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

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Applied Soil Ecology

13. Mar. 2024

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

2 **sugarcane fields**

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ABSTRACT

Dissolved organic nitrogen (DON) represents an important soluble nutrient pool in soil, however, little is known about the dynamics of DON in the litter and topsoil of Brazilian sugarcane (*Saccharum* spp.) fields, particularly those that are harvested mechanically, without burning. Therefore, the aim of this study was to determine the microbial mineralisation and sorption affinity of DON compounds in litter and soil from the litter-soil transition zone of two sugarcane plantations located in southeastern Brazil. We directly measured the C mineralisation of ^{14}C-labelled amino acids (mix of 17 amino acids), peptides (L-Ala-Ala and L-Ala-Ala-Ala), urea, and protein (isolated from tobacco leaves) by capturing $^{14}CO_2$ evolved from the litter and soil over 168 h. A sorption assay was performed using the same treatments. We found differences in the organic and mineral N pools of the litter and soil, as well as in microbial community composition. Except for protein in the soil, the DON compounds were taken up rapidly by microbes. However, the C use efficiency was higher for the amino acid mix than for the peptides and urea, indicating more rapid post-uptake catabolism (with subsequent mineralisation as $^{14}CO_2$) of both compounds. In addition, protein had the highest sorption affinity, especially in soil, and the weak sorption affinity of the amino acids, peptides, and urea indicates moderate bioavailability of these fractions to microbes and plants. We conclude that strong sorption of protein to the solid phase limits its bioavailability and represents a rate limiting step in DON turnover.

Keywords: Mineralisation, Sorption, Amino acid, Peptide, Urea, Protein.

1. Introduction

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

The last 25 years has seen a progressive shift in our understanding of terrestrial N cycling. In particular, and in contrast to the traditional paradigm of N cycling, it has been shown that a wide range of low molecular weight dissolved organic N (DON) compounds can be directly taken up by plant roots, along with inorganic forms of N (NH_4^+, NO_2^-, and NO_3^-; Barak et al., 1990; Schimel and Bennett, 2004; Jones et al., 2005; Nannipieri and Paul, 2009; Kuzyakov and Xu, 2013). Although mineralisation and immobilisation processes drive nutrient availability to plants in the classical N cycle model, the depolymerisation of organic N compounds plays a key role in the N cycling in the new conceptual paradigm (Schimel and Bennett, 2004). Depolymerisation occurs through extracellular enzymes that are produced by microbes and are capable of cleaving polymers to smaller polymers or monomers. As a consequence, these low molecular weight DON compounds (e.g., amino acids and oligopeptides) can be rapidly mineralised and nitrified, or even taken up by plants in their intact forms (Schimel and Bennett, 2004; Jones et al., 2005; Hill et al., 2011). For this reason, the contribution of organic N from litter to the N supply of growing sugarcane might have been underestimated and should be investigated more fully (Brackin et al., 2015).

The net N mineralisation of low molecular weight DON compounds by microbes also has an important effect on the bioavailability of inorganic N forms. Rapid cycling of amino acids and peptides has been extensively observed in temperate soils, using ^{14}C tracers to measure C mineralisation (Jones and Kielland, 2002; Jones et al., 2004; Jones et al., 2009; Farrell et al., 2011; Glanville et al., 2012; Wilkinson et al., 2014). The rapid mineralisation of oligopeptides is explained by its intact uptake by soil microbes, including mycorrhizas, especially in N-limited ecosystems (Farrell et al., 2011; Hill et al., 2012). However, the mineralisation of urea and protein, a low and high molecular weight DON compound, respectively, is still unclear. Although the behaviour of urea as an N fertiliser has been broadly studied and recognized (Bremner, 1995), measurements of its turnover are restricted to temperate soils, where high rates of urea catabolism have been described (Nielsen et al., 1998; Glanville et al., 2012). In contrast, Jones and Kielland (2012) reported low 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 ^{14}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_2-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^{-1} sand, 23 g kg^{-1} silt, and 152 g kg^{-1} 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^{-1} (29-year average). The site has a long history of annual vinasse, press mud, and

147 organo-mineral fertiliser (100 kg N ha^{-1} y^{-1}) 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^{-1} sand, 87 g

151 kg^{-1} silt, and 617 g kg^{-1} clay; 0.0–2.5 cm soil depth) is classified as a Rhodic Eutrustox (Soil Survey

152 Staff, 2014). The mean annual temperature is 21.6 °C, and the mean annual precipitation is 1230 mm

153 y^{-1} (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.

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2.2. Sampling procedure <mark>and characterisation of the litter and soil</mark>

Litter and soil were sampled in early Nov. 2013 from control plots (no N applied; *n* = 4) at both sites. Nine individual samples of litter or soil were randomly taken at 20 cm from plant rows in each control plot and were then combined and mixed to generate a composite sample. Litter samples were collected by hand, discarding the newest superficial litter and sampling the <mark>partially decomposed</mark> plant material at 2.5 cm above the litter-soil transition zone, and were cut into small pieces (~0.75 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. stainless steel corer, and the soil cores were gently passed through an 8-mm mesh sieve to remove any plant matter. For subsequent experimentation, <mark>litter and soil</mark> samples were pre-incubated at a water holding capacity of 60% for 14 d at 20 ± 1 °C (mean ± SEM) and were rewetted when necessary. Except for total C, total N, moisture content, and phospholipid fatty acid (PLFA) analysis, all the remaining determinations were evaluated on a volume basis (expressed in cm^3 or dm^3) due to the difference in bulk density of litter and soil.

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

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2.3. <mark>Extract preparation and chemical analysis</mark>

182 For the extraction procedure, 6 cm^3 of litter or soil were shaken with either 30 mL of distilled

183 water or 0.5 M K$_2$SO$_4$ for 15 min on a reciprocating shaker (Unimax 2010; Heidolph Elektro GmbH

184 & Co. KG, Kelheim, Germany) at 200 rev min^{-1}. 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 $8000 \times g$ 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$_4^+$-N and NO$_3^-$-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$_3^-$-N

193 and NH$_4^+$-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*-phthialdehyde-β-mercaptoethanol procedure (Jones et al., 2002), and total dissolved

197 phenolics were determined *via* reaction with Folin-Ciocalteu reagent (Swain and Hillis, 1959).

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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 µm 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): 16:1ω5c (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: 18:2ω6c (Frostegård and

215 Bååth, 1996); and vii) anaerobic bacteria: 14:1ω7cDMA, i15:0DMA, 16:1ω7cDMA, 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 2×4 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^3) 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^{-3}. 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) $^{14}CO_2$ 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 $^{14}CO_2$, the traps were changed at 2, 6, 12, 24,

237   48, 72, 96, 120, 144, and 168 h after the addition of ^{14}C-labelled compounds. After removal, the

238   amount of trapped $^{14}CO_2$ 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}C sorbed on colloid surfaces, litter

241   and soil were shaken with 25 mL of chilled 0.5 M K_2SO_4 (4 °C, 15 min, 200 rev min^{-1}), as described

242   in Rousk and Jones (2010), and centrifuged for 5 min at 18,000 × g. ^{14}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                 $f = (a_1 \times exp^{-k_1 t}) + (a_2 \times exp^{-k_2 t})$ (1)

249   in which f is the amount of ^{14}C remaining in the sample, a_1 and a_2 represent the amount of ^{14}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 ($t_{\frac{1}{2}}$) of the first

252   mineralisation pool a_1 was calculated as follows

253                $t_{\frac{1}{2}} = \ln(2) / k_1$ (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}C label not recovered in either the NaOH

258   traps or K_2SO_4 extracts was assumed to have been immobilised by microbes. The effective ability of

the 0.5 M K_2SO_4 to displace amino acids, peptides, and urea sorbed to the solid phase was postulated by other authors (Joergensen and Brookes, 1990; Rousk and Jones, 2010; Farrell et al., 2011). Based on the low recovery (<3% of the total) of added ^{14}C in the K_2SO_4 extracts, a sorption component was not used in the kinetic model. Thus, the proportion of ^{14}C used for microbial growth, termed C use efficiency (*CUE*), was calculated as follows

$$CUE = a_2 / (a_1 + a_2) \qquad (3)$$

In contrast, macromolecules, such as proteins, can be aggregated and precipitated through the salting-out phenomenon when a high concentration salt (e.g., 0.5 M K_2SO_4) is added in an aqueous solution, decreasing their solubility (Grover and Ryall, 2005). We therefore tested if 0.5 M K_2SO_4 could be used effectively as extractant of ^{14}C-labelled protein from litter and soil, and a poor recovery (<22% of the total) of added ^{14}C was recorded, regardless the medium (Suppl Info. S1). Due to K_2SO_4 interference, high uncertainty is involved in estimate the proportion of ^{14}C-labelled protein immobilised and sorbed, not being possible to calculate the *CUE* for this DON compound.

2.6. Heat sterilisation prior to sorption assay

A sterilisation experiment was performed as a possible pre-treatment to a sorption study, using a fully randomised 2×2 factorial design, with four replicates. The first factor was kept constant (litter and soil), whereas the second factor comprised the two following levels: control (without sterilisation) and heat sterilisation. The method is essentially identical to that described above, except that glucose (D-glucose-1-^{14}C) was used as the source of labile C. Sterilisation was achieved by heating subsamples of litter and soil contained in sealed polypropylene bottles in an oven at 80 °C for 90 min. Then incubations were conducted by adding 0.1 mL of a ^{14}C-labelled glucose solution (10 mM; 41.7 kBq dm^{-3}) to 1.6 cm^3 of litter or soil, and the amount of evolved $^{14}CO_2$ was measured after 1 h. We found that heat sterilisation strongly reduced microbially-mediated mineralisation, regardless of the medium at both sites ($P < 0.001$). Overall, 99% of the CO_2 evolution from the addition of ^{14}C-glucose

284 <mark>to the litter and soil samples was suppressed through heat sterilisation, thus enabling the use of the</mark>

285 <mark>protocol as a pre-treatment for the sorption assay.</mark>

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}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}C-labelled DON solution was added to 0.8 cm^3 of sterilised litter or soil in 50-mL

294 sterile centrifuge tubes (sample activity of 41.7 kBq dm^{-3}). The samples containing sorption solutions

295 were shaken for 30 min at 200 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}C determination, as

297 described above, and a linear sorption isotherm equation was fitted to the experimental data, using

298 the following equation:

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

300 where *S* is the amount of solid phase sorption (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         $K_d = S / ESC$ (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 NH_4^+-N was detected in the water and K_2SO_4 extracts of the litter than in extracts of the

319   soil at Site 1 (Table 2). However, the NO_3^--N content was higher in the soil at both experimental sites,

320   regardless of the extractant (water or K_2SO_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 K_2SO_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 K_2SO_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 K_2SO_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 K_2SO_4 extracts constituted 56 and 49% of the DON.

332   The comparison of extractants indicated a higher (134% increase) NO_3^--N soil content at Site 2

333   when using K_2SO_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 K_2SO_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 (i17:1ω9c 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 cy17:0ω7c 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,

the double first-order exponential decay equation generally fitted well to the experimental data of ^{14}C

mineralisation [$r^2 = 0.990 \pm 0.001$ (mean ± SEM)]. At the end of the experiment, the amount of ^{14}C

remaining in the amino acid mix (Fig. 3a) differed only at Site 2 and was 14% higher in the soil than

in the litter ($P < 0.01$). Remarkable differences in peptide mineralisation occurred at both sites (Fig.

3b), in which ^{14}C remaining in the soil was 151% higher, on average, than in the litter ($P < 0.01$ for

Site 1; $P < 0.001$ for Site 2). At Site 1, for the urea treatment, 132% more ^{14}C was remaining in the

soil than in the litter ($P < 0.05$; Fig. 3c). Similarly, the remaining ^{14}C from protein was, on average,

38% greater in the soil than in the litter ($P < 0.01$ for Site 1; $P < 0.001$ for Site 2; Fig. 3d). Overall,

the remaining ^{14}C content of the DON compounds exhibited the following trend in the litter: amino

acid mix ≈ protein >> peptides > urea ($P < 0.001$). For the soil, however, the pattern was slightly

different: protein > amino acid mix > peptides >> urea ($P < 0.001$).

Most of the amino acid mix was immobilised into the microbial biomass ($CUE \geq 0.75$),

regardless of the medium (Fig. 4a). In contrast, the majority of the added peptides and urea was

rapidly respired by microbes ($CUE \leq 0.50$). At both sites, there was no difference between the litter

and soil for the CUE of the amino acid mix ($P > 0.05$), whereas higher CUE was observed in the soil

than in the litter for peptides (increased by 85%; $P < 0.001$) and urea (increased by 87%; $P < 0.001$).

Overall, the CUE of urea was the lowest (varying from 0.17 to 0.35) among the DON compounds.

The mean half-lives ($t_{1/2}$) of the amino acid mix, peptides, urea, and protein were 1.3 ± 0.1 (mean ±

SEM); 0.6 ± 0.2; 0.8 ± 0.2, and 5.6 ± 1.1 h, respectively (Fig. 4b). For the peptides, urea, and protein,

the $t_{1/2}$ values were higher in the soil (increased by 60%, on average) than in the litter at both

experimental sites ($P < 0.05$). For the amino acid mix, however, no differences were found ($P > 0.05$).

3.4. Sorption

The linear isotherm equation fitted well to the experimental data describing the sorption of

different DON compounds to the solid phase of litter and soil ($r^2 \geq 0.945$; Fig. 5; Table 3). There

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 NH_4^+-N content found in the litter and soil at both sites was extremely low (< 1 mg dm^{-3}),

398 indicating high consumption of this inorganic N fraction, most likely due to 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 NO_3^--N content compared to 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 NO_3^- and NH_4^+ cannot be neglected,

405 although it should be noted that sugarcane often has a general preference for NH_4^+ over NO_3^- (Hajari

406 et al., 2014). In addition, the higher NO_3^--N recovery from soil of Site 2 when K_2SO_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 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 NO_3^- extracted with K_2SO_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

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

445 The ^{14}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 $^{14}CO_2$. Overall, a lower

450 amount of ^{14}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}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 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}C label of peptides used

471 here may have influenced results, other authors have also verified the rapid mineralisation of ^{14}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}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}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 $t_{½}$ 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

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

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

Sorption of DON compounds on the solid phase was not concentration-dependent and followed a similar pattern in the litter and soil, only differing in sorption potential (Fig. 5; Table 3). The higher sorption capacity of the soil than litter is probably related to the high charge density of Fe and Al oxyhydroxides, which are the largest sorbents for DOM in soils (Guggenberger and Kaiser, 2003). However, as previously discussed for NO_3^-, sorption of DOM to variable charge minerals occurs essentially in subsoil horizons (Guggenberger and Kaiser, 2003). No compound exhibited saturating sorption tendencies over the concentration range (0-1 mM), which was expected, since the concentrations were relatively low. The linear sorption isotherm equation was used, rather than the Freundlich exponential equation, since the Freundlich coefficient (K_r) is exponential-dependent and may overestimate K_d values (Soares, 2005). According to our results, protein can readily bind to the solid phase of soil, as proposed by Baron et al. (1997) and Jones and Kielland (2012). Thus, protein leaching through the soil profile may be low or even negligible. Since the linear equation fitted well to the urea sorption data (Fig. 5c; Table 3), heat sterilisation was apparently effective in inhibiting urease activity. The amino acid mix, peptides, and urea were only weakly sorbed to the litter and soil 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 depolymeralised 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

phase, are more biodegradable but also susceptible to be leached to subsoil horizons. Based on the ^{14}C mineralisation assay, the fate of the DON compounds was essentially driven by biotic (respiration and C immobilisation by microbes) rather than abiotic factors (sorption or chemical oxidation of DON). The DON compounds had different metabolic pathways: while peptides and urea showed rapid turnover (lower half-life) and were mainly used by microbes in catabolic processes (i.e. respired to CO_2), most of the amino acid mix was used in anabolic processes (i.e. C used for microbial growth), thus exhibiting higher turnover rate. This suggest that models of DON turnover in soil may perform better if groups of N-containing substrates are considered separately.

Acknowledgments

Appendix A. Supplementary data

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

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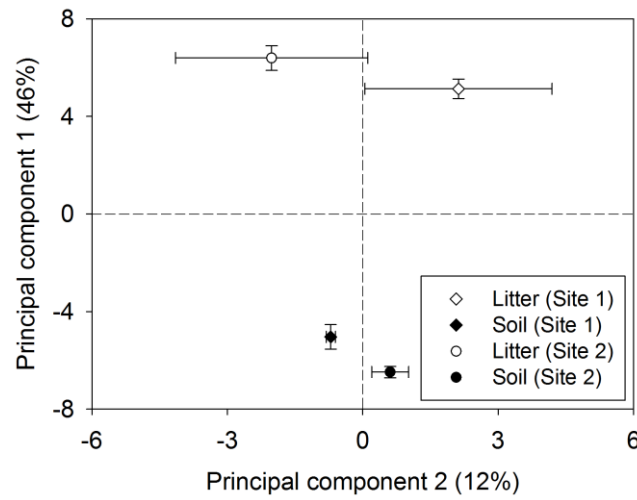
Fig. 1. Principal component analysis (PCA) of the microbial community structure based on the relative concentrations (mol %) of individual phospholipidic fatty acid biomarkers of litter and soil. Percentages in the axis labels indicate the amount of variance explained by each principal component. Symbols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

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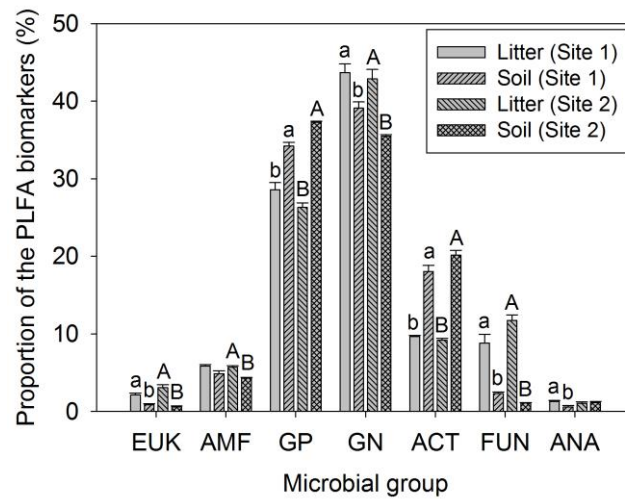
795 **Fig. 2.** Proportional phospholipidic fatty acid (PLFA) biomarkers of litter- and soil-derived PLFA held within

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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Fig. 3. Amount of ^{14}C-remaining after application of amino acid mix (A), L-Ala-Ala and L-Ala-Ala-Ala peptides (B), urea (C), and tobacco leaf protein (D) in litter and soil following 168 h incubation. Symbols represent mean values, and the error bars represent the SEM ($n = 4$). Lines represent fits to a double first-order exponential decay equation. Different lowercase letters indicate differences between the litter and soil at Site 1, whereas different capital letters indicate differences between the litter and soil at Site 2, according to Fisher's LSD test ($P \leq 0.05$).
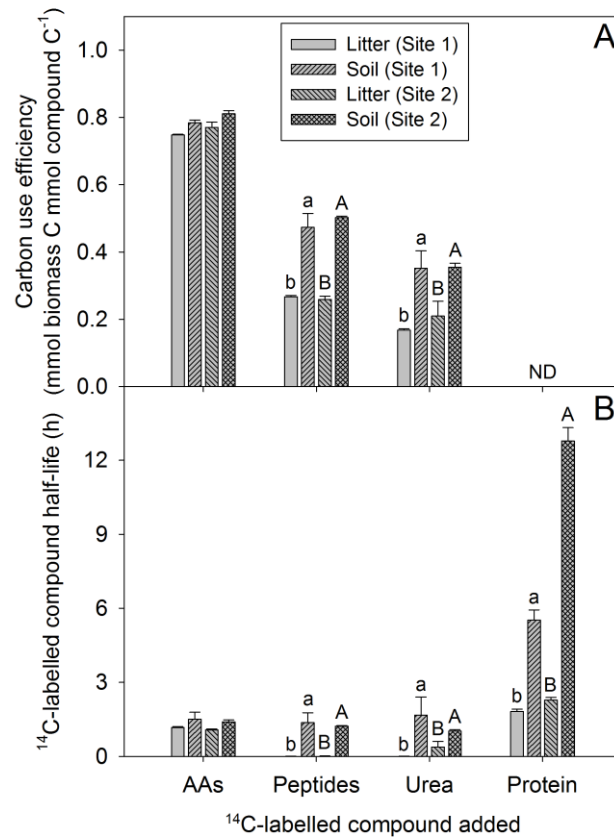
Fig. 4. Carbon use efficiency (A) and half-life of the first mineralisable pool (B) determined by a double first-order exponential decay equation for the ^{14}C-labelled amino acid mix (AAs), peptides (L-Ala-Ala and L-Ala-Ala-Ala), urea, and protein (isolated from tobacco leaves) applied to litter and soil following 168 h incubation. The error bars indicate the SEM ($n = 4$). Different lowercase letters indicate differences between the litter and soil at Site 1, whereas different capital letters indicate differences between the litter and soil at Site 2, according to Fisher's LSD test ($P \leq 0.05$). ND: not determined due to poor recovery of ^{14}C-labelled protein sorbed to the litter and soil solid phases when 0.5 M K_2SO_4 is used (Suppl Info. S1).
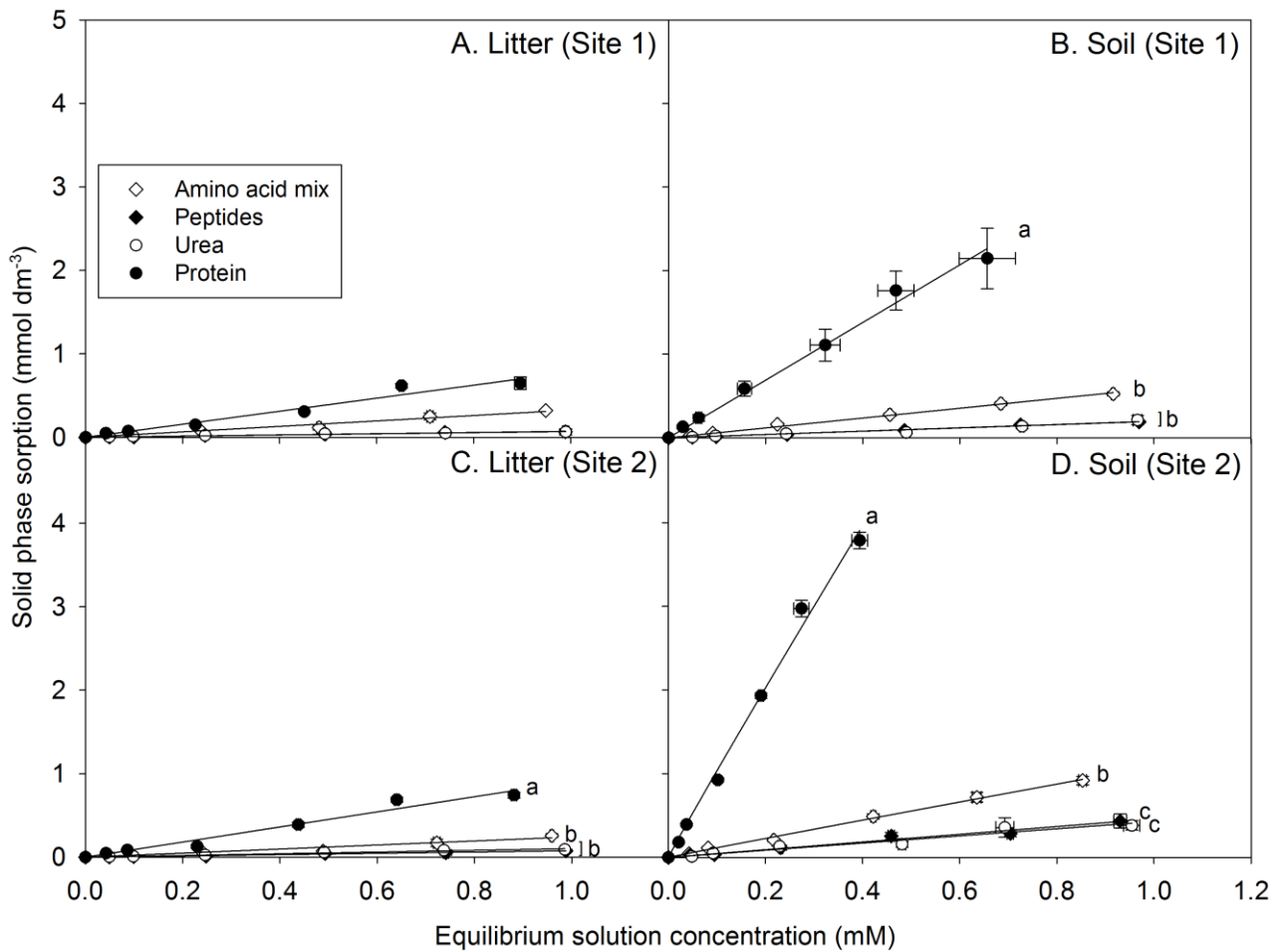
Fig. 5. Sorption of ^{14}C-labelled amino acid mix, peptides (L-Ala-Ala and L-Ala-Ala-Ala), urea, and protein (isolated from tobacco leaves) to the solid phase of litter (A - Site 1; C - Site 2) and soil (B - Site 1; D - Site 2). Bi-directional error bars indicate the SEM ($n = 4$) for sorption and solid solution concentrations. Different lowercase letters indicate differences between the ^{14}C-labelled DON compounds in the litter or soil from each site, according to Fisher's LSD test ($P \leq 0.05$).

A. N in the soluble and exchangeable pools

TIN: ~6%

DON: ~94%

TIN: ~81%

DON: ~19%

Litter

Soil

B. Reactions of DON in the litter and soil

Soil > Litter

Sorption affinity (K_d)

Protein >> Amino acid mix ≈ Peptides ≈ Urea

■ DON desorbed and assimilated by microbes

Half-life for respiration

Protein > Amino acid mix > Urea ≈ Peptides

■ Carbon immobilisation into microbial cells

Carbon use efficiency

Amino acid mix >> Peptides > Urea

858

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}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 K_2SO_4. Soluble plus exchangeable pool of the litter and soil (considering both sites; $n =$

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

869 and soil solid phases when 0.5 M K_2SO_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 ± SEM ($n = 4$), and different lowercase letters indicate differences between the litter and soil of each site, according to Fisher's LSD
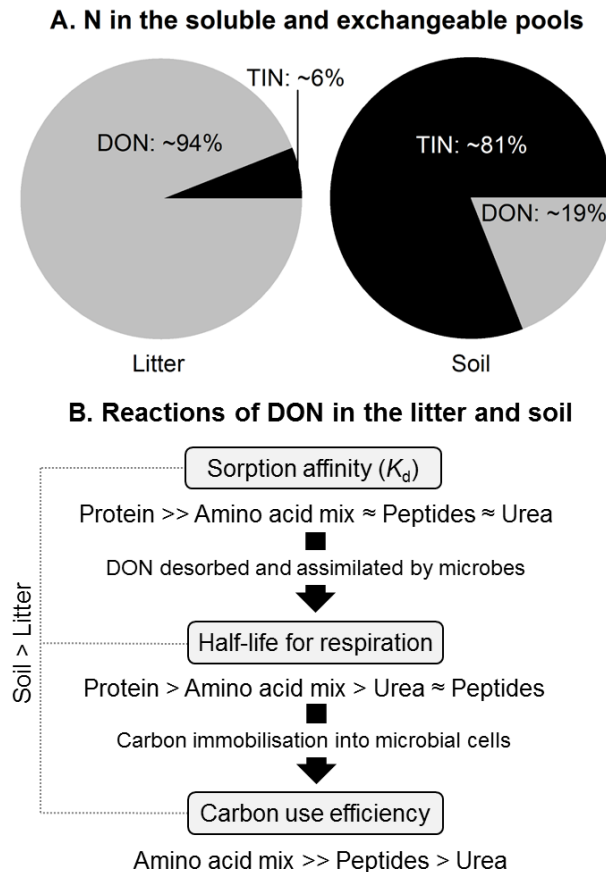
877 test ($P \le 0.05$). EC, electrical conductivity; CEC, cation exchange capacity; MC, moisture content after adjustment to 60% of maximum water holding capacity; and

878 POXC, permanganate oxidisable C.

Treatment	pH 1:2.5 water	EC µS cm^{-1}	CEC mmol$_c$ dm^{-3}	MC	Total C	Total N	C-to-N ratio	Basal respiration µmol CO$_2$-C dm^{-3} h^{-1}	POXC mg C dm^{-3}
				——————— g kg^{-1} ———————					
Site 1									
Litter	7.3 ± 0.2	213 ± 18	39.3 ± 2.7 a	1202 ± 61 a	271.8 ± 12.3	6.28 ± 0.17 a	43.3 ± 1.7 a	126.0 ± 12.6 a	1192 ± 62 a
Soil	6.6 ± 0.5	240 ± 26	27.6 ± 2.3 b	138 ± 2 b	6.7 ± 0.4 b	0.89 ± 0.03 b	7.5 ± 0.4 b	13.1 ± 0.5 b	540 ± 37 b
P value	0.229	0.429	0.017	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Site 2									
Litter	7.0 ± 0.1 a	102 ± 1	23.8 ± 2.5 b	1551 ± 47 a	421.8 ± 4.7 a	7.86 ± 0.57 a	54.5 ± 3.7 a	158.7 ± 5.2 a	1256 ± 54 a
Soil	5.7 ± 0.1 b	120 ± 10	62.4 ± 1.5 a	254 ± 3 b	13.8 ± 0.3 b	1.66 ± 0.03 b	8.3 ± 0.1 b	11.6 ± 0.2 b	546 ± 14 b
P 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_2SO_4 extracts of litter and soil. The nitrogenous compounds

885 included ammoniacal nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--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 ($P \leq 0.05$).

887 DOC, dissolved organic C; BDL, below the detection limit; N/A, not available.

Treatment	NH_4^+-N	NO_3^--N	TIN	DON	TFAAs	Phenolics	DOC	DOC-to-DON
			——————— mg N dm^{-3} ———————			mg dm^{-3}	mg C dm^{-3}	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
P 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
P value	0.208	0.012	0.012	0.165	0.009	< 0.001	0.004	< 0.001
				——— K_2SO_4 extract ———				
Site 1								
Litter	0.33 ± 0.11 a	0.04 ± 0.02 b	0.33 ± 0.11 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
P 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
P 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}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 ± 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^{-3})	r^2	K_d (L dm^{-3})	r^2	K_d (L dm^{-3})	r^2	K_d (L dm^{-3})	r^2
Amino acid mix	0.33 ± 0.01	0.974	0.59 ± 0.02 b	0.995	0.24 ± 0.02 b	0.945	1.10 ± 0.03 b	0.996
Peptides	0.08 ± 0.01	0.980	0.20 ± 0.01 b	0.988	0.07 ± 0.01 b	0.974	0.46 ± 0.05 c	0.979
Urea	0.10 ± 0.01	0.962	0.23 ± 0.02 b	0.956	0.10 ± 0.01 b	0.976	0.50 ± 0.07 c	0.947
Protein	0.78 ± 0.07	0.960	3.67 ± 0.86 a	0.991	0.91 ± 0.03 a	0.964	9.99 ± 0.53 a	0.994

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