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POSITION AND OPINION PAPER

Amino sugars as specific indices for fungal and bacterial residues in soil

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Abstract

Amino sugars are important indices for the contribution of soil microorganisms to soil organic matter. Consequently, the past decade has seen a great increase in the number of studies measuring amino sugars. However, some uncertainties remain in the interpretation of amino sugar data. The objective of the current opinion paper is to summarize current knowledge on amino sugars in soils, to give some advice for future research objectives, and to make a plea for the correct use of information. The study gives an overview on the origin of muramic acid (MurN), glucosamine (GlcN), galactosamine (GalN), and mannosamine (ManN). Information is also provided on measuring total amino sugars in soil but also on compound-specific $\delta^{13}C$ and $\delta^{15}N$ determination. Special attention is given to the turnover of microbial cell-wall residues, to the interpretation of the GlcN/GalN ratio, and to the reasons for converting fungal GlcN and MurN to microbial residue C. There is no evidence to suggest that the turnover of fungal residues generally differs from that of bacterial residues. On average, MurN contributes 7% to total amino sugars in soil, GlcN 60%, GalN 30%, and ManN 4%. MurN is highly specific for bacteria, GlcN for fungi if corrected for the contribution of bacterial GlcN, whereas GalN and ManN are unspecific microbial markers.

Keywords Microbial residues · Microbial biomass · Fungi · Bacteria · Cell walls · EPS

Introduction

As plants do not produce amino sugars (Parsons 1981), their accumulation in soil indicates the contribution of microbial residues to SOC (Liang et al. 2007; Joergensen and Wichern 2008; Bai et al. 2017). Microbial residues comprise non-biomass microbial metabolites such as exo-enzymes, extracellular polymeric substances (EPS) and dead cell remains (Joergensen and Wichern 2018). As a characteristic component of microbial residues, amino sugars in soil integrate environmental effects on microbial community composition over time (Glaser et al. 2004; Khan et al. 2016). Only a minor percentage of amino sugars in soil is still part of the microbial biomass. This is different in freshly colonized plant material, such as excised roots (Appuhn and Joergensen 2006), straw and crop residues (Liang et al. 2007; Indorf et al. 2011), leaf litter (Tremblay and Benner 2006), feces (Jost et al. 2011) and probably also particulate organic matter (POM) (Six et al. 2006; Potthoff et al. 2008). In these partly decomposed materials, bacterial muramic acid (MurN) and fungal glucosamine (GlcN) are also useful indicators of bacterial and fungal biomass.

Up to 26 amino sugars have been identified in microorganisms, but only GlcN, galactosamine (GalN), MurN, and mannosamine (ManN) have been commonly quantified in soil (Amelung 2001), contributing 5–12% to soil total N (Stevenson 1982) and 2–5% to SOC (Joergensen and Meyer 1990). This means that amino sugar contents in soil are relatively high in comparison with other microbial components, such as PLFA (Joergensen and Wichern 2008). In addition, amino sugar extraction and determination is not seriously hampered by methodological constraints (Joergensen and Wichern 2008). For these reasons, a considerable increase in the number of studies measuring amino sugars has been observed over the past decade in comparison with the previous decade.

However, some uncertainties in interpretation have remained, making it difficult to gain the full and current informational value from amino sugar data. Some uncertainties are linked to true restrictions in knowledge, and others are due to an incomplete reading or understanding of published information. This is especially a problem for

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GalN, but to some extent also for GlcN and ManN. Similar problems have been reported for soil enzyme activities (Nannipieri et al. 2018), underlining the possibility that this may be a general problem in soil biology and biochemistry. The objective of the current opinion paper is to summarize the current knowledge on amino sugars in soils, to give some advice for future research objectives, and to make a plea for the correct use of information in times in which fake news has become increasingly fashionable.

Origin and occurrence of amino sugars

Muramic acid

MurN occurs exclusively in the murein layers of bacterial cell walls (Table 1). This peptidoglycan sacculus is a unique and essential structural element of almost all bacteria (Vollmer et al. 2008). MurN contributes approximately 30% to the murein of Gram-positive and Gram-negative bacteria. According to Appuhn and Joergensen (2006), cultured Gram-positive species contain on average 13.9 mg MurN g^{-1} dry mass (95% confidence limits 12.9–21.1 mg g^{-1} dry mass) and Gram-negative only 3.7 mg g^{-1} dry mass (95% confidence limits 3.6–4.7 mg g^{-1} dry mass). Within these two groups, especially in the Gram-negative bacteria, the MurN concentration is relatively constant. However, starving soil bacteria may contain higher MurN concentrations than cultured bacteria, due to their smaller cell size.

There is no evidence that MurN occurs in microbial components other than murein, e.g., in bacterial EPS (Lee et al. 2015; Squillaci et al. 2016) or in teichoic acids of Gram-positive bacteria (Mikusová et al. 1996). MurN does not occur in the pseudo-murein of archaea (Kandler and König 1998) or the chitin of fungi (Parsons 1981). However, MurN is present in the photosynthetic organelle envelopes (chloroplasts) of the protist *Cyanophora paradoxa* as a relict of their cyanobacterial ancestors (Pfanzagl et al. 1996). However, this freshwater alga usually does not occur in soil. Consequently, MurN is the most specific biomarker for bacteria in soil, contributing between 3 and 16% to the amino sugar content (Table 2 and Fig. 1).

Glucosamine

GlcN contributes between 47 and 68% to amino sugars in soil (Table 2 and Fig. 1) and between 58 and 93% to amino sugars in plant material (Table 3). Cell walls of higher fungi are the major source of GlcN in soil (Parsons 1981). The main component of these cell walls is chitin, a polymer formed by *N*-acetyl-GlcN. The contribution of chitin from the exoskeleton of micro-arthropods to the GlcN content of soils is negligible. Their biomass (Schaefer and Schauermann 1990) and especially their chitin content (Finke 2007) are much lower than that of fungi, even in forest soils with large micro-arthropod communities. Macro-arthropods, e.g., termites, millipedes, isopods, and beetles, are usually removed by sieving < 2 mm during sample processing.

Table 1 Occurrence, dominant polymers, and remarks on the specificity of amino sugars

	Occurrence	Polymer	Remarks
MurN	Bacterial cell walls	Murein	Specific for bacteria
GlcN	Fungal cell walls and Fungal EPS	Chitin	Specific for fungi after correcting for bacterial GlcN
	Bacterial cell walls	Murein	MurN/GlcN (mol/mol) = 1/2[a]
	Gram-positive bacteria	Teichoic acids	Included[a]
	Bacterial EPS		Included[a]
	Archaeal cell walls	Pseudo-murein	Negligible in soil
	Invertebrate exoskeleton	Chitin	Negligible in soil
GalN	Bacterial EPS		Unspecific
	Fungal EPS		Unspecific
	Fungal cell walls	Galactosaminogalactan	Traces
	Archaeal EPS	Pseudo-murein	Negligible in soil
	Archaeal cell walls		Negligible in soil
ManN	Bacterial EPS		Unspecific
	Gram-positive bacteria	Teichoic acids	Gram-positive bacteria
	Fungal EPS		Unspecific

aThis conversion value includes GlcN derived from bacterial EPS and Gram-positive bacterial teichoic acid; in pure murein, the MurN/GlcN ratio is 1 (mol/mol)

Table 2 Concentrations of amino sugars in different arable and forest soils observed in the last decade

Sample	Comment	GlcN	Fungal GlcN	GalN	ManN	MurN	Source	Method
		[μg g^{-1} DW] (% total amino sugars)						
Sediment (0–24 cm)	Site 29MC	1330 (47)	1030 (42)	1000 (42)	200 (8)	73 (3)	Niggemann and Schubert (2006)	GC
Arable soil (0–15 cm)	Saline, MIN	127 (54)	88 (37)	83 (35)	0 (0)[a]	27 (11)	Khan et al. (2016)	HPLC
Arable soil (0–10 cm)	Irrigated soil, MIN	102 (68)	68 (45)	25 (17)	0 (0)[a]	24 (16)	Sradnick et al. (2014a)	HPLC
Arable soil (0–10 cm)	MIN	1180 (58)	980 (48)	690 (34)	34 (2)	140 (7)	Indorf et al. (2011)	HPLC
Arable soil (0–5 cm)	MIN	660 (62)	600 (56)	210 (20)	150 (14)[a]	45 (4)	Joergensen et al. (2010)	HPLC
Arable soil (0–25 cm)	MIN	490 (61)	450 (57)	270 (34)	11 (1)[a]	27 (3)	Sradnick et al. (2014b)	HPLC
Arable soil (0–25 cm)	FYM + BD	510 (59)	450 (53)	300 (35)	13 (2)[a]	40 (5)	Sradnick et al. (2014b)	HPLC
Arable soil (0-5 cm)	FYM + BD	780 (59)	680 (52)	300 (23)	170 (13)[a]	68 (5)	Joergensen et al. (2010)	HPLC
Arable (0–20 cm)	Plowed	420 (62)	310 (46)	164 (24)	11 (2)	79 (12)	Ding et al. (2011)	GC
Arable soil (0–20 cm)	No tillage, rotation	730 (64)	570 (51)	280 (25)	15 (1)	110 (10)	Ding et al. (2011)	GC
Forest (0–15 cm)	Sedimentary	500 (62)	410 (51)	230 (28)	20 (3)	62 (8)	Moritz et al. (2009)	GC
Forest (0–15 cm)	Ultrabasic	440 (65)	350 (51)	170 (25)	1 (0)	65 (10)	Moritz et al. (2009)	GC
Forest (0–10 cm)	Beech	2730 (55)	2430 (49)	1840 (37)	160 (3)	210 (4)	Indorf et al. (2011)	HPLC
Forest (0–10 cm)	Beech	2640 (60)	2440 (56)	1550 (35)	49 (1)	140 (3)	Indorf et al. (2011)	HPLC
Mean		(60)	(50)	(30)	(4)	(7)		

MIN inorganic fertilizer, *FYM* farmyard manure, *BD* biodynamic preparations

[a] Not published in the respective reference

More important is the contribution of bacterial cell-wall murein to the GlcN content of soil (Appuhn and Joergensen 2006). However, bacterial GlcN can be simply estimated (Engelking et al. 2007), assuming that MurN and GlcN occur at a molar 1-to-2 ratio in bacteria. Then, fungal GlcN can be calculated using the following formula: μg fungal C g^{-1} soil = ((μg GlcN g^{-1} soil / 179.17) − (2 × μg MurN g^{-1} soil / 253.23)) × 179.17 (Faust et al. 2017), where 179.17 is the molecular weight of GlcN and 253.23 that of MurN. This calculation indicates that 17% of total GlcN is on average of bacterial origin (Table 2 and Fig. 1), and as much as 31% in a saline soil with a high presence of bacterial residues (Khan et al. 2016).

It has been observed that the molar concentration of bacterial GlcN exceeds the theoretical 1-to-1 ratio for bacterial murein (Engelking et al. 2007). This indicates that some bacterial GlcN is present in other components, e.g., in bacterial EPS outside the cell walls (Lin et al. 2012) or in teichoic acids of Gram-positive bacteria (Mikusová et al. 1996). Teichoic acids contain GlcN linked by phosphate esters to a variety of glycolipids, which are covalently bonded to the MurN of murein layers (Kojima et al. 1985; Mikusová et al. 1996). In addition, the cell-wall peptidoglycan, i.e., pseudo-murein (Niemetz et al. 1997; Kandler and König 1998) and EPS (Squillaci et al. 2016) of archaea contain GlcN. However, the contribution of archaea to the soil microbial biomass is most likely negligible (Joergensen and Wichern 2008). The same is most likely true for the contribution of GlcN derived from the photosynthetic organelle envelopes of algae on the soil surface (Yamagishi et al. 2015).

Galactosamine

GalN is the second most common amino sugar in soil (Amelung 2001) and plant material (Liang et al. 2007). GalN contributes between 17 and 42% to amino sugars in soil (Table 2 and Fig. 1) and between 2 and 42% to amino sugars in plant material (Table 2 and Fig. 1). However, only 4% of the total amino sugar concentration in bacterial and 15% in fungal cultures consists of GalN (Engelking et al. 2007), i.e., considerably less than in soil. GalN is usually not a cell-wall component of bacteria, especially

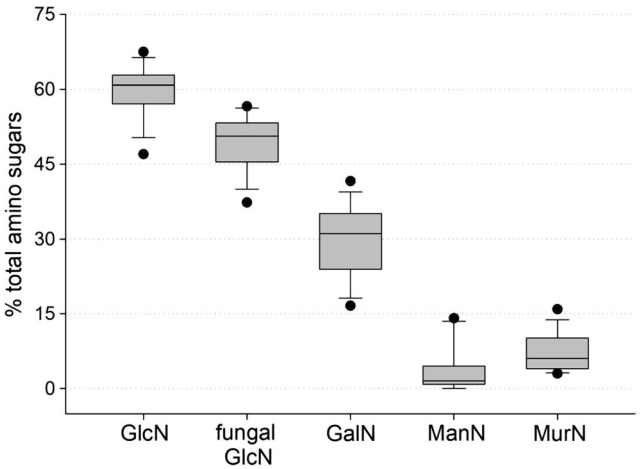

Fig. 1 Contribution of GlcN, fungal GlcN, GalN, ManN, and MurN to total amino sugars in different arable and forest soils observed in the last decade

not of actinobacteria (Glaser et al. 2004). Traces of GalN have been found in fungal cell walls (Bardalaye and Nordin 1976) and are also present in the cell walls of some rare archaeal species (Niemetz et al. 1997).

It is possible that GalN is an important component of EPS in soil, which has been repeatedly stated (Sradnick et al. 2014a; Redmile-Gordon et al. 2014), but not yet proven for soil. Soil EPS has similar functions to mucins, exuded, e.g., in the ileum of many vertebrate animals, i.e., protecting the function of exo-enzymes. These mucins are a family of high-molecular-weight, heavily glycosylated and glycol-conjugated proteins, containing high concentrations of GalN (Macfarlane et al. 2005). Similar mucous substances, such as EPS and capsular polysaccharides, but also lipopolysaccharides attached to microbial cell walls are likely the dominating source of GalN in soil (Lee et al. 2015; Squillaci et al. 2016). The extraction method of Redmile-Gordon et al. (2014) gives the unique possibility to investigate directly the contribution of amino sugars, especially GalN and ManN, to freshly formed EPS, using stable isotopes, such as ^{15}N (Liu et al. 2016) or ^{13}C (Dippold et al. 2014). If successful, this would allow a meaningful ecological interpretation of GalN concentration, considering its high contribution to organic N components.

Mannosamine

Guggenberger et al. (1999) found significant ManN amounts of microbial origin in different soils. However, ManN is unspecific for a certain microbial group, as bacteria and fungi both produce ManN (Glaser et al. 2004) similar as GalN (Table 1). In fungi, ManN has been detected as a component of sialic acids, e.g., on the conidial surface of *Aspergillus fumigatus* (Wasylnka et al. 2001). In bacteria, ManN has not only been found in sialic acids, but also in protective capsular components of Gram-negative bacteria (Lewis et al. 2016) or in teichoic acids (Kojima et al. 1985) and in EPS of Gram-positive bacteria (Patten et al. 2014). MurN contributes on average 4% to amino sugars in soil (Table 2 and Fig. 1) and between 0 and 23% to amino sugars in plant material (Table 3). Due to strong variation, low concentration, and low specificity, ManN data have not been presented in many recent studies (Sradnick et al. 2014ab; Bai et al. 2017; Peltre et al. 2017; Zhang et al. 2017).

Amino sugar determination

Hydrolysis

The basis of most amino sugar analysis used today is the hydrolysis of samples with 6 M HCl for 3 to 8 h, depending on the resistance to hydrolysis (Zhang and Amelung 1996; Appuhn et al. 2004). According to Appuhn et al. (2004), 500 mg samples are mixed with 10 ml 6 M HCl and heated for 6 h (soil) or 3 h (plant material) at 105 °C. For high-performance liquid chromatography (HPLC) analysis, a 0.5 to 1 ml aliquot from the filtered hydrolysate was evaporated at 40 °C to dryness. Then, the sample was rinsed with 0.5 ml water that was evaporated again. Finally, the residue was taken up again in 1 ml water and centrifuged at 5000×*g*. The supernatant was transferred to vials and frozen at − 18 °C before the HPLC measurements (Appuhn et al. 2004). For GC analysis, the hydrolysate was filtered, purified, neutralized, and freeze-dried (Zhang and Amelung 1996).

High-performance liquid chromatography

In most cases, reversed-phase HPLC has been used combined with derivatization of the amino sugars (Appuhn et al. 2004). A typical approach is pre-column derivatization with, for example, 9-fluorenylmethyl-chloroformate (FMOC-Cl) (Ekblad and Näsholm 1996) or ortho-phthaldialdehyde (OPA) (Zelles 1988), followed by fluorescence detection. OPA derivatization can be automatically carried out by the HPLC system (Appuhn et al. 2004; Indorf et al. 2011). Another possibility is post-column derivatization after high-performance cation exchange chromatography (HPCEC) with ninhydrin (Joergensen and Meyer 1990) or after high-performance anion-exchange chromatography (HPAEC) with OPA (Indorf et al. 2013).

Some HPLC techniques for determining amino sugars in hydrolysates do not require derivatization. Examples are HPAEC combined with amperometric (Indorf et al. 2013) and isotope-ratio mass-spectrometric (IRMS) detection (Dippold et al. 2014). The same is true for reversed-phase zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC), combined with electrospray ionization and tandem mass-spectrometric (ESI-MS/MS) detection (Olofsson and Bylund 2016) or ultra-high-performance liquid chromatography combined with high-resolution mass-spectrometric (UPLC/HRMS) detection (Hu et al. 2017).

Gas chromatography

GC analysis of amino sugars in soil is usually carried out according to Zhang and Amelung (1996) as presented by Liang et al. (2012). After hydrolysis, methanol was added to remove amino sugars from the freeze-dried hydrolysates and freeze-dried again. Finally, the purified amino sugars were converted into aldononitrile derivatives and extracted with dichloromethane from the aqueous solution. The amino sugar derivatives were detected on a GC system equipped with a fused silica column (30 m × 0.25 mm × 0.25 μm) and flame ionization detector (FID).

Table 3 Concentrations of amino sugars in different plant and other organic material observed in the last decade

Sample	Fungal					Source	Method
	GlcN	GlcN	GalN	ManN	MurN		
	[μg g^{-1} DW] (% total amino sugars)						
Shiitake (*Lentinula edodes*)	1240 (93)	1240 (93)	20 (2)[a]	64 (5)[a]	3 (0)[a]	Indorf et al. (2012)	HPLC
Oyster mushroom (*Pleurotus ostreatus*)	1210 (93)	1210 (93)	21 (2)[a]	64 (5)[a]	3 (0)[a]	Indorf et al. (2012)	HPLC
Golden oyster mushroom (*P. citrinopileatus*)	1150 (93)	1150 (93)	18 (2)[a]	61 (5)[a]	3 (0)[a]	Indorf et al. (2012)	HPLC
Maize (*Zea mays*) leaves	36 (58)	36 (58)	26 (42)	0 (0)	0 (0)	Indorf et al. (2011)	HPLC
Maize (*Z. mays*) straw	1120 (74)	1060 (70)	190 (13)	155 (10)[a]	43 (3)[a]	Potthoff et al. (2008)	HPLC
Maize (*Z. mays*) stalk	1700 (73)	1340 (57)	193 (8)	200 (9)	250 (11)	Liang et al. (2007)	GC
Amaranth (*Amaranthus caudatus*) straw	1440 (86)	1380 (82)	170 (10)	23 (1)	42 (3)	Indorf et al. (2011)	HPLC
Wheat (*Triticum aestivum*) straw	1290 (93)	1250 (90)	76 (6)	0 (0)	27 (2)	Indorf et al. (2011)	HPLC
Soybean (*Glycine max*)	240 (72)	170 (50)	26 (8)	16 (5)	52 (16)	Liang et al. (2007)	GC
Pea (*Pisum sativum*) leaves	98 (66)	98 (66)	16 (11)	34 (23)	0 (0)	Indorf et al. (2011)	GC
Sugarcane (*Saccharum officinarum*) filter cake	1070 (79)	990 (73)	210 (16)	20 (2)	55 (4)	Indorf et al. (2011)	HPLC
Cow (*Bos primigenius taurus*) feces	2080 (54)	1420 (37)	1270 (33)	76 (2)[a]	460 (12)	Jost et al. (2011)	HPLC

[a] Not published in the respective reference

Stable isotopes

Amino sugar-specific $\delta^{13}C$ and ^{15}N analysis makes it possible to determine the origin of newly formed fungal and bacterial residues after application of highly labeled and ^{13}C and ^{15}N substrates, using gas chromatography (GC) equipped with a quadruple mass spectrometer (He et al. 2006; Liu et al. 2016). GC combustion isotope ratio mass spectrometry (GC-C-IRMS) also makes it possible to measure amino sugar-specific $\delta^{13}C$ in the range of natural abundance (Glaser et al. 2006; Gunina et al. 2017). GC-C-IRMS has not been described for $\delta^{15}N$ analysis in amino sugars until now, although this seems to be possible (Charteris et al. 2016). GC-C-IRMS analysis may cause isotope fractionation of $\delta^{13}C$, due to the derivatization of the amino sugars (Decock et al. 2009). Consequently, high-performance anion-exchange liquid chromatography (HPAE-LC-IRMS) has been developed as a promising alternative for compound-specific $\delta^{13}C$ analysis, since derivatization is not necessary (Bai et al. 2013; Indorf et al. 2012, 2015).

However, recent comparison of GC-C-IRMS and HPAE-LC-IRMS suggested that both systems are reliable techniques for compound-specific ^{13}C analysis, if derivatization is complete and the calibration requirements are met for the GC-C-IRMS method (Moerdijk-Poortvliet et al. 2015). The GC-C-IRMS systems are faster and technically less delicate. HPAE-LC-IRMS systems have the drawback that the mobile phase contains NaOH, which is sensitive to CO_2 contamination and requires a metal-free liquid handling system. HPAE-LC-IRMS systems have the advantages of simpler sample preparation and a more straightforward isotopic calibration (Moerdijk-Poortvliet et al. 2015). Some HPAE-LC-IRMS systems need two separate runs for analyzing MurN on the one hand and GlcN, GalN, and ManN on the other (Indorf et al. 2015), whereas the Dionex ICS-5000 SP ion chromatography system coupled to the LC IsoLink was able to separate all four amino sugars in one run (Dippold et al. 2014). However, the problem remains that the precision of amino sugar separation is still too low in comparison with the large pool size of each amino sugar, although the coefficients of variation for $\delta^{13}C$ values are excellent. This is especially a problem for ManN (Indorf et al. 2015) but also for MurN (Bai et al. 2013).

Turnover of microbial cell-wall residues

In comparison with microbial cell-membrane or cytoplasmic components, cell walls have a slower turnover even within living cells for the following three reasons (Gunina et al. 2017): (1) cell-wall components require a rather complex biosynthesis of amino sugars, (2) cell-wall components polymerize extracellularly, and (3) the synthesis of cell-wall components is not required unless microorganisms multiply. It is commonly held that the turnover of bacterial cell-wall residues is faster than that of fungal cell walls, especially those containing melanin (Guggenberger et al. 1999; Amelung 2001). This view was supported by the observation that microbial residues of a long-term tillage experiment were dominated by fungi, based on amino sugar analysis (Guggenberger et al. 1999), whereas the microbial biomass was dominated by bacteria, based on direct microscopic measurements. However, Guggenberger et al. (1999) used calcofluor white as a stain for fungal biomass, which leads to serious underestimation of fungi as shown by Joergensen and Wichern

(2008). In contrast to this common view, fungal residues responded more dynamically with respect to land-use change from grassland to arable or to bare soil than bacterial residues (Six et al. 2006; Ding et al. 2017). It should always be considered that legacy effects of SOC accumulation in the past may control or mask microbial responses to recent management changes (Khan et al. 2016).

Differences in the turnover of fungal GlcN and bacterial MurN should be reflected by a disproportionate decrease or increase in one of these two amino sugars, comparing their concentration in freshly colonized organic material and aged organic matter or bulk soil. However, these differences were not observed in roots (Appuhn and Joergensen 2006), leaf litter (Tremblay and Benner 2006), and POM (Potthoff et al. 2008). This is in line with the observation that fungal and bacterial residues added to soil were decomposed at similar rates (Jenkinson 1976).

Derrien and Amelung (2011) calculated mean residence times (MRT) for different biomarkers with published data, including amino sugars. They showed that MRT of fungal and bacterial amino sugars are similar with an estimated MRT of 4 years, using a two-successive C pool model. The MRT of GlcN and MurN calculated by Derrien and Amelung (2011) were based on the data of Glaser et al. (2006), who presented MRT values of 6 years for ^{13}C in GlcN and even 90 years for ^{13}C in MurN, using a single-pool approach. Liu et al. (2016) used a first-order one-pool model for calculating MRT values for ^{15}N in total amino sugars. They calculated MRT values of approximately 75 years at 0–10 cm depth and approximately 160 years at 10–20 cm, without detecting significant effects of maize residue application. Liu et al. (2016) suggested different utilization pathways of amino sugar C and N, but erratic results of the one-pool model cannot be excluded. In addition, the pool size of GalN and GlcN is often too large for a significant response of $\delta^{13}C$ values in the natural abundance range, considering the variation between replicate measurements (Indorf et al. 2015).

Additional information on amino sugar turnover can be depicted from long-term field experiments (Joergensen et al. 2010; Gillespie et al. 2014; Faust et al. 2017; Schmidt et al. 2017), from model ecosystems in open top chambers (Griepentrog et al. 2014), and particularly from chrono-sequence studies (Amelung et al. 2002; Roth et al. 2011). Most information on amino sugar turnover can be obtained by combining changes from C3 to C4 vegetation or addition of ^{13}C depleted CO_2 (Glaser et al. 2006; Griepentrog et al. 2014) with compound-specific $\delta^{13}C$ analysis.

Despite the likely general similarity in the turnover of bacterial and fungal residues, changes in the environmental conditions often had specific effects on these two microbial organic matter fractions. Warming and increased atmospheric CO_2, for example, increased specifically the MurN contents and decreased the GlcN/MurN ratio (Liang et al. 2015). In arable cropping systems, long-term fertilization of manure increased MurN contents in soil (Gillespie et al. 2014; Faust et al. 2017; Peltre et al. 2017). This was also true for application of compost and sewage sludge (Peltre et al. 2017). No-tillage shifts the microbial community composition towards fungi, thereby enhancing the accumulation of fungal-derived cell-wall residues (Six et al. 2006; Ding et al. 2011). However, a common source of error in studies dealing with the effects of environmental conditions on amino sugars is that the authors discuss the accumulation or loss of microbial residues merely from changes in contents and not in stocks, ignoring the fact that soil mass matters. Equivalent soil mass corrections significantly affect the calculation of microbial C and N sequestration within their residues (Amelung et al. 2001). Another important source of error is that for example, land-use effects were derived from pseudo-replicates, i.e., comparing several samples taken from one arable, one grassland, and one forest site.

In most studies, a decrease in the GlcN/MurN ratio has been observed with depth (Moritz et al. 2009; Roth et al. 2011; Sradnick et al. 2014b; Banfield et al. 2017), explained by the higher sensitivity of fungi to the lack of fresh plant residues. However, increases in the GlcN/MurN ratio with depth have also been observed (Ding et al. 2017). The GlcN/MurN ratio may reflect depth-specific differences in the turnover of fungal and bacterial residues (Roth et al. 2011). Another possibility is that the GlcN/MurN simply represents the legacy of periods close to the surface during the sedimentation of a profile (Joergensen and Wichern 2018).

Interpretation of the GlcN/GalN ratio

A better understanding of the function of GalN and consequently the processes behind GalN formation in soil during decomposition processes is required. Although the two largest functional microbial groups both produce GalN (Glaser et al. 2004; Engelking et al. 2007), its production has been attributed mainly to bacteria in the past (Cheshire 1979; Parsons 1981). In the 60s and 70s of the last century, only GlcN and GalN could be quantified by HPCEC (Joergensen and Meyer 1990; Amelung 2001). During that period, it was common belief that bacteria dominate the soil microbial biomass and that this must be reflected by the amino sugar composition. As bacteria produce GalN, it was used as an indicator for bacterial residues. This view is still held by some today (Peltre et al. 2017; Schmidt et al. 2017), sometimes supported by correlation analysis (Joergensen et al. 2010), which does not provide any evidence.

In recent papers, GalN was even used as an indicator for fungal biomass (Banfield et al. 2017; Gunina et al.

2017). However, the authors did not provide convincing evidence for their view, which was based on the misinterpretation of changes in $\delta^{13}C$ values (Gunina et al. 2017) or misinterpretation of previously published papers. Wood-decaying fungi, for example, do not produce any GalN during growth (Indorf et al. 2012). Engelking et al. (2007) emphasized that fungi and bacteria both produce GalN, so that this amino sugar can only be used as an unspecific indicator of microbial residues on its own.

It might be possible that a shift in microbial community composition towards fungi leads to a stronger increase in GlcN than in GalN. In this case, the GlcN/GalN ratio must moderately increase, concomitantly with the GlcN/MurN ratio, usually used as a main index for the ratio of fungal to bacterial residues (Amelung et al. 2002; Lauer et al. 2011). Such concomitant increases can be calculated from the data of Joergensen et al. (2010) and Khan et al. (2016) with correlation coefficients of $r = 0.84$ ($n = 32$, $P < 0.01$) and $r = 0.48$ ($n = 66$, $P < 0.01$), respectively, between the GlcN/GalN and GlcN/MurN ratios. However, these two ratios did not consistently change in many studies (Amelung et al. 2002; Liang et al. 2007, 2015; Moritz et al. 2009; Lauer et al. 2011). Shifts in the ratio of Gram-positive to Gram-negative bacteria may weaken the relationship between the GlcN/GalN and GlcN/MurN ratios. However, the ratio of Gram-positive to Gram-negative bacteria in soil might be as constant as the ratio of fungi to bacteria (Joergensen and Wichern 2008). Liang et al. (2015) suggested using the GlcN/GalN ratio to describe overall amino sugar accumulation patterns, i.e., long-term microbial residue turnover. This idea of Liang et al. (2015) is convincing, as Appuhn and Joergensen (2006) and Engelking et al. (2007) failed to provide an idea on how to evaluate the GalN development in soil.

Sometimes, GalN has the lowest turnover of all microbial amino sugars (Gunina et al. 2017), which seems to contradict the idea that this amino sugar is mainly derived from microbial mucins, such as EPS. In the gut, animal mucins have a very high turnover (Johansson 2012). This might be different for microbial mucins in soil, which might be strongly adsorbed by organic soil colloids and clay. For example, glomalin, an EPS of arbuscular mycorrhizal fungi, seems to be highly resistant to microbial decomposition (Zhang et al. 2017). For this reason, there is an urgent need to gain more information on the chemical composition of microbial EPS in soil.

Conversion of amino sugars to microbial residue C

Appuhn and Joergensen (2006) and Engelking et al. (2007) present an approach for calculating fungal and bacterial residue C from amino sugar data. This approach consists of two steps. The calculation of fungal GlcN by subtracting bacterial GlcN (Engelking et al. 2007) is based on bacterial MurN (Appuhn and Joergensen 2006). In most studies, these corrections have not been carried out, which is in most cases not a serious bias, as fungal biomass contains higher concentrations of GlcN than bacteria (Appuhn and Joergensen 2006) and fungal residues usually dominate the microbial organic matter (Joergensen and Wichern 2008; Khan et al. 2016). However, not only in saline soils (Khan et al. 2016) but also in cattle feces (Jost et al. 2011) bacterial GlcN make a significant contribution to total GlcN of 31 and 50%, respectively.

Fungal GlcN is converted to fungal C by multiplying by 9 (Appuhn and Joergensen 2006). The narrow range of 8 to 11 of the confidence limits indicates that cultured fungi had rather constant GlcN concentrations (Appuhn and Joergensen 2006). This must be especially true for soil fungi, as the ratio of cell-wall biomarkers to cytoplasm of starving soil organisms is usually more constant (Joergensen and Wichern 2008). Bacterial C can be calculated by multiplying MurN by 45, assuming a ratio of 66% Gram-positive bacteria to 33% Gram-negative bacteria (Appuhn and Joergensen 2006). This assumption leads to underestimation of bacterial residues if the soil bacterial community shifts towards Gram-negative bacteria. Another problem is that the conversion values are derived from amino sugar concentrations in cultured organisms, presumably living biomass. In feces (Jost et al. 2011) and roots (Appuhn and Joergensen 2006), these conversion values have been used to estimate microbial biomass. In soil, these conversion values have been used to estimate microbial residues, which might be dominated in the long term by microbial necromass. The problem is that biomass is the sum of cytoplasm and cell envelope (membrane + cell wall), but residue mass is the sum of decayed exo-enzymes, EPS, cell-wall remains, etc. (Joergensen and Wichern 2018), which differ in their long-term turnover. However, the use of conversion values (1) draws attention to the uncertainties of a specific method, (2) forces the uniform use of a method without modifications, and (3) draws quantitative relationships between microbial residues and SOC, being an independent quality check.

The approach of Appuhn and Joergensen (2006) and Engelking et al. (2007) has rarely been used in the past decade but with an increasing tendency (Xiao et al. 2014; Zhang et al. 2016; Bai et al. 2017). Microbial residues, i.e., the sum of fungal and bacterial residues, contribute on average approximately 50% to SOC in arable and grassland soils (Khan et al. 2016). This percentage is in line with Simpson et al. (2007), using NMR spectroscopy, with Liang et al. (2011), using an Absorbing Markov Chain approach, with Miltner et al. (2012), applying ^{13}C labeled bacteria to soil, and with Ludwig et al. (2015), analyzing density fractions of SOM by in-source pyrolysis-field ionization mass spectrometry. The microbial contribution seems to be a unifying principle of

decomposition processes in soil, similar to homeostatic C/N, CP, and C/S ratios in soil organic matter (Khan et al. 2016). The same is probably true for the fungal dominance of microbial residues, which varies around 70% in most soils, because fungi are the principal decomposers of cellulose, hemicellulose, and lignin-containing polymers (Schneider et al. 2012). Even ecosystems with reduced oxygen supply such as coastal sediments (Niggemann and Schubert 2006) and paddy rice fields (Roth et al. 2011) are dominated by fungal residues.

Conclusions

Amino sugar analysis is not affected by methodological differences and constraints, giving confidence in the reliability of the methods used. MurN is a highly specific biomarker for bacterial residues in soil and for bacterial biomass in freshly colonized organic substrates. GlcN is a highly specific biomarker for fungal residues if corrected for the contribution of bacterial GlcN. GalN and ManN are unspecific microbial markers most likely for the contribution of fungal and bacterial EPS to SOC. However, this view needs experimental evidence. There are no indications that the turnover of fungal residues generally differs from that of bacterial residues. However, more information on amino sugar turnover can be obtained from long-term studies and chrono-sequences, combined with further improvement of amino-sugar-specific $\delta^{15}N$ and $\delta^{13}C$ analysis. The conversion of fungal GlcN and bacterial MurN to fungal and bacterial residue C is an important independent means for testing the reliability of other methods. More quantitative information on the proportion of Gram-negative to Gram-positive bacteria, such as firmicutes and actinobacteria, will reduce existing uncertainties in the conversion of MurN to bacterial residue C.

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