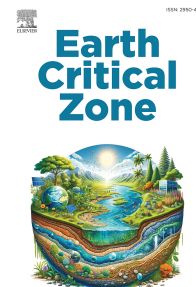


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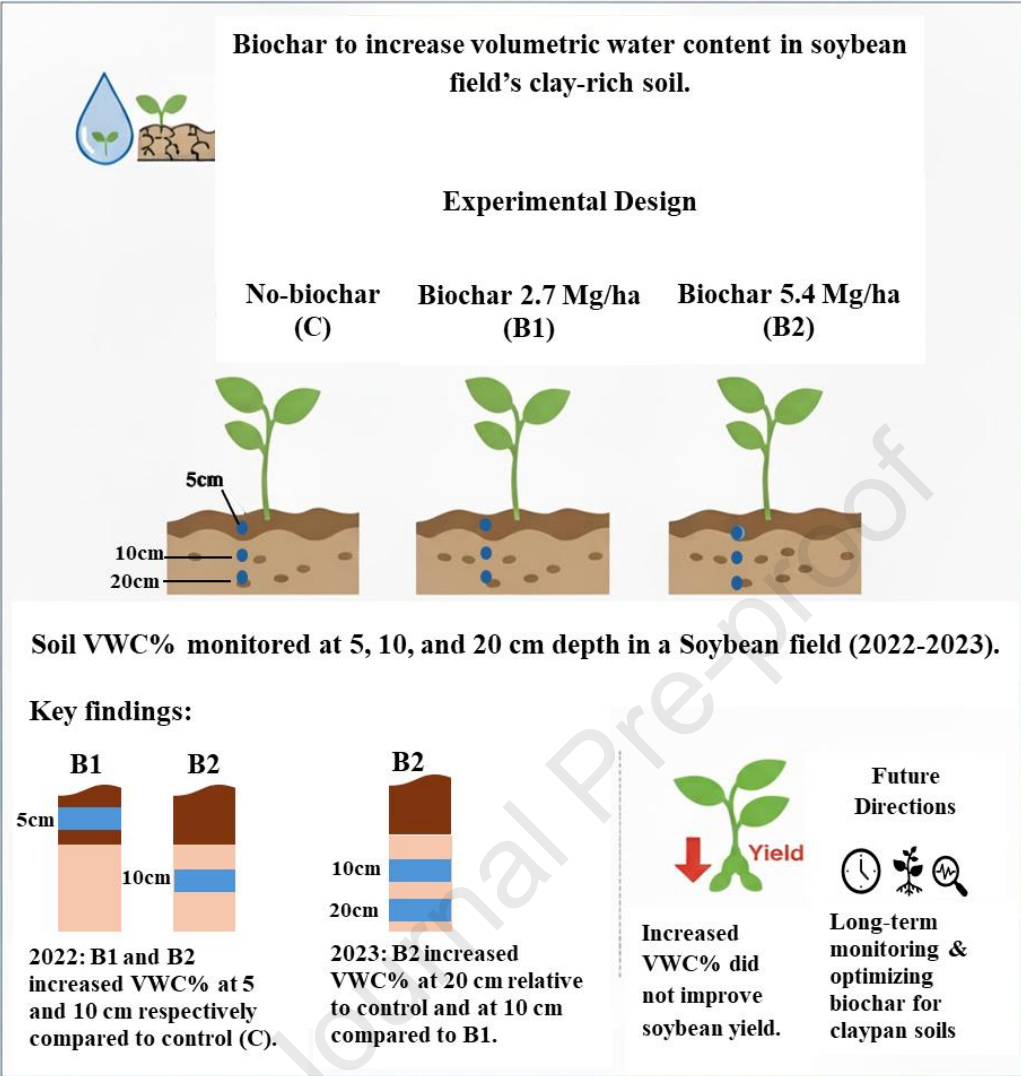
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# 1 Biochar Effects on Soil Moisture and Soybean Yield in Claypan Soils

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## 15 Abstract

16 The role of biochar application in water storage and utilization in the soil has the potential to  
17 influence crop productivity and hydrological processes. These properties contribute to enhancing  
18 the resilience of agricultural systems against drought and have been the focus of numerous  
19 studies. However, biochar application is not well studied in claypan soils in the Midwestern  
20 United States. This research compared the effect of biochar application, at rates of 2.7 Mg ha<sup>-1</sup>  
21 (B1) and 5.4 Mg ha<sup>-1</sup> (B2), relative to a no biochar control (C) on soil moisture content in a  
22 soybean [*Glycine max* (L.) Merr.] field in central Missouri, USA. Soil volumetric moisture  
23 content (VWC%) was monitored at depths of 5, 10, and 20 cm using water reflectometer sensors  
24 from June 2022 to December 2023 (57 weeks), at three replicate locations in a completely  
25 randomized plot design. The results indicated that in 2022, B1 significantly ( $p < 0.02$ ) increased  
26 VWC% at 5 cm depth compared to both C and B2. Throughout both years, B2 treatments

27 consistently increased ( $p < 0.001$ ) soil moisture at 10 and 20 cm depths relative to control.  
28 However, these increases in surface moisture did not translate into improved soybean yield.  
29 These findings suggest that while biochar can modify soil moisture dynamics in claypan soils,  
30 increased VWC alone may not directly translate to yield benefits under current application rates  
31 and conditions. Future studies should incorporate additional soil and plant indicators beyond  
32 VWC and consider long-term monitoring and focus on optimizing biochar use in claypan soils to  
33 enhance agronomic performance.

34 Keywords: land management, soil water, soil porosity, soil amendment

### 35 1. Introduction

36 Soil hydraulic properties play a central role in sustaining agricultural productivity and ecosystem  
37 functions. Soil moisture content can be significantly influenced by both agronomic activities  
38 (e.g., rotation, tillage, fertilizer, and amendments) and natural factors (e.g. rainfall, which affect  
39 water infiltration, retention, and evaporation) ultimately shaping soil health and agricultural  
40 productivity [1-5]. The role of biochar application on various soil physicochemical properties  
41 such as soil carbon sequestration and hydraulic properties has been explored by many  
42 agricultural studies [6-10]. Biochar's highly porous structure and low bulk density directly  
43 increase the total pore volume of the soil matrix, consequently reducing soil bulk density. This  
44 modification to the soil's physical structure is the primary mechanism driving improvements in  
45 soil hydraulic properties [11].

46 Current research on how biochar influences soil hydraulic properties shows considerable  
47 variability, mirroring the mixed results observed in field studies worldwide [1,10]. Such  
48 inconsistency is understandable given the inherently complex nature of biochar–soil interactions,

49 shaped by multiple environmental and material parameters [10,12-14]. Tokova et al. (2023) [12]  
50 reported that biochar application showed a positive trend in improving the hydraulic and physical  
51 properties of silty loam soil. In contrast, Jeffery et al. (2015) [13] found that biochar application  
52 did not enhance soil hydraulic function in sandy soils. In a ten-year study, Pranagal and Kraska  
53 (2020) [14] reported that biochar additions (10-30 Mg ha<sup>-1</sup>) on loamy sand soil had no  
54 meaningful effect on soil texture. Conversely, Layek et al. (2023) [15] documented the long-term  
55 persistence of biochar's benefits, reporting that a single, moderate application (5-25 Mg ha<sup>-1</sup>) on  
56 silt loam soil maintained and enhanced the soil's physical and hydraulic characteristics over a  
57 six-year period. This inconsistency, driven by the complex interaction of biochar type and soil  
58 texture, means that the physical outcome on soil quality improvement remains unpredictable,  
59 making it difficult to issue reliable recommendations for its widespread adoption in global  
60 agriculture [1,7]. To overcome this unpredictability and issue reliable recommendations, further  
61 investigation is crucially required across diverse soil types, especially within the challenging  
62 clay-rich and claypan soils which have not been sufficiently studied.

63         There are few studies that investigated the effect of biochar on soil moisture content in  
64 clay-rich soil. To identify studies examining the impact of biochar application on soil moisture  
65 content, we searched the Web of Science database using the keywords 'biochar', 'soil',  
66 'moisture', and 'agriculture', and further refined the results with the term 'clay'. This search  
67 returned 50 relevant articles, of which only 19 specifically addressed biochar's effect on soil  
68 moisture in clayey soils, and only 2 research [16,17] conducted on soybean [*Glycine max* (L.)  
69 Merr.] fields (similar to our field study) indicating that relatively few studies have specifically  
70 examined the effects of biochar on soil moisture content in clay-rich or claypan soils. Mannan et  
71 al. (2021) [16] reported that despite increased biomass production, the application of biochar to

72 clayey soil did not enhance soybean water uptake. However, Jahan et al. (2020) [17] reported  
73 that biochar application to clayey soil planted with soybean led to an increase in soil water  
74 content. This contrast highlights a gap in literature and underscores the need for more focused  
75 research in this area.

76         The claypan soils in north-central and northeastern Missouri feature a restrictive high-  
77 clay subsoil layer known as the "claypan". These soils are composed of weathered loess over  
78 glacial till and are characterized by poor to very poor drainage, slow to very slow permeability,  
79 and a tendency to result in surface runoff [18]. Sustainable agricultural practices, and monitoring  
80 soil moisture are methods farmers in this region can use to accurately regulate soil moisture and  
81 soil water availability to plants while reducing the risk of runoff and nutrient loss. Biochar is  
82 known to modify soil pore architecture, which can improve infiltration and water storage  
83 capacity, while simultaneously introducing micropores that retain water more tightly. However,  
84 in restrictive claypan soils, where drainage is inherently limited, these contrasting effects may  
85 lead to increased total water retention without proportionally improving plant-available water.  
86 Instead, moisture may be held for longer periods near saturation, potentially altering hydrological  
87 behavior in ways that differ from coarser soils. This uncertainty highlights the need for field-  
88 based research that evaluates how biochar influences water dynamics specifically within claypan  
89 systems.

90         Soil volumetric water content (VWC) measurements inherently capture the integrated  
91 effect of biochar interacting with local climate inputs (precipitation and temperature), soil  
92 physicochemical properties (e.g., soil porosity, bulk density and hydraulic conductivity), and  
93 plant root system [19,20]. Effective management of water, including infiltration, retention, and  
94 plant-available water is arguably the most critical function of a healthy soil ecosystem affecting

95 soil productivity [1,10]. Therefore, the evaluation of soil hydraulic properties, such as VWC as  
96 affected by biochar application, can be considered a primary index of soil health and productivity  
97 in water-limited or flood-prone environments. While this study does not provide a  
98 comprehensive analysis of all soil health parameters and characterization before and after  
99 biochar application, it focuses robustly on soil water content (VWC%) and productivity (yield).  
100 This dual focus allows VWC to serve as the ultimate integrated indicator that reflects the  
101 cumulative outcome of complex hydrological dynamics and soil-environment interactions,  
102 providing essential functional assessment.

103         The primary objective of this two-year field study was to investigate the influence of  
104 fresh biochar application on the hydrological dynamics and agronomic productivity of restrictive  
105 claypan soils in central Missouri, USA. Specifically, the study aimed to 1) evaluate the effect of  
106 two contrasting biochar application rates (2.7 and 5.4 Mg ha<sup>-1</sup>) on soil volumetric water content  
107 (VWC%) across various soil depths (5 cm, 10 cm, and 20 cm) over two growing seasons, 2)  
108 determine if any observed hydrological benefits from biochar application translate into improved  
109 final soybean yield compared to an unamended control.

## 110         2. Materials and Methods

### 111         2.1 Experimental site

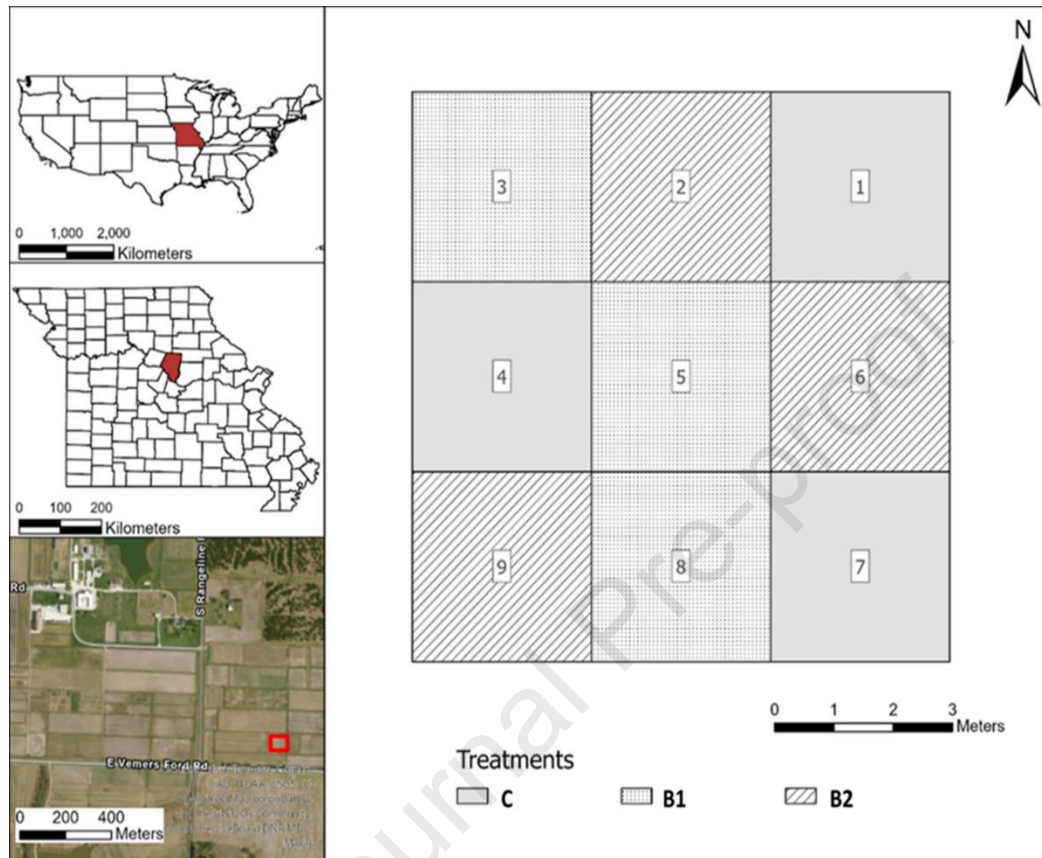
112         The study was conducted at the University of Missouri's Bradford Research Farm, Boone  
113 County, Missouri (38°53'39.3"N 92°12'20.9"W). The average annual precipitation in the last 5  
114 years was 95 cm with the average annual mean air temperature is approximately 12.5°C. The  
115 claypan soils are Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualfs) generated from  
116 glacial till and loess. The area used for this study has been used in agricultural research for

117 several decades, primarily in corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], and Wheat  
118 (*Triticum aestivum* L.). The site was planted to soybean in 2022 and 2023 operating as a rainfed  
119 agricultural system with no supplemental irrigation. Biochar (Table S1) was applied by hand at  
120 two rates, 2.7 Mg ha<sup>-1</sup> and 5.4 Mg ha<sup>-1</sup>, one day following tillage and incorporated within the 0-5  
121 cm surface layer.

122         The application of biochar at 0-5 cm depth is critical not only for infiltration but also for  
123 water redistribution among horizons. Biochar's high microporosity and large surface area  
124 primarily increase capillary water retention. When biochar is concentrated at the surface (0-5  
125 cm), it enhances the continuity of fine capillary pores. This structural improvement in the  
126 surface aggregates maintains pore continuity crucial for efficient downward capillary flow within  
127 deeper soil profile [21]. In addition, the change in the pore size distribution in the soil surface  
128 layer (0-5 cm) can effectively increase clay soil hydraulic conductivity which facilitates efficient  
129 water redistribution among horizons (5-20 cm) and minimizes surface runoff [1,21,22].

130         The rates were deliberately selected to represent the low-to-moderate, cost-effective  
131 doses that maximize the potential for successful economic translation to agricultural practice.  
132 Several studies have utilized similar biochar rates (2.5, 5.0 Mg ha<sup>-1</sup>) and documented a  
133 significant improvement in VWC%. Specifically, research by Krapfl et al. (2014), Reyes-Cabrera  
134 et al. (2020), and Layek et al. (2023) confirmed substantial increases in soil moisture content and  
135 hydraulic properties across diverse soil textures, including clayey, sandy, and silt loam soils, with  
136 applications ranging from 5-25 Mg ha<sup>-1</sup> [8,9,15]. The experiment was designed in a 9 m × 9 m  
137 area, plots measuring 3 m x 3m, using a completely randomized design (CRD) with three  
138 replications per treatment (Fig. 1). The impact of biochar on soil properties was not the focus of

139 this study; therefore, we did not investigate or report soil physicochemical characteristics beyond  
 140 soil moisture.



150  
 151 Figure 1. Study site at University of Missouri's Bradford Research Farm, Missouri, U.S. (a, b,  
 152 and e). Compared with control (C), two biochar rates, 2.7 Mg ha<sup>-1</sup> (B1) and 5.4 Mg ha<sup>-1</sup> (B2),  
 153 were applied one day after tillage, following a completely randomized design with three  
 154 replications per treatment. Numbers inside the treatment boxes are plot numbers representing  
 155 replications.

## 156 2.2 Soil moisture content measurement

157 Water Scout SM100 soil moisture sensors from Spectrum Technologies (Aurora, IL) were  
 158 installed at depths of 5, 10, 20, and 40 cm to monitor soil moisture levels. For each plot,  
 159 measurements were taken at four distinct locations. Soil moisture content, represented as percent  
 160 volumetric water content (VWC%), was recorded at 15-minute intervals throughout the study

161 period. The sensors are equipped with a 2.5mm stereo pin connector and a measuring range of  
162 0% VWC to saturation. The resolution and accuracy of the sensors are 0.1% VWC and 3%VWC  
163 (at  $EC < 8 \text{ ms cm}^{-1}$  and  $0.5\text{--}80 \text{ }^\circ\text{C}$  temperature) (Spectrum technologies, Inc., 2019). Data were  
164 downloaded monthly either via a 2000 series Watchdog Data Shuttle (Spectrum Technologies,  
165 Aurora, IL) or directly to a laptop using Spectrum Technologies' SpecWare 9 Pro software.  
166 Throughout the study period, a total of 27 soil moisture sensors (3 treatment  $\times$  3 replicates  $\times$  3  
167 depths) were monitored. The average soil moisture content for each week was calculated using  
168 the 15-minute interval data and plotted for the entire study duration. Soil moisture sensors were  
169 removed during field management and planting activities. Local climate and weather data were  
170 obtained from the Bradford Research and Extension Center located in Columbia, Boone County,  
171 Missouri.

### 172 2.3 Yield Data

173 Yield data were collected using a plot harvester (ALMACO, Nevada, IA, USA) equipped with  
174 the VantageHD data collection system (Version 1.3.2.2) and the Seed Spector LRX (Version  
175 3.06C.23, E201009). The VantageHD software was used to identify individual plots via GPS and  
176 control harvester functions, while the Seed Spector LRX system recorded real-time grain weight  
177 and moisture content for each plot. Data was automatically logged, linked to plot identifiers, and  
178 later exported for analysis.

### 179 2.4 Statistical Analysis

180 Statistical analysis was performed using SAS University Edition (V 9.4) Software and  
181 RStudio. Principal Component Analysis (PCA) was conducted using VWC% data categorized by  
182 treatment and depths. The PCA was utilized as an exploratory data analysis step before relying  
183 solely on the  $p$ -value from the ANOVA test. The PCA visualization (e.g., the separation/overlap

184 of the 95% confidence ellipses) of the VWC data provides a non-parametric visual confirmation  
185 of whether the treatments induced differences in the VWC distribution at that specific depth,  
186 thereby guiding the interpretation of the subsequent statistical tests. Mixed Models (Random  
187 Slope) and General Linear Model (GLM) were used at 95% confidence interval to evaluate the  
188 effects of the treatments on weekly soil moisture values. The fixed effect structure included all  
189 management factors (Treatment, Depth) and environmental factors (Rainfall, Temperature),  
190 along with their highest-order interaction (Treatment  $\times$  Depth). Week was included as a random  
191 intercept. Applying Linear Mixed Model and Type III Analysis of Variance (ANOVA) method  
192 allows us to determine the most effective driver of VWC. Correlation analysis was performed to  
193 analyze the relationship between soil moisture content at selected depths and soybean yield in  
194 growing season. Correlation analyses were conducted between soybean yield ( $n= 3$  per  
195 treatment) and VWC. Yield was measured as a single value for each treatment (C, B1, and B2),  
196 while VWC was measured at three depths (5, 10, and 20 cm) within each treatment. For the  
197 correlation analysis, VWC values corresponding to each treatment ( $n= 9$ ) were paired with the  
198 yield value of the respective treatment to assess treatment-level associations. Treatments were  
199 considered significant at  $p$ - value  $< 0.05$ .

### 200 3. Results and Discussion

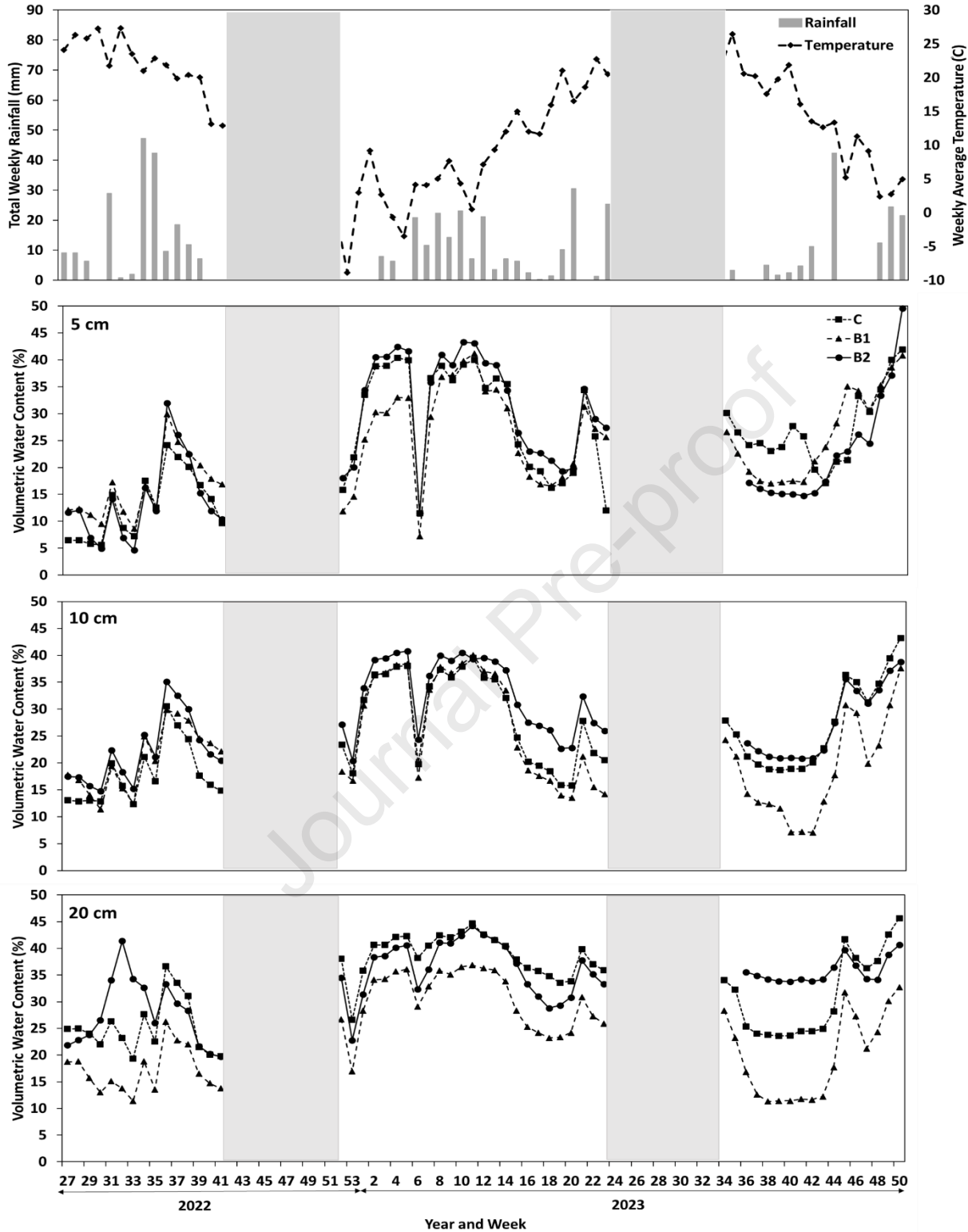
#### 201 3.1 Soil Moisture Dynamics

202 Temporal patterns of volumetric water content (VWC%) across 5 cm, 10 cm, and 20 cm  
203 soil depths (Fig. 2) reflected seasonal fluctuations in rainfall and temperature throughout the  
204 2022–2023 period. Volumetric water content increased following rainfall events and declined  
205 during dry periods, with more noticeable fluctuations observed at the surface (5 cm) compared to  
206 deeper layers. Peak VWC% was observed in week 36 (early September) of 2022, and again

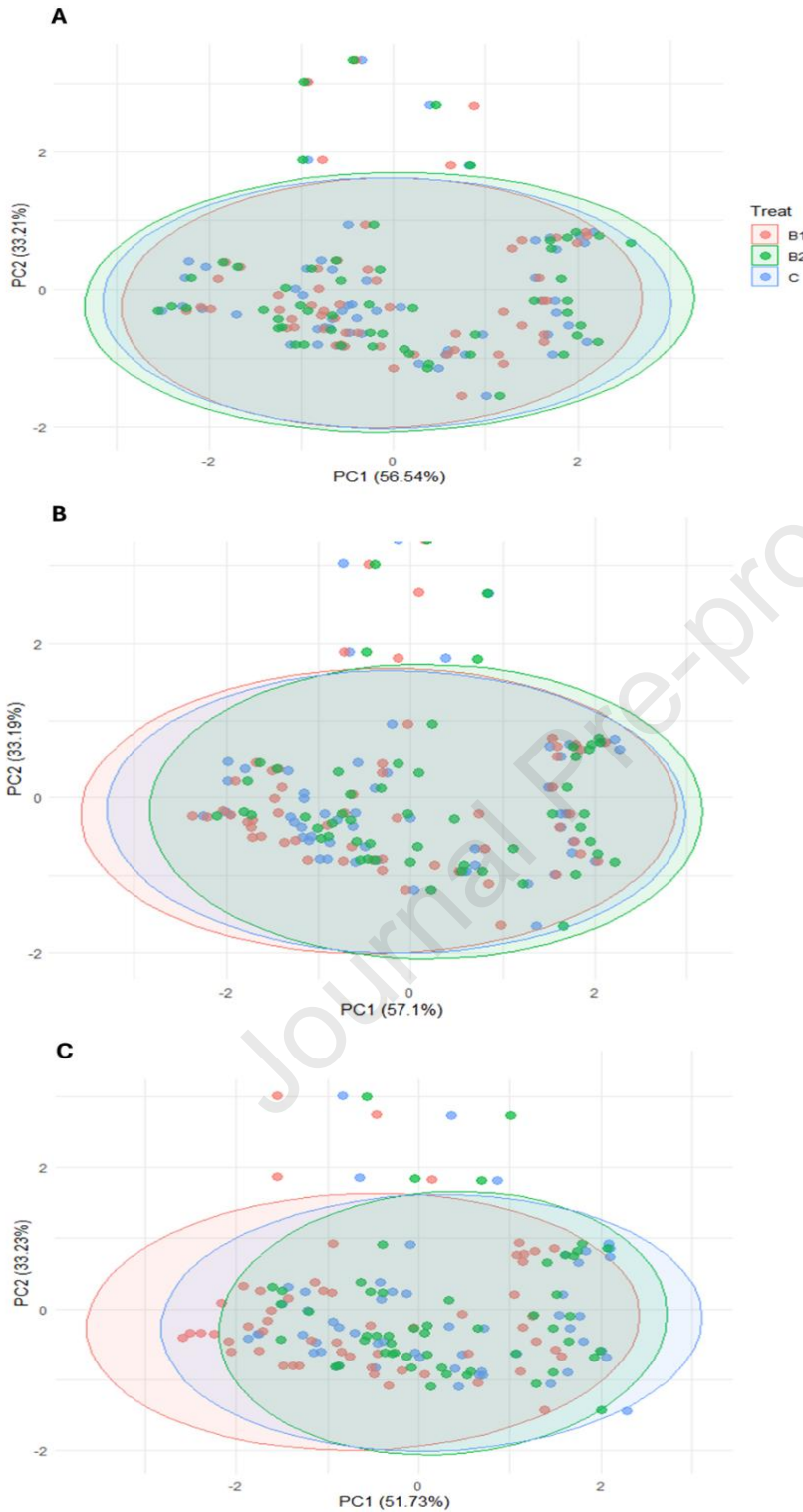
207 during weeks 10 to 11 (March 2023), as well as in week 50 of 2023, aligning with periods of  
208 moderate temperatures and rainfall (Fig. 2).

209 Principal Component Analysis (PCA) was performed at each depth to visualize the  
210 separation of treatments. At shallow (5 cm) and intermediate (10 cm) depths, all three treatment  
211 ellipses showed heavy overlap (Fig. 3). This pronounced overlap indicates that the management  
212 practices failed to create robustly distinct soil moisture regimes in the shallow profile, explicitly  
213 suggesting a limitation in the efficacy of the biochar treatments to significantly separate  
214 hydrological conditions at these depths. In contrast, the PCA for the 20 cm depth showed clear  
215 and significant separation among treatments (especially B2 vs. B1). This separation in the deeper  
216 layer was mainly influenced by VWC%, suggesting that the management practices effectively  
217 resulted in differing levels of VWC% retention in the deepest layer.

218 The linear mixed model results substantiate these visual findings by quantifying the  
219 unique variance explained by each factor (Table 1 and Fig. 4). The effects of Depth, Treatment,  
220 and their interaction were all highly significant ( $p \leq 0.0004$ ), demonstrating that these factors  
221 were the primary and strongest drivers explaining the variability in soil moisture content  
222 (VWC%). After accounting for week-to-week variability (as a random factor), the most  
223 pronounced and significant increases in VWC% are confined to the deeper depth layers (10-20  
224 cm) when combined with treatments B2 and C. The B2 treatment at Depth 20 cm results in a  
225 VWC% higher ( $p < 0.0001$ ) than control at the soil surface (5 cm depth), after accounting for all  
226 other factors. This had the strongest positive effect on the entire model. Also, B2 treatment at  
227 Depth 10 cm significantly increased ( $p < 0.0001$ ) VWC% compared to the control at soil surface.  
228 Control treatment at Depth 20 cm showed a higher VWC% than at the soil surface ( $p < 0.0001$ ).



229 Figure 2. Precipitation, temperature, and volumetric water content (VWC%) for three treatments  
 230 corresponding to two biochar treatments, 2.7 Mg ha<sup>-1</sup> (B1), and 5.4 Mg ha<sup>-1</sup> (B2), and a control  
 231 (C) plot at 5, 10, and 20 cm soil depth during the study period. Soil moisture measurements did  
 232 not perform for the shaded areas due to field management and planting activities.



233

234 Figure 3. Principal Component Analysis (PCA) of soil moisture based on mean weekly  
235 measurements. The plots illustrate the variance in moisture content across two biochar  
236 treatments, 2.7 Mg ha<sup>-1</sup> (B1) and 5.4 Mg ha<sup>-1</sup> (B2), and a control (C) plot. Panels represent data

237 at three depth intervals: (A) 5 cm, (B) 10 cm, and (C) 20 cm. The analytical basis for the PCA  
238 consists of mean weekly soil moisture values recorded throughout the experimental period.

239 In contrast, at the soil surface (5 cm depth), none of the treatments had a significant impact on  
240 VWC%. These results demonstrate that biochar application, particularly at higher rates, can  
241 significantly improve soil moisture retention in clay-rich soils, specifically at 10 to 20 cm depths  
242 [19,20]. This benefit is most evident during dry periods and at 10 to 20 cm soil depths, where  
243 water availability is crucial for plant growth [23].

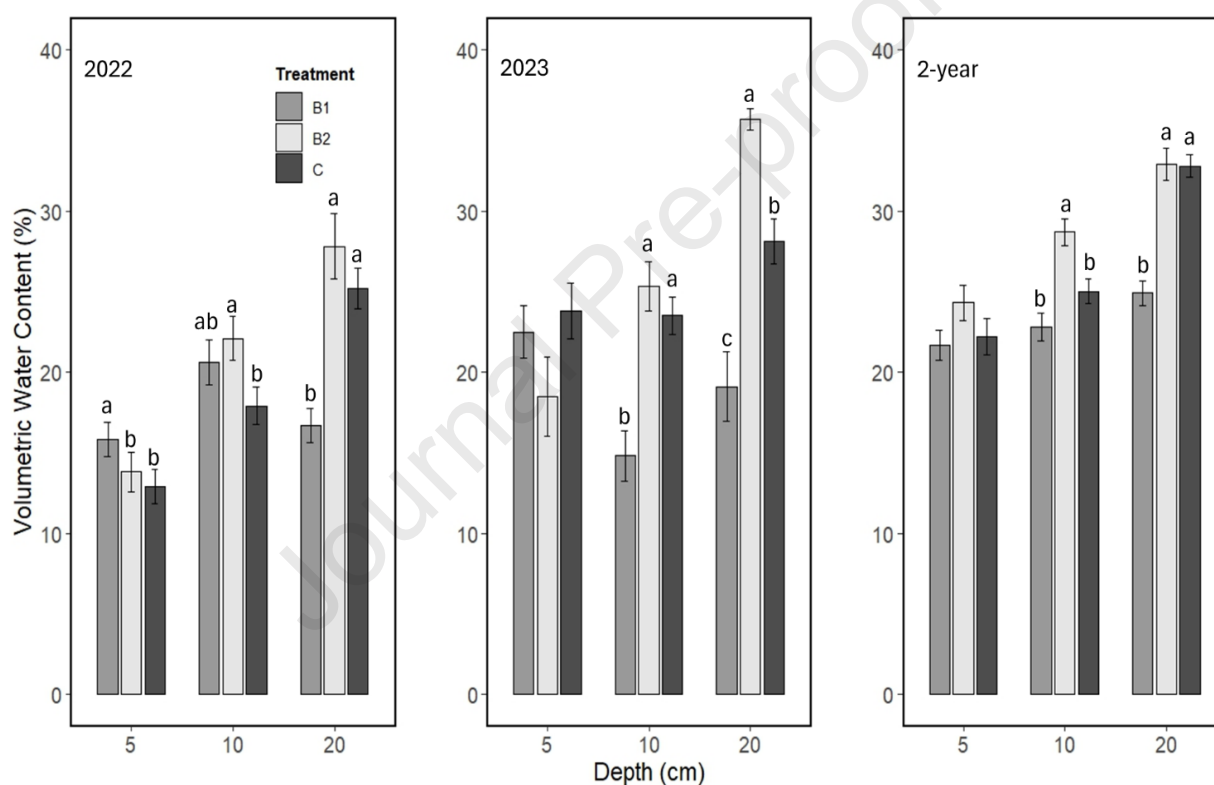
244 In 2022 and 2023, the higher biochar rate (B2) consistently and significantly maintaining  
245 higher VWC% values at 10 and 20 cm depths relative to both the lower biochar rate (B1) and  
246 control (C) (Fig. 4). In 2022, After accounting for variability in treatment effects across weeks  
247 (via random slopes analysis) at the 5cm depth, B1 consistently performs best, with significantly  
248 higher VWC% than Control ( $p = 0.0004$ ) and B2 ( $p = 0.01$ ). This is aligned with findings that  
249 application rate and soil depth influence the extent of moisture retention [24]. The enhanced  
250 moisture retention likely results from the high porosity and surface area of biochar, which  
251 improves soil structure, increases pore space, and enhances capillary water retention [10]. Such  
252 improvements in soil water dynamics have direct implications for sustainable agricultural  
253 management, especially in systems vulnerable to water scarcity [11,17]. Incorporating biochar  
254 into soil management practices may enhance drought resilience, reduce irrigation needs, and  
255 contribute to more stable crop productivity in variable climates [9,25]. At 5 cm depth, VWC%  
256 for B2 treatment is greater (not significantly) than the one of the control plots ( $p = 0.2046$ ) in  
257 2022 (Fig. 4). There is no significant effect of treatments on soil VWC% in 2023 at 5cm depth  
258 (Fig. 4). Between weeks 32 and 33 in 2022 and weeks 17–19, 36–37, and 45–47 in 2023, the  
259 higher biochar application rate (B2) demonstrated its ability to enhance soil water retention at a

260 10 cm depth under moisture stress caused by limited rainfall. In particular, the B2 treatment  
 261 preserved greater VWC% during the late summer 2023 dry period (weeks 36–41), especially at  
 262 10 cm and 20 cm depths (Fig. 2 and 4). These results suggest a cumulative or time-dependent  
 263 effect of biochar on soil water retention. The observed soil moisture content patterns reveal  
 264 distinct rate- and depth-dependent responses to biochar application, challenging the assumption  
 265 that all rates provide similar hydrological benefits. The significant differences detected at the 10  
 266 cm and 20 cm depths highlight an important trade-off between the lower (B1) and higher (B2)  
 267 biochar application rates. Two meta-analysis by Edeh et al. (2020) and Jiang et al. (2025)  
 268 demonstrates that soil moisture responses to biochar depend on both soil texture and the  
 269 application rate, as well as the interaction between these factors [10,26]. Improvements in soil  
 270 moisture content appear to depend on achieving an optimal rate where soil texture, biochar  
 271 properties, and rate interact favorably [27]. Biochar amendments are known to modify pore size  
 272 distribution in clay soils, enhancing the dominant pore spaces that store and transmit water [28].  
 273 This is reflected in our findings at the 10 cm depth, where the clay-rich layer exhibited increased  
 274 moisture content under the higher biochar rate (B2), indicating that sufficient application levels  
 275 may be necessary to improve water retention in fine-textured soils.

276 Table 1. Summary of the Mixed-Model Analysis of Variance (ANOVA) assessing the effects of  
 277 biochar treatment (2.7 Mg ha<sup>-1</sup> (B1), and 5.4 Mg ha<sup>-1</sup> (B2), control (C)) and soil depths (5, 10,  
 278 and 20cm) on volumetric water content (VWC%) over the study period.

Treatment*depth	B1 at 5cm	B1 at 10cm	B1 at 20cm	B2 at 5cm	B2 at 10cm	B2 at 20cm	C at 5cm	C at 10cm	C at 20cm
B1 at 5cm		0.99	0.33	0.61	<0.0001	<0.0001	1.00	0.25	<0.0001
B1 at 10cm	0.99		0.73	0.94	<0.0001	<0.0001	1.00	0.63	<0.0001
B1 at 20cm	0.33	0.73		0.99	0.06	<0.0001	0.56	1.00	<0.0001

B2 at 5cm	0.61	0.94	0.99		0.009	<0.0001	0.83	0.99	<0.0001
B2 at 10cm	<0.0001	<0.0001	0.06	0.009		0.03	<0.0001	0.04	0.01
B2 at 20cm	<0.0001	<0.0001	<0.0001	<0.0001	0.03		<0.0001	<0.0001	1.00
C at 5cm	1.00	1.00	0.56	0.83	<0.0001	<0.0001		0.46	<0.0001
C at 10cm	0.25	0.63	1.00	0.99	0.04	<0.0001	0.46		<0.0001
C at 20cm	<0.0001	<0.0001	<0.0001	<0.0001	0.01	1.00	<0.0001	<0.0001	



279

280 Figure 4. Mean volumetric water content (VWC%) corresponding to two biochar treatments, 2.7  
 281  $\text{Mg ha}^{-1}$  (B1), and 5.4  $\text{Mg ha}^{-1}$  (B2), and a control (C) plot in three depth intervals (5, 10, and 20  
 282 cm) for 2022 (left panel) and 2023 (right panel) over the study period. Bars are error bars and  
 283 letters represent significant differences between treatments at  $p < 0.05$ .

284 3.2 Soybean Yield Response to Soil Moisture

285           The relationship between soil moisture (VWC%) and soybean yield was explored across  
286 treatments and soil depths. It is important to note that the correlation analysis between VWC%  
287 and yield was conducted with limited replication of yield per treatment (n=3), and no statistically  
288 significant relationships were observed in any treatment or year (Table 2). Therefore, the  
289 following observations regarding the calculated  $R^2$  values must be interpreted with extreme  
290 caution and are strictly viewed as exploratory, descriptive trends that indicate potential areas for  
291 future investigation, rather than confirmed or robust statistical relationships.

292           A consistent negative correlation was observed between soil moisture and yield for all  
293 treatments in 2022 and 2023 except for C in 2023. This descriptive trend hypothesizes that  
294 increased soil moisture in the second year of the study, roughly between 20-25 (VWC%) in  
295 upper layers could be beneficial for yield in the absence of soil amendments.

296           Despite biochar's observed ability to modify soil water content, our results demonstrated  
297 a clear decoupling between soil water content and crop yield. While the higher application rate of  
298 biochar significantly increased volumetric soil water content (VWC), this hydrological benefit  
299 did not translate into enhanced crop performance. Similar findings were reported by Reyes-  
300 Cabrera et al. (2021) [8] that neither the 2.5 Mg ha<sup>-1</sup> nor the 10 Mg ha<sup>-1</sup> biochar treatment  
301 resulted in benefits or to final yield or any measured aboveground and belowground plant  
302 characteristics compared to the unamended control. This finding suggests that, in this system,  
303 soil water availability was not the primary limiting factor governing plant productivity, or that  
304 the biochar application induced other changes (e.g., nutrient immobilization or altered microbial  
305 function) that offset the gains in water retention.

306 Some studies that investigate post-application nitrogen dynamics primarily highlight  
307 biochar's strong capacity to retain or immobilize N rather than release it, particularly in the early  
308 stages after incorporation [9]. In line with this understanding, our findings demonstrate that  
309 biochar application can reduce the proportion of nitrogen available for plant uptake. This  
310 suggests that, rather than functioning as an immediate N source, biochar may initially act as an N  
311 sink, slowing N release through physical adsorption or microbial immobilization pathways  
312 resulting in lower biomass production.

313 Katterer et al. (2019) [29] reported that biochar benefits often develop gradually, as aging  
314 processes modify and enhance its capacity for water and nutrient retention. Therefore, a key  
315 reason for the limited effects observed in our study may be the relatively short experimental  
316 duration. Over longer periods, this improved retention may result in more pronounced impacts  
317 on soil function and plant productivity. This emphasizes the necessity of conducting long-term  
318 assessments in intensive annual cropping systems to accurately quantify the duration required to  
319 achieve sustained agronomic benefit. For example, a 10-year field study demonstrated the  
320 powerful effect of aged biochar, reporting that a high application rate ( $100 \text{ Mg ha}^{-1}$ ) significantly  
321 increased the water-holding capacity and reduced the bulk density in the root zone (20cm depth)  
322 of a maize–soybean system [29]. This long-term evidence suggests that the positive VWC  
323 modifications observed in B2 treatment in our study may require several growing seasons to  
324 translate into tangible yield improvements.

325 The ultimate agronomic outcome is dependent not only on total soil moisture retention  
326 but also on the temporal alignment of moisture availability with the crop's highest physiological  
327 demand. During the critical R4 (Full Pod) through R6 (Full Seed) stages (week 33-36 in 2023),  
328 when soybean water use is maximal [30], our analysis indicated a mean VWC% of 27% in the

329 Control plots, which was higher than in both biochar treatments (B2=25%, B1=21%). This  
330 specific moisture regime in the control plots may have provided the agronomically optimal  
331 condition during the peak reproductive period, serving as the likely basis for the observed  
332 positive correlation trend between VWC% and yield in the control plots (though statistically  
333 non-significant) in 2023. This demonstrates that, regardless of biochar amendment, optimal root-  
334 zone moisture supports nutrient uptake and biomass production [28,31,32]. Conversely, the  
335 comparatively lower mean VWC% observed across treatments during this critical period is  
336 hypothesized to have limited their yield potential. In 2022, all treatments struggled to maintain  
337 high VWC levels (C=24%, B1=20%, and B2=18%). This pattern suggests that the failure of the  
338 biochar amendments to enhance final yield may be linked to insufficient moisture availability  
339 precisely when the crop's water requirements were highest, potentially offsetting any benefits  
340 gained from earlier hydrological modifications. This aligns with findings by Krapfl et al. (2014)  
341 [9], who found that unamended soils maintaining approximately 30% VWC produced greater  
342 biomass than soils amended with biochar (5–50 Mg ha<sup>-1</sup>), with the highest application rate (50  
343 Mg ha<sup>-1</sup>) resulting in the lowest biomass production. This collective evidence suggests that the  
344 highest attainable moisture level (27% VWC) under the control treatment could have resulted in  
345 the most favorable condition during the critical reproductive phases.

346 The observation that our biochar applications yielded no significant agronomic gain in  
347 soybean is consistent with the high degree of variability and limited short-term response  
348 frequently reported in the literature [33,34]. While biochar is proven to boost some soil  
349 properties such as poor fertility or adverse pH, its effect on final crop yield has been documented  
350 in meta-analysis studies to be negligible compared to the unamended control [35,36]. The  
351 considerable divergence in reported biochar effects, whether beneficial, neutral, or negative

352 underscores how difficult it is to determine the mechanisms driving its behavior in soil systems  
 353 [37,38].

354 Table 2. correlation analysis parameters between soil moisture content (average of all three  
 355 depths per treatment) and soybean yield (average of three replicates per treatment) corresponding  
 356 to two biochar treatments, 2.7 Mg ha<sup>-1</sup> (B1), and 5.4 Mg ha<sup>-1</sup> (B2), and control (C) plot in  
 357 growing seasons in 2022, 2023 and the two-year.

Year	Treatment	R	<i>p</i> -value
2022	B1	-0.91	0.266
	B2	-0.58	0.606
	C	-0.97	0.136
2023	B1	-0.79	0.421
	B2	-0.83	0.380
	C	0.83	0.367
2-year	B1	-0.72	0.105
	B2	-0.66	0.150
	C	0.40	0.430

358 Limitation of the study

359 The primary focus of this study was the direct effect of biochar treatment on soil moisture  
 360 content (VWC%), meaning the analysis of several underlying soil physicochemical properties  
 361 fell outside our scope and therefore, it is important to interpret these findings with caution. While  
 362 our findings established a clear relationship between treatment and VWC%, a more complete  
 363 understanding of the mechanism would be beneficial. Therefore, future studies should look to  
 364 complement these results by including measurements of relevant soil properties like soil bulk  
 365 density and porosity. Since biochar is known to alter these characteristics, quantifying them  
 366 would provide valuable, mechanistic insight into how the product changes water infiltration and  
 367 retention, allowing for a more comprehensive discussion of its overall influence on soil health.

## 368 Conclusions

369 The application of biochar significantly influenced soil hydrology at the study site. The  
370 B2 treatment (5.4 Mg ha<sup>-1</sup>) demonstrated a robust increase in volumetric water content (VWC%)  
371 in the deeper soil profile (10 to 20 cm depths), suggesting structural modification. While the  
372 surface layer (5 cm) remained highly sensitive to rainfall fluctuations, the deeper profile  
373 confirmed a treatment effect. However, the findings clearly highlight a complex and non-linear  
374 relationship between soil moisture and soybean yield that is dependent on growth stage timing.  
375 The hydrological improvement achieved by the B2 amendment did not translate into enhanced  
376 soybean yield across the two-year period, nor did the lower B1 (2.7 Mg ha<sup>-1</sup>) rate provide  
377 agronomic benefits. Analysis of the critical R4 (Full Pod) through R6 (Full Seed) stages revealed  
378 that the unamended control plots maintained the highest mean VWC% (27% in 2023) during this  
379 period of peak water demand. This specific moisture availability in the control provided the most  
380 agronomically favorable condition, which is hypothesized to be the basis for the observed  
381 positive correlation trend (not significant though) between VWC% and yield in that treatment.  
382 This confirms that higher water retention alone is insufficient to guarantee increased yield if that  
383 retention does not align temporally with the crop's highest physiological demand, or if the  
384 retention level is suboptimal for aeration in clay-rich soils during high moisture periods.

385 The primary value of biochar in this context may not be an immediate, short-term yield  
386 increase, but rather its contribution to essential ecosystem services and long-term soil resilience.  
387 Biochar's role in improving soil physical conditions, stabilizing porosity and increasing water  
388 availability is likely most beneficial for mitigating drought risk and enhancing long-term carbon  
389 sequestration and overall soil health. To fully understand the potential of biochar in intensive  
390 agricultural systems, future research must be long-term (beyond two growing seasons) to capture

391 the benefits of aged biochar. Crucially, studies should include comprehensive soil and biochar  
 392 characterization (including bulk density, porosity, pH, nutrient availability, and microbial  
 393 activity) to mechanistically explain the observed VWC patterns and identify the true limiting  
 394 factors governing the yield response, allowing for the strategic use of biochar to match water  
 395 availability with root zone demand.

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- Biochar increased soil water content in clay rich soils.
- Different biochar application rates affect soil VWC% at specific soil depths.
- Higher soil moisture does not result in increased soybean yield.

Journal Pre-proof

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Journal Pre-proof