https://doi.org/10.17221/199/2023-PSE

Biochar application influences the stability of soil aggregates and wheat yields

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Citation: Yang W.J., Wang Z.L., Zhao H.M., Li D.P., Jia H.T., Xu W.L. (2024): Biochar application influences the stability of soil aggregates and wheat yields. Plant Soil Environ., 70: 125–141.

Abstract: In the present study, a field establishment was initiated in 2018 with eight treatment conditions using biochar application rates of 0, 10, 20, or 30 t/ha and nitrogen application rates of 0 or 150 kg/ha. After two years, the impact of biochar on carbon-nitrogen distributions, soil aggregate stability, and wheat yields was then assessed. The predominant mechanical aggregates after two years were > 5 mm and 2–5 mm granular aggregates, with notable increases in the amounts of these aggregates following the application of biochar with or without nitrogen that coincided with an increase in soil aggregate mechanical stability. Relative to control conditions, aggregate mean weight diameter (MWD) and geometric weight diameter (GMD) values rose by 17.6% and 24.3% for biochar with nitrogen treatment (N: 150 kg/ha; biochar: 20 t/ha), respectively. Biochar application alone and the application of both biochar and nitrogen fertiliser were associated with 6.4–20.2% and 20.7–42.7% increases in spring wheat yields, respectively. Overall, the results of these analyses highlight the value of applying biochar to improve soil quality and boost crop yields proximal to the study site. This study provided the scientific basis for the rational fertilisation and scientific management of biochar combined with nitrogen fertiliser in the irrigation area of Northern Xinjiang, China.

Keywords: soil structure; C/N ratio; sustainable agriculture; carbon fixation

Soil aggregates are fundamental structural units that influence key physical, chemical, and biological processes within the soil (Peng et al. 2015). These aggregates can stabilise and shield soil organic matter from decomposition, serving as a key indicator to evaluate soil quality and measure the sustainable development of the local ecological microenvironment (Islam et al. 2021). Aggregate-rich soil can achieve long-term carbon absorption conducive to improved crop yields while simultaneously helping to mitigate the process of global climate change (Zhao et al. 2018). Soil organic carbon (SOC) is a cementitious material that can stimulate aggregate formation through its ability to enhance soil particle agglomeration (Six et al. 2006). Soil aggregate size distributions significantly affect SOC turnover, with agglomeration enhancing SOC stability by shielding sequestered carbon from breakdown by microbes (Tisdall and Oades 1982). Higher levels of SOC are found within larger soil aggregates, and more stable

Supported by the National Natural Science Foundation of China, Project No. 32260326, and by the Earmarked Fund for the Xinjiang Agriculture Research System, Project No. XJARS-01.

https://doi.org/10.17221/199/2023-PSE

aggregates are better able to resist disintegration (Gupta and Germida 1988), increasing the long-term carbon sequestration and the soil's overall structural stability (Ouyang et al. 2013). Thus, soil aggregates and SOC levels have a strong relationship.

Biochar, an organic carbon-source material known for its environmentally friendly nature and distinctive physicochemical characteristics, has garnered significant interest in its potential to enhance soil quality and functions (Hansen et al. 2023). The porosity and large surface area of biochar can adsorb soil organic carbon, sequester microorganisms and their extracellular enzymes from the organic carbon, and then slow down the decomposition of organic carbon (Abbruzzini et al. 2017). Data published regarding the impact of biochar on soil aggregates are inconsistent. Several reports have suggested that biochar amendment can enhance soil aggregation in sandy or clay-based soils (Abdelhafez et al. 2014, Burrell et al. 2016, Dong et al. 2016, Amoakwah et al. 2017, Pituello et al. 2018, Fu et al. 2019). In contrast, in other soil types, biochar has been found to have no impact or even to reduce soil aggregation (Peng et al. 2011, Mukherjee et al. 2014, Lusiba et al. 2016, Rahman et al. 2017, Zhou et al. 2018). These contradictory or inconsistent results to biochar application have been observed across a range of studies and may be attributable to differences in the soil properties, biochar characteristics, and experimental conditions used therein. The present study was developed as a field experiment with the goal of assessing the effects of biochar application on soil aggregate distributions and stability in agricultural soil through measurements of soil aggregates present in a wheat field located in the northern Xinjiang irrigation area.

The irrigation lands in North Xinjiang exemplify a prototypical agricultural oasis characterised by irrigation practices within the Xinjiang Uygur Autonomous Region. Although these regions exhibit elevated levels of grain productivity, they are confronted with certain obstacles, such as the decline in organic matter content within the soil and inadequate soil fertility to sustain the growing demands for higher yields. Consequently, there has been an increasing focus on doing research that seeks to improve farmland fertility and promote the fixation of soil organic carbon. Numerous research endeavours have been conducted to explore the application of biochar as a means to enhance soil fertility and augment crop productivity in agricultural regions characterised by irrigation oases (Xiao et al. 2016, Li et al. 2017, Zhao et al. 2020), but these studies were subject to certain constraints and shortcomings. The effects of biochar application on soil aggregate distributions and stability in agricultural soil still need to be better understood, and there is also uncertainty over the extent to which biochar may effectively enhance farmed soil quality. Hence, it is imperative to conduct a thorough assessment to ascertain biochar's possible applicability in the ecosystems of agriculture irrigated in oasis regions.

We have investigated the impact of nitrogen fertiliser combined with biochar (using a normal nitrogen fertiliser level (300 kg/ha), a 15% reduced nitrogen fertiliser level (255 kg/ha) and a high biochar level applied at a rate of 30 t/ha) on the wheat soil nutrients, and yield in a previous study. The findings indicate that applying reduced nitrogen in conjunction with biochar enhanced soil nutrient levels and significantly improved wheat yield (Yang et al. 2023). In an effort to better understand the effects of biochar application on soil aggregation dynamics and crop yields, a two-year field experiment was herein conducted with the following goals: (*i*) to assess soil aggregate size distributions in response to different rates of biochar amendment with nitrogen reduction; (*ii*) to evaluate the impact of biochar application on the stability of soil aggregates, and (*iii*) to determine how biochar amendment rates influence wheat yields. The results of this study will contribute to formulating strategies to reduce fertiliser usage and implement biochar application practices in the irrigation regions of North Xinjiang. Additionally, it will offer a comprehensive assessment of the overall effectiveness and benefits of biochar application in agricultural lands inside irrigation areas.

MATERIAL AND METHODS

Site description. A fixed position experiment was conducted at the Qitai Wheat Experiment Station at the Xinjiang Academy of Agricultural Sciences (42°45'–45°29'N, 89°13'–91°22'E, altitude: 1 174 m a.s.l.) from 2018 to 2020. Qitai County has a temperate continental semi-arid climate with an average annual temperature of 5.5 °C, an average temperature of 23.7 °C in July, a maximum temperature of 39 °C, an average temperature of –18.9 °C in January, and an annual average relative humidity of 60%. The average duration of the frost-free period in this region spans 153 days, commencing in late April and concluding in early October. Additionally, the average annual precipitation in this area amounts to 269.4 mm.

https://doi.org/10.17221/199/2023-PSE

The Arenosols at the test site have a soil organic carbon content (0–20 cm) of 9.09 g/kg, a total nitrogen level of 0.93 g/kg, an available phosphorus level of 7.10 mg/kg, an available potassium level of 35.1 mg/kg, and a pH of 8.25.

Experimental design. The study employed a randomised block design. The experimental design involved the application of two different amounts of nitrogen fertiliser to the plots: a control group with no nitrogen fertiliser (N0: 0 kg/ha) and a group with a lowered nitrogen fertiliser level (N1: 150 kg/ha). The plots had four levels of biochar that were applied: no biochar (B0: 0 t/ha); low biochar (B1: 10 t/ha); medium biochar (B2: 20 t/ha), and high biochar (B3: 30 t/ha). There were 8 treatment groups, including B0N0, B0N1, B1N0, B1N1, B2N0, B2N1, B3N0, and B3N1. Each group had three replicates. The wheat was sown using drill seeding at a density of 450 × 10^3/ha with row spacing of 0.2 m, and each plot had an area of 9 m^2 (3 × 3 m). The application of biochar as a basal fertiliser occurred in 2018 at a depth of 20 cm within the plough layer. Subsequent applications of biochar were not carried out. Nitrogen fertiliser was applied as urea (46% pure nitrogen) once before wheat was sown. The remaining management practices conformed with the respective domains' established norms.

Materials. The biochar utilised in the experiment was derived from corn straw and was obtained from the Xinjiang Academy of Agricultural Sciences, China. The straw was carbonised at a temperature of 450 °C for 4 h, and biochar had the following basic properties: H/C – 0.65, pH – 9.3, total nitrogen content – 21.8 g/kg, total phosphorus content – 10.6 g/kg, total potassium content – 21.5 g/kg, alkaline hydrolysis nitrogen content – 5.4 mg/kg, and rapidly available phosphorus content – 200.9 mg/kg. The wheat cultivar employed in this study was Xinchun 37.

Soil sampling. Before the wheat harvest in July 2020, the soil samples were collected from the surface soil layer (0–20 cm). Five sites were chosen at random to collect soil samples from each plot, and about 2 kg of soil sample was obtained for each plot; after being composed, the soil samples were air-dried for two weeks, from which visible stones and plant roots were hand-removed, and all samples were sieved through a 2-mm filter for the measurements described below.

Soil aggregate separation and analysis.

(1) Dry sieving method. 500 g samples of air-dried soil were weighed and sequentially placed atop sleeve sieved with pore sizes of 5, 2, 1, 0.5 and 0.25 mm. Sieves were manually shaken for 2–3 min, allowed to stand for 1 min, and soil aggregates on the surface of each sieve were then collected and weighed.

(2) Wet sieving method. Soil aggregates obtained *via* the above dry sieving approach were used to prepare 100 g soil samples in the corresponding proportions to those identified through dry sieving, and they were sequentially placed atop sieves with pore sizes of 5, 2, 1 and 0.5 mm. After soil had been placed a top these sieves, the sieves were submerged in water in a bucket for 10 min, manually oscillated up and down for 5 min (30 times/min), and water-stable soil aggregates from each sieve layer were transferred to an aluminium box. The upper layer of water was then removed, and aggregates were dried and weighed.

Calculations were performed with the following equations:

$$w_i = \frac{m_i}{m} \times 100 \quad (1)$$

$$\bar{d_\iota} = \frac{Nmax + Nmin}{2} \quad (2)$$

$$MWD = \sum_{i=1}^{n} (\bar{d_\iota} w_i) \quad (3)$$

$$GMD = exp\left[\frac{\sum_{i=1}^{n} m_i \, ln\, \bar{d_\iota}}{\sum_{i=1}^{n} m_i}\right] \quad (4)$$

$$D = 3 - \frac{lg(\bar{d_\iota}/\bar{d}_{max})}{lg\,[m(\delta < \bar{d_\iota})/m]} \quad (5)$$

$$R_{>0.25} = 1 - \frac{m_{<0.25}}{m} \quad (6)$$

$$PAD = \frac{DR_{0.25} - WR_{0.25}}{DR_{0.25}} \times 100\% \quad (7)$$

$$WSAR = \frac{WR_{0.25}}{DR_{0.25}} \times 100\% \quad (8)$$

where: $\bar{d}_1$ – average diameter (mm) of soil aggregates of grade *i*; N_{max} – upper size limit of an aggregate grade; N_{min} – lower size limit of an aggregate grade; *n* – number of aggregate grades; w_1 – percentage (%) of the total soil sample of composed of grade *i* soil aggregates; m_i – weight of the grade *i* soil aggregates (g); $\bar{d}_{max}$ – average diameter of the largest particle grade soil aggregate (mm); m ($\delta < \bar{d}_1$) – cumulative mass of soil aggregates with a particle size less than $\bar{d}_1$; m – total soil sample weight (g); $R > 0.25$ is the proportion of > 0.25 mm aggregates; PAD and WSAR – soil aggregate destruction and stability rates, respectively; $DR_{0.25}$ – proportion of mechanically stable aggregates > 0.25 mm; $WR_{0.25}$ – proportion of water-stable aggregates > 0.25 mm; and $R > 0.25$ is the proportion of large aggregates.

https://doi.org/10.17221/199/2023-PSE

Carbon and nitrogen analyses. Initially, the five soil aggregate samples (> 5, 2–5, 1–2, 0.5–1, 0.25–0.5, and < 0.25 mm) derived from dry sieving were ground and passed through a sieve with a pore size of 0.25 mm.

Organic carbon content. Organic carbon content in each set of aggregate particles was measured *via* the potassium dichromate-sulfuric acid dilution heat method (Magdoff et al. 1996). The total nitrogen content in these aggregates was measured *via* the Semi-Micro Kjeldahl method (Bremner 1965).

$$W = \frac{C_i \times W_i}{T} \times 100\% \qquad (9)$$

where: W – carbon and nitrogen contribution rates of each particle-level aggregate; C_i – carbon and nitrogen content of a particle-level aggregate; W_i – same description as above; T – total soil carbon and nitrogen content.

$$\text{SOC and } N_{-stock} = D \times \rho b \times \text{SOC and } N_{-con} \times 0.00 \qquad (10)$$

where: SOC and N_{-stock} – SOC and nitrogen stocks of aggregates; D – depth of the soil layer (mm); ρb – soil bulk density (g/cm^3); SOC and N_{-con} – soil of SOC and nitrogen content (g/kg).

Other soil physicochemical properties analyses. Total phosphorus was measured using the $HClO_4$-H_2SO_4 digestion-molybdenum antimony colourimetric method, and available phosphorus was measured using the 0.5 mol/L $NaHCO_3$ extraction-molybdenum antimony colourimetric method (Bao 2020). Total potassium was determined using the sodium hydroxide fusion flame photometric method, and available potassium was determined using the ammonium acetate extraction flame photometric method (Faithfull 2002).

Spring wheat yield measurements. After spring wheat had reached maturity, a 1 m^2 area exhibiting uniform growth from each plot was selected. Wheat from these plots was manually harvested, and the number of spikes was counted, after which the yield was measured.

Statistical analysis. Effects of treatments on soil aggregate size distributions, stability, SOC concentrations, and nitrogen concentrations were compared through one-way ANOVAs in SPSS 19.0, with means being separated using the *LSD* (least significant difference) test. $P < 0.05$ was the threshold for significance unless otherwise noted.

RESULTS

The impact of biochar application on soil aggregate stability

(1) The impact of biochar amendment on soil aggregate distributions. Significant differences in aggregate size distributions were evident among treatment conditions ($P < 0.05$). The dry sieving method was initially used to assess aggregate fraction sizes for each treatment condition (Table 1), revealing that the predominant aggregates in CK soil samples were those > 5, 2–5 and 0.5–1 mm. Nitrogen-biochar treatment conditions resulted in even more pronounced increases in the 2–5 mm aggregate fraction than in biochar amendment alone. The 0.5–1 mm aggregates levels were also higher

Table 1. Soil aggregate content (%) by treatment as measured *via* dry sieving

Treatment	The particle size of soil aggregate (mm)					
	> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	21.1 ± 0.01^{a}	25.3 ± 0.00^{b}	11.2 ± 0.01^{b}	18.5 ± 0.00^{bc}	13.8 ± 0.01^{a}	10.1 ± 0.01^{a}
B1N0	25.7 ± 0.00^{b}	26.6 ± 0.01^{bc}	9.0 ± 0.00^{a}	17.5 ± 0.00^{b}	13.1 ± 0.00^{a}	8.1 ± 0.01^{a}
B2N0	29.8 ± 0.02^{c}	28.2 ± 0.01^{cd}	9.2 ± 0.00^{a}	15.9 ± 0.00^{a}	10.6 ± 0.01^{a}	6.3 ± 0.03^{a}
B3N0	23.5 ± 0.00^{b}	27.6 ± 0.00^{c}	9.3 ± 0.01^{a}	17.8 ± 0.01^{b}	13.7 ± 0.06^{a}	8.1 ± 0.01^{a}
B0N1	24.9 ± 0.01^{b}	22.6 ± 0.01^{a}	11.4 ± 0.00^{b}	19.6 ± 0.00^{cd}	12.8 ± 0.00^{a}	8.7 ± 0.01^{a}
B1N1	19.1 ± 0.01^{a}	28.5 ± 0.00^{cd}	9.8 ± 0.00^{ab}	21.3 ± 0.01^{de}	13.2 ± 0.01^{a}	8.1 ± 0.00^{a}
B2N1	20.5 ± 0.02^{a}	28.1 ± 0.00^{cd}	10.0 ± 0.01^{ab}	20.3 ± 0.00^{de}	14.0 ± 0.00^{a}	7.1 ± 0.00^{a}
B3N1	24.2 ± 0.02^{b}	29.5 ± 0.00^{d}	10.1 ± 0.01^{ab}	18.2 ± 0.01^{b}	11.5 ± 0.01^{a}	6.5 ± 0.01^{a}
Biochar (B)	ns	ns	*	ns	ns	ns
Nitrogen (N)	ns	ns	*	ns	ns	ns
B × N	**	**	ns	**	ns	ns

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

https://doi.org/10.17221/199/2023-PSE

Table 2. Soil aggregate stability (%) by treatment as measured *via* wet sieving

Treatment	The particle size of soil aggregate (mm)					
	> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	0.64 ± 0.09[a]	2.19 ± 0.06[a]	2.69 ± 0.72[a]	4.39 ± 0.51[a]	40.97 ± 2.40[b]	49.12 ± 3.42[c]
B1N0	1.05 ± 0.27[ab]	3.32 ± 0.37[b]	3.8 ± 0.40[b]	5.48 ± 0.43[ab]	33.79 ± 3.18[a]	52.55 ± 3.09[c]
B2N0	1.71 ± 0.12[c]	4.12 ± 0.66[c]	4.15 ± 0.20[b]	8.7 ± 0.56[cd]	45.15 ± 2.74[c]	36.17 ± 2.89[b]
B3N0	2.24 ± 0.25[d]	4.42 ± 0.11[c]	4.39 ± 0.51[b]	9.35 ± 1.26[de]	54.58 ± 1.49[e]	25.01 ± 1.37[a]
B0N1	2.33 ± 0.16[d]	3.43 ± 0.18[b]	2.27 ± 0.09[a]	7.60 ± 0.59[c]	50.13 ± 1.54[d]	34.24 ± 1.54[b]
B1N1	1.46 ± 0.21[bc]	3.25 ± 0.26[b]	3.60 ± 0.21[b]	6.36 ± 0.17[b]	53.27 ± 1.57[d]	32.06 ± 1.57[b]
B2N1	1.52 ± 0.38[c]	4.52 ± 0.34[c]	6.36 ± 0.17[c]	8.27 ± 0.69[cd]	55.16 ± 2.33[e]	24.17 ± 2.82[a]
B3N1	2.36 ± 0.31[d]	4.16 ± 0.43[c]	6.29 ± 0.65[c]	9.95 ± 0.55[e]	54.86 ± 0.36[e]	22.38 ± 0.57[a]
Biochar (B)	ns	ns	ns	ns	ns	ns
Nitrogen (N)	ns	ns	ns	ns	ns	*
B × N	**	*	**	**	**	**

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha;B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

under nitrogen-biochar amendment conditions than biochar amendment alone ($P < 0.05$).

Biochar addition also impacted the stability of soil aggregates in water (Table 2). The water-stable aggregates under CK conditions primarily consisted of 0.25–0.5 mm (40.97%) and < 0.25 mm (49.12%) aggregates, with aggregates > 5 mm being the least abundant (0.64%). Except for the < 0.25 mm aggregates, aggregate concentrations increased under B3N0 treatment conditions relative to CK conditions ($P < 0.05$). Under nitrogen-biochar treatment conditions, the 0.25–0.5 mm aggregate fractions increased by 6.2% (B1N1), 10.03% (B2N1), and 9.0% (B3N1) relative to B0N1 conditions. Significant differences were evident among these various treatments ($P < 0.05$). Nitrogen and biochar addition primarily increased the 0.5–1 mm and 0.25–0.5 mm aggregate fractions by 4.4% and 22.2%, respectively, relative to the biochar-only treatment conditions ($P < 0.05$). A significant interaction effect between biochar and nitrogen fertiliser was evident regarding soil aggregate formation ($P < 0.01$).

(2) The impact of biochar amendment on soil aggregate size. The mean weight diameter (MWD) and geometric weight diameter (GMD) values measured *via* the dry-sieving method were greater than those measured using the wet-sieving approach (Figure 1). Biochar amendment increased the MWD and GMD values under all treatment conditions, with this effect being particularly pronounced under B2N0 treatment conditions with significant increases of 21.3% and 32.2% relative to CK ($P < 0.05$), respectively. The MWD and GMD under B3N1 conditions were also increased by 10.6% and 20.1%, respectively, relative to CK conditions ($P < 0.05$). Differences among these different treatment conditions were more evident when using the wet sieving approach than the dry sieving approach, and both MWD and GMD values rose with increases in the rates of biochar amendment. Across all tested conditions, B3N1 treatment was associated with the highest MWD (60%) and GMD (52%) values as compared to CK conditions. Consistent trends in MWD and GMD values were also observed when using the dry-sieving method when it comes to nitrogen-biochar treatments. In addition, significant interaction effects on aggregate size were observed between biochar and nitrogen addition ($P < 0.01$).

(3) The impact of biochar amendment on soil aggregate destruction and stability rates. Biochar amendment was associated with increased aggregate stability and/or reduced aggregate degradation (Figure 2). The soil aggregate destruction rate (PAD) under each treatment condition declined with biochar addition, while the opposite trend was observed for the soil aggregate stability rate (WASR). The PAD under the B3N0 amendment conditions was the least of all tested treatments without nitrogen addition, being 58.1% below that for under CK conditions, whereas the WASR value was highest at 81.9%, which was 44.1% higher than CK ($P < 0.05$). With nitrogen application, PAD values declined further while WASR values trended upwards. A significant interaction effect was evident between biochar-only and biochar-nitrogen treatments concerning PAD values ($P < 0.01$).

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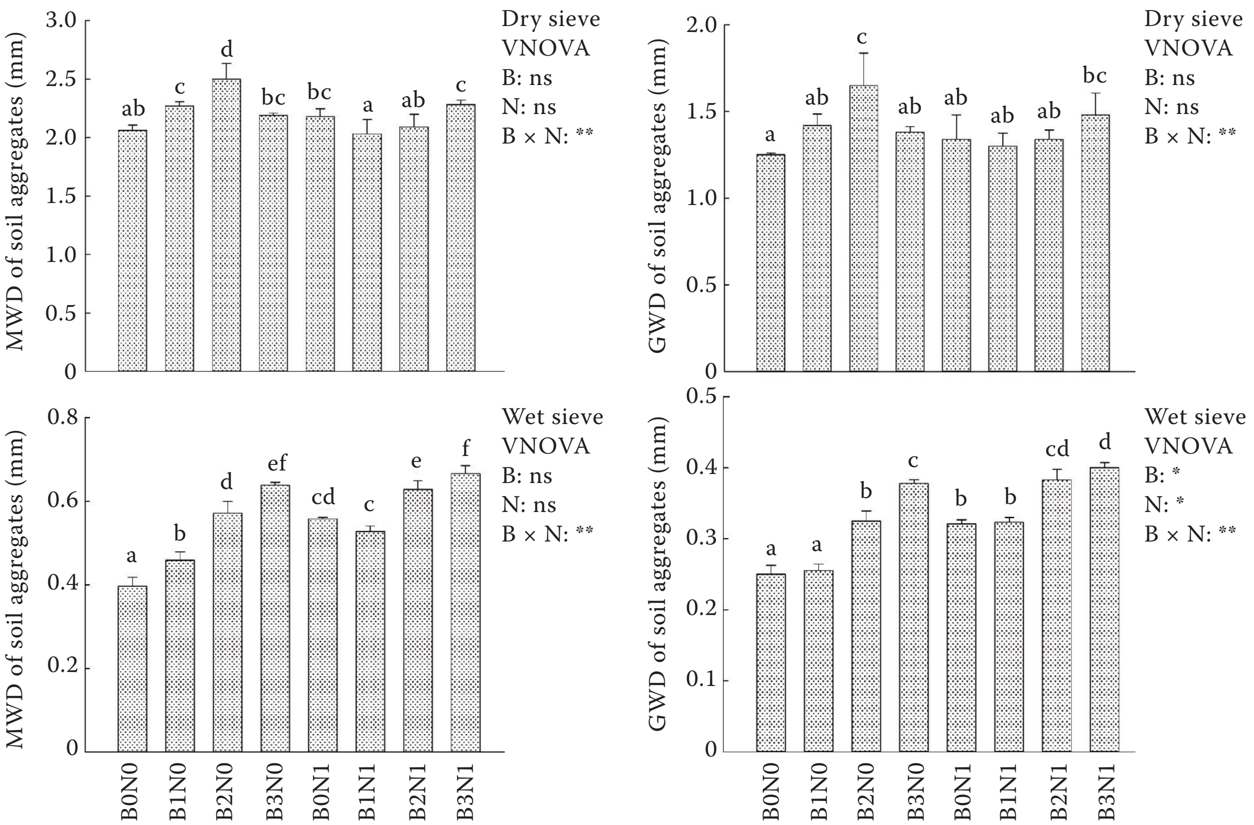

Figure 1. Soil aggregate stability indices under different treatments. Different lowercase letters indicate significant differences between treatments (P < 0.05). *P < 0.05; **P < 0.01; ns – not significant; MWD – mean weight diameter; GWD – geometric weight diameter; CK (B0N0) – control; nitrogen (N): N0 – 0 kg N/ha; N1 – 150 kg N/ha; biochar (B): B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

Carbon and nitrogen distributions within soil aggregates

(1) Organic carbon distribution within soil aggregate fraction. Based on measurements made using the dry-sieving method, biochar amendment was associated with the general enhancement of the organic carbon content present within soil aggregates. Organic carbon levels were lower in aggregates under biochar-nitrogen amendment conditions than in biochar-only treatment conditions (Table 3). In the absence of nitrogen, the organic carbon levels

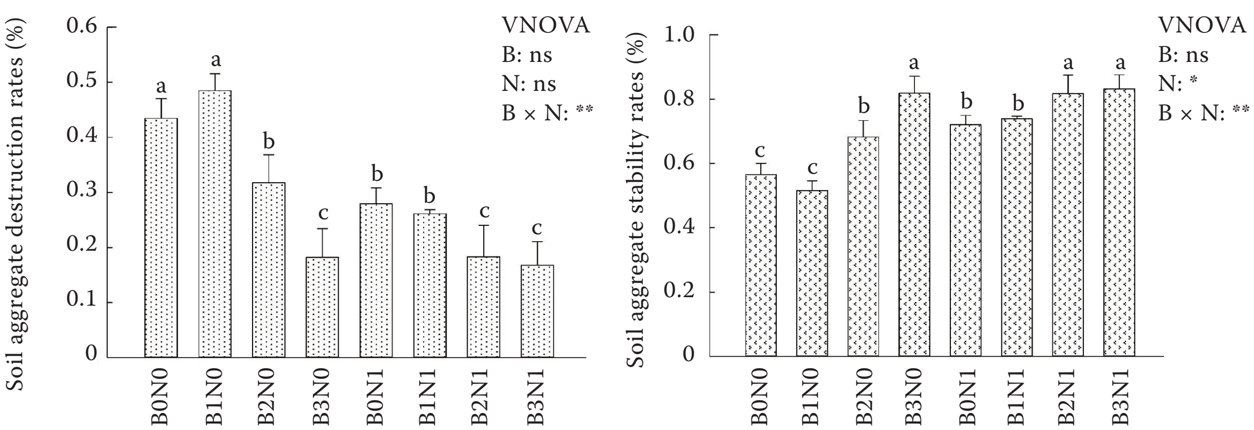

Figure 2. Soil aggregate stability indices under different treatments *via* wet sieving, CK (B0N0) – control; nitrogen (N): N0 – 0 kg N/ha; N1 – 150 kg N/ha; biochar (B): B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

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Table 3. Soil organic mean content and carbon (C) content (g/kg) in individual soil particles

Treatment	Organic carbon mean content	Particle size of soil aggregate (mm)					
		> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	8.46 ± 0.18[a]	8.91 ± 0.12[bc]	7.85 ± 0.11[ab]	7.58 ± 0.20[b]	7.78 ± 0.40[a]	8.05 ± 0.23[a]	9.38 ± 0.20[bc]
B1N0	9.17 ± 0.15[b]	9.18 ± 0.2[c]	8.31 ± 0.12[bc]	7.71 ± 0.31[bc]	8.84 ± 0.31[a]	9.31 ± 0.31[d]	10.84 ± 0.42[ef]
B2N0	11.73 ± 0.10[c]	11.17 ± 0.60[d]	11.37 ± 0.35[d]	11.77 ± 0.35[e]	11.84 ± 0.12[b]	11.97 ± 0.10[e]	12.17 ± 0.87[g]
B3N0	8.39 ± 0.25[a]	8.45 ± 0.23[ab]	7.32 ± 0.83[a]	7.85 ± 0.23[bc]	7.45 ± 0.12[a]	9.11 ± 0.46[cd]	10.17 ± 0.20[de]
B0N1	8.15 ± 0.32[a]	8.18 ± 0.20[a]	7.91 ± 0.12[ab]	7.05 ± 0.11[a]	7.25 ± 0.23[a]	8.71 ± 0.31[bc]	8.68 ± 0.17[ab]
B1N1	8.43 ± 0.07[a]	8.71 ± 0.12[bc]	8.11 ± 0.30[bc]	7.85 ± 0.31[bc]	7.58 ± 0.20[a]	8.38 ± 0.35[ab]	8.51 ± 0.31[a]
B2N1	8.61 ± 0.36[a]	8.91 ± 0.12[bc]	8.41 ± 0.06[bc]	8.11 ± 0.31[c]	8.45 ± 2.71[a]	8.51 ± 0.23[ab]	9.84 ± 0.41[cd]
B3N1	9.12 ± 0.40[b]	9.18 ± 0.20[c]	8.68 ± 0.10[c]	8.78 ± 0.34[d]	9.11 ± 0.23[a]	9.38 ± 0.35[d]	11.11 ± 0.12[f]
Biochar (B)	ns	ns	ns	ns	ns	ns	ns
Nitrogen (N)	ns	ns	ns	ns	ns	ns	ns
B × N	**	**	**	**	**	**	**

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significan. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

were highest under the B2N0 conditions, peaking at 11.73 g/kg, which was a 38.7% increase relative to control conditions. Under these conditions, organic carbon levels rose markedly by 55.2% in 1–2 mm aggregates ($P < 0.05$). Organic carbon levels in all aggregate fractions under B3N1 amendment conditions were higher than under any other tested nitrogen-biochar treatment conditions. Organic carbon content in soil aggregates tended to vary irregularly with increasing aggregate size across different tested treatment conditions.

The major aggregate contributors to SOC content under each treatment condition were the > 5 mm and 2–5 mm fractions, which accounted for over 40% of these contributions, while the contributions of the 1–2 mm and < 0.25 mm fractions were relatively minor (Table 4). Significant differences in the interaction effect between biochar and nitrogen application and aggregate contributions to SOC content in these fractions were evident for the > 5 mm and 2–5 mm fractions.

When using the wet-sieving method, relative to CK conditions, the levels of organic carbon in soil aggregates rose with biochar amendment under all treatment conditions ($P < 0.05$) (Table 5). The larg-

Table 4. Organic carbon contribution rates (%) for soil aggregates of different sizes as measured *via* dry sieving

Treatment	Particle size of soil aggregate (mm)					
	5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	22.25 ± 0.01[abc]	23.51 ± 0.01[a]	10.05 ± 0.00[b]	17.01 ± 0.01[a]	13.17 ± 0.00[a]	10.78 ± 0.01[a]
B1N0	25.74 ± 0.00[de]	24.17 ± 0.01[a]	7.59 ± 0.01[a]	16.91 ± 0.01[a]	13.37 ± 0.01[a]	9.15 ± 0.01[a]
B2N0	28.39 ± 0.02[e]	27.37 ± 0.02[b]	9.21 ± 0.00[b]	16.02 ± 0.00[a]	10.79 ± 0.01[a]	6.18 ± 0.03[a]
B3N0	23.66 ± 0.02[bcd]	23.98 ± 0.02[a]	8.69 ± 0.01[ab]	15.82 ± 0.02[a]	14.8 ± 0.06[a]	9.53 ± 0.01[a]
B0N1	25.01 ± 0.01[cd]	21.98 ± 0.02[a]	9.88 ± 0.01[b]	17.47 ± 0.01[a]	13.64 ± 0.01[a]	9.11 ± 0.04[a]
B1N1	19.75 ± 0.01[a]	27.42 ± 0.01[b]	9.14 ± 0.00[ab]	19.14 ± 0.00[a]	13.14 ± 0.01[a]	7.85 ± 0.04[a]
B2N1	21.31 ± 0.03[ab]	27.45 ± 0.02[b]	9.44 ± 0.01[b]	19.92 ± 0.06[a]	13.85 ± 0.00[a]	7.73 ± 0.00[a]
B3N1	24.37 ± 0.02[cd]	28.09 ± 0.02[b]	9.82 ± 0.02[b]	18.21 ± 0.02[a]	11.83 ± 0.01[a]	8.03 ± 0.02[a]
Biochar (B)	ns	ns	ns	ns	ns	ns
Nitrogen (N)	ns	ns	ns	*	ns	ns
B × N	**	*	ns	ns	ns	ns

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significan. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

https://doi.org/10.17221/199/2023-PSE

Table 5. Organic carbon content (g/kg) in soil aggregates for each treatment as analysed *via* wet sieving

Treatment	Organic carbon content	Particle size of soil aggregate (mm)					
		> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	6.90 ± 0.36^{e}	4.65 ± 0.42^{e}	8.44 ± 0.5^{c}	7.98 ± 0.35^{e}	5.78 ± 1.38^{e}	5.18 ± 0.35^{e}	9.38 ± 0.36^{ef}
B1N0	9.43 ± 0.17^{b}	9.37 ± 0.69^{c}	9.77 ± 0.76^{b}	9.11 ± 0.35^{cd}	8.31 ± 1.04^{cd}	9.17 ± 0.35^{b}	10.84 ± 0.19^{bc}
B2N0	10.98 ± 0.11^{a}	13.16 ± 0.2^{a}	11.17 ± 0.2^{a}	9.90 ± 0.23^{bc}	10.10 ± 0.61^{ab}	9.37 ± 0.35^{ab}	12.17 ± 0.17^{a}
B3N0	10.84 ± 0.10^{a}	10.17 ± 0.8^{c}	11.77 ± 0.91^{a}	12.10 ± 1.04^{a}	10.97 ± 0.8^{a}	9.84 ± 0.35^{a}	10.17 ± 0.12^{cd}
B0N1	7.51 ± 0.19^{d}	4.32 ± 0.6^{e}	9.11 ± 0.2^{bc}	8.17 ± 0.46^{de}	7.78 ± 0.5^{d}	6.98 ± 0.35^{d}	8.68 ± 0.11^{fg}
B1N1	8.47 ± 0.12^{c}	7.78 ± 0.69^{d}	8.77 ± 0.35^{c}	8.97 ± 0.6^{cd}	8.97 ± 0.12^{cd}	7.78 ± 0.30^{c}	8.51 ± 0.12^{g}
B2N1	9.51 ± 0.12^{b}	11.17 ± 0.2^{b}	9.17 ± 0.53^{bc}	9.57 ± 0.64^{c}	9.24 ± 0.35^{bc}	8.04 ± 0.30^{c}	9.84 ± 0.1^{de}
B3N1	10.68 ± 0.21^{a}	12.43 ± 0.61^{a}	10.97 ± 0.35^{a}	10.64 ± 0.23^{b}	9.57 ± 0.2^{bc}	9.37 ± 0.35^{ab}	11.11 ± 0.21^{b}
Biochar (B)	*	*	ns	*	ns	ns	ns
Nitrogen (N)	ns	ns	ns	ns	ns	ns	ns
B × N	**	**	**	**	**	**	**

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

est increases in organic carbon content were evident for the > 5 mm and 0.5–1 mm fractions under B2N0 conditions relative to CK conditions ($P < 0.05$). The highest increase in organic carbon content relative to CK conditions was observed under B3N1 conditions, with each fraction exhibiting a similar increase trend when nitrogen was combined with biochar. Overall, the SOC levels in these different aggregate fractions decreased in the following rank order: < 0.25 mm, 2–5 mm, and 0.25–0.5 mm.

Unlike under dry-sieving conditions, the primary soil aggregate fraction contributors to SOC content when using the wet-sieving method were the 0.25–0.5 mm and < 0.25 mm fractions, which accounted for over 70% of the SOC, whereas the contribution of the > 5 mm fraction was relatively limited (2%) (Table 6). Organic carbon contributions for the < 0.25 mm fraction declined as the level of biochar amendment increased, while the contributions of 0.25–0.5 mm aggregates increased significantly. Interaction effects between biochar and nitrogen were evident with respect to soil aggregate contributions to organic carbon and the contributions for different fractions.

(2) Total nitrogen distributions within soil aggregate fractions. When using the dry-sieving method,

Table 6. Organic carbon contribution rates (%) for soil aggregates of different sizes as measured *via* wet sieving

Treatment	Particle size of soil aggregate (mm)					
	> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	0.43 ± 0.06^{e}	2.69 ± 0.36^{c}	3.10 ± 0.73^{cd}	3.66 ± 0.92^{c}	30.77 ± 2.01^{c}	59.36 ± 3.22^{a}
B1N0	1.05 ± 0.31^{d}	3.45 ± 0.46^{bc}	3.68 ± 0.5^{c}	4.82 ± 0.25^{c}	32.90 ± 3.60^{c}	54.10 ± 3.28^{b}
B2N0	2.04 ± 0.1^{b}	4.18 ± 0.59^{ab}	3.74 ± 0.11^{c}	8.00 ± 0.28^{ab}	38.59 ± 3.51^{b}	43.45 ± 3.86^{c}
B3N0	2.10 ± 0.21^{b}	4.80 ± 0.21^{a}	4.90 ± 0.61^{b}	9.50 ± 1.62^{a}	49.60 ± 3.07^{a}	29.10 ± 2.90^{e}
B0N1	1.34 ± 0.23^{cd}	4.18 ± 0.65^{ab}	2.47 ± 0.15^{d}	7.91 ± 1.47^{ab}	46.64 ± 3.11^{a}	37.46 ± 2.22^{d}
B1N1	1.34 ± 0.27^{cd}	3.35 ± 0.24^{c}	3.83 ± 0.61^{c}	6.74 ± 0.57^{b}	48.91 ± 0.42^{a}	35.82 ± 0.56^{d}
B2N1	1.78 ± 0.39^{bc}	4.36 ± 0.33^{a}	6.40 ± 0.29^{a}	8.04 ± 0.66^{ab}	46.73 ± 3.82^{a}	32.69 ± 4.6^{de}
B3N1	2.74 ± 0.29^{a}	4.27 ± 0.34^{a}	6.27 ± 0.73^{a}	8.93 ± 0.60^{a}	48.13 ± 0.82^{a}	29.67 ± 0.36^{e}
Biochar (B)	ns	ns	ns	ns	ns	ns
Nitrogen (N)	ns	ns	ns	ns	ns	ns
B × N	**	**	**	**	**	**

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

https://doi.org/10.17221/199/2023-PSE

Table 7. Soil total nitrogen content (g/kg) and total nitrogen distribution in soil aggregates of different sizes as measured *via* dry sieving

Treatment	Soil total nitrogen	Particle size of soil aggregate (mm)					
		> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	1.2 ± 0.02[a]	1.13 ± 0.05[a]	1.13 ± 0.04[a]	1.12 ± 0.02[a]	1.18 ± 0.02[a]	1.26 ± 0.01[a]	1.36 ± 0.02[a]
B1N0	1.63 ± 0.01[e]	1.53 ± 0.01[d]	1.5 ± 0.01[bc]	1.5 ± 0.03[c]	1.62 ± 0.04[de]	1.68 ± 0.02[d]	1.93 ± 0.02[d]
B2N0	1.69 ± 0.00[f]	1.6 ± 0.01[e]	1.58 ± 0.01[d]	1.57 ± 0.02[e]	1.65 ± 0.01[e]	1.78 ± 0.01[e]	1.98 ± 0.01[e]
B3N0	1.49 ± 0.01[b]	1.5 ± 0.01[d]	1.53 ± 0.05[cd]	1.53 ± 0.01[de]	1.36 ± 0.05[b]	1.38 ± 0.03[b]	1.66 ± 0.03[b]
B0N1	1.57 ± 0.01[c]	1.37 ± 0.01[b]	1.5 ± 0.04[bc]	1.45 ± 0.02[b]	1.49 ± 0.01[c]	1.75 ± 0.05[e]	1.87 ± 0.02[c]
B1N1	1.56 ± 0.00[c]	1.42 ± 0.02[c]	1.45 ± 0.05[b]	1.5 ± 0.01[c]	1.51 ± 0.01[c]	1.56 ± 0.01[c]	1.92 ± 0.01[d]
B2N1	1.61 ± 0.02[d]	1.48 ± 0.03[d]	1.44 ± 0.04[b]	1.52 ± 0.01[cd]	1.59 ± 0.02[d]	1.65 ± 0.01[d]	1.96 ± 0.02[de]
B3N1	1.7 ± 0.01[f]	1.52 ± 0.01[d]	1.52 ± 0.04[cd]	1.56 ± 0.02[e]	1.69 ± 0.02[f]	1.85 ± 0.02[f]	2.04 ± 0.04[f]
Biochar (B)	ns	ns	ns	ns	ns	ns	ns
Nitrogen (N)	ns	ns	ns	ns	ns	ns	ns
B × N	**	**	**	**	**	**	**

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

clear differences in total nitrogen levels were evident among different soil aggregate fractions and treatment conditions (Table 7). Soil total nitrogen levels rose in response to all biochar and biochar-nitrogen amendment conditions as compared to CK conditions, with the highest levels being evident in the < 0.25 mm fraction, followed by the 0.25–0.5 mm fraction, whereas these values were lowest in the > 5 mm fraction.

The contributions of soil aggregates to total soil nitrogen content were dominated by the >5 mm and 2–5 mm fractions, whereas the < 0.25 mm fraction exhibited the smallest contributions at 10% of total nitrogen (Table 8). Increases in biochar dose were associated with increases in the contributions of the > 5 m and 1–2 mm fractions, with a pronounced 19.6% increase in these contributions under B2N0 conditions relative to CK conditions ($P < 0.05$). Biochar amendment alone was associated with a reduction in the total nitrogen contributions of the > 5 mm aggregate fraction relative to B0N1 conditions and increased contributions for the 2–5 mm fraction ($P < 0.05$), with a significant 18.3% increase under B3N1

Table 8. Total nitrogen contribution rate (%) in soil aggregates of different sizes under different treatment conditions as measured *via* dry sieving

Treatment	Particle size of soil aggregate (mm)					
	> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	19.94 ± 1.28[bc]	23.93 ± 0.86[b]	10.5 ± 0.54[b]	18.18 ± 1.04[c]	14.54 ± 0.76[a]	11.06 ± 0.61[a]
B1N0	24.15 ± 0.27[e]	24.57 ± 0.80[bc]	8.31 ± 0.19[a]	17.46 ± 0.48[bc]	13.59 ± 0.49[a]	9.17 ± 0.94[a]
B2N0	28.22 ± 2.08[f]	26.32 ± 1.82[cd]	8.52 ± 0.66[a]	15.45 ± 0.11[a]	11.11 ± 0.95[a]	7.08 ± 3.59[a]
B3N0	23.57 ± 0.59[de]	28.23 ± 0.03[d]	9.51 ± 0.94[ab]	16.22 ± 1.06[ab]	12.67 ± 5.16[a]	8.73 ± 0.77[a]
B0N1	21.72 ± 0.93[cd]	21.58 ± 1.12[a]	10.52 ± 0.31[b]	18.63 ± 0.78[cd]	14.19 ± 0.84[a]	10.22 ± 4.14[a]
B1N1	17.44 ± 0.48[a]	26.45 ± 0.88[cd]	9.45 ± 0.52[ab]	20.61 ± 1.29[e]	13.23 ± 0.03[a]	9.57 ± 5.18[a]
B2N1	19.01 ± 1.90[ab]	25.2 ± 0.76[bc]	9.48 ± 0.92[ab]	20.09 ± 0.42[de]	14.37 ± 0.19[a]	8.25 ± 0.75[a]
B3N1	21.67 ± 1.41[cd]	26.42 ± 1.18[cd]	9.3 ± 1.17[ab]	18.13 ± 1.28[c]	12.54 ± 1.12[a]	7.95 ± 1.79[a]
Biochar (B)	ns	ns	ns	ns	ns	ns
Nitrogen (N)	ns	ns	ns	ns	ns	ns
B × N	**	*	ns	*	ns	ns

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

https://doi.org/10.17221/199/2023-PSE

Table 8. Total nitrogen contribution rate (%) in soil aggregates of different sizes under different treatment conditions as measured *via* dry sieving

Treatment	Particle size of soil aggregate (mm)					
	> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	19.94 ± 1.28^{bc}	23.93 ± 0.86^{b}	10.5 ± 0.54^{b}	18.18 ± 1.04^{c}	14.54 ± 0.76^{a}	11.06 ± 0.61^{a}
B1N0	24.15 ± 0.27^{e}	24.57 ± 0.80^{bc}	8.31 ± 0.19^{a}	17.46 ± 0.48^{bc}	13.59 ± 0.49^{a}	9.17 ± 0.94^{a}
B2N0	28.22 ± 2.08^{f}	26.32 ± 1.82^{cd}	8.52 ± 0.66^{a}	15.45 ± 0.11^{a}	11.11 ± 0.95^{a}	7.08 ± 3.59^{a}
B3N0	23.57 ± 0.59^{de}	28.23 ± 0.03^{d}	9.51 ± 0.94^{ab}	16.22 ± 1.06^{ab}	12.67 ± 5.16^{a}	8.73 ± 0.77^{a}
B0N1	21.72 ± 0.93^{cd}	21.58 ± 1.12^{a}	10.52 ± 0.31^{b}	18.63 ± 0.78^{cd}	14.19 ± 0.84^{a}	10.22 ± 4.14^{a}
B1N1	17.44 ± 0.48^{a}	26.45 ± 0.88^{cd}	9.45 ± 0.52^{ab}	20.61 ± 1.29^{e}	13.23 ± 0.03^{a}	9.57 ± 5.18^{a}
B2N1	19.01 ± 1.90^{ab}	25.2 ± 0.76^{bc}	9.48 ± 0.92^{ab}	20.09 ± 0.42^{de}	14.37 ± 0.19^{a}	8.25 ± 0.75^{a}
B3N1	21.67 ± 1.41^{cd}	26.42 ± 1.18^{cd}	9.3 ± 1.17^{ab}	18.13 ± 1.28^{c}	12.54 ± 1.12^{a}	7.95 ± 1.79^{a}
Biochar (B)	ns	ns	ns	ns	ns	ns
Nitrogen (N)	ns	ns	ns	ns	ns	ns
B × N	**	*	ns	*	ns	ns

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

conditions. In general, these results indicated that biochar amendment positively impacted the soil total nitrogen contributions of soil aggregation fractions.

Using the wet-sieving method, marked increases in soil total nitrogen content and nitrogen levels in soil aggregate fractions were elevated under all treatment conditions relative to CK conditions, with a notable 39.8% increase under B2N0 conditions (Table 9) ($P < 0.05$). Biochar-nitrogen addition was associated with a 26.1% increase in the total nitrogen content under B3N1 conditions relative to CK conditions. Overall, the highest levels were observed for the < 0.25 mm soil aggregate fraction, with the largest increase in total nitrogen content relative to control conditions with a 22–50% increase, followed by > 5 mm and 0.25–0.5 mm aggregates, which exhibited the lowest total nitrogen content.

The total nitrogen contribution rates in soil aggregates tended to decrease with increasing aggregate size (Table 10). Except for the < 0.25 mm fraction, aggregate fraction contributions increased with rising levels of biochar addition. Except for the > 5 mm and < 0.25 mm fractions,

Table 9. Soil total nitrogen content (g/kg) and total nitrogen distribution in soil aggregates of different sizes as measured *via* wet sieving

Treatment	Soil total nitrogen	Particle size of soil aggregate (mm)					
		> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	1.23 ± 0.02^{g}	1.35 ± 0.02^{e}	1.27 ± 0.05^{e}	1.25 ± 0.05^{f}	1.16 ± 0.03^{d}	0.99 ± 0.03^{d}	1.36 ± 0.02^{f}
B1N0	1.59 ± 0.01^{b}	1.65 ± 0.03^{c}	1.64 ± 0.05^{b}	1.55 ± 0.06^{b}	1.54 ± 0.02^{a}	1.26 ± 0.06^{bc}	1.93 ± 0.01^{c}
B2N0	1.72 ± 0.02^{a}	2.05 ± 0.02^{a}	1.77 ± 0.02^{a}	1.6 ± 0.04^{ab}	1.57 ± 0.12^{a}	1.33 ± 0.02^{ab}	1.98 ± 0.02^{b}
B3N0	1.70 ± 0.01^{a}	2.09 ± 0.03^{a}	1.8 ± 0.02^{a}	1.64 ± 0.04^{a}	1.62 ± 0.07^{a}	1.38 ± 0.01^{a}	1.66 ± 0.02^{e}
B0N1	1.38 ± 0.01^{f}	1.34 ± 0.06^{e}	1.31 ± 0.03^{e}	1.31 ± 0.04^{ef}	1.27 ± 0.04^{c}	1.19 ± 0.04^{c}	1.87 ± 0.02^{d}
B1N1	1.44 ± 0.02^{e}	1.46 ± 0.02^{d}	1.37 ± 0.04^{d}	1.34 ± 0.03^{de}	1.34 ± 0.01^{bc}	1.21 ± 0.04^{c}	1.92 ± 0.03^{c}
B2N1	1.52 ± 0.01^{d}	1.70 ± 0.06^{bc}	1.42 ± 0.03^{cd}	1.41 ± 0.03^{cd}	1.37 ± 0.01^{bc}	1.23 ± 0.05^{c}	1.96 ± 0.01^{bc}
B3N1	1.55 ± 0.02^{c}	1.71 ± 0.04^{b}	1.45 ± 0.02^{c}	1.44 ± 0.05^{b}	1.37 ± 0.02^{b}	1.27 ± 0.06^{bc}	2.04 ± 0.04^{a}
Biochar (B)	ns	*	ns	ns	ns	ns	ns
Nitrogen (N)	ns	ns	ns	ns	ns	ns	ns
B × N	**	**	**	**	**	**	**

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

https://doi.org/10.17221/199/2023-PSE

Table 10. Total nitrogen contribution rate (%) in soil aggregates of different sizes under different treatment conditions as measured *via* wet sieving

Treatment	Particle size of soil aggregate (mm)					
	> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	0.70 ± 0.10^{c}	2.26 ± 0.06^{b}	2.74 ± 0.81^{a}	4.14 ± 0.52^{b}	32.96 ± 1.90^{a}	57.20 ± 2.46^{bc}
B1N0	1.09 ± 0.27^{c}	3.42 ± 0.27^{ab}	3.70 ± 0.5^{a}	5.29 ± 0.38^{ab}	26.68 ± 2.40^{a}	59.82 ± 2.84^{c}
B2N0	2.04 ± 0.18^{ab}	4.25 ± 0.68^{ab}	3.87 ± 0.14^{a}	7.95 ± 0.78^{ab}	35.02 ± 2.66^{a}	46.86 ± 2.55^{c}
B3N0	2.76 ± 0.32^{d}	4.68 ± 0.11^{b}	4.24 ± 0.56^{a}	8.91 ± 1.38^{b}	44.43 ± 1.00^{a}	34.98 ± 0.95^{a}
B0N1	2.26 ± 0.05^{cd}	3.25 ± 0.12^{ab}	2.15 ± 0.13^{a}	7.02 ± 0.74^{ab}	42.97 ± 0.38^{a}	42.35 ± 0.5^{a}
B1N1	1.47 ± 0.22^{b}	3.09 ± 0.21^{a}	3.36 ± 0.2^{a}	5.92 ± 0.20^{ab}	44.72 ± 1.67^{a}	41.43 ± 1.27^{bc}
B2N1	1.71 ± 0.44^{a}	4.25 ± 0.36^{a}	5.92 ± 0.18^{a}	7.46 ± 0.65^{a}	44.80 ± 0.43^{a}	35.85 ± 0.83^{bc}
B3N1	2.62 ± 0.32^{b}	3.89 ± 0.35^{ab}	5.85 ± 0.72^{a}	8.83 ± 0.25^{ab}	45.02 ± 1.29^{a}	33.80 ± 1.14^{ab}
Biochar (B)	ns	ns	ns	ns	ns	*
Nitrogen (N)	ns	ns	ns	ns	ns	ns
B × N	**	**	**	**	**	**

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

total nitrogen contribution rates rose significantly relative to CK ($P < 0.05$), with the largest increases being evident under B3N1 treatment conditions.

(3) Carbon-to-nitrogen ratio values. The observed trends in C/N ratio values varied among aggregates of different sizes and across treatment conditions (Table 11). Under the B1N0 and B2N0 conditions, notable increases in the C/N ratio were evident for each aggregate fraction, whereas these values decreased under B3N0 conditions relative to CK conditions. Overall, the C/N ratio for the > 5 mm aggregate fraction was the largest, followed by that for the 2–5 mm aggregate fraction.

Similar trends in the C/N ratio values in different aggregate fractions were observed when using the wet-sieving approach compared to the dry-sieving method (Table 12). The C/N ratio rose as the biochar application dose increased, with maximal values evident in 0.25–0.5 mm aggregates under biochar-only amendment conditions, followed by 2–5 mm aggregates. In contrast, the C/N ratio in the > 5 mm aggregate fraction was the lowest.

Table 10. Total nitrogen contribution rate (%) in soil aggregates of different sizes under different treatment conditions as measured *via* wet sieving

Treatment	Particle size of soil aggregate (mm)					
	> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	0.70 ± 0.10^{c}	2.26 ± 0.06^{b}	2.74 ± 0.81^{a}	4.14 ± 0.52^{b}	32.96 ± 1.90^{a}	57.20 ± 2.46^{bc}
B1N0	1.09 ± 0.27^{c}	3.42 ± 0.27^{ab}	3.70 ± 0.5^{a}	5.29 ± 0.38^{ab}	26.68 ± 2.40^{a}	59.82 ± 2.84^{c}
B2N0	2.04 ± 0.18^{ab}	4.25 ± 0.68^{ab}	3.87 ± 0.14^{a}	7.95 ± 0.78^{ab}	35.02 ± 2.66^{a}	46.86 ± 2.55^{c}
B3N0	2.76 ± 0.32^{d}	4.68 ± 0.11^{b}	4.24 ± 0.56^{a}	8.91 ± 1.38^{b}	44.43 ± 1.00^{a}	34.98 ± 0.95^{a}
B0N1	2.26 ± 0.05^{cd}	3.25 ± 0.12^{ab}	2.15 ± 0.13^{a}	7.02 ± 0.74^{ab}	42.97 ± 0.38^{a}	42.35 ± 0.5^{a}
B1N1	1.47 ± 0.22^{b}	3.09 ± 0.21^{a}	3.36 ± 0.2^{a}	5.92 ± 0.20^{ab}	44.72 ± 1.67^{a}	41.43 ± 1.27^{bc}
B2N1	1.71 ± 0.44^{a}	4.25 ± 0.36^{a}	5.92 ± 0.18^{a}	7.46 ± 0.65^{a}	44.80 ± 0.43^{a}	35.85 ± 0.83^{bc}
B3N1	2.62 ± 0.32^{b}	3.89 ± 0.35^{ab}	5.85 ± 0.72^{a}	8.83 ± 0.25^{ab}	45.02 ± 1.29^{a}	33.80 ± 1.14^{ab}
Biochar (B)	ns	ns	ns	ns	ns	*
Nitrogen (N)	ns	ns	ns	ns	ns	ns
B × N	**	**	**	**	**	**

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

https://doi.org/10.17221/199/2023-PSE

Table 11. Carbon-nitrogen ratios in soil aggregates for different treatment conditions as measured *via* dry sieving

Treatment	Mean C/N ratio	Particle size of soil aggregate (mm)					
		> 5	2~5	1~2	0.5~1	0.25~0.5	< 0.25
CK (B0N0)	5.64 ± 0.09[b]	6.01 ± 0.10[ab]	5.55 ± 0.07[bc]	5.15 ± 0.18[ab]	5.46 ± 0.18[ab]	5.54 ± 0.18[b]	5.64 ± 0.09[c]
B1N0	6.93 ± 0.09[c]	6.98 ± 0.50[c]	7.21 ± 0.06[d]	7.51 ± 0.31[e]	7.18 ± 0.03[c]	6.73 ± 0.21[c]	6.93 ± 0.09[d]
B2N0	7.07 ± 0.22[c]	7.90 ± 0.34[a]	6.95 ± 0.27[d]	6.77 ± 0.28[d]	6.62 ± 0.40[bc]	6.41 ± 0.20[c]	7.07 ± 0.22[e]
B3N0	5.61 ± 0.16[b]	5.64 ± 0.21[a]	4.78 ± 0.50[a]	5.13 ± 0.17[ab]	5.46 ± 0.18[ab]	6.59 ± 0.28[c]	5.61 ± 0.16[d]
B0N1	5.18 ± 0.22[a]	5.96 ± 0.18[ab]	5.26 ± 0.09[b]	4.86 ± 0.17[a]	4.85 ± 0.16[a]	4.98 ± 0.23[a]	5.18 ± 0.22[a]
B1N1	5.41 ± 0.02[ab]	6.12 ± 0.14[b]	5.61 ± 0.37[bc]	5.24 ± 0.23[abc]	5.03 ± 0.28[a]	5.37 ± 0.22[ab]	5.41 ± 0.02[a]
B2N1	5.36 ± 0.21[ab]	6.0 ± 0.11[ab]	5.84 ± 0.20[c]	5.35 ± 0.17[bc]	5.33 ± 1.74[a]	5.17 ± 0.18[ab]	5.36 ± 0.21[b]
B3N1	5.37 ± 0.23[ab]	6.03 ± 0.07[ab]	5.71 ± 0.13[bc]	5.63 ± 0.25[c]	5.38 ± 0.07[a]	5.07 ± 0.23[a]	5.37 ± 0.23[c]
Biochar (B)	ns	ns	ns	ns	ns	ns	ns
Nitrogen (N)	ns	ns	ns	ns	ns	*	ns
B × N	**	**	**	**	ns	**	**

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

The association between biochar amendment and spring wheat yield

Biochar application was associated with an enhanced spring wheat yield, with this increase being more pronounced in response to biochar-nitrogen amendment as compared to biochar application only ($P < 0.05$) (Figure 3). Relative to CK conditions, the B1N0 and B2N0 conditions were associated with 24.6% and 25.5% increases in wheat yields, respectively. The highest yield across treatments was observed for B2N1 amendment conditions, followed by B1N1 and B3N1.

Soil aggregate stability indicators, SOC levels, total nitrogen levels, and spring wheat yields were correlated. Specifically, a positive correlation was observed between wheat yields and MWD, GMD, and WSAR ($P < 0.05$). There was also a significant positive correlation between yield, SOC, and nitrogen content and a significant negative correlation with PAD, indicating that higher levels of SOC and nitrogen were associated with improvements in the stability of soil aggregates, increasing the availability of these nutrients in the soil while also supporting better crop yields.

Table 12. Carbon-nitrogen ratios in soil aggregates for different treatment conditions as measured *via* wet sieving

Treatment	Carbon to nitrogen ratio	Particle size of soil aggregate (mm)					
		> 5	2–5	1–2	0.5–1	0.25–0.5	< 0.25
CK (B0N0)	5.61 ± 0.20[d]	3.45 ± 0.27[e]	6.64 ± 0.58[bc]	6.41 ± 0.38[bc]	4.98 ± 0.11[c]	5.24 ± 0.38[d]	6.88 ± 0.17[a]
B1N0	5.91 ± 0.08[c]	5.69 ± 0.22[c]	5.95 ± 0.27[c]	5.88 ± 0.08[c]	5.39 ± 0.34[bc]	7.30 ± 0.56[a]	5.62 ± 0.21[c]
B2N0	6.40 ± 0.10[b]	6.43 ± 0.36[b]	6.31 ± 0.07[bc]	6.19 ± 0.34[bc]	6.46 ± 0.49[ab]	7.05 ± 0.19[ab]	6.15 ± 0.39[b]
B3N0	6.39 ± 0.01[b]	4.87 ± 0.13[d]	6.55 ± 0.37[bc]	7.38 ± 0.24[a]	6.80 ± 0.38[a]	7.12 ± 0.29[ab]	6.12 ± 0.06[d]
B0N1	5.43 ± 0.11[d]	3.22 ± 0.54[e]	6.95 ± 0.72[ab]	6.24 ± 0.20[bc]	6.11 ± 0.82[ab]	5.89 ± 0.29[cd]	4.65 ± 0.05[e]
B1N1	5.88 ± 0.11[c]	5.34 ± 0.47[cd]	6.39 ± 0.61[bc]	6.67 ± 0.71[b]	6.71 ± 0.67[a]	6.44 ± 0.34[bc]	4.44 ± 0.11[e]
B2N1	6.27 ± 0.13[b]	6.57 ± 0.61[b]	6.45 ± 0.27[bc]	6.79 ± 0.37[ab]	6.76 ± 0.11[a]	6.54 ± 0.54[bc]	5.03 ± 0.20[d]
B3N1	6.91 ± 0.12[a]	7.26 ± 0.27[a]	7.58 ± 0.17[a]	7.40 ± 0.11[a]	6.98 ± 0.21[a]	7.39 ± 0.29[a]	5.45 ± 0.07[c]
Biochar (B)	*	ns	ns	ns	ns	ns	ns
Nitrogen (N)	ns	ns	ns	ns	ns	ns	*
B × N	**	**	ns	ns	ns	*	**

Different lowercase letters indicate significant differences between treatments ($P < 0.05$). *$P < 0.05$; **$P < 0.01$; ns – not significant. CK – control; N0 – 0 kg N/ha; N1 – 150 kg N/ha; B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

https://doi.org/10.17221/199/2023-PSE

DISCUSSION

In the present study, biochar application can impact soil aggregate stability and crop productivity, which is the same as other findings (Blanco-Canqui 2017, Weng et al. 2017, Zhang et al. 2017, Baiamonte et al. 2019). The biochar application rate is a primary factor influencing soil aggregates. Biochar doses from 10–30 t/ha were associated with improvements in soil aggregate content in the present study (Table 1), which is in line with results published in previous reviews (Blanco et al. 2017, Zhang et al. 2017). The application of greater quantities of biochar has been linked to improved microbial activity owing to the formation of macro- and micro-aggregates owing to the production of mucilage and hyphae at the interface between biochar and soil particles (Jien and Wang 2013, Herath et al. 2014). This results in the binding of soil organic matter and multivalent ions, with fungal hyphae and roots serving to hold together micro-aggregates such that larger aggregates can develop (Tisdall and Oades 1982). The disruption and breakage of these macro-aggregates, in turn, can contribute to soil aggregate turnover (Six et al. 2000). Increases in the proportions of 1–2 mm aggregates following biochar application may thus be attributable to the rearrangement or dissolution of larger aggregates. Given that applying biochar under field conditions can also have long-lived positive effects on soil aggregation, there are clear time-dependent shifts in the impact of biochar application on soil aggregate content (Zhang et al. 2015, Du et al. 2016).

Biochar application was found to be associated with an increase in the aggregate stability index. This may be at least partially attributable to the slight increase in SOC content evident in biochar-amended plots, as organic compounds are conducive to better soil quality, more conducive to the development of mycorrhizal fungi (Fletcher et al. 2013) and the improvement of soil aggregate stability (Sun and Lu 2013, Tsai and Chen 2013, Soinne et al. 2014, Hartley et al. 2016). The characteristics of biochar may enable it to link soil particles effectively. The approaches most commonly used to assess the structural stability of soil based on WSA, MWD, and/or GMD values generally rely on both dry and wet sieving performed in the laboratory. However, dry and wet sieves differ markedly in their ability to assess the stability of soil aggregates such that they have the potential to yield contradictory or inconsistent results. The wet-sieving method, first described by Yoder (1936), is the most commonly used method to study microbial communities in soil aggregates and involves immersing soil in water for several minutes to break down aggregates. Dry sieving usually involves shaking air-dried soil on top of a nest of sieves. Thus, the energy applied to the soil differs greatly between dry- and wet-sieving, directly affecting the amount of stable soil aggregates obtained (Blaud et al. 2017). A study comparing the effect of dry- and wet-sieving on microbial enzymatic activity showed that wet-sieving overestimated the potential microbial enzymatic activity compared to dry-sieving (Bach and Hofmockel 2014). However,

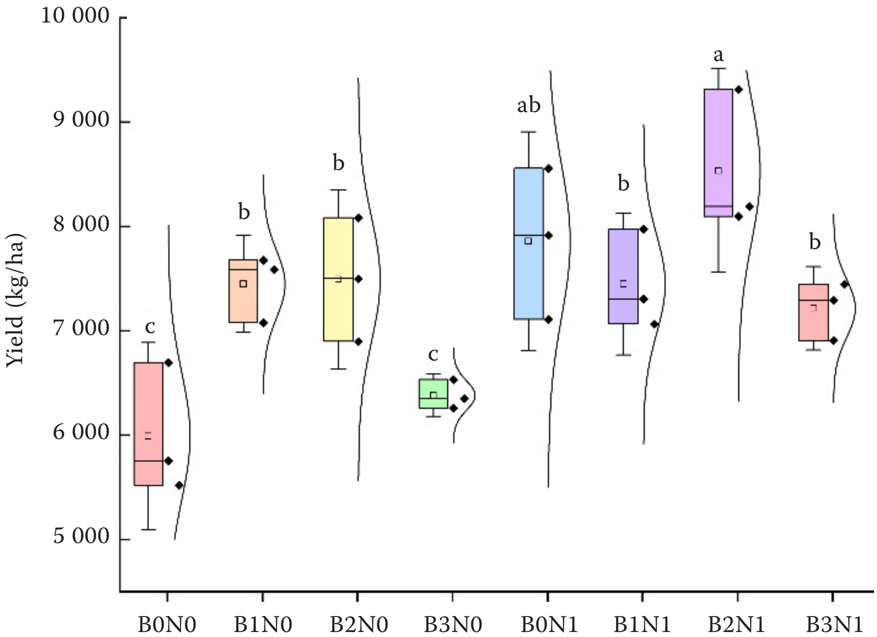

Figure 3. Grain yield of spring wheat under different treatment conditions. CK – control; nitrogen (N): N0 – 0 kg N/ha; N1 – 150 kg N/ha; biochar (B): B0 – 0 t/ha; B1 – 10 t/ha; B2 – 20 t/ha; B3 – 30 t/ha

https://doi.org/10.17221/199/2023-PSE

only the enzymatic activity differed between the sizes of soil aggregates with wet sieving and not with dry sieving. Few analyses to date have focused explicitly on the impact of biochar application on the stability of both wet and dry aggregates. While a recent review noted that biochar amendment was associated with 4–22.6% increases in wet aggregated stability in 24 of 34 tested soils (Blanco-Canqui 2017), it failed to affect dry aggregated stability in 5 of 8 tested soils. Wet sieving thus offers advantages over dry sieving when seeking to gauge the impact of biochar on soil aggregates reliably. Biochar has also been demonstrated to more readily promote the stability of wet aggregates in sandy soils than silt- or clay-based soils (Ouyang et al. 2013, Burrell et al. 2016).

Applying biochar can result in changes in the levels and availability of specific nutrients in the soil, thereby influencing the growth of crops cultivated therein (Sohi et al. 2010, Yuan et al. 2016). This may be the result of enhanced soil nutrient cycling in response to biochar application, with processes such as nitrification being enhanced as a result of the retention, adsorption, and desorption of nutrients within the porous structure of the applied biochar (Shaaban et al. 2018). The specific responses of crops to biochar amendment vary based on the physiochemical characteristics of the biochar, climatic conditions, soil conditions, and the crop type in question (Van Zwieten et al. 2009, Gaskin et al. 2010, Yamato et al. 2010, Haefele et al. 2011). Here, wheat yields were found to be enhanced following the application of varying levels of biochar after a two-year interval. Relative to applying nitrogen fertiliser alone, biochar application enhanced wheat grain weight by 5.1% and crop productivity by 7.5%. This enhanced wheat productivity may result from improvements in the availability of nutrients and/or the physical quality of the soil. However, yields were not proportional to biochar amendment rates in the present study, indicating that the application of excessively high biochar levels may reduce N availability (Lehamann et al. 2003). Applying biochar to acidic paddy soil containing high SOC levels at rates of 10–40 t/ha has been shown to enhance the efficiency of N utilisation by rice and associated rice productivity (Zhang et al. 2010). Biochar can facilitate the activation of residual nitrogen present within the soil, thereby influencing effective nitrogen content. The timing of biochar ap-

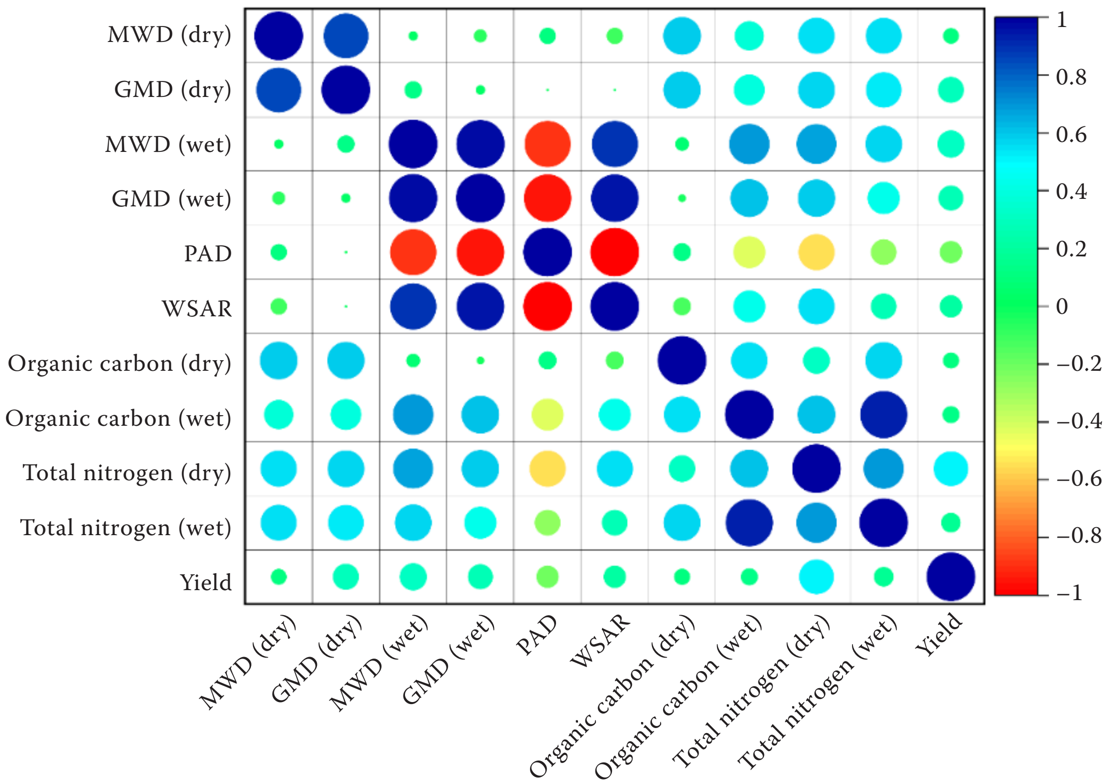

Figure 4. Correlations between soil aggregate stability, carbon and nitrogen content, and spring wheat yields. MWD – mean weight diameter GMD – geometric weight diameter; PAD – soil aggregate destruction rate; WSAR – soil aggregate stability rate

https://doi.org/10.17221/199/2023-PSE

plication also influences crop yields, with relatively limited short-term effects but far more pronounced long-term benefits to crop outputs. Hussain et al. (2016) previously determined that biochar application was associated with improved agricultural yields in infertile or highly degraded soils. In contrast, it had no similar effect when applied to healthy or fertile soils. This suggests that biochar does not uniformly enhance crop yields when used for soil amendment. Moreover, while biochar is highly stable in the soil, its ability to enhance soil quality and ecological integrity inevitably diminishes somewhat with time. Thus, long-term studies must fully explore the effects of biochar on soil aggregates, soil carbon sequestration, and crop yields in detail (Dong et al. 2016).

Our findings demonstrated that applying biochar for two years, in conjunction with nitrogen fertiliser, resulted in the development of substantial soil aggregates, bolstered the stability of organic carbon in the soil, and reinforced the process of carbon fixation by the soil. In the context of wheat fields in the irrigation areas of North Xinjiang, the application of reduced nitrogen combined with biochar (nitrogen:150 kg/ha, biochar: 20 t/ha) is deemed a favourable approach for stimulating the organic carbon levels within soil aggregates and augmenting carbon fixation. The above results provide significant contributions to the understanding of sustainable agriculture methods involving the utilisation of biochar.

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Received: May 16, 2023
Accepted: January 23, 2024
Published online: February 16, 2024