Biol Fertil Soils (2013) 49:949–954
DOI 10.1007/s00374-012-0768-0

SHORT COMMUNICATION

Long-term impacts of manure, straw, and fertilizer on amino sugars in a silty clay loam soil under temperate conditions

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Received: 11 September 2012 / Revised: 8 December 2012 / Accepted: 14 December 2012 / Published online: 6 January 2013

Abstract There is increasing evidence that microorganisms participate in soil C sequestration and stabilization in the form of resistant microbial residues. The type of fertilizers influences microbial activity and community composition; however, little is known about its effect on the microbial residues and their relative contribution to soil C storage. The aim of this study was to investigate the long-term impact (21 years) of different fertilizer treatments (chemical fertilizer, crop straw, and organic manure) on microbial residues in a silty clay loam soil (Udolls, USDA Soil Taxonomy). Amino sugars were used to indicate the presence and origin of microbial residues. The five treatments were: CK, unfertilized control; NPK, chemical fertilizer NPK; $NPKS_1$, NPK plus crop straw; $NPKS_2$, NPK plus double amounts of straw; and NPKM, NPK plus pig manure. Long-term application of inorganic fertilizers and organic amendments increased the total amino sugar concentrations (4.4–8.4 %) as compared with the control; and this effect was more evident in the plots that continuously received pig manure ($P<0.05$). The increase in total amino sugar stock was less pronounced in the straw-treated plots than the NPKM. These results indicate that the accumulation of soil amino sugars is largely influenced by the type of organic fertilizers entering the soil. Individual amino sugar enrichment in soil organic carbon was differentially influenced by the various fertilizer treatments, with a preferential accumulation of bacterial-derived amino sugars compared with fungal-derived glucosamine in manured soil.

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Keywords Fertilizer type · Microbial residues · Biomarker · Amino sugar · Long-term fertilization

Introduction

Soil organic matter (SOM) as a key attribute of soil fertility is crucial for the sustainability of agroecosystems (Manlay et al. 2007). The influences of agricultural management practices on SOM dynamics are closely related to soil microorganisms because they play central roles in turnover of organic matter and nutrient cycling (Chaparro et al. 2012; Fließbach and Mäder 2000; Paul and Clark 1989). It has been frequently reported that fertilization influences SOM dynamics and also exerts considerable impact on soil microbial biomass, activity, and community structure (Ferreras et al. 2006; Heinze et al. 2010). Little is known, however, about the response of microbial residues to soil fertilization practices (Guggenberger et al. 1999; Scheller and Joergensen 2008). It is suggested that the turnover time of these microbial residues is much longer than that of the living microbial biomass in soil (Simpson et al. 2007). There is increasing evidence that microorganisms participate in long-term sequestration of C in soils in the form of resistant microbial residues (Kiem and Kögel-Knabner 2003; Liang and Balser 2008). Therefore, microbial residues can be used as a time-integrated biomarker to indicate agricultural management effects on microorganisms (Glaser and Gross 2005; Van Groenigen et al. 2010). To acquire a more complete understanding of how fertilization practice influences SOM, it is necessary to consider its effect on the concentration and stabilization of microbial residues.

Amino sugar has been used as an important indicator to quantify microbial residues because of their minor amounts in plants (Parsons 1981) and stability after microbial cell death (Chantigny et al. 1997; Guggenberger et al. 1999). The

majority of amino sugars (>90 %) occur in dead microbial cells (Amelung et al. 2001). Analysis of amino sugars, therefore, can provide insights into the fate and sequestration of C and N in microbial residues and also into the long-term shift in SOM quality (Amelung et al. 2008; Joergensen et al. 2010). There are four amino sugars that can be detectable, i.e., glucosamine (GluN), galactosamine (GalN), muramic acid (MurA), and mannosamine (ManN) (Zhang and Amelung 1996). The origin of GalN in soil is still unclear, although it accounts for 30–50 % of the amino sugar pool (Amelung et al. 2001; Engelking et al. 2007). The chitin of fungal cell walls is the major source of GluN, although bacterial cell walls and the exoskeletons of soil invertebrates also make some contribution (Parsons 1981; Amelung 2001). MurA exclusively originates from bacteria, as a component of the peptidoglycan of the bacterial cell wall (Parsons 1981; Chantigny et al. 1997). Ratios of GluN to MurA have been widely used to illustrate the relative contribution of fungi and bacteria to microbial residues and SOM on account of their specificity (Amelung 2001; Six et al. 2006; Joergensen and Wichern 2008).

Microbial responses to fertilization regimes are largely conditioned by the type and rate of fertilizers irrespective of the soil type and texture (Ferreras et al. 2006; Heitkamp et al. 2009; Paustian et al. 1997). According to the basis of amino sugar analysis, Scheller and Joergensen (2008) and Joergensen et al. (2010) reported that the long-term application of farmyard manure integrated with organic farming practices can promote the accumulation of bacterial residues compared to inorganic fertilizer application in a haplic Luvisol, Switzerland. Our recent research showed that 10-year continuous application of pig manure at various rates stimulated the accumulation of microbial residues in a temperate Mollisol (Ding et al. 2012). However, less information is available for the effects of fertilization with different fertilizer inputs (chemical fertilizer, crop straw, and organic manure) on the microbial residues and their contribution to SOM in temperate agroecosystems based on long-term experiment. The aim of this study was to identify the effects of different fertilizer treatments on the fractions of microbial residues using a 21-year-old fertilization trail in China. This work may contribute to our understanding of how long-term different fertilizer applications influence fungal- and bacterial-derived residues and their contribution to SOM in a fertile soil (organic C content >27 $g kg^{-1}$) under temperate conditions.

Materials and methods

Site description

A long-term field experiment was established in April 1990 at the Hailun National Field Station (47°26′ N, 126°38′ E), Chinese Academy of Sciences, in Heilongjiang Province, China. The climate of the experimental site is typical continental monsoon. The mean annual temperature is 1.5 °C with the lowest and highest mean monthly values of −23.5 °C in January and 21.0 °C in July, respectively. Mean annual rainfall is about 530 mm, more than 88 % of the rain which occurs between May and September. The field was continuously cultivated for at least 100 years before 1990. The soil was classified as Udolls according to the USDA Soil Taxonomy (Soil Survey Staff 2010), derived from loamy loess, with silty clay loam texture. The soil had a clay loam texture with 257 $g kg^{-1}$ sand, 324 $g kg^{-1}$ silt, 408 $g kg^{-1}$ clay, 27.1 g organic C kg^{-1} soil, 2.08 g total N kg^{-1} soil, and an average pH (H_2O) of 6.3 (soil/water=1:2.5) for topsoil layer (0–20 cm) at outset of experiment.

Experimental design

The experiment included one crop per year, with a 3-year rotation sequence of maize (*Zea mays* L.)–soybean [*Glycine max* (L.) Merrill]–wheat (*Triticum aestivum* L.) and five treatments: (1) CK (unfertilized control), (2) NPK, (3) $NPKS_1$ (crop straw + NPK fertilizers), (4) $NPKS_2$ (double crop straw + NPK fertilizers), and (5) NPKM (pig manure + NPK fertilizers). The treatments were arranged in a randomized complete block design with three replicates. Each plot size was 12×5.6 m, and the space between adjacent plots was 0.7 m. In all treatments except for the CK, N fertilizer was added as urea and ammonium hydrogen phosphate at annual rates of 75 kg N ha^{-1} for wheat, 120 kg N ha^{-1} for maize, and 20 kg N ha^{-1} for soybean. Ammonium hydrogen phosphate was applied as P fertilizer at annual rates of 26, 26, and 22 kg P ha^{-1} for wheat, maize, and soybean, respectively. Potassium sulfate as potassium fertilizer was added at annual rate of 25 kg K ha^{-1} for all crops. These fertilizers were applied as basal fertilizers. Crop straw was added at annual rates of 2,250 and 4,500 kg ha^{-1} in the $NPKS_1$ and $NPKS_2$, respectively, and pig manure was applied at rate of 3,750 kg ha^{-1} $year^{-1}$ (on a dry weight basis). The total annual C input was equivalent to 910, 1,819, and 1,203 kg C ha^{-1} in the $NPKS_1$, $NPKS_2$, and NPKM treatments, respectively; and the amounts of added N were 27, 53, and 90 kg N ha^{-1}, respectively. Organic material addition was conducted by complete removal in the previous year and subsequent return of defined amounts in the next year. For each year, these organic materials were evenly broadcast onto the soil surface by hand, then integrated into the 0–20-cm layer by shovel and followed by rake before spring sowing. Crop was planted on top of a preformed ridge by hand. The plot was ridged again at about 15 days after sowing. Deep loose operation was conducted using a chisel before the rainy season. The aboveground crop residues in all plots were removed and soil was plowed to 20 cm depth and harrowed following crop harvest in October.

Sampling and analysis

Soil samples were collected from 0 to 20 cm soil layer at six locations in each plot using a 7-cm diameter stainless soil core sampler in April 2011 (before crop planting). All soil samples from each plot were pooled together to form a composite. The soil samples were immediately transported to the laboratory. Visible plant and organic debris were removed with forceps. One part of the moist soil sample was passed through a 2-mm mesh for determination of soil microbial biomass. The other part was air dried and passed through a 2-mm mesh for determination of soil pH and other chemical analysis. Subsamples of <2 mm air-dried soil were further ground to pass through a 0.25-mm mesh for amino sugar and soil total C and N analysis.

Total C and N content in soil were determined by dry combustion with a VarioEL CHN elemental analyzer (Heraeus Elementar Vario EL, Hanau, Germany). Because these soils are free of carbonates, the total C content is equivalent to soil organic carbon (SOC) content. Soil pH was measured in a 1:2.5 soil–water suspension with a combination reference glass electrode. Soil microbial biomass C was determined by chloroform fumigation–extraction method (Brookes et al. 1985). Some selected soil properties under different fertilizer treatments were listed in Table 1.

Determination of amino sugars was conducted by gas chromatography after their conversion to aldononitrile acetates according to Zhang and Amelung (1996). Briefly, ground samples were hydrolyzed with 6 M HCl at 105 °C for 8 h and the solution was filtered, adjusted pH to 6.6–6.8, and then centrifuged (1,006×*g*, 10 min). The supernatant solution was freeze dried, and then, amino sugars were washed out from the residues with methanol. The recovered amino sugars were transformed into aldononitrile derivatives and extracted with dichloromethane from the aqueous solution. Excess anhydride was removed with 1 M HCl and water. The amino sugar derivatives were redissolved in a hexane and ethyl acetate solvent (*v*/*v*=1:1) for final analysis after removal of dichloromethane with N_2 stream. The amino sugar derivatives were separated on an Agilent 6890A gas chromatography (GC, Agilent Tech. Co. Ltd., USA) equipped with a flame ionization detector. Amino sugars were quantified based on the internal standard *myo*-inositol which was added prior to purification. Methylglucamine was used as a recovery standard to monitor recovery efficiency. The amount of total amino sugars was calculated by summarizing four detectable amino sugars (GluN, GalN, MurA, and ManN).

Statistical analysis

Statistical analyses were performed using the SPSS 13.0 software package for windows (SPSS Inc., Chicago, USA). Data were tested for normality and log transformed as needed to normalize the distributions prior to statistical analysis. The differences in amino sugar concentrations and SOC among treatments were examined using one-way analysis of variance, followed by a Tukey's honestly significant difference test at $P<0.05$. Figures were plotted using Sigmaplot 10.0 (Systat Software Inc.).

Result

Individual amino sugar concentrations and biomarker ratios

The proportion that individual amino sugars contributed to the total followed the order of GluN > GalN > MurA across the five treatments (Table 1). The amounts of GluN, GalN, and MurA in fertilized treatments were increased by 2.8–8.1, 4.4–

Table 1 Soil properties at 0–20 cm depth of the experimental plots (April 2011)

Microbial properties	CK	NPK	$NPKS_1$	$NPKS_2$	NPKM
pH (H_2O)	6.12	6.05	5.89	5.78	6.10
Total C (g kg^{-1})	27.23	27.77	28.79	28.44	29.79
Total N (g kg^{-1})	2.18	2.13	2.22	2.17	2.41
Microbial biomass C (mg kg^{-1})	286 c	275 c	288 c	329 b	361 a
Muramic acid (mg kg^{-1})	58.6 b	64.4 b	66.6 ab	71.0 ab	88.9 a
Glucosamine (mg kg^{-1})	1,089 a	1,135 a	1,167 a	1,120 a	1,179 a
Galactosamine (mg kg^{-1})	565.4 b	590.9 ab	594.8 ab	620.6 a	590.6 ab
Total amino sugar (mg kg^{-1})	1,713 b	1,790 ab	1,829 ab	1,812 ab	1,858ab
Glucosamine/muramic acid	18.60 a	17.62 ab	17.52 ab	15.77 ab	13.25 b

Different letters within a row indicate a significant difference ($P<0.05$)

CK unfertilized control, *NPK* chemical fertilizer, *$NPKS_1$* crop straw added at 2,250 kg ha^{-1} $year^{-1}$ plus chemical fertilizer, *$NPKS_2$* crop straw added at 4,500 kg ha^{-1} $year^{-1}$ plus chemical fertilizer, *NPKM* pig manure added at 3,750 kg ha^{-1} $year^{-1}$ plus chemical fertilizer

9.7, and 13–51 %, respectively, over that in the CK plot. Crop and manure treatments had higher MurA concentrations, while only the manure treatment resulted in significantly higher MurA than the CK and NPK ($P<0.05$). No significant differences were observed for the concentrations of GluN within the five treatments (Table 1). GalN concentration was significantly higher in the $NPKS_2$ than the CK ($P<0.05$). The ratios of GluN to MurA were affected by different fertilizer application (Table 1). Specifically, the GluN to MurA ratios were lowest in the NPKM (13.2), with an increasing order of the $NPKS_2$, $NPKS_1$, NPK, and CK ($P<0.05$).

Concentrations of total amino sugars and its proportion in SOC

Concentrations of total amino sugars varied differentially in the different fertilizer treatments (Table 1). Compared with the CK, the NPK treatment increased total amino sugar concentrations by 4.4 %, and the increases were up to 5.7–8.4 % for crop residue and manure treatments. Concentrations of total amino sugars in the NPKM significantly exceeded that of the CK ($P<0.05$). Neither inorganic fertilizer nor crop straw treatments significantly changed the total amino sugar compared to the control. There was no significant treatment effect for the contribution of total amino sugar to SOC (Fig. 1). Nevertheless, significantly higher contribution of MurA to the SOC was observed in the NPKM relative to the CK, and the highest proportion of GalN in the SOC occurred in the $NPKS_2$ ($P<0.05$). The enrichment of GluN in the SOC was not prominently affected by different fertilizer treatments.

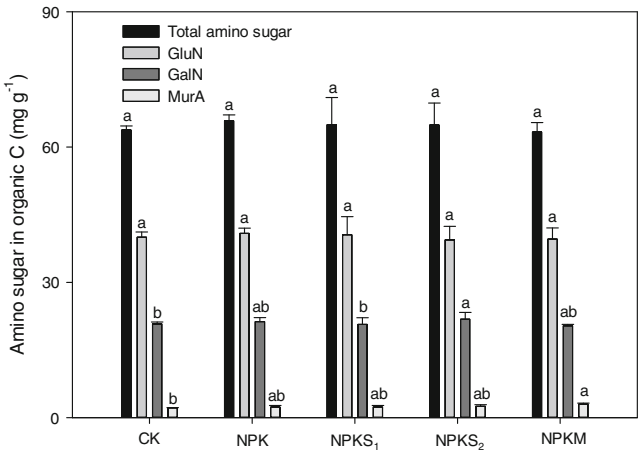

Fig. 1 Concentration of amino sugars in total organic C. *Different letters* indicate significant differences in the proportions of total amino sugar, glucosamine, galactosamine, and muramic acid to total organic C among treatments ($P<0.05$). *Bars* represent standard errors ($n=3$). *GluN*, glucosamine, *GalN*, galactosamine, *MurA*, muramic acid, *CK*, unfertilized control, *NPK*, chemical fertilizer, *$NPKS_1$*, crop straw added at 2,250 kgha^{-1} year^{-1} plus chemical fertilizer, *$NPKS_2$*, crop straw added at 4,500 kgha^{-1} year^{-1} plus chemical fertilizer, *NPKM*, pig manure added at 3,750 kgha^{-1} year^{-1} plus chemical fertilizer

Discussion

The present results of amino sugar as microbial residue biomarker reflected the general positive effects of organic amendment on soil biological properties as observed in the other studies (Ferreras et al. 2006; Heitkamp et al. 2009). The fertilizer treatments with organic additions increased total amino sugars by 5.7–8.4 % compared to the unfertilized control. Meanwhile, we found that manure and straw addition stimulates microbial biomass C more than the total amino sugar concentration (Table 1). This could be interpreted as an indication of more sensitive responses of microbial biomass to organic inputs, associated with higher rates of nutrient cycling. This result confirmed that energy supply is a key factor for maintaining a higher content of microbial biomass (Nannipieri et al. 1983). Nevertheless, total amino sugar concentrations were five- or sixfold higher than microbial biomass C, indicative of higher contribution from microbial residues to SOM enrichments. Amino sugar concentrations represent an accumulation of microbial residues in soil, which are relative stable, thus their fluctuations are not necessarily consistent with living microbial biomass (Amelung et al. 2001; Turrión et al. 2002). This also explained the smaller changes of amino sugars in the straw treatments despite larger variations of microbial biomass. The increase in the amino sugar concentration was only significant in the manure treatment. Solomon et al. (2001) and Joergensen et al. (2010) also observed that long-term application of manure significantly enhanced total amino sugar concentration in a sandy soil. This could mainly be due to readily metabolizable substrates for soil microorganisms present in organic manure (Ferreras et al. 2006). In this case, microbial biomass production and nutrient cycling may be enhanced, associated with intensive processes of cell generation and subsequent death (Chander and Joergensen 2001; Ding et al. 2011). The straw treatments produced lower amino sugars than the NPKM, suggesting the production and accumulation of microbial residues are closely related to the type of organic inputs. The quality of organic matter differ in different types of amendments (e.g., *C*/*N* ratio), which can change the composition of microbial community (Börjesson et al. 2012), as a result of different accumulation of microbial metabolites (Guggenberger et al. 1999).

Manure application significantly ($P<0.05$) increased MurA concentration compared to the control, suggesting a positive effect of manure addition on bacterial metabolic processes. This result was consistent with the finding of Joergensen et al. (2010) that long-term applications of farmyard manure increased bacterial cell wall residues under organic farming systems. Moreover, we found a marked decrease in the ratios of GluN to MurA with the pronounced increase in MurA and constant GluN. This implies a shift in

composition of amino sugar pool towards bacterial residues in long-term manured soil. Sixty-three percent of the total amino sugars was due to GluN as an average for the five treatments, indicating a dominant role of fungi in microbial-sequestered nutrients in the arable soil. GluN concentration did not differ significantly between the treatments (Table 1). This was in accordance with results by Börjesson et al. (2012) that application of different organic fertilizers did not affect the fungal biomass. However, the lack of a significant effect of the crop straw on GluN contradicts the observations that regular application of straw can promote saprotrophic fungi (Scheller and Joergensen 2008; Heinze et al. 2010). Probably this discrepancy may be due to the impact of soil disturbance by tillage on the studied agro-ecosystem. Our test soil had been subjected to frequent tillage of at least three times per year, which could have diminished the effect of organic inputs on GluN accumulation mainly through the breakdown of fungal hyphae (Frey et al. 1999; Guggenberger et al. 1999). GalN made up a high portion of total amino sugar pool (31.7–34.2 %), and continuous straw addition significantly stimulated the accumulation of GalN in the soil of $NPKS_2$. The increased GalN is important for the stabilization of SOM due to its stability in soil (Glaser and Gross 2005; Ding et al. 2011). However, we could not link the GalN accumulation pattern with specific microbial residue responses to fertilizer additions since its dynamics was different from that of GluN and MurA (Table 1). More research is required to clarify the origin and function of GalN in future study (Joergensen et al. 2010).

To further assess the long-term impact of different fertilizer inputs on the source and quality of SOM, the concentrations of amino sugars were given on an SOC basis. Different fertilizer treatment did not have significant influences on the contribution of total amino sugar to SOM. Contradictory to this result, manure application at high rate (>15,000 $kg\,ha^{-1}\ year^{-1}$) led to a significant enrichment of amino sugar in SOC in the same agricultural system (Ding et al. 2012). We proposed that the lack of pronounced differences in this study probably depended on the fact that the net increase of amino sugar (0.98–1.45 $g\,kg^{-1}$) caused by smaller organic inputs was diminished when expressed on the large SOC background of the tested soil (SOC >27 g kg^{-1}). Our results revealed that long-term manure addition caused a preferential accumulation of bacterial-derived MurA in SOM compared to fungal-derived GluN, and that continuous crop straw addition greatly stimulated GalN enrichment in SOM. The individual amino sugars played different roles in SOM turnover and nutrient cycling due to their distinct turnover rates (Amelung et al. 2008; Paul and Clark 1989). Thus, the differential accumulation of individual amino sugar in SOC reflected a change of SOM quality after 21 years of various fertilization treatments.

Conclusions

Amino sugar analysis revealed that 21 years of inorganic fertilizer and organic amendments influences the microbial residue accumulation in the silty clay loam soil under temperate agroecosystems. Inorganic fertilizer application had minor effects on the microbial residues. Long-term application of pig manure stimulated accumulation of total amino sugar more than crop straw treatments. This indicates that microbial residue accumulation was strongly affected by the type of organic inputs when they were applied at similar rates in our tested soil. Considerable differences in the individual amino sugar enrichment in SOC characterized a change in the quality of microbial-derived organic matter as a result of long-term different fertilizer inputs.

Acknowledgments This work was financially supported by the National Natural Science Foundation of China (41101282) and the National Basic Research Program of China (2011CB100506-01). We are deeply grateful to the editor and two anonymous reviewers for their insightful and valuable comments for the manuscript.

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