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

3

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31 **Abstract**

32 Microbial communities living in *Sphagnum* are known to constitute early indicators of  
33 ecosystem disturbances, but little is known about their response (including their trophic  
34 relationships) to climate change. A microcosm experiment was designed to test the  
35 effects of a temperature gradient (15, 20 and 25°C) on microbial communities including  
36 different trophic groups (primary producers, decomposers and unicellular predators) in  
37 *Sphagnum* segments (0-3 cm and 3-6 cm of the *capitulum*). Relationships between  
38 microbial communities and abiotic factors (pH, conductivity, temperature, and  
39 polyphenols) were also studied.

40 The density and the biomass of testate amoebae in *Sphagnum* upper segments increased  
41 and their community structure changed in heated treatments. The biomass of testate  
42 amoebae was linked to the biomass of bacteria and to the total biomass of other groups  
43 added and, thus, suggests that indirect effects on the food-web structure occurred.  
44 Redundancy analysis revealed that microbial assemblages differed strongly in  
45 *Sphagnum* upper segments along a temperature gradient in relation to abiotic factors.  
46 The sensitivity of these assemblages made them interesting indicators of climate  
47 change. Phenolic compounds represented an important explicative factor in microbial  
48 assemblages and outlined the potential direct and/or indirect effects of phenolics on  
49 microbial communities.

50

51 **Keywords:** *Sphagnum*, testate amoebae, temperature gradient, phenolic compounds,  
52 bioindicators

53

54

55 **Résumé**

56 Les microorganismes des sphaignes sont connus comme indicateurs précoces des  
57 perturbations environnementales. Or, peu d'études portent sur l'influence de la  
58 température sur ces communautés. Dans cette étude, l'effet d'un gradient de  
59 températures (15, 20 et 25°C) sur les microorganismes des sphaignes, incluant différent  
60 groupes trophiques (producteurs primaires, décomposeurs, micro-prédateurs), a été testé  
61 dans les différentes parties des sphaignes (0 à 3 cm et 3 à 6 cm du *capitulum*). Les  
62 relations entre les microorganismes et les facteurs abiotiques (pH, conductivité,  
63 température, polyphénols) ont également été analysées.

64 La densité et la biomasse des amibes à thèque ont augmenté dans les parties supérieures  
65 des sphaignes. Une modification de la structure de leur communauté a aussi été mise en  
66 évidence. La biomasse des amibes à thèque est apparue liée à celle des bactéries et à la  
67 biomasse additionnée des autres groupes, suggérant des effets indirects de la  
68 température sur les relations trophiques. Les analyses par redondance ont révélé que la  
69 structure des communautés microbiennes variait entre les différents traitements dans les  
70 parties supérieures des sphaignes. Ainsi, les communautés microbiennes des sphaignes  
71 apparaissent comme un outil intéressant quant au suivi *in situ* des changements  
72 climatiques. Les polyphénols ont également été identifiés comme un facteur explicatif  
73 important de la structure des communautés microbiennes, montrant ainsi leurs effets  
74 potentiels sur les microorganismes des sphaignes.

75

76 **Mots-clés** : Sphaignes, amibes à thèque, gradient de température, composés  
77 phénoliques, bioindicateurs

78

## 79 **Introduction**

80 Climatic change models cover a wide range of temperature-increase scenarios (2°C up  
81 to 8°C), especially at high latitudes where the majority of *Sphagnum*-peatlands occurs  
82 (Gorham, 1991; IPCC, 2007). Peatlands are complex ecosystems, because of the  
83 diversity of habitats and micro-habitats allowing the establishment of diverse  
84 communities (Rydin and Jeglum, 2006). Studying the impact of climate change on these  
85 ecosystems remains particularly difficult and complex because of several biological and  
86 chemical parameters interacting (Davidson and Janssens, 2006). Some microcosm  
87 studies were elaborated to test the impact of a temperature increase on *Sphagnum* plant  
88 communities (Breuwer et al., 2008, 2009). However, such studies were carried out at  
89 the scale of simple organisms or populations and did not incorporate the interactions  
90 between different populations. Thus, a simplified ecosystem including different trophic  
91 levels could be then an interesting approach to better understand the effect of a  
92 temperature increase. In this context, the use of microbial communities living in  
93 *Sphagnum* represents a simplified ecosystem (Nguyen-Viet et al., 2007; Meyer et al.,  
94 2009). *Sphagnum* mosses are ubiquitous, cosmopolitan, and characterized peatlands  
95 (Shaw et al., 2003; Rydin and Jeglum, 2006). Moreover, *Sphagnum* mosses shelter a  
96 large number of microbial species belonging to the different trophic groups, including  
97 bacteria, algae, testate amoebae, ciliates, or fungi, which have a short generation time  
98 (hours up to few weeks) (Schönborn, 1986; Gilbert et al., 1998).

99         Some studies have demonstrated that the richness and density of single  
100 microbial groups (e.g. testate amoebae, microalgae) and/or the structure of microbial  
101 communities living in *Sphagnum* are affected by environmental perturbations such as  
102 nitrogen addition and atmospheric pollution (Howell and South, 1981; Gilbert et al.,  
103 1998; Mitchell et al., 2003; Nguyen et al., 2007, 2008). In addition, the integration of

104 testate amoebae into an experimental model is interesting because they feed on a wide  
105 range of preys, (i.e. bacteria, fungi, organic matter, algae, other protozoa) and could  
106 integrate ecosystem perturbations (Gilbert et al., 2000, 2003). Thus, such a simplified  
107 system not only would notably give insight into the interactions between species but  
108 also would incorporate biotic and abiotic factors (including phytochemicals)  
109 interactions between *Sphagnum* and its associated microbial communities.

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

127         In this study, we assessed the response of vertical patterns of microbial  
128 communities living in *Sphagnum* and the relationships among microbial communities,

129 temperature, and total phenolic compounds along a temperature gradient. The  
130 abundance and the structure of microbial communities and the concentrations of  
131 phenolic compounds were quantified in *Sphagnum* peat cores placed at 3 different  
132 temperatures (15, 20, and 25°C) after 8 weeks in a growth chamber. According to the  
133 gradient of temperature, we addressed the following hypotheses: (i) that the structure  
134 and biomass of microbial communities would vary among temperature treatments and  
135 *Sphagnum* layers (upper and lower), (ii) that the effect of temperature would be  
136 different for each microbial group according to their trophic position, and (iii) that total  
137 phenolic compound concentrations quantified in *Sphagnum fallax* would change along  
138 the temperature gradient and *Sphagnum* layers, and would explain a similar fraction of  
139 the community data as other environmental factors.

140

## 141 **Materials and Methods**

### 142 *Sphagnum* sampling and experimental setup

143 *Sphagnum fallax* was collected in a peat bog in the Jura Mountains (Sur-les-Seignes,  
144 Frambouhans-Les Ecorces, France, 47°18'N, 6°79'E) at an altitude of 846 m above sea  
145 level on 19<sup>th</sup> October 2007 (temperature 10°C). The climate of the area is characterized  
146 by cold winters (an average of -2.4°C in January) and mild summers (an average of  
147 14.6°C in July). The annual mean temperature of the region is about 6.6°C. The annual  
148 amount of precipitations is 1417 mm, and the duration of snow cover is an average of  
149 50 day. year<sup>-1</sup>. Surfaces of *S. fallax* as homogenous and pure as possible were selected.  
150 Sixteen *Sphagnum* peat cores (25 cm x 15 cm) were randomly sampled with a knife.

151 Four peat cores were assigned in quadruplicates to described the initial stage (IS)  
152 and 12 peat cores for the three treatments 15, 20 and 25°C (designated T15, T20 and

153 T25, respectively). A large gradient of temperature was chosen to observe the response  
154 of communities. Peat cores were randomly placed between the different levels of the  
155 growth chamber (Cryonext RTH600). General conditions selected were a relative  
156 humidity of > 70%, a light intensity of  $120 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ , and a photoperiod of 12h  
157 (light)/12h (dark). Each treatment corresponded to 1 level of the growth chamber. For  
158 each treatment, peat cores were placed in 1 big tray full of water (water level = 20 cm)  
159 and were humidified with a standard nutrient solution (Volvic water). The water depth  
160 was kept at 2 cm below the top of *Sphagnum* and readjusted with demineralized water  
161 every 2 days. The average relative humidity of *Sphagnum* was around 90% in  
162 *Sphagnum* segments for each treatment during the experiment. The 15°C temperature  
163 corresponded to the temperature of the growth chamber. The 20°C and 25°C  
164 temperatures were obtained by heating the water of the tray with respectively 2 thermo-  
165 divers of 50 and 200 W and 2 thermo-divers of 200 W. Moreover, aquarium pumps  
166 ( $5\text{L}\cdot\text{min}^{-1}$ ) were installed in the warm trays to obtain homogeneous temperatures. The  
167 position of peat cores was changed in each tray every 2 days to avoid experimental  
168 differences. The temperature was recorded several times a day during the experiment in  
169 each peat cores. The water chemistry (pH, conductivity and Eh potential) was also  
170 measured in each peat cores every 2 days.

171 Before and after 8 growing weeks 20 *S. fallax* were sampled in each peat cores  
172 and cut into 2 layers from the capitulum 0-3 cm (upper segments) and 3-6 cm (lower  
173 segments). For microbial analysis, the samples were fixed in 20 mL of glutaraldehyde  
174 (2% final concentration). No mosses were taken from the 5 cm bordering peat cores to  
175 avoid a border effect.

176



177 Total phenolic compounds assay

178 Phenolic compounds were extracted from lyophilized mosses following two methods as  
179 described in Jassey et al. (2011) and commonly used in phytochemical interactions  
180 (Waterman et al., 1994; Macheix et al., 2005). Water-soluble phenolics (hereafter “free  
181 phenolics”) were extracted using distilled water and corresponded to phenolics  
182 susceptible to directly interact with microorganisms. Primarily bound phenolics  
183 (hereafter “bound phenolics”) were extracted using ethanol/distilled water solution  
184 (80:20, v/v) and mainly corresponded to phenolic acids slightly bound to cell-wall.  
185 Thereafter, free and bound total phenolic contents were quantified with the Folin-  
186 Ciocalteu reagent and were expressed in milligrams of equivalent gallic acid ( $A_{760}$ )  
187 (Gallet and Lebreton, 1995).

188 Microbial Communities analyses

189 Microbial organisms were extracted from *S. fallax* with the standard method of Nguyen-  
190 Viet et al. (2007). Each sample was shaken for 1 min on a vortex and then *Sphagnum*  
191 mosses were pressed to extract microorganisms (first solution). The mosses were then  
192 soaked again with 20 mL of glutaraldehyde (2% final concentration), shaken a second  
193 time on a vortex, and pressed to extract *Sphagnum* leachate. The leachate was settled for  
194 12h, and afterward the supernatant was added to the *Sphagnum* and the material on the  
195 bottom was added to the first solution. The process was repeated 6 times, and all  
196 fractions were combined to obtain a final composite sample of 40 mL. The remaining  
197 fraction was dried at 80°C for 48h and weighted to express microbial density in grams  
198 of dry weight of *Sphagnum*.

199 Primary producers, fungi and unicellular predators: a 3 mL subsample of the  
200 final composite sample was settled for 2h in a plankton chamber and analyzed at x 400

201 magnification by inverted microscopy (Olympus IX71) following Uthermöhl's method  
202 (1958). The whole slide was analyzed and the different groups of cyanobacteria,  
203 microalgae, unicellular predators (flagellates, testate amoebae and ciliates) and fungi  
204 hyphae and spores were counted and measured. For testate amoebae, only living tests  
205 were counted.

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

214         The biovolume of each community was estimated by assuming geometrical  
215 shapes and converted to carbon using the following conversion factors: bacteria,  $1\mu\text{m}^3$   
216  $= 5.6 \times 10^{-7} \mu\text{gC}$  (Bratbak, 1985); cyanobacteria and microalgae,  $1 \mu\text{m}^3 = 1.2 \times 10^{-7} \mu\text{g C}$   
217 (Borsheim and Bratbak, 1987); ciliates and testate amoebae,  $1 \mu\text{m}^3 = 1.1 \times 10^{-7} \mu\text{g C}$   
218 (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  
219 were expressed as micrograms of Carbon per gram of *Sphagnum* dry mass per square  
220 centimeter ( $\mu\text{g C} \cdot (\text{g DW})^{-1} \cdot \text{cm}^{-2}$ ).

## 221 Numerical analysis

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

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

242 We assessed the relationships among the microbial communities in the upper  
243 and lower sampling depth and the 3 sets of environmental variables: (i) water chemistry  
244 (pH, Eh potential and conductivity), (ii) temperature and (iii) phenolic compounds  
245 (bound and free). The ordination patterns of microbial communities and their causal  
246 relationships to environmental data-sets were assessed using redundancy analysis  
247 (RDA) (Ter Braak and Simlauer, 1998). A forward selection of explanatory variables  
248 was computed for each of the *Sphagnum* segments, using automatic stepwise model  
249 building for constrained ordination methods ("ordistep" function). The proportion of  
250 variance explained by environmental variables was quantified using variance

251 partitioning. Adjusted  $R^2$  were used in all RDA to estimate the proportion of explained  
252 variance by each environmental variable (Peres-Neto et al., 2006).

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

256

## 257 **Results**

### 258 Environmental variables

259 During the experiment, temperatures recorded were stable in both *Sphagnum* segments  
260 with  $14.8 \pm 0.4^\circ\text{C}$  for T15,  $19.4 \pm 0.3^\circ\text{C}$  for T20 and  $23.9 \pm 0.5^\circ\text{C}$  for T25. The  
261 conductivity and the Eh potential were significantly lower ( $P < 0.05$ ) in T15 ( $30 \pm 9.4$   
262  $\mu\text{S}\cdot\text{cm}^{-1}$  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  
263  $335.5 \pm 20$  mV, respectively). The pH was stable during the experiment ( $\text{pH} = 4.1 \pm 0.5$   
264 in IS, T15, T20 and T25).

265

### 266 Temperature effect on total phenolic compounds

267 The concentrations in the 3 treatments were 1.5 times higher in upper segments (bound:  
268 an average of  $2.6 \text{ mg}\cdot\text{g}^{-1}$  DW, free: an average of  $1.8 \text{ mg}\cdot\text{g}^{-1}$  DW) than in lower  
269 segments (bound: an average of  $1.6 \text{ mg}\cdot\text{g}^{-1}$  DW, free: an average of  $0.7 \text{ mg}\cdot\text{g}^{-1}$  DW)  
270 (Table 1). Bound phenolics produced by *S. fallax* did not significantly differ between  
271 the upper and the lower segments, with the different temperatures. Free phenolics were  
272 significantly higher in upper segments ( $P < 0.04$ ) for T25 than for the other treatments.  
273 A significant relation was also found between bound and free phenolics ( $P < 0.001$ ,  $r =$   
274  $0.62$ ).

275 Microbial communities

276 The main change in the total biomass of microbial communities occurred in the upper  
277 segments of *Sphagnum* whereas it was quite stable in lower segments (Fig. 1). Indeed  
278 the total biomass in upper segments was nearly twice as high with elevated temperature  
279 (T15: 1400  $\mu\text{g C.}(\text{g DW})^{-1}.\text{cm}^{-2}$ , T20 and T25: 1700 and 2700  $\mu\text{g C.}(\text{g DW})^{-1}.\text{cm}^{-2}$ ,  $P <$   
280 0.01).

281 Primary Producers. The biomass of primary producers did not significantly vary  
282 between T15, T20, and T25 (Fig. 1). The biomass of microalgae was  $85 \pm 31 \mu\text{g C.}(\text{g DW})^{-1}.\text{cm}^{-2}$  in upper segments and  $106 \pm 31 \mu\text{g C.}(\text{g DW})^{-1}.\text{cm}^{-2}$  in lower segments. The  
283 community was dominated by the same species along *Sphagnum* segments in any  
284 treatments. *Chlorophyceae* (e.g. *Cylindrocystis* sp and *Penium* sp) and *Bacillariophyceae*  
285 (e.g. *Pinnularia viridis*) were the main identified genus. Cyanobacteria represented a  
286 small proportion ( $< 5\%$ ) of the total biomass along *Sphagnum* segments in any  
287 treatments with an average biomass of  $50 \pm 17 \mu\text{g C.}(\text{g DW})^{-1}.\text{cm}^{-2}$  in upper segments  
288 and  $31 \pm 10 \mu\text{g C.}(\text{g DW})^{-1}.\text{cm}^{-2}$  in lower segments. Cyanobacteria were dominated by  
289 *Chroococcales* (e.g. *Chroococcus giga*) and *Nostocales* (e.g. *Anabaenae cylindrica* and  
290 *Aphanothece nidulans*) along *Sphagnum* segments.

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

298 Unicellular predators. The biomass of testate amoebae increased from T15 ( $380$   
299  $\mu\text{g C.}(\text{g DW})^{-1}.\text{cm}^{-2}$ ) to T25 ( $600 \mu\text{g C.}(\text{g DW})^{-1}.\text{cm}^{-2}$   $P < 0.01$ ; Fig. 1). At the IS the

300 most frequent testate amoebae were *Archerella (Amphitrema) flavum* (29%), *Nebela*  
301 *tincta* (18%) and *Hyalosphenia papilio* (18%) in upper segments and *A. flavum* (30%),  
302 *N. tincta* (28%) and *Diffflugia bacilliarum* (27%) in lower segments (Table 2). The  
303 same species were dominant at T15 with *H. papilio* (42%), *A. flavum* (16%), and *N.*  
304 *tincta* (16%) in upper segments, and *A. flavum* (29%), *E. strigosa* (27%), and *N. tincta*  
305 (16%) in lower segments. For T20-T25 communities, the upper segments were  
306 dominated by *H. papilio* (35% and 43%, respectively) and *E. strigosa* (30% and 27%,  
307 respectively) (Table 2). The biomass of testate amoebae was correlated with the total  
308 biomass of other groups added ( $r = 0.63$ ,  $P < 0.01$ ) in upper segments but not in lower  
309 segments ( $r = 0.15$ ,  $P = 0.57$ ). The density of *E. strigosa* was significantly correlated to  
310 the temperature increase ( $r = 0.62$ ), the density of bacteria ( $r = 0.49$ ) and free phenolics  
311 ( $r = 0.58$ ) in upper segments of *Sphagnum* ( $P < 0.01$ ). In lower segments, no species  
312 was significantly influenced by the temperature gradient nor free phenolics.

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

315

316 Relationships between environmental parameters and *Sphagnum* biota

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

321         In the separate RDAs, T20 and T25 microbial assemblages and T15 microbial  
322 assemblage were clearly separated in upper segments (Fig. 3a), while in lower segments  
323 the separation between treatments was unclear (Fig. 3b). The models explained

324 respectively 56.4% and 27.6% of the variability in microbial data in upper and lower  
325 segments analysis ( $P < 0.01$  for each axes, Monte-Carlo permutation test, 999  
326 permutations). T20 and T25 microbial assemblages (particularly bacteria, fungi and  
327 testate amoebae) in upper segments were related with temperature and free phenolics,  
328 while microbial assemblage in T15 was related to Eh potential. In lower segments,  
329 microbial community structures along the temperature gradient were not clearly  
330 separated, except some T20 and T25 replicates correlated with elevated temperatures  
331 and the biomass of bacteria.

332         The successive RDAs on individual environmental variables revealed that the  
333 proportion of microbial data explained by each explanatory variable varied among  
334 variables both between the environmental data sets and along the 2 vertical positions  
335 (Table 3). The overall RDA (upper and lower segments) showed that free phenolics and  
336 temperature data sets explained, independently of the other 2 data sets, respectively  
337 11.9% ( $P = 0.004$ ) and 7.6% ( $P = 0.02$ ) of the microbial data variance. However, the  
338 variance explained by these variables was however much higher in the partial RDA's of  
339 upper and lower segments (Table 3). For example, temperature explained 42.1% of the  
340 microbial data variance in upper segments ( $P < 0.001$ ) and decreased to 2.5% in lower  
341 segments, while the variance explained by free phenolics increased from 5.2% to 15.7%  
342 ( $P < 0.05$ ) between the upper and lower segments.

343

344

345

346

347 **Discussion**

348 Temperature gradient effect on microbial food web

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

360         Along the temperature gradient, the density and the biomass of testate amoebae  
361 in *Sphagnum* upper segments increased and their community structure changed in  
362 heated treatments. These results combined with the significant relation between the  
363 biomass of testate amoebae and the total biomass of other groups added suggested that  
364 indirect effects on the food-web structure occurred. The significant link between  
365 *Euglypha strigosa* and bacteria along the temperature gradient seemed to confirm this  
366 hypothesis. Indeed, *Euglypha* is described as bacterivorous in literature (Gilbert et al.,  
367 2000; Beyens et al., 2009). The increase of bacteria density in *Sphagnum* upper  
368 segments probably influenced the abundance of *E. strigosa*. Beyens et al. (2009) also  
369 observed similar results for the correlation between testate amoeba communities and  
370 temperature effect. For example, *Trachelocorythion* and *Euglypha* genera reacted



371 positively with heatwave and were linked to the increase of bacteria. The high density  
372 and high relative abundance of *H. papilio* in heated treatments also suggested indirect  
373 effects on the food-web structure, since this species feed on a wide range of prey,  
374 notably small euglyphids (Gilbert et al., 2000, Meisterfeld, 2000a, b). Conversely, the  
375 biomass and the density of *N. tinctoria* did not vary along the temperature gradient. This  
376 species was recognized to essentially feed on autotrophic microorganisms during  
377 summer (Gilbert et al., 2003), and the biomass of these groups was not affected by the  
378 temperature increase. Microbial primary producers (microalgae and cyanobacteria)  
379 living in *Sphagnum* are well known to be sensitive to water chemistry, and to N and P  
380 addition (Hooper, 1981; Howell and South, 1981; Gilbert et al., 1998).

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

389

390 *Sphagnum* biota – environmental factors relationships

391 Among environmental factors, temperature appeared as the principal factor that  
392 determines microbial community variations. Direct gradient analysis (RDA) revealed  
393 that in upper segments temperature, Eh potential and conductivity significantly  
394 explained a high proportion of microbial data set variations. This result was not  
395 surprising since water chemistry generally contributed to a change in microbial

396 community distribution that occurred at the top of *Sphagnum* carpet (Howell and South,  
397 1981; Mitchell et al., 2003; Mieczan, 2009). Conversely, in lower segments, only free  
398 phenolics significantly explained microbial data variations. Studying both upper and  
399 lower segments along a temperature gradient demonstrated that the response of  
400 microbial communities to abiotic factors differed between *Sphagnum* segments. These  
401 results illustrate (i) how vertical gradients lead to ecological niches separations in  
402 *Sphagnum* and (ii) the potential influence of phenolic compounds on microbial  
403 community distribution.

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

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

437

#### 438 Conclusion

439 This original approach of studying *Sphagnum* upper and lower segments revealed a  
440 strong relationship between microbial community structures and rising temperatures in  
441 upper segments of *Sphagnum*. Because of the different biotic and abiotic gradients in  
442 *Sphagnum*-dominated peatlands, our study highlighted the need to study together  
443 different microbial groups along the living parts of *Sphagnum*. A destabilization of the

444 microbial food web by elevated temperatures (5°C) through their trophic relationships is  
445 suggested in upper segments. Therefore, microbial assemblages and/or testate amoebae  
446 may be useful indicator to monitor climate change in peatlands. The relationships  
447 between water-soluble phenolics compounds and microbial communities living in  
448 *Sphagnum* remained an interesting result. Further *in situ* investigations characterized by  
449 a realistic warming and moisture variations should be undertaken.

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451

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617 **Tables**

618 **Table 1.** Total concentrations ( $\text{mg}\cdot\text{g}^{-1}$  DW) of *Sphagnum fallax* free and bound  
619 phenolics in two layers (upper and lower segments) at initial stage (IS) and at the  
620 temperatures 15, 20, and 25°C (T15, T20, and T25) ( $n = 4$ ). *Letters* indicate significant  
621 differences of the total phenolic concentrations between the upper and the lower  
622 segments for a same treatment ( $P < 0.05$ ) and *Asterisk* indicates significant difference  
623 between temperatures ( $P < 0.05$ ).

624 **Table 2.** Abundance of 12 testate amoebae species found in upper and lower segments  
625 of *Sphagnum fallax* at initial stage (IS) and grown over an 8 weeks period at 15, 20 and  
626 25°C (T15, T20, and T25) ( $n=4$ ). *Asterisks* indicate significant differences of a same  
627 species related to the gradient of temperature in upper and lower segments ( $P < 0.05$ ).

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

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644 **Figures**

645

646 **Figure 1.** Total carbon biomass ( $\mu\text{gC.g}^{-1}.\text{cm}^{-2}$ , mean  $\pm$  S.E.) of all microbial groups and  
647 Carbon biomass ( $\mu\text{gC.g}^{-1}.\text{cm}^{-2}$ , mean  $\pm$  S.E.) of microbial groups in *Sphagnum* samples  
648 analyzed after 8 weeks related to the gradient of temperature. *Letters* indicate significant  
649 differences of biomass between temperatures in upper and lower segments (Friedman  
650 test,  $P < 0.01$ ). IS, initial stage

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

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

661