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# Effect of a temperature gradient on *Sphagnum fallax* and its associated living microbial communities: a study under controlled conditions

Vincent E.J. Jassey, Daniel Gilbert, Philippe Binet, Marie-Laure Toussaint, and Geneviève Chiapusio

**Abstract:** Microbial communities living in *Sphagnum* are known to constitute early indicators of ecosystem disturbances, but little is known about their response (including their trophic relationships) to climate change. A microcosm experiment was designed to test the effects of a temperature gradient (15, 20, and 25 °C) on microbial communities including different trophic groups (primary producers, decomposers, and unicellular predators) in *Sphagnum* segments (0–3 cm and 3–6 cm of the capitulum). Relationships between microbial communities and abiotic factors (pH, conductivity, temperature, and polyphenols) were also studied. The density and the biomass of testate amoebae in *Sphagnum* upper segments increased and their community structure changed in heated treatments. The biomass of testate amoebae was linked to the biomass of bacteria and to the total biomass of other groups added and, thus, suggests that indirect effects on the food web structure occurred. Redundancy analysis revealed that microbial assemblages differed strongly in *Sphagnum* upper segments along a temperature gradient in relation to abiotic factors. The sensitivity of these assemblages made them interesting indicators of climate change. Phenolic compounds represented an important explicative factor in microbial assemblages and outlined the potential direct and (or) indirect effects of phenolics on microbial communities.

**Key words:** *Sphagnum*, testate amoebae, temperature gradient, phenolic compounds, bioindicators.

**Résumé :** Les microorganismes des sphaignes sont connus comme indicateurs précoces des perturbations environnementales. Or, peu d'études portent sur l'influence de la température sur ces communautés. Dans cette étude, l'effet d'un gradient de températures (15, 20 et 25 °C) sur les microorganismes des sphaignes, incluant différents groupes trophiques (producteurs primaires, décomposeurs, micro-prédateurs), a été testé dans les différentes parties des sphaignes (0 à 3 cm et 3 à 6 cm du capitulum). Les relations entre les microorganismes et les facteurs abiotiques (pH, conductivité, température, polyphénols) ont également été analysées. La densité et la biomasse des amibes à thèque ont augmenté dans les parties supérieures des sphaignes. Une modification de la structure de leur communauté a aussi été mise en évidence. La biomasse des amibes à thèque est apparue liée à celle des bactéries et à la biomasse additionnée des autres groupes, suggérant des effets indirects de la température sur les relations trophiques. Les analyses par redondance ont révélé que la structure des communautés microbiennes variait entre les différents traitements dans les parties supérieures des sphaignes. Ainsi, les communautés microbiennes des sphaignes apparaissent comme un outil intéressant quant au suivi in situ des changements climatiques. Les polyphénols ont également été identifiés comme un facteur explicatif important de la structure des communautés microbiennes, montrant ainsi leurs effets potentiels sur les microorganismes des sphaignes.

**Mots-clés :** sphaignes, amibes à thèque, gradient de température, composés phénoliques, bioindicateurs.

## Introduction

Climatic change models cover a wide range of temperature-increase scenarios (2 °C up to 8 °C), especially at high latitudes where the majority of *Sphagnum*–peatlands occurs (Gorham 1991; IPCC 2007). Peatlands are complex ecosystems because of the diversity of habitats and microhabitats allowing the establishment of diverse communities (Rydin and Jeglum 2006). Studying the impact of climate change on these ecosystems remains particularly difficult and complex because of several biological and chemical parameters interacting (Davidson and Janssens 2006). Some microcosm

studies were elaborated to test the impact of a temperature increase on *Sphagnum* plant communities (Breeuwer et al. 2008, 2009). However, such studies were carried out at the scale of simple organisms or populations and did not incorporate the interactions between different populations. Thus, a simplified ecosystem including different trophic levels could be an interesting approach to better understand the effect of a temperature increase. In this context, the use of microbial communities living in *Sphagnum* represents a simplified ecosystem (Nguyen-Viet et al. 2007; Meyer et al. 2010). *Sphagnum* mosses are ubiquitous, cosmopolitan, and

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characterized peatlands (Shaw et al. 2003; Rydin and Jeglum 2006). Moreover, *Sphagnum* mosses shelter a large number of microbial species belonging to the different trophic groups, including bacteria, algae, testate amoebae, ciliates, or fungi, which have a short generation time (hours up to few weeks) (Schönborn 1986; Gilbert et al. 1998).

Some studies have demonstrated that the richness and density of single microbial groups (e.g., testate amoebae, microalgae) and (or) the structure of microbial communities living in *Sphagnum* are affected by environmental perturbations, such as nitrogen addition and atmospheric pollution (Howell and South 1981; Gilbert et al. 1998; Mitchell et al. 2003; Nguyen-Viet et al. 2007, 2008). In addition, the integration of testate amoebae into an experimental model is interesting because they feed on a wide range of prey (i.e., bacteria, fungi, organic matter, algae, other protozoa) and could integrate ecosystem perturbations (Gilbert et al. 2000, 2003). Thus, such a simplified system not only would notably give insight into the interactions between species but also would incorporate biotic and abiotic factors (including phytochemicals) interactions between *Sphagnum* and its associated microbial communities.

The potential phytochemical interactions between *Sphagnum* and microbial communities are rarely studied. However, phenolic compounds (secondary metabolites) produced by plants play an important role in the interaction of vegetation with its environment (Hättenschwiler and Vitousek 2000; Chiapusio et al. 2005). For example, in humus spruce forests, such compounds are implicated in the increase of several microbial communities (i.e., cellulose hydrolysers) and in the decrease of others (i.e., bacteria) (Souto et al. 2000, 2001). Some studies have addressed phenolic production by *Sphagnum* (e.g., Rasmussen et al. 1995), more specifically the phenolics weakly and primarily bound to the cell wall (Verhoeven and Liefveld 1997). Because of *Sphagnum* morphology and anatomy, water-soluble phenolics can be easily released into *Sphagnum* environments. Recently, Jassey et al. (2011) demonstrated that such compounds were involved in the fine-scale microdistribution of testate amoebae along an ecological gradient. A multitude of environmental factors cause significant shifts in the quantity of phenolic compounds in vascular plants (e.g., UV-B; Spitaler et al. 2008), but they are understudied in *Sphagnum*. The patterns of phenolic compounds at the surface of *Sphagnum* layers may be susceptible to temperature increase and to interactions with microbial communities living in *Sphagnum*.

In this study, we assessed the response of vertical patterns of microbial communities living in *Sphagnum* and the relationships among microbial communities, temperature, and total phenolic compounds along a temperature gradient. The abundance and structure of microbial communities and the concentrations of phenolic compounds were quantified in *Sphagnum* peat cores placed at 3 different temperatures (15, 20, and 25 °C) after 8 weeks in a growth chamber. According to the gradient of temperature, we addressed the following hypotheses: (i) that the structure and biomass of microbial communities would vary among temperature treatments and between *Sphagnum* layers (upper and lower), (ii) that the effect of temperature would be different for each microbial group according to their trophic position, and (iii) that total phenolic compound concentrations quanti-

fied in *Sphagnum fallax* would change along the temperature gradient and *Sphagnum* layers and would explain a similar fraction of the community data as other environmental factors.

## Materials and methods

### *Sphagnum* sampling and experimental setup

*Sphagnum fallax* was collected in a peat bog in the Jura Mountains (Sur-les-Seignes, Frambouhans-Les Ecorces, France, 47°18'N, 6°79'E) at an altitude of 846 m above sea level on 19 October 2007 (temperature 10 °C). The climate of the area is characterized by cold winters (an average of -2.4 °C in January) and mild summers (an average of 14.6 °C in July). The annual mean temperature of the region is about 6.6 °C. The annual amount of precipitation is 1417 mm, and the duration of snow cover is an average of 50 days·year<sup>-1</sup>. Surfaces of *S. fallax* as homogeneous and pure as possible were selected. Sixteen *Sphagnum* peat cores (25 cm × 15 cm) were randomly sampled with a knife.

Four peat cores were assigned in quadruplicates to described the initial stage (IS) and 12 peat cores for the 3 treatments 15, 20, and 25 °C (designated T15, T20, and T25, respectively). A large gradient of temperature was chosen to observe the response of communities. Peat cores were randomly placed between the different levels of the growth chamber (Cryonext RTH600). General conditions selected were a relative humidity of >70%, a light intensity of 120 μmol·s<sup>-1</sup>·m<sup>-2</sup>, and a photoperiod of 12 h (light) : 12 h (dark). Each treatment corresponded to 1 level of the growth chamber. For each treatment, peat cores were placed in 1 big trayfull of water (water level = 20 cm) and were humidified with a standard nutrient solution (Volvic water). The water depth was kept at 2 cm below the top of *Sphagnum* and readjusted with demineralized water every 2 days. The average relative humidity of *Sphagnum* was around 90% in *Sphagnum* segments for each treatment during the experiment. The 15 °C temperature corresponded to the temperature of the growth chamber. The 20 and 25 °C temperatures were obtained by heating the water of the tray with, respectively, 2 thermo-divers of 50 and 200 W and 2 thermo-divers of 200 W. Moreover, aquarium pumps (5 L·min<sup>-1</sup>) were installed in the warm trays to obtain homogeneous temperatures. The position of peat cores was changed in each tray every 2 days to avoid experimental differences. The temperature was recorded several times a day during the experiment in each peat core. The water chemistry (pH, conductivity, and Eh potential) was also measured in each peat core every 2 days.

Before and after 8 growing weeks, 20 *S. fallax* were sampled in each peat core and cut into 2 layers from the capitulum: 0–3 cm (upper segments) and 3–6 cm (lower segments). For microbial analysis, the samples were fixed in 20 mL of glutaraldehyde (2% final concentration). No mosses were taken from the 5 cm bordering peat cores to avoid a border effect.

### Total phenolic compounds assay

Phenolic compounds were extracted from lyophilized mosses following 2 methods, as described in Jassey et al. (2011), and commonly used in phytochemical interactions

(Waterman et al. 1994; Macheix et al. 2005). Water-soluble phenolics (hereafter “free phenolics”) were extracted using distilled water and corresponded to phenolics susceptible to directly interact with microorganisms. Primarily bound phenolics (hereafter “bound phenolics”) were extracted using ethanol–distilled water solution (80:20, v/v) and mainly corresponded to phenolic acids slightly bound to cell wall. Thereafter, free and bound total phenolic contents were quantified with the Folin-Ciocalteu reagent and were expressed in milligrams of equivalent gallic acid ( $A_{760}$ ) (Gallet and Lebreton 1995).

### Microbial communities analyses

Microbial organisms were extracted from *S. fallax* with the standard method of Nguyen-Viet et al. (2007). Each sample was shaken for 1 min on a vortex, and then *Sphagnum* mosses were pressed to extract microorganisms (first solution). The mosses were then soaked again with 20 mL of glutaraldehyde (2% final concentration), shaken a second time on a vortex, and pressed to extract *Sphagnum* leachate. The leachate was settled for 12 h, and afterward the supernatant was added to the *Sphagnum* and the material on the bottom was added to the first solution. The process was repeated 6 times, and all fractions were combined to obtain a final composite sample of 40 mL. The remaining fraction was dried at 80 °C for 48 h and weighed to express microbial density in grams of dry mass of *Sphagnum*.

### Primary producers, fungi, and unicellular predators

A 3 mL subsample of the final composite sample was settled for 2 h in a plankton chamber and analyzed at  $\times 400$  magnification by inverted microscopy (Olympus IX71) following Uthermöhler's method (Uthermöhler 1958). The whole slide was analyzed and the different groups of cyanobacteria, microalgae, unicellular predators (flagellates, testate amoebae, and ciliates), and fungi hyphae and spores were counted and measured. For testate amoebae, only living tests were counted.

### Bacteria

A 1 mL subsample of the final composite sample was stained with 50  $\mu\text{L}$  of 4,6 diamino-2-phenylindol (DAPI, 0.2% of final concentration) for 15 min in the dark, filtered through 0.2  $\mu\text{m}$  black membrane filters, and examined by epifluorescence microscopy at  $\times 1000$  magnification (Porter and Feig 1980). Bacteria numbers and size were estimated with an image analysis program (LUCIA 4.0). For each sample, between 8 and 35 photographic grips were observed. The number of bacteria cells counted and measured was situated between 514 and 1132. This direct counting using DAPI gave the total density of bacteria.

The biovolume of each community was estimated by assuming geometrical shapes and converted to carbon using the following conversion factors: bacteria,  $1 \mu\text{m}^3 = 5.6 \times 10^{-7} \mu\text{g C}$  (Bratbak 1985); cyanobacteria and microalgae,  $1 \mu\text{m}^3 = 1.2 \times 10^{-7} \mu\text{g C}$  (Borsheim and Bratbak 1987); ciliates and testate amoebae,  $1 \mu\text{m}^3 = 1.1 \times 10^{-7} \mu\text{g C}$  (Weisse et al. 1990); fungi,  $1 \mu\text{m}^3 = 2.5 \times 10^{-7} \mu\text{g C}$  (Gilbert et al. 1998). The data were expressed as micrograms of carbon per gram of *Sphagnum* dry mass per square centimetre ( $\mu\text{g C} \cdot (\text{g DW})^{-1} \cdot \text{cm}^{-2}$ ).

### Numerical analysis

Since the data distributions of microbial communities, phenolic compounds, and water chemistry were not normal and statistically dependant, a nonparametric 2-way analysis of variance test (Friedman test) was used to determine the differences among the temperatures (T15, T20, and T25).

To reduce the influence of dominant taxa in the multivariate analysis, carbon biomass of microbial communities was transformed using the relation  $\text{Ln}(x+1)$  (Nguyen-Viet et al. 2007). Multiple factor analysis (MFA) was used to assess the general structure of the data and to determine the relationships among the 2 microbial community's data sets (upper and lower) and the 3 environmental variables data sets (water chemistry, temperature, and phenolics). MFA was chosen because it allows the simultaneous coupling of several groups or subsets of variables defined on the same objects (Escofier and Pagès 1994). The MFA was performed in 2 steps. Firstly, a first principal component analysis (PCA) was applied to the whole set of variables in which each subset was weighted by dividing all its elements by the first eigenvalue obtained from its PCA. Secondly, the normalized subsets were assembled to form a unique matrix and a second PCA was performed on this matrix. Euclidean distances of global PCA were then used in MFA to perform cluster analysis according to the Ward method, and the resulting dendrogram was projected in the MFA ordination space. This analysis revealed the main differences in the structure of the data described by all biotic and abiotic subsets of variables (Lamentowicz et al. 2010).

We assessed the relationships among the microbial communities in the upper and lower sampling depths and the 3 sets of environmental variables: (i) water chemistry (pH, Eh potential, and conductivity), (ii) temperature, and (iii) phenolic compounds (bound and free). The ordination patterns of microbial communities and their causal relationships to environmental data sets were assessed using redundancy analysis (RDA) (Ter Braak and Similauer 1998). A forward selection of explanatory variables was computed for each of the *Sphagnum* segments, using automatic stepwise model building for constrained ordination methods (“ordisep” function). The proportion of variance explained by environmental variables was quantified using variance partitioning. Adjusted  $R^2$  were used in all RDAs to estimate the proportion of explained variance by each environmental variables (Peres-Neto et al. 2006).

All multivariate analyses were performed with the software R (R Development Core Team 2008) using the vegan (Oksanen et al. 2010) and FactoMineR (Husson et al. 2009) packages.

## Results

### Environmental variables

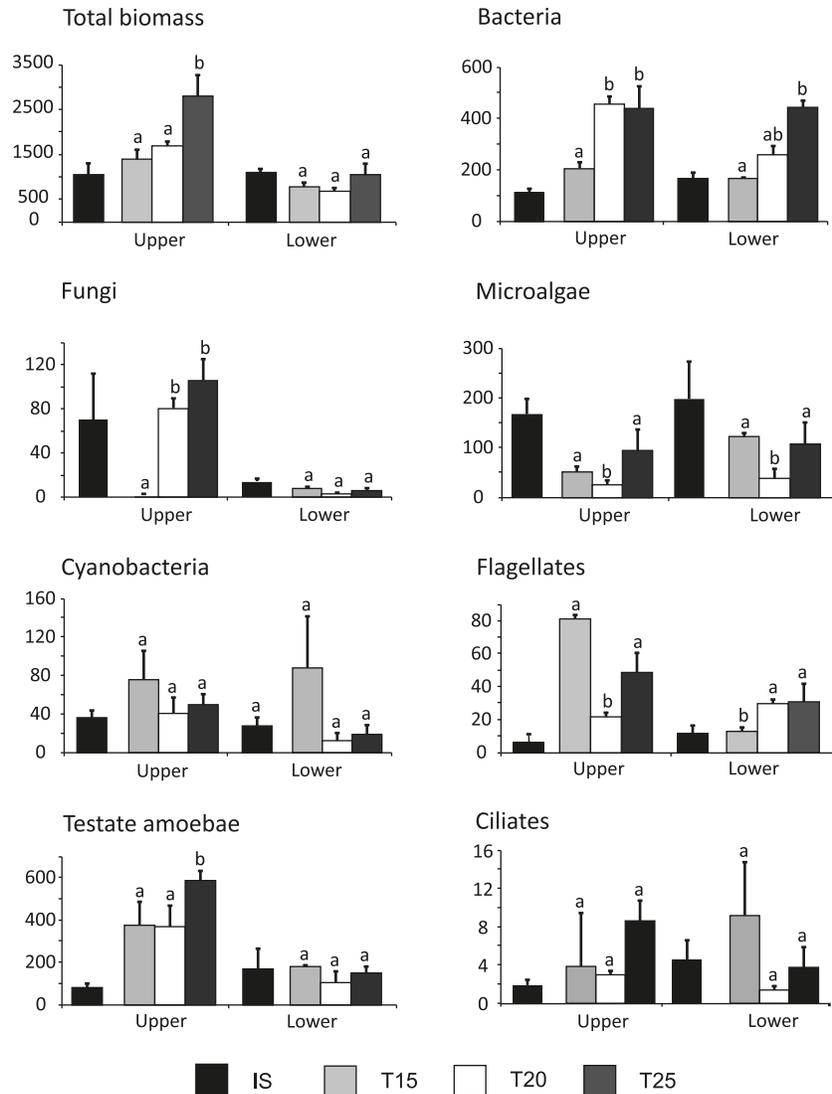
During the experiment, temperatures recorded were stable in both *Sphagnum* segments, with  $14.8 \pm 0.4$  °C for T15,  $19.4 \pm 0.3$  °C for T20, and  $23.9 \pm 0.5$  °C for T25. Conductivity and Eh potential were significantly lower ( $P < 0.05$ ) in T15 ( $30 \pm 9.4 \mu\text{S} \cdot \text{cm}^{-1}$  and  $370.1 \pm 16.2$  mV, respectively) than in T20 and T25 ( $19.1 \pm 3 \mu\text{S} \cdot \text{cm}^{-1}$  and  $335.5 \pm 20$  mV, respectively). The pH was stable during the experiment (pH =  $4.1 \pm 0.5$  in IS, T15, T20, and T25).

**Table 1.** Total concentrations (mg·(g DW)<sup>-1</sup>) of *Sphagnum fallax* free and bound phenolics in upper and lower segments at initial stage (IS) and at the temperatures 15, 20, and 25 °C (T15, T20, and T25) (n = 4).

Time		Bound phenolics		Free phenolics	
		Upper segment	Lower segment	Upper segment	Lower segment
0 weeks	IS	2.5±0.1a	1.7±0.1b	1.1±0.1a	0.7±0.0b
	T15	2.4±0.3a	1.8±0.2b	1.5±0.3a	0.8±0.1b
8 weeks	T20	3.4±1.1a	1.7±0.1b	1.6±0.1a	0.6±0.1b
	T25	2.8±0.9a	1.6±0.1b	2.4±0.3a*	0.7±0.0b

**Note:** Letters indicate significant differences of the total phenolic concentrations between the upper and the lower segments for the same treatment (P < 0.05). The asterisk indicates significant difference between temperatures (P < 0.05).

**Fig. 1.** Total carbon biomass (µg C·(g DW)<sup>-1</sup>·cm<sup>-2</sup>, mean ± SE) of all microbial groups and carbon biomass (µg C·(g DW)<sup>-1</sup>·cm<sup>-2</sup>, mean ± SE) of microbial groups in *Sphagnum* samples analyzed after 8 weeks related to the gradient of temperature (15, 20, and 25 °C designated T15, T20, and T25). Letters indicate significant differences of biomass between temperatures in upper and lower segments (Friedman test, P < 0.01). IS, initial stage.



**Temperature effect on total phenolic compounds**

The concentrations in the 3 treatments were 1.5× higher in upper segments (bound: an average of 2.6 mg·(g DW)<sup>-1</sup>; free: an average of 1.8 mg·(g DW)<sup>-1</sup>) than in lower seg-

ments (bound: an average of 1.6 mg·(g DW)<sup>-1</sup>; free: an average of 0.7 mg·(g DW)<sup>-1</sup>) (Table 1). Bound phenolics produced by *S. fallax* did not significantly differ between the upper and lower segments, with the different tempera-

**Table 2.** Abundance of 12 testate amoebae species found in upper and lower segment of *Sphagnum fallax* samples at initial stage (IS) and grown over an 8 week period at 15, 20, and 25 °C (T15, T20, and T25) ( $n = 4$ ).

	Relative abundance <sup>a</sup>		Abundance per gram (DW)		
	IS	IS	T15	T20	T25
<b>Upper segments</b>					
<i>Archerella flavum</i>	28.7±8.1	287±85	574±358	300±170	683±471
<i>Nebela tinctoria</i>	18.4±3.9	191±30	587±161	465±127	370±87
<i>Hyalosphenia papilio</i>	17.9±8.9	191±72	1489±451	1188±298	2063±425
<i>Euglypha strigosa</i>	12.2±8.3	123±34	423±130	1025±248	1291±297*
<i>Diffflugia bacillariarum</i>	6.2±25.0	55±30	7±7	137±103	55±22
<i>Assulina muscorum</i>	4.8±12.2	48±24	205±52	82±35	164±87
<i>Euglypha cristata</i>	3.4±25.0	34±34	14±8	0±0	41±24
<i>Centropyxis acuelata</i>	3.1±18.9	34±26	7±7	41±26	55±55
<i>Trinema complanatum</i>	2.0±16.8	21±13	0±0	0±0	27±16
<i>Hylosphenia subflava</i>	1.4±14.6	14±8	130±21	41±26	55±40
<i>Arecella arenaria</i>	1.2±25.0	14±14	27±19	0±0	0±0
Total abundance	—	1020±87	3600±525	3400±500	4860±1000*
<b>Lower segments</b>					
<i>Archerella flavum</i>	30.1±5.4	200±50	246±84	153±120	297±242
<i>Nebela tinctoria</i>	28.1±9.4	170±61	136±50	110±35	59±16
<i>Diffflugia bacillariarum</i>	27.5±14.0	166±31	30±4	21±4	110±53
<i>Assulina muscorum</i>	8.4±6.5	51±10	21±11	17±17	4±4
<i>Euglypha strigosa</i>	1.4±0.8	9±5	230±26	221±97	110±16
<i>Centropyxis acuelata</i>	1.4±25.0	9±9	9±5	9±5	13±8
<i>Hylosphenia elegans</i>	0.0±0.0	0±0	21±16	0±0	17±17
<i>Hyalosphenia papilio</i>	0.0±0.0	0±0	34±14	9±5	81±55
Total abundance	—	600±20	850±80	680±240	800±250

**Note:** Values are the mean ± SE. Asterisks indicate significant differences of a same species related to the gradient of temperature in upper and lower segments ( $P < 0.05$ ).

<sup>a</sup>Relative abundance =  $100 \times (\text{abundance}/\text{total number of individuals in the sample})$ .

tures. Free phenolics were significantly higher in upper segments ( $P < 0.04$ ) for T25 than for the other treatments. A significant relation was also found between bound and free phenolics ( $P < 0.001$ ,  $r = 0.62$ ).

### Microbial communities

The main change in the total biomass of microbial communities occurred in the upper segments of *Sphagnum*, whereas it was quite stable in lower segments (Fig. 1). Indeed the total biomass in upper segments was nearly twice as high with elevated temperature (T15:  $1400 \mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$ ; T20 and T25: 1700 and 2700  $\mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$ ,  $P < 0.01$ ).

### Primary producers

The biomass of primary producers did not significantly vary among T15, T20, and T25 (Fig. 1). The biomass of microalgae was  $85 \pm 31 \mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$  in upper segments and  $106 \pm 31 \mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$  in lower segments. The community was dominated by the same species along *Sphagnum* segments in any treatments. *Chlorophyceae* (e.g., *Cylindrocapsa* sp. and *Penium* sp.) and *Bacillariophyceae* (e.g., *Pinnularia viridis*) were the main identified genera. Cyanobacteria represented a small propor-

tion (<5%) of the total biomass along *Sphagnum* segments in any treatments, with an average biomass of  $50 \pm 17 \mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$  in upper segments and  $31 \pm 10 \mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$  in lower segments. Cyanobacteria were dominated by *Chroococcales* (e.g., *Chroococcus giga*) and *Nostocales* (e.g., *Anabaena cylindrica* and *Aphanothece nidulans*) along *Sphagnum* segments.

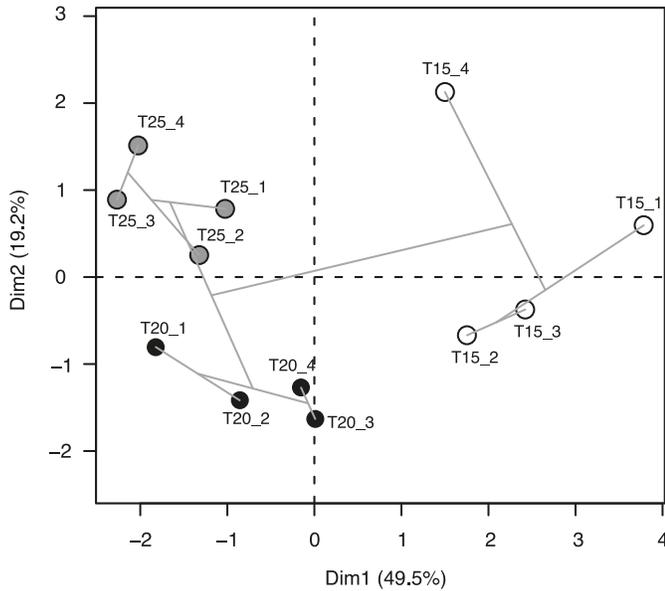
### Decomposers

The biomass of bacteria was 2 times higher in upper and lower segments with elevated temperature (T20 and T25) than in T15. For example, bacterial biomass increased from  $200 \mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$  in T15 to  $440 \mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$  at T25 in upper segments ( $P < 0.01$ ; Fig. 1). Fungal biomasses were not significantly different among treatments ( $70 \mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$  in upper segments and  $10 \mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$  in lower segments) (Fig. 1).

### Unicellular predators

The biomass of testate amoebae increased from T15 ( $380 \mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$ ) to T25 ( $600 \mu\text{g C}\cdot(\text{g DW})^{-1}\cdot\text{cm}^{-2}$ ;  $P < 0.01$ ; Fig. 1). At the IS, the most frequent testate amoebae were *Archerella (Amphitrema) flavum* (29%), *Nebela tinctoria* (18%), and *Hyalosphenia papilio* (18%) in upper

**Fig. 2.** Multiple factor analysis (MFA) of the 2 *Sphagnum* biota communities and environmental (chemical, physical, and phenolics) data sets. Biplot of axes 1 and 2 (both significant at  $P = 0.001$ ) is given together with the result of a hierarchical agglomerative clustering (grey solid lines) obtained by the Ward method on the Euclidean distance matrix between MFA site scores, showing 3 main groups of temperature treatment (T15 (white circles), T20 (black circles), T25 (grey circles)).



segments, and *A. flavum* (30%), *N. tincta* (28%), and *Diffugia bacilliarum* (27%) in lower segments (Table 2). The same species were dominant at T15 with *H. papilio* (42%), *A. flavum* (16%), and *N. tincta* (16%) in upper segments, and *A. flavum* (29%), *Euglypha strigosa* (27%), and *N. tincta* (16%) in lower segments. For T20 and T25 communities, the upper segments were dominated by *H. papilio* (35% and 43%, respectively) and *E. strigosa* (30% and 27%, respectively) (Table 2). The biomass of testate amoebae was correlated with the total biomass of other groups added ( $r = 0.63$ ,  $P < 0.01$ ) in upper segments but not in lower segments ( $r = 0.15$ ,  $P = 0.57$ ). The density of *E. strigosa* was significantly correlated to the temperature increase ( $r = 0.62$ ), the density of bacteria ( $r = 0.49$ ) and free phenolics ( $r = 0.58$ ) in upper segments of *Sphagnum* ( $P < 0.01$ ). In lower segments, no species was significantly influenced by the temperature gradient nor free phenolics.

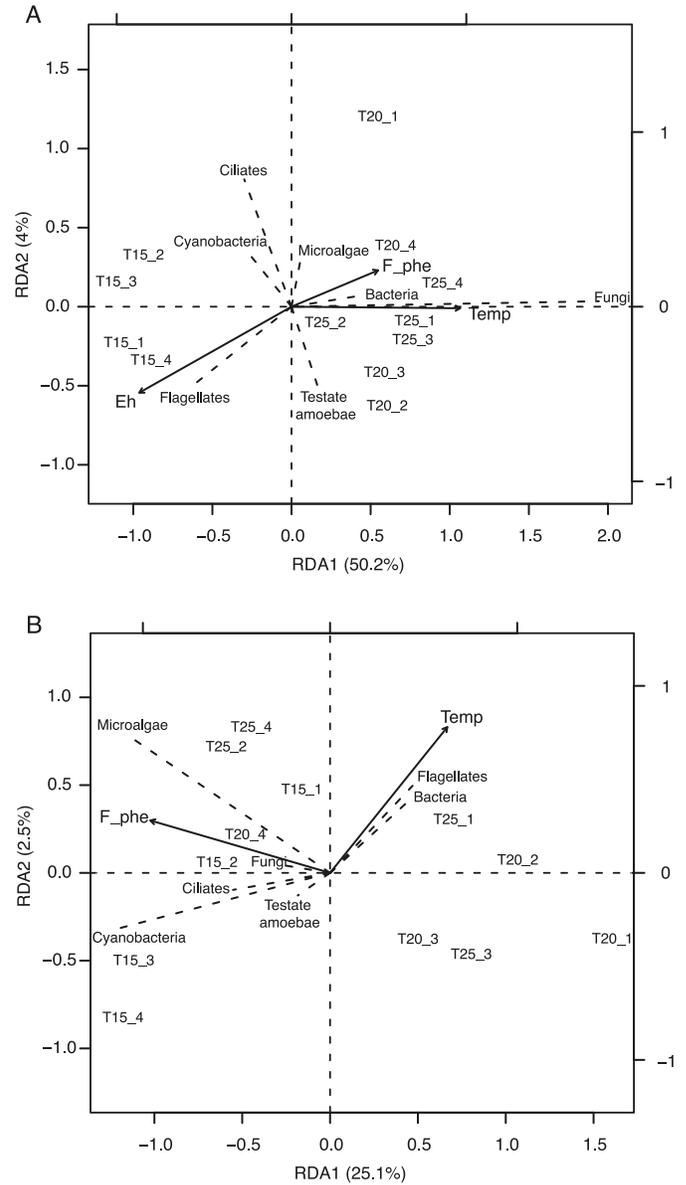
The results obtained for flagellates and ciliates strongly varied among treatments in upper and lower segments, but no temperature effect was clearly observed.

**Relationships between environmental parameters and *Sphagnum* biota**

The MFA of the 3 environmental matrices (water chemistry, temperature, and phenolics) and the 2 microbial community (upper and lower segments) data sets confirmed the existence of an overall division among the temperature treatments (i.e., T15, T20, and T25) with a different global structure of microbial communities (Fig. 2).

In the separate RDAs, T20 and T25 microbial assemblages and T15 microbial assemblage were clearly separated

**Fig. 3.** Redundancy analyses (axes 1 and 2) of microbial data in upper segments (A) and lower segments (B). Environmental variables retained after model selection are represented by arrows. Microbial groups are represented by dotted lines. F\_phe: free phenolics; Temp: temperature.



in upper segments (Fig. 3A), whereas in lower segments the separation between the treatments was unclear (Fig. 3B). The models explained, respectively, 56.4% and 27.6% of the variability in microbial data in upper and lower segments analysis ( $P < 0.01$  for each axes, Monte-Carlo permutation test, 999 permutations). T20 and T25 microbial assemblages (particularly bacteria, fungi, and testate amoebae) in upper segments were correlated with temperature and free phenolics, while microbial assemblage in T15 was related to Eh potential. In lower segments, microbial community structures along the temperature gradient were not clearly separated, except some T20 and T25 replicates correlated with elevated temperatures and the biomass of bacteria.

**Table 3.** Summary of redundancy analyses (RDA) on microorganisms and environmental variables: fraction of variance explained (%) and significance of individual variables taken alone or grouped.

Variable in RDA model	Overall RDA		Top segments		Low segments	
	% <sup>a</sup>	<i>P</i>	% <sup>a</sup>	<i>P</i>	% <sup>a</sup>	<i>P</i>
Eh	3.4	0.13	33.1	0.01	3.3	0.55
Conductivity	2.9	0.12	17.1	0.03	3.1	0.51
pH	0.4	0.87	0.8	0.95	0.4	0.64
Water chemistry (pH, Eh, conductivity)	1.6	0.57	23.9	0.08	4.4	0.87
Temperature	7.6	0.02	42.1	0.003	2.5	0.25
Free phenols	11.9	0.004	5.2	0.02	15.7	0.05

<sup>a</sup>Percentage of variance explained (adjusted  $R^2$ ).

The successive RDAs on individual environmental variables revealed that the proportion of microbial data explained by each explanatory variable varied among variables both between the environmental data sets and along the 2 vertical positions (Table 3). The overall RDA (upper and lower segments) showed that free phenolics and temperature data sets explained, independently of the other 2 data sets, respectively, 11.9% ( $P = 0.004$ ) and 7.6% ( $P = 0.02$ ) of the microbial data variance. However, the variance explained by these variables was much higher in the partial RDAs of upper and lower segments (Table 3). For example, temperature explained 42.1% of the microbial data variance in upper segments ( $P < 0.001$ ) and decreased to 2.5% in lower segments, while the variance explained by free phenolics increased from 5.2% to 15.7% ( $P < 0.05$ ) between the upper and lower segments.

## Discussion

### Temperature gradient effect on microbial food web

This study is unique in that it addresses the effects of experimental high temperature gradient on microbial communities living in *Sphagnum*, including different trophic groups. MFA confirmed the existence of 3 microbial community assemblages along the temperature gradient (T15, T20, and T25 microbial assemblages) and the validity of using *Sphagnum* and its associated microbial communities as an indicator of climate change. Interestingly, the results revealed different behavior of the trophic groups related to the temperature gradient. The biomass of the microbial primary producers (microalgae and cyanobacteria) did not vary at any temperature, while an increase in the biomass of the microorganisms (bacteria and testate amoebae) involved in the microbial loop was recorded (dissolved organic matter → bacteria → heterotrophic protists (ciliates, flagellates, testate amoebae) → macrozoorganisms; Gilbert et al. 1998).

Along the temperature gradient, the density and the biomass of testate amoebae in *Sphagnum* upper segments increased and their community structure changed in heated treatments. These results combined with the significant correlation between the biomass of testate amoebae and the total biomass of other groups added suggested that indirect effects on the food-web structure occurred. The significant link between *E. strigosa* and bacteria along the temperature gradient seemed to confirm this hypothesis. Indeed, *Euglypha* is described as bacterivorous in literature (Gilbert et al.

2000; Beyens et al. 2009). The increase of bacteria density in *Sphagnum* upper segments probably influenced the abundance of *E. strigosa*. Beyens et al. (2009) also observed similar results for the correlation between testate amoeba communities and temperature effect. For example, *Trachelocorythion* and *Euglypha* genera reacted positively with heat-wave and were linked to the increase of bacteria. The high density and high relative abundance of *H. papilio* in heated treatments also suggested indirect effects on the food-web structure, since this species feeds on a wide range of prey, notably small euglyphids (Gilbert et al. 2000; Meisterfeld 2000a, 2000b). Conversely, the biomass and the density of *N. tinctoria* did not vary along the temperature gradient. This species was recognized to essentially feed on autotrophic microorganisms during summer (Gilbert et al. 2003), and the biomass of these groups was not affected by the temperature increase. Microbial primary producers (microalgae and cyanobacteria) living in *Sphagnum* are well known to be sensitive to water chemistry, and to N and P addition (Hooper 1981; Howell and South 1981; Gilbert et al. 1998).

Because testate amoebae are at the end of the microbial food web and they feed on a wide range of prey, their community integrates the variations of the food web due to environmental perturbations (Gilbert et al. 1998, 2003; Nguyen-Viet et al. 2007; Mitchell et al. 2008). Thus, they could be interesting candidates for monitoring the impact of elevated temperatures in *Sphagnum* peatlands. On the other hand, to deepen our knowledge about the changes in the microbial food web with elevated temperatures, complementary experiments focusing on the feeding habit progression of testate amoebae would be necessary.

### *Sphagnum* biota — environmental factors relationships

Among environmental factors, temperature appeared as the principal factor that determines microbial community variations. Direct gradient analysis (RDA) revealed that in upper segments, temperature, Eh potential, and conductivity significantly explained a high proportion of microbial data set variations. This result was not surprising, since water chemistry generally contributed to a change in microbial community distribution that occurred at the top of *Sphagnum* carpet (Howell and South 1981; Mitchell et al. 2003; Mieczan 2009). Conversely, in lower segments, only free phenolics significantly explained microbial data variations. Studying both upper and lower segments along a tempera-

ture gradient demonstrated that the response of microbial communities to abiotic factors differed between *Sphagnum* segments. These results illustrate (i) how vertical gradients lead to ecological niche separations in *Sphagnum* and (ii) the potential influence of phenolic compounds on microbial community distribution.

The *Sphagnum* upper segments were characterized by a higher phenolic content (bound and free) compared with the lower segments, and constituted original results about phenolic repartition in *Sphagnum*. These results were in accordance with *Sphagnum* biology, given that capitula constituted the dominant life parts of *Sphagnum* where main metabolic processes occurred (Clymo and Hayward 1982). The metabolism of phenolic compounds in *Sphagnum* was more important at the capitulum layer than in lower segments. The observed correlation between free and bound phenolic compounds demonstrated that their production by *Sphagnum* was linked and similar. However, only free phenolics were affected by elevated temperature through an increase in top segments. Such result suggested that phenolic content was also affected qualitatively by elevated temperature. Different kinds of phenolics (e.g., phenolic acids and flavonoids) are known to be present in the *Sphagnum* genus (Rudolph and Samland 1985; Opelt et al. 2007). To better understand phenolic *Sphagnum* metabolism under elevated temperatures, it would be necessary to characterize both qualitatively and quantitatively the phenolic extracts from the different segments by using a high-pressure liquid chromatography technique.

Free phenolics were related to microbial communities and explained a large part of the variance observed, particularly in the lower segments. This relation outlines (i) the potential chemical interaction between *Sphagnum* and the microbial communities living in *Sphagnum* and (ii) the role of such compounds in the structure of microbial communities regardless of their increase at 25 °C in upper segments. Similar results were found with the testate amoebae community structure (Jassey et al. 2011). Nonetheless, the significant relation between *E. strigosa* and free phenolics in upper segments suggested a potential direct effect of these compounds on this species, since its density did not increase in lower segments while the density of bacteria increased. Although this result does not allow a conclusion to be drawn on a possible direct positive effect of water-soluble phenolic compounds on *E. strigosa*, it raises the issue of the possible role of such compounds. Recently, *p*-hydroxyl phenolic acids released by *Sphagnum* mosses have been shown to possess antibacterial and antifungal activity (Opelt et al. 2007; Mellegård et al. 2009). Thus, it is possible that water-soluble phenolic compounds released by *Sphagnum* play a role in microbial assemblages through direct (e.g., physiological) and (or) indirect (e.g., through impact on prey-predator relationships) effects.

## Conclusion

This original approach of studying *Sphagnum* upper and lower segments revealed a strong relationship between microbial community structures and rising temperatures in upper segments of *Sphagnum*. Because of the different biotic and abiotic gradients in *Sphagnum*-dominated peatlands, our

study highlighted the need to study together different microbial groups along the living parts of *Sphagnum*. A destabilization of the microbial food web by elevated temperatures (5 °C) through their trophic relationships is suggested in upper segments. Therefore, microbial assemblages and (or) testate amoebae may be a useful indicator to monitor climate change in peatlands. The relationships between water-soluble phenolics compounds and microbial communities living in *Sphagnum* remained an interesting result. Further in situ investigations characterized by a realistic warming and moisture variations should be undertaken.

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