



# Mineralisation and sorption of dissolved organic nitrogen compounds in litter and soil from sugarcane fields



Eduardo Mariano <sup>a, b, \*</sup>, Davey L. Jones <sup>b</sup>, Paul W. Hill <sup>b</sup>, Paulo C.O. Trivelin <sup>a</sup>

<sup>a</sup> Laboratory of Stable Isotopes, Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil

<sup>b</sup> Environment Centre Wales, School of Environment, Natural Resources and Geography, Bangor University, Bangor, Gwynedd, UK

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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 <sup>14</sup>C-labelled amino acids (mix of 17 amino acids), peptides (L-dialanine and L-trialanine), urea, and protein (isolated from tobacco leaves) by capturing <sup>14</sup>CO<sub>2</sub> 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 <sup>14</sup>CO<sub>2</sub>) 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.

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## 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<sup>-1</sup> (dry weight) of crop residue in sugarcane fields (Leal et al., 2013). However, along with crushed bagasse, sugarcane litter can also be 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

\* Corresponding author. Laboratory of Stable Isotopes, Center for Nuclear Energy in Agriculture, University of São Paulo, Av. Centenário, 303, CEP 13400-970, CP 96, Piracicaba, SP, Brazil.

E-mail addresses: [emariano@cena.usp.br](mailto:emariano@cena.usp.br), [dumariano@gmail.com](mailto:dumariano@gmail.com) (E. Mariano).

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 ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$ ; 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 an intact form (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  $^{14}\text{C}$  tracers to measure C mineralisation (Jones and Kielland, 2002; Jones et al., 2004, 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 required for its cleavage into monomers. Alongside the variable mineralisation of different DON compounds, the uptake of DON by microbes from the sugarcane litter may primarily provide them with C to fuel respiration, thus resulting in lower C use efficiency (CUE) and consequent higher C mineralisation, since the crop residue has a greater C-to-N ratio than the underlying soil (Sinsabaugh et al., 2013). In addition, distinct microbial communities between litter and soil can also affect the C and N turnover (Creamer et al., 2015).

Alongside mineralisation, sorption to the solid phase plays an important role in regulating the dynamics of DON in soil. There is ample evidence that sorption of DON can stabilise and promote the accumulation of organic matter in subsoil horizons, although it has also been proposed that biofilms covering mineral surfaces may counteract this to some extent (Guggenberger and Kaiser, 2003; Marschner and Kalbitz, 2003). Most amino acids and peptides are weakly sorbed to the soil solid phase, thus exhibiting relatively high bioavailability (Amelung et al., 2002; Roberts et al., 2007; Ge et al., 2012). On the other hand, the sorption of urea is variable and occurs through hydrogen bonding mainly from amino hydrogens, whereas protein is suggested to readily sorb to the colloid solid phase (Mitsui et al., 1960; Said, 1972; Baron et al., 1997). Meanwhile, the

sorption affinity of DON compounds in the litter layer is entirely unknown. However, when the sorption equilibrium between the solid and liquid phase is changed through DON depletion, part of the sorbed fraction may be released back into solution, in order to restore the previous equilibrium. Thus, if the litter layer has a significant sorption capacity, its presence may also mitigate losses of DON which would otherwise be leached down the soil profile.

On the basis of the recently proposed model of the N cycle, we believe that understanding the dynamics of DON compounds in the litter and soil of sugarcane fields is essential to increasing the sustainability of sugarcane production in Brazil, as well as in other countries. Research regarding this topic could also close gaps in our current knowledge by providing additional information about the role of litter in terrestrial N cycling. Accordingly, the aim of the present study was to evaluate the reactions (C mineralisation and sorption) of  $^{14}\text{C}$ -labelled DON compounds (amino acids, peptides, urea, and protein) in litter and soil from two sugarcane fields located in Brazil. We hypothesised (i) that the C mineralisation of amino acids, peptides, and urea by litter and soil microbes would be more rapid than the mineralisation of protein, (ii) that DON compounds would be taken up more slowly in soil than in litter, and (iii) 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  $\text{N}_2$ -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  $\text{kg}^{-1}$  sand, 23 g  $\text{kg}^{-1}$  silt, and 152 g  $\text{kg}^{-1}$  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  $\text{y}^{-1}$  (29-year average). The site has a long history of annual vinasse, press mud, and organo-mineral fertiliser (100 kg N  $\text{ha}^{-1}$   $\text{y}^{-1}$ ) 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  $\text{kg}^{-1}$  sand, 87 g  $\text{kg}^{-1}$  silt, and 617 g  $\text{kg}^{-1}$  clay; 0.0–2.5 cm soil depth) is classified as a Rhodic Eutruxox (Soil Survey Staff, 2014). The mean annual temperature is 21.6 °C, and the mean annual precipitation is 1230 mm  $\text{y}^{-1}$  (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.

### 2.2. Sampling procedure and characterisation of the litter and soil

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 partially decomposed plant material at 2.5 cm above the litter-soil transition zone, and were cut into small pieces ( $\sim 0.75 \text{ cm}^2$ ) to ensure

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