frontiers in Agronomy

TYPE Original Research
PUBLISHED 07 April 2026
DOI 10.3389/fagro.2026.1752201

OPEN ACCESS

EDITED BY
Younsuk Dong,
Michigan State University, United States

REVIEWED BY
Ali Ahmad,
Ayub Agriculture Research Institute,
Pakistan
Danilo Lucio,
University of São Paulo, Brazil

*CORRESPONDENCE
Antonio Giovino
✉ antonio.giovino@crea.gov.it

RECEIVED 22 November 2025
REVISED 20 February 2026
ACCEPTED 16 March 2026
PUBLISHED 07 April 2026

CITATION
Tortorici N, Giovino A, Mosca C, Sarno M, Tuttolomondo T and Iacuzzi N (2026) Potential use of treated wastewater for sustainable management of durum wheat under water-scarce environments. *Front. Agron.* 8:1752201. doi: 10.3389/fagro.2026.1752201

Potential use of treated wastewater for sustainable management of durum wheat under water-scarce environments

Noemi Tortorici[1], Antonio Giovino[2]*, Carmelo Mosca[1], Mauro Sarno[1], Teresa Tuttolomondo[1] and Nicolò Iacuzzi[1]

[1]Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo, Italy, [2]Council for Agricultural Research and Economics (CREA)—Research Centre for Plant Protection and Certification (CREA-DC), Palermo, Italy

Water scarcity increasingly threatens agricultural sustainability, particularly in arid and semi-arid regions. Treated wastewater (TWW) represents a promising non-conventional water resource for irrigation, offering economic and environmental benefits while contributing to freshwater conservation. Despite concerns over anthropogenic contaminants, its physiological effects on key crops such as durum wheat remain underexplored. In this study, durum wheat (*Triticum turgidum* L. var. *durum*) was irrigated with TWW or freshwater (FW) and subjected to four levels of water stress (100%, 70%, 50%, and 30% of full irrigation), with or without the addition of a microbial consortium, to assess growth, physiological traits, and stress adaptation mechanisms. Overall, TWW produced vegetative, physiological, and productive performances comparable to or slightly higher than FW, without evidence of phytotoxicity. The microbial consortium showed variable effects, including occasional negative interactions under severe water deficit, highlighting the importance of soil–plant–microbe interactions and local pedo-climatic conditions. Controlled water stress reduced yield even at moderate levels, although gas exchange data indicate that moderate deficit irrigation (70%) could be physiologically tolerated, but did not translate into higher yield under pot conditions. These findings support the potential use of TWW for durum wheat cultivation under water-limited conditions and provide new insights into plant physiological responses under combined irrigation and microbial treatments. Further studies should evaluate these effects across multiple seasons and in open-field conditions.

KEYWORDS

deficit irrigation, drought stress, irrigation, Mediterranean environment, non-conventional water, reclaimed water, triticum durum, water reuse

1 Introduction

Drought stress represents one of the main limiting factors for global agricultural productivity (Schulze et al., 1987). Irrigation has enabled cultivation in many otherwise unproductive areas (Kramer and Boyer, 1995) and is still considered an essential agronomic practice to achieve satisfactory yields and economically sustainable crops (Xu et al., 2024).

However, the current water crisis, exacerbated by climate change, population growth, and the intensification of anthropogenic activities, requires the adoption of water-saving strategies aimed at conserving freshwater resources (Liao et al., 2025). This increasing scarcity threatens the sustainability of many agricultural productions, particularly low-income crops such as cereals (Saleh et al., 2025).

In this context, treated wastewater (TWW) represents one of the most important sources among non-conventional water resources (Angelakis et al., 2024). Its use offers a viable alternative to traditional irrigation, especially in arid and semi-arid areas where natural water resources are limited. Compared to other solutions such as desalination, TWW requires significantly lower energy input, positively impacting costs (Wellmann et al., 2025) and thus contributing to the promotion of sustainable production and consumption patterns (Chen et al., 2021).

Globally, several countries have already integrated TWW into their agricultural systems: in Israel, about 90% of treated wastewater is reused for irrigation (Angelakis et al., 2024), in Jordan 80% (Mainardis et al., 2022), while increasing use is also reported in China, Japan, Australia, and the United States (Cui et al., 2019). In contrast, in Europe, despite recurrent drought episodes, the adoption of non-conventional waters remains limited, with significant use concentrated mainly in Spain, Cyprus, and Malta (Cui et al., 2019; Mainardis et al., 2022).

Numerous studies have highlighted the positive effects of irrigation with TWW on various crops, showing benefits on growth, yield, and quality parameters, such as increased protein content in cereal grains due to the supply of macro- and micronutrients (Alderfasi, 2009; Alvarez-Holguin et al., 2022; Cui et al., 2019; Elfanssi et al., 2018; Mainardis et al., 2022). However, the widespread adoption of this practice is still limited by potential negative effects linked to the presence of anthropogenic contaminants, such as heavy metals, microplastics, and emerging pollutants. Even at low concentrations, repeated use of TWW may lead to bioaccumulation in the soil, causing soil quality degradation, phytotoxicity, accumulation in edible parts, and potential entry into the food chain (Cui et al., 2019; Elfanssi et al., 2018; Mainardis et al., 2022).

An integrated management of the irrigation system, considering the quality of treated water, the type of treatment, soil characteristics, and the cultivated species, can help mitigate these critical issues and improve overall system efficiency.

In the existing literature, particular attention has been paid to the effects of heavy metals and their mobilization in the soil-plant system. Studies on cereal crops, such as wheat, have shown a significant capacity of roots to absorb metals, with limited transport to edible parts, suggesting a possible protective role of roots (Chen et al., 2016; Rezapour et al., 2019; Singh et al., 2010; Qureshi et al., 2016; Xiao-Rui et al., 2016). This feature may help mitigate the risks of food chain contamination, highlighting the importance of crop selection when adopting irrigation practices with non-conventional waters. Heavy metal concentrations detected in grains are mostly below FAO/WHO limits (Rezapour et al., 2019).

From a regulatory perspective, the European Commission has recently proposed a revision of Directive 91/271/EEC on urban wastewater treatment, with a horizon set for 2040. The proposal (COM (2024) 123 final) introduces new standards for decentralized plants, stricter limits for nutrients and micropollutants, and the implementation of monitoring and traceability systems for anthropogenic contaminants (European Commission, 2024).

To date, research has mainly focused on the effects of traditional and emerging pollutants resulting from irrigation with non-conventional waters, considering aspects such as soil physicochemical properties, groundwater quality, and human health implications. However, studies on crop physiological responses remain limited, particularly for species such as durum wheat, which could benefit from regular (rather than rescue) irrigation in areas subjected to prolonged water stress (Tortorici et al., 2024).

In this study, the application of four levels of water stress allowed for a deeper understanding of the mechanisms involved in plant responses, highlighting effects on growth, physiology, and adaptive strategies. Moreover, the use of a microbial consortium was evaluated to explore its potential role in mitigating stress effects.

These results represent an important step toward the development of more sustainable irrigation practices in the Mediterranean context, enriching the scientific literature with new experimental evidence on the physiological responses of durum wheat (*Triticum turgidum* L. var. *durum* Desf.) to different levels of water stress, including in the presence of a microbial consortium. For such solutions to be effective and long-lasting, they must be adopted conscientiously, through an integrated analysis of the entire agro-environmental system, considering the interactions between water quality, soil characteristics, crop requirements, and microbial dynamics.

2 Materials and methods

2.1 Experimental site, plant material and design

The study was conducted in pots at the “Orleans” teaching and experimental farm of the Department of Agricultural, Food and Forestry Sciences, University of Palermo, Palermo, Italy (38°06’15”N, 13°20’58”E; 70 m a.s.l.), during the period from January 14 to June 6, 2025. Round pots, measuring 22 × 22 cm with a capacity of 6.84 liters, were placed inside a rainout shelter (12.0 m length, 8.0 m width, 2.80 m height) to exclude the influence of rainfall on soil water content (SWC). Each pot was filled with 6 kg of soil collected from a field in Collesano, Palermo, Italy (37°55’16”N, 13°56’17”E; 475.11 m a.s.l.), which had been managed under organic farming for over 15 years. The soil was sampled during the last decade of November 2024 from the 0–25 cm profile, and the previous crop had been sulla (*Sulla coronaria* (L.) Choi and Ohashi, 2003). The physicochemical properties of the soil are reported in Table 1.

For the experimental trial, the medium-early variety Antalis (Semìa) of *Triticum turgidum* subsp. *durum* (Desf.) Husn. was used, as it is a commercially recommended variety in the hot-arid environments of Southern Italy due to its high agronomic and technological grain qualities. On January 14, 2025, 20 seeds were sown per pot at a depth of 2 cm and at the one-leaf stage (BBCH 11), and pots were later thinned to maintain 13 plants per pot. No fertilization was applied during the trial, while weed control was carried out manually throughout the crop cycle. Fungal diseases were managed through the application of a systemic fungicide based on prothioconazole and tebuconazole. Harvest was conducted at commercial maturity (June 6, 2025), when the grains reached a moisture content of 12%.

The experimental protocol included the study of three factors: (i) type of irrigation water — fresh water (FW) and treated wastewater (TWW); (ii) level of water stress — soil water content restored to 100% of field capacity (FC) (I1); 70% FC (I2); 50% FC (I3); and 30% FC (I4); (iii) presence (M+) or absence (M−) of a microbial consortium.

A completely randomized design (CRD) was adopted, with three replicates per treatment. In total, 16 treatments were considered, corresponding to 480 pots (16 treatments × 3 replicates × 10 pots).

2.2 Meteorological data

Meteorological data were collected from an on-site ATMOS 41 weather station (METER Group, Pullman, WA, USA). The ATMOS 41 measures twelve meteorological variables, including air temperature, relative humidity, vapor pressure, barometric pressure, wind speed and direction, solar radiation, precipitation, and lightning. The station was connected to a ZL6 datalogger (METER Group, Pullman, WA, USA), specifically designed to collect data from environmental sensors. The datalogger transmits data to the cloud via a Subscriber Identity Module (SIM) and is powered by six NiMH batteries recharged by solar panels. Figure 1

TABLE 1 Soil physico-chemical and hydrological properties.

Soil Texture				
Sandy [%]	Silt [%]	Clay [%]		
88	9	4		
Nutrients and Organic Matter				
TN [%]	P_2O_5 [mg kg^{-1}]	K_2O [mg kg^{-1}]	Organic carbon [g kg^{-1}]	Organic matter [%]
0.28	17.1	153	12.1	1.21
Chemical Properties				
Electrical conductivity [μS cm^{-1}]	pH			
609.0	8.32			
Hydrological Constants				
θfc Ψ = 0.1 [bar]	θ Ψ = 0.33 [bar]	θ Ψ = 1 [bar]	θ Ψ = 3 [bar]	θwp Ψ = 15 [bar]
0.319	0.264	0.238	0.204	0.191
θp				
0.250				

TN, total nitrogen; θ, volumetric water content; θfc, water content at field capacity; θwp, water content at the wilting point; θp, threshold water content for irrigation (readily available water); Ψ, soil matric potential.

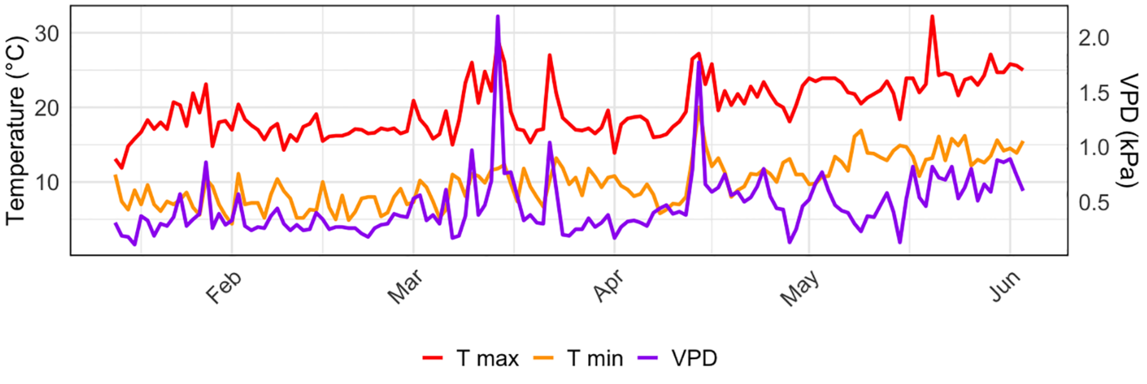


FIGURE 1
Daily air temperature (°C) and vapor pressure deficit (kPa) recorded during the experimental period. T max, maximum temperature; T min, minimum temperature; VPD, vapor pressure deficit.

shows the trends of temperature and vapor pressure deficit (VPD) during the experimental period.

2.3 Determination of soil hydrological properties

The volumetric soil water content, θ (m^3 m^{-3}), at a pressure head, h (m), of −1 m, corresponding to field capacity (FC), was determined using pressure plate extractors (Dane et al., 2002). For each treatment, two replicate samples were prepared by compacting the 2 mm soil fraction into cylindrical samplers with a diameter of 5 cm and a height of 1 cm, at the bulk density values measured in the undisturbed soil. Equilibrium with the applied pressure head was assumed when the samples ceased draining for at least 24 hours. The volumetric water content at equilibrium was determined by the thermogravimetric method, after oven-drying the samples at 105 °C until constant weight, typically achieved within 24 h. All measurements were performed under controlled conditions, with the temperature set at 22 ± 1 °C. Table 1 reports five values of volumetric soil water content, ranging from field capacity (θfc) to the wilting point (θwp), along with the corresponding matric potential values, also obtained using the pressure plate method.

2.4 Irrigation management

Four irrigation regimes were studied: restoration of soil water content to 100% of field capacity (FC) (I1); 70% FC (I2); 50% FC (I3); and 30% FC (I4). For each replicate, the available water content was monitored daily using TEROS 12 capacitive and tensiometric sensors (METER Group, Inc., Munich, Germany; formerly Decagon Devices, Inc., Pullman, WA, USA) placed at a depth of 0–0.20 m along the soil profile inside each pot. In addition, the soil matric potential at a depth of 0–0.20 m was monitored using a TEROS 21 sensor (METER Group, Inc., Munich, Germany; formerly Decagon Devices, Inc., Pullman, WA, USA). The TEROS 12 sensors provide an accuracy of ± 0.03 m^3 m^{-3} for volumetric water content (VWC), ± 0.5 °C for temperature, and ± (5% of reading + 10 µS/cm) for bulk electrical conductivity. The TEROS 21 sensors provide an accuracy of ± (10% of reading + 2 kPa) for water potential (typically ranging from −9 to −100 kPa) and ±1.0 °C for temperature. All sensors were connected to a ZL6 datalogger (METER Group, Pullman, WA, USA), specifically designed to collect data from environmental sensors. The datalogger transmits data to the cloud via a Subscriber Identity Module (SIM) and is powered by six NiMH batteries recharged by solar panels. The capacitive, tensiometric, and matric potential probes recorded data continuously, with measurements taken every 15 minutes. The soil water content at field capacity (θfc) and at the wilting point (θwp) were 0.319 and 0.191 m^3 m^{-3}, respectively. Considering a rooting depth of 200 mm, the total available water (TAW) was calculated as 25.7 mm, corresponding to 0.88 L per pot. The readily available water (RAW), calculated using a depletion coefficient of p = 0.55 (Allen et al., 1998), was 14.2 mm, equivalent to 0.48 L per pot.

The irrigation threshold (θp) was determined using the equation provided by Allen et al. (1998):

$$\theta p = \theta fc - (p \times TAW)$$

Irrigation was carried out manually based on the achievement of the irrigation threshold (θp). The volume of replenishment water was determined as the difference between θp and θfc. Irrigation was always maintained within the limits of field capacity.

After sowing, all pots were saturated with fresh water and maintained under full irrigation conditions until February 3 to ensure proper germination and emergence and to provide a uniform starting point. Subsequently, the deficit irrigation treatments and the use of treated wastewater (TWW) were initiated. Irrigation continued until the BBCH 89 stage, corresponding to 21 days before harvest. In the I4 treatments, irrigation was stopped on April 30 due to plant death. Table 2 reports the total irrigation volumes applied for each treatment.

TABLE 2 Monthly irrigation volumes (L per plot; 1 plot = 10 pots) applied to each treatment.

	FW							
	I1 × M+ [L]	I2 × M+ [L]	I3 × M+ [L]	I4 × M+ [L]	I1 × M- [L]	I2 × M- [L]	I3 × M- [L]	I4 × M- [L]
January	20.00	20.00	20.00	20.00	15.00	15.00	15.00	15.00
February	21.90	15.30	10.95	6.60	31.40	22.00	15.70	9.40
March	41.45	28.90	20.70	12.50	54.40	38.05	27.20	16.25
April	48.70	34.05	24.35	8.90	64.10	44.75	32.15	12.10
May	25.90	18.10	12.95	0.00	35.40	24.80	17.00	0.00
Total	157.95	116.35	88.95	48.00	200.30	144.60	107.05	52.75
	TWW							
	I1 × M+ [L]	I2 × M+ [L]	I3 × M+ [L]	I4 × M+ [L]	I1 × M- [L]	I2 × M- [L]	I3 × M- [L]	I4 × M- [L]
January	20.00	20.00	20.00	20.00	15.00	15.00	15.00	15.00
February	25.40	17.80	12.70	7.60	21.60	15.10	10.80	6.50
March	45.75	31.95	22.80	13.65	48.50	33.87	24.20	14.60
April	47.50	33.30	23.75	8.90	52.90	37.00	26.55	9.80
May	24.80	17.35	12.40	0.00	29.50	20.65	14.75	0.00
Total	163.45	120.40	91.65	50.15	167.50	121.62	91.30	45.90

FW, fresh water; TWW, treated wastewater; M+, presence of microbial consortium; M−, absence of microbial inoculum; I1, 100% irrigation; I2, 70% irrigation; I3, 50% irrigation; I4, 30% irrigation.

2.5 Type of water

The fresh water (FW) used in the experiment originated from groundwater extracted directly at the experimental site. Its physicochemical characteristics are reported in Supplementary Table S1 (Supplementary Materials). The treated wastewater (TWW) was collected as needed from the Municipal Wastewater Treatment Plant of Campofelice di Roccella, Palermo, Italy (37° 59'23"N, 13°53'03"E; 68 m a.s.l.). Samples were collected at the final collection point of the plant, after all treatment stages and before discharge into the sea, ensuring that the water used for irrigation reflected the quality of the fully treated effluent. The treatment facility consists of two main sections: a water line and a sludge line. In the water line, the influent enters a receiving chamber and is then conveyed into two channels equipped with mechanical screw screens. Each screen has a treatment capacity of up to 400 m^3 h^{-1}, corresponding to the plant's maximum influent flow. Following screening, where coarse solids are removed, the wastewater passes through an aerated grit and grease removal unit ("pista" type). The effluent from this stage then flows into the distribution channel feeding the biological treatment section, where probes for measuring electrical conductivity and pH are installed. The biological treatment system consists of three identical and parallel lines, each comprising a pre-denitrification tank followed by an oxidation-nitrification tank. The influent entering the pre-denitrification tank is mixed using a submerged electromechanical mixer. The effluent then flows through an opening and an overflow weir into the oxidation-nitrification tank, where aeration is provided by disk diffusers supplied by individual blowers. A dissolved oxygen sensor in each tank regulates a motorized valve, thereby controlling the airflow rate. The effluent from the oxidation-nitrification tank is partially recycled to the pre-denitrification unit through a return channel and partially sent to the secondary sedimentation unit. A flow meter measures the recirculated flow rate and controls a motorized gate valve on the pressure pipeline. The secondary sedimentation unit consists of two radial-flow clarifiers, fed by a central distribution chamber that receives effluent from the three oxidation tanks and directs it to the two clarifiers. The clarified effluent from each sedimentation tank is conveyed to a filtration bypass channel, together with effluent pumped from the sedimentation outlet. A flow meter measures the volume of effluent passing through the bypass channel, while a total suspended solids (TSS) sensor controls the opening of gates that direct the clarified water either to the UV disinfection unit or through the bypass. In all cases, the clarified effluent flows into a final collection well and from there into the discharge basin leading to the Tyrrhenian Sea. In the sludge line, the sludge is separated from the clarified effluent in the secondary sedimentation tanks. It is extracted from the bottom of the circular basins and conveyed to a collection and lifting chamber equipped with two sets of pumps: one for recycling sludge back to the biological treatment stage, and one for removing excess sludge. The amount of recirculated sludge is controlled by a valve and a flow meter, according to the influent flow and the established recirculation ratio. Excess sludge is sent to the aerobic digester, depending on process management and sludge quality. The aerobic digestion system comprises two tanks receiving sludge from the sedimentation units. Floating materials removed from the grit chamber and sedimentation tanks are also pumped into the digestion section. Each oxidation tank contains two submerged aerators, whose operation is controlled by dissolved oxygen sensors. The digested sludge is then transferred to the dewatering unit. For each TWW sampling event, physicochemical and microbiological analyses were performed by the Regional Environmental Protection Agency (ARPA Sicilia). The results are presented in Supplementary Table S1 (Supplementary Materials). The treated wastewater was used as supplied by the municipal treatment plant, and no additional filtration was performed prior to irrigation.

2.6 Microbial consortium

The day before sowing, half of the durum wheat seeds were coated using a liquid spray treatment with a commercial microbial consortium (Coveron, ITALPOLLINA S.p.A., Rivoli Veronese, Verona, Italy). This consortium consists of *Glomus intraradices* (300 spores g^{-1}), *Glomus mosseae* (200 spores g^{-1}), rhizosphere bacteria (10^7 CFU g^{-1}), and *Trichoderma atroviride* (3×10^8 CFU g^{-1}), applied at the manufacturer's recommended rate of 150 g q^{-1} seed.

2.7 Physiological parameters

Physiological data were collected during the phenological stages of stem elongation (BBCH 37), heading (BBCH 51), and flowering (BBCH 69) on fully light-exposed flag leaves, randomly sampled from five plants per replicate. Leaf gas exchange parameters, including net photosynthetic rate (Pn) and stomatal conductance (gs), were measured using a portable photosynthesis system (CIRAS-3, PP Systems, Hitchin, UK). Measurements were taken on the central portion of the leaf. The CO_2 concentration inside the leaf chamber was set at 400 µmol mol^{-1}, with an air flow rate of 200 mL min^{-1}. The photosynthetically active radiation (PAR) within the cuvette was adjusted to match ambient light levels recorded prior to each measurement using the system's integrated sensor. Measurements were performed under ambient temperature and relative humidity conditions. The measured leaf area was 6 cm^2 (2 cm × 3 cm). When the leaf did not completely cover the cuvette surface, the actual leaf area was measured and used for subsequent data normalization.

Chlorophyll content was estimated on the same leaves using a chlorophyll meter (SPAD-502, Konica Minolta, Inc., Tokyo, Japan).

Leaf temperature was measured using a portable infrared thermal camera equipped with MSX® technology (FLIR E8 Pro, Teledyne FLIR LLC, USA). Images were taken from four different angles for each replicate and subsequently averaged. The instrument was positioned to minimize soil interference and maximize canopy coverage within the frame.

The normalized difference vegetation index (NDVI) was measured using a portable active sensor (RapidScan CS-45, Holland Scientific, Lincoln, NE, USA). Measurements were performed by moving the sensor across the crop canopy in each plot, maintaining a constant height of 1 m above the ground and a perpendicular angle (90°) to the canopy, at a constant speed. NDVI values were automatically calculated by the device according to the following equation:

$$NDVI = \frac{RNIR - RRED}{RNIR + RRED}$$

where R_{NIR} is the reflectance in the near-infrared band and R_{RED} is the reflectance in the red band.

For the determination of leaf relative water content (RWC), the same leaves used for gas exchange and chlorophyll content measurements were sampled. Leaves were immediately placed in plastic Falcon tubes, kept in a dark insulated cooler to minimize water loss, and transported to the laboratory. The fresh weight (fw) was measured immediately upon arrival. Subsequently, the leaves were immersed in distilled water in Petri dishes for 24 h and kept in a cool, dark environment. Afterward, leaves were gently blotted with absorbent paper to remove surface water and weighed to determine the turgid weight (tw). Finally, they were oven-dried at 70 °C until constant weight to obtain the dry weight (dw). The RWC was calculated as a percentage according to Barrs and Weatherley (1962), using the following equation:

$$RWC = \frac{fw - dw}{tw - dw} \times 100$$

All measurements were carried out on a single clear day, between 11:00 a.m. and 2:00 p.m.

The xylem water potential (ψ_{xylem}) was measured under pre-dawn conditions (03:00–06:00 a.m.) using a Scholander-type pressure chamber (EcoSearch S.r.l., Italy) supplied with compressed nitrogen gas. Three leaves per replicate were used for each measurement.

2.8 Agronomic parameters

At physiological maturity, the following traits were recorded on 25 randomly selected plants per replicate: number of tillers, number of nodes, culm height, spike length, and number of spikes per pot. In addition, grain yield (g) and harvest index were determined for each replicate.

2.9 Data analysis

All statistical analyses were performed according to a completely randomized design, considering each treatment combination. Data were processed using Minitab Statistical Software 22 (Minitab, LLC, State College, PA, USA).

A multifactorial analysis of variance (ANOVA) was conducted, with standard errors of the means and Pearson correlation coefficients calculated. For parameters measured at multiple time points during the crop cycle, a repeated measures ANOVA was applied. To verify the assumptions of ANOVA, data were tested for sphericity (Mauchly's test, $\alpha = 0.05$), normality (Ryan–Joiner test, $\alpha = 0.05$), and homogeneity of variances (Levene's test, $\alpha = 0.05$). When ANOVA revealed significant effects, treatment means were compared using Tukey's test ($p = 0.05$).

3 Results

3.1 Soil water content trends

Figure 2 shows the daily dynamics of soil volumetric water content (SWC) recorded throughout the experimental period by the sensors for each combination of water type (FW, TWW), water level (I1, I2, I3, I4), and microbial consortium application (M+, M−). Overall, the four graphs display a similar trend: SWC progressively decreases from I1 to I4, reflecting the expected effects of the different irrigation strategies.

Until February 10, all pots were irrigated to maintain SWC within the Readily Available Water (RAW) range. The I1 treatment (full crop water requirement) remained, with rare exceptions, between field capacity ($\theta = 0.319$ m^3 m^{-3}) and the irrigation threshold ($\theta = 0.25$ m^3 m^{-3}) throughout the experiment. Soil matric potential (Ψ) ranged from -0.001 to -0.54 bar, with occasional peaks up to -1.47 bar (data not shown) (Figure 2A).

Under I2 conditions (Figure 2B), the lower limit of SWC fluctuations was around $\theta \approx 0.23$ ($\Psi \approx -4.04$ bar, data not shown), while maximum values were consistently lower than those observed in the control treatment. In the I3 treatment (Figure 2C), SWC mainly ranged between 0.20 ($\Psi \approx -18.06$ bar, data not shown) and 0.25, except for the TWW × M+ combination, which showed higher values with fluctuations exceeding the irrigation threshold (θp). Conversely, the I4 treatment (Figure 2D) exhibited a pronounced reduction in SWC across all cases, with values remaining below θp from the early stages of stress. Under FW conditions, SWC continued to decrease until reaching the wilting point around the second decade of March (37 BBCH) and remained at this threshold until plant death. During this period, mean Ψ values were -21.41 bar, with peaks up to -27.13 bar (data not shown). For TWW × M−, SWC declined to approximately 0.15, whereas in TWW × M+ (Figures 2, 3) the reduction was more gradual: values remained around 0.23 until March and fell below the wilting point only after the first decade of April (51 BBCH). Finally, after irrigation was stopped on May 16, SWC continued to decline in all treatments, although plants were already near complete wilting.

3.2 Agronomic responses

The analysis of variance (ANOVA) (Table 3) revealed highly significant effects ($p < 0.01$) resulting from the interaction between water type, water level, and microbial consortium application (WT × WL × MC) on all observed morphological and yield-related parameters, including plant height, number of nodes, spike length, total aboveground biomass, and grain yield (Figure 3).

The harvest index (HI), calculated as the ratio between grain weight and total aboveground biomass, instead showed significant differences at a lower level of interaction (two-way interaction) (Figure 4).

Plant height ranged from 33.7 to 71.1 cm, with the highest values recorded in the TWW × I1 × M− treatment. However, other treatments irrigated at 100%, regardless of water type or microbial consortium application, did not differ statistically from this, as did some treatments irrigated at 70%. As expected, the most severe water stress (I4) resulted in the greatest reduction in plant height, with the minimum value observed in FW × I4 × M +. Overall, plants irrigated with treated wastewater (TWW) exhibited heights equal to or slightly greater than those irrigated with freshwater (FW). Under the same conditions, microbial consortium application did not lead to notable differences, except for FW × I3 × M− and FW × I4 × M−,

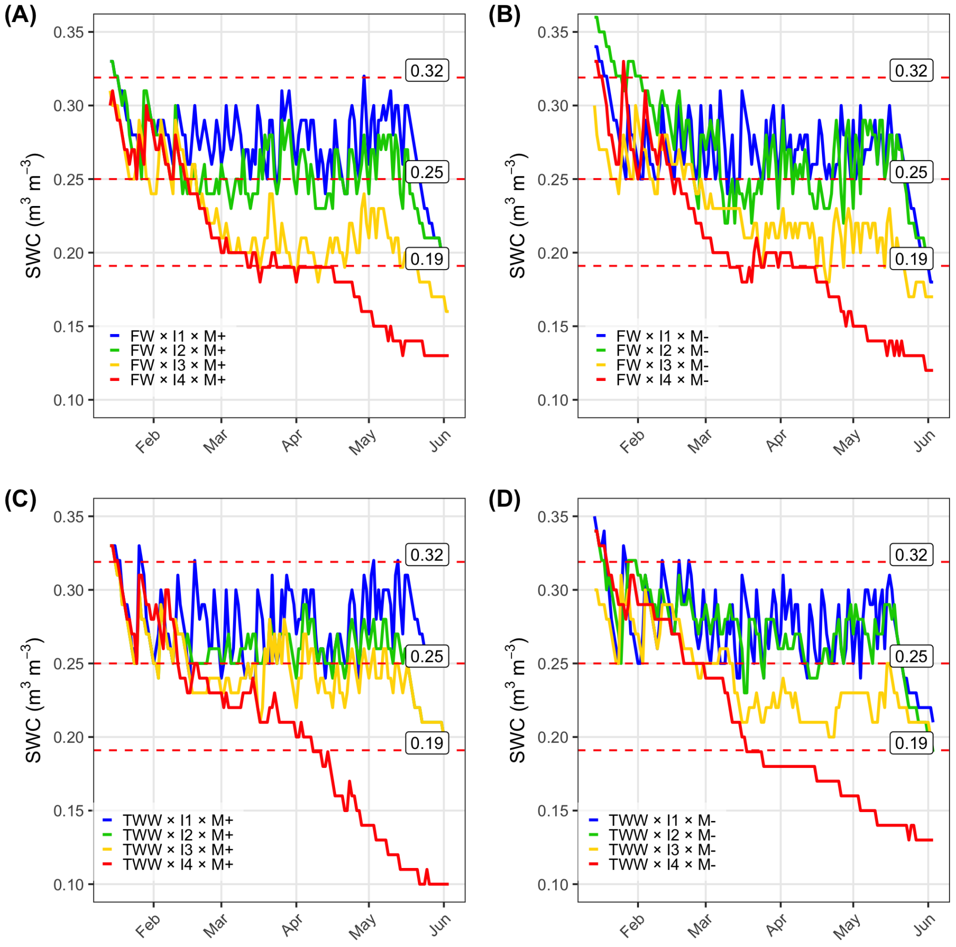


FIGURE 2
SWC over time for each combination of water type and microbial consortium, under four irrigation treatments. **(A)** FW × I × M+; **(B)** FW × I × M–; **(C)** TWW × I × M+; **(D)** TWW × I × M–. SWC, Soil water content. Water type (FW, fresh water; TWW, treated wastewater), water level (I1, 100% FC; I2, 70% FC; I3, 50% FC; I4, 30% FC), microbial consortium (M–, absence; M+, presence).

which showed significantly greater heights compared to the corresponding inoculated treatments (Figure 3A).

Regarding the number of culm nodes, although highly significant differences were observed among treatment combinations ($p< 0.001$), the overall variability between groups was limited. The highest mean values (4 nodes) were mainly recorded with TWW, although some FW-irrigated treatments showed similar values. The most pronounced differences were observed under severe stress conditions (I4), where TWW × I4 × M– and FW × I4 × M+ exhibited the lowest values (3.2 nodes), statistically distinct from the other treatments (Figure 3B).

Spike length reached its maximum in TWW × I1 × M– (14.20 cm), followed by TWW × I2 × M– (12.87 cm). Other treatments, including those with lower water levels, did not differ significantly, belonging to partially overlapping significance groups. The minimum value (9.67 cm) was observed in FW × I3 × M– (Figure 3A).

For yield parameters, treatments at the I4 water level were excluded from the ANOVA because the plants did not survive until the end of the experiment. The highest biomass and grain yields were recorded in TWW × I1 (both M+ and M–) and FW × I1 × M–, ranging from 66.4 to 77.9 q ha^{-1} of biomass and 17.9–24.3 q ha^{-1} of grains (Figure 3C). A progressive decline in productivity was observed from the I2 level onwards. The lowest biomass yields were recorded in FW × I3 × M+ (21.93 q ha^{-1}), followed by TWW × I3 × M+ (23.53 q ha^{-1}) and TWW × I2 × M– (27.54 q ha^{-1}) (Figure 3). The lowest grain yield (2.8 q ha^{-1}), observed in TWW × I2 × M–, was attributed to the reduced number of plants per pot, as confirmed during the measurements. Physiological parameters did not show abnormal values, as described in the following paragraph.

In general, microbial consortium application did not enhance yield; in several cases, M– treatments performed better. Overall, reducing irrigation from 100% to 70% resulted in yield decreases exceeding 60%, while reduction to 50% led in some cases to losses of 80% or more.

Finally, the harvest index (Figure 4) showed significant differences in the WT × WL and WT × MC interactions, with values ranging from 16% to 29%. In the WT × WL interaction, the main differences were observed between TWW × I1 and TWW × I2, while the other treatments were statistically similar (Figure 4A). In

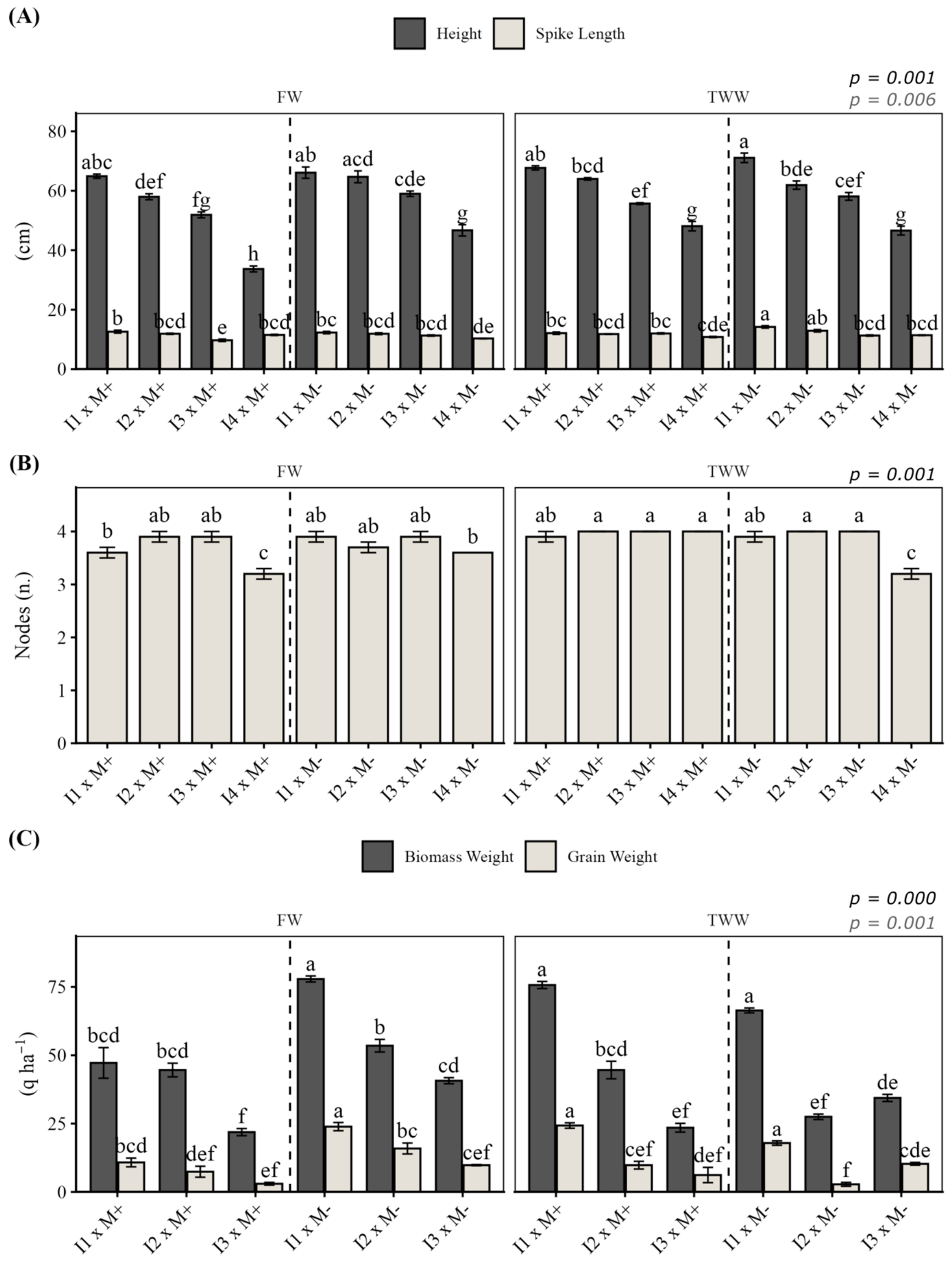


FIGURE 3
Effect of treatments on morphological and yield traits. **(A)** Height and Spike Length; **(B)** Nodes; **(C)** Biomass Weight and Grain Weight. For each data series, values with different letters are significantly different at p ≤ 0.05, according to Tukey's test. Error bars represent standard error. Water type (FW, fresh water; TWW, treated wastewater), water level (I1, 100% FC; I2, 70% FC; I3, 50% FC; I4, 30% FC), microbial consortium (M−, absence; M+, presence). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at p ≤ 0.05.

the WT × MC interaction, the highest value was observed in TWW × M+, and the lowest in FW × M+ (Figure 4B).

3.3 Physiological responses

The four-way interaction, including the sampling date (BBCH 37, 51, and 69), was not statistically significant for any of the analyzed physiological variables ($p > 0.05$). Significant effects emerged at the three-way interaction level, with the exception of relative water content (RWC), which was significant only for the WL × PS interaction (Table 4). The WL × MC × PS interaction was significant exclusively for SPAD. Since this effect followed a pattern similar to those observed in the other significant interactions and did not alter the overall interpretation, it is not discussed further.

The WT × WL × MC interaction (Figure 5) significantly influenced SPAD, stem water potential (SWP), stomatal conductance (Gs), and NDVI, each showing distinct response patterns.

SPAD ranged from 30.4 to 42.6 (Figure 5A). The highest mean occurred in FW × I1 × M+, although several other treatments at the I1 irrigation level, as well as TWW × I2 × M− and FW × I3 × M+, were

TABLE 3 **Analysis of variance (ANOVA) of main effects and interactions on morphological and yield traits.**

Source of variation		Parameters					
	df	Plant height	Spike length	Nodes	Biomass weight	Grain weight	Harvest index
WT	1	28.67 ***	17.97 ***	23.21 ***	2.97 ns	0.01 ns	0.61 ns
WL	3	237.87 ***	40.95 ***	31.21 ***	260.02 ***	78.76 ***	5.32 *
MC	1	32.58 ***	7.67 **	0.47 ns	28.82 ***	14.34 **	2.24 ns
WT × WL	3	4.12 *	1.57 ns	0.47 ns	21.59 ***	10.87 ***	4.37 *
WT × MC	1	24.01 ***	6.47 *	23.21 ***	84.95 ***	55.52 ***	11.74 **
WL × MC	3	1.82 ns	3.9 *	3.84 *	18.58 ***	2.58 ns	1.13 ns
WT × WL × MC	3	7.22 **	4.98 **	16.47 ***	12.03 ***	9.19 **	1.92 ns

Values are given as F of Fisher. ***, ** and * indicate significant at p< 0.001, p< 0.01 and p< 0.05, respectively. ns, not significant; df, Degrees of Freedom; WT, water type; WL, water level; MC, microbial consortium.

statistically comparable. The lowest SPAD values were generally associated with the most severe water stress (I4), reflecting an overall decline with increasing stress, despite some exceptions (e.g., FW × I3 × M− and FW × I3 × M+). Water type had a limited effect when the other factors were held constant. A slight increase linked to microbial inoculation was evident under FW at I1 and I3 compared with non-inoculated treatments and TWW at the same irrigation levels.

No clear differences in SWP (Figure 5B) were found between TWW and FW at equivalent irrigation and microbial treatments. SWP declined progressively with increasing water stress, reaching a minimum in FW × I4 × M+ (−16.6 bar) and a maximum in FW × I1 × M− (−2.1 bar). Treatments at the I1 and I2 irrigation levels, irrespective of microbial inoculation, as well as TWW/FW × I3 × M−, showed values close to the maximum. Non-inoculated plants generally exhibited higher (less negative) SWP, particularly under FW. Under severe stress (I3 and I4), inoculated plants irrigated with TWW showed less negative SWP compared with those irrigated with FW.

Stomatal conductance (Gs; Figure 5C) ranged from 0.1 to 0.7 mol $H_2O\ m^{-2}\ s^{-1}$. The highest value occurred in FW × I1 × M+, although TWW at I1 and I2 without inoculation and FW × I1 × M− were statistically similar. All treatments at the most severe stress level (I4), regardless of water type or microbial inoculation, exhibited the lowest Gs. Overall, consistent with SWP, non-inoculated plants tended to show slightly higher Gs.

NDVI (Figure 5D) revealed a clearer differentiation between water types, with higher values generally under TWW compared with FW at the same irrigation and microbial conditions. The highest NDVI occurred in TWW × I1 × M− (0.473) and TWW × I1 × M+

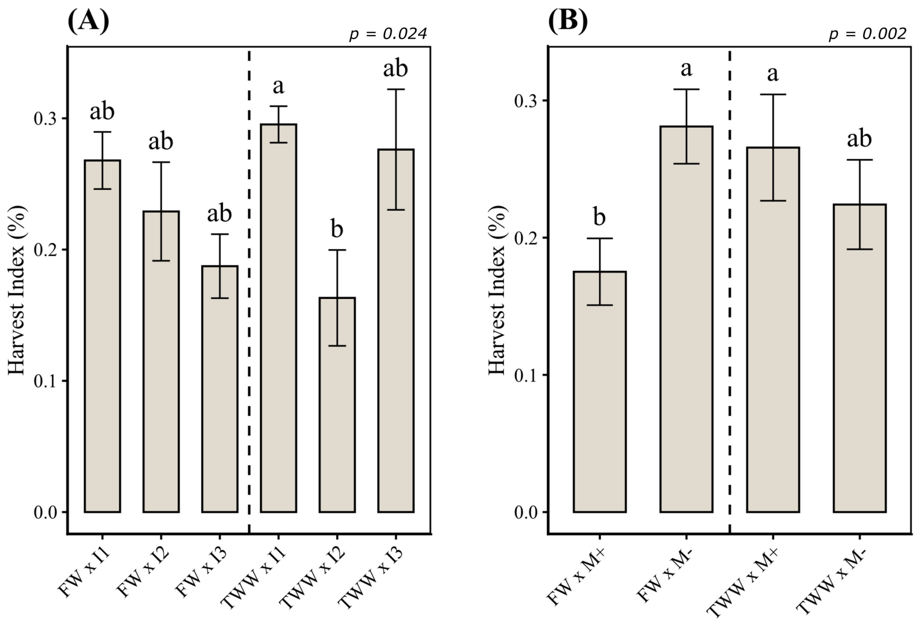


FIGURE 4
Effect of treatments on harvest index. **(A)** WT × WL; **(B)** WT × MC. For each data series, values with different letters are significantly different at p ≤ 0.05, according to Tukey's test. Error bars represent standard error. Water type (FW, fresh water; TWW, treated wastewater), water level (I1, 100% FC; I2, 70% FC; I3, 50% FC; I4, 30% FC), microbial consortium (M−, absence; M+, presence). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at p ≤ 0.05.

TABLE 4 Analysis of variance (ANOVA) of main effects and interactions on physiological responses.

Source of variation		Parameters						
	df	SPAD	RWC	SWP	Pn	Gs	Tc - Ta	NDVI
WT	1	3.07 ns	1.41 ns	6.70 *	20.58 ***	3.02 ns	133.48 ***	35.96 ***
WL	3	79.05 ***	22.81 ***	200.80 ***	14.62 ***	118.67 ***	73.96 ***	149.38 ***
MC	1	8.07 **	5.16 *	10.30 **	4.33 *	24.69 ***	8.53 **	0.14 ns
PS	2	788.18 ***	37.13 ***	111.17 ***	213.85 ***	76.40 ***	48.31 ***	205.52 ***
WT × WL	3	0.47 ns	0.25 ns	5.61 ***	5.96 ***	8.57 ***	3.69 *	2.10 ns
WT × MC	1	20.87 ***	1.10 ns	2.55 ns	0.86 ns	2.82 ns	3.67 ns	18.23 ***
WT × PS	2	0.92 ns	1.03 ns	0.38 ns	28.31 ***	13.86 ***	30.37 ***	1.00 ns
WL × MC	3	5.70 **	0.82 ns	0.45 ns	0.76 ns	7.60 ***	0.18 ns	4.51 **
WL × PS	6	85.22 ***	2.62 *	57.99 ***	18.30 ***	12.13 ***	46.86 ***	4.17 **
MC × PS	2	10.87 ***	0.17 ns	17.37 ***	3.86 *	1.47 ns	2.11 ns	2.90 ns
WT × WL × MC	3	3.28 *	0.85 ns	4.09 **	0.57 ns	3.02 *	0.09 ns	4.83 **
WT × WL × PS	6	2.70 *	1.64 ns	1.27 ns	8.62 ***	11.08 ***	2.91 *	2.55 ns
WT × MC × PS	2	17.38 ***	0.50 ns	3.30 *	2.26 ns	5.83 **	3.08 ns	15.50 ***
WL × MC × PS	6	9.61 ***	1.22 ns	1.20 ns	0.69 ns	0.77 ns	1.50 ns	0.94 ns
WT × WL × MC × PS	6	1.82 ns	0.56 ns	1.14 ns	0.54 ns	1.81 ns	0.82 ns	1.09 ns

Values are given as F of Fisher. ***, ** and * indicate significant at $p< 0.001$, $p< 0.01$ and $p< 0.05$, respectively. ns, not significant; df, Degrees of Freedom; WT, water type; WL, water level; MC, microbial consortium; PS, phenological stage. RWC, relative water content; SWP, stem water potential; Pn, net photosynthesis; Gs, stomatal conductance; Tc – Ta, difference between canopy and air temperature; NDVI, normalized difference vegetation index.

(0.434), followed by FW × I1 × M− (0.395). NDVI declined progressively with increasing water stress, reaching the lowest values in TWW × I4 × M− (0.160) and FW × I4 × M+ (0.175). Microbial inoculation had a positive effect on NDVI only under TWW and water stress conditions (I3 and I4), where inoculated plants showed higher values compared with non-inoculated ones, whereas no beneficial effect was observed under FW.

The WT × WL × PS interaction (Figure 6) significantly affected SPAD, net photosynthesis (Pn), stomatal conductance (Gs), and canopy–air temperature difference (Tc–Ta).

SPAD values (Figure 6A) reached their maximum during the intermediate phenological stage (BBCH 51), particularly in TWW × I3 × 2 (46.12 SPAD units) and FW × I3 × 2 (46.23 SPAD units). The lowest values occurred at BBCH 69, declining to 6.52 SPAD units under severe water stress (I4). Clear differences among treatments were mainly evident at this final stage, where water deficit became the dominant factor influencing SPAD.

Net photosynthesis (Pn) ranged from 7.2 to 32.4 µmol m^{-2} s^{-1} (Figure 6B). The highest rates were observed under FW at BBCH 51, regardless of irrigation level. Some TWW treatments, such as TWW × I3 × 2 and TWW × I4 × 2, also exhibited elevated Pn. The lowest values (Pn< 8 µmol m^{-2} s^{-1}) were recorded in TWW × I4 × 3 and FW × I4 × 3. Within the same water type, no clear reduction in Pn was detected between I1 and I2, indicating that moderate water restriction did not markedly limit photosynthetic activity.

For stomatal conductance (Gs; Figure 6C), the highest values were recorded at stage 1 in TWW treatments at I1 and I2, as well as in FW × I1 at BBCH 37 and 51. The lowest Gs values were observed in TWW × I4 × 3 and FW × I4 during the later stages (BBCH 51 and 69). Most other treatments clustered within intermediate, partially overlapping significance groups, generally with values below 0.5 mol H_2O m^{-2} s^{-1}.

The canopy–air temperature difference (Tc–Ta; Figure 6D) ranged from −0.47 °C (TWW × I1 × 1) to 9.87 °C (FW × I4 × 3). Overall, TWW resulted in lower Tc–Ta values compared with FW. This pattern was evident during the first two measurement dates. Within TWW treatments, significant differences were mainly observed between I1 at stage 1 (minimum value) and I4 at stage 3 (maximum value), whereas the remaining combinations were statistically similar. In contrast, a clear increase in Tc–Ta with increasing water stress was observed under FW only at the first measurement stage. At comparable irrigation levels, stage 2 generally exhibited higher Tc–Ta values, except for I4, where the maximum occurred at stage 3. Some deviations from the general trend were observed: FW × I2 × 3 displayed an unexpectedly low Tc–Ta (1.85 °C) despite moderate stress, while FW × I1 × 2 showed a relatively high value (6.47 °C) under optimal irrigation.

The WT × MC × PS interaction (Figure 7) was significant for SPAD, SWP, Gs, and NDVI, showing patterns partly consistent with those described for the previous interaction.

For SPAD (Figure 7A), no clear differences emerged between water types or microbial treatments across most phenological stages. A noticeable divergence was observed only at the final stage (BBCH 69), where FW × M+ showed higher SPAD values compared with the other treatments evaluated at the same stage.

Regarding SWP (Figure 7B), no consistent differences were detected between TWW and FW when microbial treatment and phenological stage were comparable, except at BBCH 37 with inoculation, where TWW resulted in less negative SWP values. At

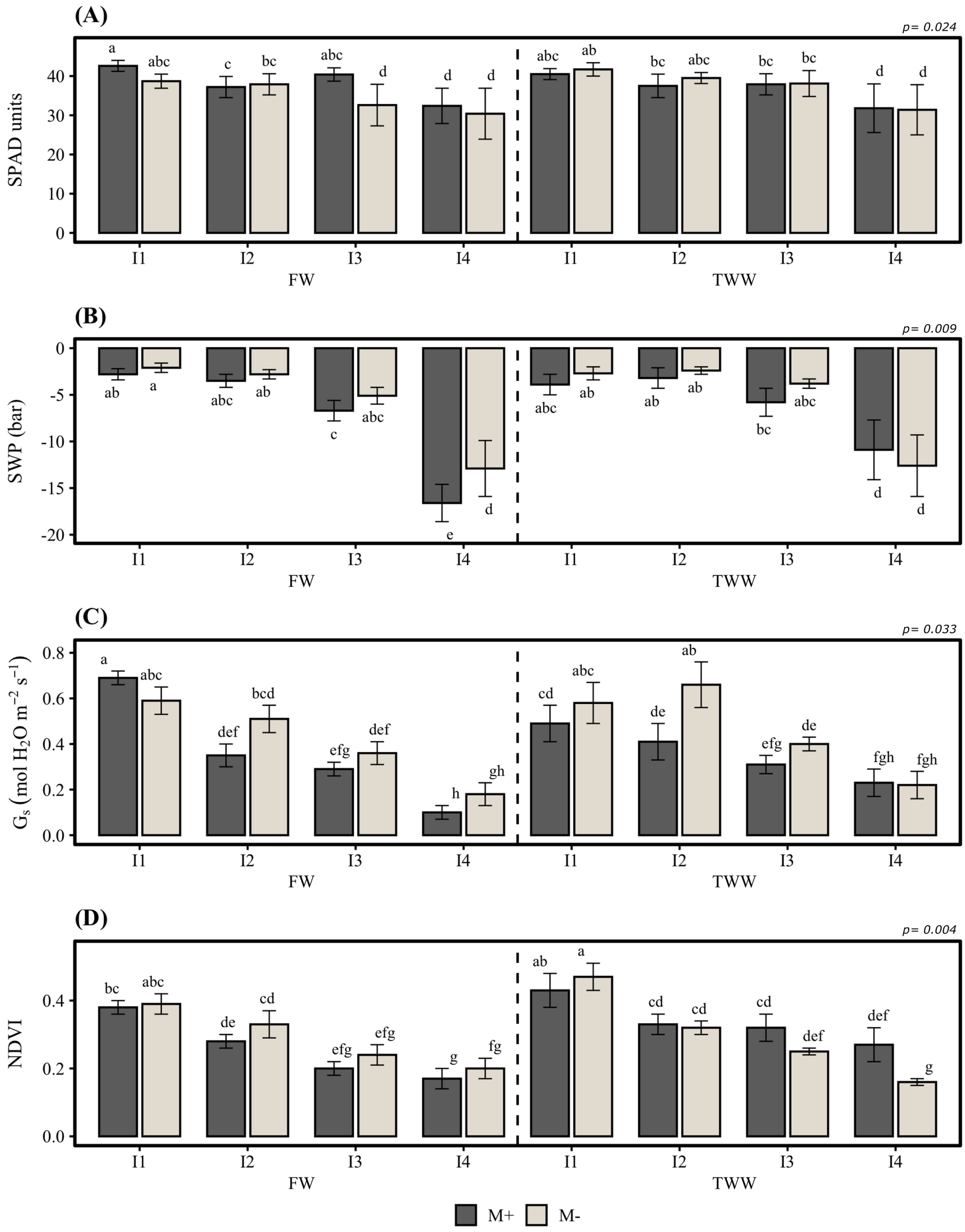


FIGURE 5
Effect of water type × water level C microbial consortium on SPAD, SWP, Gs, NDVI. **(A)** SPAD; **(B)** SWP; **(C)** Gs; **(D)** NDVI. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Error bars represent standard error. SWP, stem water potential; Gs, stomatal conductance; NDVI, normalized difference vegetation index. Water type (FW, fresh water; TWW, treated wastewater), water level (I1, 100% FC; I2, 70% FC; I3, 50% FC; I4, 30% FC), microbial consortium (M−, absence; M+, presence). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at $p \leq 0.05$.

BBCH 51, non-inoculated plants exhibited higher (less negative) SWP than inoculated ones, whose values were comparable to those recorded at BBCH 69 under more severe stress conditions.

The highest stomatal conductance (Gs; Figure 7C) was recorded at BBCH 37 in plants irrigated with TWW (both M+ and M−) and in FW × M−.

NDVI (Figure 7D) displayed a clearer differentiation among treatments. The highest mean value was recorded in TWW × M+ at the intermediate stage, whereas the lowest occurred in FW × M+ at the final stage. Overall, plants irrigated with TWW appeared to benefit from microbial inoculation only at BBCH 51.

Finally, RWC was significantly influenced only by the WL × PS interaction ($p < 0.05$) (Figure 8). Leaf relative water content declined with increasing water stress, ranging from 93.2% (I1, BBCH 51) to 64.8% (I4, BBCH 69). At the intermediate stage, differences among irrigation levels were less pronounced.

3.4 Correlations between the parameters

To explore the relationships among the analyzed physiological and agronomic parameters, a correlation matrix was constructed by aggregating the data collected across the three phenological stages

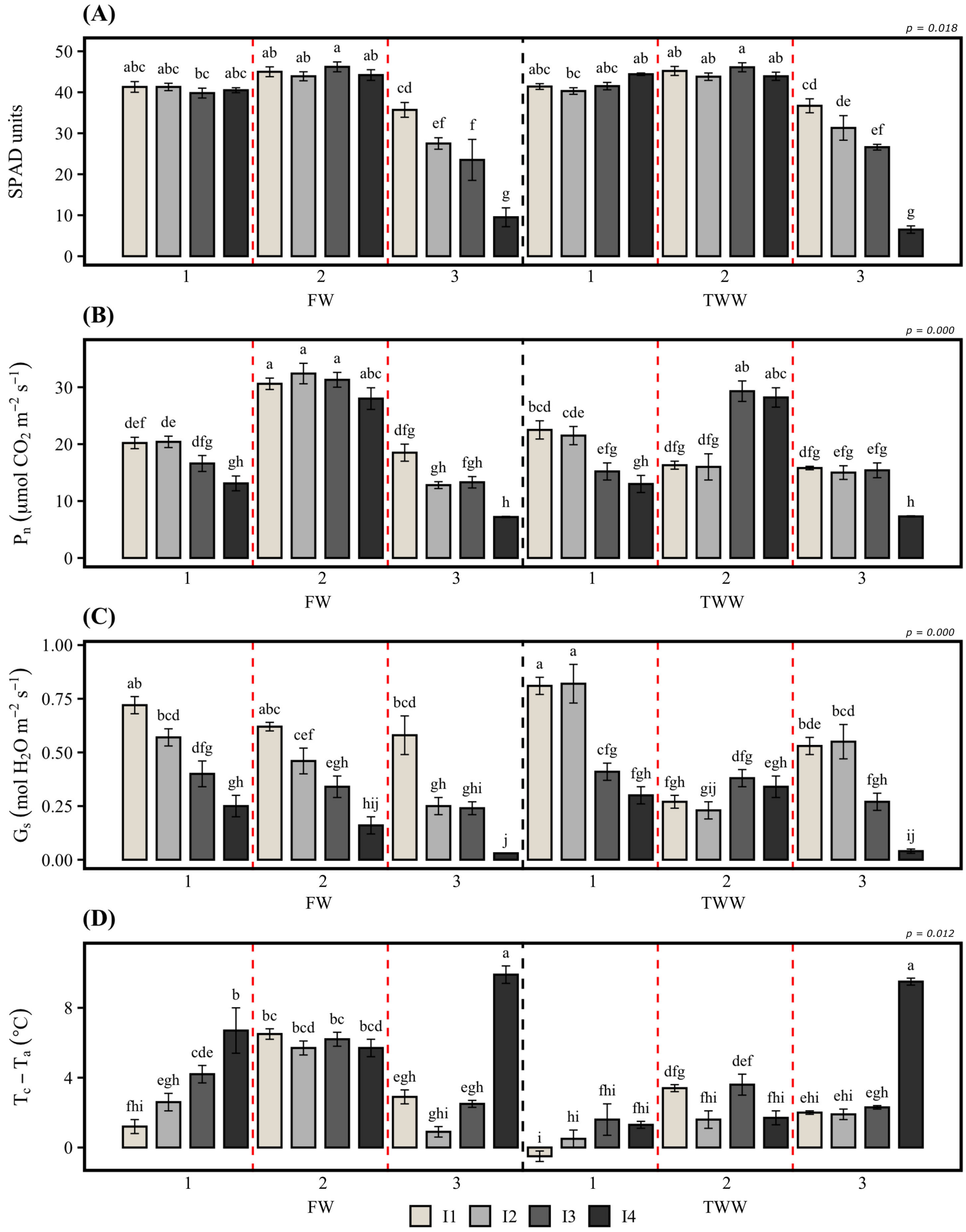


FIGURE 6
Effect of water type × water level × phenological stage on SPAD, Pn, Gs, Tc - Ta. **(A)** SPAD; **(B)** Pn; **(C)** Gs; **(D)** Tc − Ta. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Error bars represent standard error. Pn, net photosynthesis; Gs, stomatal conductance; Tc − Ta, difference between canopy and air temperature. Water type (FW, fresh water; TWW, treated wastewater), water level (I1, 100% FC; I2, 70% FC; I3, 50% FC; I4, 30% FC), phenological stage (1, 37 BBCH; 2, 51 BBCH; 3, 69 BBCH). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at $p \leq 0.05$.

(BBCH 37, 51, and 69) (Figure 9). This approach allowed the evaluation of covariation among the considered variables on a global scale, independently of the temporal effects already highlighted by repeated-measures ANOVA. Correlations were considered significant at $p < 0.05$.

Strong positive relationships ($r > 0.7$) were observed among morphological traits. In particular, plant height was strongly correlated with spike length ($r = 0.80$) and grain yield ($r = 0.77$), while grain yield also showed a strong association with total biomass yield ($r = 0.92$). Except for the number of nodes, the main morphological traits were moderately to strongly correlated with physiological indices, with the exception of net photosynthesis (Pn), which generally showed weak correlations.

Grain yield was strongly correlated with NDVI ($r = 0.71$), moderately with RWC and Gs ($r \approx 0.50$), and with SWP ($r = 0.57$).

Analysis of the physiological variables revealed strong linear relationships, as confirmed by high Pearson correlation coefficients, indicative of a tight functional connection among the measured

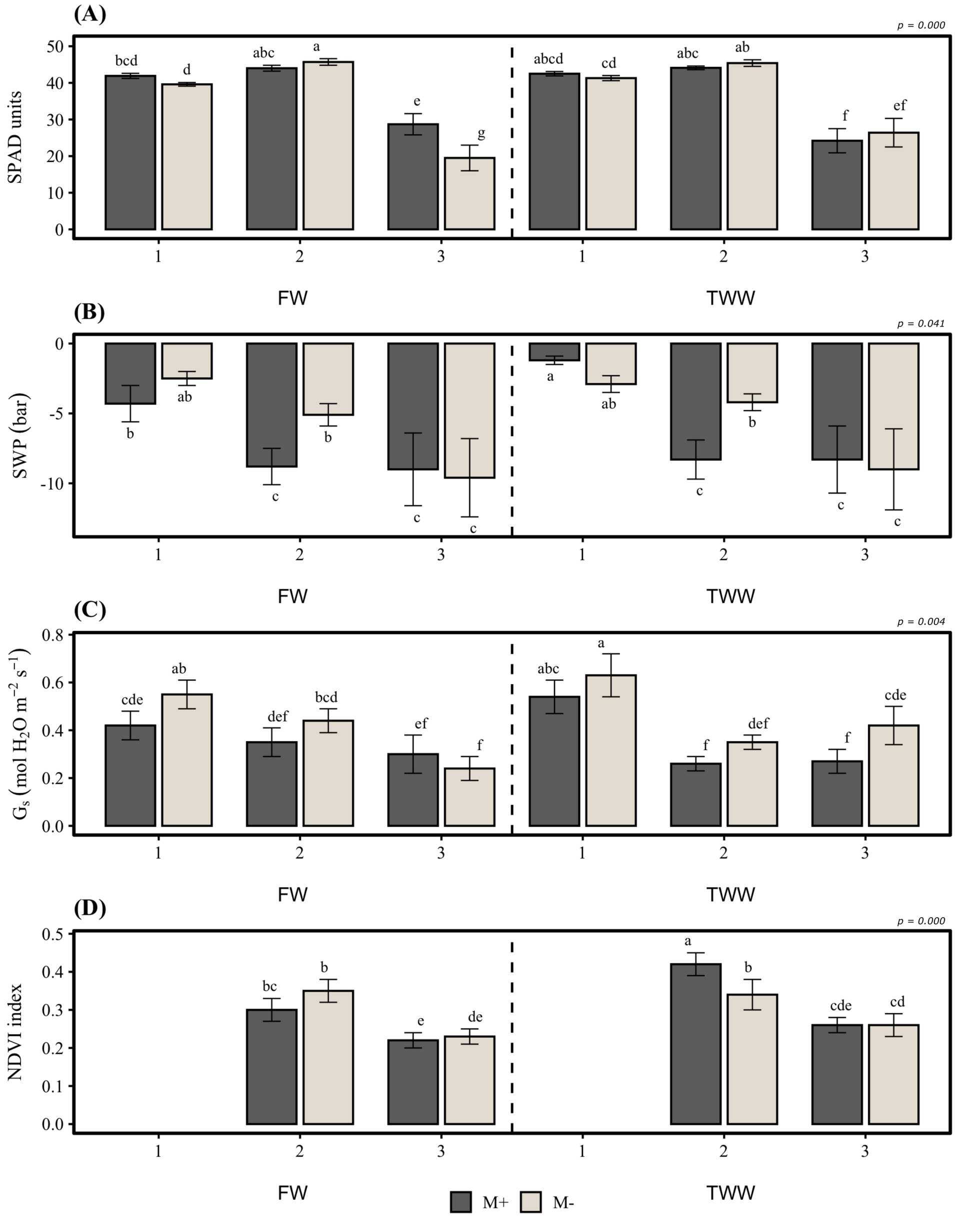


FIGURE 7
Effect of water type × microbial consortium × phenological stage on SPAD, SWP, Gs, NDVI. **(A)** SPAD; **(B)** SWP; **(C)** Gs; **(D)** NDVI. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Error bars represent standard error. SWP, stem water potential; Gs, stomatal conductance; NDVI, normalized difference vegetation index. Water type (FW, fresh water; TWW, treated wastewater), microbial consortium (M–, absence; M+, presence), phenological stage (1, 37 BBCH; 2, 51 BBCH; 3, 69 BBCH). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at $p \leq 0.05$.

parameters. Net photosynthesis, however, generally showed weak correlations, except for a moderate positive correlation with Gs ($r = 0.62$) and SWP ($r = 0.58$).

Stomatal conductance showed strong associations with the other physiological indicators, for example exhibiting a very strong positive correlation with SWP ($r = 0.81$). In this case, regression analysis indicated that a high proportion of Gs variability was explained by SWP ($R^2 = 0.657$).

Finally, the difference between leaf and air temperature (Tc–Ta) showed strong negative correlations with Gs ($r = -0.66$) and SWP ($r = -0.77$). Regression analysis also revealed that a substantial proportion of the variability in Tc–Ta was explained by SWP ($R^2 = 0.585$).

4 Discussion

The results of this study, conducted on potted durum wheat plants, clearly demonstrated that the use of treated wastewater (TWW) did not cause physiological disturbances nor compromise plant performance compared to conventional freshwater (FW) irrigation, regardless of the water supply level or the presence of the microbial complex. On the contrary, for most of the parameters analyzed, TWW showed comparable, or in some cases slightly superior, effects relative to FW.

In the literature, the use of TWW in agriculture has produced contrasting results, often related to variables such as the type and

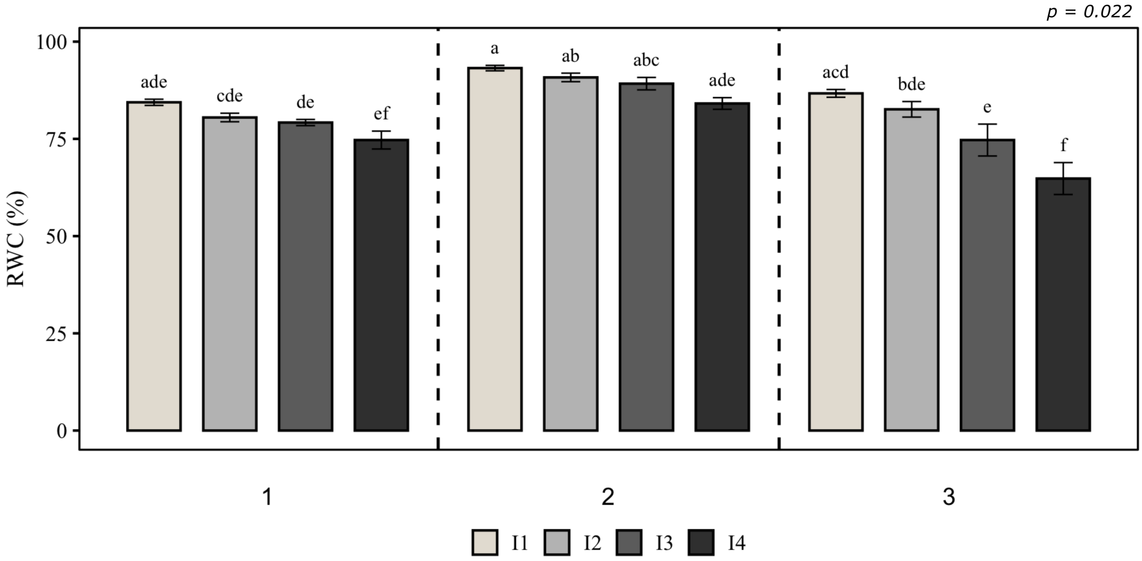


FIGURE 8
Effect of water level × phenological stage on relative water content (RWC). For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Error bars represent standard error. Water level (I1, 100% FC; I2, 70% FC; I3, 50% FC; I4, 30% FC), phenological stage (1, 37 BBCH; 2, 51 BBCH; 3, 69 BBCH). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at $p \leq 0.05$.

treatment level of the wastewater, soil characteristics, environmental conditions, and irrigation practices (Saleh et al., 2025; Rezapour et al., 2019).

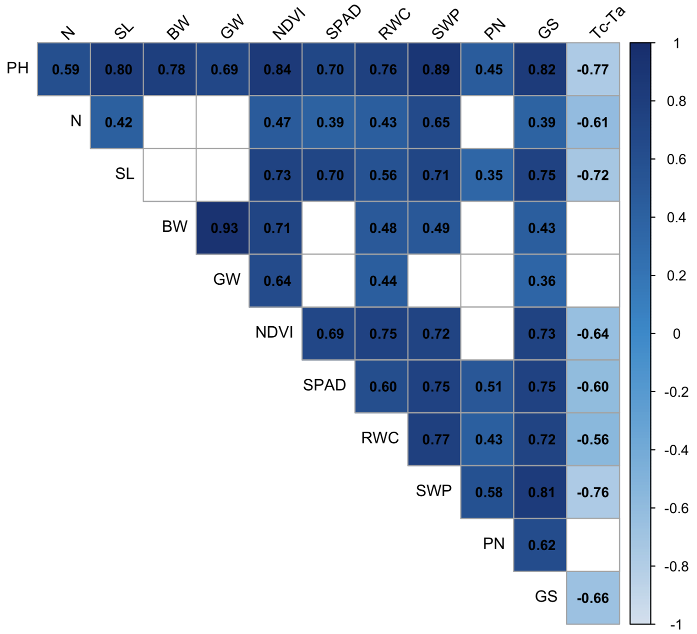
In our study, pots irrigated with TWW exhibited electrical conductivity (EC) levels similar to or lower than those irrigated with FW, remaining almost always below 3 mS cm^{-1}. This level does not pose a risk for wheat, a species moderately tolerant to salinity, whose yield reduction threshold is 6 mS cm^{-1} (Gómez-Bellot et al., 2015; Maas and Hoffman, 1977; Malipatil et al., 2025). Except for one observation, plants did not show signs of osmotic stress, altered water uptake, or decoupling between stem and matric potential, unlike what has been reported in previous studies (Hassena et al., 2021; Janda et al., 2016). Furthermore, heavy metal analysis (Cr, Ni, Pb, Cu, Zn) revealed concentrations below 0.1 mg L^{-1} (Supplementary Table S1, Supplementary Materials), well within the limits established by DM 185/2003 (Ministero Della Salute, 2003) for Category B water currently in force in Italy. These levels are sufficiently low to avoid physiological imbalances in the plants (Almuktar et al., 2015).

Irrigation with TWW induced, at the same water level, slight improvements in certain vegetative parameters, such as plant height, spike length, and node number. These effects were also supported by higher NDVI values. These results cannot be attributed to the microbial complex, as no substantial differences were observed between M– and M +. Conversely, FW-irrigated plants tended to show slightly higher values in M– under the most stressed conditions; for example, culm length was greater in I2, I3, and I4, node number in I4, and spike length in I3.

Similar studies report positive effects of TWW on vegetative growth (Cakmakci and Sahin, 2021; Elfanssi et al., 2018; Rezapour et al., 2019). Although these effects are often attributed to a higher availability of macronutrients (mainly N and P) in wastewater, in our case this hypothesis appears unlikely, given the low concentrations detected (< 5.0–17.3 mg L^{-1} N; 0.3–1.8 mg L^{-1} P). Additionally, SPAD values, which reflect chlorophyll concentration and thus the nitrogen nutritional status of plants, generally did not differ significantly between TWW and FW. Therefore, we suggest that the observed increases may instead be attributed to the presence of microelements or essential cations (Na^+, K^+, Ca^{2+}, Mg^{2+}), which were not directly analyzed in our waters but are frequently reported in the literature as present in TWW (Elfanssi et al., 2018; Singh et al., 2012). In particular, K^+ and Mg^{2+} are known to support stomatal regulation and photosynthetic activity (Hasanuzzaman et al., 2018; Li et al., 2020), which is consistent with the modest increases in stomatal conductance observed in this study.

At the productive level, the main differences between TWW and FW were observed in inoculated plants. Under FW conditions, microbial inoculation (M+) was consistently associated with lower total yield across irrigation levels. Under TWW, differences among treatments were limited and not statistically significant. However, a 26% increase in grain yield was observed at I1 in mycorrhizal plants (TWW × I1 × M+), whereas a 25% reduction occurred at I3. This trend was also reflected in the harvest index (HI).

Other studies on wheat and other cereal crops have reported yield increases associated with TWW compared to FW (Khaskhoussy et al., 2022; Rezapour et al., 2019; Samarah et al., 2020; Singh et al., 2012). However, the use of TWW does not always result in significant production gains, likely due to variability in the chemical composition of these waters, particularly regarding micro- and macronutrient content. For instance, in a study conducted in Iran by Saleh et al. (2025) on various crops (wheat, maize, barley, and alfalfa), the effects of TWW irrigation were variable and, in some cases, not significantly different from those obtained with FW. Conversely, other authors (Hajihashemi et al., 2020; Nawaz et al., 2021; Werfelli et al., 2021), in experiments on Triticum aestivum, reported negative effects from the use of untreated or insufficiently treated wastewater, characterized by high salt and Cd and Cr concentrations. Under these conditions, photosynthetic activity and chlorophyll biosynthesis



	N	SL	BW	GW	NDVI	SPAD	RWC	SWP	PN	GS	Tc-Ta
PH	0.59	0.80	0.78	0.69	0.84	0.70	0.76	0.89	0.45	0.82	-0.77
N		0.42			0.47	0.39	0.43	0.65		0.39	-0.61
SL					0.73	0.70	0.56	0.71	0.35	0.75	-0.72
BW				0.93	0.71		0.48	0.49		0.43	
GW					0.64		0.44			0.36	
NDVI						0.69	0.75	0.72		0.73	-0.64
SPAD							0.60	0.75	0.51	0.75	-0.60
RWC								0.77	0.43	0.72	-0.56
SWP									0.58	0.81	-0.76
PN										0.62	
GS											-0.66

FIGURE 9
Correlation matrix between agronomic and physiological variables. PH, plant height; N, nodes; SL, spike length; BW, biomass weight; GW, grain weight; NDVI, Normalized Difference Vegetation Index; RWC, relative water content; SWP, stem water potential; Pn, net photosynthesis; Gs, stomatal conductance; Tc − Ta, difference between canopy and air temperature. Pearson's r values are shown only for statistically significant correlations ($p \leq 0.05$).

were impaired, resulting in reduced growth and productivity parameters. These findings highlight the importance of TWW treatment level, as water quality can determine either beneficial or detrimental effects depending on the case.

Regarding the responses to microbial inoculation (AMF), these were not consistent. Under certain conditions (FW with moderate/severe water stress), a slight decrease in vegetative and productive parameters was observed. This effect could result from competition between inoculated mycorrhizae and native populations: the former may colonize roots more effectively but are more sensitive to stress (Baslam et al., 2014). Moreover, the higher salinity of FW compared to TWW might have reduced the effectiveness of the symbiosis.

Although the literature widely reports the use of AMF to mitigate the negative effects of abiotic stress (Akensous et al., 2022; Hassena et al., 2021; Ould Amer et al., 2023), it is known that AMF responses vary depending on the fungal strain, stress level, and soil conditions. Studies show reduced mycorrhizal colonization under continuous irrigation with saline wastewater or during prolonged stress (Gómez-Bellot et al., 2015). Other studies (Al-Karaki et al., 2004; Baslam et al., 2014) indicate that colonization peaks at heading and then declines under stress. Yaghoubian et al. (2014) observed that drought stress at −5 bar favors AMF, but at −10 bar the symbiosis is compromised due to reduced photosynthesis. This negative effect is attributed to decreased plant photosynthetic rate under drought, leading to lower carbon availability for symbiotic fungi and reduced symbiotic efficiency and intensity.

Analysis of wheat physiological responses at different phenological stages revealed complex interactions between the studied factors. As expected, physiological parameters generally declined with increasing water stress.

For example, both SWP and Gs markedly decreased in M+ treatments under high stress (I3 and I4), particularly when using FW. In I4, SWP dropped from −12.9 bar (M−) to −16.5 bar (M+). Conversely, in FW × I1 × M+ higher SPAD and Gs values were recorded compared to M−. In TWW, M− generally exhibited higher stomatal conductance than M+, except under I4, where no significant differences were observed.

During stem elongation (BBCH 37), there was a clear distinction between less stressed treatments (I1 and I2) and those under severe stress (I3 and I4), as well as between TWW and FW. Physiological values were clearly differentiated, particularly stomatal conductance, which ranged from 0.82 to 0.30 mol m^{-2} s^{-1}. Although ANOVA did not reveal statistically significant differences in canopy–air temperature difference (Tc–Ta) among TWW-irrigated treatments, particularly across irrigation levels, these variations are worth noting. For instance, Tc–Ta ranged from −0.47 °C in TWW × I1 to 1.55 °C in TWW × I3, in line with literature reports showing that even small thermal differentials can affect stomatal conductance and photosynthesis (Baker et al., 2007). FW × I2, I3, and I4 treatments showed higher Tc–Ta values (4.2–6.7 °C), associated with lower stomatal conductance; for example, FW × I2 experienced a 30% reduction compared to the same treatment irrigated with TWW, decreasing from 0.82 to 0.57 mol m^{-2} s^{-1}. This suggests that, at this stage, FW-irrigated plants exhibited signs of osmotic stress (Hassena et al., 2021).

During heading (BBCH 51), SPAD, photosynthesis (Pn), and RWC were high and similar across water levels, consistent with peak vegetative development (Lawrence et al., 2019; Rezzouk et al., 2022). However, stomatal conductance remained sensitive to stress, dropping to 0.16 mol m^{-2} s^{-1} in FW × I4. Unexpectedly, TWW × I1 and I2 showed reduced Gs and Pn despite high SWP (~5 bar), suggesting possible atypical physiological stress, potentially related to water quality.

The high photosynthetic rates observed at this stage (27.97–31.25 μmol m^{-2} s^{-1}) may appear inconsistent with the severe water stress conditions recorded in I3 and I4, where soil moisture approached the wilting point and SWP and Tc–Ta values reached −7.25 bar and 6.16 °C in I3, and −11.91 bar and 5.68 °C in I4. However, this apparent discrepancy can be explained by a predominantly stomatal limitation that had not yet translated into a substantial biochemical impairment of the photosynthetic machinery. Stomatal conductance, which is closely related to plant water status and widely considered a direct indicator of water stress (Schulze et al., 1987), declined markedly under stress, consistent with the well-established effect of stomatal closure in limiting CO_2 uptake and modifying leaf energy balance (Blonquist et al., 2009; Kettani et al., 2023). Despite this reduction in Gs, SPAD and RWC values remained high, indicating preserved chlorophyll content and maintained leaf turgor. Elevated RWC under stress conditions may reflect a conservative stomatal regulation strategy aimed at maintaining leaf hydration during drought (Hassena et al., 2021). Such a response is typical of isohydric species like wheat, in which early stomatal adjustment stabilizes plant water status despite progressive soil drying (Jones, 2007). Under these conditions, the stress signal was evident at the hydraulic and stomatal level but had not yet induced a significant metabolic limitation of carbon assimilation, allowing photosynthetic rates to remain relatively high at this phenological stage.

Although physiological parameters were not yet critical during BBCH 37 and 51, the flowering stage (BBCH 69) showed the most pronounced differences between water levels, with decreasing values, particularly in I3 and I4. This pattern confirms wheat's capacity to tolerate water stress up to a threshold, mainly through avoidance strategies such as stomatal regulation that reduce water consumption (Pantha et al., 2024).

At this stage, I4 showed indicators of severe stress: Gs 0.03 mol $H_2O\ m^{-2}\ s^{-1}$, Pn 7.22 µmol $m^{-2}\ s^{-1}$, and Tc–Ta 9.87 °C, the highest value recorded. I3 also showed reduced Gs (0.24–0.27 mol $m^{-2}\ s^{-1}$). Low SPAD values in I3 and I4 indicate accelerated leaf senescence (Pantha et al., 2024).

Therefore, the limited physiological differentiation observed during the second measurement phase, despite reduced soil water content and water potential, likely reflects the plant's temporary buffering capacity under moderate stress. However, the cumulative effect of prolonged water deficit eventually exceeded this compensatory threshold, leading to pronounced physiological impairment at flowering.

Overall, the strong correlation between Tc–Ta, Gs, and SWP confirms that Tc–Ta is a reliable indicator of plant water status (Blonquist et al., 2009; Kettani et al., 2023). This index allows rapid assessment of stress conditions, showing consistency with direct measurements at all phenological stages. Several studies also report its correlation with soil water availability (Ko and Piccinni, 2009). Similarly, NDVI proved useful for indirectly evaluating plant vigor in response to different irrigation and mycorrhizal treatments (Tortorici et al., 2025).

Although many studies report a strong correlation between net photosynthesis (Pn) and stomatal conductance (Gs), the moderate correlation observed in this study (r = 0.62) likely reflects the predominance of stomatal limitations under mild to moderate water stress. Under more severe deficit, non-stomatal limitations (e.g., metabolic downregulation) may increase, but in our experimental context, stomatal control and water-status regulation were the primary drivers of photosynthetic response at the intermediate stages.

Despite this experiment was conducted over a single growing season in pots, the factorial design and multifactorial ANOVA provide robust insight into the interactions among irrigation level, water quality, and microbial inoculation. The observed decoupling between high photosynthetic rates and final yield under severe water stress highlights that physiological performance does not always predict productivity. These results stress the importance of considering not only water source and irrigation intensity, but also the context-dependent effects of microbial inoculation and the potential interactions with native soil microbiota. Integrative, systems-based approaches will be essential for designing irrigation strategies that are both efficient and resilient under increasing water scarcity.

5 Conclusion

Overall, the results of the present experiment support the use of treated wastewater (TWW) as a sustainable strategy for irrigating durum wheat, as it showed vegetative, physiological, and productive effects comparable to, and in some cases slightly higher than, those observed with conventional freshwater (FW), without evidence of phytotoxicity. These findings reinforce the potential of TWW in water-scarce environments, contributing to more efficient water resource management in agriculture.

The integration of water stress and the microbial consortium provided further insight into the physiological response mechanisms of the crop. The absence of significant differences in SPAD values up to the heading stage (BBCH 51), particularly across irrigation levels, indicates that the photosynthetic system was not severely compromised during the early growth stages. During this phase, plants maintained effective stomatal regulation, with relatively high values of net photosynthesis (Pn) and leaf water potential (SWP). Gas exchange parameters suggest that moderate deficit irrigation (70%) could theoretically be implemented without compromising the physiological potential of the crop. However, under the experimental conditions adopted — characterized by limited soil volume — this physiological potential did not translate into a corresponding increase in yield, as also reflected by NDVI values.

The effect of the microbial consortium proved to be heterogeneous and, in some cases, negative, particularly under severe water stress and under FW conditions. This highlights the need for targeted evaluations based on pedo-climatic characteristics, water quality, and potential interactions with native soil microbiota, as the introduction of microbial consortia does not always result in agronomic benefits and may, under certain conditions, be neutral or even detrimental.

Further long-term field studies will be necessary to validate these findings and to define integrated irrigation and microbial management strategies that are truly sustainable and applicable in Mediterranean agricultural systems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

NT: Formal analysis, Writing – original draft, Conceptualization, Investigation, Software. AG: Supervision, Methodology, Data curation, Formal analysis, Writing – original draft. CM: Investigation, Writing – original draft, Software. MS: Validation, Writing – review & editing. TT: Project administration, Writing – review & editing, Conceptualization. NI: Formal analysis, Writing – review & editing, Writing – original draft, Data curation, Validation, Supervision, Conceptualization, Methodology, Project administration.

Funding

The author(s) declared that financial support was not received for this work and/or its publication.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

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