Complex microbial consortia improve yield and physiological performance of leafy greens under deficit irrigation

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Abstract

Water scarcity is an increasing constraint on agricultural productivity and demands scalable strategies that improve crop performance under reduced irrigation. As soil microorganisms regulate key processes at the soil–plant interface, microbial inoculants may help sustain plant growth and physiological function during water limitation. Here, we assembled five functionally diverse microbial consortia containing taxa selected to support rhizosphere colonization, soil structural stabilization, and fungal-mediated nutrient and water foraging. These consortia were evaluated in greenhouse trials with lettuce and spinach grown under full irrigation or a 30% deficit irrigation regime (70% of crop water requirement). Crop responses were assessed using yield, harvest delay, root length, wilting incidence, chlorophyll content, and Water Band Index (WBI). Across both crops, microbial consortium treatments improved performance under deficit irrigation relative to untreated water-stressed controls. In lettuce, yield increased by 3–9%, while in spinach yield increased by 4–13%, with several treatments restoring performance to levels not significantly different from the fully irrigated control. Microbial treatments also reduced harvest delay by an average of three to four days, improved root length, lowered wilting incidence, and reduced WBI, indicating reduced plant water stress. In several cases, these physiological responses approached those observed under full irrigation despite 30% lower water input. Higher application rates (500 vs 250 g h^{-1}) generally produced stronger responses, although this trend was not always statistically significant. Together, these results show that complex microbial consortia can buffer the negative effects of deficit irrigation and improve crop performance in leafy greens. These findings support the development of microbial inoculants as biologically based tools to enhance agricultural resilience under increasing water scarcity.

Teaser: Microbial soil inoculants help crops maintain yield and harvest synchrony under reduced irrigation.

Introduction

Agricultural productivity is increasingly constrained by water scarcity, soil degradation, and climate variability, with drought emerging as a primary limitation across global cropping systems (Intergovernmental Panel on Climate Change (IPCC) 2023; Lesk, Rowhani, and Ramankutty 2016; Daryanto, Wang, and Jacinthe 2016). Reduced water availability disrupts essential plant physiological processes, including photosynthesis, nutrient uptake, and growth, ultimately leading to yield instability and economic losses (Blum 2010). Leafy vegetables represent roughly 18% of the EU's fresh vegetable cultivation area according to the EU's statistical overview reports in 2024. This is a sector facing intensifying irrigation pressure as water scarcity deepens across European growing regions. In addition to climatic drivers, soil structure strongly influences plant-available water, as aggregation, porosity, and organic matter content regulate water retention and root access to moisture under stress (Six et al. 2002; Tisdall and Oades 1982). Soil microorganisms are central regulators of the soil–plant water interface, influencing both soil physical properties and plant responses to water stress through a range of mechanisms (Ngumbi and Kloepper 2016; Vurukonda et al. 2016). For example, microbial production of extracellular polymeric substances (EPS) promotes soil aggregation and can enhance pore connectivity and water retention (Costa, Raaijmakers, and Kuramae 2018; Chenu 1993). Symbiotic fungi and filamentous soil bacteria also extend the effective root system through hyphal networks, increasing plant access to water and nutrients beyond rhizosphere depletion zones (Nadeem et al. 2014).

Most studies exploring the potential of microbial inoculants to support plant physiology during stress have focused on single- or dual-species formulations, which may perform inconsistently across heterogeneous soil environments and variable stress conditions (Ocallaghan, Ballard, and Wright 2022). In contrast, natural soil microbiomes function as complex, interacting communities (Zhalnina et al. 2018). This has motivated the development of complex multi-species inoculants that more closely reflect the functional diversity of natural microbial systems (Beattie et al. 2026; Zhang, Jing, et al. 2025; Alzate Zuluaga et al. 2024; Zhang, Gilbert, et al. 2025; Coker et al. 2022). Such microbial consortia, including both bacteria and fungi, may provide broader functional coverage, with different members contributing to soil aggregation, metabolite production, nutrient exchange, and access to water beyond rhizosphere depletion zones (Liu et al. 2024). As a result, these complex multi-species consortia may improve plant-available water and enhance stress

resilience at the system level, particularly under challenging climatic conditions and in degraded soils.

To capture a broad functional diversity, we assembled microbial consortia from a diverse culture collection of beneficial soil microorganisms, including spore-forming bacteria (with emphasis on *Streptomyces*), complementary non-*Streptomyces* bacteria, and mycorrhizal fungi (**Table 1**). Selected strains were assembled into five distinct consortia, comprising between 12 and 28 taxonomic units, to increase functional redundancy. Standardized production and formulation workflows were applied, including separate bacterial and fungal propagation, quality control of viable cells and spores, and final blending into multi-organism products (**Fig. 1**). To evaluate whether this functional complementarity translates into improved crop performance under water limitation, we conducted greenhouse experiments in leafy greens (lettuce and spinach) in Spain, grown under full and 30% reduced irrigation regimes.

Microbial culture collection

Streptomyces

Non-Streptomyces

Mycorrhiza fungi (AMF and EMF)

Strain selection criteria

1. Prioritize spore-forming microbes
2. Build streptomyces-rich core consortium
3. Combine with compatible non-streptomyces bacteria (e.g. water use and nutrient use efficiency)
4. Select fungi for nutrient exchange and stress

Formulation and quality control

• Bacterial production: seed culture → agar plate fermentation → biomass harvest → drying → live cell and spore count QC, flow cytometry
• Fungal production: fruiting body collection / commercial spore purchase → spore processing → drying → spore count QC (hemocytometer)
• Mixing bacteria and fungi with dextrose to formulate final products

OSL consortium for field testing

Figure 1. Assembly and production of microbial consortia for field evaluation. Oath Biome's culture collection comprises three organism groups: *Streptomyces* spp. (filamentous Actinobacteria), non-S*treptomyces* bacteria (including members of Bacillales), and mycorrhizal fungi (arbuscular mycorrhizal fungi, AMF; and ectomycorrhizal fungi, EMF). Strain selection followed four criteria: (1) prioritization of spore-forming microbes for product stability; (2) construction of a Streptomyces-rich core consortium; (3) incorporation of compatible non-*Streptomyces* bacteria with complementary functional traits; and (4) inclusion of mycorrhizal fungi to support nutrient acquisition and drought tolerance. Selected strains underwent separate production pipelines: bacteria were produced via agar plate fermentation, followed by biomass harvest, drying, and grinding, with live cell and spore counts verified by flow cytometry; fungal material was obtained through field collection of fruiting bodies or commercial spore purchase, followed by spore processing, drying, and grinding, with spore counts verified by hemocytometer. Bacterial and fungal components were then mixed with dextrose as an osmoprotectant to formulate five wettable powder products (OSL consortia A–E; viable counts $\geq 10^7$ CFU g^{-1}; composition detailed in Table 1), which were evaluated in greenhouse and field trials across lettuce and spinach under full and deficit irrigation.

Materials and Methods

Taxonomic classification of bacteria and fungi. Genomic sequences used for the identification of bacterial strains (all derived from Oath Inc.'s strain collection) were obtained through whole-genome sequencing of the strains listed in **Table 1**, using either Illumina paired-end or Oxford Nanopore sequencing. Illumina paired-end reads were preprocessed using Fastp (Chen et al. 2018) and preprocessed reads were assembled using SPAdes (Bankevich et al. 2012) through VEBA (Espinoza and Dupont 2022; Espinoza et al. 2024). Oxford Nanopore reads were preprocessed using Fastplong (Chen 2023), preprocessed reads were assembled with Flye (Wick et al. 2021), and assemblies were polished using Medaka implemented through VEBA. Each assembly met high-quality genome standards via CheckM2 (Chklovski et al. 2023; Sorensen et al. 2026), and taxonomy classified using GTDB-Tk (release 226) (Chaumeil et al. 2022). Arbuscular mycorrhiza fungal spores from *Rhizophagus irregularis, Cloroideoglomus etunicotum, Funneliformis mosseae* were classified by the supplier Mycointech, Tarragona, Spain. *Glomus aggregatum* was

characterized by the supplier UmaHari LLC, New York, USA. Ectomycorrhiza fungi spores were obtained from *Pisolithus tinctorius, Rhizopogon amylopogon, Rhizopogon fulvigleba, Rhizopogon luteolus, Rhizopogon villosullus* and *Scleroderma cepa, Scleroderma citrinum* by collecting whole fruiting bodies in the southern Oregon Cascades, the United States during 2024 and 2025 by Dr. Michael Amaranthus at Mycorrhizal Applications Inc., OR, USA. Fruiting body morphological characteristics for each species were carefully examined in the field. In the laboratory, spores were microscopically examined for size, shape and color. Spore counts were quantified with hemocytometer (Parker and Barnes 1967). To process the fruiting bodies the sterile bases of whole fruiting bodies were removed, and the remaining spore bearing tissue cleaned and sliced for drying. The spores containing tissues were dried at 54°C for 100 hrs in a dehydrator and ground into a fine powder with a steel food grade grinder. Finished concentrated powders were tagged and sealed in a sterile plastic bag for each specimen.

Table 1. Counts of strains per species composing each product. Strains described as 'spp.' represent novel or recently discovered species for which there is no full species name currently available. (PCT patent publication WO2025097154, Microbial Compositions and Methods)

Species	**A**	**B**	**C**	**D**	**E**
Number of Bacterial Strains	28	55	41	12	19
Arthrobacter spp.		2	2	3	1
Bacillus haynesii		1	1	1	1
Bacillus inaquosorum		2	1	1	1
Bacillus licheniformis		1	1		1
Bacillus paralicheniformis					1
Bacillus safensis		2	2		1
Bacillus spizizenii		1			1
Bacillus subtilis		2	3		1
Bacillus velezensis	1		2		
Kocuria oxytropis		1			1
Lysinibacillus capsici		1		1	
Lysinibacillus sphaericus	1				
Microbacterium spp.	1	1	1		
Paenibacillus cellulositrophicus	2	1	2	1	2
Peribacillus frigoritolerans		2	2		3
Priestia aryabhattai		1	2	1	1
Priestia endophytica		1	1		1
Priestia megaterium		1	1		1
Pseudomonas spp.		1			
Rhodococcus baikonurensis	1	1	1		
Streptomyces achromogenes	2				1
Streptomyces albogriseolus		1	1		

Streptomyces bacillaris	2	2	1		
Streptomyces bikiniensis		2			
Streptomyces eurythermus	1	1			
Streptomyces fimbriatus		1	1		
Streptomyces globosus	4	7	5	1	
Streptomyces griseoaurantiacus		1	1		
Streptomyces griseoincarnatus	2	1			
Streptomyces griseoviridis		1			1
Streptomyces misionensis	2	6	5	1	
Streptomyces murinus	3	1	1		
Streptomyces nogalater	1				
Streptomyces olivaceus		1		1	
Streptomyces rameus	2	2		1	
Streptomyces rochei		2	4		
Streptomyces sennicomposti	3	3			
Number of Fungal Strains	0	11	4	8	11
Cloroideoglomus etunicatum		1	1	1	1
Funneliformis mosseae		1	1	1	1
Glomus aggregatum		1	1	1	1
Pisolithus tinctorius		1			1
Rhizopogon amylopogon		1		1	1
Rhizopogon fulvigleba		1		1	1
Rhizopogon luteolus		1		1	1
Rhizopogon villosulus		1		1	1
Rhizophagus irregularis		1	1	1	1
Scleroderma cepa		1			1
Scleroderma citrinum		1			1

Microbial consortium formulation. The bacterial components (**Table 1**) of products A-E were produced using agar growth surface fermentation. The workflow comprised media preparation (liquid media and agar media), seed culture generation, plate-scale biomass production, harvesting biomass from the top of the plates, drying of biomass, grinding dried biomass into a powder, mixing with dextrose sugar to provide an osmoprotectant and a mixing agent, and quality control that included visual inspection of growing bacteria on agar plates at multiple timepoints during incubation in 28 °C. All bacterial strains were cultivated on ISP Medium No. 3 agar (19 g L^{-1}; HiMedia M358). Seed cultures were grown in glucose broth containing glucose (10 g), yeast extract (4 g), peptone (4 g), and calcium chloride dihydrate (0.5 g) in 500 mL of distilled water. Both agar and liquid media were sterilized by autoclaving at 121 °C for 15 min and cooled before inoculation of bacteria. Working stock cultures preserved in 20% glycerol at −80 °C were retrieved in batches to minimize freeze–thaw cycles. Using a sterile 10 µL loop, frozen stocks were transferred into 3 mL glucose broth in sterile 14 mL culture tubes. Tubes were loosely capped to permit aeration and incubated in a shaking incubator (aniCell NB-206CXXL) at 28 °C and 240

155 rpm for 36–48 hrs. Turbidity was assessed visually and recorded as growth-positive (T) or no
156 growth (NG); cultures lacking turbidity after 24 hrs of incubation were returned for an additional
157 24 hrs. For each isolate, one ISP-3 plate labeled "Purity" was inoculated using a sterile swab dipped
158 in the turbid seed culture, streaked for isolation with a sterile loop, and incubated at 28 °C. Seed
159 cultures were advanced only when purity plates showed a single, morphologically consistent
160 colony type; slow-growing isolates were allowed extended incubation when broth turbidity was
161 present. For each qualified isolate, ISP-3 production plates were inoculated under aseptic
162 conditions. A sterile swab dipped in seed culture was used to apply a central streak, followed by
163 uniform surface distribution using a standardized side-to-side "landscaping" pattern with
164 sequential ~60° rotations. Eighty plates were inoculated per isolate and incubated in a walk-in
165 incubator at 28 °C for isolate-specific durations, with periodic inspection for growth and
166 contamination. At the designated endpoint (visible sporulation on top of colonies), plates were
167 visually inspected and any showing aberrant morphology or suspected contamination were
168 excluded. Under sterile conditions, 50 g sterile dextrose was dispensed onto parchment-lined trays
169 as an osmotic protectant and carrier. Microbial biomass was scraped from agar surfaces using
170 sterile scalpels, transferred onto the dextrose, and homogenized while minimizing agar carryover.
171 Biomass–dextrose mixtures were transferred to sterile trays covered with autoclaved wrap (not
172 contacting the material) and dried on enclosed racks at ambient conditions for ≥7 days until no
173 longer tacky by sterile glove assessment. Grinding was performed in a contained room with
174 enhanced PPE (lab coat, gloves, safety glasses, N95 respirator). The grinder (Vevor Model 1000)
175 was disinfected with 70% ethanol and air-dried before use. Dried material was milled to a
176 homogeneous powder while avoiding excessive repeated grinding to limit heat exposure. The
177 finished product was transferred to 907 g opaque bags and labeled with batch number and date.
178 Microbial contamination was monitored throughout production, and cultures that failed purity or
179 growth criteria were excluded from downstream mixing. Live cell and spore count for each strain
180 component were quantified by flow cytometry, performed by trained staff at BioForm Solutions
181 Inc. (San Diego, CA) using the standard protocol ISO 19344 Part B. A 1 g replicate sample was
182 shipped overnight for analysis to the BioForm Solutions facility. To formulate the final products,
183 bacterial and fungal spores were combined and diluted with dextrose to a final viable count of $\geq 10^7$
184 CFU g^{-1}, and the wettable powder was packaged in non-transparent, sealable bags.

Experimental Design and Trial Locations. All trials were conducted under Good Experimental Practice and followed EPPO standards for efficacy trial design and assessment. Greenhouse trials in leafy greens were performed in Spain during the 2025 spring season at two independent sites using randomized complete block designs with six replicates per treatment and minimum plot sizes of 10 m^2 (**Fig. 2**).

Irrigation Treatments. Irrigation regimes were designed to simulate water-limitation scenarios in both annual and perennial cropping systems. The fully irrigated control received 100% of calculated crop water requirements, whereas the deficit irrigation treatment received 70% of crop water requirements. Deficit irrigation was initiated 3–5 days after the first microbial application and was maintained throughout the duration of the experimental period.

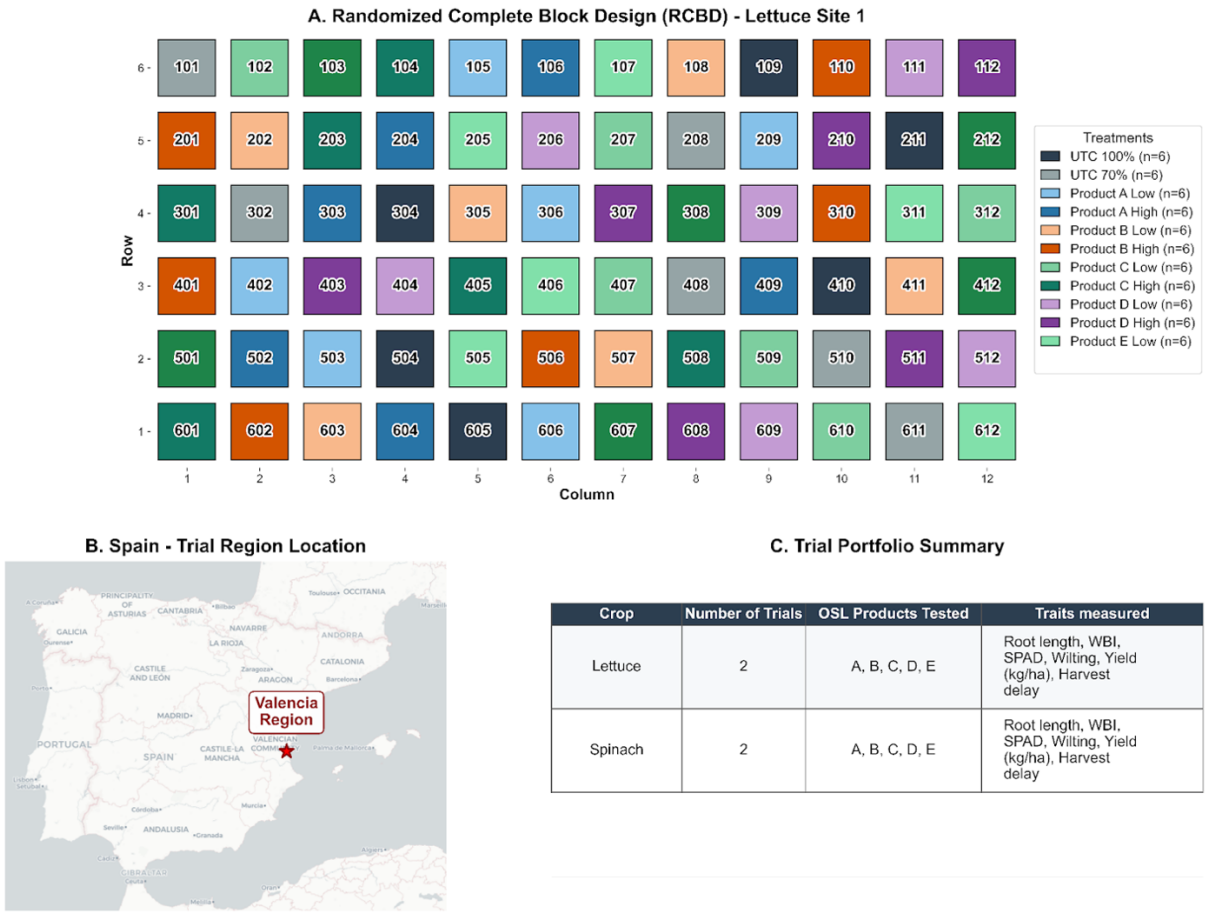
Microbial Inoculant Application Protocol. Microbial consortia were applied as soil-directed treatments through the irrigation system. The first application was made within 24 hours after transplanting, and the second application was performed 14-16 days later. Application rates ranged from low (250 g ha^{-1}) to high (500 g ha^{-1}) depending on treatment and trial protocol. All applications were aligned with early crop establishment and subsequent developmental stages to maximize potential effects on root colonization, rhizosphere function, and plant performance under water-limited conditions.

Plant phenotypic measurements. A broad set of agronomic, physiological, and spectral measurements was collected to evaluate crop performance under full and deficit irrigation. Across trials, these measurements included health indices, total yield, root length, harvest-delay time, and other crop-specific harvest traits. Plant physiological status was assessed using chlorophyll measurements (SPAD) and spectral indices associated with water content, including the Water Band Index (WBI). Spectral measurements were collected using a CI-710S leaf spectrometer. Together, these variables were used to assess treatment effects on crop productivity, stress physiology, and harvest quality under contrasting irrigation regimes.

Statistical analysis framework. To enable comparison of product performance across trials, crops, and irrigation regimes. For each endpoint within a given trial, treatment effects were estimated using the block-structured models. These endpoint-specific effects were then standardized as z-scores using the within-trial residual standard deviation. Sign conventions were assigned so that positive values consistently reflected improved performance under hydric stress, such as increased

yield or SPAD values and reduced WBI, wilting incidence and harvest delay. When standard errors were available, product-level estimates were further aggregated across crops using inverse-variance weighting, allowing the WUE index to capture a cross-system summary of treatment benefit under water limitation.

Statistical analysis of field trial data. Field trial data were analyzed using mixed-effects models to account for spatial variation and site-level differences. For continuous physiological traits spatial variation within each site was removed using Gaussian Process (GP) regression with a radial basis function kernel applied to plot coordinates. Across-site analyses for lettuce and spinach employed linear mixed-effects models with Site as a random effect, allowing for generalization of treatment effects beyond the specific trial locations. Treatment comparisons were conducted against two untreated reference controls: UTC at 100% irrigation, and UTC at 70% irrigation, representing optimal and deficit irrigation baselines respectively. P-values were calculated from model contrasts, with multiple testing corrections applied using the False Discovery Rate (Benjamini-Hochberg) method.



Crop	Number of Trials	OSL Products Tested	Traits measured
Lettuce	2	A, B, C, D, E	Root length, WBI, SPAD, Wilting, Yield (kg/ha), Harvest delay
Spinach	2	A, B, C, D, E	Root length, WBI, SPAD, Wilting, Yield (kg/ha), Harvest delay

Figure 2. Locations and experimental design of greenhouse trials. (A) Example of a randomized complete block design (RCBD) layout for the lettuce trial showing plot arrangement across rows and columns. Each plot represents an individual treatment replicate, including untreated controls (100% and 70% irrigation) and microbial consortium treatments (Products A-E at low (250 g ha^{-1}) and high (500 g ha^{-1}) application rates), with six replicates per treatment to account for spatial variability. (B) Geographic location of the trial region in Spain, highlighting the Valencia region where experiments were conducted. (C) Table showing details of the field trials included in this study.

Results

Microbial consortia improve yield under deficit irrigation in leafy greens.

Across the replicated greenhouse trials, the microbial consortium treatments (A-E) consistently improved yield in both lettuce and spinach under deficit irrigation (70% of crop water requirement) relative to untreated 70% irrigation (UTC 70%), and in multiple cases restored productivity to levels comparable with full irrigation (UTC 100%; **Fig. 3**). However, performance varied across the five consortia and between application rates. In lettuce, yield increased by between 3-9% under deficit irrigation relative to UTC 70%. In spinach, yield increased by between 4–13% under deficit irrigation relative to UTC 70%. For both crops, these increases corresponded to gains of between 1.0–3.2 t ha^{-1} depending on the consortium and application rate (Fig. 3).

The high application rate (500 g ha^{-1}) generally trended to a greater yield output when compared to the low application rate (250 g ha^{-1}) across all five consortia for both crops, but this trend was not significant ($P > 0.05$). The five different consortia (A-E) differed in the magnitude of their response. Consortium 'C' showed the greatest yield gains for lettuce under deficit irrigation when compared to the other five, and this gain was not statistically significantly different from UTC 100% ($P > 0.05$). Consortium 'A' showed the least improvement for lettuce ($P > 0.05$ vs. UTC 70%). In spinach, under deficit irrigation, the high application rate (500 g ha^{-1}) for consortia 'A', 'B', 'C', and 'E' generated yield gains comparable to, and not significantly different from, full irrigation controls ($P > 0.05$ vs. UTC 100%; **Fig. 3**). These findings indicate that microbial inoculation can compensate for reduced water availability in controlled production systems.

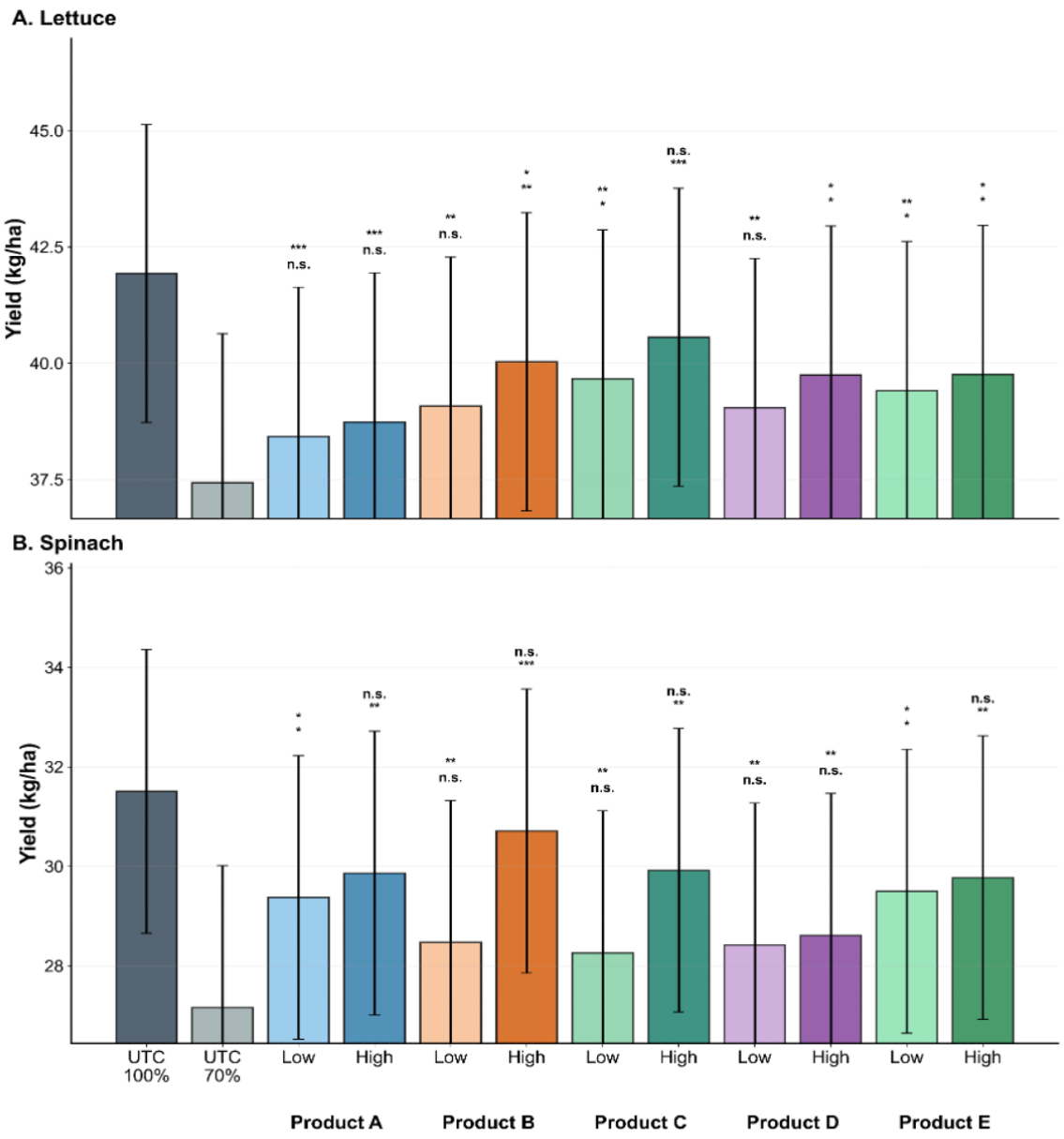


Figure 3. Yield responses of leafy greens under deficit irrigation. A) Lettuce yield and (B) spinach yield across replicated greenhouse trials in Spain (2025). The crops were treated with a low (250 g ha^{-1}) and high (500 g ha^{-1}) dose rate of the products. A trend for better performance with the higher dose rate across the products tested was observed. Bars represent treatment means; error bars indicate standard error across replicate blocks; significance is reported above bars for comparisons between products and UTC 100% irrigated control (top value), and products and UTC 70% irrigated (bottom value). Asterisk symbols correspond to ***P < 0.001, **P < 0.01, *P < 0.05, and n.s. (not significant).

Harvest dynamics and physiological responses reveal improved stress resilience in leafy greens

Microbial consortium treatments also influenced harvest timing under deficit irrigation. Across both lettuce and spinach, all consortia treatment plots and dose rates exhibited significantly reduced harvest delay and tighter maturity distributions with deficit irrigation compared to UTC 70% ($P < 0.05$), with a reduction in harvest delay of three to four days on average relative to UTC 70%. Across all five consortia, the high dose rate (500 g ha^{-1}) led to consistently reduced harvest delay compared to the low rate (250 g ha^{-1}; **Fig. 4**). However, this relationship was not significant. In lettuce, UTC 100% showed no harvest delay, whereas UTC 70% experienced approximately five days of delay. The lack of any delay for UTC 100% meant that all deficit irrigation experimental systems showed a significantly greater delay in comparison ($p<0.01$), albeit significantly less of delay when compared against UTC 70%. In spinach, UTC 100% showed a 0.5-day harvest delay, while UTC 70% showed a 4.5-day delay. Consortia 'A', 'B', 'D' and 'E' treatments all resulted in no significant difference in harvest delay compared to UTC 100% despite the irrigation deficit ($p>0.05$; **Fig. 4**). Notably, consortia 'C' and 'E' treatments eliminated harvest delay, thereby performing significantly better than UTC 100% even with deficit irrigation.

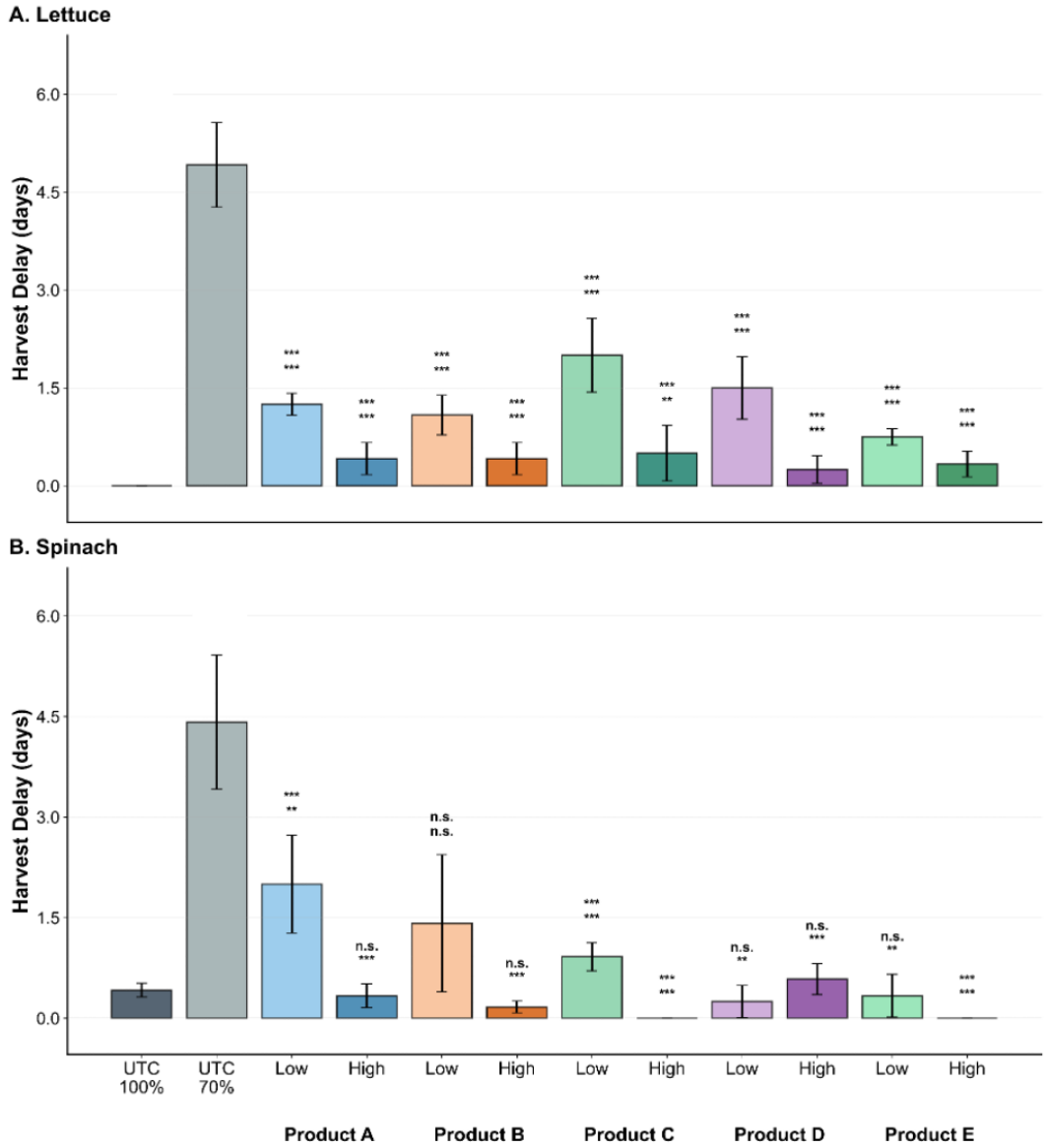
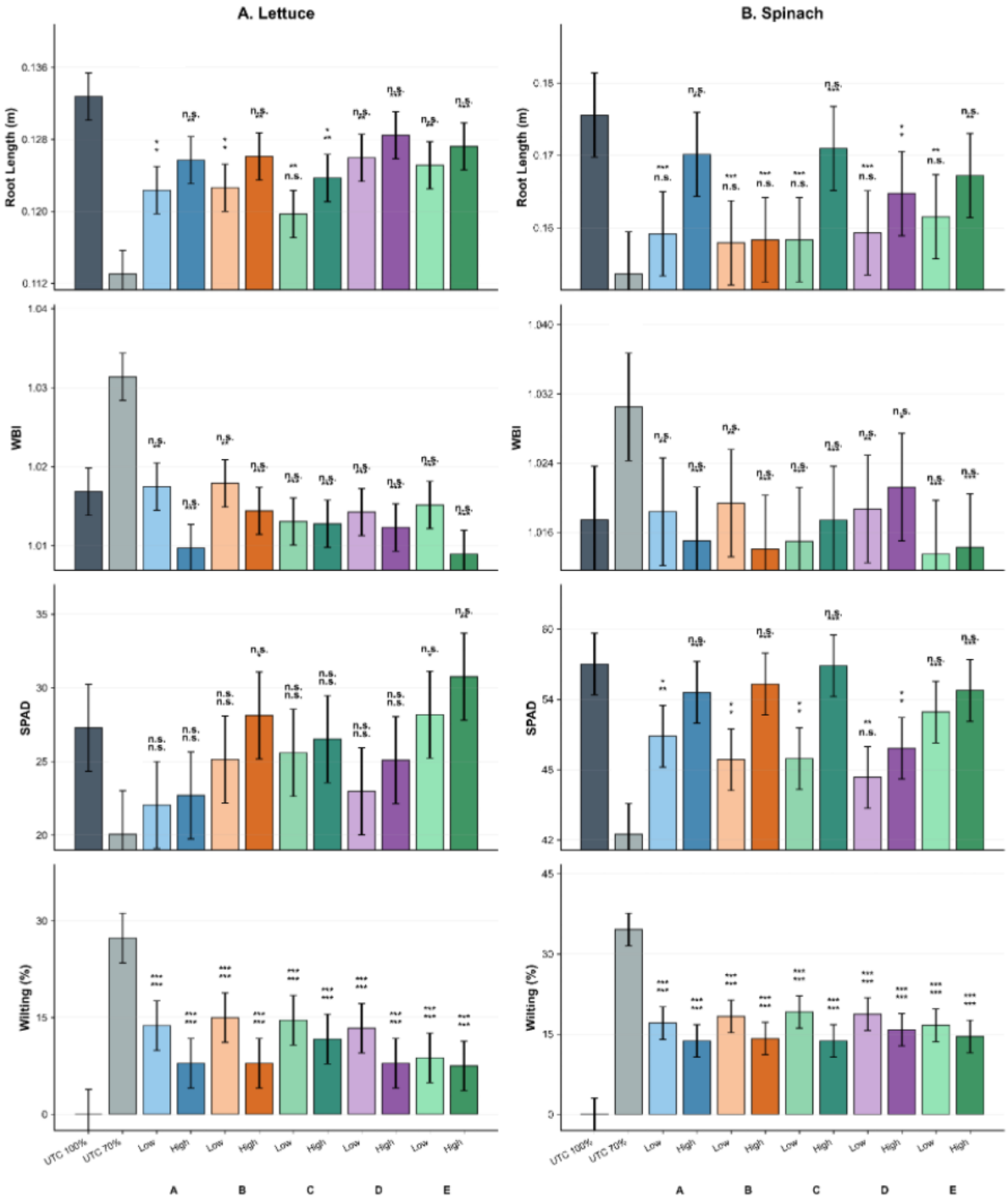


Figure 4. Harvest synchronicity in leafy greens under deficit irrigation. (A) Spinach and (B) lettuce harvest delay (days) across treatments under deficit irrigation, including full irrigation control (UTC 100%), deficit irrigation control (UTC 70%), and microbial product treatments. Microbial treatments reduced harvest delay and improved harvest synchronicity relative to untreated stressed controls, with several treatments approaching the timing observed under full irrigation. High = 500 g ha^{-1}. Low = 250 g ha^{-1}. Bars show model-estimated means ± SEM; significance is reported above bars for comparisons between products and UTC 100% irrigated control (top value), and products and UTC 70% irrigated (bottom value). Asterisk symbols correspond to ***P < 0.001, **P < 0.01, *P < 0.05, and n.s. (not significant).

The physiological status of both lettuce and spinach at harvest were also analyzed, including root length, chlorophyll level (SPAD), water band index (WBI), and wilting % (**Fig. 5**). Compared to UTC 70%, consortium treatments led to root length increases of 11% and 13% for lettuce and spinach, respectively. In lettuce, most treatments (except consortium 'C') produced root lengths that were not statistically different from UTC 100% even with deficit irrigation, especially at high dose (500 g h^{-1}; $p>0.05$). In spinach, only consortia 'A', 'C' and 'E' at high rate of application (500 g h^{-1}) had root lengths that were not significantly different from UTC 100%. Suggesting that different crops respond differently to each consortium. Water Band Index (WBI), a spectral indicator of plant water stress, was significantly lower in both lettuce and spinach for all 5 consortia, irrespective of application rate at deficit irrigation when compared to UTC 70% ($P < 0.001$; **Fig. 5**). Importantly, all consortium treatments produced WBI scores that were not significantly different to UTC 100%, despite the deficit irrigation. Chlorophyll (SPAD) responses to consortium treatments differed considerable between lettuce and spinach (**Fig. 5**). For example, in lettuce only consortia B and E showed significantly greater SPAD scores compared to UTC 70% ($P < 0.01$). However, SPAD scores were also not significantly different from 100% UTC ($P > 0.05$). In spinach, all consortia treatments except 'D', resulted in a significantly greater SPAD score than UTC 70%, and at high application rate, no significant difference to UTC 100% (**Fig. 5**). Across both lettuce and spinach, irrespective of the consortium treatment or application rate, wilting incidence was significantly lower than UTC 70%, but significantly greater than UTC 100% ($P < 0.001$; **Fig. 5**) during deficit irrigation.



315

316 **Figure 5. Physiological responses at harvest in leafy greens under deficit irrigation.**
317 Physiological traits were evaluated at harvest stage in lettuce and spinach. Microbial treatments
318 showed improved measures when compared to stressed untreated control. This was especially so
319 in wilting effects and Water Band Index (WBI). Bars show model-estimated means ± SEM;

significance is reported above bars for comparisons between products and UTC 100% irrigated control (top value), and products and UTC 70% irrigated (bottom value); asterisk symbols correspond to ***P < 0.001, **P < 0.01, *P < 0.05, and n.s. (not significant). High = 500 g ha^{-1}. Low = 250 g ha^{-1}.

Discussion and Conclusion

This study shows that complex microbial consortia can partially offset the effects of reduced irrigation in leafy greens by improving both agronomic performance and plant physiological status. Across lettuce and spinach, inoculated plants grown under 30% reduced irrigation consistently outperformed untreated deficit-irrigated controls, with gains observed in yield, harvest timing, root development, wilting, and spectral indicators of water stress. In several treatment combinations, crop performance under deficit irrigation was restored to levels statistically indistinguishable from the fully irrigated control, indicating that microbial inoculation can help preserve productivity under water limitation.

One of the most practically relevant outcomes was the reduction in harvest delay. Across both crops, microbial treatments reduced delay by approximately three to four days relative to untreated deficit-irrigated controls. This is important not only as an indicator of improved stress tolerance, but also because tighter and more predictable harvest windows have direct operational value for growers through improved labor planning, harvest efficiency, and crop uniformity (Lindemann-Zutz, Fricke, and Stützel 2016). In high-value leafy green production systems, even modest shifts in harvest timing can influence marketability and postharvest handling.

The physiological measurements further support the interpretation that the microbial consortia improved plant performance under hydric stress rather than merely shifting biomass allocation. Root length was generally greater in inoculated plants than in untreated deficit-irrigated controls, suggesting improved belowground development and potentially enhanced access to water and nutrients. Likewise, the lower Water Band Index values observed across consortium treatments indicate reduced plant water stress, while decreases in wilting and, in several cases, improved chlorophyll status point to better maintenance of plant function during water limitation (Qiao et al. 2024; Wang et al. 2025). The convergence of these physiological and agronomic responses

strengthens the conclusion that the observed effects reflect a meaningful improvement in crop resilience under deficit irrigation.

Although the overall response was positive, treatment effects were not uniform across all consortia or between crops. Some products performed better in lettuce, whereas others were more effective in spinach, indicating that consortium composition likely interacts with crop-specific physiology and rhizosphere ecology. These differences may reflect variation in microbial establishment, compatibility with host plants, functional complementarity among consortium members, or differential performance under specific soil and irrigation conditions. Future research will interrogate how the species composition of each consortium influences this variability. The fact that higher application rates generally produced stronger outcomes further suggests that inoculum density may influence the consistency or magnitude of rhizosphere colonization and downstream plant benefit, even if the dose effect was not always statistically significant in the present dataset.

The mechanisms underlying these responses were not directly tested here, but the consortium design provides plausible biological explanations. The selected taxa were intended to contribute complementary functions, including rhizosphere colonization, soil aggregation, and fungal exploration beyond root depletion zones. Such traits may improve plant-available water indirectly through enhanced soil structure and water retention, or directly through improved root access to water and nutrients. However, the present study was designed to evaluate performance outcomes rather than resolve mechanism. Future work should therefore link agronomic responses to direct measurements of microbial establishment, soil physical properties, plant hydraulic traits, and molecular indicators of stress response. Integrating metagenomics, metatranscriptomics, metatranslatomics and metabolomics (Moyne et al. 2023), with soil biophysical measurements will be especially valuable for determining which functions are most predictive of improved irrigation deficit stress response.

Several limitations should be considered when interpreting these findings. First, the study reflects greenhouse trials conducted over a single growing season and at a limited number of sites, so the durability and reproducibility of the response under broader field conditions remain to be established. Second, while the results consistently show improved crop performance under reduced irrigation, the composite physiological response likely integrates multiple interacting processes, including soil structure, microbial activity, host physiology, and environmental context. To further

interrogate hydrology and plant physiology under deficit irrigation responses, future studies would benefit from explicit measurements of water productivity per unit input.

Despite these limitations, the results provide strong evidence that complex microbial consortia can help mitigate the effects of water limitation in leafy green production systems. By improving yield retention, reducing harvest delay, and moderating physiological stress under deficit irrigation, these consortia show promise as biologically based tools for climate-resilient agriculture. More broadly, the findings support the idea that functionally diverse microbial inoculants can become an important component of resource-efficient crop management strategies as water scarcity intensifies across agricultural regions.

Conclusion

In summary, complex microbial consortia improved the performance of lettuce and spinach under 30% reduced irrigation by supporting yield retention, reducing harvest delay, and improving multiple indicators of plant physiological status. While consortium performance varied across crops and treatments, the overall pattern was consistent. Microbial inoculation reduced the severity of water-deficit effects relative to untreated controls. These findings support continued development of multi-species microbial inoculants as scalable tools to enhance crop resilience and sustain productivity under increasing water limitation.

Acknowledgements: The authors also thank Martin Voss, Chief Innovation Officer at Oath Biome, for his advice on the execution of the trial. We are grateful to the microbiology laboratory staff for producing the microbial inoculant.

Competing interests: The authors declare potential competing interests as follows: AE, SSB, JM, JLE, VB, AG are employees of Oath Inc. JAG is a compensated member of the scientific advisory board of Oath Inc. AE, SSB, JM, JLE, VB, AG are employed by the company that funded this study. AE is an inventor on patents related to the microbial consortia described in this study (WO2025240938, WO2025097154).

Data Availability: Datasets generated during the current study are available upon request.

Author Contributions: VB conceived the study. SSB, JLE, AG, JM, AE compiled, analyzed, and visualized the data for the manuscript. AE, SSB, AG, JM, JAG wrote the initial draft of the manuscript. All authors interpreted the results and contributed to the writing and editing of the manuscript.

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