months (Liang, personal communication). While GalN, in theory, behaves similarly to GluN and ManN due to similar molecular structure, GalN is actually somewhat more stable because it can occur in association with recalcitrant glomalin from arbuscular mycorrhizal fungi (Wright and Upadhyaya, 1996). Here, we use both the ratios of GluN/GalN and GluN/MurA together to differentiate the relative contribution of bacteria and fungi. We found that patterns of amino sugars were considerably influenced by the canopy species (Figure 3). The ratios of both GluN/GalN and GluN/MurA increased in the order BA < SM < HL. The significantly higher GluN/GalN and GluN/MurA ratios observed in HL compared with BA and SM indicate that the fungal : bacterial ratio over time is higher in the HL site. This can either be due to greater fungal biomass over time or because of lower bacterial biomass. Because fungal GluN was not significantly ($P > 0.1$) different among the three sites, the higher ratio is likely due to reduced bacterial biomass. It is interesting therefore to note that differences among tree species appear to be driven by bacterial abundance. This may reflect the influence of litter quality on soil pH or substrate availability. For example, it may be that the BA and SM sites particularly favour the accumulation of bacterial amino sugars compared with HL due to the higher pH promoted by BA and SM litter. Higher soil pH favours a microbial population dominated by bacteria, while fungi prefer acid soils (Turrión *et al.*, 2002). In this study, the pH in HL was significantly ($P < 0.05$) lower than that in BA and SM (Table 1). It may be that soil pH less than five in undisturbed forest soils has a small influence on fungi, but suppresses bacterial growth to some extent. Alternatively, reduced bacterial abundance may be due to the poor quality of HL litter compared with that of BA and SM. Hemlock litter has a higher concentration of waxes and other complex aliphatic and aromatic compounds that favour the degradation by fungi rather than bacteria (Swift *et al.*, 1979). It may be that fungi are able to use each litter type equally, while bacteria are disadvantaged in a HL site.

In addition to information about relative abundance of fungal or bacterial biomass, total amino sugars can be used to indicate the

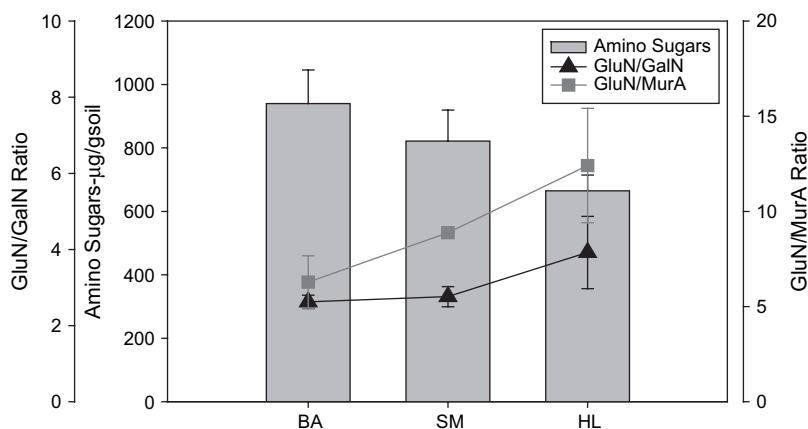


Figure 3. Amount of amino sugars and their patterns beneath three tree species. Error bar represents the standard error of the mean for the total amount of amino sugars.

contribution of microbial residues to stable SOM pools (Amelung, 2001). The extant, or standing, amino sugar pool (composed of GluN, GalN, ManN and MurA) is determined both by the build-up rate of living microbial cells and the decomposition rate of residues from senesced cells. These rates are dependent on microbial metabolism (and thus biomass growth) and the degree of physical and chemical protection of the amino sugars within the soil matrix. In addition, decomposition of microbial residues is carried out by the microbes themselves and amino sugars are not a preferred substrate (i.e. microbes prefer to utilize C and N derived from plant litter rather than microbial amino sugars) (Amelung *et al.*, 2002). In a typical forest floor, with elevated plant litter availability, decomposition of amino sugars by the microbial community is relatively minor and can be neglected to some extent. In the Michigan old-growth forest sites studied here, the total amount of amino sugars decreased ($P < 0.05$) in the order BA > SM > HL (Figure 3). This indicates less total accumulation of microbial residues at HL sites relative to BA and SM. This may indicate the dominance of selective preservation of waxes and lignins rather than more microbially dominated humification pathways in HL soils (Balsler, 2005). Selective preservation is a common humification pathway in forest soils (Kögel-Knaber, 1993).

In addition to soil pH or substrate availability influencing the turnover time of amino sugars in soils, the difference in the total amino sugars pool among the three forest sites may also be due to differences in actual microbial growth over time. In a soil with high stabilization potential (e.g. fine texture and high organic matter), even a slow-growing microbial community with low biomass can be highly preserved and show elevated accumulation over time. For example, Turrión *et al.* (2002) suggested the high amino sugars content in forest floor is due to extreme stabilization of amino sugars in the SOM rather than high microbial biomass. In this study, active microbial biomass measured by phospholipids fatty acid is not significantly different ($P > 0.05$) among the three sites (Liang, unpublished data), in contrast to the significant ($P < 0.05$) differences among amino sugars. Amino sugars in the forest floor are preserved rather than decomposed, and differences in amino sugars pools in soils beneath different canopy trees with similar levels of living microbial biomass indicate differing mechanisms of preservation or differential growth and turnover of the community.

Finally, we assessed the contribution of amino sugars to soil C and N pools. The proportions of amino sugars-C in total soil C (mg g^{-1}) and amino sugars-N in total soil N (mg g^{-1}) have been used in the past to indicate the relative importance of senesced microbial residues in soil C and N cycles.

In our study, we found that the amino sugars-C to total soil C ratio was significantly higher ($P < 0.05$) in BA relative to SM and HL. Thus, microbial biomass may play a more important role in the active carbon cycle under BA than under SM or HL. This is not unexpected; BA litter decomposes more rapidly than SM or HL (Fujinuma *et al.*, 2005), indicating lower preservation of litter-derived carbon in BA dominated stands. Again, because HL litter is recalcitrant, the relative contribution of microbial carbon to total soil carbon is lower. In contrast, the ratio amino sugars-N to total soil N was not significantly different among the three sites (Figure 4). This suggests amino sugars contribute differently to the N pool in soils compared with the C pool. It may be that N is preserved in SOM from multiple sources such as enzymes and nucleotides immobilized by humic substances. Thus, we suggest caution when interpreting the results of amino sugars analysis as a way to explain soil C and N cycling; in the forest floor, microbial residues relative to SOM and SON are diluted by plant-derived materials.

In summary, microbial residue accumulation can ultimately be regarded only as the ‘effect’ of competing factors in soils. The reason that amino sugars vary is always obscure and may be attributed to several factors. Amino sugar dynamics can reflect a relative change in the actual production of microbial biomass, the abundance of some dominant microbial species or different residence

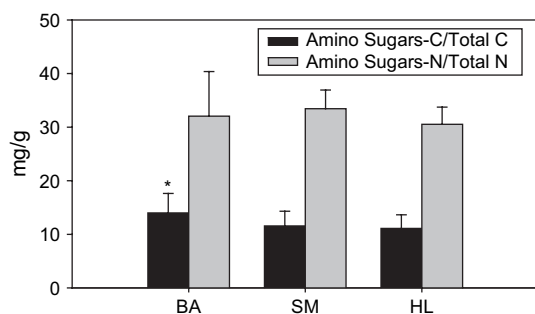


Figure 4. Concentration of amino sugars-carbon and nitrogen in soil total carbon and nitrogen. Asterisk on the bar denotes the significant difference ($P < 0.05$) among three tree species sites. Error bars show standard deviations.

time in the soils due to soil textural or litter quality differences. In future research, additional microbial biomarkers should be investigated along with amino sugars if detailed information regarding microbes is needed.

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