Biomass and Bioenergy 176 (2023) 106914

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com/locate/biombioe

Effect of wood gasification biochar on soil physicochemical properties and enzyme activities, and on crop yield in a wheat-production system with sub-alkaline soil

Nora Baldoni [a], Matteo Francioni [a,*], Laura Trozzo [a], Marco Toderi [a], Flavio Fornasier [b], Paride D'Ottavio [a], Giuseppe Corti [a,c], Stefania Cocco [a]

[a] Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy
[b] Council for Agricultural Research and Economics, Gorizia, Italy
[c] Council for Agricultural Research and Economics, Florence, Italy

ARTICLE INFO

Keywords:
Soil fertility
Mediterranean climate
Soil organic matter
Soil mesofauna

ABSTRACT

Biochar may have beneficial effects on soil depending on its properties and pedoclimatic conditions. Highly sloping soils are prone to erosion and organic matter depletion, and biochar can be useful to restore soil fertility and quality, and crop yields. To test the effect of wood gasification biochar (WGB), we conducted a field experiment applying 0 and 60 Mg ha^{-1} of WGB only (no fertilizer) to a sub-alkaline and fine-textured soil under Mediterranean climate conditions. The effect of WGB on the soil physicochemical properties and on 12 enzyme activities involved in the C, N, P, and S cycles was monitored during a wheat-growing season along with its effect on grain yield. The results show that WGB was rather recalcitrant, and the application of a high dose of it had no effect on most of the soil physicochemical properties, enzyme activities and wheat grain yield. Since enzyme activities involved in the C cycle were similar in WGB-treated and not-treated soils, WGB failed to stimulate organic matter mineralization during the monitored period, with no contribution to N and P supply. Since WGB can contribute to soil C stock with no detrimental effects on wheat yield, wood gasification can allow recycling waste woody materials of urban origin to produce energy and return biochar back to agricultural soils. We suggest that future studies on WGB focus on the effect of its aging in soil on soil physicochemical and biochemical properties, and on crop performances.

1. Introduction

Biochar is an organic carbon (C) rich material (from 40 to 80% C) obtained from thermochemical conversion of biomass at temperatures above 250 °C in the absence of or with limited oxygen that is often produced to be used as soil amendment [1,2]. Biochar is composed of recalcitrant (aromatic) and labile (amorphous) C fractions and ashes whose ratios vary depending on its original feedstock and pyrolysis temperature: generally, a wood-base material produces biochar richer of recalcitrant C with respect to herbaceous materials like straw, while the higher the pyrolysis temperature, the higher the prevalence of recalcitrant compared to the labile C [3,4].

In the last decade, biochar has received much attention due to its beneficial effects on soil fertility and quality, greenhouse gas mitigation, and crop yields [5–9]. Because of its high specific surface area and porosity [10,11], biochar is considered a valuable soil conditioner as it can adsorb pollutants [12,13], increase soil nutrient retention [14,15], improve soil biological properties [16,17], and increase the pH of acidic soils [18,19]. However, some studies indicated that biochar application may have negative effects on crop growth and soil health, such as limiting the development of plant roots, affecting soil organisms (earthworms, fungi, etc.), and enhancing weed growth [9,20]. Furthermore, biochar has an estimated persistence in the soil and a positive C balance that, for the most recalcitrant fractions, account for decades or centuries [21]. Because of this, biochar has been proposed as a C-offsetting tool, provided that it is intimately interspersed in the soil [22]. However, biochar properties like persistence in soil, water retention, C release, and availability of nutrients such as nitrogen (N) and phosphorus (P) mainly depend on the nature of the feedstock [19]. Thus, biochar effect depends on soil properties, climatic conditions, and its

* Corresponding author. Postal address: Via Brecce Bianche 10, 60131, Ancona, Italy.
E-mail address: m.francioni@staff.univpm.it (M. Francioni).

https://doi.org/10.1016/j.biombioe.2023.106914
Received 17 August 2022; Received in revised form 18 June 2023; Accepted 26 July 2023
Available online 11 August 2023

granulometry [5,6,9,10,14,21,23]. Therefore, biochar production process and particle-size are key features when the main purpose of the biochar use in agriculture is to reduce the inorganic fertilizer rate and/or increase crop yield [6,9,21]. In fact, when the biochar is applied to acidic soils, the effects of the addition are maximized mainly because of the increased soil pH, with the consequent increased availability of nutrients and activity of enzymes involved in the C, N, P, and S cycles [8,20,24]. Instead, in alkaline and sub-alkaline soils, a poor effect is expected even for high application rates of biochar, because of the alkalinity reaction of this latter [5]. In these cases, a possible indicator of the effects of the biochar application can be the activity of a wide pool of enzymes, which are frequently used as indicators of soil functional changes [25–27], although the use of the enzyme activities as the sole indicators of soil perturbations is problematic since enzymatic assays determine potential and not real enzyme activities [28,29]. Authors evidenced that biochar could improve soil physicochemical properties like soil aeration, specific surface area, and soil water holding capacity, with tangible enhancement of soil enzyme activities [3,20,30–32]. Other authors, in both long and short-term experiments conducted under field or laboratory conditions, reported that biochar application can increase the activity of soil extracellular and intracellular enzymes involved in C, N, and P cycles [30,33–36]. The effect of biochar addition on enzyme activities is controlled by two main factors: *i*) operations of biochar incorporation (e.g., tillage type, depth, and timing), and *ii*) biochar persistence in the soil. Therefore, the direct and indirect effects of biochar application on soil enzyme activities in both short and long term appear to be due to biochar type and soil characteristics [37].

Thermal gasification of woody biomass is a promising technology that combines production of bioenergy (syngas) and biochar that could be used in agriculture [13,38], but information on the effect of the wood gasification biochar (WGB) on soil morphology and fertility, and on crop performance in open field, especially alkaline or sub-alkaline soils, is still scarce, also because of the scant yield of the gasification method [39]. With the aim to assess the effects of WGB on soil properties and wheat production, we conducted a field experiment by applying a high dose (60 Mg ha^{-1}) of a WGB obtained from a mix woody feedstock to a sub-alkaline soil under a Mediterranean type of climate (central Italy). While the application of WGB can valuably contribute to soil C stock, we hypothesized that WGB applied to this type of soil scarcely affects the soil functional activity with no detrimental effect on wheat production. The hypothesis was tested by monitoring the effect of the applied WGB on physicochemical soil properties and on 12 enzyme activities involved in the C, N, P, and S cycles during a wheat-growing season. The effect of WGB on grain yield was also evaluated.

2. Materials and methods

2.1. Study site

The experiment was performed in the Gallignano experimental field, a hilly area of the Marche region, central Italy, at about 100 m above sea level, on a SW exposure, along a slope with a general inclination of about 15%. In the area, these slopes are used to cultivate cereals (mainly winter wheat) in rotation with alfalfa meadows that can last up to ten years and are often grazed by transhumant sheep flocks during winter [40,41].

The climate of the area is a sub-Mediterranean variant of the temperate oceanic climate [42], which is characterized by a mean annual precipitation of 788 mm and a mean annual air temperature of 14.6 °C, with July as the warmest month (23.3 °C) and January as the coldest one (5.4 °C). Over the study period (September 2018–September 2019), both precipitation and air temperature were monitored by a weather station located about 0.3 km away from the experimental field. The decadal mean of temperature and precipitation of the experimental period and those registered during a 15-year observation period are reported in Fig. 1. The soil of the experimental field developed from

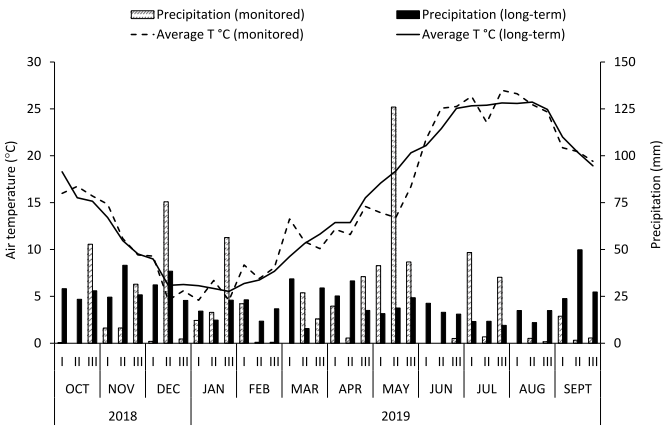

Fig. 1. Decadal mean precipitation and air temperature during the experimental period (August 2018–September 2019) and of the 1998–2012 period as reference for the study area. Gallignano experimental field (central Italy).

thinly layered marine sediments and was poorly drained.

In September 2017, before alfalfa termination, a soil survey was made in the area where the randomized block design had to be established. The slope was slightly affected by erosion, with small rills running along the maximum gradient. The upper belt of the slope (about 23% of inclination) appeared rather uniformly affected by erosion, while the lower belt (about 10% of inclination) showed a greater soil spatial morphological diversity with eroded areas alternated with areas affected by sedimentation of upslope eroded material. Point 1 and Table S1 of the Supplementary Materials report the results of the soil survey, whose results suggested to select the upper belt of the slope to install the experimental field in order to avoid bias due to soil spatial variability. In the experimental field, the upper 25 cm soil displayed a clay loam texture (with 29 ± 2% sand, 41 ± 3% silt, and 30 ± 2% clay) and a bulk density of 1.34 kg dm^{-3} (for the determination of soil texture and bulk density see Point 2 of the Supplementary Materials).

2.2. Characterization of the WGB used

The WGB was obtained from a feedstock made of 1/3 beech, 1/3 pine, and 1/3 fir woods submitted to an industrial gasification process comprising the following steps: *i*) drying of the wood chips at 100–150 °C; *ii*) pyrolysis, from 250 to 450 °C; *iii*) partial oxidation, from 650 to 850 °C; *iv*) reduction, from 850 to 1000 °C. The crude WGB obtained as a by-product of syngas production was roughly sieved mechanically to eliminate the excess of ash and ground to less than 0.5 mm to improve reactivity and soil incorporation. The ground WGB was submitted to the following analyses of characterization. The particle-size distribution was determined by dry and water sieving at 0.25 and 0.10 mm. The pH was determined using a combined glass-calomel electrode in water after the suspension (1:100 w:v) was heated in a water bath to 90 °C, stirred for 20 min to allow dissolution of the soluble components, and cooled to 25 ± 2.5 °C [43]. The content of carbonate-C was quantified by dissolution in 2 M HCl solution and successive titration of the evolved CO_2 [44]. Biochar volatile matter was determined by weight loss after heating [8,45,46]; the muffle furnace was set to 950 °C, and the sample containing crucible was heated for 2 min on the outer edge of the furnace with the door open (about 300 °C), and then for 3 min on the edge of the furnace with the door closed (about 500 °C). Thus, the crucible was left in the muffle for the night at 750 °C and the ash content of the biochar was determined as the remaining weight. Total organic carbon (TOC) after the specimens were treated with a 2 M HCl solution, total nitrogen (TN), and total S (TS) were determined using a CHNS–O analyzer (EA1110, Carlo Erba Instruments, Italy), following the protocol reported by Laberge et al. [47] and Calvelo Pereira et al. [48]. The easily oxidizable organic carbon (EOOC) was estimated by the Walkley-Black

method [49]. Total P (TP) was extracted by heating 0.500 g of sample at 500 °C in a muffle furnace for 16 h and dissolving the ashes in a 5 M HCl solution [50]. Water-extractable P was obtained by forming a suspension with 0.100 g of sample and 30 mL of water; the suspension was shaken at 120 rpm for 48 h in 50 mL centrifuge tubes, centrifuged (3000 *g*, 15 min), and the supernatant filtered using Whatman No. 42 filter paper [51]. A colorimetric method based on ascorbic acid reduction of the ammonium phosphomolybdate complex [52] was used to measure P in the solutions for both TP and water-extractable P. The cation exchange capacity (CEC) was determined as the summation of the exchangeable cations displaced by a 0.2 M $BaCl_2$ solution (1:10 w:v) and shaken for about 10 min [53]. The mixture was left to rest for a while and then gently shaken for other 10 min before centrifugation. The extracted solution was filtered through Whatman 42 filter paper and analyzed for Ca, Mg, K, and Na by atomic absorption with a Shimadzu AA-6300 (Tokyo, Japan) spectrophotometer. All the determinations were run in triplicate. The WGB main physicochemical characteristics are reported in Table 1.

The WGB contained only about 29 g kg^{-1} easily oxidizable organic C and small contents of nutrients like total N (TN) (0.38 g kg^{-1}), water extractable P (about 35 mg kg^{-1}), and exchangeable Ca, Mg, K (all less than 2 cmol + kg^{-1}) (for reference see Refs. [8,54]).

2.3. Experimental design and management practices

The experimental field was a six-year-old alfalfa (*Medicago sativa* L.) stand, which was tilled in October 2017 and then sown with durum wheat [*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.] in 2017–2018 and 2018–2019 cropping years. The present work was carried out during the second year of wheat cultivation, from September 2018 to September 2019. The sowing of wheat for one or two cropping years after alfalfa termination is a common practice in semi-arid and rainfed regions, both in Italy and other countries with a Mediterranean type of climate [55]. In our experimentation, the crops were rainfed and no fertilization was applied in the two wheat cropping years to isolate the effect of WGB.

A complete randomized block design with three replicates and individual plots of 4.0 m^2 (2.0 m × 2.0 m) was established. We are aware that plots are of small scale, but their dimensions was restricted to avoid any bias due to soil spatial variability. The treatments were *i*) durum wheat (Control, W_C) and *ii*) durum wheat with WGB added (W_B). WGB was manually distributed in September 2018 (before the wheat sowing) at a rate of 60 Mg ha^{-1} and was buried with a rotary harrow to 25 cm depth, following the method reported by Castaldi et al. [56].

The six-year-old stand of alfalfa field was terminated in October 2017 using a spading machine, followed by two passages of a rotary harrow in October and November 2017 before sowing wheat. In the following year, the plots were subjected to the same tillage before the wheat sowing. This practice provided approximately 2.53 ± 0.14 Mg ha^{-1} of alfalfa (in 2017) and 3.27 ± 0.50 Mg ha^{-1} wheat straw (in 2018) dry matter that were incorporated into the soil. In both crop years, the durum wheat was sown along rows (sowing rate was 400 seeds m^{-2} of the cv. 'Antalis') at the end of November and harvested at the beginning of July. The management practices adopted from 2017 to the end of the second cropping year are illustrated in Fig. 2.

Table 1
Main physicochemical characteristics of the biochar obtained from wood sources (1/3% beech, 1/3 pine, and 1/3 fir) used in the field experiment.

Parameter	Mean ± Standard deviation
0.50–0.25 mm fraction (g kg^{-1})	78 ± 8
0.25–0.10 mm fraction (g kg^{-1})	579 ± 14
<0.10 mm fraction (g kg^{-1})	343 ± 22
pH_{water}	8.85 ± 0.07
Moisture (%)	14.2 ± 1.2
Volatile matter (%)	84.6 ± 1.3
Ash after biochar combustion (%)	11.36 ± 2.08
Electrical conductivity (dS m^{-1})	2.42 ± 0.11
Total organic carbon (g kg^{-1})	795.4 ± 22.5
Easily oxidizable organic carbon (g kg^{-1})	29.15 ± 4.11
Carbonates-carbon (g kg^{-1})	1.65 ± 0.17
Total nitrogen (g kg^{-1})	0.38 ± 0.05
Total sulphur (g kg^{-1})	10.62 ± 1.27
Total phosphorous (g kg^{-1})	1.74 ± 0.31
Water-extractable phosphorous (mg kg^{-1})	35.3 ± 2.5
Exchangeable Ca ($cmol_+$ kg^{-1})	1.65 ± 0.14
Exchangeable Mg ($cmol_+$ kg^{-1})	1.94 ± 0.21
Exchangeable K ($cmol_+$ kg^{-1})	1.33 ± 0.13
Exchangeable Na ($cmol_+$ kg^{-1})	0.26 ± 0.05
Exchangeable Al ($cmol_+$ kg^{-1})	0.00 ± 0.00
Cation exchange capacity ($cmol_+$ kg^{-1})	5.18 ± 0.09
Base saturation (%)	100.0 ± 0.0

2.4. Soil morphology and sampling

Soil morphological observations were achieved per Schoeneberger et al. [57], while inspections on soil fauna were made by sight with the aim to recognize the type of animals, with no quantitative determination. All the soil observations were conducted in late August and early September 2018 (T0 and T1of Fig. 2, respectively) and in February and July 2019 (T3 and T6 of Fig. 2, respectively) for the six plots with wheat and three plots with alfalfa that were left as a reference for soil morphology only (Table S3 of the Supplementary Materials). The morphological observations were restricted to the Ap horizons generated by the mechanical works since the beginning of the field experiment.

Following crop operations and phenological stages, soil samples were collected from the six wheat plots at T0, T1, T2, T3, T4, T5, T6, and T7 (Fig. 2) by a 5-cm diameter manual auger for the depth of 0–25 cm, so to collect Ap1 and Ap2 horizons together. The total amount of samples collected was 48: 8 sampling dates (T0–T7) × 2 treatments (with and without WGB) × 3 replicates. Soil samples were maintained in a refrigerated bag and in the dark during the field activities; once in the laboratory, they were subdivided into two aliquots: one was maintained at a temperature of −20 °C until the analyses of enzyme activity, the other was allowed to air-dry. The dry samples were sieved through a 2-mm sieve to be submitted to physicochemical analyses.

2.5. Soil analyses

Soil pH was determined in water (1:2.5 w:v) using a combined glass-calomel electrode. Since most of the determined enzyme activities were involved in the C, N, and P cycles, on specimens ground to less than 0.5 mm, the total organic C (TOC) content was estimated by the Walkley-Black method without the application of heat [58], TN content was determined by the semi-micro Kjeldahl method, and available P (Pav)

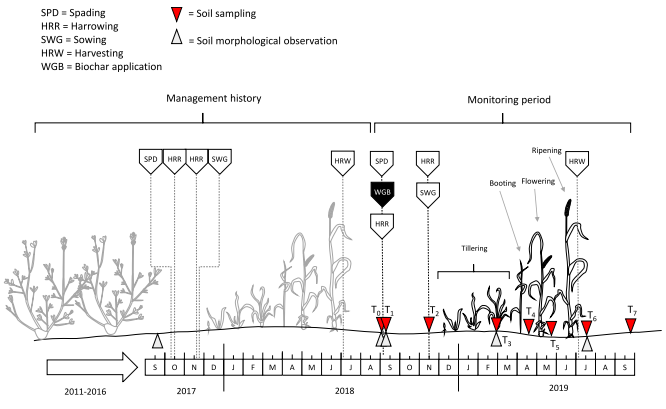

Fig. 2. Crop succession and management practices from 2011 to 2019 in the Gallignano experimental field (central Italy).

was determined according to the protocol of Olsen [59].

The activities of 12 hydrolytic soil enzymes involved in the principal nutrient cycles were determined following the method reported by Cowie et al. [60] which consists in the desorption of enzymes by heteromolecular exchange using lysozyme as desorbing protein. Aliquots of 250 mg for each soil samples were placed in 2-mL Eppendorf tubes together with glass and ceramic beads and 1.4 mL of 3% lysozyme at pH 6. The tube was subjected to bead-beating for 3 min at 30 strokes s^{-1} using a Retsch MM400 mill (Haan, Germany), and then centrifuged for 3 min at 20,000 g. Enzyme activity was assayed fluorometrically in microplates using 4- methylumbelliferyl and L-Leucine-7-amino-4-methylcoumarin derivatives. The activities of xylosidase, β-glucuronidase, β-galactosidase, β-glucosidase, chitinase, acid phosphomonoesterase, and arylsulfatase were determined in 200 mmol L^{-1} 2-(*N*-morpholino) ethanesulfonic acid buffer solution (pH 5.8), while the activities of nonanoate-esterase, leucine aminopeptidase, phosphodiesterase, and pyrophosphatase-phosphodiesterase were determined in a 200 mmol L^{-1} tris-HCl buffer solution at pH 7.5; alkaline phosphomonoesterase activity was determined in a 200 mmol L^{-1} tris-HCl buffer solution at pH 9.0. Table S3 of the Supplementary Materials reports details on the main activity of each enzyme in soil.

2.6. Crop sampling and analysis

At the beginning of July 2019, 10 wheat plants were manually harvested in each plot to assess yield components: number of ears per spike, number of caryopses per ear, weight of the straw, and weight of the chaff and of the caryopses [41]. Since plants were collected on a dry summer day, a low crop moisture was assumed and samples were not oven dried [61]. Grain yield was estimated by counting the total number of wheat plants in 1 m^2 subplot and multiplying it for the weight of the caryopses of the ten sampled plants.

2.7. Data mining and statistical analysis

All data were tested for normality distribution (Shapiro-Wilk's test), homogeneous variances (Levene's test) and, when necessary, for sphericity (Mauchly's test) prior to analysis. When data were not normal distributed and/or not homoscedastic, each numerical variable was transformed by the Box and Cox procedure [62]. A paired-sample T-Test was used to determine the differences within sampling dates. The effect of time, treatments, and their interactions were analyzed through a repeated measure ANOVA. When assumptions were not met, Wilcoxon signed-rank tests were used instead of repeated measures ANOVA. In all the tests, the differences were considered significant at $P < 0.05$. All analyses were performed with IBM SPSS Statistics version 25.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Soil morphology

At T0, the state of aggregation of Ap1, Ap2, and Ap3 horizons under alfalfa was slightly more developed than at the moment of the first survey, and was maintained until T6 (Table S2 of the Supplementary Materials). In the wheat plots, the three Ap horizons present at T0 and T1 (Ap1, Ap2, and Ap3) became two at T3 and T6 (Ap1 and Ap2). The soil color was similar in all the plots at T0 and assumed darker tinges in the Ap1 and Ap2 horizons of the WGB-treated plots. For these horizons, the general color was the result of the presence of soil particles and WGB, often in form of varnish; this is the reason for the reported 'salt-&pepper' effect in Table S2 of the Supplementary Materials. The state of aggregation under wheat was similar or slightly less developed than in alfalfa at T0; farther, especially in the WGB-treated plots, the state of aggregation increased. Worthy to note is that aggregate consistence was hard under alfalfa at T0, became friable at T1 and T3, to change back to a firm consistence at T6. Under W_C, the aggregate consistence was friable at T0, T1, and T3, to become firm at T6; in contrast, under W_B the aggregates were very friable. Ants were observed only in two of the three plots under W_B (plots 2 and 7), while earthworms were ubiquitous in all the wheat plots; other soil mesofauna (spiders, Collembola, insects, etc.) was observed only in the Oi and Ap1 horizons under alfalfa (Table S2 of the Supplementary Materials).

3.2. Effect of WGB on the soil physicochemical properties

No interaction between time and WGB application was found for the main soil characteristics considered (Table 2). Thus, while the addition of WGB had no effect on the sub-alkaline soil pH, it significantly increased TOC, which remained larger in W_B than in W_C from T1 until the end of the experimentation (Table 3). In contrast, TN did not show any variation between W_C and W_B, over time. Soil Pav decreased constantly during the monitoring period (Tables 1 and 2), with a marked decrease after the main tillage (between T0 and T1) for both W_C and W_B. Differences between W_C and W_B were observed only at the end of the monitoring period (T7, more than two months after the wheat harvest), when W_B showed a slightly higher content of Pav than W_C (Table 3).

3.3. Effect of WGB on enzyme activity

No interaction between time and WGB application emerged for the 12 considered enzyme activities (Table 2). Neither the effect of WGB application was significant for the 12 enzyme activities, except for three punctual occasions: T5 for chitinase and T3 for acid phosphomonoesterase and phosphodiesterase. Instead, five enzymes (nonanoate-esterase, β-glucuronidase, β-glucosidase, leucine-aminopeptidase, and acid phosphomonoesterase) showed significant changes over time (Table 2). In particular, the activity of nonanoate-esterase tended to increase during the monitored period, while that of leucine-aminopeptidase increased from T3 to T5-T6, namely from the end of the wheat tillering to the harvesting, to farther decrease (Fig. 3). In contrast, β-glucuronidase and acid phosphomonoesterase activities showed a decreasing trend during the monitored period. β-glucosidase activity decreased from T1 to T3, increased until T5 (wheat flowering), and decreased again farther.

3.4. Effect of WGB on wheat yield

As reported in Table 4, WGB addition had no effect on the production of straw and chaff, on the number of spikelets and caryopses per spike, neither on the caryopses mean weight. Therefore, the wheat yield, which was below the local average production for organic systems (i.e., 3–5 Mg ha^{-1}) [41], was the same between W_C and W_B.

4. Discussion

4.1. Effect of WGB on soil morphology

In the wheat plots, the reduction from three to two Ap horizons in the first 25–26 cm of soil was mainly due to the fusion of the previous Ap1 and Ap2 horizons into one horizon much probably due to the pre-sowing harrowing made in November 2018. As a support for this, the soil color and structure of the Ap1 horizon found in February 2019 appeared formed by the homogenization of the previous Ap1 and Ap2 horizons. As further support for this, in the alfalfa plots where no mechanical work was applied, the horizons remained the same for the whole monitored period. The 'salt&pepper' color displayed by the intact aggregates of the W_B-treated plots was due to the presence of WGB, which appeared not incorporated into the aggregates but adsorbed/laid on their surface. Even though in the literature no indication was found about the effect of WGB on soil aggregates, it is well-known that, in both field and laboratory experiments, biochars derived from non-woody biomass are more

Table 2
Statistical parameters for the main soil properties and enzyme activities for the monitored period under wheat (W_C) and wheat amended with WGB (W_B) at the Gallignano experimental field (central Italy). The means values were compared by a repeated measure ANOVA approach except for leucine-aminopeptidase, which was analyzed by the Wilcoxon test (assumption of normality failed even after the Box-Cox transformation).

Soil properties	Time		Treatment		Time × Treatment	
	F	*P*	*F*	*P*	*F*	*P*
pH	5.09	0.11	0.25	0.67	0.01	0.99
Total organic carbon	6.02	0.08	240.78	0.01	5.52	0.09
Total nitrogen	4.89	0.10	1.50	0.35	1.67	0.30
Available phosphorous	46.15	0.01	20.32	0.05	1.99	0.26

Element cycle	Enzyme activities	Time		Treatment		Time × Treatment	
		F	*P*	*F*	*P*	*F*	*P*
C	Nonanoate-esterase	20.54	0.01	1.02	0.42	1.22	0.39
C	Xylosidase	3.90	0.15	2.87	0.23	0.76	0.50
C	β-glucuronidase	15.51	0.02	1.29	0.37	1.28	0.37
C	β-galactosidase	5.73	0.12	0.89	0.46	0.64	0.53
C	β-glucosidase	12.23	0.03	3.04	0.22	0.16	0.82
C and N	Chitinase	5.23	0.08	3.44	0.21	3.22	0.15
N	Leucine-aminopeptidase	159.78	0.00	0.02	0.87	1.95	0.26
P	Acid phosphomonoesterase	21.57	0.02	0.01	0.93	1.13	0.41
P	Alkaline phosphomonoesterase	1.50	0.33	0.93	0.44	0.96	0.46
P	Phosphodiesterase	0.90	0.47	0.01	0.97	1.26	0.34
P	Pyrophosphatase-phosphodiesterase	5.01	0.10	1.16	0.40	0.86	0.45
S	Arylsulfatase	4.24	0.13	0.01	0.98	0.95	0.50

Table 3
Main soil properties for the monitored period under wheat (W_C) and wheat amended with WGB (W_B) at the Gallignano experimental field (central Italy). Means with different lowercase letters differed significantly between W_C and W_B for $P < 0.05$ (two tails paired T-Test). Numbers after ± are the standard deviation (n = 3).

Soil properties	Treatment	T0	T1	T2	T3	T4	T5	T6	T7
pH	W_C	8.20 ± 0.01	8.21 ± 0.01	8.40 ± 0.05	8.42 ± 0.04	8.40 ± 0.05	8.35 ± 0.02	8.45 ± 0.03	8.51 ± 0.03
	W_B	8.30 ± 0.02	8.21 ± 0.07	8.42 ± 0.07	8.33 ± 0.07	8.47 ± 0.17	8.46 ± 0.12	8.41 ± 0.09	8.48 ± 0.06
Total organic C (g kg^{-1})	W_C	9.45 ± 0.12	9.45 ± 0.09 b	9.73 ± 0.12 b	9.47 ± 0.08 b	10.15 ± 0.12 b	10.47 ± 0.09 b	10.18 ± 0.13 b	9.37 ± 0.07 b
	W_B	8.50 ± 0.06	23.87 ± 0.38 a	24.85 ± 0.56 a	24.20 ± 0.36 a	32.83 ± 0.89 a	37.43 ± 0.59 a	27.33 ± 0.65 a	31.93 ± 0.78 a
Total N (g kg^{-1})	W_C	1.03 ± 0.01	1.02 ± 0.01	1.08 ± 0.01	1.07 ± 0.00	1.08 ± 0.01	1.12 ± 0.01	1.15 ± 0.01	1.03 ± 0.00
	W_B	0.95 ± 0.01	1.07 ± 0.01	1.11 ± 0.00	1.13 ± 0.01	1.08 ± 0.00	1.28 ± 0.01	1.13 ± 0.01	1.10 ± 0.01
Available P (mg kg^{-1})	W_C	16.53 ± 0.07	9.60 ± 0.04	8.65 ± 0.05	7.70 ± 0.05	6.07 ± 0.10	5.70 ± 0.06	3.37 ± 0.05	2.37 ± 0.08 b
	W_B	14.17 ± 0.05	11.63 ± 0.26	9.22 ± 0.45	9.23 ± 0.47	7.50 ± 0.90	7.47 ± 0.36	4.50 ± 0.39	4.77 ± 0.50 a

efficiently incorporated into soil aggregates [31,63–67]. After six months since WGB distribution, the lack of its incorporation into the aggregates was taken as an indication of the recalcitrance of this WGB. However, the age of biochar in soil is also important since, in some cases, no effect was observed during the first year biochar addition [67–69], especially for biochars obtained from woody biomass [8,70,71]. The addition of WGB made the aggregates more friable, probably because of decrease in tensile strength induced by its higher moisture holding capacity [70,72].

The absence of effect of WGB on the presence of earthworms agreed with the findings obtained in open field by Hansen et al. [73], who used straw gasification biochar, and Tammeorg et al. [74], who used wood-pyrolyzed biochar. Instead, the use of biochar was found to induce loss of earthworms, especially in short term, by Briones et al. and Weyes and Spokas [75,76]. This is another indication of the chemical recalcitrance of the used WGB, which appeared to have no negative impact on the earthworms that, instead, seemed to have been favored probably by WGB moisture holding capacity [77].

4.2. Effect of WGB on soil physicochemical properties

4.2.1. Soil pH

The absence of effect on soil pH was another indication of the recalcitrance of WGB used during our field experiment. Our results contrasted with those of Hansen et al. [73], who work with straw gasification biochar in sub-acid soils, and with those of Macdonald et al. [5], who reported significant increments of soil pH after biochar addition in both acid (Arenosol and Ferralsol) and alkaline (Calcisol) soils. However, no significant effect on soil pH was reported by Foster et al. [78] for maize cropping system with woody (pine) pyrolyzed biochar at 30 Mg ha^{-1} rate and by Ventura et al. [79] in an apple orchard using 10 Mg ha^{-1} of wood pyrolyzed biochar. Castaldi et al. [56] incorporated 30 and 60 Mg ha^{-1} biochar in a silty-loam soil with pH 5.4 cultivated with wheat and observed an increase of pH in short term (3 months since biochar incorporation), but no variation after 14 months. In the meta-analysis carried out by Lehmann and Joseph [2], the greatest positive response of biochar occurred in soils with pH values from <4.0 to 5.5. Since our WGB was mechanically sieved to eliminate the excess of ash, which has an alkalizing effect especially in the short term [54], it is conceivable that the recalcitrance of this WGB has prevented consistent changes of the soil pH used for the experimentation. This reinforces the assessment that pH of soil treated with biochar depends on both biochar and soil properties, as reported by Kelly et al. [66] and Rafael et al. [54].

4.3. Total organic C, total N, and available P

In our experimental field, the incorporation of WGB significantly increased TOC content in the treated plots, even though it did not correspond to the theoretical increase of about 14.5 g of TOC per kg of soil derived from the application of 60 Mg ha^{-1} WGB containing about

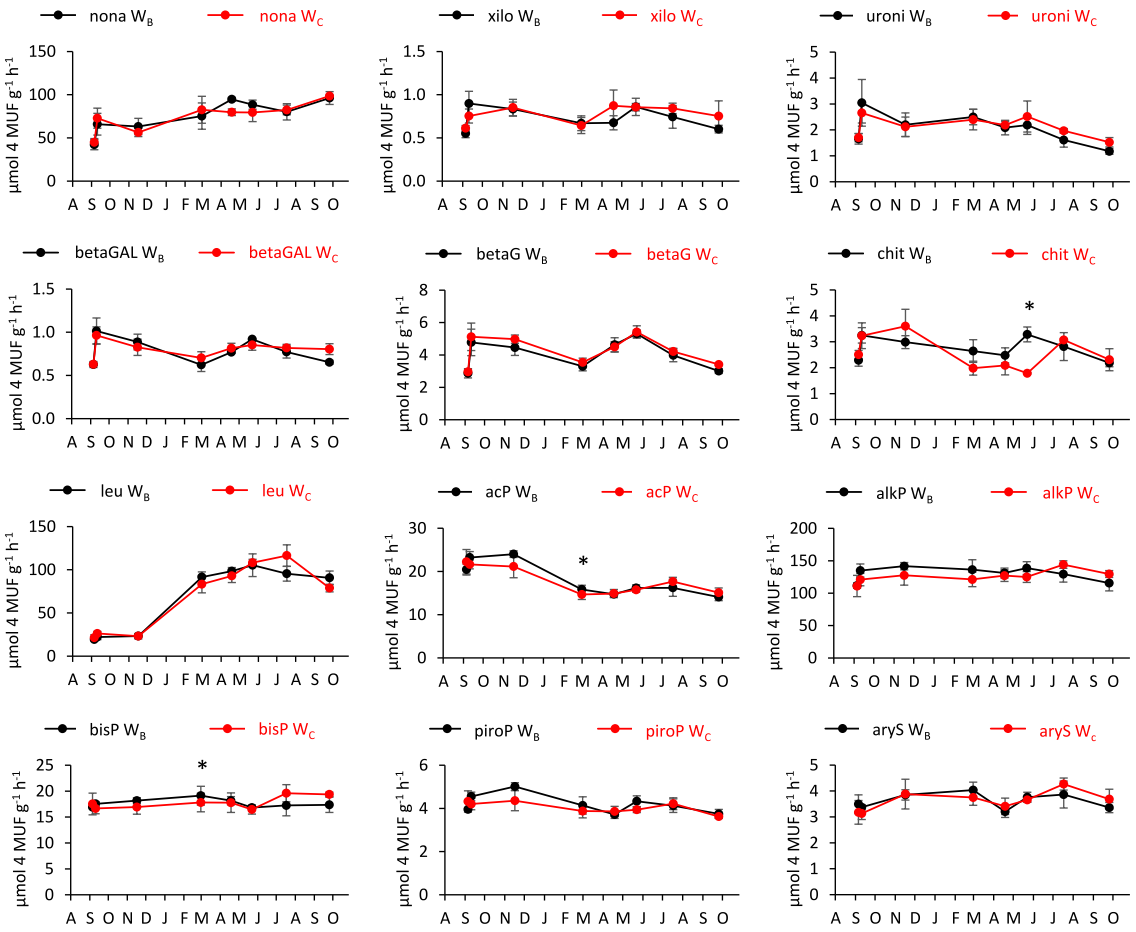

Fig. 3. Enzyme activities during the monitored period under wheat (W_C) and under wheat amended with WGB (W_B) at the Gallignano experimental field (central Italy). * = statistically significant difference at $P < 0.05$ (two tails paired T-Test). Data showed are not Box Cox-transformed. Error bars represent the standard deviation (n = 3). Nona = nonanoate-esterase, xylo = xylosidase, uroni = β-glucuronidase, betaGAL = β-galactosidase, betaG = β-glucosidase, chit = chitinase, leu = leucine-aminopeptidase, acP = acid phosphomonoesterase, alkP = alkaline phosphomonoesterase, bisP = phosphodiesterase, pyroP = pyrophosphatase-phosphodiesterase, aryS = arylsulfatase.

Table 4
Main parameters of wheat yield in the control soil (W_C) and in the soil amended with WGB (W_B). Means with different lowercase letters differed significantly between W_C and W_B for $P < 0.05$ (two tails paired T-Test). Numbers after ± are the standard error of the mean (n = 3).

Treatment	Straw weight per plant (g)	Chaff weight per plant (g)	Spikelets per spike (No.)	Caryopses per spike (No.)	Caryopsis weight per plant (g)	Grain yield (Mg ha^{-1})
W_C	0.81 ± 0.03	0.32 ± 0.02	12.93 ± 0.24	21.17 ± 1.73	0.39 ± 0.04	1.59 ± 0.18
W_B	0.82 ± 0.14	0.34 ± 0.05	13.07 ± 0.84	20.43 ± 2.73	0.38 ± 0.04	1.65 ± 0.25

80% TOC (Table S2) for a soil thickness of 25 cm with 1.34 kg dm^{-3} of bulk density. This condition is rather frequent in field experimentations. For example, Hansen et al. [73] added from 0.8 to 16 Mg ha^{-1} of straw gasification biochar to a 15 cm soil thickness, thus expecting a theoretical increment of roughly 4 g of TOC per kg of soil, but did not observe any TOC increment. In the experimentation of Hansen et al. [73], the occurrence of TOC contents lower than the theoretical increase could be ascribed to the addition of N fertilizer that induced a priming effect able to promote the mineralization rate of organic matter previously present in the soil [80] and, possibly, part of the C added with the biochar even over a short term. In our case, this explanation was not considered totally pertinent because of three reasons: *i*) we did not add any N fertilizer; *ii*) at T1, T2, and T3, the frankly lower increase with respect to the theoretical increment occurred after only one day since WGB incorporation, a time too short to induce a considerable priming effect; *iii*) at T4, T5, and T7, there was a difference W_B-W_C larger than the theoretical increase, for which there is no explanation ascribable to soil conditions. Because of this, the lower increase with respect to the theoretical increment was attributed to a deepening of the smallest WGB particles toward soil anfractuosities deeper than 25 cm produced by spading and harrowing, while the increase larger than the theoretical increment was ascribed to the presence of wheat straw residues incorporated in 2018 and that were presumably collected during the soil sampling. Both explanations would support the recalcitrance of the WGB used. As a partial support of this, Zimmerman et al. [81] found that biochars produced at low pyrolysis temperatures (250–400 °C) degrade faster than those produced at higher temperatures (525–650 °C) and that biochars made using grass feedstocks generally degrade faster than those made with hard woods. Since our WGB was obtained from wood feedstock at the high temperatures required for gasification, there is a high possibility that WGB will be a recalcitrant product.

Since no N fertilizer was applied in the wheat cropping to isolate the

effect of the WGB and because of its scarce content of N, no change of soil TN was expected between W_C and W_B. In fact, the incorporation of 60 Mg ha^{-1} WGB containing 0.38 g kg^{-1} N (Table S2) to a 25-cm soil thickness with 1.34 kg dm^{-3} of bulk density represents a theoretically negligible contribution to the soil TN (about 7 mg N per kg of soil). Consequently, neither the deepening of the smallest WGB particles in the soils, nor the accidental collection of N poor wheat straw during soil sampling were expected to produce changes in the soil TN. However, according to Brtnicky et al. [82], pyrolysis biochar can reduce N bioavailability for plants and yields if N fertilizer is not co-applied. Since in our case no N fertilization was added, the absence of any effect on wheat production indicated that the WGB used had no detrimental effects on wheat yield.

As for TN, soil Pav content was not affected by the addition of 60 Mg ha^{-1} WGB containing a very low amount of water-extractable P: 35.3 mg kg^{-1} (Table S2). The constant decrease of soil Pav during the monitored period was ascribed to the absorption of P by wheat in both W_C and W_B. Instead, the difference between W_C and W_B observed only at T7 was mainly due to the relatively strong reduction of Pav in W_C. Since T7 was more than two months after harvesting, we attributed this difference to the capacity of WGB to maintain P in available forms because of its higher moisture holding capacity [77], as all the biochars do [8,83]. Except for this difference at T7, our results generally agreed with those of Gao et al. [84], who showed that the application of wood pyrolysis biochar and biochars produced at temperatures >600 °C had no effect on soil Pav.

4.4. Effect of WGB on soil enzyme activities

Even though enzymatic assays determine potential enzyme activities [28,29], because of their sensitivity to biotic factors like organic matter content and microbial substrate efficiency [85–87] and abiotic factors like soil temperature and wetting-drying cycles [1,88,89], enzyme activities showed dynamics that may help to understand changes in general soil conditions [90]. Below we discuss the scarce difference between W_C and W_B and the general behavior of the enzyme activities during the wheat-growing season.

4.4.1. Enzyme activities involved in the C cycle

The effect of WGB addition on the soil potential enzyme activities involved in the C cycle was scarce. Working with straw gasification biochar, Imparato et al. [91] found similar results, with only two enzymes (phenol oxidase and cellulase) out of 10 tested that slightly changed their activity with an addition of 6–8 Mg ha^{-1} of gasification biochar to a sub-acid soil. Khadem and Raiesi [92] found that pyrolysis biochar application improved enzyme activities in calcareous soils with low organic matter content, but the increase of pyrolysis temperature adversely affected soil enzymatic functions, especially in soils with fine texture. As a partial support for this, an increase in the ***β***-glucosidase and chitinase activities were observed by Awad et al. [93] after an incubation experiment with commercial biochar (67% C) and plant residues using sandy and sandy loam soils. Since the effect of biochar on soil enzyme activities depends on the properties of both biochar and soil [94], the general lack of differences in our experimentation was attributed to both the recalcitrance of WGB and the sub-alkaline and fine-textured soil used. Indeed, Pokharel et al. [94] analyzed the results of 72 articles and reported that, in general, enzymes involved in the C cycle like β-glucuronidase, β-galactosidase, urease, and xylosidase are scarcely influenced by biochar addition; in contrast, Foster et al. [78] found a decrease of C cycle enzyme activities due to a denaturation or inactivation of the enzymes once adsorbed onto the biochar surfaces. Instead, Lopes et al. [95] observed an increased β-glucosidase activity after the application of biochar obtained from pyrolysis of eucalyptus residues up to the dose of 30 Mg ha^{-1}, whereas a decrease of the activity of the same enzyme was observed with higher doses. In an incubation experiment, Li et al. [96] reported an increased ***β***-glucosidase activity after apple-branch pyrolysis biochar was incorporated to a silt-clay soil, but only when urea was also added. Therefore, it appears rather normal that the addition of a poorly reactive WGB to a sub-alkaline and fine-textured soil can sort scarce effect on the soil potential enzyme activity.

For the three enzyme activities that showed a trend during the wheat-growing season, an explanation of their trend follows. The nonanoate-esterase reflects the contribution of several enzymes involved in the hydrolysis of ester bonds [97] and its activity is stimulated, as for many esterases, by the presence in soil of fatty acids and proteins [98,99]. Further, as reported by Rafael et al. [8,54], the major activity of nonanoate-esterase seems to occur in sub-acid soils. Because of this, the progressive increase of the nonanoate-esterase activity during the crop cycle was attributed to the release in soil of ester bearing moieties by wheat roots, probably following the excretion of rhizogenic stimulants produced by roots and associated rhizospheric microorganisms [100–102]. In case of β-glucuronidase, which is involved in the hydrolysis of hemicellulose, we excluded that its progressive decrease could be due to denaturation or inactivation of the enzyme adsorbed onto WGB, as reported by Foster et al. [78]; therefore, the decreasing trend was ascribed to the decrease of hemicellulose coming from the wheat straw of the past crop. In fact, several enzymes including β-glucuronidase synergistically co-operate in straw degradation starting since residues are left on and in the soil, mostly because of enzymes released by fungi [103,104]. The β-glucosidase, which is involved in the final step of cellulose utilization [97], increased its activity from wheat tillering to flowering probably because of a stimulation of the rhizosphere microbial community promoted by plant growth during this latter phonologic phase.

4.4.2. Enzyme activities involved in the N cycle

The absence of effect on enzyme activities involved in the N cycle was considered a further demonstration of the recalcitrance of the WGB used for the monitored period. Bailey et al. [105] found a reduction of leucine-aminopeptidase after the addition of switchgrass-biochar produced by a fast pyrolysis and linked this reduction to the adsorption of substrate by the biochar. On the contrary, Ventura et al. [79] observed an increase of the leucine-aminopeptidase after a small rate (10 Mg ha^{-1}) of wood pyrolysis biochar application, and Chen et al. [106] found an increase of N cycle enzymes due to the high soil C/N ratio that promotes and stimulates the microbial N mineralization.

Independently from WGB added, leucine-aminopeptidase activity showed a remarkable increase from wheat tillering to harvesting, corresponding to the period lasting from late February to May–July. Because of the increment occurred during warm and moist soil conditions due to air temperature increases and soil rewetting for spring precipitations (Fig. 1), it was attributed to a renewed microbic organic matter breakdown that made available proteins able to stimulate the activity of N enzymes like leucine-aminopeptidase [1,88,107].

4.4.3. Enzyme activities involved in the P cycle

The absence of any remarkable difference between W_C and W_B for P-related enzymes indicated that the added WGB was neither active as a source nor as a sink of P-bearing organic and inorganic molecules [3,108]. Similar results were obtained by Elzobair [109], who did not observe effects on phosphatases after wood pyrolysis biochar was incorporated in a silt loam soil under aridic conditions. Instead, Li et al. [96] observed a significant increase of alkaline phosphatase activity in pot experiments after the addition of apple branch pyrolysis biochar to a silty-clay soil.

The slow decreasing activity of acid phosphomonoesterase with time was attributed to the small increase of soil pH that, from T0 to T7, passed from 8.2 to 8.3 to about 8.5. Even if this change was not statistically significant because the samples were small, it is also true that, in both agricultural and forest soils, the acid phosphatase has its optimum pH in the range 4–6.5 [110,111] and, even though it is possible that at

subalkaline pHs the enzyme maintains a certain activity [90], this latter will decrease with increasing pH.

4.4.4. Enzyme activities involved in the S cycle

In our experimentation, the absence of differences between W_C and W_B for the arylsulfatase activity agreed with the results of an incubation experiment of Paz-Ferreiro et al. [36] and of a field experiment of Sun et al. [112] where birch wood feedstock was used to produce pyrolysis biochar. In contrast, Ventura et al. [79] found that wood-derived biochar had an increasing effect on arylsulfatase. However, as Khadem et al. [113] observed, soil texture is a determinant factor in soil arylsulfatase response to biochar application, with a greater effect in clayey than in sandy loam soil. Since, according to Khadem et al. [113], our soils had a rather appropriate clay loam texture, the null effect of the added WGB was considered as a further proof of its recalcitrance.

4.5. Effect of WGB on wheat yield

The wheat grain yield was not affected by the addition of WGB, as also reported by different authors for more than one year experimentation on cereals using both gasification and pyrolysis biochars obtained from woody feedstocks [73,74,114]. Hansen et al. [73] explained their results with the already high fertility of the soil, where biochar did not improve N and P uptake. In their meta-analysis, Lehmann et al. [2] suggested that biochar applications below 5 Mg ha^{-1} might not be sufficient to generate effects on the crop yield, while applications around 50 Mg ha^{-1} increased the wheat yield by 17%. However, the data reported in this meta-analysis give an idea of the general expectation of wheat and other crops on a global scale, with no specification of the biochar type or soil conditions. Indeed, the effect of biochar on crop-yield appears to depend on the biochar properties and on the pedoclimatic conditions, even though their interactions are not fully understood yet [2]. For example, in a study involving various types of soil (Arenosol, Ferralsol, Vertisol, and Calcisol), Macdonald et al. [5] observed different wheat responses in function of biochar type (poultry litter and wheat straw) and rates (1, 5, and 10 Mg ha^{-1}); moreover, with the same type and rate of biochar, soil type may suppress or enhance wheat production. An additional factor affecting crop yield is the fertilization level, whose interactions with biochar type and soil are numerous [8,115]. For example, working on a Cambisol, Kloss et al. [116] observed no difference in wheat crop yield at diverse wood pyrolysis biochar rates (0, 24, 72 Mg ha^{-1}) if 120 kg ha^{-1} of N was provided, while a decrease occurred when biochar was provided at 72 Mg ha^{-1} without N fertilization. In our study, no fertilizer was applied, but the alfalfa incorporation provided an amount of *N*-bearing biomass that, according to Heuzé et al. [117], was estimated to be about 83 kg ha^{-1} of N [118]. Evidently, the biomass was not sufficiently mineralized to improve the level of available N, nor did the WGB improve the activity of the enzymes involved in the C and N cycles.

5. Conclusions

In the present study, we found that the application of 60 Mg ha^{-1} of WGB to a sub-alkaline soil under a Mediterranean type of climate had no effect on most of the soil physicochemical properties, enzymatic activities, and wheat yield parameters considered. As indicated by the similar activity of the C related enzymes for W_C and W_B, the application of WGB only to a subalkaline and fine-textured soil has had negligible effect on the stimulation of the organic matter mineralization during the monitored period; as a consequence, the wheat experienced a similar N shortage on both W_C and W_B soils.

Since WGB can contribute to soil C stock with no detrimental effects on wheat yield at least over the short-term, wood gasification can allow recycling waste woody materials of urban origin to produce energy and return organic C back to agricultural soils in the form of biochar. However, because the performances of biochars vary according to both biochar and soil characteristics, we suggest that future studies on WGB focus on the effect of its aging in soil on soil physicochemical and biochemical properties, and on crop performances.

Funding

This study was conducted with the support of two projects funded by the Università Politecnica delle Marche: 'Effects of biochar application on crop yields and soil' (2019) and 'Greenhouse gas emissions in temporary and permanent pastures' (2020). Open Access Funding was provided by the Università Politecnica delle Marche through the Elsevier and CRUI agreement.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biombioe.2023.106914.

References

[1] R.G. Burns, J.L. DeForest, J. Marxsen, R.L. Sinsabaugh, M.E. Stromberger, M. D. Wallenstein, M.N. Weintraub, A. Zoppini, Soil enzymes in a changing environment: current knowledge and future directions, Soil Biol. Biochem. 58 (2013) 216–234, https://doi.org/10.1016/j.soilbio.2012.11.009.
[2] J. Lehmann, S. Joseph, Biochar for Environmental Management, Routledge, 2015, https://doi.org/10.4324/9780203762264.
[3] J. Lehmann, M.C. Rillig, J. Thies, C.A. Masiello, W.C. Hockaday, D. Crowley, Biochar effects on soil biota – a review, Soil Biol. Biochem. 43 (2011) 1812–1836, https://doi.org/10.1016/j.soilbio.2011.04.022.
[4] M.W.I. Schmidt, A.G. Noack, Black carbon in soils and sediments: analysis, distribution, implications, and current challenges, Global Biogeochem. Cycles 14 (2000) 777–793, https://doi.org/10.1029/1999GB001208.
[5] L.M. Macdonald, M. Farrell, L. Van Zwieten, E.S. Krull, Plant growth responses to biochar addition: an Australian soils perspective, Biol. Fertil. Soils 50 (2014) 1035–1045, https://doi.org/10.1007/s00374-014-0921-z.
[6] R. Subedi, C. Bertora, L. Zavattaro, C. Grignani, Crop response to soils amended with biochar: expected benefits and unintended risks, Ital. J. Agron. 11 (2017) 161–173, https://doi.org/10.4081/ija.2017.794.
[7] L. Han, K. Sun, Y. Yang, X. Xia, F. Li, Z. Yang, B. Xing, Biochar's stability and effect on the content, composition and turnover of soil organic carbon, Geoderma 364 (2020), 114184, https://doi.org/10.1016/j.geoderma.2020.114184.
[8] R.B.A. Rafael, M.L. Fernández-Marcos, S. Cocco, M.L. Ruello, F. Fornasier, G. Corti, Benefits of biochars and NPK fertilizers for soil quality and growth of cowpea (*Vigna unguiculata* L. Walp.) in an Acid Arenosol, Pedosphere 29 (2019) 311–333, https://doi.org/10.1016/S1002-0160(19)60805-2.
[9] B. Kavitha, P.V.L. Reddy, B. Kim, S.S. Lee, S.K. Pandey, K.-H. Kim, Benefits and limitations of biochar amendment in agricultural soils: a review, J. Environ. Manag. 227 (2018) 146–154, https://doi.org/10.1016/j.jenvman.2018.08.082.
[10] H.M. Anawar, F. Akter, Z.M. Solaiman, V. Strezov, Biochar: an emerging panacea for remediation of soil contaminants from mining, industry and sewage wastes, Pedosphere 25 (2015) 654–665, https://doi.org/10.1016/S1002-0160(15)30046-1.
[11] P. Blackwell, S. Joseph, P. Munroe, H.M. Anawar, P. Storer, R.J. Gilkes, Z. M. Solaiman, Influences of biochar and biochar-mineral complex on Mycorrhizal colonisation and nutrition of wheat and sorghum, Pedosphere 25 (2015) 686–695, https://doi.org/10.1016/S1002-0160(15)30049-7.
[12] A. Saravanan, P.S. Kumar, A.A. Renita, Hybrid synthesis of novel material through acid modification followed ultrasonication to improve adsorption capacity for zinc removal, J. Clean. Prod. 172 (2018) 92–105, https://doi.org/10.1016/j.jclepro.2017.10.109.
[13] P.R. Yaashikaa, P.S. Kumar, S. Varjani, A. Saravanan, A critical review on the biochar production techniques, characterization, stability and applications for circular bioeconomy, Biotechnol. Rep. 28 (2020), e00570, https://doi.org/10.1016/j.btre.2020.e00570.
[14] Z.M. Solaiman, H.M. Anawar, Application of biochars for soil constraints: challenges and solutions, Pedosphere 25 (2015) 631–638, https://doi.org/10.1016/S1002-0160(15)30044-8.

[15] M. Uchimiya, K.T. Klasson, L.H. Wartelle, I.M. Lima, Influence of soil properties on heavy metal sequestration by biochar amendment: 1. Copper sorption isotherms and the release of cations, Chemosphere 82 (2011) 1431–1437, https://doi.org/10.1016/j.chemosphere.2010.11.050.

[16] C. Bamminger, N. Zaiser, P. Zinsser, M. Lamers, C. Kammann, S. Marhan, Effects of biochar, earthworms, and litter addition on soil microbial activity and abundance in a temperate agricultural soil, Biol. Fertil. Soils 50 (2014) 1189–1200, https://doi.org/10.1007/s00374-014-0968-x.

[17] J. Chen, X. Liu, L. Li, J. Zheng, J. Qu, J. Zheng, X. Zhang, G. Pan, Consistent increase in abundance and diversity but variable change in community composition of bacteria in topsoil of rice paddy under short term biochar treatment across three sites from South China, Appl. Soil Ecol. 91 (2015) 68–79, https://doi.org/10.1016/j.apsoil.2015.02.012.

[18] C.R. Anderson, L.M. Condron, T.J. Clough, M. Fiers, A. Stewart, R.A. Hill, R. R. Sherlock, Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus, Pedobiologia 54 (2011) 309–320, https://doi.org/10.1016/j.pedobi.2011.07.005.

[19] C.J. Atkinson, J.D. Fitzgerald, N.A. Hipps, Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review, Plant Soil 337 (2010) 1–18, https://doi.org/10.1007/s11104-010-0464-5.

[20] L. Ouyang, Q. Tang, L. Yu, R. Zhang, Effects of amendment of different biochars on soil enzyme activities related to carbon mineralisation, Soil Res. 52 (2014) 706–716, https://doi.org/10.1071/SR14075.

[21] J. Wang, Z. Xiong, Y. Kuzyakov, Biochar stability in soil: meta-analysis of decomposition and priming effects, GCB Bioenergy 8 (2016) 512–523, https://doi.org/10.1111/gcbb.12266.

[22] S. De Gryze, M. Cullen, D. Durschinger, Evaluation of the Opportunities for Generating Carbon Offsets from Soil Sequestration of Biochar, 2010. http://terraglobalcapital.com/press/Soil_Sequestration_Biochar_Issue_Paper1.pdf.

[23] G. Agegnehu, A.M. Bass, P.N. Nelson, M.I. Bird, Benefits of biochar, compost and biochar-compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil, Sci. Total Environ. 543 (2016) 295–306, https://doi.org/10.1016/j.scitotenv.2015.11.054.

[24] L. Van Zwieten, S. Kimber, S. Morris, K.Y. Chan, A. Downie, J. Rust, S. Joseph, A. Cowie, Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility, Plant Soil 327 (2010) 235–246, https://doi.org/10.1007/s11104-009-0050-x.

[25] D.E. Allen, B.P. Singh, R.C. Dalal, Soil health indicators under climate change: a review of current Knowledge, in: B.P. Singh, A.L. Cowie, K.Y. Chan (Eds.), Soil Health and Climate Change, Springer Berlin Heidelberg, Berlin, Heidelberg, 2011, pp. 25–45, https://doi.org/10.1007/978-3-642-20256-8_2.

[26] M. Schloter, O. Dilly, J.C. Munch, Indicators for evaluating soil quality, Agric. Ecosyst. Environ. 98 (2003) 255–262, https://doi.org/10.1016/S0167-8809(03)00085-9.

[27] R. Lai, A. Lagomarsino, L. Ledda, P.P. Roggero, Variation in soil C and microbial functions across tree canopy projection and open grassland microenvironments, Turk. J. Agric. For. 38 (2014) 62–69, https://doi.org/10.3906/tar-1303-82.

[28] P. Nannipieri, L. Giagnoni, G. Renella, E. Puglisi, B. Ceccanti, G. Masciandaro, F. Fornasier, M.C. Moscatelli, S. Marinari, Soil enzymology: classical and molecular approaches, Biol. Fertil. Soils 48 (2012) 743–762, https://doi.org/10.1007/s00374-012-0723-0.

[29] M.A. Rao, R. Scelza, F. Acevedo, M.C. Diez, L. Gianfreda, Enzymes as useful tools for environmental purposes, Chemosphere 107 (2014) 145–162, https://doi.org/10.1016/j.chemosphere.2013.12.059.

[30] W. Demisie, Z. Liu, M. Zhang, Effect of biochar on carbon fractions and enzyme activity of red soil, Catena 121 (2014) 214–221, https://doi.org/10.1016/j.catena.2014.05.020.

[31] S. Gul, J.K. Whalen, B.W. Thomas, V. Sachdeva, H. Deng, Physico-chemical properties and microbial responses in biochar-amended soils: mechanisms and future directions, Agric. Ecosyst. Environ. 206 (2015) 46–59, https://doi.org/10.1016/j.agee.2015.03.015.

[32] S.G. Lu, F.F. Sun, Y.T. Zong, Effect of rice husk biochar and coal fly ash on some physical properties of expansive clayey soil (Vertisol), Catena 114 (2014) 37–44, https://doi.org/10.1016/j.catena.2013.10.014.

[33] G. Gascó, J. Paz-Ferreiro, P. Cely, C. Plaza, A. Méndez, Influence of pig manure and its biochar on soil CO_2 emissions and soil enzymes, Ecol. Eng. 95 (2016) 19–24, https://doi.org/10.1016/j.ecoleng.2016.06.039.

[34] S. Kumar, S. Chaudhuri, S.K. Maiti, Soil dehydrogenase enzyme activity in natural and mine soil - a review, Middle East J. Sci. Res. 13 (2013) 898–906, https://doi.org/10.5829/idosi.mejsr.2013.13.7.2801.

[35] R.E. Masto, S. Kumar, T.K. Rout, P. Sarkar, J. George, L.C. Ram, Biochar from water hyacinth (*Eichornia crassipes*) and its impact on soil biological activity, Catena 111 (2013) 64–71, https://doi.org/10.1016/j.catena.2013.06.025.

[36] J. Paz-Ferreiro, G. Gascó, B. Gutiérrez, A. Méndez, Soil biochemical activities and the geometric mean of enzyme activities after application of sewage sludge and sewage sludge biochar to soil, Biol. Fertil. Soils 48 (2012) 511–517, https://doi.org/10.1007/s00374-011-0644-3.

[37] K.N. Palansooriya, Y.S. Ok, Y.M. Awad, S.S. Lee, J.-K. Sung, A. Koutsospyros, D. H. Moon, Impacts of biochar application on upland agriculture: a review, J. Environ. Manag. 234 (2019) 52–64, https://doi.org/10.1016/j.jenvman.2018.12.085.

[38] V. Hansen, D. Müller-Stöver, J. Ahrenfeldt, J.K. Holm, U.B. Henriksen, H. Hauggaard-Nielsen, Gasification biochar as a valuable by-product for carbon sequestration and soil amendment, Biomass Bioenergy 72 (2015) 300–308, https://doi.org/10.1016/j.biombioe.2014.10.013.

[39] X. Yang, A. Tsibart, H. Nam, J. Hur, A. El-Naggar, F.M.G. Tack, C.-H. Wang, Y. H. Lee, D.C.W. Tsang, Y.S. Ok, Effect of gasification biochar application on soil quality: trace metal behavior, microbial community, and soil dissolved organic matter, J. Hazard Mater. 365 (2019) 684–694, https://doi.org/10.1016/j.jhazmat.2018.11.042.

[40] M. Francioni, R. Lai, P. D'Ottavio, L. Trozzo, A.W. Kishimoto-Mo, K. Budimir, N. Baldoni, M. Toderi, Soil respiration dynamics in forage-based and cereal-based cropping systems in central Italy, Sci. Agric. 77 (2020) 10, https://doi.org/10.1590/1678-992x-2018-0096.

[41] E. Monaci, S. Polverigiani, D. Neri, M. Bianchelli, R. Santilocchi, M. Toderi, P. D'Ottavio, C. Vischetti, Effect of contrasting crop rotation systems on soil chemical, biochemical properties and plant root growth in organic farming: first results, Ital. J. Agron. 11 (2017) 364–374, https://doi.org/10.4081/ija.2017.831.

[42] A. Agnelli, M. Allegrezza, E. Biondi, S. Cocco, G. Corti, F. Pirchio, Pedogenesis and vegetal landscape: the role of the exposure | Pedogenesi e paesaggio vegetale: il ruolo dell'esposizione, Fitosociologia 45 (2008).

[43] M. Ahmedna, M.M. Johns, S.J. Clarke, W.E. Marshall, R.M. Rao, Potential of agricultural by-product-based activated carbons for use in raw sugar decolourisation, J. Sci. Food Agric. 75 (1997) 117–124, https://doi.org/10.1002/(SICI)1097-0010(199709)75:1<117::AID-JSFA850>3.0.CO;2-M.

[44] L.G. Bundy, J.M. Bremner, A simple titrimetric method for determination of inorganic carbon in soils, Soil Sci. Soc. Am. J. 36 (1972) 273–275, https://doi.org/10.2136/sssaj1972.03615995003600020021x.

[45] K. Jindo, H. Mizumoto, Y. Sawada, M.A. Sanchez-Monedero, T. Sonoki, Physical and chemical characterization of biochars derived from different agricultural residues, Biogeosciences 11 (2014) 6613–6621, https://doi.org/10.5194/bg-11-6613-2014.

[46] R. Zhao, N. Coles, Z. Kong, J. Wu, Effects of aged and fresh biochars on soil acidity under different incubation conditions, Soil Tillage Res. 146 (2015) 13–138, https://doi.org/10.1016/j.still.2014.10.014.

[47] G. Laberge, B.I.G. Haussmann, P. Ambus, H. Høgh-Jensen, Cowpea N rhizodeposition and its below-ground transfer to a co-existing and to a subsequent millet crop on a sandy soil of the Sudano-Sahelian eco-zone, Plant Soil 340 (2011) 369–382, https://doi.org/10.1007/s11104-010-0609-6.

[48] R. Calvelo Pereira, J. Kaal, M. Camps Arbestain, R. Pardo Lorenzo, W. Aitkenhead, M. Hedley, F. Macías, J. Hindmarsh, J.A. Maciá-Agulló, Contribution to characterisation of biochar to estimate the labile fraction of carbon, Org. Geochem. 42 (2011) 1331–1342, https://doi.org/10.1016/j.orggeochem.2011.09.002.

[49] M. Pansu, J. Gautheyrou, Handbook of Soil Analysis, Mineralogical, Organic and Inorganic Methods, Springer Berlin Heidelberg, New York, 2006.

[50] M.J. Lambert, Preparation of Plant Material for Estimating a Wide Range of Elements, Wood Technology and Forest Research Division, Forestry Commission, NSW, Sidney, 1976.

[51] H. Zhang, C. Chen, E.M. Gray, S.E. Boyd, H. Yang, D. Zhang, Roles of biochar in improving phosphorus availability in soils: a phosphate adsorbent and a source of available phosphorus, Geoderma 276 (2016) 1–6, https://doi.org/10.1016/j.geoderma.2016.04.020.

[52] S. Kuo, Phosphorus, Methods of Soil Analysis, vol. 3, 1996, pp. 869–919. Part.

[53] G. Corti, A. Agnelli, F.C. Ugolini, Release of Al by hydroxy-interlayered vermiculite and hydroxy-interlayered smectite during determination of cation exchange capacity in fine earth and rock fragments fractions, Eur. J. Soil Sci. 48 (1997) 249–262, https://doi.org/10.1111/j.1365-2389.1997.tb00545.x.

[54] R.B.A. Rafael, M.L. Fernández-Marcos, S. Cocco, M.L. Ruello, F. Fornasier, G. Corti, Increased phosphorus availability to corn resulting from the simultaneous applications of phosphate rock, calcareous rock, and biochar to an acid sandy soil, Pedosphere 30 (2020) 719–733, https://doi.org/10.1016/S1002-0160(20)60034-0.

[55] M. Toderi, P. D'Ottavio, M. Francioni, A.W. Kishimoto-Mo, R. Santilocchi, L. Trozzo, Short-term response of soil greenhouse gas fluxes to alfalfa termination methods in a Mediterranean cropping system, Soil Sci. Plant Nutr. 68 (2021) 1–9, https://doi.org/10.1080/00380768.2021.1983869.

[56] S. Castaldi, M. Riondino, S. Baronti, F.R. Esposito, R. Marzaioli, F.A. Rutigliano, F. P. Vaccari, F. Miglietta, Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes, Chemosphere 85 (2011) 1464–1471, https://doi.org/10.1016/j.chemosphere.2011.08.031.

[57] P.J. Schoeneberger, D.A. Wysocki, E.C. Benham, Field Book for Describing and Sampling Soils, National soil Survey Center, Lincoln, NE, USA, 2012. Version 3.0.

[58] S. McLeod, Studies on wet oxidation procedures for the determination of Organic C in soils, in: Notes on Soil Techniques, CSIRO Division of Soils, Adelaide, Australia, 1975, pp. 73–79.

[59] S.R. Olsen, Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate, US Department of Agriculture, 1954.

[60] A.L. Cowie, V.E. Lonergan, S.M.F. Rabbi, F. Fornasier, C. Macdonald, S. Harden, A. Kawasaki, B.K. Singh, Impact of carbon farming practices on soil carbon in northern New South Wales, Soil Res. 51 (2013) 707, https://doi.org/10.1071/SR13043.

[61] J. Walter, J. Edwards, G. McDonald, H. Kuchel, Photogrammetry for the estimation of wheat biomass and harvest index, Field Crop. Res. 216 (2018) 165–174, https://doi.org/10.1016/j.fcr.2017.11.024.

[62] J.W. Osborne, Improving your data transformations: applying the box-cox transformation, practical assessment, Res. Eval. 15 (2010), https://doi.org/10.7275/qbpc-gk17.

[63] H.M.S.K. Herath, M. Camps-Arbestain, M. Hedley, Effect of biochar on soil physical properties in two contrasting soils: an Alfisol and an Andisol, Geoderma 209–210 (2013) 188–197, https://doi.org/10.1016/j.geoderma.2013.06.016.

[64] Y. Zong, Q. Xiao, S. Lu, Acidity, water retention, and mechanical physical quality of a strongly acidic Ultisol amended with biochars derived from different feedstocks, J. Soils Sediments 16 (2016) 177–190, https://doi.org/10.1007/s11368-015-1187-2.
[65] Z.L. Du, J.K. Zhao, Y.D. Wang, Q.Z. Zhang, Biochar addition drives soil aggregation and carbon sequestration in aggregate fractions from an intensive agricultural system, J. Soils Sediments 17 (2017) 581–589, https://doi.org/10.1007/s11368-015-1349-2.
[66] C.N. Kelly, J. Benjamin, F.C. Calderon, M.M. Mikha, W. R.D, C.E. Rostad, Incorporation of biochar carbon into stable soil aggregates: the role of clay mineralogy and other soil characteristics, Pedosphere 27 (2017) 694–704, https://doi.org/10.1016/S1002-0160(17)60399-0.
[67] Q. Zhang, Y. Song, Z. Wu, X. Yan, A. Gunina, Y. Kuzyakov, Z. Xiong, Effects of six-year biochar amendment on soil aggregation, crop growth, and nitrogen and phosphorus use efficiencies in a rice-wheat rotation, J. Clean. Prod. 242 (2020), 118435, https://doi.org/10.1016/j.jclepro.2019.118435.
[68] N. Borchard, M. Schirrmann, M.L. Cayuela, C. Kammann, N. Wrage-Mönnig, J. M. Estavillo, T. Fuertes-Mendizábal, G. Sigua, K. Spokas, J.A. Ippolito, J. Novak, Biochar, soil and land-use interactions that reduce nitrate leaching and N2O emissions: a meta-analysis, Sci. Total Environ. 651 (2019) 2354–2364, https://doi.org/10.1016/j.scitotenv.2018.10.060.
[69] G. Haider, D. Steffens, G. Moser, C. Müller, C.I. Kammann, Biochar reduced nitrate leaching and improved soil moisture content without yield improvements in a four-year field study, Agric. Ecosyst. Environ. 237 (2017) 80–94, https://doi.org/10.1016/j.agee.2016.12.019.
[70] H. Blanco-Canqui, Biochar and soil physical properties, Soil Sci. Soc. Am. J. 81 (2017) 687–711, https://doi.org/10.2136/sssaj2017.01.0017.
[71] T.J. Kinney, C.A. Masiello, B. Dugan, W.C. Hockaday, M.R. Dean, K. Zygourakis, R.T. Barnes, Hydrologic properties of biochars produced at different temperatures, Biomass Bioenergy 41 (2012) 34–43, https://doi.org/10.1016/j.biombioe.2012.01.033.
[72] T. Dan, Q. Zhong-Yi, G. Mang-Mang, L. Bo, L. Yi-Jia, Experimental study of influence of biochar on different texture soil hydraulic characteristic parameters and moisture holding properties, Pol. J. Environ. Stud. 24 (2015) 1435–1442. Pol. J. Environ. Stud. 2015;24(3):1435–1442.
[73] V. Hansen, D. Müller-Stöver, V. Imparato, P.H. Krogh, L.S. Jensen, A. Dolmer, H. Hauggaard-Nielsen, The effects of straw or straw-derived gasification biochar applications on soil quality and crop productivity: a farm case study, J. Environ. Manag. 186 (2017) 88–95, https://doi.org/10.1016/j.jenvman.2016.10.041.
[74] P. Tammeorg, T. Parviainen, V. Nuutinen, A. Simojoki, E. Vaara, J. Helenius, Effects of biochar on earthworms in arable soil: avoidance test and field trial in boreal loamy sand, Agric. Ecosyst. Environ. 191 (2014) 150–157, https://doi.org/10.1016/j.agee.2014.02.023.
[75] M.J.I. Briones, P. Panzacchi, C.A. Davies, P. Ineson, Contrasting responses of macro- and meso-fauna to biochar additions in a bioenergy cropping system, Soil Biol. Biochem. 145 (2020), 107803, https://doi.org/10.1016/j.soilbio.2020.107803.
[76] S.L. Weyers, K.A. Spokas, Impact of biochar on earthworm populations: a review, Appl. Environ. Soil Sci. 2011 (2011) 1–12, https://doi.org/10.1155/2011/541592.
[77] V. Hansen, H. Hauggaard-Nielsen, C.T. Petersen, T.N. Mikkelsen, D. Müller-Stöver, Effects of gasification biochar on plant-available water capacity and plant growth in two contrasting soil types, Soil Tillage Res. 161 (2016) 1–9, https://doi.org/10.1016/j.still.2016.03.002.
[78] E.J. Foster, N. Hansen, M. Wallenstein, M.F. Cotrufo, Biochar and manure amendments impact soil nutrients and microbial enzymatic activities in a semi-arid irrigated maize cropping system, Agric. Ecosyst. Environ. 233 (2016) 404–414, https://doi.org/10.1016/j.agee.2016.09.029.
[79] M. Ventura, C. Zhang, E. Baldi, F. Fornasier, G. Sorrenti, P. Panzacchi, G. Tonon, Effect of biochar addition on soil respiration partitioning and root dynamics in an apple orchard, Eur. J. Soil Sci. 65 (2014) 186–195, https://doi.org/10.1111/ejss.12095.
[80] S. Luo, S. Wang, L. Tian, S. Li, X. Li, Y. Shen, C. Tian, Long-term biochar application influences soil microbial community and its potential roles in semiarid farmland, Appl. Soil Ecol. 117–118 (2017) 10–15, https://doi.org/10.1016/j.apsoil.2017.04.024.
[81] A.R. Zimmerman, B. Gao, M.-Y. Ahn, Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils, Soil Biol. Biochem. 43 (2011) 1169–1179, https://doi.org/10.1016/j.soilbio.2011.02.005.
[82] M. Brtnicky, R. Datta, J. Holatko, L. Bielska, Z.M. Gusiatin, J. Kucerik, T. Hammerschmiedt, S. Danish, M. Radziemska, L. Mravcova, S. Fahad, A. Kintl, M. Sudoma, N. Ahmed, V. Pecina, A critical review of the possible adverse effects of biochar in the soil environment, Sci. Total Environ. 796 (2021), 148756, https://doi.org/10.1016/j.scitotenv.2021.148756.
[83] R. Chintala, T.E. Schumacher, L.M. McDonald, D.E. Clay, D.D. Malo, S. K. Papiernik, S.A. Clay, J.L. Julson, Phosphorus sorption and availability from biochars and soil/biochar mixtures, Clean 42 (2014) 626–634, https://doi.org/10.1002/clen.201300089.
[84] S. Gao, T.H. DeLuca, C.C. Cleveland, Biochar additions alter phosphorus and nitrogen availability in agricultural ecosystems: a meta-analysis, Sci. Total Environ. 654 (2019) 463–472, https://doi.org/10.1016/j.scitotenv.2018.11.124.
[85] S.D. Frey, J. Lee, J.M. Melillo, J. Six, The temperature response of soil microbial efficiency and its feedback to climate, Nat. Clim. Change 3 (2013) 395–398, https://doi.org/10.1038/nclimate1796.
[86] S. Manzoni, P. Taylor, A. Richter, A. Porporato, G.I. Ågren, Environmental and stoichiometric controls on microbial carbon-use efficiency in soils, New Phytol. 196 (2012) 79–91, https://doi.org/10.1111/j.1469-8137.2012.04225.x.
[87] N. Zhang, W. Liu, H. Yang, X. Yu, J.L.M. Gutknecht, Z. Zhang, S. Wan, K. Ma, Soil microbial responses to warming and increased precipitation and their implications for ecosystem C cycling, Oecologia 173 (2013) 1125–1142, https://doi.org/10.1007/s00442-013-2685-9.
[88] M. Wallenstein, R.G. Burns, Ecology of extracellular enzyme activities and organic matter degradation in Soil: a complex community-driven process, in: Methods of Soil Enzymology, John Wiley & Sons, Ltd, 2011, pp. 35–55, https://doi.org/10.2136/sssabookser9.c2.
[89] J.M. Steinweg, J.S. Dukes, M.D. Wallenstein, Modeling the effects of temperature and moisture on soil enzyme activity: linking laboratory assays to continuous field data, Soil Biol. Biochem. 55 (2012) 85–92, https://doi.org/10.1016/j.soilbio.2012.06.015.
[90] V. Cardelli, M. De Feudis, F. Fornasier, L. Massaccesi, S. Cocco, A. Agnelli, D. C. Weindorf, G. Corti, Changes of topsoil under *Fagus sylvatica* along a small latitudinal-altitudinal gradient, Geoderma 344 (2019) 164–178, https://doi.org/10.1016/j.geoderma.2019.01.043.
[91] V. Imparato, V. Hansen, S.S. Santos, T.K. Nielsen, L. Giagnoni, H. Hauggaard-Nielsen, A. Johansen, G. Renella, A. Winding, Gasification biochar has limited effects on functional and structural diversity of soil microbial communities in a temperate agroecosystem, Soil Biol. Biochem. 99 (2016) 128–136, https://doi.org/10.1016/j.soilbio.2016.05.004.
[92] A. Khadem, F. Raiesi, Influence of biochar on potential enzyme activities in two calcareous soils of contrasting texture, Geoderma 308 (2017) 149–158, https://doi.org/10.1016/j.geoderma.2017.08.004.
[93] Y.M. Awad, E. Blagodatskaya, Y.S. Ok, Y. Kuzyakov, Effects of polyacrylamide, biopolymer, and biochar on decomposition of soil organic matter and plant residues as determined by 14C and enzyme activities, Eur. J. Soil Biol. 48 (2012) 1–10, https://doi.org/10.1016/j.ejsobi.2011.09.005.
[94] P. Pokharel, Z. Ma, S.X. Chang, Biochar increases soil microbial biomass with changes in extra- and intracellular enzyme activities: a global meta-analysis, Biochar 2 (2020) 65–79, https://doi.org/10.1007/s42773-020-00039-1.
[95] É.M.G. Lopes, M.M. Reis, L.A. Frazão, L.E. da Mata Terra, E.F. Lopes, M.M. dos Santos, L.A. Fernandes, Biochar increases enzyme activity and total microbial quality of soil grown with sugarcane, Environ. Technol. Innov. 21 (2021), 101270, https://doi.org/10.1016/j.eti.2020.101270.
[96] S. Li, C. Liang, Z. Shangguan, Effects of apple branch biochar on soil C mineralization and nutrient cycling under two levels of N, Sci. Total Environ. 607 (2017) 109–119, https://doi.org/10.1016/j.scitotenv.2017.06.275. –608.
[97] M. Mattarozzi, J. Di Zinno, B. Montanini, M. Manfredi, E. Marengo, F. Fornasier, A. Ferrarini, M. Careri, G. Visioli, Biostimulants applied to maize seeds modulate the enzymatic activity and metaproteome of the rhizosphere, Appl. Soil Ecol. 148 (2020), 103480, https://doi.org/10.1016/j.apsoil.2019.103480.
[98] D. Komeil, R. Padilla-Reynaud, S. Lerat, A.M. Simao-Beaunoir, C. Beaulieu, Comparative secretome analysis of *Streptomyces scabiei* during growth in the presence or absence of potato suberin, Proteome Sci. 12 (2014) 1–16, https://doi.org/10.1186/1477-5956-12-35.
[99] A. Sidibé, A.-M. Simao-Beaunoir, S. Lerat, L. Giroux, V. Toussaint, C. Beaulieu, Proteome analyses of soil bacteria grown in the presence of potato suberin, a recalcitrant biopolymer, Microb. Environ. 31 (2016) 418–426, https://doi.org/10.1264/jsme2.ME15195.
[100] L. Gianfreda, Enzymes of importance to rhizosphere processes, J. Soil Sci. Plant Nutr. 15 (2015) 283–306, https://doi.org/10.4067/s0718-95162015005000022.
[101] P. Hinsinger, C. Plassard, B. Jaillard, Rhizosphere: a new frontier for soil biogeochemistry, J. Geochem. Explor. 88 (2006) 210–213, https://doi.org/10.1016/j.gexplo.2005.08.041.
[102] D.C. Naseby, J.M. Lynch, Enzymes and microorganisms in the rhizosphere, enzymes in the environment, in: Activity, Ecology and Applications, Marcel Dekker, New York, 2002, pp. 109–123.
[103] J. Arnthong, C. Chuaseeharonnachai, N. Boonyuen, C. Tachaapaikun, D. Chimchana, L. Eurwilaichitr, V. Champreda, D. Chantasingh, Cooperative decomposition of rice straw by co-cultivation of cellulolytic Fungi, Chiang Mai J. Sci. 45 (2018) 645–652.
[104] A. Rodriguez, F. Perestelo, A. Carnicero, V. Regalado, R. Perez, G. de La Fuente, M.A. Falcon, Degradation of natural lignins and lignocellulosic substrates by soil-inhabiting fungi imperfecti, FEMS Microbiol. Ecol. 21 (1996) 213–219, https://doi.org/10.1016/S0168-6496(96)00057-8.
[105] V.L. Bailey, S.J. Fansler, J.L. Smith, H. Bolton, Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization, Soil Biol. Biochem. 43 (2011) 296–301, https://doi.org/10.1016/j.soilbio.2010.10.014.
[106] J. Chen, S. Li, C. Liang, Q. Xu, Y. Li, H. Qin, J.J. Fuhrmann, Response of microbial community structure and function to short-term biochar amendment in an intensively managed bamboo (Phyllostachys praecox) plantation soil: effect of particle size and addition rate, Sci. Total Environ. 574 (2017) 24–33, https://doi.org/10.1016/j.scitotenv.2016.08.190.
[107] R.G. Burns, Soil Enzymology, Springer Berlin Heidelberg, Berlin, Heidelberg, 2011, https://doi.org/10.1007/978-3-642-14225-3.
[108] G. Xu, J.N. Sun, H.B. Shao, S.X. Chang, Biochar had effects on phosphorus sorption and desorption in three soils with differing acidity, Ecol. Eng. 62 (2014) 54–60, https://doi.org/10.1016/j.ecoleng.2013.10.027.
[109] K.A. Elzobair, M.E. Stromberger, J.A. Ippolito, R.D. Lentz, Contrasting effects of biochar versus manure on soil microbial communities and enzyme activities in an

Aridisol, Chemosphere 142 (2016) 145–152, https://doi.org/10.1016/j.chemosphere.2015.06.044.
[110] R.M. Niemi, M. Vepsäläinen, Stability of the fluorogenic enzyme substrates and pH optima of enzyme activities in different Finnish soils, J. Microbiol. Methods 60 (2005) 195–205, https://doi.org/10.1016/j.mimet.2004.09.010.
[111] K. Rejsek, V. Vranova, M. Pavelka, P. Formanek, Acid phosphomonoesterase (E.C. 3.1.3.2) location in soil, J. Plant Nutr. Soil Sci. 175 (2012) 196–211, https://doi.org/10.1002/jpln.201000139.
[112] Z. Sun, E.W. Bruun, E. Arthur, L.W. de Jonge, P. Moldrup, H. Hauggaard-Nielsen, L. Elsgaard, Effect of biochar on aerobic processes, enzyme activity, and crop yields in two sandy loam soils, Biol. Fertil. Soils 50 (2014) 1087–1097, https://doi.org/10.1007/s00374-014-0928-5.
[113] A. Khadem, H. Besharati, M.A. Khalaj, Biochar application changed arylsulfatase activity, kinetic and thermodynamic aspects, Eur. J. Soil Biol. 95 (2019), 103134, https://doi.org/10.1016/j.ejsobi.2019.103134.
[114] V. Nelissen, G. Ruysschaert, D. Manka'Abusi, T. D'Hose, K. De Beuf, B. Al-Barri, W. Cornelis, P. Boeckx, Impact of a woody biochar on properties of a sandy loam soil and spring barley during a two-year field experiment, Eur. J. Agron. 62 (2015) 65–78, https://doi.org/10.1016/j.eja.2014.09.006.
[115] M.F. Dominchin, R.A. Verdenelli, M.G. Berger, A. Aoki, J.M. Meriles, Impact of N-fertilization and peanut shell biochar on soil microbial community structure and enzyme activities in a Typic Haplustoll under different management practices, Eur. J. Soil Biol. 104 (2021), https://doi.org/10.1016/j.ejsobi.2021.103298.
[116] S. Kloss, F. Zehetner, B. Wimmer, J. Buecker, F. Rempt, G. Soja, Biochar application to temperate soils: effects on soil fertility and crop growth under greenhouse conditions, J. Plant Nutr. Soil Sci. 177 (2014) 3–15, https://doi.org/10.1002/jpln.201200282.
[117] V. Heuzé, G. Tran, D. Bastianelli, H. Archimede, D. Sauvant, Feedipedia: an Open Access International Encyclopedia on Feed Resources for Farm Animals, 2013.
[118] L. Trozzo, M. Francioni, A.W. Kishimoto-Mo, L. Foresi, M. Bianchelli, N. Baldoni, P. D'Ottavio, M. Toderi, Soil N_2O emissions after perennial legume termination in an alfalfa-wheat crop rotation system under Mediterranean conditions, Ital. J. Agron. 15 (2020) 229–238, https://doi.org/10.4081/ija.2020.1613.