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Acacia and *Eucalyptus* plantations modify the molecular composition of density organic matter fractions of subtropical native pasture soils

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ABSTRACT

In Southern Brazil, exotic species as Acacia (A) and Eucalyptus (E) are often planted over native pasturelands and may change bulk soil organic matter (SOM) composition as verified in our previous study with Cambisols (0–5 cm). Here we aimed to follow the impact of seven-year A and E plantation on the composition of the free light- (FLF), occluded light- (OLF) and heavy fraction (HF) of SOM along the soil profile. We hypothesized that A and E may have shifted the molecular composition and carbon (C) stocks (C_s) of SOM fractions, at least at 0–5 cm; with stronger shifts caused by A due to greater E litter recalcitrance. Litter and soil samples (0–20 cm) were collected at A and E and neighboring native pasturelands without A (WA) and without E (WE). Litter, FLF, OLF and HF samples were subjected to C, nitrogen (N), pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS) and lipid biomarkers analysis. In E soil, the C_s of FLF at 0–5 cm (0.5 Mg ha^{-1}) and OLF at 5–10 cm (1.7 Mg ha^{-1}) were 194 and 70 % greater than in WE, whereas in A soil the C_s of OLF at 0–5 cm (0.2 Mg ha^{-1}) was 44 % lower than in WA. Nevertheless, A changed more remarkably the composition of SOM fractions, confirming our hypothesis partially, likely due to greater A litter biodegradability (<C:N ratio, <aromatic and polyaromatic and >polysaccharides abundance) compared to E. The contribution of A litter to FLF (0–10 cm) was evidenced by abundance of long chain and the predominance of odd-over-even n-alkanes (particularly >C29), and to OLF (0–20 cm) by the greatest abundance of n-alkanes at C31, resembling A litter. Loss of C and N of OLF in A compared to WA (0–5 cm) was compensated by fresh A litter additions to FLF and OLF and microbial-derived compounds association to soil minerals, equaling soil C_s in A and WA. The lower soil N stock in A compared to WA likely resulted from depletion of occluded microbial-derived N-compounds, supposedly reflecting the breakdown of soil aggregates at forest plantation. The increase of Cs in FLF and OLF of E compared to WE soil was associated with increased abundance of aromatics and n-alkane/alkenes and decrease of fatty acids. Similar patterns of n-alkanes observed for OLF of E and WE soil confirmed the incipient contribution of E litter to OLF. Conversion of these pastures to A and E modifies SOM composition and protection, requiring policies in view of the highly invasive potential and possible negative implications of A and E to native pasture regeneration.

1. Introduction

In Brazil, the area occupied by silviculture in 2021 (9.5 million ha) was 30 % greater than that of 2012, and it is mostly concentrated (71 %) in the Southeast and Southern regions of the country, having eucalyptus (70 %), pinus (23 %) and acacia (2 %) as main species cultivated (Abraf, 2016; IBGE, 2022).

The expansion of silviculture in Southern Brazil (subtropical climate) often includes plantation of exotic forests as eucalyptus and acacia over native pasturelands (composed mainly of grasses). In the southernmost Brazilian state named Rio Grande do Sul State (RS), these native pastures cover 193,836 km^2 (69 and 2.3 % of RS and Brazilian territory, respectively) and characterize the Pampa biome, which extends to Argentina and Uruguay totaling 750,000 km^2 (MMA, 2023). From 2000

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to 2021, 34,000 km^2 of the Brazilian Pampa (restricted to RS) vegetation has been removed (Kuplich et al., 2023), raising concerns on depletion of soil organic matter (SOM) (Oliveira et al., 2017), also because A and E can be highly invasive (Koutika and Richardson, 2019).

Physical SOM fractions inform on the mechanism of soil organic carbon (SOC) protection (as chemical recalcitrance of molecules, physical protection intro aggregates, and organo-mineral interactions) and can sensitively detect changes in soil use, particularly labile-SOM fractions (Plaza et al., 2019). Depletion of SOC stocks in newly established eucalyptus plantations, especially at superficial layers, has been reported for Brazilian soils (Lima et al., 2008; Leite et al., 2010). Generally, this is associated with rupture of soil aggregates caused by mechanical preparation of the soil for forest plantation, which favors depletion of occluded SOC (Zinn et al., 2002). Interestingly, Zinn et al. (2011) observed that the SOC stocks (0–1 m layer) of tropical Brazilian soils cultivated for 14 years with eucalyptus were equivalent to that of Cerrado, likely due to the high and constant input of biomass to soil along with reduced soil disturbance in stablished eucalyptus plantations (compared to newly planted forests). These processes are known to favor the accumulation of particulate SOM and the formation and stabilization of aggregates and organo-mineral complexes (Marques et al., 2015).

Rangel and Silva (2007) reported greater SOC content in light SOM fractions (0–5 cm layer) of a tropical Brazilian Oxisol cultivated for 29 years with eucalyptus compared to pasture and maize. This was attributed to the high litter and biomass input to soil by eucalyptus and to the slow decomposition of these materials in soil. Lima et al. (2008) also found higher SOC stock (0–20 cm layer) in a tropical Brazilian Oxisol cultivated for 34 years with eucalyptus compared to pasture. This was assigned to greater (115–227 %) accumulation of SOC associated to minerals in the soil with eucalyptus. Overall, these quantitative studies attribute changes in SOC stocks after forest plantation to forest age and species, previous soil use, soil practices and type. However, these changes are less understood at a molecular and mechanistic level, especially in subtropical soils.

Since the litter of eucalyptus and acacia differ chemically, their incorporation into SOM fractions may follow different processes (Man et al., 2022). In Chinese red subtropical soils, Zhang et al. (2018) observed slower SOC accumulation in a degraded grassland converted to eucalyptus compared to acacia. The authors suggested that acacia enhances soil N status, thereby favoring organo-mineral interactions, as similarly reported for agricultural soils (Cotrufo et al., 2019; Veloso et al., 2020).

Specific lipid compounds of litter can be used as sensitive biomarkers to associate litter chemistry with litter decomposition and SOC accumulation pathways (Quenea et al., 2006). Wiesenberg et al. (2010) found that microbial-derived lipids were mostly abundant in light SOM fractions, whereas plant-derived and long-chain lipids were relatively stabilized by interaction with minerals after four years of conversion of green fallow to wheat in a temperate Cambisol. In this way, molecular proxies of n-alkanes and n-fatty acids are useful to identify C sources (e.g., plant and microbial compounds) in soil (Jansen and Wiesenberg, 2017) but may provide limited conclusions on SOM depletion and preservation pathways if employed alone (Li et al., 2018). Pyrolysis–gas chromatography–mass spectrometry (Py-GC/MS) is a state-of-art technique for fast, direct, and detailed molecular analysis of litter and SOM (San-Emeterio et al., 2023).

In Santana et al. (2015) the effects of eucalyptus and acacia plantation on the SOC content and SOM chemistry of subtropical Cambisols in Brazil were studied, reasoning the present work. The SOC content (0–80 cm) of native pasture soil was not altered by seven years of eucalyptus and acacia cultivation. However, Py-GC/MS and lipid biomarkers revealed faster incorporation of acacia litter into the soil (0–5 cm) due to its greater biodegradability compared to eucalyptus, agreeing with Koutika et al. (2019).

Given the above considerations, in this sequential study we aimed to follow the impact of acacia and eucalyptus on the molecular composition and C stocks of density SOM fractions along the profile of subtropical Cambisols previously under Pampa by means of Py-GC/MS and lipid biomarkers. We hypothesized that acacia and eucalyptus may have shifted the molecular composition and C stocks of SOM fractions at least at 0–5 cm. We also hypothesized that light SOM fractions may be more sensitive to these changes than mineral-associated SOM, especially in the acacia plantation soil.

2. Materials and methods

2.1. Study area, litter and soil sampling

The study area is located in Encruzilhada do Sul city, RS state, Southern Brazil. The climate is subtropical humid (Cfa) according to Köppen's classification. The annual mean temperature is 17 °C and annual mean precipitation is 1,500 mm. The soil is classified as Cambisol (IUSS Working Group WRB, 2015) with a sandy clay loam texture (495, 244 and 261 g kg^{-1} of sand, silt and clay, respectively, at 0–20 cm layer).

Before planting, the eucalyptus (30°09′16.86″ S, 52°37′54.37″ W) and the acacia area (30°38′01.68″ S, 52°29′10.92″ W) were prepared using a subsoiler (0.6 m working depth), weeding pre-planting and 100 g $plant^{-1}$ of NPK 06:30:06 + 200 kg ha^{-1} reactive phosphate. In the eucalyptus area, 2 Mg ha^{-1} of dolomitic limestone was applied. Uninoculated *Acacia mangium* (A) and *Eucalyptus grandis* (E) were planted at 3 x 1.5 m and 3 x 2 m spacing, totaling 2,222 and 1,667 plants ha^{-1}, respectively. After planting, 100 g $plant^{-1}$ of NPK 15:05:30 and post-emergent herbicide (glyphosate) were applied.

Seven years after A and E plantation, soil sampling was performed in these areas and in respective adjacent areas: without A (WA) and without E (WE). The WA and WE areas were defined as references as they exhibited the same native pasture with predominance of grasses (typical Pampa) present in A and E sites prior to their plantation.

Three plots of 1 ha of similar slope position were identified in A, WA, E and WE area. In each plot, three soil trenches were opened and one soil sample (subsample) was collected in each trench at 0–5, 5–10 and 10–20 cm layer. These subsamples were collected in soil blocks using a shovel and a spatula to preserve soil aggregates in view of the SOM fractionation procedure. The soil blocks were carefully disaggregated by hand to pass through a 9.51 mm sieve, and then air-dried. The three soil subsamples collected in the same plot were mixed to compose one replicate, resulting in three replicates per soil layer. In the same way, soil volumetric steel rings were collected to determine soil bulk density (Bd). Soil pH was determined in distilled water (1:1, w:v) according to Tedesco et al. (1995). Litter subsamples were collected at A, WA, E and WE area before opening each soil trench. The three subsamples of each plot were dried at 60 °C, mixed and ground to form one composite replicate, resulting in three replicates per area.

Soil and litter C and N concentration were determined by dry combustion (CHNS analyzer, FlashEA 1112, Thermo Finnigan). Soil C and N stocks (Mg ha^{-1}) were calculated based on the soil Bd using the equivalent soil mass method and WA and WE as reference sites (Ellert and Bettany, 1995). These and other general soil attributes (Table 1) were discussed in our previous work (Santana et al., 2015).

2.2. SOM physical fractionation

For the density fractionation of SOM (Conceição et al., 2008) 10 g of 9.51-sieved soil were placed into a 100 mL centrifuge tube together with 80 mL of sodium politungstate (SPT, Sometu – 99.9 %) solution (2.0 g cm^{-3}). The tube, closed with a rubber stopper, was gently inverted five times to release the free light fraction (FLF). The suspension was centrifuged at 2000 g for 90 min and the supernatant containing the FLF was filtered (Whatman GF/A, 0.45 μm) under vacuum. The material retained on the filter was rinsed with distilled water and 0.01 M $CaCl_2$ solution to remove excess of SPT, and with distilled water (200 mL) to remove the $CaCl_2$. Thereafter, 80 mL of SPT solution was added to the

Table 1
Litter carbon (C) and nitrogen (N) content and C:N ratio, and soil bulk density (Bd), pH, C and N stocks and C:N ratio at Acacia (A), without Acacia (WA), Eucalyptus (E) and without Eucalyptus (WE) site.

Sample	Litter C g kg^{-1}	N	C:N	Soil Bd g cm^{-3}	pH	C Mg ha^{-1}	N	C:N
Acacia	500.6	28.2	18					
0–5 cm				1.2	4.5	17.0	1.4	12
5–10 cm				1.3	4.4	13.1	1.1	12
10–20 cm				1.6	4.4	28.2	2.3	12
without Acacia	307.9	9.2	34					
0–5 cm				1.5	4.9	17.2	1.6	11
5–10 cm				1.5	5.1	12.4	1.1	11
10–20 cm				1.6	5.1	29.0	2.6	11
Eucalyptus	341.0	9.8	35					
0–5 cm				1.3	5.1	9.0	0.8	12
5–10 cm				1.4	5.0	11.0	1.0	11
10–20 cm				1.5	4.7	22.5	1.9	12
without Eucalyptus	145.3	6.3	23					
0–5 cm				1.0	4.9	9.0	0.8	11
5–10 cm				1.4	4.8	11.0	1.0	11
10–20 cm				1.5	4.8	21.7	1.9	11

soil into the tube, and the suspension was sonicated (Vibracel. VC, 750) at 630 J mL^{-1}, to release the occluded light fraction (OLF). The suspension was centrifuged, filtered, and rinsed as described for the FLF. The material remaining in the tube (heavy fraction, HF), was washed with distilled water-$CaCl_2$-distilled water, as described for FLF. The FLF, OLF and HF were oven-dried at 50 °C, weighed and ground. The average rate of material recovery after the fractionation procedure was 96 % (±4 standard deviation).

The C and N concentrations in FLF and OLF were determined by dry combustion (CHNS analyzer, FlashEA 1112, Thermo Finnigan), and that in HF were calculated by the difference between the C and N content of bulk soil and FLF + OLF. The C and N stocks of SOM fractions were obtained on equivalent soil mass (Ellert and Bettany, 1995) and also expressed in percentage as $C_{fraction}/C_{soil}$ and $N_{fraction}/N_{soil}$.

2.3. Analytical pyrolysis (Py-GC/MS)

Composite samples (of the three field replicates) of litter, FLF, OLF, and HF were analyzed by Py-GC/MS. Prior to analysis, FLL, OLF and HF samples were treated three to five times with 10 % hydrofluoric acid to remove minerals and concentrate SOM (Gonçalves et al., 2003).

Pyrolysis was performed using a pyrolyzer (Frontier Laboratories, model 2020i) attached to a GC–MS system (Agilent 6890 N). The density fractions and litter samples were placed in the micro-oven and pyrolyzed at 500 °C for 30 s. The gas chromatograph was equipped with a capillary column DB17-01 (30 m, 0.25 mm i.d., 0.25 μm film thickness). The oven temperature program was: 50 °C (1 min) to 100 °C at 30 °C min^{-1}, 100 °C to 300 °C at 10 °C min^{-1}, then 300 °C for 1.0 min. The carrier gas was helium at a controlled flow rate of 1 mL min^{-1}. The detector was an Agilent 5973 mass selective detector and the mass spectra were acquired with 70 eV ionizing energy. The pyrolysis compounds were identified by analysis of the mass fragments, retention time and comparison of the mass spectra with computerized libraries (Wiley and NIST). Compounds that could not be identified, appeared only once, or with abundance <0.3 % were excluded from analysis. According to the chemical structure and precursors of the identified compounds they were grouped into the following chemical families (Santana et al., 2015): unspecific/other aromatics (Oar); polyaromatics (Par); polysaccharides (Pol); lignin-derived methoxyphenols (Mph); N-compounds (N-comp); phenols (Phe); n-alkane/alkenes (n-a/a); branched-chain alkanes/alkenes (Bra); fatty acids (Fac); and terpenoids (Ter). The sum of the areas of all identified peaks was defined as 100 % of the total ion current (TIC). The relative abundance (%) of the identified compounds was calculated based on its peak area relative to TIC.

2.4. Free lipids extraction

Free lipids of FLF, OLF, HF and litter composite samples (made of the three field replicates) were extracted via Soxhlet with dichloromethane–methanol (3:1 v/v) for 10 h (González-Vila et al., 2003). The total lipid extract content (Lp) was determined gravimetrically. The GC–MS analysis was performed with a HP5 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) applying an oven temperature program increasing from 50 to 100 °C at 30 °C min^{-1} and then to 300 °C at 10 °C min^{-1}. Helium was used as carrier gas at a flow rate of 1.0 mL min^{-1} and the mass spectra were measured at 70 eV ionizing energy. Individual compounds were identified by low resolution mass spectrometry and by comparison with published mass spectra libraries (NIST and Wiley). Traces corresponding to selected homologous series of biomarkers families were obtained by single ion monitoring of ions characteristic, such as ion at *m/z* 85 for n-alkanes and ion at *m/z* 74 for n-fatty acids (Santana et al., 2015).

The compound abundance was obtained using single ion monitoring traces on a semi-quantitative basis, assuming a constant relationship between the re-constructed ion current response and the amount of a molecule in the corresponding sub-fraction. Relative abundance for each peak within each series was calculated as a percentage of TIC. Lipid molecular proxies were calculated to evaluate the contribution of different sources to the SOM: average chain length (ACL) described as [(Σzn × n)/Σzn], where zn is the relative amount of the n-alkane or n-fatty acid with n-carbons; and carbon preference index (CPI) that informs about the predominance of odd-over-even in alkanes chains (Σcodd/Σceven) and even-over-odd in n-fatty acids chains (Σceven/Σcodd) (Wiesenberg et al., 2010). High values of ACL and CPI are associated to plant biomass because of the selective preservation of long chain lipids (Eglinton and Hamilton, 1967).

2.5. Statistical analysis

The means of C and N stocks in FLF, OLF and HF within paired sites (A vs WA, E vs WE) and soil layer (0–5, 5–10 and 10–20 cm) were compared by *t*-test ($p < 0.05$). Principal component analysis (PCA) was performed to ordinate and illustrate shifts in the chemical composition of litter and SOM fractions along the soil profile detected by Py-GC/MS after conversion of native pasture (WA and WE) to A and E. Chemical groups with less abundance and relevance for the differentiation of litter and SOM fractions (polyaromatics and branched-chain alkanes/alkenes) were excluded from PCA.

Table 2
Average (n = 3) carbon (C) and nitrogen (N) stocks and C:N ratio of free-light (FLF), occluded-light (OLF) and heavy-fraction (HF) in Acacia (A), without Acacia (WA), Eucalyptus (E) and without Eucalyptus (WE) soils at 0–5, 5–10 and 10–20 cm layer.

Area		C			N			C:N		
		FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF
		Mg ha^{-1}								
A	0–5 cm	1.6	2.2 *	13.2	0.11	0.16 *	1.1	14	13	12
WA		1.1	3.9	12.3	0.08	0.31	1.2	13	13	10
A	5–10 cm	0.49	1.2	11.4	0.03	0.09	1.0	15	14	11
WA		0.29	0.8	11.3	0.02	0.07	1.0	14	12	11
A	10–20 cm	0.31	1.7	26.2	0.02	0.12	2.2	15	14	12
WA		0.52	1.8	26.7	0.04	0.16	2.4	14	12	11
E	0–5 cm	0.47 *	1.6	7.0	0.03 *	0.11	0.62	15	15	11
WE		0.16	1.3	7.6	0.01	0.10	0.70	20	14	11
E	5–10 cm	0.20	1.7 *	9.5	0.01	0.12 *	0.89	15	14	11
WE		0.27	0.7	10.2	0.02	0.05	0.95	17	14	11
E	10–20 cm	0.16	1.7	20.7	0.01	0.11	1.8	16	16	11
WE		0.25	1.3	20.2	0.01	0.09	1.8	18	14	11

* = significant difference ($p < 0.05$) for means of C and N in SOM fractions within each soil layer for "A vs WA" or "E vs WE".

3. Results

3.1. C and N stocks of SOM fractions

In all soils and layers, the C and N stocks of SOM fractions (Table 2) and their respective contribution to C_{soil} and N_{soil} (Fig. S1) increased in the order FLF < OLF < HF. In general, the C:N ratio of SOM fractions varied from 10 to 20 and tended to decrease in the order FLF > OLF > HF (Table 2).

In A and WA soil, the C stocks of FLF and OLF varied from 1.1 to 3.9 Mg ha^{-1} and those of N from 0.10 to 0.31 Mg ha^{-1} at 0–5 cm layer (Table 2) and contributed to 20–30 % of C_{soil} and N_{soil} stocks (Fig. S1). In both A and WA soil, the C and N stocks of FLF and OLF decreased abruptly below 5 cm depth (Table 2). The C and N stocks of FLF in A soil did not differ statistically from that of WA soil, regardless of soil layer (Table 2). However, the C and N stocks of OLF at 0–5 cm layer in A soil were 44 and 48 % lower, respectively, compared to WA soil (Table 2).

The C and N stocks of FLF at 0–5 cm in E soil were 194 and 200 % higher, respectively, compared to WE soil (Table 2). Furthermore, the C and N stocks of OLF at 5–10 cm in E soil were 140 and 142 % greater, respectively, compared to WE soil (Table 2). Nevertheless, bulk SOC and N contents of E soil remained statistically equivalent to that of WE soil (Santana et al., 2015). Together, the C and N stocks of FLF and OLF in E soil contributed to 30–38 % of the C_{soil} and N_{soil} stocks (0–10 cm). In WE soil these values were 17–22 % (Fig. S1).

The C and N stocks of HF in A and E soil did not differ from that of WA and WE soil, regardless of soil layer (Table 2). The C and N stocks of HF mostly contributed (75–95 %) to C_{soil} and N_{soil}, regardless of soil and layer (Fig. S1).

3.2. Molecular composition of litter and SOM fractions assessed by Py-GC/MS

3.2.1. Py-GC/MS of litter

Litter pyrograms (Fig. S2) revealed that A and E altered remarkably the molecular composition of litter compared to WA and WE. Litter samples in general were relatively rich in lignin-derived methoxyphenols (17–40 %), n-alkane/alkenes (9–25 %), polysaccharides (8–24 %) and unspecific aromatics (6–19 %) (Tables 3 and 4).

Compared to WA, A litter had considerably lower abundance of unspecific aromatics, polysaccharides, lignin-derived methoxyphenols and N-compounds, and higher abundance of phenols, and especially of terpenoids (Table 3). Compared to WE, E litter had considerably lower abundance of polysaccharides and lignin-derived methoxyphenols, and higher abundance of unspecific aromatics, n-alkane/alkenes, and terpenoids (Table 4). Particularly, terpenoids were relatively more abundant in A (20 %) and E (11 %) compared to WA (0 %) and WE (5 %) litter (Tables 3 and 4). The terpenoids group was composed mostly of steroids (Table S1), indicating the contribution of A and E plant-derived materials to litter composition.

3.2.2. Py-GC/MS of SOM fractions

The pyrograms of SOM fractions are displayed in Figs. S3–S8. Unspecific aromatics, n-alkane/alkenes, and polysaccharides were, in general, the most abundant groups in all SOM fractions, regardless of soil and layer, comprising together >50 % of the TIC (Tables 3 and 4). On the other side, branched-chain alkanes/alkenes, terpenoids and fatty acids contributed less than 6, 9 and 15 % to the TIC, respectively (Tables 3 and 4). Regardless of soil and layer, the abundance of unspecific

Table 3
Relative abundance (%) of chemical groups obtained by Py-GC/MS analysis of the free-light (FLF), occluded-light (OLF) and heavy-fraction (HF) at 0–5, 5–10 and 10–20 cm layer and litter samples of without Acacia (WA) and Acacia (A) soils. Composite samples made of the three field replicates were used for Py-GC/MS analysis.

Groups	WA			A			WA			A			WA			A			WA	A
	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF		
%	0–5 cm						5–10 cm						10–20 cm						Litter	
Oar	18	24	32	11	14	26	17	16	23	15	16	23	12	17	22	24	24	28	12	3
Par	2	2	3	1	3	0	2	2	3	1	2	4	1	4	3	2	5	4	2	2
Pol	16	8	13	27	19	14	13	11	7	7	12	5	26	15	9	14	7	6	24	19
Mph	12	4	0	9	9	0	5	3	0	4	3	0	12	5	1	2	2	0	22	17
N-comp	9	14	9	12	10	12	8	8	5	8	8	10	6	10	10	6	7	12	8	1
Phe	10	5	2	15	12	2	6	7	0	11	6	2	8	7	2	7	3	2	4	16
n-a/a	26	39	41	20	28	46	32	42	60	33	40	52	28	36	53	40	45	48	25	18
Bra	1	1	0	2	2	0	1	1	0	1	1	0	1	2	0	0	1	0	0	1
Fac	5	1	0	1	2	0	12	8	0	12	8	3	5	4	0	4	3	0	2	3
Ter	2	2	0	2	1	0	4	2	2	8	2	0	0	0	0	2	4	1	0	20

Oar = unspecific/other aromatics; Par = polyaromatics; Pol = polysaccharides; Mph = lignin-derived methoxyphenols; N-comp = N-compounds; Phe = phenols; n-a/a = n-alkane/alkenes; Bra = branched-chain alkanes/alkenes; Fac = fatty acids; Ter = terpenoids.

Table 4
Relative abundance (%) of chemical groups obtained by Py-GC/MS analysis of the free-light (FLF), occluded-light (OLF) and heavy-fraction (HF) at 0–5, 5–10 and 10–20 cm layer and litter samples of without Eucalyptus (WE) and Eucalyptus (E) soils. Composite samples made of the three field replicates were used for Py-GC/MS analysis.

	WE			E			WE			E			WE			E			WE	E
Groups	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF		
%	0–5 cm						5–10 cm						10–20 cm						Litter	
Oar	10	20	17	13	15	20	na	15	23	20	15	17	11	19	19	32	21	21	6	19
Par	2	3	4	2	4	6	na	3	0	3	2	4	1	2	3	0	6	4	0	3
Pol	30	17	21	30	18	21	na	9	17	12	9	14	36	20	21	11	14	19	24	8
Mph	13	6	1	9	10	3	na	4	0	5	5	1	10	6	0	1	6	0	40	23
N-comp	5	9	10	5	8	10	na	7	7	4	8	7	3	5	8	7	10	9	0	1
Phe	9	4	1	9	9	2	na	3	1	5	7	1	6	9	1	2	2	2	12	15
n-a/a	26	33	43	28	29	34	na	38	47	33	42	48	24	30	43	43	29	41	9	19
Bra	2	4	2	1	2	2	na	3	2	0	2	1	1	4	1	0	5	0	0	0
Fac	4	3	1	3	4	2	na	14	2	14	5	6	7	5	1	1	3	3	2	1
Ter	1	1	0	0	1	0	na	3	1	3	5	2	1	1	1	1	3	1	5	11

Oar = unspecific/other aromatics; Par = polyaromatics; Pol = polysaccharides; Mph = lignin-derived methoxyphenols; N-comp = N-compounds; Phe = phenols; n-a/a = n-alkane/alkenes; Bra = branched-chain alkanes/alkenes; Fac = fatty acids; Ter = terpenoids. na = not analyzed.

aromatics and n-alkane/alkenes (except for E at 10–20 cm) increased in the order FLF < OLF < HF accompanied by a relative decrease of polysaccharides abundance (FLF > OLF > HF) (Tables 3 and 4).

3.2.2.1. Light SOM fractions (FLF and OLF). The light SOM fractions of A and E soil were enriched in N-compounds compared to A (Table 3) and E litter (Table 4). In the FLF of A soil, the increase of unspecific aromatics and n-alkane/alkenes abundance with depth (from 11 to 24 %) was accompanied by reduction of polysaccharides (from 27 to 14 %) abundance (Table 3). Furthermore, a large abundance of unspecific aromatics in FLF of A soil (11–24 %) was observed compared with A litter (3 %) (Table 3). The abundance of N-compounds in the FLF of A soil was greater (0–5 cm) or equivalent (5–20 cm) to that of WA soil (Table 3). In the OLF, an opposite pattern was noticed: the abundance of N-compounds in WA soil was greater (0–5 and 10–20 cm) or equivalent (5–10 cm) to that in A soil (Table 3). Also, at 0–5 cm, the abundance of unspecific aromatics in the OLF of WA soil (24 %) was greater than that of A soil (14 %), whereas the abundance of polysaccharides in the OLF of WA soil (8 %) was lower compared to A soil (19 %). At 10–20 cm, the relative abundance of unspecific aromatics (24 %), polysaccharides (7 %) and n-alkane/alkenes (45 %) remarkably differed from that of E soil, which were 17, 15 and 36 %, respectively (Table 3).

The FLF of E and WE soil at 0–5 cm layer exhibited quite similar or identical abundance of the main chemical families: polysaccharides (30 %), n-alkane/alkenes (26–28 %), unspecific aromatics (10–13 %), phenols (9 %) and N-compounds (5 %) (Table 4). The FLF of E soil at 5–10 cm was dominated by n-alkane/alkenes (33 %), unspecific aromatics (20 %), fatty acids (14 %) and polysaccharides (12 %), similarly to 10–20 cm layer: n-alkane/alkenes (43 %), unspecific aromatics (32 %) and polysaccharides (11 %) (Table 4). These abundances differed from that of the FLF of WE soil at this same layer: n-alkane/alkenes (24 %), unspecific aromatics (11 %) and polysaccharides (36 %) (Table 4).

The OLF of E soil at 0–5 cm exhibited slightly greater abundance of lignin-derived methoxyphenols (10 %) compared to WE (6 %) with accumulation of more degraded lignin-derived methoxyphenols probably of E plant, as guaiacol (Lg1), 4-methylguaiacol (Lg2), 4-ethylguaiacol (Lg3) and 4-acetylguaiacol (Lg12) (2.6–3.8 %) (Table S1). For this same fraction and layer, the abundance of unspecific aromatics was interestingly lower in E (15 %) than in WE (20 %) soil (Table 4).

The OLF of E soil at 5–10 cm was slightly enriched in n-alkane/alkenes (42 %) compared to WE (38 %) soil, likely resembling the greater abundance of n-alkane/alkenes in E (19 %) compared to WE litter (9 %) (Table 4). Furthermore, at 5–10 cm, the abundance of fatty acids in the OLF of E soil (5 %) was remarkably lower compared to WE soil (14 %) (Table 4). At 10–20 cm, the most outstanding differences were the abundance of polyaromatics (6 %), polysaccharides (14 %) N-compounds (10 %) and phenols (2 %) in E soil compared to that of WE soil (2, 20, 5 and 9 %, respectively, Table 4).

3.2.2.2. HF fraction. Py-GC/MS indicated minimal effects of A and E on the molecular composition of HF (Tables 3 and 4). In general, the HF was composed mainly of unspecific aromatics (22–32 % for A and WA, 17–23 % for E and WE), n-alkane/alkenes (41–60 % for A and WA, 34–48 % for E and WE) and polysaccharides (5–14 % for A and WA, 14–21 % for E and WE). The n-alkane/alkenes group of HF was dominated by long chain n-alkane/alkenes, probably reflecting the selective preservation of epicuticular waxes from vascular plants (Jiménez-Morillo et al., 2022). Microbial-derived compounds as furan 2-methyl (Ps1), furfural (Ps8), 2-furancarboxaldehyde, and 5-methyl (Ps19) were detected in the polysaccharides group of HF (Table S1), evidencing that SOM underwent microbial decomposition and association to soil minerals (van Bergen et al., 1997; Buurman and Roscoe, 2011). The abundance of typical plant-derived compounds as terpenoids (composed mostly of steroids) was <3 % in HF, which is below the abundance observed in light SOM fractions (up to 8 %) and litters (up to 20 %) (Tables 3 and 4).

Despite the greater abundance of N-compounds in WA litter (8 %) compared to A litter (1 %), the abundance of this group in HF along the soil profile was higher in A soil (10–12 %) than in WA soil (5–10 %) (Table 3). Particularly, the N-compounds group of HF in A soil was enriched mainly with 1H-Pyrrole 3-methyl and benzonitrile (Table S1) in comparison to WA, indicative of more decomposed materials (Marques et al., 2015).

3.2.3. Ordination of main Py-GC/MS families in litter and SOM fractions by PCA

The FLF, OLF and HF samples were ordinated towards the right, center, and left portion of the PCA plot, respectively, with few exceptions (Fig. 1a and 1b). This ordination was clearer for WA and A (Fig. 1a) than for WE and E samples (Fig. 1b). Furthermore, HF samples of WA and A or WE and E soil, within a same soil layer, were always grouped close to each other and within the same quadrant in their respective PCA plots (Fig. 1a and 1b), mostly driven by their relative enrichment with N-compounds, unspecific aromatics and n-alkane/alkenes compounds compared to light SOM fractions, as separated by PCA component 1.

3.2.3.1. PCA ordination of litter and light SOM fractions of WA and A. Component 1 and 2 of PCA explained 56.0 and 19 %, respectively, of the variation of chemical families abundance in the litter and SOM fractions of WA and A (Fig. 1a). Component 1 revealed clear shifts in litter composition after A plantation, mostly driven by enrichment in phenols and mainly terpenoids and reduction in polysaccharides, unspecific

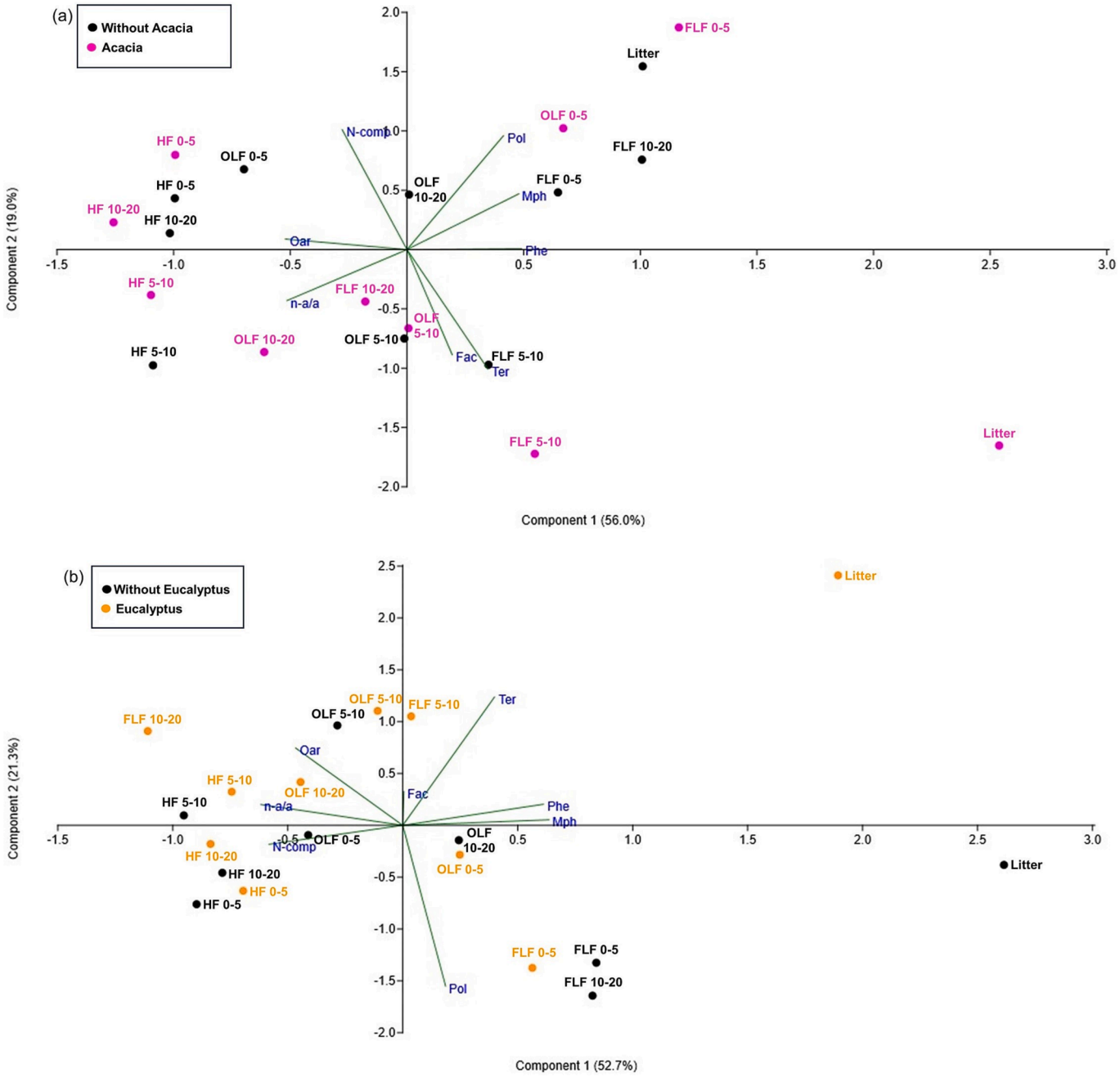

Fig. 1. Principal component analysis biplot of litter and organic matter free-light (FLF), occluded-light (OLF) and heavy (HF) fraction at soil depths (0–5, 5–10, 10–20 cm) in the without Acacia and Acacia sites (a) and without Eucalyptus and Eucalyptus sites (b) analyzed by Py-GC/MS. Eigenvectors indicate the main chemical groups of litter and organic matter fractions: unspecific/other aromatics (Oar), polysaccharides (Pol), lignin-derived methoxyphenols (Mph), N-compounds (N-comp), phenols (Phe), n-alkane/alkenes (n-a/a), fatty acids (Fac), and terpenoids (Ter).

aromatics and lignin-derived methoxyphenols (Fig. 1a). PCA showed that terpenoids, highly abundant in A litter, mostly disappeared in soil, but terpenoids-group vector is still more strongly projected towards the FLF of A compared to WA at 5–10 cm (Fig. 1a). The projection of FLF of A closer to polysaccharides compared to FLF of WA at 0–5 cm (Fig. 1a), despite the greater abundance of polysaccharides in WA litter (Table 3), indicates intense alteration of A litter at surface soil. On the other side, the projection of litter and FLF (0–5 cm) of WA close to lignin-derived methoxyphenols (Fig. 1a) reveals possible persistence of lignin compounds derived from litter in FLF. Interestingly, at 0–5 cm, the OLF of A soil is projected at the right (positive) side of component 1, close to litter and FLF samples, mostly driven by enrichment of polysaccharides, lignin-derived methoxyphenols and phenols, whereas the OLF of WA is projected at the left (negative) side of component 1, close to HF samples, mostly driven by enrichment of unspecific aromatics, N-compounds and n-alkane/alkenes (Fig. 1a). Noteworthily, at 10–20 cm, the composition of OLF in A soil is distinguished from that of WA more clearly by component 2, due to its relative enrichment with n-alkane/alkenes and unspecific aromatics and reduction of polysaccharides abundance (Fig. 1a).

3.2.3.2. PCA ordination of litter and light SOM fractions of WE and E. Component 1 and 2 of PCA explained 52.7 and 21.3 %, respectively, of the variation of chemical families abundance in the litter and SOM fractions of WE and E (Fig. 1b). Component 1 revealed clear shifts in litter composition after E plantation, mostly driven by relative

enrichment in unspecific aromatics, n-alkane/alkenes and terpenoids and reduction of lignin-derived methoxyphenols and mainly polysaccharides in E litter (Fig. 1b). Abundant terpenoids in E litter mostly disappeared in soil, as also observed for A litter (Figs. 1a and 1b). The composition of FLF at 0–5 cm in E soil is quite similar to that of WE soil, as verified along component 1 and 2 of PCA (Fig. 1b), apparently mirroring less the E litter, which appears in the upper (opposite) part of component 2 (Fig. 1b). Below 5 cm depth, FLF seems to resemble more E litter, as revealed by components 1 and 2, which separate FLF samples of E soil (relatively enriched in unspecific aromatics and n-alkane/alkenes, as E litter) from FLF samples of WE soil (relatively enriched in polysaccharides, as WE litter) (Fig. 1b). Compared to OLF samples of WA and A, that of WE and E are more centered around the intersection of component 1 and 2 (Fig. 1a and 1b), indicating less remarkable shifts in OLF composition after E plantation compared to A. Nevertheless, component 1 shows that at 0–5 cm the OLF of E soil differs from that of WE soil mainly due to its relative enrichment with lignin-derived methoxyphenols and phenols, and reduction in unspecific aromatics and n-alkane/alkenes compared to WE (Fig. 1b). These shifts are opposite to E litter composition, which differed from WE litter exactly by its relative enrichment with unspecific aromatics and n-alkane/alkenes and reduction of polysaccharides and lignin-derived methoxyphenols abundance (Fig. 1b). At 10–20 cm, the OLF of WE and E are ordinated in diagonal quadrants, but relatively close to the intersection of component 1 and 2 (Fig. 1b), showing that slight shifts occurred in the abundance of several chemical families. Litter and OLF samples of WE are projected more strongly towards polysaccharides vector, whereas that of E towards unspecific aromatics (Fig. 1b).

3.3. General lipid content and composition in litter and SOM fractions

The n-alkanes of A and E litter differed largely from that of WA and WE litter, respectively (Figs. 2 and 3), whereas patterns of n-fatty acids did not (Figs. S9 and S10). Furthermore, the Lp of A and E litter was 39 and 329 % superior to that of WA and WE litter, respectively (Table 5). Contrarily, the Lp in SOM fractions was consistently higher in WA and WE soil compared to A and E soil, regardless of SOM fraction and soil layer (exception was HF at 10–20 cm: WA = 1.9 and A = 2.1 mg g^{-1} fraction) (Table 5).

The Lp in SOM fractions varied from 1.1 to 66.2 mg g^{-1} and decreased as follows OLF > FLF > HF, regardless of soil and layer (Table 5). Depending on soil layer, Lp in WA soil compared to A soil was greater by about 59–156 % in FLF, 67–145 % in OLF, and 7–200 % in HF (Table 5). Compared to E soil, the Lp in the profile of WE soil was greater by about 36–232 % in FLF, 78–299 % in OLF and 17–27 % in HF (Table 5).

3.3.1. n-alkanes

3.3.1.1. n-alkanes in litter samples. The distribution of n-alkanes was unimodal asymmetric for all litter samples (Figs. 2 and 3), with long n-alkanes chains (Rs/l $\leq$ 0.3) and predominance of odd-over-even homologues (CPI $\geq$ 6.4 and $\leq$ 12.5) (Table 5), typical of higher plants (van Bergen et al., 1997). In both WA and WE litter, the greatest relative n-alkane abundance occurred at C33 (40 and 56 %, respectively), whereas in A and E litter it occurred at C31 (53 %) and C29 (39 %), respectively (Figs. 2 and 3). The ACL in A litter (30.1) and E litter (27.5) were lower compared to WA litter (32.1) and WE litter (31.7) (Table 5). Similarly, the CPI in A litter (9.7) and E litter (6.4) were lower compared to WA litter (12.5) and WE litter (10.7) (Table 5). The Rs/l varied only from 0.0 to 0.3 across all litter samples (Table 5).

3.3.1.2. n-alkanes in SOM fractions of WA and A soil. The number of carbons of n-alkanes in the FLF of WA soil ranged from C17 to C35 (Fig. 2). The contribution of microbial activity to FLF of WA soil at 0–10 cm layer is suggested by the abundance of short n-alkanes chains (Rs/l $\geq$ 1.0) and the predominance of even-over-odd n-alkanes (CPI < 1.0) (Table 5). Contrastingly, at 10–20 cm, the most abundant n-alkane in the FLF of WA soil was C33 (22 %), followed by C31 (13 %) and C35 (12 %) (Fig. 2) suggesting the inheritance of plant-derived compounds (Wiesenberg et al., 2010).

In the FLF of A soil, C31 predominated largely (38 %) at 0–5 cm and less remarkably at 5–10 cm layer (12 %) (Fig. 2). Within 0–10 cm layer, long chain n-alkanes and odd-over-even chains (particularly for >C29) predominated in the FLF of A soil (Table 5). Opposite to the FLF of WA soil, the FLF of A soil at 10–20 cm was dominated by short and even-over-odd n-alkanes, resulting in higher Rs/l (1.0) and lower CPI (0.3) compared to WA soil (Rs/l:0.4, CPI: 2.6) (Table 5). Furthermore, lower Rs/l values were observed not only for FLF but also for OLF (especially within 0–10 cm) in A soil (0.2–0.8) compared to WA soil (1.0–1.3) (Table 5).

The OLF of WA soil was characterized by a highest relative abundance of C33 (10 %) at 0–5 and 10–20 cm layer, similarly to WA litter, and at C28 (9.5 %) at 5–10 cm layer, (Fig. 2). Strong microbial contribution to n-alkanes series of OLF in WA soil is suggested by Rs/l values ranging from 0.7 to 1.2 in the soil profile (Table 5). In the OLF of A soil, the highest relative abundance of n-alkanes occurred at C31, exactly as A litter, at 0–5 (18 %), 5–10 (11 %) and 10–20 cm (12 %) layer (Fig. 2).

The HF of WA and A soil consisted mainly of long chain n-alkanes (ACL $\geq$ 25.0 and $\leq$ 30.0) with odd-over-even predominance (CPI $\geq$ 1.2 and $\leq$ 3.6) (Table 5). The HF of WA soil (0–20 cm) was clearly dominated by C33 (18–29 %) and C35 (13–19 %), likely WA litter (C33: 53 % and C35: 15 %) (Fig. 2). In the profile of A soil, the abundance of these typical WA litter alkanes declined to 7–24 % (C33) and 3–15 % (C35) (Fig. 2). Contrary to the Rs/l of light SOM fractions, the Rs/l ratio of HF of A soil was greater than that of WA soil at both 0–5 (1.8 and 0.4, respectively) and 5–10 cm (1.1 and 0.8, respectively) (Table 5). Concomitantly, the CPI of HF clearly decreased after A plantation, from 2.8 to 1.2 at 0–5 cm, from 2.5 to 1.7 at 5–10 cm, and from 3.6 to 2.2 at 10–20 cm (Table 5).

3.3.1.3. n-alkanes in SOM fractions of WE and E soil. The n-alkane C31 was the most abundant in the FLF of WE (0–5 cm, 16 %) and E (0–5 and 5–10 cm, 19 %) soil (Fig. 3), mirroring WE litter (C31: 36 % and C33: 40 %) rather than E litter, which exhibited highest abundance at C29 (39 %) (Fig. 3). However, as an effect of E litter, it is noteworthy the higher relative abundance of C29 in the FLF of E soil (13.4 %) compared to WE soil (8.6 %) (Fig. 3), which occurred alongside with increase of CPI from 1.3 to 2.7 and reduction of Rs/l from 0.9 to 0.4 compared to the FLF of WE soil at 0–5 cm (Table 5). At 5–10 cm, the FLF of E soil exhibited higher abundance of long n-alkanes chains (ACL: 29.1) and predominance of odd-over-even homologues (CPI: 2.1) compared to the FLF of WE soil (ACL: 26.2 and CPI: 0.8) (Table 5).

The OLF of E and WE soil (0–20 cm) exhibited similar patterns of n-alkanes (Fig. 3), indicating incipient contribution of E to this fraction. This resulted in mild variations of ACL (1.1–2.1) and Rs/l (0.7–1.0) of OLF across WE and E soil profiles (Table 5).

Interestingly, in the HF of E soil the abundance of typical n-alkanes of WE litter (C35 and C33) drastically dropped to 7–15 % (C35) at 0–20 cm layer and to 15–25 % (C33) at 0–10 cm layer (Fig. 3). Alongside, minimal increases in the relative abundance of C23, C25, C27 and C29, more abundant in E litter, were noticed in the HF of E soil, especially at 0–5 cm layer (Fig. 3). Consequently, the HF of E soil exhibited greater Rs/l and lower CPI values at 0–10 cm compared to WE soil.

3.3.2. n-fatty acids in litter and SOM fractions

In general, the n-fatty acids series of litter and SOM fractions ranged from C12 to C32 with even-over-odd predominance and exhibited highest relative abundance of the saturated n-fatty acid C16 in all litter (33–42 %) and in the majority of FLF, OLF and HF samples (Figs. S9 and

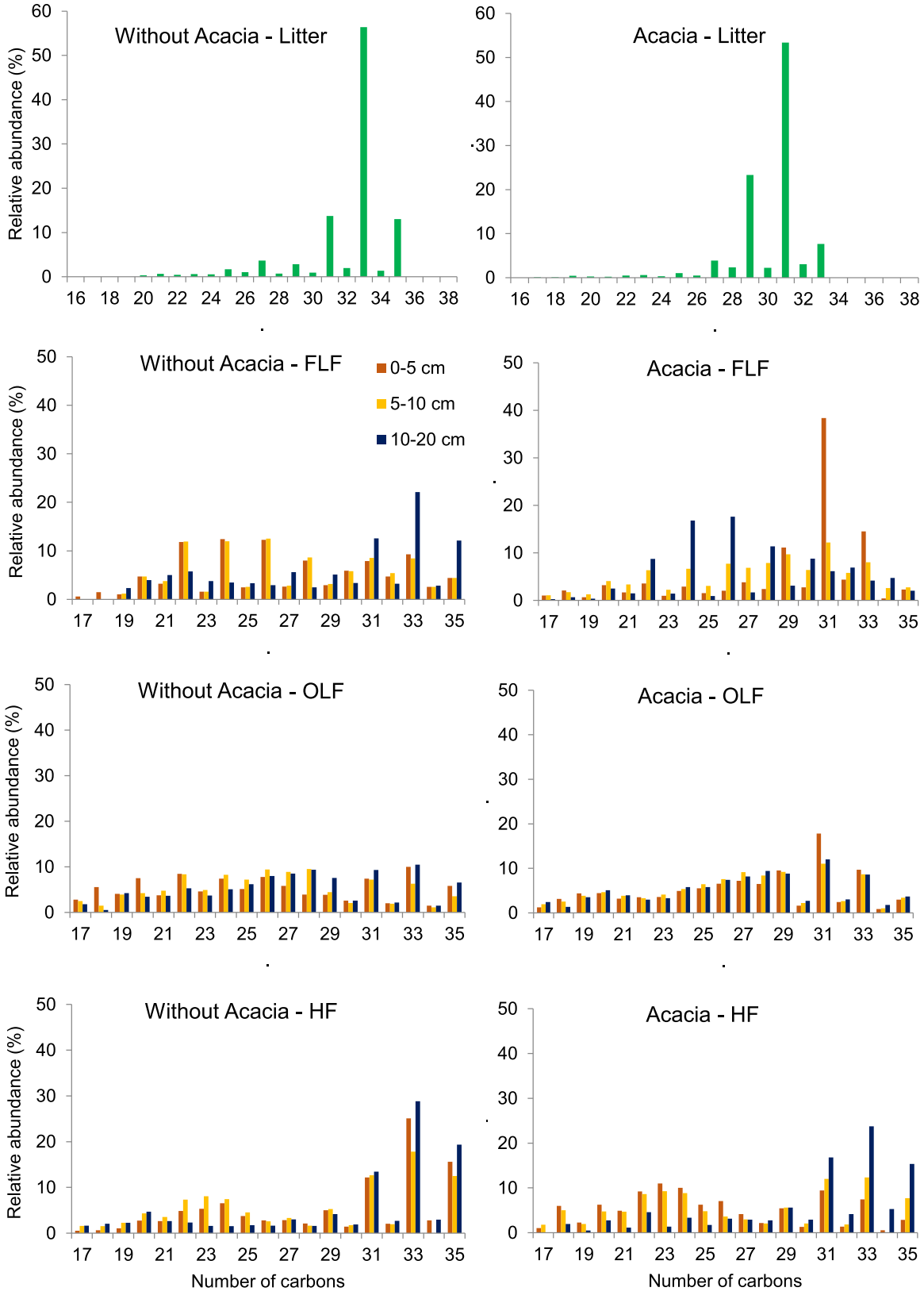

Fig. 2. Relative abundance (%) of n-alkanes (*m/z* 85) in litter, free-light (FLF), occluded-light (OLF) and heavy-fraction (HF) samples of Acacia (A) and without Acacia (WA) sites.

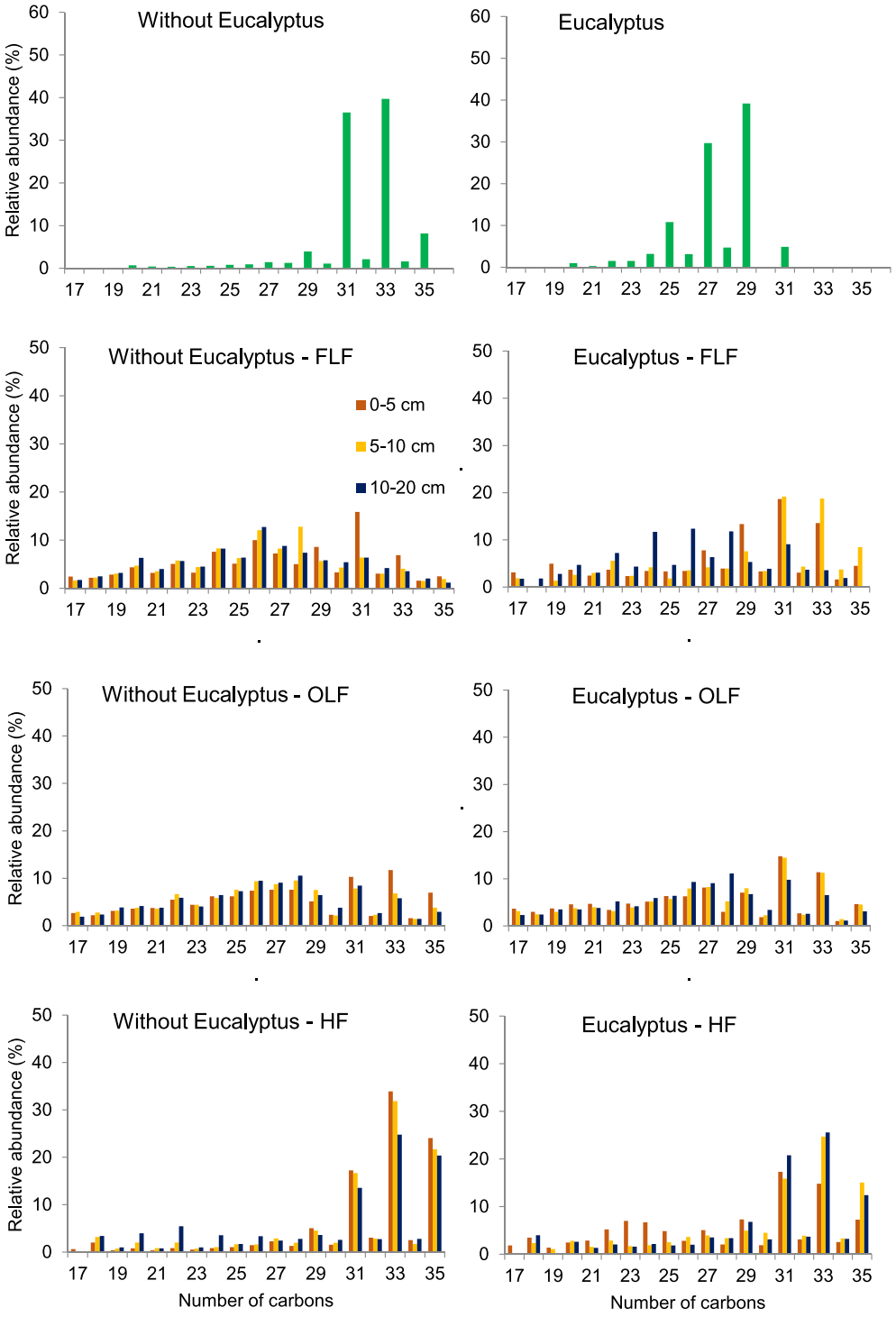

Fig. 3. Relative abundance (%) of n-alkanes (*m/z* 85) in litter, free-light (FLF), occluded-light (OLF) and heavy-fraction (HF) samples of Eucalyptus (E) and without Eucalyptus (WE) sites.

S10). Fatty-acids with long and even-numbered chains are typical of higher plants (here grasses, A and E), while C16 fatty acids are ubiquitous in plants and microorganisms hindering to define their origin (Quenea et al., 2006). The second most abundant n-fatty acid in FLF and OLF (0–10 cm) was C18, for all soils (Figs. S9 and S10). Even-long chain saturated fatty acids (mainly <C28) attributed to plant material (Wiesenberg et al., 2010) were also abundant in FLF and OLF of all soils (Figs. S9 and S10). Comparatively, HF exhibited higher abundance of longer even-chain fatty acids ≥C28 (Figs. S9 and S10), attributed to the selective preservation of epicuticular waxes of higher plants (Eglinton and Hamilton, 1967). The n-fatty acids of litter and SOM fractions were mostly similar between WA and A, and WE and E. Thus, n-fatty acids molecular proxies (ACL, CPI, Rs/l) did not distinguish the composition and source of n-fatty acids in soil. Therefore, n-fatty acids are not further discussed.

Table 5
Total lipid extracts (Lp, mg lipid g^{-1} fraction), average chain length (ACL), carbon preference index (CPI) and ratio between short and long chain (Rs/l) of n-alkanes in the free-light (FLF), occluded-light (OLF) and heavy-fraction (HF) and litter of without Acacia (WA), Acacia (A), without Eucalyptus (WE) and Eucalyptus (E) sites. Composite samples made of the three field replicates were used for lipid extraction.

	WA			A			WA			A			WA			A		
	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF
	0–5 cm						5–10 cm						10–20 cm					
Lp	9.7	45.2	4.2	6.1	27.1	1.4	17.4	66.2	1.6	6.8	27.0	1.5	16.1	25.1	1.9	8.1	10.6	2.1
ACL	27.0	25.9	29.4	29.1	26.9	25.0	27.1	26.0	27.8	27.5	26.7	26.5	29.0	27.3	30.0	27.2	27.0	30.2
CPI	0.6	1.1	2.8	3.1	1.9	1.2	0.6	1.1	2.5	1.0	1.6	1.7	2.6	1.6	3.6	0.3	1.5	2.2
Rs/l	1.1	1.3	0.4	0.2	0.7	1.8	1.0	1.2	0.8	0.6	0.8	1.1	0.4	0.7	0.3	1.0	0.7	0.3
	WE			E			WE			E			WE			E		
	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF	FLF	OLF	HF
	0–5 cm						5–10 cm						10–20 cm					
Lp	19.9	48.9	2.3	6.0	27.2	1.9	22.5	50.3	1.4	7.7	12.6	1.2	22.0	31.4	1.4	16.2	11.7	1.1
ACL	26.9	27.1	31.7	28.0	26.8	27.9	26.2	26.4	31.0	29.1	27.2	30.1	26.0	26.4	30.0	26.1	26.6	30.1
CPI	1.3	1.5	5.9	2.7	2.1	2.2	0.8	1.2	4.4	2.1	1.9	2.5	0.8	1.1	2.3	0.7	1.2	2.8
Rs/l	0.9	0.8	0.1	0.4	0.8	0.6	1.1	1.0	0.2	0.4	0.7	0.3	1.2	1.0	0.3	1.2	0.9	0.2
	WA Litter	A	WE	E			WA Litter	A	WE	E								
Lp	26.4	36.6	15.7	67.4		CPI	12.5	9.7	10.7	6.4								
ACL	32.1	30.1	31.7	27.5		Rs/l	0.1	0.0	0.0	0.3								

4. Discussion

4.1. Changes on C and N stocks of SOM fractions associated to shifts in their chemical composition revealed by Py-GC/MS

The C and N stocks (FLF < OLF < HF) and C:N ratio (FLF > OLF > HF) of SOM fractions observed in all studied soils, reflect the level of protection against degradation of each fraction. While protection of light (labile) fractions is attributed to inherent chemistry (FLF) and occlusion in aggregates (OLF), HF contains chemical moieties already decomposed by microorganisms, explaining its lower C:N ratio and sensitivity to land use change compared to the light fractions (Christensen, 2001; Lehmann and Kleber, 2015). This was confirmed by PCA plots (Fig. 1a and 1b), which grouped FLF samples towards polysaccharides, lignin-derived methoxyphenols and phenols; and HF samples towards unspecific aromatics, n-alkane/alkenes and N-compounds vectors, while OLF was mostly a mix of these compounds. For instance, the PCA plots clearly indicated that HF composition along soil profile remained practically unaltered even after seven years of A and E plantation and strong shifts in litter composition (Fig. 1a and 1b).

Specifically, statistically significant changes in C and N stocks of SOM fractions after forest plantation occurred only at A soil (OLF, 0–5 cm) and E soil (FLF, 0–5 cm; OLF, 5–10 cm). These changes are here discussed together with shifts in the chemical composition (Py-GC/MS) of these fractions.

4.1.1. Acacia plantation

The reduction of C (<44 %) and N (<48 %) stocks in the OLF of A compared to WA at 0–5 cm layer (Table 2) possibly occurred via preferential decomposition of endogenous compounds of OLF following the breakdown of soil aggregates upon soil preparation for A plantation. This is a well-known process controlling SOC storage in forestry and agriculture (Mayer et al., 2020). The composition of OLF of A and WA soil at 0–5 cm is mostly distinguished by relative enrichment of polysaccharides with plant-derived compounds as Ps26: 2-cyclopentenone, and Ps28: acetophenone (Table S1) (Fuentes et al., 2010; San-Emeterio et al., 2023) and reduction of N-compounds (N10: indole, N18: nonadecanenitrile, N19: pyridine, N20: benzonitrile and N23: pyridinone) possibly of microbial origin (De la Rosa et al., 2012; Carr et al., 2013) in A soil, as shown by PCA (Fig. 1a). These findings suggest that despite the greater biodegradability of A litter compared to WA litter, the OLF of WA has more microbial inputs, possibly due to further intra-aggregate processing of organic compounds by microorganisms (Buurman and Roscoe, 2011). This process may have been favored by the preservation of soil aggregates in WA soil leading to accumulation of microbial compounds deposited intra-aggregate. Whereas in A soil, the typical breakdown of soil aggregates because of forest plantation may have prevented this process to occur in the same magnitude as in WA soil. According to Jiménez-Morillo et al. (2016), the higher relative abundance of unspecific aromatics in the OLF of WA (24 %) compared to A (14 %) at 0–5 cm (Table 3) is indicative of more evolved organic matter altered by microorganisms. The aggregate-size (9.51 mm sieve) used for SOM fraction in our experiment may have been important to visualize this process. Larger aggregates may contain more labile (>C:N ratio) organic compounds and be more easily accessible by microbial decomposers compared to smaller aggregates, favoring the accumulation of microbial derived N-compounds intra-aggregates (Méndez-López et al., 2023). This is evidenced by the higher abundances of microbial derived N-compounds previously mentioned (N10, N18, N19, N20, N23) observed in OLF compared to FLF samples in our study (Table S1). These findings differ from that of Schellekens et al. (2017), who used 2 mm-sieved soil for SOM fractionation and found that N-compounds were largely abundant in all SOM fractions, except for OLF, in Brazilian soils under tropical forest. According to the authors, small aggregates are deficient in nutrients and oxygen and offer a physical barrier for microorganisms and their enzymes, resulting in lower abundance of microbial-derived compounds deposited intra-aggregates. The common disruption of larger aggregates by soil mechanization (Kasper et al., 2009), possibly explains partially the lower relative abundance of the microbial derived N-compounds in the OLF of A compared to WA soil. For instance, utilization of 2 mm- instead of 9.51 mm-sieved soil samples for density SOM fractionation has been shown to underestimate C and N stocks of OLF (Tomazi et al., 2011) and select for relative abundance of SOM compounds (Wagai et al., 2009). Further explanation for reduced microbial-derived compounds (N-comp) in the OLF of A compared to WA soil (0–5 cm) is possibly a deleterious effect of A plantation on soil microbial functions leading to reduced microbial activity and efficiency to convert plant biomass to soil C and N stocks, as reported by Boudiaf et al. (2013) for native vegetation soils planted with Acacia in North Africa. Accordingly, Bilgo et al. (2012) reported decrease of soil microbial biomass, respiration rate, and enzymatic activity followed by depletion of C and N contents in a ferruginous tropical soil after conversion of grasslands to acacia plantation. The authors assigned the greater microbial activity to the higher activity in the rhizosphere of

grassland soil compared to acacia, which is essential for microbial functionating and soil C and N acquisition. This may have been favored by non-inoculation of acacia with mycorrhiza in the referenced and present studies.

4.1.2. Eucalyptus plantation

The increase of C and N in the FLF of E compared to WE (0–5 cm) is possibly associated to a greater amount and recalcitrance of E litter (>C:N ratio and unspecific aromatics and n-alkane/alkenes abundance) compared with WE litter (Table 4), which hinders its decomposition by microorganisms (Gatto et al., 2010). Likewise, Pulrolnik et al. (2009) found that the C and N stocks in the FLF of a Brazilian Oxisol (0–10 cm) cultivated for 20 years with eucalyptus were 56–229 % greater than those of Cerrado and pasture soils, probably due to: greater eucalyptus's litter dry mass (>84–176 %) and C:N ratio (91) compared to Cerrado (60) and pasture (42); and lower nutrient content in the eucalyptus litter. Similarly, Montero and Delitti (2018) found that high C:N ratio of eucalyptus and pinus litter led to SOC accumulation compared to Brazilian Cerrado. The recalcitrance of E litter along with possible inhibition of microbial activity caused by soil acidity and aluminum saturation observed in E soil (Santana et al., 2015) probably prevented major contributions of E litter to SOM fractions chemistry. This is supported by only minimal relative increase in the abundance of unspecific aromatics in the FLF of E compared to WE (Table 4). Equivalent abundance of polysaccharides in the FLF of E and WE soil at 0–5 cm, despite the 3-fold greater polysaccharides abundance in WE litter (Table 4), seems to result from redistribution of the relative abundance of Py-GC/MS families after E litter enters soil and representative families in litter as Ter (11 %) practically disappear. Otherwise, if polysaccharides abundance in FLF of E soil would be associated to E litter decomposition, this would be expectedly accompanied by increase in the relative abundance of microbial derived N-compounds, which was not evidenced by individual Py-GC/MS products of N-compounds (Table S1). Likely, the limited decomposition of E litter led to its accumulation as FLF, explaining the increments of C and N stocks (Table 2) in this fraction after E plantation. For instance, Pereira et al. (2018) reported increased metabolic quotient and dehydrogenase activity in soils with 3.3-year-old eucalyptus, indicating stress of microorganisms and its low efficiency to convert C of E litter to stabilized SOC. In this way, based on the contrasting composition of WE and E litter, and on E litter recalcitrance, it would be expected that E litter accumulation as FLF at 0–5 cm would have shifted FLF of E soil towards E litter in the PCA plot, but it remained oriented closer to litter and FLF of WE (Fig. 1b). This expected shift in FLF composition may have been masked by disappearance of relatively abundant compounds in E litter (e.g., Ter) after entering the soil as previously mentioned. In fact, the contribution of E litter to FLF composition at 0–5 cm is confirmed by lipid biomarkers in section 4.3.2.

The 2.4-fold greater C and N stock of OLF in E soil compared to WE soil at 5–10 cm (Table 2) contradicts Zinn et al. (2002), who reported decrease of C stocks in the OLF after eucalyptus plantation due to rupture of soil aggregates and depletion of occluded C. In our study, it seems that breakdown of soil aggregates at E soil was compensated by the subsequent seven years of continuous forest cultivation without further soil mechanization, which combined promote soil aggregation. Also, this seems related to preferential occlusion of more stable compounds as n-alkane/alkenes (which were more abundant in E litter) and relative loss of more labile compounds as fatty acids in the OLF of E soil at 5–10 cm (Table 4). Consumption of occluded fatty acids in this case may have been favored after breakdown of soil aggregates at E plantation and due to recalcitrant E litter entering soil (Prescott and Grayston, 2013). According to Yang et al. (2020) soil microorganisms can use fatty acids as a high energy density source seeking the decomposition of recalcitrant materials. Possibly, this process was stimulated by the presence of more recalcitrant FLF in subsurface in E soil, particularly at 10–20 cm, which was ordinated closer to HF samples in the PCA of E soil (Fig. 1b).

4.2. Other relevant shifts in FLF and OLF composition detected by Py-GC/MS not conditioned to changes in their C and N stocks

Gains in relative abundance of unspecific aromatics in the FLF occurred along A soil profile (not in WA soil) and this was accompanied by decrease in the relative abundance of polysaccharides (Table 3). Together with the lower abundance of unspecific aromatics in A litter compared to WA litter (Table 3), this suggests a preferential depletion of polysaccharides from A litter and relative accumulation of unspecific aromatics in FLF of A soil profile (Buurman et al., 2007). Possibly, this occurs with contributions of persisting endogenous unspecific aromatic compounds derived from WA litter, which exhibited higher unspecific aromatics abundance (12 %) (Table 3). Similarly, at 10–20 cm of E soil, the FLF is substantially enriched relatively with unspecific aromatics and n-alkane/alkenes compared to WE soil, likely influenced by the higher abundance of these compounds in E litter (Table 4). This suggests that E litter, in the form of slightly biologically processed and recalcitrant fragments was possibly transported downwards, thereby shifting the molecular composition of FLF at 10–20 cm, as evidenced by PCA (Fig. 1b). However, the FLF represented only <10 % of SOC in all soils (Fig. S1). Thus, these molecular shifts in FLF after A and E plantation did not lead to changes in C and N stocks of FLF below 5 cm depth (Table 2). Nevertheless, these shifts may affect the dynamics and stability of SOM at subsurface in later stages of forest cultivation.

4.3. Lipid content and n-alkane molecular proxies of litter and SOM fractions

Considerably greater Lp observed in practically all SOM fractions and layers of WA and WE soils compared to A and E soil did not result in greater stocks of C in these fractions, as lipids represent usually <10 % of C_{soil} (Naafs et al., 2004). Nevertheless, alterations on Lp of SOM fractions after land use change may reveal modifications in SOM dynamics and protection mechanisms. For instance, lipids can be easily decomposed if free in soil, but stabilized via occlusion in aggregates (Lützow et al., 2006). As the Lp contents were substantially greater in A and E litter compared to WA and WE litter, respectively, but lower in the OLF of A and E compared with WA and WE soil at 0–20 cm layer (Table 5), this supports the hypothesis that lipids and possibly other organic compounds protected into aggregates were depleted because of aggregates disruption.

Decrease of n-alkanes ACL in A and E litter (Table 5) was expected as woody species exhibit lower ACL compared with grasses (Cranwell, 1973) and prove shifts in the molecular composition of litter after A and E plantation. This is also confirmed by the lower CPI in A and E litter compared to WA and WE litter (Table 5) (Wiesenberg et al., 2010). Following this change in litter composition, the higher CPI values observed in the light SOM fractions of A and E soil compared to WA and WE soil (Table 5), along with higher CPI values observed in WA and WE compared to A and E litter, clearly indicate that lipids of FLF and OLF of WA and WE soil are relatively more degraded and that incorporation of less degraded lipids derived from forest litter into light SOM fractions has started at molecular level. These results are in line with Li et al. (2018), who evaluated soils from 30 different locations in South Island, New Zealand, and found higher odd-over-even predominance (herein CPI) in forest soils compared to grass/herbs soils, and assigned it to the greater level of degradation of n-alkanes in grass/herbs soils.

4.3.1. Shifts in n-alkanes of SOM fractions after A plantation

The greater stage of lipid degradation in the FLF of WA compared to A soil at 0–10 cm is confirmed by its lower Rs/l (≥1.0) and CPI (0.6) (Table 5). According to Buurman et al. (2007) short n-alkanes chains are assigned to fragments of microbial lipids or parts of longer chains degraded by microorganisms. Contrastingly, at 10–20 cm, the most abundant n-alkane in the FLF of WA was C33, followed by C31 and C35 (Fig. 2) suggesting the inheritance of plant-derived compounds. On the

other side, the less degraded stage of lipids in the FLF of A soil (0–10 cm) is confirmed by the predominance of long- and odd-over-even n-alkane chains in these samples (Table 5) (Jansen and Wiesenberg, 2017). This is further confirmed by the dominance of C31 in the FLF of A soil (0–10 cm), which was typical of A litter (Fig. 2). Similar patterns of greater lipid degradation stage were observed in the OLF of WA compared to A soil (0–10 cm), as inferred from decrease of Rs/l and increase of CPI in this fraction after A plantation (Table 5). This is further verified by the inheritance of C31 as the most abundant n-alkane in the OLF along the profile of A soil, typical of A litter (Fig. 2). Moreover, together with the lower C and N stocks observed in the OLF of A compared to WA soil at 0–5 cm (Table 2) these findings indicate that endogenous and occluded organic C may have been lost and partially replaced with A-derived compounds.

The increase of Rs/l (0–10 cm) and reduction of CPI (0–20 cm) in the HF after A plantation, indicate enhanced microbial inputs to HF. This is in line with our Py-GC/MS data indicating relative enrichment of this fraction with microbial-derived N-compounds after A plantation (Table 3). Furthermore, the decrease of ACL observed in the HF (0–10 cm) after A plantation (Table 5), may also be a response to the (partial) replacement of grass-derived litter (with higher ACL) with woody-derived litter (with lower ACL), (Table 5) (Wiesenberg and Gocke, 2015).

4.3.2. *Shifts in n-alkanes of SOM fractions after E plantation*

The predominance of typical WE litter alkanes (C31) in the FLF of E soil (0–10 cm) confirms the inheritance of native-pasture lipids and the slow incorporation of E litter into soil. Nevertheless, the increased abundance of C29 (typical of E litter) in the FLF of E soil compared to WE soil at 0–5 cm (Fig. 3) indicates that incorporation of E litter to soil initiated. This agrees with our Py-GC/MS data showing increments in the relative abundance of unspecific aromatics and n-alkane/alkenes (abundant in E litter) to FLF at 0–5 cm layer (Table 4) and also support the hypothesis that stronger shifts in FLF composition at 0–5 cm after E plantation may have occurred but were probably masked by modification of relative abundance of Py-GC/MS families of E litter after entering soil.

Similarly to A soil, in the FLF of E soil, the increase of CPI and decrease of Rs/l compared to WE soil (0–10 cm) (Table 5) confirm the advanced degradation of lipids in WE soil and incorporation of less degraded lipids in E soil. According to Assis et al. (2011) the increase of CPI and ACL observed in the FLF after E plantation (0–10 cm) may also indicate the presence of relatively preserved grasses-derived n-alkanes and the inheritance of n-alkanes of WE in FLF of E soil. Minimal shifts in ACL, CPI and Rs/l in OLF after E plantation (Table 5) point out to incipient occlusion of lipids derived from E litter. In fact, OLF samples of WE and E soil were not as scattered in the PCA plot of Py-GC/MS data (Fig. 1b) as that of WA and A samples for example (Fig. 1a).

The remarkable increase in the relative abundance of n-alkane/alkenes in the HF of E soil at 0–5 cm compared to WE soil revealed by Py-GC/MS (Table 4) is here attributed to enrichment with degraded lipids. This is inferred from the strong reduction of CPI in the HF of E soil (2.2) compared to WE soil (5.9) (Table 5). According to Peters et al. (2004), degradation of lipids evens the proportion of even and odd homologues, resulting in decreased CPI values. The decline of CPI of HF at 0–5 cm after E plantation is accompanied by a decline of ACL and increase of Rs/l, indicating that plant-derived lipids were specially preserved by association with soil minerals in WE compared to E soil, as similarly observed in the HF of A and WA soils (Table 5). These findings are in line with Wiesenberg et al. (2010), who found increasing stabilization of plant-derived lipids with increasing association with soil minerals. As the Lp in the HF of WE and E soil were similar at 0–20 cm (Table 5), it seems that more plant derived n-alkanes in the HF were proportionally replaced by more degraded microbial-derived lipids after E plantation.

5. Conclusions

Shifts in C stocks and molecular composition of light SOM fractions were caused mainly by E and A, respectively, partially confirming our hypotheses. The loss of C and N stocks in the OLF of A compared to WA soil (0–5 cm) supposedly reflects the breakdown of soil aggregates after forest plantation followed by exposure and degradation of previously occluded microbial derived N-compounds. These compounds were partially replaced with A litter-derived less degraded compounds in OLF. Additionally, fresh A litter inputs to FLF and OLF and association of microbial-derived compounds to soil minerals (HF) occurred in A soil, explaining equivalent bulk SOC contents found in A and WA soil profile. The increase of C stocks in the FLF (0–5 cm) and OLF (5–10 cm) of E compared to WE soil was associated to introduction of recalcitrant compounds derived from E litter (aromatics and n-alkane/alkenes) and depletion of more labile endogenous compounds (fatty acids). We suggest the analysis of n-alkanes proxies combined with Py-GC/MS of size-resolved aggregates in response to soil mechanization and cultivation of A and E. This may reveal aggregate-size-dependent selective preservation of organic compounds with different stability driving C and N stocks in OLF and soil.

Conversion of Pampa to acacia and eucalyptus changes SOM composition and degree of protection, requiring further long-term studies on SOC stability as well as policies in view of the highly invasive potential of these exotic species and possible negative effects on Pampa regeneration.

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CRediT authorship contribution statement

Otávio dos Anjos Leal: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Graciele Sarante Santana:** Conceptualization, Data curation, Formal analysis, Writing – original draft. **Heike Knicker:** Conceptualization, Methodology, Supervision, Writing – original draft. **Francisco J. González-Vila:** Conceptualization, Methodology, Supervision. **José A. González-Pérez:** Conceptualization, Methodology, Supervision. **Deborah Pinheiro Dick:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2023.116745.

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