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Effects of winter waterlogging on apple root and soil summer microbiome

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Abstract

Background

Winter waterlogging is an increasingly common abiotic stress factor in temperate horticulture. While the physiological responses of apple (*Malus* × *domestica*) to waterlogging during the active growing season have been investigated, the impact of waterlogging on the soil and root microbiome, especially during the winter dormant period, remains unknown. This study investigated the legacy effects of winter waterlogging on apple root and soil microbiota 6–9 months after treatment. Potted MM106 rootstocks were subjected to four weeks of continuous waterlogging in December across two independent experimental replicates, with one cohort subjected to two waterlogging treatments over two consecutive winters. Fungal (ITS) and bacterial (16S rRNA gene) communities were assessed using amplicon sequencing to determine community size, diversity, structure, and taxonomic responses to waterlogging.

Results

Results indicated that community assembly was primarily driven by experimental replicate and temporal succession rather than the waterlogging treatment. However, a specific anaerobic legacy was identified. The anaerobic bacterial genera *Geomonas*, *Geobacter*, and *Desulfuromonas* were consistently enriched across experiments, with an increase in the abundance of *Geomonas*

associated with single and repeated waterlogging events. Conversely, aerobic saprotrophs were depleted, and potential fungal pathogens, *Gibellulopsis* and *Plectosphaerella*, were also depleted.

Conclusions

These findings suggest that winter waterlogging induces a stable shift in the microbiota characterised by a persistent anaerobic bacterial signature and a simplified fungal community. This legacy effect may influence nutrient cycling and stress resilience when trees resume active growth in the spring.

Keywords

Malus × *domestica*, winter waterlogging, microbiota response

Background

Waterlogging is a major abiotic stress in horticulture in the UK, impairing plant growth and productivity, and is projected to worsen with climate change in temperate regions [1]. Waterlogging creates a hypoxic environment in the soil, starving roots of oxygen and forcing them to rely on anaerobic respiration [2]. The resulting energy deficit leads to impaired osmotic balance, reduced photosynthetic rate, and leaf senescence [3,4]. Hypoxia and changes in pH and nutrient availability during waterlogging, as well as changes in the amount and type of root exudates, result in large changes in the root and soil microbiota composition which contributes to plant stress and disease outcomes [5].

Apple (*Malus* × *domestica*) is sensitive to waterlogging during the growing season which suppresses growth and reduces fruit yield [6,7]. While the physiological response of apple to active-season waterlogging has been investigated, the seasonal timing of waterlogging is an important but overlooked factor. Research suggests that flooding during summer is more detrimental to growth and yield than during autumn or spring [8]. However, winter waterlogging remains unstudied in apple despite being a common occurrence in temperate orchards. During winter dormancy, although the metabolic activity of the host is reduced, the soil remains a dynamic environment. To fully understand the impact of waterlogging during winter dormancy in apple, the response of microbial

communities associated with roots and soil needs to be investigated. These communities are associated with nutrient cycling and pathogen defence when active growth resumes in the spring.

Waterlogging impacts bulk soil and rhizosphere microbiota directly, primarily by gradually increasing hypoxia which favours anaerobes, but also by changing pH and nutrient levels in the soil [9]. Together with hypoxia-induced changes in root exudates by the host plant, waterlogging drives major restructuring of soil and root microbial communities [5]. Waterlogging-induced hypoxia acts as a selective filter, consistently depleting aerobic plant growth-promoting rhizobacteria (PGPR) such as *Bacillus* and *Pseudomonas* while facilitating the expansion of anaerobic or acidophilic taxa [10,11]. This restructuring often favours bacteria detrimental to plant growth and opportunistic fungal pathogens, thereby predisposing the host to disease even after reoxygenation [11,12]. While waterlogging directly favours waterborne pathogens like *Phytophthora* and *Fusarium*, leading to worse disease outcomes [13,14], its long-term suppression of beneficial microbiota may erode the key microbiome functions and consequently reduce the fitness of the associated plant host. Although the plant waterlogging response can temporarily upregulate global defences, protecting it from disease [15], the loss of protective or beneficial microbes could represent a persistent vulnerability. This was evidenced in *Malus hupehensis*, where stimulation of rhizosphere microbiota by application of melatonin and dopamine reduced the effects of waterlogging on the microbiome and improved the host's recovery after waterlogging, highlighting the microbiome's role as a critical component of stress resilience [16,17]. Characterising long term effects of winter waterlogging on apple microbiota is therefore essential to understand whether waterlogging-induced disruptions during dormancy lead to vulnerabilities during the growing season, ultimately informing better orchard soil management and site selection in a changing climate.

This study aimed to determine the effects of winter waterlogging on the root and soil microbiota of apple rootstocks (*Malus* × *domestica*). We investigated whether microbial shifts induced by winter waterlogging persist until the summer growing season and if these responses are consistent across

different experimental years and repeated waterlogging treatments of the same plants. Potted MM106 rootstocks were waterlogged continuously for 4 weeks in December, and root and soil samples were collected the following summer (6–9 months after treatment) to capture the long-term community disruption. We performed two experimental replicates in two consecutive years, starting in 2022 and 2023. To assess the impact of recurring stress, the trees from the 2022 experiment were subjected to a second waterlogging treatment in the winter of 2023/2024. Using ITS/16S rRNA gene amplicon sequencing, we assessed community size, alpha diversity, beta diversity, and the differential abundance of genera in response to waterlogging treatments. We hypothesised that winter waterlogging induces a stable shift in microbial communities that is reproducible across independent experiments and characterised by the persistent enrichment of anaerobic waterlogging-tolerant taxa.

Methods

Experimental design and sampling

Two independent experimental replicates were performed in consecutive years. The schedule for planting, waterlogging, and sampling is summarised in table 1. One-year-old MM106 rootstocks were planted in March of 2022 and 2023 in low-fertility sandy loam topsoil amended with Osmocote® slow-release fertiliser pellets according to manufacturer's recommendations of 10 g L^{-1}. Three rootstocks were planted in each 20 L pot and randomly arranged into 5 blocks of 5 plots with 4 pots (12 rootstocks) per plot. Rootstocks were inoculated with *N. ditissima* conidial suspension at leaf fall after the first growing season. Waterlogging treatments (Control and 1-, 2-, 4-, and 8-weeks) were randomly assigned to each plot with one plot per treatment in each block. Pots were waterlogged by placing in a bucket and submerging in water approximately 3 cm above the soil continuously for the assigned duration in December/January after the first growing season (2022/2023 for the 2022 experiment and 2023/2024 for the 2023 experiment). Root and soil samples were collected from 0- and 4-week waterlogging treatment plots (the longest treatment that didn't cause excessive mortality) from each block 6–9 months after treatment (June 2023 for the 2022 experiment,

September 2024 for the 2023 experiment) by extracting a core to a depth of approximately 10 cm using a cork borer from each pot in the plot and mixing them together. In the 2022 experiment a second waterlogging treatment was applied in winter 2023/2024, following the same durations and protocol as the first waterlogging treatment, and a second batch of samples collected form the same plots in September 2024. Samples were frozen after collection and stored at –20 ℃ until use. Frozen samples were freeze dried, manually separated into root and soil components, and ground in a Geno/Grinder2010 (SPEX SamplePrep, Stanmore, UK) with sterilised steel ball bearings before DNA extraction.

Table 1. Schedule for planting, watering, and sampling of the 2022 and 2023 experimental replicates.

Experiment	Planting	First waterlogging	First sampling	Second waterlogging	Second sampling
2022 experiment	Mar 2022	Dec 2022	Jun 2023	Dec 2023	Sep 2024
2023 experiment	Mar 2023	Dec 2023	Sep 2024	-	-

DNA extraction, qPCR, and sequencing

DNA was extracted from powdered root and soil samples using the E.Z.N.A Soil DNA Kit (Omega Bio-tek, Norcross, Georgia, USA). DNA was quantified and assessed for purity spectrophotometrically (Nanodrop™ 1000, ThermoFisher Scientific, USA). PCR amplification, library preparation, and metabarcoding sequencing were carried out by Novogene UK (Cambridge, UK) on the Illumina NovaSeq platform in the 250 nt paired end mode. The target regions were ITS1-1F: [ITS1-1F-F: 5'-CTTGGTCATTTAGAGGAAGTAA-3'[18]; ITS1-1F-R: 5'-GCTGCGTTCTTCATCGATGC-3' [19]] and 16S V5-V7: [799F: 5'-AACMGGATTAGATACCCKG-3' [20], 1193R: 5'-ACGTCATCCCCACCTTCC-3' [21]].

Fungal and bacterial community sizes were estimated using quantitative PCR (qPCR) based on a published protocol [22]. To ensure accuracy and account for potential inhibition, samples were analysed in triplicate at two dilutions (20 × and 200 ×). A standard curve was made using a six-step 10-fold serial dilution of DNA pooled from all samples. Each 384-well plate included the standard curve and a non-template control in triplicate. Reactions (10 µL) contained 2 µL of template DNA and 5 µL SsoAdvanced Universal SYBR Green Supermix (Bio-Rad). Primers targeted the same regions used

for metabarcoding: ITS1-1F (100 nM final concentration) and 16S V5-V7 (200 nM). The thermal cycling profile was: initial denaturation for 3 min at 98 ℃, followed by 40 cycles of 15 s at 98 ℃, 30 s at 55 ℃, and 1 min at 72 ℃, followed by a final extension of 10 min at 72 ℃ and melt curve analysis. Total ITS or 16S rRNA gene theoretical copy number per gram of sample were calculated using plate-specific standard curves. To standardise quantification, theoretical copy numbers were calibrated such that the quantification cycle (C_q) intercepts were approximately 35 (the detection limit estimated from standard curves). Quality control was strictly maintained; samples with amplification efficiencies outside the 0.8–1.2 range were re-run. Final downstream analyses used the mean efficiency-corrected theoretical copy number from the three technical replicates across both dilutions.

Sequence processing and ASV calling

The bioinformatics and computational analyses were performed on CropDiversity-HPC [23]. FASTQ reads were filtered to remove those with ambiguous primers or mismatched primer pairs and primers and barcodes were removed. Cleaned reads were processed using the DADA2 (v1.34) pipeline in R [24]. Reads were filtered and trimmed to remove unknown bases (N) and low-quality sequences using a maximum expected error of 2. Error models were constructed from the filtered reads and used to infer amplicon sequence variants (ASVs) using pseudo-pooling. Forward and reverse reads were merged, chimeras were identified and removed, and the final frequency tables were constructed.

Fungal and bacterial taxonomy was assigned to ASVs at the 80% confidence level using SINTAX [25]. Fungal taxonomy was assigned using a modified version of the RDP Warcup V2 database which was updated for consistency with NCBI taxonomy as of 2022-09-02. Bacterial taxonomy was assigned using the ‘RDP training set v18’ database for the 16S rRNA gene [26]. ASVs identified as chloroplast or mitochondria were removed and remaining ASVs were filtered to only include ASVs with at least 10 reads in two or more samples.

Statistical analysis

All statistical analyses were carried out using R 4.4.3 [27]. Because samples were collected after a single waterlogging for the 2022 and 2023 experimental replicates and after repeated waterlogging only for experiments set up in 2022, we carried out separate analysis on 1) the effects of single waterlogging across two independent experimental years (replicates), and 2) the effects of single and repeated waterlogging on the same plants in the 2022 experiment.

Fungal and bacterial community estimated from qPCR-derived theoretical copy numbers was log transformed to achieve normality and homoskedasticity of residuals before analysis of variance (ANOVA) was used to assess the effects of design factors on community size. To account for repeated measures in the repeated waterlogging analysis, plot identity was included as an error term in the model.

Alpha diversity indices Chao1, Shannon, and Simpson were calculated using R package vegan [28]. Permutation ANOVA was performed on the rank of each alpha diversity index to assess the effects of design factors using the R package lmPerm [29]. To account for repeated measures in the repeated waterlogging analysis, plot identity was included as an error term in the model.

For beta diversity, ASV counts were normalised by community size using the qPCR-derived theoretical copy number. The proportional differences in theoretical copy number between samples and the mean across samples for ITS and 16S rRNA gene were used as size factors for DESeq2 normalisation [30]. Bray-Curtis dissimilarity was calculated from normalised ASV counts using R package vegan [28]. Permutational multivariate analysis of variance (PERMANOVA) with 1000 permutations was used to assess the statistical significance of design factors on the Bray-Curtis dissimilarity matrix using the adonis2 function of the vegan package. To account for repeated measures in the repeated waterlogging analysis, plot identity was included as a strata term in adonis2. Principal component (PC) analysis was used to assess the key axes of variation in the community. ANOVA was used to test the effect of design factors on each PC and the variance

accounted for by each factor was adjusted by the total variance in the community accounted for by each PC.

Differential abundance of each fungal and bacterial genus in response to waterlogging was assessed using DESeq2. Normalised ASV counts were summed by genus at the 50% confidence level and filtered to remove genera only detected in < 4 samples. Genera were assessed for their response to waterlogging over two independent experimental replicates and separately for single and repeated waterlogging in the 2022 experiment. This approach was used because 16S rRNA gene sequencing lacks the taxonomic resolution for confident species-level identification and grouping by taxonomic units (genus) rather than ASVs ensured biological relevance and reduced the impact of sparsity and noise due to the presence of many low frequency ASVs across a limited number of samples.

Results

Sequencing and ASV summary

There were 1224 total fungal ASVs. The top 29, 262, and 853 most abundant ASVs accounted for 50%, 90%, and 99% of total reads, respectively. Of the total fungal ASVs, 89%, 79%, 67%, 59%, 54%, and 37% could be assigned to the rank of phylum, class, order, family, genus, and species respectively at ≥ 50% confidence. The top two ASVs were both *Plectosphaerellaceae* and accounted for 9.2% and 4.7% of reads respectively.

There were 4359 total bacterial ASVs. The top 247, 1903, and 3760 most abundant ASVs accounted for 50%, 90%, and 99% of total reads, respectively. Of the total bacterial ASVs, 95%, 88%, 73%, 58%, and 49% could be assigned to the rank of phylum, class, order, family, and genus respectively at ≥ 50% confidence. 16S rRNA gene sequences do not achieve meaningful species resolution and is not included. The top two ASVs were *Streptomyces* and *Actinobacteria* and accounted for 1.5% and 1.3% of reads respectively.

Community size and alpha diversity

Single waterlogging across experimental replicates

Experiment year and spatial variation (block within experiment) were the primary factors affecting community size and alpha diversity across experimental replicates, while single waterlogging treatment had small but significant effects on some alpha diversity measures (figure 1, table 2).

Fungal root communities did not respond significantly to waterlogging across experimental replicates. Fungal soil communities, however, had significantly decreased richness (Chao1: $p = 0.006$, var = 1.47%), diversity (Shannon: $p = 0.005$, var = 2.44%), and evenness (Simpson: $p = 0.001$, var = 3.64%) in response to waterlogging across experimental replicates. Conversely, bacterial root communities had significantly increased richness (Chao1: $p = 0.004$, var = 1.92%) and diversity (Shannon: $p = 0.006$, var = 12.6%) in response to waterlogging, and bacterial soil communities had significantly increased richness (Chao1: $p = 0.006$, var = 2.44%) in response to waterlogging. The response to waterlogging was experiment-dependent for evenness of fungal root (Simpson: $p = 0.001$, var = 4.33%), bacterial root (Simpson: $p = 0.005$, var = 0.75%), and bacterial soil (Simpson: $p = 0.005$, var = 1.08%) communities.

Fungal root communities were significantly more diverse (Shannon) and even (Simpson) in the 2023 experiment than in the 2022 experiment (all $p < 0.05$). Fungal soil communities were significantly larger, richer (Chao1), more diverse (Shannon), and more even (Simpson) in the 2023 experiment (all $p < 0.05$). Bacterial root communities were significantly larger, but less rich, less diverse, and less even in the 2023 experiment, and bacterial soil communities were larger, richer, and more diverse, but less even in the 2023 experiment (all $p < 0.05$). The differences between experiments were most pronounced in fungal soil communities and bacterial root communities where it explained up to 76.7% of variation in community size and alpha diversity. Block effects within experiments represented a major source of variation in alpha diversity but not community size and were significant for all alpha diversity indices for all groups (all $p < 0.05$). This was most pronounced in fungal root communities where block-within-experiment accounted for 92.1%, 55.4%, and 34.0% of

variation in Chao1, Shannon, and Simpson indices, respectively. Block effects were also pronounced in bacterial soil communities where block-within-experiment accounted for 31.2%, 55.3%, and 83.0% of variation in richness, diversity, and evenness respectively.

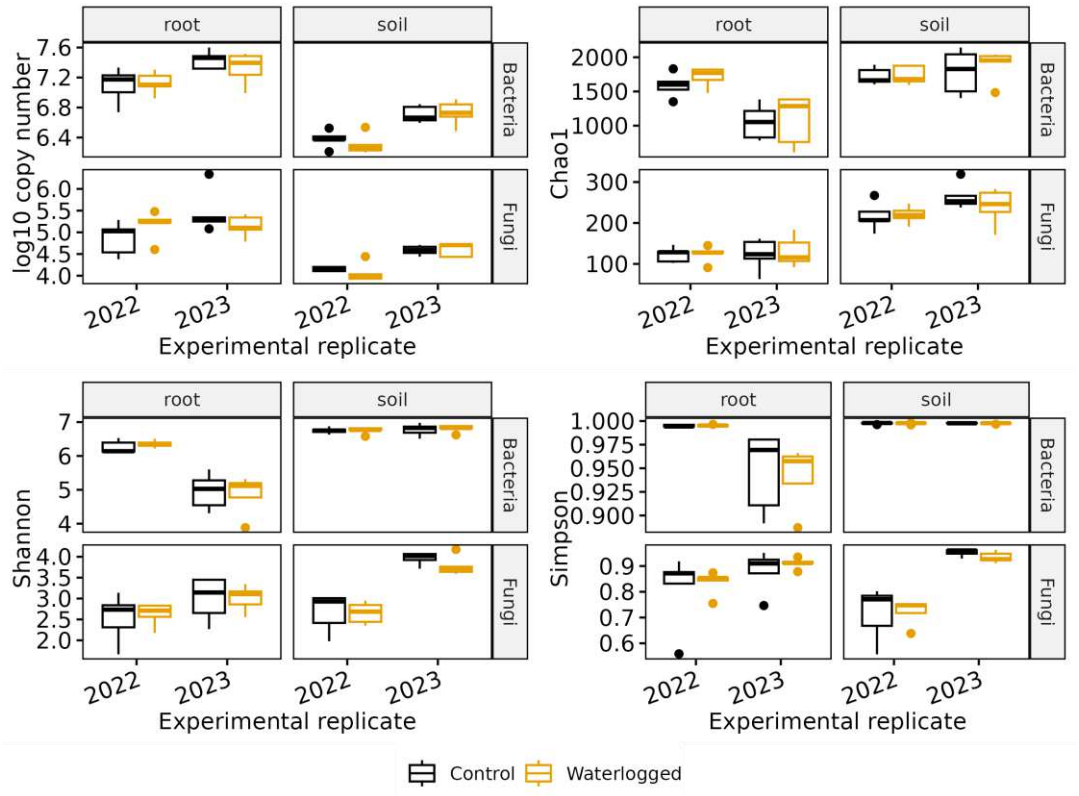


Figure 1. Community size (log_{10} theoretical copy number), and alpha-diversity measures of richness (Chao1), diversity (Shannon), and evenness (Simpson) of fungal and bacterial root and soil communities for the 2022 and 2023 experimental replicates after single waterlogging compared to non-waterlogged control.

Table 2. Effects of design factors on community size (log_{10} theoretical copy number) and alpha diversity indices (Chao1, Shannon, Simpson) of fungal and bacterial root and soil communities for single waterlogging across the 2022 and 2023 experimental replicates based on ANOVA of copy

number and permutation ANOVA of alpha diversity index ranks. Variance (%) for each measure explained by each factor is reported.

		Root				Soil			
	df	Size	Chao1	Shannon	Simpson	Size	Chao1	Shannon	Simpson
Fungi									
experiment	1	13.0	0.3	21.9 **	32.8 ***	76.7 ***	23.6 ***	75.2 ***	75.2 ***
waterlogging	1	<0.1	<0.1	<0.1	0.1	0.4	1.5 **	2.4 **	3.6 ***
exp[1] × water[2]	1	15.5	<0.1	0.1	4.3 ***	0.8	3.0	0.5	0.5
exp[1] × block	8	37.1	92.1 ***	55.4 ***	34.0 **	10.8	46.8 **	13.2 **	13.8 **
residuals	8	34.4	7.6	22.6	28.8	11.4	25.1	8.7	6.9
Bacteria									
experiment	1	38.3 *	66.4 ***	75.2 ***	75.2 ***	70.7 ***	8.7 **	7.7 **	0.8 **
waterlogging	1	0.8	1.9 **	0.3 **	0.5	0.3	2.4 **	0.8	0.1
exp[1] × water[2]	1	2.6	0.5	0.5	0.8 **	1.1	0.8	0.5	1.1 **
exp[1] × block	8	22.0	11.8 **	12.6 **	13.1 **	13.6	31.2 **	55.3 **	83.0 ***
residuals	8	36.3	19.4	11.4	10.5	14.3	57.0	35.8	15.0

*, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. [1]experiment factor. [2]waterlogging factor.

Temporal dynamics and repeated waterlogging

Temporal succession (timepoint) was the major driver of community size and alpha diversity for all communities across sampling timepoints in the 2022 experiment, while waterlogging treatments had limited effects on alpha diversity that were largely dependent on timepoint (figure 2, table 3).

Only bacterial soil communities responded to waterlogging across single and repeated treatments where waterlogged plots had significantly decreased evenness compared to the non-waterlogged control (Simpson: $p = 0.001$, var = 17.3%). The interaction between waterlogging and timepoint was significant for fungal soil diversity (Shannon: $p = 0.041$, var = 8.69%), fungal soil evenness (Simpson: $p = 0.012$, var = 7.70%), and bacterial root richness (Chao1: $p = 0.043$, var = 5.08%). Simple effects analysis, with each timepoint analysed independently, revealed that fungal soil evenness significantly increased in response to repeated waterlogging only (Simpson: $p = 0.018$, var = 43.8%).

Community size significantly increased over time for both fungi and bacteria in both root and soil compartments (all $p < 0.05$). Fungal and bacterial root communities both experienced a significant decline in richness (Chao1), diversity (Shannon), and evenness (Simpson) over time (all $p < 0.05$). In contrast, fungal soil communities experienced a significant increase in richness, diversity, and

evenness over time, and bacterial soil communities experienced a significant increase in evenness over time (all $p < 0.05$). Block effects across timepoints in the 2022 experiment significantly influenced diversity and evenness of root fungi, diversity of root bacteria, and richness of soil bacteria (all $p < 0.05$). This was most pronounced in root fungi where block effects accounted for 20.7% and 28.5% of variation in diversity and evenness respectively.

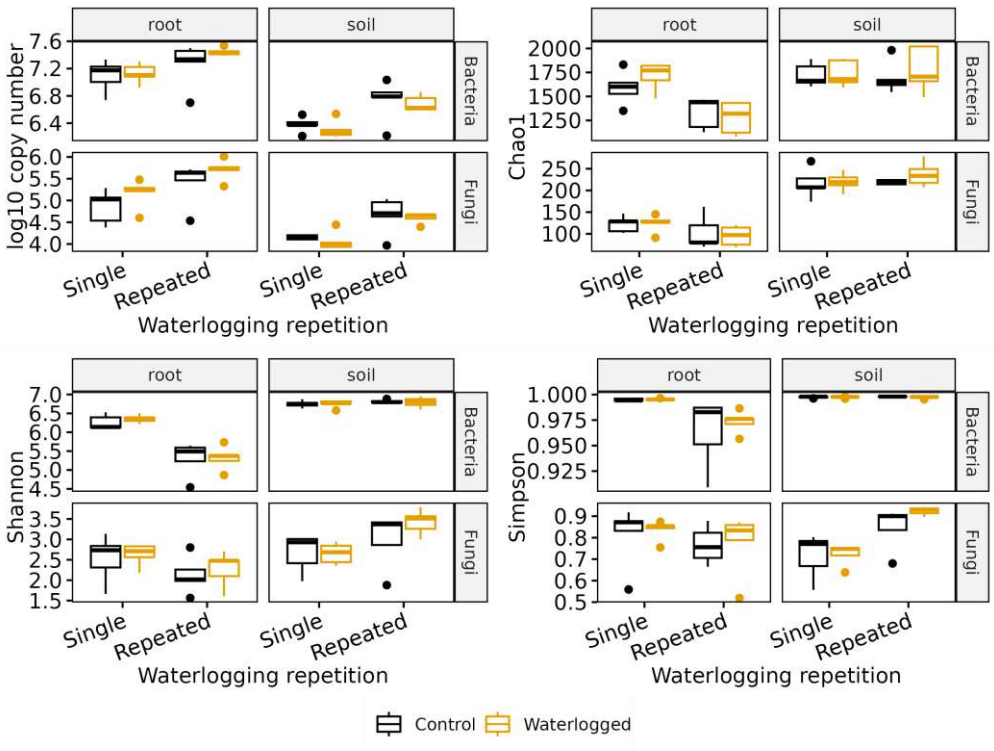


Figure 2. Community size (log_{10} theoretical copy number), and alpha-diversity measures of richness (Chao1), diversity (Shannon), and evenness (Simpson) of fungal and bacterial root and soil communities in the 2022 experiment after first waterlogging in 2022/2023 (single) and second waterlogging in 2023/2024 (repeated) compared to non-waterlogged controls at each timepoint.

Table 3. Effects of design factors on community size (log_{10} theoretical copy number) and alpha diversity indices (Chao1, Shannon, Simpson) of fungal and bacterial root and soil communities for single and repeated waterlogging in the 2022 experiment based on ANOVA of copy number and permutation ANOVA of alpha diversity index ranks. Variance (%) for each measure explained by each factor is reported. Residuals are partitioned into between subject residual (b) and within subject residual (w) error terms to account for the repeated measures ANOVA design.

		Root				Soil			
	df	Size	Chao1	Shannon	Simpson	Size	Chao1	Shannon	Simpson
Fungi									
block	4	17.9	16.3	20.7 **	28.5 ***	6.4	28.6	8.9	6.9
waterlogging	1	11.3	0.5	1.1	<0.1	1.2	5.9	1.9	3.0
residual (b)	4	17.6	8.9	6.1	5.9	8.7	17.0	21.1	15.3
timepoint	1	35.1 **	27.1 ***	25.3 ***	9.7 ***	57.8 **	5.1 ***	34.8 ***	55.6 ***
time[1] × water[2]	1	<0.1	0.8	0.1	1.5	<0.1	0.5	8.7 *	7.7 *
residual (w)	8	18.1	46.5	46.8	54.3	26.0	43.0	24.7	11.4
Bacteria									
block	4	27.6	3.5	7.3 *	5.9	9.8	20.2 *	13.6	10.0
waterlogging	1	5.3	<0.1	0.3	<0.1	1.4	3.6	<0.1	17.3 **
residual (b)	4	29.8	17.0	1.3	4.9	7.7	13.3	21.2	15.1
timepoint	1	26.1 **	60.9 ***	75.2 ***	75.2 ***	54.5 **	<0.1	8.7	9.7 **
time[1] × water[2]	1	2.6	5.1 *	0.5	0.1	0.1	1.5	1.5	6.8
residual (w)	8	8.5	13.4	15.5	13.9	26.6	61.4	54.9	41.1

*, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. [1]timepoint factor. [2]waterlogging factor.

Beta diversity

Single waterlogging across experimental replicates

Experimental year was the dominant factor affecting fungal and bacterial community structure across experimental replicates, while waterlogging had small, specific changes in some of the communities which was partly dependent on experimental year (figure 3, table 4).

Fungal root community structure significantly responded to single waterlogging (PERMANOVA: F = 1.42, p = 0.043) explaining 6.2% of total variation. However, this treatment effect was not captured by PC1–3. Fungal root communities responded to waterlogging significantly differently between experiments (PERMANOVA: F = 1.49, p = 0.017) explaining 6.6% of total variation. Fungal soil communities did not significantly respond to waterlogging across the two experiments. Bacterial root and soil communities did not have significant global community shifts (PERMANOVA) in response to

waterlogging. However, PCA revealed that a subset of the bacterial soil community represented by PC3 responded to waterlogging (PC3: $F = 5.73$, $p = 0.044$) explaining 0.40% of total community variation.

Community structure was significantly different between experiment years for all community compartments (PERMANOVA: $p < 0.05$). This effect was most pronounced in soil communities, where it explained 22.1% of total variation for fungi and 55.9% for bacteria. These experimental replicate effects were primarily captured by PC1 across all groups. Block effects significantly influenced community structure of fungal soil communities explaining 39.5% of total variation which was reflected in PC2. Fungal root communities had significant block effects in PC1 and PC2, and bacterial soil communities had significant block effects in PC3 (all $p < 0.05$).

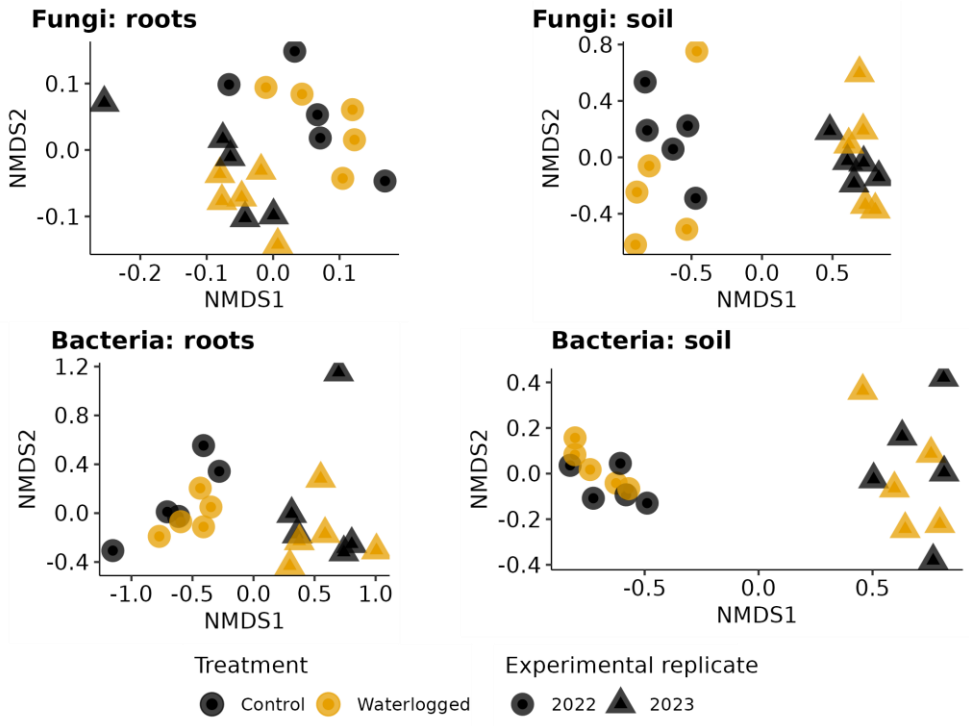


Figure 3. NMDS plots of Bray-Curtis dissimilarity of fungal and bacterial root and soil communities across samples from the 2022 and 2023 experimental replicates after single waterlogging compared to non-waterlogged control.

Table 4. Effect of design factors on community structure from Bray-Curtis dissimilarity (B-C) and principal components (PC1–3) of fungal and bacterial root and soil community structure for single waterlogging across the 2022 and 2023 experimental replicates based on PERMANOVA for B-C and ANOVA for PCs. Percentage of variance in the communities explained by each factor is reported. For PCs the variances are adjusted for the variance in the community explained by each PC.

		Root							Soil							
	df	B-C		PC1		PC2		PC3	B-C		PC1		PC2		PC3	
Fungi																
experiment	1	11.7	***	16.0	***	0.5	**	0.2	22.1	***	19.9	***	<0.1		<0.1	
waterlogging	1	6.2	*	<0.1		0.1		<0.1	4.6		0.1		<0.1		<0.1	
exp[1] × block	8	40.4		2.3	***	8.2	***	4.7	39.5	*	0.3		10.9	***	4.2	
exp[1] × water[2]	1	6.6	*	<0.1		<0.1		0.5	4.0		<0.1		0.1		0.6	
residual	8	35.1		0.2		0.2		1.7	29.8		0.1		0.2		1.5	
Bacteria																
experiment	1	36.1	***	34.2	***	0.5		<0.1	55.9	***	43.9	***	0.0		<0.1	
waterlogging	1	4.0		<0.1		<0.1		<0.1	2.6		0.2		0.1		0.4	*
exp[1] × block	8	28.0		1.7		3.5		4.2	20.8		0.7		2.6		4.5	**
exp[1] × water[2]	1	2.7		<0.1		0.1		<0.1	1.9		<0.1		0.1		<0.1	
residual	8	29.2		1.3		4.5		1.3	18.7		0.7		3.3		0.6	

*, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. [1]experiment factor. [2]waterlogging factor.

Temporal dynamics and repeated waterlogging

Temporal succession (timepoint) and spatial variability (block) were the dominant factors affecting fungal and bacterial community structure across sampling timepoints in the 2022 experiment, while waterlogging had small, specific changes in all communities (figure 4, table 5).

Fungal root and soil communities consistently responded to waterlogging across single and repeated treatments (PERMANOVA root: $F = 0.972$, $p = 0.023$; PERMANOVA soil: $F = 0.937$, $p = 0.004$) explaining 4.8% and 4.5% of total community variation, respectively. However, these treatment effects were not captured by PC1–3. Bacterial communities consistently responded to waterlogging across single and repeated treatments (PERMANOVA root: $F = 1.70$, $p = 0.004$; PERMANOVA soil: $F = 1.27$, $p = 0.004$) explaining 5.8% and 4.1% of total community variation respectively. These treatment effects were reflected in PC3 (PC3 root: $F = 7.88$, $p = 0.048$; PC3 soil: $F = 10.8$, $p = 0.030$) explaining

0.43% and 0.62% of total community variation respectively. The interactions between waterlogging and timepoint were not significant.

Temporal community succession was a major driver of fungal and bacterial community structure (PERMANOVA: $p < 0.05$), explaining between 8% and 13% of total variation in fungal communities and between 31.9% and 36.3% of total variation in bacterial communities. This temporal effect was primarily captured by PC1 across all groups. Block effects significantly influenced community structure for all communities (PERMANOVA: $p < 0.05$) and explained between 21.0% and 21.7% of total variation for fungi and between 16.5% and 17.1% of total variation for bacteria.

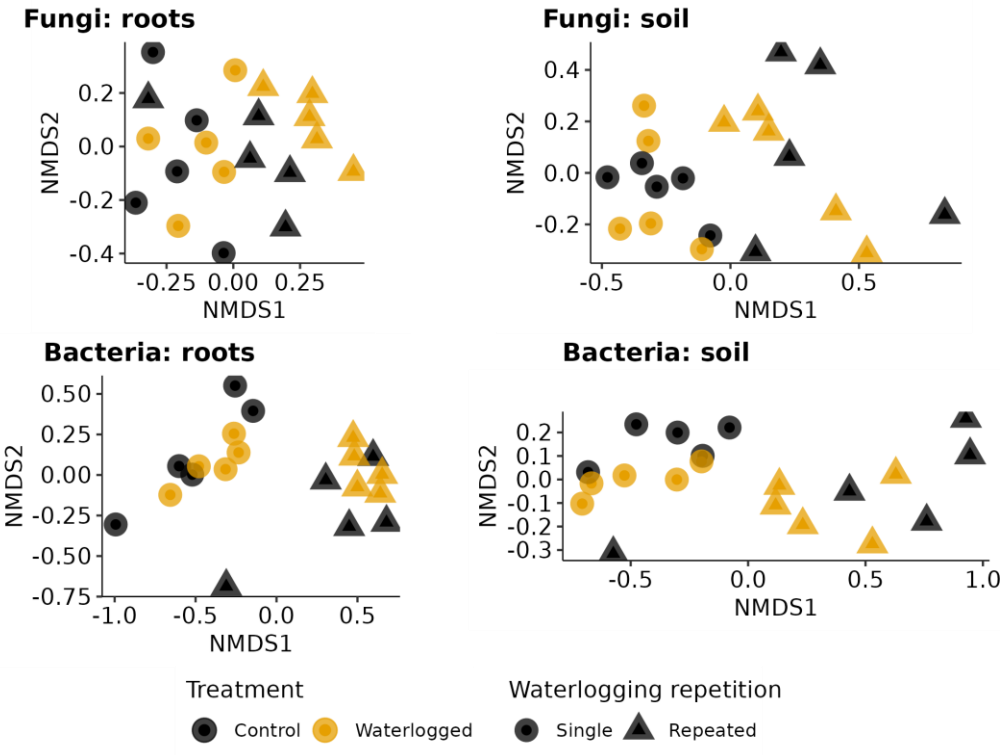


Figure 4. NMDS plots of Bray-Curtis dissimilarity of fungal and bacterial root and soil communities in the 2022 experiment after first waterlogging in 2022/2023 (single) and second waterlogging in 2023/2024 (repeated) compared to non-waterlogged controls at each timepoint.

Table 5. Effect of design factors on community structure from Bray-Curtis dissimilarity (B-C) and principal components (PC1–3) of fungal and bacterial root and soil community structure across single and repeated waterlogging in the 2022 experiment based on PERMANOVA for B-C and ANOVA for PCs. Percentage of variance in the communities explained by each factor is reported. For PCs the variances are adjusted for the variance in the community explained by each PC.

		Root				Soil			
	df	B-C	PC1	PC2	PC3	B-C	PC1	PC2	PC3
Fungi									
block	4	21.7 *	2.8 *	2.1 *	2.9	21.0 **	4.4 **	0.9	3.2
waterlogging	1	4.8 *	<0.1	<0.1	0.7	4.5 **	<0.1	0.1	0.3
timepoint	1	8.0 **	8.9 ***	0.8	1.0	13.0 **	1.7	7.3 ***	0.3
time[1] × water[2]	1	5.5	<0.1	0.1	0.4	4.5	<0.1	0.1	0.3
residual	12	59.8	2.2	7.8	3.3	57.0	8.9	1.3	3.5
Bacteria									
block	4	17.1 **	2.9	3.4	1.4	16.5 **	1.5	4.2	3.0 *
waterlogging	1	5.8 **	0.1	0.2	0.4 *	4.1 **	0.4	0.1	0.6 *
timepoint	1	31.9 **	24.6 ***	1.6 *	<0.1	36.3 **	22.7 ***	1.9	<0.1
time[1] × water[2]	1	4.0	<0.1	<0.1	0.1	4.2	0.2	0.0	0.2
residual	12	41.1	2.2	5.1	4.6	38.9	5.4	4.9	4.1

*, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. [1]timepoint factor. [2]waterlogging factor.

Abundance of fungal and bacterial genera

Single waterlogging across experimental replicates

Differential abundance analysis identified a small subset of ten genera across microbiome compartments that responded consistently to waterlogging across experimental replicates (table 6, figure 5, table S1). Fungal responses were especially limited. No fungal genera in the soil showed a consistent response across experiments. In the roots, only two fungal genera (together representing 1.16% of reads) significantly responded across experiments: *Juxtiphoma* decreased in abundance overall, but its presence was sporadic across plots and experiments and was only found in control samples, while *Serendipita*, predominantly found in experiment 2, increased in abundance overall.

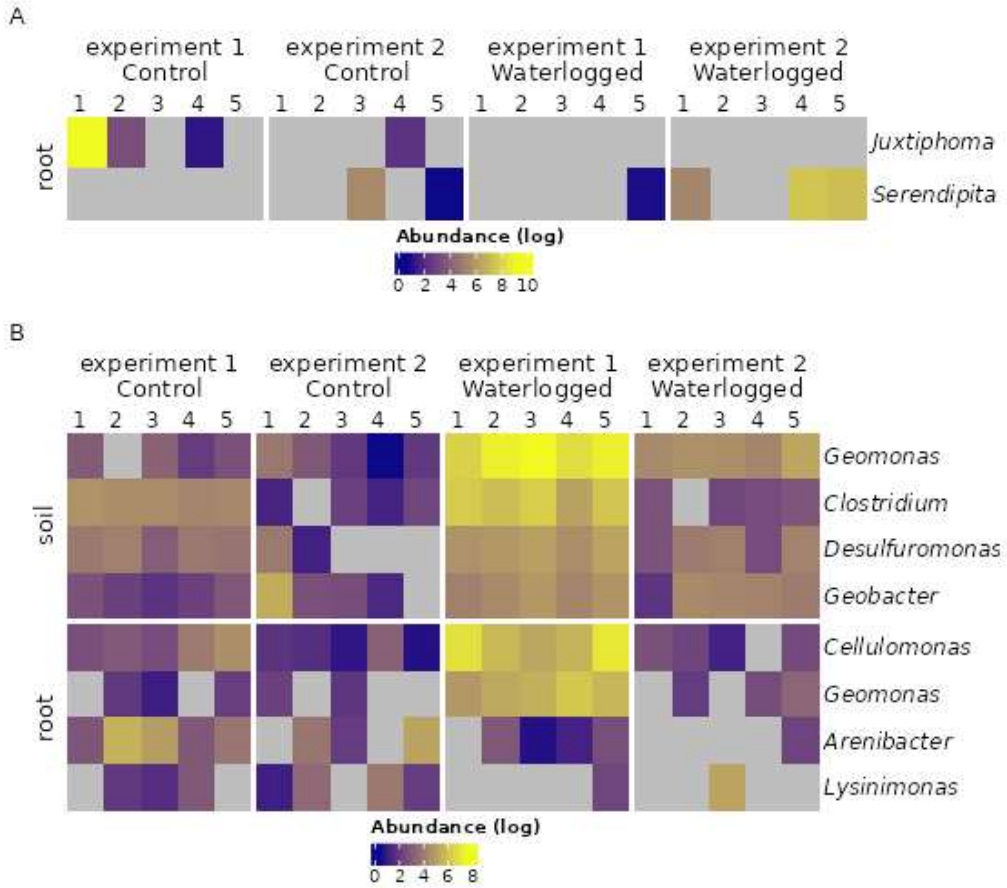
In contrast, the bacterial response was more pronounced and functionally consistent. A group of four soil bacterial genera (together representing 0.51% of reads) consistently increased in abundance in

response to waterlogging across experimental replicates: the anaerobes *Geomonas*, *Desulfuromonas*, *Geobacter*, and *Clostridium*. In the root compartment, four bacterial genera (together representing 1.18% of the reads) consistently responded to waterlogging across experimental replicates. The anaerobic *Cellulomonas* and *Geomonas* increased in abundance, while the strictly aerobic *Arenibacter* and *Lysinimonas* decreased. Analysing each experiment separately revealed 19 genera in the 2022 experiment and six genera in the 2023 experiment that significantly responded to waterlogging (table S2).

Table 6. Fungal and bacterial genera with significant ($p < 0.05$) differential abundance between control and waterlogged samples across experimental replicates. For each genus, mean normalised abundance across samples, percentage of the total population (reads), log-2-fold-change (log2FC) where a positive value indicates an increase in abundance in response to waterlogging, and *p*-values adjusted for multiple testing are included.

Genus	Mean abundance	Population (%)	log2FC	*p* (adj)	
Root Fungi					
Juxtiphoma	494	0.85	-29.30	< 0.0001	***
Serendipita	186	0.32	11.34	0.0044	**
Soil Bacteria					
Geomonas	340	0.29	5.41	< 0.0001	***
Clostridium	124	0.13	1.85	< 0.0001	***
Desulfuromonas	55	0.07	2.88	0.0137	*
Geobacter	50	0.03	3.09	0.0014	**
Root Bacteria					
Cellulomonas	155	0.70	2.67	0.0111	*
Geomonas	70	0.26	5.92	< 0.0001	***
Arenibacter	36	0.11	-4.00	0.0050	**
Lysinimonas	14	0.10	-7.51	0.0081	**

*, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.



362

363 **Figure 5**. Fungal (A) and bacterial (B) genera with significant differential abundance between control
364 and waterlogged samples across the experimental replicates in 2022 (experiment 1) and 2023
365 (experiment 2). Heatmap values show the natural log of abundance + 1 with grey cells indicating the
366 genus was not identified in the sample.

367 ## Temporal dynamics and repeated waterlogging

368 Analysis of the 2022 experiment revealed distinct responses to single and repeated waterlogging
369 (table 7, figure 6, table S1).

370 Of the four soil fungal genera that responded to waterlogging (together representing 2.53% of
371 reads), *Cadophora* and *Chaetomium* increased in abundance after single waterlogging only, while
372 *Thelonectria* and *Hirsutella* increased in abundance after repeated waterlogging only. Of the six root
373 fungal genera that responded to waterlogging (together representing 14.79% of reads), *Gibellulopsis*,
374 *Juxtiphoma*, and *Cephalotrichum* decreased in abundance after single waterlogging only, while

375 *Thelonectria* increased and *Plectosphaerella* and *Rhizoctonia* decreased in abundance in response to
376 repeated waterlogging only. No fungal genera responded to both single and repeated waterlogging.

377 Of the six soil bacterial genera that responded to waterlogging (together representing 1.33% of
378 reads), all six increased in abundance to single waterlogging. Of the ten root bacterial genera that
379 responded to waterlogging (together representing 1.18% of reads), eight increased and two
380 decreased in abundance in response to single waterlogging. Only *Geomonas* responded to repeated
381 waterlogging where it increased in abundance in both root and soil communities.

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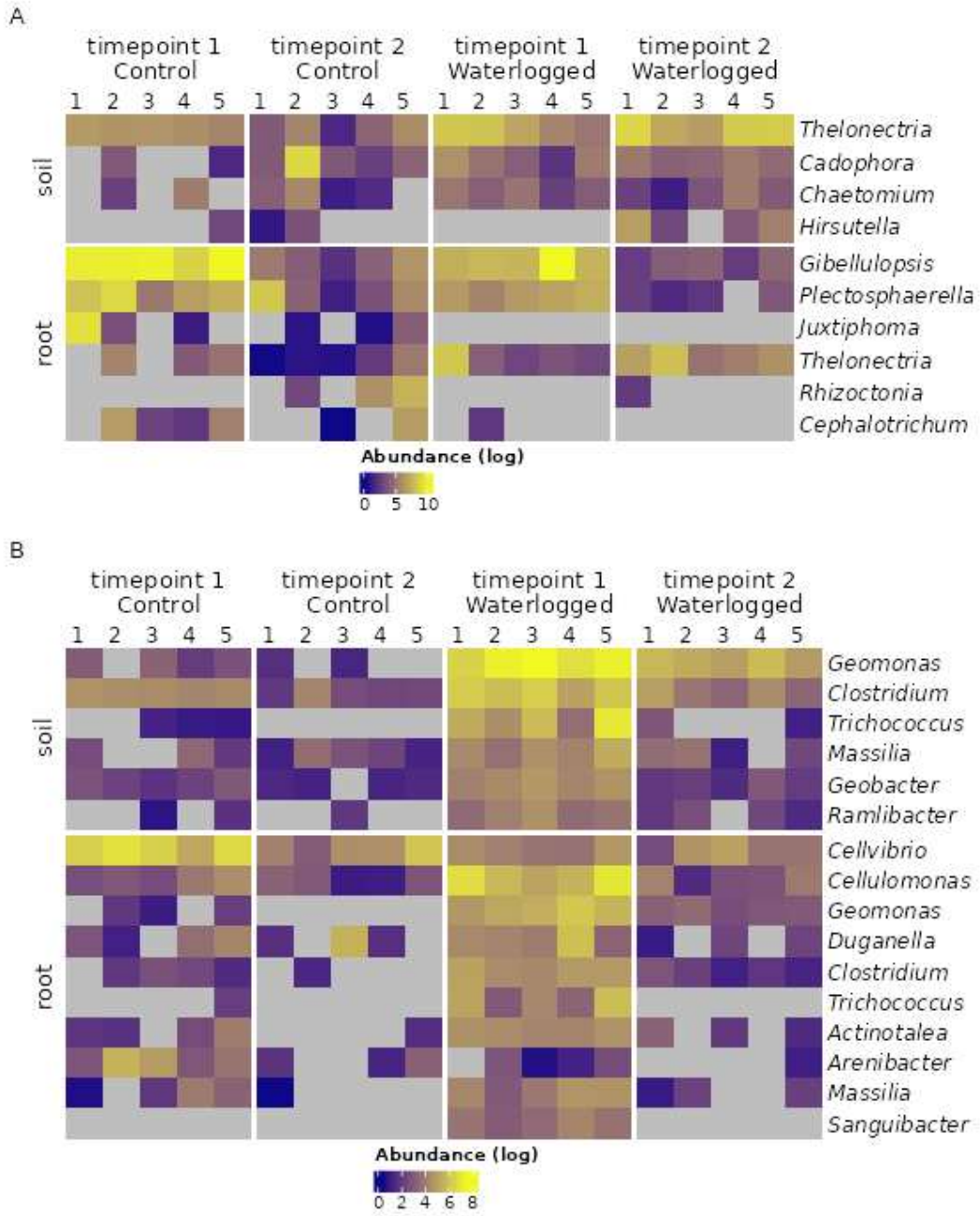
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Table 7. Fungal and bacterial genera with significant ($p < 0.05$) differential abundance between control and waterlogged samples in the 2022 experiment after single and repeated winter waterlogging. For each genus, mean normalised abundance across samples, percentage of the total population (reads), log-2-fold-change (log2FC) where a positive value indicates an increase in abundance in response to waterlogging, and *p*-values adjusted for multiple testing are included for each timepoint and overall across all samples.

	Single waterlogging				Repeated waterlogging				Overall	
Genus	**Abundance**	**log2FC**	***p* (adj)**		**Abundance**	**log2FC**	***p* (adj)**		**Abundance**	**Total (%)**
Soil Fungi										
Thelonectria	1190	0.96	0.9755		2073	5.44	< 0.0001	***	1631	1.83
Cadophora	99	5.61	0.0045	**	872	0.82	0.9805		486	0.54
Chaetomium	70	5.54	0.0318	*	75	2.88	0.5388		72	0.08
Hirsutella	2	-0.66	0.9755		123	6.95	0.0023	**	63	0.07
Root Fungi										
Gibellulopsis	13120	-2.77	0.0260	*	117	-1.51	0.8624		6619	10.74
Plectosphaerella	1902	-0.83	0.5265		477	-4.47	0.0374	*	1190	1.93
Juxtiphoma	1170	-27.87	< 0.0001	***	6	-2.92	0.9605		588	0.95
Thelonectria	478	0.32	0.8733		518	6.96	< 0.0001	***	498	0.81
Rhizoctonia	0				252	-13.55	0.0001	***	126	0.20
Cephalotrichum	107	-5.44	0.0160	*	77	-1.78	0.9820		92	0.15
Soil Bacteria										
Geomonas	642	6.78	< 0.0001	***	115	7.59	< 0.0001	***	378	0.72
Clostridium	250	2.18	< 0.0001	***	44	1.91	0.4716		147	0.28
Trichococcus	188	7.78	< 0.0001	***	2	2.00	0.9893		95	0.18
Massilia	55	4.42	0.0001	***	15	-0.10	0.9893		35	0.07
Geobacter	51	3.15	0.0001	***	5	2.12	0.9893		28	0.05
Ramlibacter	27	5.82	< 0.0001	***	4	4.83	0.7249		15	0.03
Root Bacteria										
Cellvibrio	353	-3.15	< 0.0001	***	109	-0.86	0.9436		231	0.41
Cellulomonas	297	4.03	< 0.0001	***	21	1.48	0.7810		159	0.28
Geomonas	130	6.93	< 0.0001	***	10	6.67	0.0158	*	70	0.12
Duganella	75	3.19	0.0102	*	29	-1.99	0.7491		52	0.09
Clostridium	62	4.43	< 0.0001	***	4	4.01	0.7057		33	0.06
Trichococcus	65	7.13	< 0.0001	***	0				33	0.06
Actinotalea	51	3.57	0.0013	**	4	2.11	0.9919		27	0.05
Arenibacter	50	-4.10	0.0010	***	3	-2.94	0.9436		27	0.05
Massilia	45	3.39	0.0090	**	2	2.05	0.9919		23	0.04
Sanguibacter	20	7.07	< 0.0001	***	0				10	0.02

*, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.



404

405 **Figure 6**. Fungal and bacterial genera with significant differential abundance between control and

406 waterlogged samples after single (timepoint 1) or repeat (timepoint 2) waterlogging. Heatmap values

407 show the natural log of abundance + 1 with grey cells indicating the genus was not identified in the

408 sample.

Discussion

We analysed the effects of winter waterlogging on the soil and apple root microbiota that persisted for 6–9 months after waterlogging treatments. Fungal and bacterial root and soil community size, alpha diversity, community structure, and relative abundance of genera was investigated after single winter waterlogging across experimental replicates and after single and repeated waterlogging in consecutive winters for the 2022 experiment.

It should be noted that sampling intervals varied between experimental replicates: In the 2022 experiment samples were collected 9-months after the first treatment in September, but after the second treatment and in the 2023 experiment samples were collected 6-months after treatment in June. While this confounds experimental replicate and treatment repetition with precise sampling time, it does not alter the fundamental observation of a treatment response. Instead, this variation likely contributes to the observed differences between experimental replicates.

Community dynamics

Community size, diversity, and structure were primarily driven by experimental year (replicate) and temporal succession rather than the waterlogging treatment. Community size, alpha diversity, and community structure (beta diversity) of most communities varied significantly between experimental years. Community size grew over time in the 2022 experiment (tables 3). Alpha diversity decreased over time in fungal and bacterial root communities and increased over time in fungal and bacteria soil communities in the 2022 experiment (table 3). Community structure (beta diversity) shifted consistently over time in the 2022 experiment (figure 3) which was captured by the major axes of variation (PC1–3, table 4) in each community. Most communities had very high variation in alpha diversity and community structure between blocks within experiment (tables 2 and 4). The high variation between experimental years and the strong temporal development of microbial communities resulted in limited overlap in community structure between experimental years or sampling timepoints and therefore limited effect of waterlogging that across experimental years and timepoints. The dominance of the experimental replicate effect over the waterlogging treatment

suggests that the microbial response to waterlogging is a small change in trajectory determined in part by the community's initial composition. This explains why consistent responses in community structure were only observed in a small, specialised subsets of the root fungal and soil bacterial populations, likely the obligate aerobes and anaerobes identified by differential abundance analysis.

Community changes due to single waterlogging

The fungal response to single waterlogging was different between root and soil compartments. Fungal root communities were more resilient, and their alpha diversity was mostly unaffected, although evenness changed in an experimental replicate-dependent manner (table 2). Their shift in community structure (beta diversity) was also partly dependent on experimental replicate (table 4). Conversely, fungal soil communities experienced a consistent decline in richness, diversity, and evenness independent of experimental replicate, but their community structure was not significantly affected. This suggests that the roots act as a buffer, preventing loss of fungal diversity but allowing communities to shift in response to waterlogging depending on their initial composition, while fungal soil communities are less protected by the host and the exposure to the harsh chemical shifts experienced in the bulk soil during waterlogging results in a decline in richness, diversity, and evenness regardless of their initial composition. This decline in soil fungal diversity was much smaller than the differences between experimental years and spatial variability (block within experiment), but its persistence 6–9 months after winter waterlogging and through the subsequent growing season indicates that the communities struggle to fully recover and stay in a stable simplified state. The trajectory of fungal root communities after waterlogging is likely modulated by the plant host, which was not significantly affected by 4-weeks of winter waterlogging in terms of growth in height or stem diamteter.

In contrast, bacterial communities experienced a consistent increase in richness and diversity in roots and richness in soil independent of experimental replicate, but their changes in evenness were dependent on experimental replicate. Their community structures were largely unaffected by single waterlogging, but small sub-population of soil bacteria consistently responded to waterlogging

independent of experimental replicate (PC3, table 4). The observed increase in richness and diversity in response to waterlogging suggests an expansion of anaerobic niches that results in long term diversification of bacterial communities. This appeared to lead to a targeted shift in a small population of soil bacteria that persisted throughout the subsequent growing season. Differential abundance analysis confirmed that this sub-population consists primarily of anaerobes (table 6) which increased in abundance in response to waterlogging, suggesting that while the broader community is dominated by spatial and temporal variability, winter waterlogging favours a specific anaerobic niche that persists long after the stress is alleviated.

Together, these findings contrast with previous findings in rapeseed rhizosphere where fungal richness and diversity increased and bacterial richness and diversity decreased 5–10 days after waterlogging under controlled indoor conditions during active growth [12]. This discrepancy is likely due to sampling timing in relation to waterlogging, and host and microbiome phenology at sampling. While Wasim *et al* captured the immediate microbiota response (5–10) days after waterlogging, our 6–9-month post-treatment sampling reveals a persistent legacy effect. While waterlogging during active growth may initially trigger increased fungal and decreased bacterial diversity, our findings indicate that the long-term effect of waterlogging during dormancy is a simplified fungal community and richer bacterial community.

Community changes due to repeated waterlogging

Alpha diversity had very limited responses across single and repeated waterlogging in the 2022 experiment and were largely dependent on waterlogging repetition (timepoint, table 3). Changes in fungal root evenness, fungal soil diversity and evenness, and bacterial root richness depended on waterlogging repetition, while bacterial soil evenness decreased in response to waterlogging independent of waterlogging repetition. This contrasts with the consistent decline in soil fungal alpha diversity and increase in bacterial alpha diversity independent of experimental replicate in response to single waterlogging. This suggests that microbial communities adapt to waterlogging and do not

experience the same declines or increases in alpha diversity after subsequent winter waterlogging treatments.

Despite substantial changes in community structure (beta diversity) over time (figure 4), all fungal and bacterial root and soil communities in the 2022 experiment shifted consistently in response to single and repeated waterlogging independent of treatment repetition. The shift in the bacterial root and soil communities affected specific sub-populations (PC3, table 5) also independent of waterlogging repetition. Differential abundance analysis revealed an increase in *Geomonas* in bacterial root and soil communities after both single and repeated waterlogging treatments. This is in contrast with the limited, experimental replicate-dependent, effects in response to single waterlogging, suggesting that changes in community structure are dependent on their ecological background and are reinforced by subsequent waterlogging treatments. While only small sub-populations responded to waterlogging, the persistence of these effects 6–9 months after treatment highlights that the effects of waterlogging during dormancy carry over into the following growing season, potentially impacting nutrient cycling, stress tolerance, and disease outcomes when the trees resume active growth.

Our findings are broadly consistent with literature which shows that, within a specific experimental context, waterlogging has a minimal effect on alpha diversity and results in consistent shifts in community structure, especially in bacterial communities [9–12]. However, our findings identified dominant effects of experimental year, spatial (block) variation, and temporal succession, leading to the conclusion that the long-term effects of winter waterlogging on the microbiota depended on its ecological background, which is not well represented in the literature. These findings relied on experimental replication in subsequent years which is rarely investigated in similar experiments in the literature. Studies have found high spatial variability in waterlogging response between periodically waterlogged fields [9] and variability in waterlogging response between growth stages in sugarcane (*Saccharum officinarum*) [11]. This tentatively support our findings that the microbiota

response to waterlogging is dependent on its initial community but are inconclusive without further investigation. Our work highlights the need for experimental replication in studies where the microbiota response to a treatment may be dependent on its initial structure.

Future studies should include full experimental replication and high temporal resolution to track the trajectories of distinct microbiota from the onset of hypoxia throughout the following growing season. Waterlogging treatments should also be applied in different seasons to compare the microbiota response to waterlogging during different host phenological and climate conditions.

Taxonomic legacy of waterlogging

Bacterial communities exhibited an anaerobic legacy that persisted long after re-oxygenation. While this could be due to detection of DNA from organisms that died after re-oxygenation, our sampling 6–9 months after reoxygenation makes this unlikely. Instead, this was likely caused by the persistence of anaerobic bacteria within anaerobic microsites or biofilms which have been demonstrated in agricultural soils [31,32].

The uniform enrichment of *Geomonas*, *Geobacter*, and *Desulfuromonas* after single waterlogging across experimental years identifies these as core indicators of winter waterlogging in apple orchards. These genera belong to the *Desulfuromonadales* family and are strictly anaerobic, explaining their enrichment after waterlogging [33,34]. Most significantly, the persistence of *Geomonas* 6–9 months after waterlogging across experimental years and after single and repeated waterlogging treatments suggests that once anaerobic niches establish in the soil or roots, they may form a stable component of the community that is not easily displaced even after re-oxygenation. Other studies have identified *Geobacter* as a waterlogging biomarker in bulk soil and wheat (*Triticum aestivum*) rhizosphere soil [9,10]. Some *Geobacter* species have been found to fix nitrogen and are potentially beneficial to plant hosts [34,35].

While immediate sampling after waterlogging often reveals an increase in abundance of water-borne fungal pathogens such as *Fusarium* [12], our longer-term results revealed a decline in potential

pathogens with a decrease in abundance of the fungi *Gibellulopsis* and *Plectosphaerella* which are associated with diseases in a range of horticultural crops including melon, tomato, bell pepper, and cucurbits [36–38]. This suggests that winter waterlogging does not necessarily lead to persistent fungal pathogen dominance. Instead, the enrichment of *Thelonectia* points towards an enrichment of saprotrophs which are likely responding to waterlogging-induced root senescence.

The enrichment of waterlogging-tolerant facultative anaerobic bacteria including *Clostridium* and the *Micrococcales* (*Cellulomonas* and *Sanguibacter*) after single waterlogging across both experimental replicates suggests an opportunistic expansion into newly formed niches and highlights a shift towards a community capable of anaerobic metabolism. Enrichment of *Clostridium* immediately after waterlogging has been well-documented in rapeseed (*Brassica napus*) [12] and wheat [10] rhizosphere soil and we have demonstrated that this enrichment can persist through the growing season. The effect on the host is unclear. *Clostridium* is pathogenic in kiwifruit (*Actinidia chinensis*) [39] and *Cellulomonas* and *Sanguibacter* contain members with plant growth-promoting activity [40,41]. However, this opportunistic expansion was diminished after repeated waterlogging with fewer genera maintaining their elevated abundance. Together with the findings that community structure (beta diversity) were affected by waterlogging independently of waterlogging repetition (timepoint, table 5), this suggests a transition from targeted enrichment of anaerobic bacteria after single waterlogging to a more diffuse shift in the community after repeated treatment, and a potential adaptation of the microbiome to a more resilient state. The disruption caused by the initial waterlogging event likely allowed opportunists to dominate in newly formed niches, but repeated waterlogging may broadly select for a community adapted to fluctuating oxygen levels that no longer exhibits large, erratic shifts in response to a waterlogging event.

We observed an unexpected enrichment of strictly aerobic taxa (table 7) including the fungal *Chaetomium* and bacterial Burkholderiales (*Ramlibacter* and *Massilia*). *Chaetomium* is a known biocontrol agent of apple scab (*Venturia inaequalis*) [42] and apple valsa canker (*Cytospora mali*) [43]

and is antagonistic to European apple canker (*Neonectria ditissima*) *in vitro* [44]. *Ramlibacter* and *Massilia* include members with plant growth-promoting activity [45,46]. While this enrichment contrasts with previous findings which showed that *Massilia* was depleted immediately after waterlogging in wheat rhizosphere [10], because our sampling occurred 6–9 months after waterlogging, it likely represents a host-mediated recovery recruitment. As the soil reoxygenates, the host may actively recruit these aerobic plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF) in response to hypoxic stress. We did not observe this enrichment after repeated treatment, suggesting the recruitment diminishes with successive waterlogging events as the host and microbiota adapts. This is supported by previous findings that *Chaetomium* is enriched after single waterlogging [12] but is depleted in periodically waterlogged fields [11].

The long-term depletion of strictly aerobic saprotrophs *Arenibacter* and *Cellvibrio* highlights a protracted recovery period after waterlogging. Depletion of aerobes immediately after waterlogging has been previously reported [10], however the inability of these taxa to reestablish their populations within a single growing season suggests that the disruption caused by waterlogging can result in a suppression of the aerobic saprotroph community persisting for months. The discrepancy between our findings and studies reporting a depletion of aerobic PGPRs like *Pseudomonas*, *Streptomyces*, and *Sphingomonas* either immediately after waterlogging [10] or after persistent waterlogging over many years [11] is likely due to recovery time. While these bacteria may be depleted immediately after waterlogging, they have the potential to recover after the return to normal conditions, but periodic waterlogging prevents this recovery.

Conclusions

Collectively, these results show that the long-term legacy of winter waterlogging are small changes in fungal and bacterial root and soil communities characterised by an enrichment of anaerobic bacteria (*Geomonas*) and a simplified fungal community where potential pathogens have declined. The enrichment of beneficial opportunists after single but not repeated waterlogging suggests a

vulnerability of the microbiome where the recruitment of beneficial microbes is suppressed after successive wet winters which could reduce plant growth, impair stress tolerance, and exacerbate disease.

List of abbreviations

ANOVA: analysis of variance

ASV: amplicon sequence variant

B-C: Bray-Curtis

PC: principal component

PCR: polymerase chain reaction

PERMANOVA: permutational multivariate analysis of variance

PGPR: plant growth-promoting rhizobacteria

qPCR: quantitative polymerase chain reaction

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets supporting the conclusions of this article are available in the European Nucleotide Archive (ENA) repository, PRJEB113178. ASV sequences, ASV count tables, taxonomy tables, and analysis code are available in the zenodo repository, https://zenodo.org/records/19915941.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HM, MPR, AM, and XX designed the experiments and developed the research. HM analysed and interpreted the data and wrote the manuscript. DG processed the sequence data and advised the analysis. HM, MPR, and XX refined the analysis and the manuscript. All authors contributed to the research.

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760 Supplementary information

761 **Table S1**. Full taxonomy of genera with significant differential abundance between control and

762 waterlogged samples.

Kingdom	Phylum	Class	Order	Family	Genus
Fungi	*Ascomycota*	*Dothideomycetes*	*Pleosporales*	*Didymellaceae*	*Juxtiphoma*
Fungi	*Ascomycota*	*Leotiomycetes*	*Helotiales*	*Helotiales(o)*	*Cadophora*
Fungi	*Ascomycota*	*Sordariomycetes*	*Glomerellales*	*Plectosphaerellaceae*	*Gibellulopsis*
Fungi	*Ascomycota*	*Sordariomycetes*	*Glomerellales*	*Plectosphaerellaceae*	*Plectosphaerella*
Fungi	*Ascomycota*	*Sordariomycetes*	*Hypocreales*	*Nectriaceae*	*Thelonectria*
Fungi	*Ascomycota*	*Sordariomycetes*	*Hypocreales*	*Ophiocordycipitaceae*	*Hirsutella*
Fungi	*Ascomycota*	*Sordariomycetes*	*Microascales*	*Microascaceae*	*Cephalotrichum*
Fungi	*Ascomycota*	*Sordariomycetes*	*Sordariales*	*Chaetomiaceae*	*Chaetomium*
Fungi	*Basidiomycota*	*Agaricomycetes*	*Cantharellales*	*Ceratobasidiaceae*	*Rhizoctonia*
Fungi	*Basidiomycota*	*Agaricomycetes*	*Sebacinales*	*Serendipitaceae*	*Serendipita*
Bacteria	*Actinobacteria*	*Actinobacteria*	*Micrococcales*	*Cellulomonadaceae*	*Cellulomonas*
Bacteria	*Actinobacteria*	*Actinobacteria*	*Micrococcales*	*Cellulomonadaceae*	*Actinotalea*
Bacteria	*Actinobacteria*	*Actinobacteria*	*Micrococcales*	*Jonesiaceae*	*Sanguibacter*
Bacteria	*Actinobacteria*	*Actinobacteria*	*Micrococcales*	*Microbacteriaceae*	*Lysinimonas*
Bacteria	*Bacteroidetes*	*Flavobacteriia*	*Flavobacteriales*	*Flavobacteriaceae*	*Arenibacter*
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Carnobacteriaceae*	*Trichococcus*
Bacteria	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Ramlibacter*
Bacteria	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Massilia*
Bacteria	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Duganella*
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Desulfuromonadales*	*Desulfuromonadaceae*	*Desulfuromonas*
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Desulfuromonadales*	*Geobacteraceae*	*Geomonas*
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Desulfuromonadales*	*Geobacteraceae*	*Geobacter*
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Cellvibrionales*	*Cellvibrionaceae*	*Cellvibrio*

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Table S2. Fungal and bacterial genera with significant ($p < 0.05$) differential abundance between control and waterlogged samples in the 2022 experiment or 2023 experiment separately. For each genus, mean normalised abundance across samples, percentage of the total population (reads), log-2-fold-change (log2FC) where a positive value indicates an increase in abundance in response to waterlogging, and *p*-values adjusted for multiple testing are included for each experiment and overall across all samples.

	2022 Experiment				2023 Experiment				Overall	
Genus	**Abundance**	**log2FC**	***p* (adj)**		**Abundance**	**log2FC**	***p* (adj)**		**Abundance**	**Total (%)**
Soil Fungi										
Cadophora	84	5.60	0.0032	**	29	-0.07	0.9808		57	0.08
Chaetomium	59	5.53	0.0309	*	25	-2.86	0.7574		42	0.06
Serendipita	0				69	6.96	0.0315	*	34	0.05
Root Fungi										
Ceratobasidium	76	0.97	0.6757		1081	5.67	0.0120	*	579	0.99
Juxtiphoma	988	-26.70	< 0.0001	***	1	0.55	0.8905		494	0.85
Protoventuria	0				382	6.18	0.0120	*	191	0.33
Serendipita	0	-0.54	0.8889		371	10.58	0.0003	***	186	0.32
Soil Bacteria										
Geomonas	621	6.78	< 0.0001	***	59	3.67	0.0017	**	340	0.70
Clostridium	242	2.18	< 0.0001	***	7	1.22	0.9978		124	0.26
Trichococcus	181	7.78	< 0.0001	***	0	0.78	0.9978		91	0.19
Desulfuromonas	84	1.58	0.1045		25	5.78	0.0036	**	55	0.11
Geobacter	49	3.15	0.0001	***	52	3.03	0.3588		50	0.10
Massilia	53	4.41	0.0001	***	35	0.75	0.9978		44	0.09
Ramlibacter	26	5.82	0.0001	***	12	-0.91	0.9978		19	0.04
Root Bacteria										
Cellvibrio	361	-3.15	< 0.0001	***	152	0.17	0.9932		256	0.47
Cellulomonas	303	4.03	< 0.0001	***	7	0.89	0.9932		155	0.29
Geomonas	133	6.93	< 0.0001	***	6	3.98	0.9932		70	0.13
Duganella	76	3.19	0.0101	*	30	-4.98	0.9932		53	0.10
Massilia	46	3.39	0.0087	**	29	-1.25	0.9932		37	0.07
Arenibacter	51	-4.10	0.0009	***	22	-3.43	0.9932		36	0.07
Trichococcus	67	7.13	< 0.0001	***	0				33	0.06
Actinotalea	52	3.57	0.0013	**	12	-2.49	0.9932		32	0.06
Clostridium	63	4.43	< 0.0001	***	0				32	0.06
Sanguibacter	21	7.06	0.0001	***	19	-6.56	0.9932		20	0.04