Experimental warming differentially affects microbial structure and activity in two contrasted moisture sites in a *Sphagnum*-dominated peatland

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1. Introduction

The impact of climate change and its consequences on global air temperature are still debated as some uncertainties remain (IPCC, 2007; Otto et al., 2013), in particular concerning the potential feedback of the terrestrial carbon (C) cycle to climate warming (Davidson and Janssens, 2006; Friedlingstein et al., 2006). Understanding the fate of the C stored in peatlands is crucial since these ecosystems contain about one-third of the world's soil organic C as peat (Gorham, 1991), the equivalent of about 60% of atmospheric C.

The C sink function of peatlands is mainly the result of persistent anoxic conditions, low temperature and water acidity that reduce microbial decomposition and promote the accumulation of organic matter (OM) as peat. As a result, peat organic C is expected to be particularly sensitive to climate warming because of the higher intrinsic temperature sensitivity of this type of organic soil (Davidson and Janssens, 2006). Thus, a 1°C increase in air temperature has been estimated to enhance C fluxes from heterotrophic respiration in northern peatlands by about 38–100 Mt of C per year (Dorrepaal et al., 2009). Other studies indicated that climate change can further diminish C sequestration by promoting the growth of vascular plants which, in turn, depress the productivity of peat mosses (Breeuwer et al., 2009; Bragazza et al., 2013). In contrast to these studies, Loisel et al. (2013) and Charman et al. (2013) indicated that the C accumulation rate of many northern peatlands could increase in response to a warmer climate in the future, as long as moisture is not a limiting factor. These examples illustrate the ongoing debate on the fate of C in peatlands in response to climate

change. Several studies demonstrated the complex interaction between peat moisture and air temperature in regulating peat decomposition (Delarue et al., 2011a; Jassey et al., 2011; Bokhorst et al., 2013) but few experimental studies have specifically addressed this topic, notwithstanding the close relationship between moisture, temperature and C cycling for peat decomposition (McNeil and Waddington, 2003). It was demonstrated that an increased water evaporation in peat soils and a drying out of the surface layer can decrease the soil's thermal conductivity which, in turn, can prevent heat from propagating deeper into the soil and can therefore keep them colder (Dabros and Fyles, 2010). Other authors also reported that evaporation was associated to a cooling of the upper moss layers, inducing a condensation of vapor (Carleton and Dunham, 2003).

The peculiar environmental conditions in peatlands favour the establishment of *Sphagnum* mosses, which are known to produce recalcitrant litters enriched in polyphenolic compounds (van Breemen, 1995; Abbott et al., 2013; Swain et al., 2013). Although polyphenols inhibit extracellular enzymatic activity (Freeman et al., 2001; Freeman et al., 2004), the enzymes belonging to the phenoloxidase (PO) group have the ability to degrade recalcitrant polyphenols accumulating in peatlands (McLatchey and Reddy, 1998; Freeman et al., 2001). In a perspective of climate change, PO activity is expected to increase as a consequence of more frequent drought events and associated oxygenation of peat soils (Fenner and Freeman, 2011). Due to a decrease in soluble phenols with increasing activity of PO, hydrolytic enzymes such as leucine amino-peptidase (LAP), β-glucosidase (BG) and acidic phosphatase (AP) are no longer inhibited so that the breakdown of OM can start. Such a pattern is known as the enzymatic latch theory (Freeman et al., 2001). Following such cascading effects, various studies suggested that peat OM decomposition will be enhanced by climate warming (Dorrepaal et al., 2009; Fenner and Freeman, 2011; Jassey et al., 2013). However, the studies were mainly conducted during the summer months, when environmental constraints, *i.e.* water

level drawdown and air temperature, were less limiting for microbial metabolism. Therefore, two major questions remain to be clarified: (1) how air temperature and water level interact to affect both soil temperature and moisture under conditions of water saturated peat and (2) how this interaction can affect the soil C cycle.

In this study we investigate the interactive effects of air warming which was experimentally induced by Open-Top Chambers (OTCs), and water level by comparing two habitats characterized by contrasted soil moisture conditions. The study was performed in early summer when both soil water level and air temperature had not yet reached their annual minimum and maximum values respectively. Specifically, we explored peat moisture changes as a function of soil water level, and air and peat temperatures. The impact of air warming and peat moisture on the soil C cycle was assessed using phospholipidic fatty acids as an index of microbial biomass, various enzymatic activities (leucine aminopeptidase, β-glucosidase, acidic phosphatase and phenol oxidase) as indexes of microorganism activities and the corresponding chemical quality of water-extractable OM (WEOM).

2. Material and Methods

2.1. Study site, experimental design and sampling

The study site is an undisturbed ombrotrophic *Sphagnum*-dominated peatland situated in the Jura Mountains (Le Forbonnet, France; 46°49’35”N, 6°10’20”E), at an altitude of ca. 840 m a.s.l. The annual mean temperature at the site is ca. 6.5°C, and the annual precipitation is about 1200 mm (Delarue et al., 2011b). Cold winters (mean monthly temperature ca. 1.4 °C) and mild summers (ca. 14.6°C) characterize the climate.

Peat samples were collected in late June 2011 across a vegetation gradient corresponding to a narrow transitional minerotrophic to ombrotrophic area. The transition from the minerotrophic

to the ombrotrophic area was characterized by a shift from an area of relatively flat and homogeneous surface dominated by *Sphagnum fallax* with a low abundance of vascular plants (i.e. *Eriophorum vaginatum*, *Vaccinum oxycoccus* and *Andromeda polifolia*) to a surface with a patterned vegetation of hummocks, where *S. magellanicum*, *V. oxycoccus*, *E. vaginatum* and *Calluna vulgaris* developed, and hollows mainly occupied by *S. fallax*, *Carex rostrata* and *A. polifolia*. The main change between the minerotrophic and the ombrotrophic areas was the occurrence of *S. magellanicum* in the latter, entailing a change in the microtopography (considered as a site effect). For simplicity's sake, we will call the minerotrophic and ombrotrophic areas, respectively, Fen and Bog sites hereafter.

The experimental design was described in detail in previous work (Delarue et al., 2011a; Jassey et al., 2011). Briefly, OTCs are passive warming chambers designed following the International Tundra Experiment (ITEX) to obtain quasi-natural transmittance of visible wavelengths and to minimize the transmittance of re-radiated infrared wavelengths (Marion et al., 1997; Aronson and McNulty, 2009). The hexagonal chambers are made of transparent polycarbonate and are 50 cm high, 1.7 m wide at the top and 2.4 m wide at the base. They were raised 10 cm above the soil surface to allow air to circulate. Six plots were equipped with OTCs in May 2008, and 6 other plots were used as controls (CTLs). For this study, the plots were named as follows: Bog-OTC and Bog-CTL for plots in the Bog site with and without OTCs respectively, and Fen-OTC and Fen-CTL for plots in the Fen site with and without OTCs respectively.

Temperature of the peat (7 cm deep) and air (10 cm above *Sphagnum* capitulum) was automatically measured every 30 minutes using thermocouple probes in each plot and a data logger (CR-1000 Campbell). Monthly mean, minimum and maximum temperatures, for both peat and air, were then calculated for the period from January 2011 to June 2011. The ground water level was automatically measured in one randomly selected plot at both Bog and Fen

sites (mid-May 2011 to late June 2011). Lastly, peat moisture and temperature were measured at ca. 5 cm depth by Decagon® sensors only during the growing season (from early May 2011 to October 2011) in two randomly selected plots at both the Bog and Fen sites. Twelve peat cores 30 cm long were sampled in June 2011, after 3 years of experimental warming. The peat cores were cut into five slices (0 to 5, 5 to 10, 10 to 15, 15 to 20 and 20-25 cm interval depth) and frozen. Within two weeks after sampling, each slice was subdivided in two parts. For each part, water was gently extracted following the procedure described by Delarue et al. (2011a) modified by the use of a PTFE filter (0.45 µm pore size). After WEOM extraction, peat samples were dried at 105°C during 24 hours in order to obtain the peat dry mass. The water content was calculated by considering the peat dry mass and the peat wet mass measured before WEOM extraction.

2.2. Structure of microbial communities - PLFAs

Phospholipid-fatty acids (PLFAs) were extracted on freeze-dried peat samples using the Bligh and Dyer method (1959), modified for peat (Andersen et al., 2010). Peat samples (250 mg) were shaken during 2 hours in a phosphate-buffer (0.1M; pH 7):$CHCl_3$:MeOH (0.9:1:2 v/v/v) solution and the supernatant was then transferred to a phosphate buffer/ chloroform solution. After separation of the organic phase overnight, the $CHCl_3$-lipid phase was split into neutral, glyco- and phospholipids in a silicic acid column by eluting chloroform, acetone and methanol respectively. Phospholipids were then transesterified into fatty acid methyl esters (FAMEs) after incubation at 40 °C in a methanolic KOH (1M):toluene (1:1 v/v) solution. The solution was neutralized with acetic acid (1M) and FAMEs were extracted by adding a hexane: $CHCl_3$ (4:1) solution. The hexane fraction was then passed through an $MgSO_4$ column before evaporation to dryness under an N_2 flux.

FAMEs were analysed by means of GC-MS and quantified using a GC apparatus (Trace GC, Thermo Finnigan) equipped with a Supelco Equity 5-fused silica column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) coupled to a mass spectrometer (Quadrupole DSQ II, Thermo Finnigan). Helium was employed as the carrier gas at a constant flow rate. Methyl nonadecanoate ($C_{19}O_2Me$) was used as internal standard. Strict location of double bonds was realized by derivatization of FAMEs into picolinyl esters on representative samples (Wretensjö et al., 1990).

We used the PLFAs i15:0, a15:0, i16:0, i17:0 and a17:0 as markers of G+ Bacteria (Frostegård and Bååth, 1996); 16:1ω7c et cy17:0 as markers of G- Bacteria (Wilkinson 1988 ; Zelles, 1999); 18:2ω6,9 as marker of Fungi (Bardgett et al., 1996; Frostegård and Bååth, 1996; Zelles, 1999); 10Me16:0 and 10Me18:0 as markers of actinobacteria and sulfate-reducing bacteria (Kroppenstedt, 1985) and 20:4ω6,9,12,15 as markers of Protozoa (Ringelberg et al., 1997). Other PLFAs detected in the samples were not specific to one particular functional group so were not used in the comparisons. PLFA concentrations are expressed as µg $C.g^{-1}$ of dry peat.

2.3. Water-extractable organic matter analyses

WEOM analyses were performed on the first part of each peat slice. The WEOM was divided into three aliquots for analyses of organic C (WEOC), total sugars and $SUVA_{280}$, an index of the aromaticity of WEOM (Kalbitz et al., 2003). To calculate the WEOC, the dissolved organic carbon (DOC in mg l^{-1}) was first determined after acidification with H_3PO_4 (pH = 4) and N_2 purging. DOC was then measured with a Shimadzu SSM-5000A total carbon analyser. Finally, the mass of dissolved C was calculated and divided by the initial sample dry mass to obtain the WEOC expressed in $mg.g^{-1}$ of dry peat. Total sugars were determined on the

second aliquot following the phenol–sulfuric method with glucose as standard to allow the calculation of sugar content (Dubois et al., 1956). Total sugar contents were expressed in mg of carbon g^{-1} of dry peat, since we assumed that the weight ratio of C in sugars was that of glucose (2.5). For $SUVA_{280}$, the third aliquot was adjusted to a pH ranging from 6 to 7 following the recommendation of Weishaar et al. (2003). UV absorbance was then measured at 280 nm using a UV spectrophotometer. Finally, $SUVA_{280}$ was calculated as absorbance divided by WEOC concentration (Hansson et al., 2010) and is expressed as g of dry peat per mg $C^{-1}cm^{-1}$.

2.4. Extracellular enzymatic assays

Enzymatic activities were measured on the second subsample of each slice. The activity of extracellular phenol oxidase was determined spectrophotometrically by using 10 mML-dopa (dihydroxyphenylalanine) solution as substrate (Pind et al., 1994). The activity of phenol oxidase (PO) was expressed in µmol of 2,3-dihydroindole-5,6-quinone-2-carboxylate (dicq) min^{-1} $g^{-1}$of dry peat.

The activity of extracellular hydrolytic enzymes was measured by adding 4-methylumbelliferyl-ß-D-glucoside for ß-glucosidase (BG), L-leucine-7-amido-4-methycoumarinhydrochloride for leucine aminopeptidase (LAP) and 4-MUF-phosphate for the activity of acidic phosphatase (AP) to about 1 g of fresh soil. After incubation (1 h for BG, and LAP, and 45 min for AP), the fluorescence of the supernatant after centrifugation was measured on a microplate reader (BioTekSynergyMX) at 450-nm emission and 330-nm excitation wavelength. To quantify product release and account for quenching effects, a set of standards was prepared using methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (MCU) mixed with peat extract (Freeman et al., 1995; Saiya-Cork et al., 2002). Hydrolytic

enzyme activity was expressed as µmol of substrate (MUF) converted per minute and per gram of dry peat.

2.5. Statistics

To study the impact of air warming upon water content, PLFAs, WEOM features and extracellular enzymatic activities resulting from each depth, slices were pooled in order to obtain an overall response for the 25 cm peat column in each plot. All statistical analyses were performed using xlstat software (addinsoft®). Data were tested for normality using the Kolmogorov–Smirnov test and for homogeneity of variance using the Levene test. Data were log10-transformed when non-normality and/or no homogeneity of the variance were found. Variations in air and peat temperatures were examined through Repeated Measures ANalysis Of VAriance (MANOVA) in order to test the singular impact and interactions of sites, air warming and time (i.e., months for air and peat temperatures). Following significant MANOVA tests (p-value< 0.05), significant differences were determined with Fisher's LSD tests. Variations in water content, PLFAs, WEOM features and extracellular enzymatic activities were analysed using ANOVA (i) to test the overall impact of air warming on these variables, (ii) to test the impact of air warming within the Bog and Fen sites and (iii) to investigate the impact of air warming on the initial differences distinguishing the Bog and Fen sites.

3. Results

3.1. Air and Peat temperatures

Continuous measurements of air temperature during the period from January to June 2011 in both control and OTC plots indicated significant effects related to the site type, warming

treatment and time (Table 1). Minimum air temperature was significantly higher at the Fen site (-3.8°C) than at the Bog site (-4.2°C; Table 2). Conversely, maximum air temperature was significantly higher at the Bog site (19.3°C) than at the Fen site (17.9°C). The single effect of air warming treatment also led to a rise in mean (+0.8°C), minimum (+0.4°C) and maximum (+2.3°C) air temperatures (Table 2). More specifically, the experimental air warming treatment increased the mean (+0.9°C), minimum (+0.6°C) and maximum (+2°C) air temperatures at the Bog site (Table 2). At the Fen site, OTCs were also associated with a rise in mean (+0.7°C), minimum (+0.3°C) and maximum (+2.6°C) air temperatures (Table 2).

Few specific effects of site or of the experimental warming were recorded on peat temperatures at 7cm depth (Table 1). With OTC treatment, the minimum peat temperature decreased by 0.9°C (Table 2). Two significant differences were also observed due to the interaction between site and air warming: the minimum peat temperature was lower at the Bog-OTC (3.3°C) than at the Bog-CTL plot (5.0°C) (Table 2), and the mean peat temperature was higher at the Fen-OTC (5.7°C) than at the Bog-OTC plot (4.9°C). A week before the sampling, the pattern was similar (Fig. 1), with no significant effect of experimental warming on peat temperature at the Fen site but a significant decrease in minimum peat temperature at the Bog site (from 11.1 to 10.4°C). Additionally, experimental warming also induced a decrease of mean peat temperature in the Bog-OTC site (11.3°C) as compared to the Fen-OTC (12.9°C) site.

3.2. Ground water level and peat moisture changes

From mid-May to late June 2011, the ground water level was systematically higher at the Fen than at the Bog site (+3 cm; Fig. 2) and it was strongly correlated with peat moisture from early May 2011 to late June 2011 (Fig. 3A). During this period, no significant relationship

was found between peat moisture and air and peat temperature (Fig. 3B and 3C.). Instead, there was a positive correlation between air and peat temperature ($p < 0.05$) at both the Bog and Fen sites (Fig. 3D).

No overall effect of warming treatment was observed on peat moisture (Table 3). More specifically, no significant changes were recorded at the Fen site, but water content was significantly higher in the Bog-OTC (94.5%) site than in the corresponding control plots (93.3%; Table 3). Peat moisture in the control plots at the Bog and Fen sites were not significantly different ($p = 0.07$), but this trend disappeared under the effect of air warming.

3.3. Water-extractable organic matter features and phospholipid fatty acids

No impact of air warming treatment was recorded on WEOC, sugar content and $SUVA_{280}$ (Table 3). With respect to PLFAs, significant changes occurred only when comparing the effect of air warming treatment in Bog and Fen sites (Table 3), while no significant differences were observed between their control sites. In warmed plots, PLFAs from G-positive and G-negative bacteria became significantly higher at the Fen site compared to the Bog site (respectively 47.3 and 18.8 µg $C.g^{-1}$ of dry peat in the Fen site and 12.3 and 4.2 µg $C.g^{-1}$ of dry peat in the Bog site - Table 3). The Fen control plots also had higher Protozoan contents as compared to the Bog control plots, but this difference did not persist under warming treatment.

3.4. Enzymatic activities

There was no overall effect of warming treatment on enzymatic activity (Table 3). Nevertheless, at the Bog site, warming treatment significantly enhanced AP activity. In

warmed plots, the activity of LAP and AP were significantly higher at the Bog site as compared to the Fen site (respectively. 5.4 and 1.4 µmol MUF min^{-1} g^{-1} at the Bog-site and 4.7 and 1.0 µmol MUF min^{-1} g^{-1} at the Fen site).

4. Discussion

4.1. Experimental air warming enhances the discrepancy of peat temperatures between Bog and Fen sites

From January to June 2011, the OTCs enhanced mean air temperature up to 0.9 °C and 0.7 °C in the Bog and the Fen sites, respectively. Such a temperature rise is in accordance with other *in situ* warming experiments with OTCs (Sullivan et al., 2008; Dorrepaal et al., 2009; Weedon et al., 2012). The increase in air temperature was associated with a decrease in minimum peat temperature under the impact of warming, only at the Bog site (Tables 1 and 2). At the Bog site, the increase in air temperature was also associated with an increase in peat water content (Table 3). This result is surprising since most studies on experimental warming reported a decrease or no effect on peat moisture (Hollister et al., 2006; Dorrepaal et al., 2009; Bokhorst et al., 2011; Delarue et al., 2011a; Jassey et al., 2013). The question is therefore whether this effect was due to an experimental artefact or whether it results from a thermodynamic constraint. It was demonstrated that OTCs can stop wind blowing, thus reducing evaporation (de Boeck et al., 2012). As no relationship was found between wind speed and peat moisture (at 5 cm depth) in the control plots (data not shown), we assume that no significant reduction of evaporation by OTCs occurred at 5 cm depth. However, we cannot rule out the effect of wind at the surface of the *Sphagnum* carpet. In both sites, peat moisture was mainly controlled by ground water level rather than by air temperature (Fig. 3A, B and C). Therefore, such a rise

in peat moisture at the Bog site may result from an interaction between air temperature and ground water level owing to capillary strength. Water capillary flow is the main mass flux within peat (Price et al., 2009) so if the capillary flow is not strong enough to compensate for the evaporation rate, mosses start to dry out. Conversely, if the capillary flow compensates for the evaporation rate (Yazaki et al., 2006), then the vapour diffusion through evaporation can cool the upper peat layer (Carleton and Dunham, 2003). In addition, this can lead to the condensation of vapour in the upper peat layer, which causes a slight increase in peat moisture (Price et al., 2009). This mechanism can partially explain the observed increase in peat moisture and the decrease in minimum peat temperature at the Bog site. At the Fen site, no temperature changes were recorded. Due to the different effect of experimental warming in the two sites, it can be concluded that there is a thermal discrepancy, as indicated by the lower peat temperature at the Bog-OTC as compared to the Fen-OTC site (Table 2). Such a discrepancy was also measured the week before sampling (Fig. 1) and was associated with the disappearance of the peat moisture discrepancy between the warmed Bog and Fen sites (Table 3)

Overall, this discrepancy suggests that a slightly higher ground water level (about 3 cm; Fig. 2) may prevent any effect of experimental warming on both peat temperature and moisture, suggesting that a potential thermodynamic threshold occurs as a function of groundwater level.

4.2. Air warming can simultaneously lead to an increase in bacterial community in the fen and microbial activity in the bog.

At the Bog site, air warming treatment led to higher AP enzymatic activity. This enzyme is produced by both soil microorganisms and plants and is involved in the mineralization of

phosphate from phospholipids (Turner et al., 2002; Toor et al., 2003). AP changes underpin a higher breakdown of organically bound phosphate at the Bog site in the course of air warming. Particular attention must be paid to the impact of roots which are considered as key controlling factors of AP activity (Robroek et al., 2013). Indeed, air warming favoured vascular plant abundance rather than *Sphagnum* mosses (Jassey et al. 2013). Roots are lacking in *Sphagnum* species, and therefore it can be expected that the root activity increase of vascular plants triggers AP activity. Jassey et al. (2013) also indicated that such a shift of vascular plants was associated with a decrease of *Sphagnum*-polyphenols, a strong microbial breakdown inhibitor, stimulating, in turn, bacterial and microbial enzymatic activities (Fenner and Freeman 2011). Here, the lack of changes upon POA, BG and LAP provide no evidence for such a phenomenon.

Air warming also increased discrepancies between the Bog and the Fen sites when comparing first the control plots and then, the warmed plots of both sites. Thus, the increase in enzymatic activities at the warmed Bog site could be linked to higher temperature fluctuations in both the air and the soil, which may have triggered their kinetics (Davidson and Janssens, 2006). Additionally, PLFAs indicated that bacterial biomass increased in the warmed Fen site (Table 3), suggesting that air warming can alter the microbial structure and enzyme hydrolytic activities in opposite directions at the scale of the Bog and the Fen sites. Thus, air warming might induce the emergence of differential peat C dynamics in Bog and Fen sites. Following the soil C cycle scheme of Schimel and Weintraub (2003), one could hypothesize that C uptake by microbial cell biomass was favoured at the warmed Fen site, whereas it was hydrolytic enzyme production that was favoured at the warmed Bog site. However, it was also demonstrated in a snow removal experiment that differential timing of peat defrosting or snow melting can induce delays in the microbial community response (Robroek et al. 2013). Thus, the predominance of fungi upon bacterial biomass was used as an indicator of the winter state

of the microbial community (Robroek et al. 2013). At the warmed Fen site, the relative shift of microbial structure to bacterial biomass could indicate that the microbial community was in a more advanced seasonal stage than at the Bog site. Moreover, an increase in enzymatic activities can also take place as a physiological adjustment to survive cold temperature (Beales, 2004). In any case, this advocates a more careful observation of the early spring period after snow melting.

Peat moisture was defined as the main controlling factor differentiating OM decomposition in the Bog and Fen sites (Delarue et al. 2011b). A change in moisture condition should therefore induce a change in peat C cycle. Here, we saw that water content did not differ in warmed plots nor in the control bog and fen plots. Such a change was strengthened by PLFAs from protozoa and indeed, it is known that testate amoebae are positively correlated to peat moisture and water-table depths (Woodland et al., 1998). The peat C cycle can be roughly divided into 4 components: soil organic C; dissolved organic C (WEOC in this study), microbial cell biomass and exoenzymes (Schimel and Weintraub, 2003). WEOC is an intermediate product between solid and gas phases in the course of decomposition (Schimel and Weintraub, 2003) and is considered as an indicator of the portion of dissolved OM which is the most active and mobile fraction within the OM (Akagi and Zsolnay, 2008; Zaccone et al., 2009). No significant change between control and warmed plots or between warmed Bog and Fen sites was recorded (Table 3). Moreover, WEOC mainly depended on sugar content which is known to be ubiquitous, occurring within both peat and microorganisms (results not shown). WEOC and sugar content were not discriminating enough to draw any conclusions about OM decomposition under the impact of OTCs in Bog and Fen sites. Additionally, since no change occurred upon $SUVA_{280}$, which should reflect the decomposition of recalcitrant aromatic moieties, our results suggest that air warming impacted neither recalcitrant nor labile OM pools in this study.

5. Conclusion

We have highlighted the limitation of the use of single environmental factors to assess the degree of OM decomposition. Instead, the use of multiple chemical, biological and microclimatic factors can provide more reliable information. For example, the single use of enzymatic activities would suggest that air warming indirectly favoured peat decomposition at the Bog sites, whereas our multidisciplinary approach emphasises a change in microbial structure and activities as a consequence of complex interactions between groundwater level and air warming.

In addition, we have demonstrated that the enzymatic latch theory does not apply to cases where the water content does not decrease and consequently, where the oxygen level does not increase. This implies that a better definition of the environmental framework governing the enzymatic latch theory is needed, especially under future climatic conditions. Future investigations should aim at characterizing the seasonal pattern of these interactions, taking also into consideration soil microtopography, since this will greatly affect the impact of global warming on peat decomposition.

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Figure captions

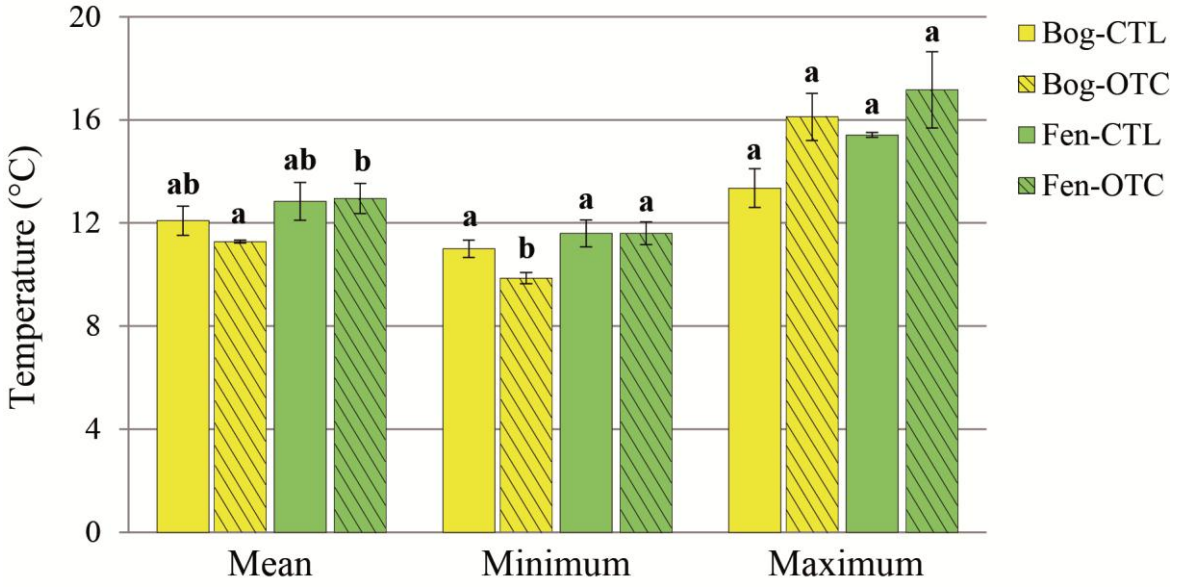


Fig. 1: Effect of experimental warming on mean, minimum and maximum peat temperatures at both Bog and Fen sites during the week before sampling. Each value corresponds to the weekly mean temperature, and to the minimum and maximum peat daily temperature. Error bars are indicative of standard error between replicates (n =3). Significant differences were tested using one-way ANOVA and are indicated by different letters.

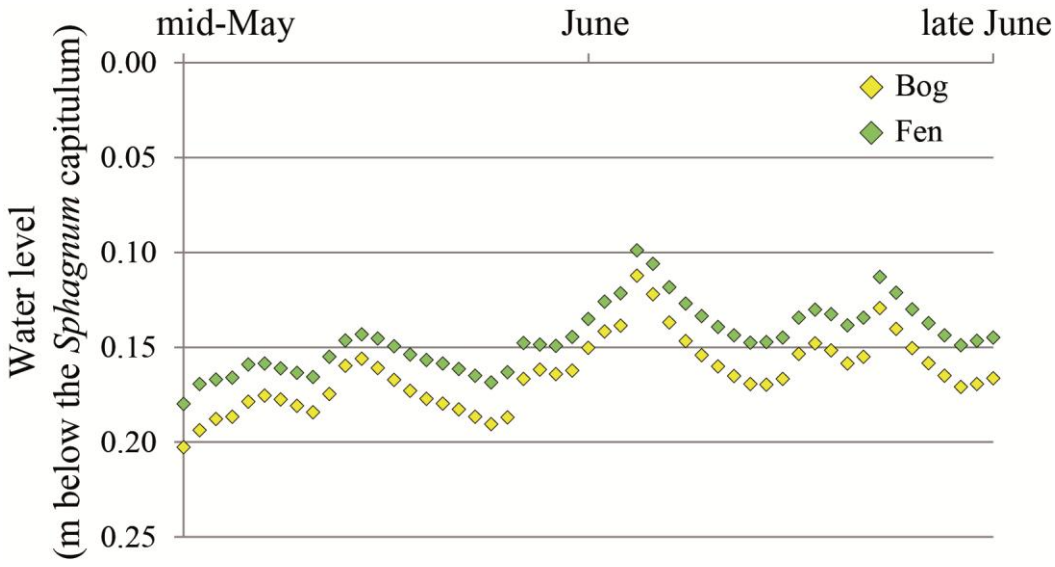


Fig. 2: Ground water level (below the *Sphagnum* capitulum) measured at both Bog and Fen sites from mid-May to late June.

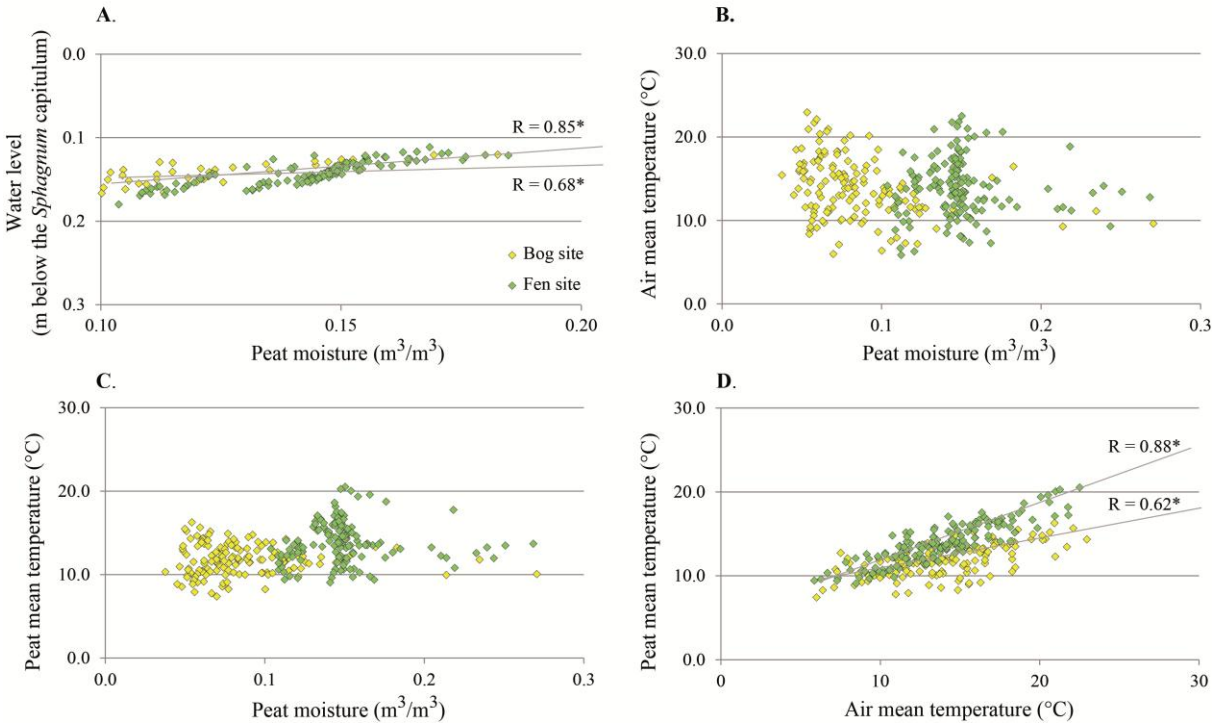


Fig. 3: Relationships between ground water level and peat moisture (A), peat moisture and air mean temperature (B), peat moisture and peat mean temperature (C), air and peat mean temperatures (D) at the Bog and Fen site. Measurements were performed from mid-May 2011 to October 2011. Correlations were based on the Pearson's test. Each value corresponds to the daily mean (n = 146). Significant correlation is indicated by an asterisk ($p < 0.05$).

Table 1: Results of a Repeated Measures ANOVA's to test the overall and interaction effects of time (n = 5), site (n = 6) and experimental warming (n = 6) on air and peat temperatures. Mean, minimum and maximum monthly temperatures from January 2011 to June 2011 were used as repeated measures. Significant differences are indicated by a *p*-value below 0.05.

Air temperatures

		Mean		**Minimum**		**Maximum**	
Effect	***df***	***F***	***p***	***F***	***p***	***F***	***p***
Site	1	2.44	0.16	11.65	< **0.05**	5.77	< **0.05**
Treatment	1	42.94	< **0.05**	15.71	< **0.05**	16.02	< **0.05**
Time	5	25639.89	< **0.05**	4220.60	< **0.05**	3492.06	< **0.05**
Site × Treat.	1	0.36	0.56	1.78	0.22	0.23	0.64
Site × Time	5	4.23	< **0.05**	2.74	< **0.05**	5.09	< **0.05**
Treat. × Time	5	21.16	< **0.05**	1.26	0.30	15.20	< **0.05**
Site × Treat. × Time	5	0.29	0.92	0.38	0.86	0.19	0.97

Peat temperatures

		Mean		**Minimum**		**Maximum**	
Effect	***df***	***F***	***p***	***F***	***p***	***F***	***p***
Site	1	2.81	0.13	0.01	0.93	1.77	0.22
Treatment	1	0.01	0.91	6.69	< **0.05**	2.01	0.19
Time	5	1389.12	< **0.05**	969.23	< **0.05**	391.76	< **0.05**
Site × Treat.	1	3.28	0.11	6.90	< **0.05**	0.01	0.92
Site × Time	5	1.37	0.26	1.01	0.42	1.14	0.35
Treat. × Time	5	1.03	0.41	6.08	< **0.05**	2.01	0.10
Site × Treat. × Time	5	0.32	0.90	3.63	< **0.05**	0.28	0.92

Table 2: Air and peat temperatures (°C) according to sites (n = 6), warming treatment (n = 6) and their interactions (n = 3). Mean, minimum and maximum temperatures were calculated according to monthly temperatures from January 2011 to June 2011. Significant differences were determined with the Fisher's LSD test and are indicated by a *p*-value below 0.05.

	Air temperatures			**Peat temperatures**		
Site (n=6)	Mean temperature	Min. temperature	Max. temperature	Mean temperature	Min. temperature	Max. temperature
Bog	6.2	-4.2	19.3	5.1	4.2	6.2
Fen	6.0	-3.8	17.9	5.5	4.1	7.2
***p*-value**	0.16	< **0.05**	< **0.05**	0.13	0.93	0.22
Treatment (n=6)	Mean temperature	Min. temperature	Max. temperature	Mean temperature	Min. temperature	Max. temperature
Control	5.7	-4.2	17.4	5.3	4.6	6.1
Warmed	6.5	-3.8	19.7	5.3	3.7	7.2
***p*-value**	< **0.05**	< **0.05**	< **0.05**	0.91	< **0.05**	0.19
Site × Treatment (n=3)	Mean temperature	Min. temperature	Max. temperature	Mean temperature	Min. temperature	Max. temperature
Bog-CTL	5.7	-4.5	18.3	5.3	5.0	5.7
Bog-OTC	6.6	-3.9	20.3	4.9	3.3	6.7
Fen-Control	5.6	-4.0	16.6	5.3	4.1	6.6
Fen-OTC	6.3	-3.7	19.2	5.7	4.2	7.8
***p*-value**						
Bog-CTL *vs.* Bog-OTC	< **0.05**	< **0.05**	< **0.05**	0.27	< **0.05**	0.38
Fen-CTL *vs.* Fen-OTC	< **0.05**	< **0.05**	< **0.05**	0.21	0.98	0.31
Bog-CTL vs. Fen-CTL	0.52	< **0.05**	0.08	0.93	0.09	0.41
Bog-OTC vs Fen-OTC	0.16	0.18	0.21	< **0.05**	0.11	0.34

Table 3: Effect of warming treatment on PLFAs (G+bacteria, G-bacteria, Fungi, Actinobacteria and Protozoan in µg C g^{-1} of dry peat), water content, WEOM characteristics (water extractable organic carbon in $mg.g^{-1}$ of dry peat, sugar content in $mg.g^{-1}$ of dry peat and $SUVA_{280}$ in g of dry peat per mg $C^{-1}cm^{-1}$) and extracellular enzymatic activities (phenoloxidase-PO in µmol of dicq min^{-1} g^{-1} of dry peat, ß-glucosidase-BG, leucine aminopeptidase-LA and acidic phosphatase-AP in µmol of substrate). The impact of warming was tested with Bog-CTL vs. Bog-OTC (n = 3), Fen-CTL vs. Fen-OTC (n = 3), Bog-CTL vs. Fen CTL, Bog-OTC vs. Fen-OTC (n = 3) and CTL vs. OTC (n = 6). Significant differences were tested using one-way ANOVA and are indicated by bold characters.

PLFAs (Average value)	G+ bacteria	G- bacteria	Fungi	Actinobacteria	Protozoan
CTL	19.5	7.3	2.7	6.7	5.7
OTC	29.8	11.5	6.1	9.5	5.9
Bog-CTL	14.1	5.7	1.4	3.9	2.3
Bog-OTC	12.3	4.2	6.0	7.2	4.3
Fen-CTL	25.0	8.8	4.1	9.5	9.0
Fen-OTC	47.3	18.8	6.1	11.7	7.5

p-value	G+ bacteria	G- bacteria	Fungi	Actinobacteria	Protozoan
CTL *vs.* OTC	0.38	0.37	0.28	0.41	0.91
Bog-CTL *vs.* Bog-OTC	0.89	0.74	0.43	0.48	0.49
Fen-CTL *vs.* Fen-OTC	0.14	0.14	0.59	0.65	0.18
Bog-CTL *vs.* Fen CTL	0.38	0.51	0.11	0.23	**0.04**
Bog-OTC *vs.* Fen-OTC	**0.05**	**0.05**	0.99	0.40	0.15

Water content and WEOM features (Average value)	Water content	WEOC	Sugar content	$SUVA_{280}$
CTL	93.8	2.9	1.2	0.066
OTC	94.2	2.7	1.1	0.068
Bog-CTL	93.3	2.9	1.2	0.073
Bog-OTC	94.5	2.8	1.1	0.073
Fen-CTL	94.3	2.9	1.1	0.059
Fen-OTC	93.9	2.6	1.2	0.062

p-value	Water content	WEOC	Sugar content	$SUVA_{280}$
CTL *vs.* OTC	0.27	0.38	0.81	0.84
Bog-CTL *vs.* Bog-OTC	**0.04**	0.89	0.44	0.98
Fen-CTL *vs.* Fen-OTC	0.40	0.32	0.92	0.62
Bog-CTL *vs.* Fen CTL	0.07	0.84	0.77	0.23

Bog-OTC *vs.* Fen-OTC	0.21	0.55	0.77	0.09

Extracellular enzymatic activities

(Average value)	PO	BG	LAP	AP
CTL	0.0041	0.7	5.2	1.3
OTC	0.0034	0.7	5.0	1.2
Bog-CTL	0.0042	0.7	4.8	1.3
Bog-OTC	0.0044	0.8	5.4	1.4
Fen-CTL	0.0040	0.8	5.6	1.3
Fen-OTC	0.0024	0.6	4.7	1.0

p-value	PO	BG	LAP	AP
CTL *vs.* OTC	0.45	0.84	0.72	0.60
Bog-CTL *vs.* Bog-OTC	0.91	0.33	0.32	**0.03**
Fen-CTL *vs.* Fen-OTC	0.13	0.14	0.10	0.13
Bog-CTL *vs.* Fen CTL	0.75	0.34	0.29	0.84
Bog-OTC *vs.* Fen-OTC	0.26	0.08	**0.03**	**0.01**