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Stable soil moisture promotes shoot performance and shapes the root-rhizosphere microbiome

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ABSTRACT

Soil moisture is a key factor limiting crop productivity and has been widely studied to optimize agriculture production. However, the majority of previous studies focus only on the soil moisture content and ignore its temporal variation. This study investigates the impact of different soil moisture conditions, specifically fluctuating soil moisture (FSM) and stable soil moisture (SSM), on the rhizosphere microbiome and the plant performance of romaine lettuce. Plants were grown in natural and sterilized soils, which were subjected to SSM through negative pressure irrigation to achieve high, mid, and low moisture levels and FSM through manual irrigation. Shoot performance parameters, such as plant height, leaf count, -size, and biomass, were significantly enhanced under SSM compared to FSM. The findings reveal SSM enhances shoot performance and crop water productivity (WPc) independent of root size, as indicated by a lower root/shoot ratio. Analyses of the soil microbiome showed that the root-associated rhizosphere microbial community composition differs for SSM and FSM conditions, while the bulk soil microbial community was unaffected. This suggests that the response of the romaine lettuce rhizosphere microbial community to soil moisture temporal variation is driven by root microbiome interactions. This study indicates that stable soil moisture, together with the recruited root microbiome, induces shoot performance without enhancing root growth. Overall, the findings highlight the importance of optimizing soil moisture dynamics to improve plant growth and resource efficiency, offering valuable implications for sustainable agricultural practices.

1. Introduction

The changing climate leads more often to extreme weather events such as drought and floods, which have detrimental effects on the security of crop production (Kreibich et al., 2022; Lesk et al., 2016). Optimizing soil moisture conditions through irrigation may be essential to guarantee a reliable food supply. The soil moisture content is constantly and dynamically changing, and these dynamic fluctuations over time are referred to as "soil moisture temporal variation" (Niu et al., 2022). Soil moisture conditions can be categorized as either fluctuating soil moisture (FSM) or stable soil moisture (SSM) based on their temporal variance. The majority of natural conditions and artificial

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Abbreviations: NPI, Negative Pressure Irrigation; SSM, Stable Soil Moisture; FSM, Fluctuating Soil Moisture; WPc, Crop water productivity; NMDS, Non-metric multidimensional scaling; ANOSIM, Analysis of similarities; LEfSe, Linear discriminant analysis Effect Size; Cv, The coefficient of variation.

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irrigation methods provide FSM, characterized by periodic drying and re-watering. SSM conditions were hardly researched until a series of studies on negative pressure irrigation (NPI) technologies demonstrated that the consistent water supply provided by NPI could create SSM. SSM was beneficial for shoot performance, yield, and crop water productivity (WPc) compared to FSM (Yang et al., 2022). The WPc-promoting effect of SSM has been observed across a variety of crops and soil types, suggesting that this effect could be universal (Niu et al., 2022; Wang et al., 2019; Yang et al., 2020; Zhang et al., 2022b, 2022a).

Soil moisture is one of the major environmental factors affecting root development, in which the root system changes its spatial distribution and extended morphology to fit the heterogeneous soil moisture regime (Dinneny, 2019). For example, when subjected to drought conditions, plants could adjust their root development towards deep-rooting, larger root angles, or increased branching to optimize the pursuit of water, and eventually shape a larger and deeper root system (Schneider et al., 2022; Uga et al., 2013; Zhang et al., 2024). Intriguingly, in the monocotyledon crop Maize, it has been reported that the root-to-shoot ratio was reduced while the shoot was enlarged under SSM compared to FSM (Zhang et al., 2022b, 2022a). This suggests that SSM provides better growing conditions for monocotyledon crops, enabling them to focus on shoot performance and yield by minimizing investments in root growth.

The root system architecture of monocotyledons is characterized by a fibrous root system, which includes a wide network of roots: a primary root, multiple seminal roots, lateral roots, and crown roots, which vary in function and anatomy (Dixon et al., 2022). In contrast, dicotyledons have a tap root system that consists of a primary root that grows deeper into the soil and has side branches called lateral and adventitious (lateral) roots (Ge et al., 2019; Sheng et al., 2017). Notably, annual dicotyledons develop secondary growth, a feature not present in monocotyledons. Secondary growth minimizes the radial transport of water across the root (Strock et al., 2021). To date, no research has been done on the impact of soil moisture temporal variation on dicotyledon root systems. Investigating how soil moisture fluctuation affects dicotyledon species is essential since monocotyledon and dicotyledon root systems differ significantly in their anatomy and root architecture.

The root system is the major organ of water and nutrient uptake from the soil and interacts with the rhizosphere, which represents a microzone surrounding the plant root (usually considered within 2 mm) (Edwards et al., 2015). The microorganisms that colonize the rhizosphere are collectively referred to as the rhizosphere microbiome, which serves a vital role in growth promotion, stress resistance, water, and nutrient acquisition of their host plant (Trivedi et al., 2020). It is now well-established from a variety of studies, that the rhizosphere microbiome plays a crucial role when facing varying soil moisture conditions (De Vries et al., 2020; Wang et al., 2024). For instance, the production of osmolytes and exopolysaccharide by microbes could provide a stable osmotic environment for plant roots (Khan et al., 2024); plant growth-promoting rhizobacteria (PGPR) are capable of producing phytohormones (such as IAA and ABA) and mineral nutrients (such as nitrate, ammonium or phosphate) (Gifford et al., 2024; Vurukonda et al., 2016); modulating the root system architecture to approach water sources (Verbon and Liberman, 2016); arbuscular mycorrhizal fungi and plant symbionts that could improve the relation between plant and soil (Kakouridis et al., 2022). The rhizosphere microbial response to soil moisture temporal variation is rarely discussed, with the exception of a few NPI-related studies that demonstrated that rhizosphere microbial community was altered by SSM condition provided by NPI (Gao et al., 2019; Zhao et al., 2019). These studies only address the irrigation methods and have not taken "temporal variation" into account. It is unknown whether the rhizosphere microbiome plays a role in how plants respond to changes in soil moisture variations. Investigating how the rhizosphere affects root and shoot performance to SSM and FSM conditions is of major importance for improving the understanding of crop improvement under different water regimes.

Romaine lettuce (Lactuca sativa L. var. longifolia), a dicotyledon vegetable, has high water demands during its growth resulting in sensitivity to soil moisture conditions (Damerum et al., 2021). This characteristic makes it an ideal plant to study the impact of soil moisture on plant performance. This study focuses on roman lettuce to investigate: (1) the impact of soil moisture temporal variation on root and shoot performance, yield, and WPc; (2) whether the performance of the host plant roots and shoots under SSM and FSM conditions is influenced by the rhizosphere microbiome; (3) the rhizosphere bacterial and fungal composition and diversity between SSM and FSM and identify the key differential species with functional potential in host plant shoot and root response. The obtained results will shed light on the impact of the soil moisture temporal variation on rhizosphere microbiome and plant performance, providing key insights for sustainable agricultural practices.

2. Material and methods

2.1. Field site description

2019 experiment was conducted from August 2019 to October 2019 in a net house of the Chinese Academy of Agricultural Sciences (39°57'37'' N, 116°20'0.9'' E), Beijing, China. Sandy loam potting soil was collected from the 0–20 cm surface layer of cultivated soil from the International High-tech Industrial Park, Chinese Academy of Agricultural Sciences (39°36'53'' N, 116°36'89'' E), Langfang, Hebei Province. The soil bulk density was 1.43 g cm^3, pH was 8.25, organic matter content was 10.06 g kg^{-1}, available N content was 58.4 mg kg^{-1}, P content was 20.4 mg kg^{-1}, and K content was 112.4 mg kg^{-1}.

From August 2023 to October 2023 a verification experiment, to analyze the effect of the microbiome on plant performance under of soil moisture temporal variation, was performed using natural and sterile soil conditions under the same aforementioned experimental conditions (same net house and identical experimental soil). The soil used for sterile treatment was autoclaved twice for 120 min at 120°C with an interval of 24 h.

2.2. Experimental design

Four treatments were designed in the 2019 experiment: three SSM treatments (high, medium and low moisture content) and a control FSM treatment. Manual irrigation with upper and lower limits (70–90 % field capacity, which could be consider as suitable for lettuce) was used for treatment FSM (Sutton and Merit, 1993). Different NPI water pressures of −3, −6, and −9 kPa were used to achieve SSM conditions for high (SSM^H), medium (SSM^M), and low (SSM^L), respectively. The three SSM treatments were designed to generate a water content gradient to investigate the effect of soil moisture content under SSM including one SSM treatment that matched the average soil moisture content used in the FSM treatment.

To verify the effect of microbiome, only two comparable soil moisture condition SSM^L and FSM with two different soil conditions, natural and sterile, were established resulting in four treatments: N-SSM^L, N-FSM, S-SSM^L and S-FSM were used for the 2023 experiment. For this experiment three pots with 6 plants were used per treatment .

Considering the spatial distribution of soil moisture, we used a specific size of pot (length 420 mm, width 26 mm, and height 25 mm), to make sure that the irrigation water can attain an uniform distribution within two hours (Wang et al., 2019). The NPI device consisted of three parts: a negative pressure generator, a water bucket (inner diameter of 13.1 cm and height of 80 cm), and a capillary water emitter (length 250 mm, outer diameter 19 mm, and inner diameter 10 mm); each part was connected via silica gel pipes (Fig. S1a). The water emitter of NPI device was placed at the central of the pot, in order to ensure the uniform distribution of the water. Absorption of water by the plant reduces the soil water matric potential. Thus, once the soil water matric potential is lower than the negative water pressure set, the soil actively absorbs water from the water bucket to prevent a further decline in soil water matric potential, thereby maintaining soil moisture stability (Yang et al., 2022).

Romaine lettuce (Lactuca sativa L. var. Longifolia. cv 'Meilijian') was used as the experimental romaine lettuce cultivar. The sowing of seeds (for the 2019 experiment) started on August 20, 2019, and six holes per pot and five seeds per hole were planted; For 2023 experiment, seeds were sowed on August 28, 2023, with four holes per pot and five seeds per hole. Before sowing, 8.35 g of urea, 4.57 g of calcium superphosphate, and 2.56 g and potassium sulphate powder were mixed and applied to each pot. Each pot contained 23 kg of soil and received 5 L of water to fully wet the soil. The initial volumetric soil moisture content (θ_v) was about 28.3 %. Once seedlings grew four leaves, the seedlings were thinned to one per hole (and selected for comparables sizes), with six respectively four plants per pot. The NPI system was used to control pot water from the time at which most plants had grown four leaves (September 16, 2019, for the 2019 experiments, September 19, 2023, for the 2023 experiment) until harvest (October 12, 2019, for the 2019 experiments, October 19, 2023, for the 2023 experiment) (Fig. S1b).

2.3. Plant and soil sampling

Plant and pot soil samples were collected when the lettuce was mature (just before bolting, at 15 leave stage) in 2019 experiment. The whole plant (including roots) was removed from the pot (including soil), the above-ground and below-ground parts were isolated to keep the roots attached to the soil. Soil removal began with gentle shaking of large clods until a 1–2 mm soil layer was left on the roots, the remaining soil was placed into a centrifuge tube filled with phosphate buffered saline and vibrated until the rhizosphere soil fell off. Roots were removed from the centrifuge tube, and the centrifuge tube was placed in a high-speed centrifuge at 4 ℃ and 10,000 rpm for 10 min; any sediment remaining in the tube was classed as rhizosphere soil (Edwards et al., 2015). The bulk soil was collected from the soil remaining in the pot after rhizosphere soil collection (Yuan et al., 2018). The bulk soil, serving as control, was sampled at same depth and 3–4 cm away from the rhizosphere soil sampling point.

2.4. DNA extraction, PCR amplification, and sequencing

DNA was extracted from 0.5 g samples of rhizosphere and bulk soil using a Fast DNA SPIN Kit (MP Biomedicals, USA). The concentration and quality of DNA were determined using NanoDrop 2000 (Thermo Scientific, USA) and 1 % agarose gel electrophoresis (Molecular biology grade, Invitrogen, USA), respectively. The v3–v4 region of the bacterial 16S rRNA gene was amplified by PCR using the upstream primers 338 F and downstream primer 806 R (Zeng and An 2021). The v5–v7 region of the fungal ITS gene was amplified by PCR using the upstream primer ITS1F and the downstream primer ITS2R (Lemons et al., 2017). Each DNA sample was amplified by PCR with three replications, and PCR-amplified products were tested by agarose gel electrophoresis (2 %). The PCR products were purified using an Axyprep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and a Quantus™ Fluorometer (Promega, USA) for quantitative analysis. Purified PCR products were sequenced using high-throughput Illumina MiSeq (Illumina, USA). The obtained sequences were submitted to the NCBI database (accession numbers PRJNA744013 and PRJNA744028 for bacterial and fungal sequences, respectively).

2.5. Measurements

2.5.1. Plant growth and yield

In the 2019 experiment, plant growth parameters were measured every 10 days in terms of plant height, number of leaves, and the largest leaf length and width: 2 plants per pot were measured. The biomass of the above and below ground was determined by measuring the fresh weight of every plant after harvest. The fresh weight (biomass) of above-ground parts served as yield.

2.5.2. Water parameters

An AZS-100 soil moisture meter (Beijing Aozuo Ecology Instrumentation Ltd.) was used to measure the soil volumetric moisture content every two days during the irrigation period in both 2019 and 2023 experiments. The depth of soil moisture measuring is 110 mm below the soil surface. The temporal variance of soil moisture was evaluated using the coefficient of variation (C_V). C_V was calculated according to (Niu et al., 2022):

$$C_V = \sigma/\mu$$

where σ and μ represent the standard deviation and average value of the soil moisture content, respectively. A weak temporal variation in soil moisture exists when $C_v \leq 0.1$, a medium temporal variation exists at $0.1 < C_v < 1$, and strong variation exists when $C_v \geq 1$.

The water consumption of a single plant was calculated according to (Niu et al., 2022):

$$ET_k = \frac{[M_k - (\theta m_k - \theta m_{k-1}) \times m_s/\rho_w]}{N_P}$$

where ET_k is the crop evapotranspiration (amount of water consumed by the crop) in the K^{th} period, M_k is the amount of irrigation in the K^{th} period, θm_k is the soil mass moisture content at the K^{th} moment, θm_{k-1} is the soil mass moisture content at the previous K^{th} moment, m_s is the weight of soil in the pot, and ρ_w is the density of water (1 g cm^{-3}), N_P is the number of plants in the pot.

The WPc of a single plant was calculated as (Fernández et al., 2020):

$$WPc = Y/ET_k$$

where Y is the single plant yield (g), and ET_k is the crop evapotranspiration per plant (L) (amount of water consumed by the crop).

2.6. Statistical analyses

Raw sequences from 16S and ITS were merged, trimmed, filtered, aligned, and clustered by operational taxonomic unit (OTU) using USEARCH v.7.0 software. A total of 1201387 merged sequences were generated, with an average of 50058 sequences for each sample in 16S sequencing; while 1571099 merged sequences were generated, with an average of 65462 sequences for each sample in ITS sequencing. Sequences with ≥ 97 % similarity were assigned to the same OTU by UPARSE in USEARCH, and chimeras were filtered during OTU clustering using the cluster_otus command. OTUs of every sample were rarefied to the minimum sample in order to minimize the impact of sequencing depth on subsequent analysis. OTU were analyzed on Majorbio Cloud Platform (https://cloud.majorbio.com) as follow: Mothur was used in calculation of alpha diversity. Non-metric multidimensional scaling (NMDS) based on weighted-UniFrac was used to test the similarity of microbial community composition among samples and combined with Analysis of similarities (ANOSIM) to determine whether the difference between sample groups was significant. Linear discriminant analysis Effect Size (LEfSe) was used to find the taxon which are mostly difference between treatments.

ANOVA of all data was performed using the IBM SPSS Statistics 21. Duncan tests were conducted to compare these data. If the variances of the data were not equal, a Games-Howell test was used. Growth parameters, biomass and WPc data were analyzed on a single plant level (24 plants for the shoot parameters, 65 plants for the biomass and WPc). At a statistical level of $p < 0.05$, significant differences were accepted. Origin 2022 pro and Adobe illustrator 2021 were used for data visualizing.

3. Results

3.1. Dynamic soil moisture changes under SSM and FSM conditions

To study how dynamic changes in soil moisture over time- also known as soil moisture temporal variation- affect plant performance, two different conditions were created. The first condition, FSM, was created by manual irrigation, while the second condition, SSM, was created via NPI. To obtain the FSM condition, only one soil moisture level was used, while for the SSM condition, three different levels of soil moisture were established: low (SSM^{L}), medium (SSM^{M}) and high (SSM^{H}). The three levels of SSM were created to guarantee that one of those SSM treatments was comparable to FSM treatment. The impact of soil moisture temporal variation on plant performance under these different conditions was investigated from the fourth leaf up to harvest. The irrigation volume and volumetric soil moisture content were monitored daily to determine the total water volume used for irrigation and the average volumetric soil moisture content during the treatment (Fig. 1a and b, Table 1). There was a significant difference in the soil moisture content between SSM conditions: SSM^{H}, SSM^{M}, and SSM^{L} ($P < 0.05$), whereas there was no significant difference between SSM^{L} and FSM ($P > 0.05$), allowing them to be comparable. (Table 1). According to Niu et al. (2022), the coefficient of variation (C$_{v}$) was used to analyze the temporal variance of different soil moistures and the lower the C$_{v}$ the more stable the soil moisture conditions. The C$_{v}$ for SSM^{H}, SSM^{M}, and SSM^{L} showed weak temporal variation ($C_v < 0.1$) whereas the C$_{v}$ for FSM was significantly higher ($P < 0.05$) and showed a medium temporal variation ($C_v > 0.1$) (Table 1). This finding is in line with the reported prediction that soil moisture conditions are more stable when the C$_{v}$ is smaller. Interestingly, SSM and FSM had distinct temporal variances, despite having comparable soil moisture content and water supply.

3.2. SSM conditions promote shoot growth and WPc independently from root growth

To study the plant performance and WPc of romaine lettuce under different soil moisture temporal variance conditions, we compared different shoot performance parameters (plant height, leaf number, longest leaf length and width), shoot and root biomass, root/shoot ratio and WPc. Significant differences were observed for SSM^{L}, SSM^{M} and SSM^{H} ($P < 0.05$) for plant height (1.6 ^{L}, 1.6 ^{M}, 2.0^{H}-fold), number of leaves (1.3 ^{L}, 1.2 ^{M}, 1.3^{H}-fold), largest leaf length (2.0 ^{L}, 2.1 ^{M}, 2.0^{H}-fold) and width (1.3 ^{L}, 1.3 ^{M}, 1.3^{H}-fold) when compared to FSM at the moment of harvest (Fig. 2a-d). The increase of the total shoot parameters revealed a rise of 2.2 ^{L}, 2.7 ^{M} and 3.2^{H}-fold in the shoot biomass and 2.0 ^{L}, 2.2 ^{M} and 2.0^{H}-fold of WPc of SSM^{L}, SSM^{M} and SSM^{H} compared to FSM (Table 2). To ascertain whether the positive impact on shoot performance in SSM conditions was due to a larger root system, the root biomass was analyzed in the different SSM and FSM conditions. However, no significant difference could be observed in root biomass of SSM^{L}, SSM^{M} and SSM^{H} compared to FSM, indicating that the enhanced shoot performance was not due to a larger root system. Similarly, the root/shoot ratio of SSM^{L} SSM^{M} and SSM^{H} was significantly lower (0.5 ^{L}, 0.4 ^{M}, 0.4^{H}-fold, $p < 0.05$) than that of FSM, indicating that under SSM conditions, the romaine lettuce shoot performance and WPc improved regardless of the size of the root system.

Table 1
Soil moisture contents and temporal variance under SSM and FSM conditions.

Treatment	Average volumetric soil moisture content (%)	Variation range of soil moisture content (%)	C$_{V}$
SSM^{H}	35.12 ± 1.67^{a}	30.40 ~ 38.05	0.079 ± 0.012^{a}
SSM^{M}	28.59 ± 1.65^{b}	25.63 ~ 30.40	0.065 ± 0.013^{a}
SSM^{L}	22.53 ± 1.75^{c}	19.85 ~ 24.48	0.071 ± 0.016^{a}
FSM	22.05 ± 0.44^{c}	18.53 ~ 25.20	0.105 ± 0.013^{b}

Note: Different lowercase letters in the same column indicate significant differences among treatments (Duncan test; $p < 0.05$).

3.3. Soil moisture conditions specifically shape the root associated rhizosphere microbiome

In SSM conditions the root system was smaller than under FSM conditions. To examine how a smaller root system might be sufficient to support a bigger shoot, we analyzed the rhizosphere microbiome. The rhizosphere microbiome plays a crucial role in promoting nutrient uptake by the root system, and might vary when roots are subjected to varying soil moisture conditions (Bandopadhyay et al., 2024; Goswami and Deka, 2020; Pereira et al., 2019). We analyzed the microbial communities of root-associated rhizosphere against non-rhizosphere bulk soil samples (control condition) under FSM and SSM conditions. Among all treatments, the bacterial community of the bulk soil and rhizosphere soil consists of the phyla *Actinobacteria* (19.1–35.1 %), *Proteobacteria* (17.8–28.6 %), *Chloroflexi* (9.7–23.8 %), *Cyanobacteria* (0.5–32.6 %), *Firmicutes* (3.8–16.3 %), *Gemmatimonadetes* (1.1–4.2 %), *Acidobacteria* (1.1–3.9 %), *Patescibacteria* (1.3–6.8 %), *Deinococcus-Thermus* (0.6–2.7 %), *Bacteroidetes* (1.2–2.1 %), and *Planctomycetes* (0.3–1 %), which collectively accounted for 97.9–99.5 % of all bacterial sequences

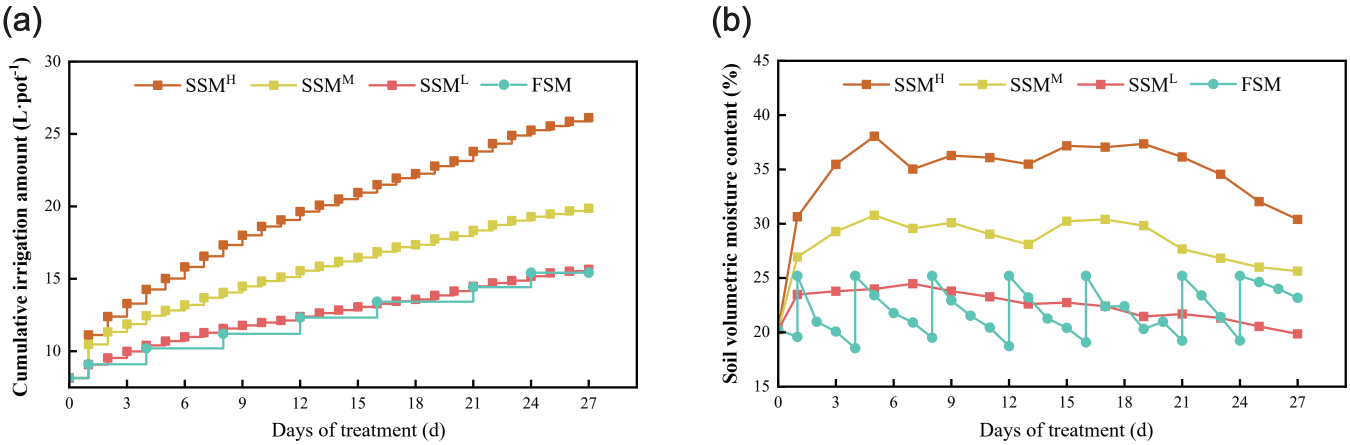

Fig. 1. Dynamic changes of soil moisture under stable and fluctuating soil moisture conditions: cumulative irrigation amount (a) and soil volumetric moisture content (b) during days of treatment.

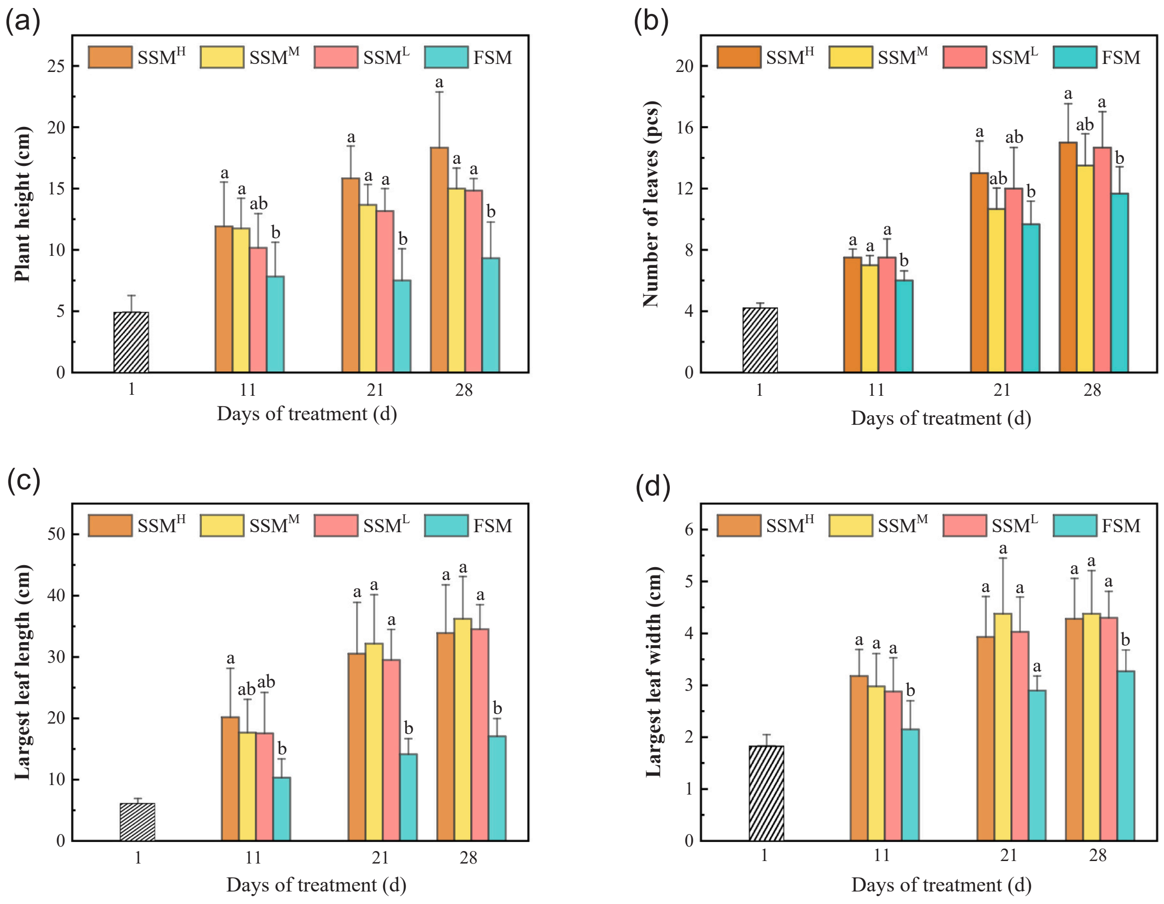

Fig. 2. Dynamic changes in shoot performance of romaine lettuce under stable and fluctuating soil moisture conditions: plant height (a), number of leaves (b), largest leaf length (c) and largest leaf width (d) measured at different time points. Different letters indicate statistically significant differences (Duncan test for equal variances and Games-Howell test for not equal variances; $p < 0.05$).

Table 2
Fresh biomass allocation and WPc of romaine lettuces under SSM and FSM conditions.

Treatment	Shoot biomass (g)	Root biomass (g)	Root/Shoot ratio (%)	WPc (g kg^{-1})
SSM^{H}	37.40 ± 21.76^{a}	1.75 ± 0.88^{a}	5.5 ± 2.1^{b}	8.23 ± 5.53^{ab}
SSM^{M}	31.53 ± 14.04^{a}	1.59 ± 0.89^{a}	4.8 ± 1.7^{b}	8.82 ± 3.90^{a}
SSM^{L}	25.91 ± 15.32^{a}	1.43 ± 0.95^{a}	5.4 ± 1.6^{b}	8.13 ± 4.28^{a}
FSM	11.74 ± 7.66^{b}	1.41 ± 0.95^{a}	12.2 ± 2.4^{a}	4.05 ± 2.56^{b}

Note: Different lowercase letters in the same column indicate significant differences among treatments (Shoot biomass and WPc using Games-Howell test, Root biomass and Root/Shoot ratio using Duncan test; $p < 0.05$).

(Fig. 3a). The overall rhizosphere fungal community was dominated by *Ascomycota* (91.2–98.5 %), *unclassified_k_Fungi* (0.9–7.0 %), *Mortierellomycota* (0.1–2.0 %), and *Basidiomycota* (0.1–1.3 %), which collectively accounted for 98.5–99.9 % of all fungal sequences (Fig. 3b). We observed that the taxonomic profiles between root associated rhizosphere and bulk soil microbiome differed between SSM and FSM, which required further investigation.

To further clarify the difference between root associated rhizosphere and bulk soil microbial community between SSM and FSM, their alpha and beta diversity were analyzed. Alpha diversity reflects the species variance within samples, while the beta diversity represents how samples vary between each other. For alpha diversity, we used the Chao1 index to represent richness and the Shannon index to represent diversity (Willis., 2019). The alpha diversity of both bacterial and fungal communities showed no significant difference between the different bulk soil samples ($p > 0.05$; Fig. 3c-f). In rhizosphere samples, significant differences could only be found using Chao1 index for bacterial community, which showed that the Chao1 index for FSM is significantly lower than for all the SSM conditions ($p < 0.05$; Fig. 3e). To analyze the beta diversity, we used Non-metric Multidimensional Scaling (NMDS) analysis based on weighted-UniFrac distances to determine whether treatments were associated with changes in the root associated rhizosphere and bulk soil microbial communities. These analysis showed that bulk soil samples of the different treatments clustered together, while they separated from root associated rhizosphere samples for both bacterial and fungal communities (Fig. 3g and h). Among the rhizosphere samples, only FSM samples were found to be separated from other SSM samples, in both bacterial and fungal communities.

Taken together, these data show that soil moisture temporal variation specifically affects the root associated rhizosphere microbial community, while the bulk soil microbiome remains unaffected. This suggests that the response of the root associated rhizosphere microbiome to soil moisture temporal variation might be due to the specific interaction with the host plant root.

3.4. *Different root associated taxa are recruited upon SSM conditions*

To better understand how the rhizosphere microbiota can affect the root system differently depending on the soil moisture conditions, a thorough investigation of the microbiota was carried out. Linear discriminant analysis Effect Size (LEfSe) was used to (i) identify the

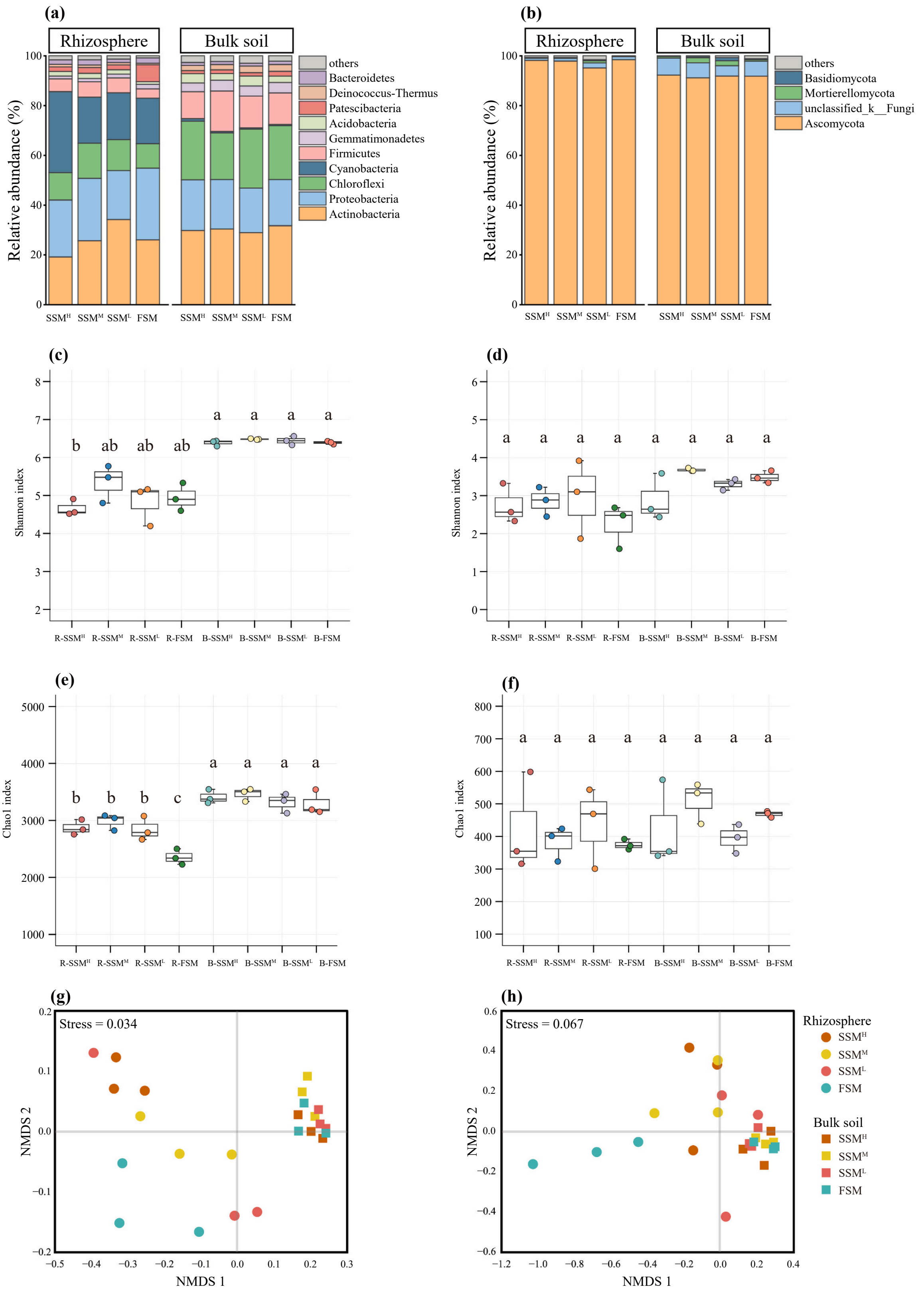

Fig. 3. Taxonomic profiles (at the phylum level) of the rhizosphere and bulk soil microbial community under stable and fluctuating soil moisture conditions: bacterial (a) and fungal (b). Shannon index of bacterial (c) and fungal (d) community and Chao1 index of bacterial (e) and fungal (f) community represent Alpha diversity, Chao1 index of bacteria using Duncan test, others using Games-Howell test; $p < 0.05$. Non-metric Multidimensional Scaling (NMDS) of romaine lettuce rhizosphere microbial communities under SSM and FSM conditions: bacterial (g) and fungal (h). Ordinations were based on weighted-UniFrac distances. Different colors and shapes include all plots within a treatment, circle dots represent rhizosphere samples and square dots represent bulk soil samples. Stress of NMDS and R and P-value from ANOSIM were marked in the figure.

specific phylotypes between different soil moisture temporal variation conditions (where SSM^L represents SSM), and (ii) determine the features that most likely explain the differences between biological groups, also called biomarker (Segata et al., 2011). At the genus level, 25 taxa were identified as biomarkers between SSM and FSM (Fig. 4). Out of these, four bacterial genera, namely *Bacillus*, *Nitrolancea*, *orank_f_Caldilineaceae* and *unclassified_f_Burkholderiaceae*, were significantly enriched at SSM condition, while 13 genera including *Devosia*, *Pseudomonas*, *Blastococcus*, *Pontibacte*, *Geodermatophilus* were significantly less abundant in the SSM condition compared to FSM. (LDA ≥ 3.0; Fig. 4a). For fungi, 5 taxa including *Aspergillus*, *Chaetomium*, *Talaromyces, Arthrobotrys* and *unclassified_f_Lasiosphaeriaceae* were significantly more abundant in SSM than in FSM, while *Alternaria*, *Stemphylium* and *unclassified_f_Ascobolaceae* were less abundant (LDA ≥ 3.0; Fig. 4b). These differences in recruitment of microbiota under the SSM condition- microbiota that might support nutrient acquisition- compared to FSM conditions could explain the enhanced shoot performance without the need of increasing the root system.

3.5. *The rhizosphere microbiome together with soil moisture conditions regulates plant growth*

To determine if SSM by itself or in combination with the rhizosphere microbiome is required for improving shoot performance, independent of root growth, an additional experiment was carried out. The experiment compared the growth of romaine lettuce in natural (N; unsterilized) and sterile (S; sterilized) soils under stable soil moisture (SSM^L) and fluctuating soil moisture (FSM) conditions (Table. S1). In each condition, the shoot and root performance of romaine lettuce was measured and analyzed. Natural soil treatments showed significantly reduced root/shoot ratio for N-SSM^L compared to the N-FSM, confirming the results of the previous experiment (Table 2; Table 3). In contrast to the sterile conditions (S-SSM^L and S-FSM), the natural conditions showed a noticeably increased shoot and root size compared (Table 3), although the effect on the root/shoot ratio of the N-SSM^L and N-FSM was consistent with the sterile conditions (S-SSM^L and S-FSM). This indicates that while the rhizosphere microbiome is important for the overall

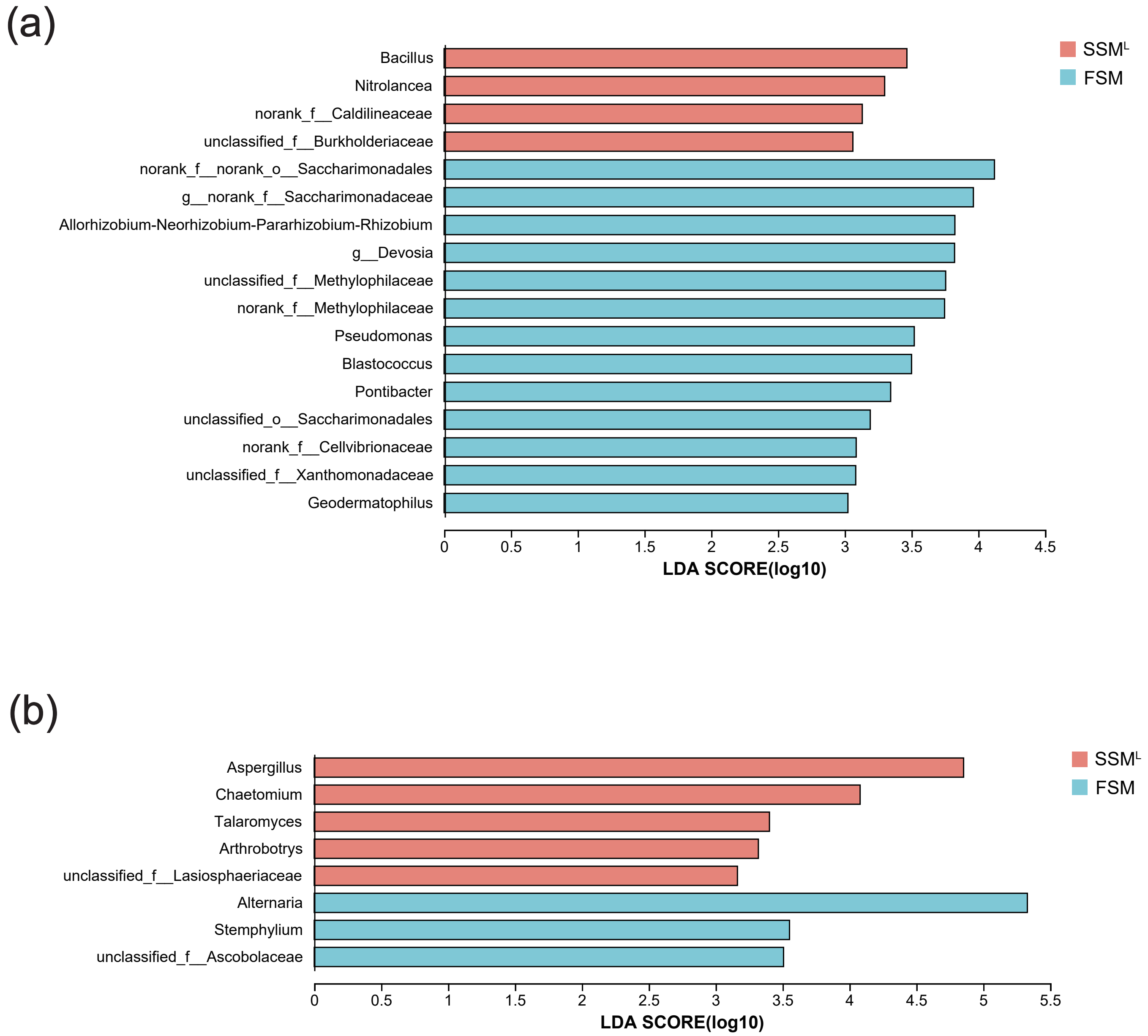

Fig. 4. Key phylotypes of rhizosphere microbiota in response to soil moisture temporal variation identified using the Linear discriminant analysis Effect Size (LEfSe) (LDA score ≥ 3.0): bacterial taxa (a); fungal taxa (b).

Table 3
Fresh biomass allocation of romaine lettuce using SSM^{L} and FSM under natural and sterile conditions.

Treatment	Shoot biomass (g)	Root biomass (g)	Root/Shoot ratio (%)
N-SSM^{L}	35.20 ± 8.56^{a}	2.23 ± 0.71^{a}	6.5 ± 1.8^{b}
N-FSM	15.89 ± 3.25^{c}	2.25 ± 0.40^{a}	14.3 ± 1.4^{a}
S-SSM^{L}	24.13 ± 3.40^{b}	1.22 ± 0.29^{b}	5.2 ± 1.9^{b}
S-FSM	8.25 ± 2.27^{d}	0.81 ± 0.19^{b}	10.6 ± 4.4^{ab}

Note: Different uppercase letters in the same column indicate significant differences among treatments (Games-Howell test; $p < 0.05$).

performance of plants, SSM conditions specifically improves shoot performance. Taken together, our findings show that temporal variation in soil moisture and the rhizosphere microbiome both promotes optimal shoot performance, the rhizosphere microbiome has a greater influence on the size of the root system than temporal variation in soil moisture.

4. Discussion

4.1. SSM improves the shoot growth and WPc

Watering regimes are of major importance and one of the most restrictive factors in agricultural production, as appropriate soil moisture levels often lead to higher crop yield (Moradgholi et al., 2022). Our results show that, even at the same average soil moisture content and irrigation volume (SSM^{L} and FSM), SSM conditions promote better shoot growth and WPc compared to FSM. The link between SSM conditions and higher WPc of plants is supported by several studies on irrigation frequency. These studies demonstrated that appropriately increasing the irrigation frequency can lead to higher WPc in crop production (Bao et al., 2024; Liu et al., 2023; Puértolas et al., 2020). Together with our results, these studies indicate that the SSM conditions, as well as the SSM-like conditions derived from high-frequency irrigation, can improve crop performance and yield while using the same or even reduced irrigation volumes. This creates opportunities to limit the input water to obtain a higher yield, as observed under SSM conditions, to a broader range of facility agriculture. This approach addresses the challenges of unbalanced temporal and spatial water distribution caused by climate change and the reduced availability of vital water resources.

4.2. Stable soil moisture reduces root/shoot ratio

A larger shoot is normally associated with a larger root system. However, in our experiments, the larger shoot obtained under SSM conditions did not result from a larger root system. The SSM conditions led to a smaller root/shoot ratio for romaine lettuce. The findings from our study on the dicotyledon romaine lettuce, along with the reported results on the monocotyledon maize, indicate that a decline in root/shoot ratio is common across different crops, regardless of their root system architecture and anatomy, including both monocotyledons and dicotyledons (Chen et al., 2018; Pagès, 2016; Zhang et al., 2022b, 2022a). One of the explanations for the larger root systems under FSM conditions might be because the repeating drying and re-watering process under FSM conditions forces the root system to adjust its growth range and architecture for better exploration of the soil resources. As a result, the root system may exhibit a trend to deep-rooting, higher lateral root density, and increased root diameters to expand its possible contact area with water, resulting in a higher root/shoot ratio (Gao et al., 2023; Redillas et al., 2012; Schneider et al., 2022; Uga et al., 2013). This is supported by a meta-analysis published recently demonstrating that under drought stress, the decrease in shoot biomass was significantly higher than in root biomass, resulting in root/shoot ratio increasing by 19 % (Li et al., 2024). The water supply under SSM conditions is always stable, which guarantees continuous access to water supply and might make a larger root unnecessary, and plants probably benefit more from investing in a larger shoot instead of investing in a larger root system.

Another explanation could be that rhizosphere microbiome is also involved in the root and shoot performance under SSM conditions. Our findings indicate that only in natural soils the root/shoot ratio was decreased under SSM conditions, while no significant difference in the root/shoot ratio can be observed in sterilized soils. This indicates that the rhizosphere microbiome is responsible for this reduction of root/shoot ratio. Analyzing the microbiome in the different conditions showed that certain bacterial taxa were significantly enriched under FSM conditions, which have been proven to promote overall plant growth rather than specifically enhancing shoot growth. For example, *Devosia* and *Pseudomonas* are referred to as members of plant growth-promoting rhizobacteria (PGPR), which facilitate plant growth by promoting secondary root formation (Chhetri et al., 2022; Li et al., 2021; Zheng et al., 2024). This is in line with our results that showing enrichment of *Pseudomonas* under FSM conditions, which resulted in an enhancement of a larger root/shoot ratio compared to SSM^{L}. This significant enrichment of beneficial plant promoting taxa that enhance root growth in the rhizosphere of romaine lettuce under FSM condition might indicate a "water deficit compensation effect" (Goswami and Deka, 2020; Pereira et al., 2019). In contrast, under SSM conditions, romaine lettuce experiences more stable and optimal soil moisture levels. As a result, its root system probably receives less or even no drought stress signals compared to a fluctuating condition. This could explain why it, under stable conditions, does not require plant promoting beneficial taxa to colonize the root system and enhance its root system (Munoz-Ucros et al., 2022).

Taken together, SSM conditions lead to a decreased root/shoot ratio in romaine lettuce, potentially due to stable water supply and the influence of rhizosphere microbiome, while FSM conditions promote deeper rooting and larger root systems to adapt to water stress.

4.3. Plants selectively recruit rhizosphere microbiome in response to temporal variation of soil moisture

By comparing the microbial community of rhizosphere to non-rhizosphere (bulk) soil samples under SSM and FSM conditions, we found that only rhizosphere microbiome was affected. Plant roots can alter their secretion patterns to recruit a favorable rhizosphere microbial community under various environmental conditions, such as drought (Santos-Medellín et al., 2021; Wang et al., 2024; Yue et al., 2024; Zhalnina et al., 2018). This suggests that in response to the soil moisture temporal variation, plants might selectively recruit specific microbial taxa by changing their root exudation pattern. Consequently, this results in changes in the rhizosphere microbial community composition, as seen in the enrichment of specific taxa in the rhizosphere of romaine lettuce grown at the same soil moisture content (SSM^{L} and FSM). In addition to the above discussed PGPR that are enriched under FSM conditions, there are also various microorganisms recruited specifically under SSM conditions which may explain the improved shoot performance and yield. For instance, the enriched genre *Bacillus* has been reported to play a role in nitrogen fixation, phosphate solubilization, and production of growth-promoting hormones, which could contribute to the growth improvement of the host plant (Ge et al., 2024; He et al., 2023; Valencia-Marin et al., 2024)., Likewise, the nitrite-oxidizing bacterium *Nitrolancea* may increase the nitrogen availability of rhizosphere soil, which is conducive to better nitrogen acquisition and absorption in plants (Spieck et al., 2020). Some enriched fungal species like *Aspergillus* and *Talaromyces* have phosphate-solubilizing functions, which could support plant phosphorus uptake (Michellin Kiruba N et al., 2024; Xu et al., 2024). It appears that SSM conditions create a healthier rhizosphere habitat for plant roots by the recruitment of well-known nematode-trapping fungi *Arthrobotrys*, as well as the reduction of pathogenic fungi *Alternaria* (Fernandes et al., 2023; Fischer and Requena, 2022).

Taken together, the recruited rhizosphere microbiome under SSM plays a role in host plant responses in both shoots and roots, along with adaptive development. This ultimately results in an improved shoot performance, yield, and WPc without enlarging the root size. The findings of this study provide a deeper insight into the interaction between the root system and the rhizosphere under stable soil moisture conditions, and its effect on plant performance offers opportunities for the utilization of rhizosphere microbial resources and watering regimes in increasing crop production in a sustainable manner.

5. Conclusion

Our results show that all SSM conditions (L, M, and H) are more beneficial for shoot growth, yield, and WP_C of romaine lettuce compared to the FSM condition without enlarging the root system. This indicates that the stability of the soil moisture, rather than its level, is more crucial for plant performance. The different soil moisture temporal variances altered the composition of the microbial community of both rhizosphere bacteria and fungi. However, this effect was not directly caused by the level of soil moisture itself, but via an indirect effect from interactions with the host plant root system. Under different soil moisture temporal variance conditions, the romaine lettuce rhizosphere selectively recruits specific microbial taxa. This research provides valuable insights into optimizing watering strategies, which could lead to a reduction in water usage; a critical consideration in climates with limited water resources. Taken together, our study aims to shed light on how to improve watering regimes for optimizing crop performance, and yield, contributing to sustainable agricultural practices.

Statements and declarations

None

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CRediT authorship contribution statement

Liu Dichuan: Writing – original draft, Visualization, Validation, Methodology, Investigation. **Willemsen Viola:** Writing – original draft, Supervision, Conceptualization. **Drohan Patrick J.:** Writing – review & editing. **Bryant Ray:** Writing – review & editing. **Long Huaiyu:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Zhu Guolong:** Investigation, Conceptualization. **Wang Zhuan:** Investigation, Formal analysis, Conceptualization. **Zhang Renlian:** Methodology, Conceptualization. **Xu Aiguo:** Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approval

This study does not involve animals.

Consent to participate

This study does not involve human participants.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agwat.2025.109354.

Data availability

Data will be made available on request.

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