



Tansley insight

The *Sphagnum* microbiome: new insights from an ancient plant lineage

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Summary

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Peat mosses of the genus *Sphagnum* play a major role in global carbon storage and dominate many northern peatland ecosystems, which are currently being subjected to some of the most rapid climate changes on Earth. A rapidly expanding database indicates that a diverse community of microorganisms is intimately associated with *Sphagnum*, inhabiting the tissues and surface of the plant. Here we summarize the current state of knowledge regarding the *Sphagnum* microbiome and provide a perspective for future research directions. Although the majority of the microbiome remains uncultivated and its metabolic capabilities uncharacterized, prokaryotes and fungi have the potential to act as mutualists, symbionts, or antagonists of *Sphagnum*. For example, methanotrophic and nitrogen-fixing bacteria may benefit the plant host by providing up to 20–30% of *Sphagnum* carbon and nitrogen, respectively. Next-generation sequencing approaches have enabled the detailed characterization of microbiome community composition in peat mosses. However, as with other ecologically or economically important plants, our knowledge of *Sphagnum*–microbiome associations is in its infancy. In order to attain a predictive understanding of the role of the microbiome in *Sphagnum* productivity and ecosystem function, the mechanisms of plant–microbiome interactions and the metabolic potential of constituent microbial populations must be revealed.

I. Introduction

Peat mosses in the genus *Sphagnum* represent an ancient, early branching lineage of land plants that is largely responsible for the complex adaptive characteristics of northern peatlands (Dise, 2009; Shaw *et al.*, 2010). *Sphagnum* can dominate primary productivity in northern ecosystems, including boreal forests and especially

peatlands (Turetsky *et al.*, 2010, 2012). Because *c.* 50% of peat volume in boreal peatlands is comprised largely of *Sphagnum* remains (Turetsky, 2003), it has been said that more carbon is stored in *Sphagnum* than in any other genus of plant (Clymo & Hayward, 1982; Van Breemen, 1995).

Sphagnum mosses are keystone species that shape their habitat through unique biochemical and morphological adaptations that

together result in an acidic, permeable and nutrient-poor environment. These adaptations appear to favor *Sphagnum* growth relative to vascular plants (Van Breemen, 1995), as well as allowing *Sphagnum* to strongly influence ecosystem function, including the cycling of water, nutrients, energy, and carbon in northern ecosystems (Turetsky *et al.*, 2012). Peatlands are thought to store one-third of Earth's soil carbon because degradation is inhibited by the acidic, nutrient-poor, cold, water-saturated and largely anoxic conditions. *Sphagnum* creates or at least contributes to many of these conditions. Without roots, the moss efficiently holds water in nonphotosynthetic, porous hyaline cells that can account for 80% of the plant's stem and leaf volume (Turetsky *et al.*, 2012). The moss intercepts and retains nutrients efficiently through direct uptake and exchange of cations with H⁺, thus reducing cation availability to vascular plants, while at the same time acidifying its environment to a pH lower than most other plants can withstand (Lamers *et al.*, 2000; Limpens *et al.*, 2011).

Sphagnum wages chemical biowarfare to outcompete other plants in its ecosystem. The decomposition of peat carbon is thought to be inhibited by antimicrobial properties of *Sphagnum* (Verhoeven & Liefveld, 1997). Biomass of the living plant consists mainly of polysaccharides made up of glucose and galacturonic acid units. Galacturonic acid is rich in carboxylic acid groups that give *Sphagnum* its high cation exchange capacity (Spearing, 1972). Acidity generally retards microbial metabolism. *Sphagnum* biomass is also recalcitrant or resistant to degradation as a result of other organic constituents. Although the plant lacks lignin, polyphenolic polymers termed sphagnic acid chemically protect cell wall polysaccharides from being degraded (Freeman *et al.*, 2001). A pectin-like compound, sphagnan, may also suppress microbial activity by inactivating extracellular enzymes and strongly binding to nitrogen and micronutrients (Hajek *et al.*, 2011).

While *Sphagnum* manufactures an inhospitable surrounding environment, it simultaneously cultivates a diverse microbial community, or microbiome, associated with its tissues. The microbiome may be at least partially responsible for the ecological dominance of the moss. The objective of this Tansley insight is to summarize the current state of knowledge on the *Sphagnum* microbiome and to provide a perspective for future research directions. For the purposes of this review, we define the

microbiome broadly as those microorganisms that live inside (endosphere) or on (ectosphere) living *Sphagnum* plants.

II. What defines the peat moss microbiome?

Plant microbiome research is in its infancy. Analogous to human microbiome research a decade ago, we are still in the discovery phase, characterizing the community composition of microbiomes associated with a variety of economically and ecologically important plants. Tens of thousands of microbial species associate with plants, and plant–microbe interactions are crucial to plant health (Lundberg *et al.*, 2012; Ofek-Lalzar *et al.*, 2014). Microbes have the potential to benefit plants through nutrient acquisition, disease suppression, and modulation of host immunity (Mendes *et al.*, 2011; Berendsen *et al.*, 2012). In particular, arbuscular mycorrhizas and root nodulation are examples of symbioses with well-described benefits to plants. Until recently, characterization of plant-associated microbial communities was hampered by methodologies lacking phylogenetic resolution and sequencing depth. The development and availability of next-generation sequencing technologies have facilitated rapid advances in the field.

In order to define the microbiome, one first has to confirm the identity and genotype of the plant host. *Sphagnum* plants provide additional challenges in this area because of an unresolved phylogeny (Shaw *et al.*, 2010). Moreover, as for many plant hosts, the definition of what is plant-associated has varied in previous studies of the *Sphagnum* microbiome. Viewpoints on what is 'Sphagnum-associated' range from microbes of the bulk peat in the surface layer of peatlands where active photosynthesis occurs (Putkinen *et al.*, 2014) to those associated with the living vegetative part of the plant (gametophyte) along with associated soil (Raghoebarsing *et al.*, 2005). As we strive to establish the structure–function relationships of the microbiome that benefit the plant host, there is a need to verify the taxonomy of *Sphagnum* individuals using genotyping methods in parallel with morphological taxonomy and microbiome interrogation.

Sphagnum microbiomes are hypothesized to differ from those of most host plants because mosses have no roots and hence microbial inhabitants are mainly detected in the hyaline cells of leaf tissues (Fig. 1; Bragina *et al.*, 2012). The water-filled hyaline cells, or hyalocytes, are dead, hollow and often pore-containing cells in the

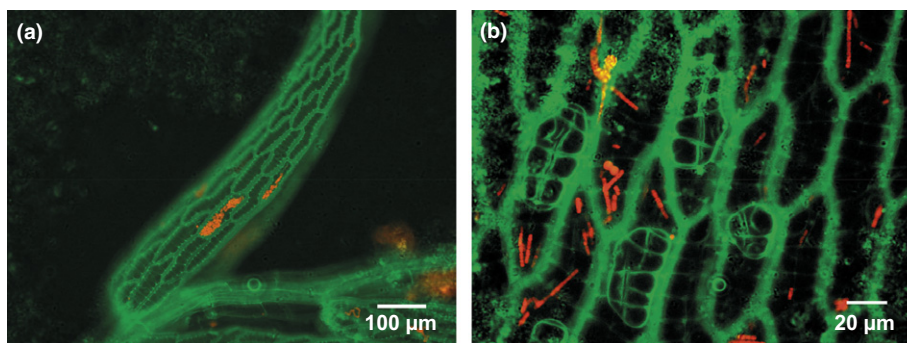


Fig. 1 Composite epifluorescent micrographs of *Sphagnum fallax* colonized with *Nostoc muscorum*. Images are of: (a) *S. fallax* leaf depicting live chlorophyllous cells (green) and *N. muscorum* (red) in hyaline cells; (b) *S. fallax* hyaline cells colonized by motile *N. muscorum* homogonia visible in close-up. Panel (a) is reprinted with permission from Weston *et al.* (2015).

leaves, stems and branches of the gametophyte. Hyaline cells allow for storage of water as well as the exchange of water between these cells and adjacent photosynthetic cells. Chlorophyllose cells were thus far shown to contain few to no bacteria. With an elevated pH, hyaline cells could act as 'oases' for microbes in the acidic peatland pore water. One could imagine these hyalocytes as tiny chemostats that cultivate microbes, which in turn benefit the peat moss host.

Plants are thought to contain a 'core microbiome' that consists of microbial taxa that are common to plant species or habitat (Vandenkoornhuysen *et al.*, 2015). The diversity and function of microbial groups that comprise the microbiome have been shown in model plants such as *Arabidopsis* to be specific to soil type or plant genotype (Lundberg *et al.*, 2012) as well as to correlate with plant fitness or health (Kembel *et al.*, 2014). However, for most plants, the microbiome has not been characterized in sufficient detail to resolve differences between plant species or functional traits. Metagenomic studies foster hypotheses on how specific microbially catalyzed functions may be linked to plant fitness, including studies of *Sphagnum* (Bragina *et al.*, 2014). However, further research is needed to determine the role of specific microbial groups in plant health at the ecosystem scale.

III. Structure and function of the *Sphagnum* microbiome

The majority of cultivation-independent molecular studies of *Sphagnum* microbiomes to date have targeted small subunit ribosomal RNA (SSU rRNA) genes or functional genes of prokaryotes extracted from unwashed gametophytes (Bragina *et al.*, 2014, 2015). Microbial cells were often separated from homogenized plant biomass using a centrifugation method. Evidence at this time points to a predominance and high diversity of *Bacteria* in the microbiome, with few to no members of the Archaea detected (Bragina *et al.*, 2014). Of the *Bacteria*, members of the *Proteobacteria* and *Acidobacteria* phyla are by far the most abundant, comprising the majority of SSU rRNA gene sequences obtained, with a lower relative abundance of other taxa (*Bacteroidetes*, *Cyanobacteria*, *Planctomycetes*, *Verrucomicrobia*, *Actinobacteria*) (Bragina *et al.*, 2013; Lin *et al.*, 2014a,b).

The ecological factors controlling microbiome structure and function are just beginning to be explored. A fairly large body of research suggests that microbiome community composition is distinct to the *Sphagnum* host species, and evidence points to environmental factors such as pH and nutrient availability as ecological drivers of microbiome community structure (Opelt *et al.*, 2007; Bragina *et al.*, 2013; Jassey *et al.*, 2014; Leppänen *et al.*, 2015). However, other studies challenge this view, showing that microbiome composition does not vary significantly between different peat moss species (Bragina *et al.*, 2012; Putkinen *et al.*, 2012). Further studies are needed at the ecosystem scale to understand the role of the environment vs plant host in the population dynamics of *Sphagnum* microbiomes.

As already described, *Sphagnum*-dominated peatlands represent extreme habitats for microbial life, as the prevailing conditions are acidic, cold, nutrient-poor, water-saturated and therefore anoxic below the surface. These conditions are believed to inhibit

microbial metabolism, leading to carbon storage. These extreme conditions also mean that the constituent microorganisms tend to be slow-growing, oligotrophic and more difficult to obtain in pure culture. As demonstrated by cultivation-independent studies, *Sphagnum* microbiomes contain a large diversity of as yet uncultivated bacteria with unknown physiologies (Dedysh, 2011; Leppänen *et al.*, 2015; Lin *et al.*, 2014a,b). Cultivation work has largely focused on microorganisms isolated from bulk peat or those from gametophytes along with adhered soil, and a few studies have used surface sterilization to select for endophytes. Dedysh (2011) provided a comprehensive review of microbes that have been cultivated from bulk peat, many of which have also been detected using molecular techniques in the *Sphagnum* microbiome. Bacterial isolates from the ectosphere or endosphere of *Sphagnum* tissues have demonstrated the potential for a number of plant growth promotion capabilities, including nutrient acquisition, antagonism toward fungal or bacterial plant pathogens, and production of plant hormones (Table 1; Opelt & Berg, 2004; Opelt *et al.*, 2007; Shcherbakov *et al.*, 2013). Although not dominant in sequence libraries, the bacterial genus *Burkholderia*, in particular, shows potential to impact the growth and health of *Sphagnum* hosts. *Burkholderia* species from a plant-beneficial cluster have been isolated from *Sphagnum* tissues collected from geographically wide-ranging peatlands. Many of these isolates produce antifungal compounds, solubilize phosphate, and possess genes for nitrogen fixation (Table 1). *Burkholderia* were present in the *Sphagnum* sporophyte and gametophyte, suggesting that they are part of a core microbiome transmitted from generation to generation if they occur within the spores (Bragina *et al.*, 2013, 2015).

Although we have learned much about the community composition of *Sphagnum*-associated microbes through molecular approaches, the metabolic potential or function of the microbiome and benefits to the moss host remain mostly unknown. Nearly all *Sphagnum* microbiome studies to date have relied on the cultivation of relatively few species or the PCR amplification of single genes, mostly SSU rRNA genes, from DNA extracts. Bragina *et al.* (2014) provided a first glimpse into the metabolic potential of microbiome communities by generating metagenomes from *Sphagnum magellanicum*. Adaptation to extreme conditions was revealed in abundant microbial genes or subsystems linked to oxidative and drought stress, mobile genetic elements, resistance to environmental factors and DNA repair mechanisms. The metagenomes also provided evidence of microbe–microbe and plant–microbe interactions such as biofilm formation, quorum sensing and nutrient acquisition. These DNA-based approaches have been effective in describing microbiome composition and generating hypotheses on plant–microbiome–environment interactions. However, RNA-based targeted and transcriptome approaches should be explored to further link microbiome structure with ecosystem function and biogeochemical cycles (Tveit *et al.*, 2015).

IV. Functional guilds of microbes that benefit *Sphagnum*

Certain functional guilds of microbes have been shown to closely associate with *Sphagnum*, and evidence indicates that these

Table 1 Representative bacterial species associated with living *Sphagnum*, including their putative beneficial phenotype

Bacterial genus	Taxonomy ^a	Host	Beneficial phenotype ^b	Citation
<i>Burkholderia</i>	Proteobacteria, Betaproteobacteria	<i>S. fallax</i> , <i>S. magellanicum</i> , <i>S. rubellum</i>	N, fungicidal, hormones	Opelt & Berg (2004); Opelt <i>et al.</i> (2007); Shcherbakov <i>et al.</i> (2013)
<i>Collimonas</i>	Proteobacteria, Betaproteobacteria	<i>S. fallax</i> , <i>S. magellanicum</i>	N, fungicidal, bactericidal	Shcherbakov <i>et al.</i> (2013)
<i>Chryseobacterium</i>	Bacteroidetes, Flavobacteria	<i>S. fallax</i> , <i>S. magellanicum</i>	Fungicidal, hormones	Shcherbakov <i>et al.</i> (2013)
<i>Dyella</i>	Proteobacteria, Gammaproteobacteria	<i>S. magellanicum</i>	N, fungicidal	Opelt & Berg (2004)
<i>Flavobacterium</i>	Bacteroidetes, Flavobacteria	<i>S. fallax</i> , <i>S. magellanicum</i>	N, P, bactericidal	Shcherbakov <i>et al.</i> (2013)
<i>Hafnia</i>	Proteobacteria, Gammaproteobacteria	<i>S. magellanicum</i>	P, fungicidal	Opelt <i>et al.</i> (2007)
<i>Micrococcus</i>	Actinobacteria, Actinobacteria	<i>S. rubellum</i>	Fungicidal	Opelt & Berg (2004)
<i>Microbacterium</i>	Actinobacteria, Actinobacteria	<i>S. magellanicum</i>	N, fungicidal	Opelt <i>et al.</i> (2007)
<i>Paenibacillus</i>	Firmicutes, Bacilli	<i>S. fallax</i> , <i>S. rubellum</i>	N, fungicidal, bactericidal	Opelt & Berg (2004); Opelt <i>et al.</i> (2007)
<i>Pedobacter</i>	Bacteroidetes, Sphingobacteria	<i>S. fallax</i> , <i>S. magellanicum</i>	N, fungicidal, bactericidal	Shcherbakov <i>et al.</i> (2013)
<i>Pseudomonas</i>	Proteobacteria, Gammaproteobacteria	<i>S. fallax</i> , <i>S. magellanicum</i> , <i>S. rubellum</i>	P, fungicidal, bactericidal, hormones	Opelt & Berg (2004); Opelt <i>et al.</i> (2007); Shcherbakov <i>et al.</i> (2013)
<i>Rahnella</i>	Proteobacteria, Gammaproteobacteria	<i>S. fallax</i> , <i>S. rubellum</i>	P, fungicidal, bactericidal	Opelt & Berg (2004); Opelt <i>et al.</i> (2007)
<i>Serratia</i>	Proteobacteria, Gammaproteobacteria	<i>S. fallax</i> , <i>S. magellanicum</i> , <i>S. rubellum</i>	Fungicidal, bactericidal, hormones	Opelt & Berg (2004); Shcherbakov <i>et al.</i> (2013)
<i>Stentrophomonas</i>	Proteobacteria, Gammaproteobacteria	<i>S. fallax</i> , <i>S. magellanicum</i>	ND ^c	Shcherbakov <i>et al.</i> (2013)
<i>Streptomyces</i>	Actinobacteria, Actinobacteria	<i>S. magellanicum</i>	N, fungicidal, bactericidal	Opelt <i>et al.</i> (2007)

This listing includes only genera for which pure cultures have been isolated from *Sphagnum* gametophytes.

^aTaxonomic classification of bacterial isolate at the Phylum and Class level.

^bPotential for beneficial phenotypes determined by *in vitro* assays: P, phosphorus solubilization; N, nitrogen fixation; 'fungicidal', antagonism toward fungal plant pathogens; 'bactericidal', antagonism toward bacterial plant pathogens; 'hormones', production of plant hormones auxin or indole acetic acid (IAA).

^cND, no beneficial phenotype detected.

microbes contribute to the health and productivity of the plant host (Fig. 2). For example, the association of methanotrophic bacteria with peat mosses may provide an additional source of carbon to the plant along with regulating methane emissions to the atmosphere. While peat mosses mainly acquire carbon through the fixation of atmospheric CO₂, methanotrophic bacteria were shown to provide 5–20% of *Sphagnum*'s cellular CO₂ needs through methane oxidation, which would be especially important under water-saturated conditions where CO₂ diffusion is slow (Raghoebarsing *et al.*, 2005; Kip *et al.*, 2010). This phenomenon has been detected in a range of *Sphagnum* species and geographically distant peatlands (Putkinen *et al.*, 2012). *Sphagnum*-associated methanotrophs were shown to be members of the *Alpha*- and *Gammaproteobacteria*. *Alphaproteobacteria*, including *Methylocystis*-*Methylosinus* genera and acidophilic methanotrophs of the genera *Methylocella*, *Methylocapsa* and *Methyloferula* (Dedysh, 2011), appear to comprise the most active methanotrophs in peatlands (Kip *et al.*, 2010; Putkinen *et al.*, 2014; Esson *et al.*, 2016).

Another vital microbiome-mediated process is N₂ fixation or diazotrophy (Fig. 2). *Sphagnum*-dominated peatlands are extremely nutrient-poor ecosystems, and nitrogen budgets have long suggested that N₂ fixation comprises a substantial source of nitrogen to these ecosystems (Hemond, 1983). Recent research has shown that nitrogen accumulation far exceeds nitrogen deposition rates from precipitation in peatlands and *Sphagnum* growth is not nitrogen-limited, suggesting that N₂ fixation is more important than previously perceived. Early studies presented microscopic evidence for the presence of heterocystous photosynthetic cyanobacteria in *Sphagnum* tissues (Granhall & Hofsten, 1976),

and more recently diazotrophic ('nitrogen-eating', N₂-fixing) cyanobacteria were shown to contribute up to 35% of cellular nitrogen to the *Sphagnum* host (Berg *et al.*, 2013; Lindo *et al.*, 2013). Surprisingly, molecular analyses targeting genes in the nitrogen fixation pathway (nitrogenase, *nifH*) tell a different story; namely that *Sphagnum*-associated N₂-fixing communities are dominated by members of the *Alphaproteobacteria*, which includes phototrophic and heterotrophic genera, rather than by cyanobacteria (Bragina *et al.*, 2013; Leppanen *et al.*, 2015). By applying a quantitative PCR approach to *Sphagnum* samples collected from boreal peatlands in Canada, Vile *et al.* (2014) further observed an order of magnitude higher expression of *nifH* genes associated with *Alphaproteobacteria* in comparison to those associated with cyanobacteria. As many known aerobic methanotrophs are found within the *Alphaproteobacteria*, this suggested that methanotrophs are the most active *Sphagnum*-associated nitrogen fixers. Studies based on gene sequences are corroborated by those that incorporate stable isotope tracers to show a direct linkage between methanotrophy and diazotrophy (Larmola *et al.*, 2014). Lastly, recent results suggest a third alternative: that the dominant diazotrophs in *Sphagnum*-dominated peats include methanogenic archaea (Lin *et al.*, 2014b). However, fungi associated with plants have also been shown to produce methane under aerobic conditions (Lenhart *et al.*, 2012). Thus, the specific microbial groups that transfer nitrogen or carbon to *Sphagnum* and their physiological ecology remain controversial (Ho & Bodelier, 2015). Oxygen is depleted close to the surface in peatlands and we suspect that most methane is produced by archaea in anoxic microzones on the surface of living *Sphagnum* plants or in the decomposing peat layers close to the

Fig. 2 Schematic illustrating functional guilds of microorganisms (methanotrophs, phototrophs and nitrogen-fixing bacteria) that have been shown to benefit *Sphagnum* growth and productivity, all of which have been located inside nonphotosynthetic, hyaline cells. Methanotrophic bacteria couple the oxidation of methane (CH_4) to aerobic respiration and these microbes may act as a source of CO_2 for *Sphagnum*. Nitrogen-fixing bacteria mediate the conversion of N_2 gas into ammonium and may aid *Sphagnum* by acting as a nitrogen source. Cyanobacteria are capable of photosynthesis and nitrogen fixation. Thus, this group may act as a source of carbon or nitrogen to the plant.

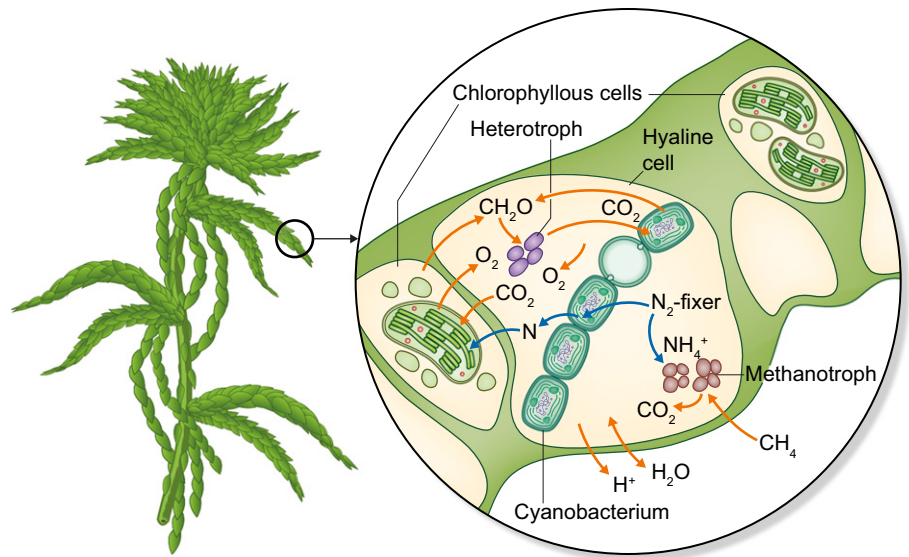


Table 2 Representative fungal species associated with living *Sphagnum*, including their known hosts and putative trophic relationship

Fungal species	Taxonomy	Host	Relationship	Citation
<i>Capnobotryella renispora</i> Sugiy.	Ascomycota, Capnodiales	<i>S. fuscum</i>	Parasite	Hambleton <i>et al.</i> (2003)
<i>Scleroconidioma sphagnicola</i> Tsuneda, Currah, & Thormann	Ascomycota, Dothideaceae	<i>S. fuscum</i>	Pathogen	Tsuneda <i>et al.</i> (2001)
<i>Bryophytomyces sphagni</i> (Navashin) Cif.	Ascomycota, Helotiaceae	<i>Sphagnum</i> spp.	Pathogen, replaces spores in capsule, explosively dispersed	Redhead & Spicer (1981)
<i>Helotium schimperii</i> Navashin	Ascomycota, Helotiaceae	<i>S. squarrosum</i> , <i>S. magellanicum</i>	Pathogen	Redhead & Spicer (1981); Filippova (2012)
<i>Lasio-sphaeria</i> spp.	Ascomycota, Lasiosphaeriaceae	<i>Sphagnum</i>	Parasite	Döbbeler (1997)
<i>Epibryon turfoso-rum</i>	Ascomycota, Pseudoperisporiaceae	<i>Sphagnum</i>	Parasite	Döbbeler (1997)
<i>Trizodia acrobia</i> Laukka	Ascomycota, Pezizomycotina, Incertae sedis	<i>S. angustifolium</i> , <i>S. capillifolium</i> , <i>S. flexuosum</i> , <i>S. girgensohnii</i> , <i>S. magellanicum</i> , <i>S. rubellum</i> , <i>S. russowii</i> and <i>S. squarrosum</i>	Symbiont, possible mutualist. Tripartite interaction with <i>Sphagnum</i> and <i>Nostoc</i>	Stenroos <i>et al.</i> (2010)
<i>Absoconditella sphagno-rum</i> Vezda & Poelt	Ascomycota, Stictidaceae	<i>S. compactum</i> , <i>S. fuscum</i> and <i>S. magellanicum</i>	Parasite/pathogen	Stenroos <i>et al.</i> (2010)
<i>Sphagnurus paluster</i> (Peck) Redhead & V. Hofstetter	Basidiomycota, Lyophyllaceae	<i>S. fallax</i> , <i>S. magellanicum</i> , <i>S. papillosum</i>	Pathogen	Untiedt & Müller (1985); Limpens <i>et al.</i> (2003)
<i>Galerina paludosa</i>	Basidiomycota, Strophariaceae	<i>S. capillaceum</i>	Parasite	Redhead & Spicer (1981)

water table depth where carbon substrates are plentiful. At this depth, nitrogen is extremely limited and the ability to fix nitrogen would confer a selective advantage to methanotrophic bacteria or methanogenic archaea. The metabolic strategy and physical location of the nitrogen fixers (associated with living or decomposing *Sphagnum*) will bear directly on the impacts of climate change drivers on biogeochemical cycles. For example, increases in temperature would have an immediate impact on aerobic nitrogen fixers of the microbiome located in the surface peat, whereas anaerobes in the deep peat would take longer to respond and the response may be decoupled from plant growth.

V. Fungi in the *Sphagnum* microbiome

Although we have focused on bacteria present in the microbiome, *Sphagnum* species host a broad spectrum of fungi as well, many of which are found only on this genus or on single *Sphagnum* species. These include saprotrophs, parasites, pathogens, and possibly mutualists (Table 2). Decaying *Sphagnum* peat supports diverse communities of free-living saprotrophs and root associates of vascular plants (Artz *et al.*, 2007; Thormann & Rice, 2007; Lin *et al.*, 2014a). Unique *Sphagnum* tissue chemistry may have selected for a suite of fungi well suited to decomposing their

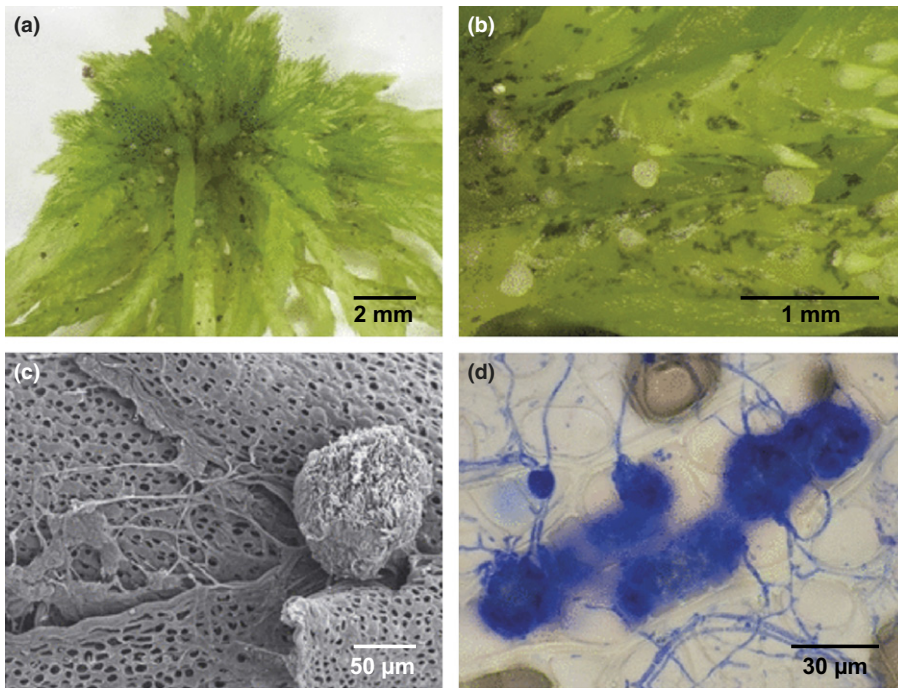


Fig. 3 Fungi associated with *Sphagnum* tissues. *Trizodia acrobia*, gen. et sp. nov. (a) Shoot apex of *Sphagnum girgensohnii* with cyanobacteria (small dark green patches) and apothecia (white globules). (b) Close-up photograph showing cyanobacteria and *T. acrobia* associated with *S. girgensohnii*. (c) Scanning electron microscopy image showing an apothecium (right) from which hyphae run to adjacent cyanobacterial colony (left). (d) Light microscope image of a cyanobacterial colony inside a hyaline cell of *Sphagnum*. Fungal hyphae enter the cell through round pores and envelop the colony. Dyed with cotton blue.

tissues. Living *Sphagnum* host a community of fungal parasites with high specificity (Döbbeler, 1997). In some cases, parasites are limited to specific tissues, e.g. *Helotium schimperi* is specific to the tip of slime hairs on the stem of *Sphagnum squarrosum* (Redhead & Spicer, 1981). Some of these interactions suggest sophisticated evolutionary mechanisms, e.g. *Bryophytomyces sphagni*, which colonizes spore capsules of *Sphagnum*, using them for its own ballistic dispersal (Redhead & Spicer, 1981). Fungal infection is believed to be ecologically important in peatlands, creating disturbances that can lead to a reversal in peatland succession and the development of microtopography (Karofeld & Pajula, 2005). Future studies should incorporate the health of *Sphagnum* tissues and fungal infection in microbiome studies at the ecosystem scale.

One of the most interesting open questions is whether *Sphagnum* has any undiscovered nonmycorrhizal mutualistic interactions with fungi. Although mutualistic mycorrhizal interactions are widespread in most plants, including liverworts and hornworts, the moss clade is not shown to form arbuscular mycorrhizal symbioses (Read *et al.*, 2000). In *Sphagnum*, the absence of root-like structures (rhizoids) would seem to preclude the possibility of mycorrhizas. However, recent evidence of a tripartite symbiosis among *Sphagnum*, the cyanobacterium *Nostoc*, and an ascomycete named *Trizodia acrobia* found in foliar hyaline cells suggests the presence of ancient yet poorly characterized symbiotic interactions that could be involved in regulating N fixation and N transport (Stenroos *et al.*, 2010; Fig. 3).

VI. Conclusions and future directions

With unique physiological and morphological adaptations, *Sphagnum* mosses dominate primary production and the carbon cycle in many climatically sensitive northern ecosystems. We

hypothesize that microbial groups that form close associations with *Sphagnum*, such as methanotrophic and diazotrophic bacteria, have the potential to act as keystone species that regulate carbon and nitrogen flow in peatlands. These functional guilds are generally considered to be endophytes that share a mutualistic or symbiotic relationship with the plant host (Raghoebarsing *et al.*, 2005; Kip *et al.*, 2010), although alternative evidence suggests a less defined association (Putkinen *et al.*, 2012). While empirical observation and model simulations suggest that microbes clearly benefit the plant host by providing a source of carbon or nitrogen that enhances productivity (Weston *et al.*, 2015), the mechanisms and controls of these interactions, as well as the benefit to the microbes, are less clear. The implication is that peat mosses provide shelter, oxygen, and perhaps carbon substrate to the microbes. More research is needed to understand the exact genetic and physiological mechanisms that define these plant–microbe interactions in peat mosses.

In order to project the impact of climate change drivers on biogeochemical cycles mediated by peat mosses, an improved mechanistic understanding of plant–microbe interactions is needed that is site- and organism-specific. The most pressing knowledge gap is to link the community composition of the *Sphagnum* microbiome to host and ecosystem function. Specific microbial groups must be linked to specific processes that benefit or adversely impact the plant through a close coupling of plant and microbial biology. Given that species of *Sphagnum* have broad geographic ranges, microbiome research should be linked with both reliable taxonomy of *Sphagnum* species and analyses of genetic variation across intraspecific ranges. The ultimate goal will be to constrain the ‘interactome’ of *Sphagnum*–microbe associations and then to link metabolome models to biogeochemical and Earth system models. For example, the *Sphagnum* microbiome was proposed to comprise a ‘missing link’ or unaccounted input in the

nitrogen cycle of boreal peatlands (Ho & Bodelier, 2015). In order to account for this input, data on the structure and function of nitrogen-fixing members of the microbiome should be incorporated into models of photosynthesis/CO₂ uptake and net primary production.

To constrain models, research is needed on the abundance and identity of specific microbial groups in plant tissues, quantitative rates of microbiome processes, and the efficiency with which carbon or nutrients are transferred from specific microbes to the host. We suggest two experimental approaches to address these knowledge gaps. First, stable isotopes can be used in conjunction with next-generation sequencing to elucidate the specific microbial groups and processes that mediate important ecosystem functions. Methane and nitrogen gas are incorporated into microbial biomass during methanotrophy and nitrogen fixation, respectively. Thus, stable isotope probing could be employed with isotopically labeled methane and/or N₂ to explore the linkage between methanotrophy and nitrogen fixation (Buckley *et al.*, 2008; Chen *et al.*, 2008). Incorporation of isotopically labeled methane or N₂ into microbial DNA or RNA extracted from plant tissues would provide direct evidence that these processes are mediated by the microbiome, and sequencing of labeled nucleic acid would identify the microbial groups involved. Moreover, stable isotope labeling and phylogenetic hybridization probes could be used in combination with nanoscale secondary ion mass spectrometry to directly link diazotrophy and methanotrophy to specific microbial cells at the cellular to subcellular level in plant host tissues (Thompson *et al.*, 2012).

Second, for plants such as *Sphagnum* that can be grown gnotobiotically (or germ-free), hypothesis-based experiments can be conducted by inoculating the sterile plant with specific microbial populations. The impact of the microbe on plant host fitness can then be directly determined by quantifying plant traits along with microbial parameters before and after inoculation. Such experiments have begun to be conducted with *Sphagnum* (Weston *et al.*, 2015).

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