

Article

Utilization of Inorganic Nanoparticles and Biochar as Additives of Agricultural Waste Composting: Effects of End-Products on Plant Growth, C and Nutrient Stock in Soils from a Mediterranean Region

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Abstract: This study was conducted to evaluate the effect of compost produced with agricultural residues and oat-based biochar, iron oxide and halloysite nanoparticles as additives of the process of composting on soil chemical properties, nutrient status and growth of ryegrass *Lolium perenne* L. For this, a 90-day mesocosm experiment was carried out under greenhouse conditions. Bare soil and a basal fertilization treatment were compared to soils amended with nonadditive compost (NA compost), compost supplied with oat-based biochar (Bioch compost), iron oxide nanoparticles (Fe compost), and halloysite nanoparticles (Ha compost). Compost supplied with nanoparticles and biochar combined were also considered. The incorporation of compost with or without additives increased the content of total C and N in soil, with N diminishing (total and mineral forms) and C/N modifications after 90 days. The addition of compost and co-composted treatments also increased the total contents of main nutrients such as Ca, K, P and S. Furthermore, the supply of additives into composting did not increase the concentration of trace toxic elements. At the end of the experiment, plant biomass increased by the addition of the different organic amendments, with the highest shoot biomass in soils amended with compost supplied with nanoparticles. These results suggest that the addition of compost based on agricultural residues with additives such as halloysite or biochar improves chemical properties and nutritional status of soil that favor and increase plant growth of *Lolium perenne* established in soils from the Mediterranean Region.

Keywords: compost; iron oxide; amendments; pyrogenic carbon; grassland soil

1. Introduction

Crop production generates a significant volume of residues including straw, roots, shafts and other tissues of the main crops (i.e., corn, wheat, rice, among others), which

comprise up to 3.7 Pg dry organic matter (OM) and mineral components [1,2]. In general, these materials are scarcely or not properly used; except for a limited utilization as pulp, animal feed, and for other purposes [3,4]. In wide areas and regions, stubble burning is one of the most common management practices [5]. The direct burning of the residues from crop production in the open field is an important source of atmospheric pollution with significant impacts on atmospheric chemistry and global climate change, and with great threat to human health [1,6]. Several consequences on soils are negative, affecting the soil organic matter (SOM) and the activity and colonization of soil's microorganisms, reducing biological diversity and nutrient dynamics [7,8]. Nowadays, to avoid these adverse effects on the environment, the reutilization practices appear as an essential biotechnological approach under food security and climate global change scenarios [1,9]. As a common practice, the direct utilization and incorporation of these residues into the soil have positive impacts in the physical, chemical, and biological properties of the soil that may also influence crop yields [10]. However, the direct incorporation of fresh and immature residues can also inhibit plant growth and negatively affect the SOM dynamic as a consequence of its composition.

Composting biologically decomposes, transforms and stabilizes the OM and turns it into humified-OM, which is potentially suitable as an amendment for agricultural, degraded, and contaminated soils [11,12]. The end-product is beneficial for soil fertility by stimulating plant growth and promoting the complexation with metallic ions [13]. Nevertheless, despite the fact that compost is defined as a stabilized material, some types of organic residues and compost have a higher rate of decomposition, lower stabilization of C and may affect the environment by serving as sources of greenhouse gas (GHG) emissions during their production or even after being incorporated into soils. For example, Gao et al., (2019) [14] described different levels of stability and humification in mixtures composted under the same conditions, as a consequence of the diverse microbes and raw material composition, producing dissimilar mechanisms for humins formation [14]. In the context of C sequestration, compost can be a source of CO₂ emissions from soils instead of being a sink, unlike other more recalcitrant organic amendments such as biochar [15,16]. Long-term observations showed that frequent compost application may increase soil C content [17], although a large part of the applied OM may be subject to a quick decomposition.

Metallic oxides and clays as constituents of soil matrix are well known to have an important role in SOM stabilization [18] and have currently gained attention for their application during composting processes [15,19]. Applying these inorganic additives to the system can improve the physical properties of the composting mixtures and some of them, such as clays or nanoclays, are more often used to reduce greenhouse gas (GHG) emissions, N losses, and to enhance the carbon (C) stabilization by increasing the activities of certain enzyme activities associated to C compounds breakdown during composting, which can lead to the production of amendments oriented to C sequestration [15,20–25]. Despite the increasing interest in using these materials as additives, little is known about the main mechanisms involved in the C stabilization in compost, with the organo–mineral associations as one of most proposed to explain the enhancing in C stabilization [15,26]. In this sense, Calabi-Floody et al., (2011) isolated allophanic nanoclays (also reported as an additive in some composting studies) from andisols and described a significant amount (11.8%) of C strongly held by nanoclay, suggesting an important role in the C stabilization [27]. Moreover, Calabi-Floody et al., (2015) suggested that OM stability was ascribed to interactions and physical protection within the micropores of clay and interlayers. Nevertheless, notwithstanding the important number of articles associated with the study of C stabilization in soils and composting mixtures, the impact of the nanoparticles of clays and metallic oxides on composting end-products once the organic amendments are incorporated into the soils has been barely analyzed [28].

Several studies have suggested that biochar, a C enriched material obtained by pyrolysis [29], could be useful as a composting additive, improving the stability and quality of the mature composts [30–32]. Prost et al., (2013) and Vandecasteele et al., (2016) showed

that biochar reduced, and adsorbed leachates produced during conventional composting processes [33,34]. Furthermore, Hagemann et al., (2018) stated that biochar did not alter the C speciation in compost OM under conditions optimized for aerobic decomposition of compost feedstock [35]. The authors also suggested that this pyrolyzed material might be an attractive strategy to produce slow release fertilizer. In general, the combination of biochar and compost has shown synergistic effects and could improve C sequestration as well as some other properties of soils [36]. Barthod et al., (2016) described the potential effects of biochar combined with clays (montmorillonite) on CO₂ emissions from compost amended soils. The authors concluded that the biochar/clay mixture may have a synergistic effect on compost amended soils; nevertheless, the effects of the amendments on soil fertility, plant growth, N dynamics (including losses by leaching), elemental composition in soils and gas emissions were not analyzed and need to be studied for composting systems and compost amended soil–plant systems [37].

In general, as described above, the addition of compost can promote soil quality by enhancing the physical, chemical, and biological properties of soils, such as structure, water holding capacity, aggregate stability, cation exchange capacity and nutrition, contributing macro- and micronutrients [38]. Moreover, the compost supply increases the microbial biomass, which plays a pivotal role in nutrient cycling [39]. Therefore, the use of these organic amendments increases the soil productivity, so it should be considered as a valid alternative to the use of synthetic fertilizers [40]. The beneficial effects of compost depend on its quality [41]. Negative effects on plant growth have been observed when immature compost is used [42], due to its high content of dissolved organic carbon and salt concentrations [43]. As we mentioned above, co-composting with biological (microorganisms), organic (e.g., biochar) and inorganic additives (lime, clays, ash, etc.) could improve compost quality [38,39], stabilizing organic carbon [44], and increasing nutrient availability [45], which could increase plant biomass [46,47]. However, some studies reported that compost with inorganic additives such as steel slag (high in content of metallic oxides) or nanomaterials may have significant contents of trace and toxic elements in leachates or solid samples. Hence, considering the above and the lack of information associated with the effects of co-composted mixtures of agricultural residues, inorganic nanoparticles (i.e., iron oxides and halloysite) and biochar into the soil, greenhouse studies to analyze the potential synergistic or deleterious effect of the end-products on plant growth, elemental composition and N dynamic need to be conducted. Therefore, the aim of this study was to evaluate the impact of end-products of co-composting (agricultural residues, inorganic nanoparticles, and biochar) on plant biomass yield, elemental composition and N dynamics in a grassland soil system with *Lolium perenne* growing under greenhouse conditions.

2. Materials and Methods

2.1. Site Description and Soil Sampling

The soil samples for this greenhouse experiment were obtained and derived from the Mediterranean region at Sierra de Anzácollar, which is close to Seville, Southern Spain. The sampled soils were obtained and characterized by López-Martín et al., (2016) [48]. Briefly, the soil was classified as a Calcic Cambisol (IUSS Working Group WRB 2014) and developed on slate, sandstones, and quartzite with occasional carbonate outcrops. Composite samples of soil were obtained by mixing material. As antecedently reported by the authors, site and soil were affected during 2004 by a strong fire that largely destroyed the vegetation. According to López-Martín (2016), the remaining vegetal material, such as tree trunks and their roots, were removed after the fire and the area was terraced for restoration. Presently, the site is used as pasture. Soil material was taken from the first 0–20 cm. After drying at 60 °C and removal of the fine roots, soil sampled were sieved through a 2-mm mesh and stored for further analysis [48]. Soil was previously characterized and described by Lopez-Martín et al., (2016) and some properties were as follows: pH = 6.1; electrical conductivity EC = 350 µS cm⁻¹; C = 42.5 mg g⁻¹; N = 2.6 mg g⁻¹; and NO₃⁻ = 0.9 mg kg⁻¹ [48].

2.2. Composting Procedure

Agricultural waste-based composts were produced in the dark under controlled temperature (25–28 °C) and moisture content (~60%) for 128 days. The initial composting mixture was prepared by using wheat straw as the main component that was collected after crop harvest and mixed with cattle manure and lupine grains with a C/N ratio of ~25 for the mixture. The straw was milled to pieces of a size of 1–2 cm and then prepared for composting. Cattle manure was previously dried at room temperature for 2 days in order to achieve a final moisture content of 65%. The composting units consisted of individual plastic containers containing 5 kg of the composting mixture, that were adjusted by applying water, weekly. The agricultural waste mixtures were co-composted with additives corresponding to oat hull-based biochar [29]; iron oxide (Fe₂O₃; Sky Spring materials[®]) and/or halloysite (Sigma Aldrich[®]) nanoparticles. The additives were applied in a proportion of 2% (*w/w*) of the total compost mixture for nanoparticles and 7% (*w/w*) for biochar. There were two mixtures that contained both biochar and nanoparticles. Composting units were manually turned every week to assure an adequate aeration of the system. The trial was a completely randomized experiment and experimental units were monitored by analyzing changes in physical and chemical properties (e.g., pH, EC, CIC, Water holding capacity, C/N atomic ratio among others) and greenhouse gases (GHG) emissions following the methodology reported by Sánchez-Monedero et al., (2010). Results of the process are detailed in Medina et al. (in preparation). Briefly, inorganic materials significantly ($p < 0.05$) affected the process and all the analyzed properties. The addition of additives reduced the emissions of GHG and influenced the changes associated with the stabilization of OM in composted material. Temperatures were slightly lower (~45–50 °C) than those observed in conventional compost systems. Final compost was obtained after 128 days of composting.

2.3. Greenhouse Assay

For the greenhouse experiment, plastic beakers (150 mL volume) which were perforated at bottom to control the excess of water by irrigation, were prepared and filled with a mixture of soil and compost. The different compost end-products were applied to 100 g of soil one week before the establishment of plants. Produced compost was supplied at a rate of 50 g C kg⁻¹ soil, corresponding to approximately 25 Mg per ha of the organic C to a depth of 5 cm [15] a common application dose for organic compost. Ten certified grass seeds (*Lolium perenne*) were sowed per each pot at the beginning of the experiment. Plants were not thinned and were grown during 90 days under greenhouse conditions at temperature ranging from 28 ± 3 °C per day to 15 ± 3 °C at night. The plants were manually irrigated with distilled water twice a week or as plants needed during the whole experiment. One treatment (Fertilization) was supplied with a starter N doses at the establishment and received an equivalent amount of 0.113 g N kg⁻¹ soil that was supplied by KNO₃. A control treatment of soil with no addition of compost or fertilization was included (Bare soil). The other treatments were defined as follows: (1) soil amended with compost end-product without additives (NA compost); (2) soil amended with compost end-product that was co-composted with biochar as additive (Bioch comp); (3) soil amended with compost end-product co-composted with iron oxide nanoparticles as additive (Fe compost); (4) soil amended with compost end-product co-composted halloysite nanoparticles as additives (Ha compost); (5) soil amended with compost end-product co-composted with biochar and iron oxide nanoparticles as additives (Bioch+Fe compost); (6) soil amended with compost end-product that was co-composted with biochar and halloysite nanoparticles as additives (Bioch+Ha compost). Each mixture was prepared in four replicates giving a total number of 32 pots. One harvest was considered at the end of the experiment corresponding to 90 days after sowing (DAS).

2.4. Chemical Analyses

Once supplied the compost end-products to soil and at the end of greenhouse assay, C and N content, C/N atomic ratio and NH_4^+ NO_3^- concentration were conducted in soils. For this, the total C and total N contents were measured in triplicate via dry combustion using an elemental analyzer (Carlo-Erba EA- 1108-CHNS). The presence of inorganic forms of C was not included due to the acidity of soil. The inorganic nitrogen (NO_3^- and NH_4^+) was analyzed after an extraction from soil with 2 M KCl and determined colorimetrically [49]. At the end of greenhouse experiment, in order to study the different elements in soil, the inductively coupled plasma optical emission spectrometry (ICP-OES; DV 5300, Perkin Elmer, Waltham, MA, USA) was performed by using modified aqua regia (nitrohydrochloric acid) and a microwave extraction/digestion.

2.5. Plant Growth

Plant height was measured as the experiment progressed for monitoring the evolution of the assay. (Data not shown). At the end of the experiment, the harvested plants of *Lolium p.* were separated into roots and shoots, which were dried at 65 °C in a forced-air oven for 48 h and then weighed. Plants were also previously weighted as fresh material before the dry process.

2.6. Statistical Analyses

The experiment was established as a two-way factorial design, with four replicates per treatment (N = 32). Data were analyzed using a two-way ANOVA after corroborating normality and homoscedasticity to determine the main effects of different compost applications and their interactions followed by orthogonal contrasts to identify significant differences among treatment means. The correlation among the different variables were analyzed using the Pearson correlation coefficient (r). Additionally, a principal component analysis (PCA) was performed to evaluate multivariate ordination of treatments according to response variables. The analysis was performed in R statistic version 3.5.1.

3. Results

3.1. Influence of Factors in Soil and Plant Growth

The different analyzed variables were significantly ($p < 0.05$) influenced by the addition of compost (amendments), time, and the interactions between them (Table 1). The addition of compost significantly affected C, N and the concentration of NO_3^- and NH_4^+ in soils. Time was the main factor explaining the variability of the N content, C/N atomic ratio and the concentrations of NO_3^- and NH_4^+ in soils. All factors and interactions influenced the content of C where the addition of the amendment represents the main factor explaining total C content variability (Table 1). The addition of the amendment also influenced and affected significantly ($p < 0.001$) the growth of plants represented as dry shoot weight and dry root weight.

Table 1. *F-Values* and significance for the effects and factors and their interactions for the variables analyzed by a two-way ANOVA.

Variable	Factor	<i>F-Value</i>
C (%)	Time	19.79 ***
	Amendments	61.20 ***
	Time × Amendments	9.99 ***
N (%)	Time	102.58 ***
	Amendments	55.74 ***
	Time × Amendments	11.39 ***
C/N	Time	37.56 ***
	Amendments	17.61 ***
	Time × Amendments	3.67 **

Table 1. Cont.

Variable	Factor	F-Value
NH ₄	Time	1608.92 ***
	Amendments	15.56 ***
	Time × Amendments	11.97 ***
NO ₃	Time	4735.10 ***
	Amendments	336.20 ***
	Time × Amendments	271.10 ***
Dry shoot weight (g/pot)	Amendments	40.42 ***
Dry root weight (g/pot)	Amendments	12.36 ***

Significant codes are based on *p*-values as follows: 0 ****/ 0.001 ***/ 0.01. Plants show the effects of Amendments since results were only obtained in Day 90. **/ 0.05 / 0.1.

3.2. Chemical Analysis of Soils

3.2.1. Elemental Analysis and C/N Atomic Ratio

For soil incubations, the C content ranged between 4.5 and 7.5% of the dry weight of the samples from the treatments without compost addition and amended with compost at the beginning of the greenhouse experiment, respectively (Figure 1). In this sense, differences between the compost amended soil treatments and those not supplied with compost were observed. Additionally, slight differences were observed between the treatments, where soils amended with Fe compost presented the highest C content at day 0 (7.6%; 76 mg g⁻¹). At the end of the experiment (day 90), there was a diminution of C content with the exception of biochar treated compost (Bioch, Bioch+Fe compost and Bioch+Ha compost). The most important decrease in C content for compost amended soils was observed for Ha and Fe compost (33% and 20%, respectively). The N content showed a similar trend where soils amended with compost were higher than bare soil (0.25%) and fertilized soil (0.30%) at day 0. The compost amended soils varied from 0.4 to 0.6% and soil treated with Fe compost showed the higher N concentration at the beginning of the experiment. After 90 days of the greenhouse assay, the N content varied from 0.2% to 0.5% in the mean value of bare soil and compost amended soil, respectively (Figure 1). Differences were also observed between treatments of soils amended with the different compost, and a similar trend to the C contents was detected for the N concentration, where a clear diminution was observed at the end of the experiment, with Ha treatment presenting the most important decrease in N. Accordingly, the C/N atomic ratio differed among the studied soil samples at the beginning of the greenhouse assay, with values that varied from 12 to 16, with biochar compost and bare soil being the treatments with the highest atomic ratio. Due to the latter variation of C and N concentrations, the C/N atomic ratio of the different treatments were different after 90 days of greenhouse assay accounted ~15 for the bare soil, fertilization, and Fe compost treatments; ~17 for Ha compost and >18 for the biochar compost, Bioch+Fe compost, and Bioch+Ha compost (Figure 1). Analysis by ICP-OES spectroscopy showed that compost increased the content of some essential plant nutrients such as Ca, K, P and S as well as decreasing some trace toxic elements, such as Pb, in comparison to the bare soil and the basal fertilization (Tables 2 and 3). In the case of additives in compost, their addition barely increased the content of nutrients and trace elements in the soil treatments according to aqua regia (estimation of total content). Differences were observed for Fe in soil treatment amended with compost co-composted with iron oxide nanoparticles in comparison to the other treatment amended with compost (Table 2).

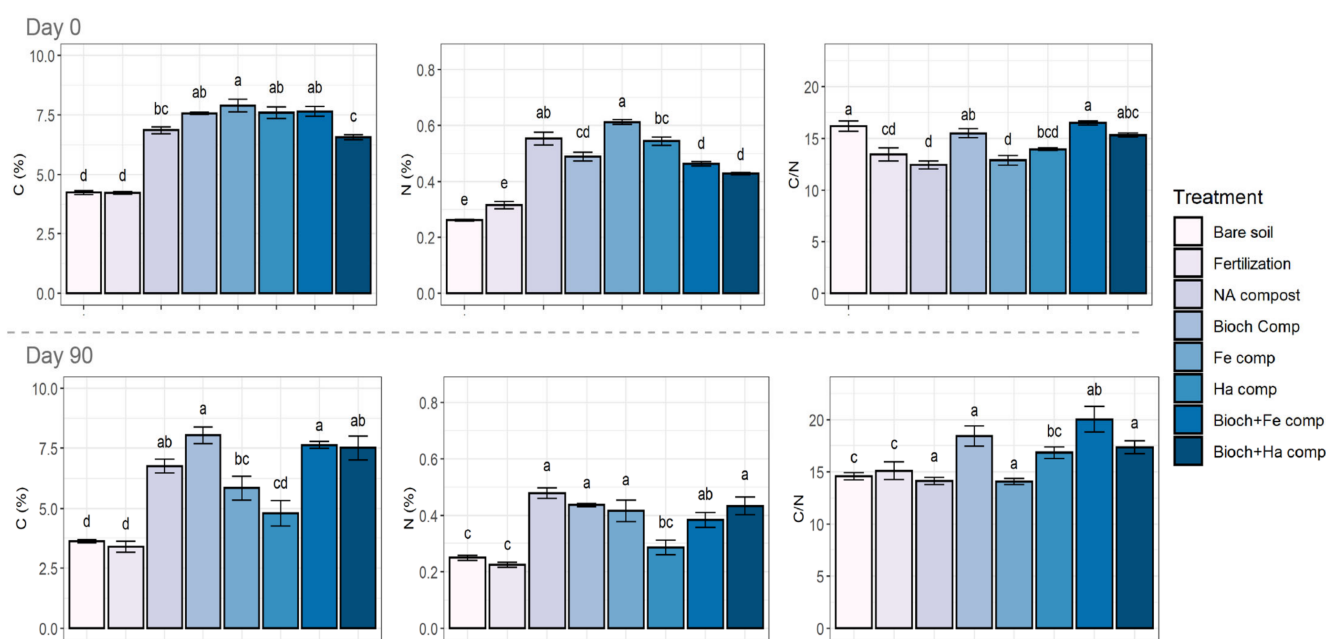


Figure 1. Carbon, nitrogen contents and C/N ratio of different treatments at the beginning and end of the greenhouse experiment (day 0 and 90). Lower-case letters indicate significantly different means at $p < 0.05$.

3.2.2. NH_4^+ and NO_3^- Concentrations in Soil

The concentration of inorganic forms of N were affected by the addition of compost and time as factors. At the starting of the assay, the NH_4^+ concentration varied from 30 and 32 mg kg^{-1} for the bare soil and fertilization treatments, respectively. The incorporation of compost without additives (NA compost) accounted for 50 mg kg^{-1} at day 0. Differences between the treatments amended with compost were also found. In this sense, Bioch+Ha compost, Bioch+Fe compost and Fe compost represented the lower NH_4^+ concentration at day 0. At the end of the greenhouse experiment, a decrease in the NH_4^+ concentration was observed in all treatments ranging from 12 mg kg^{-1} in the fertilization trial to 2.5 mg kg^{-1} associated with biochar and nanoparticle treatments (Bioch+Fe and Bioch+Ha compost) (Figure 2). Nitrate presented a concentration at the beginning of the experiment that ranged from 25 (bare soil) to 520 mg kg^{-1} (Ha compost). Differences between control and compost amended soils were found. The major values were observed by NA and HA (500 and 520 mg kg^{-1} , respectively) compost treatments at the beginning of the assay. As the experiment progressed, an important decrease in nitrate was observed after 90 days. In this context, all the treatment achieved values under 5 mg kg^{-1} except for Ha compost, which showed 50 mg kg^{-1} at the end of the experimental analysis (Figure 2).

Table 2. Elemental composition of soil and compost amended soils after the greenhouse experiment according to ICP-OES spectroscopy.

Treatment	B	Ca	Cu	Fe	K	Mg	P	S
Bare soil	3.8 ± 0.6 ab	1755.4 ± 22 d	37.0 ± 0.4 abc	35,518.0 ± 33.1 c	2225.1 ± 293.7 c	2579.4 ± 12.9 d	382.6 ± 1.2 e	216.5 ± 2.8 e
Fertilization	3.7 ± 0.4 ab	1778.1 ± 13.6 d	36.9 ± 0.1 abcd	35,621.9 ± 13.7 c	3138.3 ± 146.0 b	2541.5 ± 18.8 d	382.5 ± 1.7 e	224.6 ± 1.2 e
NA compost	4.7 ± 0.2 a	2909.3 ± 14.7 b	37.5 ± 0.1 ab	35,148.6 ± 347.5 cd	4694.1 ± 161.0 a	3082.9 ± 42.2 a	861.8 ± 0.1 b	543.7 ± 1.5 bc
Biochar comp	3.9 ± 0.0 ab	2702.4 ± 6.8 c	37.1 ± 0.1 ab	33,869.3 ± 34.9 de	4206.3 ± 95.4 a	2778.4 ± 0.9 c	831.7 ± 0.3 c	498.2 ± 3.9 d
Fe comp	4.0 ± 0.0 ab	3091.1 ± 25.4 a	38.0 ± 0.0 a	40,019.8 ± 142.6 a	4667.5 ± 17.5 a	2927.3 ± 12.4 b	864.6 ± 5.7 ab	634.4 ± 1.8 a
Ha comp	2.9 ± 0.3 b	2762.8 ± 13.3 c	35.8 ± 0.1 cd	33,137.2 ± 135.0 e	4053.5 ± 173.7 a	2795.0 ± 35.4 bc	825.5 ± 0.8 c	531.7 ± 4.5 c
Bioch+Fe com	2.8 ± 0.0 b	2684.6 ± 25.8 c	35.7 ± 0.1 d	38,301.0 ± 103.4 b	4252.3 ± 55.1 a	2811.2 ± 1.4 bc	800.3 ± 2.9 d	488.7 ± 4.6 d
Bioch+Ha com	3.3 ± 0.4 ab	2870.7 ± 10.8 b	36.6 ± 0.6 bcd	33,263.0 ± 794.5 e	4686.2 ± 269.2 a	2771.1 ± 47.0 c	878.5 ± 5.9 a	547.9 ± 1.9 b

Mean ± standard error. Lower-case letters indicate significantly different means at $p < 0.05$.

Table 3. Elemental composition of soil and compost amended soils after the greenhouse experiment according to ICP-OES spectroscopy.

Treatment	Al	As	Cd	Co	Cr	Mn	Na	Ni	Pb	Zn
Bare soil	19,567.9 ± 1285 b	23.2 ± 0.6 ab	0.19 ± 0.00 a	13.5 ± 0.1 bc	32.1 ± 1.1 a	1134.9 ± 4.8 ab	117.8 ± 32.3 c	31.7 ± 0.2 ab	43.9 ± 0.3 a	70.5 ± 0.2 cd
Fertilization	20,732.9 ± 582 ab	23.5 ± 1.0 a	0.11 ± 0.01 cd	13.5 ± 0.1 bc	33.1 ± 0.5 a	1158.8 ± 2.3 ab	160.7 ± 15.2 c	32.8 ± 0.2 a	44.5 ± 0.1 a	69.8 ± 0.4 d
NA compost	23,382.1 ± 547.6 ab	21.7 ± 0.2 ab	0.13 ± 0.00 bc	13.7 ± 0.1 b	32.9 ± 0.3 a	1120.1 ± 3.1 ab	664.3 ± 25.6 b	32.2 ± 0.8 ab	40.0 ± 0.0 c	77.5 ± 1.2 a
Biochar comp	21,444.8 ± 391.1 ab	21.6 ± 0.1 ab	0.13 ± 0.0 b	12.8 ± 0.0 c	33.2 ± 1.4 a	1065.7 ± 0.9 c	599.4 ± 15.0 b	30.9 ± 0.3 ab	40.2 ± 0.2 bc	72.3 ± 0.1 cd
Fe comp	21,362.0 ± 113.5 ab	21.3 ± 0.3 ab	0.14 ± 0.00 b	14.8 ± 0.0 a	32.4 ± 0.2 a	1144.6 ± 5.3 ab	852.6 ± 4.6 a	30.9 ± 0.1 ab	41.2 ± 0.1 b	76.6 ± 0.8 ab
Ha comp	21,630.7 ± 803.4 ab	20.9 ± 0.1 b	0.09 ± 0.00 de	12.7 ± 0.0 c	31.3 ± 0.2 a	1061.5 ± 2.2 c	620.3 ± 21.1 b	31.7 ± 0.2 ab	38.2 ± 0.2 d	72.6 ± 0.5 cd
Bioch+Fe com	20,706.3 ± 1428 ab	21.2 ± 0.1 ab	0.09 ± 0.01 e	13.5 ± 0.1 bc	32.3 ± 0.7 a	1112.4 ± 9.6 bc	655.5 ± 4.4 b	30.2 ± 0.1 b	40.9 ± 0.2 bc	73.8 ± 0.0 bc
Bioch+Ha com	24,108.6 ± 111.9 a	21.9 ± 0.5 ab	0.10 ± 0.00 de	13.5 ± 0.5 bc	33.5 ± 1.5 a	1184.4 ± 32.9 a	687.0 ± 40.7 b	31.5 ± 0.8 ab	40.9 ± 0.4 bc	72.0 ± 1.2 cd

Mean ± standard error. Lower-case letters indicate significantly different means at $p < 0.05$.

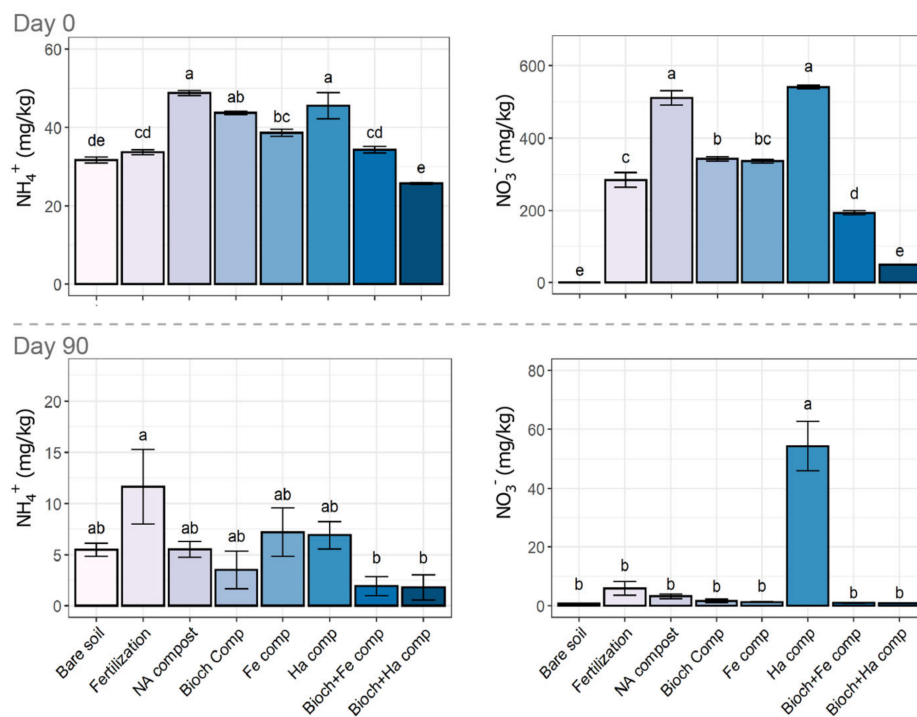


Figure 2. Mineral forms of nitrogen (NH_4^+ and NO_3^-) of the different treatments at the beginning and finish of the greenhouse experiment (day 0 and 90). Lower-case letters indicate significantly different means at $p < 0.05$.

3.3. Shoot and Root Biomass Production

The greenhouse experiment indicated that the addition of compost and compost treated with additives resulted in an increase in dry matter yield and that the levels were significantly higher than for the bare soil or fertilization treatments after 90 days of experiment (Figure 3). A difference between the treatments was found in the shoot dry weight (DW), the root DW, and the shoot/root ratio (Figure 3). The shoot DW was higher in plants growing in amended soil with Fe compost and Ha compost, ranging from 2.15- to 2.22-fold higher with respect to the bare soil, from 1.95- to 2.0-fold higher respect to the fertilization treatment, and 1.2- to 1.23-fold higher with respect to amended soil with Bioch+Ha compost. The highest value of root DW was noted in plants growing in amended soil with Bioch+Ha compost, which had values ranging from 1.72-fold with respect to soil amended with Ha compost and bare soil, and 1.79-fold respect to the fertilized soil. In addition, the soils amended with Ha compost, compost, and Bioch compost, increased shoot/root ratio in plants with values of 1.67, 1.51 and 1.36, respectively. On the contrary, the shoot/root ratio was lower in plants growing in fertilized soil, bare soil and soil amended with Bioch+Ha compost, with values of 0.86, 0.74 and 0.79, respectively.

3.4. Multivariate Relationships

Highly significant correlations were observed between C and N contents ($r = 0.8$) and consequently with the C/N atomic ratio ($r = 0.6$). The content of C and the dry weight of shoots were also highly correlated ($r = 0.7$), and a moderate correlation between C and the dry weight of roots ($r = 0.6$) was observed (Figure 4). Moderate ($r = 0.6$) and negative correlations were also observed between C and NH_4^+ contents. Nitrogen content showed a moderate correlation with the dry weight of shoots ($r = 0.6$). In general, it can be stated that total C and N showed high to moderate correlations with those parameters associated with biomass production (Figure 4).

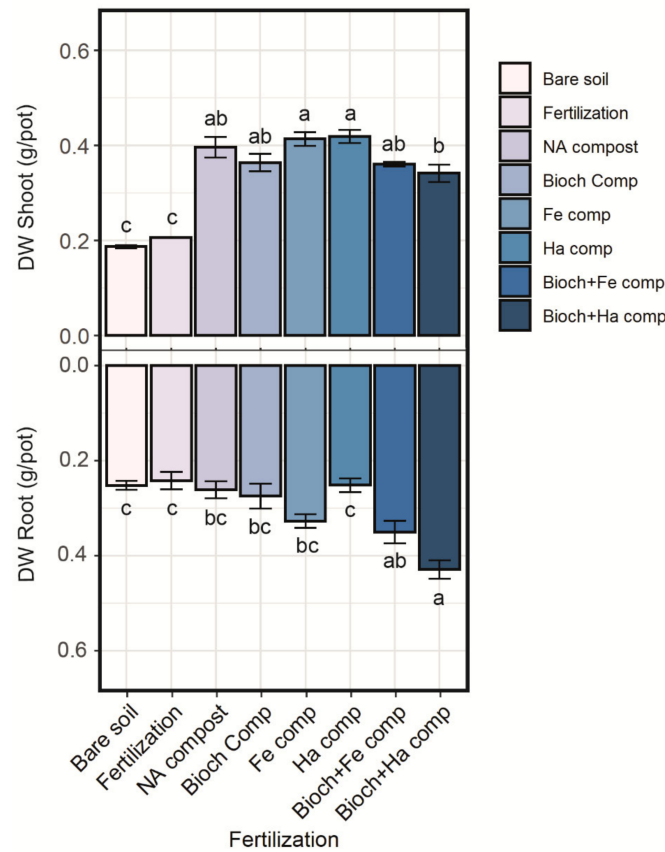


Figure 3. Effect of different compost and co-compost on plant biomass presented as dry weight (g) of root (DW Root) and shoots (DW shoot). Lower-case letters indicate significantly different means at $p < 0.05$.

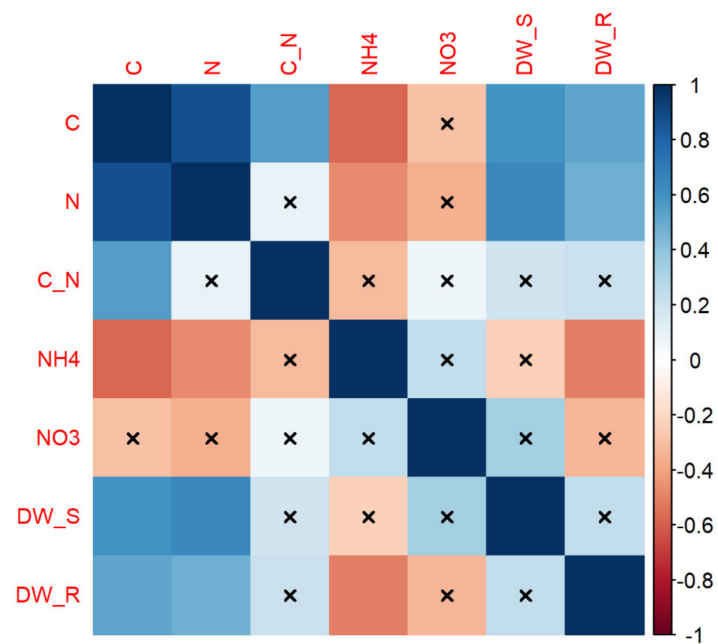


Figure 4. Bivariate correlations (r Pearson) between of the analyzed variables corresponding to total C, N and C/N atomic ratio, NH_4^+ and NO_3 concentration, and dry weight roots and shoots (DW_R and DW_S, respectively). Crossed boxes represent nonsignificant correlations. Red boxes show negative correlations, while blue boxes show positive correlations.

The PCA resulted in two principal components explaining 68.1% of total variance (Figure 5). In this sense, as we mentioned before, the C and N contents were closely correlated to those biomass parameters and were grouped in the same quadrant as dry weight roots. In the case of the analyzed mineral forms of N, the NO_3^- and NH_4^+ contents were independently grouped at a different quadrant (Figure 5). Interestingly, Ha compost treatment was plotted at PC2 associated to the mineral forms of N and biochar co-composted treatments plotted together at PC1 where chemical changes associated to biomass and C/N atomic ratio are correlated (Figure 5).

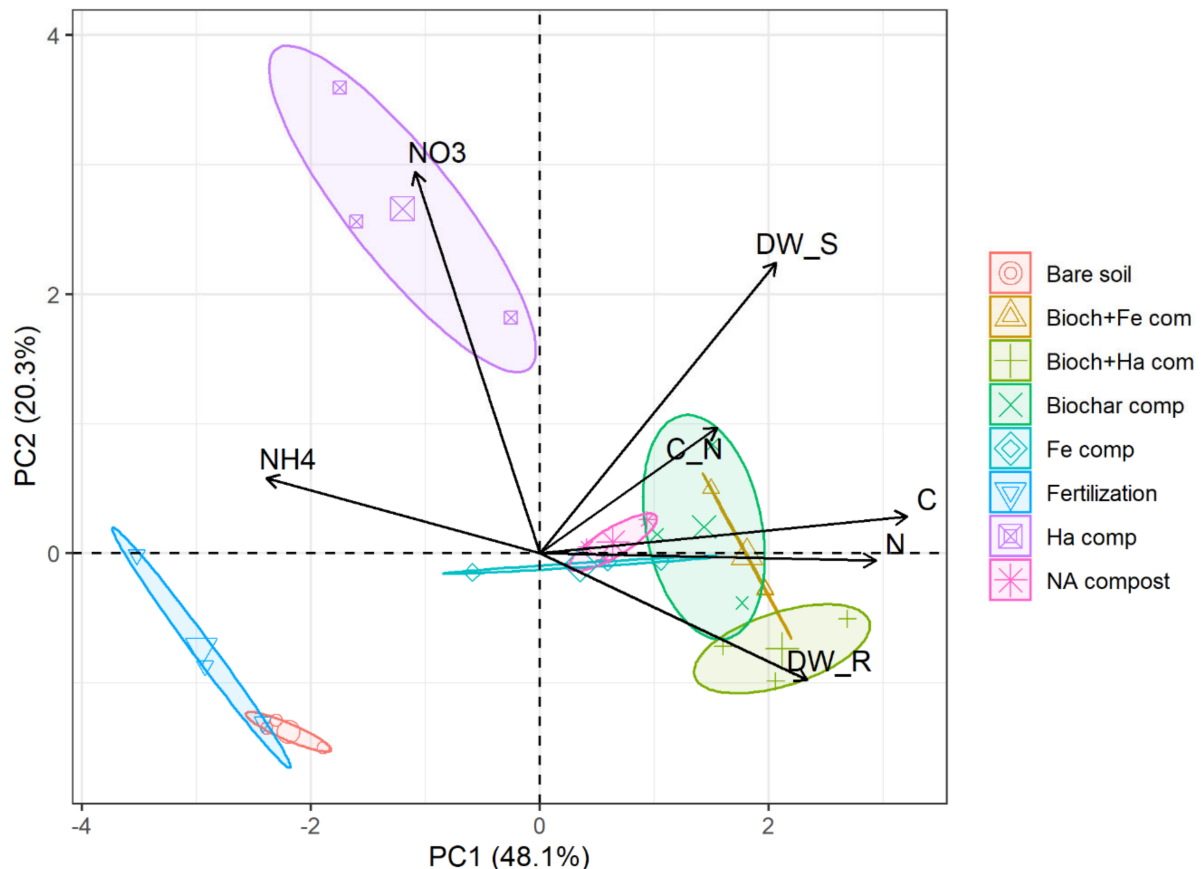


Figure 5. Principal component (PC) analysis of the response variables total C, N and C/N atomic ratio, NH_4^+ and NO_3^- concentration, and dry weight roots and shoots (DW_R and DW_S, respectively).

4. Discussion

4.1. Effect of Compost on Elemental Composition and Nutrient Status on Soil

The addition of compost into soil increased the content of C and N affecting the biomass of plants. Moreover, the addition of all the compost treatments with or without additives also increased the mineral N values in the soil (Figure 2), which may have an impact on the nutritional status of the plants. However, there was a significant difference in terms of total and inorganic forms of N in soils treated with Bioch+Ha compost compared to the other treatments. The loss of N has been mostly regarded as the volatilization of NH_3 at high pH, such as the one recorded during some composting phases [24]. The pH has also been suggested as one of the main factors that explain the volatilization of NH_3 and losses of N in soils, together to lixiviation process. Despite no results related to the pH values being presented in this study, the characterization previously recorded of the produced compost showed that the supply of biochar and halloysite combined as additives increase the pH of the end-products to pH 8.5 (data not shown), which may be related to the N losses during composting. In this regard, Jolanun and Towpravoon, (2010)

suggested that the utilization of some clay materials (i.e., granulated residues of the marble industry) as bulking agents in composting systems tended to increase the matrix porosity and the pH (over 7.5), which could promote the loss of N by the NH₃ volatilization [50]. On the contrary, Mahimairaja et al., (1994) found a diminution around 60% of NH₃ losses when absorbent materials such as zeolite and soil were added to composting mixtures [51]. Therefore, considering the dual nature of our compost additives (absorption capacities and high pH of the biochar, high pH of the HA particles) the results of this research should be interpreted with care and other studies are still necessary for a better understanding of the N dynamic in soils amended with the studied composts.

The newly added compost-derived OM at the beginning of the experiment increased the C content; however we measured a slight decrease in the C content in the soils after 90 days of the greenhouse experiment. In comparison, the major diminution of C content among the treatments of the compost amended soil was recorded by treatments Fe and Ha compost (~30% less than the initial content). This reduction of C content is the opposite to that reported in the literature. For example, Bolan et al., (2012) have demonstrated that co-composting of poultry manure with different additives, such as clay minerals, enhances the stability of its C fractions, both in composting mixtures and soil amended soils [15]. These treatments were also associated with the higher production of shoot biomass. In this context, both treatments Fe and Ha compost were also related with the higher biomass in our study. There is a clear evidence that suggests that exudates from living roots stimulate a quick response of soil microbes with the potential acceleration of the native and stabilized soil organic C mineralization. Therefore, plants may alter dynamics of microbial C fluxes and C use efficiency by balancing the catabolic and anabolic metabolism in the rhizosphere [52]. There is also some evidence that silicates and Fe oxides can catalyze redox reactions and promote, for example, de SOM oxidation [52]. On the other hand, the addition of biochar to the different treatments seems to maintain the content of C at the end of the greenhouse assay. In this sense, the utilization of biochar as a soil amendment have been widely related with C sequestration in soil due to this material's ability to be mineralized 10–100 times more slowly than uncharred biomass [53]. The application of biochar can also reduce the mineralization of native soil organic carbon (SOC). According to some reports, the decrease in SOC mineralization ranges from 3-fold to 21%. Nevertheless, our results must also be interpreted with care since more detailed studies of soil incubations that analyze the mineralization of the different forms of C need to be performed.

4.2. Effect of Compost on Plant Biomass and Growth

Our results showed that the use of organic amendments added to soil increased the shoot biomass production compared to the bare soil and fertilization treatments, which is in agreement with several other studies [54–56]. Furthermore, the soil application of organic amendments co-composted with halloysite or iron oxides as mineral additives produced the highest increases in shoot biomass expressed as dry weight. Similarly, Vidal et al., (2020) showed that the soil application of organic amendments produced with montmorillonite as mineral additive increased significantly the biomasses of *Lolium perenne* and *Phaseolus vulgaris* [57] under greenhouse-controlled conditions. In this sense, it has been reported that organic amendments produced by cattle manure and co-composted with zeolite have positive effects on soil properties, reducing the N leaching, increasing the plant-available N, increasing N efficiency and the final yield of plants [58]. The inorganic additives (e.g., zeolite, montmorillonite, among others) seems to have the capacity to retain nutrients and release them gradually into the soil. Another advantage is the ability to hydrate and rehydrate, which may have a significant impact on maintaining appropriate water balance in the soil and prevent the drying of soils [58,59], which may directly affect plant yields.

There are some differences in the co-composting processes of organic amendments that depend on the types of additives added (organic or inorganic), affecting compost quality and its effect on plant growth [38]. Chowdhury et al., (2016), reported that co-

composts produced by the mixture of poultry manure and inorganic alkaline additives such as red mud, lime and fluidized bed boiler ash improved soil fertility and revegetation of a landfill site, which increased nutrient availability and raised the dry matter yield of Indian mustard [21]. Conversely, several studies have shown that the presence of mineral additives such as bentonite and alkaline materials such lignite and lime can inhibit plant growth [60,61].

The co-composting of organic amendments with biochar has shown promising results to improve plant growth [35] due to biochar promoting plant productivity through changing the physical conditions of the soil, as well as which it may improve soil's water-holding capacity and slow nutrient release [62–64]. In addition, during the composting process, biochar amendments reduce N₂O emissions and nitrogen leaching [65]. Moreover, the increases in crop yield by the addition of biochar-amended composts have been explained by the slow nutrient release from co-composted biochar and its improved properties as an organic fertilizer [35,64]. In our study, the co-composting with additions of biochar, Bioch+Fe compost and Bioch+Ha compost increased biomass production were compared to bare soil and fertilization soil treatments. Kammann et al., (2015) showed that co-composting with biochar had positive effects on increasing the biomass yield of quinoa [64]. Conversely, the Bioch+Ha additive produced the lowest growth of shoot and reached the highest growth of roots, which was correlated with a lower shoot:root ratio. Moreover, the treated Bioch+Ha compost evidenced the lowest concentration of NH₄⁺ at the start of experiment. In this sense, nitrogen deficiency can increase the carbon allocation to the roots, which may inhibit the shoot growth and increase the root growth [66], which can finally result in a significant decrease in the shoot:root ratio [67]. Therefore, the results of this study support the search for inorganic materials and biochar as additives of composting for improving the end-products on nutrient status and plant biomass without significant presence of toxic trace elements. Further research is warranted to elucidate the effect of the co-composted mixture in terms of C sequestration once applied to soil and the potential physiological changes in plants species established on compost-amended soils.

5. Conclusions

Our study highlights the use inorganic additives such as nanoparticles of iron oxide and halloysite and pyrogenic carbon (biochar) during the co-composting of agricultural wastes, and presents the preliminary results associated with the effects of end-products in soil and plant growth. The addition of compost based on agricultural residues significantly affected the total contents of C, N and mineral nutrients. In this regard, the addition of composted materials both with or without additives increased the plant biomass of *Lolium perenne*. The contents of C and N were strongly correlated to the biomass production of the studied plants. According to the factorial analysis, two chemical properties were independently distributed and together corresponded to NO₃⁻ and NH₄⁺, which accounted for 20% of the total experimental variance represented in the PCs. Under our experimental conditions, the addition of the inorganic materials did not alter or increase the concentration of toxic trace elements in the compost-amended soils, and also caused an increase in the generation of biomass, especially those treatments supplied with iron oxide and halloysite nanoparticles. Therefore, the utilization of additives of composting both nanoparticles and biochar appears to be suitable technological tool during composting to produce amendments that preserve their nutritional status and improve soil quality with no contribution of toxic trace elements.

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