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Selective stabilization of soil fatty acids related to their carbon chain length and presence of double bonds in the Peruvian Andes

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ABSTRACT

Recent studies show increasing evidence for perseveration of soil organic matter (SOM) controlled by interactions with the soil matrix (i.e. mineral surfaces and aggregates) rather than chemical recalcitrance of the SOM. However, a consensus is still absent for potential controls of SOM chemical composition on SOM stabilization and persistence. Soil fatty acids (FAs), which form an important SOM component, can be used to investigate the effects of chemical properties on SOM stabilization because they are easily degraded by microorganisms but can be stabilized by the soil matrix against decomposition. Here we investigated whether inherent molecular properties of FAs control their stability in soils and their interactions with the soil matrix. Soil samples were collected from alpine grasslands of the Peruvian Andes (Andosols, Umbrisols and Phaeozems), as they are characterized by high carbon stocks and abundant aliphatics. We applied pyrolysis - gas chromatography/mass spectrometry analyses assisted by tetramethylammonium hydroxide (TMAH-pyrolysis-GC/MS) to determine the chemical composition of bulk SOM and FAs before and after a 76-day incubation experiment, comparing a situation with intact versus crushed soil aggregates. The results showed that the TMAH-pyrolysis-GC/MS yielded a large proportion of FAs (> 60% relative abundance of identified compounds), with a major contribution of free FAs. FA stability was controlled by the presence of double bonds (unsaturated vs. saturated FAs) and carbon chain length. Unsaturated FAs significantly ($P < 0.05$) predicted soil organic carbon mineralization rates and were more depleted after the incubation compared to saturated FAs. The depletion of unsaturated FAs is likely explained by their easier degradation compared to saturated FAs. The easier degradation might be explained by the smaller extent of stabilization through association with mineral surfaces and/or chemical properties rather than stabilization through occlusion in aggregates. In terms of carbon chain length, FA stability decreased from short-chain to long-chain FAs. A possible explanation for this is that short-chain FAs received more protection by occlusion in aggregates compared to long-chain FAs or that short-chain FAs were produced during the incubation as a result of microbial transformation of FAs. Such microbial transformation has limited effects on the prediction of FA stability using double bonds and carbon chain length. However, we observed that soil types and horizons did influence the controls of double bonds and carbon chain length on FA stability. Our results corroborate the hypothesis that the inherent properties of soil FAs control their interactions with the soil matrix and indirectly govern their stabilization and persistence in the Peruvian Andean soils under study.

1. Introduction

The persistence of soil organic matter (SOM) is crucial to sustaining the large soil organic carbon (SOC) pool in the context of global change. New-emerging paradigms indicate a shift from SOM persistency controlled by chemical recalcitrance of SOM due to the bond strength between C atoms to that controlled by decreased SOM bioavailability due to interactions between SOM and soil matrix (Lehmann and Kleber, 2015; Schmidt et al., 2011). The underlying mechanisms of the interactions are specified as (1) SOM occlusion in soil aggregates to decrease the accessibility of microbial decomposers, and (2) formation of organo-mineral associations by functional groups of SOM molecules and mineral surfaces (Lützow et al., 2006; Wiesmeier et al., 2019). Although chemical recalcitrance of SOM is considered to be less-important to SOM persistence than previously assumed (Dungait et al., 2012; Kleber, 2010), the consensus on the effects of SOM chemical characteristics on SOM persistency is still absent (Kögel-Knabner, 2017). An important reason is that influences of SOM molecular

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composition on interactions between organic matter (OM) and the soil matrix are still not well-understood.

Soil lipids are one of the significant SOM components, and are increasingly applied as the molecular proxy to investigate the origins of SOM and to reconstruct changes in vegetation, climate and human activities (Jansen and Wiesenberg, 2017; Wiesenberg et al., 2008). In general, soil lipids are separated into: (1) free-lipids that are to a large extent derived from wax esters of plants, (2) bound-lipids such as those that are part of macromolecules like cutin and suberin, and (3) phospholipid fatty acids (FAs) that originate from membrane structures of microorganisms and other microbial-derived lipids (Frostegård and Bååth, 1996; Jansen and Wiesenberg, 2017). The reliability of the application of lipids as molecular proxy for the origins of SOM is largely dependent on persistence and preservation of lipids, which is further controlled by their interactions with the soil matrix (i.e. mineral surfaces and aggregates) (Lützow et al., 2006). However, it is still poorly understood whether inherent properties of lipids, including lipid types, carbon chain length and free vs. bound lipids, have significant influences on the interaction with the soil matrix and further impact compound preservation (e.g. Angst et al., 2018).

Soil FAs, which originate from both plant materials and microorganisms, are one of the most abundant lipid fractions in soils (Jandl et al., 2004; Kögel-Knabner, 2002). In general, FAs in soils are vulnerable to decomposition. This knowledge is based on both residential time (Jansen and Wiesenberg, 2017; Schmidt et al., 2011) and decomposition experiments (Angst et al., 2016; Moucawi et al., 1981). In addition, FAs are usually used as energy sources because of their higher energy density (38 kJ g^{-1}) than carbohydrates and proteins (17 kJ g^{-1}) (Sadava et al., 2008). The decomposition of FAs in soils is controlled by β-oxidation, an intercellular enzyme-dependent process in which FAs are totally decomposed into CO_2 and H_2O (Dinel et al., 1990; Rustan and Drevon, 2005). Although soil FAs are common energy sources, they are reported to accumulate in some soils in alpine grasslands and in Antarctica (Matsumoto et al., 1981; Nierop et al., 2007, 2006). The persistence of FAs in soils is not only controlled by their origin and molecular properties, but also controlled by their interactions with mineral surfaces and occlusion in soil aggregates (Lützow et al., 2006). The amphiphilicity of FA molecules, characterized by hydrophilic carboxyl groups, hydrophobic alkyl chains and hydrophobic methyl groups, is crucial for their interaction with soil mineral surfaces (Lützow et al., 2006). The carboxyl groups of the FAs can interact with charged mineral surfaces via ligand exchange and polyvalent cation bridges, whereas the methyl groups and alkyl chains can promote the formation of hydrophobic zones (Kleber et al., 2015; Lützow et al., 2006). This is further corroborated by models of OM-mineral interaction, in which FAs play an important role in the formation of OM-mineral associations due to their amphiphilicity (Kleber et al., 2007; Wershaw, 1993). In addition, FAs can be stabilized by physical protection of soil aggregates (Angst et al., 2018; Lehmann et al., 2007). Because aggregate-protected OM is reported to have distinguished molecular composition compared to other fractions (Golchin et al., 1994; Wagai et al., 2009), aggregate-protected FAs might be potentially controlled by their inherent chemical properties.

Soils in alpine grasslands of the Andes accumulate large quantities of SOC. SOC stocks of the entire soil profiles are reported to range from 118 ± 15 to 215 ± 21 Mg ha^{-1} in the Peruvian puna grassland (e.g. Yang et al., 2018; Zimmermann et al., 2009), and up to 530 ± 40 Mg ha^{-1} in the Ecuadorian páramo grassland (Tonneijck et al., 2010). In the Andean grasslands, SOC is preserved by SOM adsorbed on mineral surfaces (Rolando et al., 2017a; Tonneijck et al., 2010), which is further controlled by environmental factors like precipitation and lithology (Yang et al., 2020a). These soils received limited studies on the molecular composition of SOM, which shows a large contribution of lipids to the bulk SOM (García and Cano, 2012; Nierop and Jansen, 2009).

The paradox that FAs are common energy sources yet able to interact with the soil matrix to gain protection against decomposition suggests that we need a better understanding of soil FA persistence and the underlying mechanisms. The lipid-rich soils in the Andes give an opportunity to investigate the potential relationships between soil FA persistence and their molecular structures. For this, we applied a combination of a pyrolysis-GC/MS analysis assisted by tetramethylammonium hydroxide (TMAH-pyrolysis-GC/MS) and a 76-day incubation of soil aggregates (intact and crushed) to address the stability of FAs in relation to their chemical properties and the soil matrix. The objectives of this study were to investigate (1) whether inherent properties (e.g. carbon chain length and double bonds) of the FAs control their stability in soils, and (2) whether the potential controls of FA stability can be explained by the chemical recalcitrance of the FAs or their interactions with the soil matrix.

2. Materials and methods

2.1. Site description and soil sampling

The study area was located in alpine grasslands of the Peruvian Andes, corresponding to the ecosystem of the Jalca or the wet Puna (Rolando et al., 2017b). Sampling sites have an altitudinal ranging from 3490 to 3720 m a.s.l. and an annual average temperate around 11 °C. Major land use type in this region is grassland together with cultivation, grazing and plantation of pine trees and eucalyptus (Rolando et al., 2017b; Vega et al., 2005). The sampling sites were selected based on the combination of two bedrock types (limestone and acid igneous rocks) and two precipitation levels (1100 mm and 680 mm). Soils developed on the limestone have dark A horizons above argic B horizons, and are classified as Phaeozems (WRB, 2014). Soils on acid igneous rocks have vitric or umbric A horizons overlaying C horizons, and correspond to Andosols or Umbrisols (WRB, 2014). Detailed soil properties are presented in Table 1.

Soil samples were collected by horizon from two replicated profiles in each combination of bedrock types and precipitation levels. Soil samples were transferred in sealed plastic bags with the protection of hard plastic boxes for the transportation. All soil samples were air-dried at 40 °C before laboratory analyses. Materials from different A subhorizons were merged to one A horizon (e.g. Ah1, Ah2 and Ah3 merged to Ah horizon) based on the weight distribution of each horizon. Limestone soils (LSs) had one merged A horizon and one B horizon, whereas acid igneous rock soils (ASs) only had one merged A horizon.

2.2. Soil properties and incubation

A dry-sieving method was applied to fractionate the soil samples (n = 12) into three fractions, being large macroaggregates (LM, > 2 mm), small macroaggregates (SM, 0.25–2 mm) and microaggregates (Mi, < 0.25 mm). We separated 170–230 g of air-dried bulk soil sample on a horizontal shaker (30 Hz for 20 s) with two mesh sieves (diameter 2 and 0.25 mm). Total C contents of each fraction were measured using a Vario EL Elementar Analyzer (Elementar, Germany). As carbonate was absent in all samples (Yang et al., 2020a), total C contents were equal to organic carbon (OC) contents.

A 76-day incubation experiment was applied for both macroaggregate (LM and SM) fractions to investigate potential changes in SOM molecular composition after a short-term microbial decomposition. Intact and crushed aggregates were used for the incubation. Aggregates were crushed by grinding the fractions using a porcelain mortar and passing through a 0.125 mm sieve. Ten-gram materials (dry-weight equivalent) were rewetted at pF = 2.0 for 10 days to activate microbial communities. The moistened materials were incubated at 20 °C for 76 days in sealed jars (120 cm^3). CO_2 concentrations of the headspace were measured to calculate SOC mineralization rates at the days of 1, 2, 6, 9, 13, 20, 28, 48 and 76. The CO_2 concentrations were analyzed using a gas chromatography flame ionization detector (GC-

Table 1
Sampling site description and basic soil properties.

	Site 1	Site 2	Site 3	Site 4
Site description				
ID	Wet-LS	Wet-AS	Dry-LS	Dry-AS
Location	7.16° S, 78.61° W	7.17° S, 78.63° W	9.25° S, 77.59° W	9.19° S, 77.60° W
Precipitation (mm)	1100 mm	1100 mm	680 mm	680 mm
Temperature (°C)	11	11	11	11
Altitude (m a.s.l.)	3510–3720	3570–3590	3520–3580	3490–3670
Lithology	Limestone	Granite or ignimbrite (rhyodacitic composition)	Limestone	Granodiorite or glacier materials rich in granodiorite
Land use	Grassland/abundant cultivation	Grassland/abundant cultivation	Grassland/abundant cultivation	Grassland
Number of replicated plots	2	2	2	2
Soil properties				
Soil classification (WRB 2014)	Phaeozems	Andosols	Phaeozems	Andosols/Umbrisols
Soil horizons	Ah-Btg-C	Ah-C	Ah-Btg-C	Ah-C
Soil depth (cm)	61 ± 3	49 ± 10	61 ± 6	51 ± 5
SOC stock accessed to parent materials ($Mg\,ha^{-1}$)	405 ± 42	226 ± 6	153 ± 27	172 ± 13
SOC content (%)	A horizons: 9.95 ± 1.29B horizons: 2.84 ± 0.45	A horizons: 5.95 ± 1.06	A horizons: 2.75 ± 0.37B horizons: 1.48 ± 0.37	A horizons: 4.88 ± 0.65
pH	A horizons: 5.97 ± 0.18B horizons: 6.56 ± 0.22	A horizons: 5.09 ± 0.09	A horizons: 5.26 ± 0.12B horizons: 5.34 ± 0.21	A horizons: 5.29 ± 0.15

Mean ± SE, $n = 3$. SOC: soil organic carbon, Wet: the wet site, Dry: the dry site, LS: limestone soil, AS: acid igneous rock soil. Soil classification and data of SOC stocks, SOC contents and pH values originate from Yang et al. (2020a).

FID with a methanizer, Thermo Scientific, Trace GC Ultra) and packed columns. SOC mineralization rates (mg CO_2-C g^{-1} SOC) with intact aggregates were used to estimate the SOM stabilization controlled by association with mineral surfaces and occlusion in aggregates, whereas SOC mineralization rates with crushed aggregates were to estimate the SOM stabilization controlled by association with mineral surfaces only. The differences in SOC mineralization rates between intact and crushed aggregates were used as measures of the stability of aggregate-occluded SOM (Wang et al., 2014). After the incubation, all samples were dried at 40 °C for molecular analyses using a TMAH-pyrolysis-GC/MS.

2.3. TMAH-Pyrolysis-GC/MS

Three grams of dried subsamples before and after incubation were homogenized for TMAH-pyrolysis-GC–MS analyses. Between 20 and 50 mg of samples were weighed depending on the SOC contents. The samples were subsequently mixed with 20–60 μL of the derivatizing reagent (25% tetramethylammonium hydroxide (TMAH) in water). A small amount of the mixture for each sample was spread on a ferromagnetic wire and dried under a halogen lamp for the pyrolysis. The pyrolysis was performed using a Curie-point pyrolyzer (Horizon Instruments; 600 °C; 5 s), which was coupled to a ThermoQuest Trace GC gas chromatograph (Waltham, USA). Helium was used as the carrier gas. The initial temperature of the oven was 40 °C for 1 min and heated to 320 °C with a rate of 7.0 °C min^{-1} and a hold time of 10 min. The analytes were separated on a ZB1-MS column (Phenomenex: 30 m, 0.25 mm i.d, 0.50 μm df). The column was connected to a Finnigan Trace MS mass spectrometer (Waltham, USA; *m/z*: 47–500, ionization energy: 70 eV, cycle time: 0.45 s).

2.4. Data analyses and statistics

Peak identification was based on retention times and mass spectra using the NIST library (Gaithersburg, USA), as well as the information from Brock et al. (2019), Buurman et al. (2007) and Nierop et al. (2007). A peak was identified as a compound when the ratio of signal to noise was larger than three and when the spectra of the target compound could be clearly identified from the peak. The sum of peak areas of all identified compounds was set to 100% and the relative abundance (RA) of each compound was calculated. Relative abundance shifts (Fig. 2) were calculated by subtracting RAs after the incubation from RAs before the incubation. Changes in compound relative abundances after the incubation of intact aggregates (RAC_{In}) and crushed aggregate (RAC_{Cr}) were used to estimate the compound stability against decomposition. Increased relative abundances indicated that the compounds were more stable against decomposition or produced during incubation, whereas decreased relative abundances indicated that the compounds were depleted and labile against decomposition. Differences in changes in the relative abundance between intact and crushed aggregates ($RA_{Cr\text{-}In}$) were used to evaluate how much the compounds were likely protected by aggregates. The RAC_{In}, RAC_{Cr} and $RAC_{Cr\text{-}Or}$ were calculated by the following equation:

$$RAC_{In} = (RA_{In} - RA_{Or}) / RA_{Or} \times 100\% \quad (1)$$

$$RAC_{Cr} = (RA_{Cr} - RA_{Or}) / RA_{Or} \times 100\% \quad (2)$$

$$RAC_{Cr-In} = (RA_{Cr} - RA_{In}) / RA_{Or} \times 100\% \quad (3)$$

In the equation, RAC_{In} = change in relative abundance after incubation of intact aggregates, RAC_{Cr} = change in relative abundance after incubation of crushed aggregates, $RAC_{Cr\text{-}In}$ = difference in change in relative abundance between intact and crushed aggregates after incubation, RA_{Or} = original relative abundance, RA_{Cr} = relative abundance after incubation of crushed aggregates, RA_{In} = relative abundance after incubation of intact aggregates.

An independent *t*-test was applied to identify differences in relative abundances of compounds and SOC mineralization rates between different aggregate sizes, and between intact and crushed aggregates. When data normality could not be assumed (checked using a Shapiro-Wilk test), a Mann-Whitney *U* test was used instead of the *t*-test. Linear regressions were applied to find significant predictors for the SOC mineralization rates from different compounds. Univariate linear regressions were applied to predict SOC mineralization rates using relative abundance (shifts) of individual FAs. Principal component regressions (PCR) were applied to predict SOC mineralization rates using relative abundance (shifts) of all FAs. The application of PCR instead of multiple linear regression was to solve the problem of collinearity. Briefly, principal component analyses were firstly conducted to create up to 12 orthogonal principal components (PCs). The large number of calculated PCs was to maximize to the variation (> 95%) that the PCs explained for the original independent variables. These PCs were used as

independent variables to predict SOC mineralization rates using multiple linear regressions. All PCs that were significant predictors ($P < 0.05$) were included in the models and regression coefficients of all significant PCs and all FAs were calculated. The analysis of covariance (ANCOVA) was applied to predict relative abundance changes (RAC) using the presence of double bonds and carbon chain length of the FAs. The ANCOVA models were conducted when all FAs were included and when microbial-produced FAs were removed. Through this we aimed to investigate whether the microbial transformation of FAs during the 76-day incubation had clear effects on our prediction of RAC. The microbially produced FAs were selected based on publications (Barré et al., 2018; Jansen and Wiesenberg, 2017; Kaneda, 1991) and our observation (C16:0 and C18:0, see Section 4.4). All statistical analyses were performed using SPSS 24.0 (SPSS Inc., USA).

3. Results

3.1. Incubation and chemical composition of SOM

Data on aggregate-size distribution and SOC mineralization originated from Yang et al. (2020a). The LM and SM fractions accounted for 61.3% and 26.5% of bulk soil samples by weight respectively, whereas the microaggregate fraction was only 12.2% of the bulk soil (Fig. 1). After the 76-day incubation, 1.0% to 1.3% of the SOC was mineralized into CO_2 (Fig. 1). No significant difference in SOC mineralization rates was observed between LM and SM fractions or between intact and crushed aggregates (Fig. 1).

Compounds yielded from TMAH-pyrolysis-GC/MS analyses were comprised of FAs, α,ω-diacids (DAs), ω-hydroxy alkanoic acids (ω-HAs), *n*-alkanes, *n*-alkenes, esters, lignin-derived compounds, polysaccharide-derived compounds and N-containing compounds, as well as other unspecified compounds (Fig. 2). Lipids (sum of FAs, DAs, ω-HAs, *n*-alkanes, *n*-alkenes and esters) were more abundant than polysaccharide-derived, lignin-derived and N-containing compounds (Fig. 2). Relative abundances of FAs were 62.4 ± 4.7% for the LM fraction and 61.5 ± 2.9% for the SM fraction (Fig. 2). After the 76-day incubation, compounds such as DAs, ω-HAs, *n*-alkenes, *n*-alkanes and esters were depleted except for ω-HAs in the SM fraction (Fig. 2).

3.2. Changes of fatty acids after incubation

Twenty-seven different FAs were identified with carbon chain length ranging from 12 to 32. FAs with a carbon chain length of 16 (C16) were the most abundant, followed by compounds with a carbon chain length of 18. Most of the FAs were saturated, except for FAs of C16:1, C18:1 and cyclopropane C19:0 (Fig. 3).

In general, relative abundances of unsaturated FAs (C16:1 and C18:1) and FAs having more than 25 carbons declined after the incubation, whereas saturated FAs of C12:0, C16:0 and C18:0 accumulated after the incubation (Fig. 3). After the incubation, crushed aggregates generally were less abundant in short-chain FAs (e.g. C16:0 and C17:0) and more abundant in long-chain FAs (e.g. C29:0 and C30:0) compared to intact aggregates (Fig. 3).

The PCR models explained 23.4% to 45.0% of the total variation in SOC mineralization rates for intact and crushed aggregates (Fig. 4-A and -B). When using relative abundances before incubation to predict SOC mineralization rates, unsaturated FAs (C16:1 and C18:1) as well saturated FAs of C20:0 and C22:0 were significant predictors that had positive relationships with SOC mineralization rates (Fig. 4-A and -B). In addition, they had higher regression coefficients (2.39–5.12) than other FAs in the PCR models (Fig. 4-A and -B). When using relative abundance shifts after the incubation to predict SOC mineralization rates, unsaturated FAs (C16:1 and C18:1) and saturated FAs of C31:0 and C32:0 were significant predictors that had negative correlations with SOC mineralization rates (Fig. 4-C and -D). They also had the lowest regression coefficients compared to other FAs (Fig. 4-C and -D). In contrast, C16:0 and C18:0 were significant predictors having positive regression coefficients to predict SOC mineralization rates (Fig. 4-C and 4-D). Notably, the prediction models for both PCR and linear regressions had no clear differences in significant predictors between intact and crushed aggregates (Fig. 4). In contrast, R^2 values of the PCR models were higher for intact aggregates than crushed aggregates (Fig. 4).

3.3. Predictions of relative abundance changes of fatty acids

Table 2 illustrates the prediction of relative abundance changes (RAC) after the incubation for both averaged RACs and RACs of different soil samples, using double bonds and carbon chain lengths of FAs as the predictors. For the overall situation (averaged RACs), unsaturated FAs significantly predicted low RAC_{In} and RAC_{Cr} in both LM and SM fractions ($P < 0.05$). In contrast, they were not significant predictors for $RAC_{Cr\text{-}In}$ (Table 2). Long-chain FAs were significant predictors for low RAC_{In} in both LM and SM fractions, for low RAC_{Cr} in the LM fraction, and for high $RAC_{Cr\text{-}In}$ in the SM fraction (Table 2). When microbial FAs were removed, significant predictors were not changed in the ANCOVA models, whereas R^2 values slightly increased ($\Delta R^2 = 0.049 \pm 0.029$, Table 2).

When comparing different soil samples and horizons, general findings were that: (1) the presence of double bonds was not a significant predictor ($P > 0.05$) for $RAC_{Cr\text{-}In}$ for all soils; (2) long-chain FAs significantly predicted low RAC_{In} and RAC_{Cr} except for the B horizons of the wet-LSs; and (3) short-chain FAs were significant predictors for low $RAC_{Cr\text{-}In}$ except for the B horizons of the wet-LSs (Table 2). In addition, Table 2 shows that the R^2 values only slightly changed ($\Delta R^2 = 0.057 \pm 0.018$) after the removal of microbial FAs (see Section 4.4). However, differences in predictive models were found between soil samples and horizons. The presence of double bonds were more

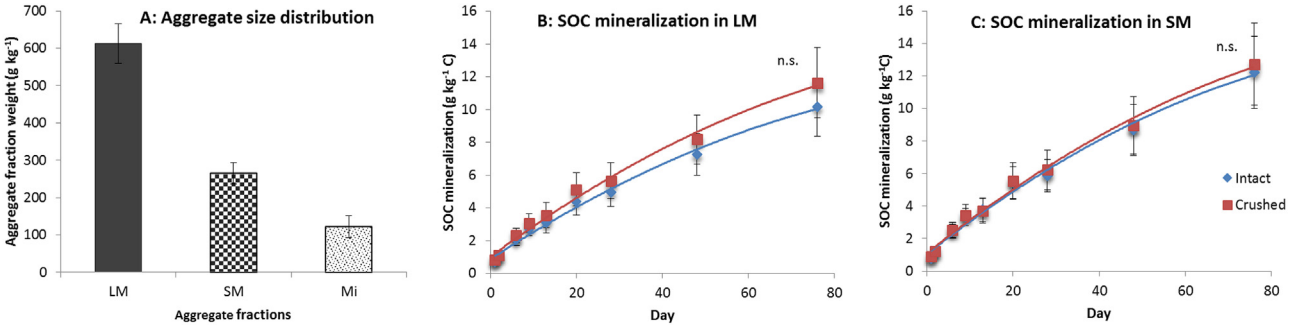

Fig. 1. Soil aggregate size distribution (A) and specific soil organic carbon (SOC) mineralization rates in a 76-day incubation (B, C) ($n = 12$, mean ± SE). LM: large macroaggregates (> 2 mm), SM: small macroaggregates (0.25–2 mm), Mi: microaggregates (< 0.25 mm), Intact: intact aggregates, Crushed: crushed aggregates, n.s.: not significant. Data originate from Yang et al. (2020a).

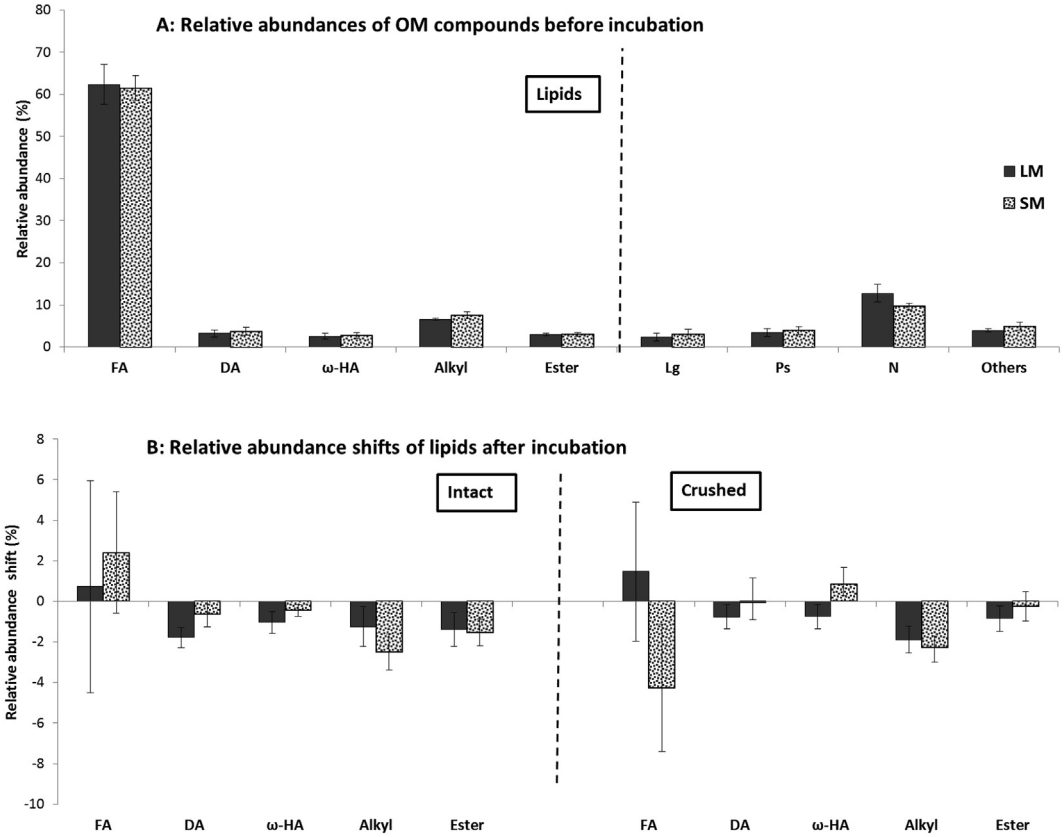

Fig. 2. Relative abundances of detected compounds before the incubation and relative abundance shifts for lipids after the incubation (n = 12, mean ± SE). LM: large macroaggregates, SM: small macroaggregates. Intact: incubating intact aggregates, Crushed: incubating crushed aggregates, FA: fatty acids, DA: α,ω-dioic acids, ω-HA: ω-hydroxy alkanoic acids, Alkyl: *n*-alkanes and *n*-alkenes, Lg: lignin-derived and aromatic compounds, Ps: polysaccharide-derived compounds, N: nitrogen-containing compounds.

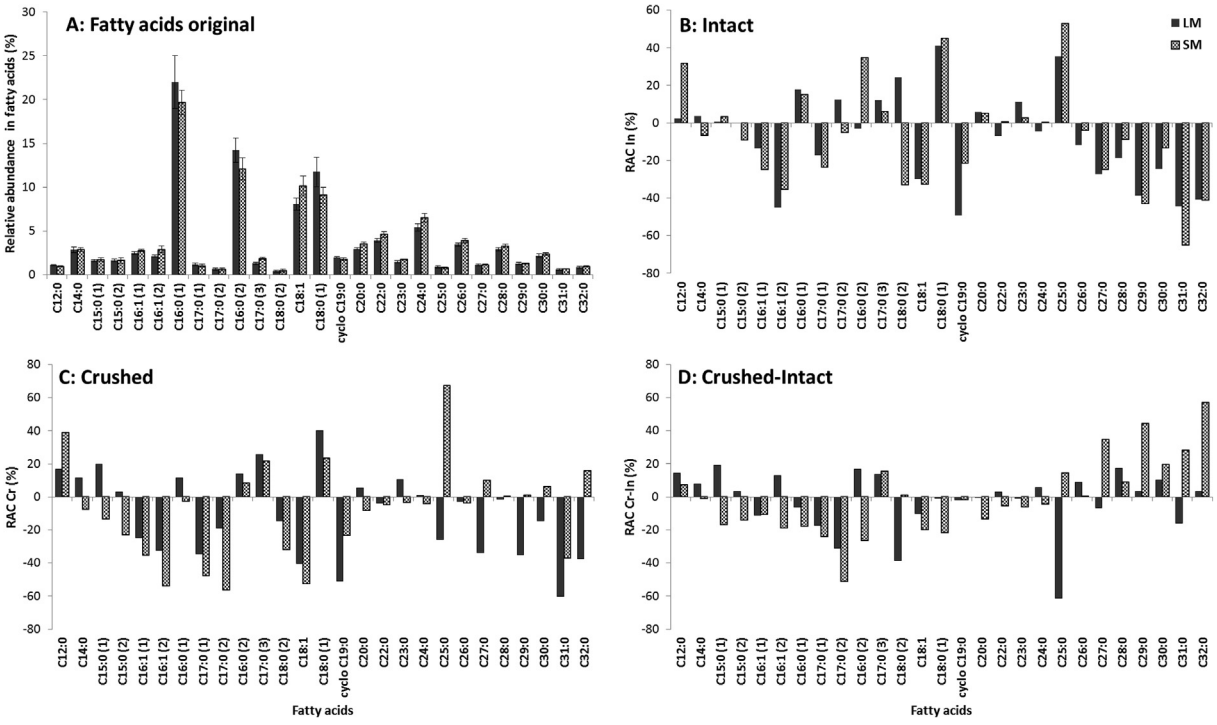

Fig. 3. Relative abundances and changes in relative abundances (RAC) of fatty acids after the 76-day incubation (means of n = 12). RAC In: change in relative abundances after incubation of intact aggregates, RAC Cr: change in relative abundances after incubation of crushed aggregates, RAC Cr-In: difference in change in relative abundances after incubation between crushed and intact aggregates (indication of protection of fatty acids by occlusion in aggregates), LM: large macroaggregates, SM: small macroaggregates, cyclo C19:0: the fatty acid of C19:0 cyclopropanated form.

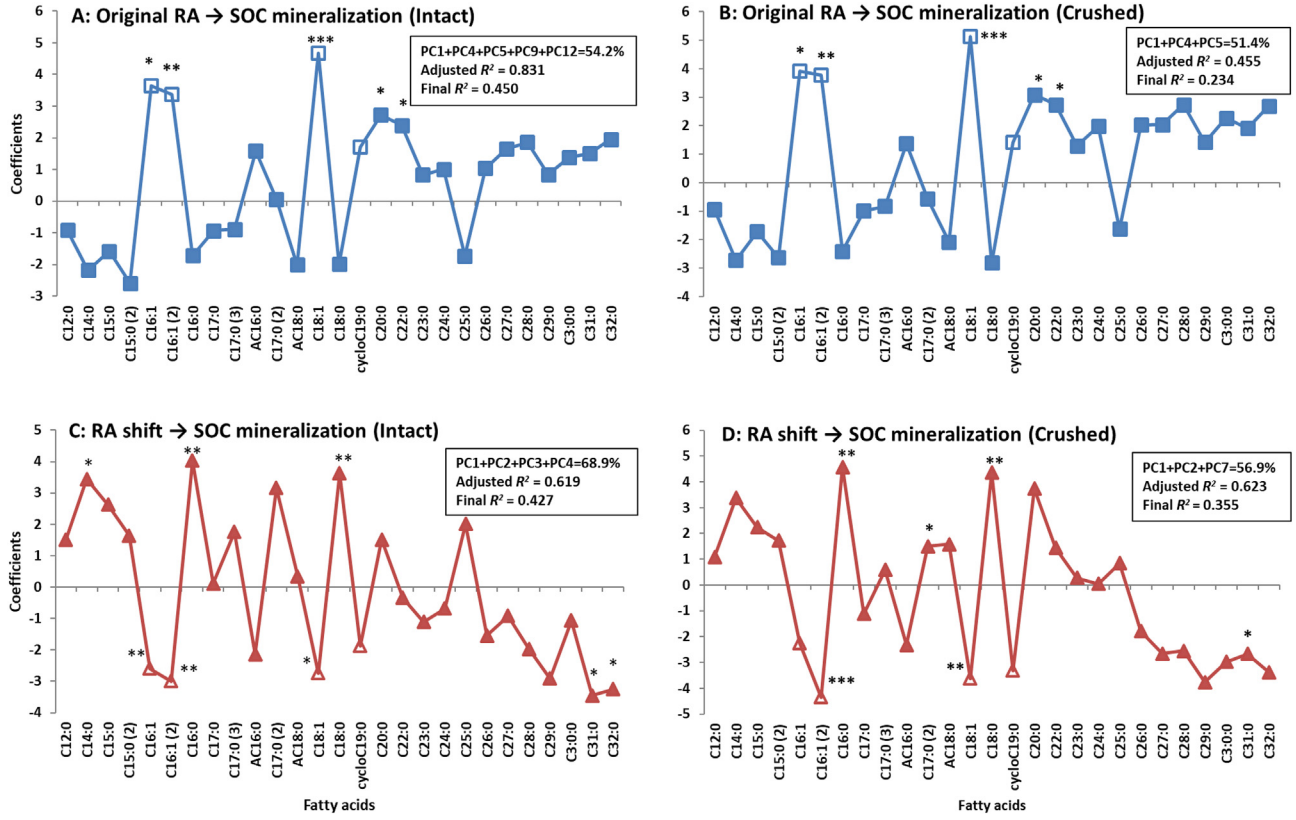

Fig. 4. Soil organic carbon (SOC) mineralization rates predicted by relative abundances (RA) of fatty acids and relative abundance shifts during the incubation using principal component regressions (PCR) and linear regressions. Regression coefficients of the PCRs are present as the values of Y axis. The box on the right of each graph presents principal components (PCs) used as the significant predictors and the total variation explained by them. In addition, the box gives information of adjusted R^2 when using PCs to predict SOC mineralization rates and final R^2 when using all fatty acids to predict SOC mineralization rates. The *, ** and*** for each compound present the significant levels of 0.05, 0.01 and 0.001 when SOC mineralization rates are predicted by individual fatty acids using univariate linear regressions. For graphs A and B, high coefficients indicate that the compounds have higher contribution to the SOC mineralization. For graphs C and D, positive coefficients suggest that the compounds are accumulated or produced after the incubation; negative coefficients suggest the compounds are depleted after the incubation. The significant level of each fatty acid was estimated by predicting SOC mineralization rates using only one fatty acid (univariate linear regressions). n = 24, RA: relative abundance, PC: principal component, intact: incubation of intact aggregates, crushed: incubation of crushed aggregates.

Table 2
Changes in relative abundances (RAC) of fatty acids after the incubation predicted by the presence of double bonds (DB) and carbon chain length (CCL) using the ANCOVA models. For significant predictors, the types (i.e. Uns, LC, and SC) corresponding to lower RAC values (more vulnerable) were presented. Microbial-produced FAs are C15:0 (1), C15:0 (2), C16:0 (1), C17:0 (1), C17:0 (2), C17:0 (3), C18:0 (1) and cyclo C19:0. Averaged RACs of all soil samples were used as dependent variables in the ANCOVA models for overall situation.

		Overall			Wet-AS-A			Dry-AS-A			Wet-LS-A			Dry-LS-A			Wet-LS-B			Dry-LS-B		
		DB	CCL	R^2	DB	CCL	R^2	DB	CCL	R^2	DB	CCL	R^2	DB	CCL	R^2	DB	CCL	R^2	DB	CCL	R^2
		(1) All FAs																				
RAC In	**LM**	**Uns****	**LC****	0.52	**Uns****	**LC****	0.46	n.s	n.s	0.04	n.s	**LC****	0.43	**Uns***	n.s	0.33	n.s	**SC****	0.73	n.s	n.s	0.22
	SM	**Uns***	**LC****	0.34	n.s	n.s	0.16	n.s	n.s	0.03	n.s	n.s	0.14	**Uns***	n.s	0.22	n.s	**LC***	0.23	n.s	n.s	0.2
RAC Cr	**LM**	**Uns****	**LC****	0.55	**Uns****	n.s	0.34	**Uns****	n.s	0.24	n.s	**LC****	0.52	**Uns****	n.s	0.3	n.s	n.s	0.05	n.s	n.s	0.05
	SM	**Uns***	n.s	0.25	**Uns****	n.s	0.37	n.s	n.s	0.12	n.s	n.s	0.05	**Uns****	**LC***	0.36	n.s	**SC****	0.46	n.s	n.s	0.22
RAC Cr-In	**LM**	n.s	n.s	0.01	n.s	n.s	0.16	n.s	n.s	0.04	n.s	n.s	0.01	n.s	**SC****	0.27	n.s	**LC****	0.71	n.s	**SC****	0.49
	SM	n.s	**SC****	0.53	n.s	**SC***	0.23	n.s	n.s	0.11	n.s	**SC***	0.26	n.s	n.s	0.04	n.s	**SC****	0.77	n.s	n.s	0.06
		(2) Microbial FAs removed																				
RAC In	**LM**	**Uns****	**LC****	0.48	**Uns***	**LC***	0.4	n.s	n.s	0.04	n.s	**LC****	0.53	n.s	n.s	0.19	n.s	**SC****	0.71	n.s	n.s	0.02
	SM	**Uns***	**LC***	0.39	n.s	**LC***	0.3	n.s	n.s	0.02	n.s	**LC***	0.27	**Uns****	n.s	0.49	n.s	**LC***	0.39	**Uns***	**LC***	0.35
RAC Cr	**LM**	**Uns****	**LC****	0.64	**Uns****	n.s	0.46	n.s	n.s	0.24	n.s	**LC****	0.66	n.s	n.s	0.17	n.s	n.s	0.1	n.s	n.s	0.23
	SM	**Uns***	n.s	0.42	**Uns****	n.s	0.62	n.s	n.s	0.12	n.s	n.s	0.17	**Uns****	**LC****	0.75	n.s	**SC***	0.37	**Uns***	n.s	0.34
RAC Cr-In	**LM**	n.s	n.s	0.01	n.s	n.s	0.15	n.s	n.s	0.01	n.s	n.s	0.13	n.s	n.s	0.2	n.s	**LC***	0.69	n.s	**SC***	0.49
	SM	n.s	**SC****	0.57	n.s	**SC****	0.49	n.s	n.s	0.15	n.s	n.s	0.24	n.s	n.s	0.09	n.s	**SC****	0.76	n.s	n.s	0.19

n = 27 (27 fatty acids). Wet: the wet site, Dry: the dry site, LS: limestone soil, AS: acid igneous rock soil, A: A horizon, B: B horizons, In: intact aggregate, Cr: crushed aggregates, Cr-In: differences between intact and crushed aggregates, LM: large macroaggregates, SM: small macroaggregates, DB: double bounds, CCL: carbon chain length, Uns: unsaturated fatty acids, LC: long-chain fatty acids, SC: short-chain fatty acids, *: $P < 0.05$, **: $P < 0.01$, n.s.: not significant.

important predictors for the A horizons of the wet-ASs and the dry-LSs (Table 2). For the B horizons of the wet-LSs, short-chain FAs significantly predicted low RAC_{In} and RAC_{Cr}, whereas long-chain FAs were a significant predictor for low $RAC_{Cr\text{-}In}$ (Table 2).

4. Discussion

4.1. Chemical composition

It is important to note that the OM molecular composition yielded by TMAH-pyrolysis-GC/MS does not reveal the quantitative composition of bulk SOM because the methodology is not fully quantitative. Nevertheless, it is still possible to compare our results to those of other studies using the same methodology under similar conditions. When compared to other studies using TMAH-pyrolysis-GC/MS (e.g. Barré et al., 2018; Brock et al., 2019; Nierop and Verstraten, 2003), our results highlighted a large proportion of aliphatic compounds (lipids) and a small proportion of lignin- and polysaccharide-derived compounds (Fig. 2). This suggests that lipids are selectively preserved in the Peruvian Andean soil under study. Similar to our results, Matsumoto et al. (1981) found a large contribution of FAs in Antarctic soils, whereas Nierop et al. (2007) reported lipid-rich soils in the Andean grassland of Northern Ecuador. As Nierop et al. (2007) found poor relationships between SOM molecular composition and vegetation (i.e. grassland vs. forest), the selective preservation of lipids is unlikely to be attributed to differences in SOM input related to the vegetation. The small proportions of compounds derived from lignin and polysaccharides in our soils agree with the results of Nierop et al. (2007), who found lignin- and polysaccharide-depleted soils in the Andean grasslands of Ecuador. In contrast, soils in other systems have larger amounts of lignin- and polysaccharide-derived compounds than our soils (Barré et al., 2018; Brock et al., 2019; Nierop and Verstraten, 2003). This suggests that compounds considered in former times as recalcitrant (e.g. lignin) do not always persist in soils, which corroborates the emerging view that chemical recalcitrance is less important to control long-term SOM stabilization (Dungait et al., 2012; Schmidt et al., 2011).

Soil lipids are separated into free lipids derived from leaf wax, bound lipids derived from macromolecules (e.g. cutin and suberin) and lipids derived from microorganisms (Jansen and Wiesenberg, 2017). The results allow for an estimation of soil lipids of plant origins. In general, α-,ω-dioic acids and ω-hydroxyl alkanoic acids are mostly derived from bound lipids as part of plant macromolecules (Feng et al., 2010; Nierop et al., 2006). The small proportions of α-,ω-dioic acids and ω-hydroxyl alkanoic acids in our soils (Fig. 2-A) suggest a minor contribution of bound lipids. This is further supported by (1) the absence of cutin or suberin biomarkers (e.g. 9,10,ω-trihydroxy alkanoic acids and x,ω-dihydroxy alkanoic acids), and (2) the depletion of α-,ω-dioic acids and ω-hydroxyl alkanoic acids after the incubation (Fig. 2 and S2). Thus, free lipids derived from plants rather than bound lipids are particularly important in our soils. The preferential accumulation of free lipids in our soils can be explained by the chemical properties of free lipids (not part of macromolecules), which make them easier to interact with the soil matrix compared to bound lipids (Angst et al., 2018). In addition, differences in functional groups (e.g. carboxyl groups vs. ester groups) between free and bound lipids can also affect their interactions with the soil matrix and further control their stability (Kleber et al., 2007; Lützow et al., 2006). Because of the abundant FAs and the major contribution of free FAs, the following discussions on FA stabilization are representative for free FAs.

4.2. Unsaturated and saturated fatty acids

The results indicated that unsaturated FAs were more depleted than saturated FAs after the incubation (Figs. 3, 4 and Table 2). This is consistent with the general view that unsaturated FAs are less persistent than saturated FAs (Ayala et al., 2014). The depletion of unsaturated after the incubation might be attributed to: (1) microbial decomposition, and (2) association with mineral surfaces or macromolecules during the incubation and being undetectable. We propose that microbial decomposition most likely causes the depletion of unsaturated FAs because SOC mineralization rates were significantly predicted by the abundances of unsaturated FAs before the incubation and the changes of unsaturated FAs during the incubation (Fig. 4). In addition, it is logical that microbes utilize unsaturated FAs when other energy sources (e.g. carbohydrates in our soils) are rare. The depletion of unsaturated FAs is unlikely to be explained by the association of unsaturated FAs with soil minerals during the incubation because (1) natural soils should reach a balance between association and dissociation of unsaturated FAs with mineral surfaces; and (2) the balance was unlikely to shift significantly to allow for more unsaturated FAs associated with mineral surfaces during the 76-day incubation. Thus, unsaturated FAs were less stable against microbial decomposition compared to saturated FAs with similar carbon chain length. Similar to our results, Moucawi et al. (1981) found higher decomposition rates for oleic acid (C18:1) compared to stearic acid (C18:0) in acid soils only, whereas Dent et al. (2004) reported the accumulation of saturated FAs and the depletion of unsaturated FAs during the necromass decomposition in soils.

The stabilization of both unsaturated and saturated FAs in soils is controlled by: (1) FAs occluded in aggregates, (2) FAs association with mineral surfaces, and (3) chemical recalcitrance of the FAs (Lehmann and Kleber, 2015; Lützow et al., 2006). As the presence of double bonds was not a significant predictor for the $RAC_{Cr\text{-}In}$ (Table 2), it is likely that saturated and unsaturated FAs with similar carbon chain lengths are protected by aggregates to a similar extent. Therefore, the depletion of unsaturated FAs can be explained by their reduced stabilization controlled by their chemical properties and/or association with mineral surfaces. Considering chemical properties, the unsaturated FAs are more vulnerable to lipid peroxidation than saturated FAs due to their double bonds. Briefly, the unsaturated FAs are susceptible to the attack of reactive radicals at the position of double bonds and are vulnerable to biotic and abiotic rancidification. These processes allow for easier degradation of unsaturated FAs as compared to saturated FAs (Ayala et al., 2014; Gardner, 1989). In addition, incorporation within macromolecules may also control the bioavailability of FAs in the early stages of decomposition (Schmidt et al., 2011). This may also affect the stability of unsaturated and saturated FAs in our study because the incubation was conducted for only 76 days. For the association with mineral surfaces, unsaturated and saturated FAs might have different affinities to the association sites of mineral surfaces. Saturated FAs are straight molecules, whereas unsaturated FAs generally have one or multiple bent structures as cis-double bonds have an angle of 120° (Rustan and Drevon, 2005). In the models of OM-mineral association, straight FAs and compounds with similar structures are important with respect to interaction with mineral surfaces and the formation of hydrophobic zones (Kleber et al., 2007; Wershaw, 1993). If the saturated FAs were replaced by unsaturated FAs, unsaturated FAs are unable to pack as tightly as saturated FAs due to the bent structures introduced by their cis configuration (Rustan and Drevon, 2005). This can potentially weaken the association of unsaturated FAs with minerals when compared to saturated FAs. However, this situation might not be applicable for soils in which OM complexation with Fe and Al is the dominant stabilization mechanism (e.g. Andosols).

4.3. Carbon chain length

In general, carbon chain length is a significant predictor for changes in relative abundances, and short-chain FAs were more stable than long-chain FAs except for the B horizons of wet-LSs (Table 2). To our knowledge, only a few studies have investigated the stability of soil FA related to carbon chain length. Mueller et al. (2013) and Otto and Simpson (2007) found that carbon chain length is crucial to soil lipids

distribution, however, the stability of the lipids against decomposition was not investigated. Otto and Simpson (2007) also found more contribution of free-lipids in long-chain lipids, which might affect the stability of FAs with different chain lengths in our soils. However, the influences are probably limited in our soils because of the minor contribution of bound-FAs (see discussion 4.1).

In general, the increasing FA stability with decreasing carbon chain length might be related to: (1) the affinity of FAs to the mineral surfaces, (2) the possibility that FAs were stabilized by occluded in aggregates, and (3) chemical recalcitrance. We observed that short-chain FAs were consistently characterized by lower $RAC_{Cr\text{-}In}$ values compared to long-chain FAs except for the B horizons of Wet-LSs (Table 2). A possible explanation is the selective stabilization of short-chain FAs by occlusion in aggregates when compared to long-chain FAs. This might be attributed to the fact that long-chain FAs are generally distributed in large-sized aggregates (Lützow et al., 2006). As large-sized aggregates can only provide weak protection and can have high microbial activities (Six et al., 2004, 2000), long-chain FAs are potentially easily decomposed. In contrast, small-size aggregates provide OM with strong and long-term protection (Six et al., 2004, 2000) that allows for short-chain FAs more stable than long-chain FAs. In addition, it is also possible that differences in short-chain FAs production during the incubation cause the different $RAC_{Cr\text{-}In}$ values between intact and crushed aggregates, as short-chain FAs can be produced by microbes during the incubation (Dinel et al., 1990; Rustan and Drevon, 2005).

4.4. Microbial transformation

During incubation, soil microbes are likely to mineralize unsaturated FAs into CO_2 and H_2O to gain energy through β-oxidation coupled with the tricarboxylic acid cycle, and they metabolize microbial (short-chain) FAs to maintain cell structures (Dinel et al., 1990; Rustan and Drevon, 2005). As these processes are a “black box” for our study, we only observed the depletion of unsaturated FAs (Fig. 3 and Table 2) and the accumulation of microbial FAs. In our study, microbial FAs are probably C16:0 and C18:0 because they accumulated after the incubation and their accumulation was positively correlated to SOC mineralization rates (Figs. 3 and 4). Also, other studies reported that the occurrence and accumulation of C16:0 and C18:0 in soils are closely related to the microbial activity (Kögel-Knabner, 2002; Wiesenberg et al., 2008). In addition to C16:0 and C18:0, we detected *iso*- or *anteiso*-C15:0, *iso*- or *anteiso*-C17:0 and cyclopropane C19:0 (Fig. 3), which are generally considered as microbial-derived FAs and unlikely occur in high plants (Barré et al., 2018; Jansen and Wiesenberg, 2017; Kaneda, 1991).

It is important to know whether the microbial transformation of FAs affects the prediction of FA stability using the presence of double bonds and carbon chain length. When these microbial FAs (C16:0, C18:0, cyclopropane C19:0 and isomers of C15:0 and C17:0) were removed from the ANCOVA models, the unchanged or slightly changed significance of predictors (Table 2) suggested that the removal of microbial FAs does not clearly affect the prediction of FAs stability. This is further supported by the fact that R^2 values were only slightly changed after the removal of microbial FAs (Table 2). Thus, although the microbial transformation of FAs occurred during the incubation, it has a limited impact on our estimation of FA stability using the presence of double bonds and carbon chain length.

4.5. Differences between soil samples

Before generalizing our findings to other soils, it is important to know if our findings are consistently applicable for all soil samples under study. We observed differences between soil samples. For example, the B horizons in wet-LSs showed higher stability of long-chain FAs, which is not consistent with the situations in other soils of our study (Table 2). This might be attributed to the clay-rich subsoil (Yang et al., 2020b) and the low availability of oxygen due to the high moisture and clay contents. The clay-rich subsoil and the low oxygen availability can potentially make the interactions between microbial decomposers and FAs different from other soil horizons. In the dry-ASs, we found a lack of effects of carbon chain length on RACs. A possible explanation is that the lack of good soil structure (aggregation) in the dry-ASs (Yang et al. 2020a) provided limited FAs stabilization by occlusion in aggregates. With regard to unsaturated FAs, the significant control of RAC by double bonds was mainly observed in the wet-ASs and the dry-LSs (Table 2). This might be attributed to the abundance of unsaturated FAs in the dry-LSs and the wet-ASs and their higher depletion after the incubation (Fig. S3). In addition, the dry-LSs had the lowest SOC stability (Yang et al., 2020a) and the highest abundance of unsaturated FAs compared to other soils. This is consistent with our findings and the general view that unsaturated FAs were less stable compared to saturated FAs (Ayala et al., 2014).

4.6. Implications to bulk soil organic matter

The results indicate an accumulation of free lipids and FAs as well as a minor contribution of bound lipids and FAs (see 4.1). The selective preservation of free FAs suggests the origins of FAs are crucial to FA persistency. Our results also give direct evidence that chemical properties of the FAs control their interaction with the soil matrix and indirectly influence their persistence in soils. This addresses the current discussion on the importance of SOM chemical properties on SOM persistence in soils (Dungait et al., 2012; Schmidt et al., 2011), and is supported by recent findings that SOM chemical properties control the interaction with the soil matrix and further influence OM stabilization (Angst et al., 2018, 2017). As our study showed a large proportion of FAs when compared to other studies using similar methods (Fig. 2), the findings highlight that chemical properties of SOM are also important factors controlling SOM persistence and stabilization in the Peruvian Andes. When applied to SOM turnover in general, we should pay attention to soil properties. Our results showed clear differences in the effects of double bonds and carbon chain length on relative abundance changes of FAs between different soil profiles and horizons (Table 2). As these soil profiles and horizons had large differences in soil mineralogy and aggregation (Yang et al., 2020a,b), the differences can be potential factors regulating the interactions between SOM composition and SOM stability.

5. Conclusions

The TMAH-pyrolysis-GC/MS analyses for the soils under study revealed large relative abundances of FAs, with a major contribution of free FAs. Our results highlighted that the presence of double bonds and carbon chain length are key factors controlling FA stability in the studied Peruvian soils. Unsaturated FAs were depleted after the incubation and this is likely explained by their easier decomposition by soil microbes when compared to saturated FAs. The easy degradation of unsaturated FAs is likely explained by their less stabilization by association with mineral surfaces and/or chemical properties rather than stabilization by occlusion in aggregates. In terms of carbon chain length, FA stability decreased from short-chain to long-chain FAs. A possible explanation is that short-chain FAs received more protection by occlusion in aggregates compared to long-chain FAs, and/or that short-FAs were produced by microorganisms during the incubation. Our results also suggest microbial transformation of FAs during the incubation. However, the transformation has limited effects on the controls of double bonds and carbon chain length on FA stability. Finally, the controls of double bonds and carbon chain length on FA stability were different between soil samples and horizons.

Our study provides direct evidence that the chemical properties of FAs influence their interaction with the soil matrix, which supports the view that the chemical composition of SOM has an impact on SOM

stabilization controlled by the soil matrix. However, more studies are needed before generalizing our findings to other soils because of the differences between soil samples and horizons. Our results were insufficient to explain whether the differences in stability between unsaturated and saturated FAs are attributed to chemical recalcitrance or interactions with mineral surfaces. Thus, future research should compare the affinity to mineral surfaces between unsaturated and saturated FAs through sorption experiments.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114414.

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