

REVIEW PAPER

A scoping review of bryophyte microbiota: diverse microbial communities in small plant packages

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Abstract

Plant health depends not only on the condition of the plant itself but also on its diverse community of microbes, or microbiota. Just like the better-studied angiosperms, bryophytes (mosses, liverworts, and hornworts) harbor diverse communities of bacteria, archaea, fungi, and other microbial eukaryotes. Bryophytes are increasingly recognized as important model systems for understanding plant evolution, development, physiology, and symbiotic interactions. Much of the work on bryophyte microbiota in the past focused on specific symbiont types for each bryophyte group, but more recent studies are taking a broader view acknowledging the coexistence of diverse microbial communities in bryophytes. Therefore, this review integrates studies of bryophyte microbes from both perspectives to provide a holistic view of the existing research for each bryophyte group and on key themes. The systematic search also reveals the taxonomic and geographic biases in this field, including a severe under-representation of the tropics, very few studies on viruses or eukaryotic microbes beyond fungi, and a focus on mycorrhizal fungi studies in liverworts. Such gaps may have led to errors in conclusions about evolutionary patterns in symbiosis. This analysis points to a wealth of future research directions that promise to reveal how the distinct life cycles and physiology of bryophytes interact with their microbiota.

Keywords: Bryophyte, hornwort, liverwort, microbiome, microbiota, moss.

Introduction

All plants harbor microbiota (i.e. microbial communities) whose collective genetic materials are referred to as the microbiome (Schlaeppli and Bulgarelli, 2015). The importance of specific mutualistic microbiota members has long been recognized, for example the nutritional symbioses with mycorrhizal fungi and nitrogen-fixing rhizobia (Box 1). Plant pathology has also long investigated the devastating effects of various microbial diseases on plants. However, advances

in biological methods, especially the development of high-throughput sequencing (HTS; Box 1) of DNA, have revealed that plants are inhabited by much more complex communities of microbes (Turner *et al.*, 2013). A plant's microbiota includes members that are diverse phylogenetically, in where they live on the plant, and in their functions. Plants can host bacteria, archaea, viruses, and many types of eukaryotes (Turner *et al.*, 2013). Eukaryotic symbionts include fungi,

Abbreviations: HTS, high-throughput sequencing; ITIS, Integrated Taxonomic Information System.

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Box 1. Glossary.**Microbiology**

Diazotrophs. Prokaryotes that fix nitrogen; that is, they take atmospheric N_2 and convert it into biologically useful nitrogen-containing compounds such as ammonia.

Heterocysts. The nitrogen-fixing cells in filamentous cyanobacteria. These cells are thick walled and lack photosynthetic activity in order to create the oxygen-poor environment needed for the nitrogen-fixing enzyme nitrogenase.

Hormogonia. The motile forms of filamentous cyanobacteria. These filaments are made exclusively of photosynthetic cells, so they lack the nitrogen-fixing ability of stationary filaments.

Methyloolithotrophs. Prokaryotes that use compounds containing a one-carbon molecule as the carbon source for their metabolism. One example is **methanotrophs** which use methane.

Microbiota. The community of microorganisms found in a particular habitat or host. Taxon-specific variants of this term also appear, such as **mycobiota** to refer to all the fungi in a habitat.

Microbiome. In the strict sense, the genetic material of a given microbiota community. The term is often also used more broadly to refer to the microbiota.

Associations

Endophytes. Microorganisms that live inside the healthy tissues of plants. They can have a variety of effects on the host, even pathogenic, as long as they can at some point be found in asymptomatic tissues.

Epiphytes. In the microbiota context, microorganisms that live on the surface of a plant host.

Mycorrhiza. A mutualistic association between a fungus and the roots of a plant. In plants lacking roots (i.e. bryophytes), the fungi can be in rhizoids, stems, or thalli. The plant provides carbon-containing compounds to the fungus and the fungus provides mineral nutrients that it can access better than the plant's roots. There are various types of this symbiosis, categorized by the plant and fungal taxa and the morphology of the mycorrhiza, including the following.

- **Arbuscular mycorrhizae.** Association with fungi of the Glomeromycotina. The fungi are obligate symbionts and form arbuscules (tree-like structures) or coils between the cell walls and plasma membranes of plant root cells. Some arbuscule-forming fungi belong to Mucoromycotina and are referred to as 'fine root endophytes'.
- **Ectomycorrhizae.** Associations with either fungi of Basidiomycota or Ascomycota in which the fungi grow in between root cells without penetrating them. The fungi generally form a sheath around the root.
- **Ericoid mycorrhizae.** Associations between plants in the family Ericaceae and fungi of Ascomycota or Basidiomycota, in which the hyphae penetrate between plant cell walls and membranes.

Mycoheterotrophy. A type of plant trophic mode in which a non-photosynthetic plant uses a fungal partner to obtain nearby carbon resources.

Methodologies

High-throughput sequencing (HTS). Sequencing techniques that can produce a large amount of sequence data for DNA and RNA without much prior knowledge of the focal organisms. Platforms for HTS include the now discontinued 454 pyrosequencing, Illumina, PacBio SMRT, IonTorrent, and direct sequencing with Oxford Nanopore.

Clone library. A technique that copy and insert unknown nucleotide fragments into vectors with known genetic backgrounds. The sequence of the unknown fragment can be obtained by sequencing the vector. Before HTS advancement, clone library was a widely used method to study the microbiome.

Stable isotope analysis. Stable isotope ratios can be used to infer nutrient and element translocation among organisms and substrates. Carbon, nitrogen, and phosphorus are the most commonly studied elements in microbiome-related studies.

oomycetes, algae, and a wide variety of other 'protists' (Turner *et al.*, 2013; Dastogeer *et al.*, 2020; Dumack *et al.*, 2021; Lee and Ryu, 2021). These microbes may live on the surface of plants (epiphytes) or inside their tissues (endophytes) (Box 1). Endophytes can be intercellular or in intimate intracellular associations (Rodriguez *et al.*, 2009). Microbiota mem-

bers can be mutualistic, commensal, or parasitic, and many microbes can shift between these relationships depending on the conditions (Partida-Martinez and Heil, 2011). Beneficial microbes have a wide range of possible functions for the host: providing nutritional supplements, protecting against pathogens, deterring herbivores, enhancing tolerance of abiotic

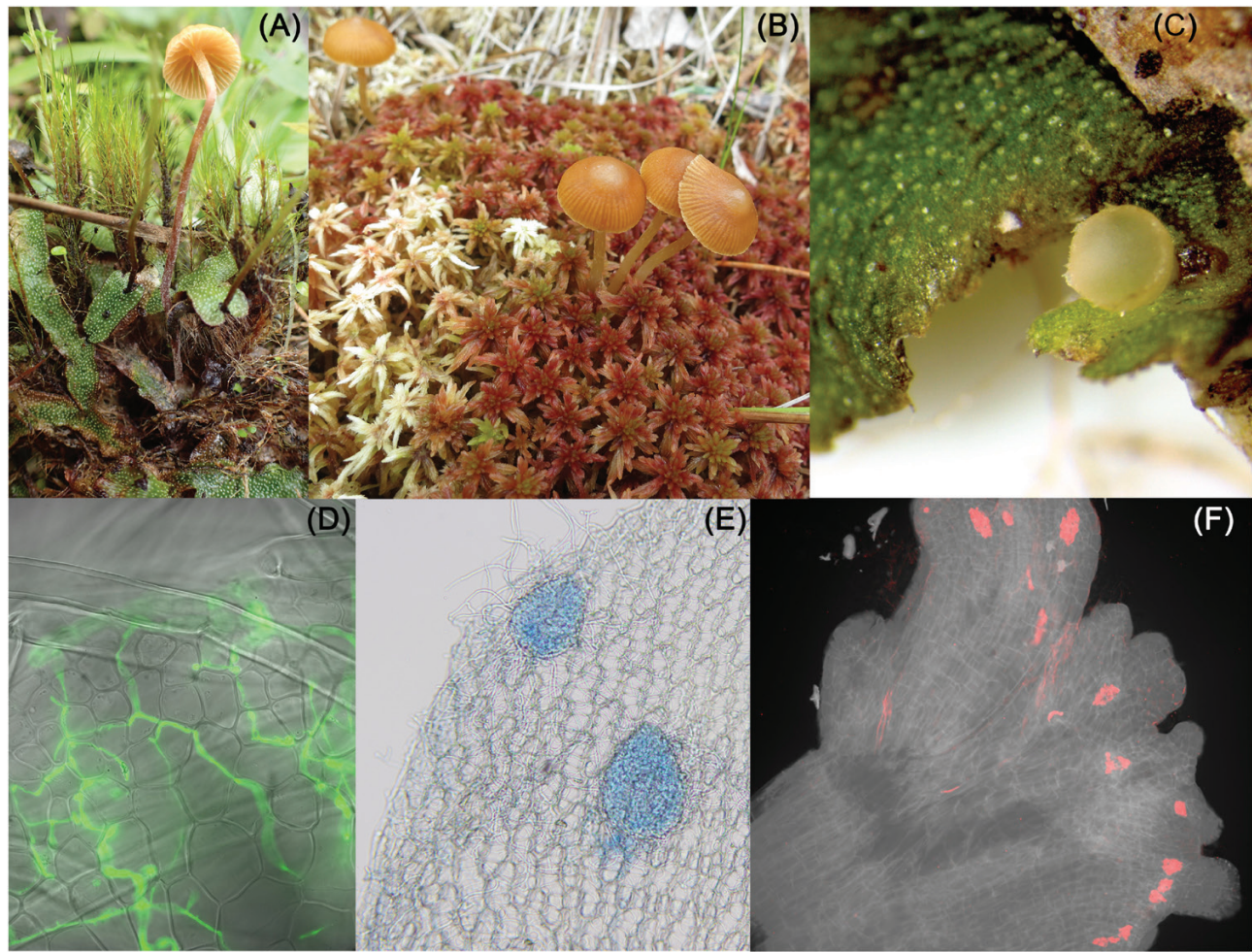


Fig. 1. Examples of bryophyte-microbe association. (A) Fungal fruiting body (*Galerina* sp., Basidiomycota) emerging from a liverwort thallus (*Marchantia* sp.). (B) Fungal fruiting bodies (*Galerina* sp., Basidiomycota) associated with moss gametophytes (*Sphagnum* sp.). (C) Apothecium associated with a liverwort thallus (*Marchantia polymorpha*). (D) Hyphae of endophytic fungi in liverwort thallus; green color, fluorescently stained fungal hyphae. (E) Microscopic fungal fruiting body (*Epibryon* sp.) associated with leaf of *Sphagnum* sp. (F) Colonies of cyanobacteria associated with a hornwort. Red color, cyanobacteria colonies.

stress, and accelerating growth (Porras-Alfaro and Bayman, 2011; Jat *et al.*, 2021; Chialva *et al.*, 2022).

The members of plant microbiota work in combination to impact host health and success. These interactions within plant microbiota have been demonstrated to change plant traits and responses to pathogens (Harrison *et al.*, 2021; Mahdi *et al.*, 2021). Plant microbes have additionally been shown to shape the diversity of plant communities so, by extension, they can help determine the composition of the whole ecosystem (Afkhani and Strauss, 2016; Aguilar-Trigueros and Rillig, 2016). Therefore, a view incorporating these complex microbiota is necessary moving forward in understanding plant physiology and ecology. This microbiota view of plants is becoming more prevalent and has now been adopted for research of the plant model *Arabidopsis thaliana* (Bergelson *et al.*, 2019) and for commercially important plants such as corn (Jat *et al.*, 2021), rice (Kim and Lee, 2020), and timber and crop trees

(Mercado-Blanco *et al.*, 2018; Terhonen *et al.*, 2019). The plant microbiome has now become an important target for developing more sustainable agricultural systems (Singh *et al.*, 2020; dos Santos and Olivares, 2021).

While studies with this microbiota context have been applied to non-seed plants to a lesser degree, bryophytes (mosses, liverworts, and hornworts) are also associated with diverse microbes (Fig. 1) and have been increasingly recognized as important models to reveal evolutionary patterns in plant function and symbiotic interactions (Wood *et al.*, 2000; Carella and Schornack, 2018; Delaux and Schornack, 2021; Naramoto *et al.*, 2022). The field of microbial interactions in bryophytes has traditionally focused on a limited number of specific symbiont types for each bryophyte group, for example the nitrogen-fixing symbiosis of cyanobacteria with hornworts or the mycorrhiza-like fungal associations in many liverworts. Microbial association research in mosses has traditionally focused more

on parasites and epiphytes (Davey and Currah, 2006; Warshan *et al.*, 2017) since mosses lack the morphologically obvious internal symbioses found in the other two groups. Some studies have begun to more broadly characterize bryophyte microbial diversity and currently focus on two key model genera: *Sphagnum* mosses (Bragina *et al.*, 2015; Kostka *et al.*, 2016; Carrell *et al.*, 2019) and *Marchantia* liverworts (Alcaraz *et al.*, 2018; Marks *et al.*, 2018; Nelson and Shaw, 2019; Poveda, 2020; Rich *et al.*, 2021).

In this scoping review, we seek to compile a holistic view of the existing bryophyte–microbe interaction research, integrating both studies that focus on individual symbionts and those expanding into broader microbiota contexts. We investigate research hot-spots and gaps in bryophyte taxa, microbial groups, and geographical areas. Based on this systematic review, we also discuss the current state of knowledge of microbiota in each of the three bryophyte groups, and highlight common themes across bryophyte microbe research.

Scoping review methods

Search strategy

We used a systematic search strategy to collect articles pertaining to relationships between bryophytes and microbes, including prokaryotes, eukaryotes, and viruses. We searched all databases on the Web of Science with terms to find bryophytes (moss OR mosses OR liverwort* OR hornwort* OR bryophyt*) in combination with terms about microbes (fung* OR bacteri* OR cyanobacteri* OR protist* OR virome OR microb*) and terms associated with symbiosis and microbiome studies (symbio* OR microbiome* OR microbiota OR microflora OR endophyt*). All three sets of terms were searched as topics connected by AND. The search was conducted on 22 July 2021. To fill in articles published in the rest of 2021, a second search was run on 7 January 2022 covering index dates from 22 July to 31 December 2021. These searches yielded a total of 1049 articles.

In this review we focus on the natural diversity of microbes belonging to modern bryophyte microbiota. We therefore excluded any studies that did not specifically sample live bryophyte tissues (e.g. only sampled soil or dead bryophytes) or for which the provided methods make this ambiguous. We also excluded studies about extinct members of bryophyte lineages and those that conducted experiments to test artificial associations with bryophytes, for example adding mycorrhizal inocula to a non-mycorrhizal bryophyte host. We included both studies that directly identified microbes and those that used an indirect method such as assessing their metabolic activity or chemical markers.

We checked the abstracts of all articles to assess their relevance for our topic. Then the main text of all the remaining articles was checked and data recorded from each relevant study. We removed reviews so the data presented below reflect only

primary research articles. In all, we found 266 relevant research articles (Fig. 2; Table S1 at https://github.com/koshroom/bryophyte-microbiome-review/blob/main/Table_S1.txt).

Data coding

For articles that fit our criteria, we recorded the type of study (e.g. diversity assessment or genetic experiment), notes about methodologies (e.g. whether culture morphologies or DNA sequencing were used to identify microbes), the bryophyte taxa studied, what tissues of the plants the microbes were found in, whether the microbes were epiphytes or endophytes, what types of microbes were targeted, and the location of sample collection (if relevant).

Study locations were grouped into climate zones according to the following latitudinal criteria when GPS coordinates were available (tropical, <23.5°; subtropical, 23.5–35°; temperate, 35–50°; subarctic and subantarctic, 50–66.5°; and arctic and antarctic, >66.5°). For those studies without GPS coordinates but with location names (e.g. a province), a google doc plugin ‘geocode’ was used to assign GPS coordinates. We manually checked geocode output to ensure the GPS coordinates matched the original locations. QGIS v3.16 (QGIS Development Team 2022) was used to produce maps.

Bryophyte taxa were grouped based on taxonomy in the Integrated Taxonomic Information System (ITIS) database. Numbers of genera for each bryophyte order were obtained from this database in order to calculate taxonomic coverage. Any genera that have been subsequently synonymized were updated. In the few cases that species were not in the ITIS database, the Global Biodiversity Information Facility (GBIF) taxonomy database was used to check whether the genus name was still correct. Phylogenetic relationships between the orders were based on the 2021 bryophyte phylogeny poster (Cole *et al.*, 2021) with information from additional bryophyte phylogeny research papers (Villarreal *et al.*, 2016; Liu *et al.*, 2019; Bonfim Santos *et al.*, 2021).

Microbial taxa were grouped into major categories. Prokaryotes were divided into two functional categories that were popular research topics, diazotrophs and methylotrophs (Box 1), and ‘other prokaryotes’ which includes all other studies focusing on bacteria or archaea. Eukaryotes were grouped into Mucoromycota, which includes the arbuscular mycorrhizal Glomeromycotina (Box 1), all other fungi, and ‘protists’ (i.e. other microbial eukaryotes). The category ‘mix’ was used for any study that investigated more than one of our designated categories.

Scoping review results

Geographic coverage

The studies show a strong bias towards the northern hemisphere (188 studies) compared with the southern hemisphere (36 studies) (Fig. 3). Studies in the northern hemisphere

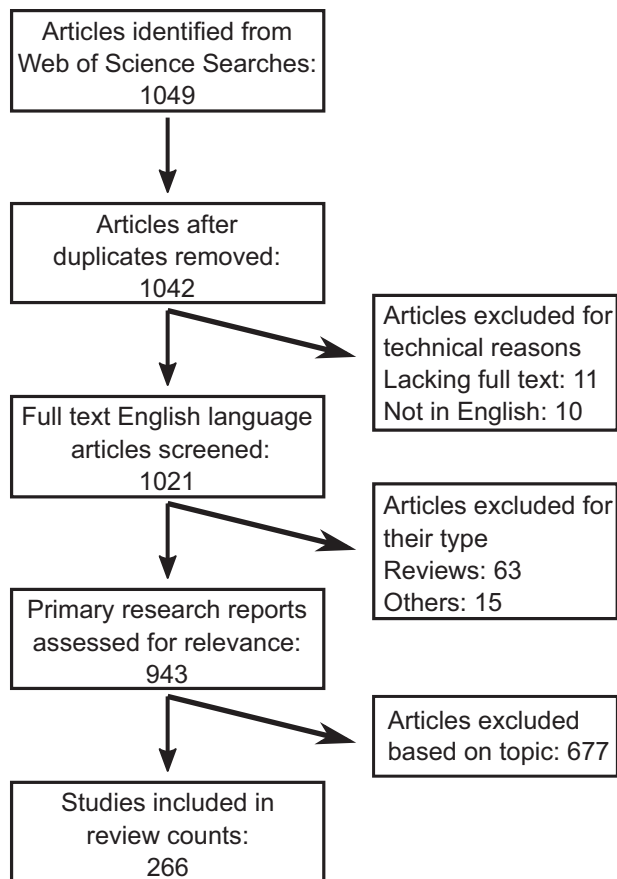


Fig. 2. Systematic search strategy and results.

concentrated in Europe, eastern North America, and east Asia (Fig. 3A). In the southern hemisphere, most studies sampled liverworts, with a particular concentration in New Zealand (Fig. 3A). Areas lacking studies include parts of central North America, the Amazon rainforest, most of Africa, most of Australia, the middle east, and Siberia (Fig. 3A).

For both liverworts and hornworts, the temperate zone has the highest number of studies (Fig. 3B). With >70 studies, the subarctic and arctic region is the most studied climate zone for moss (Fig. 3B). Among these moss studies, 23 had sampled *Sphagnum* and 10 sampled *Pleurozium*. Mosses have more studies than the other bryophyte groups in all climate zones except for the tropics which only has three studies (Fig. 3B). Despite the rising awareness of climate change and interest in high-latitude research, the arctic and antarctic regions have only 18 and 10 studies in mosses and liverworts, respectively. In the temperate zone liverworts were the most extensively sampled when considering the number of plant samples investigated (Fig. 3C). With 15 studies, liverworts were the most studied bryophyte type in the tropics (Fig. 3B).

Similar to our findings here, a recent plant endophyte review (Harrison and Griffin, 2020) also showed Europe as the research hotspot and the unbalanced sampling concentrated in

the northern hemisphere. However, the number of endophyte studies in 'tropical/subtropical wet broadleaf forest' was ranked third out of the 17 categories in Harrison and Griffin (2020) while the tropical area was the least studied climate zone for bryophyte-microbiota surveyed here (Fig. 3), highlighting the discrepancies of plant taxa sampling across climate zones.

Coverage of bryophyte taxa

Of the 266 articles we found, 33 (12%) included hornworts, 109 (41%) included liverworts, and 144 (54%) included mosses. Many articles only investigated one of the three bryophyte groups: 123 (46%) studied only mosses, 84 (32%) only liverworts, and 28 (11%) only hornworts (Fig. 4B).

The studies covered all 15 liverwort orders, four out of five hornwort orders, and 16 of the >30 moss orders (Fig. 4A). Many of the liverwort and hornwort orders have a high percentage of their genera studied for microbiome research. In contrast, the only moss orders with high generic coverage are the two studied orders containing single genera: Sphagnales and Takakiales.

Among hornworts, the Anthocerotales were represented most frequently (28) (Fig. 4A). This comes as no surprise since the model *Anthoceros punctatus* has been popular for studying cyanobacterial symbiosis for decades (Li et al., 2020) and thus appeared in 21 studies in our review, 64% of all studies that included hornworts.

For liverworts, the Marchantiales and Jungermanniales were most often studied. These two orders are some of the most genus rich among liverworts. However, genus number and studies do not correlate across all liverwort orders since Porellales has many genera but is only covered by four studies (Fig. 4A). The concentration of studies in Marchantiales is unsurprising since *Marchantia polymorpha* is the model liverwort. Eleven species of *Marchantia* are represented across 24 articles, and *M. polymorpha* specifically is studied in 17 of the total 31 articles investigating Marchantiales.

For mosses, the most studied orders by far are Sphagnales and Hypnales (Fig. 4A). There have been many resources devoted to studying *Sphagnum* because of the climatic importance of peatlands, and the research on the microbiome of this genus has been well reviewed elsewhere (Kostka et al., 2016). Hypnales is the order containing the majority of pleurocarpous mosses, with 204 genera. The 60 studies focusing on this order are dominated by those investigating *Hylocomium splendens* and *Pleurozium schreberi* (36 studies), feather mosses that are important hosts of nitrogen-fixing bacteria in boreal forests (Rousk et al., 2013b).

Many of the moss orders are not represented at all in the reviewed literature on microbiota (Fig. 4A). This is understandable for orders with low taxonomic diversity or limited habitat or geographic ranges. However, a couple of cases stand out where reasonably diverse and widespread groups have no or solitary microbiota studies: Orthotrichales and Hookeriales. Of these, perhaps

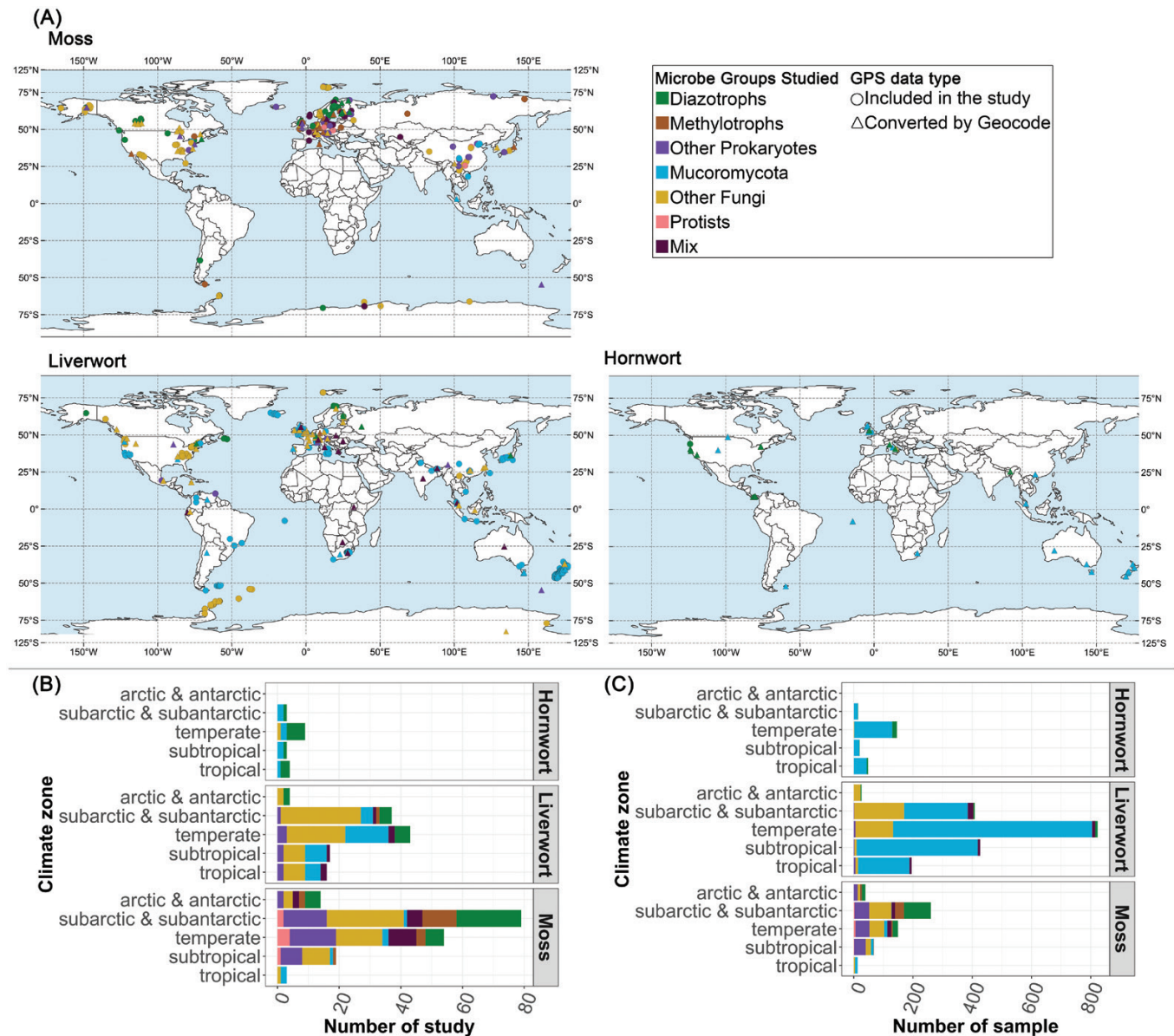
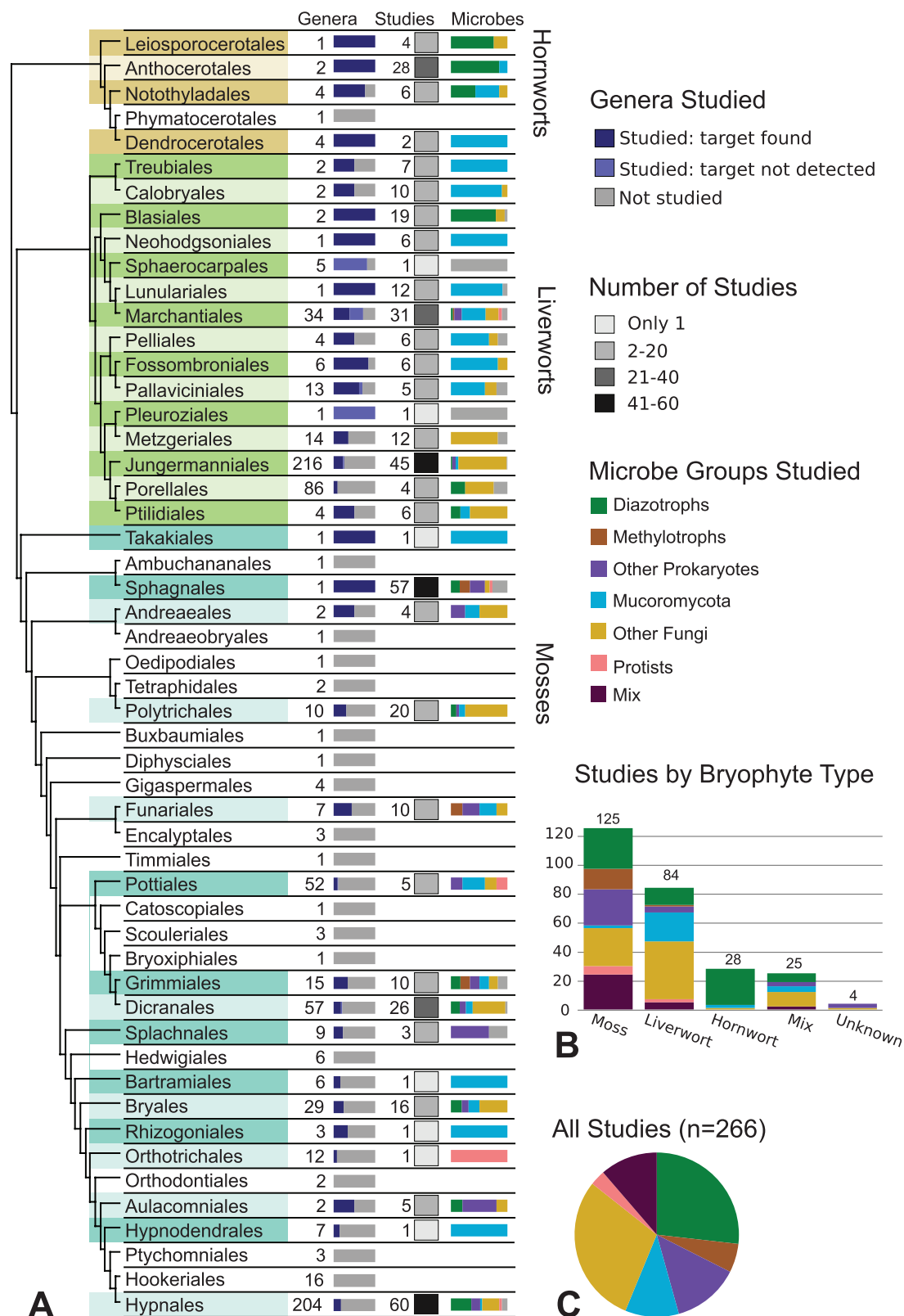


Fig. 3. Locations of bryophyte samples in the reviewed studies. (A) World map of bryophyte collections. Each dot corresponds to one bryophyte sample. The color code indicates the symbiont type studied for each sample. (B) Summary of studies across climate zones. (C) Summary of bryophyte samples across climate zones.

Hookeriales can be explained by having a more restricted habitat in humid forests (Pokorný *et al.*, 2012). For Orthotrichales, perhaps the lack of studies is related to these mosses often being epiphytic or epilithic, as many microbiota studies are interested in connections to the soil. The one recent study investigating Orthotrichales focused on protistal diversity, thereby adding a survey of understudied microbes, but not allowing for comparisons with many other studies (Walden *et al.*, 2021).

In a few cases, genera were studied without the target microbes being found. This was the case for three genera in Sphaerocarpaceae, 10 in Marchantiales, the only genus in Pleuro-

roziales, one in Pallaviciniales, four in Jungermanniales, and two genera in Dicraniales (Fig. 4A). Many of these genera have not been thoroughly investigated, with most of them only represented by one study. These studies employed various methods to detect microbial diversity including microscopy, culturing, and clone libraries (Box 1). All but one of the studies that report failing to find the target were searching for fungi, usually of a particular type, for example arbuscular mycorrhizal fungi. The remaining study was looking for cyanobacteria. Therefore, these negative results do not indicate that these plants lack microbiota generally.



Coverage of microbial partners

The majority of studies in hornworts focus on bacteria, particularly the nitrogen-fixing cyanobacteria that live in the thalli of these plants (Fig. 4A, B). A few studies explore fungi in hornworts. In liverworts, with the exception of Blasiales in which the two species host cyanobacteria as hornworts do, the focus is overwhelmingly on fungi (Fig. 4A, B). Many liverwort orders are dominated by studies of fungi in Mucoromycota, which includes the arbuscular mycorrhizal Glomeromycotina (Fig. 4A). The greatest variety of studies are for mosses, with studies focusing on Archaea, Bacteria, prokaryotes with particular metabolism (diazotrophs or methylotrophs), fungi, protists, and even one study on viruses. Studies on Mucoromycota are much rarer for mosses, which is in line with their loss of key symbiosis genes involved in forming mycorrhizal associations (Wang *et al.*, 2010; Radhakrishnan *et al.*, 2020).

Across all reviewed studies, articles that investigated fungi not specifically belonging to Mucoromycota ('other fungi') were most common (Fig. 4C). The next most common were studies focusing on diazotrophs (Fig. 4C). The focus on members of fungal microbiota is in line with the trend for plant endophyte research in general (Harrison and Griffin, 2020). The prominence of studies focused on diazotrophs reflects the focus on the very obvious cyanobacterial colonies in hornwort and Blasiales thalli and the epiphytic associations in feather mosses.

Geographically, the vast majority of studies using samples from the southern hemisphere investigated fungi (Fig. 3A). This is in line with the bias towards liverwort samples from this hemisphere since fungal associations have been the most studied for this group.

Location of symbionts in bryophytes

Many studies did not include information about where in the bryophyte host the microbes occurred, and many methods use whole plants, making it difficult to distinguish if the symbionts were in rhizoids, leaves, stems, etc. When specified, most studies used gametophyte tissues or, presumably, a mix of gametophyte and sporophyte. Few studies (Bragina *et al.*, 2012, 2013b; Vesty *et al.*, 2020) specifically separated sporophytes.

Studies did not always give enough methodological information to indicate whether the microbes were inside or on the surface of the plants, but there is still enough information on this to comment on some general trends. Only endophytic microbes were indicated for studies of hornworts. For liverworts, only one study specified that epiphytes were sampled; the majority studied endophytes. For mosses, studies of endophytes were also the most common, but 12 studies looked at epiphytes or a combination of both.

Development of the research field over time

The earliest articles in our review are from 1983, and arranging them by year shows that scientific interests on bryophyte-mi-

crobe interactions have shifted over the past 40 years (Fig. 5). Of the 35 articles from the 1980s and 1990s, many focused on cyanobacterial symbioses with hornworts or *Blasia* (19 studies). The others investigated mycorrhiza-like fungal associations, fungal parasites, and diazotrophs on other hosts (Fig. 5). These studies use microscopy, genetics, functional, and field experiments to investigate interactions, mostly between individual hosts and symbionts. Between 1990 and 2010, with >40 studies, liverworts were the most studied group, especially for fungi (Fig. 5). In addition to mycorrhiza-like fungal associations, thallus-inhabiting endophytic fungi started to gain attention. In the early 2000s, studies used direct Sanger sequencing (Costa *et al.*, 2001), culture collections (Opelt *et al.*, 2007; Davis and Shaw, 2008; Rikkinen and Virtanen, 2008; U'Ren *et al.*, 2010), and clone libraries (Kausarud *et al.*, 2008) to begin describing the diversity of fungal and bacterial communities in mosses and liverworts.

Studies on moss increased dramatically after 2010, probably due to the rising awareness of climate change. In 2020 alone, >20 studies were published on diverse moss-microbe interactions (Fig. 5). While hornworts received proportionally more attention early on, relevant studies have not increased in number like those for liverworts and mosses, a trend that is especially clear after 2010 (Fig. 5). This explosion of research in the last decade was also probably caused in part by new technologies. Studies using 454 pyrosequencing began to appear in 2011 (Kip *et al.*, 2011; Bragina *et al.*, 2013a; Davey *et al.*, 2013; Park *et al.*, 2013; U'Ren *et al.*, 2014), and studies using Illumina sequencing for metagenomic and amplicon studies began appearing in 2014 (Bragina *et al.*, 2014; Knack *et al.*, 2015; Graham *et al.*, 2017). In the last few years, the diversity of sequencing methods has continued to expand, now also including metatranscriptomes (Stough *et al.*, 2018; Chen *et al.*, 2019) and newer sequencing technologies such as PacBio long read sequencing (Nelson *et al.*, 2021a).

Research themes and current state of knowledge

In the following subsections, we summarize the current state of knowledge for prokaryotic and eukaryotic microbiota in each of the three bryophyte groups. We then highlight some common themes across multiple groups.

Mosses

Prokaryotes

Of all moss genera, *Sphagnum*, which makes up a significant portion of the essential carbon stock in northern peatlands, is the most studied system concerning microbiome and nutrient cycling (Kostka *et al.*, 2016). Northern peatlands contribute profoundly to global methane emission, probably owing to archaea in the anaerobic environments associated with submerged plants and soil (Lai, 2009; Kostka *et al.*, 2016).

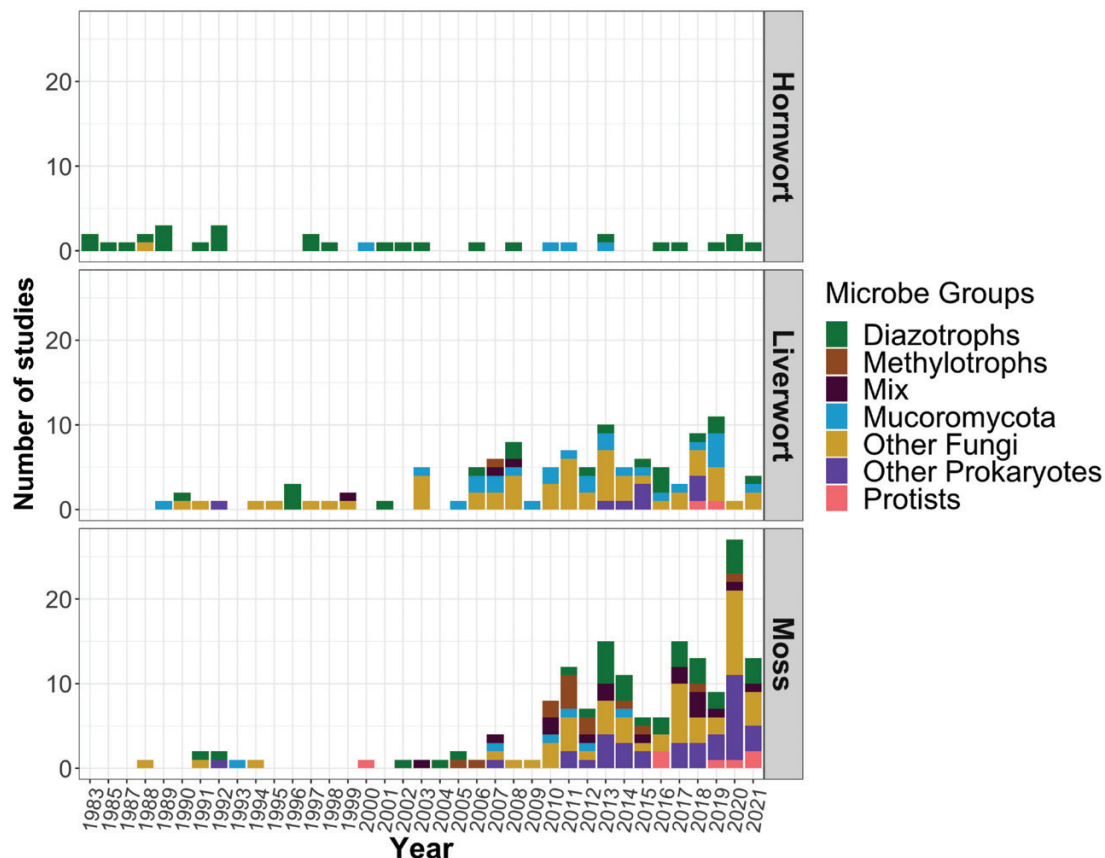


Fig. 5. Distribution of the reviewed studies over time by bryophyte type. Each bar corresponds to the number of studies per year. The color corresponds to the studied symbiont type.

Sphagnum plants harbor diverse methanotrophs and diazotrophs in their non-photosynthetic hyaline cells and the tissue surface (Kostka *et al.*, 2016). The carbon dioxide generated through methanotrophs' methane oxidation can be used by *Sphagnum*'s photosynthesis, completing the methane recycling cycle (Raghoebarsing *et al.*, 2005; Kip *et al.*, 2010). While cyanobacteria are often the dominant microbial group with nitrogen-fixing potential in *Sphagnum* microbiomes, members of Rhizobiales (Alphaproteobacteria), such as the methanotrophs of *Methyloferula*, have been shown to be the main nitrogen fixers in the bog system (Leppanen *et al.*, 2013; Warren *et al.*, 2017; Kolton *et al.*, 2022). Microbiota-assimilated carbon and nitrogen can influence both local and global nutrient cycling.

Recently, studies have aimed to identify key abiotic and biotic factors affecting *Sphagnum* microbiome assembly. *Sphagnum* species have been shown to play a more significant role in shaping the bacterial community compared with environmental factors (Bragina *et al.*, 2012, 2013a; Kox *et al.*, 2020; Tveit *et al.*, 2020). However, abiotic factors such as warming and hydrology can also have a significant impact on prokaryotes of *Sphagnum* (Carrell *et al.*, 2019; Basińska *et al.*, 2020). Plant traits and compartments harbor distinct microbiomes, such as epiphytic and endophytic space, photosynthetic and senescent parts, and sporophytes and gametophytes

(Bragina *et al.*, 2012, 2013a; Tian *et al.*, 2019, 2020). The interaction between *Sphagnum* and the associated microbes also depends on a variety of chemical and metabolic features. For example, Carrell *et al.* (2021) showed that the *Sphagnum*–cyanobacteria symbiosis was pH dependent. They also identified trehalose to be the main carbohydrate released by *Sphagnum*; in exchange, purines and amino acids were released by cyanobacteria. Vesty *et al.* (2020) showed that moss-associated bacteria probably synthesize chemical components that promote moss spore germination. More recently, a metagenomic dataset of *Sphagnum* bacteria highlighted signals of plant–microbe co-evolution in such ecosystems (Wicaksono *et al.*, 2021).

In addition to the bog ecosystem, mosses in boreal forests and tundra are of primary interest, partly due to limited nitrogen resources and concerning climate conditions (Fig. 4A). Unlike hornworts and some liverworts, the cyanobacteria of mosses are generally epiphytic and do not use specialized cavities to contain cyanobacteria. Nitrogen fixing of moss-associated cyanobacteria can contribute significantly to nitrogen content in boreal forests (Rousk *et al.*, 2013a) and tundra (Rousk and Michelsen, 2017). While less studied, the terricolous and epiphytic bryophytes in temperate forests and subtropical cloud forests also contribute to nitrogen fixation, but their net nitrogen input remains to be clarified (Han *et al.*, 2010;

Lindo and Whiteley, 2011; Deane-Coe *et al.*, 2016; Markham and Fernández-Otárola, 2021; Fan *et al.*, 2022). Nitrogen-fixing ability and distribution of cyanobacteria can vary across moss species, moss color (e.g. greenness), and carbon to nitrogen ratio of plant tissues, and differ according to the senescence variation (Chen *et al.*, 2019; Darnajoux *et al.*, 2019). By studying symbiotic versus non-symbiotic *Nostoc* strains, Warshan *et al.* (2017) showed that nitric oxide and aliphatic sulfonates are currencies in establishing feather moss–*Nostoc* symbiosis. Opposite to what was observed in the *Sphagnum*-dominated ecosystem that detected an increase of Nostocales after warming, declined Nostocales was detected in *Racomitrium lanuginosum* of arctic tundra (Klarenberg *et al.*, 2021).

Compared with high latitudinal areas, studies on moss microbiomes in lower latitudinal areas are scarce. Cao *et al.* (2020) revealed that moss species are the main drivers of the bacterial community in karst rocky desertification regions. In the montane cloud forest of subtropical regions, research showed the high nitrogen fixation rate of epiphytic moss in the rainy season compared with the dry season (Fan *et al.*, 2022).

Fungi, other eukaryotes, and viruses

Unlike all other major plant lineages, mosses are not generally considered to form typical mycorrhiza-like associations. Arbuscular mycorrhizal structures (e.g. vesicles, spores, or hyphal coils) have been reported from some mosses (Zhang and Guo, 2007), and *Takakia* is reported to form mycorrhizae (Wang and Qiu, 2006), but whether mycorrhizal fungi can establish symbiosis with moss alone (i.e. without a nearby potential plant host), and whether there is a reciprocal nutrient exchange between mosses and mycorrhizal fungi, remain unclarified. Comparative plant genomics have revealed the presence of mycorrhizal symbiotic genes in moss genomes (Delaux *et al.*, 2013; Radhakrishnan *et al.*, 2020), suggesting the mycorrhizal potentials or the neofunctionalization of these genes in mosses. While ectomycorrhizal fungi (Box 1) are detectable in the senescent or rhizoid part of mosses, these ectomycorrhizal fungi are likely to be associated with nearby tree hosts. By an isotopic tracing technique, Carleton and Read (1991) showed that ectomycorrhizal fungi might be saprotrophic on mosses, decomposing and scavenging for carbon and phosphorus to deliver to their mycorrhizal tree hosts.

Although the mycorrhizal status of mosses remains unclear, mosses harbor diverse endophytic and epiphytic fungal communities. Compared with prokaryotic community composition, which often changes drastically in the face of environmental alterations such as warming and drought, fungal community compositions are relatively static (Carrell *et al.*, 2019; Cao *et al.*, 2020). Mosses' fungal communities have been shown to be stratified by plant senescent conditions (Davey *et al.*, 2017; Lamit *et al.*, 2017; Chen *et al.*, 2018). The fungal community was also largely determined by host identity, but elevational patterns and seasonal turnover were detected (Davey *et al.*, 2012, 2013). Compared with the fungal endophyte com-

munity of co-occurring vascular plants, the endophytic fungal community of moss more closely resembles those of lichens (U'Ren *et al.*, 2010).

Besides plant–fungal interactions, microbe–microbe interactions have also been detected in mosses. Chen *et al.* (2022) revealed that *Paenibacillus* bacteria were co-isolated with the endophytic fungi in *Dicranum scoparium*. The hyphae of the ascomycetous fungus *Trizodia acrobata* surrounded *Nostoc* on and inside *Sphagnum*, suggesting potential tripartite interactions (Stenroos *et al.*, 2010). The often overlooked moss–fungal association is linked with nearby vegetation, microbes, and nutrient transfers, presenting a wealth of interactions that await discovery.

Mosses are the most well-studied bryophytes for eukaryotic microbes. Feng *et al.* (2016) detected that some moss-associated algae had lost their ability to form exterior scales, probably because the moss can provide physical protection. Protists can be phototrophs, predators, and saprotrophs in the food web of the bog system (Jassey *et al.*, 2013, 2015; Lizonova *et al.*, 2019). Jassey *et al.* (2015) carried out warming experiments and revealed drastic reduction of the dominant mixotrophic protists in response to warming, leading to a reduction of total carbon production. Finally, moss represents the only bryophyte lineages with viruses reported. A high abundance of viruses was revealed from a *Sphagnum* bog, highlighting *Sphagnum* and other mosses as future resources to unearth virus diversity (Quaiser *et al.*, 2015; Stough *et al.*, 2018).

Liverworts

Prokaryotes

In contrast to the research in mosses, prokaryotic members of liverwort microbiomes have received much less attention than fungi. The main exception is in *Blasia pusilla* and *Cavicularia densa* which form a nitrogen-fixing symbiosis with cyanobacteria in specialized cavities (Rikkinen and Virtanen, 2008). Diazotrophs without these specialized associations have been found on *Frullania asagrayana* (Cornejo and Scheidegger, 2016), *Ptilidium ciliare* (Holland-Moritz *et al.*, 2021), and *M. polymorpha* (Deane-Coe and Sparks, 2016). Methylophiles can also be found on liverworts and have been shown to promote thallus growth in *M. polymorpha* (Kutschera *et al.*, 2007). Only a few studies have surveyed prokaryotic microbiomes of liverworts more broadly, focusing mostly on the Marchantiales (Knack *et al.*, 2015; Koua *et al.*, 2015; Alcaraz *et al.*, 2018; Marks *et al.*, 2018).

Fungi and other eukaryotes

Fungal associations have been the main focus of microbiome research for liverworts, especially as an evolutionary comparison with mycorrhizae in vascular plants. As such, arbuscular mycorrhizal fungi in the Mucoromycota have received the most attention, followed by Ascomycete fungi that form ericoid mycorrhizae (Box 1) and Basidiomycete fungi of the

Sebacinales which can form a wide variety of mycorrhizae (Kottke and Nebel, 2005; Pressel *et al.*, 2010; Weiss *et al.*, 2011). Since the model liverwort *M. polymorpha* has lost key parts of the genetic system used for mycorrhizal symbiosis (Radhakrishnan *et al.*, 2020), other thalloid liverworts have been developed as models for liverwort mycorrhizae instead, including *Marchantia paleacea* (Humphreys *et al.*, 2010; Radhakrishnan *et al.*, 2020; Rich *et al.*, 2021) and *Lunularia cruciata* (Fonseca *et al.*, 2008, 2014; Delaux *et al.*, 2012b; Nobre *et al.*, 2013; Kamel *et al.*, 2017). Experiments have demonstrated that liverworts can exchange nutrients mutualistically with their Mucoromycota and Ascomycota fungi, with the plant exchanging photosynthates for phosphorus or nitrogen (Humphreys *et al.*, 2010; Kowal *et al.*, 2018; Field *et al.*, 2019; Rich *et al.*, 2021). Fungi of Basidiomycota probably have a similar role, but this has not yet been functionally demonstrated (Duckett *et al.*, 2006). In addition, the liverwort *Aneura mirabilis* is the only known example of a mycoheterotrophic bryophyte and has accordingly been studied for its parasitic relationship with fungi (Duckett *et al.*, 1990; Bidartondo *et al.*, 2003). Despite this mycorrhizal focus for the field, a recent review of mycorrhizal associations in bryophytes and lycophytes estimated that fewer than 30% of liverwort species actually engage in these nutritional symbioses (Rimington *et al.*, 2020).

Studies investigating liverwort fungi more generally indicate that these plants can also host a diverse array of endophytes that do not form mycorrhiza-like structures, with dozens of fungal taxa sharing space in a single plant (Davis *et al.*, 2003; Davis and Shaw, 2008; Nelson and Shaw, 2019). However, the impacts of these other fungi on their liverwort hosts have only begun to be tested (Nelson *et al.*, 2018).

Outside of fungi, the only eukaryotic microbes that have been studied in liverworts are oomycetes (Turnau *et al.*, 1999; Carella *et al.*, 2018, 2019). A study using general eukaryote primers does give a glimpse of a potentially rich protist community beyond fungi and oomycetes inhabiting *M. polymorpha*, including green algae and Alveolates (Nelson *et al.*, 2019), but no studies to date have specifically targeted protist diversity in liverworts.

Hornworts

Prokaryotes

All hornworts host nitrogen-fixing cyanobacteria in their thalli (Adams and Duggan, 2008), so this symbiosis has been the focus of most microbial interaction research in hornworts. The cyanobacteria found in hornworts (and liverworts) usually belong to Nostocales and live in specialized cavities formed in the plant thalli (Adams and Duggan, 2008). Hornworts release an as yet unidentified compound that induces free-living cyanobacteria to differentiate into motile hormogonia (Box 1; Adams and Duggan, 2008). These filaments are attracted to the slime cavities of the hornwort (channels in *Leiosporoceros dussii*) by chemotaxis (Adams and Duggan, 2008). Once inside a

cavity, the cyanobacteria are induced to make a high density of nitrogen-fixing heterocysts (Box 1) and the plant supports their specialization in nitrogen fixation by providing carbon compounds to compensate for reduced photosynthesis (Adams and Duggan, 2008).

Members of the genus *Anthoceros* in particular have been used as laboratory models for this interaction. More is known about the bacterial genetics and physiology in the association since the cyanobiont has been easier to manipulate (Steinberg and Meeks, 1991; Campbell *et al.*, 1998, 2003; Wong and Meeks, 2002; Ekman *et al.*, 2013), but transformation systems for hornworts have recently been developed (Frangedakis *et al.*, 2021b; Neubauer *et al.*, 2022) so the coming years should reveal the plant side of the equation in more detail. In addition to functional studies in the laboratory, the diversity of cyanobionts has been explored by various culture-based and genetic methods (West and Adams, 1997; Costa *et al.*, 2001; Bouchard *et al.*, 2020; Nelson *et al.*, 2021b). However, only one study so far has investigated the broader bacterial community in a hornwort, *Leiosporoceros dussii* (Bouchard *et al.*, 2020).

Fungi and other eukaryotes

Like liverworts, hornworts also form mycorrhiza-like associations with fungi and have conserved symbiosis genes (Wang *et al.*, 2010). In hornworts, the studies on fungal associations have been more limited and have only addressed Mucoromycota fungi (Schussler, 2000; Desiro *et al.*, 2013), and functional or mechanistic studies for this symbiosis are still lacking. A few eukaryotic algae have been isolated from hornworts when cyanobacteria were the target (Yang *et al.*, 2021) but, to our knowledge, no research has yet been published to assess the diversity of eukaryotic microbes that might be found in hornworts.

The role of microbial symbiosis in plant evolution and genetics

The fact that the divergence of bryophytes from the rest of land plants is the most ancient plant phylogenetic split has prompted an increasing interest in the fields of plant physiology, genetics, cell biology, and symbiosis in studying bryophyte models in order to compare them with vascular models. Symbioses, especially with fungi, are of particular interest since they were probably key in the terrestrialization of plants (Delaux *et al.*, 2012a). While simply comparing *M. polymorpha* or *Physcomitrium patens* with *A. thaliana* does not say something about plant evolution or the distinctions between early and modern plants (as is often mistakenly stated), it can suggest conservation over evolutionary time (McDaniel, 2021). Comparative genetics and genomics, along with mutant rescue experiments, across a larger number of plant lineages can more fully demonstrate such conservation, or lack thereof. These approaches have been applied to the genes involved in mycorrhizal symbioses and immunity in vascular plants to reveal patterns of conservation, co-option, and convergence, with the benefits

of symbiotic interactions shaping plant evolution (Delaux and Schornack, 2021). Liverworts and hornworts retain very conserved genes that mediate mycorrhizal symbioses in vascular plants, and both bryophyte groups form mycorrhiza-like associations (Wang *et al.*, 2010; Radhakrishnan *et al.*, 2020; Delaux and Schornack, 2021).

Trophic status of bryophyte fungi

Many fungi inhabiting mosses and liverworts are only known to be endophytes without harmful effects on their bryophyte hosts. However, many species of bryophilous fungi that fruit on liverworts or mosses have also been identified. These have generally been classified as parasites. Examples include the Ascomycetes *Mniaecia jungermanniae* (Pressel and Duckett, 2006) and *Epibryon* (Stenroos *et al.*, 2010; Döbbeler and Hertel, 2013), and the Basidiomycete *Galerina* (Redhead, 1981; Davey and Currah, 2006). The hypothesis of parasitism has only been tested in a few studies investigating trophic modes of fungi associated with bryophytes. Biotrophic growth similar to mycorrhizal fungi and saprotrophic behavior have both been supported for a variety of fungi on mosses and liverworts using stable isotope (Box 1) and transcriptomic analyses (Korotkin *et al.*, 2018; Chen *et al.*, 2022). Fungi known from other hosts and habitats are frequently detected in bryophytes. Some species that can be severe pathogens in vascular plants show up as endophytes in bryophytes (Nelson and Shaw, 2019). Fungi that only fruit after wildfire (e.g. *Pholiota carbonaria*) were reported to be hidden in mosses as endophytes as part of their life cycle (Raudabaugh *et al.*, 2020, 2021). In Antarctica, a browning ‘fairy ring’ symptom at the center of moss patches was reported, and diverse fungi known to be endophytes or pathogens of other plants were isolated from the symptomatic moss tissues (Rosa *et al.*, 2020).

Bioprospecting and applications

Some bryophyte endophytic fungi have also been explored for bioprospecting, investigating compounds that are toxic to cancer cells (Guo *et al.*, 2008; Wei *et al.*, 2015; Xie *et al.*, 2015; Ali *et al.*, 2017; Anaya-Eugenio *et al.*, 2019), antifungal (Guo *et al.*, 2008; Peng *et al.*, 2012), immunosuppressant (Song *et al.*, 2013), anti-inflammatory (Wang *et al.*, 2021), and herbicidal (Jiao *et al.*, 2013). Some bacterial symbionts have also been tested and show antifungal properties (Opelt and Berg, 2004). The genes revealed by metagenomics in moss-associated bacterial communities hint at a variety of potentially useful metabolic abilities (Bragina *et al.*, 2014; Mueller *et al.*, 2015). In addition, bacteria isolated from *Sphagnum* show promise for biotechnology applications (Stepniewska and Kuzniar, 2014). Bryophyte microbiomes may also be applied in agriculture. Liverwort-associated fungi have been shown to be a natural mycorrhizal inoculum for vascular plants (Kowal *et al.*, 2016), and the abundant cyanobacteria of bryophytes can fix nitrogen and be used as a natural fertilizer (Pathak *et al.*, 2018).

Recommendations for future directions

This review brings into focus a wide range of open questions and directions for the growing field of bryophyte microbiota research.

Bryophyte host locations and habitats

Bryophyte–microbe relationships are known to be important for ecosystem function in certain cases, most notably in northern *Sphagnum* bogs (Bragina *et al.*, 2014) and boreal forest moss mats (Rousk *et al.*, 2013b). Research on such ecological roles has been limited to only a few habitats, and knowledge of bryophyte microbiota is very biased toward the northern temperate zone in general. Concerns for climate change have stimulated bryophyte–microbe research to concentrate on higher latitudinal regions. Despite the mountainous regions in the subtropical and tropical areas also being bryophyte-rich ecosystems (e.g. cloud forest or alpine tundra) that are vulnerable to climate change, these ecosystems were scarcely studied for bryophyte microbiota (Sheldon, 2019). To increase knowledge of diversity, core microbiota, and ecological functions, more studies should investigate habitats in the tropics and southern hemisphere. This of course must be done with care, involving, and ideally led by, researchers and local communities in the sampling locations to ensure this geographic expansion of knowledge is not exploitative.

On the smaller scale, bryophytes’ lack of roots allows them to inhabit a wider range of substrates than vascular plants. However, this diversity and its possible impacts on the microbiota have not been well explored. The best studied bryophyte microbiota belong to terricolous taxa such as boreal feather mosses, *Sphagnum*, *Marchantia*, and *Anthoceros*. In fact, even the microbiota of epiphytic vascular plants have been relatively ignored (Eskov *et al.*, 2021; Li *et al.*, 2021). The diversity of bryophyte substrates and the occurrence of closely related bryophyte taxa on different substrates present good opportunities to investigate the assembly of plant microbiota and how their functions may contribute to plant adaptation to different habitats.

Diversity, functions, and interactions of bryophyte microbiota

A small percentage of our reviewed studies investigate both bacterial and fungal diversity, and very few venture to investigate archaea, non-fungal eukaryotic microbes, or viruses. This reflects trends in microbiota research in general, but evidence is mounting that other types of microbes are important in plant microbiota (Moissl-Eichinger *et al.*, 2018; Xiong *et al.*, 2020; Lee and Ryu, 2021). Future studies of bryophyte microbes should more often look beyond the standard symbionts, for example non-diazotroph bacteria, non-mycorrhizal fungi, or protists, to discover the functions of these other

microbes and reveal possible interactions between obvious symbionts and other microbial partners. Studies in vascular plants indicate that interactions between different microbial taxa may hold the key to regulating the health of the plant host (Durán *et al.*, 2018; Griffiths *et al.*, 2020). Improving methodologies and a growing community of researchers should allow more studies and collaborations that investigate how the diversity and functions of different taxa and guilds of microbes interact in bryophyte microbiota. Further development of laboratory gnotobiotic systems (sterile plants with known microbes) for bryophytes (Kutschera, 2007; Nelson *et al.*, 2018) and increased use of transcriptomics, metabolomics, and systems biology methods will support investigations of the complex interactive functions of bryophyte microbes (Warshan *et al.*, 2017; Chen *et al.*, 2019; Carrell *et al.*, 2021; Frangedakis *et al.*, 2021a).

Microbiota in different life stages and tissue types

The existing literature overwhelmingly sampled bryophyte gametophytes, usually the shoots of moss or the thallus of hornworts and liverworts, because these structures are the most conspicuous and persisting tissues of bryophytes in nature. However, to understand how bryophyte-associated microbes disperse and establish in nature, we need to sample sporophytes and other bryophyte tissues. While many of the bryophytes are believed to acquire their microbes from the environment, studying microbes of reproductive propagules, including spores, gemmae, and the structures containing them (e.g. the capsule and gemma cup), can clarify if bryophyte-associated microbes can be transmitted vertically (Bragina *et al.*, 2013b).

Some gametophytes have a prolonged protonema stage which exists as undifferentiated filaments. Knowledge of the protonema-associated microbiota can provide critical information regarding how bryophytes adapt to the environment and if microbes can trigger bryophyte development (Wiklund and Rydin, 2004; Carella *et al.*, 2018; Vesty *et al.*, 2020). At the gametophyte stage, many bryophytes have specific tissue types such as elongated rhizomes, tomentous layers, and parallel lamellae which have the potential to host specific microbes (Kausrud *et al.*, 2008). Because of the general small size of bryophytes, sampling specific tissue for microbial examination has been difficult. With the aid of advanced microscopy, such as laser dissecting microscopes and the ever-decreasing tissue quantity requirement for HTS library preparation, in-depth studies on different life stages and distinct tissue types are possible now and will probably uncover novel bryophyte–microbe associations. An integral view of what microbes are shared among various life stages and tissues can provide insights on the core microbiota of bryophytes and the different ecological roles in which each microbe may engage.

Finally, study designs that allow separation of endophytes and epiphytes can help to better understand the functions of these two groups of microbes. The rhizosphere microbiome of

vascular plants has been well studied, revealing many complex interactions that are key to plant health (Mendes *et al.*, 2013). While bryophytes lack roots and therefore have less of a connection to their substrate microbiota, it is likely that these external microbes are still key in bryophyte ecology.

Microbiota responses to bryophyte physiology

Many bryophytes are poikilohydric and very desiccation tolerant, being able to withstand far more extreme drying than drought-tolerant vascular plants (Proctor *et al.*, 2007). At the very least, microbes living in bryophytes must be able to tolerate the extreme changes in hydration, but may also contribute to plant resilience to these changes. Little work exists thus far on this topic, but metagenome work in *Sphagnum* does indicate a wide range of potential microbial functions that could act to support the moss during changes such as drying (Bragina *et al.*, 2014).

Bryophytes are also popular bioindicators for pollutants, especially heavy metals, and many have high metal tolerance (Stanković *et al.*, 2018). In bioremediation efforts using vascular plants, involving the microbiota has been suggested as a way to optimize the process (Thijs *et al.*, 2016). Studies in vascular plants indicate that microbiota respond to metal stress and can help plants tolerate it (Navarro-Torre *et al.*, 2017; Guarino *et al.*, 2020; Zadel *et al.*, 2020). Bryophyte microbiota studies on metal-tolerant host species could reveal how microbes and hosts may cooperate to tolerate abiotic stresses.

Conclusion

While there is still much work left to be done on individual symbionts, the field can also move forward by studying bryophytes from a broader microbiota perspective. The investigated connections of bryophyte microbes to broader ecosystem functioning have so far been limited in taxa, habitat, and geographic location. More studies of bryophyte physiology and ecology should take their microbiota into account. This should include reductionist methods such as gnotobiotic laboratory experiments and field studies that investigate multiple microbial taxa and functional guilds, and will be helped by the growing ease of HTS and other omics methods.

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Author contributions

JN and K-HC jointly carried out this systematic review, including conceptualization, data curation, analysis, visualization, and writing.

Conflict of interest

The authors declare no conflicts of interest.

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Data availability

Table S1 and full-size images of Fig. 3A are available at <https://github.com/koshroom/bryophyte-microbiome-review/tree/main>.

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