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REGULAR ARTICLE

Co-application of wood distillate and biochar improves soil quality and plant growth in basil (*Ocimum basilicum*)

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Abstract

Background: Nowadays, an ever-increasing attention toward eco-friendly and sustainable agricultural practices, such as the use of biological fertilizers that do not alter the ecological balance of soil, has been grown worldwide.

Aim: To evaluate the effect of wood distillate (WD), biochar (B), and their combination (BWD) in increasing soil biomass, soil biological activities, and plant growth in basil (*Ocimum basilicum* L.) by determination of plant biomass.

Methods: Plants of basil were cultivated in greenhouse with three different amendments. WD was applied at a 1% dilution through fertigation. B was applied at a rate of 2% (w/w) corresponding to 34 t ha^{-1}. Organic carbon (OC), dissolved organic carbon (DOC), soil microbial biomass carbon (MB-C), and enzymatic activities (dehydrogenase, phosphatase, arylsulfatase, β-glucosidase, and urease) were determined at the end of the cultivation period (4 weeks) in bulk soil and in the rhizospheric soil. The alteration index three (AI3), which calculates the balances between three enzymes and potentially allows to quantify in relative terms the differences between soils due to different management practices, was also determined.

Results: WD and B significantly increased OC and MB-C content, whereas B preferentially retains soil DOC species and the organic substance of the WD in the BWD treatment. Phosphatase and urease activities were the most increased with the combination of the two amendments. AI3 in controls suggests that the application of WD and B at the right doses promotes the activity of soil microorganisms, enhancing the soil biological quality.

Conclusion: The presence of the two organic sources increased the availability of nitrogen and phosphorus, with a positive response in terms of basil dry biomass. WD and B have been confirmed as suitable and sustainable amendments for potential application in crops cultivation.

KEYWORDS
alteration index three (AI3), nitrogen and phosphorus availability, preharvest treatments, soil enzymatic activities, soil organic carbon

1 | INTRODUCTION

Nowadays, good and sustainable agricultural practices aim to obtain abundant and high-quality harvests without altering the ecological balance. Over the past few years, biochar (B) has increased its popularity as a new multifunctional soil fertilizer, due to its rich porosity, abundant surface functional groups, strong adsorption capacity, and the presence of recalcitrant carbon (Xiao et al., 2018). B as a major source of carbon has been identified as a soil improver that can immobilize organic pollutants and potentially toxic elements (Xia et al., 2020) and improve soil fertility through its unique surface properties. Many studies have shown that adding B to soil can improve aeration and soil structure (Zheng, Wang, Luo, et al., 2018), increase water holding capacity and nutrient availability (Zheng, Wang, Luo, et al., 2018), and alter community soil microbial (Zheng, Wang, Chen, et al., 2018), thus promoting crop growth and increasing crop yield (Yu et al., 2019). Furthermore, due to the alkaline nature of B (Wu et al., 2019), its application might increase the pH of the soil–water system (Sha et al., 2019). However, evidences have shown that B might negatively affect plant growth (Borchard et al., 2014; Sigua et al., 2016), in dependence of the soil and B composition, the plant species, and their interactions (Zheng, Wang, Luo, et al., 2018).

Wood distillate (WD) is widely recognized as a promising multifunctional amendment in agriculture (Simma et al., 2017). WD, also known as pyroligneous acid or wood vinegar, is a by-product originated from the condensation of gases during B production (Grewal et al., 2018). WD also has a good yield, which can reach up to 7.26% of the total biomass of the wood used (Mopoung & Udeye, 2015). WD is composed mainly of organic acids, but also aldehydes, ketones, phenols, and furans can be detected (Souza et al., 2012). WD has recently attracted interest for its acid attributes and the presence of N, P, and K (Zheng, Sun, et al., 2018). In recent years, research on WD has focused on studying its antioxidant, plant growth regulating, bacteriostatic, and soil additive effects on crops and plants of food interest, with particular attention for the environment. It has also been reported that the application of WD can promote plant growth (Simma et al., 2017), increasing the content of mineral and nutritional elements in the soil (Polthanee et al., 2014). Furthermore, the acidic nature of WD can regulate soil pH (Lashari et al., 2015), improve nutrient availability (Lashari et al., 2013), inhibit ammonium loss (Grewal et al., 2018), mitigate N_2O and CH_4 emissions (Sun et al., 2018), and influence the soil microbial community (Cardelli et al., 2020).

Modern agriculture has started moving toward a co-application of WD with other soil improvers to enhance its efficiency, such as chemical fertilizers, manure (Polthanee et al., 2014), herbicides (Seo et al., 2015), rhizosphere microorganisms, B (Luo et al., 2019), and B manure compost (Lashari et al., 2015). Thus, the simultaneous application of B and WD can be a good strategy for sustainable agriculture. Besides, due to the acidic and alkaline nature of WD and B, respectively, their simultaneous use in agricultural soil allows to achieve a good acid–base balance in the soil–water system. Furthermore, the porous properties of B help to increase the absorption of some organic compounds and nutrients of the WD, promoting the plant growth (Paustian et al., 2016). Finally, as an effective soil improver, WD can produce significant economic and environmental benefits when applied correctly in the agricultural production process (Lashari et al., 2013).

Recently, Vannini et al. (2021) demonstrated the effectiveness of foliar application of 0.2%-diluted chestnut WD on photosynthetic performance and lettuce growth.

At present, no attempt has been made to investigate the effects of the individual and the co-application of B and WD in crop production, limiting the development of B and WD technology in soil restoration.

The aim of this research was to investigate the effect of B and WD, alone and in combination, on the soil enzymatic activities and soil properties, and on the growth of basil plants in a greenhouse-like cultivation. The findings of this work would provide useful information for the development of B–WD-based technologies for reclaiming degraded soils for horticultural production.

2 | MATERIALS AND METHODS

2.1 | Experimental setup

The experiment was conducted twice, in May and September 2020, at the Department of Agriculture, Food and Environment of the University of Pisa. The same growing conditions for basil plants, as well as the same WD and B treatments, were adopted to collect more data and improve the robustness of statistical analysis.

Seeds of the basil plants (*Ocimum basilicum* var. Genovese) were purchased from Gargini Sementi snc. (Capannori, Italy; https://www.garginisementi.it/) and sterilized with 5% sodium hypochlorite solution for 20 min, washed four times with sterile, deionized water, and allowed to germinate at room temperature on filter paper in petri dishes. Seedlings were transplanted into pots with blond peat, weekly whetted with Hoagland solution (pH ≈ 6), and placed in a climate chamber at 24 ± 2°C, 16 h light/8 h dark photoperiod, 228 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) supplied by blue/red/green (3/6/1 ratio) LEDs combination (C-LED, Imola, Italy). When the second pair of leaves was fully expanded, the plants were carefully explanted, and the roots were gently washed with running water to remove peat residues. Finally, they were individually transplanted into pots (576 cm^3) with 380 g of soil or soil/B mixture and placed in a climate chamber with the same temperature and a 16 h light/ 8 h dark photoperiod for the whole experimental period (4 weeks). Twenty-eight plants for each experiment were organized in four groups to create a randomized experiment: a control, untreated group (CTR), a group fertirrigated once a week with 1:100 WD, a group transplanted in soil amended with 2% (w/w) B, and a group subjected to the combination of B and WD treatments (BWD). Soil and soil/B mixtures were not fertilized either at the start of the test or during the experimental period to avoid masking the effects of our amendments, and all the groups received the same amount of water through irrigation (60% of the WHC soil) to ensure the best microorganisms' activity.

2.2 | Soil recovering and sampling

The surface (0–15 cm) of a loamy sand soil, classified as Typic Xerorthent (USDA Soil Taxonomy), was collected from a dedicated agricultural area at Pontasserchio (Pisa, Italy), located ≈9 km from the sea (Latitude 43°39′38.96″N; Longitude 10°18′22.17″E) and 1 m asl. The soil samples were collected in May 2020 and consisted of 10 cores measuring 5 cm diameter/15 cm depth. The soil was air-dried and passed through a 2-mm sieve to remove larger residues. The main soil characteristics were determined by standard methods (Colombo & Miano, 2015): 77% sand (2–0.05 mm), 14% silt (0.05–0.002 mm), and 9% clay (<0.002 mm) by the pipette method; 7.9 pH; 40.0 cmol kg^{-1} H^+ hydrolytic acidity by titration of 0.5 M calcium acetate extract (ratio 1:2.5) with 0.1 M NaOH; 10.0 g kg^{-1} total organic carbon (TOC) by dry combustion (induction furnace 900 CS, Eltra); 1.03 g kg^{-1} total N by the Kjeldahl procedure after acid digestion; 16.4 mg kg^{-1} available P measured in 0.5 N $NaHCO_3$ extract at pH 8.5; 43.8 mg kg^{-1} available K determined in 1 N CH_3COONH_4 extract at pH 7.0; 13.7 $cmol^{(+)}$ kg^{-1} cation exchange capacity (CEC) determined by Ba^{2+} saturation and subsequent complete replacement of Ba^{2+} with Mg^{2+}; 89% base saturation calculated as the percentage of CEC occupied by base cations; and 44% maximum water-holding capacity.

Soil sampling was carried out both during and at the end of the experimental period (4 weeks). The soil subsample for each treatment was collected at 0.03-m depth in each pot, and the subsamples were amalgamated to have a homogeneous sample. Rhizosphere and bulk soils were fractioned as reported by Barillot et al. (2013). The samples were stored in dark conditions at 4°C to interrupt the N mineralization and, subsequently, rapidly air-dried and sieved through a 2.0-mm mesh.

2.3 | Organic materials

B was applied at a rate of 2% (w/w) corresponding to 34 t ha^{-1} in dry weight, which was produced from woodchips (30–50 g) from pristine forests (*Abies* sp., *Alnus* sp., *Castanea sativa*, *Fraxinus* sp., *Quercus* sp., *Robinia pseudoacacia*) by pyrogasification (BIODEA–RM Group, Arezzo, Italy; https://www.romamamacericentroitalia.it). The average heating rate before reaching a peak of 1100°C was 75–80°C min^{-1}. The parameters for the characterization of B were analyzed through certified methods approved by Italian regulations (D.lgs. 75/2015): 9.8 pH, 400% water holden capacity, 8.7% organic carbon (OC), <0.5% total N, and 0.034% total P. The TOC content was Class 1 following the Guidelines for Certification of the International Biochar Initiative (IBI; http://www.european-biochar.org/en/ebc-ibi).

WD, also called wood vinegar or pyroligneous acid, was produced by BIODEA–RM Group (Arezzo, Italy) and was obtained from native forest plant essences with the same physiological water through pyrolysis. The manufacturer recommends using the product in the open field by a fertigation system at a 0.5% dilution. Previous studies analyzed the chemical composition of WD, which is quite variable depending on the starting woody material and the production temperature (Grewal et al., 2018; Mathew & Zakaria, 2015). The main characteristics of WD used in this study were as follows: pH 2.8, density 1.037 g mL^{-1}, TOC 33.8 g L^{-1}, total N 0.43 g L^{-1}, organic acid 3.23%, phenolic compounds 13.0 g L^{-1}, and methanol 13.4 g L^{-1}. Polychlorobiphenyls and polycyclic aromatic hydrocarbons (PAHs) were also determined using solid-phase microextraction prior to their analysis by gas chromatography coupled to tandem mass spectrometry, but none of these toxic compounds were present in relevant concentrations. Among PAHs, only acenaphthylene and phenanthrene reached 0.09 ng L^{-1}, well below the most restrictive legislative limits. In this study, the WD was applied at a 1% dilution in order not to interfere with the soil enzyme activities, according to previous investigation by the same research group (Cardelli et al., 2020).

2.4 | Soil analyses

TOC was determined by dry combustion (induction furnace 900 CS, Eltra), after removing carbonate carbon. Dissolved organic carbon (DOC) was determined by stirring soil samples 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 0.45-mm glass fiber, determining the carbon with an OC analyzer for liquid samples (Hach QbD1200).

Soil microbial biomass carbon (MB-C) was determined according to Vance et al. (1987) with the extraction of OC from fumigated and unfumigated soils by 1 N K_2SO_4. The OC was then measured using a QBD1200 Laboratory TOC Analyzer (Hach Company, USA). An extraction efficiency coefficient (K_c) of 0.45 was used to convert the difference in soluble carbon between the fumigated and unfumigated soils into MB-C.

Dehydrogenase (DHase) activity was assayed following Tabatabai (1994), based on a colorimetric assay of 2,3,5-triphenylformazan produced by the microorganism reduction of 2,3,5-triphenyltetrazolium chloride.

β-Glucosidase activity was assayed by a colorimetric method, using 4-nitrophenyl-ß-D-glucopiranosyde as a substrate: soil samples were incubated at 37°C for 60 min; the reaction product *p*-nitrophenol was determined at 410 nm (Eivazi & Tabatabai, 1988).

Following Eivazi and Tabatabai (1977), alkaline phosphatase (APase) activity was based on the hydrolysis of *p*-nitrophenyl phosphate added to the soil samples. This phosphate releases *p*-nitrophenol, which can be detected colorimetrically.

Arylsulfatase activity was determined by a colorimetric method, using *p*-nitrophenyl sulfate as a substrate: soil samples were incubated at 37°C for 1 h and the reaction product (*p*-nitrophenol) was extracted by dilute alkali ($CaCl_2$ 0.5 M and NaOH 0.5 M) and determined at 400 nm (Tabatabai & Bremner, 1970).

Urease activity was determined according to Kandeler and Gerber (1988), based on the spectrophotometric measurement of released ammonia after a 2-h incubation of soil samples with urea substrate at 37°C.

The alteration index three (AI3) was used to evaluate the influence of the several treatments on the quality and alteration degree of the soil. As reported in Puglisi et al. (2006), AI3 was determined

TABLE 1 Amount of total organic carbon (TOC) and dissolved organic carbon (DOC) in the bulk soil and in the rhizosphere in the different treatments

	TOC		DOC		DOC	
	(g kg^{-1})		(mg kg^{-1})		(% TOC)	
Treatments	Bulk	Rhizo	Bulk	Rhizo	Bulk	Rhizo
CTR	9.4 ± 0.1^{c}	9.5 ± 0.1^{c}	188 ± 8^{d}	209 ± 8^{cd}	2.00 ± 0.06^{d}	2.20 ± 0.06^{c}
WD	9.3 ± 0.1^{c}	9.4 ± 0.1^{c}	189 ± 8^{d}	227 ± 8^{c}	2.03 ± 0.06^{d}	2.41 ± 0.06^{bc}
B	10.7 ± 0.1^{b}	11.4 ± 0.1^{ab}	281 ± 8^{b}	330 ± 8^{a}	2.63 ± 0.06^{b}	2.89 ± 0.06^{a}
BWD	11.3 ± 0.1^{ab}	11.7 ± 0.1^{a}	286 ± 8^{b}	331 ± 8^{a}	2.53 ± 0.06^{b}	2.83 ± 0.06^{a}
p—treatments	**		**		***	
p—soils	n.s.		***		***	
p—treatments × soils	*		**		***	

Note: Data are mean ± SE of seven replicates. Considering each parameter separately, different letters and different asterisks correspond to statistically significant differences according to two-way ANOVA followed by Tukey–Kramer post hoc test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; n.s., not significant). Abbreviations: B, biochar; BWD, combination of biochar and wood distillate; CTR, control; WD, wood distillate.

through the conversion of the enzyme activity data in the following relationship:

$$\text{AI3} = (7.87 \times \beta - \text{glucosidase}) - (8.22 \times \text{phosphatase}) - (0.49 \times \text{urease}), \quad (1)$$

where enzyme activities were expressed in micromoles of *p*-nitrophenol per gram of soil per hour (for β-glucosidase and phosphatase), and in micrograms of urea per gram of soil per hour (urease).

2.5 | Plant sampling and analysis

Immediately after the end of the experiment, leaves and roots from each plant were separated, carefully washed, dried at 70°C for 72 h, and weighed for the determination of total nitrogen and phosphorus of plant and the nutrient use efficiency (NUE). Seven biological replicates (seven different plants) of each group from both the experiments conducted were used for chemical and biometrical analysis.

Total nitrogen and total phosphorus contents were measured through the Kjeldahl (Bremner & Mulvaney, 1982) and Olsen methods (Olsen et al., 1954), respectively, adapted for vegetal tissues. The plant nitrogen and phosphorus uptake were calculated by multiplying the element concentration to plant dry matter yield (Cao et al., 2019).

The NUE is the ratio of the quantity of element removed from the plant and the quantity of element supplied, reported as a percentage (Brentrup & Palliere, 2010).

$$\text{NUE} = (N_T - N_{CK}) / N_S, \quad (2)$$

where N_T is the amount of nutrient content in plant tissues of WD, B, and BWD treatments, N_{CK} is the nutrient content in plant tissues of CTR, and N_S is the nutrient supply derived from soil (including nutrient from the amendments).

2.6 | Statistical analysis

Data were expressed based on the oven-dried weight of the soil and plants. Results were the means of determinations carried out on five replicates considering the soil measurements and seven replicates considering the plant analyses for each experiment. Two-way ANOVA was used to analyze the differences among treatments and between rhizosphere and bulk soil, using JMP software (SAS Institute, Inc., Cary, NC), and significantly different means were separated at $p < 0.05$ using the Tukey–Kramer post hoc test.

3 | RESULTS

3.1 | Soil OC and biomass

As expected, the addition of B to the soil increased the TOC content ($p < 0.05$). Where B was present, TOC increased significantly compared to CTR and WD treatments (Table 1) from 18% to 20% both in the rhizosphere and bulk soil. In the soil where B and WD were applied together (BWD), the OC value was even higher than B treatment, although not significantly.

The DOC content was comparable between bulk and rhizosphere of CTR soil (Table 1). However, the addition of B and the combined BWD treatment resulted in significantly increasing the DOC concentration by about 50% in both bulk soil and rhizosphere. A significant difference can also be seen between bulk soil treatments and those in the rhizosphere, with an increase in DOC ranging from 15% to 19% (Table 1). If we consider the DOC data as percentage of TOC (Table 1), B and BWD treatments exhibited the greatest values, significantly higher compared to the respective soil control (bulk or rhizosphere). Regarding the soil biomass, Figure 1 shows changes in the amount of soil microbial biomass considering the different treatments in bulk and rhizosphere soils, expressed as mg of carbon per kilogram of soil (Figure 1A) and as a percentage of TOC (Figure 1B). Biomass carbon was

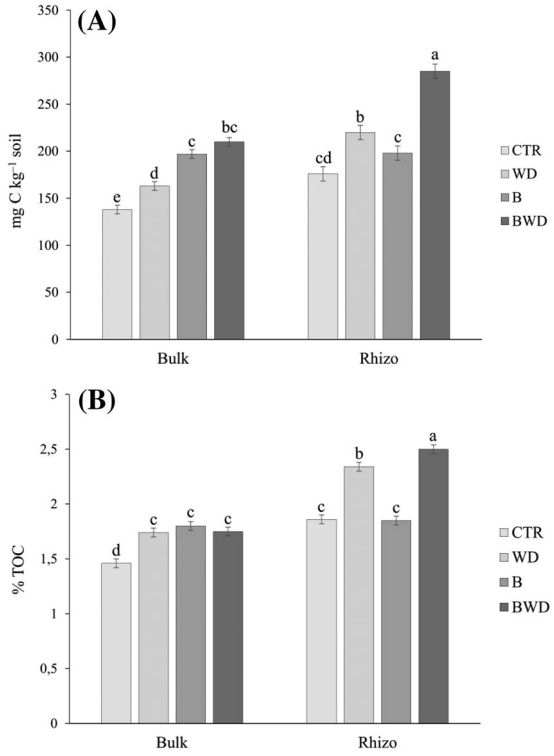

FIGURE 1 Amount of soil biomass carbon (A) and as a percentage of total organic carbon (B) in the bulk soil and in the rhizosphere in the different treatments. Different letters correspond to statistically significant differences according to two-way ANOVA followed by Tukey–Kramer post hoc test ($p \leq 0.05$). B, biochar; BWD, combination of biochar and wood distillate; CTR, control; WD, wood distillate

higher in WD, B, and BWD treatments compared with CTR both in bulk (by 18%, 43%, and 52%, respectively) and rhizospheric soil (by 25%, 13%, and 62%, respectively), clearly indicating the improvement in soil biological quality due to the organic amendments (Figure 1A).

In our study, it is evident that soil biomass is greatly influenced where WD was present, clearly indicating a biostimulating effect of such amendment. The expression of the data as a percentage of TOC (Figure 1B) confirms this positive effect, especially in the rhizosphere. Finally, as expected, a significantly greater biomass (+27%) was observed in the control rhizospheric soil than in the bulk soil, regardless the expression unit (mg kg^{-1} soil or % TOC).

3.2 | Soil enzymatic activities

An overall increase in the soil enzymatic activities tested was detected after the addition of WD (Table 2), although it was significant for phosphatase in bulk soil and for arylsulfatase in both bulk and rhizospheric soils.

In general, all activities turned out to be higher in rhizospheric than in bulk soil except for the β-glucosidase, which was higher in the bulk soil (+19% for CTR and +15% for B). The presence of B depressed the DHase activity (from −45% to −75%) and, contrarily, phosphatase and urease activities were significantly stimulated by the presence of B and, partially, of WD (Table 2). The increase of APase and urease by B treatment ranged from 28% to 47% and 27% to 32%, respectively. For WD, there was a significant difference only in APase, with a range from 13% to 62%. Our results showed that APase activity in soil treated with B was enhanced by the addition of WD, with an increase from 38% to 55% compared to the control (Table 2). However, it is important to state that APase is substrate specific, thus its extracellular activity in soil might not reflect the total microbial status of the soil. For N biogeochemical cycle, we consider the trend of urease because this is an activity that often behaves differently depending on the soil amendment. In our study, the effect of B on urease activity is more evident than the slightly stimulating action of WD. The urease values are higher (about 35%) in the rhizosphere of the basil compared to the bulk soil, with a B = BWD > WD = CTR trend. The values of both β-glucosidase and arylsulfatase activities followed a similar trend, being negatively affected by the presence of B both in bulk and rhizospheric soils (from −12% to 31% and 30% to 42%, respectively). For AI3, the bulk CTR AI3 medium was markedly higher than all the others (Table 3), suggesting that the application of B, WD, and BWD at the right doses increases biological quality of soil.

3.3 | Nitrogen and phosphorus in soil and plant

As shown in Table 4, B and BWD treatments determined an increase in soil total nitrogen in bulk (+8% and +7%) and rhizospheric soil (+5% and +18%), respectively.

Considering the soil available phosphorus, all the treatments tested, except for the WD application in rhizosphere, had a marked positive effect. For the ion forms of N and P, Figure 2 shows the trend of nitric nitrogen and available phosphorus in rhizospheric soil. It is possible to note that, at the end of experiment, the treated soils presented higher quantities of the available anionic forms than control soil (from 38% to 200%) both for nitrate and phosphate.

Regarding plants results, B improved the dry weight of basil from 36% to 43%, and both WD and B improve N and P contents in basil tissue, ranging from 16.4% to 31.5% and 55.5% to 83.3%, respectively (Table 5). B treatment, both alone and in combination with WD, increased dry plant biomass and the uptakes of N and P (Table 5). Nutrient uptake by basil plants was enhanced from 29% to 40% considering N, and more than double considering P. The results of NUE (Table 5) showed B as the key treatment that leads to an improvement of the soil/plant system into the employ of nutrients in the cultivation of basil.

4 | DISCUSSION

4.1 | Soil OC and biomass

Several studies reported the ability of B to interact with the organic compounds of soil. Similar to our findings (Table 1), the ability of B to absorb organic molecules has been also reported by Schulz and Glaser (2012), who demonstrated that the labile organic matter can be stabilized by B. In addition, Becagli et al. (2021) showed the aptitude

TABLE 2 Enzymatic activities of bulk and rhizospheric soil in the different treatments

	Dehydrogenase (μg TTF g^{-1} h^{-1})	
Treatment	**Bulk**	**Rhizo**
CTR	2.8 ± 0.1^{bc}	3.3 ± 0.1^{ab}
WD	3.5 ± 0.1^{ab}	4.5 ± 0.1^{a}
B	0.7 ± 0.1^{d}	0.9 ± 0.1^{d}
BWD	1.5 ± 0.1^{cd}	1.6 ± 0.1^{cd}
p—treatments	***	
p—soils	**	
p—treatments × soils	***	
	Phosphatase (μg *p*-nitrophenol g^{-1} h^{-1})	
Treatment	**Bulk**	**Rhizo**
CTR	181 ± 5^{d}	236 ± 5^{c}
WD	292 ± 5^{ab}	266 ± 5^{bc}
B	266 ± 5^{bc}	302 ± 5^{ab}
BWD	281 ± 5^{abc}	327 ± 5^{a}
p—treatments	***	
p—soils	***	
p—treatments × soils	***	
	β-Glucosidase (μg *p*-nitrophenol g^{-1} h^{-1})	
Treatment	**Bulk**	**Rhizo**
CTR	136 ± 4^{a}	114 ± 4^{b}
WD	140 ± 4^{a}	122 ± 4^{b}
B	94 ± 4^{c}	97 ± 4^{c}
BWD	100 ± 4^{c}	101 ± 4^{c}
p—treatments	***	
p—soils	***	
p—treatments × soils	***	
	Arylsulfatase (μg *p*-nitrophenol g^{-1} h^{-1})	
Treatment	**Bulk**	**Rhizo**
CTR	23.6 ± 0.2^{c}	26.4 ± 0.2^{b}
WD	26.5 ± 0.2^{b}	31.6 ± 0.2^{a}
B	13.8 ± 0.2^{f}	18.6 ± 0.2^{e}
BWD	18.3 ± 0.2^{e}	20.8 ± 0.2^{d}
p—treatments	***	
p—soils	***	
p—treatments × soils	**	
	Urease (mg NH_4^+-N g^{-1} 2 h^{-1})	
Treatment	**Bulk**	**Rhizo**
CTR	22 ± 1^{c}	31 ± 1^{b}
WD	22 ± 1^{c}	30 ± 1^{b}
B	28 ± 1^{bc}	41 ± 1^{a}
BWD	30 ± 1^{b}	46 ± 1^{a}
p—treatments	**	
p—soils	**	
p—treatments × soils	*	

TABLE 3 Alteration index three (AI3) of bulk and rhizosphere soil in the different treatments

	AI3	
Treatment	**Bulk**	**Rhizo**
CTR	-3.3 ± 0.6^{a}	-7.9 ± 0.6^{b}
WD	-9.7 ± 0.6^{bcd}	-9.5 ± 0.6^{bc}
B	-10.9 ± 0.6^{bcd}	-12.9 ± 0.6^{d}
BWD	-11.5 ± 0.6^{cd}	-14.5 ± 0.6^{e}
p—treatments	**	
p—soils	**	
p—treatments × soils	*	

Note: Data are mean ± SE of seven replicates. Considering each parameter separately, different letters and different asterisks correspond to statistically significant differences according to two-way ANOVA followed by Tukey–Kramer post hoc test (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; n.s., not significant).
Abbreviations: B, biochar; BWD, combination of biochar and wood distillate; CTR, control; WD, wood distillate.

of charred biomass to catch the humic substances of vermicompost leachate. Likewise for DOC analysis results, B could be an essential amendment to preserve soil organic matter thanks to its ability to retain carbon compounds. According to Hailegnaw et al. (2019), the impact of B on DOC depends on both soil and B factors, for example, the pyrolysis temperature, the feedstock typologies (herbaceous, woody, or animal-derived by-products), the B/soil ratio, and the soil characteristics (texture, pH, CEC, nutrient content). This has been confirmed by several studies reporting the higher capacity of wood chip B produced with high-temperature pyrolysis, like the one used in our experiment (Hailegnaw et al., 2019; Kasozi et al., 2010; Mukherjee & Zimmerman 2013). Besides, we have seen a significant difference between bulk and rhizospheric soils (Table 1). The highest values of rhizosphere could be due to the contribution of generally easily decomposable polysaccharides (O/N-alkyl carbon) to its clay fractions, compared to the clay fractions of the bulk soil, probably derived from root exudates (Angst et al., 2018). Besides, regardless of the treatment considered, rhizosphere soil showed a higher % TOC with respect to the bulk soil. Thus, it is evident that the addition of B within the cultivation soil determined an increase in both DOC and TOC, probably because the bioavailable OC released by roots (rhizodeposition) was added.

Furthermore, we have seen that the incorporation of both B and WD in bulk soil, and WD in rhizospheric soil, increased MB-C (Figure 1). This increase may be due to the growth of soil microbiota in response to the easily available carbon. Indeed, the highest increase in biomass carbon content occurred in BWD samples for both bulk and rhizosphere soils (210 and 285 μg biomass carbon g^{-1}, respectively). Most studies indicate that B increases microbial biomass (Lehmann et al., 2011; Zhou et al., 2017). Several authors found that the application of WD up to 2% results in increasing the microbial biomass of the soil (Koç et al., 2019), although WD does not change the characteristics of the soil bacterial composition (Zhang et al., 2014). Moreover, Liang et al. (2010) reported an increase in microbial biomass related to an increase in labile OC, such as DOC, which represents a substrate for microbial nutrition.

4.2 | Soil enzymatic activities

Concerning APase and urease activities (Table 2), our results agree with studies reporting that the activity of APase increased after B (Lehmann et al., 2011; Masto et al., 2013; Trupiano et al., 2017) and WD (Koç et al., 2019) applications. Lu et al. (2015) found that phenylalanine activity was significantly enhanced in bulk and rhizosphere of maize after B–manure compost and WD solution amendment.

TABLE 4 N and P in soil and plant at the end of experimentation

	Soil total N (g kg^{-1})		Soil available P (mg kg^{-1})			
Treatments	**Bulk**	**Rhizo**	**Bulk**	**Rhizo**	**Plant N (mg g^{-1})**	**Plant P (mg g^{-1})**
CTR	1.09 ± 0.02^{cd}	1.06 ± 0.02^{d}	17 ± 1^{e}	23 ± 1^{d}	7.3 ± 0.4^{b}	1.8 ± 0.1^{c}
WD	1.10 ± 0.02^{bcd}	1.13 ± 0.02^{bcd}	24 ± 1^{cd}	21 ± 1^{de}	9.6 ± 0.4^{a}	2.8 ± 0.1^{b}
B	1.18 ± 0.02^{b}	1.12 ± 0.02^{bcd}	26 ± 1^{cd}	28 ± 1^{c}	8.5 ± 0.4^{ab}	2.8 ± 0.1^{b}
BWD	1.17 ± 0.02^{bc}	1.25 ± 0.02^{a}	41 ± 1^{a}	35 ± 1^{b}	8.7 ± 0.4^{ab}	3.3 ± 0.1^{a}
p—treatments	*		*		**	**
p—soils	**		*			
p—treatments × soils	*		*			

Note: Data are mean ± SE of seven replicates. Considering each parameter separately, different letters and different asterisks correspond to statistically significant differences according to one- and two-way ANOVA followed by Tukey–Kramer post hoc test (*$p \leq 0.05$; **$p \leq 0.01$; **$p \leq 0.001$; n.s., not significant).
Abbreviations: B, biochar; BWD, combination of biochar and wood distillate; CTR, control; WD, wood distillate.

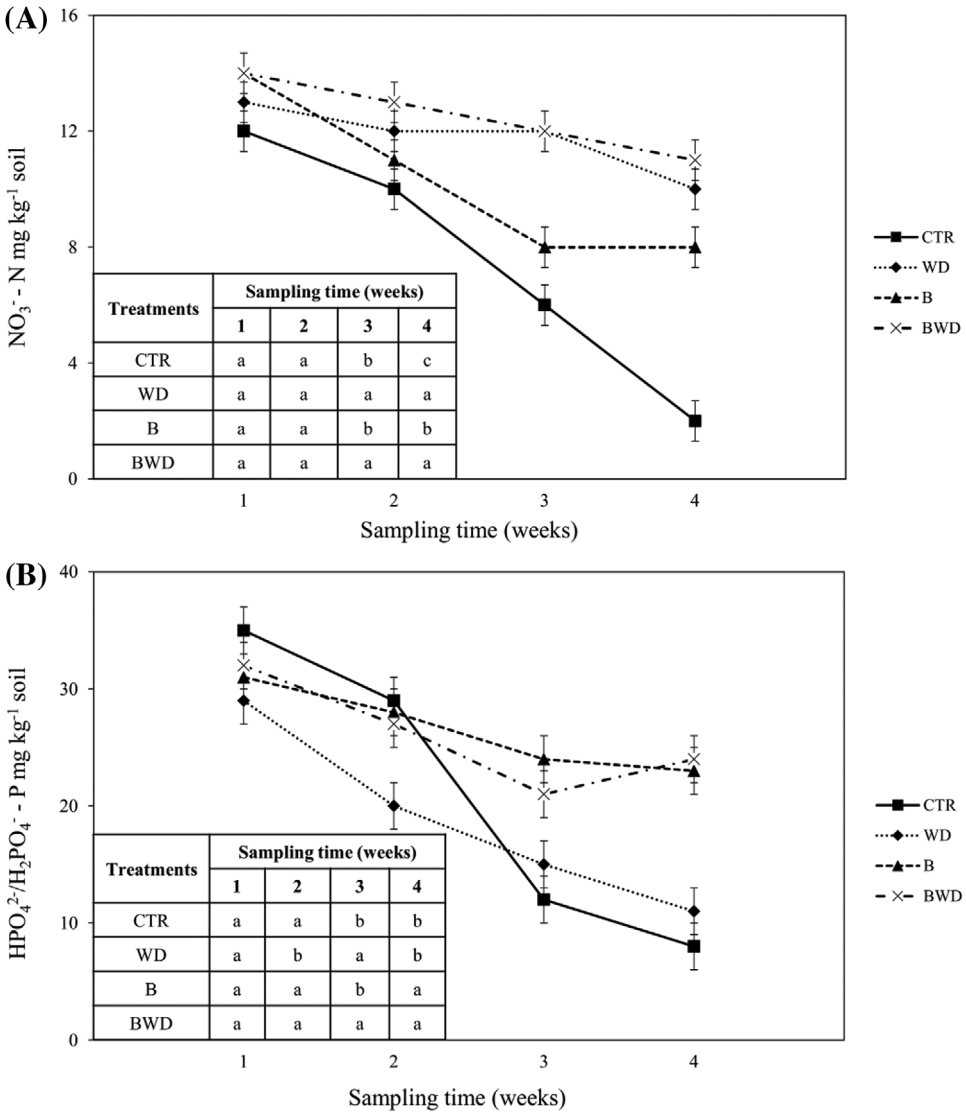

FIGURE 2 Trend of nitric nitrogen and available phosphorus in rhizospheric soil. Different letters in the table correspond to statistically significant differences according to two-way ANOVA followed by Tukey–Kramer post hoc test ($p \leq 0.05$). B, biochar; BWD, combination of biochar and wood distillate; CTR, control; WD, wood distillate

TABLE 5 Dry biomass, N uptake, P uptake, nutrient use efficiency (NUE)-N, and NUE-P response of basil plants to the B, WD, and BWD treatments

Treatment	Dry biomass (mg plant^{-1})	Plant N uptake (mg plant^{-1})	Plant P uptake (mg plant^{-1})	N-NUE (%)	P-NUE (%)
CTR	125 ± 8^b	0.91 ± 0.12^c	0.27 ± 0.06^b	–	–
WD	128 ± 8^b	1.23 ± 0.12^b	0.36 ± 0.06^b	1.4 ± 0.7^b	0.7 ± 0.04^b
B	179 ± 8^a	1.52 ± 0.12^a	0.54 ± 0.06^a	5.8 ± 0.7^a	2.3 ± 0.04^a
BWD	170 ± 8^a	1.47 ± 0.12^a	0.56 ± 0.06^a	5.1 ± 0.7^a	2.5 ± 0.04^a

Note: Data are mean ± SE of seven biological replicates. Considering each parameter separately, different letters correspond to statistically significant differences according to one-way ANOVA followed by Tukey–Kramer post hoc test ($p \leq 0.05$).
Abbreviations: B, biochar; BWD, combination of biochar and wood distillate; CTR, control; WD, wood distillate.

The lowest level of DHase activity in B treatments (Table 2) was also found by Swaine et al. (2013), who reported that the presence of B led to a significant reduction in substrate and extractable product concentrations in soil DHase assays, thus limiting the identification of B's effects on soil enzymatic activity. Although DHase losses in blends can be attributed to decreased effects of B on enzyme activity, the values can also be underestimated due to the impact of B on test components (Cardelli et al., 2019).

Günal et al. (2018) found that the responses of β-glucosidase enzyme activity varied largely depending on soil and B type, B application rate, and fertilizer application rate. Luo and Gu (2016) obtained an increase in β-glucosidase activity with the addition of B to the soil. Contrary to our results, Lu et al. (2020) observed a significant increase of β-glucosidase and sulfatase activities following a single amendment of B in paddy soil.

AI3 is a crucial parameter reflecting the soil quality based on the activities of β-glucosidase, phosphatase, and urease, which represents an indication of the degree of soil alteration. Meyer et al. (2014) reported that AI3 was able to correlate different tests by making a good distinction between treatments, and by evaluating soil organic matter content and yield performance. AI3 has no target values, and ranges from negative to positive scores (Puglisi et al., 2006). In our study (Table 3), AI3 was used to compare treated soils (WD, B, and BWD) with untreated one (CTR). Similarly, other studies have used AI3 to evaluate altered and unaltered soil, such as Puglisi et al. (2006), who reported that treatments with negative AI3 scores had higher TOC content. Previous works confirmed this observation, such as Paz-Ferreiro et al. (2009), Ghosh et al. (2020), and van Huyssteen et al. (2020) who confirmed the correlation between negative AI3 scores and higher soil quality. In our study, BWD treatment considering the rhizospheric soil obtained the lowest AI3 score, likely due to the B's ability to absorb and retain organic molecules and acids from WD, as previously reported. Although not significantly for WD and B treatments, the AI3 values in rhizosphere soil are lower than that in loose soil, suggesting that the combined use of B and WD can improve the growing environment for the root system.

4.3 | Nitrogen and phosphorus in soil and plant

The ability of B to enhance the concentration of nitrogen and available phosphorus through its physicochemical and biological characteristics is reported in several previous manuscripts (Glaser & Lehr, 2019; Liao et al., 2020). Regarding total N, the slight increase of its concentration could be due to both the B's ability to retain the mineral nitrogenous forms from fertilization practices and the WD-induced stimulation of nitrogen-fixing bacteria community of soil (Zhang et al., 2019). The ability of B to adsorb anions could be due to the presence of base functional groups such as chromones, ketones, and pyrones that ensure positive charge or via H-bonding between NO_3^- ions and the surface of B (Kammann et al., 2015; Nguyen et al., 2017). Also, Kameyama et al. (2012) indicated a possible absorption and release of NO_3^--N in the rhizosphere with an increase of N uptake by plants. The increase of the mobile phosphorus forms (Figure 2B) observed in WD treatment could be also ascribed by its low pH value, as suggested by Togoro et al. (2014), who reported an enhancement of phosphate in the leachate of oxisols wetted with high doses of WD [4%–8% (v/v)]. Generally, the use of organic amendments on soil enhances the availability of phosphorous (Ara et al., 2018) and there are no exceptions for B (Glaser & Lehr, 2019). However, there are few studies on the effects on P dynamics by the co-application of B and WD. Our results are in line with Zhang et al. (2020) who reported an increase of soil available P with the co-application of B and WD on a blueberry crop. This could be due to several factors, for example, providing a stimulating environment for specific microbial populations and/or increasing the bioavailability and retention of mobile P forms.

As shown in the results, WD and B seems to increase the capacity of plants to uptake nutrients from the soil (Table 5). Our observations agree with the results by Lashari et al. (2015), who reported an increase of *Zea mays* leaf bioactivity and its nutrient concentrations in 2-year cultivation with the co-application of B–manure compost and pyroligneous solution. A possible explanation might be the high concentration of acetic acid and phenols within the WD, which contribute to create a suitable soil environment for plants growth. It has been reported that acetic acid has several beneficial effects during crops cultivation, including an enhanced drought tolerance (Utsumi et al., 2019). Furthermore, phenols (e.g., guaiacols and exogenous catechol) can also positively influence plant physiology and the rhizosphere (Sun et al., 2013).

There are many studies reporting an increase of dry plant biomass of several cultivated species (i.e., bean, peanut, cucumber) when woody B is applied to soil (Palansooriya et al., 2019). Our results are in line with Ding et al. (2020), who observed a 29% increase in dry biomass of cropped basil after the application of banana leaves and false stems, together with a 2% B. Similar results were also found by Pandey et al. (2016), showing an increase of basil crop biomass cultivated in a pot system with soil and two woody B doses.

The increase of nutrient uptake may be due to the higher bioavailability of these elements within the soil, as shown in Table 4 and Figure 2, due to the amorphous character, surface area, surface acidity or alkalinity, and biological functional groups of surrounding solution of B (Li & Delvaux, 2019), improving the dynamics of nutrients in soil–plant systems, likely due to the chemical and biochemical traits of B and its ability to improve the soil/plant interactions. This is in agreement with Zahedifar and Najafian (2017), who noted a positive influence of B application (up to 3%) on plant's uptakes of N and P in pot-cropped basil.

The increase of NUE (Table 5) found in B treatments is reported in several studies (Becagli et al., 2021; Pereira et al., 2017), and this can be due to two mechanisms: (1) the N and P were ensured to plants by the growth of microbial biomass (Figure 1), making the aforementioned elements more bioavailable; (2) B may enhance directly the availability of elements by its surface characteristics, which improve the retention time of mobile element forms (Figure 2).

5 | CONCLUSIONS

The co-application of WD and B increased OC and microbial biomass, both in bulk soil and rhizosphere. In rhizosphere soil, the pyroligneous acid solution seems to be the best promoter for the increase of microbial biomass.

Generally, WD had positive or no effects on soil enzymes. We did not find overall synergistic effects between the two materials in increasing enzymatic activity, except for phosphatase. However, AI3 indicated that the application of WD and B at the right doses is useful for soil microorganisms, resulting in an increase of the biological quality of the soil.

The simultaneous application of the two organic materials increased the availability of nitrogen and phosphorus, with a consequent positive response in the basil plant, corresponding to an increase of the concentrations of N and P in dry biomass and an increase of the dry weight of basil plants.

Further studies are needed to better understand the links between plant and soil.

The co-application of B and WD could be a potential strategy to improve efficiency in the use of N and P fertilizers. In addition, WD treatment revealed a potential application in sustainable agriculture, due to its origin, availability, fertilizer reliability, and environmental compatibility, but its combined use with B can be a better strategic tool for preserving soil quality. Deeper studies considering other plant species, different soil types, and other cultivation techniques, also in open-field conditions, are highly encouraged.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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