Synergic Effect of Nutrient-Enriched Biochar and Zeolite on Soil-Tomato System Irrigated with Urban Treated Wastewater

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Abstract. This study investigates the impact of nutrient-enriched biochar and zeolite amendments, combined with irrigation with treated wastewater (TWW), on soil fertility and tomato plant growth at the Water Resource Recovery Facility of Palermo University. We conducted a pot experiment with tomato plants to test the combined effect of PO_4^{3-}-enriched biochar and NH_4^+-enriched zeolite, with or without using TWW for irrigation. Tomato plants irrigated with TWW matched the growth rates of those irrigated with tap water. The enriched amendments improved total nitrogen and phosphorus in the soil (23.2 and 9.3%, respectively). They seemed to promote a more salinity-tolerant microbial community, as indicated by the slight increase of microbial biomass carbon (MBC) in the combined treatment. The enriched amendments and the irrigation with TWW also led to a higher amount of metagenomic DNA extracted from soil microbiota parallel to the increase of MBC. Electrical conductivity levels suggested that TWW increased soil salinity; however, biochar and zeolite amendments effectively counteracted this effect. These results support the integrated use of TWW and nutrient-enriched amendments as a sustainable agricultural practice, offering a strategy to improve crop productivity while maintaining ecological balance. Future research should focus on the long-term results of these treatments and their influence on the soil microbiome.

Keywords: nutrient recovery · irrigation with TWW · enriched biochar · enriched zeolite

1 Introduction

Rapid global population growth is placing severe pressure on the agricultural sector, requiring a significant increase in food production to meet growing needs. This scenario has led to greater dependence on fertilisers to improve crop productivity. The increasing

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use of chemical fertilisers, while effective in stimulating crop growth, raises concerns about long-term sustainability, soil health and ecological balance (Pahalvi et al. 2021). Consequently, there is a pressing need to transition towards more sustainable methods of nutrient management. One such method is the recovery and reuse of nutrients from waste materials. The wastewater from secondary treatment plants is an often overlooked yet promising source for nutrient recovery (Mannina et al. 2021a; Mannina et al. 2021b). Although the nutrient concentration in urban treated wastewater (TWW) is relatively low, it contains sufficient quantities of essential nutrients for plant growth as N and P. Treated wastewater represents an opportunity for crop irrigation and nutrient recovery. Thanks to treatments with adsorbent material, it is possible to recover N and P from this water and apply them to crops when necessary. To this end, biochar and zeolite are increasingly recognised for their role in wastewater treatment and potential as a soil amendment (Muscarella et al. 2023a; Wang et al. 2022). When used in TWW treatment, biochar and zeolite can mainly adsorb PO_4^{3-} and NH_4^+, respectively. Therefore, when applied to soil, the main characteristics of these two natural amendments can improve water retention, soil structure, and nutrient retention and reduce soil erosion (Zhang et al. 2021; Girijaveni et al. 2018). Then, PO_4^{3-} enriched biochar and NH_4^+ enriched zeolites can be applied to the soil with the dual purpose: soil amendment and slow-release fertilisers.

Therefore, this study aims to evaluate the impact on soil fertility and tomato plant growth of nutrient-enriched biochar and zeolite applied in combination, with or without the use of TWW for irrigation. The focus is primarily on the dynamics of nitrogen (N) and phosphorus (P), microbial biomass, and on the biometric properties of tomato as a model crop. The plant growth-promoting bacteria (PGPB) are beneficial in maintaining soil fertility by increasing phosphorus and nitrogen availability and removing heavy metals from the soil (Singh et al. 2018). Therefore, understanding the microbial community's impact on plant growth in response to different treatments is necessary to analyse rhizospheric soils.

2 Materials and Methods

2.1 Experimental Design

For the purpose of this study, a pot experiment was carried out in a greenhouse at the Department of Agricultural, Food and Forest Sciences of the University of Palermo (38°10′66.6″N, 13°35′03.9″E). The experimental design involved five treatments: i) control soil, irrigated with tap water; ii) soil irrigated with TWW; iii) soil amended with enriched biochar and enriched zeolite, irrigated with tap water; iv) soil amended with enriched biochar and enriched zeolite, irrigated with TWW; v) soil amended with natural biochar and natural zeolite, irrigated with treated wastewater. The soil used for the experiment had a sandy-loam texture and was amended with 10% mature manure. The main characteristics of soil after manure amendment were 8.1 and 0.91 dS m^{-1} of pH and electrical conductivity (1:2.5, w/v, in water), respectively, 31 g kg^{-1} of TOC, 3.76 g kg^{-1} of TN and 129 mg kg^{-1} of available P, and 35 $cmol_+$ kg^{-1} of cation exchange capacity. Moreover 10% in volume of perlite was added to soil to avoid compaction. The treated wastewater used to irrigate the plants was obtained from the University of

Palermo's water resource recovery plant (Mannina et al. 2021a). A pilot plant with an advanced membrane bioreactor produced treated wastewater (TWW) (Mofatto et al. 2024; Mannina et al. 2022a). The biochar and zeolite used in the experimental test were enriched with PO_4^{3-} and NH_4^+ using a full-scale column pilot plant at the urban wastewater treatment plant in Marineo, Sicily (Mannina et al. 2022b). Tables 1 and 2 report the main characteristics of water, biochar, and zeolite, enriched or not.

Biochar and zeolite, natural or enriched, were mixed with soil at a concentration of 10 g kg^{-1} each, corresponding to 25 t ha^{-1}. Each pot was filled with 1.5 kg of soil, and one tomato plant was transplanted into each pot. Plants were irrigated twice weekly to maintain the soil at 50% of the field water capacity, with tap water (TAP) and treated wastewater (TWW) according to the experimental design. After 80 days from transplanting, the tomato plants were harvested and analysed to assess their biometrical and chemical properties. Simultaneously, the soil of each pot was sampled for the biological analysis, and the rest was air-dried, sieved at 2 mm, and then analysed to determine the main chemical and biochemical properties. In particular, metagenomic DNA was extracted and purified from 1g of rhizospheric soil of three soil replicates followed by NGS (Next Generation Sequencing) of a conserved marker gene, the 16S rRNA gene (Presti et al. 2021). DNA quality and quantity were evaluated by electrophoretic and spectrophotometric analysis. The high variable DNA fragments, termed V3–V4 regions of the 16S rDNA gene, were amplified using the DreamTaq DNA polymerase (ThermoFisherScientific) following the manufacturer's instructions. Amplification products were sequenced on an Illumina MiSeq platform at BMR Genomics (Padua, Italy).

Table 1. Main characteristics of water used for tomato plant irrigation. Tap water (TAP); Treated Wastewater (TWW)

Parameters	pH	EC	NH_4^+	NO_3^-	Total P	Fe	Mn	Ca	K	Mg	Na
u.m		dS m^{-1}	mg L^{-1}								
TAP	7.5	0.1	0.1	0.1	0.02	0.10	0.001	14.0	1.0	2.1	3.0
TWW	8.4	0.9	10.1	0.9	4.95	0.12	0.006	127.5	17.5	11.9	87.8

3 Results and Discussion

The dry weight of the plants showed no significant differences between the treatments, although plants irrigated with TWW showed a lower weight than those irrigated with tap water (Fig. 1). However, roots of plants grown in soil irrigated with TWW and not amended with biochar and zeolite showed lower weight than all other treatments and in particular were 39% lower than the control (TAP). These results suggest a potential negative effect of wastewater on plant growth, which can be reasonably attributed to the high electrical conductivity and soluble Na^+ concentration in wastewater.

Table 2. Main characteristics of biochar natural and enriched (NB and EB, respectively) and zeolite natural and enriched (NZ and EZ, respectively)

Parameters	Ca^{2+}	K^{+}	Na^{+}	Mg^{2+}	Total P	Available P	Total N
u.m.	(exchangable cations) mEq 100 g^{-1}				mg g^{-1}	mg kg^{-1}	mg g^{-1}
NB	19.0	7.0	5.0	4.3	1.2	66.0	-
EB	42.7	36.2	16.8	4.0	2.7	72.9	1.99
NZ	53.0	38.1	8.65	0.4	1.0	82.3	-
EZ	23.0	20.9	7.1	5.3	0.3	23.9	13.10

These potential negative effects of TWW on plant growth are reflected in the values of total nitrogen and phosphorus assimilated by the plants, which were lower in those irrigated with TWW than in those irrigated with tap water (Fig. 1). However, these negative effects of TWW had less impact on plants grown in soils amended with enriched biochar and zeolite than those grown in unamended soils or soils amended with unenriched biochar and zeolite. The concentration of total N in the soil irrigated with TWW was on average 22% higher than that in the control soil and similar to that in the soil irrigated with tap water and amended with enriched biochar and zeolite (Fig. 2). The available P in the soil showed the same trend as the total N, with the exception of the soil irrigated with tap water and amended with natural biochar and zeolite, which showed lower values than the control (Fig. 2). These results, combined with those for plant dry weight and total N and P, suggest that tomato plants are unable to absorb available nutrients despite their presence in the soil. Microbial biomass C was not negatively affected by TWW. On the contrary, it showed the highest values in soils irrigated with TWW and amended with enriched biochar and zeolite (Fig. 2). These results indicate that enriched biochar and zeolite can counteract the negative effect of TWW.

Moreover, the occurrence of bacteria in the analysed rhizospheric soils was demonstrated by a fragment of the expected size (500 bp) obtained after amplifying the V3–V4 regions of the 16S rDNA gene. The enriched amendments and the irrigation with TWW also led to a higher amount of metagenomics DNA extracted from soil microbiota that parallels the increase MBC. The amplicons were sequenced and bioinformatics analysis revealed the number of Amplicon Sequence Variants (ASVs). The abundance percentages related to the different bacterial species, orders, classes, and families were determined for each sample. The main components of the soil microbiota, including the plant-promoting bacteria, were found.

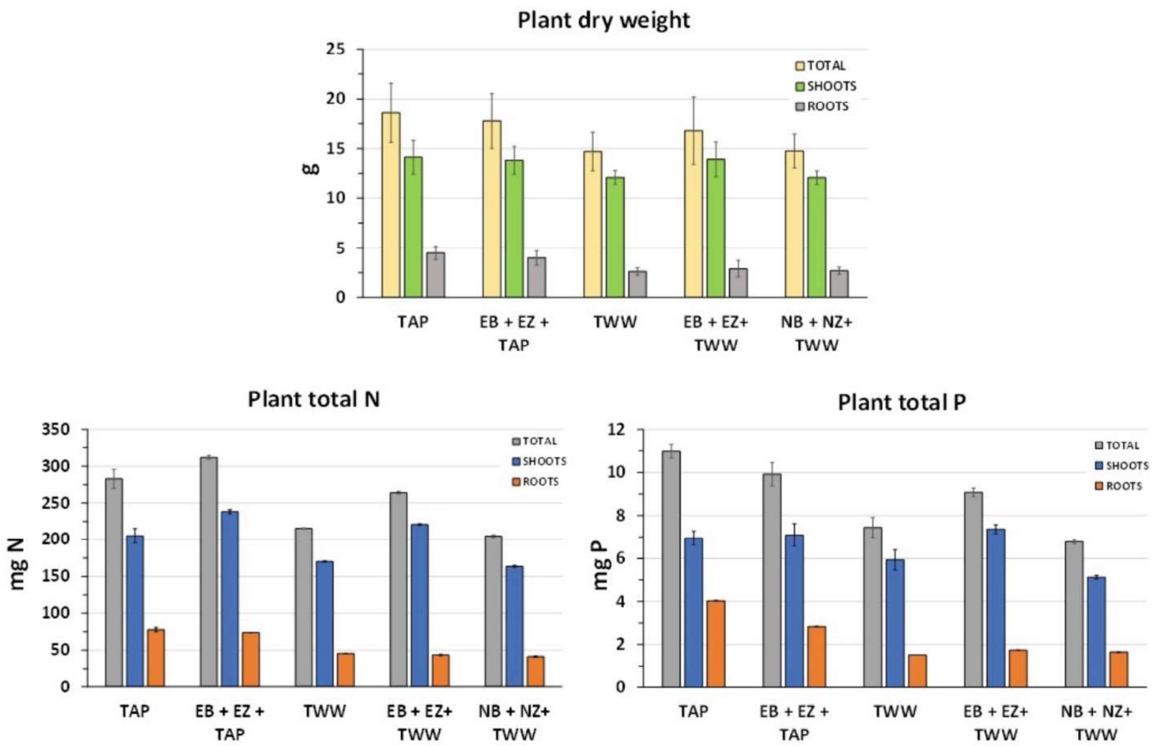

Fig. 1. Dry weight, total N and P of plants subjected to different irrigation treatments (TAP and TWW) and different amendments (EB, EZ, NB and NZ).

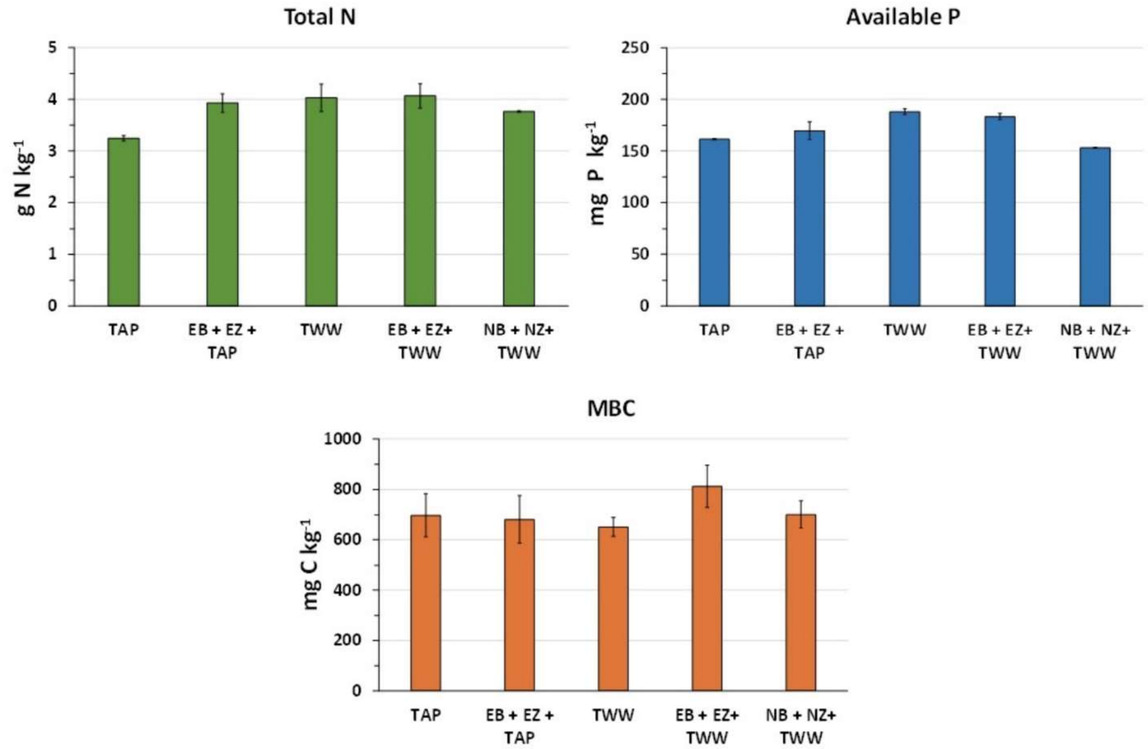

Fig. 2. Total N, available P and microbial biomass C (MBC) of soils subjected to different irrigation treatments (TAP and TWW) and different amendments (EB, EZ, NB and NZ)

Table 3. Chemical characteristics of soils subjected to different irrigation treatments (TAP and TWW) and different amendments (NB, EB, NZ and EZ)

	pH	Electrical conductivity	Cation exchange capacity	Ca^{2+}	K^{+}	Mg^{2+}	Na^{+}
	-	ds m^{-1}	mEq 100 g^{-1}				
TAP	8.0 ± 0.1	1.31 ± 0.02	35.8 ± 1.3	29.5 ± 1.1	1.9 ± 0.1	2.9 ± 0.1	1.6 ± 0.0
EB + EZ + TAP	8.0 ± 0.1	1.41 ± 0.02	37.0 ± 0.5	30.3 ± 0.5	2.2 ± 0.1	2.8 ± 0.0	1.7 ± 0.0
TWW	8.1 ± 0.2	1.67 ± 0.01	36.5 ± 0.9	29.5 ± 0.7	2.2 ± 0.1	2.5 ± 0.1	2.2 ± 0.1
EB + EZ + TWW	7.9 ± 0.1	1.60 ± 0.01	38.3 ± 1.9	31.2 ± 1.6	2.2 ± 0.2	2.6 ± 0.2	2.4 ± 0.1
NB + NZ + TWW	8.0 ± 0.1	1.50 ± 0.05	35.4 ± 1.0	29.1 ± 1.0	1.9 ± 0.1	2.4 ± 0.1	2.1 ± 0.1

4 Conclusions

Reusing treated wastewater for crop irrigation and enriched biochar and zeolite as slow-release fertilisers are desirable to move towards a circular economy approach. The results of this study suggested that treated wastewater can be used but under controlled conditions. Indeed, the adverse effect of treated wastewater was alleviated when combined with phosphorus-enriched biochar and ammonium-enriched zeolite. Moreover, phosphorus-enriched biochar and ammonium-enriched zeolite acting as slow-release N and P fertiliser enhanced soil microbial biomass.

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