

# Impact of plant species and de-icing salt on microbial and fungal communities in cold climate bioretention

## Effets des espèces végétales et du sel de déglacage sur les communautés bactériennes et fongiques dans les biorétentions en climat froid

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### RÉSUMÉ

Les microorganismes (bactéries et champignons) jouent un rôle important dans la performance des biorétentions (BR) pour la gestion des eaux pluviales. Les bénéfices apportés par les microorganismes du sol et leur activité varient selon la composition de la communauté microbienne, la diversité et l'abondance des taxons présents. Ces caractéristiques dépendent de l'environnement biotique et abiotique du sol. Ainsi, les BR en climat froid sont confrontées à des conditions particulières telles que les basses températures et l'utilisation de sels de déglacage. Les espèces végétales présentes peuvent aussi avoir un impact. Ce projet se concentre sur l'effet de ces deux derniers facteurs sur la diversité ainsi que sur l'abondance observée (nombre de séquences lues) des classes de bactéries et de champignons dans les biorétentions en climat froid. Une expérience en mésocosmes sur 4 espèces végétales utilisées en BR et sur des contrôles non plantés a été réalisée suivie d'un test de salinité. Une analyse d'ADN a été effectuée sur le microbiome des échantillons de substrat prélevés avant expérience, après deux ans en serre et après le test de salinité. Les espèces végétales ont eu un effet sur la diversité ou l'abondance observée des communautés bactériennes et fongiques (nombre de séquences lues) contrairement à la salinité.

### ABSTRACT

Microorganisms (bacteria and fungi) play an important role in the performance of bioretention cells (BR) in managing stormwater. Activity of soil microorganisms varies with the composition of the microbial community, and diversity and abundance of taxa present. These characteristics depend on the biotic and abiotic soil environment. As such, BR in cold climates face particular abiotic conditions, such as low temperature and the use of de-icing salt. Plant species, a biotic factor, may also have an impact. This project focuses on the effect of these last two factors on the diversity and abundance of bacteria and fungi at the class level in BR in cold climates. A mesocosm scale experiment including a salinity test was conducted with four plant species used in BR and unplanted controls. A DNA analysis was performed on the microbiome of the substrate samples taken before experiment, after two years in the greenhouse and after the salinity test. Plant species influenced the diversity or abundance (number of reads) of the bacterial and fungi communities present, but salt did not.

### KEYWORDS

Bacterial diversity, Bioretention, Fungal diversity, Green Infrastructures, Microbiota, Plant species, Salinity

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## 1 INTRODUCTION

Bioretention cells (BR), which are systems composed of plants, soil and microorganisms, have demonstrated performance in managing stormwater, both in terms of hydraulics and water quality (Kratky et al., 2017). The microbial community plays an important role in BR performance. Bacteria are the main drivers of nitrogen removal, hydrocarbon degradation, and metal sequestration (Fevre et al., 2012; Mehmood et al., 2021). Fungi can contribute to phosphorus filtration directly, or indirectly by improving plant or bacterial contribution (Poor et al., 2018). Indirectly, mycorrhiza can also increase plant tolerance to stresses, such as drought, contaminants, cold temperatures or salinity (Latef et al., 2016). Microbial activities and benefits vary with the composition of the microbial community, as well as the diversity and abundance of the taxa present. These characteristics depend on the biotic and abiotic soil environment (Waller et al., 2018; Zuo et al., 2019). As such, BR in cold climates face particular abiotic conditions, such as low temperatures and use of de-icing salt. The composition of the microbial community could also vary depending on the plant species present. Therefore, the choice of plant species or the effect of salt could have implications for the processes performed by microorganisms and consequently on the performance of the BR itself. This project focuses on the effect of these two factors on the diversity and abundance of bacteria and fungi in BR mesocosms in cold climatic conditions.

## 2 MATERIALS AND METHODS

### 2.1 Site conditions and experiments

The mesocosm-scale experiment on species contribution to BR performance has been published in Beral et al. (2023). It was carried out at the Montreal Botanical Garden and lasted 2 years: one acclimatization year during which mesocosms were fed rainwater, and one during which they received semi-synthetic run-off. Four species (*Cornus sericea* (CS), *Iris versicolor* (IV), *Juncus effusus* (JE), *Sesleria autumnalis* (SA)) and unplanted controls were included in this study. The substrate was a commercial sandy loam (6% organic matter) BR media (NATUREAUSOL®) topped with 3 cm of ramial chipped wood as a mulch layer. Mycorrhizae (MYKE® PRO Landscape) were added to the substrate at the time of planting. The salinity experiment was performed subsequently on the same mesocosms. De-icing salt (NaCl) was added to the semi-synthetic run-off on four occasions, from March 12 to March 23, 2020, to simulate spring salt flushes. Each mesocosm received one of four NaCl concentrations as 0 mgCl/L (control), 250 mgCl/L, 1000 mgCl/l or 4000 mgCl/L. After the flushes, the mesocosms were irrigated with regular semi-synthetic runoff without de-icing salt until the end of the experiment (August 25, 2020).

### 2.2 Sampling

An initial substrate sample was collected from 3 replicates per plant species just before planting in mesocosms as well as from the bulk soil representative of unplanted mesocosms (a total of 15 samples in June 2018). We collected the next samples from each mesocosm, for a total of 25 samples before and 16 after salt flushes (February 28, 2020; August 25, 2020, respectively). The columns were pierced, and then horizontal cores were collected with a brass pipe (diameter 3.8cm and length 15cm), washed with bleach and rinsed 3 times. All cores were stored at -20°C within one minute of collection, until DNA extraction was performed. At that time, a 0.25 g soil subsample including the rhizosphere for planted mesocosms, was prepared from thawed and homogenized core samples.

### 2.3 DNA preparation, sequencing and analysis

DNA extraction and purification were performed with DNeasy PowerSoil Pro Kit following the manufacturer's protocol (QIAGEN, Hilden, Germany), then repurified with ethanol precipitation of nucleic acids protocol (OpenWetWare contributors, 2012), and resuspended in 50 µL TE (1M Tris-HCl, pH 7.5, 1 mM EDTA). The library was prepared by GenomeQC after the target enrichment via PCR with primer (Dopheide et al. 2019). The Illumina-sequenced paired-end FASTQ files were demultiplexed by sample. Barcodes and adapters were removed by the sequencing platform. The amplicon sequence variant (ASV) table was obtained following DADA2 pipeline tutorial version 1.16 (Callahan et al., 2016) to trim, merge, and remove chimeras from reads. Taxonomy for global and singletons ASV were assigned using the UNITE database version 8.3 (Abarenkov et al., 2021) for ITS and the Silva database version 138.1 (McLaren and Callahan, 2021) for 16S, as training set. Phyloseq package in R (McMurdie and Holmes, 2013) was used to analyze communities, and graphically display complex phylogenetic sequencing data. Two technical replicates, plus a negative control of DNA extraction and sequencing, were done. Shannon and Chao1 indices of diversity and Bray-Curtis dissimilarity were calculated.

### 3 RESULTS

#### 3.1 Bacterial community

After two years in the greenhouse, 2 classes (Acidobacteriae, Chloroflexia) did not remain on the list of the 20 most abundant classes while 2 new classes appeared (Limnochordia, Methyloirabilia). Initially, CS and SA showed less bacterial alpha diversity than the other plant species and the unplanted treatment (Shannon index below 7.25). After 1 year however, the diversity became more similar between treatments and both CS and SA increased in diversity to the same level as the other plant species (IV and JE), reaching a Shannon index close to 8.30. The Bray-Curtis dissimilarity between mesocosms also decreased during that period. We did not observe plant species effects on bacterial read numbers at the class level before or after two years, except for SA. Abundance was very low for SA initially, but increased after two years, reaching the levels observed for the other plant species (Fig. 1). Salt had no effect on abundance or diversity as measured by the Shannon index.

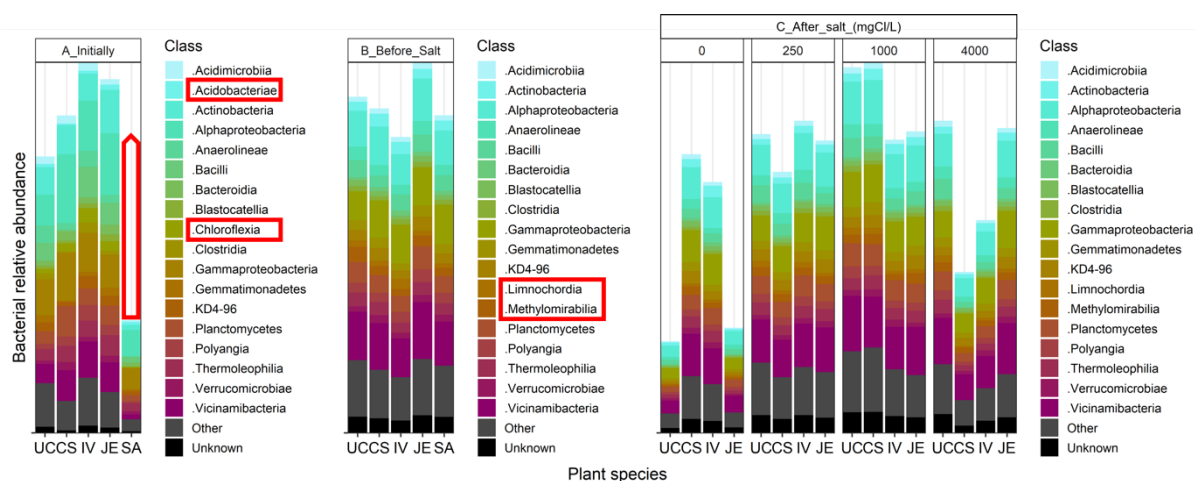


Figure 1. Bacterial diversity and observed abundance (number of reads), at the start of the experiment (Initial), after two years in the greenhouse (Before salinity), and after the salt experiment (NaCl). In red, classes which left or joined the list of most observed classes. Number of samples per treatment: Initial n = 3; Before salinity n=5; NaCl n = 1.

#### 3.2 Fungal community

The Glomeromycetes were not present initially. However, they were present after two years, possibly because MYKE® PRO Landscape, presumably containing *Glomus interadices*, was added to the substrate at planting time. When representing the fungal alpha diversity for the plant species, the Shannon index showed a high variability (between 2 to 5.5). The fungal community became more homogeneous over time, except for the SA and 4000mgCl/L NaCl mesocosms.

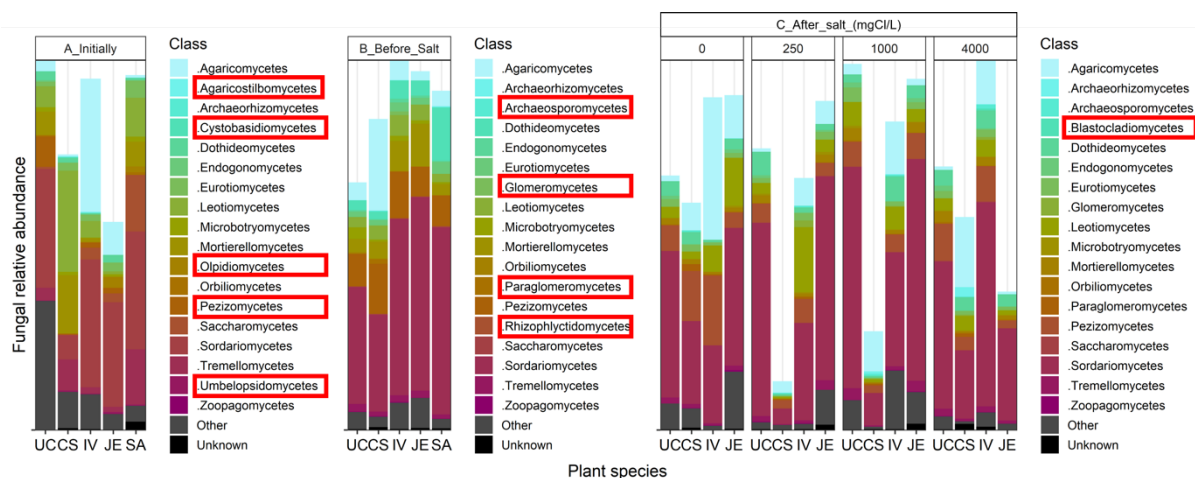


Figure 2 Fungal diversity and observed abundance (number of reads), at the start of the experiment (Initial), after two years in the greenhouse (Before salinity) and after the salt experiment (NaCl). In red, classes which left or joined the list of most observed classes. Number of samples per treatment: Initial n = 3; Before salinity n=5; NaCl n = 1.

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## 4 DISCUSSION AND CONCLUSION

In this BR experiment, plant species influenced bacterial and fungal communities but salt did not. Endreny et al. (2012) obtained similar results for the impact of salt on bacterial communities in BR. Plant species have been shown to affect microbial communities in a range of environments (e.g. Sweeney et al., 2021). Further analysis and interpretation of our data is in progress.

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