sustainability

MDPI

Article

Impact of Biochar on Fusarium Wilt of Cotton and the Dynamics of Soil Microbial Community

Mamoona Asif, Muhammad Saleem Haider and Adnan Akhter *

Department of Plant Pathology, Faculty of Agricultural Sciences, Quaid-e-Azam Campus, University of the Punjab, Lahore P.O. Box 54590, Pakistan; mamoona.rehan@gmail.com (M.A.); dean.fas@pu.edu.pk (M.S.H.)
* Correspondence: adnanakhter.iags@pu.edu.pk

Abstract: The effects of biochar on leaf and soil-borne diseases of plants can be seen in addition to its ability to sequester carbon, improve soil quality, and enhance plant performance. However, the mechanisms by which soil-borne pathogens are suppressed and plant performance is enhanced are not well understood. The present work aims to comprehensively establish the links between biochar-induced changes in the richness of the rhizosphere microbial population, in association with the reduction of soil-borne Fusarium wilt disease (*Fusarium oxysporum* f. sp. *vasinfectum*), in cotton (*Gossypium hirsutum*), with improved plant performance. Biochar made from organic waste significantly decreased the colonization and survival of Fusarium in soil, raised the culture-able counts of numerous microbes with biocontrol potential (microorganisms that boost plant growth and development), and inhibited Fusarium wilt of cotton. The biochar amendment significantly enhanced the cotton plant development and physiological parameters such as chlorophyll content, etc. Overall, 9% organic waste biochar had shown a significant impact on cotton growth as compared to other treatments with or without biochar. Compared to the soil-only control, the disease index was considerably reduced in all biochar-amended treatments. In terms of the plant's resistance to Fusarium wilt, biochar-induced increases in the level of overall chlorophyll content and biochemicals such as phenolics, flavonoids, etc. Additionally, cotton plants grown with a 9% biochar composition had considerably greater NPK levels than other treatments with or without biochar. The biochar addition resulted in increased counts of *Pseudomonas* spp., *Actinomycetes* spp., and *Trichoderma* spp., while Acidobacteriales, Rhodospirillales, and Frankiales were less when compared with an un-amended (without biochar) soil control. Thus, the composition of rhizosphere bacteria in the treatments with and without modified biochar was found to differ significantly.

Keywords: disease protection; *Fusarium oxysporum* f. sp. *vasinfectum*; microbial biodiversity; defense chemicals

Citation: Asif, M.; Haider, M.S.; Akhter, A. Impact of Biochar on Fusarium Wilt of Cotton and the Dynamics of Soil Microbial Community. *Sustainability* **2023**, *15*, 12936. https://doi.org/10.3390/su151712936

Academic Editor: Chenggang Gu

Received: 31 July 2023
Revised: 19 August 2023
Accepted: 24 August 2023
Published: 28 August 2023

1. Introduction

Emissions of greenhouse gases need to be decreased if we are going to combat global warming. Pollution caused by fossil fuels is the main cause of the anthropogenic greenhouse effect, making a decrease in the usage of fossil fuels a top concern [1]. Yet, a responsible plan also includes actively removing carbon dioxide from the atmosphere because some emissions will be inevitable [2]. Such carbon sequestration confronts a variety of difficulties, including the need for a long-term, significant net removal of carbon dioxide. For the soil to retain its chemical, physical, and biological integrity to carry out its agricultural production and environmental functions, a certain level of organic matter must be maintained in the soil [3]. Attention has also been drawn to the idea that adding biochar to soil could be a way to control plant diseases. Biochar sequestration is a short-term solution that can balance the increasing production of carbon. It is a renewable energy technique that sequesters carbon and decreases emissions when paired with bioenergy generation [4]. Growing plants that store carbon dioxide in their biomass or in soil organic matter is an established method of

extracting carbon from the atmosphere [2]. In fact, under the Kyoto Protocol, techniques for sequestering CO_2 through vegetation have previously been approved as trade "carbon offsets". Nevertheless, low-temperature pyrolysis—a method of heating plant material without adding oxygen—can advance this sequestration [5]. Black carbon production is thought to be in the range from 50 million tons to 270 million tons annually globally, with up to 80% of this persisting as soil leftovers [6,7]. The authors of [8] predicted that a variety of biochar application programs may possibly store 9.5 billion tons of carbon in soils by the year 2100. Biochar is a carbon-rich substance that is created from organic feedstock by thermal burning methods with minimum oxygen. According to the worldwide Biochar project, biochar is a high-carbon byproduct of pyrolysis which leads to the development of plants and often a decrease in the progression of plant diseases [9]. To create biochar, biomass is pyrolyzed in an environment with regulated oxygen levels. When a mixture of organic waste is heated to high temperatures between 200 and 900 °C with little to no oxygen or air, the process is known as pyrolysis [10]. Researchers found that adding biochar increased soil permeability by up to 51% when compared to nearby agricultural soils [11]. However, it was discovered that the color, value, and chroma of the charcoal kilns' black soil were reduced by 8, 20, and 20%, respectively [12]. The reduction in soil degradation and overland flow was shown to be caused by a higher flow rate of soil altered with biochar [13]. The pH increased significantly after biochar was mixed with the soil. According to [14], biochar prepared from chicken litter enhances liming in soil, raising the pH of acidic or neutral soils. In multiple trials, it was discovered that adding biochar with the compost application improved the soil's physicochemical characteristics, making the nutrients more easily assimilated by plants. The degraded soils, especially in locations that are favorable for farming, have been rejuvenated by the combined use of biochar with compost [15]. Crop production and increased fertilizer efficiency are both considerably improved by the addition of biochar to the soil [16]. Also, according to researchers, there has been a considerable shift in the crop's development and production [17]. Characteristics of early staged soil and crop development would react differentially to the biochar additions dependent on the feedstock and amount of biochar applied. The application of both applied and natural nutrients was significantly impacted by biochar with compost. The addition of compost and inorganic fertilizers had a considerable influence on growth, grain output, and plant biomass [18].

Cotton (*Gossypium hirsutum* L.) is a leading cash crop in Asia, Australia, Africa, and America [19]. It plays a primary role as the source of fiber worldwide, providing income to industrialists and farmers. It includes the Malvaceae family with the genus Gossypium. In approximately 60 countries worldwide, it acts as an integral component of the farming system. For thousands of years, it has been cultivated throughout the world to make fabrics [20–24]. Yield losses and other situations that damage the quality of fiber are caused by a diversity of biotic factors [25]. Pathogenic fungi, viruses, and bacteria are examples of biotic factors that cause diseases in plants. Cotton is prone to more than sixty diseases causing significant yield losses [26]. Diseases and symptoms such as leaf spots, seedling and boll rot, reduction in the leaf size, stunting of the crop, premature boll opening, blights caused by bacteria, nematode attack, leaf curling by viruses, etc., are responsible for decreasing the quality and economic value of cotton crops. The degree of damage is influenced by the genotype of the cultivar and environmental conditions [27]. Fusarium wilt and Verticillium wilt are two destructive wilts of cotton plants. The pathogen infects the vascular system of the host. They infect the cotton root and then spread throughout the plant by penetrating and replicating within the xylem tissues [28]. Vascular wilt fungi are soil-dwelling diseases, yet they can develop thick-walled resting structures on crop residues for an extended time without the host. In optimal circumstances, the fungus sporulates and induces infection by obstructing the cotton plant's vascular system [29]. Cotton wilt is triggered by soil-borne pathogens, *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) [30]. Ref. [31] was the first to report Fusarium wilt in America. Fusarium wilt in Mexico has since expanded to South America, the USA, and many other Asian countries [32–34]. Fusarium

symptoms can arise at any phase of the plant's progress, i.e., from the seedling to the maturity stage. Darkening of the veins, yellowing in leaves, and withering of new young leaves provide an initial sign of infection in seedlings. Cotton seedlings may wilt and eventually die as their cotyledons become necrotic and start shedding. Marginal yellowing of lower leaves is one of the first indications of disease in older plants. Ever-increasing worry about pesticides' potential to contaminate the environment has drawn attention towards the need for risk-free alternative disease control approaches. The basic objective of this present study was to study the influence of soil biochar additions on the onset of cotton wilts disease and the establishment of disease resistance. Therefore, it is predicted that using organic soil alterations would improve the physiochemical characteristics of the soil, plant health, and suppress the Fusarium wilt of cotton.

2. Materials and Methods

2.1. Isolation of Fusarium oxysporum f. sp. *Vasinfectum (FOV)*

At the University of the Punjab in Lahore, Pakistan (31.29′42.2664″ N, 74.17′49.1316″ E), infected cotton plants that were growing in field conditions and displaying the typical signs of Fusarium wilt were selected for fungus isolation. Characteristics of the fungus culture, including mycelium topography, color, and colony border on Potato dextrose agar medium, were noted [35].

2.2. Preparation of Organic Waste Biochar (OWB)

For the manufacture of the biochar, the agricultural organic waste was pyrolyzed at 450 °C for three hours [36]. For the purpose of creating biochar, the organic agricultural waste was collected from the University of the Punjab's experimental field regions. For the production of biochar, TLUD (Top-lit Updraft), a portable kiln technique, was used with a few minor changes [37]. This method involves drilling around 40 holes in the bottom of the burner or primary container (a 200 L drum) that are 6–8 mm in diameter to allow air to flow freely [38].

During the whole pyrolysis process, organic waste was slowly heated for 45 min with no oxygen. After pyrolysis by spraying water, the biochar was left to cool, and the biochar was pulverized to a fineness of 1 mm using a sieve [39].

2.3. Preparation of Soil Substrate

The soil from the farmland was used in this research. With additional organic additions like biochar and compost, sandy loam soil with a bulk density of 1.31 g/cm3, 5.7% clay (2 mm), 42.8% silt (>2 mm), and 52.1% sand (>63 mm) was used as the base material for planting [40]. Compost that was purchased from a nearby retailer under the Nutri brand was used with biochar. Treated soil was used as the main ingredient to make different composting potting combinations with OWB (3%, 6%, and 9% v/v) for growing plants (Table 1).

2.4. Experiment Plan

In the experimental setup, various volume-based percentages (3, 6, and 9%) of biochar were utilized as soil amendments, either inoculated or not with *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). The complete randomized (CRD) design included ten replicates for each treatment, each replicate represented one pot with one plant. In the experimental design, there were four different treatment combinations: soil alone (S), and soil combined with 3%, 6%, and 9% (v/v) of organic waste biochar. As a source of nutrients, the compost was consistently blended at 20% (v/v) in all of the treatments. The surface of the cotton seeds was treated by immersing them in 50% sodium hypochlorite (3.8% NaOCl) for 10 min, followed by three showers with autoclaved water [41].

Table 1. Characteristics of soil, organic waste biochar (OWB), and compost.

Parameter	Soil	OWB	Compost
N (%)	0.068	0.91	1.19
P (%)	2.11	0.74	0.383
K (%)	1.80	0.62	0.51
C (%)	1.18	45.61	28.42
C/N Ratio	15.16	48.53	23.66
Cu	0.24 ppm	0.14 (%)	74 mg/kg
Zn	1.15 ppm	0.025 (%)	4459 mg/kg
Fe	1.51 ppm	0.55 (%)	-----
CEC (mcq/100 g)	125	13.11	-----
EC (mS/cm)	0.49	1.61	1.29
Organic matter (%)	0.588	60.11	17.11
pH	7.88	9.23	7.31

----- Parameters were not checked.

2.5. Inoculation of Pathogen

The FOV was grown on PDA and kept for 7 days at 26 °C in the dark in an incubator for preparation of injection. Conidia were collected by carefully scraping the colony's surface with a spatula while submerging the FOV culture plates in autoclaved water to create suspension for inoculation. Four layers of cheesecloth were used to filter the suspension after that, each measuring 50 m thick. Using a hemocytometer, the conidial suspension's final concentration was calculated and adjusted to 1×10^6 conidia/mL [42]. Conidial suspension of FOV (106 conidia/mL) was applied by piping 25 mL of a conidial suspension onto the soil around the plants' bases during the 1st true-leaf stage. In order to retain the necessary moisture (about 70%) for infection growth, the infected cotton was sprayed with sterilized water for two days. To grow a decent yield, the plants were cared for in a greenhouse in a random order using all the suggested cotton growth techniques [43].

2.6. Cotton Plant Analysis

2.6.1. Growth Parameters Assessment

The plants were harvested 65 days after inoculation by carefully uprooting them and then cleaning the roots to measure parameters such as plant height and dry weight of root and shoot [44]. Stem to its peak, the plant height was measured. Also, both root and stems were cut apart and then dried in an air flow oven at 60 °C over 8 to 11 days (so if no change in the overall weight was noted) in order to compute the dry weights.

2.6.2. Biochemical Assays

Protein content

A 0.5 g sample of fully emerging leaves was powdered in a cold extraction buffer. The supernatant was separated after being centrifuged at 15,000 RPM for 8 min at 4 °C. Bradford's dye binding test was used to measure the protein content [45].

Flavonoid content

Leaf samples (250 mg) were mixed in 3 mL of 80% water-based ethanol and stored at 37 °C without light for 40 min. After the centrifugation, the resulting product was taken away and 4.3 mL of each of the following solutions were added: 10% aluminum nitrate and 80% aqueous ethanol, plus 1 M sodium acetate solution was added. The mixture was then gently shaken to combine the ingredients. After 30 min of incubation, the absorbance measurement of 495 nm was recorded [46]. Flavonoid content was measured in mg/g of cotton leaf fresh weight.

Catalase

In order to evaluate CAT activity, leaves from plants that had been infected with FOV and plants that had not were well mixed together in 50 mM of a potassium phosphate buffer, pH 7.0, and 1 mM DTT (for the estimate of CAT). In total, 5.9 mM of H_2O, 0.1 mL of enzyme extract, and 50 mM of phosphate buffer (pH 7.0) were all included in the assay

solution's 3 mL volume. Every 20 s, a reduction in the reaction solution's absorbance at 240 nm was noted. An alteration in absorbance of 0.01 units a min was used to express a single unit of CAT activity. Expression of enzyme activity was based on fresh weight [47].

Phenolic

The modified Folin–Ciocalteu technique was used to quantify the total quantity of phenolic compounds [48]. The extracts were mixed with Na_2CO_3, the Folin–Ciocalteu reagent, and 0.5 mL of Na_2CO_3. The absorbance of 760 nm was measured using a spectrum analyzer after the mixture had been incubated for 10 min. Gallic acid equivalents (GAEs) were utilized to quantify the samples' total phenolic content.

Chlorophyll content

The total chlorophyll content of cotton leaf was measured three days before harvest using a movable chlorophyll meter SPAD-502 [49]. Each figure received reflects the average of three measurements to reduce the possibility of inaccuracies.

2.6.3. Plant Nutrient (NPK) Contents Determination

The amounts of nitrogen, phosphate, and potassium in cotton shoots were measured and the Kjeldhal technique was used for NPK analysis [50].

The dry plants were crushed into a fine powder for nitrogen analysis. In total, 0.5 g of the material was processed with 10 mL sulfuric acid at 420 °C for 2 h while using a 9:1 ratio of potassium and copper sulfate as the catalysts. The three principal processes of the Kjeldhal technique for the estimation of N are digestion, distillation, and titration.

2.6.4. Cotton Plant Disease Assessment

Measurement of AUDPC

By using the following formula, the area under the disease progress curve (AUDPC) was determined:

$$\text{AUDPC} = \sum_{i=1}^{n} [(X_{i+n1} + X_i)/2][Y_{i+1} - Y_i]$$

where Y_i is the time (Days) at the ithobservation, n is the total number of observations, and X_i is the disease severity (per unit) at the ith observation [51].

Disease Incidence and Severity measurement

Plants that were infected were enumerated, and the formula below provided the % incidence of the disease [52].

$$\text{Disease incidence } (\%) = \frac{\text{Cotton Plants Showing Disease symptoms}}{\text{Total Plants}} \times 100$$

The given formula was used to determine the severity of the disease [40].

$$\text{Disease severity } (\%) = \frac{\text{Length of infected stem [cm]}}{\text{Total length of stem [cm]}} \times 100$$

Additionally, the percent disease index (PDI) was determined using the formulas provided by [53].

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all disease rating}}{\text{Total no. of observations} \times \text{Maximum rating grade}} \times 100$$

2.6.5. Identifying Culturable Microbes

Roots of plants were manually shaken after being taken out of their pots. To count 6 different kinds of culturable bacteria, samples of the soil mixture adhering to the cotton root surfaces, which had either been left unaltered or had been amended with, 3, 6, or 9% organic waste biochar (OWB), were taken. In total, 1 ppm of benomyl with agar [54] was used to count the number of bacteria, and a PDA medium with chloramphenicol (250 ppm) (Sigma Aldrich, Beijing, China) and a Rose Bengal (50 ppm) stain was used to count the number of fungi, specifically yeast or *Trichoderma* spp. [55]. Fluorescent *Pseudomonas*

spp. and *Actinomycetes* spp. were examined on a KSTR medium and Kings B medium, respectively [56,57]. In order to quantify CFUs g^{-1} dry potting mixture, microbe colonies were enumerated.

For gene sequencing, 16S rRNA was used and the universal bacteriological 11F and 1392R primer pair, unique Pseudomonas strains, were taxonomically identified [58]. Following the removal of chimeric sequences using DECIPHER-Find Chimaeras, partial sequences of 16S rRNA genes were compared with sequences retrieved from the NCBI database by employing Blast and the RDP classifier (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed on: 12 November 2022).

2.6.6. FOV Survival and Root Colonization in Soil

By using the root maceration technique, *Fusarium* spp. in the roots of infected cotton was quantified. Cotton roots were taken from plants that were asymptomatic at 1 and 3 days after pathogen inoculation, as well as symptomatic plants at 5 and 8 days following inoculation (when symptoms had typically begun to develop). The roots were divided and carefully cleaned in three separate batches of 30 mL of saline water. Prior to macerating the roots with 10 mL of sterile saline water, the roots were cleaned, blotted, and measured. The resultant paste was then plated using the plating dilution technique on an altered peptone PCNB (Pentachloronitrobenzene) medium [59]. At 25 °C, the plates were incubated for 5 days. In order to produce log CFU values, the data were logarithmically converted. The findings were expressed as CFU per gram of fresh root dry weight.

To determine how biochar affected Fusarium's survival in the potting mixture, the FOV inoculums were varied with the mixture for potting, both with and without biochar. Fusarium populations were assessed over a period of 25 days on an altered peptone PCNB medium using a serial dilution method. CFU per gram of dry material was used to express the results. After logarithmically transforming the CFU per gram of dry potting soil mixture data to produce log CFU values, a Fusarium persistence curve was shown. The apparent decline rate was calculated using the survival curve's slope after logit transformation [60].

2.6.7. Potting Mixture DNA Extraction

Prior to the FOV inoculation, samples of the potting mixture adhered to root surfaces from cotton that had been treated with 0% (the un-amended control), and 3, 6, and 9% organic waste biochar were taken from the rhizosphere. For DNA extraction, each treatment included four biological replicates, and to lower variability, each replication had mixed potting mixture materials from five different plants. The samples were kept at 80 °C until DNA extraction. Using a commercially available soil DNA kit for extraction and obtaining the DNA from 0.4 g of the root zone potting soil mixture using FastPrep FP120 and two cycles of beads beat for 45 s at 4.5 speed, nanodrop evaluated the yield and purity of the extracted DNA, and 1.5% agarose gel electrophoresis was used to determine the integrity of the sample. Finally, before PCR amplification, isolated DNA was diluted to 10 ng μL^{-1}.

2.6.8. 16S RNA Gene Sequencing

The primer sets [61] (CS1_515F and CS2_806R) which amplify the 16S rRNA gene's V4 region and contain for preparing libraries for the next-generation sequencings adaptors were used to perform PCR amplification on the DNA samples. There were the following elements in PCR reactions, which had a final volume of 50 µL: 2.5 µL (10 µM) of each primer, 25 µL of Dream Taq PCR master mix from Thermo Scientific, Waltham, MA, USA, 1.5 µL of (10 µg μL^{-1}) bovine serum albumin, and 3 µL (10 ng μL^{-1}) of DNA template. Up until they were sent for sequencing, the sections were kept at −20 °C. The sequencing steps used Illumina MiSeq technology in accordance with the amplicon sequencing procedure of the DNA service competence.

2.6.9. Analysis of Bioinformatics Data and Sequence Processing

PEAR was used to integrate pair-end FASTQ files. For reduction of length and quality, CLC software (version 8.5) was employed. Except where otherwise noted, the remaining analysis was carried out using the Quantitative Insights into Microbial Ecology (QIIME; version 1.9.1) pipeline37. UCLUST was used to classify the sequences into operational taxonomic units (OTUs; 97% similarity threshold) [62]. Each OTU's representative sequence was chosen and it was aligned with the Silva 16S rRNA bacterial database using PyNAST [(https://www.arb-silva.de/ (accessed on: 12 January 2023)]. Using the Silva database and the UCLUST method, taxonomy was assigned to each representative sequence. An OTU table was made using the taxonomic designations and the arrangement of the typical sequences.

Estimating the diversity was performed using the Bray–Curtis distances matrix and the most prevalent OTUs. Nonmetric multidimensional scaling (NMDS) was then used to visualize the data using the PAST software (version 4). The most prevalent OTUs in QIIME (at least 40–50 sequences overall) were used to build the heatmap.

2.6.10. Bacterial CLPP

Clarifying the impact of biochar on the functional capacity of rhizosphere microbial communities required the use of the community-level physiological profiles (CLPP) approach employing Biolog GN plates. A 2.5 g aliquot of soil taken from the cotton root zones and either biochar or a non-amended control was added to 22.6 mL of a 0.86% solution of NaCl and shaken for one hour at 25 °C. The suspensions of potting soil mixtures were diluted at 1:1000 and then added (150 µL) to obtain 96 wells in the microplate after settling for 10 min. The microplate was further incubated in the dark at 25 °C. Throughout the 120 h incubation period, color improvement in wells was monitored at 595 nm using a reader (Microplate reader, Thermo Fisher Scientific, Waltham, MA, USA). Using the Garland technique, the ordinary well color improvement for each plate was measured [63].

To check the potential change in CPPL, NMDS ordination was used after standardizing the data [64]. Maximum utilization rates were calculated for each functional group from which the substrates on Biolog plates were divided for the determination of maximal consumption rates [64]. According to Garland, the Shannon diversity index H′ was used to describe the metabolic functional diversity of the rhizosphere microbial population based on the absorbance measurement at 72 h. The amount of oxidized C substrates was used to determine the richness (R) values, with an OD of 0.25 serving as the cutoff for a positive response [63].

2.6.11. Statistical Analysis

The Statistix 8.1 program (Statistix, Tallahassee, FL, USA) was used to analyze data. Prior to analysis, percentage data were transformed. With soil substrate compositions and FOV as the main factors in the analysis of variance (ANOVA), the data were subjected to a two-way analysis of variance, and the means were compared using the HSD test at $p \leq 0.05$.

3. Results

3.1. Plant Growth Parameters

The examined plant agronomic parameters of cotton are significantly impacted by *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) and the nature of the soil substrate (S). However, the combination between the soil's composition and FOV had a significant impact on plant biomass results (Table 2).

Table 2. Results of a two-way ANOVA were used to assess the importance of the association between FOV and the soil substrate composition (S) on cotton plant development and physiological parameters.

Treatment	Total Phenolic	Catalase	Flavonoids	Total Proteins	Chlorophyll	N	P	K	SL	RL	SDW	RDW
S	***	**	***	*	***	**	***	**	***	***	***	***
FOV	*	***	**	***	**	***	*	***	***	***	***	**
S × FOV	***	**	**	***	**	*	***	*	***	**	**	*

*** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$.

Overall, plant growth indicators, including root weight, shoot weight, and height, were significantly impacted by the addition of soil biochar and the FOV inoculation. Plant growth measurements were often inhibited by the pathogen infection.

3.1.1. Shoot Length

In all the FOV-infected treatments, plant height was drastically decreased. The maximum plant stem length (131.8 cm), which was 48.92% greater than the soil with no biochar, was found in the un-inoculated (−FOV), 9% OWB soil amendment (Figure 1). The minimum shoot length (73.2 cm) was recorded in non-amended soil control under pathogen (+FOV) stress.

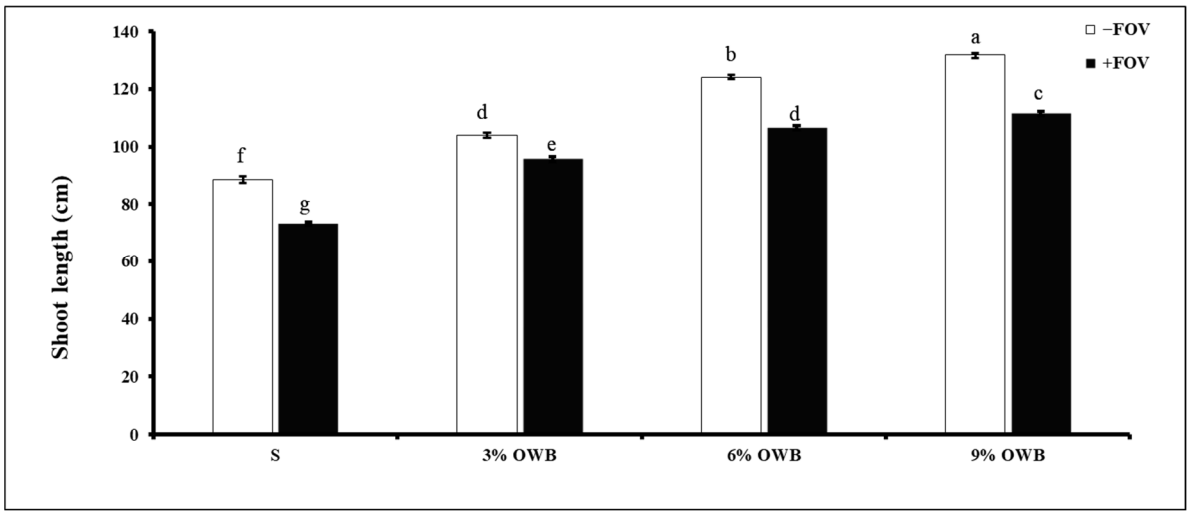

Figure 1. Organic waste biochar (3, 6, and 9%) and FOV effect on shoot length of cotton plant, including soil-only control, soil amended with 3% OWB, soil amended with 6% OWB, and soil amended with 9% OWB, either with (+FOV) or without (−FOV) pathogen. Bars followed by same letters are not significantly different.

3.1.2. Root Length

Figure 2 shows that the treatment with 6% organic waste biochar amendment (S + 6% OWB) had the highest root length (35.2 and 44.3 cm) when infected with (+FOV) and without (−FOV) pathogen inoculation, respectively. The control group, on the other hand, had the lowest root length, with measures of 15.3 cm under disease stress

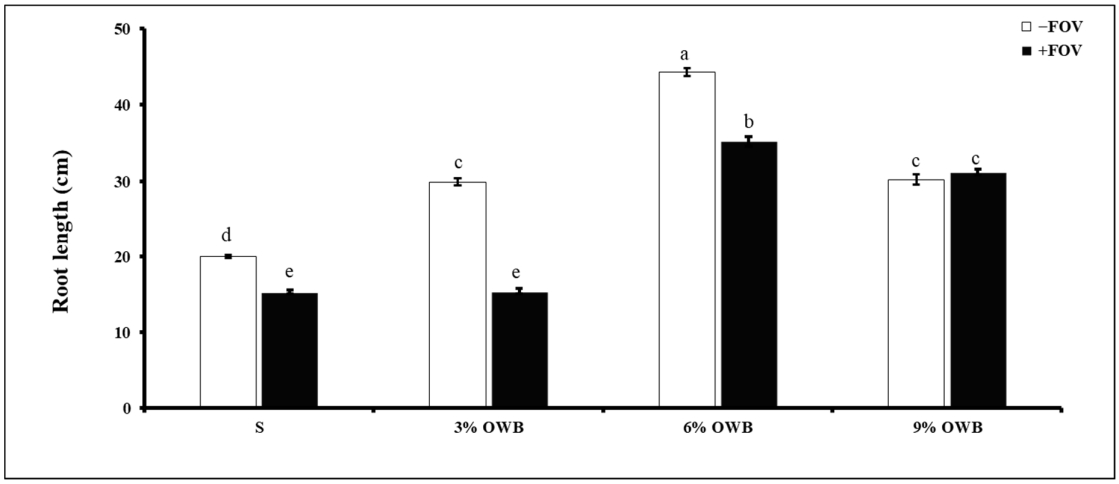

Figure 2. Organic waste biochar (3, 6, and 9%) and FOV effect on root length of cotton plant, including soil-only control, soil amended with 3% OWB, soil amended with 6% OWB, and soil amended with 9% OWB, either with (+FOV) or without (−FOV) pathogen. Bars followed by same letters are not significantly different.

3.1.3. Shoot Dry Weight

The lowest dry shoot weight (11.7 g) among the biochar-amendment treatments was found in the 3% OWB treatment (S + 3% OWB + FOV) with pathogen-induced disease stress (+FOV), while the highest (20.9 g) was found in the plants grown in the 9% organic waste biochar treatment (S + 9% OWB-FOV) without pathogen inoculation. It was significant ($p \leq 0.001$) in all treatments when the pathogen (FOV) caused a decrease in the shoot dry weight (Figure 3). In the presence of pathogen stress, the 9% organic waste biochar treatment (S + 9% OWB + FOV) produced the highest shoot dry weight (16.2 g), whereas the soil control with no biochar amendments produced the lowest (7.3 g).

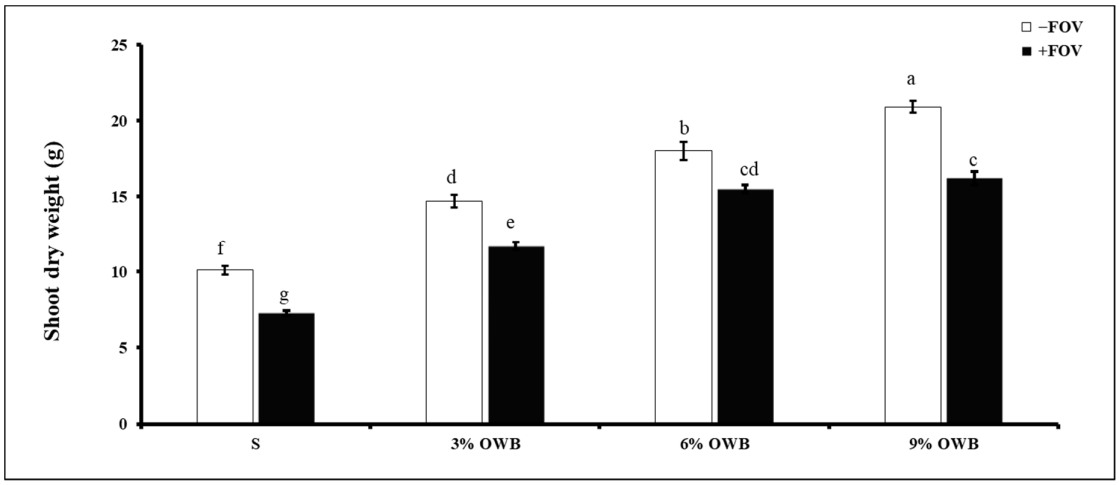

Figure 3. Organic waste biochar (3, 6, and 9%) and *Fusarium oxysporum* f. sp. *vasinfectum (FOV)* effect on shoot dry weight of cotton plant, including soil-only control, soil amended with 3% OWB, soil amended with 6% OWB, and soil amended with 9% OWB, either with (+FOV) or without (−FOV) pathogen. Bars followed by same letters are not significantly different.

3.1.4. Root Dry Weight

In all treatments, with or without the addition of biochar, FOV inoculation decreased the dry weights of the plant's above- and below-ground portions (Figures 3 and 4). However, in the 6% organic waste biochar modified treatment (S + 6% OWB), either inoculated (+FOV) or un-inoculated (−FOV), respectively, the highest root dry weight (2.25 and 2.95 g)

was found (Figure 4). The lowest root dry weights (0.66 and 1.23 g) were found in unaltered soil controls under disease stress and without disease stress, respectively. For plants cultivated in 6% OWB (S + 9% OWB + FOV), there was an increase in root dry biomass of (57.34 and 30.03%) compared to 3% OWB (S + 3% OWB + FOV) and 9% OWB (S + 6% OWB + FOV) treatments

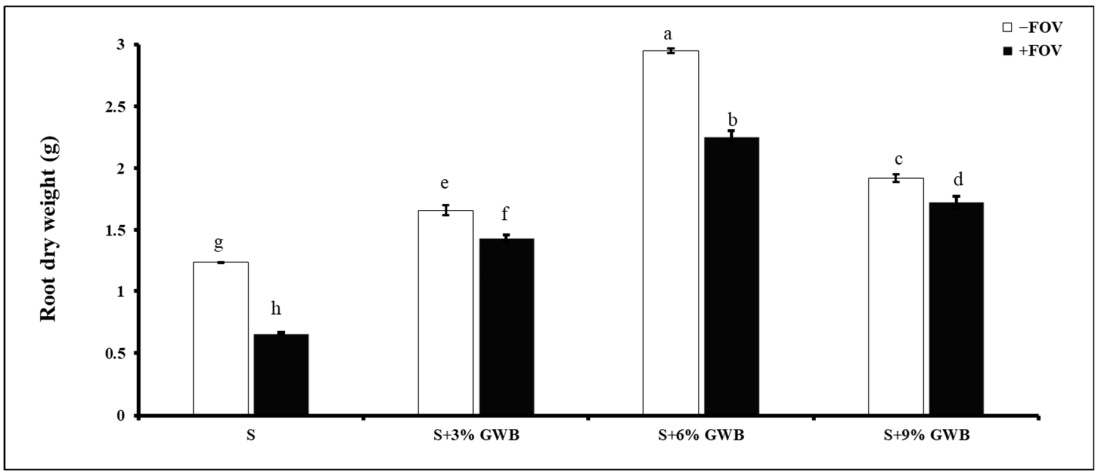

Figure 4. Organic waste biochar (3, 6, and 9%) and *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) effect on root dry weight of cotton plant, including soil-only control, soil amended with 3% OWB, soil amended with 6% OWB, and soil amended with 9% OWB, either with (+FOV) or without (−FOV) pathogen. Bars followed by same letters are not significantly different.

3.2. Nutritional Contents [Nitrogen (N), Phosphorus (P) and Potassium (K)] of Cotton Plants

On the N, P, and K contents of cotton plants, a very significant two-way ($p \leq 0.001$) interaction impact was found between soil compositions (S) and *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) (Table 1). Overall, the 9% OWB amended soil had a good effect on cotton plants' nutritional makeup both under disease stress and without it.

For plants cultivated in 9% OWB modified treatment that were either inoculated (+FOV) or un-inoculated (−FOV) with *Fusarium oxysporum* f. sp. *vasinfectum*, respectively, greater percentages of nitrogen (N) (4.19 and 4.38%) are shown. For the unaltered soil control under disease stress, the minimal (2.77%) N contents were assessed (Figure 5).

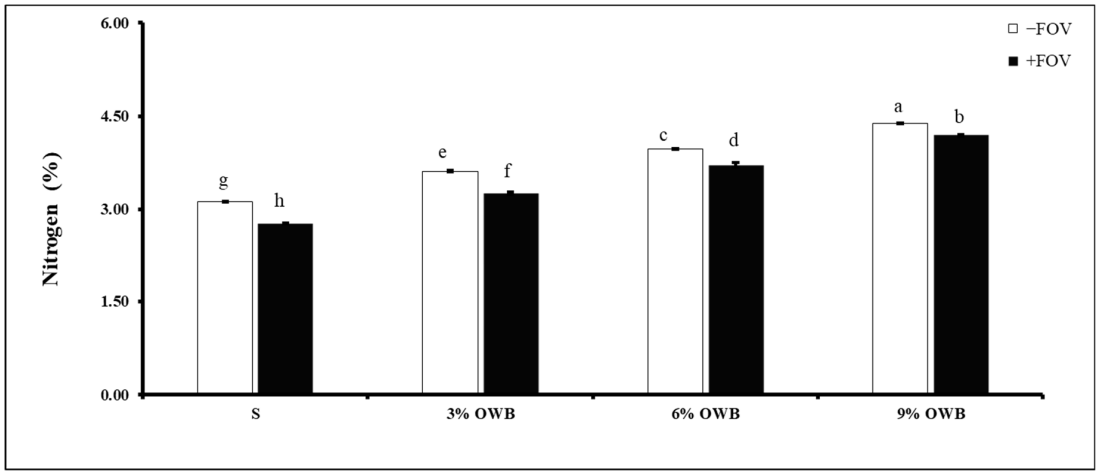

Figure 5. Organic waste biochar (3, 6, and 9%) and FOV effect on nitrogen of cotton plant, including soil-only control, soil amended with 3% OWB, soil amended with 6% OWB, and soil amended with 9% OWB, either with (+FOV) or without (−FOV) pathogen. Bars followed by same letters are not significantly different.

The highest significant values of phosphorus (P) content (0.83 and 0.91%) were found for plants growing in 6% OWB modified treatments that were either infected (+FOV) or not inoculated (−FOV) with *Fusarium oxysporum* f. sp. *vasinfectum* (Figure 6). While the unaltered soil control under disease stress had the lowest P levels (0.61%).

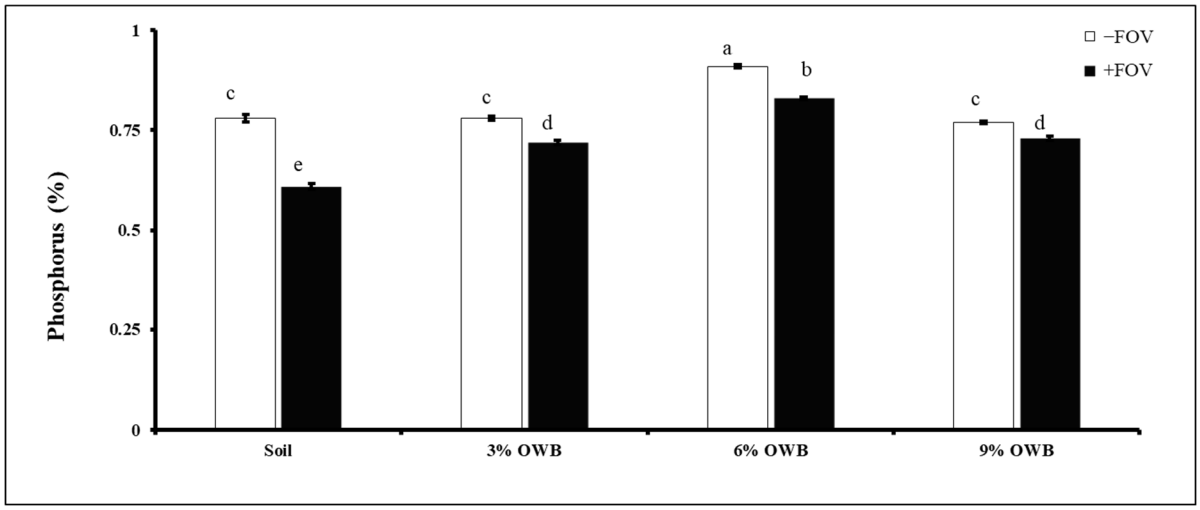

Figure 6. Organic waste biochar (3, 6, and 9%) and FOV effect on phosphorus of cotton plant, including soil-only control, soil amended with 3% OWB, soil amended with 6% OWB, and soil amended with 9% OWB, either with (+FOV) or without (−FOV) pathogen. Bars followed by same letters are not significantly different.

FOV significantly decreased the potassium (K) content of cotton plants, as seen in Figure 7. For plants cultivated in 9% OWB modified treatment, either un-inoculated (−FOV) or inoculated (+FOV) with *Fusarium oxysporum* f. sp. *vasinfectum*, respectively, the maximal K content was 2.18 and 1.97%. On the other hand, the unaltered soil control under disease stress had a minimum K content of 1.22%.

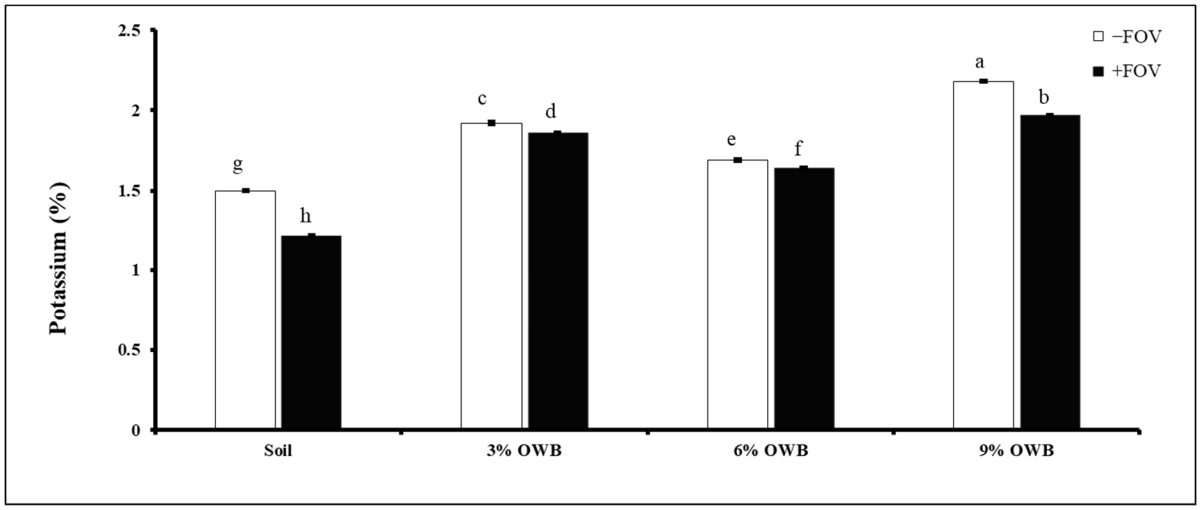

Figure 7. Organic waste biochar (3, 6, and 9%) and FOV effect on potassium of cotton plant, including soil-only control, soil amended with 3% OWB, soil amended with 6% OWB, and soil amended with 9%OWB, either with (+FOV) or without (−FOV) pathogen. Bars followed by same letters are not significantly different.

3.3. Effect of FOV and Organic Waste Biochar on Chlorophyll Contents of Cotton Plants

The amount of chlorophyll in cotton plants was considerably impacted by all major parameters, including soil composition and FOV, in addition to the interacting effect of

SC × FOV ($p \leq 0.001$). The amount of chlorophyll in cotton plants was, however, greatly enhanced by the soil composition containing 9% OWB, as shown in Table 3. In the absence of FOV, plants produced in the "S + 9% OWB" and "S + 6% OWB" treatments, respectively, had maximum chlorophyll levels of 45.2 ± 0.63 and 43.5 ± 0.52. The amount of chlorophyll was reduced overall as a result of FOV inoculation. However, regardless of the concentration, the plants infected with FOV and cultivated in biochar-enhanced treatments have maintained the level of chlorophyll contents. The maximum chlorophyll contents under Fusarium wilt stress (44.69 ± 0.33) were found in the "S + 9% OWB" treatment, whereas the lowest levels (30.9 ± 0.73) were found in the soil control in the absence of biochar amendment.

Table 3. Effect of organic waste biochar (3, 6, and 9%) and FOV on chlorophyll contents (SPAD value) of cotton plant in different treatments, including soil-only control, soil amended with 3% OWB, soil amended with 6% OWB, and soil amended with 9% OWB, either inoculated (+FOV) or un-inoculated (−FOV) pathogen. The mean values followed by different letters in each column represent significant differences among treatments.

Treatments		Chlorophyll Contents (SPAD Value)
Soil	−FOV	36.2 ± 0.42 ^{f}
	+FOV	30.9 ± 0.73 ^{g}
S + 3% OWB	−FOV	41.6 ± 0.51 ^{d}
	+FOV	39.65 ± 0.47 ^{e}
S + 6% OWB	−FOV	43.5 ± 0.52 ^{b}
	+FOV	42.5 ± 0.52 ^{c}
S + 9% OWB	−FOV	45.2 ± 0.63 ^{a}
	+FOV	44.4 ± 0.69 ^{a}

3.4. Plant Disease Assessment

The response of cotton plants to FOV inoculation was classified as highly sensitive or resistant depending on the type of soil (Figure 8). For plants cultivated in soil (S + FOV) treatment, a highly susceptible (HS) disease response was seen, with a maximum percent disease index (PDI) of 80% and a 100% disease incidence (Figure 9). However, disease growth was suppressed in soil that had been modified with biochar. Only a highly resistant (HR) plant response to Fusarium wilt development was seen, with the lowest 10% PDI and disease incidence of 20% being reported in the 9% biochar-altered treatment (S + 9% OWB + FOV). PDI and disease incidence fell dramatically for all biochar-altered treatments.

In the unaltered soil control, the highest disease severity was observed at 68.33% (Figure 8). However, plants cultivated in the 9% OWB altered treatment (S + 9% OWB + FOV) showed a minimal disease severity of 7.75%.

FOV-infected plants that were grown in a variety of soil substrate compositions had AUDPC values that ranged from 209.37 to 1785.07 overall (Figure 9). The highest AUDPC score represents a cotton plant's severe susceptibility to infections, while the lowest value reflects a plant's resistance to Fusarium wilt. The soil control (S + FOV) without any amendment resulted in the highest AUDPC (1785.07) and the lowest AUDPC (209.37) was calculated for plants grown in the S + 9% OWB + FOV treatment. Although the AUDPC of cotton plants was significantly decreased by biochar at 3 and 6%, the greatest reduction in AUDPC was seen for plants cultivated in 9% OWB, followed by 6% and 3% (Figure 9).

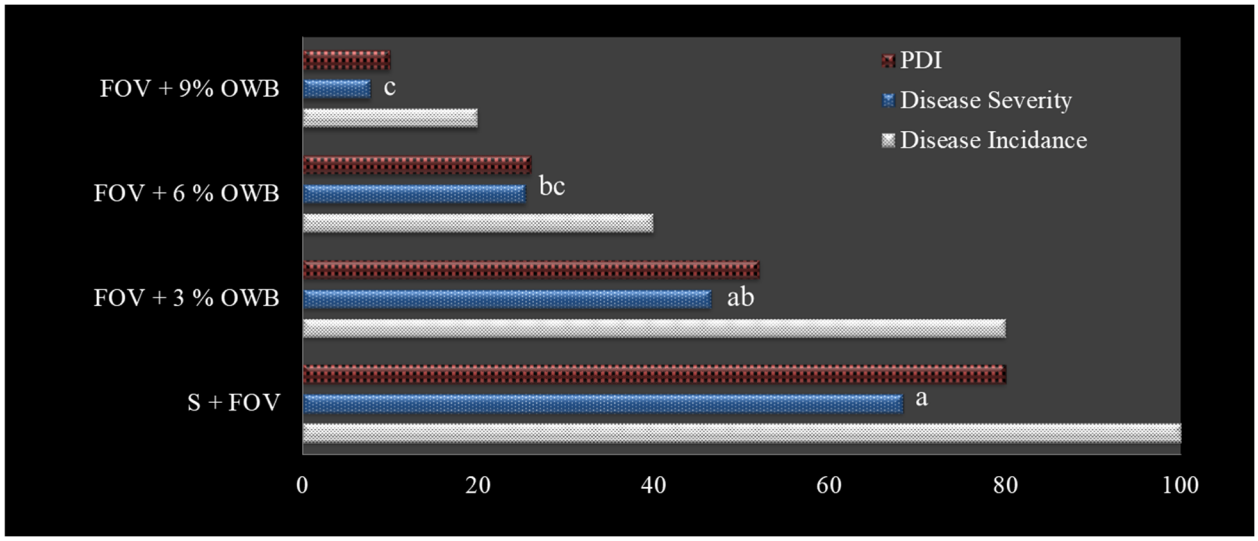

Figure 8. Effect of organic waste biochar (3, 6, and 9%) and FOV on percent disease index (PDI), Disease severity (DS), and disease incidence in different treatments, including soil-only control, soil with 3% OWB, soil with 6% OWB, and soil with 9% OWB under pathogen stress (+FOV). Bars representing disease severity followed by different letters are significantly different.

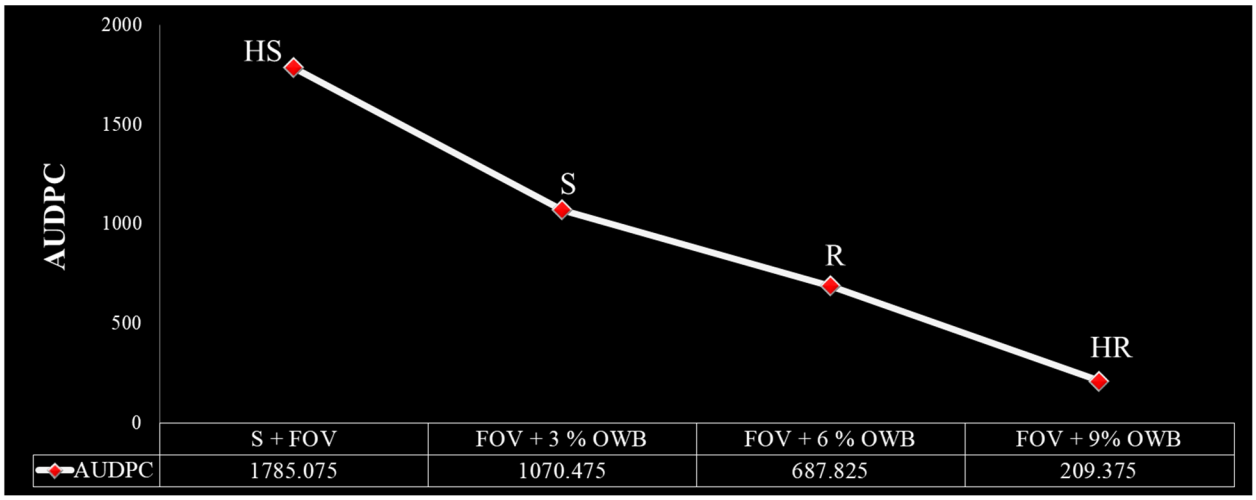

Figure 9. Effect of organic waste biochar (3, 6, and 9%) and FOV on area under disease progress curve (AUDPC) and disease response (DR) in different soil compositions, including soil, soil with 3% OWB, soil with 6% OWB, and soil with 9% OWB under pathogen stress (+FOV). HS, S, R and HR corresponds to Highly susceptible, Susceptible, Resistant and Highly Resistant plant response to Fusarium wilt, respectively.

3.5. Biochemical Analysis of Cotton Plants

The amount of phenol in the cotton plant, measured in mg/g of fresh weight, was assessed. Between soil composition (S) and FOV, phenol activity was shown to be significantly influenced. The plants cultivated in a 6% organic waste biochar in the presence of pathogen (+FOV) had the highest phenol concentrations of any treatment (35.3 ± 0.67 mg/g of f.wt) (Table 4). The soil-only control treatment had the lowest 21.2 ± 0.91 mg/g of f.wt phenol levels measured.

Table 4. Organic waste biochar (3, 6, and 9%) and *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) effects on biochemical compound of cotton plant, including soil-only control, soil amended with 3% OWB, soil amended with 6% OWB, and soil amended with 9% OWB, either with (+FOV) or without (-FOV) pathogen. The mean values followed by different letters in each column represent significant differences among treatments.

Treatment	Total Phenolic (mg/g of f.wt)	Catalase (mg/g of f.wt)	Flavonoids (mg/g of f.wt)	Total Proteins (mg/g of f.wt)
S	21.2 ± 0.91^{f}	118.7 ± 0.94^{h}	16.9 ± 1.19^{f}	12.5 ± 1.17^{f}
S + FOV	24.6 ± 0.45^{e}	129.9 ± 1.37^{f}	17.5 ± 0.70^{f}	16.9 ± 1.19^{e}
S + 3% OWB	29.7 ± 0.82^{d}	123.8 ± 1.03^{g}	19.3 ± 1.33^{e}	17.5 ± 0.70^{e}
S + 3% OWB + FOV	32.5 ± 0.52^{c}	138.8 ± 0.78^{c}	20 ± 1.33^{e}	21.7 ± 0.94^{d}
S + 6% OWB	33.8 ± 0.78^{bc}	133.7 ± 0.82^{e}	24.8 ± 0.78^{d}	28.2 ± 1.39^{b}
6% OWB + FOV	35.3 ± 0.67^{a}	147.7 ± 0.67^{a}	27.2 ± 0.63^{c}	35.3 ± 0.67^{a}
S + 9% OWB	34.3 ± 0.82^{ab}	137.4 ± 1.07^{d}	30.1 ± 1.19^{b}	24.8 ± 0.78^{c}
9% OWB + FOV	34.9 ± 1.79^{ab}	142 ± 0.94^{b}	33.4 ± 1.34^{a}	27.2 ± 0.63^{b}

All tasting factors had a significantly substantial interaction influence on the catalase activity in cotton plants. There was a difference in catalase concentration of 8.8 and 1.70 percent in treatments with 6% biochar modified (+FOV) compared to 3% OWB and 9% OWB, respectively (Table 4). When compared with the soil-only treatment under pathogen stress, there was a 36.27% percentage difference in the 6% biochar-amended treatment. The lowest catalase content was noted in the non-amended soil-only control without pathogen stress.

The quantity of flavonoids in cotton plants was calculated as mg/g of fresh weight. The plants cultivated in 9% OWB, either infected (+FOV) or un-inoculated (−FOV), respectively, had the highest flavonoid levels (33.4 and 30.1 mg/g of f.wt) of any of the treatments (Table 4). While the soil control in the absence of biochar had the lowest 16.91 ± 0.19 mg/g of f.wt flavonoids concentrations on record. In cotton plants, there was a discernible interaction between soil composition and FOV that affected phenol activity.

FOV significantly affected the total protein levels of all the treatments, both with and without the addition of biochar (Table 2). The protein levels for un-inoculated (−FOV) plants grown in potting soil devoid of biochar and the 6% organic waste biochar-comprising substrate under pathogen stress, respectively, ranged from 12.5 mg/g f.wt to 35.3 mg/g f.wt (Table 4).

3.6. Biochar Effect on Fusarium in Potting Mixture

Figure 10A depicts the effects of organic waste biochar at higher concentrations (3, 6, and 9%) on Fungus root colonization of cotton plants sown, amended, or un-amended, with biochar for 2 and 4 days following inoculation (beforehand symptoms appeared) and 5, 7, 9, 11, 13, and 15 days following inoculation (when symptoms typically appeared). However, after three days, Fusarium colonization was dramatically reduced in treatments with the 9% biochar amendment (reduction in CFU by up to 29.57%). Biochar had no impact on Fusarium colonization on the first day of inoculation.

Figure 10B depicts the impact of biochar on Fusarium survival in the potting mixture during a 45-day period. The Fusarium population's ultimate concentrations in the charcoal treatments were 4.95, 4.44, 4.19, and 3.8 CFU Log^{-1} in control, 3, 6, and 9% OWB, respectively. This is despite the fact that after 5 days, Fusarium abundance in all biochar-amended treatments was found to be considerably lower than in the control treatment ($p \leq 0.001$).

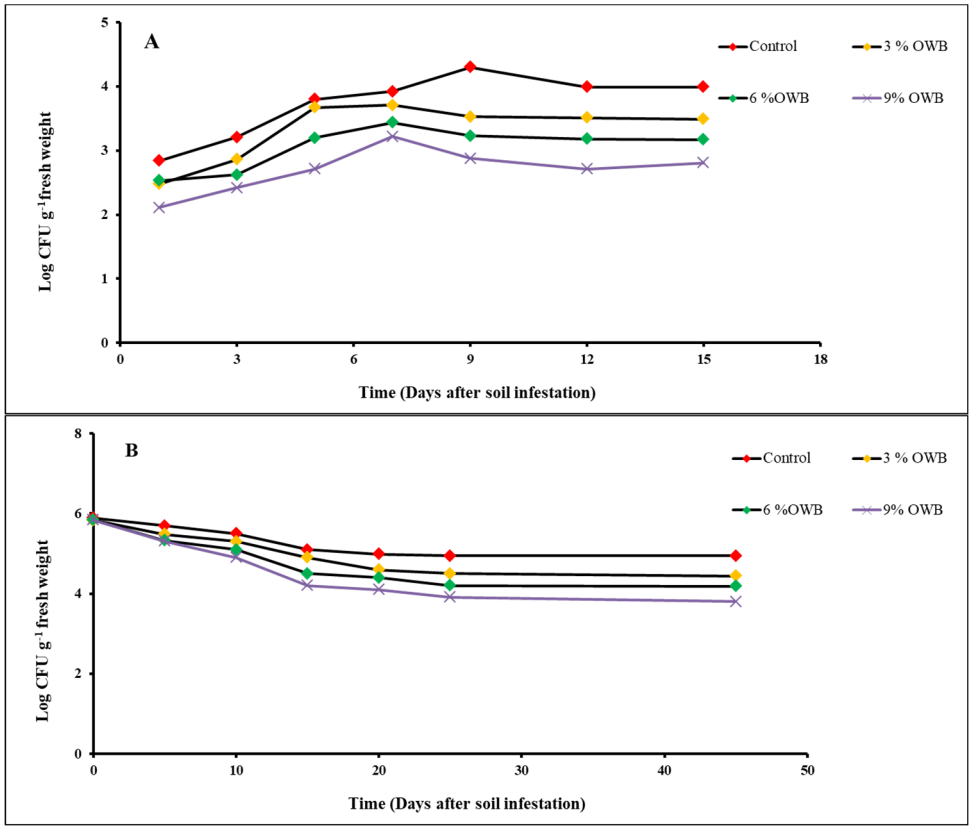

Figure 10. Effect of organic waste biochar at concentrations of 3, 6, and 9% with compost on *Fusarium* root colonization (**A**) and survival in soil potting mixture (**B**). As log CFU g^{-1} fresh root or dry potting media, *Fusarium* counts are displayed.

3.7. Biochar's Impact on the Diversity of Culturable Populations of Microbes

At all biochar doses (3, 6, and 9%), fluorescent *Pseudomonas* spp., *Actinomycetes* spp., and *Trichoderma* spp. counts increased in comparison to the unmodified control (Table 5). The amount of yeast did not significantly alter after the addition of charcoal.

Table 5. Effect of organic waste biochar (OWB) with compost on culturable rhizosphere bacteria at increasing concentrations (3, 6, and 9%).

Microorganism	Stress	Soil	S + 3% OWB	S + 6% OWB	S + 9% OWB
		CFU g^{-1} dry potting mixture			
General Bacteria	−FOV	6.52×10^7	1.38×10^8	1.24×10^8	7.38×10^9
	+FOV	5.93×10^7	1.25×10^8	1.31×10^8	6.79×10^9
Fluorescent Pseudomonas	−FOV	3.14×10^4	3.11×10^6	4.13×10^6	6.92×10^6
	+FOV	3.09×10^4	2.83×10^6	2.99×10^6	4.33×10^6
Actinomycetes spp.	−FOV	1.07×10^5	1.01×10^5	1.69×10^5	4.13×10^5
	+FOV	0.95×10^5	0.98×10^5	1.19×10^5	2.11×10^5
Filamentous Fungi	−FOV	3.93×10^5	3.91×10^5	3.92×10^5	4.12×10^5
	+FOV	3.06×10^5	3.12×10^5	3.39×10^5	3.18×10^5
Trichoderma spp.	−FOV	3.74×10^5	3.41×10^5	4.18×10^5	6.99×10^5
	+FOV	3.18×10^5	3.11×10^5	3.68×10^5	5.48×10^5
Yeasts	−FOV	1.03×10^4	1.03×10^4	1.11×10^4	1.21×10^4
	+FOV	1.07×10^4	0.95×10^4	0.96×10^4	1.13×10^4

3.8. Diversity and Composition of the Rhizosphere's Bacterial Population Effected by Biochar

To investigate the biochar's effect on the diversity and quantity of bacterial communities in the rhizosphere, 16S rRNA gene amplicons were sequenced using the Illumina platform. The Proteobacteria, Bacteroidetes, Acidobacteria, and Actinobacteria made up the bulk of the bacteria in both the biochar-amended (90%) and non-amended (91%) rhizospheres. Proteobacteria, Bacteroidetes, Verrucomicrobia, and Firmicutes, which constitute 52.3, 16.7, 2.92, and 0.58% of the total phyla, respectively, showed substantially greater relative abundances in the biochar-amended rhizosphere ($p \leq 0.05$). Actinobacteria and Gemmatimonadetes were similarly prevalent, making up 5.92, 6.23, 0.2, and 0.53% of the rhizospheres that had not been altered by biochar and those that had. On the other hand, Planctomycetes 1.2% and Acidobacteria 8.1% had significantly lower relative abundances in the biochar-altered rhizosphere (Table 6) than in the non-amended rhizosphere.

Table 6. Phylum-level relative abundances of the bacterial composition of the rhizosphere as determined by the Illumina sequencing of 16S rRNA gene amplicons are affected by the organic waste biochar.

Phylum	Stress	Soil	3% OWB	6% OWB	9% OWB
Proteobacteria	−FOV	44.1	51.2	51.4	52.3
	+FOV	39.2	41.2	43.4	45.2
Bacteroidetes	−FOV	9.5	12.3	14.3	16.7
	+FOV	7.3	9.7	11.4	14.9
Acidobacteria	−FOV	18	7.2	6.4	8.1
	+FOV	15	6.3	5.0	7.8
Actinobacteria	−FOV	5.92	6.12	6.01	6.23
	+FOV	5.31	5.43	5.39	5.89
Verrucomicrobia	−FOV	1.3	2.2	2.78	2.92
	+FOV	1.1	2.2	2.24	2.43
Firmicutes	−FOV	0.19	0.25	0.46	0.58
	+FOV	0.07	0.09	0.21	0.31
Gemmatimonadetes	−FOV	0.2	0.4	0.48	0.53
	+FOV	0.1	0.33	0.38	0.49
Planctomycetes	−FOV	2.01	1.11	1.03	1.2
	+FOV	1.71	1.04	1.13	1.15

The relatively large quantity of the major microbial orders was considerably changed by biochar alterations, as shown by the heatmap analysis in Figure 11 and the association findings in Figure 12. The most prevalent bacterial orders included the Burkholderiales, Xanthomonadales, and others mentioned in the table. However, in the biochar-modified rhizosphere community, the relative quantity of Acidobacteriales, Rhodospirillales, and Frankiales was much lower.

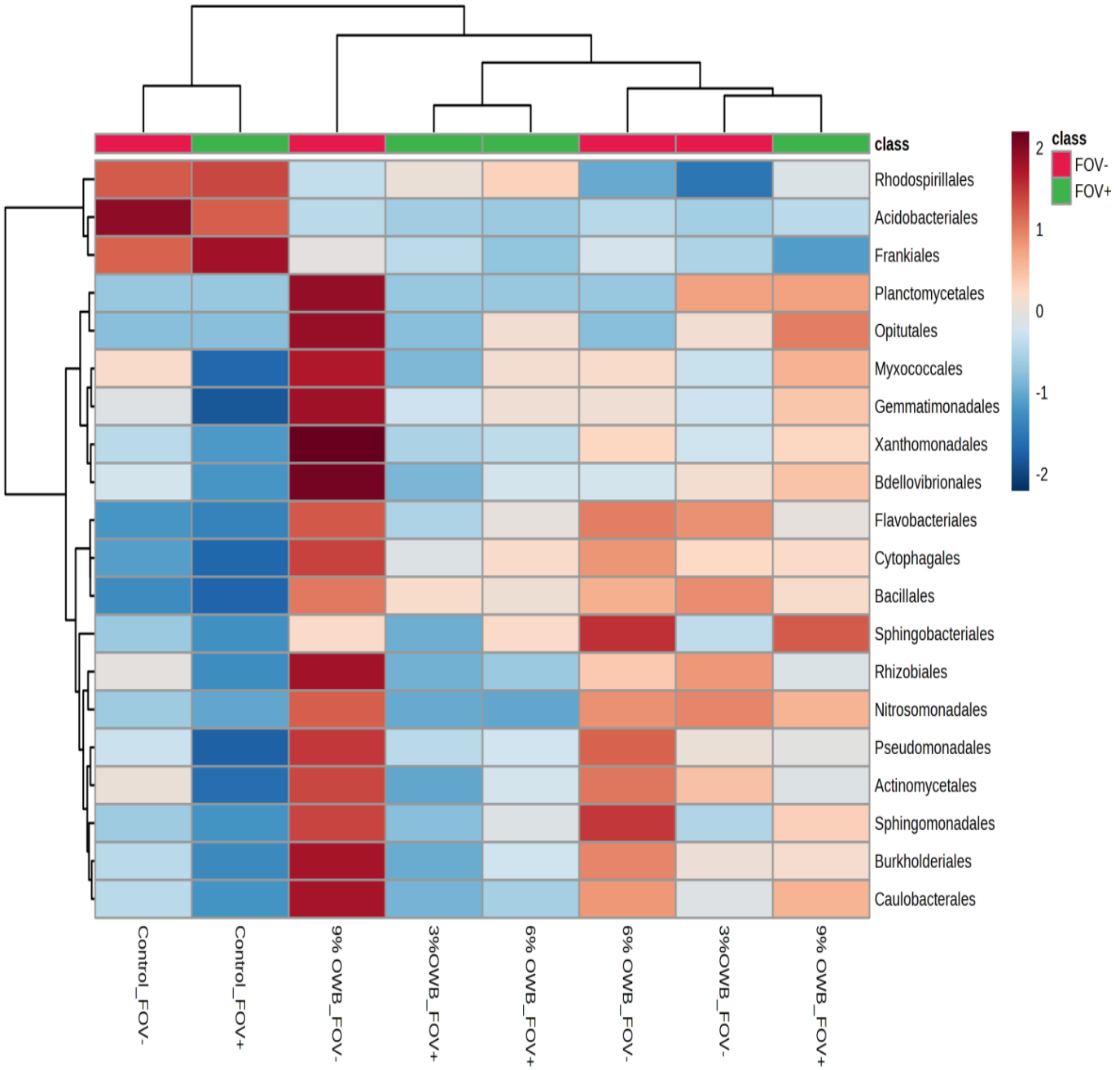

Figure 11. Clustering results in the form of a heatmap of relative abundances of the major bacterial orders in different treatments, including soil only, soil amended with 3% OWB, soil amended with 6% OWB, and soil amended with 9% OWB, either inoculated (+FOV) or un-inoculated (−FOV) with a pathogen.

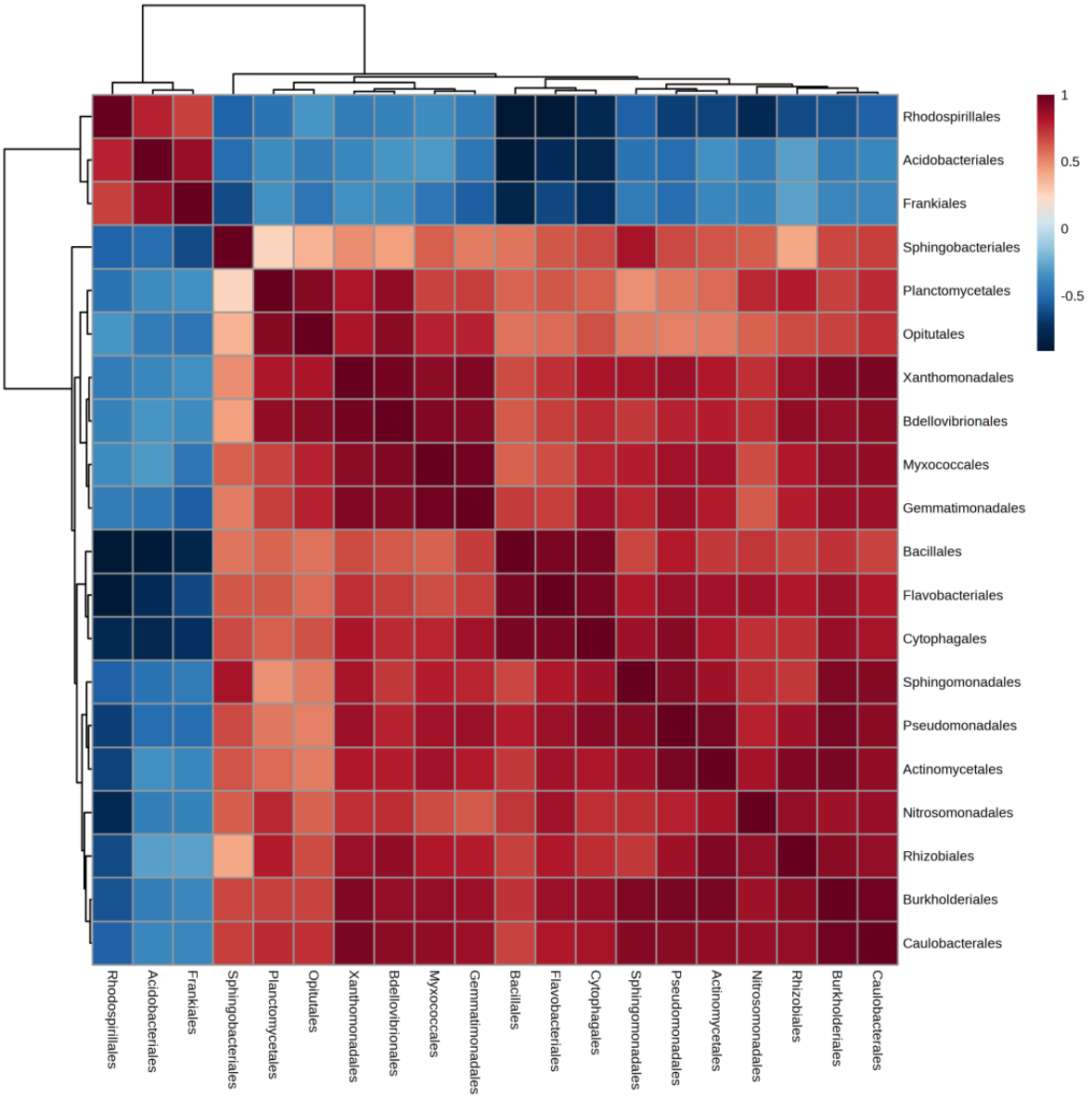

Figure 12. Correlation of relative abundances of the major bacterial orders in different soil treatments, including soil-only control, soil amended with 3% OWB, soil amended with 6% OWB, and soil amended with 9% OWB, either inoculated (+FOV) or un-inoculated (−FOV) with a pathogen.

4. Discussion

Emissions of greenhouse gases need to be decreased if we are going to combat global warming. Pollution caused by fossil fuels is the main cause of the anthropogenic greenhouse effect, making a decrease in the usage of fossil fuels a top concern [1]. Yet, a responsible plan also includes actively removing carbon dioxide from the atmosphere because some emissions will be inevitable [2]. Such carbon sequestration confronts a variety of difficulties, including the need for a long-term, significant net removal of carbon dioxide, as well as the need for accountability and a low danger of large-scale or quick leakage. For the soil to retain its physical, chemical, and biological integrity, as well as to carry out its agricultural production and environmental functions, a certain threshold level of organic matter must be maintained in the soil [3].

Biochar is a carbon-rich substance that is created from organic feedstock using thermal burning methods with minimum oxygen. According to the worldwide biochar project [65], biochar is a high-carbon byproduct of pyrolysis which leads to the growth of plants and a

decrease in plant diseases [9]. To create biochar, biomass is pyrolyzed in an environment with regulated oxygen levels. When a diversity of organic wastes are heated to temperatures between 200 to 900 °C with little to no oxygen or air, the process is known as pyrolysis [10]. Compost and biochar have been recognized as potent pesticide-free solutions for boosting agricultural output/yield in the twenty-first century. Although adding biochar to the soil is an age-old method of improving soil quality [66], it has only been in the past two decades that researchers have shown a substantial interest in biochar as an organic plant protection agent and a carbon sequestration tool [67]. We believe that the findings presented here indicate for the first time the effectiveness of compost, and biochar used in combination in reducing the impact of the soil-born pathogen *Fusarium oxysporum* f. sp. *Vasinfectum* in cotton and linking the soil microbial biodiversity in response to biochar. According to an analysis conducted after one year of the biochar application, the population of Acidobacteria and Gemmatimonadetes were observed to be enhanced in the treatment plots with biochar [68]. While in another research biochar increased the quantity of Actinobacteria, Proteobacteria, and Planctomycetes, however, the increase in the number of particular microbes varied depending on the kind of biochar used [69].

The activation of plant defense systems is connected to the bacteria in the rhizosphere, also known as the second genome of the plants [70]. Additionally, prior studies demonstrated that repeated monoculture could significantly boost the population of harmful pathogens while reducing the abundance of potentially advantageous diversity in the rhizosphere soil [71]. In this work, we showed how dynamic variations in the microscopic communities of the rhizosphere under the stress of Fusarium wilt were influenced by the addition of biochar with compost. According to the recent research findings [72], using biochar and *B. subtilis* together resulted in the improvement of soil texture and decreased Fusarium wilt incidence while positively influencing radish growth. There was a significant rise in the number of advantageous microorganisms, thus having a competition for space and food with pathogenic microbes in the rhizosphere. The findings demonstrated that the application of biochar increased the total amount of bacteria and fungus, but clearly had a detrimental impact on pathogenic *F. oxysporum*. Additionally, following biochar amendment, the contents of possibly beneficial microorganisms increased. Our findings are in line with those of [60] who found a considerable increase in the variety of tomato rhizobacteria that may promote plant development following amendment with biochar. The rhizosphere's microbial community's structure and functional capabilities were considerably altered by biochar inputs. Increased microbial activity and the abundance of numerous groups strongly associated with biocontrol and PGPR were also brought about by the addition of biochar to the soil [73]. The strains with known growth-promoting effects or biocontrol potential were increased in biochar-amended soils. Among these strains were *Pseudomonas fluorescens*, *P. putida*, *P. koreensis*, *P. moraviensis*, and *P. monteilii*, all of which have previously been identified as PGPR, and *Trcihoderma* spp. with potential biocontrol activity [74,75]. *In vitro*, we observed that biochar considerably reduced the development of *F. oxysporum*, which is consistent with past findings regarding bacterial and Fusarium wilt [76,77]. The root zone of the biochar-altered treatments showed a greater comparative quantity of Proteobacteria, Bacteroidetes, and Firmicutes phyla and lower relative abundances of Acidobacteria and Planctomycetes, following trends seen in previous research in biochar-altered bulk soils and rhizospheres [78,79].

The effect of biochar on the spread of soil-borne diseases has been the subject of some very conflicting earlier research. While some studies found very modest [80] or no impact [81] on disease suppression, some found a rise in disease severity [82] as a result of biochar alteration. Others who used either a U-shaped dose-response arc [83] or a linear dose-response curve [84] observed a reduction in disease severity. Similarly, in our study, we observed the reduction of disease severity and incidence of FOV with the increase in the concentration of biochar with compost as well. According to [85], at much lower biochar concentrations, the pathogens that cause powdery mildew and grey mold on strawberries demonstrate reduced disease dominance. While pinewood biochar was shown

to be effective against replant disease in sensitive peach rootstock by [86] at significantly higher treatment rates, i.e., 10 and 20% (*v*/*v*), biochar-based stimuli sufficiently trigger the defense response at the molecular level. Previous studies suggested that induced resistance in plants was influenced by the signaling pathways created by SA, JA, and ET [87]. The soil's porosity, pH, CEC, organic carbon content, and easily available nutrients were all increased by the addition of biochar, while its bulk density was lowered. Our research demonstrated that OWB amendment raised soil pH and amounts of bioavailable P and K regardless of pathogen inoculation.

The use of biochar separately and in combination with compost significantly improved the examined parameters. This could be because biochar has a great potential for retaining nutrients due to its vast pore spaces, which contribute to the enhancement of nutrient availability for plants. According to [88], using biochar made from cow dung resulted in a 98–150% increase in production. The authors of [18] Found that compost application to soil results in greater biomass output, indicating that the compost supplies enough nutrients. In his further studies, he found that biochar and compost may be a source of exchangeable and readily accessible phosphorus by either making the natural phosphorus available or applying it directly. Higher phosphorus concentrations in shoot, grain, and soil supported the positive effects of organic inputs on plant growth since there was enough phosphorus available [89]. The addition of biochar and compost improved crop stand, development, and yield, which boosted plant uptake of potash [16]. Improved nitrogen and phosphorus accessibility may have contributed directly or indirectly to the increased potassium content in plants and soil. It is reasonable to believe that the greater concentration of nutrients found in biochar is a result of nutrient saturation during the pyrolysis of biomass. Similar to many other studies, in this study we found a significant increase in the percentage of N, P, and K in cotton plant shoots with the increasing organic waste biochar concentration. Furthermore, the increased availability of N, P, and K may have an impact on the enzymatic activity of soil microbes, which would greatly boost soil fertility [90].

In general, FOV decreases the effectiveness of photosynthesis and other physiological processes in the plant because conductive arteries are blocked by fungal mycelium or because of the toxicity of chemicals released by Fusarium [91]. Reduced water flow slows down the rates of photosynthesis, CO_2 fixation, and transpiration as well as the activity of the chloroplasts' electron-transport chain and stomatal conductance [92]. In the current study, biochar increased the chlorophyll content in both the pathogen-free and pathogen-containing conditions, and it even partially offset the negative impacts of FOV on the development and physical state of the cotton plant. This could be because the usage of biochar has reduced the colonization of FOV in roots as demonstrated in the results. In the presence of biochar, [93] discovered significant activation of plant genes controlling plant development hormones and the photosynthetic apparatus in Arabidopsis. Although the Fusarium survival rates were decreased in the biochar-altered treatment, both in the soil mixture and to a greater extent in the vicinity of the cotton roots, biochar was not directly harmful to the pathogen, as demonstrated by the absence of biochar influence on radial mycelium development. A similar outcome has already been documented in other pathosystems [94]. This supports the hypothesis that the noticed reduction in disease may be caused by processes besides direct toxicity, such as an increase in beneficial bacteria abundance, variety, and activity, in addition to the development of immune systems, either by chemicals related to biochar or by microbes encouraged by biochar.

In our study, biochar application also increases the concentration of different bio-chemical like phenol protein in plant shoots. By producing lignin and suberin, which are necessary for the production of physical barriers that can stop the spread of infections, phenolic compounds may help to improve the mechanical strength of host cell walls [95]. It has been shown that changing the concentration of phenolic chemicals in plants might alter their susceptibility to disease [96]. These results are in line with research on other plant-pathogenic bacteria, viruses, and fungi interactions, which showed that a number of common phenols and phenolic compounds are poisonous to pathogens and have long been

recognized as crucial defense-related molecules. These chemicals are naturally present in high concentrations in resistant crop types, and they build up in plants following infection, especially in resistant kinds [97]. Numerous plant pathogenic interactions have shown evidence of the significance of specific proteins in plant disease resistance [98]. Our results demonstrated the significantly higher total protein content in 9% organic waste biochar amendment in both with (+FOV) and without (−FOV) pathogen stress. Similar outcomes in mollicutes-infected apple, grapevine, tomato, and maize have also been documented [98,99]. In this study, we also observed increases in other biochemical flavonoids and catalase content. The plants cultivated in 9% organic waste biochar, either infected (+FOV) or un-inoculated (−FOV), respectively, had the highest flavonoid levels (33.4 and 30.1 mg/g of f.wt) of any of the treatments. CAT is an oxygen-scavenging enzyme that performs the unique peroxidative function of shielding developing cells from the harmful effects of substrates (H_2O_2) that would otherwise be fatal [100,101]. Our understanding of how biochar helps to the emergence of disease resistance and the recycling of waste materials into carbon-rich soil amendments has substantially increased as a result of the study's findings. Organic alterations perhaps boosted the soil's organic carbon concentrations, making the soil's structure finally porous. As a result, it made it easier for roots to grow deeper into the soil to acquire more nutrients. These findings might potentially be utilized to create organic materials that can be used in place of conventional disease management techniques for preventing Fusarium wilt in cotton crops.

5. Conclusions

Plants can benefit from soil microorganisms like bacteria and fungi directly or indirectly. While other functions, such as those that promote plant development, are impacted by disease stress. Sequencing examination of cotton's rhizosphere microbes revealed that under normal and Fusarium-like conditions, a wide range of bacterial communities are produced. A reduction in soil-borne illness and increased plant performance has been shown to be significantly correlated with the modifications in microbial community organization, taxonomic variety, and microbial activity brought about by biochar. These results position the microbiome of the rhizosphere at the core of the elaborate, multi-mechanism model that postulates that the impact of biochar on establishing well-being and productivity is a result of complex interactions among an assortment of physical, chemical, and physiological components that make up the soil-plant-pathogen system.

Author Contributions: A.A., M.A. and M.S.H. conceptualized this research. A.A. planned the methodology, while M.A. performed the formal analysis as well as was responsible for writing—original draft preparation. A.A., M.S.H. and M.A. were responsible for visualization and validation of data. A.A. and M.S.H. were also responsible for writing—review and editing. M.S.H. and A.A. provided resources and raw material for research and also supervised the research. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data will be shared if required.

Acknowledgments: The authors are thankful to the University of the Punjab Lahore, Pakistan for providing consumables as well as laboratory and greenhouse facility for the experimentation.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. McKinstry, R.B., Jr.; Unterberger, G.L.; Mandelbaum, D.G.; Brown, C.B. State Global Climate Change Policy And Business Opportunity. *Oil Gas Energy Law* **2007**, *3*, 1–10.
2. Lackner, K.S. A Guide to CO_2 Sequestration. *Science* **2003**, *300*, 1677–1678. [CrossRef]

3. Izaurralde, R.C.; Rosenberg, N.J.; Lal, R. Mitigation of Climatic Change by Soil Carbon Sequestration: Issues of Science, Monitoring, and Degraded Lands. *Adv. Agron.* **2001**, *70*, 1–75.
4. Lehmann, J. Bio-Energy in the Black. *Front. Ecol. Environ.* **2007**, *5*, 381–387. [CrossRef]
5. Lehmann, J. A Handful of Carbon. *Nature* **2007**, *447*, 143–144. [CrossRef]
6. Suman, D.O.; Kuhlbusch, T.A.J.; Lim, B. Marine Sediments: A Reservoir for Black Carbon and Their Use as Spatial and Temporal Records of Combustion. In *Sediment Records of Biomass Burning and Global Change*; Springer: Berlin/Heidelberg, Germany, 1997; pp. 271–293.
7. Kuhlbusch, T.A.J. Black Carbon and the Carbon Cycle. *Science* **1998**, *280*, 1903–1904. [CrossRef]
8. Lehmann, J.; Gaunt, J.; Rondon, M. Bio-Char Sequestration in Terrestrial Ecosystems—A Review. *Mitig. Adapt. Strateg. Glob. Chang.* **2006**, *11*, 403–427. [CrossRef]
9. Elad, Y.; Cytryn, E.; Meller Harel, Y.; Lew, B.; Graber, E.R. The Biochar Effect: Plant Resistance to Biotic Stresses. *Phytopathol. Mediterr.* **2011**, *50*, 335–349.
10. Bonanomi, G.; Ippolito, F.; Scala, F. A "Black" Future for Plant Pathology? Biochar as a New Soil Amendment for Controlling Plant Diseases. *J. Plant Pathol.* **2015**, *97*, 223–234.
11. Chen, Z.; Kamchoom, V.; Chen, R.; Prasittisopin, L. Investigating the Impacts of Biochar Amendment and Soil Compaction on Unsaturated Hydraulic Properties of Silty Sand. *Agronomy* **2023**, *13*, 1845. [CrossRef]
12. Chandrasekaran, A.; Subbiah, S.; Ramachandran, S.; Narayanasamy, S.; Bartocci, P.; Fantozzi, F. Natural Draft-Improved Carbonization Retort System for Biocarbon Production from Prosopis Juliflora Biomass. *Energy Fuels* **2019**, *33*, 11113–11124. [CrossRef]
13. Sombroek, W.I.M.; Ruivo, M.D.L.; Fearnside, P.M.; Glaser, B.; Lehmann, J. Amazonian Dark Earths as Carbon Stores and Sinks. In *Amazonian Dark Earths: Origin Properties Management*; Springer: Dordrecht, The Netherlands, 2003; pp. 125–139.
14. Van Zwieten, L.; Kimber, S.; Morris, S.; Chan, K.Y.; Downie, A.; Rust, J.; Joseph, S.; Cowie, A. Effects of Biochar from Slow Pyrolysis of Papermill Waste on Agronomic Performance and Soil Fertility. *Plant Soil* **2010**, *327*, 235–246. [CrossRef]
15. Barrow, C.J. Biochar: Potential for Countering Land Degradation and for Improving Agriculture. *Appl. Geogr.* **2012**, *34*, 21–28. [CrossRef]
16. Steiner, C.; Glaser, B.; Teixeira, W.G.; Lehmann, J.; Blum, W.E.H.; Zech, W. Nitrogen Retention and Plant Uptake on a Highly Weathered Central Amazonian Ferralsol Amended with Compost and Charcoal. *J. Plant Nutr. Soil Sci.* **2008**, *171*, 893–899. [CrossRef]
17. Abiven, S.; Hund, A.; Martinsen, V.; Cornelissen, G. Biochar Amendment Increases Maize Root Surface Areas and Branching: A Shovelomics Study in Zambia. *Plant Soil* **2015**, *395*, 45–55. [CrossRef]
18. Agegnehu, G.; Bird, M.I.; Nelson, P.N.; Bass, A.M. The Ameliorating Effects of Biochar and Compost on Soil Quality and Plant Growth on a Ferralsol. *Soil Res.* **2015**, *53*, 1–12. [CrossRef]
19. Ozyigit, I.I.; Dogan, I.; Kaya, Y.; Bajrovic, K.; Gozukirmizi, N. Cotton Biotechnology: An Efficient Gene Transfer Protocol via Agrobacterium Tumefaciens for a Greater Transgenic Recovery. *J. Nat. Fibers* **2022**, *19*, 11582–11596. [CrossRef]
20. Ahmad, S.; Abbas, Q.; Abbas, G.; Fatima, Z.; Atique-Ur-rehman; Naz, S.; Younis, H.; Khan, R.J.; Nasim, W.; Rehman, M.H.U.; et al. Quantification of Climate Warming and Crop Management Impacts on Cotton Phenology. *Plants* **2017**, *6*, 7. [CrossRef]
21. Abbas, Q.; Ahmad, S. Effect of Different Sowing Times and Cultivars on Cotton Fiber Quality under Stable Cotton-Wheat Cropping System in Southern Punjab, Pakistan. *Pakistan J. Life Soc. Sci.* **2018**, *16*, 77–84.
22. Tariq, M.; Yasmeen, A.; Ahmad, S.; Hussain, N.; Afzal, M.N.; Hasanuzzaman, M. Shedding of Fruiting Structures in Cotton: Factors, Compensation and Prevention1. *Trop. Subtrop. Agroecosyst.* **2017**, *20*, 251–262.
23. Tariq, M.; Afzal, M.N.; Muhammad, D.; Ahmad, S.; Shahzad, A.N.; Kiran, A.; Wakeel, A. Relationship of Tissue Potassium Content with Yield and Fiber Quality Components of Bt Cotton as Influenced by Potassium Application Methods. *Field Crops Res.* **2018**, *229*, 37–43. [CrossRef]
24. Usman, M.; Ahmad, A.; Ahmad, S.; Arshad, M.; Khaliq, T.; Wajid, A.; Hussain, K.; Nasim, W.; Chattha, T.M.; Trethowan, R.; et al. Development and Application of Crop Water Stress Index for Scheduling Irrigation in Cotton (*Gossypium hirsutum* L.) under Semiarid Environment. *J. Food Agric. Environ.* **2009**, *7*, 386–391.
25. Karaş, E. Sustainable and Effective Management Strategies in Cotton Cultivation. Available online: https://www.intechopen.com/chapters/81421 (accessed on 23 June 2022).
26. Majeed, S.; Rana, I.A.; Mubarik, M.S.; Atif, R.M.; Yang, S.-H.; Chung, G.; Jia, Y.; Du, X.; Hinze, L.; Azhar, M.T. Heat Stress in Cotton: A Review on Predicted and Unpredicted Growth-Yield Anomalies and Mitigating Breeding Strategies. *Agronomy* **2021**, *11*, 1825. [CrossRef]
27. Ahmad, S.; Hasanuzzaman, M. *Cotton Production and Uses: Agronomy, Crop Protection, and Postharvest Technologies*; Springer Nature: Singapore, 2020.
28. Uluhan, E.; Keleş, E.N.; Tufan, F. Analysis of WRKY Transcription Factors in Barley Cultivars Infected with Fusarium Culmorum. *Int. J. Life Sci. Biotechnol.* **2019**, *2*, 165–174. [CrossRef]
29. Mohiddin, F.A. *Studies on the Development of Certain Fungal and Bacterial Biopesticides for the Management of Wilt Disease Complex of Chickpea Caused by Fusarium and Meloidogyne Species*; Aligarh Muslim University: Aligarh, India, 2007.

30. Liu, N.; Zhang, X.; Sun, Y.; Wang, P.; Li, X.; Pei, Y.; Li, F.; Hou, Y. Molecular Evidence for the Involvement of a Polygalacturonase-Inhibiting Protein, GhPGIP1, in Enhanced Resistance to Verticillium and Fusarium Wilts in Cotton. *Sci. Rep.* **2017**, *7*, 39840. [CrossRef] [PubMed]
31. Atkinson, G.F. Some Diseases of Cotton. 3. Frenching. *Ala. Agric. Exp. Stn. Bull.* **1892**, *41*, 19–29.
32. Dastur, R.H.; Arora, R.D.; Sawhney, K.; Sikka, S.M. Resource Productivity and Resource Use Efficiency in Sugarcane Production. *J. Agric. Res. Technol.* **2015**, *40*, 176.
33. Cook, R.J. Fusarium Diseases in the People's Republic of China. In *Fusarium Diseases, Biology, and Taxonomy*; Nelson, P.E., Tousson, T.A., Cook, R.J., Eds.; Penn State University Press: University Park, PA, USA, 1981.
34. Vandermeer, J.H. *The Ecology of Intercropping*; Cambridge University Press: Cambridge, UK, 1992.
35. Chohan, S.; Perveen, R.; Abid, M.; Naz, M.S.; Akram, N. Morpho-Physiological Studies Management and Screening of Tomato Germplasm against Alternaria Solani the Causal Agent of Tomato Early Blight. *Int. J. Agric. Biol.* **2015**, *17*, 111–118.
36. Akmal, M.; Maqbool, Z.; Khan, K.S.; Hussain, Q.; Ijaz, S.S.; Iqbal, M.; Aziz, I.; Hussain, A.; Abbas, M.S.; Rafa, H.U. Integrated Use of Biochar and Compost to Improve Soil Microbial Activity, Nutrient Availability, and Plant Growth in Arid Soil. *Arab. J. Geosci.* **2019**, *12*, 232. [CrossRef]
37. Mitchell, P.J.; Dalley, T.S.L.; Helleur, R.J. Preliminary Laboratory Production and Characterization of Biochars from Lignocellulosic Municipal Waste. *J. Anal. Appl. Pyrolysis* **2013**, *99*, 71–78. [CrossRef]
38. Aftab, Z.-H.; Aslam, W.; Aftab, A.; Shah, A.N.; Akhter, A.; Fakhar, U.; Siddiqui, I.; Ahmed, W.; Majid, F.; Wróbel, J.; et al. Incorporation of Engineered Nanoparticles of Biochar and Fly Ash against Bacterial Leaf Spot of Pepper. *Sci. Rep.* **2022**, *12*, 8561. [CrossRef]
39. Xi, J.; Li, H.; Xi, J.; Tan, S.; Zheng, J.; Tan, Z. Effect of Returning Biochar from Different Pyrolysis Temperatures and Atmospheres on the Growth of Leaf-Used Lettuce. *Environ. Sci. Pollut. Res.* **2020**, *27*, 35802–35813. [CrossRef] [PubMed]
40. Rasool, M.; Akhter, A.; Soja, G.; Haider, M.S. Role of Biochar, Compost and Plant Growth Promoting Rhizobacteria in the Management of Tomato Early Blight Disease. *Sci. Rep.* **2021**, *11*, 6092. [CrossRef] [PubMed]
41. Davoudpour, Y.; Schmidt, M.; Calabrese, F.; Richnow, H.H.; Musat, N. High Resolution Microscopy to Evaluate the Efficiency of Surface Sterilization of Zea Mays Seeds. *PLoS ONE* **2020**, *15*, e0242247. [CrossRef]
42. Zhu, Y.; Lujan, P.A.; Wedegaertner, T.; Nichols, R.; Abdelraheem, A.; Zhang, J.F.; Sanogo, S. First Report of *Fusarium oxysporum* f. Sp. *Vasinfectum* Race 4 Causing Fusarium Wilt of Cotton in New Mexico, USA. *Plant Dis.* **2020**, *104*, 588. [CrossRef]
43. Naika, S.; van Lidt de Jeude, J.; de Goffau, M.; Hilmi, M. *AD17E Cultivation of Tomato*; Agromisa Foundation: Wageningen, The Netherlands, 2005; ISBN 9085730392.
44. Awan, Z.A.; Shoaib, A.; Khan, K.A. Variations in Total Phenolics and Antioxidant Enzymes Cause Phenotypic Variability and Differential Resistant Response in Tomato Genotypes against Early Blight Disease. *Sci. Hortic.* **2018**, *239*, 216–223. [CrossRef]
45. Bradford, N. A Rapid and Sensitive Method for the Quantitation Microgram Quantities of a Protein Isolated from Red Cell Membranes. *Anal. Biochem.* **1976**, *72*, e254. [CrossRef]
46. Chia-Chi, C.; Ming-Hua, Y.; Hwei-Mei, W.; Jiing-Chuan, C. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colometric Methods. *J. Food Drug Anal.* **2002**, *10*, 3.
47. Tan, M. Analysis of DNA Methylation of Maize in Response to Osmotic and Salt Stress Based on Methylation-Sensitive Amplified Polymorphism. *Plant Physiol. Biochem.* **2010**, *48*, 21–26. [CrossRef] [PubMed]
48. Singleton, V.L.; Rossi, J.A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158. [CrossRef]
49. Saleh, B. Effect of Salt Stress on Growth and Chlorophyll Content of Some Cultivated Cotton Varieties Grown in Syria. *Commun. Soil Sci. Plant Anal.* **2012**, *43*, 1976–1983. [CrossRef]
50. Estefan, G. *Methods of Soil, Plant, and Water Analysis: A Manual for the West Asia and North Africa Region*; International Center for Agricultural Research in the Dry Areas (ICARDA): Beirut, Lebanon, 2013.
51. Shaner, G. The Effect of Nitrogen Fertilization on the Expression of Slow-Mildewing Resistance in Knox Wheat. *Phytopathology* **1977**, *67*, 1051–1056. [CrossRef]
52. Hage-Ahmed, K.; Krammer, J.; Steinkellner, S. The Intercropping Partner Affects Arbuscular Mycorrhizal Fungi and *Fusarium oxysporum* f. sp. *Lycopersici* Interactions in Tomato. *Mycorrhiza* **2013**, *23*, 543–550. [CrossRef]
53. Wheeler, B.E.J. *An Introduction to Plant Diseases*; John Wiley & Sons Ltd.: London, UK, 1969.
54. Dhingra, O.D.; Sinclair, J.B. *Basic Plant Pathology Methods*, 2nd ed.; Lewis Publishers: Boca Raton, FL, USA, 1995.
55. Elad, Y.; Chet, I.; Henis, Y. Biological Control of *Rhizoctonia solani* in Strawberry Fields by *Trichoderma harzianum*. *Plant Soil* **1981**, *60*, 245–254. [CrossRef]
56. Kritzman, G.; Shani-Cahani, A.; Kirshner, B.; Riven, Y.; Bar, Z.; Katan, J.; Grinstein, A. Pod Wart Disease of Peanuts. *Phytoparasitica* **1996**, *24*, 293–304. [CrossRef]
57. Elad, Y.; Baker, R. The Role of Competition for Iron and Carbon in Suppression of Chlamydospore Germination of *Fusarium* Spp. by *Pseudomonas* spp. *Phytopathology* **1985**, *75*, 1053–1059. [CrossRef]
58. Cytryn, E.; Minz, D.; Gieseke, A.; van Rijn, J. Transient Development of Filamentous Thiothrix Species in a Marine Sulfide Oxidizing, Denitrifying Fluidized Bed Reactor. *FEMS Microbiol. Lett.* **2006**, *256*, 22–29. [CrossRef]
59. Gamliel, A.; Katan, J. Involvement of Fluorescent Pseudomonads and Other Microorganisms in Increased Growth Response of Plants in Solarized Soils. *Phytopathology* **1991**, *81*, 494–502. [CrossRef]

60. Jaiswal, A.K.; Elad, Y.; Paudel, I.; Graber, E.R.; Cytryn, E.; Frenkel, O. Linking the Belowground Microbial Composition, Diversity and Activity to Soilborne Disease Suppression and Growth Promotion of Tomato Amended with Biochar. *Sci. Rep.* **2017**, *7*, 44382. [CrossRef]
61. Moonsamy, P.V.; Williams, T.; Bonella, P.; Holcomb, C.L.; Höglund, B.N.; Hillman, G.; Goodridge, D.; Turenchalk, G.S.; Blake, L.A.; Daigle, D.A. High Throughput HLA Genotyping Using 454 Sequencing and the Fluidigm Access Array™ System for Simplified Amplicon Library Preparation. *Tissue Antigens* **2013**, *81*, 141–149. [CrossRef]
62. Lee, K.C.; Archer, S.D.J.; Boyle, R.H.; Lacap-Bugler, D.C.; Belnap, J.; Pointing, S.B. Niche Filtering of Bacteria in Soil and Rock Habitats of the Colorado Plateau Desert, Utah, USA. *Front. Microbiol.* **2016**, *7*, 1489. [CrossRef] [PubMed]
63. Garland, J.L. Analysis and Interpretation of Community-Level Physiological Profiles in Microbial Ecology. *FEMS Microbiol. Ecol.* **1997**, *24*, 289–300. [CrossRef]
64. Garland, J.L.; Mills, A.L. Classification and Characterization of Heterotrophic Microbial Communities on the Basis of Patterns of Community-Level Sole-Carbon-Source Utilization. *Appl. Environ. Microbiol.* **1991**, *57*, 2351–2359. [CrossRef] [PubMed]
65. Pratt, K.; Moran, D. Evaluating the Cost-Effectiveness of Global Biochar Mitigation Potential. *Biomass Bioenergy* **2010**, *34*, 1149–1158. [CrossRef]
66. Khan, N.; Bolan, N.; Jospeh, S.; Anh, M.T.L.; Meier, S.; Kookana, R.; Borchard, N.; Sánchez-Monedero, M.A.; Jindo, K.; Solaiman, Z.M. Complementing Compost with Biochar for Agriculture, Soil Remediation and Climate Mitigation. *Adv. Agron.* **2023**, *179*, 1–90.
67. Lehmann, J.; Joseph, S. Biochar for Environmental Management: An Introduction. In *Biochar for Environmental Management: Science, Technology and Implementation*; Routledge: London, UK, 2015.
68. Xu, W.; Xu, H.; Delgado-Baquerizo, M.; Gundale, M.J.; Zou, X.; Ruan, H. Global Meta-Analysis Reveals Positive Effects of Biochar on Soil Microbial Diversity. *Geoderma* **2023**, *436*, 116528. [CrossRef]
69. Ali, N.; Khan, S.; Yao, H.; Wang, J. Biochars Reduced the Bioaccessibility and (Bio)Uptake of Organochlorine Pesticides and Changed the Microbial Community Dynamics in Agricultural Soils. *Chemosphere* **2019**, *224*, 805–815. [CrossRef]
70. Berendsen, R.L.; Pieterse, C.M.J.; Bakker, P.A.H.M. The Rhizosphere Microbiome and Plant Health. *Trends Plant Sci.* **2012**, *17*, 478–486. [CrossRef]
71. Chen, J.; Wu, L.; Xiao, Z.; Wu, Y.; Wu, H.; Qin, X.; Wang, J.; Wei, X.; Khan, M.U.; Lin, S. Assessment of the Diversity of *Pseudomonas* Spp. and *Fusarium* Spp. in Radix Pseudostellariae Rhizosphere under Monoculture by Combining DGGE and Quantitative PCR. *Front. Microbiol.* **2017**, *8*, 1748. [CrossRef]
72. Chen, W.; Wu, Z.; Liu, C.; Zhang, Z.; Liu, X. Biochar Combined with Bacillus Subtilis SL-44 as an Eco-Friendly Strategy to Improve Soil Fertility, Reduce Fusarium Wilt, and Promote Radish Growth. *Ecotoxicol. Environ. Saf.* **2023**, *251*, 114509. [CrossRef]
73. Song, Y.; Yuan, G.; Wu, Q.; Situ, G.; Liang, C.; Qin, H.; Chen, J. Change in Microbial Metabolic Quotient Under Biochar Amendment Was Associated with Soil Organic Carbon Quality, Microbial Community Composition, and Enzyme Activity in Bulk and Rhizosphere Soils in an Acid Rice Paddy. *J. Soil Sci. Plant Nutr.* **2023**, *23*, 3149–3162. [CrossRef]
74. Bakker, P.A.H.M.; Pieterse, C.M.J.; Van Loon, L.C. Induced Systemic Resistance by *Fluorescent pseudomonas* spp. *Phytopathology* **2007**, *97*, 239–243. [CrossRef] [PubMed]
75. Gerbore, J.; Benhamou, N.; Vallance, J.; Le Floch, G.; Grizard, D.; Regnault-Roger, C.; Rey, P. Biological Control of Plant Pathogens: Advantages and Limitations Seen through the Case Study of Pythium Oligandrum. *Environ. Sci. Pollut. Res.* **2014**, *21*, 4847–4860. [CrossRef]
76. Akhter, A.; Hage-Ahmed, K.; Soja, G.; Steinkellner, S. Potential of Fusarium Wilt-Inducing Chlamydospores, in Vitro Behaviour in Root Exudates and Physiology of Tomato in Biochar and Compost Amended Soil. *Plant Soil* **2016**, *406*, 425–440. [CrossRef]
77. Gu, Y.; Hou, Y.; Huang, D.; Hao, Z.; Wang, X.; Wei, Z.; Jousset, A.; Tan, S.; Xu, D.; Shen, Q. Application of Biochar Reduces Ralstonia Solanacearum Infection via Effects on Pathogen Chemotaxis, Swarming Motility, and Root Exudate Adsorption. *Plant Soil* **2017**, *415*, 269–281. [CrossRef]
78. Caroline, A.; Debode, J.; Vandecasteele, B.; D'Hose, T.; Cremelie, P.; Haegeman, A.; Ruttink, T.; Dawyndt, P.; Maes, M. Biological, Physicochemical and Plant Health Responses in Lettuce and Strawberry in Soil or Peat Amended with Biochar. *Appl. Soil Ecol.* **2016**, *107*, 1–12.
79. Jenkins, J.R.; Viger, M.; Arnold, E.C.; Harris, Z.M.; Ventura, M.; Miglietta, F.; Girardin, C.; Edwards, R.J.; Rumpel, C.; Fornasier, F. Biochar Alters the Soil Microbiome and Soil Function: Results of Next-generation Amplicon Sequencing across Europe. *Gcb Bioenergy* **2017**, *9*, 591–612. [CrossRef]
80. Matsubara, Y.; Hasegawa, N.; Fukui, H. Incidence of Fusarium Root Rot in Asparagus Seedlings Infected with Arbuscular Mycorrhizal Fungus as Affected by Several Soil Amendments. *J. Jpn. Soc. Hortic. Sci.* **2002**, *71*, 370–374. [CrossRef]
81. Gravel, V.; Dorais, M.; Ménard, C. Organic Potted Plants Amended with Biochar: Its Effect on Growth and Pythium Colonization. *Can. J. Plant Sci.* **2013**, *93*, 1217–1227. [CrossRef]
82. Copley, T.R.; Aliferis, K.A.; Jabaji, S. Maple Bark Biochar Affects Rhizoctonia Solani Metabolism and Increases Damping-off Severity. *Phytopathology* **2015**, *105*, 1334–1346. [CrossRef]
83. Zwart, D.C.; Kim, S.H. Biochar Amendment Increases Resistance to Stem Lesions Caused by *Phytophthora* spp. in Tree Seedlings. *HortScience* **2012**, *47*, 1736–1740. [CrossRef]
84. Elmer, W.H.; Pignatello, J.J. Effect of Biochar Amendments on Mycorrhizal Associations and Fusarium Crown and Root Rot of Asparagus in Replant Soils. *Plant Dis.* **2011**, *95*, 960–966. [CrossRef]

85. Meller Harel, Y.; Elad, Y.; Rav-David, D.; Borenstein, M.; Shulchani, R.; Lew, B.; Graber, E.R. Biochar Mediates Systemic Response of Strawberry to Foliar Fungal Pathogens. *Plant Soil* **2012**, *357*, 245–257. [CrossRef]
86. Atucha, A.; Litus, G. Effect of Biochar Amendments on Peach Replant Disease. *HortScience* **2015**, *50*, 863–868. [CrossRef]
87. Mehari, Z.H.; Elad, Y.; Rav-David, D.; Graber, E.R.; Meller Harel, Y. Induced Systemic Resistance in Tomato (*Solanum lycopersicum*) against Botrytis Cinerea by Biochar Amendment Involves Jasmonic Acid Signaling. *Plant Soil* **2015**, *395*, 31–44. [CrossRef]
88. Uzoma, K.C.; Inoue, M.; Andry, H.; Fujimaki, H.; Zahoor, A.; Nishihara, E. Effect of Cow Manure Biochar on Maize Productivity under Sandy Soil Condition. *Soil Use Manag.* **2011**, *27*, 205–212. [CrossRef]
89. Nigussie, A.; Kissi, E.; Misganaw, M.; Ambaw, G. Effect of Biochar Application on Soil Properties and Nutrient Uptake of Lettuces (*Lactuca sativa*) Grown in Chromium Polluted Soils. *Am. J. Agric. Environ. Sci.* **2012**, *12*, 369–376.
90. Alkorta, I.; Aizpurua, A.; Riga, P.; Albizu, I.; Amézaga, I.; Garbisu, C. Soil Enzyme Activities as Biological Indicators of Soil Health. *Rev. Environ. Health* **2003**, *18*, 65–73. [CrossRef]
91. Garcia-Perez, M.; Wang, X.S.; Shen, J.; Rhodes, M.J.; Tian, F.; Lee, W.-J.; Wu, H.; Li, C.-Z. Fast Pyrolysis of Oil Mallee Woody Biomass: Effect of Temperature on the Yield and Quality of Pyrolysis Products. *Ind. Eng. Chem. Res.* **2008**, *47*, 1846–1854. [CrossRef]
92. Flexas, J.; Escalona, J.M.; Medrano, H. Water Stress Induces Different Levels of Photosynthesis and Electron Transport Rate Regulation in Grapevines. *Plant. Cell Environ.* **1999**, *22*, 39–48. [CrossRef]
93. Viger, M.; Hancock, R.D.; Miglietta, F.; Taylor, G. More Plant Growth but Less Plant Defence? First Global Gene Expression Data for Plants Grown in Soil Amended with Biochar. *Gcb Bioenergy* **2015**, *7*, 658–672. [CrossRef]
94. Jaiswal, A.K.; Frenkel, O.; Elad, Y.; Lew, B.; Graber, E.R. Non-Monotonic Influence of Biochar Dose on Bean Seedling Growth and Susceptibility to Rhizoctonia Solani: The "Shifted Rmax-Effect". *Plant Soil* **2015**, *395*, 125–140. [CrossRef]
95. Singh, H.P.; Kaur, S.; Batish, D.R.; Kohli, R.K. Ferulic Acid Impairs Rhizogenesis and Root Growth, and Alters Associated Biochemical Changes in Mung Bean (*Vigna radiata*) Hypocotyls. *J. Plant Interact.* **2014**, *9*, 267–274. [CrossRef]
96. Yao, K.; De Luca, V.; Brisson, N. Creation of a Metabolic Sink for Tryptophan Alters the Phenylpropanoid Pathway and the Susceptibility of Potato to Phytophthora Infestans. *Plant Cell* **1995**, *7*, 1787–1799. [CrossRef] [PubMed]
97. Gogoi, R.; Singh, D.V.; Srivastava, K.D. Phenols as a Biochemical Basis of Resistance in Wheat against Karnal Bunt. *Plant Pathol.* **2001**, *50*, 470–476. [CrossRef]
98. De Carvalho, D.; Ferreira, R.A.; de Oliveira, L.M.; de Oliveira, A.F.; Gemaque, R.C.R. Proteins and Isozymes Electroforesis in Seeds of Copaifera Langsdorffii Desf.(Leguminosae Caesalpinioideae) Artificially Aged. *Rev. Árvore* **2006**, *30*, 19–24. [CrossRef]
99. Favali, M.A.; Vestena, C.; Fossati, F.; Musetti, R.; di Toppi, L.S.; Martin, R.R. Phytoplasmas Associated with Tomato Stolbur Disease. In Proceedings of the Ninth International Symposium on Small Fruit Virus Diseases, Canterbury and HRI, East Malling, UK, 9–15 July 2000.
100. Hameed, A.; Iqbal, N. Chemo-Priming with Mannose, Mannitol and H_2O_2 Mitigate Drought Stress in Wheat. *Cereal Res. Commun.* **2014**, *42*, 450–462. [CrossRef]
101. Choodamani, M.S.; Hariprasad, P.; Sateesh, M.K.; Umesha, S. Involvement of Catalase in Bacterial Blight Disease Development of Rice Caused by Xanthomonas Oryzae Pv. Oryzae. *Int. J. Pest Manag.* **2009**, *55*, 121–127. [CrossRef]