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, P. R. China

[c]Institute of Agricultural Engineering and Soil Science, Faculty of Agricultural Sciences, Universidad Austral de Chile, Valdivia, Chile

[1] **Equal contribution of both authors**

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

* Corresponding author: Tel. +32 9 264 60 00, pascal.boeckx@ugent.be

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.

Key words

Amino sugar, Kinetics, Organic residue, Tillage, Carbon-13, LC-IRMS

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;

58 Amelung et al. 2001, 2008; He et al. 2005). While muramic acid can be directly
59 attributed to bacterial residue, the glucosamine content has to be corrected for the
60 bacterial glucosamine contribution in order to use it as an estimate of fungal residues
61 (Amelung et al. 2008; He et al. 2011a). The origin of galactosamine is less clear and is
62 typically considered to be nonspecific, as actinomycetes, bacteria and fungi all likely
63 contain considerable galactosamine amounts (He et al. 2005; Ding et al. 2010). Amino
64 sugars have been used to investigate soil microbial residues. However, little is known
65 about the kinetics of amino sugar formation. Albeit the mean age of amino sugar
66 carbon might be similar to or even older than bulk SOM (Derrien et al. 2006), we
67 anticipate that the new, residue derived, amino sugar formation rate might be fast,
68 considering the high turnover rates of microbial cell walls (Mauck et al. 1971; Park
69 2001).
70 Crop residues provide resources for soil microbial metabolism thereby stimulating
71 amino sugar buildup in soil (Mauck et al. 1971; Park 2001), and vary in their relative
72 amounts of easily decomposable and more recalcitrant compounds. The easily
73 decomposable compounds are exhausted in a very short time period and induce a
74 quick build-up of microbial biomass. Rousk and Bååth (2007) showed that soil CO_2
75 flux peaked between day two and four while fungal and bacterial growth rates reached
76 maxima between day three and seven after residue incorporation. Sauheitl et al. (2005)
77 demonstrated an exponential incorporation of plant-derived carbon into microbial
78 sugars reaching a maximum within 4 days after substrate addition. Marx et al. (2010)
79 showed that almost half of total soil microbial biomass C was replaced by
80 substrate-derived C two days after the incubation of ^{13}C-labeled organic compounds,
81 which suggests a very rapid turnover of the microbial biomass.
82 Therefore, the aim of this study is to elucidate residue derived amino sugar formation
83 kinetics during the peak CO_2 respiration following plant residue incorporation. We
84 tested the following hypotheses: (1) Since bacteria are thought to play an important
85 role in early stage degradation of new carbon sources, i.e. ‘fast energy channel’ *sensu*
86 Rousk and Bååth (2007) we expect a faster incorporation of residue carbon into
87 bacterial amino sugar than fungal amino sugar; (2) Given that fungi are thought to be

88 better adapted to degrade more recalcitrant carbon sources compared to bacteria
89 (Myers et. al 2001; Waldrop and Firestone 2004) we expect a larger effect of residue
90 quality on the formation of bacterial amino sugars compared to fungal amino sugars;
91 and (3) for the same reasons we expect that a higher fungal/bacteria ratio will result in
92 higher amino sugars formation from low quality residues.
93 To test these hypotheses we carried out a laboratory incubation experiment in which
94 uniformly ^{13}C-labeled crop residues of different quality (wheat grain, leaves and roots)
95 were incubated in two soils with a distinct tillage management affecting the
96 fungi-to-bacteria ratio. The amino sugar formation dynamics were determined by
97 measuring the evolution of the ^{13}C content of individual amino sugars via liquid
98 chromatography - isotope ratio mass spectrometry (LC-IRMS).
99

100 ## 2. Materials and methods

101 ### 2.1. Soil description, sampling and incubation

102 ***Site description***

103 The study site was located in Maulde, Belgium (50°37'N, 3°34'E). The climate is
104 characterized as temperate and humid marine with a 30-year mean precipitation of
105 780 mm per year and a mean maximum and minimum temperature of 13.5 and 6.3^{o}C,
106 respectively. The soil is classified as a Luvisol (FAO 2006). The field site has been
107 under arable land over 100 years and was converted from conventional tillage
108 (moldboard plowing until 30 cm and harrowing of the top 10 cm) to reduced tillage
109 (harrowing of the top 10 cm) in 1995. In 2006, one third of the field was re-converted
110 to conventional tillage, another third to "no-till" (no soil disturbance and direct
111 seeding).

112 ***Soil sampling strategy and pre-incubation***

113 On November 9, 2010, topsoils (0-10 cm) of the conventional tilled soil (CT) and
114 "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.

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 hours, 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 hours 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).

Respiration rate (ν) was determined using the slope of a linear regression of the CO_2 concentrations as a function of time and the isotopic composition of the respired CO_2 was calculated as:

$$\delta^{13}C_{resp} = \frac{n_1 \cdot \delta^{13}C_1 - n_2 \cdot \delta^{13}C_2}{n_1 - n_2} \qquad \textbf{(1)}$$

With n_1, n_2, $\delta^{13}C_1$ and $\delta^{13}C_2$ are the amount and isotopic composition of the CO_2 in the airtight glass jar measured at two different sampling points.

The fraction of CO_2 derived from the added residue ($f\,M_{R,t}$) was calculated as:

$$f\,M_{R,t} = \left(\frac{\delta^{13}C_{resp,t} - \delta^{13}C_{resp,0}}{\delta^{13}C_R - \delta^{13}C_{SOM}} \right) \times 100 \qquad \textbf{(2)}$$

With $\delta^{13}C_{resp,t}$ and $\delta^{13}C_{resp,0}$, being the $\delta^{13}C$ values of the respired CO_2 at time point t and by a control soil, the respectively and $\delta^{13}C_R$ and $\delta^{13}C_{SOM}$ are respectively the $\delta^{13}C$ of the added residues and SOM. The CO_2 respiration rate attributed to the added plant residue (ν_R) was calculated as:

$$\nu_R = \nu \cdot f\,M_{R,t} \qquad \textbf{(3)}$$

with ν the total measured CO_2 respiration. To estimate the amount of residue carbon readily available for mineralization (C_M) the area under the residue-derived CO_2 mineralization peak (Fig 1) was determined, assuming a constant change in CO_2 production rate between two adjacent sampling points.

2.3. Amino sugar analysis

The amino sugar extraction procedure was based on the method described by Bodé et al. (2009). Briefly, c.a. 0.2 g soil was hydrolyzed for 8 hours at 105 °C using 10 mL 6M HCl. Thereafter, the soil suspension was filtered (GF/C 25mm, Whatman) using a

reusable syringe filter device (Millipore, SWINNEX). Water and HCl were removed by evaporating under reduced pressure at 45 °C, and the concentrated amino sugar sample was re-dissolved in MilliQ water. After purification by a cation exchange resin, the amino sugar solution was dried and re-dissolved with 1.5 mL MilliQ water. Concentration and $\delta^{13}C$ of amino sugar were determined by liquid chromatography-isotope ratio mass spectrometry (LC-IRMS) (Thermo Electron, Bremen, Germany). We refer to Bodé et al. (2009) for more details on the amino sugar extraction procedure.

The fraction of amino sugar originated from the ^{13}C-labeled residues at a time point t was calculated as:

$$fAS_{R,t} = \left(\frac{\delta^{13}C_{AS,t} - \delta^{13}C_{AS,t_0}}{\delta^{13}C_{R} - \delta^{13}C_{SOM}} \right) \quad \textbf{(4)}$$

whereby $\delta^{13}C_R$ and $\delta^{13}C_{SOM}$ are the ^{13}C isotopic composition of the added residues and original SOM respectively. The $\delta^{13}C_{AS,t}$ and $\delta^{13}C_{AS,t0}$ are respectively the isotopic composition of the amino sugar of interest at time t and at the start of the incubation experiment. It has to be noted that the $\delta^{13}C_{AS,t0}$ was not identical to the original isotopic composition of the soil amino sugar (AS_0) due the presence of ^{13}C labeled amino sugars in the plant residues. Since plants do not produce amino sugars (Amelung et al. 2008), this is likely explained via the presence of endophytic bacteria and fungi (Appuhn et al. 2004, Reinhold-Hurek and Hurek 2011) in the labeled plant material (Table 2).

An important fluctuation on the measured concentration of the unlabeled amino sugar pool between sampling time points was observed (see SI1), however these fluctuations did not show any trend (except a very slight increase for glucosamine in NG). Since the amino sugar pool is known to be rather stable (Glaser and Gross 2005) and although priming (positive and negative) cannot be excluded (Bell et al. 2003, Blagodatskaya et al. 2007) we expect these fluctuation to be mainly due to variability in extraction efficiency or bias in analytical response, the later was also supported by a very similar deviations for galactosamine and glucosamine. Therefore in order to have the best possible estimation of the amount residue derived amino sugar produced,

the newly formed amino sugar concentration was standardized to the unlabeled amino sugar pool (see SI2).

2.4. Bulk soil isotopic analysis

Subsamples of air-dried soil samples were ground by a planetary ball mill (PM400, Retsch, Germany) for total C and N, and ^{13}C and ^{15}N analysis by an elemental analyzer (EA) (ANCA-SL, SerCon, UK) coupled to an IRMS (20-20, SerCon, UK).

2.5. Statistical analysis

Statistical analysis was performed using SPSS 19.0. A three-way analysis of variance (ANOVA) procedure with Tukey's HSD (Honestly Significant Difference) post hoc test was used to analyze the effects of plant residue quality, amino sugar identity and tillage on amino sugar formation and C mineralization using a general linear model. When a significant interactions between factors was observed this interaction was investigated by repeating the statistical test for the different levels of the interacting factors individually. Unless otherwise stated significant level of difference was set at $\alpha = 0.05$. Non-linear regression analysis was used to determine *k* and maxima in non-linear equation (5).

3. Results

3.1. Carbon mineralization

Residue mineralization started immediately after residue addition and CO_2 flux peaked between day 1 and 3 across treatments (Fig. 1) after which it decreased again to reach constant "steady state mineralization rate" at day 10. The amount of carbon readily available for mineralization (C_M) ranged from 23 till 84 μmol g^{-1} dry soil with highest C_M for grain incubated soils and lowest for root incubated ones. At the end of the experiment the amount of residue mineralized (total cumulative amount mineralized during the 21 days incubation) was between 10% (root) and 26% (grain) of the added plant residue (Fig. 1).

3.2. Original amino sugar concentration

The original concentration of amino sugar was higher for no-till site compared to

conventional tilled one (F = 14, P < 0.001) (Table 4). The highest amounts were found for glucosamine (3781 ± 66 nmol g^{-1} dry soil for the no-till treatment and 2985 ± 199 nmol g^{-1} dry soil for the conventional till treatment) followed by galactosamine (1937 ± 102 (no-till) and 1742 ± 110 nmol g^{-1} dry soil (conventional till))(Table 3) and lowest for muramic acid (657 ± 240 (no-till) and 522 ± 171 nmol g^{-1} dry soil (conventional till)) (F = 240, P <0.001) (Table 4). Additionally, there was a small but significant effect of the tillage treatment on amino sugar type (F = 4.13, P<0.05), with larger differences in concentration for the different amino sugars in the no-till soil (F = 217) compared to conventional tilled soil (F = 73) (Table 4).

3.3. Model approach for parameter estimation of residue-derived amino sugar formation kinetics

Similarly to the residue mineralization, the incorporation of ^{13}C- carbon derived from the added residues into the amino sugar pool was detected from the first sampling point (9 hours) on and it increased exponentially during the first days of the incubation after which residue derived amino sugars formation reached a steady state (Fig. 2).

A first-order kinetic model was fitted to the formation dynamics of residue-derived glucosamine and galactosamine (Fig. 2):

$$AS_R = AS_{R,Max} \cdot (1 - e^{-k \cdot t}) \quad \textbf{(5)}$$

with $AS_{R,Max}$ being the maximum of the exponential residue derived amino sugar formation, $AS_{R(t)}$ is the amount residue derived amino sugar at time t and *k* the formation rate constant of the exponential formation of residue derived amino sugars. The inverse of *k* is the mean production time (MPT), which is the average time needed to form "*de novo*" residue derived amino sugars during the microbial peak activity (see Appendix):

$$MPT = \frac{1}{k} \quad \textbf{(6)}$$

The $AS_{R,Max}$ of glucosamine and galactosamine ranged from 40 till 319 nmol g^{-1} and 14 till 98 nmol g^{-1} respectively. MPT of glucosamine and galactosamine ranged from 1.6 to 3.7 days and from 2.1 to 8.3 days respectively (Table 3).

Unfortunately the high ^{13}C enrichment of muramic acid at start of the incubation (due to considerable amount of muramic acid present in the labeled plant residue (Table 2) relative to the low soil muramic acid concentration) and higher variability on concentration and isotopic measurements of muramic acid, impeded the determination of the dynamics of residue derived formation of muramic acid in this incubation experiment (Fig. 2).

3.4. Effect of residue quality, amino sugar type and tillage treatment

The effect of amino sugar type, residue quality and tillage history of the soil on parameters describing the dynamics of residue derived amino sugar formation ($AS_{R,Max}$, k (equation 4) and on $AS_{R,Max}$ relative to the original amino sugar concentration) was investigated using a multi-way ANOVA approach (Table 4).

Maxima of residue derived amino sugar formation ($AS_{R,Max}$)

There was a significant effect of amino sugar type, residue quality and tillage history on $AS_{R,Max}$. The formation of the different amino sugars followed dissimilar kinetics, with greatest $AS_{R,Max}$ values obtained for glucosamine (40 to 319 nmol g^{-1} dry soil) than for galactosamine (14 to 98 nmol g^{-1} dry soil) (Table 3 and 4). The comparison of $AS_{R,Max}$ values among residue quality indicated that the values were greater for grain (198 nmol g^{-1} dry soil, averaged over all amino sugar type-tillage combinations) followed by leaf (137 nmol g^{-1} dry soil) and root (45 nmol g^{-1} dry soil) (Table 3 and 4). The no-till soil (134 nmol g^{-1} dry soil, averaged over all amino sugar type-residue combinations) generally showed greater $AS_{R,Max}$ values than conventional tilled one (119 nmol g^{-1} dry soil). Additionally, significant interactive effects between amino sugar type and residue quality (F = 130), residue quality and tillage history of the soil sample (F= 19.1) (Table 4) were observed. The interaction between amino sugar type and residue quality indicated that the differences in $AS_{R,Max}$ between glucosamine and galactosamine were more prominent for grain incubations (F = 722) than for leaf (F = 337) and root (F =45.6), while quality of residue had a greater effect on glucosamine

(F = 287) than on galactosamine (F = 221) (Table 3 and 4). The interaction of residue quality and tillage treatment of the soil sample revealed a greater effect of residue quality in the conventional tilled soil (F = 391) compared to no-till soil (F = 121). Tillage history of the soil sample had a larger effect for root (F = 28.5) than for leaf (F = 19.3) while the effect of tillage history was inverted for grain incubated samples $AS_{R,Max}$ was highest for the conventional tilled soil (F = 7.4) (Table 4).

Formation rate constant (k) and mean production time (MPT)

A significant effect of residue quality and amino sugar type on the formation rate was observed. There was no effect of the tillage history. Glucosamine was formed at a greater rate compared to galactosamine (Table 4) with an average MPT of 3.2 and 4.9 days (Table 3), respectively. Comparing the formation rates among residue quality, indicated that the values were greatest for grain (0.40 day^{-1}, averaged over all amino sugar type-tillage combinations), followed by root (0.27 day^{-1}) and leaf (0.19 day^{-1}), which did not differ significantly (Table 4). No interactive effects were observed.

$AS_{R,Max}$ relative to the original amino sugar concentration

The effects of the tested factors on the ratio $AS_{R,Max}$ to original amino sugar were, in general, similar to the effects observed on $AS_{R,Max}$ (Table 4). Exceptions were the absence of the effect of tillage history and an additional interaction between amino sugar type and tillage history (F = 32.3) with a larger tillage effect on glucosamine (F = 5.52) formation than on galactosamine (F = 0.562) (Table 4).

4. Discussion

In contrast to most previous works, this study was able to successfully quantify newly formed amino sugars during peak microbial activity following plant residue addition. *De novo* formed amino sugars could be approximated by the residue derived amino sugars as the unlabeled amino sugar pool did not vary significantly of over time (SF1). Noticeably, soil microorganisms prefer to feed on fresh organic residues rather than

on endogenous SOM during exponential microbial activity following residue addition (Amelung et al. 2008).

The MPT of the amino sugar, defined here as the average time needed to form "*de novo*" residue derived amino sugar during peak microbial activity following plant residue addition, varied between 2.1 and 9.3 days. Maxima of the exponential microbial amino sugars formation were attained within ca. one week after which the residue derived amino sugars formation reached a steady state (Table 3, Fig. 2). Decock et al. (2009) also revealed a maximum $\delta^{13}C$ in glucosamine and galactosamine within one week after incubation using ^{13}C-labeled wheat residues. Liang et al. (2007) reported that amino sugar content in black soil reached a maximum within 3 weeks upon incubation with maize residue, thereby increasing the original amino sugar content with one third.

4.1. Effect of amino sugar type

As muramic acid exclusively originates from bacterial cell wall, while glucosamine and galactosamine are present in both bacterial as fungal residues (Amelung et al. 2001 and 2008; Glaser and Gross 2005; Engelking et al. 2007), muramic acid is the preferred biomarker to differentiate bacterial and fungal activity for incorporation of residue-derived carbon. Unfortunately, due to a higher uncertainty on the muramic acid measurements and a high ^{13}C-muramic acid contamination of the plant residues the formation dynamics of muramic acid could not be determined.

The $AS_{R,Max}$ relative to the original soil amino sugar concentration ranged from 1.3 to 11% for glucosamine followed by galactosamine (0.8 to 5.7%). A similar trend was also observed in other studies (Glaser and Gross 2005; Engelking et al. 2007; He et al. 2011b).

Bacteria and fungi both produce glucosamine and galactosamine (Amelung et al. 2001 and 2008; Glaser and Gross 2005). However, the strong amino sugar-type effect on $AS_{R,Max}$ relative to the original amino sugar concentration, and on the MPT, indicate that these amino sugars are formed through dissimilar processes. Engelking et al. (2007) reviewed the available literature on amino sugar concentrations in cultured bacteria and fungi, which revealed that the galactosamine/glucosamine ratio appeared

to be on average almost 3 times higher in fungi compared to bacteria, making residue derived galactosamine a 'more' fungal marker than glucosamine when considering microbial activity. The higher formation rate constant of glucosamine compared to galactosamine thus most likely indicates that bacteria play a more important role for early stage incorporation of residue-derived carbon, i.e. 'fast energy channel' *sensu* Rousk et al., 2007). The slower formation of galactosamine, corroborates with the slower turnover of fungi compared to bacteria; for which fungi are involved in the slow energy channel through the soil food web (Rousk et al. 2007).

4.2. Effect of Residue quality

Exponential residue derived amino sugar formation during the first days after residue addition was accompanied with high carbon mineralization rates (Fig. 1 and 2). Carbon mineralization and microbial growth upon residue decomposition is largely determined by organic matter quality (Liang et al. 2007; Rousk and Bååth 2007). The high C:N and lignin content of root indicates its low quality while grain had the highest quality. This difference in quality was also revealed by the amount of readily available carbon. Altogether this resulted in different $AS_{R,Max}$ values between residues: grain>leaf>root ($P < 0.001$).

The interaction between residue quality and amino sugar type revealed that the difference between the $AS_{R,Max}$ for glucosamine and galactosamine was much more pronounced for grain compared to leaf and root (both absolute as relative to the original amino sugar concentration). Considering the higher fungal origin of galactosamine, this interaction indicates that, at least during peak microbial activity, fungi seem to be less dependent on the quality of the residue than bacteria for *de novo* amino sugar formation. This is in accordance with what we expected since it is generally believed that bacteria especially rely on easily available C compounds while the fungal community is better adapted to colonize more recalcitrant sources (Myers et al. 2001; Waldrop and Firestone 2004).

4.3. Effect of the site's tillage history

The increased ability of the no-till soil samples to enhance mineralization and amino sugars formation out of residues of lower quality may be explained by microbial

community differences typically found in not tilled soil. Fungi, showing a great ability to decompose more recalcitrant substrates (Acosta-Martinez et al. 2003; Ding et al. 2010; Werth and Kuzyakov 2010), are typically more abundant in the no till soils (Fu et al. 2000; Thiet et al. 2006; White and Rice 2009).

Furthermore comparing the amount amino sugars formed during peak microbial activity ($AS_{R,Max}$ of glucosamine + $AS_{R,Max}$ of galactosamine) relative to the readily available carbon of the residue (estimated by C_M), the no-till soil showed an enhanced ability to form more amino sugars from the readily available carbon of the lower quality residues (leaf and root) while this was not different for grain.

The difference in microbial community in both soils was, however, not significantly reflected in the ratio of the masses of fungal and bacterial residues per g of soil, estimated according to van Groenigen et al. (2010). This ratio turned out to be equal (0.53) for both soils with different tillage history, probably due to the relative short time lap since the conversion of tillage management compared to the relative long residence time of amino sugars in soils after cell death (Glaser and Gross 2005) and to the larger uncertainty on the muramic acid concentrations.

Crop residue input is not different between conventional and no-till treatment (Table 1), but tillage redistribute the residue input over the plow depth. Therefore, the annual C input in the 0-10 cm topsoil is around 0.7 mg C g^{-1}dry soil higher for the not tilled site, assuming an evenly distributed of the crop residue over the entire plowing depth in the tilled site. The annual net difference of amino sugar content between the tilled and the no-till site was calculated as 6.7 ± 1.8% for glucosamine, 2.8 ± 2.2% for galactosamine and 6.5 ± 14% for muramic acid. Meanwhile, the average proportion of $AS_{R,Max}$ to the original amino sugar content for leaf and root (grain was left out since it is not incorporated *in situ*) was respectively 3.9 % for glucosamine, 2.6 % for galactosamine upon crop residues in this study.

Both from field measurement and laboratory incubations we observed that the relative change of galactosamine was significantly smaller than for glucosamine (Table 4), indicating that the more conservative response of galactosamine upon shift in tillage was (at least partially) due to a lower formation of residue derived galactosamine

compared to glucosamine after receiving increased residue input in the no-till treatment.

5. Conclusions:

A first-order kinetic model could describe residue derived amino sugar formation, which reached a maximum and steady state several days after residue addition.

During peak microbial activity *de novo* residue derived amino sugar formation was surprisingly fast, giving shorter mean production times (MPT) for glucosamine (2.1 – 5.0 days) than for galactosamine (2.5 – 9.3 days). The faster incorporation of residue carbon into glucosamine compared to the dominantly fungal galactosamine, underpinned the role of bacteria as 'fast energy channel' described by Rousk and Bååth (2007). In addition the *de novo* amino sugar formation relative to original amino sugar pool was higher for glucosamine than for galactosamine, however this difference declined strongly with decreasing residue quality, confirming the better adaptation of fungal communities to colonize more recalcitrant C sources. Finally, the influence of tillage history on *de novo* amino sugar formation indicated a better adaptation of soil microbial community in the no-till treatment compared to conventional tillage to incorporate carbon originating from more recalcitrant plant residues.

6. Acknowledgements

We thank Katja Van Nieuland and Jan Vermeulen for ^{13}C analysis of bulk soil and CO_2. We would like to acknowledge Karolien Denef and Joan Six for providing the labeled plant material. Dries H. is a postdoctoral fellow of the Fund for Scientific Research - Flanders (FWO). Zhen Bai's scientific mission was financed by the National Natural Science Foundation of China and the "Departement Onderwijs en Vorming" of the Flemish Government. Finally the authors would like to express their gratitude to Prof. Dr. J. Schimel and two anonymous reviewers for their very valuable remarks and comments.

7. References

Acosta-Martinez, V., Zobeck, T.M., Gill, T.E., Kennedy, A.C., 2003. Enzyme activities and microbial community structure in semiarid agricultural soils. Biology and Fertility of Soils 38, 216-227.

Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3, 336-340.

Amelung, W., Brodowski, S., Sandhage-Hofmann, A., Bol, R., 2008. Combining Biomarker with Stable Isotope Analyses for Assessing the Transformation and Turnover of Soil Organic Matter. Advances in Agronomy, Vol 100 100, 155-250.

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.

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-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 167, 17-21.

Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.H., Kuzyakov, Y., 2007. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. Applied Soil Ecology 37, 95-105.

Bell, J.M., Smith, J.L., Bailey, V.L., Bolton, H., 2003. Priming effect and C storage in semi-arid no-till spring crop rotations. Biology and Fertility of Soils 37, 237-244.

Bodé, S., Denef, K., Boeckx, P., 2009. Development and evaluation of a high-performance liquid chromatography/isotope ratio mass spectrometry methodology for delta(13)C analyses of amino sugars in soil. Rapid Communications in Mass Spectrometry 23, 2519-2526.

Boeckx, P., Van Nieuland, K., Van Cleemput, O., 2011. Short-term effect of tillage intensity on N2O and CO2 emissions. Agronomy for Sustainable Development 31, 453-461

Decock, C., Denef, K., Bode, S., Six, J., Boeckx, P., 2009. Critical assessment of the applicability of gas chromatography-combustion-isotope ratio mass spectrometry

463 to determine amino sugar dynamics in soil. Rapid Communications in Mass
464 Spectrometry 23, 1201-1211.
465 Denef, K., Six, J., 2006. Contributions of incorporated residue and living roots to
466 aggregate-associated andmicrobial carbon in two soilswith different
467 claymineralogy. European Journal of Soil Science 57, 774–786 .
468 Derrien, D., Amelung, W., 2011. Computing the mean residence time of soil carbon
469 fractions using stable isotopes: impacts of the model framework. European Journal
470 of Soil Science 62, 237-252.
471 Derrien, D., Marol, C., Balabane, M., Balesdent, J., 2006. The turnover of
472 carbohydrate carbon in a cultivated soil estimated by (13)C natural abundances.
473 European Journal of Soil Science 57, 547-557.
474 Ding, X.L., Zhang, X.D., He, H.B., Xie, H.T., 2010. Dynamics of soil amino sugar
475 pools during decomposition processes of corn residues as affected by inorganic N
476 addition. Journal of Soils and Sediments 10, 758-766.
477 Engelking, B., Flessa, H., Joergensen, R.G., 2007. Shifts in amino sugar and
478 ergosterol contents after addition of sucrose and cellulose to soil. Soil Biology &
479 Biochemistry 39, 2111-2118.
480 FAO, 2006. World reference base for soil resources. Report 013. FAO, Rome, p 86
481 Farkas, V., 1979. Biosynthesis of Cell-Walls of Fungi. Microbiological Reviews 43,
482 117-144.
483 Fu, S.L., Coleman, D.C., Schartz, R., Potter, R., Hendrix, P.F., Crossley, D.A., 2000.
484 C-14 distribution in soil organisms and respiration after the decomposition of crop
485 residue in conventional tillage and no-till agroecosystems at Georgia Piedimont.
486 Soil & Tillage Research 57, 31-41.
487 Glaser, B., Gross, S., 2005. Compound-specific delta C-13 analysis of individual
488 amino sugars - a tool to quantify timing and amount of soil microbial residue
489 stabilization. Rapid Communications in Mass Spectrometry 19, 1409-1416.
490 Glaser, B., Turrion, M.B., Alef, K., 2004. Amino sugars and muramic acid -
491 biomarkers for soil microbial community structure analysis. Soil Biology &
492 Biochemistry 36, 399-407.

He, H.B., Li, X.B., Zhang, W., Zhang, X.D., 2011a. Differentiating the dynamics of native and newly immobilized amino sugars in soil frequently amended with inorganic nitrogen and glucose. European Journal of Soil Science 62, 144-151.

He, H.B., Xie, H.T., Zhang, X.D., Wang, Y.H., Wu, Y.Y., 2005. A gas chromatographic/mass spectrometric method for tracing the microbial conversion of glucose into amino sugars in soil. Rapid Communications in Mass Spectrometry 19, 1993-1998.

He, H.B., Zhang, W., Zhang, X.D., Xie, H.T., Zhuang, J., 2011b. Temporal responses of soil microorganisms to substrate addition as indicated by amino sugar differentiation. Soil Biology & Biochemistry 43, 1155-1161.

Liang, C., Balser, T.C., 2010. Mass spectrometric characterization of amino sugar aldononitrile acetate derivatives used for isotope enrichment assessment of microbial residues. Soil Biology & Biochemistry 42, 904-909.

Liang, C., Zhang, X.D., Rubert, K.F., Balser, T.C., 2007. Effect of plant materials on microbial transformation of amino sugars in three soil microcosms. Biology and Fertility of Soils 43, 631-639.

Marx, M., Buegger, F., Gattinger, A., Zsolnay, A., Munch, J.C., 2010. Determination of the fate of regularly applied (13)C-labeled-artificial-exudates C in two agricultural soils. Journal of Plant Nutrition and Soil Science 173, 80-87.

Mauck, J., Chan, L., Glaser, L., 1971. Turnover of the cell wall of Gram-positive bacteria. J Biol Chem 246, 1820-1827.

Myers, R.T., Zak, D.R., White, D.C., Peacock, A., 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal 65, 359-367.

Park, J.T., 2001. Identification of a dedicated recycling pathway for anhydro-N-acetylmuramic acid and N-acetylglucosamine derived from Escherichia coli cell wall murein. Journal of Bacteriology 183, 3842-3847.

Perelo, L.W., Munch, J.C., 2005. Microbial immobilisation and turnover of (13)C labelled substrates in two arable soils under field and laboratory conditions. Soil Biology & Biochemistry 37, 2263-2272.

Rousk, J., Bååth, E., 2007. Fungal and bacterial growth in soil with plant materials of different C/N ratios. Fems Microbiology Ecology 62, 258-267.

Reinhold-Hurek, B., Hurek, T., 2011. Living inside plants: bacterial endophytes. Current Opinion in Plant Biology 14, 435-443.

Sauheitl, L., Glaser, B., Bol, R., 2005. Short-term dynamics of slurry-derived plant and microbial sugars in a temperate grassland soil as assessed by compound-specific delta C-13 analyses. Rapid Communications in Mass Spectrometry 19, 1437-1446.

Thiet, R.K., Frey, S.D., Six, J., 2006. Do growth yield efficiencies differ between soil microbial communities differing in fungal: bacterial ratios? Reality check and methodological issues. Soil Biology & Biochemistry 38, 837-844.

van Groenigen, K.J., Bloem, J., Bååth, E., Boeckx, P., Rousk, J., Bode, S., Forristal, D., Jones, M.B., 2010. Abundance, production and stabilization of microbial biomass under conventional and reduced tillage. Soil Biology & Biochemistry 42, 48-55.

Waldrop, M.P., Firestone, M.K., 2004. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138, 275-284.

Werth, M., Kuzyakov, Y., 2010. (13)C fractionation at the root-microorganisms-soil interface: A review and outlook for partitioning studies. Soil Biology & Biochemistry 42, 1372-1384.

White, P.A., Rice, C.W., 2009. Tillage Effects on Microbial and Carbon Dynamics during Plant Residue Decomposition. Soil Science Society of America Journal 73, 138-145.

White, R.J., 1968. Control of Amino Sugar Metabolism in Escherichia Coli and Isolation of Mutants Unable to Degrade Amino Sugars. Biochemical Journal 106, 847-858.

8. Appendix 1: mean production time

Similarly to the mean residence time (MRT) of a litter cohort in the soil reservoir introduced by Derrien and Amelung (2011) the mean production time (MPT) defined here as the average time needed to form "*de novo*" amino sugars following plant residue addition can be estimated by equation (6), this can be found by rewriting equation (5) as :

$$(AS_{R,Max} - AS_R)_{(t)} = AS_{R,Max} \cdot e^{-k \cdot t} \qquad (7)$$

The difference $(AS_{R,Max} - AS_R)$ follows an exponential decrease and the change of $(AS_{R,Max} - AS_R)$ in function of time can be written as:

$$\frac{d(AS_{R,Max} - AS_R)}{dt} = -k \cdot (AS_{R,Max} - AS_R) \qquad (8)$$

Since the change in this difference $[d(AS_{R,Max} - AS_R)]$ is equal to the negation of the change in amount residue derived amino sugar $[-d(AS_R)]$. The amount residue derived amino sugar formed during a time interval from t to t+dt is thus equal to:

$$d(AS_R) = k \cdot (AS_{R,Max} - AS_R) dt \qquad (9)$$

The time needed to form a portion $d(AS_R)_{(t)}$ out of the added residue, is equal to t and this portion is a fraction $d(AS_R)_{(t)}/AS_{R,Max}$ of the total amount $AS_{R,Max}$ that will be formed during the exponential *de novo* amino sugar formation. So that the mean production time (MPT) can be calculated by integration from start of the incubation till end of the exponential *de novo* amino sugar formation (t_{Max}):

$$MPT = \frac{1}{AS_{R,Max}} \int_0^{t_{Max}} t \times d(AS_R)_{(t)} \qquad (10)$$

$$MPT = \frac{1}{AS_{R,Max}} \int_0^{t_{Max}} t \times k (AS_{R,Max} - AS_R) dt \qquad (11)$$

$$MPT = \frac{1}{AS_{R,Max}} \int_0^{t_{Max}} t \cdot k \cdot AS_{R,Max} \cdot e^{-k t} dt = k \int_0^{t_{Max}} t \cdot e^{-k t} dt \qquad (12)$$

And by integration by parts.

$$MPT = \frac{1}{k} \qquad (13)$$