The Sphagnome Project: enabling ecological and evolutionary insights through a genus-level sequencing project

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Summary

Considerable progress has been made in ecological and evolutionary genetics with studies demonstrating how genes underlying plant and microbial traits can influence adaptation and even 'extend' to influence community structure and ecosystem level processes. Progress in this area is limited to model systems with deep genetic and genomic resources that often have negligible ecological impact or interest. Thus, important linkages between genetic adaptations and their consequences at organismal and ecological scales are often lacking. Here we introduce the Sphagnome Project, which incorporates genomics into a long-running history of *Sphagnum* research that has documented unparalleled contributions to peatland ecology, carbon sequestration, biogeochemistry, microbiome research, niche construction, and ecosystem engineering. The Sphagnome Project encompasses a genus-level sequencing effort that represents a new type of model system driven not only by genetic tractability, but by ecologically relevant questions and hypotheses.

Keywords: ecological genomics, ecosystem engineering, evolutionary genetics, genome sequencing, genomics, niche construction, peatlands, Sphagnome, *Sphagnum*

I. Introduction

The discovery, characterization, and prediction of genes associated with traits, and how those traits influence ecosystem function, are key challenges, especially in the face of changing climatic conditions (Whitham *et al.*, 2006). Climate-driven alteration of biological processes occurs across all levels of organization, and is expected to impact a wide range of ecosystem goods and services including biodiversity, nutrient cycling, climate feed-back regulation, and productivity (Rockström *et al.*, 2009). However, our ability to associate genes with traits of ecological interest is generally restricted to plant model systems primarily developed for crop and bioenergy feedstocks, and further limited by the sheer complexity of applying genetic and genomic approaches to multiple species or communities. Yet the need to apply system genetic approaches in complex communities is paramount as evolution takes place within a complex web of genetic interactions among species (Whitham *et al.*, 2006).

Here we argue that the genus *Sphagnum* (peat moss) represents an unparalleled - model system for ecological and evolutionary genomics, empowered by its contribution to global carbon cycling and emerging genomic resources. *Sphagnum* species play a major role in peatland formation, a prime example of ecosystem engineering, whereby the organism manipulates its surrounding habitat. *Sphagnum* primary production influences carbon and nutrient cycling, such as methane production and soil carbon storage, in many boreal forests and peatlands (Turetsky *et al*., 2012). *Sphagnum* ecosystem engineering involves the accumulation of peat that facilitates its own growth while making the surrounding environment hostile for vascular plants (van Breemen, 1995). Ultimately these multi-level processes lead to peatland formation that occupy nearly 3% of the land surface and store 25% of the world's soil carbon as recalcitrant peat (Yu *et al*., 2010). The latter point has led to the assertion that *Sphagnum* has a greater impact on global carbon fluxes, and therefore climate, than any other single genus of plants (Clymo & Hayward, 1982; van Breemen, 1995).

The *Sphagnum* sequencing project provides a novel non-food crop or non-bioenergy feedstock example for a plant-based genome sequencing project aimed specifically at carbon cycling. The project is developing resources for within-species genetic associations with ecologically relevant functional traits, and the extension of those gene-to-trait relationships to additional species within the *Sphagnum* genus. We refer to this effort collectively as the Sphagnome Project. In the following sections, we provide a brief introduction to the ecology and evolution of this unique plant genus. We then outline a research roadmap that highlights scientific questions relevant to the disclosure and use of a genus-wide genomic resource for *Sphagnum* in two major areas of distinct but overlapping research: (a) carbon sequestration and global biogeochemistry, and (b) niche construction, ecosystem engineering, and microbial associations. We demonstrate that the Sphagnome Project is an example of a novel model system aimed at addressing ecologically relevant questions and hypotheses across levels of organizations.

II *Sphagnum* ecology and evolution

1. Functional traits and ecosystem function

Sphagnum has a remarkable ability to create and then uniquely thrive in nutrient-poor, acidic, and waterlogged conditions. The suite of morphological, physiological, and life history traits that affect *Sphagnum* fitness, herein termed functional traits, enable this 'ecosystem engineer' (Jones *et al*., 1994) to gain a competitive advantage over other co-occurring species and therefore flourish under relatively harsh environmental conditions. For example, the ability of *Sphagnum* to store and transport water is controlled largely by three distinct morphological adaptations – branching architecture, leaf size and arrangement on branches, and hyaline cells (Fig. 1a,b; Rydin & Jeglum, 2013). These traits differ considerably among species, and are associated with highly partitioned microhabitat preferences where *Sphagnum* species coexist within a peatland. Hummock-forming species, growing ca. > 30 cm above the water table, have small close-set leaves forming numerous interconnected small capillary spaces (Fig. 1). Spreading branches allow lateral movement of water through the capillary continuum, while numerous close-set pendant branches appressed to the stem form an efficient vertical water-transport system. Consequently, *Sphagnum* species growing on hummocks can wick moisture and maintain metabolic activity even during drought (Rice & Giles, 1996). In all species, dead hyaline cells in the leaves and the outer cortex of the stems and branches act as water-storage structures.

The capitula at the top of the stem are alive, but a few (~5) cm down 99 % of the light has been absorbed and most of the *Sphagnum* cells die (Hayward & Clymo, 1983). From there down to the water table the carpet structure is permeable to water and gases (particularly O_2) and the damp plant substrates begin to decay in this oxic zone, termed the acrotelm (Ingram, 1978; Clymo & Hayward 1982). The consequent loss of stem strength and increasing weight eventually result in collapse of the plant structure. This reduces the pore size so water can no longer flow easily through it, and from this point downwards the peat is permanently waterlogged and this is what determines the depth of the water table. In this waterlogged zone, oxygen is consumed by aerobic respiration more rapidly than it can be replenished by diffusion (which is 10,000 times slower in water than it is in air), creating the anoxic catotelm (Clymo, 1983). Hence, through distinct traits, *Sphagnum* generates environmental conditions that are suitable for its own

growth but hostile for the vast majority of other plants (e.g., van Breemen, 1995; Rydin & Jeglum, 2013).

The mechanisms by which *Sphagnum* inhibits fungal and microbial decomposition -- and hence promotes peat accumulation -- are not fully understood, but involve both the external environment engineered by the species, as well as the internal biochemistry of its plant tissue, particularly the low N:C ratio (a reflection of the unusually efficient use of N in producing new biomass) (Bragazza *et al.*, 2006). A passive mechanism for intrinsic decay resistance in the oxic acrotelm layer is suggested by the correlation of microbial decomposition of *Sphagnum* litter with the relative amounts of structural versus metabolic carbohydrates (Turetsky *et al.*, 2008). Active mechanisms of antimicrobial activity are also implicated, mainly through acid hydrolysis of cell-wall polysaccharides, fragments of which are released into the soil water as 'sphagnan' (Hájek *et al.*, 2011). The precise mechanisms for the antimicrobial activity of sphagnan are still under investigation, but may involve lowering soil pH, reducing availability of nitrogen and carbon, or interfering with extracellular enzymes by immobilizing them in a polyelectrolyte complex (Hájek *et al.*, 2011). Soluble phenolic compounds, either leached directly from *Sphagnum* tissue or produced during its breakdown, may play a more minor role in tissue preservation, physically protecting polysaccharides through the formation of humic substances (Hájek *et al.*, 2011). While environmental factors such as soil oxygen profiles serve as important regulators of peat decomposition (cf Freeman *et al.*, 2001) it is clear that a variety of mechanisms contribute to slow decomposition of *Sphagnum* tissue, thereby retarding the turnover of organic biomass in peatlands and sequestering carbon in the form of peat for centuries.

2. Phylogeny and evolution

Like all mosses, the haploid gametophyte is the dominant life cycle stage for *Sphagnum* (Fig. 1). Haploid spores germinate into a filamentous protonema, quickly followed by a thalloid protonemal phase, before transitioning into mature haploid gametophytes. A single spore can result in a large clonal biomass through vegetative growth. Furthermore, the ability to propagate clonally is ubiquitous in *Sphagnum* and typical clone sizes vary

among species (Cronberg, 1991). In *S. austinii*, one clone occurs throughout North America and the same dominates in Europe (Kyrkjeeide *et al.*, 2016). A single clone of *S. subnitens* extends from Oregon to the westernmost Aleutian Islands (Karlin *et al.*, 2011). Reproductive seasons are species-specific and sperm require water to access the egg cell in the archegonial venter to form the zygote. The formation of the zygote marks the beginning of the brief diploid stage of development and at maturity meiosis occurs within the capsule, producing haploid spores.

Sphagnum is one of four genera in the class Sphagnopsida (phylum Bryophyta: mosses), an ancient lineage of land plants. Molecular phylogenies suggest the Sphagnopsida diverged from other mosses more than 250-350 mya (Shaw *et al.*, 2010), and fossils of peat moss-like fragment, which are the oldest known land plant macrofossils to date, have been found in the Ordovician rocks (~500 mya, Cardona-Correa *et al.*, 2016). Fossil *Sphagnum* and close relatives are recognized by the unique cell pattern in leaves. Three of the genera in the Sphagnopsida contain just one or two species each, and none of them form extensive peats nor do they dominate wetlands as do species of *Sphagnum*. With 200-300 species, *Sphagnum* is by far the largest genus in the Sphagnopsida and the most important for peatlands. *Sphagnum* species share a common ancestor in the late Tertiary, a surprisingly recent radiation considering the great antiquity of Sphagnopsida (Shaw *et al.*, 2010). This recent radiation, which may have occurred following the mid-Miocene climatic optimum, coincides with the rise of boreal peatlands in the northern hemisphere (Greb *et al.*, 2006).

Today, *Sphagnum* occurs on all continents aside from Antarctica (Crum, 1984). The genus dominates wetland habitats throughout the boreal zone of the Northern Hemisphere but is also diverse at tropical latitudes, especially in South America (as well as in tropical Africa and Asia). At tropical latitudes, *Sphagnum* sometimes occurs in high altitude peatlands, but in lower altitude tropical regions they typically grow on wet soil banks, along streams, and on dripping rocks, and do not accumulate substantial amounts of peat. *Sphagnum* comprises five major subgenera (Fig. 2a; Shaw *et al.*, 2016a). The small subgenus *Rigida* (ca. 2-4 species), sister to the four other subgenera, sometimes occur in peatlands, but its species are never dominant and are not major peat-formers. Most *Sphagnum* species belong to the remaining two clades, both of which include

important peat-forming species. The species in one clade (subgenera *Cuspidata* + *Subsecunda*) generally occupy hollows close to or at the water table, whereas those in the other clade (subgenera *Sphagnum* + *Acutifolia*) generally create lawns and raised hummocks more distant from the water table (Fig. 2b). For decades, peatland ecologists have noted that individual *Sphagnum* species have narrow realized niches along this hydrological gradient—from low hollow to high hummock (Vitt & Slack, 1984). *Sphagnum* species also exhibit narrow preferences along a chemical gradient, with some species preferring acidic ombrotrophic bogs and other species preferring fens with more neutral pH. Unlike preferences along the hydrological gradient, species preferences along the chemical gradient do not exhibit a strong phylogenetic signal (Johnson *et al.,* 2015). During the rapid radiation of modern *Sphagnum*, microhabitat preferences along the chemical gradient plausibly evolved simultaneously in unrelated groups, creating natural experiments with which the genetic basis of microhabitat preferences can be disentangled from phylogenetic history.

III. Developing resources for a tractable *Sphagnum* model system with evolutionary and ecological relevance

Genomic resources for *Sphagnum* are rapidly expanding (https://phytozome.jgi.doe.gov). The Sphagnome Project will provide two high quality reference genomes (*S. magellanicum* and *S. fallax*), sequences for 15 additional species across the *Sphagnum* phylogeny (Fig. 2), and shallow sequencing of ca. 200 individual members from a haploid-sib pedigree. A draft genome for *S. fallax* is now available on https://phytozome.jgi.doe.gov. These Sphagnome Project resources are motivated by two overarching aims: 1) identifying genetic associations with ecologically relevant functional traits within species, and 2) extending those gene-to-trait relationships to additional species within genus.

1. *Sphagnum* pedigree sequencing and gene to trait mapping

The Sphagnome Project is producing high-quality reference genomes for *Sphagnum magellanicum* Brid. and *S. fallax* H. Klinggr (Shaw *et al*., 2016b). These two peat-forming species are in different subgenera, occupy very different microhabitats in boreal peatlands, and will provide strong contrasts for investigating phylogenetic and ecological differences (Fig. 2, Johnson *et al*., 2015). To fulfill the first aim focusing on within-species variation, the Sphagnome Project will conduct re-sequencing of ca. 200 individuals from a *S. fallax* pedigree to generate a high quality genetic linkage map that will facilitate gene-to-trait experimental approaches (Fig. 3) and genome assembly. The pedigree was developed from single stem descent propagation using sporelings germinated from a single field collected sporophyte; all individuals are haploid sibs. Because *Sphagnum fallax* has separate gametophytic sexes, pedigree individuals can be maintained in clonal culture without risk of intra-gametophytic selfing. Preliminary data show vast phenotypic variation among haploid siblings in response to laboratory growth conditions, temperature and pH (Shaw *et al,* 2016). *Sphagnum* is haploid in its dominant life cycle stage, which eliminates the confounding heterozygosity which can mask allele expression. Therefore, the F_1 (gametophytic) generation can be used in trait mapping, which is not possible for genetic studies in diploid non-bryophyte organisms where, at a minimum, a segregating F_2 pedigree is required. Furthermore, the paternal genotype can be reconstructed by subtracting the progeny genetic markers from the maternal markers. This latter point is especially important, as controlled crosses are currently difficult to perform in *Sphagnum*. As recently shown in the *Sphagnum* moss-relative *Physcomitrella patens* (Stevenson *et al.*, 2016), the simplified genetics of mosses coupled with linkage-analysis can provide a powerful means of predicting phenotypes from DNA markers and their underlying causal alleles (Fig. 3).

Recent advances in maintaining *Sphagnum* tissue cultures (Beike *et al.*, 2015) have improved the reliability of producing axenic cultures that produce *Sphagnum* plants that are morphologically similar to field-collected specimens. The Sphagnome Project encompasses a developing germplasm collection that includes culture material for all species being sequenced and a *S. fallax* haploid-sib pedigree. The low stature of *Sphagnum* and ease of establishing populations in trans-well culture plates that have

relatively small ‘bench top’ space requirements enable rapid phenotyping that is necessary for gene-to-trait studies (Fig. 3). Further, this germplasm collection can be used to test responses of *Sphagnum* genotypes to different environmental conditions. Because the complete genomes of these genotypes will already be known as a result of re-sequencing, genetic associations can be made as soon as phenotypic data are collected. Due to the small size of *Sphagnum* and other mosses, imaging-based phenotyping will be especially useful in this effort. Single images can capture data on hundreds of individuals, entire populations, and mixed communities, simultaneously aiding the linkage of genes to traits. The broader collection of gene to trait associations can be integrated in network models to form a systems biology view of the trait combinations and their correlations underlying phenotype expression and adaptation (Chitwood & Topp, 2015).

2. A genus-wide approach

Extending gene-to-trait relationships beyond a single species is necessary for understanding the evolution of ecosystem function in *Sphagnum*-dominated peatlands. Traits important for ecosystem function differ among species, including productivity and resource acquisition, resource allocation such as production of secondary compounds, and decomposition rates (Bengtsson *et al*., 2016, Limpens *et al*., 2017). Therefore, in addition to the intensive within-species resequencing approach described above, the Sphagnome Project includes the sequencing of 31 individuals across 15 species representing the five major clades within *Sphagnum* (Fig. 2). This information, combined with ongoing and existing transcriptome resources (Devos *et al*., 2016), will provide the basis for genus-level phylogenomics and comparative genomic analyses in *Sphagnum* (Fig. 3). This approach is especially useful for the majority of traits in *Sphagnum* where interspecific variation seems to be greater than intraspecific variation (e.g. Bengtsson *et al.*, 2016). Genetic associations will be tested using models that incorporate phylogenetic comparative methods (e.g. Blomberg & Garland, 2002; Revell *et al*., 2009) to account for phylogenetic distance when identifying gene-to-trait relationships.

Through this sequencing effort, gene-to-trait relationships of multiple species will be placed within a broader phylogenomic landscape thereby identifying evolutionary

patterns associated with microhabitat preferences and functional traits (Fig. 2b & Fig. 4). While a few recent studies have taken a genus-wide approach to genetic associations (e.g., Haudry *et al*., 2013; Pease et al. 2016; Novikova *et al.*, 2016) the Sphagnome Project encompasses species that co-occupy and engineer the same ecosystem. We anticipate that these genus-wide sequences, phenotype data, and comparative gene-to-trait relationships will enable the detection of genes under purifying or positive selection as well as gene family evolution associated with major ecological and biogeographic shifts.

IV. Facilitating new ecological and evolutionary understanding

1. What is the biological basis of unique *Sphagnum* traits or combinations of traits, and how do these trait combinations extend beyond the organism?

Tissue chemistry is a noted functional trait for *Sphagnum* (Clymo & Hayward, 1982). Polyuronic acids (cell-wall polysaccharides that form a pectin-like polymer) comprise 10-30% of *Sphagnum* dry mass. They have a high cation exchange capacity (CEC) initially satisfied with H+, which is rapidly exchanged for cations in rainwater, thus making the water around the plants acidic (Clymo & Hayward, 1982) and make cation nutrients unavailable to microbes and other plants (Stalheim *et al.*, 2009). However, the question of a possible link between unique organic compounds and niche engineering by *Sphagnum* remains a matter of active research (Hájek, 2009; Limpens *et al.*, 2017). It has long been speculated that living *Sphagnum* benefits from peat formed over time through the accumulation of dead *Sphagnum* biomass (van Breemen, 1995). Should this be viewed as one type of extended phenotype, where the phenotype of vertically accumulating peat (dead *Sphagnum* material) changes the function of living *Sphagnum* at the surface? *Sphagnum* plants clearly modify their environment in several important ways, but how this influences selection on future offspring and other recipient organisms is unknown. We believe that the *Sphagnum* genomic resource offers one of the best opportunities to explore these questions and ultimately identify the genetic basis for the traits responsible for ecosystem engineering in *Sphagnum*. For example, what is the

genetic basis of tissue chemistry traits, and do these traits impart a fitness advantage from a nutrient competition perspective? Furthermore, how do these traits extend beyond the organism? For example, do hummock formation traits covary with tissue chemistry and decomposition rates, and how will these currently adapted trait combinations influence fitness to changing environmental conditions? In regard to niche engineering, is there evidence for an extended phenotype in *Sphagnum*, and if so, what is the unit of selection, and at which level does selection occur (Whitham *et al.*, 2003)? Do neighborhood effects, such as the genetic effect of an individual on trait values of neighboring individuals influence how *Sphagnum* traits interact with the environment? How important is clonality to the extended *Sphagnum* phenotype? These important questions extend into much broader spheres of the Sphagnome Project (Fig. 4) and general ecological and evolutionary theory.

2. Did adaptation to spatially or temporally varying climate variation spark *Sphagnum* species radiations?

Genus-wide phylogenetic analyses of geographic ranges support the view that the two major peat-forming, crown clades within *Sphagnum* (*Acutifolia*+*Sphagnum*; *Cuspidata*+*Subsecunda*) (Fig. 2a,b) originated and first diversified in the Northern Hemisphere (Shaw *et al.*, unpublished). In contrast, phylogenetic analyses of large seed plant clades that span tropical and Northern Hemisphere ranges usually reveal tropical origins and rare expansions into cold northern climates (Jansson *et al.*, 2013). *Sphagnum* represents one of a small minority of groups that appear to have initially diversified at northern latitudes and subsequently extended their ranges into the tropics. Phylogenetic patterns indicate that southward range expansions were followed by evolutionary radiations that gave rise to groups of tropical species nested within larger boreal clades.

Moreover, non-boreal radiations occurred in each of the four large subgenera of *Sphagnum*, providing phylogenetic patterns that can be used as replicated natural experiments to account for shared ancestry when investigating the genetic basis of adaptation and the evolution of functional traits associated with range expansions. In addition to these radiations, a few individual boreal *Sphagnum* species have extended

their ranges into tropical habitats, presumably more recently. Inter- and intraspecific comparative analyses can be harnessed to address several questions. What genes, gene families, and genomic regions underwent changes associated with range expansions from boreal to tropical climate zones? Are the same genomic features associated with intra- and with interspecific range changes across climate zones? Are the same or similar genomic changes associated with climate adaptation in different *Sphagnum* subgenera, associated with independent range changes? Clarifying functional trait and genomic changes associated with migrations into warmer climates can provide informative analogies to how *Sphagnum* mosses and, perhaps, other plants may respond to current climate warming.

3. What are the factors that limit or facilitate local-scale adaptive evolution?

There has been much interest regarding the importance of phenotypic plasticity relative to local adaptation in response to environmental heterogeneity, and how such responses can ultimately extend to influence ecosystem function (Miner *et al.*, 2005). The sequenced haploid-sib pedigree, coupled with phenotype screening will provide the resources necessary for quantitative genetics to determine the extent to which a phenotypic change has a quantitative genetic basis (Section III). Plasticity is inferred as the proportion of phenotypic variance not explained by genetics (Merilä *et al.*, 2014). The use of common gardens, especially when established among multiple environments with appropriate replication and controls, provides a powerful approach to disentangle genetic from plastic contributions to phenotype. The sequenced *Sphagnum* haploid-sib pedigree and emerging research community surrounding the Sphagnome Project make the establishment of common gardens with characterized genotypes a reality. Finally, the demonstration that allele frequency shifts occur confirms that evolution has occurred, with the challenge being the need to determine if changes in specific allele frequencies are relevant to the traits and phenomena being investigated. The sequencing of 15 *Sphagnum* species and nearly 200 progeny individuals provides an ideal system to determine shared and species-specific components of the collective genome and relationships that co-occur with phylogenetic signals. For example, does a gene family expansion coincide with the

lineage diversification to novel environments? Together with common garden experiments we will begin to address questions centering on the relative importance of local adaptation versus phenotypic plasticity in *Sphagnum* responses to environmental heterogeneity.

4. What is the role of *Sphagnum* and its interacting microbiome in ecosystem carbon and nitrogen cycling?

Hyaline cells not only play a vital function as water storage organs, but also create a novel and safe habitat for a diverse microflora spanning all domains of life (Fig. 1b; Bragina *et al*., 2012; 2014; Kostka *et al*., 2016). The *Sphagnum*-associated microbiome seems to be divided into two broad categories. Those that are host species specific, with specificity maintained across both the sporophyte and gametophyte generations (Bragina *et al*., 2012), and those that are host species agnostic with environmental factors such as pH and nutrient availability explaining much of the community structure (Larmola *et al.*, 2014). With a raised pH, hyaline cells may serve as 'oases' for microbes in acidic peatland pore waters. The ecological function of *Sphagnum* symbionts is just beginning to be explored, with evidence pointing to strong linkages with the cycling of both carbon (i.e., methane oxidation) and nitrogen (i.e., nitrogen fixation). For example, diazotrophic cyanobacteria were shown to contribute up to 35% of cellular N to the *Sphagnum* host (Berg *et al*., 2013; Lindo *et al*., 2013) while methanotrophic bacteria can provide 5–20% of *Sphagnum*'s CO_2 demand through CH_4 oxidation (Raghoebarsing *et al*., 2005; Kip *et al*., 2010). Together, methanotrophy and N_2 fixation are tightly linked and was estimated to provide over one-third of the new N input in a coastal peatland (Larmola *et al.* 2014), although see Ho & Bodelier (2015). Therefore, a number of critical questions concerning the *Sphagnum* microbiome remain, for example what are the signaling and communication pathways between *Sphagnum* and its microbiome, and do these interactions represent true beneficial symbioses. How do protists and miroeukayotes influence peatland C and N cycles (Jassey *et al.*, 2015)? More questions than answers remain, and achieving a comprehensive understanding of the *Sphagnum* microbiome will benefit greatly from the application of comparative and functional genomics to evaluate

microbial community profiles across *Sphagnum* lineages and environments, and meta-transcriptomics to evaluate symbiotic pathways and metabolism.

5. How do we model *Sphagnum* genotype-by-environment interactions?

The understanding of *Sphagnum* trait characteristics and the population genetics underlying trait distributions may have important implications for modeling biogeochemistry and vegetation dynamics, both within an ecosystem and across regions up to a global scale. However, the *Sphagnum* trait characterization needed to inform these models is lacking for many high-latitude process-based models (Turetsky *et al*., 2012). Many ecosystem and regional models have adopted the concept of plant functional types (PFTs), where PFTs are defined as groupings of plant species that share similar characteristics and roles in ecosystem function. However, recent work suggests that parameterization of PFTs with current trait values may not be valid under future environmental conditions because trait values and trait-trait relationships may change under future environmental conditions (Scheiter *et al.*, 2013, van Bodegom *et al*., 2012). In this regard, we will benefit from population genomics programs – like the Sphagnome Project – where population genetics, genomics and phenotype analysis can be used to statistically model genome features (such as single nucleotide polymorphism (SNP) distributions) to trait value predictions. The 'trait values' are then entered as parameter values in physiological models. An elegant example of this approach was presented by Reuning *et al*. (2014), where QTL analysis was used to genetically parameterize a physiological model to predict transpiration of specific *Arabidopsis* genotypes. An intriguing question is whether such 'genome informed' ecophysiological models can be used to decipher the mechanisms of local adaptation, which provides deeper insights into heritable variation and trait covariances (and trade-offs) responsible for evolutionary dynamics (Weinig *et al.*, 2014).

V. Conclusions

The Sphagnome Project seeks to resolve important and general issues in ecology and evolution including (1) the niche differentiation and co-occurrence of many closely

related *Sphagnum* species within the same wetland habitat, (2) the genetic regulation of the unique chemical traits that define the central role of *Sphagnum* species in engineering those habitats, 3) the importance of *Sphagnum* in determining biodiversity patterns of other organisms, including microbes, and (4) The role of *Sphagnum* genetics and physiology on biogeochemistry and hydrology at ecosystem to global scales. With new genomic resources already available, and growing rapidly, we are poised to utilize the *Sphagnum* system for linking genomes and phenotypic traits to community assembly, ecosystem function, and evolutionary processes. Moreover, the *Sphagnum* system can provide unique insights into the phylogenetic history of genome and trait evolution, and allow predictions about how these organismal features are likely to respond to future environmental change.

Author Contributions:

DJW, AJS, MRT conceived the Sphagnome project and solicited community input; DJW, AJS, MRT, MGJ, and GG wrote the paper; ZL, LRB, SKR, DTH, KAME, ED, ESK, RJN, JEK, JBG, HR, JL, EST, AC, BWB, TAO, MBN, EAL and RSC conceived of and contributed to the ecological, physiology and modeling section; HKS, PS, MJ, BTP, developed the evolutionary genetic sections; JS, WM KKU, JGC, PR, DJ, contributed to the bioinformatics and quantitative genetics.

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Figures Legends

Figure 1: Morphological traits of *Sphagnum*. Left, four representative species (modified from Crum, 1984), A. Plant habit showing differences in branch density. B. Branch leaf cross sections showing arrangements of larger hyaline cells. As in most mosses, *Sphagnum* leaves consist of a single layer of cells, but unlike in other mosses, the leaf

cells are dimorphic, comprising large hyaline cells, dead and empty at maturity, alternating with narrow photosynthetic chlorophyllose cells. In some species (e.g., top), those chlorophyllose cells are not exposed at the leaf surface and in other species they are exposed at the inner or outer surface. C. Surface view of branch leaf cells, showing variously arranged pores on hyaline cells. The chlorphyllose cells are very narrow, forming a network around each hyaline cell. D. Branch fascicles, each including so-called spreading and pendent branches. E. Branch leaf. F. Stem cross section showing variously developed, sometimes enlarged outer cortex cells. Right, one (haploid) gametophyte plant with stalked capsules releasing spores (modified from Weston *et al.*, 2015). Far right, detail of branch leaf cells showing differentiation of chlorophyllose and hyaline cells.

Figure 2: Distribution, phylogeny and habitat preference of species within the Sphagnome Project. A recent phylogeny based on Shaw et al. 2016a with colored branches representing subgenus designations (brown = *Rigida*, yellow = *Subsecunda*, green = *Cuspidata*, blue = *Sphagnum*, purple = *Acutifolia*) and colored circles next to species being sequenced with the Sphagnome Project (2A); generalized habitat preferences for *Sphagnum* species typical of boreal peatlands, in relation to pore water pH and height above water table (2B); global distribution of *S. fallax* (green) and *S. magellanicum* (blue) (2C). Note that *S. affine* (*Sphagnum*), *S. cribrosum* (*Subsecunda*), *S. fimbriatum* (*Acutifolia*), and *S. molle* (*Acutifolia*) are not in the figure because they are not boreal peatland species, but have been sequenced as part of the Sphagnome Project.

Figure 3: Schematic of the proposed depth and breadth genetic approaches. In gene-to-trait studies, linkage-based and association mapping are main approaches used to discover (or map) the genetic basis of quantitative phenotypic variation. Both assume that there is variation for the traits of interest within the population being studied. The linkage-based method relies on individuals with known relationships to each other and DNA variants (termed genetic markers) that segregate through the population. The genetic marker is ‘linked’ through proximity to the causal loci and they therefore segregate together. Association mapping does not require known relationships among

individuals within the population, but instead relies on historical recombination from many generations of random mating. Together these methods constitute the 'genetic depth' approach discussed in text aimed at identifying candidate genes (bottom) that are then included in phylogenomic and comparative genome analyses (top). These analyses are simplified by the fact that *Sphagnum* gametophytes are typically haploid. Two allopolyploid species (*S. palustre*, *S. papillosum*) are included to address subsidiary issues related to the evolution of polyploid genomes.

Figure 4: An integrated approach for *Sphagnum* as a model system linking genetic information on genes underlying functional traits (depth) with phylogenomic analyses (breadth) to large-scale, emergent properties at the level of the ecosystem. Increases in the availability of genomic resources and recent developments of germplasm resources can facilitate collaborative research across multiple disciplines. Understanding the genetic basis of integrated traits will facilitate our understanding of trait-trade-offs, fitness and selection, and response to environmental change.

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