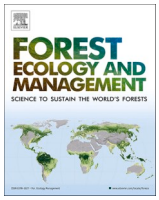
Forest Ecology and Management 581 (2025) 122554

Contents lists available at ScienceDirect

Forest Ecology and Management

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



Conifer epiphytic phyllosphere bacterial communities respond more strongly to rain exclusion and host species identity than to soil water content

Rim Khlifa [a], Marie Renaudin [a], Daniel Houle [a,b], Loïc D'Orangeville [c], Louis Duchesne [d], Steven W. Kembel [a,*]

[a] Université du Québec à Montréal, Département des Sciences Biologiques, Montréal, QC H2X 1Y4, Canada
[b] Environment and Climate Change Canada, Science and Technology Branch, Montréal, QC H2Y 2E7, Canada
[c] Université Laval, Département des sciences du bois et de la forêt, Québec, QC G1V 0A6, Canada
[d] Ministère des Ressources naturelles et des Forêts du Québec, Direction de la recherche forestière, Québec, QC G1P 3W8, Canada

ARTICLE INFO

Keywords:
Boreal forest
Coniferous trees
Epiphytic bacteria
Phyllosphere
Soil water
Drought

ABSTRACT

With global warming, the frequency and intensity of drought episodes are projected to increase worldwide, especially in the boreal forest. This represents a serious threat to the boreal forest ecosystem's productivity and environmental services. It is thus crucial to better understand how drought or water limitation could affect boreal forest ecosystems functioning, and to be prepared to overcome damage caused by drought events. Studies suggest that microbes may mitigate the negative effects of drought or water shortage on plants. However, most of these studies focused on soil microbes and on agricultural ecosystems. Here, we used a rainout shelters and soil irrigation experimental design to study the response to rain exclusion and soil water content of epiphytic phyllosphere bacterial communities associated with four boreal conifer tree species. Our results showed only a weak response of phyllosphere bacterial communities to variation in soil water content. On the other hand, host tree species identity and rain exclusion were the main drivers of epiphytic phyllosphere bacterial communities' structure and diversity. This suggests that fewer rain events, in the context of climate change, would impact boreal trees phyllosphere microbiome composition.

1. Introduction

Drought episodes are projected to become more frequent and intense as global warming accelerates, making many ecosystems around the globe significantly more vulnerable (Lee et al., 2023). In agricultural systems, drought represents a critical obstacle to meeting the food demands of the coming century (Lesk et al., 2016), while in forest ecosystems, severe drought seriously threatens productivity and carbon sequestration (Ciais et al., 2005; Pan et al., 2024). Drought strongly limits plant growth by inducing changes in their physiology, nutrient acquisition, and metabolism (Evelin et al., 2009). In this context, a growing body of studies has focused on understanding mutualistic relationships between plants and other organisms (e.g., animal, arbuscular mycorrhiza), since positive interactions under drought conditions have been reported (Pringle et al., 2013; Augé et al., 2015; Angelini et al., 2016;).

Experiments focusing specifically on microbes have suggested that, in addition to multiple other beneficial effects on plant-host fitness (Berg, 2009; Laforest-Lapointe et al., 2017; Zheng et al., 2018), they may help mitigate negative effects of drought on plants (Lau and Lennon, 2012; Marasco et al., 2012; Ortiz et al., 2015). For example, mutualistic associations with fungi can help lettuce plants maintain growth under stress conditions and permit more efficient water usage (Ruiz-Lozano et al., 1995). Similarly, *Achromobacter piechaudii* ARV8 (i.e., an ACC deaminase-producing bacterium) significantly decreases the growth inhibition of peppers and tomatoes under drought (Glick et al., 2007). However, drought can affect microbes by decreasing their biomass and diversity, as well as inducing community shifts towards specific taxa (Hueso et al., 2012; Castaño et al., 2018; Preece et al., 2019). Some bacterial taxa can be more tolerant to water stress and therefore be advantaged during drought or limited water availability periods. For example, Gram-positive bacteria are generally more resistant to drought

* Corresponding author.
E-mail address: kembel.steven_w@uqam.ca (S.W. Kembel).

https://doi.org/10.1016/j.foreco.2025.122554
Received 5 December 2024; Received in revised form 4 February 2025; Accepted 5 February 2025
Available online 13 February 2025

than Gram-negative bacteria, perhaps due to their thicker cell walls (Schimel et al., 2007). In European coniferous forest soils, drought and rewetting stress was shown to increase relative abundance of *Actinobacteria* and *Firmicutes* while decreasing relative abundance of *Gammaproteobacteria* and *Bacteroidetes* (Chodak et al., 2015). Soil moisture was also shown to impact soil bacterial communities' composition as it was negatively correlated with *Caulobacterales* and *Rhizobiales* abundance on a 500-km regional climate gradient in North American hardwood forests (Romanowicz et al., 2016).

Drought can affect soil microbes through physical alteration of their environment. It can decrease soil water content (Schimel et al., 2007; Manzoni et al., 2012), reduce soil nutrient mobility (Schjønning et al., 2003; Schimel et al., 2007), and ultimately, alter microbial physiological activity and biogeochemical cycles (Deng et al., 2021; Patel et al., 2021; Li et al., 2024). For example, in forest soils, lack of water can reduce microbial litter decomposition, nitrification, and C and N mineralization (Deng et al., 2021). Drought can also indirectly alter soil microbial communities by affecting plants and modifying plant-microbes interactions (Berendsen et al., 2012). On the contrary, several studies reported small effects or no effect of drought on soil microbial communities (Homyak et al., 2017; Cole et al., 2019; Hammerl et al., 2019).

While the majority of plant-microbes research has focused on the rhizosphere, less is known about phyllosphere microbial communities (i.e., microorganisms living on plant leaves or needles; Lindow and Brandl, 2003; Vorholt, 2012), especially for non-model organisms such as forest trees. Drought or reduced water availability can affect phyllosphere bacteria (i) directly, as rain quantity received by leaves and immediately available to epiphytic bacteria decreases, (ii) or indirectly, by decreasing soil water content which can impact plant growth and development but can also modify phyllosphere morphological and chemical properties (e.g., foliar nutrients, wax layer) with potential consequences on bacterial communities' structure. For example, leaf wax thickness is negatively correlated with phyllospheric bacterial biomass (Tang et al., 2023). On the other hand, the soil microbiome may be a reservoir for the phyllosphere microbiome (Copeland et al., 2015; Truchado et al., 2018). Therefore, any alteration in soil bacterial communities induced by drought or lower soil water content could ultimately modify phyllospheric bacterial communities' composition.

Similarly to soil microbes, drought can impact phyllosphere bacterial communities' structure and diversity. Rain events were shown to influence tomato and cucumber phyllosphere bacterial communities' composition (Allard et al., 2020) and rain exclusion treatment modified Holm oak phyllosphere epiphytic bacterial richness in a Spanish forest (Rico et al., 2014). Drought also caused an increase of *Gammaproteobacteria* abundance and a decrease of bacterial diversity in temperate and tropical forage grass species phyllospheres (Bechtold et al., 2021). However, it is still unclear how boreal forest tree phyllosphere microbes respond to drought or water limitation. Given their roles in forest nutrient cycling, it is crucial to investigate the effects of water availability on tree phyllosphere bacterial communities.

In the present study, we aimed to determine how rain exclusion and variation in soil water content affect the epiphytic bacterial communities of four host tree species' phyllosphere, using a rainout shelters and soil irrigation experiment in the eastern Canadian boreal forest, where models predict that warmer and drier soil conditions during the growing season will have significant impacts on forest growth and biogeochemical cycles (Houle et al., 2012; Sherwood and Fu, 2014). We hypothesized that: i) as there is a potential relationship between soil and phyllosphere microbiomes, soil water content would indirectly influence the overall epiphytic phyllosphere bacterial community composition and would be positively correlated with its diversity; ii) epiphytic phyllosphere bacterial communities would vary with the host tree species identity, as observed in previous studies (Kembel et al., 2014; Laforest-Lapointe et al., 2016); iii) rain exclusion would modify phyllosphere bacterial communities and reduce their diversity, as rain represents a source of inoculation for tree foliage.

2. Material and methods

2.1. Study site

The study site is located at the Montmorency research forest (47°15'N 71°11'W) which is located at 800 m of altitude and approximately 70 km north of Quebec City in the province of Quebec, Canada, within the balsam fir – white birch bioclimatic domain (Fig. 1). The site is characterized by a cold and moist climate. Mean annual temperature and precipitation measured between 1971 and 2000 are −0.5°C and 1605 mm, respectively. During the experiment, no specific rain pattern was observed as daily precipitation was relatively evenly distributed throughout the snow-free period (Fig. S1). However, summer 2017 totalled 454 mm of rain and was therefore drier than 2015 and 2016 which totalled 542 and 570 mm of precipitation, respectively.

The experimental site was clearcut harvested in summer 2013 following a hemlock looper (*Lambdina fiscellaria* [Guenée]) outbreak. The soil has a sandy loam texture and is classified as Orthic Humo-Ferric Podzol (Soil Classification Working Group, 1998). Humus layer is ~16.8 cm thick and is classified as Mor type. Soil pH is 4.2 and average C:N is 23.

2.2. Experimental design

The experimental design consisted of 3 blocks, divided into 6 plots of 4.9 m × 7.6 m each (Fig. 1, Fig. S2). The three blocks were characterized by a natural slope of about 12 % north orientation, and one of them was separated from the other two by a road. Each plot was divided again into 3 sub-plots where 10 seedlings of black spruce (*Picea mariana* (Mill.) BSP), white spruce (*Picea glauca* (Moench) Voss), jack pine (*Pinus banksiana* Lamb.), and balsam fir (*Abies balsamea* (L.) Mill.) were planted in June 2014 in two contiguous rows of five seedlings for a total of 8 rows and 120 seedlings per plot. In total, the experiment included 18 plots and 2160 seedlings.

Each plot corresponded to a soil irrigation treatment. The six treatments were arranged randomly within each block and consisted in a gradient of four levels of altered precipitation quantity distributed through a soil irrigation system (40 %, 60 %, 80 % and 150 % of received precipitation) and two controls (positive: 100 +% and negative: 100-%). All treatments except treatments 150 % and 100-% were covered with a transparent polyethylene rainout shelter of 37 m^2 (7.6 m × 4.9 m, and 0.15 mm thick) (Harnois Industries, Saint-Thomas-de-Joliette, Canada) maintained at 2–2.5 m above the ground using a galvanized steel structure. Rainout shelters had minimal impacts on light conditions as the material allowed transmission of 88–91 % of photosynthetically active radiation, according to the manufacturer's specifications. The 150 % treatment corresponded to plots without rainout shelters receiving additional water volume corresponding to precipitation intercepted by 50 % of the surface of another rainout shelter. The controls 100 + % and 100-% represented plots receiving the totality of rainfall (or equivalent water volume through soil irrigation) with and without the presence of rainout shelters, respectively.

Rain used for the soil irrigation treatments was collected by a rain interceptor system (Fig. 1) located near the experimental set up. This system consisted of tarps catching rain and redirecting it in plastic barrels through gutters. The collected rain was then transferred to plots with perforated pipes.

Soil irrigation treatments started in August 2014 and stopped in October 2014 and were then maintained only during the snow-free period from June to September/October in 2015, 2016 and 2017. Rainout shelters were also only installed during the snow-free period, when soil irrigation treatments were performed.

Soil water content (%) was measured during the growing season (June to September) using a FieldScout TDR-300 soil moisture meter of

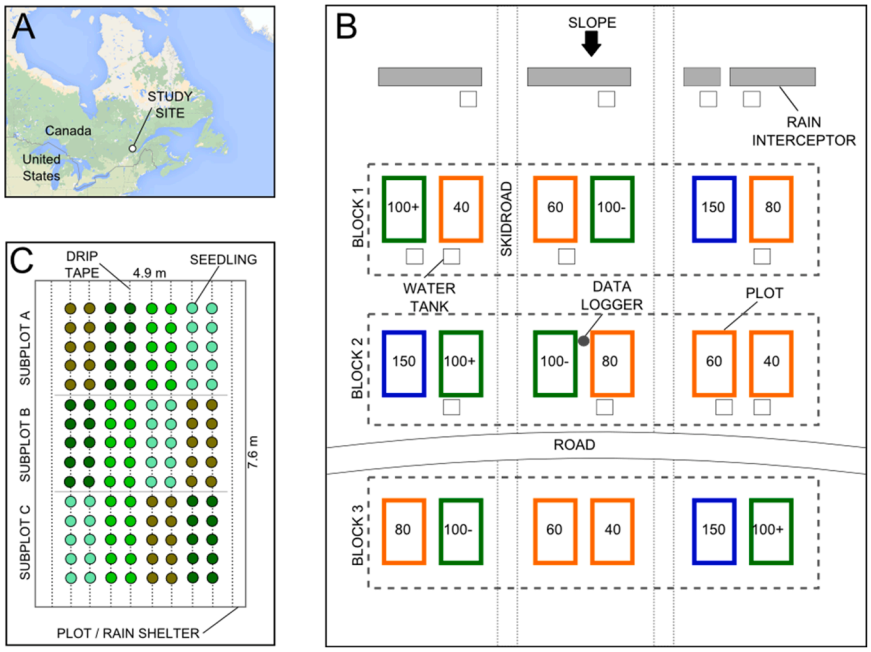


Fig. 1. (A) Study site location in the province of Quebec, Canada. (B) Experimental design showing blocks (dashed lines) and plots (colour lines) set up. Values in each plot indicate the soil irrigation treatment applied, which corresponds to a percentage of received precipitation volume. Treatments 100 + and 100- represent negative and positive controls receiving the totality of precipitation volume with and without shelter, respectively. Colours correspond to treatment type (orange for reduced precipitation, blue for excess precipitation and green for controls). (C) Example of seedlings distribution in each plot. Colours correspond to a tree species.

standard calibration with a 20-cm rod (accuracy ± 3 % vol; Spectrum Technologies Inc., Plainfield, USA). Systematic measurements along rows of seedlings were repeated four to seven times during the summer, before and during treatments.

2.3. Sample collection and DNA sequencing

At the end of September 2017, we randomly selected a total of 200 seedlings (i.e., 50 seedlings from each tree species) across all the experimental plots. For each seedling, we collected and mixed branches from 3 distinct locations (top-, mid- and bottom-phyllosphere) in order to control for spatial variation in bacterial community structure. We used previously described protocols to collect, amplify and quantify epiphytic bacteria living on leaves (Kembel et al., 2014). Briefly, for each sample, we collected microbial communities from the surface of approximately 50 g of needles with a five minute agitation wash in 100 mL of diluted Redford buffer solution (1 M Tris-HCl, 0.5 M Na EDTA, and 1.2 % CTAB) (Kadivar and Stapleton, 2003). We removed plant tissues from the buffer solution and centrifuged the samples at 4000 rpm for 20 min at 4°C to form a pellet. We then removed the supernatant and resuspended the pellet in 500 µL of PowerSoil bead solution (MoBio PowerSoil DNA Isolation kit, Carlsbad, CA, USA). We extracted DNA using the PowerSoil kit according to the manufacturer's instructions with the exception that the samples were vortexed for 15 min instead of 10. We amplified and barcoded the samples using a one-step PCR approach to prepare them for Illumina sequencing following a protocol adapted from Fadrosh et al. (2014). We used primers which target the V5-V6 region of the bacterial 16S rRNA gene (799 F and 1115 R; Redford et al., 2010). These primers exclude cyanobacteria which avoids amplification of plant chloroplast DNA. We performed the PCR using 25 µl reactions prepared with 1 µl genomic DNA, 5 µl 5x HF buffer (Thermo Scientific), 0.5 µl dNTP's (10 µM each), 0.5 µl forward and reverse primer (10 µM each), 0.75 µl DMSO, 0.25 µl Phusion HotStart II polymerase (Thermo Scientific), and 16.5 µl molecular-grade water. Each reaction began with 30 seconds of denaturation at 98°C, followed by 35 cycles of: 15 s at 98°C, 30 s at 64°C, 30 s at 72°C, and a final elongation step at 72°C for 10 minutes. We included a positive control and a negative control in each PCR run, that were verified using gel electrophoresis on an agarose gel prior to sequencing. We used a SequalPrep Kit (Invitrogen) to clean and normalize PCR products following manufacturer's instructions. We then purified normalized products using Agencourt AMPure XP beads (New England Biolabs) according to the manufacturer's recommendations. We prepared multiplexed 16S libraries by mixing equimolar concentrations of DNA and subsequently sequenced the DNA library using Illumina MiSeq platform (Claesson et al., 2010). We included all negative controls in the sequencing run to ensure the absence of contamination by confirming that they yielded no sequences.

2.4. Bioinformatics

We demultiplexed raw DNA sequence reads into separate files using QIIME 1.9.1 software (Caporaso et al., 2010). We used the R package DADA2 version 1.8.0 (Callahan et al., 2016) to turn demultiplexed paired reads FASTQ files into quality checked, filtered, and trimmed sequences. We used default filtering parameters to filter and trim the reads. We removed the primers and truncated the forward reads at position 210 and the reverse reads at position 175 to remove low quality tails. Sequences that passed quality control filtering were dereplicated and subjected to the DADA2 algorithm to identify error-corrected unique sequences (amplicon sequence variants; ASVs). After merging paired-end reads and removing chimeric sequences, the ASVs were identified and annotated taxonomically using the SILVA rRNA database version 123 (Quast et al., 2013). Finally, chloroplast and eukaryote sequences were removed.

2.5. Statistical analyses

After the first year of the experiment, we found that the study site's slope and its bedrock structure influenced soil thickness and water holding capacity, creating a natural soil water gradient that was maintained despite the irrigation treatments. We thus used each block as a statistical unit and used the variation in soil water content as a predictive variable. More precisely, the soil irrigation treatments were grouped

as presence or absence of rainout shelters for subsequent analysis.

We performed all statistical analyses in R version 3.4.4 (http://www.R-project.org) and built all figures using the package *ggplot2* (Wickham, 2016). To analyse bacterial community composition for each rain exclusion treatment and tree species, taxa relative abundances were calculated and plotted using the package *vegan* (Oksanen et al., 2016).

Prior to bacterial alpha-diversity analysis, ASVs were rarefied to the minimum number of reads per sample using the *phyloseq* package (McMurdie and Holmes, 2013) to permit comparison of diversity among samples. Then, to estimate bacterial alpha-diversity, we used Shannon index calculated from ASVs relative abundances for each treatment and tree species. We tested the potential differences in Shannon index means between treatments with and without rain exclusion and tree species using ANOVA followed by Tukey's post hoc test. To evaluate the relationship between bacterial diversity and soil water content, we performed Pearson's correlations for each tree species.

We investigated relationships between bacterial community composition, soil water content, tree host species identity, and the presence of rainout shelters by conducting a permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) of Bray-Curtis dissimilarities among samples using the adonis function from the package *vegan*. Since we had an unbalanced design (71 seedlings without shelters vs. 129 seedlings with shelters) and because PERMANOVA is more robust to heterogeneity in balanced designs (Anderson and Walsh, 2013), we randomly selected 71 samples from the seedlings with shelters in order to balance our design for this analysis.

We performed a principal coordinates analysis (PCoA) ordination on Bray-Curtis dissimilarities with the package *vegan* to visualize patterns of bacterial community composition of the four tree species in presence or absence of rainout shelter.

Finally, for each tree species, we used the univariate DESeq2 method (Love et al., 2014) to identify ASVs that showed significant differential relative abundance in presence vs. in absence of rainout shelters and between samples collected in plots with soil water content < 35 % vs. > 35 %. This threshold of 35 % was determined as the soil water content percentage for which there was the overall highest number of significantly different ASVs.

3. Results

3.1. Sequence quality and rarefaction

After sequencing, we identified a total of 3423,807 sequences from 199 samples. After quality filtering, we obtained 2613,196 sequences and one sample was excluded from subsequent analyses due to insufficient sequence reads. The number of sequences per sample ranged from 2301 to 27,366. We rarefied each sample to the minimum sample count over all samples (i.e., 2301 sequences) (Fig. S3). In total, we used 455,598 sequences and 4867 ASVs from 198 samples for the analyses.

3.2. Taxonomic composition of epiphytic phyllosphere bacterial communities

Whether between host tree species or in the presence or absence of rainout shelters, we observed that the overall most abundant taxa belonged to the phyla *Proteobacteria* (*Alphaproteobacteria* and *Gammaproteobacteria* classes), *Acidobacteria*, and *Actinobacteria*. In absence of shelters, the genus *1174–901–12*, which belongs to the *Beijerinckiaceae*, had the highest relative abundance for all tree species and accounted for 25–35 % of the assigned taxa (Fig. 2). *Sphingomonas* had the second highest relative abundance for black spruce, white spruce, and balsam fir (~10–12 % of bacterial communities) while *Endobacter* had the second highest relative abundance for jack pine (~12 % of bacterial communities). The most abundant genera varied much more in presence of shelters. *Frondihabitans*, *Serratia*, *Erwinia*, and *Sphingomonas* had the highest relative abundances for white spruce, black spruce, jack pine and balsam fir respectively, while *1174–901–12* accounted for less than 10 % of bacterial communities of all tree species.

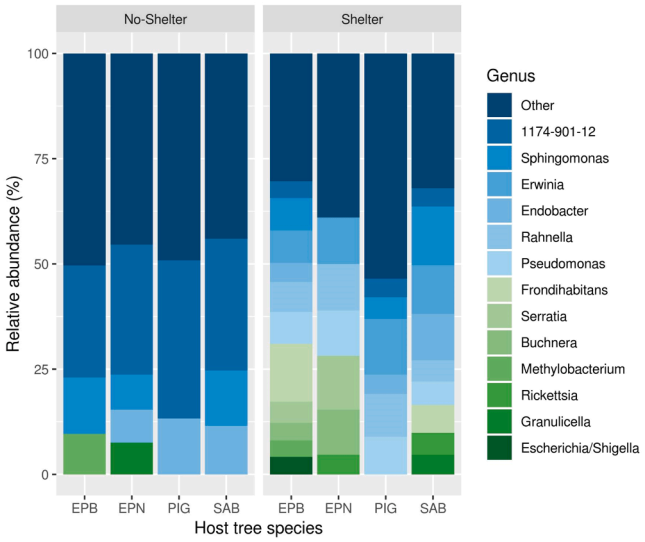


Fig. 2. Epiphytic bacterial community structure in the phyllosphere of white spruce (EPB), black spruce (EPN), jack pine (PIG), and balsam fir (SAB) trees grown in presence or absence of rainout shelters.

3.3. Effects of soil water content, tree species and rain exclusion on the diversity of epiphytic phyllosphere bacterial communities

We found a significant negative Pearson's correlation between the overall (i.e., considering all samples) epiphytic phyllosphere bacterial diversity and soil water content ($r = -0.145$; $p = 0.042$). Among the four host tree species, jack pine was the only one whose bacterial diversity responded significantly negatively to an increase in soil water content ($r = -0.28$, $p = 0.046$) (Fig. 3).

We also found that bacterial alpha-diversity was significantly higher in balsam fir samples compared to black spruce in absence of shelters ($p = 0.016$) (Fig. 4). Bacterial diversity was also significantly higher in

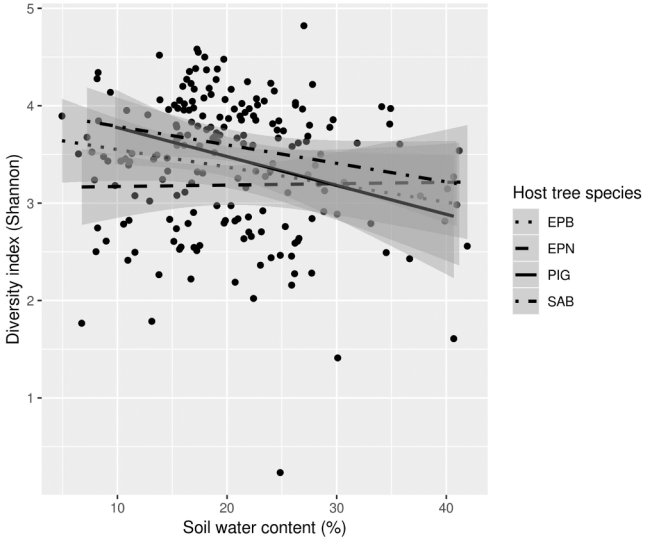


Fig. 3. Pearson's correlations between soil water content and phyllosphere bacterial diversity of white spruce (EPB; $r = -0.19$, $P = 0.190$), black spruce (EPN; $r = 0.02$, $P = 0.882$), jack pine (PIG; $r = -0.28$, $P = 0.046$), and balsam fir (SAB; $r = -0.24$, $P = 0.081$). Dots represent observations, lines represent linear regressions, and grey zones represent 95 % confidence intervals.

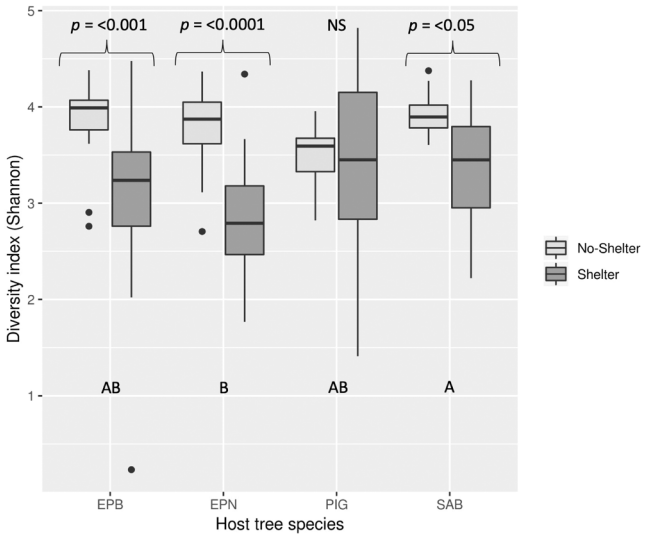


Fig. 4. Epiphytic bacterial diversity estimated with Shannon index in the phyllosphere of white spruce (EPB), black spruce (EPN), jack pine (PIG), and balsam fir (SAB) trees grown in presence ($n = 32$) or absence ($n = 17$) of rainout shelters. Capital letters represent ANOVAs significant differences between tree species and *p* indicate ANOVAs p-values between shelter treatments for each species.

absence of rainout shelters for all host tree species ($p = 6.7 \times 10^{-6}$ for black spruce; $p = 0.003$ for white spruce; $p = 0.032$ for balsam fir), except jack pine.

3.4. Epiphytic phyllosphere bacterial community response to soil water content, tree species, and rain exclusion

The epiphytic phyllosphere bacterial community structure responded significantly to soil water content, host tree species and the presence of rainout shelters (Table 1). Among these three variables, the presence of rainout shelters was the most important factor explaining variation in bacterial community structure ($R^2 = 13$ %), followed by host tree species ($R^2 = 7$ %) and soil water content ($R^2 = 1$ %). The interaction between the host tree species and the presence of rainout shelters, as well as the interaction between the host tree species and soil water content also slightly influenced epiphytic phyllosphere bacteria, explaining 4 % and 2 % respectively of the variation in bacterial community structure.

Supporting these results, the PCoA ordination revealed that phyllosphere bacterial communities differed in presence or absence of rainout shelters whereas the effect of tree species was less visible (Fig. 5).

3.5. Effects of rain exclusion and tree species on epiphytic phyllosphere bacterial communities at the ASV level

Using differential abundance analysis, we identified ASVs whose

Table 1
PERMANOVAs of Bray-Curtis dissimilarities on bacterial community structure explained by host tree species, the presence of rainout shelter, soil water content, and their interactions.

Model	df	R^2 (%)	P^a
Species	3	0.07343	0.001 *
Rainout shelter	1	0.13452	0.001 *
Soil water content	1	0.01112	0.013 *
Species * Rainout shelter	3	0.04242	0.001 *
Species * Soil water content	3	0.02173	0.042 *
Rainout shelter * Soil water content	1	0.00571	0.289 n.s.
Species * Rainout shelter * Soil water content	3	0.01443	0.732 n.s.

[a] *, $P < 0.05$; n.s., not significant

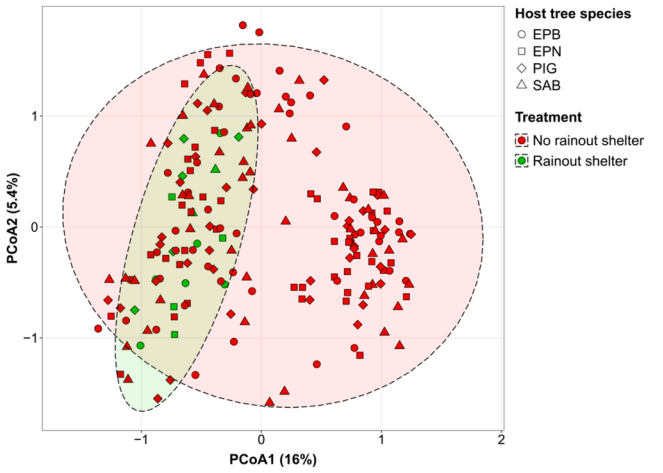


Fig. 5. Principal coordinates analysis (PCoA) ordination on Bray-Curtis dissimilarities showing structure variation in phyllosphere bacterial communities of white spruce (EPB), black spruce (EPN), jack pine (PIG), and balsam fir (SAB) trees grown in presence or absence of rainout shelters. The PCoA regroups 198 observations. Points represent observations and ellipses represent 95 % confidence intervals. Point shapes were assigned to host tree species and colors were assigned to rainout shelter conditions.

abundances differed significantly in the presence versus absence of rainout shelters (Table 2) and between tree species (Fig. S4). We found that bacterial communities in absence of shelters had more ASVs from genera *1174–901–12* (*Proteobacteria*), *Massilia* (*Proteobacteria*), *Terriglobus* (*Acidobacteria*), *Bdellovibrio* (*Proteobacteria*), *Jatrophihabitans* (*Actinobacteria*), *Caedibacter* (*Proteobacteria*), *Novosphingobium* (*Proteobacteria*), *Acidicaldus* (*Proteobacteria*), *Bryocella* (*Acidobacteria*), *Rhizobacter* (*Proteobacteria*), *Mucilaginibacter* (*Bacteroidetes*), *Rhodovastum* (*Proteobacteria*) compared to epiphytic phyllosphere bacterial communities in presence of rainout shelters. On the other hand, epiphytic

Table 2
ASVs relative abundance differential analysis between rainout shelter conditions (presence vs. absence). Only ASVs whose abundances differed significantly between treatments ($P \leq 0.05$) are shown.

No Shelter		Shelter	
ASVs associated taxa (Genus level)	Relative Abundance of ASVs (%)	ASVs associated taxa (Genus level)	Relative Abundance of ASVs (%)
1174–901–12	13.88	Sphingomonas	6.98
Granulicella	11.43	Acidothermus	6.05
Sphingomonas	8.16	Rickettsia	5.58
Acidiphilium	5.31	Granulicella	5.12
Massilia	4.08	Pseudomonas	5.12
Terriglobus	3.67	Acidiphilium	2.79
Bdellovibrio	3.27	Clostridium_sensu_stricto_1	2.79
Endobacter	3.27	Acidisoma	2.33
Jatrophihabitans	3.27	Erwinia	2.33
Caedibacter	2.86	Frigoribacterium	2.33
Novosphingobium	2.86	Aureimonas	1.86
Methylobacterium	2.45	Bacillus	1.86
Methylocella	2.45	Burkholderia-Caballeronia-Paraburkholderia	1.86
Acidicaldus	2.04	Endobacter	1.86
Bryocella	2.04	Enterococcus	1.86
Pandoraea	2.04	Methylobacterium	1.86
Rhizobacter	2.04	Pandoraea	1.86
Rickettsia	2.04	Conexibacter	1.40
Acidisoma	1.63	Methylocella	1.40
Mucilaginibacter	1.63	Pantoea	1.40
Pseudomonas	1.22	Serratia	1.40
Rhodovastum	1.22	Shewanella	1.40
Others (< 1 %)	17.14	Wolbachia	1.40
		Others (< 1 %)	37.21

phyllosphere bacterial communities in presence of rainout shelters had more ASVs from genera *Acidothermus*, *Clostridium*, *Erwinia*, *Frigoribacterium*, *Aureimonas*, *Bacillus*, *Burkholderia-Caballeronia-Paraburkholderia*, *Enterococcus*, *Conexibacter*, *Pantoea*, *Serratia*, *Shewanella* and *Wolbachia* compared to bacterial communities without shelters.

Soil water content had much less impacts on ASVs differential abundance compared to the presence of rainout shelters. Between soils with water content < 35 % and > 35 %, there were only a few different ASVs abundances, and there was even no difference detected for white spruce (Fig. S4). All the ASVs whose abundance differed were more abundant for soil water content < 35 %. Black spruce was the tree species with the highest number of ASVs abundance differences. For black spruce, jack pine and balsam fir, most of these ASVs belonged to the phyla *Proteobacteria* and *1174–901–12* was the only genus that displayed differential ASVs abundance for all three tree species.

4. Discussion

The aim of the present study was to explore the epiphytic phyllosphere bacterial community response to rain exclusion and variation in soil water content in four coniferous tree species of the eastern Canadian boreal forest. We hypothesized that decreasing soil water content would induce changes in the composition of epiphytic phyllosphere bacterial communities and lower their diversity, and that microbial communities would differ among host tree species. We also investigated a potential effect of rain exclusion on epiphytic phyllosphere bacterial communities since rainout shelters have been criticized for creating confounding effects on the plant microenvironment (Fay et al., 2000; English et al., 2005; Vogel et al., 2013).

While we were expecting a lower bacterial diversity associated with lower soil water content, we found that soil water content only had weak effects on epiphytic phyllosphere bacterial community structure (Table 1) and diversity (Fig. 3), which partly contradicted our first hypothesis, but was in accordance with other studies reporting small or no effects of drought on soil (Homyak et al., 2017; Cole et al., 2019; Hammerl et al., 2019) and phyllosphere bacterial communities (Lin et al., 2023; Hoefle et al., 2024). This suggests that phyllosphere microbes are not significantly impacted by soil water availability, potentially because there is no direct contact between soil water and foliage bacteria.

As expected, we found significant variation in phyllosphere bacterial community structure depending on host tree species identity (Fig. 2), as well as a difference of bacterial diversity between black spruce and balsam fir (Fig. 4), which is in agreement with our second hypothesis and other studies (Kembel et al., 2014; Laforest-Lapointe et al., 2016). This reinforces the idea that host tree species identity is an important driver of phyllosphere bacterial communities' structure. We also observed that the effects of soil water content differed among species. In contrast with our hypothesis, jack pine bacterial diversity significantly increased as soil water content decreased. Since jack pine usually grows in dry soils, the higher soil water contents measured in our study are unusual for this species, which may explain this observation if the elevated water levels lead to changes in the ecophysiology of jack pine hosts. The effects of rainout shelter also differed among tree species. We observed differences in epiphytic phyllosphere bacterial community structure with and without rainout shelters (Fig. 2), as well as a higher bacterial diversity in absence of rainout shelters (Fig. 4). However, we found that, unlike other tree species, jack pine bacterial diversity did not vary in presence of rainout shelter. This could suggest that jack pine phyllosphere bacterial communities could be more sensitive to the internal physiological state of the host tree species (here, sensitivity to soil water content potentially affecting root nutrient acquisition) than to external environmental factors.

Among all the studied variables (soil water content, host tree species identity and rain exclusion), we found that rain exclusion was the variable explaining the most variation in phyllosphere bacterial communities (Table 1). This effect may have several causes since phyllosphere bacterial communities have been shown to be sensitive to various environmental factors (Kadivar and Stapleton, 2003; Lindow and Brandl, 2003; Beattie, 2011; Gomes et al., 2018; Haas et al., 2018). However, lower solar radiation and higher temperature under the shelters are unlikely to be involved here, since the materials used in this study allowed transmission of 88–91 % of photosynthetically active radiation (manufacturer's specifications) while the structure insured good air circulation. Moreover, a recent study on soil microbial communities (which reported a high resilience of soil microbes to drought) showed that unanticipated artefacts of rain shelters were unlikely to have impacted their results (Cole et al., 2019). Another important potential impact of rainout shelters on phyllosphere bacteria is the fact that they prevent contact between rain and needle's surface, leading to changes in the likelihood of colonization by bacterial taxa that are dispersed via rain, as well as changes in the leaf/needle surface microclimate. Rainwater could represent an additional source of bacterial inoculation of tree needles, as well as a source of liquid water that could activate some dormant bacteria. Moreover, rain is also a source of nutrients directly available for phyllosphere microbes. In this N-limited ecosystem, canopy uptake represents an important sink for atmospheric N deposition and contributes significantly to tree nutrition, as it was demonstrated by previous work at the study site (Houle et al., 2015). Bacterial community composition was more diverse for trees exposed directly to rain versus those growing under rainout shelters, further suggesting that exposure to rainwater leads to more stochastic colonization of the phyllosphere by microbes arriving from the environment. Interestingly, many of the phyllosphere bacteria found in presence of rainout shelters are taxa that are commonly associated with human and animal hosts, including *Clostridium, Enterococcus, Pantoea, Shewanella,* and *Wolbachia*. To explain this, we suggest that the lack of direct contact between rain and needles in presence of rainout shelters could lead to decrease needles' colonization by rainborne bacteria and ultimately increase the relative abundance of animal-associated bacterial taxa (e.g., from wildlife or people who carried out maintenance and measurements at the study site) that are dispersing from the atmosphere. As the frequency of rain events is predicted to decrease during summer in the boreal forest, boreal tree phyllosphere bacterial communities' composition and diversity could change in the future. A decline of phyllosphere bacterial diversity caused by lower water availability, as demonstrated in this study, could impact microbial beneficial services towards their host plants, such as growth promotion or protection against pathogens. This may reduce overall tree growth and increase tree disease outbreaks, ultimately affecting forestry activities and management in boreal ecosystems.

Further work is needed to assess if these changes would also imply bacterial activity modifications and ultimately impact boreal forest trees' physiology and health.

Overall, our results highlight that the use of rainout shelters for studies of drought or water limitation effects on plants and their associated microbes may have important unintended effects due as shelters can affect the leaf-surface microclimate and colonization by microbes. We suggest that future studies investigating drought/water availability impacts on plants and their associated microbes should evaluate the effects of different irrigation and water-exclusion methods and should be designed to ensure that rainout shelter effects *per se* are not incorrectly attributed to drought/water limitation. Such studies should also determine whether these impacts are mediated by effects on microbial colonization dynamics versus direct effects on leaf surface microclimate or host tree ecophysiology.

5. Conclusion

In summary, contrary to our expectations, we found that soil water content only weakly influenced epiphytic phyllosphere bacterial communities of coniferous trees. On the other hand, our results showed that

epiphytic phyllosphere bacteria were more sensitive to host tree species identity and rain exclusion. The strong effect of rain exclusion on phyllosphere bacterial structure and diversity highlights the importance of considering the direct impacts of rainout shelters when conducting drought or water limitation experiments. Overall, our results suggest that a diminution of rain events predicted by climate change models could lead to changes in phyllosphere bacterial biodiversity of boreal trees. Future studies will be needed to forecast the exact impacts of drought or rain reduction on phyllosphere microbes' activity and tree health in boreal forest ecosystems.

CRediT authorship contribution statement

Khlifa Rim: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Renaudin Marie:** Writing – review & editing, Visualization, Validation, Software, Formal analysis, Data curation. **Houle Daniel:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **D'Orangeville Loïc:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Duchesne Louis:** Writing – review & editing, Resources, Methodology, Investigation, Data curation, Conceptualization. **Kembel Steven W.:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of Competing Interest

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

Acknowledgements

This research was supported by the Ministère des Ressources naturelles et des Forêts du Québec and by the Climate Change and Atmospheric Pollutants program of Environment and Climate Change Canada, Science and Technology branch. We would like to thank E. Brazeau Goupil, P. Desjardins, J. Gagné, J.-P. Mottard, and M. St-Germain for field assistance.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2025.122554.

Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2025.122554.

Data availability

Data will be made available on request.

References

Allard, S.M., Ottesen, A.R., Micallef, S.A., 2020. Rain induces temporary shifts in epiphytic bacterial communities of cucumber and tomato fruit. Sci. Rep. 10 (1), 1765.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.

Anderson, M.J., Walsh, D.C.I., 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecol. Monogr. 83, 557–574. https://doi.org/10.1890/12-2010.1.

Angelini, C., Griffin, J.N., van de Koppel, J., Lamers, L.P.M., Smolders, A.J.P., Derksen-Hooijberg, M., van der Heide, T., Silliman, B.R., 2016. A keystone mutualism underpins resilience of a coastal ecosystem to drought. Nat. Commun. 7. https://doi.org/10.1038/ncomms12473.

Augé, R.M., Toler, H.D., Saxton, A.M., 2015. Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza 25, 13–24. https://doi.org/10.1007/s00572-014-0585-4.

Beattie, G.A., 2011. Water Relations in the Interaction of Foliar Bacterial Pathogens with Plants. Annu. Rev. Phytopathol. 49, 533–555. https://doi.org/10.1146/annurev-phyto-073009-114436.

Bechtold, E.K., Ryan, S., Moughan, S.E., Ranjan, R., Nüsslein, K., 2021. Phyllosphere community assembly and response to drought stress on common tropical and temperate forage grasses. Appl. Environ. Microbiol. 87 (17), e00895-21.

Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. Trends Plant Sci. 17, 478–486. https://doi.org/10.1016/j.tplants.2012.04.001.

Berg, G., 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biotechnol. 84, 11–18. https://doi.org/10.1007/s00253-009-2092-7.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336. https://doi.org/10.1038/nmeth.f.303.

Castaño, C., Lindahl, B.D., Alday, J.G., Hagenbo, A., Martínez de Aragón, J., Parladé, J., Pera, J., Bonet, J.A., 2018. Soil microclimate changes affect soil fungal communities in a Mediterranean pine forest. N. Phytol. 220, 1211–1221. https://doi.org/10.1111/nph.15205.

Chodak, M., Gołębiewski, M., Morawska-Płoskonka, J., Kuduk, K., Niklińska, M., 2015. Soil chemical properties affect the reaction of forest soil bacteria to drought and rewetting stress. Ann. Microbiol. 65, 1627–1637.

Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogée, J., Allard, V., Aubinet, M., Buchmann, N., Bernhofer, Chr, Carrara, A., Chevallier, F., De Noblet, N., Friend, A. D., Friedlingstein, P., Grünwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J.M., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J.F., Sanz, M.J., Schulze, E.D., Vesala, T., Valentini, R., 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437, 529–533. https://doi.org/10.1038/nature03972.

Claesson, M.J., Wang, Q., O'Sullivan, O., Greene-Diniz, R., Cole, J.R., Ross, R.P., O'Toole, P.W., 2010. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. e200–e200 Nucleic Acids Res. 38. https://doi.org/10.1093/nar/gkq873.

Cole, A.J., Griffiths, R.I., Ward, S.E., Whitaker, J., Ostle, N.J., Bardgett, R.D., 2019. Grassland biodiversity restoration increases resistance of carbon fluxes to drought. J. Appl. Ecol., 1365-2664.13402 https://doi.org/10.1111/1365-2664.13402.

Copeland, J.K., Yuan, L., Layeghifard, M., Wang, P.W., Guttman, D.S., 2015. Seasonal community succession of the phyllosphere microbiome. Mol. Plant-Microbe Interact. 28 (3), 274–285.

Deng, L., Peng, C., Kim, D.-G., Li, J., Liu, Y., Hai, X., Liu, Q., Huang, C., Shangguan, Z., Kuzyakov, Y., 2021. Drought effects on soil carbon and nitrogen dynamics in global natural ecosystems. Earth-Sci. Rev. 214, 103501. https://doi.org/10.1016/j.earscirev.2020.103501.

English, N.B., Weltzin, J.F., Fravolini, A., Thomas, L., Williams, D.G., 2005. The influence of soil texture and vegetation on soil moisture under rainout shelters in a semi-desert grassland. J. Arid Environ. 63, 324–343. https://doi.org/10.1016/j.jaridenv.2005.03.013.

Evelin, H., Kapoor, R., Giri, B., 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann. Bot. 104, 1263–1280. https://doi.org/10.1093/aob/mcp251.

Fadrosh, D.W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R.M., Ravel, J., 2014. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome 2, 1–7.

Fay, P.A., Carlisle, J.D., Knapp, A.K., Blair, J.M., Collins, S.L., 2000. Altering Rainfall Timing and Quantity in a Mesic Grassland Ecosystem: Design and Performance of Rainfall Manipulation Shelters. Ecosystems 3, 308–319.

Glick, B.R., Cheng, Z., Czarny, J., Duan, J., 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur. J. Plant Pathol. 119, 329–339. https://doi.org/10.1007/s10658-007-9162-4.

Gomes, T., Pereira, J.A., Benhadi, J., Lino-Neto, T., Baptista, P., 2018. Endophytic and Epiphytic Phyllosphere Fungal Communities Are Shaped by Different Environmental Factors in a Mediterranean Ecosystem. Microb. Ecol. https://doi.org/10.1007/s00248-018-1161-9.

Haas, J.C., Street, N.R., Sjödin, A., Lee, N.M., Högberg, M.N., Näsholm, T., Hurry, V., 2018. Microbial community response to growing season and plant nutrient optimisation in a boreal Norway spruce forest. Soil Biol. Biochem. 125, 197–209. https://doi.org/10.1016/j.soilbio.2018.07.005.

Hammerl, V., Kastl, E.-M., Schloter, M., Kublik, S., Schmidt, H., Welzl, G., Jentsch, A., Beierkuhnlein, C., Gschwendtner, S., 2019. Influence of rewetting on microbial communities involved in nitrification and denitrification in a grassland soil after a

prolonged drought period. Sci. Rep. 9, 2280. https://doi.org/10.1038/s41598-018-38147-5.
Hoefle, D., Sommer, M., Wassermann, B., Faticov, M., Serra, D., Berg, G., Tack, A.J.M., Abdelfattah, A., 2024. Oak seedling microbiome assembly under climate warming and drought. Environ. Micro 19, 62. https://doi.org/10.1186/s40793-024-00602-4.
Homyak, P.M., Allison, S.D., Huxman, T.E., Goulden, M.L., Treseder, K.K., 2017. Effects of Drought Manipulation on Soil Nitrogen Cycling: A Meta-Analysis. J. Geophys. Res.: Biogeosciences 122, 3260–3272. https://doi.org/10.1002/2017JG004146.
Houle, D., Bouffard, A., Duchesne, L., Logan, T., Harvey, R., 2012. Projections of Future Soil Temperature and Water Content for Three Southern Quebec Forested Sites. J. Clim. 25, 7690–7701. https://doi.org/10.1175/JCLI-D-11-00440.1.
Houle, D., Marty, C., Duchesne, L., 2015. Response of canopy nitrogen uptake to a rapid decrease in bulk nitrate deposition in two eastern Canadian boreal forests. Oecologia 177, 29–37. https://doi.org/10.1007/s00442-014-3118-0.
Hueso, S., García, C., Hernández, T., 2012. Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. Soil Biol. Biochem. 50, 167–173. https://doi.org/10.1016/j.soilbio.2012.03.026.
Kadivar, H., Stapleton, A.E., 2003. Ultraviolet Radiation Alters Maize Phyllosphere Bacterial Diversity. Microb. Ecol. 45, 353–361. https://doi.org/10.1007/s00248-002-1065-5.
Kembel, S.W., O'Connor, T.K., Arnold, H.K., Hubbell, S.P., Wright, S.J., Green, J.L., 2014. Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. Proc. Natl. Acad. Sci. 111, 13715–13720. https://doi.org/10.1073/pnas.1216057111.
Laforest-Lapointe, I., Messier, C., Kembel, S.W., 2016. Tree phyllosphere bacterial communities: exploring the magnitude of intra- and inter-individual variation among host species. PeerJ 4, e2367. https://doi.org/10.7717/peerj.2367.
Laforest-Lapointe, I., Paquette, A., Messier, C., Kembel, S.W., 2017. Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. Nat. Publ. Group 546, 145–147. https://doi.org/10.1038/nature22399.
Lau, J.A., Lennon, J.T., 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. Proc. Natl. Acad. Sci. 109, 14058–14062. https://doi.org/10.1073/pnas.1202319109.
Lee, H., Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P., et al., 2023. IPCC, 2023: Climate Change 2023: Synthesis Report, Summary for Policymakers. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland.
Lesk, C., Rowhani, P., Ramankutty, N., 2016. Influence of extreme weather disasters on global crop production. Nature 529, 84–87. https://doi.org/10.1038/nature16467.
Li, P., Sun, Y., Hou, X., 2024. Drought stress impacts soil microbial nutrient limitation more strongly than O_3 pollution. Appl. Soil Ecol. 201, 105496. https://doi.org/10.1016/j.apsoil.2024.105496.
Lin, T., Tang, J., Li, Shuying, Li, Shujiang, Han, S., Liu, Y., Yang, C., Chen, G., Chen, L., Zhu, T., 2023. Drought stress-mediated differences in phyllosphere microbiome and associated pathogen resistance between male and female poplars. Plant J. 115, 1100–1113. https://doi.org/10.1111/tpj.16283.
Lindow, S.E., Brandl, M.T., 2003. Microbiology of the Phyllosphere. Appl. Environ. Microbiol. 69, 1875–1883. https://doi.org/10.1128/AEM.69.4.1875-1883.2003.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550. https://doi.org/10.1186/s13059-014-0550-8.
Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930–938. https://doi.org/10.1890/11-0026.1.
Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S., Abou-Hadid, A.F., El-Behairy, U.A., Sorlini, C., Cherif, A., Zocchi, G., Daffonchio, D., 2012. A Drought Resistance-Promoting Microbiome Is Selected by Root System under Desert Farming. PLoS ONE 7, e48479. https://doi.org/10.1371/journal.pone.0048479.
McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE 8, e61217. https://doi.org/10.1371/journal.pone.0061217.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H., 2016. vegan: Community Ecol. Package.
Ortiz, N., Armada, E., Duque, E., Roldán, A., Azcón, R., 2015. Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: Effectiveness of autochthonous or allochthonous strains. J. Plant Physiol. 174, 87–96. https://doi.org/10.1016/j.jplph.2014.08.019.
Pan, Y., Birdsey, R.A., Phillips, O.L., Houghton, R.A., Fang, J., Kauppi, P.E., Keith, H., Kurz, W.A., Ito, A., Lewis, S.L., Nabuurs, G.-J., Shvidenko, A., Hashimoto, S., Lerink, B., Schepaschenko, D., Castanho, A., Murdiyarso, D., 2024. The enduring world forest carbon sink. Nature 631, 563–569. https://doi.org/10.1038/s41586-024-07602-x.
Patel, K.F., Fansler, S.J., Campbell, T.P., Bond-Lamberty, B., Smith, A.P., RoyChowdhury, T., McCue, L.A., Varga, T., Bailey, V.L., 2021. Soil texture and environmental conditions influence the biogeochemical responses of soils to drought and flooding. Commun. Earth Environ. 2, 127. https://doi.org/10.1038/s43247-021-00198-4.
Preece, C., Verbruggen, E., Liu, L., Weedon, J.T., Peñuelas, J., 2019. Effects of past and current drought on the composition and diversity of soil microbial communities. Soil Biol. Biochem. 131, 28–39. https://doi.org/10.1016/j.soilbio.2018.12.022.
Pringle, E.G., Akçay, E., Raab, T.K., Dirzo, R., Gordon, D.M., 2013. Water Stress Strengthens Mutualism Among Ants, Trees, and Scale Insects. PLoS Biol. 11, e1001705. https://doi.org/10.1371/journal.pbio.1001705.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, 590–596.
Redford, A.J., Bowers, R.M., Knight, R., Linhart, Y., Fierer, N., 2010. The ecology of the phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree leaves. Environ. Microbiol. 12, 2885–2893. https://doi.org/10.1111/j.1462-2920.2010.02258.x.
Rico, L., Ogaya, R., Terradas, J., Peñuelas, J., 2014. Community structures of N_2-fixing bacteria associated with the phyllosphere of a Holm oak forest and their response to drought. Plant Biol. 16 (3), 586–593.
Romanowicz, K.J., Freedman, Z.B., Upchurch, R.A., Argiroff, W.A., Zak, D.R., 2016. Active microorganisms in forest soils differ from the total community yet are shaped by the same environmental factors: the influence of pH and soil moisture. FEMS Microbiol. Ecol. 92 (10), fiw149.
Ruiz-Lozano, J.M., Azcon, R., Gomez, M., 1995. Effects of Arbuscular-Mycorrhizal Glomus Species on Drought Tolerance: Physiological and Nutritional Plant Responses. Appl. Environ. Microbiol. 61, 5.
Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial Stress-Response Physiology and Its Implications for Ecosystem Function. Ecology 88, 1386–1394.
Schjønning, P., Thomsen, I.K., Moldrup, P., Christensen, B.T., 2003. Linking Soil Microbial Activity to Water- and Air-Phase Contents and Diffusivities. Soil Sci. Soc. Am. J. 67, 156–165.
Sherwood, S., Fu, Q., 2014. A Drier Future? Science 343, 737–739. https://doi.org/10.1126/science.1247620.
Soil Classification Working Group, 1998. The Canadian System of Soil Classification, 3rd ed., 1646. Agriculture and Agri-Food Canada Publication, Ottawa, ON, Canada, p. 187.
Tang, G., Xu, L., Wang, X., Zhang, J., 2023. Effects of leaf morphological and chemical properties on the population sizes of epiphytes. Microb. Ecol. 85 (1), 157–167.
Truchado, P., Gil, M.I., Suslow, T., Allende, A., 2018. Impact of chlorine dioxide disinfection of irrigation water on the epiphytic bacterial community of baby spinach and underlying soil. PLoS One 13 (7), e0199291.
Vogel, A., Fester, T., Eisenhauer, N., Scherer-Lorenzen, M., Schmid, B., Weisser, W.W., Weigelt, A., 2013. Separating Drought Effects from Roof Artifacts on Ecosystem Processes in a Grassland Drought Experiment. PLoS ONE 8, e70997. https://doi.org/10.1371/journal.pone.0070997.
Vorholt, J.A., 2012. Microbial life in the phyllosphere. Nat. Rev. Microbiol. 10, 828–840. https://doi.org/10.1038/nrmicro2910.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.
Zheng, M., Wang, W., Hayes, M., Nydell, A., Tarr, M.A., Van Bael, S.A., Papadopoulos, K., 2018. Degradation of Macondo 252 oil by endophytic *Pseudomonas putida*. J. Environ. Chem. Eng. 6, 643–648. https://doi.org/10.1016/j.jece.2017.12.071.