



HAL
open science

Cropping systems including legume cover crops favour mineral–organic associations enriched with microbial metabolites in no-till soil

Murilo G Veloso, Deborah Pinheiro Dick, Janaina Berne Da Costa, Cimélio Bayer

► To cite this version:

Murilo G Veloso, Deborah Pinheiro Dick, Janaina Berne Da Costa, Cimélio Bayer. Cropping systems including legume cover crops favour mineral–organic associations enriched with microbial metabolites in no-till soil. *Soil Research*, 2019, 57 (8), pp.851. 10.1071/SR19144 . hal-04260754

HAL Id: hal-04260754

<https://normandie-univ.hal.science/hal-04260754v1>

Submitted on 26 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Cropping systems including legume cover crops favour mineral–organic associations enriched with microbial metabolites in no-till soil

Murilo G. Veloso^{id} A,C, Deborah Pinheiro Dick^B, Janaina Berne da Costa^B, and Cimélio Bayer^{id} A

^ADepartment of Soil Science, Federal University of Rio Grande do Sul, 7712 Bento Gonçalves Avenue, CEP 91540-000, Porto Alegre, RS, Brazil.

^BInstitute of Chemistry, Federal University of Rio Grande do Sul, 9500 Bento Gonçalves Avenue, CEP 91501-970, Porto Alegre, RS, Brazil.

^CCorresponding author. Email: murilo.veloso@ufrgs.br

Abstract. Long-term carbon (C) stabilisation in tropical and subtropical soils under no-tillage (NT) rests on the formation of mineral–organic associations (MOAs) that can be enriched with microbial metabolites. In this work, we assessed the role of long-term tillage and cropping systems and mineral N fertilisation in enriching MOAs with microbial metabolites in a subtropical soil. For this purpose, we sampled a sandy clay loam Acrisol up to 1 m depth involved in an ongoing 30-year-old experiment under two different tillage systems (conventional tillage and NT) in the presence and absence of legume cover crops and mineral nitrogen (N) fertilisation. The soil samples were subjected to particle size fractionation and *n*-alkane analysis. The NT and the presence of legume cover crops in the surface soil layer (0–5 cm) increased the abundance of plant-derived lipids (i.e. compounds with *n*-alkane chains of 25–33 C atoms) in the whole soil. Microbial-derived lipids (i.e. compounds with shorter *n*-alkane chains (15–24 C atoms)) were more abundant in the clay fraction of the surface (0–5 cm) and sub-surface soil layers (20–30 and 75–100 cm) in NT soil receiving high-quality residues of legume cover crops. However, N fertilisation decreased the abundance of microbial-derived lipids in the clay fraction of the 0–5 and 20–30 cm soil layers. Our findings highlight the role of N-rich residues of legume cover crops, but not of mineral N fertilisation, in the long-term stabilisation of C in MOAs in NT soils through the action of microbial residues.

Additional keywords: *n*-alkanes, no-tillage, soil carbon accumulation, soil lipids.

Received 30 May 2019, accepted 13 August 2019, published online 19 September 2019

Introduction

No-tillage (NT) is a widespread method of facilitating storage of organic carbon (C) in tropical and subtropical soils (Bayer *et al.* 2006; Veloso *et al.* 2018). Legume cover cropping is widely recognised as a key practice to favour storage of organic C in NT soils; however, the specific mechanisms behind C stabilisation in soils are incompletely understood. Veloso *et al.* (2019) recently found legume cover crops to play a major role in stabilising C by association with the silt–clay fraction in a kaolinitic Acrisol. This C stabilisation mechanism is especially important in tropical and subtropical soils, where mineral–organic associations (MOAs) account for 65–92% of total soil organic carbon (SOC; Amado *et al.* 2006).

In addition to increasing soil nitrogen (N) input, legume cover crops may favour C storage by increasing the efficiency of microbial metabolism in stabilising C in plant residues enriched with N and biochemically labile structures. This hypothesis is supported by the fact that MOAs consist mainly of microbial

residues (Puget *et al.* 1998; Kögel-Knabner *et al.* 2008; Rumpel *et al.* 2010). Also, labile residues are known to increase microbial biomass and hence to facilitate C accumulation through formation of MOAs (Rumpel *et al.* 2010; Cotrufo *et al.* 2013, 2015).

The contribution of microbes to stabilising C in MOAs can be assessed with lipid analyses. Soil lipids represent the net result of processes such as the addition of plant residues or microbial synthesis and degradation (Dinel *et al.* 1990). Aliphatic compounds containing long chains of *n*-alkanes (25–33 C atoms but especially C₂₇, C₂₉, C₃₁ and C₃₃) derive largely from plants, whereas those with shorter chains (usually 15–24 C atoms, but especially C₁₆, C₁₈ and C₂₀) are usually of microbial origin (van Bergen *et al.* 1998; Wiesenberg *et al.* 2004; Jandl *et al.* 2007). Not only the pattern distribution of extracted soil *n*-alkanes according to chain length, but also molecular proxies calculated from it, can be useful to elucidate soil organic matter (SOM) formation processes. Thus, the

degradation of long-chain *n*-alkanes in fresh biomass reduces the amount of odd-chain homologues – and hence the ratio of odd-to-even *n*-alkane homologues (i.e. the so-called ‘Carbon Preference Index’, CPI) is a measure of degradation in soil (Peters *et al.* 2005; Wiesenberg *et al.* 2010). Despite its potential to elucidate SOM formation process, studies involving the distribution of soil *n*-alkanes and molecular proxies in Brazil are scant.

In this work, we sought evidence that highly labile N-enriched crop residues in combination with NT promote the accumulation of microbial residues in MOAs, thus facilitating SOC stabilisation. As the first proposal in determining *n*-alkanes in soil under pedoclimatic conditions of southern Brazil, we assessed the effect of tillage (conventional tillage (CT) vs NT), legume cover crops and the mineral N fertiliser on C and *n*-alkanes in whole soil and the clay-size fraction of a sandy clay loam Acrisol involved in an ongoing long-term (30 years) field experiment in southern Brazil.

Material and methods

Study site and pedoclimate

The study was based on soil samples from a long-term (30 years) field experiment in Eldorado do Sul, RS, Brazil (30°06'S, 51°40'W, elevation 96 m). The climate is subtropical (Cfa as per Köppen's classification), with a mean annual temperature and rainfall of 19.4°C and 1440 mm respectively. The soil is a sandy clay loam granite-derived Acrisol (FAO 2002) and its clay content ranges from 217 g kg⁻¹ in the 0–5 cm layer to 394 g kg⁻¹ in the 20–30 cm layer to 511 g kg⁻¹ in the 75–100 cm layer. The main clay minerals are kaolinite (720 g kg⁻¹) and iron oxides (109 g kg⁻¹) (Bayer *et al.* 2001).

Description of the long-term experiment

The ongoing experiment follows a randomised block experimental design with split-split plots and three replicates. Two tillage systems (CT and NT) were arranged in main plots of 15 m × 20 m. The CT plots were ploughed to a furrow-depth of 17 cm once a year in spring before maize sowing using a three-disc plough and harrowed twice to a depth of 10 cm using a disc harrow; while in NT, soil disturbance occurred only in the sowing line and the residues of the cover crop were left on the soil surface. Each tillage system was composed of three cropping systems in subplots of 5 m × 20 m: oat (*Avena strigosa* Schreb)/maize (*Zea mays* L.) (O/M), vetch (*Vicia sativa* L.)/maize (V/M) and oat + vetch/maize + cowpea (*Vigna unguiculata* L. Walp) (OV/MC). These were subjected to two mineral N fertilisation rates of 0 and 180 kg N-urea ha⁻¹ (0 and 180N respectively) applied to the maize crop (sub-subplots of 5 × 10 m). A detailed description of tillage and cropping systems and of agricultural practices is found in Veloso *et al.* (2018).

Soil sampling

Because lipids analyses are especially time consuming, the present study was restricted in terms of number of treatments and soil depths. Thus, a total of four treatments (CT O/M 0N, NT/OM 0N, NT OV/MC 0N and NT OV/MC 180N) were chosen to assess the effect of tillage system (CT vs NT), legume cover crop (cereal (O/M) vs legume (OV/MC)) and mineral N

fertilisation (0N vs 180 N). Three different soil layers across the top 100 cm of the soil profile were sampled in September 2014 to assess the effect of the soil management system on *n*-alkanes: a surface layer (0–5 cm), an intermediate layer (20–30 cm) and a deep layer (75–100 cm). All soil samples were air-dried and sieved through 2-mm mesh.

Annual C input from the cropping systems

The mean annual aboveground C input was calculated from the updated data of the shoot biomass of cover crops and maize for the period 1985–2014 (Zanatta *et al.* 2007; Veloso *et al.* 2018), and assuming a C content of 40% in dry matter. The root C input was assumed to be equivalent to 30% of the shoot biomass, an average value calculated from the available literature (Fehrenbacher and Alexander 1955; Buyanovsky and Wagner 1986; Balesdent and Balabane 1992; Crozier and King 1993; Bolinder *et al.* 1997; Kisselle *et al.* 2001).

Isolation of clay-sized MOAs

Amounts of 10 g of air-dried soil samples were placed in centrifuge tubes containing 80 mL of 2.0 g cm⁻³ sodium polytungstate solution and sonicated at 240 J mL⁻¹ to facilitate soil dispersion and isolation of clay-sized MOAs (Inda Junior *et al.* 2007). Then, the soil suspensions were passed through a 53-µm mesh sieve to remove the sand-sized fraction and the portion passing through the sieve (silt + clay) was transferred to glass tubes, the volume of which was subsequently made up to 1 L with distilled water. The clay-size fraction (<2 µm) was separated according to Stokes' law. The suspension was mixed with a rod, and approximately 2 h later 5 mL of suspension was collected from the top 5 cm of each tube and the tube made up to 1 L again, the process being repeated 55 times. The clay fraction thus isolated was flocculated with 2 M CaCl₂, oven-dried at 50°C, weighed and milled in an agate mortar.

Analysis for organic C in whole soil and the clay-size fraction

Samples of whole soil and the clay-size fraction were oven-dried at 50°C and milled in an agate mortar (<250 µm) before analysis for organic C by dry combustion in a FlashEA 1112 instrument from Thermo Electron Corp. (Milan, Italy).

Extraction of free lipids and determination of *n*-alkanes

Lipids were determined in whole soil from the 0–5 cm layer, and also in the clay-size fraction from the 0–5, 20–30 and 75–100 cm layers. Analysing lipids in whole soil from the 20–30 and 75–100 cm layers was not feasible owing to their relatively low organic C contents. Lipid extraction was performed on composite samples made up of three field replicates, following the procedure adopted by other authors (e.g. Kuhn *et al.* 2010). Based on studies that showed that up to 50°C only the structural water was affected (Kučerík *et al.* 2018), and considering that lipids are regarded as the most stable structures of SOM (Dinel *et al.* 1990; Quénéa *et al.* 2006), we assumed that soil drying at room temperature and clay fraction separation did not affect *n*-alkanes.

Lipids were extracted from soil according to Wiesenberg *et al.* (2004). Thus, ~4 g of whole soil and the clay-size fraction previously ground in an agate mortar was Soxhlet-extracted with a 3:1 (v/v) dichloromethane–methanol mixture on a

Marconi MA 487 apparatus (flask capacity, 120 mL; siphon tube height, 282 mm) for 24 h to isolate free lipids. The volume of extract obtained was reduced to ~1 mL on a rotary evaporator and then fractionated by preparative liquid chromatography, using sequential solvent elution from a 30 cm long × 1 cm thick column containing 1.5 g of previously activated silica gel. The saturated hydrocarbon fraction was collected by elution with 20 mL of *n*-hexane, the extracted volume being reduced to ~1 mL on the rotary evaporator and transferred to chromatographic vials for dilution with *n*-hexane and homogenisation before injection into a gas chromatograph coupled to a mass spectrometer (a GC–MS Agilent 5975C instrument) to determine *n*-alkanes. Analyses were done with an HP5 MS fused silica capillary column (30 m × 0.25 mm i.d.; 0.25- μ m film thickness), using an initial oven temperature of 40°C that was raised to 290°C at 6°C min⁻¹. The helium carrier gas was used at a flow rate of 1.0 mL min⁻¹ and mass spectra were obtained using an ionisation energy of 70 eV. Traces corresponding to selected homologous series of lipid families were obtained by using the single-ion monitoring mode for ions such as that of *m/z* 71 for *n*-alkanes. Compounds were identified from their relative retention times and mass spectral data as compared with reported data.

Because long-chain aliphatic *n*-alkanes (25–33 C, but especially C₂₇, C₂₉, C₃₁ and C₃₃) derive largely from plants, whereas smaller-chain alkanes (15–24 C atoms but especially C₁₆, C₁₈ and C₂₀) are largely produced by microorganisms (van Bergen *et al.* 1998; Wiesenberg *et al.* 2004; Jandl *et al.* 2007), we used the ratio of short- to long-chain *n*-alkanes ($R_{S/L}$) in whole soil and the clay-size fraction to elucidate lipid origin (microbial- or plant-derived). We also assessed the extent of degradation of SOM from the CPI for short- and long-chain *n*-alkanes (CPI_S and CPI_L in Eqns 1 and 2 respectively):

$$\text{CPI}_S = \left[\left(\frac{\sum C_{17,19,21,23}}{\sum C_{16,18,20,22}} \right) + \left(\frac{\sum C_{17,19,21,23}}{\sum C_{18,20,22,24}} \right) \right] \times 0.5 \quad (1)$$

$$\text{CPI}_L = \left[\left(\frac{\sum C_{25,27,29,31,33}}{\sum C_{26,28,30,32}} \right) + \left(\frac{\sum C_{25,27,29,31,33}}{\sum C_{28,30,32}} \right) \right] \times 0.5 \quad (2)$$

According to Wiesenberg *et al.* (2010), CPI decreases with increasing decomposition of *n*-alkane chains.

Statistical analyses

Data were subjected to ANOVA after checking for normality with the Kolmogorov–Smirnov test and variance homogeneity with Levene’s test. The statistical model used to evaluate the effect of the treatments on C in the clay-size fraction (clay-C) and SOC was based on the equation $Y_{ij} = \mu + B_i + T_j + \text{Error}_{(ij)}$, where Y is the mean of treatment j in block i , μ is the overall average of the experiment, B the specific block ($i = 1, 2, 3$), T is the specific treatment ($j = 1, 2, 3, 4$) and Error the experimental error. All statistical analyses were done with the software SAS[®] ver. 9.4 (Statistical Analysis System Institute, Cary, NC, USA). When significant ($P < 0.05$), treatment means were compared by contrast analysis, and Tukey’s test was used to elucidate the effect of (a) tillage system (CT O/M 0N vs NT O/M 0N);

(b) legume cover crop (NT O/M 0N vs NT OV/MC 0N) and (c) fertilisation (NT OV/MC 0N vs NT OV/MC 180N).

Results

Annual C input by crops

The mean annual C input ranged from 4.8 to 10.2 Mg ha⁻¹ (Fig. 1) and was similar for CT O/M 0N (4.8 Mg ha⁻¹ year⁻¹) and NT O/M 0N (5.0 Mg ha⁻¹ year⁻¹); however, the inclusion of legume cover crops in NT without N fertilisation increased C inputs by 4.0 Mg ha⁻¹ year⁻¹. Combining N fertilisation with legume cover crop further increased C input, but only by 1.0 Mg ha⁻¹ year⁻¹, essentially because of the more marked contribution of maize residues.

Organic C in whole soil and clay-size fraction

The SOC concentrations ranged from 10.6 to 25.9 g kg⁻¹ in the 0–5 cm layer and were greater with NT, legume cover crops and N fertilisation than with CT and no cover crop or N fertilisation (Table 1). In the 20–30 cm soil layer, SOC was greater with than without legume cover crop but SOC decreased compared to without N fertilisation. Finally, the SOC concentration in the 75–100 cm soil layer ranged within 5.4–6.7 g kg⁻¹ and was greater under NT than CT.

The clay-C accounted for the greatest portion of total SOC, especially in the deeper soil layers (85% on average in the 20–30 and 75–100 cm layers). This was also the case in the surface soil layer, but values differed among management systems (from 39% with NT OV/MC 180N to 63% with CT O/M 0N). The clay-C was affected by management practices in all soil layers. In the surface layer, clay-C was not influenced by tillage, but increased from 7.1 to 9.6 g kg⁻¹ on average due to the presence of legume cover crops (Table 1). In the 20–30 and 75–100 cm soil layers, clay-C concentration was greater in NT than in CT soil but was not affected significantly by legumes or N fertilisation (Table 1).

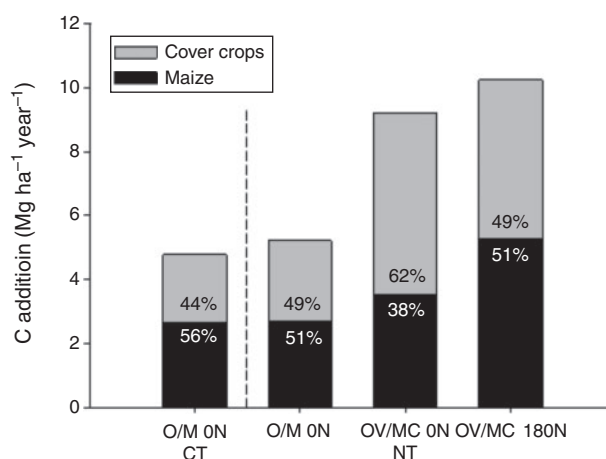


Fig. 1. Mean annual C addition by crops in a sandy clay loam Acrisol under conventional tillage (CT) or no-tillage (NT) system combined with oat/maize (O/M) and/or oat+vetch/maize+cowpea (OV/MC) cropping systems, subjected to 0 or 180 kg ha⁻¹ of N-urea (0N and 180N).

Free lipids

Figures 2 and 3 show the *n*-alkane distribution in the lipid extracts as established from the chromatograms for the ion with *m/z* 71. The alkanes spanned a broad range of C chains from C₁₆ to C₃₃.

Table 1. Concentration of soil organic C in whole soil (SOC) and in the clay fraction (Clay-C) of 0–5, 20–30 and 75–100 cm depths of a sandy clay loam Acrisol under conventional tillage (CT) and no-tillage (NT) systems, combined with oat/maize (O/M) and/or oat+vetch/maize+cowpea (OV/MC) cropping systems, subjected to 0 or 180 kg ha⁻¹ of N-urea (0N and 180N)

Tillage	Cropping systems	N fertilisation	SOC	Clay-C
			(g kg ⁻¹)	
0–5 cm				
CT	O/M	0	10.6	6.53
NT	O/M	0	15.0	7.07
	OV/MC	0	20.9	9.24
	OV/MC	180	25.9	10.01
Contrast ^A	CT vs NT		0.04	0.56
	O/M vs OV/MC		0.01	0.04
	0 vs 180		0.03	0.42
20–30 cm				
CT	O/M	0	9.1	7.42
NT	O/M	0	9.4	8.25
	OV/MC	0	10.6	8.77
	OV/MC	180	10.0	8.36
Contrast	CT vs NT		0.02	0.001
	O/M vs OV/MC		0.07	0.06
	0 vs 180		0.09	0.65
75–100 cm				
CT	O/M	0	5.4	4.70
NT	O/M	0	6.5	5.68
	OV/MC	0	6.7	5.82
	OV/MC	180	6.4	5.85
Contrast	CT vs NT		0.02	0.02
	O/M vs OV/MC		0.61	0.65
	0 vs 180		0.95	0.92

^AContrast analysis was used to test the effects of tillage (CT O/M 0 vs NT O/M 0), legume cover cropping (NT O/M 0 vs NT OV/MC 0) and N fertilisation (NT OV/MC 0 vs NT OV/MC 180) with their respective *P*-value.

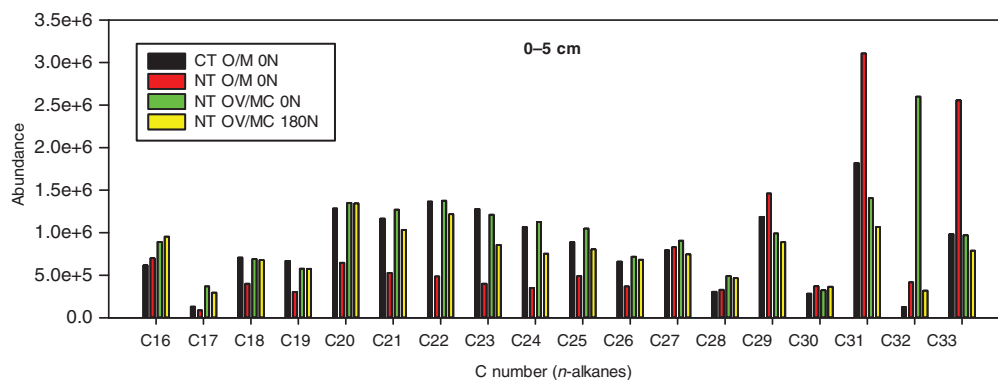


Fig. 2. Distribution of the homologous series of *n*-alkanes (*m/z* 71) from lipid extracts of the whole soil of 0–5 cm depth of a sandy clay loam Acrisol under conventional tillage (CT) or no-tillage (NT) system combined with oat/maize (O/M) and/or oat+vetch/maize+cowpea (OV/MC) cropping systems subjected to 0 or 180 kg ha⁻¹ of N-urea (0N and 180N).

There was a marked difference in *n*-alkane distribution between whole soil and the clay-size fraction in the surface soil layer. In fact, *n*-alkanes in whole soil exhibited a unimodal distribution peaking between C₂₉ and C₃₃ (Fig. 2). Those in the clay-size fraction also exhibited a unimodal distribution, albeit with a prevalence of even-chain alkanes and a peak at C₁₆–C₂₀ in the surface layer (Fig. 3a), especially under a legume cover crop. The clay-size fraction exhibited a similar trend in the 20–30 cm soil layer, with a peak at C₂₃–C₃₃ observed in the absence of legumes (Fig. 3b). A peak at C₁₈ was observed under NT O/M 0N and NT OV/MC 180N (Fig. 3c) in the 75–100 cm soil layer; and one at C₂₃–C₂₉ was also seen with NT OV/MC 0N.

The ratio of short- (C₁₆–C₂₄) to long-chain (C₂₅–C₃₃) *n*-alkanes ($R_{S/L}$) ranged within 0.37–32.84 (Table 2). The clay-size fraction exhibited greater $R_{S/L}$ values than whole soil as a result of the prevalence of short-chain *n*-alkanes ($R_{S/L} > 1$) as reflected in a unimodal distribution peaking at C₁₆, C₁₈ and C₂₀. Short-chain *n*-alkanes also prevailed at greater depths in the clay-size fraction. The $R_{S/L}$ was similar irrespective of tillage system (CT or NT), with the difference in the surface layer amounting to only 15%. The $R_{S/L}$ for the clay-size fraction in the 0–5 and 20–30 cm layers was greater with than without legume cover crop. However, N fertilisation in the presence of legume cover crops decreased $R_{S/L}$ by 38% on average in the 0–5 and 20–30 cm soil layers. Soil management practices had no influence on $R_{S/L}$ in the 75–100 cm soil layer, where *n*-alkanes of even-numbered short chains continued to prevail.

Considering separately short- and long-chain *n*-alkanes, in general, lower values were observed for CPI_S than CPI_L in whole soil and the clay-size fraction (Table 3). Differences between soil management systems were more marked in CPI_L, and especially in the surface soil layer. In the surface layer, the whole soil presented lower CPI_L with than without legume cover crops; however, higher CPI_L was observed with than without legume cover crops in the clay-size fraction. In contrast, lower CPI_L was observed with than without N fertilisation in the clay-size fraction. Both CPI_L and CPI_S tended to decrease in the 75–100 cm compared to the 0–5 cm layer.

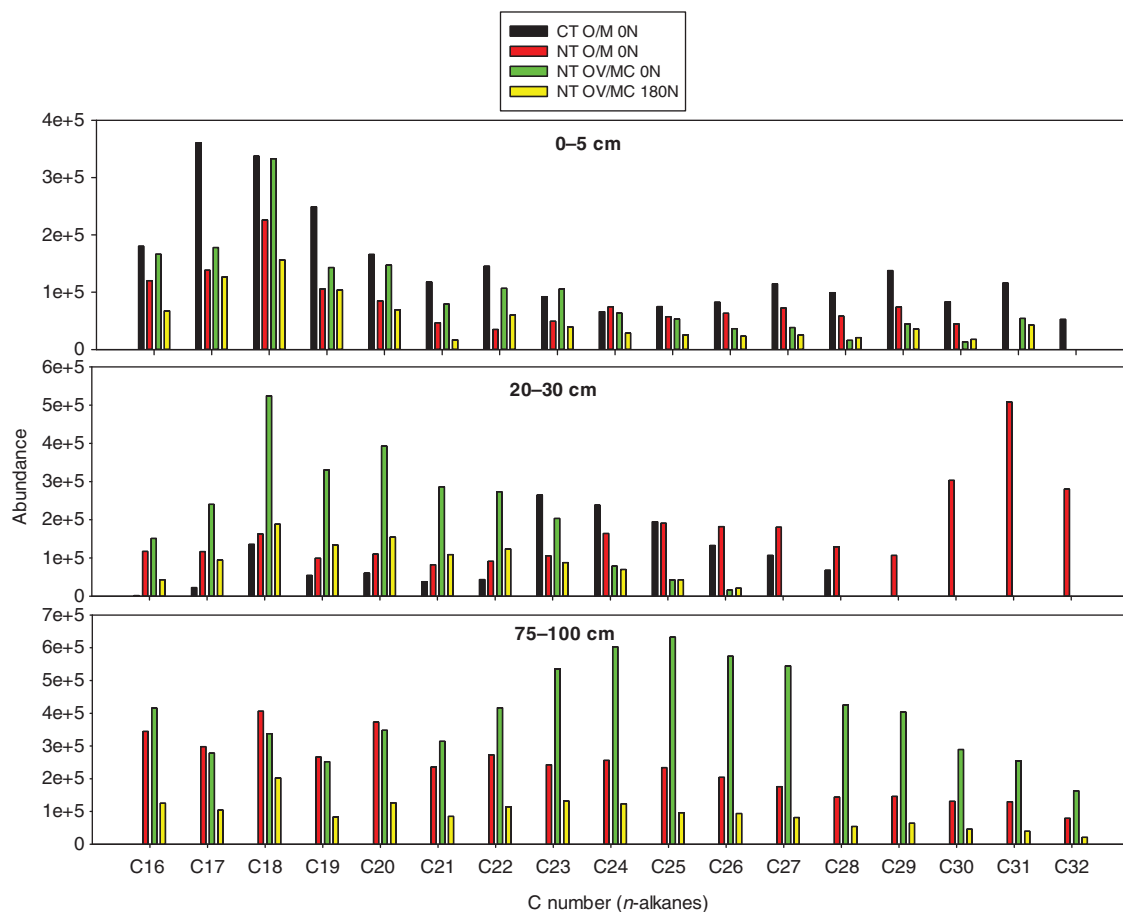


Fig. 3. Distribution of the homologous series of *n*-alkanes (m/z 71) from lipid extracts of the clay fraction of 0–5, 20–30 and 75–100 cm depths of a sandy clay loam Acrisol under conventional tillage (CT) or no-tillage (NT) system combined with oat/maize (O/M) and/or oat+vetch/maize+cowpea (OV/MC) cropping systems, subjected to 0 or 180 kg ha⁻¹ of N-urea (0N and 180N).

Table 2. Ratios of short- to long-chain *n*-alkanes ($R_{S/L}$) in the whole soil and in the clay-size fraction of 0–5, 20–30 and 75–100 cm depths of a sandy clay loam Acrisol under conventional tillage (CT) or no-tillage (NT) system combined with oat/maize (O/M) and/or oat+vetch/maize+cowpea (OV/MC) cropping systems, subjected to 0 or 180 kg ha⁻¹ of N-urea (0N and 180N)

nd, not determined; –, not detected

Tillage	Cropping System	Fertilisation	$R_{S/L}$ ^A	
			Whole soil	Clay fraction
0–5 cm				
CT	O/M	0	1.18	1.85
NT	O/M	0	0.39	1.95
	OV/MC	0	0.94	4.08
	OV/MC	180	1.26	2.83
20–30 cm				
CT	O/M	0	nd	0.62
NT	O/M	0	nd	0.37
	OV/MC	0	nd	32.84
	OV/MC	180	nd	11.32
75–100 cm				
CT	O/M	0	nd	–
NT	O/M	0	nd	1.55
	OV/MC	0	nd	0.59
	OV/MC	180	nd	1.46

^ARatio between short- and long-chain *n*-alkanes ($\Sigma C_{16-24}/\Sigma C_{25-33}$).

Discussion

The clay-C accounted for the greatest portion of total SOC, especially in the deeper soil layers (85% on average in the 20–30 and 75–100 cm layers). Clay-C concentration was affected by soil management practices in different soil layers. Thus, NT had a greater effect than CT on clay-C in the 20–30 and 75–100 cm soil layers, possibly as a result of the presence of biopores facilitating percolation of soluble compounds across the soil profile – and their subsequent adsorption and stabilisation by minerals in MOAs (Hobley and Wilson 2016). The increased clay-C concentration of the surface layer mainly resulted from increased input of N from enriched plant residues by two legume cover crops (vetch and cowpea) relative to the cereal-based cropping system (O/M).

Our results showed that the distribution of *n*-alkanes differed between whole soil and clay-size fraction; also, the greatest length of the C chain was affected by the management system, which consequently affected the C concentration in the clay-size fraction. As previously found in the lipid extracts from various types of soil (Jambu *et al.* 1985; Amblès *et al.* 1989), C number predominance in the C₂₅–C₃₃ range was observed in the surface soil layer. Long-chain, odd C numbered C₂₅–C₃₃ *n*-alkanes are typical components of epicuticular waxes in higher plants, classically used as biomarkers of higher plant input (e.g. van Bergen *et al.* 1998; Wiesenberger *et al.* 2004). By contrast, short

Table 3. Carbon preference index (CPI) in whole soil and the clay fraction of 0–5, 20–30 and 75–100 cm depths of a sandy clay loam Acrisol under conventional tillage (CT) or no-tillage (NT) system combined with oat/maize (O/M) and/or oat+vetch/maize+cowpea (OV/MC) cropping systems, subjected to 0 or 180 kg ha⁻¹ of N-urea (0N and 180N)

nd, not determined; –, not detected; *, not possible to determine due to only short-chain occurrence

Tillage	Cropping system	Fertilisation	Whole soil		Clay fraction	
			CPI _{short chain} ^A	CPI _{long chain} ^B	CPI _{short chain} ^A	CPI _{long chain} ^B
		0–5 cm				
CT	O/M	0	0.77	6.03	1.07	1.64
NT	O/M	0	0.65	6.62	0.77	1.60
	OV/MC	0	0.78	1.42	0.72	4.66
	OV/MC	180	0.68	3.04	0.86	2.74
		20–30 cm				
CT	O/M	0	nd	nd	1.18	2.97
NT	O/M	0	nd	nd	0.80	1.24
	OV/MC	0	nd	nd	0.81	*
	OV/MC	180	nd	nd	0.81	*
		75–100 cm				
CT	O/M	0	nd	nd	–	–
NT	O/M	0	nd	nd	0.77	1.59
	OV/MC	0	nd	nd	0.86	1.68
	OV/MC	180	nd	nd	0.71	1.81

^ACPI_{short chain} = $[(\Sigma C_{17, 19, 21, 23} / \Sigma C_{16, 18, 20, 22}) + (\Sigma C_{17, 19, 21, 23} / \Sigma C_{18, 20, 22, 24})] \times 0.5$.

^BCPI_{long chain} = $[(\Sigma C_{25, 27, 29, 31, 33} / \Sigma C_{26, 28, 30, 32}) + (\Sigma C_{25, 27, 29, 31, 33} / \Sigma C_{28, 30, 32})] \times 0.5$.

chains and even C numbered C₁₆, C₁₈ or C₂₀ chain *n*-alkanes prevailed in the clay fraction, consistent with prevalence of microbial residues in the fine soil fractions (Wiesenberg *et al.* 2004; Quénéa *et al.* 2006).

Unlike NT, the adoption of legume cover crop-based cropping system (OV/MC) altered the C number predominance of C atoms in *n*-alkane chains. The predominance of even C numbered short chains confirmed predominance of microbial residues by comparison of results for the 0–5 and 20–30 cm soil layers in the absence of a legume cover crop (O/M), which increased clay-C in the surface soil layer (Table 1). However, the fact that N fertilisation increased the amount of C addition but not clay-C suggests that residue quality influences conversion of the residues into stable organic matter, which is possibly associated with the negative effect of N fertilisation on the contribution of microbial-derived residue in the 0–5 and 20–30 cm soil layers. These results are consistent with those of Yu *et al.* (2016), who found lower microbial biomass with N fertilisation, and those of Spielvogel *et al.* (2008), who found lignin-derived phenols not to be stabilised in mineral soil. Our results suggest that N-enriched labile residues from legumes facilitate C stabilisation in MOAs through microbial residues. These field-based data strengthen previous results of Rumpel *et al.* (2010) and Cotrufo *et al.* (2015), who found microbial-derived C to contribute to stabilised, mineral associated organic matter to a greater extent than nonmicrobially processed plant-derived C.

As suggested by the smaller CPI_L values, legume cover crop-based cropping systems also decreased residue degradation in the clay fraction. This was probably a result of more effective protection of *n*-alkanes by association with mineral particles relative to the O/M. Therefore, the presence of legume cover crops may have a more direct impact on the formation of MOAs in the clay-size fraction by facilitating direct association through new clusters in mineral surfaces (Kopittke *et al.* 2018).

The increment of microbial residues in the clay fraction favoured by the two legume cover crop systems compared to without legumes (only grasses) under NT led to SOC accumulation in the whole soil profile, achieving rates greater than 0.9 Mg ha⁻¹ year⁻¹ (Veloso *et al.* 2018). The low values found for CPI_S and CPI_L in the sub-surface soil layers suggested that the residues were microbially degraded and that C accumulation in depth was mediated by percolation of soluble compounds, or exudation and decay of deep plant roots, which boosted microbial activity and the formation of MOAs in the deeper soil layers. Increased amounts in combination with also increased biochemical lability of the residual inputs derived from the two legume cover crops (vetch and cowpea) under NT was likely the main driver for strong SOC accumulation down to 1 m in the soil (Veloso *et al.* 2018).

Conclusions

Our findings from the long-term (30 years) field experiment support the hypothesis that organic C present in clay-size MOAs comes mainly from microbial-derived residues. The presence of legume cover crops on NT soil led to a prevalence of microbial over plant-derived residues in the clay-size MOAs. Also, the addition of plant residues enriched with N and labile compounds through legume cover crops was the main driver for C accumulation in MOAs in the NT Acrisol studied. These results were verified in the whole soil profile (down to 100 cm) and support the long-term potential of C sequestration in subtropical soils subjected to NT and legume cover crops.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors acknowledge funding from the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES), National Council

for Scientific and Technological Development (CNPq) and Foundation for Research Support of Rio Grande do Sul State (Fapergs).

References

- Amado TJC, Bayer C, Conceicao PC, Spagnollo E, de Campos BHC, da Veiga M (2006) Potential of carbon accumulation in no-till soils with intensive use and cover crops in southern Brazil. *Journal of Environmental Quality* **35**, 1599–1607. doi:10.2134/jeq2005.0233
- Amblès A, Jambu P, Ntsikoussalabongui B (1989) Evolution des lipides naturels d'un podzol forestier induite par l'apport d'engrais minéraux: hydrocarbures, cétones, alcools. *Science du Sol* **27**, 201–214.
- Balesdent J, Balabane M (1992) Maize root-derived soil organic carbon estimated by natural ^{13}C abundance. *Soil Biology & Biochemistry* **24**, 97–101. doi:10.1016/0038-0717(92)90264-X
- Bayer C, Martin-Neto L, Mielniczuk J, Pillon CN, Sangoi L (2001) Changes in soil organic matter fractions under subtropical no-till cropping systems. *Soil Science Society of America Journal* **65**, 1473–1478. doi:10.2136/sssaj2001.6551473x
- Bayer C, Martin-Neto L, Mielniczuk J, Pavinato A, Dieckow J (2006) Carbon sequestration in two Brazilian Cerrado soils under no-till. *Soil & Tillage Research* **86**, 237–245. doi:10.1016/j.still.2005.02.023
- Bolinder MA, Angers DA, Dubuc JP (1997) Estimating shoot to root ratios and annual carbon inputs in soil for cereal crops. *Agriculture, Ecosystems & Environment* **63**, 61–66. doi:10.1016/S0167-8809(96)01121-8
- Buyanovsky GA, Wagner GH (1986) Post-harvest residue input to cropland. *Plant and Soil* **93**, 57–65. doi:10.1007/BF02377145
- Cotrufo MF, Wallenstein MD, Boot CM, Deneff K, Paul E (2013) The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology* **19**, 988–995. doi:10.1111/gcb.12113
- Cotrufo MF, Soong JL, Horton AJ, Campbell EE, Haddix ML, Wall DH, Parton WJ (2015) Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nature Geoscience* **8**, 776–779. doi:10.1038/ngeo2520
- Crozier CR, King LD (1993) Corn root dry matter and nitrogen distribution as determined by sampling multiple soil cores around individual plants. *Communications in Soil Science and Plant Analysis* **24**, 1127–1138. doi:10.1080/00103629309368865
- Dinel H, Schnitzer M, Mehuys GR (1990) Soil lipids: origin, nature, content, decomposition, and effect on soil physical properties. In 'Soil biochemistry. Volume 6'. (Eds JM Bollag, G Stotzky) pp. 397–429. (Marcel Dekker: New York)
- FAO (2002) 'World reference base for soil resources.' (FAO: Rome)
- Fehrenbacher JB, Alexander JD (1955) A method for studying corn root distribution using a soil-core sampling machine and shaker-type washer. *Agronomy Journal* **47**, 468–472. doi:10.2134/agronj1955.0002196200470100006x
- Hobley EU, Wilson B (2016) The depth distribution of organic carbon in the soils of eastern Australia. *Ecosphere* **7**, e01214. doi:10.1002/ecs2.1214
- Inda Junior AV, Bayer C, Conceição PC, Boeni M, Salton JC, Tonin AT (2007) Variáveis relacionadas à estabilidade de complexos organominerais em solos tropicais e subtropicais brasileiros. *Ciência Rural* **37**, 1301–1307. doi:10.1590/S0103-84782007000500013
- Jambu P, Moucawi J, Fustec E, Amblès A, Jacquesy JC (1985) Interrelation entre le pH et la nature des composés lipidiques du sol: étude comparée d'une rendzine et d'un sol lessivé glossique. *Agrochimica* **29**, 186–198.
- Jandl G, Leinweber P, Schulten HR (2007) Origin and fate of soil lipids in a Phaeozem under rye and maize monoculture in Central Germany. *Biology and Fertility of Soils* **43**, 321–332. doi:10.1007/s00374-006-0109-2
- Kisselle KW, Garrett CJ, Fu S, Hedrix PF, Crossley DA, Coleman DC, Potter RL (2001) Budgets for roots-derived C and litter-derived C: comparison between conventional tillage and no tillage soils. *Soil Biology & Biochemistry* **33**, 1067–1075. doi:10.1016/S0038-0717(01)00012-8
- Kögel-Knabner I, Guggenberger G, Kleber M, Kandeler E, Kalbitz K, Scheu S, Eusterhues K, Leinweber P (2008) Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition and Soil Science* **171**, 61–82. doi:10.1002/jpln.200700048
- Kopittke PM, Hernandez-Soriano MC, Dallal RC, Finn D, Menzies NW, Hoeschen C, Mueller CW (2018) Nitrogen-rich microbial products provide new organo-mineral associations for the stabilization of soil organic matter. *Global Change Biology* **24**, 1762–1770. doi:10.1111/gcb.14009
- Kučerík J, Tokarski D, Demyan MS, Merbach I, Siewert C (2018) Linking soil organic matter thermal stability with contents of clay, boundwater, organic carbon and nitrogen. *Geoderma* **316**, 38–46. doi:10.1016/j.geoderma.2017.12.001
- Kuhn TK, Krull ES, Bowater A, Grice K, Gleixner G (2010) The occurrence of short chain n-alkanes with an even over odd predominance in higher plants and soils. *Organic Geochemistry* **41**, 88–95. doi:10.1016/j.orggeochem.2009.08.003
- Peters KE, Walters CC, Moldowan JM (2005) 'The biomarker guide. I. Biomarkers and isotopes in the environment and human history.' 2nd edn. (Cambridge University Press: Cambridge, UK)
- Puget P, Angers DA, Chenu C (1998) Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. *Soil Biology & Biochemistry* **31**, 55–63. doi:10.1016/S0038-0717(98)00103-5
- Quéneá K, Largeau C, Derenne S, Spaccini R, Bardoux G, Mariotti A (2006) Molecular and isotopic study of lipids in particle size fractions of a sandy cultivated soil (Cestas cultivation sequence, southwest France): Sources, degradation, and comparison with Cestas forest soil. *Organic Geochemistry* **37**, 20–44. doi:10.1016/j.orggeochem.2005.08.021
- Rumpel C, Eusterhues K, Kögel-Knabner I (2010) Non-cellulosic neutral sugar contribution to mineral associated organic matter in top- and subsoil horizons of two acid forest soils. *Soil Biology & Biochemistry* **42**, 379–382. doi:10.1016/j.soilbio.2009.11.004
- Spielvogel S, Priezel J, Kögel-Knabner I (2008) Soil organic matter stabilization in acidic forest soils is preferential and soil type-specific. *European Journal of Soil Science* **59**, 674–692. doi:10.1111/j.1365-2389.2008.01030.x
- van Bergen PF, Nott CJ, Bull ID, Poulton PR, Evershed RP (1998) Organic geochemical studies of soils from the Rothamsted Classical Experiments—IV. Preliminary results from a study of the effect of soil pH on organic matter decay. *Organic Geochemistry* **29**, 1779–1795. doi:10.1016/S0146-6380(98)00188-0
- Veloso MG, Angers DA, Tiecher T, Giacomini S, Dieckow J, Bayer C (2018) High carbon sequestration in subtropical soil profiles under no-tillage with legume cover crop. *Agriculture, Ecosystems & Environment* **268**, 15–23. doi:10.1016/j.agee.2018.08.024
- Veloso MG, Cecagno D, Bayer C (2019) Legume cover crops under no-tillage favor organomineral association in microaggregates and soil C accumulation. *Soil & Tillage Research* **190**, 139–146. doi:10.1016/j.still.2019.03.003
- Wiesenberg GLB, Schwarzbauer J, Schmidt MWI, Schwark L (2004) Source and turnover of organic matter in agricultural soils derived from n-alkane/n-carboxylic acid compositions and C-isotope signatures. *Organic Geochemistry* **35**, 1371–1393. doi:10.1016/S0146-6380(04)00122-6
- Wiesenberg GLB, Dorodnikov M, Kuzyakov Y (2010) Source determination of lipids in bulk soil and soil density fractions after

- four years of wheat cropping. *Geoderma* **156**, 267–277. doi:[10.1016/j.geoderma.2010.02.026](https://doi.org/10.1016/j.geoderma.2010.02.026)
- Yu H, Gao Q, Shao Z, Ying A, Sun Y, Liu J, Mao W, Zhang B (2016) Decreasing nitrogen fertilizer input had little effect on microbial communities in three types of soils. *PLoS One* **11**, e0151622. doi:[10.1371/journal.pone.0151622](https://doi.org/10.1371/journal.pone.0151622)
- Zanatta JA, Bayer C, Dieckow J, Vieira FCB, Mielniczuk J (2007) Soil organic carbon accumulation and carbon costs related to tillage, cropping systems and nitrogen fertilization in a subtropical Acrisol. *Soil & Tillage Research* **94**, 510–519. doi:[10.1016/j.still.2006.10.003](https://doi.org/10.1016/j.still.2006.10.003)

Handling Editor: Mark Farrell