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**Experimental climate effect on seasonal variability of
polyphenol/phenoloxidase interplay along a narrow fen-bog ecological
gradient in *Sphagnum fallax***

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Running title: phenol/phenoloxidase interplay in peatland

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Abstract

Extracellular phenoloxidase enzymes play an important role in the stability of soil carbon storage by contributing to the cycling of complex recalcitrant phenolic compounds. Climate warming could affect peatland functioning through an alteration of polyphenol/phenoloxidase interplay, which could lead them to becoming weaker sinks of carbon. Here, we assessed the seasonal variability of total phenolics and phenoloxidases subjected to 2-3°C increase in air temperature using Open Top Chambers. The measurements were performed along a narrow fen-bog ecological gradient over one growing season. Climate warming had a weak effect on phenoloxidases, but reduced phenolics in both fen and bog areas. Multivariate analyses revealed a split between the areas and also showed that climate warming exacerbated the seasonal variability of polyphenols, culminating in a destabilization of the carbon cycle. A negative relationship between polyphenols and phenoloxidases was recorded in controls and climate treatments suggesting an inhibitory effect of phenolics on phenoloxidases. Any significant decrease of phenolics through repeatedly elevated temperature would greatly impact the ecosystem functioning and carbon cycle through an alteration of the interaction of polyphenols with microbial communities and the production of extracellular enzymes. Our climate treatments did not have the same impact along the fen-bog gradient and suggested that not all the peatland habitats would respond similarly to climate forcing.

Introduction

Boreal peatlands currently represent a terrestrial sink of carbon with approximately one-third of the world's organic carbon (390-455 Pg) (Gorham, 1991; Moore, 2002). The ability of peatlands to store atmospheric carbon resides in the long-term accumulation of partially decomposed organic matter. The accumulated peat is mainly dominated by remnants of mosses of the *Sphagnum* genus, highly enriched in recalcitrant organochemical compounds such as polyphenols (van Breemen, 1995; Verhoeven & Toth, 1995). Such compounds play a role both through a polyphenolic network linked to cell walls which could directly preserve *Sphagnum*-derived organic matter from degradation, and through the release of water soluble phenolics which directly interact with the surrounding environment (van Breemen, 1995; Verhoeven & Liefveld, 1997). Phenolics produced by *Sphagnum* have a potential inhibitory effect on fungal and bacterial activity and/or on enzymes involved in organic matter decomposition (Wetzel, 1992; Fenner *et al.*, 2005; Opelt *et al.*, 2007; Mellegard *et al.*, 2009). Among the diversity of enzymatic activities recorded in peat soils, only phenoloxidases – mainly produced by fungi – are involved in the polymerization, depolymerisation and transformation of both complex and simple phenolic compounds (Pind *et al.*, 1994; Thormann *et al.*, 2002; Fenner *et al.*, 2005; Baldrian, 2006; Sinsabaugh, 2010). However, acidic conditions, waterlogging and low soil temperatures that occur in peat soils were recognized to limit phenoloxidase activity (Pind *et al.*, 1994, Williams *et al.*, 2000; Freeman *et al.*, 2001a, b; Toberman *et al.*, 2008, 2010). Thus, carbon sequestration in peatlands is thought to partly result from a suppression of phenoloxidase activity (Freeman *et al.*, 2001a, 2004).

The expected increase of air temperatures in boreal regions is predicted to lead to a destabilization of peatland carbon stores (Smith *et al.*, 2004; Strack, 2008). Owing to the temperature regimes that currently constrain biological activities, climate warming may significantly impact the stability of the carbon cycle of peatlands by the breakdown of its

recalcitrant organic matter and thus act on “the enzymatic latch” (Freeman *et al.*, 2001a, 2004). However, recent research on the effect of climate change on phenoloxidases highlight equivocal results in peatlands (Laiho, 2006; Fenner *et al.*, 2007; Toberman *et al.*, 2008, 2010).

In regions without permafrost the most fundamental distinction among peatland types is between bog and fen (Bridgham *et al.*, 1998, 2001; Rydin & Jeglum, 2006). Bogs and fens have been found to have different plant communities, hydrology, nutrient availability, and soil chemistry (Bridgahm *et al.*, 1998, 2001; Wheeler & Proctor, 2000; Rydin & Jeglum, 2006). Owing to these differences in biotic and abiotic settings, bogs and fens are likely to differ in their response to climate change, (Weltzin *et al.*, 2000, 2001, 2003). Recently, Jassey *et al.* (2011a) demonstrated that microorganisms (e.g. testate amoebae) and their interplay with polyphenols varied along a short fen-bog gradient. Accordingly, an understanding of how climate change modifies carbon cycling in peatlands by modifying the polyphenol/phenoloxidase interplay in different ecological setting is essential to assess the capacity of peatlands to continue to store carbon.

The aim of this study was to investigate the impact of experimental climate warming on seasonal variation of polyphenols, phenoloxidases and their interplay in different ecological settings. These factors were studied at two depths along the living *Sphagnum* shoot on a short ecological gradient from a transitional *Sphagnum*-dominated poor fen to a *Sphagnum* bog with more pronounced micro-topography. Temperatures were manipulated using open-top chambers placed on half of the sampling plots, and compared with control plots. We hypothesized that (1) seasonal variations of polyphenols, phenoloxidases and their interplay would be different between the structurally more complex *Sphagnum* “bog” habitat and the more uniform poor fen, and (2) the warming effect would alter the seasonal variations of these factors along the fen-bog gradient.

Materials and methods

Field site and vegetation

The study site is an undisturbed *Sphagnum*-dominated mire situated in the Jura Mountains (The Forbonnet peatland, France, 46°49'35''N, 6°10'20''E) at an altitude of 840 m a.s.l. Cold winters (on average -1.4°C) and mild summers (on average 14.6°C) characterize the site. The annual mean temperature measured at the site over a one-year period from 5th November 2008 to 30th November 2009 was 6.5°C and the annual precipitation 1200 mm.

Samples of *Sphagnum fallax* were collected within homogeneous areas of *S. fallax* carpet across two adjacent areas selected in relation to their wetness, soil micro-topography, vegetation and assessment of sources and decay of organic matter according to Delarue *et al.*, (2011). The first sampling area (called “fen”) was a transitional *Sphagnum*-dominated poor fen with a relatively flat and homogeneous topography, characterized by a moss cover dominated by *S. fallax* and by the lack of *S. magellanicum*. Vascular plants such as *Eriophorum vaginatum*, *Vaccinium oxycoccus* and *Andromeda polifolia* were recorded in very low abundance. *Scheuchzeria palustris* and *Carex limosa* occurred outside of the studied plots. The second sampling area (called “bog”) was a *Sphagnum* bog directly adjacent to the fen area. Patterns of hummocks with *S. magellanicum*, *V. oxycoccus*, *E. vaginatum* and *Calluna vulgaris*, and hollows with lawns of *S. fallax*, *Carex rostrata* and *A. polifolia* characterized the sampling area. The terms “fen” and “bog” are used for simplicity and to denote the existence of a trophic and wetness gradient inferred from the vegetation.

Environmental manipulations and data collection

In each of the two sampling areas, six plots were selected in representative surfaces. Among the 12 sampling plots, the maximal distance between the two most distant plots was

ca. 30 m. In both sampling areas, 3 plots (replicates) were randomly assigned as controls and 3 plots were assigned as climate warming treatment (begin April, 2008). An increase of air and soil temperatures was passively achieved by placing hexagonal ITEX open-top chambers (hereafter “OTC”) over the vegetation (Marion *et al.*, 1997). Since warming in OTC chambers also affects the top-soil humidity, we hereafter name this treatment “climate effect”. Hexagonal OTCs were 50 cm high, had a diameter of 1.8 m at the top and 2.5 m at the bottom, and were made of transparent polycarbonate. To reduce edge effects such as reduced precipitation in the chamber we used the OTC design described by Aerts *et al.* (2004) and Dorrepaal *et al.* (2004). In each plot, air temperature (10 cm above the *Sphagnum* surface) and soil temperature (7 cm below the *Sphagnum* surface) were recorded continuously every 30 minutes using thermocouple probes and a datalogger (CR-1000 Campbell). Moreover, in each plot, pH, conductivity, water content of *Sphagnum* and the depth to the water table (DWT) were measured at each sampling campaign.

Every month from 25th May 2009 to 25th November 2009, samples of *S. fallax* were collected in each plot for the study of phenolic compounds, fungi-producing phenoloxidasases and phenoloxidasase activities around 10 permanent markers inserted in moss carpets. The goals of this sampling design were (1) to allow for multiple sampling at the site over time, and (2) to obtain a composite sample from each plot and avoid any bias due to spatial heterogeneity. *S. fallax* shoots were cut into two pieces (sampling depth): 0-3 cm (living “top segments”) and 3-10 cm (early declining “bottom segments”) from the capitulum.

Phenolic compounds quantification

Primarily bound (hereafter “bound phenolics”) and water-soluble phenolic (hereafter “free phenolics”) compounds were extracted from lyophilized mosses as described in Jassey *et al.* (2011a). Briefly, bound phenolic compounds were extracted using ethanol / distilled water

solution (80/20 v/v) and free phenolics using distilled water. Free and bound total phenolic contents were quantified with the Folin-Ciocalteu reagent and were expressed in mg equivalent gallic acid (A_{760}) per gram of *Sphagnum* dry mass (mg g^{-1} DM).

Quantification of culturable fungi-producing phenoloxidases

Culturable fungi-producing phenoloxidases were counted as described by Criquet *et al.* (2000). Two grams fresh weight of *Sphagnum* was powdered (< 0.5 mm; SEB[®] *Optimo* compact mixer) and suspended in 250 mL of a 0.85% NaCl solution with 0.05% Tween 80. This mixture was agitated for 2h on a reciprocal shaker (120 rpm). The extract was diluted (10^{-1} to 10^{-3}) in NaCl (0.85%) solution and 0.1 mL of each dilution was used to inoculate a medium containing 5 g of malt (Sigma), 15 g of agar (Sigma), 50 mg of chloramphenicol (Sigma) and 0.5 mL of guaiacol (Sigma) per liter. The fungi-producing phenoloxidases were revealed by the red color of the environment related to the oxidation of guaiacol. Results are expressed in colony forming units per gram of *Sphagnum* dry mass (CFU g^{-1} DM).

Phenoloxidase activities quantification

Phenoloxidase activities were quantified following the method described by Criquet *et al.* (1999). Phenoloxidases were extracted by adding in a Pyrex bottle 3 g of fresh weight of powdered *Sphagnum* with 50 mL of a 0.1 M CaCl_2 solution with 0.05% Tween 80 and 20 g of polyvinylpyrrolidone. The samples were shaken at room temperature for 1h on a reciprocal shaker (120 rpm). The suspension of each extract was filtered through a double layer of gauze to remove floating debris and centrifuged at 10 000 g for 10 min at 4°C. Then the supernatant was filtrated through 1.2 μm Whatman GF / D filters and concentrated for 24h in a cellulose-dialysis tube (Medicell International Ltd.) with a 10 kDa molecular mass cut-off, covered with polyethylene glycol (PEG, Sigma-Aldrich), until a final volume of 1/10 of the initial volume. Enzymatic activities were measured using a 96 well microtiter plate with

L-DOPA (10 mM). For each sample, 8 pseudo-replicate wells were included. Assay wells received 150 μ l of extract. Phenoloxidase activities were measured by adding 100 μ L of L-DOPA. For each sample, 8 pseudo-replicate wells containing 150 μ l of boiled extract (2h at 90°C) were performed as control. Then samples were incubated at 23°C and L-DOPA oxidation rates were monitored spectrophotometrically at 460 nm for 24h using a microstation plate reader (Bioadvance).

Enzymatic activities were calculated by subtracting the mean absorbance of control wells from the mean absorbance of extract wells and by using Beers Law. The molar absorptivity coefficient for the L-DOPA product 3-dihydroindole-5,6-quinone-2-carboxylate (dicq) ($3.7 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$; Mason, 1948) was used and activities were expressed in enzymatic units (U) defined as one nmol of substrate oxidized per h^{-1} per g of dry mass.

Numerical analysis

To compare the general effects of the OTCs on environmental parameters during the 7 months of our study, daily average temperature, as well as minimum and maximum daily temperatures, pH and conductivity were calculated for spring (May-June), summer (late-June-September) and autumn (late-September-November). Then repeated measures ANOVA were computed among sampling areas to focus on the effect of OTCs on these factors with time as repeated measure (time = 3: spring, summer and autumn). The depth and climatic effect on phenolic compounds (free and bound), culturable fungi-producing phenoloxidases and phenoloxidase activities were also analysed using repeated measures ANOVA with time as repeated measure (time = 7: May-November). Each dataset was thereafter split by month to get one response matrix per month for each biological factor using one-way ANOVA. In parallel, correlations between free phenolics, fungi-producing phenoloxidases and phenoloxidase activity in controls and OTCs were determined along the fen-bog gradient

using general linear models (GLM) and one-way ANOVAs. The residuals from ANOVAs were tested for normality. Moreover, the coefficient of determination of each variable in the models (adjusted R^2) was determined with an analysis of variance.

Redundancy analyses (RDA) were applied to *Sphagnum* related biochemical variables (polyphenols, phenoloxidases, culturable fungi-producing phenoloxidases) for each *Sphagnum* segment among the fen and the bog areas using climatic treatment (a binary variable with two levels: Control and OTC), *Sphagnum* moisture content and time (months coded as classes) as explanatory variables. The interactions between climatic treatment and *Sphagnum* moisture content were also included in the model. The significance of the model and of each explanatory variable included in the model was tested using 1,000 permutations (Gillet *et al.*, 2010). Partial RDAs were also computed after removing the time effect (months) from the ordination following the same method. Additionally, variation partitioning using RDA and adjusted R^2 was applied to compare the respective effect of each explanatory variable alone (Peres-Neto *et al.* 2006).

Multiple factor analysis (MFA) was used to symmetrically link seven groups of descriptors split in seven sub-matrices: the two *Sphagnum* related biochemical matrices (phenolic compounds and phenoloxidase data sets), the two abiotic data sets describing physical (depth to water table, air and soil temperature, rainfall and *Sphagnum* moisture content) and chemical (conductivity and pH) environmental conditions, the climatic data set describing climate treatment (a binary variable with two levels: OTC coded with 1 and control with 0), and the two data sets describing the seasons (spring, summer or autumn coded as classes), and the sampling areas (fen or bog coded as classes). MFA was chosen because it allows the simultaneous coupling of several groups or subsets of variables defined on the same objects and to assess the general structure of the data (Escofier & Pagès, 1994). Briefly, MFA is basically a PCA applied to the whole set of variables in which each subset is

weighted, which balances inertia between the different groups and thus balances their influences. RV-coefficients (Pearson correlation coefficient, ranging from 0 to 1) were used to measure the similarities between two data matrices and were tested by permutations (Robert & Escoufier, 1976; Josse *et al.*, 2008). Euclidean distances of global PCA were used in MFA to perform cluster analysis according to the Ward method, and the resulting dendrogram was projected in the MFA ordination space. This allows discovering the main discontinuities among groups and/or sites described by all biotic and abiotic subsets of variables (Carlson *et al.*, 2010; Borcard *et al.*, 2011).

All multivariate analyses were performed with the software R 2.10.1 (R Development Core Team 2010) using the vegan (Oksanen *et al.*, 2010) and FactoMineR (Husson *et al.*, 2009) packages.

Results

Seasonal variation of climate variables

In spring and summer (May to September), the OTCs significantly increased the daily maximum air temperature (an average of 3°C; ANOVA $P < 0.01$) and the average air temperature (an average of 1°C; ANOVA $P < 0.01$). Climate treatment also significantly affected the daily soil temperature in spring in the bog area (an average increase of 0.6°C; ANOVA $P < 0.05$) and in summer in the fen area (an average increase of 0.8°C; ANOVA $P < 0.05$). No significant differences emerged for the minimum and maximum soil temperatures. In autumn, no significant effect of OTCs was recorded along the gradient for air and soil temperature. An indirect effect of climate treatment was also observed in *Sphagnum* mosses, since a significant decrease of *Sphagnum* water content in OTCs was recorded in summer

(August and September) in both *Sphagnum* segments in the bog area, and in top segments in the fen area (ANOVA $P < 0.05$. Fig. 1).

Rainfall significantly varied following the seasons with a decrease from June (156 mm) to August, September and October (a monthly average of 72 mm) and an increase in November (231 mm). These variations were also reflected in the depth to water table. Following the seasons and climate treatments, average monthly pH did not significantly vary in both sampling areas (Table 1). Conversely, the conductivity increased from spring to autumn in both sampling areas, with significant differences between controls and OTCs in summer (bog area, $P = 0.05$) and in autumn (fen area, $P = 0.01$).

Climate effect on phenolic compounds and seasonal variations

Regardless of seasonal variations, climate effect and fen-bog gradient, bound and free phenolic contents were significantly higher (ANOVA $P < 0.001$) in top segments as compared to bottom segments (Fig. 2), except bound phenolics in the bog area ($P = 0.16$). The two phenolics variables were also positively correlated, with respectively $r = 0.38$ and 0.37 in the bog area (ANOVA, $P < 0.01$) and $r = 0.70$ and 0.41 in the fen area (ANOVA, $P < 0.001$). The climate effect on bound phenolics resulted in a decrease of concentration of an average of $0.4 \text{ mg g}^{-1} \text{ DM}$ in the two sampling areas, particularly in spring and summer in top segments ($P = 0.04$ and 0.02 , respectively). The climate effect on free phenolics was essentially recorded in the fen area for both *Sphagnum* segments, with constantly lower concentrations in OTCs than in controls over the seasons (ANOVA, $P = 0.001$) (Fig. 2), whereas the climate effect in the bog area was more rare.

In controls, seasonal variations of bound phenolics were recorded in top segments along the fen-bog gradient ($P = 0.04$ and 0.05 , respectively) (Fig. 2a, b, c, d), especially from May to August with a significant decrease of an average of $1.5 \text{ mg.g}^{-1} \text{ DM}$. In bottom

segments of controls, no significant seasonal variations of bound phenolics were recorded along the fen-bog gradient ($P = 0.86$ and 0.66 , respectively), with an average of respectively $1.5 \text{ mg g}^{-1} \text{ DM}$ in the bog area and $1.0 \text{ mg g}^{-1} \text{ DM}$ in the fen area. As for bound phenolics, seasonal variations of free phenolics in controls were recorded in top segments with a significant decrease in summer (from 1.4 to $0.8 \text{ mg g}^{-1} \text{ DM}$ in the two sampling areas; $P < 0.01$ and 0.03 , respectively). In bottom segments, no seasonal variations of free phenolics were recorded, with an average of $0.8 \text{ mg g}^{-1} \text{ DM}$ along the fen-bog gradient (Fig. 2e, f, g, h). In addition, a significant correlation was found between the decrease of phenolics (free and bound) and the decrease of *Sphagnum* moisture content in summer (ANOVA, $P < 0.01$) in both segments in the bog area, and in top segments in the fen area.

In OTCs, the same seasonal variations as in controls were recorded in *Sphagnum* segments and for both phenolics along the fen-bog gradient ($P < 0.05$ for all) (Fig. 2). As for controls, the same significant correlations were recorded between the decrease of phenolics (free and bound) and the decrease of *Sphagnum* moisture content in summer (ANOVA, $P < 0.05$).

Climate effect on culturable fungi-producing phenoloxidas and enzymatic activity, and seasonal variations

Significant differences between top and bottom segments of *Sphagnum* were recorded with overall higher densities of fungi-producing phenoloxidas and higher phenoloxidase activities in bottom segments as compared to top segments in both sampling areas (ANOVA $P < 0.05$).

For densities of culturable fungi-producing phenoloxidas, the climate effect was only significant in the fen area in top segments (ANOVA $P = 0.03$), with a significant lower value in June in OTCs compared to control (Fig. 3a, b). Seasonal variations were recorded for

both *Sphagnum* segments in the fen and bog area, with a peak in June in controls ($P < 0.05$) (Fig. 3 a, b, c, d), while in OTCs this peak was only recorded in the bog area (Fig. 3c, d). Climate effects on phenoloxidase activity demonstrated equivocal results in the fen area, while phenoloxidase activity tended to be higher in OTCs in the bog area (Fig. 3e, g).

Significant positive correlations were also found between densities of fungi-producing phenoloxidases and extracellular phenoloxidase activities, in both sampling areas and both climate treatments (on average $r = 0.40$; ANOVA, $P < 0.05$). In parallel, significant negative correlations between free phenolic compounds and phenoloxidase activities were found for controls in the fen and bog areas when top and bottom *Sphagnum* segments were pooled (Fig. 4a, b). The same tendency was recorded in OTCs, except in the bog area (Fig. 4b). Additionally, the combination of fungi and free phenols in a general linear model explained respectively 27.4% and 10.6% of the variability of phenoloxidase activity in controls, and 29.6% and 0.6% in OTCs in the bog area (adjusted R^2 ; $P < 0.001$). For the fen area another patterns occurred since fungi and free phenolics explained respectively 13.7% and 9.8% of the variability of phenoloxidase activity in controls, and 11.3% and 25.8% in OTCs (adjusted R^2 ; $P < 0.001$).

The phenol-phenoloxidase complex and its relation to abiotic variables

The contribution of the explanatory variables in the RDA (Table. 2) showed that time (months) has a major influence on the moss biochemical patterns. In bottom segments sampling time explained between 41% and 66% of the variation. In top moss segments, biplots of partial RDAs showed that *Sphagnum* related biochemical variables were influenced by climate treatment, as shown by the separation of control and OTC plots along the first RDA axis (Fig. 5a, c). Together, OTCs and *Sphagnum* moisture content explained 20.6% (fen) and 27.1% (bog) of the variation of biochemical factors ($P < 0.05$) in top segments.

Variation partitioning and adjusted R^2 showed that OTCs alone explained a higher variation in the fen area than in the bog area, whereas *Sphagnum* moisture content has higher influence in the bog than in the fen (Table 2). On the other hand, the biochemical descriptors showed a strong opposition between phenolics and warming treatment (OTC) in all biplots, while fungi appears linked to *Sphagnum* moisture content, particularly in top segments.

If we consider all samples together along the fen-bog gradient in the multiple factor analysis (Fig. 6), a clear pattern appeared, with a split into the three seasons (spring, summer and autumn) and within each partition a subdivision into fen and bog areas, each of these subdivisions being further divided into OTC and control plots. The RV-coefficients (Table 3) indicate strongest links between *Sphagnum* related biochemical variables, sampling area, climate warming and seasons, and between sampling area and physicochemical environment.

Discussion

Polyphenol/phenoloxidase interplay in *Sphagnum* mosses and along the fen-bog gradient

Sphagnum related biochemical factors quantified in this work yielded different results according to *Sphagnum* segments. Total phenolic content (free and bound) was higher in living top segments as compared to decaying bottom segments in both sampling areas. Such differences have been also observed in *S. fallax* under controlled conditions (Jassey *et al.*, 2011b). This phenomenon is explained by a higher phenolic metabolism in capitulum than in lower part of the shoot, since *Sphagnum* capitula (top segments) constitute the living part of the moss where most of the metabolic processes occur, including the growth (Clymo & Hayward, 1982). The reduction of phenolics towards the lower part of the shoot was also accompanied by an increase of culturable fungi-producing phenoloxidases and of phenoloxidase activity, suggesting a higher degradation of recalcitrant phenolics in early

declining *Sphagnum* segments (Baldrian, 2006; Toberman *et al.*, 2010; Sinsabaugh & Follstad Shah, 2011). These results also pointed to the fact that at low concentrations free phenols may induce phenoloxidase activity, and inhibit the oxidation activity at high concentration (Sinsabaugh, 2010). Given that no clear correlation was found between fungi and free phenols, such vertical gradient also highlighted a possible direct inhibitory effect of free phenols on phenoloxidase activity (Wetzel, 1992; Freeman *et al.*, 2001a; Fenner *et al.*, 2005).

Our results likewise demonstrated a strong relationship between fungi and phenoloxidase activities. Phenoloxidase activity is essentially attributable to lignolytic fungi such as basidiomycetes (Criquet *et al.*, 2000; Thormann *et al.*, 2002; Baldrian, 2006). Fungal activity is known to be directly influenced by the supply of organic matter (Berg *et al.*, 1998; Criquet *et al.*, 2000). A study in the same experimental site demonstrated over the fen-bog gradient an increase of organic matter content in the upper 10 cm soil layer, which induced higher fungal activity (Delarue *et al.*, 2011). Thus, all of these findings emphasize that phenoloxidase activity was mainly controlled by fungi and secondarily by phenols.

Beside the differences between *Sphagnum* segments, different patterns of polyphenol content and phenoloxidase activities were recorded along the fen-bog gradient over the seasons. In particular, phenoloxidase activities were more intense in the bog area than in the fen area. Again, this result appeared linked to fungi. The abundance of vascular plants is higher in the bog area and supplies more easily decomposable organic matter, favouring fungal activity (Delarue *et al.*, 2011). A number of studies have demonstrated that fen and bog litters were characterized by distinct patterns of microfungal community, especially in the surface horizons (Thormann *et al.*, 2001, 2002, 2004; Thormann, 2006; Artz *et al.*, 2007). Thus, vegetation patchiness along the fen-bog gradient may directly affect fungal community composition, and indirectly phenoloxidase activity. In particular, the quality and quantity of plant-derived labile carbon resulting from vegetation succession may directly influence fungal

diversity, e.g. polymer- and recalcitrant polymer degraders (Thormann, 2006). On the other hand, the influence of free phenols on phenoloxidases was higher in the fen area than in the bog and this could be explained by qualitative differences of phenolics in *Sphagnum* along the gradient (Opelt *et al.*, 2007). When comparing phenolic content in *Sphagnum* from different ecological setting, Folin assay only gives a global tendency of phenolic variation, and not the quality of free phenols that may influence phenoloxidase activity. Such results clearly call for a detailed analysis of phenolic variation (e.g. phenolic acids or flavonoids).

Climate effect on polyphenols, phenoloxidases and their interactions along the fen-bog gradient

As described in previous studies (Dorrepaal *et al.*, 2004; Aerts, 2006), higher air temperatures induced higher evapotranspiration, which resulted in lower *Sphagnum* moisture content during summertime. Obviously, higher evapotranspiration also could have sometimes induced lower soil temperature by heat loss towards atmosphere and reduction of soil thermal conductivity, thus explaining the so-called marginal effect of OTCs on soil temperature (Dabros *et al.*, 2010). Despite contrasted effects of OTCs on air and soil temperature, a climate effect has been recorded on biochemical variables measured along *Sphagnum* segments.

Seasonal effects were predominant for the biochemical variation in *Sphagnum* carpet. However, multivariate analyses revealed a climate warming effect beyond the seasonal variations of *Sphagnum* biochemical related factors. As observed elsewhere (Aerts, 2006; Bragazza, 2008; Dabros and Fyles, 2010; Dabros *et al.*, 2010), the increase of air temperature associated with the reduction in rainfall led to heat waves, and the impact of these events was exacerbated in OTCs increasing drought in top-soil. Enhanced top-soil aeration as a result of water table drawdown and air temperature increase was recognized to influence

phenoloxidase activity and polyphenols (Freeman *et al.*, 1993, 2001a, b; Toberman *et al.*, 2008; Ellis *et al.*, 2009). As supported by current findings in peatlands (Pind *et al.*, 1994; Williams *et al.*, 2000; Freeman *et al.*, 2001a; Toberman *et al.*, 2008, 2010; Sinsabaugh, 2010), peat soil environmental factors (i.e. acidic pH, water table depth, and oxygen) mainly inhibit phenoloxidase activity, explaining our weak variations of phenoloxidases with climate warming.

In parallel, climate warming had greatest impact on the phenolic metabolism with a decrease of phenolics related to the decrease of *Sphagnum* moisture in OTCs and the increase of air temperatures. The level of total phenolic compounds tends to be lower in several boreal species under elevated temperatures (Veteli *et al.*, 2007). Such decrease may be explained by a diminution of carbon partitioning to phenolics (Herms & Mattson, 1992; Mattson *et al.*, 2005). Elevated temperatures are recognized to induce better growth of *Sphagnum* species (Breeuwer *et al.*, 2008). It might well be that a trade-off between growth and differentiation (i.e. the production of carbon-based secondary metabolites such as phenols) occurred, with a potential diminution of carbon skeletons allocation to phenolics (Mattson *et al.*, 2005; Veteli *et al.*, 2007). Such results imply that any repeated significant decrease of phenolics through more intense and frequent heat waves – as predicted by climate scenarios (Meehl & Tabaldi, 2004; Schär *et al.*, 2004; IPCC, 2007) – will probably lead to the opening of the enzymatic latch, as described by Freeman *et al.* (2001b).

Furthermore, our climate experiment demonstrated that climate warming has not had the same impact along the fen-bog gradient since a stronger decrease of polyphenols was recorded in the fen area. This decrease induced a switch between fungi and free phenols, leading to a reduction of the potential inhibitory effect of free phenols on phenoloxidases. However, the decrease in the density of culturable fungi-producing phenoloxidase during dryer periods could not compensate for the decrease of phenolics and lowering of their

inhibitory effect on phenoloxidase activity. Alternatively, or additionally, phenolics may also have inhibitory effects on other microbial activities with implication for the carbon cycle, such as hydrolase activity (Fenner *et al.*, 2005, 2007). Thus, the reduction of the inhibitory effect of free phenols could affect carbon cycling in the fen area through another microbial/polyphenols interplay (e.g. Jassey *et al.*, 2011a). In the bog area phenoloxidase activity remained the key factor influenced by climate treatment with a slight increase of activity in top segments, leading to potentially higher degradation of recalcitrant materials in surface horizons. In contrast to the fen area, it appeared that fungi mainly influenced phenoloxidases in OTCs, as shown by GLMs.

Although a slight increase of temperature induced by OTCs is not strong enough to significantly affect the decomposition rate of *Sphagnum* litter on short-time scale (Dabros *et al.*, 2010), our results demonstrated that already within a 7-month period key elements of the carbon cycle can be altered in surface horizons. Furthermore, our climate experiment highlights different responses of *Sphagnum* related biochemical variables along the fen-bog gradient. The main consequence is that not all the peatland habitats would respond similarly to climate forcing. Ultimately, our results suggest a destabilization of peatland ecosystems and reinforce the point that phenoloxidase/polyphenol interplay is especially critical to understanding the response of peatlands to climate change.

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Table 1: Seasonal variations of environmental variables measured in controls and OTCs in the fen and bog sampling areas in Le Forbonnet mire (French Jura). *Letters* indicate significant seasonal variations ($P < 0.05$). *Asterisks* indicate significant variations between controls and OTCs ($P < 0.05$).

Table 2: Summary of RDA on *Sphagnum* related biochemical variables and environmental explanatory variables from Le Forbonnet mire (French Jura): fraction of variance explained and significance of individual variables taken alone. *Sph* moisture = *Sphagnum* moisture content; clim treat = climate treatment.

Table 3: RV-coefficients (RV) and corresponding P -values among the six groups of variables used in the Multiple factor analysis (MFA) of the entire data set split into 6 groups of variables describing *Sphagnum* biochemistry, environmental physical and chemical conditions, climate warming treatment, seasons, depth of moss segment and bog/fen areas . Significant coefficients are in bold.

640 Figures:

641 Figure 1: Seasonal variations of *Sphagnum* moisture content in the two shoot segments (top
642 and bottom) in controls and OTCs of the fen (a, b) and bog (c, d) areas. Mean $\pm$ S.E. (n = 3).
643 *Asterisk* indicates significant difference between controls and OTCs (ANOVA tests, $P <$
644 0.05).

645 Figure 2: Seasonal variations of bound (a, b, c, d) and free (e, f, g, h) phenolics in the two
646 shoot segments (top and bottom) in controls and OTCs of the bog and fen areas. Mean $\pm$ S.E.
647 (n = 3). *Asterisk* indicates significant difference between controls and OTCs (ANOVA tests, P
648 < 0.05).

649 Figure 3: Seasonal variations of densities of fungi producing phenoloxidas (a, b, c, d) and
650 phenoloxidase activities (e, f, g, h) in the two shoot segments (top and bottom) in controls and
651 OTCs of the bog and fen areas. Mean $\pm$ S.E. (n = 3). *Asterisk* indicates significant difference
652 between controls and OTCs (ANOVA tests, $P < 0.05$).

653 Figure 4: Correlations between free phenolics and phenoloxidase activity for *Sphagnum*
654 segments (top and bottom segments pooled) in controls and OTCs in the fen (a) and bog (b)
655 areas.

656 Figure 5: Biplots of redundancy analyses (RDA) of biochemical data measured on *Sphagnum*
657 mosses (free and bound phenolics, phenoloxidas and fungi-producing phenoloxidas) in
658 top (a) and bottom (b) *Sphagnum* segments of the fen area, and in top (c) and bottom (d)
659 segments of the bog area. Climate treatments are coded with open symbol for controls and
660 with filled symbol for OTCs. Months are indicated next to the sample points by their number.
661 Season effect has been removed by giving the variable months as covariable. Environmental
662 variables are represented by vectors (arrows for quantitative or semi-quantitative variables):
663 *Sph_moist.*: *Sphagnum* moisture content; *Sph_moist:OTC*: interactions between *Sphagnum*

moisture and OTCs. Biochemical variables are given with dotted arrows: F_phen: free phenolics, B_phen: bound phenolics; Phen_oxid: phenoloxidase activity; Fungi: culturable fungi-producing phenoloxidase. Axes are significant ($P < 0.05$), except for bottom segments. Axes 3 are never significant, with less than 1% of variance). *Grey ellipses* represent S.E. of site scores around the centroid of each treatment level.

Figure 6: Multiple factor analysis (MFA) samples biplot of the entire data set split into 7 groups of variables describing *Sphagnum* biochemistry, environmental physical and chemical conditions, climate warming treatment, seasons and fen-bog areas. Biplot of axes 1 and 2 (both significant at $P = 0.001$) is given together with the result of a hierarchical agglomerative clustering (grey lines) obtained by the Ward method on the Euclidean distance matrix between MFA site scores, showing three main groups of sampling plots (circles = spring, squares = summer, triangles = autumn) and two sub-groups (white symbols = controls, black symbols = OTCs). Sampling areas are indicated with letters besides sampling plots (F: fen area; B: bog area).