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Kinetics of amino sugar formation from organic residues of different quality

Zhen Bai [a,b,1], Samuel Bodé [a,1], Dries Huygens [a,c], Xudong Zhang [b], Pascal Boeckx [a,*]

[a] Isotope Bioscience Laboratory – ISOFYS, Ghent University, Ghent, Belgium
[b] State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, PR China
[c] Institute of Agricultural Engineering and Soil Science, Faculty of Agricultural Sciences, Universidad Austral de Chile, Valdivia, Chile

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ABSTRACT

Amino sugars are key compounds of microbial cell walls, which have been widely used as biomarker of microbial residues to investigate soil microbial communities and organic residue cycling processes. However, the formation dynamics of amino sugar is not well understood. In this study, two agricultural Luvisols under distinct tillage managements were amended with uniformly ^{13}C-labeled wheat residues of different quality (grain, leaf and root). The isotopic composition of individual amino sugars and CO_2 emission were measured over a 21-day incubation period using liquid chromatography–isotope ratio mass spectrometry (LC–IRMS) and trace gas IRMS. Results showed that, the amount of residue derived amino sugars increased exponentially and reached a maximum within days after residue addition. Glucosamine and galactosamine followed different formation kinetics. The maxima of residue derived amino sugars formation ranged from 14 nmol g^{-1} dry soil for galactosamine (0.8% of the original concentration) to 319 nmol g^{-1} dry soil for glucosamine (11% of the original concentration). Mean production times of residue derived amino sugars ranged from 2.1 to 9.3 days for glucosamine and galactosamine, respectively. In general, larger amounts of amino sugars were formed at a higher rate with increasing plant residue quality. The microbial community of the no-till soil was better adapted to assimilate low quality plant residues (i.e. leaf and root). All together, the formation dynamics of microbial cell wall components was component-specific and determined by residue quality and soil microbial community.

1. Introduction

One of the most significant impacts that microbial communities have on their environment is their ability to recycle essential elements that make up their cells. Soil organic carbon (SOC) is mainly degraded by microbes and then assimilated into living matter or respired to generate energy for cellular processes (Glaser et al., 2004; Perelo and Munch, 2005). Therefore, there is a considerable interest in understanding the biological mechanisms that regulate C exchanges between the land and atmosphere, including microbial metabolism (Allison et al., 2010). Amino sugars are useful microbial biomarkers to investigate the dynamics of microbial communities due to their prevalence in the cell walls of microorganisms, their insignificant content in plant residues and their recalcitrance after cell death (White, 1968; Amelung et al., 2001; Glaser and Gross, 2005; He et al., 2005; Liang and Balser, 2010). While 26 amino sugars have been identified in microorganisms, only four of them have been quantified in soil, i.e. glucosamine, galactosamine, mannosamine and muramic acid (Amelung et al., 2008). Glucosamine is most abundant (50–65%), followed by galactosamine (30–44%) and muramic acid (4–6%), while mannosamine is typically low in soils (Engelking et al., 2007; Ding et al., 2010). Over 90% of amino sugars are found in dead cells (Amelung et al., 2001). Therefore, the amino sugar content is used to quantify microbial residues rather than a proxy for living microbial biomass and activity (van Groenigen et al., 2010). Glucosamine in soil is mainly derived from chitins of fungal cell walls, though it also occurs in bacteria. Muramic acid exclusively originates from peptidoglycans of bacterial cell walls (Farkas, 1979; Amelung et al., 2001, 2008; He et al., 2005). While muramic acid can be directly attributed to bacterial residue, the glucosamine content has to be corrected for the bacterial glucosamine contribution in order to use it as an estimate of fungal residues (Amelung et al., 2008; He et al., 2011a). The origin of galactosamine is less clear and is typically considered to be nonspecific, as actinomycetes, bacteria and fungi all likely contain considerable galactosamine amounts (He et al., 2005; Ding et al.,

* Corresponding author. Tel.: +32 9 264 60 00.
E-mail address: pascal.boeckx@ugent.be (P. Boeckx).
[1] Equal contribution of both authors.

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2010). Amino sugars have been used to investigate soil microbial residues. However, little is known about the kinetics of amino sugar formation. Albeit the mean age of amino sugar carbon might be similar to or even older than bulk SOM (Derrien et al., 2006), we anticipate that the new, residue derived, amino sugar formation rate might be fast, considering the high turnover rates of microbial cell walls (Mauck et al., 1971; Park, 2001).

Crop residues provide resources for soil microbial metabolism thereby stimulating amino sugar buildup in soil (Mauck et al., 1971; Park, 2001), and vary in their relative amounts of easily decomposable and more recalcitrant compounds. The easily decomposable compounds are exhausted in a very short time period and induce a quick build-up of microbial biomass. Rousk and Bååth (2007) showed that soil CO_2 flux peaked between day two and four while fungal and bacterial growth rates reached maxima between day three and seven after residue incorporation. Sauheitl et al. (2005) demonstrated an exponential incorporation of plant-derived carbon into microbial sugars reaching a maximum within 4 days after substrate addition. Marx et al. (2010) showed that almost half of total soil microbial biomass C was replaced by substrate-derived C two days after the incubation of ^{13}C-labeled organic compounds, which suggests a very rapid turnover of the microbial biomass.

Therefore, the aim of this study is to elucidate residue derived amino sugar formation kinetics during the peak CO_2 respiration following plant residue incorporation. We tested the following hypotheses: (1) Since bacteria are thought to play an important role in early stage degradation of new carbon sources, i.e. 'fast energy channel' *sensu* Rousk and Bååth (2007) we expect a faster incorporation of residue carbon into bacterial amino sugar than fungal amino sugar; (2) Given that fungi are thought to be better adapted to degrade more recalcitrant carbon sources compared to bacteria (Myers et al., 2001; Waldrop and Firestone, 2004) we expect a larger effect of residue quality on the formation of bacterial amino sugars compared to fungal amino sugars; and (3) for the same reasons we expect that a higher fungal/bacteria ratio will result in higher amino sugars formation from low quality residues.

To test these hypotheses we carried out a laboratory incubation experiment in which uniformly ^{13}C-labeled crop residues of different quality (wheat grain, leaves and roots) were incubated in two soils with a distinct tillage management affecting the fungi-to-bacteria ratio. The amino sugar formation dynamics were determined by measuring the evolution of the ^{13}C content of individual amino sugars via liquid chromatography–isotope ratio mass spectrometry (LC–IRMS).

2. Materials and methods

2.1. Soil description, sampling and incubation

2.1.1. Site description

The study site was located in Maulde, Belgium (50°37′N, 3°34′E). The climate is characterized as temperate and humid marine with a 30-year mean precipitation of 780 mm per year and a mean maximum and minimum temperature of 13.5 and 6.3 °C, respectively. The soil is classified as a Luvisol (FAO, 2006). The field site has been under arable land over 100 years and was converted from conventional tillage (moldboard plowing until 30 cm and harrowing of the top 10 cm) to reduced tillage (harrowing of the top 10 cm) in 1995. In 2006, one third of the field was re-converted to conventional tillage, another third to "no-till" (no soil disturbance and direct seeding).

2.1.2. Soil sampling strategy and pre-incubation

On November 9, 2010, topsoils (0–10 cm) of the conventional tilled soil (CT) and "no-till" soil (NT) of the study field were collected from randomly selected locations at each site. The fresh soil was handpicked to remove plant or animal residues, sieved (<2 mm) and stored at 4 °C for one day before the start of the pre-incubation. The fresh soil was adjusted to 20% (m/m) moisture content and pre-incubated at 24 °C under aerobic conditions for 5 days. Basic properties of collected soils are described in Table 1.

2.1.3. Incubation with uniformly ^{13}C-labeled wheat residues

The ^{13}C-labeled roots, leaves and grains originated from uniformly ^{13}C-labeled wheat (*Triticum aestivum*), which had been grown with $^{13}CO_2$ (2 atom% excess) (Denef and Six, 2006). The plant material was collected and dried at 45 °C and stored at room temperature until incubation. Plant quality was assessed on the basis of C:N, lignin:N, hemicellulose, cellulose and polyphenol content (Table 2). Residues were ground to a size <250 μm and thoroughly mixed with the soil to facilitate substrate decomposition. An application rate of 6 mg substrate-C g^{-1} dry soil was used in six treatments: NG (NT with grain residue), NL (NT with leaf residue), NR (NT with root residue), CG (CT with grain residue), CL (CT with leaf residue), and CR (CT with root residue). There were three microcosm replicates for each treatment.

The soil (15 g) with ^{13}C-residues was placed in plastic container covered by aluminum foil with small holes to allow O_2 and CO_2 exchange. The incubation temperature was maintained at 24 °C and the moisture content was kept at 20% (w/w) by adding MilliQ water every 2–3 days. After 0, 9, 24, and 45 h, and 3, 5, 10 and 21 days, mineralization rate was measured and samples were collected destructively by freezing microcosms instantaneously in liquid nitrogen followed by lyophilization. The subsamples were stored at −20 °C for subsequent analyses.

2.2. Carbon mineralization rate

CO_2 respiration rates were measured by placing the microcosms in an airtight glass jar with rubber septa to allow gas sampling. The jars were kept closed for 5 h and gas samples were withdrawn after 0 h, 0.5 h, 3 h and 5 h. The CO_2 concentration was determined with a gas chromatograph (Shimadzu 14B, Japan) equipped with a 2 m Porapak Q column (2.2 mm o.d., SS 80/100), a pre-column (1 m) of the same material, both at 55 °C, and a ^{63}Ni electron capture detector (ECD) at 250 °C. To determine the portion of the respired CO_2 originating from the mineralization of added plant residues, the isotopic composition of respired CO_2 was determined using a trace gas preparation unit (ANCA-TGII, SerCon, UK) coupled to an isotope ratio mass spectrometer (IRMS) (20-20, SerCon, UK).

Table 1
Characteristics of the conventional tillage and no-tillage soil (0–10 cm); data of soil texture, bulk density, CO_2 fluxes and crop residue input were taken from Boeckx et al. (2011) (except*).

	Convention tillage	No-till
Total carbon (%)	1.11[*b]	1.49[*b]
Total nitrogen (%)	0.120[*b]	0.152[*b]
δ^{13}C (‰)	−26.7[*c]	−27.0[*c]
δ^{15}N (‰)	6.1[*d]	6.4[*d]
Clay (%)	17.3	15.4
Silt (%)	66.5	66.9
Sand (%)	16.2	17.7
Bulk density (ton m^{-3})	1.4	1.5
Annual CO_2 fluxes (ton C ha^{-1} y^{-1})	5.9	4.8
Annual crop residues input (ton C ha^{-1})[a]	1.3	1.5

[a] Due to crop yield differences.
[b] Standard error = 1%.
[c] Standard error = 0.1‰.
[d] Standard error = 0.2‰.