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RESEARCH ARTICLE

Influences of sample storage and grinding on the extraction of soil amino sugars

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A B S T R A C T

Soil amino sugars have been widely used to evaluate the potential roles of microbes in mediating soil carbon (C) cycling and various pretreatment methods were used for its extraction. However, few studies assessed their potential influences on the soil amino sugar extraction. In this study, we investigated the effects of sample storage method and grinding on amino sugar extraction across different climatic zones and land uses. Results showed that the concentrations of soil amino sugars varied greatly among sample pretreatments and their impacts were highly dependent on climatic condition and land use. Specifically, higher concentrations of amino sugars were extracted from field-moist samples than dried samples in subtropical grassland, temperate forest and arable land with no significant differences among storage methods for the samples from subtropical forest, arable land, and temperate grassland. Moreover, grinding improved the extraction efficiency of amino sugars for the dried soils. Due to the reduced extraction concentration in dried soils, field-moist samples were recommended in priority. For the dried soils used for the long-term storage, grinding can be an option to improve the extraction efficiency. Such information will be valuable for reducing the uncertainty and improving the accuracy during the determination of soil amino sugars.

1 Introduction

Amino sugars in soils are important cell wall components of microorganisms (Roberts and Jones, 2012) and account for 5%–12% of soil organic nitrogen (Schulten and Schnitzer, 1997) and 2%–5% of soil organic carbon (Joergensen and Meyer, 1990). Since more than 90% of amino sugars originate from microbial necromass and less than 10% from living microorganisms and invertebrates (Amelung et al., 2001), amino sugars have been widely used as time-integrated biomarkers for evaluating the contribution of microbial residues to soil organic carbon turnover and accumulation (Shao et al., 2017; Joergensen, 2018). Glucosamine (GluN),

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galactosamine (GalN), mannosamine (ManN), and muramic acid (MurN) are the most important amino sugars quantified in soils (Zhang and Amelung, 1996; Indorf et al., 2011; Joergensen, 2018). Furthermore, due to the dominant fungal origin of GluN and exclusively bacterial origin of MurN in soils (Appuhn and Joergensen, 2006), the ratio of GluN to MurN is commonly used to assess the relative contribution of fungal and bacterial residues to soil organic carbon accumulation (Lauer et al., 2011; Liu et al., 2019).

Because of its ecological importance in elucidating microbial role in mediating soil C cycling, an increasing body of studies about soil amino sugars have been conducted in various habitats, climate regions, land uses and soil types (Joergensen, 2018). In current studies, gas chromatography (GC) and high-performance liquid chromatography (HPLC) were usually adopted to quantify the concentrations of GluN, GalN, ManN, and MurN in soils after hydrolysis (Zhang and Amelung 1996; Appuhn et al., 2004). However, there is still lack of consensus about the pretreatment of soil samples before hydrolysis. In previous studies, various sample pretreatments including field-moist (Murugan et al., 2019), freeze-dried (Moritz et al., 2008), and air-dried soils ground to different particle sizes have been used for amino sugar extraction (Indorf et al., 2011; Liu et al., 2019; Shao et al., 2019). However, few studies evaluated the potential impacts of sample pretreatments on the extraction and determination of amino sugars in soils.

Since soil structure and biological properties might be altered by sample storage condition, i.e., air-dried and freeze-dried, field-moist samples were recommended in priority for microbiological studies (Deacon et al., 2008; Mimmo et al., 2008). For example, air-dried procedure significantly affected the solubility and sorption of phosphorus (Peltovuori and Soinne, 2005), and soil enzymatic activities (Andres Abellan et al., 2011). Furthermore, grinding to different particle sizes also impacted soil nutrient conditions (Yang et al., 2015). In this study, we aimed to evaluate the potential impacts of sample storage condition (field-moist, freeze-dried, and air-dried) and grinding (2-mm, 0.25-mm, and 0.15-mm) on the extraction and concentrations of soil amino sugars.

2 Materials and methods

2.1 Soils

Top soils (0–10cm) were collected from forest, grassland and arable land both in subtropical and temperate region. The basic description of sampling sites and measured soil properties were presented in Table 1. The collected field-moist soil samples were sieved to 2-mm and assigned to five treatments: (a) field-moist soils were stored at 4°C in the dark; (b) freeze-dried; (c) air-dried and further ground to pass a 2-mm, (d) 0.25-mm, and (e) 0.15-mm sieves. Field-moist soils were stored at 4°C less than one week after sampling. All the pretreatments were conducted within two weeks.

2.2 Hydrolysis

Amino sugars were extracted according to Indorf et al. (2011) with minor modifications. Briefly, 1.0 g of field-moist/dried/grinding soils was hydrolyzed with 10 mL of 6 M HCl at 105°C for 6 h. After hydrolysis, samples were uniformly mixed and cooled to room temperature, and then filtered. 0.5 mL of filtrate was evaporated to dryness by nitrogen gas at 40–45°C to remove HCl. The dried residues were dissolved in 0.5 mL of deionized water, dried by nitrogen gas again, and re-dissolved in 2 mL of deionized water and stored at −20°C before analysis. Five replicates of each treatment were performed for the extraction.

2.3 Determination

According to the determination procedure of Indorf et al. (2011), four amino sugars GluN, GalN, MurN, and ManN were measured by a high-performance liquid chromatographer (Dionex Ultimate 3000, Thermo Fisher Scientific, USA) equipped with an octadecylsilylated silica (ODS) gel column (Acclaim120 C18; 150 mm × 4.6 mm, 3 μm; Thermo Fisher Scientific, USA) after procedure of pre-column derivatization with ortho-phthaldialdehyde (OPA). The mobile phase A was consisted of 50% methanol and 50% water and used to clean the column. The mobile phase B (pH 5.3) was consisted of

Table 1 Description of sampling sites and soil properties for testing.

Climate	Land use	Latitude	Longitude	pH	SOC ($g\ kg^{-1}$)	TN ($g\ kg^{-1}$)	TP ($g\ kg^{-1}$)
Subtropical	Forest	23.17°N	112.53°E	3.82±0.02	38.45±0.11	2.84±0.39	0.30±0.01
	Grassland	21.84°N	111.41°E	4.83±0.04	37.66±0.36	4.02±0.41	0.30±0.02
	Arable land	23.10°N	113.21°E	5.67±0.06	33.08±0.34	3.40±0.48	1.87±0.10
Temperate	Forest	42.23°N	128.05°E	5.82±0.02	98.11±0.72	6.83±1.04	0.96±0.04
	Grassland	49.19°N	119.55°E	6.29±0.01	34.50±0.29	3.61±0.45	0.70±0.07
	Arable land	47.27°N	126.55°E	6.15±0.01	27.52±0.55	1.92±0.53	1.15±0.10

Note: Data was presented as mean±standard error (n = 5). SOC, soil organic carbon content; TN, soil total nitrogen content; TP, soil total phosphorus content.

sodium citrate solution, methanol, and tetrahydrofuran at a ratio of 95:2:3 in volume and used as a carrier solution. The sodium citrate solution was prepared by dissolving 2.941 g sodium citrate and 0.3281 g sodium acetate into 800 mL deionized water, adjusted to pH 5.3 with 6 M HCl, and diluted to 1000 mL ultra-pure water. The amino sugar separation procedure contained a gradient change of the volume ratio of mobile phase A and B in each run. The mobile phase A and B entered the column at a flow rate of 1.5 mL min^{-1}, with a volume ratio of 5/95 (v/v) in the first 19 min. Then linearly changing to 80/20 (v/v) within 2 min and hold 3 min. Subsequently, a reverse gradient to the initial volume ratio of 5/95 (v/v) within 1 min and remained until the end of the run. The emission wavelength was 445 nm and the excitation wavelength was 330 nm (Indorf et al., 2011). The individual amino sugars (GluN, GalN and MurN) were identified and quantified according to the chromatograms of standard solutions containing mixed amino sugars. The concentrations of individual and total amino sugars were calculated as mg kg^{-1} dry soil.

2.4 Statistics

Before statistical analysis, the data were subject to the Shapiro-Wilk test for normality and the Levene test for homogeneity of variance. When data were not normally distributed, logarithmic or square-root transformation was performed. One-way analysis of variance (ANOVA) with Tukey's *post-hoc* tests was used to test the effects of soil storage conditions (field-moist, freeze-dried, and air-dried) and grinding (2-mm, 0.25-mm, and 0.15-mm) on amino sugar extraction. Nested ANOVA model was used to examine how variance in soil amino sugar concentration can be explained by climate, land use type and sample pretreatment. All statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, USA) and statistical significance was determined at $P<0.05$ level. The figures were plotted using GraphPad Prism 8.0 (GraphPad software, Inc., USA).

3 Results

3.1 Effect of soil storage condition on amino sugar extraction

The effects of sample storage condition were highly dependent on climatic conditions and land use types (Fig. 1). Soil storage conditions including field-moist, freeze-dried and air-dried did not show any significant effects on the extraction and concentrations of individual and total amino sugars in the soils from subtropical forest and arable land, and temperate grassland (Fig. 1, $P>0.05$). However, field-moist samples were found to be favorable for improving the extraction efficiency of amino sugars in the soils from the subtropical grassland, temperate forest and arable land, with the

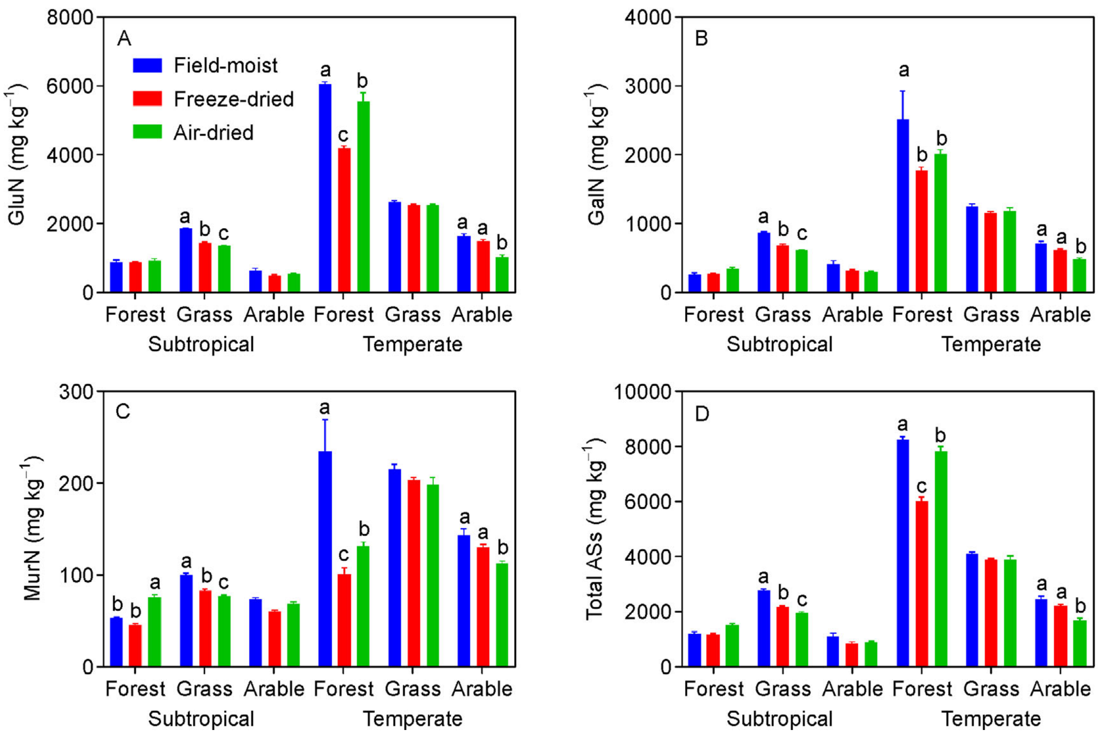

Fig. 1 Concentrations of individual and total amino sugars extracted from the field-moist, freeze-dried, and air-dried soil samples. Bars indicated mean±standard error (n = 5). Columns with different letters indicated significant differences among sample pretreatments ($P<0.05$) within the same land use type. GluN, glucosamine; GalN, galactosamine; MurN, muramic acid; Total ASs, total amino sugars.

concentrations of GluN, GalN, MurN, and total amino sugars much higher in the field-moist samples than in the dried samples (Fig. 1).

3.2 Effect of grinding on amino sugar extraction

Similar to soil storage condition, the effects of sample grinding was also dependent on climatic conditions and land use types (Fig. 2). For the samples from subtropical forest and temperate arable land, air-dried soils ground to 0.15-mm obtained higher concentrations of GluN, GalN, MurN and total amino sugars compared with other grinding pretreatments (Fig. 2, $P<0.05$). However, samples ground to 0.25-mm obtained significantly higher amino sugar concentrations in comparison with 2-mm and 0.15-mm grinding in the temperate grassland (Fig. 2, $P<0.05$). For the samples from subtropical grassland and arable land, and temperate forest, soils ground to 2-mm and 0.25-mm could obtain satisfied extraction efficiency of amino sugars with exception of MurN in the temperate forest (Fig. 2).

3.3 Effect of climate, land use type, and sample pretreatment on amino sugar concentration

Analysis of climate, land use and sample pretreatment partitioning of effect on the concentration of soil amino sugars indicated that land use explained more than 50% of the variation in the concentrations of GluN (59.30%), GalN (54.34%), and total amino sugars (56.31%) (Fig. 3). Climate played secondly strongest role in explaining the variation in soil amino sugars: GluN (38.25%), GalN (42.60%), and total amino sugars (40.83%) (Fig. 3). Compared with land use and climate, sample pretreatment explained relative small amount of variation in soil amino sugars: GluN (2.44%), GalN (3.66%), and total amino sugars (2.67%) in the contrast (Fig. 3). Interestingly, the variation in the concentration of MurN was mainly explained by climate (71.04%), followed by land use (23.75%) and sample pretreatment (5.21%) (Fig. 3).

4 Discussion

Air-drying and freeze-drying are common practices for soil storage before soil physiochemical analysis and fresh soils are preferred for soil biochemical and microbiological analysis (Stenberg et al., 1998). However, for practical reason, fresh soils are not always possible and it is necessary to find a satisfactory soil storage technique and assess the potential impacts of soil storage conditions on the measured soil properties. Different studies have showed that soil storage conditions prior to the analysis might affect the estimation of soil abiotic and biotic properties due to the altered environment when soil samples are collected and stored (Mondini et

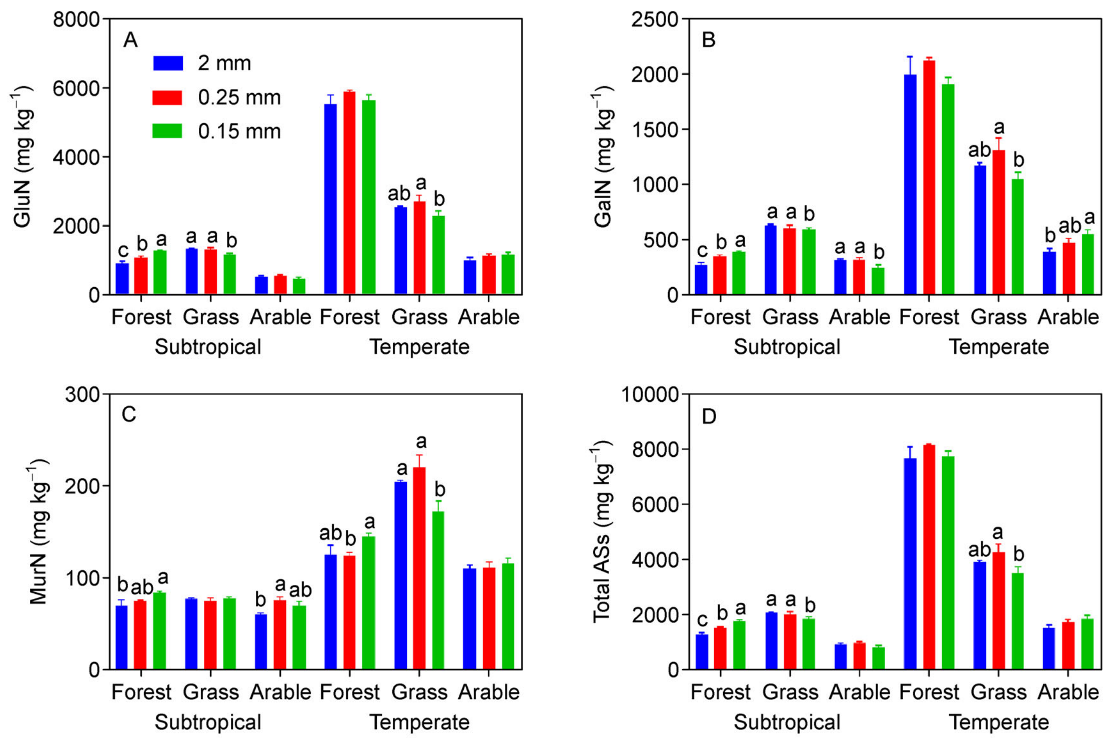

Fig. 2 Concentrations of individual and total amino sugars extracted from air-dried soils ground to 2-mm, 0.25-mm, and 0.15-mm sieves. Bars indicated mean±standard errors (*n* = 5). Columns with different letters indicated significant differences among different grinding size ($P<0.05$) in the same land use type. GluN, glucosamine; GalN, galactosamine; MurN, muramic acid; Total ASs, total amino sugars.

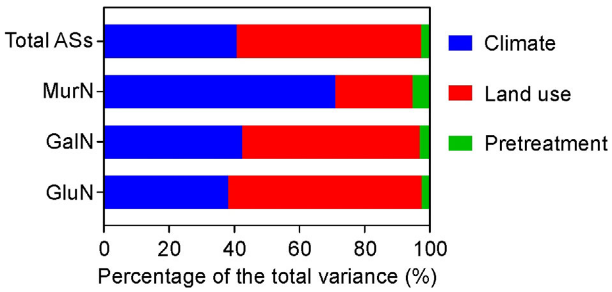

Fig. 3 Partitioning of the variance for soil amino sugars into climate, land use type and sample pretreatment.

al., 2002; De Nobili et al., 2006), which may be decisive for the results (Stenberg et al., 1998). For example, Deacon et al. (2008) suggested that freeze-drying was an appropriate way to minimize disruption of soil physical properties and community structure comparing to the field-moist soils, whereas β-glucosidase activities were reported to reduce by more than half in freeze-drying soils (Yoshikura et al., 1980). Most of the previous studies have been focused on the effects of soil storage methods on soil enzyme activities (Andres Abellan et al., 2011) and microbial community composition (Trabue et al., 2006; Zornoza et al., 2006), and less attention was paid on their effects on the estimation of soil microbial residues. In this study, we found that the concentrations of amino sugars extracted from freeze-dried and air-dried soils were significantly lower than those from the field-moist soils in subtropical grasslands, temperate forests and arable lands. The highest concentrations of amino sugars in field-moist soils were attributed to their integrated soil physicochemical and biological properties which can well indicate their real status in the nature (Deacon et al., 2008). Furthermore, plant residues containing a substantial amount of amino sugars may be hard to remove from the field-moist soils, which might overestimate the concentrations of amino sugar in soils (Appuhn et al., 2004). On the contrary, the reduced concentrations of amino sugars in dried samples can be explained by the altered environment and microbial metabolism during drying processes. Soil organo-mineral complexes (Peltovuori and Soinne, 2005) and soil pore structure (Deacon et al., 2008) were significantly altered by drying procedure, which may result in changes of amino sugar contents in soils. Besides, soil microbial activity still exists during the air-drying procedure, which may cause the depletion of amino sugars by microbial respiration (Mimmo et al., 2008). It should be pointed out that although dried samples result in lower extraction of amino sugars, the pattern of amino sugars among land use types and climate region was also most the same as those indicated by field-moist samples. Therefore, air-dried and freeze-dried samples can provide satisfactory information about the effects of land use and climate on soil microbial residues.

Although field-moist soils were recommended to obtain higher extraction efficiency of amino sugars, freeze-dried and air-dried samples were also satisfactory for amino sugar extraction in the subtropical forest and arable land, and temperate grassland (Fig. 1). Moreover, air-dried soils ground to different mesh sizes were commonly used in the previous studies (Indorf et al., 2011; Liu et al., 2019; Shao et al., 2019), we therefore investigated the influence of grinding sizes of air-dried soils on amino sugar extraction. In contrary to the studies claiming that amino sugars were mainly concentrated in finer fractions of soils (Amelung et al., 2002), we did not observe a similar trend of the effect of grinding on the amino sugar extraction. However, similar to the research reported by Ding et al. (2017), our results showed that the effect of grinding on amino sugar extraction was strongly influenced by land use types. Samples ground to 0.15 mm in the subtropical forests and samples ground to 0.25 mm in subtropical and temperate grasslands can obtain relative higher concentrations of amino sugars without significant differences observed in subtropical arable lands, temperate forests and arable lands (Fig. 2). So grinding to finer size show some potential to improve the extraction efficiency of amino sugars and can be adopted for the sample process in above region.

Since soils were sampled across different climatic conditions (subtropical and temperate) and land use types (forest, grassland and arable land) in this study (Table 1), we further investigated the effects of climate, land use types and sample treatment on the concentrations of amino sugars. Our study showed that the concentrations of individual and total amino sugars were significantly affected by climate, land use system and sample treatment (Fig. 1). These results were consistent with Khan et al. (2016), who found that the contents of amino sugars were significantly varied with land use types and climatic conditions. Besides, based on an investigation in temperate grasslands across different mean annual temperature (MAT) and mean annual precipitation (MAP), Amelung et al. (1999) also found a significant effect of climate on amino sugar concentrations. In addition, Ding et al. (2017) also reported that amino sugar accumulation was significantly impacted by land use types. Interestingly, we found that the effect of climate and land use types varied with the identity of biomarkers, with GluN, GalN, and total amino sugars were dominantly affected by land use types and MurN mainly influenced by climate (Fig. 3). Overall, the effects of sample process on soil amino sugars were minimal compared with climate and land use types.

5 Conclusions

In this study, six soils from the forests, grasslands and arable lands under subtropical and temperate region were sampled to investigate the effects of sample storage method and grinding size on amino sugar extraction. We found that soil pretreatments exerted significant effects on the extraction of amino sugars, and the impacts were highly dependent on climate condition and land use types. Field-moist samples were satisfactory for amino sugar extraction for all soil types, while soil drying techniques including freeze-drying and air-drying significantly reduced the concentrations of amino sugars in subtropical grassland, temperate forest and arable soils. Air-dried soil ground to 0.15-mm was recommended for amino sugar extraction in subtropical forest soil, while air-dried subtropical arable land and temperate grassland soils ground to 0.25-mm increased the extraction efficiency of amino sugars. Our results suggested that the selection of sample storage method and grinding size should consider the context information (climate and land use) of soil samples. Such information will be valuable for reducing the uncertainty and improving the accuracy during the determination of soil amino sugars.

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Disclosure statement

The authors declare no conflict of interest.

References

Amelung, W., Lobe, I., Preez, C.C.D., 2002. Fate of microbial residues in sandy soils of the South African Highveld as influenced by prolonged arable cropping. European Journal of Soil Science 53, 29–35.

Amelung, W., Miltner, A., Zhang, X., Zech, W., 2001. Fate of microbial residues during litter decomposition as affected by minerals. Soil Science 166, 598–606.

Amelung, W., Zhang, X., Flach, K.W., Zech, W., 1999. Amino sugars in native grassland soils along a climosequence in North America. Soil Science Society of America Journal 63, 86–92.

Andres Abellan, M., Wic Baena, C., Garcia Morote, F.A., Picazo Cordoba, M.I., Candel Perez, D., Lucas-Borja, M.E., 2011. Influence of the soil storage method on soil enzymatic activities in Mediterranean forest soils. Forest Systems 20, 379–388.

Appuhn, A., Joergensen, R.G., 2006. Microbial colonization of roots as a function of plant species. Soil Biology & Biochemistry 38, 1040–1051.

Appuhn, A., Joergensen, R.G., Raubuch, M., Scheller, E., Wilke, B., 2004. The automated determination of glucosamine, galactosamine, muramic acid, and mannosamine in soil and root hydrolysates by HPLC. Journal of Plant Nutrition and Soil Science 167, 17–21.

De Nobili, M., Contin, M., Brookes, P.C., 2006. Microbial biomass dynamics in recently air dried and rewetted soils compared to others stored air-dry for up to 103 years. Soil Biology & Biochemistry 38, 2871–2881.

Deacon, L.J., Grinev, D.V., Crawford, J.W., Harris, J., Ritz, K., Young, I.M., 2008. Simultaneous preservation of soil structural properties and phospholipid profiles: A comparison of three drying techniques. Pedosphere 18, 284–287.

Ding, X., Qiao, Y., Filley, T., Wang, H., Lü, X., Zhang, B., Wang, J., 2017. Long-term changes in land use impact the accumulation of microbial residues in the particle-size fractions of a Mollisol. Biology and Fertility of Soils 53, 281–286.

Indorf, C., Dyckmans, J., Khan, K.S., Joergensen, R.G., 2011. Optimization of amino sugar quantification by HPLC in soil and plant hydrolysates. Biology and Fertility of Soils 47, 387–396.

Joergensen, R.G., 2018. Amino sugars as specific indices for fungal and bacterial residues in soil. Biology and Fertility of Soils 54, 559–568.

Joergensen, R.G., Meyer, B., 1990. Chemical change in organic matter decomposing in and on a forest Rendzina under beech (*Fagus sylvatica* L.). Journal of Soil Science 41, 17–27.

Khan, K.S., Mack, R., Castillo, X., Kaiser, M., Joergensen, R.G., 2016. Microbial biomass, fungal and bacterial residues, and their relationships to the soil organic matter C/N/P/S ratios. Geoderma 271, 115–123.

Lauer, F., Kösters, R., du Preez, C.C., Amelung, W., 2011. Microbial residues as indicators of soil restoration in South African secondary pastures. Soil Biology & Biochemistry 43, 787–794.

Liu, X., Zhou, F., Hu, G., Shao, S., He, H., Zhang, W., Zhang, X., Li, L., 2019. Dynamic contribution of microbial residues to soil organic matter accumulation influenced by maize straw mulching. Geoderma 333, 35–42.

Mimmo, T., Ghizzi, M., Marzadori, C., Gessa, C.E., 2008. Organic acid extraction from rhizosphere soil: effect of field-moist, dried and frozen samples. Plant and Soil 312, 175–184.

Mondini, C., Contin, M., Leita, L., De Nobili, M., 2002. Response of microbial to air-drying and rewetting in soils and compost. Geoderma 105, 111–124.

Moritz, L.K., Liang, C., Wagai, R., Kitayama, K., Balser, T.C., 2008. Vertical distribution and pools of microbial residues in tropical forest soils formed from distinct parent materials. Biogeochemistry 92, 83–94.

Murugan, R., Djukic, I., Keiblinger, K., Zehetner, F., Bierbaumer, M., Zechmeister-Bolternstern, S., Joergernsen, R.G., 2019. Spatial distribution of microbial biomass and residues across soil aggregate fractions at different elevations in the Central Austrian Alps. Geoderma 339, 1–8.

Peltovuori, T., Soinne, H., 2005. Phosphorus solubility and sorption in frozen, air-dried and field-moist soil. European Journal of Soil Science 56, 821–826.

Roberts, P., Jones, D.L., 2012. Microbial and plant uptake of free amino sugars in grassland soils. Soil Biology & Biochemistry 49, 139–149.

Schulten, H.R., Schnitzer, M., 1997. The chemistry of soil organic nitrogen: a review. Biology and Fertility of Soils 26, 1–15.

Shao, P., Liang, C., Lynch, L., Xie, H., Bao, X., 2019. Reforestation accelerates soil organic carbon accumulation: Evidence from microbial biomarkers. Soil Biology & Biochemistry 131, 182–190.

Shao, S., Zhao, Y., Zhang, W., Hu, G., Xie, H., Yan, J., Han, S., He, H., Zhang, X., 2017. Linkage of microbial residue dynamics with soil organic carbon accumulation during subtropical forest succession. Soil Biology & Biochemistry 114, 114–120.

Stenberg, B., Johansson, M., Pell, M., Sjodahl, S., Stenstrom, J., Torstensson, L., 1998. Microbial biomass and activities in soil as affected by frozen and cold storage. Soil Biology & Biochemistry 3, 393–402.

Trabue, S.L., Palmquist, D.E., Lydick, T.M., Singles, S.K., 2006. Effects of soil storage on the microbial community and degradation of metsulfuron-methyl. Journal of Agricultural and Food Chemistry 54, 142–151.

Yang, J., Ma, X., Tang, G., Wang, Z., Xu, Y., 2015. Effects of particle size and leaching solution concentration on the analytical methods of soil dissolved organic carbon. Xinjiang Agricultural Sciences 52, 151–156. (in Chinese)

Yoshikura, J., Hayano, K., Tsuru, S., 1980. Effects of drying and preservation on Beta-Glucosidases in soil. Soil Science and Plant Nutrition 26, 37–42.

Zhang, X., Amelung, W., 1996. Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biology & Biochemistry 28, 1201–1206.

Zornoza, R., Guerrero, C., Mataix, S.J., Arcenegui, V., García, F., Mataix, B.J., 2006. Assessing air-drying and rewetting pre-treatment effect on some enzyme activities under Mediterranean conditions. Soil Biology & Biochemistry 38, 2125–2134.