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Different N-fertilization sources affecting the native soil organic matter mineralization on Technosols under iron ore tailing

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ABSTRACT: Accidents related to the rupture of iron ore tailings dams have dramatically impacted on the Brazilian natural ecosystem. So, the development of strategies to recover soil organic matter levels and build-up Technosols are required. This study aimed to evaluate the effect of N-mineral and -organic fertilization and the rhizospheric effect on soil organic matter mineralization from Technosols built under iron ore tailings. The experiment was carried out in a greenhouse; we used a factorial scheme 2 × 2 × 3: without and with the plant *Crotalaria juncea*; without and with mineral fertilization; and three different organic fertilization (without organic fertilization, sewage sludge, and household waste composting), with four randomized blocks. The experimental units were PVC columns installed with a top layer of iron ore tailing from a dam, where gases were sampled to determine the CO_2-C, CH_4-C concentrations, and CO_2-$\delta^{13}C$. The organic fertilization promoted a positive priming effect of 184 %. However, with mineral fertilization, no effect was observed in soil derived-CO_2-C accumulated and priming effect. Similarly to non-planted soil, the planted soils with mineral and organic fertilization promoted a steady reduction in soil derived-CO_2-C accumulated and rhizosphere priming effect. The soil with mineral fertilization improved in 5.52 and 0.45 g per column the shoot and root dry matter of Crotalaria when compared to the soil without mineral fertilization. In summary, the organic fertilization in Technosols built over iron ore tailings dams during revegetation was not enough to ensure the plant development and preservation of soil organic matter. However, the combination of mineral and organic fertilization promoted negative rhizosphere priming effect and a significant increase in plant biomass.

Keywords: priming effect, CO_2-C emissions, sewage sludge, household waste composting, *Crotalaria juncea.*

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INTRODUCTION

Brazil was responsible for 19.6 % of the iron ore world production in 2018 (U.S. Geological Survey, 2019), which highlights the importance of this activity for the Brazilian economy (Ibram, 2019). Nevertheless, the iron ore mining activity has environmental impacts, since the ore extraction process promotes the entire reshaping of the landscape and a large production of iron ore tailing (Guerra et al., 2017). This ore tailing is commonly stored in dams built with coarse material derived from iron ore mining activity (Azam and Li, 2010).

Accidents related to rupture of large iron ore tailings dams (IOTD) in Brazil, as those happened on November 5th, 2015 in Fundão, Minas Gerais (Samarco Company), and January 2019 in Brumadinho, Minas Gerais (Vale Company), had deep impacts on the natural ecosystem and on the security of human communities (Freitas et al., 2019). So, special attention should be paid to promote revegetation of areas affected by the rupture of iron ore tailings dams. Due to the physical, chemical, and biological constraints caused by tailings layer under the soil (Juwarkar et al., 2009), the attempt to build a Technosols (IUSS Working Group WRB, 2014) by topsoil deposition of adjacent areas has been thought as the first step towards reconstituting the minimum conditions required for plant development. Some studies have been focused on the construction of Technosols as a strategy for the recovery of degraded areas (Rivas-Pérez et al., 2016; Forján et al., 2019). The deposition of adjacent topsoil is not sufficient for plant establishment because, despite their excellent physical properties, most tropical soils are nutrient-poor. Thus, the improvement in soil fertility is required and it has been carried out with mineral and organic fertilizers, isolated or in combination, applied to mined soils (Hu et al., 2015; Vilas Boas et al., 2018).

The main goal of recovered strategies in lands affected by IOTD is, ideally, the reestablishment of a self-sustainable systems, so the maintenance and increase of the soil organic matter (SOM) is desirable. As the SOM mineralization is regulated by many microbiological processes, markedly influenced by soil nutrients availability (notably the N) and energy demand, the effect in SOM promoted by N additions (mineral or organic sources) should be understood. Although many studies reported the N availability effect (e.g., N fertilization) in soil CO_2 effluxes, divergent results are observed regarding the positive or negative effect on SOM mineralization (Averill and Waring, 2018; Mason-Jones et al., 2018).

On the other hand, the addition of the energy-rich compounds by organic fertilization (e.g., composts or organic waste) can stimulate the soil microbial activity and, consequently, increase the soil derived-CO_2 effluxes (“native SOM” mineralization), a phenomenon well-documented and known as *priming effect* (PE) (Kuzyakov, 2010; Mason-Jones et al., 2018). The main mechanisms responsible to induce the priming effect are the N availability (“N-mining hypothesis”) and energy requirement. According to the N-mining hypothesis, the readily available C added to the soil stimulates an N limitation in microorganisms, which mineralize the native SOM to access the N contained within. In summary, the simple N-mineral or -organic fertilization is not enough to ensure adequate SOM management in areas affected by IOTD, and the understanding of these processes need to be explored.

Roots can regulate SOM mineralization through many soil-microbial-plant interactions, often named as rhizosphere priming effect (RPE) (Kuzyakov, 2010; Cheng et al., 2014). The RPE is commonly reported as the competition between plant roots and soil microorganisms for nutrients such as N (Kuzyakov and Xu, 2013) since the readily available C released by the plant (rhizodeposits) supplies the initial microbial energy and C requirement but promotes an N limitation that is offset by SOM mineralization (“positive RPE”) (Kuzyakov and Xu, 2013). Otherwise, in N-rich soils, the supply of readily available C released by plant switch the native SOM mineralization to utilize the new C and mineral N available (“negative RPE”) (Cheng et al., 2014).

Independently of the N sources applied in the soil (mineral or organic), the N supply to plants will affect C allocation above- or below-ground and hence the SOM mineralization (Zang et al., 2016). It follows that to the recovery strategies definition of areas affected by IOTD should be considered the many intricate interactions around plant-microbe-soil for the self-sustainable system establishment, which are scarcely understood. So, in this study, we intend to test the hypothesis that the supply of N to plants via N-organic fertilization in Technosols as a soil recovery strategy relieves SOM mineralization in comparison to N-mineral fertilization.

To achieve this goal, we studied the effect of N-organic fertilization on SOM decomposition without the presence of plant, and the rhizospheric effect stimulating SOM decomposition with different fertilization (N-organic, N-mineral, or N-mineral + N-organic). The novelty of our study is to understand the intricate relation of SOM decomposition during the different recovery strategies of areas affected by iron ore tailings.

MATERIALS AND METHODS

Experiment set up

The experiment was carried out in a greenhouse according to a factorial scheme 2 × 2 × 3 [without (-Plant) and with the plant (+Plant); without (-MNF) and with mineral fertilization (+MNF); and three levels of organic fertilization – without (NONF), sewage sludge (SS), and household waste composting (HWC)], with four randomized blocks.

The experimental units were PVC columns (0.55 m height and 0.10 m diameter; Figure 1) filled with 0.30 m of iron mining tailings samples (2.36 dm^3; Table 1), 0.10 m of topsoil samples (0.78 dm^3; Table 1), and organic fertilization (no fertilization, sewage sludge, or household waste composting; Table 2) under topsoil surface. The iron mining tailing

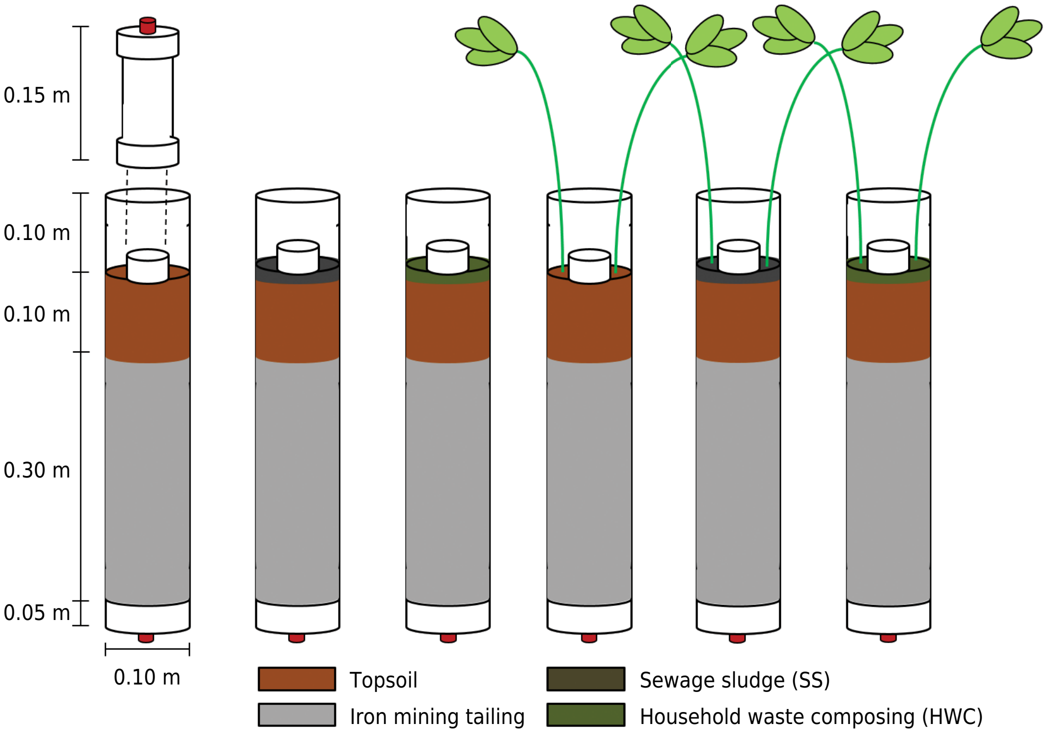

Figure 1. Scheme of experimental units (PVC columns) with the iron mining tailing, topsoil, sewage sludge (SS) or household waste composting (HWC), and plants (*Crotalaria juncea*). The PVC chambers (0.10 × 0.05 m of height and diameter, respectively) were installed centrally on the soil surface, while PVC caps (0.15 × 0.05 m of height and diameter, respectively) fitted with a central rubber septum were used to collect the gas.

samples were collected in a mining tailings dam (20° 24’ 46” S; 43° 50’ 21” W) located in Ouro Preto, Minas Gerais State, Brazil. The samples were air-dried, passed through a 4 mm mesh sieve, homogenized, and subjected to compaction to obtain a bulk density similar to the natural field condition (2.1 g cm^{-3}). The topsoil samples (0.00-0.20 m soil layer) were collected from a *Latossolo Vermelho Amarelo Distrófico típico* (Santos et al., 2018), which corresponds to a Hapludox (Soil Survey Staff, 2014), located in the southeastern part of the state of Minas Gerais - Brazil (20° 52’ 31.1” S; 42° 58’ 21.6” W, 730 m a.s.l.). This area was cultivated with *Urochloa brizantha* for over 20 years (so the SOM was predominantly derived from this C_4 photosynthesis plant; $\delta^{13}C_{PDB}$: -16.75 ‰). The coarse plant debris visible to the naked eye was manually removed from the soil samples, and the soil was air-dried and passed through a 2-mm mesh sieve.

The amount of 5.24 Mg ha^{-1} of sewage sludge (C: 21.81 %; N: 2.86 %; $\delta^{13}C_{PDB}$: -26.57 ‰) and 30 Mg ha^{-1} of household waste composting (C: 18.27 %; N: 0.5 %; $\delta^{13}C_{PDB}$: -23.47 ‰) applied under the topsoil were those necessary to achieve a level of 150 kg ha^{-1} of N, while in the mineral fertilization treatments, 150 mg dm^{-3} of NH_4NO_3-N were applied.

Table 1. Chemical and physical properties of topsoil and mining tailing

Properties	Topsoil	Mining tailing
Chemical properties		
$pH(H_2O)$	5.65	7.30
Al^{3+} ($cmol_c$ dm^{-3})	0.00	0.00
Ca^{2+} ($cmol_c$ dm^{-3})	1.76	0.79
Mg^{2+} ($cmol_c$ dm^{-3})	1.23	0.02
K^+ ($cmol_c$ dm^{-3})	85.00	19.20
P (mg dm^{-3})	3.60	8.26
S (mg dm^{-3})	2.50	17.80
Fe (mg dm^{-3})	196.90	52.78
Zn (mg dm^{-3})	4.56	1.51
Mn (mg dm^{-3})	87.50	68.70
Cu (mg dm^{-3})	1.60	0.99
CEC ($cmol_c$ dm^{-3})	9.51	1.13
Cr (mg dm^{-3})	0.00	0.78
Ni (mg dm^{-3})	0.00	1.04
Cd (mg dm^{-3})	0.00	0.08
Pb (mg dm^{-3})	0.00	0.10
SOC (dag kg^{-1})	3.01	0.09
TN (dag kg^{-1})	0.20	0.00
Physical properties		
Coarse sand (g kg^{-1})	410	270
Fine sand (g kg^{-1})	110	330
Silt (g kg^{-1})	170	350
Clay (g kg^{-1})	310	50
Dp (Mg m^{-3})	2.80	3.16
Ds (Mg m^{-3})	0.98	2.10

$pH(H_2O)$ at a soil:solution ratio of 1:2.5 (Donagema et al., 2011). Exchangeable Ca^{2+}, Mg^{2+}, and Al^{3+} in KCl 1 mol L^{-1}; available P, K, Fe, Zn, Mn, and Cu extracted by Mehlich-1; S extracted by Monocalcium phosphate in acetic acid; CEC: cation exchange capacity measured with calcium acetate 0.5 mol L^{-1} at pH 7.0; SOC: soil organic carbon - Walkley-Black (Yeomans and Bremner, 1988); TN: total nitrogen - Kjeldahl digestion (Bremner and Mulvaney, 1982); particle size analysis with the pipette method (Ruiz, 2005); Dp: soil particle density and D_S: soil bulk density. Cr, Ni, Cd, and Pb extracted by acid digestion - EPA3052 (USEPA, 1996).

A solution containing 300 mg dm^{-3} of P; 150 mg dm^{-3} of K; 40 mg dm^{-3} of S; 0.81 mg dm^{-3} of B; 0.15 mg dm^{-3} of Mo; 1 mg dm^{-3} of Cu; and 4 mg dm^{-3} of Zn (Novais et al., 1991) was applied on the topsoil of each treatment. The treatments that received organic fertilization did not receive mineral N fertilization. The NH_4NO_3-N dose was divided into three coverage applications, while the K dose was applied together on sowing fertilization. Subsequently, six crotalaria (*Crotalaria juncea*) seeds were sowed, and after germination thinning was performed, leaving two plants per column along with 78 days.

Gases collection and analyses

We performed 15 gases sampling during the experimental period (0, 5, 9, 12, 16, 19, 23, 26, 33, 40, 47, 54, 61, 68, and 78 days after sowing) using static PVC chambers installed into the columns (Figure 1). The PVC chambers were installed centrally in the soil surface, reaching a depth of 0.05 m. At the moment of each gas sampling, the PVC chambers were sealed with a PVC cap fitted with a central rubber septum. The atmosphere temperature was measured using an EC-5 sensor. Four atmospheric gas samples were collected over time (0, 3, 6, and 9 min) using syringes (60 mL) equipped with three-way valves. Tests were performed and indicated that 9-min was sufficient to adjust the CO_2-C fluxes to the linear model (R^2: 0.99).

After the gas sampling, the syringes were brought to the laboratory, and the CO_2-C, CH_4-C concentrations, and CO_2-$\delta^{13}C$ were determined using the Cavity ring-down laser spectroscopy (CRDS) equipment. The soil CO_2-C and CH_4-C fluxes were calculated according to Smith and Conen (2004) (Equation 1):

$$\text{Soil } CO_2\text{-C or } CH_4\text{-C flux} = [(\Delta Q/\Delta t) \times M \times P \times V] / (R \times T \times A) \qquad \text{Eq. 1}$$

in which the *Soil* CO_2-*C or* CH_4-*C flux* is the CO_2-C or CH_4-C flux (μg m^{-2} min^{-1}); $\Delta Q/\Delta t$ is the angular coefficient (ppm min^{-1}) obtained by adjusting gas concentration over the gas sampling

Table 2. Chemical properties of sewage sludge and household waste composting

Properties	Sewage sludge	Household waste
pH(H_2O)	4.25	6.57
Al (g kg^{-1})	8.11	6.68
Ca (g kg^{-1})	1.58	22.40
Mg (g kg^{-1})	0.19	2.06
Na (g kg^{-1})	77.69	840.17
K (g kg^{-1})	0.11	1.19
P (g kg^{-1})	1.89	0.63
S (g kg^{-1})	1.66	0.97
Fe (g kg^{-1})	10.93	8.17
Zn (mg kg^{-1})	164.95	8.84
Mn (mg kg^{-1})	22.25	571.27
Cu (mg kg^{-1})	76.01	3.90
Cr (mg kg^{-1})	2.80	14.75
Ni (mg kg^{-1})	5.47	8.37
Cd (mg kg^{-1})	0.20	0.00
Pb (mg kg^{-1})	6.11	0.00
C (%)	21.81	18.27
N (%)	2.86	0.50

pH(H_2O) at a solid:solution ratio of 1:2.5 (Donagema, 2011); Al, Ca, Mg, Na, K, P, S, Fe, Zn, Mn, Cu, Cr, Ni, Cd, and Pb extracted by acid digestion EPA3052 - (USEPA, 1996); C: carbon - Walkley-Black (Yeomans and Bremner, 1988); N: nitrogen - Kjeldahj digestion (Bremner and Mulvaney, 1982).

time; M is the molar mass of C; P is the pressure inside the chamber, it was assumed to be 1 atm; V is the chamber volume (L); R is the universal gas constant (0.0821 L atm K^{-1} mol^{-1}); T is the atmosphere temperature (K); and A is the chamber area (m^2).

The total soil CO_2-C or CH_4-C flux accumulated was calculated according to the Cai et al. (2012) (Equation 2).

$$\text{Total } CO_2\text{-C or } CH_4\text{-C accumulated } (g\ m^{-2}) = \sum_{i=1}^{n} \frac{(F_i + F_{i+1})}{2} \times (t_{i+1} + t_1) \qquad \text{Eq. 2}$$

in which: F is the soil CO_2-C or CH_4-C flux (mg m^{-2} min^{-1}); i is the i^{th} gas sampling; $(t_{i+1} + t_1)$ is the interval (days) between two gas sampling, and n is the total time of gas sampling.

The CO_2-$\delta^{13}C$ from each gas sample over time (0, 3, 6, and 9 min) allowed to calculate the soil CO_2-$\delta^{13}C$ flux according to the Keeling plot method (Keeling, 1958). Due to differences between the $\delta^{13}C$ from topsoil ($\delta^{13}C_{PDB}$: -16.75 ‰) and SS ($\delta^{13}C_{PDB}$: -26.57 ‰), HWC ($\delta^{13}C_{PDB}$: -23.47 ‰) or plant ($\delta^{13}C_{PDB}$: -27.00 ‰), it was possible partitioning the respective CO_2-C contributions to soil CO_2-C fluxes according to Millard et al. (2010) following the equations 3 and 4:

$$f_{\text{SS or HWC}} = [(CO_2\text{-}\delta^{13}C_{\text{+SS or + HWC (-Plant)}} - CO_2\text{-}\delta^{13}C_{\text{-SS or – HWC (-Plant)}}) / (\delta^{13}C_{\text{SS or HWC}} - CO_2\text{-}\delta^{13}C_{\text{-SS or – HWC (-Plant)}}) \qquad \text{Eq. 3}$$

$$f_{\text{Plant}} = [(CO_2\text{-}\delta^{13}C_{\text{+Plant (+SS, -SS + HWC or HWC)}} - CO_2\text{-}\delta^{13}C_{\text{-Plant (+SS, -SS + HWC or HWC)}}) / (\delta^{13}C_{\text{Plant}} - CO_2\text{-}\delta^{13}C_{\text{-Plant (+SS, -SS + HWC or HWC)}}) \qquad \text{Eq. 4}$$

in which: $f_{\text{SS or HWC}}$ is the proportion of soil CO_2-C flux derived from SS or HWC only in the treatments without plant (-Plant); CO_2-$\delta^{13}C_{\text{+SS or + HWC (-Plant)}}$ is the soil CO_2-$\delta^{13}C$ flux from the soil with SS or ST application without plant; CO_2-$\delta^{13}C_{\text{-SS or – HWC (-Plant)}}$ is the soil CO_2-$\delta^{13}C$ flux from the soil without SS or ST application without plant; $\delta^{13}C_{\text{SS or HWC}}$ is the $\delta^{13}C_{PDB}$ of SS (-26.57 ‰) or HWC (-23.47 ‰); f_{Plant} is the proportion of soil CO_2-C flux derived from a plant in the treatments with the plant (+Plant); CO_2-$\delta^{13}C_{\text{+Plant (+SS, -SS + HWC or HWC)}}$ is the soil CO_2-$\delta^{13}C$ flux from the soil +Plant; CO_2-$\delta^{13}C_{\text{-Plant (+SS, -SS + HWC or HWC)}}$ is the soil CO_2-$\delta^{13}C$ flux from the soil –Plant and $\delta^{13}C_{\text{Plant}}$ is -27.00 ‰.

The CO_2-C contributions derived from topsoil to soil CO_2-C fluxes were calculated following equation 5:

$$\text{Soil } CO_2\text{-C flux}_{\text{ Topsoil}} = 1 - [(f_{\text{ SS or HWC}} \text{ or } f_{\text{ Plant}}) \times \text{soil } CO_2\text{-C flux}_{\text{ treat.}}] \qquad \text{Eq. 5}$$

in which the soil CO_2-C flux $_{\text{Topsoil}}$ is the soil CO_2-C flux derived from topsoil and soil CO_2-C flux $_{\text{treat.}}$ is the soil CO_2-C flux from each respective treatment.

Priming effect estimations

The priming effect estimations were performed to organic fertilization and the rhizosphere effect separately according to equations 6 and 7.

$$\text{Priming effect}_{\text{ OF}} (\%) = [(\text{Total soil } CO_2\text{-C flux}_{\text{ Topsoil (+SS or + HWC)}} - \text{Total soil } CO_2\text{-C flux}_{\text{ Topsoil (NONF)}}) / \text{Total soil } CO_2\text{-C flux}_{\text{ Topsoil (NONF)}}] \times 100 \qquad \text{Eq. 6}$$

in which: *Priming effect* $_{\text{OF}}$ is the priming effect promoted by organic fertilization application, calculated only the soil without plants (-Plant); Total soil CO_2-C flux $_{\text{Topsoil (+SS or + HWC)}}$ is the total soil CO_2-C flux derived from topsoil in the soils with the SS or HWC application, and a Total soil CO_2-C flux $_{\text{Topsoil (NOF)}}$ is the total soil CO_2-C flux derived from topsoil in the soils without the SS or HWC application (non-organic-N fertilization – NONF).

$$\text{Rhizosphere priming effect } (\%) = [(\text{Total soil } CO_2\text{-C flux}_{\text{ Topsoil (+Plant)}} - \text{Total soil } CO_2\text{-C flux}_{\text{ Topsoil (-Plant)}}) / \text{Total soil } CO_2\text{-C flux}_{\text{ Topsoil (-Plant)}}] \times 100 \qquad \text{Eq. 7}$$

in which: *Rhizosphere priming effect* is the priming effect promoted by the plant; *Total soil CO_2-C flux* $_{\text{Topsoil (+Plant)}}$ is the total soil CO_2-C flux derived from topsoil in the soils with the plant and a *Total soil CO_2-C flux* $_{\text{Topsoil (-Plant)}}$ is the total soil CO_2-C flux derived from topsoil in the soils without the plant.

Plant biomass

After 78 days, the plants were harvested and separated into shoots and roots. The vegetal materials were washed in distilled water, dried at 60 °C under forced-air circulation until the constant weight was achieved and, then, they were weighed.

Statistical analyses

The assumptions of the parametric statistics (normal distribution of residuals and homogeneity of variances) were tested by Shapiro-Wilk and Levene's test, respectively; and when it was not confirmed, the data were transformed by quadratic inverse transformation [$y''=-2/(y'+100)^2$]. Additionally, a three-way analysis of variance (ANOVA) was performed, and the differences among the treatments were tested by pre-planned orthogonal and additional contrasts (Table 3). However, because the ANOVA assumptions were violated for total soil CH_4-C accumulated data, despite the transformation, we performed Kruskal–Wallis tests ($p<0.05$) with means pairwise comparisons using the Kruskal–Wallis multiple-comparison *Z*-value test. Linear regression model adjustment was performed to root dry matter of *Crotalaria juncea* and Rhizosphere priming effect. All statistical analyses were performed using R software (R Development Core Team, 2020).

RESULTS

Soil CO_2-C and CH_4-C emissions

The Soil CO_2-C fluxes in soils without (-Plant) and with plant (+Plant) showed similar behavior over time, with peak emissions at 47 days after planting (Figures 2a and 2b). However, in the last days (61, 68, and 78 days) was possible to observe a slight tendency to increase the soil CO_2-C fluxes in planted soils (Figure 2b). After 78 days, the total soil CO_2-C accumulated in planted soils was 501 g m^{-2} higher than unplanted

Table 3. Orthogonal (C) and additional (C_A) contrasts to different comparisons among plant, mineral-N fertilization (MNF) and organic-N fertilization (ONF)

Treatments			Orthogonal contrasts												
Plant	**ONF**	**MNF**	**C_1**	**C_2**	**C_3**	**C_4**	**C_5**	**C_6**	**C_7**	**C_8**	**C_9**	**C_{10}**	**C_{11}**	**C_{A1}**	**C_{A2}**
-	-	-	-1	-1	-2	0	0	0	0	0	0	0	0	0	0
-	SS	-	-1	-1	1	-1	0	0	0	0	0	0	0	-1	0
-	HWC	-	-1	-1	1	1	0	0	0	0	0	0	0	-1	0
-	-	+	-1	1	0	0	-2	0	0	0	0	0	0	2	0
-	SS	+	-1	1	0	0	1	-1	0	0	0	0	0	0	0
-	HWC	+	-1	1	0	0	1	1	0	0	0	0	0	0	0
+	-	-	1	0	0	0	0	0	-1	-2	0	0	0	0	0
+	SS	-	1	0	0	0	0	0	-1	1	-1	0	0	0	-1
+	HWC	-	1	0	0	0	0	0	-1	1	1	0	0	0	-1
+	-	+	1	0	0	0	0	0	1	0	0	-2	0	-2	2
+	SS	+	1	0	0	0	0	0	1	0	0	1	-1	1	0
+	HWC	+	1	0	0	0	0	0	1	0	0	1	1	1	0

-: absence; +: presence; SS: Sewage sludge; and HWC: household waste composting.

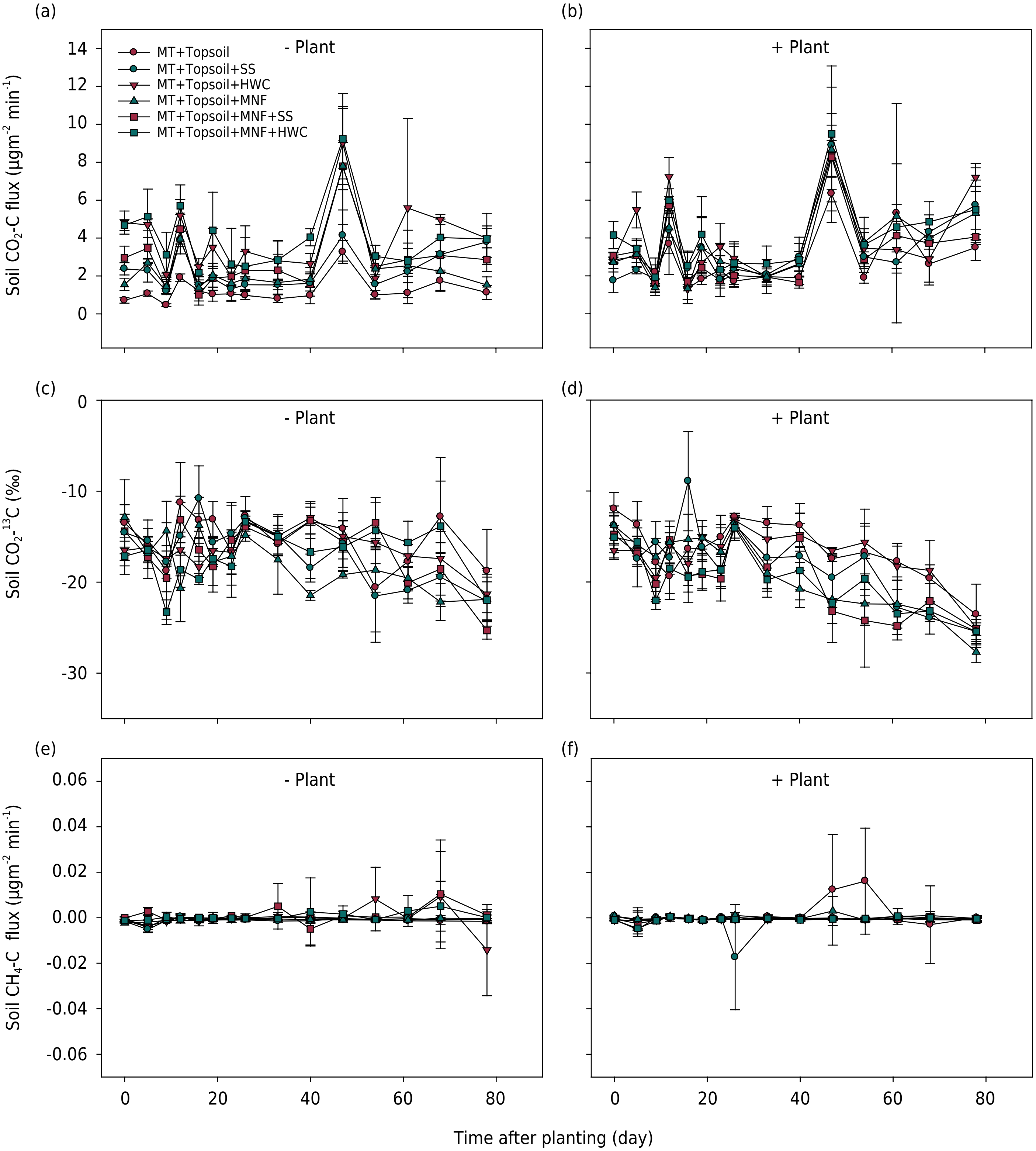

Figure 2. Soil CO_2-C flux (µg m^{-2} min^{-1}; a and b), Soil CO_2-^{13}C (‰; c and d), and Soil CH_4-C flux (µg m^{-2} min^{-1}; e and f) over the time (0, 5, 9, 12, 16, 19, 23, 26, 33, 40, 47, 54, 61, 68, and 78 days after sowing) in iron mining tailing + Topsoil (MT + Topsoil), without (-Plant) and with plant (+Plant), without (-MNF) and with mineral-N fertilization (+MNF) and different organic-N fertilization (non-organic-N fertilization, sewage sludge – SS, and household waste composting - HWC). Vertical bars are the means ± 90 % confidence interval (n = 4).

soils (Figure 3a and Table 4; C_1 $p<0.01$). When we analyzed the soils without plants, the mineral (Figure 3a and Table 4; C_2 $p<0.05$) and organic fertilization (Figure 3a and Table 4; C_3 and C_5, $p<0.01$ and $p<0.05$, respectively) stimulated a higher total soil CO_2-C fluxes. Otherwise, in planted soils, only the organic fertilization without mineral fertilization showed differences, increasing 173 g m^{-2} of total soil CO_2-C accumulated (Figure 3a and Table 4; C_8 $p<0.05$).

Low magnitudes and non-tendency behavior of soil CH_4-C fluxes were verified over the 78 days (in -Plant and +Plant; Figures 2e and 2f), showing no clear tendency of sink or source of CH_4-C in the soils studied. No significant differences were observed to the total soil CH_4-C accumulated when compared pairwise by Kruskal-Wallis multiple-comparison Z-value test ($H_{11,\ 48} = 9.0510$) (Figure 3b).

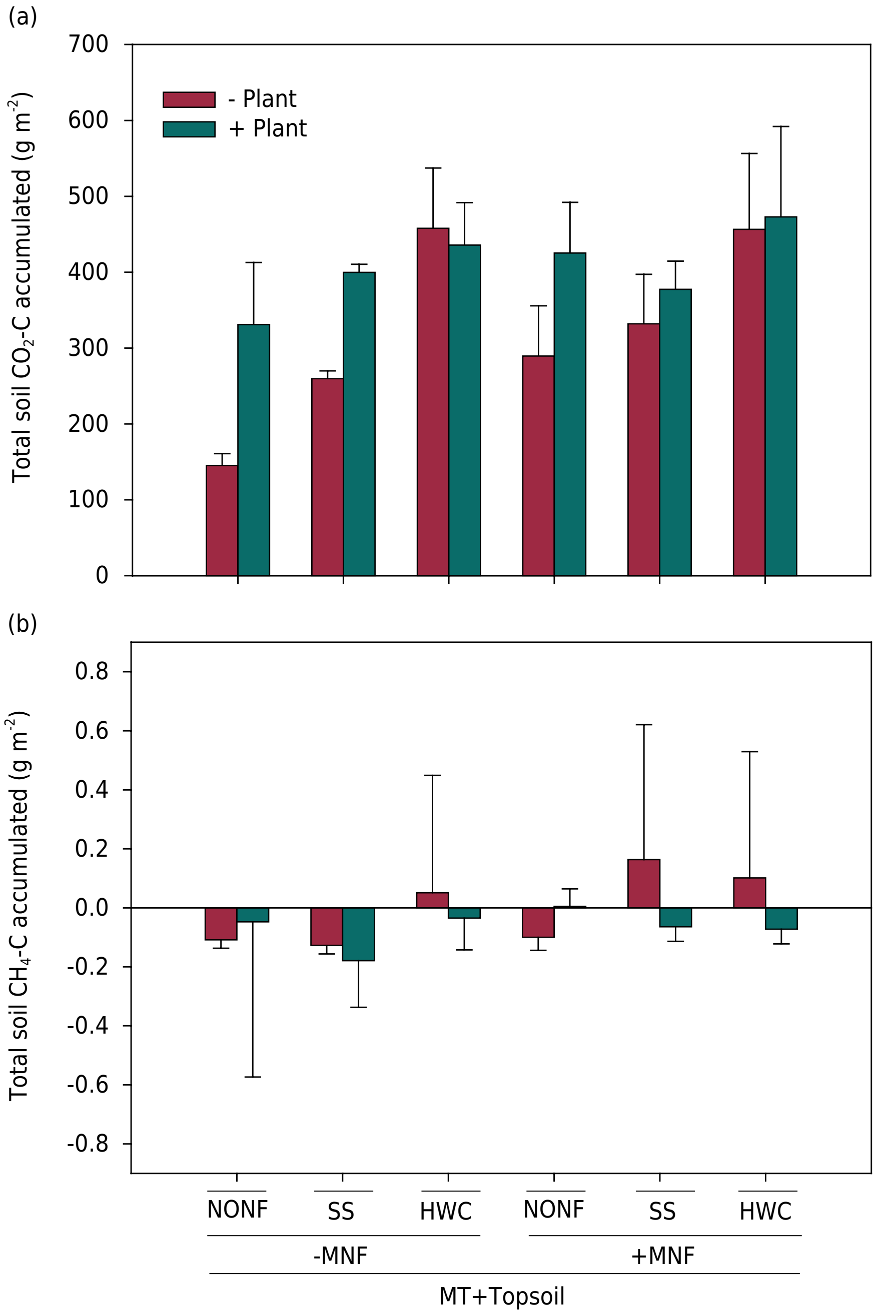

Figure 3. Total soil CO_2-C accumulated (g m^{-2}; a) and total soil CH_4-C accumulated (g m^{-2}; b) 78 days after the planting in iron mining tailing + Topsoil (MT + Topsoil), without (-Plant) and with plant (+Plant), without (-MNF) and with mineral-N fertilization (+MNF), and different organic-N fertilization (non-organic-N fertilization, sewage sludge - SS, and household waste composting - HWC). No significant differences were verified to total soil CH_4-C accumulated according to the Kruskal-Wallis multiple-comparison Z-value test. Vertical bars represent the means ± 90 % confidence interval (n = 4).

The soil CO_2-^{13}C (‰) among the different treatments over time showed enough differences to allow the partitioning calculations of the different sources of contribution to Soil CO_2-C fluxes (Figures 2c and 2d).

Organic fertilization effect on soil CO_2-C emissions

Due to the difficulty of partitioning the contribution of organic fertilization ($\delta^{13}C_{SS}$: -26.57 ‰ and $\delta^{13}C_{HWC}$: -23.47 ‰) and plant ($\delta^{13}C_{Plant}$: -27.00 ‰) to soil CO_2-C fluxes, we decided to study the organic fertilization effect to soil CO_2-C emissions only in the unplanted soils (-Plant; Figures 4a and 4b). The mineral-N fertilization promoted a reduction of the mean in the decomposition of organic-N fertilization sources (organic-N fertilization derived-CO_2-C: 55.28 g m^{-2}; Figure 4a and Table 4; C_2 $p<0.01$). However, in both situations (-MNF and +MNF), the HWC showed the largest decompositions (Figure 4a and Table 4; C_4: 47.44 g m^{-2} $p<0.01$ and C_6: 54.65 g m^{-2} $p<0.01$).

Table 4. Mean contrasts to total soil CO_2-C accumulated and organic-N fertilization derived-CO_2-C (ONF derived-CO_2-C) and soil derived-CO_2-C to the soil without plant (-Plant)

Contrasts	Total soil CO_2-C ac.	Plant		
		ONF derived-CO_2-C$^{(1)}$	Soil derived-CO_2-C$^{(1)}$	PE
	g m^{-2}	g m^{-2}		%
C_1	501.09**	-277.45**	-1669.54**	-227.10**
C_2	215.05*	-55.28**	270.33**	-140.17**
C_3	426.84**	166.36**	260.48**	183.64**
C_4	198.19**	47.44**	150.76**	105.92**
C_5	209.28*	111.09**	98.19^{ns}	43.47^{ns}
C_6	124.43*	54.65**	69.79^{ns}	23.53^{ns}
C_7	109.25^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}
C_8	173.38*	0.00^{ns}	0.00^{ns}	0.00^{ns}
C_9	36.03^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}
C_{10}	-0.13^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}
C_{11}	95.53^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}
C_{A1}	-138.43^{ns}	-166.36**	27.93^{ns}	-183.64**

**, *, and ^{ns}: significant at 1 and 5 % and no significant to F test, respectively. $^{(1)}$ Data transformed by quadratic inverse transformation [$y'' = -2 / (y'+100)^2$]. C: orthogonal contrasts; C_A: additional contrasts.

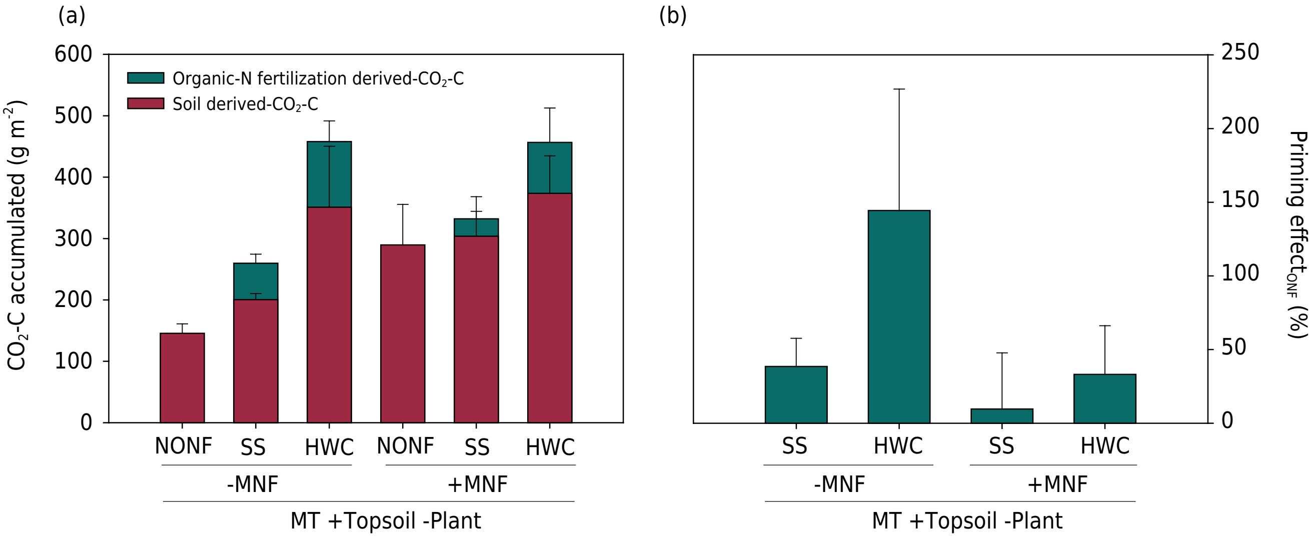

Figure 4. Organic-N fertilization derived-CO_2-C and Soil derived-CO_2-C accumulated (g m^{-2}; a) and priming effect $_{ONF}$ (%; b) 78 days after sowing in iron mining tailing + Topsoil (MT + Topsoil), without (-Plant), without (-MNF) and with mineral-N fertilization (+MNF) and different organic-N fertilization (non-organic-N fertilization, sewage sludge - SS, and household waste composting - HWC). Vertical bars represent the means ± 90 % confidence interval (n = 4).

The mineral-N fertilization stimulated higher soil derived-CO_2-C (Figure 4a and Table 4; C_2: 270 g m^{-2} $p<0.01$). Nonetheless, in soils without mineral fertilization (-MNF), the organic-N fertilization promoted an increase in soil derived-CO_2-C of 260 g m^{-2}, with the most contribution of waste composting (Figure 4a and Table 4; C_3 $p<0.01$ and C_4 $p<0.01$). Otherwise, in soils with mineral fertilization (+MNF), the organic-N fertilization did not affect the soil derived-CO_2-C (Figure 4a and Table 4; C_5: 43.47 g m^{-2} $p>0.10$). When we analyzed only N sources, it was possible to observe higher emissions of soil derived-CO_2-C in soil with +MNF (MNF, SS, and HWC; Figure 4a and Table 4, C_{A1}: 28 g m^{-2} $p<0.01$). The priming effect promoted by organic-N fertilization (priming effect ONF; %) was observed only in soils without mineral fertilization (-MNF). In this treatment, the most contribution was in the HWC (Figure 4b and Table 4; C_3: 184 % $p<0.01$ and C_4: 106 % $p<0.01$), while in the soils with mineral fertilization (+MNF) there were no significant effects (Figure 4b and Table 4; C_5: 43.47 % $p>0.10$).

Plant growth effect to soil CO_2-C emissions

The mineral N fertilizer (MNF) application promoted a mean increase of 193 g m^{-2} of plant derived-CO_2-C (Figure 5a and Table 5; C_7 $p<0.01$), while in soils without mineral fertilization (-MNF), the SS application stimulated higher plant growth Plant derived-CO_2-C than HWC (Figure 5a and Table 5; C_9: -64 g m^{-2} $p<0.05$).

However, the mineral N fertilizer application affected the soil derived-CO_2-C differently. There was a reduction in Soil derived-CO_2-C emissions when MNF was applied (Figure 5a and Table 5; C_7: -136 g m^{-2} $p<0.01$), and no differences were observed to ONF application (Figure 5a and Table 5; C_{10}: -23.57 g m^{-2} $p>0.10$ and C_{11}: 14.19 g m^{-2} $p>0.10$). Otherwise, in soils without mineral fertilization (-MNF), the organic fertilization stimulated the soil derived-CO_2-C emissions in 167 g m^{-2} (Figure 5a and Table 5; C_8 $p<0.01$), with a more significant contribution from organic waste composting source (Figure 5a and Table 5; C_9: 100 g m^{-2} $p<0.01$). Among the different N sources, the ONF (SS + HWC) promoted higher soil derived-CO_2-C emissions than MNF application (Figure 5a and Table 5; C_{A2}: 131 g m^{-2} $p<0.01$). Consequently, the rhizosphere priming effect showed similar behavior from Soil derived-CO_2-C emissions (Figure 5b and Table 5; $C_{7\text{-}A2}$).

The MNF increased in 10.03 and 1.00 g of shoot and root dry matter, respectively, per column (Figure 6a and Table 5; C_7: $p<0.01$). In soil without mineral fertilization (-MNF) the

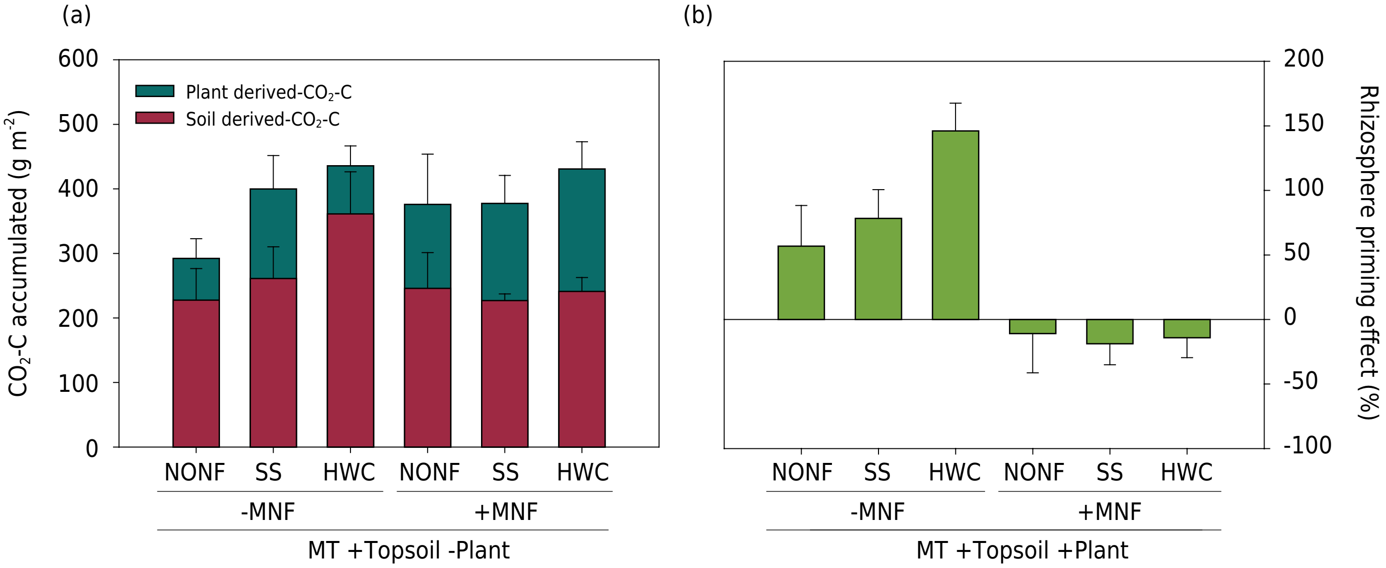

Figure 5. Plant derived-CO_2-C and Soil derived-CO_2-C accumulated (g m^{-2}; a) and Rhizosphere priming effect (%; b) 78 days after sowing in iron mining tailing + topsoil (MT + Topsoil), with (+Plant), without (-MNF) and with mineral-N fertilization (+MNF) and different organic-N fertilization (non-organic-N fertilization, sewage sludge – SS, and household waste composting - HWC). Vertical bars represent the means ± 90 % confidence interval (n = 4).

Table 5. Mean contrasts to Plant derived-CO_2-C, Soil derived-CO_2-C, Rhizosphere priming effect (RPE), shoot dry matter (Shoot DM), and root dry matter (Root DM) to the soil with the plant (-Plant)

Contrast	Plant				
	Plant derived-CO_2-C	Soil derived-CO_2-C	RPE	Shoot DM	Root DM
	g m^{-2}		%	g per column	
C_7	192.72**	-136.28**	-325.37**	10.03**	1.00**
C_8	83.70^{ns}	167.14**	110.70**	2.58**	0.12^{ns}
C_9	-64.06*	100.09**	67.79**	0.54^{ns}	0.03^{ns}
C_{10}	79.99^{ns}	-23.57^{ns}	-11.01^{ns}	0.45^{ns}	0.26**
C_{11}	39.17^{ns}	14.19^{ns}	4.61^{ns}	0.25^{ns}	-0.05^{ns}
C_{A2}	47.25^{ns}	-130.85**	-246.47**	5.52**	0.45**

**, *, and ^{ns}: significant at 1and 5 %, and no significant to F test, respectively. C: orthogonal contrasts; and C_A: additional contrasts.

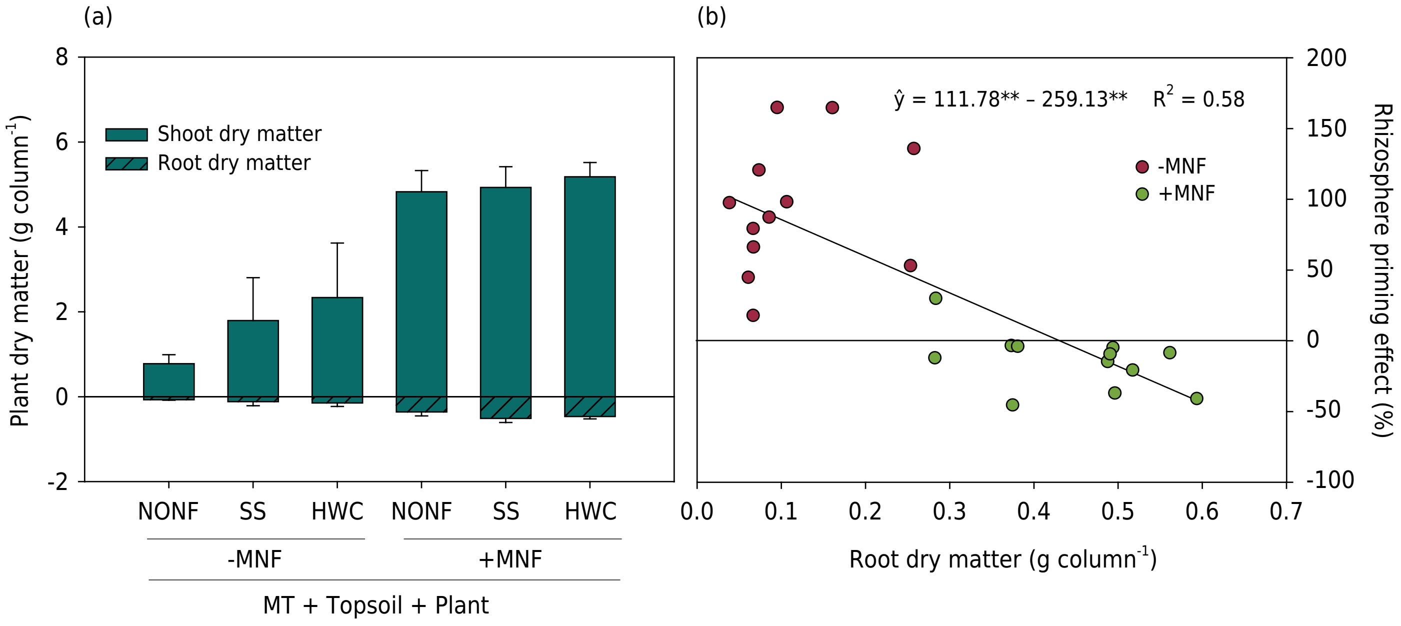

Figure 6. Shoot and root dry matter (g) of *Crotalaria juncea* after 78 days after the sowing in iron mining tailing + Topsoil (MT + Topsoil), without (-MNF) and with mineral-N fertilization (+MNF) and different organic-N fertilization (non-organic-N fertilization, sewage sludge - SS and household waste composting - HWC). Linear regression model adjustment to root dry matter of *Crotalaria juncea* and Rhizosphere priming effect (b). Vertical bars denote means ± 90 % confidence interval (n=4).

organic fertilization increased by 2.58 g of shoot dry matter per column (Figure 6a and Table 5; C_8: p<0.01), while the root dry matter was not affected by organic fertilization (Figure 6a and Table 5; C_8: 0.12 g per column p>0.10). Opposite behavior was observed in the soils with +MNF application, which the organic-N fertilization did not affect the shoot dry matter (Figure 6a and Table 5; C_{10}: 0.45 g per column p>0.10) but improved the root dry matter (Figure 6a and Table 5; C_{10}: 0.26 g per column p<0.01). The mineral-N fertilization increased the shoot dry matter (C_{A2}: 5.52 g per column p<0.01) and root dry matter (C_{A2}: 0.45 g per column p<0.01) than the organic-N fertilization sources (SS + HWC) (Figure 6a and Table 5). The Root DM showed linear adjustment with rhizosphere priming effect (%), regardless of mineral-N fertilization (-MNF and +MNF; Figure 6b).

DISCUSSION

Organic fertilization effect on soil CO_2-C emissions

The addition of organic compounds to the soil as a strategy to improve soil physical, chemical, and biological properties has been widely used in recovery strategies of degraded soil (Hu et al., 2015; Vilas Boas et al., 2018). However, complex microbially-mediated interactions may promote conflicting results regarding the increase of soil organic matter (Blagodatskaya and Kuzyakov, 2008; Cheng et al., 2014; Mason-Jones et al., 2018). Our results demonstrate an apparent effect of organic compounds application on total soil CO_2-C emissions (Figure 3a and Table 4). In contrast, the total soil CH_4-C did not show a significant difference between N-mineral and N-organic fertilization (Figure 3b). In soil without N-mineral fertilization (-MNF), the application of N-organic stimulated the native SOM mineralization. The addition of energy-rich compounds into the soil possibly supplied the energy needed to access different native SOM pools (by co-metabolism of SOM). This addition changes the microbial community and increase the production of SOM-degrading exo-enzymes (Fontaine et al., 2003; Blagodatskaya and Kuzyakov, 2008; Mason-Jones et al., 2018). This assumption is supported by the different effects in soil derived-CO_2-C emissions stimulated by sewage sludge (C:N = 7.62) and household waste composting (C:N = 36.54) (Figures 4a and 4b; and Table 4).

Since household waste composting presented the highest C:N ratio, it promoted the SOM decomposition (higher priming effect). This can be explained by the action of the K-strategists microorganism to acquire the N. These microorganisms can use energy-rich compounds as a source of energy to successfully degrade energy-poor compounds present on SOM (Fontaine et al., 2003; Cheng et al., 2014). Since divergent effect in SOM mineralization can be stimulated, it is important to consider the C:N ratio of the organic material applied to fertilization.

Some studies have reported reductions in soil CO_2 effluxes promoted by the increase of N availability (Kuzyakov et al., 2019; Li et al., 2019). This phenomenon is mainly related to microbially-mediated processes such as: (i) soil acidification promoted by N application, which can reduce the microbial growth (Averill and Waring, 2018); (ii) increase of the C use efficiency by the soil microbes (Manzoni, 2017); and (iii) decreasing the production of enzymes responsible to assure the SOM decomposition (Kumar et al., 2016). However, our results contradict these assumptions since the N-mineral fertilization stimulated the native SOM mineralization (Figure 4a and Table 4), indicating that in this case, the more limited source for soil microbial activity was the N.

In our study, the effect of N-mineral fertilization overlaps the N-organic fertilization, and no significant impact in native SOM mineralization was observed (Figure 4a and Table 4). Thereby, the N-mineral was more efficiently used by microbes than the N-organic sources. Nevertheless, a study performed with different and representative soil (from America, Australia, Asia, Africa, and Europe) observed that the energy-rich compounds inputs despite promoted the priming effect; the fraction of the added C incorporated in the soil is always higher than C losses from native SOM (Perveen et al., 2019).

Plant growth effect to soil CO_2-C emissions

The particular rhizosphere zone promotes large C inputs by plant (exudates, secretions, mucilage, and lysates), stimulating the microbiological diversity in the rhizosphere (Kuzyakov, 2002). However, the plant-microbial interactions can benefit the plant and microorganisms but it may impact the SOM dynamics (Murphy et al., 2017). In our study, the plant in soil with N-organic fertilization and without N-mineral fertilization mineralized more SOM than that cultivated in the soil without fertilization, which enabled a positive rhizosphere priming effect (Figures 5a and 5b and Table 5). It is stipulated that plants can modulate the rhizodeposition to stimulate SOM degradation by soil microorganisms and consequently mobilize soil nutrients (Fontaine et al., 2011). So, this indicates that the energy available from the N-organic fertilization (aggravated by C-to-N rate from compounds, $C:N_{SS}$ = 7.62 and $C:N_{HWC}$ = 36.54) was smaller than that needed to break down the SOM (Fontaine et al., 2011), and the rhizodeposition-C input stimulated the higher positive rhizosphere priming effect. Consistent with our finding, a study in a forest soil mesocosm experiment observed in SOM mineralization (priming effect) when both simple and complex organic compounds were added (Jackson et al., 2019).

Contrary to our hypothesis, only the N-organic fertilization (with sewage sludge or household waste composting) was not enough to provide N availability to plant and conserve the SOM. In soil with only the N-mineral fertilization (+MNF and NONF), there was an expressive reduction in SOM mineralization about N-organic fertilization (with sewage sludge or household waste composting) (Figure 5a and Table 5). The N-mineral fertilization was enough to ensure that plants to overcome competition with soil microorganisms for N (Kuzyakov and Xu, 2013), which was hampered when the only source of N was organic ($C:N_{SS} = 7.62$ or $C:N_{HWC} = 36.54$).

Otherwise, the N-mineral fertilization (+MNF) to both NONF and N-organic fertilization (SS or HWC) promoted a reduction in SOM mineralization, hence it produced a negative rhizosphere priming effect in soil without significant difference between NONF and N-organic fertilization (Figure 5b and Table 5). The reduction in priming effect by N-mineral fertilization was observed in some studies (Fontaine et al., 2011; Chen et al., 2014; Zang et al., 2016). Furthermore, the negative rhizosphere priming effect might be argued by the preference substrate utilization hypothesis, which when an energy-rich compound is available, the soil microorganisms (probability r-strategists) directs their diets to break down these organic compounds and decrease SOM mineralization (Fontaine et al., 2003; Kuzyakov, 2010). Ideally, this fact was only possible due to the N demand by plants and because the microorganisms were supplied by N-mineral fertilization.

The shoot and root dry matter production reflected adequate plant nutrition when the N-mineral fertilization was performed (Figure 6). Previously, we could expect that more root abundance would lead to a stronger rhizosphere priming effect, contradictorily our results demonstrate that not necessarily this occurs (Figure 6). So, once more, reflect the intricate mechanisms of the plant in modulating rhizodeposit release to stimulate soil microorganisms and improve nutrients availability. Zang et al. (2016) verified in rhizospheric soil that the N fertilization increases the C sequestration, not only by increasing root biomass production but mainly the reduction in SOM mineralization.

We did not observe differences to shoot and root dry matter, and rhizosphere priming effect between NONF and N-organic fertilization in soil with N-mineral fertilization (+MNF) (Figure 5b and Figure 6a). However, N-organic fertilization together with N-mineral can promote long-term improvements in the chemical, physical, and biological properties of the soil (Hu et al., 2015; Vilas Boas et al., 2018; Teixeira et al., 2019).

The soil recovery strategy that used plant and N-mineral or N-organic fertilization increased the total soil CO_2-C emissions as compared to unplanted soils (Figure 3a and Table 4). However, healthy cycling of SOM in soil recovery systems is extremely necessary for energy and matter flows, in which the C losses could be overcome in the long-term with the continually energy-rich compounds deposited by the plant roots during its growth (Perveen et al., 2019).

In particular, the total soil CH_4-C emissions showed no differences between fertilization (NOF, N-mineral, or N-organic) and the presence of plants (Figure 3b). The CH_4-C dynamics are driven mainly by the abundance of methanogenic and methanotrophic bacteria in soils, which in well-aerated soil conditions there is predominantly CH_4 consumption by methanotrophic bacteria (Boeckx et al., 1997); while in poorly-aerated soil conditions there is predominantly CH_4 production by methanogenic bacteria. So, no apparent effect of soil CH_4-C consumption or emission in our study suggests no significant effect of different fertilization (N-mineral or N-organic) during the plant growth in Technosols built under iron ore tailing. This fact is crucial because it indicates that there is no significant impediment of water drainage in the profile (anaerobic conditions) in the produced

Technosols, despite the higher bulk density of iron mining tailing (2.10 g cm^{-3}) than the topsoil (0.98 g cm^{-3}; Table 1).

In summary, the revegetation strategies of areas affected by the rupture of iron ore tailing dams ideally could promote the improvement of soil chemical, physical, and biological properties. Notably, organic fertilization is power management, but subsequent increases in SOM mineralization (priming effect) can be verified. So, in opposite to our hypothesis tested, only N-organic fertilization was not enough to conserve the SOM (Figures 5a and 5b; Table 5) and promote the plant growth efficiently (Figure 6a). Our study was the first approach based on sensitive SOM compartments (soil CO_2-C and CH_4-C dynamics) that suggests that the combination of N-organic and N-mineral fertilization to revegetation of areas affected by IOTD rupture is promising to produce plant biomass and preserve soil organic matter. Studies to investigate different SOM pools dynamics and their biochemical composition are necessary to planning SOM recovery strategies for areas affected by the rupture of IOTD.

CONCLUSIONS

Our findings clarify the importance of soil organic matter, along with the definition of recovery strategies for areas affected by the rupture of iron ore tailings dams. The use of N-organic fertilization in Technosols built in areas affected by iron ore tailings during revegetation (with *Crotalaria juncea*) was not sufficient to ensure efficient plant development and preservation of soil organic matter.

However, the combination of organic and mineral nitrogen fertilization promoted a negative rhizosphere priming effect and a significant increase in plant biomass. Therefore, intricate plant-microorganisms interactions intermediated by imbalances of nutrients (mainly N) should be considered to achieve more self-sustainable strategies recovery.

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