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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, expecially 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

126 (Pisa, Italy), was produced mainly from farmyard manure and green waste. The composition
127 of the organic materials is reported in Table 1.
128
129 **2.3 Incubation procedures**
130 In 2-L microcosms, the experiment was conducted with six treatments to differentiate
131 between the influence of amendments alone or in combination with biochar (Table 2). The
132 soil and soil-mixture parameters were monitored for 360 days through an aerobic incubation.
133 The samples were watered at appropriate intervals to maintain a constant moisture level (60%
134 maximum water holding capacity), closed with parafilm to permit a gaseous exchange, and
135 incubated at 28 ± 1 °C for 360 days. Six sampling times were selected to monitor the soil
136 parameters: at 15 (T1), 30 (T2), 60 (T3), 120 (T4), 180 (T5), and 360 (T6) days after the
137 amendments. At each sampling time, 50g of soil were taken out of each microcosm and frozen
138 at 4 °C for further analyses.
139
140 **2.4 Soil analyses**
141 The particle-size distribution of the soils was obtained by the pipette method. The pH was
142 determined according to the SISS (1995) using a soil-to-water ratio of 1:2.5; inorganic carbon
143 ($CaCO_3$) was determined with a Scheibler apparatus; TOC was determined by dry combustion
144 (induction furnace 900 CS, Eltra); total N was determined by the Kjeldahl procedure after
145 acid digestion (Bremner and Mulvaney, 1982); available P was measured on the 0.5 N
146 $NaHCO_3$ extract at pH 8.5±0.1 (Olsen et al. 1954); exchangeable K was determined on the 1
147 N CH_3COONH_4 extract at pH 7.0 (Thomas, 1982); cation exchange capacity (CEC) was
148 determined according to Bascomb (1964).
149 The DOC was determined at T1 and T6 by stirring soil samples with distilled water (soil /
150 H_2O 1:20) for 24 h at room temperature, centrifuging the suspension at 10,000 rpm for 10

151 min, and filtrating it through a 0.45 mm glass fiber. In this extract, DOC was determined with
152 an organic C analyzer for liquid samples (Hach QbD1200). Soil microbial biomass C was
153 determined at T1 and T6 according to Vance et al. (1987) with the extraction of organic C
154 from fumigated and unfumigated soils by 1 N K_2SO_4. The organic C was then measured as
155 described by Jenkinson and Powlson (1976) using dichromate digestion. An extraction
156 efficiency coefficient of 0.38 was used to convert the difference in soluble C between the
157 fumigated and the unfumigated soils into microbial biomass C (Vance et al. 1987).
158
159 **2.5 Biological activities**
160 The soil biological activity was assayed on freshly-sieved samples. Dehydrogenase activity
161 (DH-ase) was determined by a colorimetric assay of 2,3,5 triphenylformazan (TPF) produced
162 by the microorganism reduction of 2,3,5 triphenyltetrazolium chloride (TTC) (Casida et al.
163 1964). Alkaline phosphatase activity was determined by the colorimetric assay with p-
164 nitrophenol released after incubation of the soil samples with p-nitrophenyl-phosphate
165 (Eivazi and Tabatabai, 1977).
166 The specific enzyme activity was calculated by dividing the enzyme activity by total organic
167 C (Trasar-Cepeda et al. 2008). The metabolic potential (MP) was calculated as follows: MP
168 = DH-ase/ 10^{-3} DOC (Masciandaro et al. 1998).
169
170 **2.6 Amendments analyses**
171 The main characteristics of B, GC and VC were determined using standard methods according
172 to ANPA (2001).
173
174 **2.7 Statistics**

175 Statistica 7.0 software (StatSoft Inc., Tulsa, Oklahoma, USA) was used for the statistical
176 analysis. Data were expressed on the basis of the oven-dry weight of the soil. Results were
177 the means of determinations carried out on three replicates. Differences among mean replicate
178 values for treatments were compared at the 0.05 significant level by analysis of variance
179 (ANOVA).
180
181 **3 Results and discussion**
182 Figure 1 shows that at T1 B led to an increase in the soil reaction compared to the control.
183 This was expected, given the high pH values (10.2) of biochar (Table 1), due to the
184 carbonates, basic oxides and organic carboxylates produced during pyrolysis (Yuan et al.
185 2010). The alkalizing effect of B on pH could also be due to the poor soil buffering due to the
186 low level of organic matter in the system. In contrast to B, GC and VC lowered soil pH.
187 Differently, the application of alkaline biochar, which has a slightly lower pH than the soils,
188 was not found to increase the soil pH of five types of alkaline soils (Liu and Zhang
189 2012).Previous studies also indicate that organic amendments can lower soil pH.
190 Accordingly, Saviozzi et al. (2006) observed that green compost significantly decreased the
191 pH of the control (pH 8.6) already at the first sampling time. Uz et al. (2016) reported that
192 pH values of an alkaline soil receiving vermicompost decreased significantly over two growth
193 seasons. GC and BVC did not affect the alkalinizing influence of B (Table 1), with
194 significantly similar values to those induced by the material alone (Figure 1).
195 During incubation, there was a constant decrease in soil reaction in all amended soils, likely
196 attributable to the production of acidifying nitrates and/or to a release of functional groups of
197 an acidic character during the oxidation of B (Liu and Zhang 2012). According to Atkinson
198 et al. (2010), the binding of Ca to P reduces the concentration of Ca ions in a soil solution.
199 The pH elevation in B, BGC and BVC was temporary as the biochar alkali salts and functional

200 groups reacted with carbonic acid from microbial activity and atmospheric CO_2 to form
201 bicarbonates, thus lowering the soil pH below 8.4. In BGC and BVC, the pH began to be
202 lower than the control 4 months after the application of the material (T4), while in B, the same
203 effect was observed only after 6 months (T5). However, at the end of incubation (T6) the
204 differences in pH between treatments disappeared, with values being lower by about one unit
205 compared to the control.
206 Figure 2 presents the TOC changes in the soil during the experiment. As expected, at T1 the
207 addition of amendments to the soil increased the TOC content ($p < 0.05$), which was almost
208 proportional to the amounts applied. In all treatments, TOC decreased during incubation and,
209 at T6, the organic C values differed significantly from each other, without statistically
210 justified differences only between the two types of compost. In the control, the remaining
211 TOC at T6 was 94.3%, while in both GC and VC about 92% of the initial TOC was found. In
212 B 96.1% of the initial TOC content remained, indicating a more efficient stabilization of the
213 soil organic matter. In line with our findings, Zimmerman et al. (2011) reported that C
214 mineralization was generally lower than expected for soils treated with biochars produced at
215 525 and 650 °C and from hard woods, similarly to those used in our study.
216 In BGC and BVC only about 90% of the initial TOC was found, suggesting that both compost
217 additions led to higher TOC mineralization when combined with the biochar. As the TOC
218 decrease was higher in BGC and BVC compared to GC and VC, and since the biochar was
219 only slightly degraded during the experiment, the changes in TOC could be due to the
220 mineralization of the organic fraction of the composts. Schulz and Glaser (2012)
221 demonstrated, however, that thelabile organic matter of compost can be stabilized by biochar.
222 On the other hand, the decomposition of added plant residues in soil have been found to be
223 enhanced by biochar (Awad et al. 2012). This may be attributed to more favourable soil
224 aeration and porosity, induced by the biochar thus stimulating microbial growth and

225 respiration (Lei and Zhang 2013). Although the biochar is much more stable than both
226 composts, the greater TOC decrease in BGC and BVC could be also explained by the
227 increased decomposition of biochar when mixed with the two composts. Indeed, as observed
228 by Kuzyakov et al. (2009), biochar decomposition rates increase until an easily degradable
229 substrate, in our case provided by the compost, is available.
230 Table 3 reports the amount of DOC at T1 and T6 in soil systems. GC and VC led to
231 significantly increased DOC contents, with the much larger initial rise occurring in GC (Table
232 3). As suggested by Ngo et al. (2011), the vermicompost is a more decomposed and stabilized
233 organic substrate, with lower forms of C available to microorganisms. The higher content of
234 TOC in GC than VC (Table 1) could also account for the difference between the two types of
235 compost. Smith et al. (2010) demonstrated that young biochar provides significant amounts
236 of labile C. In our study, B did not change the level of DOC in the soil. At T6, lower values
237 of DOC were generally observed for each soil-system than at T1, perhaps because the water-
238 soluble C is degraded in the first stage of mineralization (Pascual et al. 1997). GC and VC
239 increased the DOC level compared to B. The DOC values of mixtures remained significantly
240 higher at T6 compared to B and the control.
241 Table 3 shows changes in the amount of soil microbial biomass at T1 and T6 in the soil
242 systems. The incorporation of both composts in soil increased the microbial biomass C, which
243 reflects the increased number of microorganisms. This increase may be due to the growth in
244 soil microbiota in response to the easily available C, and/or to the addition of foreign
245 microorganisms by the materials.
246 The highest initial increase in biomass C content occurred in VC. Similarly, Aira and
247 Dominguez (2008) found a higher microbial biomass in vermicompost than in compost.
248 Studying the impact of vermicompost on the biological characteristics of an alkaline soil, Uz
249 et al. (2016) reported a strong increase in the bacterial number. Most studies indicate that

250 biochar increases the microbial biomass (Lehmann et al. 2011; Zhang et al. 2017). However,
251 changes in the amount of microorganisms are likely connected to the intrinsic properties of
252 both biochar and the soil (Khodadad et al. 2011). Dempster et al. (2012) found a decrease in
253 soil microbial biomass with biochar addition to a coarse textured soil. In a six-year field study,
254 biochar amendment did not change soil microbial population (Tian et al. 2016).
255 Liang et al. (2010) reported an increase in microbial biomass related to an increase in labile
256 organic carbon, such as DOC, which acts as a substrate for microbial nutrition. The increase
257 in soil pH may also account for the lack of changes in the amount of microbial biomass
258 (Lehmann et al. 2011). In our research, the level of microbial biomass in B did not increase
259 and was never significantly different to that of the control. This is probably due to the increase
260 in pH value (Figure 1) and/or because the addition of biochar did not increase soil DOC (Table
261 3). Although the biomass C level was lower in BGC and BVC compared to GC and VC (Table
262 3), both compost additions to B increased the amount of biomass C compared to B and the
263 control. This suggests that native soil fertility can be likewise increased with the biochar-
264 compost amendments. Since the TOC mineralization was higher when both composts were
265 combined with B (Figure 2), it is possible that the microbial biomass of mixtures, although in
266 a lesser amount, is more active.
267 For each soil-system, we found that at T6 the biomass C values were 1.8 - 2.4 times lower
268 than at T1, perhaps because DOC, which acts as an energy source for the microorganisms and
269 contributes to their biomass, degrades rapidly. The biomass C level in B fell as sharply as it
270 did with the other treatments, in spite of the higher stability of the material. The fall in the
271 level of biomass C in the control may be due to the disturbance of the soil ecosystem in
272 laboratory conditions. Nevertheless, with the exception of B, biomass C values in amended
273 soils were higher than in the control, which clearly indicates the improvement in soil
274 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

300 calculation. As with VC, BVC consistently showed the highest AP-ase activity during
301 incubation. However, note that AP-ase is substrate specific, extracellular and active in soil,
302 and does not reflect the total microbial status of the soil.
303 Both GC and VC significantly supported more DH-ase activity than the control throughout
304 the experimental period (Figure 3). DH-ase activity was significantly higher in VC than in
305 GC at each sampling time. Arancon et al. (2006) also reported high soil DH-ase activity
306 following vermicompost applications. Lower DH-ase was found in B compared to the control
307 already at T1, and persisted throughout the experiment (Figure 3).
308 Similar results were observed by Bandara et al. (2015), while no biochar amendment effects
309 of DH-ase were found by Wu et al. (2013) in a chernozemic soil after a 100-day incubation
310 period, and by Niemi et al. (2015) in two different types of soil, each bare and cultivated,
311 during one growing season. Ameloot et al. (2015) suggested that the level of soil organic
312 matter can affect DH-ase activity in biochar amended soil, due to the increased physical
313 contact between the biochar particles and microorganisms. They observed no changes in DH-
314 ase in soil with 0.89% C, however they found higher enzyme activity than control in soil with
315 a higher C content (1.61%). Thus, the amount of soil organic C (1.47%) (see Materials and
316 Methods) would have supported a higher enzymatic activity.
317 The response of DH-ase activity in B might be from toxic compounds in the material
318 (Moeskops et al., 2010). The poor level of DH-ase activity in B could also be explained by
319 the results of Swaine et al. (2013), who reported that biochar amendments led to significant
320 reductions in concentrations of substrate and extractable product in soil DH-ase assay, thus
321 limiting the identification of biochar effects on soil enzyme activity. Since DH-ase actsin the
322 biological oxidation of organic matter in the soil, the low level of the enzyme in B is consistent
323 with the low tendency of its organic matter to mineralize, which was already inferred from
324 the TOC values (Figure 2) and the microbial quotient (Table 3). When green compost and

325 vermicompost were mixed with biochar, the DH-ase activity increased, with values that did
326 not differ substantially from the control during trials. However, DH-ase in mixtures never
327 reached the GC and VC levels. This confirms the reducing effect induced by B on DH-ase.
328 Since DH-ase is considered as a respiratory enzyme, this result seems inconsistent with the
329 high mineralization rate of organic matter in BGC and BVC, revealed by the TOC trends
330 (Figure 2). Again, although losses of DH-ase in mixtures may be attributed to decreasing
331 effects of B on the enzyme activity, values may be underestimated because of the impact of
332 biochar on assay constituents.
333 If alkaline phosphatase activity is expressed in relation to TOC (specific enzyme activity, AP-
334 ase TOC^{-1}), lower values were found in each treated soil at T1 than the control (Table 4). The
335 specific AP-ase activities in B were about one third that of the control. Note that the reducing
336 effect of B on the AP-ase activity, already highlighted by the results for absolute values, was
337 emphasized by expressing DH-ase per unit C. As reported by Bastida et al. (2012),
338 extracellular enzymes can be stabilized via the formation of enzyme-clay or enzyme–humus
339 complexes. Thus, the lower specific AP-ase in the amended soil may reflect the
340 immobilisation of enzymes following the biochar addition. GC and VC did not significantly
341 change the specific AP-ase activity in B, both at T1 and T6 (Table 4). At T6, the specific AP-
342 ase activity did not change in the control but increased in all the amended soils, due to the
343 reduction in soil organic C (Figure 2) and the concurrent increase in enzyme activity (Figure
344 3). Only in GC and even more in VC did values exceed that of the control.
345 Regarding DH-ase activity in relation to TOC (specific enzyme activity, DH-ase TOC^{-1}), a
346 value was found which was about three times lower in B than in the control, both at T1 and
347 T6 (Table 4). The observed decline in the specific activities of soil DH-ase following the
348 biochar amendment was not attributable to a lower microbial biomass content (Table 3).
349 These results may indicate a worse nutritional status of the organic matter of B and/or a toxic

350 effect of compounds present in the material. As for the absolute values of DH-ase, the specific
351 enzyme activities were higher in GC and VC than in the control, however this happened only
352 at T1, while at T6, differences disappeared.
353 Similarly to the results related to the absolute values of DH-ase, values of the specific DH-
354 ase activity in B were not increased by the addition of either of the two composts. Unlike DH-
355 ase, the specific DH-ase in mixtures never reached the levels of the control, thus indicating
356 the strong influence of B on the enzyme activity. Similarly to findings for the specific AP-
357 ase activity, the lowering effect of B on the DH-ase activity was emphasized by expressing
358 DH-ase as specific activity. These results suggest that specific enzyme activity may be a more
359 suitable indicator than the absolute values in detecting the effect of the B amendment on soil
360 microbial activity.
361 The dynamics of soil biological activity can also be described by the metabolic potential index
362 (MP) (Masciandaro et al. 1998). Unlike absolute and specific DH-ase, the MP was not
363 changed by B compared to the control. Of the two composts, the MP increased at T1 only for
364 the vermicompost treatment with respect to the control (Table 4), thus revealing less evident
365 soil responses to amendments than AP-ase and DH-ase TOC^{-1} indexes. The MP in VC was
366 also found to be the highest at T6, which is consistent with Masciandaro et al. (2000) who
367 found an increase in MP in a soil amended with vermicompost one year after the treatment.
368 This confirms the stimulation of soil metabolism by VC, already observed for biomass C
369 (Table 3), AP-ase and DH-ase (Figure 3). The results are probably due to an increase in
370 available organic substrates and/or the fact that the water-soluble organic carbon of
371 vermicompost is particularly effective in stimulating enzyme activity. In spite of the high MP
372 in VC, the addition of vermicompost did not significantly change the MP in B, either at T1
373 or T6. The MP of the B treatment was more than halved by when it was mixed with green
374 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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591 **Table 1**. Selected characteristics of the organic materials

		green compost	vermicompost	biochar
pH		8.5	7.1	10.2
Inorganic C %		22.8	10.5	12.7
Organic C %		30.0	27.0	86.0
Total N %		2.5	1.9	0.48
C to N ratio		12	14	179
Available P $\mu g \cdot g^{-1}$		452	349	443
Exchangeable K $mg \cdot g^{-1}$		11.2	10.7	12.5

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595 **Table 2**. Experimental setup

Treatment	Soil	Biochar	Green Compost	Vermicompost
	g	% by weight		
Control	1000	0	0	0
Soil + green compost (GC)	1000	0	2.5	0
Soil + vermicompost (VC)	1000	0	0	2.5
Soil + biochar (B)	1000	2.5	0	0
Soil + biochar + green compost (BGC)	1000	2.5	2.5	0
Soil + biochar + vermicompost (BVC)	1000	2.5	0	2.5

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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

Treatment	T1	T6
	DOC (μg g^{-1})	
Control	174 e	132 fg
GC	350 a	256 b
VC	226 cd	159 e
B	157 ef	124 g
BGC	368 a	248 bc
BVC	209 d	161 e
	Microbial biomass C (μg g^{-1})	
Control	173.6 d	95.4 e
GC	511.6 b	256.9 c
VC	875.4 a	490.4 b
B	170.4 d	91.2 e
BGC	296.2 c	168.0 d
BVC	492.6 b	200.3 d
	Microbial quotient (microbial biomass C TOC^{-1} 10^2)	
Control	1.23 c	0.72 cde
GC	2.36 b	1.28 c
VC	4.17 a	2.54 b
B	0.48 de	0.27 e
BGC	0.69 cde	0.43 e
BVC	1.17 cd	0.53 de

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

Treatment	T1	T6
	Specific enzyme activity (AP-ase TOC^{-1})	
Control	60.6 cd	64.5 c
GC	41.1 f	105.0 b
VC	48.3 ef	123.9 a
B	22.9 g	58.8 cde
BGC	21.0 g	50.9 def
BVC	21.3 g	58.9 cd
	Specific enzyme activity (DH-ase TOC^{-1})	
Control	0.52 c	0.47 c
GC	0.66 b	0.37 d
VC	0.75 a	0.49 c
B	0.18 e	0.15 e
BGC	0.14 e	0.16 e
BVC	0.21 e	0.18 e
	MP (DH-ase DOC^{-1})10^3	
Control	4.25 c	4.77 c
GC	4.09 c	2.89 d
VC	6.95 a	5.91 b
B	4.08 c	4.19 c
BGC	1.66 e	2.50 de
BVC	4.31 c	4.16 c

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.