CHARACTERIZATION OF MICROBIAL COMMUNITIES ASSOCIATED WITH THE RHIZOSPHERE OF WETLAND PLANTS FROM THE SUDBURY REGION

by

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Abstract

The role of microbial communities within the plant rhizosphere is a rapidly developing area of research. Specificity of microbial community structure to plant species and environmental drivers of this relationship are not yet well understood in natural communities, particularly in wetlands. In the present thesis, species-specific differences in those communities as well as environmental influences on those differences were examined separately. In an experiment, six different wetland species taken from field sites around Sudbury, Ontario were grown in mesocosms in a wetland garden for two growing seasons. The species included two species from two genera of the family Cyperaceae each, one species of Poaceae and one species of Ericaceae. Mesocosms were inoculated with a mixture of field rhizosphere soils from all the collected plants. A field study was simultaneously conducted on the rhizospheres of two of those species from wetlands along an industrial disturbance gradient. The microbial community structures of the rhizospheres of the selected wetland plants were determined using next generation 454-pyrosequencing techniques. Microbial community structure in the garden experiment showed specificity to plant taxa which was related to the phylogeny of the host plant, the differences increasing with decreasing taxonomic relatedness of the plants. Differences in the microbial community structure between the investigated plant species were also found in the field, but were secondary to site-specific effects. I conclude that the microbial community structure of the rhizosphere does differ between plant species and that environmental conditions were stronger than plant-microbe interactions in the scale of influence over microbial community structure.

Keywords

Microbial community structure, bacteria, fungi, 454-pyrosequencing, rhizosphere, species specifity, industrial disturbance, taxonomic relatedness

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Table of Contents

List of Tables

List of Figures

List of Appendix

Chapter 1

1.1 Background and Rationale

The rhizosphere, which is comprised of the fine roots and their exudates along with microbial organisms, soil and other chemical compounds immediately surrounding plant roots, is populated by an immense load of bacterial and fungal microbes (Uroz et al. 2010; Gottel et al., 2011). Within this region, complex interactions between microbes, plants, minerals, nutrients, gases and liquids are occurring constantly. This region is therefore both complex and fascinating, with many unanswered questions remaining. The structure of this microbial community may be the result of a number of different factors, including the species of the host plant and the physical and chemical properties of the soil.

Several studies have examined the extent to which these factors influence the microbial community of the rhizosphere (Berg and Smalla, 2009). Kuske et al. (2002) identified differences between microbial communities in soil interspaces and rhizosphere, and between rhizosphere soils of the invading plant *Bromus tectorum* and native species, as well as between soil depths. Finer levels of differentiation can also be seen, such as between the microbial rhizosphere communities of different genotypes and even life stages of maize (Aira et al., 2010). Maize plants of different inbred genetic groups may also produce their own unique microbial communities (Bouffaud et al., 2012). In addition to that, bacterial communities in biological soil crusts in the Kalahari Desert in Africa varied according to the grasses, shrubs or trees present (Elliott et al., 2014). Batten et al. (2006) found that rhizosphere microbial community of native plant species changed after

invasive plants invaded the site, with recent invasions having greater similarity to native microbial communities than areas that had been invaded for longer time periods (two years). Several studies examining only fungal communities have shown that host plants play a stronger role in differentiating communities than habitat does (Becklin et al., 2012; Mouhamadou et al., 2013). Furthermore, interspecific differences in root traits specific to plant species (mass, length, surface area of roots) have been identified as having control on soil fungal communities (Mouhamadou et al., 2013).

Some examples show that bacterial community composition and diversity strongly respond to soil pH, land use, and plant species (Nacke et al., 2011). It is also influenced by restoration status (agriculturally affected vs. restored wetlands) whereas it does not respond as strongly to nutrient concentrations and not at all to wetland type or soil carbon (Hartman et al., 2008). Similarly Rousk et al. (2010) suggest that the strong correlations with pH are a result of narrow growth ranges for bacteria with respect to pH, which is corroborated by Bååth (1996) and Rosso et al. (1995). However, the relative importance of these factors is not fully understood, nor is the interactions between these communities and the plants themselves.

Soil microorganisms are an important component of freshwater wetlands. Wetlands represent the transition from terrestrial to aquatic ecosystems (McCaig et al., 2001) and their microbial community influences central internal processes such as the cycling of nutrients and carbon. These processes affect water quality and the global carbon cycle (Richardson and Marshall, 1986; Roulet, 2000). Also, wetland microbial communities are not completely understood (Hartman et al., 2008). Microbial communities have a high diversity of phenotypes and genotypes. DNA renaturation studies showed that there are a

billion bacterial cells, at least 10^6 16S rRNA gene sequences, and 10^3 bacterial species per 1 g of soil sample (Gans et al., 2005). However, little is known about the abundance and structure of different microbial communities in different habitats.

Hence, rhizosphere microbial communities are an important functional component of ecosystems, and should also be considered when ecosystem functions are being restored after industrial damage, such as in the region of Sudbury, Ontario. In this region, more than a century of mining related activities led to a major disturbance of the existing ecosystems, but with improvements in mining practices and restoration activities the ecosystems are beginning to recover. Industrial ore roasting and smelting dispersed sulphur and particulate matter containing metals over regionally larger areas until the late 1960s. By this time, in the Sudbury area, 80 000 ha of forest had been destroyed along with acidification of nearly 7000 lakes (Gunn and Keller, 1990). Sudbury had become the largest point source of sulphur emissions in the world. Since then, tougher regulations and improved technology have led to a reduction of the emissions by 95 % and a slow recovery of the terrestrial and aquatic ecosystems (Keller et al., 1992a, Gunn and Keller, 1990).

The mining and smelting activities which deposit metals and acidify the soils can strongly influence the natural structure and functioning of soil microbial communities (Ramsey et al., 2012). Soil acidification can lead to a decrease in community richness and changes in ecosystem function. Other factors which may influence this are changes in phosphorus concentration and metals including cadmium, and aluminium (Ramsey et al., 2012).
The loss of beneficial soil arbuscular mycorrhizal fungi (AMF) communities leads to damaged ecosystem functioning as AMF reduce the severity of environmental stresses on

plant communities (Alqarawi et al., 2014). Soil geomorphological properties as well as soil physical and chemical characteristics are critical to the formation of soil bacterial community structure during restoration from mining (Preem et al., 2012). As such, soil microbial communities are a useful indicator for assessment of the progress of restoration efforts (Waterhouse et al., 2014).

Culture-independent sequence analysis is a current technology which is used to understand microbial diversity, function and processes, such as pyrosequencing and Illumina high-throughput sequencing (Stahl et al., 1984; Hugenholtz and Pace, 1996). These techniques use the sequences to qualify relative abundances of the microbial taxa present in a given sample. Comparing these techniques showed that pyrosequencing has a higher error rate (Margulies et al., 2005; Quince et al., 2009) and Illumina high-throughput sequencing has its own systematic base calling biases (Erlich et al., 2008). Even though Illumina high-throughput sequencing might provide greater depth for sequencing effort, pyrosequencing is still an efficient, high throughput, and cost-effective method for studying the microbial community profile. In recent years, pyrosequencing of 16S and 18S rRNA gene fragments were used to analyse soil microbial communities in depth (Roesch et al., 2007; Acosta-*Martínez* et al., 2008). Recent studies have also been specifically targeting microbial communities associated with plants. For example, Uroz et al. (2010) emphasized the diversity of microbial community of oak rhizosphere and surrounding soil and Gottel et al. (2011) highlighted the microbial communities from *Populus deltoids* roots based on 454-pyrosequencing libraries.

1.2 Objectives

The overall aim of this thesis was to characterize the microbial community within the rhizosphere of wetland plants along a pollution gradient in the Sudbury region. For understanding the relationship between environmental conditions and rhizosphere micro-organisms, the relationship between plant taxa and microbial communities has to be understood. Hence, the approach was two-pronged: 1) to determine to what extent the microbiome is plant species-specific, and 2) to determine to what extent the microbial communities differ along a gradient of environmental disturbance. These specific objectives of my study were achieved by combining an experiment under semi-controlled conditions, and field measurements. The species-specificity of microbial communities of rhizosphere was investigated with a mesocosm experiment over two growing seasons (Chapter 2), and the variation of the microbial communities along a gradient of environmental disturbance with a field-survey of the microbial rhizosphere communities in selected wetland plant species (Chapter 3). The study was conducted in the Sudbury region, which has over 100 years of logging, mining and smelting history, with environmental restoration occurring over the past 40 years.

1.3 Organization of thesis

The first part of this study described in Chapter 2 used an experimental setup to examine species specificity of microbes under semi-controlled conditions. Six common Northern Ontario wetland plant species (*Chamaedaphne calyculata* (L.) (Moench), *Eriophorum vaginatum* (L.), *Eriophorum virginicum* (L.), *Carex utriculata (*Boott), *Carex oligosperma* (Michx.) and *Glyceria canadensis* (Michx.)) were used. The microbial flora in all mesocosms was equalized at the beginning of the experiment by adding an

inoculum derived from the rhizosphere soils from all the samples mixed together. Analyses were conducted to test the degree to which differences in the microbiome community could be attributed to the host plant species.

The second part of the study described in Chapter 3 the rhizosphere microbiome community compared among four sites which previous studies have shown to differ with respect to exposure to metal pollution; Lake Laurentian (LU), Silver Lake (SLV), Rockcut Lake (RCK) and Daisy Lake (D5). Rhizosphere samples were collected from the rhizosphere of two wetland plant species common in the region and also studied in the common garden experiment; *Chamaedaphne calyculata* (leatherleaf) and *Eriophorum vaginatum* (cottongrass). Microbial community structure was determined by to what degree sites were different and which environmental factors had an effect on microbial community structure.

The first part (Chapter 2) of my study will examine how the microbial structure of the rhizosphere differs between different levels of taxa of wetland plants under controlled conditions and the importance of their functional role in wetland plant ecological strategies in relation to their phylogenetic relationships. I demonstrate that bacterial and fungal community composition in the rhizosphere of wetland plants is related to the host plant species and also that the microbiomes of plants are related at different taxonomic levels. The second part (Chapter 3) uses field sites to explore differences in species composition and diversity of microbial communities associated with the wetland plant rhizosphere along a gradient of environmental disturbance in the Sudbury area. This will expand the understanding of particular diversity in the rhizobiome of different wetlands within the context of environmental disturbance.

Chapter 2

Revealing the microbial diversity between the rhizospheres of six wetland plant species

Abstract

Microbial rhizosphere communities of crop plants have been shown to display species-specific relationships. To investigate the degree to which these interactions occur in the natural environment, the microbial rhizosphere communities of six wetland plant species (*Chamaedaphne calyculata, Eriophorum vaginatum, Eriophorum virginicum, Carex utriculata, Carex oligosperma* and *Glyceria canadensis*) were examined using pyrosequencing. The study was conducted in Sudbury, Ontario. Plants were transplanted into mesocosms in an experimental garden. The mesocosms were inoculated with a suspension made of rhizosphere soil samples from all plant collection sites to provide for each mesocosm the same microbial inoculum. After two growing seasons, rhizosphere samples were collected from each mesocosm in late summer before the onset of plant senescence. Root rhizosphere samples and root fragments were used for total microbial community analysis using 454-pyrosequencing. Additionally, CO_2 and CH_4 production were determined for each mesocosm. Differences occurred in the bacterial community between plant phylogeny with the microbial community of different plant families displaying greater differences than between different plant genera, and different groups (monocot to eudicot) showing greater differences than between different plant families. There were also interspecific differences in the bacterial communities between species of the same genera. Similar patterns of relationships between fungal groups were observed between plant species but they were not as distinct as between the bacterial communities

in the rhizospheres of different plant species. So, even under controlled conditions in mesocosms using the same inoculum, microbial communities showed plant-taxa specific relationships.

2.1 Introduction

The rhizosphere is the volume of soil surrounding plant roots which includes root exudates and is where soil microorganisms including bacteria and fungi interact with plant roots (Barea et al., 2005). It is known to have high microbial activity when compared to soil from the interspaces between plant roots (Kuske et al., 2002; Uroz et al., 2010). The microbial community of the rhizosphere is linked to many benefits for plants, such as translocating nutrients and minerals from soils to plants (Hawkins et al., 2000; Miransari, 2011; Adeleke et al., 2012), promoting plant growth and supporting development (Jetiyanon and Kloepper, 2002), enhancing the production of secondary metabolites (van de Mortel et al., 2012), protecting the plants against pathogen attacks (Raaijmakers et al., 2009) and adapting to a variety of temperatures and water availability to help plants survive under stressed conditions (Bordeleau and Prevost, 1994). They can also modulate plant immune systems; some bacterial strains induce systemic resistance via jasmonic acid, ethylene and salicylic acid pathways (Zamioudis and Pieterse, 2012).

Exudates released from the plant roots, which may be specific to plant species (Gransee and Wittenmayer, 2000) or even genotypes (Corrales et al., 2007), are substrates for the microbes. Changes to these exudates therefore can alter the conditions under which microbial communities develop, which may influence the microbial community. For example; the quality or composition of plant exudates may drive changes in the microbial community associated with different maize genotypes (Aira et al., 2010). Maize plants of different inbred genetic groups may also produce their own unique microbial communities (Bouffaud et al., 2012).

Differences between the rhizosphere soils of native and invading plant species have been found previously (Kuske et al., 2002). Bacterial communities in biological soil crusts in the Kalahari Desert in Africa varied according to whether the site was occupied by grasses, shrubs or trees (Elliott et al., 2014). Batten et al. (2006) determined microbial communities in rhizospheres of native plant species after invasive plants invaded, differences to native microbial communities increasing over time since the invasion. Plant species have also been shown to influence rhizospheric fungal communities in two alpine grasses grown in microcosms (Mouhamadou et al., 2013). Moreover, Becklin et al. (2012) showed that the host plants had more influence on fungal community in the rhizosphere of alpine plants than did the habitat.

Collectively, these finding suggest that microbial communities may differ according to the plant species (Kuske et al., 2002; Batten et al., 2006; Mouhamadou et al., 2013; Elliott et al., 2014) and genotypes present (Aira et al., 2010; Bouffaud et al., 2012). However, microbial community structure may also differ according to soil properties including pH, water availability, and nutrient limitation. A possible association of plant species with microsites with certain soil characteristics may contribute to relationships between plants and microbes (Kuske et al., 2002; Frey et al., 2004; Uroz et al., 2010). Rhizosphere microbial communities express different ecological strategies (*r* vs. *K* strategy selection) depending on the plant species they are associated with as well as the specific stage of plant growth, which means these differences may reflect the plants' ecological strategy as well (Blagodatskaya et al., 2004).

Little research has been done to date with respect to microbial rhizosphere communities in wetlands and the mechanisms determining microbial community structure from plant

to plant are not known. The aim of the present study was to determine if wild plant species are able to select for distinct rhizosphere communities from a common soil substrate. Understanding how the microbial community structure of the rhizosphere differs between different levels of taxa of wetland plants under controlled conditions will shed light on the importance of their functional role in wetland plant ecological strategies in relation to their phylogenetic relationships. Ultimately, my goal was to determine if bacterial or fungal community composition in the rhizosphere of wetland plants is related to the host plant species, and to compare microbiomes of plants related at different taxonomic levels.

I collected plants which were readily available from several wetlands in the Sudbury region to distinguish between species-specific and potential site-specific effects on microbial communities. Cyperaceae are prevalent in wetlands of the region with a large number of species. Two species each of two genera within the family of Cyperaceae were chosen for the study (4 species). Additionally, to expand the level of phylogenetic comparisons, one species from another family of monocots (Poaceae), and one eudicot species of the family of Ericaceae were chosen. Collection of plants from different sites made it possible to test if their origin had an effect on the microbial community structure, or if the plant species themselves were the stronger contributing factor. I hypothesized that different plant species grown under similar conditions will have different microbial community structures after two growing seasons.

A soil inoculum composed of soils from all sites added to the mesocosm of each plant provided a common initial microbial community to each plant mesocosm. As the distinguishing effect of plant species on the soil microbial community is increasing over

time (Batten et al., 2006), the plant-microbiome associations in the mesocosms were allowed to develop for two growing seasons. I used next generation sequencing techniques to determine if there was evidence of species specificity within bacterial and fungal communities to specific wetland plants under controlled growth conditions. Also, the microbial community is known to influence the degradation of organic matter (Sinsabaugh, 2010; Godin et al., 2012), a process which involves enzymes including hydrolases and lignases and produces CH_4 and CO_2. Blagodatskaya et al. (2004) have shown different CO_2 production rates for rhizosphere microbial communities of different plant species. Therefore, measuring differences in these enzyme activities and the production of CH_4 and CO_2 should indicate functional differences in the microbial community structure in the plant rhizosphere.

2.2 Material and Methods

2.2.1 Experimental setup

In order to investigate the extent to which the bacterial and fungal rhizosphere microbiome is species-specific among different wetland plant species, six different common Northern Ontario wetland plant species – *Chamaedaphne calyculata* (L.) Moench, *Eriophorum vaginatum* L., *Eriophorum virginicum* L., *Carex utriculata* Boott, *Carex oligosperma* Michx. and *Glyceria canadensis* (Michx.) Trin. – were used. *C. calyculata* is a eudicot and belongs to the family of Ericaceae whereas the other plants are monocots. *G. canadensis* is a member of Poaceae family while *Eriophorum* and *Carex* belong to the Cyperaceae family. In spring 2013, vegetative tillers of the species were collected in wetlands around Sudbury, Ontario. In this study, plants were propagated vegetatively as the propagation of many wetland plant species by seed is difficult. The local microbiome was therefore included in the collected plants. To distinguish species effects on rhizosphere microbial communities from site-specific effects, the species were collected from two different wetlands each. However, for three of the species, plants from only one wetland could be analysed due to mortality after planting or limited availability (Table 2.1).

One wetland was common for all the Cyperaceae species, a floating, nutrient poor fen near Cartier, ON, 60 km NW of Sudbury. One wetland North of Sudbury (Rockcut Lake) was the second collection site for the two *Eriophorum* species, and the only site for *G. canadensis* collection. The second site for *C. utriculata* was a wetland in Sudbury with soil contaminated by emissions from a Cu-Ni smelter, which also was the only site for *C. calyculata* (Lake Laurentian). The floating nutrient poor fen was dominated by species of

Cyperaceae and *Sphagnum* sp., whereas in the two other wetlands, *C. calyculata* was the dominant species. At collection, plants were dug out by cutting the roots around the plant with a serrated knife and lifted out, after which the roots of all plants were shaken at the site to remove the bulk soil before storing them in sealable plastic bags. Within a day after collection, on 29 and 31 May 2013, the roots were rinsed and the tillers planted into 10-liter, 25 cm diameter mesocosms in an experimental garden with pools filled to 20 cm depth of water. The mesocosms were filled using 1 cm sieved commercially obtained wetland soil from a former beaver meadow with a pH of 4.5 and an organic matter content of 26% (Bainbridge Constructions, North Bay, Ontario).

All mesocosms were inoculated with a mixture of rhizosphere soils (soil closest to the roots) of all collected plants in order to provide each plant an opportunity to have a similar microbial community. Samples of approximately 5 g rhizosphere soil from all sites and species (3 from each plant for each site and 3 control samples, 32 in total) were mixed together with 4 L of de-ionized water and thoroughly mixed. All pots were inoculated with 50 ml of this suspension. Control mesocosms were composed of soil only and no plants, but they did receive the inoculum as well. Also, regular weeding was performed to prevent other plant species from influencing the mesocosms. Soils in natural wetlands of the region mostly do not freeze during the winter, so, during the winter months, the plants in the mesocosms were covered with straw to avoid below-zero temperatures. Temperature was monitored using iButtons. Pot temperatures approached 0°C but never went below during the winter.

2.2.2 Microbial Community Analyses

After two growing seasons in the mesocosms, between 20 and 22 August 2014, rhizosphere soil of each experimental plant was sampled for molecular and enzyme analyses by removing the bulk soil, shaking the roots, and then collecting the soil from the closest part of the root, including the roots themselves. Hence, the endorhizosphere (microbial community within the roots) is included in the data.

Total microbial community DNA was extracted from each of the 32 samples using the PowerSoil® DNA Isolation Kit (Mobio, California) according to the manufacturer's protocols. Purified DNA was quantified on a Nanodrop 8000 Spectrophotometer (Thermo Scientific, Delaware, USA; 14.81 - 50.23 ng/µl). Samples were sent for SSU gene pyrosequencing analyses at MR. DNA Molecular Research LP (Shallowater, Texas) using the Roche 454 GS-FLX platform (Roche 454, Branford, CT, USA). Samples were targeted for 16S rRNA gene (28F-5'-GAGTTTGATCNTGGCTCAG-3', 519R-5'GTNTTACNGCGGCKGCTG-3') (Dowd et al., 2008a), and eukaryal/fungal 18S rRNA (ISSU) (F-5'-TGGAGGGCAAGTCTGGTG-3', R-5'-TCGGCATAGTTTATGGTTAAG-3') (Foster et al., 2013), pyrosequencing. Sequencing was performed using titanium amplicon pyrosequencing (bTEFAP) with a one-step PCR (Dowd et al., 2008b). Pyrotagged samples were amplified with ePCR using Hot Start and HotStar high-fidelity *Taq* (Qiagen) to generate ~400bp reads for each of the above primer libraries at a sequence depth of 3000 reads per sample. All sequencing and quality filtering were conducted by the sequencing facility and included removing primer sequences, reads < 200bp long, sequences with ambiguous base calls, homopolymer repeats greater than 6bp, and chimera removal (Dowd et al., 2008b).

Analysis of pyrosequencing data was performed for all rhizosphere datasets (32 in total). Filtered quality sequences were analyzed using the QIIME software package (Quantitative Insights into Microbial Ecology (Caporaso et al., 2010)); using the RDP (version 8.15.13) reference database http://pyro.cme.msu.edu/index.jsp) for the bacterial 16S rRNA libraries, and using the Silva SSU reference alignments (Pruesse et al., 2007) for the eukaryotic/fungal 18S rRNA libraries. Operational taxonomic units (OTUs) were assigned using complete linkage clustering at 97% similarity (Cole et al., 2009). Operational taxonomic units were taxonomically classified using the Silva database (Release 110) and verified using the RDP classifier (Wang et al., 2007). One sample (*Eriophorum vaginatum* from Cartier - EvaC3) returned only one OTU, and so was assumed to be an error and was discounted from further analysis. Operational taxonomic unit diversity was estimated using the Shannon indices, as well as Unifrac distances, calculated using the Qiime pipelines (Caporaso et al., 2010). OTUs accounting for greater than 0.1 % of the total reads in each dataset were used in generating community taxonomic profiles. The representative sequences for the >0.1% OTUs for each data set were trimmed to the shortest sequence length, aligned using ClustalW with ambiguously aligned positions removed, and the alignments used to generate maximum likelihood (Tamura et al., 2011). Comparison of community profiles and the site/soil characteristics were completed with multivariate analyses using Qiime and the R vegan package (Oksanen et al., 2013).

2.2.3 Enzyme analyses

Activity of hydrolases (β- glucosidase and phosphatase) and lignases (phenoloxidase and peroxidise) were determined in the rhizosphere soil following published protocols

(Sinsabaugh, 2009; Hendel and Marxsen, 2005; Saiya-Cork et al., 2002). For both lignase and hydrolase assays 0.5 to 1 g rhizosphere soil was mixed with 60 ml of 50 mM acetate buffer (pH: 5) and blended for 1-2 min to homogenize the sample. The lignase assay medium contained 50 mM acetate buffer (pH: 5), 25 mM L-DOPA and 0.3% H_2O_2 (for peroxidise wells). Lignase assay was followed at 460 nm. Activity was calculated using the extinction coefficient of L-DOPA (7.9 μM−1). The hydrolase assay medium contained 50 mM acetate buffer (pH: 5), 10 μM MUB (4-Methylumbelliferone), 10 μM MUB β-D-glucopyranoside (for β-glucosidase wells) and 10 μM MUB-phosphate (for phosphatase wells) in a final assay volume of 250 μl. Microplates were incubated for 4 h at 20℃. At the end of incubation of hydrolase assay 10 μL of 0.5 N NaOH was added to each of the wells to raise them to pH above 7.5 to make the fluorescence reading obtainable. Hydrolase assay was followed at 365 nm, emission Quench coefficients were calculated for each sample independently and used to calculate β- glucosidase and phosphatase activities.

2.2.4 Soil measurements

Soil pH was determined for the original soil used as a substrate, along with rhizosphere soil samples from each plant and soil from the control mesocosms at the end of the experiment, using pH indicators accurate to the nearest 0.5 on the pH scale (Hellige – Truog Soil pH Tester Kit) following the manufacturer's protocol.

CH_4 and CO_2 production from the soil of each mesocosm were measured on 18 August 2014. Before rhizosphere soil collection, 118 mL plastic jars with the bottoms cut off were placed in the pots near the plant itself, without their lids. After 5 minutes of equilibration, the lids of the jars, which had a stopper inserted through the center, were

placed back on the jars, sealing them. Gas sampling was performed at 0, 30 and 60 minutes. During sampling, 3 mL of air was injected into the closed jar, mixed by pumping the syringe for 3 times, and then 3 mL of air was extracted. From these samples, CH_4 and CO_2 concentrations were measured using an SRI 8610C gas chromatograph (SRI Instruments, Torrance, CA) fitted with a 1 ml sample loop and a column temperature of 105 °C.

2.2.5 Measurement of RDMC

Root dry matter content (RDMC) was measured to describe the species' position along plant economics spectrum (Fort et al., 2015). One replicate root sample of approximately 1 g was collected from each mesocosm, cleaned and its fresh mass and dry mass (after 96 h at 75 °C) were determined. RDMC was calculated as the ratio of dry mass over fresh mass.

2.2.6 Statistical analysis

Statistical analyses were completed using R (R Core Team 2012, version 3.0.1) along with the phyloseq package (McMurdie and Holmes, 2013, version 1.6.1). Sequencing data were transformed from absolute abundance to relative abundance for all OTUs within the samples. A threshold of > 0.1 % relative abundance was used to select for data which would have an appreciable contribution to the microbiome and eliminate rare species. Vegan package (Oksanen et al., 2013, version 2.0-10) was used to perform multivariate analysis, which included Detrended Correspondence Analysis (DCA).

Hierarchical clustering dendrogram figures were generated by the gplots package (Wagnes et al., 2014, version 2.13.0) using hclust in R based on the most abundant

OTU's in both bacterial and fungal data sets. Spearman and Pearson correlation matrices were generated to be used for *Ward's* minimum variance method to organize the matrix into a series of compact spherical clusters presented as a dendrogram. Using Pearson correlation matrix enables correlations using the original abundance data, whereas Spearman correlations capture relationships based on the abundance ranks. Mantel tests were performed using the ade4 package (Dray and Dufour, 2007; Chessel and Dufour, 2004; Dray et al., 2007, version 1.6-2) with DCA scores of > 0.1 % abundant OTUs for differences between plant species. The Mantel statistic estimates the correlation between two proximity matrices and *p* value represents the significance of the Mantel regression coefficients from zero following 9,999 permutations of DCA 1 and DCA 2 after transformation of plant species values to ordinal ranks. The indicspecies (Cáceres and Legendre, 2009, version 1.7.4) package was used to determine indicator species for >1% abundant bacterial and fungal OTUs. First, the multipatt function was used to find associations between plant species and combination of those plant species with 10,000 permutations. Then, OTUs that were significantly associated with a plant species (*p*-value < 0.05) were considered as indicator species for the matched taxa (family for bacterial data or class for fungal data). These were then plotted in a phylogenetic tree with the bootstrap values (500 re-samplings). I also performed Analysis of Variance (ANOVA) on the abundances of the individual bacterial families between each plant species and repeated the same analysis for fungal classes between each plant species.

Table 2. 1 Plants were collected from indicated sites in order to get rhizobiome samples in August 2014. Numbers in the cells indicate how many plants were collected from those sites. *, indicates those plants did not survive.

	Rockcut	Laurentian	Cartier
Chamaedaphne calyculata	3*	3	
Eriophorum vaginatum	3		3
Eriophorum virginicum	3		3
Carex utriculata		3	3
Carex oligosperma			5
Glyceria canadensis	3		

2.3 Results

2.3.1 Distribution of Bacterial Taxa across the Plants

Structure of bacterial rhizosphere communities

In total, 7,418 different bacterial OTUs were found in the 31 rhizosphere samples of the experimental mesocosms. There were 5,662 OTUs in the rare biosphere (> 0.1 % abundant community) and 1,756 OTUs were present in > 0.1 % population. Shannon-Wiener diversity index was calculated both with and without the OTUs in the rare biosphere. The highest bacterial Shannon-Wiener and Simpson diversities among the total and among the > 0.1% abundant populations were found in the control mesocosms without any plants, and the lowest values in *Carex* mesocosms (Table 2.2), but the differences were not significant (Kruskal-Wallis test, $p = 0.2$ and $p = 0.2$) (Table 2.2). For Simpson diversity index of the total bacterial communities there was a slight indication of differences among the mesocosms (Kruskal-Wallis test, $p = 0.083$), but not for > 0.1 % abundant communities (Kruskal-Wallis test, $p = 0.2$, Table 2.3). Both bacterial Shannon-Wiener and Simpson diversities among the total and among the > 0.1% abundant populations were not significant among the sites ((Kruskal-Wallis test, $p > 0.05$).

166,306 sequences classified within a domain were affiliated to 38 bacterial phyla. The dominant phyla across all samples were Proteobacteria, Acidobacteria, Actinobacteria and TM7 clade, representing 49.1, 14.1, 6.5 and 3.1 %, respectively, in total abundance (Appendix B). Additionally, I found chloroplast and mitochondria sequences in my data set (Appendix B). Proteobacteria was the most abundant phylum with over 50% abundance in *C. calyculata, E. vaginatum* and *E. virginicum* (Appendix B). Acidobacteria

was more abundant in control mesocosms (19.9 ± 3.0 %) compared with plant mesocosms, whereas Actinobacteria was more abundant in *G. canadensis* (10.7 ± 1.8%) compared to other mesocosms (Appendix B). Also, clade TM7 was more abundant in *G. canadensis* (6.4 ± 0.8 %) and control (4.6 ± 0.9 %) mesocosms. *C. oligosperma* and *C. utriculata* samples had high chloroplast abundance, 19.7 ± 7.3 % and 24.2 ± 8.7 %, respectively (Appendix B).

Specificity of bacterial rhizosphere communities

The abundance of several bacterial taxa showed positive associations with the types of mesocosms and with certain plant species and genera, with mesocosms of *C. calyculata* being the most distinct (Table 2.4). *Bacteroidales* (Bacteroidetes) and *Thermogemmatisporaceae* (Chloroflexi) were significantly more abundant in mesocosms of *C. calyculata* compared to all other plant mesocosms, while *Hyphomicrobiaceae*, *Acetobacteraceae* and *Methylocystaceae* (Alphaproteobacteria) were most abundant in *C. calyculata* mesocosms. Specifically, abundance of *Hyphomicrobiaceae* was significantly higher in *C. calyculata* mesocosms than *Carex* mesocosms, whereas abundance of *Methylocystaceae* was significantly higher in mesocosms of *C. calyculata* than those of *G. canadensis* and the controls. For the abundance of *Acetobacteraceae,* ANOVA showed significance difference between the plants but Tukey test did not determine which plant species was different than other. *Gaiellaceae* (Actinobacteriaceae) was significantly more abundant in *G. canadensis* mesocosms than in all other mesocosms except for the control mesocosms. Control mesocosms could be distinguished from all others by having higher abundances of *Rhodospirillaceae* (Alphaproteobacteria) and the highest abundance of *Rhodocyclaceae* (Betaproteobacteria) when compared to *Carex* and *Eriophorum*

mesocosms (Table 2.4). Control and *G. canadensis* mesocosms had significantly higher abundances of the clade SC-I-85 and unclassified family group belonging to *Betaproteobacteria* compared to other mesocosms. Abundance of clade TM7-1 was significantly higher in control mesocosms than *Eriophorum* and *Carex* mesocosms, and lower in *C. calyculata* mesocosms than control and *G. canadensis* mesocosms. The clade SBla14 in *Eriophorum* mesocosms was significantly higher, compared to other mesocosms. Additionally, chloroplast sequences were found in higher abundances in *Carex* and *G. canadensis* mesocosms, but these differences were not significant. For the remaining abundant bacterial families there was considerable variation among the replicate mesocosms of a single plant species resulting in overlap of the microbial community structure for 60 % of the rhizosphere microbiomes (> 0.1% abundant data) across all plants.

Hierarchical clustering analysis was performed to analyse similarities among the different plant mesocosms. The samples were compared using the *Ward's* linkage method both with Spearman and Pearson correlation coefficients. In the hierarchical clustering analysis based on Spearman correlation coefficient *C. calyculata* mesocosms were, as a first step, separated from all other mesocosms (Figure 2.1.). The next level of clustering separated *G. canadensis* and control mesocosms from the Cyperaceae mesocosms. *G. canadensis* and control mesocosms formed their own subclusters, equally to *Carex* mesocosms and *Eriophorum* mesocosms (Figure 2.1). All but three *C. utriculata* and two *C. oligosperma* mesocosms were placed in the corresponding clusters. Also the hierarchical cluster analysis using Pearson correlation coefficients resulted in groupings with definite plant taxa-specific patterns (Figure 2.2). One cluster consisted of *Carex* mesocosms, with

subclusters for each of the two *Carex* species. The other main branch divided in clusters comprised of *G. canadensis* and *C. calyculata* mesocosms, and mainly *Eriophorum* and control mesocosms (Figure 2.2). The latter cluster consisted of two subclusters, one with mainly *Eriophorum* mesocosms, and one with all control mesocosms and a mixture of several mesocosms with different plant species.

A DCA ordination of abundance of bacterial OTUs in all the mesocosms showed that *C. calyculata* mesocosms are clearly distinct from all other mesocosms along DCA1 (Figure 2.3). According to a Mantel test, plant species differentiated significantly along DCA1, but no differences were found on DCA2. A second DCA was conducted without *C. calyculata* in order to better see the relations among the other mesocosms. In this second DCA the areas covered by all the species partially overlapped (Figure 2.4), but the different species differentiated along separate axes of the DCA. *Carex* differentiated along DCA1 and *Eriophorum* along DCA2 from the control mesocosms. The two *Eriophorum* species displayed different ranges along the axes, with *E. vaginatum* being much broader in its distribution along DCA 2, *E. virginicum* along DCA 1. *G. canadensis* and control mesocosms separated from all other species by a combination of differences along both first and second DCA axes (Figure 2.4). The patterns of the ellipses are not as distinct as was shown in the hierarchical cluster analysis. This is because DCA's method does not first calculate spearman correlation coefficients, and order the samples using these, but rather, places each sample in 2 dimensional spaces based on the eigenvalues which may overlap rather than simply be ordered.

Indicator species analysis (Cáceres, & Legendre, 2009) identified the following bacterial families as being significantly ($p < 0.05$) associated to rhizospheres of specific plant taxa

(Figure 2.5). *Hyphomicrobiaceae* and *Thermogemmatisporaceae* were indicative of *C. calyculata* while clade SBla14 (*Betaproteobacteria*) was indicative of *Eriophorum vaginatum* and *Eriophorum virginicum* plants. The OTU described as chloroplast DNA was indicative of *Glyceria canadensis, Carex utriculata* and *Carex oligosperma*. The family *Methylocystaceae* was indicative of *Eriophorum vaginatum, Eriophorum virginicum* and *Carex oligosperma* and *Acidobacteriaceae* members were indicative of rhizospheres of all plants except *C. calyculata* (Figure 2.5). It is curious to note that two different groups from separate branches of the phylogenetic tree belonged to chloroplast. This could only be explained by difficulties with accurately identifying bacterial OTUs. The relative abundance of indicator species coverage in the total bacterial community is 2.8 ± 0.5 %, Appendix A shows relative abundance of those bacterial indicator species within the plant mesocosms. Then those indicator species were plotted into their bacterial phylogenetic tree, with information on which plant species each bacterial family were indicative of.

2.3.2 Distribution of fungal taxa across plant species

Fungal taxa could not be distinguished as finely as bacterial taxa and their description is mainly restricted to the class level or phylum level.

474,715 sequences classified within a domain were affiliated to 11 fungal phyla (Appendix E). 714 OTUs were present in abundance of > 0.1 %. Shannon-Wiener diversity index calculated based on the total rhizosphere fungal communities and on populations with > 0.1 % abundance showed no significant differences among the different plant species (Kruskal-Wallis test, $p = 0.103$, and $p = 0.215$, respectively; Table 2.2). Simpson diversity indices did not show significant differences among the types of

mesocosms either (Kruskal-Wallis test, p= 0.153 (total community); p = 0.285 (> 0.1 % abundant data), Table 2.3).

Control mesocosms had higher abundance of *Ascomycota* in total community than the rhizosphere samples of any plant species (82.6 ± 1.3%) (Appendix E). The *C. calyculata* rhizosphere had a higher abundance of *Basidiomycota* in total community (16.2 ± 12.5%) than that of other plants including control samples and had the lowest abundance of *Ascomycota* (66.3 ± 13.7%) (Appendix E).

Many fungal OTUs were not classified beyond phyla and were designated unclassified at the class level. Nevertheless, *Leotiomycetes*, *Sordariomycetes* and *Dothideomycetes* were the most abundant fungal classes, with average abundances of 30.0 ± 2.4 %, 8.9 ± 1.3 % and 5.6 ± 1.6%, respectively. Abundance of *Sordariomycetes* was significantly higher in control mesocosms than others (Table 2.5). Abundance of *Entorrhizomycetes* was significantly higher in *E. virginicum* than *Carex, G. canadensis* and *C. calyculata* mesocosms (Table 2.5). Abundance of *Mucoromycotina* was significantly higher in control mesocosms than *C. calyculata* and *Eriophorum* mesocosms (Table 2.5).

Hierarchical clustering analysis of fungal community based on Spearman and Pearson correlation shows less clear separation among the types of mesocosms and plant taxa than the analysis based on bacterial communities (Figure 2.6 and 2.7). Nevertheless, some patterns can be observed. An analysis with Spearman correlation coefficients results in a cluster dominated by control and *Carex* mesocosms, the other of *Eriophorum* and *C. calyculata* mesocosms with some *Carex utriculata*. In the analysis based on Pearson

correlation coefficients, one of the two main clusters consists almost exclusively of *Eriophorum* mesocosms.

DCA ordination of fungal communities did not distinguish mesocosms with the different plant species as clearly as the bacterial communities did, but in a DCA including all species, mesocosms with *E. virginicum* distinguished from the other mesocosms along the DCA2 axis (Figure 2.8). A Mantel test did not show significant differences among mesocosms with different plant species neither along DCA1 nor DCA2 (Figure 2.8).

To be consistent with the analysis of bacterial communities, a second DCA was run without *C. calyculata* for the fungal data. Differentiation among the species was less clear than in the case of bacterial communities, all species overlapped in a central area coinciding with the control mesocosms. Additionally, there was some indication of a slight differentiation between the genera of *Eriophorum* and *Carex* (Figure 2.9).

The relative abundance of indicator species coverage in the total fungal community was 3.6 ± 0.4 %, Appendix F shows relative abundance of those indicator species within the plant mesocosms. An analysis of fungal indicator species showed that *Sordariomycetes* was indicative of control, *Carex* and *G. canadensis* rhizosphere microbiomes, and *Saccharomyces* was indicative of control and *G. canadensis* rhizosphere microbiomes, whereas *Eurotiomycetes* and unclassified *Chytridiomycota* were indicative of *C. calyculata* (Figure 2.10). Of the fungal classes *Entorrhizomycetes* was indicative of *Eriophorum* plants. The other fungal classes showed far too much variation to distinguish meaningful patterns. In contrast to the bacterial phylogenetic tree, the fungal phylogenetic tree does not show any relationship with plant phylogeny.

2.3.3 Gas Fluxes

Along with enzyme activity, total gas fluxes were used as a measure of overall microbial respiration activity between plant species. The effect of plant species on methane production was significant ($p< 0.05$, $F= 3.76$, Figure 2.11a). This effect was caused by *Chamaedaphne calyculata* the mesocosms of which had higher methane production than those of the other plant species. CO_2 production did not significantly differ among the mesocosms of the different plant species ($p> 0.05$, Figure 2.11b).

2.3.4 Enzyme activities

The effect of plant species on rhizosphere phosphatase activity was significant due to the high values in *C. calyculata* mesocosms (ANOVA, $p <0.001$, $F=10.2$, Figure 2.12d). Plant species effects had no effect on other enzyme activities with no significant differences (β-glucosidase $p=0.07$, $F=2.3$, Figure 2.12a; peroxidase, $p=0.06$, $F=1.71$, Figure 2.12b; phenoloxidase $p = 0.17$, $F=1.71$, Figure 2.12c).

2.3.5 Rhizosphere pH

Initial pH of the mesocosm soil was 4.5. Control pots had pH-values around 5.0 at the end of the experiment. Rhizosphere soil pH values for most plant species were around 4.5, while *C. calyculata* rhizosphere soil had a significantly higher pH at 5.5 compared to the other plant species (ANOVA, $p < 0.001$, $F=12.2$, Figure 2.13). Tukey post hoc tests showed pH of the control samples were significantly higher than *E. vaginatum*, *E. virginicum* and *G. canadensis* mesocosms.

2.3.6 Root DMC

Root DMC showed significant variation among the species (ANOVA, F=6.15, p=0.001). *C. calyculata* had the highest root DMC, while *E. vaginatum* had the lowest (Table 2.6)

Table 2. 2 Shannon- Wiener diversity index (mean ± 1 SD) of mesocosms based on total and > 0.1 % abundant populations of bacterial and fungal communities. Cca: Chamaedaphne calyculata, Gca: Glyceria canadensis, Col: Carex oligosperma, Cut: Carex utriculata, Eva: Eriophorum vaginatum and Evi: Eriophorum virginicum.

Shannon- Wiener diversity index

	bacterial		**fungal**	
Plants	whole community	> 0.1% abundant	whole community	> 0.1% abundant
Control	6.4 ± 0.0	5.1 ± 0.1	4.5 ± 0.4	3.8 ± 0
Cca	5.8 ± 0.1	4.7 ± 0.2	4.9 ± 0.1	3.5 ± 0.2
Gca	6.0 ± 0.1	4.6 ± 0.2	3.8 ± 0.4	3.6 ± 0.1
Col	5.7 ± 0.2	4.4 ± 0.3	3.6 ± 0.3	3.6 ± 0.2
Cut	5.7 ± 0.4	4.3 ± 0.3	4.1 ± 0.3	4.0 ± 0.1
Eva	6.1 ± 0.1	4.8 ± 0.1	4.4 ± 0.3	3.6 ± 0.2
Evi	6.2 ± 0.1	4.9 ± 0.1	4.5 ± 0.1	3.4 ± 0.1

Table 2. 3 Simpson diversity index (mean ± 1 SD) of mesocosms based on total and > 0.1 % abundant populations of bacterial and fungal communities. For mesocosm abbreviations see Table 2.2

Simpson diversity index

	bacterial		**fungal**	
Plants	total community	> 0.1% abundant	total community	> 0.1% abundant
Control	0.995 ± 0	0.988 ± 0.001	0.923 ± 0.046	0.954 ± 0.005
Cca	0.987 ± 0.003	0.965 ± 0.001	0.975 ± 0.001	0.910 ± 0.028
Gca	0.987 ± 0.003	0.969 ± 0.007	0.866 ± 0.041	0.939 ± 0.004
Col	0.977 ± 0.011	0.953 ± 0.021	0.901 ± 0.022	0.931 ± 0.023
Cut	0.967 ± 0.021	0.977 ± 0.005	0.932 ± 0.017	0.957 ± 0.006
Eva	0.993 ± 0	0.950 ± 0.033	0.936 ± 0.022	0.912 ± 0.020
Evi	0.993 ± 0.001	0.984 ± 0.002	0.961 ± 0.004	0.908 ± 0.017

Table 2. 4 Abundance (mean ± 1 SD) of bacterial taxa among control mesocosms and rhizospheres of the plant species (> 0.1% abundant community). For mesocosm abbreviations see Table 2.2.* indicates where family is significantly different among/between plants. Mean values followed by different letters are significantly different ($p < 0.05$) from each other.

Phylum	**Class**	**Family**	**Control**	**Cca**	**Gca**	**Col**	**Cut**	**Eva**	**Evi**
Acidobacteria	Acidobacteriia	Acidobacteriaceae	11.5 ± 2.0	2.3 ± 0.7	8.0 ± 2.8	8.7 ± 1.1	6.5 ± 1.7	9.1 ± 1.7	8.1 ± 0.9
	Holophagae	Holophagaceae	0.2 ± 0.1	1.2 ± 0.3	0.2 ± 0.3	0.8 ± 0.2	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.2
	Acidobacteriia	Koribacteraceae	1.6 ± 0.3	1.2 ± 0.3	1.1 ± 0.9	1.1 ± 0.3	0.9 ± 0.2	1.0 ± 0.2	0.9 ± 0.1
Actinobacteria	Thermoleophilia	Gaiellaceae*	1.8 ± 0.3 ^{a}	0 ^{b}	2.3 ± 0.5^{a}	0.6 ± 0.1 ^{b}	1.0 ± 0.4 ^{b}	0.6 ± 0.1 ^{b}	0.2 ± 0.1 ^{b}
Bacteroidetes	Bacteroidia	Bacteroidales*	0.1 ± 0.1	1.1 ± 0.5^{a}	0.2 ± 0.4	0.2 ± 0.1^{b}	0.1 ± 0.1 ^{b}	0.2 ± 0.1 ^{b}	0.2 ± 0.1 ^{b}
Chloroflexi	Ktedonobacteria	Thermogemmatisporaceae*	0^{b}	1.4 ± 0.6^{a}	0 ^{b}	0 ^{b}	0 ^{b}	0 ^{b}	0 ^{b}
Chloroplast			0	0.5 ± 0.2	0.2 ± 0.6	0.1 ± 0.0	0.2 ± 0.2	0.2 ± 0.1	1.1 ± 0.5
			0	0.8 ± 0.3	10.1 ± 10.1	18.4 ± 7.2	22.6 ± 8.8	1.1 ± 0.4	3.4 ± 1.5
Proteobacteria	Alphaproteobacteria	Acetobacteraceae*	0.9 ± 0.2	4.6 ± 2.0	0.6 ± 0.5	0.8 ± 0.3	1.5 ± 0.8	0.5 ± 0.2	2.7 ± 0.6
		Beijerinckiaceae	0.9 ± 0.2	0.4 ± 0.0	0.9 ± 0.0	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	1.0 ± 0.3
		Bradyrhizobiaceae	5.1 ± 1.0	7.2 ± 2.1	5.4 ± 1.6	4.4 ± 0.4	4.3 ± 0.7	4.4 ± 0.6	5.1 ± 0.9
		Ellin330	1.6 ± 0.5	0.6 ± 0.3	0.3 ± 0.0	0.9 ± 0.2	0.4 ± 0.1	0.8 ± 0.2	0.5 ± 0.2
		Hyphomicrobiaceae*	6.7 ± 0.5	13.6 ± 4.6^{a}	5.5 ± 2.5	3.9 ± 0.5^{b}	4.0 ± 0.8 ^{b}	6.2 ± 1.4	5.9 ± 1.0
		Methylocystaceae*	0.9 ± 0.1 ^{b}	9.9 ± 1.7 ^{a}	0.9 ± 0.3 ^{b}	4.4 ± 1.7	2.5 ± 1.2	4.8 ± 1.8	3.2 ± 0.8
		mitochondria	0	0.2 ± 0.1	1.9 ± 1.8	1.1 ± 0.8	0.6 ± 0.2	0	0.4 ± 0.2
		Rhodospirillaceae*	3.9 ± 0.9 ^{a}	0.8 ± 0.1 ^{b}	1.7 ± 0.4 ^{b}	1.7 ± 0.4 ^{b}	1.7 ± 0.3 ^{b}	1.5 ± 0.3 ^{b}	1.5 ± 0.2 ^{b}
	Betaproteobacteria	Gallionellaceae*	0.3 ± 0.1	0^{b}	1.0 ± 0.7^{a}	0.2 ± 0.1	0.3 ± 0.1	0.9 ± 0.2^{a}	0.4 ± 0.1
		Rhodocyclaceae*	1.5 ± 0.4 ^{a}	0.6 ± 0.3	0.6 ± 0.6	0.3 ± 0.1 ^{b}	0.4 ± 0.2 ^{b}	0.5 ± 0.1 ^{b}	0.2 ± 0.1 ^{b}
		SBla14*	0.5 ± 0.4 ^{b}	0.3 ± 0.1 ^{b}	1.5 ± 1.5 ^{b}	3.6 ± 0.9 ^{b}	2.0 ± 0.4 ^{b}	7.1 ± 1.0 ^{a}	6.9 ± 1.4 ^{a}
		SC-I-85*	3.0 ± 0.5^{ab}	0 ^{ac}	1.9 ± 0.5 ^{ab}	0.8 ± 0.2 ^{ac}	0.9 ± 0.3 ^{ac}	0.9 ± 0.3 ^{ac}	0.8 ± 0.1 ^{ac}
		unlassified Betaproteo*	3.0 ± 1.0	0.3 ± 0.1	4.3 ± 1.4	1.3 ± 0.2	1.9 ± 0.6	1.9 ± 0.4	1.7 ± 0.2
	Deltaproteobacteria	Myxococcales	0.2 ± 0.1	0.4 ± 0.2	0.4 ± 0.4	0.5 ± 0.2	0.1 ± 0.1	1.9 ± 1.0	0.8 ± 0.2
TM7	TM7-1	TM7-1*	2.8 ± 0.8^{c}	0.1 ± 0.0^{ab}	4.4 ± 0.4^{c}	0.9 ± 0.2^{ab}	1.7 ± 0.5 ^{ab}	1.6 ± 0.3 ^{ab}	1.0 ± 0.2 ^{ab}

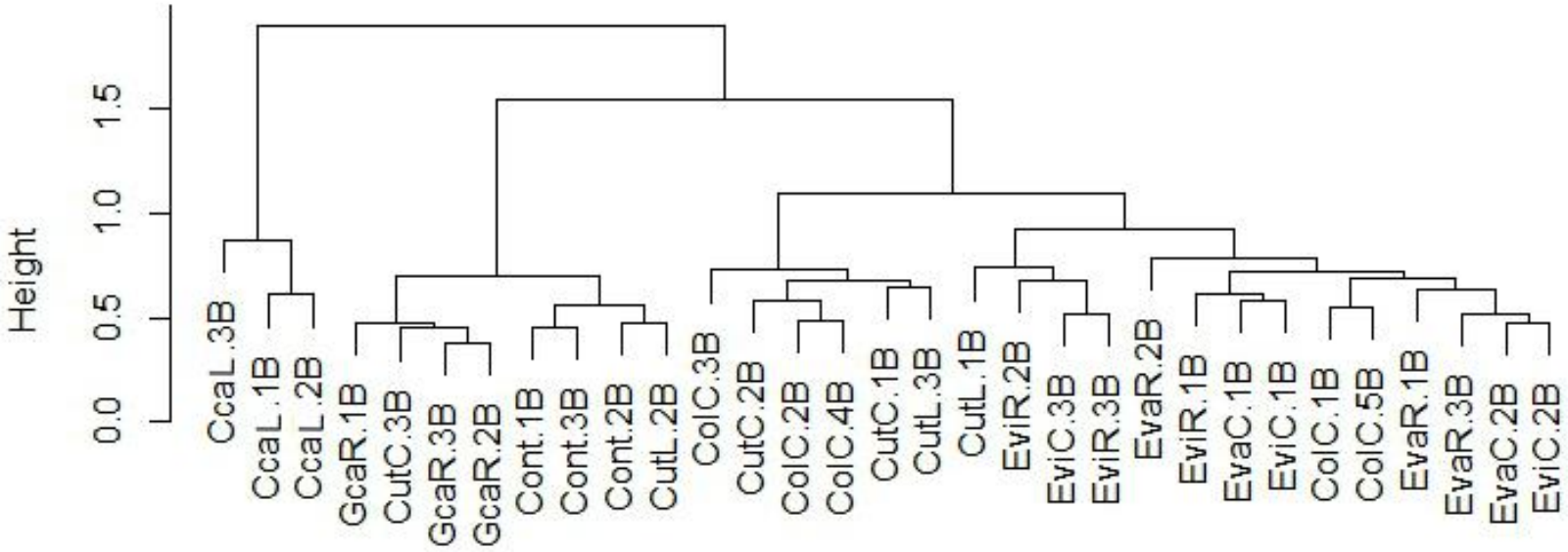

Figure 2. 1 Hierarchical clustering of the mesocosms based on bacterial community structure of the > 0.1 % abundant OTUs using the Spearman correlation coefficient. For mesocosm abbreviations see Table 2.2. The letter after species abbreviation indicates site of collection: C, Cartier; L: Lake Laurentian; R, Rockcut Lake.

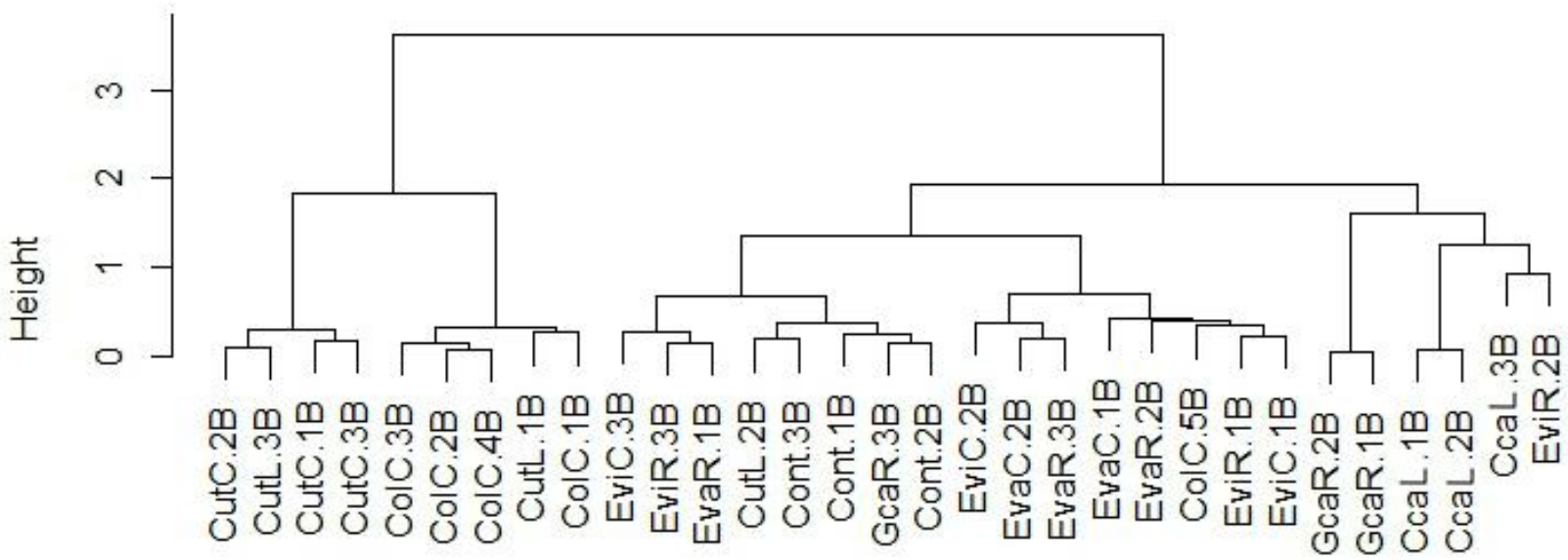

Figure 2. 2 Hierarchical clustering of mesocosms based on the bacterial community structure of the > 0.1 % abundant OTUs using the Pearson correlation coefficient. For mesocosm abbreviations see Table 2.2 and Figure 2.1.

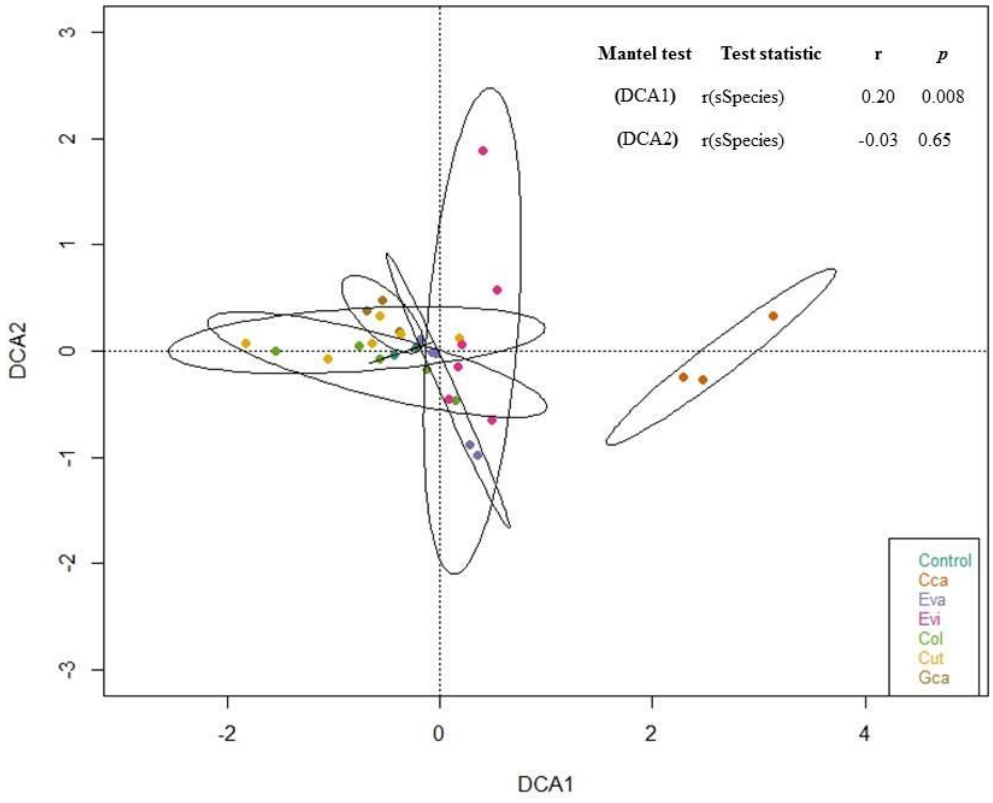

Figure 2. 3 DCA ordination of > 0.1 % abundant bacterial communities present in the rhizosphere of plant samples. Each dot represents bacterial community structure within a mesocosm. Ellipses represent 95% confidence. For mesocosm abbreviations see Table 2.2.

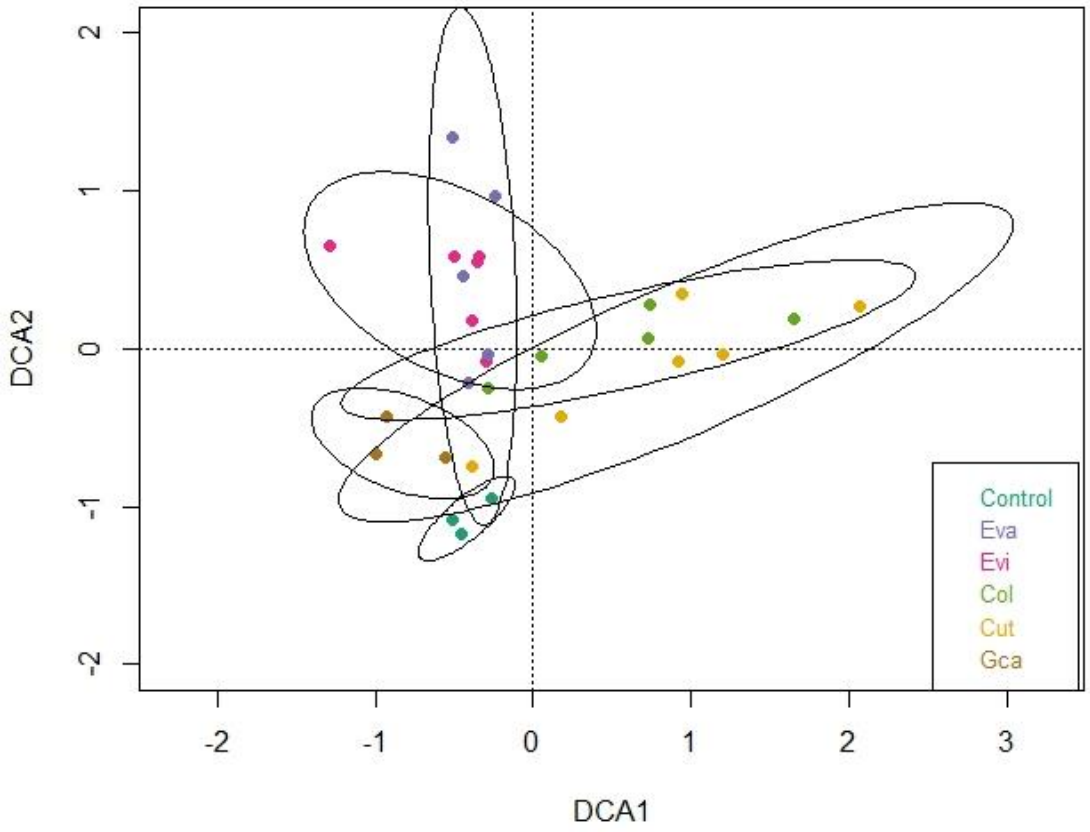

Figure 2. 4 DCA ordination of > 0.1 % abundant bacterial communities present in the rhizosphere of plant samples. This ordination does not include the Chamaedaphne calyculata in order to plot monocots. Each dot represents bacterial community structure within a mesocosm. Ellipses represent 95% confidence. For mesocosm abbreviations see Table 2.2.

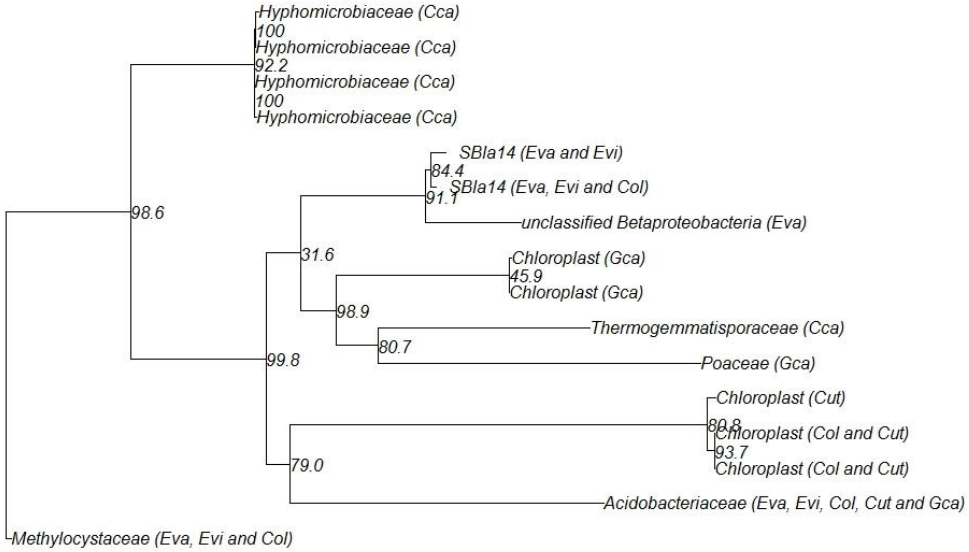

Figure 2. 5 Phylogenetic tree of bacterial indicator species matched to their families labeled by their significantly delineated plant species as determined by indicator species analysis (>1% abundant community). The numbers for the interior branches are bootstrap percentages. For mesocosm abbreviations see Table 2.2.

Table 2. 5 Abundance (mean ± 1 SD) of fungal taxa among control mesocosms and rhizosphere of the plant species (> 0.1% abundant community). For mesocosm abbreviations see Table 2.2.* indicates where value is significantly different from other plants. * indicates where family is significantly different among/between plants. Mean values followed by different letters are significantly different from each other.

Phylum	**Class**	**Control**	**Cca**	**Gca**	**Col**	**Cut**	**Eva**	**Evi**
Ascomycota	Dothideomycetes	1.1 ± 0.1	2.5 ± 0.9	3.4 ± 3.5	1.7 ± 0.7	6.7 ± 1.1	2.2 ± 0.8	18.8 ± 6.6
	Eurotiomycetes	3.0 ± 1.3	3.8 ± 1.2	2.0 ± 1.9	1.2 ± 0.3	2.2 ± 0.4	0.7 ± 0.2	1.0 ± 6.4
	Leotiomycetes	24.4 ± 3.5	33.8 ± 10.7	29.6 ± 7.5	28.1 ± 6.8	27.1 ± 3.0	40.7 ± 4.0	26.8 ± 6.7
	Pezizomycetes	0.3 ± 0.3	0	0	0	0	1.8 ± 1.6	0.2 ± 0.1
	Saccharomycetes	4.2 ± 1.2	0.2 ± 0.1	2.9 ± 2.7	2.2 ± 0.7	4.7 ± 2.3	0.6 ± 0.2	0.2 ± 0.1
	Sordariomycetes*	16.4 ± 0.5 [b]	2.0 ± 0.6 [a]	13.1 ± 8.6	16.1 ± 3.9 [b]	9.9 ± 2.2	6.8 ± 1.1	1.7 ± 0.3 [a]
	unclassified_Ascomy*	3.6 ± 1.2[a]	0.1 ± 0.0 [b]	0.8 ± 0.9 [b]	0.6 ± 0.3 [b]	0.5 ± 0.1 [b]	0.1 ± 0.1 [b]	0.1 ± 0.0 [b]
Basidiomycota	Entorrhizomycetes*	1.2 ± 0.9	0.1 ± 0.1 [b]	0.1 ± 0.2 [b]	0.1 ± 0.0 [b]	0.2 ± 0.1 [b]	4.6 ± 2.3	7.9 ± 1.5 [a]
	Agaricomycetes	0.9 ± 0.1	0	0.4 ± 0.4	0.6 ± 0.4	0.8 ± 0.3	0.1 ± 0.1	0
	Tremellomycetes	0.9 ± 0.3	0.1 ± 0.0	0.5 ± 0.3	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.0 ± 0.1
Blastocladiomycota	Blastocladiaceae	0.5 ± 0.4	0	0	0	0	0	0
Chytridiomycota*		0.6 ± 0.3	3.1 ± 1.3	4.0 ± 5.3	0.6 ± 0.3	0.9 ± 0.4	1.0 ± 0.2	1.3 ± 0.3
Glomeromycota		0	0	1.3 ± 3.3	0	0	0	0
LKM11		2.2 ± 1.1	6.8 ± 0.9	5.8 ± 12.9	9.0 ± 2.8	4.9 ± 1.2	3.0 ± 0.6	12.7 ± 8.3
LKM15		0.2 ± 0.2	2.3 ± 0.5	0.1 ± 0.1	1.2 ± 1.0	2.5 ± 1.7	0.9 ± 0.4	1.3 ± 1.0
Mucoromycotina*		2.1 ± 0.6 [a]	0.1 ± 0.0 [b]	1.3 ± 1.5	0.9 ± 0.3	1.0 ± 0.2	0.4 ± 0.1 [b]	0.1 ± 0.0 [b]
Nucletmycea	Fonticula	0	0.3 ± 0.1	0.2 ± 0.4	0.8 ± 0.5	0.1 ± 0.0	0.2 ± 0.1	0.6 ± 0.3

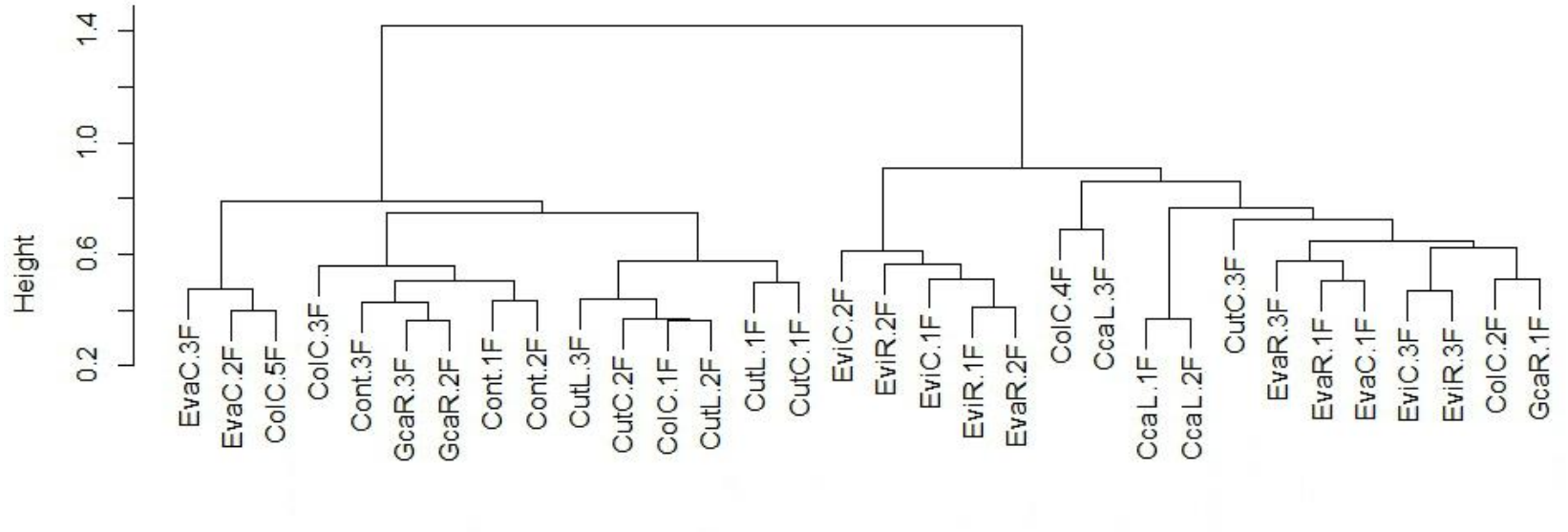

Figure 2. 6 Hierarchical clustering of the fungal community using the > 0.1 % abundant OTUs using the Spearman correlation coefficient. For mesocosm abbreviations see Table 2.2 and Figure 2.1.

Figure 2. 7 Hierarchical clustering of the fungal community using the > 0.1 % abundant OTUs using the Pearson correlation coefficient. For mesocosm abbreviations see Table 2.2 and Figure 2.1.

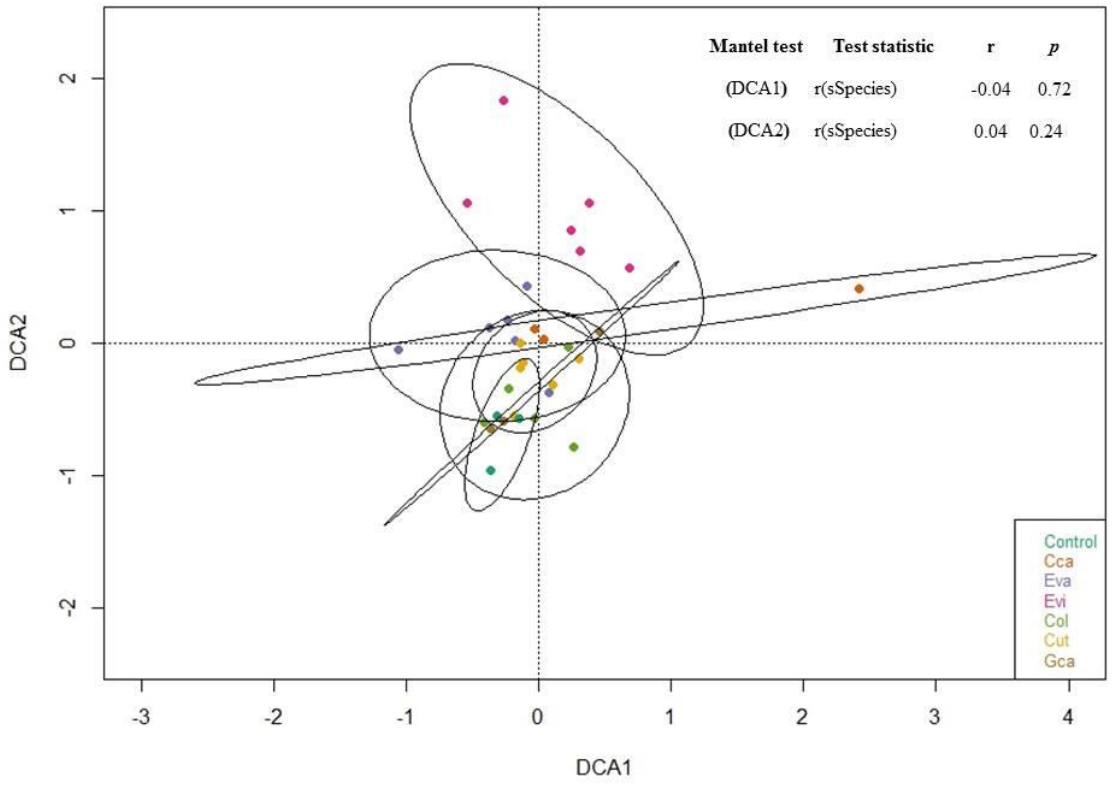

Figure 2. 8 DCA ordination of > 0.1 % abundant fungal communities present in the rhizosphere of plant samples. Each colored dot represents fungal community structure within each sample. Ellipses represent 95% confidence. For mesocosm abbreviations see Table 2.2. Differences in fungal community composition in DCA was not explained by differentiation along either of the DCA axes ($P > 0.05$).

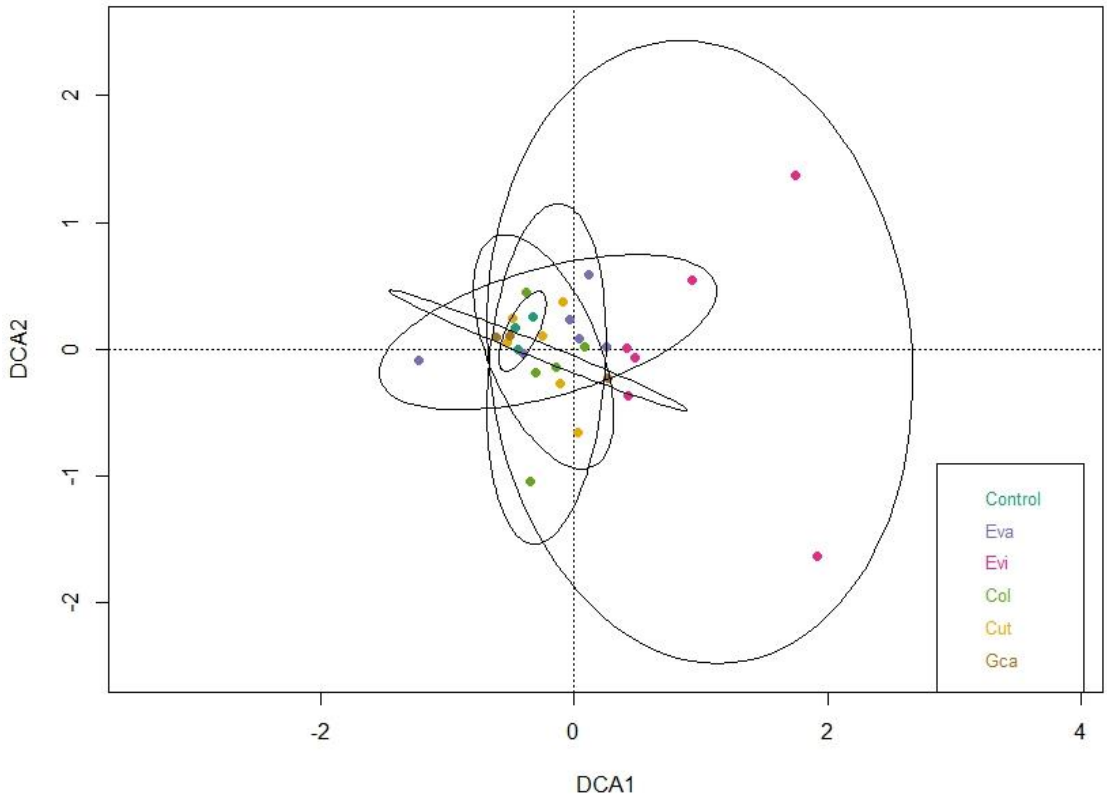

Figure 2. 9 DCA ordination of > 0.1 % abundant fungal communities present in the rhizosphere of plant samples. Each colored dot represents fungal community structure within each sample. Ellipses represent 95% confidence. For mesocosm abbreviations see Table 2.2.

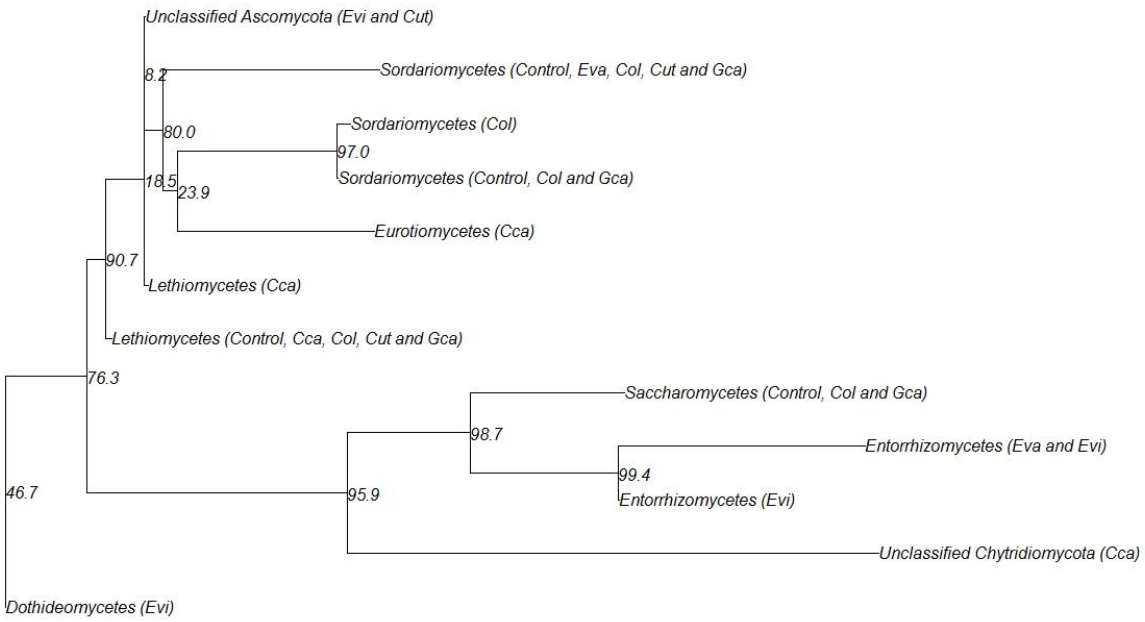

Figure 2. 10 Phylogenetic tree of fungal class labelled by their significantly delineated plant species as determined by indicator species analysis (> 1% abundant community). The numbers for the interior branches are bootstrap fractions. For mesocosm abbreviations see Table 2.2.

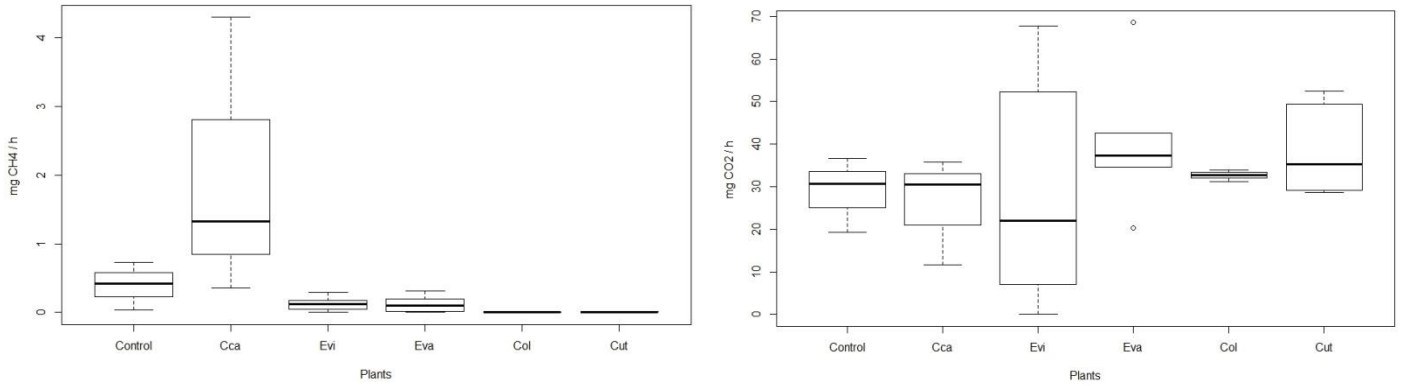

Figure 2. 11 Methane (a) and CO2 (b) production before the final harvest in August 2014 in the different mesocosms. For mesocosm abbreviations see Table 2.2.

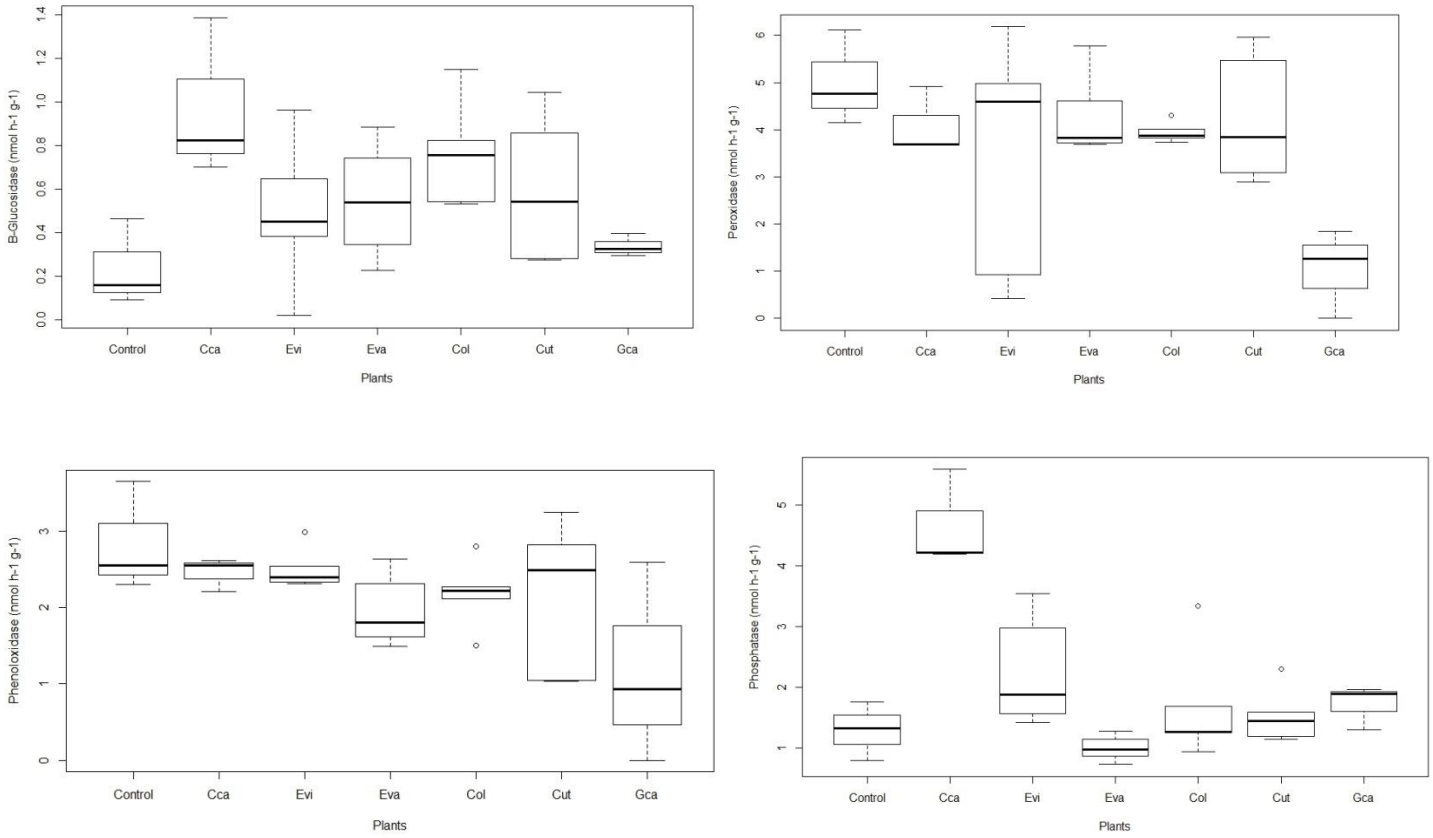

Figure 2. 12 Box plots of β-glucosidase (a), peroxidase (b), phenoloxidase (c), and phosphatase (d) levels within the rhizospheres of the different plants and control mesocosms. For mesocosm abbreviations see Table 2.2.

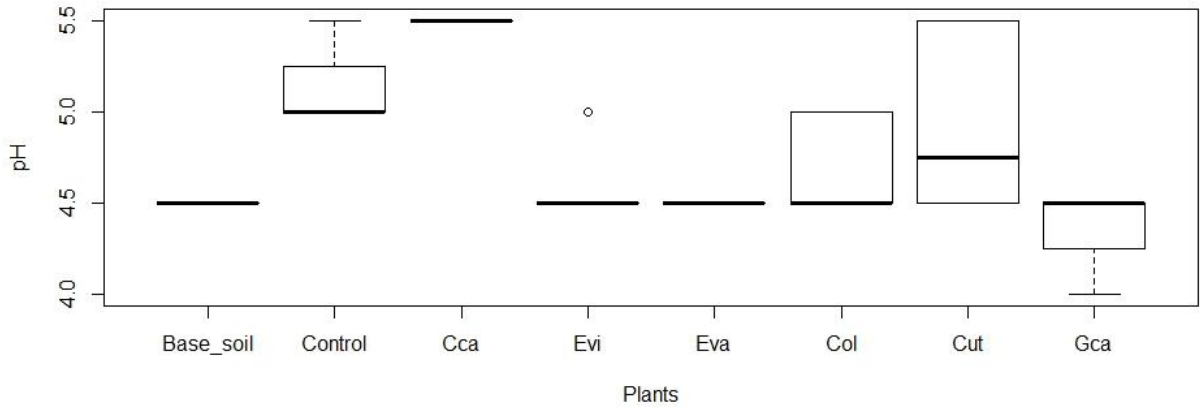

Figure 2. 13 Box plots of soil pH levels of the original soil (base soil), and at the end of the experiment in control mesocosms and within rhizosphere of the different plant species. For mesocosm abbreviations see Table 2.2.

Table 2. 6 Root dry matter content of each plant species investigated to characterize the plants (mean ± 1 SE).

Plant species	Root DMC (g g^{-1})
C. calyculata	0.30 ± 0.02
G. canadensis	0.20 ± 0.01
C. oligosperma	0.24 ± 0.01
C. utriculata	0.20 ± 0.02
E. vaginatum	0.14 ± 0.01
E. virginicum	0.19 ± 0.02

2.4 Discussion

Differences in microbial community structure among the different mesocosms

Rhizosphere microbiomes of bacterial and fungal communities differ among wetland plant species with respect to their composition after two years of growth in experimental mesocosms. To my knowledge this is the first study on species specificity of bacterial rhizosphere microflora using an experimental setting with wild plants collected from different locations. Findings indicate plant taxa-specific relations between the microbial communities of plant rhizospheres after two years of growth under controlled growth conditions, and given the same microbial inoculum. These patterns indicate that the diversity and composition of the rhizosphere-associated bacterial communities is to some extent specific to plant taxa, with a smaller number of fungal groups (compared to bacterial) showing this as well. Microbial community differences of rhizospheres among the plant species were present regardless of plants being from different sites. However, after two years under controlled conditions in an experimental garden, no differences between rhizosphere microbial communities of conspecific wetland plants originating from different sites could be found.

For a variety of agricultural crop species, (e.g., wheat, fava bean and maize) it has been demonstrated that rhizosphere bacterial communities in crop systems are species specific (Kuske et al., 2002; Mouhamadou et al., 2013; Becklin et al., 2012). Many studies have also shown genotype-specific differences in rhizosphere communities among varieties and inbred lines of maize (Aira et al., 2010; Bouffaund et al., 2012; Peiffer et al., 2013). Differences in rhizosphere communities may become more pronounced depending on environmental conditions (Feng et al., 2015). Studies of bacterial rhizosphere

communities of wild plants are not as common, but it has been found in dry grasslands that invasive species have different rhizosphere microbial communities than native plants (Kuske et al., 2002; Batten et al., 2006). Fungal communities have been more studied than bacterial ones in natural systems, and arbuscular mycorrhizal fungi (AMF) richness and diversity has been shown to vary among alpine host plants *Taraxacum ceratophorum, Taraxacum officinale,* and *Polemonium viscosum* (Becklin et al., 2012) as well as between *Agrostis capillaris* and *Trifolium repens* co-occurring in the same grassland ecosystem (Vandenkoornhuyse et al., 2002). Most of the studies of wild plants so far describe rhizosphere microbial communities as observed in the field; however, a 4-week experiment using microcosms by Mouhamadou et al. (2013) found that fungal communities in the rhizospheres of *Festuca paniculata* and *Dactylis glomerata* differed with respect to abundances of the OTUs.

The DCA and hierarchical clustering data shows different degrees of specificity among the microbial communities at different taxonomic levels of the plants. Specifically in the DCA of the bacterial community, each of the ellipses which describe the mesocosm positions with respect to the two first DCA axes are distinct, which suggests that rhizosphere bacterial communities of plant species are different. The slight overlap of ellipses with each other and with control mesocosms without plants indicates that the differences develop over time, with variation in the speed. However, differences between *K*- strategy bacteria and the faster growing r-strategy bacteria have been shown to reduce plant-specific differences between the microbial rhizosphere communities of older roots as compared to young, immature roots (Chiarini et al., 1998). Differences between plants of the same species that came from different sites were not obvious, which means that site

effects which may have been present at the very beginning had disappeared. The phylogenetic tree of individual plant taxa branched similarly to the phylogenetic tree of the bacterial species identified by indicator species analyses for those plants. However, only 2.8 ± 0.5 % of the bacterial community could be distinguished significantly according to indicator species analysis ($p<0.05$). The only eudicot species, *C. calyculata*, clearly distinguished in its rhizosphere microbial community composition from the monocots, the only Poaceae species differed from the species of Cyperaceae, and within Cyperaceae, the genus *Eriophorum* clearly distinguished itself from the genus *Carex*. Within *Carex*, rhizosphere microflora of *C. oligosperma* showed a trend to differentiate from that of *C. utriculata*. By contrast, plant-specificity of the fungal communities did not display the phylogenetic relations as clearly, but there was evidence of differences between fungal communities, for sample between the genera of *Carex* and *Eriophorum*.

Previous investigations among agricultural plants have found specifity of root microbiome at species and genotype level (Feng et al., 2015; Manter et al., 2010; Inceoglu et al., 2011; Aira et al., 2010; Elliott et al., 2014). The data in the present thesis for the first time shows that differences among the microbiomes of different taxa are phylogenetically connected: the observed differences increased when the taxonomic relation level of the plants was increased from species to genera to families, The question arises, to what extent do the differences in community composition among the plant taxa reflect ecological and functional differences among the plant taxa. All investigated species can co-occur in nutrient poor fens indicating certain ecological similarity. However, the ranges of the species are variable, for example *C. oligosperma* occurring in nutrient poor fens and bogs, whereas the characteristic habitats of *C. utriculata* are more-

nutrient rich marshes (Harris et al., 1996). Both *Eriophorum* species occur in nutrient poor fens and bogs, but show distinct growth form and phenology. *E. vaginatum* forms dense tussocks with filiform leaves, while *E. virginicum* has broader leaves and is colonial with long-creeping rhizomes (Flora of North America Editorial Committee 1993). Also, *Eriophorum* sp. had lower root DMC than *Carex* sp., indicating higher potential growth rates, possibly being able to colonize fast after a disturbance (Fort et al., 2015). *C. calyculata* had clearly the highest root DMC of all investigated species indicating the most conservative strategy with respect to nutrient economy (Fort et al., 2015), this possibly contributing to the distinctness of its rhizosphere community. The variation of microbial rhizosphere communities among the species closely matching their phylogenetic relations could indicate that the differences in rhizosphere community composition reflect rather phylogeny-related evolutionary history than ecological and functional differences among the taxa. This is supported by the phylogenetic relations among the bacterial OTUs indicative to the different plant species, which to some extent seem to match the phylogenetic relations among the plants. Four of the five bacterial OTUs indicative of *C. calyculata* rhizospheres were phylogenetically distinct from OTUs indicative to monocot rhizospheres, and three out of six OTUs indicative to *Carex* sp. were distinct to OTUs indicative to *Eriophorum*. Over the duration of the experiment, the microbiomes in the mesocosms differentiated enough that plant-specific communities developed which were significantly different from each other. The patterns of differentiation between the bacterial microbiomes seemed to follow similar branching to the phylogenetic tree of the plant species. This could indicate that at least some of the microbiome specificity is a result of coevolution between the bacteria and plants similar to how the soil bacteria community structure in the rhizosphere of maize plant seedlings

was shaped by genetic shifts that occurred during crop diversification (Bouffaud at al., 2012).

C. calyculata is widely spread in the area wetlands and it was the only plant widely present in all investigated wetlands. It had the most distinct bacterial rhizosphere community, with higher abundance of *Bradyrhizobiaceae, Hyphomicrobiaceae* and *Methylocystaceae* members and the only occurrence of *Chloroflexi.* These differences may be partially attributable to it being the only eudicot in the study, and the only woody species. *Chloroflexi* has an ability to function under a wider range of environmental conditions (Elliott et al., 2014), and it has been found to be involved in the anaerobic processes of photoheterotrophy and chemoheterotrophy (Steven et al., 2013). I observed bacteria belonging to the *Bradyrhizobiaceae* family, (which include nitrogen-fixing bacteria) present across all plants including control samples, whereas the family *Methylocystaceae*, which include methane-oxidizing bacteria, were found in the rhizospheres of *C. calyculata, Eriophorum* and *Carex* plants.

Results of the study showed differences in fungal rhizosphere communities among species and genera of wetland Cyperaceae. Cyperaceae are commonly assumed to be non-mycorrhizal (Gerdemann, 1968; Powell, 1975; Brundrett, 1991; Smith and Read, 1997). However, among 23 species of *Carex* surveyed from both upland and wetland habitats, arbuscular mycorrhizal fungi were found in the roots of 16 species (Miller et al., 1999), with wetland habitats being less conducive to mycorrhizal infections than upland habitats. Also, fungal communities are known to respond negatively to high water levels, with dry or well-drained regimes showing the best fungal growth and colonization by AMF of semiaquatic grasses as compared to water-saturated regimes (Miller and Sharitz, 2000).

All plant species in my study frequently occur in wetlands with completely submersed root systems, such as in the nutrient poor floating fen where all the Cyperaceae were collected. Kachalkin et al. (2008) found species-specific associations between phyllosphere yeasts and plants, specifically among *Sphagnum* and bog vascular plants including *Carex limosa* and *C. calyculata*. Other studies have shown that below-ground fungal communities in fens and bogs are primarily influenced by leaf-litter (Andersen et al. 2013).

C. calyculata are a member of the Ericaceae family, which are associated with Ericoid mycorrhizal fungi (Smith and Read, 1997). Selosse et al. (2007) were unable to produce any successful amplification of ericoid mycorrhizal fungi from samples of *C. calyculata* using PCR. Thus, this plant species may not have any ericoid mycorrhizal fungi, unlike other members of the Ericaceae family.

Ecological significance of the specificity

Differences in bacterial and fungal taxa in rhizospheres of the different species may give some indication of their ecological significance. My study showed that soil pH, phosphatase activity and methane production of *C. calyculata* mesocosms were higher than that of other pots, and the low abundance of *Acidobacteria* in *C. calyculata* pots may be associated with the pH sensitivity of this bacterial family (Jones et al., 2009). From my results the question arises; to what extent is microbiome specificity functional in an ecological context? Examples demonstrating ecological linkages have been found previously, such as symbioses of nitrogen-fixing bacteria with plant species giving plants access to atmospheric nitrogen (Oldroyd and Downie, 2008). Alternatively, root exudates

may be playing a role in manipulating the bacterial communities in the rhizosphere of these plants (Sorensen, 1997; Berg et al., 2005; Bever et al., 2012; Berg and Smalla, 2009), which is playing out in coevolution. The plant species in the present study occupy very similar habitats and often co-occur close to each other, thus, the expected ecological differences among their microbiomes would be small, compared to plants with different ecological requirements.

2.5 Conclusion

The compositions of bacterial communities show distinct trends for different taxa of wetland plants even under controlled conditions. The difference between the investigated monocots and the one investigated eudicot was especially pronounced, and within the monocots the phylogenetically more distinct plant taxa had more distinct bacterial rhizosphere communities. Based on these findings, the question about the relative importance of phylogenetic and ecological contributions for this differentiation arises, indicating that investigations of the co-evolutionary patterns between plant species and their rhizosphere micro-organisms could merit interesting results.

Chapter 3

Characterization of microbial communities associated with wetland plants along a gradient of industrial disturbances around Sudbury, Ontario

Abstract

Soil microorganisms are an important component of the rhizosphere of wetland plants. Microorganisms play important roles in many processes including nutrient and carbon cycling. Pollution, including trace metals, caused by mining may have negative effects on this community and throw these ecosystems out of balance. It is therefore critical that we understand how rhizosphere communities are influenced by pollution. Understanding the factors affecting the composition of the microbial community is a first step towards this goal. Therefore, it is important to understand how the microbial community varies between plant species as well as between sites. There is a lack of knowledge regarding the impacts associated with over a century of air pollution in the Sudbury area on the microbial community of the rhizosphere. This study aims to describe differences in species composition and diversity of microbial communities associated with the wetland plant rhizospheres along a gradient of environmental disturbance in the Sudbury area. Characterization of the microbial community included 16S/18S rRNA pyrosequencing to identify microbial diversity, and enzymatic analyses (hydrolases and lignases) to characterize microbial community function. Microbial communities within the rhizospheres were compared for two wetland plant species, *Chamaedaphne calyculata* and *Eriophorum vaginatum*, across four sites along a range of a historic industrial disturbance including metal contamination and soil acidity. Bacterial community structure was different among the sites, but within the sites, there were differences between the

rhizosphere communities of the two plant species as well. These patterns were less distinct for fungal rhizosphere communities.

3.1 Introduction

The abundance and diversity of soil microorganisms is an essential component of freshwater wetland ecosystems. Wetlands represent a transition from terrestrial to aquatic ecosystems (McCaig et al., 2001) and their microbial community influences valuable internal processes such as the cycling of nutrients and carbon, which, in turn, affect water quality and the global carbon cycle (Richardson and Marshall, 1986; Roulet, 2000). However, the structures of microbial communities in wetlands are not well understood (Hartman et al., 2008). DNA renaturation studies showed that there are a billion bacterial cells, up to 10^6 16S rRNA gene sequences, and 10^3 bacterial species per 1 g soil sample (Gans et al., 2005). The significance of microbes within the rhizosphere has long been recognized. For instance, the rhizosphere microbiome interacts with plants in many ways including promoting growth (Jetiyanon and Kloepper, 2002), protection from pathogens (Raaijmakers et al., 2009) or facilitating uptake of nutrients (Hawkins et al., 2000; Miransari, 2011; Adeleke et al., 2012). Rhizosphere bacteria may also be involved in nitrogen fixation, which is highly important in wetland ecosystems which may be nutrient poor (Khan, 2005). Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with roots in most land plants; root exudates are often essential nutrients for microbes. However, little is known about the abundance and structure of different microbial communities specifically in association with plant species in wetland ecosystems.

In the past century, the Sudbury area has been affected by industrial emissions of sulfur and metals deposited over the landscape (Potvin and Negusanti, 1995). Since the decrease of SO_2 and metal emissions in the 1960's, it has been shown that the quality of lake water and biological communities have improved (Gunn and Keller, 1990; Keller et al., 1992a;

Keller et al., 1992b; Havas et al., 1995), but the legacy of metals remains in the wetland soils that have acted as sinks for these metals and accumulated them over time. Recent studies have shown that Sudbury's peatlands still contain high levels of metals (Pennington, 2014) and that these metals can be re-mobilized following drought events (Szkokan-Emilson et al., 2013; Szkokan-Emilson, 2014). A recent survey on plant diversity and plant health showed that plant cover was negatively related to metal concentrations in the surface peat (total metal content, g kg^{-1}), as well as the Sphagnum cover (Barrett et al., 2014). Meanwhile, Luke et al. (2015) found that bacterial communities shared similar taxa across a metal contamination gradient, while fungal communities varied much more but microbial respiration in these sites generally responded similarly to C substrate additions.

Improving our knowledge of the microbial community is the first step to understand how the environment affects soil function (Sessitsch et al., 2001). Soil structure provides a heterogeneous habitat for microorganisms specified by altered concentrations of substrates, nutrients, oxygen, water, and pH values (Ladd et al, 1996). Diversity of the microbial community depends on the structure of soil and its pH (Hartman et al., 2008; Nacke et al., 2011 Preem et al., 2012), as well as the availability of nitrogen (Frey et al., 2004) and phosphorus (Richardson and Simpson, 2011) in the soil. Abiotic stresses including pH (Rousk et al., 2010) affect the community and abundance of microorganisms (Pineda et al., 2013). Metals such as Cu, Ni, Cd and Zn have been shown to have negative impacts on microbial diversity including damage to mycorrhizae (Koomen et al., 1990), reductions to number of rhizobia (Chaudri et al., 1992) and lowered microbial biomass in general, and shifts in microbial community structure

(Sandaa et al., 1999). Root exudates including sugars (fructose, glucose, sucrose), organic acids (succinic acid, malic acid) and amino acids (arginine, serine and cysteine) change the structure and activity of the root- associated microbial community (Griffiths et al., 1999; Berg and Smalla, 2009; Aira et al., 2010). Therefore the structure of the particular rhizobiome microbial community depends on the type of soil and plant species (Mahaffe and Klöpper, 1997; Duineveld et al., 1998).

Gaps remain in scientific knowledge about how rhizosphere communities are influenced by industrial pollution, and what effects this might have on wetlands in ecosystems recovering from industrial impacts. The purpose of this study was to evaluate differences in species composition and diversity of microbial communities associated with the wetland plant rhizosphere along a gradient of industrial disturbance (mining and smelter effect) in four sites within the Sudbury area. The study was conducted by measuring microbial diversity within the rhizosphere using high-throughput sequencing methods of two different wetland plants which were present throughout the studied sites; *Chamaedaphne calyculata* L. Moench (leatherleaf) and *Eriophorum vaginatum* L. (cottongrass). Ultimately, my goal was to expand the understanding of particular diversity in the rhizobiome of these two specific wetland plants along a gradient of pollution in Sudbury area. It was anticipated that microbial community level analyses would be able to distinguish between the rhizobiomes of these specific wetland plants across a range of peatland types and Cu and Ni concentrations.

3.2 Methods

3.2.1 Study sites, plant, and rhizosphere soil collection

Four wetlands in Sudbury, Ontario, Canada were chosen, along a gradient of metal concentrations and a range of physical and chemical characteristics: Rockcut Lake (RCK), Silver Lake (SLV), Lake Laurentian (LU) and Daisy Lake (D5). The surface peat of the sites (Table 3.1), ranged with respect to metal concentrations for Ni between 915 and 242 mg/kg, for Cu between 466 and 105 mg/kg and for Fe between 16 and 2.5 g/kg (Pennington, 2014; Barrett, 2014) (Table 3.1). Rockcut site is a nutrient poor, shrub dominated fen, 0.46 ha in size and surrounded by a mixed conifer forest dominated by black spruce (Barrett, 2014). This site is located to the North of Sudbury, next to Rockcut Lake. Silver site is a nutrient poor, sedge dominated fen, 1.07 ha in size, surrounded by birch stands. It is located South of Sudbury, next to Silver Lake. Laurentian site is a nutrient poor, shrub dominated fen, 3.08 ha in size. It is surrounded by mixed conifers and deciduous trees and is close to Lake Laurentian on the university campus in the South end of Sudbury. Daisy site is a nutrient poor, sedge/grass dominated fen, 2.05 ha in size. Water table in the site was the lowest of all. This site is surrounded by mixed conifers and deciduous trees and is located close to Daisy Lake in the South-East end of Sudbury.

Two common wetland plant species were chosen that were present at each of the four sites: *Chamaedaphne calyculata* L. Moench and *Eriophorum vaginatum* L. *C. calyculata* had 100 % frequency of occurrence from eighteen $1m^2$ quadrats per wetland across Laurentian, Silver and Rockcut sites and 12.5 % at Daisy site from Barrett, 2014 (Table 3.1) whereas, *E. vaginatum* had varying (93.75 % - 0.0^* %) frequency of occurrence from

eighteen $1m^2$ quadrats per wetland across four sites (Table 3.1, Barrett, 2014). Each species was collected in triplicate from each site on 22-25 of July, 2013.

For the determination of the microbial composition, soil samples were collected from the rhizosphere by removing the bulk soil and collecting only the soil adhering to the roots. Rhizosphere soil was pooled from numerous roots until ~10 g had been collected from each individual plant, for 4 locations, 2 species, and 3 replicates making 24 samples in total. The samples were stored at –20 °C until DNA extraction was performed within a week of collection.

3.2.2 Microbial Community Analyses

Total microbial community DNA was extracted using the PowerSoil® DNA Isolation Kit (Mobio, California) according to the manufacturer's protocols. Purified DNA was quantified on a Nanodrop 8000 Spectrophotometer (Thermo Scientific). Samples were sent for SSU gene pyrosequencing analyses at Mr. DNA-Molecular Research LP (Shallowater, Texas) using the Roche 454 GS-FLX platform (Roche 454, Branford, CT, USA). Samples were targeted for 16S rRNA gene (28F-5'-GAGTTTGATCNTGGCTCAG-3', 519R-5'GTNTTACNGCGGCKGCTG-3'), and (Dowd et al., 2008a), and eukaryal/fungal 18S rRNA (ISSU) (F-5'-TGGAGGGCAAGTCTGGTG-3', R-5'-TCGGCATAGTTTATGGTTAAG-3') (Foster et al., 2013), pyrosequencing. Sequencing was performed using titanium amplicon pyrosequencing (bTEFAP) using a one-step PCR as previously described (Dowd et al., 2008b). Pyrotagged samples were amplified using ePCR using Hot Start and HotStar high-fidelity *Taq* (Qiagen) to generate ~400bp reads for each of the above primer libraries at a sequence depth of 3000 reads per sample. All sequencing and quality filtering were

conducted by the sequencing facility and included removing primer sequences, reads < 200bp long, sequences with ambiguous base calls, homopolymer repeats greater than 6bp, and chimera removal (Dowd et al., 2008b).

Analysis of pyrosequencing data was performed for all rhizosphere samples from Daisy, Laurentian, Silver and Rockcut datasets. Filtered quality sequences were analyzed using the QIIME software package (Quantitative Insights into Microbial Ecology (Caporaso et al., 2010); http://pyro.cme.msu.edu/index.jsp) for the bacterial 16S libraries, and using the Silva SSU reference alignments (Pruesse et al., 2007) for the eukaryotic/fungal 18S rRNA libraries. Operational taxonomic units (OTUs) are assigned using complete linkage clustering at 97% similarity (Cole et al., 2009). OTUs were taxonomically classified using the Silva database (Release 110) and verified using the RDP classifier (Wang et al., 2007). Diversity was estimated using the Shannon and Chao indices, as well as Unifrac distances, calculated using the Qiime pipelines (Caporaso et al., 2010). OTUs accounting for greater than 0.1% of the total reads in each dataset were used in generating community taxonomic profiles. The representative sequences for the > 0.1% OTUs for each data set were trimmed to the shortest sequence length, aligned using ClustalW with ambiguously aligned positions removed, and the alignments used to generate maximum likelihood (Tamura et al., 2011). Comparison of community profiles and the site/soil characteristics were completed with multivariate analyses using Qiime and the R vegan package (Oksanen et al., 2013).

3.2.3 Enzyme analyses

Activity of hydrolases (β- glucosidase and phosphatase) and lignases (phenoloxidase and peroxidise) were determined in the rhizosphere soil (Sinsabaugh, 2009; Hendel et al.

2005; Saiya-Cork et al., 2002). For both lignase and hyrolase assays 0.5 to 1 g rhizosphere soil was mixed with 60 ml of 50 mM acetate buffer (pH: 5) and blended for 1-2 min to homogenize the sample. The lignase assay medium contained 50 mM acetate buffer (pH: 5), 25 mM L-DOPA and 0.3% H_2O_2 (for peroxidise wells). Lignase assay was followed at 460 nm. Activity was calculated using the extinction coefficient of L-DOPA (7.9 μM−1). The hydrolase assay medium contained 50 mM acetate buffer (pH: 5), 10 μM MUB (4-Methylumbelliferone), 10 μM MUB β-D-glucopyranoside (for β-glucosidase wells) and 10 μM MUB-phosphate (for phosphatase wells) in a final assay volume of 250 μl. Microplates were incubated for 4 h at 20℃. At the end of incubation of hydrolase assay 10 μL of 0.5 N NaOH was added to each wells to raise to pH above 7.5 to make the fluorescence reading obtainable. Hydrolase assay was followed at 365 nm, emission Quench coefficients were calculated for each sample independently and used to calculate β- glucosidase and phosphatase activities.

3.2.4 Soil measurements

Soil organic matter (SOM) is influenced by climate, management, mineral composition, topography, soil biota and interaction between those factors (Krull et al., 2004). Also, degradation of soil organic matter is mainly driven via microbial respiration (Lutzow et al., 2006). Soil organic matter content was determined by loss on ignition. Dried 2 g samples were heated to 150 °C by 0.7 °C per minute, where the temperature remained for half an hour. Temperature was increased to 450 °C by 0.3 °C per minute where it remained for 2 hours. Percentage of the weight loss was considered to be organic matter content of the samples (Hughes et al., 2009; Riley, 2012).

Soil pH was measured using pH indicators (Hellige – Truog Soil pH Tester Kit). The method was completed as outlined by the manufacturer's protocol. Briefly, a pinch of wet soil sample was placed onto the color reaction plate. Four drops of Reagent N triplex Indicator were added in soil and mixed with a spatula. Reagent M Reaction Powder was added by using the spatula, as soil sample surface was covered with Reagent M Reaction Powder. After 2 minutes, pH can be determined (+/- 0.5) depending on the colour change of the indicator. The remaining bulk soil was stored at -20 °C until further processing.

3.2.5 Assessment of site conditions based on in-situ plant characteristics

Intraspecific comparisons of several plant characteristics allowed for a relative comparison of the growth conditions at the different study sites. Leaf dry matter content (LDMC), shows a sensitive response to various stresses, increasing with decreasing growth rates (Ryser and Lambers, 1995). LDMC was determined by the method of Vile et al. (2005). Leaf samples were cut from the plants, wrapped with moist paper towel and put in a plastic box and stored in a refrigerator for 24 h. Then, the leaves' fresh weight was measured and the leaves were oven-dried at 75 °C for 4 d and dry weight was measured. Each species had 5 replicate plants. The equation for LDMC follows:

$$LDMC = Leaf_{dry\ mass} / Leaf_{saturated\ fresh\ mass}$$

LDMC also helps to describe the ecological strategy of the investigated species, as it is related to vital functions (e.g. leaf lifespan, relationship between rapid production of biomass and conservation of nutrients) (Grime et al., 1997; Poorter and Garnier, 1999; Ryser and Urbas, 2000).

Plant size is a coarse indicator of how conducive the site conditions are to growth. To describe plant size, single stem tiller size was determined from 3 replicates per plant and per site. Each *Eriophorum* tiller length was measured from the longest tip to bud scar. Each *Chamaedaphne* plant stem length was measured from the shoot tip to roots of last year. After length determination, plants were cleaned of soil and dust, put in envelopes and oven dried at 75 °C until they were dry. Then, each plant's dry mass was recorded.

3.2.6 Phytometer experiment in the laboratory

Soil productivity of the study sites was described using phytometers (Wheeler et al. 1992). This technique allowed us to compare relative potential plant growth on different growth substrates and under controlled growth conditions. Bulk soil from each study site was added into 15 ml pots (four sites; LU, SLV, D5 and RCK with 6 replicates each for a total of 24 pots) and planted with one *Phalaris arundinacea* seed each pot in a controlled growth chamber (16 h day/8 h night) under 23 ºC for about 3 weeks. After harvest the shoots were dried for at least 24 h at 75 °C and their weights were recorded.

3.2.7 Statistical analysis

R (version 3.0.1) was used along with the phyloseq (version 1.6.1) package (McMurdie and Holmes, 2013, version 1.6.1) to transform absolute abundance to relative abundance for the phylogenetic groups within the samples. The Shannon - Wiener diversity index was calculated based on total bacterial and fungal community. A threshold of > 0.1% relative abundance was used to select for data which would have an appreciable contribution to the microbiome and eliminate rare species. The vegan package (Oksanen et al., 2013, version 2.0-10) was used to perform multivariate analysis. ANOVA was used to compare the relative abundance of bacterial families and fungal classes of the

rhizospheres of the two plants, pooled for all sites. Because there was no difference between the plants, ANOVA was again used to compare the relative abundance of bacterial families and fungal classes of the rhizosphere samples by sites, pooled for plants. Hierarchical clusters were generated by the gplots package (Warnes et al., 2014, version 2.13.0) using hclust function. A Spearman correlation matrix was first generated then *Ward's* minimum variance method was used to organize the matrix into a series of compact spherical clusters presented as a dendrogram. ade4 package (Dray and Dufour, 2007; Chessel and Dufour, 2004; Dray et al., 2007, version 1.6-2) was used to perform mantel tests using DCA scores of Unifrac values for differences between sites and plants. The Mantel statistic estimates the correlation between two proximity matrices. *p* value represents the significance of the Mantel regression coefficients distance from zero following 9,999 permutations of DCA 1 and DCA 2 after transformation of site and plant values to ordinal ranks. Indicspecies (Cáceres, & Legendre, 2009, version 1.7.4) package was used to determine indicator species for >1% abundant bacterial and fungal OTUs. First, multipatt function was used to find associations between sites/plants and combination of those sites/plants with 10,000 permutations. Then, OTUs that were significantly associated with sites or plants (p-value < 0.05) were considered as indicator species and matched taxa (family for bacterial data or class for fungal data) were plotted in phylogenetic tree with the bootstrap values. DCA was performed using bacterial family and fungal class abundances with environmental variables included into the ordination.

Table 3. 1 Sample site coordinates, peat soil metal content and relative abundance of target wetland plants (Barrett, 2014). *: Previous survey indicated that there was no *Eriophorum vaginatum* in Daisy Lake wetland areas, but during the field visits we found very small tussocks of *Eriophorum vaginatum*.

Drainage Waterbody	Laurentian	Silver	Rockcut	Daisy
Wetland Code	LU	SLV	RCK	D5
Latitude	46.455146	46.431819	46.727457	46.45354
Longitude	-80.967763	-81.017149	-80.926603	-80.8893
***Acid extractable peat Al, g/kg* (SD)**	4 ± 0	6 ± 1	7 ± 1	19 ± 1
***Acid extractable peat Fe, g/kg* (SD)**	7 ± 0	7 ± 1	5 ± 1	17 ± 1
***Acid extractable peat Mn, mg/kg* (SD)**	59 ± 7	19 ± 1	37 ± 2	115 ± 10
***Acid extractable peat Ni, mg/kg* (SD)**	918 ± 63	618 ± 47	181 ± 26	570 ± 175
***Acid extractable peat Cu, mg/kg* (SD)**	801 ± 206	895 ± 325	254 ± 20	489 ± 53
Eriophorum vaginatum L. *(% frequency of occurrence)*	18.75	93.75	12.5	0.0*
Chamaedaphne calyculata L. Moench *(% frequency of occurrence)*	100	100	100	12.5

3.3 Results

3.3.3 Distribution of bacterial communities between plant species

In total, 7,822 different bacterial OTUs were found in the rhizosphere of the two plant species from Daisy Lake, Lake Laurentian, Silver Lake and Rockcut Lake sites and 2,194 OTUs were present in the > 0.1 % abundant bacterial population (Appendix G). The < 0.1 % abundant bacterial population (rare biosphere) comprised 12.1 to 49.9 % of the total bacterial population. Shannon- Wiener diversity of the bacterial communities of the rhizospheres did not show any differences between the two plant species (ANOVA, $F=0.0586$, $p = 0.811$), whereas there was a significant difference among the Shannon-Wiener diversity of the bacterial communities of the soils of the rhizosphere samples from the sites (ANOVA, $F= 13.064$, $p < 0.0001$) with rhizosphere communities from Rockcut and Daisy sites having the most diverse communities (Figure 3.1).

116,160 sequences classified within the bacterial domain were affiliated to 29 phyla (Appendix H). The dominant phyla across all rhizosphere samples were Acidobacteria, Proteobacteria, Chloroflexi and Actinobacteria, representing 55.4, 33.1, 1.9 and 1.7 %, respectively, in total abundance (Figure 3.1).

The most abundant families among the cottongrass plants were *Koribacteraceae* (29.3 ± 3.6 %), *Acidobacteriaceae* (10.2 ± 2.0 %), *Hyphomicrobiaceae* (4.5 ± 0.9 %), *Bradyrhizobiaceae* (2.8 ± 0.5 %), *Rhodospirillaceae* (1.8 ± 0.6 %), *Methylocystaceae* (1.6 ± 0.4), and *Sinobacteraceae* (1.4 ± 0.3 %), and whereas the most abundant families in leatherleaf were *Koribacteraceae* (17.6 ± 3.3 %), *Acidobacteriaceae* (16.8 ± 3.1 %) followed by *Bradyrhizobiaceae* (5.5 ± 1.4), *Hyphomicrobiaceae* (3.1 ± 0.6 %)

Methylocystaceae (1.7 ± 0.5), *Sinobacteraceae* (1.7 ± 0.3 %), and *Ktedonobacteraceae* (0.9 ± 0.4 %) (Table 3.2). *Koribacteraceae* was the only bacterial family significantly differentiated between the rhizosphere of the plants (ANOVA, F = 5.24, p = 0.032). Hierarchical cluster analysis of soil samples based on their bacterial community structure showed the plants branch out separately within each of four site clusters (Figure 3.2). Leatherleaf and cottongrass could not be distinguished using DCA with no significant differences looking at the communities as a whole (Figure 3.3).

3.3.4 Distribution of fungal communities among plant species

In total, 2,881 fungal OTUs were found in the rhizosphere samples from Daisy, Laurentian, Silver and Rockcut Lake sites (Appendix M). The > 0.1 % abundant fungal population (rare biosphere) comprised 6.1 to 37.1 % of the total fungal population. Shannon- Wiener diversity index was calculated based on total fungal community and there were no significant differences between the fungal communities of the two plant rhizosphere (ANOVA, F = 0.06, p = 0.81) as well as among sites (ANOVA, F = 0.678, p = 0.576, Figure 3.4). I found 629 OTUs present in the > 0.1% abundant fungal community (Appendix M).

186,180 sequences classified within a domain were affiliated to 13 fungal phyla (Appendix N and Appendix O). The dominant phyla across all rhizosphere samples from the sites were *Ascomycota*, *Basidiomycota*, LKM11, *Chytridiomycota* and *Mucoromycotina*, representing 89.8, 4.3, 2.3, 1.3 and 1.3 %, of all sequences that were assigned to Fungi, respectively.

The dominant fungal phyla in the leatherleaf samples were *Ascomycota* (84.0 ± 2.9 %) followed by *Basidiomycota* (3.1 ± 1.6 %), LKM11 (2.3 ± 0.7 %), *Mucoromycotina* (1.6 ± 0.8 %), and *Chytridiomycota* (1.0 ± 0.3 %), where as in cottongrass, the dominant phyla were *Ascomycota* (83.1 ± 2.5 %), *Basidiomycota* (4.5 ± 2.1 %), LKM11 (1.8 ± 0.6 %), *Chytridiomycota* (1.0 ± 0.2 %), and *Mucoromycotina* (0.6 ± 0.2 %) (Table 3.3). Cottongrass plants had higher abundances of the fungal classes *Leotiomycetes* and *Agoricomycetes* than leatherleaf. Leatherleaf plants had higher abundance of *Dothideomycetes, Sordariomycetes* and *Eurotiomycetes* than cottongrass plants did (Table 3.3). Hierarchical cluster analysis of soil samples based on their fungal community structure did not show any distinct pattern for plants (Figure 3.5). DCA of fungal communities did not differentiate between plant species (Figure 3.6).

3.3.1 Rhizosphere bacterial community distribution across study sites

There were several differences between rhizosphere communities from Daisy and the other sites. In general, it was composed of a larger percentage of *Proteobacteria* (58.8 ± 2.5 %) and a smaller percentage of *Acidobacteria* (23.5 ± 2.3 %) (Figures 3.1 and Appendix I). Also, there were 0.1 to 0.6 % of chloroplast sequences which will be filtered out in later publications.

Acidobacteria was the most abundant class in the rhizosphere samples from Laurentian (71.6 ± 2.3) and Silver (68.4 ± 2.5), with decreasing abundance in Rockcut (58.3 ± 4.8), and Daisy (23.5 ± 2.3) (Appendix H and Appendix J). There were two families from this class; *Acidobacteriaceae* and *Koribacteraceae* (Appendix J and Appendix K). Rhizosphere samples from Silver had the highest abundance of *Acidobacteriaceae* (23.0 ± 2.2 %), then Laurentian (18.3 ± 3.5 %), and Rockcut (11.0 ± 1.9 %). Rhizosphere samples

from Daisy had the significantly lowest abundance of *Acidobacteriaceae*. *Koribacteraceae* was the most abundant family in Laurentian (34.6 ± 4.8 %), Silver (26.2 ± 3.4 %), Rockcut (23.6 ± 6.6 %), and Daisy (9.5 ± 0.8 %) (Appendix J and Appendix K).

Alphaproteobacteria members were most abundant in the rhizosphere samples from Daisy, Rockcut, Laurentian and Silver sites, in that order. There were six families from this class; *Acetobacteraceae, Bradyrhizobiaceae,* Ellin330, *Hyphomicrobiaceae, Methylocystaceae* and *Rhodospirillaceae* (Appendix J and Appendix K). *Acetobacteraceae* family was mostly present in the rhizosphere of Silver, Laurentian, Rockcut and Daisy, in that order (Appendix J). Daisy (9.4 ± 1.9 %) displays significantly higher abundances of the *Bradyrhizobiaceae* family than Silver (3.1 ± 0.6 %), Rockcut (2.1 ± 0.3 %), and Laurentian (2.0 ± 0.5 %). *Hyphomicrobiaceae* was significantly higher in the rhizosphere of Daisy (7.8 ± 1.5 %) than Laurentian (3.4 ± 0.3 %), Rockcut (2.1 ± 0.2 %), and Silver (2.1 ± 0.3 %). Laurentian, Silver and Rockcut were sites which had most abundant *Methylocystaceae* in the rhizosphere, representing 2.5, 1.7 and 2.0 % abundance, respectively. *Rhodospirillaceae* family was significantly higher in the rhizosphere samples from Daisy (5.0 ± 0.6 %, Appendix J).

Betaproteobacteria were mostly abundant in the rhizosphere from Daisy site with 6.5 ± 0.7 % abundance. There were two families from this class; Ellin6067 and *Oxalobacteraceae* (Appendix J and Appendix K). Both of those families were significantly associated to Daisy.

The only family of the class *Gammaproteobacteria, Sinobacteraceae*, was found in the rhizosphere samples from Daisy (2.3 ± 0.3 %), Rockcut (2.1 ± 0.3 %), Laurentian (1.1 ± 0.3 %) and Silver (0.6 ± 0.2 %) sites (Appendix J and Appendix K).

Ktedonobacteria members were most abundant in the rhizosphere at Silver site. Families of this class were *Ktedonobacteraceae* and *Thermogemmatisporaceae* (Appendix J and Appendix K).

Hierarchical clustering analysis (using the total community profile/unifrac measures) showed that bacterial communities clearly differed within the rhizosphere samples among the sites (Figure 3.2). The first branch of the hierarchical clustering dendrogram contained all the Daisy samples, with the other branch separating into Rockcut, Laurentian and Silver sites. This indicates that sites were a major determinant of bacterial community structure but within the sites the bacterial community structure appears more similar within the rhizosphere of individual plant species.

Sites were effectively differentiated through detrended correspondence analysis. Daisy had considerably lower variation along DCA1 than the other sites. Rockcut Lake, which as the least polluted site had the greatest variation along DC1 compared to the other three sites. A mantel test examining differences between sites confirmed that differences between the sites along DCA1 and DCA2 were significant ($p<0.001$, Figure 3.7) however, Laurentian and Silver were indistinguishable from each other.

The phylogenetic tree of bacterial indicator species within the >1 % abundant communities shows that there are a number of bacteria that effectively represents the individual sites. Several OTUs belonging to the *Koribacteriaceae* family were unique to

rhizosphere at Daisy, Laurentian and Silver sites. Daisy site also had a member of the *Bradyrhizobiaceae* significantly associated with it (Figure 3.8 and Appendix L).

3.3.2 Rhizosphere fungal community distribution across study sites

Ascomycota was, by far, the most abundant phylum in all rhizosphere samples from the sites; Rockcut (95.6 ± 0.8 %), Silver (93.5 ± 1.33 %), Laurentian (87.3 ± 4.2%), and Daisy (82.7 ± 4.3%) (Figure 3.4 and Appendix N). *Ascomycota* had six classified classes; *Dothideomycetes, Eurotiomycetes, Lecanoromycetes, Leotiomycetes, Orbiliomycetes* and *Sordariomycetes*. *Leotiomycetes* had the highest abundance of all classes. It was highest in the rhizosphere from Rockcut (73.7 ± 3.8), Silver (71.4 ± 1.7), Laurentian (63.0 ± 6.5) and Daisy (47.8 ± 5.3), in that order (Appendix P and Appendix Q). *Dothideomycetes* was the second most abundant class among all rhizosphere samples from the sites with highest abundance in Daisy (13.6 ± 4.5), Silver (9.2 ± 1.0), Laurentian (8.7 ± 1.7) and Rockcut (8.5 ± 1.5), in that order. *Eurotiomycetes* was most abundant in Daisy site (4.5 ± 3.5).

Basidiomycota had two assigned classes; *Agaricomycetes* and *Tremellomycetes* (Appendix P and Appendix Q). *Agaricomycetes* was mostly present in rhizosphere samples from Laurentian site. Whereas, *Tremellomycetes* was only present in rhizosphere from Laurentian and Rockcut sites (Appendix P and Appendix Q). Also, some *Basidiomycota* members were not possible to identify further than to phylum level.

The *Glomaceae* family was only found in the rhizosphere samples from Daisy site (Appendix P). LKM11 abundance was highest in Laurentian samples (2.5 ± 1.2 %) followed by Silver (2.5 ± 1.0 %), Daisy (2.4 ± 0.5 %), and then Rockcut (0.8 ± 2.5 %).

Hierarchical cluster analysis using the total community profile/unifrac measures of the rhizosphere samples showed that fungal communities grouped Daisy in a separate cluster, but all other sites were mixed in another cluster (Figure 3.5).

DCA of rhizosphere fungal communities showed that different sites varied in the variability of their DCA scores, and a mantel test determined that differences between sites were significant along DCA1 (p=0.001, Figure 3.9). Rockcut and Silver sites had low variability along both DCA1 and DCA2 whereas, Laurentian and Daisy sites varied to a larger extent compared to the least metal contaminated site.

The phylogenetic tree of fungal indicator species shows that there are a number of fungi which belong to the *Ascomycota* phylum and *Leotiomycetes* family, present in Rockcut, Laurentian and Silver sites. In fact, Daisy site only had *Basidiomycota* and LKM11 members significantly associated with it (Figure 3.10 and Appendix S).

3.3.5 Enzymatic activities of rhizosphere soils

Hydrolase and lignase enzyme activity of the rhizosphere microbial communities showed that peroxidase activity was the only enzyme to significantly differ in activity across sites (range 0.56 to 5.07 nmol $h^{-1}g^{-1}$, ANOVA, p = 0.032, F=3. 8, Figure 3.11d). Other enzyme activities did not significantly differ among the sites (β-glucosidase, range 0.80 to 3.50 nmol $h^{-1}g^{-1}$, p=0.07, F=2.8, Figure 3.11a; phosphatase, range 3.18 to 7.70 nmol $h^{-1}g^{-1}$, p=0.82, F=0.30, Figure 3.11b; phenoloxidase, range 0.03 to 1.60 nmol $h^{-1}g^{-1}$, p = 0.10, F=2.5, Figure 3.11c). Hydrolases (β-glucosidase and phosphatase) were the only enzymes which showed plant-specific differences. β-glucosidase activity in leatherleaf and cottongrass plants were significantly different only in the sites Laurentian and Silver.

Phosphatase activity showed a significant difference, but only between cottongrass from Silver compared with cottongrass from either Daisy or Laurentian, suggesting it is an interaction effect of both plant and site.

3.3.6 Soil Properties of rhizosphere soil and plant

pH values across the sites were in the range of 5.0 to 6.7 and were significantly different (ANOVA p = 0.01, F=7.6). Daisy was significantly higher (6.7) compared to Laurentian (5.3) and Rockcut (5.0) and, Silver (6.3) was significantly lower compared to Rockcut (Figure 3.12 and Table 3.4). Percentage of soil organic matter content (77 to 85 range) (ANOVA p = 0.56, F=0.71), LDMC of leatherleaf (ANOVA p = 0.34, F=1.21), LDMC of cottongrass (ANOVA p = 0.34, F=1.19) and above ground dry plant mass (phytometric experiment results) (ANOVA p = 0.06, F=2.96) did not show any significant differences among the sites (Table 3.4).

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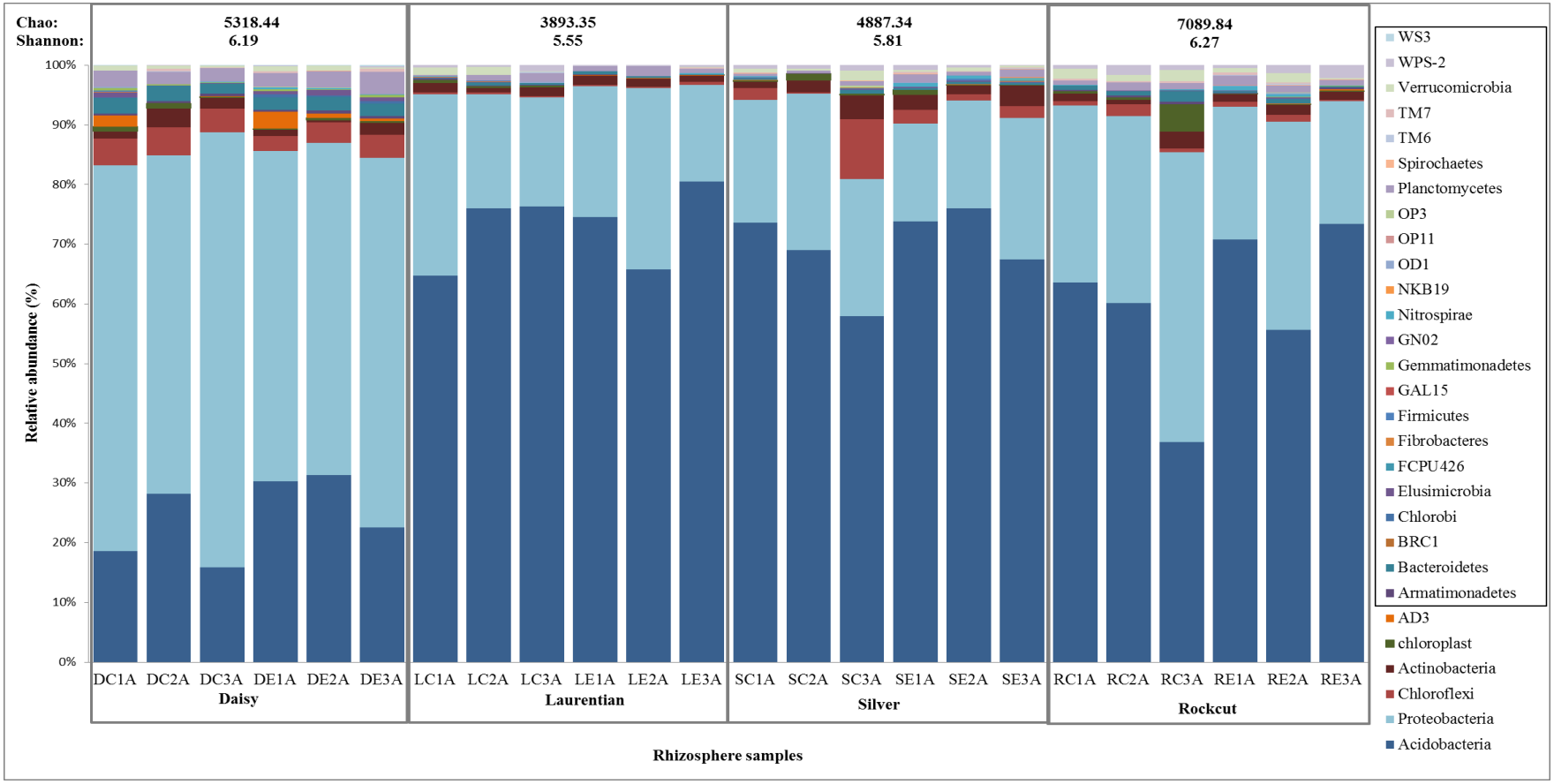

Figure 3.1 Relative abundance of bacterial phylum in rhizosphere soil from different samples. DC: Daisy & Leatherleaf, DE: Daisy & Cottongrass, LC: Laurentian & Leatherleaf, LE: Laurentian & Cottongrass, RC: Rockcut & Leatherleaf, RE: Rockcut & Cottongrass, SC: Silver & Leatherleaf and SE: Silver & Cottongrass.

Table 3. 2 Average abundance and standard deviation of bacterial taxa among leatherleaf and cottongrass plants (> 0.1% abundant community). * indicates where value is significantly different from other plant.

Phylum	Class	Family	Cottongrass	Leatherleaf
Acidobacteria	Acidobacteriia	Acidobacteriaceae	10.2 ± 2.0	16.8 ± 3.1
	Acidobacteriia	Koribacteraceae	29.3 ± 3.6*	17.6 ± 3.3
	DA052	Ellin6514	3.0 ± 0.4	2.8 ± 0.9
	Holophagae	Holophagaceae	0.1 ± 0.0	0.1 ± 0.1
	Solibacteres	Solibacterales	0.2 ± 0.1	0.3 ± 0.1
Actinobacteria	Actinobacteria	Actinomycetales	0.3 ± 0.1	0.3 ± 0.1
Chloroflexi	Ktedonobacteria	Ktedonobacteraceae	0.1 ± 0.0	0.9 ± 0.4
		Thermogemmatisporaceae	0.1 ± 0.1	0.2 ± 0.2
Proteobacteria	Alphaproteobacteria	Acetobacteraceae	1.5 ± 0.4	3.2 ± 0.7
		Bradyrhizobiaceae	2.8 ± 0.5	5.5 ± 1.4
		Ellin330	0.2 ± 0.1	0.5 ± 0.2
		Hyphomicrobiaceae	4.5 ± 0.9	3.1 ± 0.6
		Methylocystaceae	1.6 ± 0.4	1.7 ± 0.5
		Rhodospirillaceae	1.8 ± 0.6	1.4 ± 0.6
		Unclassified Alpha	0.2 ± 0.1	0.4 ± 0.2
	Betaproteobacteria	Ellin6067	0.2 ± 0.1	0.1 ± 0.1
		Oxalobacteraceae	0.2 ± 0.1	0.1 ± 0.1
		Unclassified Beta	0.5 ± 0.3	0.2 ± 0.1
	Deltaproteobacteria	Myxococcales	0.2 ± 0.1	0.2 ± 0.1
	Gammaproteobacteria	Sinobacteraceae	1.4 ± 0.3	1.7 ± 0.3
Verrucomicrobia	Opitutae	Opitutaceae	0.2 ± 0.1	0.5 ± 0.1
WPS-2	WPS-3	WPS-5	0.3 ± 0.1	0.2 ± 0.1

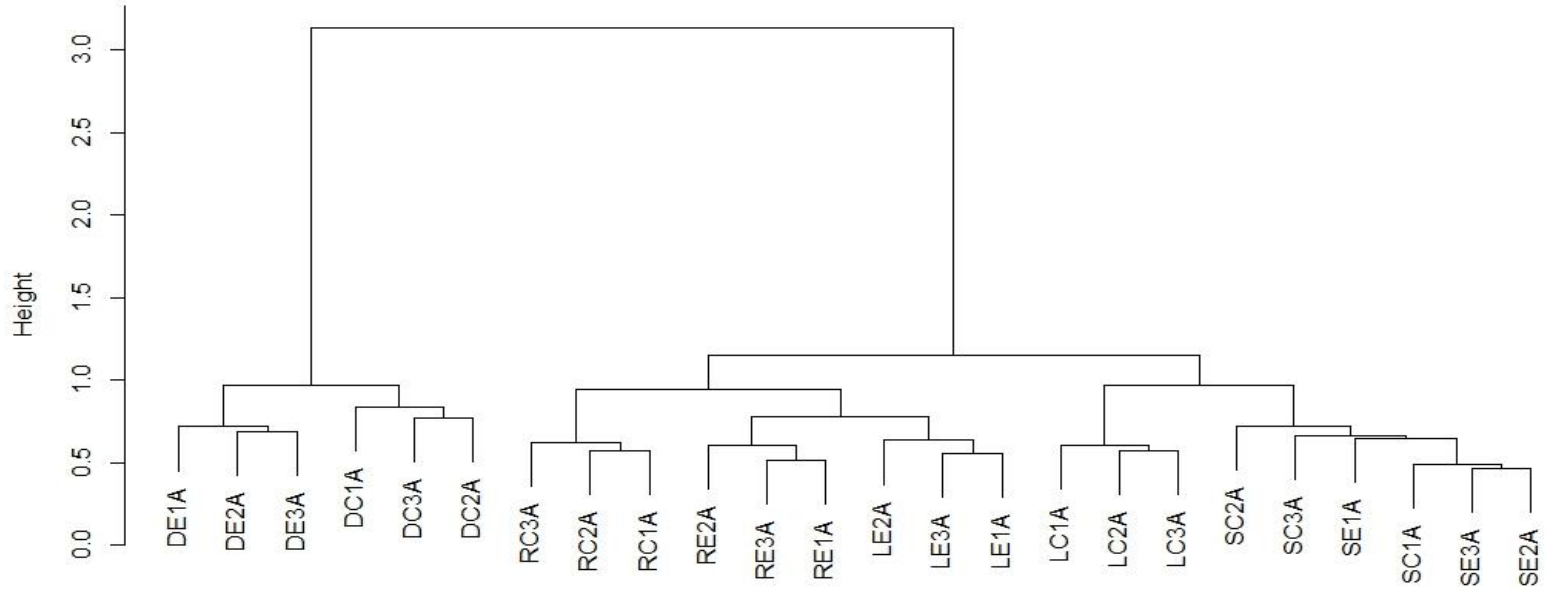

Figure 3. 2 Hierarchical cluster analysis based on Spearman correlation coefficients of bacterial community in the population with > 0.1 % abundance. DC: Daisy & Leatherleaf, DE: Daisy & Cottongrass, RC: Rockcut & Leatherleaf, RE: Rockcut & Cottongrass, LC: Laurentian & Leatherleaf, LE: Laurentian & Cottongrass, SC: Silver & Leatherleaf and SE: Silver & Cottongrass.

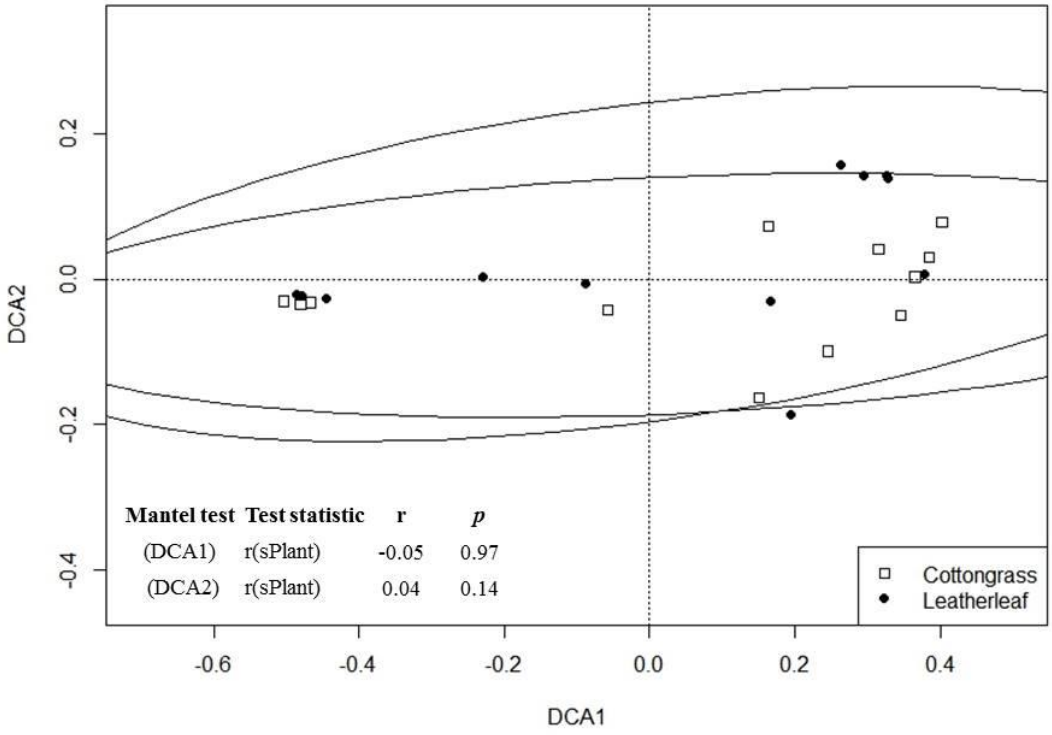

Figure 3. 3 Detrended correspondence analysis plot of bacterial unifrac values by plant species. Ellipses represent extent of 95% confidence intervals. Mantel test results are presented in the top right corner of DCA.

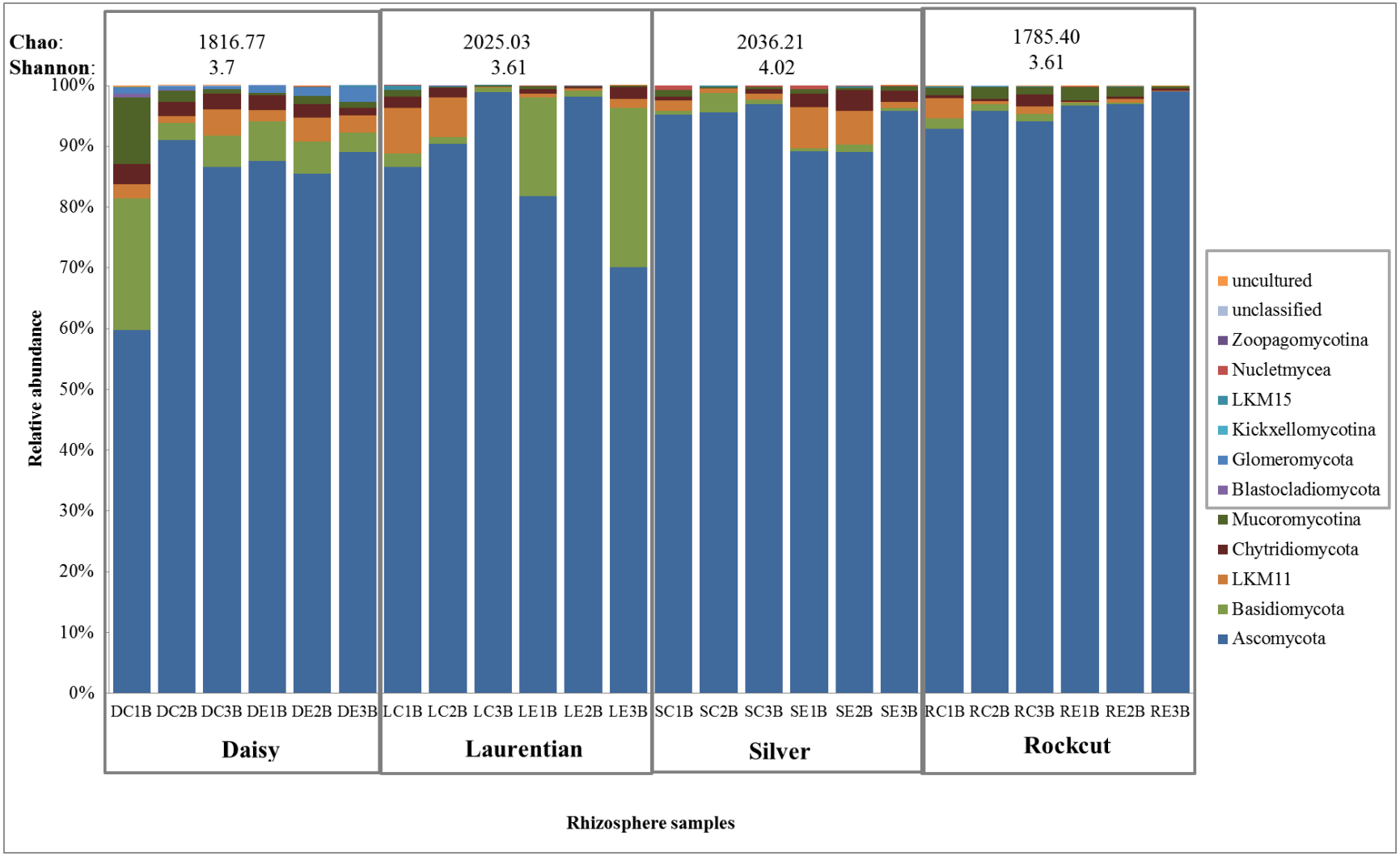

Figure 3. 4 Relative abundance of fungal phyla in soil from different sites. For sample abbreviations see Figure 3.1.

Table 3. 3 Average abundance and standard deviation of fungal taxa among leatherleaf and cottongrass plants (> 0.1% abundant community).

Kingdom	Phylum	Class	Cottongrass	Leatherleaf
Fungi	Ascomycota	Dothideomycetes	8.4 ± 0.8	11.6 ± 2.5
		Eurotiomycetes	0.3 ± 0.1	3.9 ± 2.0
		Lecanoromycetes	0.9 ± 0.3	0.5 ± 0.1
		Leotiomycetes	67.2 ± 3.3	60.8 ± 5.1
		Orbiliomycetes	0	0.1 ± 0.1
		Sordariomycetes	2.3 ± 0.4	4.0 ± 0.8
	Basidiomycota	Agaricomycetes	3.9 ± 2.2	0.7 ± 0.2
		Tremellomycetes	0	0.3 ± 0.2
		unclassified Basidio	0.6 ± 0.4	2.0 ± 1.4
	Chytridiomycota		1.0 ± 0.3	1.0 ± 0.3
	Glomeromycota	Glomaceae	0.3 ± 0.2	0.1 ± 0.1
	LKM11		1.8 ± 0.6	2.3 ± 0.7
	LKM15		0	0.1 ± 0.1
	Mucoromycotina		0.6 ± 0.2	1.6 ± 0.8

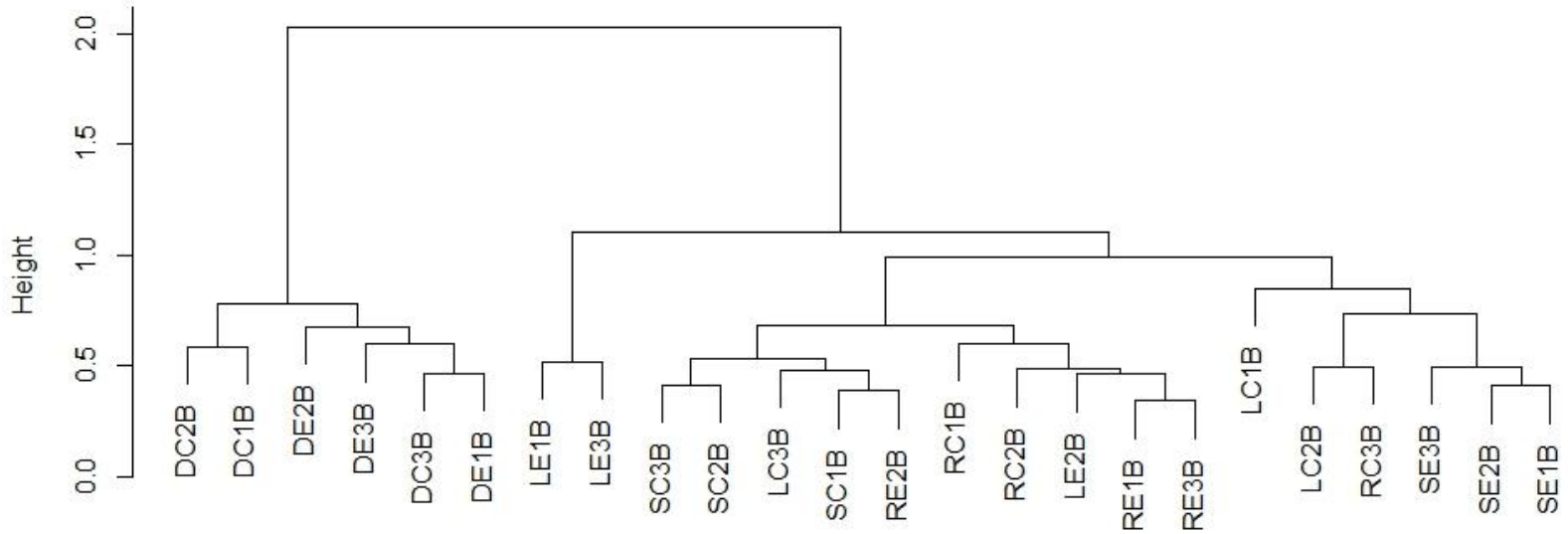

Figure 3. 5 Hierarchical cluster analysis based on Spearman correlation coefficients of fungal community in the population with > 0.1 % abundance. For sample abbreviations see Figure 3.1. DC: Daisy & Leatherleaf, DE: Daisy & Cottongrass, LC: Laurentian & Leatherleaf, LE: Laurentian & Cottongrass, RC: Rockcut & Leatherleaf, RE: Rockcut & Cottongrass, SC: Silver & Leatherleaf and SE: Silver & Cottongrass

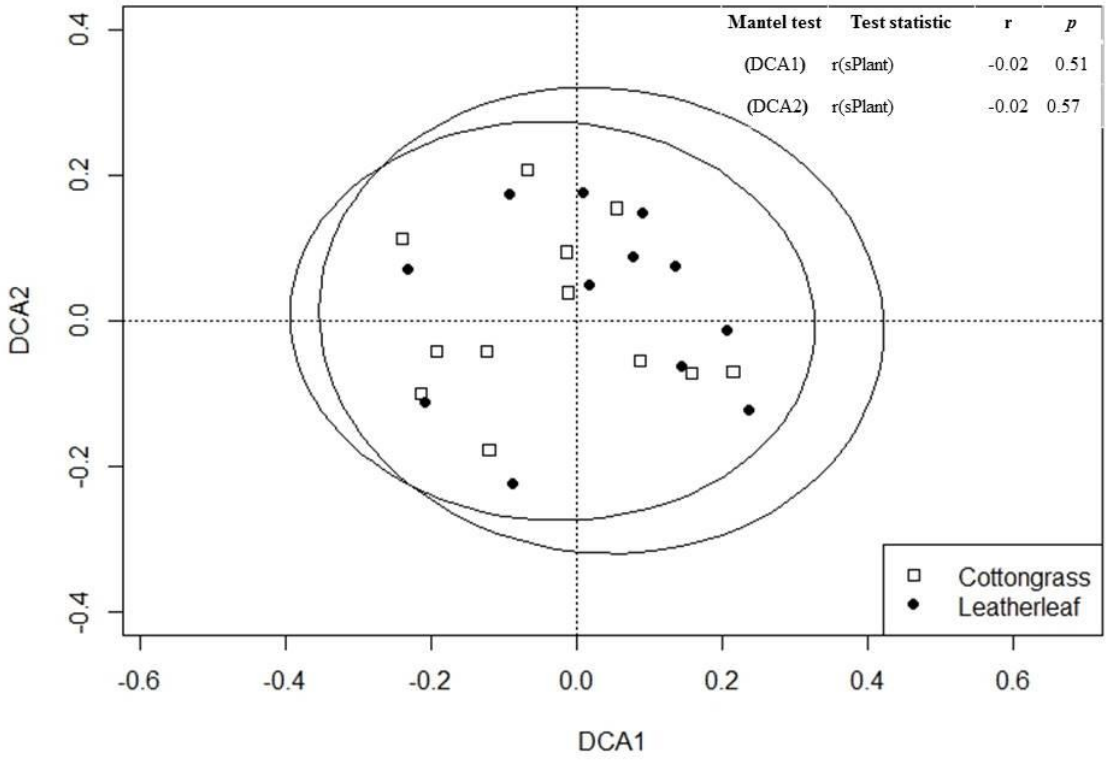

Figure 3. 6 Detrended correspondence analysis plot of fungal unifrac values by plant species. Ellipses represent extent of 95% confidence intervals. Mantel test results are presented in the top right corner of DCA.

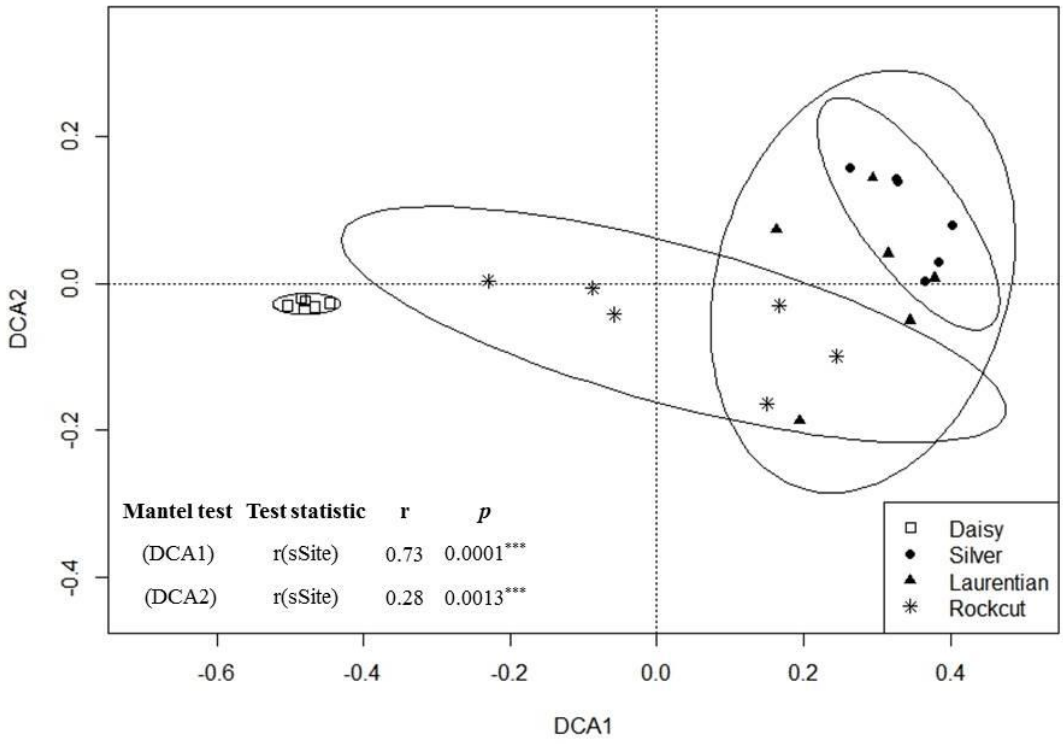

Figure 3. 7 Detrended correspondence analysis plot of bacterial unifrac values by sites. Ellipses represent extent of 95% confidence intervals. Mantel test results are presented in the bottom left corner of the DCA.

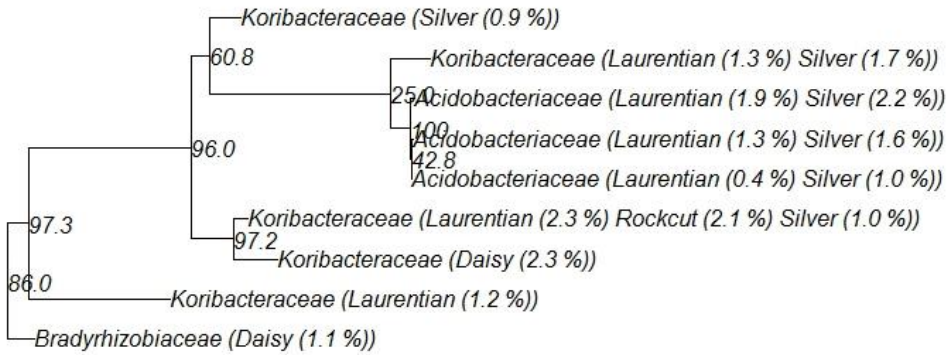

Figure 3. 8 Phylogenetic tree of indicator species matched to bacterial families and their significantly delineated sites as determined by indicator species analysis (> 1 % abundant community). Percent abundance of each species at their respective sites is included in brackets. The numbers for the interior branches are bootstrap values.

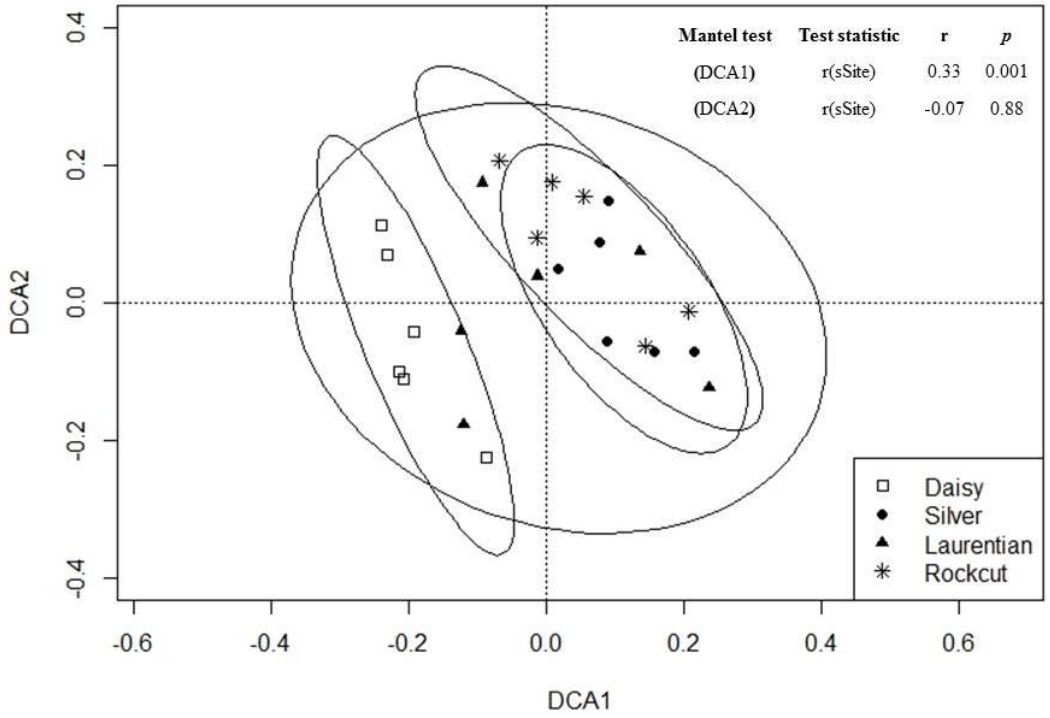

Figure 3. 9 Detrended correspondence analysis plot of fungal unifrac values by sites. Ellipses represent extent of 95% confidence intervals. Mantel test results are presented in the top right corner of the DCA.

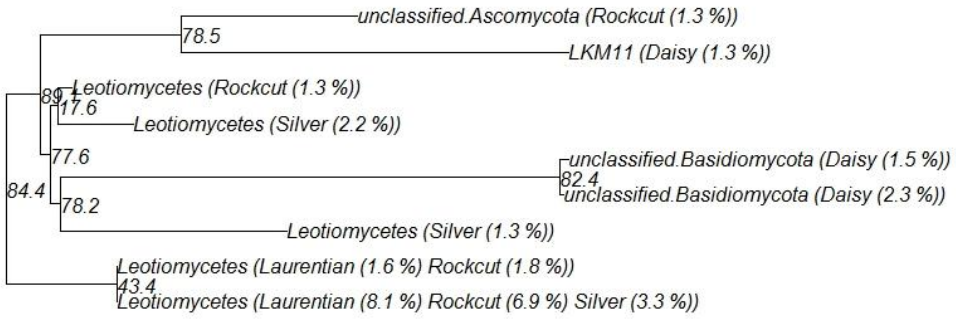

Figure 3. 10 Phylogenetic tree of indicator species matched to fungal families and their significantly delineated sites as determined by indicator species analysis (> 1 % abundant community). The numbers for the interior branches are bootstrap values.

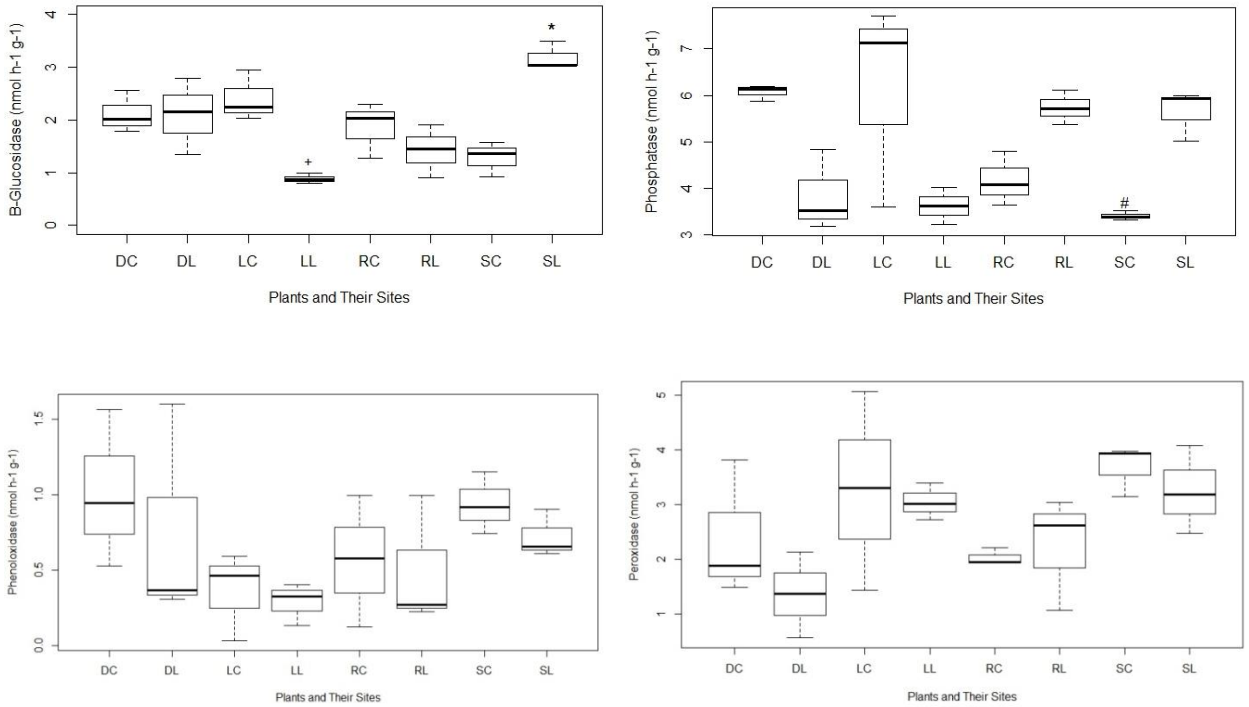

Figure 3. 11 Box plots of β-glucosidase (a), phosphatase (b), phenoloxidase (c), and peroxidase (d) levels within soil samples across sites and plants. DC: Daisy/Cottongrass, DL: Daisy/ Leatherleaf, LC: Laurentian/Cottongrass, LL: Laurentian/Leatherleaf, RC: Rockcut/Cottongrass, RL: Rockcut/Leatherleaf, SC: Silver/Cottongrass and SL: Silver/Leatherleaf.*: β-glucosidase activity of Silver/Leather leaf sample was significantly different compared to LL, RC, RL and SC. +: β-glucosidase activity of Laurentian/Leatherleaf was significantly different compared to LC. #: phosphatase activity of Silver/Cottongrass sample was significantly different compared to DC and LC.

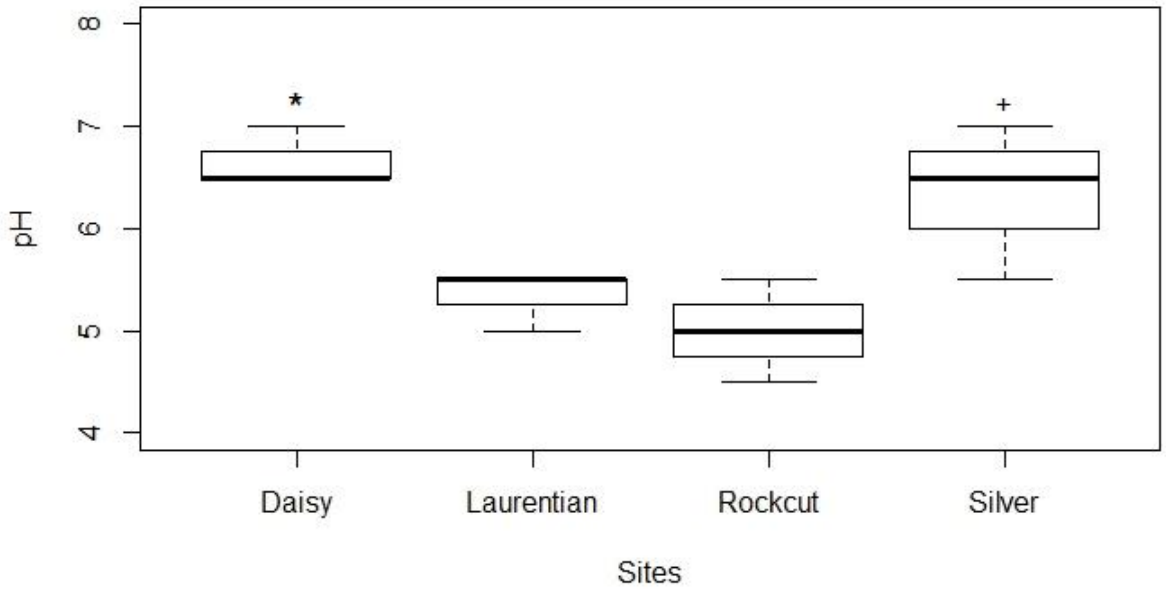

Figure 3. 12 Box plots of pH levels within soil samples across the sites.*: Daisy was significantly differed compared to Laurentian and Rockcut sites. +: Silver was significantly differed compared to Rockcut.

Table 3. 4 Environmental variables measured during the summer of 2013 that were included in ANOVA models to determine if environment influences microbial communities. Mean ± SE of each environmental variable for 2013 summer survey, and respective P-value for ANOVA of each variable against the response variable of sites. LDMC: Leaf Dry Matter Content. a: Daisy is significantly higher compared to Laurentian and Rockcut (ANOVA). b: Silver is significantly higher compared to Rockcut (ANOVA).

Variable	Mean ± SE				*p*-value (α = 0.05)
	Rockcut	Silver	Laurentian	Daisy	
pH	5.0 ± 0.2	6.3 ± 0.4[b]	5.3 ± 0.1	6.7 ± 0.1[a]	0.01*
Soil Organic Matter (%)	80 ± 11.7	81 ± 5.5	85 ± 5.3	77 ± 10.6	0.56
Leatherleaf LDMC (g dry matter/g fresh matter)	0.47 ± 0.02	0.50 ± 0.003	0.52 ± 0.02	0.49 ± 0.01	0.34
Cottongrass LDMC (g dry matter/g fresh matter)	0.21 ± 0.01	0.24 ± 0.02	0.21 ± 0.02	0.26 ± 0.01	0.34
Phytometric experiment (mg dry matter)	2.8 ± 0.2	2.8 ± 0.4	3.6 ± 0.2	2.6 ± 0.1	0.06
Leatherleaf size (cm)	57.2 ± 6.4	41.6 ± 2.2	39.5 ± 4.0	55.8 ± 1.2	0.07
Cottongrass size (cm)	42.6 ± 3.9	43.7 ± 2.0	35.7 ± 2.5	32.0 ± 3.5	0.17

3.4 Discussion

I found evidence of site-specific differences in the rhizosphere microbial communities. The purpose of this study was to explore differences in species composition and diversity of bacterial and fungal communities in the rhizosphere of two wetland plants *Chamaedaphne calyculata* L. Moench (leatherleaf) and *Eriophorum vaginatum* L. (cottongrass) across a gradient of environmental disturbance.

3.4.1 Rhizosphere bacterial and fungal community distribution across plant species

According to Barrett (2014), *Chamaedaphne calyculata* had 100 % frequency of occurrence at Laurentian, Silver and Rockcut sites and 12.5 % frequency of occurrence at Daisy site whereas *Eriophorum vaginatum*'s frequency of occurrence was only high at Silver (93.8 %) with low frequency of occurrence at Laurentian (18.8 %) and Rockcut (12.5 %), and none found at Daisy in Barrett's survey (2014) (Table 2.1). Overall, patterns in rhizosphere microbial community composition were less prominent between plant species than among sites. However, some instances of plant specificity could be observed in the community data. For instance, *Koribacteraceae* abundance was significantly higher in the rhizosphere of *Eriophorum vaginatum.* As in the bacterial data, there was no obvious or significant difference between the abundance of fungal classes in the rhizosphere of cottongrass and leatherleaf samples.

When looking for plant specific differences, plants differed from each other within the individual sites, but not when averaged across the sites. In other words, the plant-specific differences are nested inside and masked by the site-differences. In support of this, there are slight similarities between two plant species within each site evident from the hierarchical cluster analysis. Also, a number of bacterial families had differing

abundances between the plant species within each site, but analysis showed these differences were smaller than the site effects.

3.4.2 Rhizosphere bacterial community distribution across study sites

There are a number of ways in which rhizosphere samples from Daisy Lake site differed from the other sites. Physically, this site was dry, with no standing water. It had the most different plant community of all the sites (Barrett, 2014). Between the rhizosphere samples among the four sites, it was clear that the microbial community of the rhizosphere samples from Daisy had numerous differences from those of other sites. OTU's from the family *Bradyrhizobiaceae*, came out as a strong indicator of rhizosphere soils at Daisy, which matches findings from Preem et al. (2012), in which *Bradyrhizobiaceae* abundance was influenced by soil chemistry and physical factors including pH. This bacterial family is capable of N_2 fixation and denitrification. Since Daisy had low levels of NO_3^- and was rich with PO_4^{3-} (Pennington, 2014), Daisy may have an environment which favours N-fixing bacteria, possibly explaining the abundance of *Bradyrhizobiacea*e in the rhizosphere soils at Daisy site.

As mentioned above, the rhizosphere samples from Daisy site were the driest of all, which may have made a more favorable habitat for bacterial families linked to leaf-litter such as *Hyphomicrobiaceae* and *Rhodospirillaceae* (Pfeiffer et al., 2013). It is also known that these family members are involved in nitrogen fixing (Madigan et al., 1984).

The class *Betaproteobacteria*, which was almost exclusively found in the rhizosphere samples from Daisy, is known to be more abundant in agricultural soils and displays responses to land-use change and eutrophication (Hartman et al., 2008). This class

includes root-associated *Oxalobacteraceae*, which breaks down complex soil compounds by biodegradation (Green et al., 2006; Ofek et al., 2012).

Several studies (Serkebaeva et al., 2013; Sait et al., 2006; Lauber et al., 2008; Jones et al., 2009; Mapelli et al., 2011) found that the phylum *Acidobacteria* had the highest abundance in acidic peat soils. Likewise, *Acidobacteria* was lowest in the rhizosphere samples from Daisy, which had the highest pH (6.7) but it was the most abundant phylum in the rhizosphere samples from Silver (pH 6.3). Members of *Acidobacteria* have been revealed to be important in soil ecosystems but little is known about acidobacterial species as well as in anoxic environments (Nacke et al., 2011; Serkebaeva et al., 2013).

Surface peat samples from Laurentian and Silver had the highest overall metal concentrations (Ni and Cu) (Pennington, 2014) with a range of rhizosphere pH (5.3 – 6.3), making them the most impacted of the four sites. Although significant differences between the abundances of the bacterial families in these sites were few, hierarchical cluster analysis based on Spearman correlations showed that the sites clearly separated according to bacterial communities. Correspondingly; Cu concentrations were highest at Silver. Other studies have found positive correlations between *Acidobacteria* and pH (Nacke et al., 2011; Hartman et al., 2008). Correlations between total N and *Acidobacteria* were found in Nacke et al. (2011). Likewise, concentration of N was high in Silver (Pennington, 2014). Indicator species analysis was able to differentiate the rhizosphere soil from Laurentian and Silver by members of the family *Koribacteraceae*. Also, *Methylocystaceae* family members were more abundant in the rhizosphere samples from Laurentian, Rockcut and Silver sites. Also, this family has the ability to thrive in a wide range of methane and nitrogen availabilities and was most abundant in the

rhizosphere samples from Laurentian, which was also the wettest site, whereas Daisy, which was the driest site, had the least abundance of *Methylocystaceae* in the rhizosphere soil. *Acetobacteraceae* were significantly more abundant in the rhizosphere samples from Rockcut site than in the rhizosphere samples from Daisy site, and corresponding to a previous cultivation-independent study (Nacke et al., 2011) these family members were more abundant in low pH than in high pH environments. It has also been found that this family has N-fixing members (Saravanan et al., 2007; Pedraza, 2008; Donn et al., 2014).

3.4.3 Rhizosphere fungal community distribution across study sites

Overall, there was less site-specific differentiation of the fungal communities indicating the fungal members were more ubiquitous and less affected by the range of geochemical parameters that distinguish the sites. Luke et al. (2015) also found that fungal communities varied without consistency. The fungal microbiome of the rhizosphere samples from Daisy were again the most distinct from those of the other sites. Fungal sequences were dominated by the phylum *Ascomycota* which is known to be included in plant-associated ectomycorrhizal communities (Dickie and Reich, 2005; Smith et al., 2007). However, the rhizosphere samples from Daisy had the least *Ascomycota* abundance. Phylum *Chytridiomycota*, which has been associated with high extracellular enzyme activities (Kivlin and Treseder, 2014), was most abundant in the rhizosphere samples from Daisy. Phylum *Glomeromycota* was found exclusively in the rhizosphere samples from Daisy. Kivlin and Treseder (2014) found that this phylum was positively correlated with P-degrading extracellular enzyme activities.

My results demonstrated that Daisy, which was the driest site with the highest pH, had the second highest diversity of the microbial community within the rhizospheres (next to

Rockcut). This site also had the greatest diversity of both vascular and non-vascular vegetation (Barrett, 2014). It's also important to note that Daisy had some of the lowest soluble metals (pore water [Ni] 121 ppb, [Cu] 85 ppb from Pennington, 2014). Whereas, Rockcut, Silver and Laurentian had higher Cu and Ni concentrations, in that order (pore water [Ni] 174 - 737 ppb, [Cu] 174 - 272 ppb from Pennington, 2014). So, overall, Daisy's higher microbial community diversity could be linked to one or all of these factors.

In general, the environmental disturbance gradient across the sites was uneven, with lower pH found at Rockcut site, which was supposed to be the least disturbed site, while the most neutral pH was found in Daisy (which was understood to be more disturbed), and large differences between the metal concentrations in the surface peat and those from pore water samples. Wetlands have naturally low pH values in Sudbury region, and liming has strongly influenced the soil pH of some disturbed sites.

3.5 Conclusion

This study examined wetland sites along a gradient of environmental disturbance in Sudbury. Rockcut and Daisy had the highest diversity within the microbial community, but interestingly, the community structure of these sites was distinct. The intermediate sites had the most similarity in both site characteristics and plant rhizosphere community structure. A large portion of the identified bacterial communities were present throughout all of the sites (i.e. common communities) but a cluster of taxa could distinguish individual sites. Moreover, bacterial and fungal community structure has been found to be shaped by soil properties. Daisy stood out from the other sites in many ways. It was dry, pH neutral and had a distinct plant community. The distinct microbial communities found in the rhizosphere of plants from this site, including unique OTU's could be a result of one or many of these factors. There was also evidence that the bacterial community structure showed some plant-specificity within the sites but to a much lesser extent. There are still many unclassified bacterial and fungal taxa which require further study in order to illuminate how plant-microbe interactions are affected by the surrounding environment. Greater ability to classify these organisms beyond the family level will aid in determining specific relationships between plants and site characteristics.

Chapter 4

4.1 General conclusions

The findings presented in this thesis suggest that both the species of the host plant and the site play a role in determining the composition of rhizosphere microbial community. The garden experiment strongly indicated that plant species have distinct bacterial rhizosphere communities, even when provided with a common inoculum and soil, while the field experiment broadened this understanding to show that these differences, while important, exist within a context of larger site differences. This demonstrates that environmental conditions were stronger than plant species-microbe interactions in the scale of influence over microbial community structure. This concept extends to differences between the plant groups of monocots and eudicots, with decreasing differentiation between plant species with increasing taxonomic relatedness. This is important given what is known about the interactions between the microbial rhizosphere and plant species, such as the roles microbes play in nutrient uptake, pathogen defences and promoting plant growth.

Sudbury was an interesting setting for this study due to the history of metal mining and smelting in this region. Rhizosphere samples from Lake Laurentian and Silver Lake were expected to show the strongest differences due to the high metal concentrations of those sites, but rhizosphere samples from Daisy Lake turned out to be the most distinct ones. This site was the driest of the studied locations with a low water table. Its pH was the closest to neutral, whereas the other sites were acidic. Cluster analysis of bacterial communities separated Lake Laurentian and Silver Lake from Rockcut Lake, but even so, they were more similar than would have been expected based on their environmental history and all were highly distinct from Daisy Lake.

In general, neither the bacterial nor the fungal community profiles were clearly distinguished along a gradient of pollution. Similarly, fungal communities in other studies have been found to be less affected by plant specificity than bacterial communities (Berg and Smalla, 2009). The dynamics of metal concentration vs. bioavailability as a result of pH are likely to be important at these sites as many metals become more bioavailable at lower pH. Although bioavailable metals were not measured directly, the higher pH at the Daisy Lake wetland suggests less metal bioavailability at Daisy.

In conclusion, this thesis was the first to investigate the dynamics of microbial communities within the rhizosphere of wetland plants within the Sudbury region using deep sequencing techniques. The findings presented here provide evidence for species-specific associations among rhizosphere bacteria and wetland plants but also highlights that these differences are less pronounced than the effect of environmental conditions driving the community structure at specific sites. Fungal communities did not show either plant or site specificity. Future studies should examine more specific plant-microbe interactions and attempt to identify interactions with ecological functionality and their importance; for example, using different metal treatments across mesocosms of same plant species could justify only metal effects on rhizosphere microbial community without other environmental effects. This study also raises questions regarding the relative importance of phylogenetic relationships for differentiation among microbial rhizosphere communities and if they are tied to evolutionary history. Additional studies might also attempt to classify bacterial and fungal taxa to lower taxonomic ranks and determine the functional relationships between the environment, microbes and plants. Improving the level of taxonomic resolution to lower ranks will make it possible to link

the specific effects to specific organisms. Alternatively, characterizing the community-level physiological profile of a microbial community could provide an understanding of its basic ecology including carbon utilization patterns, which can be tracked over time to monitor responses to stressors including environmental changes (Weber et al., 2008).

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Appendices

Appendix B Sequence numbers of bacterial data in raw, filtered data and, >1%, > 01% abundant data for each sample. For mesocosm abbreviations see Table 2.2. R: Rockcut Lake, C: Cartier and L: Lake Laurentian

Samples	Number raw input seqs	Number filtered input seqs	< 1 %	< 0.1 %
Cont.1	7660	6559	4	210
Cont.2	6234	5832	5	251
Cont.3	6270	5845	5	244
CcaR.1	5094	4610	8	194
CcaR.2	5236	4617	6	199
CcaR.3	4428	3759	9	249
GcaR.1	6527	6136	7	180
GcaR.2	8241	7734	7	197
GcaR.3	6116	5782	6	239
ColC.1	6327	5696	7	214
ColC.2	7760	6958	10	185
ColC.3	2227	2150	13	196
ColC.4	10598	9854	9	108
ColC.5	4071	3732	8	255
CutC.1	6366	5827	6	205
CutC.2	4473	4289	9	177
CutC.3	5753	5396	6	219
CutL.1	6806	5891	7	209
CutL.2	6586	6043	6	187
CutL.3	2741	2687	13	111
EvaC.1	7923	7108	10	148
EvaC.2	6978	6344	10	199
EvaR.1	4229	3907	7	262
EvaR.2	2618	2464	7	249
EvaR.3	6406	5773	9	198
EviC.1	5329	4879	5	251
EviC.2	4641	4274	12	190
EviC.3	7603	6785	4	220
EviR.1	3043	2904	12	262
EviR.2	7592	7016	11	178
EviR.3	5935	5455	5	217

Appendix C Average abundance in % (± 1 SE) of bacterial taxa among control and rhizosphere of plant species in total community. For mesocosm abbreviations see Table 2.2.

Phylum	Control	Cca	Gca	Col	Cut	Eva	Evi
Acidobacteria	19.9 ± 3.0	8.2 ± 1.4	14.0 ± 3.0	14.5 ± 1.4	11.8 ± 2.5	16.0 ± 2.2	14.5 ± 1.0
Actinobacteria	9.5 ± 1.2	3.2 ± 0.5	10.7 ± 1.8	5.8 ± 0.4	6.6 ± 1.2	5.7 ± 1.0	5.7 ± 0.6
AD3	0.4 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
Armatimonadetes	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.6 ± 0.1
Bacteroidetes	1.6 ± 0.1	3.7 ± 1.0	1.4 ± 0.8	1.0 ± 0.2	1.5 ± 0.3	1.6 ± 0.1	2.4 ± 0.4
BRC1	0.1 ± 0.0	0	0	0	0	0	0
Chlorobi	1.6 ± 0.2	0.6 ± 0.3	0.6 ± 0.3	0.8 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.1
Chloroflexi	2.9 ± 0.3	6.7 ± 1.8	2.5 ± 0.4	2.2 ± 0.3	1.9 ± 0.3	2.8 ± 0.5	2.3 ± 0.3
Chloroplast	0.2 ± 0.1	4.5 ± 2.1	10.7 ± 10.5	19.7 ± 7.3	24.2 ± 8.7	1.9 ± 0.5	6.5 ± 2.4
Elusimicrobia	0.3 ± 0.1	0.1 ± 0.1	0.6 ± 0.5	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	0.3 ± 0.1
Fibrobacteres	0.2 ± 0.0	0.1 ± 0.0	0.5 ± 0.5	0.7 ± 0.4	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.0
Firmicutes	2.0 ± 0.3	0.9 ± 0.3	0.6 ± 0.1	1.2 ± 0.2	0.7 ± 0.1	1.6 ± 0.7	0.8 ± 0.2
GAL15	0	0	0	0	0	0	0
Gemmatimonadetes	0.6 ± 0.0	0.1 ± 0.1	0.9 ± 0.3	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.0
Nitrospirae	0.2 ± 0.1	0.9 ± 0.3	0.1 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
NKB19	0	0	0	0	0	0	0
OD1	1.9 ± 0.5	0.6 ± 0.2	1.1 ± 0.2	1.4 ± 0.4	1.8 ± 0.4	1.9 ± 0.3	1.1 ± 0.2
OP11	0	0.1 ± 0.0	0	0	0	0.1 ± 0.0	0
OP3	0.1 ± 0.0	0	0.3 ± 0.2	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Planctomycetes	1.2 ± 0.2	3.9 ± 0.9	1.4 ± 0.9	0.9 ± 0.3	1.3 ± 0.3	1.5 ± 0.3	2.4 ± 0.5
Proteobacteria	47.0 ± 1.7	61.6 ± 2.8	43.5 ± 7.8	43.0 ± 3.9	40.5 ± 4.3	56.1 ± 1.8	54.7 ± 2.0
Spirochaetes	0.5 ± 0.2	0.7 ± 0.1	0.3 ± 0.1	0.6 ± 0.3	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
SR1	0.1 ± 0.0	0	0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
TM6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0	0.1 ± 0.0	0.1 ± 0.0
TM7	4.6 ± 0.9	0.5 ± 0.1	6.4 ± 0.8	2.1 ± 0.4	3.1 ± 0.7	3.5 ± 0.4	2.6 ± 0.2
Verrucomicrobia	0.9 ± 0.1	0.6 ± 0.3	1.1 ± 0.5	1.1 ± 0.4	0.6 ± 0.1	1.0 ± 0.1	1.4 ± 0.2
WPS-2	1.2 ± 0.1	0.5 ± 0.1	0.9 ± 0.5	0.7 ± 0.2	0.8 ± 0.3	0.6 ± 0.1	0.7 ± 0.1
WS1	0.1 ± 0.0	0.1 ± 0.0	0	0	0	0	0
WS4	0	0	0.0 ± 0.1	0	0	0	0
WS5	0.1 ± 0.0	0	0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0

Appendix D Average abundance in % (±1 SE) of indicator bacterial taxa among control and rhizosphere of plant species. For mesocosm abbreviations see Table 2.2. * indicates where OTU is significantly indicative of that plant.

indicator OTU ID	Family name of the OTU	Control	Cca	Gca	Col	Cut	Eva	Evi
5	Methylocystaceae	0	0.2 ± 0.0	0.1 ± 0.2	1.4 ± 0.4*	0.5 ± 0.1	3.1 ± 1.4*	1.1 ± 0.4*
4	Acidobacteriaceae	2.2 ± 0.5*	0.4 ± 0.1	1.7 ± 0.6*	1.7 ± 0.3*	1.5 ± 0.3*	1.9 ± 0.4*	1.5 ± 0.2*
0	Chloroplast	0	0	0	10.4 ± 3.6*	3.9 ± 1.5*	0.4 ± 0.2	0.6 ± 0.2
7051	Chloroplast	0	0	0	2.4 ± 0.9*	1.6 ± 0.6*	0	0
4586	Chloroplast	0	0	0	1.1 ± 0.6	10.9 ± 4.8*	0.1 ± 0.1	0.2 ± 0.1
31	mitochondria	0	0	1.6 ± 1.5*	0	0	0	0
122	Thermogemmatisporaceae	0	0.9 ± 0.4*	0	0	0	0	0
152	Chloroplast	0	0	7.8 ± 7.7*	0	0	0	0
7373	Chloroplast	0	0	2.0 ± 2.1*	0	0	0	0
4457	Betaproteobacteria	0	0.1 ± 0.0	0.0 ± 0.1	0.7 ± 0.2	0.3 ± 0.1	1.9 ± 0.5*	0.8 ± 0.2
7291	SBla14	0.1 ± 0.0	0.2 ± 0.1	0.5 ± 0.2	1.7 ± 0.4*	0.6 ± 0.1	2.1 ± 0.3*	1.8 ± 0.3*
6	SBla14	0	0	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	1.0 ± 0.2*	0.7 ± 0.2*
7112	Hyphomicrobiaceae	0	0.7 ± 0.3*	0	0	0	0	0
7263	Hyphomicrobiaceae	0	1.2 ± 0.5*	0	0.2 ± 0.1	0	0.4 ± 0.1	0.4 ± 0.2
7	Hyphomicrobiaceae	0	2.5 ± 1.0*	0	0.1 ± 0.1	0	0.2 ± 0.1	0.1 ± 0.0
336	Hyphomicrobiaceae	0	6.8 ± 2.5*	0	0.4 ± 0.1	0	0.6 ± 0.3	0.4 ± 0.1

Appendix E Sequence numbers of fungal data in raw, filtered data and, >1%, > 01% abundant data for each sample. For mesocosm abbreviations see Table 2.2 and Appendix A.

Samples	Number raw input seqs	Number filtered input seqs	< 1 %	< 0.1 %
Cont.1	9785	8580	22	97
Cont.2	4564	3990	17	121
Cont.3	16013	13831	18	120
CcaR.1	15547	13248	18	129
CcaR.2	12430	10756	20	99
CcaR.3	15126	13108	16	113
GcaR.1	13769	11825	15	104
GcaR.2	14556	13218	18	115
GcaR.3	13401	11449	16	118
ColC.1	17646	15591	18	115
ColC.2	17904	16095	18	141
ColC.3	6255	5488	21	135
ColC.4	26202	23183	22	44
ColC.5	21359	18938	12	71
CutC.1	12168	10728	13	125
CutC.2	20891	18195	20	129
CutC.3	5153	2778	22	184
CutL.1	9272	8000	22	127
CutL.2	20560	17995	16	121
CutL.3	17875	15864	22	135
EvaC.1	13959	12084	11	98
EvaC.2	19572	16738	17	108
EvaC.3	16355	14495	16	92
EvaR.1	19913	17108	16	161
EvaR.2	15812	13797	15	106
EvaR.3	7300	5087	18	208
EviC.1	17038	14679	22	103
EviC.2	10912	9291	14	100
EviC.3	13145	11604	14	79
EviR.1	17546	15306	15	88
EviR.2	14813	12743	22	119
EviR.3	17874	15634	16	105

Appendix F Average abundance in % (± 1 SE) of fungal taxa among control and rhizosphere of plant species in total community. For mesocosm abbreviations see Table 2.2.

Phylum	Control	Cca	Gca	Col	Cut	Eva	Evi
Ascomycota	87.6 ± 1.3	66.3 ± 13.7	82.8 ± 22.8	83.0 ± 4.1	85.0 ± 2.5	85.0 ± 2.6	72.5 ± 8.8
Basidiomycota	3.5 ± 0.7	16.2 ± 12.5	1.5 ± 0.5	1.4 ± 0.4	1.9 ± 0.7	5.4 ± 2.4	8.8 ± 1.5
Blastocladiomycota	0.5 ± 0.4	0	0	0	0	0	0
Chytridiomycota	0.9 ± 0.3	3.6 ± 1.4	4.2 ± 5.3	0.8 ± 0.3	1.1 ± 0.4	1.3 ± 0.2	1.7 ± 0.3
Glomeromycota	0	0	1.4 ± 3.4	0	0	0	0
Kickxellomycotina	0	0	0.0 ± 0.1	0	0	0	0.1 ± 0.1
LKM11	2.7 ± 1.1	7.3 ± 1.0	6.2 ± 13.1	9.2 ± 2.7	5.3 ± 1.3	3.3 ± 0.7	11.7 ± 8.3
LKM15	0.2 ± 0.2	2.3 ± 0.5	0.1 ± 0.1	1.2 ± 1.0	2.6 ± 1.8	1.0 ± 0.4	2.0 ± 0.9
Mucoromycotina	2.1 ± 0.6	0.2 ± 0.1	1.4 ± 1.5	1.0 ± 0.3	1.0 ± 0.2	0.4 ± 0.1	0.1 ± 0.0
Nucletmycea	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.4	0.9 ± 0.5	0.1 ± 0.1	0.3 ± 0.1	0.7 ± 0.3
Zoopagomycotina	0.3 ± 0.1	0	0.2 ± 0.2	0	0	0	0

Appendix G Average abundance in % (± 1 SE) of indicator fungal taxa among control and rhizosphere of plant species. For mesocosm abbreviations see Table 2.2. * indicates where OTU is significantly indicative of that plant.

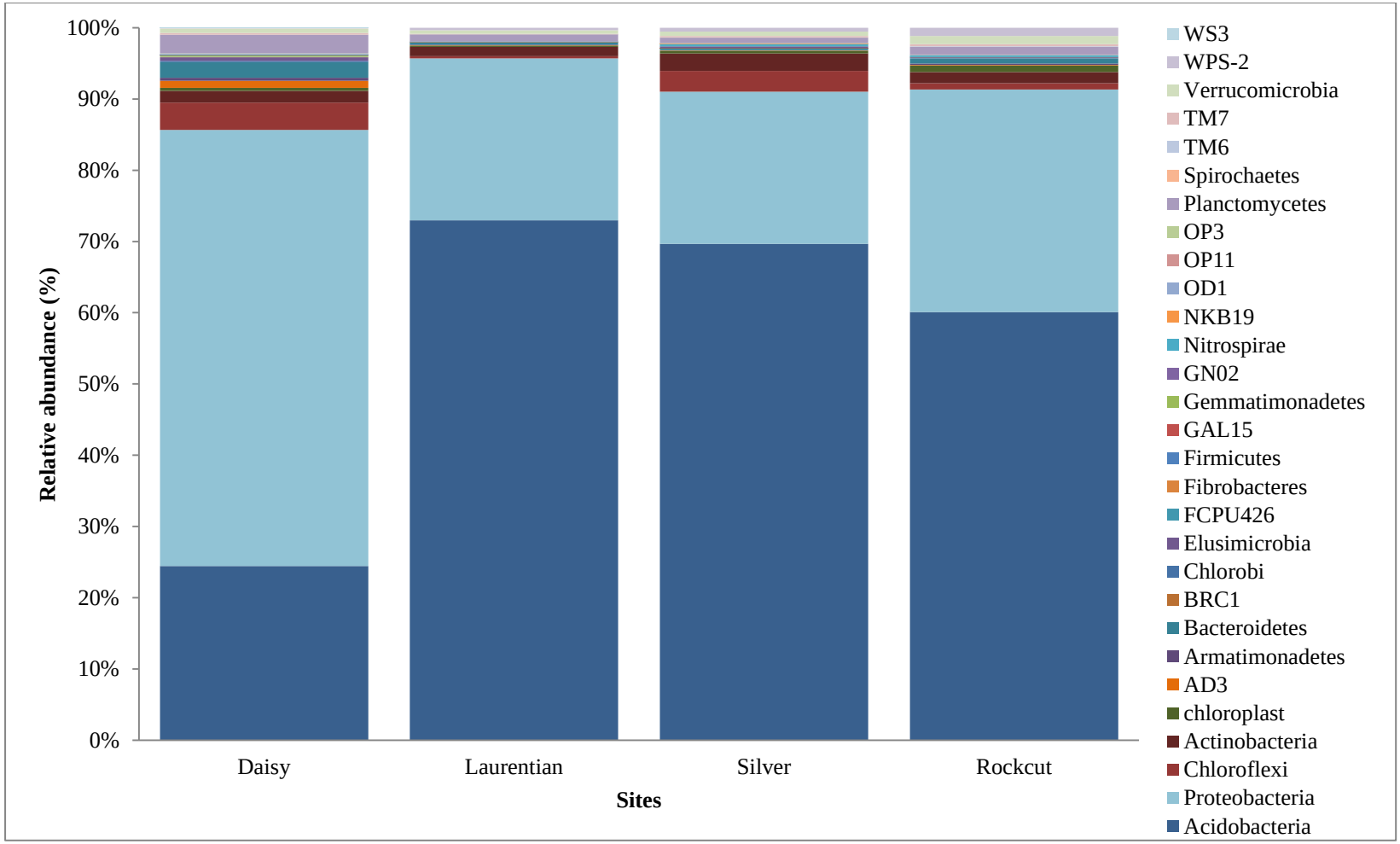
indicator OTU ID	Family name of the OTU	Control	Cca	Gca	Col	Cut	Eva	Evi
2577	Dothideomycetes	0	0	0	0	0	0	1.1 ± 0.4*
91	Chytridiomycota	0	1.4 ± 0.6*	0	0	0	0.3 ± 0.3	0
2088	Entorrhizomycetes	0	0	0	0	0	0.4 ± 0.4	2.0 ± 0.5*
267	Entorrhizomycetes	0	0	0	0	0	1.6 ± 0.9*	1.6 ± 0.5*
36	Saccharomycetes	4.1 ± 1.7*	0	2.7 ± 3.9*	1.2 ± 0.5*	1.0 ± 0.6	0	0
3654	Leotiomycetes	4.9 ± 1.3*	7.4 ± 3.3*	4.6 ± 5.8*	4.5 ± 1.5*	2.5 ± 0.9*	0.5 ± 0.4	0
4132	Leotiomycetes	0.7 ± 0.6	6.8 ± 2.9*	0	0.4 ± 0.3	0.3 ± 0.3	0	0
4043	Sordariomycetes	6.5 ± 0.5*	0	3.6 ± 5.3*	3.5 ± 0.5*	1.5 ± 0.9	0.3 ± 0.3	0
3542	Sordariomycetes	0	0	0	1.8 ± 0.7*	0	0	0
55	Eurotiomycetes	0	4.1 ± 1.7*	0	0	0	0	0
13	Sordariomycetes	6.2 ± 1.1*	0	4.6 ± 3.6*	7.8 ± 4.3*	4.1 ± 1.5*	2.1 ± 0.8*	0
2616	unclassified.Ascomycota	0	0	0	0	1.1 ± 0.5*	0	1.5 ± 0.5*

Appendix H Sequence numbers of bacterial data in raw, filtered data and, >1%, > 01% abundant data for each sample. For sample abbreviations see Figure 3.1.

Samples	Number raw input seqs	Number filtered input seqs	> 0.1 %	> 1 %
DC1A	4468	2638	309	4
DC2A	5739	3592	274	4
DC3A	6347	3656	250	3
DE1A	4710	2651	310	9
DE2A	6011	3479	264	5
DE3A	6791	4459	240	4
LC1A	6201	4063	185	17
LC2A	6935	4779	183	19
LC3A	8421	5965	190	11
LE1A	4415	1236	200	16
LE2A	4915	1719	270	13
LE3A	7181	3163	161	15
RC1A	6450	3105	203	8
RC2A	16263	11315	172	6
RC3A	14251	8824	209	7
RE1A	14773	10354	166	14
RE2A	12422	7644	200	6
RE3A	7985	5662	204	12
SC1A	7867	5430	178	13
SC2A	3072	711	325	12
SC3A	13676	8996	201	13
SE1A	4195	1314	217	11
SE2A	8748	5398	181	15
SE3A	8870	6007	186	11

Appendix I Average abundance in % (± 1 SE) of bacterial taxa among rhizosphere samples in total community.

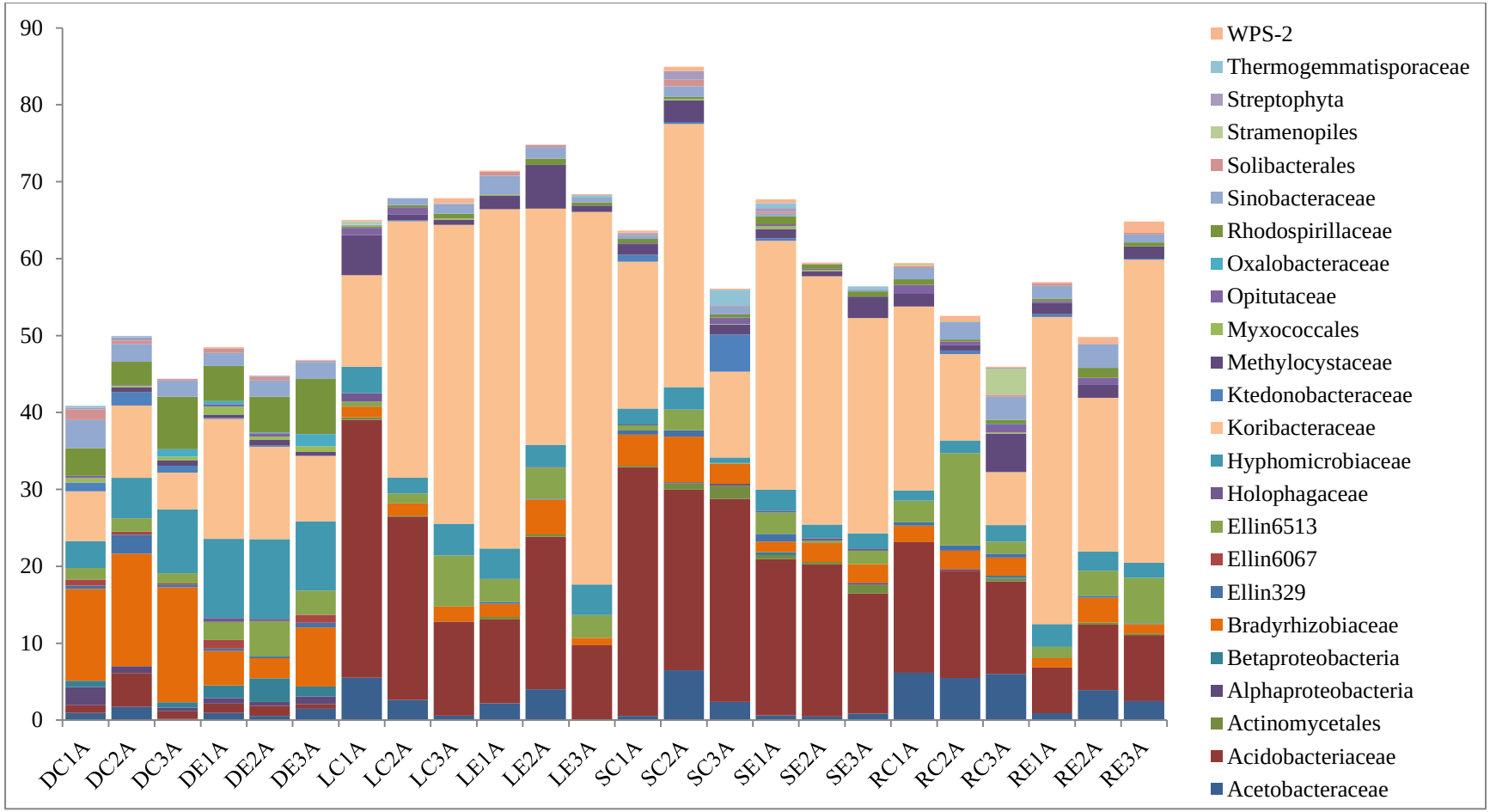
	Daisy	Laurentian	Rockcut	Silver
Acidobacteria	23.5 ± 2.3	71.6 ± 2.3	58.3 ± 4.8	68.4 ± 2.5
Actinobacteria	1.6 ± 0.3	1.3 ± 0.1	1.5 ± 0.3	2.4 ± 0.4
AD3	1.0 ± 0.4	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
Armatimonadetes	0.4 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.0 ± 0.0
Bacteroidetes	2.1 ± 0.1	0.3 ± 0.1	0.7 ± 0.2	0.3 ± 0.1
BRC1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Chlorobi	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Chloroflexi	3.7 ± 0.3	0.3 ± 0.0	0.9 ± 0.2	2.9 ± 1.3
Cyanobacteria	0.4 ± 0.1	0.2 ± 0.1	0.9 ± 0.6	0.4 ± 0.2
Elusimicrobia	0.5 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
FCPU426	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fibrobacteres	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Firmicutes	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
GAL15	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Gemmatimonadetes	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GN02	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Nitrospirae	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.3 ± 0.1
NKB19	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
OD1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
OP11	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
OP3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Planctomycetes	2.5 ± 0.2	1.0 ± 0.2	1.1 ± 0.1	0.8 ± 0.1
Proteobacteria	58.8 ± 2.5	22.3 ± 2.3	30.2 ± 3.6	20.9 ± 1.4
Spirochaetes	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
TM6	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
TM7	0.2 ± 0.1	0.0 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
Verrucomicrobia	0.6 ± 0.1	0.5 ± 0.2	1.1 ± 0.2	0.6 ± 0.2
WPS-2	0.1 ± 0.0	0.4 ± 0.1	1.1 ± 0.2	0.5 ± 0.1
WS3	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0



Appendix J Relative abundance of bacterial phylum in the rhizosphere samples from different sites. Colours indicate bacterial phylum.

Appendix K Average abundance and standard deviation of bacterial taxa among Daisy, Rockcut, Laurentian and Silver sites (> 0.1% abundant community). * indicates where value is significantly different from other sites.

Phylum	Class	Family	Daisy	Laurentian	Silver	Rockcut
Acidobacteria	Acidobacteriia	Acidobacteriaceae	1.6 ± 0.5*	18.3 ± 3.5	23.0 ± 2.2	11.0 ± 1.9
	Acidobacteriia	Koribacteraceae	9.5 ± 0.8*	34.6 ± 4.8	26.2 ± 3.4	23.6 ± 6.6
	DA052	Ellin6514	2.4 ± 0.5	3.1 ± 0.8	1.4 ± 0.4	4.5 ± 0.7
	Holophagae	Holophagaceae	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0	0
	Solibacteres	Solibacterales	0.5 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Actinobacteria	Actinobacteria	Actinomycetales	0	0.2 ± 0.1	0.8 ± 0.2*	0.1 ± 0.1
Chloroflexi	Ktedonobacteria	Ktedonobacteraceae	0.7 ± 0.3	0	1.1 ± 0.7	0.2 ± 0.1
		Thermogemmatisporaceae	0.1 ± 0	0.1 ± 0	0.5 ± 0.3	0
Cyanobacteria	Chloroplast	Stramenopiles	0	0.1 ± 0.1	0	0.6 ± 0.5
		Streptophyta	0.5 ± 0.1	0	0.3 ± 0.2	0
Proteobacteria	Alphaproteobacteria	Acetobacteraceae	1.0 ± 0.2	2.5 ± 0.8	1.9 ± 0.9	4.2 ± 0.8
		Bradyrhizobiaceae	9.4 ± 1.9*	2.0 ± 0.5	3.1 ± 0.6	2.1 ± 0.3
		Ellin330	0.7 ± 0.3	0.1 ± 0	0.4 ± 0.2	0.3 ± 0.1
		Hyphomicrobiaceae	7.8 ± 1.5*	3.4 ± 0.3	2.1 ± 0.3	2.1 ± 0.2
		Methylocystaceae	0.5 ± 0.1	2.5 ± 0.9	1.7 ± 0.3	2.0 ± 0.6
		Rhodospirillaceae	5.0 ± 0.6*	0.4 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
		Unclassified Alpha	1.0 ± 0.3	0	0.1 ± 0	0
	Betaproteobacteria	Ellin6067	0.6 ± 0.2*	0	0	0
		Oxalobacteraceae	0.5 ± 0.2*	0	0	0
		Unclassified Beta	1.3 ± 0.4	0	0.1 ± 0.1	0
	Deltaproteobacteria	Myxococcales	0.6 ± 0.1*	0.1 ± 0	0.1 ± 0.1	0
	Gammaproteobacteria	Sinobacteraceae	2.3 ± 0.3	1.1 ± 0.3	0.6 ± 0.2	2.1 ± 0.3
Verrucomicrobia	Opitutae	Opitutaceae	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.6 ± 0.2
WPS-2	WPS-3	WPS-5	0	0.2 ± 0.1	0.3 ± 0.1	0.6 ± 0.2



Appendix L Relative abundance of bacterial families in soil from different sites (> 0.1% abundant community). For sample abbreviations see Figure 3.1.

Appendix M Average abundance in % (±1 SE) of indicator bacterial taxa among rhizosphere samples. * indicates where OTU is significantly indicative of that site.

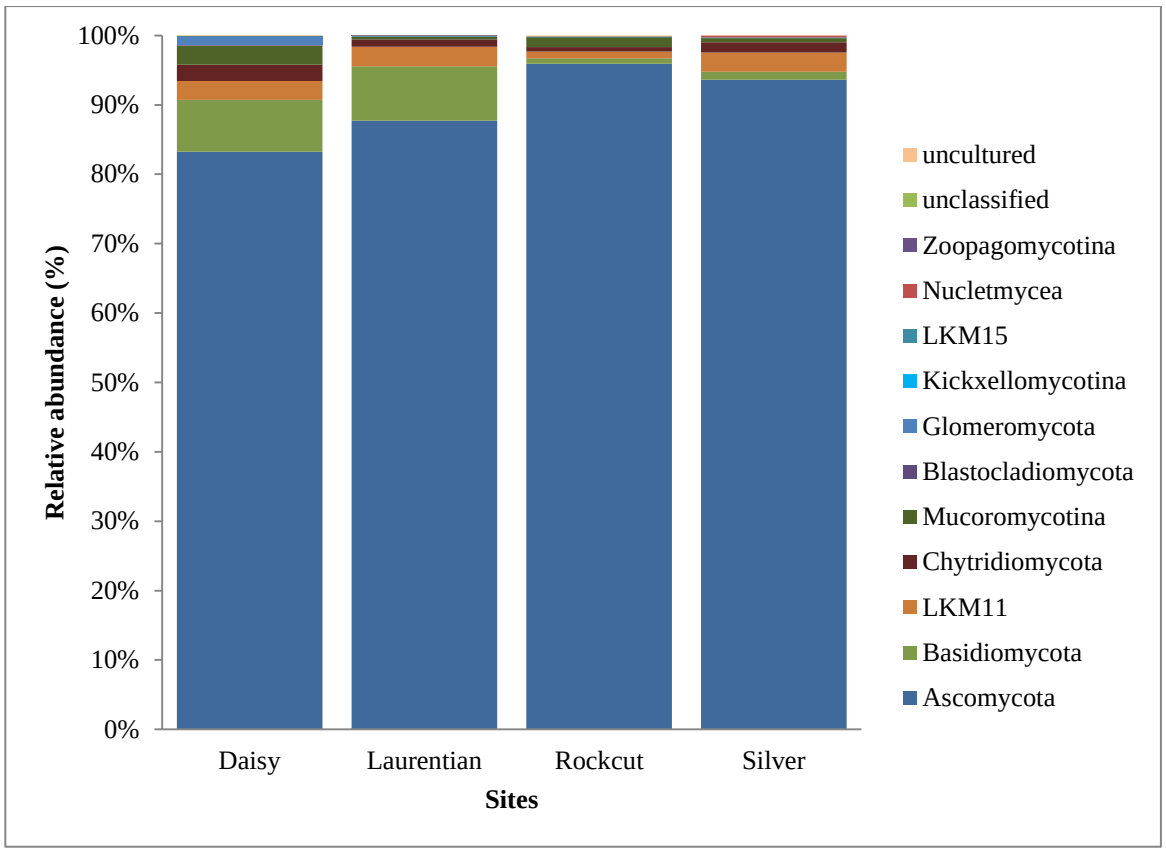
indicator OTU ID	Family name of the OTU	Daisy	Laurentian	Rockcut	Silver
2104	Bradyrhizobiaceae	1.1 ± 0.4*	0	0	0
5397	Koribacteraceae	0	1.2 ± 0.3*	0	0
17	Koribacteraceae	2.3 ± 0.3*	0	0	0
1	Koribacteraceae	0	2.3 ± 0.9*	2.1 ± 0.5*	1.0 ± 0.4*
7741	Acidobacteriaceae	0	0.4 ± 0.2*	0	1.0 ± 0.3*
6994	Acidobacteriaceae	0	1.3 ± 0.4*	0	1.6 ± 0.5*
2776	Acidobacteriaceae	0	1.9 ± 0.4*	0.3 ± 0.2	2.2 ± 0.3*
4478	Koribacteraceae	0	1.3 ± 0.6*	0	1.7 ± 0.2*
52	Koribacteraceae	0	0	0.2 ± 0.2	0.9 ± 0.4*

Appendix N Sequence numbers of fungal data in raw, filtered data and, >1%, > 01% abundant data for each sample. For sample abbreviations see Figure 3.1.

Samples	Number raw input seqs	Number filtered input seqs	> 0.1 %	> 1 %
DC1B	3656	2759	122	18
DC2B	8100	6914	96	10
DC3B	11344	9537	91	7
DE1B	14658	11660	87	8
DE2B	9167	5997	133	14
DE3B	15459	13504	84	10
LC1B	8831	7309	136	20
LC2B	7198	6226	95	12
LC3B	8937	7975	52	6
LE1B	9447	7732	75	12
LE2B	9604	7849	71	8
LE3B	7007	5602	106	17
RC1B	5128	4408	90	12
RC2B	6711	5311	73	10
RC3B	9840	7624	107	11
RE1B	10481	9277	61	11
RE2B	15293	13295	70	10
RE3B	15065	12114	64	12
SC1B	9575	8296	83	11
SC2B	7220	5789	87	10
SC3B	11278	9046	79	7
SE1B	6256	4642	119	14
SE2B	7633	5919	104	14
SE3B	10260	7395	113	17

Appendix O Average abundance in % (± 1 SE) of fungal taxa among rhizosphere samples in total community.

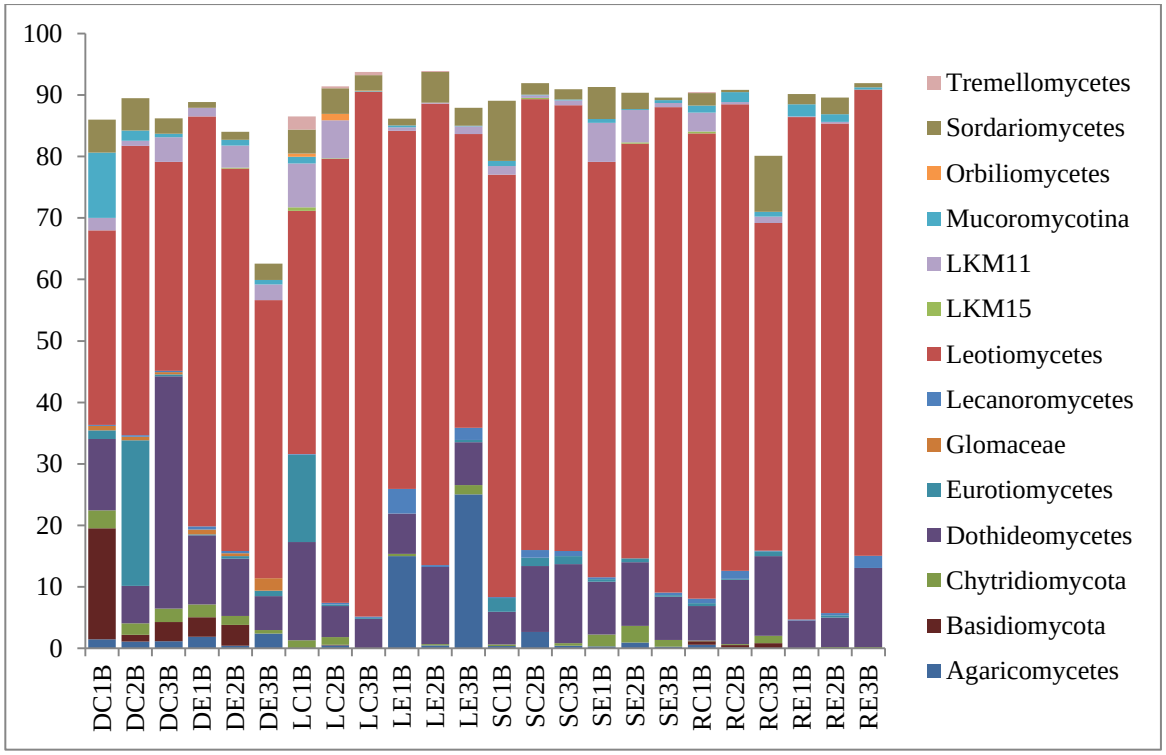
	Daisy	Laurentian	Silver	Rockcut
Ascomycota	82.7 ± 4.3	87.3 ± 4.2	93.5 ± 1.3	95.6 ± 0.8
Basidiomycota	7.4 ± 2.6	7.8 ± 4.0	1.1 ± 0.4	0.8 ± 0.2
Blastocladiomycota	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Chytridiomycota	2.4 ± 0.3	1.1 ± 0.3	1.5 ± 0.5	0.6 ± 0.2
Glomeromycota	1.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Kickxellomycotina	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
LKM11	2.7 ± 0.5	2.8 ± 1.2	2.8 ± 1.0	1.0 ± 0.5
LKM15	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
Mucoromycotina	2.7 ± 1.5	0.4 ± 0.2	0.6 ± 0.1	1.4 ± 0.2
Nucletmycea	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.0 ± 0.0
unclassified	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
uncultured	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
Zoopagomycotina	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0



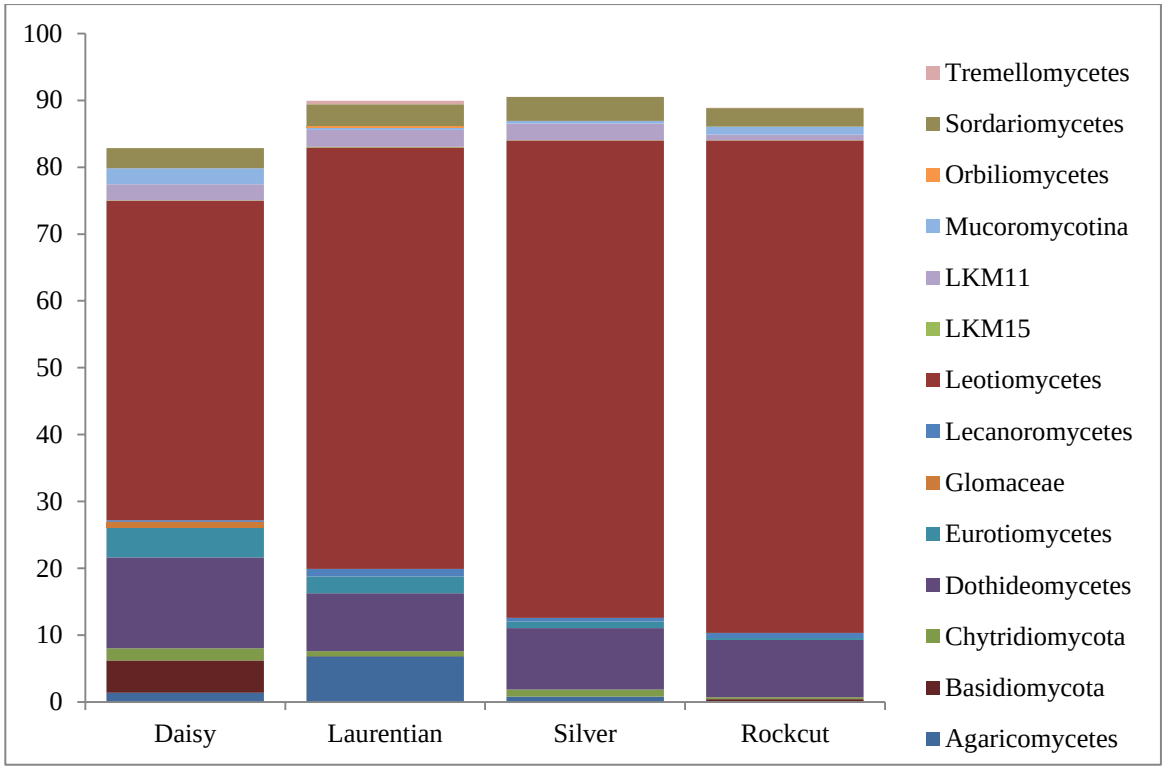
Appendix P Relative abundance of fungal phylum in rhizosphere soil from different sites. Colours indicate fungal phylum.

Appendix Q Average abundance and standard deviation of fungal taxa among Daisy, Rockcut, Laurentian and Silver sites (> 0.1% abundant community). * indicates where value is significantly different from other sites.

Kingdom	**Phylum**	**Class**	**Daisy**	**Laurentian**	**Silver**	**Rockcut**
Fungi	Ascomycota	Dothideomycetes	13.6 ± 4.5	8.7 ± 1.7	9.2 ± 1.0	8.5 ± 1.5
		Eurotiomycetes	4.5 ± 3.5	2.5 ± 2.2	1.0 ± 0.3	0.3 ± 0.1
		Lecanoromycetes	0.2 ± 0.1	1.2 ± 0.6	0.5 ± 0.2	0.8 ± 0.3
		Leotiomycetes	47.8 ± 5.3	63.0 ± 6.5	71.4 ± 1.7	73.7 ± 3.8
		Orbiliomycetes	0	0.3 ± 0.2	0	0
		Sordariomycetes	3.0 ± 0.7	3.3 ± 0.5	3.6 ± 1.3	2.8 ± 1.2
	Basidiomycota	Agaricomycetes	1.4 ± 0.3	6.8 ± 4.0	0.8 ± 0.3	0.1 ± 0.1
		Tremellomycetes	0	0.5 ± 0.3	0	0
		unclassified Basidio	4.8 ± 2.5	0	0	0.3 ± 0.1
	Chytridiomycota		1.9 ± 0.3	0.8 ± 0.3	1.1 ± 0.4	0.3 ± 0.2
	Glomeromycota	Glomaceae	0.8 ± 0.2*	0	0	0
	LKM11		2.4 ± 0.5	2.5 ± 1.2	2.5 ± 1.0	0.8 ± 0.4
	LKM15		0	0.1 ± 0.1	0.1 ±	0.1 ± 0
	Mucoromycotina		2.4 ± 1.5	0.3 ± 0.2	0.4 ± 0.1	1.2 ± 0.2



Appendix R Relative abundance of fungal classes in soil from different sites (> 0.1% abundant community). Colours indicate fungal class. For sample abbreviations see Figure 3.1.



Appendix S Relative abundance of fungal classes in soil from different sites (> 0.1% abundant community). Chao richness and Shannon-Wiener diversity are presented on top of each site.

Appendix T Average abundance in % (±1 SE) of indicator fungal taxa among rhizosphere samples. * indicates where OTU is significantly indicative of that site.

indicator OTU ID	Family name of the OTU	Daisy	Laurentian	Silver	Rockcut
983	Leotiomycetes	0	8.1 ± 4.0*	3.3 ± 1.4*	6.9 ± 1.8*
2853	Leotiomycetes	0	1.6 ± 0.6*	0.3 ± 0.2	1.8 ± 0.4*
581	Leotiomycetes	0.2 ± 0.2	0	1.3 ± 0.7*	0
12	unclassified.Basidiomycota	2.3 ± 1.3*	0	0	0
421	unclassified.Basidiomycota	1.5 ± 1.0*	0	0	0
2689	Leotiomycetes	0	0	2.2 ± 1.0*	0.3 ± 0.3
232	Leotiomycetes	0	0.3 ± 0.2	0	1.3 ± 0.4*
14	LKM11	1.3 ± 0.4*	0	0	0
461	unclassified.Ascomycota	0	0.2 ± 0.2	0	1.3 ± 0.4*