View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Ghent University Academic Bibliography

1 # Kinetics of amino sugar formation from organic residues
2 # of different quality

3

4 Zhen Bai[a,b,1], Samuel Bodé[a,1], Dries Huygens[a,c], Xudong Zhang[b], Pascal Boeckx[a*]
5

6 [a] Isotope Bioscience Laboratory - ISOFYS, Ghent University, Ghent, Belgium

7 [b]State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of
8 Sciences, Shenyang, P. R. China

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

11 **[1] Equal contribution of both authors**

12

13 ## Abstract

14 Amino sugars are key compounds of microbial cell walls, which have been widely
15 used as biomarker of microbial residues to investigate soil microbial communities and
16 organic residue cycling processes. However, the formation dynamics of amino sugar
17 is not well understood. In this study, two agricultural Luvisols under distinct tillage
18 managements were amended with uniformly ^{13}C-labeled wheat residues of different
19 quality (grain, leaf and root). The isotopic composition of individual amino sugars and
20 CO_2 emission were measured over a 21-day incubation period using liquid
21 chromatography - isotope ratio mass spectrometry (LC-IRMS) and trace gas IRMS.
22 Results showed that, the amount of residue derived amino sugars increased
23 exponentially and reached a maximum within days after residue addition.
24 Glucosamine and galactosamine followed different formation kinetics. The maxima of
25 residue derived amino sugars formation ranged from 14 nmol g^{-1} dry soil for
26 galactosamine (0.8% of the original concentration) to 319 nmol g^{-1} dry soil for
27 glucosamine (11% of the original concentration). Mean production times of residue

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

28 derived amino sugars ranged from 2.1 to 9.3 days for glucosamine and galactosamine,
29 respectively. In general, larger amounts of amino sugars were formed at a higher rate
30 with increasing plant residue quality. The microbial community of the no-till soil was
31 better adapted to assimilate low quality plant residues (i.e. leaf and root). All together,
32 the formation dynamics of microbial cell wall components was component-specific
33 and determined by residue quality and soil microbial community.

34 **Key words**

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

36 1. Introduction

37 One of the most significant impacts that microbial communities have on their
38 environment is their ability to recycle essential elements that make up their cells. Soil
39 organic carbon (SOC) is mainly degraded by microbes and then assimilated into
40 living matter or respired to generate energy for cellular processes (Glaser et al. 2004,
41 Perelo and Munch 2005). Therefore, there is a considerable interest in understanding
42 the biological mechanisms that regulate C exchanges between the land and
43 atmosphere, including microbial metabolism (Allison et al. 2010). Amino sugars are
44 useful microbial biomarkers to investigate the dynamics of microbial communities
45 due to their prevalence in the cell walls of microorganisms, their insignificant content
46 in plant residues and their recalcitrance after cell death (White 1968; Amelung et al.
47 2001; Glaser and Gross 2005; He et al. 2005; Liang and Balser 2010). While 26
48 amino sugars have been identified in microorganisms, only four of them have been
49 quantified in soil, i.e. glucosamine, galactosamine, mannosamine and muramic acid
50 (Amelung et al. 2008). Glucosamine is most abundant (50-65 %), followed by
51 galactosamine (30-44 %) and muramic acid (4-6%), while mannosamine is typically
52 low in soils (Engelking et al. 2007; Ding et al. 2010). Over 90% of amino sugars are
53 found in dead cells (Amelung et al. 2001). Therefore, the amino sugar content is used
54 to quantify microbial residues rather than a proxy for living microbial biomass and
55 activity (van Groenigen et al. 2010). Glucosamine in soil is mainly derived from
56 chitins of fungal cell walls, though it also occurs in bacteria. Muramic acid
57 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

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

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).

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

115 at each site. The fresh soil was handpicked to remove plant or animal residues, sieved
116 (< 2 mm) and stored at 4 °C for one day before the start of the pre-incubation. The
117 fresh soil was adjusted to 20 % (m/m) moisture content and pre-incubated at 24 °C
118 under aerobic conditions for 5 days. Basic properties of collected soils are described
119 in Table 1.

120 ***Incubation with uniformly ^{13}C-labeled wheat residues***

121 The ^{13}C-labeled roots, leaves and grains originated from uniformly ^{13}C-labeled wheat
122 (*Triticum aestivum*), which had been grown with $^{13}CO_2$ (2 atom% excess) (Denef and
123 Six 2006). The plant material was collected and dried at 45 °C and stored at room
124 temperature until incubation. Plant quality was assessed on the basis of C:N, lignin:N,
125 hemicellulose, cellulose and polyphenol content (Table 2). Residues were ground to a
126 size <250 μm and thoroughly mixed with the soil to facilitate substrate decomposition.
127 An application rate of 6 mg substrate-C g^{-1} dry soil was used in six treatments: NG
128 (NT with grain residue), NL (NT with leaf residue), NR (NT with root residue), CG
129 (CT with grain residue), CL (CT with leaf residue), and CR (CT with root residue).
130 There were three microcosm replicates for each treatment.
131 The soil (15 g) with ^{13}C-residues was placed in plastic container covered by aluminum
132 foil with small holes to allow O_2 and CO_2 exchange. The incubation temperature was
133 maintained at 24 °C and the moisture content was kept at 20% (w/w) by adding
134 MilliQ water every 2-3 days. After 0, 9, 24, and 45 hours, and 3, 5, 10 and 21 days,
135 mineralization rate was measured and samples were collected destructively by
136 freezing microcosms instantaneously in liquid nitrogen followed by lyophilization.
137 The subsamples were stored at -20 °C for subsequent analyses.

138 **2.2. Carbon mineralization rate**

139 CO_2 respiration rates were measured by placing the microcosms in an airtight glass jar
140 with rubber septa to allow gas sampling. The jars were kept closed for 5 hours and gas
141 samples were withdrawn after 0 h, 0.5 h, 3 h and 5 h. The CO_2 concentration was
142 determined with a gas chromatograph (Shimadzu 14B, Japan) equipped with a 2 m

143 Porapak Q column (2.2 mm o.d., SS 80/100), a pre-column (1 m) of the same material,
144 both at 55 °C, and a ^{63}Ni electron capture detector (ECD) at 250 °C. To determine the
145 portion of the respired CO_2 originating from the mineralization of added plant
146 residues, the isotopic composition of respired CO_2 was determined using a trace gas
147 preparation unit (ANCA-TGII, SerCon, UK) coupled to an isotope ratio mass
148 spectrometer (IRMS) (20-20, SerCon, UK).

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

152 $$\delta^{13}C_{resp} = \frac{n_1 \cdot \delta^{13}C_1 - n_2 \cdot \delta^{13}C_2}{n_1 - n_2} \quad \textbf{(1)}$$

153 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
154 airtight glass jar measured at two different sampling points.

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

156 $$f\,M_{R,t} = \left(\frac{\delta^{13}C_{resp,t} - \delta^{13}C_{resp,0}}{\delta^{13}C_R - \delta^{13}C_{SOM}} \right) \times 100 \quad \textbf{(2)}$$

157 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
158 and by a control soil, the respectively and $\delta^{13}C_R$ and $\delta^{13}C_{SOM}$ are respectively the $\delta^{13}C$
159 of the added residues and SOM. The CO_2 respiration rate attributed to the added plant
160 residue (ν_R) was calculated as:

161 $$\nu_R = \nu \cdot f\,M_{R,t} \quad \textbf{(3)}$$

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

166

167 ## 2.3. Amino sugar analysis

168 The amino sugar extraction procedure was based on the method described by Bodé et
169 al. (2009). Briefly, c.a. 0.2 g soil was hydrolyzed for 8 hours at 105 °C using 10 mL
170 6M HCl. Thereafter, the soil suspension was filtered (GF/C 25mm, Whatman) using a

171 reusable syringe filter device (Millipore, SWINNEX). Water and HCl were removed
172 by evaporating under reduced pressure at 45 °C, and the concentrated amino sugar
173 sample was re-dissolved in MilliQ water. After purification by a cation exchange resin,
174 the amino sugar solution was dried and re-dissolved with 1.5 mL MilliQ water.
175 Concentration and $\delta^{13}C$ of amino sugar were determined by liquid
176 chromatography-isotope ratio mass spectrometry (LC-IRMS) (Thermo Electron,
177 Bremen, Germany). We refer to Bodé et al. (2009) for more details on the amino sugar
178 extraction procedure.
179 The fraction of amino sugar originated from the ^{13}C-labeled residues at a time point t
180 was calculated as:

$$f\mathrm{AS}_{\mathrm{R,t}} = \left(\frac{\delta^{13}\mathrm{C}_{\mathrm{AS,t}} - \delta^{13}\mathrm{C}_{\mathrm{AS,t_0}}}{\delta^{13}\mathrm{C}_{\mathrm{R}} - \delta^{13}\mathrm{C}_{\mathrm{SOM}}} \right) \quad \textbf{(4)}$$

181 whereby $\delta^{13}C_R$ and $\delta^{13}C_{SOM}$ are the ^{13}C isotopic composition of the added residues
182 and original SOM respectively. The $\delta^{13}C_{AS,t}$ and $\delta^{13}C_{AS,t0}$ are respectively the
183 isotopic composition of the amino sugar of interest at time t and at the start of the
184 incubation experiment. It has to be noted that the $\delta^{13}C_{AS,t0}$ was not identical to the
185 original isotopic composition of the soil amino sugar (AS_0) due the presence of ^{13}C
186 labeled amino sugars in the plant residues. Since plants do not produce amino sugars
187 (Amelung et al. 2008), this is likely explained via the presence of endophytic bacteria
188 and fungi (Appuhn et al. 2004, Reinhold-Hurek and Hurek 2011) in the labeled plant
189 material (Table 2).
190 An important fluctuation on the measured concentration of the unlabeled amino sugar
191 pool between sampling time points was observed (see SI1), however these
192 fluctuations did not show any trend (except a very slight increase for glucosamine in
193 NG). Since the amino sugar pool is known to be rather stable (Glaser and Gross 2005)
194 and although priming (positive and negative) cannot be excluded (Bell et al. 2003,
195 Blagodatskaya et al. 2007) we expect these fluctuation to be mainly due to variability
196 in extraction efficiency or bias in analytical response, the later was also supported by
197 a very similar deviations for galactosamine and glucosamine. Therefore in order to
198 have the best possible estimation of the amount residue derived amino sugar produced,

199 the newly formed amino sugar concentration was standardized to the unlabeled amino
200 sugar pool (see SI2).
201
202 **2.4. Bulk soil isotopic analysis**
203 Subsamples of air-dried soil samples were ground by a planetary ball mill (PM400,
204 Retsch, Germany) for total C and N, and ^{13}C and ^{15}N analysis by an elemental
205 analyzer (EA) (ANCA-SL, SerCon, UK) coupled to an IRMS (20-20, SerCon, UK).
206
207 **2.5. Statistical analysis**
208 Statistical analysis was performed using SPSS 19.0. A three-way analysis of variance
209 (ANOVA) procedure with Tukey's HSD (Honestly Significant Difference) post hoc
210 test was used to analyze the effects of plant residue quality, amino sugar identity and
211 tillage on amino sugar formation and C mineralization using a general linear model.
212 When a significant interactions between factors was observed this interaction was
213 investigated by repeating the statistical test for the different levels of the interacting
214 factors individually. Unless otherwise stated significant level of difference was set at α
215 $= 0.05$. Non-linear regression analysis was used to determine *k* and maxima in
216 non-linear equation (5).
217 **3. Results**
218 **3.1. Carbon mineralization**
219 Residue mineralization started immediately after residue addition and CO_2 flux
220 peaked between day 1 and 3 across treatments (Fig. 1) after which it decreased again
221 to reach constant "steady state mineralization rate" at day 10. The amount of carbon
222 readily available for mineralization (C_M) ranged from 23 till 84 µmol g^{-1} dry soil with
223 highest C_M for grain incubated soils and lowest for root incubated ones. At the end of
224 the experiment the amount of residue mineralized (total cumulative amount
225 mineralized during the 21 days incubation) was between 10% (root) and 26% (grain)
226 of the added plant residue (Fig. 1).
227 **3.2. Original amino sugar concentration**
228 The original concentration of amino sugar was higher for no-till site compared to

229 conventional tilled one ($F = 14$, $P < 0.001$) (Table 4). The highest amounts were found
230 for glucosamine (3781 ± 66 nmol g^{-1} dry soil for the no-till treatment and 2985 ± 199
231 nmol g^{-1} dry soil for the conventional till treatment) followed by galactosamine (1937
232 ± 102 (no-till) and 1742 ± 110 nmol g^{-1} dry soil (conventional till))(Table 3) and
233 lowest for muramic acid (657 ± 240 (no-till) and 522 ± 171 nmol g^{-1} dry soil
234 (conventional till)) ($F = 240$, $P < 0.001$) (Table 4). Additionally, there was a small but
235 significant effect of the tillage treatment on amino sugar type ($F = 4.13$, $P < 0.05$), with
236 larger differences in concentration for the different amino sugars in the no-till soil (F
237 = 217) compared to conventional tilled soil ($F = 73$) (Table 4).

238 **3.3. Model approach for parameter estimation of residue-derived amino sugar**
239 **formation kinetics**

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

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

247 $AS_R = AS_{R,Max} \cdot (1 - e^{-k \cdot t})$ **(5)**

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

254 $MPT = \frac{1}{k}$ **(6)**

255 The $AS_{R,Max}$ of glucosamine and galactosamine ranged from 40 till 319 nmol g^{-1} and
256 14 till 98 nmol g^{-1} respectively. MPT of glucosamine and galactosamine ranged from
257 1.6 to 3.7 days and from 2.1 to 8.3 days respectively (Table 3).

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

264

265 **3.4. Effect of residue quality, amino sugar type and tillage treatment**

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

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

271 There was a significant effect of amino sugar type, residue quality and tillage history
272 on $AS_{R,Max}$. The formation of the different amino sugars followed dissimilar kinetics,
273 with greatest $AS_{R,Max}$ values obtained for glucosamine (40 to 319 nmol g^{-1} dry soil)
274 than for galactosamine (14 to 98 nmol g^{-1} dry soil) (Table 3 and 4). The comparison of
275 $AS_{R,Max}$ values among residue quality indicated that the values were greater for grain
276 (198 nmol g^{-1} dry soil, averaged over all amino sugar type-tillage combinations)
277 followed by leaf (137 nmol g^{-1} dry soil) and root (45 nmol g^{-1} dry soil) (Table 3 and 4).
278 The no-till soil (134 nmol g^{-1} dry soil, averaged over all amino sugar type-residue
279 combinations) generally showed greater $AS_{R,Max}$ values than conventional tilled one
280 (119 nmol g^{-1} dry soil). Additionally, significant interactive effects between amino
281 sugar type and residue quality (F = 130), residue quality and tillage history of the soil
282 sample (F= 19.1) (Table 4) were observed. The interaction between amino sugar type
283 and residue quality indicated that the differences in $AS_{R,Max}$ between glucosamine and
284 galactosamine were more prominent for grain incubations (F = 722) than for leaf (F =
285 337) and root (F =45.6), while quality of residue had a greater effect on glucosamine

286 (F = 287) than on galactosamine (F = 221) (Table 3 and 4). The interaction of residue
287 quality and tillage treatment of the soil sample revealed a greater effect of residue
288 quality in the conventional tilled soil (F = 391) compared to no-till soil (F = 121).
289 Tillage history of the soil sample had a larger effect for root (F = 28.5) than for leaf (F
290 = 19.3) while the effect of tillage history was inverted for grain incubated samples
291 $AS_{R,Max}$ was highest for the conventional tilled soil (F = 7.4) (Table 4).

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

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

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

301 The effects of the tested factors on the ratio $AS_{R,Max}$ to original amino sugar were, in
302 general, similar to the effects observed on $AS_{R,Max}$ (Table 4). Exceptions were the
303 absence of the effect of tillage history and an additional interaction between amino
304 sugar type and tillage history (F = 32.3) with a larger tillage effect on glucosamine (F
305 = 5.52) formation than on galactosamine (F = 0.562) (Table 4).

306

307 **4. Discussion**

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

313 on endogenous SOM during exponential microbial activity following residue addition
314 (Amelung et al. 2008).
315 The MPT of the amino sugar, defined here as the average time needed to form "*de*
316 *novo*" residue derived amino sugar during peak microbial activity following plant
317 residue addition, varied between 2.1 and 9.3 days. Maxima of the exponential
318 microbial amino sugars formation were attained within ca. one week after which the
319 residue derived amino sugars formation reached a steady state (Table 3, Fig. 2).
320 Decock et al. (2009) also revealed a maximum $\delta^{13}C$ in glucosamine and
321 galactosamine within one week after incubation using ^{13}C-labeled wheat residues.
322 Liang et al. (2007) reported that amino sugar content in black soil reached a maximum
323 within 3 weeks upon incubation with maize residue, thereby increasing the original
324 amino sugar content with one third.

325 **4.1. Effect of amino sugar type**

326 As muramic acid exclusively originates from bacterial cell wall, while glucosamine
327 and galactosamine are present in both bacterial as fungal residues (Amelung et al.
328 2001 and 2008; Glaser and Gross 2005; Engelking et al. 2007), muramic acid is the
329 preferred biomarker to differentiate bacterial and fungal activity for incorporation of
330 residue-derived carbon. Unfortunately, due to a higher uncertainty on the muramic
331 acid measurements and a high ^{13}C-muramic acid contamination of the plant residues
332 the formation dynamics of muramic acid could not be determined.
333 The $AS_{R,Max}$ relative to the original soil amino sugar concentration ranged from 1.3 to
334 11% for glucosamine followed by galactosamine (0.8 to 5.7%). A similar trend was
335 also observed in other studies (Glaser and Gross 2005; Engelking et al. 2007; He et al.
336 2011b).
337 Bacteria and fungi both produce glucosamine and galactosamine (Amelung et al. 2001
338 and 2008; Glaser and Gross 2005). However, the strong amino sugar-type effect on
339 $AS_{R,Max}$ relative to the original amino sugar concentration, and on the MPT, indicate
340 that these amino sugars are formed through dissimilar processes. Engelking et al.
341 (2007) reviewed the available literature on amino sugar concentrations in cultured
342 bacteria and fungi, which revealed that the galactosamine/glucosamine ratio appeared

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

351 **4.2. Effect of Residue quality**

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

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

370 **4.3. Effect of the site's tillage history**

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

373 community differences typically found in not tilled soil. Fungi, showing a great ability
374 to decompose more recalcitrant substrates (Acosta-Martinez et al. 2003; Ding et al.
375 2010; Werth and Kuzyakov 2010), are typically more abundant in the no till soils (Fu
376 et al. 2000; Thiet et al. 2006; White and Rice 2009).
377 Furthermore comparing the amount amino sugars formed during peak microbial
378 activity ($AS_{R,Max}$ of glucosamine + $AS_{R,Max}$ of galactosamine) relative to the readily
379 available carbon of the residue (estimated by C_M), the no-till soil showed an enhanced
380 ability to form more amino sugars from the readily available carbon of the lower
381 quality residues (leaf and root) while this was not different for grain.
382 The difference in microbial community in both soils was, however, not significantly
383 reflected in the ratio of the masses of fungal and bacterial residues per g of soil,
384 estimated according to van Groenigen et al. (2010). This ratio turned out to be equal
385 (0.53) for both soils with different tillage history, probably due to the relative short
386 time lap since the conversion of tillage management compared to the relative long
387 residence time of amino sugars in soils after cell death (Glaser and Gross 2005) and to
388 the larger uncertainty on the muramic acid concentrations.
389 Crop residue input is not different between conventional and no-till treatment (Table
390 1), but tillage redistribute the residue input over the plow depth. Therefore, the annual
391 C input in the 0-10 cm topsoil is around 0.7 mg C g^{-1}dry soil higher for the not tilled
392 site, assuming an evenly distributed of the crop residue over the entire plowing depth
393 in the tilled site. The annual net difference of amino sugar content between the tilled
394 and the no-till site was calculated as 6.7 ± 1.8% for glucosamine, 2.8 ± 2.2% for
395 galactosamine and 6.5 ± 14% for muramic acid. Meanwhile, the average proportion of
396 $AS_{R,Max}$ to the original amino sugar content for leaf and root (grain was left out since
397 it is not incorporated *in situ*) was respectively 3.9 % for glucosamine, 2.6 % for
398 galactosamine upon crop residues in this study.
399 Both from field measurement and laboratory incubations we observed that the relative
400 change of galactosamine was significantly smaller than for glucosamine (Table 4),
401 indicating that the more conservative response of galactosamine upon shift in tillage
402 was (at least partially) due to a lower formation of residue derived galactosamine

403 compared to glucosamine after receiving increased residue input in the no-till
404 treatment.
405
406 **5. Conclusions:**
407 A first-order kinetic model could describe residue derived amino sugar formation,
408 which reached a maximum and steady state several days after residue addition.
409 During peak microbial activity *de novo* residue derived amino sugar formation was
410 surprisingly fast, giving shorter mean production times (MPT) for glucosamine (2.1 –
411 5.0 days) than for galactosamine (2.5 – 9.3 days). The faster incorporation of residue
412 carbon into glucosamine compared to the dominantly fungal galactosamine,
413 underpinned the role of bacteria as 'fast energy channel' described by Rousk and
414 Bååth (2007). In addition the *de novo* amino sugar formation relative to original
415 amino sugar pool was higher for glucosamine than for galactosamine, however this
416 difference declined strongly with decreasing residue quality, confirming the better
417 adaptation of fungal communities to colonize more recalcitrant C sources. Finally, the
418 influence of tillage history on *de novo* amino sugar formation indicated a better
419 adaptation of soil microbial community in the no-till treatment compared to
420 conventional tillage to incorporate carbon originating from more recalcitrant plant
421 residues.
422
423 **6. Acknowledgements**
424 We thank Katja Van Nieuland and Jan Vermeulen for ^{13}C analysis of bulk soil and
425 CO_2. We would like to acknowledge Karolien Denef and Joan Six for providing the
426 labeled plant material. Dries H. is a postdoctoral fellow of the Fund for Scientific
427 Research - Flanders (FWO). Zhen Bai's scientific mission was financed by the
428 National Natural Science Foundation of China and the "Departement Onderwijs en
429 Vorming" of the Flemish Government. Finally the authors would like to express their
430 gratitude to Prof. Dr. J. Schimel and two anonymous reviewers for their very valuable
431 remarks and comments.
432

433 **7. References**
434

435 Acosta-Martinez, V., Zobeck, T.M., Gill, T.E., Kennedy, A.C., 2003. Enzyme
436 activities and microbial community structure in semiarid agricultural soils. Biology
437 and Fertility of Soils 38, 216-227.
438 Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to
439 warming dependent on microbial physiology. Nature Geoscience 3, 336-340.
440 Amelung, W., Brodowski, S., Sandhage-Hofmann, A., Bol, R., 2008. Combining
441 Biomarker with Stable Isotope Analyses for Assessing the Transformation and
442 Turnover of Soil Organic Matter. Advances in Agronomy, Vol 100 100, 155-250.
443 Amelung, W., Miltner, A., Zhang, X., Zech, W., 2001. Fate of microbial residues
444 during litter decomposition as affected by minerals. Soil Science 166, 598-606.
445 Appuhn, A., Joergensen, R.G., Raubuch, M., Scheller, E., Wilke, B., 2004. The
446 automated determination of glucosamine, galactosamine, muramic acid, and
447 mannosamine in soil and root hydrolysates by HPLC. Journal of Plant Nutrition
448 and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 167, 17-21.
449 Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.H., Kuzyakov, Y., 2007. Priming
450 effects in Chernozem induced by glucose and N in relation to microbial growth
451 strategies. Applied Soil Ecology 37, 95-105.
452 Bell, J.M., Smith, J.L., Bailey, V.L., Bolton, H., 2003. Priming effect and C storage in
453 semi-arid no-till spring crop rotations. Biology and Fertility of Soils 37, 237-244.
454 Bodé, S., Denef, K., Boeckx, P., 2009. Development and evaluation of a
455 high-performance liquid chromatography/isotope ratio mass spectrometry
456 methodology for delta(13)C analyses of amino sugars in soil. Rapid
457 Communications in Mass Spectrometry 23, 2519-2526.
458 Boeckx, P., Van Nieuland, K., Van Cleemput, O., 2011. Short-term effect of tillage
459 intensity on N2O and CO2 emissions. Agronomy for Sustainable Development 31,
460 453-461
461 Decock, C., Denef, K., Bode, S., Six, J., Boeckx, P., 2009. Critical assessment of the
462 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.

493 He, H.B., Li, X.B., Zhang, W., Zhang, X.D., 2011a. Differentiating the dynamics of
494 native and newly immobilized amino sugars in soil frequently amended with
495 inorganic nitrogen and glucose. European Journal of Soil Science 62, 144-151.
496 He, H.B., Xie, H.T., Zhang, X.D., Wang, Y.H., Wu, Y.Y., 2005. A gas
497 chromatographic/mass spectrometric method for tracing the microbial conversion
498 of glucose into amino sugars in soil. Rapid Communications in Mass Spectrometry
499 19, 1993-1998.
500 He, H.B., Zhang, W., Zhang, X.D., Xie, H.T., Zhuang, J., 2011b. Temporal responses
501 of soil microorganisms to substrate addition as indicated by amino sugar
502 differentiation. Soil Biology & Biochemistry 43, 1155-1161.
503 Liang, C., Balser, T.C., 2010. Mass spectrometric characterization of amino sugar
504 aldononitrile acetate derivatives used for isotope enrichment assessment of
505 microbial residues. Soil Biology & Biochemistry 42, 904-909.
506 Liang, C., Zhang, X.D., Rubert, K.F., Balser, T.C., 2007. Effect of plant materials on
507 microbial transformation of amino sugars in three soil microcosms. Biology and
508 Fertility of Soils 43, 631-639.
509 Marx, M., Buegger, F., Gattinger, A., Zsolnay, A., Munch, J.C., 2010. Determination
510 of the fate of regularly applied (13)C-labeled-artificial-exudates C in two
511 agricultural soils. Journal of Plant Nutrition and Soil Science 173, 80-87.
512 Mauck, J., Chan, L., Glaser, L., 1971. Turnover of the cell wall of Gram-positive
513 bacteria. J Biol Chem 246, 1820-1827.
514 Myers, R.T., Zak, D.R., White, D.C., Peacock, A., 2001. Landscape-level patterns of
515 microbial community composition and substrate use in upland forest ecosystems.
516 Soil Science Society of America Journal 65, 359-367.
517 Park, J.T., 2001. Identification of a dedicated recycling pathway for
518 anhydro-N-acetylmuramic acid and N-acetylglucosamine derived from Escherichia
519 coli cell wall murein. Journal of Bacteriology 183, 3842-3847.
520 Perelo, L.W., Munch, J.C., 2005. Microbial immobilisation and turnover of (13)C
521 labelled substrates in two arable soils under field and laboratory conditions. Soil
522 Biology & Biochemistry 37, 2263-2272.

523 Rousk, J., Bååth, E., 2007. Fungal and bacterial growth in soil with plant materials of
524 different C/N ratios. Fems Microbiology Ecology 62, 258-267.
525 Reinhold-Hurek, B., Hurek, T., 2011. Living inside plants: bacterial endophytes.
526 Current Opinion in Plant Biology 14, 435-443.
527 Sauheitl, L., Glaser, B., Bol, R., 2005. Short-term dynamics of slurry-derived plant
528 and microbial sugars in a temperate grassland soil as assessed by
529 compound-specific delta C-13 analyses. Rapid Communications in Mass
530 Spectrometry 19, 1437-1446.
531 Thiet, R.K., Frey, S.D., Six, J., 2006. Do growth yield efficiencies differ between soil
532 microbial communities differing in fungal: bacterial ratios? Reality check and
533 methodological issues. Soil Biology & Biochemistry 38, 837-844.
534 van Groenigen, K.J., Bloem, J., Bååth, E., Boeckx, P., Rousk, J., Bode, S., Forristal,
535 D., Jones, M.B., 2010. Abundance, production and stabilization of microbial
536 biomass under conventional and reduced tillage. Soil Biology & Biochemistry 42,
537 48-55.
538 Waldrop, M.P., Firestone, M.K., 2004. Microbial community utilization of recalcitrant
539 and simple carbon compounds: impact of oak-woodland plant communities.
540 Oecologia 138, 275-284.
541 Werth, M., Kuzyakov, Y., 2010. (13)C fractionation at the root-microorganisms-soil
542 interface: A review and outlook for partitioning studies. Soil Biology &
543 Biochemistry 42, 1372-1384.
544 White, P.A., Rice, C.W., 2009. Tillage Effects on Microbial and Carbon Dynamics
545 during Plant Residue Decomposition. Soil Science Society of America Journal 73,
546 138-145.
547 White, R.J., 1968. Control of Amino Sugar Metabolism in Escherichia Coli and
548 Isolation of Mutants Unable to Degrade Amino Sugars. Biochemical Journal 106,
549 847-858.
550

551 ## 8. Appendix 1: mean production time

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

557 $(AS_{R,Max} - AS_R)_{(t)} = AS_{R,Max} \cdot e^{-k \cdot t}$ (7)

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

560 $$\frac{d(AS_{R,Max} - AS_R)}{dt} = -k \cdot (AS_{R,Max} - AS_R) \quad (8)$$

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

564 $$d(AS_R) = k \cdot (AS_{R,Max} - AS_R) dt \quad (9)$$

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

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

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

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

570 And by integration by parts.

571 $$MPT = \frac{1}{k} \quad (13)$$

572