OPEN ACCESS
phytobiomesjournal.org
Phytobiomes Journal
A Transdisciplinary Journal of Sustainable Plant Productivity

RESEARCH

Biotic and Abiotic Stress Factors Induce Microbiome Shifts and Enrichment of Distinct Beneficial Bacteria in Tomato Roots

Burkhardt Flemer,[1] Sneha Gulati,[1] Alessandro Bergna,[2,3] Manuela Rändler,[2] Tomislav Cernava,[2] Katja Witzel,[1] Gabriele Berg,[2,4,5] and Rita Grosch[1,†]

[1] Leibniz Institute of Vegetable and Ornamental Crops (IGZ) e.V., Plant-Microbe Systems Grossbeeren, Germany
[2] Graz University of Technology, Institute of Environmental Biotechnology, Graz, Austria
[3] Austrian Centre of Industrial Biotechnology, Graz, Austria
[4] Leibniz Institute for Agricultural Engineering and Bioeconomy, Microbiome Biotechnology, Potsdam, Germany
[5] University of Potsdam, Institute for Biochemistry and Biology, Potsdam, Germany

Accepted for publication 23 August 2022.

ABSTRACT

Crops are often simultaneously threatened by abiotic and biotic stress factors but the stress response of the plant holobiont is not well understood, despite the high importance of this response to ensure future plant production. Therefore, the aim of this study was to assess the impact of individual and combined abiotic (ionic and osmotic) and biotic (*Verticillium dahliae* and *Fusarium oxysporum*) stress factors on plant performance and on the bacterial composition of the root endosphere in tomato. Structure and function of the microbiota was analyzed by 16S ribosomal RNA gene amplicon sequencing and a complementary cultivation approach, including in vitro and in vivo assays. Under all stress conditions, tomato growth and photosynthetic activity was reduced. Combined abiotic stressors with *F. oxysporum* but not with *V. dahliae* infection led to an additive negative effect on plant performance. All stress conditions induced a microbiome shift, and changed the relative abundance of phyla such as *Firmicutes* and classes of *Proteobacteria*. Endophytes identified as *Bacillus*, *Paenibacillus*, and *Microbacterium* spp. showed tolerance to abiotic stress conditions and plant beneficial effects. Stressor-specific enrichments of beneficial bacteria in the root were discovered (e.g., *Paenibacillus* in roots infected with *F. oxysporum* and *Microbacterium* in roots infected with *V. dahliae*). Interestingly, endophytes that were able to promote plant growth were obtained only from roots exposed to individual biotic and combined abiotic and biotic stress conditions but not individual abiotic stressors. Our study revealed stressor-specific enrichment of beneficial bacteria in tomato roots, which has implications for novel plant protection strategies.

Keywords: combined stress factors, *Fusarium*, microbiome, osmotic stress, salinity, soilborne pathogens, *Verticillium*

[†]Corresponding author: R. Grosch; grosch@igzev.de

B. Flemer, S. Gulati, G. Berg, and R. Grosch contributed equally to this work.

Current address of B. Flemer: Institute of Clinical Molecular Biology, Kiel University and University Medical Center Schleswig-Holstein, Campus Kiel, Rosalind-Franklin-Strasse 12, 24105 Kiel, Germany.

Author contributions: B.F. provided study design, data analysis, manuscript writing, and supervision; S.G. provided performance of the plant experiments and assessment of plant characteristics, isolation of bacteria and preparation for their classification, performance of in vitro and in vivo tests, and manuscript writing (introduction, Materials and Methods); A.B. provided data analysis; M.R. provided isolation of bacteria and support in vitro assays for plant growth promotion; K.W. provided data analysis; T.C. provided study design and supervision; G.B. provided study design, supervision, and manuscript writing; and R.G. provided study design, supervision, and manuscript writing.

The demand for agricultural products is steadily increasing due to the growing world population. However, the discrepancy between the required food and the global agricultural output is alarming and has dramatically worsened (Fróna et al. 2019). In addition, intensive agricultural management practices expose plants to enhanced stress conditions due to abiotic factors such as osmotic stressor and salinity, with negative effects on crop yield and quality (Francini and Sebastiani 2019). Due to climate change, crops were more frequently subjected to both abiotic and biotic stress factors, which has led to an increase in the frequency and severity of disease outbreaks

Funding: Funding of the project was provided by the Indian Council of Agricultural Research (ICAR) (ICAR-IF 2014-15 and F. No. 29-1/2009-EQR/Edn [Pt. III]), the German Federal Ministry of Food and Agriculture (BMEL), and the Brandenburgian Ministry of Science, Research and Culture, Germany.

e-Xtra: Supplementary material is available online.

The author(s) declare no conflict of interest.

(Pandey et al. 2017). Approximately 50 and 30% of yield loss in recent years in global agricultural production systems have been attributed to abiotic and biotic stress conditions, respectively (Kumar and Verma 2018).

An abiotic stress factor of increasing importance for agriculture is salinity; yield loss has been predicted to surpass US$27 billion per year worldwide (Qadir et al. 2014). Elevated levels of salt (electrical conductivity [EC] > 4 dS/m) such as Ca^{2+}, Mg^{2+}, SO_4^{2-}, and NaCl reduce the ability of most plants to take up water. Similar effects on cellular and metabolic processes are observed under dry conditions (osmotic stressor) (Fathi and Tari 2016). Therefore, salinity exerts a dual-type of stressor on plants by acting as (i) ionic stressor and (ii) osmotic stressor (Munns 2005). Osmotic stressors are considered to be one of the major climatic constraints for crop yield (Lesk et al. 2016). The limited availability of nutrients in dry conditions negatively affects plant development and growth, and results in oxidative stress (Ma et al. 2019). Nutrients used for plant growth and biomass production generally require water for their solubility and translocation (Singh and Singh 2004). Furthermore, biotic stress conditions induced by pests and pathogens have been estimated to provoke 20 to 30% of global yield losses (Delgado-Baquerizo et al. 2020). Hence, plant protection has an important role in securing the growing food demand. Diseases due to soilborne fungal pathogens such as *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium dahliae* in tomato (*Solanum lycopersicum* L.) are difficult to control. Tomato is the most important vegetable in the world (Akköprü and Demir 2005), and both pathogens cause vascular wilt disease by entering the host via the roots and subsequent colonization of the vascular system. As a result, the vascular system is plugged with fungal material and host reaction products, restricting water transport to the upper part of the plants (Essarioui et al. 2016; Song et al. 2020). The pathogens can survive for a long time in soil by employing different strategies. *V. dahliae* forms microsclerotia and *F. oxysporum* forms chlamydospores as survival structures. Current agricultural practice, usually characterized by high inputs of synthetic pesticides, does not provide the means to efficiently reduce the yield loss caused by soilborne pathogens (Trivedi et al. 2017). Therefore, novel plant and stress protection strategies are urgently required.

Plant-associated microorganisms are involved in plant protection against abiotic and biotic stress factors (Xu and Coleman-Derr 2019). Members of the plant microbiota facilitate nutrient uptake by the plant, improve nutrient use efficiency, interact with phytohormones, and induce systemic resistance in plants, resulting in promotion of plant growth and health (Brader et al. 2014; Santoyo et al. 2016). Endophytes are a central part of the plant microbiota and belowground tissues are commonly associated with a higher number of endophytes compared with aboveground parts (Hardoim et al. 2015). Endophytes with inherent capability of salt or osmotic tolerance and plant growth promotion (PGP) traits are expected to be useful for sustainable treatments to support plants in stress mitigation (Prittesh et al. 2020). Previous findings indicate that a fraction of belowground or root-colonizing endophytes may be selected by the plant from the rhizosphere and contribute to functional traits of the phytobiome (İnceoğlu et al. 2012). For example, abiotic stressors have been shown to weaken plant immune function and, thus, lead to an increased susceptibility against pathogens (Bostock et al. 2014; Suzuki et al. 2014). In modern plant production systems, cultivated plants are often simultaneously exposed to both abiotic and biotic stress factors. Little is known about their response to such conditions.

Therefore, this study aimed to evaluate the impact of individual and combined abiotic (ionic and osmotic) and biotic (*F. oxysporum* f. sp. *lycopersici* and *V. dahliae*) stress factors on tomato growth, on the plant's sensitivity to pathogens, and on the composition of the tomato root endosphere microbiota. We focused on bacterial endophytes and, especially, on their plant-beneficial parts that promote plant growth or confer tolerance to biotic and abiotic stress factors (Bergna et al. 2018). The mentioned stress factors can induce several metabolic alterations in the plants and, eventually, influence plant growth and resilience to diseases (Ma et al. 2020). It is assumed that these alterations in plant response also affect plant microbiota composition and function. Because of the similar effects induced by salinity and osmotic stressors on metabolic processes such as nutrient and water uptake, comparable impacts of these stress factors on the root endosphere microbiota were expected. Furthermore, the colonization of the vascular system of the plant by both pathogens has an influence on the host response to the pathogens that may change as well the root microbiota composition. In addition, we hypothesized that (i) combined abiotic and biotic stress conditions should result in synergistic effects on plant growth and susceptibility to pathogens and (ii) the plant will enrich microbes in the root endosphere adapted to the respective stress conditions imposed, including microbes that support the plant in stress mitigation. Hence, plants exposed to stress factors can serve as a source for beneficial microbes useful for plant growth promotion (PGP) and protection.

MATERIALS AND METHODS

Design of the plant experiment. Cultivation of the tomato cultivar Hildares (Hild Samen GmbH, Marbach, Germany) was performed as described previously (Buhtz et al. 2017). Briefly, tomato seeds were surface sterilized by immersion in 0.7% sodium hypochlorite solution for 3 min and rinsed seven times with sterile distilled water. Surface-sterilized seeds were sown in trays filled with sterile quartz sand (0.5- to 1.0-mm particle size) and maintained in a growth chamber (York, Mannheim, Germany) until the two-leaf stage. Seedlings (one per pot) were transferred into pots (10 by 10 by 12 cm) containing a mixture of coarse sand (2.0- to 3.0-mm particle size), quartz sand (0.5- to 1.0-mm particle size), and sandy soil in a ratio of 9:2:2. The sandy soil was obtained from an uncultivated field site at the Institute of Vegetable and Ornamental Crops (IGZ), Grossbeeren, Germany (52°33′N, 13°22′E). The seedlings were grown under a 16-h photoperiod (light at 400 μmol m^{-2} s^{-1}) with day and night temperatures of 25 and 20°C, respectively, and a relative humidity (RH) of 72 to 80%. Each treatment included 20 replicates (one plant per replicate) which were arranged in a Latin-square design. The pots were watered regularly according to the water-holding capacity of the substrate (40%) by weighing the pots. A nutrient solution, consisting of $Ca(NO_3)_2 \cdot 4H_2O$ at 590.4 μg ml^{-1}, KNO_3 at 253.0 μg ml^{-1}, KH_2PO_4 at 68.1 μg ml^{-1}, $MgSO_4 \cdot 7H_2O$ at 246.5 μg ml^{-1}, H_3BO_3 at 2.9 μg ml^{-1}, $MnCl_2 \cdot 4H_2O$ at 1.8 μg ml^{-1}, $ZnSO_4 \cdot 7H_2O$ at 0.2 μg ml^{-1}, $CuSO_4 \cdot 5H_2O$ at 0.1 μg ml^{-1}, $Na_2MoO_4 \cdot 2H_2O$ at 0.1 μg ml^{-1}, and ferric EDTA at 41.5 μg ml^{-1}; EC = 2.0 dS m^{-1}; and pH 5.8, was regularly used to water the plants.

Plants were harvested 3 weeks after stress induction, corresponding to the 16- to 30-leaf stage, depending on the type of stress imposed. Before harvest, the net photosynthesis rate was measured at day cultivation conditions (25°C, 72% RH) and a photosynthetically active radiation of 400 μmol m^{-2} s^{-1} using the gas analyser LICOR 6400. At harvest, shoot and root biomass were recorded. Roots were also used for metagenomic DNA extraction and isolation of bacterial root endophytes.

Induction of abiotic and biotic stress conditions. For abiotic stress experiments, plants were exposed to following conditions: salinity, referred to as ionic stressor (I) in the following sections, osmotic stressor (O), and combined ionic-osmotic stressors (IO). The stress conditions were induced at the two- to three-leaf stage

of the tomato plants after transplanting the seedlings into the pots. Control plants (C) were watered with half-strength nutrient solution (see above, EC = 2 dS m^{-1}). Ionic stress condition was introduced by watering with nutrient solution supplemented with NaCl (EC = 5 dS m^{-1}) until the salinity of the substrate reached 10 dS m^{-1}. This level of salinity in the substrate was achieved within 7 days of watering, which was then maintained throughout the experiment. Osmotic stress condition was induced by adjusting and maintaining 40% of saturated water content using nutrient solution. Combined ionic and osmotic stress factors (IO) were induced by watering with NaCl-supplemented nutrient solution (EC = 5 dS m^{-1}) to 40% of saturated water content and until the salinity in the pots reached 10 dS m^{-1}. This level was achieved after watering with saline nutrient solution for 9 days and was maintained thereafter.

For the biotic stress experiments, plants were inoculated with 20 ml of spore suspension of 10^7 spores ml^{-1} for *V. dahliae* (V) or 10^5 conidia ml^{-1} for *F. oxysporum* (F) at the one- to two-leaf stage. The success of root colonization by the pathogens was confirmed by PCR with extracted root DNA using respective pathogen-specific primers and conditions described earlier (Buhtz et al. 2017; Ma et al. 2010).

Preparation of the fungal pathogen inoculum. *Verticillium dahliae*. The pathogen *V. dahliae* GU060637 was maintained on potato dextrose agar (PDA) (Merck, Darmstadt, Germany). Mycelial suspensions for plant inoculation were prepared as described previously (Buhtz et al. 2017). Briefly, 100 ml of sucrose-sodium nitrate (SSN) media was inoculated with six agar discs (size = 5 mm) and shaken at 25°C for 1 week. Afterward, 200 ml of fresh SSN media was added to the flask and the fungal suspension was cultivated for two additional weeks under described conditions. The mycelium was filtrated, blended, and centrifuged for 2 min at 13,000 × *g*. The pellet was rinsed twice by resuspension in sterile distilled water. The inoculum density was determined using a Thoma chamber and adjusted to 10^7 spores ml^{-1}.

Fusarium oxysporum. Isolate Fol007 was grown on PDA for 5 days, then overlayed with sterile distilled water. Within this time period, the implemented isolate produced mainly microconidia. To separate the microconidia from the mycelium, the suspension was passed through filter paper. The number of microconidia was counted using a Thoma chamber and adjusted to 10^5 conidia ml^{-1}.

High-throughput amplicon sequencing of 16S ribosomal RNA gene fragments. To analyze the impact of individual and combined abiotic and biotic stress factors on the composition of bacterial root endophytes of tomato, amplicon sequencing of the 16S ribosomal RNA (rRNA) gene was carried out. For each treatment, 5 of the 20 plants were merged to continue analysis, with four replicates per treatment. Root DNA (500 mg of root material) was extracted using the FastDNA Spin kit for soil (MP Biomedicals, Irvine, CA, U.S.A.) according to the manufacturer's instructions. Bead beating was performed on a Precellys 24 tissue homogenizer (Bertin GMBH, Frankfurt am Main, Germany) following general operation recommendations. Purity and concentration of the extracted DNA was evaluated with a Nanodrop1000 (Thermo Fisher Scientific, Waltham, MA, U.S.A.). For the generation of gene fragment amplicons via PCR, the following reaction mix (10 µl) was used: 5 µl Taq&GO (2×), 0.1 µl of 10 µM each primer (F515 and R806) (Caporaso et al. 2011), 0.25 µl of 50 µM mPNA (ggcaagtgttcttcgga) (PNA Bio, Newbury Park, CA, U.S.A.), 0.25 µl of 10 µM pPNA (ggctcaaccctggacag) (PNA Bio), and 3.3 µl of molecular biology-grade water. The following PCR program was used: 94°C for 3 min; 35 cycles at 94°C for 45 s, 50°C for 60 s, and 72°C for 90 s; and a final extension for 10 min at 72°C. Library preparation and sequencing on an Illumina MiSeq instrument was conducted by a commercial sequencing provider (GATC Biotech, Germany). Data analysis of 16S rRNA gene fragment sequences was performed using QIIME 2 (version 2019.4) (Bolyen et al. 2019). Demultiplexing was conducted with the "demux emp-paired" plugin. Quality control and chimera removal was performed with the DADA2 pipeline (dada2 denoise-paired) (Callahan et al. 2016). Taxonomical assignment was executed using SILVA 16S database (version 128; feature-classifier classify-sklearn).

Isolation of bacterial endophytes from roots. Bacterial endophytes were isolated from tomato roots 3 weeks after stress treatment. Following surface sterilization in 70% ethanol for 30 s and in 2.5% sodium hypochlorite for 3 min, roots were rinsed seven times with sterile water. Only roots for which surface sterility was confirmed by imprinting the roots on tryptic-soy agar (TSA) (Carl Roth GmbH, Karlsruhe, Germany) were included in further analysis. The root samples were cut into nine equally sized sections, which were used for isolation of bacteria and total community DNA extraction. The root sections were homogenized in a Mixer Mill MM40 (Retsch GmbH, Haan, Germany) in 2-ml Eppendorf tubes containing six metal beads (2.4 mm) until a fine powder was obtained. Then, 250 mg of homogenized root material was diluted in 0.1 M phosphate-buffered saline (PBS) and 100 µl of the suspension was spread on TSA (10%, supplemented with cycloheximide at 100 mg $liter^{-1}$ and thiabendazole at 50 mg $liter^{-1}$). Agar plates were incubated for 4 to 6 days at 20°C. Colonies were counted for the full plate and all colonies from one-quarter of each plate as well as bacteria with distinct visible morphological characteristics were picked and restreaked on fresh TSA until pure cultures were obtained. Stocks were prepared by growing the isolates in tryptic-soy broth (TSB) (Carl Roth GmbH) and subsequently storing them at −80°C in 25% glycerol.

Identification of cultivated bacterial root endophytes by 16S rRNA gene sequencing. First classification of isolated bacterial root endophytes was done using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (Maldi Biotyper, Bruker Daltonics, Germany) as described previously (Elsawey et al. 2020), to include only unique strains in further analyses. The isolates were further identified by amplification and sequencing of the 16S rRNA gene. Briefly, a colony PCR (25 µl of total volume) was carried out in the following reaction mix: 12.5 µl of 2× MyTaq Mix (Bioline, Luckenwalde, Germany), 2.5 µl each of forward (27F: 5′-AGA GTTT GAT CMT GGC TCAG-3′) and reverse (1492R: 5′-GRT ACC TTG TTA CGA CTT-3′) primers (Kocher et al. 1989; Weisburg et al. 1991), 5.5 µl of molecular biology-grade H_2O, and 2 µl of glycerol stock as DNA template. The PCR was carried out in a Biometra thermocycler as follows: initial denaturation for 5 min at 95°C; 30 cycles of 15 s at 95°C, 15 s at 55°C, and 30 s at 72°C; followed by a final extension for 10 min at 72°C. Successful amplification was confirmed using agarose gel electrophoresis. PCR products were sequenced by Eurofins (Eurofins, Berlin, Germany) using the forward primer 27F. Only isolates for which sequences of at least 500 bp in length were obtained were kept for further analysis. Classification of reads was done using BLAST (Altschul et al. 1990) and the NCBI nucleotide database. To assess the α-diversity of isolates, operational taxonomic units (OTUs) of 97% sequence similarity were obtained using USEARCH (Edgar 2010).

In vitro characterization of endophytes. To assess whether cultivable tomato root endophytes adapted to the respective stress factors, the obtained isolates were tested for tolerance to ionic and osmotic stress factors in vitro. Traits tested for in vitro included antagonism against *F. oxysporum*; production of siderophores, indole-3-acetic acid (IAA), chitinase, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase; and phosphate solubilization.

Ionic stress tolerance was assessed by growing bacterial isolates on nutrient broth medium (Carl Roth GmbH) supplemented with

0, 2.5, 5, 7.5, and 10% NaCl (Sigma-Aldrich, Taufkirchen, Germany). Osmotic stress tolerance was assessed on nutrient broth medium supplemented with 0, 10, 20, and 30% polyethylene glycol 6000 (Sigma-Aldrich). The isolates were grown in flat-based 96-well plates (Sarstedt, Nümbrecht, Germany) with three replicates of each isolate and incubated for 7 days at 28°C. The growth rate of the bacteria was determined by measurement of the optical density (OD) of the broth at 600 nm (OD_{600}) after 7 days.

The ability of the isolates to produce IAA was determined based on the colorimetric method described by Gordon and Weber (1951) as well as Khabbaz et al. (2015), with some modifications. A preculture was initiated by inoculating 5 ml of TSB with overnight cultures of the respective isolate and then incubating for 48 h at 28°C and 175 rpm. The precultures (100 μl) were inoculated with 3 ml of supplemented nutrient broth (SNB) medium (Setlow and Kornberg 1969) and incubated for a further 72 h in the dark. The IAA levels were determined from the supernatant as described by Tang and Bonner (1948).

The production of siderophores was qualitatively detected using the method described previously (Schwyn and Neilands 1987). Briefly, bacterial isolates were grown on agar plates containing the ferric complex chromeazurol S at 28°C for 7 days. Isolates with an orange-to-yellow halo were considered to be siderophore producers.

The ability to solubilize phosphate was tested on Pikovskaya agar medium (Pikovskaya 1948). Isolates were spotted onto the agar and incubated for 7 to 10 days at 28°C. Phosphate-solubilization activity was indicated by the development of clearing zones around the bacterial colonies (Naik et al. 2008).

ACC deaminase production was tested as described by Dworkin and Foster (1958). Isolates were grown in Dworkin Foster medium which contained ACC as sole nitrogen source. Precultures of bacterial isolate in SNB (5 ml) were incubated for 16 h at 28°C and 150 rpm; then, 10 μl of the respective culture was used to inoculate 200 μl of the modified DF medium in 96-well plates. The production of ACC deaminase was estimated qualitatively after another incubation for 16 h at 28°C and 150 rpm by measuring the OD_{600} before and after incubation. An increased OD_{600} after incubation indicated growth and, thus, the ability to utilize ACC as nitrogen source.

The antagonistic potential to inhibit mycelial growth of *F. oxysporum* was tested using a dual-culture assay. TSA agar plates (10%) were streaked with the bacteria along four orthogonal lines in the center. Then, the plates were incubated for 24 h at 28°C. Subsequently, agar plugs (diameter = 5 cm) with *F. oxysporum* were placed between the streaked lines. After another incubation for 96 h at 28°C, clearing zones indicated inhibition of fungal growth.

Extracellular chitinolytic activity of the endophytes was determined by the use of colloidal chitin agar medium, which consisted of 10% TSA supplemented with 4% (wt/vol) colloidal chitin. After an incubation for 5 to 10 days at 28°C, clearing zones around the bacterial colonies indicated the production of extracellular chitinases (Nagpure and Gupta 2013).

The number of isolates with functional traits tested in vitro was calculated in relation to the total number of endophytes obtained from the roots of plants exposed to the respective stress conditions.

PGP assay in vivo. All root-derived bacterial isolates were tested for their ability to promote plant growth under growth conditions as described above. For this, tomato plants (Hildares) were transferred to pots at the one- to two-leaf stage and arranged in a randomized block design, with four replicates for each tested isolate and three plants per replicate. Bacterial isolates were grown overnight at 28°C in 3 ml of TSB and centrifuged for 5 min at 4,000 × *g*; then, the supernatant was discarded and the pellet was resuspended in 5 ml of 0.1 M PBS. This procedure was repeated three times to wash the cells. The density of bacterial cells was measured using a spectrophotometer at 600 nm and plants were inoculated with 1 ml of bacterial suspension with 10^6 cells ml^{-1}. Control plants were inoculated with 1 ml of 0.1 M PBS. Plants were harvested 1 week after inoculation with bacterium.

Testing selected endophytes on plant growth under individual and combined stress conditions. The root endophytes were scored based on their tolerance to osmotic and ionic stress and the potential for PGP in vitro and in vivo. Each in vitro and in vivo trait counted as one toward the rating. The six highest-scoring isolates were tested for PGP under individual and combined abiotic and biotic stress factors. For this, surface-sterilized seeds (50 seeds) were coated by vortexing the seeds with 50 μl of the bacterial suspension at 10^8 cells ml^{-1} prior to seeding in sterile quartz sand. The tomato plants were cultivated as described above. At the one- to two-leaf stage, plants were treated again with 5 ml of bacterial solution (10^8 cells ml^{-1}). The biotic stress conditions in plants were induced by inoculation of 20 ml of a conidial suspension of *V. dahliae* (10^7 conidia ml^{-1}) at the two- to three-leaf stage. Additional ionic stress condition (I) in the biotic-stressed plants was induced after 24 h of pathogen inoculation by regularly watering with nutrient solution, EC of 5.0 dS m^{-1} and pH 5.8. Under combined abiotic and biotic stress treatments, plants were exposed to salinity stressor after 24 h of pathogen inoculation by regularly watering with nutrient solution, EC of 5.0 dS m^{-1} and pH 5.8. The plants were harvested after 21 days of exposure to stress conditions.

Statistical analysis. Statistical analysis was carried out using R version 3.5.0 (R Core Team). Data were tested for normality and comparisons were carried out using Dunnett's test or Dunn's test for normal and not-normal data, respectively. When applicable, Fisher's exact test was used together with Cramer's V (Cv) and the odds ratio (Or), which were reported in addition to the *P* value. Comparisons were made between individual abiotic or biotic treatments with controls (i.e., F, V, I, O, and IO versus C) and between combined treatments and their respective abiotic control (e.g., IF and IV versus I). Statistically differentially abundant amplicons between these groups were detected using DESeq2 (Love et al. 2014) using raw counts and the default procedure described in the package's vignette. Analyses of α- and β-diversity was carried out using rarified sequence counts (i.e., random subsamples of sequencing reads were taken in a way that, for the sample set, each sample had the same number of sequencing reads). The analyses of α-diversity were carried out using the package vegan using functions diversity and species number (Oksanen et al. 2020). Results for the Gini-Simpson and Shannon-Wiener indices were reported using the expected number of species calculated with the exponential function of R's base package. Phylogenetic trees and UniFrac distances were obtained using QIIME2 (Bolyen et al. 2019). Statistical analyses on distance matrices were carried out using the function adonis of the vegan package.

RESULTS

Effects of biotic and abiotic stress factors on plant characteristics. Plant growth (root and shoot) was significantly reduced by individual biotic (*F. oxysporum* and *V. dahliae)* and abiotic (ionic and osmotic) stress conditions compared with controls 3 weeks posttreatment (wpt), with the exception of individual *F. oxysporum* stress factor on shoot biomass (Table 1). Treatment of plants under ionic or osmotic stress conditions with *F. oxysporum* led to further significant reduction of shoot and root mass compared with individual ionic or osmotic stress treatment (IF versus I, OF versus O), whereas this was not the case for *V. dahliae* (Table 1). Simultaneous

treatment of tomato plants with biotic stress under combined abiotic conditions had no additional negative effect on plant growth (root and shoot mass, shoot length) (IOF versus IO, IOV versus IO).

Using the LI-6400 Photosynthesis System (LI-COR Biosciences, Lincoln, NB, U.S.A.) we also measured net photosynthesis rate 3 wpt. Tomato leaf net photosynthesis rate did not change significantly under individual ionic stress conditions compared with controls. Contrastingly, a decrease in leaf net photosynthesis rate was observed for tomato plants infected with the pathogens (*F. oxysporum* or *V. dahliae*) and for plants subjected to osmotic (O) and combined abiotic stress factors (IO) (Table 1). An additive effect on photosynthetic activity was found under ionic and both abiotic stressors (IO) with *F. oxysporum* (IF and IOF) while combined abiotic stress factors with *V. dahliae* (IV and IOV) did not lead to reduced leaf net photosynthesis rate.

Composition of the bacterial root endosphere microbiota. 16S rRNA gene fragment sequencing (Illumina MiSeq, 2 × 300 bp) of the four replicates per treatment (i.e., 48 sequencing libraries in total) resulted in 1.38×10^5 sequences per replicate on average. One replicate from treatment OF was removed due to low sequence read count (118 reads). Across all samples, reads were primarily classified as *Alphaproteobacteria* (33.5% of all assigned reads), *Betaproteobacteria* (19.6%), *Gammaproteobacteria* (14.7%), *Firmicutes* (11%), and *Bacteroidetes* (6.1%) (Fig. 1A). The relative abundance of several bacterial phyla in tomato roots was significantly ($P < 0.05$, DESeq) decreased under ionic stress conditions (candidate division WPS, *Planctomycetes*, *Verrucomicrobia*) and inoculation with *V. dahliae* (*Chlamydiae*, *Deltaproteobacteria*, *Firmicutes*, *Planctomycetes*, and *Verrucomicrobia*) whereas the relative abundance of other taxa increased under ionic

TABLE 1
Plant parameters of Hildares tomato plants 3 weeks after stress treatment[x]

Treatment[y]	Shoot fresh mass (g/plant)		Root fresh mass (g/plant)		Photosynthesis rate (µmol CO_2/m s)	
	Mean	P^z	Mean	P^z	Mean	P^z
C	17.3	–	5.4	–	21.1	–
F	14.3	0.157	3.2	<0.0001	12.3	<0.0001
V	11.6	0.003	3.7	0.021	15.2	0.009
I	7.6	<0.0001	2.8	<0.0001	18.3	0.370
IF	4.7	0.030	1.0	<0.0001	9.3	0.000
IV	9.4	0.104	3.0	0.989	18.2	0.993
O	8.8	<0.0001	2.7	0.001	14.3	0.004
OF	7.4	0.037	1.3	<0.0001	16.3	0.182
OV	7.9	0.224	2.4	0.184	14.5	0.969
IO	7.9	<0.0001	1.9	<0.0001	12.6	<0.0001
IOF	7.3	0.723	1.3	0.330	9.7	0.170
IOV	7.4	0.805	2.0	0.426	16.2	0.071

[x] Plants were grown in pots and subjected to individual and combined abiotic and biotic stress conditions.
[y] Statistical test: Dunnett's test. C = controls, F = inoculation with *Fusarium oxysporum*, V = inoculation with *Verticillium dahliae*, I = ionic stress factor, and O = osmotic stress factor.
[z] Comparisons made by Dunnett's test: F, V, I, O, and IO to C; IF and IV to I; OF and OV to O; and IOF and IOV to IO.

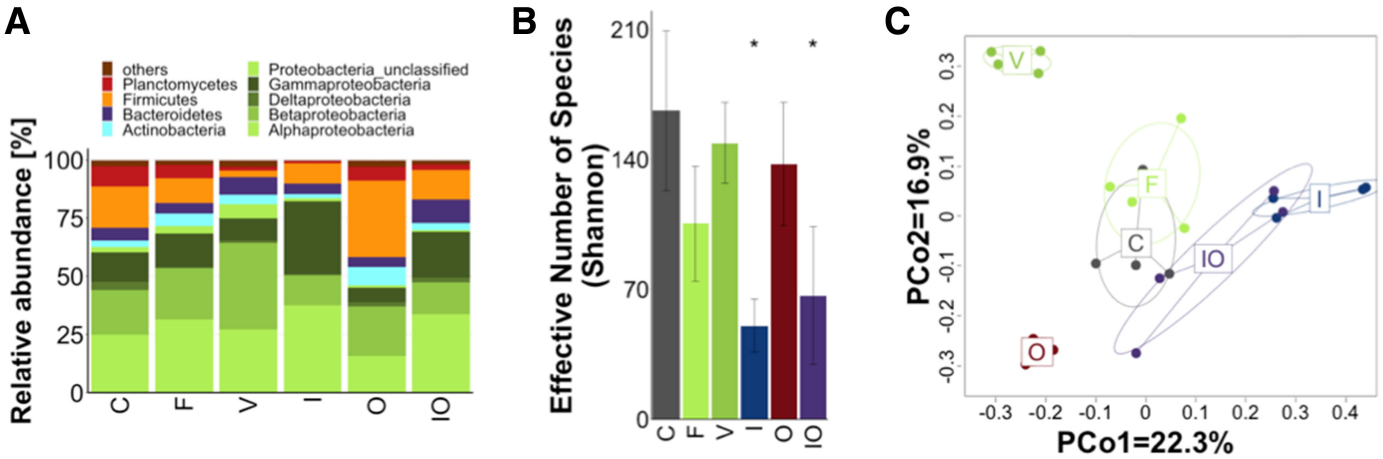

Fig. 1. Effect of individual abiotic and biotic treatment on root-associated bacterial communities of Hildares tomato plants 3 weeks posttreatment. **A,** Phylum-level composition; **B,** α-diversity (effective number of species based on the Shannon index); and **C,** β-diversity (Bray-Curtis dissimilarity) of the tomato root microbiota. Significance in B: Dunnett's test; asterisk (*) indicates $P < 0.05$. C = nontreated plants, F = inoculation with *Fusarium oxysporum*, V = inoculation with *Verticillium dahliae*, I = ionic stress factor, O = osmotic stress factor, and PCo = principal coordinate.

stress conditions (*Alphaproteobacteria*, *Bacteriodetes*, *Betaproteobacteria*, and *Gammaproteobacteria*) and in the exclusive treatment with *V. dahliae* (*Acidobacteria*, *Chloroflexi*, and *Oligoflexia*) (Supplementary Table S1). Only one phylum (candidate division WPS) was significantly differentially abundant for condition IO while no significantly differentially abundant phyla were detected for F and O (Supplementary Table S1).

Significant changes of root bacterial community profiles upon treatment of tomato plants with individual and simultaneous abiotic and biotic stress factors were found. A tendency for decreased α-diversity upon subjecting plants to individual biotic and abiotic stress factors was detected (Fig. 1B). This effect was most consistent for individual ionic stress factors (I) because it was statistically significant for all three indices and least pronounced for inoculation with *V. dahliae*; statistical significance was not reached for either index (Fig. 1B; Supplementary Fig. S1). The overall root microbiota composition (β-diversity) was significantly influenced by all single treatments except for inoculation with *F. oxysporum* (Fig. 1C; Table 2). Interestingly, root endophytic β-diversity was most strongly affected by *V. dahliae*, as indicated by the highest R^2 value for each considered β-diversity index (Bray-Curtis, Jaccard, unweighted UniFrac, and weighted UniFrac) (Table 2).

Additionally, differential abundance of bacterial amplified sequence variants (ASVs) upon application of individual biotic and abiotic stress factors (F, V, I, and O) and IO compared with non-treated controls (C) was tested using DESeq2 with 70 (F), 366 (V), 51 (I), 113 (O), and 158 (IO) differentially abundant ASVs being detected, respectively. Most of the ASVs were only statistically significantly different in one treatment (Supplementary Fig. S2).

The effect of combined biotic and abiotic treatment on the plant root microbiota (i.e., IF and IV versus I, OF and OV versus O, IOF and IOV versus IO) showed that abiotic stress factors with *V. dahliae* (IV, OV, and IOV) had a significant effect on the relative abundance of several bacterial phyla. Specifically, it led to a statistically significant decrease in relative abundance of the phyla *Firmicutes*, *Actinobacteria*, *Planctomycetes*, *Verrucomicrobia* (OV), and *Chlamydiae* (IOV) (Supplementary Table S1). These were similar phyla observed for inoculation with *V. dahliae* alone (*Firmicutes*, *Planctomycetes*, and *Chlamydiae*), *Proteobacteria* (class *Deltaproteobacteria*), and *Verrucomicrobia*. No statistically significant differences were detected for simultaneous exposure to two abiotic stress factors with *F. oxysporum* compared with the respective individual abiotic stress conditions (Fig. 2A). However, the combination of abiotic stressors and inoculation with *F. oxysporum*

TABLE 2
Influence of individual abiotic and biotic stress factors and combined abiotic and biotic stress conditions on the overall bacterial community composition within the root microbiota of Hildares tomato plants compared with controls using permutational multivariate analysis of variance[w]

Metric	Individual stress[x]	R^2	*P* adj[y]	Combined stress	R^2	*P* adj[z]
Bray-Curtis	***V***	***0.54***	*<0.05*	IF	0.27	0.085
Bray-Curtis	*O*	*0.39*	*<0.05*	IV	0.25	0.085
Bray-Curtis	*I*	*0.37*	*<0.05*	*OF*	*0.24*	*<0.05*
Bray-Curtis	*IO*	*0.29*	*<0.05*	***OV***	***0.63***	*<0.05*
Bray-Curtis	F	0.19	0.059	*IOF*	*0.23*	*<0.05*
Bray-Curtis				*IOV*	*0.32*	*<0.05*
Jaccard	***V***	***0.42***	*<0.05*	*IF*	*0.29*	*<0.05*
Jaccard	*IO*	*0.34*	*<0.05*	*IV*	*0.22*	*<0.05*
Jaccard	*O*	*0.30*	*<0.05*	*OF*	*0.28*	*<0.05*
Jaccard	*F*	*0.26*	*<0.05*	***OV***	***0.31***	*<0.05*
Jaccard	*I*	*0.25*	*<0.05*	IOF	0.29	0.059
Jaccard				IOV	0.22	0.059
Unweighted UniFrac	***V***	***0.35***	*<0.05*	IF	0.16	0.202
Unweighted UniFrac	*I*	*0.28*	*<0.05*	IV	0.19	0.116
Unweighted UniFrac	*IO*	*0.28*	*<0.05*	OF	0.19	0.171
Unweighted UniFrac	*O*	*0.23*	*<0.05*	**OV**	**0.33**	0.060
Unweighted UniFrac	F	0.16	0.168	IOF	0.19	0.087
Unweighted UniFrac				IOV	0.21	0.087
Weighted UniFrac	***V***	***0.58***	*<0.05*	IF	0.19	0.171
Weighted UniFrac	*I*	*0.43*	*<0.05*	IV	0.19	0.171
Weighted UniFrac	*O*	*0.38*	*<0.05*	*OF*	*0.28*	*<0.05*
Weighted UniFrac	IO	0.27	0.071	***OV***	***0.80***	*<0.05*
Weighted UniFrac	F	0.19	0.204	IOF	0.12	0.691
Weighted UniFrac				IOV	0.12	0.168

[w] Values in bold indicate treatment with *V. dahliae* and italics indicate significant effects.
[x] F = inoculation with *Fusarium oxysporum*, V = inoculation with *Verticillium dahliae*, I = ionic stress factor, and O = osmotic stress factor.
[y] Adjusted *P* value (*P* adj) comparisons made with F, V, I, O, and IO to C.
[z] Comparisons made: IF and IV to I, OF and OV to O, and IOF and IOV to IO.

(IF, OF and IOF) significantly increased bacterial α-diversity in roots of OF and IOF plants compared with the respective abiotic treatment alone (O and IO) (Fig. 2B). This effect was only statistically significant for species richness from roots of OF and IOF plants (Fig. 2B).

Indices of β-diversity were most consistently influenced by combined osmotic and biotic stressors (Fig. 2C; Table 2). A particularly strong effect was detected for the combination of osmotic stress conditions with *V. dahliae* (R^2 values from 0.31 to 0.80 depending on the index; permutational multivariate analysis of variance).

Composition of the cultivable bacterial root endosphere microbiota. The effect of individual and combined biotic and abiotic stress conditions on the composition of the bacterial root endosphere microbiota was assessed by isolation of 683 root-bacterial endophytes. The highest number of isolates was obtained in treatment IOV (86 isolates), followed by C ($n = 76$), IV ($n = 75$), OV ($n = 70$), O ($n = 69$), V ($n = 56$), F ($n = 46$), IO ($n = 46$), I ($n = 45$), OF ($n = 40$), IOF ($n = 39$), and IF ($n = 35$). The majority of isolates was assigned to the genera *Bacillus* (219 isolates, 32.1%), *Paenibacillus* ($n = 118$, 17.3%), *Microbacterium* ($n = 114$, 16.7%), *Rhizobium* ($n = 20$, 2.9%), and *Methylobacterium* ($n = 17$, 2.5%) using partial 16S rRNA gene sequencing (Table 3). The enrichment of certain bacterial genera within isolates obtained from the different treatments revealed a higher percentage of the genus *Paenibacillus* from tomato roots exposed to individual (F) and, interestingly, combined stress treatments with *F. oxysporum* (IF, OF, and IOF) (Table 3). However, this was only statistically significant for F and IF tested using Fisher's exact test (Table 4). In contrast, endophytes assigned to the genus *Microbacterium* were significantly more frequently isolated from the roots infected with *V. dahliae* (V) (Table 4). For individual treatments compared with controls, the fraction of isolates classified as *Agrobacterium* was significantly lower in most libraries (Tables 3 and 4).

To assess bacterial α-diversity, isolates were clustered based on their 16S rRNA gene sequences into OTUs. OTUs of 97% sequence similarity were obtained using USEARCH (Edgar 2010). OF yielded the most diverse set of isolates (effective number of species [ENS] based on the Shannon-Wiener index = 18.3], while IOV had the most detrimental effect on bacterial α-diversity (ENS = 7.7) (Supplementary Fig. S3B). Strikingly, inoculation with *F. oxysporum* often led to an increase in the diversity of cultivable bacteria (F versus C, IF versus I, OF versus O).

Functional characteristics of cultivable root endophytes. An altered number of bacterial root endophytes with ionic stress tolerance was only detected under individual ionic stressor (I) (enrichment; $P < 0.05$, Cv = 0.24, Or = 2.7) and in treatment with *V. dahliae* (V) (depletion; $P < 0.05$, Cv = 0.22, Or = 0.35). Combination of ionic stress condition with *V. dahliae* (IV) negated this effect (Fig. 3A; Supplementary Table S2). In contrast, tomato root endophytes tolerant to osmotic stress condition were enriched under abiotic treatments I, O, and IO ($P < 0.05$, Fisher's exact test; Supplementary Table S2). Combination of abiotic and biotic stress conditions (IF and IV versus I, IOF and IOV versus IO) negated this effect (Fig. 3B).

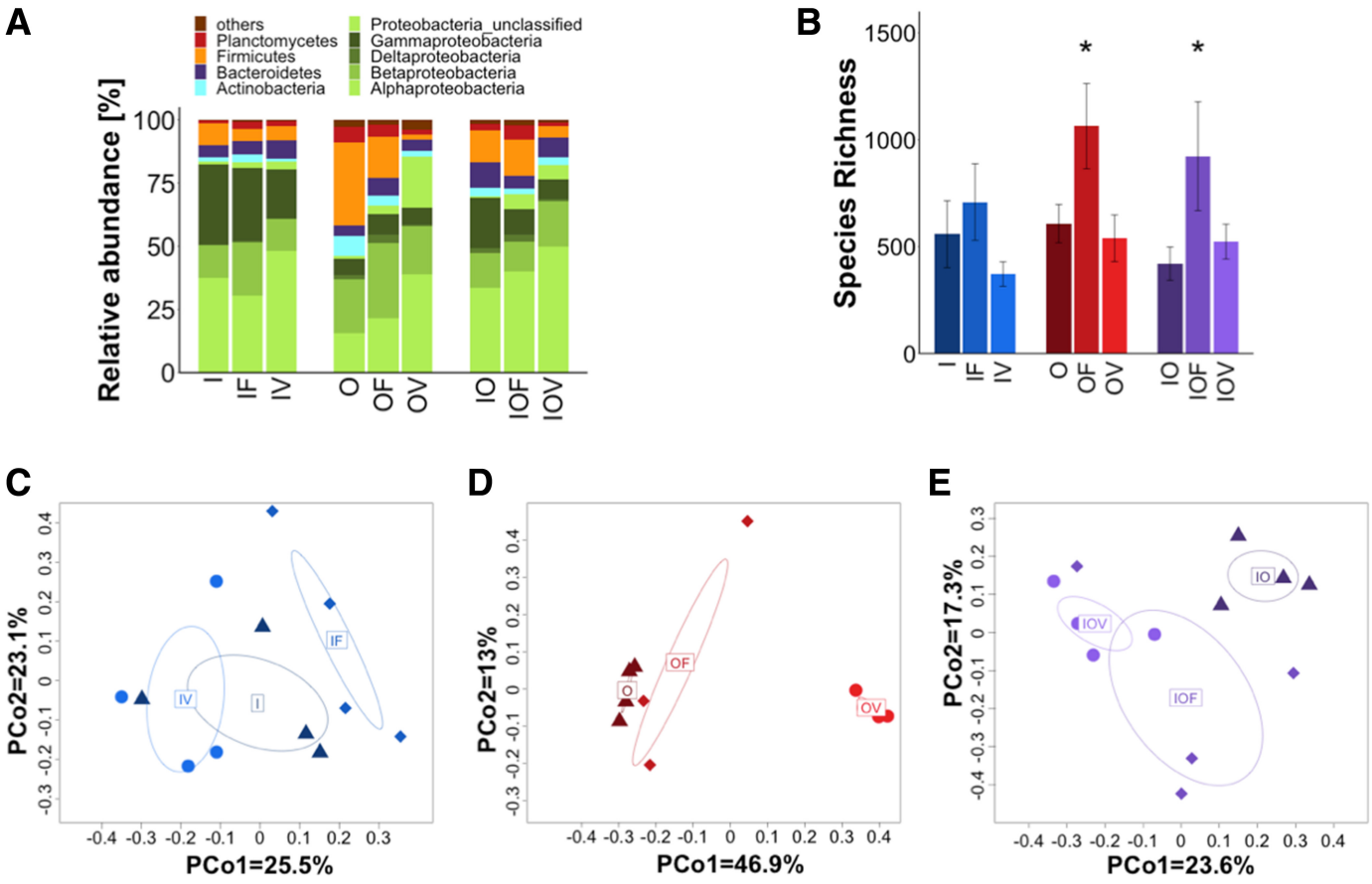

Fig. 2. Effect of combined biotic and abiotic treatment on root-bacterial microbiota in Hildares tomato plants 3 weeks posttreatment. **A,** Phylum-level composition, **B,** α-diversity; and **C to E,** β-diversity (Bray-Curtis dissimilarity) of the tomato root-associated microbiota. Significance in B: Dunnett's test; asterisk (*) indicates $P < 0.05$. F = inoculation with *Fusarium oxysporum*, V = inoculation with *Verticillium dahliae*, I = ionic stress factor, O = osmotic stress factor, and PCo = principal coordinate.

TABLE 3
Percentage of root bacterial endophytes at genus level obtained from Hildares tomato plants exposed to individual and combined biotic and abiotic stress conditions[z]

Genus	C	F	V	I	IF	IV	O	OF	OV	IO	IOF	IOV	IO	IOF	IOV	Avg
Achromobacter	1.3	0	5.4	0	0	1.3	0	0	0	0	0	0	0	0	0	0.7
Aeromicrobium	0	0	0	0	0	1.3	0	0	0	0	0	0	0	0	0	0.1
Agrobacterium	**14.5**	**2.2**	0	0	0	**1.3**	0	0	0	0	0	**2.3**	0	0	0	**2.2**
Agromyces	0	0	0	0	0	2.7	0	0	0	0	0	0	0	0	0	0.3
Arthrobacter	0	0	5.4	0	0	0	2.9	0	0	4.3	0	0	2.9	0	0	1
Azospirillum	0	0	0	0	0	0	0	0	0	4.3	0	0	0	0	0	0.3
Bacillus	**28.9**	**21.7**	**19.6**	**53.3**	**31.4**	**10.7**	**15.9**	**30**	**24.3**	**28.3**	**51.3**	**69.8**	**15.9**	**30**	**24.3**	**32.1**
Beijerinckia	0	0	0	0	0	1.3	0	0	0	0	0	0	0	0	0	0.1
Bradyrhizobium	0	0	0	0	0	0	2.9	0	1.4	0	0	0	2.9	0	1.4	0.4
Brevibacillus	0	0	0	0	0	0	0	2.5	0	0	0	0	0	2.5	0	0.1
Brevibacterium	0	0	0	0	0	0	1.4	0	0	0	0	0	1.4	0	0	0.1
Burkholderia	1.3	0	0	0	0	0	0	0	1.4	0	0	0	0	0	1.4	0.3
Castellaniella	0	0	0	0	0	1.3	0	0	0	0	0	0	0	0	0	0.1
Cellulosimicrobium	0	2.2	0	4.4	0	1.3	0	0	1.4	0	0	0	0	0	1.4	0.7
Cohnella	1.3	6.5	0	0	0	0	1.4	0	0	0	10.3	0	1.4	0	0	1.3
Comamonas	2.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3
Cupriavidus	2.6	2.2	0	0	0	0	0	0	0	6.5	0	0	0	0	0	0.9
Curtobacterium	0	0	0	0	0	0	0	0	0	2.2	0	0	0	0	0	0.1
Devosia	0	0	0	2.2	0	4	5.8	0	0	0	0	0	5.8	0	0	1.2
Ensifer	2.6	2.2	1.8	0	0	4	1.4	0	0	2.2	0	2.3	1.4	0	0	1.6
Enterobacter	0	0	0	0	0	13.3	0	0	0	2.2	0	0	0	0	0	1.6
Fontibacillus	0	4.3	0	0	5.7	2.7	0	0	0	0	0	0	0	0	0	0.9
Herbaspirillum	1.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1
Leifsonia	0	4.3	1.8	0	0	0	0	0	5.7	0	0	1.2	0	0	5.7	1.2
Lysinibacillus	1.3	0	0	0	2.9	0	1.4	5	1.4	4.3	0	0	1.4	5	1.4	1.3
Mesorhizobium	0	2.2	0	0	0	2.7	1.4	0	0	0	0	0	1.4	0	0	0.6
Methylobacterium	2.6	2.2	0	2.2	0	0	4.3	2.5	4.3	6.5	2.6	2.3	4.3	2.5	4.3	2.5
Microbacterium	**11.8**	**15.2**	**39.3**	**20**	**2.9**	**16**	**21.7**	**15**	**27.1**	**17.4**	**0**	**7**	**21.7**	**15**	**27.1**	**16.7**
Nocardia	0	0	0	0	0	0	0	0	2.9	0	0	0	0	0	2.9	0.3
Ochrobactrum	0	0	0	0	0	2.7	0	0	0	0	0	0	0	0	0	0.3
Paenibacillus	**7.9**	**34.8**	**16.1**	**15.6**	**51.4**	**5.3**	**14.5**	**40**	**20**	**4.3**	**23.1**	**8.1**	**14.5**	**40**	**20**	**17.3**
Pantoea	0	0	0	0	0	18.7	0	0	0	4.3	0	0	0	0	0	2.3
Pedobacter	0	0	0	0	0	0	1.4	0	0	2.2	0	0	1.4	0	0	0.3
Phyllobacterium	1.3	0	1.8	0	0	0	1.4	0	0	0	0	0	1.4	0	0	0.4
Pimelobacter	0	0	0	0	0	0	0	0	0	0	0	1.2	0	0	0	0.1
Pseudomonas	3.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4
Pseudoxanthomonas	1.3	0	0	0	0	1.3	0	0	2.9	0	0	0	0	0	2.9	0.6
Psychrobacillus	1.3	0	0	0	2.9	0	0	2.5	1.4	0	5.1	0	0	2.5	1.4	0.9
Ralstonia	3.9	0	1.8	2.2	0	1.3	5.8	0	0	6.5	0	0	5.8	0	0	1.9
Rhizobium	5.3	0	1.8	0	0	1.3	11.6	2.5	0	4.3	0	3.5	11.6	2.5	0	2.9
Shinella	0	0	1.8	0	0	0	0	0	0	0	5.1	0	0	0	0	0.4
Sphingobium	0	0	0	0	0	0	1.4	0	0	0	0	0	1.4	0	0	0.1
Sphingomonas	0	0	0	0	0	0	2.9	0	1.4	0	0	0	2.9	0	1.4	0.4
Sphingopyxis	0	0	0	0	0	5.3	0	0	0	0	0	0	0	0	0	0.6
Sporosarcina	0	0	0	0	2.9	0	0	0	0	0	0	0	0	0	0	0.1
Stenotrophomonas	0	0	0	0	0	0	0	0	1.4	0	0	0	0	0	1.4	0.1
Streptomyces	2.6	0	1.8	0	0	0	0	0	1.4	0	0	0	0	0	1.4	0.6
Tardiphaga	0	0	0	0	0	0	0	0	1.4	0	0	0	0	0	1.4	0.1
Thermomonas	0	0	0	0	0	0	0	0	0	0	0	2.3	0	0	0	0.3
Viridibacillus	0	0	1.8	0	0	0	0	0	0	0	0	0	0	0	0	0.1

[z] C = controls, F = inoculation with *Fusarium oxysporum*, V = inoculation with *Verticillium dahliae*, I = ionic stress factor, O = osmotic stress factor, and Avg = average. Values in bold indicate more frequently isolated endophytes.

Additionally, isolates classified as *Bacillus* ($P = 1.04 \times e^{-06}$, Cv = 0.21, Or = 2.53) and *Pantoea* ($P < 0.1$, Cv = 0.10, Or = 3.49) were more likely to be tolerant to ionic stress condition compared with the average of all other isolates (Supplementary Table S3). Strains of the genera *Agrobacterium* ($P = 1.63 \times e^{-04}$, Cv = 0.16, Or = 0), *Paenibacillus* ($P = 1.62 \times e^{-04}$, Cv = 0.16, Or = 0.41), and *Enterobacter* ($P = 0.025$, Cv = 0.10, Or = 0.17) were significantly depleted for isolates with tolerance to osmotic stress condition compared with all other isolates combined (Supplementary Table S4). In contrast, strains of the genera *Bacillus* ($P = 0.0089$, Cv = 0.12, Or = 1.67) and *Microbacterium* ($P = 0.012$, Cv = 0.11, Or = 1.87) comprised more isolates with tolerance to osmotic stressor (Supplementary Table S3).

The prevalence of PGP traits (IAA, siderophore, and ACC deaminase production and phosphate solubilization) and antagonistic potential (chitinase production and inhibition of *F. oxysporum*) in the isolate collection was quite different for each property, with only 4.6% of endophytes inhibiting mycelial growth of *F. oxysporum* compared with 87% of endophytes producing ACC deaminase. Many of the treatments enriched for one or more PGP traits, most often for IAA or ACC deaminase (Table 5; Supplementary Table S5). Interestingly, the overall number of PGP traits per isolate was increased upon inoculation with *F. oxysporum* (F compared with C, IF compared with I, IOF compared with IO) (Fig. 4; Supplementary Table S5). Moreover, enrichment of specific PGP traits was found for certain bacterial genera. For example, isolates classified as

TABLE 4
Proportion of bacterial genera isolated from roots of Hildares tomato plants exposed to individual or combined abiotic and biotic stress conditions based on a cultivation dependent approach[y]

Treatment[z]	Genus	*P* adj	Cv	Or
IOF	*Microbacterium*	0.0039	0.4788	0
O	*Agrobacterium*	0.0079	0.3185	0
I	*Agrobacterium*	0.0151	0.3035	0
V	*Agrobacterium*	0.0151	0.3072	0
IO	*Agrobacterium*	0.0400	0.2787	0
IV	*Bacillus*	0.0004	0.4485	0.1369
IOV	*Microbacterium*	0.0122	0.3212	0.1721
IOV	*Bacillus*	0.0452	0.2567	3.4949
V	*Microbacterium*	0.0070	0.3678	5.2368
IF	*Paenibacillus*	0.0012	0.4389	6.8434
F	*Paenibacillus*	0.0070	0.4005	6.9812
IV	*Pantoea*	0.0004	0.3971	Inf
IV	*Enterobacter*	0.0041	0.3269	Inf

[y] *P* adj = adjusted *P* value (Benjamini-Hochberg) Fisher's exact test (only significant results are shown), Cv = Cramer's V, Or = odds ratio, and Inf = infinite.
[z] C = controls, F = inoculation with *Fusarium oxysporum*, V = inoculation with *Verticillium dahliae*, I = ionic stress factor, and O = osmotic stress factor. Comparisons made by Fisher's exact test: F, V, I, O and IO to C; IF and IV to I; OF and OV to O; and IOF and IOV to IO.

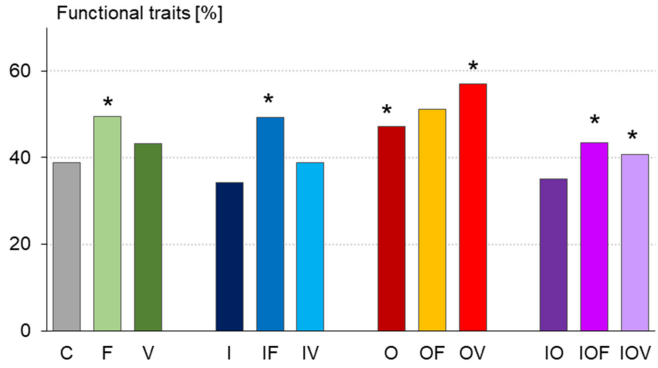

Fig. 4. Functional traits in bacterial root endophytes from Hildares tomato plants exposed to individual and combined abiotic and biotic stressors. Traits tested for (in vitro): antagonism against *F. oxysporum*; production of siderophores, indole-3-acetic acid (IAA), chitinase, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase; and phosphate solubilization. Individual treatments (F, V, I, O, and IO) were compared with C. Combined biotic and abiotic treatments were compared with their respective abiotic control (e.g., IF versus I). Significance (Dunnett's test): asterisk (*) indicates $P < 0.05$. C = nontreated plants, F = inoculation with *Fusarium oxysporum*, V = inoculation with *Verticillium dahliae*, I = ionic stress factor, and O = osmotic stress factor.

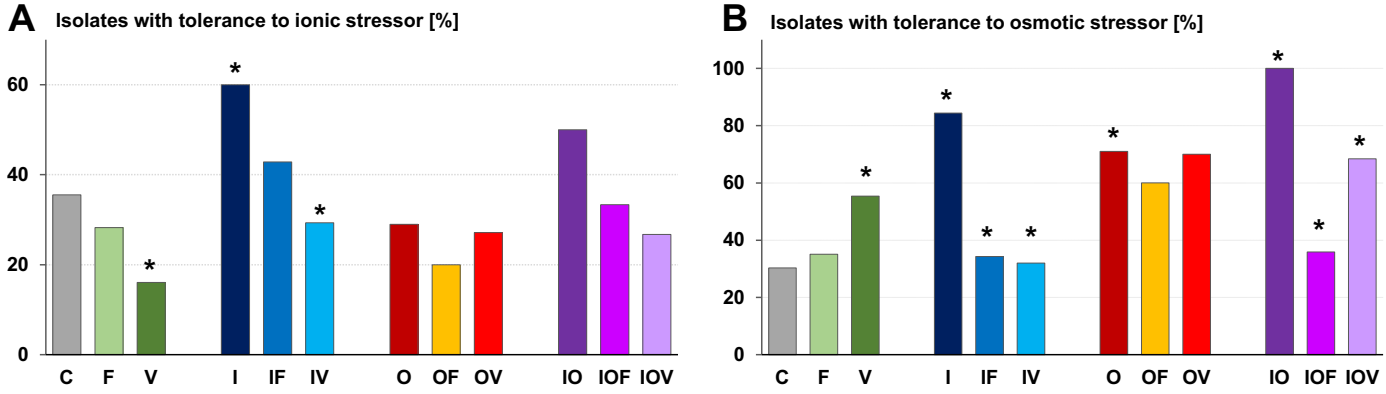

Fig. 3. Effect of individual and combined abiotic and biotic stress conditions on tolerance of root endophytes from Hildares tomato plants to **A,** ionic (7.5% NaCl) and **B,** osmotic (30% polyethylene glycol) stressors. Asterisks (*) indicate significance of individual treatments (F, V, I, O, and IO) with the control (C) and combined biotic and abiotic treatments to their respective abiotic control (e.g., IF versus I) according to Fisher's exact test ($P < 0.05$). C = nontreated plants, F = inoculation with *Fusarium oxysporum*, V = inoculation with *Verticillium dahliae*, I = ionic stress factor, and O = osmotic stress factor.

Microbacterium were more likely to have the potential for phosphate solubilization ($P < 0.001$, Cv = 0.19, Or = 2.74, Fisher's test) while *Agrobacterium* isolates were depleted for siderophore production ($P < 0.001$, Cv = 0.20, Or = 0.083) (Supplementary Table S6). Finally, presence of tolerance for ionic stress was predictive for the number of PGP traits detected in an isolate (linear regression, $P < 0.001$).

Effect of root endophytes on plant growth in vivo. In total, 199 root endophytes significantly modulated tomato growth in greenhouse experiments ($P \leq 0.1$; 58 promoted growth and 141 inhibited growth). Endophytes which promoted plant growth were obtained only from individual biotic and combined biotic and ionic stress treatments (Fig. 5). Plant-growth-inhibiting isolates were found in isolate libraries from all treatments (Fig. 5). Isolates classified as *Pantoea* were enriched for PGP traits ($P < 0.05$, Cv = 0.15, Or = 5.14, Fisher's exact test). Isolates with tolerance to ionic stressor were more likely to promote plant growth. Of the 187 isolates with tolerance to ionic stressor, 30 (16.04%) showed PGP in the greenhouse experiment, compared with 8.51% of nontolerant isolates ($P < 0.05$, Cv = 0.11, Or = 2.05, Fisher's exact test).

Effect of selected root endophytes on plant growth under abiotic stress conditions. Although none of the isolates had a statistically significant effect on plant growth under individual abiotic stress conditions, all root endophytes improved growth of tomato plants inoculated with *V. dahliae* (Fig. 6A). Two isolates, IV30 and IV95, were chosen for testing their growth-promoting effect under combined biotic (*V. dahliae*) and abiotic (ionic) stress factors (Table 6). IV30 only showed a trend for promoting plant growth ($P \leq 0.1$, Dunnett's test) while IV95 statistically significantly improved plant growth ($P < 0.05$, Dunn's test) (Fig. 6B).

DISCUSSION

Stress conditions affects plant performance differently and combined stress factors are not necessarily additive. In the present study, we detected additive effects on tomato growth under

TABLE 5
Number of cultivable root endophytes isolated from Hildares tomato with plant-growth-promoting traits depending on individual and combined abiotic and biotic stress conditions[z]

	ACC deaminase production			IAA production			Phosphate solubilization			Siderophore activity			Antagonism against *Fusarium oxysporum*			Chitinase activity		
TRT	+	−	+ (%)	+	−	+ (%)	+	−	+ (%)	+	−	+ (%)	+	−	+ (%)	+	−	+ (%)
C	59	17	77.6 B	27	49	35.5	28	41	40.6 A	42	18	70.0 B	3	73	3.9 B	4	65	5.8 B
F	28	2	93.3 A	29	8	78.4	16	18	47.1 A	11	12	42.3 B	6	31	16.3 A	5	21	19.2 A
V	49	4	92.5 A	23	30	43.4	20	33	37.7 A	30	15	71.4 C	3	50	5.7 B	4	46	8.0 B
I	34	11	75.6 B b	11	34	24.4	10	35	22.2 B a	27	12	69.2 B a	2	43	4.4 B a	4	40	9.1 B a
IF	23	4	85.2 a	29	6	82.9	12	19	38.7 a	15	6	71.4 a	2	33	5.7 a	3	23	11.5 a
IV	73	2	97.3 a	33	41	44.6	23	48	32.4 a	33	27	55.0 b	0	75	0.0 a	3	68	4.2 a
O	44	7	86.3 B a	29	22	56.9	21	26	44.7 A a	41	5	89.1 A b	0	51	0.0 B a	3	44	6.4 B b
OF	22	2	91.7 a	25	7	78.1	11	17	39.3 b	14	6	70.0 c	2	32	5.9 a	5	18	21.7 a
OV	37	1	97.4 a	33	5	86.8	18	18	50.0 a	37	0	100.0 a	2	36	5.3 a	1	32	3.0 b
IO	27	19	58.7 C b	13	33	28.3	20	24	45.5 A a	24	7	77.4 B b	0	46	0.0 B b	0	44	0.0 b
IOF	36	1	97.3 a	7	32	17.9	17	21	44.7 a	23	10	69.7 b	6	33	15.4 a	0	42	0.0 b
IOV	71	5	93.3 a	18	58	23.7	15	59	20.3 b	68	5	93.2 a	2	74	2.6 b	8	65	11.0 a

[z] TRT = treatment, ACC = 1-aminocyclopropane-1-carboxylate, IAA = indole-3-acetic acid, F = inoculation with *Fusarium oxysporum*, V = inoculation with *Verticillium dahliae*, I = ionic stress factor, O = osmotic stress factor, and symbols + and − indicate with and without the respective trait, respectively. Numbers followed by uppercase letters indicate significance by individual treatments (F, V, I, O, and IO) with the control (C), and lowercase letters indicate combined biotic and abiotic treatments to their respective abiotic control (e.g., IF versus I) according to Fisher's exact test ($P < 0.05$).

TABLE 6
Comparison of bacterial root endophytes from Hildares tomato plants with plant-growth-promotion (PGP) potential using in vitro and in vivo assays[z]

		In vivo PGP			In vitro PGP						Tolerance (%) to stress		
Code	Classification	1st	2nd	3rd	IAA	S	PS	ACC	Chit	Ant	Ionic	Osmotic	Rating
IV95	*Bacillus licheniformis*	1	1	1	1	1	–	1	1	1	10	30	8
V71	*Bacillus* sp. KJ-16	1	1	1	1	1	–	1	1	1	10	30	8
IV22	*Pantoea agglomerans*	1	1	1	1	1	1	1	–	–	7.5	30	7
IV30	*Microbacterium paraoxydans*	1	1	1	1	1	1	1	–	–	7.5	30	7
OV31	*Microbacterium oleivorans*	1	1	1	1	1	1	1	–	–	7.5	30	7

[z] Each in vitro and in vivo trait was scored with 1 for the rating. First, second, and third in vivo experiments; indole-3-acetic acid (IAA) production; S = siderophore activity; PS = phosphate solubilization; 1-aminocyclopropane-1-carboxylate (ACC) deaminase production; chitinase (Chit) activity; and Ant – antagonism against *Fusarium oxysporum*.

combined abiotic stress conditions with *F. oxysporum* (treatments IF and OF) but not for simultaneous challenge of plants with *V. dahliae* (IV and OV) (Table 1). Recent findings suggest that an abiotic stressor in combination with a pathogen can either limit or increase disease susceptibility of the plant (Pandey et al. 2015; Ramu et al. 2016; Suzuki et al. 2014). The interaction between abiotic and biotic stressors is known to be mediated by hormone-signaling pathways in the plant that alter the host–pathogen relationship (Atkinson and Urwin 2012). This can include plant responses at the molecular level or affect pathogenicity factors of the pathogen such as the production of cell-wall-degrading enzymes or secretion of effectors to suppress basal defense in plants (Essarioui et al. 2016; Song et al. 2020). For instance, a downregulation of genes associated with plant response against abiotic stress was shown to be induced in potato by *Rhizoctonia solani* (Zrenner et al. 2021). An abiotic stressor can weaken plant defenses and enhance their susceptibility to pathogens and, thus, result in additive effects on plant performance (Atkinson and Urwin 2012; Suzuki et al. 2014); we have shown this for *Fusarium* but not for *Verticillium*. The latter is an ubiquitously occurring endophyte, and pathogenic behavior in plants is often associated with additional abiotic stressors such as drought and high temperature (Rybakova et al. 2020).

Stress altered the composition of the root endosphere microbiota and induced a stress-specific shift. We confirmed our primary hypothesis that both abiotic and biotic stressors induce alterations in the composition of the tomato root endosphere microbiota. It can be assumed that limited photosynthetic activity in stressed plants also alters the pattern of root exudation (Gargallo-Garriga et al. 2018) and, thus, affects the composition of the rhizosphere microbiota (Sasse et al. 2018). Impacts on the rhizosphere microbiota likely also translate into compositional changes of the root endosphere microbiota, as revealed by α- and β-diversity analyses in the present study. The lowest α-diversity was found in ionically stressed plants. Previous studies showed contrary results and reported an increase in bacterial root diversity in plants exposed to an ionic stressor (Yaish et al. 2016; Yang et al. 2016). In this context, the tolerance of the plant species itself and its microbiota to an ionic stressor seems to play a crucial role. The majority of bacterial root endophytes were assigned to the phylum *Proteobacteria*, the most representative phylum within the plant microbiome (Bergna et al. 2018; Bulgarelli et al. 2013). However, the relative abundance of some proteobacterial classes (e.g., *Gammaproteobacteria* and *Deltaproteobacteria*) varied depending on the stress factor.

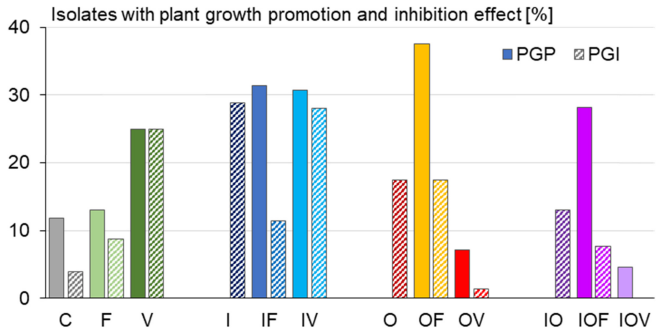

Fig. 5. Percentage of bacterial root endophytes with plant growth promotion (PGP) and plant growth inhibition (PGI) effect on Hildares tomato plants. Isolates were isolated from Hildares tomato plants grown under individual and combined abiotic and biotic stress conditions. C = non-treated plants, F = inoculation with *Fusarium oxysporum*, V = inoculation with *Verticillium dahliae*, I = ionic stress factor, and O = osmotic stress factor.

The overall shift in bacterial root endosphere diversity (β-diversity) was substantially more pronounced when exposed to *V. dahliae* in comparison with *F. oxysporum* (Fig. 1C). A shift in the plant microbiome due to pathogen infection was also found by Erlacher et al. (2014) and Köberl et al. (2017). Contrary to our results, Erlacher et al. (2014) observed an increased α-diversity after pathogen infection in lettuce, whereas Köberl et al. (2017) also detected a lower root endosphere diversity in banana plants infected with *Fusarium* spp. Interestingly, in our study, an increase of α-diversity of the tomato root endosphere community was found under simultaneous abiotic stress conditions with *F. oxysporum* but not with *V. dahliae* compared with individual abiotic stress. Hence, root endosphere diversity seems to be affected by the nature of plant–pathogen interactions.

We observed an impact of stress conditions on the relative abundance of distinct taxa in the tomato root endosphere, including a reduced relative abundance of *Firmicutes* in the pathogen treatments and under individual ionic stress conditions. Lee et al. (2021) also found a lower relative abundance of *Firmicutes* in the tomato rhizosphere of diseased compared with healthy plants. Members of this phylum are known to protect plants against pathogens. A substantially higher relative abundance of the class *Betaproteobacteria* was found in roots infected with *V. dahliae* compared with roots colonized by *Fusarium* spp. It is known, for instance, that *Burkholderia phytofirmans* can produce ACC deaminase. Tiwari et al. (2018) demonstrated that this compound can contribute to enhanced plant stress tolerance against *V. dahliae*. Therefore, future studies should include analysis of microorganism- and plant-produced metabolites involved in mitigating effects of biotic and abiotic stressors. In ionic-challenged plants, the root endosphere microbiota was dominated by the class *Gammaproteobacteria*, which contains crucial constituents of the plant microbiota (Erlacher et al. 2014; Köberl et al. 2017). However, their contribution to plant protection depends on the abundance of potentially plant-beneficial species such as *Pseudomonas* spp. In contrast to biotic and ionic stressors, an increased relative abundance of *Firmicutes* and *Actinobacteria* was detected in the roots exposed to the individual osmotic stressor (Fig. 1A). Xu and Coleman-Derr (2019) reported that an osmotic stressor induced a near-universal enrichment of Gram-positive bacteria in the roots, as was observed in this study. Karlowsky et al. (2018) noted that the enrichment of monoderm bacteria is mostly driven by an interaction within the plant host rather than by the ability of monoderms to withstand dry environments.

In all abiotic treatments that included *V. dahliae* (IV, OV, and IOV) an increase in the relative abundance of the class *Alphaproteobacteria* was found compared with the respective control or *Fusarium* treatment (Fig. 2A). A number of *Alphaproteobacteria*, including *Rhizobium* and *Azospirillum* spp., provide functions that are beneficial for their interaction with plants such as traits involved in nitrogen fixation (Pini et al. 2011). Under ionic and osmotic stress conditions, the availability of nutrients to the plants is limited. An increase in relative abundance of *Alphaproteobacteria* might be involved in the observed enhanced plant stress tolerance in the *V. dahliae*-infected plants compared with the *Fusarium* treatments.

Treatments resulted in an enrichment of stress-specific beneficial bacteria. It was hypothesized that tomato plants under stress conditions enrich for bacterial endophytes with plant-growth-promoting traits. The characterization of cultivable root endophytes confirmed that stressors affected the composition of the root endosphere microbiota. Based on 16S rRNA gene amplicon sequences, most endophytes were classified as members of the phylum *Proteobacteria*. In contrast, the majority of cultivable root endophytes were assigned to the phylum *Firmicutes*. This contrasting observation may be explained by the low proportion of plant endophytes

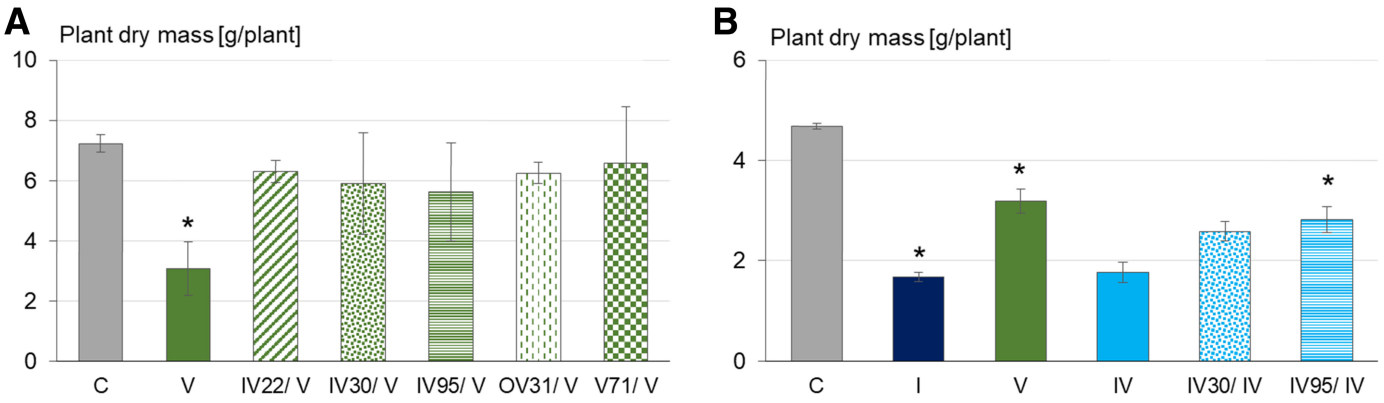

Fig. 6. Effect of selected root endophytes (IV22 = *Pantoea agglomerans*, IV30 = *Microbacterium paraoxydans*, IV95 = *Bacillus licheniformis*, OV31 = *M. oleivorans*, and V71 = *Bacillus* sp. KJ-16) on plant dry mass of Hildares tomato plants **A,** inoculated with *Verticillium dahliae* (V) and **B,** under combined ionic and biotic stress conditions (IV). C = the control treatment. Plant dry mass 3 weeks after stress treatment was analyzed. Error bars = standard error of means. Significance of each treatment or endophyte to the control (C) or of the endophytes to the combined stress treatment (IV) is indicated by asterisks (Dunn's or Dunnett's test, as appropriate; $P < 0.05$).

that are cultivable. Distinct genera, including *Bacillus, Paenibacillus*, and *Microbacterium*, were isolated from all conducted treatments. Although the cultivation-independent approach indicated a reduced relative abundance of *Firmicutes* under the ionic stressor, a high number of *Bacillus* isolates was obtained from this treatment and most of them showed tolerance to both abiotic stressors applied (Table 3). Various root-associated *Bacillus* spp. have been developed as biocontrol agents due to their plant-protection ability (Andrić et al. 2020; Chowdhury et al. 2015). Beneficial effects on plants were also shown for *Paenibacillus* strains (Hussain et al. 2020), which were obtained at a higher rate from treatments with *F. oxysporum* (F, IF, IOF, and OF). This suggests that colonization of tomato roots by *F. oxysporum* favors members of this genus within the root endosphere microbiota. Moreover, a significantly higher number of the cultivable endophytes was obtained, especially, from plants infected with *F. oxysporum* that showed antagonistic properties and functional traits that are likely involved in PGP, based on in vitro assays (Fig. 4). This indicates that the plant enriched those beneficial microbes under stress conditions and highlights the role of endophytes in plant stress mitigation. In contrast, *Microbacterium* strains were obtained at higher rates from treatments with *V. dahliae* (IV, OV, and OIV), although the phyla *Actinobacteria* accounted for a low relative abundance, based on cultivation-independent analysis. A high proportion of these endophytes were tolerant to the osmotic stressor. In accordance with findings of Li et al. (2017), a high rate of tomato root endophytes showed tolerance to the respective ionic or osmotic stressors in vitro (Fig. 3). Hence, abiotic stressors seem to enrich the fraction of root endophytes with the respective tolerance. The support of the host plant's tolerance to biotic and abiotic stressors by beneficial microorganisms partially depends on their traits related to plant stress mitigation. Interestingly, all endophytes able to promote plant growth in vivo were isolated from tomato plants subjected to biotic stress factors (Fig. 5). The overall number of plant-growth-promoting traits tested in vitro was often higher in these endophytes (F versus C, IF versus I, OV versus O, and IOF and IOV versus IO) (Fig. 4). However, the presence of PGP traits in vitro had no predictive power for successful PGP in vivo and highlighted the importance of in vivo experiments. Using a screening score (Krechel et al. 2002), five root endophytes assigned to the genera *Bacillus*, *Microbacterium*, and *Pantoea* with PGP and disease suppression effects were selected for additional experiments in this study. PGP effects under abiotic stress were previously shown for *Bacillus* and *Pantoea* strains (Nautiyal et al. 2013; Panwar et al. 2016; Wang et al. 2012). However, all selected endophytes were not able to confer plant stress tolerance under the tested conditions (I and O), although the strains were able to produce ACC deaminase, which can likely confer stress tolerance in various crops (Saikia et al. 2018; Vurukonda et al. 2016).

Overall, the results of this study provided new insights into stress-factor-dependent effects on the composition of the tomato root endosphere microbiota and linked them to potential consequences for plant health. Our results indicate that tomato plants can specifically adapt to prevalent environmental or stress conditions by enrichment of beneficial microorganisms which support the plant in stress mitigation. Shifts in the root endosphere microbiota can induce changes in plant–microbe interactions, including alteration of plant metabolism, depending on the applied stressor. Furthermore, plants grown under biotic or abiotic stress condition can be a source for beneficial bacterial strains, which alleviate the impact of biotic stressor. For the selection of beneficial strains, in vivo experiments are always necessary. Microbiome as well as genome analyses are valuable tools to support such selection processes.

Data availability. The Illumina 16S rRNA gene fragment library was deposited at the European Nucleotide Archive under bioproject number PRJEB48124. The samples can be accessed directly via accession numbers ERS7695290 to ERS7695337.

LITERATURE CITED

Akköprü, A., and Demir, S. 2005. Biological control of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF *Glomus intraradices* and some rhizobacteria. J. Phytopathol. 153:544-550.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Andrić, S., Meyer, T., and Ongena, M. 2020. *Bacillus* responses to plant-associated fungal and bacterial communities. Front. Microbiol. 11: 1350.

Atkinson, N. J., and Urwin, P. E. 2012. The interaction of plant biotic and abiotic stresses: From genes to the field. J. Exp. Bot. 63:3523-3543.

Bergna, A., Cernava, T., Rändler, M., Grosch, R., Zachow, C., and Berg, G. 2018. Tomato seeds preferably transmit plant beneficial endophytes. Phytobiomes J. 2:183-193.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37:852-857.

Bostock, R. M., Pye, M. F., and Roubtsova, T. V. 2014. Predisposition in plant disease: Exploiting the nexus in abiotic and biotic stress perception and response. Annu. Rev. Phytopathol. 52:517-549.

Brader, G., Compant, S., Mitter, B., Trognitz, F., and Sessitsch, A. 2014. Metabolic potential of endophytic bacteria. Curr. Opin. Biotechnol. 27:30-37.

Buhtz, A., Hohe, A., Schwarz, D., and Grosch, R. 2017. Effects of *Verticillium dahliae* on tomato root morphology considering plant growth response and defence. Plant Pathol. 66:667-676.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E. V. L., and Schulze-Lefert, P. 2013. Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant Biol. 64:807-838.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W. Johnson, A. J. A., and Holmes, S. P. 2016. DADA2: High resolution sample inference from Illumina amplicon data. Nat. Methods 13:581-583.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., and Knight, R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U.S.A. 108:4516-4522.

Chowdhury, S. P., Uhl, J., Grosch, R., Alquéres, A., Pittroff, S., Dietel, K., Schmitt-Kopplin, P., Borriss, B., and Hartmann, A. 2015. Cyclic lipopeptides of *Bacillus amyloliquefaciens* subsp. *plantarum* colonizing the lettuce rhizosphere enhance plant defence responses towards the bottom rot pathogen *Rhizoctonia solani*. Mol. Plant-Microbe Interact. 28:984-995.

Delgado-Baquerizo, M., Guerra, C. A., Cano-Díaz, C., Egidi, E., Wang, J.- T., Eisenhauer, N., Singh, B. S., and Maestre, F. T. 2020. The proportion of soil-borne pathogens increases with warming at the global scale. Nat. Clim. Change 10:550-554.

Dworkin, M., and Foster, J. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. J. Bacteriol. 75:592.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460-2461.

Elsawey, H., Patz, S., Nemr, R. A., Sarhan, M. S., Hamza, M. A., Youssef, H. H., Abdelfadeel, M. R., Daanaa, H.-S. A., El-Tahan, M., Abbas, M., Fayez, M., Witzel, K., Ruppel, S., and Hegazi, N. A. 2020. Plant broth- (not bovine-) based culture media provide the most compatible vegan nutrition for in vitro culturing and in situ probing of plant microbiota. Diversity 12:418.

Erlacher, A., Cardinale, M., Grosch, R., Grube, M., and Berg, B. 2014. The impact of the pathogen *Rhizoctonia solani* and its beneficial counterpart *Bacillus amyloquifaciens* on the indigenous lettuce microbiome. Front. Microbiol. 5:175.

Essarioui, A., Mokrini, F., and Afechtal, M. 2016. Molecular interactions between tomato and its wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici*. Rev. Mar. Sci. Agron. Vét. 4:66-74.

Fathi, A., and Tari, D. B. 2016. Effect of drought stress and its mechanism in plants. Int. J. Life Sci. 10:1-6.

Francini, A., and Sebastiani, L. 2019. Abiotic stress effects on performance of horticultural crops. Horticulturae 5:67.

Fróna, D., Szenderák, J., and Harangi-Rákos, M. 2019. The challenge of feeding the world. Sustainability 11:5816.

Gargallo-Garriga, A., Preece, C., Sardans, J., Oravec, M., Urban, O., and Peñuelas, J. 2018. Root exudate metabolomes change under drought and show limited capacity for recovery. Sci. Rep. 8:12696.

Gordon, S. A., and Weber, R. P., 1951. Colorimetric estimation of indoleacetic acid. Plant Physiol. 26:192.

Hardoim, P. R., Van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., and Sessitsch, A. 2015. The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol. Mol. Biol. Rev. 79:293-320.

Hussain, A., Ahmad, M., Nafeed, M., Iqbal, Z., Luqman, M., Jamil, M., Maqsood, A., Mora-Poblete, F., Ahmar, S., Chen, J.-T., Alyemeni, M. N., and Ahmad, P. 2020. Plant-growth-promoting *Bacillus* and *Paenibacillus* species improve the nutritional status of *Triticum aestivum* L. PLoS One 15:e0241130.

İnceoğlu, Ő., Salles, J. F., and van Elsas, J. D. 2012. Soil and cultivar type shape the bacterial community in the potato rhizosphere. Microbiol. Ecol. 63:460-470.

Karlowsky, S., Augusti, A., Ingrisch, J., Akanda, M. K. U., Bahn, M., and Gleixner, G. 2018. Drought-induced accumulation of root exudates supports post-drought recovery of microbes in mountain grassland. Front. Plant Sci. 9:1593.

Khabbaz, S. E., Zhang, L., Cáceres, L. A., Sumarah, M., Wang, A., and Abbasi, P. A. 2015. Characterisation of antagonistic *Bacillus* and *Pseudomonas* strains for biocontrol potential and suppression of damping-off and root rot diseases. Ann. Appl. Biol. 166:456-471.

Köberl, M., Dita, M., Matinuz, A., Staver, C., and Berg, G. 2017. Members of *Gammaproteobacteria* as indicator species of healthy banana plants on Fusarium wilt-infested fields in Central America. Sci. Rep. 7:45318.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., and Wilson, A. C., 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. U.S.A. 86:6196-6200.

Krechel, A., Faupel, A., Hallmann, J., Ulrich, A., and Berg, G. 2002. Potato-associated bacteria and their antagonistic potential towards plant pathogenic fungi and the plant parasitic nematode Meloidogyne incognita (Kofoid & White) Chitwood. Can. J. Microbiol. 48:772-786.

Kumar, A., and Verma, J. P. 2018. Does plant–microbe interaction confer stress tolerance in plants: A review? Microbiol. Res. 207:41-52.

Lee, S. M., Kong, H. G., Song, G. C., and Ryu, C.-M. 2021. Disruption of *Firmicutes* and *Actinobacteria* abundance in tomato rhizosphere causes the incidence of bacterial wilt disease. ISME J.15:330-347.

Lesk, C., Rowhani, P., and Ramankutty, N. 2016. Influence of extreme weather disasters on global crop production. Nature 529:84.

Li, X., Meng, D., Li, J., Yin, H., Liu, H., Liu, X., Cheng, C., Xiao, Y., Liu, Z., and Yan, M. 2017. Response of soil microbial communities and microbial interactions to long-term heavy metal contamination. Environ. Pollut. 231:908-917.

Love, M. I., Huber, W., and Anders, S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15:550.

Ma, L.-J., Van Der Does, H. C., Borkovich, K. A., Coleman, J. J., Daboussi, M.-J., Di Pietro, A., Dufresne, M., Freitag, M., Grabherr, M., and Henrissat, B. 2010. Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. Nature 464:367-373.

Ma, Q. J., Sun, M. H., Lu, J., Kang, H., You, C. X., and Hao, Y. J. 2019. An apple sucrose transporter MdSUT2. 2 is a phosphorylation target for protein kinase *MdCIPK22* in response to drought. Plant Biotechnol. J. 17:625-637.

Ma, Y., Dias, M. C., and Freitas, H. 2020. Drought and salinity stress responses and microbe-induced tolerance in plants. Front. Plant Sci. 11:591911.

Munns, R. 2005. Genes and salt tolerance: Bringing them together. New Phytol. 167:645-663.

Nagpure, A., and Gupta, R. K. 2013. Purification and characterization of an extracellular chitinase from antagonistic *Streptomyces violaceusniger*. J. Basic Microbiol. 53:429-439.

Naik, P. R., Raman, G., Narayanan, K. B., and Sakthivel, N. 2008. Assessment of genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads isolated from rhizospheric soil. BMC Microbiol. 8:230.

Nautiyal, C. S., Srivastava, S., Chauhan, P. S., Seem, K., Mishra, A., and Sopory, S. K. 2013. Plant growth-promoting bacteria *Bacillus amyloliquefaciens* NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. Plant Physiol. Biochem. 66:1-9.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H., Szoecs, E., and Wagner, H. 2020. vegan: Community Ecology Package, version 2.5-7. https://github.com/vegandevs/vegan

Pandey, P., Irulappan, V., Bagavathlannan, M. V., and Senthil-Kumar, M. 2017. Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. Front. Plant Sci. 8:537.

Pandey, P., Ramegowda, V., and Senthil-Kumar, M. 2015. Shared and unique responses of plants to multiple individual stresses and stress combinations: Physiological and molecular mechanisms. Front. Plant Sci. 6:723.

Panwar, M., Tewari, R., Gulati, A., and Nayyar, H. 2016. Indigenous salt-tolerant rhizobacterium *Pantoea dispersa* (PSB3) reduces sodium uptake and mitigates the effects of salt stress on growth and yield of chickpea. Acta Physiol. Plant. 38:278.

Pikovskaya, R. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiology 17:362-370.

Pini, F., Galardini, M., Bazzicalupo, M., and Mengoni, A. 2011. Plant-bacteria association and symbiosis: Are there common genomic traits in *Alphaproteobacteria*? Genes 2:1017-1032.

Prittesh, P., Avnika, P., Kinjal, P., Jinal, H. N., Sakthivel, K., and Amaresan, N. 2020. Amelioration effect of salt-tolerant plant growth-promoting bacteria on growth and physiological properties of rice (*Oryza sativa*) under salt-stressed conditions. Arch. Microbiol. 202:2419-2428.

Qadir, M., Quillérou, E., Nangia, V., Murtaza, G., Singh, M., Thomas, R. J., Drechsel, P., and Noble, A. D. 2014. Economics of salt-induced land degradation and restoration. Nat. Resour. Forum 38:282-295.

Ramu, V. S., Paramanantham, A., Ramegowda, V., Mohan-Raju, B., Udayakumar, M., and Senthil-Kumar, M. 2016. Transcriptome analysis

of sunflower genotypes with contrasting oxidative stress tolerance reveals individual- and combined- biotic and abiotic stress tolerance mechanisms. PLoS One 11:e0157522.

Rybakova, D., Wikström, M., Birch-Jensen, F., Postma, J., Ehlers, R. U., Schmuck, M., Kollmann, R., Köhl, J., and Berg, G. 2020. Verticillium wilt in oilseed rape—The microbiome is crucial for disease outbreaks as well as for efficient suppression. Plants 9:866.

Saikia, J., Sarma, R. K., Dhandia, R., Yadav, A., Bharali, R., Gupta, V. K., and Saikia, R., 2018. Alleviation of drought stress in pulse crops with ACC deaminase producing rhizobacteria isolated from acidic soil of Northeast India. Sci. Rep. 8:3560. https://www.nature.com/articles/s41598-018-21921-w

Santoyo, G., Moreno-Hagelsieb, G., del, C. Orozco-Mosqueda, M., and Glick, B. R. 2016. Plant growth-promoting bacterial endophytes. Microbiol. Res. 183:92-99.

Sasse, J., Martinoia, E., and Northen, T. 2018. Feed your friends: Do plant exudates shape the root microbiome? Trends Plant Sci. 23:25-41.

Schwyn, B., and Neilands, J. 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160:47-56.

Setlow, P., and Kornberg, A. 1969. Biochemical studies of bacterial sporulation and germination XVII. Sulfhydryl and disulfide levels in dormancy and germination. J. Bacteriol. 100:1155-1160.

Singh, B., and Singh, G. 2004. Influence of soil water regime on nutrient mobility and uptake by *Dalbergia sissoo* seedlings. Tropical Ecol. 45:337-340.

Song, R., Li, J., Xie, C., Jian, W., and Yang, X. 2020. An overview of the molecular genetics of plant resistance to the Verticillium wilt pathogen *Verticillium dahliae*. Int. J. Mol. Sci. 21:1120.

Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., and Mittler, R. 2014. Abiotic and biotic stress combinations. New Phytol. 203:32-43.

Tang, Y., and Bonner, J. 1948. The enzymatic inactivation of indole acetic acid. II. The physiology of the enzyme. Am. J. Bot. 35:570-578.

Tiwari, G., Duraivadivel, P., Sharma, S., and Hariprasad, P. 2018. 1-Aminocyclopropane-1-carboxylic acid deaminase producing beneficial rhizobacteria ameliorate the biomass characters of *Panicum maximum* Jacq. by mitigating drought and salt stress. Sci. Rep. 8:17513.

Trivedi, P., Schenk, P. M., Wallenstein, M. D., and Singh, B. K. 2017. Tiny Microbes, Big Yields: Enhancing food crop production with biological solution. Microb. Biotechnol. 10:999-1003.

Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M., and SkZ, A. 2016. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol. Res. 184:13-24.

Wang, X., Hu, M., Xia, Y., Wen, X., and Ding, K. 2012. Pyrosequencing analysis of bacterial diversity in 14 wastewater treatment systems in China. Appl. Environ. Microbiol. 78:7042-7047.

Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J. 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173: 697-703.

Xu, L., and Coleman-Derr, D. 2019. Causes and consequences of a conserved bacterial root microbiome response to drought stress. Curr. Opin. Microbiol. 49:1-6.

Yaish, M. W., Al-Lawati, A., Jana, G. A., Vishwas Patankar, H., and Glick, B. R. 2016. Impact of soil salinity on the structure of the bacterial endophytic community identified from the roots of caliph medic (*Medicago truncatula*). PLoS One 11:e0159007.

Yang, H., Hu, J., Long, X., Liu, Z., and Rengel, Z. 2016. Salinity altered root distribution and increased diversity of bacterial communities in the rhizosphere soil of Jerusalem artichoke. Sci. Rep. 6:20687.

Zrenner, R., Verwaaijen, B., Genzel, F., Flemer, B., and Grosch, R. 2021. Transcriptional changes in potato sprouts upon interaction with *Rhizoctonia solani* indicate pathogen-induced interference in the defence pathways of potato. Int. J. Mol. Sci. 22:3094.