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# Effects of *Bacillus subtilis* and *Pseudomonas fluorescens* Inoculation on Attributes of the Lettuce (*Lactuca sativa* L.) Soil Rhizosphere Microbial Community: The Role of the Management System

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**Abstract:** Inoculation with beneficial microbes has been proposed as an effective practice for the improvement of plant growth and soil health. Since soil acts as a physicochemical background for soil microbial communities, we hypothesized that its management will mediate the effects of microbial inoculants on the indigenous soil microbes. We examined the effects of bacterial inoculants [*Bacillus subtilis* (Ba), *Pseudomonas fluorescens* (Ps), and both (BaPs)] on the growth of *Lactuca sativa* cultivated in soils that originated from an organic maize (OS) and a conventional barley (CS) management system. Moreover, the biomass and the community structure of the rhizosphere microbial communities and the soil enzyme activities were recorded. The root weight was higher in CS than OS, while the foliage length was greater in OS than CS treatments. Only in OS pots, inoculants resulted in higher biomasses of bacteria, fungi, and actinomycetes compared to the control with the highest values being recorded in Ps and BaPs treated soils. Furthermore, different inoculants resulted in different communities in terms of structure mainly in OS soils. For soil enzymes, the effect of the management system was more important due to the high organic matter existing in OS soils. We suggest that for microbial inoculation to be effective it should be considered together with the management history of the soil.

**Keywords:** microbial inoculants; soil enzyme activities; soil microbes

## 1. Introduction

Several conventional farming management practices, such as extensive use of inorganic fertilizers and pesticides, have a significant impact on the environment [1,2]; increasing the greenhouse effect [3], reducing biodiversity [4], and enhancing toxicity in the food chain [5]. Such practices could have serious impacts on the soil environment and more specifically on soil microbial communities. Insecticides, such as pyrethroids altered the composition of the soil microbial community [6], fungicides lowered the abundance of *Bacillus* species by 63% [7] or affected negatively the populations of soil fungi and bacteria [8]. Moreover, pesticide residues could remain in the soil for a long period (even for eight years) after their application [9]. On the contrary, organic farming practices, such as manure application and lack of tillage could improve soil fertility, increase microbial biomass [10] and activity [11], and elevate soil microbial diversity [12], thus affecting the soil microbial community structure [13].

The need for more environmentally friendly viable alternatives to traditional fertilizers for enhancing plant productivity and improving soil quality is growing [14]. The use of bacteria and fungi as inoculants for enhancing crop production is a sustainable approach that has gained ground over the last years, as inoculation with beneficial microorganisms can reduce the requirements for chemical fertilizers and pesticides [15]. Inoculation of soil with plant growth-promoting rhizobacteria (PGPR) proved to have beneficial effects on plant growth, as PGPR have a wide range of activities such as the enhancement of nutrient availability, the biocontrol of soil-borne pathogens, the release of plant hormones, and the alleviation of various types of abiotic stress [16,17]. PGPR are either exogenous bacteria introduced into agricultural ecosystems or autochthonous bacteria that are being enhanced. Both types of bacteria act positively upon plant development in annual and perennial crops and under different abiotic conditions [18,19]. The genera of PGPR that are most often used in sustainable agriculture are *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Rhizobium* [20,21].

Among the PGPR genera, several *Pseudomonas* and *Bacillus* species are the most widely known ones and those that have been frequently commercialized due to their survival within a diverse range of biotic and abiotic environments [22]. *Pseudomonas* is one of the most abundant Gram-negative soil bacterial genus in rhizosphere soil [23]. *Pseudomonas* species can either suppress the growth of soil pathogens by inducing the plant's systemic resistance [24] and by producing antibiotics and siderophores [23] or play a vital role in plant growth by modifying plant phytohormone concentrations [25]. The *Bacillus*-based inoculants have demonstrated significant biocontrol properties by inhibiting the growth of plant pathogens (e.g., *Rhizoctonia solani* and *Botrytis cinerea*) due to the secretion of antifungal compounds [26], or toxins [27], or by inducing the plant's defense systems through the synthesis of plant growth hormones [28]. *Bacillus* species can also increase the concentration of soil essential nutrients, such as P and N, by converting complex nutrient compounds to more simple available forms easily accessible by the plant roots [29].

Over the last 10 years, many studies have been conducted to investigate the effect of PGPR inoculants on various lettuce varieties aiming at increasing productivity and nutritional quality, improving plant abiotic stress tolerance, facilitating nutrient uptake, and controlling pests [30,31]. *Bacillus* and *Pseudomonas*-based inoculants have presented promising results for lettuce crop yield [32,33]. Nevertheless, only a few studies have focused on the effect of PGPR inoculants' application on the indigenous soil microbial communities [33,34]. The incorporation of PGPR inoculants into the soil could have a significant impact on indigenous soil microbial community because these exogenous microbes are involved in a wide range of interactions with the autochthonous microbes such as competition, synergy, or prey-predator interactions. For that reason, PGPR may increase, decrease, or not affect the indigenous microorganisms [35].

The present study aimed to assess the effect of *Pseudomonas fluorescens* and *Bacillus subtilis* inoculation on the biomass, community composition, and functionality of the lettuce rhizosphere microbial community in plants cultivated in soils that originated from two different management systems (conventional barley and organic maize cultivations). Since soil acts as a physicochemical template for microbial communities due to nutrient supply (availability and movement of nutrients to microbes) and by providing refugia for them, we expect that inoculants applied in the conventional system will impose suppressing effects on microbial biomass, community, and enzyme activity resulting in a narrower range of responses of microbial attributes compared to relevant effects recorded in the organic management system. We hypothesize that in the conventional system, exogenous microbes would be involved mainly in competitive interactions due to limitations in resource availability. In contrast, the lack of nutrient limitation and tillage effect in the organic system would enable inoculants to express their influence, a fact that would be identified by a wide range of inoculant-specific responses of soil microbial community structure, biomass, and functionality in this system.

## 2. Materials and Methods

### 2.1. Experimental Design and Sampling

The experimental design consisted of two management systems [organically (OS) and conventionally (CS) managed soils] × four inoculation treatments [*Bacillus subtilis* (Ba), *Pseudomonas fluorescens* (Ps), *Bacillus* and *Pseudomonas* consortia (BaPs), and non-inoculated (control, C)], with four replicates per treatment, giving a total of 32 pots arranged in a randomized block design. The soils used in the experiment were collected from two different fields in Lithia, Kastoria, Western Macedonia, Greece (40.5208° N, 21.4097° E). The OS originated from an organically managed maize crop cultivation, involving the application of sheep manure (1 t ha<sup>-1</sup>) every 2 years, no-tillage, and no use of pesticides for more than 10 years, while plant residues were left on the soil surface after harvest. The CS originated from a conventionally managed barley crop cultivation that involved the use of inorganic fertilizers (NPK 20-10-0; 20–25 kg ha<sup>-1</sup>), extensive tillage, and extensive use of pesticides (Phenylpyrazolines), insecticides (Pyrethroids), and fungicides (Triazoles and Pyrazoles) for more than 10 years. We selected these specific fields as they presented similar soil texture properties [CS: SL soil texture (S: 44%, C: 6%, Si: 50%), pH: 6.60 and EC: 0.93 mS cm<sup>-1</sup> and OS: SL soil texture (S: 24%, C: 14%, Si: 62%), pH 7.81 and EC 1.19 mS cm<sup>-1</sup>]. The soil collected from the top 15 cm from each management system was passed through a 2 mm sieve and 1500 g of either OS or CS were put into plastic pots (15 cm diameter and 11 cm depth) that have been previously disinfected with ethanol. Before the experiment, lettuce seeds (*Lactuca sativa* var. *longifolia*) were surface sterilized with sodium hypochlorite 10% (v/v) for 20 min and then washed repeatedly with deionized water; then the sterilized seeds were sowed in seedbeds containing soil from either CS or OS to grow for 60 days, before being transplanted in the experimental pots. The last watering was 24 h before their transplantation. Five seeds of lettuce were planted in each seedbed. After their germination, plants were thinned, leaving only one lettuce plant per seedbed and these were transplanted to pots. The experiment was conducted in outdoor conditions under natural light and temperature conditions. The experimental period was November to December with mean temperatures 12.1 and 6.3 °C, respectively (Florina Meteorological Station). Both seedling trays and the plastic pots (after the transplantation) were watered every 3 days with 50 mL of deionized/distilled water to keep the soil water content around 10% w/w that imposes no limitation to plant growth [36]. A destructive sampling was conducted 60 days after the transplantation.

We manually separated the roots from the soil, while the rhizosphere soil attached to the roots was collected on a sterilized surface. Fresh rhizosphere soil samples were sieved to remove small roots and then were stored at 4 °C until further analysis. We analyzed the rhizosphere soil samples for microbial community structure, abundance, and extracellular enzyme activity as well. We also determined lettuce root weight, foliage weight, and foliage length.

### 2.2. Inoculum Preparation

*Bacillus subtilis* and *Pseudomonas fluorescens* were isolated from different soil samples collected from the rhizosphere of the *Solanum lycopersicum* (tomato) and the *Gossypium hirsutum* (cotton), respectively. The rhizobacteria were isolated on their respective media; *B. subtilis* on nutrient agar and *P. fluorescens* on King's B agar. The pure isolates were further cultured on new plates for colony morphology. The colony morphological characteristics including margins, shape, raised, and pigmentation were observed. Biochemical studies such as Gram reaction, endospore staining catalase production, oxidase, indole production, citrate utilization, methyl red test were carried out for the confirmation of *Pseudomonas fluorescens* and *Bacillus subtilis* [37]. The preliminary identification was confirmed by molecular classification with polymerase chain reaction (PCR), using ERIC1f/ERIC2 (ERIC 1: 5'-ATGTAAGCTCCTGGGGATTAC-3', ERIC2: 5'-AAGTAAGTGACT GGGGTGAGCG-3') oligonucleotide primers [38], and based on the sequence of the 16S rDNA gene. The preparation of the inocula involved the growth of the bacteria in nutrient broth (100 mL) for 48 h on a rotary shaker at 28–30 °C. After incubation, the inoculum reached the final cell density of about 10<sup>10</sup> cfu cm<sup>-3</sup> by

diluting the bacterial cultures with a 0.85% NaCl water solution. Bacterial inoculation was applied once. Bacteria were applied by drenching of the soil in a dose of 10 cm<sup>3</sup> of bacterial suspension per 1 cm<sup>3</sup> of soil. The suspensions of *B. subtilis*, *P. fluorescens*, or their mixture (1:1 v/v) were used for inoculation. The concentration of the suspensions of B and P was 5 mL of the inoculant in 45 mL of water, while the consortia suspension of Ba/Ps was constituted of 2 × 2.5 mL and 45 mL of water. The control was treated with 50 mL of water.

### 2.3. Analyses of Soil Chemical and Biochemical Variables

Soil pH was determined by the 1:2 soil/water suspension method. Soil texture was estimated by the Bouyoucos method. Soil organic C was determined by a wet oxidation titration procedure using an acid dichromate system. NO<sub>3</sub>-N concentration was determined by distillation and titration. Mg and K were estimated in soil extracts by the atomic absorption spectrophotometer (Perkin-Elmer 2380). For inorganic P the Olsen method was used. All soil chemical variables were determined as described in Allen [39].

### 2.4. Phospholipid Fatty Acid Analysis

Soil samples were analyzed for phospholipid (PLFAs) bioindicators according to the method presented in Ntalli et al. [40]. A Trace GC Ultra gas chromatograph (Thermo Finnigan, San Jose, CA, USA) coupled with a Trace ISQ mass spectrometry detector, an autosampler with a split-splitless injector, and an Xcalibur MS platform was used for the chromatographic separation and identification of the main components. We quantified each fatty acid (in nmol/g) by one-point calibrating against the GC response of the internal standard (19:0 methyl ester). Overall, in all the samples, we consistently found 24 fatty acid methyl esters which were included in all further analyses. These fatty acids were assigned to functional groups as follows [40,41]: i-15:0, a-15:0, 15:0, i-16:0, i-17:0, 17:0 (Gram-positive bacteria); 16:1 $\omega$ 9c, 16:1 $\omega$ 9t, cy17:0 (Gram-negative bacteria); 10Me16:0, 10Me17:0, 10Me18:0 (actinomycetes); 18:2 $\omega$ 9,12 (fungi); 20:0, 22:0, and 24:0 (microeukaryotes, e.g., algae, nematodes). The remaining PLFAs may derive from several sources and were considered only for the estimation of total microbial biomass. For example, 18:1 $\omega$ 9t, 18:1 $\omega$ 9c may derive from both Gram-negative bacteria and fungi, 16:0 from bacteria and fungi, while 11:0, 13:0, 14:0, 18:0, 18:2 $\omega$ 6 are mainly of microbial origin. We also estimated the bacteria/fungi (B/F) and Gram<sup>+</sup>/Gram<sup>-</sup> ratios.

### 2.5. Enzymatic Activity Analysis

Urease,  $\beta$ -glucosidase, and acid phosphatase play key roles in the N, C, and P cycles, respectively [42]. We determined the acid phosphatase (AP) and  $\beta$ -glucosidase (BG) activity according to the procedures of Allison and Jastrow [43], modified for 96-well microplates. We used 5 mM p-nitrophenyl-phosphate and 5 mM p-nitrophenyl- $\beta$ -glucopyranoside substrate solutions for AP and BG, respectively. The p-nitrophenol (PNP) reaction product from the AP and BG assays was measured at 405 nm. The method of Sinsabaugh et al. [44] was used for the estimation of the urease activity. The urea concentration in the wells was 20 mM. The ammonium released by the reaction was measured spectrophotometrically at 610 nm.

### 2.6. Data Analysis

We applied a two-way analysis of variance (ANOVA) to determine the effect of the management system, inoculum type, and their interaction, on plant variables, microbial biomasses, and enzyme activity data. In the case of significant effects, a post hoc test was performed at  $p < 0.05$ . An independent t-test was used to compare the mean soil physicochemical variables between the two management systems (CS and OS). Before analyses, we logarithmically transformed the data when considered necessary, to meet the assumptions of t-test and ANOVA.

To further explore whether the management system or inoculum type exerted the greatest influence on microbial community structure, we applied a principal component analysis (PCA). Moreover,

we applied the analysis of similarities (ANOSIM), based on the similarity index of Bray-Curtis, to the data of individual PLFAs to detected similarities in community structure between the treatments.

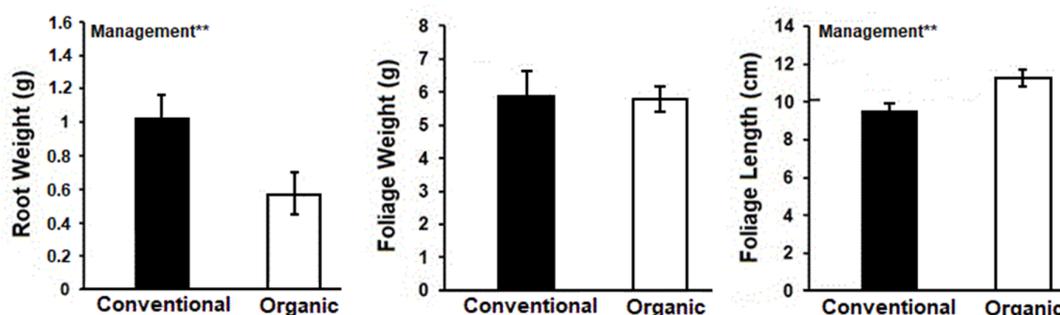
### 3. Results

A *t*-test revealed that the OS pots presented significantly higher values of pH, organic matter, nitric nitrogen, P, K, and Mg compared to the CS. On the contrary, the concentration of Zn, Mn, and B did not differ significantly (Table 1).

**Table 1.** Mean values ( $\pm$ SE) of the soil physicochemical variables in conventionally and organically managed systems. The independent *t*-test was used for the comparison of the mean values. (\*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ; ns: Non-significant).

	Conventionally Managed System	Organically Managed System	<i>p</i> -Value
pH	6.60 $\pm$ 0.054	7.81 $\pm$ 0.035	***
EC (mS cm <sup>-1</sup> )	0.93 $\pm$ 0.107	1.19 $\pm$ 0.049	ns
Organic matter (%)	2.29 $\pm$ 0.159	3.24 $\pm$ 0.351	**
Nitric Nitrogen (mg kg <sup>-1</sup> )	14.13 $\pm$ 6.955	32.36 $\pm$ 2.377	**
Pext (mg kg <sup>-1</sup> )	26.33 $\pm$ 0.609	133.75 $\pm$ 3.609	***
K (mg kg <sup>-1</sup> )	153.66 $\pm$ 9.769	347.33 $\pm$ 10.170	***
Mg (mg kg <sup>-1</sup> )	188.33 $\pm$ 7.264	352.33 $\pm$ 7.838	***
Zn (mg kg <sup>-1</sup> )	5.64 $\pm$ 0.065	5.61 $\pm$ 0.196	ns
Mn (mg kg <sup>-1</sup> )	28.04 $\pm$ 2.256	26.40 $\pm$ 1.307	ns
B (mg kg <sup>-1</sup> )	0.84 $\pm$ 0.070	0.81 $\pm$ 0.051	ns

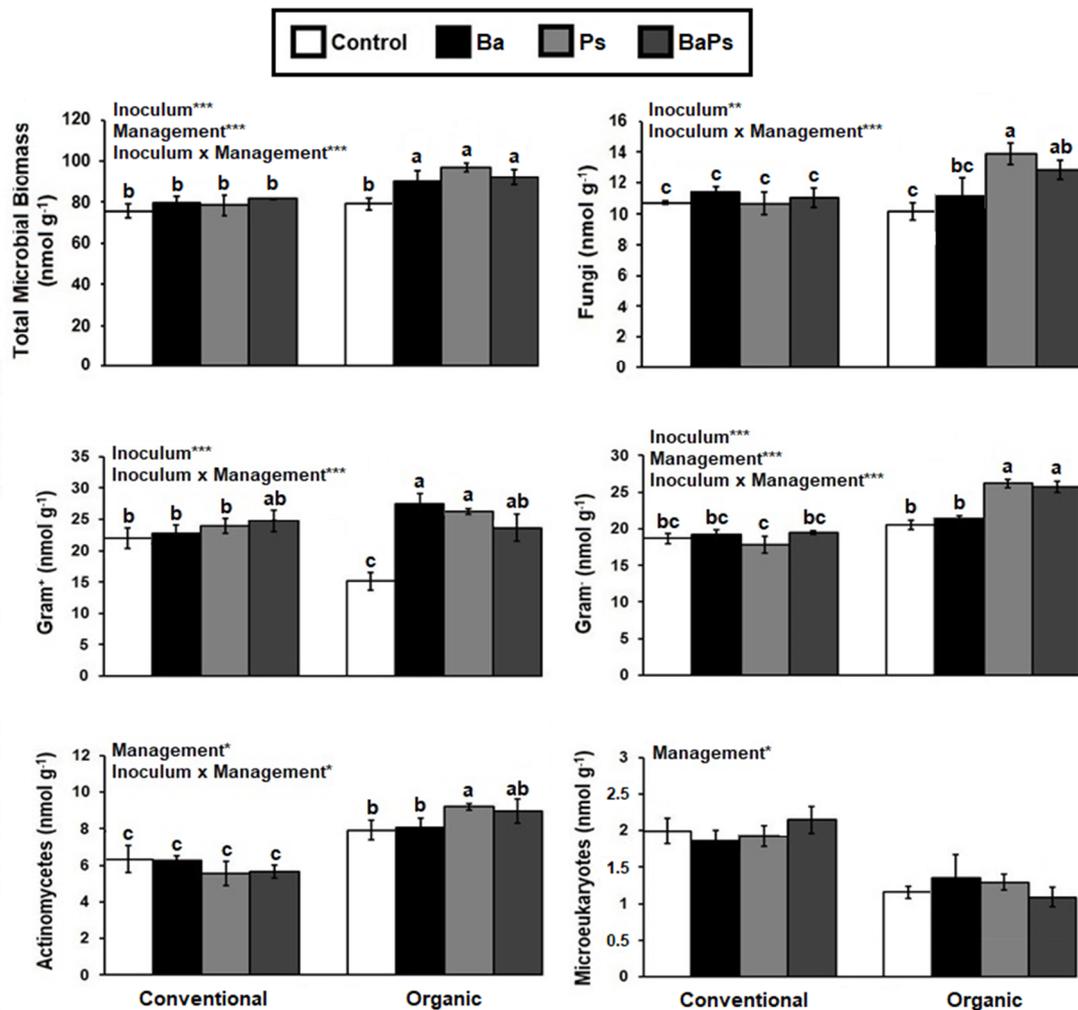
The effects of the management system, inoculum type, and their interactions on plant growth are shown in Supplementary Materials Table S1 and Figure 1. The root weight and the foliage length were affected only by the management type. The root weight was higher in CS than in OS, while the foliage length was greater in OS than in CS.



**Figure 1.** Mean values ( $\pm$ SE) of root weight, shoot weight, and shoot length in conventionally and organically managed systems. “Management” on the top of the graphs, indicates a significant effect of the management type, as revealed by two-way analysis of variance (ANOVA). (\*\*:  $p < 0.01$ )

For the abundance of most microbial groups (Gram-positive and Gram-negative bacteria, actinomycetes, fungi), the interaction of management system  $\times$  inoculum type was significant (Table S2). The same was held also for the total microbial biomass. The effect of inoculum type was much more pronounced in OS pots, while in the CS pots no differences in biomasses were recorded between the different inoculants (Figure 2). Ba or Ps inoculants resulted in significantly higher biomasses of Gram-positive bacteria in the OS pots compared to CS ones, while the BaPs inoculant induced similar effects in both management systems. The biomass of Gram-negative bacteria was higher in OS pots inoculated with Ps or with both inoculants (Figure 2). For actinomycetes, the highest biomass was recorded in Ps inoculated organic pots. The highest value of fungal biomass was in Ps OS pots, while the biomass in BaPs pots was similar to Ba and Ps ones. Finally, the total microbial biomass showed non-significant differences in organic pots inoculated with Ba, Ps, or BaPs. The abundance of

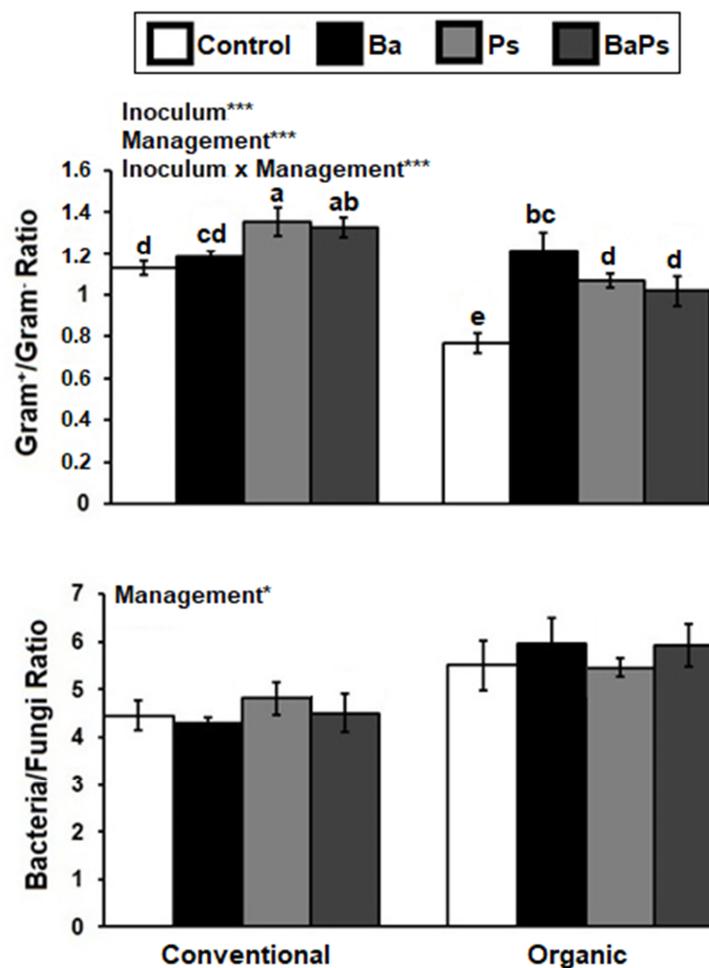
microeukaryotes was higher in the CS samples, regardless of the inoculum type. The bacteria/fungi ratio was affected only by the management system; the organic pots presented significantly higher values than the conventional ones (Figure 3). On the contrary, inoculants resulted in increased values of Gram<sup>+</sup>/Gram<sup>-</sup> ratios compared to the controls in both CS and OS pots. The ratios recorded in the Ps and BaPs conventional pots were the highest, whereas in organic pots the Ba inoculant exhibited the highest ratios.



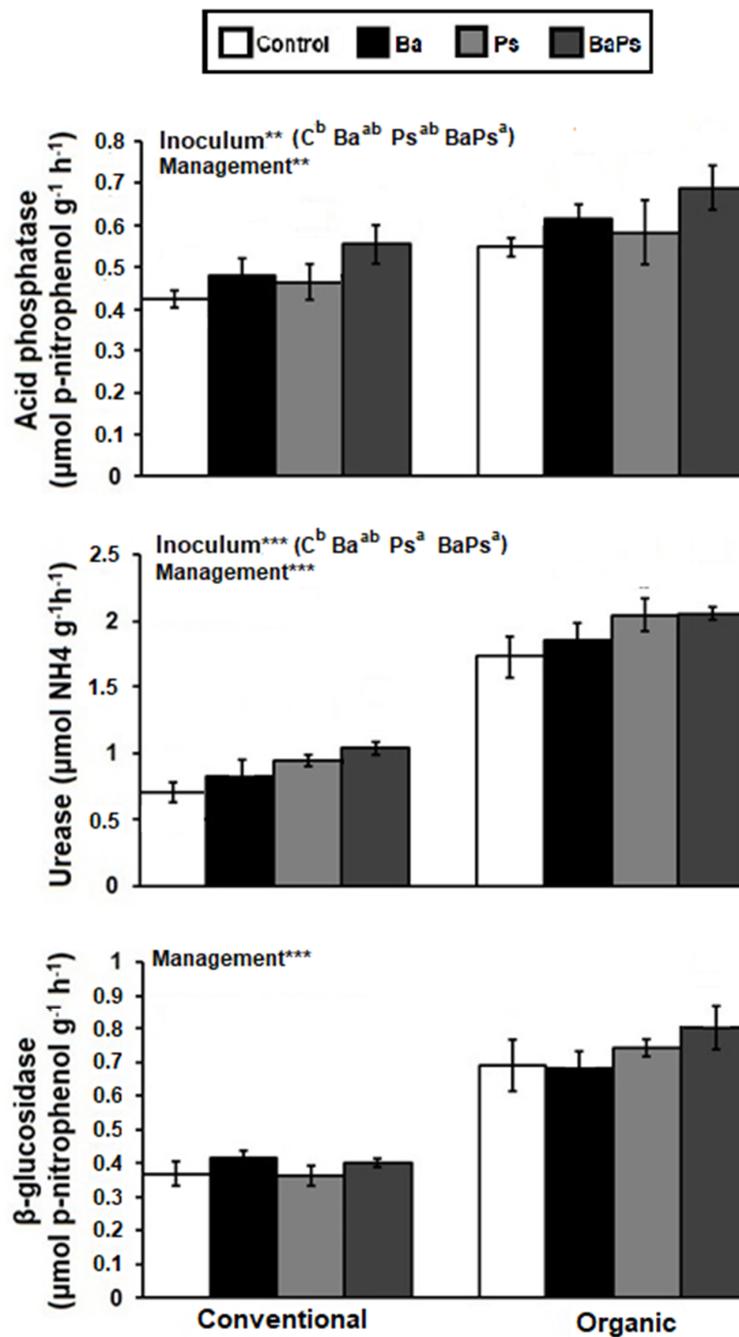
**Figure 2.** Mean biomass ( $\pm$ SE) of total microbes, fungi, Gram<sup>+</sup> and Gram<sup>-</sup> bacteria, actinomycetes, and microeukaryotes in the conventionally and organically managed systems, at the four treatments. “Inoculum”, “Management”, and “Inoculum  $\times$  Management” at the top of the graphs, indicate a significant effect of these factors and their interaction, respectively, as revealed by two-way ANOVA (\*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , for all cases  $n = 4$ ). The different letters above bars represent statistically significant differences between treatments described by the inoculum type  $\times$  management system as emerged from Fisher’s test (Fisher post hoc; a: Corresponds to the highest value).

For acid phosphatase and  $\beta$ -glucosidase, the independent effect of the management system and inoculum type was significant (Table S3), while for urease only the effect of management system was significant. All enzymes presented higher activity values in the OS compared to the CS pots (Figure 4). While for phosphatase and urease the co-inoculated Ba pots exhibited significantly higher activity compared to the controls in both management systems.

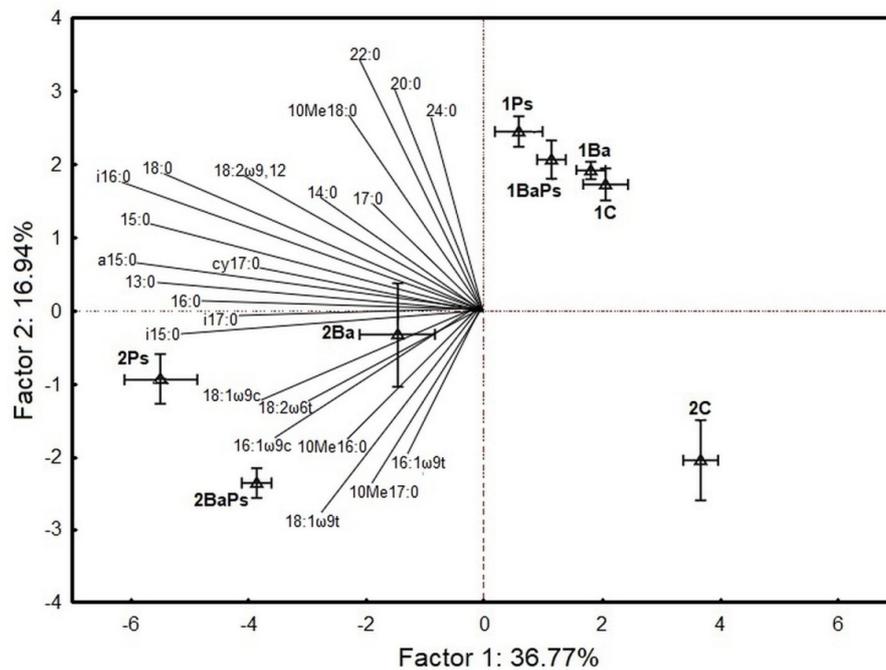
PCA depicting the ordination of samples and individuals PLFAs is presented in Figure 5. The first axis explained 36.7% of the data variability and the second one explained 16.9% (53.6% in total). The OS control samples were ordinated at the right end of the first axis, while all the other samples from the OS treatments (Ba, Ps, BaPs) were ordinated at the left side of the first axis showing a positive correlation to all PLFA biomarkers, that correspond to Gram-positive, Gram-negative bacteria, actinomycetes, and fungi. Along the second axis, the samples of CS are ordinated in the upper part showing a positive correlation to PLFA biomarkers describing microeukaryotes (20:0, 22:0, 24:0) and were clearly separated from the OS samples that are ordinated in the lower part. The classification of all the CS samples close to each other indicated that the addition of any type of inoculum did not affect significantly the structure of the soil microbial community. On the contrary, the distance between the control and the inoculated OS samples showed a great impact of the inoculants on the microbial community structure.



**Figure 3.** Mean values ( $\pm$ SE) of microbial ratios in the conventionally and organically managed systems, at the four treatments. “Inoculum”, “Management”, and “Inoculum  $\times$  Management” at the top of the graphs, indicate a significant effect of these factors and their interaction, respectively, as revealed by two-way ANOVA (\*:  $p < 0.05$ , \*\*\*:  $p < 0.001$ , for all cases  $n = 4$ ). The different letters above bars represent statistically significant differences between treatments described by the inoculum type  $\times$  management system as emerged from Fisher’s test (Fisher post hoc; a: Corresponds to the highest value).



**Figure 4.** Mean values ( $\pm$ SE) of acid phosphatase, urease, and  $\beta$ -glucosidase enzymes in the conventionally and organically managed systems, at the four treatments. “Inoculum”, “Management” at the top of the graphs, indicate a significant effect of these factors, as revealed by two-way ANOVA (\*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , for all cases  $n = 4$ ). Superscript letters in parentheses following the term “Inoculum” denote the significant differences between inoculum types (C: Control, Ba: *B. subtilis*, Ps: *P. fluorescens* and BaPs: *B. subtilis* and *P. fluorescens*) as emerged from Fisher’s test (Fisher post hoc; a: Corresponds to the highest value).



**Figure 5.** Ordination of the soil samples and the phospholipid (PLFA) biomarkers on a principal component analysis (PCA) biplot. Each point corresponds to the mean value of the loadings of the four samples belonging to the same treatment at the first and second axis (the first symbol corresponds to the management system (1: Conventional system (CS), 2: Organic system (OS)) and the second symbol corresponds to the inoculum treatment; Ba: *B. subtilis*; Ps: *P. fluorescens*; BaPs: Both inoculants; C: Control). Error bars indicate standard errors at both axes ( $n = 4$ ).

To get a deeper insight into the differences in microbial community structure between treatments, we applied an ANOSIM analysis on individual PLFAs. As shown in Table 2, in organic pots, the Ps community structure differed significantly from the Ba community and both differed from the control. Moreover, the community in co-inoculated organic pots differed from the control. On the contrary, in conventional pots, Ba and Ps communities' structure was similar to the control community. In this management system, when both inoculants were added together (BaPs), a significantly different microbial community structure emerged compared to the control.

**Table 2.** Results of ANOSIM analysis ( $p$ -values) based on the similarity index of Bray-Curtis applied to the data of individual PLFAs. The numbers in bold ( $p < 0.05$ ) indicate statistically significant differences (the first symbol is the acronym for the management system (CS: Conventional system; OS: Organic system) and the second symbol corresponds to the inoculum type; Ba: *B. subtilis*; Ps: *P. fluorescens*; BaPs: Both inoculants; C: Control).

CS-C	<b>0.174</b>	<b>0.116</b>	<b>0.024</b>	<b>0.030</b>	<b>0.027</b>	<b>0.026</b>	<b>0.061</b>
CS-Ba	0.174	0.318	0.284	<b>0.029</b>	0.061	<b>0.030</b>	<b>0.027</b>
CS-Ps	0.116	0.318	0.055	<b>0.030</b>	<b>0.028</b>	<b>0.030</b>	<b>0.027</b>
CS-BaPs	<b>0.024</b>	0.284	0.055	<b>0.029</b>	<b>0.028</b>	<b>0.029</b>	<b>0.028</b>
OS-C	<b>0.030</b>	<b>0.029</b>	<b>0.030</b>	<b>0.029</b>	<b>0.028</b>	<b>0.031</b>	<b>0.033</b>
OS-Ba	<b>0.027</b>	0.061	<b>0.028</b>	<b>0.028</b>	<b>0.028</b>	<b>0.026</b>	0.057
OS-Ps	<b>0.026</b>	<b>0.030</b>	<b>0.030</b>	<b>0.029</b>	<b>0.031</b>	<b>0.026</b>	0.222
OS-BaPs	<b>0.061</b>	<b>0.027</b>	<b>0.027</b>	<b>0.028</b>	<b>0.033</b>	0.057	0.222

## 4. Discussion

### 4.1. Plant Growth

The effects of different microbial inoculants on lettuce growth and attributes of the indigenous microbial community were examined in two soils of similar soil texture but of different management histories (organic maize and conventional barley).

The root biomass of the lettuce plants grown in the conventional soil was increased significantly in relation to the organic one while lettuce's growth was unaffected by inoculation. As the conventional soil had lower soil nutrient concentrations (e.g., organic matter, nitric N, P, K, and Mg), plants may have allocated more photosynthetic products to their roots to increase their exploitative capacity [45]. Previous studies showed that the lettuce plant's inoculation with *Bacillus* and *Pseudomonas* strains, either separately or as co-inoculants resulted in increased plant growth parameters compared to the control [33,46]. In those studies, different results were recorded, as the temperature of the experimental areas was higher than 20 °C while the pots had been fertilized before the plant transplantation either with chemical fertilizers or compost and green manure. In our experiment, the poor plant growth performance in the inoculated treatments could be related to the low winter air and soil temperatures (6 to 12 °C). Plant inoculation during winter has frequently resulted in restricted PGPR colonization [47,48]. Specifically, *Bacillus* species are favored by temperatures above 20 °C, enhancing plant growth through hormone production and nutrient solubilization [48]. Along this line, Nguyen et al. (2019) [47] reported that the root growth of the inoculated plants with a *B. velezensis* strain was lower in October when the temperature was low and the natural daytime was short, in comparison to the root growth in May. Further, due to the short daytime in this experiment, plants grown in organically managed system pots with no limitation in nutrient availability probably increased significantly their foliage length to increase their photosynthetic surface. Contrary to *B. subtilis*, Lynch [49] detected functional growing cells of the psychrotrophic *P. fluorescens* at a temperature from 3 to 35 °C. Based on these studies, although there is a difference in the response of the two microbial species to the temperature spectrum, this had no consequence for the lettuce plant performance.

### 4.2. Microbial Biomass, Composition, and Activity

The inoculants in the conventionally managed pots had a limited effect on the biomass of all of the microbial groups; in most cases, the abundances did not differ significantly compared to the control. This may be attributed to the pesticide residues of the conventional management practice before this experiment. More specifically, the conventional management of barley crops included the extensive use of triazole fungicides (propiconazole), which reduced significantly the bacterial and fungal populations, even after long incubation periods [50]. On the contrary, in the organically managed pots, the application of the Ps and BaPs inoculants increased the abundance of most rhizosphere microbial groups, while the abundances in *B. subtilis*-inoculated pots were similar to the control.

The co-inoculation with *B. subtilis* and *P. fluorescens* enhanced the biomass of Gram-positive and Gram-negative bacteria in the organic system. This suggests a lack of competitive interactions between the inoculants and the indigenous microbes, which could be due to the enhanced availability of resources. Similarly, *P. fluorescens per se* increased the size of the populations of both Gram-positive and Gram-negative bacteria in the organic system. *P. fluorescens* is a Gram-negative opportunistic bacterium that adapts rapidly to the environment and becomes the dominant species [51]. Moreover, according to Ke et al. [52], *P. fluorescens* could affect positively the indigenous diazotroph populations, the majority of which are Gram-negative bacteria, while Kozdroj et al. [53] found that pots inoculated with *Pseudomonas* sp. presented higher populations of the slow-growing Gram-positive bacterial classes compared to the untreated controls. Apart from *P. fluorescens*, *B. subtilis per se* increased the biomass of Gram-positive bacteria in the organic system. This increase could be attributed to the increased *B. subtilis* biomass. However, the low temperatures prevailing during the experiment, compared to the optimum temperature for this species (30–35 °C, makes this explanation less possible. Alternatively,

*B. subtilis* could act synergistically with the other Gram-positive bacteria supporting their growth. The lack of *B. subtilis* effect on Gram-negative bacteria contradicts the results of Han et al. [54] who mentioned the antibacterial activity of *B. subtilis* against specific Gram-negative phytopathogens.

The abundance of microeukaryotes was affected only by the management type and exhibited higher values in conventional pots. This finding contradicted the idea that the increased biomass of microeukaryotes is strongly associated with an increased number of bacteria, due to the prey-predator relationship [55]. Nevertheless, our results may be explained by the increased plant's root biomass in the conventional pots, which may have led to an enhanced number of plant-feeding nematodes (parasitic and non-parasitic), which are associated with plant roots.

The addition of any of the three inoculants in the organic pots increased the fungal abundance, while the Ps treatment presented the highest values; similar results were recorded by Viollet et al. [56]. Inoculation of the organic soil with Ba resulted in increased fungal biomass, but not to the levels of the Ps treatment. This may be explained by the negative relationship between the *Bacillus* species and some strains of fungi, either by synthesizing chitinases, which are hydrolytic enzymes that break down glycosidic bonds in chitin, a component of the fungal cell walls or by producing antifungal lipopeptides [57]. The fact that the co-inoculated pots presented intermediate fungal biomass between the other inoculated treatments further supports our suggestion.

The Gram<sup>+</sup>/Gram<sup>-</sup> ratio showed an idiosyncratic response to the management type × inoculum type treatment. The Ps and BaPs treatments in the pots with conventionally managed soil and the Ba ones of the organically managed pots had the highest values. According to Moeskops et al. [58], Gram-negative bacteria are negatively affected by chemical fertilizers. Another possible reason could be the capacity of the Gram-positive for spore formation under extreme conditions. Further in Ps conventional pots, *Pseudomonas* may have developed competitive interactions with the other Gram-negative bacteria suppressing their biomass and leading to the increase of the Gram<sup>+</sup>/Gram<sup>-</sup> ratio. The dominance of Gram-positive bacteria when young roots are colonized with the inoculant containing the *Pseudomonas* genus has been reported by other researchers as well [59] and was attributed to the vulnerability of r-strategy microbes to the environmental perturbation caused by the *P. fluorescens* inoculant. These negative relationships were not developed in the organically managed pots probably due to the greater presence of resources. Specifically, in the organic system, the incorporation of *B. subtilis* seemed to result in synergistic relationships with the other Gram-positive bacteria.

Apart from the ratio among bacterial groups, dissimilarities in the structure of microbial communities were revealed. Nutrient adequacy in the organically managed pots facilitated the development of distinct microbial assemblages; community structure in the Ps inoculated pots differed significantly from the community in the Ba pots and both differed from the control. Moreover, the microbial community in the BaPs organic pots differed from the control. This result looks similar to the results of Nunan et al. [60] who demonstrated that the intrinsic differences in microbial communities of different structures and/or diversities were revealed under conditions of high nutrient availability. In contrast, the limited nutrient availability in the conventional system together with the pesticide residues may have weakened the effect of the inoculants. In the conventionally managed pots, only in the case of co-inoculation did a significantly different microbial community structure emerge.

Although microbial biomasses and community structure were affected by the interaction of management type × inoculum, the soil enzyme activity was affected separately by each one of the independent variables. For all enzymes, a higher activity was recorded in the organically managed pots. Extracellular enzymes can be bound on clay minerals and stabilized by soil organic matter via the formation of enzyme-clay or enzyme-humus complexes [61]. This binding protects enzymes from decomposition by microbial proteases. For acid phosphatase and urease, the lowest and highest activity was recorded in the control and BaPs inoculated pots, respectively. Kumar et al. [23] also reported that the co-inoculation with *Bacillus* and *Pseudomonas* strains showed a synergistic effect, resulting in increased enzymatic activity. The increased urease activity in treatments that involved inoculation with *P. fluorescens* (Ps and BaPs) could be explained by the increased values of Gram-negative bacteria

(at least in the organically managed pots) since most urease-producing bacteria are Gram-negative [62]. In contrast, the activity of  $\beta$ -glucosidase was not affected by any of the inoculants indicating the independence of the carbon cycle on inoculation.

## 5. Conclusions

This study confirmed the central role of the soil management system for microbial inoculation. The management system exerted the only significant effect on plant growth and an independent significant effect on enzyme activity. However, it mediated the effects of inoculants on soil rhizosphere microbial biomass and community structure confirming partly our initial hypothesis. Specifically, *P. fluorescens* inoculation and co-inoculation with *B. subtilis* resulted in increased microbial biomasses only in the organic system. Further, in the organic system, new microbial assemblages emerged because of the inoculants. To conclude, we suggest that for microbial inoculation, which has been promoted as a suitable method for sustainable agriculture, to be beneficial for plant growth and soil health, it should be considered together with the management history of the soil. Our results suggest that microbial inoculants are most effective when applied to soils that were previously cultivated using more environmentally friendly methods such as organic matter incorporation, no tillage, and with limited use of pesticides.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4395/10/9/1428/s1>, Table S1: The effects of management system, inoculum type, and their interaction on plant growth parameters as revealed by two-way ANOVA. (\*\*:  $p < 0.01$ , ns: non-significant); Table S2: The effects of management system, inoculum type, and their interaction on microbial groups and PLFAs ratios as revealed by Two-way ANOVA (\*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , ns: non-significant); Table S3: The effects of management system, inoculum type, and their interaction on microbial groups and PLFAs ratios as revealed by Two-way ANOVA (\*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , ns: non-significant)

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## References

1. Tang, W.; Shan, B.; Zhang, H. Phosphorus buildup and release risk associated with agricultural intensification in the estuarine sediments of Chaohu Lake Valley, Eastern China. *Clean Soil Air Water* **2010**, *38*, 336–343. [[CrossRef](#)]
2. Ju, X.T.; Xing, G.X.; Chen, X.P.; Zhang, S.L.; Zhang, L.J.; Liu, X.J.; Cui, Z.L.; Yin, B.; Christie, P.; Zhu, Z.L.; et al. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 3041–3046. [[CrossRef](#)] [[PubMed](#)]
3. Edenhofer, O.; Pichs-Madruga, R.; Sokona, Y.; Farahani, E.; Kadner, S.; Seyboth, K.; Adier, A.; Baum, I.; Brunner, S.; Eickemeier, P. *Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2014.
4. Tilman, D.; Cassman, K.G.; Matson, P.A.; Naylor, R.; Polasky, S. Agricultural sustainability and intensive production practices. *Nature* **2002**, *418*, 671–677. [[CrossRef](#)] [[PubMed](#)]
5. Laetz, C.A.; Baldwin, D.H.; Collier, T.K.; Hebert, V.; Stark, J.D.; Scholz, N.L. The synergistic toxicity of pesticide mixtures: Implications for risk assessment and the conservation of endangered Pacific salmon. *Environ. Health Perspect.* **2009**, *117*, 348–353. [[CrossRef](#)] [[PubMed](#)]
6. Dou, R.; Sun, J.; Deng, F.; Wang, P.; Zhou, H.; Wei, Z.; Chen, M.; He, Z.; Lai, M.; Ye, T.; et al. Contamination of pyrethroids and atrazine in greenhouse and open-field agricultural soils in China. *Sci. Total Environ.* **2020**, *701*, 134916. [[CrossRef](#)] [[PubMed](#)]
7. Baćmaga, M.; Wyszowska, J.; Kucharski, J. Response of soil microorganisms and enzymes to the foliar application of Helicur 250 EW fungicide on *Horderum vulgare* L. *Chemosphere* **2020**, *242*, 125163. [[CrossRef](#)]

8. Satapute, P.; Kamble, M.V.; Adhikari, S.S.; Jogaiah, S. Influence of triazole pesticides on tillage soil microbial populations and metabolic changes. *Sci. Total Environ.* **2019**, *651*, 2334–2344. [[CrossRef](#)] [[PubMed](#)]
9. Neuwirthová, N.; Trojan, M.; Svobodová, M.; Vašíčková, J.; Šimek, Z.; Hofman, J.; Bielská, L. Pesticide residues remaining in soils from previous growing season(s)—Can they accumulate in non-target organisms and contaminate the food web? *Sci. Total Environ.* **2019**, *646*, 1056–1062. [[CrossRef](#)]
10. Birkhofer, K.; Bezemer, T.M.; Bloem, J.; Bonkowski, M.; Christensen, S.; Dubois, D.; Ekelund, F.; Fließbach, A.; Gunst, L.; Hedlund, K.; et al. Long-term organic farming fosters below and aboveground biota: Implications for soil quality, biological control and productivity. *Soil Biol. Biochem.* **2008**, *40*, 2297–2308. [[CrossRef](#)]
11. Zhang, Q.; Zhou, W.; Liang, G.; Wang, X.; Sun, J.; He, P.; Li, L. Effects of different organic manures on the biochemical and microbial characteristics of albic paddy soil in a short-term experiment. *PLoS ONE* **2015**, *10*, e0124096. [[CrossRef](#)]
12. Hartmann, M.; Frey, B.; Mayer, J.; Mäder, P.; Widmer, F. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME J.* **2015**, *9*, 1177–1194. [[CrossRef](#)] [[PubMed](#)]
13. Li, F.; Chen, L.; Zhang, J.; Yin, J.; Huang, S. Bacterial community structure after long-term organic and inorganic fertilization reveals important associations between soil nutrients and specific taxa involved in nutrient transformations. *Front. Microbiol.* **2017**, *8*, 187. [[CrossRef](#)] [[PubMed](#)]
14. Ierna, A.; Mauromicale, G. Sustainable and profitable nitrogen fertilization management of potato. *Agronomy* **2019**, *9*, 582. [[CrossRef](#)]
15. Bizos, G.; Papatheodorou, E.M.; Chatzistathis, T.; Ntalli, N.; Aschonitis, V.G.; Monokrousos, N. The role of microbial inoculants on plant protection, growth stimulation, and crop productivity of the olive tree (*Olea europaea* L.). *Plants* **2020**, *9*, 743. [[CrossRef](#)] [[PubMed](#)]
16. Mirshad, P.P.; Puthur, J.T. Drought tolerance of bioenergy grass *Saccharum spontaneum* L. enhanced by arbuscular mycorrhizae. *Rhizosphere* **2017**, *3*, 1–8. [[CrossRef](#)]
17. Vurukonda, S.S.K.P.; Vardharajula, S.; Shrivastava, M.; SkZ, A. Multifunctional *Pseudomonas putida* strain FBKV2 from arid rhizosphere soil and its growth promotional effects on maize under drought stress. *Rhizosphere* **2016**, *1*, 4–13. [[CrossRef](#)]
18. Deshmukh, Y.; Khare, P.; Patra, D. Rhizobacteria elevate principal basmati aroma compound accumulation in rice variety. *Rhizosphere* **2016**, *1*, 53–57. [[CrossRef](#)]
19. Bhattacharyya, P.N.; Jha, D.K. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1327–1350. [[CrossRef](#)]
20. Barea, J.M. Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. *J. Soil Sci. Plant Nutr.* **2015**, *15*, 261–282. [[CrossRef](#)]
21. Kamou, N.N.; Karasali, H.; Menexes, G.; Kasiotis, K.M.; Bon, M.C.; Papadakis, E.N.; Tzelepis, G.D.; Lotos, L.; Lagopodi, A.L. Isolation screening and characterisation of local beneficial rhizobacteria based upon their ability to suppress the growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and tomato foot and root rot. *Biocontrol Sci. Technol.* **2015**, *25*, 928–949. [[CrossRef](#)]
22. Ruiu, L. Microbial biopesticides in agroecosystems. *Agronomy* **2018**, *8*, 235. [[CrossRef](#)]
23. Kumar, M.; Mishra, S.; Dixit, V.; Kumar, M.; Agarwal, L.; Chauhan, P.S.; Nautiyal, C.S. Synergistic effect of *Pseudomonas putida* and *Bacillus amyloliquefaciens* ameliorates drought stress in chickpea (*Cicer arietinum* L.). *Plant Signal. Behav.* **2016**, *11*, 1–9. [[CrossRef](#)] [[PubMed](#)]
24. Haas, D.; Défago, G. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat. Rev. Microbiol.* **2005**, *3*, 307–319. [[CrossRef](#)] [[PubMed](#)]
25. Leveau, J.H.J.; Lindow, S.E. Utilization of the plant hormone Indole-3-Acetic Acid for growth by. *Society* **2005**, *71*, 2365–2371.
26. Srivastava, S.; Bist, V.; Srivastava, S.; Singh, P.C.; Trivedi, P.K.; Asif, M.H.; Chauhan, P.S.; Nautiyal, C.S. Unraveling aspects of *Bacillus amyloliquefaciens* mediated enhanced production of rice under biotic stress of *Rhizoctonia solani*. *Front. Plant Sci.* **2016**, *7*, 1–16. [[CrossRef](#)]
27. Palma, L.; Muñoz, D.; Berry, C.; Murillo, J.; Caballero, P.; Caballero, P. *Bacillus thuringiensis* toxins: An overview of their biocidal activity. *Toxins* **2014**, *6*, 3296–3325. [[CrossRef](#)]
28. Choudhary, D.K.; Johri, B.N. Interactions of *Bacillus* spp. and plants—With special reference to induced systemic resistance (ISR). *Microbiol. Res.* **2009**, *164*, 493–513. [[CrossRef](#)]

29. Alori, E.T.; Glick, B.R.; Babalola, O.O. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Front. Microbiol.* **2017**, *8*, 1–8. [[CrossRef](#)]
30. Aini, N.; Yamika, W.S.D.; Ulum, B. Effect of nutrient concentration, PGPR and AMF on plant growth, yield and nutrient uptake of hydroponic lettuce. *Int. J. Agric. Biol.* **2019**, *21*, 175–183.
31. Hsu, C.K.; Micallef, S.A. Plant-mediated restriction of *Salmonella enterica* on tomato and spinach leaves colonized with *Pseudomonas* plant growth-promoting rhizobacteria. *Int. J. Food Microbiol.* **2017**, *259*, 1–6. [[CrossRef](#)]
32. Chowdhury, S.P.; Dietel, K.; Rändler, M.; Schmid, M.; Junge, H.; Borriss, R.; Hartmann, A.; Grosch, R. Effects of *Bacillus amyloliquefaciens* FZB42 on lettuce growth and health under pathogen pressure and its impact on the rhizosphere bacterial community. *PLoS ONE* **2013**, *8*, 1–10. [[CrossRef](#)]
33. Cipriano, M.A.P.; Lupatini, M.; Lopes-Santos, L.; da Silva, M.J.; Roesch, L.F.W.; Destéfano, S.A.L.; Freitas, S.S.; Kuramae, E.E. Lettuce and rhizosphere microbiome responses to growth promoting *Pseudomonas* species under field conditions. *FEMS Microbiol. Ecol.* **2016**, *92*. [[CrossRef](#)] [[PubMed](#)]
34. Cucu, M.A.; Gilardi, G.; Pugliese, M.; Matić, S.; Gisi, U.; Gullino, M.L.; Garibaldi, A. Influence of different biological control agents and compost on total and nitrification-driven microbial communities at rhizosphere and soil level in a lettuce—*Fusarium oxysporum* f. sp. *lactucae* pathosystem. *J. Appl. Microbiol.* **2019**, *126*, 905–918. [[CrossRef](#)] [[PubMed](#)]
35. Castro-Sowinski, S.; Herschkovitz, Y.; Okon, Y.; Jurkevitch, E. Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms. *FEMS Microbiol. Lett.* **2007**, *276*, 1–11. [[CrossRef](#)] [[PubMed](#)]
36. Troelstra, S.R.; Wagenaar, R.; Smant, W.; Peters, B.A.M. Interpretation of bioassays in the study of interactions between soil organisms and plants: Involvement of nutrient factors. *New Phytol.* **2001**, *150*, 697–706. [[CrossRef](#)]
37. Kapali, S.; Gade, R.M.; Shitole, A.V.; Swathi, S. Isolation and characterization of *Pseudomonas fluorescens* and *Bacillus subtilis* and their in vitro evaluation. *Adv. Life Sci.* **2016**, *5*, 5856–5859.
38. Versalovic, J.; Schneider, M.; de Bruijn, F.J.; Lupski, J.R. Versalovic\_MMCB 1994\_Genomic Fingerprinting.pdf. *Methods Mol. Cell. Biol.* **1994**, *5*, 25–40.
39. Allen, E. *Chemical Analysis of Ecological Materials*; Blackwell Scientific Publishing: Oxford, UK, 1976.
40. Ntalli, N.; Monokrousos, N.; Rumbos, C.; Kontea, D.; Zioga, D.; Argyropoulou, M.D.; Menkissoglu-Spiroudi, U.; Tsiropoulos, N.G. Greenhouse biofumigation with *Melia azedarach* controls *Meloidogyne* spp. and enhances soil biological activity. *J. Pest Sci.* **2018**, *91*, 29–40. [[CrossRef](#)]
41. Ntalli, N.; Menkissoglu-Spiroudi, U.; Doitsinis, K.; Kalomoiris, M.; Papadakis, E.N.; Boutsis, G.; Dimou, M.; Monokrousos, N. Mode of action and ecotoxicity of hexanoic and acetic acids on *Meloidogyne javanica*. *J. Pest Sci.* **2020**, *93*, 867–877. [[CrossRef](#)]
42. Panayiotou, E.; Dimou, M.; Monokrousos, N. The effects of grazing intensity on soil processes in a Mediterranean protected area. *Environ. Monit. Assess.* **2017**, *189*, 441. [[CrossRef](#)]
43. Allison, S.D.; Jastrow, J.D. Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. *Soil Biol. Biochem.* **2006**, *38*, 3245–3256. [[CrossRef](#)]
44. Sinsabaugh, R.L.; Reynolds, H.; Long, T.M. Rapid assay for amidohydrolase (urease) activity in environmental samples. *Soil Biol. Biochem.* **2000**, *32*, 2095–2097. [[CrossRef](#)]
45. Poorter, H.; Nagel, O. Poorter and Nagel 1999 The role of biomass allocation in the growth response of plants to different levels of light, CO<sub>2</sub>, nutrients and water: A quantitative review. *Aust. J. Plant Physiol.* **2000**, *27*, 1191.
46. Khosravi, A.; Zarei, M.; Ronaghi, A. Effect of PGPR, Phosphate sources and vermicompost on growth and nutrients uptake by lettuce in a calcareous soil. *J. Plant Nutr.* **2018**, *41*, 80–89. [[CrossRef](#)]
47. Nguyen, M.L.; Glaes, J.; Spaepen, S.; Bodson, B.; du Jardin, P.; Delaplace, P. Biostimulant effects of *Bacillus* strains on wheat from in vitro towards field conditions are modulated by nitrogen supply. *J. Plant Nutr. Soil Sci.* **2019**, *182*, 325–334. [[CrossRef](#)]
48. Mohite, B. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *J. Soil Sci. Plant Nutr.* **2013**, *13*, 638–649. [[CrossRef](#)]
49. Lynch, W.H. Effect of temperature on *Pseudomonas fluorescens* chemotaxis. *J. Bacteriol.* **1980**, *143*, 338–342. [[CrossRef](#)]

50. Faucon, M.P.; Houben, D.; Lambers, H. Plant Functional Traits: Soil and Ecosystem Services. *Trends Plant Sci.* **2017**, *22*, 385–394. [[CrossRef](#)]
51. Thirup, L.; Ekelund, F.; Johnsen, K.; Jacobsen, C.S. Population dynamics of the fast-growing sub-populations of *Pseudomonas* and total bacteria, and their protozoan grazers, revealed by fenpropimorph treatment. *Soil Biol. Biochem.* **2000**, *32*, 1615–1623. [[CrossRef](#)]
52. Ke, X.; Feng, S.; Wang, J.; Lu, W.; Zhang, W.; Chen, M.; Lin, M. Effect of inoculation with nitrogen-fixing bacterium *Pseudomonas stutzeri* A1501 on maize plant growth and the microbiome indigenous to the rhizosphere. *Syst. Appl. Microbiol.* **2019**, *42*, 248–260. [[CrossRef](#)]
53. Kozdrój, J.; Trevors, J.T.; Van Elsas, J.D. Influence of introduced potential biocontrol agents on maize seedling growth and bacterial community structure in the rhizosphere. *Soil Biol. Biochem.* **2004**, *36*, 1775–1784. [[CrossRef](#)]
54. Han, L.; Wang, Z.; Li, N.; Wang, Y.; Feng, J.; Zhang, X. *Bacillus amyloliquefaciens* B1408 suppresses *Fusarium* wilt in cucumber by regulating the rhizosphere microbial community. *Appl. Soil Ecol.* **2019**, *136*, 55–66. [[CrossRef](#)]
55. Zhang, L.; Lueders, T. Micropredator niche differentiation between bulk soil and rhizosphere of an agricultural soil depends on bacterial prey. *FEMS Microbiol. Ecol.* **2017**, *93*, 1–11. [[CrossRef](#)] [[PubMed](#)]
56. Viollet, A.; Pivato, B.; Mougel, C.; Cleyet-Marel, J.C.; Gubry-Rangin, C.; Lemanceau, P.; Mazurier, S. *Pseudomonas fluorescens* C7R12 type III secretion system impacts mycorrhization of *Medicago truncatula* and associated microbial communities. *Mycorrhiza* **2017**, *27*, 23–33. [[CrossRef](#)]
57. El Arbi, A.; Rochex, A.; Chataigné, G.; Béchet, M.; Lecouturier, D.; Arnauld, S.; Gharsallah, N.; Jacques, P. The Tunisian oasis ecosystem is a source of antagonistic *Bacillus* spp. producing diverse antifungal lipopeptides. *Res. Microbiol.* **2016**, *167*, 46–57. [[CrossRef](#)]
58. Moeskops, B.; Buchan, D.; Sleutel, S.; Herawaty, L.; Husen, E.; Saraswati, R.; Setyorini, D.; De Neve, S. Soil microbial communities and activities under intensive organic and conventional vegetable farming in West Java, Indonesia. *Appl. Soil Ecol.* **2010**, *45*, 112–120. [[CrossRef](#)]
59. Nacamulli, C.; Bevivino, A.; Dalmastri, C.; Tabacchioni, S.; Chiarini, L. Perturbation of maize rhizosphere microflora following seed bacterization with *Burkholderia cepacia* MCI 7. *FEMS Microbiol. Ecol.* **1997**, *23*, 183–193. [[CrossRef](#)]
60. Nunan, N.; Leloup, J.; Ruamps, L.S.; Pouteau, V.; Chenu, C. Effects of habitat constraints on soil microbial community function. *Sci. Rep.* **2017**, *7*, 1–10. [[CrossRef](#)]
61. Nannipieri, P.; Ascher, J.; Ceccherini, M.T.; Landi, L.; Pietramellara, G.; Renella, G. Microbial diversity and soil functions. *Eur. J. Soil Sci.* **2003**, *54*, 655–670. [[CrossRef](#)]
62. Fujita, M.; Nakashima, K.; Achal, V.; Kawasaki, S. Whole-cell evaluation of urease activity of *Pararhodobacter* sp. isolated from peripheral beachrock. *Biochem. Eng. J.* **2017**, *124*, 1–5. [[CrossRef](#)]

