Plant Physiology and Biochemistry 221 (2025) 109646

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Waterlogging alone and combined with other abiotic stresses provides unique metabolic signatures at the plant-rhizosphere interface: A multi-omics perspective on root metabolome, root exudation and rhizomicrobiome

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ARTICLE INFO

Keywords:
Abiotic stress
Combined stresses
Rhizosphere community
Metabolomics
Metagenomics
Multi-omics
Plant holobiont

ABSTRACT

Despite the growing evidence on unique and unpredictable impact of stress combination over plants, waterlogging-combined stresses effects are still underexplored. Under those conditions, besides the impairment of plant aerial parts, the root system is particularly vulnerable, leading to consequences on plant survival. Here, we report on the short-term exposure of soil-grown *Arabidopsis thaliana* L. to waterlogging alone and combined with cold, heat, and salinity to inspect their antagonistic, additive or synergistic effects in the rhizosphere. To this aim, root metabolic changes, exudation profiles, and microbial diversity were investigated using a combination of metabolomics and metagenomics, and their interaction was analysed through multi-omics data integration. In roots, waterlogging strongly affected metabolism compared to other single stresses, causing a down-accumulation of targeted classes of compounds including, phenylpropanoids, sterols, terpenoids, and alkaloids. Additive and synergistic effects were reported in roots under waterlogging combined with heat and cold stresses, respectively. Regarding root exudates, flavonoids, terpenoids, and alkaloids were the main classes of compounds affected. Waterlogging caused a down-accumulation of all classes except for coumarins, and mixed trends were observed in waterlogging-combined stresses, with waterlogging-salinity stresses resulting in an ameliorating effect. Even though microbial communities' alpha- and beta-diversity remained stable, suggesting their resilience under short-term exposure, specific taxa modulation was recorded under each condition. Overall, these results contribute to understanding the hierarchical impact of waterlogging on root metabolism and exudation, influencing rhizosphere interactions. This multi-omics approach advances our understanding of plant stress responses and microbial dynamics, paving the way for future studies on adaptive mechanisms.

1. Introduction

Warming, changing precipitation patterns, and climatic extremes such as floods, heatwaves, droughts, and storms induced by anthropogenic climate change have risen in frequency and intensity since the 1950s (Calvin et al., 2023; Hirabayashi et al., 2013). Among the adverse impacts and damages reported worldwide, agricultural production is being significantly hampered, with consequences on food security (OECD-FAO Agricultural Outlook, 2024–2033, 2024) as plenty of environmental stresses threaten plant growth and survival, harming both qualitative and quantitative crop yield (Kumar, 2016).

In this context, water submergence stands out as a deathly threat to

Abbreviations: CNT, (Control, unstressed plants); H, (Heat); C, (Cold); S, (Salinity); W, (Waterlogging); W + H, (Waterlogging + Heat); W + C, (Waterlogging + Cold); W + S, (Waterlogging + Salinity).

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https://doi.org/10.1016/j.plaphy.2025.109646
Received 29 November 2024; Received in revised form 3 February 2025; Accepted 11 February 2025
Available online 11 February 2025

plant survival, with approximately 16% of the fertile areas of the world affected by soil waterlogging and a 40–80% average yield loss due to spring floods or excess rainfall (Phukan et al., 2016). Besides causing variations in soil physico-chemical characteristics and microbial diversity (Randle-Boggis et al., 2018), plant roots are susceptible to flooding due to the decrease in soil oxygen levels by replacement of gas-filled pores with water (Daniel and Hartman, 2024), which is accelerated by aerobic microbial respiration (Bhaduri et al., 2017). While O_2 levels typically decline both vertically and radially within the root tissues and in the surrounding soil (Lee et al., 2011), the concentrations of gases such as ethylene, carbon dioxide (CO_2), and nitric oxide (NO) increase (Bailey-Serres and Voesenek, 2008). Moreover, O_2 deficiency induce the production of ethylene via the oxidation of 1-Aminocyclopropane-1-carboxylate (ACC) (Pan et al., 2021). By regulating transcriptional, translational and protein turnover, ethylene accumulation in submerged plant tissue is crucial for inducing a plethora of submergence-adaptive responses that promote hypoxia tolerance. Reduced O_2 levels stabilize the ethylene response factor of the family VII (ERFVII), which binds to hypoxia-responsive promoters, primarily enhancing a shift towards anaerobic ATP production via glycolysis and fermentation to counteract lack of O_2 for respiration (Licausi et al., 2010). This cellular energy crisis further affects nutrient uptake and hydraulic conductance, eventually resulting in wilting symptoms (Ahmed et al., 2013). In addition to changes in carbohydrate and energy status, plant hormones, accumulation of reactive oxygen species (ROS), and altered cytosolic pH contribute to hypoxia signalling thus inducing stress acclimation (Sasidharan et al., 2018). Overall, ethylene and hypoxia control most of the waterlogging-adaptive response in roots through a metabolic and morphologic adaptation (Voesenek and Sasidharan, 2013). Although energetically demanding processes, such as DNA and mRNA synthesis or protein degradation are significantly reduced, the control of genes and proteins involved in anaerobic metabolism and antioxidant metabolism is maintained to counteract ROS formation and improve oxidative stress tolerance. As a mechanism to conserve energy and limit O_2 dispersion, hypoxic conditions and ethylene induce a decline in root growth rate (Huang et al., 1997; Van Dongen et al., 2009), primarily by modulating the expression and transport of auxins. Furthermore, to escape critical hypoxic soil zones, lateral root formation and density are reduced under submergence, while root bending is modified, showing an upward slanting trend in both primary and lateral roots.

Unfortunately, flooding co-occurs with other abiotic stresses under field conditions, and a greater attempt is needed to unravel the plant response under this framework (Mittler and Blumwald, 2010; Suzuki et al., 2014). In fact, excessive water naturally occurs concurrently with soil toxicity, including salinity or thermal stresses, as well as following prolonged drought periods (Barrett-Lennard, 2003; Renziehausen et al., 2024). Notably, the co-occurrence of different stresses does not merely sum up but rather create a new state of stress condition with its effect on the plant molecular and metabolic response with differential consequences on plant tissues (Mittler, 2006; Zandalinas et al., 2022). These can be antagonistic, additive, or synergistic, depending on the intensity of the multiple impacts compared to the single one (Renziehausen et al., 2024; Zandalinas and Mittler, 2022). Recently, a higher number of research have tried to unravel the plant response under multiple stress conditions, but in-depth attempts to elucidate the mechanistic nature of stress integration are rare, owing to investigating the plant-soil system (Rillig et al., 2021).

In fact, although plants are often regarded as standalone entities, by living in the soil interphase, they form the holobiont together with their microbiota, from which their metabolic response strongly depends on (Vandenkoornhuyse et al., 2015). As such, soils, as plants, face numerous simultaneous anthropogenic pressures, including abiotic stresses, which impact their properties and functionality, including microbial communities, that remain understudied (Rillig et al., 2019; Zhou et al., 2020). Flooding induces changes in microorganisms due to variations in pH, O_2 concentration and availability of nutrients, such as Fe or Mn. Specifically, submerged soils may favor anaerobic and methanogenic microorganisms, which usually live in soil aggregates adapted to oxygen-free environments (Šibanc et al., 2014). Thus, anaerobiosis can modify soil functions, affecting nutrient availability and microbial activities, which can result in the accumulation of toxic compounds that inhibit root growth and eventually predispose to root diseases (Bhaduri et al., 2017).

The interaction with root and soil systems is driven by root exudates that play a vital role in shaping the rhizosphere bacterial community (Tiziani et al., 2022). Being formed by energy sources and carbon in the form of carbohydrates, amino acids, and organic acids, these substances attract microorganisms living within the rhizosphere, which undertake crucial ecological roles like nutrient solubilization (Parasar et al., 2024). Despite advancements in understanding the metabolomic changes in plants in response to hypoxia, the molecular mechanisms and changes in exudate composition due to flooding are still underexplored, as well as their contribution in shaping the soil microbiome (García et al., 2024). Deciphering the interplay between roots, exudates, and their modulation at the rhizosphere level through a multi-omics approach is crucial for unravelling the complex modulation affecting the holobiont. This approach is part of the "systems biology" approach which aims for a deeper understanding of physiologically complex processes (Cramer et al., 2011).

This study used the model plant *Arabidopsis thaliana* L. to understand the impact of single and co-occurring abiotic stresses, including cold, heat, salinity, waterlogging, and its combination on the complex interlink at the root and soil interface. The tripartite interaction between the root metabolism, the composition of root exudates, and the modulation of the soil microorganisms characterizing the plants' rhizosphere has been explored. Specifically, this work aimed at investigating (i) the metabolic profile of roots via untargeted metabolomics coupled with multivariate statistics and pathway analysis to unravel the distinct impact of each stress applied separately and in combination, (ii) the metabolic characterization of root exudates, (iii) the microbial population characterizing the related rhizosphere through 16S amplicon sequencing, and (iv) the determination of the main features outlining the interaction between these factors through an integrated multi-omics approach.

2. Material and methods

2.1. Plant growth and stress treatments

Arabidopsis thaliana L. Col-0 plants were grown in the facilities of Università Cattolica del Sacro Cuore (Piacenza, Italy). Seeds were kept for 48 h at 4 °C in dark condition for vernalization, then sowed in polystyrene seed trays and kept in a growth chamber at 20 ± 2 °C, 8/16 h light/dark photoperiod, 150 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) (Ambralight, Ambra Elettronica, Bolzano Vicentino, Italy). Once at 2-leaves stage, seedlings were transplanted in pots (6 × 6 × 9.5 cm) filled with 250 g of agricultural soil sampled during the off-season (bare soil) in the area around Cremona, Po Valley, Italy (45°12′24.1″ N, 9°53′23.3″ E; 52 m above sea level). After field collection, the soil was air-dried, sieved to 1 cm and homogenized. Physico-chemical characterization indicated that soil was fine loamy, mixed, semiactive, mesic, Oxyaquic Haplustalfs. The initial specific composition was clay, 15%; sand, 61%; silt, 24%; pH-KCl, 5.06; organic matter, 1.43 g kg^{-1}; total N, 0.10%; available P, 98 mg kg^{-1}; exchangeable K, 464 mg kg^{-1}; electric conductibility, 137.6 μs cm^{-1}. Pots watered one day before transplant to maximum soil water holding capacity. Plants were watered every other day until reaching 3.7 stage, 45 Days After Sowing (Boyes et al., 2001), corresponding to the beginning of the experiment. Then, plants were randomly divided into eight groups corresponding to the following conditions: CNT (Control, untreated plants), H (Heat), C (Cold), S (Salinity), W (Waterlogging), W + C

(Waterlogging + Cold), W + H (Waterlogging + Heat), W + S (Waterlogging + Salinity). Specifically, Cold stress was induced by keeping plants at 4 °C for 16 h (dark period); Heat stress was applied to plants by rising the temperature at 30 °C for 8 h (light period); Salinity stress was induced by watering plants with a 100 mM NaCl solution until full water holding capacity; Waterlogging was applied by placing pots in containers filled with enough water to reach the soil surface, thus keeping roots submerged in water. Plants selected for combined stresses were exposed to the simultaneous application of two treatments. Overall, these treatments were repeated for three days.

2.2. Plant harvest and samples preparation

After three days of treatments, each group of plants was split into two subgroups: (i) five replicates were used to collect soil rhizosphere and roots; (ii) three replicates were destined for exudate collection. Roots and rhizosphere samples were prepared by separating the above-ground part, then by gently removing soil with tweezers and collecting the particles adjacent to the roots. One gram of the rhizosphere from each sample was collected for the microbiome analysis. Afterwards, roots were thoroughly washed with tap water, rinsed with distilled water, and snapped frozen to stop the metabolism. Both the roots and rhizosphere were stored at −20 °C before subsequent analysis.

Root exudates were collected after taking the soil away, washing the roots using tap water, and rinsing them with distilled water until any trace of soil was removed. The plants were then transferred into 30-mL tubes containing 20 mL of distilled water and kept under the light. Root exudates were collected for 1 h; then the exudate solutions were centrifuged at 10,000×*g* for 10 min at 4 °C (Eppendorf 5430R, Hamburg, Germany), filtered with 0.22 μm syringe filters, and then lyophilized.

2.3. Roots and root exudates metabolomics analysis

Root material was homogenized by pestle and mortar with liquid nitrogen, then 0.2 g was weighed and mixed with 2 mL of the extraction solution (80% (v/v) methanol acidified with 0.1% (v/v) formic acid; LC-MS grade, Sigma-Aldrich, St. Louis, MO, USA). The solution was mechanically homogenized with an UltraTurax (Polytron PT 1200 E, Kinematica AG, Switzerland) and centrifuged at 10,000×*g* for 15 min at 4 °C (Eppendorf 5430R, Hamburg, Germany). The supernatant was then filtered into vials with a 0.22 μm cellulose for liquid chromatography analysis. Freeze-dried root exudates were resuspended in 1 mL of 50% (v/v) methanol aqueous solution (LC-MS grade, Sigma-Aldrich, St. Louis, MO, USA), extracted in an ultrasonic bath (ArgoLab DU-32; Carpi (MO), Italy) for 15 min at maximum power to facilitate resuspension.

The untargeted profiling of roots and root exudates samples was performed using a 1290 Infinity II ultra-high performance liquid chromatography system coupled with a 6560-drift tube-ion mobility-Q-ToF (Agilent Technologies, Santa Clara, CA, USA) as previously described in (Pardo-Hernández et al., 2024). Briefly, the chromatographic separation was performed using an Agilent Zorbax Eclipse Plus C18 analytical column (100 × 2.1 mm, 1.8 μm particle size), and the mobile phase consisted of a binary mixture of water and acetonitrile (from 6% to 94% in 35 min), both acidified with 0.1% (v/v) formic acid. The injection volume was 6 μL and 19 μL respectively for roots and exudates samples, and elution was operated at 200 μL/min flow rate. Mass spectrometry was conducted in positive polarity, utilizing full SCAN mode (100–1200 m/z) with a nominal mass resolution of 30,000 FWHM. The injection sequence was randomized, with five replicates per treatment for root samples and three replicates per treatment, with two technical replicates per sample for root exudates. Both for root samples and root exudates, Quality Controls (QC) samples, prepared by pooling the same aliquots of each extract, were randomly injected throughout the chromatographic sequence and acquired in a data-dependent (TOP N = 8) MS/MS mode, and the Top N ions were selected for fragmentation under stepped (10, 20, 40 eV) Normalized Collisional Energy.

The raw spectral data underwent processing using MS-DIAL software (version 4.90) (Tsugawa et al., 2015) for post-acquisition procedures and data filtering, with minor modifications (Sarv et al., 2023). Specifically, mass features were acquired in the 100–1200 m/z mass range, with a minimum peak height of 1000 counts. Accurate mass tolerance for peak centroiding and identification was set at 0.05 Da and 0.1 Da for MS and MS/MS analysis, respectively. Notably, retention time information was excluded from calculating the total identification score. The identification process depended on mass accuracy, isotopic pattern (considering isotopic distribution, space, and abundance), and spectral matching. The total identification cut-off score was set at 60%, retaining the most common ESI + adducts. Annotation of roots and exudates metabolites was performed using the comprehensive Vanija (available at CompMS | MS-DIAL (systemsomicslab.github.io), accessed on April 18, 2024) and BMDMS (Lee et al., 2020) databases. Additionally, the software MS-Finder (Tsugawa et al., 2016) ver. 3.50 was employed for *in-silico* identification of non-annotated MS/MS features by using FooDB, PlantCyc, HMDB, CheBi, NPA, NANPDB, Coconut, KnapSack, Pub-chem, UNPD Libraries, operating at a Level 2 confidence in annotation referring to COSMOS standards in metabolomics (i.e., putatively annotated compounds with spectral matching) (Salek et al., 2013).

2.4. 16S amplicon sequencing

Microbiological analyses were performed on *Arabidopsis thaliana* rhizosphere samples. Total DNA was extracted using FastDNA™ SPIN Kit for Soil (MP Biomedicals, Santa Ana, California; US) and quantified using Quant-iTTM HS ds-DNA assay kit (Invitrogen, Paisley, UK) with a QuBit 2.0 fluorometer (Invitrogen, Paisley, UK). V3-V4 region of 16S ribosomal RNA (rRNA) gene was amplified from rhizosphere bacteria, using the universal primers 343F (5′-TACGGRAGGCAGCAG-3′) and 802R (5′-TACNVGGGTWTCTAATCC-3′), as previously described in detail by (Bandini et al., 2021; Vijver et al., 2003). The fungal communities were analysed using the universal primers ITS-1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS-2 (5′-GCTGCGTTCTTCATCGATGC-3'. The amplified products were then subjected to nested PCR and indexed for multiplexing. High Throughput Sequencing was performed in Novogene facilities (Novogene Cambridge, UK), with the TruSeq DNA sample preparation kit for amplicon preparation (REF 15026486, Illumina Inc, San Diego, CA). Novaseq 6000 Illumina instrument (Illumina Inc, San Diego, CA) was used to obtain 250 bp paired end reads. Illumina barcode demultiplexing and base calling were performed with the MiSeq Control Software version 2.3.0.3, RTA v1.18.42.0, and CASAVA v1.8.2) (Bortolini et al., 2016). Raw sequences were aligned with the 'pandaseq' script (Bartram et al., 2011) with a minimum overlap of 30 bp between read pairs and a maximum of two mismatches allowed. After demultiplexing, sequences were imported into QIIME™ 2. Further filtration, trim, denoising of the sequences, and removal of chimeric sequences was performed with the QIIME™ 2 vsearch plugin and the feature table of amplicon sequence variants (ASV) was produced. The QIIME™ 2 feature-classifier plugin was then used to align the ASV sequences with a pre-trained Silva database (trimmed to the V3-V4 region bound by the 338F/806R primer pair) to produce the bacterial taxonomy table. Fungal taxonomy table was produced by aligning sequences to a custom UNITE (https://unite.ut.ee/) based database. Thermal cycling conditions, primer concentrations and volumes are provided in Supplementary Document.

2.5. Data processing and statistical analysis

The statistical elaboration of putatively annotated metabolomic data was performed using Mass Profiler Professional 15.1 software (Agilent Technologies). Briefly, data were filtered, Log2-transformed, and normalized at the 75th percentile. Additionally, detected features were baselined against the median of all samples. An overview of sample

clustering was obtained by unsupervised hierarchical cluster analysis (HCA) (Euclidean distance, Ward's linkage method). Additionally, the supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) was performed, and the validation of the output models was inspected by recording the model parameter (goodness-of-fit R^2Y and goodness-of-prediction Q^2Y). Besides, the models were cross-validated (CV-ANOVA), as well as inspected for outliers (Hotelling's T2), and a permutation test (n = 200) was applied to exclude overfitting. The Variable Importance in Projection (VIP) compounds (VIP score >1.0) were selected to identify metabolites having a discriminant potential ascribed to different treatments with respect to the control condition. Finally, significantly modulated compounds were identified via Volcano Analysis, setting a fold-change threshold of >2 with Benjamini-Hochberg multiple testing correction and p-value <0.05 with respect to the control condition. Statistically significant compounds were selected for further interpretation, including pathway analysis. To get an overview of significantly impacted metabolite clusters as a consequence of treatments, a chemical enrichment approach (ChemRICH) (Barupal and Fiehn, 2017) was carried out on each single and combined stresses vs control condition. Besides, a Venn diagram was carried out on Volcano significant compounds ($p < 0.05$ and FC > 2) out using jVenn platform (Bardou et al., 2014) to discern among significant markers for discrimination between each abiotic condition as well as to highlight markers common to more than one stress. Statistical analysis on sequencing data was performed with R supplemented with Vegan package (Dixon, 2003) and MicrobiomeAnalyst (Chong et al., 2020).

2.6. Multi-omics data integration

Data integration analysis was performed on four datasets, including bacteria and fungi metagenomics data obtained from rhizosphere and UHPLC-HRMS omics datasets of roots and exudates to identify key variables that could discriminate between single and combined abiotic stress conditions. The analysis was conducted on R software (version 4.1.3) using a MixOmics-derived framework called Data Integration Analysis for Biomarker discovery using Latent variable approaches for Omics studies (DIABLO) (http://mixomics.org/mixdiablo/) (Rohart et al., 2017). DIABLO aims to model and maximise correlations between omics datasets (the 16S rRNA gene sequence of fungi and bacteria and LC-MS datasets of roots and exudates), while identifying key multi-omics markers (taxa and metabolites) which reliably predict the classification of the groups of interest. The integration approach was based on multivariate supervised sparse Partial Least Squares Discriminant Analysis (sPLS-DA) with a sparse Generalized Canonical Correlation Analysis method (sGCCA) following the DIABLO framework (Singh et al., 2019). The total number of components for the DIABLO model and the number of variables per dataset and per component were calculated with the tune function in the DIABLO package. The aim of this tuning was to maximise the model's performance without overfitting. Five-fold validation (repeated 10 times) using the "leave-one-out" algorithm was undertaken to optimize the model's performance over the chosen set of parameters. The model's prediction performance was based on the balanced classification error rate, setting the optimal number of significant components.

3. Results and discussion

3.1. Root metabolomics

An untargeted metabolomics-based approach was first applied to comprehensively investigate the effect of 3-day stress application on roots metabolome. In our study, a total of 1295 chemical entities were putatively annotated across samples (Table S1). Besides, the dedicated MS-MS approach applied to Quality Control (QC) samples allowed the recording of 92 unique structures, of which 10 were based on the *in-silico* characterization. A hierarchical cluster analysis (HCA) was performed to group the samples via an unsupervised approach based on metabolome-wide similarities (Fig. S1). The cluster essentially indicated the hierarchical effect of waterlogging over other stresses. In detail, H and C exhibited the most similar profile to CNT, whereas waterlogging and its combination branched together, showing a different profile from other conditions. This result suggested that root submergence broadly impacted their metabolic profiles, thus causing a marked differential reprogramming compared to other treatments.

To better differentiate the effect of each stress condition from the CNT, a supervised OPLS-DA modelling approach was performed. All the models showed good performance parameters (R^2Y and Q^2Y), and a significant CV-ANOVA *p*-value <0.05 was recorded for W and W + H models (Fig. S2), providing the associated VIP markers (VIP score <1). Alongside, metabolites resulting from the Volcano analysis (FC > 2; $p < 0.05$) between each treatment against CNT were identified, conferring the differentially accumulated compounds (DACs) (Table S2), which were further subjected to pathway analysis and chemical enrichment approach.

Pathway analysis on DACs was first divided into primary and specialised metabolisms (Fig. S3). Concerning primary metabolism, "growth factors and regulators" covered a group of metabolites acting as phytohormones (cytokinins and auxins), carriers, and vitamins (such as biotin and α-tocopherol). In general, the stress treatments caused a mild impact on primary metabolism, except for amino acids and fatty acids (Fig. S3A). Notably, the lipid metabolism resulted in a complex modulation due to W applied alone and in combination, leading to the accumulation of long-chain FAs. These metabolites have been strictly related to hypoxia response by regulating acyl-CoA-binding protein–Group VII ethylene response factor (Xie et al., 2021), confirming the key role of lipids in response to submergence stress, as recently reviewed by (Xie et al., 2021). In parallel, specialised metabolites biosynthesis was differentially modulated in response to specific stresses (Fig. S3B), reporting a higher impact on phenylpropanoids, terpenoids, and alkaloids biosynthesis. A slight accumulation was found under single H, C, and S stresses, due to the short duration and low intensity of the stress applied. In particular, regarding S-treated samples, the elicitation of specialised metabolism indicates the plants' responses to mitigate the stresses, including the accumulation of antioxidants, such as polyphenols and terpenoids, to counteract root oxidative stress (Zandalinas et al., 2022). Meanwhile, a down-accumulation was shown for W and stress combinations, especially on phenylpropanoids, terpenoids and steroids. This pronounced reaction to W stress has been hypothesized to be mediated by the induction of a quiescence stage to face submergence (Bashar et al., 2019; Voesenek and Bailey-Serres, 2013). The hypoxic conditions associated with W dramatically induce roots anaerobic metabolism, thereby disrupting the energy supply in plants (Hofmann et al., 2020). As a result, a basal metabolic rate to minimize unnecessary metabolic reactions is adopted at a systemic level to conserve energy and extend their survival underwater (Gui et al., 2024; Zhou et al., 2020). However, there is still a lack of consensus regarding the metabolic effect of W duration, showing contrasting results in either the short or long term, as reported in rice (Fukushima et al., 2020) and orchard grass (Shang et al., 2023). The W combination with other stresses reported different effects concerning the individual contribution of both stressors, following previous evidence (Rivero et al., 2022). Terpenoids were strongly down accumulated, especially in W + H within all submerged samples (Fig. S3B). Moreover, the synthesis of phenylpropanoids followed a similar trend, being promoted inhibited under waterlogging combined treatments. Interestingly, a decrease in alkaloids was highlighted in W + H and W + S stress combination samples, while an increase of these compounds was disclosed with single S application and above all in W + C treatment.

To further inspect metabolic modulation, DACs were subjected to chemical enrichment analysis (ChemRICH) to detect the chemical classes mainly affected by the impact of single and combined stresses on *Arabidopsis* root metabolome (Fig. 1; Table S3). The enrichment plot

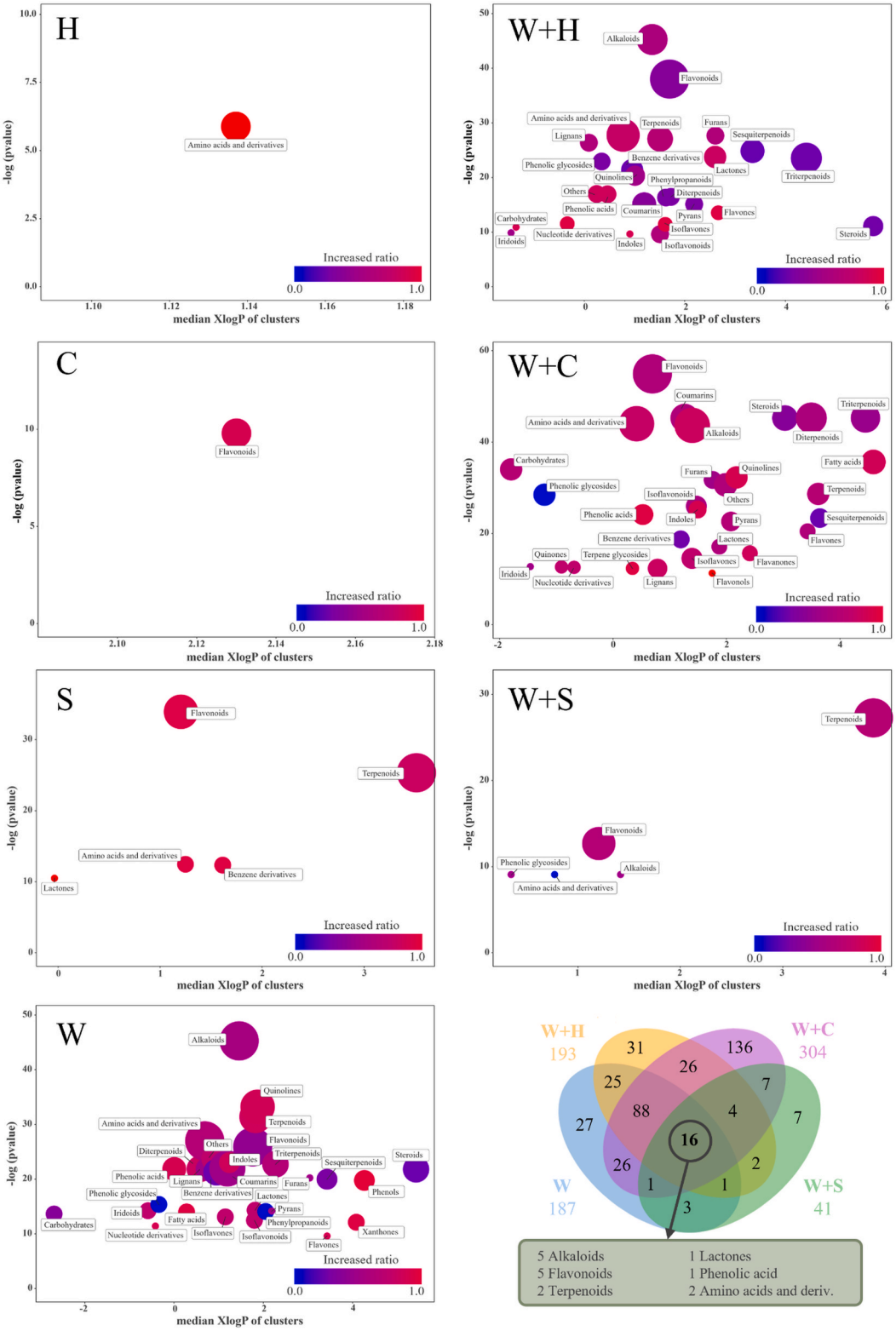

Fig. 1. Chemical similarity enrichment analysis (ChemRICH) of the compounds characterizing Arabidopsis roots differentially modulated after treatment with single and combined abiotic stresses as indicated by each label. A log-fold-change score ≥2 (p-value <0.05) and VIP ≥1 was used as the identification criterion of metabolites discriminating control vs stress-treatments. Each node indicates a significantly altered cluster of compounds; Enrichment p-values are given by the Kolmogorov–Smirnov test; node sizes is proportionate to the total number of metabolites in each cluster while the node color scale represents the increased (red), decreased (blue), or mixed trend (purple) of classes significant altered in treated plants compared to control condition. The y-axis shows the most significantly altered clusters on the top while the x-axis indicates the growing polarity of the cluster. The Venn analysis indicates roots metabolites specific and common to all W-treated plants.

confirmed the mild effect of H, C, and S single stresses on root metabolome, confirming the outcome from pathway analysis. Except for the up-accumulation of amino acids and derivatives in H-treated samples, and of flavonoids under single C condition, no significant changed were reported. Alongside, NaCl application elicited flavonoids, amino acid and benzene derivatives, while terpenoids followed a mixed trend. Accordingly, W severely impacted specialised metabolism compared to CNT, as shown by the high number of significantly altered clusters. A wide range of classes, including flavonoids, terpenoids, lignans, and alkaloids, showed a marked down-accumulation, whereas less representative classes, i.e. xanthones, fatty acids, phenols and phenolic acids, were accumulated (Table S3). This observation confirms the results on orchard grass, where W led to a down-regulation of genes involved in flavonoid biosynthesis, although less representative families of compounds were accumulated (Shang et al., 2023). Interestingly, W + H and W + C showed a similar trend to W, essentially reflecting a down-accumulation of di-tri-sesquiterpenoids, sterols, alkaloids and flavonoids, constituted almost half of the modulated compounds. While flavonoids up-accumulation under C, and S single stress aligns with antioxidant-stress coping response in plants (Wu et al., 2023), submergence differentially modulated polyphenolic compounds, including phenolic acids, xanthones, flavones, flavonols, isoflavonoids, and coumarins. This modulation aligns with findings in orchard grass, where most genes linked to flavonoid biosynthesis were down-regulated after submergence, despite some compounds being up-accumulated (Shang et al., 2023). Moreover, accumulation of carboxylic acids and phenols supports modulation reported for sensitive varieties (Pang et al., 2007). Interestingly, the down-accumulation of flavonoid glycosides (i.e.: kaempferol 3-glucuronide and quercetin 3-O-beta-D-glucose-7-O-beta-D-gentiobioside) coupled to the accumulation of flavonoid aglycones (i.e., myricetin, kaempferol, and pinoquercetin) in W, W + H and W + C (Table S3) suggests an efficient mobilization of flavonoids as part of their defensive role against abiotic stress (Mierziak et al., 2014). In addition, the absence of 6-hydroxyl groups makes these flavonoids act as analogues of the auxin inhibitor N-1-naphthylphthalamic acid (NPA), which is known to block polar auxin transport in roots (Dixon et al., 1996; Zhang et al., 2021) favouring the accumulation of auxins in this tissue, thus suggesting a dual role of flavonoids on stress mitigation at root level (Mierziak et al., 2014; Wu et al., 2023).

Alongside, the cytokinin (CK) kinetin was found to accumulate by W, W + H, and W + C, supporting the previous findings by (Shang et al., 2023), who reported the accumulation of the CK zeatin in submerged orchard grass. It is well-known that CKs promote cell division and plant growth and act as inductors of ethylene biosynthesis via a post-transcriptional modification of one isoform of ACC synthase (Vogel et al., 1998). Thus, the accumulation of kinetin reported here may contribute to W tolerance, thanks to the involvement of ethylene in the management of hypoxic conditions (Khan et al., 2020; Voesenek and Sasidharan, 2013). In parallel, sterols and terpenoids were generally down-accumulated by W, suggesting that hypoxic conditions contribute to the shift of pyruvate towards anaerobic metabolism, thus impairing mevalonate biosynthetic pathway (Jardine and McDowell, 2023; Sun et al., 2023). Although alkaloids have been reported to decrease in roots under W and combined stresses as well due to reduced photosynthetic flux (Honório et al., 2024), little information is available on their regulation in flooded roots.

With respect to W + H and W + C, the combined W + S stress caused a milder metabolic outcome on *Arabidopsis* roots, reflecting an opposite trend as compared to the S single stress: while S mostly induced the accumulation of flavonoids, alkaloids, and amino acids, accordingly to literature (Hill et al., 2013; Zhu et al., 2021), W + S promoted the down-accumulation of terpenoids, alkaloids, and flavonoids (Fig. 1). This was further confirmed by a Venn analysis, showing only 7 specific compounds for W + S, meanwhile 27, 31, and 136 were specific to W, W + H, and W + C, respectively. Due to the frequency and high diffusion of seawater intrusion in coastal regions and waterlogging in saline lands, the combinatorial effect of W and S has been relatively well-characterized, highlighting very diversified effects across plants with contrasting results (Martins et al., 2024; Tahjib-Ul-Arif et al., 2023). By reporting a reduced growth inhibition, activation of antioxidant enzymes, and regulation of ion concentrations, some research outlined that the combined effects of W + S were less detrimental than the impact of each stressor individually (Haddadi et al., 2016; Liu et al., 2020; Striker et al., 2015). Generally, the adverse effect of W + S stress was much more significant in salt-sensitive than in water-sensitive varieties, and moderate salinity stress could increase the tolerance to waterlogging stress (Martins et al., 2024), thus suggesting a driving role of salinity in dually stressed plants. This is also in accordance with the number and type of modulated clusters under S and W + S reported in our results.

Interestingly, only 16 metabolites were found to be modulated in roots under all W conditions either alone and in combination, including alkaloids, flavonoids, terpenoids, and amino acids derivatives, among other compound classes (Fig. 1). Among these, 9 were also shared with S-treated roots, including the alkaloids haplamine and lappaconitine, the flavonoids dehydrovariabilin and 5-hydroxy-2-phenyl-10-(thiophen-3-yl)-9,10-dihydropyrano[2,3-f]chromene-4,8-dione and the isoflavones demethyltexasin and glycitein. This can be ascribed to the activation of a common regulatory pathway under both stresses, which plays a role in the outcome of stresses' integration (Lamichhane et al., 2020). The remaining compounds modulated only under W include the alkaloids isocycloheximide, liriodenine, corynoxine, the phenolic compounds ginkgetin and chlorogenic acid, and two amino acids derivatives, which were all significantly down-accumulated. Their homogenous regulation suggests a specific effect of waterlogging on their metabolic pathway and possibly identifies them as negative markers of this stress in *Arabidopsis*. Anyway, the low number of compounds common to W-treated plants emphasises the very diverse effect of submergence applied alone and in combination with other stresses and confirms the necessity to untangle the impact of dual stresses by considering them as a new standalone stress condition (Zandalinas et al., 2022).

Our findings show that W applied alone strongly alters root metabolism, particularly downregulating targeted classes of phenylpropanoids (e.g., phenolic glycoside), steroids, terpenoids, and alkaloids. However, when combined with C or H, these effects driven by hypoxic conditions were amplified (additive/synergistic), exhibiting unique modulations not observed under individual stress, conditions. On opposite, the impact of W in combination with S created an intermediate effect, which may indicate a metabolic shift to counteract ionic stress rather than oxygen deprivation alone.

3.2. Root exudation profiles

The untargeted metabolomics analysis of root exudates allowed for the putative annotation of 1204 chemical entities, from which 121 compounds were MS/MS confirmed and 34 were detected *in silico* (Table S4). Overall, numerous plant primary metabolites were detected, including sugars (arabitol and mannitol), amino acids and derivatives (betaine, γ-aminobutyric acid, L-citrulline), as well as a wide range of organic acids (such as citric, fumaric, syringic, and isoferulic acids), in good agreement with the nature of the matrix under investigation (Dakora and Phillips, 2002; Dennis et al., 2010). Among the secondary metabolites identified, flavonoids, lignans, stilbenes, and coumarins were detected, together with terpenoids, steroids, and alkaloids. In addition, growth factors and vitamins, including thiamine and carnitine, were also reported, typically in exudate profiles (Vives-Peris et al., 2020a). It is well-known that abiotic stresses significantly alter both the quantitative and qualitative composition of root exudates (Vives-Peris et al., 2020b) and despite their involvement in rhizosphere signalling, the composition of root exudates under adverse conditions has been understudied. In fact, the modulation of secreted molecules under

abiotic stresses, including submerged non-wetland species, and the consequent effects on soil microbial communities remain relatively unexplored (Hartman and Tringe, 2019).

The application of HCA on exudates revealed two main clusters (Fig. S4): the first cluster included CNT and H grouped together with C, whereas the second cluster gathered all W-containing treatments together with S. Interestingly, S and W + S branched together, indicating a substantial similarity between these two treatments. Afterwards, metabolites were used to perform a supervised OPLS-DA analysis combined with Volcano analysis to provide the DACs to determine the impact of treatments on exudate composition with respect to CNT (Table S5). According to the number of DACs, W exhibited the richest composition (80 compounds), followed by W + H (74), and W + C (57). Meanwhile, H and C samples were less strongly affected than the CNT, with <30 modulated compounds. This impact was reflected in the corresponding ChemRICH analysis (Fig. 2 and Table S6). H and C led to an overall up-accumulation of polyphenols in H and phenolic compounds and organic acids in C-treated plants. Under H-stress, the increased exudation of flavonoids, which play a role in root colonization (Liu et al., 2021; Tian et al., 2021), could be a plant strategy to enhance the solubilization and uptake of nutrients whose mobility is restricted. Similarly, the coordinate exudation of flavonoids, coumarins, and organic acids under C stress has been demonstrated to facilitate the solubilization and uptake of Fe and P (Chai and Schachtman, 2022), which can be restricted under low-temperature conditions (Soualiou et al., 2022; Yan et al., 2012). Alongside, due to their allelopathic properties, they strictly interact with root microbiome promoting plant tolerance to cold (Stringlis et al.). However, in both conditions, the exudation pattern remained similar to the control condition due to the short duration of stress application.

Contrarily, W strongly impacted root exudation, with a significant down-accumulation of all classes except for coumarins (Fig. 2). Notably, the most altered classes were terpenoids, flavonoids and organic acids. The impact of W on root exudation remains poorly understood (Martínez-Arias et al., 2022). While Henry et al. (2007) observed a 45% increase in total organic carbon exuded by flooded *Agropyron cristatum,* Meng et al. (2022) reported that 10-h flooded roots of the wetland mangrove *K. obovate* did not secrete additional inorganic P and N, dissolved organic C or organic acids. However, these finding should be interpreted with caution due to the experiment's low replication, varying submergence duration, and the ecological diversity of the studied plants. According to other authors, soil anoxia reduces net photosynthesis, limiting the amount of root-derived carbon in the rhizosphere following waterlogging (Hamonts et al., 2013), thus decreasing sediment organic carbon stocks under prolonged stress conditions. Indeed, hypoxic root systems change the quantity and quality of exudates, affecting the soil microbiome and reducing potentially beneficial plant microorganisms (Francioli et al., 2021; Gladkov et al., 2023).

With respect to W + H, stress combination caused a down accumulation of all clusters, and in particular terpenoids, flavonoids, and alkaloids; only coumarins showed a mixed trend (Fig. 2). Under W + C, modulation of the exudation composition induced a down-accumulation for all clusters except for phenylpropanoids and derivatives, organic acids and amino acids. Besides highlighting the dominant effect of waterlogging on root exudation (Renziehausen et al., 2024), the impact of temperature stresses on modulated classes is also present, suggesting that waterlogging-induced metabolic quiescence may prevail with other typical stress responses. Depending on species and plant traits, waterlogging can prioritize plant's hypoxia-related metabolic adjustments, potentially suppressing other stress response pathways (Lin et al., 2015). The opposite trend was observed for S and W + S, with S showing a more robust modulation than its combined counterpart, in which coumarins were found significantly accumulated with respect to control conditions (Fig. 2). NaCl can change the composition of root exudates according to its concentration range, plant growth stage, and species (Kumar et al., 2023), although the mechanism of action of salt stress on root exudate composition is yet to be defined. Our results suggest a complex modulation of the exuded phenolic profile under salinity, in line with previous evidence in soybean (Dardanelli et al., 2010). Regarding the combination W + S, the results on *S. mariqueter* showed that total organic carbon levels did not significantly change (Bi et al., 2024; Xue et al., 2020), requiring further research in this field to provide robust evidence on the effect of combined stresses on root exudate composition.

Considering the universal signature of W on root exudation, the corresponding Venn diagram containing W singles and combined treatments showed eight commonly modulated metabolites (Fig. 2). Accordingly, two coumarins were reported, i.e. bergapten and 3,4-dihydro-6,8-dihydroxy-3-(10-hydroxyundecyl)isocoumarin, together with, two other polyphenols (salidroside, eupomatenoid 5), citric acid, two alkaloids and the terpenoid veronicoside. All common markers, except for bergapten, were found down-accumulated under all waterlogging conditions, identifying these as negative W-stress markers in *Arabidopsis* exudates. However, these common metabolites had a limited contribution in all W-treated plants, confirming the distinct effects of submergence applied alone and combined, underscoring the need to study dual stresses as a unique condition (Zandalinas et al., 2022). Notably, the coumarin bergapten was accumulated across all W conditions, suggesting a pivotal role in counteracting abiotic stresses in *A. thaliana*. Interestingly, the positive effect of bergapten on the assembly and structure of the microbial community in the rhizosphere by inhibiting the soil-borne fungal pathogens and promoting beneficial ones has been reported (Stringlis et al., 2019). These findings support the identification of bergapten, and in general coumarins, as promising candidates for *Arabidopsis* exudation markers under either single or combined abiotic stress.

3.3. 16S amplicon sequencing

A total of 2991115 reads were identified for bacteria after quality check, an average count per sample of 74777 with a minimum count per sample of 43771 reads. For fungal communities, the results obtained after quality check and filtering were a total number of reads of 705633, with an average count of 17640 per sample, with the minimum count for a sample at 5893.

The bacterial community analysis at the genus level revealed that the most abundant genera in the rhizosphere samples were *Bacillus*, *Thermoactinomyces*, and members of the Solirubrobacteriaceae family (Fig. 3A). These genera did not exhibit significant modulation across the different treatments. Notably, at least 30% of the relative abundance in all samples was comprised of "Other" bacterial genera, which individually represented less than 5% of the total abundance. This indicates a high level of diversity within the rhizosphere environment with a specific modulation of specific less abundant taxa by the different treatments. A three-dimensional PCoA analysis with PERMANOVA ($p < 0.05$) was performed to assess the beta diversity among the treatments, showing no overall shift in microbial population structures (Fig. 3B), consistent with results on alpha diversity, measured by Simpson's index (Fig. 3C). LefSe analysis on differential abundances (Fig. 3D) showed that the treatments significantly modulate specific bacterial genera. The genera *Rubrobacter* and *Skermanella* were significantly more abundant under H and W + H, indicating stronger resistance to higher temperatures. In contrast, the relative abundance of *Nitrospira* increased under both S and W + S treatments, suggesting enhanced resistance to saline soil conditions. The genus *Pseudomonas* showed increased abundance in treatments involving C, S, and their combinations with waterlogging (W + C and W + S), demonstrating a higher tolerance to both cold and saline environments even under submergence, reducing its abundance under W. This indicates that *Pseudomonas* has a higher tolerance for cold and saline soils independently of waterlogging stress.

For fungal populations, the genera *Mortierella* and *Solicoccozyma* were particularly abundant, with the family Orbiliaceae showing high variability between replicates (Fig. 4A). With respect to the fungal community, beta diversity analysis and PCoA analysis with

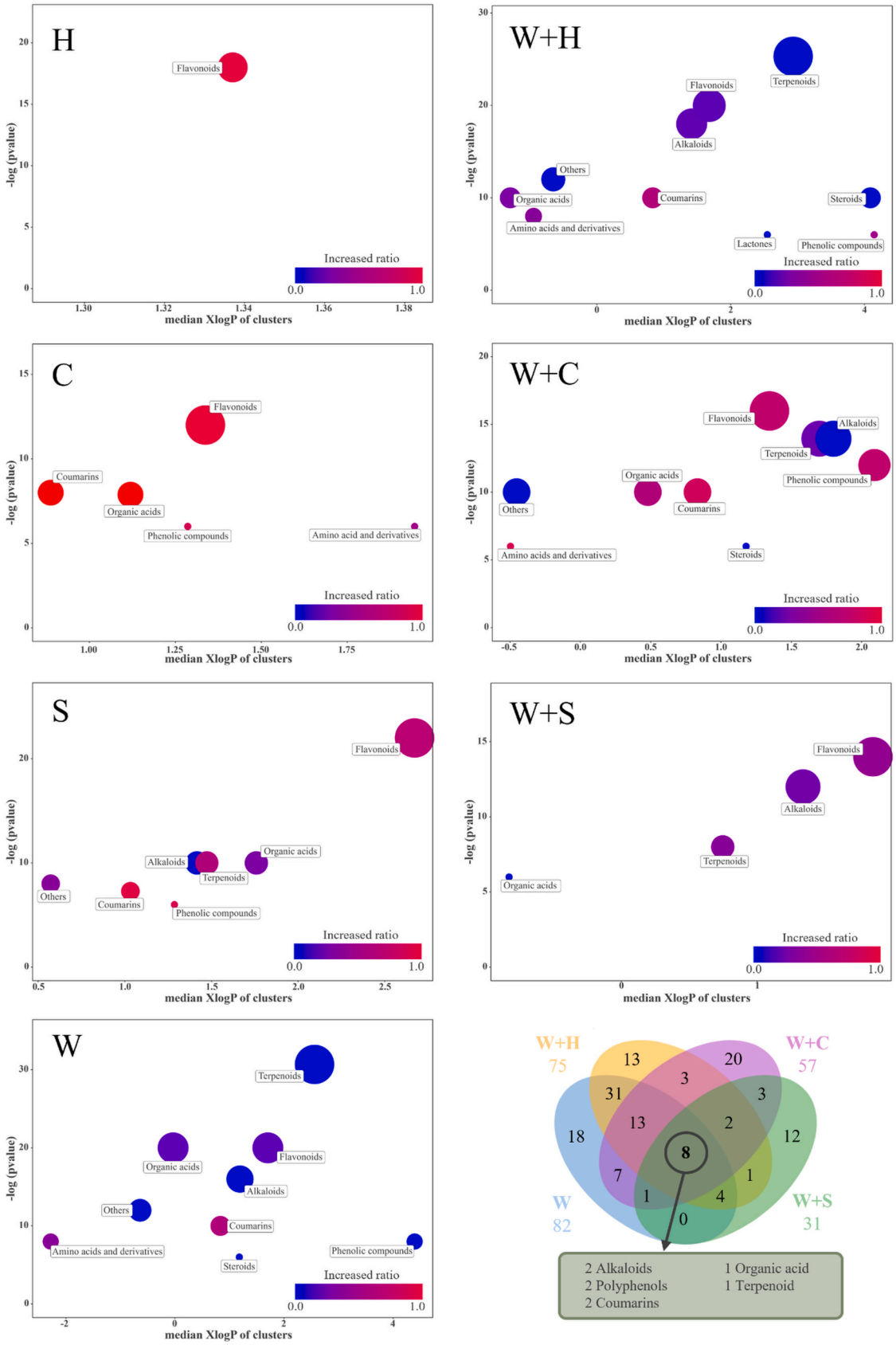

Fig. 2. Chemical similarity enrichment analysis (ChemRICH) of the Arabidopsis root exudates differentially modulated after treatment with single and combined abiotic stresses as reported by the corresponding labels. A fold-change score ≥2 (p-value <0.05) and VIP score ≥1 was used as the identification criterion of metabolites discriminating control vs stress-treatments. For the graph interpretation, see explanation at Fig. 1.

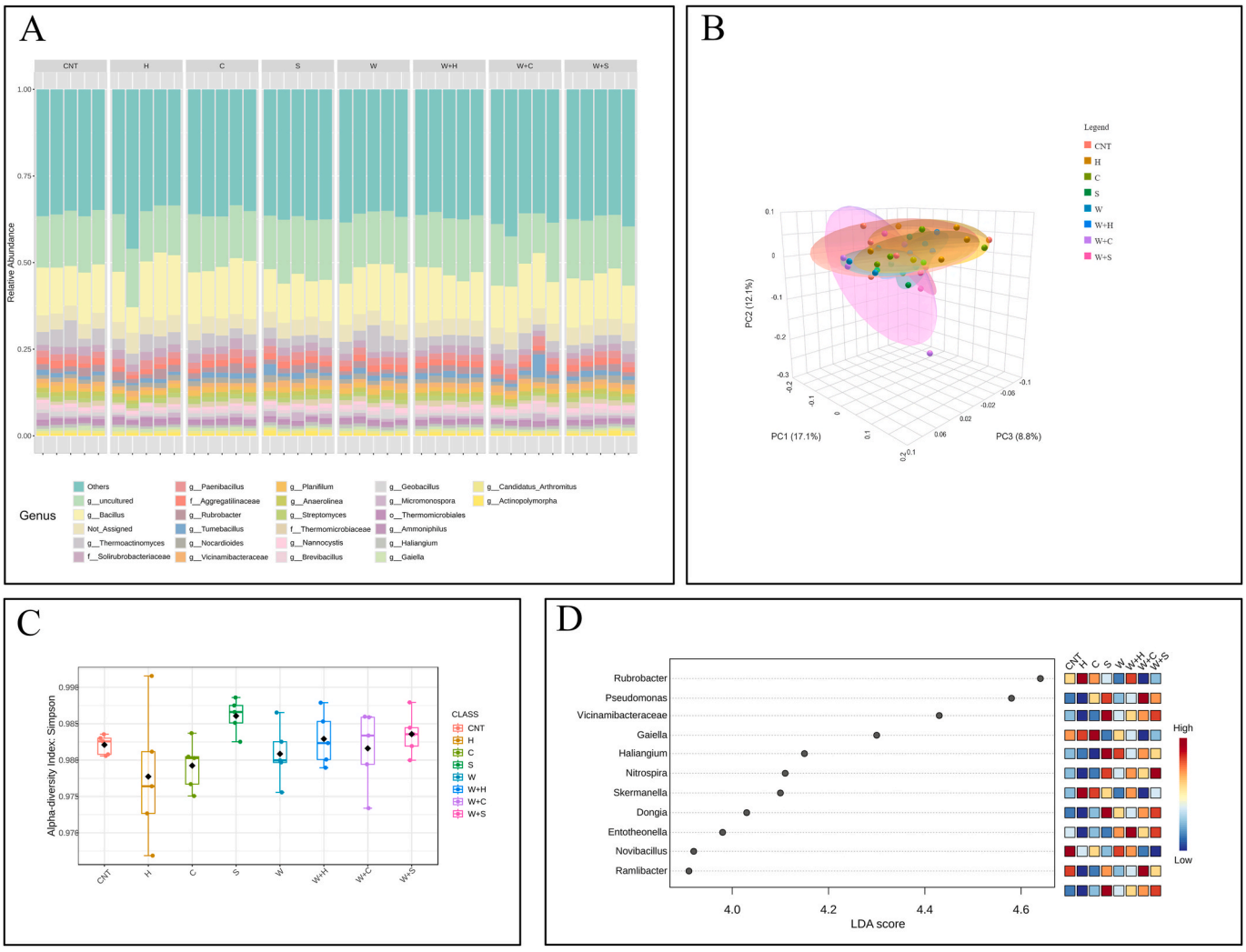

Fig. 3. Results from bacterial amplicon sequencing analysis: A) Taxa-barplot at the Genus level B) Beta diversity profiling at three components (p-value <0.05) C) Alpha-diversity graph of the treatments measured with Simpon's index. D) LefSe Analysis for differential abundances of bacterial genera. The image shows all genera significantly modulated by the treatments (LDA>2; p-value <0.05).

PERMANOVA ($p < 0.05$) indicated an overall homogeneity across treatments (Fig. 4B). This suggests that the fungal population is generally conserved, with differences confined to less abundant taxa. As assessed by Simpson's index (Fig. 4C), Alpha diversity did not show significant differences between treatments, reflecting an overall stability within the fungal community. When looking at differential abundance in fungal communities (Fig. 4D), few genera were found to be significantly influenced by treatments. The genera *Rhizophlyctis* and *Syncephalis* were more abundant in treatments involving C and W + C, suggesting a higher adaptation to cold environments irrespective of waterlogging. In contrast, *Cladosporium* appeared to be more abundant under H. The genus *Phialemonium* was less abundant in the CNT but showed increased abundance in both C and W + S treatments.

16S amplicon sequencing results indicate that the rhizosphere maintains an overall stable microbial community structure, with only minor shifts that do not affect the most abundant taxa present in the rhizosphere in response to the stress conditions of this experiment. In fact, the applied short-term stress exposure was insufficient to induce pronounced changes in the rhizosphere community structure. Only minor shifts were confined to less abundant genera, consistent with previous studies pointing out that microbial communities are resilient to short-term abiotic stresses (Bandopadhyay et al., 2024). Similarly to our study, recent research examined the microbiome structure and function in the rhizosphere of Jerusalem artichoke grown in saline soils and found that, while the short-term changes induced by stress affected microbial activities and some properties, the core microbial diversity in the rhizosphere remained stable, thus suggesting that microbial communities can exhibit resilience to short-term environmental stressors (Yue et al., 2020). This is noteworthy because much of the research on stress effects on rhizosphere communities typically involves longer stress exposure, including heat, cold, saline, and waterlogging stresses, which result in more significant changes in rhizosphere microbial composition (Francioli et al., 2021; Liao et al., 2023). For example, long-term studies on saline stress have shown a deep modulation in the rhizosphere microbial communities, particularly favoring salt-tolerant bacteria such as *Pseudomonas* and *Nitrospira*, which play essential roles in maintaining plant health under saline conditions. Interestingly, both genera were positively modulated in our study, confirming the relation between these taxa and salinity stress applied alone and in combination with waterlogging. Particularly, *Nitrospira* has demonstrated resistance to saline environments, where its role in nitrification under stress conditions is crucial (Shao et al., 2019; Wang et al., 2017; Yue et al., 2020). Alongside, *Pseudomonas* species, when co-inoculated with other microbes, significantly alleviated salt stress in plants, altering the rhizosphere microbial community to favor salt-tolerant species (Egamberdieva et al., 2016). Moreover, existing literature indicates an increased abundance of *Pseudomonas* under cold conditions as well. In fact, being psychrotrophic, long-term cold stress studies have shown

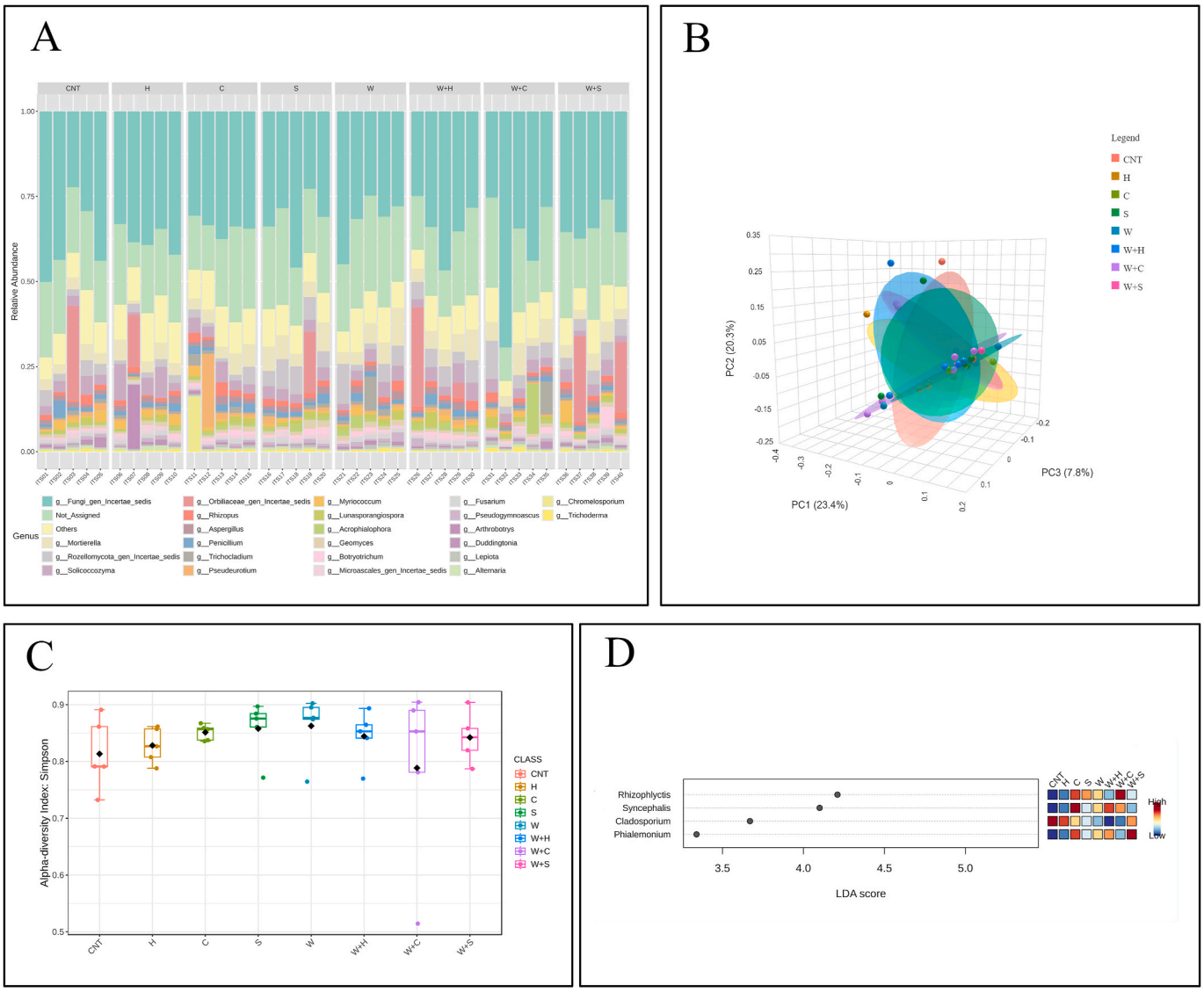

Fig. 4. Results from fungal amplicon sequencing analysis: A) Taxa-barplot at the Genus level B) Beta diversity profiling at three components (p-value <0.05) C) Alpha-diversity graph of the treatments measured with Simpon's index. D) LefSe Analysis for differential abundances of fungal genera. The image shows all genera significantly modulated by the treatment (LDA>2; p-value <0.05).

their ability to survive and function in low-temperature environments, promoting plant growth even under adverse conditions (Mishra et al., 2011; Reva et al., 2006; Suyal et al., 2017). In general, *Pseudomonas* are species well-known for their resilience in various stress environments, where they promote plant growth and plant stress resistance by modulating root microbiota and improving nutrient uptake under adverse conditions (Egamberdieva et al., 2016). Additionally, other specific shifts in microbial genera observed in this experiment align with previous reports on stress tolerance in specific genera. For example, *Rubrobacter* and *Skermanella*, which have been detected in H and W + H treatments, have been documented to thrive in heat-stressed environments due to their thermophilic and stress-resistant properties, suggesting that these genera are well-adapted to heat stress (Liu et al., 2021). With respect to fungi, few community shifts were observed. Even though, among these, *Cladosporium* showed an increased abundance under heat treatment, which is consistent with its known thermotolerant properties, supporting its survival and proliferation in heat-stressed soils.

Overall, despite the stability of the microbial population, differentially enriched taxa imply a specific role of certain microorganisms, under combined stress conditions, which require further investigation. For example, the enrichment of *Nitrospira* and *Pseudomonas* under W + S, or *Rubrobacter* and *Skermanella* under W + H, indicates stress-specific microbial adaptation mechanisms and suggests that certain microbial groups may be selectively recruited based on the dominant stress factor.

3.4. Multi-omics data integration

A DIABLO data integration workflow was further applied to integrate the metabolomics and metabarcoding datasets from *Arabidopsis* with aim of determining the influence of the stress treatments on both the root metabolome and rhizosphere metagenomics. The individual effect of four datasets, i.e.: root metabolomics, exudates metabolomics, bacteria metagenomics, fungi metagenomics, was firstly evaluated through sPLS-DA (Fig. 5A). In general, H and C were grouped together with CNT, whereas S, W, and combined stresses were separated according to first component. In parallel, according to the second component, W + C clustered independently from the rest of the treatments, suggesting a slight discrimination for this treatment. These results support the previous evidence from single datasets, with H and C playing a mild effect with respect to CNT, while W + C exhibited a differential. The integrated outcome of treatments is shown in the arrow plot (Fig. 5B), reporting a

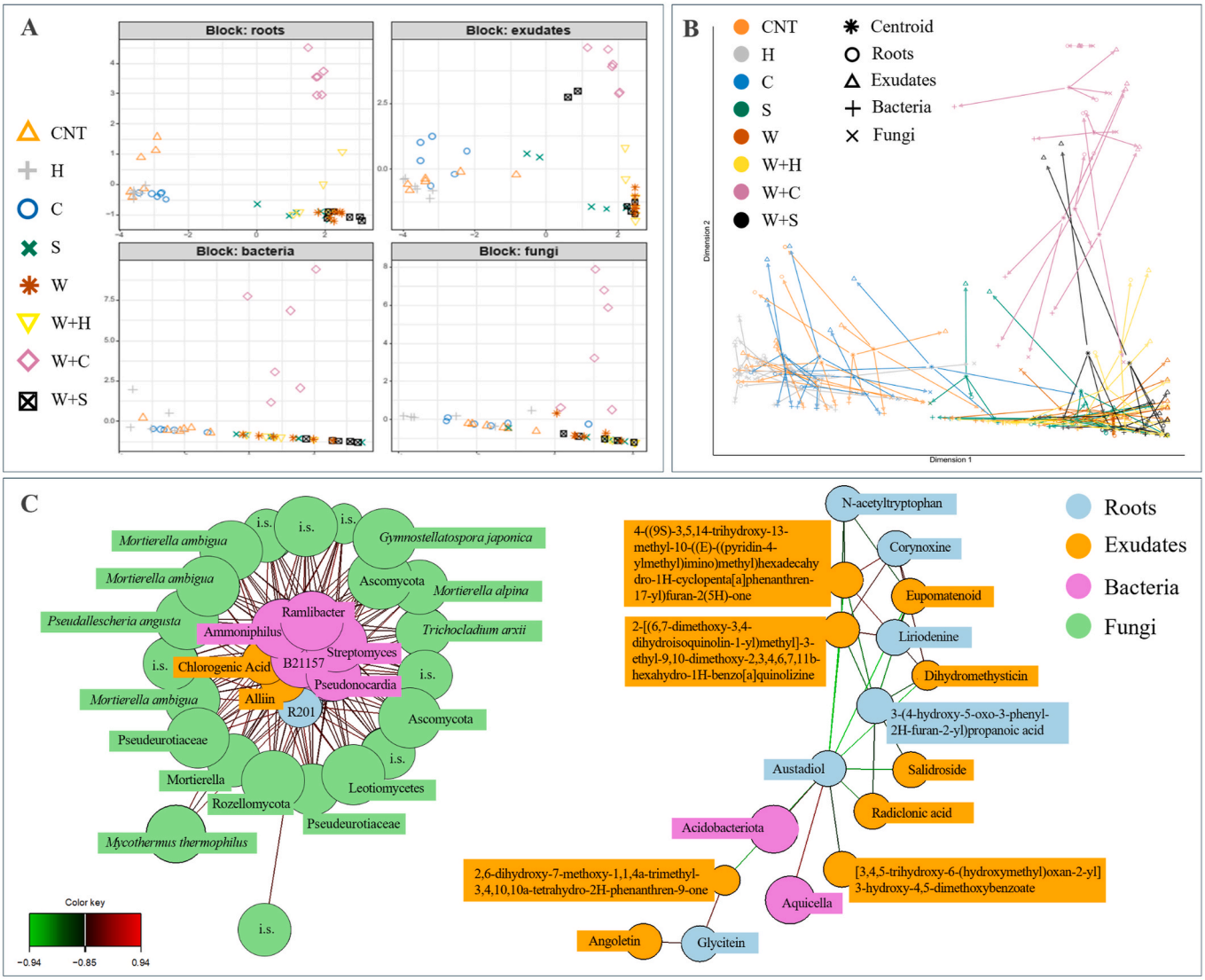

Fig. 5. DIABLO-based data integration models for the metabolomics of Arabidopsis plants' roots and exudates and metagenomics of soil rhizosphere. (A) Block contributions for metabolomics of roots and exudates and metagenomics of bacteria and fungi discriminating among CNT, single (H, C, S, W) and combined (W + H, W + C, W + S) stress treatments. (B) Arrow plot from multiblock sPLS-DA performed on the four datasets integration. The start of the arrow indicates the centroid between all datasets for a given sample and the tip of the arrow's location of the same sample in each block. (C) Network diagram showing the correlations between the featured markers identified by the model (cut-off = 0.94; green lines, negative correlation; red lines, positive correlation). R201: 2-O-methylbutyrolactone II; B21157: Vicinamibacterales.

close relationship between metabolomics and metagenomics in response to the stress treatments involved in this study, thus indicating a marked correlation between microbial taxa and specific metabolites, with exudates being the more diverse. Beyond the first two components, up to five components were predicted by DIABLO model tuning to achieve minimal generalization error, all of them showing high-quality of prediction and statistical robustness (Fig. S6). These findings highlight the significance of integrating results from various -omics analyses rather than relying on individual -omics studies, as already highlighted in other studies (Pardo-Hernández et al., 2024).

Moreover, thanks to the supervised nature of DIABLO modelling, the identification of critical markers for each block was performed for each component, providing direct associations between metabolite markers and critical taxa to understand better the integral impact of single and combined stresses in *Arabidopsis* (Table S7). With respect to the first component, the integrative markers provided mostly insight into the effect of W + S. The polyketides curvularin, austadiol, and 3-(4-hydroxy-5-oxo-3-phenyl-2H-furan-2-yl)propanoic acid were detected, together with the endophytic fungal genus *Phialemonium* and a wide number of bacteria. The most relevant were the genera *Azoarcus* and *Nitrospira,* an endophytic nitrogen fixing bacteria and a nitrite oxidizer, respectively and Woeseia. Curvularin, a compound known for its antibacterial and antifungal properties, is produced by various fungi, including symbiont species typical of marine environments (Dai et al., 2010; de Castro et al., 2016). The positive correlation suggests a potential interaction between this compound and the endophytic *Phialemonium*. Interestingly, the presence of *Woeseia*, commonly found in marine sediment ecosystems (Mußmann et al., 2017), supports this fungi genus as a marker of W + S treatment.

The results from the second component shed light into the integrative markers ascribed to W + C. Most markers for both roots and exudates were polyphenols, including the phenolic acids erionic and chlorogenic acids, the flavonoid (−)-vestitol, and the lignan podophyllin acetate. Alongside, many microorganisms correlated to W + C. These included the bacterial genera *Paenibacillus*, *Ammoniophilus*, *Ramlibacter*, *Pseudonocardia* and *Streptomyces* and the fungal genera *Mycothermus*,

Solicoccozyma, *Mortierella* and *Trichocladium*. Interestingly, the exuded compounds include recognized phytoalexins with different effects, such as the antipathogenic vestitol, and the antioxidant and cytotoxic podophyllin acetate (M. Li et al., 2012; Shimada et al., 2000). Notably, also the alpha-amino acid alliin was spotted as a marker of root exudates, together with chlorogenic acid (CGA), whose role is increasing resistance to bacterial infection has been thoroughly demonstrated by acting both as a defence molecule and signalling compound for beneficial microorganism (Rizaludin et al., 2021). Indeed, many beneficial microbes were recorded under the selected condition. These include the fungi *Solicoccozyma* and *Mortierella and* the bacteria *Ammoniophilus* and *Ramlibacter,* which are involved in various processes that promote nutrient solubilization and improve plant growth under adverse conditions (Wolińska et al., 2022). Moreover, plant growth promoting rhizobacteria (PGPR), such as *Paenibacillus* and *Streptomyces* were identified, whose role in nutrients acquisition and in the detoxification and degradation of pathogenic factors, promotes the induced systemic resistance (ISR) response which enhances plant defences against pathogens (Glick, 2014; Mishra et al., 2017). Similarly, *Pseudonocardia* has been shown to thrive in stress-affected soils, further highlighting the selective recruitment of beneficial microbes by plants through their exudation profile (Li et al., 2010), in close relation to the "cry-for-help" hypothesis (Rolfe et al., 2019).

Overall, the release of antipathogenic compounds and the presence of PGPR suggest a synergistic effect in alleviating the stressful condition induced by W + C. Notably, both *Streptomyces* and *Solicoccozyma* have an ACC deaminase activity involved in the protection against the damage caused by flooding, cold and other abiotic stresses, thus improving plant resilience to multiple stress (Carvajal et al., 2024; Chandwani and Amaresan, 2022; Harman et al., 2021).

The third component, essentially describing the C-associated markers, many fatty acids and steroids were associated with roots, whereas specialised metabolites, such as alkaloids, terpenoids, and polyphenols were ascribed to exudates. Overall, these results confirm the independent outcome found for the chemical exudates profiles, suggesting their interaction with the rhizosphere by acting as a robust phytoalexin source (Mushtaq and Fauconnier, 2024). Alongside, bacterial genera positively associated with C treatments have been identified in: *Aquicella*, *Bacillus*, *Georgenia*. Notably, the phylum *Ascomycota* as well as the genera *Trichocladium* were linked to C-mediated stress. In the same way, the fourth component essentially reported W-associated markers. In the case of exudates, lipids (9-methoxycarbonyldec-9-enoic acid and sarmentoside B) and nucleotide derivatives were mainly represented. Concerning microbial taxa, W positively influenced the bacterial genera *Marininema*, *Bacillus*, *Conexibacter*, *Geobacillus*, *Alcaligenes*, as well as the fungal communities of the genera *Calvatia*, *Mortierella*, and *Gymnostellatospora*. Interestingly, *Marininema* has been found in the sediment, suggesting an adaptation to submerged soil environment, as it was previously associated with marine and aquatic environments (J. Li et al., 2012). Belonging to the family Thermoactinomycetaceae, it can biosynthesize bioactive compounds, potentially contributing to soil health. Considering metabolomics and soil metagenomics integration, the model identified the W-associated marker sarmentoside B, which was originally reported from the plant *Strophanthus sarmentosus,* proceeding from *Streptomyces cavourensis*, isolated from sea cucumbers (Wibowo et al., 2019) and has been found correlated with microbial communities associated with high salinity sediment environment (Zamora-Quintero et al., 2022).

The integration of DIABLO-derived markers was jointly achieved by the establishment of two multiblock networks (Fig. 5C), providing new perspectives for exploring the potential interactions in the rhizosphere by elucidating the interplay of the different microbial community members and metabolites (Ge et al., 2023). Specifically, the first network integrated a wide range of roots and exudates features primarily ascribed to the first component (Fig. 5C, right), highlighting both positive and negative correlation of amino acids, isoprenoids, and flavonoids biosynthesis in root metabolism and terpenoids, phenolic compounds, and sterols from exudates. Interestingly, besides negatively correlating with other identified chemical features, austdiol, a fungus-produced compound with antibacterial properties, correlated with the bacteria *Aquicella* and Acidobacteriota. The positive correlation of this compound with bacteria may indicate its inhibitory effect under adverse conditions, although further research is needed to elucidate this interaction.

The second network (Fig. 5C, left), confirmed the correlation between microorganisms and molecules already highlighted by the W + C component, identifying, among others, *Mortierella, Gymnostellatospora, Pseudallescheria, Mycothermus* fungi, as taxa strongly correlated to the biosynthesis of fatty acids (2-*O*-methylbutyrolactone II) in roots, and the exudation of alliin and CGA. These fungi also positively correlated to a wide range of bacteria, including *Ammoniophylus, Streptomyces, Ramilbacter* and *Pseudonocardia*. This correlation further supports the significant functional role of CGA as a defence and signalling compound even under stress conditions. Specifically, *Ammoniophilus, Ramilbacter, Streptomyces*, and *Pseudonocardia* genera may have mechanisms to tolerate or metabolize CGA, allowing them to colonize the root environment during stress conditions (Li et al., 2010; Patil et al., 2011). Moreover, CGA antifungal influences fungal dynamics in the rhizosphere, modulating plant growth-promoting species and endophytes, which alter rhizosphere microbial composition (Chen et al., 2010). Overall, the increase in CGA secretion under abiotic stress serves as an adaptive mechanism by which plants recruit specific rhizomicroorganisms that not only help to tolerate stress but also contribute to long-term plant health and resilience by modulating the microbiome of the rhizosphere.

Even though the applied integrated multi-omics approach successfully identified correlations between root metabolites, exudates, and microbial taxa, the mechanistic links driving plant stress resilience remain unclear. The observed metabolic shifts and microbial community modulations suggest potential interactions in stress adaptation, but direct causal relationships require experimental validation. For example, our data indicate that flavonoids, terpenoids, and alkaloids in root exudates correlate with microbial shifts, suggesting their role in microbial recruitment. However, it remains to be confirmed whether these compounds actively enhance microbial stress tolerance or if microbial communities modulate plant metabolism in response to stress. Similarly, the enrichment of *Pseudomonas* and *Nitrospira* under W + S points out their possible role in stress mitigation, but functional assays are necessary to establish their precise contribution.

Overall, although this study offers insights into metabolic and microbial responses to combined abiotic stresses, further research is needed to pinpoint molecular and physiological mechanisms driving distinctions between single vs combined W-stresses, particularly at the rhizosphere level, which remains poorly investigated. By integrating transcriptomic and proteomic data, regulatory networks governing hypoxia, osmotic stress, and temperature extremes interactions could be revealed at the gene expression level. Additionally, time-course analyses would help clarify whether metabolic shifts under combined stresses represent an adaptive strategy or a transient imbalance before stabilization, as similar trends have been reported by other studies under W alone (Fukushima et al., 2020; Herzog et al., 2018; Shang et al., 2023). By addressing these mechanistic aspects, we can better understand how plants orchestrate responses to multiple simultaneous stresses, ultimately informing strategies to enhance crop resilience in dynamic environmental conditions.

4. Conclusion

This study investigated the roots and exudates composition of *A. thaliana* and the community structure characterizing its rhizosphere under submergence applied alone and in combination with heat, cold, and salinity. Metabolomics analysis showed the hierarchical effect of

submergence over other stresses in inhibiting root metabolism, especially of phenylpropanoids, terpenoids and steroids. By creating new environmental conditions, stresscombinations showed different effects, with W + C exacerbating metabolic modulation or W + S likely ameliorating plant response. Except for the increase in coumarins, the exudate signatures followed a similar modulation to roots, with down-accumulation under W and W + H, and a mixed trend under W + S. The three-day stress application did not shift the overall rhizosphere community, suggesting the resilience of microorganisms to short stresses. Multi-omics analysis revealed a strong correlation between metabolomics and metabarcoding data, pointing out specific markers for each stress combination not detectable under separated dataset analysis. Further studies of targeted metabolomics and transcriptomics, stable isotopic labelling, soil enzymatic assays and microbial inoculation are required to comprehensively investigate the dynamics of plant-exudate-microbe interactions, focusing on the revealed markers under combined abiotic stresses. This will help uncover the underlying mechanisms of stress interaction and enhancement of those few metabolic pathways similarly altered under single and compound environmental threats to develop crops with improved tolerance to field conditions under the current climatic change. By moving beyond correlative analyses, we can uncover causal relationships in plant-microbe interactions under combined abiotic stresses. Alongside, beside the identification of microorganism taxa resistant to more than one stress, the molecular and biochemical processed underlying the plant-microorganisms association could contribute the development of biostimulant formulation with plant protection effects.

Author contribution statement

Conceptualization: LL, ES, MADG; Formal Analysis: ES, MADG, FV, PGP; Investigation: ES, MADG, FV; Methodology: ES, MADG, FV, PGP; Resources: LL, EP; Visualization: ES, FV, PGP; Writing–Original Draft Preparation: ES, MADG, FV, PGP; Writing – Review & Editing: LL, EP.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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.

Acknowledgements

Pascual García-Pérez thanks the finantial support through the Ramón y Cajal program (reference: RYC2023-044123-I) by the Spanish Ministry of Science, Innovation and Universities, the National Research Agency (MCIU/AEI/10.13039/501100011033) and the European Social Fund Plus (FSE+). This paper and related research have been conducted during and with the support of the Italian national inter-university PhD course in Sustainable Development and Climate change (www.phd-sdc.it) which founded the fellowship of ES and of the PhD course in Agro-Food System, which founded the fellowships of MADG and FV respectively.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2025.109646.

Data availability

The metabolomics data that support the findings of this study are openly available in METABOLIGHTS at https://www.ebi.ac.uk/metabolights/, reference number ID: MTBLS 11108.

References

Ahmed, F., Rafii, M.Y., Ismail, M.R., Juraimi, A.S., Rahim, H.A., Asfaliza, R., Latif, M.A., 2013. Waterlogging tolerance of crops: breeding, mechanism of tolerance, molecular approaches, and future prospects. BioMed Res. Int. 1–10. https://doi.org/10.1155/2013/963525, 2013.

Bailey-Serres, J., Voesenek, L.A.C.J., 2008. Flooding stress: acclimations and genetic diversity. Annu. Rev. Plant Biol. 59, 313–339. https://doi.org/10.1146/annurev.arplant.59.032607.092752.

Bandini, F., Hchaichi, I., Zitouni, N., Missawi, O., Cocconcelli, P.S., Puglisi, E., Banni, M., 2021. Bacterial community profiling of floating plastics from South Mediterranean sites: first evidence of effects on mussels as possible vehicles of transmission. J. Hazard Mater. 411. https://doi.org/10.1016/j.jhazmat.2021.125079.

Bandopadhyay, S., Li, X., Bowsher, A.W., Last, R.L., Shade, A., 2024. Disentangling plant- and environment-mediated drivers of active rhizosphere bacterial community dynamics during short-term drought. Nat. Commun. 15. https://doi.org/10.1038/s41467-024-50463-1.

Bardou, P., Mariette, J., Escudié, F., Djemiel, C., Klopp, C., 2014. SOFTWARE Open Access Jvenn: an Interactive Venn Diagram Viewer.

Barrett-Lennard, E.G., 2003. The interaction between waterlogging and salinity in higher plants: causes, consequences and implications. Plant Soil.

Bartram, A.K., Lynch, M.D.J., Stearns, J.C., Moreno-Hagelsieb, G., Neufeld, J.D., 2011. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. Appl. Environ. Microbiol. 77, 3846–3852. https://doi.org/10.1128/AEM.02772-10.

Barupal, D.K., Fiehn, O., 2017. Chemical Similarity Enrichment Analysis (ChemRICH) as alternative to biochemical pathway mapping for metabolomic datasets. Sci. Rep. 7. https://doi.org/10.1038/s41598-017-15231-w.

Bashar, K.K., Tareq, MdZ., Amin, MdR., Honi, U., Tahjib-Ul-Arif, Md, Sadat, MdA., Hossen, Q.MdM., 2019. Phytohormone-mediated stomatal response, escape and quiescence strategies in plants under flooding stress. Agronomy 9, 43. https://doi.org/10.3390/agronomy9020043.

Bhaduri, D., Mandal, A., Chakraborty, K., Chatterjee, D., Dey, R., 2017. Interlinked chemical-biological processes in anoxic waterlogged soil - a review. Indian J. Agric. Sci. https://doi.org/10.56093/ijas.v87i12.76483.

Bi, Y., Gao, X., Su, L., Lei, Y., Li, T., Dong, X., Li, X., Yan, Z., 2024. Unveiling the impact of flooding and salinity on iron oxides-mediated binding of organic carbon in the rhizosphere of Scirpus mariqueter. Sci. Total Environ. 908. https://doi.org/10.1016/j.scitotenv.2023.168447.

Bortolini, C., Patrone, V., Puglisi, E., Morelli, L., 2016. Detailed analyses of the bacterial populations in processed cocoa beans of different geographic origin, subject to varied fermentation conditions. Int. J. Food Microbiol. 236, 98–106. https://doi.org/10.1016/j.ijfoodmicro.2016.07.004.

Boyes, D.C., Zayed, A.M., Ascenzi, R., McCaskill, A.J., Hoffman, N.E., Davis, K.R., Görlach, J., 2001. Growth stage–based phenotypic analysis of arabidopsis. Plant Cell 13, 1499–1510. https://doi.org/10.1105/TPC.010011.

Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P.W., Trisos, C., Romero, J., Aldunce, P., Barrett, K., Blanco, G., Cheung, W.W.L., Connors, S., Denton, F., Diongue-Niang, A., Dodman, D., Garschagen, M., Geden, O., Hayward, B., Jones, C., Jotzo, F., Krug, T., Lasco, R., Lee, Y.-Y., Masson-Delmotte, V., Meinshausen, M., Mintenbeck, K., Mokssit, A., Otto, F.E.L., Pathak, M., Pirani, A., Poloczanska, E., Pörtner, H.-O., Revi, A., Roberts, D.C., Roy, J., Ruane, A.C., Skea, J., Shukla, P.R., Slade, R., Slangen, A., Sokona, Y., Sörensson, A.A., Tignor, M., van Vuuren, D., Wei, Y.-M., Winkler, H., Zhai, P., Zommers, Z., Hourcade, J.-C., Johnson, F.X., Pachauri, S., Simpson, N.P., Singh, C., Thomas, A., Totin, E., Alegría, A., Armour, K., Bednar-Friedl, B., Blok, K., Cissé, G., Dentener, F., Eriksen, S., Fischer, E., Garner, G., Guivarch, C., Haasnoot, M., Hansen, G., Hauser, M., Hawkins, E., Hermans, T., Kopp, R., Leprince-Ringuet, N., Lewis, J., Ley, D., Ludden, C., Niamir, L., Nicholls, Z., Some, S., Szopa, S., Trewin, B., van der Wijst, K.-I., Winter, G., Witting, M., Birt, A., Ha, M., 2023. IPCC, 2023: climate change 2023: synthesis report. In: Lee, H., Romero, J. (Eds.), Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team. IPCC, Geneva, Switzerland. https://doi.org/10.59327/IPCC/AR6-9789291691647.

Carvajal, M., Godoy, L., Gebauer, M., Catrileo, D., Albornoz, F., 2024. Screening for indole-3-acetic acid synthesis and 1-aminocyclopropane-carboxylate deaminase activity in soil yeasts from Chile uncovers Solicoccozyma aeria as an effective plant growth promoter. Plant Soil 496, 83–93. https://doi.org/10.1007/s11104-023-05906-x.

Chai, Y.N., Schachtman, D.P., 2022. Root exudates impact plant performance under abiotic stress. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2021.08.003.

Chandwani, S., Amaresan, N., 2022. Role of ACC deaminase producing bacteria for abiotic stress management and sustainable agriculture production. Environ. Sci. Pollut. Control Ser. 29, 22843–22859. https://doi.org/10.1007/s11356-022-18745-7.

Chen, X., Sang, X., Li, S., Zhang, S., Bai, L., 2010. Studies on a chlorogenic acid-producing endophytic fungi isolated from Eucommia ulmoides Oliver. J. Ind. Microbiol. Biotechnol. 37, 447–454. https://doi.org/10.1007/s10295-010-0690-0.

Chong, J., Liu, P., Zhou, G., Xia, J., 2020. Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. Nat. Protoc. 15, 799–821. https://doi.org/10.1038/s41596-019-0264-1.

Cramer, G.R., Urano, K., Delrot, S., Pezzotti, M., Shinozaki, K., 2011. Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biol. https://doi.org/10.1186/1471-2229-11-163.

Dai, J., Krohn, K., Flörke, U., Pescitelli, G., Kerti, G., Papp, T., Kövér, K.E., Bényei, A.C., Draeger, S., Schulz, B., Kurtán, T., 2010. Curvularin-type metabolites from the fungus *curvularia* sp. isolated from a marine alga. Eur. J. Org Chem. 2010, 6928–6937. https://doi.org/10.1002/ejoc.201001237.

Dakora, F.D., Phillips, D.A., 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil.

Daniel, K., Hartman, S., 2024. How plant roots respond to waterlogging. J. Exp. Bot. https://doi.org/10.1093/jxb/erad332.

Dardanelli, M.S., Manyani, H., González-Barroso, S., Rodríguez-Carvajal, M.A., Gil-Serrano, A.M., Espuny, M.R., López-Baena, F.J., Bellogín, R.A., Megías, M., Ollero, F. J., 2010. Effect of the presence of the plant growth promoting rhizobacterium (PGPR) Chryseobacterium balustinum Aur9 and salt stress in the pattern of flavonoids exuded by soybean roots. Plant Soil 328, 483–493. https://doi.org/10.1007/s11104-009-0127-6.

de Castro, M.V., Ióca, L.P., Williams, D.E., Costa, B.Z., Mizuno, C.M., Santos, M.F.C., de Jesus, K., Ferreira, É.L.F., Seleghim, M.H.R., Sette, L.D., Pereira Filho, E.R., Ferreira, A.G., Gonçalves, N.S., Santos, R.A., Patrick, B.O., Andersen, R.J., Berlinck, R.G.S., 2016. Condensation of macrocyclic polyketides produced by *penicillium* sp. DRF2 with mercaptopyruvate represents a new fungal detoxification pathway. J. Nat. Prod. 79, 1668–1678. https://doi.org/10.1021/acs.jnatprod.6b00295.

Dennis, P.G., Miller, A.J., Hirsch, P.R., 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol. Ecol. https://doi.org/10.1111/j.1574-6941.2010.00860.x.

Dixon, M.W., Jacobson, J.A., Cady, C.T., Muday, G.K., 1996. Cytoplasmic orientation of the naphthylphthalamic acid-binding protein in zucchini plasma membrane vesicles. Plant Physiol. 112, 421–432. https://doi.org/10.1104/pp.112.1.421.

Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x.

Egamberdieva, D., Li, L., Lindström, K., Räsänen, L.A., 2016. A synergistic interaction between salt-tolerant Pseudomonas and Mesorhizobium strains improves growth and symbiotic performance of liquorice (Glycyrrhiza uralensis Fish.) under salt stress. Appl. Microbiol. Biotechnol. 100, 2829–2841. https://doi.org/10.1007/s00253-015-7147-3.

Francioli, D., Cid, G., Kanukollu, S., Ulrich, A., Hajirezaei, M.R., Kolb, S., 2021. Flooding causes dramatic compositional shifts and depletion of putative beneficial bacteria on the spring wheat microbiota. Front. Microbiol. 12. https://doi.org/10.3389/fmicb.2021.773116.

Fukushima, A., Kuroha, T., Nagai, K., Hattori, Y., Kobayashi, M., Nishizawa, T., Kojima, M., Utsumi, Y., Oikawa, A., Seki, M., Sakakibara, H., Saito, K., Ashikari, M., Kusano, M., 2020. Metabolite and phytohormone profiling illustrates metabolic reprogramming as an escape strategy of deepwater rice during partially submerged stress. Metabolites 10. https://doi.org/10.3390/metabo10020068.

García, P., Singh, S., Graciet, E., 2024. New insights into the connections between flooding/hypoxia response and plant defenses against pathogens. Plants 13, 2176. https://doi.org/10.3390/plants13162176.

Ge, J., Li, D., Ding, J., Xiao, X., Liang, Y., 2023. Microbial coexistence in the rhizosphere and the promotion of plant stress resistance: a review. Environ. Res. 222, 115298. https://doi.org/10.1016/j.envres.2023.115298.

Gladkov, E.A., Tereshonok, D.V., Stepanova, A.Y., Gladkova, O.V., 2023. Plant–microbe interactions under the action of heavy metals and under the conditions of flooding. Diversity (Basel). https://doi.org/10.3390/d15020175.

Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol. Res. 169, 30–39. https://doi.org/10.1016/j.micres.2013.09.009.

Gui, G., Zhang, Q., Hu, W., Liu, F., 2024. Application of multiomics analysis to plant flooding response. Front. Plant Sci. https://doi.org/10.3389/fpls.2024.1389379.

Haddadi, B.S., Hassanpour, H., Niknam, V., 2016. Effect of salinity and waterlogging on growth, anatomical and antioxidative responses in Mentha aquatica L. Acta Physiol. Plant. 38. https://doi.org/10.1007/s11738-016-2137-3.

Hamonts, K., Clough, T.J., Stewart, A., Clinton, P.W., Richardson, A.E., Wakelin, S.A., O'Callaghan, M., Condron, L.M., 2013. Effect of nitrogen and waterlogging on denitrifier gene abundance, community structure and activity in the rhizosphere of wheat. FEMS Microbiol. Ecol. 83, 568–584. https://doi.org/10.1111/1574-6941.12015.

Harman, G., Khadka, R., Doni, F., Uphoff, N., 2021. Benefits to plant health and productivity from enhancing plant microbial symbionts. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.610065.

Hartman, K., Tringe, S.G., 2019. Interactions between plants and soil shaping the root microbiome under abiotic stress. Biochem. J. 476, 2705–2724. https://doi.org/10.1042/BCJ20180615.

Henry, A., Doucette, W., Norton, J., Bugbee, B., 2007. Changes in crested wheatgrass root exudation caused by flood, drought, and nutrient stress. J. Environ. Qual. 36, 904–912. https://doi.org/10.2134/jeq2006.0425sc.

Herzog, M., Fukao, T., Winkel, A., Konnerup, D., Lamichhane, S., Alpuerto, J.B., Hasler-Sheetal, H., Pedersen, O., 2018. Physiology, gene expression, and metabolome of two wheat cultivars with contrasting submergence tolerance. Plant Cell Environ. 41, 1632–1644. https://doi.org/10.1111/pce.13211.

Hill, C.B., Jha, D., Bacic, A., Tester, M., Roessner, U., 2013. Characterization of ion contents and metabolic responses to salt stress of different arabidopsis AtHKT1;1 genotypes and their parental strains. Mol. Plant 6, 350–368. https://doi.org/10.1093/mp/sss125.

Hirabayashi, Y., Mahendran, R., Koirala, S., Konoshima, L., Yamazaki, D., Watanabe, S., Kim, H., Kanae, S., 2013. Global flood risk under climate change. Nat. Clim. Change 3, 816–821. https://doi.org/10.1038/nclimate1911.

Hofmann, A., Wienkoop, S., Harder, S., Bartlog, F., Lüthje, S., 2020. Hypoxia-responsive class III peroxidases in maize roots: soluble and membrane-bound isoenzymes. Int. J. Mol. Sci. 21, 8872. https://doi.org/10.3390/ijms21228872.

Honório, A.B.M., De-la-Cruz-Chacón, I., da Silva, G.C., Mimi, C.O., Campos, F.G., da Silva, M.R., Boaro, C.S.F., Ferreira, G., 2024. Differential tolerance of primary metabolism of annona emarginata (schltdl.) H. Rainer to water stress modulates alkaloid production. Horticulturae 10, 220. https://doi.org/10.3390/horticulturae10030220.

Huang, B., Johnson, J.W., Box, J.E., NeSmith, D.S., 1997. Root characteristics and hormone activity of wheat in response to hypoxia and ethylene. Crop Sci. 37, 812–818. https://doi.org/10.2135/cropsci1997.0011183X003700030020x.

Jardine, K.J., McDowell, N., 2023. Fermentation-mediated growth, signaling, and defense in plants. New Phytol. 239, 839–851. https://doi.org/10.1111/nph.19015.

Khan, M.I.R., Trivellini, A., Chhillar, H., Chopra, P., Ferrante, A., Khan, N.A., Ismail, A. M., 2020. The significance and functions of ethylene in flooding stress tolerance in plants. Environ. Exp. Bot. https://doi.org/10.1016/j.envexpbot.2020.104188.

Kumar, M., 2016. Impact of climate change on crop yield and role of model for achieving food security. Environ. Monit. Assess. 188, 465. https://doi.org/10.1007/s10661-016-5472-3.

Kumar, N., Haldar, S., Saikia, R., 2023. Root exudation as a strategy for plants to deal with salt stress: an updated review. Environ. Exp. Bot. https://doi.org/10.1016/j.envexpbot.2023.105518.

Lamichhane, S., Alpuerto, J.B., Han, A., Fukao, T., 2020. The central negative regulator of flooding tolerance, the PROTEOLYSIS 6 branch of the n-degron pathway, adversely modulates salinity tolerance in arabidopsis. Plants 9, 1–17. https://doi.org/10.3390/plants9111415.

Lee, S., Hwang, S., Seo, M., Shin, K.B., Kim, K.H., Park, G.W., Kim, J.Y., Yoo, J.S., No, K. T., 2020. BMDMS-NP: a comprehensive ESI-MS/MS spectral library of natural compounds. Phytochemistry 177. https://doi.org/10.1016/j.phytochem.2020.112427.

Lee, S.C., Mustroph, A., Sasidharan, R., Vashisht, D., Pedersen, O., Oosumi, T., Voesenek, L.A.C.J., Bailey-Serres, J., 2011. Molecular characterization of the submergence response of the *Arabidopsis thaliana* ecotype Columbia. New Phytol. 190, 457–471. https://doi.org/10.1111/j.1469-8137.2010.03590.x.

Li, J., Zhang, G.-T., Yang, J., Tian, X.-P., Wang, F.-Z., Zhang, C.S., Zhang, S., Li, W.-J., 2012. Marininema mesophilum gen. nov., sp. nov., a thermoactinomycete isolated from deep sea sediment, and emended description of the family Thermoactinomycetaceae. Int. J. Syst. Evol. Microbiol. 62, 1383–1388. https://doi.org/10.1099/ijs.0.035071-0.

Li, J., Zhao, G.-Z., Huang, H.-Y., Zhu, W.-Y., Lee, J.-C., Kim, C.-J., Xu, L.-H., Zhang, L.-X., Li, W.-J., 2010. Pseudonocardia rhizophila sp. nov., a novel actinomycete isolated from a rhizosphere soil. Antonie Leeuwenhoek 98, 77–83. https://doi.org/10.1007/s10482-010-9431-7.

Li, M., Zhou, L., Yang, D., Li, T., Li, W., 2012. Biochemical composition and antioxidant capacity of extracts from Podophyllum hexandrum rhizome. BMC Compl. Alternative Med. 12. https://doi.org/10.1186/1472-6882-12-263.

Liao, Z., Fan, J., Lai, Z., Bai, Z., Wang, H., Cheng, M., Zhang, F., Li, Z., 2023. Response network and regulatory measures of plant-soil-rhizosphere environment to drought stress. In: Advances in Agronomy. Academic Press Inc., pp. 93–196. https://doi.org/10.1016/bs.agron.2023.03.002

Licausi, F., Van Dongen, J.T., Giuntoli, B., Novi, G., Santaniello, A., Geigenberger, P., Perata, P., 2010. HRE1 and HRE2, two hypoxia-inducible ethylene response factors, affect anaerobic responses in Arabidopsis thaliana. Plant J. 62, 302–315. https://doi.org/10.1111/j.1365-313X.2010.04149.x.

Lin, H.-H., Lin, K.-H., Chen, S.-C., Shen, Y.-H., Lo, H.-F., 2015. Proteomic analysis of broccoli (Brassica oleracea) under high temperature and waterlogging stresses. Bot. Stud. 56, 18. https://doi.org/10.1186/s40529-015-0098-2.

Liu, J., He, X., Sun, J., Ma, Y., 2021. A degeneration gradient of poplar trees contributes to the taxonomic, functional, and resistome diversity of bacterial communities in rhizosphere soils. Int. J. Mol. Sci. 22. https://doi.org/10.3390/ijms22073438.

Liu, X., Chen, C., Liu, Yun, Liu, Yanlu, Zhao, Y., Chen, M., 2020. The presence of moderate salt can increase tolerance of Elaeagnus angustifolia seedlings to waterlogging stress. Plant Signal. Behav. 15. https://doi.org/10.1080/15592324.2020.1743518.

Martínez-Arias, C., Witzell, J., Solla, A., Martin, J.A., Rodríguez-Calcerrada, J., 2022. Beneficial and pathogenic plant-microbe interactions during flooding stress. Plant Cell Environ. https://doi.org/10.1111/pce.14403.

Martins, T.S., Da-Silva, C.J., Shabala, S., Striker, G.G., Carvalho, I.R., de Oliveira, A.C.B., do Amarante, L., 2024. Understanding plant responses to saline waterlogging: insights from halophytes and implications for crop tolerance. Planta. https://doi.org/10.1007/s00425-023-04275-0.

Meng, H., Yan, Z., Li, X., 2022. Effects of exogenous organic acids and flooding on root exudates, rhizosphere bacterial community structure, and iron plaque formation in Kandelia obovata seedlings. Sci. Total Environ. 830. https://doi.org/10.1016/j.scitotenv.2022.154695.

Mierziak, J., Kostyn, K., Kulma, A., 2014. Flavonoids as important molecules of plant interactions with the environment. Molecules. https://doi.org/10.3390/molecules191016240.

Mishra, J., Singh, R., Arora, N.K., 2017. Plant growth-promoting microbes: diverse roles in agriculture and environmental sustainability. In: Probiotics and Plant Health.

Springer Singapore, Singapore, pp. 71–111. https://doi.org/10.1007/978-981-10-3473-2_4.
Mishra, P.K., Bisht, S.C., Ruwari, P., Selvakumar, G., Joshi, G.K., Bisht, J.K., Bhatt, J.C., Gupta, H.S., 2011. Alleviation of cold stress in inoculated wheat (Triticum aestivum L.) seedlings with psychrotolerant Pseudomonads from NW Himalayas. Arch. Microbiol. 193, 497–513. https://doi.org/10.1007/s00203-011-0693-x.
Mittler, R., 2006. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 11, 15–19. https://doi.org/10.1016/j.tplants.2005.11.002.
Mittler, R., Blumwald, E., 2010. Genetic engineering for modern agriculture: challenges and perspectives. Annu. Rev. Plant Biol. 61, 443–462. https://doi.org/10.1146/annurev-arplant-042809-112116.
Mushtaq, W., Fauconnier, M.-L., 2024. Phenolic profiling unravelling allelopathic encounters in agroecology. Plant Stress 13, 100523. https://doi.org/10.1016/j.stress.2024.100523.
Mußmann, M., Pjevac, P., Krüger, K., Dyksma, S., 2017. Genomic repertoire of the *Woeseiaceae*/JTB255, cosmopolitan and abundant core members of microbial communities in marine sediments. ISME J. 11, 1276–1281. https://doi.org/10.1038/ismej.2016.185.
OECD, 2024. FAO Agricultural Outlook 2024-2033. OECD. https://doi.org/10.1787/4c5d2cfb-en.
Pan, J., Sharif, R., Xu, X., Chen, X., 2021. Mechanisms of waterlogging tolerance in plants: research progress and prospects. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.627331.
Pang, J., Cuin, T., Shabala, L., Zhou, M., Mendham, N., Shabala, S., 2007. Effect of secondary metabolites associated with anaerobic soil conditions on ion fluxes and electrophysiology in barley roots. Plant Physiol. 145, 266–276. https://doi.org/10.1104/pp.107.102624.
Parasar, B.J., Sharma, I., Agarwala, N., 2024. Root exudation drives abiotic stress tolerance in plants by recruiting beneficial microbes. Appl. Soil Ecol. https://doi.org/10.1016/j.apsoil.2024.105351.
Pardo-Hernández, M., García-Pérez, P., Lucini, L., Rivero, R.M., 2024. Multi-omics exploration of the involvement of ABA in identifying unique molecular markers for single and combined stresses in tomato plants. J. Exp. Bot. https://doi.org/10.1093/jxb/erae372.
Patil, H.J., Srivastava, A.K., Singh, D.P., Chaudhari, B.L., Arora, D.K., 2011. Actinomycetes mediated biochemical responses in tomato (Solanum lycopersicum) enhances bioprotection against Rhizoctonia solani. Crop Prot. 30, 1269–1273. https://doi.org/10.1016/j.cropro.2011.04.008.
Phukan, U.J., Mishra, S., Shukla, R.K., 2016. Waterlogging and submergence stress: affects and acclimation. Crit. Rev. Biotechnol. 36, 956–966. https://doi.org/10.3109/07388551.2015.1064856.
Randle-Boggis, R.J., Ashton, P.D., Helgason, T., 2018. Increasing flooding frequency alters soil microbial communities and functions under laboratory conditions. Microbiologyopen 7. https://doi.org/10.1002/mbo3.548.
Renziehausen, T., Frings, S., Schmidt-Schippers, R., 2024. 'Against all floods': plant adaptation to flooding stress and combined abiotic stresses. Plant J. https://doi.org/10.1111/tpj.16614.
Reva, O.N., Weinel, C., Weinel, M., Böhm, K., Stjepandic, D., Hoheisel, J.D., Tümmler, B., 2006. Functional genomics of stress response in Pseudomonas putida KT2440. J. Bacteriol. 188, 4079–4092. https://doi.org/10.1128/JB.00101-06.
Rillig, M.C., Lehmann, A., Orr, J.A., Waldman, W.R., 2021. Mechanisms underpinning nonadditivity of global change factor effects in the plant–soil system. New Phytol. 232, 1535–1539. https://doi.org/10.1111/nph.17714.
Rillig, M.C., Ryo, M., Lehmann, A., Aguilar-Trigueros, C.A., Buchert, S., Wulf, A., Iwasaki, A., Roy, J., Yang, G., 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. https://doi.org/10.1126/science.aay2832.
Rivero, R.M., Mittler, R., Blumwald, E., Zandalinas, S.I., 2022. Developing climate-resilient crops: improving plant tolerance to stress combination. Plant J. 109, 373–389. https://doi.org/10.1111/tpj.15483.
Rizaludin, M.S., Stopnisek, N., Raaijmakers, J.M., Garbeva, P., 2021. The chemistry of stress: understanding the 'cry for help' of plant roots. Metabolites 11, 357. https://doi.org/10.3390/metabo11060357.
Rohart, F., Gautier, B., Singh, A., Lê Cao, K.A., 2017. mixOmics: an R package for 'omics feature selection and multiple data integration. PLoS Comput. Biol. 13. https://doi.org/10.1371/journal.pcbi.1005752.
Rolfe, S.A., Griffiths, J., Ton, J., 2019. Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. Curr. Opin. Microbiol. 49, 73–82. https://doi.org/10.1016/j.mib.2019.10.003.
Salek, R.M., Steinbeck, C., Viant, M.R., Goodacre, R., Dunn, W.B., 2013. The role of reporting standards for metabolite annotation and identification in metabolomic studies. GigaScience 2. https://doi.org/10.1186/2047-217X-2-13.
Sarv, V., Kerner, K., Rimantas Venskutonis, P., Rocchetti, G., Paolo Becchi, P., Lucini, L., Tänavots, A., Bhat, R., 2023. Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs. Food Chem. X 19. https://doi.org/10.1016/j.fochx.2023.100761.
Sasidharan, R., Hartman, S., Liu, Z., Martopawiro, S., Sajeev, N., van Veen, H., Yeung, E., Voesenek, L.A.C.J., 2018. Signal dynamics and interactions during flooding stress. Plant Physiol. 176, 1106–1117. https://doi.org/10.1104/pp.17.01232.
Shang, P., Shen, B., Zeng, B., Bi, L., Qu, M., Zheng, Y., Ye, Y., Li, W., Zhou, X., Yang, X., Jiang, Y., 2023. Integrated transcriptomic and metabolomics analysis of the root responses of orchardgrass to submergence stress. Int. J. Mol. Sci. 24. https://doi.org/10.3390/ijms24032089.
Shao, T., Gu, X., Zhu, T., Pan, X., Zhu, Y., Long, X., Shao, H., Liu, M., Rengel, Z., 2019. Industrial crop Jerusalem artichoke restored coastal saline soil quality by reducing salt and increasing diversity of bacterial community. Appl. Soil Ecol. 138, 195–206. https://doi.org/10.1016/j.apsoil.2019.03.003.
Shimada, N., Akashi, T., Aoki, T., Ayabe, S., 2000. Induction of isoflavonoid pathway in the model legume Lotus japonicus: molecular characterization of enzymes involved in phytoalexin biosynthesis. Plant Sci. 160, 37–47. https://doi.org/10.1016/S0168-9452(00)00355-1.
Šibanc, N., Dumbrell, A.J., Mandić-Mulec, I., Maček, I., 2014. Impacts of naturally elevated soil CO2 concentrations on communities of soil archaea and bacteria. Soil Biol. Biochem. 68, 348–356. https://doi.org/10.1016/j.soilbio.2013.10.018.
Singh, A., Shannon, C.P., Gautier, B., Rohart, F., Vacher, M., Tebbutt, S.J., Cao, K.A.L., 2019. DIABLO: an integrative approach for identifying key molecular drivers from multi-omics assays. Bioinformatics 35, 3055–3062. https://doi.org/10.1093/bioinformatics/bty1054.
Soualiou, S., Duan, F., Li, X., Zhou, W., 2022. Crop production under cold stress: an understanding of plant responses, acclimation processes, and management strategies. Plant Physiol. Biochem. 190, 47–61. https://doi.org/10.1016/j.plaphy.2022.08.024.
Striker, G.G., Teakle, N.L., Colmer, T.D., Barrett-Lennard, E.G., 2015. Growth responses of Melilotus siculus accessions to combined salinity and root-zone hypoxia are correlated with differences in tissue ion concentrations and not differences in root aeration. Environ. Exp. Bot. 109, 89–98. https://doi.org/10.1016/j.envexpbot.2014.08.006.
Stringlis, I.A., de Jonge, R., Pieterse, C.M.J., 2019. The age of coumarins in plant–microbe interactions. Plant Cell Physiol. 60, 1405–1419. https://doi.org/10.1093/pcp/pcz076.
Sun, L., Wang, J., Cui, Y., Cui, R., Kang, R., Zhang, Y., Wang, S., Zhao, L., Wang, D., Lu, X., Fan, Y., Han, M., Chen, C., Chen, X., Guo, L., Ye, W., 2023. Changes in terpene biosynthesis and submergence tolerance in cotton. BMC Plant Biol. 23. https://doi.org/10.1186/s12870-023-04334-4.
Suyal, D.C., Kumar, S., Yadav, A., Shouche, Y., Goel, R., 2017. Cold stress and nitrogen deficiency affected protein expression of psychrotrophic Dyadobacter psychrophilus B2 and Pseudomonas jessenii MP1. Front. Microbiol. 8, 430. https://doi.org/10.3389/fmicb.2017.00430.
Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E., Mittler, R., 2014. Abiotic and biotic stress combinations. New Phytol. 203, 32–43. https://doi.org/10.1111/nph.12797.
Tahjib-Ul-Arif, M., Hasan, M.T., Rahman, M.A., Nuruzzaman, M., Rahman, A.M.S., Hasanuzzaman, M., Haque, M.R., Hossain, M.A., Abdel Latef, A.A.H., Murata, Y., Brestic, M., 2023. Plant response to combined salinity and waterlogging stress: current research progress and future prospects. Plant Stress. https://doi.org/10.1016/j.stress.2023.100137.
Tian, B., Pei, Y., Huang, W., Ding, J., Siemann, E., 2021. Increasing flavonoid concentrations in root exudates enhance associations between arbuscular mycorrhizal fungi and an invasive plant. ISME J. 15, 1919–1930. https://doi.org/10.1038/s41396-021-00894-1.
Tiziani, R., Miras-Moreno, B., Malacrinò, A., Vescio, R., Lucini, L., Mimmo, T., Cesco, S., Sorgonà, A., 2022. Drought, heat, and their combination impact the root exudation patterns and rhizosphere microbiome in maize roots. Environ. Exp. Bot. 203. https://doi.org/10.1016/j.envexpbot.2022.105071.
Tsugawa, H., Cajka, T., Kind, T., Ma, Y., Higgins, B., Ikeda, K., Kanazawa, M., VanderGheynst, J., Fiehn, O., Arita, M., 2015. MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. Nat. Methods 12, 523–526. https://doi.org/10.1038/nmeth.3393.
Tsugawa, H., Kind, T., Nakabayashi, R., Yukihira, D., Tanaka, W., Cajka, T., Saito, K., Fiehn, O., Arita, M., 2016. Hydrogen rearrangement rules: computational MS/MS fragmentation and structure elucidation using MS-FINDER software. Anal. Chem. 88, 7946–7958. https://doi.org/10.1021/acs.analchem.6b00770.
Van Dongen, J.T., Fröhlich, A., Ramírez-Aguilar, S.J., Schauer, N., Fernie, A.R., Erban, A., Kopka, J., Clark, J., Langer, A., Geigenberger, P., 2009. Transcript and metabolite profiling of the adaptive response to mild decreases in oxygen concentration in the roots of arabidopsis plants. Ann. Bot. 103, 269–280. https://doi.org/10.1093/aob/mcn126.
Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., Dufresne, A., 2015. The importance of the microbiome of the plant holobiont. New Phytol. https://doi.org/10.1111/nph.13312.
Vijver, M.G., Vink, J.P., Miermans, C.J., van Gestel, C.A., 2003. Oral sealing using glue: a new method to distinguish between intestinal and dermal uptake of metals in earthworms. https://doi.org/10.1016/S0038-0717(02)00245-6.
Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., Pérez-Clemente, R.M., 2020a. Root exudates: from plant to rhizosphere and beyond. Plant Cell Rep. https://doi.org/10.1007/s00299-019-02447-5.
Vives-Peris, V., López-Climent, M.F., Pérez-Clemente, R.M., Gómez-Cadenas, A., 2020b. Root involvement in plant responses to adverse environmental conditions. Agronomy. https://doi.org/10.3390/agronomy10070942.
Voesenek, L., Bailey-Serres, J., 2013. Flooding tolerance: O2 sensing and survival strategies. Curr. Opin. Plant Biol. 16, 647–653. https://doi.org/10.1016/j.pbi.2013.06.008.
Voesenek, L.A.C.J., Sasidharan, R., 2013. Ethylene – and oxygen signalling – drive plant survival during flooding. Plant Biol. 15, 426–435. https://doi.org/10.1111/plb.12014.
Vogel, J.P., Schuerman, P., Woeste, K., Brandstatter, I., Kieber, J.J., 1998. Isolation and Characterization of Arabidopsis Mutants Defective in the Induction of Ethylene Biosynthesis by Cytokinin.
Wang, Y., Chen, J., Zhou, S., Wang, X., Chen, Y., Lin, X., Yan, Y., Ma, X., Wu, M., Han, H., 2017. 16S rRNA gene high-throughput sequencing reveals shift in nitrogen conversion related microorganisms in a CANON system in response to salt stress. Chem. Eng. J. 317, 512–521. https://doi.org/10.1016/j.cej.2017.02.096.

Wibowo, J.T., Kellermann, M.Y., Versluis, D., Putra, M.Y., Murniasih, T., Mohr, K.I., Wink, J., Engelmann, M., Praditya, D.F., Steinmann, E., Schupp, P.J., 2019. Biotechnological potential of bacteria isolated from the sea cucumber Holothuria leucospilota and Stichopus vastus from lampung, Indonesia. Mar. Drugs 17, 635. https://doi.org/10.3390/md17110635.

Wolińska, A., Podlewski, J., Słomczewski, A., Grządziel, J., Gałązka, A., Kuźniar, A., 2022. Fungal indicators of sensitivity and resistance to long-term maize monoculture: a culture-independent approach. Front. Microbiol. 12. https://doi.org/10.3389/fmicb.2021.799378.

Wu, J., Lv, S., Zhao, L., Gao, T., Yu, C., Hu, J., Ma, F., 2023. Advances in the study of the function and mechanism of the action of flavonoids in plants under environmental stresses. Planta. https://doi.org/10.1007/s00425-023-04136-w.

Xie, L.J., Zhou, Y., Chen, Q.F., Xiao, S., 2021. New insights into the role of lipids in plant hypoxia responses. Prog. Lipid Res. https://doi.org/10.1016/j.plipres.2020.101072.

Xue, L., Jiang, J., Li, X., Yan, Z., Zhang, Q., Ge, Z., Tian, B., Craft, C., 2020. Salinity affects topsoil organic carbon concentrations through regulating vegetation structure and productivity. J Geophys Res Biogeosci 125. https://doi.org/10.1029/2019JG005217.

Yan, Q., Duan, Z., Mao, J., Li, X., Dong, F., 2012. Effects of root-zone temperature and N, P, and K supplies on nutrient uptake of cucumber (*Cucumis sativus* L.) seedlings in hydroponics. Soil Sci. Plant Nutr. 58, 707–717. https://doi.org/10.1080/00380768.2012.733925.

Yue, Y., Shao, T., Long, X., He, T., Gao, X., Zhou, Z., Liu, Z., Rengel, Z., 2020. Microbiome structure and function in rhizosphere of Jerusalem artichoke grown in saline land. Sci. Total Environ. 724. https://doi.org/10.1016/j.scitotenv.2020.138259.

Zamora-Quintero, A.Y., Torres-Beltrán, M., Guillén Matus, D.G., Oroz-Parra, I., Millán-Aguiñaga, N., 2022. Rare actinobacteria isolated from the hypersaline Ojo de Liebre Lagoon as a source of novel bioactive compounds with biotechnological potential. Microbiology (N. Y.) 168. https://doi.org/10.1099/mic.0.001144.

Zandalinas, S.I., Balfagón, D., Gómez-Cadenas, A., Mittler, R., 2022. Plant responses to climate change: metabolic changes under combined abiotic stresses. J. Exp. Bot. https://doi.org/10.1093/jxb/erac073.

Zandalinas, S.I., Mittler, R., 2022. Plant responses to multifactorial stress combination. New Phytol. 234, 1161–1167. https://doi.org/10.1111/nph.18087.

Zhang, X., Huang, X., Li, Y., Tao, F., Zhao, Q., Li, W., 2021. Polar auxin transport May Be responsive to specific features of flavonoid structure. Phytochemistry 185, 112702. https://doi.org/10.1016/j.phytochem.2021.112702.

Zhou, Z., Wang, C., Luo, Y., 2020. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. Nat. Commun. 11. https://doi.org/10.1038/s41467-020-16881-7.

Zhu, Y., Wang, Q., Wang, Y., Xu, Y., Li, J., Zhao, S., Wang, D., Ma, Z., Yan, F., Liu, Y., 2021. Combined transcriptomic and metabolomic analysis reveals the role of phenylpropanoid biosynthesis pathway in the salt tolerance process of Sophora alopecuroides. Int. J. Mol. Sci. 22, 2399. https://doi.org/10.3390/ijms22052399.