Application of biochar and sulfur-modified biochar in a saline-sodic and calcareous soil: Effects on soil water content, soil biochemical properties and millet (Panicum miliaceum) yield

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Research Article

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Abstract

Aims

Soil microbial and enzymatic activity have important roles in soil health, nutrient cycle, and plant growth. Biochar is excellent biomass for increasing soil water content, but some of the biochar compounds due to high pH are harmful to the soil. This study aimed to investigate the effect of biochar and sulfur-modified biochar on improving soil water content and biochemical properties of soil in a millet cropping system.

Methods

The field experiment was performed in a completely random design with three replications under field conditions. Experimental treatments included control, 15 t ha$^{-1}$ sunflower biochar (B), and 15 t ha$^{-1}$ sulfur-modified biochar (BS).

Results

Soil water content in B treatment was higher than that in BS treatment. B and BS treatments improved soil biological properties (MBC, BR, CAT, DHA, UA and ALP) compared to control. Application of B and BS treatments decreased soil EC and SAR compared to the control. The highest (8.26) and lowest (7.83) values of soil pH were related to B and BS treatments, respectively. Treatment of soil with B and BS enhanced soil DOC (90% and 33%), AN (74% and 48%), and AP (60% and 96%) compared to control. Application of B and BS treatments enhanced plant nutrients such as N, P and K and increased RWC (40% and 14%) and plant yield (58% and 115%) compared to the control.

Conclusions

The sulfur increased the efficiency of biochar on amending saline and calcareous soil and enhance plant yield through improving chemical properties (pH and CEC) of biochar.

Introduction

Iran has an arid and semi-arid climate with an average annual precipitation of less than 250 mm. The lack of rainfall and high evaporation have caused Iran's agricultural lands to have problems such as saline-sodic, and calcareous soil. More than 30% of agricultural land in Iran has a salinity problem (Emadi et al. 2018). Today, soil salinity is one of the essential agricultural problems in the world, which has limited agricultural production. Soil salinity decreases plant yield by limiting the available water of the plant, ion toxicity, reduction of available elements in the soil, and decreasing the microbial activity of the
soil (Safdar et al. 2019). Soil microorganisms have a key role in the cycle of soil nutrients, so the reduction of microbial activities in saline soils can decrease the growth of plants (Andronov et al. 2012).

More than 70% of Iran's agricultural lands have less than 1% organic matter (Rezaei et al. 2020). Lack of organic matter reduces the soil water content and accelerates soil salinization (Wichern et al. 2020). In order to have sustainable agriculture, much attention should be paid to the soil's organic matter. One of the materials that can help to improve unfavorable soil conditions is biochar. Biochar is a black substance that is produced from organic residues during the pyrolysis process in the absence of oxygen (Wang et al. 2020).

Many studies have been done on the effect of biochar on soil quality. The results of these studies show that biochar enhanced: 1- soil organic carbon (Glaser et al. 2002), 2- water holding capacity (Abel et al. 2013), 3- soil aeration, 4- nutrient availability, 5- stimulation of soil microbial and enzymatic activity (Joseph and Lehmann 2009), 6- cation exchange capacity (CEC).

Sunflower is one of the main plants widely cultivated in Iran to produce cooking oil. Every year, a large amount of sunflower residue is left in the fields and buried in the soil by plowing. A high C/N ratio of sunflower residues disrupts the cycle of soil nutrients and delays soil fertility. The structure of the sunflower stem is fibrous. The micro and macro pores in the sunflower pith fiber have created a lot of space that can hold a significant amount of water. Water retention in plant residues is affected by physical and chemical processes, for example, the presence of micro and mesa-sized pores on the surface of the cell wall, which is occasionally open or closed, controlling water retention (Almeida and Hernández 2006). In wood and composite technology, it is established that compounds such as cellulose and hemicellulose are hydrophilic and compounds such as lignin are hydrophobic, and the ratio of these two groups influences the characteristics of water retention (Pejic et al., 2008). Sunflower stalk pith has a lot of pectin and cellulose and contains a small amount of lignin. The sunflower pith is different from the contents of other crops, such as sorghum and corn. Marechal and Rigal, (1999) reported that sunflower stalk pith contains 45% cellulose and 3% lignin.

The physical properties of biochar, such as water holding capacity (WHC), surface area, and surface functional groups, are closely related to the feedstock biomass of biochar (Ahmad et al. 2014). Sunflower stalk residues contain pith fibrous that have more water-holding capacity compared to other agricultural residues due to their porous structure. The water-holding capacity of the pith fiber of sunflower stalk is between 20 to 40 g water / g fiber (Qi 2017). Previous studies show that the WHC of sunflower fibers is much higher than that of other crop fibers. For example, the WHC of fiber from peach pulp and cocoa husk is 12 and 5 ml water / g fiber, respectively (Mrabet et al. 2012). One of the advantages of biochar for the soil is soil water retention. Biochar materials have a high water-holding capacity due to their high porosity, which depends on their raw materials (Streubel et al. 2011). Yu et al., (2013) reported that the application of 1−5% biochar in loamy soil increased the soil water holding capacity by 4−10%, respectively.
Biochar has many benefits for soil, but the high pH of biochar can be problematic for soil fertility. The availability of phosphate, iron, boron, zinc, and manganese has been shown to decrease at high pH (Cheng et al. 2018). Increasing pH also increases microbial nitrification, which results in losses of nitrate and limited availability of ammonium, the preferred nitrogen source for plants (Xiao and Meng 2020). In some cases, the pH-raising effect of biochar can create unfavorable conditions for plants, particularly in calcareous soils; this can result in yield losses (Xiao & Meng, 2020; Bachmann et al., 2016). Some types of biochar have a high pH; it cannot be used in soil, it seems that with substances such as sulfur, the pH of biochar can be reduced and used. This study hypothesizes that 1) Sunflower stalk biochar improves soil water content and biochemical properties of soil. 2) Sulfur–modified biochar has a more significant effect on improving soil biological and chemical properties than biochar. This study aimed to investigate the effect of biochar and sulfur-modified biochar on soil water content, and soil biological and chemical properties under saline-sodic and calcareous soil with millet plants.

**Materials And Methods**

**Biochar and sulfur-modified biochar preparation**

The dried residual of sunflower stalk was used to produce biochar. The sunflower stalk was cut into pieces 10 cm and produced biochar during the pyrolysis process under the temperature of 400 °C and in the absence of oxygen (Fu et al. 2019). To produce sulfur-modified biochar (BS), sunflower biochar (B) was mixed with a 5% inorganic sulfur solution (Purity of 80%). After 24 hours, the sulfur and biochar suspension was filtered and dried. Biochar and sulfur-modified biochar were first air-dried and then dried in an oven at 70 °C for 24 hours.

**Analyses of biochar (B and BS)**

B and BS after passing a 0.5 mm sieve were used for chemical analysis. Carbon, hydrogen, nitrogen, and sulfur were measured by a CHNS analyzer (SERIES 1112 FLASH EA). Alkaline cations (Ca$^{2+}$, Mg$^{2+}$, K$^{+}$, and Na$^{+}$), ash content and CEC were measured according to Singh et al., (2017) procedure (Table 1). The water-holding capacity of biochar was measured by weighting method as shown in Fig. 1. For FTIR analysis, 10 mg of B and BS samples were mixed with 190 mg of spectroscopic-grade KBr and prepared for analysis after grinding. The FTIR measurements were performed with a Nicolet 6700 FTIR (Thermo Nicolet), the model of the device was AVATAR 370 FT-IR (USA). The scans were obtained in the range from 400 to 4000 cm$^{-1}$ with a resolution of 4 cm$^{-1}$.
### Table 1

Characteristics of biochar (B) and sulfur-modified biochar (BS)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>B</th>
<th>BS</th>
<th>Reference of measurement methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECe</td>
<td>(dS m$^{-1}$)</td>
<td>6.74</td>
<td>6.81</td>
<td>(Jackson 1973)</td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>10.31</td>
<td>7.56</td>
<td>(Jackson 1973)</td>
</tr>
<tr>
<td>C</td>
<td>(%)</td>
<td>61</td>
<td>57</td>
<td>(Walkley and Black 1934)</td>
</tr>
<tr>
<td>H</td>
<td>(%)</td>
<td>2.9</td>
<td>4.5</td>
<td>-</td>
</tr>
<tr>
<td>O</td>
<td>(%)</td>
<td>8.6</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>N</td>
<td>(%)</td>
<td>0.32</td>
<td>0.43</td>
<td>(Subbaiah 1956)</td>
</tr>
<tr>
<td>S</td>
<td>(%)</td>
<td>0.11</td>
<td>1.4</td>
<td>-</td>
</tr>
<tr>
<td>Ash</td>
<td>(%)</td>
<td>22</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>Volatile matter</td>
<td>(%)</td>
<td>4.43</td>
<td>5.76</td>
<td>-</td>
</tr>
<tr>
<td>H:C</td>
<td>-</td>
<td>0.047</td>
<td>0.078</td>
<td>-</td>
</tr>
<tr>
<td>O:C</td>
<td>-</td>
<td>0.14</td>
<td>0.24</td>
<td>-</td>
</tr>
<tr>
<td>P</td>
<td>(g kg$^{-1}$)</td>
<td>0.68</td>
<td>0.44</td>
<td>(Olsen 1954)</td>
</tr>
<tr>
<td>K$^+$</td>
<td>(g kg$^{-1}$)</td>
<td>22</td>
<td>10.8</td>
<td>(Hanway and Heidel 1952)</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>(g kg$^{-1}$)</td>
<td>15.5</td>
<td>12.6</td>
<td>-</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>(g kg$^{-1}$)</td>
<td>54</td>
<td>42</td>
<td>-</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>(g kg$^{-1}$)</td>
<td>21.6</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>CEC</td>
<td>[Cmol (+) kg$^{-1}$]</td>
<td>36</td>
<td>39</td>
<td>-</td>
</tr>
<tr>
<td>Water holding capacity</td>
<td>(% w/w)</td>
<td>400</td>
<td>250</td>
<td>-</td>
</tr>
</tbody>
</table>

### Experimental site

This study was conducted in Shahrabad village (58° 29’ N, 36° 0’ E), located in the middle of the Neyshabur plain of northeast Iran, in the summer of 2021 (June - August) under field conditions. The average rainfall of this city is less than 250 mm with relatively hot summers (max 42° C). The soil used in this experiment had a clay texture and had the problem of salinity, sodic, and calcareous. A soil sample was taken from 0–30 cm surface soil depth for physicochemical analysis of the soil (Table 2).
Table 2
Physicochemical properties of experimental soil

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Values</th>
<th>Reference of measurement methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical analysis</td>
<td>-</td>
<td>-</td>
<td>(Bouyoucos 1962)</td>
</tr>
<tr>
<td>Sand</td>
<td>%</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Silt</td>
<td>%</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>Clay</td>
<td>%</td>
<td>42</td>
<td>-</td>
</tr>
<tr>
<td>Texture</td>
<td>-</td>
<td>Clay</td>
<td>-</td>
</tr>
<tr>
<td>Chemical analysis</td>
<td>-</td>
<td>-</td>
<td>(Richards et al. 1956)</td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>8.05</td>
<td>-</td>
</tr>
<tr>
<td>ECe</td>
<td>dS.m(^{-1})</td>
<td>9.1</td>
<td>-</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>meq.l(^{-1})</td>
<td>12.6</td>
<td>-</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>meq.l(^{-1})</td>
<td>8.4</td>
<td>-</td>
</tr>
<tr>
<td>Na(^{+})</td>
<td>meq.l(^{-1})</td>
<td>81</td>
<td>-</td>
</tr>
<tr>
<td>SO(_{4}^{2-})</td>
<td>meq.l(^{-1})</td>
<td>2.03</td>
<td>-</td>
</tr>
<tr>
<td>SAR</td>
<td>-</td>
<td>17.6</td>
<td>-</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>%</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>Organic C</td>
<td>%</td>
<td>0.12</td>
<td>(Walkley and Black 1934)</td>
</tr>
<tr>
<td>Available N</td>
<td>mg kg(^{-1})</td>
<td>19.4</td>
<td>(Subbaiah 1956)</td>
</tr>
<tr>
<td>Available P</td>
<td>mg kg(^{-1})</td>
<td>6.2</td>
<td>(Olsen 1954)</td>
</tr>
<tr>
<td>Available K</td>
<td>mg kg(^{-1})</td>
<td>150</td>
<td>(Hanway and Heidel 1952)</td>
</tr>
<tr>
<td>CEC</td>
<td>Cmol (+) kg(^{-1}) soil</td>
<td>27</td>
<td>(Jackson 1973)</td>
</tr>
</tbody>
</table>

**Experimental design and treatments**

This experiment was performed in a completely random design with three replications under field conditions. Experimental treatments were control, 15 t ha\(^{-1}\) sunflower stem biochar (B) and 15 t ha\(^{-1}\) sulfur-modified biochar (BS). The experiment was carried out in one square meter plot with a border distance of 50 cm. B and BS treatment was added to the plot before planting (0–30 cm). In each plot, 10 millet plants (Proso variety) were grown as an experimental crop and protected until the seeding stage (4 months). The experiment plots were irrigated every 15 days with saline and sodic water (EC: 5 dS m\(^{-1}\)).
and SAR: 15). The millet plant was planted on the 12th of June 2021 and harvested on the 4th of November 2021 (Fig. 2).

**Post-harvest soil analysis**

After the millet seeds ripened, the plant was harvested. The fresh weight of the plant was measured. The relative water content of a leaf (RWC) is calculated from the following formula. The plant was dried in an oven at 70° C for 48 hours and was used for chemical analysis after to powder. Soil samples were taken from 0 to 30 cm of experimental plots. The soil samples were divided into two parts, one part was kept for biological analysis at a temperature of 4° C, and the other part of the soil sample was used for chemical analysis after drying and passing through a 2 mm sieve.

\[
RWC = \frac{(FW - DW)}{(TW - DW)}
\]

**Soil water content**

After the millet plant was fully established, in the flowering stage, after irrigation plots, every day at 10:00 AM, samples were taken from the soil of the experiment plots (0–20 cm) for 20 days (Irrigation period: 20 days), and their water content was measured by oven at a temperature of 105° C and time 24 hours.

**Biological analysis of soil**

The activity of alkaline phosphatase (ALP) was measured using the PNP-PO4 method (p-nitro phenyl phosphate) and reported as µg PNP (p-nitro phenol) in one gram of soil for one hour. (Tabatabai & Bremner, 1969). Basal respiration (BR) was measured by Anderson & Domsch (1990) method, soil samples closed containers at a temperature of 25° C were kept and the amount of CO₂ produced was absorbed by sodium hydroxide and determined by titration with HCL. Microbial biomass carbon (MBC) was measured according to the fumigation and incubation method (Jenkinson 1976). Urease activity (UA) was measured by using 10% urea solution as a substrate and the amount of NH₄ released was determent by the colorimetric method at 578 nm (Hoffmann and Teicher 1961). Catalase activity (CAT) was determined by titration using kmno4 (Quan et al. 1986). The activity of dehydrogenase (DHA) was determent according to TTC (Triphenyl tetrazolium chloride) method and reported as ug TPF (Triphenyl formazan) in one g of soil for 24 hours. (Burns 1978).

**Chemical analysis of soil**

The dissolved organic carbon (DOC) was determined using the ferrous sulfate titration method (Herbert and Bertsch 1995). Available phosphorus (AP) was determined by the Olsen method (Olsen 1954). Available nitrogen (AN) was measured using the colorimetric method (Øien and Selmer-Olsen 1980). Soil ECE and pH, soluble cations, and anions were measured in soil saturated paste extract (Richards et al. 1956).

**Statistical analysis**
One-way ANOVA and Tukey HSD test at a 95% confidence level were used to analyze significant differences among treatments by JMP software. The relative importance analysis was done by the R package “relaimpo” to find the key soil properties that control millet yield.

Results

Characteristics of biochar (B) and sulfur-modified biochar (BS)

Modification of biochar with sulfur increased sulfate ions in BS treatment and decreased its pH by about 3 units compared to B treatment (Table 1). Sulfur-modified biochar (BS) had less carbon but more hydrogen and oxygen than biochar (B). H/C and O/C ratios in sulfur-modified biochar were higher than in biochar. The amount of CEC in sulfur-modified biochar was higher than in biochar. The concentration of $K^+$, $Ca^{2+}$, $Mg^{2+}$, and $Na^+$ cations in sulfur-modified biochar were lower than in biochar. The water-holding capacity of biochar was 60% higher than that of sulfur-modified biochar (Table 1).

FTIR analysis of sunflower biochar (B and BS)

The sunflower biochar FTIR spectra result showed in Fig. 3. The number of peaks in sulfur-modified biochar is more than B treatment. Also, the peaks in BS are sharper than the B peaks. Peaks of 3419 cm$^{-1}$ in B treatment and 3402 cm$^{-1}$ in BS correspond to O-H hydroxyl groups of phenol (Goswami et al. 2016). The O-H hydroxyl group in BS is sharper than to B peak which shows its high hydroxyl groups in BS. The peak of 2929 cm$^{-1}$ in biochar treatment represents C-H stretching that corresponds to methyl and methylene groups (Liu et al. 2018). The 1589 cm$^{-1}$ peak in the BS treatment and the 1576 cm$^{-1}$ peak in the B treatment are related to the C = O bending of oxygen functional groups in carboxylic groups that was sharper than in BS compared to the B treatment (Mahmoud et al. 2016). The peaks of 1433 cm$^{-1}$ in BS and B treatment represent the C = C stretching, indicative of lignin and aromatic C that it was sharper than in B compared to BS treatment. The 1140 and 1192 cm$^{-1}$ peaks which there are only in BS treatment refer to the sulfur component, C = S stretching of thiocarbonyl groups (Burke and Fackler Jr 1972). Peaks of 1102 cm$^{-1}$ in BS and 1106 cm$^{-1}$ in B treatment correspond to the C-O stretching of secondary alcohol such as cyclohexane. The C-O peak in BS is sharper than to C-O peak of B treatment. The 1033 cm$^{-1}$ peak in BS and B treatment represents of S = O bending of sulfoxide that is very sharp in BS compared to B treatment. The 875 cm$^{-1}$ peaks in BS and B treatment refer to C = C bending alkene vinylidene that is sharper than in B compared to BS treatment. The 780 and 718 cm$^{-1}$ peaks in B treatment and 755 cm$^{-1}$ peak in BS correspond to C-H stretching that is 1,2,3-trisubstituted and 1,2-disubstituted, respectively. The peak of 660 cm$^{-1}$ in sulfur-enriched biochar refers to the C-S stretch of thioethers (Burke and Fackler Jr 1972). Peaks of 620 cm$^{-1}$ in the B treatment and 602 cm$^{-1}$ in the BS treatment represent to S-S stretch which is a disulfide component that in BS was sharper than in the B treatment. The 467 cm$^{-1}$ peaks in BS and 469 cm$^{-1}$ peaks in the B treatment corresponded to the S-S stretch of aryl disulfides that was very sharper than in the BS treatment (Fig. 3).
Soil water content

Soil treated with 15 t ha$^{-1}$ biochar (B) and sulfur-modified biochar (BS) increased soil water content by 47% and 35%, respectively, in the time of 20 days after irrigation compared to the control treatment. Adding 15 t ha$^{-1}$ biochar to soil enhanced soil water content by 18% more than 15 t ha$^{-1}$ sulfur-modified biochar (Fig. 4).

Soil microbial and enzymatic activities

The application of 15 t ha$^{-1}$ biochar (B) and sulfur–modified biochar (BS) enhanced MBC by 113% and 50% compared to without biochar (Control), respectively. Sulfur-modified biochar increased MBC by 63% less compared to biochar ($p < 0.05$, Fig. 5). Soil BR in presence of B and BS was 75% and 108% higher than that in absence of biochar. Biochar and sulfur–modified biochar application stimulated CAT activity by 13% and 30% compared to without biochar, respectively. Soil treated with 15 t ha$^{-1}$ biochar and sulfur–modified biochar increased ALP activity by 12% and 4% compared to the control treatment, respectively. Sulfur-modified biochar increased ALP by 7% less compared to biochar ($p < 0.05$, Fig. 5). Soil amended with 15 t ha$^{-1}$ B and BS promoted UA activity by 70% and 28% compared to without biochar, respectively. Sulfur-modified biochar increased UA by 40% less compared to biochar treatment. Biochar and sulfur–modified biochar treatments increased DHA activity by 38% and 82% compared to control treatment, respectively ($p < 0.05$, Fig. 5).

Soil chemical properties

Soil amended with 15 t ha$^{-1}$ biochar (B) enhanced soil pH compared to without biochar (Control), but soil amended with 15 t ha$^{-1}$ sulfur-modified biochar (BS) decreased soil pH compared to control ($p < 0.05$, Table 3). Soil treated with biochar and sulfur-modified biochar decreases soil EC by 17% and 5% compared to control treatment, respectively. Soil sodium adsorption ratio (SAR) in presence of 15 t ha$^{-1}$ biochar and sulfur-modified biochar was 14% and 7% lower than that compared to the control treatment, respectively. Biochar application increased DOC by 93% and sulfur-modified biochar application enhanced DOC by 33% compared to without biochar (Control). BS increased DOC by 60% less compared to B treatment ($p < 0.05$, Table 3). Biochar and sulfur-modified biochar promoted AN by 74% and 48% compared to control treatment, respectively. Sulfur-modified biochar increased AN by 25% less compared to biochar treatment. B and BS treatments addition enhanced AP by 60% and 95% compared to without biochar (Control), respectively ($p < 0.05$, Table 3).
Table 3
The effect of treatments on soil chemical properties

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH</th>
<th>ECe</th>
<th>SAR</th>
<th>DOC</th>
<th>AN</th>
<th>AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.96 ± 0.03b</td>
<td>9.66 ± 0.2a</td>
<td>16.83 ± 0.3a</td>
<td>8.12 ± 0.7c</td>
<td>13.88 ± 2c</td>
<td>3.06 ± 0.5c</td>
</tr>
<tr>
<td>B</td>
<td>8.26 ± 0.06a</td>
<td>7.95 ± 0.1c</td>
<td>14.5 ± 0.1c</td>
<td>15.70 ± 0.5a</td>
<td>24.16 ± 0.9a</td>
<td>4.92 ± 0.9b</td>
</tr>
<tr>
<td>BS</td>
<td>7.83 ± 0.04c</td>
<td>9.20 ± 0.08b</td>
<td>15.7 ± 0.15b</td>
<td>10.82 ± 0.3b</td>
<td>20.60 ± 1b</td>
<td>6.01 ± 0.3a</td>
</tr>
</tbody>
</table>

B: 15 t ha⁻¹ biochar, BS: 15 t ha⁻¹ sulfur-modified biochar. DOC: Dissolved organic carbon, AN: Available nitrogen, AP: Available phosphorus. Different letters show significant differences among treatments at p < 0.05. Error bars indicate standard deviation (n = 3).

Millet plant properties

Experimental treatments improved plant nutrients (N, P, and K). Application of biochar (B and BS) increased plant nitrogen content by 7% and 11% compared to the control, respectively. Soil treated with B and BS treatments enhanced plant phosphorus content by 75% and 92% compared to the control treatment, respectively. B and BS treatments promoted plant potassium content by 56% and 35% compared without biochar (control), respectively (p < 0.05, Table 4). Soil treated with biochar and sulfur-modified biochar improved RWC by 40% and 13% compared to the control treatment, respectively. Sulfur-modified biochar increased RWC by 27% less compared to biochar treatment. B and BS treatment increased plant yield by 58% and 115% compared to the control, respectively (p < 0.05, Table 4).

Table 4
The effect of treatments on plant properties.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>RWC</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.37 ± 0.04c</td>
<td>1.11 ± 0.2c</td>
<td>1.50 ± 0.09c</td>
<td>81 ± 4c</td>
<td>1.53 ± 0.1c</td>
</tr>
<tr>
<td>B</td>
<td>2.53 ± 0.06b</td>
<td>1.96 ± 0.05b</td>
<td>2.34 ± 0.06a</td>
<td>113 ± 7a</td>
<td>2.43 ± 0.3b</td>
</tr>
<tr>
<td>BS</td>
<td>2.64 ± 0.03a</td>
<td>2.14 ± 0.02a</td>
<td>2.07 ± 0.04b</td>
<td>92 ± 2b</td>
<td>3.30 ± 0.07a</td>
</tr>
</tbody>
</table>

B: 15 t ha⁻¹ biochar, BS: 15 t ha⁻¹ sulfur-modified biochar. N: Nitrogen content, P: Phosphorus content, K: Potassium content. RWC: relative water content. Yield: Total dry weight plant. Different letters show significant differences among treatments at p < 0.05. Error bars indicate standard deviation (n = 3).

Correlation between soil and plant parameters
The Pearson correlation coefficient between soil and plant factors showed in Fig. 5. Soil EC and SAR had a negative and significant correlation (p < 0.01) with most of the measured parameters, especially with soil microbial and enzymatic activity (MBC, ALP, and UA) and plant properties (Yield, RWC, and plant nutrients). Soil pH had a positive correlation with RWC and DOC, and a negative correlation with ALP and AP (p < 0.01, Fig. 6). There is a positive correlation (p < 0.01) between DOC and MBC, ALP, UA, and AN. Soil available nitrogen (AN) had a positive correlation with MBC, UA, and RWC (p < 0.01, Fig. 6). AP has the highest positive correlation (p < 0.01) with BR, DHA, CAT, and plant yield. There is a positive correlation between UA activity and MBC, and RWC (p < 0.01). ALP activity had a positive correlation with MBC. CAT and DHA activity has the highest positive correlation with plant yield and BR (p < 0.01, Fig. 6). MBC had a positive correlation with RWC. There are positive correlations between plant yield and AP, CAT, DHA, BR, plant N, and plant P (p < 0.01).

**Relative importance index**

The results show that BR, AP, DHA, and CAT had the highest on millet yield, and respectively the EC, pH, and AN had the secondary relative important index on the millet plant. According to the result, biological parameters (CAT, DHA and BR) had a more impact on millet yield compared to chemical parameters (Fig. 7).

**Discussion**

**Biochar Characteristics**

The presence of sulfate ions in sulfur-modified biochar increased CEC compared to biochar. This change in CEC can be due to the alteration in the number of functional groups of biochar after modification with sulfur (Fig. 3). The enhancement of hydroxyl and carboxyl functional groups in biochar increases negative surface charge and boosts CEC (Janu et al. 2021). The modification of biochar with sulfur has increased functional groups (Fig. 3) and enhanced active sites on the surface of biochar, which can improved ion absorption power of BS treatment (Nguyen et al. 2019). According to the result concentration of H in BS is more than in the B treatment (Table 1). The higher molar ratio of O:C and H:C in sulfur-modified biochar compared to biochar reduces carbon stability and lower aromatic carbon content so that its organic compounds are more easily decomposed (Domingues et al. 2017). The water-holding capacity of BS is higher than B treatment (Table 1), according to figure one, the drainage water from the water-holding capacity test of biochar is black, but the water from sulfur-modified biochar is yellow. The black color of the biochar extract can be due to carbon compounds such as humic acid. It is possible that in BS treatment, sulfur reacted with carbon and caused carbon deposition. The inactivation of carbon in the pore wall disrupts the empty pore structure and reduces its water-holding capacity compared to biochar. Liu et al. (2020) reported that modified biochar with H$_2$SO$_4$ decreases specific surface area and total pore volume but increases average pore size. The low acidity of biochar modified with sulfuric acid has weakened the structure of biochar and blocked its pores. According to the result of FTIR (Fig. 3) in BS treatment C = C stretching (peak 1433 cm$^{-1}$), that indicative of lignin and aromatic C
shifted to $\text{C} = \text{S}$ stretching (pecks 1140 and 1192 cm$^{-1}$) that corresponding to increases carbon-sulfur components such as thiocarbonyl groups.

**Soil enzymatic activity (DHA, ALP, CAT and UA)**

Sulfur-modified biochar increased DHA more than biochar (Fig. 5). In the biochar component, the higher the molar ratio of O/C and H/C, the lower carbon stability and aromatic carbon content so that the organic compounds are easily decomposed and increase the enzymatic activity of the soil (Domingues et al. 2017). The molar ratio of O/C and H/C in BS is more than B treatment (Table 1). Correlation results (Fig. 6) between the data showed that dehydrogenase activity had a positive correlation with soil microbial respiration (BR) and available phosphorus (AP). Biochar provides the available substrate (carbon and nutrients) to the microbial population and stimulates soil enzymatic activity (Palansooriya et al. 2019). In general, the results of this study showed the positive effect of biochar and sulfur-modified biochar on increasing the activity of dehydrogenase enzyme as an intracellular enzyme effective in cellular metabolism of soil microorganisms and cell protection against reactive oxygen species such as hydrogen peroxide. According to results B and BS stimulated ALP activity (Fig. 5). The application of sulfur-modified biochar reduced the increasing trend of alkaline phosphatase activity compared to biochar treatment. Sulfur-modified biochar reduced soil pH and subsequently increased soil phosphorus solubility (Table 3). Increasing phosphorus in soil solution will reduce alkaline phosphatase activity (Khadem and Raiesi 2017). Also, the results of the correlation between the data showed that there is a positive correlation between alkaline phosphatase activity and soil pH (Fig. 6). Ghoularata et al (2008) indicated that the available phosphorus in soil solution is a controlling factor in the synthesis and release of phosphatases by microorganisms and plants, which reduces the enzymatic content of phosphatases in the soil. The use of sulfur reduces ALP due to lower soil pH (Gupta and Germida 1988). The results show that sulfur-modified biochar reduced the increasing trend of urease activity, probably sulfur has an inhibitory role in the urease activity. Gupta and Germida, (1988) reported that the application of sulfur in soil reduced the activity of the enzyme urease due to the reduction of the population of protozoans and the reduction of the nitrification process. Baligar et al (2005) reported that there is a positive relationship between urease activity and soil organic carbon. The results show that, biochar treatments (B and BS) increased catalase activity (Fig. 5). Sulfur-modified biochar treatment increased catalase activity more than biochar. Zhao et al (2008) reported that long-term soil treatment with sulfur fertilizers increased catalase activity. In general, catalase activity is higher in high-quality soils but decreases in soils with high pH or temperature (Wang et al. 2016). Accordingly, since biochar treatment has increased soil pH (Table 3), it has less catalase activity than biochar modified with sulfur.

**Microbial activity (MBC and BR)**

According to the results biochar and sulfur-modified biochar increased MBC (Fig. 5). The increase in MBC indicates the growth of soil microorganisms, which could be due to increased carbon and nutrients. Due to its porous structure, biochar creates a suitable environment for the growth of soil microorganisms, especially bacteria (Zhu et al. 2017). Sulfur-modified biochar, in contrast to biochar, reduced the increasing trend of MBC (Fig. 5), this result may be due to the reduction of dissolved carbon in soil
(Table 3). The use of sulfur reduces the pH of the soil, acidification of soil increases the solubility of soil organic matter and causes leaching of dissolved carbon from the biologically active points (Stroo and Alexander 1986). Correlation analysis also showed a positive relationship between MBC, soil pH, and DOC (Fig. 6). The porous structure of biochar, its high specific surface area, and its ability to absorb soluble organic matter, gases, and minerals provide a suitable place for the habitat and growth of soil microorganisms (Lehmann et al. 2011). In biochar pores, there is water, and a variety of gases, including carbon dioxide and oxygen, which are either dissolved in solution or adsorbed to the biochar surface (Shinogi and Kanri 2003). Application of B and BS treatment enhanced BR (Fig. 5). Soil microbial respiration depends on soil porosity, gas concentration, diffusion rate, and soil aerobic or anaerobic conditions. (Lehmann et al. 2011). Biochar compounds have the ability to absorb volatile substances on their surface and slowly provide them as an accessible substrate to soil microorganisms and increase the population and soil microbial respiration (Rutigliano et al. 2014). The amount of volatiles in sulfur-modified biochar was higher than in biochar (Table 1).

**Soil water content**

Sunflower biochar (B and BS) enhanced soil water content, BS has a lower effect on soil water content compared to B treatment (Fig. 4). The biochar used in this study (B and BS) has a high water-holding capacity (400% and 250%) compared to other biochar compounds, for example, the water-holding capacity of coconut shell biochar is 60% (Pituya et al., 2017; Paul and Harikumar, 2022). This WHC of sunflower biochar is due to the superabsorbent materials of the sunflower stalk, (pith fibrous and hemicellulose). The water-holding capacity of the pith fiber of a sunflower stalk is 40 g water/g fiber (Qi 2017). Modification of biochar with sulfur decreases WHC of BS and soil water content compared to B treatment. Sulfur has probably destroyed the internal spaces of the biochar by loosening the carbon bonds of the biochar and reducing the water-holding capacity of sulfur-modified biochar. Liu et al, (2020) reported that modified biochar with H$_2$SO$_4$ decreases specific surface area and total pore volume, the low acidity of biochar modified with sulfuric acid has weakened the structure of biochar and blocked its pores. The peak intensities of C-C stretching (1576 and 1433 cm$^{-1}$) in B treatment is more than BS (Fig. 3). Functional groups of C-C that indicated lignin in BS shifted to the C=S stretching of sulfur component (1140 and 1192 cm$^{-1}$).

**Soil properties**

Biochar (B) increased soil pH, but sulfur-modified biochar decreased soil pH (Table 3). Also, the pH of sulfur-modified biochar was lower than that of biochar (Table 1). Increased sulfur oxidation, increased sulfate ions, and decreased soil pH. There is a negative correlation between sulfate ions and soil pH (Maynard et al. 1986). Biochar is a moisture-absorbing compound, porous, and has a high specific surface area, so combining sulfur with biochar will provide a suitable environment for the activity of sulfur-oxidizing microorganisms and reduce soil pH. Grafe et al, (2021) reported that sulfur-modified biochar reduced soil pH. Biochar and sulfur-modified biochar treatments decreased soil EC and SAR, biochar decreased soil EC and SAR more than sulfur-modified biochar. Biochar had a water-holding capacity more than sulfur-modified biochar (Table 1) it’s an enhanced separation of salts such as Na$^+$.
from the surface of soil colloids, and leaching salt from the root area, in addition, the CEC of sulfur-modified biochar is more than biochar (Table 1) than its can absorbing some harmful ions such as sodium, it reduces soil salinity (Yang et al. 2020). The correlation results in Fig. 5 showed a negative correlation of soil EC and SAR with all measured parameters, especially with soil biological parameters. Biochar increased soil-dissolved organic carbon (DOC), but sulfur-modified biochar reduced the increasing trend of DOC (Table 3). Sulfur-modified biochar has increased sulfate ions and decreased pH in the soil. Soil acidification increases the solubility of carbon and increases the leaching of dissolved carbon (Stroo and Alexander 1986). The presence of sulfur in the soil solution can be complex with dissolved carbon and cause carbon deposition (Koch et al. 2017). There was a positive correlation between DOC and the pH of the soil (Fig. 6). Biochar and sulfur-modified biochar treatments increased soil available phosphorus (Table 3). Sulfur-modified biochar increased phosphorus availability more than biochar. Sulfur-modified biochar decreased soil pH (Table 3) and thus improved soil phosphorus availability. Biochar and sulfur-modified biochar treatments increased the available nitrogen in the soil. BS lower than B enhanced AN (Table 3). Biochar increased the urease enzyme by providing suitable conditions for the growth of soil microorganisms (Fig. 5), followed by an enhancement in the concentration of ammonium and nitrate in the soil solution. Although the total nitrogen in sulfur-modified biochar was higher than in biochar (Table 1), the available nitrogen (AN) in the soil treated with BS is lower than in the B treatment. Sulfur probably has an inhibitory effect on the enzyme urease (Fig. 5) and reduces the availability of nitrogen concentration in the soil (Crusciol et al. 2019).

**Plant parameters**

Based on the results biochar (B, BS) treatments increased the yield of millet plants (Table 4). Biochar improves the physical and chemical conditions of the soil, such as soil water content (Fig. 4), and solubility of nutrients such as phosphorus, and creates a suitable environment for the increasing beneficial soil microorganisms, and subsequent development of plant yield. Based on the correlation results, biological parameters had the highest effect on increasing millet yield (Fig. 6). The result shows that RWC increased in presence of B and BS treatments and BS enhanced RWC lower than B treatment. There is a positive correlation between RWC and soil water content (Huang et al. 2020). Biochar has increased the RWC in the plant by increasing the soil water content of the soil (Fig. 4). According to the result of the relative important index (Fig. 7) biological parameters such as DHA and CAT, and soil-available phosphors had a high effect on plant yield. Since the soil of this study is calcareous and deficient in phosphorus (Table 2), sunflower biochar especially sulfur-modified biochar by decreases soil pH, and the phosphorus content it's have enhanced plant phosphors and followed promoted plant yield (Table 4). In saline and sodic soils with high salt concentrations, the presence of toxic ions such as sodium and the accumulation of reactive oxygen species (ROS) in microbial biomass reduces the microbial and enzymatic activities of the soil (Kumawat et al. 2022). Strengthening oxidoreductase activities such as catalase and dehydrogenase can protect the microbial community from ROS compounds and by improved the activity of other enzymes such as urease and phosphatase can help to provide nutrients and plant growth (Zandi and Schnug 2022). There are positive correlations between CAT and DHA with soil AP and AN (Fig. 6).
Conclusions

This study showed that the application of sunflower biochar (B and BS) promoted soil water content by 47% and 35% compared to the control, respectively. B and BS treatments by reducing soil EC and SAR stimulated soil microbial and enzymatic activity and its followed enhanced soil available nutrient (AN and AP) and millet yield. Modification of biochar with sulfur, although decreased the water-holding capacity of BS (60%) but also decreased the pH of BS by 3 units compared to the B treatment, and by providing more nutrients to the plant, enhanced plant yield by 57% more than the simple biochar treatment (B). This study demonstrates that using sunflower biochar (B and BS) in saline-sodic and calcareous soil increased millet yield by improving soil water content, and soil nutrients (especially phosphorus), decreasing soil salinity and stimulating soil microbial and enzymatic activity (especially DHA and CAT).

Declarations

Acknowledgment

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Conflict of interest

The authors declare that there is no conflict of interest

References

https://doi.org/10.1126/science.aam6328
41. Olsen SR (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture


43. Paul NM, Harikumar VS (2022) Pyrolytic transformation of indigenous biomass wastes into biochar: an insight into char structure and physicochemical characteristics


46. Qi M (2017) Sunflower stalk pith fibre: Investigation on oil holding capacity, oil-fibre interaction, and related application in food


Figures
Figure 1

*Water holding capacity of biochar (B) and sulfur-modified biochar (BS)*
Figure 2

*Field experiment*
Figure 3

FT-IR spectra of biochar (B) and sulfur-modified biochar (BS)
Figure 4

Soil water content. B: 15 t ha$^{-1}$ biochar; BS: 15 t ha$^{-1}$ sulfur-modified biochar.
Figure 5

Soil microbial and enzymatic activities. B: 15 t ha\(^{-1}\) biochar; BS: 15 t ha\(^{-1}\) sulfur-modified biochar. CAT: catalase activity, ALP: alkaline phosphatase activity, UA: urease activity, DHA: dehydrogenase activity, MBC: microbial biomass carbon, BR: basal respiration. Different letters show significant differences among treatments at p<0.05. Error bars indicate standard deviation (n=3).

Figure 6

Figure 7


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