Supplementary Information (SI)

Vegetation and Microbes Interact to Preserve Carbon in many Wooded Peatlands.

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Supplementary Note 1

Brief introduction and results for separating the relative contributions of fungi and bacteria to peat decomposition

Fungi are more tolerant to low pH and low nutrients[1-4] than bacteria and are more efficient in degrading complex polymers[5] like recalcitrant cellulose, hemicellulose and polyphenols[6]. Many studies have suggested that fungi are the principal decomposers in ombrotrophic bogs with low pH and high C/N ratios[7,8], and thus playing a dominant role over bacteria in peat decomposition[5,9]. Much higher fungal than bacterial biomass has been observed in bogs globally[10,11]. While three studies have shown that bacteria had a significant, even dominant, impact on the glucose-induced respiration[12-14] that was extended to peat decomposition. Such conclusion in these studies was based mainly on a selective inhibition (SI) of glucose-induced respiration[15], in which bactericides (e.g. streptomycin sulphate) and fungicides (e.g. cycloheximide), were separately used to inhibit protein synthesis in prokaryotic and eukaryotic cells. The SI technique works well in most organic-poor mineral soils[16] where glucose solution was used as substrate for both bacteria and fungi. However, the chemistry and complexity of carbon in organic-rich peatlands are distinctively different from organic-poor mineral soils. There are plenty of carbon sources for microbes in peat, while most of them are recalcitrant compounds. The addition of labile glucose to peats using the SI technique may quickly favor the fast growth of labile-carbon craving bacteria that are present but are not active initially in peatlands. Thus, in a short-incubation period this would lead to a false conclusion that bacteria play a major role in soil respiration, which cannot represent the dominant decomposing activities in the recalcitrant peat that initially lacks labile carbon. We also noticed that, without adding glucose, the added streptomycin and cycloheximide did not inhibit soil respiration across 3

ecologically and hydrologically diverse and spatially dispersed peatlands in Canada[12]. Hackney et al. (2000) also found that bacteria contributed only about 30% of fresh litter (that is less recalcitrant than peat) decomposition in the Everglades peatland[17]. To parse out fungi and bacterial contribution to peat decomposition, we first conducted SI-technique experiments without addition of glucose, but all tests failed to separate the fungal and bacterial contributions. We then physically separated bacteria from fungi based on their body sizes using porous filters. as noted earlier.

The results are showed in Supplementary Fig. 1. The soil respiration in the *Sphagnum*-formed peat was always >3 times higher than that in the shrub-formed peat treated with the inoculum through the same pore-size filter. Both the *Sphagnum*- and the shrub-formed peats inoculated by the 0.22-μm and 0.45-μm filtrates emitted CO_2 at low rates similar to the control, which means 1) the bacteria with a diameter <0.45-μm in our sites only had minor contribution to peat decomposition, 2) and the low soil respiration we detected in the controls likely came from air-borne contamination microbes during the operation. The difference of soil respiration between 1.2 or 1.5-μm filtrate and control was considered the major bacterial contribution to peat decomposition, which was 6.6% and 4.8% in the *Sphagnum*- and the shrub-formed peats, respectively. As macro-decomposers (insects and worms) were removed, the remaining respiration (>90%) was therefore contributed by the fungi that we had earlier measured in both our sites. Therefore, our results, consistent with many previous studies[5,9], suggest that fungi, not bacteria, dominantly control decomposition of acidic and highly recalcitrant peat, particularly in the unsaturated surficial layer.

Supplementary Methods

Tree Height, Vegetation Cover, and Biomass Estimates

In the Dajiuhu peatland, we set up quadrates of 2m×2m and 1m×1m for shrubs and non-shrub plants survey in the shrubbed areas in the summer of 2014. Abundance, height and cover of plants were surveyed. The abundance of non-*Sphagnum* plants was counted while that of *Sphagnum* was estimated by counting for 3 10 cm × 10 cm sub-quadrates. Vascular plant height was measured from their top to 12 cm (where stem and leave color change from white to brown) below moss surface with a steel tap and *Sphagnum* height was recorded as 15 cm. Plant species cover was estimated by visual observation.

For all other study sites, we obtained data from field sampling plots in conjunction with satellite photo to estimate plant height, vegetation cover, and biomass in a vast scale of land area. Based on our ground truth survey in Pocosin site, land covers are classified into four vegetation types, tree, shrub, herb and moss. Trees include arborous hardwoods and conifers; shrubs are muli-stemmed woody plants; herbs are other low stature vascular plants including broadleaf flowering plants, graminoids and ferns; and mosses include mosses and lichens.

Tree Height

Tree height measurement was carried out by interpreting satellite photo images. We measured tree shadow directions of the interest areas in Google Earth. Because trees do not always grow vertically toward zenith, we first average a dozen of tree shadow directions, clockwise from north. Using information of the shadow direction in conjunction with image date and location, we could figure out what time the image was taken. At the same time, what azimuth of the sun position from the north and what the solar angle above horizon can be obtained. Once the solar altitude, angle α, is available, a simple trigonometry equation can back calculate to estimate the tree height, H, from the shadow length, l, on ground.

$$H = l \times \tan(\alpha)$$

A total of 66 tree height estimates were taken from Pocosin and 22 each from Marcell and Calvert sites. Although efficient, the method is not without limitation. Tree shadows require a relatively even ground to cast a complete tree crown to be measured. In Pocosin site tree crowns are distinct from one another and the shadows are obvious. In other sites, trees are crowded. Individual tree shadow is not obvious. Measurements of shadows need to be done in off-site vegetation with comparable texture, where individual tree shadows were casted on a bog, fen, or open water surface. We applied a factor of ×0.75 for Marcell and ×0.85 for Calvert as correction because trees located on the edge of ecotone received less competition and grew to larger sizes compared with those in the inland sampling sites. As a result, average tree height we calculated in Marcell Forest is comparable to that reported in Weishampel et al. (2009)[18].

Vegetation Cover

Like tree height estimate, we first used satellite photos from Google Earth (dated 3/22/2017 for Pocosin, 8/21/2013 for Marcell and 9/20/2017 for Calvert images) to estimate areal covers of the four vegetation types. Depending on neighboring vegetation around sampling site, by avoiding artificial structures, roads and buildings and open water, we chose an area of image 900 m × 900 m for Pocosin, 700 m × 700 m for Marcell, and 500 m × 500 m for Calvert sites. Aided by Google Earth 3-D option, we first identified standing trees and erased the areas of tree crown from the image with Photoshop Elements software. The color image was then converted to grey scale in NIH's ImageJ processor. After making the grey scale into binary, a histogram was then created. The relative count of value = 0 (white) is the portion of tree crown areal cover, [C_t]. The same process is repeated to obtain area for both tree and shrub, [$C_t + C_s$], then total tree and shrub plus herb cover, [$C_t + C_s + C_h$]. Taking differences among them could yield individual relative cover of each vegetation type, including the remaining moss cover, C_m.

$$[C_s] = [C_t + C_s] - [C_t]$$

$$[C_h] = [C_t + C_s + C_h] - [C_t + C_s]$$

$$[C_m] = 1 - [C_t + C_s + C_h]$$

With ground survey experience, we add a factor of ×0.3 area under the tree crown as additional shrub cover since their presence is not mutually exclusive in the same location. The overlapped stratification is particularly obvious in Marcell and Dajiuhu sites, where *Sphagnum* spp. is dominant ground cover.

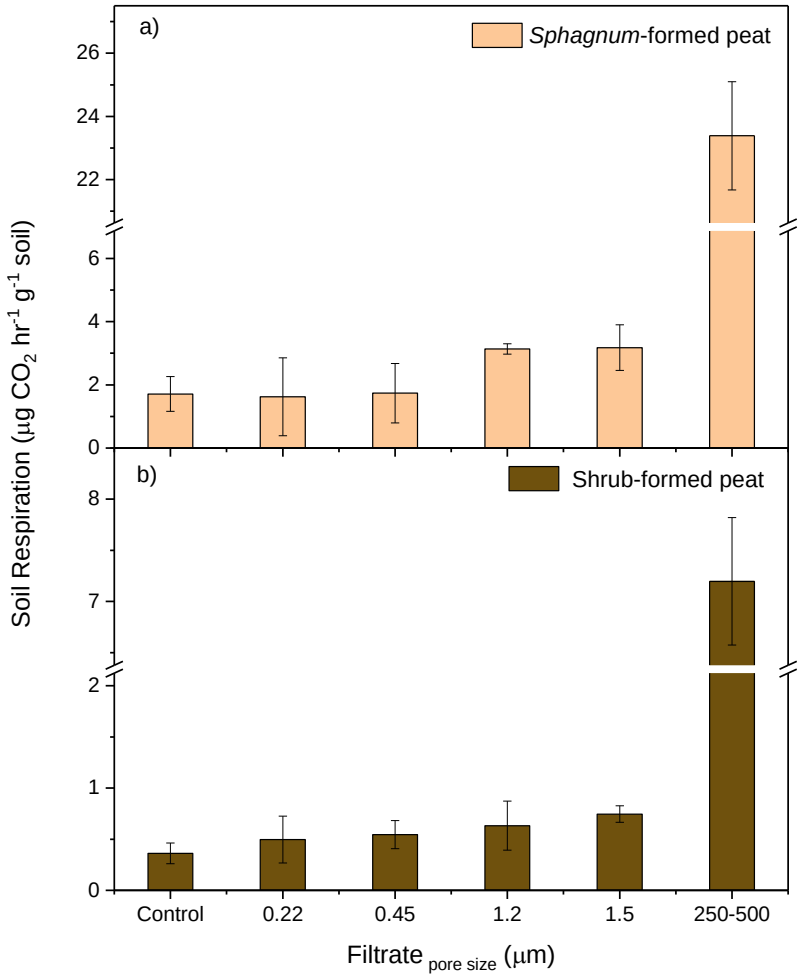
Biomass

To obtain above biomass, we measured individual trees' DBH (diameter at breast height) of each species and their canopy vertical projection areal covers in Pocosin site. We selected trees that had individual canopy covering about 100 m^2 or a number of individuals of the same species to amount to 100 m^2. Once the DBH are tallied, equations are used to calculate tree species biomass per unit canopy area. For large woody shrub such as *Cyrilla racemiflora*, *Morella cerifera*, we first estimate its canopy areal cover, then harvest the entire standing stock and obtain oven-dry weight in lab. For small shrub such as *Ilex glabra*, *Lyonia lucida*, *Zenobia pulverulenta*, we collected entire plant materials, separating different species, within 2 m × 2 m quadrat for biomass estimate. Data of large shrubs and small shrubs then combine based on proportions of different species occurrence on site. Multiple species shrub biomass per unit is then calculated. Land covers other than trees and shrubs are mostly occupied by the widely distributed *Pteridium aquilinum*, *Woodwardia virginica* and *Carex striata* in Pocosin site. We measured this fern biomass from 4 replicates of 1 m × 1 m quadrat. We assumed the majority of moss type vegetation consisted of *Sphagnum* spp. mosses although *Cladonia* spp. lichens were widespread in Pocosin site as well. By using biomass enumerated in Pocosin site, where prior ground survey

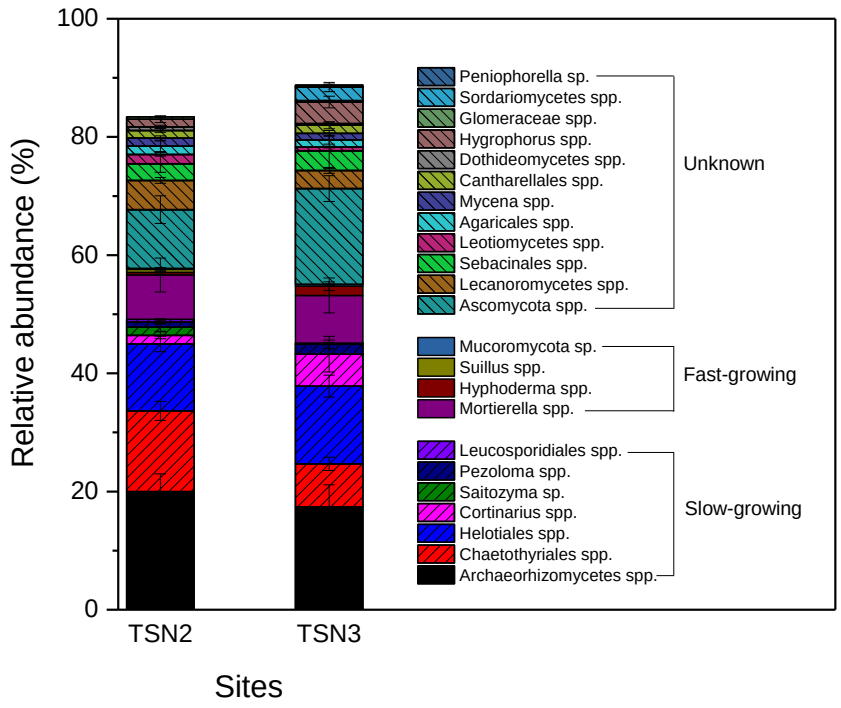
had been conducted, we extrapolated it to estimate biomass of three other sites. Proportional to tree height and relative cover, a biomass estimate can be extrapolated for each vegetation type at different site with high confidence. Again we compared the biomass estimate from Marcell site with published data[18] and the results are fairly close. Measurement of moss and other cryptogam biomass has not been an easy task. Since the main components of moss type vegetation are *Sphagnum*, we employed Clymo (1970) method[19] to make an estimate. The top 1 cm of capitulae weighed 2 g and the rest of *Sphagnum* body weighed 2 g per ~10 cm length in 1 m^2 area.

Bioinformatic analysis

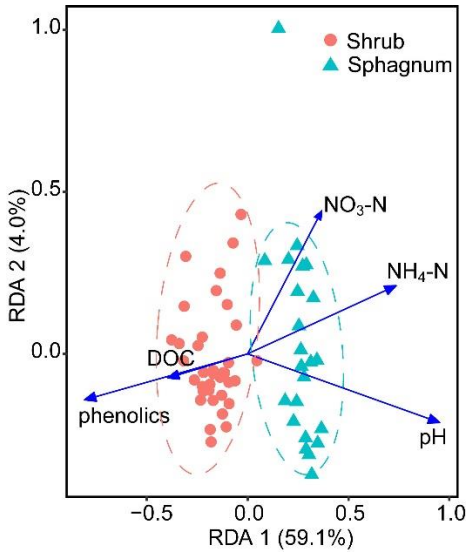
The trimming, sorting and quality filtering of reads were processed using the QIIME Pipeline[20]. All sequences were demultiplexed into samples based on unique identification barcodes using the split_libraries_fastq.py script. As ITS1f and ITS4 primers are too long to pair-end, the sequences from both primers were processed separately. Sequences were sorted by gene region and primers were removed using cutadapt, and then reads were trimmed to 220 bp using -fastq_filter with a q10 minimum value as a quality filter. Sequences were dereplicated and singletons were removed. The ITS1 and ITS2 region from the remaining sequences was identified and extracted using the fungal ITSx software[21]. Chimeras were detected and eliminated using the UCHIME program[22] referenced with the UNITE database (https://unite.ut.ee/)[23]. Non-chimera sequences that passed the filtering processes were binned into operational taxonomic units (OTUs) at a 97% similarity cutoff using the UPARSE pipeline[22]. All singletons OTU were excluded from the dataset. Subsequently, the taxonomic identity of each OTU was determined based on BLAST search against the UNITE reference database. The quality and depth of coverage of both primers reads were not significant difference, thus libraries from ITS4 reads were used for further analysis of fungal communities.



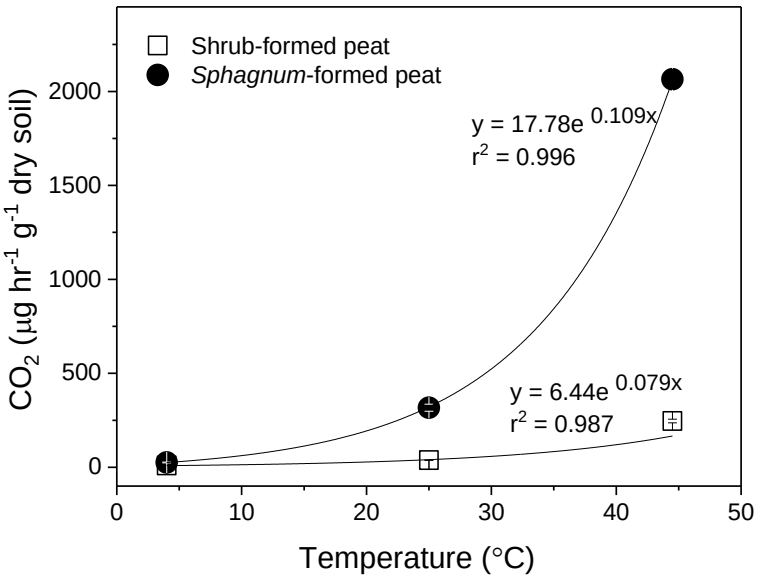
Supplementary Fig. 1 Soil respiration from sterilized *Sphagnum*-formed and shrub-formed peats inoculated by its own filtrate through different pore sizes of filters. Panel a) and b) represent *Sphagnum*- and shrub-form peats, respectively. The filtrate through 0.22(nylon), 0.45 (nylon), both 1.2 (glass fiber) and 1.5-μm (glass fiber), and 250-500- μm filters contain no-bacteria/ fungi, nano-bacteria & non-fungi, most bacteria & non-fungi, and all bacteria & fungi without macro-decomposers like insects and worms. Error bar represents S.E.



Supplementary Fig. 2 Distribution of dominant fungal community in the bog forest and *Sphagnum*-shrub mixed peat bog in Canada. Relative abundance (mean ± S.E.) of the dominant fungi (OTU relative abundance >0.1%) in a bog forest (TSN2) and a *Sphagnum*-shrub mixed peat bog (TSN3) in the Pacific coastal temperate rainforest in Canada. The ecological traits of the dominant fungi present in Supplementary Table 3. The relative abundances were recalculated from raw amplicon reads in the European Nucleotide Archive [ITS(ERS1798771-ERS1799064)] (ref.24).



Supplementary Fig. 3 Redundancy analysis (RDA) of the relationship between soil variables (constraining variable) and the weighted Bray-Curtis distances between fungal communities. Dots indicate individual shrub-formed peat samples; triangles indicate individual *Sphagnum*-formed peat samples.



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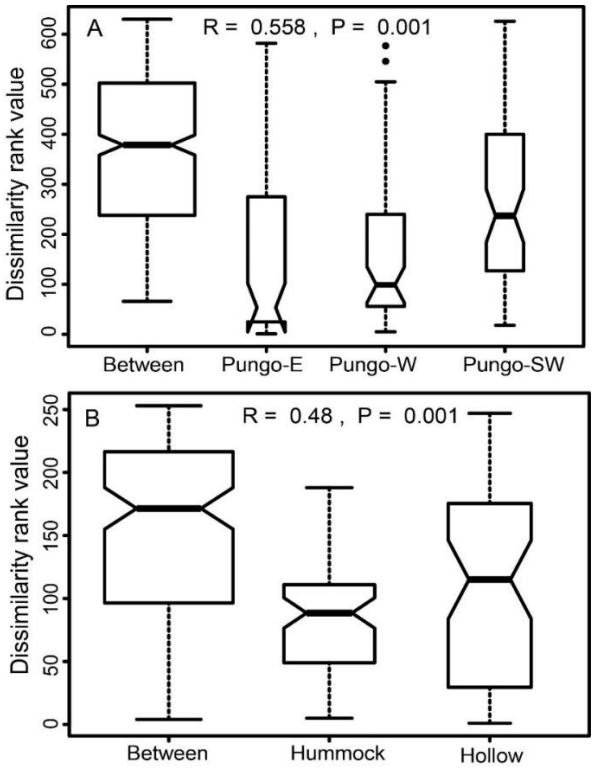
189 **Supplementary Fig. 4 Temperature sensitivity of soil respiration in the subtropical shrub-**

190 **formed and the boreal *Sphagnum*-formed peats through a lab incubation experiment.**

191 Regression analyses were run using $R = \alpha e^{\beta T}$, where R is soil respiration, coefficient α is the

192 intercept of soil respiration when temperature is zero, coefficient β represents the temperature

193 sensitivity of soil respiration and T is soil temperature. Error bar represents S.E.



Supplementary Fig. 5 Analysis of similarities (ANOSIM) plot showing dissimilarity between and within sites at (A) the shrub-dominated peatlands in NC and (B) the *Sphagnum*-dominated peatlands in MN. Bold horizontal bar in the box indicates median; bottom of the box indicates 25th percentile; top of the box indicates 75th percentile; whiskers extend to the most extreme data point, which is no more than the range (i.e. 1.5) times the interquartile range from the box; width of the bar is directly proportional to sample size.

Supplementary Table 1 Characterization of research sites at Pocosin Lakes National Wildlife Refuge, Marcell Experimental Forest and Dajiuhu, and Canada

	Pocosin Lakes National Wildlife Refuge, USA			Marcell Experimental Forest, USA		Dajiuhu Peatlands,China	Calvert Island Field Station (ref. [24]),Canada	
Sites	Pungo West	Pungo Southwest	Pungo-East	S1H4S1 (Hollow)	S1H4L1 (Hummock)	N/A	TSN2 (Bog forest)	TSN3 (*Sphagnum*/shrub bog)
Coordinate	35.68882°, -76.56437°	35.673478°, -76.5581°	35.69078°, -76.52856°	47.50871°, -93.45240°	47.5084°, -93.45163°	31.48333°, 109. 98333°	51.65222°, -128.19272°	51.65139°, -128.12861°
Dominant Species	*Ilex glabra, Lyonia lucida*	*Ilex glabra, Lyonia lucida, Woodwardia virginica*	*Lyonia lucida, Ilex glabra, Zenobia pulverulenta, Gaylussacia frondosa, Vaccinium formosum, Pinus serotina*	*S. fallax S. angustifolium, Picea mariana, Pinus resinosa, Larix laricina,*	*S. angustifolium, S. magellanicum, Rhodendron groenlandicum, Chamaedaphne calyculata, Vaccinium oxycoccos, Picea mariana, Pinus resinosa, Larix laricina,*	*Sphagnum palustre Betula albosinensis Spiraea salicifolia*	*Pinuscontorta, Chamaecyparis nootkatensis, andThuja plicata.*	Abundant ericaceous shrubs and *Sphagnum* spp
Mean Water Level	*-39.6**	*-22.0**	*-30.5**	N/A	N/A	N/A	*-31.7*	*-40.7*
MAT(°C)	16.8			3.3		7.2	N/A	N/A
Soluble Phenolics in soil (μg C/g dry soil)	858±64	1171±98	658±137	114±11	107±21	89±26	N/A	N/A
TIN (μg N/g dry soil)	76±8	37±10	49±4	202±28	130±23	90±16	30.1±4.5	55.7±8.2
TN	1.89±0.08	1.88±0.07	1.11±0.04	1.42±0.14	1.35±0.24	N/A	1.13±0.04	1.40±0.05
TC (%)	55.91±0.78	51.33±0.71	50.09±0.93	43.69±1.02	44.52±2.58	38.0±7.4	55.5±0.9	57.6±1.1
pH	4.06±0.05	4.19±0.04	3.86±0.08	5.00±0.06	4.76±0.09	4.7±0.12	3.76±0.04	3.72±0.06
SM (%)	241±33	322±15	253±29	1214±91	1328±117	398±104	`966±43	820±54

MAT = Mean Annual Temperature
TIN =Total Soil Inorganic Nitrogen (NO_x-N + NH_4-N)
TN =Total Soil Nitrogen
SM =Soil Moisture
TC = Total Soil Carbon
N/A = not available
* mean water level from 3/17/2015 to 10/05/2017 at Pocosin Lakes National Wildlife Refuge

Supplementary Table 2 Vegetation information of research sites at Pocosin Lakes National Wildlife Refuge (PLNWR), Marcell Experimental Forest (MEF) and Dajiuhu (DJH), and Calvert Island Field Station in Canada (CIFS)

	Height (m)				Coverage (%)				Aboveground Biomass (Mg/ha)			
Site	PLNWR	MEF	DJH	CIFS	PLNWR	MEF	DJH	CIFS	PLNWR	MEF	DJH	CIFS
Trees	11.85	16.82	0.00	19.07	1.40	68.50	0.00	13.80	2.45	164.44	0.00	39.76
Shrubs	0.95	0.54	1.42	1.63	93.00	16.40	41.50	52.30	35.14	6.19	15.68	19.77
Herbs	0.55	0.73	0.38	0.50	4.20	17.50	40.00	21.90	0.44	1.83	4.15	2.29
Mosses	0.15	0.25	0.10	0.30	1.80	97.80	60.00	16.20	0.09	6.65	2.28	1.26

Supplementary Table 3 Growth rates of dominant fungi in the subtropical shrub-dominated and the boreal *Sphagnum*-dominated peatlands

Division	Lower taxa	Growth rates	Shrub sites	*Sphagnum* site	Ref.
Ascomycota	*Archaeorhizomyces* spp.	Slow	+	−	[25,26]
Ascomycota	*Pseudogymnoascus roseus*	Slow	−	+	[27]
Ascomycota	Helotiales sp.	Slow	+	−	[28,29]
Basidiomycota	*Cryptococcus* sp.= *Saitozyma* sp.	Slow	+	−	[30-32]
Basidiomycota	*Solicoccozyma* sp.	Slow	+	−	[30]
Basidiomycota	*Guehomyces* sp.	Slow	+	−	[31]
Basidiomycota	*Cystofilobasidium* sp.	Slow	+	−	[30-32]
Basidiomycota	*Leucosporidiella* sp.	Slow	+	−	[32]
Ascomycota	*Calycina* sp.	Slow	+	−	[33]
Ascomycota	*Chaetothyriales* spp.	Slow	+	−	[34,35]
Basidiomycota	*Cortinarius* spp	Slow	+	−	[36]
Ascomycota	*Pezoloma* spp.	Slow	+	−	[37]
Ascomycota	*Pseudeurotium* sp.	Fast	−	+	[38]
Ascomycota	*Penicillium* sp.	Fast	−	+	[39,40]
Ascomycota	Saccharomycetales sp.	Fast	−	+	[41]
Basidiomycota	*Rhodotorula* sp.	Fast	−	+	[42]
Zygomycota	*Mortierella* spp.	Fast	−	+	[43,44]
Basidiomycota	*Bullera* sp.	Fast	+	−	[45,46]
Ascomycota	*Aspergillus* sp.	Fast	+	−	[5]
Basidiomycota	*Hyphoderma* spp.	Fast	+	−	[47]
Basidiomycota	*Suillus* spp.	Fast	+	−	[48]
Zygomycota	Mucoromycota sp.	Fast	+	−	[49]

Supplementary Table 4 Mantel test were conducted to compare the relative effect of environment distance on soil fungal compositional dissimilarity. Bold value indicated statistical significance.

	r	*P*
Soluble phenolics	**0.53**	0.001
pH	**0.50**	0.001
Dissolved organic carbon	**0.14**	0.004
NO_3-N	**0.29**	0.001
NH_4-N	**0.28**	0.001

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