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Plant Tissue Associated Microbial Community Composition in a Permafrost Thaw Zone

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Plant Tissue Associated Microbial Community Composition in a Permafrost Thaw Zone

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Due to climate change, thawing permafrost may cause a release of stored carbon which can increase concentrations of greenhouse gases in the atmosphere. Global warming has been most extreme at northern latitudes. In northern Sweden, warming already has impacted the ecosystems and communities that make up this area. To address how microbial community is affected by the changing landscape, I collected longitudinal samples of roots and leaves of seven different plant species across three permafrost thaw zones and profiled the microbial communities using high throughput sequencing. The comparative analysis of the data will be used to answer questions about ecological secession and community formation in these permafrost environments, and to begin characterizing microorganisms involved in mediating carbon fluxes in wetland ecosystems. This study integrates with a larger project on how microbial systems respond to and contribute to global change. In my talk, I will explain the observed colonization patterns and their significance, and how these communities fit in a dynamic and vulnerable permafrost system.

“Hope and the future for me are not in lawns and cultivated fields, not in towns and cities, but in the impervious and quaking swamps.”

Walking

Henry David Thoreau
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1. Introduction

Permafrost, soil that remains frozen for more than two years, accounts for more than one-third, of the Earth’s terrestrial carbon (Tarnocai et al., 2009). Much of this carbon is located in subarctic peatlands, which cover 12% of the earth’s surface, and are one of the ecosystems most vulnerable to climate change. The southern border of northern permafrost has thawed drastically in the past thirty years, allowing carbon immobilized since the last glaciation to become biologically available and prompting the transition to wetland environments (McGuire, 2009). This pattern of thawing and carbon release will continue as warming progresses northward. Biologically available carbon mobilized from permafrost is metabolized by bacteria and transformed into methane and carbon dioxide, two potent greenhouse gases (Figure 1). These gases are then released from the water-logged soils into the atmosphere, causing a positive climate feedback (Zimov et al., 2006). In this study, the plant-associated microbial communities were characterized, and their relevance to the carbon cycle evaluated.
Figure 1: Plants and microorganisms play a role in the transport of carbon in permafrost thaw ecosystem. Plant-associated microbiota transport carbon fixed during photosynthesis to the pool of peat organic matter, and later control the speciation of carbon emissions. Asterisks indicate processes mediated by phyllosphere and rhizosphere microbiota.

1.1 Carbon in a permafrost thaw wetland

The rate of carbon cycling in permafrost thaw zones is predicted to increase as warming continues due to the increased amount of carbon available to microbes and increased metabolic activity of plants and microbes. The increase in carbon availability is hypothesized to come from organic material released from permafrost and the increase in carbon fixed through photosynthesis (McGuire, 2009). The rate of carbon cycling in the permafrost region is expected to increase due to more soil microbiota becoming metabolically active as permafrost thaws, and the increase of metabolic rates with temperature (Leahy and Colwell, 1990; Price and Sowers, 2004).

1.1.1 Magnitude of flux

The Arctic has experienced a faster rate of warming due to climate change than anywhere else on the globe (Hansen et al., 2006). In the Arctic, carbon levels in wetlands are increasing quickly and variably (Kennedy et al., 2008). This is occurring because the warming climate drastically changes permafrost zones by inducing thaw when the average yearly temperature is above 0 °C (Schuur and Abbott, 2011). In wetland areas, this organic carbon is available to microbes for metabolism mostly as dissolved organic carbon (Evans et al., 2005). As permafrost thaws, the biological availability of dissolved organic matter increases, increasing the carbon flux out of the wetland (Turetsky et al., 2000).
1.1.2 Speciation of flux

Much of the permafrost that has begun to thaw is located in wetland areas, which means that the influx of newly thawed carbon has a more unpredictable fate (Schuur et al., 2008). Unpredictability arises because both CO₂ and methane cycling occur in these ecosystems (Anderson et al., 2010). The nutrient-rich and water-saturated environment of a wetland creates an anoxic environment, which is an ideal setting for methanogenic archaea that metabolize excess organic matter left over after more energetically favorable electron acceptors, like oxygen and sulfate, are consumed by other bacteria (Burgin et al., 2011; Segers, 1998). Methane is a more potent greenhouse gas than carbon dioxide, providing 28 times more of a greenhouse forcing per molecule over 100 years (Anderson et al., 2010; Keller et al., 2009). This means that carbon within wetland environments could have variety of climate forcings after it is metabolized and emitted, depending on how its speciation when emitted.

1.2 Thawing permafrost peatlands

The change in habitat structure as permafrost thaws can be divided up into three stages: palsa, bog and fen (Figure 2). The palsa habitat consists of grasses, lichens, and moss-dominated peat mounds, which rise above the surrounding wet area due to a supportive core of ice (Kujala et al., 2008). The process of palsa formation is well characterized, since the formation of palsa often occurs in annual cycles due to differences in snow coverage and freezing depth. Ice forces the bog surface to rise above the water table, creating a dry environment (Seppala, 1982). This is a naturally occurring process, but the rate of collapse has increased with climate change and permafrost thaw (Luoto et al., 2004). Because of the well-studied pattern of palsa formation and collapse, the secession of plants that colonize this region as permafrost thaws is also well
understood (Kuhry, 2008; Zuidhoff and Kolstrup, 2005). While plant species-specific colonization patterns after collapse differ from wetland to wetland, they share a characteristic pattern. Woody plants with complex root systems dominate the dry palsa. After thaw begins roots have much better access to the water table (Sugimoto et al., 2002). Hydrophilic species like sphagnum and sedges begin to dominate these waterlogged soils. Because of the cation uptake by *Sphagnum spp.* and the lack of mixing with groundwater, the bog becomes acidified (Mitsch and Gosselink, 1993). This process makes it more challenging for characteristic palsa communities of dicot plants to live. As permafrost fully thaws, the bog continues to sink, and peat now rests at the water table, creating a groundwater fed, nutrient-rich fen.

![Figure 2: Mire topography and plant colonization patterns changes as permafrost thaws (Johansson et al., 2006).](image-url)
1.3 Carbon movement in a permafrost thaw wetland

Through photosynthesis, plant communities act as a mediator of the flux of carbon from the atmosphere to belowground pools. This occurs as plant tissue accumulates biomass through carbon fixation and then senesces and is decomposed (Raich and Schlesinger, 1992). The flux of biomass into the belowground environment is accompanied by an input of the microbes that colonize this plant tissue. These microbial communities, along with what previously existed in the peat, support movement of the plant carbon back into the atmospheric pool through the process of decomposition (Kögel-Knabner, 2002). While permafrost and bulk peat communities have been well characterized, communities mediating decomposition of plant material in these ecosystems are relatively unknown.

The first communities that have the opportunity to decompose plant material are the microbes colonizing plant itself (Thomaz and Wetzel, 1995). These communities exist on leaves and roots, and are known as phyllosphere and rhizosphere microbial communities respectively. In addition to mediating the carbon flux by decomposing plant material, they may also metabolize products produced by other organisms, serving as a dampener of methane emissions (Deng et al., 2015; Jansson and Taş, 2014; Raghoebarsing et al., 2005).

1.4 Microbiology of the phyllosphere

Microbial communities associated with a phyllosphere are defined as all bacterial communities on plant surfaces above the ground, though in this study they are defined solely as leaf tissue in angiosperms and currently photosynthetically active tissue in bryophytes. These communities can be made up of endophytes, which are microorganisms living within the plant tissues, or epiphytes, which are microorganisms living on the host plant (Ruinen, 1956). Early in the study
of microbiology, bacterial colonization of plant tissue was only considered when it resulted in pathogenesis. Now it is understood that most often these organisms function commensally, not impacting the health of the host plant (Baldotto and Olivares, 2008; Lindow and Brandl, 2003). Several factors make colonization of plant leaves difficult for microorganisms, and contribute to the distinction of these communities. Plant anatomy, which is designed to keep moisture in, and pathogens and predators out, uses a waxy cuticular layer to keep water in the leaf (Leveau and Lindow, 2001). This contributes to a dry and nutrient poor environment for microorganisms colonizing the leaf (Leveau and Lindow, 2001; Miller et al., 2001). An additional challenge for microbes inhabiting this zone is the ephemeral nature of a leaf environment. Unlike roots, leaves are shed on a regular basis. The formation of a core phyllosphere microbiome has been demonstrated to occur via vertical transmission, meaning the seed provides initial microbial colonizers, as well as horizontal transmission of microbes, in which soil and water continually contribute to the microbial community composition present on the plant’s above ground surfaces (Knief et al., 2010; Vorholt, 2012).

The microbial communities associated with the phyllosphere are often closely related to the communities present in the surrounding environment, but are enriched in the phylum Proteobacteria, which often survive using host metabolites, and Actinobacteria (Delmotte et al., 2009; Knief et al., 2012). While trends exist at the phylum and class level to differentiate the phyllosphere from other habitats, at higher resolution, there is high variance between different plants (Delmotte et al., 2009). Differences in colonization patterns can be shaped by environmental variables, host plant health or genotype, or differences in surrounding microbial communities (Redford et al., 2010). One significant process selected for within phyllosphere microbial communities is methano and methylotrophy (Fall and Benson, 1996). These processes
allow microbes to obtain energy from single-carbon molecules, such as methane or methanol. While present elsewhere in the environment, these organisms are able to live on leaves due to the methane present at the leaf’s surface due to the breakdown of the plant’s own organic matter, cell wall synthesis, and anaerobic production in the soil (Keppler et al., 2006; Nadalig et al., 2011).

1.5 Microbiology of the rhizosphere

Rhizosphere, or root associated, microbial communities have been more extensively studied than phyllosphere communities, due to their more direct connection to plant nutrition (Philippot et al., 2013). The rhizosphere is the name for all belowground areas impacted by the growth of roots, including the roots themselves, and the thin layer of soil around them (Berendsen et al., 2012; Bonfante and Anca, 2009; Mendes et al., 2011; Philippot et al., 2013). The size of this zone depends on the structure of the roots, including density and size of filaments, and on fungal associations of the host plant (Parniske, 2008). Roots contain or exude rhizodeposits, nutritive molecules or complete cells that can serve to attract motile, chemotaxic bacteria, or allow populations of stationary bacteria to multiply (Hannula et al., 2012; Vandenhoornhuyse et al., 2007).

The makeup of root exudates depends both on the species of plant and on the growing conditions of the plant. Carbon-containing exudates most often get used by bacteria with close plant associations, specifically Burkholderia spp. and other related groups of Proteobacteria (Vandenhoornhuyse et al., 2007). The influence of root exudates causes rhizosphere communities of plants in different soil to be more similar to each other than the soil communities are (Costa et al., 2006). These root communities are often less diverse than the microbial communities in the surrounding soil matrix (Berg and Smalla, 2009). Because of differing root
architecture and exudates, plants impact microbial communities in the rhizosphere generally and
on a species-specific level (Bergsma-Vlami et al., 2005; Kowalchuk et al., 2002).

An additional impact of plants on the microenvironment occurs as roots uptake water and
undergo cellular respiration. The uniqueness of the energy and nutritive resources around these
roots leads to the presence of distinct taxa in root-associated material (DeAngelis et al., 2009).
The presence of specific microorganisms, as well as the unique chemical structure of this zone,
can play a role in nutrient cycling, specifically of nitrogen and carbon. Much of this research has
been done on agricultural plants, which have been bred to not have features that maximize
microbial associations, since nutrients are provided via fertilization (Germida and Siciliano,
2001; Smith et al., 1999). It is hypothesized that in natural ecosystems, the surrounding plants,
fungi, and animal interactions are a more significant driving force than in agricultural
ecosystems, where that signal is washed out by heavy mechanical and chemical intervention
(Bezemer et al., 2010; De Deyn et al., 2004).

Because of the close association between microbial communities and the host plant, the
communities are temporally variable. While resembling bulk soil communities more than that of
microbes initially living on the seed, the rhizosphere microbial community is influenced by the
community on the seed, especially early in development (Nelson, 2004). Throughout the process
of root growth, faster growing bacteria are more likely to be found at the root tip, while slower
growing bacteria are more likely to be found in zones where growth has reached equilibrium
(Folman et al., 2001). Additionally, as root growth slows, microbe-microbe interactions become
more significant, acting as an initial defense against pathogens or biofilm formation to maximize
efficiency of nutrient transfers in the belowground environment (Cook et al., 1995; Raaijmakers
and Mazzola, 2012).
1.6 Significance of phyllosphere and rhizosphere microbiota to elemental cycling in a permafrost thaw ecosystem

Leaf and root microbial communities both have the potential to assist in the decomposition process which mediates the flow of carbon between atmospheric, above, and below ground pools (Strickland et al., 2009). By understanding the microbial biogeography of this area, the first steps of the decomposition process and carbon flow can be understood. Additionally, the hosting or inhibition of methanogens or methanotrophs on plant tissue can shape the speciation of carbon emissions. Many details of colonization patterns are dependent on plant-specific characteristics, which will be described in the “Study species” section below.

1.7 Experimental questions

To examine the rhizosphere and phyllosphere communities of plants in a permafrost thaw zone, and how they interact with the dynamic carbon fluxes of this environment, I chose to examine microbial communities associated with seven plant species, *Andromeda polifolia*, *Rubus chamaemorus*, *Eriophorum vaginatum*, *Carex rotundata*, *Eriophorum angustifolium*, *Sphagnum fuscum*, and *Sphagnum balticum* (Table 1). These species have highly variable morphologies and colonization patterns. Species were collected in different states of permafrost thaw to determine if habitat type, as well as species type plays a role in determining microbial colonization patterns. Microbial community makeup was determined by sequencing of the 16S rRNA gene, a highly conserved gene used for the classification and identification of both bacteria and archaea. With this study, I hope to address the following questions:

- What microorganisms colonize phyllosphere and rhizosphere of permafrost plants?
• Do leaves and roots facilitate the colonization by unique microbiota within the rhizosphere and phyllosphere?

• How does diversity compare between the phyllosphere, rhizosphere, and bulk peat microbiota?

• To what extent do host plant species and habitat impact the microbial colonization in the phyllosphere and rhizosphere?

• Do phyllosphere- and rhizosphere-associated microbiota contribute to methane cycling and impact the greenhouse gas emissions of the mire?

### Table 1. Study plants, sampling location, and materials collected for each plant.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Plant Type</th>
<th>Habitat</th>
<th>Tissue Type</th>
<th>Total samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rubus chamaemorus</em> (Cloudberry)</td>
<td>Herbaceous</td>
<td>palsa</td>
<td>Leaf, Root, Associated Peat</td>
<td>39</td>
</tr>
<tr>
<td><em>Andromeda polifolia</em> (Bog rosemary)</td>
<td>Herbaceous</td>
<td>palsa, bog</td>
<td>Leaf, Root, Associated Peat</td>
<td>78</td>
</tr>
<tr>
<td><em>Eriophorum vaginatum</em></td>
<td>Sedge</td>
<td>palsa, bog</td>
<td>Leaf, Root, Associated Peat</td>
<td>78</td>
</tr>
<tr>
<td><em>Carex rotundata</em></td>
<td>Sedge</td>
<td>bog, fen</td>
<td>Leaf, Root, Associated Peat</td>
<td>78</td>
</tr>
<tr>
<td><em>Eriophorum angustifolium</em></td>
<td>Sedge</td>
<td>fen</td>
<td>Leaf, Root, Associated Peat</td>
<td>39</td>
</tr>
<tr>
<td><em>Sphagnum fuscum</em></td>
<td>Bryophyte</td>
<td>palsa</td>
<td>Photosynthetic, Non-photosynthetic</td>
<td>30</td>
</tr>
<tr>
<td><em>Sphagnum balticum</em></td>
<td>Bryophyte</td>
<td>bog</td>
<td>Photosynthetic, Non-photosynthetic</td>
<td>30</td>
</tr>
</tbody>
</table>
1.8 Plant communities and study species

As previously discussed, the plant communities in permafrost thaw wetlands follow a predictable pattern of habitat succession of dry palsa to rainwater fed bog to groundwater fed fen (Hodgkins et al., 2014). Most diversity exists in zones where permafrost thaw has not yet begun, though primary productivity is greatest in thawed, mineral rich fen environment.

1.8.1 Sphagnum species

Sphagnum moss is a dominant species in partially thawed palsa and all bog sites. Stordalen mire is home to roughly ten species of sphagnum (Sonesson and Kvillner, 1980). *Sphagnum fuscum* and *Sphagnum balticum* were examined in this study (Figure 3). *S. fuscum* is more prominent in microhabitats under which permafrost has just begun to thaw, and *S. balticum* is more common in ecosystems where permafrost thaw is more complete, since it thrives in wetter environments (Rydin and McDonald, 1985). Sphagnum plays an important role in the successional process of palsa to fen, as it acidifies the environment and allows for more free movement of water (Giller and Wheeler, 1988; Zoltai, 1993). Sphagnum serves as one of the biggest carbon sinks in

---

Figure 3: Morphologically distinct species of sphagnum moss grow at different stages of permafrost thaw. A) *Sphagnum fuscum* grows in palsa, and B) *Sphagnum balticum* in bog habitats.
wetland ecosystems (Heijmans et al., 2002). This ecosystem function occurs because of their large primary productivity, as well as their inhibition of decomposition due to lowering the pH (Hájek et al., 2011). They also outcompete vascular plants like sedges that speed methane transport away from anoxic zones (Benavides and Vitt, 2014).

Sphagnum mosses have been shown to host a complex microbiota which differs from other plants and communities present in surrounding peat (Jassey et al., 2013). This microbial community, as characterized by a metagenomic profiling, is dominated by Proteobacteria and is enriched in motile bacteria and bacteria with a high metabolic diversity (Bragina et al., 2014). Microbial communities in sphagnum also show variation with warming. The number of trophic levels present in the habitat decreases with an increase in temperature, which leads carbon and nutrients to become more labile. This result indicates a potential for reduction in diversity as the growing season passes. Microbial communities vary above and belowground, due to different light and temperature conditions (Jassey et al., 2013).

One component of the sphagnum microbiome that serves an important ecological role is the presence of endophytic methanogens (Chen and Murrell, 2010). Sphagnum associated microorganisms, primarily on plants that live in wetter areas, have the potential to oxidize up to 30 µmol of methane per gram dry weight of plant each day, thus decreasing wetland’s contributions to atmospheric methane (Liebner et al., 2011). The presence of methanotrophs has been found within the tissue of Sphagnum balticum, which is found more often in wetter environments (Putkinen et al., 2014). Bacterial species that are carrying out this oxidation are likely related to Methylocella palustris and Methylocapsa acidiphilia (Raghoebarsing et al., 2005). This methanotrophy is beneficial to the sphagnum since it provides additional carbon
dioxide from microbial respiration to the plant to use during photosynthesis (Parmentier et al., 2011).

1.8.2 Sedge species

Sedge species are prominent across the thaw gradient (Rawat and Adhikari, 2005). Sedges are monocot angiosperms characterized by long, grass-like leaves, triangular cross sections, and spirally arranged leaves and they are found in both palsa and fen habitats (Figure 4). *Eriophorum vaginatum* colonizes drier, completely frozen to slightly thawed areas (Hopkins and Sigafoos, 1951). *Eriophorum angustifolium* colonizes wetter completely or almost completely thawed areas, as well as the edges of lakes (Jorgenson and Osterkamp, 2005). *Carex rotundata* colonizes partially thawed areas (Sonesson and Kvillner, 1980). These species serve an important ecological role in a wetland ecosystem. They account for a significant proportion of the primary productivity in the ecosystem, and also act as a means of transport for methane produced by microbial anaerobic respiration (Figure 1). This process allows the insoluble methane to travel through fibrous tissue of the plant stem to the atmosphere without being oxidized. However, the

---

**Figure 4. Dominant species of sedge shifts as permafrost thaws.** A) *Eriophorum vaginatum*, B) *Carex rotundata*, and C) *Eriophorum angustifolium* growing in a palsa, fen and fen habitat, respectively.
scope of this pattern may be limited because of the oxidation that can occur when roots exude oxygen into the peat. The presence of exuded oxygen can act to inhibit the anaerobic respiration that results in methanogenesis (Ström et al., 2003).

Methane transport has been documented in the species *Eriophorum angustifolium*, *Eriophorum scheuchzeri*, and *Carex aquatilis* (Joabsson et al., 1999). There has been no work specifically demonstrating the function of *Eriophorum vaginatum* or *Carex rotundata*, two of this study’s species of interest, as methane transporters. Along with methane, sedge leaves can function as a source of water vapor due to evapotranspiration. These leaf evaporation-based exudates likely will impact microbial communities that colonize the leaves of these species.

Leaves on sedge study species plants range from 20 cm (in *E. vaginatum*) to 100 cm (in *E. angustifolium*), with *C. rotundata* growing to approximately 60 cm. Leaves follow a typical graminoid growth strategy in which leaves grow and senesce simultaneously, about every two months (Shaver and Laundre, 1997). A portion of green leaves overwinter each year, indicating that microbial colonization may not involve new primary succession each year (Shaver and Laundre, 1997). Growth rates were found to depend mostly on nutrient abundance and water table depth, with phosphorus often acting as a limiting nutrient. Leaf tissue in sedge species have been shown to be unique habitats for microbial colonization. The plant tissue of sedge species have been found to contain a smaller fraction of common plant associated microbes than the microbiota associated with peat mosses or dwarf shrubs, the other two plant groups that are examined in this study (Bragina et al., 2015).

The root structures of these species are made up of a thick taproot and smaller root hairs. While no conclusive molecular analysis has been completed analyzing microbes that may
colonize these roots, research has been done to examine the bulk geochemical changes that root microbes may mediate. Sedge species have high phosphorus demands, and concentrations of phosphorus are often low in peat environments (Chapin III and Slack, 1979). Phosphatase exoenzymes of microbial origin are important part of balancing a wetland’s nutrient budget. Enzyme activities, modeled by Michaelis-Menten kinetics, were shown to be temporally variable, and not in synch with plant growth (Moorhead et al., 1993). This activity varies depending on CO$_2$ level (Moorhead and Linkins, 1997). This indicates a potential for coevolution of microbes and plant, due to the capability of the plant to integrate nutrients across the growing season. Nutrient acquisition interactions between sedges and microbes have been shown to be partially mediated by mychorizal fungi (Peters et al., 2011). The other important role of the root microbiome is to mediate the decomposition process.

1.8.3 Dicot species

Two annually recurrent dicot plant species dominate Stordalen’s drier areas and these are Rubus chamamorus, or cloudberry, and Andromeda polifolia, or bog rosemary. Historically, these two species have been found in both palsa and bog habitats, though they make up a larger portion of biomass in palsa (Haapanen et al., 2013). When these plant’s growth patterns were analyzed to determine co-occurrence, cloudberry was found to be most closely associated with S. fuscum, while bog rosemary was equally closely associated with other characteristic palsa species, such as E. vaginatum and various lichens (Sonesson and Kvillner, 1980). When a thorough inventory was taken in the 1970s, bog rosemary was found in 11.9% of experimental quadrants, while cloudberry was found in 9.8% of experimental sites. While this information gives an idea of relative abundances, the elevated water table due to permafrost melting over the past decades has
shrunk the palsa portion of the mire. This means that the overall plant communities is now likely dominated by species that thrive in wet areas, so data given may be an overestimate.

Cloudberry leaves likely act as a harsh environment for microbial colonization, as the leaves are widely thought to possess antimicrobial properties (Thiem and Goślińska, 2004). The butanolic and ellagic acid fractions of leaf extracts were found to be an effective antimicrobial agent against gram-positive species, indicating that colonization will likely be dominated by gram-negative taxa, while the berries were found to have more antimicrobial activity against gram-negative taxa (Puupponen-Pimiä et al., 2001). The ellagic acid present in leaves, which comes from the decomposition of plant tannins, is known to serve as an insect deterrent. The berries of the cloudberry plant are known to be infected with fungus, including members of the *Botrytis* and *Penicillium* genera, a potential source for antibiotic activity (Thiem, 2003).

To our knowledge, no research has been conducted to examine the microbiota associated with bog rosemary leaves, though knowledge of the leaves chemical composition can offer hints to what microbes may colonize them. However, they are known to contain grayanotoxin, which is known to cause digestive, respiratory, and nervous disorders in livestock (FDA, 2012). The presence of this compound is likely to impact microbes, since it functions by binding and deactivating sodium channels, which bacteria use to modulate their osmotic pressure. Additionally, their small surface area and thick cuticle may make these leaves a challenging habitat for bacteria.

Both species were found to have similarly small above ground biomass per unit area, concentrating carbon allocation belowground (Flower-Ellis, 1975; Wallén, 1986). Differences were seen in belowground growth, which could strongly influence microbial colonization.
Cloudberry roots were found to go much deeper, accumulating significant biomass past 25 cm beneath the surface of the peat. Roughly half of root mass is found in the rhizome and half in fine root hairs. Bog rosemary appears to allocate much more energy into creating fine root hairs to maximize surface area. A significantly greater portion of root biomass was found in fine root hairs than within the rhizome, and almost no roots extended past 12 cm (Wallén, 1986).

By integrating knowledge about the physiology and chemical makeup of these plants with phyllosphere and rhizosphere microbiota data, microbial patterns can be predicted, and then evaluated.
2. Materials and methods

2.1 Sample Collection. Samples were collected from Stordalen Mire in Abisko, Sweden on five different days in 2015 (20 June, 21 June, 20 July, 23 July and 2 September; Figure 5A). The site is managed by the University of Stockholm and the Integrated Carbon Observation System. It is located 10 km east of Abisko, at 68°21' N, 18°49' E; altitude is 363 m above sea level. Within the mire, three sampling sites were selected, each roughly 100 m apart. At each site, samples were collected from all three sampled habitats, palsa, bog and fen (Figure 5B). Within Stordalen, 49% of the area is made up of intact palsa habitat, 37% is made up of partially thawed bog habitat, 12% is made up of fully thawed fen habitat. Within recent years, thawing areas have increased, with bog sites expanding by 3% and fen sites expanding by 54% from 1970 to 2000 (Hodgkins et al., 2014; McCalley et al., 2014).

Figure 5. Sampling location and habitat types. A) Location of Stordalen Mire outlined in purple is located in Sweden on the southern border of permafrost thaw. B) Study site is composed of a patchwork of three habitat types, fen, bog and palsa.
Figure 6: Sampling Scheme. Quintuplicate samples of leaf, root, and associated peat for seven different plant species were collected in June, July, and September of 2015 across palsa, bog and fen.

Plants were selected to be representative species across the mire’s three habitats (Figure 6). Leaves were collected from target plants wearing latex gloves and using forceps sterilized with ethyl alcohol and rinsed twice with water. The amount of sampled material varied by species and is described in Table 2. When all phyllosphere sample material had been collected, a serrated edge knife was used to cut around the base of the plant. The plant was then pulled out with associated peat still attached. Fifteen ml of peat from 2-3 cm deep was collected, from as close to the root as possible without touching the root. The root was collected by snipping small pieces of the fibers into a 1.5 ml Eppendorf tube. Both the primary root and lateral roots were included; the root tip was excluded. Three replicates of each sample were taken at the first site, and one replicate was taken at the second and third sites. For peat samples at the triplicate sites, peat from all three replicates was combined in one tube for a bulk analysis. At bog and fen sites, a 30 mL sample of surface water was also collected. After collection, samples were stored in a cooler, and then transferred to a -80 °C freezer within 4 hours of collection. The samples were shipped on dry ice to Wellesley College and, upon their arrival, were immediately stored at -80 °C until further processing.
2.2 Matrix and Plant Characterization. Un-dried, homogenized peat samples were mixed with DI water in a 2:1 ratio by weight and, pH was measured. Collected surface water was tested for pH. For each plant, in both June and July, leaves were collected, dried, and ground. The homogenized plant tissue was analyzed for the carbon to nitrogen ratio.

2.3 Sample Processing. Each peat, root, and leaf sample was processed using the MOBIO Power Soil DNA extraction kit (MOBIO Laboratories, Carlsbad, CA, USA) according to manufacturer’s instructors. Between 50 and 100 mg of sample was processed (Table 2). Briefly, following mechanical and chemical lysis, cell and environmental debris were removed. Two purification steps were completed to remove carbohydrates, humic acids, phenolic compounds, and other PCR inhibitors. The purified sample was run through a spin column, precipitated by ethanol, and eluted in 50 µL of sterile elution buffer to a new 1.5 ml collection tube. Upon extraction, DNA was quantified by NanoDrop (Thermo Scientific, Inc., Wilmington, DE, USA) and sent to Argonne National Laboratories on dry ice.

From each sample, 16S rRNA genes were amplified using the following:

- A forward primer containing the following components (5’ to 3’)
  - An illumina adaptor: AATGATACGGCGACCACCGAGATCTACAC
  - A barcode sequence, which varied depending on the sample
  - Forward primer pad and linker: TATGGTAATT GT
- 515F primer GTGYCAGCMGCGCGGTAA
- A reverse primer containing the following components (5’ to 3’)
  - A reverse complement of 3’ illumina adaptor:
    CAAGCAGAAGACGGCATACGAGAT
  - Reverse primer pad and linker: AGTCAGTCAG CC
  - 806R reverse primer: GGACTACNVGGGTWTCTAAT (Apprill et al., 2015; Caporaso et al., 2012; Jiang et al., 2006).

Sequences greater than 250 base-pairs in length were assigned to a taxonomy using open reference OTU picking at 97% sequence identity. Each cluster was assigned taxonomy by blast analysis and using the GreenGenes reference database (McDonald et al., 2012). Chloroplast and mitochondrial sequences were removed. Samples were rarefied to 1,000 sequences and only samples ≥1000 sequences were used in subsequent analyses, and diversity metrics were analyzed using QIIME (Caporaso et al., 2012).

After OTU table was assembled, QIIME was also used to calculate alpha and beta diversity using chao1 and Bray Curtis dissimilarity metrics. The sample distance was calculated by Unifrac analysis, and QIIME scripts were used to create PCoA and boxplots plots (Lozupone and Knight, 2005). OTU tables were pruned to address specific experimental questions, then LefSe biomarker analysis was completed (Segata et al., 2011).
3 Results

3.1 Sample Collection and Processing

In order to understand microbial ecology of the phyllosphere and rhizosphere of plants living in a permafrost thaw zone wetland, I collected samples from seven different plant species and associated peat across the permafrost thaw gradient before, during, and after the growing season in Stordalen Mire, located in Abisko, Sweden (Table 1). Four plant species grew in only one thaw state, and three grew in two thaw states (Figure 6). I characterized the microbial community composition through 16S rRNA gene amplicon sequencing. From 402 processed samples, 252 samples (89 leaf, 132 root, and 31 peat samples) had more than 1000 16S rRNA gene sequences and sequences from these samples were used in subsequent analyses.

3.2 Microbial community composition

To compare microbial communities across samples, 252 samples were rarefied to include 1000 high-quality 16S rRNA gene sequences per sample. Out of all sequences, 99.6% belonged to domain Bacteria and 0.4% to Archaea. We detected a total of 58 different phyla, the four most dominant being Proteobacteria (44%), Acidobacteria (22.9%), Bacteroidetes (8.9%), and Verrucomicrobia (7.8%). Proteobacteria and Acidobacteria, the two most dominant phyla also displayed the highest levels of diversity within each phylum. Examining sequences at the genus level (97% sequence identity), there were 684 genera. We performed rarefaction analysis to determine how completely the 16S rRNA gene libraries were sampled if only 1000 sequences were included in the analyses. While the libraries samples from palsa and bog habitats reached an asymptote, samples from the fen were undersampled (Figure 7).
3.2.1 Microbial community structure of the rhizosphere

To characterize the microbial community composition, we sequenced the 16S rRNA gene sequences sampled from roots and directly associated peat. The microbial community composition was compared to microorganisms obtained from the surrounding peat. The rhizosphere differed from the rest of the peat environment; the composition of rhizosphere communities was influenced by permafrost state (Figure 8). The most abundant phylum in the rhizosphere was Proteobacteria with 43.3% of the sequences, compared to only 31% in associated peat. Within this phylum, 20.4% sequences clustered within the class Alphaproteobacteria. Other abundant phyla included Bacteroidetes 9.6%, Actinobacteria 4.1%, and Verrucomicrobia 8.5%. At the order level, the order Acidobacteriales was the most abundant, followed by Rhodospirales. Most sequences belonging to Rhodospirales were from the family Acetobacteraceae. The 30 orders found in a relatively high abundance in at least one sample make up the bulk of all samples (Figure 8).
Figure 8. Phyla level composition of microbial community in all samples. Each pie chart represents all samples of that sample type. Taxa with a relative abundance of <1% in at least one sample type are included in the graph.

3.2.1 Microbial structure of the phyllosphere

To characterize the structure of the phyllosphere microbial communities, we analyzed 89 samples belonging to six different species of plants. The plants sampled were bog rosemary, *E. vaginatum*, *C. rotundata*, *E. angustifolium*, *S. fuscum* and *S. balticum*. Cloudberry leaves were also sampled, but no samples yielded 1000 sequences. *E. angustifolium* had the most diverse phyllosphere community with 232 OTUs, operational taxonomic units, or phylogenetic groups
made up of organisms sharing 97% of their 16S rRNA gene. While bog rosemary phyllosphere communities were the least diverse with 126 OTUs. Sphagnum communities were distinct from angiosperm phyllosphere communities, being heavily colonized by Cyanobacteria. Leaf communities were characterized by being dominated by Alphaproteobacteria from the phylum Proteobacteria (Figure 8). Acidobacteria, shown in green in Figure 9, were also common, making up on average 15% of organisms within the population. The only other organism making up 10% of the average sampled leaf community was Acidobacterialales. While the majority of organisms were accounted for by the 31 orders that were present in at least 10% abundance in one sample, the majority of taxa were found in low abundances, indicating that phyllosphere samples had a few dominant and many rare taxa.

3.3 Distinct communities form within the phyllosphere and rhizosphere

To understand the major variables that drive the differentiation between bacterial communities, a multi dimensional distance matrix was created to represent the distance between each sample. This distance matrix was used for principal coordinate analysis (Figure 9). This analysis revealed significant clustering both within permafrost thaw state and within tissue type. While points also clustered together based off of associated plant species, this pattern occurred within the other two patterns in the top ten dimensions of difference in the distance matrix. Clustering by habitat type according to the largest three axes of difference was tighter in palsa and fen habitats, and more spread out in bog communities. Roots and associated peat clustered close together, while leaves were distinct but more dispersed. This information was used to structure further analyses: indicating which variables should be seen as primary and secondary within the nested analysis.
Figure 9. **PCoA plot depicting separation of microbial communities** by A) habitat and B) tissue type. Plot created by weighted UniFrac analysis (Lozupone et al. 2012). Each point represents a microbial community of a sample collected from plant tissue or associated peat.

To assess initial differences between main experimental classes, the alpha diversity of these groups was measured by the chao1 diversity index (Kuczynski et al., 2012), an alpha diversity metric that is designed to include rare taxa (Figure 10). Samples had an average chao1 diversity of 443. By comparing the chao1 diversity of the tissue types, phyllosphere communities had the least bacterial diversity, with rhizosphere communities being slightly more diverse, and peat
communities being the most diverse. When habitats were compared, palsa and bog had similar levels of alpha diversity, while the fen was significantly more diverse (Figure 10B).

**Figure 10.** Chao1 Diversity of A) tissue type and B) habitat show significant enrichment of diversity in fen derived tissue, and significant reduction of diversity in leaf tissue shown by mean ± standard deviation.

To ascertain which taxa are involved in creating the separation between different variable groups, the core microbiome of each permafrost thaw state and tissue type was determined (Figure 11). To be considered a part of a group’s core microbiota in this study, an OTU, or operational taxonomic unit must be found in at least 70% of the samples of that variety. This analysis takes in to account prevalence, the number of samples a taxa was detected in, but not abundance, the amount of a sequences belonging to that taxa within each sample. While most taxa were ubiquitous across all tissue types, some were unique to one or two tissue types. Root tissue, which possessed the fewest unique taxa, was uniquely colonized by taxa belonging to the orders Sphingobacterales, Caulobacterales, and Xanthomondales. Leaf communities were the
most unique, with less than half of the taxa in the leaf core microbiome being a part of the core microbiota of another group. These taxa were found elsewhere in the environment, but not at a high enough rate to be considered part of the core microbiome. Much of this differentiation comes from the presence of unique proteobacterial taxa belonging to the orders of Rhodospiralles, Sphingomonadales, and Burkholderiales, and these were consistently present on leaves and not on roots or in peat. Additional taxa in the leaf core microbiome and not in other core microbiomes are members of the Acidobacteriaceae family. The peat core microbiome was the largest core microbiome, and strongly overlapped with the root core microbiome. Unique peat taxa were primarily Acidobacteria, of the Solibacterales, and Acidobacteriales orders, as well as one OTU each from the order Saprospirales, Rhizobiales, Xanthomondales, and Chthoniobacterales. Core microbiome analysis indicates the separation of root, peat, and leaf microbial communities when abundance is not accounted for. This analysis indicates the uniqueness of phyllosphere and peat microbiota, and the similarity of rhizosphere and peat microbiota.
Figure 11. Distinctive taxa form the core microbiomes of root, peat, and leaf associated microbiota demonstrated by taxa present in each sample type. A) Diagram indicating the presence of a genus within at least 70% of the samples of that type (orange). B) Venn diagram shows overlap of taxa at the genus level between core microbiomes.

Core microbiome analysis was determined for permafrost thaw state habitats palsa, fen and bog (Figure 12). One OTU, associated with the alphaproteobacterial family Methylocystaceae was ubiquitously found across all three thaw states. The core palsa microbiome was dominated by Acidobacteriales, and also included Actinobacteria, Bacteroidales, Rhizobiales, Burkholderiales, Xanthomondales, and Spartobacteria. The core bog microbiome overlapped substantially with the palsa microbiome, and slightly with the fen core microbiome. Unique bog taxa belonged to the orders Sphingobacteriales, Caulobacterales, Rhizobiales, Rhodospirillales, Sphingomonadales, and Opitualas. Taxa that were found to overlap between palsa and bog habitats were members of the orders Acidobacteriales, Saprospirales, Sphingobacteriales, Rhizobiales, Rhodospirales, Methylacidiphilales, Xanthomondales, and WPS-2. The core fen microbiome was the smallest, but had the most distinct community composition. The fen was the only thaw state in which archeal taxa were detected in all samples, with Methanobacteriales...
making up a part of the core microbiome. Additionally, the orders Bacteroidales,
Desulfomondales and Holophagales were uniquely prevalent within the fen. The small overlap
between bog and fen core microbiomes consisted of members belonging to the orders of
Pedosphaerales and Rhizobiales. There was no overlap in OTUs present in palsa and fen habitat,
though they both contained multiple OTUs of the Burkholderiales order, which were never
present in the bog core microbiome.

Figure 12. Distinctive taxa form the core microbiomes of palsa, bog, and fen associated
microbiota demonstrated by taxa present in each sample type. A) Diagram indicating the
presence of a genus within at least 80% of the samples of that type (orange). B) Venn diagram
shows overlap between core microbiomes.

3.4 Habitat acts as a primary driver of differentiation in the rhizosphere and phyllosphere

A plant’s habitat is a key driver in structuring the composition of its phyllosphere and
rhizosphere microbial communities (Figure 8). To better understand which specific taxa
contribute to this differentiation, I examined the diversity and taxa relative abundance of three
plants that grew in two different permafrost thaw states. *E. vaginatum* (EV) is a sedge species and grows in fully frozen palsa and acidic bog habitats. *A. polifolia*, or bog rosemary (BR), is a small woody shrub like plant, which colonizes the palsa and bog habitats. *C. rotundata* (CR) is a larger sedge plant species that colonizes the bog and fully thawed fen habitats. By comparing colonization patterns between different thaw states and within plant species and tissue type, the impact of a specific habitat on the colonization patterns of leaf or root communities of a plant can be understood.

### 3.4.1 Habitat based differences in alpha diversity

Comparing palsa and bog colonization, habitat did not significantly impact the diversity present in rhizosphere microbial communities (Figure 13). Within the phyllosphere, the diversity present in *E. vaginatum* leaves was not impacted by the habitat in which the plant was growing in (Figure 14). The diversity of bog rosemary leaves is enriched within the bog habitat.

The impact of the bog to fen transition on phyllosphere and rhizosphere microbial alpha diversity.
diversity was more consistent. Within the rhizosphere, if outliers outside of the 95% confidence interval were excluded, the fen was significantly more diverse (t-test, p=0.012). However, if the two samples outside of this confidence interval were included, enrichment of fen diversity was not significant (p=0.23). A similar pattern exists in the phyllosphere communities (Figure 13). When samples outside of the 95% confidence interval were excluded, the fen was significantly more diverse (p=0.031), but including two outliers, the difference was no longer significant (p=0.48).

3.4.2 The rhizosphere microbiota across the three habitats

Differences at all phylogenetic levels, from phyla to genera, played a role in creating the separation of rhizosphere microbial communities by habitat by colonizing one permafrost state at a higher relative abundance (Figure 14). On *E. vaginatum* roots, less differentiation occurred, with 13 taxa more abundant in plants growing in the palsa, while 24 were more abundant in the bog (calculated by linear discriminant analysis). On bog rosemary roots, 39 taxa were significantly enriched in the palsa habitat, while 95 taxa were significantly more abundant in the bog. On *C. rotundata* roots 63 taxa were significantly more abundant in roots in the bog, while 93 were more abundant on roots in the fen.

When plants colonizing both palsa and bog habitats were compared, more differentiation was found in bog rosemary roots than in *E. vaginatum* roots. Different genera were selectively abundant between habitats in *E. vaginatum* and bog rosemary. On the phylum level, bog rosemary roots in the palsa were significantly elevated in Acidobacteria and Actinobacteria, and roots in the bog were enriched in Armatimonadia, Verrucomicrobia and Proteobacteria. In *E. vaginatum* roots, Verrucomicrobia was significantly more abundant in the bog, which was the
only phylum level difference. Differences between habitats at a finer phylogenetic scale were more pronounced in bog rosemary roots, though the magnitude of difference between bog rosemary and *E. vaginatum* was less pronounced than at the phylum level. The differentiation between bog and fen on *C. rotundata* roots was shaped by colonization difference at many taxonomic and abundance levels. On the phylum level, roots in the bog were enriched in members of the Armatimonadia phylum. At a finer phylogenetic resolution, verrucomicrobial taxa are more abundant in the bog while proteobacterial taxa are more abundant in the fen. Notably, there were more microorganisms, capable of metabolizing complex organic molecules in the fen enriched with Proteobacteria. Rhizosphere communities differ between habitats, but the magnitude of this difference varies between species.
Figure 14: Taxa identified as drivers of habitat based separation are shown though within-plant comparisons of microbial communities colonizing roots of bog rosemary (BR), *Eriophorum vaginatum* (EV), and *Carex rotundata* (CR). Asterisks indicate significance via Bonferroni Hochberg corrected Kruskal Walis test.

3.4.3 Within-habitat comparison of phyllosphere microbiota

Leaf communities demonstrated similar patterns of separation by habitat as root communities.

The division of palsa and bog communities was driven by 11 taxa which favored BR leaf colonization in the palsa, 49 which favored BR root colonization in the bog, 3 taxa which favored EV leaf colonization in the palsa, and 19 taxa which favored EV leaf colonization in the bog (Figure 16). Most of these taxa (629) were present at low abundances, but 19 were present at abundances on average above one percent a habitat. Four bog rosemary genera were both
abundant and found significantly more in one habitat than another, while no taxa on *E. vaginatum* leaves were both selective and abundant. In *E. vaginatum*, taxa present significantly more in one habitat than another were not present at an abundance of over one percent. On a phylum level, bog rosemary leaves were significantly enriched in Bactreiodetes when growing in a bog environment. On the genus level, *Acidilobus*, genus belonging to Acidobacteria, proteobacterial genus *Acidocella* and *Sphingomonas*, were found significantly more in leaves growing in palsa environments than leaves in bog environments.

The differentiation of *C. rotundata* leaf colonization patterns was driven by colonization patterns at all phylogenetic levels. Twenty-eight taxa were significantly enriched on roots that grow in bog habitats, and 38 were significantly enriched on roots in the fen, but only some represented a significant population size. Of those taxa found at abundances above one percent in any habitat, Acidobacteria was more likely to be found within the bog, and colonization by Deltaproteobacteria was favored in the fen. At a finer phylogenetic resolution, proteobacterial genera *Acidocella*, *Novosphingobium*, *Burkholderia*, and *Propionivibrio* were found in the rhizosphere more often in the fen than in the bog, and *Crenothrix*, an iron precipitating bacterium, was found significantly more on leaves in the bog (Figure 15).
Within habitat comparison of phyllosphere and rhizosphere microbiota indicate importance of plant type

We also examined the microbiota of the phyllosphere and rhizosphere associated with several plant species co-existing within the same habitat. In palsa habitats, we compared two non-sedge plants bog rosemary (*A. polifolia*, BR) and cloudberry (*R. chamaemorus*, CB). In the bog habitat, two sedge plants *E. vaginatum* (EV) and *C. rotundata* (CR) were compared, and within the fen, two sedge species *C. rotundata* and *E. angustifolium* (EA) were compared to each other. By controlling for habitat and tissue type, the impact of species on the formation of microbial communities can be isolated.
3.5.1 Richness of rhizosphere and phylosphere microbiota within the three habitats.

As an initial method to understand the impact of plant species on the microbial colonization patterns alpha diversity (chao 1 index) was compared within each habitat and tissue type (Figure 16). In rhizosphere microbial communities, plant species did not make a significant impact on the alpha diversity of a sample, though previously noted patterns of increased habitat diversity as thaw occurs was confirmed when habitat changes are examined on a per species basis. Within the phyllosphere, patterns were more variable. No cloudberry phyllosphere samples were successfully sequenced, so no comparison can be drawn for comparable palsa plants. Within the bog, *E. vaginatum* leaves were shown to host more diverse microbial communities than *C. rotundata* leaves. No significant species based differences were observed in phyllosphere fen communities.

Figure 16. Chao1 diversity analysis, shown my bars representing the mean ± standard deviation. comparing the alpha diversity of the microbial communities on the tissue of different plants grown the same permafrost thaw state.

3.5.2 Plant species cause impact colonization within the rhizosphere

In order to understand how plants impact microbial assemblage within root communities, the associated rhizosphere microbiota from similar species from the same habitats were compared to each other (Figure 17). When bog rosemary was compared to cloudberry, bog rosemary was preferentially colonized by Alphaproteobacteria. All genera present above one percent that
selectively colonized either habitat where members of the Alphaproteobacteria class. When the
two sedge species, *C. rotundata* and *E. vaginatum* were compared in the bog, differences were
found primarily in less abundant taxa. The one exception to this pattern was how *C. rotundata*
roots favored colonization by Gammaproteobacteria. *E. vaginatum* roots were colonized
significantly more by organisms belonging to Actinobacteria, Plantomycetes, Verrucomicrobia,
and Spartobacteria (Verrucomicrobia). *C. rotundata* were colonized significantly more by
Bacteroidia, Firmicutes (including members of the class Clostridia), and Spirochaetes. In the fen,
no taxa were present at an abundance of over 1% that were significantly more or less likely to
colonize the roots of *C. rotundata* and *E. angustifolium*.

### 3.5.3 Plant species impact colonization patterns within the phyllosphere

While roots and leaves respond similarly to changes in thaw state, the changes observed
across different host plant species was different above and belowground. Members
Acidobacteria, Rhodospirillales, and Legionellales were more abundant on *E. vaginatum* leaves,
while verrucomicrobial Opitutae were preferentially found in the phyllosphere colonizing *C.
rotundata* (Figure 17). Like in the roots, there were less significant differences in the
composition of microbial communities in fen colonizing plants phyllosphere than in the plants

![Figure 17](image)

**Figure 17.** Taxa which act as drivers of plant species based separation are shown though
within-habitat comparisons of microbial communities colonizing leaves of *E. vaginatum (EV)*
vs *C. rotundata* (CR), and *C. rotundata* (CR) vs. *E. angustifolium* (EA). Asterisks indicate
colonizing the bog. Two significant differences were the preferential colonization of Actinobacteria and the Acidobacteria class on *E. angustifolium* over *C. rotundata*.

### 3.6 The sphagnum microbiome

Bryophyte tissue, has photosynthetic tissue, which we treated as a leaf tissue, and non-photosynthetic tissue capable of conducting water and chemicals, which, for the purposes of this study, we treated as a root. As habitat changes, so does the dominant species of *Sphagnum* spp. colonizing the area. In palsa environments, *S. fuscum* is a dominant colonizer. In partially thawed bog areas, a variety of species cover the majority of the ground’s area. This study focused on the dominant species, *S. balticum*.

The sphagnum microbiome bears some similarity to both the peat microbiome and the plant associated microbiome, but is significantly different from angiosperm colonizing communities (Figure 7,8). The most significant difference between the bryophyte and angiosperm microbiomes is the reliable and substantial colonization of cyanobacteria on sphagnum tissue, and the reduction of diversity when bog-colonizing sphagnum is compared to other microbial communities in the bog.

### 3.6.1 Richness of the sphagnum microbiome

As an initial effort to characterize the differences between above and belowground tissue, as well as between species, the chao1 alpha diversity was calculated for each species in each photosynthetic state (Figure 18). *S. balticum*’s associated microbial communities are more diverse in both photosynthetic and non-photosynthetic tissue (Figure 18). Alpha diversity does not vary by photosynthetic capability in either sphagnum study species.
Figure 18: Chao1 Diversity of photosynthetic leaf-like and non-photosynthetic root like tissue of Sphagnum spp. Brown bars indicate mean chao1 alpha diversity of Sphagnum fuscum sampled in a palsa environment, while green bars represent mean Sphagnum balticum sampled in a bog environment. Error bars represent standard error.

3.6.2 Taxa specific differentiation in Sphagnum spp. communities

Taxa specific colonization patterns were examined to ascertain if specific taxa reliably respond to species change or a photoactive habitat. Three taxa demonstrated consistent responses to a change in species or photosynthetic activity. Cyanobacterial taxa were found to be enriched in the samples of photosynthetic tissue of both sphagnum species. Acidocella, an abundant genus of Proteobacteria, preferentially colonized the photosynthetic tissue of sphagnum in both habitats as well. Acidobacteriaceae was significantly more likely to be found colonizing both photosynthetic and non-photosynthetic tissue of S. fuscum, in the palsa habitat, than colonize S. balticum in the bog (Figure 19). When these variables were examined separately, much more variation is evident. When just comparing communities colonizing photosynthetic tissue, 12 taxa were more likely to be found on S. fuscum, and 18 were more likely to be found on S. balticum. When non-
photosynthetic tissue was examined, these numbers increased to 86 and 43. When comparing within *S. fuscum*, 17 taxa were found to preferentially colonize the photosynthetic tissue, while 85 taxa were found to preferentially colonize the non-photosynthetic tissue. When *S. balticum* was examined these numbers change to 20 and 43, respectively.

Figure 19: Taxa that act as drivers of sphagnum microbiome differentiation between habitats and tissue location. Sphagnum colonization patterns split up by habitat and photosynthetic and non-photosynthetic tissue types. Asterisk indicates the a significant enrichment based off the Wilcoxon test.
3.7 Methane cycling bacterial populations

Nutrient cycling lies at the nexus of microbial ecology and global change biology, and for that reason is of special interest to this project. Because of the wetland nature of this study site, and the influx of formerly frozen organic carbon as substrates, anoxic methanogenesis is an important and dynamic feature of the ecosystem services these bacterial communities provide. Because of the relatively high methane emissions, methanotrophy is thought to be increasingly abundant in this area as well, since it too is a substrate for bacterial respiration. In order to better understand the ecological distribution and balance between methanogenesis and methanotrophy, the relative abundances of these organisms have been assessed and compared.

3.6.1 Archaeal colonization patterns

Since all methanogenic bacteria are of the phylum Archaea, by examining the archaeal diversity, both methanogenic capabilities and community assemblage can be better understood. Archaeal populations were found at a rate of .5% or greater in rhizosphere and peat associated microbial communities in bog and fen ecosystems (Figure 20). The majority of archaeal organisms are part of the genus Methanobacterium, a methanogenic taxa. Fen communities were preferentially colonized by Methanobacterium over bog communities, while peat was preferentially colonized over roots. Methanobacterium were found to be significantly more associated with the peat surrounding E. angustifolium roots than the roots of C. rotundata. Besides methanotrophic bacteria, the only other significant archaeal taxa are the Parvarchaeota, who are most dominant in the fully thawed fen ecosystem.
Figure 20 Archaeal taxa found across all rhizosphere and peat samples. Significant differences in Methanobacterium relative abundance between habitats and plants by Wilcoxon analysis indicated using asterisks.

3.6.2 Colonization patterns of methanotrophic bacteria

To assess the potential for impact of bacteria on the speciation of carbon emissions from the mire, the relative abundance of methanotrophic, or methane eating, bacteria was measured across all samples. Unlike methanogenesis, methanotrophy is not confined to one phylum. Taxa in both Verrucomicrobia and Proteobacteria phyla can be methanotrophic. Three groups of methanotrophic bacteria were examined in this study: Methylacidiphilales, Methylococcaceae, and Methylocystaceae. These families and orders are entirely methanotrophic, meaning they can all subsist on only methane as a carbon source.

Methanotrophic bacterial DNA sequences were detected in phyllosphere, rhizosphere, and peat samples. Methylococcaceae were preferentially found colonizing plant associated tissue, and found significantly less in peat communities. Methylacidiphilales was abundant in both palsa and bog habitats, but rare in all three tissue types in the fen. Methylocystaceae was
significantly abundant in all sample types, and did not have a visible strictly defined colonization pattern (Figure 21).

To isolate the impact of habitat on levels of phyllosphere and rhizosphere community methanotrophy, *C. rotundata* was examined. This plant grows in the sites where anoxic production of methane is the highest. Methanotrophs make up a higher relative abundance of both the phyllosphere and rhizosphere microbial communities. However, this does not indicate that more methanogenesis is occurring in bog leaf and root microbial communities because population density is not known.

![Figure 21](image_url)  
**Figure 21** Relative abundances of methanotrophs suggest methane availability across the thaw gradient. Plant-associated tissues were enriched by Methyllococcaceae.
4. Discussion

Permafrost stores ~30% of Earth’s organic carbon (Tarnocai et al., 2009). As permafrost thaws, the biological availability of dissolved organic matter increases, increasing the carbon flux out of the wetland (Turetsky et al., 2000). Microbiota associated with the peat and its contribution to carbon cycling has been previously characterized (Jansson and Taş, 2014). However, little is known about microorganisms-associated with the most abundant permafrost plants and how these interactions are contributing to the carbon cycling in the area. Here, we identified microbial communities associated with rhizosphere and phyllosphere of seven different plants and associated peat and compared the communities across different plants and habitats.

4.1 Evaluating patterns of rhizosphere and phyllosphere microbiota

4.1.1 Study both supports and contradicts the findings of previous studies of rhizosphere microbiota

The majority of the studies on rhizosphere were completed in silt or clay based soils, not in peat (Philippot et al., 2013). Additionally, this study is unique because of the relatively high range of soil characteristics, like moisture and pH (from 4 to 6) in a small spatial rage (Hodgkins et al., 2014). Rhizosphere microbiota of plants growing in non-peat soils were enriched with Firmicutes, Actinobacteria, Alphaproteobacteria, Pseudomonadaccae, or Burkholderiaceae (DeAngelis et al., 2009; Uroz et al., 2010). The relative abundance of Firmicutes was twice as high within the rhizosphere as in the bulk peat material. However, the entire phyla comprised on average, only 1% of the phyllosphere microbial community, so this difference may not be ecologically significant. The difference is likely a result of the selection for anaerobic bacteria around the roots, which is indicated by the higher representation of anaerobic Clostridia class in the rhizosphere and the higher representation of the aerobic Bacilli class in the bulk peat. Alphaproteobacteria and Burkholderiaceae are also more abundant. The prevalence of the
Burkholderiaceae order may be especially significant because of the documented rapid uptake of root exudates this order is capable of (Vandenkoornhuyse et al., 2007).

Actinobacteria did not show the predicted enrichments within the rhizosphere, with more of these taxa present within the peat than in root-associated material. Additionally, because of extremely low abundances, there was no significant trend in the abundance of Pseudomonadaceae. One trend apparent through the core microbiome not previously discussed was the over dominance of some *Bacteriodetes* spp., especially from the class Sphingobacteria, in the rhizosphere compared to the bulk peat (Philippot et al., 2013).

4.1.2 Phyllosphere data supports “Seeding” hypothesis

Phyllosphere communities are formed by the initial seeding of soil or water microbiota followed by the subsequent selection due to the unique environmental conditions of the leaf environment (Vorholt, 2012). Phyllosphere microbiota are more tolerant to dry conditions and are capable of metabolizing complex carbon sources and single-carbon based compounds such as methane (Boch et al., 2002; Gourion et al., 2006; Lindow et al., 1993; Marco et al., 2005). While published research on the phyllosphere stems from characterizing plants colonizing habitats other than permafrost thaw wetlands, we observed some similar patterns in our data.

Previous research regarding the Alphaproteobacteria abundance on leaves relative to the surrounding environment (Delmotte et al., 2009) was also observed in our dataset. The relative abundance Alphaproteobacteria in the phyllosphere microbiota was twice as high as the relative abundance of this bacterium in the surrounding peat. This difference indicates a strong selective pressure towards alphaproteobacterial Acetobacteria within the phyllosphere. This bacterial family obtains energy by the fermentation of organic molecules (Cleenwerck et al., 2002). The
second most abundant family within Alphaproteobacteria was Methylocystaceae, a Type II methanotroph (Eller et al., 2001). Both Methylocystaceae and Acetobacteria have adapted to the life on leaf surfaces, metabolizing the carbon compounds exuded by their host.

The expected actinobacterial enrichment on leaf tissue did not occur. No Actinobacteria was found in the leaf core microbiome, and abundances of this bacterium were less in phyllosphere communities than in either rhizosphere or bulk peat communities. This could potentially be caused by the lack of the genus *Streptomyces* in the environment, which is very abundant in most non-peat soil (Blagodatskaya and Kuzyakov, 2013). Because peat communities are not significantly colonized by this genus, it is possible that the exact taxa of Actinobacteria are not present to colonize the leaves. This trend indicates the importance of understanding the soil microbiota before making predictions about leaf colonizing microbiota, since if a seeding matrix community lacks a member that is traditionally a strong colonizer, then the colonization pattern will be different. Additionally, it demonstrated the importance of knowing the phylogenetic level of selection pressures.

Methanogens were also detected in the phyllosphere, but only at low levels in the fen. Methanogens are almost always outcompeted in aerobic environments, since methanogenesis yields substantially less energy for metabolic function than aerobic respiration (Nealson, 1997), so their detection was surprising. It is likely that the presence of these sequences is coming not from the presence of living methanogens on leaves, but instead from the presence of cellular debris of dead organisms on the leaves. This within environment genomic mixing is more likely in the wet environment of the bog and fen, where anaerobic methanogenesis is occurring (Groffman et al., 1996).
4.2 Bacterial microbiota distinctions between the rhizosphere and phyllosphere

Analyses of the microbiota sampled from the phyllosphere and rhizosphere revealed that the microbial communities primarily grouped by site and then by the plant species (Figures 9, 11, 12), similar to what has been observed in other studies (Bailey, 2004; Berg and Smalla, 2009). Phyllosphere community composition is typically driven by the dry and nutrient poor environment of the leaf, as well as the chemical composition of individual host species (Bailey, 2004). Rhizosphere communities are shaped by the root structure, exudates, and compounds taken up by the roots (Lugtenberg and Bloemberg, 2004). We postulate that observed differences in the composition of phyllosphere and rhizosphere microbiota are largely due to differences in niche environments.

In order to understand how these microbial communities differ, principal component and core microbiome analysis were performed.

4.2.1 Evidence phyllosphere and rhizosphere formation is a key process of differentiation

Core microbiome analysis indicates that more taxa are specific to one tissue type’s (peat, root, or leaf) core microbiome than there are taxa shared between two or all three tissue types. This is a preliminary indicator that there indeed are distinct patterns of community formation that rely on the association to specific plant tissues. The presence and ubiquity of several taxa above but not below ground indicates that colonization by those taxa were potentially spread from above ground sources. Additionally, the overlap between phyllosphere and rhizosphere core microbiomes that is different from the peat microbiome indicates that specific plant associated taxa may exist, that are transmitted by seed or from other spatially close plants. These pieces of
evidence support the hypothesis that phyllosphere and rhizosphere are distinct, rather than just peat communities that have undergone a selection process. If this hypothesis holds true, then the addition of this litter would likely cause at least a temporary change in the composition of the peat microbiota. This could hold implications for the decomposition of the recently fallen litter, and therefore, the carbon cycle within the wetland.

4.2.2 Evidence phyllosphere and rhizosphere formation is a secondary pattern

The interactions between habitat and plant tissue association occurs differently in the phyllosphere than in the rhizosphere. In the phyllosphere, the selective pressure of colonization acts to bring plant species’ disperse, habitat-based microbiomes closer together. This effect is secondary- it depends on the microbes available to seed the community. In the rhizosphere, communities more closely resemble those of the peat. Instead of different habitats becoming more closely related, they differ from peat in similar ways. The microbes in the peat are more related to roots of the same habitat than those roots are to each other. This result is indicated by the calculation of UniFrac distances, illustrated visually by PCoA analysis. If it is true that the impact of the phyllosphere and rhizosphere environments is secondary to the impact of permafrost thaw state, then the falling of litter would likely not make a significant impact on the peat microbiota, and would only impact the decomposition if the colonizing bacteria significantly increase the microbial biomass, and therefore rate of decomposition.

4.3 Explaining observed differences in alpha diversity
Alpha diversity, or diversity within a sample, is a first order way to understand the microbial ecology occurring in an environment. The significant differences in Alpha diversity between habitat types and tissue types indicates the role of these factors on general trends of microbial colonization.

4.3.1 Plant-associated microbiomes are less diverse than bulk peat microbiome

Comparison of plant-associated microbiomes to the bulk peat microbiomes indicated that the microbial diversity associated with plants was lower than diversity associated with the peat. This finding contradicted my hypothesis that the overall diversity in the roots would be greater than in the peat, because of the additional carbon sources supplied by plants available for microbial metabolism. The fact that bulk peat microbiota is the most diverse, even though it does not contain some of the unique compounds exuded directly by plant tissue supports the hypothesis that phyllosphere and rhizosphere communities are formed due to selection from the peat communities, and therefore would be less diverse.

4.3.2 Plant- and peat-associated microbiota are more diverse in fen than bog and palsa

Across the permafrost thaw gradient, our analysis revealed that fen has the most diverse microbial communities associated with plants and the surrounding peat when compared to bog and palsa. Microbial diversity is shaped by moisture, nutrient levels, and pH. One of the possible explanation for the fen’s observed microbial diversity is that the palsa’s habitat is limited by a lack of moisture and and the bog’s habitat have low pH (Hodgkins et al., 2014). Both habitats where permafrost is present are not connected to the water table, and instead are fed only by rainwater (Svensson and Rosswall, 1984). This leads them to be very nutrient poor. This is likely another cause of the lowered diversity in the palsa and bog and the raised diversity in the fen.
The fen’s diversity is likely raised by the wide variety of oxidation states present in that environment, as oxygen and other terminal electron acceptors are used up by cellular respiration (Megonigal et al., 2005). Interestingly, the trend of diversity for plants is reversed, with the frozen palsa being the most diverse habitat and the fully thawed fen the least diverse (Sonesson and Kvillner, 1980). Potentially, this could be due to the fact that increased moisture is often associated with less plant diversity, while being associated with high microbial diversity. This is an important reminder that broad principals of macroecology can be applicable to microbial ecology, but only when the details of both patterns are understood.

### 4.4 Addressing hypothesis regarding variables driving community formation in the phyllosphere and rhizosphere

A main goal of this experiment was to ascertain which variables must be known about a sample to begin to predict composition of the associated microbial community. I hypothesized that the makeup of the rhizosphere microbiota is more related to the host plant’s habitat, while the leaf microbiota is more related to the plant species. In fact, both community types respond more to changes in habitat than to the identity of the host plant. However, on an individual taxa scale, these changes are much more complex and dynamic.

#### 4.4.1 Habitat based differentiation follows three patterns

Microbial taxa, whether in the phyllosphere or the rhizosphere demonstrated three unique patterns of habitat-based colonization. Some taxa were very specific to one habitat, only found at significant abundance in the palsa, bog, or fen. Others were found in two adjacent habitat types. This pattern was more common for organisms colonizing the palsa and bog than the bog and fen. The third pattern is more difficult to detect. Several taxa which, while found in all three
environments, were significantly more prevalent in the palsa and fen, where the acidic environment is less harsh (Hodgkins et al., 2014). This result indicates there is a group of organisms whose abundance is most strongly correlated to acidity, not thaw state progression.

4.4.2 Impact of host plant on differentiation is specific to plant type rather than to species

This study indicates species’ associated microbiota’s dissimilarity does not vary reliably by habitat, but may by plant type. While all comparison species had some significantly different taxa, species comparisons separations by Unifrac analysis was variable (Figure 10). Separation between two species’ associated microbial communities in the phyllosphere does not predict the magnitude of separation between the rhizosphere, and the converse is also true. It is possible that habitat acts as a predictor of species-specific separation in phyllosphere or rhizosphere communities, though because for most habitats only one comparison was made, this data set is not sufficient to answer that question. The one pattern evident is that the amount of difference in plant type is a predictor of the amount of separation occurring— that is phyllosphere and rhizosphere microbial communities of a sedge will be more different from a woody shrub or dicot plant than another sedge.

4.5 Phyllosphere and rhizosphere microbiota impact carbon cycling

A main motivation of studying microbiology in a permafrost thaw wetland is how the microbial communities you seek to better understand play a role in a complex carbon cycle. These communities interact with the carbon cycle in a few key ways. First, they act as habitats for microbes that control if carbon emissions are in the form of carbon dioxide or methane, through methanogenesis or methanotrophy. Second, they may act as a mechanism for preventing leaf or
root carbon from getting to the anoxic zone where methanogenesis can occur, since they initiate decomposition in an oxygenated environment (Chapin et al., 2002; Leis and Flemming, 2002).

4.5.1 Methanogenic taxa abundances depend on tissue type, thaw state, and plant species. The presence of methanogenic Archaea can be thought of as a function of the concentrations of other terminal electron acceptors, like oxygen, nitrate, iron, or sulfate, only colonizing zones where these electron acceptors are present at very low concentrations (Kiener and Leisinger, 1983). Because above ground environments are too aerobic to facilitate the colonization by methanogens, this discussion will be focused on the inhibition or encouragement of the roots in a wetland on the colonization by methanogens. Whether roots serve to make an environment more aerobic depends on how aerobic the environment is to start with. In an aerobic zone, like the palsa and upper bog, they serve to deplete the soil of oxygen due to cellular respiration (Blossfeld et al., 2011). However, in the anoxic soil of the fen, they can serve the function of adding small amounts of air to the peat, since oxygen transported aboveground for cellular respiration may escape through the semi permeable root hairs (Bodelier et al., 1996). For this reason, more methanogenesis is expected on the roots than in the peat in the palsa and bog environments, while more methanogenesis is expected on peat in the fen.

In this study’s data set, there was negligible methanogenic Archaea found in the palsa. This result indicates that the drawdown of oxygen caused by the roots is not enough to create a substantially anaerobic environment for these Archea to compete with other organisms that use metabolism strategies with higher energy yields. In the bog, no significant difference was found between the roots and peat. This indicates that the partial or micro aerobic environment is sufficient for partial colonization by methanogens, but roots do not impact aerobic conditions. In
the fen, substantially more of the microbes colonizing the peat were methanogenic than the microbes within the rhizosphere. This result could indicate roots make the environment less hospitable to methanogens, due to oxygenation, nitrification, or other chemical or physical properties. It could also indicate that the roots encourage the colonization of other microbes, which then out compete the methanogenic Archea. For this reason, it would be interesting to do quantitative PCR using archeal primers, to assess population density along with relative abundance.

4.5.2 Methanotrophy is ubiquitous throughout the phyllosphere and rhizosphere

After a molecule of acetate or carbon dioxide is converted to methane through anaerobic methanogenesis, it becomes available to bacteria as another carbon source. The bacteria that can use this methane as a source of energy are known as methanotrophs. In order for a molecule of methane created in the anaerobic environment to be emitted as a greenhouse gas, it must travel through the peat matrix containing methanotrophs without being oxidized. This process of upward bubbling and diffusion can be slow, or expedited by transport through the vascular tissue of plants (Ström et al., 2003). A primary control over the likelihood that a methane molecule gets oxidized to carbon dioxide or fixed as biomass by a methanotrophic organism is the speed of egress, so plant colonization correlates positively with methane emissions (Joabsson et al., 1999).

Since roots and leaves act as transport mechanisms for methane, it is likely that the phyllosphere and rhizosphere will be sites for colonization by methanotrophs. However, because methane transport is faster within plant tissue than in peat, the relative abundance will be less. This hypothesis was confirmed by data seen in this study. Methanotrophic taxa were detected in all sample types throughout the phyllosphere, rhizosphere, and associated peat.
The transport of methane through plant vascular tissue and out of leaves, and the increased methanogenesis in the waterlogged fen predicts the greater presence of methane on roots and leaf surfaces in the bog than the fen. To assess this question, the relative abundance of methanotrophic bacteria in the rhizospheres and phyllospheres of *C. rotundata*. I hypothesize that methanotrophy would be greater in communities colonizing *C. rotundata* leaves and roots in the fen than in the bog. This hypothesis was not supported in the data, since both leaves and roots were more heavily colonized by methanotrophic bacteria in the bog than in the fen. This pattern was repeated in the peat as well. While methanotrophy should occur more in environments where there is more methane, it is also limited by the presence of oxygen, since methanotrophy is an oxidative process. The explanation that methanotrophy is limited by oxygen levels is not sufficient for explaining why less methanotrophy is occurring on leaves in the fen, since oxidation levels above ground are not impacted by habitat change. This difference could be explained by the “seeding” formation of phyllosphere microbial communities. If fewer methanotrophs are present in the soil and water, since those environments are less oxic, then they are less available to colonize leaves. Another mechanism to explain the increased abundance of methanotrophs in the bog is that relative, not total, abundance is being measured. Because of this, there could be more methanotrophic organisms in the fen phyllosphere, but relative abundance may appear lower because of higher population density.

4.5.3 Decomposition potential of rhizosphere and phyllosphere tissue

Bacteria, even when acting commensally, can still be complicit in the breakdown of the material that they colonize. This potential is indicated by the large amount of bacteria on leaves that can break down complex carbon molecules, like Proteobacterial taxa and cellulose. It would be
helpful to know about the metagenomic data of phyllosphere communities, so decomposition mediating proteins’ activities can be better quantified. Within the rhizosphere, Burkholderia are known to rapidly assimilate root exudates that are produced. Since these compounds are not being used as an energy source, but instead as a carbon source, it means that root carbon exudates could be fated for eventual anaerobic methanogenesis. If we knew that root exudate carbon was used as an energy source rather than to increase biomass, we could say that root exudate carbon’s final fate will be as carbon dioxide, a less powerful greenhouse gas. This process could be further explored through experimental stable isotope probing- the input of heavier carbon meant to track the movement of carbon through a system.

4.6 Conclusion: This study has lead to a better understanding of the methane cycling and decomposition potentials of phyllosphere and rhizosphere microbiota by demonstrating the inhibitory effect of roots on methanogenic colonization and the increase of gammaproteobacterial methanogenesis on plant tissues. Additionally, it has contributed to the knowledge of plant associated communities by exploring how microhabitats are shaped in a across a permafrost thaw gradient in many species. Future work includes examining the impact of small scale spatial variation on microbial colonization variability, and the process of secession throughout the growing season.
5. Key Terms

**Bog:** A wetland area that accumulates decomposing plant material, often sphagnum mosses.

**Endophyte:** A microorganism that lives within a plant without causing disease

**Epiphyte:** A microorganism that lives on a plant without reducing the host plant’s fitness.

**Fen:** A wetland area fed by groundwater with a neutral or alkaline pH.

**Methanogenesis:** The process of formation of methane by Achaea, during the reduction of small carbon compounds, often using carbon dioxide or acetic acid as the terminal electron acceptor. Methanogenesis is final step of the breakdown of organic biomass in most environments.

**Methanotrophy:** A type of methylotrophy, where microorganisms use methane as a carbon source. Methanotrophs can be obligate, when methane is the only carbon source they can use, or facultative, when it is one of many carbon sources they can metabolize.

**Methylotrophy:** The use of carbon compounds with no carbon-carbon bonds by microorganisms as a source for growth.

**Mire:** A peatland or wetland area dominated by peat-forming plants.

**Palsa:** Mounds of dry peat that are raised above the water table, often by permafrost.

**Peat:** Slowly decomposing organic matter that has accumulated in an anoxic environment.

**Permafrost:** Rock, soil, or peat that is below 0 °C temperature for two or more years.

**Phyllosphere:** Aboveground plant tissue, including leaves, flowers, and stems.

**Rhizosphere:** Belowground root tissue.
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