Soil organic matter formation is controlled by the chemistry and bioavailability of organic carbon inputs across different land uses

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Abstract

Soil organic matter (SOM) formation involves microbial transformation of plant materials of various quality with physico-chemical stabilisation via soil aggregation. Land use and vegetation type can affect the litter chemistry and bioavailability of organic carbon (OC), and consequently influence the processing and stabilisation of OC into SOM. We used ^{13}C nuclear magnetic resonance (^{13}C-NMR) and hot-water extraction to assess the changes in chemical composition and labile OC fractions during the transformation processes from leaf to litter to SOM depending on land use and vegetation type. The hot-water-extractable OC (HWEOC) decreased from leaf (43–65 g kg^{-1}) to litter (19-23 g kg^{-1}) to SOM (8–16 g kg^{-1}) similar in four land use types: grassland, sugarcane, forest and banana. These trends demonstrated the uniform converging pathways of OC transformation and increasing stability by SOM formation. The preferential decomposition and decrease of labile OC fractions (∑% di-O-alkyl, O-alkyl and methoxyl) from leaf (54-69%) to SOM (41-43%) confirmed the increasing stability of the remaining compounds. Despite differences in the biochemical composition of the leaf tissues among the vegetation types, the proportions of labile OC fractions in SOM were similar across land uses. The OC content of soil was higher in forest (7.9%) and grassland (5.2%) compared to sugarcane (2.3%) and banana (3.0%). Consequently, the HWEOC per unit of soil weight was higher in forest and grassland (2.0 and 1.2 g kg^{-1} soil, respectively) compared to sugarcane and banana (0.3 and 0.4 g kg $soil^{-1}$, respectively). The availability of labile SOM is dependent on the quantity of SOM not the chemical composition of SOM. In conclusion, labile OC fractions in SOM, as identified by ^{13}C-NMR, were similar across land use regardless of vegetation type and consequently, SOM formation leads to convergence of chemical composition despite diversity of OC sources.

Keywords: ^{13}C CPMAS NMR, Hot water extractable C and N, Carbon sequestration.

1. Introduction

Plants fix carbon dioxide (CO_2) and convert it into biochemical compounds through photosynthesis and deposit organic residues onto and in soil, that in turn, become a primary source of energy for a wide range of organisms (Baldock et al. 1997). The rate of soil organic matter (SOM) decomposition is highly dependent on the chemical composition of the organic inputs such as plant litter and organic amendments (Almendros et al. 2000; Gunina et al. 2017). The chemically reactive and refractory nature of organic inputs in different land uses contribute to their persistence in soils as well as to their important role in nutrient flows through ecological systems, and carbon (C) emissions to the atmosphere (Guimarães et al. 2013).

The complex interactions between plants, soil microorganisms, SOM and the mineral matrix and their dependency on land use and vegetation type make it extremely difficult to disentangle the processes and mechanisms that are responsible for SOM formation and stabilization (Paul 2016). Plant biomass is the dominant source of organic matter input into soil, however, microbial necromass makes a significant contribution to SOM formation (Kindler et al. 2009; Ma et al. 2018). Although microbial necromass comprises a small fraction of SOM (1-4%), the majority of organic matter input is cycled via microbial metabolism (Kindler et al. 2009; Cui et al. 2020).

The traditional view on SOM processing is that SOM is gradually decomposed by soil organisms to synthesise their own tissues and metabolic products. During this process, the most readily available fractions of plant residues (*e.g.,* proteinaceous and carbohydrate materials) are utilised first, while the more recalcitrant proportion of organic matter (*e.g.,* aromatic-C) tends to accumulate in soil (Baldock et al. 1997). The aromatic-C, presumably derived from lignin, is further decomposed to leave structures with high alkyl-C content in soil (Baldock et al. 1997).

Any changes in land use, vegetation type and the associated management practices may alter soil aggregates and SOM composition, which subsequently impact the plant-derived organic matter degradation through alterations in soil structure (Li et al. 2014; Guo et al. 2016). For example, land use conversion from native forest to cropland and following long-term intensive management system depleted soil organic carbon (OC) and decreased the MBC and labile OC fractions (Li et al. 2014). Land use change from forest to hoop pine plantations also changed the quantity and quality of SOM. Soils under hoop pine plantations had lower SOC content, labile OC fractions (*e.g.,* O-alkyl C) and higher recalcitrant OC fractions (*e.g.,* alkyl C) compared to native forest (Chen, Xu, and Mathers 2004). A recent study showed that the variation in the chemical composition of SOM in different land uses was only pronounced in their particulate organic fractions that was dependent on vegetation type and chemical composition of plant litter input (Yeasmin et al. 2020). Particulate organic matter in soils have a smaller proportion of alkyl C and a larger proportion of O-alkyl C than the organic matter occluded in aggregates (Helfrich et al. 2006). The OC associated with mineral particles could also have a significant proportion of O-alkyl C which is due to a selective stabilisation of microbially derived carbohydrate on mineral surfaces (Yeasmin et al. 2020; Helfrich et al. 2006).

The aim of this study was to provide an improved understanding of the mechanisms that drive the shifts in chemical composition from leaf tissue through to SOM across different land uses; including intensive farming systems (sugarcane and banana plantation) and grasslands and native forest. We hypothesised that the bioavailability of OC decreased along with increasing chemical stability as the OC moved towards SOM. Further, we hypothesise that land use and vegetation type will have an effect of the OC quality from leaf litter through to SOM.

2. Materials and methods

2.1 Catchment description and sampling sites

The Johnstone River catchment is in the Wet Tropics region of north east (NE) Queensland, Australia and covers an area of 2624 km^2 (Fig. 1). This catchment is dominated by native vegetation (forest) covering around 52% of the area with grassland (21%), sugarcane (14%) and horticulture (mainly bananas, 4%) being the main agricultural industries (Bahadori et al. 2020). Four dominant land uses including grassland, native forest, sugarcane and banana plantations were identified and sampled in July 2016. Land uses on the Johnstone catchment were located on various soil types. The upper part of this catchment comprised a mixture of land uses including native forest, grassland and some sugarcane productions with Red Ferrosol as the dominant soil type. Brown Dermosol was the main soil type in the lower part of the Johnstone catchment which was mostly under banana plantations and sugarcane (Bahadori et al. 2019). To collect a representative group of soil samples, the targeted land uses were randomly selected throughout the Johnstone catchment. Surface soil samples (0-10 cm) were collected from three points within each sampling site with an auger after ground vegetation was carefully removed. For sugarcane, soil samples were collected before harvesting from the shoulder of the cane rows.

For each land use, the leaf samples were taken from dominant plant species following standard procedures (Jones Jr and Case 1990), while litter samples were collected from plant leaves accumulated on the ground. Leaf and litter samples were stored in paper bags, protected from heat, and placed in a refrigerator before being transported to the laboratory. Leaf and litter samples were thoroughly washed using tap water, and a homogenous subsample of each of the fresh plant materials (leaf and litter) was oven-dried for one week at 65 ˚C, before being finely ground (<150 μm) prior to analysis.

2.2 Chemical analysis of leaf, litter and soil samples

Total organic C (TOC) and total N (TN) in soil and plant material were measured with a Sercon Hydra 20-22 Europa EA-GSL mass spectrometer. To analyse the TOC content of soils, the inorganic C was removed by adding 2 ml of hydrochloric acid (HCl, 10%) into 2-5 g soil and allowing the suspension to stand overnight. Then, the samples were oven-dried at 65 °C and finely ground (<150 μm) prior to pelletizing them in silver capsules (Bahadori et al. 2019). For TN analysis, no HCl pre-treatment was applied, and soil samples were pelletized in tin capsules. SOM was estimated using a multiplication of 1.724 (Pribyl 2010). The hot-water extractable organic carbon (HWEOC) and hot-water extractable nitrogen (HWEN) concentrations were determined by incubation of soil (40 g) with a 1:5 ratio and plant material (3 g) with a 1:20 ratio in DI water at 70 °C for 16 h (Butler, Lewis, and Chen 2017; Yao et al. 2019). The samples were then centrifuged at 10,000 rpm (~17,500 × g) for 10 min and filtered with a Whatman 42 filter paper. The filtrates were analysed using a Shimadzu TOC-VCPH/CPN analyser. The chloroform fumigation extraction method was used to measure microbial biomass C and N (MBC and MBN) (Brookes et al. 1985). Briefly, 10 g fresh weight of fumigated and non-fumigated soils were extracted by 40 mL of 0.5 M K_2SO_4 (soil:extractant ratio of 1:4). Soil samples were shaken at 70 rpm on an end-to-end shaker for 30 min and filtered through a Whatman 42 filter paper. Concentrations of soluble OC and total soluble N in K_2SO_4 extracts were measured using a TOC-VCPH/CPN analyser fitted with a TN unit (Shimadzu Scientific Instruments, Japan). The particle size distribution of the soil samples was determined by laser diffraction particle size analyser, Master sizer 3000 (Malvern Instruments). Soil pH and electrical conductivity (EC) were measured in 1:5 (w:v) soil to water mixture using labCHEM-Cond/pH glass electrode. The moisture content of soil samples was measured by oven-drying samples (105 °C) for 48 h.

2.3 Solid-state ^{13}C cross polarisation magic angle spinning nuclear magnetic resonance (^{13}C CPMAS NMR) spectroscopy.

In order to minimise the influence of paramagnetic species (*e.g.* Fe^{3+} and Mn^{2+}) present in soil samples in the NMR experiments, and to concentrate the organic matter of the bulk soil samples, the samples were pre-treated with hydrofluoric acid (HF) (Mathers et al. 2002). Briefly, 5 g soil was added into a Nalgene high speed centrifuge tube (40 ml) and then 30 ml of 10% HF was added to each centrifuge tube. The tube was shaken on an end-to-end shaker (2 h), and then centrifuged (10,000 rpm or ~17,500 × g, 15 min). Following the centrifugation step, the supernatant was discarded. The HF treatment procedure was repeated a further four times for each sample, however for the final extraction the mixture was shaken for 16 h (overnight). The final HF-treated soil samples were oven-dried at 65 °C and gently ground using mortar and pestle. The ^{13}C CPMAS NMR spectra of soil and litter samples were acquired using a 300 MHz Varian VNMRS spectrometer (Varian Inc., CA) operating at a frequency of 75.4 MHz (^{13}C). Samples were packed in a 7 mm diameter silicon nitride rotor and spun at 5 kHz at the magic angle. The CPMAS sequence tancpx, contained within the VnmrJ 3.1A software package, was used in all experiments. A total of 20,000 transients were collected for soil samples and 2,000 transients were collected for litter samples. A contact time of 1.2 ms, an acquisition time of 20 ms, a recycle delay of 2.5 s and a sweep width of 36 kHz was used in all cases. The ^{13}C CPMAS NMR spectra of leaf samples were acquired using a 400 MHz Varian INOVA spectrometer (Varian Inc., CA) operating at a frequency of 100.6 MHz (^{13}C). The cross polarisation sequence xpolar1, contained within the VnmrJ 2.1B software package was used. A total of 2,000 transients were collected for leaf samples. A contact time of 2 ms, an acquisition time of 14 ms, a recycle delay of 2.5 s and a sweep width of 50 kHz was

used in all cases. Spectra were processed using the MestReNova v11.0 software package (Mestrelab Research S.L.). Lorentzian line broadening functions of 50 Hz and 20 Hz were applied to all spectra acquired at 75 MHz and 100 MHz NMR, respectively. ^{13}C chemical shift values were referenced relative to external hexamethylbenzene (HMB; δ_{CH3}, 17.4 ppm).

In this study, the resonance regions assignments for leaf, litter and soil samples (Fig. 2) closely followed those used in previous ^{13}C CPMAS NMR studies of plant materials and SOM (Wang et al. 2019; Rashti et al. 2016). Where chemical shift boundaries were moved in this work, in comparison to previous studies, this has been to facilitate the intensity corrections necessitated by spinning side bands (SSBs) appearing in the spectra acquired in this work. The labile OC fractions was calculated as ∑di-O-alkyl, O-alkyl and methoxyl (45-110 ppm) and recalcitrant fractions was calculated as ∑carboxyl, O-aryl, aryl and alkyl C functionalities. The alkyl C/ O-alkyl C ratio (A/O-A) was the ratio of alkyl C region (0-45 ppm) to O-alkyl C region (45-110 ppm). The A/O-A ratio was calculated as an index of the extent of decomposition and changes in organic matter composition from plant materials to SOM (Mathers et al. 2003). Representative solid-state ^{13}C CPMAS NMR spectra for leaf, litter and SOM collected from sugarcane land use are provided in Fig. 2. The ^{13}C CPMAS NMR spectra for other land uses are provided in the supplementary material (Figs. S1-S3).

3. Results

3.1 Chemical composition of soil organic matter, litter and leaf depending on land use

The soil TOC and TN in native forest and grassland were significantly higher than sugarcane and bananas (Table 1). Sugarcane and forest soils had higher TOC:TN ratios

than grassland. Soils collected from forest and grassland had higher HWEOC and HWEN than sugarcane and bananas. Forest and grassland soils also had higher microbial biomass C and N (MBC and MBN) than sugarcane and banana soils (Table 1). The labile OC fractions and A/O-A ratios were similar in SOM collected from the four land uses (Fig. 3A, B and Table S1). While the principal component analysis (PCA) could not visualise the difference in the soil OC functional groups among land uses, it clearly demonstrated that soils under grazing and forest had different SOM content (*e.g.,* TOC, TN, HWEOC and HWEN) than those under sugarcane and bananas (Figs. 3C and D).

Banana leaf samples had the highest TN and lowest TOC:TN ratio compared to other leaf materials. Forest leaf samples had significantly higher HWEOC compared to sugarcane leaf samples. The TOC content and the HWEOC:HWEN ratio of litter were higher in forest compared to banana land use (see Table S2). The leaf samples collected from the four land uses were clearly separated from each other based on their TOC, TN, TOC:TN ratio, HWEOC, HWEN and HWEOC:HWEN ratios (Fig. 4A).

^{13}C NMR results showed that O-alkyl was the dominant OC functional group ranging from 34% to 46% in leaf and 35% to 43% in litter samples. Grass and sugarcane leaves were more enriched in labile OC fractions (∑di-O-alkyl, O-alkyl and methoxyl) compared to bananas. Forest and banana leaves were more enriched in recalcitrant OC fractions (∑aryl, O-aryl, carboxyl and alkyl) compared to sugarcane (see Table S3). The OC functional groups in banana and forest leaves were clearly distinguished from those of sugarcane and grass (Fig. 4B).

3.2 Changes in chemical composition of organic matter from leaf to litter and to soil organic matter

Changes in the HWEOC and HWEN from leaf to litter to SOM was only presented for sugarcane, forest and banana land uses, as there was no litter from the grassland. The HWEOC continually decreased from leaf to litter to SOM collected from sugarcane and banana. In the forest, HWEOC in litter was lower than leaf samples. HWEN decreased from leaf to litter in sugarcane and bananas. Moreover, HWEN comprised a similar fraction of SOM and litter in all land uses. The HWEOC:HWEN ratio had a gradual decrease in forest and banana, while this ratio was lower in SOM compared to leaf and litter samples collected from sugarcane farms (Table 2).

The A/O-A ratio (as an indication of the extent of organic matter decomposition) corresponded to a decrease in the labile OC fractions (∑ methoxyl, O-alkyl and di-O-alkyl C) by transformation from leaf to litter and then to SOM (Fig. 5). These changes were comparable between leaf, litter and SOM collected from the same land uses. The A/O-A ratio of SOM (ranging from 0.52 to 0.60) increased compared to plant materials (e.g., A/O-A ratio ranging from 0.17 to 0.37 in leaf samples) collected from all land uses (Fig. 5). The labile fractions of OC also decreased from plant material (*e.g.,* ranging from 54% to 69% in leaf samples) to SOM (ranging from 41% to 43%). Further details of the chemical composition of organic matter in plant materials and soil samples are provided in the supplementary material (see Table S4).

Regardless of land use, all the leaf, litter and SOM samples were clearly separated from each other due to the HWEOC and HWEN content as well as HWEOC:HWEN ratios (Fig. 6A). The PCA plot provided the distribution of leaf, litter and SOM samples according to their OC functionalities over two axes (explaining 97% of the variation). The primary separation (88%, axis 1) largely represented the difference between the proportion of labile OC fractions in SOM (methoxyl, O-alkyl and di-O-alkyl C) and that in plant material (leaf and litter) (Fig. 6B).

4. Discussion

4.1 The effect of vegetation type on chemical composition of soil organic matter

The chemical composition of sugarcane and grass leaf samples had a greater portion of labile OC fractions, while more recalcitrant fractions were found in forest and banana leaves (Fig. 4B and Table S3). The quality of plant material for decomposition is mainly dependent on species, with grass detritus having faster decomposition rates compared to woody plants under the same environmental conditions (Wang et al. 2004). Generally, the decomposition rate of plant material in soil is controlled by their initial chemical composition, with a faster decomposition rate for higher cellulose to lignin ratio and higher nutrient content (McKee et al. 2016). In contrast to the recalcitrant fractions, labile OC fractions are stabilised via microbial metabolism and incorporation as microbial necromass (Buckeridge et al. 2020).

Although the proportion of labile OC fractions varied in leaves, the vegetation type did not have major impacts on the proportion of labile OC fractions and the A/O-A ratio in the remaining organic matter in soil. Irrespective of this, the overall quantity of HWEOC per unit of soil weight was higher in forest and grassland soils compared to sugarcane and banana plantations (Table 1 and Fig. 3). It is unlikely that the labile SOM fractions (*e.g.,* O-alkyl C) completely disappear during decomposition, because a part of the C assimilated by the microbial community ends up in the labile fraction of microbial necromass (Chen et al. 2019; Cui et al. 2020). Indeed, the O-alkyl C gradually shifts by SOM decomposition towards a greater dominance of microbially-derived, rather than plant-derived organic matter (Baldock et al. 1992). Moreover, the extent of SOM decomposition is controlled by the level of protection provided by mineral particles

through their interactions with the organic components (Mikutta et al. 2006; Apostel et al. 2017) as well as via aggregation (Beare et al. 1994).

SOM accumulation is ongoing when the rates of OC inputs and incorporation are greater than the rates of decomposition/transformation and environmental loss (Torn et al. 2009). TOC and TN contents of the forest and grassland soils were higher than in sugarcane and bananas (Table 1). Forests have significant plant-derived organic matter contributions to SOM from above and below ground biomass, and typically maintain high levels of OC inputs relative to decomposition (Billings 2006; McLauchlan, Hobbie, and Post 2006; Yimer, Ledin, and Abdelkadir 2007). There is also evidence of organic matter accumulation under grassland (Conrad et al. 2017).

The higher HWEOC in grassland and forest soils, compared to sugarcane and banana plantations, was due to the higher non-humified particulate organic material content of these soils. This part of the SOM pool is extensively extractable by hot-water (Wang and Wang 2007). It also reflected the higher MBC and MBN content of forest and grassland compared to sugarcane and banana soils (Table 1). Many studies have shown the strong correlation between HWEOC, TOC and MBC in forest and grasslands, and our study supported these findings (Wang and Wang 2007; Ghani, Dexter, and Perrott 2003; Haynes 2000). The low TOC and TN content of sugarcane soils was also due to the cultivation and tillage practices which promoted soil OC loss (Jaiyeoba 2003; Yimer, Ledin, and Abdelkadir 2007). High inputs of leaf litter into these sugarcane soils has been shown to result in priming of SOC to satisfy the microbial demand for N (Weng et al. 2020). Tillage practice also changes the soil moisture and temperature regimes and exposes more micro-aggregate OC to microbial decomposition by deep mixing of the SOM during ploughing (Yimer, Ledin, and Abdelkadir 2007; Belmonte et al. 2018).

4.2 Impact of vegetation types on shifts in the chemical composition of organic matter from leaf to litter and to soil

The HWEOC of organic matter decreased from leaf to litter and to SOM in all land uses and vegetation types, except forest (Table 2). The similarity between HWEOC of forest litter and SOM was due to high content of non-humified organic material in forest soil (*e.g.,* root exudates), as these are extractable with hot water. Given the evidence that it is readily decomposable, HWEN has drawn more attention as an indicator of potentially mineralizable N (Curtin et al. 2006). Except for native forest, HWEN decreased from leaf to SOM which was due to C and N mineralisation from microbial activity.

The decrease in the percentage of the labile OC fractions in SOM compared to plant material was in agreement with the increase in the percentage of alkyl-C and A/O-A ratios (Figure 5). These changes indicated that SOM was mainly composed of the remaining and more resistant plant tissues that had already lost part of their polysaccharide structures. This trend was consistent with the general plant-derived nature of SOM postulated by Golchin et al. (1994b). In many studies, a decrease of O-alkyl C and an increase of alkyl C fractions in SOM has been attributed to cross-linking of the long-chain alkyl C compounds during the humification processes, and selectively preserving more resistant aliphatic macromolecules (Golchin et al. 1994a; Baldock et al. 1997; Kögel-Knabner, de Leeuw, and Hatcher 1992). Additionally, changes in the relative compositions of O-alkyl and alkyl fractions have been attributed to *in situ* synthesis of some organic compounds within the soil (Kölbl and Kögel-Knabner 2004; Helfrich et al. 2006; Golchin et al. 1994a; Winkler, Haumaier, and Zech 2005).

Understanding the mechanisms that control SOC storage and release is becoming important, particularly due to predicted global climate changes (Trumbore 1997). The biological stability of SOC is influenced by soil characteristics, the chemical structure of

SOC (susceptibility to decomposition) and physical accessibility of SOM to microbes and enzymes (Gleixner et al. 2001). Due to the heterogeneity and structural complexity of SOC, rates of decomposition vary in land uses under different vegetation types (Krull, Baldock, and Skjemstad 2003).

In this study, a new conceptual model for C flow during transformation of organic matter from leaf to litter and to SOM was proposed (Fig. 7) with the relative contribution of plant derived organic matter and microbial necromass to the SOM. In this model, fresh organic matter is decomposed through a stepwise process starting with the initial loss of soluble components in fresh litter, followed by the removal of labile OC fractions such as carbohydrates in soil, while more recalcitrant compounds such as aliphatic (*e.g.,* alkyl C) compounds are selectively preserved (McKee et al. 2016). Microbial cell wall material was considered a significant source of SOM (Paul 2016; Cotrufo et al. 2013; Kindler et al. 2009). The stabilisation of organic matter in soil through microbial necromass was as important as the recalcitrance of plant residues and should be reflected in soil C turnover models (Kindler et al. 2009). Highly-degradable substrates input into soil (e.g., plant litter containing a high proportion of labile fractions) could boost microbial growth and result in increasing SOM content in cases where turnover of the microbial necromass was faster than the microbial degradation of SOM (Kindler et al. 2009; Cui et al. 2020). Considering the number of factors that control SOM turnover, it is difficult to predict the final composition of organic C compounds in soil. However, the factors such as deposition of chemically distinct plant-derived organic matter in soil (Wang et al. 2004), decomposition of organic matter input by soil microorganisms (Waldrop and Firestone 2004), redeposition of microbial necromass in soil as microbes die (Grandy and Neff 2008) and the physical stabilization of C in soils by mineral particle surfaces and aggregation play critical roles in this process (Schimel and Weintraub 2003; Mikutta et al. 2006). We

argued here that the shift in chemical composition and bioavailability of organic matter from leaf to litter and to SOM, and its variation across land uses could be explained through the interaction of these factors. This argument was based on the hypothesis that regardless of variation in vegetation types, the shift in chemical composition and bioavailability of organic matter from leaf to litter and to soil was predictable and followed a consistent path.

5. Conclusions

The shifts in the chemical composition of organic matter from leaf to litter to SOM followed a similar trend across different land uses. This trend was characterised by decreasing labile OC fractions and increasing A/O-A ratios. The proportion of labile OC fractions in the remaining SOM was similar in different land uses, indicating the convergence of organic compounds by SOM formation and stabilisation. It also showed that our hypothesis that plant quality/ type would control the process of SOM formation was not confirmed. As forest and grasslands had a higher TOC content, the overall quantity of HWEOC per unit of soil weight was higher in these land uses relative to the others. Further investigations are required to determine the potential impact of different vegetation types on the composition and diversity of the soil microbial communities involved in the process of SOM decomposition.

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Fig. 1 Location of the study region and soil and plant sampling sites (Johnstone catchment, Queensland, Australia).

Fig. 2 ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra acquired for sugarcane soil (75 MHz, 20,000 scans, 5 kHz), litter (75 MHz, 2,000 scans, 5 kHz) and leaf (100 MHz, 2,000 scans, 5 kHz). Assignments of the ^{13}C chemical shift regions are illustrated. The NMR spectra for other land uses were presented in the supplementary material (Figs. S1-S3).

Fig. 3 A) comparison of the proportion of labile organic carbon (OC) fractions (∑di-O-alkyl, O-alkyl and methoxyl); and B) comparison of the ratio of alkyl C region intensity (0–45 ppm) to O-alkyl C region intensity (45–110 ppm) of soil organic matter (A/O-A ratio) depending on land use. The principal component analysis (PCA) for differentiating soils depending on land use based on C) the OC functional groups as revealed by ^{13}C nuclear magnetic resonance (^{13}C NMR); and D) soil OC and nitrogen (N) parameters [total organic carbon (TOC), total nitrogen (TN), hot-water extractable organic carbon (HWEOC), hot-water extractable nitrogen (HWEN)].

Fig. 4 The principal component analysis (PCA) for differentiating leaf samples collected from grassland, sugarcane, forest and banana land uses based on A) leaf organic carbon (OC) and nitrogen (N) parameters [total organic carbon (TOC), total nitrogen (TN), TOC:TN ratios, hot-water extractable organic carbon (HWEOC), hot-water extractable nitrogen (HWEN) and HWEOC:HWEN ratios]; and B) the OC functional groups of leaf samples as revealed by ^{13}C nuclear magnetic resonance (^{13}C NMR).

Fig. 5 Shifts in the proportion of labile organic carbon (OC) fractions (∑di-O-alkyl, O-alkyl and methoxyl) and the ratio of alkyl C region intensity (0–45 ppm) to O-alkyl C region intensity (45–110 ppm) of organic matter (A/O-A ratio) in leaf, litter and soil

samples collected from grassland, sugarcane, forest and banana land uses (no litter sample was collected from grassland).

Fig. 6 The principal component analysis (PCA) for differentiating leaf, litter and soil organic matter collected from grassland, sugarcane, forest and banana land use based on A) hot-water extractable organic carbon (HWEOC), hot-water extractable nitrogen (HWEN) and HWEOC:HWEN ratios; and B) the organic carbon (OC) functional groups as revealed by the ^{13}C nuclear magnetic resonance (^{13}C NMR).

Fig. 7 Conceptual model of the organic carbon flow during degradation of organic matter from leaf to litter to soil organic matter (SOM), and the contribution of plant derived organic matter and microbial necromass to the SOM (A/O-A ratio: the ratio of alkyl C region intensity (0–45 ppm) to O-alkyl C region intensity (45–110 ppm)).

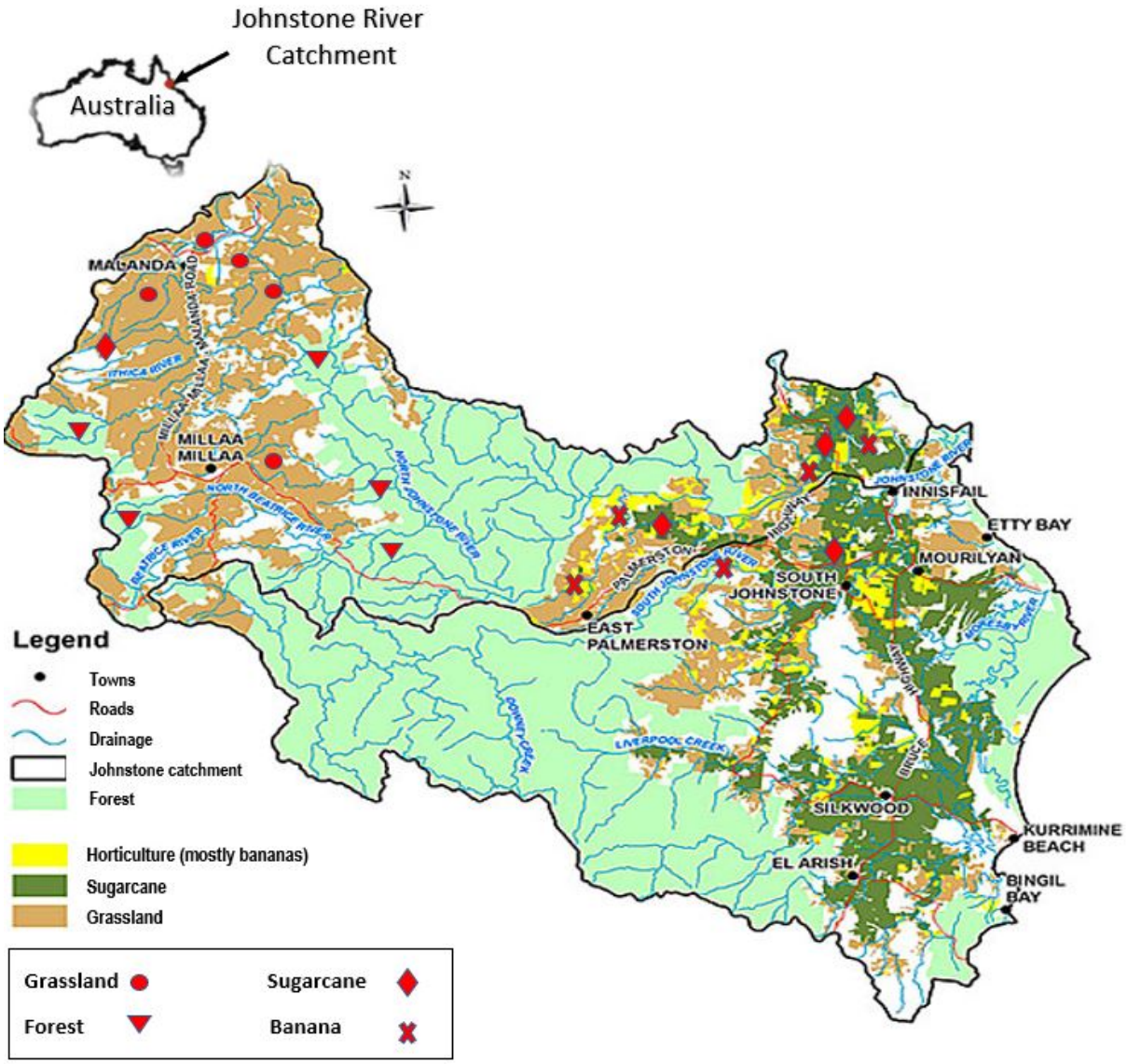
Johnstone River
Catchment
Australia
N
MALANDA
MILLAA
MILLAA
INNISFAIL
ETTY BAY
MOURILYAN
SOUTH
JOHNSTONE
EAST
PALMERSTON
SILKWOOD
KURRIMINE
BEACH
EL ARISH
BINGIL
BAY
Legend
Towns
Roads
Drainage
Johnstone catchment
Forest
Horticulture (mostly bananas)
Sugarcane
Grassland
Grassland
Sugarcane
Forest
Banana

Fig. 1

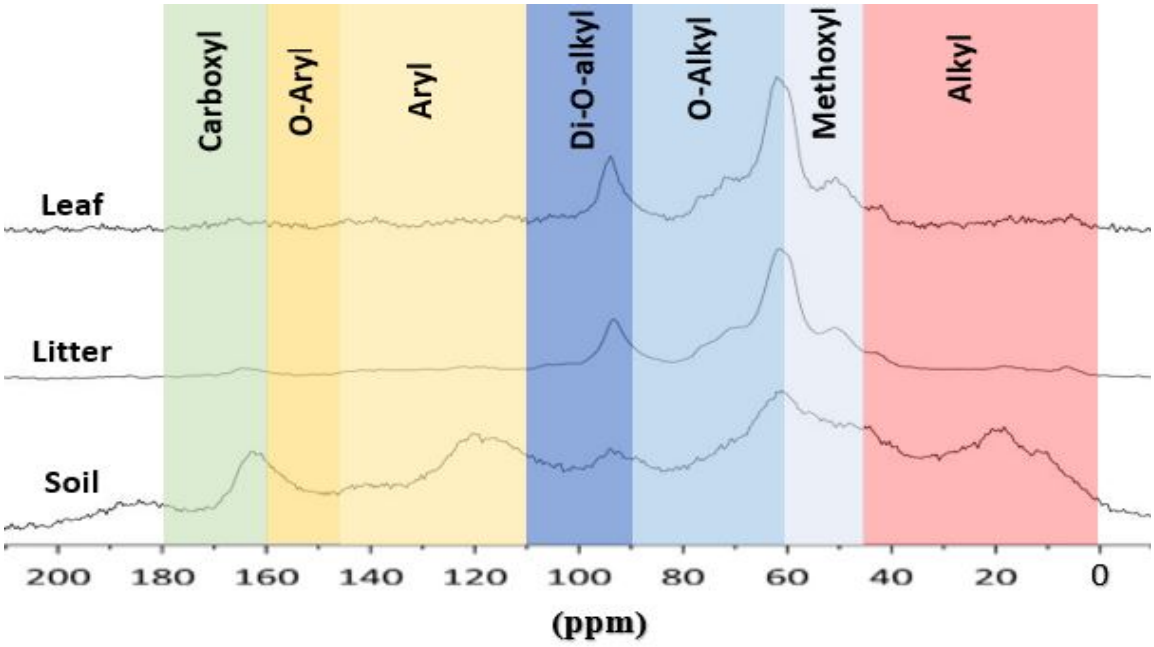
Carboxyl
O-Aryl
Aryl
Di-O-alkyl
O-Alkyl
Methoxyl
Alkyl
Leaf
Litter
Soil
200 180 160 140 120 100 80 60 40 20 0
(ppm)

Fig. 2

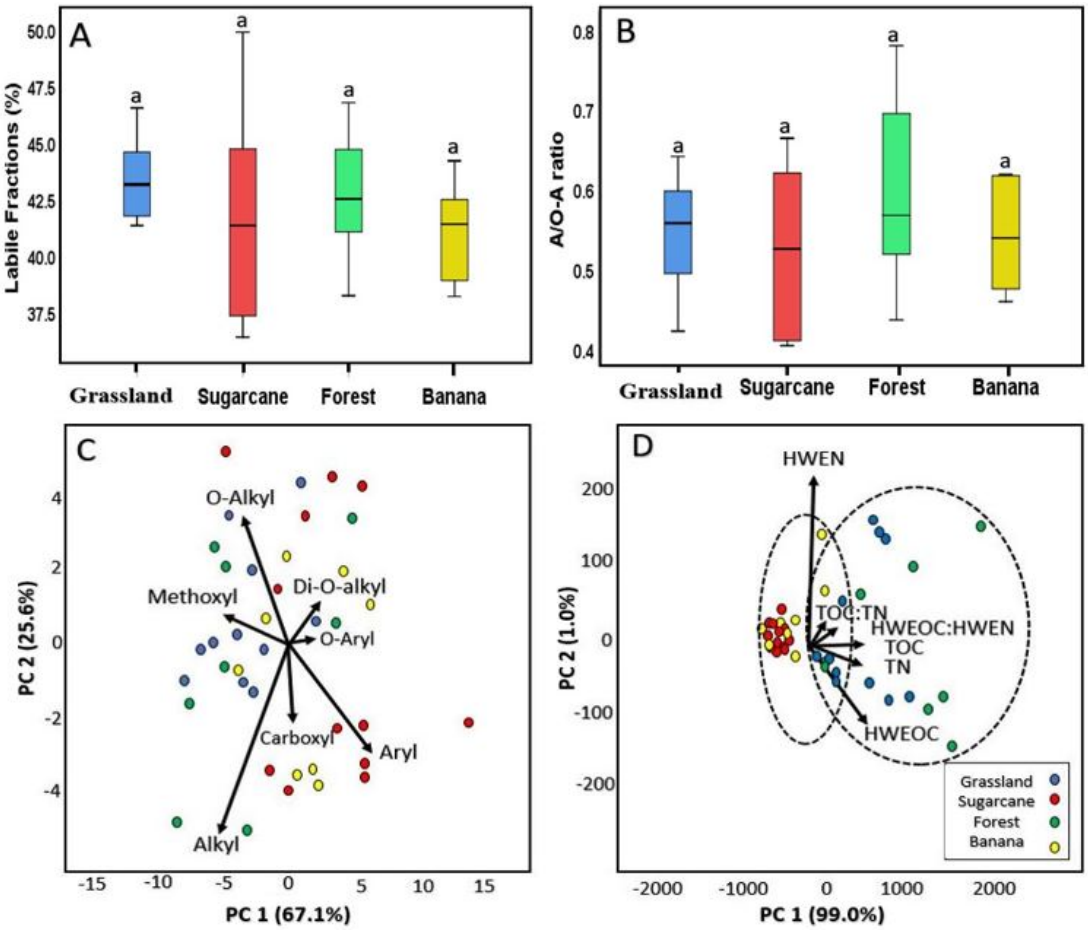
A
Labile Fractions (%)
50.0
47.5
45.0
42.5
40.0
37.5
a
Grassland
Sugarcane
Forest
Banana
B
A/O-A ratio
0.8
0.7
0.6
0.5
0.4
C
PC 2 (25.6%)
PC 1 (67.1%)
O-Alkyl
Di-O-alkyl
Methoxyl
O-Aryl
Carboxyl
Aryl
Alkyl
D
PC 2 (1.0%)
PC 1 (99.0%)
HWEN
TOC:TN
HWEOC:HWEN
TOC
TN
HWEOC
Grassland
Sugarcane
Forest
Banana

Fig. 3

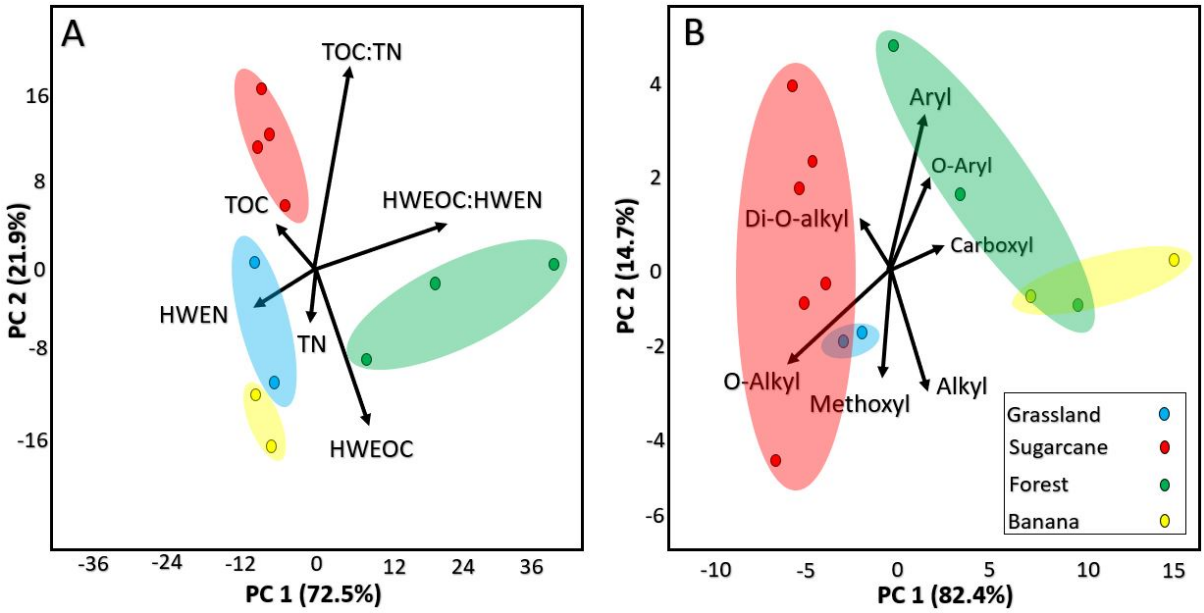
A
TOC:TN
TOC
HWEOC:HWEN
HWEN
TN
HWEOC
PC 2 (21.9%)
PC 1 (72.5%)
B
Aryl
O-Aryl
Di-O-alkyl
Carboxyl
O-Alkyl
Methoxyl
Alkyl
Grassland
Sugarcane
Forest
Banana
PC 2 (14.7%)
PC 1 (82.4%)

Fig. 4

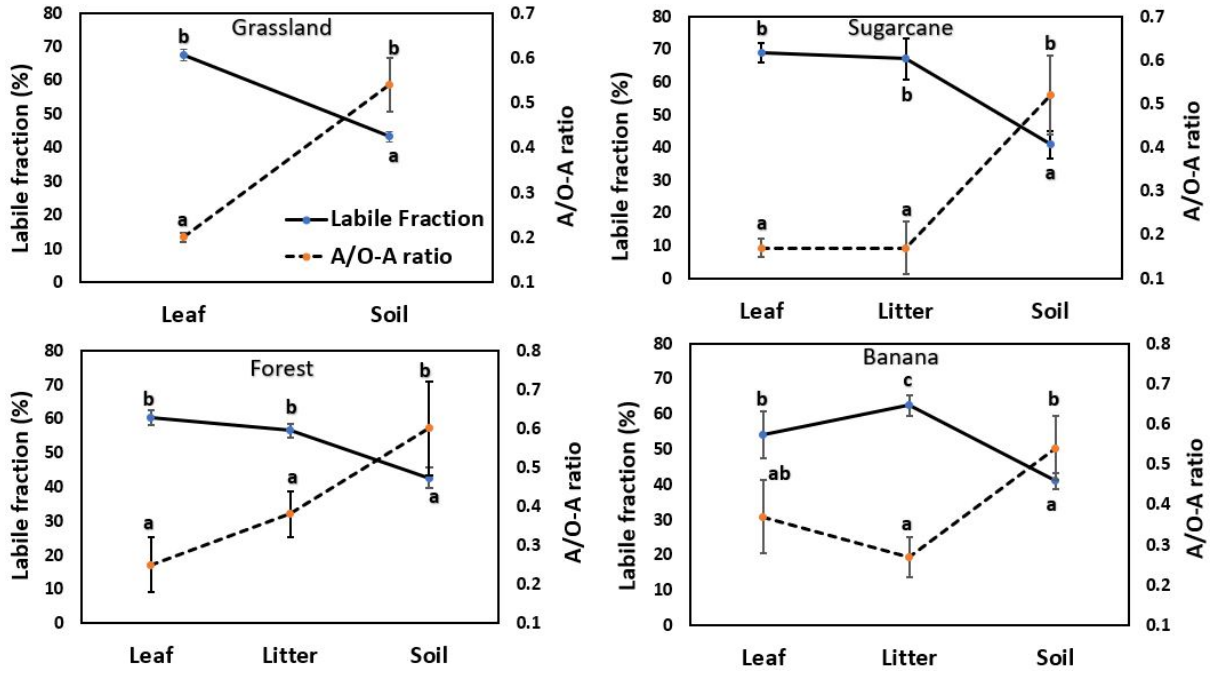
Grassland
Sugarcane
Forest
Banana
Labile fraction (%)
A/O-A ratio
Labile Fraction
A/O-A ratio
Leaf
Litter
Soil

Fig. 5

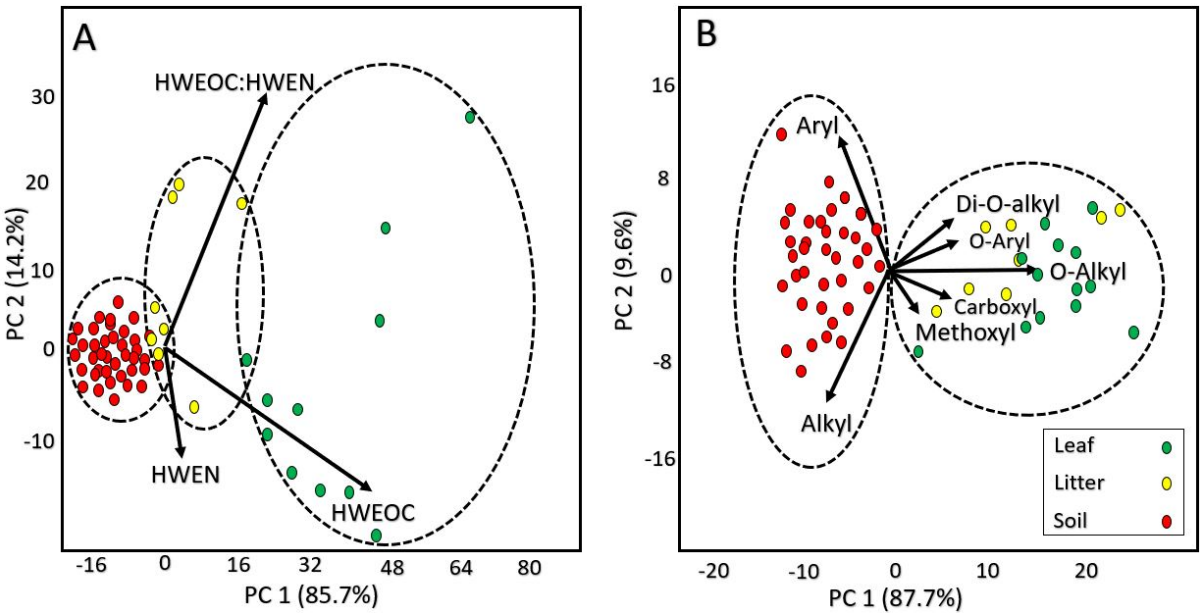
A
HWEOC:HWEN
HWEN
HWEOC
PC 2 (14.2%)
PC 1 (85.7%)
B
Aryl
Alkyl
Di-O-alkyl
O-Aryl
O-Alkyl
Carboxyl
Methoxyl
Leaf
Litter
Soil
PC 2 (9.6%)
PC 1 (87.7%)

Fig. 6

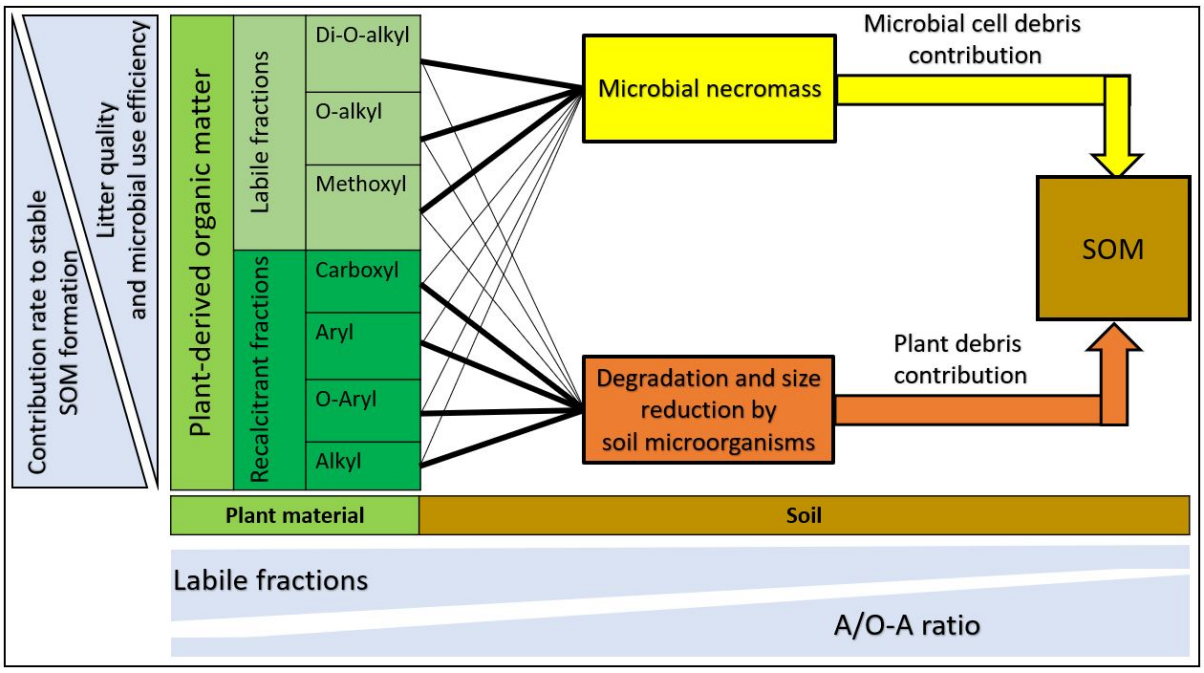
Contribution rate to stable SOM formation
Litter quality and microbial use efficiency
Plant-derived organic matter
Labile fractions
Recalcitrant fractions
Di-O-alkyl
O-alkyl
Methoxyl
Carboxyl
Aryl
O-Aryl
Alkyl
Microbial necromass
Microbial cell debris contribution
SOM
Degradation and size reduction by soil microorganisms
Plant debris contribution
Plant material
Soil
Labile fractions
A/O-A ratio

Fig. 7

Table 1 Properties (Mean ± SD) of soils in different land uses (grassland, sugarcane, forest and bananas). Total organic carbon, TOC; total nitrogen, TN; soil organic matter, SOM; hot-water extractable organic carbon, HWEOC; hot-water extractable nitrogen, HWEN; microbial biomass carbon, MBC; and microbial biomass nitrogen, MBN; electrical conductivity, EC.

Land uses	n	TOC (%)	TN (%)	TOC:TN ratio	SOM (%)	HWEOC (g kg soil^{-1})	HWEN (g kg soil^{-1})	HWEOC:HWEN ratio	MBC (mg kg soil^{-1})	MBN (mg kg soil^{-1})	MBC:MBN ratio	pH	EC	Clay	Silt	Sand
Grassland	11	5.2 ±1.8^{b}	0.4 ±0.2^{b}	11.4 ±0.9^{a}	8.9 ±3.2^{b}	1.2 ±0.4^{b}	0.22 ±0.11^{b}	6.4 ±1.8^{a}	77.6 ±39.5^{b}	17.1 ±6.2^{b}	4.2 ±1.1^{a}	4.7 ± 0.5^{a}	105.9 ± 59.4^{a}	13.0 ±4.7^{a}	64.6 ±6.2^{ab}	22.4 ± 8.6^{ab}
Sugarcane	12	2.3 ±0.4^{a}	0.2 ±0.0^{a}	13.6 ±1.8^{b}	3.9 ±0.8^{a}	0.3 ±0.1^{a}	0.05 ±0.01^{a}	6.5 ±1.4^{a}	22.9 ±9.5^{a}	4.0 ±1.6^{a}	6.1 ±2.4^{a}	4.5 ± 0.6^{a}	68.5 ± 47.4^{a}	12.1 ±3.2^{a}	69.6 ±9.4^{b}	18.4 ± 9.4^{a}
Forest	8	7.9 ±1.8^{c}	0.5 ±0.1^{b}	14.5 ±1.7^{b}	13.7 ±3.1^{c}	2.0 ±0.5^{c}	0.33 ±0.16^{b}	6.9 ±2.9^{a}	155.8 ±61.3^{c}	27.2 ±6.0^{c}	5.6 ±1.3^{a}	4.3 ± 0.3^{a}	70.3 ± 20.7^{a}	9.9 ±5.3^{a}	53.8 ±18.3^{a}	36.2 ±23.4^{b}
Banana	8	3.0 ±1.1^{a}	0.2 ±0.1^{a}	12.5 ±1.7^{ab}	5.1 ±1.9^{a}	0.4 ±0.2^{a}	0.10 ±0.09^{a}	5.6 ±1.5^{a}	19.2 ±6.5^{a}	3.2 ±0.4^{a}	6.2 ±2.4^{a}	5.2 ± 0.7^{a}	333.9 ±206.6^{b}	14.8 ±1.9^{a}	69.2 ±3.0^{b}	16.0 ± 3.6^{a}

- Means within a column followed by the same letter are not different at $p < 0.05$.

Table 2 Changes in the hot-water extractable organic carbon (HWEOC), hot-water extractable nitrogen (HWEN) and HWEOC:HWEN ratio from leaf to litter to soil organic matter (SOM) in grassland, sugarcane, forest and banana land uses.

	Land use	n	HWEOC (g/kg plant dry mass or SOM)	HWEN (g/kg plant dry mass or SOM)	HWEOC:HWEN ratio
	Grassland				
Leaf		2	55.6±8.3^{b}	6.6±1.1^{b}	8.5±0.2^{a}
Soil		11	14.4±3.1^{a}	2.4±0.8^{a}	6.4±1.8^{a}
	Sugarcane				
Leaf		4	43.5±5.3^{c}	3.8±1.0^{b}	12.1±2.5^{b}
Litter		4	18.7±3.7^{b}	1.4±0.6^{a}	15.8±7.4^{b}
Soil		12	8.8±2.8^{a}	1.4±0.4^{a}	6.5±1.4^{a}
	Forest				
Leaf		3	64.9±4.7^{b}	1.7±0.8^{a}	43.1±20.7^{b}
Litter		2	23.1±11.4^{a}	0.8±0.3^{a}	27.2±3.7^{ab}
Soil		8	16.4±1.7^{a}	2.7±1.1^{a}	6.9±2.8^{a}
	Banana				
Leaf		2	56.8±3.6^{c}	6.0±0.4^{b}	9.4±0.1^{b}
Litter		2	20.8±9.1^{b}	2.7±1.4^{a}	8.0±0.7^{ab}
Soil		8	8.4±2.3^{a}	1.7±1.1^{a}	5.6±1.5^{a}

- Means within a column followed by the same letter are not different at $p < 0.05$.
- No litter sample was collected from grassland.