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► To cite this version:

M. Veloso, D. Angers, M. Chantigny, C. Bayer. Mineral–organic associations are enriched in both microbial metabolites and plant residues in a subtropical soil profile under no-tillage and legume cover cropping. *Soil Research*, 2022, 60 (6), pp.590-600. 10.1071/SR21151 . hal-03684015

HAL Id: hal-03684015

<https://hal.science/hal-03684015>

Submitted on 19 Sep 2022

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**Mineral-organic associations are enriched in both microbial metabolites and plant residues
in a subtropical soil profile under no-tillage and legume cover cropping**

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Long-term carbon stabilisation in tropical and subtropical soils rests on the formation of mineral–organic associations (MOAs), compounded by residues of plants and microorganisms. We sampled soil profiles in a long-term field experiment to evaluate if different soil managements affect these residues and consequent impact on the MOAs. Our findings highlight that the carbon accumulation is due to MOAs formation which is composed of both plant and microbial residues and is positively influenced by no-tillage and legume cover crops.

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Abstract

The implementation of no-tillage (NT) coupled with legume cover cropping results in high carbon sequestration rates in soils of Southern Brazil. We hypothesized that this effect is due to the formation of mineral-organic associations (MOAs) through the enrichment in plant and microbial residues, arising from the absence of tillage and the presence of high-quality legume residues. We sampled soil profiles in a long-term field experiment, and used density and particle size fractionation in combination with carbohydrate analyses to compare the effect of conventional tillage (CT) vs. NT, combined or not with legume cover cropping, and combined or not with mineral N fertilization. Both NT and the presence of legume cover crops favoured the accumulation and enrichment in plant-derived carbohydrates in the surface soil layer (0–5 cm), due to the accumulation of plant residues. The ratio of microbe-derived carbohydrates (galactose, manose, fucose and rhamnose) to plant-derived carbohydrates (arabinose and xylose) increased with soil depth indicating that the soil C is more microbially processed at depth than at the surface. Conservation management systems also increased soil C at depth and this was most visible in the clay fraction, suggesting that additional C is relatively stable (mineral-organic associations). The additional clay-size C accumulating at depth under conservation treatments is of both microbial and plant origin. Our results support, with field-based data, the current hypothesis that mineral-associated C is composed of both plant and microbial residues, and is positively influenced by conservation management practices.

Keywords: organo-mineral associations, soil carbohydrates, soil organic carbon accumulation, no-till, cover crops, plant residues, microbial residues, soil management, legumes

Introduction

The Paris climate agreement aims at holding global warming below 2 °C with efforts to limit it to 1.5 °C (Rogelj et al., 2016). The soil organic carbon (SOC) pool might function as a significant sink for atmospheric carbon dioxide (CO₂) and contribute to mitigate anthropogenic greenhouse gas emissions, provided that soil be properly managed (Lal, 2004). Changing agricultural practices represents one of the low-cost solutions proposed by Brazil to sequester carbon in soil and mitigate global warming (Brazil Ministry of the Environment, 2015).

Conversion from CT to NT is an important measure resulting in mean SOC sequestration rates of 0.32 and 0.48 Mg ha⁻¹ year⁻¹ in tropical and subtropical regions, respectively, in the top 20 cm of soil (Bayer et al., 2006). Recently, it was demonstrated that combining legume cover cropping with NT and N fertilization may result in SOC sequestration rates up to 1.15 Mg ha⁻¹ year⁻¹ in subtropical soils down to 100 cm in the soil profile (Velooso et al., 2018).

Previous studies have shown that mineral-organic associations (MOAs) are composed largely of microbial residues (Puget et al., 1998; Kogel-Knabner et al., 2008; Rumpel et al., 2010). Moreover, labile plant residues can induce microbial growth and have high microbial use efficiency, thereby favoring C accumulation through the formation of stable MOAs (Rumpel et al., 2010; Cotrufo et al., 2013). Formation of MOAs appears to be an important mechanism of C stabilization in tropical and subtropical soils as 65 to 92% of SOC in these soils is associated with kaolinite and Fe/Al oxides as the major clay constituents (Amado et al., 2006). However, recent studies have also shown that the persistent SOC is not composed exclusively of compounds derived from microbial activity but that plant-derived compounds contribute directly to MOAs even after several decades of degradation (e.g. Barré et al., 2018; Angst et al., 2021).

Carbohydrate analysis can be used to investigate the contribution of microbial products to SOC in MOAs. Carbohydrates containing hexoses such as galactose (G), mannose (M), fucose (F) and rhamnose (R) are considered to be synthesized predominantly by microorganisms, whereas carbohydrates containing pentoses such as arabinose (A) and xylose (X) are synthesized predominantly by plants (Oades and Wagner, 1971; Chesire, 1979). Thus, the GMFR/AX and GM/AX ratios have been used to estimate the relative contribution of microbial vs. plant derived carbohydrates in soils (Rumpel et al., 2010; Martins et al., 2012; Samson et al., 2020).

Our goal was to investigate the impact of cropping systems involving a cover crop of high residue lability associated with no-tillage (NT) on the accumulation of mineral-associated C and its associated carbohydrates, allowing to distinguish plant and microbe-derived residues. Using a long-term field experiment, we evaluated the effect of CT vs. NT, with or without legume cover cropping, on carbohydrate in SOC (in whole soil and MOAs), and in surface and sub-surface soil layers of a subtropical Acrisol in Southern Brazil.

Material and methods

Description of the experiment

The study was conducted in a long-term experiment (30 years) located at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul, in Eldorado do Sul-RS (30°06'S, 51°40'W and altitude of 96 m). The climate is subtropical (Cfa according to the Köppen classification), with mean annual temperature and annual rainfall of 19.4 °C and 1440 mm, respectively. The soil of the experiment is classified as sandy clay loam granite-derived Acrisol (FAO, 2015). However, there is an increase in clay content down the soil profile, from 217 g kg⁻¹ at 0–5 cm, 394 g kg⁻¹ at 20–30 cm and reaching 511 g kg⁻¹ at 75–100 cm. The main clay minerals are kaolinite (720 g kg⁻¹) and iron oxides (109 g kg⁻¹) (Bayer et al., 2001).

Until 1969, the area was under native grassland (mainly *Paspalum* and *Andropogon*). In the following 16 years, the soil was under intensive plowing and disking operations with annual cropping, and was seriously degraded at the time of establishment of a factorial experiment in 1985.

The experimental treatments comprised two tillage practices, CT and NT, applied in main plots of 15 × 20 m, which were divided in three 5 × 20 m subplots to compare three crop succession: black oats (*Avena strigosa* Schreb) – maize (*Zea mays* L.) (O/M); vetch (*Vicia sativa* L.) – maize (V/M); oats + vetch – maize + cowpea (*Vigna unguiculata* (L.) Walp) (OV/MC). Each subplot of the six tillage-cropping combinations were divided in two 5 × 10 m sub-subplots receiving either 0 or 180 kg ha⁻¹ of urea-N (0N and 180N), band-applied in the maize crop. The experimental design was a split-split plot with three replicates arranged in randomized complete blocks.

Winter cover crops were sown in April-May, at a seed rate of 80 kg ha⁻¹ for oats, 70 kg ha⁻¹ for vetch, and 30+40 kg ha⁻¹ for the oats+vetch combination. The cover crops were cut with a knife roller at the flowering stage (September-October). Tillage treatments and maize sowing were usually performed 1 to 3 weeks later. In CT, operations included plowing to 17-cm depth to incorporate cover crop residues, and two disking passes to 10 cm to prepare seedbed. In NT, soil was not disturbed and crop residues left on the surface, except on plant rows for seeding. Maize was planted at a row-spacing of 90 cm, with a targeted density of 50–70 thousand plants per hectare. Fifty kg ha⁻¹ of P₂O₅ and 50 kg ha⁻¹ of K₂O were applied at planting. In OV/MC, cowpea was sown 20–30 days after maize planting, in maize inter-rows with 40 cm between plants on the row. After maize harvesting, cowpea reached full development and was terminated with a knife roller immediately before the establishment of oats and vetch.

Annual C inputs in the cropping systems were calculated using a database compiling information since inception of the experiment in 1985 (Zanatta et al., 2007), and updated until year 2014 (Veloso et al., 2018). Annual C input from roots was estimated to be 30% of C input from aboveground plant parts (Zanatta et al., 2007). The C input in CT O/M 0N, NT O/M 0N, NT OV/MC 0N and NT OV/MC 180N was 4.8, 5.2, 9.2 and 10.2 Mg ha⁻¹ yr⁻¹.

Soil Neutral Carbohydrates

Carbohydrates extractable with strong acid are considered to represent the total carbohydrates in the soil (Lowe, 1993). The method described by (Chantigny and Angers, 2008) was used for this determination. Specifically, 2 g of air-dried and finely ground (<0.15-mm) whole soil, and 1 g of fine silt and clay-sized MOAs were placed in 50-mL centrifuge tubes. Eight mL of 12 mol L⁻¹ H₂SO₄ were added to whole soil, and 4 mL to fine silt and clay fractions. The soil and acid were gently mixed with a glass rod to ensure that the soil was completely moistened with the acid. The tubes were loosely capped and left on the bench for 2 h. After this period, the slurry were transferred into a 250-mL centrifuge bottles by washing the tube with 184 mL of deionized water to bring the solution to 0.5 mol L⁻¹ H₂SO₄. The bottles were sealed and placed in an oven set at 85°C for 24 h. After cooling, the bottles were centrifuged at 16,000 × g for 10 min. A 15-mL aliquot of the supernatant was transferred to plastic vials and stored at -20 °C for subsequent analysis.

Soil carbohydrates were analyzed based on the methods of Martens and Loeffelmann (2002) and (Chantigny and Angers, 2008). Briefly, 5 mL of soil hydrolysate was transferred in a 50-mL beaker and neutralized to pH 6.5-7.0 by dropwise addition of 0.5 mol L⁻¹ NaOH solution. The volume of NaOH solution used was recorded to adjust calculation of carbohydrate

concentrations. The neutralized solution was filtered using Whatman no. 42 filter paper. Approximately 2.5 mL of this filtrate was passed through a strong cation solid-phase exchange resin and collected in a 3-mL polypropylene Eppendorf vial. The cation-purified solution was then passed through a strong anion solid-phase exchange resin and collected in a new Eppendorf vial.

The monosaccharides present in the purified solutions were quantified using a liquid anion-exchange chromatograph with pulsed amperometric detection (Model Dionex ICS 5000+, Thermo Scientific, Sunnyvale, CA) equipped with automated sampler. The monosaccharide separation was performed with a CarboPac-PA1 pre-column (4 by 50 mm) in series with an analytical column (4 by 250 mm) and 10 μ L injection loop. As eluents, we used 20 and 500 mmol L⁻¹ NaOH solutions for monosaccharide analyses (0.8 mL min⁻¹ flow rate) and conditioning of the column (1.0 mL min⁻¹ flow rate), respectively. Monosaccharides (fucose, rhamnose, galactose, glucose, mannose, arabinose, xylose) were identified based on their retention time and co-elution with spiked standards (Martens and Loeffelmann, 2002). Blank samples were included during the extraction and purification processes to correct for the small amounts of arabinose, xylose, and glucose yielded by filter papers.

Statistical analyses

After analysis for normality by the Kolmogorov-Smirnov test and homogeneity of variance by the Levene test, the data were submitted to analysis of variance (ANOVA). The statistical model used to evaluate the effect of treatments on soil neutral carbohydrate concentrations and SOC stock and accumulation was: $Y_{ij} = \mu + B_i + T_j + \text{Error}_{(ij)}$, where Y = mean of treatment j in block i ; μ = general average of the experiment; B = block ($i = 1, 2, 3$); T = treatments ($j = 1, 2, 3, 4$); Error =

experimental error. All analyses were performed using SAS ® v.9.4 (Statistical Analysis System Institute, Cary, North Carolina).

When significant ($p < 0.05$), treatment means were compared by contrast analysis using the Tukey's test ($p < 0.05$):

- i) No-tillage effect: CT O/M 0N vs. NT O/M 0N;
- ii) Legume effect: NT O/M 0N vs. NT OV/MC 0N;
- iii) Fertilization effect: NT OV/MC 0N vs. NT OV/MC 180N.

Concentrations of individual neutral carbohydrates were expressed on C basis. The sum of arabinose and xylose (AX) was taken as a proxy of plant-derived soil carbohydrate-C, whereas the sum of galactose, mannose rhamnose and fucose (GMRF) was taken as a proxy of microbe-derived soil carbohydrate-C. Regression analyses were performed (SigmaPlot for Windows v. 13.0, Systat Software, Inc., San Jose, CA) to explore the relationships between GMFR or AX and previously published C concentrations in whole soil and in fine silt- and clay-sized MOAs (Veloso et al., 2020).

Results

Plant-derived carbohydrates (AX values in Table 1) ranged from 658 to 2096 mg kg⁻¹ in whole soil and fractions, and markedly declined down the soil profile. In the surface soil layer (0-5 cm), the concentration of plant-derived carbohydrates was 1.5 times greater under NT than CT in the fine silt fraction, and 1.3 times greater under NT in the clay fraction; the same trend (not significant) was found in the whole soil. Plant-derived carbohydrates were also 1.8 times more abundant, on average, in whole soil where legumes were included in the crop rotation, and 1.4

times more abundant with than without N fertilization. Similar effects of legumes and fertilization were also found in the clay and fine-silt fractions. The treatments did not influence plant-derived carbohydrates in the 20–30 cm depth, but a positive effect of legumes was found in the fine silt and clay fractions at the 75–100 cm depth.

As compared to the whole soil, concentrations of plant carbohydrates were generally greater in the clay fractions (1.26 to 1.88-fold), except in the surface layer of NT soils with legumes where concentrations were similar to whole soil (Table 1). The concentration of plant-derived carbohydrates in the fine silt fraction were generally similar to or lower than the whole soil at all soil depths.

Microbe-derived carbohydrates (GMFR values in Table 1) ranged from 880 to 3398 mg kg⁻¹ in whole soil and fractions in the surface soil layer, and declined more gradually than plant-derived carbohydrates down the soil profile, with values ranging from 554 to 1845 mg kg⁻¹ at 20–30 cm depth, and from 131 to 1034 mg kg⁻¹ at 75–100 cm depth. In the surface soil layer, microbe-derived carbohydrates were more abundant under NT than CT in the fine silt and clay fractions; a similar trend (not significant) was found for the whole soil. Microbe-derived carbohydrates were also more abundant in the presence of legumes and with N fertilization in whole soil and fractions of the surface soil layer. A positive effect of NT and a negative effect of N fertilization were found in the 20–30 cm soil layer in the clay fraction only. A negative effect of NT was found in the whole soil and the clay fraction at the 75–100 cm depth, whereas a positive effect of legumes was found in whole soil and both fractions, and a positive effect of fertilization was found in whole soil. As compared to the whole soil, the clay fraction was enriched in microbe-derived carbohydrates in all soil layers, but this relative enrichment was greater in surface soil and declined at depth (Table 1). The fine silt fraction was enriched in microbial carbohydrates in the

surface soil layer compared to the whole soil, especially in NT systems, but was clearly impoverished at greater depths (Table 1).

The microbe-to-plant-derived carbohydrate ratio (GMFR/AX values in Table 1) ranged from 0.90 to 2.85 and was generally narrower in surface than in deeper soil layers in all fractions. In the clay fraction of the surface soil layer, the ratio was narrower with than without legumes; the same trend (not statistically significant) was found in the whole soil and the fine-silt fraction. The ratio was not significantly influenced by treatments at greater depths. While the GMFR/AX ratio was wider in the clay and fine-silt fraction than in whole soil in the surface layer, the ratio was in the order: whole soil > clay fraction > fine-silt fraction at greater depths.

Significant and positive relationships were found between the concentration of neutral-sugars and whole soil C, fine silt-C and clay-C (Figs. 1-3) concentrations. The concentration of GMFR was positively related to whole soil C concentration in the 0–5 and 75–100 cm layer (Fig. 1 a,c), and to fine silt-C (Fig. 2 a,b,c) and clay-C at all depths (Fig. 3 a,b,c). A positive relationship was also found between the concentration of AX and whole soil C (Fig. 1 d) and clay-C in the top soil layer only (Fig. 3d), and fine silt-C at all depths (Fig. 2d, e, f).

Discussion

Surface soil layer

Our previous report at this site showed that implementing NT and legumes cover cropping significantly increased SOC storage in Southern Brazil (Veloso et al., 2018). After 30 y, this effect was visible in the whole soil profile (100 cm) with SOC stock being 20 (NT alone) to 35 (NT + legumes) Mg ha⁻¹ greater than in the CT system.

In NT soils, the narrower microbe-to-plant carbohydrate ratio in systems including legumes (with and without N fertilizer) reflected the large increase in LF-C in the surface soil layer (Veloso et al., 2020) that led to a dominant contribution of plant-derived carbohydrates. As compared to whole soil, the clay and fine-silt fractions were slightly enriched or had comparable plant-derived carbohydrate concentration in the surface soil layer. In contrast, both fractions were generally highly enriched in microbe-derived carbohydrates, resulting in wider microbe-to-plant carbohydrate ratio in fractions than in whole soil, especially with the clay fraction. These findings support our previous results that plant-derived light-fraction (LF) C is a dominant SOC source in the surface soil layer (Veloso et al., 2020), and further indicate that part of this LF-C was heavily colonized with microbes and had initiated decomposition and encrustation with soil particles, resulting in enrichment of SOC with microbial carbohydrates in fine silt- and clay-sized MOAs.

Subsurface soil layers

Deeper in the soil profile, the direct influence of crop residues on MOA formation was much smaller (much less LF-C as observed in Veloso et al., 2020). In these soil layers, it is likely that microbial activity was stimulated by percolation of soluble substrates from surface crop residues and/or diffusion of root exudates, leading to direct formation of fine MOAs. This hypothesis is supported by the markedly wider microbe-to-plant carbohydrate ratio in whole-soil and clay-sized MOA (> 2), in subsurface than in surface soil layers (Table 1), suggesting that the contribution of microbially-derived compounds to SOM increases with soil depth (Angst et al., 2018). Our results further suggest that the contribution of microbial-derived compounds to stabilized MOAs may also increase with soil depth.

The positive effect of NT on SOC accumulation was more visible in clay fraction in the 20–30 cm depth (Fig. 3b), where this fraction represented 87-91% of SOC (Veloso et al., 2020), showing that SOC accumulation is more directly associated with the clay fraction in deeper soil layers. This suggests these systems also stimulated the accumulation of microbial metabolites at depth and the formation of presumably stable fine MOAs. This C accumulation at depth can be hypothesized to be mediated by percolation of soluble compounds through the biopores, as discussed earlier, or by exudation and decay of deep roots, which stimulated microbial activity and formation of fine MOAs at depth (Hobley et al., 2016). This hypothesis would also explain that while both plant-derived and microbe-derived carbohydrates were closely related to SOC, fine-silt-C and clay-C concentrations in the surface soil layer and to fine-silt-C at greater depths, microbe-derived but not plant-derived carbohydrate concentration explained variations in clay-C concentration in the 20–30 and 75–100 cm depths (Fig. 3). Our results are in agreement with Angst et al. (2018) who suggested that subsoil SOC accumulation was highly influenced by OM input and its retention by the soil mineral matrix through the contribution of microbial-derived compounds.

The lower concentration of microbial residue in the NT O/M treatment in whole soil, fine silt and clay fraction of the 75–100 cm soil layer compared to CT O/M was not completely understood, as this trend was not observed for C concentration (Veloso et al., 2020). However, the inclusion of the legume cover crop under NT (NT OV/MC) increased the concentration of both microbial and plant carbohydrates at that depth suggesting that legume cover crops are a greater driver of carbohydrate accumulation at depth than NT alone. Both plant- and microbial-derived carbohydrates could be leached from the topsoil layer or deposited directly at depth by the roots, and then colonized by microbes. These two mechanisms correspond to the “ex vivo modification”

and “in vivo turnover” pathways described by Liang et al. (2017) and evidenced by Angst et al. (2017) and Córdova et al. (2018), and likely drive MOA formation and accumulation at depth. In the ex vivo modification pathway, extracellular enzymes release depolymerized products of plant compounds that are not readily assimilated by microbes and thus susceptible to rapid adsorption to mineral surfaces and stabilization in MOAs. In contrast, in the in vivo turnover pathway, microbes assimilate plant-derived organic matter and synthesize new compounds that subsequently interact with the mineral soil matrix to form MOAs. Overall, our results are in agreement with Barré et al. (2018) and Angst et al. (2021), who demonstrated that persistent SOC is not composed exclusively of compounds derived from microbial activity but that plant-derived compounds also directly contribute to SOC even after several decades of degradation. Our results contribute to the current debate on the composition of persistent SOC related to substrate quality and microbial turnover (Kallenbach et al. 2016; Liang et al. 2017; Barré et al., 2018; Córdova et al., 2018), suggesting that MOAs are enriched in both microbial metabolites and plant residues in a subtropical soil profile under no-tillage and legume cover cropping.

Conclusions

Using a long-term field experiment, we found a dominant effect of accumulation of crop residues as influenced by conservation management systems, that led to a dominant contribution of plant-derived carbohydrates in surface soil layer. The widening in microbe-to-plant carbohydrate ratio with depth in whole soil and the clay fraction indicate that the soil C is more microbially processed at depth than at surface. The conservation management systems increased soil C at depth and this was most visible in the clay fraction, suggesting that the additional C accumulated in these systems is fairly stable (mineral-organic associations). However, the additional clay-C accumulating at

depth under conservation treatment comprised both microbe- and plant-derived materials. These results strengthen our understanding of how carbon accumulates and MOAs are formed at different depths in subtropical soils after adoption of no-tillage and legume cover cropping.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgement

The authors are grateful to the staff of the Experimental Station and Department of Soil Science of the Federal University of Rio Grande do Sul for their help with field and laboratory activities, respectively. They also thank the National Council for Scientific and Technological Development (CNPq) and Foundation for Research Support of Rio Grande do Sul State (Fapergs) for funding this work and the Coordination for the Personnel Improvement of Graduate Education (CAPES) together with to Agriculture and Agri-Food Canada (AAFC) for award of a sandwich doctorate scholarship to M. Veloso in AAFC.

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SUMMARY TEXT FOR THE TABLE OF CONTENTS

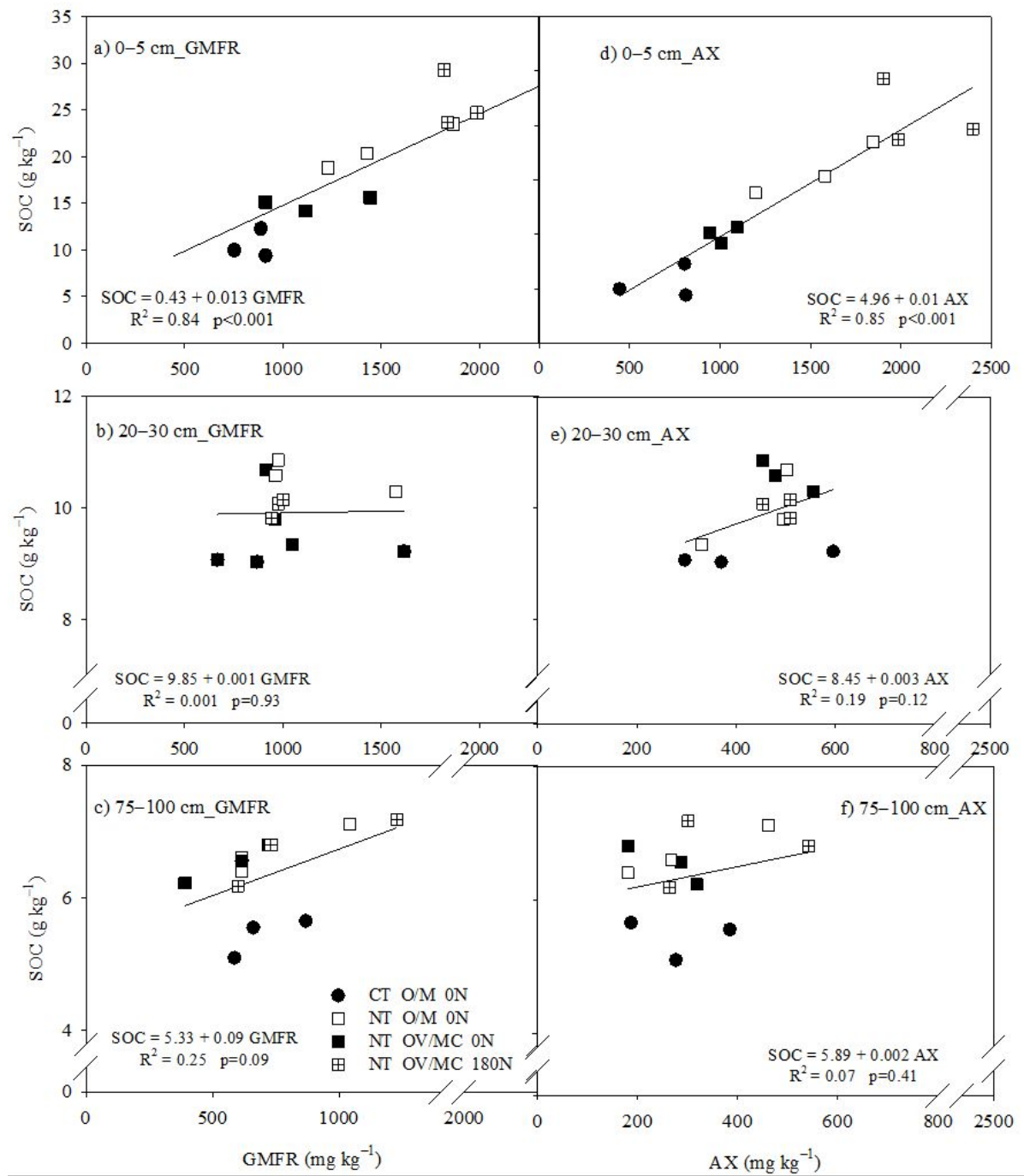
Long-term carbon stabilisation in subtropical soils rests on the formation of mineral–organic associations (MOAs), that is compounded by residues of plants and microorganisms. We sampled soil profiles in a long-term field experiment to evaluate if different soil managements affect these residues and consequent impact on the MOAs. Our findings highlight that the carbon accumulation is due to MOAs formation which is composed of both plant and microbial residues and is positively influenced by no-tillage and legume cover crops.

Figures

Figure 1. Regression of GMFR concentration (a,b,c) and AX concentration (d,e,f) as function of SOC concentration of whole-soil in 0–5, 20–30 and 75–100 cm soil layer the from Acrisol submitted to conventional tillage (CT) and no tillage (NT) with oat/maize (O/M) and oat+vetch/maize+cowpea (OV/MC); and two nitrogen fertilization rates, 0 e 180 kg of urea ha⁻¹ (0N and 180N).

Figure 2. Regression of GMFR concentration (a,b,c) and AX concentration (d,e,f) as function of carbon concentration in fine-silt fraction in 0–5, 20–30 and 75–100 cm soil layer the from Acrisol submitted to conventional tillage (CT) and no tillage (NT) with oat/maize (O/M) and oat+vetch/maize+cowpea (OV/MC); and two nitrogen fertilization rates, 0 e 180 kg of urea ha⁻¹ (0N and 180N).

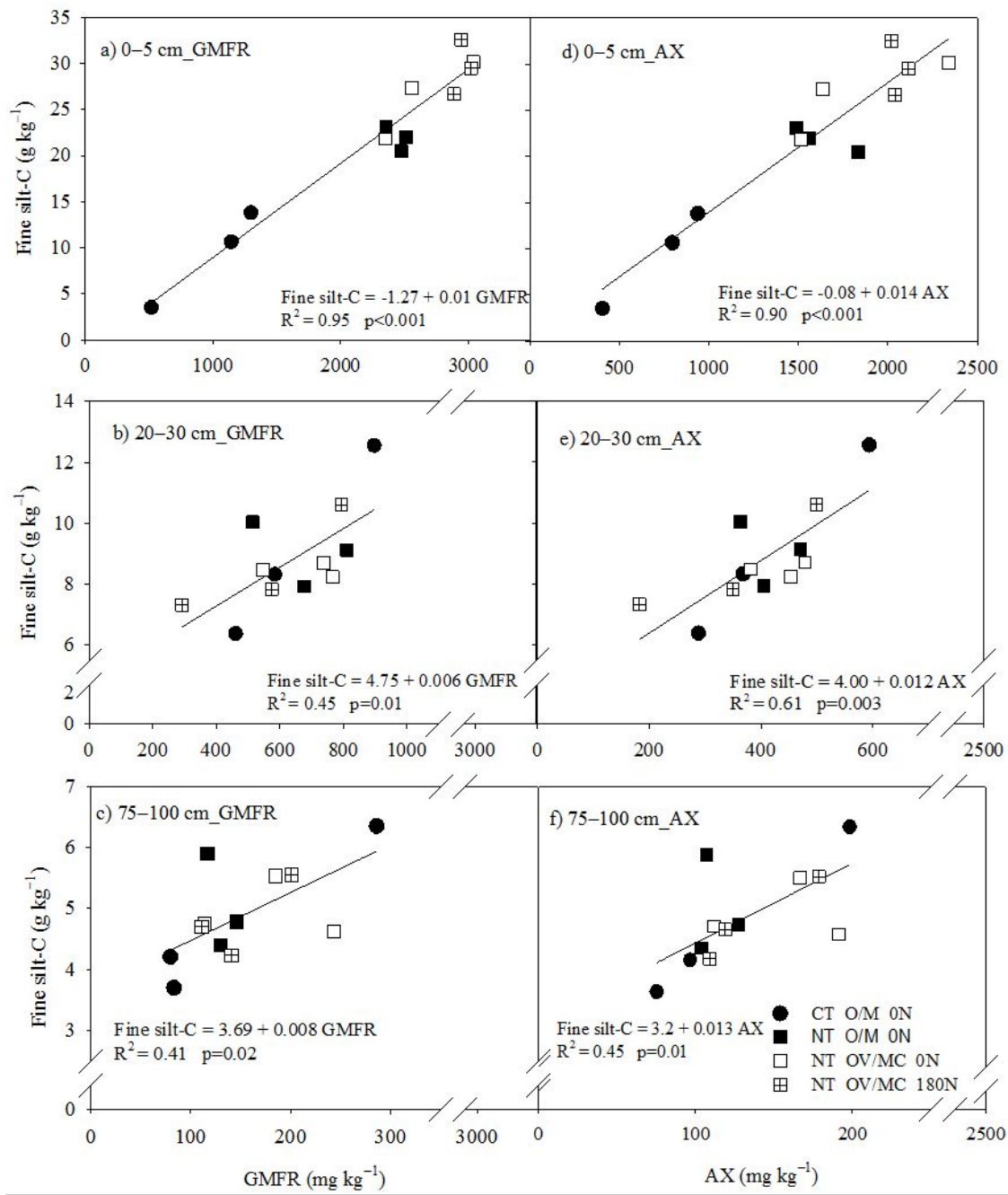
Figure 3. Regression of GMFR concentration (a,b,c) and AX concentration (d,e,f) as function of carbon concentration in clay fraction in 0–5, 20–30 and 75–100 cm soil layer the from Acrisol submitted to conventional tillage (CT) and no tillage (NT) with oat/maize (O/M) and oat+vetch/maize+cowpea (OV/MC); and two nitrogen fertilization rates, 0 e 180 kg of urea ha⁻¹ (0N and 180N).



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23 **Figure 1.**



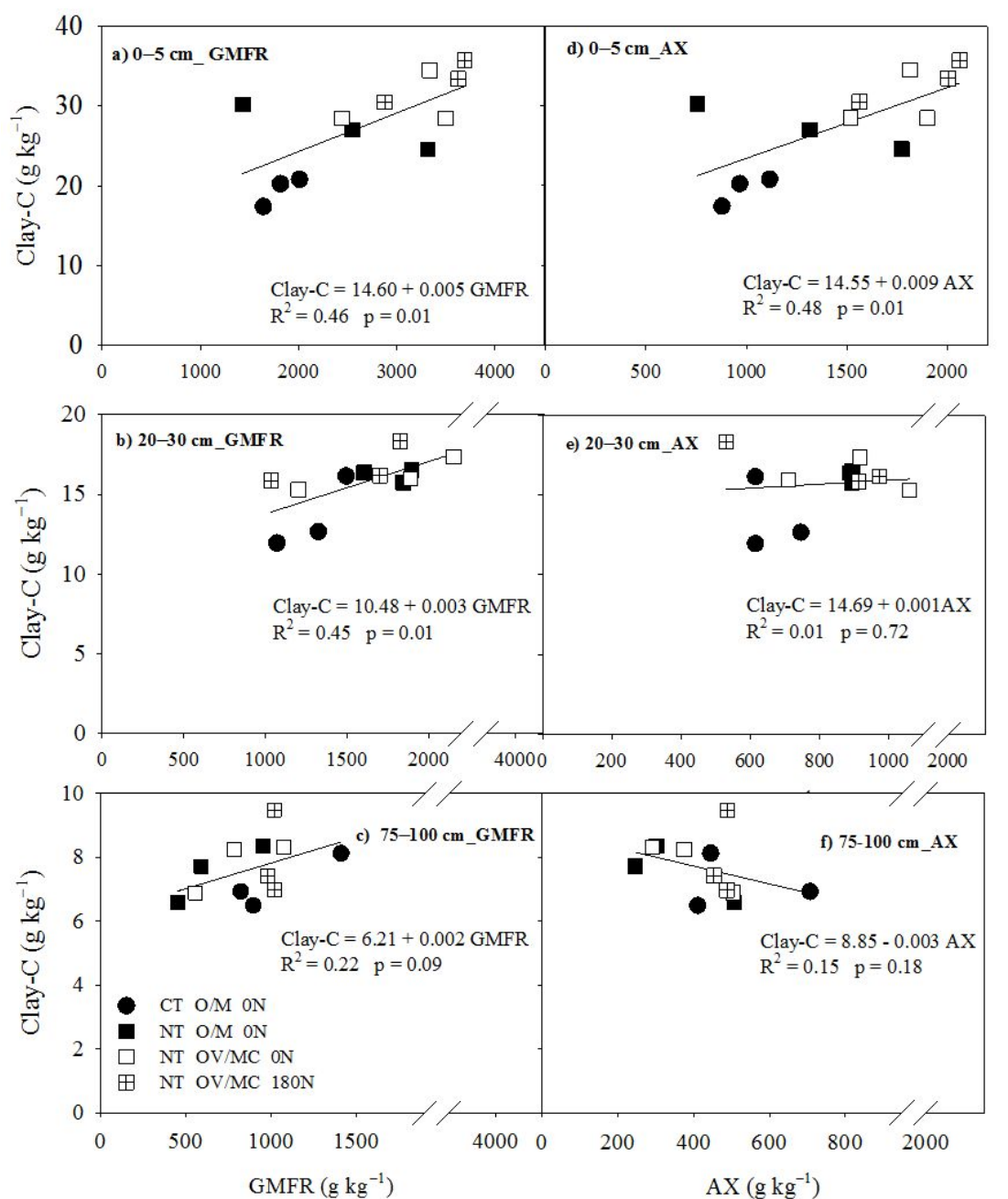


Figure 3.

Table 1. Total plant- (AX) and microbe-derived (GMRF) carbohydrates and GMFR/AX ratio in whole soil (WS) and clay- and fine silt-sized fractions in 0–5, 20–30 and 75–100 cm layers of an Acrisol submitted to conventional tillage (CT) and no tillage (NT), with oat/maize (O/M) or oat+vetch/maize+cowpea (OV/MC) succession, and with (180 kg of urea ha⁻¹ [180N]) or without (0N) fertilization.

Tillage	Crop succession	Fertilization	AX‡			GMFR‡			GMRF/AX ratio		
			WS	Clay	FS	WS	Clay	FS	WS	Clay	FS
			mg kg ⁻¹								
0 – 5 cm											
CT	O/M	0	657.6	988.8	709.6	880.1	1813.2	981.6	1.39	1.83	1.38
NT	O/M	0	1015.4	1283.3	1628.1	1156.5	2443.2	2445.3	1.13	1.90	1.50
	OV/MC	0	1541.2	1744.1	1833.8	1506.5	3100.4	2655.0	0.98	1.78	1.45
	OV/MC	180	2096.0	1875.7	2059.0	1880.5	3398.2	2949.9	0.90	1.81	1.43
Contrast†	No-tillage		0.09	0.006	<0.001	0.10	0.002	<0.001	0.14	0.19	0.08
	Legumes		0.04	<0.001	0.01	0.05	0.001	0.07	0.34	0.04	0.37
	Fertilization		0.03	0.03	0.007	0.04	0.04	0.02	0.63	0.49	0.82
20 – 30 cm											
CT	O/M	0	421.0	690.9	416.4	1051.6	1317.6	647.4	2.44	1.92	1.52
NT	O/M	0	473.2	891.7	413.1	909.6	1686.7	667.8	1.93	1.89	1.60
	OV/MC	0	506.0	917.2	438.0	1184.9	1845.4	683.1	2.33	2.05	1.55
	OV/MC	180	491.2	805.9	347.1	972.4	1551.7	553.6	1.98	2.02	1.54
Contrast	No-tillage		0.42	0.19	0.92	0.54	0.02	0.91	0.12	0.51	0.52
	Legumes		0.59	0.86	0.70	0.25	0.22	0.93	0.14	0.20	0.85
	Fertilization		0.81	0.44	0.19	0.37	0.05	0.49	0.19	0.90	0.94
75 – 100 cm											
CT	O/M	0	282.6	393.4	125.2	704.7	823.9	149.6	2.65	2.17	1.11
NT	O/M	0	262.3	351.6	112.7	575.0	666.5	130.9	2.28	2.01	1.16
	OV/MC	0	303.4	484.1	159.7	767.4	1034.3	180.7	2.85	2.17	1.12
	OV/MC	180	369.5	476.9	138.6	853.9	1008.6	150.6	2.49	2.12	1.09
Contrast	No-tillage		0.72	0.43	0.48	0.01	0.001	0.75	0.38	0.23	0.87
	Legumes		0.47	0.03	0.02	0.001	<0.001	0.41	0.20	0.24	0.91
	Fertilization		0.26	0.89	0.24	0.03	0.50	0.61	0.41	0.58	0.90

† Contrast analysis was used to test the effects of no-tillage (CT O/M 0 vs. NT O/M 0), legume cover cropping (NT O/M 0 vs. NT OV/MC 0) and nitrogen fertilization (NT OV/MC 0 vs. NT OV/MC 180) with their respective *P* value.

‡ AX, sum of arabinose and xylose concentrations; GMFR, sum of galactose, mannose, fucose and rhamnose concentrations.