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## Mineral nitrogen forms alter C-14-glucose mineralisation and nitrogen transformations in litter and soil from two sugarcane fields

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1 **Mineralisation and sorption of dissolved organic nitrogen compounds in litter and soil from**  
2 **sugarcane fields**

3

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27 **ABSTRACT**

28 Dissolved organic nitrogen (DON) represents an important soluble nutrient pool in soil, however,  
29 little is known about the dynamics of DON in the litter and topsoil of Brazilian sugarcane (*Saccharum*  
30 spp.) fields, particularly those that are harvested mechanically, without burning. Therefore, the aim  
31 of this study was to determine the microbial mineralisation and sorption **affinity** of DON compounds  
32 in litter and soil from the litter-soil transition zone of two sugarcane plantations located in  
33 southeastern Brazil. We directly measured the C mineralisation of <sup>14</sup>C-labelled amino acids (mix of  
34 17 amino acids), peptides (L-Ala-Ala and L-Ala-Ala-Ala), urea, and protein (isolated from tobacco  
35 leaves) by capturing <sup>14</sup>CO<sub>2</sub> evolved from the litter and soil over 168 h. A sorption assay was  
36 performed using the same treatments. We found differences in the organic and mineral N pools of the  
37 litter and soil, as well as in microbial community composition. Except for protein **in the soil**, the DON  
38 compounds were taken up rapidly by microbes. However, the C use efficiency was higher for the  
39 amino acid mix than for the peptides and urea, indicating more rapid post-uptake catabolism **(with**  
40 **subsequent mineralisation as <sup>14</sup>CO<sub>2</sub>) of both compounds**. In addition, protein had the highest sorption  
41 **affinity**, especially in soil, and the weak sorption affinity of the amino acids, peptides, and urea  
42 indicates moderate bioavailability of these fractions **to microbes and plants**. We conclude that **strong**  
43 **sorption of protein** to the solid phase limits its bioavailability and represents a rate limiting step in  
44 DON turnover.

45  
46 *Keywords:* Mineralisation, Sorption, Amino acid, Peptide, Urea, Protein.

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## 52 1. Introduction

53 In the southeast region of Brazil, the burning and manual harvesting of sugarcane (*Saccharum*  
54 spp.) has been extensively replaced by mechanical harvesting, without burning, owing to  
55 environmental, economic, social, and human health concerns (Galdos et al., 2013). This modern  
56 harvest system promotes the deposition of leaves and other debris on the soil surface, which can result  
57 in an annual input of between 10 and 20 Mg ha<sup>-1</sup> (dry weight) of crop residue in sugarcane fields  
58 (Leal et al., 2013). However, along with crushed bagasse, sugarcane litter can also be used in the  
59 cogeneration of heat and electricity in mills (Leal et al., 2013), and the ever-growing possibility of  
60 second generation bioethanol production from the enzymatic hydrolysis of lignocellulosic materials,  
61 including sugarcane litter, has also raised important discussions regarding the removal of the residue  
62 from sugarcane fields (Cantarella et al., 2013; Leal et al., 2013; Sordi and Manechini, 2013). The  
63 main benefits of litter deposition are related to increases in soil microbial activity, soil moisture  
64 content, soil C storage, nutrient cycling, stability of soil temperature, and erosion control (Dourado-  
65 Neto et al., 1999; Sparovek and Schnug, 2001; Cerri et al., 2011; Franco et al., 2013; Azevedo et al.,  
66 2014), whereas the disadvantages include increased incidence of some plant pests (as the litter  
67 provides a more conducive habitat for pathogen persistence; Dinardo-Miranda and Fracasso, 2013)  
68 and ammonia volatilisation from urea fertiliser (higher urease activity is reported in plant residues  
69 than in soil; Barreto and Westerman, 1989). In addition, the influence of litter deposition on the supply  
70 of N to sugarcane is another subject that has attracted interest from both scientists and farmers, owing  
71 to its role in proper N fertiliser management (Fortes et al., 2011, 2012, 2013; Trivelin et al., 2013). It  
72 is generally assumed that plant litter and humus are the two most important sources of dissolved  
73 organic matter (DOM) in soils, and its release into solution occurs through physicochemical  
74 decomposition and leaching from litter and formation of humic substances (Kalbitz et al., 2000).  
75 However, despite the low net N mineralisation of sugarcane residue (Fortes et al., 2012), studies  
76 characterizing organic N fractions contained in the litter layer that can be mineralised in the short  
77 term, to our knowledge, are scarce.

78 The last 25 years has seen a progressive shift in our understanding of terrestrial N cycling. In  
79 particular, and in contrast to the traditional paradigm of N cycling, it has been shown that a wide  
80 range of low molecular weight dissolved organic N (DON) compounds can be directly taken up by  
81 plant roots, along with inorganic forms of N ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$ ; Barak et al., 1990; Schimel and  
82 Bennett, 2004; Jones et al., 2005; Nannipieri and Paul, 2009; Kuzyakov and Xu, 2013). Although  
83 mineralisation and immobilisation processes drive nutrient availability to plants in the classical N  
84 cycle model, the depolymerisation of organic N compounds plays a key role in the N cycling in the  
85 new conceptual paradigm (Schimel and Bennett, 2004). Depolymerisation occurs through  
86 extracellular enzymes that are produced by microbes and are capable of cleaving polymers to smaller  
87 polymers or monomers. As a consequence, these low molecular weight DON compounds (e.g., amino  
88 acids and oligopeptides) can be rapidly mineralised and nitrified, or even taken up by plants in their  
89 intact forms (Schimel and Bennett, 2004; Jones et al., 2005; Hill et al., 2011). For this reason, the  
90 contribution of organic N from litter to the N supply of growing sugarcane might have been  
91 underestimated and should be investigated more fully (Brackin et al., 2015).

92 The net N mineralisation of low molecular weight DON compounds by microbes also has an  
93 important effect on the bioavailability of inorganic N forms. Rapid cycling of amino acids and  
94 peptides has been extensively observed in temperate soils, using  $^{14}\text{C}$  tracers to measure C  
95 mineralisation (Jones and Kielland, 2002; Jones et al., 2004; Jones et al., 2009; Farrell et al., 2011;  
96 Glanville et al., 2012; Wilkinson et al., 2014). The rapid mineralisation of oligopeptides is explained  
97 by its intact uptake by soil microbes, including mycorrhizas, especially in N-limited ecosystems  
98 (Farrell et al., 2011; Hill et al., 2012). However, the mineralisation of urea and protein, a low and  
99 high molecular weight DON compound, respectively, is still unclear. Although the behaviour of urea  
100 as an N fertiliser has been broadly studied and recognized (Bremner, 1995), measurements of its  
101 turnover are restricted to temperate soils, where high rates of urea catabolism have been described  
102 (Nielsen et al., 1998; Glanville et al., 2012). In contrast, Jones and Kielland (2012) reported low  
103 protein mineralisation rates in a taiga forest soil, owing to the wide range of extracellular enzymes

104 required for its cleavage into monomers. Alongside the variable mineralisation of different DON  
105 compounds, the uptake of DON by microbes from the sugarcane litter may primarily provide them  
106 with C to fuel respiration, thus resulting in lower C use efficiency (*CUE*) and consequent higher C  
107 mineralisation, since the crop residue has a greater C-to-N ratio than the underlying soil (Sinsabaugh  
108 et al., 2013). In addition, distinct microbial communities between litter and soil can also affect the C  
109 and N turnover (Creamer et al., 2015).

110       Alongside mineralisation, sorption to the solid phase plays an important role in regulating the  
111 dynamics of DON in soil. There is ample evidence that sorption of DON can stabilise and promote  
112 the accumulation of organic matter in subsoil horizons, although it has also been proposed that  
113 biofilms covering mineral surfaces may counteract this to some extent (Guggenberger and Kaiser,  
114 2003; Marschner and Kaiser, 2003). Most amino acids and peptides are weakly sorbed to the soil  
115 solid phase, thus exhibiting relatively high bioavailability (Amelung et al., 2002; Roberts et al., 2007;  
116 Ge et al., 2012). On the other hand, the sorption of urea is variable and occurs through hydrogen  
117 bonding mainly from amino hydrogens, whereas protein is suggested to readily sorb to the colloid  
118 solid phase (Mitsui et al., 1960; Said, 1972; Baron et al., 1997). Meanwhile, the sorption affinity of  
119 DON compounds in the litter layer is entirely unknown. However, when the sorption equilibrium  
120 between the solid and liquid phase is changed through DON depletion, part of the sorbed fraction  
121 may be released back into solution, in order to restore the previous equilibrium. Thus, if the litter  
122 layer has a significant sorption capacity, its presence may also mitigate losses of DON which would  
123 otherwise be leached down the soil profile.

124       On the basis of the recently proposed model of the N cycle, we believe that understanding the  
125 dynamics of DON compounds in the litter and soil of sugarcane fields is essential to increasing the  
126 sustainability of sugarcane production in Brazil, as well as in other countries. Research regarding this  
127 topic could also close gaps in our current knowledge by providing additional information about the  
128 role of litter in terrestrial N cycling. Accordingly, the aim of the present study was to evaluate the  
129 reactions (C mineralisation and sorption) of <sup>14</sup>C-labelled DON compounds (amino acids, peptides,

130 urea, and protein) in litter and soil from two sugarcane fields located in Brazil. We hypothesised 1)  
131 that the C mineralisation of amino acids, peptides, and urea by litter and soil microbes would be more  
132 rapid than the mineralisation of protein, 2) that DON compounds would be taken up more slowly in  
133 soil than in litter, and 3) that the sorption **affinity** of protein would be higher than that of the other  
134 DON compounds.

135

## 136 **2. Material and methods**

### 137 *2.1. Site characteristics*

138 Litter and soil samples were collected from two sugarcane N rate-response experiments located  
139 in São Paulo, Brazil. At both experimental sites, sugarcane is planted *ca.* every six years and is  
140 harvested annually. Before crop replanting, soil tillage (ploughing, harrowing, and furrow opening),  
141 lime and gypsum application, and the sowing of atmospheric N<sub>2</sub>-fixing legume plants are usually  
142 performed.

143 Site 1 was located in Novo Horizonte (21°32'S, 49°20'W), where the sandy loam soil (825 g  
144 kg<sup>-1</sup> sand, 23 g kg<sup>-1</sup> silt, and 152 g kg<sup>-1</sup> clay; 0.0–2.5 cm soil depth) is classified as a Typic Hapludox  
145 (Soil Survey Staff, 2014). The mean annual temperature is 23.2 °C, and the mean annual precipitation  
146 is 1134 mm y<sup>-1</sup> (29-year average). The site has a long history of annual vinasse, press mud, and  
147 organo-mineral fertiliser (100 kg N ha<sup>-1</sup> y<sup>-1</sup>) application. The experiment was established in Oct. 2010,  
148 and the harvest of the 2012/2013 season crop was performed using a mechanical harvester in Aug.  
149 2013.

150 Site 2 was located in Piracicaba (22°41'S, 47°38'W), where the clay soil (297 g kg<sup>-1</sup> sand, 87 g  
151 kg<sup>-1</sup> silt, and 617 g kg<sup>-1</sup> clay; 0.0–2.5 cm soil depth) is classified as a Rhodic Eutruxox (Soil Survey  
152 Staff, 2014). The mean annual temperature is 21.6 °C, and the mean annual precipitation is 1230 mm  
153 y<sup>-1</sup> (81-year average). There is no recent record of by-product or manure amendment at this site. The  
154 experiment was established in Apr. 2010, and the harvest of 2012/2013 season crop was performed  
155 by hand in Sep. 2013.

156

157 *2.2. Sampling procedure and characterisation of the litter and soil*

158 Litter and soil were sampled in early Nov. 2013 from control plots (no N applied;  $n = 4$ ) at both  
159 sites. Nine individual samples of litter or soil were randomly taken at 20 cm from plant rows in each  
160 control plot and were then combined and mixed to generate a composite sample. Litter samples were  
161 collected by hand, discarding the newest superficial litter and sampling the **partially decomposed**  
162 plant material at 2.5 cm above the litter-soil transition zone, and were cut into small pieces ( $\sim 0.75$   
163  $\text{cm}^2$ ) to ensure homogeneity. Soil samples were collected to a depth of 0.0-2.5 cm using a 5.5 cm i.d.  
164 stainless steel corer, and the soil cores were gently passed through an 8-mm mesh sieve to remove  
165 any plant matter. For subsequent experimentation, **litter and soil** samples were pre-incubated at a  
166 water holding capacity of 60% for 14 d at  $20 \pm 1$  °C (mean  $\pm$  SEM) and were rewetted when necessary.  
167 Except for total C, total N, moisture content, and phospholipid fatty acid (PLFA) analysis, all the  
168 remaining determinations were evaluated on a volume basis (expressed in  $\text{cm}^3$  or  $\text{dm}^3$ ) due to the  
169 difference in bulk density of litter and soil.

170 Both pH and electrical conductivity of the **litter and soil** were determined from a single sample  
171 [1:2.5 (v/v) ratio of litter or soil to distilled water]. Cation exchange capacity was determined by the  
172 unbuffered salt extraction method of Sumner and Miller (1996). Moisture content was determined  
173 gravimetrically by drying the litter and soil at 80 and 105 °C, respectively, for 24 h. Total C and N  
174 were determined by dry combustion with a CHN-2000 analyser (LECO Corp., St. Joseph, MI, USA).  
175 Basal respiration was measured using an automated SR1-IRGA multichannel soil respirometer (PP-  
176 Systems, Hitchin, UK) at 20 °C. Active C, represented by permanganate oxidisable C (Culman et al.,  
177 2012), was determined *via* oxidation with  $\text{KMnO}_4$  and colour measurement with a 96-well microplate  
178 spectrophotometer (Biotek PowerWave HT; Biotek Instruments Inc., Winooski, VT, USA). Basic  
179 properties of the litter and soil from the two experimental sites are presented in Table 1.

180

181 *2.3. Extract preparation and chemical analysis*



182 For the extraction procedure, 6 cm<sup>3</sup> of litter or soil were shaken with either 30 mL of distilled  
183 water or 0.5 M K<sub>2</sub>SO<sub>4</sub> for 15 min on a reciprocating shaker (Unimax 2010; Heidolph Elektro GmbH  
184 & Co. KG, Kelheim, Germany) at 200 rev min<sup>-1</sup>. To **minimise** losses in DOC and DON from  
185 microbial transformation during the extraction process, extraction was performed at 4 °C (Rousk and  
186 Jones, 2010). After shaking, the extracts were centrifuged at 8000 × g for 10 min **to remove suspended**  
187 **solids**. The supernatant was filtered using Whatman GD/X syringe filters (PTFE membrane, pore size  
188 of 0.2 µm; GE Healthcare Life Sciences, Buckinghamshire, UK) to remove particulate material,  
189 including microbial cells, and was stored in polypropylene bottles at 4 °C until analysis.

190 The NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N content of the extracts was determined colorimetrically using the  
191 previously mentioned microplate spectrophotometer, following the protocols of Mulvaney (1996) and  
192 Miranda et al. (2001), respectively. Total inorganic N (TIN) was calculated as the sum of NO<sub>3</sub><sup>-</sup>-N  
193 and NH<sub>4</sub><sup>+</sup>-N. Dissolved organic C (DOC) and total dissolved N (TDN) were measured using a multi  
194 N/C 2100S TOC-TN analyser (Analytik Jena AG, Jena, Germany), and DON was calculated as the  
195 difference between TDN and TIN. Total free amino acids (TFAAs) were quantified by the  
196 fluorometric *o*-phthalaldehyde-β-mercaptoethanol procedure (Jones et al., 2002), and total dissolved  
197 phenolics were determined *via* reaction with Folin-Ciocalteu reagent (Swain and Hillis, 1959).

198

#### 199 2.4. Microbial phospholipid fatty acid

200 The microbial community structure of the soil and litter samples was measured by PLFA  
201 analysis, according to the MIDI-FA protocol (Buyer and Sasser, 2012). Briefly, 2 g of litter and soil  
202 samples (15 g) that had been stored at -20 °C since collection were placed in test tubes, dried  
203 overnight, and then subject to a Bligh-Dyer lipid extraction. The extract was sonicated, centrifuged,  
204 dried, dissolved in chloroform, and loaded onto a 96-well solid phase extraction plate (Phenomenex,  
205 Torrance, CA, USA). To form the fatty acid methyl esters required for further analysis, the extracted  
206 phospholipids were eluted into glass vials in a 96-well format, dried, and transesterified. The resulting  
207 fatty acid methyl esters were then detected using capillary gas chromatography with a flame

208 ionization detector (Agilent 6890; Agilent Technologies, Wilmington, DE, USA) and separated on  
209 an Agilent Ultra 2 column (25 m long  $\times$  0.2 mm internal diameter  $\times$  0.33  $\mu$ m film thickness).

210 The **PLFAs** were summed into the following biomarker groups: i) eukaryotes: polyunsaturated  
211 fatty acids (Zelles, 1999); ii) arbuscular mycorrhizal fungi (AMF): 16:1 $\omega$ 5c (Frostegård et al., 2011);  
212 iii) gram-positive bacteria: iso- and anteiso-series saturated branched fatty acids (Zelles, 1999); iv)  
213 gram-negative bacteria: monounsaturated fatty acids and cyclopropyl 17:0 and 19:0 (Zelles, 1999);  
214 v) actinobacteria: 10-methyl branched fatty acids (Zelles, 1999); vi) fungi: 18:2 $\omega$ 6c (Frostegård and  
215 Bååth, 1996); and vii) anaerobic bacteria: 14:1 $\omega$ 7cDMA, i15:0DMA, 16:1 $\omega$ 7cDMA, 18:0DMA,  
216 18:2DMA, and 19:0cyclo9,10DMA (Frostegård et al., 1991; Zelles, 1997, 1999). **However, these**  
217 **biomarkers are not entirely specific for their taxonomic groups and therefore must be interpreted with**  
218 **some caution (Frostegård et al., 2011).**

219

#### 220 *2.5. Mineralisation and microbial uptake of dissolved organic nitrogen compounds*

221 To investigate the C mineralisation of DON compounds in litter and soil from sugarcane fields,  
222 we performed a fully randomised 2  $\times$  4 factorial design experiment, with four replicates. The first  
223 factor comprised the media (litter and soil), and the second factor comprised four  $^{14}$ C-labelled DON  
224 compounds, which included a mix of 17 amino acids (U- $^{14}$ C), peptides (50% L-Ala-Ala and 50% L-  
225 Ala-Ala-Ala: 1- $^{14}$ C), urea (U- $^{14}$ C), and protein isolated from tobacco leaves (U- $^{14}$ C; American  
226 Radiolabeled Chemicals, St Louis, MO, USA). Litter and soil (4 cm<sup>3</sup>) were transferred to 50-mL  
227 polypropylene centrifuge tubes and treated with 0.1 mL of the  $^{14}$ C-labelled DON compounds (0.1  
228 mM), which each had specific activity of 41.7 kBq dm<sup>-3</sup>. The amount of DON was chosen based upon  
229 the average size of this pool in the soil (Jones et al., 2005). The amino acid mixture contained the  
230 following L-isomeric amino acids in equimolar proportions: alanine, arginine, aspartic acid, cysteine,  
231 glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline,  
232 serine, threonine, tyrosine, and valine.

233 To trap any respired (evolved)  $^{14}\text{CO}_2$  from the samples, a polypropylene vial containing 1 mL  
 234 of 1 M NaOH was placed above the litter or soil, and the tubes were hermetically sealed and  
 235 maintained at 20 °C. Loss of water from the tubes during the incubation period was negligible and,  
 236 therefore, not corrected for. To quantify the collected  $^{14}\text{CO}_2$ , the traps were changed at 2, 6, 12, 24,  
 237 48, 72, 96, 120, 144, and 168 h after the addition of  $^{14}\text{C}$ -labelled compounds. After removal, the  
 238 amount of trapped  $^{14}\text{CO}_2$  was determined using a scintillation cocktail (Scintisafe 3; Fisher Scientific  
 239 UK Ltd., Leicestershire, UK) and a Wallac 1404 liquid scintillation counter (EG&G Wallac, Milton  
 240 Keynes, UK). At the end of the incubation period, to quantify  $^{14}\text{C}$  sorbed on colloid surfaces, litter  
 241 and soil were shaken with 25 mL of chilled 0.5 M  $\text{K}_2\text{SO}_4$  (4 °C, 15 min, 200 rev  $\text{min}^{-1}$ ), as described  
 242 in Rousk and Jones (2010), and centrifuged for 5 min at  $18,000 \times g$ .  $^{14}\text{C}$  in the supernatant was  
 243 measured by scintillation counting, as described above.

244 Previous studies have indicated that the organic C substrate mineralisation of soil follows a  
 245 biphasic pattern (Chotte et al., 1998; Roberts et al., 2007; Jones et al. 2009; Farrell et al. 2011), which  
 246 can be estimated by fitting a double first-order exponential decay equation in SigmaPlot (version  
 247 11.0, 2008, Systat Software Inc., Chicago, IL, USA) where

$$248 \quad f = (a_1 \times \exp^{-k_1 t}) + (a_2 \times \exp^{-k_2 t}) \quad (1)$$

249 in which  $f$  is the amount of  $^{14}\text{C}$  remaining in the sample,  $a_1$  and  $a_2$  represent the amount of  $^{14}\text{C}$   
 250 partitioned into catabolic processes (i.e. microbial respiration) and biomass production, respectively,  
 251  $k_1$  and  $k_2$  are the rate constants for these two components, and  $t$  is time. The half-life ( $t_{1/2}$ ) of the first  
 252 mineralisation pool  $a_1$  was calculated as follows

$$253 \quad t_{1/2} = \ln(2) / k_1 \quad (2)$$

254 However, calculating the half-life for the slower second phase  $a_2$  is subject to uncertainty, since  
 255 the connectivity between pools  $a_1$  and  $a_2$  is unknown (Saggar et al., 1999; Boddy et al., 2007;  
 256 Glanville et al., 2016).

257 For the low molecular weight DON compounds, any  $^{14}\text{C}$  label not recovered in either the NaOH  
 258 traps or  $\text{K}_2\text{SO}_4$  extracts was assumed to have been immobilised by microbes. The effective ability of

259 the 0.5 M K<sub>2</sub>SO<sub>4</sub> to displace amino acids, peptides, and urea sorbed to the solid phase was postulated  
 260 by other authors (Joergensen and Brookes, 1990; Rousk and Jones, 2010; Farrell et al., 2011). Based  
 261 on the low recovery (<3% of the total) of added <sup>14</sup>C in the K<sub>2</sub>SO<sub>4</sub> extracts, a sorption component was  
 262 not used in the kinetic model. Thus, the proportion of <sup>14</sup>C used for microbial growth, termed C use  
 263 efficiency (*CUE*), was calculated as follows

$$264 \quad CUE = a_2 / (a_1 + a_2) \quad (3)$$

265 In contrast, macromolecules, such as proteins, can be aggregated and precipitated through the  
 266 salting-out phenomenon when a high concentration salt (e.g., 0.5 M K<sub>2</sub>SO<sub>4</sub>) is added in an aqueous  
 267 solution, decreasing their solubility (Grover and Ryall, 2005). We therefore tested if 0.5 M K<sub>2</sub>SO<sub>4</sub>  
 268 could be used effectively as extractant of <sup>14</sup>C-labelled protein from litter and soil, and a poor recovery  
 269 (<22% of the total) of added <sup>14</sup>C was recorded, regardless the medium (Suppl Info. S1). Due to K<sub>2</sub>SO<sub>4</sub>  
 270 interference, high uncertainty is involved in estimate the proportion of <sup>14</sup>C-labelled protein  
 271 immobilised and sorbed, not being possible to calculate the *CUE* for this DON compound.

272

### 273 2.6. Heat sterilisation prior to sorption assay

274 A sterilisation experiment was performed as a possible pre-treatment to a sorption study, using  
 275 a fully randomised 2 × 2 factorial design, with four replicates. The first factor was kept constant (litter  
 276 and soil), whereas the second factor comprised the two following levels: control (without sterilisation)  
 277 and heat sterilisation. The method is essentially identical to that described above, except that glucose  
 278 (D-glucose-1-<sup>14</sup>C) was used as the source of labile C. Sterilisation was achieved by heating  
 279 subsamples of litter and soil contained in sealed polypropylene bottles in an oven at 80 °C for 90 min.  
 280 Then incubations were conducted by adding 0.1 mL of a <sup>14</sup>C-labelled glucose solution (10 mM; 41.7  
 281 kBq dm<sup>-3</sup>) to 1.6 cm<sup>3</sup> of litter or soil, and the amount of evolved <sup>14</sup>CO<sub>2</sub> was measured after 1 h. We  
 282 found that heat sterilisation strongly reduced microbially-mediated mineralisation, regardless of the  
 283 medium at both sites (*P* < 0.001). Overall, 99% of the CO<sub>2</sub> evolution from the addition of <sup>14</sup>C-glucose

284 to the litter and soil samples was suppressed through heat sterilisation, thus enabling the use of the  
285 protocol as a pre-treatment for the sorption assay.

286

## 287 2.7. Sorption of dissolved organic nitrogen compounds

288 The sorption of organic N compounds to the solid phases of litter and soil was determined using  
289 heat-sterilised samples to prevent microbial mineralisation during the assay (Kuzyakov and Jones,  
290 2006), and the experimental design and treatments were identical to those used to measure the C  
291 mineralisation of DON compounds. Briefly, different concentrations of  $^{14}\text{C}$ -labelled DON compound  
292 solutions (amino acid mix, peptides, urea, and protein; 0-1 mM) were prepared using distilled water,  
293 and 5 mL of each  $^{14}\text{C}$ -labelled DON solution was added to  $0.8\text{ cm}^3$  of sterilised litter or soil in 50-mL  
294 sterile centrifuge tubes (sample activity of  $41.7\text{ kBq dm}^{-3}$ ). The samples containing sorption solutions  
295 were shaken for 30 min at  $200\text{ rev min}^{-1}$ , and immediately after shaking, the litter and soil suspensions  
296 were centrifuged for 5 min at  $18,000 \times g$ . The supernatant was recovered for  $^{14}\text{C}$  determination, as  
297 described above, and a linear sorption isotherm equation was fitted to the experimental data, using  
298 the following equation:

$$299 \quad S = K_d \times ESC \quad (4)$$

300 where  $S$  is the amount of solid phase sorption ( $\text{mmol dm}^{-3}$ ),  $K_d$  is the partition coefficient, and  $ESC$  is  
301 the equilibrium solution concentration at the end of the experiment (mM). Partition coefficients ( $K_d$ )  
302 were calculated as:

$$303 \quad K_d = S / ESC \quad (5)$$

304

## 305 2.8. Statistical analysis

306 Differences between the physicochemical characteristics of litter and soil (C, N, and phenolics  
307 in the extracts) and microbial community structure were determined using one-way ANOVA (fixed  
308 effects models). The  $F$ -test was used, followed by Fisher's LSD as a post-hoc test, with significance  
309 defined at  $P \leq 0.05$ , unless otherwise stated. To elucidate major variation patterns, the relative

310 concentrations (mol %) of individual PLFAs were subjected to principal component analysis. Two-  
311 way ANOVA (fixed effects models), followed by Fisher's LSD as a post-hoc test ( $P \leq 0.05$ , unless  
312 otherwise stated), was conducted to compare differences in the results of the C mineralisation,  
313 sorption affinity, and heat sterilisation assays. All statistical analyses were performed using SAS  
314 (version 9.3, 2011, SAS Institute Inc., Cary, NC, USA).

315

### 316 **3. Results**

#### 317 *3.1. Chemistry of water and potassium sulphate extracts*

318 More  $\text{NH}_4^+$ -N was detected in the water and  $\text{K}_2\text{SO}_4$  extracts of the litter than in extracts of the  
319 soil at Site 1 (Table 2). However, the  $\text{NO}_3^-$ -N content was higher in the soil at both experimental sites,  
320 regardless of the extractant (water or  $\text{K}_2\text{SO}_4$ ), as was also the case for the TIN. Much higher DOC,  
321 DON, DOC-to-DON ratio, and phenolic values were observed in the water extracts of litter than in  
322 the water extracts of soil at Site 1 (Table 2). Similar differences were also detected in the  $\text{K}_2\text{SO}_4$   
323 extracts, with the exception of the DON content, which did not differ between the extracts of litter  
324 and soil. It was not possible to compare the TFAA values at Site 1, for either extractant, owing to  
325 extremely low values that were below the detection limit. At Site 2, higher DOC, DOC-to-DON ratio,  
326 TFAA, and phenolic values were observed in the water extracts of litter than in the water extracts of  
327 soil. However, only the phenolic content was higher in the  $\text{K}_2\text{SO}_4$  extract of litter. On average, the  
328 DON represented 95 and 22% of the TDN (TIN + DON) in the water extracts of litter and soil,  
329 respectively, whereas DON represented 94 and 19% of the TDN in the  $\text{K}_2\text{SO}_4$  extracts. TFAAs  
330 represented, on average, 31 and 5% of the DON in the water extracts of litter and soil, respectively,  
331 whereas the TFAAs in the  $\text{K}_2\text{SO}_4$  extracts constituted 56 and 49% of the DON.

332 The comparison of extractants indicated a higher (134% increase)  $\text{NO}_3^-$ -N soil content at Site 2  
333 when using  $\text{K}_2\text{SO}_4$ , rather than water ( $P < 0.01$ ), and in the same soil, higher DOC, DON, DOC-to-  
334 DON ratio, and TFAA values were also found when using  $\text{K}_2\text{SO}_4$  ( $P < 0.01$ ), especially for DOC  
335 content, which was 472% higher than that of the water extract. In addition, greater TFAA content

336 was measured in the litter at both sites when extracting with  $K_2SO_4$ , rather than water ( $P < 0.01$ ).  
337 However, there were no significant differences in the values obtained by the two extractants for any  
338 of the soil attributes at Site 1 ( $P > 0.05$ ).

339

### 340 3.2. Microbial community structure

341 Principal component analysis revealed that the relative concentrations of individual PLFAs  
342 from litter and soil were compositionally distinct from each other, and together, the first and second  
343 principal components (PC1, PC2) accounted for 58% of the variation in PLFA levels (Fig. 1).  
344 Negative loadings by Gram-positive bacteria and actinobacteria biomarkers (i17:1 $\omega$ 9c and 10Me16:0  
345 fatty acid, respectively) were the most important for the strict separation of litter from soil (regardless  
346 of site) along the PC1 axis, which explained 46% of the variation. The PC2 axis described 12% of  
347 the variation, wherein negative loading by the cy17:0 $\omega$ 7c fatty acid (Gram-negative bacteria  
348 biomarker) appeared to differentiate litter of Sites 1 and 2.

349 The proportion of litter- and soil-C held by microbial groups was highest in both Gram-positive  
350 and -negative bacteria, which accounted for 72% of the total litter- and soil-derived PLFA, on average  
351 (Fig. 2). At both experimental sites, higher proportions of eukaryotes, Gram-negative bacteria, and  
352 fungi were observed in the litter than in the soil, whereas slightly greater proportions of anaerobic  
353 bacteria and AMF were detected in the litter at Sites 1 and 2, respectively. On the other hand, no  
354 differences were found for the proportions of AMF or anaerobic bacteria in the litter- and soil-derived  
355 PLFA biomarkers of Sites 1 and 2, respectively. For both sites, litter-derived PLFA showed lower  
356 proportional contributions from Gram-positive bacteria and actinobacteria to the microbial  
357 community than in soil.

358

### 359 3.3. Mineralisation and microbial uptake

360 The  $^{14}CO_2$  evolution from the  $^{14}C$ -labelled DON compounds demonstrated a biphasic pattern,  
361 which was characterized by a rapid initial phase and a slower secondary phase (Fig. 3). In addition,

362 the double first-order exponential decay equation generally fitted well to the experimental data of  $^{14}\text{C}$   
 363 mineralisation [ $r^2 = 0.990 \pm 0.001$  (mean  $\pm$  SEM)]. At the end of the experiment, the amount of  $^{14}\text{C}$   
 364 remaining in the amino acid mix (Fig. 3a) differed only at Site 2 and was 14% higher in the soil than  
 365 in the litter ( $P < 0.01$ ). Remarkable differences in peptide mineralisation occurred at both sites (Fig.  
 366 3b), in which  $^{14}\text{C}$  remaining in the soil was 151% higher, on average, than in the litter ( $P < 0.01$  for  
 367 Site 1;  $P < 0.001$  for Site 2). At Site 1, for the urea treatment, 132% more  $^{14}\text{C}$  was remaining in the  
 368 soil than in the litter ( $P < 0.05$ ; Fig. 3c). Similarly, the remaining  $^{14}\text{C}$  from protein was, on average,  
 369 38% greater in the soil than in the litter ( $P < 0.01$  for Site 1;  $P < 0.001$  for Site 2; Fig. 3d). Overall,  
 370 the remaining  $^{14}\text{C}$  content of the DON compounds exhibited the following trend in the litter: amino  
 371 acid mix  $\approx$  protein  $\gg$  peptides  $>$  urea ( $P < 0.001$ ). For the soil, however, the pattern was slightly  
 372 different: protein  $>$  amino acid mix  $>$  peptides  $\gg$  urea ( $P < 0.001$ ).

373 Most of the amino acid mix was immobilised into the microbial biomass ( $CUE \geq 0.75$ ),  
 374 regardless of the medium (Fig. 4a). In contrast, the majority of the added peptides and urea was  
 375 rapidly respired by microbes ( $CUE \leq 0.50$ ). At both sites, there was no difference between the litter  
 376 and soil for the  $CUE$  of the amino acid mix ( $P > 0.05$ ), whereas higher  $CUE$  was observed in the soil  
 377 than in the litter for peptides (increased by 85%;  $P < 0.001$ ) and urea (increased by 87%;  $P < 0.001$ ).  
 378 Overall, the  $CUE$  of urea was the lowest (varying from 0.17 to 0.35) among the DON compounds.  
 379 The mean half-lives ( $t_{1/2}$ ) of the amino acid mix, peptides, urea, and protein were  $1.3 \pm 0.1$  (mean  $\pm$   
 380 SEM);  $0.6 \pm 0.2$ ;  $0.8 \pm 0.2$ , and  $5.6 \pm 1.1$  h, respectively (Fig. 4b). For the peptides, urea, and protein,  
 381 the  $t_{1/2}$  values were higher in the soil (increased by 60%, on average) than in the litter at both  
 382 experimental sites ( $P < 0.05$ ). For the amino acid mix, however, no differences were found ( $P > 0.05$ ).

383

### 384 3.4. Sorption

385 The linear isotherm equation fitted well to the experimental data describing the sorption of  
 386 different DON compounds to the solid phase of litter and soil ( $r^2 \geq 0.945$ ; Fig. 5; Table 3). There  
 387 were no differences in the  $K_d$  of the four DON compounds in the litter layer at Site 1 ( $P > 0.05$ ; Fig.



388 5a; Table 3). In contrast, the  $K_d$  value for protein was, on average, 979, 569, and 1356% higher than  
389 the  $K_d$  values of the other DON compounds for the topsoil at Site 1 ( $P < 0.001$ ), and litter at Site 2 ( $P$   
390  $< 0.05$ ), and soil at Site 2 ( $P < 0.001$ ), respectively (Fig. 5bcd; Table 3). At both sites, a higher protein  
391  $K_d$  value was observed in the soil than in the litter ( $P < 0.001$ ), whereas the  $K_d$  value of the amino  
392 acid mix was greater in the soil than in the litter at Site 2 ( $P < 0.01$ ). For the remaining compounds  
393 (peptides and urea), no differences were found ( $P > 0.05$ ) between the soil and litter at either site.

394

## 395 4. Discussion

### 396 4.1. Litter and soil characteristics

397 The  $\text{NH}_4^+$ -N content found in the litter and soil at both sites was extremely low ( $< 1 \text{ mg dm}^{-3}$ ),  
398 indicating high consumption of this inorganic N fraction, most likely due to  $\text{NH}_4^+$  immobilisation in  
399 the high C-to-N ratio litter, and also due to nitrification in the soil. The contrasting C-to-N ratio of  
400 the litter, compared to the soil (Table 1), supports this hypothesis (Mary et al., 1996). In contrast, the  
401 much higher  $\text{NO}_3^-$ -N content compared to  $\text{NH}_4^+$ -N detected in the soil may be explained by high rates  
402 of nitrification (Mariano et al., 2015). Raison et al. (1987) also reported an increase in mineralisation  
403 and nitrification rates, owing to disturbance of soil samples and further incubation in the absence of  
404 plants. However, the lack of plant roots depleting both soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  cannot be neglected,  
405 although it should be noted that sugarcane often has a general preference for  $\text{NH}_4^+$  over  $\text{NO}_3^-$  (Hajari  
406 et al., 2014). In addition, the higher  $\text{NO}_3^-$ -N recovery from soil of Site 2 when  $\text{K}_2\text{SO}_4$  was used as  
407 extractant rather than water might indicated that this anion was weakly adsorbed on the positively  
408 charged surface of colloids, and a salt solution was required for its complete displacement (Hingston  
409 et al., 1974). A note of caution arises from the fact that  $\text{NO}_3^-$  sorption in acid soils is particularly  
410 important in subsoil horizons than topsoil (Cahn et al., 1992). Thus, the mechanism involved in the  
411 higher amount of  $\text{NO}_3^-$  extracted with  $\text{K}_2\text{SO}_4$  remains unclear for the current study.

412 The DOC, DON, DOC-to-DON ratio, and phenolic values of the litter and soil extracts are  
413 similar to other agricultural soils, whereas the TFAA content is greater (Roberts et al., 2007; Ge et

414 al., 2012; Versini et al., 2014). The higher DOC and phenolic contents in the litter than soil, regardless  
415 of the site or extractant (with exception of DOC extracted with  $K_2SO_4$  at Site 2, which is explained  
416 below) is congruent with the thought that decomposing litter is a potential source of DOM in soils  
417 (Kalbitz et al., 2000; Versini et al., 2014). The DOC-to-DON ratios, which were lower than the C-to-  
418 N ratio in the litter at both sites ( $P < 0.001$ ), indicate a greater proportion of soluble organic N than  
419 C compounds released from the crop residue, or faster microbial breakdown (biodegradability) of  
420 DOC than DON (Kalbitz et al., 2000). In addition, the higher DOC, DON, DOC-to-DON ratio, and  
421 TFAA values when using  $K_2SO_4$  than water extract in the soil of Site 2 indicate the salt solution  
422 induced displacement of DOM from solid-phase exchange sites (Jones et al., 2012). Thus, the much  
423 higher DOC content than DON found in the  $K_2SO_4$  extract suggests that high C-to-N ratio  
424 hydrophobic compounds (i.e. DOC) were strongly sorbed to soil minerals in comparison to low C-  
425 to-N ratio hydrophilic compounds (i.e. DON), which likely possess weaker or nonspecific bondings  
426 (Kalbitz et al., 2000; Guggenberger and Kaiser, 2003).

427 In terms of microbial community structure, the clear differences between sugarcane litter and  
428 soil (Fig. 1) is probably related to differences in moisture content, chemistry properties (e.g., relative  
429 proportions of lignin, cellulose, hemicellulose, and protein), total C, total N, and C-to-N ratio. The  
430 broad dominance of Gram-positive and -negative bacteria as C decomposers in the soil microbial  
431 community reported here (70% of the total soil-derived PLFA) is slightly higher than in other studies,  
432 where both microbial groups accounted for 48-60% of the total PLFA (Buyer et al., 2010; Buyer and  
433 Sasser, 2012). The bacterial dominance observed in the litter microbial community is also consistent  
434 with previous knowledge, in which plant litter with greater C-to-N ratio is dominated by bacteria,  
435 whereas fungi are the dominant decomposers of recalcitrant litter (Strickland and Rousk, 2010;  
436 Creamer et al., 2015). In addition, the greater occurrence of fungi in litter (10%, on average) than in  
437 soil (2%, on average; Fig. 2) is probably related to their abilities in degrading more recalcitrant  
438 compounds (e.g., lignin; de Boer et al., 2005) during mineralisation of plant residues. As related to  
439 the PLFA method, it should be noted that the biomarker commonly used for fungal identification

440 (18:2ω6c) is not unique to organisms from this kingdom, but is also common to other eukaryotic  
441 organisms, such as plants (Zelles, 1999; Frostegård et al., 2011). Nevertheless, Kaiser et al. (2010)  
442 demonstrated that the influence of plant sources (e.g., plant roots) is minimal.

443

#### 444 4.2. Dynamics of dissolved organic nitrogen compounds in the litter and soil

445 The  $^{14}\text{C}$  remaining and the *CUE* values for the amino acid mix (Fig. 3a; Fig. 4a) indicate that  
446 this DON compound was mostly immobilised, being used either in increasing microbial biomass or  
447 in energy storage, rather than in respiration. In contrast, peptides and urea, which exhibited lower  
448 *CUE* values (Fig. 3bc; Fig. 4a), may have followed different metabolic pathways once inside the  
449 microbial cells, being rapidly catabolised and subsequently mineralised as  $^{14}\text{CO}_2$ . Overall, a lower  
450 amount of  $^{14}\text{C}$  remained in the litter at the end of the incubation and produced a lower *CUE* than in  
451 the underlying soil. As suggested by Creamer et al. (2015), such differences in the C mineralisation  
452 of  $^{14}\text{C}$ -labelled DON compounds in litter and soil may possibly be driven by N availability (C-to-N  
453 ratio; Table 1) and microbial community structure (Figs. 1 and 2). In N-limited systems, such as  
454 sugarcane litter, *CUE* tends to decrease as the C-to-N ratio increases, since excess C is respired as  
455  $\text{CO}_2$ , rather than being used to build more microbial biomass (Sinsabaugh et al., 2013). Furthermore,  
456 the higher availability of labile C (measured as permanganate oxidisable C) in the litter than in the  
457 soil may have enhanced the growth rate of faster-growing microbes (i.e. copiotrophs), which usually  
458 dominate unstable and unpredictable environments and exhibit lower growth yield (i.e. *CUE*) than  
459 slower-growing microbes (i.e. oligotrophs), predominant in more stable ecosystems, where organic  
460 C quality and/or quantity is low (Fierer et al., 2007).

461 The  $t_{1/2}$  of the low molecular weight compounds (amino acid mix, peptides, and urea) was very  
462 short, especially for the peptides and urea in the litter, indicating that the DON pool can be very low  
463 in some situations, owing to faster uptake by microbes or plants (Jones et al., 2009; Farrell et al.,  
464 2011; Hill et al., 2011, 2012; Wilkinson et al., 2014). The remarkable rapid mineralisation of L-Ala-  
465 Ala and L-Ala-Ala-Ala suggests that the peptides were probably not deaminated before their uptake

466 by microbes. This assumption is consistent with the findings of Payne (1980), who demonstrated that  
467 some bacteria have the ability to take up intact peptides using oligopeptide-specific transport systems  
468 (Farrell et al., 2011). Thus, peptide uptake by litter and soil microbes is sufficient to dominate the  
469 flux of protein-derived N through the soil solution, without further cleavage to amino acid monomers  
470 (Hill et al., 2012). Although it should be noted that the non-homogeneous  $^{14}\text{C}$  label of peptides used  
471 here may have influenced results, other authors have also verified the rapid mineralisation of  $^{14}\text{C}$ -  
472 labelled peptides in temperate soils (Farrell et al., 2011; Ge et al., 2012; Wilkinson et al., 2014). The  
473 intense release of  $^{14}\text{CO}_2$  from the urea during the rapid mineralisation phase is associated with  
474 hydrolysis by urease and urea amidolyase (UALase; Solomon et al., 2010). In soil, urease is derived  
475 from microbes and is also found extracellularly in plant residues (Frankenberger and Tabatabai,  
476 1982). In addition, its activity is reported as being greater in plants and plant residues than in the soil  
477 (Barreto and Westerman, 1989). However, UALase is found within microbial cells, and its activity  
478 requires energy in the form of ATP (Antia et al., 1991; Hausinger, 2004). Thus, the higher urease  
479 activity and more rapid urea assimilation by microbes *via* UALase in the litter than in the soil may  
480 explain the consequent lower remaining  $^{14}\text{C}$  and *CUE* of urea in the crop residue.

481 Unlike low molecular weight DON compounds, which do not usually require catalysis by  
482 extracellular enzymes before being taken up by the microbial biomass (Boddy et al., 2007; Ge et al.,  
483 2012; Hill et al., 2012), effective protein cleavage requires a suite of enzymes, thus reducing its  
484 mineralisation rate (Jones and Kielland, 2012). This is evident in the higher  $t_{1/2}$  value of protein than  
485 that of the other DON compounds used in the present study (Fig. 4b). In addition, phenolics have  
486 been proposed to block protein breakdown by binding to the substrate and proteases (Jones and  
487 Kielland, 2012). However, the high phenolic content of the sugarcane litter apparently did not affect  
488 its breakdown, since protein mineralisation occurred more quickly in the crop residue than in the soil,  
489 which had a lower phenolic content. As suggested by Jones and Kielland (2012), the ability of  
490 phenolics to inhibit protein depolymerisation is dependent on polyphenol type, their solubility and

491 their degree of exposure (i.e. whether they are on the outside of SOM and thus capable of reaction  
492 with proteins, or whether they are soluble and can diffuse and react with insoluble proteins).

493 The much higher  $t_{1/2}$  for peptides, urea, and protein in the soil than in the litter could be attributed  
494 to the possible large proportion of oligotrophs in the topsoil, which are characterised by the long  
495 latency after substrate addition and enzyme induction rather than their constitutive production,  
496 whereas copiotrophs in plant litter have brief latency before growth and enzymes are produced  
497 constitutively (Fierer et al., 2007). The greater  $t_{1/2}$  of the protein in the soil (primarily at Site 2) than  
498 in the litter is probably also linked to the higher sorption affinity of this DON compound with soil  
499 minerals rather than organic matter (Figure 5, Table 3), being more slowly desorbed to the solution.  
500 On the other hand, the lack of difference between soil and litter for the amino acid mix is explained  
501 by the ubiquitous uptake and internal partitioning of substrate-C by the soil microbial community.

502 Sorption of DON compounds on the solid phase was not concentration-dependent and followed  
503 a similar pattern in the litter and soil, only differing in sorption potential (Fig. 5; Table 3). The higher  
504 sorption capacity of the soil than litter is probably related to the high charge density of Fe and Al  
505 oxyhydroxides, which are the largest sorbents for DOM in soils (Guggenberger and Kaiser, 2003).  
506 However, as previously discussed for  $\text{NO}_3^-$ , sorption of DOM to variable charge minerals occurs  
507 essentially in subsoil horizons (Guggenberger and Kaiser, 2003). No compound exhibited saturating  
508 sorption tendencies over the concentration range (0-1 mM), which was expected, since the  
509 concentrations were relatively low. The linear sorption isotherm equation was used, rather than the  
510 Freundlich exponential equation, since the Freundlich coefficient ( $K_f$ ) is exponential-dependent and  
511 may overestimate  $K_d$  values (Soares, 2005). According to our results, protein can readily bind to the  
512 solid phase of soil, as proposed by Baron et al. (1997) and Jones and Kielland (2012). Thus, protein  
513 leaching through the soil profile may be low or even negligible. Since the linear equation fitted well  
514 to the urea sorption data (Fig. 5c; Table 3), heat sterilisation was apparently effective in inhibiting  
515 urease activity. The amino acid mix, peptides, and urea were only weakly sorbed to the litter and soil  
516 solid phases and are, therefore, relatively bioavailable for microbes and plants. These results are in

517 agreement with other studies (Said, 1972; Baron et al., 1997; Amelung et al., 2002; Roberts et al.,  
518 2007; Ge et al., 2012). As postulated by Kalbitz et al. (2000), a major limitation of sorption assays  
519 performed under laboratory conditions relates to the use of disturbed soil samples. Under these  
520 conditions, DOC often exhibits rapid sorption with a high affinity for the solid phase, suggesting low  
521 transport rates of this organic fraction in the soil profile. However, under field conditions, the soil  
522 aggregation and flow regime can lead to low contact times between liquid and solid phase, thus  
523 diminishing DOC retention. Further work is therefore required to translate our results to the field  
524 under a range of hydrological conditions. Finally, the sorption affinity of DON compounds verified  
525 here is also supported by a previous comparison between extractants, where the  $K_2SO_4$  was able to  
526 desorb exchangeable TFAAs, DOC, and DON in the litter and soil. Jones and Willet (2006) also  
527 verified the ready desorption of DOC and DON compounds from the soil solid phase with 2 M KCl  
528 and 0.5 M  $K_2SO_4$ .

529

#### 530 *4.3. Implications for stabilisation and decomposition of dissolved organic nitrogen*

531 The results from the present study provided interesting insights regarding abiotic and biotic  
532 factors controlling the origin and transformation of DON in litter and topsoil of sugarcane fields (Fig.  
533 6). As proposed by other authors (Kalbitz et al., 2000; Guggenberger and Kaiser, 2003), we also  
534 suggest that the litter is a source of DON, whereas the soil predominantly acts as a sink, sorbing  
535 organic and inorganic compounds to the solid phase, thus reducing the potential to be leached (Fig.  
536 6). In addition, we demonstrate the dual role of the microbial community DON dynamics, namely, 1)  
537 as agents of SOM decomposition, which are important for the production of DON in the litter layer  
538 (Fig. 2); and 2) as a transient pool of organic N through which labile DON rapidly passes (Fig. 4b).  
539 The strong sorption affinity of protein with soil minerals can promote the stabilisation (preserve) of  
540 organic N (Guggenberger and Kaiser, 2003), however, its subsequent low rate of desorption may limit  
541 its potential to be depolymerised leading to a slowing of the N cycle. In contrast, the  
542 depolymerisation products (e.g., amino acids, peptides) and urea were weakly sorbed to the solid

543 phase, are more biodegradable but also susceptible to be leached to subsoil horizons. Based on the  
544  $^{14}\text{C}$  mineralisation assay, the fate of the DON compounds was essentially driven by biotic (respiration  
545 and C immobilisation by microbes) rather than abiotic factors (sorption or chemical oxidation of  
546 DON). The DON compounds had different metabolic pathways: while peptides and urea showed  
547 rapid turnover (lower half-life) and were mainly used by microbes in catabolic processes (i.e. respired  
548 to  $\text{CO}_2$ ), most of the amino acid mix was used in anabolic processes (i.e. C used for microbial growth),  
549 thus exhibiting higher turnover rate. This suggest that models of DON turnover in soil may perform  
550 better if groups of N-containing substrates are considered separately.

551

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558

## 559 **Appendix A. Supplementary data**

560 **Supplementary data related to this article can be found at [URL].**

561

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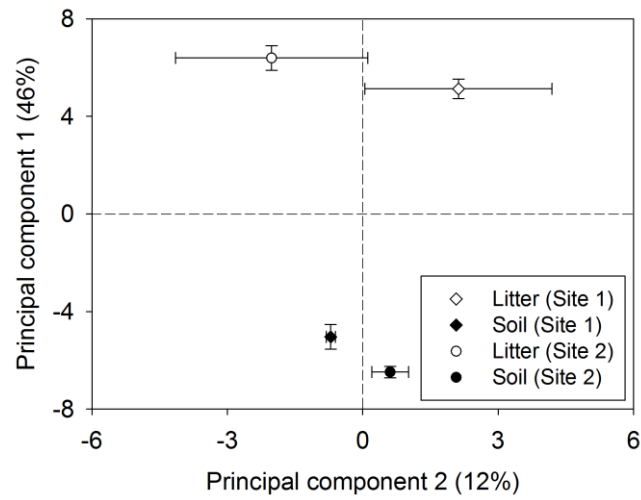
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774 **Figure captions**

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777 **Fig. 1.** Principal component analysis (PCA) of the microbial community structure based on the relative  
778 concentrations (mol %) of individual phospholipidic fatty acid biomarkers of litter and soil. Percentages in the  
779 axis labels indicate the amount of variance explained by each principal component. Symbols represent mean  
780 values, derived from the PCA with individual samples ( $n = 16$ ), whereas bi-directional error bars indicate the  
781 SEM ( $n = 4$ ) for principal components 1 and 2.

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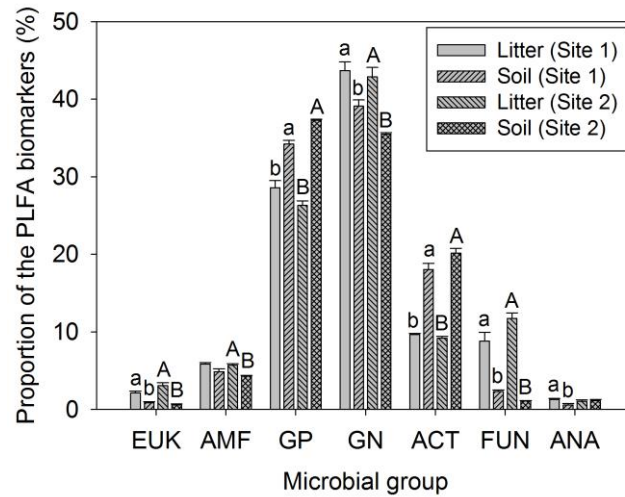
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795 **Fig. 2.** Proportional phospholipidic fatty acid (PLFA) biomarkers of litter- and soil-derived PLFA held within  
 796 microbial groups. EUK: eukaryotes; AMF: arbuscular mycorrhizal fungi; GP: gram-positive bacteria; GN:  
 797 gram-negative bacteria; ACT: actinobacteria; FUN: fungi; ANA: anaerobic bacteria. The error bars indicate  
 798 the SEM ( $n = 4$ ). Different lowercase letters indicate differences between the litter and soil at Site 1, whereas  
 799 different capital letters indicate differences between the litter and soil at Site 2, according to Fisher's LSD test  
 800 ( $P \leq 0.05$ ).

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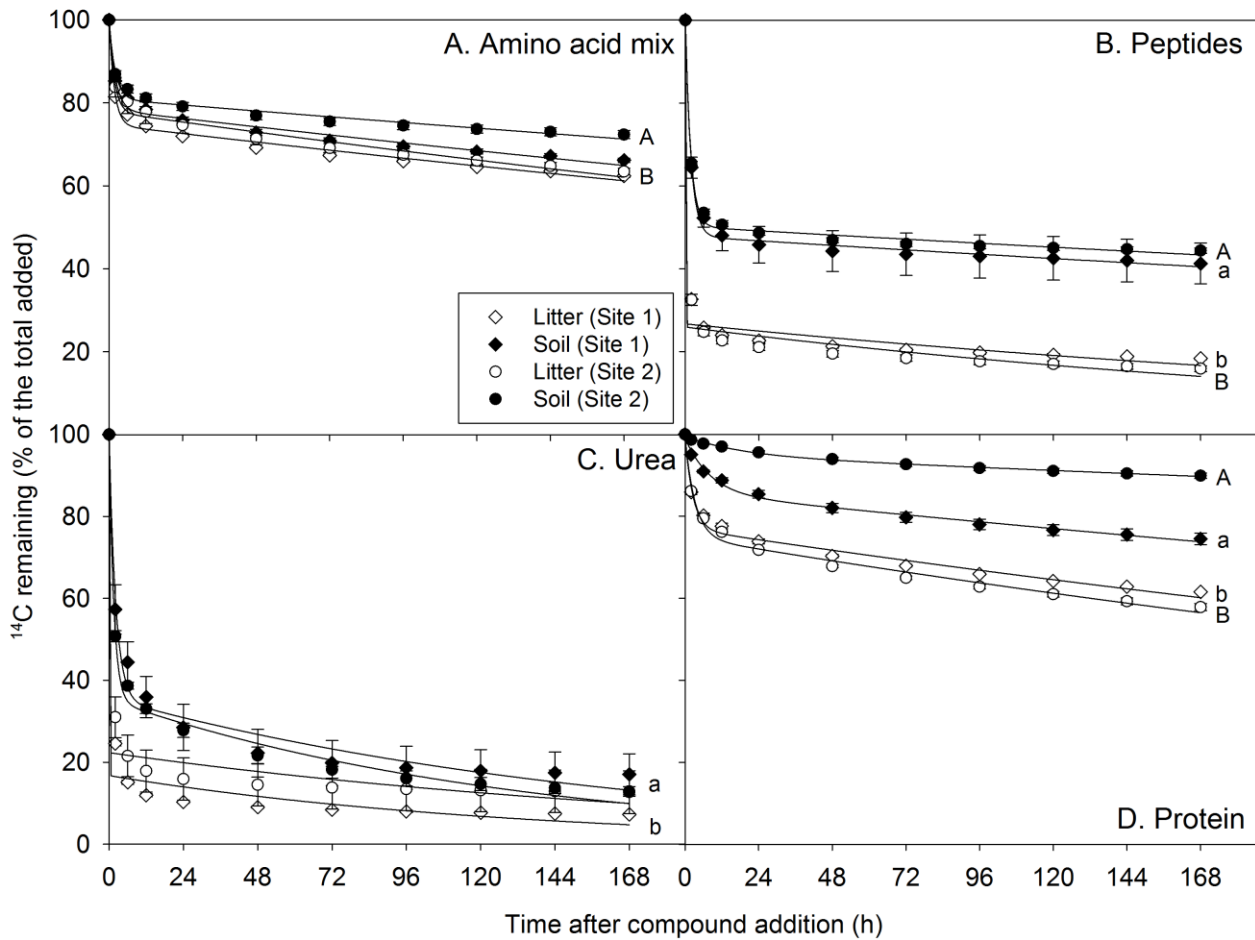
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815 **Fig. 3.** Amount of  $^{14}\text{C}$ -remaining after application of amino acid mix (A), L-Ala-Ala and L-Ala-Ala-Ala  
 816 peptides (B), urea (C), and tobacco leaf protein (D) in litter and soil following 168 h incubation. Symbols  
 817 represent mean values, and the error bars represent the SEM ( $n = 4$ ). Lines represent fits to a double first-order  
 818 exponential decay equation. Different lowercase letters indicate differences between the litter and soil at Site  
 819 1, whereas different capital letters indicate differences between the litter and soil at Site 2, according to Fisher's  
 820 LSD test ( $P \leq 0.05$ ).

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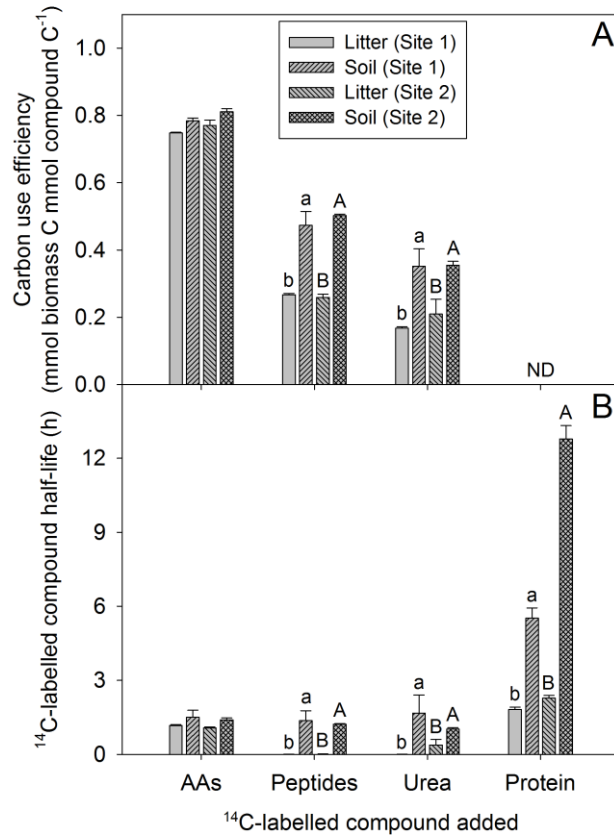
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829 **Fig. 4.** Carbon use efficiency (A) and half-life of the first mineralisable pool (B) determined by a double first-  
830 order exponential decay equation for the <sup>14</sup>C-labelled amino acid mix (AAs), peptides (L-Ala-Ala and L-Ala-  
831 Ala-Ala), urea, and protein (isolated from tobacco leaves) applied to litter and soil following 168 h incubation.  
832 The error bars indicate the SEM ( $n = 4$ ). Different lowercase letters indicate differences between the litter and  
833 soil at Site 1, whereas different capital letters indicate differences between the litter and soil at Site 2, according  
834 to Fisher's LSD test ( $P \leq 0.05$ ). **ND: not determined due to poor recovery of <sup>14</sup>C-labelled protein sorbed to the**  
835 **litter and soil solid phases when 0.5 M K<sub>2</sub>SO<sub>4</sub> is used (Suppl Info. S1).**

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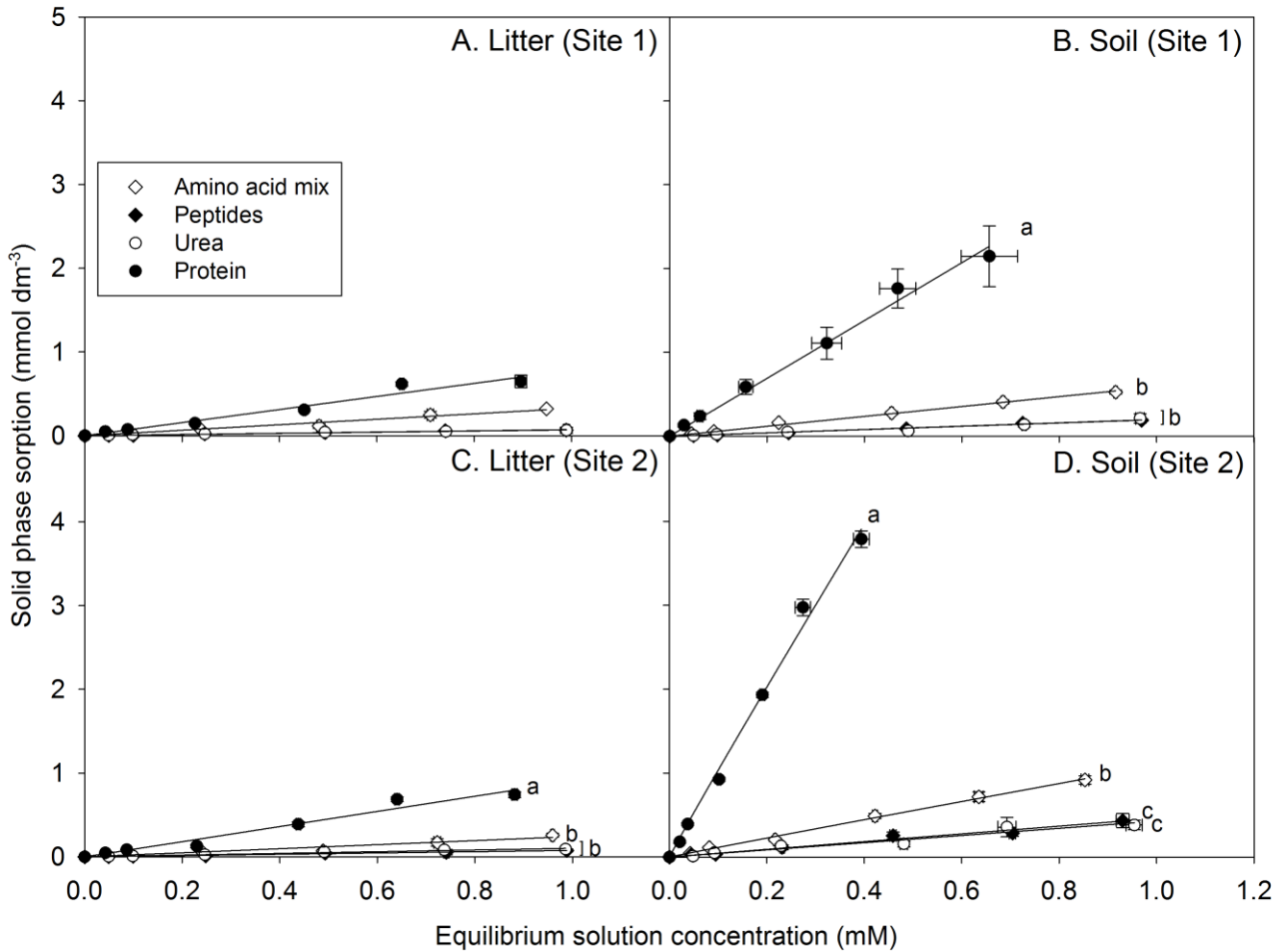
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845 **Fig. 5.** Sorption of  $^{14}\text{C}$ -labelled amino acid mix, peptides (L-Ala-Ala and L-Ala-Ala-Ala), urea, and protein  
 846 (isolated from tobacco leaves) to the solid phase of litter (A - Site 1; C - Site 2) and soil (B - Site 1; D - Site  
 847 2). Bi-directional error bars indicate the SEM ( $n = 4$ ) for sorption and solid solution concentrations. Different  
 848 lowercase letters indicate differences between the  $^{14}\text{C}$ -labelled DON compounds in the litter or soil from each  
 849 site, according to Fisher's LSD test ( $P \leq 0.05$ ).

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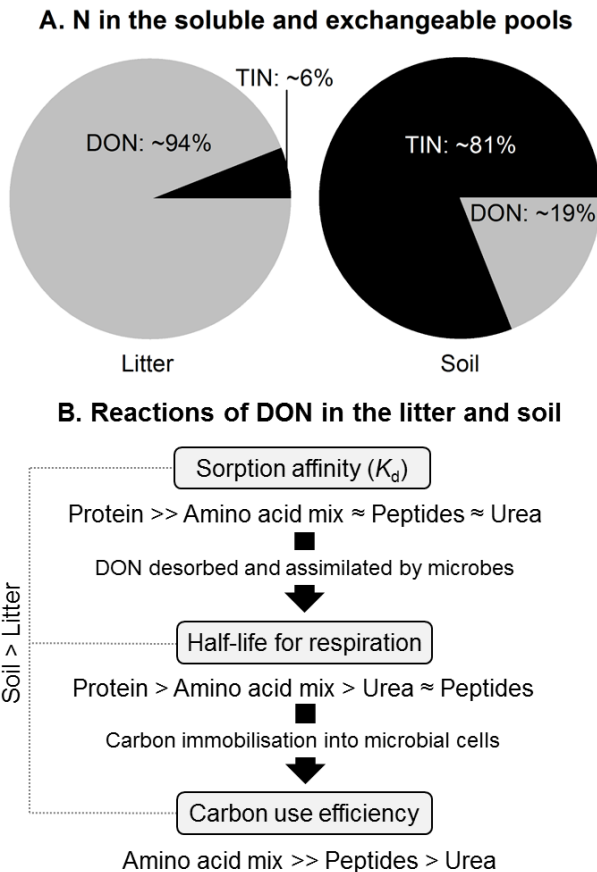
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859 **Fig. 6.** Schematic representation of the proportion of dissolved organic N (DON) and total inorganic N (TIN)

860 associated with the soluble and exchangeable pools of litter and soil from two sugarcane fields (A), and the

861 possible link between sorption, turnover rate, and immobilisation into the microbial biomass of  $^{14}\text{C}$ -labelled

862 amino acid mix, peptides (L-Ala-Ala and L-Ala-Ala-Ala), urea, and protein (isolated from tobacco leaves)

863 applied to the litter and soil (B). We suggest that the partially decomposed litter is an important source of DON

864 to the solution, whereas the soil acts as a sink, sorbing organic and inorganic compounds to the solid phase,

865 thus reducing N losses in leaching. In addition, the high sorption affinity and low mineralisation rate of the

866 protein limits its bioavailability and represents a rate limiting step in DON turnover. Exchangeable pool was

867 extracted with 0.5 M  $\text{K}_2\text{SO}_4$ . Soluble plus exchangeable pool of the litter and soil (considering both sites;  $n =$

868 6) were 6.7 and 31.4  $\text{mg N dm}^{-3}$ , respectively. Due to poor recovery of  $^{14}\text{C}$ -labelled protein sorbed to the litter

869 and soil solid phases when 0.5 M  $\text{K}_2\text{SO}_4$  is used (Suppl Info. S1), the C use efficiency of this DON compound

870 was therefore not determined.

871

872 **Tables**

873

874 **Table 1**

875 Selected properties of litter and soil (both sampled from the litter-soil transition zone at 0.0-2.5 cm depth) from under sugarcane at two experimental sites in southeastern  
 876 Brazil. Values represent means  $\pm$  SEM ( $n = 4$ ), and different lowercase letters indicate differences between the litter and soil of each site, according to Fisher's LSD  
 877 test ( $P \leq 0.05$ ). EC, electrical conductivity; CEC, cation exchange capacity; MC, moisture content after adjustment to 60% of maximum water holding capacity; and  
 878 POXC, permanganate oxidisable C.

Treatment	pH 1:2.5 water	EC $\mu\text{S cm}^{-1}$	CEC $\text{mmol}_c \text{ dm}^{-3}$	MC _____ $\text{g kg}^{-1}$ _____	Total C	Total N	C-to-N ratio	Basal respiration $\mu\text{mol CO}_2\text{-C dm}^{-3} \text{ h}^{-1}$	POXC $\text{mg C dm}^{-3}$
Site 1									
Litter	$7.3 \pm 0.2$	$213 \pm 18$	$39.3 \pm 2.7 \text{ a}$	$1202 \pm 61 \text{ a}$	$271.8 \pm 12.3$	$6.28 \pm 0.17 \text{ a}$	$43.3 \pm 1.7 \text{ a}$	$126.0 \pm 12.6 \text{ a}$	$1192 \pm 62 \text{ a}$
Soil	$6.6 \pm 0.5$	$240 \pm 26$	$27.6 \pm 2.3 \text{ b}$	$138 \pm 2 \text{ b}$	$6.7 \pm 0.4 \text{ b}$	$0.89 \pm 0.03 \text{ b}$	$7.5 \pm 0.4 \text{ b}$	$13.1 \pm 0.5 \text{ b}$	$540 \pm 37 \text{ b}$
<i>P</i> value	0.229	0.429	0.017	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Site 2									
Litter	$7.0 \pm 0.1 \text{ a}$	$102 \pm 1$	$23.8 \pm 2.5 \text{ b}$	$1551 \pm 47 \text{ a}$	$421.8 \pm 4.7 \text{ a}$	$7.86 \pm 0.57 \text{ a}$	$54.5 \pm 3.7 \text{ a}$	$158.7 \pm 5.2 \text{ a}$	$1256 \pm 54 \text{ a}$
Soil	$5.7 \pm 0.1 \text{ b}$	$120 \pm 10$	$62.4 \pm 1.5 \text{ a}$	$254 \pm 3 \text{ b}$	$13.8 \pm 0.3 \text{ b}$	$1.66 \pm 0.03 \text{ b}$	$8.3 \pm 0.1 \text{ b}$	$11.6 \pm 0.2 \text{ b}$	$546 \pm 14 \text{ b}$
<i>P</i> value	< 0.001	0.102	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

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883 **Table 2**

884 Concentrations of nitrogenous compounds, dissolved organic carbon (DOC), and phenolics in water and K<sub>2</sub>SO<sub>4</sub> extracts of litter and soil. The nitrogenous compounds  
 885 included ammoniacal nitrogen (NH<sub>4</sub><sup>+</sup>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N), total inorganic N (TIN), dissolved organic N (DON), and total free amino acids (TFAA). Values  
 886 represent means ± SEM (*n* = 4), and different lowercase letters indicate differences between the litter and soil of each site, according to Fisher's LSD test (*P* ≤ 0.05).  
 887 DOC, dissolved organic C; BDL, below the detection limit; N/A, not available.

Treatment	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	TIN mg N dm <sup>-3</sup>	DON	TFAAs	Phenolics mg dm <sup>-3</sup>	DOC mg C dm <sup>-3</sup>	DOC-to-DON ratio
Water extract								
Site 1								
Litter	0.34 ± 0.04 a	0.03 ± 0.03 b	0.37 ± 0.06 b	7.8 ± 1.0 a	2.53 ± 0.43	8.02 ± 0.90 a	131.6 ± 13.6 a	17.1 ± 1.2 a
Soil	0.18 ± 0.03 b	20.09 ± 3.12 a	20.27 ± 3.10 a	4.2 ± 0.3 b	BDL	0.40 ± 0.05 b	31.1 ± 4.3 b	7.6 ± 1.2 b
<i>P</i> value	0.021	< 0.001	< 0.001	0.014	N/A	< 0.001	0.001	0.002
Site 2								
Litter	0.13 ± 0.03	0.18 ± 0.10 b	0.31 ± 0.13 b	5.8 ± 0.9	1.76 ± 0.36 a	12.23 ± 0.87 a	135.0 ± 23.9 a	23.4 ± 1.8 a
Soil	0.20 ± 0.04	8.11 ± 2.22 a	8.32 ± 2.26 a	3.8 ± 0.9	0.21 ± 0.20 b	1.85 ± 0.21 b	25.9 ± 2.4 b	7.7 ± 1.4 b
<i>P</i> value	0.208	0.012	0.012	0.165	0.009	< 0.001	0.004	< 0.001
K <sub>2</sub> SO <sub>4</sub> extract								
Site 1								
Litter	0.33 ± 0.11 a	0.04 ± 0.02 b	0.33 ± 0.11 <b>b</b>	6.0 ± 0.9	3.76 ± 0.13	8.15 ± 0.61 a	121.3 ± 4.3 a	21.4 ± 2.6 a
Soil	0.01 ± 0.01 b	31.10 ± 5.55 a	31.11 ± 5.55 a	4.2 ± 1.2	BDL	0.82 ± 0.26 b	32.7 ± 2.1 b	9.2 ± 1.9 b
<i>P</i> value	0.023	0.001	0.001	0.297	N/A	< 0.001	0.001	0.010
Site 2								
Litter	0.11 ± 0.02	0.30 ± 0.30 b	0.41 ± 0.30 b	6.7 ± 1.0	3.40 ± 0.39	9.75 ± 0.59 a	139.7 ± 7.8	22.2 ± 3.2
Soil	0.65 ± 0.30	19.00 ± 1.22 a	19.65 ± 1.01 a	7.8 ± 0.7	3.84 ± 0.29	2.37 ± 0.59 b	148.2 ± 8.8	19.2 ± 0.7
<i>P</i> value	0.124	< 0.001	< 0.001	0.366	0.401	< 0.001	0.495	0.389

888



889 **Table 3**

890 Partition coefficient ( $K_d$ ) estimated using a linear isotherm sorption equation for the  $^{14}\text{C}$ -labelled amino acid mix, peptides (L-Ala-Ala and L-Ala-Ala-Ala), urea, and  
 891 protein (isolated from tobacco leaves) sorbed on the solid phase of litter and soil. Values represents means  $\pm$  SEM ( $n = 4$ ).  $r^2$  is the coefficient of determination for the  
 892 linear regression, and different lowercase letters indicate differences within columns, according to Fisher's LSD test ( $P \leq 0.05$ ).

DON compound	Litter (Site 1)		Soil (Site 1)		Litter (Site 2)		Soil (Site 2)	
	$K_d$ (L dm <sup>-3</sup> )	$r^2$	$K_d$ (L dm <sup>-3</sup> )	$r^2$	$K_d$ (L dm <sup>-3</sup> )	$r^2$	$K_d$ (L dm <sup>-3</sup> )	$r^2$
Amino acid mix	0.33 $\pm$ 0.01	0.974	0.59 $\pm$ 0.02 b	0.995	0.24 $\pm$ 0.02 b	0.945	1.10 $\pm$ 0.03 b	0.996
Peptides	0.08 $\pm$ 0.01	0.980	0.20 $\pm$ 0.01 b	0.988	0.07 $\pm$ 0.01 b	0.974	0.46 $\pm$ 0.05 c	0.979
Urea	0.10 $\pm$ 0.01	0.962	0.23 $\pm$ 0.02 b	0.956	0.10 $\pm$ 0.01 b	0.976	0.50 $\pm$ 0.07 c	0.947
Protein	0.78 $\pm$ 0.07	0.960	3.67 $\pm$ 0.86 a	0.991	0.91 $\pm$ 0.03 a	0.964	9.99 $\pm$ 0.53 a	0.994

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