agriculture

MDPI

Article

Effects of Reclaimed Wastewater Containing Pharmaceutical Active Compounds (PhACs) and Tomato–Wheat Crop Succession on Soil Microbial Communities and Crop Productivity

Luciano Beneduce [1,*], Federica Carucci [2], Marcella Michela Giuliani [1], Anna Gagliardi [1], Carlo Salerno [3], Michele Denora [4], Michele Perniola [4], Francesco De Mastro [5], Gennaro Brunetti [5], Martina Totaro [1], Lorenzo Brusetti [6,7], Federica Piergiacomo [6], Luigimaria Borruso [6,7] and Giuseppe Gatta [1]

[1] Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, 71122 Foggia, Italy; marcella.giuliani@unifg.it (M.M.G.); anna.gagliardi@unifg.it (A.G.); martina.totaro@unifg.it (M.T.); giuseppe.gatta@unifg.it (G.G.)
[2] Department of Agricultural and Forestry scieNcEs (DAFNE), University of Tuscia, 01100 Viterbo, Italy; federica.carucci@unitus.it
[3] National Research Council of Italy, Water Research Institute (CNR IRSA), 70132 Bari, Italy; carlo.salerno@cnr.it
[4] Department of Agriculture, Forest, Food and Environmental Sciences, University of Basilicata, 85100 Potenza, Italy; michele.denora@unibas.it (M.D.); michele.perniola@unibas.it (M.P.)
[5] Department of Soil, Plant, and Food Science, University of Bari, 70121 Bari, Italy; francesco.demastro@uniba.it (F.D.M.); gennaro.brunetti@uniba.it (G.B.)
[6] Faculty of Agricultural, Environmental and Food Sciences, Free University of Bolzano-Bozen, 39100 Bolzano, Italy; lorenzo.brusetti@unibz.it (L.B.); federica.piergiacomo@unibz.it (F.P.); luigimaria.borruso@unibz.it (L.B.)
[7] Competence Centre for Plant Health, Free University of Bozen-Bolzano, 39100 Bolzano, Italy
* Correspondence: luciano.beneduce@unifg.it

Abstract

Water scarcity is driving increased use of treated wastewater in agriculture while also leading to an increase in concerns about the presence of active pharmaceutical active compounds (PhACs) and their impact on soil ecosystems. This study provides novel field-scale evidence on the combined impact of tertiary-treated wastewater (TWW) irrigation and short-term tomato/wheat crop succession on soil microbial communities, nitrogen-cycling functional groups, and crop productivity. Over two consecutive years in southern Italy, TWW was compared with freshwater (FW) using integrated chemical, microbiological, and metagenomic approaches. TWW irrigation significantly increased tomato and wheat yields (+14% and +20%, respectively) without negatively affecting crop quality. Several PhACs were detected in soil and showed moderate accumulation under TWW, particularly sitagliptin and flecainide, which reached 10 ng/g. However, limited effects were observed in terms of total microbial abundance, nitrogen-cycle gene markers, or overall microbiome structure. The fungal population of *Bionectria* was found to be a potential biomarker since it was negatively affected by TWW (−56%). In contrast, time and crop succession emerged as the primary driver of microbial dynamics, inducing marked shifts in bacterial and fungal community composition and diversity, with wheat promoting higher diversity than tomato. Nitrogen-fixing bacteria were higher in tomato crop seasons. Ammonia oxidizing increased in the wheat crop season, while denitrifiers were more influenced by sampling time. These findings demonstrate that, under compliant treatment conditions, TWW reuse can enhance crop productivity with limited short-term ecological risks, supporting sustainable agricultural water management.

Check for updates

Academic Editor: Mario Licata

Received: 14 May 2026
Revised: 24 June 2026
Accepted: 26 June 2026
Published: 30 June 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

Keywords: wastewater reuse; emerging contaminants; soil microbiome; nitrogen-cycling; soil microbial indicators

1. Introduction

The increasing scarcity of freshwater resources has led to the growing adoption of treated wastewater for agricultural irrigation, particularly in Mediterranean and semi-arid regions.

Treated wastewater reuse is considered to be a sustainable strategy for ensuring crop productivity, recycling nutrients, and reducing pressure on conventional water resources [1,2]. In recent years, several long-term and field-scale studies have demonstrated the agronomic feasibility of treated wastewater irrigation, highlighting its potential to sustain yields and improve water-use efficiency in horticultural and herbaceous crops [3–5].

Nevertheless, treated effluents may contain residual micropollutants, including pharmaceutically active compounds (PhACs), personal care products, and other emerging contaminants that persist after treatment [6–8].

Field investigations carried out under Mediterranean conditions have shown that, despite the presence of such compounds, their effects on soil quality and crop performance are often limited when treated wastewater complies with current European regulatory standards [3]. While laboratory and field studies frequently report measurable impacts of reclaimed wastewater and individual PhACs on soil microorganisms, field-scale evidence is inconsistent and requires further investigation [9].

In agricultural systems, the response of soil microbiomes is shaped by multiple interacting drivers, including crop species, seasonal dynamics, soil management, and crop rotation, which interact and may compound the effects of irrigation water quality [4]. Therefore, disentangling the relative contributions of irrigation water and agronomic factors is essential for accurately assessing the risks and benefits of wastewater reuse under field conditions.

Crop species exert strong selective pressure on soil microbial communities through processes such as rhizodeposition, nutrient uptake, and crop residue quality, leading to the enrichment of specific bacterial and fungal populations in the rhizosphere [10]. Together, root architecture, phenology, and soil carbon content play a crucial role in determining both the quantity and quality of root exudates, thereby shaping the composition of microbial communities and their functional capabilities [11].

Tomato and wheat represent two different cropping systems, particularly with respect to their root traits and rhizosphere chemistry. Tomato plants, for instance, actively shape their rhizosphere microbiome by releasing bioactive secondary metabolites such as steroidal glycoalkaloids. These metabolites selectively promote the growth of specific bacterial taxa [12,13]. Wheat, by contrast, possesses a markedly different root architecture and exudation patterns, giving rise to distinct microbial communities and functional profiles, with particular relevance to nutrient cycling and organic matter turnover.

Crop succession can therefore induce pronounced shifts in microbial community structure, abundance, and diversity, even within a short time-frame. Experimental evidence demonstrates that shifts in crop identity across successive seasons rapidly modify soil microbiota composition and diversity metrics, often exceeding the effects that can be attributed to other management factors [13,14].

Changes in soil microbial functioning can indirectly affect crop quality by modulating nutrient availability, plant nutrition, and plant stress responses, primarily through micro-

bially mediated processes such as nitrogen mineralization, nitrification, and rhizosphere interactions [15,16].

The transfer of PhACs to soil via irrigation water can exert two contrasting effects on microbial communities. Microbial populations sensitive to specific pharmaceuticals may undergo inhibition or experience toxic effects. Conversely, tolerance mechanisms may be activated, and biochemical degradation pathways—carried out by individual microbial populations or consortia—may be promoted either as a detoxification strategy or as an active metabolic process to derive energy from these compounds [17,18]. Irrigation water quality can affect influence N-cycling populations, overall community structure, and crop yield and quality. Several field studies have reported that reclaimed wastewater irrigation can affect the soil microbial functioning and crop quality parameters [1,19,20]. Understanding these soil–plant–microbiome interactions is therefore essential for the safe and sustainable implementation of reclaimed wastewater irrigation. Integrated soil and crop management strategies that account for crop choice, rotation schemes, and soil biological functioning can enhance system resilience and mitigate the risks associated with water reuse, supporting long-term agricultural sustainability [2,21].

Despite the growing interest in this topic, there are only a limited number of studies involving multi-season, open-field experiments that systematically evaluate crop productivity, the fate of PhACs, and their effects on the soil microbiome. In particular, a knowledge gap still exists regarding the impact of treated wastewater containing PhACs on the soil microbiome under full-scale crop succession. The objective of the present study was to test the following hypotheses: (i) irrigation with reclaimed wastewater containing PhACs may influence soil microbial indicators, community structure, and functional traits related to nitrogen cycling; (ii) sensitive microbial groups can represent potential bioindicators of soil PhACs accumulation; (iii) irrigation water can affect crop quality, with limited safety concerns resulting from the use of tertiary-treated wastewater; and (iv) short-term crop rotation and seasonal variation in environmental conditions have complex effects on the soil microbiome that can buffer the impact of differentially treated irrigation wastewater.

2. Materials and Methods

2.1. Site Description and Crop Management

The study was conducted over two experimental years (2020–2021, Y_1 and 2021–2022, Y_2) in Trinitapoli, Apulia, southern Italy (41°21′ N; 16°03′ E; 10 m a.s.l.), within a 2000-hectare irrigation district. The area has a Mediterranean climate with hot summers (with temperatures exceeding 40 °C), mild winters, and average annual rainfall of 590 mm, mostly between October and April [22]. The soil is classified as loam (USDA), and its main chemical–physical characteristics are as follows: pH 8.5 (1:2.5 *w*/*v*), organic matter 1.1 (% dry weight); available phosphorus as P_2O_5 240 (mg kg^{-1}); total potassium 11.2 (g kg^{-1}); N-NO_3^- 11.2 (mg kg^{-1}); N-NH_4^+ 3.3 (mg kg^{-1}); soil electrical conductivity 0.6 (dS m^{-1}, 1:2 *w*/*v*).

During the two experimental years (Y_1 and Y_2), processing tomato (*Solanum lycopersicum* L.) and durum wheat (*Triticum turgidum* spp. durum) were grown in succession. Processing tomato (cv. "Taylor") was transplanted on 11 May 2020, and 17 June 2021, in Y_1 and Y_2, respectively, in single rows (plant density 2.77 plants m^{-2}). Durum wheat (cv. "Saragolla") was sowed on 15 November 2020, and 10 December 2021, in Y_1 and Y_2, respectively, at a seeding rate of 350 germinable seeds m^{-2}. The other agricultural techniques used for the two crops were implemented in accordance with standard management practices (Table S1).

Two irrigation treatments were compared: freshwater (FW, control) and tertiary-treated wastewater (TWW) from a nearby municipal WWTP with activated sludge and

settler as secondary treatments, sand filtration, chlorine and UV disinfection and membrane filtration as tertiary treatments.

The experimental field was arranged according to a randomized complete block design (RCBD) which was replicated four times for a total of 8 plots (2 irrigation treatments × 4 replicates). Each plot was 12.5 m × 5.0 m (Figure S1).

Drip irrigation was used with 2 L h^{-1} drippers spaced 20 cm apart, and water volumes were monitored using flow meters. Tomato irrigation scheduling was guided by the BluleafTM DSS model (Sysman Srl, Ivrea-TO-Italy) [23,24] to maintain 30% soil water depletion [25]; evapotranspiration was calculated via the FAO Penman–Monteith method and a single-crop coefficient (Kc) approach. Wheat received supplementary irrigation only in the second year, when no rainfall occurred, in accordance with Mediterranean agronomic practices [26]. The total amount of irrigation water applied to tomato was 5900 m^3 ha^{-1} (Y_1) and 6800 m^3 ha^{-1} (Y_2), and 1100 m^3 ha^{-1} to crop wheat in Y_2.

Harvesting occurred on 24 August 2020 and 4 October 2021 for tomato, and on 7 June 2021 and 11 June 2022 for durum wheat.

2.2. Chemical Parameters of Irrigation Water and PhACs Soil Content

During the two growing seasons, water samples were collected ten times (Figure 1) from three randomly selected emitters per irrigation treatment, replicated four times, and stored at 4 °C until analysis.

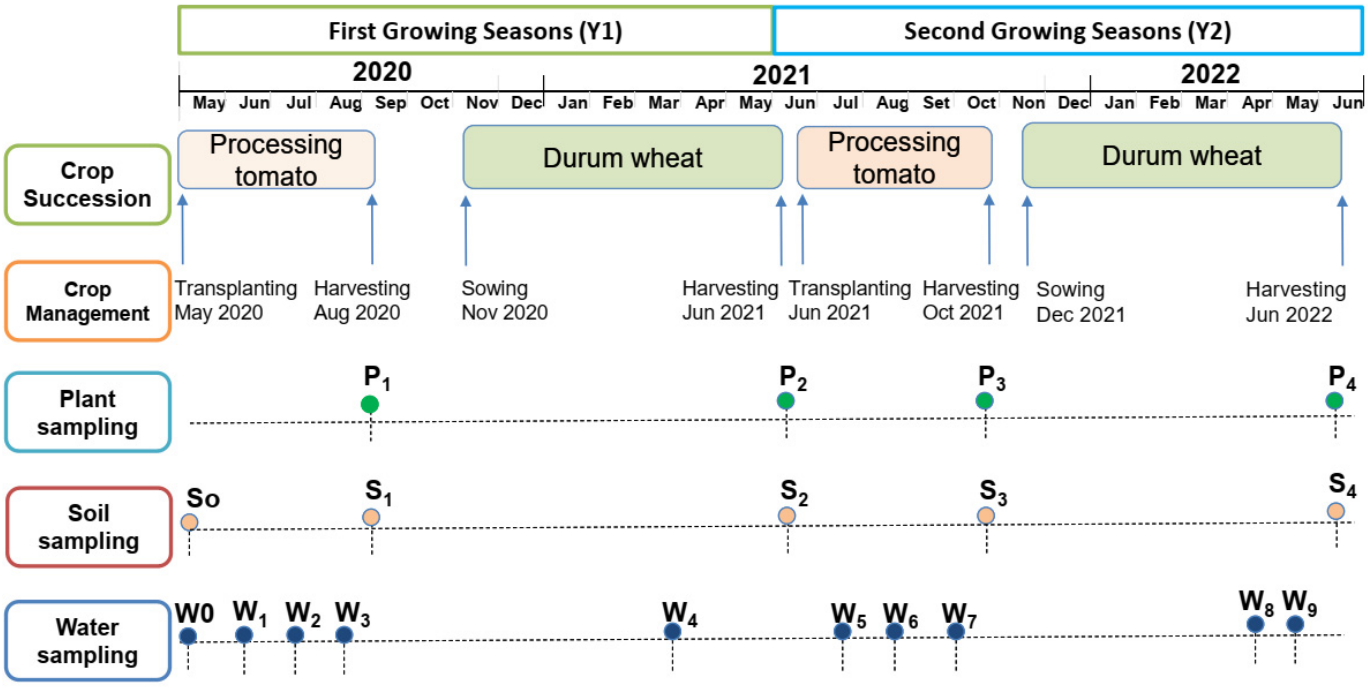


Figure 1. Temporal sampling scheme of the experimental field.

Irrigation water samples were analyzed in triplicate according to Italian standard methods [27] and international procedures [28]. Measured parameters (Table 1) included pH, electrical conductivity (EC_w), biochemical oxygen demand over 5 days (BOD_5), chemical oxygen demand (COD), total nitrogen (TN), ammonium nitrogen (NH_4–N), nitrate nitrogen (NO_3–N), and orthophosphate phosphorus (PO_4–P). The SAR was calculated using the formula (with concentrations in meq L^{-1}) [27]: $SAR = Na^+/[(Ca^{2+}Mg^{2+})/2]^{1/2}$.

To determine the main physicochemical parameters of the soil, in addition to the PhACs content, and to perform a microbiological analysis, soil samples were collected from a sub-sampling area (1 m × 1 m, Figure S1) within each experimental plot before the start

of the trial (S_0), at the end of the growth periods of the first tomato (S_1), first wheat (S_2), second tomato (S_3), and second wheat (S_4) (Figure 1).

Table 1. Main chemical parameters for water sources used for crop irrigation.

Parameters	Irrigation Water Source †	
	FW	TWW
COD (mg L^{-1})	10.0 ± 3.5	22.9 ± 9.0
BOD_5 (mg L^{-1})	5.3 ± 1.1	3.7 ± 1.5
TN (mg L^{-1})	5.5± 2.1	11.9 ± 7.9
NH_4-N, (mg L^{-1})	0.0 ± 0.0	0.5 ± 0.4
NO_3-N (mg L^{-1})	0.9 ± 0.1	7.6 ± 4.4
PO_4-P (mg L^{-1})	0.2 ± 0.01	3.1 ± 2.4
pH (-)	7.5± 0.1	7.4 ± 0.3
EC_w (mS cm^{-1})	0.60 ± 0.1	1.21 ± 0.23
SAR (-)	0.68 ± 0.15	0.89 ± 0.34

† FW—fresh water; TWW—tertiary-treated wastewater. The data are the means ± standard errors from 40 samples (10 sampling times × 4 replications) collected during the field experimental trials.

Soil samples for the physicochemical analyses and PhACs determination were collected from the sub-sampling area at a depth of 0–0.30 m (bulk soil). In contrast, soil samples for microbiological analyses were obtained from each sub-sampling area by combining two samples taken far away from (bulk soil) and near to the roots (soil surrounding roots), pooled in a single sample per plot and per replicate. Total DNA was extracted separately from soil aliquots obtained from each field replicate. The same DNA extracts were used for bacterial 16S rRNA gene and fungal ITS amplicon sequencing. Thus, the sequenced samples represented biological replicates associated with the replicated field design, rather than technical sequencing replicates.

The electrical conductivity and pH were measured in 1:2 (*w*/*v*) and 1:2.5 (*w*/*v*) aqueous soil extracts, respectively. Phosphorus soil content was determined using the sodium bicarbonate method [29]. The organic matter content of soil (OM) is estimated by multiplying the organic carbon percentage by 1.724 [30]. The soluble NO 3 -N and NH 4 -N were determined according to [31]. The modified QuEChERS approach developed by De Mastro et al. (2022) was used to extract pharmaceutical active compounds (PhACs) from experimental soils [32].

PhACs in both soil extracts and water samples were quantified using an optimized analytical method based on online solid-phase extraction coupled with ultra-high-performance liquid chromatography and high-resolution tandem mass spectrometry (SPE–UPLC–HRMS/MS). Analyses were performed using a UHPLC system (Ultimate 3000, Thermo Fisher Scientific, Waltham, MA, USA) interfaced with a TripleTOF 5600+ high-resolution mass spectrometer (AB Sciex, Framingham, MA, USA) equipped with a DuoSpray™ ion source operating in electrospray ionization mode. Detailed LC and MS conditions are reported in the work of De Mastro et al. (2022) [33]. Aliquots of each soil sample (0 to 30 cm, removing the first 2 cm of soil crust) were aseptically stored and transported at 4 °C for microbiological analyses. Triplicate samples (25.0 g) were resuspended in buffered peptone water (Merck Millipore, Darmstadt, Germany) for 30 min to allow microbial cell recovery. Then, serial decimal dilutions were plated on TSA (Oxoid, Thermo Fisher Scientific) to measure the total mesophilic heterotrophic count (THC), RBCA (Biolife Italia, Milan, Italy) for total fungal count, C-EC chromogenic agar (Biolife Italia) for fecal coliforms and TBX chromogenic agar (Oxoid) for the enumeration of *E. coli*. Incubation temperatures were 25 °C for THC and fungal counts and 44 °C for fecal coliforms and *E. coli* enumerations after 48 h incubation.

DNA extraction from soil was conducted on 0.25 g aliquots from each replicate, aided by DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA samples were quantified by using Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) on a Qubit® 2.0 Fluorometer then stored at −80 °C prior to molecular analyses.

Quantitative PCR (qPCR) was conducted, targeting the nitrogen-cycle key bacterial groups, namely nitrogen-fixing bacteria (target gene *nifH*), ammonia-oxidizing bacteria (target gene *amoA*), and denitrifying bacteria (target genes *nosZ* and *nirK*). Table S2 summarizes the primer sequences and literature references used to establish the methods.

All qPCR analyses were conducted in the Applied BioSystem 7300 Real-Time PCR cycler (Foster City, CA, USA. PowerUp™ SYBR™ Green Master Mix (Thermo-Fisher), primers, and DNase-free deionized water were added in defined volumes based on the target gene detected. In this study, 2 µL of DNA was added to the reaction mixture, and linearized plasmids harboring each of the target genes (GeneArt gene synthesis–Thermo Fisher) were used as internal quantitative standards. The quantification of N-cycle populations was expressed as the gene copy number (GCN) per gram of dry soil.

The same DNA samples were sent to Stab Vida, Lda. (Caparica, Portugal) for bacterial 16S and fungal ITS region Metagenomic Sequencing using an Illumina MiSeq platform (San Diego, CA, USA, 300 bp paired-end sequencing reads).

The analysis of raw sequence data was conducted using QIIME2 v2021.4 [34] and denoised using DADA2 plugin [35].

The reads were organized in features, which are essentially units of observation—in this particular case, amplicon sequence variants (ASVs)—and then classified using the SILVA (release 138 QIIME) database, with a clustering threshold of 99% similarity. For classification purposes, only ASVs containing at least 10 sequence reads were considered significant.

2.3. Statistical Analysis

The dataset was tested in accordance with the basic assumptions of ANOVA. The Shapiro–Wilk and Bartlett tests were used to verify the experimental error's normal distribution and common variance, respectively. If required, Box–Cox transformations [36] were applied prior to analysis.

For qualitative and quantitative analysis of tomato and wheat crops, ANOVA was conducted according to a randomized complete block design (four replicates), with irrigation source combined (over time) with growing seasons (Y_1 and Y_2). The irrigation source factor and growing seasons were considered as fixed effects, whereas replicates were considered as random effects.

For the PhACs' soil content and microbial enumeration (plate count and qPCR results), ANOVA was conducted using a randomized complete block design (four replicates), with the irrigation source factor combined (over time) with sampling time (S_0–S_4). The irrigation source and sampling time were treated as fixed effects, whereas the replicates were treated as random effects.

The statistical significance of the differences in the means was determined using Tukey's honest significance difference *post hoc test* at the 5% probability level.

The statistical analysis of metagenomic data included weighted UniFrac multivariate method [37] and plots generated by using Qiime2 [34] To statistically support the beta-diversity patterns observed in the ordination plots, PERMANOVA was performed on the UniFrac distance matrix using the adonis2 function of the vegan package in R (version 2.8.0), with 9999 permutations. Since crop and sampling time were not fully crossed in the experimental design, they were not included as independent predictors in the same

model. Instead, a combined crop-time factor was created to represent the main crop/time structure of the experiment. The final model tested the effects of the combined crop-time group and irrigation water source on UniFrac beta-diversity. Homogeneity of multivariate dispersion among groups was assessed using the Betadisper function. Alpha diversity indexes were calculated with Past statistical software, version 4.07b. LEfSe was performed using relative abundance data normalized between 0 and 1. Differentially abundant features were identified using an LDA score threshold of >2.0, with a significance threshold of $p < 0.05$.

3. Results

3.1. Effect of Irrigation Water Source on the Main Quali-Quantitative Parameters of Crops

The mean values of quali-quantitative parameters related to processing tomato and durum wheat crops under irrigation sources are reported in Table 2.

Table 2. Effect of irrigation water source (FW and TWW) and growing seasons (Y_1 and Y_2) on main quantitative and qualitative traits of the processing tomato and durum wheat. The data shown are the means ± standard errors (n = 4). For each parameter and factor, different letters indicate significantly different means at $p \leq 0.05$, according to Tukey's test. ns = not significant.

Quali-Quantitative Parameters	Experimental Factors				Significance
	Irrigation Source (IS)		Growing Seasons (GS)		IS × GS
	FW	TWW	Y_1	Y_2	
Processing tomato					
Marketable yield (t ha^{-1})	96.26 ± 3.51 b	110.02 ± 1.6 a	101.2 ± 2.8 a	105.1 ± 3.8 a	ns
Total yield (t ha^{-1})	111.3 ± 3.4 b	122.1 ± 1.20 a	111.9 ± 2.3 a	121.4 ± 3.0 a	ns
Dry matter of fruits (% fresh weight)	5.80 ± 0.17 a	5.70 ± 0.19 a	5.9 ± 0.19 a	5.6 ± 0.23 a	ns
pH (-)	4.06 ± 0.02 a	3.96 ± 0.03 a	4.02 ± 0.03 a	4.01 ± 0.04 a	ns
Titratable acidity (g citric acid 100 mL^{-1} fresh juice)	0.34 ± 0.03 a	0.33 ± 0.01 a	0.32 ± 0.04 a	0.35 ± 0.02 a	ns
Soluble solids content (°Brix)	4.7 ± 0.2 a	4.6 ± 0.2 a	4.8 ± 0.3	4.5 ± 0.36	ns
Color index (-)	1.20 ± 0.09 a	1.17 ± 0.1 a	1.17 ± 0.01 a	1.19 ± 0.01 a	ns
Durum Wheat					
Grain yield (t ha^{-1})	4.76 ± 0.56 b	5.71 ± 0.33 a	5.14 ± 0.33 a	5.32 ± 0.41 a	ns
Grain protein content (%)	11.72 ± 0.43 b	13.26 ± 0.46 a	12.11 ± 0.27 a	12.7 ± 0.76 a	ns
Thousand kernel weight (g)	81.55 ± 0.52 a	80.85 ± 0.65 a	81.13 ± 0.72 a	81.26 ± 0.30 a	ns

The FW and TWW irrigation sources significantly affected ($p \leq 0.05$) the productive parameters, while the mean values were not significantly different between the two growing seasons ($p > 0.05$); in addition, the irrigation sources and the growing season factors did not show a significant interaction effect.

In processing tomato, the marketable yield obtained with TWW was higher than with FW (110.02 ± 1.6 vs. 96.26 ± 3.51 t ha^{-1}). The same trend was observed for total yield (121.0 ± 1.20 vs. 110.0 ± 3.4 t ha^{-1}).

Under our experimental conditions, the average values of tomato qualitative parameters (dry matter of fruits, pH, titratable acidity, soluble solids content, and color index) were not significantly affected ($p > 0.05$) by different irrigation water sources and growing season factors (Table 2).

The yield and grain protein content were higher with TWW compared to FW (5.71 ± 0.33 vs. 4.76 ± 0.56 t ha^{-1}; 13.26 ± 0.46 vs. 11.72 ± 0.43%). For the thousand-kernel weight, no significant differences were observed ($p > 0.05$). Also, for the wheat crop, the irrigation source and growing season factors did not show any significant interaction effects (Table 2).

3.2. Irrigation Water and Soil-PhACs Content

The PhACs naturally found in both irrigation water sources and their concentrations are reported in Table S3. Seventeen compounds found in the wastewater belong to ten drug classes, including antibiotics, antifungals, anti-inflammatories, beta-blockers, antidepressants, antiarrhythmics, antiepileptics, antidiabetics, antihypertensives, and lipid-regulating agents.

Figure 2 presents the mean PhACs soil concentrations across soil sampling (S_0–S_4) and irrigation water sources (FW and TWW). Of the compounds identified in the TWW used for irrigation, only the eight reported (i.e., clarithromycin, carbamazepine, fluconazole, climbazole, flecainide, sitagliptin, telmisartan, and venlafaxine) were detected in the soil profile. At the beginning of the experiment, the PhACs' concentrations in soil ranged from 5.5 ng g^{-1} for climbazole to 0.7 ng g^{-1} for venlafaxine, likely resulting from prior use of treated wastewater or biosolids in the area.

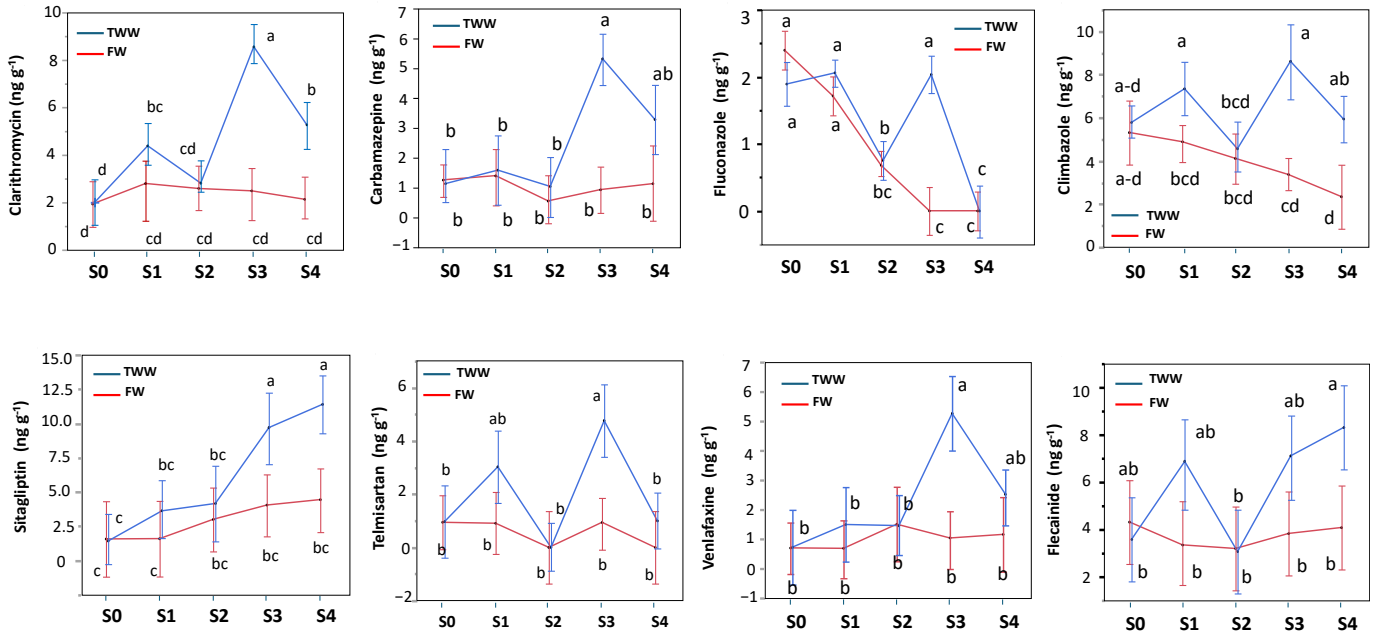


Figure 2. PhACs soil content of the two irrigation water types applied (FW and TWW) as a function of water sampling date (S_0–S_4), showing the irrigation water source × soil sample date interaction. The data are presented as means ± standard errors from four replicates. The mean values followed by different letters are significantly different ($p \leq 0.05$; Tukey's test).

During the second tomato cycle (S_3), significant differences between FW and TWW-irrigated soils were observed for clarithromycin, carbamazepine, fluconazole, climbazole, sitagliptin, telmisartan, and venlafaxine. In contrast, no significant differences were detected by the end of the first tomato and wheat cycle (S_1 and S_2). Carbamazepine, climbazole sitagliptin, flecainide, and clarithromycin showed accumulation in TWW-irrigated soils over the two tomato–wheat cycles.

3.3. Soil Microbial Indicators

The viable cell count of the main soil microbial indicators is listed in Table 3. The total heterotrophic bacterial count showed no significant effect according to the irrigation water, while the crop effect was found to be significant, particularly during tomato cultivation, with lower counts reported in both crop seasons. No interaction was found among the two variables considered (two-way ANOVA $p > 0.05$). The fungal population exhibited different behavior: with both crops, the fungal population was significantly reduced compared to the control (S_0). In the second year (Y_2), the fungal population decreased independently for both

crops. On the contrary, the level of fecal coliforms increased during crop seasons compared to the control (S_0), but the increase was not significantly related to the type of crop, nor to the irrigation water. Finally, for the *E. coli* levels, the concentration was generally very low or below the detection limits. The only exception was for the first wheat crop cycle (S_2), for which a higher level of this fecal indicator was found for both irrigation theses.

Table 3. Soil microbial indicators quantified by viable cell plate count (Log CFU g^{-1} dry soil). FW = fresh water; TWW = tertiary-treated wastewater. The represented data are the mean values ± standard deviations (s.d.). Significant differences and interactions are reported with different letters according to ANOVA and Tukey's post hoc test. ns = not significant.

Experimental Factor	Microbial Counts			
	Bacteria	**Fungi**	**Fec. Coliforms**	***E. coli***
Main effect				
Irrigation source (IS)				
FW	5.87 ± 0.39 a	3.94 ± 0.63 a	1.66 ± 1.01 a	0.96 ± 0.25 a
TWW	5.86 ± 0.43 a	3.78 ± 0.62 a	1.53 ± 1.33 a	0.99 ± 0.26 a
Sampling time (ST)				
S_0 (No crop)	6.11 ± 0.18 ab	4.77 ± 0.31 a	0.43 ± 0.32 b	0.38 ± 0.80 b
S_1 (Tomato 1)	5.56 ± 0.43 c	3.90 ± 0.32 bc	0.98 ± 0.33 ab	0.34 ± 0.82 b
S_2 (Wheat 1)	6.39 ± 0.11 a	3.32 ± 0.26 b	2.14 ± 0.26 a	1.91 ± 0.99 a
S_3 (Tomato 2)	5.52 ± 0.05 c	3.95 ± 0.38 d	2.25 ± 0.28 a	0.0 ± 0.0 b
S_4 (Wheat 2)	5.72 ± 0.20 bc	3.37 ± 0.47 cd	2.18 ± 0.48 a	0.0 ± 0.0 b
Interaction IS × ST	ns	ns	ns	ns

3.4. Nitrogen Cycling Microbial Populations

The evaluation of the influence of the experimental theses on the key microbial groups involved in the nitrogen cycle of soil is reported in Table 4. For all of the microbial groups, no significant effect was found according to the different irrigation waters and no interaction was found with the distinct crop. The main effect observed were all related to the different type of crops: the nitrogen-fixing bacterial populations were affected by the crops, with a higher level for tomato and a lower level for wheat ($p < 0.05$). The same effect was found for the nitrifying population measured by quantification of *amoA* gene. Tomato hosted a higher population of this microbial group compared to the wheat, particularly in the first crop cycle. The denitrifying populations were assessed by *nirK* and *nosZ* genes to account for different populations that can conduct partial or complete denitrification. In this case there was no significant difference with crops. Namely *nosZ* was decreasing over time, while *nirK* was increasing.

Table 4. Quantification of the main bacterial groups related to the nitrogen cycle through qPCR analysis. The represented data are the mean values and respective standard deviations (s.d.). Significant differences are reported with different letters according to two-way ANOVA analysis and Tukey's post hoc test. * = significant interaction; ns = not significant. *nifH* = nitrogen-fixing bacteria; *amoA* = ammonia-oxidizing bacteria; *nirK* and *nosZ* = denitrifying bacteria target genes.

Experimental Factor	qPCR Nitrogen Cycling Bacterial Genes			
	nifH	***amoA***	***nirK***	***nosZ***
Main effect				
Irrigation source (IS)				
FW	5.69 ± 0.46 a	6.33 ± 0.44 a	6.23 ± 0.34 a	6.90 ± 0.46 a
TWW	5.80 ± 0.38 a	6.28 ± 0.41 a	6.25 ± 0.28 a	6.83 ± 0.52 a

Table 4. *Cont.*

Experimental Factor	qPCR Nitrogen Cycling Bacterial Genes			
	nifH	***amoA***	***nirK***	***nosZ***
Sampling time (ST)				
S_0 (No crop)	5.53 ± 0.54 bc	6.64 ± 0.06 a	6.29 ± 0.09 b	7.39 ± 0.11 a
S_1 (Tomato 1)	5.77 ± 0.48 ab	6.59 ± 0.15 a	6.31 ± 0.12 ab	7.42 ± 0.11 a
S_2 (Wheat 1)	5.42 ± 0.18 c	6.47 ± 0.11 c	5.73 ± 0.15 c	6.65 ± 0.08 b
S_3 (Tomato 2)	6.08 ± 0.08 a	5.60 ± 0.17 a	6.49 ± 0.11 a	6.56 ± 0.10 b
S_4 (Wheat 2)	5.95 ± 0.25 a	6.21 ± 0.30 b	6.38 ± 0.28 ab	6.32 ± 0.32 c
Interaction IS × ST	*	ns	ns	ns

3.5. Soil Microbiome Structure and Dynamics

The metataxonomic data for bacteria and fungi were used for an in-depth investigation of which microbial populations were influenced either by treatment (irrigation water type) or by the crop cultivated in the surveyed soils. Figure S3 reports the relative abundance plots at the class level for both bacterial and fungal communities. The most abundant taxa were shared across all samples, but an interesting trend related to sampling time and type of crop was revealed based on the variations in the relative abundance of each taxon. To support a more robust statement, the beta-diversity of bacterial and fungal populations was evaluated by non-metric multidimensional scaling (NMDS). Figure 3 graphically summarizes the differences in bacterial (A) and fungal (B) populations.

With regard to bacterial populations, the communities clearly clustered according to the time and the corresponding cultivated crop. In particular, the first-season tomato crop co-clustered with the control (red and blue dots) and was adjacent to the tomato crop of the second season (green dots). Wheat seasons 1 and 2 clustered separately (yellow and purple dots). Irrigation water did not exert a measurable effect on the bacterial community.

Fungal populations showed similar results. In this case, the clustering was broader but analogous to that of the bacterial community. Tomato crop seasons 1 and 2 were more similar to one another and did not differ from time 0, while wheat crops 1 and 2 gave rise to separate clusters.

The clustering observed in the UniFrac-based ordination was confirmed by PERMANOVA. The combined crop-time factor was highly significant and explained most of the variation in microbial beta-diversity (R^2 = 0.669, F = 7.40, p = 0.0001), whereas the irrigation water source was not significant (R^2 = 0.037, F = 0.81, p = 0.583). Homogeneity of multivariate dispersion was verified using Betadisper and was not significant for the crop-time groups (p = 0.180), indicating that the observed PERMANOVA result was not driven by differences in within-group dispersion.

The soil physicochemical parameters changed according to crop type during the 24-month experimental period (Figure S2) and contributed to the microbial community shifts, consistent with the NMDS results. As shown in Figure 3A,B, both bacterial and fungal communities clustered under the influence of different variables. Specifically, the first two sampling points (S_0 and S_1) were influenced by soil pH and E.C., while total P, N-NO_3, and, to a lesser extent, N-NH_3 influenced S_1–S_3 tomato crop-associated communities. By contrast, the durum wheat-associated communities were mostly affected by soil organic matter.

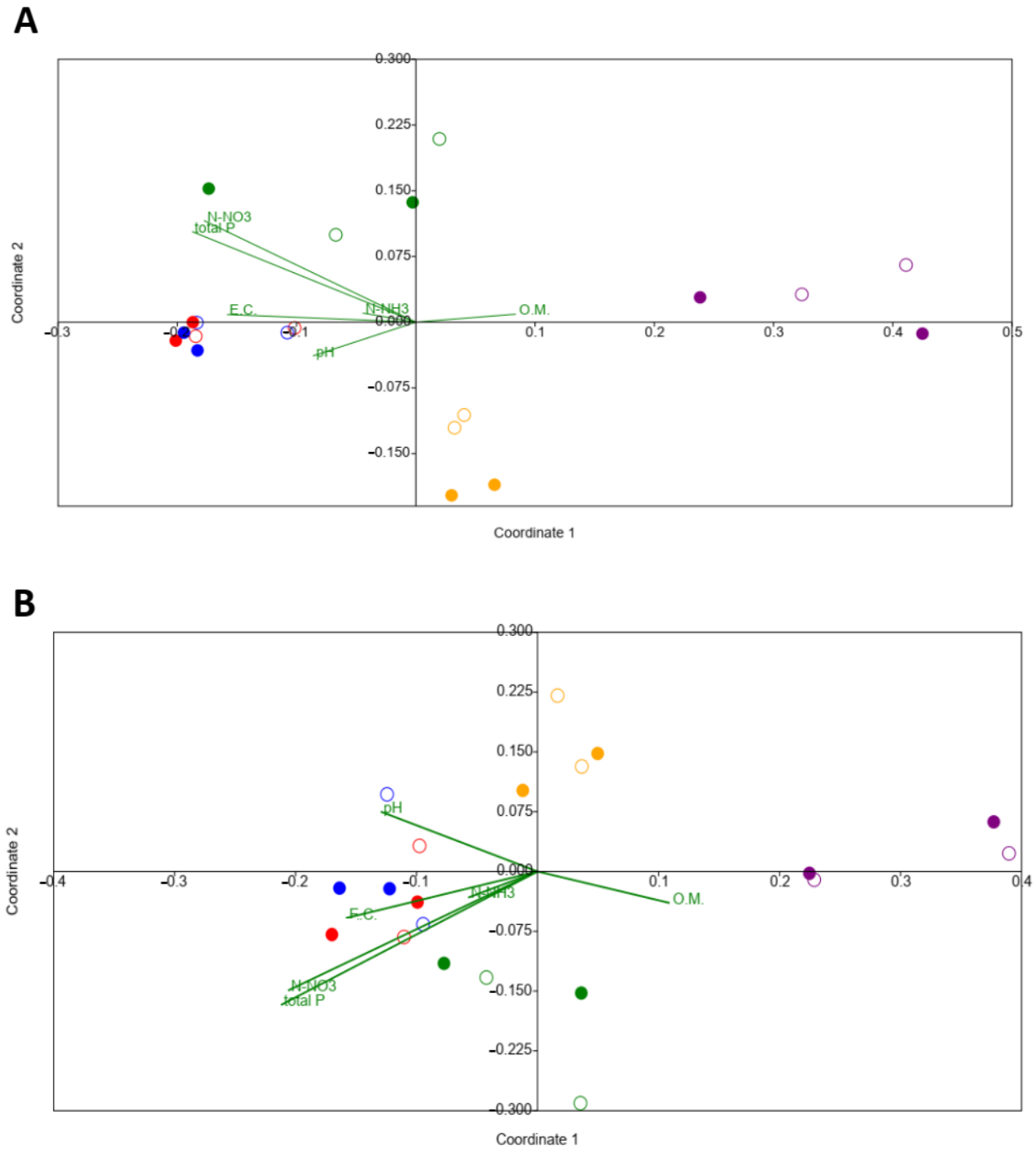


Figure 3. Beta-diversity of bacterial (**A**) and fungal (**B**) populations in the experimental soils. The NMDS multivariate analysis was conducted basing on the weighed Unifrac distance matrix. Filled dots represent the TWW thesis; empty dots represent the FW thesis. The colors represent the crops as follows: red: $S_{0,}$ before the start of the trials (no crop); blue: S_1, first tomato cycle; yellow: S_2, first wheat cycle; green: S_3, second tomato cycle; purple: S_4, second wheat cycle.

3.6. Alpha Diversity

The alpha diversity was evaluated through the main diversity indices, namely taxon abundance (n. ASV), the Simpson and Shannon diversity, Shannon Evenness and Chao1. Figure 4 shows the calculated indexes and their comparison according to the crop and the irrigation treatment. The abundance of taxon (number of ASVs) in soil was influenced by the presence of both crops compared to the control, irrespective of crop or water type. No significant difference was found for the Simpson index, while the Shannon index showed an increment in diversity for cultivated soil, with a significantly higher value for

wheat crop. Evenness was found to be higher for wheat, while tomato showed the lowest values. In this case, the type of irrigation water was not found to have any influence. The Chao1 index reflected the same trend observed for the Shannon index, where wheat crops showed the highest diversity. Regarding irrigation water, a general increase in all diversity indexes was observed but no significant effect of the tertiary-treated water compared to the conventionally treated water was observed.

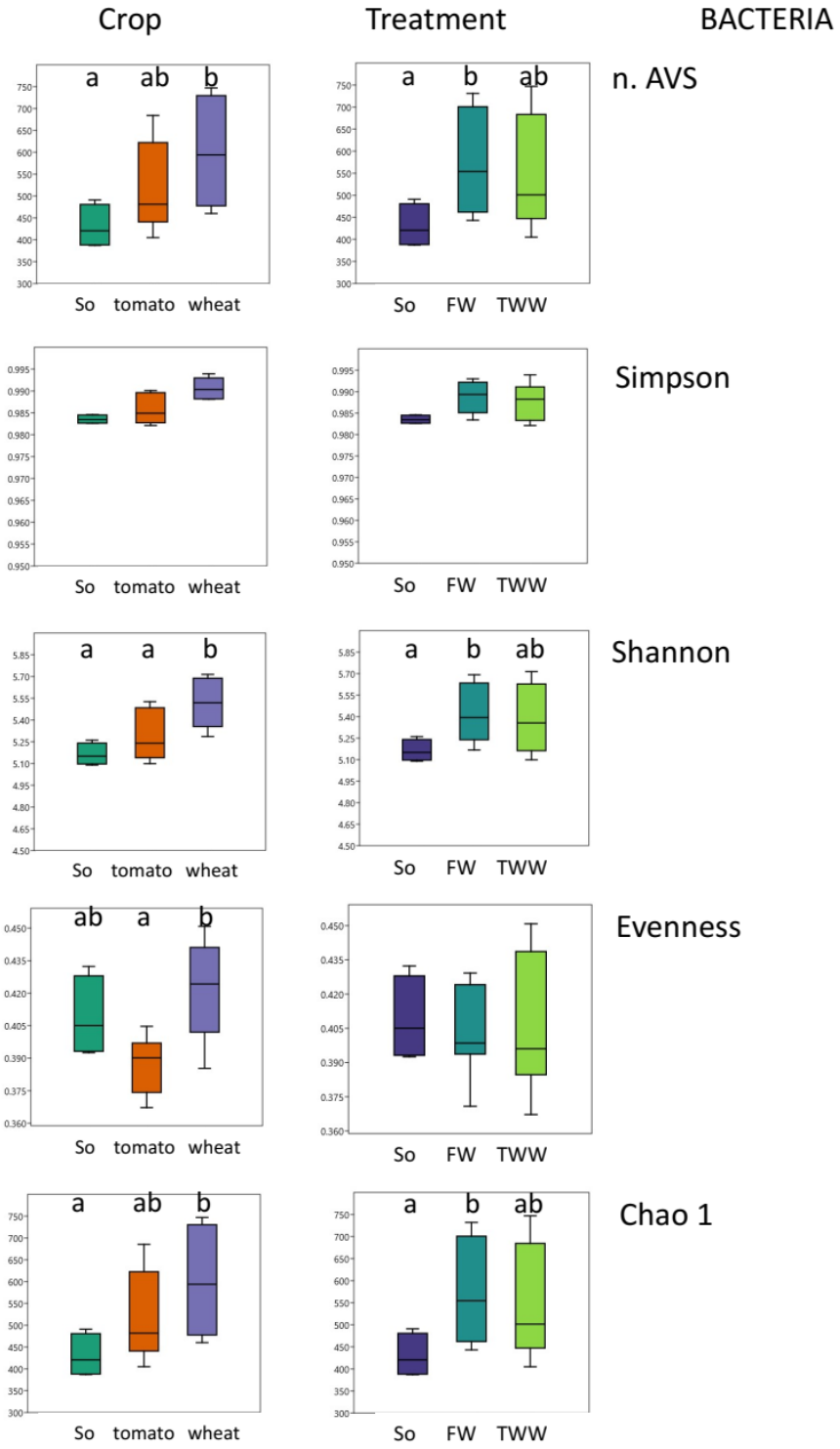


Figure 4. Alpha-diversity indexes of bacterial communities. The plots are grouped according to the crops and treatments. No = no crop (s0). Significant differences are evidenced by letters ($p < 0.05$, Tukey's post hoc).

The observed differences were less evident in the alpha diversity of the fungal community. Figure 5 shows that abundance and Chao1 were positively influenced by both types of crops and both irrigation water types, regardless of treatment. Interestingly, for the fungal population, a significant decrease in evenness was found compared to the control soil.

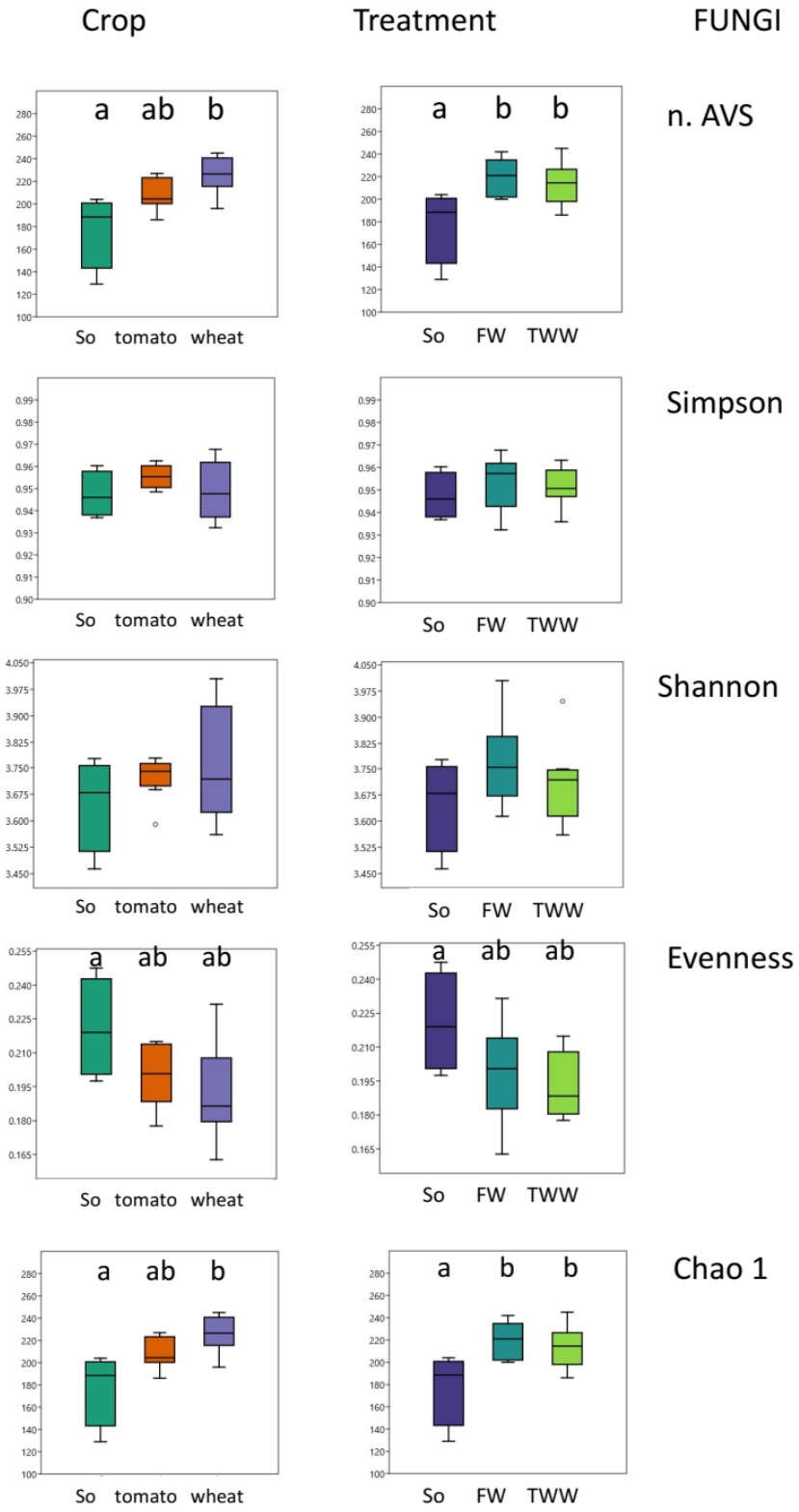


Figure 5. Alpha-diversity indexes of fungal communities. The plots are grouped according to the crops and treatments. No = no crop (S_0). Significant differences are evidenced by letters ($p < 0.05$, Tukey's post hoc).

3.7. LEfSe Analysis

The Linear discriminant analysis Effect Size (LEfSe) analysis was conducted to identify genomic biomarkers that characterize statistical differences among biological groups. The focus was on the crop type and irrigation water treatment. Figure 6A,B show the linear discriminant analysis (LDA) score of bacterial and fungal taxa of the soil that were significatively affected by the different crops. Regarding bacteria (Figure 6A), prior to cultivation, the soil was characterized by *Streptosporangiaceae*, *Alyciclobacillaceae* and *Sferoidobacteriaceae*. The tomato crop bacterial community distinguished *Haliangiaceae* and *Mixococcaceae* (both from the phylum *Mixococcota*) plus *Alphaproteobacteria*. The durum wheat crops' distinct communities were represented by a higher number of taxa, including *Blastocatellaceae*, *Saprospiraceae*, *Herpetosiphonaceae* and *Pseudohongiellaceae* from different phyla. Also, several taxa belonging to families of *Verrucomicrobiota* phylum (*Chthoniobacteriaceae*, *Opitutaceae*, *Pedosphaeraceae*, *Verrucomicoribaceae*) were distinctively affected by the wheat rhizosphere.

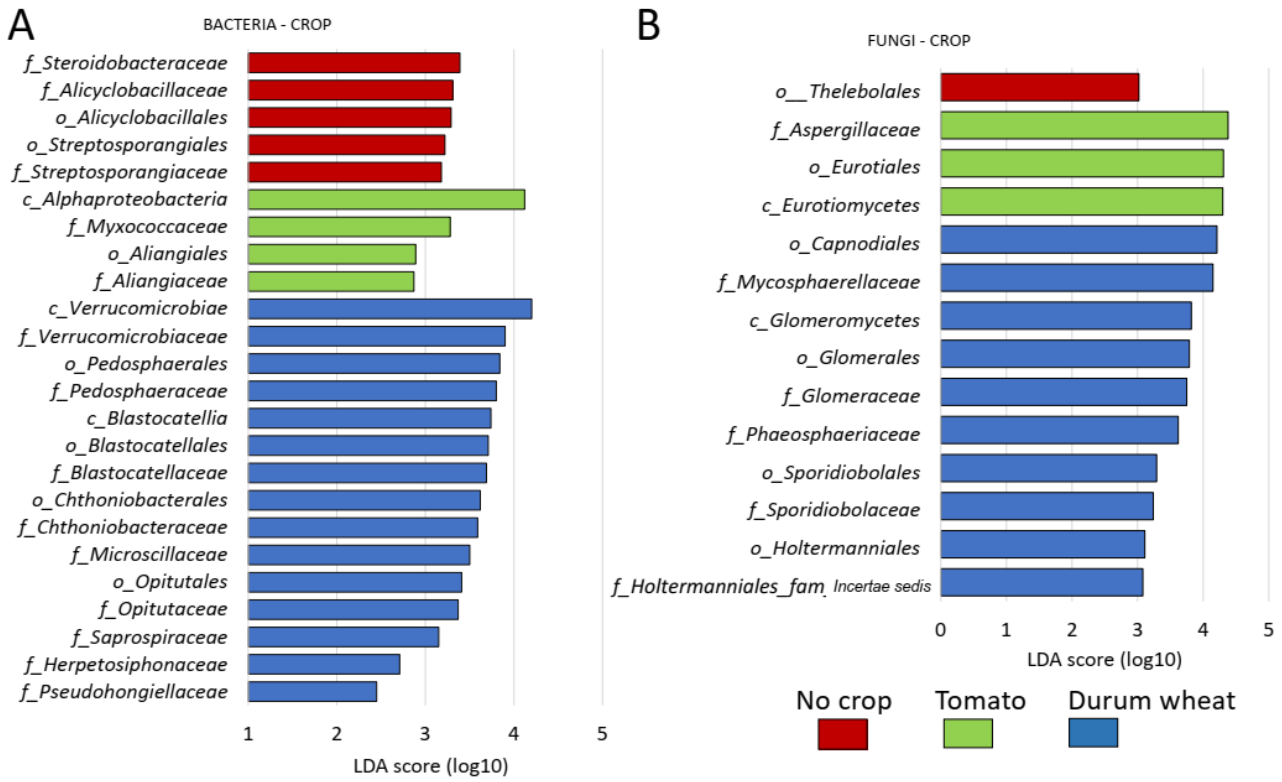


Figure 6. LDA score plots generated from the Lefse analysis related to (**A**) bacterial communities and (**B**) fungal communities, according to the crop. No crop = (S_0). Tomato = (S_1 and S_3). Durum wheat = (S_2 and S_4).

Regarding the fungal communities (Figure 6B), the soil prior to crop cultivations had only one significantly differentially represented taxa, namely the *Thelebolales* order from *Leotiomycetes* class. The tomato crop showed differential *Eurotiomycetes* and *Aspergillaceae*. Also, in the case of fungi, the wheat crop affected a broader range of taxa, including *Mycosphaerellaceae*, *Phaeosphaeriaceae*, *Sporidiobolaceae* and *Holtermanniales*. The *Glomeromycetes* taxa that was also affected and includes mycorrhizal fungi is also worthy of interest.

When evaluating the different treatment with irrigation water, the LEfSe analysis did not show any bacterial taxa affected by this variable. On the other hand, in the case of Fungi (Figure 7), the *Bionectria* population was found to be characteristic of the FW (conventional irrigation water)-treated soil. A significant reduction in this fungal population was found in TWW.

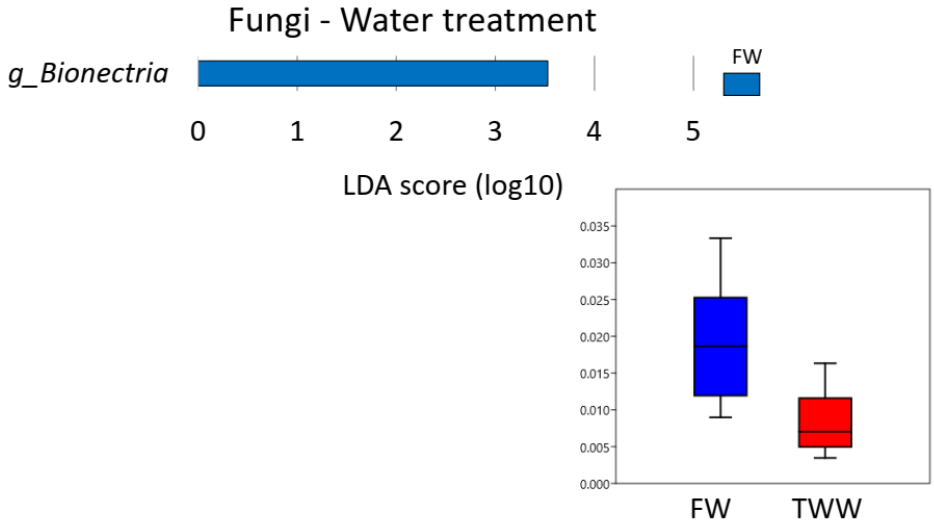


Figure 7. LDA score plots generated from the Lefse analysis related to fungal communities influenced by irrigation water type. The box plot shows a detailed report of the diverse abundance of *Bionectria* ASV found in the dataset among FW and TWW treatments.

4. Discussion

This research focused on evaluating the impact of TWW containing PhACs on crop quality and yield and the possible alteration of the soil microbiome. The starting scenario under investigation was a field soil that had received secondary treated wastewater for 20 consecutive years and, as a consequence, had accumulated an intrinsic baseline content of PhACs. The possibility of exploiting advanced tertiary wastewater treatments for fertigation was evaluated with the aim of achieving sustainable water management without affecting crop safety and quality. The crop rotation scheme adopted was representative of sound agronomic practice aimed at achieving better productivity in both summer and winter seasons.

4.1. The Impact of the TWW Reuse on Crop Yield and Quality

The results of our study confirm that the reuse of TWW processed through advanced tertiary systems could represent an effective agronomic strategy to address water scarcity and support sustainable agricultural practices. Beyond ensuring water availability, TWW may act as a genuine fertigation tool, providing essential macronutrients such as nitrogen (N) and phosphorus (P) in forms readily assimilable by crops. In our trials, irrigation with TWW led to a significant increase in the marketable yield of tomato (110.02 t ha^{-1} vs. 96.26 t ha^{-1} in the freshwater (FW) plot) and an increase in the yield and protein content of durum wheat. This fertilizing effect is consistent with recent findings [21,38–41], where the supply of mineral nitrogen through TWW improved tomato biometric parameters (shoot and root dry weights) relative to conventional water. The yield increase is likely due to the cumulative nutrient load delivered through the irrigation water over the growing season. Notably, despite the increase in productivity, fruit quality parameters (pH, dry matter, titratable acidity) were not negatively affected. Moreover, the microbial indicators (fecal coliforms and *E. coli*) were generally below the level permitted under European Regulation for wastewater reuse in agriculture [42] or not detected, irrespective of the use of TWW or FW (see Table 3), confirming the agronomic suitability and safety of water reuse in the short-to-medium term. These observations support the hypothesis that TWW can potentially substitute for or supplement groundwater and/or freshwater irrigation in sustainable agriculture without compromising crop quality and safety.

4.2. Fate and Persistence of PhACs in the Soil–Plant System

Although the analyzed TWW contained traces of 17 pharmaceutically active compounds, only eight were detected within the soil profile, and clarithromycin and carbamazepine were two of the most persistent. Their presence in the soil is well documented due to their high resistance to microbial degradation and strong adsorption onto organic matter [43]. Carbamazepine, in particular, is often considered a recalcitrant marker for wastewater contamination because it is mainly metabolized by human or animal enzymes not typically present in soil and its reversible sorption on soil organic matter can make it available for plant uptake [44].

The dissipation of many other PhACs observed during the experiment can be attributed to natural biodegradation and photodegradation processes. Indigenous microbial activity plays a crucial role in the bioremediation of emerging contaminants, with decay rates potentially exceeding 70–80% for certain compounds such as diclofenac or ibuprofen within 14 days [45]. In our study, the baseline level of PhACs also found in FW-irrigated soils suggests a substantial legacy of wastewater exposure at the site, where the soil acted as a biological filter capable of stabilizing additional inputs. However, the persistent detection of compounds such as carbamazepine highlights the need for continuous monitoring, given their potential for long-term accumulation and translocation from soil into edible plant tissues [17].

A more detailed examination of the degradation, persistence, and accumulation patterns of the eight detected PhACs in soil allows them to be classified into distinct behavioral groups. Clarithromycin, carbamazepine, venlafaxine, and flecainide exhibited persistent behavior in freshwater (FW)-irrigated plots, maintaining very low background concentrations (1–2 ng g^{-1}). Their concentrations increased only under treated wastewater (TWW) irrigation, particularly during summer tomato cropping periods, and showed signs of progressive soil accumulation, most notably for clarithromycin and carbamazepine. Fluconazole and climbazole displayed a similar temporal pattern characterized by stable concentration declines under FW irrigation and pronounced concentration peaks associated with the summer TWW irrigation regime. Following the irrigation season, these compounds underwent slow but consistent degradation, with no evidence of long-term accumulation.

Telmisartan exhibited a distinct pattern, maintaining a stable baseline concentration in both FW- and TWW-irrigated soils, with only transient concentration increases during summer cropping periods when higher volumes of TWW were applied.

In contrast, sitagliptin exhibited a unique pattern, showing a tendency toward accumulation in both soil types. This behavior is likely driven by TWW inputs combined with enhanced compound mobility within the soil matrix, potentially facilitated by root system development and agronomic soil management practices during crop rotation.

Based on our observation, the intrinsic biotic and abiotic features of the soil retained only part of the PhACs received from irrigation water, and the majority of the molecules that were not completely depleted by physical or biological activities could persist at very low concentrations over extended periods. Only clarithromycin, carbamazepine, and sitagliptin showed an accumulation pattern after 2 years of experimentation, indicating that the prolonged use of treated wastewater could pose a threat to the environment and human health for some specific PhACs that exhibit a greater tendency to resist biological and physicochemical degradation. Although the present work was not intended to provide an in-depth ecotoxicological evaluation, we made a general assumption to roughly estimate the risk level to the soil ecosystem for persistent compounds found to be accumulating in soil. Based on the NORMAN database reporting Predicted No-Effect Concentrations (PNECs) for pharmaceuticals compounds aquatic ecosystems and the environment [46,47], we estimated that the compounds found in our study associated with higher risk may affect

soil ecosystems within the range of 20–200 ng/g of soil. As shown in Figure 2, sitagliptin and flecainide showed an accumulation trend when TWW was applied, which, after 2 years and four crop cycles, reached levels at or above 10 ng/g in the soil. This accumulation over a relatively short period of time may be cause for concern regarding the effects of these PhACs on plant and microbial ecosystems. Further studies will be needed to draw more robust conclusions over the medium-to-long term.

4.3. Microbial Ecology and Assembly Processes in the Soil

A central finding of this research is that the soil microbial community structure was influenced more by time and crop succession (tomato–wheat) than by the quality of the irrigation water. This aligns with several studies indicating that the "crop rotation stage" or "crop identity" acts as a primary driver of soil microbiome assembly, often exceeding the effects of fertilization or water quality [14,48]. In our study, the stronger effect exerted by the crop and seasonal variations was demonstrated by both cultural and molecular methods, as will be discussed in further detail in the following sections.

4.3.1. Microbial Indicators

As reported in the results, both bacterial and fungal counts were mostly affected by sampling time and crop type, while irrigation water did not significantly affect their levels. This result is in accordance with previous research [49] which demonstrated that water spiked with 15 different PhACs did not change its bacterial and fungal biomass over a single cropping season. Looking at the impact of rotation cultures, recent studies have confirmed that crop rotation may influence the microbial abundance more than the type of irrigation water [14,50]. Regarding the fecal indicators, we could not find a significant increase when TWW was used. While a long-term previous study reported consistent higher level of fecal indicators in treated-wastewater irrigated soils [51], other studies that accounted for the effect of the rainfall on the leaching and dispersion of fecal indicators showed that this effect is negligible even after prolonged treatments with TWW [52]. Despite some methodological differences and differing environments surveyed, both studies were consistent with our findings, indicating that irrigation water, even over extended periods, is only one of the many factors that may influence the level of fecal indicators in agricultural soils.

4.3.2. Functional Genes and Nitrogen Cycle Dynamics

The qPCR analysis of functional genes (*nifH*, *amoA*, *nosZ*, *nirK*) confirmed that nitrogen-cycling communities are closely linked to the crop phase rather than water origin. Tomato hosted a higher population of nitrogen-fixing bacteria (*nifH*) and nitrifiers (*amoA*) compared to wheat. This well-known "rhizosphere effect" [52] is likely driven by the specific metabolites released by tomato roots, which selectively promote beneficial bacterial taxa particularly related to nitrogen uptake [53]. Our observations confirmed that while TWW adds mineral nitrogen with positive effects on the crop yield and quality, it did not drastically influence the relative nitrogen-cycling microbial populations of the soil compared to the effect exerted by crop type.

Interestingly, with regard to denitrifying populations, our results showed that *nosZ* (complete denitrification) decreased over time while *nirK* (partial denitrification) increased, irrespective of the crop type and the irrigation water. This trend may be influenced by seasonal shifts in soil moisture and oxygen availability rather than irrigation water quality.

4.3.3. Microbial Community Dynamics

The metataxonomic analyses of bacterial communities in treated soils confirmed the trends suggested by culture-based methods and qPCR. Overall, the major drivers of the microbial community dynamics in soil were the crop type used and the sampling time,

while the use of differentially treated wastewater had a minimal effect on both bacterial and fungal populations.

Both microbial populations (bacteria and fungi) exhibited progressive evolution with time, as shown by the abundance plots (Figure S3A,B) and beta-diversity reported in Figure 2. From the figure, it is clearly visible that time zero and first crop season (tomato) clustered together, while after the second crop season beta-diversity was increasingly shaped by the combined effect of time and crop type.

Interestingly, fungi formed less distinct clusters than bacteria and responded more slowly to time and crop. The PERMANOVA results further support this interpretation, showing that the crop-time structure was the main factor explaining UniFrac beta-diversity, whereas irrigation water source had no significant effect. This confirms that the observed microbial community shifts were primarily associated with crop succession and sampling stage rather than with the use of tertiary-treated wastewater. Therefore, under the tested field conditions, the soil microbiome appeared more responsive to crop-related and temporal drivers than to irrigation water quality. Our findings are consistent with the findings of [54], who showed, in a high frequency sampling experiment, that bacteria exhibit shorter temporal fluctuations compared to fungi within a single crop-growing season, being more responsive to the changes in physical soil conditions and weather. Similar evidence of strong differences between and within microbial domains in response to environmental variation and changing soil conditions was reported in another recent study [55].

Regarding the crop-rotation impact on bacterial and fungal communities, a recent comprehensive meta-analysis [51] demonstrated that soil bacterial and fungal alpha and beta diversities are differentially affected. In particular, bacteria were found to be more dynamic and sensitive to the differential root environment than fungi, confirming our findings (see also Figures 4 and 5 for the alpha diversity) and supporting our hypotheses regarding the differential responses of bacteria and fungi to crop rotation-induced changes in soil.

The substantial stability of the microbial community despite differing water treatment can be explained by deterministic assembly processes, which typically dominate in agricultural systems, as suggested by a recent study that evaluated microbial community changes under different fertilization regimes in a wheat/rice rotation for two seasons [48]. The presence of PhACs in both soils, despite the lower concentration in FW relative to TWW, did not generate measurable effects on the relative abundance of microbial taxa (Figure S2) or on beta-diversity (Figure 3). The soil microbial community response to differing irrigation water did not allow us to draw robust conclusions regarding possible selection mechanisms or potential degradative capabilities related to the PhACs detected. It is likely that the baseline PhACs content in both soils may act as a driver for adaptation mechanisms, and that the relatively low concentrations are not sufficient to generate inhibition or toxicity effects in the 24-month duration of the experiment.

The soil microbiome not affected by crop rotation and irrigation treatments (S_0) had distinct populations of bacteria such as *Streptosporangiaceae*, *Alicyclobacillaceae*, and *Steroidobacteraceae* and fungal order *Thelebolales*. The first two families both include spore-forming species and are generally resistant to soil pH shifts and high-temperature stress, conditions typical of agricultural soil in arid climates [56,57]. *Streptosporangiaceae* are often isolated from upper soil layers and are important contributors to soil restoration after wildfires or during revegetation [58,59], while *Alicyclobacillaceae* includes species involved in the decomposition of soil organic matter. Regarding *Steroidobacteraceae*, this family includes species that are not only soil organic matter decomposers but also potential denitrifiers and possess rhizosphere competence [60]. Overall, the control soil (S_0) was characterized by higher populations of taxa associated with upper-soil-layer decomposition, typical of

uncultivated agricultural soil and able to withstand soil stress in arid lands, including the fungus belonging to the order *Thelebolales*.

In our crop rotation, the specific root exudates of tomato and wheat created distinct "novel microbial environments". For instance, as shown by LEfSe analysis, the tomato crop selected different bacterial populations such as *Alphaproteobacteria*, *Haliangiaceae* and *Myxococcaceae* (both from the *Myxococcota* phylum). *Myxococcaceae* was found to be one of the families usually associated with tomato plant roots [56,61] and was sensitive to saline and other stresses. Interestingly both *Myxococcaceae* and *Haliangiaceae* include species that are natural predators of soil pathogens, including tomato-associated ones [62,63]. In the case of fungi as well, the tomato crop selected *Aspergillaceae* (and other *Eurotiales* order taxa) that are typically enriched in tomato–wheat successions as reported by other studies [64].

The wheat rhizosphere promoted greater taxonomic diversity (Figure 6A), recruiting a larger number of taxa belonging to families within the *Verrucomicrobiota* phylum, namely, *Chthoniobacteriaceae*, *Verrucomicrobiaceae*, *Opitutaceae*, *Pedosphaeraceae*.

Verrucomicrobiota has been reported as a phylum associated with the plant rhizosphere, particularly that of wheat and other cereals, and especially modern cultivars [65–67].

Other taxa associated with the wheat crop season microbiome belonged to *Bacteroidota*, *Chloroflexota*, *Acidobacteriota*, and *Pseudomonadota*, which have been found among the most represented phyla in wheat rhizosphere environments [66]. This effect of wider diversity selection was confirmed for fungal populations, as reported in the Section 3. Although partially affected by the intrinsic seasonal variations, we confirmed that the crop effect on the soil microbiome is significant and that different crops exerted a specific influence in promoting certain microbial taxa. Irrigation water type did not contribute significantly to shaping different microbial communities, with the exception of the fungal taxon *Bionectria*, which was found to be more represented in FW irrigated soil. Further studies will be needed to confirm whether this taxon can represent a potential biomarker for monitoring the effect of treated wastewater on the soil microbiome.

5. Conclusions and Implications for Sustainability

In conclusion, integrating tertiary-treated TWW irrigation into a tomato–wheat rotation system is a sustainable practice that enhances agricultural productivity without compromising soil health. The soil can act as a filter, and the resilience of its microbial communities mitigates risks associated with PhACs in the short term. The crop rhizosphere effect, along with inherent seasonal variations, identified as the main drivers of microbial community dynamics, support the hypothesis that proper agronomic management (i.e., crop rotation) can contribute to preserving diversity and functional robustness. To further evaluate these aspects and separate crop-related effects from time- and season-related effects, future studies utilizing controlled environments (e.g., greenhouses with climate manipulation) or split-root designs may be necessary to isolate the specific contributions of climatic seasonality versus crop identity.

Regarding functional diversity, future research should specifically address the impact of wastewater used for irrigation and the inherent presence of emerging pollutants, through metagenomic and meta-transcriptomic approaches. Although our observations were limited to the taxonomic dimension, the intriguing dynamics observed in bacterial and fungal communities represent an encouraging starting point for deeper investigation and broader conclusions.

However, it is important to recognize that the short-term benefits observed do not guarantee the safety of long-term continuous irrigation, given the potential progressive accumulation of recalcitrant PhACs such as carbamazepine and clarithromycin. Thus, there is a need for a precautionary approach that integrates the upgrading of wastewater

treatment through advanced technologies (e.g., oxidation, adsorption, membranes), smart irrigation management strategies (e.g., alternating TWW with freshwater), soil-based mitigation practices (e.g., biochar and organic amendments), and structured monitoring programs to ensure long-term environmental sustainability and food safety.

Regarding the presence of PhACs in the soil prior to the start of the experiments, our study was not designed to separate historical legacy effects from those associated with the current experimental treatments. Future studies comparing soils with different irrigation histories will be necessary to fully disentangle these effects. In our study, only one fungal taxon (*Bionectria* sp.) was found to be sensitive to TWW treatment, indicating a possible role as a biomarker. Although our findings show a minor disturbance of soil microbial communities when wastewater that naturally contains PhACs is used for irrigation, important knowledge gaps remain regarding the potential effects of different type and amount of PhACs and their metabolites on both soil microbiology and their uptake and transport into plants.

A possible strategy to minimize risk would be to alternate TWW and FW in order to reduce soil contamination or apply TWW only during the early stage of the crop cycle for annual crops. Future studies integrating soil, plant, and food safety assessments are needed to fully characterize PhACs' dynamics within the soil–plant system.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture16131426/s1.

Author Contributions: Conceptualization, L.B. (Luciano Beneduce) and G.G.; methodology, M.T. and A.G.; validation, M.M.G. and L.B. (Lorenzo Brusetti); formal analysis, F.C., F.P. and A.G.; investigation, M.D. and C.S.; data curation, L.B. (Luigimaria Borruso); writing—original draft preparation, L.B. (Luciano Beneduce) and G.G.; writing—review and editing, C.S., L.B. (Luciano Beneduce), F.D.M. and G.G.; visualization, C.S.; supervision, M.P.; funding acquisition, G.B. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Ministero dell'Istruzione, dell'Università e della Ricerca, Italy, grant number 2017C5CLFB.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Pedrero, F.; Kalavrouziotis, I.; Alarcón, J.J.; Koukoulakis, P.; Asano, T. Use of Treated Municipal Wastewater in Irrigated Agriculture—Review of Some Practices in Spain and Greece. *Agric. Water Manag.* **2010**, *97*, 1233–1241. [CrossRef]
2. Van Opstal, J.; Droogers, P.; Kaune, A. *Reuse of Water in Agricultural Systems*; FAO: Bangkok, Thailand, 2025.
3. Libutti, A.; Gatta, G.; Gagliardi, A.; Vergine, P.; Pollice, A.; Beneduce, L.; Disciglio, G.; Tarantino, E. Agro-Industrial Wastewater Reuse for Irrigation of a Vegetable Crop Succession under Mediterranean Conditions. *Agric. Water Manag.* **2018**, *196*, 1–14. [CrossRef]
4. Gatta, G.; Libutti, A.; Gagliardi, A.; Beneduce, L.; Brusetti, L.; Borruso, L.; Disciglio, G.; Tarantino, E. Treated Agro-Industrial Wastewater Irrigation of Tomato Crop: Effects on Qualitative/Quantitative Characteristics of Production and Microbiological Properties of the Soil. *Agric. Water Manag.* **2015**, *149*, 33–43. [CrossRef]
5. Beneduce, L.; Gatta, G.; Bevilacqua, A.; Libutti, A.; Tarantino, E.; Bellucci, M.; Troiano, E.; Spano, G. Impact of the Reusing of Food Manufacturing Wastewater for Irrigation in a Closed System on the Microbiological Quality of the Food Crops. *Int. J. Food Microbiol.* **2017**, *260*, 51–58. [CrossRef] [PubMed]
6. aus der Beek, T.; Weber, F.-A.; Bergmann, A.; Hickmann, S.; Ebert, I.; Hein, A.; Küster, A. Pharmaceuticals in the Environment—Global Occurrences and Perspectives. *Environ. Toxicol. Chem.* **2015**, *35*, 823–835. [CrossRef] [PubMed]
7. Michael, I.; Rizzo, L.; McArdell, C.S.; Manaia, C.M.; Merlin, C.; Schwartz, T.; Dagot, C.; Fatta-Kassinos, D. Urban Wastewater Treatment Plants as Hotspots for the Release of Antibiotics in the Environment: A Review. *Water Res.* **2013**, *47*, 957–995. [CrossRef] [PubMed]

8. Gatta, G.; De Mastro, F.; Carucci, F.; Perniola, M.; Denora, M.; Brunetti, G.; Gagliardi, A.; Giuliani, M.M. Soil Accumulation and Plant Uptake of Pharmaceutical Active Compounds and Related Metabolites from Irrigation Water in Fennel (*Foeniculum vulgare* Mill.). *Front. Plant Sci.* **2026**, *17*, 1664441. [CrossRef] [PubMed]
9. Grenni, P.; Ancona, V.; Barra Caracciolo, A. Ecological Effects of Antibiotics on Natural Ecosystems: A Review. *Microchem. J.* **2018**, *136*, 25–39. [CrossRef]
10. Paterson, E.; Gebbing, T.; Abel, C.; Sim, A.; Telfer, G. Rhizodeposition Shapes Rhizosphere Microbial Community Structure in Organic Soil. *New Phytol.* **2007**, *173*, 600–610. [CrossRef] [PubMed]
11. Araujo, A.S.F.; Pereira, A.P.A.; de Medeiros, E.V.; Mendes, L.W. Root Architecture and the Rhizosphere Microbiome: Shaping Sustainable Agriculture. *Plant Sci.* **2025**, *359*, 112599. [CrossRef] [PubMed]
12. Nakayasu, M.; Ohno, K.; Takamatsu, K.; Aoki, Y.; Yamazaki, S.; Takase, H.; Shoji, T.; Yazaki, K.; Sugiyama, A. Tomato Roots Secrete Tomatine to Modulate the Bacterial Assemblage of the Rhizosphere. *Plant Physiol.* **2021**, *186*, 270–284. [CrossRef] [PubMed]
13. Matthews, A.; Pierce, S.; Hipperson, H.; Raymond, B. Rhizobacterial Community Assembly Patterns Vary Between Crop Species. *Front. Microbiol.* **2019**, *10*, 581. [CrossRef] [PubMed]
14. Zverev, A.O.; Kurchak, O.N.; Orlova, O.V.; Onishchuk, O.P.; Kichko, A.A.; Eregin, A.V.; Naliukhin, A.N.; Pinaev, A.G.; Andronov, E.E. Dynamic of the Soil Microbiota in Short-Term Crop Rotation. *Life* **2023**, *13*, 400. [CrossRef] [PubMed]
15. Falkowski, P.G.; Fenchel, T.; Delong, E.F. The Microbial Engines That Drive Earth's Biogeochemical Cycles. *Science* **2008**, *320*, 1034–1039. [CrossRef] [PubMed]
16. Kuypers, M.M.M.; Marchant, H.K.; Kartal, B. The Microbial Nitrogen-Cycling Network. *Nat. Rev. Microbiol.* **2018**, *16*, 263–276. [CrossRef] [PubMed]
17. Pérez-Lucas, G.; Navarro, S. How Pharmaceutical Residues Occur, Behave, and Affect the Soil Environment. *J. Xenobiot.* **2024**, *14*, 1343–1377. [CrossRef] [PubMed]
18. Chauhan, A.; Jain, A.; Kolton, M.; Pathak, A. Impacts of Long-Term Irrigation of Municipally-Treated Wastewater to the Soil Microbial and Nutrient Properties. *Sci. Total Environ.* **2025**, *959*, 178143. [CrossRef] [PubMed]
19. Christou, A.; Papadavid, G.; Dalias, P.; Fotopoulos, V.; Michael, C.; Bayona, J.M.; Piña, B.; Fatta-Kassinos, D. Ranking of Crop Plants According to Their Potential to Uptake and Accumulate Contaminants of Emerging Concern. *Environ. Res.* **2019**, *170*, 422–432. [CrossRef] [PubMed]
20. Christou, A.; Antoniou, C.; Christodoulou, C.; Hapeshi, E.; Stavrou, I.; Michael, C.; Fatta-Kassinos, D.; Fotopoulos, V. Stress-Related Phenomena and Detoxification Mechanisms Induced by Common Pharmaceuticals in Alfalfa (*Medicago sativa* L.) Plants. *Sci. Total Environ.* **2016**, *557–558*, 652–664. [CrossRef] [PubMed]
21. Denora, M.; Mehmeti, A.; Candido, V.; Brunetti, G.; De Mastro, F.; Murgolo, S.; De Ceglie, C.; Gatta, G.; Giuliani, M.M.; Fiorentino, C.; et al. Fate of Emerging Contaminants in the Soil-Plant System: A Study on Durum Wheat Irrigated with Treated Municipal Wastewater. *Front. Soil Sci.* **2024**, *4*, 1448016. [CrossRef]
22. Caliandro, A.; Lamaddalena, N.; Stelluti, M.; Steduto, P. *Caratterizzazione Agro-Ecologica Della Regione Puglia in Funzione Della Potenzialità Produttiva*; Ideaprint: Bari, Italy, 2005.
23. Buono, V.; Gatta, G.; Riezzo, E.; Manes, A.; Nardella, E.; Gagliardi, A.; Carucci, F.; Giuliani, M.M. Experimental Testing of a Model-Based Decision Support System Integrated with Smart Sensors to Optimize Irrigation Strategies for Processing Tomato: A Case Study in Southern Italy. *Acta Hortic.* **2021**, *1311*, 485–492. [CrossRef]
24. Abi Saab, M.T.; Houssemeddine Sellami, M.; Giorio, P.; Basile, A.; Bonfante, A.; Rouphael, Y.; Fahed, S.; Jomaa, I.; Stephan, C.; Kabalan, R.; et al. Assessing the Potential of Cereal Production Systems to Adapt to Contrasting Weather Conditions in the Mediterranean Region. *Agronomy* **2019**, *9*, 393. [CrossRef]
25. Carucci, F.; Gagliardi, A.; Giuliani, M.M.; Gatta, G. Irrigation Scheduling in Processing Tomato to Save Water: A Smart Approach Combining Plant and Soil Monitoring. *Appl. Sci.* **2023**, *13*, 7625. [CrossRef]
26. Si, Z.; Qin, A.; Liang, Y.; Duan, A.; Gao, Y. A Review on Regulation of Irrigation Management on Wheat Physiology, Grain Yield, and Quality. *Plants* **2023**, *12*, 692. [CrossRef] [PubMed]
27. APAT Agenzia per La Protezione Dell'ambiente e per i Servizi Tecnici; IRSA-CNR Istituto Di Ricerca Sulle Acque Consiglio Nazionale Delle Ricerche. *APAT Metodi Analitici per Le Acque*; Volume Primo Sezione 1000-Parte Generale Sezione 2000-Parametri Chimico-Fisici Sezione 3000-Metalli; APAT Agenzia per La Protezione Dell'ambiente e per i Servizi Tecnici: Rome, Italy, 2003.
28. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; APHA: Washington, DC, USA, 2025.
29. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. *Estimation of Available Phosphorus in Soil by Extraction with Sodium Bicarbonate*; Circular no. 939; USDA: Washington, DC, USA, 1954; pp. 1–19.
30. Walkley, A.; Black, I.A. An examination of the Degtjareff method for deter-mining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci.* **1934**, *63*, 251–263.
31. Keeney, D.R.; Nelson, D.W. Nitrogen inorganic forms. In *Methods of Soil Analysis: Part 2*, 2nd ed.; Agronomy Monographs 9; ASA: Madison, WI, USA; SSSA: Madison, WI, USA, 1982; pp. 643–698.

32. De Mastro, F.; Cocozza, C.; Traversa, A.; Cacace, C.; Mottola, F.; Mezzina, A.; Brunetti, G. Validation of a Modified QuEChERS Method for the Extraction of Multiple Classes of Pharmaceuticals from Soils. *Chem. Biol. Technol. Agric.* **2022**, *9*, 49. [CrossRef]
33. De Mastro, F.; Brunetti, G.; De Mastro, G.; Ruta, C.; Stea, D.; Murgolo, S.; De Ceglie, C.; Mascolo, G.; Sannino, F.; Cocozza, C.; et al. Uptake of Different Pharmaceuticals in Soil and Mycorrhizal Artichokes from Wastewater. *Environ. Sci. Pollut. Res.* **2022**, *30*, 33349–33362. [CrossRef] [PubMed]
34. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef] [PubMed]
35. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef] [PubMed]
36. Box, G.E.P.; Cox, D.R. An Analysis of Transformations. *J. R. Stat. Soc. Ser. B Stat. Methodol.* **1964**, *26*, 211–243. [CrossRef]
37. Lozupone, C.; Knight, R. UniFrac: A New Phylogenetic Method for Comparing Microbial Communities. *Appl. Environ. Microbiol.* **2005**, *71*, 8228–8235. [CrossRef] [PubMed]
38. Gatta, G.; Libutti, A.; Gagliardi, A.; Disciglio, G.; Tarantino, E.; Beneduce, L.; Giuliani, M.M. Wastewater Reuse in Agriculture: Effects on Soil-Plant System Properties. In *Interaction and Fate of Pharmaceuticals in Soil-Crop Systems*; Springer: Cham, Switzerland, 2020. [CrossRef]
39. Muscarella, S.M.; Alduina, R.; Badalucco, L.; Capri, F.C.; Di Leto, Y.; Gallo, G.; Laudicina, V.A.; Paliaga, S.; Mannina, G. Water Reuse of Treated Domestic Wastewater in Agriculture: Effects on Tomato Plants, Soil Nutrient Availability and Microbial Community Structure. *Sci. Total Environ.* **2024**, *928*, 172259. [CrossRef] [PubMed]
40. De Mastro, F.; Brunetti, G.; Cocozza, C.; Murgolo, S.; Mascolo, G.; Salerno, C.; Ruta, C.; De Mastro, G. Dynamics of Pharmaceuticals in the Soil–Plant System: A Case Study on Mycorrhizal Artichoke. *Soil Syst.* **2025**, *9*, 51. [CrossRef]
41. Denora, M.; Candido, V.; Brunetti, G.; De Mastro, F.; Murgolo, S.; De Ceglie, C.; Salerno, C.; Gatta, G.; Giuliani, M.M.; Mehmeti, A.; et al. Uptake and Accumulation of Emerging Contaminants in Processing Tomato Irrigated with Tertiary Treated Wastewater Effluent: A Pilot-Scale Study. *Front. Plant Sci.* **2023**, *14*, 1238163. [CrossRef] [PubMed]
42. The European Parliament; The Council of the European Union. Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on Minimum Requirements for Water Reuse (Text with EEA Relevance). *Off. J. Eur. Union* **2020**, *177*, 33–55.
43. Kodešová, R.; Kočárek, M.; Klement, A.; Golovko, O.; Koba, O.; Fér, M.; Nikodem, A.; Vondráčková, L.; Jakšík, O.; Grabic, R. An Analysis of the Dissipation of Pharmaceuticals under Thirteen Different Soil Conditions. *Sci. Total Environ.* **2016**, *544*, 369–381. [CrossRef] [PubMed]
44. Paz, A.; Tadmor, G.; Malchi, T.; Blotevogel, J.; Borch, T.; Polubesova, T.; Chefetz, B. Fate of Carbamazepine, Its Metabolites, and Lamotrigine in Soils Irrigated with Reclaimed Wastewater: Sorption, Leaching and Plant Uptake. *Chemosphere* **2016**, *160*, 22–29. [CrossRef] [PubMed]
45. Guedes, P.; Martins, C.; Couto, N.; Silva, J.; Mateus, E.P.; Ribeiro, A.B.; Pereira, C.S. Irrigation of Soil with Reclaimed Wastewater Acts as a Buffer of Microbial Taxonomic and Functional Biodiversity. *Sci. Total Environ.* **2022**, *802*, 149671. [CrossRef] [PubMed]
46. Dulio, V.; Koschorreck, J.; van Bavel, B.; van Leerdam, J.A.; von der Ohe, P.C.; Schlabach, M.; Slobodnik, J.; Thomaidis, N.S.; Aalizadeh, R. The NORMAN Association: A network approach to address emerging environmental substances. *Environ. Sci. Eur.* **2018**, *30*, 16.
47. NORMAN Network. NORMAN Ecotox Database: Ecotoxicology Module. NORMAN Database System (NDS). 2026. Available online: https://www.norman-network.com/ (accessed on 14 June 2026).
48. Xie, Y.; Ouyang, Y.; Han, S.; Se, J.; Tang, S.; Yang, Y.; Ma, Q.; Wu, L. Crop Rotation Stage Has a Greater Effect than Fertilisation on Soil Microbiome Assembly and Enzymatic Stoichiometry. *Sci. Total Environ.* **2022**, *815*, 152956. [CrossRef] [PubMed]
49. Gallego, S.; Montemurro, N.; Béguet, J.; Rouard, N.; Philippot, L.; Pérez, S.; Martin-Laurent, F. Ecotoxicological Risk Assessment of Wastewater Irrigation on Soil Microorganisms: Fate and Impact of Wastewater-Borne Micropollutants in Lettuce-Soil System. *Ecotoxicol. Environ. Saf.* **2021**, *223*, 112595. [CrossRef] [PubMed]
50. Mahjoub, O.; Mauffret, A.; Michel, C.; Chmingui, W. Use of Groundwater and Reclaimed Water for Agricultural Irrigation: Farmers' Practices and Attitudes and Related Environmental and Health Risks. *Chemosphere* **2022**, *295*, 133945. [CrossRef] [PubMed]
51. Li, C.; Shi, L.; Wang, K.; Liu, B.; Liao, J.; An, Z.; Chang, S.X. Crop Rotation Differentially Increases Soil Bacterial and Fungal Diversities in Global Croplands: A Meta-Analysis. *Nat. Commun.* **2025**, *16*, 11686. [CrossRef]
52. Berg, G.; Smalla, K. Plant Species and Soil Type Cooperatively Shape the Structure and Function of Microbial Communities in the Rhizosphere. *FEMS Microbiol. Ecol.* **2009**, *68*, 1–13. [CrossRef] [PubMed]
53. Maleva, M.; Borisova, G.; Tugbaeva, A.; Malakheeva, A.; Voropaeva, O.; Ermoshin, A.; Betekhtina, A. Rhizobacteriome Diversity and Morphophysiological Features of Three Tomato Plant Varieties Under Nitrogen Deficiency. *Nitrogen* **2025**, *6*, 102. [CrossRef]

54. Leitner, Z.R.; Daigh, A.L.M.; DeJong-Hughes, J. Temporal Fluctuations of Microbial Communities within the Crop Growing Season. *Geoderma* **2021**, *391*, 114951. [CrossRef]
55. Wang, J.; Niu, W.; Song, X.; Han, J. Response of Tomato Root Endophytic Bacterial Communities to Water-Oxygen Coupling under Micro/Nanoaerated Drip Irrigation in Slightly Saline Soils. *Sci. Hortic.* **2023**, *321*, 112281. [CrossRef]
56. Otoguro, M.; Yamamura, H.; Quintana, E.T. The family *Streptosporangiaceae*. In *The Prokaryotes*; Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer: Berlin/Heidelberg, Germany, 2014; pp. 1011–1045. [CrossRef]
57. Kim, H.W.; Kim, N.K.; Phillips, A.P.R.; Parker, D.A.; Liu, P.; Whitaker, R.J.; Rao, C.V.; Mackie, R.I. Genomic insight and physiological characterization of thermoacidophilic *Alicyclobacillus* isolated from Yellowstone National Park. *Front. Microbiol.* **2023**, *14*, 1232587. [CrossRef] [PubMed]
58. Nelson, A.R.; Narrowe, A.B.; Rhoades, C.C.; Fegel, T.S.; Daly, R.A.; Roth, H.K.; Chu, R.K.; Amundson, K.K.; Young, R.B.; Steindorff, A.S.; et al. Wildfire-dependent changes in soil microbiome diversity and function. *Nat. Microbiol.* **2022**, *7*, 1419–1430. [CrossRef] [PubMed]
59. Ghaly, T.M.; McPherson, V.J.; Rajabal, V.; Ghaly, M.E.; Taws, N.; Gallagher, R.V.; Le Roux, J.J.; Tetu, S.G. Soil microbial traits shift on contrasting timescales following revegetation of former grazing lands. *bioRxiv* **2026**. [CrossRef]
60. Wu, X.; Bei, S.; Zhou, X.; Luo, Y.; He, Z.; Song, C.; Yuan, H.; Pivato, B.; Liesack, W.; Peng, J. Metagenomic insights into genetic factors driving bacterial niche differentiation between bulk and rhizosphere soils. *Sci. Total Environ.* **2023**, *891*, 164221. [CrossRef] [PubMed]
61. Chen, J.; Liu, S.-S.; He, L.-X.; Cheng, Y.-X.; Ye, P.; Li, J.; Ying, G.-G.; Wang, Y.-J.; Yang, F. The Fate of Sulfonamides in the Process of Phytoremediation in Hydroponics. *Water Res.* **2021**, *198*, 117145. [CrossRef] [PubMed]
62. Groß, V.; Reinhard, A.; Petters, S.; Pichler, M.; Urich, T. Adding complexity to soil food webs: Myxobacteria have broad predation spectra with bacteria, yeasts and filamentous fungi in vitro. *Eur. J. Soil Biol.* **2023**, *117*, 103508. [CrossRef]
63. Dong, H.; Xu, X.; Gao, R.; Li, Y.; Li, A.; Yao, Q.; Zhu, H. *Myxococcus xanthus* R31 suppresses tomato bacterial wilt by inhibiting the pathogen *Ralstonia solanacearum* with secreted proteins. *Front. Microbiol.* **2022**, *12*, 801091. [CrossRef] [PubMed]
64. De Corato, U.; Patruno, L.; Avella, N.; Salimbeni, R.; Lacolla, G.; Cucci, G.; Crecchio, C. Soil management under tomato-wheat rotation increases the suppressive response against Fusarium wilt and tomato shoot growth by changing the microbial composition and chemical parameters. *Appl. Soil Ecol.* **2020**, *154*, 103601. [CrossRef]
65. Gruet, C.; Abrouk, D.; Börner, A.; Muller, D.; Moënne-Loccoz, Y. Wheat Genome Architecture Influences Interactions with Phytobeneficial Microbial Functional Groups in the Rhizosphere. *Plant Cell Environ.* **2023**, *46*, 1018–1032. [CrossRef] [PubMed]
66. Dilla-Ermita, C.J.; Lewis, R.W.; Sullivan, T.S.; Hulbert, S.H. Wheat Genotype-Specific Recruitment of Rhizosphere Bacterial Microbiota Under Controlled Environments. *Front. Plant Sci.* **2021**, *12*, 718264. [CrossRef] [PubMed]
67. Naziębło, A.; Pytlak, A.; Furtak, A.; Dobrzyński, J. Advances and hotspots in research on Verrucomicrobiota: Focus on agroecosystems. *Microb. Ecol.* **2025**, *89*, 1. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.