Journal of Forest and Ecosystem Sciences Vol 1, № 4, 2025

Harnessing Soil Ecology through Biochar and AMF Integration for Improved Growth and Enzymatic Dynamics in *Swiss Chard*

Ghulam Murtaza[1], Muhammad Usman[2], and Rashid Iqbal[3]

[1] School of Agriculture, Yunnan University, Kunming, Yunnan 650504, China;
[2] School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, 200240, China;
[3] Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan;

Received: 03.11.2025 Accepted: 13.11.2025 Published: 25.12.2025

https://doi.org/10.54414/UUXO5275

Abstract

Biochar and arbuscular mycorrhizal fungi (AMF) are increasingly recognized for their potential to improve soil quality and plant productivity. This study evaluated the individual and combined effects of non-wood biochar and AMF on growth performance, root morphology, physiological attributes, and soil enzymatic activity in Swiss chard (*Beta vulgaris* L.) under pot conditions. A randomized block design was employed with four treatments and three replications: A_1 (control, no amendment), A_2 (biochar alone), A_3 (AMF alone), and A_4 (combined AMF and biochar). Results demonstrated that biochar application alone significantly enhanced plant growth parameters, including shoot length, leaf dimensions, leaf number, and biomass accumulation (fresh and dry weight), compared to the control. Improvements were also observed in root morphological traits, physiological performance, and soil enzymatic activities. AMF inoculation further contributed to plant and soil improvements; however, the combined application (A_4) produced the most pronounced effects across all measured variables. The synergistic interaction between AMF and biochar resulted in superior plant growth, enhanced root system architecture, improved physiological efficiency, and elevated soil enzymatic activity and microbial biomass. Overall, the findings highlight the synergistic potential of integrating AMF with biochar as a sustainable soil management strategy to enhance crop productivity and soil biological functioning.

Keywords: Non-wood biochar, AMF, Swiss chard, root morphological, soil enzymatic activities, microbial activity

1. Introduction

Biochar is obtained by pyrolysis of different types of carbonaceous feedstocks. The utilization of biochar is a means of mitigating climate change and decreasing atmospheric carbon dioxide levels, while also enhancing agricultural soil quality (Emenike et al., 2024; Iwuozor et al., 2024; Sieradzka et al., 2022). The use of biochar has been shown to enhance soil microbial activity and improve soil physiochemical traits (H Pangaribuan et al., 2022; Zhao et al., 2022). A study conducted by Khadem et al. (Khadem et al., 2021) found that the application of biochar enhanced soil productivity and health. According to Teodoro et al. (Teodoro et al., 2020), the application of biochar resulted in a substantial enhancement of the specific surface area, cation exchange capacity, and water holding capacity (WHC) of hostile soils. Biochar derived from rice husk substantially enhanced the organic matter content in soil by 50% as compared to the control group (Ayaz et al., 2022). According to several studies, biochar has been shown to enhance the functions of esterase, lipase-esterase, phosphohydrolase, trypsin, protease, alkaline phosphomonoesterase, phosphomonoesterase acid, phosphatase acid, alkaline phosphatase, dehydrogenase, and chymotrypsin enzymes when used alone (Antonia Sindesi et al., 2022). Biochar facilitates the enrichment of soil with essential nutrients like total carbon, sodium, magnesium, calcium, potassium, and nitrogen (Verma &

Reddy, 2020). Egamberdieva et al. (Egamberdieva et al., 2019) found that the incorporation of biochar into soil substantially enhanced the potassium, phosphorus, and nitrogen levels.

Biochar could be shown to improve productivity and nutritional composition of different plant species (Jatuwong, et al., 2024). Previous studies have demonstrated that the use of biochar resulted in enhanced germination of seeds and growth of plants and soybean production (Arshad et al., 2023). After biochar was added, the rate of germination was much higher than in the control group (Osei et al., 2023).

The levels of magnesium and calcium in maize (*Zea mays*) leaves were markedly greater when a high rate of biochar was applied compared to the control group (Gunes et al., 2023). The use of biochar enhanced the biomass of both the roots and shoots of buckhorn (Nirukshan et al., 2022). The application of biochar derived from rice straw showed a substantial enhancement in plant height, per plant bolls, average boll weight, and cotton seed output compared to the control group (Huang et al., 2024). According to Niu et al. (Niu et al., 2024), the application of biochar obtained from rice husk resulted in an increase in the final biomass, plant height, root biomass, and leaf numbers of cabbage and lettuce compared to plants grown without biochar. Biochar exerts a beneficial influence on the biochemical and physiological characteristics of plants. Multiple studies have demonstrated that the use of biochar enhanced transpiration rate, plant photosynthesis, and levels of chlorophyll (Lalay et al., 2024).

AMF are a prominent contributor to the rhizosphere microflora in ecological systems and have a crucial function in nutrient cycling within these ecosystems (Ishaq et al., 2023). Mycorrhiza is a symbiotic microorganisms that stimulate root development and significantly contribute to improving plant nutrition (Khaliq et al., 2022). AMF has a beneficial relationship with crops (Huang et al., 2024). The introduction of mycorrhiza into plant roots enhances the absorption of essential nutrients, including Mg, Ca, P, K, and N (Bhattacharyya & Furtak, 2022). Inoculated plants with mycorrhiza exhibited elevated levels of carotenoids and chlorophyll, as well as enhanced levels of antioxidant enzymes, including ascorbate peroxidase, peroxidase, catalase, dismutase, and superoxide (Nazari et al., 2023). The application of mycorrhiza enhanced the root system of plants and facilitated the growth and production of numerous field crops (Bhale et al., 2018). Previous studies have indicated that the use of both PGPR (Plant Growth-Promoting Rhizobacteria) and mycorrhiza through inoculation may have a positive impact on cultivation (Uwamungu et al., 2022). Mycorrhiza and biochar have demonstrated their efficacy in improving plant productivity while mitigating the severity of diseases. Singh et al. (Singh et al., 2022) found that inoculating maize with both biochar and AMF simultaneously greatly enhanced the growth of plants and phosphorus level.

Swiss chard is rich in essential components that have positive effects on human health, such as bioactive chemicals, minerals, and vitamins. The substance is abundant in bioactive chemicals that possess anticancer, hypolipidemic, hypoglycemic, and antiobesity traits (Kaparapu et al., 2020; Ivanović et al., 2019). The cultivation of chard is strongly influenced by both biotic and abiotic stress factors. Furthermore, the concurrent use of AMF and biochar is beneficial in mitigating the adverse effects of biotic stress, irrespective of abiotic stress (Tan et al., 2017). However, there is no data on the combined impact of AMF and non-wood biochar on chard. Non-wood biochar can be attractive when agricultural waste and side streams are used as raw materials. The objective of this work was to study the effect of simultaneously applying AMF and biochar to the soil of growing Swiss chard, compared with Jabborova et al. (Jabborova et al., 2020). The authors postulated that the concurrent use of AMF and non-wood biochar will cause positive impacts on soil characteristics and plant nutrients and thereby on the growth of plants and their physiological characteristics.

2. Materials and Methods

2.1. Soil and biochar description

The experiments were conducted using soil from the Agricultural Research field of Islamia University Bahawalpur, and the soil properties were noticed (EC: 0.33 μS/cm, pH: 7.66, SOC(soil organic carbon): 8.32 g/kg, CEC(cation exchange capacity): 19.11 cmol kg^{-1}, Available N: 54.19 mg/kg, Available P: 0.47 mg/kg, Available K: 80.1 mg/kg, Total N: 0.69 g/kg, Total P: 0.41 g/kg, Total K: 20.2 g/kg, Texture; silty loam). The biochar considered in this work was synthesized at 450°C using non-wood feedstock (corncobs). The biochar had a particle size smaller than 2 mm, and the following properties: pH: 8.7, VC: 22%, BET-surface area: 123 m^2g^{-1}; C: 44%, H: 2.80%, N: 2.69%, O: 12.46%, bulk density; 0.349 g/cm^3; CEC: 37.88 cmol kg^{-1}, EC: 5470 µS/cm, ash content: 1.3%, VM (volatile matter): 33.8%). Swiss chard seeds were obtained from Yunnan Zhuoyu Seeds Industry, China. AMF was obtained from the Microbiology research center at the same institute.

2.2. Experimental design

The experiments were planned as pot tests conducted in a shade house in a randomized block design (4 treatments, 3 replications). These treatments comprised a control A_1 (no biochar/AMF added into the soil), A_2 (only addition of biochar), A_3 (only addition of AMF), and A_4 (biochar combined with AMF). Permissions or licenses were obtained to collect Swiss chard seeds from the Regional Agricultural Research Institute (RARI) before starting the research. The seeds were planted in plastic containers with a depth of 15 cm and 15 cm in diameter, holding a soil mass of 4.5 kg. Every pot received irrigation every 3 days. At harvest, after 35-days, the length of the shoot, leaf width, number of leaves, fresh weight of shoot and root, dry weight of root and shoot were all measured, compared to Jatuwong et al (Jatuwong et al., 2024). Every pot received irrigation every 3 days. After that 35-day period, physiological data, including RWC (relative water content), transpiration rate, net photosynthetic rate, and photosynthetic pigment contents, were also measured.

2.3. Analysis of Swiss chard root morphological characteristics

After harvesting, the root system was meticulously rinsed with water. The complete root system was dissected and examined with an imaging system against a blue background. Analysis of digital photographs of the roots was conducted using the programme Win RHIZO (Regent Instruments Inc., Canada). Evaluated were the root length, total root surface area, root diameter, projected area, and root volume.

2.4. Quantification of physiological characteristics

Relative water content was determined using the technique described by Boussora et al. (Boussora et al., 2024). A quantity of 100 mg of fresh leaf biomass was promptly transferred into petri plates containing deionized water and incubated at ambient temperature for four hours as described by Jatuwong et al. (Jatuwong et al., 2024). Next, the samples were pulled from the soil, washed, and dried by blotting, and the weight was quantified. The samples were stored in a 75°C oven for >10h, and then the mass of the dry sample was measured, as described in Jabborova et al. (Jabborova, et al., 2021). Quantification of RWC was performed as:

$$RWC\,\% = [(FW - DW)/(TW - DW)] \times 100 \quad (1)$$

Here, FW (fresh leaf sample), DW (dry weight), and TW (turgid weight) are used.

The photosynthetic pigment contents were quantified using the modified approach by Hashemi et al. (Victoria et al., 2023). Fresh leaves were gathered in the early hours of the day. 50 mg of finely chopped fresh leaf samples were placed into test vials filled with 5 ml of dimethyl sulfate (DMS, CAS no. 77-78-1). Incubation was done at 37 °C for four hours in the absence of light and then prolonged until tissue rendered entirely devoid of colour was obtained. The extract's absorbance was measured at wavelengths of 470, 645, and 663 nm with a spectrophotometer, with a dimethyl sulfate blank as a calibrating reference; compare the procedure by Shahraki et al. (Shahraki et al., 2024). The concentrations of chlorophyll a, chlorophyll b, carotenoid, and total chlorophyll were calculated with the following formulas provided by Soman and Shetty (Soman & Shetty, 2018).

$$\text{Chlorophyll a (mg/g)} = [12.7(A663) - 2.69(A645)] \times V/W \quad (2)$$

$$\text{Chlorophyll b (mg/g)} = [22.9(A645) - 4.68(A663)] \times V/W \quad (3)$$

$$\text{Total chlorophyll (mg/g)} = [20.2(A645) + 8.02(A663)] \times V/W \quad (4)$$

$$\text{Carotenoid (mg/g)} = [(1000 \times A470) - (3.27 \times \text{Chlorophyll a} + 104 \times \text{Chlorophyll b})] \times V/W \quad (5)$$

Where A represents optical density, V represents the volume of dimethyl sulfate (in mL), and W represents the sample weight.

The net photosynthetic rate and the transpiration rate were quantified with a portable analytical instrument between 10:00 a.m. and 11:30 a.m. The measurement was conducted using the completely elongated youngest leaf. The measured value for temperature was 30°C, the concentration of CO_2 was 400 ppm, and photosynthetic active radiation (PAR) was 300 mmol m^{-2} s^{-1}.

2.4. Analysis of AMF spores

The spores of AMF were collected from soil samples weighing 10 grams employing a wet sieve and decanting technique. The sample of soil was passed through a sequence of soil sieves organized in a hierarchy of decreasing sieve diameters. The sterile spores were sifted through a mesh sieve and gently rinsed with deionized water multiple times before being put into a clean petri dish filled with water. The spores of AMF were enumerated using a stereomicroscope (Yusif et al., 2018).

2.5. Evaluation of soil microbial biomass

The biomass carbon measurement techniques were derived from those outlined by Tackenberg (Tackenberg, 2007). Three out of six 17.5-gram duplicates of each soil sample were subjected to 24-hour fumigation with finely purified chloroform. Following the elimination of chloroform, the carbon was obtained from both fumigated and unfumigated samples by subjecting them to a 0.5 M potassium sulphate solution for 1 hour under shaking. Sequential filtration of unfumigated and fumigated samples was performed using filter paper (Whatman no. 42). The supernatant was quantified at a wavelength of 280 nm via a spectrophotometer.

2.6. Soil Enzymes analysis

The activities of alkaline phosphatase were measured using the technique reported by Sugawara et al. (Sugawara et al., 2002). For each soil sample, 1 gram of the soil sample was tested in duplicate. A single set was employed as a control. Next, 0.2 mL of $C_6H_5CH_3$ (toluene) and 4 mL of buffer solution with a pH of 11 were applied. Additionally, 1 mL of $C_6H_6NO_6P$ (p-nitrophenyl phosphate) solution was introduced to the second set of samples, compared to Jabborova et al. (Jabborova et al., 2021). After agitating both flasks briefly to ensure thorough mixing of the contents, they were then positioned in an incubator set at 37 °C for around one hour. Then, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were administered. Flasks were agitated briefly, and then 1 mL of a solution of $C_6H_6NO_6P$ was introduced to the remaining assemblage of samples. Rapid filtration of all suspensions via Whatman No. 1 filter paper was followed by measuring the absorbance at 440 nm.

The hydrolytic activity of fluorescein diacetate (FDA) was measured using the technique described by Adam and Duncan (Baloch et al., 2024). A quantity of 0.5 grams of soil was introduced into 25 ml of Na_3PO_4 (pH = 7.6; 0.06 mM). A 0.25 mL volume of a 4.9 mM fluorescein diacetate substrate solution was incorporated into each test vial. Each vial was vigorously agitated and then placed in a water bath set at 37 °C for 5 hours. The soil suspension was then centrifuged at 8000 rpm for 5 minutes. The spectrophotometer was used to probe the clear supernatant at 490 nm compared to a reagent blank solution as described in Jabborova et al. (Jabborova et al., 2021).

The activity of dehydrogenase was measured according to the procedure outlined by Tan et al. (Tan et al.,2017). 5 grams of freshly homogenized soil specimens were transferred into test tubes, followed by the addition of 5 milliliters of a 3% volume/weight substrate containing $C_{19}H_{15}C_lN_4$ (TTC, 2,3,5-

triphenyltetrazolium chloride). Incubation lasted 24 hours at 25 °C. For the blank, 1 mL of a phosphate buffer solution containing 3% 2, 3, 5-Triphenyltetrazolium chloride was used. Once incubated, the samples were subjected to centrifugation at 4500 revolutions per minute for 10 minutes. The synthetic triphenyl-formazan was isolated via methanol extraction. 5 ml of methanol was added to each tube, followed by vigorous shaking of the tubes for 10 minutes. The procedure was done twice, with 10 mL of CH_3OH dedicated to extraction. The tubes were once more subjected to centrifugation. The supernatant was transferred into a sterile tube, and the absorbance of the solution was quantified at a wavelength of 485 nm, based on the protocol by Jabborova et al. (Jabborova et al., 2021).

2.7. Statistical data analysis

Statistical analysis of experimental data was performed with ANOVA employing Stat-View Software (SAS Institute Inc.). The significance of treatment impact was assessed based on the level of the p-value ($p < 0.05$).

3. Results

Figure 1 shows that the application of AMF greatly enhanced both the leaf width and leaf numbers. The leaf length, length of shoot, leaf width, and leaf number were all considerably enhanced by 44%, 80%, 51%, and 49%, respectively, as compared to the control plant. The synergistic application of AMF and biochar resulted in a substantial 56% and 55% increase in leaf length and shoot length, respectively, compared to the control group. Furthermore, the application of AMF and biochar together resulted in a positive impact on both leaf width and leaf number, exhibiting a 40% and 29% enhancement, respectively, in comparison to the control group.

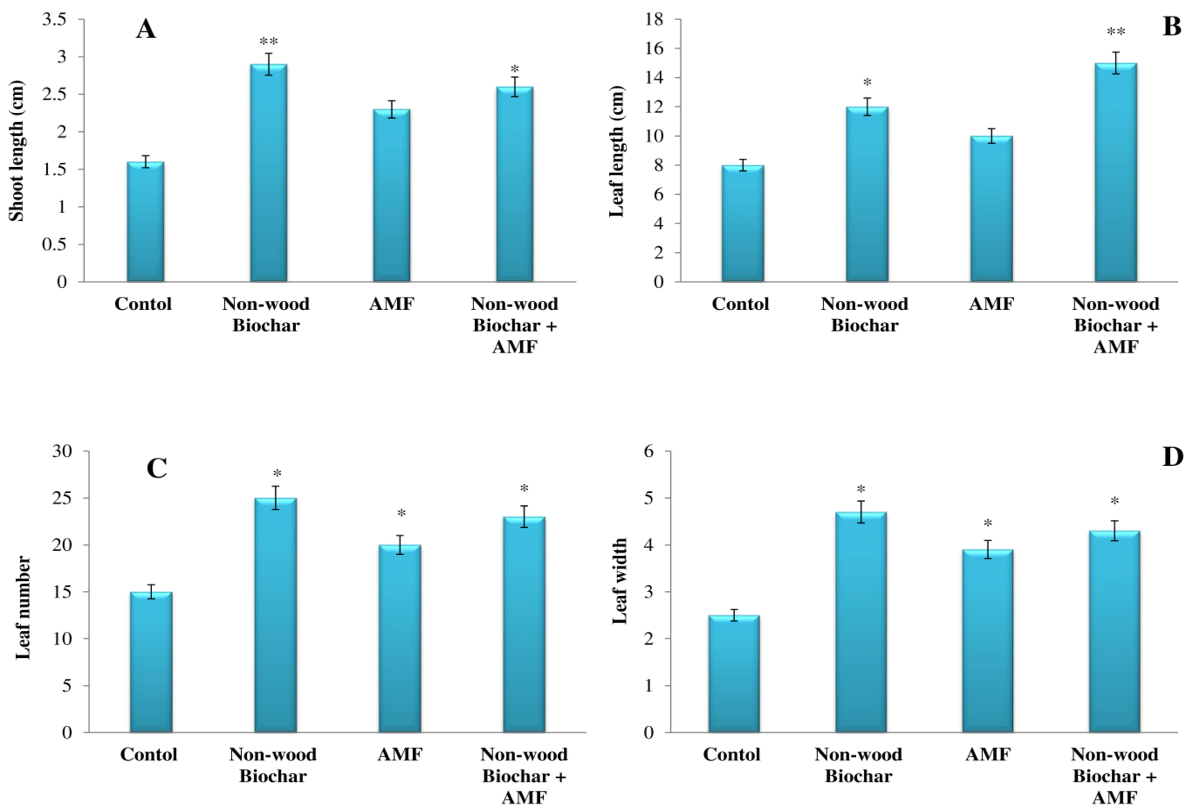

Figure 1: *AMF and biochar for the increment of (A) length of shoot, (B) length of leaf, (C) leaf numbers, and (D) leaf width of Swiss chard. Data are the means of 3 replicates (n = 3); * differed significantly at p < 0.05 *, p < 0.01 ***

In Figure 2, the biochar administration and the combination of AMF and biochar showed the greatest recorded values for shoot and root fresh weight as well as dry weight. The application of biochar demonstrated a notable enhancement in both the dry weight (49%) and fresh weight (61%) of roots over the control group (Figure 2). The biochar application resulted in a substantially higher fresh weight of the shoot by 40% and dry weight by 40%. The use of AMF gradually enhanced both the fresh and dry weight of the shoot. The application of AMF substantially enhanced the dry weight of both the roots and shoots in comparison to the control. Comparing the control to the simultaneous use of AMF and biochar, fresh and dry weight roots were considerably increased by 47% and 48%, respectively. In comparison to the control, the simultaneous use of AMF and biochar considerably increased the shoot's fresh weight (30%) and dry weight (31%).

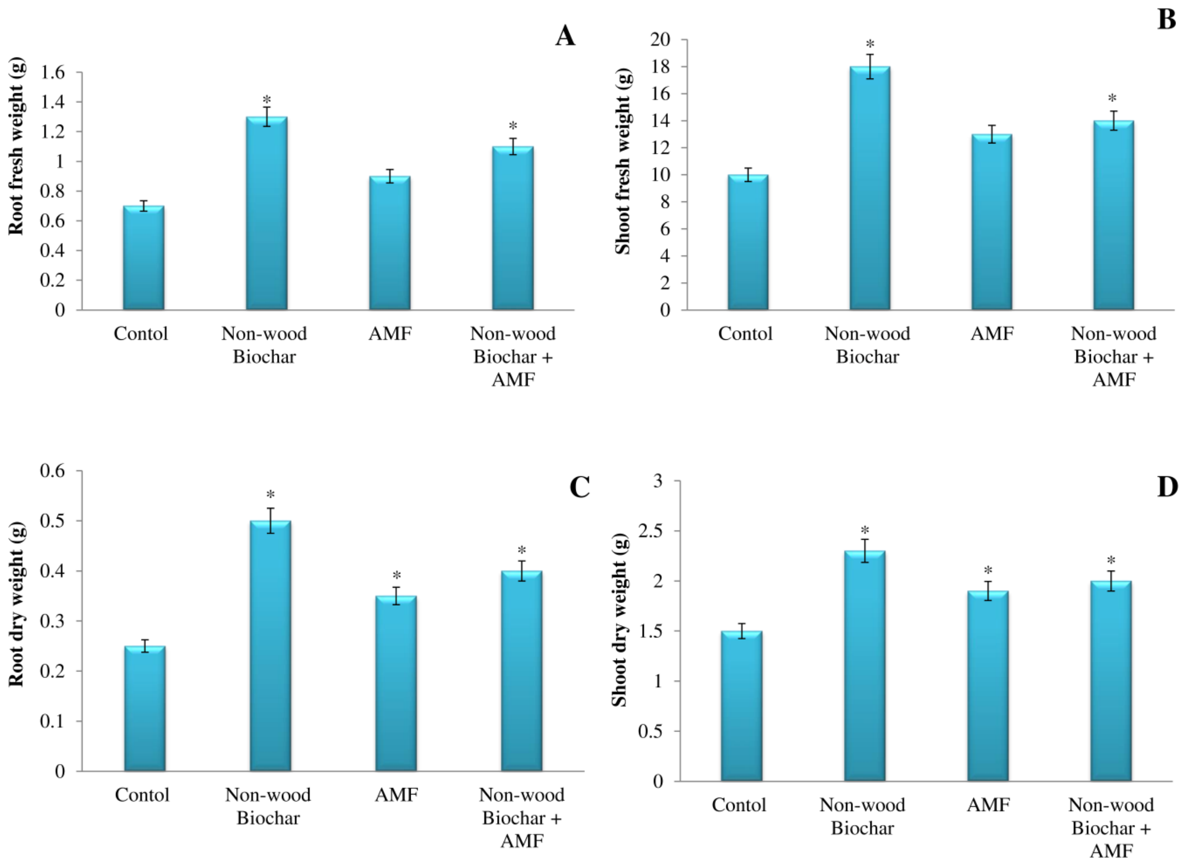

Figure 2: *AMF and biochar for the increment of (A) fresh weight of root, (B) fresh weight of shoot, (C) dry weight of root, (D) dry weight of shoot of Swiss chard. Data are the means of three replicates (n = 3); * differed significantly at $p < 0.05$**

Application of AMF increased the total length, diameter of root, projected area, and volume of the roots by 45%, 49%, 37%, and 38%, respectively, in comparison to the control group (Figure 3). Biochar application substantially enhanced the root volume and projected area by 60% and 65%, respectively, in comparison to the control. A 78% increase in total root length and 79% increase in the diameter of the root were observed after the biochar administration vs. the control. The simultaneous application of AMF and biochar resulted in a substantial 80% increase in total root length and an 89% increase in the diameter of roots compared to the control group. The mixed application of AMF and biochar resulted in a 49% increase in root volume and a 55% increase in the projected area.

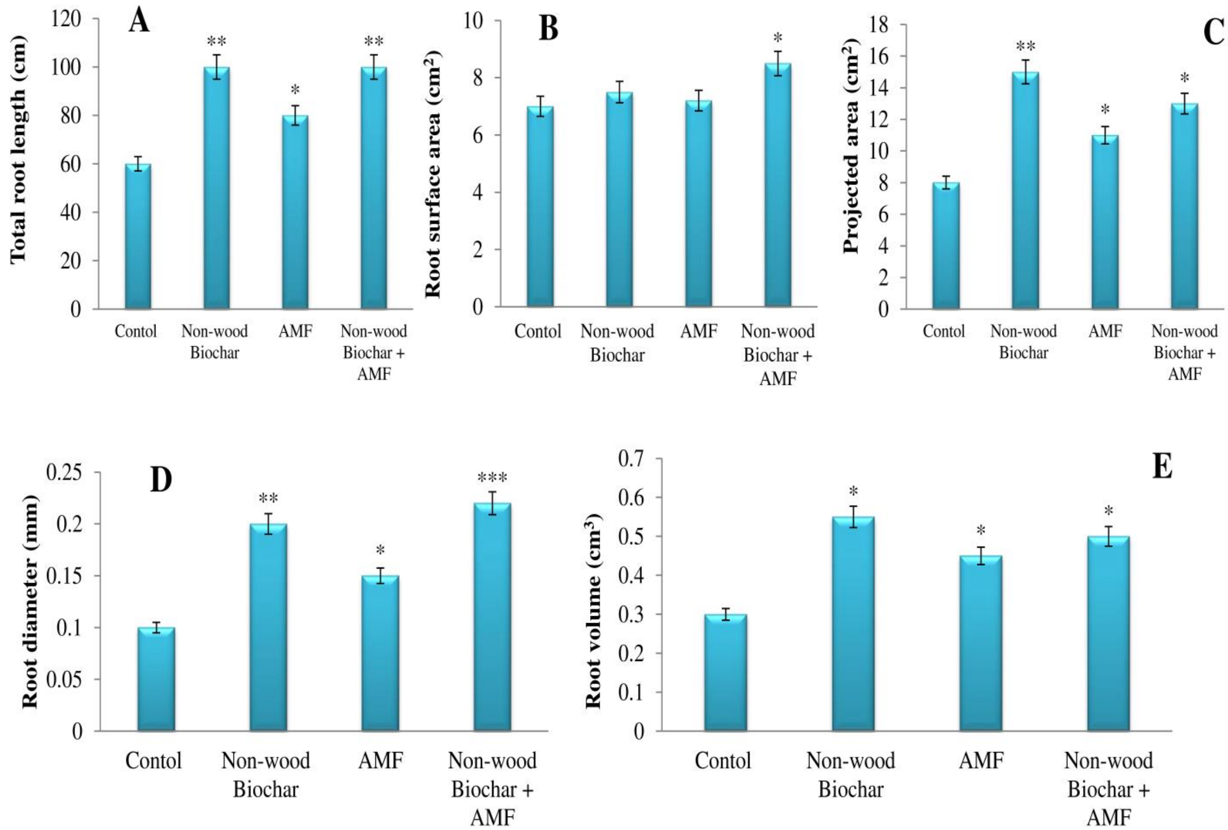

Figure 3: *AMF and biochar for the increment of (A) Total root length, (B) surface area of root, (C) projected area, (D) diameter of root, and (E) volume of root of Swiss chard. Data are the means of three replicates (n = 3); * differed significantly at $p < 0.05$*, $p < 0.01$**, $p < 0.001$****

The photosynthetic rate was significantly enhanced by 49% and 76%, respectively, when biochar was used alone and when combined with AMF, in comparison to the control group (Figure 4). The combination of AMF and biochar exhibited a substantial enhancement in stomatal conductance vs. the control. The rate of transpiration exhibited a substantial increase across all treatments in comparison to the control. The biochar administration yielded the highest value. A single application of biochar substantially increased the rate of transpiration by 47% in comparison to the control.

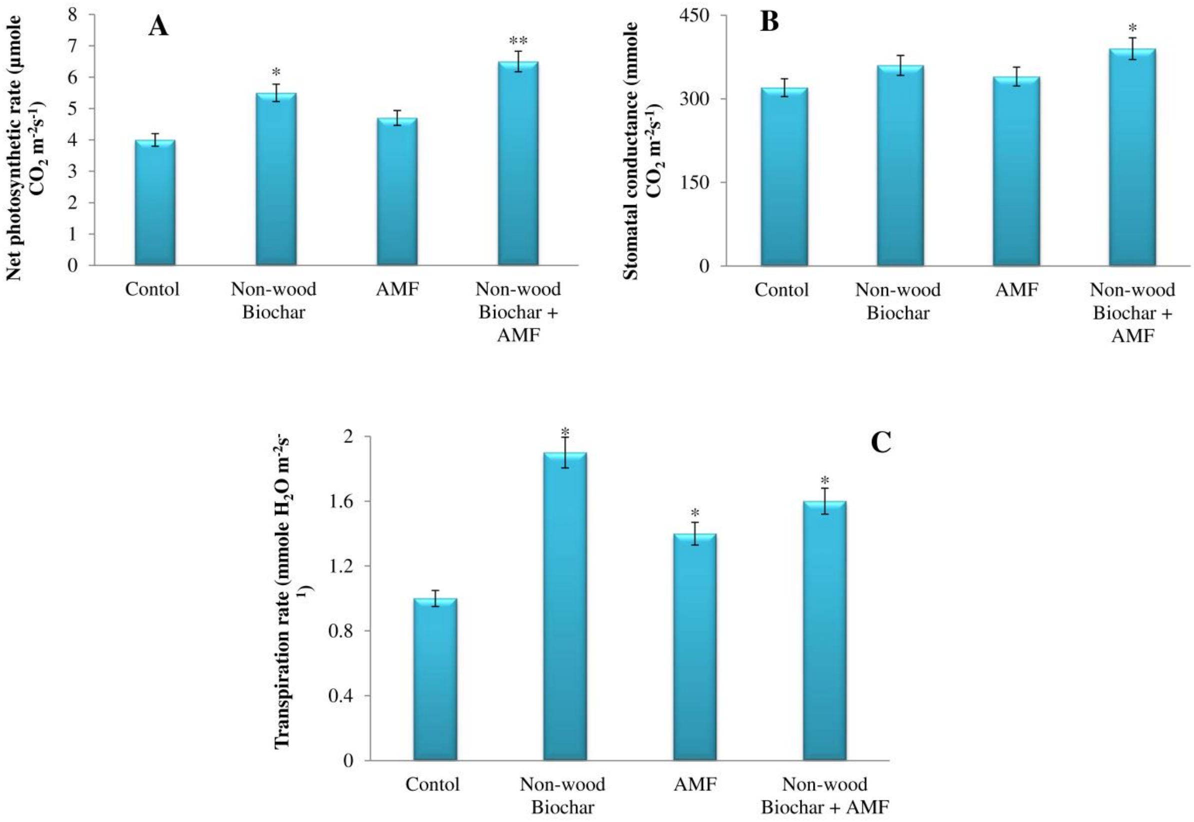

Figure 4: *AMF and biochar for the increment of (A) Net photosynthetic rate, (B) Stomatal conductance, and (C) Transpiration rate of Swiss chard. The averages of the three replicates (n = 3) are shown; * differed significantly at $p < 0.05$ *, $p < 0.01$ ***

Each of the treatments enhanced the levels of photosynthetic pigments in the leaf vs. the control (Figure 5). The biochar substantially enhanced the levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid in the leaf by 14%, 30%, 19%, and 51%, respectively, compared to the control group (Figure 5). The sole application of AMF resulted in a substantial increase of 19% in the overall level of total chlorophyll, 27% in chlorophyll, and 11% in chlorophyll b, and 29% in the carotenoid level. The simultaneous application of AMF and biochar caused a substantial increase in the total chlorophyll level, chlorophyll a and chlorophyll b levels, and carotenoid level, with respective increases of 27%, 19%, 20%, and 40% compared to the control.

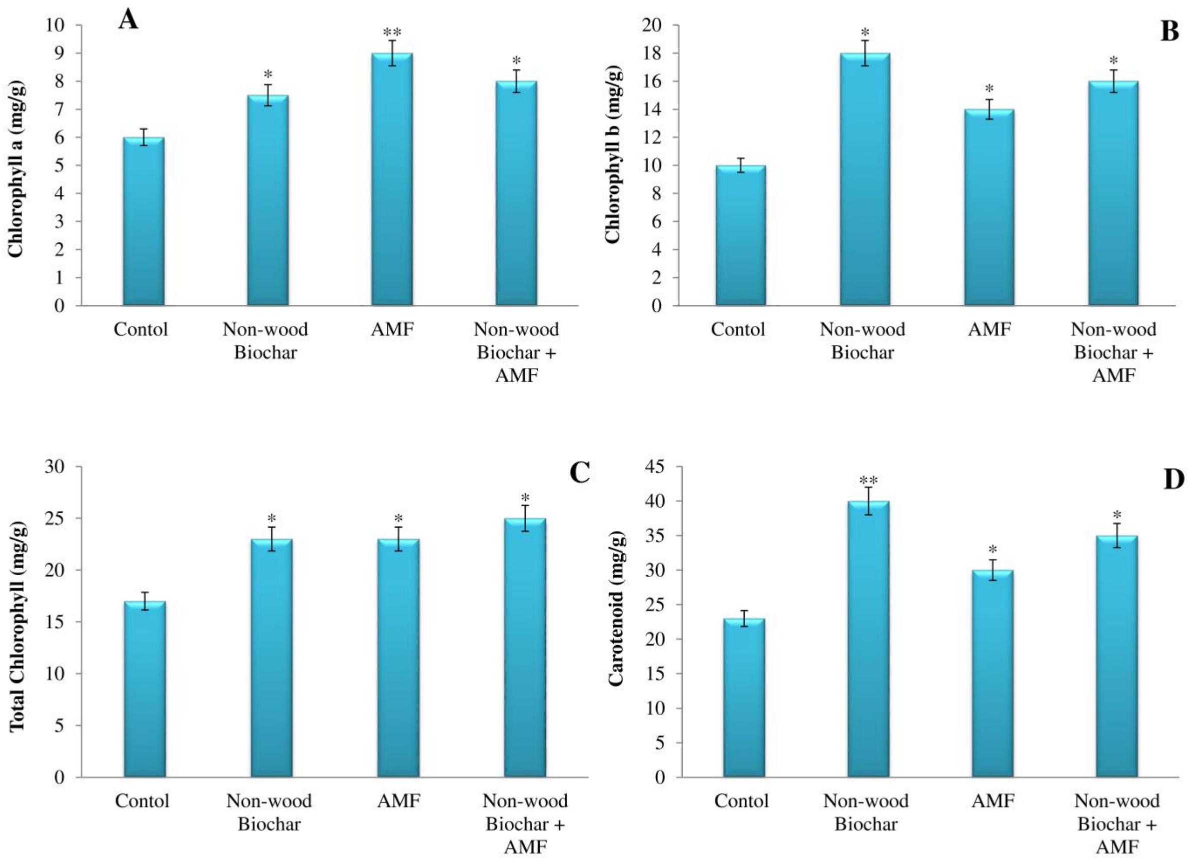

*__Figure 5:__ AMF and biochar for the increment of (A) chlorophyll a, (B) chlorophyll b, (C) Total chlorophyll, and (D) carotenoid level of Swiss chard. The three replicates (n = 3) were averaged; *differed significantly at p < 0.05 *, p < 0.01 ***

Each treatment, namely only AMF, only biochar, and the use of AMF plus biochar, led to higher values of the RWC (relative water content) vs. the control group (Figure 6). In the mixture of AMF and biochar treatment, the leaf exhibited the highest RWC level, which was 19% greater than that of the control. In the application including AMF alone or biochar alone, the RWC increased by 22% and 17%, respectively, in comparison to control plants.

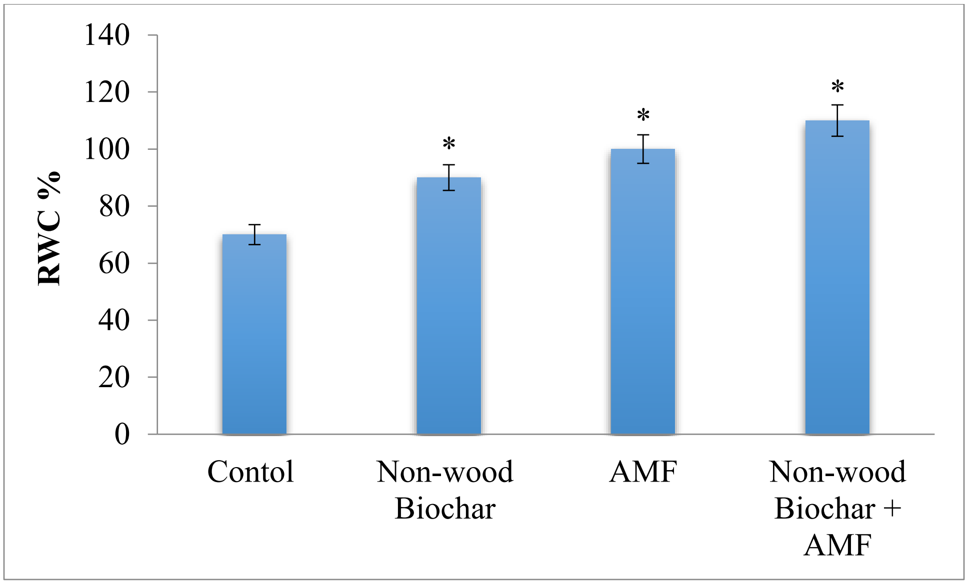

Figure 6: *AMF and Biochar for enhancement of the leaf RWC. Data of three replicates (n = 3) were averaged; *differed significantly at p < 0.05**

Both the application of AMF alone and the combination of biochar and AMF showed greater efficacy in enhancing the quantities of AMF spores in the soil compared to the control group (Figure 7). The population of AMF spores in soil exhibited a significant increase, ranging from 130% to 149% when exposed to AMF alone and when mixed with AMF and biochar, above the control group. The biochar application resulted in a 79% enrichment of AMF spores in soil compared to the control.

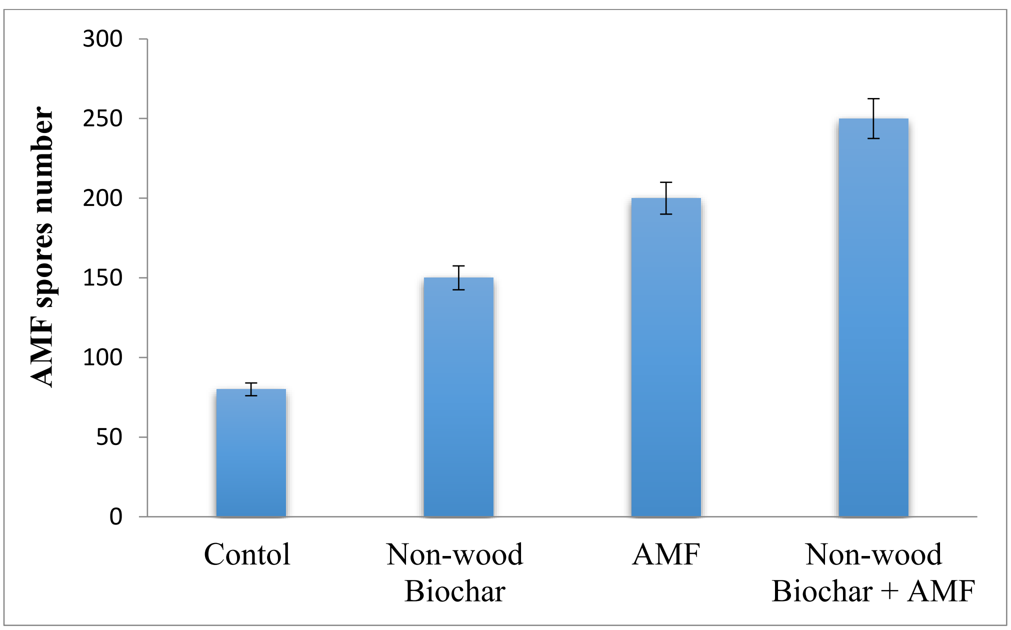

Figure 7: *AMF and Biochar for the enhancement of AMF spores in the soil. Data are the averages of the three replicates (n = 3); *differed significantly at p < 0.05 *, p < 0.01***

Both the application of only biochar and the combination of AMF and biochar treatment resulted in an increase in carbon content within the microbial biomass in the soil vs. the control (Figure 8). The mix of AMF and biochar treatment yielded the greatest carbon content among all the treatments, surpassing the control by 29%.

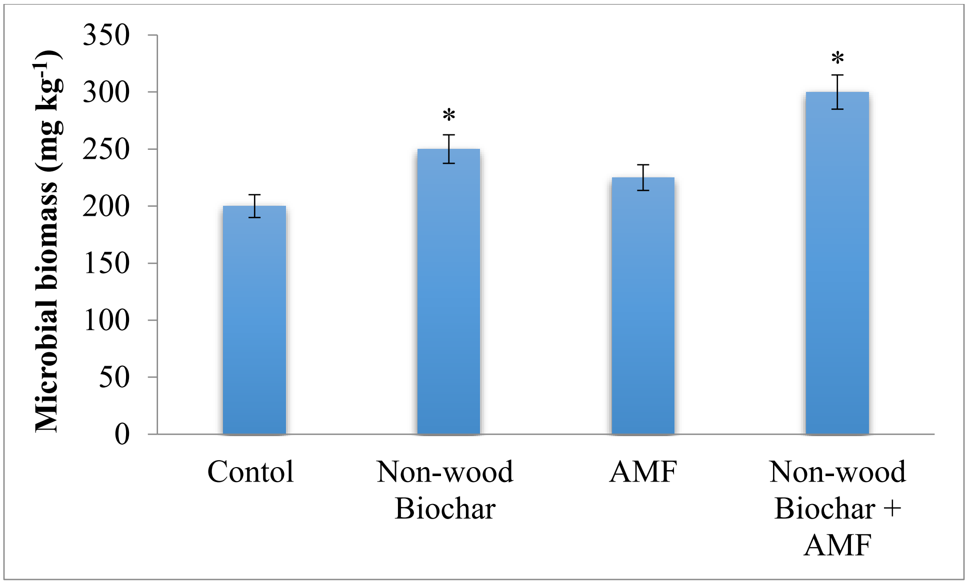

Figure 8: *AMF and Biochar for the enhancement of microbial biomass in the soil. Data are the means of three replicates (n = 3), * asterisk differed significantly at p < 0.05 **

All treatments exhibited a positive impact on alkaline phosphomonoesterase activity, resulting in an increase in enzymatic activity within a range of 59% to 91% (Figure 9). The combination treatment of AMF and biochar resulted in the highest observed alkaline phosphomonoesterase activity in soil. The administration of AMF alone (61%), as well as the combination of AMF and biochar (49%), had a more positive impact on the activity of dehydrogenase in the soil than the control. Moreover, the application of biochar and the combination of AMF and biochar treatment showed a positive impact on the activity of fluorescein diacetate in the soil in comparison to the control treatments. The combined use of AMF and biochar resulted in the greatest enhancement in the activity of fluorescein diacetate, surpassing the control by 59%.

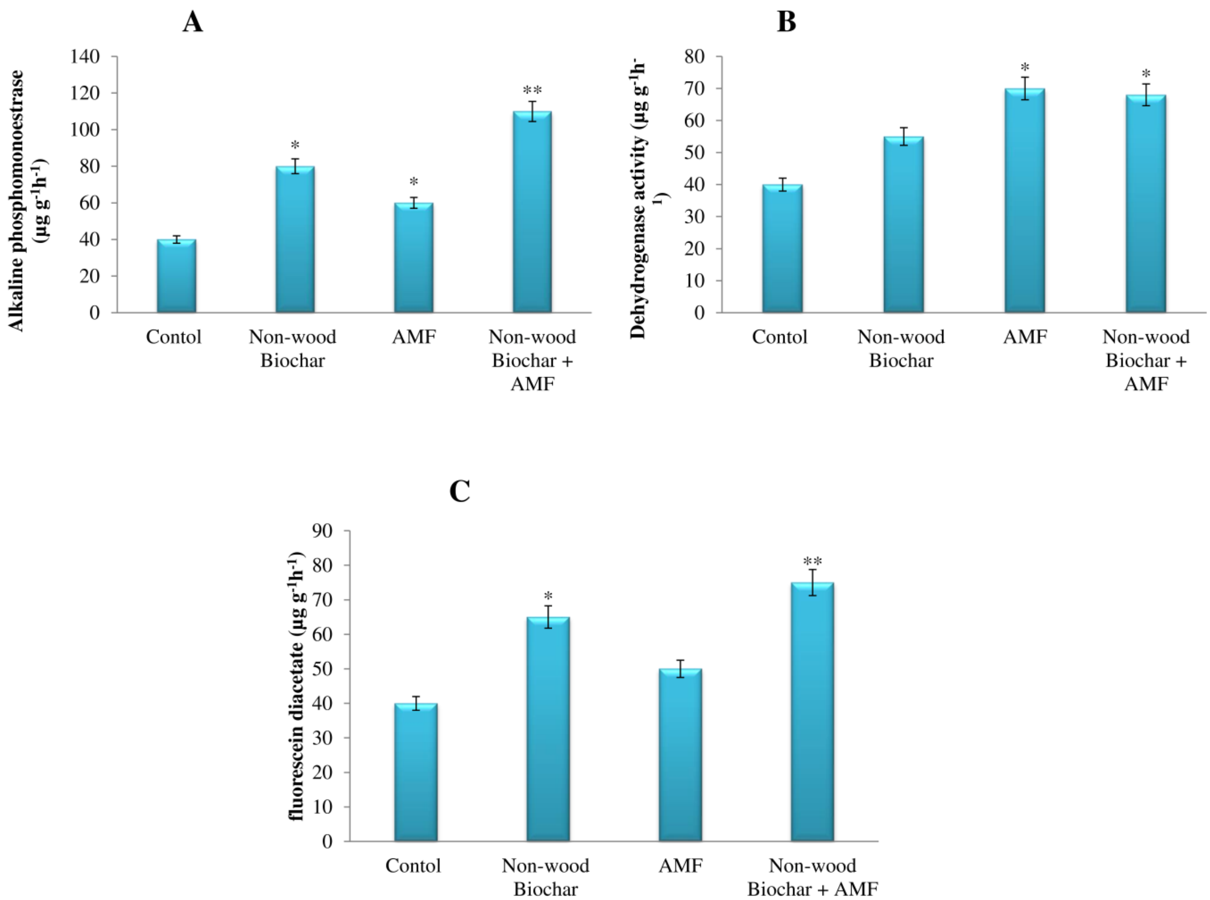

Figure 9: *AMF and Biochar for enhancement of soil enzymes (A) alkaline phosphomonoestrase (B) dehydrogenase (C) fluorescein diacetate. The three replicates (n = 3) are shown as means; *differed significantly at $p < 0.05$ *, $p < 0.01$ ***

4. Discussion

4.1. Impact of AMF and biochar on the Swiss chard growth

Overall, the application of biochar resulted in notable improvements in several aspects of plant growth-related parameters, including length of leaf, shoot length, leaf width, and leaf number, in comparison to the control group. In a similar vein, the application of biochar caused a substantial increase in both the fresh and dry weights of the roots and shoots compared to the control group. This result aligns with the study conducted by Zhang et al. (Zhang et al., 2021), which reported a notable improvement in the development of black locusts when exposed to biochar. Furthermore, Dobariya et al. (Dobariya et al., 2022) revealed that the application of biochar derived from castor waste increased the castor crop biomass. Multiple studies have documented that biochar is effective in enhancing growth and productivity in various crops (Murtaza et al., 2024). Results have been reported by Shoudho et al. (Shoudho et al., 2024) regarding the positive impact of biochar addition on the beans' shoot length, root length, shoot biomass, root biomass, and total yield. Ye et al. (Ye et al., 2020) found that the use of biochar derived from rice husk resulted in higher levels of final biomass, plant height, root biomass, and leaf numbers in cabbage and lettuce plants than the controls. Farrar et al. (Farrar et al., 2021) also documented an increase in dry weight, root biomass, and leaf biomass when biochar was applied. According to Jin et al. (Jin et al., 2024), biochar from rice straw showed a substantial enhancement in the height of the plant, per plant bolls, weight of boll, and seed output when compared to the control plants. The leaf width and number, fresh shoot weight, and dry root and shoot weight all exhibited a notable increase following AMF administration. Several studies have documented that the use of AMF enhanced plant growth characteristics (Begum et al., 2019). In their study, Khajeeyan et al. (Khajeeyan et al., 2024) observed a significant enhancement in plant growth indices of tea plants when AMF were applied. These parameters included the leaf area, leaf numbers, root length, shoot length, plant height, and weight of shoot and root. The simultaneous addition of AMF and biochar yielded positive results in terms of leaf length and numbers, and dry and fresh weights of root and shoot compared to the control. The research conducted by Malik (Malik, et al., 2019) revealed that both AMF and biochar enhanced the growth performance of maize. Ndiate et al. (Ndiate et al., 2021) also had similar findings; their research demonstrated that AMF and biochar substantially enhanced the height of the plants, the diameter of the plants, the dry weight of the roots, and the shoots of the plants in comparison to the control.

4.2. Root morphology changes through AMF and biochar

Significant enhancements in root morphological indices, including total root length, root volume, root diameter, and projected area, were seen after biochar addition in comparison to the control. Several studies have documented that the application of biochar enhanced the growth of plant roots (Kumar et al., 2024), thereby validating our findings. A notable augmentation in root length, root volume, and surface area of the root was documented by Yin et al. (Yin et al., 2024) after the utilization of biochar derived from woodchips and rice residue. Similar findings of substantial enhancement in root development resulting from the incorporation of biochar were also documented by (Ghorbani & Amirahmadi, 2024). Mahmoud et al. (Mahmoud et al., 2022) reported that the use of biochar enhanced root volume, taproot length, and total root area for absorption in tobacco plants. Mona et al. (Mona et al., 2024) reported that the inclusion of biochar had a substantial impact on the structure of the roots at both 40% and 60% field water capacity. AMF administration resulted in a substantial increase in the projected area, total root length, root volume, and root diameter compared to the control. The application of AMF to tomato seedlings resulted in a greater total root length and an increased number of root tips (Liu et al., 2024). The study conducted by Anli et al. (Anli et al., 2020) revealed that AMF have the ability to mitigate root stress by altering the root integrity. Seedlings of Melia azedarach treated with *Gigaspora margarita* exhibited markedly increased plant diameter, height, and dry weight of roots and shoots (Meng et al., 2023). Results on the combined application of AMF and biochar treatment revealed a notable enhancement in both the diameter and total length of the roots compared to the control. This result validates a previous study conducted by Chen et al .(Chen et al., 2020), which demonstrated that the application of both AMF and biochar administration resulted in a substantial enhancement of the chickpea root length. The consumption of underground nutrients and water by plants is significantly influenced by the combined addition of AMF and biochar, and this dependence is also contingent upon the root morphology (Thanni et al., 2024).

4.3. Impact of AMF and biochar on plant physiological attributes

Research demonstrated that the incorporation of biochar had a beneficial impact on the physiological characteristics of chard. Treatment with biochar alone resulted in a substantial increase in both the net transpiration rate and photosynthesis rate. The treatment with biochar also substantially enhanced the levels of chlorophyll a and b, carotenoid, total chlorophyll, and leaf RWC compared to the control group. Several studies have shown that the application of biochar enhances photosynthesis, transpiration rate, and chlorophyll content in several plant species (Duan et al., 2024). In their study, Isik and Ortas (Işik & Ortaş, 2024) found that the use of biochar was associated with a substantial enhancement in both photosynthetic rate and the concentration of chlorophyll in C3 plants. The study conducted by Zulfiqar et al. (Zulfiqar et al., 2021) revealed a significant and beneficial impact of biochar addition on the rate of photosynthesis in okra. Gharred et al. (Gharred et al., 2022) showed that the use of biochar increased the levels of chlorophyll a and chlorophyll b, and total photosynthetic pigments. Treatment with AMF alone increased the net photosynthetic rate, transpiration rate, and stomatal conductance. The inclusion of alone AMF greatly enhanced the levels of chlorophyll a and b, carotenoid, total chlorophyll, and leaf RWC.

Similar findings have been recorded by Cong et al. (Cong et al., 2023), indicating that the introduction of AMF greatly enhanced the activity of antioxidant enzymes and net photosynthetic rate in maize. Inoculation of AMF enhanced the chlorophyll concentration and rate of photosynthesis in chickpeas and maize (Gale & Thomas, 2019). The simultaneous use of AMF and biochar provided beneficial outcomes on the net photosynthesis rate, transpiration rate, stomatal conductance, photosynthetic pigments, and RWC in comparison to the control group (Figures 4, 5, 6). Loo et al. (Loo et al., 2022) have also revealed similar findings, demonstrating that simultaneous use of biochar and AMF substantially enhanced the chickpea RWC, photosynthetic rate, chlorophyll a and b, and total chlorophyll level under control conditions. Rehman et al. (Rehman et al., 2024) found similar results that confirm the substantial increase in photosynthetic rate and chlorophyll levels in corn. As depicted in Figure 10, it is indicated that biochar and AMF can boost siderophore synthesis and nitrogen fixation, simultaneously improving the absorption and availability of nutrients. Furthermore, they stimulate the synthesis of endogenous phytohormones and the creation of antioxidants.

4.4. Impact of AMF and biochar on AMF spore count, microbial biomass, and enzymatic activity of soil

The use of biochar markedly enhanced the activity of fluorescein diacetate and alkaline phosphomonoesterase in comparison to the control. Similar results showing increased enzymatic activity in soil resulting from the incorporation of biochar derived from soybeans were documented by Benaffari et al. (Benaffari et al., 2022). Soussani et al. (Soussani et al., 2023) similarly reported upregulated soil enzymatic activity as a result of biochar treatment. Previous research has documented an elevation in the levels of esterase, protease, lipase-esterase, phosphohydrolase, trypsin, and chymotrypsin enzymes when biochar is applied (Kakabouki et al., 2023). By biochar application, Lopes et al. (Lopes et al., 2021) observed a substantial rise in the activity of urease, phosphatase, and invertase. The highest dose (12 t ha^{-1}) could be observed at depths of the soil ranging from 0 m to 0.1 m. AMF spores and microbial biomass in the soil also exhibited an increase in comparison to the control. Similarly, Aziz et al. (Aziz et al., 2024) reported a comparable rise in AMF spores as a result of biochar addition. Multiple experiments have demonstrated that the administration of biochar enhanced the rates of AMF colonization (Jaffar et al., 2024). In comparison to the control, alone AMF administration increased the activity of dehydrogenase and alkaline phosphomonoesterase activity, AMF spores, and microbial biomass. Yang and Lu (Yang & Lu, (2022) revealed similar results that biochar upregulated phosphatase and urease activities as well as the microbial biomass in soil. The simultaneous addition of AMF and biochar greatly enhanced the enzymatic activity of fluorescein diacetate, dehydrogenase, and alkaline phosphomonoesterase, and also increased AMF spore yield and microbial biomass. The microbial activity in the corn rhizosphere was shown to be greatly enhanced by the simultaneous use of biochar and AMF, as described by Dobo (Dobo, 2022). Figure 10 provides a summary of the mechanism via which biochar and AMF exhibit their combined impact.

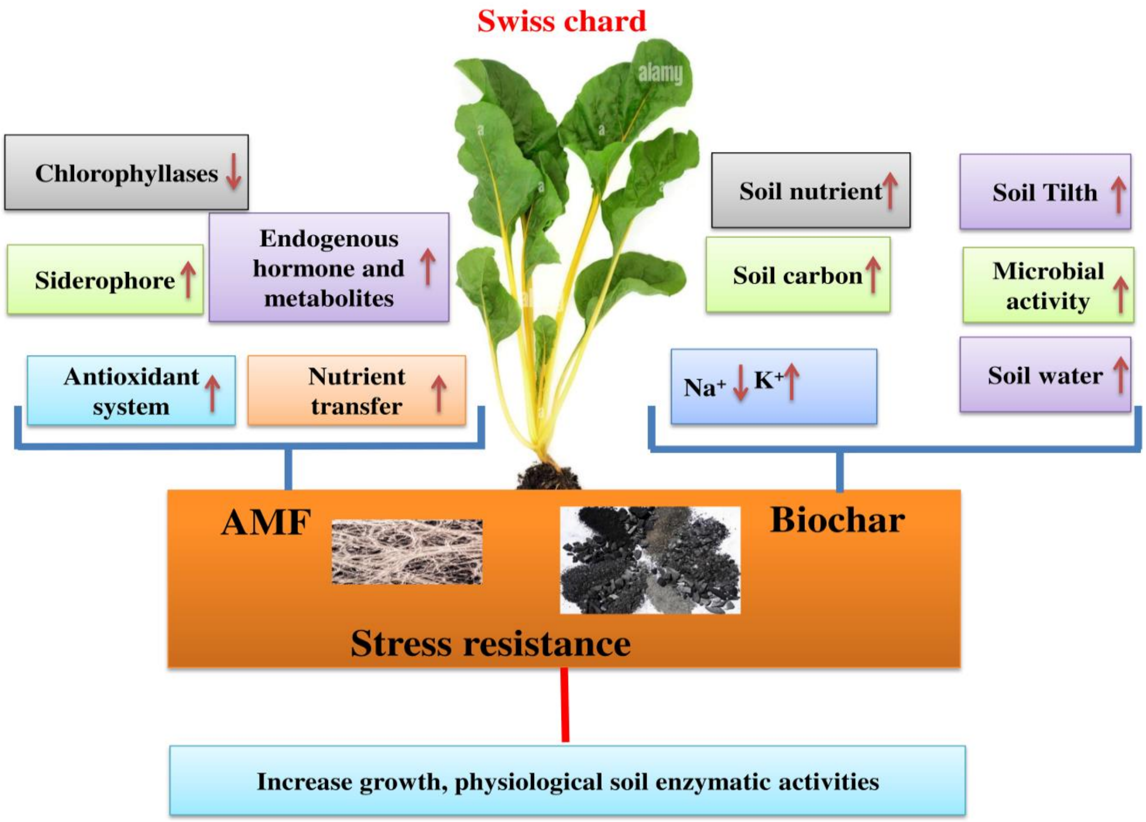

Figure 10: *Mechanism summaries for the synergistic impact of AMF and biochar on enhancing the growth of plants and enzymatic activity in soil*

5. Conclusion

Applying biochar has enhanced the development of root morphological characteristics and promoted plant growth. Furthermore, it has a beneficial impact on enzymatic activity in soil. The synergistic use of AMF and biochar had a notably beneficial effect on the Swiss chard growth, the morphological characteristics of their roots, the physiological attributes, and the enzymatic activity observed in the soil. Therefore, we figured out that conducting more research on the precise interactions among biochars can reduce the need for mineral-based fertilizers. The synergistic usage of AMF and biochar can serve as a highly effective biofertilizer to enhance the growth and productivity of Swiss chard plants in a field trial.

Authors Contributions

G.M. and M.U. designed the sampling strategy. G.M. designed the experiments. G.M performed the experiment. M.U. provided materials and supervision. G.M wrote the manuscript. R.I reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding

No Funding.

Data Availability Statement

All the raw data in this research can be obtained from the corresponding authors upon reasonable request.

Ethics declarations

Ethics approval

Permissions or licenses were obtained to collect Swiss chard (*Beta vulgaris*) seeds from the Regional Agricultural Research Institute (RARI) before starting the research. All experimental studies and experimental materials involved in this research are in full compliance with relevant institutional, national, and international guidelines and legislation.

Consent to participate

Not applicable.

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors stated that they had no interest that might be perceived as posing a conflict or bias.

References

1. Anli, M., Kaoua, M. E., Ait-el-Mokhtar, M., Boutasknit, A., Ben-Laouane, R., Toubali, S., ... & Meddich, A. (2020). Seaweed extract application and arbuscular mycorrhizal fungal inoculation: a tool for promoting growth and development of date palm (Phoenix dactylifera L.) cv «Boufgous». *South African journal of botany*, *132*, 15-21.
2. Antonia Sindesi, O., Ncube, B., Nike Lewu, M., Reckson Mulidz, A., & Bayo Lewu, F. (2022). Cabbage and Swiss chard yield, irrigation requirement and soil chemical responses in zeolite-amended sandy soil. *Asian Journal of Agriculture and Biology*, (Online).
3. Arshad, U., Raheel, M., Ashraf, W., Ur Rehman, A., Zahid, M. S., Moustafa, M., & Ali, M. A. (2023). Influence of Biochar Application on morpho-physiological attributes of tomato (Lycopersicon esculentum Mill) and soil Properties. *Communications in Soil Science and Plant Analysis*, *54*(4), 515-525.
4. Ayaz, M., Feizienė, D., Feiza, V., Tilvikienė, V., Baltrėnaitė-Gedienė, E., & Khan, A. (2022). The impact of swine manure biochar on the physical properties and microbial activity of loamy soils. *Plants*, *11*(13), 1729.
5. Aziz, M. A., Khan, K. S., Khalid, R., Shabaan, M., Alghamdi, A. G., Alasmary, Z., & Majrashi, M. A. (2024). Integrated application of biochar and chemical fertilizers improves wheat (Triticum aestivum) productivity by enhancing soil microbial activities. *Plant and soil*, *502*(1), 433-448.
6. Baloch, H., Sabir, I. A., Leghari, S. K., Saddiq, M. S., Alam, P., Khan, S., ... & Iqbal, R. (2024). AJAB. *Asian J Agric & Biol*, (4).
7. Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., ... & Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in plant science*, *10*, 1068.
8. Benaffari, W., Boutasknit, A., Anli, M., Ait-El-Mokhtar, M., Ait-Rahou, Y., Ben-Laouane, R., ... & Meddich, A. (2022). The native arbuscular mycorrhizal fungi and vermicompost-based organic amendments enhance soil fertility, growth performance, and the drought stress tolerance of quinoa. *Plants*, *11*(3), 393.
9. Bhale, U. N., Bansode, S. A., & Singh, S. (2018). Multifactorial role of arbuscular mycorrhizae in agroecosystem. In *Fungi and their role in sustainable development: Current perspectives* (pp. 205-220). Singapore: Springer Singapore.
10. Bhattacharyya, S. S., & Furtak, K. (2022). Soil–Plant–Microbe interactions determine soil biological fertility by altering rhizospheric nutrient cycling and biocrust formation. *Sustainability*, *15*(1), 625.

11. Boussora, F., Triki, T., Bennani, L., Bagues, M., Ben Ali, S., Ferchichi, A., ... & Guasmi, F. (2024). Mineral accumulation, relative water content and gas exchange are the main physiological regulating mechanisms to cope with salt stress in barley. *Scientific Reports*, *14*(1), 14931.
12. Chaudhari, V. M., Singh, O. B., Gouthami, N. S., Thakur, N., Singh, R., Singh, S., ... & Nagar, B. L. (2024). Unlocking the nutritional power of vegetables: A guide to vibrant health. *Eur. J. Nutr. Food Saf*, *16*, 247-261.
13. Chen, W., Meng, P., Feng, H., & Wang, C. (2020). Effects of arbuscular mycorrhizal fungi on growth and physiological performance of Catalpa bungei CA Mey. under drought stress. *Forests*, *11*(10), 1117.
14. Cong, M., Hu, Y., Sun, X., Yan, H., Yu, G., Tang, G., ... & Jia, H. (2023). Long-term effects of biochar application on the growth and physiological characteristics of maize. *Frontiers in Plant Science*, *14*, 1172425.
15. Dobariya, U. D., Gojiya, D. K., Makavana, J. M., Kelaiya, S. V., Gadhiya, G. A., Dulawat, M. S., ... & Chauhan, P. M. (2022). Influence of Temperature on the Production of Biochar from Cotton and Castor Feed Stalk in a Pyrolysis Process. *Current World Environment*, *17*(3), 634.
16. Dobo, B. (2022). Effect of arbuscular mycorrhizal fungi (AMF) and rhizobium inoculation on growth and yield of Glycine max L. varieties. *International Journal of Agronomy*, *2022*(1), 9520091.
17. Duan, S., Al-Huqail, A. A., Alsudays, I. M., Younas, M., Aslam, A., Shahzad, A. N., ... & Hong Yong, J. W. (2024). Effects of biochar types on seed germination, growth, chlorophyll contents, grain yield, sodium, and potassium uptake by wheat (Triticum aestivum L.) under salt stress. *BMC Plant Biology*, *24*(1), 487.
18. Egamberdieva, D., Li, L., Ma, H., Wirth, S., & Bellingrath-Kimura, S. D. (2019). Soil amendment with different maize biochars improves chickpea growth under different moisture levels by improving symbiotic performance with Mesorhizobium ciceri and soil biochemical properties to varying degrees. *Frontiers in Microbiology*, *10*, 2423.
19. Emenike, E. C., Iwuozor, K. O., Ighalo, J. O., Bamigbola, J. O., Omonayin, E. O., Ojo, H. T., ... & Adeniyi, A. G. (2024). Advancing the circular economy through the thermochemical conversion of waste to biochar: a review on sawdust waste-derived fuel. *Biofuels*, *15*(4), 433-447.
20. Farrar, M. B., Wallace, H. M., Xu, C. Y., Joseph, S., Dunn, P. K., Nguyen, T. T. N., & Bai, S. H. (2021). Biochar co-applied with organic amendments increased soil-plant potassium and root biomass but not crop yield. *Journal of Soils and Sediments*, *21*(2), 784-798.
21. Gale, N. V., & Thomas, S. C. (2019). Dose-dependence of growth and ecophysiological responses of plants to biochar. *Science of the Total Environment*, *658*, 1344-1354.
22. Gharred, J., Derbali, W., Derbali, I., Badri, M., Abdelly, C., Slama, I., & Koyro, H. W. (2022). Impact of biochar application at water shortage on biochemical and physiological processes in medicago ciliaris. *Plants*, *11*(18), 2411.
23. Ghorbani, M., & Amirahmadi, E. (2024). Biochar and soil contributions to crop lodging and yield performance-A meta-analysis. *Plant Physiology and Biochemistry*, *215*, 109053.
24. Gunes, H., Demir, S., Erdinc, C., & Furan, M. A. (2023). Effects of arbuscular mycorrhizal fungi (AMF) and biochar on the growth of pepper (Capsicum annuum L.) under salt stress. *Gesunde Pflanzen*, *75*(6), 2669-2681.
25. H Pangaribuan, D., Widagdo, S., Muhammad Hariri, A., & Siregar, S. (2022). The effect of rice straw mulch and cow urine on growth, yield, quality on sweet corn and pest population density. *Asian Journal of Agriculture and Biology*, (Online).
26. Huang, P., Huang, S., Ma, Y., Danish, S., Hareem, M., Syed, A., ... & Wong, L. S. (2024). Alleviation of salinity stress by EDTA chelated-biochar and arbuscular mycorrhizal fungi on maize via modulation of antioxidants activity and biochemical attributes. *BMC Plant Biology*, *24*(1), 63.
27. Ishaq, L., V Simamora, A., O Bako, P., & Airthur, M. M. (2023). Abundance of arbuscular mycorrhizal fungi in the rhizosphere of healthy and declining citrus in East Nusa Tenggara, Indonesia. *Asian Journal of Agriculture and Biology*.

28. Işik, M., & Ortaş, İ. (2024). Effect of biochar and mycorrhiza inoculation on maize growth, photosynthesis activity, and water use efficiency under deficient irrigation conditions. *Communications in Soil Science and Plant Analysis*, *55*(19), 2952-2965.
29. Ivanović, L., Milašević, I., Topalović, A., Đurović, D., Mugoša, B., Knežević, M., & Vrvić, M. (2019). Nutritional and phytochemical content of Swiss chard from Montenegro, under different fertilization and irrigation treatments. *British Food Journal*, *121*(2), 411-425.
30. Iwuozor, K. O., Emenike, E. C., Bakare, B. F., Eleregbe, F. O., Aransiola, F. T., Omonayin, E., ... & Adeniyi, A. G. (2024). A review on the conversion of plant husk-based biomass into biochar. *Biofuels*, *15*(10), 1331-1345.
31. Jabborova, D., Wirth, S., Halwani, M., Ibrahim, M. F., Azab, I. H. E., El-Mogy, M. M., & Elkelish, A. (2021). Growth response of ginger (Zingiber officinale), its physiological properties and soil enzyme activities after biochar application under greenhouse conditions. *Horticulturae*, *7*(8), 250.
32. Jaffar, M. T., Chang, W., Zhang, J., Mukhtar, A., Mushtaq, Z., Ahmed, M., ... & Siddique, K. H. (2024). Sugarcane bagasse biochar boosts maize growth and yield in salt-affected soil by improving soil enzymatic activities. *Journal of Environmental Management*, *363*, 121418.
33. Jatuwong, K., Aiduang, W., Kiatsiriroat, T., Kamopas, W., & Lumyong, S. (2024). Effects of biochar and arbuscular mycorrhizal fungi on soil health in Chinese kale (Brassica oleracea var. alboglabra L.) cultivation. *Microbiology Research*, *15*(1), 404-421.
34. Jin, W., Liu, Z., Wang, Q., Cheng, Z., Zhang, Y., Cao, N., ... & Zhao, W. (2024). Straw-derived biochar incorporation improves seedcotton yield and fiber quality by optimizing photosynthetic carbon and nutrients partitioning and boll formation patterns. *Industrial Crops and Products*, *214*, 118617.
35. Kakabouki, I., Stavropoulos, P., Roussis, I., Mavroeidis, A., & Bilalis, D. (2023). Contribution of arbuscular mycorrhizal fungi (AMF) in improving the growth and yield performances of flax (Linum usitatissimum L.) to salinity stress. *Agronomy*, *13*(9), 2416.
36. Kaparapu, J., Pragada, P. M., & Geddada, M. N. R. (2020). Fruits and vegetables and its nutritional benefits. In *Functional foods and nutraceuticals: bioactive components, formulations and innovations* (pp. 241-260). Cham: Springer International Publishing.
37. Khadem, A., Raiesi, F., Besharati, H., & Khalaj, M. A. (2021). The effects of biochar on soil nutrients status, microbial activity and carbon sequestration potential in two calcareous soils. *Biochar*, *3*(1), 105-116.
38. Khajeeyan, R., Salehi, A., Movahhedi Dehnavi, M., Hamidian, M., & Hazrati, S. (2024). Evaluation of the benefits of plant growth-promoting rhizobacteria and mycorrhizal fungi on biochemical and morphophysiological traits of Aloe barbadensis Mill under water deficit stress. *Scientific Reports*, *14*(1), 14480.
39. Khaliq, A., Perveen, S., Alamer, K. H., Zia Ul Haq, M., Rafique, Z., Alsudays, I. M., ... & Attia, H. (2022). Arbuscular mycorrhizal fungi symbiosis to enhance plant–soil interaction. *Sustainability*, *14*(13), 7840.
40. Kumar, A., Bhattacharya, T., Shaikh, W. A., & Roy, A. (2024). Sustainable soil management under drought stress through biochar application: Immobilizing arsenic, ameliorating soil quality, and augmenting plant growth. *Environmental Research*, *259*, 119531.
41. Lalay, G., Ullah, A., Iqbal, N., Raza, A., Asghar, M. A., & Ullah, S. (2024). The alleviation of drought-induced damage to growth and physio-biochemical parameters of Brassica napus L. genotypes using an integrated approach of biochar amendment and PGPR application. *Environment, Development and Sustainability*, *26*(2), 3457-3480.
42. Liu, C. Y., Hao, Y., Wu, X. L., Dai, F. J., Abd-Allah, E. F., Wu, Q. S., & Liu, S. R. (2024). Arbuscular mycorrhizal fungi improve drought tolerance of tea plants via modulating root architecture and hormones. *Plant Growth Regulation*, *102*(1), 13-22.
43. Loo, W. T., Chua, K. O., Mazumdar, P., Cheng, A., Osman, N., & Harikrishna, J. A. (2022). Arbuscular mycorrhizal symbiosis: a strategy for mitigating the impacts of climate change on tropical legume crops. *Plants*, *11*(21), 2875.

44. Lopes, É. M. G., Reis, M. M., Frazão, L. A., da Mata Terra, L. E., Lopes, E. F., Dos Santos, M. M., & Fernandes, L. A. (2021). Biochar increases enzyme activity and total microbial quality of soil grown with sugarcane. *Environmental Technology & Innovation*, *21*, 101270.
45. Mahmoud, A. W. M., Samy, M. M., Sany, H., Eid, R. R., Rashad, H. M., & Abdeldaym, E. A. (2022). Nanopotassium, nanosilicon, and biochar applications improve potato salt tolerance by modulating photosynthesis, water status, and biochemical constituents. *Sustainability*, *14*(2), 723.
46. Malik, Z., Shah, Z., & Tariq, M. (2019). Biochar improves viability of Arbuscular Mycorrhizal Fungi (AMF) in soil and roots of wheat (Triticum aestivum) and maize (Zea mays L.) under various cropping systems. *Sarhad J. Agric*, *35*(3), 834-846.
47. Meng, L. L., Xu, F. Q., Zhang, Z. Z., Alqahtani, M. D., Tashkandi, M. A., & Wu, Q. S. (2023). Arbuscular mycorrhizal fungi, especially Rhizophagus intraradices as a biostimulant, improve plant growth and root columbin levels in Tinospora sagittata. *Horticulturae*, *9*(12), 1350.
48. Mona, S., Saini, N., Kumar, S., Sharma, A., Yadav, A., Yadav, N., & Deepak, B. (2024). Characterization and utilization of algal and wheat husk biochar as biofertilizers for sustainable soil amelioration. *Bioresource Technology Reports*, *27*, 101893.
49. Murtaza, G., Usman, M., Iqbal, J., Tahir, M. N., Elshikh, M. S., Alkahtani, J., ... & Gruda, N. S. (2024). The impact of biochar addition on morpho-physiological characteristics, yield and water use efficiency of tomato plants under drought and salinity stress. *BMC Plant Biology*, *24*(1), 356.
50. Nazari, M., Hemati, A., Backer, R., Lajayer, B. A., & Astatkie, T. (2023). The role of arbuscular mycorrhizal fungi (AMF) in rhizosphere soil and plant growth regulation. In *The Role of Growth Regulators and Phytohormones in Overcoming Environmental Stress* (pp. 101-111). Academic Press.
51. Ndiate, N. I., Saeed, Q., Haider, F. U., Liqun, C., Nkoh, J. N., & Mustafa, A. (2021). Co-application of biochar and arbuscular mycorrhizal fungi improves salinity tolerance, growth and lipid metabolism of maize (Zea mays L.) in an alkaline soil. *Plants*, *10*(11), 2490.
52. Nirukshan, G. S., Ranasinghe, S., & Sleutel, S. (2022). The effect of biochar on mycorrhizal fungi mediated nutrient uptake by coconut (Cocos nucifera L.) seedlings grown on a Sandy Regosol. *Biochar*, *4*(1), 68.
53. Niu, M., Chen, X., Pan, Y., Wang, S., Xue, L., Duan, Y., ... & Peng, D. (2024). Biochar effectively promoted growth of Ardisia crenata by affecting the soil physicochemical properties. *Plants*, *13*(13), 1736.
54. Osei, A. F., Jin, X. L., Abdullah, W. Z. B. W., & Sidique, S. N. M. (2023). Silicon improves strawberry plants nutrient uptake and epicuticular wax formation in a rhizosphere cooling system.
55. Rehman, M. M. U., Zhu, Y., Abrar, M., Khan, W., Wang, W., Iqbal, A., ... & Xiong, Y. C. (2024). Moisture-and period-dependent interactive effects of plant growth-promoting rhizobacteria and AM fungus on water use and yield formation in dryland wheat. *Plant and Soil*, *502*(1), 149-165.
56. Shahraki, S. H., Javar, F. M., Jamali, B., & Sargazi, F. (2024). Beneficial role of Coronatine on the morphological and physiological responses of Cress Plants (Lepidium sativum) exposed to Silver Nanoparticle. *Botanical Studies*, *65*(1), 17.
57. Shoudho, K. N., Khan, T. H., Ara, U. R., Khan, M. R., Shawon, Z. B. Z., & Hoque, M. E. (2024). Biochar in global carbon cycle: Towards sustainable development goals. *Current Research in Green and Sustainable Chemistry*, *8*, 100409.
58. Sieradzka, M., Kirczuk, C., Kalemba-Rec, I., Mlonka-Mędrala, A., & Magdziarz, A. (2022). Pyrolysis of biomass wastes into carbon materials. *Energies*, *15*(5), 1941.
59. Singh, H. V., Singh, U. B., Sahu, P. K., Malviya, D., Singh, S., & Saxena, A. K. (2022). Arbuscular mycorrhizal fungal symbiosis for mutual benefit: More than expectation. In *Re-Visiting the Rhizosphere Eco-System for Agricultural Sustainability* (pp. 105-128). Singapore: Springer Nature Singapore.
60. Soman, K. N., & Shetty, R. (2018). Potential of cow urine as plant growth enhancer, its antimicrobial activity and presence of cellulolytic and polipolytic activity. *Glob. Scientific J*, *6*, 73-82.
61. Soussani, F. E., Boutasknit, A., Ben-Laouane, R., Benkirane, R., Baslam, M., & Meddich, A. (2023). Arbuscular mycorrhizal fungi and compost-based biostimulants enhance fitness, physiological responses, yield, and quality traits of drought-stressed tomato plants. *Plants*, *12*(9), 1856.

62. Sugawara, Y., Suzuki, K., Koshikawa, M., Ando, M., & Iida, J. (2002). Necessity of enzymatic activity of alkaline phosphatase for mineralization of osteoblastic cells. *Japanese Journal of Pharmacology*, *88*(3), 262-269.
63. Tackenberg, O. (2007). A new method for non-destructive measurement of biomass, growth rates, vertical biomass distribution and dry matter content based on digital image analysis. *Annals of botany*, *99*(4), 777-783.
64. Tan, X., Liu, Y., Yan, K., Wang, Z., Lu, G., He, Y., & He, W. (2017). Differences in the response of soil dehydrogenase activity to Cd contamination are determined by the different substrates used for its determination. *Chemosphere*, *169*, 324-332.
65. Teodoro, M., Trakal, L., Gallagher, B. N., Šimek, P., Soudek, P., Pohořelý, M., ... & Mohan, D. (2020). Application of co-composted biochar significantly improved plant-growth relevant physical/chemical properties of a metal contaminated soil. *Chemosphere*, *242*, 125255.
66. Thanni, B., Merckx, R., Hauser, S., Soretire, A., & Honnay, O. (2024). Multiple taxa inoculants of arbuscular mycorrhizal fungi enhanced colonization frequency, biomass production, and water use efficiency of cassava (Manihot esculenta). *International Microbiology*, *27*(4), 1219-1230.
67. Uwamungu, J. Y., Shi, G., Wang, Y., Paliwal, A., Jadhav, R. R., & Wani, A. W. (2022). Arbuscular mycorrhizal fungi (AMF) for sustainable soil and plant health. In *Microbial and Biotechnological Interventions in Bioremediation and Phytoremediation* (pp. 135-152). Cham: Springer International Publishing.
68. Verma, B., & Reddy, M. S. (2020). Biochar augmentation improves ectomycorrhizal colonisation, plant growth and soil fertility. *Soil Research*, *58*(7), 673-682.
69. Victoria, O., Idorenyin, U. D. O., Asana, M., Shuoshuo, L., & Yang, S. (2023). Seed treatment with 24-epibrassinolide improves wheat germination under salinity stress. *Asian Journal of Agriculture and Biology*.
70. Yang, C., & Lu, S. (2022). Straw and straw biochar differently affect phosphorus availability, enzyme activity and microbial functional genes in an Ultisol. *Science of the Total Environment*, *805*, 150325.
71. Ye, L., Camps-Arbestain, M., Shen, Q., Lehmann, J., Singh, B., & Sabir, M. (2020). Biochar effects on crop yields with and without fertilizer: A meta-analysis of field studies using separate controls. *Soil Use and Management*, *36*(1), 2-18.
72. Yin, Y., Li, J., Zhu, S., Chen, Q., Chen, C., Rui, Y., & Shang, J. (2024). Effect of biochar application on rice, wheat, and corn seedlings in hydroponic culture. *Journal of Environmental Sciences*, *135*, 379-390.
73. Yusif, S. A., Dare, M. O., Babalola, O. A., Popoola, A. R., Sharif, M. R., & Habib, M. Y. (2018). The roles of biochar and arbuscular mycorrhizal inoculation on selected soil biological properties and tomato performance. *FUTY Journal of the Environment*, *12*(2), 1-8.
74. Zhang, X., Yu, J., Huang, Z., Li, H., Liu, X., Huang, J., ... & Zhu, Y. (2021). Enhanced Cd phytostabilization and rhizosphere bacterial diversity of Robinia pseudoacacia L. by endophyte Enterobacter sp. YG-14 combined with sludge biochar. *Science of the Total Environment*, *787*, 147660.
75. Zhao, Y., Wang, X., Yao, G., Lin, Z., Xu, L., Jiang, Y., ... & Ping, L. (2022). Advances in the effects of biochar on microbial ecological function in soil and crop quality. *Sustainability*, *14*(16), 10411.
76. Zulfiqar, F., Chen, J., Younis, A., Abideen, Z., Naveed, M., Koyro, H. W., & Siddique, K. H. (2021). Biochar, compost, and biochar–compost blend applications modulate growth, photosynthesis, osmolytes, and antioxidant system of medicinal plant Alpinia zerumbet. *Frontiers in Plant Science*, *12*, 707061.