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# Carbon and Mycorrhizal Fungi for Improving Sustainable Ecosystems and Mitigate Greenhouse Gases

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*Article*

# Carbon and Mycorrhizal Fungi for Improving Sustainable Ecosystems and Mitigate Greenhouse Gases

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**Abstract:** While composting of organic wastes is increasingly adopted in sustainable agriculture, continued interest on biochar application, dark soils, and compost, showed morphological aspects and functionality of biochar as a challenge to solve the CO2 aspects related to climatic change, and carbon stability in sustainable soils, as Black carbon presents long chemical permanency due to incomplete combustion of biomass. The objective of this work was to study the characteristics of soil amendments and to review the state of the art of biochar, showing differences in composition and main microorganisms with relevant presence in the soils, such as Arbuscular mycorrhizal spores (Scutellospora sp., Glomus like, Funneliformis geosporus, Glomus crenatum, and Acaulospora excavata, obtained from TPI soil samples. New strategies are highlighted such as nutrient supplementation of compost, which can constitute the basis for improved compost-based biofertilizers in the future. Thus, the most important strategies for C sequestration are underlined to diminish substantial organic agro- and municipal wastes, increasingly disposed in the fields, landfills or either released by burning or discarding, specially to highlight sustainable technologies to use biochar and compost amendments.

**Keywords:** biochar; mycorrhizae; dark soil; compost; soil nutrients; ecological sustainability indicators

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## 1. Introduction

Elucidating the morphological aspects and the functionality of biochar is a challenge to solve the $CO_2$ aspects related to climatic change, carbon (C) stability in soils and effects of biochar addition on plants and soil microorganisms, aiming to agro-ecosystem, sustainability. Increasing interest for sustainable agriculture is based on the use of biochar, natural dark soils, and compost, pointing the need to consider the structure of C and composition of soils containing C as charcoal. As substantial organic agro- and municipal wastes are increasingly disposed in the fields, landfills or either released by burning or discarding, which results in air, water, and soil pollution (Gabhane et al., 2020) [1], thus, composting of organic wastes is increasingly adopted in sustainable farming activities. As waste is a large source of green-house gases ($GHG_s$) emission, together with deforestation, the characteristics of biochar were deeply studied and tested to be used for improving plant cultivation and at the same time, to mitigate carbon emissions trough carbon sequestration in soil. Therefore, biochar and compost can face Climate change via reduction of $CO_2$ emissions and improving soil organic matter. Black carbon is the result of incomplete combustion of biomass (slow pyrolysis), presenting long chemical stability [2], constituting a sustainability indicator. However, its application shows limitations besides its known benefits as compiled by Kavitha et al [3]. Among benefic services, enhancement of crop yield as fertilizer, and soil quality as soil conditioner, influence biochar application to soils and its numerous benefits for sustainable agriculture and land restoration. However, the biomass source, pyrolysis temperature, and application rate are detailed variables to

control, which produce advantages of biochar addition for soil quality, plant growth and yield, besides removal of pollutants retained in the microporous matrix of biochar, and greenhouse gases (GHGs) mitigation; however, the role of biochar in agricultural soils is still debatable. It is also believed that biochar was used in TPI formation (dark soil presenting an anthropic horizon with high levels of nutrients, mainly Ca and P, in comparison with the main soils of Amazonia, Brazil [4]. Terra Preta de Indio are dark soils in the humid tropics of South America, intensively used by farmers to this day. In this sense, the historical accumulation of biochar as a soil amendment was recently compiled by Glaser et al. [5]. Compost also recovers the soil physico-chemical and microbiological properties, through aerobic fermentation, resulting in stable organic materials [6]. In recent years the use of new products derived from compost, such as compost tea (liquid organic preparation obtained using the aqueous extraction of composted materials), were also tested for their positive effects on crops [7] due to the microporous matrix of biochar. Biochar, a pyrogenic carbonaceous material produced against biomass is promising for carbon sequestration and climate change mitigation. While biomass degrades in the soil simply, biochar can be stored in the soil for extreme long longevity. Biochar C stability is fundamental to its longevity as well as biochar carbon sequestration potential. higher temperature produced biochar of higher stability [8]; however, controversial results highlighted the intricate interactions between biochar, soil, and environmental conditions. The Amazonians. utilized biochar probably to improve crop production, performing the TPI dark soils, as biochar can be considered “charcoal given life” as explained by Singh et al. [9], therefore, farmers used to burn their harvested crops in the recent past until it was appreciated that biochar could increase soil microbial activity. In this sense, biochar contrasts from charcoal (naturally occurring substance, burned to generate heat [10], One of biochar's best qualities is that it conserves carbon for several years. Additionally, Chemical properties of biochar improve soil pH, which decreases soil acidity (due to its higher pH) and improves fertilizer and nutrient retention.[9]. Moreover, mycorrhizae can grow on biochar mobilizing P from its surface, as investigated by Hammer et al. [11]; however, as several questions have not been totally answered, in the present study, different samples were selected to investigate soils, amendments and their carbon properties. Changes in the molecular form of Carbon in dark soil (*terra preta do Índio* (TPI), turf and commercial biochar, were detected. Some microorganisms associated to the TPI) samples were reported previously (Pagano et al. [4]; however, more studies are continuously performed, to reveal the attributes associated to different biochar and to mimic TPI soil). We investigated the characteristics of different biochar/char samples, including those originated from natural compost, in rural and urban areas from Brazil, as there is a need to
better study and determine the availability of nutrients in the microporous matrix of biochar grains and other soil amendments for improving sustainable plant- soil systems. We compared different samples (natural or synthetic), to select novel soil conditioners, and to indicate better methods of soil amendments for greenhouse gases mitigation. Increasing number of reviews emphasizes the need for systematic research on biochar stability, such as that from Tsolis and Barouchas [12]. Moreover, controversial results highlighted the intricate interactions between biochar, soil, and environmental conditions. Thus, the objective of this work was to point out the characteristics of selected soil amendments to potentialize their application. Microorganisms with relevant presence in the soils are showed, such as Arbuscular mycorrhizal fungi (spores) obtained from soil samples. AMF contribute to soil C via hyphae and glycoproteins (Glomalin). The higher contribution of AMF hyphal biomass in C sequestration can be explained by the larger amount of remains after decomposition and presence of chitinous cell walls from fungi, thus, SOM storage is related to hyphal architecture, and glomalin production, as pointed by Parihar et al. [13]. We hypothesize that TPI contains the highest fertility and carbon levels, but other soil amendments, such as compost and turf can sequester more carbon in its composition. We also aim to contribute data for finding indicators of sustainable development.

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## 2. Material and Methods

The samples of charcoal examined were: Activated charcoal Synth® (São Paulo, Brazil) and Vegetal charcoal Synth®, Vegetal compost, and samples of soils were Turf, peat, and terra preta do Índio (Figure 1), collected using a transect. To evaluate the variations in carbon and nutrient contents, a reference sample from soil with native vegetation was selected (control sample) and sampled. This evaluation was used only for descriptive comparison between the soils and amendments. The following chemical and microbiological characteristics were observed in the reference area: 4.7 of pH $H_2O$, 1.0 mg $dm^{-3}$ of P, 45.6 mg $dm^{-3}$ of $K^+$, 1.1 $cmol_c$ $dm^{-3}$ of $Al^{3+}$, 0.3 $cmol_c$ $dm^{-3}$ of $Ca^{2+}$+$Mg^{2+}$, 0.15 $cmol_c$ $dm^{-3}$ of H+$Al^{3+}$, 1.3 g $dm^{-3}$ of TOC, 253.1 mg $kg^{-1}$ and 40 mg $g^{-1}$ of EE-GRSP. For mycorrhizal assessment, only spore density of AMF was evaluated, which presented an AMF spore density of 151.0 spores 50 $g^{-1}$ soil. Native Forest soil: (0-20 cm depth) originated from northern Brazil, Amazonas State, Lat. 1º 54' S, long. 59º 28'O, Altitude: 60 m.a.s.l.) Turf was originated from a farmland close to Belo Horizonte Municipality, Minas Gerais State. The Compost (leaves, stems, cut grass) was collected from the Federal University of Minas Gerais-Campus after 6 months of maturity, conditioned in compost piles (Figure 1), Peat (7 m depth) was collected from Federal University of Minas Gerais- Campus; and Vermicompost+ biochar, was prepared in South Kazakhstan (Vermicomposting was produced against cattle manure by *Eisenia foetida* (Red Californian earthworm, and contained 50% moisture, total N (1.02%) $P_2O_5$ (1.05%), $K_2O$ (1.27%). Soil amendment analysis was performed at Minimax® Agropecuary Laboratory, Belo Horizonte, Minas Gerais State, Brazil. TPI (Terra Preta do Indio samples (0-20 cm) (Takará velho and Poropu sites, Amazonia, Brazil were obtained in 2011 and analyzed at the Laboratory.

AMF spores were separated by wet sieving [14] decanting and sucrose centrifugation, and the analysed data were expressed as number of spores 20$g^{-1}$ of dry soil. Only healthy spores were counted. Each spore type was mounted sequentially in polyvinyl-lacto-glycerol (PVLG) and Melzer's reagent for identification, which was based on spore color, size, surface ornamentation and wall structure, with reference to the descriptions provided by the original species descriptions. AMF nomenclature and authorities are those of IMA fungus [15] and mycobank (www.mycobank.org).

Easily extractable soil glomalin Bradford (PSRB-FE) and total (PSRB) glomalin content was evaluated, and C from glomalin was estimated as GC (mg $g^{-1}$soil), according to Wright and Upadhyaya [16]. The easily extractable GRSP (EE-GRSP) was extracted from 1 g of soil in 8 mL of citrate buffer (20 mM and pH 7.0) and autoclaving at 121°C for 30 min. Total GRSP was extracted from 1 g soil in 8 mL of 50 mM citrate buffer at pH 8.0 and autoclaving for 1 h at 121°C, repeating this procedure several times on the same sample until the reddish-brown color typical of GRSP disappeared [17]. Bio-Rad Protein Assay (Catalog 500-0006) analyzed in a 'VariosKan' plate reader. Controls and extracts were repeated (n= 3) and measured at Varioskan Flash Multireader Thermo Scientific® in Laboratory of Cellular Signalization and Nanobiotechnology, UFMG, with bovine serum albumin as standard, glomalin concentration (mg glomalin $g^{-1}$ soil (Table 1). Glomalin-C was estimated from literature as (C-G), varies between 27.9 +/- 3.3 and 43.1 +/-1.4 (n=8) % of glomalin depending on extraction and soil type [16]. We prefer to use 40% according to Rillig et al. [16]. and Borie et al. [18]. Microscopic analysis of carbon was achieved by Raman measurements performed in aleatory selected carbon grains in the samples of soils with spectrometer, (60 objective. The samples (0.15 g) were diluted in ultrapure water, 60 μL of the solution was placed on a cover slip and measured after dried, and charcoal samples were previously grounded. Thirty Raman spectra of randomly selected C grains of each sample were accomplished. For statistical analysis, all spectra were submitted to exclusion of a linear baseline between the Raman shifts of 800 $cm^{-1}$ and 1900 $cm^{-1}$ according to Pagano et al. [4]. Data were analyzed using Origin Pro 8 software. To evaluate the chemical and physical properties, soil samples were obtained from three composite samples at each site (Figure 1). Means of three composite samples were calculated. Morphological, chemical and physical methods were applied in this study to distinguish samples. Principal component analysis (PCA), evaluated on the samples (Figure 1) showed that the VC sample and compost were associated with K, thus diverging from the TPI and Turf (associated with Fe) and from peat (associated to Fe and Cu (Figure 2). AMF occurrence and glomalin content. AMF spores occurring in the soil samples

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(20g) obtained in 2011 were extracted by wet sieving [13] identified, and their diversity was estimated. For statistical Analysis, data on AMF propagules, and glomalin content were analyzed in 2011 for each sample (three replicates each). The significance of differences between samples in glomalin content and AMF occurrence was tested by using analysis of variance (one-way ANOVA). Tukey post hoc test was used to conduct pairwise tests for significant effects. Principal component analysis (PCA) was evaluated on the three samples of each material using data from soil chemical properties and statistical analysis were performed with PAST 4.08 (2021) [21].

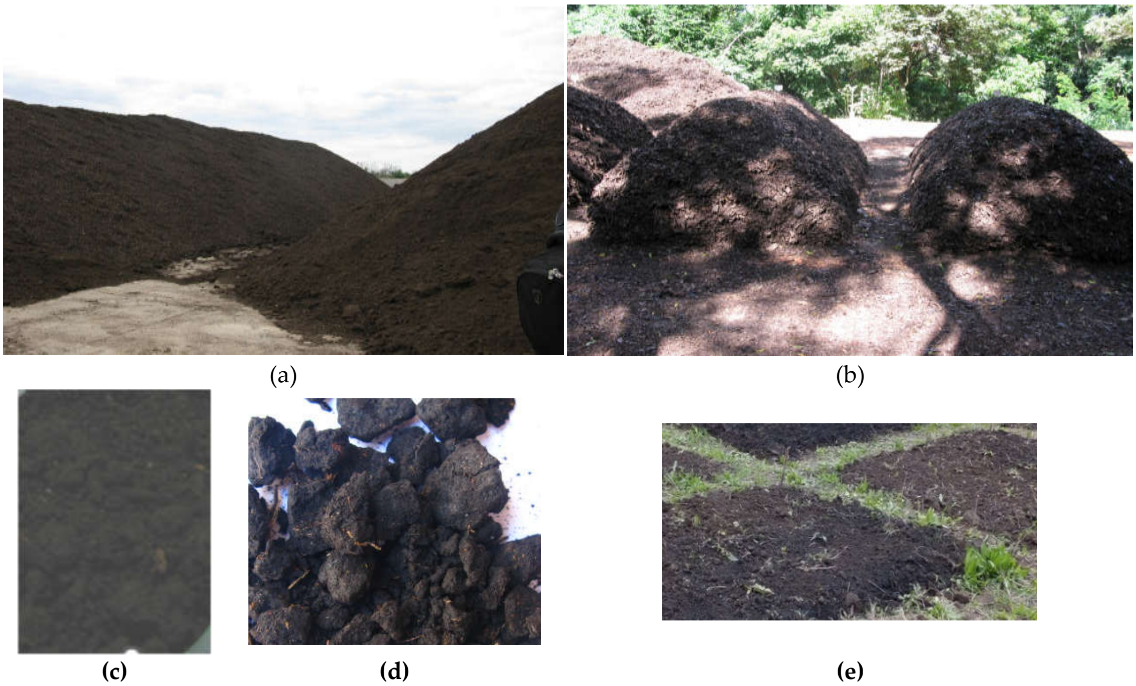


**Figure 1.** Compost (domestic wastes) produced in Italy at Biovegetal plant production (www.biovegetal.it) (A), Compost produced at UFMG - Campus, Belo Horizonte, Brazil (B) TPI (C, D) Vermicompost plus biochar produced in Kazakhstan (E) (photos by M.P., and B.Y, with permission).

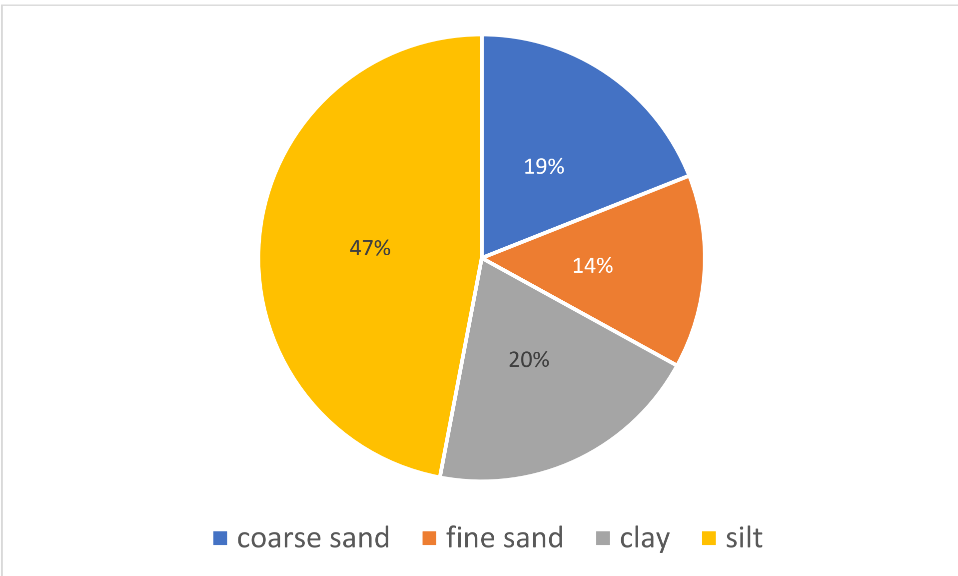


**Figure 2.** Characterization of TPI soil. (% sand, clay, silt.

**Table 1.** Chemical properties of (a) "Terra Preta do Índio" (TPI) (0-20cm), compositional analysis of different soil amendments, and control.

| Sample | pH ($H_2O$) | K | Fe | Ca | Mg | Al | P | C | Cu | Mn | Zn | GC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| # TPI | 5.8 | 14 | 61.26 | 2.90 | 3.1 | 0.74 | 15.5 | 6.42 | 0.001 | 22.12 | 3.72 | 0.6 |
| Compost | 7.8 | 3100 | 60.15 | 14.225 | 4.3 | 0.01 | 41 | 14.2 | 0.85 | 81.3 | 12.1 | NE |
| VC | | 2900 | | 4.5 | | - | 162.9 | 4.5 | 1 | 10.2 | 1.1 | NE |
| Peat | 2.3 | 72 | 4.33 | 4.835 | 2.6 | 1.75 | 3 | 4.83 | 1.9 | 2.65 | 1.45 | NE |
| Turf | 5.3 | 3.5 | 8.4 | 8.095 | 0.65 | 0.6 | 20.95 | 8.4 | 0.001 | 18.55 | 1.45 | NE |
| Control | 4.6 | 6 | 316.6 | 6 | 0.01 | 0.2 | 1 | 1.3 | 0.001 | 0.1 | 1.1 | NE |

# TPI (Terra Preta Do Índio samples: (Takará velho, Poropu sites, Amazonia, Brazil (0-20 cm). GC (mg. $g^{-1}$soil); = Glomalin (EEG) Carbon (cmolc $kg^{-1}$). C (%). #Mean of three samples. Fe, K, Ca, Mg, Al (cmolc $kg^{-1}$). C (dag.$kg^{-1}$). Available P (mg· $kg^{-1}$) NE: not evaluated.

## 3. Results

Compositional analysis of selected soils and amendments (Figure 4) is depicted in Table 1. The chemical analysis revealed high Levels of K, Ca, Mg, Al, P and Zn, whereas the pH varied in different TPI samples from acid (4.3) or slightly acid (6.3) to neutral (7.0) The P level was very high (>30 mg.$kg^{-1}$ in TPI samples. (Table 1); Levels of K, Ca, Mg, P and Zn outstand in the Compost sample. The Ph of Peat resulted acid (5.3), opposite to the alkaline (9.6). VC sample. Table 1 presents the chemical analysis of the samples. Vegetal charcoal presented higher K (31,000mg.$kg^{-1}$) and C (4.6 dag.$kg^{-1}$) contents.

In general, the level of available nutrients decreases in the following order: Compost > TPI > Peat > turf > VC for Zn and Mn. The C content was significantly higher in Compost (14.2 dag $Kg^{-1}$) than in TPI.>Turf > Peat> VC. (Table 1, Figure 3).

Soil Analysis of the studied samples. showed significant higher carbon content (%) in compost than in the samples of VC, Peat, Turf, and control **(Figure 3)**.In particular, TPI soil is composed of organic and inorganic fractions, with predominant silt content (47%), followed by clay>coarse sand> fine sand (Table 2, Figure 4).

**Table 2.** Physical properties of "*Terra Preta de Índio*" (TPI, 0-20 cm) soil samples.

| Sample | Coarse sand | Fine sand | Clay | Silt (%) |
|---|---|---|---|---|
| TPI | 19 | 14 | 20 | 47 |

The TPI soil was comprised of 20% clay (2 μm), 47% silt (2–50μm), and 33% sand (50–2000 μm) at a depth of 0–20 cm (Figure 2). The Textural composition of the examined TPI soil was predominantly silty; opposite, peat and turf samples presented more clay content. TPI showed abundant silt> clay >coarse sand > fine sand (Figure 2). Compost presented the highest levels of K, Zn, P, OM, C, Mg and Mn; however, the pH (water) of Compost (7.8) was moderately alkaline. Fe content of peat was higher than that of the other samples (Table 1). Vegetal charcoal presented higher K and strongly alkaline pH, compared to than the other samples. The Soil analysis of TPI showed high nutrient content (K, Ca, Mg, Al, Zn and Fe) while low level of B and Cu (vestiges). The pH (water) of TPI was strongly acid (5.2) and Soil texture, coarse sand. The P level, Percent OM, N and C were also high. Mn and K content was higher in topsoil (0-20 cm). (Table 1). Compost presented superior levels of elements (Ca, K, Mg, Mn, P, C) as it is formed against tanned twigs, leaves, mixed with little feces; however, Ph is usually high. Control soils (natural local soils) present low elemental content, except for Fe and Al, which are common in those sites, depending on the region. Therefore, the level of available nutrients follows the order: Compost > TPI > Peat > turf > VC for Zn and Mn. Compost carries micro and macronutrients, providing a wider range of nutrients than inorganic fertilizers. Interestingly, compost showed the highest carbon content (14.2 dag.$kg^{-1}$, (Table 1). Biochar (of varied quality) and compost presented high pH (alkaline). In this sense, for agronomic plant cultivation macronutrients (N, P, K, Ca, Mg, S, Si) (obtained from soil) and micronutrients (Fe, Cl, B, Mn, Na, Zn, Cu, Ni, Mo), obtained against water and $CO_2$, are required [19].

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*3.2. Spectroscopic Analysis of Carbon*

Data on the microstructure of carbon grains (Figure 3B) demonstrated that samples of different origin showed different nanocrystallite size (La), as obtained by Raman spectroscopy analysis. The evaluation of the crystallites showed smaller crystallite size in compost, forest, peat, and turf (~ 4nm) than in VC, and AC (10 nm). (Figure 5, Bars represent means accompanied by standard errors.

The samples of TPI showed a lower (6-8 nm) crystallite size (Figure 4a-b). According to the results of the PCA (Figure 5), the major chemical parameters of sampled soils (soil organic matter (SOM) and K) were in a positive correlation with VC and Compost. In contrast, TPI and Turf were associated to other elements (P, Fe, and Mn). Regarding mycorrhizal occurrence, abundance of arbuscular mycorrhizal fungi (AMF) (spores) was measured by morphological methods. For analysis of AMF, spores were recovered from soil samples (20 g), separated by wet sieving [13], decanting and sucrose centrifugation, data were annotated as number of spores/20 gram of dry soil, to preserve soil for sampling. AMF identification was performed using current papers and AMF spores occurring in the soil samples (20g soil) were extracted by wet sieving, according to standard methods and were identified using updated descriptions of AMF species. Arbuscular mycorrhizal spores obtained from TPI samples are showed in Figure 6.

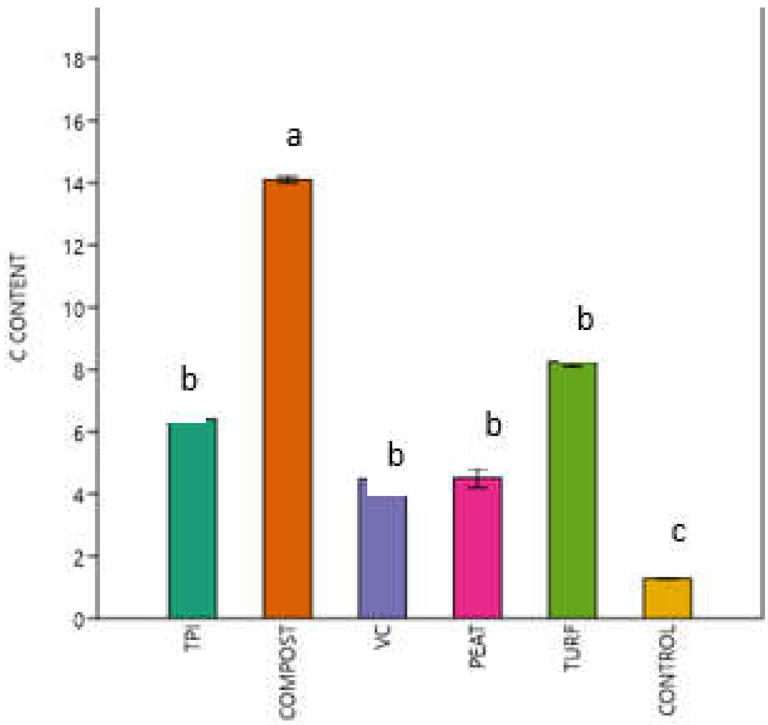


**Figure 3.** Carbon content (%) in the samples. Bars represent means accompanied by standard errors. Means followed by different letter(s), within each specie, are statistically significant, P< 0.005 0 (Tukey's multiple-range test).

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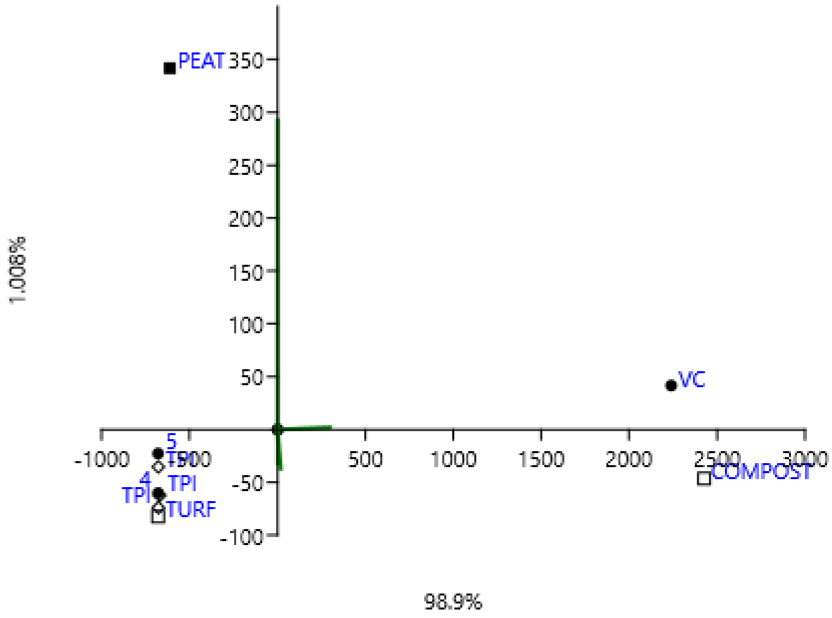


**Figure 4.** PCA ordination diagram for the chemical properties of the samples: turf, vegetal carbon (VC), peat, compost and TPI (*Terra preta do Índio*). Soil parameters evaluated (Iron; Aluminum, Phosphorus level; calcium, magnesium, and potassium levels. Size and orientation of vectors represent correlation among them and with the axes. Eigenvalues are indicated on each axis. Relation between nutrients in natural compared to commercial samples of biochar.

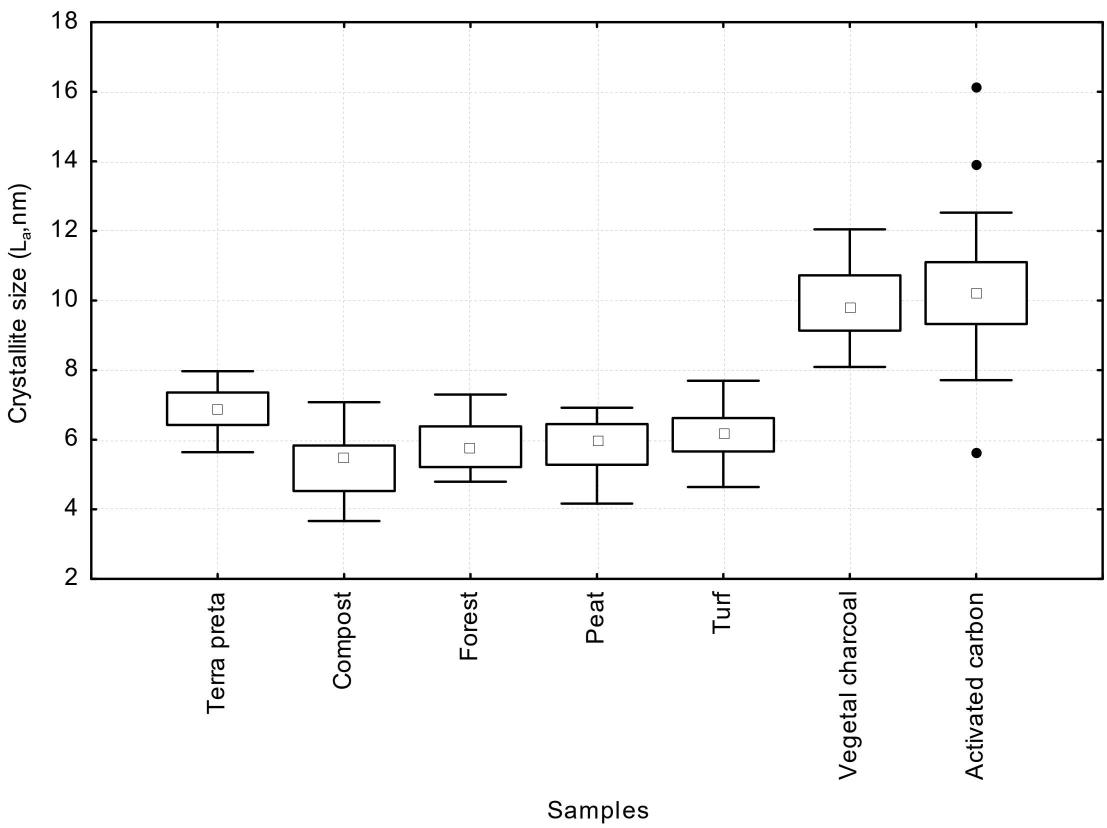


**Figure 5. A.** Average crystallite size La (nm) of carbon in natural compared to commercial samples; = Vegetal charcoal Synth; TPI = Terra preta do Índio (0 - 20 cm); Turf (0-20 cm) and Peat (7 m depth), Compost. Bars represent means accompanied by standard errors.

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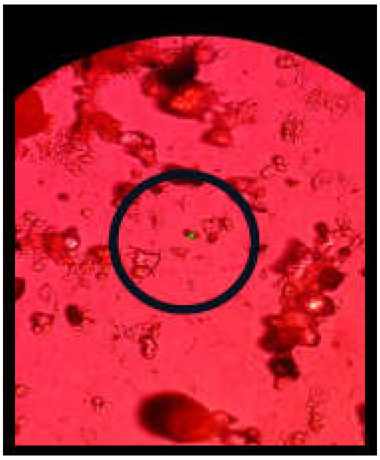
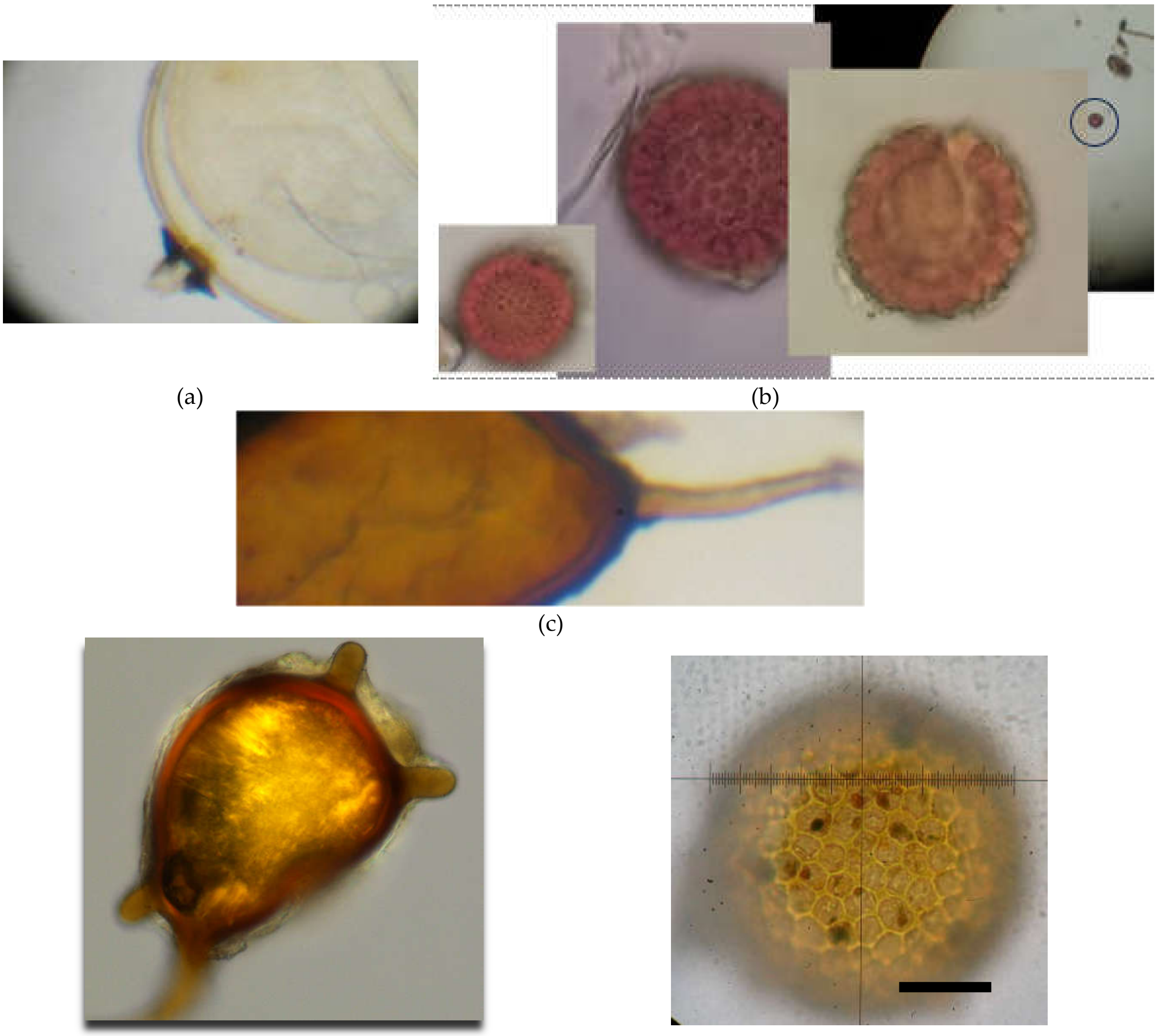


**Figure 5. B.** Image of a C grain. The circle highlights a black carbon grain of TPI being analyzed.



(a) (b)

(c)

(d) (e)

**Figure 6.** Arbuscular mycorrhizal spores obtained from TPI samples (0-20cm (A-C) A= S*cutellospora* sp.; B= *Glomus* like; detailed views, C= *Funneliformis geosporus;* D =*Glomus crenatum. E =Acaulospora excavata* (photos by M. Pagano).

**Table 3.** AMF species in the TPI (0-20cm).

| AMF species | |
|---|---|
| *Acaulospora bireticulata* | |
| *Acaulospora mellea* | |
| *Acaulospora rhemii* | |
| *Acaulospora scrobiculata* | |
| *Ambispora appendicula* | |
| *Claroideoglomus etunicatum* | |
| *Glomus crenatum* | |
| *Funneliformes geosporus* | |
| *Glomus rubiforme* | |
| *Pacispora franciscana* | |
| **Species richness (number of species):** | 10 |

Data obtained for 0-20 cm, 20 g soil samples.

## 4. Discussion

Biochar is a common soil conditioner, besides contributing to plant fertilization, biochar application to soils has multiple benefits improving the soil environment; however, some features such as the biomass source, pyrolysis temperature, application percentage, particle size, and its economic viability modulate the advantages for soil quality, crop yield, removal of pollutants, and greenhouse gases mitigation in agricultural soils, which still remains controversial. The temperature of formation (pyrolysis) is the main factor which determines the properties of biochar, thus, the samples of produced biochar (VC) showed different characteristics than natural soils (TPI, turf, peat, and compost). In the present study, in general, the level of available nutrients in the soils and amendments decreases in the following: order: Compost > TPI > Peat > turf > VC, especially for C, Ca, Zn and Mn. This agrees with findings by Gabhane et al. [1], and Sanchez et al. [6]. The samples of turf, peat, dark soil, and vegetal compost showed different characteristics desirable for C sequestration. In this work, most important strategies for C sequestration are underlined. We showed that TPI contains high fertility levels, but compost showed the highest C content, thus sequestering more carbon in its composition, compared to the other samples. Additionally, it is estimated that TPI Anthropogenic dark soil had a slow formation time (10 years/cm (Glaser and Birk (2012) [5], thus, to reveal the conditions for creating t*erra preta nova* is a desired great plan. Regarding microbiological analysis, TPI contains unique characteristics, besides high microbial abundance, especially, the fungal diversity, which can assist improving plant biofertilization and soil aggregation. In this sense, *Ambispora appendicula*; *Acaulospora rhemii*; *Acaulospora scrobiculata*; *Acaulospora bireticulata*; *Acaulospora mellea*; *Acaulospora spinosa; Scutellospora calospora*; *Claroideoglomus etunicatum*; *Funneliformes geosporus*; and *Pacispora franciscana* were previously found in TPI soils (Pagano et al., 2016) [4]. Contrarily, compost presented vestiges of spores (*Glomus* spp.), which is expected for an amendment not yet integrated to the plant-soil system. The occurrence of AMF species in TPI showed a relatively high richness, including some common and unusual species (spores) (Pagano et al, 2016[4], this study). Further studies in this direction, including the nutritional modes of different species of soil fungi associated with different C materials are needed to elucidate the biochar effect. As it was showed that the crystallite size of carbon from compost, forest, peat, turf, resulted smaller (4 nm) than that of VC, and AC (10 nm). And the small crystallite size indicates that the material is softer, thus, to mimic the TPI, a crystallite size between 6 and 8 nm is desired. Based on these analyses, we can point out that the best strategy for C sequestration and to minimize $GHG_s$ is compost amendment on topsoil; however, nutrient and microorganism supplemented compost has great biofertilizer potential, besides accelerate C sequestration. Additionally, nutrient supplementation of compost can constitute the basis for improved compost-based biofertilizers in the future. Thus, biochar in combination with compost, can adsorb nutrients from this rich amendment, and maintain them inside the holes for slow release and availability for plants. Fortunately, this will reduce the need of chemical fertilizer application. Moreover, the inoculation of AMF can supply more C such as through glomalin-C Therefore, in the future, innovative commercial or manipulated products including biochar could counteract low soil fertility, a common problem worldwide, and this technology would be available to farmers. Therefore, composting of organic wastes and functionality of biochar continue to be a challenge to solve the $CO_2$ aspects of carbon stability in sustainable ecosystems. The occurrence of AMF species in TPI have showed a relatively high richness until 1 m depth [4], which can improve biofertilization of plants and soil structure. Contrarily, compost presented vestiges of spores (*Glomus* species), as no plant was growing in the piles. The occurrence of AMF species in TPI showed a relatively high richness, which could be more investigated. Thus, to mimic TPI, not only soil and biochar are required, but AMF (spores and hyphae) and their secreted glomalin protein are also present and must be considered. Moreover, Turf has been also considered as an appropriate Substrate for inoculated microorganisms, thus, it can be more studied. The temperature of formation is the factor that most influence biochar properties; thus, the structure of C associated with turf, peat, dark soil, and vegetal compost showed different characteristics related to C content and desirable for soil C sequestration, as indicator for Climate change $CO_2$ emissions in sustainable ecosystems. Regarding microbiological analysis, TPI contains high microbial abundance, especially, high fungal diversity. Further studies in this direction, including the nutritional modes of different species of soil fungi associated with different C materials, are needed to elucidate the biochar effect. As it was showed that the crystallite size of compost, forest, peat, and turf, resulted smaller (4 nm) than that of VC, and AC (10 nm). The crystallite size indicates whether the material is soft (small crystallite size), accordingly to the present study, to mimic the TPI fertile soil, a crystallite size between 6 and 8 nm is desired. Among strategies for C sequestration and to minimize $GHG_s$ and $CO_2$ emissions responsible for Climate change, compost amendment on topsoil; however, nutrient and microorganisms-supplemented compost has great biofertilizer potential, besides accelerate soil C sequestration in sustainable ecosystems. Supplements including associated AMF biofertilizers need to be better investigated.

## 5. Conclusions

The best and easiest strategy for C sequestration and to minimize $GHG_s$ content and Climate change $CO_2$ emissions is compost amendment on topsoil; however, nutrient and microorganisms-supplemented compost has great biofertilizer potential, besides accelerate soil C sequestration in sustainable ecosystems. Thus, those supplements could be better investigated. Moreover, Arbuscular mycorrhizae occurring in dark soils prevailed as an important component contributing to soil carbon content via hyphal networks and secretion of glomalin.

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All authors have read and agreed to the published version of the manuscript.

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**Conflict of interest.** The authors declare that they have no conflict of interest.

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