Effect of young biochar, green compost and vermicompost on the quality of a calcareous soil: a one-year laboratory experiment

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Abstract

Purpose: Biochar addition has been recognized as a potential way to improve soil quality. However, questions remain regarding the influence of biochar on soil biological activity. In order to mitigate the possible negative effects of biochar on soil biological activities, it can be enriched by amendments such as compost. Since there is no unanimity on the advantages of biochar when mixed with amendments, it is important to ascertain how the impacts of biochar on soil biological activity could be changed by the addition of compost.

Materials and methods: A 360-d aerobic incubation was carried out of a soil treated with biochar, green compost, vermicompost, biochar+green compost and biochar+vermicompost. The biochar was produced from pruning residues of fruit trees by slow pyrolysis at 550 °C. The green compost was taken to the CERMEC facility (Massa Carrara, Italy) and the vermicompost was produced mainly from farmyard manure and green waste by the Centro di Lombricoltura Toscano (Pisa, Italy). The pH, total and dissolved organic C, microbial biomass, dehydrogenase and alkaline phosphatase were monitored. The metabolic quotient, specific enzyme activities and the metabolic potential were calculated.

Results and discussion: After 360-d incubation the green compost and vermicompost significantly lowered the alkaline soil pH by about one unit, increased total and dissolved organic C, microbial biomass, microbial quotient, alkaline phosphatase and specific alkaline phosphatase, dehydrogenase and specific dehydrogenase, and metabolic potential. The improvement in the biological activity was more notable and permanent with vermicompost than green compost. The biochar lowered soil pH by about one unit, showed the lowest loss of the total organic C (3.9%), did not change the amounts of dissolved organic C and microbial biomass, induced scarce effects on biological activities. When mixed with biochar, composts significantly induced higher C mineralization, dissolved organic C, microbial biomass, dehydrogenase, and did not change the metabolic quotient, specific alkaline phosphatase and specific dehydrogenase activities. The metabolic potential of control was more than halved by the green compost (2.89) and was not changed by the vermicompost.

Conclusions: The mixing of green compost, and especially vermicompost with biochar increased some biological parameters in the used calcareous soil compared with the biochar-only treatment. Biochar could have benefits for carbon sequestration. The specific enzyme activities (alkaline phosphatase and dehydrogenase) were more suitable indicators than the respective absolute activities and metabolic potential for detecting the effects of amendments on soil microbial activity.

Keywords: Biochar • Calcareous soil • Green compost • Soil biological activity • Vermicompost


1 Introduction

Concerns regarding the productivity of agro-ecosystems have stressed the need to develop management practices capable of maintaining soil resources. In the Mediterranean area, soils are degraded due to the loss of organic matter (Albaladejo and Diaz 1990). Methods to reverse this degradation include the addition of amendments. Xie et al. (2016) summarized the characteristics of biochar, a carbon-rich product created from different feedstocks, and identified the potential of this material to maintain soil quality and sequester carbon. They concluded that biochar performed well in terms of the improvement in organic carbon, pH and cation exchange capacities of soil, but they also recommended additional studies. Igalavithana et al. (2016) have shown that biochar addition enhances the soil fertility, especially for poor, acidic soils. Agegnehu et al. (2016) reported an increase of the carbon stock, available P, exchangeable Ca and cation-exchange capacity in soil after biochar addition. In contrast, the meta-analysis by Jeffery et al. (2011) mentioned the negative effects of young (artificially prepared) biochar addition, such as nutrient immobilization, especially due to the adsorption of mineral N and water-soluble organic carbon (Graber and Elad 2013). Non significant effects of biochar on soil characteristics have also been reported. Yamato et al. (2006) reported non significant increases in soil pH, N, available P and cation-exchange capacity following the biochar amendment of an infertile soil. The meta-analysis of Biederman and Harpole (2013) highlights the non significant effects of biochar in soil under a temperate climate. Biederman et al. (2017) found that biochar and manure treatments did not change soil pH, inorganic nitrogen concentrations and extractable soil K, and Cardelli et al. (2016) reported no interactions with native soil C, that is priming effect.

Soil biological characteristics have been proposed as sensitive indicators of soil changes which can thus be used to predict trends in soil quality. Bailey et al. (2011) observed variable effects of biochar on enzyme activities in soils, which depended on the reactions between
biochar and the substrate. Chintala et al. (2014) observed a decrease in dehydrogenase, β-
glucosidase, protease and arylsulphatase activities in soils amended with biochar. Zhang et
al. (2017) reported increases of soil microbial biomass and no significant effect in alkaline
phosphatase with biochar application. Luo et al. (2013) reported microbial colonizations
following biochar addition, while Biederman et al. (2017) observed a lack of influence of
biochar on soil microbial biomass carbon.

Although the effect of biochar in acidic soils has been studied extensively, insufficient
research has been carried out on calcareous soils. Recently, El-Naggar et al. (2015) reported
that the biochar addition to calcareous soils may improve carbon sequestration and soil
fertility. However, questions remain regarding the influence of biochar on soil biological
activities (Kolb et al. 2009) or soil processes (Granatstein et al. 2009).

In order to mitigate the possible negative effects of young biochar, it can be enriched by
organic and/or mineral nutrients (Gathorne-Hardy et al. 2009; Joseph et al. 2013). However,
there is no unanimity on the advantages of biochar when mixed with amendments. The
biochar and compost combination increased soil organic C and the activity of enzymes
(Trupiano et al., 2017). The quality of amendments is of major importance in the regulation
of microbiological properties. Some research has related the quality and stability of compost
and vermicompost to their effects on biological properties (Diacono and Montemurro 2010;
Yakushev et al. 2011). Vermicomposts are usually more stable than composts, with a higher
availability of mineral nutrients and improved biological properties (Pramanik et al. 2007;
Yakushev et al. 2011). We hypothesize that biochar may have benefit for carbon sequestration
and that mixing biochar with green compost or vermicompost may change the biological
activity in soil. The objectives of this study were i) to evaluate the impacts of green compost,
vermicompost and biochar on a calcareous soil, and ii) to test whether the biochar effects on
soil quality could be changed by the addition of green compost or vermicompost. A 360-d
aerobic incubation was carried out of a soil (Control) treated with biochar (B), green compost (GC), vermicompost (VC), biochar + green compost (BGC) and biochar + vermicompost (BVC). Changes in chemical properties and biological activities were monitored.

2 Materials and methods

2.1 Soil sampling

Surface (0–15 cm) soil was collected from a dedicated agricultural area at the Interdepartmental Centre E. Avanzi, which is located at a distance of approximately 4 km from the sea (43°40′N, 10°19′E) and 1 m above sea level (Pisa, Italy). The soil sample was air-dried and passed through a 2-mm sieve to remove large residue fragments. The main soil characteristics were: 73.3% sand (2 - 0.05 mm), 12.2% silt (0.05 - 0.002 mm), 14.5% clay (< 0.002 mm), 8.2 pH, 7.7% inorganic C, 1.42 g kg⁻¹ total organic C (TOC), 0.17 g kg⁻¹ dissolved organic C (DOC), 1.30 g kg⁻¹ total N, 40.4 mg kg⁻¹ available P, 350.3 mg kg⁻¹ available K, 10.3 cmol (+) kg⁻¹ cation exchange capacity (CEC). The soil was classified as a Xerorthent.

2.2 Organic materials

The young biochar was produced from orchard pruning residues of fruit trees (Pirus communis, Malus domestica, Persica vulgaris, Vitis vinifera) by slow pyrolysis process with a transportable ring kiln (215 cm in diameter and holding around 2t of hardwood). The average heating rate before reaching the peak of 550 °C was 15-18 °C min⁻¹. The green compost was taken to the CERMEC facility (Massa Carrara, Italy), which is designed to take green waste from neighbouring producers. The composting process was designed as an initial forced-air, in-vessel composting process, over two weeks. The composted material is removed from the tunnels and placed in "windrows" in a maturation area, for twelve weeks before being screened. The vermicompost, taken to the Centro di Lombricoltura Toscano
(Pisa, Italy), was produced mainly from farmyard manure and green waste. The composition of the organic materials is reported in Table 1.

2.3 Incubation procedures

In 2-L microcosms, the experiment was conducted with six treatments to differentiate between the influence of amendments alone or in combination with biochar (Table 2). The soil and soil-mixture parameters were monitored for 360 days through an aerobic incubation. The samples were watered at appropriate intervals to maintain a constant moisture level (60% maximum water holding capacity), closed with parafilm to permit a gaseous exchange, and incubated at 28 ± 1 °C for 360 days. Six sampling times were selected to monitor the soil parameters: at 15 (T1), 30 (T2), 60 (T3), 120 (T4), 180 (T5), and 360 (T6) days after the amendments. At each sampling time, 50g of soil were taken out of each microcosm and frozen at 4 °C for further analyses.

2.4 Soil analyses

The particle-size distribution of the soils was obtained by the pipette method. The pH was determined according to the SISS (1995) using a soil-to-water ratio of 1:2.5; inorganic carbon (CaCO₃) was determined with a Scheibler apparatus; TOC was determined by dry combustion (induction furnace 900 CS, Eltra); total N was determined by the Kjeldahl procedure after acid digestion (Bremner and Mulvaney, 1982); available P was measured on the 0.5 N NaHCO₃ extract at pH 8.5±0.1 (Olsen et al. 1954); exchangeable K was determined on the 1 N CH₃COONH₄ extract at pH 7.0 (Thomas, 1982); cation exchange capacity (CEC) was determined according to Bascomb (1964).

The DOC was determined at T1 and T6 by stirring soil samples with distilled water (soil / H₂O 1:20) for 24 h at room temperature, centrifuging the suspension at 10,000 rpm for 10
min, and filtrating it through a 0.45 mm glass fiber. In this extract, DOC was determined with an organic C analyzer for liquid samples (Hach QbD1200). Soil microbial biomass C was determined at T1 and T6 according to Vance et al. (1987) with the extraction of organic C from fumigated and unfumigated soils by 1 N K_{2}SO_{4}. The organic C was then measured as described by Jenkinson and Powlson (1976) using dichromate digestion. An extraction efficiency coefficient of 0.38 was used to convert the difference in soluble C between the fumigated and the unfumigated soils into microbial biomass C (Vance et al. 1987).

2.5 Biological activities

The soil biological activity was assayed on freshly-sieved samples. Dehydrogenase activity (DH-ase) was determined by a colorimetric assay of 2,3,5 triphenylformazan (TPF) produced by the microorganism reduction of 2,3,5 triphenyltetrazolium chloride (TTC) (Casida et al. 1964). Alkaline phosphatase activity was determined by the colorimetric assay with p-nitrophenol released after incubation of the soil samples with p-nitrophenyl-phosphate (Eivazi and Tabatabai, 1977). The specific enzyme activity was calculated by dividing the enzyme activity by total organic C (Trasar-Cepeda et al. 2008). The metabolic potential (MP) was calculated as follows: MP = DH-ase/ 10^{-3} DOC (Masciandaro et al. 1998).

2.6 Amendments analyses

The main characteristics of B, GC and VC were determined using standard methods according to ANPA (2001).

2.7 Statistics
Statistica 7.0 software (StatSoft Inc., Tulsa, Oklahoma, USA) was used for the statistical analysis. Data were expressed on the basis of the oven-dry weight of the soil. Results were the means of determinations carried out on three replicates. Differences among mean replicate values for treatments were compared at the 0.05 significant level by analysis of variance (ANOVA).

3 Results and discussion

Figure 1 shows that at T1 B led to an increase in the soil reaction compared to the control. This was expected, given the high pH values (10.2) of biochar (Table 1), due to the carbonates, basic oxides and organic carboxylates produced during pyrolysis (Yuan et al. 2010). The alkalizing effect of B on pH could also be due to the poor soil buffering due to the low level of organic matter in the system. In contrast to B, GC and VC lowered soil pH. Differently, the application of alkaline biochar, which has a slightly lower pH than the soils, was not found to increase the soil pH of five types of alkaline soils (Liu and Zhang 2012). Previous studies also indicate that organic amendments can lower soil pH. Accordingly, Saviozzi et al. (2006) observed that green compost significantly decreased the pH of the control (pH 8.6) already at the first sampling time. Uz et al. (2016) reported that pH values of an alkaline soil receiving vermicompost decreased significantly over two growth seasons. GC and BVC did not affect the alkanizing influence of B (Table 1), with significantly similar values to those induced by the material alone (Figure 1).

During incubation, there was a constant decrease in soil reaction in all amended soils, likely attributable to the production of acidifying nitrates and/or to a release of functional groups of an acidic character during the oxidation of B (Liu and Zhang 2012). According to Atkinson et al. (2010), the binding of Ca to P reduces the concentration of Ca ions in a soil solution. The pH elevation in B, BGC and BVC was temporary as the biochar alkali salts and functional
groups reacted with carbonic acid from microbial activity and atmospheric CO\(_2\) to form bicarbonates, thus lowering the soil pH below 8.4. In BGC and BVC, the pH began to be lower than the control 4 months after the application of the material (T4), while in B, the same effect was observed only after 6 months (T5). However, at the end of incubation (T6) the differences in pH between treatments disappeared, with values being lower by about one unit compared to the control.

Figure 2 presents the TOC changes in the soil during the experiment. As expected, at T1 the addition of amendments to the soil increased the TOC content (p < 0.05), which was almost proportional to the amounts applied. In all treatments, TOC decreased during incubation and, at T6, the organic C values differed significantly from each other, without statistically justified differences only between the two types of compost. In the control, the remaining TOC at T6 was 94.3%, while in both GC and VC about 92% of the initial TOC was found. In B 96.1% of the initial TOC content remained, indicating a more efficient stabilization of the soil organic matter. In line with our findings, Zimmerman et al. (2011) reported that C mineralization was generally lower than expected for soils treated with biochars produced at 525 and 650 °C and from hard woods, similarly to those used in our study.

In BGC and BVC only about 90% of the initial TOC was found, suggesting that both compost additions led to higher TOC mineralization when combined with the biochar. As the TOC decrease was higher in BGC and BVC compared to GC and VC, and since the biochar was only slightly degraded during the experiment, the changes in TOC could be due to the mineralization of the organic fraction of the composts. Schulz and Glaser (2012) demonstrated, however, that the labile organic matter of compost can be stabilized by biochar.

On the other hand, the decomposition of added plant residues in soil have been found to be enhanced by biochar (Awad et al. 2012). This may be attributed to more favourable soil aeration and porosity, induced by the biochar thus stimulating microbial growth and
respiration (Lei and Zhang 2013). Although the biochar is much more stable than both comports, the greater TOC decrease in BGC and BVC could be also explained by the increased decomposition of biochar when mixed with the two comports. Indeed, as observed by Kuzyakov et al. (2009), biochar decomposition rates increase until an easily degradable substrate, in our case provided by the compost, is available.

Table 3 reports the amount of DOC at T1 and T6 in soil systems. GC and VC led to significantly increased DOC contents, with the much larger initial rise occurring in GC (Table 3). As suggested by Ngo et al. (2011), the vermicompost is a more decomposed and stabilized organic substrate, with lower forms of C available to microorganisms. The higher content of TOC in GC than VC (Table 1) could also account for the difference between the two types of compost. Smith et al. (2010) demonstrated that young biochar provides significant amounts of labile C. In our study, B did not change the level of DOC in the soil. At T6, lower values of DOC were generally observed for each soil-system than at T1, perhaps because the water-soluble C is degraded in the first stage of mineralization (Pascual et al. 1997). GC and VC increased the DOC level compared to B. The DOC values of mixtures remained significantly higher at T6 compared to B and the control.

Table 3 shows changes in the amount of soil microbial biomass at T1 and T6 in the soil systems. The incorporation of both comports in soil increased the microbial biomass C, which reflects the increased number of microorganisms. This increase may be due to the growth in soil microbiota in response to the easily available C, and/or to the addition of foreign microorganisms by the materials.

The highest initial increase in biomass C content occurred in VC. Similarly, Aira and Dominguez (2008) found a higher microbial biomass in vermicompost than in compost. Studying the impact of vermicompost on the biological characteristics of an alkaline soil, Uz et al. (2016) reported a strong increase in the bacterial number. Most studies indicate that
biochar increases the microbial biomass (Lehmann et al. 2011; Zhang et al. 2017). However, changes in the amount of microorganisms are likely connected to the intrinsic properties of both biochar and the soil (Khodadad et al. 2011). Dempster et al. (2012) found a decrease in soil microbial biomass with biochar addition to a coarse textured soil. In a six-year field study, biochar amendment did not change soil microbial population (Tian et al. 2016).

Liang et al. (2010) reported an increase in microbial biomass related to an increase in labile organic carbon, such as DOC, which acts as a substrate for microbial nutrition. The increase in soil pH may also account for the lack of changes in the amount of microbial biomass (Lehmann et al. 2011). In our research, the level of microbial biomass in B did not increase and was never significantly different to that of the control. This is probably due to the increase in pH value (Figure 1) and/or because the addition of biochar did not increase soil DOC (Table 3). Although the biomass C level was lower in BGC and BVC compared to GC and VC (Table 3), both compost additions to B increased the amount of biomass C compared to B and the control. This suggests that native soil fertility can be likewise increased with the biochar-compost amendments. Since the TOC mineralization was higher when both composts were combined with B (Figure 2), it is possible that the microbial biomass of mixtures, although in a lesser amount, is more active.

For each soil-system, we found that at T6 the biomass C values were 1.8 - 2.4 times lower than at T1, perhaps because DOC, which acts as an energy source for the microorganisms and contributes to their biomass, degrades rapidly. The biomass C level in B fell as sharply as it did with the other treatments, in spite of the higher stability of the material. The fall in the level of biomass C in the control may be due to the disturbance of the soil ecosystem in laboratory conditions. Nevertheless, with the exception of B, biomass C values in amended soils were higher than in the control, which clearly indicates the improvement in soil biological quality due to the organic amendment.
After one year (T6), the amount of biomass C was 1.8-fold (for GC) and about 4-fold (for VC) higher than that of the control. Although biomass C was expressed on the basis of TOC (microbial quotient) (Table 3), the values decreased between T1 and T6, indicating a true decline in the microbial biomass. After one year, a higher level of the microbial quotient compared to the control was found only for VC.

Vermicompost therefore appears to be the best amendment, of those tested, to stimulate the growth of soil microorganisms. The lowest metabolic quotient found was for B. The value found for the biochar treatment explains the low tendency of its organic matter to mineralize (Pascual et al. 1997). This indicates a higher stabilization of the organic matter of biochar compared to both composts, both at the beginning and the end of the incubation experiment. The result confirms the TOC trends (Figure 2) which were characterized by the lowest decrease for B. GC and VC did not increase the metabolic quotient of B, both at T1 and T6.

Figure 3 shows that B had significantly more AP-ase activity than the control from T4, after which it increased further up to T5 and then stabilized. These results are in agreement with studies reporting that the activity of alkaline phosphatase increased with biochar applications (Jin, 2010; Lehmann et al. 2011; Masto et al. 2013; Trupiano et al. 2017). Similarly to B, the AP-ase in VC and GC were higher than that of control from T4, increased up to T5, after which the enzyme activity stabilized towards the end of experiment (Figure 3). VC had significantly higher AP-ase activity compared to GC. In fact, Saha et al. (2008), Doan et al. (2013) and Uz et al. 2016 observed an increase in AP-ase with vermicompost application. We observed similar patterns for BGC and BVC, which started to show significantly higher AP-ase over the control, at approximately the same time as GC and VC. Our results also show that the AP-ase activity in the soil treated with biochar was not enhanced by the addition of green compost (Figure 3). The vermicompost significantly increased the AP-ase enzyme activity in B, although it was less affected by vermicompost than expected using an additive.
calculation. As with VC, BVC consistently showed the highest AP-ase activity during incubation. However, note that AP-ase is substrate specific, extracellular and active in soil, and does not reflect the total microbial status of the soil.

Both GC and VC significantly supported more DH-ase activity than the control throughout the experimental period (Figure 3). DH-ase activity was significantly higher in VC than in GC at each sampling time. Arancon et al. (2006) also reported high soil DH-ase activity following vermicompost applications. Lower DH-ase was found in B compared to the control already at T1, and persisted throughout the experiment (Figure 3).

Similar results were observed by Bandara et al. (2015), while no biochar amendment effects of DH-ase were found by Wu et al. (2013) in a chernozemic soil after a 100-day incubation period, and by Niemi et al. (2015) in two different types of soil, each bare and cultivated, during one growing season. Ameloot et al. (2015) suggested that the level of soil organic matter can affect DH-ase activity in biochar amended soil, due to the increased physical contact between the biochar particles and microorganisms. They observed no changes in DH-ase in soil with 0.89% C, however they found higher enzyme activity than control in soil with a higher C content (1.61%). Thus, the amount of soil organic C (1.47%) (see Materials and Methods) would have supported a higher enzymatic activity.

The response of DH-ase activity in B might be from toxic compounds in the material (Moeskops et al., 2010). The poor level of DH-ase activity in B could also be explained by the results of Swaine et al. (2013), who reported that biochar amendments led to significant reductions in concentrations of substrate and extractable product in soil DH-ase assay, thus limiting the identification of biochar effects on soil enzyme activity. Since DH-ase acts in the biological oxidation of organic matter in the soil, the low level of the enzyme in B is consistent with the low tendency of its organic matter to mineralize, which was already inferred from the TOC values (Figure 2) and the microbial quotient (Table 3). When green compost and
vermicompost were mixed with biochar, the DH-ase activity increased, with values that did not differ substantially from the control during trials. However, DH-ase in mixtures never reached the GC and VC levels. This confirms the reducing effect induced by B on DH-ase. Since DH-ase is considered as a respiratory enzyme, this result seems inconsistent with the high mineralization rate of organic matter in BGC and BVC, revealed by the TOC trends (Figure 2). Again, although losses of DH-ase in mixtures may be attributed to decreasing effects of B on the enzyme activity, values may be underestimated because of the impact of biochar on assay constituents.

If alkaline phosphatase activity is expressed in relation to TOC (specific enzyme activity, AP-ase TOC$^{-1}$), lower values were found in each treated soil at T1 than the control (Table 4). The specific AP-ase activities in B were about one third that of the control. Note that the reducing effect of B on the AP-ase activity, already highlighted by the results for absolute values, was emphasized by expressing DH-ase per unit C. As reported by Bastida et al. (2012), extracellular enzymes can be stabilized via the formation of enzyme-clay or enzyme–humus complexes. Thus, the lower specific AP-ase in the amended soil may reflect the immobilisation of enzymes following the biochar addition. GC and VC did not significantly change the specific AP-ase activity in B, both at T1 and T6 (Table 4). At T6, the specific AP-ase activity did not change in the control but increased in all the amended soils, due to the reduction in soil organic C (Figure 2) and the concurrent increase in enzyme activity (Figure 3). Only in GC and even more in VC did values exceed that of the control.

Regarding DH-ase activity in relation to TOC (specific enzyme activity, DH-ase TOC$^{-1}$), a value was found which was about three times lower in B than in the control, both at T1 and T6 (Table 4). The observed decline in the specific activities of soil DH-ase following the biochar amendment was not attributable to a lower microbial biomass content (Table 3). These results may indicate a worse nutritional status of the organic matter of B and/or a toxic
effect of compounds present in the material. As for the absolute values of DH-ase, the specific enzyme activities were higher in GC and VC than in the control, however this happened only at T1, while at T6, differences disappeared. Similarly to the results related to the absolute values of DH-ase, values of the specific DH-ase activity in B were not increased by the addition of either of the two composts. Unlike DH-ase, the specific DH-ase in mixtures never reached the levels of the control, thus indicating the strong influence of B on the enzyme activity. Similarly to findings for the specific AP-ase activity, the lowering effect of B on the DH-ase activity was emphasized by expressing DH-ase as specific activity. These results suggest that specific enzyme activity may be a more suitable indicator than the absolute values in detecting the effect of the B amendment on soil microbial activity.

The dynamics of soil biological activity can also be described by the metabolic potential index (MP) (Masciandaro et al. 1998). Unlike absolute and specific DH-ase, the MP was not changed by B compared to the control. Of the two composts, the MP increased at T1 only for the vermicompost treatment with respect to the control (Table 4), thus revealing less evident soil responses to amendments than AP-ase and DH-ase TOC⁻¹ indexes. The MP in VC was also found to be the highest at T6, which is consistent with Masciandaro et al. (2000) who found an increase in MP in a soil amended with vermicompost one year after the treatment. This confirms the stimulation of soil metabolism by VC, already observed for biomass C (Table 3), AP-ase and DH-ase (Figure 3). The results are probably due to an increase in available organic substrates and/or the fact that the water-soluble organic carbon of vermicompost is particularly effective in stimulating enzyme activity. In spite of the high MP in VC, the addition of vermicompost did not significantly change the MP in B, either at T1 or T6. The MP of the B treatment was more than halved by when it was mixed with green compost, due to the very high DOC content in BGC (Table 3).
4 Conclusions

Biochar application to the used calcareous soil increased TOC but had scarce effects on biological parameters, thus confirming that the material may be beneficial mainly in C sequestration. Biochar-compost applications showed additional benefits compared to simply adding biochar, in terms of availability of water-soluble C (DOC), the amount of microbial biomass and DH-ase activity, although the values of these parameters did not reach the levels attained by VC and GC. These results suggest the limiting effect of biochar on some biological activities. Other biological parameters were not affected by mixing the compost with biochar, such as metabolic quotient, specific AP-ase activity, and specific DH-ase activity. Between composts, the improvement in the soil biological activity was more notable and permanent with VC than GC, highlighting the beneficial influence of the material. Some quality indexes were influenced by only one type of compost. The AP-ase activity increased after the addition of vermicompost, although in a non-additive way. In addition, MP was more than halved by the green compost but was not changed by the vermicompost. The specific enzyme activities (AP-ase and DH-ase) proved to be more suitable indicators than the respective absolute activities and MP for detecting the effect of amendments on soil microbial activity. However, since the influence of amendments on soil quality depends on site-specific conditions (Haefele et al. 2011), the resulting benefit of mixing biochar and compost needs to be determined in further calcareous soils, under field conditions and for longer-term monitoring. Further research on the identification and quantification of potentially toxic compounds released by the biochar may also explain its supposed negative effect on soil biological activity.

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Table 1. Selected characteristics of the organic materials

<table>
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<th>vermicompost</th>
<th>biochar</th>
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<tr>
<td>pH</td>
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<td>Inorganic C %</td>
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<td>Organic C %</td>
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<td>443</td>
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<td>Exchangeable K mg·g⁻¹</td>
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Table 2. Experimental setup

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<th>Biochar</th>
<th>Green Compost</th>
<th>Vermicompost</th>
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<td></td>
<td>g</td>
<td>% by weight</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>1000</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soil + green compost (GC)</td>
<td>1000</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Soil + vermicompost (VC)</td>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Soil + biochar (B)</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soil + biochar + green compost (BGC)</td>
<td>1000</td>
<td>2.5</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>Soil + biochar + vermicompost (BVC)</td>
<td>1000</td>
<td>2.5</td>
<td>0</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Table 3. Changes of dissolved organic C (DOC), microbial biomass C and microbial quotient in soil at the start (T1) and the end (T6) of incubation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T1</th>
<th>T6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DOC (µg g⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>174 e</td>
<td>132 fg</td>
</tr>
<tr>
<td>GC</td>
<td>350 a</td>
<td>256 b</td>
</tr>
<tr>
<td>VC</td>
<td>226 cd</td>
<td>159 e</td>
</tr>
<tr>
<td>B</td>
<td>157 ef</td>
<td>124 g</td>
</tr>
<tr>
<td>BGC</td>
<td>368 a</td>
<td>248 bc</td>
</tr>
<tr>
<td>BVC</td>
<td>209 d</td>
<td>161 e</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microbial biomass C (µg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>GC</td>
</tr>
<tr>
<td>VC</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>BGC</td>
</tr>
<tr>
<td>BVC</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microbial quotient (microbial biomass C TOC⁻¹ 10²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>GC</td>
</tr>
<tr>
<td>VC</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>BGC</td>
</tr>
<tr>
<td>BVC</td>
</tr>
</tbody>
</table>

GC = green compost treatment; VC = vermicompost treatment; B = biochar treatment; BGC = biochar+green compost treatment; BVC = biochar+vermicompost treatment

Means followed by the same letter were not significantly different (p<0.05) according to Tuckey’s test.
Table 4. Changes in biochemical properties in soil at the start (T1) and the end (T6) of incubation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T1</th>
<th>T6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific enzyme activity (AP-ase TOC⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>60.6 cd</td>
<td>64.5 c</td>
</tr>
<tr>
<td>GC</td>
<td>41.1 f</td>
<td>105.0 b</td>
</tr>
<tr>
<td>VC</td>
<td>48.3 ef</td>
<td>123.9 a</td>
</tr>
<tr>
<td>B</td>
<td>22.9 g</td>
<td>58.8 cde</td>
</tr>
<tr>
<td>BGC</td>
<td>21.0 g</td>
<td>50.9 def</td>
</tr>
<tr>
<td>BVC</td>
<td>21.3 g</td>
<td>58.9 cd</td>
</tr>
<tr>
<td></td>
<td>Specific enzyme activity (DH-ase TOC⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.52 c</td>
<td>0.47 c</td>
</tr>
<tr>
<td>GC</td>
<td>0.66 b</td>
<td>0.37 d</td>
</tr>
<tr>
<td>VC</td>
<td>0.75 a</td>
<td>0.49 c</td>
</tr>
<tr>
<td>B</td>
<td>0.18 e</td>
<td>0.15 e</td>
</tr>
<tr>
<td>BGC</td>
<td>0.14 e</td>
<td>0.16 e</td>
</tr>
<tr>
<td>BVC</td>
<td>0.21 e</td>
<td>0.18 e</td>
</tr>
<tr>
<td></td>
<td>MP (DH-ase DOC⁻¹)₁₀³</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.25 c</td>
<td>4.77 c</td>
</tr>
<tr>
<td>GC</td>
<td>4.09 c</td>
<td>2.89 d</td>
</tr>
<tr>
<td>VC</td>
<td>6.95 a</td>
<td>5.91 b</td>
</tr>
<tr>
<td>B</td>
<td>4.08 c</td>
<td>4.19 c</td>
</tr>
<tr>
<td>BGC</td>
<td>1.66 e</td>
<td>2.50 de</td>
</tr>
<tr>
<td>BVC</td>
<td>4.31 c</td>
<td>4.16 c</td>
</tr>
</tbody>
</table>

GC = green compost treatment; VC = vermicompost treatment; B = biochar treatment; BGC = biochar+green compost treatment; BVC = biochar+vermicompost treatment.

Means followed by the same letter were not significantly different (p<0.05) according to Tuckey’s test.