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Original article

Contrasting effects of treated wastewater irrigation and *Trichoderma* inoculation on tomato soil microbiomes

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ABSTRACT

Agricultural water reuse is expanding to mitigate freshwater scarcity, yet its effects on soil microbial ecology remain unclear, particularly when combined with biological inoculants. In a controlled greenhouse setting, we examined how municipal treated wastewater (TW), chlorinated treated wastewater (TW^{Cl}), and tap water (TapW) interact with *Trichoderma afroharzianum* T-22 inoculation to shape soil bacterial communities, rhizosphere potential pathobiomes, and class 1 integron abundance. Prolonged (till harvest) watering with TW^{Cl} significantly reduced bacterial community richness compared to TapW in not inoculated samples. Chlorination did not significantly lower potential pathobiome richness; however, in TW^{Cl} treatment, there was a trend for potentially pathogenic bacteria to accumulate. *Trichoderma* colonized the roots and reshaped the rhizosphere communities, increasing the potential pathobiome richness at intermediate stages, yet reducing the overall bacterial diversity at harvest. To note that, even if not significant, class 1 integron abundance tended to decrease in *Trichoderma*-associated soils, suggesting a potential beneficial effect on anthropogenic pollution markers. These findings, although based on a limited number of samples, highlight the interacting effects of irrigation water quality and microbial inoculation on soil microbial structure and potential pathobiome. This information supports the need for systematic microbial monitoring to develop sustainable water reuse strategies.

1. Introduction

Global environmental changes, including climate warming, land degradation, and intensifying water scarcity, are increasingly challenging agriculture, and ecosystem stability worldwide [1]. Agriculture accounts for most global freshwater withdrawals, increasing the need for alternative irrigation resources under conditions of growing water scarcity [2–4]. Treated wastewater (TW) represents a promising strategy to reduce pressure on freshwater resources and support circular water management [2,5]. However, TW can contain salts, nutrients, residual contaminants, and antibiotic-resistant bacteria [6,7]. These contaminants may alter soil physicochemical properties and reshape soil microbiomes [8–10].

In parallel with water scarcity challenges, agriculture must increase productivity under constraints, such as soil degradation, ecosystem disruption, and contaminant accumulation [11–13]. In this context, reclaimed municipal effluent represents a strategic resource for irrigation, particularly in arid and semi-arid regions [14–18]. However, concerns about environmental and health risks persist [5].

Understanding how TW interacts with soil biological processes and crop management practices is essential for sustainable agricultural systems that promote beneficial microbiome functions [19–22]. Although modern wastewater treatment plants (WWTPs) substantially reduce pathogen loads and organic matter, wastewater disinfection treatments such as chlorination are commonly used to improve microbiological quality before agricultural reuse. However, while chlorination reduces microbial loads, it may also selectively favor resistant microorganisms and alter microbial community composition, with possible

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consequences for soil microbial dynamics after irrigation [23].

Tomato (*Solanum lycopersicum* L.) is a globally important crop [24,25], but its productivity is increasingly constrained by water scarcity, soil degradation, and pest pressures [26,27]. Beneficial microbial inoculants, including *Trichoderma* spp., are increasingly used to enhance plant performance and modulate rhizosphere processes. *Trichoderma* spp. can colonize plant roots, stimulate nutrient acquisition and plant defenses, and influence microbial communities through competition, antimicrobial activity, and changes in plant metabolism and root exudation [28–32].

In real-world agricultural systems affected by chronic water scarcity, the application of *Trichoderma* operates within complex environments characterized by the widespread use of TW for irrigation [15,16]. Interactions between wastewater-derived microbial communities and beneficial inoculants may influence rhizosphere assembly and microbial functioning, but these processes remain poorly understood [33].

Recent studies indicate that contaminants associated with TW can alter microbial community composition and influence environmental reservoirs of antibiotic resistance and potentially pathogenic microorganisms [8,34]. However, limited information is available regarding the combined effects of reclaimed irrigation water and microbial inoculants on soil microbial communities and pathobiome dynamics. *Trichoderma* spp. can reshape rhizosphere microbial communities through root colonization and pathogen suppression mechanisms, including competition, antibiosis, mycoparasitism, and induction of plant defenses [28,29]. In contrast, TW can modify soil conditions and introduce contaminants and microbial inputs that collectively influence soil microbiome and pathobiome composition.

In this study, we conducted a controlled greenhouse experiment to investigate how irrigation water quality and the inoculation of beneficial microbes jointly influence soil microbial dynamics, crop-associated pathobiomes, and the abundance of class 1 integrons, which have been proposed to be used as a proxy for anthropogenic pollution and environmental antibiotic resistance [35,36]. Specifically, we evaluated the interactive effects of municipal TW, chlorinated TW (TW^{Cl}), and conventional irrigation water in combination with *Trichoderma afroharzianum* T-22 inoculation in a commercial tomato cultivar.

Based on previous evidence showing that *Trichoderma* can interact with resident microbial communities and influence rhizosphere assembly and microbial functions [37,38], we hypothesized that *Trichoderma* inoculation would reshape soil bacterial communities and modulate pathobiome and class 1 integron dynamics [39] under different irrigation regimes. To test this, we integrated amplicon-based characterization of soil bacterial communities with the quantification of class 1 integron abundance.

2. Materials and methods

The experiment was conducted under controlled greenhouse conditions using potted tomato plants to investigate the interactive effects of irrigation water quality and microbial inoculation on soil microbial communities and class 1 integron dynamics. Pot experiments were selected to ensure standardized environmental conditions and regulatory compliance for TW irrigation while minimizing environmental variability.

Potted systems are inherently constrained in terms of root volume, soil resource availability, and therefore can confound interpretation of growth-related parameters and do not allow for reliable assessment of yield components. As a result, standard agronomic performance metrics (*e.g.*, total biomass, fruit yield, harvest index) were not measured in this study. Also, the effects of *Trichoderma* inoculation or nutrient contributions from TW have been widely documented in the literature. Rather, our experimental design was specifically targeted at elucidating how irrigation with TW, combined with *Trichoderma* inoculation, modulates soil microbial community structure, potential pathobiome composition, and the abundance of class 1 integrons.

2.1. Plant material and growth conditions

Seeds of the F1 tomato hybrid 'Bobcat' (Syngenta, Italy) were germinated on moist cotton disks inside sterile Petri dishes kept in the dark at 20 ± 1°C. After five days, 100 germinated seeds were transferred into alveolate containers (3.5 cm diameter × 5.5 cm height) filled with sterile substrate composed of peat, coconut fiber, and perlite and maintained under controlled conditions (21 ± 2°C, 50 ± 5% RH, 16:8 h L:D photoperiod). Plants were irrigated three times per week with tap water (the main physical, chemical, and microbiological characteristics are available at the following link https://www.arpab.it/temi-ambientali/acqua/acque-dolci-destinate-alla-produzione-di-acqua-potabile/) and fertilized twice using 20 mL of NPK nutrient solution "Piante verdi" (Compo®, NKP: 7-3-6) at 3% concentration.

2.2. Fungal inoculation

Tomato plants were inoculated with *Trichoderma afroharzianum* strain T-22 (Trianum-P, Koppert B.V., Netherlands). Viability of the commercial formulation was verified by colony-forming unit (CFU) determination following Forlano et al. [40]. Inoculation was performed twice: immediately after seed transfer and after transplanting into experimental pots, using aqueous suspensions (4 g commercial product per liter of distilled water, 1×10^9 CFU/g viable spores). The first inoculation was performed immediately after placing the germinated seeds into the alveolate containers, watering each seed with 10 mL of a freshly prepared suspension. The second inoculation was carried out one month after germination, immediately after transplanting the seedlings into cylindrical pots with a volume of 1500 cc, and each inoculated plant received 75 mL. Control plants received equivalent volumes of distilled water. Based on previous studies [10,40,41], this double procedure ensured 100% fungal colonization. However, to confirm the persistence of the symbiosis, *T. afroharzianum* colonization was assessed at the end of the experiment by fungal isolation from root samples following the procedure described by Forlano et al. [40] and Trotta et al. [10]. Roots from a subset of six control and six inoculated plants (two per treatment) were carefully washed, cut into 2 cm segments, surface-sterilized by immersion in 70% ethanol followed by 1% sodium hypochlorite, and rinsed with sterile distilled water. Root fragments were placed on potato dextrose agar supplemented with streptomycin sulphate (0.05%) and incubated at 25 ± 1°C for seven days, after which fungal growth was recorded. Colonization frequency was expressed as the percentage of root fragments from which *T. afroharzianum* was successfully re-isolated. A root fragment was considered colonized when fungal colonies displaying the typical morphological characteristics of *T. afroharzianum* emerged from the root tissue after incubation.

2.3. Experimental design and irrigation treatments

One month after germination, seedlings were transplanted into 1500-cc pots (11 cm height × 9.5 cm top diameter) containing an artificial substrate (40% peat, 40% perlite, 10% sand, 10% clay; pH 7.7; EC 740 μS/cm) selected to provide standardized physicochemical conditions while minimizing background microbial variability [8,10,34].

Immediately after transplantation, the pots were placed in a greenhouse and were divided into three irrigation groups, each receiving a different water treatment: (i) control irrigation with tap water (TapW), (ii) irrigation with municipal TW, and (iii) irrigation with chlorinated TW^{Cl}. Based on this procedure, each irrigation treatment was combined with the two fungal inoculation levels (Co: Control, not inoculated plants; *Tr*: *Trichoderma* inoculated plants), resulting in six treatment combinations, each consisting of 10 plants: Co-TapW, Co-TW, Co-TW^{Cl}, *Tr*-TapW, *Tr*-TW, and *Tr*-TW^{Cl} (Fig. 1).

2.3.1. Collection and treatment of water

Municipal wastewater was collected weekly from the Vaglio WWTP

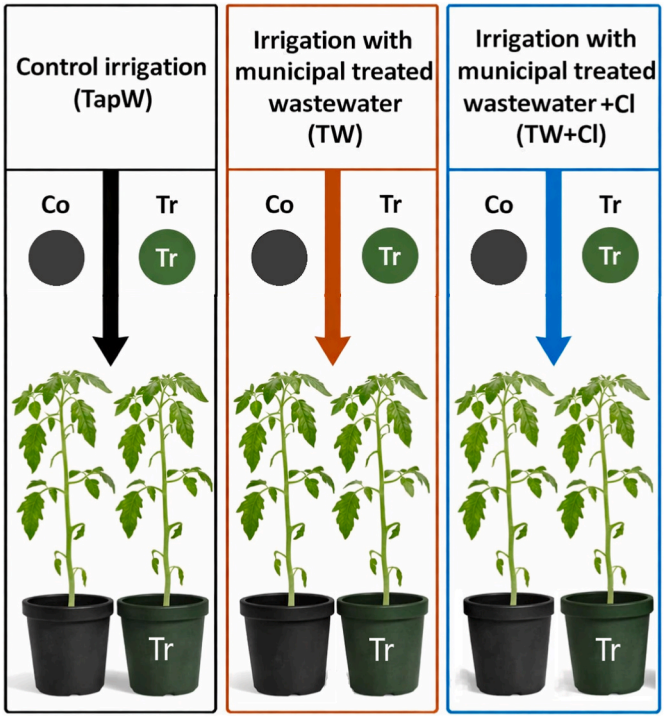

Fig. 1. Treatments and conditions. Schematic representation of experimental design.

(Potenza, Italy), which treats wastewater from 160,000 population equivalents. The plant serves the Municipality of Potenza and the industrial areas of Potenza and Tito. Wastewater samples were taken before the disinfection for the TW, or after chlorination for the TW^{Cl} treatment. Chlorination is employed to ensure effective disinfection while protecting the Basento River, into which the treated effluents are discharged. The chlorination process involves the addition of sodium hypochlorite, supplied as a commercial solution containing 12–14% w/w of active chlorine. The maximum allowable concentration of free residual chlorine in TW discharged into surface waters or to the soil in Italy is generally 0.2–0.3 mg/L, in accordance with Legislative Decree 152/2006 and subsequent implementing regulations. Accordingly, the dosage of sodium hypochlorite is adjusted based on the daily flow to ensure compliance with legal limits.

Both TapW, TW, and TW^{Cl} were stored in 25-liter barrels under identical conditions before being applied to the plants.

2.3.2. Irrigation and sampling

Tomato plants were irrigated with treatment-specific water sources at the soil surface from transplanting until fruit ripening (107 Days After Transplanting, or DAT). Soil moisture was maintained within predefined water-holding capacity ranges to ensure comparable hydric conditions across treatments. Soil samples for microbial analyses were collected at the pre-flowering stage (40 DAT, T1) and at fruit ripening (107 DAT, T2). At the onset of flowering, selected plants were transferred to larger pots containing the same soil substrate to preserve physicochemical and microbial continuity. Fertilization was applied uniformly across treatments. Additional detailed information on irrigation volumes, transplant procedures, the fertilization regimen, and sampling specifications is provided in the **Supplementary** Material, Section S1.

2.4. DNA extraction

At 40 DAT, 90 cc soil samples were collected from three distinct plants per experimental group, and stored at −20°C. This process was repeated for three plants per group, generating three independent replicates per treatment. Additionally, soil samples were independently collected at 107 DAT from three plants grown in larger pots and stored at −20°C. DNA was extracted from these soil samples using the DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

2.5. 16S rRNA gene amplicon sequencing, intI1 gene quantification, and data analysis

An aliquot of DNA extracted from the soil samples were sent under controlled conditions at IGA Technologies (Udine, Italy) for the 16S rRNA gene amplicon sequencing. Bacterial community composition was obtained by sequencing the V3–V4 hypervariable regions on the Illumina NovaSeq 6000 platform using the universal bacterial primer set S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 [42]. All the data and statistical analyses were made in the R environment v4.5.2 [43]. The DADA2 pipeline v1.16 was followed to process raw reads essentially as suggested in the online tutorial (https://benjjneb.github.io/dada2/tutorial.html). Processing was performed under *dada2* package v1.32.0 [44]. Briefly, the raw reads were, first, quality checked (showing a mean Phred score> 28 in forward and reverse) and then, trimmed applying the following parameters: trimLeft = c(17, 20), maxN = 0, maxEE = c(2, 5), truncQ = 2, rm.phix = TRUE. Then, they were merged and merged sequences used to obtain unique Amplicon Sequences Variants (ASVs). Chimeras were removed in "consensus" mode. Taxonomic assignments of ASVs were conducted against Silva database v138.2 [45]. Data were rarefied to the ASV number of the sample with the lowest ASV count (60809) (Supplementary Table S1), using *Rarefy* function of *GUniFrac* package v1.9 [46], to obtain a comparable dataset. ASVs not identified at a Phylum level or identified as chloroplasts or mitochondria were removed from the dataset. The original dataset was composed of 20375 ASVs and 18164 ASVs were retained after the above-mentioned removal steps. Raw sequences were publicly available in the NCBI database under project n° PRJNA1430414. The dynamics of soil whole bacterial community were exanimated. Moreover, we prepared, from the taxonomy annotation obtained after 16S rRNA gene amplicon sequencing, a subset of data containing only the potentially pathogenic bacterial genera (potential pathobiome). In detail, we selected those genera included in the list of established pathogenic bacteria reported by Bartlett and colleagues [47]. This approach represented an exploratory tool based on genus-level resolution rather than species-level identification and has been previously proposed for amplicon sequencing data from water samples [8,48,49].

For both datasets, richness was defined as the number of different ASVs or potentially pathogenic genera in each sample through *vegan* package v2.7-1 [50]. For the analysis of beta diversity, a matrix of all pairwise comparisons through Bray–Curtis dissimilarity index was calculated (*vegan* v2.7-1). The matrix was used to build a dendrogram connecting all samples after hierarchical cluster analysis with average linkage. First, we investigated the effect of "Date" factor, applying a negative binomial generalized linear model (NB-GLM) for richness (since they were counted data), using *glm.nb* function of MASS package v7.3.60.2 [51] according to formula (1), and a PERMANOVA (*adonis2* function in *vegan* v2.7-1) on beta diversity matrix (2).

(1) NB-GLM (richness (all samples) ~ Date)
(2) PERMANOVA (distance matrix (all samples) ~ Date)

Then, data were subset and differences in richness (through NB-GLM (3)), and beta diversity (through PERMANOVA (4)), for each time point, were assessed in relation to the inoculation with *Trichoderma* (2 levels: control and *Trichoderma* inoculated plants), the treatments (3 levels: TapW, TW, and TW^{Cl}) and their interaction. Moreover, for the potentially pathogenic bacterial genera, we calculated the total abundance for sample and tested it, using a generalized linear model (GLM) according to formula (5). If the treatment or the interaction resulted variables

significant to the models, NB-GLM and GLM were *Tukey post-hoc* tested using *emmeans* function of *emmeans* package v1.11.2.8 [52] according to the general formula (6).

(3) NB-GLM (richness (at T1 or T2) ~ Inoculation * Treatment)
(4) PERMANOVA (distance matrix (at T1 or T2) ~ Inoculation * Treatment, by = "terms")
(5) GLM (potential pathogen abundance (at T1 or T2) ~ Inoculation * Treatment, family = quasipoisson (link = "log"))
(6) emmeans ("*model*", pairwise ~ "*explanatory variable*", infer = c (TRUE, TRUE), adjust = "Tukey")

Another aliquot of DNA was analyzed to quantify class 1 integrons (targeting the integrase gene *intI*1) by quantitative real-time PCR (qPCR). The qPCR, including normalization using the 16S rRNA gene, was performed as described in Brienza et al. [8]. Inhibition test was carried out by dilution method, as previously described [53], and no PCR inhibition was detected. Standard curves ranged from 2.25 x 10^1 to 2.25 x 10^7, and from 5.0 x 10^2 to 5.0 x 10^7 gene copies μL^{-1}, for *intI*1 and 16S rRNA gene, respectively. The limit of quantification of the reactions (LOQ), determined as proposed by Bustin et al. [54], was 22.5 and 500 gene copies μL^{-1}, for *intI*1 and 16S rRNA gene, respectively. The mean value ± standard deviation of the efficiency and R2 were 91.4 ± 0.7 and 0.998 ± 0.002, respectively. Values below the LOQ but above the limit

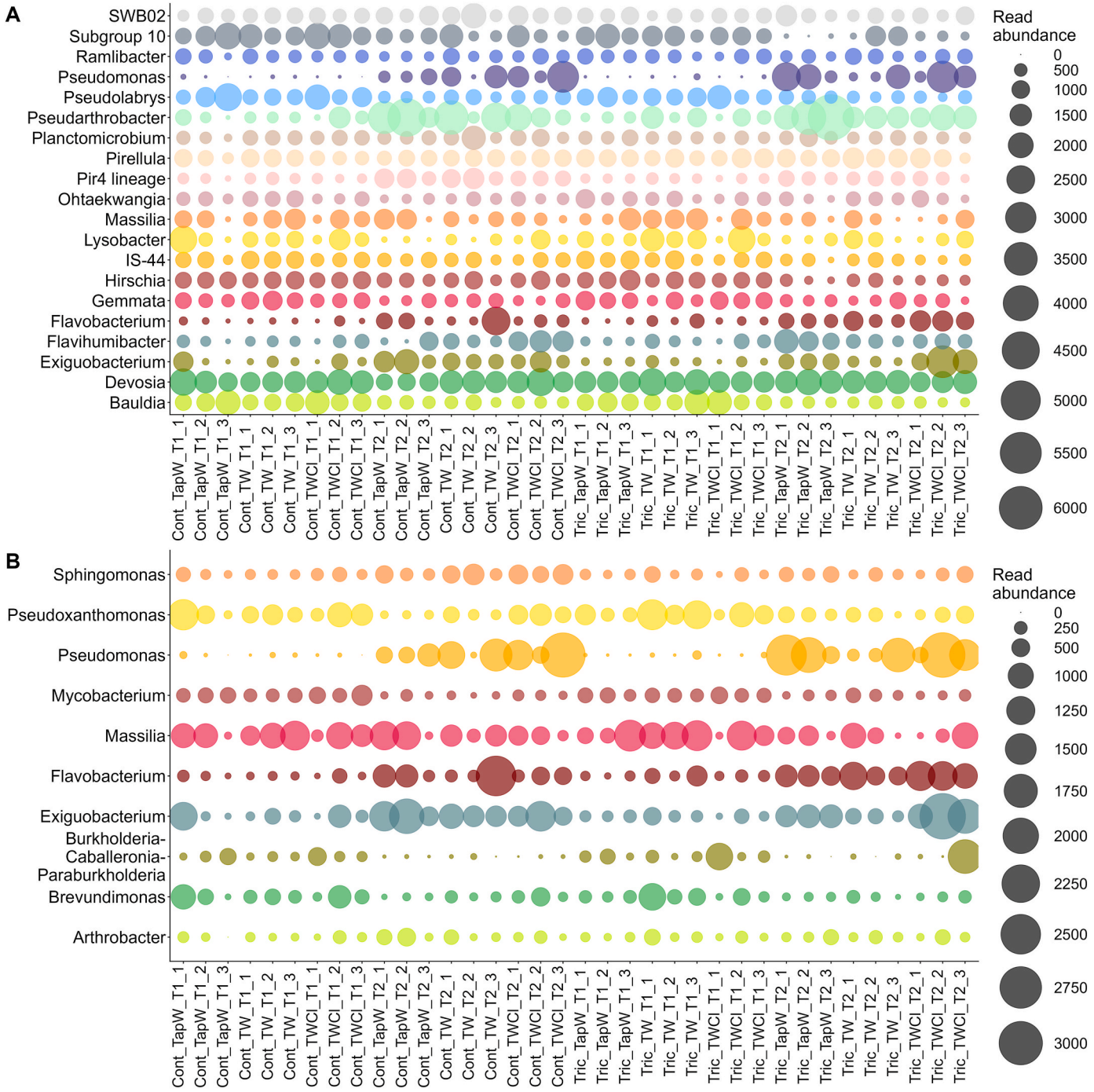

Fig. 2. Most abundant genera in soil samples. Twenty and ten most abundant genera retrieved for the whole bacterial community **A)** and potential pathobiome **B)**, respectively.

of detection (LOD) were considered positive but non quantifiable, whereas values below the LOD were considered negative. Four no-template controls were assayed in each run, resulting below the LOD. *intI*1 gene abundances were expressed by dividing the gene copy number by the corresponding 16S rRNA gene copy number. Then, as for the potential pathobiome abundance, GLM was applied to the abundance of the *intI*1 gene to assess differences in class 1 integron among samples throughout the experiment. In the statistical tests, differences were considered significant for p-value< 0.05.

3. Results

The results focus on the effects of irrigation water quality and *Trichoderma* inoculation on bacterial community structure, potential pathobiome composition, and class 1 integron abundance in soil samples.

3.1. *Trichoderma afroharzianum T-22 root colonization*

At the end of the experiment (107 DAT), successful root colonization by *Trichoderma afroharzianum* T-22 was confirmed by fungal re-isolation from the inoculated plants, whereas no fungal growth was detected in control roots.

3.2. *Soil bacterial community and potential pathobiome composition*

Raw read processing identified 18164 ASVs belonging to 324 bacterial families and 744 genera (Supplementary Table S2). At the genus level, *Devosia* and *Pseudarthrobacter* were among the three most abundant overall, regardless of the irrigation water used or the presence of *Trichoderma* inoculation (Fig. 2A). Within the potential pathobiome, *Exiguobacterium*, *Massilia*, and *Pseudomonas* were prevalent in TW^{Cl}-, TW-, and TapW-irrigated soil samples, respectively, in the presence of *Trichoderma*, whereas in the absence of *Trichoderma*, *Pseudomonas*, *Massilia*, and *Exiguobacterium* were the most abundant genera in TW^{Cl}-, TW-, and TapW-irrigated soils, respectively (Fig. 2B).

In soil, whole bacterial community and potential pathobiome richness did not significantly differ among samples when considering the time point (NB-GLM: chisq = 1.308, df = 1, p = 0.253 and chisq = 0.024, df = 1, p = 0.877, respectively) (Supplementary Table S3). On the contrary, "Date" factor drove the whole bacterial community and potential pathobiome beta diversity, explaining 34.6 and 38.1% of sample variance (Supplementary Table S3). Indeed, in both cases, samples split based on the time point (Supplementary Fig. S1A–B). At T1, the richness of the whole bacterial community showed a trend to increase in *Trichoderma* enriched soils (quasi-significant differences; NB-GLM: chisq = 3.738, df = 1, p = 0.053), becoming significantly higher in inoculated samples when considering only the diversity in potentially pathogenic genera (NB-GLM: chisq = 5.360, df = 1, p = 0.021) (Fig. 3, Supplementary Table S3). The treatment did not affect richness (whole bacterial community NB-GLM: chisq = 1.461, df = 2, p = 0.482; potential pathobiome NB-GLM: chisq = 0.926, df = 2, p = 0.629) (Supplementary Table S3).

At T2, whole bacterial community richness was significantly higher in controls than in *Trichoderma* inoculated samples (NB-GLM: chisq = 3.868, df = 1, p = 0.049) (Fig. 4A) and, particularly, in controls watered with TapW (NB-GLM: chisq = 20.376, df = 2, p = 3.763×10^{-5}; Tukey *post-hoc* test: p $\leq$ 0.004) (Fig. 4B); whereas, it did not vary according to the treatment (NB-GLM: chisq = 3.129, df = 2, p = 0.209) (Supplementary Table S3). Potential pathobiome richness showed no differences among samples, neither regarding the inoculation nor the treatment (NB-GLM: chisq = 1.508, df = 1, p = 0.219 and chisq = 3.573, df = 2, p = 0.168, respectively) (Supplementary Table S3).

At both time points, all the investigated factors (inoculation, treatment, and their interaction) had a limited effect on whole bacterial community and potential pathobiome composition, explaining from 2.95 to 13.51% of the sample variance (Supplementary Table S3). However, only at T2, PERMANOVA gave a statistically significant response (p $\leq$ 0.038), with the inoculation and treatment factor explaining 11.2 and 14.7% of whole bacterial community composition, respectively (Supplementary Table S3). As consequence, samples appeared not to follow a univocal pattern in clustering (Supplementary Fig. S1A–B). The potential pathobiome total abundance did not significantly differ among samples over time, neither regarding the inoculation (T1 NB-GLM: chisq = 0.209, df = 1, p = 0.648; T2 NB-GLM: chisq = 0.647, df = 1, p = 0.421) nor the treatment (T1 NB-GLM: chisq = 1.003, df = 2, p = 0.606; T2 NB-GLM: chisq = 4.998, df = 2, p = 0.082) (Supplementary Table S3); however, at T2, samples watered with TW^{Cl} showed a tendency to accumulate potentially pathogenic genera (Fig. 5).

For *intI*1 gene, at both time points, no significant differences among samples were found when considering the inoculation (T1 NB-GLM: chisq = 0.023, df = 1, p = 0.880; T2 NB-GLM: chisq = 3.149, df = 1, p = 0.076) and the treatment (T1 NB-GLM: chisq = 0.190, df = 2, p = 0.910; T2 NB-GLM: chisq = 2.670, df = 2, p = 0.263) as factors (Supplementary Table S3). However, we observed a tendency to decrease in inoculated samples over time (Fig. 6).

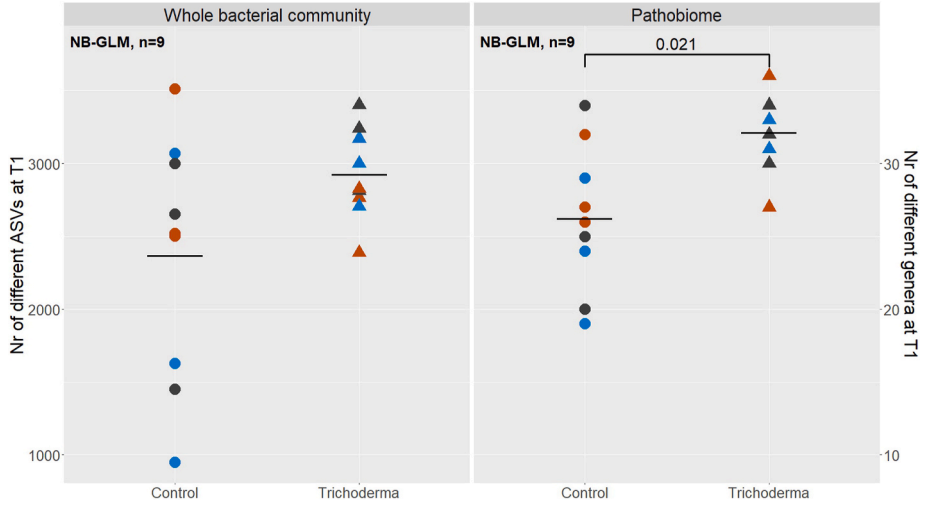

Fig. 3. Richness at T1. Richness of the whole bacterial community and potential pathobiome according to the inoculation at T1. Differences were considered significant for p $<$ 0.05. Only significant p-values were shown.

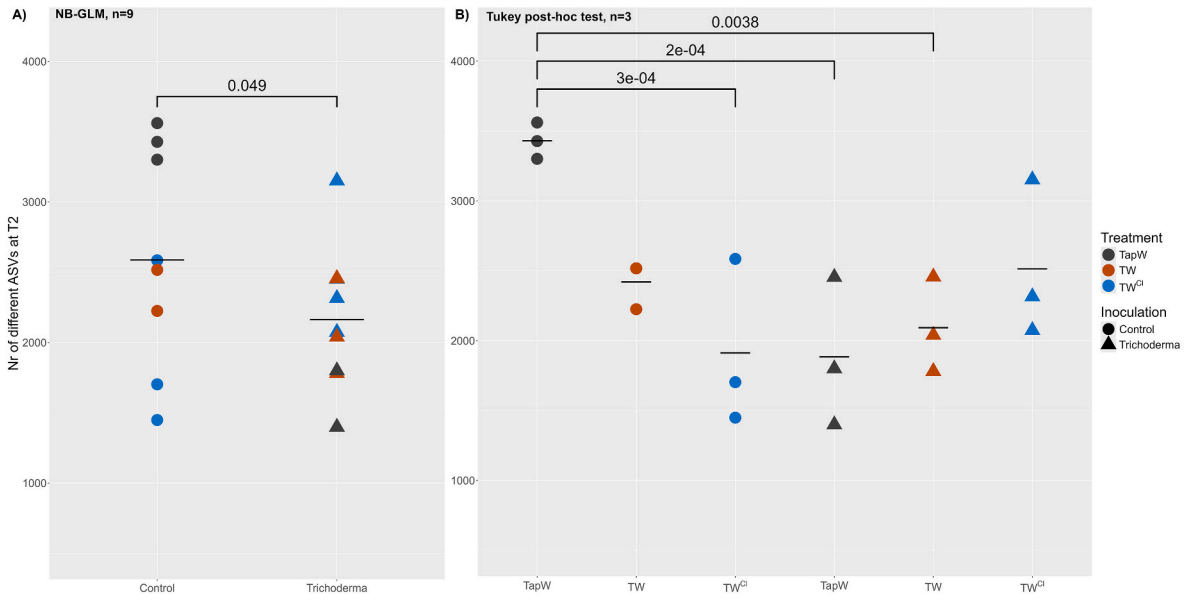

Fig. 4. Richness at T2. Overall richness of the whole bacterial community according to the inoculation **A)** and richness of control samples according to the treatment **B)** at T2. Differences were considered significant for $p < 0.05$. Only significant p-values were shown.

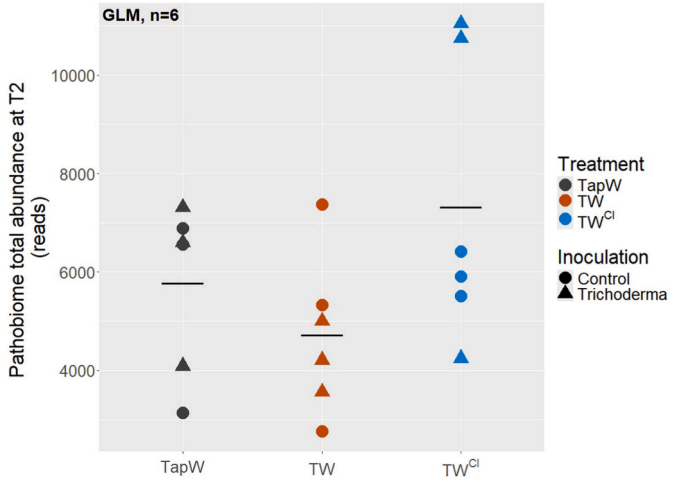

Fig. 5. Potential pathobiome abundance. Abundance of the potential pathobiome according to the inoculation at T2. Differences were considered significant for $p < 0.05$. Only significant p-values were shown.

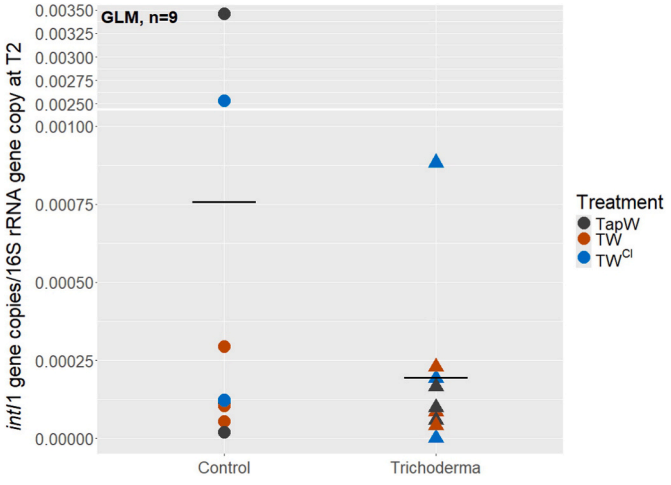

Fig. 6. *intI*1 gene abundance. Abundance of the *intI*1 gene according to the inoculation at T2. Differences were considered significant for $p < 0.05$. Only significant p-values were shown.

4. Discussion

This study provides insights into how irrigation water quality and *Trichoderma afroharzianum* T-22 inoculation jointly shape soil microbial communities, the rhizosphere potential pathobiome, and the abundance of class 1 integrons in tomato cultivation systems. By minimizing environmental and nutrient variability under controlled conditions, the experiment allowed a more direct evaluation of the effects of irrigation water quality and microbial inoculation on belowground microbial dynamics.

Our results demonstrate that, at the end of the experiment, irrigation with TW^{Cl} significantly affected bacterial community richness of control samples, which decreased compared to controls irrigated with TapW. Concerning the effects of TW on soil microbial community alpha diversity, irrespective of the tertiary disinfection processes applied, the literature does not provide a consensus. Previous studies have reported inconsistent responses of soil bacterial diversity to TW irrigation, ranging from no significant effects [55–57], to increases in bacterial community richness in soils irrigated with TW [58]. A possible explanation for these contrasting results may be the type of reference water used across studies. In contrast to previous studies that used groundwater as a control, we used TapW, which represents a higher-quality irrigation source. Therefore, the reduction in richness observed under TW irrigation may reflect differences relative to a high-quality reference rather than an overall suppressive effect of reclaimed water itself. Nevertheless, reductions in bacterial community richness may influence soil ecosystem functioning because microbial diversity is frequently associated with ecological stability and functional redundancy [59,60].

Looking deeper at the bacterial community, the two most abundant bacteria constituting the whole bacterial community were the same regardless of the irrigation water, *i.e.*, *Devosia* and *Pseudarthrobacter*. The potential pathobiome showed a higher variability, with *Exiguobacterium*, *Massilia*, and *Pseudomonas* as the most abundant bacteria. Interestingly, among these three potentially pathogenic genera, *Pseudomonas* was prevalent in soil irrigated with TW^{Cl} and TapW in absence and in presence of *Trichoderma* respectively, and it ranked as the fourth most

abundant genus within the entire bacterial community in soil samples irrigated with TapW. This suggests that also when irrigating with a high-quality but unsustainable water source like TapW, potentially pathogenic bacteria may become relatively prevalent within the overall soil bacterial community.

Notably, although differences were not statistically significant, the abundance of potentially pathogenic bacteria tended to be higher in soils irrigated with TW^{Cl}, compared with TW treatments. A possible explanation is that chlorination may exert selective pressure on microbial communities, altering their composition rather than uniformly reducing all microbial groups. Indeed, previous studies have demonstrated that chlorine-based disinfection can favor the growth of chlorine-tolerant taxa, including some opportunistic microorganisms, while simultaneously lowering the overall microbial abundance [61,62].

Based on previous findings showing the ecological role of *Trichoderma* as a rhizosphere-competent fungus capable of modulating plant physiology and root exudation patterns [39,63,64], we hypothesized that inoculation could modify soil bacterial communities. Consistent with this expectation, *Trichoderma afroharzianum* T-22 successfully colonized tomato roots and influenced the structure of the soil bacterial community.

Several mechanisms may explain the observed microbial shifts. It was reported that *Trichoderma* induced systemic responses that altered plant metabolism, hormone signaling (jasmonic acid, salicylic acid, ethylene), and nutrient uptake efficiency [29,31,63,65]. These changes, in turn, can modify root exudate composition, influencing bacterial recruitment and competition in the rhizosphere. In addition, *Trichoderma* produced antimicrobial compounds, siderophores, and cell-wall-degrading enzymes that can suppress specific bacterial taxa, including some opportunistic pathogens [28]. The increase in potential pathobiome richness at the intermediate stage together with lower bacterial community richness at the end of the experiment suggests that *Trichoderma* inoculation can influence microbial community dynamics over time. However, because only richness metrics were evaluated, the ecological implications of these changes remain uncertain. Changes in community structure could potentially affect the distribution of opportunistic or resistance-associated taxa, although this requires further investigation.

However, *Trichoderma* species are widely recognized as effective biocontrol agents and plant growth-promoting fungi. Extensive evidence supports their agronomic benefits and environmental compatibility [10,15–17,28,39–41,66]. Therefore, the present findings do not question their overall utility but rather highlight the importance of context-dependent effects. In particular, when applied under non-conventional irrigation regimes, such as with TW or under other stress-prone conditions, microbial inoculants may interact with altered physicochemical and microbiological environments in ways that reshape community dynamics. These interactions underscore the need for integrated assessments of biostimulant performance within complex agroecosystems, especially in areas where water reuse practices are implemented. In contrast, although the reduction was not statistically significant, class 1 integron abundance tended to decrease in soils associated with *Trichoderma*-inoculated plants. This pattern may indicate a potential influence of microbial inoculation on anthropogenic pollution markers; however, it should be considered as a preliminary observation that requires validation under broader experimental conditions.

5. Conclusions

Our study, although conducted in greenhouse context and relied on a small sample size, underlines the importance of systematic microbial monitoring when reclaimed wastewater is used for irrigation. We observed that prolonged (till harvest) watering with chlorinated effluents can influence the structure of soil bacterial communities, reducing their diversity. In addition, soil inoculation with *Trichoderma* as a biostimulant showed contrasting effects: on one hand, it appeared to produce a decreasing trend in class 1 integron abundance, suggesting a potentially positive outcome; while, on the other hand, it was associated with a reduction in the whole bacterial community richness and an increase in potential pathobiome richness, highlighting the need for further investigation to fully understand its impact on soil bacterial communities. Overall, our findings indicate that additional studies under real-field conditions are needed to assess whether the observed patterns occurred outside the experimental setting and to evaluate whether TW and *Trichoderma* inoculation can be beneficially applied with respect to both soil ecosystem functioning and human health.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT 3.5 from OpenAI in order to review the language (English). After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Raffaella Sabatino: Conceptualization, Writing – original draft. **Andrea Di Cesare:** Data curation, Methodology, Writing – original draft. **Monica Brienza:** Conceptualization, Funding acquisition, Methodology, Writing – review & editing. **Giulia Borgomaneiro:** Conceptualization, Methodology, Writing – original draft. **Roberto Rosamilia:** Methodology, Writing – original draft. **Samia Khadhar:** Conceptualization, Writing – original draft. **Farah Khezami:** Conceptualization, Formal analysis, Writing – original draft. **Paolo Fanti:** Methodology, Supervision, Writing – review & editing. **Vincenzo Trotta:** Conceptualization, Formal analysis, Methodology, 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2026.103852.

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