Relationships between antioxidant capacity and microbial activity in a soil amended with biochar, green compost and vermicompost.

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SUMMARY - The aim of the study was to evaluate the relationships between the antioxidant capacity and the microbial activity in a soil amended with different organic materials. In a 1-year laboratory incubation, biochar, green compost and vermicompost were added to soil at the dose of 2.5% w/w. Antioxidant capacity (TEAC), dissolved organic carbon (DOC), water- and alkali-soluble phenols and microbial activity, as measured by the fluorescein diacetate hydrolysis (FDA), were monitored during incubation. Greater TEAC was in green compost and vermicompost than in biochar treated soil and it was directly related on the TEAC values of the added materials. The application of organic materials caused a marked increase of DOC and water- and alkali-soluble phenols, with values reflecting the amount of these compounds present in the amendments. The relationships between values of TEAC and phenols in the treatments suggest that these substances may be involved in determining the antioxidant capacity of soil. Compared to control, FDA was not influenced by B at each sampling time, probably because the inhibiting activity of TEAC of the material. The two types of compost, particularly vermicompost, stimulated FDA throughout the whole experimental period. Any possible inhibition of the microbial activity induced by TEAC of vermicompost and green compost could have been masked by the considerable supply of organic soluble compounds of composts, that may have stimulated strongly the microorganisms. These results suggest that the

soil microbial activity can be defined by the balance between the stimulating activity of DOC and antioxidant activity of the phenols.

INTRODUCTION - The cultivated soils have lost considerable amounts of their original carbon stock, much of which has been oxidized upon exposure to air to become CO_2. The importance of soil carbon is the subject of intense scientific investigation, with important implications for attempts to slow the rapid increase of CO_2 in the atmosphere due to the losses of organic matter. Methods to reverse loss of organic C include the addition of organic amendments. Biochar is currently used to increase soil organic matter (SOHI, 2012). Given the porous nature and high affinity of the native soil organic matter (KASOZI *et al.*, 2010), it is assumed that biochar (B) can sequestrate organic C in the soil within its pore structure, protecting it from both biotic and abiotic decomposition. Indeed, chemical transformation and microbial decomposition of biochar are often found to be very slow (CARDELLI *et al.*, 2016). Also, the scientific literature reports a reduction in soil microbial activity due to the addition of biochar (ŠPOKAS *et al.*, 2010), which leads to a decrease in soil respiration (JONES *et al.*, 2011).

The use of compost from wastes may also be a correct solution for increasing the organic matter level in soils. Some research has related the quality and stability of compost to their effects on soil C sequestration (SAVIOZZI and CARDELLI, 2014) and biological properties (DIACONO and MONTEMURRO, 2010; YAKUSHEV *et al.*, 2011). The green waste compost (GWC) from recycled garden waste, which includes grass clippings, hedge cuttings and wood materials, may provide a worthy alternative to traditional amendments (SAVIOZZI *et al.*, 2006). Vermicomposts (VC) are usually more stable than composts and improved biological properties (PRAMANIK *et al.*, 2007; YAKUSHEV *et al.*, 2011). However, DOAN *et al.* (2013) reported that after 15 months of experiments, soils amended with vermicompost were characterized by a lower metabolic activity than compost produced from domestic buffalo manure.

Maintaining the level of soil organic matter can also be achieved by protecting the existing organic matter. It was hypothesized by RIMMER (2006) that the protection of the organic matter from degradation is linked to the soil antioxidant capacity (Trolox Equivalent Antioxidant Capacity, TEAC). Organic amendments contain large amounts of antioxidants (RIMMER *et al*., 2013) and consequently they may participate in redox-mediated reactions in the soil. The organic fraction of the extracts (dissolved organic C, DOC) apparently was responsible for the major part of the reducing capacity. This hypothesis was tested by measuring the reducing capacities of aqueous extracts of biochars and the reduction and solubilization of soil Mn and Fe oxides by the extracts (GRABER *et al*., 2014). It can be argued that amendments having a great concentration of soluble reducing agents are expected to have more impact on soil redox reactions.

Within DOC compounds, TOBERMAN *et al*. (2008), SINSABAUGH (2010) and CARDELLI *et al*. (2012) suggested an important role of dissolved phenolic compounds as antioxidants because they are able to inhibit enzymes that decompose the organic matter in soil and reduce its availability for decomposition by binding organic compound, especially proteins. Carbon sequestration in peatlands was found to be linked to low activities of phenoloxidases, leading to an accumulation of dissolved phenols able to inhibit the activity of hydrolases and microorganisms (TOBERMAN *et al*., 2008; SINSABAUGH, 2010).

Fluorescein diacetate (FDA) hydrolysis rate is widely accepted as an accurate and simple method for measurement of total microbial activity in soil because FDA hydrolysis is mediated simultaneously by lipase, protease and esterase and it can reflect the activities of these enzymes in soil (SCHNURER and ROSSWALL, 1982). FDA hydrolysis has been found to be significantly correlated with microbial biomass in both pasture and cultivated soils (VEKEMANS *et al*.,1989) and therefore could be used as alternative estimate for microflora activity in soil.

The objectives of this research were to evaluate: i) the involvement of the soluble component in antioxidant activity and ii) a possible influence of the antioxidant capacity on the microbial activity in a soil amended with biochar (B), green compost (GC) and vermicompost (VC). For this purpose, a one-year aerobic incubation was carried out and changes in soil microbial activity and the antioxidant system in soil following amendments were monitored.

MATERIALS AND METHODS - *Soil sampling*. - Soil type was a Xerorthent. 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 climate of the area is hot-summer Mediterranean (Csa) with mean annual maximum and minimum daily air temperatures of 20.2 and 9.5 °C respectively, and a mean rainfall of 971 mm per year. The soil sample was air-dried and passed through a 2-mm sieve to remove large residue fragments. The main soil characteristics were determined by standard methods (SISS, 1995): 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, 14.1 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), phenol compounds (H_2O extracted) 5 $\mu g \cdot g^{-1}$, phenol compounds (NaOH extracted) 810 $\mu g \cdot g^{-1}$, TEAC trolox equivalent 3.02 $mM.g^{-1}$. *Organic materials*. - The biochar (B) was produced from orchard pruning residues of fruit trees (*Pyrus 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}. and the temperature was maintained for 30 min (residence time). The green compost (GC) was taken to the CERMEC facility (Consortium Ecology and Resources of 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 2 weeks. The composted material is removed from the tunnels and placed in "windrows" in a maturation area, for 12 weeks before being screened. The vermicompost (VC), taken from the Centro di Lombricoltura Toscano (Pisa, Italy), was produced mainly from farmyard manure and green waste within a 4-month cycle in an open air litter with *Eisenia fetida* and *Eisenia andrei* (Oligochaeta: Lumbricidae). The main characteristics of B, GC and VC were determined using standard methods according to ANPA (2001). The composition of the organic materials is reported in Table 1.

Incubation procedures. - In 2-L microcosms, the experiment was conducted in triplicate with four treatments (12 microcosms total). The organic materials were air-dried, ground, passed through a 1 mm sieve and incorporated

into the soil at doses of 2.5% on dry matter basis. The chemical and biological parameters of the soil and soil-mixture 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, which we consider to be optimal for soil biological activities), closed with parafilm to permit a gaseous exchange, and incubated at 28±1°C. 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 refrigerated at 4°C.

Parameters monitored during the incubation. - Dissolved organic carbon (DOC) was determined by stirring samples of soil with distilled water (soil/H_2O 1:20) for 24 h at room temperature, centrifuging the suspension at 10,000 rpm for 10 min and after filtration through a 0.4 mm glass fibre. In this extract, the DOC was determined with a C analyzer for liquid samples (Hach QbD1200).

Water-soluble phenols were determined on the same extract used for DOC. Alkali-soluble phenols on a 2 M NaOH solution extracts (soil:solution 1:5). The NaOH extraction was performed under N_2 for 16 h at room temperature; after centrifuging (6000 rpm 15 min), the centrifuged was filtered on cellulose acetate (pore size 0.2 mm) and treated with a 10% solution of TCA to remove proteins. The water- and alkali-extracted phenols were determined using a Folin– Ciocalteu reagent, following the method of KUWATSUKA and SHINDO (1973).

The trolox equivalent antioxidant capacity (TEAC) was determined on the 2 M NaOH solution extract used for phenols. The method (RE et al., 1999) is based on the use of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS+), a stable colored radical in aqueous solution. The measurement of the antioxidant capacity is expressed as a decrease in absorbance of the solution of ABTS+ in the presence of an antioxidant. The NaOH extract was neutralized from approximately pH 13 to pH 7±0.2 by using 2M HCl.

The fluorescein diacetate (FDA)-hydrolytic activity was determined as the absorbance at 490 nm

of the filtrate from the soil suspension incubated with fluorescein diacetate at 24°C for 60 min (SCHNURER and ROSSWALL, 1982).

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. Results were subjected to two-way analysis of variance (ANOVA). Significantly different means were separated at the 0.05 probability level by the Tukey's test (STEEL *et al*., 2007).

RESULTS. - The values of the soil antioxidant capacity (TEAC) were shown in Figure 1. Overall, the control soil had the smallest TEAC and the amended samples the greatest during the whole experimental period. Earlier work (RIMMER and SMITH, 2009) reported greater TEAC values in

organic materials than that in soils. The Figure 2 reveals significant differences in TEAC between soils treated with the different types of amendments, which persist throughout the entire incubation period. The highest value of TEAC was in GC followed by VC, while B had the smallest. Such increases in TEAC seem depend directly on the TEAC amounts in the added materials (Table 1).

Generally, the immediate effect of the application of organic materials to soils is an increase in the dissolved organic carbon (DOC) (SAVIOZZI *et al*., 2006), which may pass into soil solution, contributing to the enhancement of the amount of substrate for the microorganisms. In our study, the composts amended soils showed a higher DOC content than the soil treated with the biochar (Figure 2), reflecting the higher amount of soluble organic compounds in these materials (Table 1).

Figures 3 and 4 report the values of the water-soluble phenols, the free and nonadsorbed form, and those of the alkali-soluble phenols, the chemically bound form. Results show that the application of GC and VC caused a marked increase in soil of both water- and alkali-soluble phenols, persisting during the 1-year experimental period. This increase was more noticeable for GC than VC. The biochar addition did not significantly change the content of phenolic substances at each sampling time, probably due to the very low amount of these compounds in the material (Table 1).

The Figure 5 shows the changes during the 1-year incubation of the soil FDA, that we used as a measure of microbial activity in soil.

DISCUSSION. - As reported by SANCHEZ-MONEDERO *et al*. (2004), LIANG *et al*. (2005) and SAVIOZZI and CARDELLI (2014), land application of organic materials may cause an increase of the microbial biomass activity. A significant increase of FDA was observed in all the compost amended samples throughout the whole experimental period. The increase of FDA, found in

our study, may be due to the growth in soil microbiota in response to the easily available C, and/or to the addition of microorganisms by the materials. SEAL *et al*. (2016) already found a significant increase in FDA in case of plots treated with compost. Studying the impact of compost on the biological characteristics of an alkaline soil, UZ *et al*. (2016) reported a strong increase in the bacterial number. ARANCON *et al*. (2006) also showed that using compost resulted in a much higher microbial biomass than unamended soil. Figure 5 reveals that the highest increase in FDA occurred in VC. Similarly, AIRA and DOMINGUEZ (2008) found a higher microbial biomass in soil receiving vermicompost than compost. No differences in FDA values were found in B compared to the control at each sampling time (Figure 5). Accordingly, no biochar amendment effects on microbial activity, as measured by the dehydrogenase activity, 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.

As reported by RIMMER and SMITH (2009) and RIMMER and ABBOTT (2011), TEAC may be due to the antioxidant activity of soluble-phenols in the organic matter. Data indicate a direct relationship between phenols compounds and antioxidant capacity in the different theses: GC and VC had great contents of water- and alkali-soluble phenols and large TEAC; B and control had low amount of phenols, corresponding to small TEAC. This suggests therefore that these compounds may play a role in determining the antioxidant capacity of the treated or the unamended soil.

RIMMER and SMITH (2009) stated that high TEAC may decrease the degree of decomposition of soil organic matter by controlling the level of microbial activity. On this basis, it should be found an inverse relationship between antioxidant capacity and microbial activity. In our study, results of TEAC and FDA confirm partially such hypothesis. The biochar, with higher TEAC than control, did not stimulate the microbial activity of soil, while more microbial activity was

constantly observed during the incubation in presence of larger antioxidant capacity in both composts (Figure 1). A possible explanation of the different patterns between biochar and composts could be the different amount of soluble organic compounds (DOC) in samples amended with the materials (Figure 2). Any possible inhibition of the microbial activity induced by TEAC of composts, exerted by their phenolic component, could have been masked by the considerable supply of organic soluble compounds of amendments (DOC), that may have stimulated strongly the microorganisms.

CONCLUSIONS. - The application of the composts caused a marked increase of dissolved organic carbon (DOC) and both water- and alkali-soluble phenols, with values reflecting the amount of these compounds present in the materials.

Significant higher antioxidant capacity (TEAC) was observed in the soils amended with all the different amendments. Greater soil TEAC was in the two types of compost than in biochar and it depended on the TEAC values of the added materials.

Soil antioxidant capacity was found to be also directly related both to water- and alkali-soluble phenols. This confirm therefore the role of these compounds reported in the literature in determining the antioxidant capacity in the amended soil.

The findings herein showed a marked influence of amendments on soil microbial activity as measured by FDA. The most significant increase of FDA was observed throughout the whole experimental period in the compost amended samples, particularly in VC treated soil. No differences in FDA values were instead found in B compared to the control at each sampling time.

As expected, an inverse relationship between antioxidant capacity and microbial activity was found, but only for biochar, while opposite results were found for the two composts. Any possible inhibition of the microbial activity induced by TEAC of VC and GC, could have been

masked by the considerable supply of organic soluble compounds of composts (DOC), that may have stimulated strongly the microorganisms. These results indicate that the soluble organic C pool may play an important role as regulator of soil microbial activity, which can therefore be the result of the balance between the stimulating activity of the DOC and the antioxidant activity of the phenolic component.

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Table 1. Selected characteristics of the green compost (GC), vermicompost (VC) and biochar (B) used in this experiment.

	GC	VC	B
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
Dissolved Organic C $mg \cdot g^{-1}$	6.77	2.46	0.56
Phenol compounds (H_2O) $\mu g \cdot g^{-1}$	374.5	1158.6	32.4
Phenol compounds (NaOH) $mg \cdot g^{-}$	14.94	19.78	0.37
TEAC trolox equivalent $mM.g^{-1}$	830	801	605

Figure captions

Figure 1. Fluorescein diacetate (FDA) hydrolysis as affected by “amendment treatments x sampling times” interaction. Vertical bars represent l.s.d. at P≤0.05.

Figure 2. Trolox equivalent antioxidant capacity (TEAC), as affected by “amendment treatments x sampling times” interaction. Vertical bars represent l.s.d. at P≤0.05.

Figure 3. Water-soluble phenols as affected by “amendment treatments x sampling times” interaction. Vertical bars represent l.s.d. at P≤0.05.

Figure 4. Alkali-soluble phenols as affected by “amendment treatments x sampling times” interaction. Vertical bars represent l.s.d. at P≤0.05.

Figure 5. Dissolved organic carbon (DOC), as affected by “amendment treatments x sampling times” interaction. Vertical bars represent l.s.d. at P≤0.05.