Chemical and Spectroscopic Investigation of Different Soil Fractions as Affected by Soil Management

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Abstract: The interaction of organic matter with the finest soil fractions (<20 μm) represents a good way for its stabilization. This study investigates the effects of conventional (CT), minimum (MT), and no (NT) tillage, fertilization, and non-fertilization, and soil depth (0–30, 30–60, and 60–90 cm) on the amount of organic carbon (OC) in four soil fractions. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) was performed to obtain information about the OC quality and the mineralogical composition of these fractions. The CT shows the highest amount of the finest fraction while the fertilization enhances the microbial community with the increase of soil micro-aggregates (250–53 μm). The coarse fraction (>250 μm) is highest in the upper soil layer, while the finest fraction is in the deepest one. The greatest OC content is observed in the topsoil layer and in the finest soil fraction. DRIFT analysis suggests that organic components are more present in the finest fraction, calcite is mainly localized in the coarse fraction, quartz is in micro-aggregates and 53–20 μm fraction, and clay minerals are in the finest fraction.

Keywords: tillage; fertilization; soil depth; organic carbon; clay minerals; diffuse reflectance; infrared Fourier transform spectroscopy

1. Introduction

Previous studies have used the particle size fractionation for obtaining information about the influence of land use and depth on the distribution of soil organic carbon (SOC) [1,2]. The various soil fractions can differently immobilize organic carbon (OC) through the formation of organo-mineral complexes [3,4]. In particular, quartz particles exhibit only weak bonding affinities to organic matter (OM), while clay size particles (i.e., sesquioxides and phyllosilicates) have a large surface area and numerous sorption sites [3,4]. The physical protection of OM through its occlusion within clay minerals limits its microbial decomposition, which reduces the C mineralization [5]. Therefore, the sand related OM represents the active pool of soil organic matter (SOM), the OM linked to the silt size fraction is the intermediate pool, and the clay related OM represents the passive and the older SOM pool [4]. The microaggregates, composed mainly of clay minerals, represents the most efficient way to stabilize the SOM [6,7] by forming bridges between the exchangeable cations of layer silicates and functional groups of organic compounds [8]. The formation of macroaggregates is favored by the decomposition of fresh plant residues and fungal hyphae [9]. The SOM in the macroaggregates is available for microbial utilization while the protected microaggregates form a long-term reserve of mineral-associated C that is not "humified" and can be attacked by microorganisms once exposed [9].

It is known that chemical fertilizers can modify soil physical, chemical, and biological properties with clear consequences on soil aggregates [10]. A different development of the root system, stimulated by fertilization, influences the production and release of root exudates, directly involved in the formation of aggregates [11]. In addition, a greater growth of the root system following the fertilization increases the SOM [11]. Several previous studies have demonstrated that tillage practices influence the content and the dynamic of SOM [7,12–14]. Soil tillage increases the turnover of macroaggregates by inhibiting the formation of microaggregates within macroaggregates and, thus, reducing the sites where the OM is stabilized [5].

In order to identify the minerals and their changes among the different soil fractions, the diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy has been utilized. This technique can be considered rapid, inexpensive, and precise, and can be applied to estimate the water-bearing minerals, such as clay minerals together to other sheet silicates as muscovite, illite, smectite, kaolinite, and chlorite [15], and the presence of organic matter [16]. DRIFT spectroscopy allows us to analyze the matrices without pressing them by avoiding the error due to scattering [17], and to have a band intensity four times greater than that of IR spectroscopy due to the non-mixing of the soil sample with KBr [18].

The objective of this study was to investigate the effects of different soil managements on the quantity of SOC associated with several soil-size fractions. In addition, with DRIFT analysis, we tried to better understand the OM interaction with the mineral parts of the different soil fractions.

2. Materials and Methods

2.1. Study Area and Experimental Design

The trial was conducted in the experimental station of the University of Bari (Italy) located at Policoro (40°10′20″ N; 16°39′04″ E; altitude: 15 m above sea level). The soil texture was classified as silt loam (sand 8%, silt 68%, clay 24%), according to the USDA [19]. Since 2005, a two-year rotation of durum wheat with faba bean in a split-block design with three field replications has been introduced. Treatments were as follows: i) no tillage and no fertilization (NT), ii) NT and crop fertilization (30 kg P_2O_5 ha^{-1}: NTF), iii) minimum tillage (20 cm deep subsoiling in late August and 15 cm deep disk harrowing in November) and no fertilization (MT), iv) MT and crop fertilization (MTF), v) conventional tillage (35 cm deep moldboard plowing in late August and 15 cm deep disk harrowing in November) and no fertilization (CT), and vi) CT and crop fertilization (CTF). More details about treatments and soil properties are reported elsewhere [20,21].

After more than a decade of trial, each faba bean plot (30 × 30 m) was sampled in July 2017 at three different depths (0–30, 30–60, and 60–90 cm) using an auger, after the harvest, and the removal of aboveground crop residues. Due to the soil homogeneity, nine sub-samples have been collected from each plot using a grid sampling scheme.

2.2. Particle-Size Physical Fractionation and Determination of Organic Carbon in Fractions

Fraction-size separation was obtained by ultrasonic dispersion, according to Amelung and Zech [22], and wet sieving, according to Bornemann et al. [23] (Figure 1).

About 150 mL of milli-Q® ultrapure water were added to 30 g of air-dried soil and the suspension was gently sonicated by placing the probe tip 15 mm below the water surface and using a probe-type sonicator Sigma Aldrich, 500-Watt model (60 J mL^{-1}). This weak sonication was used for preserving micro-aggregates from disruption [24]. The first fraction (macro-aggregates fraction, A: 2000–250 μm) was separated from the suspension by wet sieving (250 μm), and the filtered remnant was sonicated a second time at 440 J mL^{-1} and separated by wet sieving using sieves with different meshes (53 μm and 20 μm). After this step, the obtained fractions were: fraction B (microaggregates fraction, 250–53 μm), fraction C (coarse silt-sized fraction 53–20 μm), and fraction D (free fine silt plus clay fraction, <20 μm). All fractions were dried at 35 °C before elemental analysis. The water-soluble organic fraction was

isolated and discharged from each D fraction, according to Zsolnay [25], to avoid any interference. Briefly, an aliquot of each air-dried D fraction was suspended in water (1:10, w/v), and mechanically shaken for 15 min. The suspensions were then centrifuged at 6000 rpm for 15 min and the supernatant was removed.
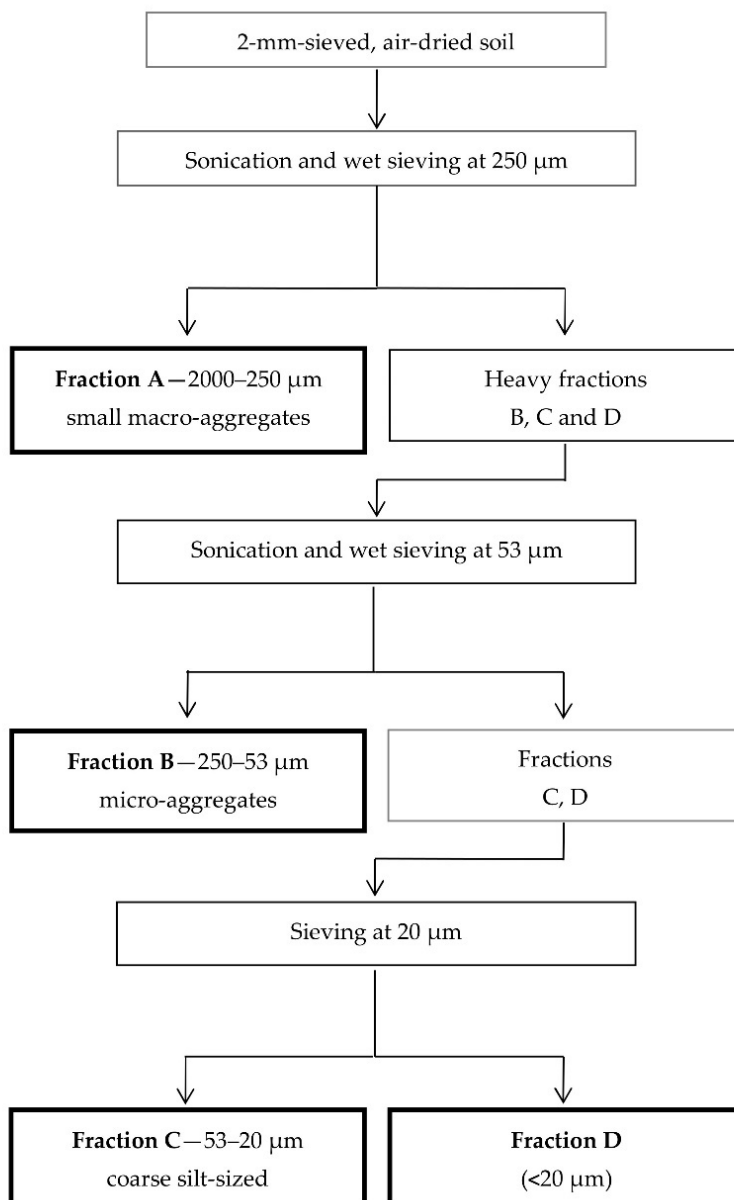
Figure 1. Fractionation scheme of soil.

The OC content of all soil fractions was determined in triplicate using a Flash 2000 CHNS-O Elemental Analyser (Thermo Scientific) calibrated by an organic analytical standard consisting of a low organic content soil with 1.55% (w/w) of carbon. About 6–7 mg of each soil fraction were dried at 40 °C and pre-treated with hydrochloric acid (HCl 1%) to dissolve carbonates. The OC stocks were calculated by multiplying the C concentrations and the corresponding particle-size weights.

2.3. Spectroscopic Analysis of Particle-Size Fractions

Diffuse Reflectance Fourier Transform (DRIFT) spectra were recorded for each fraction in triplicate and in transmittance mode using a Thermo Nicolet Nexus FT-IR spectrophotometer, which was equipped with a Nicolet Omnic 6.0 software. Before DRIFT analysis, air-dried samples were thoroughly

mixed to obtain a representative sample and then finely ground in a mill. About 200 mg of the mixture was filled in a cup and the surface was smoothed with a plastic slide. Spectra were recorded in the range of 4000 to 400 cm^{-1}, with 4 cm^{-1} resolution and 16 scans min^{-1} for each acquisition.

2.4. Statistical Analysis

All analyses performed on soil fractions were conducted in triplicate. The analysis of variance (four-way ANOVA) and the Tukey's test (R software, version 3.2.3) were used to measure the effect of fertilization, tillage, and depth on the OC content for each soil fraction.

3. Results and Discussion

3.1. Effects of Treatments on the Amount of Each Soil Fraction and on their Organic Carbon Content

The physical fractionation recovered 98% of the mass and 99% of the OC from all samples. Such percentages were comparable to those previously reported by other authors [26–28], which indicates that the loss of material was very low and confirms the efficiency of the fractionation method adopted.

Table 1 shows the amounts of soil dry matter obtained from each fraction (g kg^{-1} soil), while Table 2 reports the corresponding statistical analyses, as influenced by treatments (soil depth, tillage, and fertilization).

Table 1. Amount of soil dry matter in the size fractions (g kg^{-1} soil) (mean ± standard deviation).

Sample	Dry Matter (g kg^{-1})			
CT	**A (>250 μm)**	**B (250–53 μm)**	**C (53–20 μm)**	**D (<20 μm)**
0–30 cm	6.8 ± 0.2	89.5 ± 12.0	220.2 ± 26.2	689.0 ± 0.9
30–60 cm	3.8 ± 0.2	46.0 ± 0.9	199.0 ± 3.8	763.7 ± 0.9
60–90 cm	2.0 ± 0.0	15.2 ± 0.7	134.7 ± 3.8	867.0 ± 1.9
CTF				
0–30 cm	8.3 ± 0.5	174.7 ± 17.9	202.5 ± 11.1	604.5 ± 12.0
30–60 cm	4.8 ± 0.2	174.3± 21.2	203.7 ± 17.9	617.8 ± 6.8
60–90 cm	1.8 ± 0.7	39.7 ± 2.4	135.8 ± 4.0	835.2 ± 3.5
MT				
0–30 cm	10.3 ± 2.8	123.2 ± 28.5	311.7 ± 36.3	557.3 ± 43.4
30–60 cm	5.3 ± 0.0	95.2 ± 10.1	197.5 ± 2.6	722.5 ± 7.3
60–90 cm	1.3 ± 0.0	28.8 ± 0.2	179.2 ± 9.7	826.8 ± 10.6
MTF				
0–30 cm	17.7 ± 2.4	285.5 ± 14.4	203.3 ± 1.4	500.7 ± 9.4
30–60 cm	9.0 ± 2.8	251.5 ± 7.8	223.7 ± 9.9	534.0 ± 3.8
60–90 cm	3.8 ± 0.7	153.5 ± 2.1	190.0 ± 0.9	716.3 ± 1.4
NT				
0–30 cm	5.3 ± 0.0	70.7 ± 3.3	232.7 ± 0.5	695.3 ± 4.2
30–60 cm	4.2 ± 0.2	45.7 ± 2.4	199.2 ± 0.2	764.7 ± 10.4
60–90 cm	1.7 ± 0.0	19.0 ± 3.8	172.0 ± 10.8	826.0 ± 7.1
NTF				
0–30 cm	8.7 ± 0.9	192.3 ± 2.8	239.0 ± 2.4	563.5 ± 4.5
30–60 cm	4.2 ± 0.2	171.8 ± 8.2	260.0 ± 2.4	559.3 ± 21.7
60–90 cm	6.7 ± 0.5	269.5 ± 4.0	174.0 ± 4.7	575.3 ± 10.4

CT: Conventional tillage. CTF: Conventional tillage fertilized. MT: Minimum tillage. MTF: Minimum tillage fertilized. NT: No tillage. NTF: No tillage fertilized.

On average, 70% to 75% of the soil fractions consisted of fine silt and clay (<20 μm). The A, B, and C fractions decreased with depth (Table 1), while the D fraction showed an inverse trend with the only exception being the deepest layer of NTF treatment. The quantity of the smallest fraction (<20 μm) increased with depth and ranged from 500 to 867 mg kg^{-1}.

The fertilized plots resulted in the highest amount of B fraction and the lowest amount of D fraction with respect to the unfertilized ones. Since microaggregates are the result of microbial decomposition of SOM from the macroaggregates [9,29], the highest amount of B fraction in fertilized soils could derive from the higher microbial activity promoted by the same fertilization. For example, Liao et al. [30] found the higher fungal abundance in microaggregates (250–53 μm) regardless of the type of fertilization.

Table 2. Analysis of variance and mean values of the amount of each soil fraction subdivided by soil depth, tillage, and fertilization treatment. The standard deviation is in parentheses.

	Dry Matter (g kg^{-1})			
Size Fractions	A (>250 um)	B (250–53 um)	C (53–20 um)	D (<20 um)
Depth	***	**	**	***
Tillage	**	**	n.s.	**
Fertilization	**	***	n.s.	***
Depth				
0–30	0.28 b (0.12)	4.67 b (2.30)	7.04 b (2.01)	18.05 a (2.54)
30–60	0.15 a (0.06)	3.92 ab (2.37)	6.41 b (0.80)	19.81 b (2.96)
60–90	0.08 a (0.05)	2.62 a (2.94)	4.92 a (0.68)	23.23 c (3.15)
Tillage				
NT	0.15 a (0.06)	3.84 ab (2.80)	6.38 a (1.05)	19.92 a (3.03)
MT	0.23 b (0.17)	4.68 b (2.79)	6.52 a (2.12)	19.28 a (3.93)
CT	0.13 a (0.07)	2.69 a (2.03)	5.47 a (1.20)	21.88 b (3.16)
Fertilization				
No	0.13 a (0.08)	1.77 a (1.14)	6.15 a (1.93)	22.37 b (2.90)
Yes	0.21 b (0.14)	5.70 b (2.18)	6.10 a (1.12)	18.35 a (3.02)

CT: Conventional tillage. MT: Minimum tillage. NT: No tillage. The values in each column followed by a different letter are significantly different according to Tukey's test. n.s.: not significant. *** significant at the $P \leq 0.001$.

With regard to the tillage, the highest amount of D fraction and the lowest amount of B fractions were obtained from CT soils, which is likely due to the major physical disturbance and microbiological activity induced by the conventional tillage that increased macro-aggregates and micro-aggregates turnover [5].

Table 3 shows the analysis of variance and mean values of OC content of soil fractions, as affected by soil depth, tillage, fertilization, and size fraction. The interactions among these parameters were not significant (data not shown) except the one between the OC content of each soil fraction and soil depth ($P \leq 0.001$) since, as expected, the OC content of all fractions decreased with depth.

The significant decrease of the OC content of all fractions from the upper layer (on average, 125.9 mg OC kg^{-1} fraction) to the deepest one (54.5 mg OC kg^{-1} fraction) resembles the common stratification of SOC along the profiles. The highest value of OC was found in the D fraction due to the entrapment of organic components in the finest fractions of soil, as reported by Gregorich et al. [31]. This highlighted the role of clay particles in the OM stabilization due to their high specific surface area and charge. In fact, clay minerals are considered the most active constituents in the formation of organo-mineral complexes [32] and are responsible for long-term preservation of soil OM, even over millennia [33].

No kind of tillage significantly affected the OC content of each fraction likely due to the balance in the soil achieved because of the long-term experiment, as reported by Rita et al. [34] who found no significant difference in OC fraction content among several 30-year-old land-use trials. In addition, the main OM input, which is the above-ground crop residues, has been removed from the field at the end of the crop cycles in all treatments. With regard to the latter topic, it has been demonstrated that the aboveground crop residues are important for building up soil fertility not only as input of OM, but also because they cover the soil during the hot weather, which conserves SOM [35].

Table 3. Analysis of variance and mean values of the OC in soil fractions, subdivided by soil depth, tillage, fertilization treatment, and size of fractions. The standard deviation is in parentheses.

	Organic Carbon (mg kg^{-1})
Depth	***
Tillage	n.s.
Fertilization	n.s.
Size	***
Depth (cm)	
0–30	125.9 c (10.9)
30–60	89.8 b (7.8)
60–90	54.5 a (4.7)
Tillage	
NT	94.9 a (8.2)
MT	81.8 a (7.1)
CT	93.5 a (8.1)
Fertilization	
No	92.8 a (6.6)
Yes	87.3 a (6.2)
Size	
A	7.7 a (0.8)
B	22.0 ab (2.2)
C	54.8 b (5.5)
D	275.7 c (27.6)

CT: Conventional tillage. MT: Minimum tillage. NT: No tillage. A (>250 μm). B (250–53 μm). C (53–20 μm). D (<20 μm). The values in each column followed by a different letter are significantly different according to Tukey's test. n.s.: not significant. *** Significant at the $P \leq 0.001$.

Lastly, the fractional OC content did not differ between fertilized and not fertilized plots since the adopted fertilization was only inorganic and rather low.

3.2. Effects of Treatments on the Spectroscopic Properties of Each Soil Fraction

Figure 2 shows the DRIFT spectra of different soil fractions under various tillage.

The peak at about 3623 cm^{-1} can be ascribed to the O-H vibration in the octahedral layers of 2:1 and/or 1:1 silicates [36]. The same peak can be highlighted by removing the SOM through an appropriate procedure [37]. This peak was observed in B, C, and D fractions regardless of the type of tillage, with a slight increase of the relative intensity as the fraction size decreased. This was in line with other papers [38–41] showing that phyllosilicates (kaolinite, chlorite, smectite, illite) are the main components of the clay fraction of soils. The peak at 2927 cm^{-1}, ascribed to the stretching of the aliphatic C-H group, was evident only in the A fraction, and could be due to the signal of organic matter consisting of labile plant residues [42]. Additionally, in this case, this peak can be highlighted by removing minerals through HF treatment [43]. The peak at about 2517 cm^{-1} can be attributed to the CO_3 stretching and calcite bending, as indicated by peaks at about 1450, 867, and 698 cm^{-1} [15,44]. These peaks, especially those at 1450 and 867 cm^{-1}, were more pronounced in the spectrum of the A fraction. High percentage of calcite in the sand-sized fraction of a Mediterranean soil was also found in a previous work [3] and was ascribed to its lithogenic origin. The peaks at about 1991, 1868, 1793, and 698 cm^{-1} were related to Si-O bending of quartz minerals [15,43,44] as well as the peaks at about 791 cm^{-1} related to the Si-O stretching of quartz minerals [15,43]. Overall, these peaks were slightly more pronounced in all fractions B and C possibly because of the physical breakup of sand size quartz into silt dimension. In contrast, the reduced intensity of the same peaks in D fractions suggested a limited physical alteration of quartz minerals in the finest particles and, therefore, an intermediate stage of soil evolution [45]. The peak at about 1630 cm^{-1} was ascribed to aromatic C=C skeletal vibrations, C=O stretching of quinone and amide groups, C=O of H-bonded conjugated ketones, and it was typical of the organic components [46]. As expected, this peak was absent in the A fraction, appeared in the B fraction, and became more pronounced from fraction C to D.

The presence of the peak linked to aromatic structures in the fraction D could be due to the presence of organic matter involved in the formation of organo-mineral associations and in the coating of the mineral surface by sorption or precipitation processes [47–49]. The broad band at about 1030 cm^{-1} can be ascribed to the stretching of the carbohydrate and polysaccharides-like substances [50]. It was found mainly in the silt-clay and free fine silt plus clay fractions (fraction C and D). Many previous studies have reported high proportions of these compounds in the mineral-associated organic matter fraction [5,51–54]. Polysaccharides of microbial origin mainly bind clay particles by promoting the formation of microaggregates of <50 μm [55]. Glicoproteins of fungal origin, such as glomalin, contain about 85% of sugars and are decomposed very slowly in soil [56]. In contrast, the lower relative intensity of the previously mentioned peak in fraction A suggested the presence of polysaccharides of plant origin responsible for the formation of easily degradable macro-aggregates [56]. The bands at about 529 and 478 cm^{-1} can be ascribed to Si-O-Al and Si-O-Si vibrations, respectively, and are distinctive of phyllosilicates [41]. The relative intensity of the second band decreased in proportion to the size of the fractions. Ndzana et al. [41] reported similar results suggesting that the crystalline structure of phyllosilicates weakened in the finest soil fraction.

DRIFT spectra recorded from NT, MT, and CT samples were similar. Therefore, Figure 3 shows only the DRIFT spectra of each soil-size fraction isolated from the NT treatment along the soil profile. The only slight difference evident in all the soil-size fractions was the peak among 1493 and 1450 cm^{-1}, which increased its relative intensity with depth. This suggests a slight increase of calcite along the soil profile due to dissolution/precipitation phenomena typical of aridic climates and high soil pH [45]. In addition, a slight reduction of the relative intensity of the peak at 1628 cm^{-1} was observed only in the D fraction, according to the reduction of organic matter content with depth.

Figure 4 reports the DRIFT spectra of each soil fraction isolated from the 0–30 layer of the NT treatment fertilized and unfertilized. The spectra of fractions A, B, and C were very similar between the two levels of fertilization. The fraction D of NTF treatment showed a greater relative intensity of the peak at 1027 cm^{-1} compared to NT treatment, possibly due to the greater quantity of polysaccharides coming from microorganisms whose activity is certainly favored by fertilization, as reported by De Mastro et al. a [20].

Figure 5 shows the DRIFT spectra of each soil fraction isolated from the 0–30 layer under NT, MT, and CT practices. Even in this case, the spectra of fractions A, B, and C were more similar regardless of the kind of tillage. The peak at 1027 cm^{-1} showed a greater relative intensity in fraction D of MT and CT treatments compared to NT since the higher aeration of the formers enhanced the microbial activity.
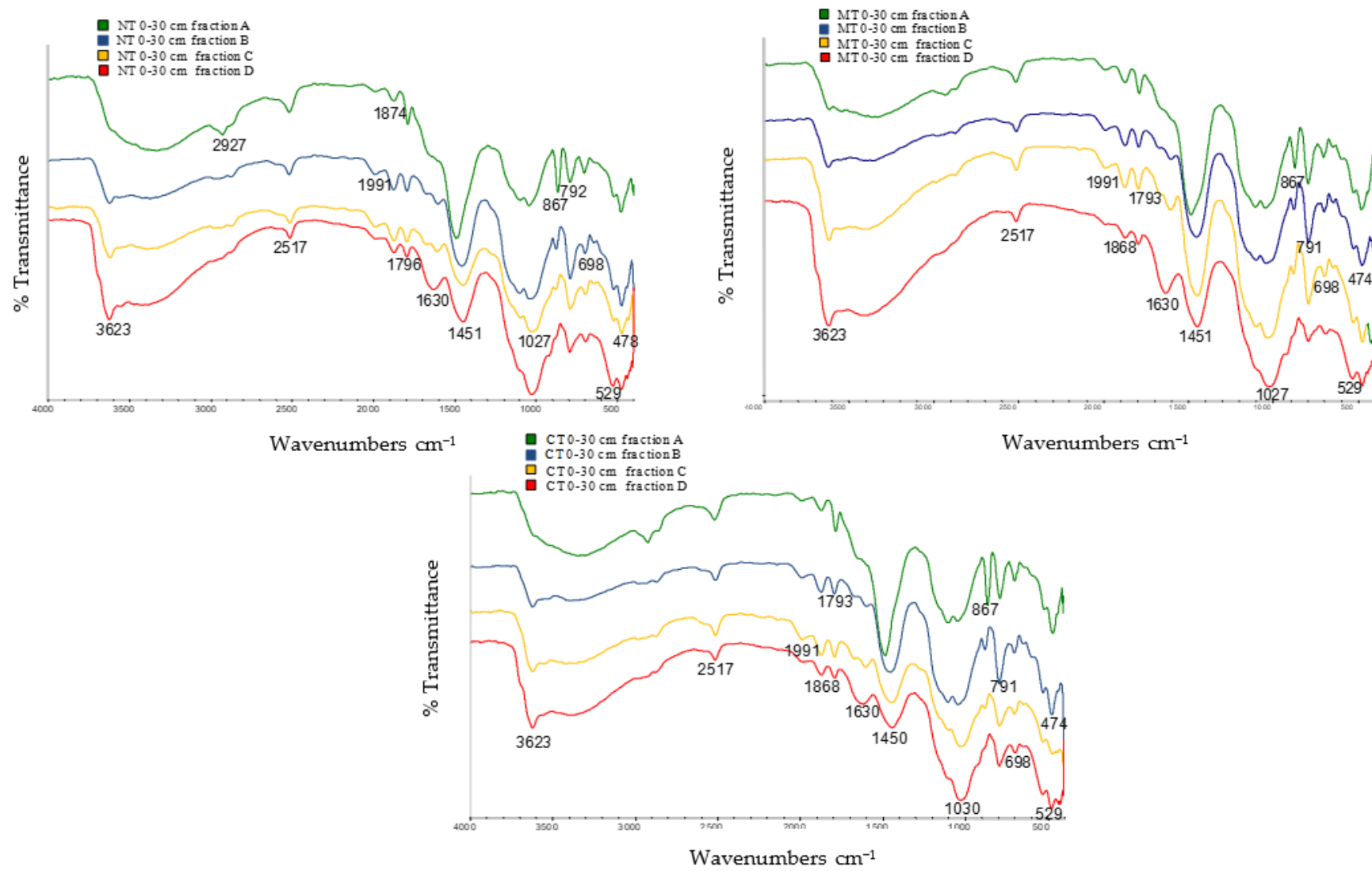
Figure 2. Diffuse reflectance infrared Fourier transform spectra of all soil fractions isolated from different tillage treatments at 0–30 cm of depth.
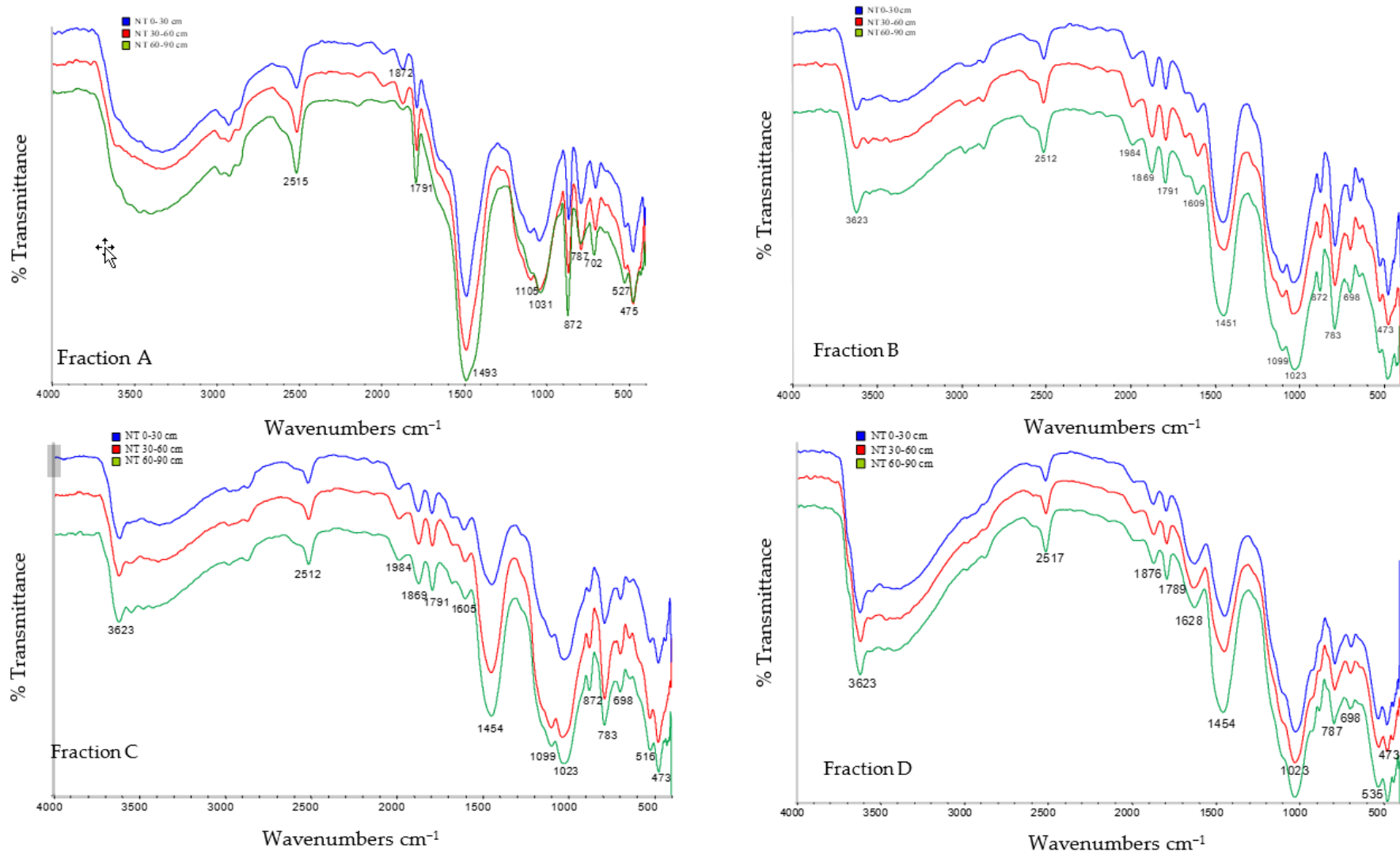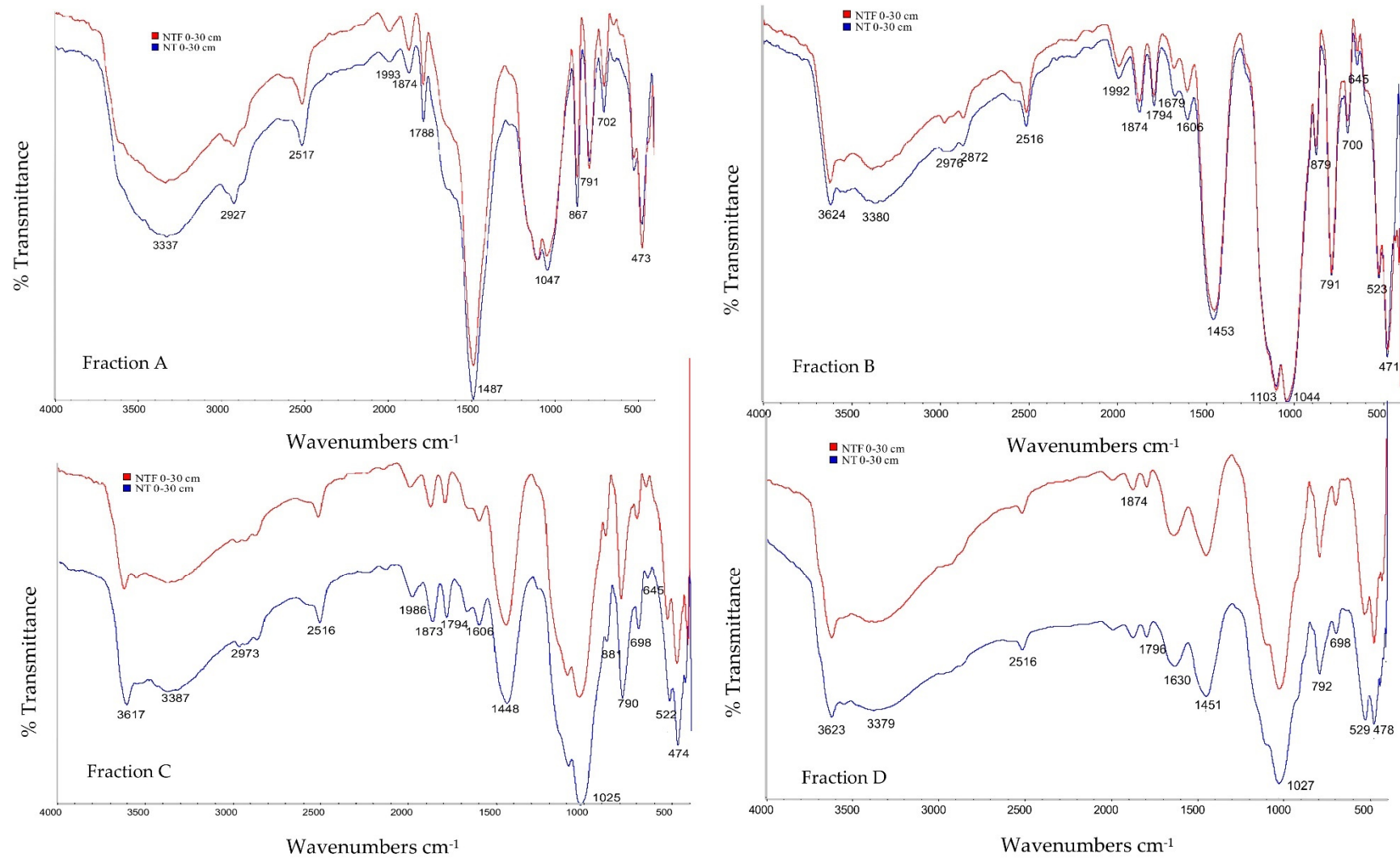
Figure 3. Diffuse reflectance infrared Fourier transform spectra of all soil fractions (A, B, C, and D) isolated from no tillage (NT) treatment at different soil depths.

Figure 4. Diffuse reflectance infrared Fourier transform spectra of the different soil fractions (A, B, C, and D) isolated from no tillage (NT) treatment (0–30 cm) fertilized and not fertilized.
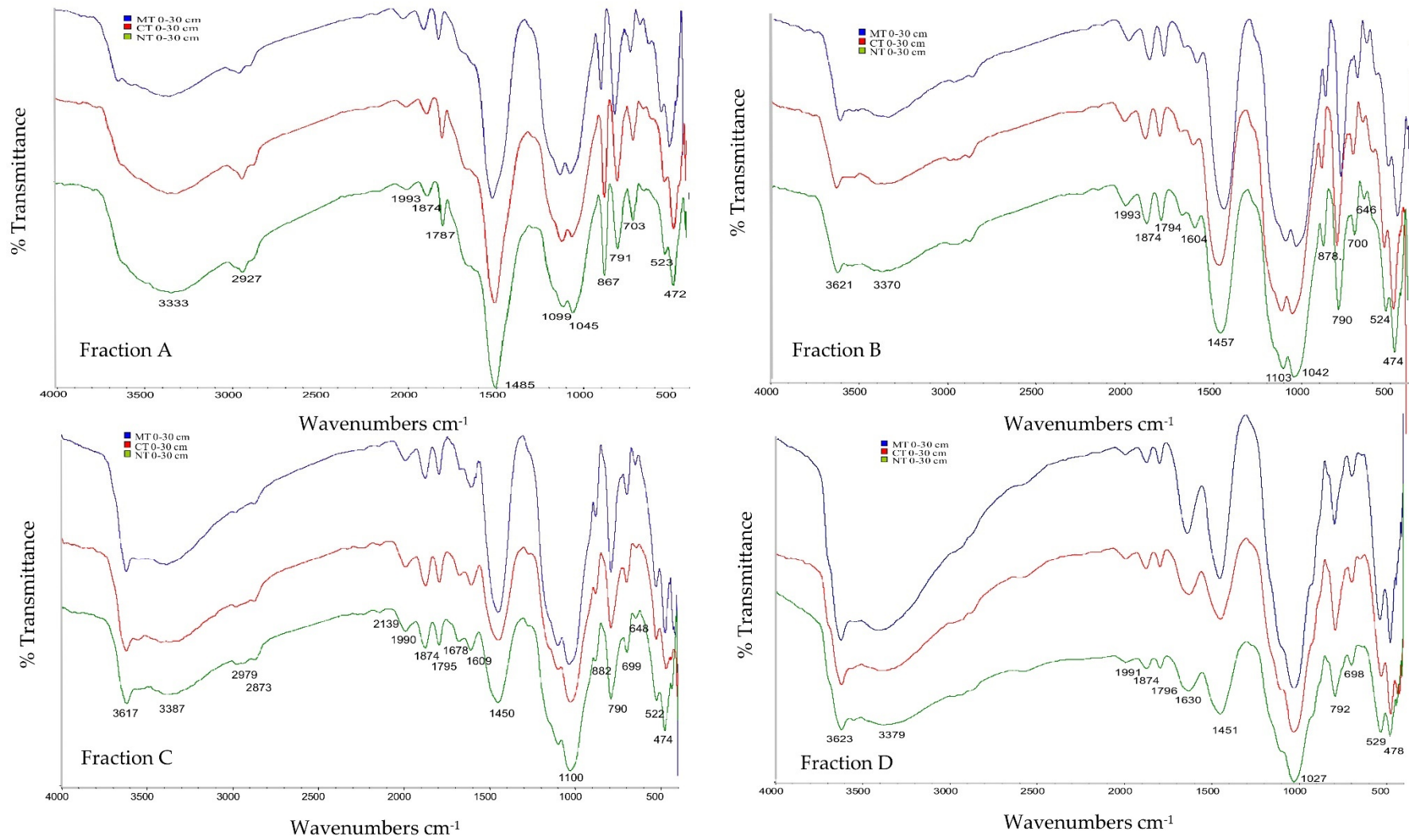
Figure 5. Diffuse reflectance infrared Fourier transform spectra of all soil fractions (A, B, C, and D) isolated from different tillage treatments at 0–30 cm of depth.

4. Conclusions

The soil management influenced the quantity of soil fractions since the CT enhanced the finest ones, whereas the fertilization increased the B fractions, which possibly fuels the development of a microbial community that fosters microaggregate formation. In general, soil depth influenced the amount of each fraction, with higher amounts of fraction A in the upper soil layer and higher amounts of the finest one is more evident in the deepest. The OC content was primarily influenced by fraction size and soil depth since higher OC content was found in the topsoil layer (0–30 cm) and in the finest soil fraction (fraction D), as confirmed by the DRIFT analysis. The different tillage may increase the mass of the soil fractions but not their OC content. However, MT and CT affected positively the quality of the OM stimulating a relative major production of polysaccharides of microbial origin that possibly stabilize the finest size fractions due to their major recalcitrance. The same result is obtained with fertilization. The DRIFT analysis can provide information about the quality of the main minerals present in the different soil-size fractions, since fraction A appeared mostly rich in calcite, fractions B and C appeared mostly rich in quartz, and the finest fraction showed the highest content of phyllosilicates. The different quality of minerals among the soil fractions suggested an early-intermediate stage of weathering and did not change with tillage and fertilization, except for calcite whose relative intensity increased with depth.

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