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Amino sugars and muramic acid—biomarkers for soil microbial community structure analysis

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Abstract

Characterizing functional and phylogenetic microbial community structure in soil is important for understanding the fate of microbially-derived compounds during the decomposition and turn-over of soil organic matter. This study was conducted to test whether amino sugars and muramic acid are suitable biomarkers to trace bacterial, fungal, and actinomycetal residues in soil. For this aim, we investigated the pattern, amounts, and dynamics of three amino sugars (glucosamine, mannosamine and galactosamine) and muramic acid in the total microbial biomass and selectively cultivated bacteria, fungi, and actinomycetes of five different soils amended with and without glucose. Our results revealed that total amino sugar and muramic acid concentrations in microbial biomass, extracted from soil after chloroform fumigation varied between 1 and 27 mg kg^{-1} soil. In all soils investigated, glucose addition resulted in a 50–360% increase of these values. In reference to soil microbial biomass-C, the total amino sugar- and muramic acid-C concentrations ranged from 1–71 g C kg^{-1} biomass-C. After an initial lag phase, the cultivated microbes revealed similar amino sugar concentrations of about 35, 27 and 17 g glucosamine-C kg^{-1} TOC in bacteria, fungi, and actinomycetes, respectively. Mannosamine and galactosamine concentrations were lower than those for glucosamine. Mannosamine was not found in actinomycete cultures. The highest muramic acid concentrations were found in bacteria, but small amounts were also found in actinomycete cultures. The concentrations of the three amino sugars studied and muramic acid differed significantly between bacteria and the other phylogenetic microbial groups under investigation (fungi and actinomycetes). Comparison between the amino sugar and muramic acid concentrations in soil microbial biomass, extracted after chloroform fumigation, and total concentrations in the soil showed that living microbial biomass contributed negligible amounts to total amino sugar contents in the soil, being at least two orders of magnitude greater in the soils than in the soil inherent microbial biomass. Thus, amino sugars are significantly stabilized in soil.

Keywords: Biomarker; Amino sugar; Muramic acid; Microbial community structure; Microbial biomass; Microbial necromass

1. Introduction

Microbial biomass has been defined as the part of soil organic matter (SOM) which constitutes living microorganisms smaller than 5–10 μm^3. It contributes between 1 and 5% of SOM (Alef and Nannipieri, 1995). The importance of estimating soil microbial biomass is related to its function as a pool for the delivery of plant nutrients, its role in soil structure formation and stabilization and as a marker for soil fertility and sustainability (Smith and Paul, 1990). Estimations of the microbial biomass have usually involved treatment of the biomass as a single component, although it is known that a diversity of populations with different biochemical characteristics are present.

Several methods have been applied to estimate the microbial biomass in soil (Alef and Nannipieri, 1995). The chloroform fumigation extraction (CFE) method has several advantages in comparison with other methods such as time and resource intensity of the analysis (Joergensen, 1996), being applicable immediately after substrate addition to a soil, in the organic layers of soils, in acid soils and in water-logged soils. With the CFE method, soil microorganisms die after their cell membranes are attacked by chloroform fume. Microbial constituents, especially in the cytoplasm, are degraded by enzymatic autolysis and transformed into extractable components.

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Strategies for community structure analysis comprise laboratory cultivation techniques and those based on direct extraction and analysis of indicator molecules (Ogram and Feng, 1997). We used amino sugars as biomarkers because these compounds are part of the cell wall of bacteria, fungi and actinomycetes (Amelung, 2001; Joergensen et al., 1995; Kandeler et al., 2000). Bacterial cell walls contain a peptidoglycan, constructed of the glucose derivatives *N*-acetyl glucosamine and muramic acid. The peptidoglycan structure is present only in prokaryotes, and has never been found in eukaryotic cells. In gram-positive bacteria, as much as 90% of the cell wall consists of peptidoglycan, whereas in gram-negative bacteria this figure is only 5–20% (Brock and Madigan, 1988). It is thought that fungi contain only glucosamine and galactosamine (Cochran and Vercellotti, 1978). Also, invertebrate exoskeletons contain chitin, an *N*-acetyl glucosamine polymer (Chantigny et al., 1997). Moreover, glucosamine has been found in earthworm gut lining, nematode egg shells, mollusc polysacharides, and in snail gelatin (Amelung, 2001). Nevertheless, the concentrations of amino sugars and muramic acid are routinely applied to indicate microbial contributions to SOM (Zhang et al., 1999; Amelung, 2001; Solomon et al., 2001; Turrión et al., 2002). Furthermore, the ratios of amino sugars and muramic acid have been used to characterize the contribution of microbial residues to SOM. As examples, the ratio of glucosamine to galactosamine was used to indicate the relative contribution of fungal-derived sugars, whilst the ratio of glucosamine to muramic acid was used to evaluate the fate of bacterial-derived SOM (Amelung et al., 1999; Amelung, 2001). However, up to now it remains unclear whether applying these ratios is valid.

Therefore, the objectives of this study were (1) to ascertain if amino sugars are suitable indicators for the determination of microbial biomass and/or necromass in soil, (2) to investigate whether amino sugar patterns allow a differentiation between bacterial, fungal and actinomycetal contributions in soil, and (3) to evaluate the variability of these indicator concentrations in microbial biomass isolated from different soil types.

2. Materials and methods

2.1. Selected soils

To cover a wide range of different soil habitats, the upper 10 cm of five well characterized soils differing in basic soil properties (Table 1) were used. Soil 1 was a Vertisol from the North American Great Plains (Amelung et al., 1997). Soils 2 and 3 were highly weathered soils of the humid tropics (Glaser et al., 2000a, 2001). Soil 4 represented a typical central European Hortisol (Glaser et al., 1998) and Soil 5 was from a high mountain ecosystem of Central Asia (Glaser et al., 2000b; Turrión et al., 2000, 2002). The amino sugar results that appear in Turrión et al. (2002) are the mean among five different soil samples under pasture vegetation, whereas in the present paper the data shown correspond only to one soil sample which was incubated with and without glucose.

2.2. Selective cultivation of bacteria, fungi, actinomycetes and soil microbial community

We consulted the bibliography about the determination of microbial biomass, and there was ambiguity about the time of pre-incubation of soils. To determine microbial biomass several authors have pre-incubated the soils only for 1 week (Beck et al., 1997; Tsai et al., 1997) observing a complete recovery of the microbial biomass. Additionally, it was not clear whether there was enough labile SOM in the non-glucose-amended soils for 2 weeks of incubation. Therefore, prior to the extraction of extractable and viable microbes, the air-dried soil (<2 mm) was incubated at field capacity and room temperature (25 °C) for 1 week. Moist soil (10 g) was then extracted with 90 ml of 50 mM Tris buffer at pH 7.5 by ultrasonication using a total energy input of 4500 J, as suggested by Ramsay (1984). An aliquot (1 ml) of each soil suspension was spread over the surface of agar plates which contained different growth media and incubated at 36 °C in five replicates. All microbes were cultivated on solid media containing 10 ml of a trace element solution per liter as proposed by Alef and Nannipieri (1995). A standard II agar with actidione (cycloheximide; 100 mg dm^{-3}) to inhibit fungal growth was used as a selective media for the cultivation of bacteria. Fungi were selectively grown on Sabouraud 2% glucose agar containing 100 mg dm^{-3} streptomycin as a bactericide. Actinomycetes were grown on a chitinous mineral salt agar (1 g dm^{-3} chitin, 1 g dm^{-3} NH_4Cl, 0.1 g dm^{-3} $MgCl_2$, 20 mM phosphate buffer adjusted to pH 6.8). Rose bengal (40 mg dm^{-3}), actidione (100 mg dm^{-3}) and streptomycin (100 mg dm^{-3}) were added to suppress bacterial and fungal growth.

2.3. Chloroform fumigation extraction (CFE)

Previous to the extraction of the microbial biomass, air dried soil (<2 mm) was incubated at field capacity and room temperature (25 °C) for 1 week. A second sample set was prepared by the addition of 100 mg glucose, to stimulate microbial activity. Chloroform fumigation of the soils released microbial cytoplasm into the soil environment. After subsequent extraction of the cell material with 0.5 M K_2SO_4 (Vance et al., 1987), total organic carbon (TOC) was quantified as a reference for the microbial biomass. The microbial biomass was calculated by biomass $C = E_C/k_{E_C}$, where E_C is the organic C extracted from fumigated soil minus that extracted from non-fumigated soil and k_{EC} is the extractable part of microbial biomass C after

Table 1
General properties of the investigated soils (0–10 cm)

Soil	Soil type (ISSS-ISRIC-FAO, 1998)	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})	Texture	pH $CaCl_2$	TOC (g kg^{-1})	N (g kg^{-1})	C/N (g kg^{-1})
1	*Haplic vertisol*	228	322	452	Clayey loam	7.6	29.9	4.5	6.6
2	*Xanthic ferralsol*	850	13	104	Clayey sand	3.8	13.6	0.9	15.1
3	*Fimic anthrosol*	879	60	50	Clayey sand	6.6	37.4	2.9	18.9
4	*Aric anthrosol*	572	237	168	Sandy loam	7.4	55.9	4.5	12.4
5	*Haplic phaeozem*	275	453	199	Sandy loam	5.6	87.7	11.5	7.6

fumigation. We used a k_{EC} value of 0.38 as recommended by Joergensen (1996).

2.4. Chemical analyses

TOC of the biomass solutions and CFE extracts were determined by wet oxidation with dichromate (Tiessen and Moir, 1993) and TOC in the soils by dry combustion on a CNS analyzer (Elementar, Vario EL).

Amino sugars in biomass suspensions, CFE extracts and in incubated soils were determined according to Zhang and Amelung (1996). Where possible, sample aliquots corresponding to a about 50 mg microbial biomass, with 100 μg myo-inositol added as internal standard, were hydrolyzed with 10 ml of 6 M HCl at 105 °C for 8 h. The CFE extracts were freeze-dried prior to hydrolysis. The released amino sugars were separated from impurities by neutralization with 0.4 M KOH. Prior to derivatization, 100 μg of methylglucamine was added as recovery standard. Derivatization was carried out according to Guerrant and Moss (1984). In brief, aldononitrile derivatives of the amino sugars were prepared by heating the samples in 0.3 ml of a derivatization reagent (32 mg hydroxylamine hydrochloride ml^{-1} and 40 mg 4-(dimethylamino)pyridine ml^{-1} in pyridine–methanol 4/1) at 75 °C for 30 min. After acetylation with 1 ml of acetic anhydride at 75–80 °C for 20 min, dichloromethane was added, and excess derivatization reagents were removed by washing with 1 ml of 1 M HCl and 1 ml of water two times each. The remaining organic phase was dried under an air stream at 45 °C and dissolved in 0.3 ml ethyl acetate–hexane (1/1). The amino sugar derivatives were separated on a HP 6890 GC equipped with a HP-5 fused silica column (30 m × 0.25 mm ID with 0.33 μm film thickness) and a flame ionization detector. Amino sugars were quantified using inositol as the internal standard and methylglucamine as recovery standard.

2.5. Statistical analyses

Data were statistically analysed using SPSS 10. Data were tested for normality using the Kolmogorov–Smirnov test and the homogeneity of the variances using the Levene test (Sokal and Rohlf, 1995). ANOVA followed by the Bonferroni test was used to detect differences between phylogenetic microbial groups and to determine differences between soils incubated with and without glucose, using as categorical factors type of soil and type of incubation.

3. Results

3.1. Microbial biomass

Incubation of the soils reduced the TOC and N concentrations (Tables 1 and 2). Glucose addition further significantly ($P < 0.05$) decreased the TOC but not the N concentrations compared to soils incubated with water only (Table 2). However, the changes were not significant with respect to the C/N ratio (Table 2). The biomass concentration, referred to the dry weight of the soil was lowest in the tropical soils (Soils 2 and 3, Fig. 1a). Glucose application significantly ($P < 0.001$) increased the microbial biomass in all soils, with a 300% increase in the Vertisol (Fig. 1a). The contribution of microbial biomass to TOC, however, was 1–2% in all soils incubated with water, and around 3% after glucose application, apart from the Vertisol (Soil 1), where the microbial biomass contribution to TOC increased from 2 to 8% upon glucose addition (Fig. 1b). These low C_{mic}/TOC ratios could be due to the remoistening of air-dried soils.

3.2. Amino sugars and muramic acid in biomass extracted after chloroform fumigation

After chloroform fumigation, total amino sugars plus muramic acid in the extracted microbial biomass ranged between 1 and 45 mg kg^{-1} soil. Glucose application

Table 2
Total organic carbon (TOC) and total nitrogen (N) concentrations and C/N ratio of the investigated soils incubated with and without glucose

Soil	Without glucose			With glucose		
	TOC (g kg^{-1})	N (g kg^{-1})	C/N (g kg^{-1})	TOC (g kg^{-1})	N (g kg^{-1})	C/N (g kg^{-1})
1	25.8	3.1	8.3	21.1	2.8	7.5
2	13.0	0.9	14.4	13.8	1.0	13.8
3	36.2	2.8	12.9	34.3	2.7	12.7
4	49.4	4.0	12.4	47.9	3.9	12.3
5	80.8	8.3	9.7	78.6	8.2	9.6

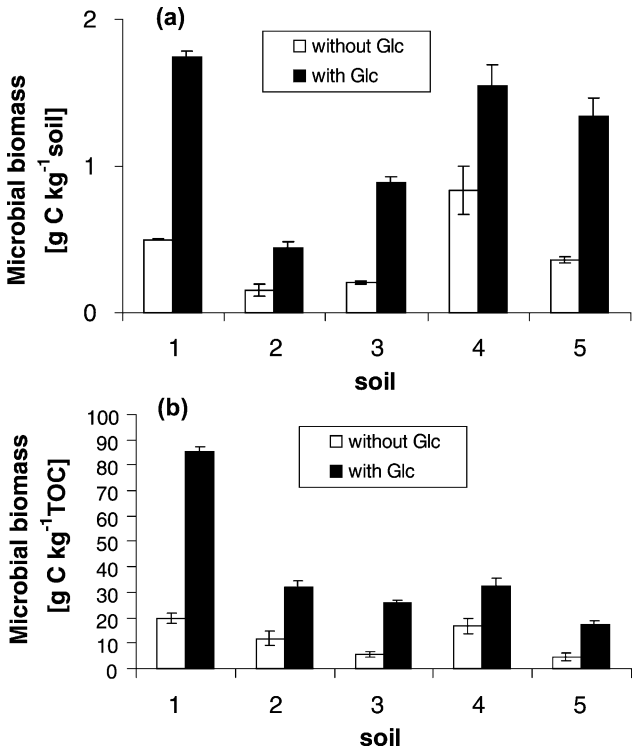

Fig. 1. Microbial biomass (a) referred to soil weight and (b) referred to total organic carbon (TOC).

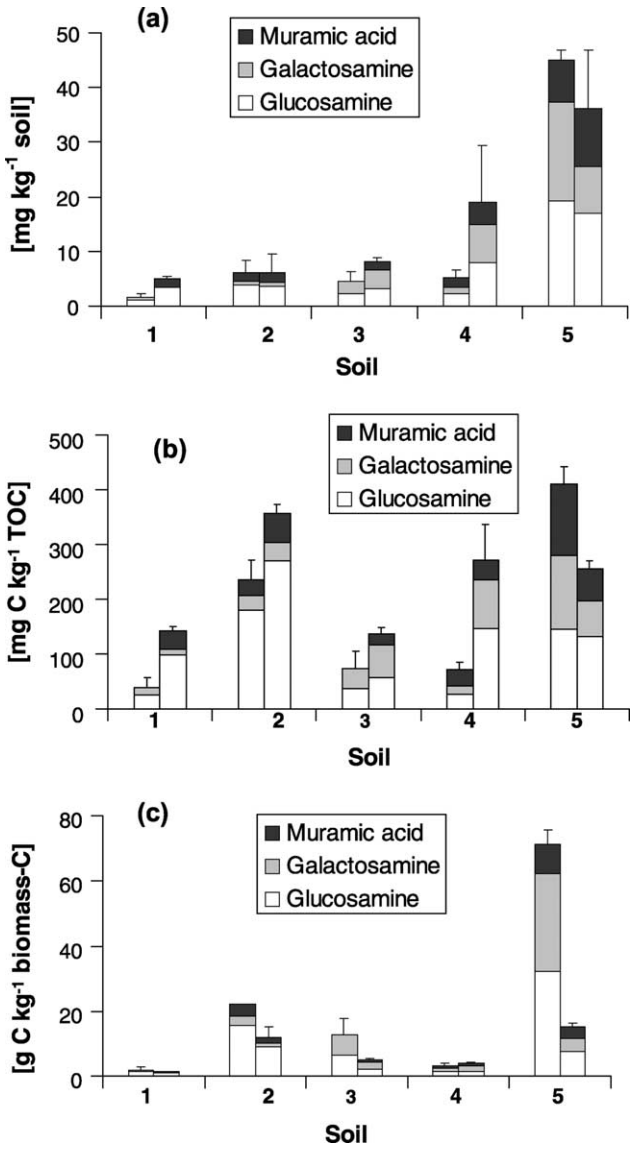

Fig. 2. Amino sugars and muramic acid in biomass extracts after chloroform fumigation of different soils without (left columns) and with (right columns) glucose application (a) referred to soil weight, (b) referred to total soil organic C, and (c) referred to extracted microbial biomass C.

increased these values by 50–360% in three of the five soils (Fig. 2a). After glucose addition, the contribution of amino sugars and muramic acid to TOC increased in all soils, except the high mountain soil (Soil 5; Fig. 2b). The total amino sugar- and muramic acid-C concentrations per unit of biomass-C are shown in Fig. 2c. No general trend was observed with the amino sugar and muramic acid concentrations after incubation both with and without glucose addition. In three of the five soils, glucose application decreased the amino sugar- and muramic acid-C per unit of extracted biomass-C. In Soils 1 and 4 it was more or less constant (Fig. 2c). However, for all soils, the amino sugar and muramic acid concentrations per unit of microbial biomass-C were about two orders of magnitude higher when compared to their contribution to TOC (Fig. 2b and c).

3.3. Amino sugars and muramic acid in cultivated microbes extracted from soil

To cover various phases of microbial replication we harvested colonies during the lag phase: immediately after the first colony appearance (sampling date 1), during the exponential growth phase (sampling date 2), and at the beginning of colony degradation (sampling date 3). Bacterial colonies were visible after 1 day of incubation, whereas fungal colonies were sampled after 3 days of incubation. The first colonies of actinomycetes appeared only after 10 days of cultivation (Fig. 3a–c). Similar trends were observed with the exponential growth phase sampled after 3, 7, and 20 days of incubation for bacteria, fungi, and actinomycetes, respectively. Colonies started to degrade and were sampled after 10 and 21 days for bacteria and fungi, respectively. Actinomycetes could not be harvested again because of excessive drying and adhesion to the culture medium.

The total amino sugar concentrations in cultivated bacteria increased during the first 4 days of cultivation to about 70 g C kg^{-1} TOC (Table 3) and remained at that level for the last 10 days of incubation (Fig. 3a). The most abundant amino sugar in bacteria was glucosamine which was present at approximately twice the concentration of galactosamine (Table 3). The concentration of muramic

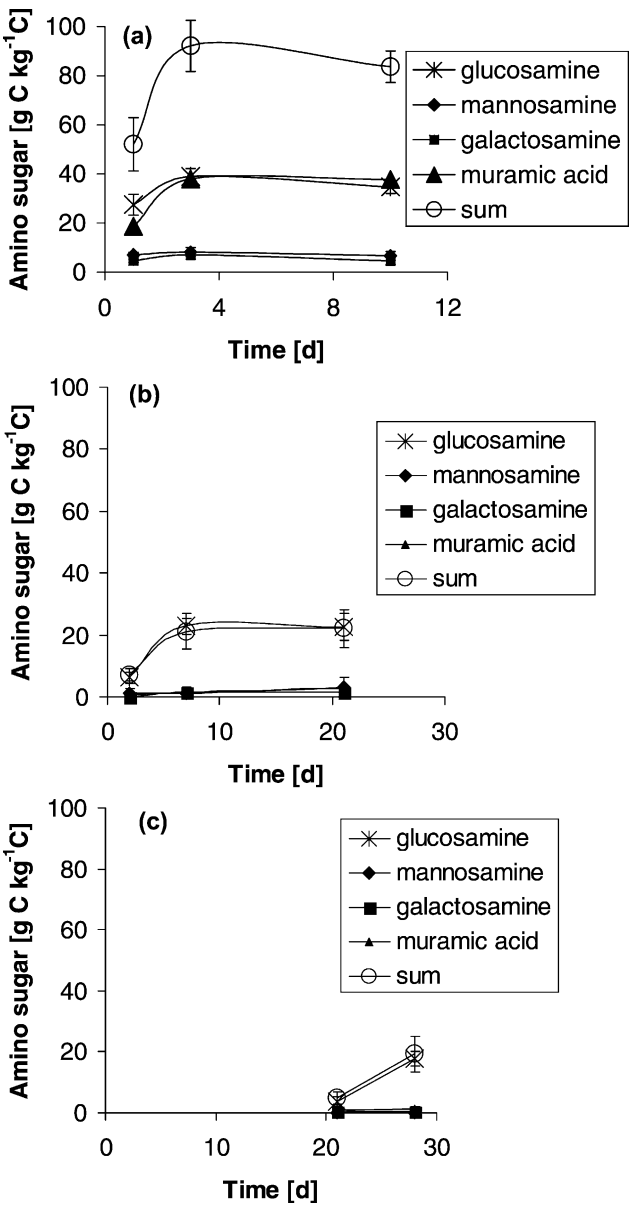

Fig. 3. Amino sugar concentration in the biomass of (a) bacteria, (b) fungi, and (c) actinomycetes.

acid, typically found in the peptidoglycan structure of bacteria (Brock and Madigan, 1988; Chantigny et al., 1997) was around 7 g C kg^{-1} TOC. Millar and Casida (1970) reported muramic acid concentrations on a dry weight basis of 3.4 ± 0.5, 9.6 ± 1.6, and 37.6 ± 3.5 g kg^{-1} in gram-negative, gram-positive bacteria, and in spores of gram-positive bacteria, respectively. Assuming the TOC content of soil bacteria to be 50% and a carbon content of muramic acid of 50%, similar values were obtained for the muramic acid-C concentrations, when referred to TOC. Thus, the mean muramic acid concentrations of our soil-bacteria isolates are in the range of the muramic acid concentrations measured by Millar and Casida (1970).

Fungal cultures showed a similar pattern of amino sugar production over time. However, the maximum amino sugar concentrations were only 27 g C kg^{-1} TOC after 7 days of cultivation, with glucosamine as the predominant amino sugar (Fig. 3b). In view of the low amounts of galactosamine and mannosamine as well as traces of muramic acid found in the fungi (Table 3), it is suggested that the total amino sugar content of fungi can be estimated by the glucosamine concentration alone. Mannosamine and galactosamine concentrations in bacteria were two to three times higher than those found in fungal cultures (Table 3). Based on the same assumptions as mentioned above (50% TOC content in the dry mass and 50% TOC of glucosamine), the glucosamine concentrations of fungi reported in the literature vary between 26 and 260 g C kg^{-1} TOC (Chen and Johnson, 1983). Thus, our glucosamine concentrations of fungi isolated from different soils lie at the lower range of these data.

Actinomycetes produced only negligible amounts of mannosamine and galactosamine but about 25 and 50% of muramic acid and glucosamine, respectively, when compared to bacterial concentrations (Table 3). Although actinomycetes could not be sampled after 30 days of incubation, it can be assumed that the amino sugar and muramic acid concentrations reached a plateau similar to bacteria and fungi.

Amino sugar and muramic acid concentrations were significantly different ($P < 0.05$) between bacteria and the other two phylogenetic groups (fungi and actinomycetes), however, no significant differences were found between fungal and actinomycetal cultures (Table 3).

3.4. Amino sugars and muramic acid concentrations in soils

The concentrations of amino sugars and muramic acid in soil are shown in Fig. 4. The concentrations in the soils amended with glucose were always higher than in the soils

Table 3
Concentrations and standard deviations ($n = 5$) of glucosamine (GlcN), mannosamine (ManN), galactosamine (GalN), muramic acid (MurAc), their sum, and ratios of GlcN:MurAc, ManN:MurAc, and GalN:MurAc in selectively cultivated bacteria, fungi and actinomycetes

	GlcN (g C kg^{-1} TOC)	ManN (g C kg^{-1} TOC)	GalN (g C kg^{-1} TOC)	MurAc (g C kg^{-1} TOC)	Sum (g C kg^{-1} TOC)	GlcN:MurAc	ManN:MurAc	GalN:MurAc
Bacteria	34.7 ± 6.3 a	7.1 ± 2.7 a	18.3 ± 4.3 a	6.6 ± 1.8 a	66.7 ± 11.7 a	5.3	1.1	2.8
Fungi	27.1 ± 6.9 b	3.1 ± 1.7 b	5.9 ± 6.0 b	0.1 ± 0.3 b	36.2 ± 11.8 b	271.0	31.0	59.0
Actinomycetes	16.8 ± 9.6 b	0 ± 0 c	0.3 ± 0.4 b	1.5 ± 1.0 b	18.6 ± 10.9 b	11.2	0.0	0.2

Different letters indicate significant differences ($P < 0.05$) between phylogenetic microbial groups.

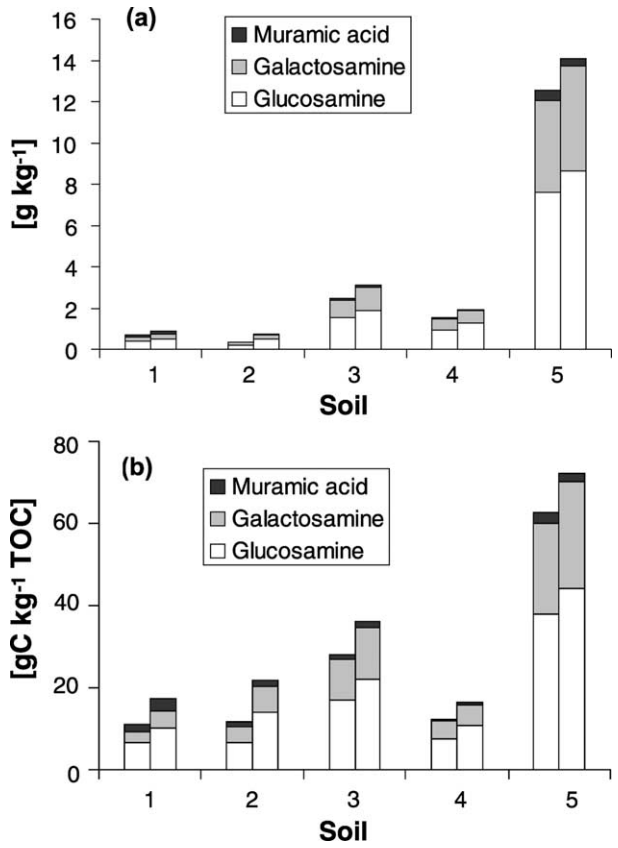

Fig. 4. Amino sugars and muramic acid in different soils without (left columns) and with (right columns) glucose application (a) referred to soil weight and (b) referred to total organic C.

without glucose addition (Fig. 4a). Thus, the contribution of each single compound increased in the order mannosamine < muramic acid ≪ galactosamine < glucosamine. It is interesting to note that the total content of amino sugars plus muramic acid (soils with glucose additions in parentheses) varied between 0.4 and 2.5 (0.8–3.1) g kg^{-1} soil for all soils apart the high mountain soil which had a concentration about five times as high (Fig. 4a).

Similar trends were observed for the amino sugar and muramic acid contributions to TOC. The concentrations for the soils amended were always higher than the soils without glucose addition (Fig. 4b).

Ratios between the amino sugars and muramic acid in the studied soils are shown in Table 4.

4. Discussion

4.1. Microbial biomass in the investigated soils

Following glucose addition, net C mineralization was evident. Only in the Ferralsol (Soil 2) which had a lower TOC level than all other soils (Table 1), immobilization dominated mineralization upon glucose addition (Table 2). On the other hand, microbial biomass-C was higher in the glucose amended soils, both in absolute and relative (referred to TOC) terms (Fig. 1a and b). Thus, net immobilization due to glucose addition can be estimated by the difference of biomass-C between the glucose amended and the non amended soils. Net immobilization was 0.3–1.3 g biomass-C per kg of soil (Fig. 1a) and 20–65 g biomass-C per kg of TOC (Fig. 1b).

4.2. Amino sugars in microbial biomass extracted from soil

With respect to amino sugar and muramic acid yields, our investigation showed that the quality of organic material extracted from soils after chloroform fumigation was highly variable (Fig. 2). Additionally, it is interesting to note that the amino sugar plus muramic acid concentrations in the extracted organic materials were smaller in the glucose amended soils throughout the whole sample set (Fig. 2c), whereas the absolute amounts of amino sugars plus muramic acid in the chloroform fumigation extracts (Fig. 2a), along with other estimates such as the amount (Figs. 2a and 4a) and contribution (Figs. 2b and 4b) to TOC of microbial biomass-C and amino sugars in soil, respectively, were higher in the glucose amended soils. These results indicate that stimulated microbial activity following glucose addition led to an enhanced extractability of microbial degraded organic matter after contact with chloroform fumes but also that the non-microbial extracted organic material which was extracted in a sample set without chloroform fumigation, was higher in the glucose amended soils (data not shown).

4.3. Amino sugars in cultivated microbes

Current estimates of microbial cultivation techniques indicate that less than 1% of the microorganisms present in

Table 4
Ratios between glucosamine (GlcN) and muramic acid (MurAc), between mannosamine (ManN) and MurAc, and between galactosamine (GalN) and MurAc in different soils

Soil	Without glucose			With glucose		
	GlcN:MurAc	ManN:MurAc	GalN:MurAc	GlcN:MurAc	ManN:MurAc	GalN:MurAc
1	4.2	0.6	1.7	3.6	0.6	1.6
2	5.8	1.0	3.6	9.6	0.3	4.4
3	14.2	0.4	8.1	15.2	0.2	8.8
4	15.7	1.2	9.4	15.2	0.9	7.3
5	13.9	0.3	8.1	23.6	0.5	13.9

many soil environments are readily extractable and cultivable. This suggests that methods based on laboratory cultivation are significantly biased (Torsvik et al., 1990). Nevertheless, a cultivation assay is necessary to test whether amino sugars and muramic acid are suitable biomarkers for characterizing soil community structure composition. The amino sugar and muramic acid composition of bacterial, fungal, and actinomycetal cultures were homogeneous in the different soils under study. Therefore, mean values and standard errors were calculated (Fig. 3, Table 3). It is interesting to note that after a short lag phase, plateau concentrations of each individual amino sugar and muramic acid were reached for each group of microorganisms until the cultivated colonies started to degrade. Due to methodological constraints, this could not fully be shown for actinomycetes. However, based on these other similarities, we assume that also this microorganism group behaves in a similar manner to bacteria and fungi with respect to changes in amino sugar and muramic acid concentrations over time. Thus, although there are certainly microorganisms in all different stages of growth (lag phase, exponential growth, steady state, degradation) for a given soil, the plateau values are useful estimates of the mean amino sugar and muramic acid contributions of viable soil microorganisms.

The data given in Fig. 3 and Table 3 yield a ratio of glucosamine to muramic acid (GlcN/MurAc) of 5. Joergensen et al. (1995) found GlcN/MurAc ratios ranging from 2 to 8 for four different bacteria. No significant differences were found among the glucosamine concentrations in fungal and actinomycetal cultures, but significant differences ($P < 0.05$) were found between bacteria and fungi and actinomycetes. Thus, glucosamine cannot be used as an indicator for fungi alone. Additionally, the contribution of insect and invertebrate chitin to the glucosamine concentration in soil is largely unknown. Furthermore, our results show that muramic acid is not a specific biomarker for soil bacteria because actinomycetal cultures showed a mean of 1.5 g muramic acid-C kg^{-1} TOC (Table 3), or about 25% of the muramic acid content of cultivable soil bacteria. Therefore, as already suggested by various authors (Joergensen et al., 1995; Chantigny et al., 1997; Amelung et al., 1999; Amelung, 2001), a better indicator for microbial community structure might be the ratio between glucosamine and muramic acid. This ratio averaged 5, 11, and 271 for bacteria, actinomycetes, and fungi, respectively (Table 3).

A better alternative for the GlcN/MurAc ratio might be the galactosamine to muramic acid (GalN/MurAc) ratio. In our study, this ratio was 59, 3, and 0.2 for fungi, bacteria, and actinomycetes, respectively, because on contrary to glucosamine, mannosamine and galactosamine could only be detected in traces in actinomycetes (Table 3).

Our results indicate that actinomycetes do not contribute significantly to the mannosamine and galactosamine content of soils and that the mannosamine to muramic acid (ManN/MurAc) and the GalN/MurAc ratios might be more valuable as indicators of the contribution of bacterial and fungal residues to SOM than the GlcN/MurAc ratio.

4.4. Amino sugars and muramic acid in soil

The amino sugar and muramic acid concentrations in the incubated soils of this study were comparable to those of other soil investigations (Amelung et al., 1999; Solomon et al., 2001; Turrión et al., 2002). A comparison of Figs. 2b and 4b shows clearly, that the amino sugar concentrations in the soils exceeded those contributed by the microbial biomass extractable after chloroform fumigation from the same soils, by at least two orders of magnitude. These results indicate unambiguously that amino sugars are stabilized to a substantial part in soil even after the death of the microbial biomass. Such a stabilization has already been assumed by Guggenberger et al. (1999) and Amelung et al. (2001). Thus, the determination of amino sugar and muramic acid concentrations in soil is more useful as an indicator of microbial necromass than microbial biomass. Characterizing microbial biomass should be carried out with more labile biomarkers such as phospholipid fatty acids (Ogram and Feng, 1997), and other biology molecular techniques.

An important application of estimating amino sugars and muramic acid concentrations in soil might be the 'memory effect' of amino sugars. An integration of the microbial community structure composition over time may therefore be possible. It is known that microbial community structure is very sensitive to changes in environmental conditions, therefore, analyzing amino sugars and muramic acid might help to investigate medium-term to long-term effects of land-use changes to soil microbial community composition. However, up to now it remains unknown how long amino sugars are stabilized in soil and how fast they are turned over. Amelung et al. (2001) investigated the concentrations of amino sugars and muramic acid in beech litter mineral mixtures incubated over a period of about 500 days. Apart from very high amino sugar concentrations at the beginning of the experiment, the authors only found an enrichment of galactosamine during the experiment.

The GlcN/MurAc ratio of the investigated soils ranged between 4.5 and 25 (Table 4) which was in the same range as reported by other authors, with values between 8 and 20 common for mineral soil (Amelung, 2001). In our study, the GlcN/MurAc ratio suggests that the soil microbial community structure of the North American Vertisol and the Brazilian Ferralsol is dominated by fungi. Adding a labile carbon source (glucose in our case) decreased the GlcN/MurAc ratio in the Vertisol but increased it in the Ferralsol. Thus, the microbial community structure rapidly switched to more bacteria in the case of the Vertisol and to more fungi in the case of the Ferralsol. These differences might be explained by the different pH values of the soils (neutral in the Vertisol, and acidic in the Ferralsol; Table 1). It is known that neutral pH values favor bacterial growth

whereas acidic conditions favor fungal growth. The high GlcN/MurAc ratio of around 15 of the Anthrosols and the high mountain soil reflected a higher contribution of actinomycetes and/or bacteria to the microbial community structure. Higher GlcN/MurAc ratios might also be indicative of a significant contribution from invertebrate, mollusk, or snail glucosamine.

On the other hand, the ManN:MurAc ratio suggested a predominance of fungi or actinomycetes in the microbial community structure favoring an increase in actinomycetal growth after glucose addition in the Anthrosols and the Brazilian Ferralsol (Table 3). The GalN:MurAc ratio indicated a predominance of fungi in the Vertisol and the Brazilian Ferralsol and a higher contribution of bacteria in all other soils. This is contrary to the evidence obtained with the GlcN:MurAc ratio (Table 3). As already mentioned above, several unpredictable sources could contribute to the glucosamine concentrations, thus making the ManN:MurAc and the GalN:MurAc ratios more reliable indicators for soil microbial residue and/or soil microbial community structure analysis.

Obviously, there is no direct link between the sorption of amino sugars to mineral and organic soil particles as no significant correlation between clay or TOC content (Table 1) and the individual amino sugar concentrations (Fig. 4a) could be observed.

5. Conclusions

Amino sugar and muramic acid analyses in microbial biomass extracted from soil, cultivated phylogenetic microbial groups and soil samples, revealed that glucosamine, mannosamine, galactosamine, and muramic acid are produced in appreciable amounts by soil microbes. None of these biomarkers alone seems to be specific for bacteria, fungi, and actinomycetes only. Additionally, glucosamine might have unknown sources in soil. Thus, it is strongly suggested to use the ratios of ManN:MurAc and GalN:MurAc to characterize the origin of microbial residues in soil rather than the GlcN:MurAc ratio. Additionally, the extractable microbial biomass contributed a negligible amount to the total amino sugar content in soil. Therefore, amino sugars and muramic acid are better biomarkers for characterizing soil microbial necromass to the medium-to-long-term instead of actual changes of soil microbial biomass which could be better traced by labile biomarkers such as phospholipid fatty acids. However, further studies should be carried out to estimate turnover times of glucosamine, mannosamine, galactosamine and muramic acid in soils, with the aim of using them as suitable indicators for characterizing the medium- or long-term composition of soil microbial community composition. Compound-specific stable isotope analysis could be an appropriate tool for this aim.

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