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An Organic Fertilizer 'Doped' with a *Bacillus* Strain Improves Melon and Pepper Yield, Modifying the Rhizosphere Microbiome with Negligible Changes in the Bulk Soil Microbiome

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Abstract: Doped compost consists of compost inoculated with *Bacillus siamensis* SCFB3-1 that is formulated in biochar and then mixed with the compost. The study objective was to analyze, at field scale, the effect of doped compost on the melon and pepper yield and on the soil microbiome, hypothesizing that the synergy between the components of doped compost confers additional benefits to the crop. Two doses of compost (2 and 5 t/ha) and two doses of the inoculant (biochar+SCFB3-1) with respect to the compost (3% and 6% *w:w*) were tested. The highest yield was observed for a reduced dose of mineral fertilization (NPK -20%) with a compost dose of 2 t/ha with 6% of the inoculant. Specifically, the yield increase compared with the control, which only received NPK, was a 47% increase in melon and 28% in pepper. The microbiome of the bulk soil was not modified by the doped compost, but the composition of the rhizosphere microbiome changed, increasing in the abundance of *Bacillus* (the inoculated strain), but also changing the relative abundance of other genera in the bacterial community. Future works will be focused on unravelling the possible effects of phytohormones on the observed results.

Keywords: *Bacillus siamensis*; biochar; compost; microbial biostimulants; soil microbiome

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1. Introduction

Nowadays, new strategies for sustainable food production are essential [1] to meet the needs of the continuously growing global population [2]. In order to address this demand for food, agricultural productivity must increase [3]. Mineral fertilizers greatly increase crop yields but, at the same time, have led to perturbations in natural environments, where nutrient over-enrichment leads to a loss of habitats, as well as species richness and functionality, which in turn alters the goods and services provided by ecosystems [4,5]. Hence, maintaining crop yield improvements whilst adhering to sustainability criteria is considered necessary to reduce the levels of chemical inputs that could potentially harm natural ecosystems [6]. Introducing the principles of the circular economy in agriculture [7] and combining fertilizer-based techniques with microbiological-based ones [4] have represented, in recent years, a promising compromise to solve the problem of resource depletion and excess chemical inputs. At the same time, the adequate combination of these factors can provide sufficient yields [8].

As part of such biological inputs, several microbial species are acknowledged to have a beneficial effect on plant growth. Among the bacteria, those that colonize plant roots and promote their growth are named plant growth-promoting rhizobacteria (PGPR) or, more broadly, plant-growth promoting bacteria (PGPB). Mutualistic fungi, mainly mycorrhizae, also promote plant growth [9,10]. Both beneficial bacteria and fungi improve plant yields in a number of different ways [11,12] and reduce the proliferation of plant pathogens [13].

According to European regulations [14], the so-called microbial plant biostimulants (MPBs) comprise a PGPB or consortia of PGPBs that act as fertilizer products independently of the nutrient content of the product, stimulating plant nutrition processes and enhancing one or more of the following: (a) nutrient use efficiency; (b) abiotic stress tolerance; (c) quality traits; and (d) the availability of nutrients confined in the soil or rhizosphere. Biocontrol of the induction of plant resistance to biotic stresses is excluded from the MPB group.

Member species of the genus *Bacillus* are acknowledged as PGPBs because they comprise a relevant number of strains used in biocontrol in agriculture; moreover, their role as MPBs is being increasingly addressed [10], and thus *Bacillus* is currently considered an important and promising tool for sustainable agriculture [15]. Scientific studies about *Bacillus* strains as MPBs have focused more on cereals than on horticultural crops; *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus cereus* and *Bacillus pumilus* are the leading species [10]. To the best of our knowledge, in contrast with other *Bacillus* species, there are very few works about the use of *Bacillus siamensis* strains as an MPB [16].

Bio-residues from agriculture or forestry pruning cause problems in the soil when they are not properly managed. Some of these bio-residues are applied to soils as crop supplements. However, predicting plant-available N from decomposing crop residues is highly challenging because of the multiple factors involved in its decomposition [17]. These factors include residue chemistry, residue placement, environmental conditions and soil characteristics [18–20]. For example, the application of bio-residues can produce the opposite results in crops as many are allelopathic, and organic matter accumulation has been demonstrated to exert an autotoxic effect on reiterated crops [21,22]. The circular economy approach involves the conditioning of bio-residues intended for use as agricultural inputs [23]. Two outstanding conditioning technologies are composting and pyrolysis; the latter is used to obtain biochar. Compost is a high-quality organic fertilizer for crops [24]. Biochar is widely used in agriculture as a soil supplement because of the benefits it confers on the soil [25–27] and the environment, e.g., sequestration of atmospheric C in soil [28–30], as well as its capacity to reduce mineral N leaching and N_2O emissions [31,32]; thus, it can be considered a mitigator of climate change. Apart from soil supplementation, biochar has also been used in combination with other agricultural inputs, e.g., through co-composting, it can increase the retention and supply of plant-available nutrients, improving the crop yield [33,34]; it has also been used as a carrier for rhizobia inoculants in legume crops [35,36].

Among the horticultural crops, Spain stands out for the production of melons and peppers. Specifically, Spain is the leading European producer of melons, as well as being the leading exporter and the eighth largest producer worldwide [37]. Spain is also a leading European producer of peppers and the fifth largest in the world [37].

To contribute to the global search for technologically improved organic fertilizers for sustainable agriculture within a circular economy approach, the objective of this work was to assess the agronomic and environmental effects of an innovative organic fertilizer based on compost inoculated with spores of *B. siamensis* formulated with biochar as a carrier (which we have called 'doped compost'), combined with a 20% reduction in the dose of mineral fertilizer in melon and pepper crops. We hypothesized that synergies between the compost, the biochar and the PGPR strain would confer additional benefits in terms of crop yield and fruit and ecological parameters. Such parameters were assessed in a field trial, analyzing the crop response and the effect of the treatment on the microbiome composition using a next-generation sequencing approach.

2. Materials and Methods

2.1. Doped Compost Production and Component Description

The 'doped compost' consisted of two components, i.e., compost and an inoculant consisting of the bacterial strain SCFB3-1 from *B. siamensis* formulated with biochar as a carrier. The strain SCFB3-1 was isolated from the rhizosphere of a sweet pepper crop in San Cristobal de Entreviñas (Zamora, Spain) and it belongs to the IQUIMAB microbial strain collection (University of León, León, Spain); it was stored at −80 °C in tryptic soy

broth (TSB) medium with 30% glycerol [16,38]. It was selected out of a collection of 186 isolates because it showed relevant growth-promoting activities in vitro, in concrete IAA production and ACC deaminase activity [38]. Moreover, in a field trial, a sweet pepper crop inoculated with a liquid formulation of SCFB3-1 in pure culture and fertilized with a reduced dose of mineral nitrogen (80%) produced a nearly 30% yield increase compared to the uninoculated control with a full N dose [16]. To prepare the inoculum, the frozen strain was first grown in tryptic soy agar (TSA) (Millipore reference number 22091) at 28 °C for 3 d. To produce a sufficient quantity of the inoculum at a concentration of 1.5×10^9 cfu mL^{-1}, a pilot fermenter (BIOSTAT Bplus-MO, Sartorius, Germany; 5 L in total capacity) was used; the growth medium was TSB, and the temperature 28 °C, under continuous stirring and aeration; the fermentation took 36 h. The resulting bacterial suspension was combined with two cell protectors, one polysaccharide and one disaccharide, at 0.5% (weight:volume) each. The biochar was obtained from the wood of vine shoots (Table S2) by slow pyrolysis in a pilot pyrolizer with an electrically heated reactor and a semi-continuous feeding system. The system for biochar production and the characteristics of the pyrolyzer are described in [39]. The compost used was a mixture of de-alcoholized grape pomace combined with vinasses of lees and crushed plant biomass (Table S1).

2.2. Field Trial Design

The crops used for the trial were the melon cultivar 'Piel de sapo' and the pepper cultivar 'Medrano'. The trials were conducted in Rambla Salada (37°2′0.11″ N, 2°16′27.095″ W; Mazarrón) for melon and El Moaire (37°2′0.11″ N, 2°16′27.095″ W; Blanca) for pepper in 2018, while trials in 2019 were conducted in Los Lorentes (37°2′0.11″ N, 2°16′27.095″ W; Mazarrón) for melon and Rambla Salada (37°2′0.11″ N, 2°16′27.095″ W; Mazarrón) for pepper. At each location, the experimental design was a randomized complete block with three blocks. For the melon trial, the elementary plot was 35 m^2, with one row 17.5 m in length, 2 m row spacing and a space between plants of 0.8 m, for a total plantation density (plants/ha) of 6250. For the pepper trial, the elementary plot was 20 m^2, with one row 20 m in length, 1 m row spacing and a space between plants of 0.4 m, for a total plantation density (plants/ha) of 25,000. The whole set of treatments and the corresponding controls are described in Table 1.

Table 1. Controls and treatments carried out in the field trial for melon and pepper crops.

Treatment	Mineral N Fertilizer		Organic Fertilizer			
					Additive	
	Dose (kg N ha^{-1})	Type	Dose (t ha^{-1})	Type	Percentage of Additive in the Compost (*w*:*w*) (%)	Description
Control 0	0		0			
Control 80	176 (80% of full dose)	NH_4NO_3 (27% N)	0			
Control 100	220 (full dose)	NH_4NO_3 (27% N)	0			
Compost 2	176	NH_4NO_3 (27% N)	2	Compost	0	-
Compost 5	176	NH_4NO_3 (27% N)	5	Compost	0	-
Compost 2 + bb3	176	NH_4NO_3 (27% N)	2	Compost + additive	3	biochar:bacterial suspension 1×10^9 cfu ml^{-1} (1:1 w:vol.)
Compost 2 + bb6	176	NH_4NO_3 (27% N)	2	Compost + additive	6	biochar:bacterial suspension 1×10^9 cfu ml^{-1} (1:1 w:vol.)
Compost 5 + bb3	176	NH_4NO_3 (27% N)	5	Compost + additive	3	biochar:bacterial suspension 1×10^9 cfu ml^{-1} (1:1 w:vol.)
Compost 5 + bb6	176	NH_4NO_3 (27% N)	5	Compost + additive	6	biochar:bacterial suspension 1×10^9 cfu ml^{-1} (1:1 w:vol.)

The organic fertilizers (Table 1) were applied by hand immediately before transplantation, and the corresponding dose was spread on each row at a distance of 25 cm at each site of the drip line and incorporated into the soil with a motorized hoe. Subsequently, the plants were transplanted. The mineral N fertilizer was applied in the form of ammonium nitrate (27% N) at the dose per treatment indicated in Table 1. The schedule of application consisted of ten different applications along the crop cycle, each one corresponding to one tenth of the full dose; the schedule of application was intended to emulate fertirrigation, in which the fertilizer is distributed along the crop cycle. Mineral P and K fertilizers were applied at the same dose for all the treatments by fertirrigation in the following doses: P_2 O_5 at 38 kg ha^{-1} in melon 2018, 126 kg ha^{-1} in pepper 2018, 100 kg ha^{-1} in melon 2019 and 42 kg ha^{-1} in pepper (2019); K_2O at 216 kg ha^{-1} in melon (2019) and 0 kg ha^{-1} in the rest. The calculation of P and K needs was based on the methodology indicated by [40], which considers, for dose calculations, the soil characteristics (Table S3) and the expected yield of 31,000 kg ha^{-1} for the melon crop and 42,000 kg ha^{-1} for the pepper crop. Transplantation was conducted on April 25th for melons and May 18th for peppers in 2018, and May 21st for melons and June 19th for peppers in 2019. The crop was drip-irrigated, keeping the soil moisture between 80% and 100% of the field capacity. The climatic conditions at each site and plot are summarized in Table S4.

2.3. Sampling, Variables Measured and Data Analysis

For both crops, the dependent variables of the trial were yield and its components, fresh and dry aerial vegetative biomass, chlorophyll content (measured in 25 leaves per treatment and plot with a portable chlorophyll meter: CCM-200, ADC BioScientific Ltd., Hoddesdon, U.K.), fruit contour (expressed in centimeters for the melon fruit and in millimeters for the pepper fruit) and conductivity (measured with a conductivity meter: PCE-CM 41, PCE instruments, Albacete, Spain) and solute concentration (measured with a portable digital meter) in the fruit juice. Twenty-five fruits were analyzed per treatment and plot. Moreover, the fruit penetrometry (with a penetrometer: PCE-PTR 200N, PCE Holding GmbH & Co, Hamburg, Germany) and flowering time were also measured in melons. The flowering time was measured by counting the number of plants in phenological stage 61, and the result was expressed as the percentage of the plant that had reached that stage. Melons were harvested at crop phenological stage 74 on the BBCH scale and ended at stage 78, and in pepper, harvesting started at stage 71 and ended at stage 75 on the BBCH scale [41].

The mean values of the dependent variables for the combined treatments (compost dose and inoculant dose) were compared with the Dunnett test, using the control 80 treatment as a reference for comparison (Figures 1–4). Moreover, ANOVA was carried out with the location, year and plot as random factors, whilst the treatments (the compost dose on the one hand and the inoculant dose on the other) were fixed factors. The effects of the compost dose, the inoculant dose and the interaction between the two were analyzed, and the Tukey test was used for post hoc mean comparisons (Tables 2–5 and Tables S5–S8), using IBM-SPSS v.26.0., IBM Corporation, Armonk-NY, USA.

2.4. DNA Extraction, Sequencing and Data Analysis

Rhizospheric and bulk soil from the melon crop were sampled to isolate total soil DNA during 2019, in the following treatments: control 0, control 80, compost 5 and compost 5+bb6. Rhizospheric soil samples were obtained from two plants per treatment belonging to the central block. In detail, plants were extracted from the soil five days after the flowering measurement, and the rhizospheric soil in contact with the root was collected using previously sterilized brushes to avoid cross-contamination, sieved (2 mm), homogenized and stored as three different samples in Falcon tubes at −80 °C for subsequent DNA extraction. Bulk soil was also collected from each treatment, sieved, homogenized and stored as three samples under the same conditions.

Three samples each of rhizospheric and bulk soil were collected per treatment, each one consisting of 300 mg of soil, and used for total microbial DNA extraction using the DNeasy Power Soil kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The composition of soil microbial communities was analyzed by sequencing paired-end amplicons of the 16S rRNA gene and the ITS region of fungal ribosomal DNA using the Illumina MiSeq high-throughput sequencing platform at Molecular Research DNA (MR DNA) (www.mrdnalab.com Shallowater, TX, USA) (accessed on 5 April 2022).Each sample was sequenced as a paired-end set of reads. The primer set used was 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), which are specific to the V4 region of 16S rRNA, and ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) for the ITS region.

Sequence data were processed using the MR DNA pipeline (MR DNA, Shallowater, TX, USA), which involves the removal of primers, short sequences <150 bp and sequences with doubtful base calls. Sequences were quality-filtered at Q25 quality prior to processing using a maximum error threshold of 1.0, and subsequently dereplicated and denoised. A removal procedure was carried out to remove sequences with PCR point errors, singletons and chimeric sequences to obtain denoised sequences or amplicon sequence variants (ASV). Taxonomy was then assigned using BLASTn against a database derived from the Ribosomal Database Project II (RDPII, http://rdp.cme.msu.edu, accessed on 6 April 2022) and the National Centre for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov, accessed on 6 April 2022). The raw data obtained from this analysis were deposited in the Sequence Read Archive (SRA) of the NCBI under nucleotide sequence accession number PRJNA860271.

Microbial community analysis and plots were obtained using the Primer v7 and PERMANOVA+ software [42]. Diversity metrics such as the ASV number, the Shannon diversity index and the number of reads of the soil microbial communities for the four treatments (control 0, control 80, compost 5 and compost 5+bb6) were calculated and boxplots used to show the distribution of diversity indices. Factorial analysis of variance (ANOVA) was run to verify the effect of soil position (rhizospheric or bulk) and treatment (control 0, control 80, compost 5 and compost 5+bb6) on the species richness, number of reads and Shannon index of the obtained ASV. Specific changes among experimental conditions were then calculated using post-hoc Tukey's HSD test. Statistical significance of treatments was assumed for p-values below 0.05. Analysis of variance and post hoc tests were carried out using IBM-SPSS v.26.0. IBM Corporation, Armonk-NY, USA.

Relative abundances of microbial taxa at the phylum level were shown by the use of stacked bar charts, while the detailed organization of both bacterial and fungal communities was presented as heat plots at the ASV level. Heat plots were organized according to hierarchical clustering based on Bray–Curtis distances among samples, while the 50 most frequent species were plotted according to hierarchical clustering based on the index of association. Non-metric multidimensional scaling (nMDS) of the Bray–Curtis dissimilarity matrix was used to assess the variation in the composition of the bacterial and fungal communities after applying the different treatments for rhizosphere and bulk soil. The significance of pairwise differences between the responses to the treatment within each type of soil for each microbial community was tested with permutational multivariate analysis of variance (PERMANOVA) for 999 permutations; dissimilarity was based on Bray–Curtis using treatment and soil as fixed factors.

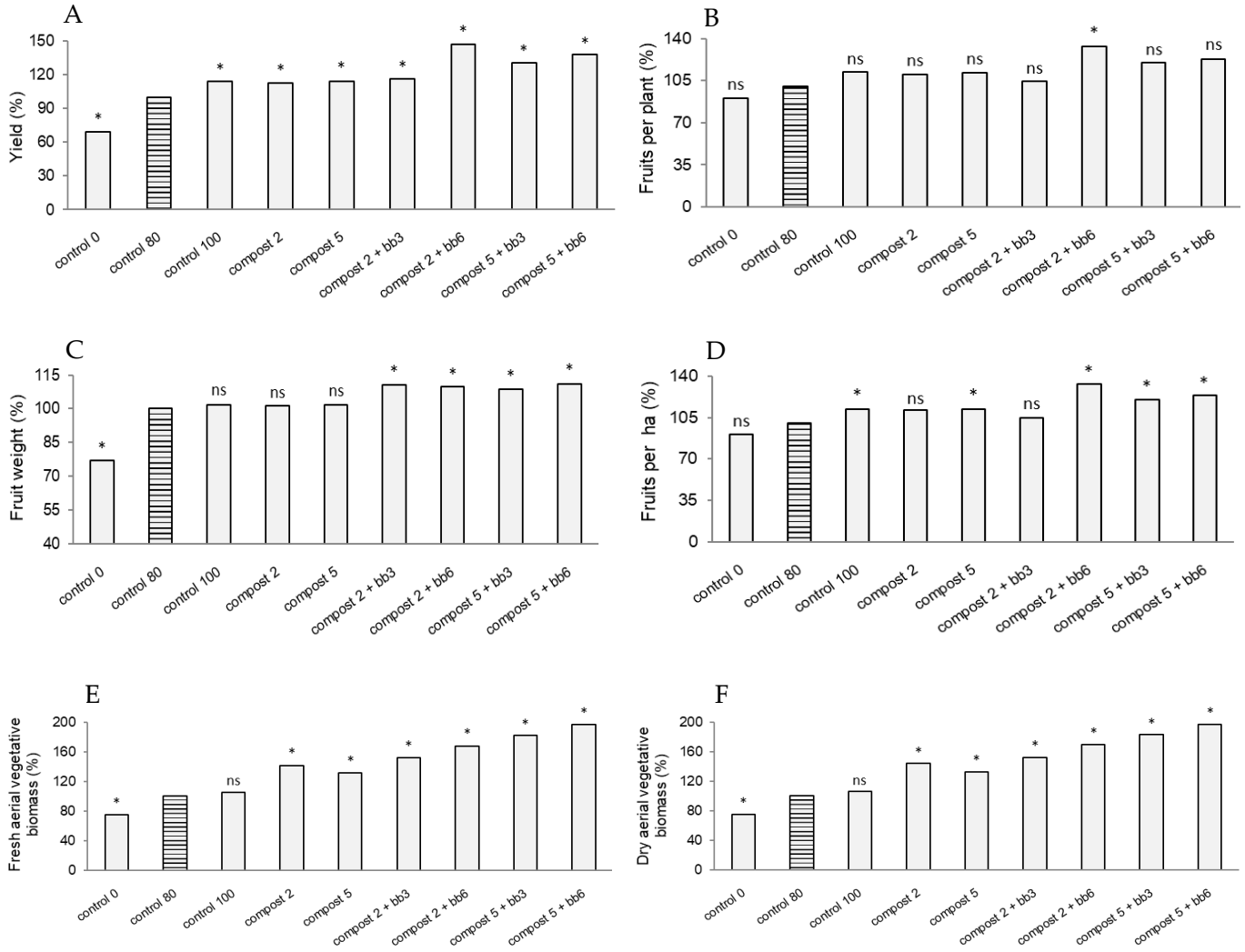

Figure 1. Responses of several agronomic variables to the treatments applied in the field trial for the melon crop: yield (**A**), its components (**B**–**D**), fresh aerial vegetative biomass (**E**) and dry aerial vegetative biomass (**F**). The values are expressed as relative values in percentage, using, as a reference (100%), the control 80. Comparison against control 80 was performed with the Dunnett test (* means significant difference at $p \leq 0.05$ with the control 80, ns means not significant difference at $p \leq 0.05$ with the control 80).

Table 2. ANOVA for yield, yield components and biomass production in the melon crop. The location, the year and the plot were considered random factors, whilst the compost dose and the additive (PGPR + biochar) dose were fixed factors (significance level: *** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; ns, not significant).

Doses	Fresh Aerial Vegetative Biomass (g Per Plant)		Dry Aerial Vegetative Biomass (g Per Plant)		Yield (kg ha^{-1})		Number Fruits Per Plant		Fruit Weight (g)		Number Fruits Per ha	
	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic
Compost dose (t/ha)	1,445,378	33.32 ***	802,250	33.43 ***	48,158,351	9.19 ***	0.23	1.13 ns	2948	0.59 ns	7,615,915	8.06 ***
Additive dose (%)	1,170,231	26.98 ***	629,323	26.22 ***	323,276,628	61.67 ***	1.01	4.94 **	151,818	30.27 ***	29,944,528	31.69 ***
Compost dose × Additive dose	283,338	6.53 ***	163,831	6.83 ***	53,606,898	10.23 ***	0.47	2.27 ns	4462	0.89 ns	12,941,871	13.69 ***

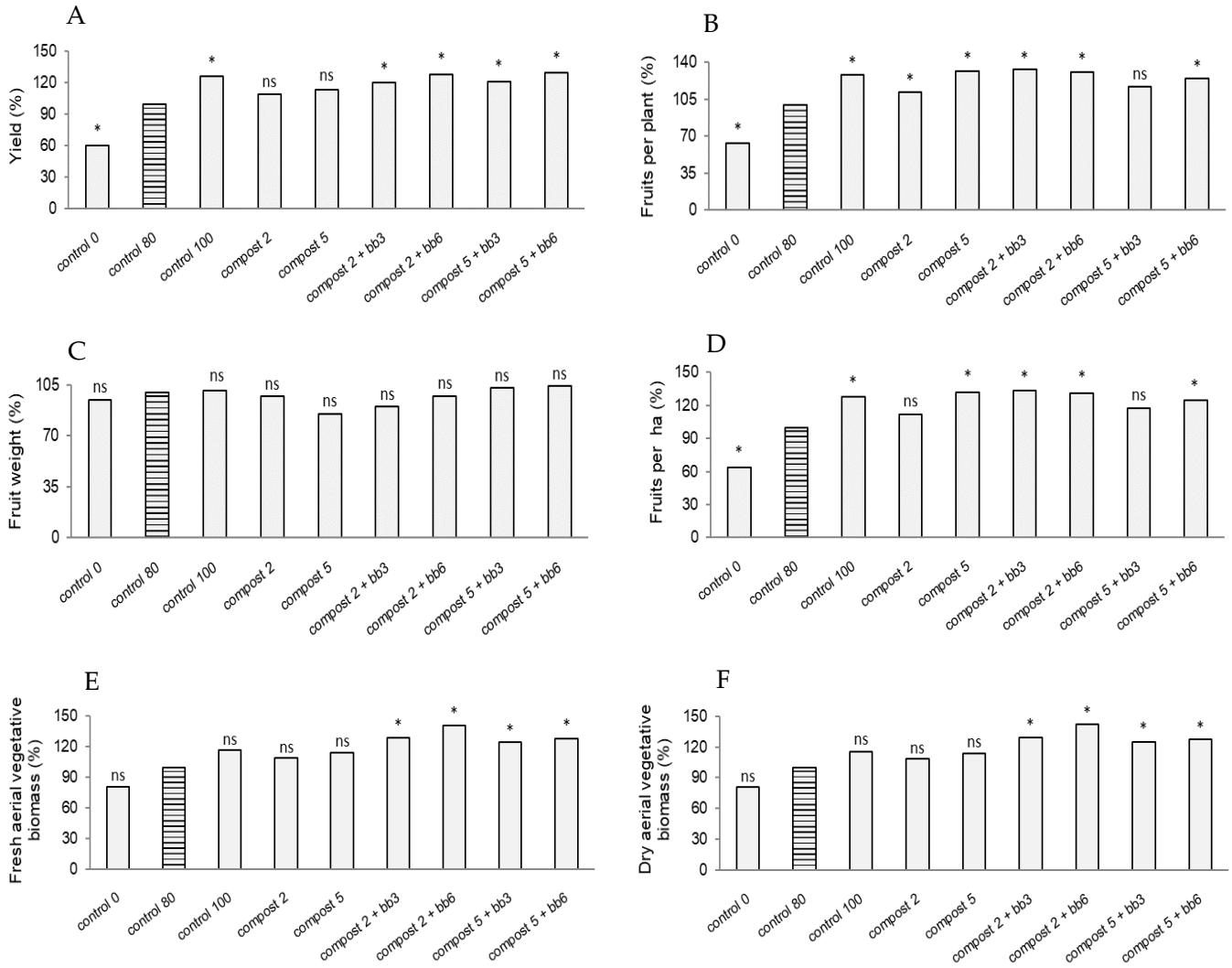

Figure 2. Responses of several agronomic variables to the treatments applied in the field trial for the pepper crop: yield (**A**), its components (**B**–**D**), fresh aerial vegetative biomass (**E**) and dry aerial vegetative biomass (**F**). The values are expressed as relative values in percentage, using, as a reference (100%), control 80. Comparison against control 80 was performed with the Dunnett test (* means significant difference at $p \leq 0.05$ with the control 80, ns means not significant difference at $p \leq 0.05$ with the control 80).

Table 3. ANOVA for yield, yield components and biomass production in the pepper crop. The location, the year and the plot were considered random factors, whilst the compost dose and the additive (PGPR + biochar) dose were fixed factors (significance level: *** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$; ns, not significant).

Doses	Fresh Aerial Vegetative Biomass (g Per Plant)		Dry Aerial Vegetative Biomass (g Per Plant)		Yield (kg ha^{-1})		Number Fruits Per Plant		Fruit Weight (g)		Number Fruits Per ha	
	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic
Compost dose (t/ha)	4938	2.42 ns	-	-	51,096,351	3.57 *	40	5.71 **	152	2.02 ns	24,859,770,931	5.71 **
Additive dose (%)	12,428	6.10 **	1284	5.43 **	171,270,302	11.95 ***	4	0.59 ns	242	3.23 *	2,582,073,179	0.59 ns
Compost dose × Additive dose	1813	0.89 ns	-	-	1,602,240	0.11 ns	45	6.47 ***	436	5.81 **	28,180,202,318	6.47 ***

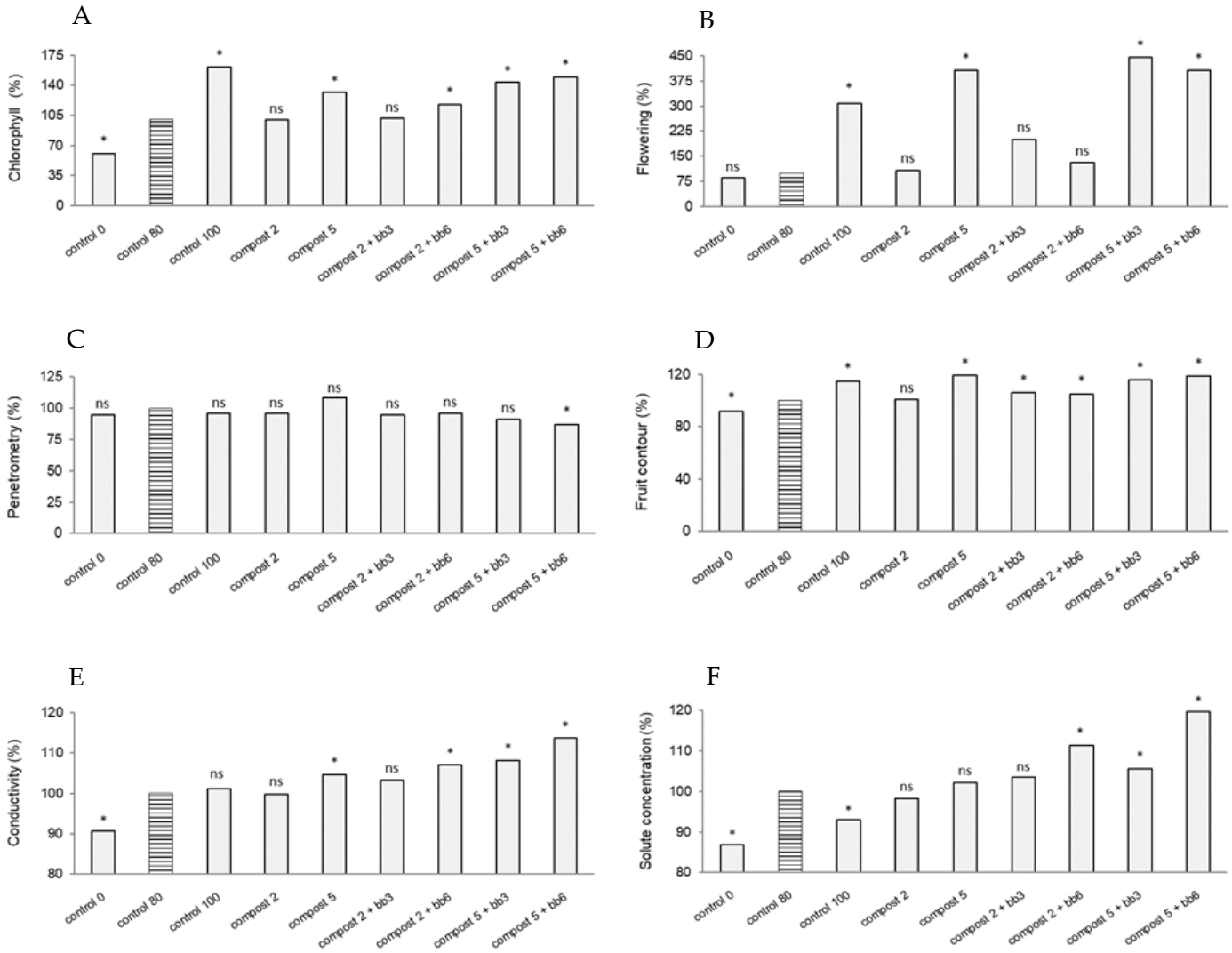

Figure 3. Responses of other variables to the treatments applied in the field trial for the melon crop. The chlorophyll (**A**), the flowering (**B**), the penetrometry (**C**), the fruit contour (**D**), the conductivity (**E**), the solute concentration (**F**).The values are expressed as relative values in percentage, using, as a reference (100%), control 80. Comparison against control 80 was performed with the Dunnett test (* $p \leq 0.05$, ns not significant).

Table 4. ANOVA for chlorophyll content, flowering and several fruit parameters, in the melon crop. The location, the year and the plot were considered random factors, whilst the compost dose and the additive (PGPR + biochar) were fixed factors (significance level: *** $p \leq 0.001$; ns, not significant).

Doses	Chlorophyll (CCI)		Flowering (%)		Penetrometry (kg)		Fruit Contour (cm)		Conductivity (μS cm^{-1})		Solute Concentration (mg l^{-1})	
	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic
Compost dose (t/ha)	460.030	9.640 ***	3724.643	37.421***	0.059	2.566 ns	222.610	31.083 ***	0.591	15.926 ***	81045.252	15.240 ***
Additive dose (%)	71.741	1.503 ns	147.024	1.477 ns	0.247	10.80 ***	1.427	0.199 ns	0.564	15.186 ***	222846.500	41.905 ***
Compost dose × Additive dose	8.190	0.172 ns	22.024	0.22 ns	0.218	9.507 ***	10.496	1.465 ns	0.009	0.231 ns	8879.786	1.670 ns

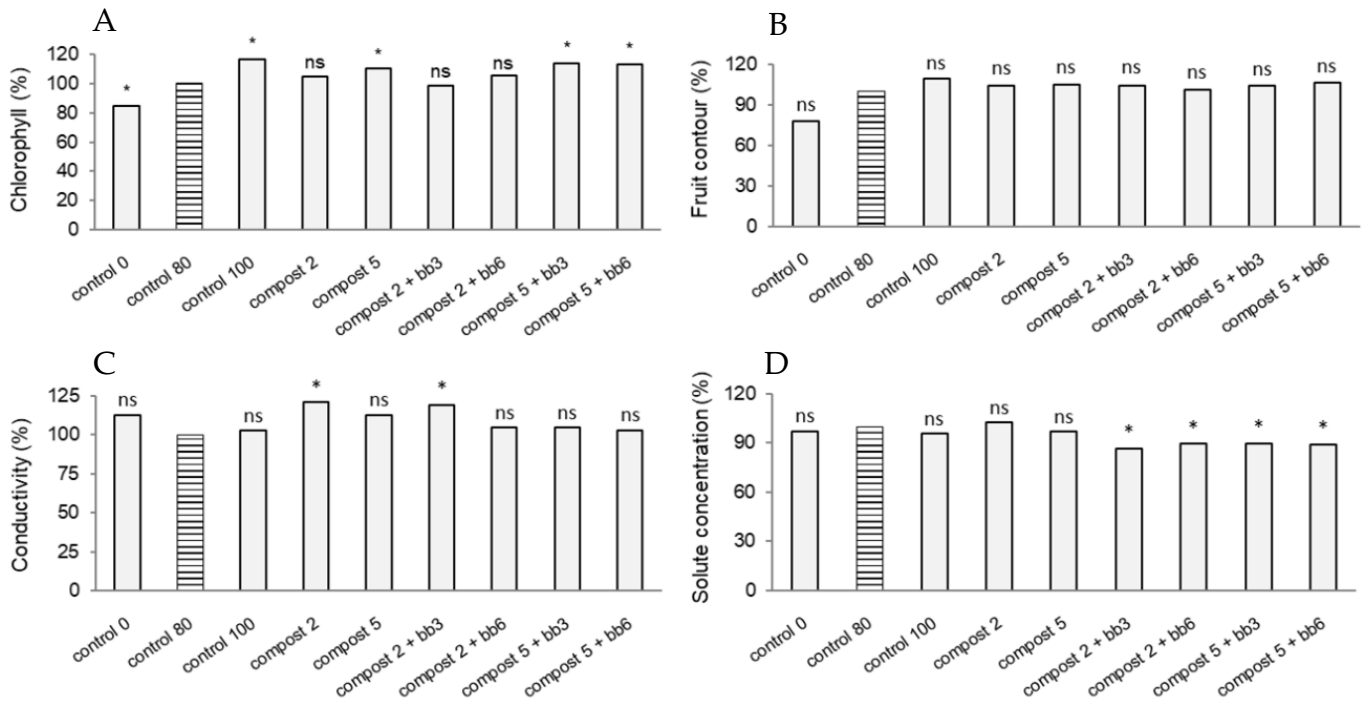

Figure 4. Responses of other variables to the treatments applied in the field trial for the pepper crop. The chlorophyll (**A**), the fruit contour (**B**), the conductivity (**C**), the solute concentration (**D**).The values are expressed as relative values in percentage, using, as a reference (100%), control 80. Comparison against control 80 was performed with the Dunnett test (* means significant difference at $p \leq 0.05$ with the control 80, ns means not significant difference at $p \leq 0.05$ with the control 80).

Table 5. ANOVA for chlorophyll content and several fruit parameters, in the pepper crop. The location, the year and the plot were considered random factors, whilst the compost dose and the additive (PGPR + biochar) were fixed factors (significance levels: *** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$; ns, not significant).

Doses	Chlorophyll (CCI)		Fruit Contour (mm)		Conductivity (µS cm^{-1})		Solute Concentration (mg l^{-1})	
	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic
Compost dose (t/ha)	383.867	6.790 **	66.404	0.051 ns	0.532	7.733 **	7926.828	15.240 ***
Additive dose (%)	18.672	0.330 ns	0.141	0.000 ns	0.357	5.194 *	180,354.056	41.905 ***
Compost dose × Additive dose	53.852	0.953 ns	6.699	0.005 ns	0.082	1.187 ns	18,287.056	1.670 ns

3. Results

3.1. Yield, Yield Components and Biomass Production

In the melon crop (Figure 1), all the treatments showed significantly higher yields and vegetative biomass than control 80. The best yield and the highest aerial biomass were achieved by the doped compost, with yields of 16%, 31%, 38% and 47% above control 80 depending on the combination between the compost dose and inoculant dose (Figure 1A); the highest yield corresponded to the compost 2+bb6 treatment, whilst the highest vegetative biomass was for compost 5+bb6 (Figure 1E,F). Regarding the number of fruits per plant, only the treatment compost 2+bb6 produced significantly higher values than control 80, with a 33% increase (Figure 1B). For fruit weight, the treatments with doped compost showed significantly higher values than control 80 (Figure 1C), and for the fruits per ha, the compost at the higher dose, irrespective of supplementation, and the compost (at 2 t/ha) at the lower dose of inoculant produced significantly higher values than control

80 (Figure 1D). In summary, the best-performing treatments were compost 2+bb6 for yield and its components and compost 5+bb6 for fresh and dry aerial vegetative biomass.

The results of ANOVA for the factors of compost dose and inoculant dose in the melon crop are presented in Table 2. The two factors produced significant differences in most of the dependent variables, and the interaction between the compost and inoculant doses was also significant; the exceptions were for the number of fruits per plant and the fruit weight, in which only the inoculant dose produced significant differences. For the compost dose 2t/ha, the inoculant dose of 6% produced significantly higher values than other inoculant doses (0% and 3%) for all the dependent variables except the fruit weight, in which there were no differences between 3% and 6%, but a difference between the inoculated and non-inoculated compost was observed (Table S5). For the compost dose 5t/ha, the two inoculant doses (3% and 6%) produced similar responses for all the dependent variables, and the obtained values were significantly higher than those in the non-inoculated compost, except for the number of fruits per plant, which was not affected by the inoculant, and the number of fruits per ha, in which only the dose of 6% produced a significant increase (Table S5).

In the pepper crop (Figure 2), the yield was significantly improved by all the treatments with doped compost; compared to control 80, the increase ranged between 21% and 30%, the highest corresponding to the treatment compost 5+bb6 (Figure 2A). Conversely, for fruit weight, there were no significant differences between the treatments and the control. For the number of fruits per plant and fruits per ha, the treatments with doped compost and the non-inoculated compost at 5 t/ha showed significantly higher values than control 80, but the highest ones were for the treatments with doped compost, reaching the maximum for compost 2+bb3. In the case of aerial vegetative biomass (fresh and dry), only the treatments with doped compost showed significant differences with respect to control 80, the highest value corresponding to compost 2+bb6 (on average 41% higher than control 80).

The ANOVA results for compost dosing and inoculant dosing in the pepper crop are presented in Table 3. Both factors produced a significant effect on crop yield, but, interestingly, compost dosing produced a significant effect on one of the yield components (the number of fruits) and an inoculant dose effect on the other one (the fruit weight). For the vegetative aerial biomass, only the inoculant dose produced a significant effect. The interaction of both doses was significant for the yield components, but neither for the yield nor for the fresh aerial biomass. For either compost dose, 2 t/ha or 5 t/ha, only the highest dose of inoculant (6%) produced a significantly higher yield compared to the non-inoculated compost, but, interestingly, both inoculant doses produced a significantly higher number of fruits for the compost dose 2 t/ha and significantly higher fruit weight for the compost dose 5 t/ha (Table S6). Regarding vegetative aerial biomass, both inoculant doses produced significantly higher biomass (Table S6).

3.2. Chlorophyll Content, Flowering and Fruit Parameters

In general terms, the treatments with doped compost produced significantly higher values than control 80, and the best results for fruit contour, conductivity and solute concentration were shown by the treatment with compost 5+bb6 (Figure 3D–F); exceptions to this general rule were found for the chlorophyll content and flowering, of which the highest values were observed for control 100, as well as the treatment with compost 5+bb3 (respectively, 61% and 346% higher with respect to control 80).

The ANOVA results for melon parameters are presented in Table 4. The compost dose produced significant differences for all the parameters except for the penetrometry, whilst the inoculant dose only produced significant differences for the penetrometry, conductivity and solute concentration. Moreover, the interaction between the two parameters was only significant for the penetrometry. For the compost dose 2t/ha, the inoculant in a dose of 3% produced significantly higher values than the non-inoculated compost, for three parameters (fruit contour, conductivity and solute concentration), whilst the dose of 6% produced higher values for all the parameters except the penetrometry (Table S7). For the compost dose 5t/ha, the inoculant in a dose of 3% produced significantly higher values

than the non-inoculated compost for the chlorophyll content and the penetrometry, as did the dose of 6% for all parameters except flowering and fruit contour (Table S7).

In the case of peppers, the response to the treatment varied depending on the treatment, and it was not possible to identify a generalized trend (Figure 4). Table 5 shows the ANOVA results for pepper fruit parameters. The compost dose produced significant differences in three out of the four parameters (the exception being the fruit contour), as did the inoculant dose in two (conductivity and solute concentration). No interaction was detected between compost and inoculant doses for any of the parameters analyzed. Interestingly, for both compost doses (2 and 5 t/ha), both doses of the inoculant (2% and 6%) significantly reduced the solute concentration (Table S8).

3.3. Bacterial and Fungal Diversity in Soil

Bacterial and fungal diversity showed a variation across the experimental samples (Figures 5 and 6). For bacteria (Figure 5), species richness (S) was significantly higher in the doped compost compared with the rest of the treatments and controls, both in bulk and in rhizospheric soil, the absolute highest being in rhizospheric soil. The absolute lowest value was for control 80 in the bulk soil, but this value did not significantly differ from control 0 in the bulk soil. Regarding the number of reads, the highest value was for non-inoculated compost in the rhizospheric soil, which did not differ from the doped compost in the rhizospheric soil; conversely, in the bulk soil, the highest value was for control 80, which did not differ from control 0. The Shannon index (H′) indicated that the greatest diversity was in rhizospheric soil in the doped compost, and the lowest was in rhizospheric soil of the non-inoculated compost; interestingly, in the bulk soil, the H′ value did not significantly differ between the doped compost and the non-inoculated compost, being, in both cases, significantly higher than in the two controls. For the fungal community, S was generally higher in the rhizospheric soil than in the bulk soil, with few differences for the other parameters (number of reads and H′) between the two locations (Figure 6). Focusing on the rhizospheric soil (Figure 6A), the highest values for species richness and H′ were for the non-inoculated compost treatment, but they did not differ from either control 80 or the treatment with the doped compost; conversely, the number of reads was significantly lower for the non-inoculated compost treatment. Regarding the bulk soil (Figure 6B), the non-inoculated control produced significantly higher S and significantly lower H′ than the rest of the treatments and controls. The effect of the soil position, the treatments and their interaction on the diversity indexes for bacterial and fungi is shown in Tables S9 and S10.

3.4. Composition of Bacterial and Fungal Soil Communities

The rhizospheric and bulk soil bacterial communities showed consistent changes in community organization when analyzed at the phylum level. The rhizospheric soil abundance of the phyla Proteobacteria, Actinobacteria and Bacteroidetes was higher, while in bulk soil, ASVs belonging to Firmicutes, Gemmatimonadetes and Acidobacteria dominated the bacterial community. Comparing communities by soil type, the differences between treatments were smaller in bulk soil than in rhizospheric soil (Figure S1). For fungal communities, non-relevant changes were observed at the phylum level for the type of soil, although the treatments produced an increased relative abundance of Basidiomycota in the rhizospheric soil (Figure S2).

At the ASV level, the bacterial and fungal communities differed between rhizospheric soil and bulk soil (Figures 7 and 8), except for the shared presence of *Ulocladium* and *Fusarium* in both soil types. Irrespective of the treatment, the bacterial community was characterized by the high presence of *Arthrobacter*, *Acinetobacter*, *Rhizobium*, *Pseudomonas*, *Sphingopyxis*, *Sphingobium* and *Novosphingobium* in the rhizospheric soil and by *Pelobacter*, *Gemmatimonas*, *Sphingomonas* and *Bacillus* in the bulk soil (Figure 7).

The genus *Bacillus* showed a much higher relative abundance in the rhizospheric soil of the doped compost treatment than in the rest of the treatments. Concomitantly, several bacterial genera, such as *Pseudoxantomonas*, *Massilia*, *Sphingopyxis*, *Sphingobium*,

Sphingomonas and *Ohtaekwangia*, increased in the doped compost treatment compared to the non-inoculated compost (compost 5), whilst the genera *Pseudomonas* and *Rhizobium* maintained their relative abundance but with a very slight increase in the latter, and the genera *Acinetobacter* and *Arthrobacter* decreased. However, few changes in relative abundance in the different genera were observed when comparing the doped compost treatment with the rest of the treatments in bulk soil. In the fungal community, irrespective of the treatment, an abundance of *Plestosphaerella* and *Phoma* in the rhizospheric soil and *Erotium*, *Mortierella*, *Microascus*, *Chaetomium*, *Chrysosporium*, *Stachybotrys* and *Aspergillus* in the bulk soil stood out (Figure 8). The treatment affected the relative abundance of the different genera in the rhizospheric soil, whilst negligible changes were observed in the bulk soil.

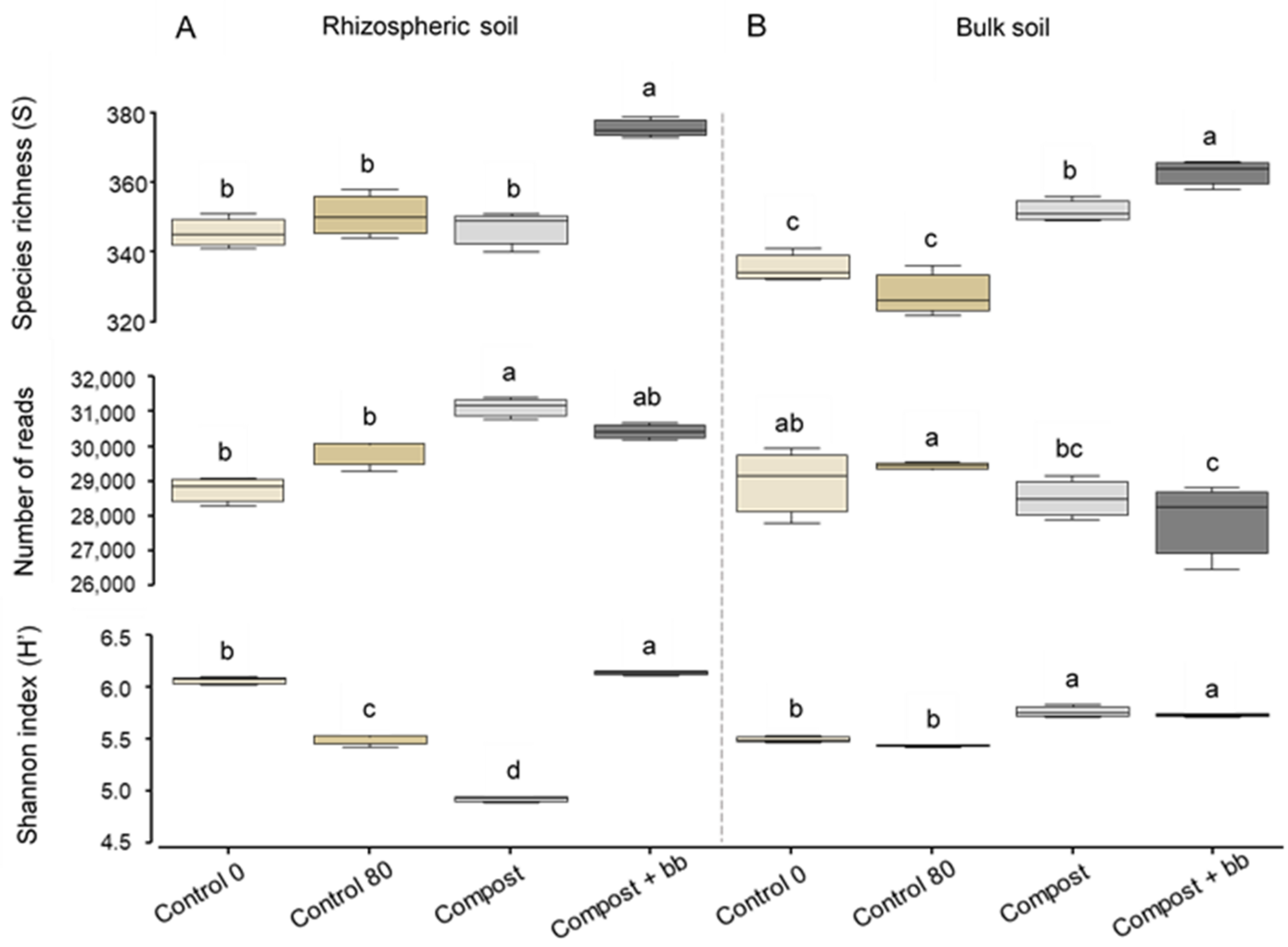

Figure 5. Boxplots showing the distribution of diversity indices for the bacterial community in each treatment in (**A**) rhizospheric and (**B**) bulk soil. S: N° of ASV/number of amplicon sequence variant; number of reads for the bacterial community; H': Shannon index. The lower and upper bounds of the boxplots show the first and third quartiles (the 25th and 75th percentiles), the middle line shows the median, and the whiskers above and below the boxplot indicate inter-quartile ranges. Different letters indicate significant differences for *p*-value below 0.05 according to Tukey's HSD test.

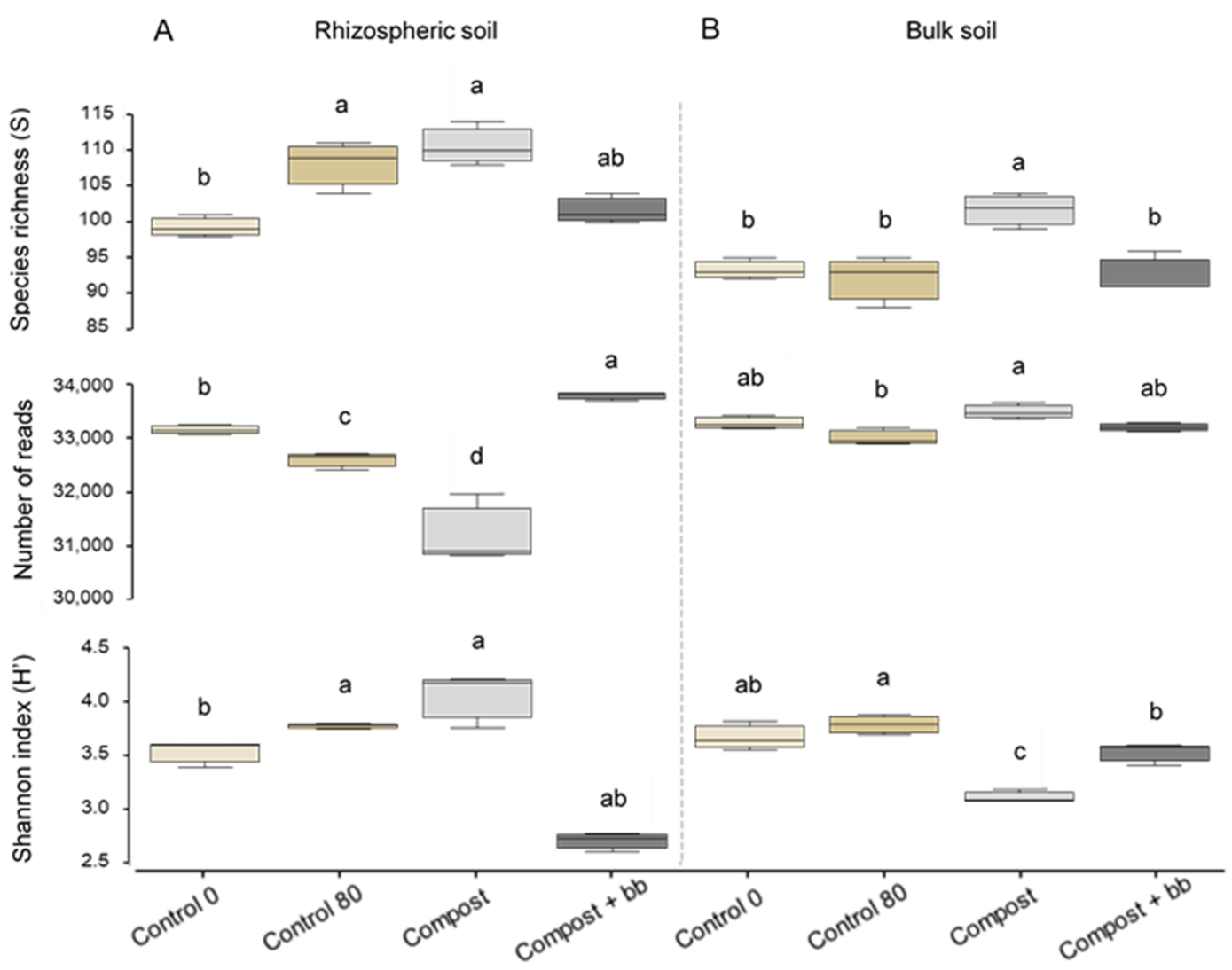

Figure 6. Boxplots showing the distribution of diversity indices for the fungal community in each treatment in (**A**) rhizospheric and (**B**) bulk soil. S, number of ASVs/number of amplicon sequence variants; number of reads for the bacterial community; H', Shannon index. The lower and upper bounds of the boxplots show the first and third quartiles (the 25th and 75th percentiles), the middle line shows the median, and the whiskers above and below the boxplot indicate inter-quartile ranges. Different letters indicate significant differences for *p*-values below 0.05 according to Tukey's HSD test.

NMDS ordination showed that the bacterial community of the rhizospheric soil showed a response to the treatments, whilst there was no response in the bulk soil, as can be confirmed by the degree of similarity (Figure 9A). Notwithstanding, for the rhizospheric soil, control 0 and the treatment with compost 5+bb6 did not show significant differences in the pairwise comparison (Figure 9A) (Table 6), and the same occurred for control 80 and the treatment with compost 5 (Figure 9A and Table 6). Moreover, some differences in the composition of the fungal community as a result of the treatments were observed in the rhizospheric soil and, to a lesser extent, in the bulk soil (Figure 9B). In the pairwise comparisons, such differences were significant for three pairs of treatments in the rhizospheric soil and only for one pair of treatments in the bulk soil (Table 6).

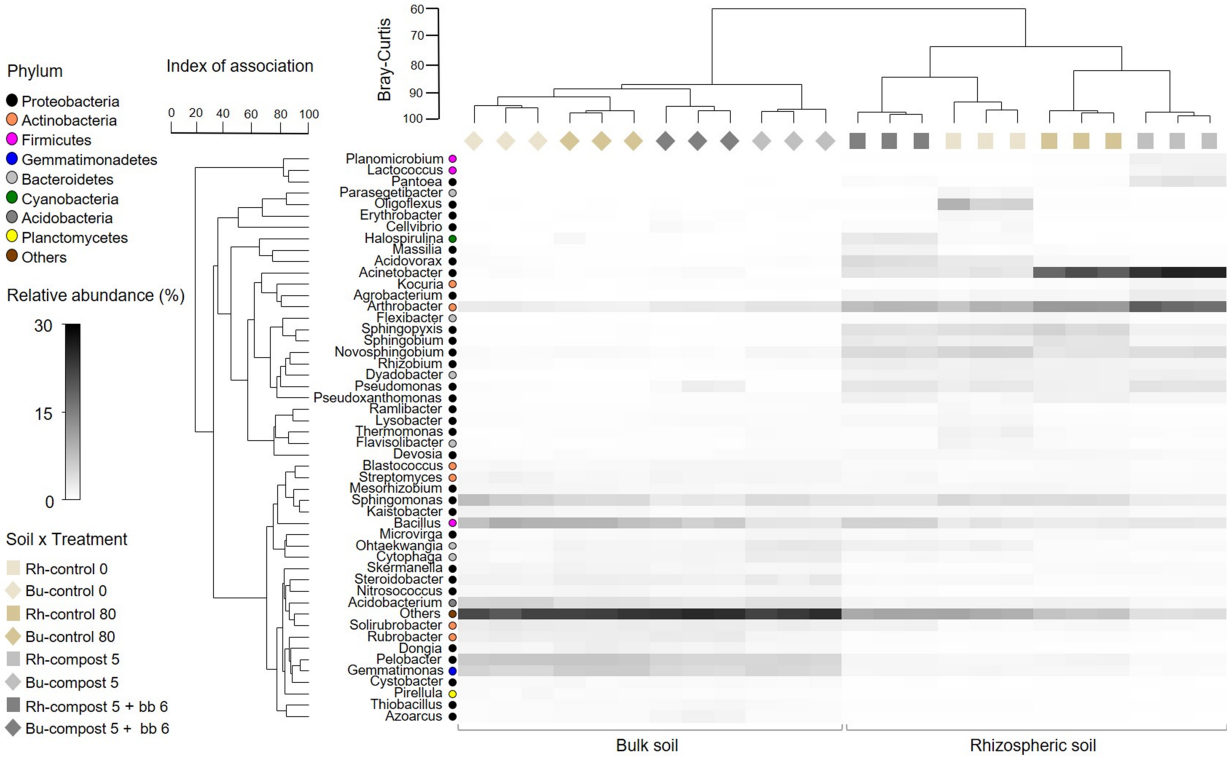

Figure 7. Heat plot showing the relative abundance of the 50 most frequent taxa in the bacterial community. In the column legend, the same color and shape indicate replicates of the same treatment and the same type of soil (Rh, rhizospheric; Bu, Bulk).

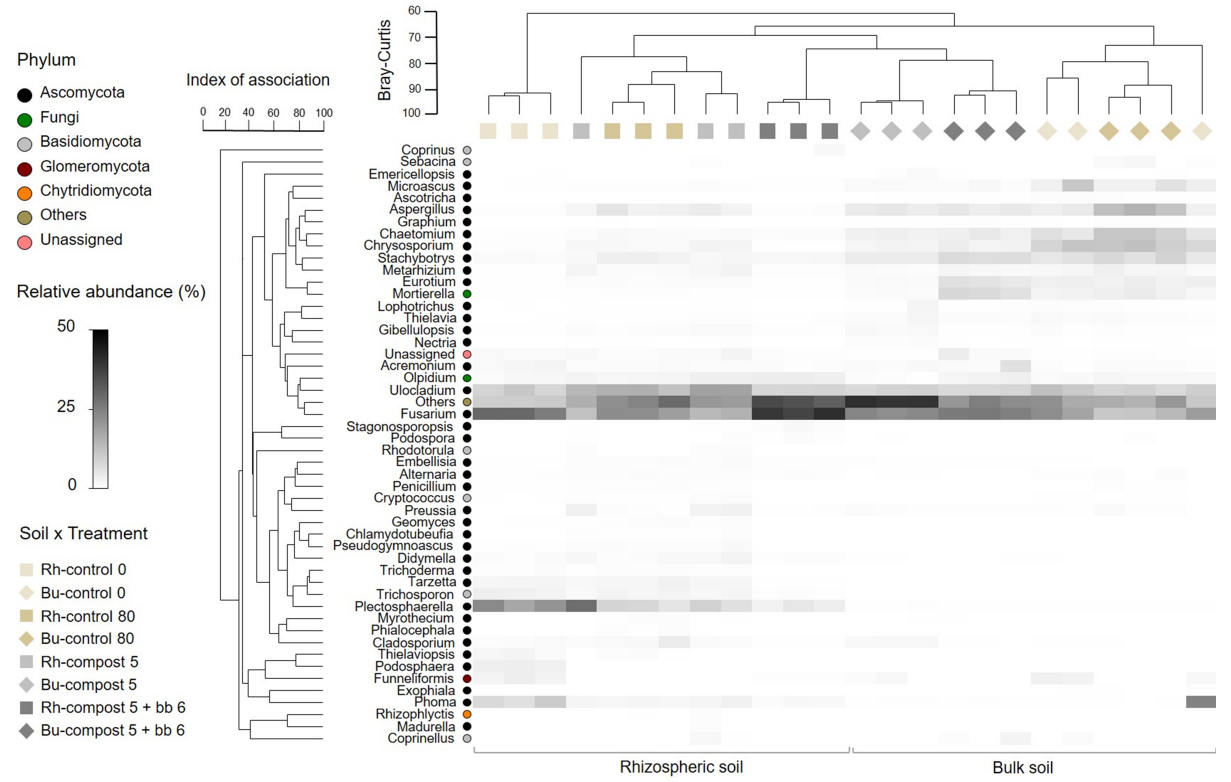

Figure 8. Heat plot showing the relative abundance of the 50 most frequent taxa in the fungal community. In the column legend, the same color and shape indicate replicates of the same treatment and the same type of soil (Rh, rhizospheric; Bu, Bulk).

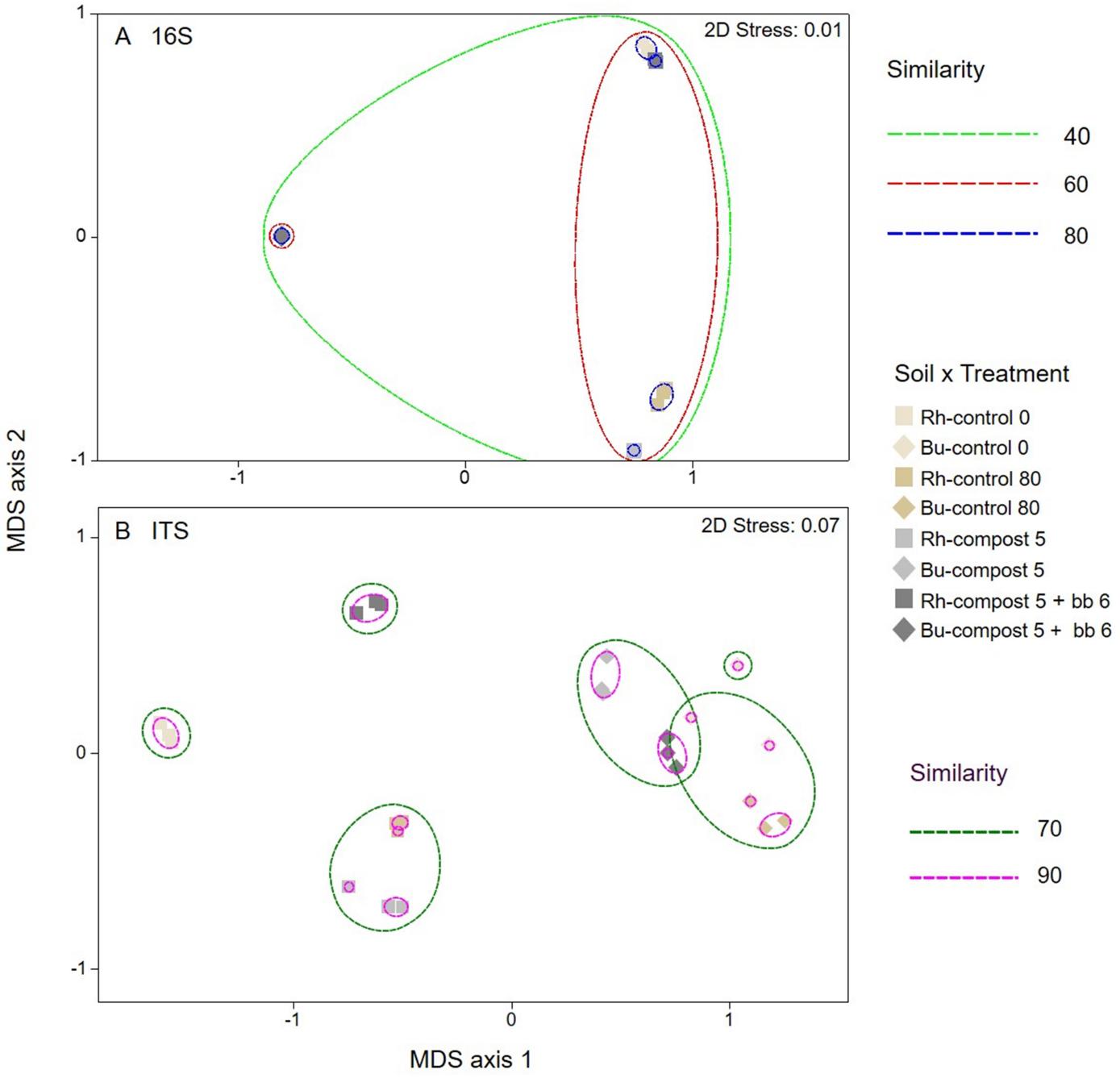

Figure 9. Non-metric multidimensional scaling (nMDS) plots of bacterial (**A**) and fungal (**B**) communities according to treatment applied. Each point represents the microbiome of one replicate of soil according to soil type. All rhombus shapes for bulk soil are placed on the left for the bacterial community (**A**) and on the right for the fungal community (**B**) in the figure. MDS axis 1 and MDS axis 2 represent the two axes of the two-dimensional ordination space. The stress level shown indicates how well the individual distances are represented (the closer to 0, the better are the original data points represented in the ordination space). All ordinations were performed using ASV-level data (p = 0.001 based on 999permutations).

Table 6. Results of PERMANOVA significance test across treatments within each type of soil in bacterial and fungal communities. Treatment and soil were used as fixed factors (number of permutations: 999). The test of significance was based on Bray–Curtis similarity values. The significance level was fixed for *p*-value equal to 0.001 (significance *p*-values are in bold).

	Bacteria				**Fungi**			
	Rhizospheric Soil		**Bulk Soil**		**Rhizospheric Soil**		**Bulk Soil**	
	Pseudo-F/t	***p*-Value**	**Pseudo-F/t**	***p*-Value**	**Pseudo-F/t**	***p*-Value**	**Pseudo-F/t**	***p*-Value**
Control 0 vs. Control 80	6.7329	**0.001**	2.8431	0.005	5.7629	**0.001**	2.2273	0.027
Control 0 vs. Compost 5	10.406	**0.001**	4.6153	0.002	4.4506	0.003	3.1415	0.009
Control 0 vs. Compost 5+bb6	5.0538	0.004	3.7117	0.004	8.5004	**0.001**	2.3973	0.025
Control 80 vs. Compost 5	8.1157	0.002	4.7808	0.002	2.1977	0.031	7.3687	**0.001**
Control 80 vs. Compost 5+bb6	9.3350	**0.001**	3.7124	0.002	5.3430	0.003	4.7638	0.003
Compost 5 vs. Compost 5+bb6	15.099	**0.001**	4.0405	0.005	5.7326	**0.001**	4.9925	0.002

4. Discussion

4.1. Yield, Yield Components and Biomass Production

For the two crops, melon and pepper, the doped compost produced a significant increase in yield and biomass production compared to the control with the same reduced dose of the mineral fertilizer (control 80). Moreover, the yield obtained in the treatment with the doped compost and a reduced dose of the mineral fertilizer was even higher than that obtained in the control without any type of compost and fertilized with the full mineral dose (control 100) (between 24% and 33% higher in melon and between 2% and 4% higher in pepper).

Furthermore, we have demonstrated that the incorporation of the inoculant, consisting of the *Bacillus* strain formulated with biochar as a carrier, improved the yield compared with the non-inoculated compost; this yield increase was up to 34% in the melon crop and up to 19% in the pepper crop. These results prove the effect of *B. siamensis* as a PGPR at field scale. Nowadays, not only is the biocontrol effect of *Bacillus* well known, but also its role as a plant growth promoter has been demonstrated. Examples of the latter are numerous for several horticultural crops and several different species of the genus *Bacillus*, e.g., red pepper [43], banana [44], radish [45], strawberry [46], Chinese cabbage [47], etc. It is known that the plant-growth-promoting effect of the MPBs belonging to the *Bacillus* species is largely due to phytohormone production [10], i.e., hormone-mediated effects are considered to be the reason for the yield increase when *B. siamensis* is inoculated onto the crop [48], and the same is true for other phylogenetically close species, such as *B. amyloliquefaciens* [49,50] and *B. subtilis* [51,52]. A possible hypothesis to explain the good field results obtained is that the mode of action of *B. siamensis* strain SCFB3-1 could be related to phytohormones. Indeed, SCFB3-1 was chosen as an MPB because, in a previous work [38], it showed a high rate of IAA production (2.8 µg IAA per each 1×10^8 cfu) and high ACC deaminase activity (6.63 µmol ACC mg prot^{-1} h^{-1}), the latter reducing the synthesis of the hormone ethylene. In the mentioned work, for both IAA and ACC, SCFB3-1 was at the beginning of the second quartile of the best producers, from a collection of 186 bacteria isolated from the rhizosphere of irrigated crops in Castilla y León (Spain) [38]. However, future research is needed to unravel the mode of action, because, according to [53], the hormone-producing microbes could improve the plant growth in two different ways: (i) direct action on the plant, or (ii) inducing changes in the rhizosphere microbiome (as observed in this work) consisting of attracting those taxa that exert a plant-growth-promotion effect.

Therefore, the observed effect of *B. siamensis* as an MPB is not a novelty, but what is truly new is the concept of the doped compost, which is a compost mixed with the MPB, using biochar as the carrier for the bacteria, which protects the bacteria and facilitates uniform mixing. Our results confirm that the addition of the formulated PGPR strain together with the compost at a final dose of 1.5×10^9 cfu mL^{-1} exerted a noticeable effect on crop yield, which was accompanied by a modification of the rhizosphere microbiome.

Although partial combinations of the components of the doped compost have been previously tested, the doped compost remains a novelty, as far as we know. In this way, biochar has been previously combined with NPK mineral fertilizers by [54], who observed crop yield increases and reduced greenhouse gas emissions compared to mineral fertilizers alone, and by [55,56], who also observed an improved nutrients' availability by the crop, as a consequence of biochar addition. Other authors, e.g., [57,58], combined compost and biochar in soil and observed a synergistic effect on the microbial biomass and activity and, as a consequence, on plant growth. PGPRs have been mixed with biochar; [59] obtained the best economic return in wheat with a reduced dose of mineral fertilizer combined with biochar and PGPR. The effect of triple mixes of compost, biochar and PGPR has been analyzed at the microcosm scale, i.e., in pots, to overcome abiotic [60] and biotic stress [61], as well as for bioremediation [62,63]. However, to the best of our knowledge, this is the first work that analyzes the effect of doped compost (triple mix of compost, PGPR and biochar) at field scale, evaluating the effect on the soil microbiome and on the crop yield.

4.2. Chlorophyll Content, Flowering and Fruit Parameters

According to the literature, the observed higher chlorophyll content in the leaves of both melon and pepper crops could be due to the effect of IAA produced by the inoculated strain [64], which was as high as 2.8 μg IAA per each 1×10^8 cfu [38]. Moreover, the chlorophyll content depends on the concentration of available nitrogen in plants [65] because N stimulates the chlorophyll biosynthesis process [66]. This is consistent with the fact that control 100 showed the highest chlorophyll content, as previously observed by [67] in tomato; however, compost is a source of N, and thus the addition of compost at the highest dose significantly improves the chlorophyll content compared to the control without compost; interestingly, supplementation of the compost also increased the chlorophyll content compared to the non-inoculated compost, and thus the inoculant, which does not provide N, must act in a different way, probably improving the N use efficiency.

For the melon crop, the treatment compost 5+bb6 produced the highest values of fruit contour, conductivity and solute concentration, which are fruit quality indexes, the latter two related to the concentration of minerals in the fruit flesh. The effect of the doped compost on the concentration of minerals in melon fruit flesh cannot be replaced by a higher dose of nutrients in mineral form, because control 100 showed significantly lower conductivity and solute concentration. A possible hypothesis to explain the observed improvement in mineral content in the fruit flesh could be related to gibberellin production [68], but the confirmation of this hypothesis would need further research. Surprisingly, for the pepper crop, the response of the crop to the treatments with doped compost was the opposite to that of the melon crop and resulted in a decreased solute concentration; moreover, the fruit contour was not modified by treatment with doped compost, indicating that the response regarding these parameters depends on the crop, and it is not possible to establish a general rule for such parameters.

In line with the results obtained for these parameters in plants and fruit, other authors, such as [69], have noted the importance and potential of PGPR for the improvement of sustainable, environmentally friendly vegetable production for healthy human nutrition, with special reference to selected vegetable species, such as tomato, pepper, melon, radish and lettuce, because of increasing food-borne illnesses.

4.3. Soil Microbiome: Bacteria

As expected, we observed sharp changes in the bacterial community in both the rhizosphere and the bulk soil. This is a common situation that has been described by other authors [70,71]. Moreover, the highest dose of doped compost (compost 5+bb6) modified the composition of the bacterial community, but only in the rhizospheric soil and not in the bulk soil; indeed, in the rhizospheric soil, the treatment compost 5+bb6 produced a greater relative abundance of the *Bacillus* genus, to which the inoculated strain also belongs; this could indicate that the crop would select the inoculated strain because it is beneficial for

the plant, given that it was selected because it is a PGPR [16,38]. Moreover, comparing the rhizosphere bacterial community of the crop treated with the doped compost (compost 5+bb6) with that of the crop treated with the non-doped compost (compost 5), other changes were observed in addition to the *Bacillus* abundance; thus, the relative abundance of the genera *Pseudoxantomonas*, *Massilia*, *Sphingopyxis*, *Sphingobium*, *Sphingomonas* and *Ohtaekwangia* increased; *Pseudomonas* and *Rhizobium* maintained their relative abundance, whilst the relative abundance of *Acinetobacter* and *Arthrobacter* decreased. In such a way, the addition of a PGPR induces a modification of the rhizospheric taxa, specifically of the rhizosphere bacterial community. However, the following questions arise: how is the change in the rhizospheric community triggered? Is it the PGPR that directly attracts or diminishes other genera? By contrast, is it the plant that attracts other genera as a result of a change in the roots' secretions in turn induced by the PGPR? Jacobs-Hoffman and Hills [72] indicated that the modification of the rhizosphere bacterial community could be induced by the inoculated PGPR, a selection taking place exerted by the roots of the crop. Moreover, [53] demonstrated that the inoculation of the PGPR *B. subtilis* stimulates the production of cytokinins in wheat, and this increases the diversity and size of the beneficial rhizospheric microbiota; thus, these authors demonstrated that the inoculated PGPR triggers biochemical changes in the plant root to attract specific bacteria. From our results, it could be hypothesized that similar processes are triggered by the inoculation of the PGPR *B. siamensis* at the highest dose that clearly modified the composition of the rhizosphere bacterial community, with a concurrent increase in bacterial diversity and richness, as could be seen in the boxplot. Consequently, the doped compost produces a positive effect on the rhizosphere compared with the non-inoculated compost. Thus, in our work, the inoculated PGPR strain could be responsible for: (i) the increase in the relative abundance of other bacteria with PGP properties, such as *Pseudoxantomonas* [73,74] and *Massilia* [75]; (ii) the maintenance of *Rhizobium* and *Pseudomonas*, which are proven PGPRs [76,77] and (iii) the increase in other bacterial genera that produce beneficial effects in the soil, which indirectly improves plant growth, e.g., *Sphingopyxis*, *Sphingobium*, *Sphingomonas* and *Ohtaekwangia*. Specifically, *Sphingopyxis*, *Sphingobium* and *Sphingomonas* degrade aromatic compounds [78,79], and could act synergistically with biochar, which favors the sorption of these compounds in soil [80]. Moreover, *Ohtaekwangia* contains nitrifying strains, and others with antifungal and insecticidal properties [81], while the *Sphingopyxis* genus has the ability to produce indolacetic acid (IAA) [82] and other secondary metabolites [79]. Therefore, an increase in or maintenance of the beneficial bacterial strains has been observed in the new rhizosphere microbiome obtained as a result of the treatment with doped compost (compost 5+bb6), and this could be related to the positive agronomic effects found for this treatment. However, interestingly, control 0 and compost 5+bb6 are shown to be similar in nMDS analysis and with no significant differences in the pairwise analysis via PERMANOVA. The reason for this could be that, in spite of the differences observed for the above-indicated genera, the rest of the bacterial genera have maintained their relative abundance in both treatments, with more species richness (S) in compost 5+bb6, but a similar Shannon index. This could be interpreted as the very low environmental impact of the use of the doped compost, i.e., the inoculated strains produce an increase in the beneficial strains whilst not altering the overall microbiome composition.

By contrast, in the bulk soil, where the plant cannot intervene directly, the doped compost did not modify the soil bacterial community, including the relative abundance of the *Bacillus* genus, compared, e.g., with control 0, which did not receive any treatment. Furthermore, in the bulk soil, there was no noticeable modification of the relative abundance of the rest of the bacterial genera in the doped treatment. These results clearly indicate that *B. siamensis* requires plant host proximity to exert its effect as a bio-stimulant and modulator of the rhizospheric community. The addition of *B. siamensis* to naked soil long before crop transplantation could otherwise bring a loss in frequency of the inoculated taxa that are otherwise overwhelmed by the extant soil microbiota.

4.4. Soil Microbiome: Fungi

The doped compost modified the fungal microbiome in the rhizospheric soil, whilst, in the bulk soil, the changes were negligible, as evidenced by the heat plot, the nMDS chart and the PERMANOVA. Accordingly, for the bulk soil, the doped compost, the non-inoculated compost and control 0 were plotted nearby in the nMDS, and the pairwise comparisons between them were not significant according to PERMANOVA. On the other hand, for the rhizospheric soil, the mentioned treatments were plotted clearly apart, and the pairwise comparison revealed significant differences. The observed differences between treatments in the rhizospheric soil were due to modifications in the microbiome, i.e., the increase in the genus *Fusarium* in the doped compost when it is compared to the other treatments; moreover, in the doped compost, *Plectosphaerella* and *Phoma* strongly decreased, *Aspergillus* and *Chrysosporium* practically disappeared and *Ulocladium* slightly decreased compared to the other treatments. The genus *Fusarium* is known for several species that are notorious soil-borne pathogens that cause serious losses in hundreds of economically important crops worldwide [83], including the melon crop. However, despite the increase in the genus *Fusarium* in both bulk and rhizospheric soils, in our field trial, there was no evidence of disease caused by *Fusarium*; conversely, there was a significantly higher yield and plant biomass, as well as better values of other parameters. Thus, the observed increase in *Fusarium* would correspond to non-pathogenic *Fusarium* strains that, interestingly, have been described as PGPRs and inducers of systemic resistance [84]. The genera *Plectosphaerella* and *Phoma* are well known as soil pathogens for plants [85], causing significant economic damage to horticultural crops [86]. Hence, the strong decrease in these genera in the treatments with doped compost is very beneficial for melon crops and could contribute to explaining the improved values of the agronomic parameters.

5. Conclusions

The doped compost, regardless of the inoculant dose (3% or 6%), produced the highest yield of all the treatments, which was, in comparison with the control that received the same mineral dose (control 80), 47% higher in melon and 30% higher in pepper. The yield components, the aerial biomass of the plant and the chlorophyll content, an indirect measurement of N assimilation, showed the same response to doped compost application. The best combination to optimize yields in melon and pepper crops was a reduced dose of mineral fertilizer (80%) combined with the lowest dose of compost (2 t/ha) doped with the highest dose of inoculant (6%). Interestingly, the yield increase did not involve a modification of the bacterial composition in the bulk soil, whilst the fungal composition was slightly modified. Conversely, the bacterial and, to a lesser extent, the fungal composition of the rhizosphere was modified by treatment with doped compost, although the modification was more evident with the inoculant dose of 6%. Thus, considering that the inoculant (biochar+PGPR) does not provide nutrients as such, the significant yield increase compared with the non-inoculated compost could be due to the inoculated strain *B. siamensis* SCFB3-1 but also to other native strains, potentially beneficial for the plant, that were attracted to the rhizosphere as a consequence of the treatment. It is relevant from the environmental side that the replacement of 20% of the mineral NPK fertilizer by the doped compost increased the yield compared to the full mineral NPK dose, contributing to mineral fertilizer savings for farmers and improvements in the environmental performance of these crops; moreover, in the case of melon, some fruit parameters were also increased, namely the fruit contour and the solute concentration in the fruit juice.

This work has demonstrated, at field scale, the agronomic value of supplementing compost with PGPRs. It has also demonstrated that the treatment mainly modified the rhizospheric microbiome. Thus, future research is necessary to unravel the mechanisms responsible for the observed agronomic effect, focusing on the effect of phytohormones, and the origin of such phytohormones, which can include not only those produced by the inoculated microbes, but also by the new components of the rhizosphere microbiome and by the plant as a response to the microbial inhabitants of its rhizosphere. Our study

opens up the understanding of the association of plant and bacterial and fungal communities in response to an organic fertilizer inoculated with biochar and a PGPR strain in agricultural systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12112620/s1, Figure S1: Stacked bar plot indicating the relative abundance of bacterial phyla for the factors: type of soil (A) and type of soil and treatment (B); Figure S2: Stacked bar plot indicating the relative abundance of fungal phyla for the factors: type of soil (A) and type of soil and treatment (B); Table S1: Composition of the compost used; Table S2: Composition of the biochar used for the experiment; Table S3: Soil analysis previous to the field trials; Table S4: Climatic conditions of the locations selected for field trials in 2018 and 2019; Table S5: Mean values for yield, yield components and biomass production, obtained in the field trial for the melon crop. Means followed by the same letter did not differ significantly at $p \leq 0.05$ in Tukey's test; Table S6: Mean values for yield, yield components and biomass production, obtained in the field trial for the pepper crop. Means followed by the same letter did not differ significantly at $p \leq 0.05$ in Tukey's test; Table S7: Mean values for leaf chlorophyll content, flowering and several fruit parameters obtained in the field trial for the melon crop. Means followed by the same letter did not differ significantly at $p \leq 0.05$ in Tukey's test; Table S8: Mean values for leaf chlorophyll content, flowering and several fruit parameters obtained in the field trial for the pepper crop. Means followed by the same letter did not differ significantly at $p \leq 0.05$ in Tukey's test; Table S9: Results of factorial ANOVA on bacterial species richness (S), number of reads (N), Shannon index (H') according to the effect of the soil position (rhizospheric or bulk), the treatments and the interaction of the two explanatory variables. Significant differences for value of p below 0.05; Table S10: Results of factorial ANOVA on fungal species richness (S), number of reads (N), Shannon index (H') according to the effect of the soil position (rhizospheric or bulk), the treatments and the interaction of the two explanatory variables. Significant differences for value of p below 0.05.

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