



## Predicting the structure and functions of peatland microbial communities from Sphagnum phylogeny, anatomical and morphological traits and metabolites

Anna Sytiuk, Régis Céréghino, Samuel Hamard, Frédéric Delarue, Amélie Guittet, Janna Barel, Ellen Dorrepaal, Martin Küttim, Mariusz Lamentowicz, Bertrand Pourrut, et al.

### ► To cite this version:

Anna Sytiuk, Régis Céréghino, Samuel Hamard, Frédéric Delarue, Amélie Guittet, et al.. Predicting the structure and functions of peatland microbial communities from Sphagnum phylogeny, anatomical and morphological traits and metabolites. *Journal of Ecology*, 2021, 10.1111/1365-2745.13728 . hal-03373855

**HAL Id: hal-03373855**

**<https://hal.science/hal-03373855>**

Submitted on 15 Nov 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1

2   **Predicting the structure and functions of peatland microbial communities**  
3   **from *Sphagnum* phylogeny, anatomical and morphological traits and**  
4   **metabolites**

5   Anna Sytiuk<sup>1\*</sup>, Regis Céréghino<sup>1</sup>, Samuel Hamard<sup>1</sup>, Frédéric Delarue<sup>2</sup>, Amélie Guittet<sup>2</sup>, Janna M.  
6   Barel<sup>1</sup>, Ellen Dorrepaal<sup>3</sup>, Martin Küttim<sup>4</sup>, Mariusz Lamentowicz<sup>5</sup>, Bertrand Pourrut<sup>1</sup>, Bjorn J.M.  
7   Robroek<sup>6,7</sup>, Eeva-Stiina Tuittila<sup>8</sup>, Vincent E.J. Jassey<sup>1</sup>

8   <sup>1</sup>Laboratoire Ecologie Fonctionnelle et Environnement, Université de Toulouse, UPS, CNRS, Toulouse,  
9   France,

10   <sup>2</sup>Sorbonne Université, CNRS, EPHE, PSL, UMR 7619 METIS, 4 place Jussieu, F-75005 Paris, France

11   <sup>3</sup>Climate Impacts Research Centre, Department of Ecology and Environmental Science, Umeå University,  
12   SE-981 07, Abisko, Sweden,

13   <sup>4</sup>Institute of Ecology, School of Natural Sciences and Health, Tallinn University, Uus-Sadama 5, 10120  
14   Tallinn, Estonia,

15   <sup>5</sup>Climate Change Ecology Research Unit, Faculty of Geographical and Geological Sciences, Adam  
16   Mickiewicz University in Poznań, Bogumiła Krygowskiego 10, 61-680 Poznań, Poland,

17   <sup>6</sup>Aquatic Ecology & Environmental Biology, Institute for Water and Wetland Research, Faculty of Science,  
18   Radboud University Nijmegen, AJ 6525 Nijmegen, The Netherlands,

19   <sup>7</sup>Biological Sciences, Faculty of Natural and Environmental Sciences, Institute for Life Sciences, University  
20   of Southampton, Southampton SO17 1BJ, UK

21   <sup>8</sup>School of Forest Sciences, Joensuu campus, University of Eastern Finland, Finland.

22

23   \*corresponding author: [anna.sytiuk@univ-tlse3.fr](mailto:anna.sytiuk@univ-tlse3.fr)

## Abstract

1. *Sphagnum* mosses are keystone species in northern peatlands. Notably, they play an important role in peatland carbon (C) cycling by regulating the composition and activity of microbial communities. However, it remains unclear whether information on *Sphagnum* phylogeny and/or traits-based composition (*i.e.* anatomical and morphological traits and metabolites) can be used to predict the structure of microbial communities and their functioning. Here we evaluated whether *Sphagnum* phylogeny and traits predict additional variation in peatland microbial community composition and functioning beyond what would be predicted from environmental characteristics (*i.e.* climatic and edaphic conditions).
2. We collected *Sphagnum* and microbial data from five European peatlands distributed along a latitudinal gradient from northern Sweden to southern France. This allowed us to assess *Sphagnum* anatomical and morphological traits and metabolites at different sites along changing environmental conditions. Using structural equation modelling (SEM) and phylogenetic distance analyses, we investigated the role of *Sphagnum* traits in shaping microbial community composition and functioning along with environmental conditions.
3. We show that microbial community composition and traits varied independently from both *Sphagnum* phylogeny and the latitudinal gradient. Specifically, the addition of *Sphagnum* traits to climatic and edaphic variables to the SEM allowed it to explain a larger proportion of the explained variance ( $R^2$ ). This observation was most apparent for the biomass of decomposers (+42%) and phototrophs (+19%), as well as for growth yield microbial traits (+10%). As such, that *Sphagnum* metabolites were important drivers for microbial community structure and traits, while *Sphagnum* anatomical and morphological traits were poor predictors.

4. *Synthesis*. Our results highlight that *Sphagnum* metabolites are more to influence peatland microbial food web structure and functioning than *Sphagnum* anatomical and morphological traits. We provide further evidence that measurements of the plant metabolome, when combined with classical functional traits, improve our understanding of how the plants interact with their associated microbiomes.

**Key-words:** Functional traits, Latitudinal gradient, Metabolomics, Microbial traits, Peatlands, Plant and microbial communities, Plant–soil (below- ground) interactions, *Sphagnum*

## Introduction

Soil microbial communities are highly diverse and make a significant contribution to many critical ecosystem functions (Crowther et al., 2019), such as the decomposition of plant litter (Geisen, 2020; Schlesinger & Andrews, 2000; Singh et al., 2010), nutrient cycling (Gui et al., 2017), and the mineralization and stabilization of soil organic matter (Liang et al., 2017, 2019). Moreover, soil microorganisms are interconnected with plants. By aiding plant nutrient acquisition (Averill et al., 2019) and drought resistance (Mariotte et al., 2015), soil microorganisms play a key role in shaping plant productivity and community dynamics (Mommer et al., 2018; Wardle et al., 2004). Plants, in turn, determine the composition of soil communities by regulating surface soil temperature and hydrology, as well as the chemical signature of organic carbon inputs (litter) and rhizodeposits (Bardgett & Wardle, 2010).

Biotic and abiotic factors influence the composition of soil microbial communities. Climatic (*e.g.* temperature, precipitation) and edaphic conditions (*e.g.* soil pH, moisture) are often seen as important determinants of microbial communities (Borowik & Wyszowska, 2016; Singh et al., 2009; Wang et al., 2020) yet they cannot completely explain the full variation observed within microbial communities (De Gruyter et al., 2020). This suggests that biotic interactions also play an important role in shaping microbial communities (Geisen, 2020). Trophic and non-trophic (*e.g.* competition) interactions among microorganisms are important, but often neglected (Gralka et al., 2020). Recently, plant species identity (Burns et al., 2015), plant phylogeny (Barberán et al., 2015) and plant community composition (de Vries et al., 2012; Robroek et al., 2015) have also been identified as important drivers of microbial communities. However, disparity remains once individual plant leaf and root traits are taken into account (Leff et al., 2018), suggesting that plant traits are poor predictors of microbial communities and microbial processes (see Sweeney et al., 2020). Alternatively, the effect of plant species identity and/or community composition on microbial communities may mostly rely on chemical interactions between plant and soil

microbes (van Dam & Bouwmeester, 2016). Plants produce a plethora of biochemicals, and can release over a hundred different metabolites in their surroundings that can attract, deter, or even kill belowground microbes (Fernandez et al., 2016; Hamard et al., 2019; Hu et al., 2018; Pinton et al., 2001). Elucidating which plant characteristics (phylogeny, morphological and anatomical traits and/or metabolites) govern microbial communities, particularly in addition to microbial interactions and climatic and edaphic conditions, is thus urgently needed to predict the structure of microbial communities, their functioning and subsequent ramifications for biogeochemical cycles (Bardgett & Wardle, 2010).

We address this knowledge gap by examining the understudied linkages between peat mosses (*i.e.* *Sphagnum* moss) and their associated microbiome. *Sphagnum*-dominated peatlands store more carbon (C) than any other terrestrial ecosystem (Nichols & Peteet, 2019). Carbon accumulation in *Sphagnum*-peatlands results from cold, acidic, nutrient-poor and water-saturated conditions that have hindered microbial decomposition of litter over millennia (Rydin & Jeglum, 2006). *Sphagnum* mosses also facilitate their own growth by creating unfavorable conditions for vascular plants, generating recalcitrant litter and changing physical and chemical properties of the soil (Turetsky, 2003; van Breemen, 1995). As *Sphagnum* do not possess roots, the leaf-associated microbiome comprises crucial functions, such as defenses against pathogen and additional nutrient supply for *Sphagnum* growth and development (Opelt, et al., 2007b). The association between *Sphagnum* moss and its microbiome, *i.e.* the bryosphere (*sensu* Lindo & Gonzalez, (2010)), plays a key role in peatland C dynamics. These *Sphagnum*-associated microbial communities include a core detrital network for C and nutrient cycling (Gilbert et al., 1998; Jassey et al., 2015; Lindo & Gonzalez, 2010). Unique anatomical and morphological traits of *Sphagnum*, especially the cell structure of leaves - with one layer of photosynthetically active cells (chlorocystes) and dead, water-filled hyaline cells, create consistent microenvironments for the microbial communities (Bragina et al., 2012a). Large hyaline cells can serve as less acidic 'oases' for microorganisms in the otherwise acidic peatland pore water (Kostka et al., 2016). *Sphagnum* also actively excretes bioactive metabolites (*i.e.*

biochemicals) to their surroundings such as polyphenols (Rasmussen et al., 1995a; Rasmussen et al., 1995b; Rudolph & Samland, 1985; Schellekens et al., 2015), flavonoids (Sytiuk et al., 2020), carbohydrates (Hájek et al., 2011; Painter, 1991; Tetemadze et al., 2018; van Breemen, 1995), and tannins (Sytiuk et al., 2020; Verhoeven & Liefveld, 1997), that have been related to the functioning of peatlands (Verhoeven & Liefveld, 1997). Many of these metabolites show antimicrobial properties (Fudyma et al., 2019). For example, *Sphagnum* phenolics have been suggested to reduce vascular plant's mycorrhization (Binet et al. 2017; Chiapusio et al., 2018), and inhibit bacterial growth (Mellegård et al., 2009), decomposition (Freeman et al., 2001; Verhoeven & Liefveld, 1997; Verhoeven & Toth, 1995) and microbial respiration (Hamard et al., 2019). As *Sphagnum* species engineer their environment (van Breemen, 1995; Bengtsson et al., 2016; Bengtsson et al., 2018), both *Sphagnum* anatomical/morphological and biochemical traits may be expected to steer the structure and function of peatland microbial communities. A better identification and comprehension of these drivers are crucial for predicting the composition of the microbial community and its functioning in peatlands.

Here, we explore how and to what extent *Sphagnum* phylogeny, anatomical and morphological traits and metabolites drive the spatial variability of microbial community composition and functioning. To do so, we conducted an observational study in five European *Sphagnum*-dominated peatlands representing a wide range of climatic and edaphic conditions. Because *Sphagnum* microbial community composition can vary across space (Mitchell et al., 2003; Robroek et al., 2021), and according to the variation of edaphic factors such as pH and nutrient richness (Bragina et al., 2013a; Jassey et al., 2014; Opelt et al., 2007a), we hypothesized that (1) microbial community composition and functional traits will show distinct patterns among the five peatlands. We expected (2) that geographical variation in microbial community composition and microbial traits is driven by climatic/edaphic conditions as well as *Sphagnum* anatomical and morphological traits and metabolites. In particular, we hypothesized that (3) *Sphagnum* traits will explain a fraction of variation in microbial community composition and microbial traits that is

127 not explained by climatic and edaphic conditions. Among *Sphagnum* traits, we predicted that (4)  
128 *Sphagnum* metabolites have a stronger effect in shaping microbial properties than anatomical and  
129 morphological traits, since metabolites are released into *Sphagnum* surroundings and can directly  
130 influence microbial community composition and/or microbial traits. Finally, as *Sphagnum* traits do not  
131 exclusively vary with climatic and edaphic conditions (Sytiuk et al., 2020), but also according to phylogeny  
132 (Laine et al., 2021), we hypothesized that (5) *Sphagnum* phylogeny is an important determinants of  
133 microbial properties, in addition to climatic and edaphic conditions and *Sphagnum* traits.



## Material and methods

### Sites, sampling design and sample collection

We selected five *Sphagnum*-dominated peatlands along a latitudinal gradient from northern Sweden to southern France to represent a wide range of edaphic and climate conditions (Table 1, Table S1, S3). In each site, a preliminary vegetation survey (see Table S2) allowed us to select five homogeneous plots (50 x 30 cm each; 5 plots x 5 sites = 25 plots in total) dominated by a single *Sphagnum* species: *S. warnstorffii* (France, FR), *S. magellanicum* (Poland, PL), *S. rubellum* (Estonia, EST), *S. papillosum* (Finland, FI) and *S. balticum* (Sweden, SE). Dominant *Sphagnum* species were site specific, potentially creating a confounding effect with climate/edaphic conditions. To overcome this issue, we measured phylogenetic differences among *Sphagnum* species at five sites, and found that *Sphagnum* phylogeny did not covary with climatic variation (*i.e.* mean annual temperature,  $F_{1,3} = 0.15$ ,  $P = 0.72$ , Fig. S1). Despite the absence of phylogeny-climate covariation, it remains true that species identity and site variation were still potentially confounding. We thus quantified metabolite plasticity (*i.e.*, water-soluble phenols) of the five *Sphagnum* species using a reciprocal transplantation along the latitudinal gradient to determine whether *Sphagnum* metabolite production is more sensitive to environmental variability than to taxonomy. We found that the concentration of water-soluble phenols increased for all *Sphagnum* species along the latitudinal gradient (Fig S2a, b), and more importantly that the variance of water-soluble phenol concentrations within the same species at different temperatures was higher than between the different species at the same temperature (Fig. S2c). Together, these findings show that water-soluble phenol concentrations in *Sphagnum* tissues vary independently of taxonomy. However, other metabolites may depend on taxonomy. To exclude this potential, we used unpublished data (Jassey, Allard and Robroek, unpublished data) on *Sphagnum* metabolomic profiling from 56 European ombrotrophic peatlands (see sites in Robroek et al., 2017). A PCoA analysis revealed that the metabolomic composition of *Sphagnum*

species was strongly determined by local and regional conditions (site effect,  $P < 0.05$ ), rather than by taxonomy (species effect,  $P = 0.46$ ; Fig. S3). Altogether, these additional analyses demonstrate that a potential confounding effect of species and site was not an important issue when referring to *Sphagnum* metabolites. However, we acknowledge that anatomical and morphological traits are used to identify *Sphagnum* moss to species (Isoviita, 1966), and that in our study anatomical and morphological traits cannot be disentangled from *Sphagnum* taxonomy. We therefore advise caution when using these data to predict microbial communities and microbial activities.

In each plot, 15-20 *Sphagnum* shoots were sampled around ten marked spots (ca. 250 g fresh weight of *Sphagnum* per plot). This sampling design allowed us to obtain a composite sample, representative of the entire plot. Upon sampling, the living top of the *Sphagnum* shoots (0-3 cm) were cut immediately, pooled, homogenized and then dispatched for the different lab analyses. Approximately 10 g of *Sphagnum* shoots were fixed in 20 mL of glutaraldehyde (2% final concentration) and stored at 4°C in the dark for microbial biomass/abundance measurements. Approximately 20 g were frozen and lyophilized for fungal and biochemical analyses. Another 10 g were frozen for analyses of microbial enzymatic activities. The remaining 10 g were stored at 4°C and used for analyses of *Sphagnum* anatomical and morphological traits. We collected *Sphagnum* samples in every site within the same week in early-July 2018.

## **Characterizing climate and site conditions**

For each site, we extracted bioclimatic data from WorldClim v2 (Fick & Hijmans, 2017): mean annual temperature, temperature seasonality, annual precipitation, and precipitation seasonality averaged over the 1960-2018 period (Table S1). Water-table depth (WTD) and pH were measured directly in the field using a ruler and a portable multimeter Elmetron CX742, respectively (Table 1). Water-extractable organic matter (WEOM) was extracted from the *Sphagnum* shoots (0-3 cm height) collected at the five sites

according to Jassey et al. (2018) (Table S3). Briefly, *Sphagnum* shoots were soaked in 30 mL of demineralized water and then shaken for 90 min at 150 rpm. *Sphagnum* shoots were then dried at 60°C for 48 hours and weighted to obtain dry mass (mg/g DW). The water extract was filtered with Whatman filter (1 µm pore size) and several physical-chemical parameters were analyzed: a TOC analyser (Shimadzu TOC-L) was used to quantify dissolved organic carbon, nitrogen and phosphate (WEOC, WEON and WEOP respectively). To measure dissolved organic matter aromatic content and molecular weight (WEOCq), we used absorbance measurements between 250 and 660 nm (15 wavelengths in total) in 200 µL sample aliquots in 96-well quartz microplate using a BioTek SynergyMX spectrofluorometer (Jaffrain et al., 2007). For a blank, we used demineralized water filtered through Whatman filter to correct our values for the potential C released from the filter. Spectral slopes ( $S_{250-660}$ , nm<sup>-1</sup>) were calculated using linear least squares regressions with Ln-transformed absorptions. High  $S_{250-660}$  values indicate low molecular weight material (Hansen et al., 2016).

We further performed a vegetation survey using two high-resolution images (25 x 15 cm) of each plot, according to Buttler et al. (2015). On each picture, we laid a grid of 336 points and identified species overlaying the grid intersects. This technique did not assess vertical biomass and could underestimate the relative abundance of certain species. However, the bias was alike in each plot, making species frequencies comparable among sites.

### ***Sphagnum* anatomical and morphological traits**

We characterized a suite of four anatomical and morphological *Sphagnum* traits determining the capacity of *Sphagnum* moss to provide shelter for microbial communities following Jassey & Signarbieux (2019): volume of the capitulum (height x diameter of capitulum), water-holding capacity of the capitulum and shoot, number of hyaline cells per leaf area (*i.e.* dead cells storing water), the surface area of hyaline cell (length x width) and width of chlorocystes (photosynthetic cells surrounding hyaline cells). In total, 125

203 individuals (25 per site) were randomly collected to estimate the volume of the capitula ( $\text{mm}^3$ ) by  
204 measuring their height and diameter using a precision ruler. Then, we used the same samples to quantify  
205 the net water content of the capitula and stem (first cm) at water saturation. Capitula and stems were  
206 submerged in water until their maximum water retention capacity was reached. Excess water was  
207 removed by allowing water to drain naturally for two minutes. Then, individual capitula and stems were  
208 weighed as water-saturated and subsequently dried for three days at  $60^\circ\text{C}$ . The net water content at  
209 water saturation of each individual was expressed in grams of water per gram of dry mass ( $\text{g H}_2\text{O/g DW}$ ).  
210 For anatomical analyses, we carefully deconstructed five *Sphagnum* capitula in each plot (in total, 125  
211 capitula) to isolate *Sphagnum* leaves. Then, we pooled all *Sphagnum* leaves, homogenized, and took three  
212 leaves from that pool to prepare microscope slides from each plot (375 leaves analyzed in total). We  
213 quantified the number of hyaline cells per leaf area (number of hyaline cells per  $\text{mm}^2$ ), their surface ( $\mu\text{m}^2$ ),  
214 as well as the width of chlorocystes ( $\mu\text{m}$ ), using a light microscope connected to a camera (LEICA ICC50  
215 HD) and the size analytic tools (LEICA suite software).

### 216 ***Sphagnum* metabolic fingerprint**

217 We assessed the metabolic fingerprint of *Sphagnum* mosses using two different approaches. First, we  
218 quantified a set of nine moss metabolites that can influence microbes. The different extractions pathways  
219 used for quantifying the various *Sphagnum* metabolites are detailed in Sytiuk et al. (2020). Briefly,  
220 *Sphagnum* mosses were frozen, lyophilized, ground and stored at  $-20^\circ\text{C}$  prior to biochemical analysis.  
221 Then, we used (i) a 99.9% methanol extraction for quantifying *Sphagnum* pigments (chlorophyll a, b and  
222 total carotenoids), (ii) a 50% methanol extraction for quantifying total polyphenols, flavonoids, tannins  
223 and carbohydrates, (iii) a water extraction for quantifying water-extractable total polyphenols, (iv) a  
224 sulfosalicylic acid extraction for quantifying proline and (v) a dosage of proteins with bovine serum  
225 albumin (BSA). All metabolites were quantified using spectroscopy at different wavelengths. Secondly, we

characterized the polysaccharides, aromatic and aliphatics content of *Sphagnum* mosses using Fourier Transform Infrared Spectroscopy (FT-IR-ATR; (Hodgkins et al., 2014). 30 mg freeze-dried and ground *Sphagnum* was placed directly on a germanium crystal and pressed down with a flat tip to improve distribution and contact. Spectra were acquired by 64 scans at a  $2\text{ cm}^{-1}$  resolution over the range  $4000\text{--}600\text{ cm}^{-1}$ . All spectra were corrected for water vapor,  $\text{CO}_2$  and for differences in depth of beam penetration at different wavelengths (ATR correction; Opus software). All spectra were then normalized. For each spectrum, normalization involved (i) a subtraction of the minimum absorption value applied to the whole spectrum followed by (ii) a multiplication - also applied on the whole spectra - to obtain a spectral maximal absorbance value of 1 for each *Sphagnum* sample. Six main absorption peaks were used as an indicator of *Sphagnum* polysaccharides, aromatics and aliphatics: 1) the  $1064\text{ cm}^{-1}$  region (combination of C-O stretching and O-H deformation) is associated to polysaccharides; 2) the  $1515\text{ cm}^{-1}$  region (C=C; aromatic compounds) is assigned to lignin/phenolic backbone; 3) the  $1610\text{ cm}^{-1}$  region (C=C stretching; aromatic compounds and/or asymmetric C-O stretch in  $\text{COO}^-$ ) is associated to lignin and other aromatics, or aromatic or aliphatic carboxylates; 4) the  $1724\text{ cm}^{-1}$ - $1710\text{ cm}^{-1}$  region (C=O stretch of  $\text{COOH}$  or  $\text{COOR}$ ) corresponds to free organic acids, carboxylic acids, aromatic esters; 5)  $2850\text{ cm}^{-1}$  region (symmetric  $\text{CH}_2$ ) is associated to aliphatics; and 6)  $2920\text{ cm}^{-1}$  region (antisymmetric  $\text{CH}_2$ ) is associated to aliphatics. We used the ratio between the relative intensities of FT-IR absorption bands, where  $1610\text{ cm}^{-1}$  region was used as denominator due to its highly recalcitrant nature, in order to evaluate *Sphagnum* fingerprints and their degree of degradability.

## **Microbial abundances and biomass**

Microbial consumers (testate amoebae, ciliates, rotifers and nematodes), phototrophs (microalgae and cyanobacteria), and decomposers (fungi and bacteria) were extracted from *Sphagnum* following Jassey et al. (2011a). For bacterial counts, a 1-mL sub-sample was stained with SYBR Green (0.1x final

concentration) and incubated in the dark for 15 minutes. Then the sub-samples were run at a speed of 2  $\mu\text{L s}^{-1}$  at a count rate not exceeding 1000 events  $\text{s}^{-1}$  in a cytometer (Guava® easyCyte). Epifluorescence microscopy was used to determine the size of bacteria: 1 mL sub-samples were stained with DAPI (4,6-diamino-2-phenylindole; 3  $\mu\text{g mL}^{-1}$  final concentration), incubated in the dark for 15 minutes, filtered on 0.2  $\mu\text{m}$  black membrane filters and examined by fluorescence microscopy at 1000x magnification. Bacterial sizes were determined manually under the microscope following Jassey et al. (2011a). The abundance of phototrophs and microbial consumers, as well as their identification to species level when possible, was carried out using a 3-mL subsample and inverted microscopy ( $\times 400$ , Utermöhl method). The abundance of bacteria, phototrophic and consumer species was then converted into biovolume ( $\mu\text{m}^3$ ), calculated based on geometrical shapes using dimensions measured under the microscope (length or diameter; width, and height). Biovolumes were converted to biomass ( $\mu\text{gC}$ ) using conversion factors as given in Gilbert et al. (1998). The biomass data were expressed in micrograms of C per gram of *Sphagnum* dry mass ( $\mu\text{g C g}^{-1} \text{DM}$ ). The biomass of fungi was quantified using ergosterol quantification according to the standard extraction procedure previously described in Gessner et al. (1991). Briefly, 50 mg of lyophilized *Sphagnum* were incubated in glass vials with 5 mL of potassium hydroxide methanol (8  $\text{g L}^{-1}$ ) for 24h at 4°C. A control vial containing 100  $\mu\text{L}$  of a 200- $\mu\text{g mL}^{-1}$  solution of ergosterol was also incubated in the same conditions to take into account the yield of the extraction. All vials were then heated at 80°C for 30 min. After cooling, 1 mL of hydrochloric acid (0.65  $\text{mol L}^{-1}$ ) was added in each sample. 3mL of each sample were filtered on Oasis HLB cartridges (60 mg sorbent, 30  $\mu\text{m}$  particle size). Cartridges were previously and successively conditioned with 1 mL of methanol and 1 mL of a mixture of 15%v methanol, 70%v potassium hydroxide methanol (8  $\text{g L}^{-1}$ ) and 15%v hydrochloric acid (0.65  $\text{mol L}^{-1}$ ). After sample filtration, cartridges were washed with 1 mL of 5%v methanol diluted in autoclaved milli-Q water. Cartridges were then dried under vacuum ( $-5 \text{ bar}$ ) for 1 h, after what they were eluted with 4\*350  $\mu\text{L}$  of isopropanol. The concentration of ergosterol in eluates was assessed by HPLC, using a calibration curve.

The yield of the extraction was assessed by comparing the measured and theoretical concentration of ergosterol in the control vial. Ergosterol concentrations in samples were corrected from the yield of the reaction and were expressed in  $\mu\text{g}$  of ergosterol per g of *Sphagnum* dry weight.

## **Microbial traits**

Following the revised life history theory for microbial traits (Malik et al., 2020), we collected microbial traits classified into three main microbial life history strategies: growth yield, resource acquisition and stress tolerance. We quantified nine traits in the growth yield strategy: biomass per cell, biovolume per cell, body size (length and width), the quantum yield of photosystem II for phototrophs, photosynthetic pigments content per cell for phototrophs, growth rate, reproduction rate, and respiration rate per cell. We classified 14 traits in the resource acquisition strategy: nine microbial enzyme activities, C uptake by phototrophs, predation rates, nitrogen fixation, methanotrophy and motility. Finally, three traits were assigned to the stress tolerance strategy: morphology, response to temperature increase, and tolerance to desiccation. A total of 26 microbial traits were either directly quantified or acquired from the literature (see Supplementary method on microbial traits for more details).

To describe the functional trait space in each site, we calculated community weighted means (CWM) of each trait calculated as the presence/absence weighted means of species trait values using the FD R package (Laliberté et al., 2015). We then created a functional distance matrix by applying Gower's distance on each pair of species described by their traits, and then computed a Principal Coordinate Analysis (PCoA) on it. Gower's distance allows mixing of different types of traits (*i.e.* qualitative and quantitative traits) while giving them equal weights. Then, the two first axes of the PCoA were selected as synthetic CWMs summarizing the microbial functional space in each site.

## Numerical analyses

All statistical analyses were performed in R 3.5.3 (R Core Team, 2019) using packages, as specified below. Linear mixed effects models were used to assess the *Sphagnum* taxonomy effect (fixed effect) on the microbial biomass of each trophic group, CWM of each microbial trait and *Sphagnum* traits. The models were fitted with plot nested within *Sphagnum* taxonomy as a random effect on the intercept (Pinheiro & Bates, 2000). Tukey's multiple comparison test was used for *post hoc* analyses of differences among the levels of the fixed effects in the final model. Normality and homogeneity assumptions of the data, as well as model residuals, were assessed using a Shapiro test and diagnostic plots. Log<sub>10</sub>-transformations of the data were applied if needed in order to meet these assumptions. To represent differences in microbial community composition, microbial trait composition and *Sphagnum* traits, we performed principal coordinate analysis (PCoA) using Gower's distance that allowed mixing of different types of data (*i.e.* qualitative and quantitative traits) while giving them equal weights. A standardization (*Sphagnum* anatomical and morphological traits and metabolites) or Hellinger transformation (microbial community composition and microbial traits) was applied on the matrices beforehand (Legendre & Legendre, 2012). We used Spearman correlations to test the potential relationships between microbial community composition, CWM of microbial traits and *Sphagnum* traits and/or climatic and edaphic factors.

We assessed the effect of *Sphagnum* phylogenetic distance on microbial biomass and microbial trait composition under the Brownian Motion model (BM). BM predicts that the variance in microbial properties increases at a constant rate proportionate to the evolutionary distance among *Sphagnum* species, with more closely related species having more similar values for microbial properties, and indicating that the variable has a phylogenetic signal (Felsenstein, 1985). We used Blomberg's K index (Münkemüller et al., 2012) to test for a *Sphagnum* phylogenetic signal among microbial variables with randomization and 1000 permutations (Table S4).



To assess whether differences in *Sphagnum* anatomical and morphological traits and metabolites predicted variation in microbial community composition and microbial traits beyond the explanatory power of climatic and edaphic conditions (Leff et al., 2018), we built a set of path diagrams subjected to structural equation modelling (Grace et al., 2010, 2014). We compared the explanatory power of the models, assessed through adjusted  $R^2$  values and Akaike Information Criterion (AIC), by framing four types of models (Fig. 1). First, we tested the effects of climatic and edaphic conditions on each (hereafter ‘single’ SEM models) trophic group (*i.e.* the biomass of either decomposers, phototrophs, predators or the total microbial biomass) and microbial trait strategy (*i.e.* either growth yield, resource acquisition and stress tolerance strategies or the overall traits composition; Fig. 1a) separately. Second, we tested the effects of climatic and edaphic conditions on the interactions (hereafter ‘interactions’ SEM model) among/within microbial community composition and microbial trait strategies (Fig. 1b). Third, we tested the effect of *Sphagnum* anatomical and morphological traits and metabolites (*i.e.* *Sphagnum* traits; in addition to climatic and edaphic conditions as in the first model) on each trophic group and microbial trait strategy separately (Fig. 1c) and in interaction (Fig. 1d). The benefits gained ( $\Delta R^2$ ) by including *Sphagnum* anatomical and morphological traits and metabolites into the models were calculated as follows:

$$\Delta R^2 = (R^2_{SEM \text{ with } Sphagnum \text{ traits}} - R^2_{SEM \text{ without } Sphagnum \text{ traits}}) * 100\%$$

We further compared AIC values between models with and without *Sphagnum* traits to check for potential overfitting (Burnham & Anderson, 2004). In these models, we used annual precipitation and mean temperature of the wettest quarter as climate variables, selected beforehand using a principal component analysis (PCA) applied on all bioclimatic variables. For edaphic peatland conditions, we used *Sphagnum* water content and the first axis of a PCA applied on WEON, WEOC, WEOP and  $S_{260-660}$ . For microbial trait strategies, we used the first axis of three PCoAs applied on the CWM of traits of each trait strategy, respectively. The paths of the SEM were fitted as previously described for ANOVAs using *piecewiseSEM*

package (Lefcheck, 2016). We selected this approach as it allowed using the Shipley's test of d-separation to assess whether direct or indirect paths are missing from the *a priori* model. The adequacy of the model was evaluated via several tests including non-significant *Fisher's C* statistic ( $P > 0.05$ ), and low Akaike information criterion (AIC) (Grace et al., 2010).

We used two strategies to validate our SEM models and generate statistics of the models' predictive power. The first strategy was inspired by 'null-model' analyses in ecology (Gotelli & Ulrich, 2012), and tests the assumption that the effects of *Sphagnum* traits in predicting microbial community composition and microbial traits are not random and driven by changes in *Sphagnum* traits. To test this assumption, we randomized *Sphagnum* trait matrices to break any structure in the data. We iteratively and randomly shuffled the *Sphagnum* trait matrices ten times before running the SEM models. The second strategy focused on the size of the data set as it can strongly influence SEM modelling (Grabowski & Porto, 2017; Grace et al., 2010). To do so, we iteratively reduced our entire data set by 20% by randomly removing one replicate from the dataset. In other words, we retained four out of five replicates before running the SEM models. We repeated this step five times, until all possible combinations were covered. For each SEM model we extracted the data relative to the adequacy of the model (*Fisher's C* statistic and *P*-value) and AIC values (see model outputs in Fig. S7 and Tables S11, S12). The sensitivity analyses were performed on the most relevant SEM models, where the benefits gained ( $\Delta R^2$ ) by including *Sphagnum* traits into the models was more than 10%: decomposers single, decomposers interactions, phototrophs single, and yield single.

## Results

Throughout this section we refer to changes in sites (see Table 1 for sites' acronyms), which nevertheless are confounded with *Sphagnum* species identity. Thus, we advise to check the Materials and methods section and Supplementary materials (Fig S1, S2, S3) where we demonstrate that a potential confounding effect of species and site was not an important issue.

### ***Sphagnum* morphological and anatomical traits and metabolites**

PCoA analysis revealed that *Sphagnum* trait composition (anatomical and morphological traits and metabolites) differed among the five sites (Fig. 2a). Three distinct groups emerged from the first PCoA axis: a first group composed of *Sphagnum* from FI, a second group composed of EST and SE and a third group with FR and PL. On the second PCoA axis, there was a gradient ranging from FI to FR/PL and then SE/EST. This gradient was not related to any particular climatic and/or edaphic trend. Instead, it showed a clear trend as *Sphagnum* from FI, PL and FR had higher capitulum sizes, water holding-capacity and/or metabolites concentrations (Fig. S4, S5, Table S5), as compared to SE and EST. Specific *Sphagnum* anatomical and morphological traits and metabolites varied between five *Sphagnum* species from three phyla (Fig. 2b; Fig. S4, S5). We found that *Sphagnum* from FR and PL produced more total and water-soluble phenols, total flavonoids and tannins than SE and FI (Fig. 2b). However, *Sphagnum* from SE, FI and EST produced more polysaccharides, organic acids, symmetric and antisymmetric CH<sub>2</sub> than FR and PL. In terms of anatomical and morphological traits, *Sphagnum* sampled from PL and FI possessed higher capitulum diameter, height and volume than EST and FR. Water holding capacities, hyaline cell surface and chlorocyste width were highest for *Sphagnum* from FI and FR. Even though *Sphagnum* from EST had highest number of hyaline cells per leaf area, its surface of hyaline cells was smallest.

## Microbial community composition and trait composition

Microbial community composition and microbial traits differed significantly among the five sites (Fig. 3; Fig. S6-S9, Tables S5), and similar to the *Sphagnum* traits composition, no particular climatic and/or edaphic trend was found neither in microbial community composition nor microbial traits (Fig. 3). The first PCoA axis showed three distinct groups of microbial community composition with FR and SE aside and a third group composed of PL, FI and EST (Fig. 3c). On the second axis, there was a clear separation between SE and the four other sites. Microbial trait composition differed markedly across the five sites (Fig. 3d). While no particular variation was observed on the second PCoA axis, sites were well separated along the first PCoA axis (Fig. 3d). Overall biomass differed with *Sphagnum* phylogeny. Specifically, the highest biomass of consumers and decomposers was observed in SE and FR (Fig. 3a, Fig. S6). Conversely, PL and EST were characterized by low biomass of most microbial groups (Fig. 3a, Fig. S6). For community weighted mean (CWM) microbial traits, we found that the microbial traits related to the growth yield strategy were the most abundant in FI and SE, and the least abundant in EST (Fig. 3b, Fig. S7). Microbial traits related to resource acquisition peaked in FI and EST, while stress tolerance traits were the most abundant in SE (Fig. 3b, Fig. S8, S9). Despite such differences in microbial biomass and CWMs of traits among sites, *Sphagnum* phylogenetic distances were weakly related to differences in microbial biomasses ( $P > 0.1$  in most cases) and in microbial trait composition ( $P > 0.1$  in all cases; Table S4). Only the biomass of flagellates ( $K = 1.1$ ,  $P = 0.03$ ; Table S4) was significantly related to *Sphagnum* phylogenetic distances.

## Predictors of microbial community and microbial traits

Differences in microbial community composition and microbial traits were related to both climatic and edaphic conditions and *Sphagnum* traits (Fig. 4). As *Sphagnum* trait composition was also correlated with climatic and edaphic conditions (*i.e.* annual precipitation and WEOM chemistry, Fig. 4), we ran structural equation models with and without *Sphagnum* anatomical and morphological traits and metabolites to

tease apart the effects attributable to *Sphagnum* traits and metabolites on microbial properties (Fig. 5, Table S6-S10). Shifts in microbial community composition and microbial traits across *Sphagnum* species were correlated with multiple climatic and edaphic variables, which together explained 27%-86% of the variation of the biomass of decomposers, phototrophs and consumers, as well as of microbial trait composition (Table S6). When *Sphagnum* anatomical and morphological traits and metabolites were added to the SEM models, prediction accuracies for most microbial biomass and trait compositions increased by 42% (Fig. 5), notably for decomposer biomass (+42%), phototrophs (+19%) and traits related to growth yield (+10%). Rigorous sensitivity analyses on SEM models revealed that  $R^2$  improvements provided by the addition of *Sphagnum* traits in SEMs were robust and without bias due to possible randomness in the estimations (Fig. S7, S11) or the size of the dataset (Fig. S7, Table S12). Our sensitivity analyses hence indicated that microbial properties can be reasonably predicted from *Sphagnum* traits, and most importantly, that such effects are complementary to environmental (*i.e.* climatic and edaphic conditions) variation.

More precisely, most *Sphagnum* metabolites were related to microbial biomasses and/or microbial trait strategies (Fig. 6). The biomass of cyanobacteria, some decomposers (*i.e.* fungi and bacteria) and rotifers was positively related to water-soluble phenolic compounds, whereas microalgae and testate amoebae tended to be negatively correlated to phenols (Fig. 6). *Sphagnum* anatomical and morphological traits, such as the width of chlorocystes and water-holding capacity, were positively correlated with the biomass of nematodes and some growth yield traits (*i.e.* respiration, biomass, biovolume) and some enzymes. However, methanotrophs were negatively correlated to the same *Sphagnum* traits. Most of the individual microbial traits, especially those related to growth yields (microbial pigments, respiration, size), were negatively correlated to water-soluble phenols, total tannins, phenols, proteins, carbohydrates and pigments while also being positively correlated to polysaccharides,

427 phenols/lignins, CH<sub>2</sub> compounds. Opposite trends were observed for some resource acquisition traits  
428 (mostly enzymes; Fig. 6).

429

## Discussion

Here we tested whether *Sphagnum* phylogeny, anatomical and morphological traits and metabolites are important determinants of peatland microbial community composition and functional traits. Contrary to our expectations which were based on earlier observations suggesting a high degree of similarity in microbial composition among closely related *Sphagnum* species (Bragina et al., 2013b; Bragina et al., 2012b; Putkinen et al., 2012), we found here that microbial community and trait composition did not vary with *Sphagnum* phylogenetic distance. Our findings may indicate that certain microbial taxa and traits are strongly related with particular *Sphagnum* anatomical and morphological traits and metabolites, while other microbial taxa and traits are more generalist and mostly influenced by environmental (climatic and edaphic) conditions. Hence, *Sphagnum* interspecific trait plasticity may drive microbial community composition and functional diversity in addition to climatic and local condition variables. Our results, however, need to be interpreted cautiously as *Sphagnum* species and sampling site co-varied in our study. Moreover, our observations were undertaken at a single date, thereby ignoring potential seasonality. Nevertheless, our study represents an important and necessary step in understanding which traits from diverse *Sphagnum* species are key in shaping the *Sphagnum* microbiome along an environmental gradient.

In contrast to peatland plant species richness and functional diversity (Robroek et al., 2017), no notable latitudinal trends, neither in microbial community composition nor trait composition, were observed. Instead, climatic (*i.e.* the mean temperature of the wettest quarter and annual precipitation) and edaphic (*i.e.* water table depth, *Sphagnum* water content, and nutrient availability) variables were identified as important drivers of microbial community and traits. This corroborates previous studies showing that global and local peatland conditions play deterministic roles in shaping microbial communities and functioning (Elliott et al., 2015; Jassey et al., 2014; Urbanová & Bárta, 2016). However, we show that *Sphagnum* traits, mostly metabolites, were as important as climatic and edaphic conditions

in driving microbial community composition and functioning (Fig. 4). Our analysis revealed that microbial consumers, as well as growth yield and resource acquisition traits, generally decreased with frequent rainfall, high *Sphagnum* water content, and low nutrient content. Phototrophs and decomposers, however, showed opposite trends. In addition, *Sphagnum* traits were negatively correlated to decomposers, but positively to growth yield and resource acquisition. While correlations between microbial traits, *Sphagnum* traits and climatic and edaphic factors enabled us to assess the direction of these relationships, the underlying mechanisms remain unknown, since here *Sphagnum* metabolites were also driven by climatic and edaphic conditions. Using the SEM approach and taking into account the response of *Sphagnum* traits to climatic and edaphic conditions, our multi-model comparisons revealed that the addition of *Sphagnum* traits generally did increase the predictive power of SEMs, especially for the biomass of decomposers, phototrophs, and growth yield traits, while avoiding overfitting the models. This suggests that *Sphagnum* anatomical and morphological traits and metabolites are important regulators of *Sphagnum*-microbial interactions.

Overall, *Sphagnum* anatomical and morphological traits were poor predictors of microbial communities. Nevertheless, we found that the biomass of cyanobacteria, large consumers, such as nematodes, and microbial traits related to growth yield (*i.e.* respiration, size and volume) and resource acquisition (*i.e.* some extracellular enzymes) were positively correlated to *Sphagnum* species with high capitulum size, and, hence, high water-holding capacities, and width of chlorocystes (Fig. 6). Water held between leaves and hyaline cells of the capitulum provides a habitat for many microorganisms (Vitt, 2000), and allows them to move freely with water exchange between hyaline cells and adjacent photosynthetic cells (Kostka et al., 2016). However, large *Sphagnum* species are known to maintain a more stable water content under unfavorable conditions thanks to the high water-holding capacity traits of their capitula (Jassey & Signarbieux, 2019). Consequently, our findings suggest that microbial communities associated with *Sphagnum* species with high water-holding capacity are better protected from desiccation



than for those living in smaller *Sphagnum* species, while the hunting space for large consumers is less limited. Indeed, habitat-size is an important factor structuring microbial communities. For example, Sweeney et al (2020) found that increased root surface area improved opportunities for mycorrhizal fungi colonization in grasslands. Moreover, Delgado-Baquerizo et al. (2018) highlighted habitat-size as a crucial driver of soil bacterial biodiversity and functional diversity.

Comparative effects between *Sphagnum* anatomical and morphological traits and metabolites provide evidence that *Sphagnum* metabolites play a central role in structuring microbial communities and their traits. We found that the biomass of cyanobacteria, fungi, and bacteria, as well as a number of resource acquisition traits, such as extracellular enzymes, were positively correlated to many metabolites, including total carbohydrates, proteins, *Sphagnum* pigments, total phenols and/or tannins (Fig. 6). In contrast, the biomass of microalgae and nematodes, and most of microbial growth yield traits (*i.e.* microbial pigments, respiration, biomass, volume and size), were negatively correlated to *Sphagnum* metabolites (Fig. 6). Our findings suggest that *Sphagnum* metabolites have diverse effects on microbial communities and their traits, supporting observations that the degree of host specificity varies despite *Sphagnum* phylogenetic distances (Bragina et al., 2012b). The positive links between decomposers and resource acquisition traits (mostly enzyme activities), and *Sphagnum* pigments, proteins and carbohydrates suggest that the activity of these microorganisms benefit *Sphagnum* growth (Kostka et al., 2016). Alternatively, *Sphagnum* also releases easy-degradable carbohydrates (*i.e.* glucose) that can stimulate decomposers' nutrient mineralization, which directly and positively feeds back to *Sphagnum* growth, the 'host'. However, such beneficial interactions between microbes and plants often involve specific metabolites (Hiruma, 2019). Our findings indeed suggest that *Sphagnum* use an array of specific metabolites to regulate microbial communities and their functions. Polyphenols (*e.g.* *Sphagnum* acids) are released by *Sphagnum* to interact with *Sphagnum* associated microbial communities (Hamard et al., 2019; van Breemen, 1995; Verhoeven & Liefveld, 1997). Polyphenols can be associated with *Sphagnum*

cell walls and prohibit microbial breakdown of *Sphagnum* litter (Freeman et al., 2001; van Breemen, 1995; Verhoeven & Liefveld, 1997; Verhoeven & Toth, 1995) or can be released into the environment to deter or kill microorganisms (Fudyma et al., 2019; Hamard et al., 2019; Mellegård et al., 2009; Opelt et al., 2007b). This likely explains the negative link between phenols and most of the microbial growth traits. Cell-wall carbohydrates (*e.g.* sphagnum) are released slowly into the environment and thus inhibit microbial activity (Stalheim et al., 2009; van Breemen, 1995) either directly by inactivation of extracellular enzymes or indirectly by limiting C and N mineralization and thus microbial growth (Balance et al., 2007; Hájek et al., 2011). However, additional chemical analyses as well as targeted experiments are required to justify this assumption for such *Sphagnum*-microbial interactions.

Our findings demonstrate how soil microbial communities can be structured by *Sphagnum* metabolites. Also, our findings highlight the need for more targeted *Sphagnum* metabolomic analyses to identify the specific compounds involved in *Sphagnum*-microbial relationships (Chiapusio et al., 2018; Fudyma et al., 2019). *Sphagnum* leachates are composed of thousands of compounds (Fudyma et al., 2019; Hamard et al., 2019), and contain not only *Sphagnum* compounds but also microbial derivative compounds (Hamard et al., 2019). We found that *Sphagnum* organic acids, symmetric and asymmetric CH<sub>2</sub> (lipids and fatty acids) were positively related to microbial phototrophic traits such as microbial photosynthetic pigments, photosynthesis efficiency, and C fixation. Microbial phototrophs are highly diverse and abundant in *Sphagnum* mosses (Jassey et al., 2015), and are an important source of lipids (Griffiths & Harrison, 2009). Hence, these findings suggest that free lipids biomarkers within the *Sphagnum* surface may indicate the activity of photosynthetic microbes associated with *Sphagnum*, which is in line with previous findings on the occurrence of cyanobacterial lipids in peat deposits (Huang et al., 2012). In addition, phototrophic lipids also possess antimicrobial properties (Leflaive & Ten-Hage, 2007), which could explain the negative relationships between lipids and microbial enzyme activities. Further studies are clearly needed to assess how well molecular-derived *Sphagnum* and microbial metabolites can

determine microbial community and trait assemblages. In particular, more attention should be given to how to extract and quantify *Sphagnum* metabolites to be able to distinguish the effects of strictly *Sphagnum*-derived metabolites from microbial metabolites on microbial community composition and functioning.

Our study showcases the key role of *Sphagnum* interspecific trait variations in driving microbial community composition and microbial traits in addition to climatic and edaphic variables. Despite the importance of these findings, some limitations have to be acknowledged and considered for further experiments. Firstly, the confounding effect between dominant *Sphagnum* species and climate (sampled one species per site), did not allow us to test for species identity and climatic effects separately. However, our additional analyses showed that such a potential confounding effect was not an issue for *Sphagnum* metabolites, and thus did not prevent us from assessing the direct and indirect effects of *Sphagnum* traits and phylogeny in driving microbial community composition and microbial traits. Secondly, despite the limited size of our dataset, sensitivity analyses showed that reducing sample size by 20 % did not influenced our SEM model outputs, providing our findings with robustness and confidence.

In summary, our findings show that *Sphagnum* metabolites prevail over *Sphagnum* morphological and anatomical traits as predictors of microbial community composition and functioning in peatlands. They further reveal the possible pathways by which *Sphagnum* interacts with its microbiome. Despite the importance of anatomical and morphological traits for determining *Sphagnum* ecophysiology (Oke et al., 2020; S  stad & Flatberg, 1993; S  stad et al., 1999) and peatland functioning (Bengtsson et al., 2016; Laing et al., 2014; Turetsky et al., 2008), we show that *Sphagnum* anatomical and morphological traits are poor predictors of microbial processes compared to *Sphagnum* metabolites. This finding echoes previous work in grasslands, where classical plant leaf and root traits leave a large fraction of variation in microbial communities unexplained (Leff et al., 2018; but see Sweeney et al., 2020), suggesting a limited role for

classic morphological traits in explaining plant-microbial interactions. This can potentially be explained by the fact that *Sphagnum* mosses grow in clumps, where they maintain uniform growth and anatomical/morphological characteristics (Oke et al., 2020) whilst their metabolome can vary according to surrounding conditions (Chiapusio et al., 2018). In addition, changes among *Sphagnum* anatomical and morphological traits can take weeks or years to become apparent (Jassey & Signarbieux, 2019; Oke et al., 2020), whereas changes in *Sphagnum* metabolite concentrations occur more quickly after an environmental stimulus (Bakhtiari et al., 2020; Callis-Duehl et al., 2017; Defosse et al., 2021; Jassey et al., 2011b) – a timescale that corresponds to microbial growth rates. As such, while the effects of climatic and edaphic factors on *Sphagnum* health can be missed in anatomical/morphological traits, they can be detectable in the *Sphagnum* metabolome.

The exact mechanisms by which *Sphagnum* mosses shape their microbiome are as yet unknown, but differences in the metabolite cocktails that *Sphagnum* release into their surrounding are likely to be an important factor. Interestingly to mention, a recent study found that repeated litter inputs resulted in directional shifts in the composition of the soil microbiome, especially fungal communities (Veen et al., 2021). The addition of grass litter to tree soils resulted in the convergence of fungal communities to those found in grass soils incubated with grass litter and vice versa. Such steering effects are more likely driven by different chemical composition of plant litter, suggesting that microbial communities can be selected by adding particular litter, and therefore particular plant metabolite cocktails (van Dam & Bouwmeester, 2016; Veen et al., 2021). These results support our findings and highlight the urgent need in new experiments to test whether plants select particular soil microbiome. The use of deeper plant metabolomic analyses would certainly shine more light into the 'black box' of plant-microbial interactions.

## Acknowledgments

This work was supported by the MIXOPEAT project (Grant No. ANR-17-CE01-0007 to VEJJ) funded by the French National Research Agency. We thank the *Plateforme Analyses Physico-Chimiques* from the Laboratoire Ecologie Fonctionnelle et Environnement (Toulouse) for their analyses (water extractable organic matter) and for the provision of an HPLC (pigments quantification). ML was supported by the National Science Foundation, Poland under grant no. UMO-2017/27/B/ST10/02228, within the framework of the project ‘Carbon dioxide uptake potential of *Sphagnum* peatlands in the context of atmospheric optical parameters and climate changes’ (KUSCO2). BJMR was supported by the British Ecological Society research grant (SR17\1427), the Stiftelsen Anna och Gunnar Vidfelts for biologisk forskning (2018-024-Vidfelts fond) and the Dutch Foundation for the Conservation of Irish bogs. Tallinn University Research Fund and the project “Life Peat Restore” supported MK. We also thank Bruno Leroux from the *Fédération Aude Claire* and the *Syndicat Forestier de Counozouls* for providing the access to the site of Counozouls.

## Author contribution

VEJJ conceived the ideas and designed methodology with the help of AS and RC. VEJJ chose the sites with the help of BJMR, ML, MK, EST and ED. VEJJ and SH collected samples with the help of MK. AS, SH and VEJJ proceeded to laboratory work with the help of BP. AS analysed the data with the help of VEJJ, JMB and BJMR. AS and VEJJ led the writing of the manuscript with the help of RC, JMB and BJMR. All authors contributed to the drafts and gave final approval for publication.

## Data availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data and R codes related to this paper will be publicly available from Figshare (10.6084/m9.figshare.c.5191493).

## References

- Averill, C., Bhatnagar, J. M., Dietze, M. C., Pearse, W. D., & Kivlin, S. N. (2019). Global imprint of mycorrhizal fungi on whole-plant nutrient economics. *Proceedings of the National Academy of Sciences of the United States of America*, 116(46), 23163–23168. doi: 10.1073/pnas.1906655116
- Bakhtiari, M., Glauser, G., Defosse, E., & Rasmann, S. (2020). Ecological convergence of secondary phytochemicals along elevational gradients. *New Phytologist*, 229(3), 1755–1767. doi: 10.1111/nph.16966
- Ballance, S., Børshheim, K. Y., Inngjerd, K., Paulsen, B. S., & Christensen, B. E. (2007). A re-examination and partial characterisation of polysaccharides released by mild acid hydrolysis from the chlorite-treated leaves of *Sphagnum papillosum*. *Carbohydrate Polymers*, 67(1), 104–115. doi: 10.1016/j.carbpol.2006.04.020
- Barberán, A., McGuire, K. L., Wolf, J. A., Jones, F. A., Wright, S. J., Turner, B. L., ... Fierer, N. (2015). Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. *Ecology Letters*, 18(12), 1397–1405. doi: 10.1111/ele.12536
- Bardgett, R. D., & Wardle, D. A. (2010). Aboveground-Belowground linkages. In *Oxford Series in Ecology and Evolution*.
- Bengtsson, F., Granath, G., & Rydin, H. (2016). Photosynthesis, growth, and decay traits in *Sphagnum* - a multispecies comparison. *Ecology and Evolution*, 6(10), 3325–3341. doi: 10.1002/ece3.2119
- Bengtsson, F., Rydin, H., & Hájek, T. (2018). Biochemical determinants of litter quality in 15 species of *Sphagnum*. *Plant and Soil*, 425(1–2), 161–176. doi: 10.1007/s11104-018-3579-8
- Borowik, A., & Wyszowska, J. (2016). Soil moisture as a factor affecting the microbiological and biochemical activity of soil. *Plant, Soil and Environment*, 62(6), 250–255. doi: 10.17221/158/2016-PSE
- Bragina, A., Berg, C., Cardinale, M., Shcherbakov, A., Chebotar, V., & Berg, G. (2012). *Sphagnum* mosses harbour highly specific bacterial diversity during their whole lifecycle. *ISME Journal*, 6(4), 802–813. doi: 10.1038/ismej.2011.151
- Bragina, A., Berg, C., Müller, H., Moser, D., & Berg, G. (2013). Insights into functional bacterial diversity and its effects on Alpine bog ecosystem functioning. *Scientific Reports*, 3, 1–8. doi: 10.1038/srep01955
- Bragina, A., Cardinale, M., Berg, C., & Berg, G. (2013). Vertical transmission explains the specific Burkholderia pattern in *Sphagnum* mosses at multi-geographic scale. *Frontiers in Microbiology*, 4(DEC), 1–10. doi: 10.3389/fmicb.2013.00394

- Bragina, A., Maier, S., Berg, C., Müller, H., Chobot, V., Hadacek, F., & Berg, G. (2012). Similar diversity of Alphaproteobacteria and nitrogenase gene amplicons on two related Sphagnum mosses. *Frontiers in Microbiology*, 2(JAN), 1–10. doi: 10.3389/fmicb.2011.00275
- Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference: Understanding AIC and BIC in model selection. *Sociological Methods and Research*, 33(2), 261–304. doi: 10.1177/0049124104268644
- Burns, J. H., Anacker, B. L., Strauss, S. Y., & Burke, D. J. (2015). Soil microbial community variation correlates most strongly with plant species identity, followed by soil chemistry, spatial location and plant genus. *AoB PLANTS*, 7(1), 1–10. doi: 10.1093/aobpla/plv030
- Buttler, A., Robroek, B. J. M., Laggoun-Défarge, F., Jassey, V. E. J., Pochelon, C., Bernard, G., ... Bragazza, L. (2015). Experimental warming interacts with soil moisture to discriminate plant responses in an ombrotrophic peatland. *Journal of Vegetation Science*, 26(5), 964–974. doi: 10.1111/jvs.12296
- Callis-Duehl, K., Vittoz, P., Defosse, E., & Rasmann, S. (2017). Community-level relaxation of plant defenses against herbivores at high elevation. *Plant Ecology*, 218(3), 291–304. doi: 10.1007/s11258-016-0688-4
- Chiapusio, G., Jassey, V. E. J., Bellvert, F., Comte, G., Weston, L. A., Delarue, F., ... Binet, P. (2018). Sphagnum Species Modulate their Phenolic Profiles and Mycorrhizal Colonization of Surrounding Andromeda polifolia along Peatland Microhabitats. *Journal of Chemical Ecology*, 44(12), 1146–1157. doi: 10.1007/s10886-018-1023-4
- Chomel, M., Guittonny-Larchevêque, M., Fernandez, C., Gallet, C., DesRochers, A., Paré, D., ... Baldy, V. (2016). Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling. *Journal of Ecology*, 104(6), 1527–1541. doi: 10.1111/1365-2745.12644
- Crowther, T. W., van den Hoogen, J., Wan, J., Mayes, M. A., Keiser, A. D., Mo, L., ... Maynard, D. S. (2019). The global soil community and its influence on biogeochemistry. *Science*, 365(6455), eaav0550. doi: 10.1126/science.aav0550
- De Gruyter, J., Weedon, J. T., Bazot, S., Dauwe, S., Fernandez-Garberí, P.-R., Geisen, S., ... Verbruggen, E. (2020). Patterns of local, intercontinental and interseasonal variation of soil bacterial and eukaryotic microbial communities. *FEMS Microbiology Ecology*, 96(3). doi: 10.1093/femsec/fiaa018
- de Vries, F. T., Manning, P., Tallowin, J. R. B., Mortimer, S. R., Pilgrim, E. S., Harrison, K. A., ... Bardgett, R. D. (2012). Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecology Letters*, 15(11), 1230–1239. doi: 10.1111/j.1461-0248.2012.01844.x
- Defosse, E., Pitteloud, C., Descombes, P., Glauser, G., Allard, P.-M., Walker, T. W. N., ... Rasmann, S. (2021). Spatial and evolutionary predictability of phytochemical diversity. *Proceedings of the*

656 *National Academy of Sciences*, 118(3), e2013344118. doi: 10.1073/pnas.2013344118  
 657 Delgado-Baquerizo, M., Eldridge, D. J., Hamonts, K., Reich, P. B., & Singh, B. K. (2018). Experimentally  
 658 testing the species-habitat size relationship on soil bacteria: A proof of concept. *Soil Biology and*  
 659 *Biochemistry*, 123(August 2017), 200–206. doi: 10.1016/j.soilbio.2018.05.016  
 660 Elliott, D. R., Caporn, S. J. M., Nwaishi, F., Nilsson, R. H., & Sen, R. (2015). Bacterial and fungal communities  
 661 in a degraded ombrotrophic peatland undergoing natural and managed re-vegetation. *PLoS ONE*,  
 662 10(5), 1–20. doi: 10.1371/journal.pone.0124726  
 663 Felsenstein, J. (1985). Phylogenies and the Comparative Method. *The American Naturalist*, 125(1), 1–15.  
 664 doi: 10.1086/284325  
 665 Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global  
 666 land areas. *International Journal of Climatology*, 37(12), 4302–4315. doi: 10.1002/joc.5086  
 667 Freeman, C., Ostle, N., & Kang, H. (2001). An enzymic “latch” on a global carbon store: A shortage of  
 668 oxygen locks up carbon in peatlands by restraining a single enzymes. *Nature*, 409(6817), 149. doi:  
 669 10.1038/35051650  
 670 Fudyma, J. D., Lyon, J., AminiTabrizi, R., Gieschen, H., Chu, R. K., Hoyt, D. W., ... Tfaily, M. M. (2019).  
 671 Untargeted metabolomic profiling of *Sphagnum fallax* reveals novel antimicrobial metabolites. *Plant*  
 672 *Direct*, 3(September), 1–17. doi: 10.1002/pld3.179  
 673 Geisen, S. (2020). Protists as catalyzers of microbial litter breakdown and carbon cycling at different  
 674 temperature regimes. *The ISME Journal*, 10–13. doi: 10.1038/s41396-020-00792-y  
 675 Gessner, M. O., Bauchrowitz, M. A., & Escutier, M. (1991). Extraction and quantification of ergosterol as  
 676 a measure of fungal biomass in leaf litter. *Microbial Ecology*, 22(1), 285–291. doi:  
 677 10.1007/BF02540230  
 678 Gilbert, D., Amblard, C., Bourdier, G., & Francez, A. J. (1998). The microbial loop at the surface of a  
 679 peatland: Structure, function, and impact of nutrient input. *Microbial Ecology*, 35(1), 83–93. doi:  
 680 10.1007/s002489900062  
 681 Gotelli, N. J., & Ulrich, W. (2012). Statistical challenges in null model analysis. *Oikos*, 121(2), 171–180. doi:  
 682 10.1111/j.1600-0706.2011.20301.x  
 683 Grabowski, M., & Porto, A. (2017). How many more? Sample size determination in studies of  
 684 morphological integration and evolvability. *Methods in Ecology and Evolution*, 8(5), 592–603. doi:  
 685 10.1111/2041-210X.12674  
 686 Grace, J. B., Adler, P. B., Stanley Harpole, W., Borer, E. T., & Seabloom, E. W. (2014). Causal networks  
 687 clarify productivity-richness interrelations, bivariate plots do not. *Functional Ecology*, 28(4), 787–



798. doi: 10.1111/1365-2435.12269

- Grace, J. B., Anderson, T. M., Olff, H., & Scheiner, S. M. (2010). On the specification of structural equation models for ecological systems. *Ecological Monographs*, 80(1), 67–87. doi: 10.1890/09-0464.1
- Gralka, M., Szabo, R., Stocker, R., & Cordero, O. X. (2020). Trophic Interactions and the Drivers of Microbial Community Assembly. *Current Biology*, 30(19), R1176–R1188. doi: 10.1016/j.cub.2020.08.007
- Griffiths, M. J., & Harrison, S. T. L. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Phycology*, 21(5), 493–507. doi: 10.1007/s10811-008-9392-7
- Gui, H., Hyde, K., Xu, J., & Mortimer, P. (2017). Arbuscular mycorrhiza enhance the rate of litter decomposition while inhibiting soil microbial community development. *Scientific Reports*, 7(February), 1–10. doi: 10.1038/srep42184
- Hájek, T., Ballance, S., Limpens, J., Zijlstra, M., & Verhoeven, J. T. A. (2011). Cell-wall polysaccharides play an important role in decay resistance of Sphagnum and actively depressed decomposition in vitro. *Biogeochemistry*, 103(1), 45–57. doi: 10.1007/s10533-010-9444-3
- Hamard, S., Robroek, B. J. M., Allard, P.-M., Signarbieux, C., Zhou, S., Saesong, T., ... Jassey, V. E. J. (2019). Effects of Sphagnum Leachate on Competitive Sphagnum Microbiome Depend on Species and Time. *Frontiers in Microbiology*, 10(September), 1–17. doi: 10.3389/fmicb.2019.02042
- Hansen, A. M., Kraus, T. E. C., Pellerin, B. A., Fleck, J. A., Downing, B. D., & Bergamaschi, B. A. (2016). Optical properties of dissolved organic matter (DOM): Effects of biological and photolytic degradation. *Limnology and Oceanography*, 61(3), 1015–1032. doi: 10.1002/lno.10270
- Hiruma, K. (2019). Roles of Plant-Derived Secondary Metabolites during Interactions with Pathogenic and Beneficial Microbes under Conditions of Environmental Stress. *Microorganisms*, 7(9), 362. doi: 10.3390/microorganisms7090362
- Hodgkins, S. B., Tfaily, M. M., McCalley, C. K., Logan, T. A., Crill, P. M., Saleska, S. R., ... Chanton, J. P. (2014). Changes in peat chemistry associated with permafrost thaw increase greenhouse gas production. *Proceedings of the National Academy of Sciences*, 111(16), 5819–5824. doi: 10.1073/pnas.1314641111
- Hu, L., Robert, C. A. M., Cadot, S., Zhang, X., Ye, M., Li, B., ... Erb, M. (2018). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications*, 9(1), 1–13. doi: 10.1038/s41467-018-05122-7
- Huang, X., Xue, J., Zhang, J., Qin, Y., Meyers, P. A., & Wang, H. (2012). Effect of different wetness conditions on Sphagnum lipid composition in the Erxianyan peatland, central China. *Organic Geochemistry*, 44,

1–7. doi: 10.1016/j.orggeochem.2011.12.005

Isoviita, P. (1966). Studies on Sphagnum L . I . Nomenclatural revision of the European taxa. *Annales Botanici Fennici*, 3(2), 199–264. Retrieved from <https://www.jstor.org/stable/23724595>

Jaffrain, J., Gérard, F., Meyer, M., & Ranger, J. (2007). Assessing the Quality of Dissolved Organic Matter in Forest Soils Using Ultraviolet Absorption Spectrophotometry. *Soil Science Society of America Journal*, 71(6), 1851–1858. doi: 10.2136/sssaj2006.0202

Jassey, V. E. J., Chiapusio, G., Gilbert, D., Buttler, A., Toussaint, M.-L., & Binet, P. (2011). Experimental climate effect on seasonal variability of polyphenol/phenoloxidase interplay along a narrow fen-bog ecological gradient in Sphagnum fallax. *Global Change Biology*, 17(9), 2945–2957. doi: 10.1111/j.1365-2486.2011.02437.x

Jassey, V. E. J., Gilbert, D., Binet, P., Toussaint, M.-L., & Chiapusio, G. (2011). Effect of a temperature gradient on Sphagnum fallax and its associated living microbial communities: a study under controlled conditions. *Canadian Journal of Microbiology*, 57(3), 226–235. doi: 10.1139/w10-116

Jassey, V. E. J., & Signarbieux, C. (2019). Effects of climate warming on Sphagnum photosynthesis in peatlands depend on peat moisture and species-specific anatomical traits . *Global Change Biology*, 25(May), 1–12. doi: 10.1111/gcb.14788

Jassey, V. E. J., Lamentowicz, L., Robroek, B. J. M., Gabka, M., Rusińska, A., & Lamentowicz, M. (2014). Plant functional diversity drives niche-size-structure of dominant microbial consumers along a poor to extremely rich fen gradient. *Journal of Ecology*, 102(5), 1150–1162. doi: 10.1111/1365-2745.12288

Jassey, V. E. J., Reczuga, M. K., Zielińska, M., Słowińska, S., Robroek, B. J. M., Mariotte, P., ... Buttler, A. (2018). Tipping point in plant–fungal interactions under severe drought causes abrupt rise in peatland ecosystem respiration. *Global Change Biology*, 24(3), 972–986. doi: 10.1111/gcb.13928

Jassey, V. E. J., Signarbieux, C., Hättenschwiler, S., Bragazza, L., Buttler, A., Delarue, F., ... Robroek, B. J. M. (2015). An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. *Scientific Reports*, 5, 1–10. doi: 10.1038/srep16931

Kostka, J. E., Weston, D. J., Glass, J. B., Lilleskov, E. A., Shaw, A. J., & Turetsky, M. R. (2016). The Sphagnum microbiome: New insights from an ancient plant lineage. *New Phytologist*, 211(1), 57–64. doi: 10.1111/nph.13993

Laine, A. M., Lindholm, T., Nilsson, M., Kutznetsov, O., Jassey, V. E. J., & Tuittila, E. (2021). Functional diversity and trait composition of vascular plant and Sphagnum moss communities during peatland succession across land uplift regions . *Journal of Ecology*. doi: 10.1111/1365-2745.13601

- Laing, C. G., Granath, G., Belyea, L. R., Allton, K. E., & Rydin, H. (2014). Tradeoffs and scaling of functional traits in Sphagnum as drivers of carbon cycling in peatlands. *Oikos*, 123(7), 817–828. doi: 10.1111/oik.01061
- Laliberté, E., Legendre, P., & Shipley, B. (2015). FD: measuring functional diversity from multiple traits, and other tools for functional ecology. *R Package*, Version 1.0-12.
- Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, 7(5), 573–579. doi: 10.1111/2041-210X.12512
- Leff, J. W., Bardgett, R. D., Wilkinson, A., Jackson, B. G., Pritchard, W. J., De Long, J. R., ... Fierer, N. (2018). Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. *ISME Journal*, 12(7), 1794–1805. doi: 10.1038/s41396-018-0089-x
- Leflaive, J., & Ten-Hage, L. (2007). Algal and cyanobacterial secondary metabolites in freshwaters: A comparison of allelopathic compounds and toxins. *Freshwater Biology*, 52(2), 199–214. doi: 10.1111/j.1365-2427.2006.01689.x
- Legendre, P., & Legendre, L. (2012). *Numerical Ecology*. Elsevier Science.
- Liang, C., Amelung, W., Lehmann, J., & Kästner, M. (2019). Quantitative assessment of microbial necromass contribution to soil organic matter. *Global Change Biology*, 25(11), 3578–3590. doi: 10.1111/gcb.14781
- Liang, C., Schimel, J. P., & Jastrow, J. D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology*, 2(8), 1–6. doi: 10.1038/nmicrobiol.2017.105
- Lindo, Z., & Gonzalez, A. (2010). The bryosphere: An integral and influential component of the Earth's biosphere. *Ecosystems*, 13(4), 612–627. doi: 10.1007/s10021-010-9336-3
- Malik, A. A., Martiny, J. B. H., Brodie, E. L., Martiny, A. C., Treseder, K. K., & Allison, S. D. (2020). Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *ISME Journal*, 14(1), 1–9. doi: 10.1038/s41396-019-0510-0
- Mariotte, P., Robroek, B. J. M., Jassey, V. E. J., & Buttler, A. (2015). Subordinate plants mitigate drought effects on soil ecosystem processes by stimulating fungi. *Functional Ecology*, 29(12), 1578–1586. doi: 10.1111/1365-2435.12467
- Mellegård, H., Stalheim, T., Hormazabal, V., Granum, P. E., & Hardy, S. P. (2009). Antibacterial activity of sphagnum acid and other phenolic compounds found in Sphagnum papillosum against food-borne bacteria. *Letters in Applied Microbiology*, 49(1), 85–90. doi: 10.1111/j.1472-765X.2009.02622.x
- Mitchell, E. A. D., Gilbert, D., Buttler, A., Amblard, C., Grosvernier, P., & Gobat, J.-M. (2003). Structure of microbial communities in Sphagnum peatlands and effect of atmospheric carbon dioxide

784 enrichment. *Microbial Ecology*, 46(2), 187–199. doi: 10.1007/BF03036882

785 Mommer, L., Cotton, T. E. A., Raaijmakers, J. M., Termorshuizen, A. J., van Ruijven, J., Hendriks, M., ...  
 786 Dumbrell, A. J. (2018). Lost in diversity: the interactions between soil-borne fungi, biodiversity and  
 787 plant productivity. *New Phytologist*, 218(2), 542–553. doi: 10.1111/nph.15036

788 Münkemüller, T., Lavergne, S., Bzeznik, B., Dray, S., Jombart, T., Schiffrers, K., & Thuiller, W. (2012). How  
 789 to measure and test phylogenetic signal. *Methods in Ecology and Evolution*, 3(4), 743–756. doi:  
 790 10.1111/j.2041-210X.2012.00196.x

791 Nichols, J. E., & Peteet, D. M. (2019). Rapid expansion of northern peatlands and doubled estimate of  
 792 carbon storage. *Nature Geoscience*, 12(11), 917–921. doi: 10.1038/s41561-019-0454-z

793 Oke, T. A., Turetsky, M. R., Weston, D. J., & Shaw, J. A. (2020). Tradeoffs between phenotypic plasticity  
 794 and local adaptation influence the ecophysiology of the moss, *Sphagnum magellanicum*. *Oecologia*,  
 795 193(4), 867–877. doi: 10.1007/s00442-020-04735-4

796 Opelt, K., Berg, C., Schönmann, S., Eberl, L., & Berg, G. (2007). High specificity but contrasting biodiversity  
 797 of *Sphagnum*-associated bacterial and plant communities in bog ecosystems independent of the  
 798 geographical region. *ISME Journal*, 1(6), 502–516. doi: 10.1038/ismej.2007.58

799 Opelt, K., Chobot, V., Hadacek, F., Schönmann, S., Eberl, L., & Berg, G. (2007). Investigations of the  
 800 astructure and function of bacterial communities associated with *Sphagnum* mosses. *Environmental*  
 801 *Microbiology*, 9(11), 2795–2809. doi: 10.1111/j.1462-2920.2007.01391.x

802 Painter, T. J. (1991). Lindow man, tollund man and other peat-bog bodies: The preservative and  
 803 antimicrobial action of Sphagnum, a reactive glycuronoglycan with tanning and sequestering  
 804 properties. *Carbohydrate Polymers*, 15(2), 123–142. doi: 10.1016/0144-8617(91)90028-B

805 Pinheiro, J. C., & Bates, D. M. (2000). *Mixed-Effects Models in S and S-PLUS*. Springer.

806 Pinton, R., Varanini, Z., & Paolo, N. (2001). *The rhizosphere: biochemistry and organic substances at the*  
 807 *soil–plant interface*. New York: Marcel Dekker, Inc.

808 Putkinen, A., Larmola, T., Tuomivirta, T., Siljanen, H. M. P., Bodrossy, L., Tuittila, E. S., & Fritze, H. (2012).  
 809 Water dispersal of methanotrophic bacteria maintains functional methane oxidation in *Sphagnum*  
 810 mosses. *Frontiers in Microbiology*, 3(JAN), 1–10. doi: 10.3389/fmicb.2012.00015

811 R Core Team. (2019). *R: A language and environment for statistical computing*. Vienna, Austria: R  
 812 Foundation for Statistical Computing. Retrieved from <http://www.r-project.org/>

813 Rasmussen, S., Peters, G., & Rudolph, H. (1995). Regulation of phenylpropanoid metabolism by exogenous  
 814 precursors in axenic cultures of *Sphagnum fallax*. *Physiologia Plantarum*, 95(1), 83–90. doi:  
 815 10.1111/j.1399-3054.1995.tb00812.x

816 Rasmussen, S., Wolff, C., & Rudolph, H. (1995). Compartmentalization of phenolic constituents in  
817 sphagnum. *Phytochemistry*, 38(1), 35–39. doi: 10.1016/0031-9422(94)00650-I

818 Robroek, B. J. M., Jassey, V. E. J., Kox, M. A. R., Berendsen, R. L., Mills, R. T. E., Cécillon, L., ... Bodelier, P.  
819 L. E. (2015). Peatland vascular plant functional types affect methane dynamics by altering microbial  
820 community structure. *Journal of Ecology*, 103(4), 925–934. doi: 10.1111/1365-2745.12413

821 Robroek, B. J. M., Jassey, V. E. J., Payne, R. J., Martí, M., Bragazza, L., Bleeker, A., ... Verhoeven, J. T. A.  
822 (2017). Taxonomic and functional turnover are decoupled in European peat bogs. *Nature*  
823 *Communications*, 8(1). doi: 10.1038/s41467-017-01350-5

824 Robroek, B. J. M., Martí, M., Svensson, B. H., Dumont, M. G., Veraart, A. J., & Jassey, V. E. J. (2021).  
825 Rewiring of peatland plant–microbe networks outpaces species turnover. *Oikos*, 130(3), 339–353.  
826 doi: 10.1111/oik.07635

827 Rudolph, H., & Samland, J. (1985). Occurrence and metabolism of sphagnum acid in the cell walls of  
828 bryophytes. *Phytochemistry*, 24(4), 745–749. doi: 10.1016/S0031-9422(00)84888-8

829 Rydin, H., & Jeglum, J. K. (2006). *The biology of peatlands*. United Kingdom Oxford: Oxford University  
830 Press.

831 Såstad, S.M., & Flatberg, K. I. (1993). Leaf morphology of *Sphagnum strictum* in Norway, related to habitat  
832 characteristics. *Lindbergia*, 18(2), 71–77. doi: 10.2307/20149834

833 Såstad, Sigurd M., Pedersen, B., & Digre, K. (1999). Habitat-specific genetic effects on growth rate and  
834 morphology across pH and water-level gradients within a population of the moss *Sphagnum*  
835 *angustifolium* (Sphagnaceae). *American Journal of Botany*, 86(12), 1687–1698. doi:  
836 10.2307/2656667

837 Schellekens, J., Bindler, R., Martínez-Cortizas, A., McClymont, E. L., Abbott, G. D., Biester, H., ... Buurman,  
838 P. (2015). Preferential degradation of polyphenols from *Sphagnum* – 4-Isopropenylphenol as a proxy  
839 for past hydrological conditions in *Sphagnum*-dominated peat. *Geochimica et Cosmochimica Acta*,  
840 150, 74–89. doi: <https://doi.org/10.1016/j.gca.2014.12.003>

841 Schlesinger, W. H., & Andrews, J. A. (2000). Soil respiration and the global carbon cycle. *Biogeochemistry*,  
842 48, 7–20. doi: 10.1023/A:1006247623877

843 Singh, B. K., Bardgett, R. D., Smith, P., & Reay, D. S. (2010). Microorganisms and climate change: Terrestrial  
844 feedbacks and mitigation options. *Nature Reviews Microbiology*, 8(11), 779–790. doi:  
845 10.1038/nrmicro2439

846 Singh, B. K., Dawson, L. A., Macdonald, C. A., & Buckland, S. M. (2009). Impact of biotic and abiotic  
847 interaction on soil microbial communities and functions: A field study. *Applied Soil Ecology*, 41(3),

239–248. doi: 10.1016/j.apsoil.2008.10.003

Stalheim, T., Ballance, S., Christensen, B. E., & Granum, P. E. (2009). Sphagnan - A pectin-like polymer isolated from Sphagnum moss can inhibit the growth of some typical food spoilage and food poisoning bacteria by lowering the pH. *Journal of Applied Microbiology*, 106(3), 967–976. doi: 10.1111/j.1365-2672.2008.04057.x

Sweeney, C. J., de Vries, F. T., van Dongen, B. E., & Bardgett, R. D. (2020). Root traits explain rhizosphere fungal community composition among temperate grassland plant species. *New Phytologist*, 53(9), 16976. doi: 10.1111/nph.16976

Sytiuk, A., Cereghino, R., Hamard, S., Delarue, F. F., Dorrepaal, E., Kuttim, M., ... Jassey, V. E. J. (2020). Morphological and biochemical responses of Sphagnum mosses to environmental changes. *BioRxiv*, 2020.10.29.360388. doi: 10.1101/2020.10.29.360388

Tetemadze, N., Bakuridze, A., Jokhadze, M., & Machutadze, I. (2018). Peculiarities of the composition of acids in Sphagnum species of the percolation bog of the Kolkheti lowland. *Annals of Agrarian Science*, 16(2), 222–225. doi: 10.1016/j.aasci.2018.04.012

Turetsky, M. R. (2003). New Frontiers in Bryology and Lichenology The Role of Bryophytes in Carbon and Nitrogen Cycling. *The Bryologist*, 106(3), 395–409. doi: 10.1016/S1364-8152(03)00155-5

Turetsky, M. R., Crow, S. E., Evans, R. J., Vitt, D. H., & Wieder, R. K. (2008). Trade-offs in resource allocation among moss species control decomposition in boreal peatlands. *Journal of Ecology*, 96(6), 1297–1305. doi: 10.1111/j.1365-2745.2008.01438.x

Urbanová, Z., & Bárta, J. (2016). Effects of long-term drainage on microbial community composition vary between peatland types. *Soil Biology and Biochemistry*, 92, 16–26. doi: 10.1016/j.soilbio.2015.09.017

van Breemen, N. (1995). How Sphagnum bogs down other plants. *Trends in Ecology & Evolution*, 10(7), 270–275. doi: 10.1016/0169-5347(95)90007-1

van Dam, N. M., & Bouwmeester, H. J. (2016). Metabolomics in the Rhizosphere: Tapping into Belowground Chemical Communication. *Trends in Plant Science*, 21(3), 256–265. doi: 10.1016/j.tplants.2016.01.008

Veen, G. F. (Ciska), ten Hooven, F. C., Weser, C., & Hannula, S. E. (2021). Steering the soil microbiome by repeated litter addition. *Journal of Ecology*, 9(1), 1365-2745.13662. doi: 10.1111/1365-2745.13662

Verhoeven, J. T. A., & Liefveld, W. M. (1997). The ecological significance of organochemical compounds in Sphagnum. *Acta Botanica Neerlandica*, 46(2), 117–130. doi: 10.1111/plb.1997.46.2.117

Verhoeven, J. T. A., & Toth, E. (1995). Decomposition of Carex and Sphagnum litter in fens: Effect of litter

880           quality and inhibition by living tissue homogenates. *Soil Biology and Biochemistry*, 27(3), 271–275.  
881           doi: 10.1016/0038-0717(94)00183-2

882 Vitt, D. H. (2000). *Peatlands : ecosystems dominated by bryophytes*.

883 Wang, C., Michalet, R., Liu, Z., Jiang, X., Wang, X., Zhang, G., ... Xiao, S. (2020). Disentangling Large- and  
884           Small-Scale Abiotic and Biotic Factors Shaping Soil Microbial Communities in an Alpine Cushion Plant  
885           System. *Frontiers in Microbiology*, 11(May), 1–17. doi: 10.3389/fmicb.2020.00925

886 Wardle, D. A., Bardgett, R. D. R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H., Wall, D. H., ... Wall,  
887           D. H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, 304(5677),  
888           1629–1633. doi: 10.1126/science.1094875

889

890 **Tables**

891 Table 1. Site conditions and climatic data of the study sites

Site	location	Longitude	Latitude	Altitude	Mean annual temperature	Annual precipitation	pH (pore water)*	Water table depth*	Trophic state	Dominant <i>Sphagnum</i> on the site
FR	Counozouls (France)	2°14'02.4"	42°41'19.7"	1374 m	7.9 °C	1027 mm	4.90	16.5 cm	poor fen	<i>Sphagnum warnstorffii</i>
PL	Kusowo (Poland)	16°35'12.1"	53°48'47.9"	145 m	7.3 °C	656 mm	3.56	60 cm	bog	<i>Sphagnum magellanicum</i>
EST	Männikjärve (Estonia)	26°15'03.6"	58°52'26.4"	82 m	4.9 °C	623 mm	4.11	20 cm	bog	<i>Sphagnum rubellum</i>
FI	Siikaneva (Finland)	24°17'17.5"	61°50'41.6"	160 m	2.9 °C	611 mm	3.86	8 cm	poor fen	<i>Sphagnum papillosum</i>
SE	Abisko (Sweden)	19°03'58.7"	68°20'43.1"	281 m	- 0.1 °C	418 mm	3.83	10 cm	bog	<i>Sphagnum balticum</i>

\*Measured in early July 2018

892



## Figure captions

**Figure 1.** *A priori* conceptual structural equation model (SEM) depicting pathways by which climate and edaphic conditions (standardized data of annual precipitation (clim1), mean temperature of the wettest quarter (clim2), *Sphagnum* water content (local1), PC1 of WEOM chemistry (local2), PCoA1 of *Sphagnum* anatomical and morphological traits (anatom) and metabolites (metab) can affect microbial community composition (PcoA1 of tot.biom=total microbial biomass; Hellinger transformation of decomp.=decomposers, consum.=consumers, phototr.=phototrophs) and traits (PCoA 1 of tot.traits=total traits, yield=growth yield, res.acq.=resource acquisition, stress=stress tolerance). (A) a single model, (B) an interaction model, (C) a single model with *Sphagnum* traits (D) an interaction model with *Sphagnum* traits. Thin lines indicate a single path, while thicker lines indicate that any climatic/edaphic parameter affected each representative of microbial community composition (or the sum of them) and/or microbial trait composition (or the sum of them).

**Figure 2.** *Sphagnum* anatomical and morphological traits and metabolites data. A) Principal coordinates analysis (PCoA) on the Gower dissimilarity matrix of *Sphagnum* anatomical and morphological traits and metabolites for five dominant species collected along a gradient. Groups are colored according to *Sphagnum* species sampled in sites spanning from south to north. B) *Sphagnum* phylogenetic tree and normalized means of *Sphagnum* anatomical and morphological traits and metabolites. The square shape represents mean values of anatomical and morphological traits, while circle shape – mean values of metabolites. The size of mean is represented from the smallest (the smallest circle/square) to the highest (the highest circle/square) values of anatomical and morphological traits and metabolites.

**Figure 3.** Microbial community composition and traits composition. Upper panels: *Sphagnum* phylogenetic tree with the corresponding heatmap showing the dissimilarities in (A) the composition of each trophic group components in which colors represent the standardized value calculated from

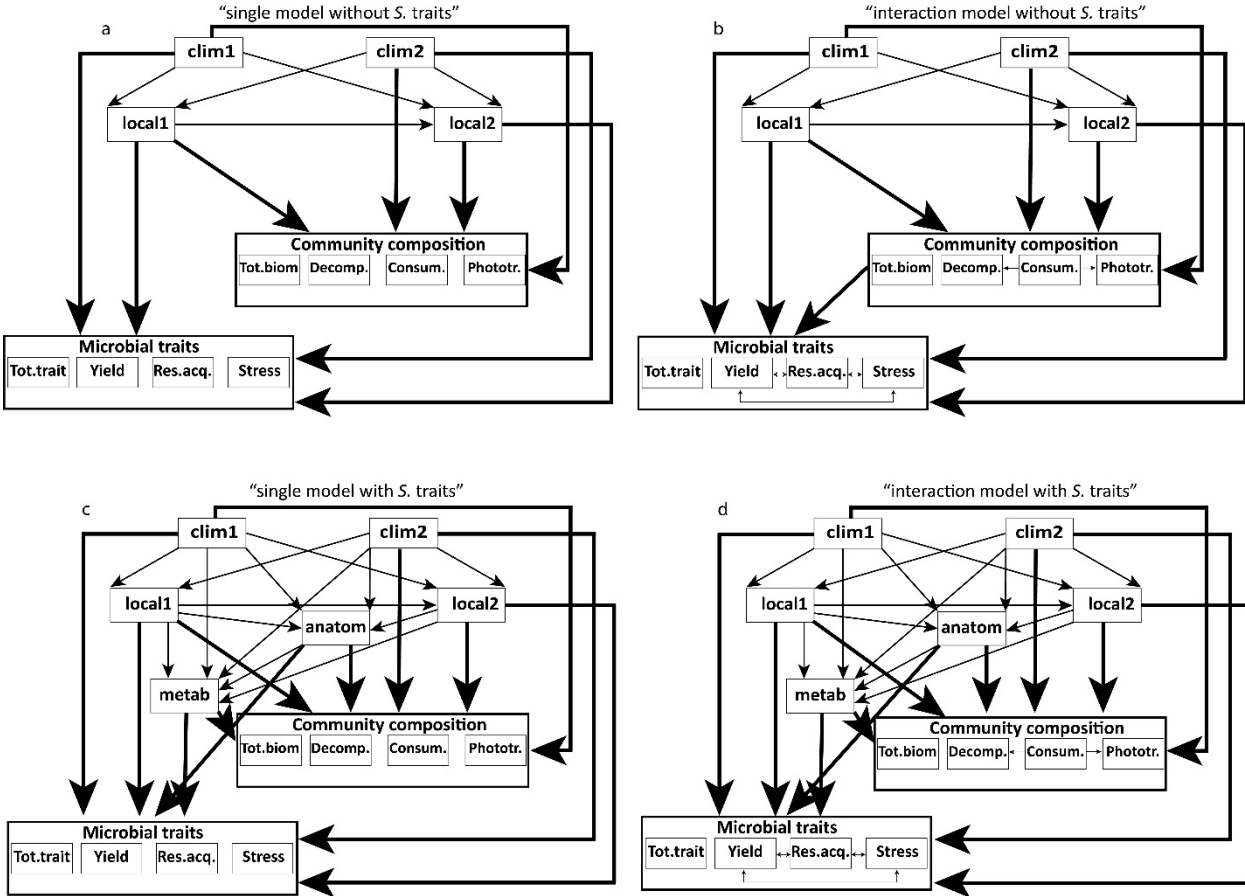
standardized means of microbial biomass, and (B) the microbial traits composition in which colors represent the standardized value calculated from the first PCoA on the Gower dissimilarity matrix of microbial trait composition. Lower panels: Principal coordinates analysis (PCoA) on the Gower dissimilarity matrix of (C) the microbial community composition based on the abundance of all microbes (micro-eukaryotic species cyanobacteria, fungi and non-photosynthetic bacteria) and (D) the microbial traits composition. Groups are colored according to *Sphagnum* species sampled in sites spanning from south to north.

**Figure 4.** Correlation table on the relationships between climatic and edaphic conditions (standardized data of annual precipitation (an. precip.), the mean temperature of the wettest quarter (war. temp.), PC1 of WEOM chemistry (WEOM. chem.), *Sphagnum* water content (*S.wat.cont.*), PCoA1 of *Sphagnum* anatomical and morphological traits and metabolites (*S. traits*) and microbial community composition and traits (PCoA1). Correlations with  $P < 0.05$  only are shown.

**Figure 5.** Outputs of the SEMs when *Sphagnum* traits were included in SEMs for microbial community composition and microbial traits composition: (A) the benefits gained ( $\Delta R^2$ ) and (B) Akaike Information Criteria (AIC) values. \*Full summed models= Full model with PCoA1 for total microbial biomass and PCoA1 for total microbial traits. All details about SEMs including  $R^2$ ,  $P$ -values, Fisher's  $C$ , path explanations are provided in Tables S6-S10.

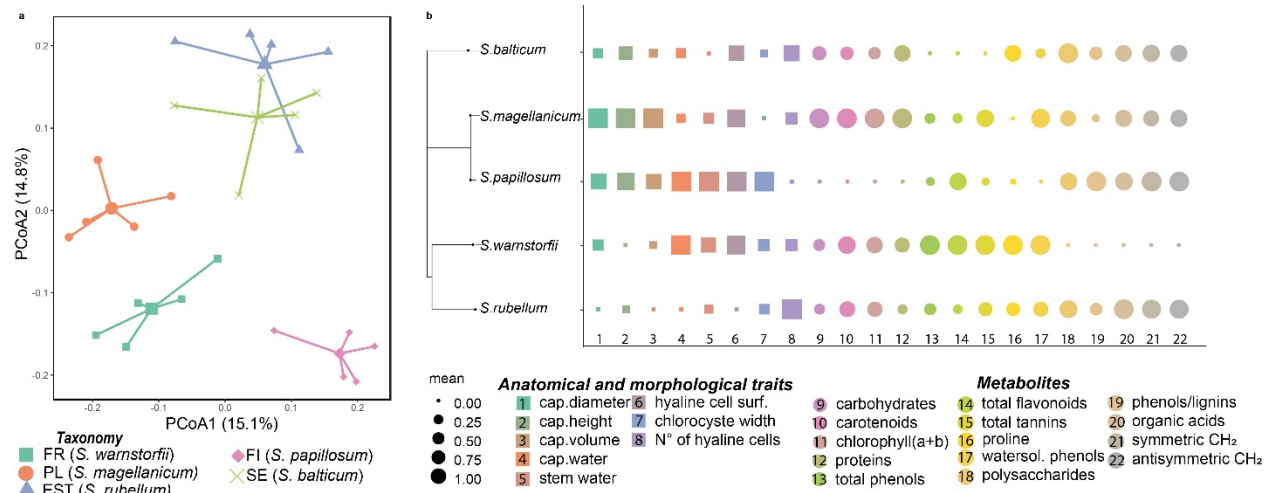
**Figure 6.** The relationship between differences in the microbial community composition (sum of Hellinger-transformed microbial biomass per trophic group) and their traits (PCoA1 axes) and individual *Sphagnum* traits. Points represent Spearman correlation coefficients (Rho) and their significance ( $P < 0.05$ ).

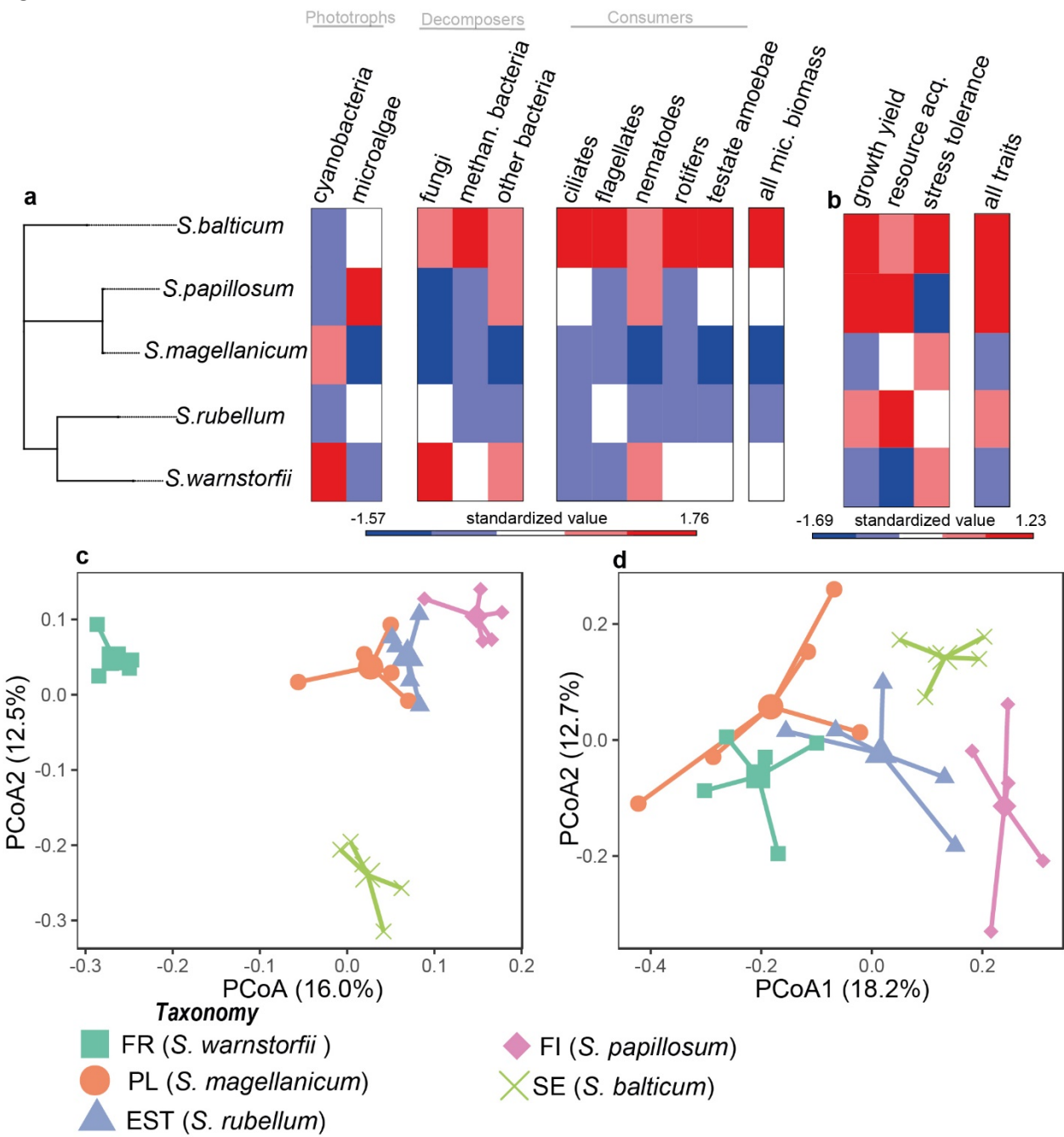
937 Figure 1



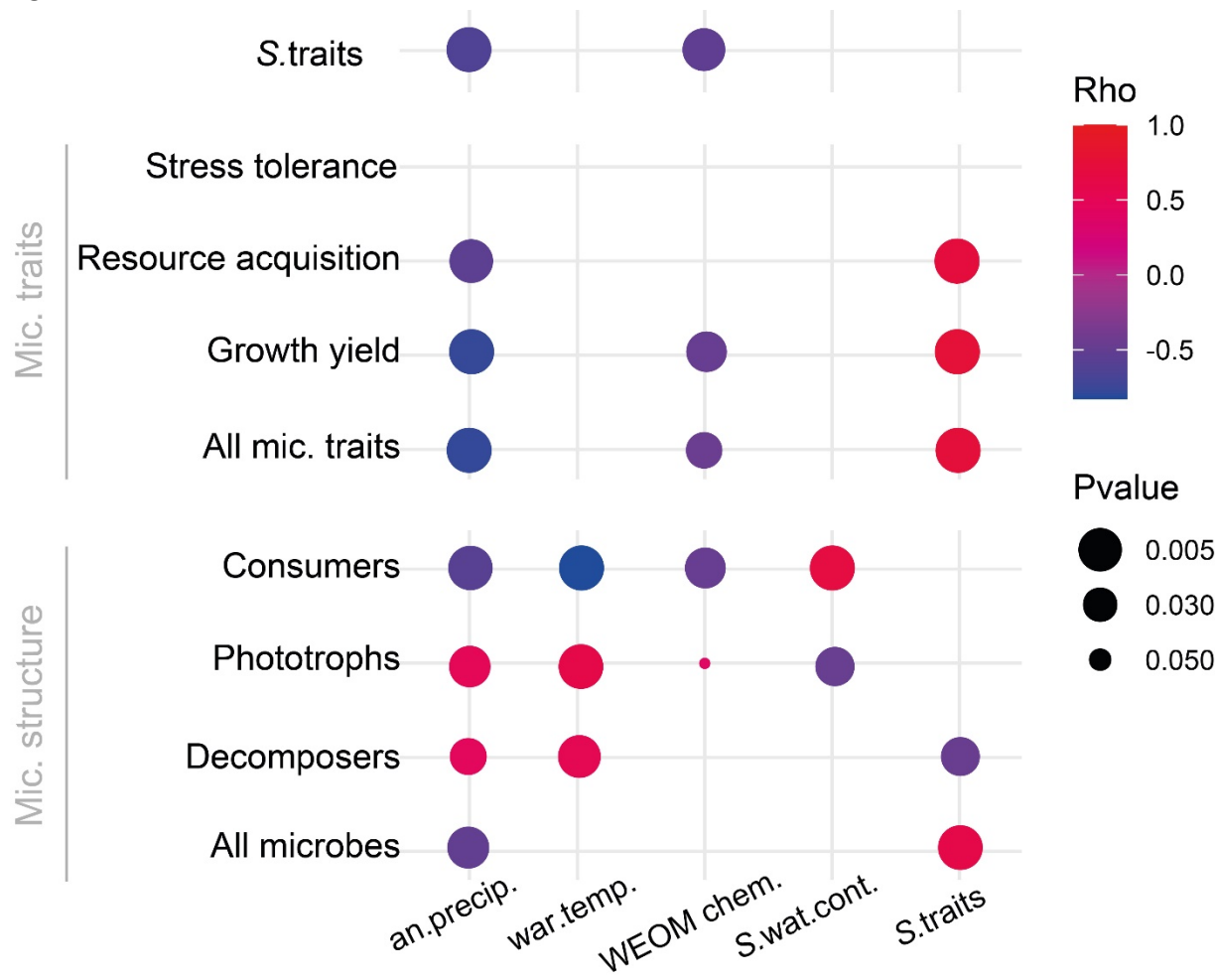
938  
939

940 Figure 2



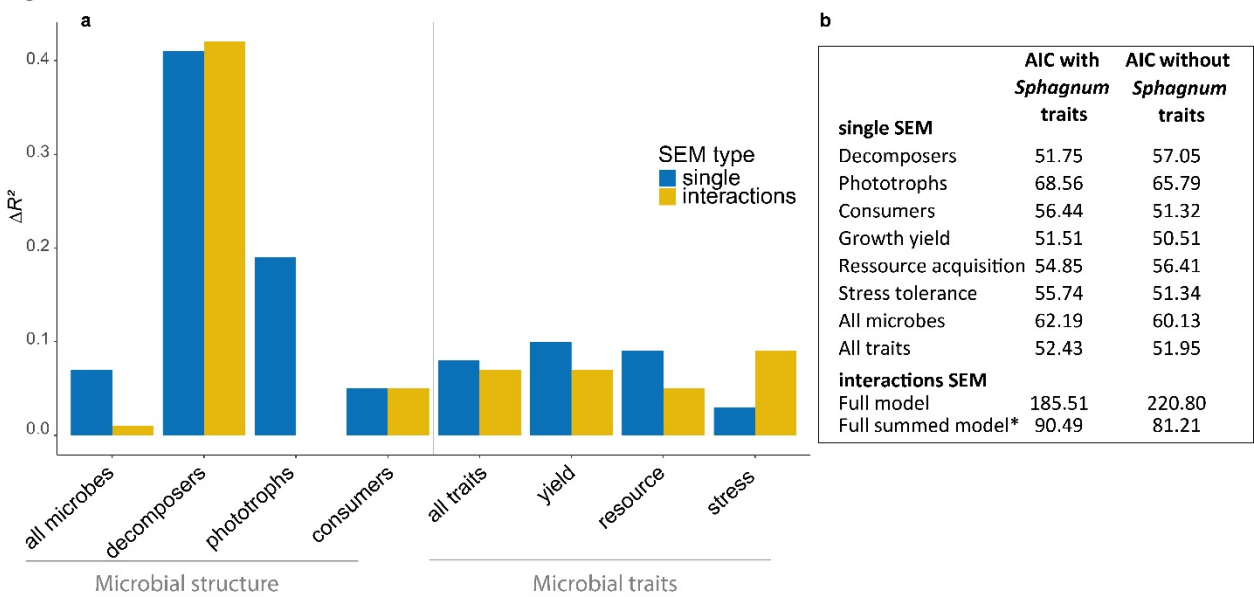


946 Figure 4



947  
948

949 Figure 5



950  
951

