

Net microbial amino sugar accumulation process in soil as influenced by different plant material inputs

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Abstract Identifying the impact of plant material inputs on soil amino sugar synthesis may advance our knowledge of microbial transformation processes in soils. In a 12-week laboratory microcosm incubation, 1, 2, 4, and 6% (w/w) soybean leaf or maize stalk were initially added to soil, respectively, whereas soil without plant addition was used as a control. The results showed that adding organic materials to the soil led to a net accumulation of amino sugars, because of greater microbial synthesis. The ratios of glucosamine to galactosamine and of glucosamine to muramic acid, two indicators differentiating the relative contribution to soil organic matter of fungi and bacteria, showed substantial variance across the gradient of substrate addition. Our results suggest that the amount of nutrients in a given substrate is the primary attribute determining microbial net accumulation of soil amino sugars, especially in the relatively short term, whereas the composition of nutrients might be more important in the relatively long term when nutrients are not sufficient. The use of the two ratios (glucosamine to galactosamine and glucosamine to muramic acid) reflects different dynamics of galactosamine and muramic acid during the decomposition of organic substrates in soils. Muramic acid, compared with galactosamine, is

more likely to accumulate in the soil active organic fraction under abundant nutrient conditions, whereas it would be decomposed along with active organic matter when the nutrients are scarce and remain in minor quantities in the clay fraction without being attacked by microbes.

Keywords Amino sugar · Organic material · Microbial accumulation · Soil

Introduction

Amino sugars can provide clues in investigating the fate and sequestration of carbon (C) and nitrogen (N) in residues of microbes, because plants do not synthesize amino sugars in significant amounts whereas fungi and bacteria do (Parsons 1981; Stevenson 1982). Moreover, the concentrations of amino sugars in living microorganisms and amino sugar-containing invertebrates are much less than those occurring in dead microbial cell walls (Curry and Good 1992; Amelung 2001; Glaser et al. 2004).

It is well known that amino sugars are rapidly synthesized during immobilization of labeled inorganic N (McGill et al. 1973) and during mineralization of many organic materials (Sowden 1968; Amelung et al. 2001; Liang et al. 2006). Lowe (1973) found that the content of amino sugars increased with humification in forest soils. Evidence from incubation experiments with arctic soils suggests that the level of amino sugars in total soil N either remained constant or increased with time (Dai et al. 2002). Recently, we have shown that the amounts and patterns of amino sugars in three soils displayed different dynamic responses during the decomposition of organic materials (Liang et al. 2006). In particular, amino sugar concentrations in all three soils increased in the first stage (approximately 15 weeks)

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of incubation, yet slowly decreased as incubation proceeded to the 30th week; moreover, the simulated models for the whole incubation course suggest that soil organic matter (SOM) is capable of maintaining a stable baseline level of amino sugars. Accordingly, the buildup and the degradation of amino sugars may not have reached equilibrium during the first 15 weeks in soils with the addition of 4% (w/w) soybean leaf or maize stalk. Amino sugar production took priority over amino sugar degradation during this period.

To date, little is known about the rate at which soil amino sugars respond to plant material additions, especially the net accumulation process of amino sugars, as well as the dynamics of individual amino sugars. We therefore designed an incubation procedure to gain more detailed knowledge of the mechanism of microbial processes during the decomposition of substrates. To optimally restrict the gradient of plant material addition in the net accumulation process, a 12-week incubation schedule was chosen with sequential additions (0% control, 1, 2, 4, and 6%, by mass) of soybean leaf or maize stalk.

Materials and methods

Soil

Brown soil (Alfisol) samples were taken at the Shenyang Experimental Ecological Station of the Chinese Academy of Sciences, Shenyang, China, located at about 31 m a.s.l. (41°31' N, 123°24' E), which has a well-documented management history in long-term soil science research. The soils at this site do not contain lime, and so the total C of the soil can be regarded as entirely organic. The sampled soils (0–20 cm) were air-dried and homogenized with any remaining visible large soil fauna, stones, and discrete pieces of undecomposed plant materials removed by a 1-mm sieve and subsequently stored under dark and cool conditions. Soil properties are presented in Table 1.

Incubation experiment

The soil incubation was conducted in the Institute of Applied Ecology, the Chinese Academy of Sciences, Shenyang, China. The plant materials for the incubation

study were Soybean leaf (*Glycine max* [L.] Merr.) and Maize stalk (*Zea mays* L.) sampled in spring, then air-dried and cut into pieces of approximately 2 mm² with properties listed in Table 2. The two plant materials were respectively mixed with soils along a gradient of ratios (0, 1, 2, 4, and 6%, by mass) in pots. Each of the mixtures was homogenized and distributed to individual plastic pots (approximately 1 liter). The pots were moved to a room kept at 25°C at which temperature soil microorganisms are at satisfying capability to utilize organic substrates. Purified water was added at approximately 25% air-dried soil weight, and pots were then covered with perforated plastic lids (9 holes, each approximately 4 mm in diameter) to ensure aeration while minimizing water loss. The soil water content in each pot was maintained by regular watering to a constant weight every 6 days. Soils without organic material additions were used as the control; meanwhile, to stimulate microbial metabolism, KH₂PO₄ (40 mg P kg⁻¹ soil) was added to each pot at start time and (NH₄)₂SO₄ (40 mg N kg⁻¹ soil) was added after 1, 3, 6, and 9 weeks. Three replicates of the soil were sampled at the 12th week. The samples were air-dried and milled (<0.25 mm) before analysis.

It should be mentioned here that amino sugars detected in the plant materials at the beginning of the incubation were likely produced by the soil and epiphyllous microbial communities before sample collection, as all previous studies demonstrated plants do not synthesize amino sugars in significant amounts (Parsons 1981; Stevenson 1982).

Chemical analysis

Soil total C and N contents of each replicate sample were determined by dry combustion and detection of the produced CO₂ and N₂ by thermal conductivity detection using a Vario EL C/N/S analyzer (Elementar, Germany). SOM was estimated by multiplying the soil organic C (equal to TOC in this study) by 1.724. The determination of four individual amino sugars, glucosamine (GluN), mannosamine (ManN), galactosamine (GalN) and muramic acid (MurA) was conducted by means of gas chromatography (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). Samples

Table 1 Some physical and chemical properties of the soil studied

Soil type	OM (g kg ⁻¹)	Total N (g kg ⁻¹)	C/N ratio	Alkaliabie N (μg g ⁻¹)	Available P (μg g ⁻¹)	Available K (μg g ⁻¹)	pH(H ₂ O) value 1:2.5
Alfisol	19.60	1.21	8.28	108.51	0.07	85.14	6.0

OM, organic matter

Table 2 Some properties of organic materials selected

Organic material	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	Total S (g kg ⁻¹)	C/N ratio	Total AS (μg g ⁻¹)	GluN (μg g ⁻¹)	ManN (μg g ⁻¹)	GalN (μg g ⁻¹)	MurA (μg g ⁻¹)
Soybean leaf	442.5	9.08	3.19	48.73	328	234.5	16.0	26.0	51.5
Maize stalk	416.0	14.38	3.87	28.93	2340	1701.5	200.5	192.5	245.5

AS, amino sugars; *GluN*, glucosamine; *ManN*, mannosamine; *GalN*, galactosamine; *MurA*, muramic acid

were hydrolyzed with 6 M HCl at 105°C for 8 h, and then the solution was filtered and purified by neutralization. After freeze-drying the supernatant, methanol was used to wash amino sugars out from the residues. The recovered amino sugars were transformed into aldonitrile derivatives. Excess anhydride was destroyed with water and 1 M 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., USA) equipped with a HP-5 (25 m by 0.32 mm by 0.25 μm)-fused silica column and flame ionization detector. The individual amino sugars were identified by comparing their retention times with those of authentic standards. Quantification was gained relative to the internal standard myo-inositol, which was added to the samples before purification and the recovery standard methyl-glucamine, which was added before derivatization. In addition to the individual amino sugar content, we calculated the total amino sugar contents as the sum of four amino sugars (Amelung 2001). All analyses were done in duplicate.

Statistical analysis

Statistical analysis of the data was carried out in triplicate by one way analysis of variance using the software package SPSS for Windows. Statistical regression analysis (correlation coefficients) and figures were performed using Microsoft Excel and Sigma Plot (SYSTAT Software).

Results and discussion

Amino sugars pool

Total amino sugar content can be used to estimate microbial cell-wall mass in soils (Parsons 1981; Chantigny et al. 1997; Amelung 2001). As reported by Zhang et al. (1998, 1999) and Guggenberger et al. (1999), *GluN* was the dominant compound, followed by *GalN*, whereas *ManN* and *MurA* were minor constituents of the amino sugars determined. The dynamics of *ManN* in soil with plant material addition is erratic in comparison with the other

three individual amino sugars; nevertheless, this did not affect the content of the total amino sugars because of its small proportion.

At the 12-week sampling time, all treatments containing added soybean leaf or maize stalk accumulated significantly more total amino sugars than the control (Figs. 1 and 2). The amino sugar accumulations in treatments with gradient addition of soybean leaf (1, 2, 4, and 6%) were 1.38, 1.36, 1.50, and 1.47 times that of the control, respectively; the corresponding numbers for maize stalk addition are 1.21, 1.18, 1.46, and 1.43. This suggests that the increased accumulation of amino sugars is determined by the soil substrate abundance, i.e., the more organic materials available in soils, the greater the microbial contributions to accumulation of soil amino sugars. Within the treatments, amino sugar production showed similar values with 1 and 2% substrate addition, as well as with 4 and 6%, which separately indicates the similar intensity of amino sugars' net production by microbes in use of the substrates and microbial product. In contrast, 1 and 2% addition treatments were significantly lower than the 4 and 6% addition treatments in terms of amino sugar content, which suggests that there is a quantitative "jump" between 2 and 4% treatment, possibly because of the relative shortage of nutrients for microbes in 1 and 2% treatment, resulting in the production of amino sugars not outweighing the degradation of amino sugars, whereas the 4 and 6% treatment provide sufficient nutrients so that the priority of decomposition of amino sugars would not be expressed in 12-week incubation.

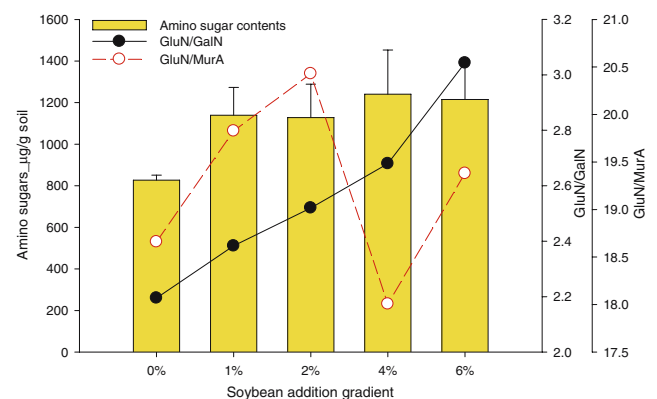


Fig. 1 Contents and corresponding ratios of amino sugars under soybean leaf addition gradient

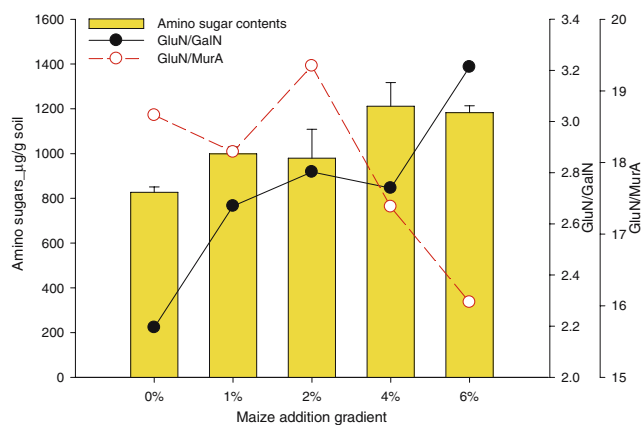


Fig. 2 Contents and corresponding ratios of amino sugars under maize stalk addition gradient

tions. Altogether, these results imply that more substrates can maintain longer net amino sugar accumulation in the soil biological process by microbes. Furthermore, it is reasonable to predict that the variation of amino sugar content among the gradient treatments would eventually become insignificant if the incubation time were prolonged far enough.

Compared with the control, the soybean leaf treatments have consistently higher net accumulation of amino sugars than the maize stalk treatments; moreover, this accumulation difference is most apparent in 1 and 2% treatments. These results agree with those from our past research results which show that the substrate component (C/N) is the key determinant factor affecting microbial amino sugar's accumulation (Liang et al. 2006). Regarding the insignificant difference between 4 and 6% treatments with soybean leaf versus maize stalk, it is possible that 4% addition of either soybean leaf or maize stalk can provide sufficient nutrients for microbial growth for 12 weeks; thus, resulting in similar predominance of amino sugar production.

Amino sugar patterns

Individual amino sugars in soil have particular microbial origins (Sharon 1965; Parsons 1981). GluN is found in most fungal cell walls and in invertebrate exoskeletons such as chitin (Parsons 1981). Ga1N frequently occurs in capsular and extracellular polysaccharides of bacteria (Sharon 1965; Parsons 1981), possibly attached to lipopolysaccharides (Parsons 1981) or teichoic acids (Ladd and Jackson 1982). Fungi may also produce Ga1N in small amounts but only by certain taxonomic classes (Sharon 1965); however, Sowden and Ivarson (1974) demonstrated that little, if any, Ga1N is synthesized by fungi during fungi-inoculated incubation experiments. MurA uniquely originates from bacteria (Kenne and Lindburg 1983; Amelung 2001) in terrestrial ecosystems. Accordingly, different amino sugar ratios can be used as qualitative indicators for changes in the

composition of the decomposer communities, which reliably reflect the fate of bacterial- and fungal-derived SOM. The ratio of GluN/Ga1N has been successfully used to evaluate the relative contributions between fungi and bacteria to SOM turnover and accumulation (Kögel and Bochter 1985; Zhang et al. 1998; Amelung et al. 1999; Amelung 2001). Furthermore, the ratio of GluN/MurA has been used to differentiate between the contributions of fungi and bacteria to the SOM in many soils (Chantigny et al. 1997; Zhang et al. 1998; Amelung et al. 2001; Dai et al. 2002).

The ratios of GluN/Ga1N and GluN/MurA varied markedly among the different addition treatments (Figs. 1 and 2), indicating that either the microbial origin or the stability of the different individual amino sugars was different among the gradient treatments. The ratio of GluN/Ga1N after 12 weeks increased significantly along the gradient addition of plant materials, and there is a close linear correlation between the GluN/Ga1N ratio and substrate addition amounts ($R^2=0.96$ for soybean leaf and $R^2=0.84$ for maize stalk). This results primarily from the more rapid accumulation of fungal-derived GluN with more substrate in comparison to bacteria-derived Ga1N (individual amino sugar data not shown here, Liang 2003). This could be because of the preferential retention and net accrual of fungal hyphae in soils with more organic substrate, or both, together with the previous suggestion that bacterial-derived Ga1N and MurA are less stable and have a fast decomposition rate compared with fungal-derived GluN in soils (Guggenberger et al. 1999; Amelung et al. 2001; Solomon et al. 2002). In sum, the increase in GluN/Ga1N ratio reflects a shift of bacterial- to fungal-derived residues in soil with substrate gradient addition, indicating more fungus contribution than bacterial in short term (12 weeks) to substrate decomposition. In contrast, the ratio of GluN/MurA is different from that of GluN/Ga1N (Figs. 1 and 2), which is possibly because of the different bacterial allocation of MurA and Ga1N or the distinction of MurA's molecular structure. Generally, the cell wall of Gram-positive bacteria is much thicker and accounts for a

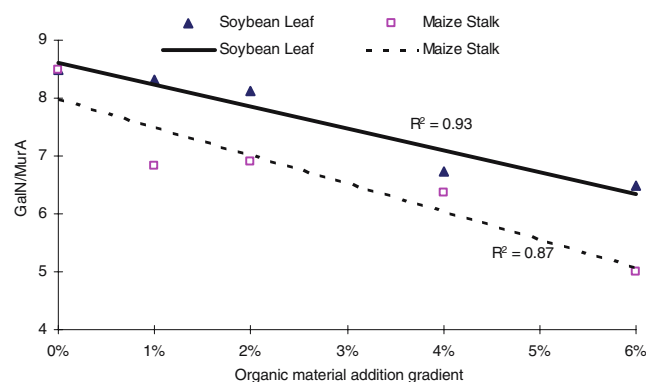


Fig. 3 Ratios of Ga1N to MurA in soil with organic material addition gradient

Table 3 Ratios of soil amino sugars to organic matter with organic material addition gradient

Substrate type	Organic material addition amount				
	0%	1%	2%	4%	6%
Soybean leaf	49.30a	46.12a	47.61a	43.96a	32.91b
Maize stalk	49.30a	49.41a	43.55a	42.92a	31.44a

Different letters in the same line represent a significant difference between treatments ($P < 0.05$).

larger percentage of MurA than in Gram-negative bacteria (Brock et al. 1994; Kögel-Knabner 2002). This difference might indicate a shift within the composition of the bacterial community in soils after organic material additions. Furthermore, MurA is larger and, unlike other hexosamines, has a carboxylic group, which may result in a different turnover time in comparison with others. Many previous studies have shown that the dynamics of Ga1N are different from those of MurA (Zhang et al. 1998, 1999; Liang et al. 2006).

Although both ratios of GluN/Ga1N and GluN/MurA generally indicate relatively bacterial residues, they have to be recognized as to belonging to two evaluation systems and serving different objectives. It is most meaningful to stick to a single ratio as much as possible when evaluating the differential contribution of bacterial and fungal amino sugars to SOM sequestration. In general, the ratio of GluN/Ga1N seems a good indicator in the long term, whereas that of GluN/MurA does better for the short term (Zhang, personal communication). This is consistent with the previous speculation from research about the influence of climate on amino sugar production in different particle size fraction of soils (Zhang et al. 1998), because low concentrations of MurA in soils may reflect a faster turnover, as indicated by the fact that MurA standard solutions are unstable in water and must be stored in a freezer compared with solutions of the other amino sugars. Generally, amino sugars in soils are relatively stable, regardless of their form (bound to soil matrices or not), whereas MurA in soils is stable only when bound.

Two ratios reflecting changes in the composition of living biomass give different measures of the extent of the shift from bacterial to fungal residues in our study; however, their internal mechanisms are uncertain. To characterize the Ga1N and MurA, especially regarding their response to substrates, the ratio of Ga1N/MurA was also investigated here (Fig. 3). Ga1N/MurA ratios decreased with the addition gradient and had markedly linear correlation with substrate amount (soybean leaf, $R^2 = 0.93$; maize stalk, $R^2 = 0.87$). These results might suggest that in the substrate decomposition process, bacterial-derived MurA is more easily enriched in soils with a high labile SOM than bacterial-derived Ga1N, i.e., MurA is most likely accumulated in the active organic

fraction more than Ga1N over the short term (12 weeks). The ratio of Ga1N/MurA responded more quickly to the addition of maize stalk than to the addition of soybean leaf, as indicated by the linear slope comparison (Fig. 3). Our maize stalk provides a greater nutrient source (N amount) for microbes than does soybean leaf; thus, the more the substrate, the greater the difference between soybean leaf and maize stalk. This nutrient dependence appeared to directly determine the relatively rapid change of Ga1N/MurA by maize stalk addition and further supports our previous suggestion that MurA has a greater ability to accumulate in highly organic soil compared with Ga1N during a 12-week incubation. On the other hand, the ratio of Ga1N/MurA was consistently higher in soybean-leaf-addition treatments than in maize-stalk-addition treatments (except in controls), suggesting that soil with added soybean leaf has a greater amount of Ga1N residue as well as relatively strong fungal activity, possibly because of the differential ratio of C to N in the substrates (C/N of soybean leaf is more than that of maize stalk). In summary, considering the accumulation of bacterial residues in soils over the short term, substrate abundance has the greatest effect within gradient additions of a specific substrate, whereas the identity of the substrate has the greatest effect between substrate addition treatments.

There have been few reports about the relationship between soil active organic matter and amino sugars to this point. Dissolved SOM has a marked linear correlation with

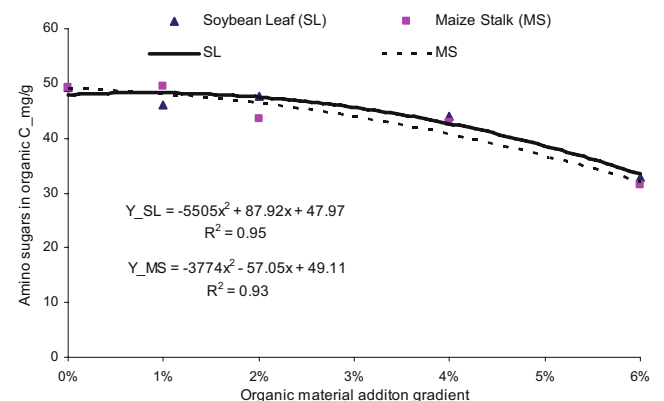


Fig. 4 Parabolic regression of soil amino sugars to organic matter with organic material addition gradient

amino sugars in polluted soils, especially MurA (Zhao 2001). Considering the instability of MurA, we can deduce that MurA is most likely enriched in soil active organic fractions when substrate is relatively abundant, whereas it will decompose together with active compound when substrate is relatively scarce, only leaving a minor proportion of MurA attached to mineral or clay fractions unattacked by microbes.

Amino sugar concentrations in SOM

For a better understanding of amino sugar dynamics, not only the absolute content but also C-related concentrations were considered. The amino sugar concentrations in SOM reflects the relative accumulation of amino sugars to soil organic C.

Amino sugar contents in soil organic C after 12-week incubation is not significantly different under different substrate types (Table 3), although the nutrient component (C/N) greatly varies between soybean leaf and maize stalk. These results verify our prior model suggestion, which states that soil amino sugar accumulation in theory is an internal characteristic of SOM and tends to keep a constant C proportion over time (Liang et al. 2006). The total amino sugar concentrations increased with the substrate addition amount in the 12-week incubation; amino sugars in soil organic C, however, showed a decreasing trend in relation to the substrate addition amount. This apparent contradiction is primarily due to the diluting effect of high inputs of plant-derived organic materials, which is accounted for by the undecomposed organic substrate in 12 weeks, i.e., the net amino sugar accumulation is less than net SOM augmentation in short-term incubations. Amino sugar content in SOM decreased bi-exponentially with increasing organic substrate, and this trend was the same for soybean leaf ($R^2=0.95$) and maize stalk ($R^2=0.93$). As shown in Fig. 4, the ratio of amino sugars in SOM will decline as the amount of plant material increases, but this decrease is not significant, as indicated by the dynamics of the regression slope. This result also supports the previous suggestion that the ability of SOM to sustain amino sugars is constant. In addition, the maximum value of the ratio of amino sugars in SOM is close to the control, which suggests the amino sugars in SOM will approach a constant value when smaller additions decompose over a short time.

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