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Distribution of soil organic carbon between particulate and mineral-associated fractions as affected by biochar and its co-application with other amendments

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A R T I C L E I N F O

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A B S T R A C T

The application of biochar to soils, either alone or combined with other amendments, represents a management practice aimed at storing carbon (C) while enhancing soil fertility. However, the long-term effects of biochar application on soil organic C protection against microbial decomposition are uncertain. This study investigated, in a 9-year-long-term field experiment, the protection of organic C by minerals and iron (Fe) in soils amended with biochar alone or combined with either municipal solid waste compost or sewage sludge. Particulate (not protected by minerals) and mineral-associated organic matter fractions were separated, quantified, and finally the Fe-mediated protection mechanisms were examined by Fe K-edge extended X-ray absorption fine structure spectroscopy. With respect to the unamended control soil, soils amended with biochar, especially when combined with municipal solid waste compost and sewage sludge, had 3 times greater content of particulate organic C. Biochar combined with municipal solid waste compost and sewage sludge also increased mineral-associated organic C content (1.5×), although the magnitude of the effect was smaller than for the particulate organic C fraction. The contents of Fe(III)-organic matter complexes in particulate and mineral-associated organic matter fractions of the amended soils were similar to those of the unamended soils. Hematite represented the main Fe oxide phase in the particulate organic matter fraction of all the soils as well as in the mineral-associated organic matter fraction of the unamended soils, whereas ferrihydrite was more abundant in the mineral-associated organic matter fraction of the amended soils. As a whole, the obtained results in general, and the positive effect on the mineral-associated organic matter possibly mediated by ferrihydrite occurrence in particular, highlight the potential of biochar, alone or in combination with other amendments, as a strategy to store and preserve C in soils.

1. Introduction

Biochar production and application to soils represents a carbon (C) sequestration strategy because of its intrinsic biochemical recalcitrance (Smith, 2016). The application of biochar combined with other amendments can induce positive synergistic effects, creating a soil environment rich in nutrients and stable organic matter (OM) with *terra preta* (*i.e.*, Anthropogenic Dark Earths) properties (Cooper et al., 2020; Fischer and Glaser, 2012; Glaser and Birk, 2012; Khan et al., 2023).

The stabilization and turnover of organic C in agricultural soils depend on both the amendment rate and quality (Sonrsi et al., 2022) and their interaction with the soil mineral matrix, which in turn provides physical and chemical protection against microbial decomposition (Six et al., 2002). Organic C stabilization in soils is also promoted by iron (Fe) (hydr)oxides, and the interaction between poorly crystalline minerals species and soil organic matter (SOM) is widely considered as one of the most important mechanisms for C preservation (Giannetta et al., 2022; Lalonde et al., 2012; Mikutta et al., 2006; Torn et al., 1997). Among Fe mineral phases, short-range-order ferrihydrite represents one of the most important Fe (hydr)oxide promoting organo-mineral associations

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(Eusterhues et al., 2008); this is due to its high specific surface area and reactivity for aromatic organic C (Kaiser, 2003; Riedel et al., 2013).

There have been several studies on biochar application on soils that have reported divergent results. Shi et al. (2021) found that biochar increased the content of the particulate organic C (POC) fraction not protected by minerals, but decreased that of mineral-associated (protected) organic C (MAOC). Cooper et al. (2020) reported an increase of POC and other soil aggregate fractions after biochar application, while municipal solid waste compost only increased the organic C storage in the fraction >53 μm. In a previous study (Plaza et al., 2016), it was found that, eight months after initial application of organic treatments, biochar accumulated organic C mainly in the mineral-free SOM pool, *i. e.*, the fraction not protected by the soil mineral matrix, and enhanced the MAOC content when co-applied with other amendments. In spite of an intense research carried out in the past decade, long-term field studies on the effects of biochar applied with other amendments on SOM stability are still scarce. Given the prominent role of minerals, and particularly of Fe phases, studies pairing SOM fractionation methods and Fe speciation can be particularly helpful to enhance our understanding on these effects.

The objectives of this work were to evaluate the effect of biochar (BC), alone or combined with municipal solid waste compost (MC) or sewage sludge (SS), on the particulate and mineral-associated organic C fractions after 9 years of amendments, and to evaluate the influence of Fe abundance and species on soil C stabilization. We hypothesize that MAOC content in soils will increase due to long-term biochar application and Fe (hydr)oxide-SOM interactions modulated by the newly-added inputs.

2. Materials and methods

2.1. Experiment and soil sampling

The field experiment was established in 2012 and described in Moreno-Jiménez et al. (2016) and Plaza et al. (2016). Briefly, the experiment was set up in the experimental farm "La Poveda", Arganda del Rey, Spain, in an area characterized by a Mediterranean climate. The experiment was a randomized block design consisting of four blocks composed of 3 × 5 m plots (Plaza et al., 2016); the treatments used for this study included unamended control, amendment with biochar at a rate of 20 t ha^{-1} $year^{-1}$, municipal solid waste compost and sewage sludge at rate equivalent to 75 kg ha^{-1} of potentially available nitrogen (to match N crop needs) and the co-amended BC+MC and BC+SS applied at the same application rates used for the single treatments (Fig. 1). Biochar was broadcasted in the fall of 2012, 2013, 2014, 2016 and 2017, while the other amendments were applied in the fall of 2012, 2013, 2014, 2016, 2017, 2019 and 2020 (plots were left fallow in 2015 and 2018). After each application, amendments were immediately incorporated into the top 15 cm of the soil using a rotary tiller. In October 2021 topsoil samples (0–15 cm depth) were collected from each plot.

2.2. Physical fractionation of soil organic matter

Soils were fractionated by size after aggregate dispersion, following the method described by Cambardella and Elliott (1992). Briefly, 10.5 g of soil, sieved at 2 mm, was shaken for 18 h in a 5 g L^{-1} sodium hexametaphosphate [$(NaPO_3)_6$] solution. After dispersion, soil samples were sieved using a vibratory sieve shaker (AS 200, Retsch). The particulate organic matter (POM) represented the fraction remaining on the sieve (>53 μm), while the mineral-associated organic matter (MAOM) represented the fraction that passed through it (<53 μm). All the fractions were oven-dried at 60 °C, weighed and ground with a zirconium ball mill (MM 400, Retsch) for analyses.

2.3. Organic C and total N analysis

Organic C and total N contents of bulk soils and corresponding SOM fractions were determined by dry combustion using an elemental analyzer (CHNS vario MACRO cube, Elementar, Germany). All samples were subject to acid (HCl) fumigation before organic C analysis to remove carbonates (Harris et al., 2001). Hereafter, POC and MAOC acronyms will refer to the organic C contents of the POM and MAOM fractions, as for particulate N (PN) and mineral-associated N (MAN) will refer to the total N contents of the same fractions, respectively.

2.4. Fe extended X-ray absorption fine structure spectroscopy

X-ray absorption spectroscopic analyses of the POM and MAOM fractions were performed at the XAFS beamline at Elettra Sincrotrone (Trieste, Italy). Samples (*ca.* 15 mg) were powdered, pressed into pellets, sealed with Kapton® tape and then mounted in a chamber at room temperature. The transmission mode was used for Fe extended X-ray absorption fine structure (EXAFS) spectra collection. A Si (111) monochromator, calibrated to the first-derivative maximum of the K-edge absorption spectrum of a metallic Fe foil (7112 eV) was used. Linear

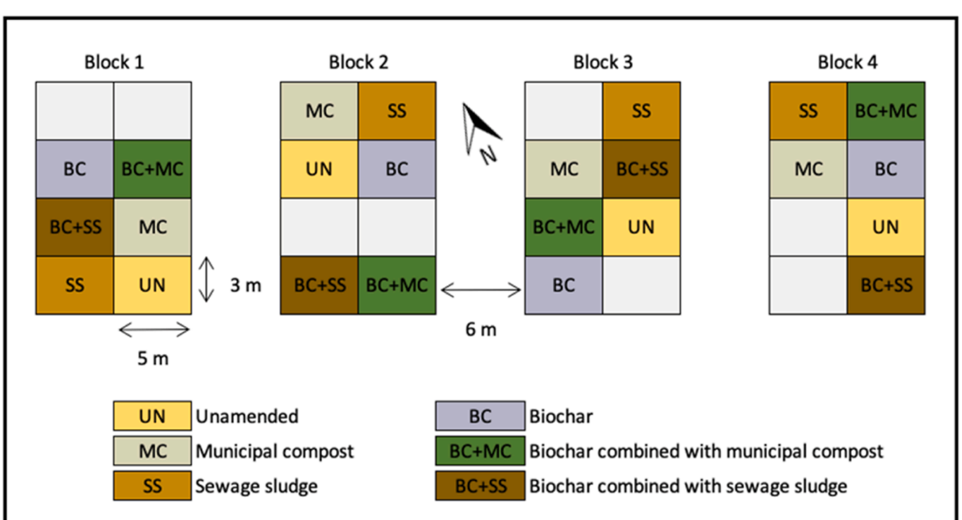

Fig. 1. Schematic representation of the experimental design. The treatments included the soil unamended (UN), amended with municipal solid waste compost (MC), sewage sludge (SS), with or without biochar (BC).

combination fitting analyses of k^3-weighted Fe and EXAFS spectra were performed over a k-range of 2–10 $Å^{-1}$. EXAFS data were processed using the software Athena (Ravel and Newville, 2005).

2.5. Data analysis

Treatment effects were examined by linear mixed effects models, with an intercept random structure and block as the random factor. Tukey tests at the 0.05 level were used for pairwise comparisons of marginal means. All data analyses were performed using R version 4.1.1 (R Core Team, 2021).

3. Results and discussion

3.1. Organic C and total N content in fractions

Nine years after the beginning of the experiment, soils amended with municipal solid waste compost, sewage sludge and biochar applied alone had significantly greater organic C contents compared to the control soil. The organic C contents of the BC+MC and BC+SS amended soils was about 2-fold higher than that of the unamended one (Fig. 2a). Compared to the control soil, the treatment with biochar co-applied with other amendments (BC+MC and BC+SS) significantly increased POC content by 3.5 and 2.9, and by 2.4 and 3.2 times, in 2021 and 2013, respectively. Therefore, during the 9-year experiment, the POC content overall increased by 1.3 times in biochar-amended soil and by 1.8 times in the BC+SS treatment. Compared to the unamended soil, the treatment with BC+MC significantly increased the MAOC content by 1.6 and 1.2 times in 2021 and 2013, respectively. Differently from Shi et al. (2021), whose results showed a decrease in MAOC following biochar addition, in the present study the same fraction increased over time by 1.7 times in both the soil amended with biochar alone and co-applied with compost (Fig. 2a and S1a).

The ratio between particulate and mineral-associated organic C ranged from 0.65 in the control soil to 1.9 in the biochar-amended soils in 2013, after only eight months from the first amendment application (Plaza et al., 2016; Figure S1a), and from 0.74 in the control soil to 1.6 in BC+MC in 2021, after 9 years (Fig. 1a). Nevertheless, the POC/MAOC ratio in the biochar-amended soils decreased from 1.9 in 2013 to 1.5 in 2021.

Thus, after 9 years of amendments, both biochar and other amendments significantly increased POC content, confirming results from Grunwald et al. (2017) who found that 80% of the total biochar-C occurred in the free light fraction. At the same time, the results from this long-term study indicate that the cumulative application of biochar and other amendments played an important role for C sequestration in the MAOC along time. Previous results showed that, after eight months from its first application, biochar stimulated the microbial transformation of the more labile organic fractions applied with other amendments and the consequent sorption of those microbial by-products on soil mineral surfaces (Plaza et al., 2016; Zaccone et al., 2018); this effect is more evident after 9 years.

While biochar had no effects on soil N contents, soils amended with sewage sludge and in particular with compost showed a significant increase in total N content (Fig. 2b). This was expected, since N content of biochar was low compared to those of the municipal solid waste compost and sewage sludge (Plaza et al., 2016). After 9 years of cumulative amendments, particulate N increased by 2.4 times in both soils amended with municipal solid waste compost and BC+MC compared to the control, and to a lesser extent in BC+SS and SS; no significant variations were observed in biochar-amended soils. The sign of these effects was similar but the extent was smaller in 2013 (Plaza et al., 2016; Figure S1b). Compared to the unamended soil, mineral associated N content was also significantly greater (1.4 times) both in municipal solid waste compost and BC+MC treated soils (Fig. 2b); like for the particulate N fraction, this trend was more evident than that observed 8 months after the first application of amendments (Plaza et al., 2016; Figure S1b). Municipal solid waste compost contains N-rich organic compounds that have a strong affinity for minerals, thus leading to an accumulation of C and N in the MAOM fraction (Samson et al., 2020).

The C/N ratio of bulk soil (C/N) and POM fractions (POC/PN) was significantly higher in soils amended with biochar, alone or combined with sewage sludge and municipal solid waste compost, with respect to the unamended soil (Fig. 2c). In particular, the application of municipal solid waste compost and sewage sludge decreased both the C/N and POC/PN ratios, whereas no effects were observed for the MAOC/MAN ratio independently of the different treatments (Fig. 2c).

Consistently with the present results, Sun et al. (2022) found that both biochar and straw application increased MAOC contents after nine successive maize crop seasons. Similarly, Greenberg et al. (2019) found that the combined application of digestate and biochar increased not only the occlusion of biochar into aggregates, but also the association with minerals, 14 months after the application into soil.

The present findings suggest that the application of both biochar and other amendments represents an effective strategy to increase in the long-term not only soil POC but also MAOC and total N. This is extremely important in light of future climate change scenarios (Rubin et al., 2023) as increasing temperature and rainfall reduction do not interact significantly with biochar effects on particulate and mineral-associated organic C contents (Giannetta et al., 2023). Biochar application improved the formation of organo-mineral interaction, contributing to stabilize other SOM sources like compost and sewage sludge. Moreover, other studies demonstrate that biochar can alter soil microenvironment by enhancing the growth and diversity of microbial communities (Siedt et al., 2021), with a consequent increase of functional microbial interactions that play a key role in the regulation of soil capacity to sequester C (Yu et al., 2018). However, more research on the long-term impact of biochar on soil microbial communities, and on the C storage in the MAOM fraction, is still needed (Wang et al., 2022).

3.2. Iron species

Linear combination fitting results revealed the four most representative species identified as major components by the fits (Table 1). The Fe EXAFS data fitting showed that Fe phases in POM and MAOM fractions of unamended soils mainly differed in Fe(III)-SOM content (12% and 31% in POM and MAOM, respectively). The contents of Fe(III)-SOM in the POM and MAOM fractions of the amended soils (12–17% and 27–38%, respectively) were rather comparable to those of the unamended soil.

In POM fractions, hematite represented the main Fe oxide phase in both unamended and amended soils (11–13%). In the MAOM fractions, hematite was also present (6–10%). However, except for the unamended soil, ferrihydrite was the main Fe oxide phase (22, 32, and 8% in soil amended with biochar, municipal solid waste compost, and with both, respectively).

Biochar can alter the interaction between native soil organic C and mineral particles and compete with native C for the surface binding sites, thus resulting in a poor stabilization in the silt and clay fraction (Shi et al., 2021). Most of the studies show how C storage occurs preferentially in unprotected C pools, such as POC (Cotrufo et al., 2019). Nevertheless, since MAOC is theorized as being more stable, storing C as mineral-associated organic C would reduce soil C losses. Moreover, as this C stabilization mechanism occurs through organo-mineral interaction, it is interesting to assess if long-term agricultural practices are affecting the reactive sites of minerals within the soil matrix. Among the reactive sites, interaction with Fe oxides from soil clays are considered as one of the most important mechanisms (Torn et al., 1997). The addition of biochar and other amendments can affect the interaction between the native SOM and the mineral surfaces, and this can be particularly true for minerals having an elevated surface area, like ferrihydrite. In all MAOM fractions from amended soils, a high affinity

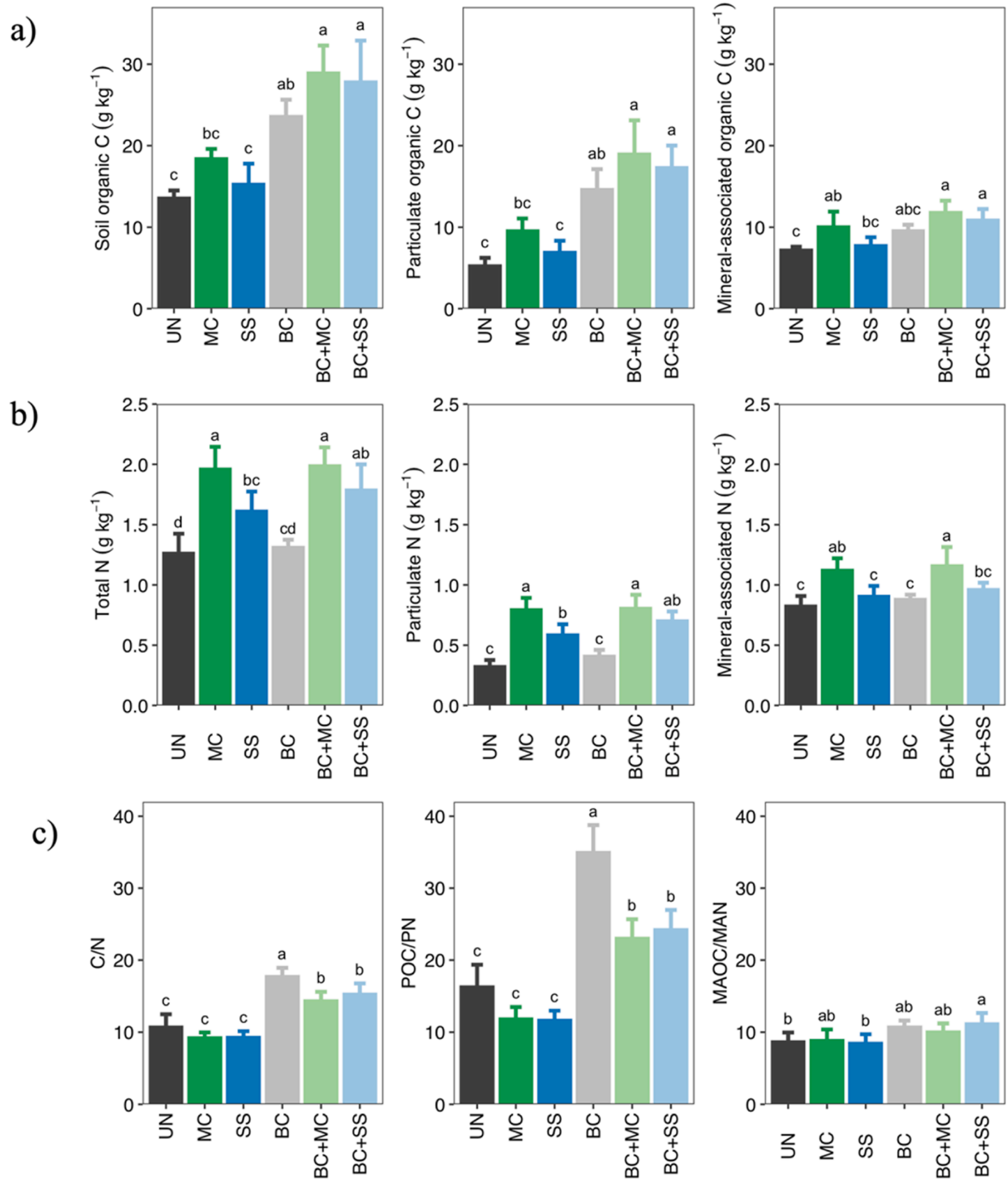

Fig. 2. Concentrations of (a) total, particulate and mineral-associated organic C and (b) total, particulate and mineral-associated N, and (c) C/N ratios in bulk soils and corresponding fractions, under different treatments: (UN) unamended; amended with municipal solid waste compost (MC), sewage sludge (SS), with or without biochar (BC), after 9 years from the first application. Error bars indicate pooled standard error. Different letters indicate statistically significant differences according to Fisher's LSD test at the 0.05 level.

Table 1
Results for the four component linear combination fitting on the k^3- weighted Fe K-edge EXAFS data of particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions in soils under different treatments: unamended (UN); amended with municipal solid waste compost (MC), sewage sludge (SS), with or without biochar (BC). Results are expressed in percentage of the fitting components corresponding to experimental spectra of model compounds.

	Component 1	%	Component 2	%	Component 3	%	Component 4	%	Sum
UN POM	Chlorite	23	Illite	54	Hematite	11	Fe(III)-SOM	12	100
MC POM	Chlorite	21	Illite	52	Hematite	13	Fe(III)-SOM	14	100
BC POM	Chlorite	20	Illite	57	Hematite	11	Fe(III)-SOM	12	100
BC+MC POM	Chlorite	21	Illite	51	Hematite	11	Fe(III)-SOM	17	100
UN MAOM	Chlorite	4	Illite	58	Hematite	6	Fe(III)-SOM	31	100
MC MAOM	Chlorite	20	Ferrihydrite	32	Hematite	9	Fe(III)-SOM	38	100
BC MAOM	Illite	43	Ferrihydrite	22	Hematite	7	Fe(III)-SOM	28	100
BC+MC MAOM	Illite	56	Ferrihydrite	8	Hematite	10	Fe(III)-SOM	27	100

between ferrihydrite and aromatic C is expected, probably due to the adsorption of coprecipitation of the carboxyl groups attached to aromatic rings (Eusterhues et al., 2011). Over time, the amorphous Fe phases are retained on the biochar surfaces by a strong organo-mineral association. The key role of short-range-order minerals for C stabilization, together with biochar application, should be corroborated taking in consideration not only the diversity of biochars, but also field conditions and long-term experiments. In a previous work (Giannetta et al., 2020), the Fe phases occurring in the fine sand and in the fine silt and clay fractions from the same agricultural soil used in the present study have been identified. The application of biochar and other amendments resulted in an increase of Fe-SOM complexes and a decrease of ferrihydrite in the fine sand fraction. In contrast, the percentage of ferrihydrite negligibly changed in the amended fine silt and clay fractions. The presence of ferrihydrite only in the MAOM fraction of amended soils indicated that, after 9 years, C storage in the MAOM fraction is mainly related to the presence of this Fe oxide form.

4. Conclusions

Long-term cumulative applications of biochar and other amendments exerted a positive effect on organic C storage. Even though biochar mainly accumulated C in the POM fraction after 9 years, it also increased the MAOM pool especially when applied in combination with other amendments (*i.e.*, municipal solid waste compost, sewage sludge). Overcoming the limitation to study samples collected from a single experimental area, future studies including other agroecosystems and amendments (including different types of biochar), a larger gradient of pedoclimatic conditions and cropping rotations might be needed. The present results showed that ferrihydrite plays a fundamental role in the organic C stabilization within the MAOM fraction, at least in these specific pedoclimatic conditions. Thus, long-term diversified C sources coming from biochar and other amendments represent a valid and sustainable agricultural practice to improve C storage, through the formation of organo-mineral interactions, while recycling waste biomass.

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

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2023.108777.

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