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RESEARCH ARTICLE

Beneficial soil fungi enhance tomato crop productivity and resistance to the leaf-mining pest *Tuta absoluta* in agronomic conditions

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Abstract

Research has shown that soil-borne beneficial microorganisms can enhance plant growth, productivity, and resistance against pests and pathogens and could thus serve as a sustainable alternative to agrochemicals. To date, however, the effect of soil-beneficial microbes under commercial crop production has been little assessed. We here investigated the effect of root inoculation with nine well-characterized bacterial and fungal strains and two consortia on tomato performance under intensive tomato crop management practices. We measured the impact of these root inoculations on plant growth, fruit quality, yield, and pest and pathogen incidence. While most microbial strains showed weak effects, we found that the fungal strains *Trichoderma afroharzianum* T22 and *Funneliformis mosseae* significantly increased marketable tomato yield. Moreover, we found that inoculation with most of the fungal strains led to a significant reduction in the incidence of the devastating leaf-mining pest *Tuta absoluta*, while this effect was not observed for bacterial inoculants. In addition, we found that microbial inoculations did not impact the incidence of introduced natural pest enemies, supporting their compatibility with well-established integrated pest management strategies in horticulture. In summary, the observed general positive effects of soil microbes on tomato yield and resistance reinforce the move toward broader adoption of microbial inoculants in future crop production, ultimately improving agricultural sustainability.

Keywords Beneficial soil-borne microorganisms · Bioinoculants · Crop protection · Field research · Microbe-induced resistance · Plant-microbe-insect interaction · *Tuta absoluta* · Yield improvement

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1 Introduction

The urgent need to enhance agricultural sustainability has compelled scientists, agroindustry, growers, and consumers to seek innovative approaches in order to mitigate the reliance on agrochemicals, all while ensuring optimal crop yields (Arora 2018). One emerging environmentally friendly biotechnology is the use of soil-beneficial microbes, applied as bioinoculants, to improve plant growth and productivity across a variety of systems (Berg 2009; Martínez-Medina et al. 2011; Barea 2015; Trivedi et al. 2017; Ab Rahman et al. 2018; Compant et al. 2019; Singh et al. 2020). Furthermore, several of these microbes have been shown to antagonize soil pathogens (Minchev et al. 2021) and boost the resistance of crops to a broad spectrum of pests and diseases, a phenomenon known as induced resistance (IR) (Pieterse et al. 2014; De Kesel et al. 2021). Therefore, the multifaceted functionality of soil-beneficial microbes could be harnessed to potentially increase crop productivity via direct boosting of resource acquisition and indirectly, by reducing yield losses due to pest and pathogen attacks (Li et al. 2022). Yet soil-beneficial microbes constitute a complex and diverse community of bacteria or fungi (Bakker et al. 2018), with their cumulative effect shown to be context- and crop-dependent (Lee Díaz et al. 2021). This context dependency and environmental impact on microbe functionality make the outcome of their application unpredictable, thereby currently limiting their competitiveness for commercial exploitation when compared to agrochemicals. Consequently, more studies in proper commercial settings are needed to test the efficacy of soil microbes for more sustainable agriculture practices.

Beneficial soil microbes used as bioinoculants include several functional groups, such as the plant growth–promoting rhizobacteria (PGPR), the plant growth–promoting fungi (PGPF), and the arbuscular mycorrhizal fungi (AMF) (Woo et al. 2014; Aamir et al. 2020; Bitterlich et al. 2020; Bamisile et al. 2021). Besides their growth-promoting properties, PGPR have been shown to act as efficient biological control agents, either by direct pathogen or disease suppression or through IR (Orozco-Mosqueda et al. 2021). Bacteria from the genera *Bacillus* and *Pseudomonas* are among the most studied and best characterized PGPR (Santoyo et al. 2012; Orozco-Mosqueda et al. 2021; Elnahal et al. 2022), as evidenced by the high number of commercial biofertilizer and biocontrol products containing them on the market (Aamir et al. 2020). Similarly, the ability of some PGPF species from the genus *Trichoderma* to promote plant growth and resistance to pests and pathogens has been widely acknowledged during the last decades (Harman et al. 2004; Martínez-Medina et al. 2014; Guzmán-Guzmán et al. 2019; Poveda 2021; Papantoniou et al. 2021; Woo et al. 2022; Modrzewska et al. 2022). However, their current success in the market as bioinoculants is primarily attributed to their mycoparasitic capacity, constituting 64.8% of available products claiming to be fungicidal (Woo et al. 2014). AMF are obligate biotrophs that establish symbiotic associations with the roots of most terrestrial plants, constituting one of the most studied plant-fungal interactions (Pozo et al. 2021). This symbiosis has been shown to improve plant nutrient uptake (van der Heijden et al. 2015; Sardans et al. 2023) and increase plant tolerance to biotic and abiotic stresses (Pozo & Azcón-Aguilar 2007; Rivero et al. 2018, 2021; Dejana et al. 2022; Ramírez-Serrano et al. 2022). Accordingly, the use of AMF has been amply proposed in sustainable agriculture (Smith & Smith 2011; Jeffries & Barea 2012; Barea 2015; Salomon et al. 2022a; Martin & van der Heijden 2024). Despite often lacking viable propagules (Salomon 2022b), commercially available AMF-based inoculants are steadily increasing on the market (Bitterlich et al. 2020). Commercial products containing AMF are mostly used in agriculture as biofertilizers, mainly for nutrient and growth promotion benefits, but also for stress alleviation (Basiru et al. 2021). Finally, entomopathogenic fungi (EPF) constitute another important group of fungi in agroecosystems because of their well-known ability to infect and kill insect and mite pests (Quesada-Moraga 2020). Besides this direct antagonism, EPF can interact and colonize plants endophytically, promoting plant growth and negatively affecting pathogens and phytophagous insects without direct contact with them (Gange et al. 2019; Quesada-Moraga 2020; Rasool et al. 2021; Bamisile et al. 2021). EPF have been used in the biological control of insects for more than 150 years. Currently, several commercial products are available, with more than 170 species formulated as biopesticides (Bamisile et al. 2021).

Besides single microbe applications, the design of synthetic microbial communities (SynComs) for improving plant growth and health is receiving increasing interest within the scientific community and on the market (Liu et al. 2020; Trivedi et al. 2020; Batista & Singh 2021; Minchev et al. 2021). SynComs can offer extended functionality compared to single-strain inoculations for the biocontrol of foliar and soil pathogens, as they can simultaneously combine different modes of action (Minchev et al. 2021). For instance, the combined application of EPF and AMF showed functional complementarity for plant protection and growth promotion (Zitlalpopoca-Hernandez et al. 2022).

Nonetheless, despite the widespread optimism for using these microbial inoculants to improve plant growth and health, most of the research has been performed under highly controlled conditions, posing challenges to the successful transfer and adoption of this technology in

agriculture (Mitter et al. 2019; Saad et al. 2020). Indeed, plant-microbe interactions and their effect on plant growth and health are often conditioned by environmental factors (Saad et al. 2020; Lee Díaz et al. 2021). For example, temperature (Di Lelio et al. 2021), nitrogen or phosphorous fertilization (Ramírez-Serrano et al. 2022; Dejana et al. 2022), soil water content (Orine et al. 2022), and light intensity (de La Hoz et al. 2021) and quality (Saha et al. 2022) have all been reported to impact plant-microbe interactions and microbial benefits for host plants. In other words, the intricate nature and context dependency of these interactions emphasize the need to consider how variable environmental conditions and agricultural practices can potentially impede the success and reproducibility of microbial inoculation results under field conditions (Compant et al. 2019). Consequently, it is essential to perform rigorous assessments of previously characterized plant-beneficial microorganisms within real production systems. Such evaluations serve not only to gauge microbial impacts on the target crop under commercial production conditions, but also to ascertain their compatibility with commonly used crop management practices.

To address this gap, we performed a comprehensive screening of microbial inoculants in order to evaluate their impact on plant protection against pathogens and pests, as well as their effects on plant growth and crop productivity under commercial production conditions (Fig. 1). We used tomato (*Solanum lycopersicum*, Fig. 1), the second most produced vegetable crop worldwide, as a model system. We selected well-characterized strains of bacteria and fungi and previously designed SynComs to test their impact on yield and pest resistance in a commercial greenhouse that uses standard tomato management practices, including integrated pest management (IPM) methods (Acebedo et al. 2022). We predicted that microbial inoculations would benefit plant performance by reducing susceptibility to biotic stressors without major costs in plant growth or production even under standard crop management practices.

2 Material and methods

2.1 Microbial treatments

We performed a large-scale experiment in a commercial greenhouse located at the Estación Experimental, Cajamar, Spain, with a total of 12 microbial treatments. Microbes were selected based on previous results obtained under controlled conditions within the Marie Skłodowska-Curie Innovative Training Network (MiRA, Nb. 765290). Our objective was to validate their reliability under commercial production conditions. Thus, the inoculants were prepared following the same methodology as in the previous studies that were conducted for their selection. The treatments included (1) two bacteria: *Bacillus amyloliquefaciens* CECT8238 (BA) and *Pseudomonas azotoformans* F30A (PA), (2) two strains of *Trichoderma afroharzianum*: T22 (T2) and *T. harzianum* T78 (T7), (3) two EPF: *Beauveria bassiana* KVL 13-39 (BB) and *Metarhizium robertsii* KVL 12-35 (MR), and (4) three AMF: *Rhizophagus irregularis* MUCL57021 (RI), *Funneliformis mosseae* BEG12 (FM), and *Claroideoglomus etunicatum* EEZ163 (CE). In addition, (5) two SynComs (M1, M2), described below, and (6) a control treatment without soil microbe addition (non-inoculated, NI) were included. BB and MR strains were obtained from a collection of entomopathogenic fungal cultures at the University of Copenhagen. RI, T2, PA, and BA strains were obtained from the microbial collection of Koppert B.V. (The Netherlands). FM and CE inoculants were obtained from the AMF collection of Estación Experimental del Zaidín–CSIC

Fig. 1 Picture showing tomato plants from the experiment conducted under commercial production conditions, which included common tomato crop management practices. Photo credits: Guadalupe Zitlalpopoca-Hernandez.

(Spain). Finally, T7 (CECT 20714) was obtained from the Spanish collection of type cultures (Valencia University).

The bacterium *B. amyloliquefaciens* was cultured on tryptone soy agar (TSA) and grown at 28 °C for 24 h. For spore production, liquid Difco sporulation medium (Nicholson & Setlow 1990) was inoculated with a single bacterial colony and incubated at 28 °C for 48 h with rotary shaking at 200 rpm. Spore concentration of the liquid culture was quantified using a Neubauer hemocytometer, and then, the culture was centrifuged for 15 min at 5000 rpm to separate the spores from the growing medium. Finally, the recovered spores were resuspended in sterile water to a concentration of 1×10^7 spores/mL. For inoculation, 1 mL of spore solution was applied to each plant in the root system during transplanting (Minchev et al. 2021). The bacterium *P. azotoformans* was cultured on TSA and grown at 28 °C for 24 h. A pre-culture was prepared in tryptone soya broth (TSB) inoculated with a single colony and incubated overnight at 28 °C with rotary shaking at 200 rpm. Next, 1 mL of pre-culture was added to 25 mL of TSB and incubated at 28 °C for 2 h 30 min with rotary shaking at 200 rpm to reach the exponential growth phase. Then, the cell concentration was quantified by measuring the optical density (620 nm) of the bacterial culture using a spectrophotometer. The bacterial culture was centrifuged for 15 min at 5000 rpm to separate the bacterial cells from the growing medium. Finally, the obtained cells were resuspended in sterile water to a concentration of 1×10^7 CFU/mL. For inoculation, 1 mL of bacterial solution per plant was applied to the root system during transplanting (Minchev et al. 2021). The fungus *T. afroharzianum* strain T22 was cultured on potato dextrose agar (PDA) and grown at room temperature for 7 days. The sporulated plates were scraped using a sterile spatula and sterile water. The resulting spore suspension was filtered using a sterile miracloth filter to remove remaining mycelia, and the spore concentration was quantified using a Neubauer hemocytometer and adjusted to 1×10^7 spores/mL. For inoculation, 1 mL of spore suspension was added to the root system of each plant during transplanting (Minchev et al. 2021). The fungus *T. harzianum* strain T78 was cultured on PDA. The fungal inoculum was prepared by adding aseptically a square piece of the fungal culture on a sterile mix of vermiculite and oat (Martínez-Medina et al. 2009) and incubated at 28 °C in the dark for 5 days. The inoculum, containing 1×10^9 spores/g, was mixed with the substrate in a proportion of 1 g per Kg of substrate (Martínez-Medina et al. 2013). The EPF *B. bassiana* and *M. robertsii* were cultured in Sabouraud dextrose agar (SDA) and grown at 24 °C in darkness for 3 weeks. The sporulated plates were scraped using a sterile spatula, and the spores were recovered in a sterile solution of Triton X (0.05 %). The spore concentration was quantified using a Neubauer hemocytometer and adjusted to 1×10^8 spores/mL. Inoculation was done by adding 1 mL of spore suspension per plant directly to the roots during transplanting (Zitlalpopoca-Hernandez et al. 2022). The AMF *R. irregularis* was grown in vitro on a minimal (M) medium with *Agrobacterium rhizogenes*-transformed carrot (*Daucus carota*) roots as host (St-Arnaud et al. 1996). Spore extraction was performed by adding citrate buffer (0.01 M, pH = 6) to the AMF culture in a proportion of 3:1 (v/v) and maintained for 1 h on a rotary shaker to dissolve the agar. The spores were recollected using sieves with mesh sizes of 250 and 53 µm and resuspended in sterile water at 1000 spores/mL. For inoculation, 1 mL of spore solution was applied to the root system of each plant (Minchev et al. 2021). The AMF *F. mosseae* and *C. etunicatum* were maintained as living inocula on mixed cultures of *Trifolium repens* and *Sorghum vulgare* in vermiculite-sepiolite substrate. The inoculants consisted of the substrate containing colonized root fragments, mycelia, and spores. For inoculation, 10% (v/v) of mycorrhizal inocula were mixed with the substrate at transplanting (Rivero et al. 2018).

Further, two SynComs were used, which were selected based on previous studies (Minchev et al. 2021; Zitlalpopoca-Hernandez et al. 2022). The M1 inoculum included *B. amyloliquefaciens*, *P. azotoformans*, and *T. afroharzianum* T22 at concentrations of 1×10^7 CFU/mL each and *R. irregularis* at a concentration of 1000 spores/mL (Minchev et al. 2021). The M2 inoculum included *M. robertsii* and *B. bassiana* both inoculated at a concentration of 1×10^8 spores/mL and *R. irregularis* at a concentration of 1000 spores/mL. For both SynComs, 1 mL/plant was applied to the root system during transplanting.

2.2 Plant material and growing conditions

Solanum lycopersicum cv Money maker seeds (Vreeken's Zaden, The Netherlands) were surface sterilized by immersion in 5% sodium hypochlorite solution for 10 min and rinsed three times in sterile water for 10 min each. The surface sterilized seeds were sown in sterile vermiculite and incubated for 7 days in a greenhouse at 24 °C:16 °C day-night with a photoperiod of 16 h:8 h light-dark and 70% of relative humidity.

2.3 Quantification of microbial colonization

Mycorrhizal colonization was assessed by quantifying the percentage of root length containing fungal structures upon staining. Briefly, the roots were washed with tap water, cleared in 10% KOH, acidified with 2% acetic acid, and stained with 5% black ink (Lamy, Germany) dissolved in 2% acetic acid (García et al. 2020). After removing the excess ink, the roots were randomly placed in a Petri dish with gridlines, and the percentage of root length colonized by AMF was quantified under a binocular microscope using

the gridline intersect method described by Giovannetti and Mosse (1980).

For PGPR and PGPF, we assessed the presence of each microbe in rhizospheric soil as described by Minchev et al. (2021). For this, we sampled 1 g of rhizospheric soil and diluted it in 9 mL of sterile tap water. Samples were then homogenized in a horizontal shaker for 1 h at 350 rpm. To detect *Trichoderma*, samples were plated on PDA + igepal (11 mL/L) + tetracycline (50 µg/mL), and for bacteria, samples were plated on TSA + natamycin (0.1 g/L). The plates were then incubated at 25 °C, and the presence or absence of the microbes was determined after 24 h for bacteria and after 48 h for *Trichoderma* (Minchev et al. 2021).

For EPF, fresh roots were cut into pieces of 1 cm and mixed. Fifteen pieces of roots were selected and homogenized with a pestle in 5 mL of sterilized Triton X (0.05%). Then, 100 µL of the homogenate were spread in Petri dishes with selective media (500 mL of SDA containing agar 6 g, glucose 10 g, peptone 5 g, dodine 0.2 mL of 0.1 g/mL, streptomycin 0.5 mL of 0.6 g/mL, tetracycline 0.5 mL of 0.05 g/mL, and cycloheximide 1 mL of 0.05 g/mL, pH 6.3–6.5) and incubated at 24 °C in darkness during 14 days. Fungal colonies with morphological features to the inoculated EPF species were quantified as colony-forming units (CFU) on the 7th and corroborated on the 14th day.

2.4 Experimental set-up

One-week-old tomato seedlings were transferred to starting trays, with cell dimensions 2.9 × 2.9 × 6.8 cm—containing blond seedling peat (Kekkilä LSM 0 R8406, Projar, Valencia, Spain)-zeolite-perlite (1:1:1) mixture and inoculated with the microbial treatments described previously. Inoculated seedlings were grown in a commercial nursery (ACRENA SAT 251, El Ejido, Spain; 36°, 47′, 52.9″ N; 2°, 43′, 36.3″ W) for 4 weeks. Before transplanting to the greenhouse, microbial colonization of all beneficial microbes was assessed in a subset of plants, confirming the successful establishment of all microorganisms in close to 100% of the plants (Table S3). On September 3, 2020, the plants were transplanted to a commercial production greenhouse (Estación experimental Cajamar, Paraje las Palmerillas, El Ejido, Almería; 36°, 47′, 36.3″ N; 2°, 43′, 15.2″ W) and maintained during the whole crop cycle from September 2020 to March 2021. The greenhouse consisted of a typical "raspa y amagado" type (Ávalos-Sánchez et al. 2022), 37.8 m long and 23.2 m wide with a total area of 877 m^2 and usable area of 720 m^2, passive ventilation (25.0% window surface) with side windows (north and south sides) and zeniths, covered with anti-trip mesh. The microbial inoculation treatments were organized following a randomized complete block design, with four blocks. Each block contained 12 treatments, and each treatment in all blocks consisted of a group of six plants (pseudo-replicates) (Fig. S1; $N = 12$ treatments × 4 blocks × 6 pseudo-replicates = 288 plants).

2.5 Application of biological control products, pheromones, and pollinators

Two weeks after transplanting, the predatory mirid bug *Nesidiocoris tenuis* (Hemiptera: Miridae) (NESIDIOcontrol, Agrobio, Spain) was released in the greenhouse with a density of 0.5–1.5 individuals/m^2 following the product label recommendation to reduce incidence of whiteflies (Hemiptera: Sternorrhyncha) and *Tuta absoluta* (Lepidoptera: Gelechiidae) on tomato plants. In addition, pheromones for the mating disruption of *T. absoluta* were released during the whole cropping season. To ensure pollination of tomato flowers from the start of flowering, bumblebees *Bombus terrestris* (Hymenoptera: Apidae) were released 3 weeks post transplantation (wpt), placing one hive (Agrobio, Spain) in the middle of the greenhouse.

2.6 Irrigation and fertilization

The irrigation scheme and nutrient supply followed throughout the cropping season correspond to realistic tomato commercial fertilization regimes in production systems in the area (detailed in Table S1). Nutrient content in soil and irrigation water (nutrient solution) was evaluated periodically to adjust to the crop needs for nutrient supply. Specifically, phosphorus was measured by visible spectrophotometry using the compound phosphorous vanadate molybdate (Tandon et al. 1968). Nitrates were measured spectrophotometrically at 220 and 275 nm (Norman & Stucki 1981). Ammonia was measured by the Nessler reagent method (Yuen & Pollard 1954). Sodium, calcium, potassium, magnesium, iron, copper, manganese, and zinc were determined by atomic absorption/emission (Isaac & Kerber 2015). Carbonates and bicarbonates were measured by titration with 0.01 N sulfuric acid (Allison et al. 1954). Chlorides were also measured by volumetry with silver nitrate between 0.01 and 1 N using potassium chromate as an indicator (Mohr's titration). Boron was determined by spectrophotometry with the azomethine reaction (John et al. 2006). Sulfates were measured by precipitation of barium sulfate.

2.7 Response variables and data collection

In total, we measured 17 response variables related to plant growth and yield and plant resistance to pathogens and insect pests (see below and Table S2). Data collection for each response variable was done on the below-mentioned specific time points, mostly for practical reasons, unless otherwise specified.

2.8 Plant growth, nutritional status, and yield

As a proxy of plant growth, plant height from the soil surface to the top of the shoot of each plant (six plants per treatment per block) was measured on December 3, 2020 (12 wpt). As a proxy for plant productivity, we quantified the number of inflorescences per plant (six plants per treatment per block) on October 26, 2020 (8 wpt), before the onset of the fruit collection period. Leaves for total leaf carbon and nitrogen content measurements were sampled on January 21, 2021 (19 wpt), evaluating three plants per treatment per block. Leaves were sampled, immediately frozen in liquid nitrogen, and lyophilized. Then, lyophilized leaves were ground in a Tissue Lyser II (Qiagen, Germany) using metal beads at a maximum speed for 3 min. Two milligrams were weighed from each sample to measure total carbon (C) and nitrogen (N) content, using a Flash 1112 Elemental Analyzer (Thermo Scientific, MA, USA).

Fruit productivity (average g/plant) was evaluated regularly, when fruits were in an optimal state of ripeness for collection, for a total of 10 time points, between November 12, 2020 (10 wpt), and February 4, 2021 (22 wpt). Fruits were sampled from six plants per treatment per block, and fruit biomass was evaluated at the block level. Tomato fruits were classified by size (size GG 82–102 mm; size G 67–82 mm; size M 57–67 mm; size MM 47–57 mm) and by categories (first, second, and non-commercial). Fruits were considered non-commercial when their size was too small (< 45 mm in diameter), when they showed the presence of pathogen damage, cracks, blossom-end rot, or blotchy ripening or when they were misshapen.

2.9 Fruit quality and nutraceutical value

Parameters such as fruit dry weight (determined after drying the fruits in a forced air stove at 70 °C for 48 h), acidity percent (acid-base volumetry using 1 N NaOH as base and phenolphthalein indicator), Brix or total soluble solids (manual refractometer), and maturity index (the ratio between the content of total soluble solids and assessable acidity) were assessed on December 16, 2020 (14 wpt) on one fruit per treatment per block ($n = 4$).

Polyphenol and carotenoid content in fruits were evaluated on February 25, 2021 (23 wpt) on one fruit per treatment per block ($n = 4$). Polyphenols were measured by the spectrophotometric method of Folin-Ciocalteau (Georgé et al. 2005) using a standard curve of gallic acid from 0 to 1000 ppm at 760 nm (double ultraviolet-visible beam, Unicam Helios Alpha) and expressed as milligrams of gallic acid/100 g dry fruit biomass. Lycopene and beta-carotene content of fruits were measured with an acetone-hexane extraction and spectrophotometric determination at 487.5 nm (Sadler et al. 1990) with modifications (Rousseaux et al. 2005) and expressed as mg/100 g fresh fruit.

2.9.1 Pest and disease incidence

The incidence of thrips, *T. absoluta*, whiteflies, and powdery mildew was evaluated on December 3, 2020 (12 wpt), when the presence of these pests or diseases was high enough to be accurately assessed simultaneously. For thrips, occurrence was assessed by counting the number of leaves per plant presenting lacerations caused by thrips, evaluating three plants per treatment per block. The incidence of *T. absoluta* was estimated as the percentage of plants per treatment displaying mines (the typical lesions caused by the larvae of this species), evaluating the six plants per treatment per block. Whiteflies were evaluated using yellow sticky traps for a period of 4 weeks until December 3, 2020 (12 wpt). One sticky trap was used per treatment per block, placed in the middle of the six plants of each block (Fig. S1). The number of whiteflies per trap was counted. Finally, powdery mildew prevalence was measured as the percentage of infected plants per treatment, evaluating three plants per treatment per block.

2.9.2 Abundance of natural enemies

The abundance of the predatory mirid bug *Nesidiocoris tenuis*, released in the greenhouse at the beginning of the cropping season for the control of whiteflies and *T. absoluta*, was evaluated on December 3, 2020 (12 wpt) at the same time point as pest incidence. As for whitefly incidence (see above), one yellow sticky trap per treatment per block was evaluated by counting the number of *N. tenuis* per trap.

2.9.3 Statistical analysis

Data were analyzed using R statistical language, version 4.1.1 (R Development Core Team 2021). Figures were produced using the package ggplot2 (Wickham 2009). The effects of the 12 microbial treatments (including the control) on each of the 17 different response variables were analyzed with linear mixed- or generalized mixed-effect models (lmer or glmer functions in the lmerTest package (Kuznetsova et al. 2017)), with treatments as fixed factors and blocks as random factors as follows: lmer(var ~ Treatment + (1|Block) + (1|Block:Plot)) (Table S2). Generalized mixed-effect models were performed when data did not meet normality assumptions as shown in Table S2. For productivity-related variables, the treatment effect was tested using a repeated measures mixed-effect model as follows: lmer(Production ~ Treatment + (1|Block) + (1|Block:Day)). If significant differences among treatments were detected, the different microbial treatments

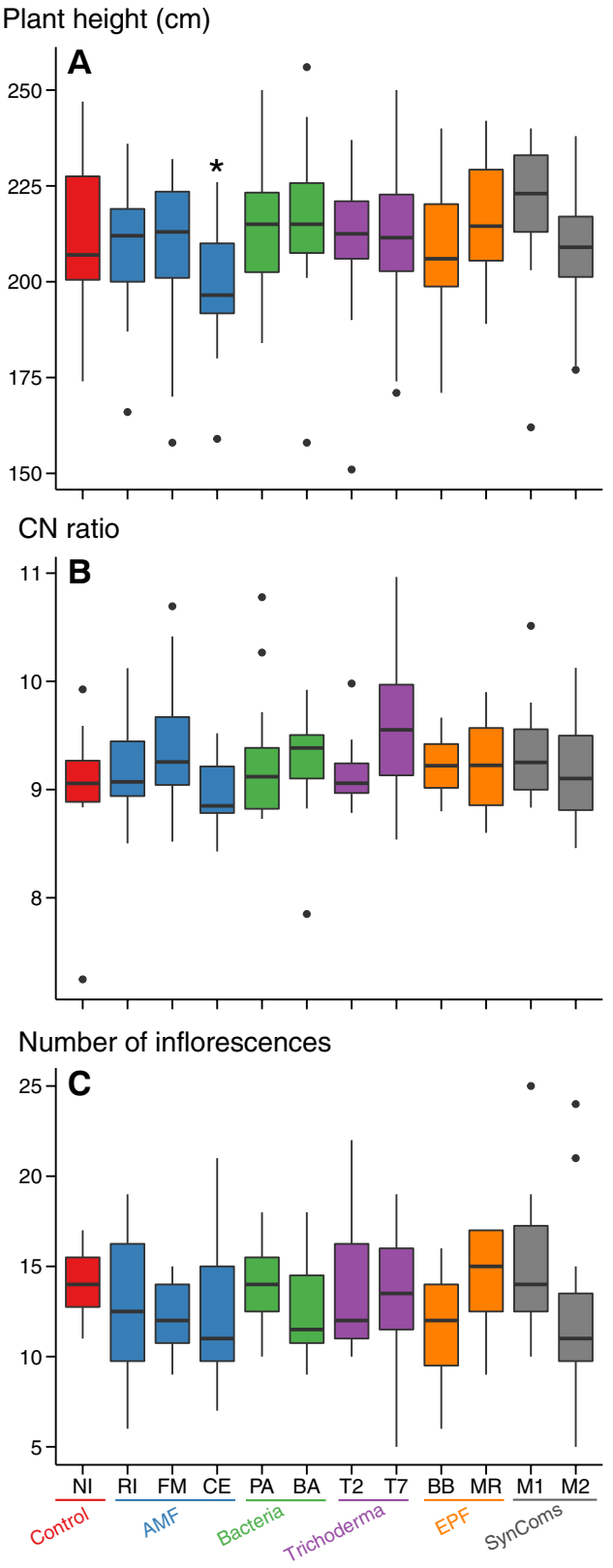


Fig. 2 Impact of microbial inoculation on **A** plant height, **B** leaf carbon-nitrogen ratio, and **C** number of inflorescences. Plants were inoculated with *R. irregularis* (RI), *F. mosseae* (FM), *C. etunicatum* (CE), *P. azotoformans* (PA), *B. amyloliquefaciens* (BA), *T. afroharzianum* T22 (T2), *T. harzianum* T78 (T7), *B. bassiana* (BB), *M. robertsii* (MR), consortium 1 (M1) including RI+PA+BA+T2, and consortium 2 (M2) including RI+BB+MR. Non-inoculated plants were included as a control (NI). Boxes represent the interquartile range, black lines represent the median, whiskers represent the maximum and minimum within 1.5 times the interquartile range, and black dots represent outliers. The asterisk indicates statistically significant differences compared to the control treatment (red boxplots) (*$p < 0.05$).

were compared to the control (non-inoculated) treatment with multiple comparisons of means using the multcomp package (Hothorn et al. 2008). Model validation was performed graphically by inspecting the residuals and fitted values (Zuur and Ieno, 2016). Moreover, to visualize the overall effect of soil microbial inoculation on plant resistance, we produced radar plots using scaled values for each of the four pests and pathogens studied (function *ggradar* in ggplot2) and calculated the area of each polygon generated for each treatment using R.

3 Results

3.1 Effect of soil-beneficial microbes on plant growth, nutritional status, and flowering

For plant height, we found weak negative effects of the soil microbial treatments, only given by *C. etunicatum.* This AMF decreased tomato plant height on average by 12.96 cm compared to the control plants (Fig. 2A, treatment effect; $Chisq_{11,284} = 25.58$, $p = 0.01$). We however found no effect of soil microbial treatments on C and N leaf content, resulting in an unaltered C/N ($Chisq_{11,144} = 16.63$, $p = 0.12$; Fig. 2B) nor on the number of inflorescences ($Chisq_{11,144} = 9.73$, $p = 0.56$; Fig. 2C).

3.2 Effect of soil-beneficial microbes on fruit yield

Soil microbial inoculations had significant effects on total tomato production ($Chisq_{11,480} = 69.85$, $p < 0.001$), as well as on commercial quality tomato production ($Chisq_{11,480} = 74.58$, $p < 0.001$). Specifically, *F. mosseae* ($z = 4.35$, $p < 0.001$) and *T. afroharzianum* T22 ($z = 4.65$, $p < 0.001$) inoculated plants showed a 13% and 15% higher total productivity than control plants, respectively (Fig. 3A). Even more so, the same soil microbes increased commercial quality

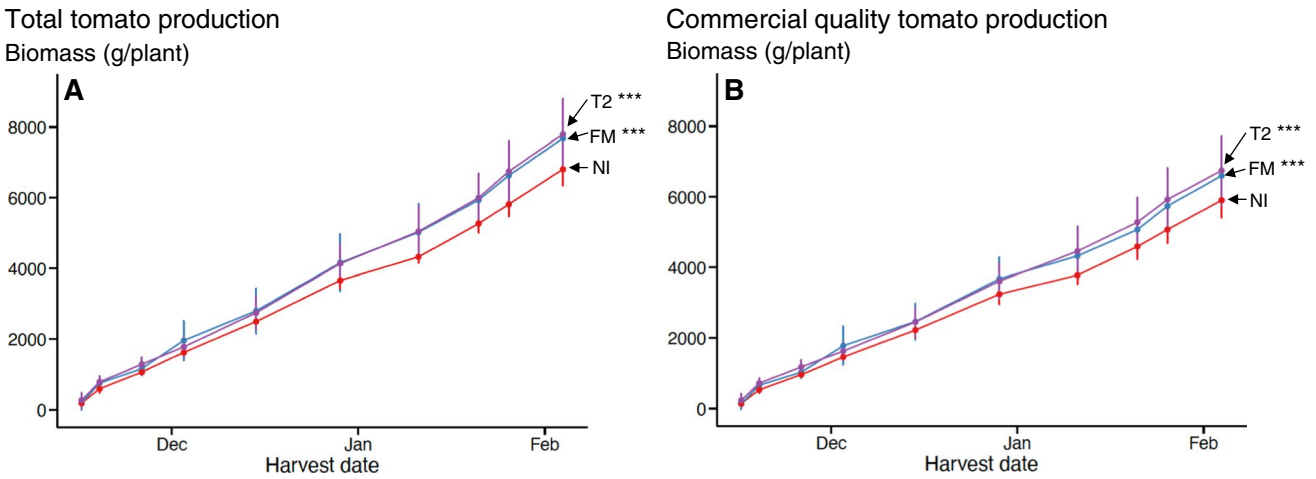


Fig. 3 Impact of microbial inoculants on tomato production. Scatter plots show only microbial treatments with a significant impact on tomato production as compared to the control treatment. **A** Total tomato production and **B** commercial quality tomato production. Plants were inoculated with *F. mosseae* (FM, blue) and *T. afroharzianum* T22 (T2, purple). Non-inoculated plants were included as a control (NI, red). Lines represent the average yield increase across time, dots represent the mean tomato biomass, and error bars represent ± one standard error. Asterisks indicate statistically significant differences compared to the control (***$p < 0.001$).

fruit production by 12% ($z = 3.88$, $p < 0.001$) and 14% ($z = 4.71$, $p < 0.001$) respectively at the end of the experiment as compared to the non-inoculated control plants (Fig. 3B).

3.3 Effect of soil-beneficial microbes on fruit quality and nutraceutical value

We did not observe any effect of the microbial inoculations on Brix ($Chisq_{11,48} = 11.02$, $p = 0.44$), maturity index ($Chisq_{11,48} = 14.19$, $p = 0.22$), and %Dry weight ($Chisq_{11,48} = 9.43$, $p = 0.58$). The only fruit quality parameter significantly affected by the microbial treatments was the %Acidity ($Chisq_{11,48} = 27.07$, $p = 0.01$), in which fruits from *C. etunicatum* inoculated plants showed an increase of 0.04% in acidity compared to the control treatment ($z = -2.25$, $p = 0.03$; Table S3).

Furthermore, regarding fruit nutraceutical value, we did not find that fruit polyphenols' content was significantly affected by the soil microbial treatment ($Chisq_{11,48} = 6.11$, $p = 0.87$, Fig. S2A), as well as carotenoids' content of fruits (lycopene, beta-carotene, and total carotenoids) were also not significantly altered by soil microbes ($Chisq_{11,48} = 16.79$, $p = 0.11$, Fig.S2B; $Chisq_{11,48} = 7.69$, $p = 0.74$, Fig. S2C and $Chisq_{11,48} = 15.70$, $p = 0.15$, Fig. S2D, respectively).

3.4 Effect of soil-beneficial microbes on natural enemies

To evaluate any potential impact of the microbial inoculants on applied beneficial insects, we evaluated the abundance of the predatory mirid bug *N. tenuis*, released in the greenhouse at the beginning of the cropping season for the control of whiteflies and *T. absoluta*. *Nesidiocoris tenuis* abundance was affected by microbial treatments ($Chisq_{11,48} = 19.82$, $p = 0.03$), but no significant differences were observed between the microbial treatments and the control plants (Fig. S3).

3.5 Effect of soil-beneficial microbes on pest and disease incidence

We found that the incidence of *T. absoluta* was significantly impacted by microbial inoculation ($Chisq_{11,284} = 19.77$, $p = 0.048$). In particular, *R. irregularis* ($z = -2.69$, $p = 0.007$), *F. mosseae* ($z = -2.30$, $p = 0.02$), *C. etunicatum* ($z = -2.30$, $p = 0.02$), *T. afroharzianum* T22 ($z = -2.30$, $p = 0.02$), *T. harzianum* T78 ($z = -1.97$, $p = 0.048$), *M. robertsii* ($z = -2.68$, $p = 0.008$), and the consortium M2 ($z = -1.97$, $p = 0.048$) treatments significantly decreased the percentage of plants damaged by the leaf miner as compared to the control treatment, with average reductions ranging from 60% for *T. harzianum* T78 up to 90% for *R. irregularis* and *M. robertsii* (Fig. 4A).

Microbial treatments also significantly affected thrip incidence ($Chisq_{11,144} = 21.95$, $p = 0.03$). This effect was evidenced by a reduction of the pest damage in plants inoculated with either *T. afroharzianum* T22 ($z = -2.31$, $p = 0.02$) or *B. bassiana* ($z = -2.15$, $p = 0.03$) compared with control plants (Fig. 4B). Whitefly incidence was not significantly affected by the microbial treatments ($Chisq_{11,48} = 11.38$, $p = 0.41$; Fig. 4C). Regarding diseases, powdery mildew was the only pathogen that naturally appeared on

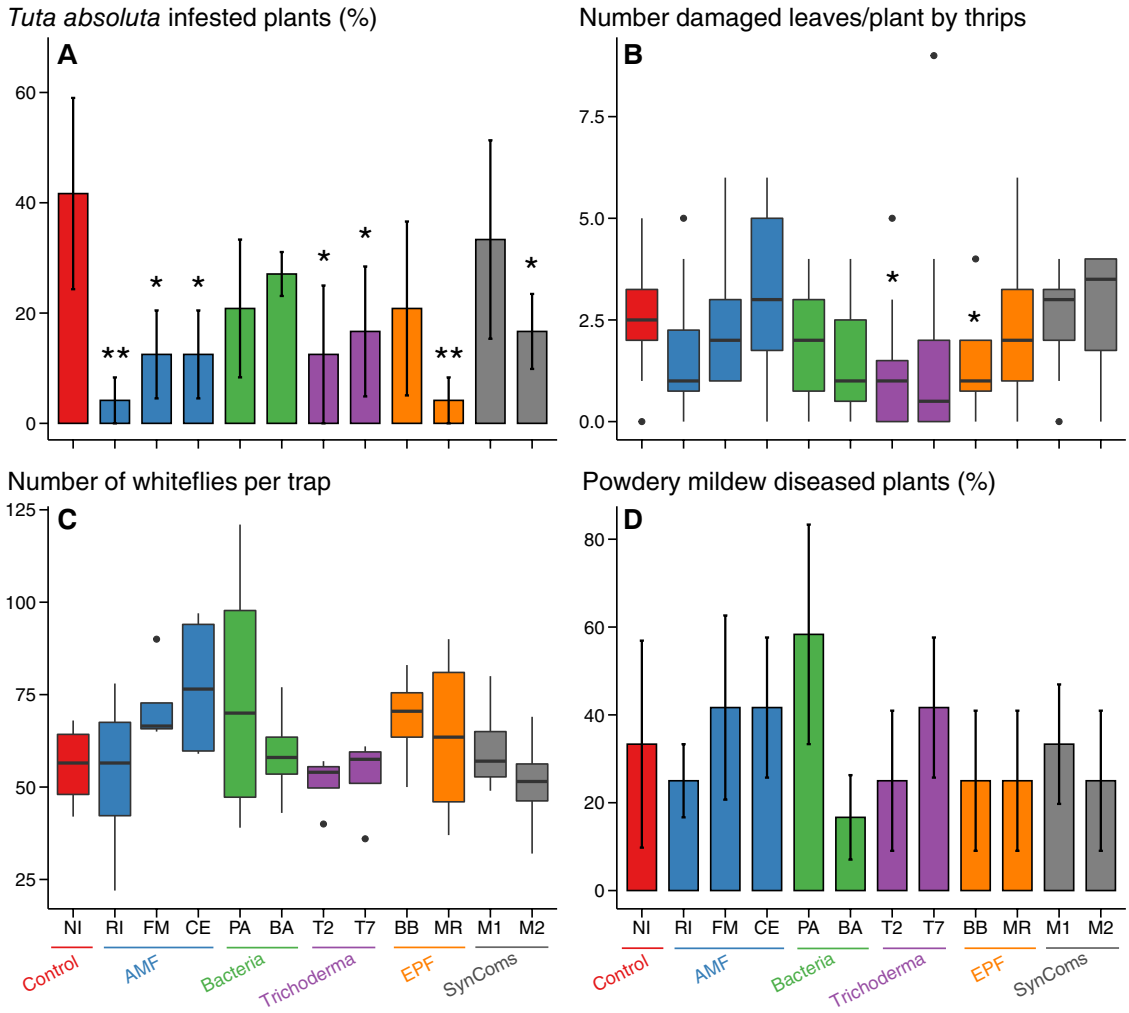


Fig. 4 Impact of microbial inoculation on **A** percent of plants infested by *T. absoluta*, **B** number of damaged leaves per plant by thrips, **C** number of whiteflies per trap, and **D** percent of plants diseased by powdery mildew. Plants were inoculated with: *R. irregularis* (RI), *F. mosseae* (FM), *C. etunicatum* (CE), *P. azotoformans* (PA), *B. amyloliquefaciens* (BA), *T. afroharzianum* T22 (T2), *T. harzianum* T78 (T7), *B. bassiana* (BB), *M. robertsii* (MR), consortium 1 (M1) including RI+PA+BA+T2, and consortium 2 (M2) including RI+BB+MR. Non-inoculated plants were included as a control (NI). Boxes represent the interquartile range, black lines represent the median, whiskers represent the maximum and minimum within 1.5 times the interquartile range, and black dots represent outliers. Asterisks indicate a statistically significant difference compared to the control (red boxplots) (*$p < 0.05$, **$p < 0.01$).

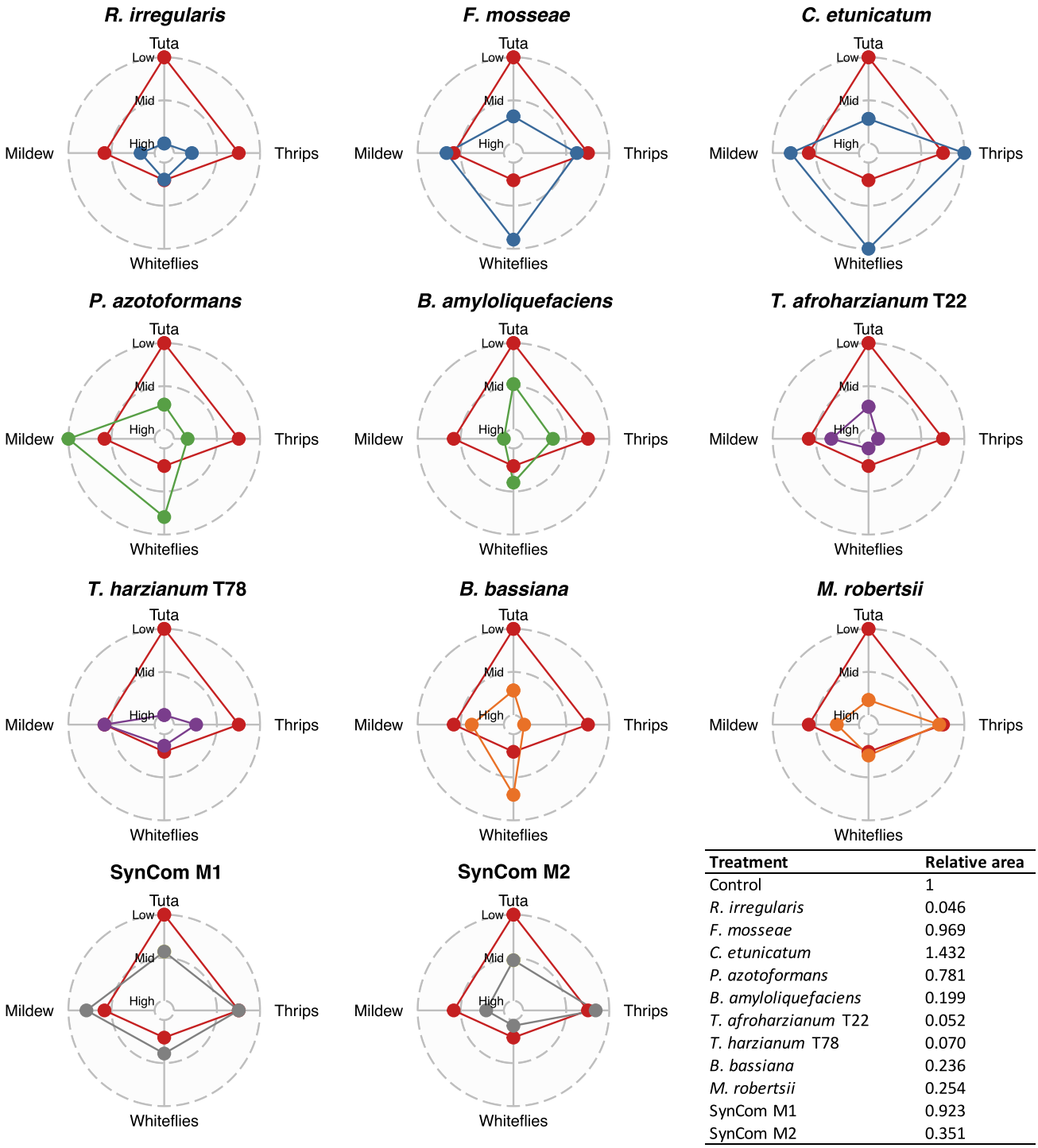
the crop. No significant differences between microbial treatments were observed in the incidence of powdery mildew ($Chisq_{11,144} = 8.22$, $p = 0.69$; Fig. 4D).

3.6 Compound effect of soil-beneficial microbes on plant resistance

As represented in radar plots, where smaller areas indicate a higher level of plant resistance, the polygons corresponding to microbial treatments exhibit reduced areas relative to control plants (represented in red in Fig. 5), except for *C. etunicatum* (see relative areas in Fig. 5). Among them, the smallest area was displayed by the AMF *R. irregularis*, with a decrease of 95% compared to the control treatment. These results suggest that besides *C. etunicatum*, soil microbial inoculations increase the general resistance of tomato plants against the four pests and pathogens studied.

4 Discussion

In this study, by testing diverse plant-beneficial microorganisms under commercial settings, we demonstrated the viability of using microbial inoculants for crop protection and yield improvement for a commercial crop production system. We have identified microbial strains that can be used



Treatment	Relative area
Control	1
R. irregularis	0.046
F. mosseae	0.969
C. etunicatum	1.432
P. azotoformans	0.781
B. amyloliquefaciens	0.199
T. afroharzianum T22	0.052
T. harzianum T78	0.070
B. bassiana	0.236
M. robertsii	0.254
SynCom M1	0.923
SynCom M2	0.351

Fig. 5 Radar plots showing the overall effects of different soil microbial inoculations on plant resistance against pests and pathogens. Each panel represents the level of resistance against *Tuta absoluta*, thrips, whiteflies, and mildew of plants inoculated with each tested microbe with respect to control plants' resistance (red polygons). Microbial treatments include *Rhizophagus irregularis*, *Funneliformis mosseae*, *Claroideoglomus etunicatum*, *Pseudominas azotoformans*, *Bacillus amyloliquefaciens*, *Trichoderma afroharzianum* T22, *Trichoderma harzianum* T78, *Beauveria bassiana*, *Metarhizium robertsii*, SynCom M1 (*R. irregularis* + *P. azotoformans* + *B. amyloliquefaciens* + *T. afroharzianum* T22), and SynCom M2 (*R. irregularis* + *B. bassiana* + *M. robertsii*). Radar plots show the scaled values for the percent damage by *T. absoluta* per plant, the number of damaged leaves per plant by thrips, the number of whiteflies per trap, and the average percent of leaves per plant infested by powdery mildew. Higher values mean that the attack is stronger, and plant resistance is low (and vice versa). The table shows the scaled area of each polygon relative to the polygon area of the control.

as biostimulants and bioprotectors under real tomato production conditions, thus confirming their potential as bioinoculants to improve agricultural sustainability. Considering all measured traits, our results point out a prominent effect of fungal inoculants (including different AMF, EPF, and *Trichoderma* strains) in promoting plant resistance and, in some cases, improving crop yield, while the tested bacterial inoculants did not show any significant effect on the evaluated parameters.

4.1 Soil-beneficial microbe effect on tomato growth and yield

Plant-beneficial microbes such as PGPR, *Trichoderma*, AMF, and EPF have been widely reported to improve plant growth and nutritional status (Quesada-Moraga 2020; Orozco-Mosqueda et al. 2021; Salomon et al. 2022a, b; Woo et al. 2022). Contrary to previous observations, the microbial inoculation did not impact plant height or leaf C/N under commercial production conditions. Although plants inoculated with the AMF *C. etunicatum* were significantly smaller than control plants, this reduction did not negatively affect tomato yield, which is the most relevant parameter for tomato producers. The lack of microbial effects on plant nutritional levels can be related to the standard periodic application of fertilization so that the plant nutritional needs were sufficiently covered, making the role of nutrient acquisition by the beneficial microbes redundant. Indeed, nutritional benefits by interaction with beneficial microorganisms are usually visible only under limiting conditions (Martínez-Medina et al. 2011). Specifically, under reduced fertilization dosage, some of the tested microbes such as *T. harzianum* T78, *R. irregularis*, and *F. mosseae* have shown to improve plant growth, nutrient acquisition, and fruit production in melon plants under field conditions, while such effects were absent under conventional fertilization conditions (Martínez-Medina et al. 2011).

Yet, when evaluating the potential of microbial inoculants as biostimulants, crop yield and fruit quality are the most relevant parameters, particularly for the economy of producers. A recent meta-analysis conducted on 97 peer-reviewed articles (69% conducted under greenhouse and 31% under field conditions) that examined the effect of different microbial inoculants—mostly PGPR—on crop productivity concluded that microbial inoculants can overall improve crop productivity, mainly by stress alleviation or by improving nutrient availability for plants (Li et al. 2022). Our findings demonstrate that while none of the treatments influenced flower production, two fungal treatments, the AMF *F. mosseae* and the fungus *T. afroharzianum* T22, increased the total and marketable tomato yield during the cropping season. Increased tomato yield after the application of microbial inoculants can result from a better alleviation of stress (Li et al. 2022). In agreement with this hypothesis, we observed that the two fungal treatments that increased yield also caused significant pest reduction of the leaf miner *T. absoluta* (see below). Of particular interest is the fact that tomato plants with increased yield did not show a trade-off by reducing fruit quality, suggesting a net benefit for the farmers under these conditions.

4.2 Soil microbial effect on tomato resistance against pests

Soil-borne beneficial microbes are widely reported to improve plant resistance by triggering defenses against a broad range of attackers, including pathogens and herbivorous insects (Pieterse et al. 2014). Here, we evaluated the impact of microbial inoculation on the incidences of powdery mildew, the phloem and cell content feeders whiteflies and thrips respectively, and the leaf miner *T. absoluta*. While we found no effect of soil microbes on powdery mildew or whiteflies, T22 and *B. bassiana* reduced thrip damage, while the percentage of plants damaged by the leafminer *T. absoluta* was significantly reduced by most of the fungal inocula. This benefit was not observed upon inoculation with PGPR, underscoring the positive outcomes associated with fungal inoculations. All three mycorrhizal strains, both *Trichoderma* strains, the EPF *M. robertsii*, and the M2 SynCom (fungal consortia including the EPF *B. bassiana* and *M. robertsii*, and the AMF *R. irregularis*) reduced the natural incidence of *T. absoluta*, in some cases (*R. irregularis* and *M. robertsii*) up to 90%. These results agree with recent studies showing induced resistance against *T. absoluta* under controlled conditions by strains of AMF (Shafiei et al. 2022), *T. afroharzianum* (Aprile et al. 2022), and by the EPF *B. bassiana* and *M. anisopliae* (Giannoulakis et al. 2023). Regarding AMF, inoculants from in planta cultures—including fungal propagules, mycorrhizal roots, and rhizospheric soil (*F. mosseae*, *C. etunicatum*)—and from in vitro monoxenic culture—including only axenic spores (*R. irregularis*)—significantly increased plant resistance. These results support the intrinsic properties of AMF in improving plant resistance against this pest.

While AMF and *Trichoderma* are widely documented to induce plant resistance against very diverse pathogens and pests (Martínez-Medina et al. 2013; Coppola et al. 2019; Sanmartín et al. 2020; Di Lelio et al. 2021; Rivero et al. 2021; Dejana et al. 2022), only few recent studies have demonstrated their negative impact on the performance of the leafminer *T. absoluta* (Aprile et al. 2022; Shafiei et al. 2022). For EPF, most studies are focused on the direct biological control action of fungal conidia resulting in the infection or reduction of the performance and fitness of *T. absoluta* (Chouikhi et al. 2022). More recently, evidence

on the induction of plant resistance by EPF against insects and pathogens is increasing (Raad et al. 2019; Rivas-Franco et al. 2020; Rasool et al. 2021; Zitlalpopoca-Hernandez et al. 2022). This effect is particularly important for concealed pest stages such as mining larvae, which are hidden from contact with sprayed biopesticides. Thus, the results illustrate the ability of AMF, *Trichoderma*, and EPF to enhance plant resistance and reduce *T. absoluta* incidence in commercial production conditions and, thus, to improve or complement current IPM practices in tomato crop protection.

Worldwide, *T. absoluta* is a major pest of tomato (Biondi et al. 2018), so the present results are encouraging for its management. The high reproductive capacity of *T. absoluta* allows for rapid population growth and widespread infestation. Moreover, due to its miner lifestyle, the use of surface-applied (bio)pesticides is generally inefficient (Abd El-Ghany et al. 2016). This insect is notorious for developing resistance toward chemical pesticides, making control measures even more challenging (Guedes et al. 2019). Hence, applying effective soil microbes that can hinder the development of this pest, without reducing fruit quality or yield—and even improving it—can be an efficient and sustainable solution for tomato crop protection.

However, under real crop production conditions, several different pests and diseases could emerge simultaneously or sequentially during the cropping season, challenging crop performance and productivity. Thus, we explored the impact of each microbial inoculant on the overall pest and disease incidence, considering all four aggressors, to gather insights into the effect of microbial inoculations on overall tomato plants' resistance against insect pests and pathogens. We found that most of the beneficial microbes used in our study increased overall plant resistance, prominently reducing the overall pest and disease incidence in the crop. Remarkably, for some microbial strains, such as the AMF *R. irregularis* and the PGPF *T. harzianum*, the increased resistance effect was as high as 95% when compared to the non-inoculated control plants. These findings agree with the general idea that IR by beneficial microbes such as AMF, and *Trichoderma* could be effective against a broad range of pests and pathogens (Martínez-Medina et al. 2013; Sanmartín et al. 2020; Di Lelio et al. 2021; Rivero et al. 2021).

The successful implementation of microbe-induced resistance in agriculture does not only rely on its effectiveness in controlling pests and pathogens but also on its compatibility with other strategies regularly used in IPM (Stenberg 2017). One point of caution would be if the microbes, by modifying plant defenses, may negatively impact auxiliary fauna, for example, biocontrol insects. As this study was performed under common pest management practices in Spanish tomato production based on IPM standards (Acebedo et al. 2022), we also evaluated the effect of microbial inoculation on the pest predator *N. tenuis*. Our results did not show any negative effects of the inoculations on the abundance of the predator, suggesting that microbe-induced resistance is compatible with the release of this predator, a generalist biocontrol agent, commonly used in IPM programs. Nonetheless, the lack of statistical significance in *N. tenuis* abundance (as well as in other parameters considered in this study) does not fully rule out a biological effect of the microbial inoculants on insect predators, or parasitoids as was previously shown (Rasmann et al. 2017). Instead, the potential presence of subtle or context-dependent effects might not have been captured due to limited statistical power ($N = 6$ blocks per treatment in this case). Accordingly, we advocate for more thorough investigations of the effect of microbial on biocontrol agents of crop pests.

4.3 Limitations of the study and future perspectives

Facilitating and accelerating a wider adoption of microbial-based products in agriculture are major goals toward improving agricultural sustainability. As microbial functionalities are highly context-dependent, the output of their inoculation can be influenced by diverse environmental factors (Lee Díaz et al. 2021). Accordingly, performing experiments under agronomic settings is essential to test microbe efficacy under the fluctuating conditions of real crop production. Our findings demonstrate a positive impact of microbial inoculation within the agronomic context of Southern Spain, a key European region for tomato production and exportation. However, we argue that our results obtained under these specific conditions and farming practices of plasticulture cannot be directly extrapolated to other key agricultural systems. Therefore, additional research across a wider range of agricultural settings is essential to support the reliability of integrating microbial-based solutions into different crop management programs. Accordingly, validation of these results would require repetition of experiments across various years, sites, and settings—such as greenhouses or open fields—and using different varieties or crops. This would allow us to assess spatiotemporal and management variability and plant-microbe specificity.

Moreover, although this study reveals phenotypes of agronomic interest, understanding the mechanisms behind the improved plant productivity and resistance against major pests is essential for improving reproducibility. The promising results from this study, along with the imperative for additional validations and mechanistic studies, encourage further research efforts toward optimizing the use of microbial-based solutions for environmentally smart farming practices.

Further limitations of our study include evaluating most parameters only at a one-time point. This may result in the loss of valuable information on the overall impact of microbial inoculants on crop performance. Comprehensive monitoring at multiple time points, throughout the entire cropping season, is desirable, as it would lead to a more detailed understanding of plant-microbe interactions and their effects on crops, and it

may additionally shed light on specific environmental factors that contribute to their context-dependent outcomes.

5 Conclusions

This study aimed to test the effect of diverse soil-beneficial microbes on improving plant health and productivity under commercial tomato production settings. We hypothesized that even under the optimized crop management conditions of commercial agriculture (using greenhouse protection, drip fertigation, and integrated pest management), the application of microbial inoculants can positively impact crop health and yield. By testing microbial strains—previously characterized under controlled lab conditions—in agronomic settings, we identified beneficial microbes that are competent and functional under commercial growing conditions for prolonged periods after their application. We show that soil-inoculated microorganisms, particularly fungi, improve tomato crop productivity and resistance to relevant pests such as the devastating leaf miner *T. absoluta* in commercial settings. Thus, our study supports the implementation of microbe-induced resistance in integrated pest management programs. Identifying microbes that effectively improve plant health and productivity in real crop production systems will contribute to faster and wider adoption of bioinoculants for environmentally safe crop protection, enhancing future agricultural sustainability.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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