

Research Paper

Influence of a multi-species bacterial biostimulant on rhizosphere dynamics, yield earliness, and fruit quality of open-field winter melon (*Cucumis melo* L. var. *inodorus*)

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ABSTRACT

The effect of a multi-species (*Bacillus tequilensis*, *Peribacillus frigoritolerans*, *Pseudomonas lini*, *Pseudomonas frederiksbergensis*, *Pseudomonas atacamensis*, *Pseudomonas reinekei*, and *Pseudomonas granadensis*) plant growth-promoting bacterial (PGPB) biostimulant on soil microbial dynamics and winter melon performance was evaluated over two growing seasons. In both trials PGPB were inoculated twice: at transplant and 30 days after transplant. Shortly after the first inoculation, total mesophilic bacteria increased by up to +1.8 log CFU g⁻¹ and total mesophilic anaerobic bacteria by +1.6 log CFU g⁻¹, while fungi rose from 4.35 to 5.15 log CFU g⁻¹ (p < 0.01). The second inoculation further enhanced bacterial abundance (+1.0 and +0.8 log units for TMB and TMAb, respectively), whereas fungal populations remained unchanged. By the end of the crop cycle, microbial counts converged between treatments. Culture-dependent tracking showed persistence of three out of the eight inoculated strains, with *B. tequilensis* and *P. reinekei* detected at harvest. Illumina profiling revealed strong early shifts in community structure, including an increase of *Pseudomonas* to 13.2 % and *Bacillus* to 13.8 %, followed by partial re-equilibration. Agronomically, inoculated plants exhibited enhanced vegetative growth (stem length +21.9 % at 20 days after transplant), earlier female flowering and accelerated fruit set. Total yield increased by 12.0 %, with early yield rising by 27.9 %, driven by a 37.6 % higher fruit number. Fruit quality also improved, showing a 15 % increase in firmness and higher soluble solid content (13.7 °Brix). These findings confirm that early microbial priming with this mixed inoculum drives lasting agronomic benefits, supporting efficient and sustainable winter melon production under field conditions.

1. Introduction

The growing global demand for food, coupled with the environmental challenges from excessive chemical inputs and the degradation of arable land due to intensive agricultural practices, highlights the urgent need for sustainable and environmentally friendly agricultural systems (Zuma et al., 2023). Among the most promising solutions, plant growth-promoting bacteria (PGPB) have emerged as biologically based tools capable of improving crop performance while reducing reliance on synthetic inputs. These soil- and rhizosphere-associated microorganisms enhance plant productivity through multiple direct and indirect mechanisms, including biological nitrogen fixation, phosphate solubilization, siderophore production, and the synthesis of phytohormones such as

auxins, gibberellins, and cytokinins (Gómez-Godínez et al., 2023; Ajijah et al., 2023). In addition, many PGPB exhibit biocontrol activity against phytopathogens through antimicrobial compound production or by inducing systemic resistance in plants (Prasad et al., 2019).

The integration of PGPB into horticultural systems represents a key strategy to improve resource use efficiency and reduce dependence on chemical fertilizers and pesticides, in line with agroecological and circular bioeconomy principles (Majeed et al., 2018; Ibáñez et al., 2023). The growth-promoting potential of many bacteria has been widely tested under controlled in vitro conditions. However, the performance of PGPB under field conditions may be deeply influenced by biotic and abiotic factors, including climatic conditions, soil characteristics, plant genotype, and the native microbial community structure (Ambrosini

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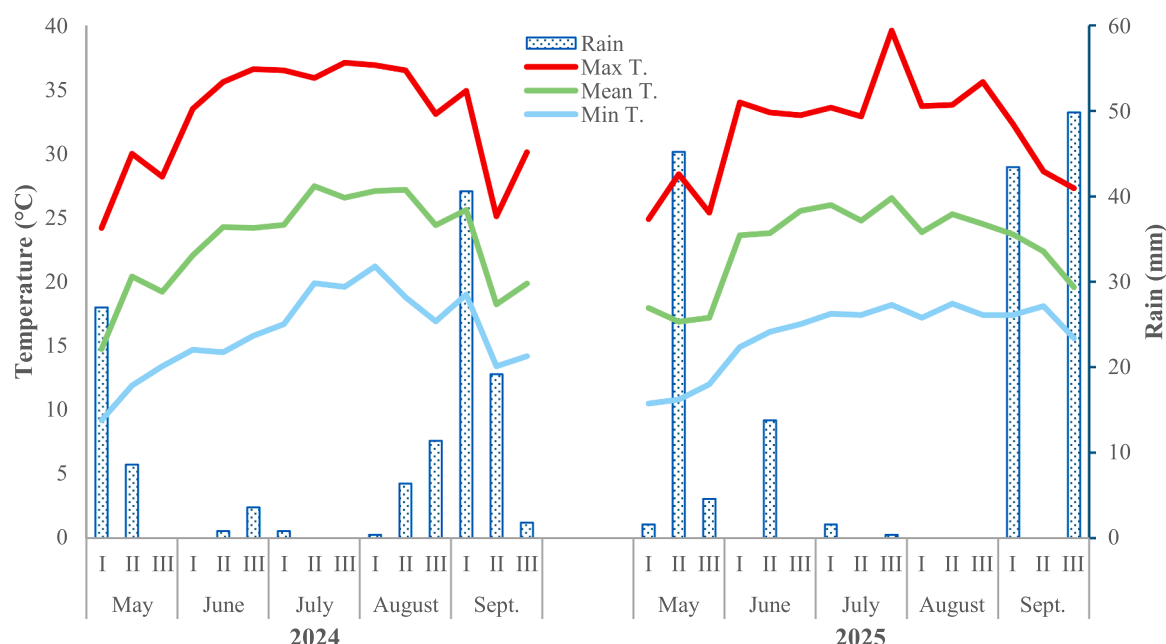


Fig. 1. Average, maximum and minimum temperatures and rainfall during the trial periods.

et al., 2016). Consequently, a comprehensive understanding of the ecological behaviour, persistence, and functional traits of PGPB in situ is critical for developing effective and reliable bioinoculants (Hossain et al., 2023).

Melon (*Cucumis melo* L.) is a globally important horticultural crop, with an annual production of approximately 27–30 million tons (Zhang et al., 2024). Originating from African and Asian domestication centers, it has diversified into numerous horticultural groups adapted to a wide range of agroclimatic conditions (Wang et al., 2021). The fruit is nutritionally valuable, providing sugars, fibre, minerals, vitamins A and C, and bioactive compounds with antioxidant properties (Lester, 1997). Despite its adaptability, melon cultivation often relies on intensive management practices, which can limit sustainability. Previous studies have demonstrated that PGPB can enhance melon biomass and nutrient uptake (Martínez et al., 2019). In particular, strains such as *Bacillus cereus* and *Pseudochrobactrum* sp. exhibit key plant growth-promoting traits, including nutrient solubilization and indole-3-acetic acid (IAA) production, which plays a central role in root development and nutrient acquisition (Amalina et al., 2023; Duca et al., 2014). Nevertheless, the effectiveness of PGPB in melon remains context-dependent, varying according to strain, environmental conditions, and cultivar. For instance, while *Bacillus* and *Pseudomonas* species are commonly effective, their performance can fluctuate significantly under field conditions (Bashandy et al., 2025). The use of bacterial consortia has been proposed to overcome these limitations. In *C. melo* var. *barattiere*, a tri-strain consortium improved yield, accelerated fruit maturation, and enhanced nutrient uptake even with reduced fertilizer inputs, indicating synergistic effects among strains (Murgese et al., 2020).

Among melon types, *C. melo* var. *inodorus* (winter melon) is of high economic importance, particularly in Mediterranean regions, Central America, and Asia. This group includes honeydew, canary, and Piel de Sapo melons, which are valued for their high sugar content, long shelf life, and suitability for transport (Carro et al., 2023). Despite its commercial relevance and nutritional value (Manchali et al., 2021), research on PGPB application has largely focused on netted melons (*C. melo* var. *reticulatus*), leaving a significant knowledge gap regarding the response of *inodorus* cultivars, especially under variable environmental conditions.

To address this gap, the present study evaluates the influence of a multi-species bacterial biostimulant on winter melon. Specifically, we

assess its effects on plant growth, yield, and quality, while also monitoring the persistence of the introduced strains in the soil and rhizosphere throughout the crop cycle. By linking agronomic performance with microbial dynamics, this work aims to contribute to the development of sustainable, bio-based strategies for winter melon cultivation.

2. Materials and methods

2.1. Experimental site, soil and climate data

A two-year field trial was conducted at “Valdibella” Agricultural Cooperative experimental site in Monreale, Palermo, Italy (37°54′35.0″N; 13°07′58.2″E; altitude 265 m a.s.l.), utilizing two different field areas within the same farm (one for each experimental year). These field areas shared identical soil conditions, agricultural management history, and previous crop rotations, ensuring that soil baseline characteristics were entirely uniform between both growing seasons while maintaining strict experimental independence. The study aimed to assess the effectiveness of PGPB on winter melon (*Cucumis melo* L. var. *inodorus*) under organic farming and irrigation conditions. According to the USDA Soil Taxonomy, the soil at the experimental site is classified as a Calcixerollic Xerochrept (corresponding to a Calcic Cambisol in the World Reference Base (WRB) for Soil Resources classification). The soil profile is characterized by a high content of clay and limestone featuring a clay loam texture, consisting of 38.40 % sand, 25.35 % silt, and 36.25 % clay (0–30 cm depth). Chemical analysis of the soil revealed a pH of 8.1, a Total Organic Carbon (TOC) of 1.01 %, and an active CaCO₃ content of 1.97 %.

The study area is characterized by a Mediterranean climate with an average annual rainfall of 580 mm, primarily occurring between September and March, outside the active growing season (Fig. 1). During the experimental periods, the temperature averaged 23.1 ± 1.0 °C in 2024 and 22.8 ± 0.8 °C in 2025; the maximum and minimum average temperatures were respectively 32.9 ± 1.1 °C and 15.9 ± 0.9 °C in 2024 and 31.8 ± 1.0 °C and 15.9 ± 0.7 °C in 2025. The minimum temperature fell below 15 °C during the initial weeks of the trial, persisting until mid-June in 2024 and until the first ten-day period of June in 2025. In both years, the maximum temperatures remained above 25 °C throughout the growing cycle and exceeded 30 °C from the beginning of June until the first ten days of September. The year 2024 was characterized by higher

thermal peaks, with maximum temperatures exceeding 35 °C from mid-June until the end of August; the hottest period occurred between late July and early August (37.0 °C on average). In contrast, during 2025, temperatures rose above 35 °C only during the second ten-day period of July (39.6 °C on average) and late August (35.6 °C on average). Total rainfall recorded from May to August was 59.0 mm in 2024 and 67.2 mm in 2025. May was the wettest month, with 35.6 mm in 2024 and 51.4 mm in 2025, followed by August (18.2 mm) in 2024 and June (13.8 mm) in 2025.

2.2. Crop management and experimental design

The trials utilized the hybrid cultivar 'Helios' (HM.Clause Italia SpA, Venaria Reale, TO, Italy), one of the most widely cultivated winter melon variety in Italy. Seedlings were obtained from a local commercial nursery and transplanted into soil covered with transparent polyethylene mulch on 22 May 2024 and 9 May 2025. Following standard regional practices, plants were spaced 1.0 m apart within rows and 3.0 m between rows. Each subplot covered an area of 150 m² across three rows and contained 60 plants. The irrigation system consisted of a single drip line per row with emitters spaced 0.33 m apart (discharge rate: 4 L h⁻¹). Two treatments were tested: (i) an inoculated treatment receiving a mixed inoculum of eight PGPB strains (IN), and (ii) a non-inoculated control receiving an equal volume of sterile distilled water (CTR). The study followed a Randomized Complete Block Design (RCBD) with four replications. To avoid edge effects, agronomic sampling and observations were conducted on 20 plants selected from the central row of each subplot.

Prior to transplanting, 1 t ha⁻¹ of organic fertilizer (Stallone NP5.5, Pollipoli s.r.l., Fasano, Italy) was broadcast and incorporated into the soil. All other cultural practices were applied uniformly across treatments according to standard requirements for winter melon organic cultivation.

2.3. Plant growth-promoting bacterial strains

The PGPB mixed inoculum comprised eight bacterial strains previously isolated from anthropogenic soils (Typic Haploxererts) located in the Agrigento province (Italy), as described by Barbaccia et al. (2022a). Taxonomic identification was performed by 16S rRNA gene sequencing, and the corresponding accession numbers are available in the NCBI GenBank database. The strains included *Bacillus tequilensis* RC-UNIPASAAFM01186 (Acc. No. OM866219), *Peribacillus frigitolerans* RC-UNIPASAAFM01212 (Acc. No. OM866202), *Pseudomonas lini* RC-UNIPASAAFM01175 (Acc. No. OM866256), *Pseudomonas frederiksbergensis* RC-UNIPASAAFM01183 (Acc. No. OM866245), *Pseudomonas atacamensis* RC-UNIPASAAFM01217 (Acc. No. OM866247), *Pseudomonas reinekei* RC-UNIPASAAFM01216 (Acc. No. OM866246), and *Pseudomonas granadensis* RC-UNIPASAAFM01209 (Acc. No. OM866248) and RC-UNIPASAAFM01218 (Acc. No. OM866249). All strains are preserved in the microbial culture collection of the Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, which is shared by the Italian MIRRI-IT infrastructure (Moretti et al., 2024).

The selected strains were previously characterized for multiple plant growth-promoting (PGP) traits, including the production of indole-3-acetic acid (IAA), ammonia (NH₃), hydrogen cyanide (HCN), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and siderophores, as well as the capacity for inorganic phosphate solubilization, as reported by Barbaccia et al. (2022b).

In the present study, these strains were combined in mixed inoculum and tested together for the first time under field conditions to evaluate their collective effect on rhizosphere microbial dynamics and crop performance. No prior optimization of strain ratios or compatibility was conducted.

2.4. Preparation and inoculation of PGPB

The eight PGPB strains were individually cultured in pure form. Each strain was reactivated from glycerol stocks (20 % v/v) stored at -80 °C by incubation in Nutrient Broth (NB) at 30 °C for 48 h to obtain the mother cultures. These cultures served as pre-inoculum, which were subsequently transferred at 2 % (v/v) into 2.5 L of NB and incubated under the same conditions to generate the required volume of bacterial suspension for field application. The individual broth cultures were then combined, to form a mixed inoculum, into a single 20-liter plastic container (Ecoplast S.r.l., Gela, Italy), previously sanitized with an isopropyl alcohol-based disinfectant (PMC 10,235, DTH 101 Isopril, SiFi, Sasso Marconi, Italy). Each bacterial strain reached a cell density ranging from 9.0×10^7 to 2.0×10^8 CFU mL⁻¹ prior to inoculum preparation. The final PGPB inoculum (approximately 1.0×10^8 CFU mL⁻¹, as determined by plate count) was obtained by combining appropriate volumes of the individual strain suspensions, thereby ensuring a comparable contribution of each strain to the mixture. A total volume of 6 L of the mixed PGPB inoculum was prepared for each inoculation.

At transplanting (designated as time zero, t₀), 300 mL of the PGPB inoculum were poured directly into each planting hole. Control plants received an equivalent volume (300 mL) of sterile distilled water. A second inoculation (t₁) was performed 30 days post-transplant using the same volume and concentration by pouring the inoculum or distilled water directly into the soil surrounding the base of each plant. All inoculations were conducted early in the morning under clear conditions, with ambient temperatures ranging between 15 and 25 °C. PGPB treatments were repeated across both experimental years.

2.5. Agronomical, morpho-physiological, yield, and quality parameters

During the vegetative stage, the length of the main shoot and the leaf number were recorded at 20, 30, and 40 days after transplanting (DAT). In the same period, flowering (male and female flowers emission) was monitored and the percentage of plants with fruit set was calculated 40 DAT.

Harvesting commenced when fruits reached full maturity (78 DAT in 2024 and 83 DAT in 2025), with the harvest period lasting 31 and 26 days, respectively. All harvested fruits were weighed individually to calculate the average fruit weight. Fruits weighing less than 1.0 kg, or those exhibiting cracks, visual defects, or significant malformations, were classified as unmarketable. Total fruit number per plant, marketable fruit yield per plant, and marketable yield per hectare were determined.

Immediately following harvest, ten fully mature marketable fruits were randomly selected per replicate to evaluate quality parameters. Fruit volume was estimated using the water displacement technique. Each fruit was submerged in a known volume of water at 25 °C, and the mass of the displaced water was recorded. The actual volume was calculated according to Miceli et al. (2016) using the following equation Actual volume (ml) = W/γ, where W is the weight of displaced water (g), and γ is the mass density of water (1.0 g mL⁻¹ at 25 °C).

Fruits were then bisected longitudinally for further analysis. Peel surface colour (two measurements at opposite equatorial points) and the flesh colour (measured on three points of one half per fruit) were determined using a colorimeter (Chroma Meter CR-400C, Minolta, Osaka, Japan) recording CIELab parameters: L* (lightness), a* (red-green axis), and b* (yellow-blue axis). From these, Chroma (C*) and Hue angle (H°) were calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$ and $H^\circ = \arctan(b^*/a^*)$. Flesh firmness was measured at three points along the longitudinal axis of one fruit half using a digital penetrometer (mod. 53205, TR Snc., Forlì, Italy) equipped with an 8 mm flat stainless steel cylinder probe. Firmness was defined as the peak force (expressed in Newtons) required to penetrate the flesh to a depth of 0.5 cm. Rind thickness was measured at two opposite equatorial points using a digital calliper. After removing the placenta and seeds, the flesh was separated and weighed to

determine the edible part percentage.

The mesocarp was homogenized and centrifuged at 3500 rpm for 10 min. The resulting supernatant was used to determine the following parameters: Soluble Solid Content (SSC), measured in Brix using a digital refractometer (MTD-045nD, Taiwan); Titratable Acidity (TA), analysed by potentiometric titration with 0.1 M NaOH (pH 8.1 target) using 10 mL of extract, and expressed as mg of citric acid 100 g⁻¹ fresh weight (FW); ascorbic acid, extracted by mixing 10 mL of juice with 100 mL of metaphosphoric acid (HPO₃). After filtration through Whatman no. 1 paper, a 10 mL aliquot was titrated with 2,6-dichlorophenol-indophenol until a stable pink coloration persisted for 15 s; values were expressed as mg 100 g⁻¹ FW (Miceli et al., 2023).

2.6. Soil sample collection and treatment

Bulk soil samples were collected at *t*₀ immediately after distilled water addition for the control treatment (CTR₀) and after application of the mixed PGPB inoculum for the inoculated treatment (IN₀). Rhizosphere soil samples were collected from three individual melon plants per replicate at two subsequent time points: *t*₁ (second inoculation) and *t*₂ (end of crop cycle), approximately 90 days after transplantation. Rhizosphere soil samples were collected from three individual melon plants per replicate at two subsequent time points: *t*₁ (corresponding to the second inoculation, 30 days after transplanting) and *t*₂ (end of the crop cycle, approximately 90 days after transplantation). Accordingly, samples were coded as follows: CTR₁ and CTR₂ for control rhizosphere soils collected at *t*₁ and *t*₂, respectively, and IN₁ and IN₂ for inoculated rhizosphere soils collected at the same time points. At each sampling point, entire plants were carefully uprooted to retain intact root systems, and the soil adhering to the roots (rhizosphere soil) was gently dislodged by manual shaking and immediately transferred into sterile stomacher bags (BagLight® 400 Multilayer®, Interscience, Saint Nom, France). All bulk and rhizosphere soil samples were immediately stored in a portable refrigerator containing reusable ice packs and transported under refrigerated conditions to the Agricultural Microbiology Laboratory of the SAAF Department.

Prior to microbiological analyses, all soil samples were air-dried under a laminar flow hood for 24 h to minimize environmental contamination. Subsequently, the dried soils were sieved using a 2 mm stainless steel sieve (Laboratory Test Sieve, London, UK), previously sterilized by autoclaving at 121 °C for 15 min, following the protocol described by Thomson et al. (2010).

2.7. Microbiological analysis

Microbiological analyses of all soil samples were performed using standard plate count methods to determine the levels of total mesophilic bacteria (TMB), total mesophilic anaerobic bacteria (TMAb), and filamentous fungi. For each analysis, 25 g of soil were transferred into sterile beakers equipped with lateral baffles, diluted with 225 mL of a 0.16 % (w/v) sodium pyrophosphate solution, used to disperse soil aggregates and release entrapped microorganisms, and homogenized by shaking on an orbital shaker at 150 rpm for 10 min (Settanni et al., 2012). Starting from the 10⁻² dilution, serial dilutions were prepared using Ringer's solution (Sigma-Aldrich, Milan, Italy), and homogenization was performed using a vortex mixer. Aliquots from the higher dilutions (10⁻⁴ to 10⁻⁷) were subsequently plated for the enumeration of the microbial groups object of investigation, to obtain countable plates within the accepted limits of the plate count method (20–200 CFU per plate for spread plating and 30–300 CFU for pour plating), thus ensuring reliable quantification across a wide range of microbial densities up to approximately 10⁹ CFU g⁻¹ dry weight (DW).

For TMB enumeration, microbial suspensions were spread onto Plate Count Agar (PCA) (Biotec, Grosseto, Italy) and incubated aerobically at 30 °C for 72 h. TMAb counts were obtained by pouring microbial suspensions into NA (Oxoid, Milan, Italy), followed by anaerobic

incubation at 30 °C for 48 h in sealed jars using the AnaeroGen AN25 system (Oxoid). To inhibit fungal growth, cycloheximide (10 mg/mL) was added to both PCA and NA media. Filamentous fungi were enumerated by spreading the suspensions onto Yeast Peptone Dextrose (YPD) agar supplemented with chloramphenicol (0.1 mg/mL), followed by aerobic incubation at 30 °C for 7 days. All colony counts were performed in duplicate. DW of the soil was determined by drying the samples at 105 °C until a constant weight was achieved.

2.8. Culture-independent analysis of bacterial communities in bulk and rhizosphere soil

Soil bacterial communities in bulk and rhizosphere samples were analysed through 16S rRNA gene sequencing using the Targeted Sequencing Microbiome Analysis Service at Zymo Research Europe (Freiburg, Germany).

DNA was extracted using the ZymoBIOMICS®–96 MagBead DNA Kit (Zymo Research, Irvine, CA), following the manufacturer's instructions, with a final elution volume of 50 µL. Targeted libraries were prepared using the Quick-16S™ NGS Library Prep Kit with V3-V4 primers. PCR amplification was monitored in real time to minimize chimera formation. Amplicons were quantified by qPCR fluorescence, pooled in equimolar concentrations, purified using the Select-a-Size DNA Clean & Concentrator™ kit, and quantified using TapeStation® (Agilent Technologies) and Qubit® (Thermo Fisher Scientific). Quality control included the ZymoBIOMICS® Microbial Community Standard and the Microbial Community DNA Standard as positive controls for DNA extraction and library preparation, respectively, while blank controls were used to assess contamination during laboratory procedures. Sequencing was performed on an Illumina® NextSeq™ 1000 platform using the P1 XLEAP-SBS™ Reagent Kit (600 cycles), with a 40 % PhiX spike-in to enhance data quality. Sequence processing was conducted using the DADA2 pipeline for amplicon inference and chimera removal (Callahan et al., 2016). Taxonomic assignment was carried out with UCLUST in QIIME v1.9.1 using the Zymo Research curated 16S rRNA database (Caporaso et al., 2010). Community composition and diversity (alpha and beta) were analysed in QIIME, and differentially abundant taxa were identified using LEfSe (Segata et al., 2011).

Absolute bacterial abundance was quantified by qPCR using standard curves generated from plasmid DNA containing a single copy of the 16S rRNA gene (10-fold serial dilutions). The same primers used for library preparation were applied. Gene copy numbers were normalized to copies per µL of DNA (based on a 4 µL input volume), converted to genome equivalents assuming an average of four 16S rRNA gene copies per genome, and expressed as DNA concentration using an estimated genome size of 4.64 × 10⁶ bp, corresponding to *Escherichia coli*.

Sequencing data were deposited in the NCBI database under accession number PRJNA1406236.

2.9. Monitoring PGPB in soil

To monitor the persistence of the inoculated PGPB strains and evaluate their potential dominance over native soil microbiota, bacterial isolations were conducted on all soil samples collected at the three time points considered (*t*₀, *t*₁, and *t*₂) under both conditions (CTR and IN). Bacteria were isolated from the highest countable serial dilutions plated on PCA. This approach was intentionally adopted to preferentially recover dominant and numerically relevant bacterial populations, although it may underestimate less abundant or subdominant taxa. Colonies were purified through repeated streaking on NA until morphologically uniform cultures were obtained. Purified isolates were first subjected to preliminary phenotypic characterization, including Gram reaction determination using the 3 % (w/v) KOH method (Gergersen, 1978) and catalase activity assessed with 3 % (v/v) H₂O₂ (Koneman et al., 1997). Morphological characterization was then carried out based on colony features such as shape, size, colour, margin,

surface texture, and elevation.

Genomic DNA was extracted from the purified isolates using the InstaGene Matrix kit (Bio-Rad, Hercules, CA), following the manufacturer's instructions. Crude cell extracts served as templates for PCR amplification. To assess the identity of the isolates relative to the mixed inoculum inoculated, randomly amplified polymorphic DNA-PCR (RAPD-PCR) technique was applied. RAPD-PCR represents a reliable compromise for rapid and cost-effective strain-level discrimination in culture-dependent studies (Settanni et al., 2012, 2013). This approach allowed comparison of genetic profiles between field isolates and the eight reference PGPB strains (run concurrently as reference control lanes on each gel), enabling the identification of inoculated strains within the dominant culturable fraction.

RAPD-PCR amplification was performed using a Swift™ MaxPro Thermal Cycler (Esco Healthcare Pte. Ltd., Singapore) with the primer M13 (5'-GAGGGTGGCGGTTCT-3'), following Stenlid et al. (1994). Each reaction mixture (30 µL) contained 0.2 mmol L⁻¹ dNTPs (Invitrogen™, Thermo Fisher Scientific, Carlsbad, CA, USA), 2 µmol L⁻¹ primer (PrimmBiotech srl, Milan, Italy), 2.5 mmol L⁻¹ MgCl₂, 0.625 U Taq DNA polymerase (MBI Fermentas, St. Leon-Rot, Germany), 3 µL of 1 × DreamTaq™ Green Buffer (Invitrogen™), 1 µL of template DNA (approximately 60 ng µL⁻¹), and sterile bi-distilled water to reach the final volume. Amplification was carried out for 40 cycles, each consisting of denaturation at 94 °C for 1 min, annealing at 45 °C for 20 s, and extension at 72 °C for 2 min, followed by a final extension step at 72 °C for 5 min (Zapparoli et al., 1998). PCR products were separated by electrophoresis on 1.5 % (w/v) agarose gels (Gibco BRL, Cergy Pontoise, France), using the GeneRuler 100 bp Plus DNA ladder (M-Medical Srl, Milan, Italy) as a molecular size marker. Gels were stained with SYBR® Safe DNA Gel Stain (Molecular Probes, Eugene, OR), and images were acquired using the Azure 200 Gel Imaging System (Azure Biosystems, Dublin, CA). Polymorphic profiles were analysed and compared using GelCompare II software, version 6.5 (Applied Maths, Saint-Martens-Latem, Belgium).

2.10. Statistical analysis

All data were checked for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively, prior to conducting the analysis of variance (ANOVA). Percentage data (i.e., plant fruit set and edible part percentage) were subjected to arcsine square-root transformation before statistical processing to satisfy the assumptions of ANOVA; non-transformed data are presented in text and tables. Microbiological determinations were subjected to ANOVA.

To evaluate the main effects and interactions of the two experimental years and the biostimulant treatments on plant morpho-physiological traits, yield components, and fruit physicochemical quality parameters, a two-way ANOVA was performed. When significant effects or interactions were detected, mean separations were determined using Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$.

3. Results and discussion

3.1. Microbiological analyses of soil

Plate count data are the average of the technical repeats of two independent experimental years (Table 1). At the first inoculation (t_0), the PGPB treatment (IN₀) produced a clear numerical increase in all microbial groups compared to the uninoculated control (CTR₀). TMB increased of approximately 1.8 log units, while TMAb increased of a little less (1.6 log units). Filamentous fungi also increased significantly ($p < 0.01$), rising from 4.35 to 5.15 log CFU g⁻¹ dry weight (DW). At the second inoculation (CTR₁ vs IN₁), the stimulatory effect of the PGPB was even more pronounced within the bacterial community. TMB increased significantly ($p < 0.01$) from 7.28 to 8.28 log CFU g⁻¹ DW, while TMAb showed a highly significant increase ($p < 0.001$) from 7.06 to 7.87 log CFU g⁻¹ DW. These changes correspond to increases of 1.0 and 0.8 log units, respectively. In contrast, fungal counts remained stable, with values of 5.11 in the control and 5.02 log CFU g⁻¹ DW in the treated samples, showing no significant difference.

By the end of the cultivation cycle (CTR₂ vs IN₂), microbial abundances converged between treatments. TMB values were 8.75 in the control and 7.97 log CFU g⁻¹ DW in the treated rhizosphere, while TMAb values were 8.23 and 7.62 log CFU g⁻¹ DW, respectively; however, none of these differences were statistically significant. Similarly, fungal counts showed no meaningful variation, with 5.10 in the control and 4.43 log CFU g⁻¹ DW in the treated samples. Although slightly higher numerical values were observed in the control at this stage, these differences fall within the expected variability of culture-dependent enumeration methods and soil heterogeneity, and therefore cannot be interpreted as biologically relevant. Rather, they reflect the dynamic and spatially variable nature of soil microbial communities. These results indicate that the initial PGPB-induced increases were progressively attenuated over time, leading to a stabilization of the rhizosphere microbiome toward the end of the melon crop cycle.

The early stimulation of rhizosphere microbial groups following PGPB inoculation aligns with the direct introduction of high densities of viable bacterial cells and their transient establishment in the soil environment (Kong et al., 2025a; Vuolo et al., 2022). The marked increases in both aerobic and anaerobic mesophilic bacteria after the first and second inoculations suggest the ability of the introduced strains to temporarily dominate culturable microbial fractions and/or contribute to short-term shifts in microbial abundance (Ambrosini et al., 2016). In contrast, the slightly higher counts of filamentous fungi observed at t_0 in the inoculated treatment cannot be attributed to the bacterial inoculum itself, nor to biological stimulation of native fungal populations, given that sampling was performed immediately after inoculation. Rather, this modest difference (<1 log unit) likely reflects the intrinsic variability of culture-dependent enumeration methods and soil heterogeneity; soil fungal populations are highly heterogeneous in space, even at small scales (Djemiel et al., 2024). Accordingly, it does not appear to represent

Table 1
Microbial loads (log CFU g⁻¹ DW) of soil samples.

Sampling time	Bacterial counts							
	TMB		TMAb		FF			
	CTR	IN	CTR	IN	CTR	IN		
t_0	6.84 ± 0.28	8.62 ± 0.32	6.39 ± 0.24	7.98 ± 0.47	4.35 ± 0.09	5.15 ± 0.22	***	**
t_1	7.28 ± 0.19	8.28 ± 0.28	7.06 ± 0.03	7.87 ± 0.12	5.11 ± 0.30	5.02 ± 0.09	**	ns
t_2	8.75 ± 0.48	7.97 ± 0.67	8.23 ± 0.65	7.62 ± 0.09	5.10 ± 0.60	4.43 ± 0.12	ns	ns

Results indicate mean values ± standard deviation of four plate counts (carried out in duplicate for each experimental year). Abbreviations: TMB, total mesophilic bacteria; TMAb, total mesophilic anaerobic bacteria; FF, filamentous fungi; CTR, control treatment; IN, soil inoculated with the mixed PGPB inoculum; t_0 , first inoculation; t_1 , second inoculation; t_2 , end of crop cycle; Significance: ns = not significant; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

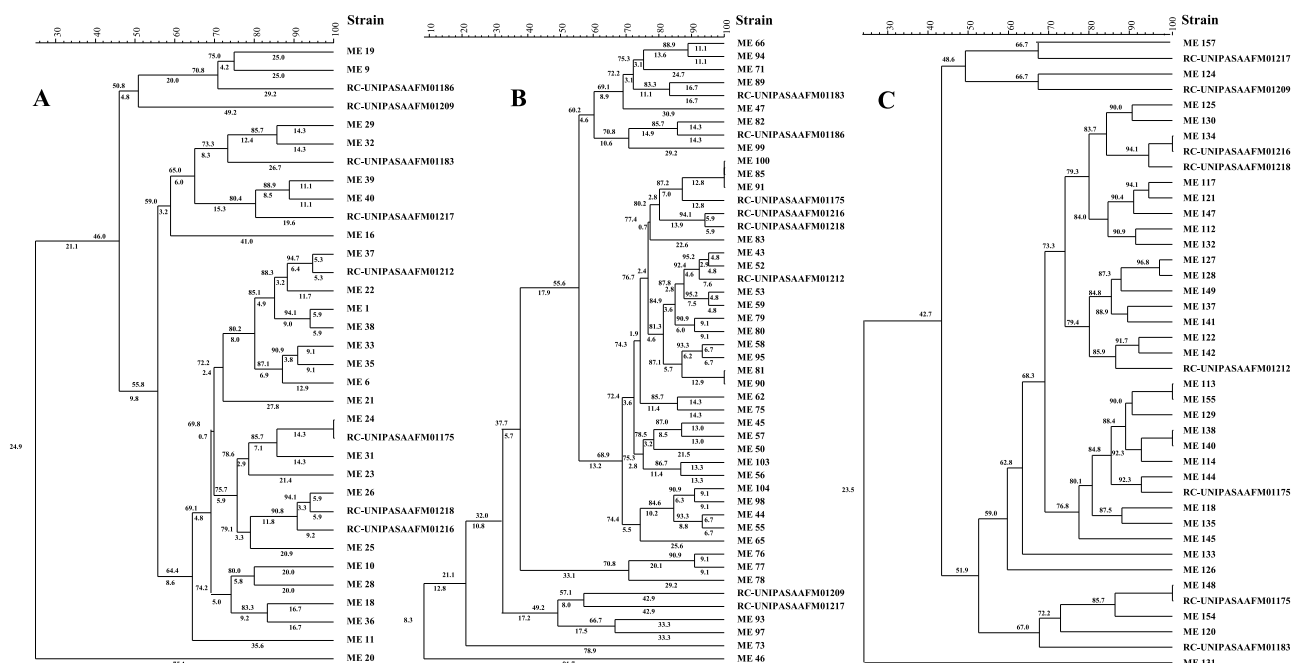


Fig. 2. Dendrograms obtained from RAPDPCR profiles using Gel Compare II. A, dendrogram generated from strains isolated at t_0 (first inoculation of the PGPB mix); B, dendrogram generated from strains isolated at t_1 (second inoculation of the PGPB mix); C, dendrogram generated from strains isolated at t_2 (end of crop cycle). The isolates with the strain codes of the official RC-UNIPASAAFM collection correspond to: *Bacillus tequilensis* RC-UNIPASAAFM01186, *Peribacillus frigiditolerans* RC-UNIPASAAFM01212, *Pseudomonas lini* RC-UNIPASAAFM01175, *Pseudomonas frederiksbergensis* RC-UNIPASAAFM01183, *Pseudomonas atacamensis* RC-UNIPASAAFM01217, *Pseudomonas reinekei* RC-UNIPASAAFM01216, *Pseudomonas granadensis* RC-UNIPASAAFM01209, *Pseudomonas granadensis* RC-UNIPASAAFM01218.

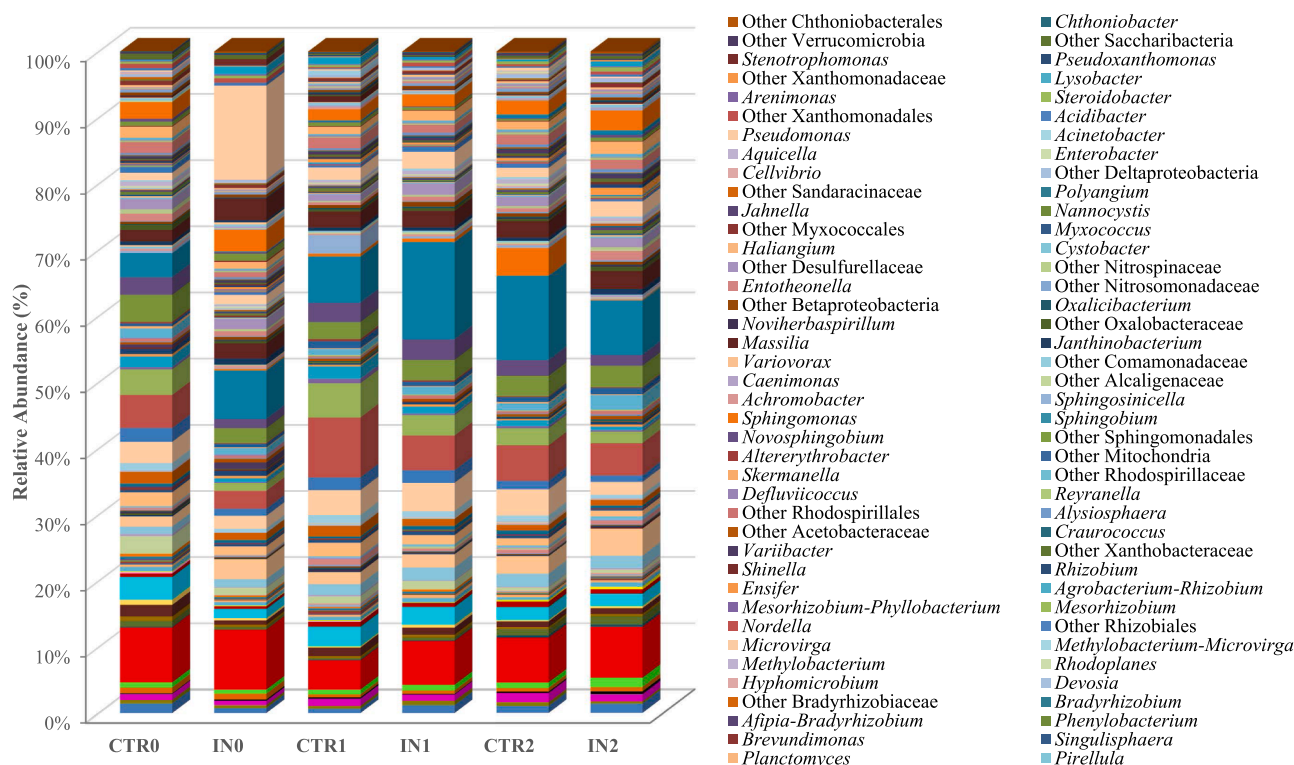


Fig. 3. Relative abundances (%) of bacterial genera identified through Illumina sequencing in topsoil. Abbreviations: CTR₀, control bulk soil collected at t_0 (first inoculation); IN₀, bulk soil inoculated with the mixed PGPB inoculum collected at t_0 ; CTR₁, rhizosphere soil collected at t_1 (first inoculation); IN₁, rhizosphere soil inoculated with the mixed PGPB inoculum collected at t_1 ; CTR₂, rhizosphere soil collected at t_2 (end of crop cycle); IN₂, rhizosphere soil inoculated with the mixed PGPB inoculum collected at t_2 .

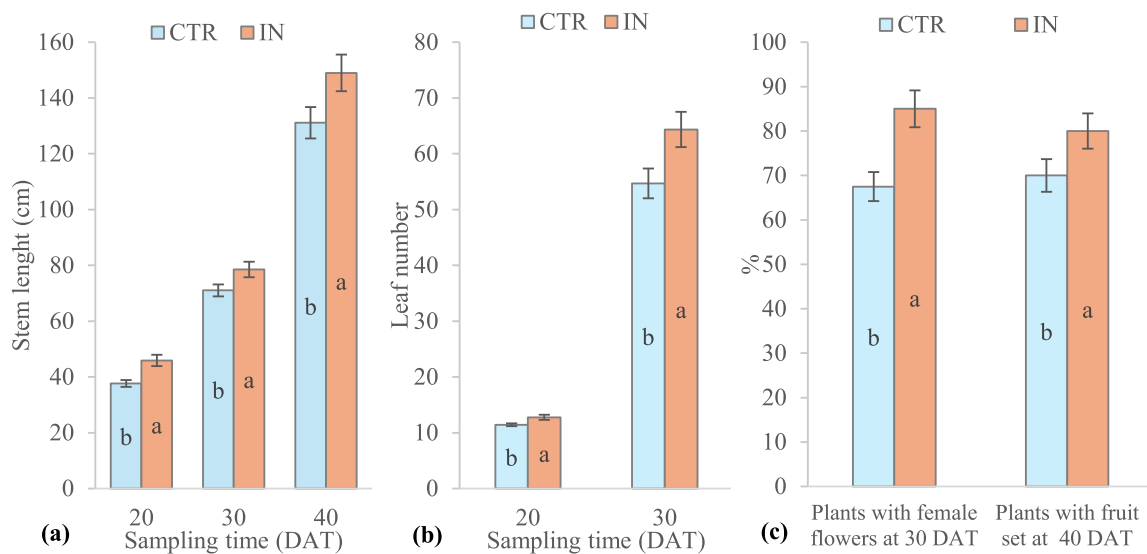


Fig. 4. Vegetative and reproductive parameter of winter melon plants grown on soil inoculated (IN) or non-inoculated (CTR) with the mixed PGPB inoculum: a) stem length at 20, 30 and 40 days after transplanting (DAT); b) leaf number; c) percentage of the plants with at least one female flower or with a fruit set (black bars represent standard errors of the means; at each sampling time, bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

a biologically meaningful effect and is not maintained at subsequent sampling times (t_1 and t_2). The absence of a significant increase in fungal populations after the second inoculation further supports the limited responsiveness of this group to the PGP bacterial treatment.

By the end of the crop cycle, the convergence of microbial abundances between treated and untreated rhizospheres indicated a progressive attenuation of the initial PGPB effect. This stabilization is commonly observed in field and greenhouse studies, where native microbiome resilience and plant developmental stage gradually override early perturbations induced by inoculants (Berendsen et al., 2012; Toju et al., 2018). Overall, these results suggest that while PGPB can transiently influence bacterial population dynamics, the rhizosphere microbiome tends to re-equilibrate over time, reflecting its dynamic but self-regulating nature.

3.2. Persistence of PGPB in soil

A total of 123 bacterial isolates from soil and rhizosphere samples were characterized phenotypically through optical microscopy, the KOH test, and the catalase test. Microscopy revealed 22 cocci and 101 bacilli, indicating a predominance of rod-shaped bacteria. The KOH test identified 96 isolates as Gram-positive and 27 as Gram-negative. Catalase testing showed that 57 isolates lacked catalase activity, while 66 were catalase positive. All isolates were purified, subjected to RAPD-PCR analysis, and isolates with distinct RAPD profiles were considered genetically different. Three dendrograms were generated using Gel Compare II (Fig. 2), each representing a different sampling stage, and were compared with the eight inoculated PGPB strains. This analysis aimed to track native microbial dynamics and evaluate the persistence of the inoculated strains throughout the crop cycle. RAPD-PCR revealed that *P. lini* RC-UNIPASAAFM01175 was present after the first inoculation, while *B. tequilensis* RC-UNIPASAAFM01186 and *P. reinekei* RC-UNIPASAAFM01216 were recovered at the end of the cultivation cycle (t_2), demonstrating their persistence in the rhizosphere. Strain comparison was conducted only in the first year (2024) to provide a comprehensive assessment of bacterial dynamics to develop the methodology of PGPB inoculation for winter melon cultivation. These findings provide insight into the ecological behaviour of the inoculated strains. Although eight PGPB were introduced, only a subset persisted or was recovered over time, reflecting the strong competitive pressures

within the rhizosphere. The early detection of *P. lini* RC-UNIPASAAFM01175 suggests rapid but transient colonization, consistent with the recognized rhizosphere competence of *Pseudomonas* spp., which often display fast growth, chemotaxis toward root exudates, and efficient root colonization. In contrast, the long-term persistence of *B. tequilensis* RC-UNIPASAAFM01186 and *P. reinekei* RC-UNIPASAAFM01216 indicates that these strains possess traits favouring ecological stability in the root environment. Persistence is a key determinant of inoculant success, as strains that remain associated with the plant are more likely to sustain beneficial functions such as nutrient mobilization, phytohormone production, or pathogen suppression (Backer et al., 2018). The selective survival of only three strains aligns with evidence that native microbiota often limits the establishment of introduced bacteria through niche exclusion and resource competition (Mallon et al., 2015). The persistence of *Bacillus* and *Pseudomonas* strains is consistent with their known adaptive traits, including biofilm formation and metabolic versatility (Rilling et al., 2018; Santoyo et al., 2017). The ability of *B. tequilensis* RC-UNIPASAAFM01186 and *P. reinekei* RC-UNIPASAAFM01216 to persist until the end of the crop cycle highlights their ecological fitness and supports their potential use in future biofertilizer formulations.

3.3. Culture-independent analysis of topsoil

Next generation sequencing analysis was conducted only in the first year (2024) to provide a comprehensive assessment of rhizosphere bacterial dynamics as reported above for strain-tracking analysis. Fig. 3 illustrates clear shifts in bacterial community composition across treatments. The rhizosphere microbiome shows clear restructuring following PGPB inoculation, with several dominant taxa responding strongly to treatment. In control soils at t_0 and t_1 (CTR₀ and CTR₁), the community is largely shaped by high-abundance Actinobacteria and Acidobacteria, particularly *Rubrobacter*, other Gaiellales, other Solirubrobacterales, and other Acidobacteria, which are typical of oligotrophic, stable soil environments (Fierer et al., 2012). These groups consistently decline in inoculated samples, especially at early time points (IN₀ and IN₁). At t_0 , this trend is primarily attributable to the immediate effect of inoculation, which introduces a high density of bacterial cells and temporarily shifts the relative abundance of microbial groups. At the subsequent sampling time (t_1), this pattern may also reflect emerging ecological

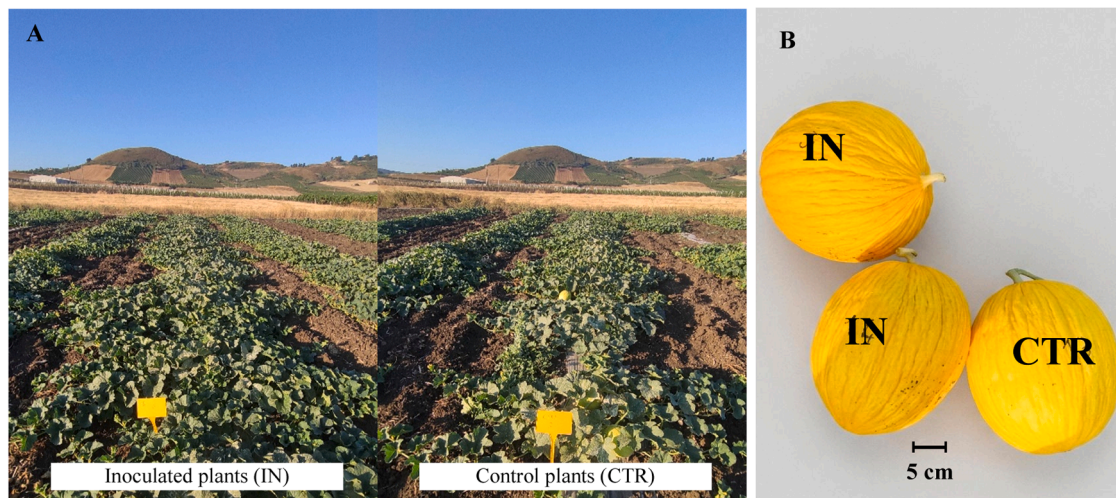


Fig. 5. A) Winter melon plants grown on soil inoculated (IN) or non-inoculated (CTR) with the mixed PGPB inoculum during the reproductive stage (70 days after transplant); B) Melon fruit sampled soon after harvest for morpho-physiological analysis.

interactions within the rhizosphere, although these effects appear transient.

A striking effect of PGPB inoculation is the dramatic increase in *Pseudomonas*, which rises from negligible levels in controls to 13.2 % in inoculated trial, making it the single most enriched genus immediately after inoculation. This surge is consistent with the well documented ability of *Pseudomonas* spp. to rapidly colonize the rhizosphere, exploit root exudates, and express plant beneficial traits such as siderophore production, phytohormone modulation, and pathogen suppression (Backer et al., 2018; Kong et al., 2025b). The dominance of *Pseudomonas* in IN_0 primarily reflects the direct introduction of these bacteria through inoculation, confirming the effectiveness of the treatment. Any ecological selection processes are more likely to occur at subsequent sampling times.

Other Proteobacteria, including *Sphingomonas*, *Microvirga*, and *Arthrobacter-Pseudarthrobacter*, also increase across treated samples, with *Microvirga* peaking in IN_1 (2.4 %) and *Arthrobacter-Pseudarthrobacter* reaching its highest abundance in IN_2 (3.8 %). These genera are associated with nitrogen cycling, stress tolerance, and plant beneficial metabolic versatility (Braglia et al., 2025). Their enrichment suggests that PGPB inoculation promotes a shift toward metabolically flexible, plant associated taxa.

Firmicutes also respond strongly; *Bacillus* increases from 3.3 % in CTR_0 to 13.8 % in IN_1 , reflecting rapid proliferation shortly after inoculation. Although its abundance declines by IN_2 (7.6 %), it remains higher than in the corresponding control, indicating a sustained but transient stimulation. This pattern aligns with the known rhizosphere competence of *Bacillus* spp. and their frequent inclusion in commercial biofertilizers (Backer et al., 2018).

By the end of the crop cycle (CTR_2 vs. IN_2), the community partially reverts toward a soil-driven structure, but treated soils retain higher levels of Proteobacteria, including *Pseudomonas*, *Sphingomonas*, and *Arthrobacter-Pseudarthrobacter*, than controls. This suggests that PGPB inoculation induces both short-term blooms (e.g., *Pseudomonas* and *Bacillus*) and longer-lasting shifts in community composition. These changes are consistent with reports that microbial inoculants can reshape rhizosphere networks, enhance nutrient cycling, and promote plant growth (Alcántara Neto et al., 2025). However, the transient nature of some responses highlights the need to assess long-term stability under field conditions.

3.4. Plant growth and fruit yield

The vegetative growth of melon plants was significantly enhanced by

the PGPB inoculum in both experimental years, with no significant difference recorded between the two seasons. At 20 DAT, the main stem of inoculated plants was 21.9 % longer than that of the control plants, maintaining a significant advantage (+13.6 %) until the final measurement at 40 DAT (Fig. 4a). Inoculated plants also exhibited a higher leaf count than the control, producing 17.7 % more leaves at 30 DAT (Fig. 4b). This significant enhancement of vegetative growth in inoculated melon plants confirmed that the bacteria tested in this study acted as a mixed PGPB inoculum.

The early increase in stem length and leaf count suggests that the inoculated strains (particularly the fast-colonizing *P. lini* RC-UNIPASAAFM01175) successfully established a beneficial relationship with the host during the critical establishment phase. These results align with previous studies where *Bacillus* and *Pseudomonas* species enhanced nutrient uptake or produced phytohormones such as auxins and gibberellins, which stimulate cell elongation and division (Han et al., 2024; Li et al., 2024; Orozco-Mosqueda et al., 2023; Sah et al., 2021; Sun and Shahrajabian, 2025). Notably, the lack of significant interannual variation in vegetative parameters suggests that microbial colonization was robust enough to overcome minor environmental fluctuations between the two growing seasons.

All the plants developed male flowers by 20 DAT, whereas female flower development was significantly accelerated by bacterial inoculation. At 30 DAT, 85.0 % of inoculated plants had developed female flowers compared to 67.5 % in the control, with all plants reaching anthesis by 40 DAT (Fig. 4c). This accelerated development of female flowers directly influenced fruit set. By 40 DAT, 80.0 % of inoculated plants had set fruit, significantly exceeding the control (70.0 %) (Fig. 5c). The early appearance of female flowers is a critical finding. In cucurbits, the transition from the vegetative to the reproductive stage is regulated by the plant's nutritional status and hormonal balance (Martínez and Jamilena, 2021). The earlier shift to female flowering likely resulted from improved carbon assimilation facilitated by the higher leaf number. This boost could have provided the energy reserves necessary for earlier reproductive onset, establishing a developmental advantage that persisted throughout the reproductive stage, showing enhanced canopy density in the soil-inoculated plots (IN) compared to the non-inoculated control plots (CTR) (Fig. 5). The fact that the transition from vegetative to reproductive stages was markedly advanced in inoculated plants indicates that the microbial inoculum may have modulated the plant's hormonal balance (likely through IAA and gibberellin production) and nutritional status to prioritize early flowering (Orozco-Mosqueda et al., 2023).

Although harvesting times shifted between the first and second years

Table 2

Effect of soil inoculation with the mixed PGPB inoculum on winter melon plant yield components.

Source of variance	Total marketable fruit			Early marketable fruit			Unmarketable fruit		
	Yield (t ha ⁻¹)	Number (fruit plant ⁻¹)	Average weight (kg)	Yield (t ha ⁻¹)	Number (fruit plant ⁻¹)	Average weight (kg)	Yield (t ha ⁻¹)	Number (fruit plant ⁻¹)	Average weight (kg)
Year									
2024	22.45	2.91	2.22	10.98	1.52	2.18	1.57	0.91	0.77
2025	23.05	2.77	2.61	11.81	1.44	2.37	1.76	0.73	0.73
Soil treatment									
CTR	21.42	2.58	2.50	10.00	1.25	2.32	1.62	0.85	0.70
IN	24.08	3.10	2.34	12.79	1.72	2.23	1.72	0.80	0.79
Significance									
Year	ns	ns	ns	ns	ns	ns	ns	ns	ns
Soil treatment	**	**	ns	**	**	ns	ns	ns	ns
Year x Soil treatment	ns	ns	ns	ns	ns	ns	ns	ns	ns

Results indicate mean values of 4 replicates of 20 plants each. Abbreviations: CTR, control treatment; IN, soil inoculated with the mixed PGPB inoculum. Significance: ns = not significant; ** significant at $p < 0.01$.

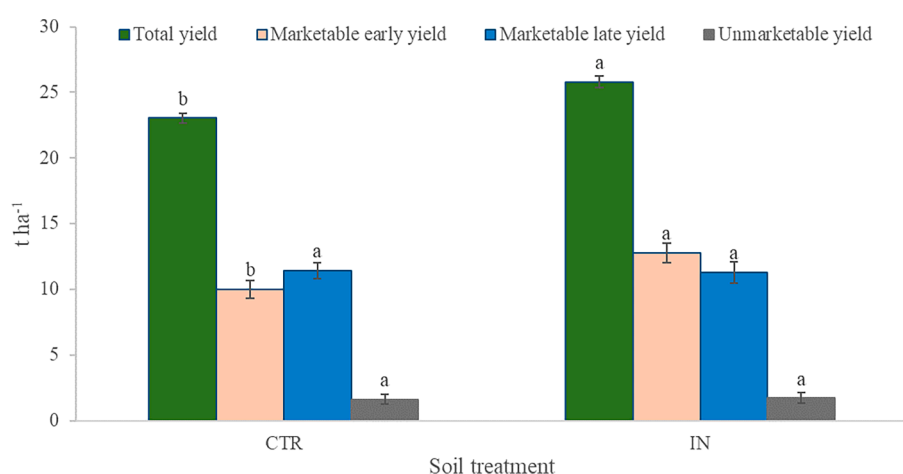


Fig. 6. Yield components of winter melon plants grown on soil inoculated (IN) or non-inoculated (CTR) with the mixed PGPB inoculum. Data represent the mean values averaged across the two consecutive growing seasons (2024 and 2025) (black bars represent standard errors of the means; bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

of the trials, fruit set and harvest did not differ significantly. Fruit production and quality followed a consistent trend over the two years, with no significant interannual differences recorded. Furthermore, two-way ANOVA revealed no significant interactions between plant inoculation and the experimental year. Consequently, results are presented as the mean values across both years for each individual feature.

Yield characteristics of melon plants were significantly affected by inoculation. Total yield was 12.0 % higher in inoculated plants than in the control (Table 2 and Fig. 6).

This increase was primarily driven by the marketable fruit fraction, as unmarketable yield remained similar across treatments (1.7 t ha⁻¹ on average). Analysis of sequential harvests revealed further differences in yield earliness; early yield (comprising the first two harvests) was 27.9 % higher in the inoculated plants compared to the control (10.0 t ha⁻¹), while no significant differences were found in subsequent harvests (11.4 t ha⁻¹ on average). This increase can be ascribed to a higher number of fruits per plant in the inoculated plants (+37.6 %) during the early stages compared to control (1.25 fruit plant⁻¹), as average fruit weight did not differ significantly (Table 2).

This indicates that the mixed PGPB inoculum did not alter the genetic size potential of the cultivar but improved fruit set efficiency, especially at an early fruit setting stage. Hence, the primary agronomic benefit of the bacterial inoculum was the promotion of yield earliness, a trait that often dictates profitability in horticultural markets (Bantis and Koukounaras, 2023). Moreover, the stability of these results across both

experimental years underscores the reliability of this microbial inoculum as a tool for yield stabilization.

Beyond altering vegetative growth and yield components, rhizosphere inoculation with the PGPB inoculum exerted a qualitative influence on harvested fruit characteristics (Table 3). Gross morphological parameters, such as fruit weight, longitudinal diameter, and equatorial diameter, did not change significantly, whereas the internal structural and chemical characteristics of the fruits were significantly improved. A prominent structural finding was the increased rind thickness and the higher percentage of edible part in the fruits from inoculated plants. Moreover, pulp firmness increased by 15.0 % in inoculated fruits compared to the non-inoculated control (12.10 N). This is a remarkable result, as firmness at harvest is a key determinant of shelf-life and transportability in melons, functioning as the primary indicator of post-harvest quality, directly dictating shelf-life, mechanical transport resistance, and consumer crispness perception (Farcuh et al., 2020). The structural integrity of melon pulp relies on the preservation of the middle lamella and primary cell walls which are linked to calcium uptake and translocation to the fruits (Gustani et al., 2024). Members of the genera *Bacillus* and *Pseudomonas*, which selectively persisted in our rhizosphere trials (*B. tequilensis* RC-UNIPASAAFM01186 and *P. reinekei* RC-UNIPASAAFM01216), are known to secrete organic acids and siderophores that chelate and solubilize mineral pools in the soil, significantly enhancing plant uptake of divalent cations, particularly calcium (Agaras et al., 2023; Nuvoli et al., 2024). Once transported to the fruit

Table 3
Effect of inoculation with the mixed PGPB inoculum on winter melon fruit characteristics during two years of experiments (SSC: soluble solid content; TA: titratable acidity).

	Fruit Volume (ml)	Fruit Diameter		P/E	Thickness		Internal space (mm)	Pulp Firmness (N)	Edible Part (%)	Rind color			SSC (°Brix)	TA (mg 100 g ⁻¹ FW)	SSC/TA	AsA (mg 100 g ⁻¹ FW)		
		Equatorial (E) (mm)	Polar (P) (mm)		Rind (mm)	Flesh (mm)				L	a*	b*					Chroma	Hue angle
Year																		
2024	2192.4	176.2	215.2	1.22	11.1	41.5	72.3	12.8	58.9	77.8	-3.9	65.0	12.6	11.2	1.13	44.4		
2025	2312.6	179.4	217.0	1.21	10.9	42.7	73.0	13.2	61.2	76.9	-4.5	65.9	13.0	11.4	1.14	45.2		
Soil																		
CTR	2331.9	172.8	212.5	1.23	10.5	41.7	72.5	12.1	57.2	78.4	-4.7	64.0	11.9	11.5	1.04	43.4		
IN	2173.1	182.7	219.7	1.20	11.4	42.4	72.6	13.9	63.1	76.5	-3.6	66.9	13.7	11.1	1.24	46.2		
Significance																		
Year	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
Soil	ns	*	ns	ns	*	ns	ns	**	*	*	ns	*	*	ns	*	ns		
treatment																		
Year x Soil	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
treat.																		

Each value is the mean of four replicates. Abbreviations: CTR, control treatment; IN, soil inoculated with the mixed PGPB inoculum; SSC, soluble solid content; TA, titratable acidity; AsA, Ascorbic acid. Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$. Titratable acidity expressed as citric acid.

via the xylem, free calcium ions accumulate within the apoplast, where they bound securely to the carboxyl groups of de-esterified linear chains of polygalacturonic acid. This cross-linking reaction forms a rigid calcium-pectate gel network that physically stabilizes the cell-wall structure and drastically restricts the accessibility of cell-wall loosening enzymes which typically catalyze protopectin degradation during the final stages of maturity (Zhi et al., 2017). The observed concomitant increase in rind thickness further corroborates this systemic reinforcement of cell structural layers, suggesting a uniform deposition of structural carbohydrates and minerals across the pericarp tissues. Furthermore, the inoculum modified rind colour, resulting in lighter, more vibrant colour (greater b^* and chroma), potentially increasing consumer appeal.

Parallel to these structural improvements, the chemical profile of the fruits also saw significant improvement due to bacterial inoculum, as evidenced by a substantial increase in soluble sugar content (SSC), reaching 13.7 °Brix. In Inodorus melons, sucrose, glucose, and fructose represent the primary components of total soluble solids (Tzuri et al., 2025), and sucrose accumulation usually occurs in the final stage during fruit maturation (Yang et al., 2019). Because these fruits lack starch reserves, they cannot synthesize sugars post-harvest; their final sweetness depends entirely on the photoassimilates translocated during the active growth phase (da Graça Barreiro et al., 2001). This marked sugar accumulation can be physiologically justified through a two-fold mechanism acting at both the source and sink levels. At the source level, the early vegetative boost stimulated by the inoculum, manifested by the 17.7 % increase in leaf number at 30 DAT, vastly expanded the active photosynthetic canopy surface, maximizing light interception and net carbon fixation during the critical vegetative-to-reproductive transition. At the sink level, the persistent rhizosphere colonization by the inoculated strains suggests a sustained biochemical stimulation of carbohydrate transport, optimizing the expression of sucrose-synthesizing enzymes in source leaves and accelerating phloem unloading into the vacuolar compartments of the melon pulp cells.

Interestingly, this intense influx of carbohydrates occurred without altering the titratable acidity (TA) of the juice, which remained perfectly stable across treatments. In melon fruit, organic acids serve as primary respiratory substrates (Batista-Silva et al., 2018). The stability of TA indicates that the PGPB-induced acceleration of fruit maturation did not trigger premature organic acid depletion or hyper-respiratory consumption within the cellular vacuoles. Consequently, the combination of elevated SSC and stable TA resulted in a significantly superior ripening index (SSC/TA ratio), representing a highly balanced and commercially desirable sensory profile. Furthermore, the ascorbic acid content remained uniform across treatments, averaging 44.8 mg 100 g⁻¹ FW. This stable pool confirms that the PGPB-induced maturity optimization avoids the oxidative depletion or dilution of key antioxidants, delivering a high-quality functional food that contributes substantially to the daily recommended dietary intake of vitamin C.

The overall improvement in physical (firmness, rind thickness) and chemical (SSC) parameters suggests that bacterial treatment provides a comprehensive benefit to fruit quality.

The significant improvements in yield and fruit quality are particularly noteworthy given the transient nature of the total microbial population described in Section 3.1. While initial inoculations triggered an initial massive numerical surge in the rhizosphere, microbial counts converged between inoculated and control rhizosphere by the end of the cultivation cycle. This suggests that the agronomic benefits were not dependent on a sustained high-density microbial population, but rather on an early-stage priming effect. The increase in early yield and the surge in fruit count during initial harvests indicate that the primary influence of the mixed PGPB inoculum occurred during the vegetative and flowering transitions. The early abundance of *P. lini* RC-UNIPASAAFM01175 likely facilitated rapid nutrient mobilization and hormonal signalling, establishing a robust plant architecture (greater stem length and leaf count) that supported higher fruit set

independently of the subsequent bacterial decline. The fact that total microbial abundances (TMB and TmAb) returned to control levels by the end of harvests indicates that the melon plants successfully re-established their native rhizosphere equilibrium once the developmental trajectory was set. Even if the total microbial counts converged and the bacterial boost in the soil faded, the long-term persistence of specific strains from the initial bacterial inoculum (*B. tequilensis* RC-UNIPASAAFM01186 and *P. reinekei* RC-UNIPASAAFM01216) until the end of the cultivation cycle provides a plausible explanation for the enhanced fruit quality. Unlike the total mesophilic population, which returned to control levels, these specific strains maintained a functional niche. Their continued presence may have exerted sustained growth-promoting effects or influenced sink-source relationships during fruit ripening, ensuring superior physical and chemical parameters at harvest, as found on other crops (Kang et al., 2019).

4. Conclusions

This study demonstrates that multi-species PGPB inoculation, including *Bacillus*, *Peribacillus*, and *Pseudomonas* species, can effectively enhance winter melon productivity primarily through early-stage rhizosphere priming rather than sustained microbial dominance. The transient microbial stimulation and selective persistence of ecologically fit strains, such as *B. tequilensis* RC-UNIPASAAFM01186 and *P. reinekei* RC-UNIPASAAFM01216 throughout the crop cycle, underline the importance of functional resilience over numerical abundance. Agonomic benefits, particularly increased yield earliness and improved fruit quality traits (flesh firmness and soluble solids content), highlight the practical value of this biostimulant for commercial production, where timing and postharvest performance are critical. These findings support the integration of bacterial consortia into sustainable organic horticultural systems and provide a scientific basis for developing biofertilizer guidelines aimed at reducing inputs while maintaining yield stability and quality standards under open-field conditions. Future research should explore the application of this multi-strain inoculum in conventional agroecosystems to evaluate its potential to compensate for reduced synthetic chemical inputs without compromising yield or quality.

CRedit authorship contribution statement

Lino Sciarba: Data curation, Investigation, Methodology, Visualization, Writing – original draft. **Alessandro Esposito:** Data curation, Investigation, Methodology, Visualization, Writing – original draft. **Gabriele Busetta:** Data curation, Investigation, Writing – original draft. **Alessandra Moncada:** Data curation, Validation, Writing – original draft. **Valentina Paci:** Investigation, Writing – original draft. **Filippo Vetrano:** Data curation, Validation, Writing – original draft. **Raimondo Gaglio:** Conceptualization, Data curation, Formal analysis, Methodology, Validation, Writing – original draft. **Alessandro Miceli:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Luca Settanni:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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