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

DOI:
10.1016/j.apsoil.2016.05.019

Published: 01/11/2016

Peer reviewed version

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

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

2. Material and methods

2.1. Site characteristics

Litter and soil samples were collected from two sugarcane N rate-response experiments located in São Paulo, Brazil. At both experimental sites, sugarcane is planted *ca.* every six years and is harvested annually. Before crop replanting, soil tillage (ploughing, harrowing, and furrow opening), lime and gypsum application, and the sowing of atmospheric N_2-fixing legume plants are usually performed.

Site 1 was located in Novo Horizonte (21°32′S, 49°20′W), where the sandy loam soil (825 g 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 (Soil Survey Staff, 2014). The mean annual temperature is 23.2 °C, and the mean annual precipitation is 1134 mm y^{-1} (29-year average). The site has a long history of annual vinasse, press mud, and organo-mineral fertiliser (100 kg N ha^{-1} y^{-1}) application. The experiment was established in Oct. 2010, and the harvest of the 2012/2013 season crop was performed using a mechanical harvester in Aug. 2013.

Site 2 was located in Piracicaba (22°41′S, 47°38′W), where the clay soil (297 g kg^{-1} sand, 87 g kg^{-1} silt, and 617 g kg^{-1} clay; 0.0–2.5 cm soil depth) is classified as a Rhodic Eutrustox (Soil Survey Staff, 2014). The mean annual temperature is 21.6 °C, and the mean annual precipitation is 1230 mm y^{-1} (81-year average). There is no recent record of by-product or manure amendment at this site. The experiment was established in Apr. 2010, and the harvest of 2012/2013 season crop was performed 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 (~0.75
163 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 ± 1 °C (mean ± 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 cm^3 or 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 $KMnO_4$ 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*

For the extraction procedure, 6 cm^3 of litter or soil were shaken with either 30 mL of distilled water or 0.5 M K_2SO_4 for 15 min on a reciprocating shaker (Unimax 2010; Heidolph Elektro GmbH & Co. KG, Kelheim, Germany) at 200 rev min^{-1}. To minimise losses in DOC and DON from microbial transformation during the extraction process, extraction was performed at 4 °C (Rousk and Jones, 2010). After shaking, the extracts were centrifuged at 8000 × *g* for 10 min to remove suspended solids. The supernatant was filtered using Whatman GD/X syringe filters (PTFE membrane, pore size of 0.2 µm; GE Healthcare Life Sciences, Buckinghamshire, UK) to remove particulate material, including microbial cells, and was stored in polypropylene bottles at 4 °C until analysis.

The NH_4^+-N and NO_3^--N content of the extracts was determined colorimetrically using the previously mentioned microplate spectrophotometer, following the protocols of Mulvaney (1996) and Miranda et al. (2001), respectively. Total inorganic N (TIN) was calculated as the sum of NO_3^--N and NH_4^+-N. Dissolved organic C (DOC) and total dissolved N (TDN) were measured using a multi N/C 2100S TOC-TN analyser (Analytik Jena AG, Jena, Germany), and DON was calculated as the difference between TDN and TIN. Total free amino acids (TFAAs) were quantified by the fluorometric *o*-phthialdehyde-β-mercaptoethanol procedure (Jones et al., 2002), and total dissolved phenolics were determined *via* reaction with Folin-Ciocalteu reagent (Swain and Hillis, 1959).

2.4. Microbial phospholipid fatty acid

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

ionization detector (Agilent 6890; Agilent Technologies, Wilmington, DE, USA) and separated on an Agilent Ultra 2 column (25 m long × 0.2 mm internal diameter × 0.33 µm film thickness).

The PLFAs were summed into the following biomarker groups: i) eukaryotes: polyunsaturated fatty acids (Zelles, 1999); ii) arbuscular mycorrhizal fungi (AMF): 16:1ω5c (Frostegård et al., 2011); iii) gram-positive bacteria: iso- and anteiso-series saturated branched fatty acids (Zelles, 1999); iv) gram-negative bacteria: monounsaturated fatty acids and cyclopropyl 17:0 and 19:0 (Zelles, 1999); v) actinobacteria: 10-methyl branched fatty acids (Zelles, 1999); vi) fungi: 18:2ω6c (Frostegård and Bååth, 1996); and vii) anaerobic bacteria: 14:1ω7cDMA, i15:0DMA, 16:1ω7cDMA, 18:0DMA, 18:2DMA, and 19:0cyclo9,10DMA (Frostegård et al., 1991; Zelles, 1997, 1999). However, these biomarkers are not entirely specific for their taxonomic groups and therefore must be interpreted with some caution (Frostegård et al., 2011).

2.5. Mineralisation and microbial uptake of dissolved organic nitrogen compounds

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

To trap any respired (evolved) $^{14}CO_2$ from the samples, a polypropylene vial containing 1 mL of 1 M NaOH was placed above the litter or soil, and the tubes were hermetically sealed and maintained at 20 °C. Loss of water from the tubes during the incubation period was negligible and, therefore, not corrected for. To quantify the collected $^{14}CO_2$, the traps were changed at 2, 6, 12, 24, 48, 72, 96, 120, 144, and 168 h after the addition of ^{14}C-labelled compounds. After removal, the amount of trapped $^{14}CO_2$ was determined using a scintillation cocktail (Scintisafe 3; Fisher Scientific UK Ltd., Leicestershire, UK) and a Wallac 1404 liquid scintillation counter (EG&G Wallac, Milton Keynes, UK). At the end of the incubation period, to quantify ^{14}C sorbed on colloid surfaces, litter 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 in Rousk and Jones (2010), and centrifuged for 5 min at 18,000 × *g*. ^{14}C in the supernatant was measured by scintillation counting, as described above.

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

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

in which f is the amount of ^{14}C remaining in the sample, a_1 and a_2 represent the amount of ^{14}C partitioned into catabolic processes (i.e. microbial respiration) and biomass production, respectively, k_1 and k_2 are the rate constants for these two components, and t is time. The half-life ($t_{½}$) of the first mineralisation pool a_1 was calculated as follows

$$t_{½} = \ln(2) \; / \; k_1 \quad (2)$$

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

For the low molecular weight DON compounds, any ^{14}C label not recovered in either the NaOH 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

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

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

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

2.7. Sorption of dissolved organic nitrogen compounds

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

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

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

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

2.8. Statistical analysis

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

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

3. Results

3.1. Chemistry of water and potassium sulphate extracts

More NH_4^+-N was detected in the water and K_2SO_4 extracts of the litter than in extracts of the soil at Site 1 (Table 2). However, the NO_3^--N content was higher in the soil at both experimental sites, regardless of the extractant (water or K_2SO_4), as was also the case for the TIN. Much higher DOC, DON, DOC-to-DON ratio, and phenolic values were observed in the water extracts of litter than in the water extracts of soil at Site 1 (Table 2). Similar differences were also detected in the K_2SO_4 extracts, with the exception of the DON content, which did not differ between the extracts of litter and soil. It was not possible to compare the TFAA values at Site 1, for either extractant, owing to extremely low values that were below the detection limit. At Site 2, higher DOC, DOC-to-DON ratio, TFAA, and phenolic values were observed in the water extracts of litter than in the water extracts of soil. However, only the phenolic content was higher in the K_2SO_4 extract of litter. On average, the DON represented 95 and 22% of the TDN (TIN + DON) in the water extracts of litter and soil, respectively, whereas DON represented 94 and 19% of the TDN in the K_2SO_4 extracts. TFAAs represented, on average, 31 and 5% of the DON in the water extracts of litter and soil, respectively, whereas the TFAAs in the K_2SO_4 extracts constituted 56 and 49% of the DON.

The comparison of extractants indicated a higher (134% increase) NO_3^--N soil content at Site 2 when using K_2SO_4, rather than water ($P < 0.01$), and in the same soil, higher DOC, DON, DOC-to-DON ratio, and TFAA values were also found when using K_2SO_4 ($P < 0.01$), especially for DOC content, which was 472% higher than that of the water extract. In addition, greater TFAA content

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

3.2. Microbial community structure

Principal component analysis revealed that the relative concentrations of individual PLFAs from litter and soil were compositionally distinct from each other, and together, the first and second principal components (PC1, PC2) accounted for 58% of the variation in PLFA levels (Fig. 1). Negative loadings by Gram-positive bacteria and actinobacteria biomarkers (i17:1ω9c and 10Me16:0 fatty acid, respectively) were the most important for the strict separation of litter from soil (regardless of site) along the PC1 axis, which explained 46% of the variation. The PC2 axis described 12% of the variation, wherein negative loading by the cy17:0ω7c fatty acid (Gram-negative bacteria biomarker) appeared to differentiate litter of Sites 1 and 2.

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

3.3. Mineralisation and microbial uptake

The $^{14}CO_2$ evolution from the ^{14}C-labelled DON compounds demonstrated a biphasic pattern, 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}C
363 mineralisation [r^2 = 0.990 ± 0.001 (mean ± SEM)]. At the end of the experiment, the amount of ^{14}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}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}C was remaining in the
368 soil than in the litter (P < 0.05; Fig. 3c). Similarly, the remaining ^{14}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}C content of the DON compounds exhibited the following trend in the litter: amino
371 acid mix ≈ protein >> peptides > urea (P < 0.001). For the soil, however, the pattern was slightly
372 different: protein > amino acid mix > peptides >> urea (P < 0.001).

373 Most of the amino acid mix was immobilised into the microbial biomass (*CUE* ≥ 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* ≤ 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 ($t_{½}$) of the amino acid mix, peptides, urea, and protein were 1.3 ± 0.1 (mean ±
380 SEM); 0.6 ± 0.2; 0.8 ± 0.2, and 5.6 ± 1.1 h, respectively (Fig. 4b). For the peptides, urea, and protein,
381 the $t_{½}$ 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 ≥ 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.

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

4. Discussion

4.1. Litter and soil characteristics

The NH_4^+-N content found in the litter and soil at both sites was extremely low (< 1 mg dm^{-3}), indicating high consumption of this inorganic N fraction, most likely due to NH_4^+ immobilisation in the high C-to-N ratio litter, and also due to nitrification in the soil. The contrasting C-to-N ratio of the litter, compared to the soil (Table 1), supports this hypothesis (Mary et al., 1996). In contrast, the much higher NO_3^--N content compared to NH_4^+-N detected in the soil may be explained by high rates of nitrification (Mariano et al., 2015). Raison et al. (1987) also reported an increase in mineralisation and nitrification rates, owing to disturbance of soil samples and further incubation in the absence of plants. However, the lack of plant roots depleting both soil NO_3^- and NH_4^+ cannot be neglected, although it should be noted that sugarcane often has a general preference for NH_4^+ over NO_3^- (Hajari et al., 2014). In addition, the higher NO_3^--N recovery from soil of Site 2 when K_2SO_4 was used as extractant rather than water might indicated that this anion was weakly adsorbed on the positively charged surface of colloids, and a salt solution was required for its complete displacement (Hingston et al., 1974). A note of caution arises from the fact that NO_3^- sorption in acid soils is particularly important in subsoil horizons than topsoil (Cahn et al., 1992). Thus, the mechanism involved in the higher amount of NO_3^- extracted with K_2SO_4 remains unclear for the current study.

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

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

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

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

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

The ^{14}C remaining and the *CUE* values for the amino acid mix (Fig. 3a; Fig. 4a) indicate that this DON compound was mostly immobilised, being used either in increasing microbial biomass or in energy storage, rather than in respiration. In contrast, peptides and urea, which exhibited lower *CUE* values (Fig. 3bc; Fig. 4a), may have followed different metabolic pathways once inside the microbial cells, being rapidly catabolised and subsequently mineralised as $^{14}CO_2$. Overall, a lower amount of ^{14}C remained in the litter at the end of the incubation and produced a lower *CUE* than in the underlying soil. As suggested by Creamer et al. (2015), such differences in the C mineralisation of ^{14}C-labelled DON compounds in litter and soil may possibly be driven by N availability (C-to-N ratio; Table 1) and microbial community structure (Figs. 1 and 2). In N-limited systems, such as sugarcane litter, *CUE* tends to decrease as the C-to-N ratio increases, since excess C is respired as CO_2, rather than being used to build more microbial biomass (Sinsabaugh et al., 2013). Furthermore, the higher availability of labile C (measured as permanganate oxidisable C) in the litter than in the soil may have enhanced the growth rate of faster-growing microbes (i.e. copiotrophs), which usually dominate unstable and unpredictable environments and exhibit lower growth yield (i.e. *CUE*) than slower-growing microbes (i.e. oligotrophs), predominant in more stable ecosystems, where organic C quality and/or quantity is low (Fierer et al., 2007).

The $t_{½}$ of the low molecular weight compounds (amino acid mix, peptides, and urea) was very short, especially for the peptides and urea in the litter, indicating that the DON pool can be very low in some situations, owing to faster uptake by microbes or plants (Jones et al., 2009; Farrell et al., 2011; Hill et al., 2011, 2012; Wilkinson et al., 2014). The remarkable rapid mineralisation of L-Ala-Ala and L-Ala-Ala-Ala suggests that the peptides were probably not deaminated before their uptake

by microbes. This assumption is consistent with the findings of Payne (1980), who demonstrated that some bacteria have the ability to take up intact peptides using oligopeptide-specific transport systems (Farrell et al., 2011). Thus, peptide uptake by litter and soil microbes is sufficient to dominate the flux of protein-derived N through the soil solution, without further cleavage to amino acid monomers (Hill et al., 2012). Although it should be noted that the non-homogeneous ^{14}C label of peptides used here may have influenced results, other authors have also verified the rapid mineralisation of ^{14}C-labelled peptides in temperate soils (Farrell et al., 2011; Ge et al., 2012; Wilkinson et al., 2014). The intense release of $^{14}CO_2$ from the urea during the rapid mineralisation phase is associated with hydrolysis by urease and urea amidolyase (UALase; Solomon et al., 2010). In soil, urease is derived from microbes and is also found extracellularly in plant residues (Frankenberger and Tabatabai, 1982). In addition, its activity is reported as being greater in plants and plant residues than in the soil (Barreto and Westerman, 1989). However, UALase is found within microbial cells, and its activity requires energy in the form of ATP (Antia et al., 1991; Hausinger, 2004). Thus, the higher urease activity and more rapid urea assimilation by microbes *via* UALase in the litter than in the soil may explain the consequent lower remaining ^{14}C and *CUE* of urea in the crop residue.

Unlike low molecular weight DON compounds, which do not usually require catalysis by extracellular enzymes before being taken up by the microbial biomass (Boddy et al., 2007; Ge et al., 2012; Hill et al., 2012), effective protein cleavage requires a suite of enzymes, thus reducing its mineralisation rate (Jones and Kielland, 2012). This is evident in the higher $t_{½}$ value of protein than that of the other DON compounds used in the present study (Fig. 4b). In addition, phenolics have been proposed to block protein breakdown by binding to the substrate and proteases (Jones and Kielland, 2012). However, the high phenolic content of the sugarcane litter apparently did not affect its breakdown, since protein mineralisation occurred more quickly in the crop residue than in the soil, which had a lower phenolic content. As suggested by Jones and Kielland (2012), the ability of 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 $t_{½}$ 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 $t_{½}$ 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_f) 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

543 phase, are more biodegradable but also susceptible to be leached to subsoil horizons. Based on the
544 ^{14}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 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
552 **Acknowledgments**

553 The first author (EM) was supported by a scholarship from the São Paulo Research Foundation
554 (FAPESP; Process n. 2013/3866-1), and this work was funded by the UK Natural Environment
555 Research Council (NERC). We would like to thank Beatriz N. Boschiero and Rafael Otto for their
556 assistance in collecting litter and soil samples, as well as Fernando D. Andreote and Thiago Gumiere
557 for their advice regarding statistical analysis of the microbial community structure.
558
559 **Appendix A. Supplementary data**
560 Supplementary data related to this article can be found at [URL].
561
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Figure captions

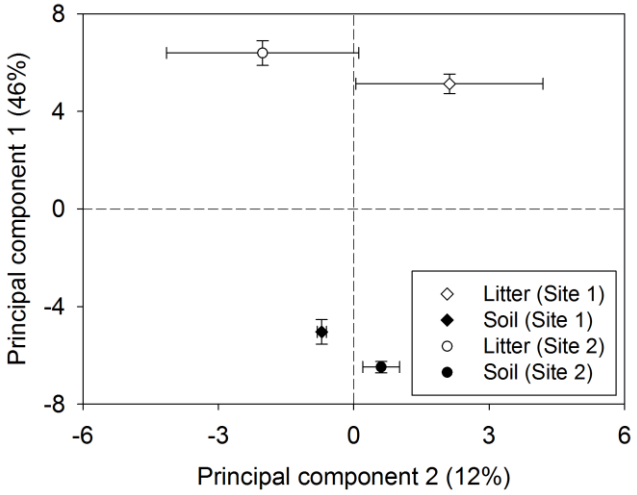
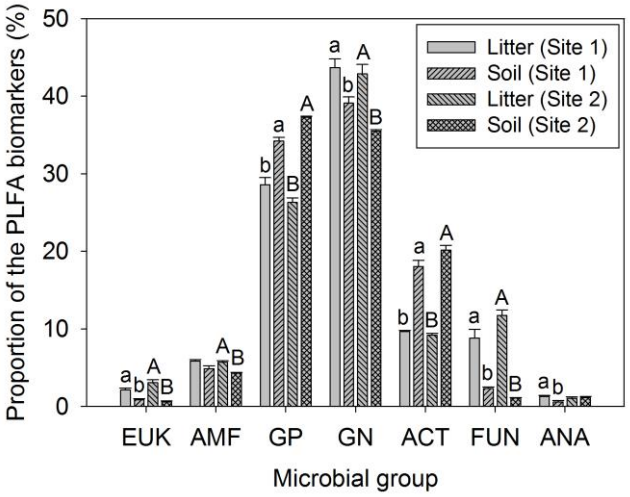


Fig. 1. Principal component analysis (PCA) of the microbial community structure based on the relative concentrations (mol %) of individual phospholipidic fatty acid biomarkers of litter and soil. Percentages in the axis labels indicate the amount of variance explained by each principal component. Symbols 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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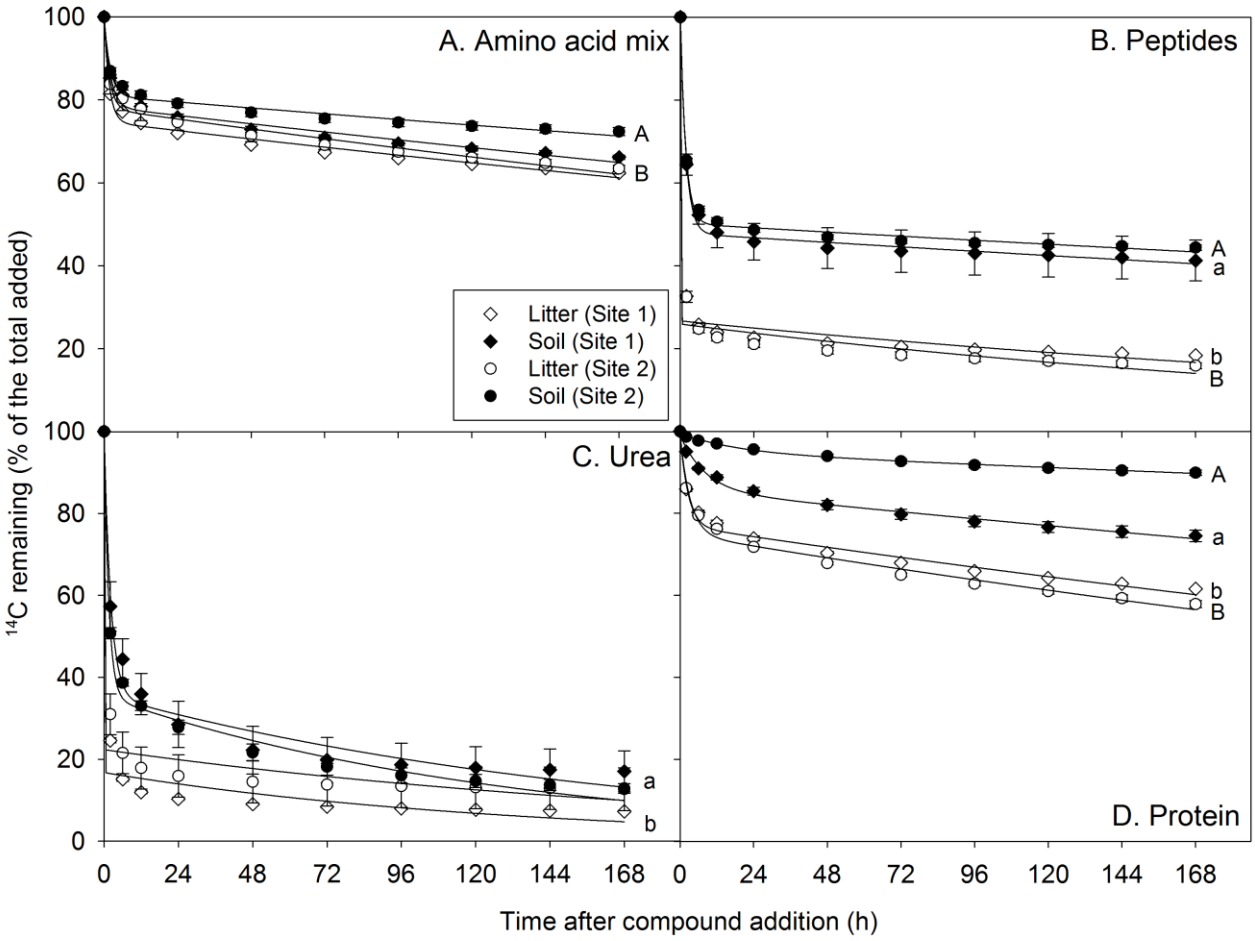


Fig. 3. Amount of ^{14}C-remaining after application of amino acid mix (A), L-Ala-Ala and L-Ala-Ala-Ala peptides (B), urea (C), and tobacco leaf protein (D) in litter and soil following 168 h incubation. Symbols represent mean values, and the error bars represent the SEM (n = 4). Lines represent fits 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 ($P \leq 0.05$).

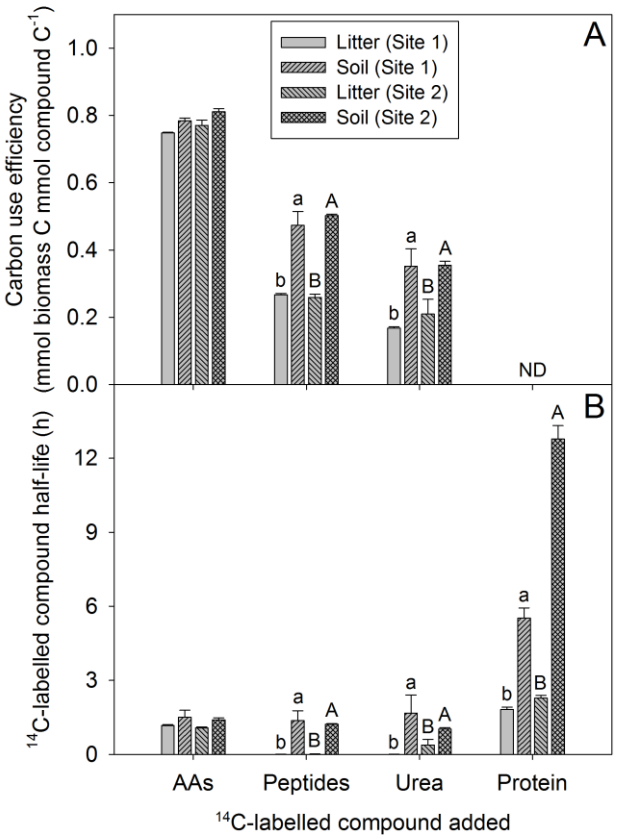
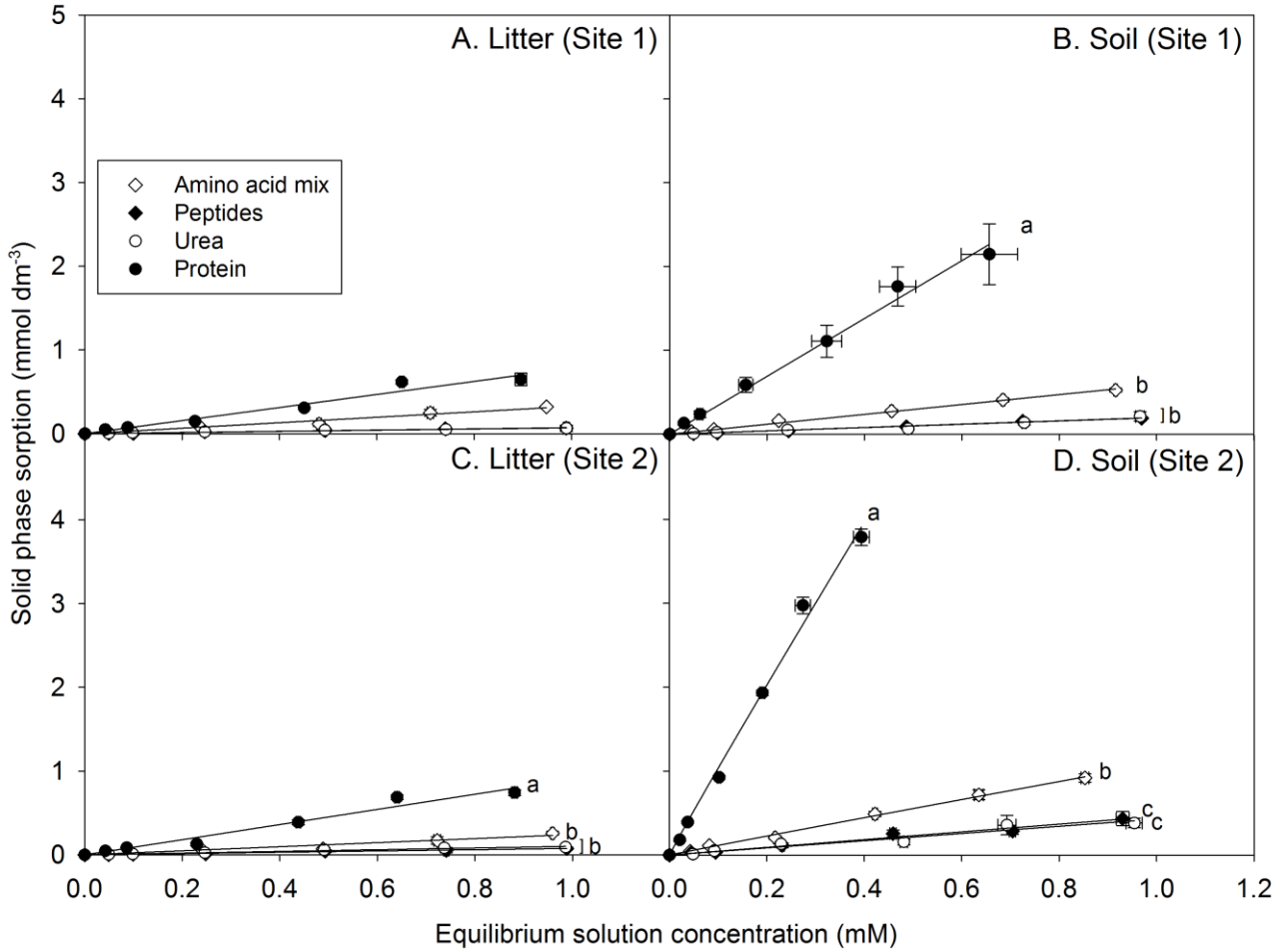


Fig. 4. Carbon use efficiency (A) and half-life of the first mineralisable pool (B) determined by a double first-order exponential decay equation for the ^{14}C-labelled amino acid mix (AAs), peptides (L-Ala-Ala and L-Ala-Ala-Ala), urea, and protein (isolated from tobacco leaves) applied to litter and 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).



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845 **Fig. 5.** Sorption of ^{14}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}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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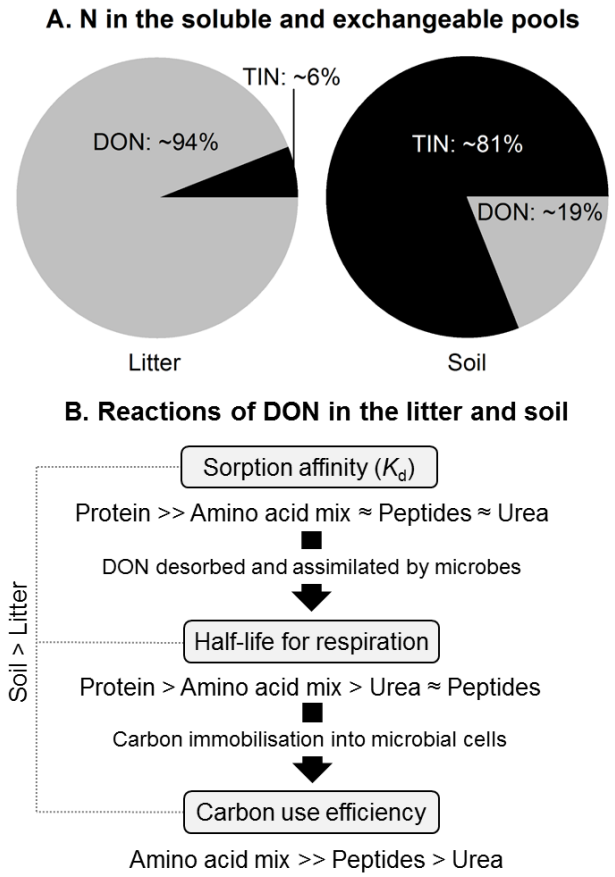

Fig. 6. Schematic representation of the proportion of dissolved organic N (DON) and total inorganic N (TIN) associated with the soluble and exchangeable pools of litter and soil from two sugarcane fields (A), and the possible link between sorption, turnover rate, and immobilisation into the microbial biomass of ^{14}C-labelled amino acid mix, peptides (L-Ala-Ala and L-Ala-Ala-Ala), urea, and protein (isolated from tobacco leaves) applied to the litter and soil (B). We suggest that the partially decomposed litter is an important source of DON to the solution, whereas the soil acts as a sink, sorbing organic and inorganic compounds to the solid phase, thus reducing N losses in leaching. In addition, the high sorption affinity and low mineralisation rate of the protein limits its bioavailability and represents a rate limiting step in DON turnover. Exchangeable pool was extracted with 0.5 M K_2SO_4. Soluble plus exchangeable pool of the litter and soil (considering both sites; n = 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 and soil solid phases when 0.5 M K_2SO_4 is used (Suppl Info. S1), the C use efficiency of this DON compound was therefore not determined.

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 ($P \leq 0.05$). 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 $\mu S\ cm^{-1}$	CEC $mmol_c\ dm^{-3}$	MC	Total C	Total N	C-to-N ratio	Basal respiration $\mu mol\ CO_2\text{-}C\ dm^{-3}\ h^{-1}$	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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Table 2

Concentrations of nitrogenous compounds, dissolved organic carbon (DOC), and phenolics in water and K_2SO_4 extracts of litter and soil. The nitrogenous compounds 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 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 ($P \leq 0.05$). 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 ratio
	mg N dm^{-3}					mg dm^{-3}	mg C dm^{-3}	
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

Table 3

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 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 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