



From kitchen to crop: The efficacy and safety of the microbial consortium treated dishwasher wastewater for the Zero Mile system

Annamaria Alabiso^{a,b}, Sara Frasca^{a,b}, Valerio Cantelmo^b, Roberto Braglia^{b,d},
Francesco Scuderi^b, Fiammetta Costa^c, Roberta Congestri^b, Luciana Migliore^{b,d,*}

^a PhD Program in Evolutionary Biology and Ecology, Tor Vergata University of Rome, 00133, Rome, Italy

^b Department of Biology, Tor Vergata University of Rome, 00133, Rome, Italy

^c Department of Design, Polytechnic of Milan, 20133, Milan, Italy

^d eCampus University, 22060, Novedrate, (CO), Italy

ARTICLE INFO

Handling Editor: Mario De Tullio

Keywords:

Autotrophic/heterotrophic consortium

Biofilter design

Metabolic and ecological functions

Wastewater upcycling

ABSTRACT

A microbial consortium, based on the functional integration of photosynthetic and heterotrophic microorganisms, is the core of the Zero Mile System. This system is designed for reusing and upcycling household greywaters, a still untapped water resource. The previous challenges of dishwasher wastewater bioremediation demonstrated the capability of an *ad hoc* consortium (including a photosynthetic cyanobacterium and three heterotrophic bacterial isolates from dishwasher wastewater) to reclaim the wastewater at small/medium scale. In this study the wastewater treatment demonstrated to be effective in nutrient recycling and upcycling at a larger scale, i.e. 4 L (in three replicates to treat the total amount of wastewater discharged by the dishwasher), by removing high percentage of N and P from the wastewater (70% nitrogen, 50% phosphorous, respectively). Again, the reclaimed wastewater successfully fertilized lettuce plants both indoor (in the Zero Mile System demonstrator) and outdoor (in open field). Plants showed a significant higher biomass productivity in fresh weight compared to control plants and comparable or better values of the pigments and quality indices (e.g., soluble solids, total phenols, total flavonoids). Furthermore, the safety of the reclaimed wastewater is demonstrated by the analysis of the metabolic/ecologically relevant functions of the microbial communities in both untreated and treated wastewater. Colonizers were mainly organic matter degraders and bacteria involved in nitrogen cycling. The human related genera are quite few and no pathogens or potential microbiological contaminants of water bodies (as *E. coli*), were found. Hence, the utilization of treated dishwasher wastewater does not imply biological risks to agricultural products, soil, or groundwater.

1. Introduction

Wastewater treatment and reuse is a pivotal issue worldwide, due to its direct link with the reduction of freshwater consumption and its potential reuse, necessary to face the freshwater scarcity (Shakir et al., 2017; Bixio et al., 2005). The biological rehabilitation made by microbial consortia has emerged as an environmentally and economically sustainable procedure (Muñoz and Guieysse, 2006), which may efficiently be applied to reduce the organic load of several different wastewaters. In particular, in the microbial consortia, the synergistic interactions between photosynthetic and heterotrophic microorganisms result in an efficient biological system capable of exploiting wastewater nutrients, effectively purifying the water (Liu et al., 2017; Posadas et al.,

2017).

The dishwasher wastewater (DWW) is classified as greywater (Raschid-Sally and Jayakody, 2008), generally flowing into blackwater. Recovery and reuse of dishwasher wastewater is of particular relevance since it is composed essentially of tap water, detergents and food leftovers. The reuse of treated wastewater in plant irrigation must be controlled to minimize risks to agricultural products, soil, and groundwater from toxic and pathogenic microorganisms, which could pose health risks to consumers (Toze, 2006). The careful consideration of environmental and human health risks, such as pathogen exposure, is essential when using wastewater for irrigation (Zhang and Shen, 2019; Shakir et al., 2017; Brambilla et al., 2007). When properly treated and managed, wastewater irrigation can significantly promote the plant

* Corresponding author. Department of Biology, Tor Vergata University of Rome, 00133, Rome, Italy.

E-mail address: luciana.migliore@uniroma2.it (L. Migliore).

<https://doi.org/10.1016/j.plaphy.2024.108967>

Received 30 March 2024; Received in revised form 11 July 2024; Accepted 22 July 2024

Available online 23 July 2024

0981-9428/© 2024 The Authors. Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

growth, being a valuable resource in water-scarce areas (Libutti et al., 2018). As already shown in Congestri et al. (2020) and Alabiso et al. (2023), proper treatment can transform dishwasher wastewater into water of quite good quality, suitable for plant irrigation. Within the framework of The Jetsons' Kitchen project, which focuses on reusing and upcycling household greywaters, starting with dishwasher wastewater, the Zero Mile System was designed. This system, as described in Costa et al. (2021a) and Alabiso et al. (2023), is based on a biological filter made up of an *ad hoc* engineered microbial consortium (specifically, a photosynthetic cyanobacterium and three heterotrophic bacteria isolated from dishwasher wastewater). This consortium has been designed for the specific purpose (*ad hoc* in Latin) to process and mineralize food leftovers in the dishwasher wastewater. To this end, besides a photosynthetic, filamentous and nitrogen-fixing cyanobacterium, *Trichormus variabilis* (known to produce EPS and biofilm), three heterotrophic aerobic bacterial strains were chosen among the colonizer of dishwasher wastewater. This composition ensures the heterotrophic partner of the consortium to thrive in dishwasher wastewater consuming the food leftovers, benefiting of the oxygen produced by the cyanobacterium. In turn, heterotrophic bacteria directly supply *Trichormus variabilis* of the CO₂ necessary for its photosynthetic activity. This design gives the consortium intrinsic properties for the bioprocessing of dishwasher wastewater (Congestri et al., 2020; Alabiso et al., 2023). The first attempt to reuse untreated dishwasher wastewater (Costa et al., 2021b) has been followed by a couple of studies aimed to treat progressively larger amounts of dishwasher wastewater: the mineralizing process of food leftovers carried out by the consortium has been tested at a lab scale (50 and 500 mL) with different dishwasher wastewaters obtained using bio-detergents (Alabiso et al., 2023, 2024). The studies demonstrated the consortium's bioremediation capability at the lab scale: a significant reduction of nitrogen and phosphorus from food leftovers in dishwasher wastewater which are mineralized, thereby making the treated wastewater suitable for plant irrigation. Nitrogen is a main component of essential compounds such as amino acids, proteins, nucleic acids, and chlorophyll, which are vital for various plant metabolic processes. It directly influences the photosynthetic capacity of plants and significantly affects plant growth (Cercioglu et al., 2012; Tsiakaras et al., 2014) by promoting vegetative development, increasing leaf area, and enhancing the rate and duration of photosynthesis (Fathi, 2022). Similarly, phosphorus plays a critical role in various physiological and biochemical plant processes. In addition to being part of energy-rich compounds like ATP, phosphorus is also crucial component of nucleic acids and phospholipids. It is essential for the development of a strong root system, necessary for the efficient uptake of water and nutrients (Malhotra et al., 2018). In fact, the treated dishwasher wastewater from the 500 mL test has been successfully utilized for plant irrigation (Alabiso et al., 2023).

Under this conceptual framework, the scope of this study has been to continue the upscaling of the Zero Mile System toward the operational volume and to reuse the treated dishwasher wastewater for watering vegetables in a demonstrator built for this purpose (Zero Mile demonstrator) and in the field. In particular, the aims of this study were: i) to challenge the microbial consortium with a large scale dishwasher wastewater volume (4 L, in three replicates to treat 12 L, the total amount of wastewater discharged by the dishwasher), maintaining the dishwasher wastewater replenishment rate every 4 days; ii) to evaluate the efficiency of the treated dishwasher wastewater for watering vegetables in the Zero Mile demonstrator and in the field; and iii) to explore the safety of dishwasher wastewater reuse by evaluating the putative ecological functions of the microorganisms present in untreated/treated wastewater and in the microbial consortium to confirm its suitability.

2. Materials and methods

2.1. Cyanobacterium/heterotrophic bacteria consortium assemblage

The *ad hoc* engineered microbial consortium developed by Congestri et al. (2020), has been assembled according to Alabiso et al. (2023, 2024). The microbial consortium was made up as a one-to-three consortium forming by the heterocytous cyanobacterium *Trichormus variabilis* (Kützing ex Bornet & Flahault) Komárek & Anagnostidis (VRUC168) from the Tor Vergata University of Rome Collection (VRUC168; ATCC Strain designation: IUCC 1444; MSU A-37) and three bacterial strains isolated from dishwasher wastewater (*Acinetobacter* sp., *Aeromonas* sp. and *Exiguobacterium* sp.).

Each of the three heterotrophic bacteria were cultured as confluent growth to produce a uniform layer of growth over the surface of the plates. These microbes were collected each in 1L TSB (Tryptic Soy Broth) to obtain three separated pure cultures, incubated under magnetic stirring for 24 h at room temperature. Then, the optical density (OD) was measured at $\lambda = 600$ nm until the value of 0.15 was reached. A suspension of *T. variabilis* was also prepared and maintained in BG11₀ (Blue-Green Medium-nitrogen-depleted) until the OD at 665 nm reached 0.15. The microbial consortium was assembled by combining *T. variabilis* and the three heterotrophic bacteria, in order to get a 0.15 OD of each microbe in the final volume of dishwasher wastewater (pH around neutrality), in this case 12 L (see section 2.3); this allows to use the same bacterial density and ratio utilized in Alabiso et al. (2023).

2.2. Dishwasher wastewater collection

The wastewater was produced by a household dishwasher (Energy Class A+++, Electrolux EES69300L) setting the 'eco' program as washing cycle and using an EU Ecolabel certified dishwasher detergent (Everdrop tablets, Germany). The dishwasher wastewater was collected from April 2023 every four days, for 40 days. The characteristics of tap water and dishwasher wastewater, are reported in Supplementary Table 2.

2.3. Testing the consortium with periodic and complete DWW refill in scaled-up volume

The semi-batch test was planned to evaluate the capability of the consortium (i) to face and operate under periodic and almost complete wastewater refill, as expected for the Zero Mile system functioning, and (ii) to modify the concentration of total nitrogen and total phosphorus in the wastewater, by consuming the food leftover.

After assembling the consortium as reported in section 2.1, the test was performed in Erlenmeyer flasks 5.0 L (*Large size-Replenish test*, 4 L medium; *L-Rep test*). The consortium has grown in undiluted dishwasher wastewater and the experiment was carried out in controlled condition in a thermostatic chamber at 25 °C, under 130 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiance, with a 12:12 L:D cycle. The experiments were set up in 3 replicates of 4 L, to treat the total amount of dishwasher wastewater discharged per washing cycle.

Every 4 days, 3.0 L of *treated* DWW (*t-DWW*) were removed from each Erlenmeyer flask without damaging the consortium and replaced with the same amount of *untreated* DWW (*u-DWW*). The test lasted 40 days, for a total of 8 refills and the production of 24.0 L of *treated* wastewater.

The consortium growth was measured at the end of the experiments as microbial biomass and quantified as dry weight, measured after 48 h in oven at 37 °C.

2.4. Nitrogen and phosphorus concentration in consortium treated/untreated wastewaters

The capability of consortium to reduce the organic load was evaluated as total nitrogen and total phosphorus concentrations in the treated DWW, collected every four days. Analyses were performed in triplicate, according to the Italian official protocol (Belli et al., 2004), as reported in Congestri et al. (2020) and Alabiso et al. (2023). The data were calculated against a calibration curve for each nutrient: nitrogen calibration curve was built with a standard solution of NaNO_3 in distilled water at 0, 0.625, 1.25, 2.5, 5, 10 and 20 mg L^{-1} N, phosphorous calibration curve was built with a standard solution of KH_2PO_4 in distilled water at 0, 6.25, 12.5, 25.0, 50, 100 and 200 $\mu\text{g L}^{-1}$ P.

2.5. Growing plants with treated dishwasher wastewater

The *L-Rep* test has been planned to evaluate the efficiency of treated wastewater in fertilizing edible plants.

Two set of tests were performed: an indoor test, using the Zero Mile demonstrator and an open field test (environmental conditions in Supplementary Table 3). In both tests *Lactuca sativa* cv. Canasta (Asteraceae) was used, particularly seedlings were used in the indoor test and four-week-old plants in the open field test. The lettuce has been selected because it holds a high commercial and economic value, being the leafy vegetable most widely used in agricultural crops in southern Europe (Marzuoli et al., 2017).

Indoor test. Twenty-four seedlings (each of 3.13 ± 0.16 g fresh weight and 0.23 ± 0.02 g dry weight) were planted in the plant dedicated structure (8 pots, $60 \times 15 \times 10$ cm) of the Zero Mile demonstrator (Fig. 1, left), 3 per pot, in commercial soil (Dorater, Vigor Plant). Plants were divided in 2 experimental batches (4 pots each), watered every second day with 40 mL of consortium treated DWW (*t*-DWW) or tap water (TW, Rome water main). The Zero Mile demonstrator was placed at indoor room temperature (April–May) under L:D cycle 12:12 artificial light (irradiance: $130 \text{ mM photons m}^{-2} \text{ s}^{-1}$). After 42 days all plants were collected and, for each experimental batch, six plants were weighted (fresh and dry weight) to quantify the final plant biomass and morphometrically analysed by means ImageJ platform (version 1.52a), while six plants were used to quantify the plant descriptors: total phenols and flavonoids, soluble solid content.

Open field test. The field experiment was conducted in May–June 2023 at the Botanic Gardens of the Tor Vergata University of Rome in soil previously subjected to organic farming practices, such as hoeing

and manual weed removal (the soil properties are reported in Supplementary Table 4). The plants were arranged in a row (9.50 m length and 0.80 m width) divided into two groups, spaced 1.5 m apart, one for the *t*-DWW group and one for the control (Fig. 1, right). Each group comprised fifteen plants, spaced 25 cm apart, divided into 3 subgroups of five plants, each representing a replicate. The soil was covered with biodegradable mulching film to prevent water evaporation and the weed growth. The plants were manually irrigated every day with 200 mL of a 1:1 (v/v) dilution of consortium *t*-DWW (in 100 mL distilled water, dH_2O) or dH_2O for the control group. The choice of dH_2O was mandatory as in the Botanic Gardens only well water is available, very rich in nutrient, metals and microbes. To evaluate the growth difference between wastewater treated lettuces and control plants, dH_2O has been necessary to use. After 56 days the entire lettuce plants were collected and, for both treated and control batches, plants were weighted (fresh weight) and measured. To determine the total length, all plants were photographed using a Canon EOS 550D camera placed 70 cm away from the countertop and the images processed using the ImageJ v1.51 software (U.S. National Institutes of Health, Bethesda, MD, USA). Sub-samples of leaf tissue from 15 plants of both treated and control batches were immediately used for photosynthetic pigment determination and the rest of the samples were stored at -20°C for total phenol and flavonoid analysis.

2.6. Soluble solid content (SSC) determination

Soluble Solid Content determination was made according to Braglia et al. (2022): 1.5 g fresh weight (FW) of each plant were homogenized in liquid nitrogen with mortar and pestle, then centrifuged at 6.089g for 10'. Aliquots of 100 μL of the supernatants obtained were collected and analysed by a digital refractometer (Hanna HI96800, Woonsocket, RI, USA) to determine (quantify) sugars and other soluble solids in the extracts (Jaywant et al., 2022).

2.7. Photosynthetic pigments contents (concentrations)

Pigments, i.e., Chlorophyll *a* (Chl *a*), *b* (Chl *b*) and Carotenoids (Car), were determined in 500 mg of each plant samples (fresh weight, FW), after homogenization in liquid nitrogen with mortar and pestle and extraction in 80% acetone for 24 h, at 4°C under dark. After centrifuging (3000g; 10') the suspensions, extract (supernatants) absorbance was measured using a spectrophotometer (Hanna HI801, Woonsocket, RI, USA) at the wavelengths of 663 nm, 644 nm, and 452 nm, respectively.



Fig. 1. Seedling of *L. sativa* cv. Canasta: planted in the Zero Mile demonstrator (left) or planted in the open field (right).

Pigments concentration was calculated according to Lichtenthaler (1987) and expressed as $\mu\text{g g}^{-1}$ of fresh weight (FW).

2.8. Total phenol and flavonoid contents

Total phenol and flavonoid contents were determined in 500 mg (fresh weight) leaf material of each plant sample), homogenized in liquid nitrogen with mortar and pestle, extracted overnight in 4 ml of absolute methanol, under continuous mixing (orbital shaker, 110 rpm) and then centrifuged (5.000g; 20'). The supernatant was utilized for both total phenol and flavonoid quantification, as reported below.

Total phenols content was determined by the Folin Ciocalteu procedure (Di Marco et al., 2014). Aliquots of 200 μL of plant extract were mixed with 1000 μL of Folin-Ciocalteu reagent (1:10 v/v in distilled water) and 800 μL of 1 M Na_2CO_3 and then the mixture was incubated (60') at room temperature. The absorbance was measured spectrophotometrically at 765 nm and results compared to a reference curve of gallic acid (0–250 mg/L). Total phenol content was expressed as mg gallic acid equivalent (GAE) g^{-1} plant fresh weight.

Total flavonoid content was determined by the aluminium chloride colorimetric method (Chang et al., 2002). Aliquots of 200 μL of plant extract were mixed with 40 μL of 10% AlCl_3 , 40 μL of 1 M CH_3COOK , 600 μL of absolute methanol and 1.120 μL of dH_2O . The reaction mixture was maintained at room temperature for 30', then absorbance was measured spectrophotometrically at 415 nm and results compared to a reference curve of quercetin (0–250 $\mu\text{g mL}^{-1}$). Total flavonoid content was expressed as mg quercetin equivalent (QE) g^{-1} plant fresh weight.

2.9. Statistical analysis

Parametric *t*-test was used to assess significant differences between plant treatments, after confirming a normal distribution of the data using the Shapiro-Wilk normality test; *p*-value <0.05 were considered statistically significant. The software Past 4 (Øyvind Hammer, Oslo, Norway) was used for all statistical analyses.

2.10. Analysis of putative microbial functional groups by FAPROTAX analysis

To get insight on the ecological functions of microorganisms present in the 3D consortium aggregate and in the dishwasher wastewater globally utilized in all experiments, the Functional Annotation of Prokaryotic Taxa (FAPROTAX) tool have been used. The tool uses amplicon-based next-generation sequencing data, associating taxa with their documented functions in microbiology research, organizing them according to their functional roles and annotations.

The tool analyses the OTU (Operational Taxonomic Unit) table of each sample, which has been previously compiled using the Greengenes or Silva databases. A Python program is utilized to align the OTU's annotated information with the corresponding taxa data in the function database (Liang et al., 2020). This alignment produces the list of potential ecological roles played by each microorganism found in each different sample. FAPROTAX has been applied to the 3D consortium aggregate and both treated and untreated dishwasher wastewater microbial communities utilized in the studies of Congestri et al. (2020), Alabiso et al. (2023, 2024): unpublished data (Perini N., personal communication; Supplementary Table 1) for the Milan dishwasher wastewater utilized in Congestri et al. (2020), and data for the Rome dishwasher wastewater, already published in Alabiso et al. (2023, 2024) were collected and used to build the OTU table; the OTUs represented by less than 10 reads were excluded.

3. Results and discussion

The efficiency in wastewater reclamation and water reuse of the *ad hoc* engineered cyanobacterium/heterotrophic bacteria consortium has

been assessed using upscaled operational condition compared to those used in Alabiso et al. (2023), although using a similar approach.

In fact, the ability and efficiency of the consortium were challenged with three batches of 4 L wastewater and an almost complete refill every 4 days, a condition that increasingly approximate the Zero Mile system which implied the treatment of 12 L of DWW approximately every 2 days.

The efficiency of the treated dishwasher wastewater for watering and fertilizing vegetables has been tested in different conditions: both indoor (Zero Mile demonstrator) and outdoor (open field); and finally, the safety of treated dishwasher wastewater has been assessed.

3.1. Consortium functioning in the zero mile system condition

Consortium assemblage was performed as in Congestri et al. (2020) and Alabiso et al. (2023), but for the largest size (4 L medium); the cyanobacterium *T. variabilis* and the heterotrophic bacteria *Acinetobacter* sp., *Aeromonas* sp. and *Exiguobacterium* sp. were assembled in triplicates and grown in semi-batch conditions using a home household dishwasher wastewater as growth medium.

As already observed in experiment of different size (50 mL, Congestri et al., 2020; 50 and 500 mL, Alabiso et al., 2023; Alabiso et al., 2024), few hours after the assemblage, the consortium formed a three-dimensional structure (Fig. 2a), visible to the naked eye. Even at this increased volume the consortium showed to adapt its shape to the container shape and to retain this shape along the entire experiment.

After 40 days, consortium biomass increases significantly (mean values from 8.01 ± 0.62 to 32.05 ± 2.46 g; Student *t*-test; *p* < 0.001) maintaining its dark green colour and 3D-aggregation structure during the whole test (Fig. 2b).

The bioremediation efficiency of the consortium, as nutrient removal capability, was evaluated every 4 day at every wastewater refill. Total nitrogen and total phosphorous were quantified in the consortium exposed wastewater, measuring the total amount of nutrients, including both the solubilised and those still embedded into the solid fraction of the wastewater. The amount of nutrient in each wastewater is highly variable, depending on the daily load of leftovers on dishes. For nitrogen, it varied from 14.69 mg/L (day 4) to 43.42 mg/L (day 30), and after four days it was reduced to 13.2 mg/L and 10.33 mg/L, respectively. For phosphorous, it varied from 1.12 mg/L (day 8) to 3.02 mg/L (day 30), and after four days it was reduced to 0.8 mg/L and 1.34 mg/L, respectively. This reduction depends on the metabolic activities of the consortium which grows 4-fold in 40 days (Fig. 2b), by utilizing the mineralized nutrients. Fig. 3 shows that the consortium efficiency to remove nutrients increased with time, reaching respectively a maximum of 70% for nitrogen and 50% for phosphorous. Hence, a reasonable amount of nutrients remains in the treated dishwasher wastewater useful for plant fertilization.

The process of mineralization of the two nutrients is quite low at the very beginning: at the first refill (day 4th), no phosphorus removal while nitrogen removal was observed, then they both increased with time, the removal of nitrogen being always higher than phosphorous. This initial difference in nitrogen or phosphorous mineralization can be explained with the growth conditions of the *T. variabilis* inoculum before the consortium assemblage: during the preparation of the *T. variabilis* inoculum BG11₀, a culture medium not containing nitrogen was used; the culture medium is conversely rich in phosphorous (K_2HPO_4). Therefore, at the consortium assemblage, *T. variabilis* has reserves of phosphorus, since it is well known that cyanobacteria accumulate phosphorus in their biomass (Gismondi et al., 2016; Guzzon et al., 2008; Voronkov and Sinetova, 2019; Wang et al., 2018). The initial low amount of nutrient removal can be both related to the (small) dimension of the consortium and to the time taken by the just assembled heterotrophic bacteria in attacking and degrading the food leftovers. As regard nitrogen, the presence of quite a high amount of N in the DWW did not activate the resource-consuming N-fixation metabolic pathway (Zhang



Fig. 2. The 3D microbial consortium aggregates in treated domestic wastewater (DWW), (a) after few hours and (b) after 40 days from the assemblage.

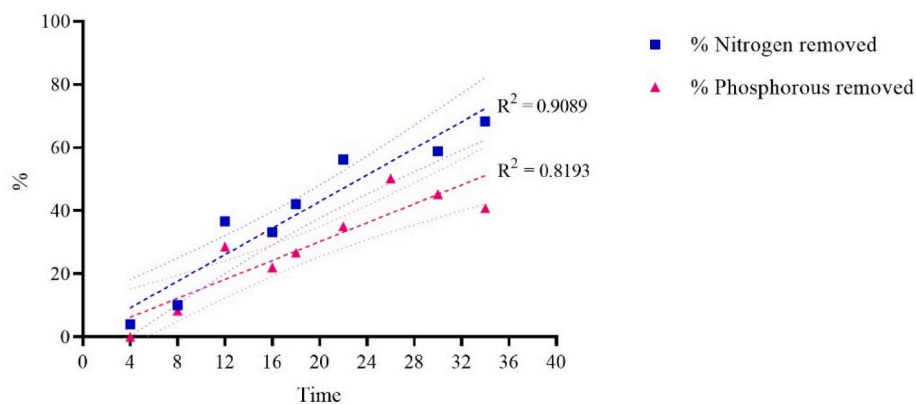


Fig. 3. Microbial consortium efficiency in removing Nitrogen and Phosphorus from DWW over time. The R-squared values indicate a strong correlation between time and the removal percentages for both elements. The light dashed lines represent the confidence intervals (95%) for the regression models.

et al., 2023). As regard phosphorous, several extra- and intra-cellular processes of P removal from the environment are known in multispecies consortia (Di Pippo et al., 2012; Xu et al., 2020), anyway the interactions among these consortium partners still remain unknown.

These results are particularly important because, notwithstanding the different experimental set up (4 L DWW instead of 50 or 500 mL and an almost complete refill instead of 1/4 DWW refill), the capability of the consortium to attach and degrade the organic matter present in the DWW is confirmed and data are consistent with those already reported (Alabiso et al., 2023).

Another result of this experimental setting is the additional insight into the design of the biofilter container, which integrate the preliminary indications reported in Alabiso et al. (2024). Indeed, the necessity to treat 12 L of DWW and simultaneously ensure the best consortium growth conditions (e.g., light, pH, nutrient supply; Alabiso et al., 2024) can steer the biofilter container design towards the following characteristics: capacity of 12 L, rectangular shape, transparent material, which can be divided into three independent sections (each of 4 L capacity), simultaneously operating to obtaining a high speed of refill, a reduced mixing rate avoiding consortium damages and, if physically separated, even a better illumination (Fig. 4). This triple structure may allow even an efficient DWW bioremediation if compared to a single structure as it enhances the consortium surface/DWW volume ratio.

3.2. Plant growing in the zero mile demonstrator or in open-field

The bioremediation efficiency of the consortium supported the use of treated DWW in plant fertilization. To assess the effect of *t*-DWW

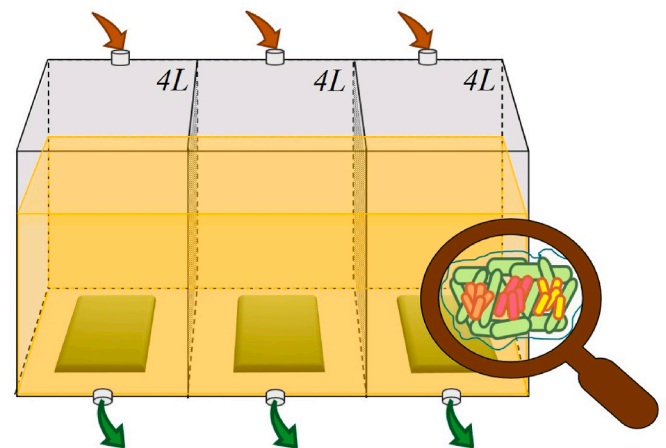


Fig. 4. Biofilter container inspired to the experimental settings. Brown arrows = input of untreated DWW; green arrows = output of treated DWW; in the lens, the inferred organization from the experimental data of the 3D microbial consortium aggregates.

watering on the growth of *L. sativa* cv. Canasta plants, morphological and biometric parameters, such as fresh weight and total length, were measured in plants both cultivated indoor in the Zero Mile demonstrator and outdoor, in open field.

As shown in Table 1, the plants grown both indoor and outdoor,

Table 1

Fresh weight of *L. sativa* cv. Canasta plants grown indoor or outdoor, watered with consortium treated dishwasher wastewater (*t*-DWW) or water (TW = tap water; dH₂O = distillate water).

Parameters	Indoor (Zero Mile demonstrator)		Outdoor (Open field)	
	Treatment (42 days)		Treatment (56 days)	
	<i>t</i> -DWW	Tap Water	<i>t</i> -DWW	dH ₂ O
Total Fresh Weight (g)	13.51 ± 1.19**	8.12 ± 0.55	631.60 ± 88.23**	471.22 ± 89.57
Total Length (cm)	22.53 ± 3.02	19.02 ± 1.24	54.17 ± 5.66*	46.56 ± 5.14

Data represents mean ± SD (n = 3); asterisks indicate significant differences between treatments (* = p < 0.05; ** = p < 0.01).

watered with *t*-DWW showed a significant higher fresh weight than control plants, with a 66.38% and 34.03% increase, respectively. Also, the total length showed an increase of 18.45% in the indoor plants and 16.34% in the outdoor plants, although the indoor growth was clearly lower than outdoor.

These data are in agreement with previous studies reporting an increase in lettuce fresh weight when irrigated with treated domestic wastewater (Urbano et al., 2017) or with our findings where the DWW was treated with the same engineered microbial consortium as in this study (Alabiso et al., 2023), which made nutrients more readily available for plants due to the metabolic activity of heterotrophic bacteria.

The high quality and the good physiological status of the treated plants was further confirmed by other parameters used to assess the effect of *t*-DWW fertigation, such as photosynthetic pigments, soluble solids (SSC), total phenols and total flavonoids content (Table 2).

The content of pigments (chlorophylls and carotenoids) in lettuce leaves treated with *t*-DWW showed no significant differences compared to plants watered with tap or distilled water, under both indoor and outdoor conditions. The analysis of photosynthetic pigments content is commonly used as indicator of plant physiological stress, by evaluating their variation in plant tissues (Peñuelas and Filella, 1998; Yang et al., 2010). Their content in plants under fertigation, from an agronomic perspective, is crucial to evaluate the productivity and the overall health conditions of plants (Shah et al., 2017). The absence of significant variations in photosynthetic pigments between *t*-DWW watered plants and control plants, indicates the healthy state of the lettuce plants, highlighting that fertigation did not cause negative effect on plants.

Also, the analysis of soluble solid content (SSC, mainly sugars) showed comparable results in all plants (indoors and outdoors grown, *t*-DWW or water irrigated). This quality index allows to evaluate the quality of fruits and vegetables (Wu et al., 2021) and the data indicated a good and comparable quality in treated and control plants. Moreover, the SSC concentration in our plants was higher than those observed in thirteen different cultivars of *L. sativa* grown in a hydroponic system (Hernandez et al., 2020) or grown in controlled environment (Sabatino et al., 2021; Consentino et al., 2022), suggesting that in lettuce this index may be affected by both cultivar and growing conditions.

Table 2

Pigments (chlorophyll *a* and *b*, Chl *a*, Chl *b*; carotenoids, CAR) and quality indices (soluble solids, SSC; total phenols, PHE; total flavonoids, FLA) in *L. sativa* cv. Canasta plants grown indoor or outdoor, watered with consortium treated dishwasher wastewater (*t*-DWW) or water (TW = tap water; dH₂O = distillate water).

Parameters	Indoor (Zero Mile demonstrator)		Outdoor (Open field)	
	Treatment		Treatment	
	<i>t</i> -DWW	TW	<i>t</i> -DWW	dH ₂ O
Chl <i>a</i> (µg g ⁻¹ FW)	120.25 ± 3.93	118.31 ± 5.21	58.22 ± 0.65	59.74 ± 0.87
Chl <i>b</i> (µg g ⁻¹ FW)	126.81 ± 2.11	123.08 ± 2.36	89.97 ± 6.35	76.58 ± 6.16
Chl <i>a</i> + <i>b</i> (µg g ⁻¹ FW)	247.06 ± 3.21	241.39 ± 4.35	148.20 ± 5.74	136.32 ± 5.37
CAR (µg g ⁻¹ FW)	67.93 ± 0.61	67.73 ± 1.21	32.13 ± 0.13	32.00 ± 0.12
SSC (%)	10.97 ± 0.15	10.80 ± 0.2	5.75 ± 0.48	5.96 ± 0.83
PHE (mg GA Eq g ⁻¹ FW)	134 ± 5.71	152.97 ± 11.21	44.98 ± 14.99	278.05 ± 14.04**
FLA (mg Q Eq g ⁻¹ FW)	11.65 ± 0.95	14.32 ± 1.82	43.96 ± 22.82	251.08 ± 20.75**

FW = Fresh weight; GA Eq = Gallic acid equivalent; Q Eq = Quercetin equivalent.

Data represents mean ± SD (n = 3); asterisks indicate significant differences between treatments (* = p < 0.05; ** = p < 0.01).

Conversely, total phenol and flavonoids concentration in plant grown outdoor, showed a significant difference between *t*-DWW and dH₂O watered plants, dH₂O showing a significantly higher concentration of total phenols and total flavonoids than *t*-DWW (83.82% and 82.49%, respectively; p < 0.01). These compounds are produced to fulfill functions related to plant growth, development, and protection (Pratyusha, 2022) and exhibit a readily detectable response to nutrient deficiency (Naikoo et al., 2019). Hence, the high phenolic compound concentration observed in the field in dH₂O treated plants, may be associated to the low availability of nutrients in plants irrigated with distilled water. These data agree with previous studies, showing increased production of phenolic compounds as a response to nutrient deficiency, in lettuce (Galieni et al., 2015; Sofu et al., 2016). Furthermore, some authors reported a reduction of phenol content in *L. sativa* (Qadir et al., 2017) and other vegetables following the application of nitrogenous fertilizers and different agricultural management (Ahmed et al., 2002; Heimler et al., 2017). Flavonoids, being phenolic compounds, follow the same trend as phenols. Their reduction can be explained by the correlation between flavonoid biosynthesis and nitrogen metabolism, as the synthesis of these compounds is influenced by the nitrogen content of the plants (Deng et al., 2019) and the flavonoid concentration in crop is inversely related to nitrogen fertilization (Nguyen and Niemeier, 2008; Becker et al., 2015; Groenbaek et al., 2016).

3.3. Functional prediction of microbial communities

The results of this study demonstrated a good growth of the plants watered with treated dishwasher wastewater, as already found in another previous study (Alabiso et al., 2023). Hence, a consequent important point is the evaluation of the safety of untreated and treated wastewater, that become a pressing information.

To achieve this goal, the NGS DNA metabarcoding of the microbial communities present in the 3D consortium aggregate (3D-AGG), in the dishwasher wastewater utilized in all experiments (dishwasher wastewater from Milan and Rome experiments, M-DWW and R-DWW, respectively) and in the treated wastewater (TR-DWW), were utilized to

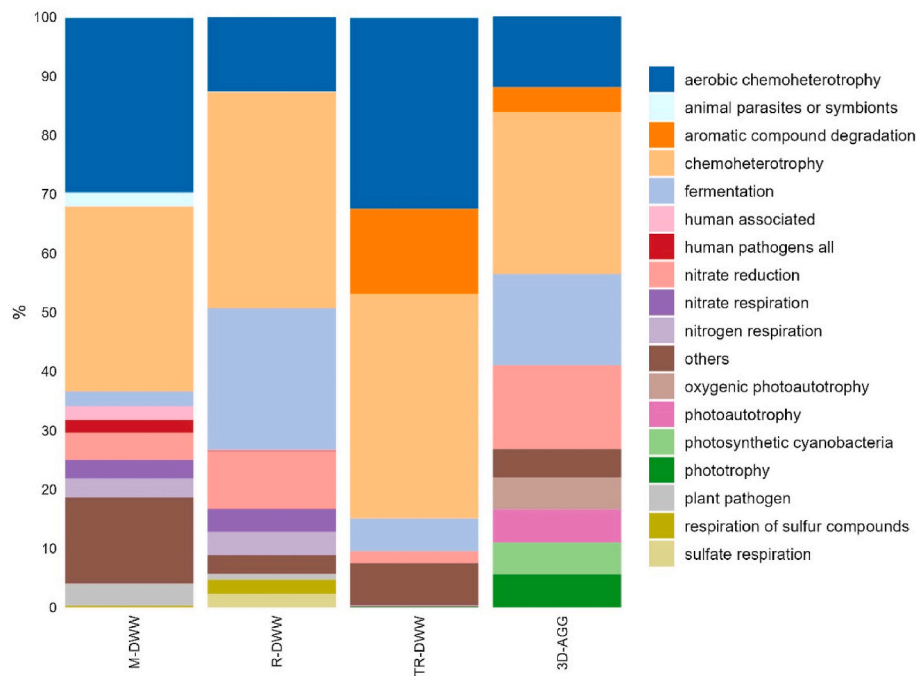


Fig. 5. Relative abundance of putative functions, in dishwasher wastewater collected in Milan or Rome (M-DWW, R-DWW), in the consortium treated dishwasher wastewater (TR-DWW) and in the 3D consortium aggregate (3D-AGG).

explore the putative microbial functions associated with the identified microorganisms. Functional prediction analysis was performed using the FAPROTAX software, a database that maps the metabolic or ecologically relevant functions (e.g., nitrogen fixation, sulfate respiration, hydrocarbon degradation) of bacterial or archaeal taxa, based on literature data about cultivated representatives. The relative abundance of functional groups per sample was calculated as the cumulative abundance of OTUs assigned to each functional group (Fig. 5).

Based on the classification results of 16S rDNA sequences, a total of 67 functional groups were identified, among these, 18 showed a relative abundance greater than 2% in at least one sample (see Fig. 5).

As expected, the predominant functional groups in all samples were chemoheterotroph/aerobic chemoheterotroph. Their abundance indicated that a large number of microbes obtain carbon and energy from the oxidation of the organic compounds (food leftovers) present in the wastewater. Concurrently, several microbes are involved in the nitrogen cycle, although they are not uniformly distributed among samples. This indicates that some microorganisms are actively involved in nitrogen transformation, a typical function in biological treatments. In these processes, organic nitrogen compounds are actively converted into inorganic nitrogen forms, including those essential for growth, such as ammonium and nitrate (Kuypers et al., 2018).

As awaited, among the functions identified there were some related to humans and stated as 'human associated' or 'human pathogen all' (Table 3). However, looking further into the analysis, only four bacterial genera were found (*Citrobacter* sp., 51 reads; *Pseudomonas* sp., 1026

reads; *Roseomonas* sp., 16 reads; *Stenotrophomonas* sp., 132 reads) differently distributed in the samples and with a general predominance of *Pseudomonas* (80.3%) and *Stenotrophomonas* (14.5%). Three of the human related genera were only found in a single sample (M-DWW), they were represented by few reads per sample that, as a total, accounted for 2.3% and 2.2% of the total, respectively for 'human associated' and 'human pathogens'. The identified genera are known colonizers of kitchens, as they have already been found in dishwasher rubber seals (Zupancić et al., 2019; Raghupathi et al., 2018) or on kitchen surfaces (Jeon et al., 2013; Flores et al., 2013; Kembel et al., 2012). Therefore, their presence in DWW is not surprising and depends on different sources, including raw foods and dishwasher users.

As a final remark, the recovered purified wastewater even complies with the limits set by the Italian Ministerial Decree 185/2003 for the microbiological parameters (*Escherichia coli*, 100 CFU/100 mL and *Salmonella* absent), as no *E. coli* were found in the dishwasher wastewaters. It is worth to note that reads may depend on the presence of just DNA, while CFUs (Colony Forming Units) imply the presence of living microorganism. In any case, wastewater can be utilized for growing lettuce plants.

To the best of our knowledge, this was the first study aimed to investigate the functional prediction analysis for bacterial communities in dishwasher wastewaters to evaluate their possible use for the irrigation of edible plants. Some studies offer insights into microbial communities in various types of wastewaters and their potential functions (Xie et al., 2021; Tong et al., 2023), while others focused on the changes

Table 3

DNA metabarcoding identified reads including the two potentially harmful functions, as identified by the FAPROTAX software (several reads are found in both groups).

Functions	'human associated'		'human pathogens'		Genus-level taxonomic identification of the reads	
	Sample	Total reads per sample	%	Total reads per sample		%
	M-DWW	1209	2.3	1158	2.2	<i>Citrobacter</i> *; <i>Pseudomonas</i> ; <i>Stenotrophomonas</i>
	R-DWW	10	0.1	10	0.1	<i>Stenotrophomonas</i>
	R-TDWW	48	0.2	48	0.0	<i>Roseomonas</i> ; <i>Stenotrophomonas</i>
	3D-AGG	11	0.2	11	0.0	<i>Roseomonas</i>

of the microbial community structure and function in wastewater-irrigated soils (Chen et al., 2017), as well as on the importance of monitoring wastewater quality for plant development, soil characteristics and human health (Ibekwe et al., 2018).

4. Conclusion

The capability of the microbial consortium to treat a dishwasher operating volume (12 L, divided into three batches), producing treated wastewater every four days, has been demonstrated. The consortium maintains, also in these operational conditions, the capability to mineralize the organic load of the wastewaters (due to food leftovers) and the mineralization increased with time, related to the increase of consortium biomass.

The treated dishwasher wastewater demonstrated to be useful for plant watering, both indoor, in the Zero Mile System demonstrator, and outdoor, in open field. While watered with treated wastewater dishwasher, lettuce plants demonstrated good growth and quality (as pigments, soluble solids, phenols, and flavonoid content), comparable or better than control plants.

A final consideration has been devoted to state the salubrity of the dishwasher wastewater: the microbial load in both untreated and treated wastewater is mainly made by organic matter degraders and bacteria involved in nitrogen cycling. The human related genera, widespread in the kitchen environment, are quite few in the dishwasher wastewater, probably counter selected by the limiting dishwasher conditions. Furthermore, no pathogens or potential microbiological contaminants of wastewater (as *E. coli*) were found. Hence, the on-site utilization of this treated dishwasher wastewater does not imply risks to agricultural products, soil, and groundwater.

The use of the synergistic interactions between photosynthetic and heterotrophic microorganisms represents an effective and convenient solution to reclaim a still untapped water resource in a regenerative circular economy framework.

Funding

Funding for Annamaria Alabiso and Sara Frasca is provided through three-year fellowships from Tor Vergata Rome University's Ph.D. School of Evolutionary Biology and Ecology, covering the XXXVI and XXXVII cycles, respectively. Alabiso's industrial doctorate is in partnership with AlgaRes s.r.l., and Frasca's PON doctorate is in collaboration with GT50 s.r.l. The IS CRA CINECA Project Class C, 'The JETSONS' Kitchen,' on the Galileo100 server, awarded to A.A. (project HP10CF8YO), supports their data analysis efforts.

CRedit authorship contribution statement

Annamaria Alabiso: Conceptualization, Investigation, Data analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. Sara Frasca: Data analysis. Valerio Cantelmo: Investigation, Data analysis. Roberto Braglia: Data analysis, Data curation, Writing – review & editing. Francesco Scuderi: Data analysis, Data curation, Writing – review & editing. Fiammetta Costa: Conceptualization, Writing – review & editing. Roberta Congestri: Conceptualization, Writing – review & editing. Luciana Migliore: Conceptualization, 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.

Data availability

Data will be made available on request.

Acknowledgments

The authors acknowledge the CINECA award under the IS CRA initiative, for the availability of high-performance computing resources and support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2024.108967>.

References

- Ahmed, A.H.H., Khalil, M.K., Farrag, A.M., 2002. Nitrate accumulation, growth, yield and chemical composition of Rocket (*Eruca vesicaria* subsp. sativa) plant as affected by NPK fertilization, kinetin and salicylic acid. *Ann. Agric. Sci.* 47, 495–508.
- Alabiso, A., Frasca, S., Cantelmo, V., D'Andrea, M.M., Braglia, R., Scuderi, F., Costa, F., Savio, S., Congestri, R., Migliore, L., 2023. A dirty job: dishwasher wastewater reuse and upcycle through an ad hoc engineered microbial consortium. *npj Clean Water* 6 (1), 66. <https://doi.org/10.1038/s41545-023-00280-8>.
- Alabiso, A., Frasca, S., Bartolini, M., Congestri, R., D'Andrea, M.M., Buratti, G., Costa, F., Meraviglia, M., Nebuloni, A., Migliore, L., 2024. Home sweet home: setting the best thriving conditions for the ad hoc engineered microbial consortium in the Zero Mile system. *Sustainability* 16 (6), 2227. <https://doi.org/10.3390/su16062227>.
- APAT IRSA-CNR Azoto totale e fosforo totale. In: Belli, M., et al. (Eds.), 2004. *Metodi analitici per le acque*. I.G.E.R. srl, pp. 537–540, 29/2003.
- Becker, C., Urlić, B., Jukić Špika, M., Kläring, H.P., Krumbein, A., Baldermann, S., Ban, S. G., Perica, S., Schwarz, D., 2015. Nitrogen limited red and green leaf lettuce accumulate flavonoid glycosides, caffeic acid derivatives, and sucrose while losing chlorophylls, β -carotene and xanthophylls. *PLoS One* 10 (11), e0142867. <https://doi.org/10.1371/journal.pone.0142867>.
- Bixio, D., De Heyder, B., Cikurel, H., Muston, M., Miska, V., Joksimovic, D., Schäfer, A.I., Ravazzini, A., Aharoni, A., Savic, D., Thoeue, C., 2005. Municipal wastewater reclamation: where do we stand? An overview of treatment technology and management practice. *Water Science and Technology. Water Supply* 5 (1), 77–85. <https://doi.org/10.2166/ws.2005.0010>.
- Braglia, R., Costa, P., Di Marco, G., D'Agostino, A., Redi, E.L., Scuderi, F., Gismondi, A., Canini, A., 2022. Phytochemicals and quality level of food plants grown in an aquaponics system. *J. Sci. Food Agric.* 102 (2), 844–850. <https://doi.org/10.1002/jsfa.11420>.
- Brambilla, G., Patrizzi, M., De Filippis, S.P., Bonazzi, G., Mantovi, P., Barchi, D., Migliore, L., 2007. Oxytetracycline as environmental contaminant in arable lands. *Anal. Chim. Acta* 586 (1–2), 326–329. <https://doi.org/10.1016/j.aca.2006.11.019>.
- Cercioglu, M., Okur, B., Delibacak, S., Ongun, A.R., 2012. Effects of tobacco waste and farmyard manure on soil properties and yield of lettuce (*Lactuca sativa* L. var. capitata). *Commun. Soil Sci. Plant Anal.* 43, 875–886.
- Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* 10 (3) <https://doi.org/10.38212/2224-6614.2748>.
- Chen, L., Feng, Q., Li, C., Wei, Y., Zhao, Y., Feng, Y., Zheng, H., Li, F., Li, H., 2017. Impacts of aquaculture wastewater irrigation on soil microbial functional diversity and community structure in arid regions. *Sci. Rep.* 7 (1), 11193 <https://doi.org/10.1038/s41598-017-11678-z>.
- Congestri, R., Savio, S., Farrotti, S., Amati, A., Krasojevic, K., Perini, N., Costa, F., Migliore, L., 2020. Developing a microbial consortium for removing nutrients in dishwasher wastewater: towards a biofilter for its up-cycling. *Water Sci. Technol.* 82 (6), 1142–1154. <https://doi.org/10.2166/wst.2020.325>.
- Consentino, B.B., Aprile, S., Roupael, Y., Ntatsi, G., De Pasquale, C., Iapichino, G., Alibrandi, P., Sabatino, L., 2022. Application of PGPB combined with variable n doses affects growth, yield-related traits, n-fertilizer efficiency and nutritional status of lettuce grown under controlled condition. *Agronomy* 12 (2), 236. <https://doi.org/10.3390/agronomy12020236>.
- Costa, F., Aureggi, M., Migliore, L., Perego, P., Pillan, M., Standoli, C.E., Vignati, G., 2021a. D Design for sustainability and ICT: a household prototype for wastewater recycling. In: Costa, F., Nebuloni, A. (Eds.), *The Jetsons' Kitchen - A Zero-Mile System for Waste Water Recycling and Cultivation*. FrancoAngeli, Milan, pp. 55–62. <https://series.francoangeli.it/index.php/oa/catalog/book/629>.
- Costa, F., Amati, A., Antonelli, M., Cocetta, G., Di Mauro, M., Ferrante, A., Krasojevic, K., Mangiarotti, R., Meraviglia, M., Nebuloni, A., Perego, P., Sironi, R., Spanu, F., Standoli, C.E., Vignati, G., Volontepi, P., Ziyaae, M., Migliore, L., 2021b. Designing the future: an intelligent system for zero-mile food production by upcycling wastewater. In: Costa, F., Nebuloni, A. (Eds.), *The Jetsons' Kitchen - A Zero-Mile System for Waste Water Recycling and Cultivation*. FrancoAngeli, Milan, pp. 11–17. <https://series.francoangeli.it/index.php/oa/catalog/book/629>.

- Deng, B., Li, Y., Xu, D., Ye, Q., Liu, G., 2019. Nitrogen availability alters flavonoid accumulation in *Cyclocarya paliurus* via the effects on the internal carbon/nitrogen balance. *Sci. Rep.* 9 (1), 2370. <https://doi.org/10.1038/s41598-019-38837-8>.
- Di Marco, G., Gismondi, A., Canuti, L., Scimeca, M., Volpe, A., Canini, A., 2014. Tetracycline accumulates in *Iberis sempervirens* L. through apoplastic transport inducing oxidative stress and growth inhibition. *Plant Biol.* 16 (4), 792–800. <https://doi.org/10.1111/plb.12102>.
- Di Pippo, F., Ellwood, N.T.W., Guzzon, A., Siliato, L., Micheletti, E., De Philippis, R., Albertano, P.B., 2012. Effect of light and temperature on biomass, photosynthesis and capsular polysaccharides in cultured phototrophic biofilms. *J. Appl. Phycol.* 24, 211–220. <https://doi.org/10.1007/s10811-011-9669-0>.
- Fathi, A., 2022. Role of nitrogen (N) in plant growth, photosynthesis pigments, and N use efficiency: a review. *Agri* 28, 1–8. <https://doi.org/10.5281/zenodo.7143588>.
- Flores, G.E., Bates, S.T., Caporaso, J.G., Lauber, C.L., Leff, J.W., Knight, R., Fierer, N., 2013. Diversity, distribution and sources of bacteria in residential kitchens. *Environ. Microbiol.* 15 (2), 588–596. <https://doi.org/10.1111/1462-2920.12036>.
- Galièni, A., Di Mattia, C., De Gregorio, M., Specca, S., Mastrocola, D., Pisante, M., Stagnari, F., 2015. Effects of nutrient deficiency and abiotic environmental stresses on yield, phenolic compounds and antiradical activity in lettuce (*Lactuca sativa* L.). *Sci. Hortic.* 187, 93–101. <https://doi.org/10.1016/j.scienta.2015.02.036>.
- Gismondi, A., Pippo, F.D., Bruno, L., Antonaroli, S., Congestri, R., 2016. Phosphorus removal coupled to bioenergy production by three cyanobacterial isolates in a biofilm dynamic growth system. *Int. J. Phytoremediation* 18, 869–876. <https://doi.org/10.1080/15226514.2016.1156640>.
- Groenbaek, M., Jensen, S., Neugart, S., Schreiner, M., Kidmose, U., Kristensen, H.L., 2016. Nitrogen split dose fertilization, plant age and frost effects on phytochemical content and sensory properties of curly kale (*Brassica oleracea* L. var. sabotica). *Food Chem.* 197, 530–538. <https://doi.org/10.1016/j.foodchem.2015.10.108>.
- Guzzon, A., Bohn, A., Diociaiuti, M., Albertano, P., 2008. Cultured phototrophic biofilms for phosphorus removal in wastewater treatment. *Water Res.* 42, 4357–4367. <https://doi.org/10.1016/j.watres.2008.07.029>.
- Heimler, D., Romani, A., Ieri, F., 2017. Plant polyphenol content, soil fertilization and agricultural management: a review. *Eur. Food Res. Technol.* 243, 1107–1115. <https://doi.org/10.1007/s00217-016-2826-6>.
- Hernandez, E., Timmons, M.B., Mattson, N.S., 2020. Quality, yield, and biomass efficacy of several hydroponic lettuce (*Lactuca sativa* L.) cultivars in response to high pressure sodium lights or light emitting diodes for greenhouse supplemental lighting. *Horticulturae* 6 (1), 7. <https://doi.org/10.3390/horticulturae6010007>.
- Ibekwe, A.M., Gonzalez-Rubio, A., Suarez, D.L., 2018. Impact of treated wastewater for irrigation on soil microbial communities. *Sci. Total Environ.* 622, 1603–1610. <https://doi.org/10.1016/j.scitotenv.2017.10.039>.
- Jaywant, S.A., Singh, H., Arif, K.M., 2022. Sensors and instruments for brix measurement: a review. *Sensors* 22 (6), 2290. <https://doi.org/10.3390/s22062290>.
- Jeon, Y.S., Chun, J., Kim, B.S., 2013. Identification of household bacterial community and analysis of species shared with human microbiome. *Curr. Microbiol.* 67, 557–563. <https://doi.org/10.1007/s00284-013-0401-y>.
- Kembel, S.W., Jones, E., Kline, J., Northcutt, D., Stenson, J., Womack, A.M., Bohannan, B.J.M., Brown, G.Z., Green, J.L., 2012. Architectural design influences the diversity and structure of the built environment microbiome. *ISME J.* 6 (8), 1469–1479. <https://doi.org/10.1038/ismej.2011.211>.
- Kuypers, M., Marchant, H., Kartal, B., 2018. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* 16, 263–276. <https://doi.org/10.1038/nrmicro.2018.9>.
- Liang, S., Deng, J., Jiang, Y., Wu, S., Zhou, Y., Zhu, W., 2020. Functional distribution of bacterial community under different land use patterns based on FaProTax function prediction. *Pol. J. Environ. Stud.* 29 (2) <https://doi.org/10.15244/pjoes/108510>.
- Libutti, A., Gatta, G., Gagliardi, A., Vergine, P., Pollice, A., Beneduce, L., Disciglio, G., Tarantino, E., 2018. Agro-industrial wastewater reuse for irrigation of a vegetable crop succession under Mediterranean conditions. *Agric. Water Manag.* 196, 1–14. <https://doi.org/10.1016/j.agwat.2017.10.015>.
- Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: *Methods in Enzymology*, vol. 148. Academic Press, pp. 350–382.
- Liu, J., Wu, Y., Wu, C., Muylaert, K., Vyverman, W., Yu, H.Q., Muñoz, R., Rittmann, B., 2017. Advanced nutrient removal from surface water by a consortium of attached microalgae and bacteria: a review. *Bioresour. Technol.* 241, 1127–1137. <https://doi.org/10.1016/j.biortech.2017.06.054>.
- Malhotra, H., Vandana, Sharma, S., Pandey, R., 2018. Phosphorus nutrition: plant growth in response to deficiency and excess. In: Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., Hawrylak-Nowak, B. (Eds.), *Plant Nutrients and Abiotic Stress Tolerance*. Springer, Singapore, pp. 171–190. https://doi.org/10.1007/978-981-10-9044-8_7.
- Marzuoli, R., Finco, A., Chiesa, M., Gerosa, G., 2017. A dose-response relationship for marketable yield reduction of two lettuce (*Lactuca sativa* L.) cultivars exposed to tropospheric ozone in Southern Europe. *Environ. Sci. Pollut. Res.* 24, 26249–26258. <https://doi.org/10.1007/s11356-016-8224-6>.
- Muñoz, R., Guieysse, B., 2006. Algal-bacterial processes for the treatment of hazardous contaminants: a review. *Water Res.* 40 (15), 2799–2815. <https://doi.org/10.1016/j.watres.2006.06.011>.
- Naikoo, M.I., Dar, M.I., Raghob, F., Jaleel, H., Ahmad, B., Raina, A., Khan, F.A., Naushin, F., 2019. Role and regulation of plants phenolics in abiotic stress tolerance: an overview. In: *Plant Signaling Molecules*. Elsevier, Amsterdam, pp. 157–168. <https://doi.org/10.1016/B978-0-12-816451-8.00009-5>.
- Nguyen, P.M., Niemeyer, E.D., 2008. Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). *J. Agric. Food Chem.* 56 (18), 8685–8691. <https://doi.org/10.1021/jf801485u>.
- Peñuelas, J., Filella, I., 1998. Visible and near-infrared reflectance techniques for diagnosing plant physiological status. *Trends Plant Sci.* 3, 151–156. [https://doi.org/10.1016/S1360-1385\(98\)01213-8](https://doi.org/10.1016/S1360-1385(98)01213-8).
- Posadas, E., Alcántara, C., García-Encina, P.A., Gouveia, L., Guieysse, B., Norvill, Z., Ación, F.G., Markou, G., Congestri, R., Koreiviene, I., Muñoz, R., 2017. Microalgae cultivation in wastewater. In: *Microalgae-based Biofuels and Bioproducts*. Woodhead Publishing, pp. 67–91. <https://doi.org/10.1016/B978-0-08-101023-5.00003-0>.
- Pratyusha, S., 2022. Phenolic compounds in the plant development and defense: an overview. In: Hasanuzzaman, M., Nahar, K. (Eds.), *Plant Stress Physiology: Perspectives in Agriculture*. IntechOpen, London.
- Qadir, O., Siervo, M., Seal, C.J., Brandt, K., 2017. Manipulation of contents of nitrate, phenolic acids, chlorophylls, and carotenoids in lettuce (*Lactuca sativa* L.) via contrasting responses to nitrogen fertilizer when grown in a controlled environment. *J. Agric. Food Chem.* 65 (46), 10003–10010. <https://doi.org/10.1021/acs.jafc.7b03675>.
- Raghupathi, P.K., Zupanić, J., Brejnrod, A.D., Jacquiod, S., Houf, K., Burmølle, M., Gunde-Cimerman, N., Sørensen, S.J., 2018. Microbial diversity and putative opportunistic pathogens in dishwasher biofilm communities. *Appl. Environ. Microbiol.* 84 (5), e02755 <https://doi.org/10.1128/AEM.02755-17>.
- Raschid-Sally, L., Jayakody, P., 2008. Drivers and Characteristics of Wastewater Agriculture in Developing Countries: Results from a Global Assessment. International Water Management Institute. Research Report 127. https://www.iwmi.cgiar.org/Publications/IWMI_Research_Reports/PDF/PUB127/RR127.pdf.
- Sabatino, L., Consentino, B.B., Roupael, Y., De Pasquale, C., Iapichino, G., D'Anna, F., La Bella, S., 2021. Protein hydrolysates and mo-biofortification interactively modulate plant performance and quality of 'canasta' lettuce grown in a protected environment. *Agronomy* 11 (6), 1023. <https://doi.org/10.3390/agronomy11061023>.
- Shah, S.H., Houborg, R., McCabe, M.F., 2017. Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agronomy* 7 (3), 61. <https://doi.org/10.3390/agronomy7030061>.
- Shakir, E., Zahraw, Z., Al-Obaidy, A.H.M., 2017. Environmental and health risks associated with reuse of wastewater for irrigation. *Egyptian Journal of Petroleum* 26 (1), 95–102. <https://doi.org/10.1016/j.ejpe.2016.01.003>.
- Sofo, A., Lundegårdh, B., Mårtensson, A., Manfra, M., Pepe, G., Sommella, E., De Nisco, M., Tenore, G.C., Campiglia, P., Scopa, A., 2016. Different agronomic and fertilization systems affect polyphenolic profile, antioxidant capacity and mineral composition of lettuce. *Sci. Hortic.* 204, 106–115. <https://doi.org/10.1016/j.scienta.2016.04.003>.
- Tong, T., Tong, J., Xue, K., Li, Y., Yu, J., Wei, Y., 2023. Microbial community structure and functional prediction in five full-scale industrial park wastewater treatment plants. *Sci. Total Environ.* 904, 166529 <https://doi.org/10.1016/j.scitotenv.2023.166529>.
- Toze, S., 2006. Water reuse and health risks - real vs. perceived. *Desalination* 187 (1-3), 41–51. <https://doi.org/10.1016/j.desal.2005.04.066>.
- Tsiakaras, G., Petropoulos, S.A., Khah, E.M., 2014. Effect of GA3 and nitrogen on yield and marketability of lettuce (*Lactuca sativa* L.). *Aust. J. Crop. Sci.* 8, 127–132.
- Urbano, V.R., Mendonça, T.G., Bastos, R.G., Souza, C.F., 2017. Effects of treated wastewater irrigation on soil properties and lettuce yield. *Agric. Water Manag.* 181, 108–115. <https://doi.org/10.1016/j.agwat.2016.12.001>.
- Voronkov, A., Sinetova, M., 2019. Polyphosphate accumulation dynamics in a population of *Synechocystis* sp. PCC 6803 cells under phosphate overplus. *Protoplasma* 256, 1153–1164. <https://doi.org/10.1007/s00709-019-01374-2>.
- Wang, X., Wang, X., Hui, K., Wei, W., Zhang, Wen, Miao, A., Xiao, L., Yang, L., 2018. Highly effective polyphosphate synthesis, phosphate removal, and concentration using engineered environmental bacteria based on a simple solo medium-copy plasmid strategy. *Environ. Sci. Technol.* 52, 214–222. <https://doi.org/10.1021/acs.est.7b04532>.
- Wu, X., Chen, F., Zhao, X., Pang, C., Shi, R., Liu, C., Sun, C., Zhang, W., Wang, X., Zhang, J., 2021. QTL mapping and GWAS reveal the genetic mechanism controlling soluble solids content in *Brassica napus* shoots. *Foods* 10 (10), 2400. <https://doi.org/10.3390/foods10102400>.
- Xie, N., Zhong, L., Ouyang, L., Xu, W., Zeng, Q., Wang, K., Zaynab, M., Chen, H., Xu, F., Li, S., 2021. Community composition and function of bacteria in activated sludge of municipal wastewater treatment plants. *Water* 13 (6), 852. <https://doi.org/10.3390/w13060852>.
- Xu, Y., Wu, Y., Esquivel-Elizondo, S., Dolfing, J., Rittmann, B.E., 2020. Using microbial aggregates to entrap aqueous phosphorus. *Trends Biotechnol.* 38, 1292–1303. <https://doi.org/10.1016/j.tibtech.2020.03.012>.
- Yang, F., Li, J., Gan, X., Qian, Y., Wu, X., Yang, Q., 2010. Assessing nutritional status of *Festuca arundinacea* by monitoring photosynthetic pigments from hyperspectral data. *Comput. Electron. Agric.* 70 (1), 52–59. <https://doi.org/10.1016/j.compag.2009.08.010>.
- Zhang, Y., Shen, Y., 2019. Wastewater irrigation: past, present, and future. *Wiley Interdisciplinary Reviews: Water* 6 (3), e1234. <https://doi.org/10.1002/wat2.1234>.
- Zhang, W., Chen, Y., Huang, K., Wang, F., Mei, Z., 2023. Molecular mechanism and agricultural application of the NifA–NifL system for nitrogen fixation. *Int. J. Mol. Sci.* 24, 907. <https://doi.org/10.3390/ijms24020907>.
- Zupanić, J., Turk, M., Crnigoj, M., Ambrožič Avguštin, J., Gunde-Cimerman, N., 2019. The dishwasher rubber seal acts as a reservoir of bacteria in the home environment. *BMC Microbiol.* 19, 1–15. <https://doi.org/10.1186/s12866-019-1674-5>.