Short-term effects on soil quality of biogas digestate applied in combination with young biochar

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Abstract

We assessed the suitability of digestate (D) from biogas production and green biochar (B) to improve soil biological activity and antioxidant capacity, and investigated whether there is an interaction between digestate and biochar applied to soil in combination. In a short-term (100-d) laboratory incubation, we monitored soil chemical and biological parameters. We compared soil amendments with 1% D (D1), 5% D (D5), 1% B (B), digestate-biochar combinations (D1+B and D5+B), and soil with no amendment. In D5, CO_2 production, antioxidant capacity (TEAC), and dehydrogenase activity (DH-ase) and the contents of microbial biomass C, DOC and alkali-soluble phenols increased to the highest level. The biochar increased the total organic C (TOC) and TEAC of soil but decreased DOC, CO_2 production, microbial biomass C and DH-ase. The addition of biochar to digestate reduced soluble compounds (DOC and phenols), thus limiting the amount and activity of the soil microbial biomass (CO_2 production and DH-ase). After 100 days of incubation D5+B showed the highest TOC content (82.8% of the initial amount). Both applied alone and in combination with digestate, the biochar appears to enrich the soil carbon sink by reducing CO_2 emissions into the atmosphere.

Keywords: digestate, biochar, short-term incubation, biological activity, soil antioxidant capacity.

Introduction

The anaerobic digestion of waste for biogas production is of great interest for livestock waste management, and is in line with EU policies concerning renewable energy production (Holm-Nielsen *et al.* 2009). Anaerobic digestion produces a residual material (digestate), whose management or disposal must be addressed in order not to constrain the development of anaerobic digestion systems. In addition, intensive agriculture has led to soil degradation and a loss of organic matter and fertility, increased production costs, and contributed to CO_2 emissions.

Recycling digestate in agricultural systems reduces mineral fertilizer applications, which then leads to resource conservation, climate change mitigation and soil quality maintenance. Several studies have shown the positive effects of digestate from agricultural biogas production on soil quality (Elste *et al.* 2010; Risberg *et al.* 2017). However, digestate can contain inorganic (Kupper *et al.* 2014) and organic pollutants (Spielmeyer *et al.* 2014), thus explaining the negative effects on the soil microbial community (Sänger *et al.* 2014; Abubaker *et al.* 2013). There is therefore a need for research in order to assess the appropriate use of digested materials in soil.

The addition of biochar to soil has been proposed as a way to improve soil quality and sequester carbon (Xie *et al.* 2016), however the value of biochar as an amendment is currently being discussed. With regard to the suitability of biochar for short-term C stabilization in soil, CO_2 emissions have been found both to increase (Scheer *et al.* 2011) and decrease (Malghani *et al.* 2013). How biochar affects soil biological processes is also very controversial. Khodadad *et al.* (2011) and O'Neill *et al.* (2009) reported the different behaviors of soil microbial biomass in biochar amended soils. Lehmann *et al.* (2011) explained the positive effect of biochar on the soil microbial biomass as its ability to increase the concentration of dissolved organic matter and soil nutrients, to remove toxic compounds from soil solution by adsorption, and to change the quality of soil water and its pH. However, Dempster *et al.* (2012) reported the toxic effect of biochar on soil microbial biomass due to its polycyclic aromatic hydrocarbons and various highly volatile organic compound substances.

The value of digestate as an amendment could be improved by combining it with other sources of organic and mineral fertilizers (Bougnom *et al.* 2012). Biochar has been mixed with digestate in order to enhance the amendment quality of the materials. Mukherjee *et al.* (2016) reported a much lower soil respiration (up to 11-fold) in soil/digestate/biochar with 1% biochar addition compared to soil/digestate mixtures without biochar. Mukherjee *et al.* (2016) also reported that dissolved organic C (DOC) can be sorbed by the biochar, reducing the microbial accessible DOC in the liquid phase and as a consequence also the CO_2 production. They suggested that more specific research is

78 needed, where the DOC production should be monitored over time. Maintaining the level of soil
79 organic matter can also be achieved by protecting the organic matter. Rimmer (2006) postulated
80 that the protection of organic matter from degradation is linked to the soil antioxidant capacity. The
81 mechanism explaining this effect has often been attributed to the antioxidant activity of phenol
82 compounds in soil organic matter, which are able to slow the rate of oxidation down, thus
83 controlling the rate of breakdown in more labile and easily degradable fractions (Cardelli *et al.*
84 2012; Saviozzi and Cardelli 2104). Thus, the accumulation of soil organic matter could be
85 stimulated using amendments with a higher phenol content, which could slow the C mineralization.
86 The aims of this study were to: i) evaluate the suitability of digestate and biochar to improve the
87 biological activity and antioxidant capacity of soil, and ii) verify the interaction effect between
88 digestate and biochar applications on soil quality.
89
90 **Materials and methods**
91 Soil sampling
92 Surface (0–15 cm) soil was collected from a dedicated agricultural area at the Interdepartmental
93 Centre E. Avanzi, which is located at a distance of approximately 4 km from the sea (43°40′N,
94 10°19′E) and 1 m above sea level (Pisa, Italy). The soil sample was air-dried and passed through a
95 2-mm sieve to remove large residue fragments. The main soil characteristics were: 73.3% sand,
96 12.2% silt, 14.5% clay, 8.3 pH, 7.7% inorganic C, 14.1 g kg^{-1} total organic C (TOC), 0.17 g kg^{-1}
97 dissolved organic C (DOC), 1.30 g kg^{-1} total N, 40.4 mg kg^{-1} available P, 350.3 mg kg^{-1} available
98 K, 12.1 cmol (+) kg^{-1} cation exchange capacity (CEC). The soil was classified as Xerorthent.
99 Organic materials
100 The biochar was produced from orchard pruning residues of fruit trees (Pirus communis, Malus
101 domestica, Persica vulgaris, Vitis vinifera) by slow pyrolysis with a transportable ring kiln (215 cm
102 in diameter and holding around 2t of hardwood). The average heating rate before reaching the peak
103 of 550°C was 15-18°C min^{-1}.

104 The digestate was the by-product of methane and heat production in a biogas plant from organic
105 waste, and was taken to a facility in northern Italy (Lodi, Italy).
106 Experimental design
107 Six treatments were tested: a) control, soil without any inorganic or organic fertilizer; b) soil
108 amended with digestate 1% w/w (D1); c) soil amended with digestate 5% w/w (D5); d) soil
109 amended with biochar 1% w/w (B); e) soil amended with digestate 1% w/w plus biochar 1% w/w
110 (D1+B); f) soil amended with digestate 5% w/w plus biochar 1% w/w (D5+B).
111 Organic materials were mixed with the soil in a concrete mixer and transferred into 2-L containers.
112 The soil and soil-mixture parameters were monitored for 100 days through aerobic incubation. The
113 samples were watered at appropriate intervals to maintain a constant moisture level (60% maximum
114 water holding capacity), closed with parafilm to permit gaseous exchange, and incubated at 28 ± 1
115 °C for 100 days. Four sampling times were selected to monitor the soil parameters: at 0 (T1), 15
116 (T2), 45 (T3), and 100 (T4) days after the amendments.
117 Soil analyses
118 The particle-size distribution of the soils was obtained by the pipette method. The pH was
119 determined according to the SISS (1995); inorganic carbon ($CaCO_3$) with a Scheibler apparatus;
120 TOC by dry combustion (induction furnace 900 CS, Eltra); total N by the Kjeldahl procedure after
121 acid digestion (Bremner and Mulvaney 1982); available P was measured on the 0.5 N $NaHCO_3$
122 extract at pH 8.5 ± 0.1 (Olsen *et al.* 1954); exchangeable K was determined on the 1 N CH_3COONH_4
123 extract at pH 7.0 (Thomas 1982), and cation exchange capacity (CEC) according to Bascomb
124 (1964).
125 The DOC was determined by stirring soil samples with distilled water (soil / H_2O 1:25) for 24 h at
126 room temperature, centrifuging the suspension at 10,000 rpm for 10 min, and filtrating it through a
127 0.45 mm glass fiber. In this extract, DOC was determined with an organic C analyzer for liquid
128 samples (Hach QbD1200).

129 Alkali-soluble phenols were determined on 2 M NaOH solution extracts (soil / solution 1:5). The
130 NaOH extraction was performed under N_2 for 16 h at room temperature; after centrifuging (6000
131 rpm x 15 min), the product was filtered on cellulose acetate (pore size 0.2 mm) and treated with a
132 10% TCA solution to remove proteins. The alkali-extracted phenols were determined using a Folin–
133 Ciocalteu reagent, following Kuwatsuka and Shindo (1973).
134 The Trolox Equivalent Antioxidant Capacity (TEAC) was determined on the 2M NaOH solution
135 extract used for phenols. Before the TEAC assay, the NaOH extract was neutralized from
136 approximately pH 13 to pH 7 ± 0.2 using 2M HCl. The method (Re *et al.* 1999) is based on the use
137 of ABTS+, a stable colored radical in aqueous solution. The antioxidant capacity measurement is
138 expressed as a decrease in absorbance of the ABTS+ solution after the addition of an antioxidant.
139 To measure the antioxidant capacity, 3 ml of a solution of ABTS+ radical, obtained by reacting an
140 ABTS stock solution (7 mM) overnight with a 24.5 mM potassium persulfate solution, were placed
141 directly into spectrophotometer cuvettes. Thirty ml of each extract were then added and, in blank
142 cuvettes, 30 ml of deionized H_2O. The absorbance of the blank was read with a spectrophotometer
143 set at a wavelength of 734 nm. Each cuvette was then sealed with parafilm, shaken and placed to
144 incubate in the dark at 25°C. After 6 min, the absorbance of the mixture was read again. The
145 decrease in absorbance due to the activity of the soil extract on the antioxidant ABTS+ radical was
146 expressed as a percentage of initial absorbance.
147 Soil microbial biomass C was determined at T4 according to Vance *et al.* (1987) with the extraction
148 of organic C from fumigated and unfumigated soils by 1 N K_2SO_4. The organic C was then
149 measured as described by Jenkinson and Powlson (1976) using dichromate digestion. An extraction
150 efficiency coefficient of 0.38 was used to convert the difference in soluble C between the fumigated
151 and unfumigated soils into microbial biomass C.
152 On samples collected at T4, the CO_2 evolution was monitored daily during a 21-d incubation period.
153 One hundred g of soil alone or soil mixtures were placed in 250-ml microcosms closed with rubber
154 stoppers, moistened at 50% of the maximum water holding capacity, and incubated at 25 ± 1 °C.

155 At appropriate time intervals, deionized water free of CO_2 was added to the samples in order to
156 maintain a constant moisture level. The CO_2 evolved was trapped in an NaOH solution and the
157 alkali excess was titrated with HCl after precipitation of carbonates with a 2N solution of $BaCl_2$.
158 Daily opening of the microcosms to replenish the NaOH for CO_2 absorption prevented any
159 decomposition inhibition owing to a lack of oxygen. The results were expressed as mg of C
160 mineralized / 100 g of dry soil.
161 The soil dehydrogenase activity (DH-ase) was assayed on freshly-sieved samples by a colorimetric
162 assay of 2,3,5 triphenylformazan (TPF) produced by the microorganism reduction of 2,3,5
163 triphenyltetrazolium chloride (TTC) (Casida *et al.* 1964).
164 Statistics
165 Statistica 7.0 software (StatSoft Inc., Tulsa, Oklahoma, USA) was used for the statistical analysis.
166 Data were expressed on the basis of the oven-dry weight of the soil. Results were the means of
167 determinations carried out on three replicates. Differences among mean replicate values for
168 treatments were compared at the 0.05 significant level by analysis of variance (ANOVA).
169 **Results and discussion**
170 Figure 1 shows the effects of the amendment applications on soil pH. Both digestate application
171 doses (pH = 8.0) immediately increased the soil reaction (8.3) by ½ unit (8.8). Then, it rapidly
172 decreased as a consequence of nitrification, showing already lower pH values (around 7.8) at day
173 15 than those found in the control (8.2). Similar results were reported by De la Fuente *et al.* (2013)
174 in a study on the addition of digestate to an alkaline soil. The pH in the D1 and D5 treatments
175 gradually decreased during incubation, likely attributable to the production of acidifying nitrates
176 and/or to a release of functional groups of an acidic character during oxidation of the organic matter.
177 Figure 1 shows that at T1 B led to a slight increase in the soil reaction compared to the control.
178 This was expected, given the high pH values (10.2) of biochar (Table 1), due to the carbonates,
179 basic oxides and organic carboxylates produced during pyrolysis (Yuan *et al.* 2010). The alkalizing
180 effect of B on pH could also be due to the poor soil buffering due to the low level of organic matter

181 in the system. Artiola *et al.* (2012) also reported an increase in soil reaction in an alkaline soil (pH
182 of 8.10-8.15) amended with 2-4% fresh biochar. The pH elevation in B was temporary as the
183 biochar alkali salts and functional groups reacted with carbonic acid from microbial activity and
184 atmospheric CO_2 to form bicarbonates, thus lowering the soil pH. The presence of biochar in
185 mixtures initially did not affect the alkalinizing influence of digestate, with significantly similar
186 values to those induced by the material alone. Towards the end of incubation, D1+B and D5+B
187 resulted in a more substantial decrease in soil pH than D1 and D5. It should also be noted that in
188 the samples in which the amount of digestate was added at the highest dose, the pH had the largest
189 decrease (pH = 6.5).
190 As expected, the addition of amendments to the soil initially increased the amount of organic carbon
191 in the soil, with values which were almost proportional to the added amount (Figure 2). During the
192 incubation period, the TOC content remained substantially unchanged but at T4, the TOC decreased
193 as a result of mineralization, however significantly only for D5, D1+B and D5+B treatments. By
194 adding biochar, there was less of a TOC reduction during incubation, particularly at the highest
195 dose of digestate. In D5, the TOC content was 8.5% lower at T4 than at T1, while it was only 7.2%
196 lower in D5+B. The ability of biochar to reduce the mineralization of the TOC of digestate could
197 be attributed to the virtually absent mineralization of TOC of the biochar and/or the lower
198 availability of the labile substrate in digestate due to its adsorption by the biochar. These two
199 hypotheses were confirmed by the respiration results (Figure 3). First, the addition of biochar to
200 soil did not change the CO_2 production compared to the control, as a consequence of the organic
201 fraction of this material being particularly stable. Other studies have reported no variations in the
202 CO_2 efflux following the biochar treatments (Kuzyakov *et al.* 2009; Novak *et al.* 2010). Second,
203 digestate-biochar mixtures showed a lower CO_2 production than in D1 and D5, both as absolute
204 values and as expressed as a percentage of applied C (3.1 and 5.3 respectively). Similar results were
205 reported by Mukherjee *et al.* (2016), who attributed the decrease in CO_2 production to the lower
206 availability of labile substrate found in digestate-biochar mixtures. The addition of digestate to soil

207 led to a significant increase in CO_2 evolution compared to untreated soil, perhaps because of the
208 higher proportion of easily degradable carbon in digestate. The cumulative respiration data of the
209 soil supplemented with digested 1% are in line with those obtained from Johansen *et al.* (2013),
210 who found double CO_2 amounts compared to the control at the same application rate of 1%.
211 Accordingly, Abubaker *et al.* (2015) also reported stimulatory short-term effects on microbial
212 respiration in soil amended with digestate. If the CO_2 values of D1 and D5 were expressed as a
213 percentage of TOC, the breakdown percentages for D1 (6.1) and D5 (9.3) were much higher than
214 the control (3.1), thus confirming the results relative to the absolute values. The higher value in D5
215 than D1 indicates that even 5-fold higher amounts of digestate did not induce negative effects on
216 mineralization by the microbial activity.
217 The application of digestate to soil immediately increased the amount of DOC (Figure 4). During
218 incubation, D1 and D5 showed a higher decrease in DOC in soil solution than the control. Due to
219 their mineralization, values at T4 were lower than 50% compared to T1, while the DOC in soil was
220 about 21% lower. This may be due to a greater lability of the soluble compounds in digestate
221 compared to soil organic matter. Zimmerman *et al.* (2011) found a higher amount of labile C
222 fractions in biochar amended soils than in the corresponding untreated soils. In our study, the DOC
223 content in B was constantly lower than the control, suggesting an adsorption of labile soil organic
224 C by biochar (Mukherjee *et al.* 2016). Since the DOC in B did not change significantly during the
225 study, it can be assumed that the microorganisms did not need soluble organic carbon for their
226 activity, in line with the lack of organic matter mineralization of biochar (Figure 3). However, the
227 behavior of samples treated with biochar was different to that observed by Su *et al.* (2017), who
228 found that the DOC of biochar was degraded in early incubation. Lower amounts of DOC in D1+B
229 and D5+B than D1 and D5 were consistently observed during incubation (Figure 4), confirming the
230 results observed for the soil-biochar treatment. This may explain the lower CO_2 production in
231 digestate-biochar mixtures than in soil-digestate without biochar (Figure 3).

232 Figure 5 presents the amendment effects on the amount of alkali-soluble phenols, a chemically
233 bound form. Results show that the application of digestate initially led to a more marked increase
234 in phenols in the soil compared to biochar. This is due to the large amount of phenols in digestate,
235 much higher than in biochar (Table 1). The alkali-soluble phenols remained virtually constant over
236 time for the biochar-amended soil. This may be due to the recalcitrance of these organic molecules
237 to microbial degradation (Kuzyakov *et al.* 2009). Phenols decreased significantly in D1, D5 and
238 their mixtures with biochar. However, in the digestate-biochar mixtures, the amounts of alkali-
239 soluble phenols decreased at a lower rate (by about 27% and 18% between T1 and T4 for D1+B
240 and D5+B respectively) than in D1 and D5 (by about 17% and 14% respectively). This could be
241 explained by the lack of mineralization of the phenols present in biochar and/or the adsorption by
242 biochar of phenols of the digestate, thus reducing their extractability. Many studies have shown the
243 ability of biochar to adsorb phenols, due to the large surface area of the micropores and the carbon
244 content of the material (Han *et al.* 2013; Hall *et al.* 2014).
245 The addition of digestate initially increased the antioxidant capacity of the soil, although significant
246 differences compared to the control were observed only at the highest rate (Figure 6). The results
247 agree with Rimmer and Smith (2009), who reported greater TEAC in organic materials than soil.
248 There were no significant differences between D5 and B in terms of the antioxidant capacities of
249 the amended soil. However, the addition of biochar to digestate did not result in significant changes
250 in TEAC, i.e. no additive values were observed. Rimmer and Abbott (2011) and Cardelli *et al.*
251 (2012) reported that TEAC is mainly due to the antioxidant activity of alkali-soluble phenols.
252 Saviozzi and Cardelli (2014) also reported positive correlations between TEAC and DOC in a study
253 on five different organic materials applied to soil. In our study, no clear trends in TEAC values over
254 time were observed, reflecting no positive relationships between TEAC and both alkali-soluble
255 phenols and DOC.
256 The size of the soil microbial biomass at T4, expressed as biomass carbon, is shown in Figure 7.
257 The microbial biomass content was significantly increased by both doses of digestate. In D1 and

D5, the percentages of soil microbial C of total soil organic C (2.4 and 2.2%) were also higher than the control (1.5%). Many macro- and micronutrients, growth promoters and hormones, provided by the material, could have supported a greater proliferation of the microbial biomass present in the soil (Makadi *et al.* 2012). Biederman *et al.* (2017) observed a lack of influence of biochar on soil microbial biomass carbon. In B, lower values of biomass than the control were observed, both as absolute values and as a percentage of TOC (0.6%). The presence of volatile compounds in the biochar, as reported by Deenik *et al.* (2010) and Dempster *et al.* (2012), could have suppressed the proliferation of the microbial population. The addition of biochar to digestate significantly decreased the amount of biomass found in D1 and D5. As for D1 and D5, the percentages of soil microbial C of total soil organic C in D1+B and D5+B were found to be similar at both digestate application rates. The low levels of microbial biomass in B, D1+B and D5+B agree well with the scarce activity of the treatments where biochar was present, as previously observed for the organic matter mineralization.

The soil dehydrogenase activity (DH-ase) is shown in Figure 8. A significant increase in soil dehydrogenase activity was found in the digestate-treated soil compared to the control. This effect may be ascribed to the substrates added to the soil by the digestate, which could have stimulated the synthesis of the enzyme (Alburquerque *et al.* 2012). On the other hand, the addition of biochar to the soil throughout the incubation period led to a lower DH-ase compared to the control, suggesting that the microorganisms are unable to use biochar as a substrate (Wu *et al.* 2012) and/or they are inhibited by the presence of volatile compounds. The addition of biochar to digestate also decreased the enzyme activity with respect to the unmixed samples. At T4, a lower DH-ase than at T1 was found in all treatments, with the control showing the lowest value. The results are in accordance with Wu *et al.* (2012) and Chintala *et al*. (2014), who explained the decline in activity with the progressive decrease in the substrate available for microorganisms.

Conclusions

284 The digestate used in the present study improved the soil chemical and biological characteristics.
285 Compared to the untreated soil, the highest application dose of 5% resulted in the highest decrease
286 in pH and led to the greatest increases in TOC, CO_2 production, DOC, alkali-soluble phenols,
287 TEAC, microbial biomass C and dehydrogenase activity. This is because of the supply of carbon,
288 which is partially in an easily available form and can be used and metabolized by soil
289 microorganisms.
290 The biochar led to a marked increase in TOC and TEAC in the soil but decreased the biological
291 properties of the soil, probably due to the phenolic compounds in the biochar originating from the
292 pyrolysis process.
293 The addition of biochar to digestate reduced the soluble organic compounds (DOC and phenols),
294 thus limiting the amount (microbial biomass C) and activity of microorganisms (CO_2 production
295 and DH-ase).
296 Both applied alone and in combination with digestate, the biochar enriched the carbon sink of the
297 soil through a reduction in soil respiration and the related mineralization activities.
298 To better evaluate these effects, more research is needed through the regular monitoring of soil pH
299 in long-term digestate and digestate-biochar applications together with further studies on the effects
300 of these amendments on soil antioxidant capacity. The quality of DOC and the alkali soluble
301 compounds need to be examined.
302 This increase in understanding should help to improve the assessment of the environmental and
303 economic benefits of digestate and biochar additions to agricultural soils.
304

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436 **Figure captions**

437

438 Figure 1. pH as affected by “amendment treatments x sampling times” interaction. Vertical bars
439 represent l.s.d. at P≤0.05.

440

441 Figure 2. Total organic carbon (TOC), as affected by “amendment treatments x sampling times”
442 interaction. Vertical bars represent l.s.d. at P≤0.05.

443

444 Figure 3. Cumulative CO2-C in studied treatments after 100 days from the addition of the
445 amendments. Columns with different letters are significantly different (P < 0.05).

446

447 Figure 4. Dissolved organic carbon (DOC), as affected by “amendment treatments x sampling
448 times” interaction. Vertical bars represent l.s.d. at P≤0.05.

449

450 Figure 5. Alkali-soluble phenols as affected by “amendment treatments x sampling times”
451 interaction. Vertical bars represent l.s.d. at P≤0.05.

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453 Figure 6. Trolox equivalent antioxidant capacity (TEAC), as affected by “amendment treatments x
454 sampling times” interaction. Vertical bars represent l.s.d. at P≤0.05.

455

456 Figure 7. Microbial biomass carbon after 100 days from the addition of the amendments. Columns
457 with different letters are significantly different (P < 0.05).

458

459 Figure 8. Dehydrogenase activity (DH-ase), as affected by “amendment treatments x sampling
460 times” interaction. Vertical bars represent l.s.d. at P≤0.05.