agronomy

MDPI

Article

Microbial Consortia versus Single-Strain Inoculants: An Advantage in PGPM-Assisted Tomato Production?

Klára Bradáčová [1,*], Andrea S. Florea [2], Asher Bar-Tal [3], Dror Minz [3], Uri Yermiyahu [4], Raneen Shawahna [3], Judith Kraut-Cohen [3], Avihai Zolti [3,5], Ran Erel [4], K. Dietel [6], Markus Weinmann [1], Beate Zimmermann [7], Nils Berger [8], Uwe Ludewig [1], Guenter Neumann [1] and Gheorghe Poşta [2]

1 Institute of Crop Science (340h), Universität Hohenheim, Fruwirthstraße 20, 70593 Stuttgart, Germany; markus.weinmann@uni-hohenheim.de (M.W.); ludewig@uni-hohenheim.de (U.L.); guenter.neumann@uni-hohenheim.de (G.N.)
2 Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" form Timişoara, Faculty of Horticulture and Forestry, Calea Aradului 119, 300645 Timişoara, România; andreeas.florea@yahoo.com (A.S.F.); posta.gheorghe@gmail.com (G.P.)
3 Institute of Soil, Water & Environmental Sciences, The Volcani Center, Agricultural Research Oraganization (ARO), Rishon LeZion 75359, Israel; abartal@volcani.agri.gov.il (A.B.-T.); minz@volcani.agri.gov.il (D.M.); raneen@volcani.agri.gov.il (R.S.); judith@volcani.agri.gov.il (J.K.-C.); avihai.zolti@mail.huji.ac.il (A.Z.)
4 Institute of Soil, Water & Environmental Sciences, Gilat Research Center, Agricultural Research Oraganization (ARO), Gilat 85280, Israel; uri4@volcani.agri.gov.il (U.Y.); ranerel@volcani.agri.gov.il (R.E.)
5 Department of Plant Pathology and Microbiology, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 85280, Israel
6 ABiTEP GmbH, Glienicker Weg 185, D-12489 Berlin, Germany; dietel@abitep.de
7 Institute of Farm Management (410b), Universität Hohenheim, Schwerzstr. 44, 70593 Stuttgart, Germany; zimmerbe@uni-hohenheim.de
8 EuroChem Agro GmbH, 8165 Mannheim, Germany; nils.berger@eurochemgroup.com
* Correspondence: klara.bradacova@uni-hohenheim.de; Tel.: +49(0)-711-459-22253

Received: 26 January 2019; Accepted: 18 February 2019; Published: 22 February 2019

Abstract: The use of biostimulants with plant growth-promoting properties, but without significant input of nutrients, is discussed as a strategy to increase stress resistance and nutrient use efficiency of crops. However, limited reproducibility under real production conditions remains a major challenge. The use of combination products based on microbial and non-microbial biostimulants or microbial consortia, with the aim to exploit complementary or synergistic interactions and increase the flexibility of responses under different environmental conditions, is discussed as a potential strategy to overcome this problem. This study aimed at comparing the efficiency of selected microbial single-strain inoculants with proven plant-growth promoting potential versus consortium products under real production conditions in large-scale tomato cultivation systems, exposed to different environmental challenges. In a protected greenhouse production system at Timisoara, Romania, with composted cow manure, guano, hair-, and feather-meals as major fertilizers, different fungal and bacterial single-strain inoculants, as well as microbial consortium products, showed very similar beneficial responses. Nursery performance, fruit setting, fruit size distribution, seasonal yield share, and cumulative yield (39–84% as compared to the control) were significantly improved over two growing periods. By contrast, superior performance of the microbial consortia products (MCPs) was recorded under more challenging environmental conditions in an open-field drip-fertigated tomato production system in the Negev desert, Israel with mineral fertilization on a high pH (7.9), low fertility, and sandy soil. This was reflected by improved phosphate (P) acquisition, a stimulation of vegetative shoot biomass production and increased final fruit yield under conditions of limited P supply. Moreover, MCP inoculation was associated with selective changes of the rhizosphere-bacterial

community structure particularly with respect to *Sphingobacteriia* and *Flavobacteria*, reported as salinity indicators and drought stress protectants. Phosphate limitation reduced the diversity of bacterial populations at the root surface (rhizoplane) and this effect was reverted by MCP inoculation, reflecting the improved P status of the plants. The results support the hypothesis that the use of microbial consortia can increase the efficiency and reproducibility of BS-assisted strategies for crop production, particularly under challenging environmental conditions.

Keywords: plant growth-promoting microorganisms (PGPM); biostimulants; microbial consortia; tomato production; organic fertilization

1. Introduction

The agricultural use of biostimulants (BS) based on microbial inoculants or bioactive natural compounds, originating, e.g., from plant, seaweed, and compost extracts or plant and animal based protein hydrolysates with plant growth-promoting and strengthening potential but without significant input of nutrients, has a long history [1,2]. Seaweed and gelatine-based. biostimulants are discussed to be a potential tool in terms of reducing the fertilizer and agrochemical inputs, which is often accompanied with negative environmental side effects [3–6]. Biostimulants could thus contribute towards more sustainable crop production. This is of particular importance for crop systems depending on intensive fertilizer input (e.g., vegetable production), associated with high risks of unwanted nutrient losses [7]. However, BS may also enable a more efficient use of organic and inorganic fertilizers based on materials and by-products of waste recycling [8–11], promoting concepts for the sustainable management of resources.

The commercial use of microbial BS in crop production was based initially on targeted selection of efficient single strain inoculants, starting with a first patent already in 1896 on Rhizobia to increase the atmospheric nitrogen fixation potential in leguminous plants [12]. Nowadays, numerous single-strain inoculants with biofertilizer functions are commercially available [1]. There is an increasing trend to use combination products based on microbial and nonmicrobial BS or microbial consortia, with the aim to exploit complementary or even synergistic interactions. Microbial consortia products (MCPs) are composed of compatible microbial strains with different modes of action to provide a broad spectrum of usage [13]. Strains of genetically diverse groups are selected, with the ability to adapt differentially to variations in environmental conditions, such as soil temperature, soil moisture, or soil pH [14]. However, due to high costs for single-strain production, frequently, strain combination products are at least partially replaced by less defined microbial populations, originating from fermentation of various natural substrates [13,15] or composting processes [16,17]. The concept behind these types of products is based on the assumption, that under variable environmental conditions, different members of the inoculated microbial communities are selectively activated by rhizosphere signals and ecophysiological responses of the host plant, to express their beneficial effects on plant growth. Examples comprise activation of phosphate solubilizing microorganisms in the absence of soluble P forms in soils, promotion of P mineralizers after supply of organic fertilizers or of chitinase-producing bacteria in response to proliferation of pathogenic fungi [13]. Various literature reviews claim that there is a clear trend showing the advantage of MCPs in comparison with single strain inoculants [1,14,18], but there are also contradictory reports [19] and particularly direct comparisons under real practice conditions are rare.

Based on the hypothesis of superior performance of microbial consortia over single-strain inoculants, in this study we present a comparative efficiency evaluation of a MCP versus a selection of fungal and bacterial single strain products and strain combinations with proven plant growth-promoting potential [11,20–23] under real production conditions.

Experiments were conducted under greenhouse and field conditions in two tomato production systems, characterized by different challenges with respect to type and amount of fertilizer supply, water availability, plant protection, and climatic conditions. In case study I, the effects of the different microbial inoculants were comparatively investigated during two seasons of commercial greenhouse tomato production in Timisoara, Romania with composted cow manure (nursery substrate), guano, hair, and feather meals (main culture) as major fertilizers frequently used in the local production practice. Case study II was conducted under more challenging environmental conditions in a drip-irrigated tomato production system in Ramat, Negev (the Negev Highlands), a desert region in Israel, on an alkaline sandy soil (pH 7.9) with very low phosphate availability (P_{Olsen}: 5 mg kg^{-1} soil DM), using microbial inoculants that were also investigated in case study I. The plants were supplied with different levels of mineral P supply (triple superphosphate), applied via band placement in combination with dicyandiamide (DCD)-stabilized ammonium sulfate to increase P availability on the alkaline soil. Microbial inoculants were applied via fertigation. In both case studies, the effects of single-strain inoculants on vegetative plant growth, yield formation, and fruit quality parameters were assessed in comparison with the consortium products.

2. Materials and Methods

2.1. Case Study 1: Large-Scale Greenhouse Tomato Production in Timisoara, Romania 2016/2017

2.1.1. Tomato Cultivation and Fertilization

The tomato (*Lycopersicum esculentum* L.) variety Primadona F1 (Hazera Seeds Ltd., Berurim M.P Shikmim, Israel) was used in the greenhouse experiment located at the horticultural research station of Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timișoara, Romania. The experiment was carried out under farmer's practice conditions. For preculture, tomato seeds were sown during February in plastic pots (1 seed pot^{-1}) containing 350–400 g of a nursery substrate mixture pH 7.3, based on 45% *v*/*v* composted cow manure, 30% *v*/*v* garden soil, 15% *v*/*v* peat, and 10% *v*/*v* sand (Supplementary Table S1). At phenological growth stage BBCH 51 in 2016, the nursery plants were transplanted for greenhouse culture into a clay loam Vertisol, pH 7.1 (Supplementary Table S1), preincorporated with an organic seabird guano (60%) and feather meal (40%) fertilizer (DIX 10N 10-3-3+1, 10% N, 1.3% P, 2.5% K, 0.6% Mg, Italpollina SpA, Rivoli Veronese, Italy) at the recommended dosage of 2.2 t ha^{-1}). Due to phytosanitary replant problems observed in 2016, in 2017 the nursery plants were transplanted into 10 L containers filled with a prefertilized clay peat substrate (SP ED63 T grob SM, 1kgNs+FE, Einheitserdewerke, Gebr. Patzer GmbH & Co. KG, Sinntal-Altengronau, Germany, N: 140 mg L^{-1}, P: 70 mg L^{-1}, K: 149 mg L^{-1}), plus 10% sand (*v*/*v*), pH 6.2. Additional organic fertilization was performed with a mixed hair/feather meal fertilizer (Monterra 13-0-0, 13% N, 0.22% P, MeMon BV, Arnhem, The Netherlands) at the recommended dose of 2 to 3 t ha^{-1} (=100 g $plant^{-1}$ in 10 L containers). In both years, supplementary foliar fertilization during the culture period according to the local practice was divided into 17 cumulative application rates with a total N, P, K application of 76.7, 1.8, and 3.3 kg ha^{-1}, respectively (details in Supplementary Table S2). The different types of commercial fertilizers used for the experiments comprised:

Lithovit® (Biofa AG, Münsingen, Germany), CO_2 foliar fertilizer: 77.9% $CaCO_3$, 8.7 % $MgCO_3$, 7.5% SiO_2, >0.25% Fe, >0.1% K_2O, >0.015% N, >0.015% P_2O_5, Al, S, >0.01% Mn, Cu, Zn.

CropMax® (Holland Farming B.V, Groenekan, The Netherlands): 0.2% N, 0.4% P, 0.02% K, 220 mg/L Fe, 550 mg/L Mg, 49 mg/L Zn, 35 mg/L Cu, 54 mg/L Mn, 70 mg/L B, 10 mg/L Ca, Mo, Co, Ni, aminoacids 2%, vitamins, enzymes, auxin, cytokinin, gibberellin.

YaraLiva Calcinit 15.5-0-0 (Yara UK Ltd., Grimsby, UK): 15.5% N (NO_3 14.4%, NH_4 1.1%), 19% Ca.

YaraVita® Universal Bio (Yara UK Ltd., Grimsby, UK): 8.5% N, 3.4% P_2O_5, 6% K_2O, 0.02% B, 0.1% Cu, 0.11% Mn, 0.003% Mo, 0.06% Zn.

Myr Calcium (Italpollina SpA, Rivoli Veronese, Italy): 3% organic N, 5% CaO, 18.5% organic C.

Myr Potassium (Italpollina SpA, Rivoli Veronese, Italy): 12% K_2O, 3% organic N, 11% organic C.

Plants were pruned after the development of 12 inflorescences (2016) and 10 inflorescences (2017), respectively. During the first eight weeks after transplanting, bumble bees (*Bombus* spp.) were deployed to facilitate pollination. Final harvest was conducted 15 weeks after transplanting.

2.1.2. Microbial Inoculation

Microbial biostimulants used in these experiments comprised Biological Fertilizer DC (Bayer CropScience Biologics GmbH, Malchow/Poel, Germany): active ingredient *Penicillium* sp. PK 112 (1×10^9 vital spores mL^{-1}); Proradix® WP (Sourcon Padena, Tübingen, Germany): active ingredient *Pseudomonas* sp. DMSZ 13134 (5.0×10^{10} colony forming units g^{-1}); RhizoVital® 42 fl. (Abitep GmbH, Berlin, Germany): active ingredient *Bacillus amyloliquefaciens* FZB42 (2.5×10^{10} spores g^{-1}), also referred as *Bacillus velezensis* FZB42; *Bacillus simplex* R41 (Abitep GmbH, Berlin, Germany, cold-tolerant strain, 2.5×10^{10} spores g^{-1}); and the microbial consortia product (MCP), (EuroChem Agro, Mannheim, Germany): declared active ingredients, twelve different beneficial bacterial strains including *Azotobacter vinlandii*, *Clostridium* sp., *Lactobacillus* sp., *Bacillus velezensis*, *B. subtilis* (SILo Sil® BS), *B. thuringiensis*, *Pseudomonas fluorescens*, Acetobacter, Enterococcus, *Rhizobium japonicum*, Nitrosomonas, and Nitrobacter, as well as fungi: Saccharomyces, *Penicillium roqueforti*, Monascus, *Aspergillus oryzae*, *Trichoderma harzianum* (TRICHOSIL®), and algae extracts from *Arthrospira platensis (Spirulina)* and *Ascophyllum nodosum* [13].

For application of the different BS products, suspensions were prepared freshly in nonchlorinated tap water: Biological Fertilizer DC (BFDC) 0.05% *w*/*w*, Proradix WP 0.02% *w*/*w*, RhizoVital 42 liquid formulation 0.04% *w*/*w* + *Bacillus simplex* (R41) 0.04% *w*/*w*, MCP 0.01325% *w*/*w*. Inoculation was performed after seedling emergence BBCH 12 (second primary leaf on main shoot unfolded, approx. 21 days after sowing) with 20 mL stock suspension of the respective microbial products per pot. Control plants (Ctrl) were treated with the respective amounts of non-chlorinated tap water. After transplanting into the greenhouse soil (2016), or into container culture (2017) at BBCH 51 (first inflorescence visible), each plant was supplied again with 250 mL of the respective BS suspensions. MCP treatments were performed by fertigation at a concentration of 0.03% *w*/*w*, as recommended by the manufacturer (details of the inoculation procedure are summarized in Supplementary Table S3). 2016: transplanting of tomato plantlets into greenhouse soil (18,940 plants ha^{-1}); 2017: transplanting of tomato plantlets into 10 L pots with peat substrate (22,000 plants ha^{-1}).

2.1.3. Plant Protection

Disease control against fungal pathogens was performed with various chemical fungicides: Mancozeb 80% (0.2% *w*/*w*); Metiram 80% (0.2% *w*/*w*); Propineb 70% (0.2% *w*/*w*); Folpet 80% (0.15% *w*/*w*), Clorotalonil 500 g/L (0.2–0.4% *w*/*w*); and Cu hydroxide + 50% Cu metallic (0.3% *w*/*w*). Control of insect pests was conducted by use of biocontrol systems (Biobest® Sustainable Crop Management, Westerlo, Belgium): plant protection system used for *Trialeurodes vaporariorum*: Encarsia system (*Encarsia formosa)* and Macrolophus system (*Macrolophus caliginosus*) + Nutrimac (of food eggs from Ephestia kuehniella); for aphids: Aphidius system (*Aphidius colemani* and *Aphidoletes aphidimyza*); for trips *Frankliniella occidentalis*: Swirskii system (*Amblyseius swirskii*) and Orius system (*Orius laevigatus*) and for spiders (*Polyphagotarsonemus latus* and *Tetranychus urticae*): *Phytoseiulus persimils* (details are summarized in Supplementary Table S4).

However, later in the season of 2016, the tomato plants showed symptoms of replant diseases, such as root rot induced by the soil-borne pathogen *Fusarium oxysporum* Schlecht f. sp. *radicis-lycopersici* Jarvis and Shoemaker. A high population density of larvae of the beetle *Agriotes lineatus* L. that can feed on the roots of tomato plants was recorded as well. Therefore, in the experiment conducted in 2017, the crop management was modified. To counteract soil-borne diseases, precultured nursery plants were not directly transplanted into the pathogen-affected greenhouse soil, but cultivation was performed in 10 L containers with a commercial clay peat substrate (see Section 2.1.1).

2.1.4. Experimental Design and Statistical Evaluations

The experiment was arranged in a randomized block design with four replicate blocks, each block comprising four treatment plots. The size of individual treatment plots was 8 m × 3.3 m (26.4 m^2) with 50 plants per plot. Plants were arranged in five rows per plot (10 plants per row). The distance between rows was adjusted to 1.5 m. The distance between plants within the rows was 33 cm. The total size of the experimental area with four variants in four replicates was about 634 m^2. Treatment variants comprised Ctrl (Control, no BS treatment), BFDC (Biological Fertilizer DC), Proradix (Proradix WP), FZB42 + R41 (Rhizovital® 42 liquid formulation + *Bacillus simplex* R41), and MCP (Microbial Consortia Product, EuroChem Agro, Mannheim, Germany). A two-way ANOVA with a Tukey test ($p \leq 0.05$) to ascertain significant differences was performed using the SAS Software package 9.4, Institute Inc., Cary, NC, USA.

2.1.5. Pre- and Postharvest Analyses

During the nursery phase, 21 days after the first application of the BS products, scoring of plant height and total leaf area (measured by a leaf area meter device) were determined for eight replicate plants per treatment group. Plant performance after transplanting was characterized for 30 plants per plot in terms of cumulative fruit yield ha^{-1}, mean weight per fruit, fruit biomass production per plant, fruit size distribution (three quality classes: II, I, and extra), and seasonal yield distribution during three months of harvest (June, July, and August), as relevant marketing factors.

2.2. Case study 2: Drip-Irrigated Field Production of Tomato (ARO Research Center), 2017

The field experiment was conducted in Ramat Negev, Israel on a sandy soil (96% sand), with low available P_{Olsen} (5.5 mg kg^{-1}), very low organic carbon (0.08%), and alkaline pH_{CaCl2} 7.9 (Supplementary Table S1). No precipitation occurred during the vegetation period as usual in the dry summer months of warm desert climates. Air temperature, relative humidity and radiation intensity are presented Supplementary Figure S1. The effects of BS applied as single-strain inoculants, as a combination product and a microbial consortium (MCP) were investigated in fertigated tomato plants with different levels of P supply applied by band placement 20 cm width and 30 cm depth along the row.

2.2.1. Tomato Cultivation and Fertilization

Tomato seeds (*Lycopersicum esculentum* L., var. Smadar, Hazera Seeds Ltd., Berurim M.P Shikmim, Israel) were sown on 10 March 2017 in a commercial nursery (Hishtil, Israel) into seedling trays containing Perlite medium (Agrekal Habonim Ind., Hof Hacarmel, Israel). During the nursery period, the seedlings were irrigated with above canopy sprinklers; irrigation was performed several times every day and in excess to allow drainage and to minimize water stress. Nutrients were delivered through the irrigation water. The concentrations of the macronutrients N, K, Ca, and Mg in the irrigating water: N: 50 mg L^{-1} (30% of N-NH_4); P: 13 mg L^{-1}; K: 62 mg L^{-1}; Ca: 6–80 mg L^{-1}; and Mg: 24 mg L^{-1}. Micronutrients were supplied at concentrations of Fe: 1 mg L^{-1}; Mn: 0.5 mg L^{-1}; Zn: 0.25 mg L^{-1}; Cu: 0.04 mg L^{-1}; and Mo: 0.03 mg L^{-1}.

At 6.5 weeks after sowing, nursery plants were transplanted to the open field on 25 April 2017. Before transplanting, potassium chloride and ammonium sulfate, stabilized with the nitrification inhibitor DCD (dicyandiamide) were applied by band placement at a soil depth of 20 cm and width of 30 cm along the center of the plots with a dosage of 47.6 g m^{-2} (ammonium sulfate), 0.48 g m^{-2} (DCD), and 50.0 g m^{-2} (KCL). Additionally, the band placement included three levels of triple superphosphate (TSP) application at 0, 1.25, and 5 g P m^{-2}, corresponding to 0, 12.5, and 50.0 kg P ha^{-1}, respectively. After transplanting, irrigation/fertigation in the field was performed by a dripper system with one lateral tube per row and drippers 25 cm apart. The distance between rows was 2 m and the distance between plants in the row was 25 cm. During field cultivation, additional fertigation without P was

employed to deliver nutrients to the plant roots into the wetted soil. The concentrations of N, K, Ca, and Mg in the fertigation solution were 80 mg L^{-1} (30% of N-NH_4), 75 mg L^{-1}, 200 mg L^{-1}, and 24 mg L^{-1}, respectively. Micronutrients were supplied at concentrations of 1 mg L^{-1} Fe, 0.5 mg L^{-1} Mn, 0.25 mg L^{-1} Zn, 0.04 mg L^{-1} Cu, and 0.03 mg L^{-1} Mo (for details see Supplementary Table S2). Irrigation was performed once a day and the amount was determined by the potential evaporation multiplied by the crop coefficient for each stage of plant development.

2.2.2. Microbial Inoculation

Inoculation was performed with two single-strain inoculants also used case study I (Proradix, FZB42), a combination product (Combifector B = CFB) based on *Trichoderma harzianum OMG16* and *Bacillus amyloliquefaciens FZB42*, enriched with Zn and Mn (Hochschule Anhalt, Bernburg, Germany, Abitep GmbH, Berlin, Germany) and the consortium product MCP (EuroChem Agro, Mannheim, Germany). The dosages of the inoculants are as follows.

Proradix WP suspension: 0.02% *w*/*w*, applied at a rate of 20 mL plant^{-1} in the nursery phase and 250 mL plant^{-1} applied after field transplanting.

Rhizovital 42 liquid formulation: 0.04% *w*/*w*, applied at a rate of 20 mL plant^{-1} in the nursery phase and 250 mL plant^{-1} applied after field transplanting.

CFB: in the nursery, each plant was supplied with 1 mL of a 1% (*w*/*w*) CFB suspension. At transplanting, each plant received 2 mL of a 2% (*w*/*w*) suspension

MCP suspension: 0.03% *w*/*w*—250 mL applied after field transplanting.

2.2.3. Plant Protection

No measures of plant protection were employed during the nursery phase. During open field culture, a range of different insecticides was repeatedly applied by canopy spraying during the culture period (Alaunt, Defender, Denim-Fit, Exirel, Floramite, Metronom, Mospilan, Oberon, and Pirate), as well as Vertimec by soil application. The major target was plant protection against various insects, especially mites and the tobacco white fly (*Bemisia tabaci*), as a vector of Tomato yellow leaf curl virus (for details see Supplementary Table S4).

2.2.4. Experimental Design and Statistical Evaluation

The experiment was arranged in a full factorial design (15 treatments with 5 BS variants × 3 P levels) in four randomized blocks. Each block included 15 plots. The length of each plot was 5 m and the distance between the centers of two adjacent plots was 2.0 m. Planting density was adjusted to 4 plants m^{-1} (2 plants m^{-2}).

Statistical analyses were performed by two-way ANOVA (treatments and blocks) with a Tukey test $p \leq 0.05$ for significance testing of treatment differences with JMP12.0 software package of SAS.

Additional statistical evaluations were performed also by three-way ANOVA (P dose, BS, and blocks) with a Tukey test $p \leq 0.05$ for significance testing of the overall major differences between the P dose treatments and the BS using the JMP13.0 software package of SAS (Supplementary Table S6).

2.2.5. Pre- and Postharvest Analysis

The experiment was terminated five months after sowing on 3 August 2017. Red fruits (approximately 80% red color) were selectively harvested on 20 July 2017 and all remaining fruits were harvested at the termination of the experiment. One representative plant was sampled from each plot on 20 July 2017. The following variables were measured; vegetative shoot (stem with leaves) biomass, root biomass and length, fruit yield (red, green, small fruits), and shoot P concentrations and content. The whole canopy including stems and leaves was removed aboveground and the rooted soil samples were collected in a diameter of 25 cm around the plant and a soil depth of 30 cm.

The roots were separated from the soil by washing with water over sieve. Separated roots segments were digitalized by scanning and root length was determined using the WinRhizo root

analysis software (Regent Instruments Inc., Quebec, QC, Canada). For determination of the P nutritional status, subsamples of the shoot tissue were oven-dried at 60 °C for three days, until the dry weight was constant, ground, and digested with concentrated sulfuric acid. The P concentration was determined with an automated photometric analyzer (Gallery plus, Thermo Fisher Scientific, Vantaa, Finland).

2.2.6. Soil Microbiome Amplicon Sequencing

DNA was extracted using the GenALL DNA extraction kit (GeneAll Biotechnology Co. Ltd., Seoul, South Korea) from root surface washings of rooted soil samples (rhizoplane, 200 mg; see Section 2.2.5) and from soil samples collected between the plant rows (300 mg), which still contained some roots. Therefore, this soil fraction was termed as "root-affected soil". The DNA was amplified with the primer pair CS1_515F (ACACTGACGACATGGTTCTACAGTGCCAGCMGCCG CGGTAA) and CS2_806R (TACGGTAGCAGAGACTTGGTCTGGACTACHVGGGTWTCTAAT), and sequence libraries were generated. An Illumina MiSeq run was performed at the University of Illinois at Chicago Sequencing Core (UICSQC). This process yielded 22 Gb of information, and overall 3511942 sequences. These sequence data have been submitted to the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) databases under the BioProject PRJNA491280. Sequencing analysis was performed as follows; the sequences were paired, quality filtered, and chimeric sequences were removed by use of the 'mothur' software package [24]. Thereafter, the resulting sequences were clustered to operation taxonomic units (OTUs) based on 97% similarity (Table S7). Alpha and Beta diversity were calculated and the taxonomic affiliation was assigned with the QIIME software package [25], based on SILVA 123 database (https://www.arb-silva.de/download/archive/qiime/). Statistical analysis was performed in JMP Pro 13 (Statistical Analysis Software, SAS, Cary, NC, USA). The OTU rarefaction curve of soil and roots samples were computed using Vegan package in R.

The alpha diversity of bacterial communities indicated by the Shannon Diversity Index was determined in the root-affected soil collected between the plant rows and from root washings of the rhizoplane. Comparisons included the treatments with MCP versus single inoculants in the unfertilized control and in the variants with moderate P fertilization (12.5 kg P ha^{-1} soil). Beta diversity for soil and roots microbial community was estimated by nonmetric multidimensional scaling (nMDS) used to visualize the distances between the bacterial communities as calculated for the Bray–Curtis distance matrices.

3. Results

3.1. Case Study 1: Greenhouse Tomato Production in Timisoara, Romania 2016 and 2017

3.1.1. Growth of Nursery Plants

The tomato experiments carried out in 2016 and 2017 in Timisoara, Romania, revealed remarkable benefits of microbial BS applications already during nursery cultivation on the standard substrate mix used in the culture system. In both years, plant height and leaf area, determined as nondestructive indicators of plant performance at 43 days after sowing, were significantly increased by 29 to 100% (leaf area) and 29 to 74% (plant height) in response to BS application at the two leaves stage (Figure 1). However, in the two-year experimental period, neither the application of the *Bacillus* strain combination or the MCP treatment was associated with any consistent additional plant growth-promoting effect, as compared with the single-strain products.

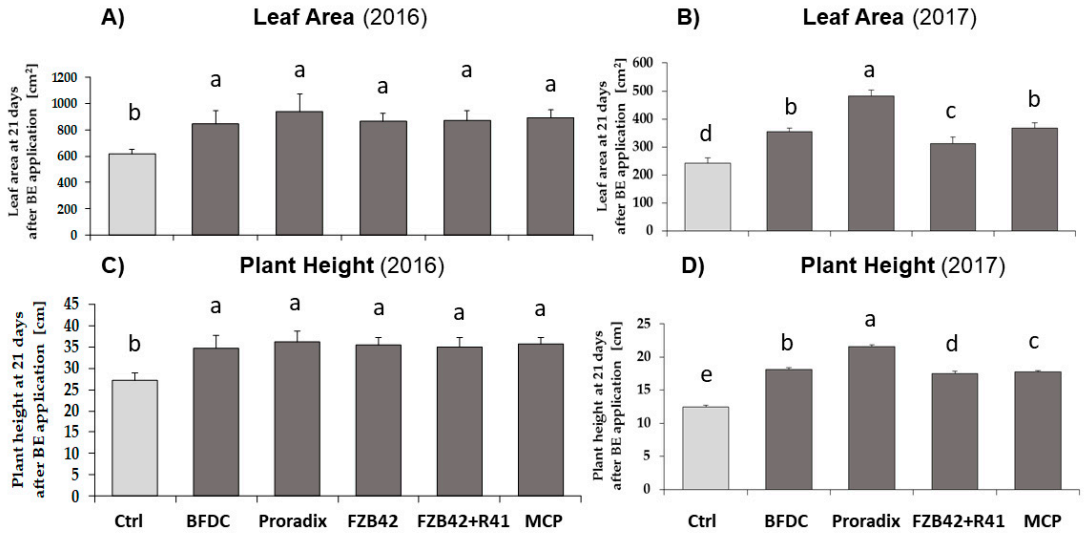

Figure 1. Leaf area (**A**,**B**) and plant height (**C**,**D**) of tomato cv Primadona F1 during the nursery phase at 43 days after sowing. Columns represent means ± standard deviation (n = 4 with each 10 plants as subsamples). Significant treatment differences (Tukey test, $p \leq 0.05$) are indicated by different characters.

3.1.2. Cumulative Fruit Yield

With approximately 70 t ha^{-1}, the control treatment did not reach the yield potential for greenhouse tomato production of 90 to 140 t ha^{-1} supplied with organic fertilizers [26]. By contrast, in both years, the cumulative yield of BS-treated plants was significantly increased by 39 to 84%, as compared with the untreated controls (Figure 2) with a yield range between 95 and 130 t ha^{-1}, which is in-line with the yield expectations. However, as compared with single strain inoculants, no additional yield improving effect was achieved by application of the *Bacillus* strains combination or the consortium product, and in 2016, even a significantly lower yield was recorded for the MCP treatment (Figure 2A).

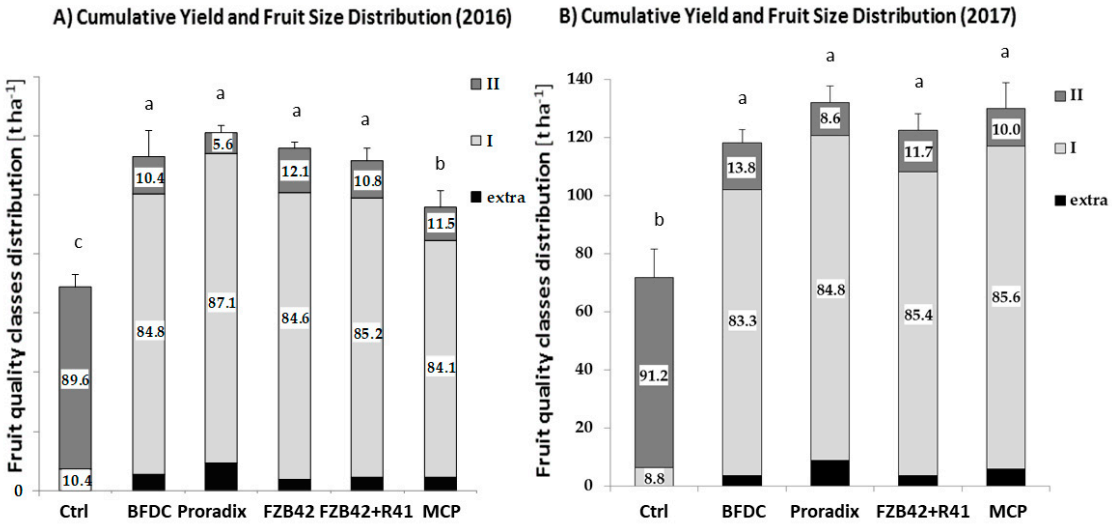

Figure 2. Cumulative yield (t ha^{-1}) and fruit size distribution (t ha^{-1} and %) for greenhouse tomato production with different BS treatments in 2016 (**A**) and 2017 (**B**). Quality classes (g FW fruit^{-1}): extra-large: >200 g, class I: 150–200 g, and class II: <150 g. Columns represent mean values of cumulative fruit yield ± SD (n = 4). Significant treatment differences in cumulative yield (Tukey test, $p \leq 0.05$) are indicated by different characters.

3.1.3. Distribution of Fruit Size and Seasonal Yield

In the control treatments, mainly class II fruits with a fresh biomass of less than 150 g were produced (approximately 90%) in both vegetation periods (Figure 2). By contrast, class I fruits (150–200 g) represented the dominant fruit size fraction (84–87%) in the BS-treated plants. The production of extra-large fruits (<200 g) was an exclusive feature of BS-treated plants (Figure 2). However, again no superior performance was detected for plants treated with MCP or the *Bacillus* strains combination as compared with the single-strain inoculants.

Regarding the seasonal yield distribution, BS inoculation promoted fruit ripening. This was reflected by 100 to 200% higher yield share during the main harvesting and marketing period in July as compared with untreated controls. This effect was similar for single strain and multiple inoculants as well (Figure 3).

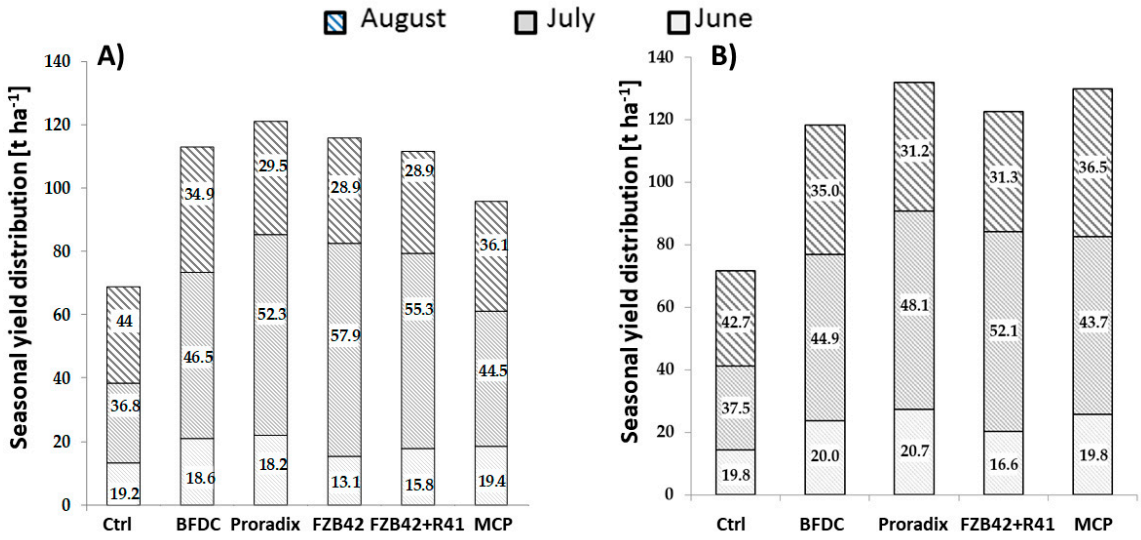

Figure 3. Seasonal yield share of tomato production over the whole harvesting period (June, July, and August) for different BS applications in Romania 2016 (**A**) and 2017 (**B**) (fruit yield in t ha^{-1}).

3.2. Case Study 2: Open Field Tomato Production with Drip Fertigation and Fertilizer Placement, Ramat Negev Desert, Israel, 2017

As expected, on the soil with alkaline pH 7.9 with low P availability (P_{Olsen} 5 mg kg^{-1} DM), P was the major limiting plant nutrient, indicated by continuously increasing shoot P concentration, plant biomass production and fruit yield with increasing levels of soluble TSP fertilization.

3.2.1. Vegetative Growth and Phosphate Status

In contrast to the greenhouse experiment in case study 1, only the *Bacillus–Trichoderma* combination product with Zn/Mn supplementation (CFB) and MCP treatments but not the single-strain inoculants exerted significant effects on early plant growth. CFB significantly increased shoot biomass production (24%) during vegetative growth (16 weeks after sowing) only in the variant with maximum P fertilization (50 kg P ha^{-1}), while exclusively, MCP significantly increased shoot biomass (113%) of the unfertilized control (Table 1). The effect of these products was not associated with corresponding changes in root biomass or root length. However, under maximum P supply, MCP significantly increased root length by 80% in comparison to the control in the investigated subsample without producing any effects on shoot growth (Table 1, Figure 4).

Table 1. Effect of banded P fertilization with DCD-stabilized ammonium sulfate and BS on the aboveground vegetative biomass production, root growth and shoot P status of tomato plants at 4 months after sowing, Ramat Negev, Israel. Data present means of four replicates. Statistical evaluation performed by two-way ANOVA. In each column, significant treatment differences (Tukey test, $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$, are indicated by different characters, n.s. = not significant, * = significant after Tukey–Kramer Honest Significant Difference (HSD) test).

P Dose	Biostimulant	Shoot	Root	Root	Shoot P	Shoot P
		Biomass		Length	Concentration	Content
kg ha^{-1}		g plant^{-1}	g plant^{-1}	m plant^{-1}	mg g^{-1}	mg plant^{-1}
0	Control	300e	50.5	54ab	0.51g	23e
0	Proradix	340e	57.0	55ab	0.61fg	31de
0	FZB42	350de	62.8	63ab	0.67efg	36cde
0	CFB	260e	62.0	71ab	0.70efg	27de
0	MCP	640bc	36.7	46b	0.72efg	69abcde
12.5	Control	420bcde	51.2	47ab	0.78defg	49cde
12.5	Proradix	630bcd	42.2	42b	0.83def	78abcde
12.5	FZB42	400cde	46.3	58ab	0.87cdef	53bcde
12.5	CFB	430bcde	65.7	59ab	0.93cde	59bcde
12.5	MCP	500bcde	78.1	60ab	0.97bcde	73abcde
50	Control	620bcd	44.5	45b	1.01bcde	103abcde
50	Proradix	670bc	62.4	62ab	1.07bcd	106abcd
50	FZB42	680ab	58.6	70ab	1.16bc	119abc
50	CFB	770a	43.3	43b	1.28b	148a
50	MCP	500bcde	67.0	81a	1.87a	139ab
		Analysis of Variance				
	df	Shoot	Root	Root Length	Shoot P	
		Fresh weight			concentration	content
Treatment	14	**	ns	*	***	***
block	3	ns	ns	ns	ns	ns

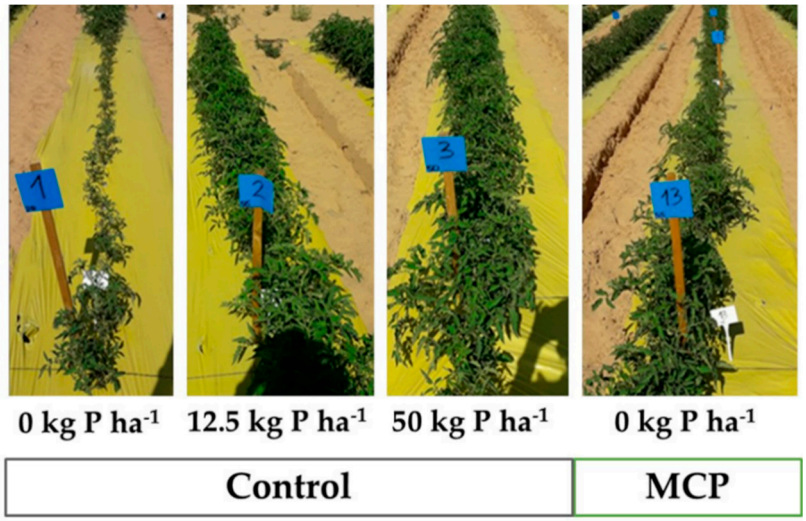

Figure 4. Effects of microbial consortia product (MCP) inoculation without external P fertilization on field performance of tomato plants at four months after sowing in comparison with different levels of P (triple superphosphate) fertilization in a field experiment at Ramat, Negev, Israel.

With a P shoot concentration of 0.05%, the control plants without P supply suffered from severe P limitation [27]. Accordingly, the application of TSP fertilizer increased the P nutritional status of the plants with a significant effect of 98% on P shoot concentration at the highest fertilization level in comparison with the unfertilized control, although the P tissue concentration was still suboptimal. A trend for a further improvement of the shoot P status was recorded for all BS treatments at all levels of P supply. However, a significant increase of 85% was obtained only for shoot P concentration of

the MCP treatment over the respective control when combined with the highest P dose of 50 kg ha^{-1} (Table 1). Analyzing the main effects of P dose and BS treatments revealed that both factors had a significant effect on shoot P ($F < 0.0001$ in both cases). The Tukey–Kramer separation test showed that MCP treatments were significantly different from all other BS treatments and CFB was significantly different as compared with the controls over all P doses (Table 1).

3.2.2. Fruit Yield

According to the improved P status, total fruit biomass significantly increased by 113% with a P supply of 12.5 kg ha^{-1} and by 232% with 50 kg P ha^{-1}, as compared with the unfertilized control (Table 2). The recorded BS effects on vegetative plant growth (Table 1) translated into a significant increase in final fruit biomass yield by 108% compared to the control only in the MCP variant without additional P fertilization, while no significant yield increase was recorded for the remaining inoculants. Similar trends were recorded for biomass and number of red fruits, although in this case the MCP effects were not significant. After supply of 12.5 kg P ha^{-1}, the yield effect of MCP was no longer significant compared with the untreated control and completely disappeared at the highest P fertilization level of 50 kg P ha^{-1} (see Table 2).

Table 2. Effect of banded P fertilization with DCD-stabilized ammonium sulfate and BS on total yield, fruit number per plant (No), and fruit quality distribution of drip-irrigated tomato between 4 and 5 months after sowing, Ramat Negev, Israel. Means represent four replicates. Statistical evaluation performed by two-way ANOVA. Significant treatment differences (Tukey test, $p \leq 0.05$ and Tukey–Kramer HD test) are indicated by different characters, n.s.: not significant, * = significant after Tukey–Kramer HD test).

P Dose	Bio Stimulant	Red Fruits		Green Fruits		Small Fruits		Total Yield	
kg ha^{-1}		t ha^{-1}	No	t ha^{-1}	No	t ha^{-1}	No	t ha^{-1}	No
0	Control	14.4e	13.5d	1.00	1.00	1.76	4.2	17.2b	187d
0	Proradix	21.7cde	19.8bcd	0.89	0.91	0.70	1.6	23.3ab	223bcd
0	FZB42	25.9 bcde	23.9abcd	0.69	0.69	0.72	1.6	27.3ab	262abcd
0	CFB	15.2de	16.1cd	0.74	0.72	1.53	3.7	17.5b	205cd
0	MCP	33.2bcde	27.5abcd	1.15	1.13	1.44	2.9	35.8a	315abcd
12.5	Control	36.1bcde	30.3abcd	0.30	0.25	0.34	0.7	36.7a	312abcd
12.5	Proradix	40.7bcd	27.8abcd	0.38	0.34	0.44	1.1	41.5a	293abcd
12.5	FZB42	33.1abcde	30.8abcd	0.00	0.00	0.00	0.0	33.1a	308abcd
12.5	CFB	31.9abcde	27.7abcd	0.29	0.31	0.57	1.6	31.7a	296abcd
12.5	MCP	45.5bc	32.4abc	0.29	0.34	0.49	1.3	46.3a	341abcd
50	Control	56.7a	40.6a	0.00	0.00	0.42	0.9	57.1a	415a
50	Proradix	57.0a	40.7a	0.35	0.44	0.43	1.1	57.8a	422a
50	FZB42	47.3abc	37.0ab	0.07	0.13	0.05	0.2	47.4a	374ab
50	CFB	52.9a	34.9ab	0.12	0.09	0.02	0.1	53.0a	350abc
50	MCP	50.8ab	36.1ab	0.08	0.09	0.13	0.6	51.1a	368ab
		Analysis of Variance							
	df	Red fruits		Green fruits		Small fruits		Total yield	
		Fresh weight	No	Fresh weight	No	Fresh weight	No	Fresh weight	No
Treatment	14	*	*	ns	ns	*	*	*	*
block	3	ns	ns	*	*	*	*	ns	ns

3.2.3. Microbiome Interactions

In face of the selective effects induced by the MCP treatments with respect to promotion of plant growth and yield formation, in case study II an amplicon sequencing approach was included to identify putative interactions of the BS with the soil microbiome, potentially related to the specific MCP effects.

Sequencing depth was adequate and as expected from highly complex environment root and soil bacterial communities (Figure S2). Although significant differences were found for the alpha diversity of root or soil samples treated with BS, no significant differences were found between the

examined treatments (two-way PERMANOVA nonparametric test) when the beta diversity of soil or root communities was analyzed using nonmetric multidimensional scaling (nMDS) calculated for the Bray–Curtis distance matrices (Figure S3).

In all treatments, the bacterial alpha diversity was lower at the rhizoplane as compared with the root-affected soil (Figure 5). In the control plants without BS inoculation, a significant decline in alpha diversity was recorded for the variant without P fertilization in comparison with the plants supplied with 12.5 kg P h^{-1}. This P fertilization effect on alpha diversity was not detectable in presence of the BS inoculants (Figure 5B). In the treatments without P supply, BS inoculation increased bacterial alpha diversity at the rhizoplane compared with the non-inoculated control, without significant differences between the different inoculants (Figure 5B). The BS inoculation effect on alpha diversity was similar to the effect of P fertilization. Moreover, the MCP inoculant significantly increased the alpha diversity also at the rhizoplane of the plants with P fertilization (Figure 5B). However, even in the root-affected soil samples, BS inoculation increased the bacterial alpha diversity with significant effects for FZB42 in the unfertilized control and for Proradix in the variant fertilized with 12.5 kg P ha^{-1} (Figure 5A).

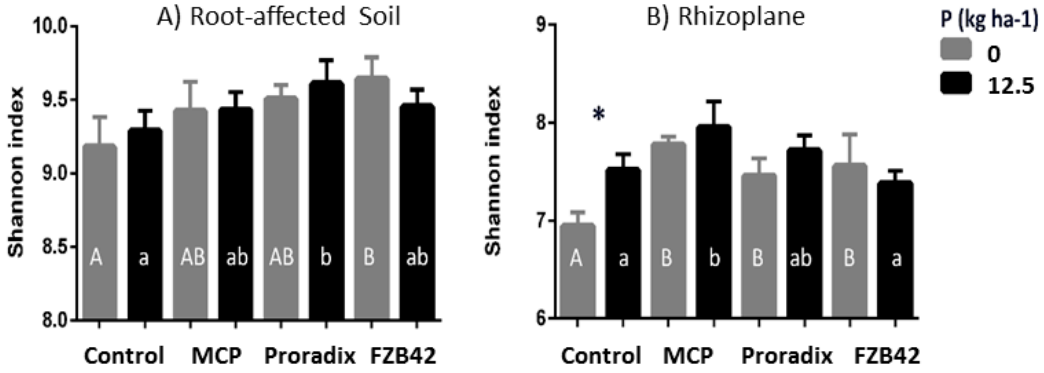

Figure 5. Shannon index for mean α-diversity of the bacterial communities in root-affected soil (**A**) and the rhizoplane (**B**) of drip-irrigated tomato plants with and without band placement of triple superphosphate (12.5 kg P ha^{-1}) and inoculation with different microbial biostimulants at 6 months after sowing, Negev Ramat, Israel. Significant differences (paired Student's *t*-test) in Shannon index between 0 and 12.5 kg P ha^{-1} dose of the same inoculant treatment are marked by *. Significant differences after pairwise comparison between inoculation treatments with the same P dose are indicated by different characters: A, B for 0 P, and a,b for 12.5 kg P ha^{-1}.

At the taxonomy level of class, *Acidobacteria*, *Nitrospira*, *Thermoleophilia*, and *Gemmatimonadetes* were detected exclusively in the root-affected soil but not at the rhizoplane, while *Flavobacteria* were detectable at the rhizoplane only. *Alphaproteobacteria* were dominant, both in the root-affected soil and in the rhizoplane–microbial communities. The abundance of *Actinobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, and *Sphingobacteriia* was higher at the rhizoplane as compared with the root-affected soil samples in noninoculated control plants, while *Bacilli* and *Deltaproteobacteria* declined (Figure 6A,B). *Bacilli, Alpha-*, *Beta-*, and *Gammaproteobacteria* increased at the rhizoplane of P-deficient plants but the abundance of *Actinobacteria* and *Deltaproteobacteria* declined (Figure 6A,B). The inoculation with biostimulants was associated with a decrease in the abundance of *Sphingobacteriia* at the rhizoplane, and this effect was particularly expressed in P-deficient plants with MCP treatment (Figure 6B), associated with plant growth-promoting and yield-increasing effects. By contrast, the abundance of *Flavobacteria* was particularly high in the respective treatment (Figure 6B).

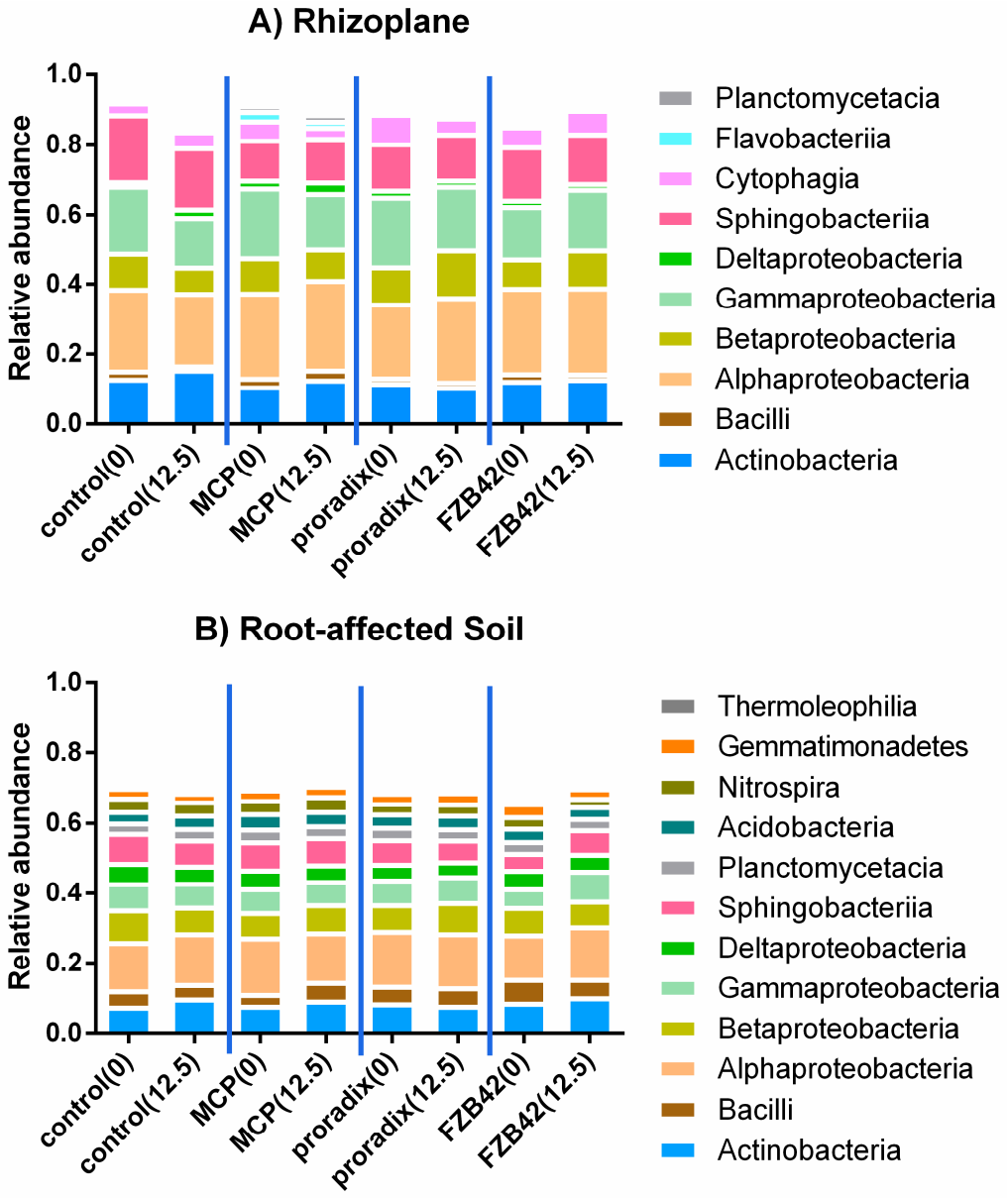

Figure 6. Relative abundance of different bacterial taxa at the rhizoplane (**A**) and in the root-affected soil (**B**) of drip-irrigated tomato plants with and without band placement of triple superphosphate (12.5 kg P ha^{-1}) and inoculation with different microbial BS at 6 months after sowing, Negev, Ramat, Israel.

4. Discussion

4.1. Case Study I: Large-Scale Greenhouse Experiments Timisoara, Romania, 2016/2017

In the large-scale greenhouse tomato production system in Romania, reproducible positive effects on the establishment of nursery plants, cumulative yield, fruit size distribution, and seasonal yield share were recorded in two successive vegetation periods.

4.1.1. Nursery and Vegetative Growth

In face of high nutrient contents of the organic nursery substrate (Supplementary Table S1), based on 45% composted cow manure amended with peat, soil and sand, the strong expression of BS-induced growth effects on nursery plants (Figure 1) was unexpected. However, in a comparative study on peat-based tomato nursery substrates, reduced plant biomass production and nutrient uptake was

associated with the application of manure fertilizers, frequently used in organic tomato production [28]. Maturation usually reduces the risk of phytotoxic effects of fresh manures and manure composts, while limitations in the availability of certain nutrients, such as Fe, Zn, and N, have been reported for mature composts [29,30]. Although the reasons for the suboptimal performance of the nursery plants in our studies are not entirely clear, the mitigation effect of BS applications is obvious (Figure 1) and may therefore, offer a perspective for optimization of nursery substrates frequently used also used in organic tomato production. Accordingly, for many of the microbial inoculants used in this study, root growth promoting and P-solubilizing properties are well documented [21,22,31–34]. The same holds true for priming effects against various abiotic and abiotic stresses [20,21,35–37] with protective effects also against potential substrate toxicities.

Inoculation with BS was performed during the nursery phase and just after transplanting into greenhouse culture. It remains to be established, whether the improved nursery plant performance after BS application (Figure 1), finally translated into the observed beneficial yield effects (Figure 2). Alternatively, this may be attributed to more direct effects, induced by long-lasting BS colonization during maturation of the host plants. Tomato is a plant species with documented ability to release root secretory acid phosphatases under P limitation [38] with potential to hydrolyze organic P forms abundant in manure-based fertilizers. Moreover, increased P deficiency-induced root extrusion of protons [39,40] can contribute to solubilization of acid-soluble mineral soil P forms. Strengthening and root growth promotion of nursery plants after BS inoculation may therefore improve the utilization of the applied organic fertilizers. On the other hand, phosphatase secretion and mobilization of sparingly soluble mineral phosphates, mycorrhizal helper functions, as well as contributions to N turnover and N fixation, are features also documented for the microbial BS used in the present study [11,15,32,33,41]. Therefore, in case of longer lasting rhizosphere survival, also direct contributions of the inoculants to plant growth promotion and nutrient acquisition from the organic fertilizers in the production phase are a likely scenario, at least in the 2017 experiment. For phytosanitary reasons, in this case, plant culture was performed in substrate containers, with a rooting volume restricted to 10 L. The basal substrate fertilization was dominated by a mixed hair/feather meal fertilizer (Monterra 13% N, 0.22% P; 10 g L^{-1} substrate), supplemented with mineral N, P, and K at a rate of 140, 70, and 149 mg L^{-1} substrate, respectively. Thus a better exploitation of the available rooting volume by BS-induced root growth promotion and improved utilization of the organic fertilizers as previously reported in the literature [9,11,23,42,43], would represent an advantage under the respective growth conditions. The same holds true for P acquisition in face of the moderate P fertilization level and low background P availability of the unfertilized substrate (Supplementary Table S1).

Organic fertilization was dominant also in the 2016 experiment and applied as a mixed guano/feather meal product (DIX-10N, 10% N, 1.3% P, 2 t ha^{-1}). Nevertheless, limitations in nutrient availability of the substrate seem to be unlikely in this case, since nutrient analysis of the greenhouse soil revealed high background levels of plant-available P and N_{min} (Supplementary Table S1). However, as an important challenge, in the 2016 experiment, the tomato plants showed symptoms of root rot induced by the soil-borne pathogen *Fusarium oxysporum* Schlecht f. sp. *radicis-lycopersici* Jarvis and Shoemaker. Additionally, increased larvae abundance of *Agriotes lineatus* L., that can feed on the roots of tomato plants was recorded as well. In this context, biocontrol properties and the ability to induce systemic resistance or improved plant vitality and root growth, as reported for the inoculated BS [20,37,41,44], could represent an additional advantage. The BS inoculants may contribute to compensation of pathogen-induced root damage, thereby determining the observed effects on plant growth promotion and yield formation. Nevertheless, independent of pathogen suppression, in all three scenarios described in case study I, microbial root growth stimulation and nutrient mobilization as documented features of the applied inoculants, would definitely represent a beneficial factor, either supporting nutrient acquisition under limited nutrient availability (in 2017) or by counteracting inhibition of root growth and activity due to nursery substrate toxicities or pathogen infection.

4.1.2. Generative Growth and Fruit Yield

The application of BS increased the individual fruit weight by 20 to 30% whereas the total fruit biomass production per plant was promoted even more strongly by approximately 40 to 75% (Supplementary Table S5). This finding suggests that BS application particularly increased the number of fruits per plant and to a lesser extent the growth of individual fruits. This may be attributed to beneficial effects on flowering and fruit setting as processes under hormonal control [45]. Experiments with exogenous application of plant growth regulators and measurements of internal changes in hormone concentrations, suggest an important role of auxins in this context [45–47]. This raises the question whether the well-documented potential of the selected inoculants for auxin production [21,48] or their interactions with plant hormonal balance might be involved in the observed BS-induced promotion of fruit setting and fruit growth. In experiments testing different single-strain and mixed BS, similar effects on tomato growth and yield formation have been recently reported by Oancea et al. [49]. Microbial BS based on *Azospirillum lipoferum* and *Brevibacillus parabrevis* proved to increase total marketable tomato yield by more than 10%. The authors speculated that the effects were due to accelerated vegetative growth and quicker development during the early growth of tomato plants. The fruits had the chance to ripe more rapidly, which improved the commercial fruit quality and the weight of marketable fruits since the earlier ripening of fruits ensures better competitiveness for the farmers, as similarly observed also in the present study (Figure 3). Although numerous studies show beneficial effects of microbial BS particularly on flowering, fruit setting and fruit development of tomato and other fruit crops [49–52], the underlying modes of action still remain to be elucidated.

Single Strains versus Microbial Consortia

Interestingly, fungal and bacterial BS of different phylogenetic origin (strains of *Penicillium*, *Bacillus*, and *Pseudomonas)* as well as single-strain inoculants versus microbial consortia exhibited very similar stimulatory effects on plant growth and yield formation (Figures 1 and 2). There was no indication for an improved performance of strain combinations in comparison with single strains, previously postulated as an advantage of consortium products in various literature reviews. As a possible explanation for this observation, the stress-protected nursery in small pots with a small soil volume, followed by protected greenhouse culture, may offer optimal conditions for effective root colonization by the selected microbial BS, as a prerequisite for the establishment of efficient plant-inoculant interactions in the rhizosphere. Environmental stress factors, such as temperature or pH extremes, limited or excess water supply, oxygen limitation, salinity, etc., were largely excluded. Under these conditions, the beneficial effects of BS inoculation may be limited rather by the genetically fixed response potential of the host plants than by the plant growth-promoting properties of the inoculants. Therefore, obviously maximum growth and yield responses, reaching the reported yield potential for organic greenhouse tomato production [26], were already induced by the single strain inoculants leaving no further scope for additional effects of combination products.

4.2. Case Study II: Open Field Tomato prOduction with Drip Fertigation and Fertilizer Placement, Ramat Negev Desert, Israel, 2017

A completely different scenario was observed under the more extreme environmental conditions in case study II. Although, similar to the experiments in Romania, nursery culture, and BS inoculation were performed under protected conditions, subsequent open field culture in the Negev desert was of course more challenging for plant growth. High temperatures and radiation intensities (daytime temperature 30–42 °C, radiation: 1000 W m^{-2}), lack of precipitation throughout the whole culture period, high soil pH, low soil fertility, and organic matter content as well as limited P availability represented major challenges in this production system (Supplementary Figure S1, Table S1). This may be related with induction of multiple stresses including nutrient deficiencies, limited plant-beneficial soil microbial activities, heat stress, excessive transpiration, and oxidative stress due to high light intensities. Although water and nutrients were supplied by fertilizer drip

fertigation and fertilizer placement, a drip irrigation system may be associated with some limitations under these challenging environmental conditions due to rapid evaporation and concentration of nutrient salts in the application zone.

4.2.1. Vegetative Plant Growth and Yield Responses

In contrast to the greenhouse experiments in Romania, only combination products successfully induced plant growth stimulation, while single strain inoculants were largely ineffective (Table 1). Stimulation of yield formation was observed exclusively for the MCP treatments, particularly under conditions of P limitation (Table 2), identified as limiting nutrient. With increasing levels of P supply, the P nutritional status, plant growth, and fruit yield increased, while the MCP effect finally disappeared (Table 2). Although tomato is a plant species with documented potential to acidify the rhizosphere under P-limited conditions [39,40], this effect was obviously not sufficient to mobilize significant amounts of acid soluble P forms on the alkaline soil. Even a further promotion of the acidification effect by placement of a stabilized ammonium fertilizer, leading to localized root proliferation and ammonium-induced proton extrusion [53], was not effective in this context. Only the additional MCP inoculation increased vegetative plant growth and fruit yield of plants without external P supply to a level comparable with a moderate P fertilization level of 12.5 kg P ha^{-1} (Table 2). MCP inoculation of plants supplied with 12.5 kg P ha^{-1} even resulted in a yield increase not significantly different from the fully fertilized positive control with 50 kg P ha^{-1} (Table 2), reflected also by a similar P nutritional status in both treatments (Table 1). This finding points to a significant contribution of the MCP inoculants to P acquisition of the host plant. Although no BS-induced promotion of root length development with beneficial effects on spatial P acquisition was detectable (Table 2), local root growth stimulation close to the ammonium fertilizer depot, as recently described also by Nkebiwe et al. [22], cannot be excluded. In this context, it must be taken into consideration that the related locally restricted root growth modifications are not easily detected by excavation of whole root systems under field conditions. Moreover, the MCP inoculant provided a wide range of microbial genera (*Bacillus*, *Pseudomonas*, *Trichoderma*, *Penicillium*, and *Aspergillus*) with documented P-solubilizing properties [32,33,54]. Phosphate limitation is also associated with a rapid inhibition of N-uptake and -assimilation [35,36]. This effect may be particularly expressed in case of ammonium-dominated fertilization due to lower soil mobility of ammonium compared with nitrate. In this context, the presence of *Nitrobacter*, *Nitrosomonas*, and *Azotobacter* in the MCP inoculant may contribute to improved N availability by nitrification and N_2 fixation. Compared with single strain inoculants, the MCP product may therefore offer a larger flexibility, by providing a whole range of root growth-promoting and/or P-solubilizing strains, which may differ in their sensitivity to environmental stress factors. This will increase the probability for the expression of beneficial effects on crop performance, even under the more adverse environmental conditions in the selected culture system.

Interestingly also the *Bacillus*/*Trichoderma* combination product, amended with Zn and Mn (CFB), exerted some growth-promoting effects during vegetative plant development (Table 1). However, in contrast to the MCP product, these effects were restricted to the treatments with the highest mineral P fertilization (50 kg P ha^{-1}). On alkaline soils, limited micronutrient availability (e.g., Zn, Mn, and Fe) is frequently a growth-limiting factor. Particularly external and internal Zn availability can be further reduced by high levels of P fertilization [55,56] and Zinc limitation is associated with shoot growth depression and impairment of defense responses against oxidative stress [27]. Although, the P nutritional status of the plants supplied with 50 kg P ha^{-1} was not extraordinarily high (Table 1), the application mode via band placement implicates high local P concentrations. Therefore, a certain degree of Zn limitation may also represent a problem in the present study on the alkaline pH 7.9 soil in the treatments with the highest level of P supply, required to overcome low soil P availability, and mitigated by supplementation of Zn with CFB inoculation.

4.2.2. Interactions with the Soil Microbiome

In face of the significant and highly selective plant growth-promoting and yield-increasing effects of MCP inoculation in case study II (Tables 1 and 2) we decided to characterize also interactions with the soil microbiome in comparison with the ineffective single-strain inoculants. The aim of these investigations was to identify potential indirect plant growth-promoting modes of action via changes in soil bacterial communities. An amplicon sequencing approach revealed a lower alpha diversity of bacterial communities at the rhizoplane as compared with the root-affected soil between the plant rows (Figure 5). Similar effects have been reported also in previous studies [57–59] and may reflect the selective impact of the root on microbial communities. Accordingly, plant-, and even cultivar-specific patterns in the composition of root exudates and rhizodeposits, as well as specific root-induced modifications of physicochemical rhizosphere properties have been described [60]. The rhizoplane alpha diversity was also lower under P limitation compared to variants with P fertilization (Figure 5). This may reflect specific adaptive modifications of the rhizosphere conditions by the host plant towards improved P acquisition, such as rhizosphere acidification, increased release of organic metal chelators and phenolic compounds, phosphatases, chitinases, etc. [61], with a selective impact on rhizosphere-microbial communities. Interestingly, inoculation of the microbial biostimulants increased the alpha diversity at the rhizoplane under P limitation, which was particularly expressed in the MCP treatments (Figure 5), and may be regarded as consequence of an improved plant P nutritional status in these variants (Table 2).

However, increased rhizoplane–microbial diversity may also increase the probability for the establishment of beneficial plant-microbial interactions and some apparent changes were detectable at the taxonomy level of class. Particularly in the MCP treatments, with the highest plant growth-promoting and yield-increasing potential, a distinctly increased abundance of *Sphingobacteriia* was recorded at the rhizoplane as compared with the root-affected soil (Figure 6). Sphingobacteriia are known as salinity indicators [62,63], and increased accumulation of salts in the rhizosphere is characteristic for plants exposed to high transpiration and/or drought [64], as stress factors affecting also the investigated tomato production system under desert conditions (Supplementary Figure S1). High water evaporation due to high temperatures, water uptake by the plants and the comparatively low water supply by drip irrigation are factors increasing the concentrations of minerals in the rhizosphere soil solution, and may promote the accumulation of salts, as indicated by a higher abundance of salinity-adapted Sphingobacteriia at the rhizoplane. Interestingly, this effect was at least partially reverted in response to microbial inoculation, particularly in the MCP variants (Figure 6A). For various PGPRs, arbuscular mycorrhizae, and also plant roots, the ability to increase aggregate stability and the water-holding capacity of the rhizosphere soil by secretion of exopolysaccharides and glomalin is well-documented [65–67]. The resulting higher rhizosphere hydration would consequently reduce the salt concentrations in the rhizosphere soil solution and may explain the lower abundance of *Sphingobacteriia* as salinity indicators. Moreover, higher water content in the rhizosphere would also improve the nutrient availability under drought stress conditions. Particularly for members of the genera *Pseudomonas* and *Bacillus* as dominant bacterial groups in the MCP inoculant, exopolysaccharide production with the potential to promote drought and salinity tolerance of host plants but also mycorrhizal helper functions have been identified [11,41,67,68]. These inoculants might therefore contribute to the superior plant growth-promoting potential of MCP under the investigated culture conditions. *Flavobacteria* represented another bacterial group, exclusively detectable at the rhizoplane particularly in MCP-inoculated plants without external P supply (Figure 6B). For *Flavobacteria*, PGPR properties [69–71] and a role as drought stress protectants [72] have been reported in the literature.

However, with the exception of bacilli in the P-fertilized control (Figure 6A), there was no indication for an increased abundance of bacterial groups that are reported to be present in the MCP inoculant, suggesting indirect effects of the BS products on the microbiome composition, rather than a direct introduction of the respective genera by BS inoculation. Moreover, the evident increase in alpha diversity at the rhizoplane of BS-treated plants suggests this indirect effect to be selective

for the root-associated microbiome, particularly under conditions of low P availability. However, the microbiome analysis was conducted approximately four months after inoculation and therefore direct interactions in the earlier growth stages with plant growth-promoting effects of a beneficial consortium (MCP) on the low fertility soil with limited microbial activity cannot be excluded. For a more accurate examination, inoculant tracing would be required during the culture period, which would be a particularly challenging task for consortium products, due to the large number of inoculated strains. However, fertigation-based culture systems may offer a suitable approach for comparing the effectiveness and economy of starter application versus repeated inoculations. Particularly with subsurface fertigation tubes, it should be possible to perform effective repeated inoculations of the rooting zone even in later stages of plant development. This could provide important information to the question whether BS treatments are more suitable to support the sensitive phase of crop establishment with indirect effects on later plant development or whether a longer lasting rhizosphere establishment would be more effective.

5. Conclusions

The results of the present study clearly indicate a plant growth-promoting and yield-increasing potential of various fungal and bacterial BS in tomato production. Although the modes of action are not entirely clear, the results suggest that direct plant growth-promoting activities providing improved start conditions already during early growth stages enabled the plants to utilize a given nutrient supply more effectively and increased the stress resistance, translating into tremendous yield increases particularly under conditions of suboptimal nutrient acquisition. Furthermore, stimulation of flowering or fruit setting and fruit size development, have been observed in response to the BS application during early growth, indicating long-lasting effects on plant development. The data also demonstrate that the performance of microbial consortia is not always superior over single-strain inoculants. In accordance with the concept of an improved adaptive potential postulated for MCPs, a clear advantage in comparison with single-strain inoculants was recorded in the drip-irrigated tomato production system in the Negev desert, exposed to various environmental challenges, such as high temperature, limitations in water availability, low soil fertility, and high soil pH. By contrast, superior MCP performance was not detectable under the more controlled and less challenging conditions in the greenhouse production system in Romania, where all inoculants showed similar plant growth-promoting effects. Since two different tomato cultivars, characteristic for the two different production systems, were used for the experiments, cultivar-specific responsiveness to BS inoculation cannot be completely excluded as an alternative explanation for the observed differences in the expression of BS effects. However, for the selected inoculants, a broad efficiency spectrum in combination with a wide range of different crops has been reported in the literature [20,21,23,31,73]. These findings do not suggest highly selective strain- and cultivar-specific plant–BS interactions at least for the investigated single strain inoculants.

The selective plant growth-promoting MCP effects in the drip-irrigated tomato production system with limited P supply were also associated with some characteristic modifications of rhizosphere–bacterial communities. MCP inoculation increased the bacterial alpha diversity at the rhizoplane of P-limited tomato plants. The abundance of *Sphingobacteriia*, known as salinity indicators, declined while the population of potentially plant growth-promoting and drought stress-protective *Flavobacteria* increased. Although the observed effects suggest some MCP-mediated interactions with the expression of stress-adaptive processes also related with alterations of the rhizosphere microbiome, it is still not clear whether these effects must be regarded as a cause or rather as a consequence of an improved stress adaptation of the MCP-inoculated tomato plants.

Nevertheless, the presented findings support the hypothesis that the use of microbial consortia can serve as a tool to increase the efficiency and reproducibility of BS-assisted strategies for crop production, particularly under challenging environmental conditions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/2/105/s1, Figure S1: Climate parameters (air temperature 0.5 m above ground, relative air humidity, and radiation) during the experiment in Ramat Hanegev, Israel; Figure S2: Sample rarefaction curves for soil (a) and root (b) samples; Figure S3: Nonparametric multidimensional scaling (nMDS) analysis of root-affected soil (a) and rhizoplane (b) microbiome; Table S1: Substrate properties; Table S2: Fertilization management; Table S3: Application of biostimulants; Table S4: Plant protection; Table S5: Fresh biomass of individual fruits and cumulative fruit biomass production per plant for greenhouse tomato production in Romania with different BS treatments in 2016; Table S6: Three-way ANOVA (P dose, biostimulants, and blocks) on effects of banded P fertilization with DCD-stabilized ammonium sulfate and biostimulants on vegetative shoot and root biomass, root length, P nutritional status, total yield, fruit number per plant (No), and fruit quality distribution of drip-irrigated tomato, Ramat Negev, Israel; Table S7: OTU tables for all soil/rhizoplane samples. In root affected soil, a total of 7815 OTUs were found: 7200 Bacterial, 252 Archaeal, and 363 unassigned. At the rhizoplane, there were 5218 OTUs overall: 4931 Bacterial, 37 Archaeal, and 250 unassigned. Sample names indicate the treatment (Control, MCP, Proradix, FZB421), phosphate level (0, 12.5), and replicate (Rep A–D).

Author Contributions: Conceptualization, G.P., M.W., G.N., K.B., U.Y., D.M., A.B.-T., and N.B.; Investigation, G.P., A.S.F., B.Z., R.S., A.Z., J.K.-C., R.E.; Resources K.D. and N.B.; Writing—Original Draft Preparation, K.B.; Writing—Review & Editing, G.N., J.K.-C., A.Z., A.B.-T., G.P., M.W., B.Z., U.L., K.D., and N.B.; Project Administration G.N. and U.L.; Funding Acquisition U.L., M.W., and G.N.

Funding: Funding was provided by the European Community's FP7 Programme/2007–2013 under grant agreement No. 312117 (BIOFECTOR).

Acknowledgments: This work is dedicated in memory to h.c. Karl Fritz Lauer († 2018) as mentor and initiator of the German-Romanian research cooperation and his outstanding contributions to planning, setup, and evaluation of the experiments and all issues related with plant protection. Thanks to the Eyal Klein and Hishtil Company for producing tomato seedlings grown both with and without application of biostimulants in the nursery and to Michal Amichai, Ofer Guy and Einan Sagy from Ramat Hanegev R&D Station for conducting the field experiment.

Conflicts of Interest: The authors declare no conflicts of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results, that could be construed as a potential conflict of interest.

References

1. Nuti, M.; Giovannetti, G. Borderline Products between Bio-fertilizers/ Bio-effectors and Plant Protectants: The Role of Microbial Consortia. *J. Agric. Sci. Technol. A* **2015**, *5*, 305–315. [CrossRef]
2. Yakhin, O.I.; Lubyanov, A.A.; Yakhin, I.A.; Brown, P.H. Biostimulants in Plant Science: A Global Perspective. *Front. Plant Sci.* **2017**, *7*, 1–32. [CrossRef] [PubMed]
3. Michalak, I.; Dmytryk, A.; Schroeder, G.; Chojnacka, K. The Application of Homogenate and Filtrate from Baltic Seaweeds in Seedling Growth Tests. *Appl. Sci.* **2017**, *7*, 230. [CrossRef]
4. Rouphael, Y.; Giordano, M.; Cardarelli, M.; Cozzolino, E.; Mori, M.; Kyriacou, M.C.; Bonini, P.; Colla, G. Plant- and Seaweed-Based Extracts Increase Yield but Differentially Modulate Nutritional Quality of Greenhouse Spinach through Biostimulant Action. *Agronomy* **2018**, *8*, 126. [CrossRef]
5. Wilson, H.T.; Amirkhani, M.; Taylor, A.G. Evaluation of Gelatin as a Biostimulant Seed Treatment to Improve Plant Performance. *Front. Plant Sci.* **2018**, *9*, 1–11. [CrossRef] [PubMed]
6. Amirkhani, M.; Netravali, A.N.; Huang, W.; Taylor, A.G. Investigation of Soy Protein—Based Biostimulant Seed Coating for Broccoli Seedling and Plant Growth Enhancement. *HortScience* **2016**, *51*, 1121–1126. [CrossRef]
7. Tilman, D.; Fargione, J.; Wolff, B.; Antonio, C.D.; Dobson, A.; Howarth, R.; Schindler, D.; Schlesinger, W.H.; Simberloff, D.; Swackhamer, D. Forecasting Agriculturally Driven Global Environmental Change. *Science* **2001**, *292*, 281–284. [CrossRef]
8. Xu, H.-L.; Wang, R.; Mridha, M.A.U. Effects of Organic Fertilizers and a Microbial Inoculant on Leaf Photosynthesis and Fruit Yield and Quality of Tomato Plants. *J. Crop Prod.* **2001**, *3*, 173–182. [CrossRef]
9. Esitken, A.; Yildiz, H.E.; Ercisli, S.; Figen Donmez, M.; Turan, M.; Gunes, A. Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. *Sci. Hortic.* **2010**, *124*, 62–66. [CrossRef]
10. Abbasi, M.K.; Musa, N.; Manzoor, M. Mineralization of soluble P fertilizers and insoluble rock phosphate in response to phosphate-solubilizing bacteria and poultry manure and their effect on the growth and P utilization efficiency of chilli (*Capsicum annuum* L.). *Biogeosciences* **2015**, *12*, 4607–4619. [CrossRef]

11. Thonar, C.; Duus, J.; Lekfeldt, S.; Cozzolino, V.; Kundel, D.; Kulhánek, M.; Mosimann, C.; Neumann, G.; Piccolo, A.; Rex, M.; et al. Potential of three microbial bio-effectors to promote maize growth and nutrient acquisition from alternative phosphorous fertilizers in contrasting soils. *Chem. Biol. Technol. Agric.* **2017**, 1–16. [CrossRef]
12. Hartmann, A.; Rothballer, M.; Schmid, M. Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant Soil* **2008**, *312*, 7–14. [CrossRef]
13. Lopez-Cervantes, J.; Thorpe, D.T. Microbial Composition Comprising Liquid Fertilizer and Processes for Agricultural Use. Agrinos, AS. United States Patent Application Publication US 2013/0255338 A1, 3 October 2013.
14. Sekar, J.; Raj, R.; Prabavathy, V.R. Microbial Consortial Products for Sustainable Agriculture: Commercialization and Regulatory Issues in India. In *Agriculturally Important Microorganisms*; Singh, H.B., Sarma, B.K., Keswani, C., Eds.; Springer Science+Business Media: Singapore, 2016; pp. 107–131.
15. Higa, T.; Parr, J.F. *Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment*; International Nature Farming Research Center Atami: Okinawa, Japan, 1994; pp. 1–16.
16. Hadar, Y. Suppressive compost: When plant pathology met microbial ecology. *Phytoparasitica* **2011**, *39*, 311–314. [CrossRef]
17. Carvalhais, L.C.; Muzzi, F.; Tan, C.-H.; Hsien-Choo, J.; Schenk, P.M. Plant growth in Arabidopsis is assisted by compost soil-derived microbial communities. *Front. Plant Sci.* **2013**, *4*, 1–15. [CrossRef] [PubMed]
18. Bashan, Y. Inoculants of plant growth-promoting bacteria for use in agriculture. *Biotechnol. Adv.* **1998**, *16*, 729–770. [CrossRef]
19. Edathil, T.T.; Manian, S.; Udaiyan, K. Interaction of multiple VAM fungal species on root colonization, plant growth and nutrient status of tomato seedlings. *Agric. Ecosyst. Environ.* **1996**, *59*, 63–68. [CrossRef]
20. Fröhlich, A.; Buddrus-Schiemann, K.; Durner, J.; Hartmann, A.; Rad, U. Von Response of barley to root colonization by *Pseudomonas* sp. DSMZ 13134 under laboratory, greenhouse, and field conditions. *J. Plant Interact.* **2012**, *7*, 1–9. [CrossRef]
21. Borris, R. Towards a New Generation of Commercial Microbial Disease Control and Plant Growth Promotion Products. In *Principles of Plant-Microbe Interactions*; Lugtenberg, B., Ed.; Springer International Publishing: Basel, Switzerland, 2015; pp. 329–337.
22. Nkebiwe, P.M.; Weinmann, M.; Müller, T. Improving fertilizer—Depot exploitation and maize growth by inoculation with plant growth-romoting bacteria: From lab to field. *Chem. Biol. Technol. Agric.* **2016**, *3*, 1–16. [CrossRef]
23. Mpanga, I.K.; Dapaah, H.K.; Geistlinger, J.; Ludewig, U. Soil Type-Dependent Interactions of P-Solubilizing Microorganisms with Organic and Inorganic Fertilizers Mediate Plant Growth Promotion in Tomato. *Agronomy* **2018**, *8*, 213. [CrossRef]
24. Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley, B.B.; Parks, D.H.; Robinson, C.J.; et al. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl. Environ. Microbiol.* **2009**, *75*, 7537–7541. [CrossRef]
25. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Peña, A.G.; Goodrich, K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [CrossRef] [PubMed]
26. Hornischer, U.; Koller, M. Biologischer Anbau von Tomaten. Bioland Beratung GmbH Kompetenzzentrum Ökolandbau Niedersachsen FiBl/KÖN/Bioland, Germany. 2005. Available online: https://www.bioland.de/fileadmin/dateien/HP_Dokumente/Verlag/MB_Tomaten.pdf (accessed on 20 December 2018).
27. Marschner, P. *Marschner's Mineral Nutrition of Higher Plants Third Edition*; Elsevier Academic Press: San Diego, CA, USA, 2012; ISBN 9780123849052.
28. Nielsen, K.L.; Thorup-Kristensen, K. Growing media for organic tomato plantlet production Archived at http://orgprints.org/00001606. *Acta Hortic.* **2001**, *664*, 183–188. [CrossRef]
29. Tiquia, S.M.; Tam, N.F.Y.; Hodgkiss, I.J. Effects of composting on phytotoxicity of spent pig-manure sawdust litter. *Environ. Pollut.* **1996**, *93*, 249–256. [CrossRef]
30. Loecke, T.D.; Liebman, M.; Cambardella, C.A.; Richard, T.L. Corn Growth Responses to Composted and Fresh Solid Swine Manures. *Crop Sci.* **2004**, *44*, 177–184. [CrossRef]

31. Gulden, R.H.; Vessey, J.K. Penicillium bilaii inoculation increases root-hair production in field pea. *Can. J. Plant Sci.* **2000**, *80*, 801–804. [CrossRef]
32. Leggett, M.; Newlands, N.; Greenshields, D.; West, L.; Inman, S.; Koivunen, M. Maize yield response to a phosphorus-solubilizing microbial inoculant in field trials Maize yield response to a phosphorus-solubilizing microbial inoculant in field trials. *J. Agric. Sci.* **2014**, 1–15. [CrossRef]
33. Nkebiwe, P.M.; Neumann, G.; Müller, T. Densely rooted rhizosphere hotspots induced around subsurface NH_4^+-fertilizer depots: A home for soil PGPMs? *Chem. Biol. Technol. Agric.* **2017**, *4*, 1–16. [CrossRef]
34. Sánchez-Esteva, S.; Muñoz, B.G.; Jensen, L.S.; De Neergaard, A.; Magid, J. The effect of *Penicillium bilaii* on wheat growth and phosphorus uptake as affected by soil pH, soil P and application of sewage sludge. *Chem. Biol. Technol. Agric.* **2016**, *3*, 1–11. [CrossRef]
35. Von Rad, U.; Mueller, M.J.; Durner, J. Evaluation of natural and synthetic stimulants of plant immunity by microarray technology. *New Phytol.* **2005**, *165*, 191–202. [CrossRef]
36. Meng, Q. Characterization of *Bacillus amyloliquefaciens* Strain BAC03 in Disease Control and Plant Growth Promotion. Ph.D. Thesis, Institute of Plant Pathology, Michigan State University, East Lansing, MI, USA, 2014.
37. Xie, S.; Jiang, H.; Ding, T.; Xu, Q.; Chai, W.; Cheng, B. Bacillus amyloliquefaciens FZB42 represses plant miR846 to induce systemic resistance via a jasmonic acid-dependent signalling pathway. *Mol. Plant Pathol.* **2018**, *19*, 1612–1623. [CrossRef]
38. Suen, P.-K.; Zhang, S.; Sun, S.S.M. Molecular characterization of a tomato purple acid phosphatase during seed germination and seedling growth under phosphate stress. *Plant Cell Rep.* **2015**, *34*, 981–992. [CrossRef] [PubMed]
39. Pilbeam, D.J.; Cakmak, I.; Marschner, H.; Kirkby, E.A. Effect of withdrawal of phosphorus on nitrate assimilation and PEP carboxylase activity in tomato. *Plant Soil* **1993**, *154*, 111–117. [CrossRef]
40. Neumann, G.; Römheld, V. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. *Plant Soil* **1999**, *211*, 121–130. [CrossRef]
41. Yusran, Y.; Roemheld, V.; Mueller, T. Effects of *Pseudomonas* sp. "Proradix" and Bacillus amyloliquefaciens FZB42 on the Establishment of AMF Infection, Nutrient Acquisition and Growth of Tomato Affected by Fusarium oxysporum Schlecht f.sp. radicis-lycopersici Jarvis and Shoemaker. In Proceedings of the International Plant Nutrition Colloquium XVI, California Digital Library, University of California, Davis, CA, USA, 31 July 2009.
42. Chen, J. The Combined Use of Chemical and Organic Fertilizers and/or Biofertilizer for Crop Growth and Soil Fertility. In Proceedings of the International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use, Land Development Department, Bangkok, Thailand, 16–20 October 2006; pp. 1–11.
43. Xu, H.; Wang, R.; Mridha, A.U. Effects of Organic Fertilizers and a Microbial Inoculant on Leaf Photosynthesis and Fruit Yield and Quality of Tomato Plants Effects of Organic Fertilizers and a Microbial Inoculant on Leaf Photosynthesis and Fruit Yield and Quality of Tomato Plants. *J. Crop Prod.* **2008**, *3*, 173–182. [CrossRef]
44. 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*, e68818. [CrossRef]
45. Srivastava, A.; Handa, A.K. Hormonal Regulation of Tomato Fruit Development: A Molecular Perspective. *J. Plant Growth Regul.* **2005**, *24*, 67–82. [CrossRef]
46. Alam, S.M. Fruit Yield of Tomato as Affected by NAA Spray. *Asian J. Plant Sci.* **2002**, *1*, 1–5. [CrossRef]
47. Sarkar, M.D.; Jahan, M.S.; Kabir, M.H.; Kabir, K.; Rojoni, R.N. Flower and Fruit Setting of Summer Tomato Regulated by Plant Hormones. *Appl. Sci. Rep.* **2014**, *7*, 117–120. [CrossRef]
48. Buddrus-Schiemann, K.E.M. Wirkung des biologischen Pflanzenstärkungsmittels Proradix® (*Pseudomonas fluorescens*) auf das Wachstum von Gerste (*Hordeum vulgare* L. cv. Barke) und auf die bakterielle Gemeinschaft in der Rhizosphäre. Ph.D. Thesis, Ludwig-Maximilians-University, Munich, Germany, 2008.
49. Oancea, F.; Raut, I.; Zamfiropol-Cristea, V. Influence of soil treatment with microbial plant biostimulant on tomato yield and quality. *Agric. Food* **2017**, 156–165.
50. Ownley, B.H.; Seth, D.; Hamilton, C.; Dee, M. Effects of Plant-Growth-Promoting-Rhizobacteria on Biomass, Flowering, and YIELD of Field Tomatoes. In *Extension—Vegetable Production*; University of Tennesee, Institute of Agriculture: Knoxville, TN, USA, 1999.

51. Murphy, J.F.; Reddy, M.S.; Ryu, C.; Kloepper, J.W.; Li, R. Rhizobacteria-Mediated Growth Promotion of Tomato Leads to Protection Against Cucumber mosaic virus. *Phytopathology* **2003**, *93*, 1301–1307. [CrossRef]
52. Karakurt, H.; Kotan, R.; Dadaşoğlu, F.; Aslantaş, R.; Şahİn, F. Effects of plant growth promoting rhizobacteria on fruit set, pomological and chemical characteristics, color values, and vegetative growth of sour cherry (*Prunus cerasus* cv. Kütahya). *Turk. J. Biol.* **2011**, *35*, 283–291. [CrossRef]
53. Jing, J.; Rui, Y.; Zhang, F.; Rengel, Z.; Shen, J. Localized application of phosphorus and ammonium improves growth of maize seedlings by stimulating root proliferation and rhizosphere acidification. *Field Crops Res.* **2010**, *119*, 355–364. [CrossRef]
54. Sharma, S.B.; Sayyed, R.Z.; Trivedi, M.H.; Gobi, T.A. Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus* **2013**, *2*, 1–14. [CrossRef] [PubMed]
55. Marschner, H.; Schropp, A. Vergleichende Untersuchungen über die Empfindlichkeit von 6 Unterlagensorten der Weinrebe gegenüber Phosphat- induziertem Zink-Mangel. *Vitis* **1977**, *16*, 79–88.
56. Cakmak, I.; Marschner, H. Mechanism of phosphorus-induced zinc deficiency in cotton. III. Changes in physiological availability of zinc in plants. *Physiol. Plant.* **1987**, *70*, 13–20. [CrossRef]
57. Marilley, L.; Vogt, G.; Blanc, M.; Aragno, M. Bacterial diversity in the bulk soil and rhizosphere fractions of Lolium perenne and Trifolium repens as revealed by PCR restriction analysis of 16S rDNA. *Plant Soil* **1998**, *198*, 219–224. [CrossRef]
58. Berg, G.; Zachow, C.; Lottmann, J.; Go, M.; Costa, R.; Icrobiol, A.P.P.L.E.N.M. Impact of Plant Species and Site on Rhizosphere-Associated Fungi Antagonistic to Verticillium dahliae Kleb. *Appl. Environ. Microbiol.* **2005**, *71*, 4203–4213. [CrossRef] [PubMed]
59. Wang, P.; Marsh, E.L.; Ainsworth, E.A.; Leakey, A.D.B.; Amy, M.; Schachtman, D.P. Shifts in microbial communities in soil, rhizosphere and roots of two major crop systems under elevated CO_2 and O_3. *Sci. Rep.* **2017**, 1–12. [CrossRef] [PubMed]
60. 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]
61. Neumann, G.; Römheld, V. The release of root exudates as affected by the plant physiological status. In *The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface*, 2nd ed.; Pinton, R., Varanini, Z., Nannipieri, Z., Eds.; CRC Press: Boca Raton, FL, USA, 2007; pp. 23–72.
62. Lydell, C.; Dowell, L.; Sikaroodi, M.; Gillevet, P.; Emerson, D. A Population Survey of Members of the Phylum Bacteroidetes Isolated from Salt Marsh Sediments along the East Coast of the United States. *Microb. Ecol.* **2004**, *48*, 263–273. [CrossRef]
63. Lucas, J.A.; Villaraco, A.G.; Ramos, B.; Garcia-Cristobal, J.; Algar, E.; Gutierrez-ma, J. Structural and functional study in the rhizosphere of *Oryza sativa* L. plants growing under biotic and abiotic stress. *J. Appl. Microbiol.* **2013**, *115*, 218–235. [CrossRef] [PubMed]
64. Riley, D.; Barber, S.A. Salt accumulation at the soybean (*Glycine max.* (L.) merr.) root-soil interface. *Soil Sci. Soc. Am. J.* **1970**, *34*, 154–155. [CrossRef]
65. Huang, B.; North, G.B.; Nobel, P.S. Soil Sheaths, Photosynthate Distribution to Roots, and Rhizosphere Water Relations for Opuntia ficus-indica. *Int. J. Plant Sci.* **1993**, *154*, 425–431. [CrossRef]
66. Kaushal, M.; Wani, S.P. Plant-growth-promoting rhizobacteria: Drought stress alleviators to ameliorate crop production in drylands. *Ann. Microbiol.* **2015**, *66*, 35–42. [CrossRef]
67. Vurukonda, S.S.K.P.; Vardharajula, S.; Shrivastava, M.; Skz, A. Rhizosphere 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]
68. Kasim, W.A.; Gaafar, R.M.; Abou-ali, R.M.; Omar, M.N.; Hewait, H.M. Effect of biofilm forming plant growth promoting rhizobacteria on salinity tolerance in barley. *Ann. Agric. Sci.* **2016**, *61*, 217–227. [CrossRef]
69. Bloemberg, G.V.; Lugtenberg, B.J.J. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Curr. Opin. Plant Biol.* **2001**, *4*, 343–350. [CrossRef]
70. Mayak, S.; Tirosh, T.; Glick, B.R. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.* **2004**, *42*, 565–572. [CrossRef] [PubMed]
71. Soltani, A.-A. Plant Growth Promoting Characteristics in Some *Flavobacterium* spp. Isolated from Soils of Iran. *J. Agric. Sci.* **2010**, *2*, 106–115. [CrossRef]

72. Gontia-Mishra, I.; Sapre, S.; Sharma, A.; Tiwari, S. Amelioration of drought tolerance in wheat by the interaction of plant growth-promoting rhizobacteria. *Plant Biol.* **2016**, *18*, 992–1000. [CrossRef]
73. Harman, G.E.; Howell, C.R.; Viterbo, A.; Chet, I.; Lorito, M.; Pathology, P. Trichoderma Species—Opportunistic, Avirulent Plant Symbionts. *Nat. Rev. Microbiol.* **2004**, *2*, 43–56. [CrossRef] [PubMed]