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The Impact of Sewage-Sludge- and Olive-Mill-Waste-Derived Biochar Amendments to Tomato Cultivation

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Abstract: This study elucidated the impact of sewage-sludge (SS) and olive-mill-waste (OMW) biochar amendments to soil using tomatoes as a test crop. Four treatments were evaluated: the "control" with no biochar amendment, two SS biochar treatments with the addition of 10 t/ha and 25 t/ha, respectively, and an OMW biochar treatment with the addition of 25 t/ha. Higher yields were observed in both SS biochar treatments, providing evidence that biochar acts as a plant biostimulant. Biochar application had positive impacts on carbon sequestration and soil structure. The uptake of heavy metals by all plant parts was very low, indicating that biochar is an appropriate product for land application. Biochar dose and type induced changes in the composition due to the different unique species and biodiversity of microbial communities. Venn diagrams revealed that the majority of the identified taxa were shared among the treatments, and only a small proportion of them were unique in bulk soil between treatments. In the rhizosphere, the OMW-biochar-treated plants showed a higher number of unique taxa. Microbiota structure plays a major role in the stimulation of plant growth; however, further research is needed to understand the impact of these shifts in the functioning of agroecosystems.

Keywords: bio-based products; biochar application; agricultural production; bio stimulant; microbial communities

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1. Introduction

Over the last century, the global population has increased, driving up the global demand for food. Concurrently, the expected impacts of climate change on agricultural production, the limited opportunities for arable land expansion, and soil degradation underline the need to maintain/enhance the productivity of existing agricultural land by adopting approaches that use soil and water resources efficiently and restore soil fertility [1]. The ultimate challenge of modern agriculture is to increase the productivity of agricultural land while enhancing the quality of produce [2]. It has been widely accepted that agroecological practices, such as recycling of organic amendments, improve soil fertility and reverse soil degradation while improving agroecosystem services [3,4]. One such organic amendment is biochar, which has long lasting beneficial effects on the soil.

Biochar is a carbon-rich, fine-grained, porous material, which is produced by the thermal processing of biomass under oxygen-limited conditions at temperatures ranging from 250 to 700 °C [5,6]. Biochar is alkaline and hydrophobic in nature and contains both aliphatic and aromatic compounds [7]. According to the International Biochar Initiative (IBI), biochar is a charcoal that can be applied to soil to offer both agricultural and environmental benefits [8]. Biochar has a strong impact on soil physical properties, altering soil structure, bulk density, porosity, macro-aggregate, and water content [9]. Biochar can be used as a soil amendment in agriculture [10], since it has the potential to improve soil fertility and quality [11], enhance crop productivity [12], and increase carbon and nutrient sequestration [13,14]. Furthermore, biochar application in soil has been associated with improved nutrient-use efficiency [15], either through nutrients contained within it or through physicochemical processes that allow better utilization of soil-inherent or fertilizer-derived nutrients. Strong interactions between nitrogen (N) availability and biochar addition have been reported [16]; for example, in reducing N fertilizer demand in maize plants [17]. Biochar is also characterized by increased biological and chemical stability [18]. Due to its porosity and large surface area, biochar can be also used as an adsorbent for the removal of organic and inorganic contaminants from wastewaters and soils [19–21].

Apart from its improvement of soil physical and chemical properties, biochar may also affect agroecosystem functioning and performance through its effect on the composition and functioning of microbial communities. Increases in the diversity and shifts in the metabolic potential of the root microbiome were reported as a result of biochar recalcitrance [22]. There are also reports that biochar increases the complexity and improves the stability of microbial networks [23]. However, most of these effects appear to be site-specific and dose-dependent [24].

So far, numerous feedstocks have been used in biochar production [25], including wood [13,26], corn cobs [27,28], wheat and maize residues [29], sugar beet tailings [30], rice straw [31], biogas residues [32], grape husks [33], olive mill solid wastes and wastewater (OMW) [34], and sewage sludge (SS) [35,36]. Europe alone produces approximately 13×10^6 tons of SS biosolids [37], while their management remains one of the most complicated tasks for wastewater treatment plants. Its use as feedstock for biochar production is a feasible management and valorization alternative [38]. The use of SS biochar as a soil amendment is considered a cost-efficient alternative, offering opportunities to reuse the essential nutrients and organic-C to the soil for crop production. In parallel, it decreases the bioavailability of toxic elements and eliminates the environmental and public health risks associated with its direct use or disposal [39]. SS-based biochars that are produced at relatively higher temperature (>500 °C) have porous structure, larger surface area, and higher pH, whereas those produced at lower temperature have high electrical conductivity (EC), cation exchange capacity, and surface functional groups; thus, they are more suitable for agricultural applications (e.g., soil conditioning and fertilization) [40]. Similarly, OMW constitutes a notable amount of waste in the Mediterranean region, and significant volumes are produced annually (3×10^7 m^3) during olive oil processing [41]. The conversion of OMW to biochar is an efficient valorization option, which apart from its environmental and economic benefits results in the elimination of the impacts caused by its improper disposal in soils and water reservoirs.

Although several studies have been conducted to investigate the impact of biochar produced from different feedstocks on soil properties, crop growth, yield, and fruit quality in greenhouse and field-grown tomatoes [34,42–45], limited information is available for SS- and OMW-produced biochar. Moreover, most published studies do not clearly assess the effect of biochar on soil microbial communities, and hence its potential use as a biostimulant. The main objective and novelty of this research was to further elucidate the impacts of SS- and OMW-derived biochar amendments to soil using tomatoes as a test crop. More specifically, the present study intended to (a) evaluate SS- and OMW-based biochar as a soil improver and bio-stimulant in agriculture, (b) investigate whether the different effects of the application of biochar to soil are mediated by the feedstock type, and (c) further examine the role of microbiota in the stimulation of plant growth.

2. Materials and Methods

2.1. Production and Properties of Biochar

The primary feedstock material used for the production of biochar was SS, which was provided by the Municipal Enterprise for Water and Sewage of Chania. In addition, the production of biochar from another local feedstock, OMW produced from a 3-phase organic olive mill in Akrotiri, Chania, was explored. The material sampled was the dried sludge remaining after the evaporation of the wastewaters disposed of in an evaporation pond.

Both feedstocks were subjected to slow pyrolysis at 400 °C in a furnace with an operating capacity of 1 m^3. The temperature was selected based on the results of earlier studies in order to achieve a reasonable yield and obtain biochar with sufficient C and N content. It is noted that pyrolysis at temperatures higher than 500 °C results in reduced yield and higher pH for almost all feedstocks. Thus, during pyrolysis, a balance between yield, quality of biochar, energy requirements, and type of soil to which the biochar will be applied is needed, in order to produce a biochar with beneficial properties and reasonable cost [5]. Oxygen-free conditions were maintained in the furnace with the flow of 99% pure nitrogen. The heating rate was 20 °C/min, and the residence time at the desired temperature was 1 h.

2.2. Field Experiment Description

The experiment was conducted between May 2021 and September 2021 in a field in the Akrotiri area of Chania, Greece (35°33′14.77″ N, 24°07′50.26″ E). Four treatments were evaluated: the "control" with no biochar amendment, the "SS_(10 t/ha)" with biochar addition of 10 t/ha, the "SS_(25 t/ha)" with biochar of 25 t/ha of biochar, and the "OMW_(25 t/ha)", also with a biochar addition of 25 t/ha. Biochar was mixed manually with the surface soil prior to cultivation (0–20 cm). The plot size of each experimental unit was 4 m^2 and included 6 tomato plants. In addition to the high value of tomato as a food crop, this plant was chosen for evaluation due to its short life cycle. The tomato variety used for the experiments was *esculenta "Bobcat F1"*, which is a hybrid, mid-early, self-pruning, low-growing, and large-fruited variety, and it is suitable for field cultivation.

All treatments received the same amount of fertilizer, which was applied in a water-soluble form during the growing season. The total amount of N-P-K added to each plant during the experiment was 29 g, 13 g, and 36 g for N, P, and K, respectively. The total amount of water added through irrigation for each plant was 161 L. It should be noted that if it is assumed that there are 1800 plants for 1000 m^2 land, this means that 288 mm water/year is needed. The irrigation rates recommended by the agricultural organizations and Ministries of most countries are in the order of 700–800 mm/year for vegetables, which is 2.5 higher than the actual needs of the plant.

An initial soil-sampling campaign was conducted during the application of the biochar in May 2021 to define the initial conditions, in which 3 soil samples were collected from each of the 4 treatments. A final soil sampling (3 samples per treatment) was also conducted at the end of the growing cycle in September 2021. Soil samples were taken from the surface (top of 20 cm), homogenized, air dried, and processed for physical and chemical analyses. Three soil and three rhizosphere samples per treatment were also taken in June (middle of the experiment) and in September (end of the experiment) to evaluate the effect of treatments on the soil microbiome. Finally, the growth of tomato plants was monitored during the growing season by monitoring plant height. Tomato yield and dry weight of roots, shoots, and leaves for each plant were also recorded at the end of the experiment.

2.3. Physicochemical Analyses of Soil, Biochars and Plants

SS and OMW biochar and soil samples were analyzed for physical and chemical properties, including pH and electrical conductivity (EC) (EPA Method 9045D/ASTM D4972-19). SS and OMW biochars were also analyzed for dry matter/moisture (APHA-AWWA-WEF 2540 B/ASTM D2216–19), volatile solids, ash (APHA-AWWA-WEF 2540 G/ASTM E1755–01), volatile matter and char. Biochar samples were analyzed before its application to soil.

The distribution of water-stable aggregates (WSA) in the different size classes was determined by wet-sieving, adopting the protocols developed by Elliott (1986) [46] and Cambardella and Elliott (1993) [47]. All samples (biochar, soils, tomatoes, leaves, stems, and roots) were also analyzed for moisture content, total organic carbon (TOC) (ASTM D6316), total nitrogen (TN) (multi N/C 2100S, Analytik Jena, Jena, Germany), nutrients, trace elements (EPA Method 3051a, EPA Method 6010b), phenols (EPA Method 1312, DIN 38409-16:1984-06), chlorides and sulfates (EPA Method 1312, EPA Method 9038, EPA Method 9251), ammonium and nitrates (ISO/TS 14256-1:2003, ISO 7890-1-2-1986, EPA Method

350.2), and available phosphorus (ISO 11263, 1994, EPA Method 365.1). The leachable (bioavailable) part of the chemical elements was also determined (EPA Method 1312).

2.4. DNA Extraction, High-Throughput Sequencing, and Bioinformatic Analysis

Soil and rhizosphere genomic DNA was extracted from a 0.3 g of dry soil using the DNeasy Power Soil kit (Qiagen). Bulk soil was homogenized in a mortar with liquid nitrogen before DNA extraction. Rhizosphere soil was sampled by using the protocol of Lakshmanan et al. (2017) [48]. The quality of DNA was checked by running 4 μL in 2% agarose gel, and its concentration was quantified in a QFX fluorometer using the Qubit dsDNA HS kit. The V4 region of 16S rRNA gene was amplified using primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Library construction and sequencing at a minimum depth of 50,000 sequences per sample was performed by the NOVOGENE UK using the NovaSeq 6000 platform (Illumina) to obtain 2 × 250 bp paired-end reads.

First, primers, adapters, and barcodes were removed from raw reads, and the remaining paired-end reads were joined to amplicon sequence variants (ASVs) using the DADA2 (v. 1.22) pipeline [49], following the typical workflow described by the authors (https://benjjneb.github.io/dada2/tutorial.html (accessed on 22 July 2022), and using R (v. 4.2.1) (R Core Team 2017). Forward and reverse reads were trimmed and filtered using default parameters (maxN = 0, maxEE = 2, truncQ = 2). The adoption of ASVs makes marker-gene sequencing more precise, reusable, and reproducible and reduces the computation costs. Chimeric ASVs were identified and removed using the "removeBimeraDenovo" function. Taxonomy was assigned to the reads by training the naïve Bayesian classifier [50] against the SILVA database [51] (v. 139) implemented within the DADA2 pipeline. Multiple alignment was performed with the DECIPHER package [52], and a maximum likelihood phylogenetic tree was constructed with the phangorn package [53], with a neighbor-joining tree as the starting point [54]. During the processing of sequences, sequences that were not assigned at the phylum level were discarded. In addition, a conservative threshold value of three was applied. α- and β-diversity: α-diversity of microbial communities was estimated by the Shannon index with the microeco package [55]. Beta diversity was assessed by Bray–Curtis dissimilarity as well as by the weighted UniFrac distances calculated by log transformation of the non-rarefied 16S rRNA data. PERMANOVA, with 999 randomizations, was performed to assess the statistical significance.

Network analysis: Microbial networks were constructed in the NetCoMi package [56] using Pearson correlation association. To avoid compositional effects, the data were clr-transformed. Zero treatment was necessary in this case. ASVs with an abundance higher than 100 were used in network construction, and a threshold of 0.6 was used as a sparsification method [54].

3. Results and Discussion

3.1. Physical and Chemical Conditions of the Experiment

The physicochemical properties of SS and OMW feedstocks and the produced biochars are summarized in Table 1.

Evidently, the yield was quite similar for both biochars, and ranged between 21% and 25% for OMW- and SS-based biochars, respectively. This was due to the condensation of aliphatic compounds and loss of CH_4, H_2, and CO during pyrolysis. At higher temperatures (500 and 700 °C), dehydration of hydroxyl groups and thermal degradation of lignocellulose structures also took place, and the yield was even lower. This is one of the reasons for the selection of 400 °C as the pyrolysis temperature [5]. Significant and opposing effects were observed in the pH of both feedstocks and the produced biochars. The pH of sewage sludge was higher (8.52) compared to that of OMW (5.57), and this was mainly due to the addition of lime, which is a registered biocide, at the wastewater treatment plant for its stabilization, the reduction of pathogens, and the elimination of odors. On the other hand, the biochar produced from OMW had a much higher pH (9.86) compared to the value measured for the SS-based biochar (6.8). The high value of the pH in the OMW-based biochar makes it suitable for application in acidic soils.

Table 1. SS and OMW feedstock and biochar properties. Parentheses show standard deviation of the replicates.

	SS		OMW	
Parameter	**Feedstock**	**Biochar**	**Feedstock**	**Biochar**
Yield (%)	-	25 (0.03)	-	21 (0.06)
pH	8.52	6.81	5.57	9.86
EC (mS/cm)	2.38 (0.22)	3.35 (0.23)	0.96 (0.11)	1.66
Dry Matter/(TS%)	32.6 (1.36)	92.01 (0.01)	61.9 (2.22)	97.85 (0.02)
Moisture (%)	67.39 (3.61)	7.98 (0.02)	38.11 (2.27)	2.14 (0.03)
Volatile Solids (%)	70.77 (2.55)	67.50 (0.65)	93.22 (4.08)	86.35 (1.20)
Ash (%)	29.23 (2.11)	32.49 (0.13)	6.78 (0.25)	13.64 (0.3)
Volatile Matter (%) (TG)	92 (2.12)	34 (0.01)	59.45 (1.39)	58.01 (0.01)
Char (%) (TG)	7.99 (0.33)	65 (0.01)	40.54 (1.21)	41.98 (0.01)
Specific Surface Area (m^2/gr)		130 (0.02)		16
S (%)	1.78 (0.12)	0.95 (0.02)	0.44	0.09 (0.01
K (g/kg)	2.3 (0.23)	3.4 (0.02)	19.8 (1.4)	45.7 (0.4)
Cr (mg/kg)	60.2 (4.7)	68.4 (1.7)	2.9 (0.2)	3.9 (0.04)
Ni (mg/kg)	31.7 (2.8)	53.5 (2.1)	3.4 (0.3)	4.3 (0.13)
Cd (mg/kg)	<DL	2.4 (0.01)	<DL	<DL
Pb (mg/kg)	144.8 (9.5)	206 (4.2)	<DL	1.2 (0.03)
Cu (mg/kg)	343.2 (26.8)	263.6 (6.6)	84.7 (8.4)	88.7 (0.8)
Zn (mg/kg)	1409 (89.8)	1647 (6.4)	56.1(7.3)	81.9 (2.1)
As (mg/kg)	2.8 (0.12)	<DL	<DL	<DL
Hg (mg/kg)	2.3 (0.08)	0.2 (0.01)	0.24(0.03)	<DL
Cl (mg/kg)	640 (220)	<800	2290 (830)	6551 (427)
SO4 (mg/kg)	12,290 (1890)	33,597(2257)	7640 (6000)	<600
Phenols (mg/kg)	128.9 (23.4)	4.4 (0.4)	460.1 (150)	163.7 (26.3)
N-NO3 (mg/kg)	35.2 (7.2)	44.1 (4.2)	167.7 (52.3)	32.7 (4.8)
N-NH4 (mg/kg)	3258 (644)	120.5 (6.9)	155.9 (39.2)	4.58 (0.93)
Olsen-P (mg/kg)	376.5 (58.2)	564.9 (143)	111.7 (27.9)	132.8 (9.9)
TOC (%)	38.9 (3.6)	20.02 (1.48)	55.4 (4.2)	58.5 (3.04)
TN (%)	6.0 (0.8)	2.49 (0.24)	6.2 (0.7)	3.9 (0.48)

The EC of biochars increased slightly compared to the respective value of the feedstocks, measuring 3.35 mS/cm and 1.66 mS/cm for the SS- and OMW-based biochar, respectively. The char content was lower (42%) in the OMW-based biochar, while the specific surface area for the SS-based biochar was much higher compared to that of OMW-based biochar (130 and 17.5 m^2/g respectively). Despite their difference, both values are considered adequate and justify their application as soil amendment.

In both biochars, a decrease in their C, H, and N content was observed compared to the respective content in the feedstocks. This decrease, which is more noticeable in the SS-based biochar, is related to the volatilization of these elements during pyrolysis [35].

The content of heavy metals, namely Cr, Ni, Cd, Pb, Cu, and Zn, increased in the SS-based biochar, due to the presence of these elements in the initial feedstock. However, these values were significantly lower than the defined thresholds in the existing regulations. In addition, the low application rates of biochars as soil amendments ensure that SS biochar does not pose any threat to soil functioning or public health [57].

The main properties of the produced biochars, both from SS and OMW, are quite similar to those reported in earlier studies [34,35,58–60].

Table S1 (Supplementary Information) presents the physicochemical characterization of the soil samples collected from each of the four treatments during the application of biochar in May 2021, in order to determine the initial experimental conditions, while

Table S2 (Supplementary Information) presents the physicochemical characterization of the soil samples collected from each of the four treatments at the end of the experiment in September 2021, in order to determine the final conditions and assess the effect of biochar addition.

The nutrients and heavy metal content in fruits in aboveground (shoots and leaves) and belowground (roots) plant tissues collected from each of the four treatments at the end of the experiment are presented in Tables S3–S6 (Supplementary Information). As expected, no differences were detected among the four treatments. Specifically, Ni, As, Pb, Se, and Cd in the tomato fruits were below detection limit, and Cr was below 0.34 mg/kg in all treatments, significantly lower than the threshold for Cr (2.3 mg/kg) in vegetables according to FAO/WHO (2001) [61].

3.2. Crop Growth and Productivity

The yield of tomato plants and the number of fruits harvested during the harvesting period are summarized in Figure 1. Overly, higher yields were observed in both SS biochar treatments ("SS_(10 t/ha)" and "SS_(10 t/ha)") (Figure 1) compared to the other treatments. Specifically, 20.0 kg and 126 tomatoes in total were produced in soils not treated with biochar ("control"). The corresponding figures for the biochar-treated soils were 39.7 kg and 218 tomatoes for "SS_(10 t/ha)", 55.7 kg and 250 tomatoes for "SS_(25 t/ha)" treatment, and 25.8 kg and 144 tomatoes for "OMW_(25 t/ha)" treatment (Figure 1). In conclusion, the yield of tomato plants treated with "SS_(10 t/ha)", "SS_(25 t/ha)", and "OMW_(25 t/ha)" biochar was 98.5%, 178.5%, and 29% higher than control treatment.

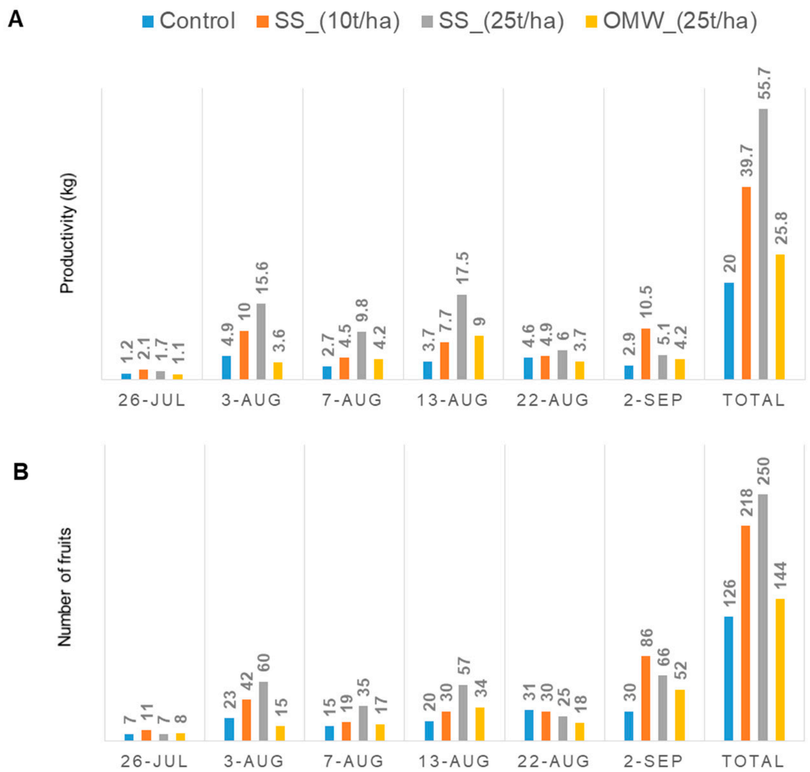

Figure 1. (**A**) Tomato yield (in kg) per treatment in each harvest, and total productivity at the end of the harvesting period (**B**) Number of tomatoes per treatment at each harvest and total number of tomatoes collected at the end of the harvesting period. Dates in the horizontal axis represent each harvest date and refer to year 2021.

Similar results were also observed for the total biomass of tomato plants and its distribution in the individual organs (leaves, shoots, and roots) (Figure 2). The biomass per plant was greater in "SS_(25 t/ha)" treatment (Figure 2). The positive effect of biochar on the dry weight of belowground and aboveground plant tissues has been speculated to be due to its structure [62], which is related to nutrient availability as well as to the presence of microbial communities. Biochar improves soil water retention and aeration through its effect on porosity. Consequently, organic matter and other nutrients were available and accessible, while a favorable environment for microorganisms and crop growth was created.

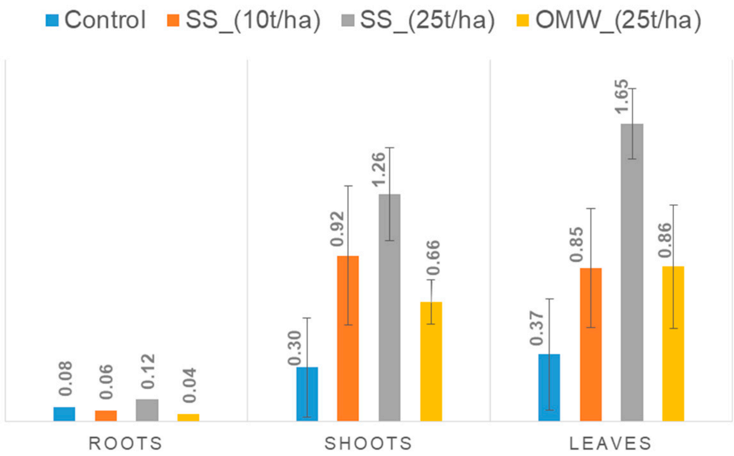

Figure 2. Biomass (in kg/plant) of roots, shoots, and leaves per plant per treatment at the end of the experiment. Lines in the Figure present the standard deviation of the replicates.

Overall, significantly higher productivity due to the SS and OMW biochar amendments is observed. The OMW biochar amendment stimulated lower productivity that the equivalent-dose SS biochar. It is believed that this was due to phenol toxicity at the early stages of plant growth; the phenol content of OMW-based biochar was 164 mg/kg, almost 40 times higher compared to the SS-based biochar. The plants during the first month of growth exhibited signs of stress and slower growth. This stress was overcome, and the plants continued to grow to higher levels of production than the control.

At the end of the experiment, the shoots of the plants subjected to the treatment with "SS_(25 t/ha)" biochar addition had a larger diameter (2.45 ± 0.42 cm) compared to the other treatments (1.62 ± 0.26 cm, 2.02 ± 0.39 cm, and 1.95 ± 0.36 cm for "control", "SS_(10 t/ha)", and "OMW_(25 t/ha)" treatment, respectively); however, the difference was not statistically significant.

3.3. Soil Fertility and Structure

Figure 3 presents the TOC and TN content of the treatments at the beginning and the end of the study period. The higher TOC content in the treatments compared to the control was consistent with the amended biochar. Following the application of biochar, the TOC in the "SS_(10 t/ha)" soil increased by approximately 6 g C/kg (from 15.18 in the control to 21.23 g/kg) and 15 g/kg in the "SS_(25t/ha)", which is 2.5 times higher than "SS_(10 t/ha)". The application of SS biochar resulted in a slightly increased C/N in the soil for the two SS biochar doses (7.5 in the "control" vs. 8.7 and 8.8 for the two SS biochar doses). The OMW biochar increased the TOC content in the 0–20 cm soil depth by 18 g/kg and increased the C/N ratio to 15.

TOC content, however, decreased in SS-biochar-treated soils at the end of the experiment, particularly for the highest dose (Figure 3). This effect implies a downward movement of SS biochar (from the upper 20 cm to deeper soil layers), which was likely stimulated by the applied irrigation, the sandy texture (90% sand) of the soil, and the very small size of biochar particles. To confirm the downward movement of SS biochar, an additional soil sampling was conducted 3 months after the end of the experiment. The results confirmed that SS biochar was present to a depth up to 60 cm. A similar trend has also been observed in greenhouse experiments (unpublished data). Even though the SS biochar was incorporated in the top 20 cm of soil in 30 L pots, at the end of the experiment it was distributed throughout the pot. A downward transport was not observed in the case of OMW treatment. In the "control" and "OMW_25 t/ha" treatments, soil TOC content remained the same at the end of the experiment (Figure 3).

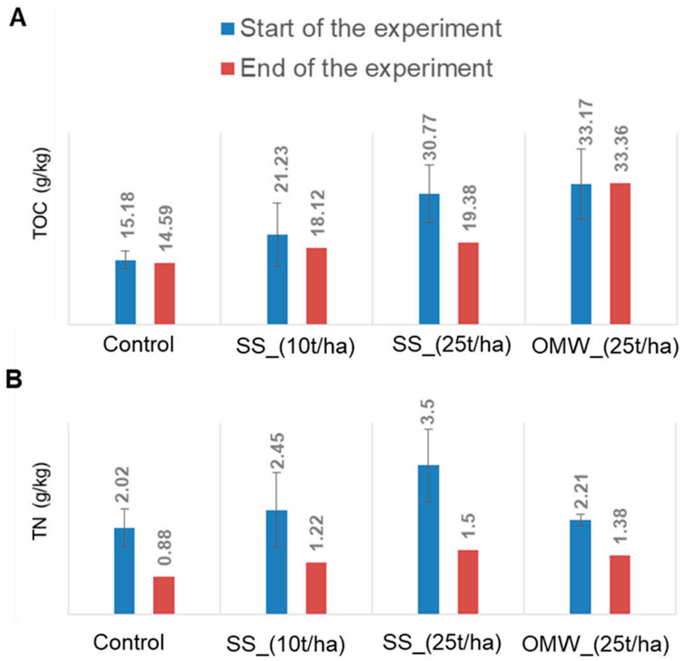

Figure 3. (**A**) Total organic carbon (TOC) and (**B**) nitrogen (TN) per treatment at the start and end of the experiment. Lines in the Figure present the standard deviation of the replicates.

TN decreased in all treatments by the end of the experiment compared to the start (Figure 3). The TN content decreased by 1.14 g/kg in the control treatment, 1.23 g/kg and 2 g/kg for the two doses of SS biochar, and 0.86 g/kg for the OMW biochar. These findings document a preferential stimulation of N mineralization, likely induced by the properties of biochar and/or the increased crop demand for N. However, this effect was milder in the case of OMW biochar. Although the mechanisms responsible for this differential effect on N among biochar treatments remain obscure, Tsiknia et al. (2014) [63] reported a lower availability of N in OMW-treated soils and lower rates of nitrification. Biochar dose and type of feedstock likely have important effects on the availability of nutrients in agroecosystems and could potentially improve its use efficiency and eliminate losses to the environment. Such an influence is consistent with the lower content of inorganic N in soils treated with OMW biochar.

The C/N ratio increased in all treatments; however, soils amended with SS biochar showed a smaller increase compared to the control (unamended).

Regarding the soil structure, the effects of dose and feedstock of biochar are summarized in Figure 4. The mass distribution of WSA shows an increase in the mass of macro-aggregates in the highest doses of both feedstocks ("SS_(25 t/ha)": 21.3% and "OMW_(25 t/ha": 27.1%) compared to control and "SS_(10 t/ha)" (10.7% and 10.6%, respectively). The difference in the mass of macro-aggregates between the highest doses of both feedstocks may partly explain the lack of difference in the TOC in OMW treatment compared to SS treatment (Figure 3A). The sandy (90%) texture of the soil was considered responsible for the small percentage of particles that corresponded in the silt-clay fraction and microaggregates. In addition, the observed feedstock-dependent effect of biochar on soil macro-aggregates was partially responsible for the lower downward movement of OMW biochar compared to SS biochar, implying probable differences in the charge or size of biochar.

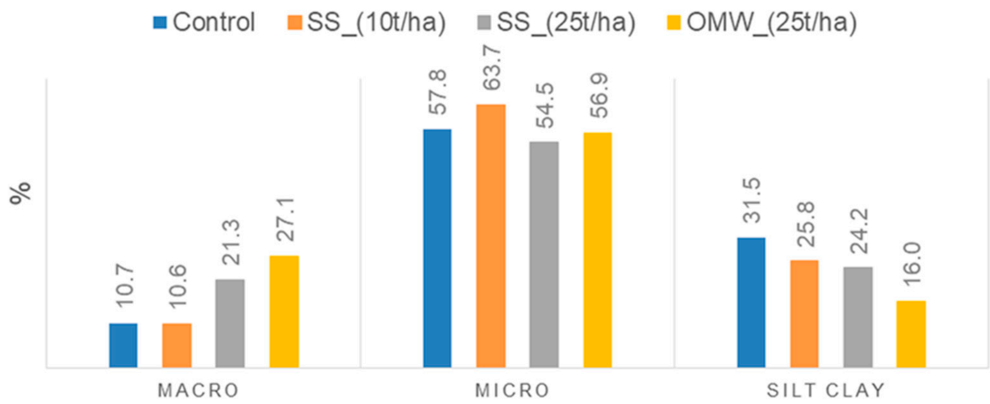

Figure 4. Macro-aggregates, micro-aggregates, and silt-clay-sized micro-aggregates per treatment at the end of the experiment.

3.4. Microbial Communities

3.4.1. Bacterial Community Composition

Similar patterns of bacterial community composition were found in the bulk soil and rhizosphere of the tomato plants treated with different types and doses of biochar. Six phylum, *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroides*, *Acidobacteriota*, and *Gemmatimonadota* accounted for more than 80% of procaryotic communities in the bulk soil and rhizosphere compartments (Figure 5A,B). However, differences were also observed between compartments. Rhizosphere communities showed lower relative abundance of *Proteobacteria* and *Actinobacteria*. More striking differences in the communities were detected between developmental stages. In the bulk soil, the relative abundance of Proteobacteria increased at the harvest period, while that of *Chloroflexi*, *Myxococcota*, and *Verrucomicrobiota* decreased (Figure S1A). In the rhizosphere the relative abundance of *Proteobacteria* showed a milder increase compared to the bulk soil. An increase was also observed for *Acidobacteria* and *Chloroflexi*, while Myxococcota decreased (Figure S1B). Venn diagrams revealed that the majority of the identified taxa were shared among the treatments, and only a small proportion of them were unique in the bulk soil between treatments (Figure 6A,B). In the rhizosphere, however, the OMW-biochar-treated plants showed a higher number of unique taxa (Figure 6B.)

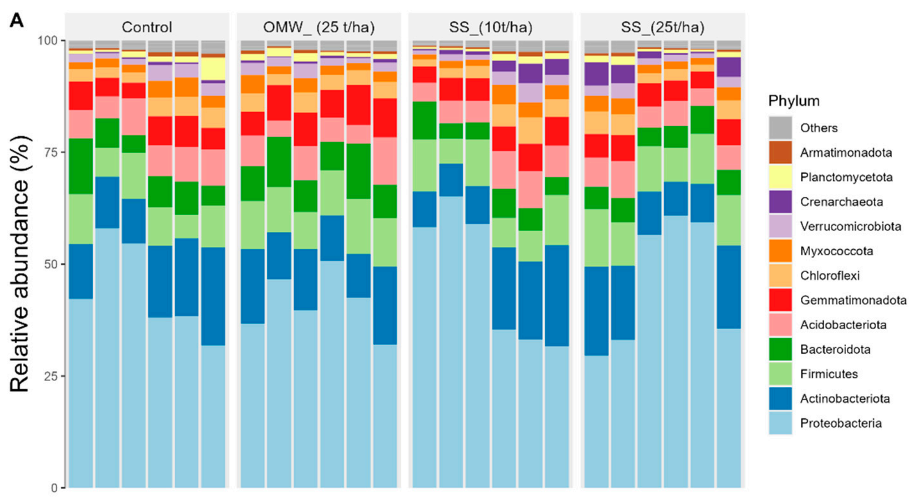

Figure 5. *Cont.*

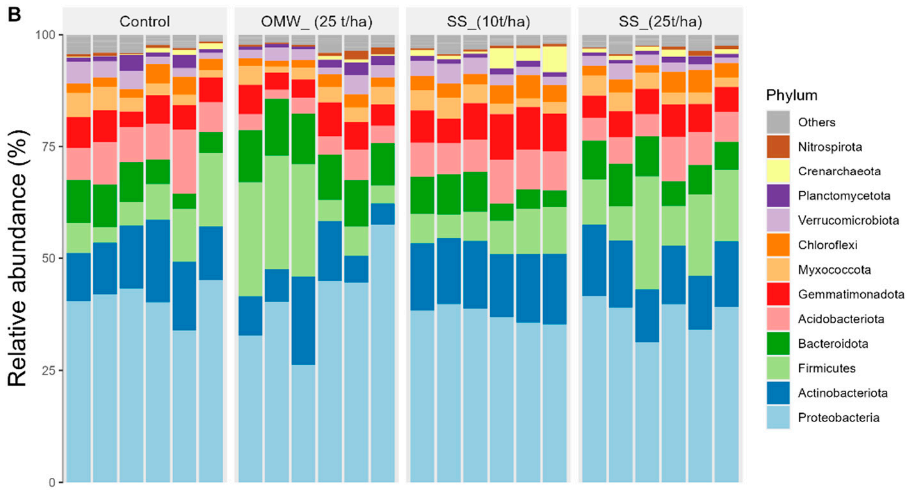

Figure 5. Composition of microbial communities in the bulk soil (**A**) and in the rhizosphere (**B**) of tomato plants treated with biochar from different sources.

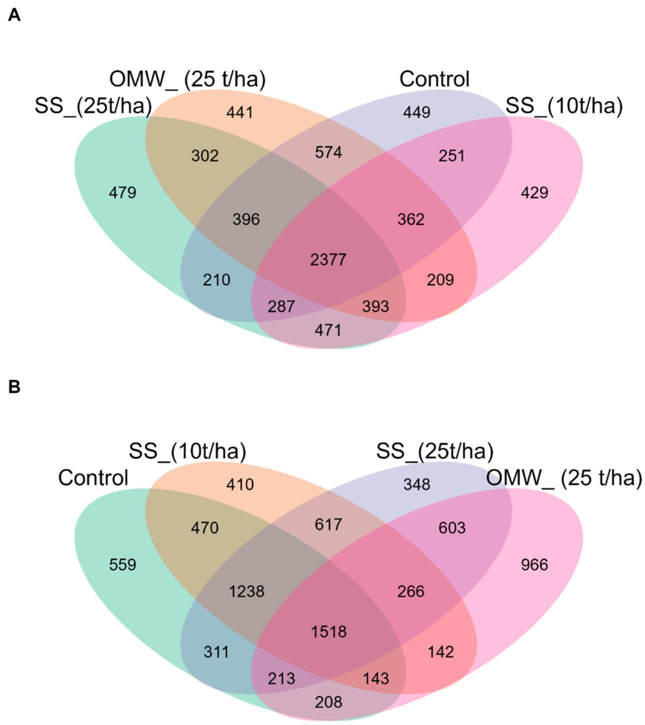

Figure 6. Venn diagrams indicating the sharing of microbial taxa between soils treated with different sources of biochar and untreated in the bulk soil (**A**) and the rhizosphere (**B**).

3.4.2. Alpha and Beta Diversity

The treatments investigated in this study did not affect a-diversity either in the bulk soil (Figure 7A) or the rhizosphere (Figure 7B). Significant effects in α-diversity were detected between samplings (fruit set vs. harvest), with soil communities sampled during the fruit set having a higher α-diversity in bulk soil compared to the sampling that took place during the harvest time (Figure S2A). By contrast no effect on a-diversity between samplings was found in the rhizosphere (Figure S2B). These findings are in agreement with those of earlier studies, which reported minor effects of biochar on α-diversity. Latini et al. (2019) [64] found no effect, or even a decrease in α-diversity of microbial communities to biochar application depending on the feedstock type or the activation of biochar. Similarly, amendment of soils with rice straw or mixed rice and corn straw biochar did not affect the α-diversity of bacterial communities [65].

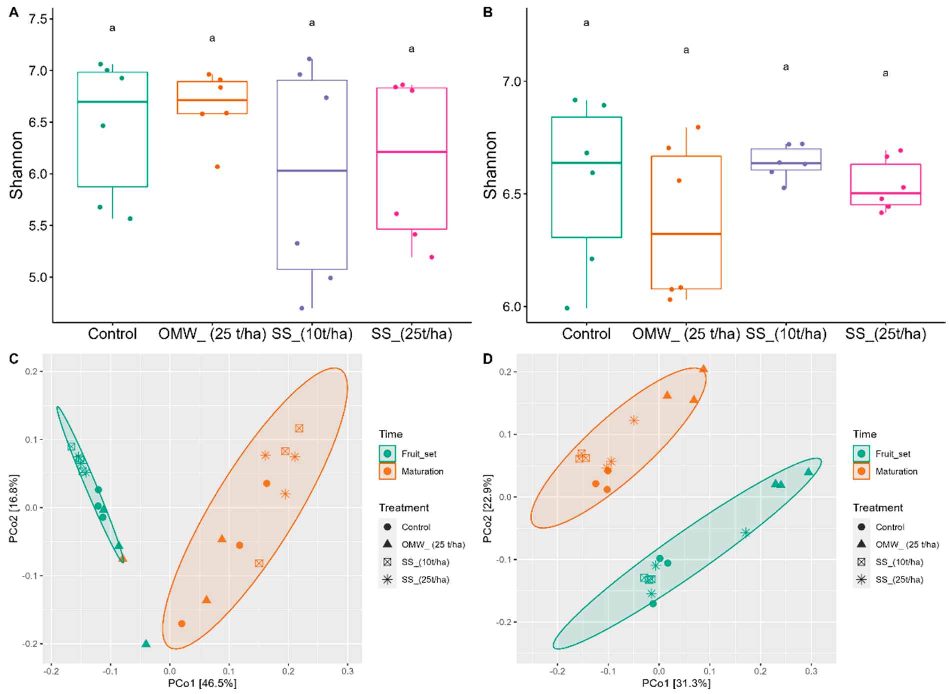

Figure 7. α- and β-diversity in soils treated with biochar from various sources and different doses in the bulk soil (**A**,**C**) and the rhizosphere (**B**,**D**) of tomato plants grown under field conditions. In panels (**A**,**B**) the colors denote the treatments; green; Control, Yellow: 25 t/ha OMW biochar, Blue: 10 t/ha SS biochar and Magenta: 25 t/ha SS biochar.

Beta diversity was evaluated separately in each soil compartment (bulk soil, rhizosphere) using both the Bray–Curtis dissimilarity and weighted UniFrac distance to assess the effect of treatment and sampling time. In the bulk soil principal coordinates analysis (PCoA) of the Bray–Curtis dissimilarity explained 31% of the variance in the first axis, which corresponded mainly to the different sampling times, while the second axis explained a lower proportion (14.9%), addressing the main effect of the applied treatments (Figure S3A). A similar pattern was observed with the weighted UniFrac distance, which explained a higher percentage of variance (46% and (Figure 7C). Regarding the β-diversity in the rhizosphere, the effect of the treatments was even more pronounced in the rhizosphere in soils treated with OMW to form a separate cluster (PERMANOVA of Adonis test ($p < 0.01$), that was mainly addressed by the first component (Figure 7D). The second component mainly addressed the effect of sampling time (Figure 7D). As in the case of bulk soil, the metrics, Bray–Curtis dissimilarity (Figure S3B) followed a similar pattern to that of weighted UniFrac distance (Figure 7D).

3.4.3. Network Analysis

Microbial networks and their topological properties have been linked to the stability of microbial communities to abiotic factors, ecosystem services, and soil functioning [66–68]. To understand whether biochar type and/or dose affect the complexity of microbial networks, the topological properties of the individual networks using Pearson correlation were evaluated. The results revealed minor differences in the topological properties of networks (clustering coefficient, modularity, positive edge percentage, edge density, edge connectivity, average path length) between treatments and control soils (Table S7). Specifically, soils treated with OMW biochar showed a higher modularity and clustering coefficient compared to the other treatments in the rhizosphere. All the biochar-treated soils were also characterized by a higher edge connectivity. In contrast, these differences nearly disappeared in bulk soil (Table S7). These findings contrast with a previous study that reported significant shifts in the structure of networks in biochar-amended soils [22,23]. Strong shifts in the topological properties of networks in soils amended with different doses of biochar were also reported in a recent study [69]. The reasons for this deviation in our study remain obscure and may be related to environmental conditions or the feedstocks used.

4. Conclusions

The aim of this research study was to provide evidence that biochar acts as an effective and safe soil improver and plant bio-stimulant in agricultural applications. The detailed assessment of the biochar produced from two bio wastes, SS and OMW, under real field conditions documented the potential for their valorization and yielded some key conclusions that can be summarized as follows:

- SS biochar is a bio-stimulant to plant growth, but this effect is mediated by the feedstock. Fruit marketable yield and crop performance increased significantly. The mechanism that stimulated plant growth was not fully identified. Microbiota structure in the presence of the SS biochar may play a major role, and further research is needed to better understand the impact of these shifts in the functioning of agroecosystems and crop performance.
- Biochar application also had positive impacts on C and P sequestration in the soil, improved soil structure, and improved soil nutrient content at deeper soil levels.
- The OMW-derived biochar did not exhibit results as positive as the SS biochar. This likely arose from the low availability of N and/or higher C/N ratio, which favored N immobilization, revealing additional criteria for biochar selection for application on agricultural lands, depending on variability.

This study may act as a guideline for farmers and agronomists for maximizing the growth and yield of agricultural crops in Mediterranean environments using tomato as reference crop, improving soil health, and contributing to the sustainable management of agroecosystems. Co-valorization of locally available by-products of agricultural or municipal origin is more advantageous, provides better opportunities for success, is in line with the principles of the circular economy, generates new knowledge, and is an added value for the local community and the environment. The significance of the experimental results has implications in all aspects of the global challenges, from climate change adaptation to food security, water management, soil ecosystem services, and environmental health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15053879/s1. Table S1: Characterization of the soil samples collected from each of the 4 treatments (initial conditions); Table S2: Characterization of the soil samples collected from each of the 4 treatments (final conditions); Table S3: Characterization of the tomato samples collected from each of the 4 treatments (final conditions); Table S4: Characterization of the leaf samples collected from each of the 4 treatments (final conditions); Table S5: Characterization of the shoot samples collected from each of the 4 treatments (final conditions); Table S6: Characterization of the root samples collected from each of the 4 treatments (final conditions); Table S7: Topological properties of microbial networks in the bulk soil and rhizosphere of soils treated with biochar (SS, OMW) and non-treated (controls); Figure S1: Effect of sampling time on the composition of microbial communities in the bulk soil (A) and rhizosphere (B); Figure S2. Effect of sampling period on the a diversity of microbial communities in the bulk soil (A) and rhizosphere (B); Figure S3. Beta diversity of microbial communities using the Bray distance in the bulk soil (A) and the rhizosphere (B).

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