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Combined biochar and nitrogen fertilizer change soil enzyme and microbial activities in a 2-year field trial

Dali Song [a,b], Lu Chen [a], Shuai Zhang [a], Qin Zheng [a], Sami Ullah [a], Wei Zhou [a,**], Xiubin Wang [a,*]

[a] Institute of Agricultural Resource and Regional Planning, Chinese Academy of Agricultural Sciences/Key Lab of Plant Nutrition and Nutrient Cycling, Ministry of Agriculture, Beijing, 100081, China
[b] College of Resources and Environmental Sciences, China Agricultural University, Beijing, 100193, China

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ABSTRACT

Treating soil with a combination of nitrogen (N) and biochar (BC) has often been suggested as an approach to enhancing soil quality. In the present study, we therefore conducted a two -year randomized two-factorial field experiment in order to explore optimal N fertilizer management strategies in the context of BC application to calcareous soil, with a focus on both microbial activities and soil nutrient levels. Maize straw BC (0 or 22.5 t ha^{-1}) was applied to the soil once prior to the planting of wheat, with four different N fertilizer concentrations (0, 150, 225, and 300 kg ha^{-1}) being applied to experimental plots. We found that N fertilizer addition resulted in significant reductions in soil pH and available phosphorus (AP) levels, whereas soil phosphatase activity was increased by such treatment. Relative to treatment with only BC or N in isolation, the combined application of both N and BC led to significant increases in soil organic carbon (SOC), total nitrogen (TN), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and available potassium (AK) levels, while also enhancing the activity of C- and N-cycling enzymes. In contrast, this combination treatment did not impact soil pH or phosphatase activity. The application of BC did not significantly affect microbial biomass, but it was associated with changes in overall microbial community structure, including a decrease in the fungi/bacteria ratio and the Gram-negative/Gram-positive bacteria ratio. These changes were also linked to increases in relative actinomycetes abundance and an elevated cy19:0/18:1ω7c ratio. These results suggested that combined N and BC application is thus not conducive to rapid fungal growth, with soil AK, pH, TN, and TDN being the primary factors that affected soil microbial community structure. While BC did significantly increase the βG:(NAG LAM) ratio, this was not associated with any N-mediated microbial restriction. Overall, our findings conclusively demonstrate that combined BC and N fertilizer application can enhance soil quality while supporting a more stable microbial community structure and more active soil biological activity.

1. Introduction

Biochar (BC) is a term used to refer to carbon-rich organic residues that are generated via the pyrolysis of organic compounds under low-oxygen conditions. Owing to the C-rich nature of BC and the fact that it can persist for extended periods of time, there has been substantial research interest in the application of BC as a means of enhancing soil structure and quality [1,2]. BC application has been shown to both directly affect soil properties owing to its mineral contents and adsorptive properties, while also indirectly altering soil pH and biological activity [3,4]. BC is generally rich in minerals including calcium, potassium, and phosphorus that are readily released into the soil during soil amendment [5]. When applied to low-pH soil, BC can also increase local soil pH, thereby altering the binding characteristics of important cationic and anionic nutrients and enhancing the availability of macronutrients including N and P [4,6]. BC application can further bolster soil anion and cation exchange capacity while reducing N leaching [7], and BC-modified soil has been additionally proposed to exhibit increased water-holding capacity that bolsters N retention [8]. However, some studies have provided contrasting evidence suggesting that BC application can adversely impact soil nutrient availability, potentially resulting in microbial N immobilization and thereby decreasing soil

* Corresponding author.
** Corresponding author.
E-mail addresses: zhouwei02@caas.cn (W. Zhou), wangxiubin@caas.cn (X. Wang).

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inorganic N levels [9,10]. These variable outcomes associated with BC application suggest that the impact of BC on soil is BC type-, soil type-, fertilizer-, and time-dependent [3,9,11].

Soil microbes are essential regulators of nutrient cycling and the transformation of organic matter [12]. BC application has been proposed to both directly and indirectly impact these microbial communities via altering key abiotic factors such as soil pH and C availability [13,14]. For example, Warnock et al. [15] proposed that BC application was able to enhance soil microbial biomass via offering a niche for fungal and bacterial growth while simultaneously providing additional C and other mineral nutrients. Other researchers have found that BC application can promote the growth of specific microbes and can thereby increase fungi/bacteria and Gram-negative/Gram-positive bacteria (G+/G−) ratios and associated microbial activities thus improving the overall microbial communities [10,16,17]. However, several other studies have suggested that BC cannot serve as a C source for soil microbes, and that its application has either no impact on these microbial communities or may even adversely affect them [2,18]. Many different parameters have been shown to impact interactions between BC and soil, including the characteristics of the applied BC [19] and N availability within the soil [8]. BC generally contains low levels of inorganic N, and must therefore often be applied together with fertilizer [6,20]. BC-induced changes in soils treated with N fertilizer have been suggested to be attributable to fertilizer-based improvements in direct soil nutrient supplies, thereby bolstering soil microbial activities owing to a lack of N restriction on these microbes [21,22]. A six-year field trial conducted by Tian et al. determined that synergistic effects of BC and NPK application resulted in increased total soil microbial biomass and bacterial biomass [20]. In contrast, Dempster et al. have suggested that BC and N application may not impact soil microbial biomass or community structure, depending on the types of BC and N fertilizer that are used [23].

Generally speaking, an altered microbial community structure is often associated with changes in underlying functional activities [24,25]. Active microbes within the soil produce extracellular enzymes that are capable of catalyzing the depolymerization of large organic molecules, breaking them down into absorbable monomers that can support bacterial growth [26,27]. For example, α-glucosidase (αG), β-xylosidase (βX), β-cellobiosidase (CBH), and β-glucosidase (βG) are important C-cycling hydrolases that serve to primarily degrade cellulose and carbohydrates [25]. In contrast, chitin and other proteins and peptides are broken down by leucine aminopeptidase (LAM) and N-acetyl-glucosaminidase (NAG) [28], while urease (U) facilitates urea degradation and phospholipids are hydrolyzed by phosphomonoesterase (Pho) [29]. These soil extracellular enzymes are essential mediators of soil mineralization [30], and as such Sinsabaugh et al. [31] have defined enzyme stoichiometry that can be used to evaluate microbial nutritional status and metabolic activity. These authors proposed that increases in the (NAG + LAM):Pho, βG:Pho, and βG:(NAG + LAM) ratios respectively correspond to microbial N limits relative to P, C limits relative to P, and C limits relative to N [31,32]. Several other studies have highlighted the relationship between BC application and soil enzyme activity, with conflicting results suggesting that BC can promote [33], inhibit [34], or fail to effect such activity [35]. Strong BC adsorption has been proposed to be the primary mediator of reduced soil enzyme activity [2,36], whereas nutrients and other compounds present within BC are believed to be the primary drivers of enhanced soil enzyme activity [37]. There is, however, additional evidence suggesting that BC can potentially impose nutritional restrictions on soil microbes [38,39], thereby altering soil enzyme activity owing to the fact that microbes utilizing complex BC-derived substrates will require additional N [20].

While BC application is capable of enhancing crop growth and altering soil nutrient availability, it functions in a manner distinct from that of mineral fertilizer owing to its low inorganic N content, leading to it most often being applied in combination with fertilizer [6,20]. In field studies, combined BC and N fertilizer application has been shown to synergistically enhance crop growth and nutrient cycling [20]. However, the specific impact of BC on soil nutrient availability and biological activity is tightly linked to both soil physicochemical properties and to the amount of fertilizer that is applied [1,40]. Field-based studies thus represent an optimal approach to more fully understanding the interaction between BC and differing N levels. The present study was thus designed to assess the combined impact of BC and different N levels on soil nutrient availability, enzyme activities, and microbial community dynamics under field conditions. We hypothesized that combined BC and N fertilizer application would significantly increase soil C and N content and enhance overall soil biological activity. We additionally hypothesized that such combination treatment will result in the imposition of N restrictions on soil microbes, with such restrictions being alleviated via the application of additional N.

2. Materials and methods

2.1. Study site

This field study was conducted at the Fertilizer Efficiency Monitoring Network Station (34°47′02″N, 113°39′25″E) in Zhengzhou, Henan Province, China. This site exhibits a warm-temperate and sub-humid monsoon climate with an annual average temperature of 14.4 °C. The average rainfall at this site was 640 mm per year, of which 60%–70% falls during the maize-growing season (June–September). Study site soil was alkaline and calcareous with a loamy texture (15.5% clay, 26.0% silt, 58.5% sand), and was classified as Aquic Ustochrept according to the American soil classification system. During the two years preceding this field study, no fertilizer was applied to the experimental site. Upon experimental initiation, soil properties were as follows (0–20 cm depth): pH, 8.17; soil organic carbon (SOC), 5.38 g kg^{-1}; total N (TN), 0.71 g kg^{-1}; available phosphorus (AP), 24.98 mg kg^{-1} and available potassium (AK), 154.65 mg kg^{-1}.

BC used in this study was obtained from Xinfa Agricultural Technology (Henan, China), and had been generated via the gradual pyrolysis of maize straw under anoxic conditions at 450 °C (heating at 5 °C min^{-1}, with the maximum temperature being maintained for 1 h). BC properties were as follows: 0.01 mL g^{-1} pore volume; 4.00 m^2 g^{-1} surface area; 23.62% ash; pH, 10.62; 45.20% organic carbon; and 1.50% total N; 1.35% total phosphorus; and 11.87% total potassium.

2.2. Experimental design

This field trial was initiated in October 2014 with the planting of winter wheat (*Triticum aestivum* L., *cv.* Zhengmai 7698) and summer maize (*Zea mays* L., *cv.* Jundan 20) in rotation. For this study, a randomized two-factor (BC and N levels) block design was employed, with all conditions being replicated in triplicate. In total, eight individual treatments were tested, comprising four different N fertilizer levels applied in combination with or without BC (Table S1). Individual plots were 20 m^2 in size, and BC was applied to the soil surface of appropriate plots by hand at 22.5 t ha−1 (1% BC by wt), after which it was rototilled to a 20 cm depth prior to the sowing of wheat. This BC application rate was selected as it is commonly utilized in other studies [41,42], and is generally considered to be an intermediate level BC application [43]. The N fertilizer for this study was applied in the form of urea at four different concentrations (per season): (0, 150, 225, and 300 kg N ha^{-1}). The 225 kg N ha^{-1} concentration is the fertilizer level that is most conventionally utilized by farmers based upon local field conditions. Plots treated with N fertilizer were additionally supplemented with P and K in the form of superphosphate (150 kg P_2O_5 ha^{-1} per season) and potassium chloride (90 kg K_2O ha^{-1} per season), respectively. Winter wheat was sown on approximately October 9th, and was harvested in early June of the following year, while maize was sown following the wheat harvest, and was in turn harvested in early October. For further details regarding these field operations, sowing and irrigation

parameters, see the study conducted by Ai et al. [44].

2.3. Soil sampling

Following maize harvesting in October 2016, soil samples from four random cores (0–20 cm deep; 2.5 cm diameter) near the center of each plot (to eliminate potential edge effects) were collected and pooled together into a single bulk sample. Samples were transferred to airtight polypropylene bags and were maintained on ice during transport to the laboratory, at which time samples were sieved through a 2.0 mm mesh to remove roots and other debris. Remaining soil in each sample was then subdivided into three samples that were air-dried at room temperature and were then utilized for appropriate chemical analyses, were stored at 4 °C for enzymatic analysis, or were frozen at −80 °C for phospholipid fatty acid (PLFA) analyses.

2.4. Measurements

To measure soil water content, samples were dried for 24 h at 105 °C. Levels of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) in fresh soil were extracted with 0.5 M K_2SO_4 and were then quantified with a total organic carbon analyzer (Multi N/C 3100/HT1300, Analytik Jena AG, Germany). Soil microbial biomass carbon (MBC) and nitrogen (MBN) were assessed via a chloroform fumigation approach as previously outlined by Vance et al. [45] and Wu [23,46]. A pH meter was employed to evaluate soil pH (soil:water ratio of 1:2.5) (PE 10, Sartorius, Goettingen, Germany). The $K_2Cr_2O_7$ titration method was used for the measurement of soil organic carbon (SOC) [47], while Kjeldahl digestion was employed for total nitrogen (TN) measurement [48]. AK was measured by displacing exchangeable cations with 1 M ammonium acetate (pH 7) [49], while the Olsen method was used to measure AP [50].

2.5. Soil enzyme activity measurement

Activities of eight different soil enzymes associated with C, N and P cycling were measured in this study, including two N-cycling enzymes (urease and leucine aminopeptidase), four C-cycling enzymes (α-glucosidase, β-xylosidase, β-cellobiosidase and β-glucosidase), one C/N-cycling enzyme (N-acetyl-glucosaminidase) and one P-cycling enzyme (phosphomonoesterase). Standard fluorometric protocols previously detailed by Elzobair et al. [51] and Wang et al. [52] were used to measure the activities of all enzymes other than urease. Soil alkalinity was approximated by suspending and homogenizing 1 g of fresh soil in 100 mL of 50 mM sodium acetate buffer (pH 8.4), with magnetic stirring being employed to maintain samples in suspension. Acetate buffer, sample suspensions, 10 μM reference solutions, and 200 μM substrates were then added to black 96-well microplates that were incubated for 4 h at 25 °C protected from light, after which 10 μL of NaOH (1 M) was added to terminate all enzymatic reactions. A microplate fluorometer (Scientific Fluoroskan Ascent FL, Thermo Fisher Scientific, USA) was then used to evaluate fluorescence in individual wells (excitation: 365 nm; emission: 450 nm). Urease activity was measured as described by Kandeler and Gerber, and is given in mg NH_4^+ g (dry soil) $^{-1}$ h^{-1} [53].

2.6. Phospholipid fatty acid extraction

A previously described PLFA method was employed to measure soil microbial community composition and microbial biomass [54]. Briefly, after soil samples had been freeze-dried, they were suspended in a one-phase mixture of chloroform:methanol:citrate buffer (1:2:0.8 volumetric ratios, pH 4.0) in order to extract fatty acids. Polar lipids were separated from glycolipids and neutral lipids with a silica-bonded phase column using chloroform and acetone, with the resultant phospholipids being eluted using methanol that was subsequently evaporated under a gentle stream of N2. As internal standards, nonadecanoic acid methyl ester (19:0) was added, after which samples were dissolved in n-hexane prior to quantification and identification with an Agilent 6890 gas chromatograph (Agilent Technologies, CA, USA) with a flame-ionization detector. The MIDI Sherlock microbial identification system version 4.5 (MIDI Inc., DE, USA) was employed for PLFA identification.

Previous data were used to differentiate biomarker PLFAs into the following specific groups [51,52]: i14:0, i15:0, i16:0, i17:0, a15:0 and a17:0 were hallmarks of Gram-positive (G^+) bacteria; 16:1ω9c, 16:1ω7c, cy17:0, 17:1ω8c, 18:1ω7c, 18:1ω5cand cy19:0 were hallmarks of Gram-negative (G^-) bacteria. The sum of G^+ and G^- bacteria together with 15:0, and 17:0 for bacteria; 15:00 and 17:00 for other bacteria. The 18:1ω9c and 18:2ω6,9c PLFAs were characteristic of fungi, with16:1ω5c corresponding to arbuscular mycorrhizal fungi (AMF), 10Me-16:0, 10Me-17:0, and 10Me-18:0 corresponding to actinomycetes, and 20:4ω6,9,12,15c and 20:5ω3,6,9,12,15c being indicative of the presence of protozoa [25,55]. The PLFAs 14:00, 16:00, 18:00, and 16:1 2OH were also summed and utilized as overall microbial community indicators, with all of the above PLFAs being incorporated into total PLFA counts [25]. We additionally calculated the ratios of PLFA relative abundances of total saturated/total monounsaturated fatty acids (sat/mono, 14:00 + 15:00 + 16:00 + 17:00 + 18:00/16:1ω5c + 16:1ω7c + 18:1ω7c + 18:1ω9c + 18:2ω6,9c) and cyclopropyl/precursor (cy19:0/18:1ω7c) [56,57].

2.7. Statistical analyses

SAS v9.4 was used for all statistical testing, with significant differences between group means being compared via the least significant difference (LSD) test at a $P = 0.05$ significance threshold. One-way ANOVA) were conducted by using individual N or BC application levels as a single factor, whereas two-way ANOVAs were used to evaluate the effects of interactions between N and BC on other soil parameters of interest. Principal component analysis (PCA) and redundancy analysis (RDA) were conducted with CANOCO (v5.0) in order to assess soil enzyme activity, changes in microbial communities, and the factors influencing these phenotypes. Permutational multivariate analysis of variance (PERMANOVA) was conducted with the Primer software (v6.0; Plymouth, UK). Stepwise multiple regression analysis was conducted to identify factors that influenced the fungi/bacteria ratio, G^+/G^- ratio, cy19:0/18:1ω7c ratio, and sat/mono ratio, with $P < 0.05$ being used as the significance threshold.

3. Results

3.1. Soil chemical properties

We began by evaluating the impact of BC and N application on soil physicochemical properties (Table 1 and Table 2). No significant impact of BC addition on soil pH, C/N or MBN was observed ($P > 0.05$), whereas such addition was associated with significant increases in SOC, TN, DOC, TDN, AP and AK concentrations relative to BC_0 treatments at a given level of N application ($P < 0.05$). N fertilizer application significantly impacted soil pH and AP (up to 73.0% and 47.3% variation, respectively) ($P < 0.05$) in both the absence and presence of BC, with these contents decreasing significantly with increasing N application rate. Significant interactive effects between BC and N were observed on soil DOC, TDN, MBC and AK contents ($P < 0.05$), with these concentrations being elevated in response to combination treatment relative to $BC_{22.5}$ N_0-treated plots. Neither BC, N, nor their combined application significantly affected MBN ($P > 0.05$).

3.2. Soil enzyme activities

Application of BC and N significantly affected N and C cycling-related soil enzyme activity ($P < 0.05$; Tables 1 and 3), with BC application being associated with increases in the significantly affected N and

Table 1
Results of two-way ANOVA of the effects of BC and N fertilizer and their interaction on soil chemical and biological parameters.

Source of variation/factors	BC		N Fertilization		Interaction	
Factors	*F* value	Contribution (%)	*F* value	Contribution (%)	*F* value	Contribution (%)
pH	0.07	0.1	**15.27**	73.0	0.28	1.3
SOC	**148.19**	87.6	0.90	1.6	0.76	1.3
TN	**198.69**	86.3	**3.45**	4.5	1.69	2.2
C/N	0.17	0.7	0.93	11.3	1.92	23.3
DOC	**124.36**	70.2	**7.79**	13.2	**4.50**	7.6
TDN	**209.79**	73.5	**14.61**	15.4	**5.25**	5.5
MBC	**8.62**	20.0	2.30	16.0	**3.88**	27.0
MBN	0.46	2.6	0.32	5.4	0.06	1.0
AP	**80.42**	39.2	**32.37**	47.3	**3.93**	5.7
AK	**818.84**	92.9	**5.22**	1.8	**10.15**	3.5
Soil enzymes						
αG	**53.36**	58.2	**3.89**	12.7	**3.55**	11.6
βG	**38.90**	48.0	**4.78**	17.7	**3.94**	14.6
CBH	**66.82**	56.1	**8.83**	22.2	**3.27**	8.2
βX	**49.37**	52.3	**5.73**	18.2	**3.94**	12.5
Pho	1.31	1.8	**18.44**	74.9	0.42	1.7
NAG	**33.38**	21.1	**8.23**	15.6	**27.96**	53.1
LAM	**1059.20**	89.2	**29.61**	7.5	**7.94**	2.0
U	**53.53**	49.7	**5.37**	15.0	**7.37**	20.5
Microbial groups						
Total PLFAs	4.22	16.6	1.59	18.7	0.15	1.8
Bacteria	0.20	0.7	**3.94**	38.8	0.82	8.1
Fungi	**75.77**	63.9	**4.50**	11.4	**4.46**	11.3
Actinomycetes	**27.79**	58.1	0.11	0.7	1.24	7.8
AM fungi	2.85	11.1	2.19	25.5	0.10	1.2
Protozoa	0.28	0.3	**9.21**	29.0	**17.17**	54.0
Other Bacteria	1.55	6.1	1.62	19.2	0.96	11.4

Bold values indicate a significant influence ($P < 0.05$ or $P < 0.01$). Contribution (%) corresponds to the percentage of the overall variance explained by the indicated factor. Abbreviations: SOC, soil organic carbon; TN, total nitrogen; C/N, ratio of soil organic carbon to total nitrogen; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; AP, available phosphorus; AK, available potassium. Enzyme abbreviations: α-glucosidase, αG; β-glucosidase, βG; β-xylosidase, βX; β-cellobiosidase, CBH; phosphomonoesterase, Pho; N-acetyl-glucosaminidase, NAG; leucine aminopeptidase, LAM; urease, U.

Table 2
Soil chemical properties of the treatments (BC_0 and $BC_{22.5}$ represent the 0 and 22.5 t ha^{-1} BC application rates, respectively. N_0, N_{150}, N_{225}, and N_{300} correspond to the 0, 150, 225 and 300 t ha^{-1} N application rates, respectively.). Data are mean ± S.D. ($n = 3$).

	Treatments	pH	SOC	TN	C/N	DOC	TDN	MBC	MBN	AP	AK
			g/kg	g/kg		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
BC_0	N_0	8.46 ± 0.01a	6.19 ± 0.4B	0.55 ± 0.05B	11.28 ± 0.4	67.51 ± 4.6	1.50 ± 0.2B	200.99 ± 9.7	38.75 ± 3.3	21.95 ± 1.9 aB	124.30 ± 0.9B
	N_{150}	8.42 ± 0.01b	6.43 ± 0.4B	0.56 ± 0.06B	11.44 ± 0.7	68.79 ± 0.0B	3.33 ± 0.6B	187.87 ± 7.6	40.36 ± 4.3	19.44 ± 1.8abB	120.45 ± 2.1B
	N_{225}	8.39 ± 0.02c	6.13 ± 0.6B	0.57 ± 0.07B	10.76 ± 1.0	68.20 ± 2.8B	3.25 ± 0.4B	190.67 ± 11.6	41.12 ± 4.2	13.14 ± 0.3 cB	115.95 ± 4.4B
	N_{300}	8.37 ± 0.00c	6.22 ± 0.5B	0.58 ± 0.06B	10.06 ± 0.8	71.37 ± 4.0B	3.34 ± 1.4B	192.19 ± 13.7B	40.75 ± 3.0	17.28 ± 1.4b	120.25 ± 10.0B
	Pr > F	**	NS	NS	NS	NS	NS	NS	NS	**	NS
$BC_{22.5}$	N_0	8.47 ± 0.04a	8.67 ± 0.6A	0.82 ± 0.07bA	11.57 ± 1.4	74.21 ± 4.5b	6.15 ± 1.2bA	194.00 ± 3.0b	40.60 ± 5.1	33.30 ± 1.1 aA	164.52 ± 4.1 cA
	N_{150}	8.41 ± 0.03 ab	9.51 ± 0.5A	0.86 ± 0.03bA	10.37 ± 1.0	87.74 ± 2.8 aA	13.44 ± 1.1 aA	200.00 ± 7.3b	41.14 ± 2.5	26.52 ± 3.1bA	183.70 ± 2.6abA
	N_{225}	8.37 ± 0.02b	9.66 ± 0.9A	0.88 ± 0.04bA	10.87 ± 0.4	85.59 ± 2.0 aA	11.12 ± 1.6 aA	200.27 ± 9.0b	42.11 ± 1.0	21.35 ± 2.4bcA	180.50 ± 7.6bA
	N_{300}	8.38 ± 0.04b	9.35 ± 0.8A	0.99 ± 0.05 aA	11.29 ± 0.4	85.67 ± 1.5 aA	13.46 ± 2.8 aA	220.56 ± 5.4 aA	40.97 ± 2.4	19.65 ± 0.8c	190.55 ± 1.9 aA
	Pr > F	*	NS	*	NS	**	**	**	NS	**	**

Different lowercase letters in a given column correspond to significant differences between N treatments at a given BC level ($P < 0.05$). Uppercase letters correspond to significant differences among BC treatments at a given N application level ($P < 0.05$). NS, * and ** indicate ANOVA results of $P > 0.05$, $P < 0.05$ and $P < 0.01$, respectively. Abbreviations: SOC, soil organic carbon; TN, total nitrogen; C/N, ratio of soil organic carbon to total nitrogen; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; AP, available phosphorus; AK, available potassium.

C cycling-related soil enzyme activity. However, BC application did not affect Pho activity ($P > 0.05$). In the absence of BC, N application only significantly affected Pho activity, increasing its activity in a dose-dependent manner ($P < 0.05$; Table 3), contributing to 74.9% of the variation in the activity of Pho (Table 1). In contrast, under $BC_{22.5}$ conditions, N application significantly impacted the activities of all

Table 3
Soil enzyme activities in treatments (BC_0 and $BC_{22.5}$ represent the 0 and 22.5 t ha^{-1} BC application rates, respectively. N_0, N_{150}, N_{225}, and N_{300} correspond to the 0, 150, 225 and 300 t ha^{-1} N application rates, respectively.). Data are means ± S.D. ($n = 3$).

	Treatments	αG	βG	CBH	βX	Pho	NAG	LAM	U
					nmol h^{-1} g^{-1}				NH_4^+-mg $g^{-1}d^{-1}$
BC_0	N_0	12.87 ± 1.7B	54.04 ± 0.4	8.39 ± 0.8B	13.84 ± 0.2	133.43 ± 13.6b	7.53 ± 0.4	224.49 ± 1.3B	0.12 ± 0.05
	N_{150}	14.91 ± 1.9	55.95 ± 7.9	8.65 ± 0.2B	13.38 ± 1.8B	163.34 ± 6.8a	6.89 ± 0.8	242.89 ± 12.7B	0.15 ± 0.02B
	N_{225}	13.93 ± 2.1B	57.89 ± 3.2B	9.70 ± 1.4B	15.74 ± 0.1B	163.46 ± 9.6a	5.99 ± 0.7B	251.51 ± 19.1B	0.11 ± 0.05B
	N_{300}	15.00 ± 1.2B	55.18 ± 5.8B	9.50 ± 0.9B	13.48 ± 1.4B	178.04 ± 1.4a	7.08 ± 0.6	223.74 ± 8.1B	0.13 ± 0.03B
	Pr > F	NS	NS	NS	NS	**	NS	NS	NS
$BC_{22.5}$	N_0	17.15 ± 0.6bA	60.75 ± 6.1c	10.69 ± 1.6 cA	14.75 ± 1.5b	137.63 ± 13.6b	6.86 ± 0.5c	354.35 ± 4.5bA	0.15 ± 0.03b
	N_{150}	17.14 ± 1.6b	63.20 ± 3.7bc	12.17 ± 1.2bcA	19.47 ± 1.3 aA	162.92 ± 5.5a	8.19 ± 0.9b	430.00 ± 1.2 aA	0.22 ± 0.01bA
	N_{225}	21.87 ± 1.9 aA	70.53 ± 6.3abA	16.35 ± 0.9 aA	19.72 ± 2.2 aA	176.54 ± 0.9a	11.37 ± 0.6 aA	414.49 ± 14.5 aA	0.32 ± 0.06 aA
	N_{300}	19.11 ± 0.7bA	79.26 ± 0.6 aA	13.63 ± 2.0abA	19.16 ± 1.6 aA	181.43 ± 20.0a	7.02 ± 0.3c	358.60 ± 15.6bA	0.31 ± 0.04 aA
	Pr > F	**	**	**	*	*	**	**	**

Different lowercase letters in a given column correspond to significant differences between N treatments at a given BC level ($P < 0.05$). Uppercase letters correspond to significant differences among BC treatments at a given N application level ($P < 0.05$).NS, * and ** indicate ANOVA results of $P > 0.05$, $P < 0.05$ and $P < 0.01$, respectively. Abbreviations: α-glucosidase, αG; β-glucosidase, βG; β-xylosidase, βX; β-cellobiosidase, CBH; phosphomonoesterase, Pho; N-acetyl-glucosaminidase, NAG; leucine aminopeptidase, LAM; urease, U.

enzymes ($P < 0.05$).

The (NAG + LAM):Pho and βG:Pho ratios were significantly elevated in BC-amended soils relative to BC0 soil samples ($P < 0.05$), and N addition significantly decreased these ratios relative to their N0 values. The βG:(NAG + LAM) ratio was also significantly decreased in samples from BC-treated plots relative to BC_0 plots, and it rose with increasing rates of N application ($P < 0.05$; Fig. 1C). A PCA approach was used to visualize and analyze changes in soil enzyme activities under different treatment conditions (Fig. 2A), with the first and second principal components (PC1 and PC2) accounting for 69.3% and 10.8% of the variance, respectively. On this PCA plot, the two BC application conditions were clearly separated from one another along the PC1 axis ($P < 0.05$), while N-amended samples were clearly separated from N_0 samples along the PC1 axis ($P < 0.05$). RDA suggested that SOC, TDN, pH,

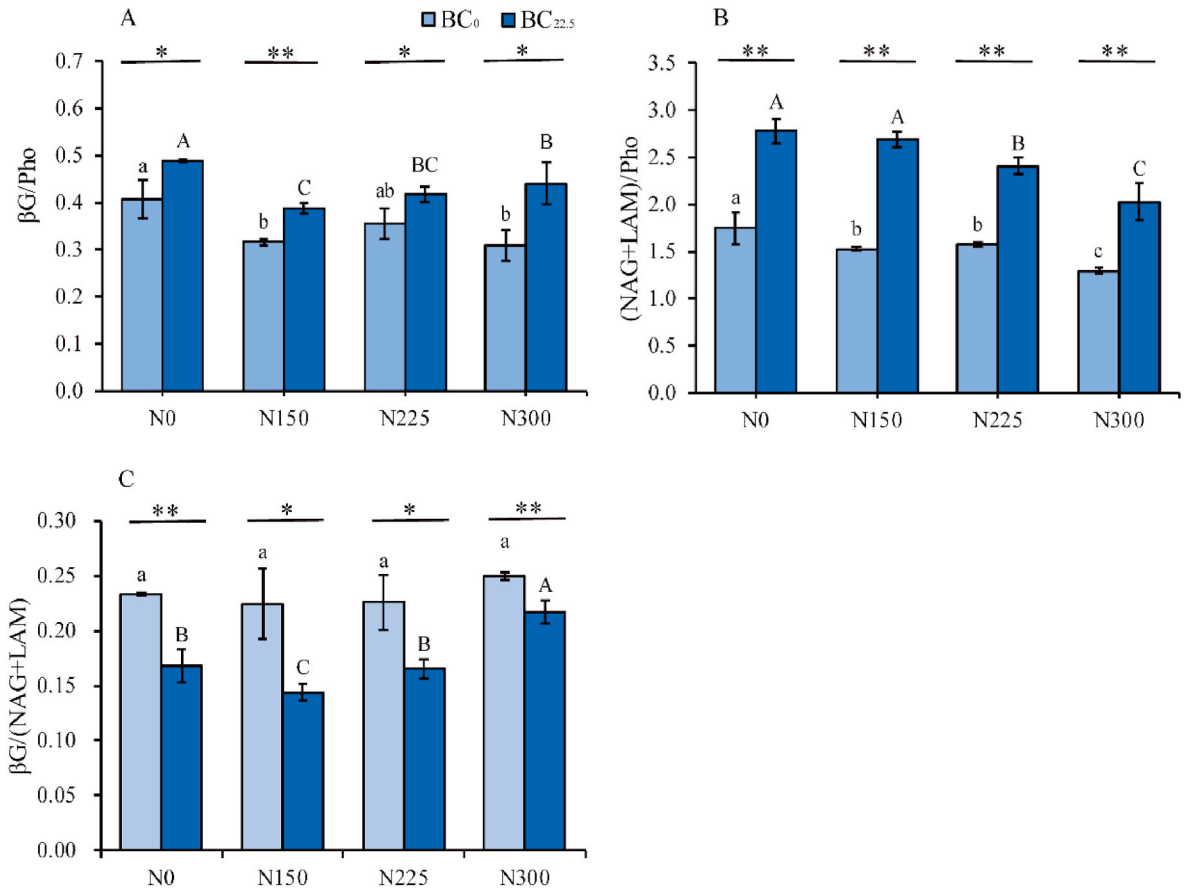

Fig. 1. Soil microbial enzyme activity stoichiometry (BC_0 and $BC_{22.5}$ represent the 0 and 22.5 t ha^{-1} BC application rates, respectively. N_0, N_{150}, N_{225}, and N_{300} correspond to the 0, 150, 225 and 300 t ha^{-1} N application rates, respectively.). Enzyme abbreviations: β-glucosidase, βG; phosphomonoesterase, Pho; *N*-acetyl-glucosaminidase, NAG; leucine aminopeptidase, LAM. Different lowercase letters correspond to significant differences among N levels under BC_0 treatment conditions ($P < 0.05$), while different uppercase letters correspond to significant differences among N levels under $BC_{22.5}$ treatment conditions ($P < 0.05$). Asterisks indicate significant differences between BC conditions at a given N level ($P < 0.05$). Data are means ± standard deviations ($n = 3$).

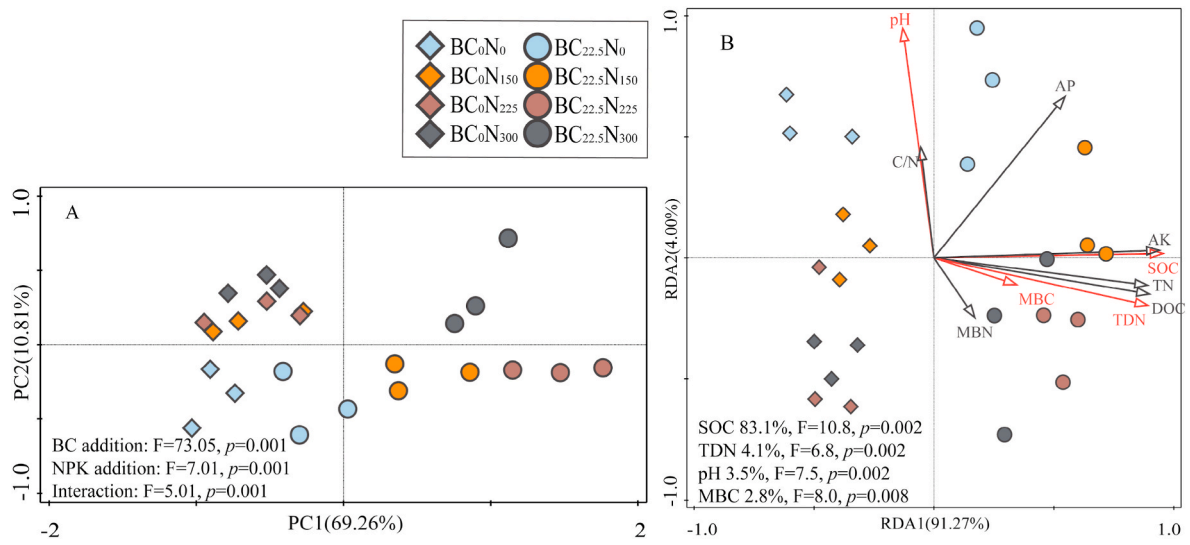

Fig. 2. Principal component analyses (PCA) of soil enzyme activities under differing treatment conditions (A), and redundancy analyses (RDA) of relationships among soil parameters and enzyme activities (B) (BC_0 and $BC_{22.5}$ represent the 0 and 22.5 t ha^{-1} BC application rates, respectively. N_0, N_{150}, N_{225}, and N_{300} correspond to the 0, 150, 225 and 300 t ha^{-1} N application rates, respectively.). Red arrows are used to identify parameters significantly correlated with enzyme activities ($P < 0.05$). Percentages in the lower right corner indicate the percentage of variability explained. Abbreviations: SOC, soil organic carbon; TN, total nitrogen; C/N, ratio of soil organic carbon to total nitrogen; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; AP, available phosphorus; AK, available potassium. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and MBC were able to significantly explain observed shifts in soil enzyme activities (Fig. 2B).

3.3. Microbial community composition

BC application did not affect total PLFA ($P > 0.05$), whereas it did more significantly fungal and actinomycete communities than did N amendment, accounting for 63.9% and 58.1% of the total variance in these two communities, respectively ($P < 0.05$; Tables 1 and 4). In BC-amended soil samples, the relative actinomycete PLFAs content was increased by 2.4%–14.4%, whereas the relative fungal PLFA content was decreased 8.0%–18.3% relative to BC_0 soil samples at a given N application level. In contrast, N fertilizer application more significantly affected protozoans than did BC application, accounting for 29.0% of the observed variation ($P < 0.05$; Table 1). Relative protozoan PLFAs levels fell significantly with rising N application rate in BC_0 soil samples ($P < 0.05$; Table 4), whereas such N application failed to affect protozoan PLFA contents in BC-amended soil samples ($P > 0.05$).

A PCA approach was used to evaluate overall shifts in community composition based upon these PLFA analyses, with PC1 and PC2 explaining 46.0% and 33.6% of the observed variation, respectively (Fig. 3). BC application ($P = 0.001$), N application ($P = 0.001$), and interactions between the two ($P = 0.003$) all significant impacted microbial community composition (PERMANOVA test; Fig. 3A), with significant differences between the BC_0 and $BC_{22.5}$ treatments ($P < 0.05$), and between the N_0 and other N application treatments also being detected ($P < 0.05$). No significant differences were detected among samples that had been subjected to different N fertilizer application rates ($P > 0.05$). RDA indicated that soil AK, pH, TN and TDN were all significantly associated with the composition of these soil microbial communities (Fig. 3B). Relative fungal abundance was negatively correlated with AK, TN, and TDN levels ($P < 0.05$), whereas relative actinomycetes abundance was positively correlated with these same levels ($P < 0.05$). In addition, protozoal abundance and AMF were positively correlated with soil pH ($P < 0.05$), and the relative abundance of bacteria was negatively correlated with soil pH ($P < 0.05$).BC application was associated with significant decreases in the G^-/G^+ and fungi/bacteria ratios relative to BC_0 treatment ($P < 0.05$; Fig. 4A and B),

Table 4
Mean concentration and relative percentage of soil biomarker phospholipid fatty acids (PLFAs) in treatments (BC_0 and $BC_{22.5}$ represent the 0 and 22.5 t ha^{-1} BC application rates, respectively. N_0, N_{150}, N_{225}, and N_{300} correspond to the 0, 150, 225 and 300 t ha^{-1} N application rates, respectively.). Data are means ± S.D. ($n = 3$).

	Treatment	Total PLFAs	Bacteria	Fungi	Actinomycetes	AM fungi	Protozoa	Other Bacteria
		nmol g dry $soil^{-1}$	The relative abundance PLFAs (mol %)					
BC_0	N_0	38.36 ± 1.9	50.44 ± 0.3	11.69 ± 0.4bA	12.25 ± 0.4B	5.18 ± 0.3	1.56 ± 0.1 aA	0.74 ± 0.07
	N_{150}	40.49 ± 0.93	51.36 ± 0.2	11.44 ± 0.0bA	12.59 ± 0.2B	5.17 ± 0.2	1.09 ± 0.0b	0.71 ± 0.04
	N_{225}	39.97 ± 4.1	51.15 ± 0.8	12.87 ± 0.4 aA	12.40 ± 0.5	4.80 ± 0.1	0.93 ± 0.0c	0.68 ± 0.04
	N_{300}	38.90 ± 1.2	51.58 ± 0.4	12.00 ± 0.4bA	12.06 ± 0.8B	4.84 ± 0.3	0.86 ± 0.1 cB	0.70 ± 0.03
	Pr > F	NS	NS	**	NS	NS	**	NS
$BC_{22.5}$	N_0	40.43 ± 0.6	49.66 ± 2.1	10.50 ± 0.0B	13.18 ± 0.3A	5.47 ± 0.4	1.11 ± 0.2B	0.69 ± 0.08
	N_{150}	41.76 ± 1.3	51.68 ± 0.4	10.52 ± 0.5B	12.99 ± 0.1A	5.27 ± 0.2	0.99 ± 0.1	0.65 ± 0.02
	N_{225}	42.19 ± 1.3	51.59 ± 0.3	10.52 ± 0.7B	13.23 ± 0.3	5.02 ± 0.2	1.11 ± 0.2	0.65 ± 0.04
	N_{300}	39.84 ± 1.9	50.99 ± 0.4	10.98 ± 0.0B	13.35 ± 0.0A	5.13 ± 0.6	1.32 ± 0.1A	0.73 ± 0.05
	Pr > F	NS	NS	NS	NS	NS	NS	NS

Different lowercase letters in a given column correspond to significant differences between N treatments at a given BC level ($P < 0.05$). Uppercase letters correspond to significant differences among BC treatments at a given N application level ($P < 0.05$).NS, * and ** indicate ANOVA results of $P > 0.05$, $P < 0.05$ and $P < 0.01$, respectively.

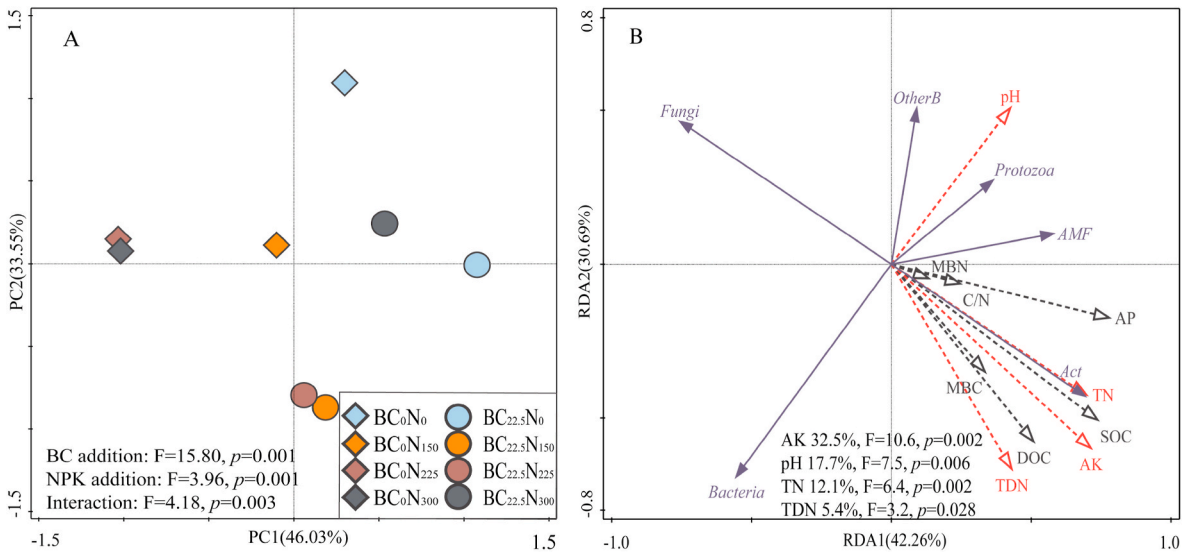

Fig. 3. Principal component analysis (PCA) of phospholipid fatty acid (PLFA) levels (A), and redundancy analyses (RDA) of the associations between soil parameters and relative microbial PLFA percentages (B) (BC_0 and $BC_{22.5}$ represent the 0 and 22.5 t ha^{-1} BC application rates, respectively. N_0, N_{150}, N_{225}, and N_{300} correspond to the 0, 150, 225 and 300 t ha^{-1} N application rates, respectively.). Red arrows indicate the soil parameters that were significantly associated with microbial community biomass ($P < 0.05$), with the percentage of variability explained being shown in the lower middle. Abbreviations: SOC, soil organic carbon; TN, total nitrogen; C/N, ratio of soil organic carbon to total nitrogen; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; AP, available phosphorus; AK, available potassium. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

whereas N fertilizer application did not significantly affect these ratios under any conditions other than the BC_0N_{225} condition ($P > 0.5$). We detected significant increases in bacterial stress indices in BC-amended samples (Fig. 4C and D), with the cy19:0/18:1ω7c and sat/mono ratios having risen by 10.95%–22.48% and 3.61%–7.10%, respectively, in $BC_{22.5}$ samples relative to BC_0 samples ($P < 0.05$). Specifically, the

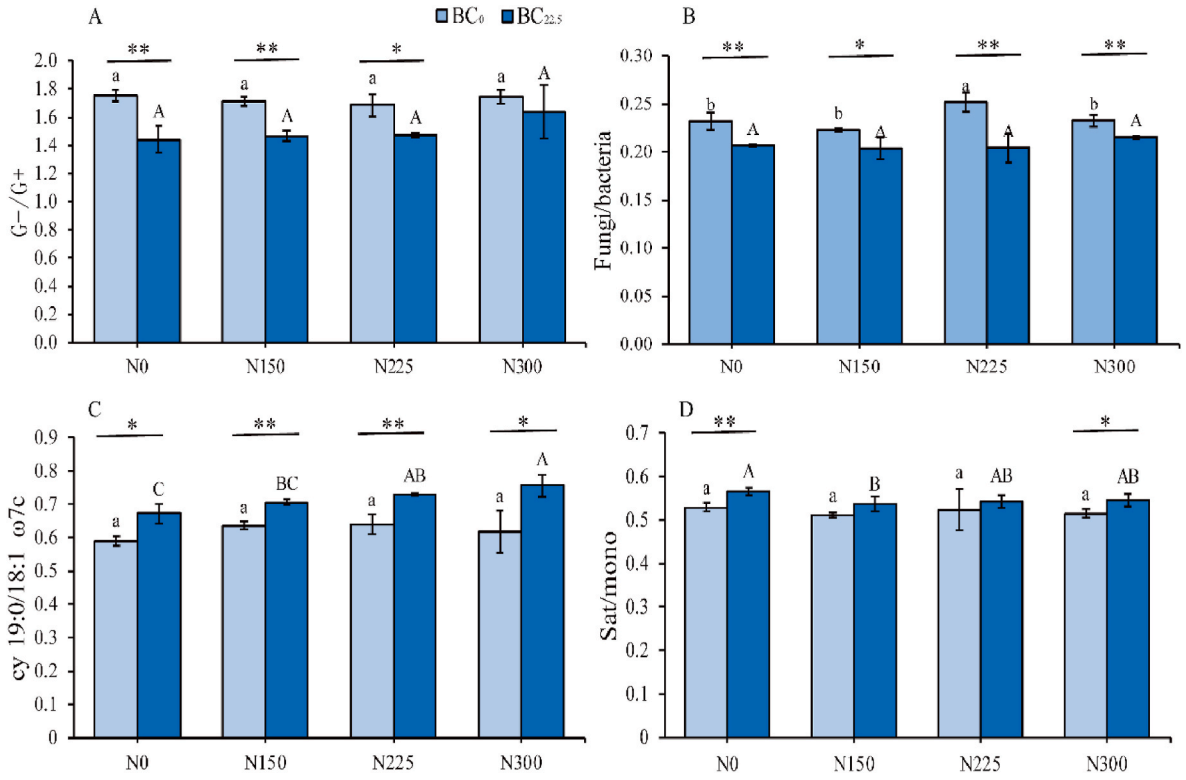

Fig. 4. Changes in the ratio of different microorganisms during treatment (BC_0 and $BC_{22.5}$ represent the 0 and 22.5 t ha^{-1} BC application rates, respectively. N_0, N_{150}, N_{225}, and N_{300} correspond to the 0, 150, 225 and 300 t ha^{-1} N application rates, respectively.). Different lowercase letters correspond to significant differences among N levels under BC_0 treatment conditions ($P < 0.05$), while different uppercase letters correspond to significant differences among N levels under $BC_{22.5}$ treatment conditions ($P < 0.05$). Asterisks indicate significant differences between BC conditions at a given N level ($P < 0.05$). Data are means ± standard deviations ($n = 3$).

fungi/bacteria and cy19:0/18:1ω7c ratios being significantly correlated with AK and AP, sat/mono ratios being significantly correlated with AK and TDN, and the G^-/G^+ ratio being associated with SOC and MBC (Table 5).

4. Discussion

4.1. The impact of BC and N application on soil nutrients

We found that BC application resulted in significant increases in soil TN and SOC levels (Table 2), confirming the ability of BC to bolster the C and N pool within treated soil [58–60]. These increases are likely attributable to the fact that the BC used in the present study was composed of 45.20% organic carbon and 1.50% total N. Lehmanna et al. [1] has similarly found that BC application can increase soil SOC and TN contents owing to the fact that it is rich in N and C, and that it can be difficult for microbes to degrade owing to the fact that it exhibits an aromatic structure. Similarly, Liang et al. [61] have proposed that applied BC can adsorb small organic compounds within the soil, thereby making soil C less amenable to microbial degradation and thus increasing SOC levels. In this study, we observed no impact of BC addition on soil pH, consistent with the findings of Cheng et al. [62] and Zhu et al. [63], who proposed that this lack of effect may be attributable to BC surface oxidation over time or to the buffering capacity of calcareous soils.

The combined BC and N application resulted in marked increases in soil DOC, TDN, and AK (Table 2), potentially due to synergistic effects between these compounds [20,64]. In prior research, the release of labile C, N, and K within BC into the soil has been proposed to explain such increases in nutrient abundance [5,52]. Furthermore, combined BC and N application can bolster soil water retention owing to the adsorptive properties of BC, thereby reducing N leaching and increasing soil TDN [8]. In one recent study, combined BC and N application was proposed to increase C and N soil contents as a result of increases in plant or root biomass and exudation, given that humus and soil litter represent key sources of dissolved organic matter [20]. We additionally found that BC application increased AP content, whereas N application had the opposite effect. In prior field studies of temperate systems, BC application was found to reduce AP levels [65], with this decrease being attributed to BC-mediated soil alkalization [66]. Given that BC failed to impact soil pH in our study, the observed increase in soil AP may have been a result of the release of phosphate ions therein [5], and may have occurred as a result of changes in soil microbe phosphorus utilization [67]. N application markedly reduced soil AP content, potentially suggesting that N application promoted soil P circulation in proximal plants and microbes [25,68].

Table 5
Multiple regression analysis of microbial properties (dependent variables) and soil properties (independent variables) in soil ($p < 0.05$).

Dependents	Constant	Parameters		R^2
Total PLFAs	= (27.055 ± 3.551)	+(0.167 ± 0.046) DOC		0.37
	(<0.0001)	(0.002)		(0.002)
G-/G+	= (1.419 ± 0.291)	-(0.083 ± 0.012) SOC	+(0.004 ± 0.002) MBC	0.696
	(0.0001)	(<0.0001)	(0.017)	(<0.0001)
Fungi/ bacteria	= (0.29 ± 0.012)	+(0.0003 ± 0.0001) AK	-(0.001 ± 0.001) AP	0.630
	(0.0001)	(0.003)	(0.02)	(<0.0001)
cy 19:0/18:1 ω7c	= (0.447 ± 0.029)	+(0.002 ± 0.0002) AK	-(0.004 ± 0.001) AP	0.813
	(<0.0001)	(<0.0001)	(0.01)	(<0.0001)
Sat/mono	= (0.397 ± 0.032)	+(0.001 ± 0.0003) AK	-(0.005 ± 0.002) TDN	0.49
	(<0.0001)	(0.001)	(0.012)	(0.001)

Values in parentheses below individual parameters in each equation correspond to *P* values. Abbreviations: SOC, soil organic carbon; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; MBC, microbial biomass carbon; AP, available phosphorus; AK, available potassium.

4.2. The impact of BC and N application on soil microbial biomass

Soil MBC is an important soil unstable C component [69]. We observed significant interactions between BC and N application on soil MBC. In a prior meta-analysis, Liu et al. [70] similarly found that BC did not significantly affect soil MBC in the absence of N fertilizer application, whereas in the presence of N, BC application was associated with up to a 42% increase in MBC. Sun et al. [40] found that under conventional or reduced fertilization conditions (100% or 50% of recommended fertilizer rates), BC did not significantly affect MBC, in line with our results. However, when BC was applied, we found that N application significantly increased soil MBC content (Table 2). Liu et al. [70] suggested that increases in MBC may be attributable to the application of large N fertilizer quantities, with this fertilizer serving as a direct source of nutrients and soil N that can modulate soil microbial activity in addition to the direct effects of BC addition. Zhou et al. [71] found that MBC changes are likely associated with changes in microbial communities, with this also likely holding true in our study. MBN changes generally reflect N competition between microbes and plants under field conditions [72]. BC addition is also believed to cause N limitation [9,73]. However, BC and its interaction with N fertilizer failed to significantly impact MBN, suggesting that significant microbial N fixation did not occur in our experimental system. This is in contrast to our hypothesis, and suggests that BC addition may not necessarily cause N limitation, in line with results from Dempster et al. [23]. Foster et al. [30] similarly found that when BC was added to a corn planting system, microbial N immobilization did not increase within one year following application, suggesting that the time post-application may influence BC and N dynamics. Prommer et al. [74] suggested that combined fertilizer and BC application would not negatively impact plants or microbes, given that such combined application would result in the decoupling of the N cycle between inorganic and organic pools, thereby ultimately promoting organic C and N retention in the soil.

Changes in soil microbial community composition may also be a consequence of soil property changes in response to BC addition [2]. Prior studies have reported increases in microbial biomass following BC addition, with these increases being attributed to BC-mediated toxic substance adsorption, to BC serving as a niche and C source to support microbial growth, and to BC providing a source of enhanced nutrient availability while also producing irritating molecules [13,14,17]. Unlike these prior studies, however, we observed no significant differences in total PLFA in any treatment conditions, consistent with a lack of change in microbial biomass (Tables 1 and 4), and regression analyses indicated that soil DOC was the primary factor that influenced such microbial biomass (Table 5). Consistent with our results, Elzobair et al. [51] performed field experiments in which they detected no significant alterations in soil microbial biomass at 1 or 4 years post-BC addition, leading them to speculate that only a relatively limited amount of the C within the applied BC was amenable to microbial degradation or that relatively labile BC–C had been degraded prior to sampling. Nelissen et al. [10] found that labile C within BC may explain why BC can stimulate microbial activity in the short-term such that when this unstable C has undergone mineralization BC is no longer to enhance microbial growth. Anders et al. [75] have also found that combined BC and N application did not significantly impact soil microbial biomass, and speculated that only soil, climate, and cultivation parameters significantly influenced observed differences in total PLFA in their study.

4.3. The impact of BC and N application on soil microbial community composition

While we failed to observe any obvious impact on microbial biomass in our experimental system, BC and N application can also alter

microbial community structure [75], and we observed changes in such microbial community composition as a function of BC, N, and BC + N application. The chemical changes that occur in soil following BC and N application tend to favor the growth of specific microbes [14,20]. We found that while BC application did not significantly impact bacteria, it did negatively impact relative fungal abundance and reduced the ratio of fungi to bacteria. In contrast, the opposite effect on actinomycetes was observed following BC application (Table 4). These findings are consistent with studies conducted by Gomez et al. [2], who found that a portion of the C within BC can support the growth of actinomycetes and other specific taxa within soil communities. Actinomycetes are capable of degrading complex substrates and aromatic compounds, making them advantageous in soil that has been treated with BC [76]. Previous studies have suggested that relative fungal abundance decreases following BC application because these fungi are not able to readily utilize BC as a substrate [77], or that BC inhibits fungal growth [78]. We also found that relative fungal abundance was negatively correlated with ATN and TDN. We hypothesize that improving soil TN and TDN levels via combined BC + N application may have further contributed to reductions in fungal abundance, as fungi are favorable in low N environments [79]. Unlike BC, N application significantly impacted bacterial communities within the soil (Table 2), largely because bacteria are closely linked to N mineralization [80]. Relative protozoan PLFA abundance also decreased significantly with increasing N application rates in the absence of BC, whereas no such effect was observed in BC amended soil (Table 4). Anderson et al. [37] suggested that BC addition can enhance the stability of microbial community structure and function, with this effect largely being attributable to BC-mediated improvements in soil physicochemical properties [2,14].

The sat/mono ratio is generally used as a nutrient transformation indicator, with the cy19:0/18:1ω7c ratio being associated with bacterial growth rates [25]. Higher cy19:0/18:1ω7c and sat/mono ratio values are thought to correspond to lower G^- bacteria growth and slower nutrient turnover rates (especially C), respectively [56,57]. We found that BC-amended soils exhibited higher cy19:0/18:1ω7c ratio and lower G^-/G^+ ratio (Fig. 4), consistent with the inhibition of G^- bacterial growth. G^- bacteria typically grow best in environments with sufficient available carbon, and tend to break down easily decomposable organic compounds [57], whereas K-strategist G^+ bacteria exhibit dominant growth under conditions of BC amendment [81]. BC is a form of inert carbon, and G^+ bacteria are better able to decompose many recalcitrant C compounds [82], including lignin and aromatic/olefin-C. Our findings further revealed that BC-N addition markedly increased the cy19:0/18:1ω7c ratio relative to BC application alone, with slight corresponding increases in the G^-/G^+ ratio, suggesting that combination BC-N addition shifted the microbial community towards a more G^+ bacteria-dominated state, in line with the results of Lu et al. [79]. Treseder et al. [83] found that G^+ bacteria are capable of secreting enzymes capable of decomposing recalcitrant C, but that they require higher N levels. Regression analyses revealed that the G^-/G^+ ratio was negatively correlated with SOC in this study. Wang et al. [84] determined that BC application can enhance soil aggregation, increase the physical protection of native organic C and BC-C, and alter microbial community composition. Our results further indicated that BC application increased the sat/mono ratio, suggesting that the soil nutrient turnover rate was altered, as in prior studies [84].

4.4. The impact of BC and N application on soil enzyme activities

We found that BC application enhanced the activity of multiple enzymes associated with C and N cycling (Table 3), consistent with the results of Pandey et al. [85]. However, in other studies, BC application has been found to reduce soil enzyme activity owing to the strong adsorptive properties of BC [2,34], though we failed to observe this effect. Lammirato et al. [34] have proposed that BC-related changes in soil enzyme activity are largely dependent upon soil type, BC type, application rate, and pyrolysis temperature. Ameloot et al. [33] found that BC generated via low-temperature pyrolysis (350–500 °C) contains higher levels of volatile compounds capable of accelerating the activity of enzymes with carbon and dehydrogenase-related activities in sandy loam. Wang et al. [52] found that adding reduced BC amounts (0.5% by mass) can enhance soil enzyme activity, whereas larger quantities of BC have the opposite effect. In addition, fertilizer application can significantly affect the activity of these soil enzymes [19]. We determined that combined BC and N fertilizer addition significantly increased the activity of soil enzymes related to C and N cycling relative to the application of BC or N in isolation, consistent with our hypothesis. Tian et al. [20] similarly found that combination BC-NPK treatment resulted in significantly increased C cycle enzyme activity, and they attributed these increases to more rapid microbial turnover as a result of NPK fertilization. BC and N addition also significantly alter soil physicochemical properties [51], and these changes can further impact enzyme activity [25]. Given that we observed significant correlations between soil enzyme activities and soil nutrient contents, our data may support such a model (Fig. 2). We further found that BC and N failed to significantly impact phosphatase activity, which may be attributable to the fact that BC did not impact soil pH [30]. Lehmann et al. [1] have suggested that BC addition-mediated changes in soil pH are the primary drivers of such altered phosphatase activity.

Herein, we found that average βG:Pho ratio (0.31–0.49) and βG: (NAG + LAM) ratio (0.14–0.25) values in our treatment conditions were lower than the global averages (0.62 and 1.43, respectively) reported by Sinsabaugh et al. [29,31], whereas our (NAG + LAM): Pho ratio (1.30–2.78) were substantially higher than the reported global average (0.44) [31]. Herein, we found that BC application significantly affected the enzyme metering ratio and significantly reduced the βG: (NAG + LAM) ratio without observing an inhibitor impact of N on microbes as a consequence of BC addition. Guo et al. [86] similarly reported that changes in microbial activity following BC application were unable to support the theory of ecological stoichiometry. Mori et al. [28] suggested that changes in the βG: (NAG + LAM) ratio were indicative of C vs. N limits only when cellulose was the predominant C source available to microorganisms, but not in the context of other C sources.

5. Conclusion

In summary, in the present study we identified interactive effects between BC and N fertilizer when applied to alkaline calcareous soil. The combined application of these two agents significantly increased soil nutrient availability in a synergistic manner. In addition, under BC application conditions we observed no significant changes in soil microbial biomass, although we did observe significant changes in overall microbial community composition. Relative to N application alone, combined BC and N application significantly increased C and N cycle-related soil enzyme activity. As such, this combination treatment offers an ideal means of bolstering C and N cycle management in agriculturally-important ecosystems. BC application does not cause limitations in soil N levels, and as such further research will be essential to establish the optimal rate of N application to BC-amended calcareous soil. In addition, further study of the long-term effects of combined BC and N application is necessary, as is research regarding the impact of this combination on N uptake by crops and soil microbes.

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.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejsobi.2020.103212.

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