Published in Global Change Biology 19, issue 3, 811-823, 2013
which should be used for any reference to this work

Above- and belowground linkages in *Sphagnum* peatland: climate warming affects plant-microbial interactions

VINCENT EJ JASSEY*†‡, GENEVIÈVE CHIAPUSIO*, PHILIPPE BINET*, ALEXANDRE BUTTLER*†‡, FATIMA LAGGOUN-DÉFARGE§¶**, FRÉDÉRIC DELARUE§¶**, NADINE BERNARD*, EDWARD AD MITCHELL††, MARIE-LAURE TOUSSAINT*, ANDRÉ-JEAN FRANCEZ‡‡ and DANIEL GILBERT*

*Laboratoire Chrono-Environnement, UMR CNRS/UFC 6249, Université de Franche-Comté, F-25211, Montbéliard cedex, France, †Ecological Systems Laboratory (ECOS), Civil and Environmental Engineering (ENAC), Ecole Polytechnique Fédérale de Lausanne EPFL, School of Architecture, Station 2, 1015, Lausanne, Switzerland, ‡Snow and Landscape Research (WSL), Swiss Federal Institute for Forest, Site Lausanne, Station 2, 1015, Lausanne, Switzerland, §ISTO, UMR 7327, Université d'Orléans, 45071, Orléans, France, ¶ISTO, UMR 7327, CNRS/INSU, 45071, Orléans, France, **ISTO, UMR 7327, BRGM, BP 36009, 45060, Orléans, France, ††Laboratory of Soil Biology, University of Neuchâtel, Rue Emile-Argand 11, CH-2000, Neuchâtel, Switzerland, ‡‡CNRS UMR 6553 ECOBIO 'Ecosystèmes, Biodiversité, Evolution' & FR 90 CAREN, Campus de Beaulieu, Université de Rennes 1, F-35042, Rennes cedex, France*

Abstract

Peatlands contain approximately one third of all soil organic carbon (SOC). Warming can alter above- and belowground linkages that regulate soil organic carbon dynamics and C-balance in peatlands. Here we examine the multi-year impact of *in situ* experimental warming on the microbial food web, vegetation, and their feedbacks with soil chemistry. We provide evidence of both positive and negative impacts of warming on specific microbial functional groups, leading to destabilization of the microbial food web. We observed a strong reduction (70%) in the biomass of top-predators (testate amoebae) in warmed plots. Such a loss caused a shortening of microbial food chains, which in turn stimulated microbial activity, leading to slight increases in levels of nutrients and labile C in water. We further show that warming altered the regulatory role of *Sphagnum*-polyphenols on microbial community structure with a potential inhibition of top predators. In addition, warming caused a decrease in *Sphagnum* cover and an increase in vascular plant cover. Using structural equation modelling, we show that changes in the microbial food web affected the relationships between plants, soil water chemistry, and microbial communities. These results suggest that warming will destabilize C and nutrient recycling of peatlands via changes in above- and belowground linkages, and therefore, the microbial food web associated with mosses will feedback positively to global warming by destabilizing the carbon cycle. This study confirms that microbial food webs thus constitute a key element in the functioning of peatland ecosystems. Their study can help understand how mosses, as ecosystem engineers, tightly regulate biogeochemical cycling and climate feedback in peatlands

Keywords: food chains, microbial food web, plant and microbial communities, polyphenols, testate amoebae, water chemistry

Introduction

Ongoing global warming is causing ecological communities to rapidly change, resulting in modifications to their interactions, and to ecosystem functioning and services. Climate warming can directly affect aboveground communities, by changing plant community composition, carbon allocation patterns, or the quality of plant-derived organic matter, which indirectly affect soil biota (Wardle *et al*., 2004, 2012; Veteli *et al*., 2007; De Deyn *et al*., 2008). The response of the microbial subsystem may in turn directly or indirectly create feedbacks on plant communities by breaking down plant-derived organic matter (Wardle *et al*., 2004; Bardgett *et al*., 2008; Singh *et al*., 2010). Unfortunately, despite recent interest in linkages between aboveground-belowground subsystems in driving ecosystem functioning (Wardle, 2006; Ward *et al*., 2007; Kardol & Wardle, 2010; Wardle *et al*., 2012), much remains unknown about the impacts of climate change on these linkages (Singh *et al*., 2010; Eisenhauer *et al*., 2012). Understanding warming-induced changes on both plants and soil organisms is thus relevant to predict future impacts on above- and belowground terrestrial ecosystem processes (Bardgett & Wardle, 2010).

Peatlands dominated by *Sphagnum* mosses store more C than any other terrestrial ecosystem owing to

Correspondence: phone: Vincent E. J. Jassey, tel. +41 (0) 21 69 35 774, fax +41 (0) 21-693-39-13, e-mail: vincent.jassey@epfl.ch

imbalance between litter inputs and C outputs from soil respiration (Clymo *et al*., 1998; Davidson & Janssens, 2006; Dise, 2009). Complex linkages between above- and belowground communities regulate this C-sequestration, which may decrease or even reverse in response to warming, leading to a positive feedback to global warming (Bardgett *et al*., 2008; Dorrepaal *et al*., 2009; Fenner & Freeman, 2011). However, despite recent interest in peatlands response to warming as potential carbon source (Dorrepaal *et al*., 2009), only a limited number of warming experiments dealing with above- and belowground linkages have been conducted (Fenner *et al*., 2007; Weedon *et al*., 2012). Knowledge of how climate warming impacts above- and belowground linkages in peatlands is currently insufficient. Indeed, changes in linkages at one level of organization (e.g. species characteristics and interactions, community composition or carbon storage) can affect above- and belowground biota, and their linkages, at other levels of organization, thus potentially destabilizing peatland functioning.

Sphagnum mosses, as well-known ecosystem engineers, create unfavourable conditions for the growth of vascular plant that they then can partly out-compete by efficiently accumulating nutrients, producing recalcitrant litter, and modifying the chemical and physical conditions of the soil (van Breemen, 1995; Turetsky, 2003). On the other hand, *Sphagnum* can also stabilize the vegetation by encroaching on vascular plant space in response to climate warming (Keuper *et al*., 2011). These mosses are also tightly linked to microbial communities through a variety of both direct and indirect mechanisms that ultimately exert control on peatland ecosystem C dynamics (Lindo & Gonzalez, 2010). Microbial communities living in *Sphagnum* mosses constitute a crucial detrital network for nutrient and C cycling, where protozoans (and especially testate amoebae) play a central role (Gilbert *et al*., 1998; Mitchell *et al*., 2003; Lindo & Gonzalez, 2010). *Sphagnum* also produces organochemical compounds such as polyphenols, which are known to have a strong inhibitory effect on microbial breakdown of organic matter, therefore favouring peat accumulation (Verhoeven & Toth, 1995). For instance, a reduction in polyphenol content may stimulate bacterial and microbial enzymatic activity (Fenner & Freeman, 2011; Jassey *et al*., 2011a). However, despite the global significance of microbial communities in SOC dynamics of peatlands, their sensitivity to climate change has so far received little attention (Jassey *et al*., 2011b; Kim *et al*., 2012; Tsyganov *et al*., 2012; Weedon *et al*., 2012) and considerable gaps remain in our understanding of the impacts of warming on aboveground-belowground relationships in peatlands following changes in microbial components. Long-term global warming is expected to considerably alter peat-forming areas, potentially involving substantial C loss due to modifications in microbial processes linked to *Sphagnum* mosses (Dorrepaal *et al*., 2009; Delarue *et al*., 2011; Fenner & Freeman, 2011; Jassey *et al*., 2011a,b). Understanding key mechanisms behind the changing *Sphagnum*–microbial–vascular plant interactions in responses to climate change, is obviously needed to better understand ongoing processes and to predict more accurately future changes in the functioning of peatlands.

Here we focus on the ways in which warming affects above- and belowground subsystems as key components of C fluxes in *Sphagnum* peatlands. Specifically, we analyse the effect of climate warming on key above- and belowground components in a *Sphagnum* peatland in the Jura Mountains (France) to explore whether changes in peatland properties will be brought by plant communities, microbial communities, or by interactions between the two. Our aims were as follows (1) to quantify over the course of two years how the soil microbial food web (biomass of bacteria, fungi, protozoans, and metazoans), plant communities (species composition and diversity), *Sphagnum*-polyphenols and soil water chemistry composition changed in response to warming, and (2) to clarify whether or not climate warming destabilized the functioning of the peatland by changing plant-soil-microbial interactions, including polyphenol phytochemical interactions. Linkages among these variables were investigated using a path-relation network and structural equation models (SEM) to gain a mechanistic understanding of how warming effects materialized on above- and belowground linkages.

Materials and methods

Field site and experimental design

The experimental site is an undisturbed *Sphagnum*-dominated peatland situated in the Jura Mountains (The Forbonnet peatland, north-eastern France, 46°49′35″N, 6°10′20″E). Above- (mosses, vascular plants, polyphenols produced by *Sphagnum*) and belowground (soil water chemistry, microbial communities living in *Sphagnum* carpet) components were measured across a transitional fen-bog area in June 2008, 2009, and 2010 (beginning of the warming experiment April 2008). The experimental site has an area of 30m x 30m and the vegetation consists of a mosaic of hummocks and lawns (Jassey *et al*., 2011a). The moss layer is dominated by *S. fallax* in lawns and by a mixture of *Sphagnum fallax*, *S. magellanicum* and *S. palustris* in hummocks whereas the evergreen dwarf shrubs *Eriophorum vaginatum* and *Andromeda polifolia*, the deciduous dwarf shrubs *Vaccinum oxycoccus* and the graminoids *Calluna vulgaris* and *Carex rostrata* characterize the field layer. We chose 12 sampling plots (six ambient and six warmed plots) across the area

placed so as to cover all the possible microforms along the gradient. Six plots were placed in lawns and six plots in hummocks, and in each area three plots were assigned as AMB and three as warmed conditions. We used open-top chambers (OTC) to simulate the regional climate expected for the coming decades (IPCC, 2007; Jassey *et al*., 2011a). The OTCs significantly increased mean air temperature in spring and summer by up to about 1.3 °C, and decreased the water content of *Sphagnum* in summer by up to about 30% (Delarue *et al*., 2011; Jassey *et al*., 2011a). The water table depth did not change with warming and was ranged from 13.4 to 20.8 cm between 2008 and 2010.

Belowground measurements

We analysed the entire microbial community using a morphotype functional group approach rather than focusing on a given group, such as bacteria or fungi. Samples of *S. fallax* were collected for the study of microbial communities from 10 permanent markers in each plot and the average altitude (microtopography) was measured. This sampling design allowed for multiple sampling over time and collection of a composite sample from each plot, avoiding any bias due to spatial heterogeneity (Mitchell *et al*., 2000a). Microbial communities were analysed from surface (0–3 cm) and litter (3–6 cm) layers of *Sphagnum* shoots. Microbial samples were fixed in glutaraldehyde (2%) and extracted following the standard method described in Jassey *et al*. (2011b). We used direct observation by inverted microscopy (x200 and x400 magnification) to determine precisely the abundance and biomass of individual microbial functional groups (fungi, microalgae, ciliates, testate amoebae, rotifers, and nematodes). Flow cytometry (FACSCalibur flow cytometer, Becton Dickinson) was used for bacterial counts. Fluorescent microbeads (Molecular probes) of diameter 1 μm were added to each sample as an internal standard. Bacterial samples were stained with SYBR Green I (1/10,000 final conc.) for 15 min in the dark and run at medium speed (ca 40 μL min^{-1}). For each specimen, the average biovolume (μm^3) was estimated by assuming geometrical shapes using image analysis and converted to biomass using conversion factors (Gilbert *et al*., 1998). Data were expressed as micrograms of carbon per gram of *Sphagnum* dry mass (μg C g^{-1} DM).

The trophic position of each testate amoeba species was estimated by measuring their body size and their shell-aperture size. We chose these two specific morphological traits because they are linked to their feeding habit. Species with a low shell-aperture size over body size ratio (shell-aperture size/body size < 0.18) were considered as having a low trophic position (i.e. primarily bacterivores and algivores), and species with a high one (shell-aperture size/body size > 0.18) as having a high trophic position (i.e. primarily predators of other protists and micro-metazoan) in the microbial food web (Yeates & Foissner, 1995; Mitchell *et al*., 2003; Jassey *et al*., 2012a).

Water chemistry (20 mL) was analysed in each sampling plot at 10 cm depth from piezometers during each sampling campaign. Total dissolved nitrogen (DN) and dissolved organic carbon (DOC) of peatland water were determined with a SHIMADZU SSM-5000A total C and N analyser (Shimadzu Schweiz, Reinach, Switzerland). Ammonium (NH_4^+), nitrates (NO_3^-) and phosphates (PO_4^{3-} and total phosphorus) were analysed colorimetrically using a continuous flow analyser (FLOWSYS; Systea, Roma) after filtering the bog water at 0.45 μm.

Aboveground measurements

We performed vegetation surveys by the point-intercept method (Buttler, 1992; Keuper *et al*., 2011). We used a 50 × 50 cm Plexiglas frame placed above a permanently marked quadrat by means of four adjustable poles. A ruler with 20 holes was moved along 20 different positions to obtain 400 measuring points. A metal pin with a 1 mm diameter tip was lowered through each hole in the ruler and each contact of the pin with green living vegetation was recorded by species until the pin reached the moss substrate. Moss and vascular plant abundances were expressed as percentage of mean number of hits (%). Moreover, we measured the average microtopography of the marked vegetation quadrat.

Total water-soluble *Sphagnum*-polyphenols were quantified in living segments of *Sphagnum* shoots (0–6 cm) during each sampling campaign using Folin methods and gallic acid as standard (see Jassey *et al*., 2011b for details). These samples were collected around the same 10 permanent markers used for microbial communities for each plot.

Numerical analyses

We carried out linear mixed effects models and ANOVAs to test for differences between treatments and years in the following variables: (i) biomass of individual microbial groups; (ii) abundance of individual testate amoeba species; (iii) individual water chemical components; (iv) total abundance of plants (mosses and vascular plants) and abundance of individual moss and vascular plant; and (v) polyphenol content in *Sphagnum*. Linear mixed effects were used to assess the effects of warming (difference of temperature between ambient and warmed plots, delta TC), time, and microtopography (fixed effects) on measured of biotic and abiotic variables while accounting for the temporal repeated measurements in each plot on three dates. We fitted all models including plot as a random effect on the intercept, i.e. we corrected for the inflation of the residual degrees of freedom that would occur if we were using repeated measurements as true replicates (Pinheiro & Bates, 2000). Then ANOVAs were applied on the different models to test the fixed effects, and differences among the levels of the fixed effects in the final model were determined using multiple comparison post hoc analyses (general linear hypothesis test). Finally, we used the *F*-values from ANOVA to quantify the impact of warming on the abundance of individual testate amoeba species. Higher *F*-values indicated stronger warming effect on the individual testate amoeba species.

We evaluated the warming effect on plant species, microbial community, and water chemistry composition using the principal response curve (PRC) method (van den Brink & ter

Braak, 1999). PRC was used to focus on the time-dependent treatment effect and applied on the Hellinger-transformed vegetation and microbial assemblages and the standardized water chemistry matrix, including the response of individual plant species, microbial groups or chemical components. In diagrams, the curves represent the time trajectory for the controls as a horizontal line maintained to 0 (dashed line) and deviation of biotic or abiotic matrices compositions in warmed plots in course of 2 years (solid line). This is achieved by taking the control treatment as the reference against which the other treatments are contrasted and by defining "time" as the horizontal axis of the diagram. With the help of the species weights on the right *Y*-axis, the PRC can be used to infer about the response of individual species to warming. The species scores on the right *Y*-axis allow an interpretation at the species level, i.e. values indicate the contribution of individual species/group/component to the deviation of the community structure observed in warmed plots. Individual group with positive scores on right *Y*-axis are inferred to show a negative effect of warming, whereas groups with negative scores show a positive response to warming. A group near zero scores either show no response or a response unrelated to the pattern shown in PRC. Higher values (positive or negative) on the right *Y*-axis indicate stronger warming effect on the individual species/group. Permutation tests with 1,000 permutations, stratified by year, were performed for every canonical axis. In addition, we compared the respective effect of microtopography, time, and warming (i.e. delta air temperature between ambient and warmed plots) on each biotic and abiotic matrices using variance partitioning in redundancy analyses (RDA) and adjusted R^2. The significance of the each explanatory variable included in RDA was tested using 1,000 permutations.

Above- and belowground data were organized into a path-relation network and subjected to structural equation modelling (Grace *et al*., 2010). This enabled us to explore simultaneous influences of several potentially important drivers of peatland functioning that may be affected by warming, and thereby identify their relative importance to obtain a better understanding of peatland response to warming. SEM was based on the overall data set and predicted causal relationships between variables were based upon prior knowledge, theory, and past experience on the role of above- and belowground factors in peatland functioning. The adequacy of the model was determined via several tests, i.e. χ^2 tests, goodness fit index (GFI), Akaike value (AIC), root square mean error of approximation (RMSEA), and root mean square residual (RMR) (Jonsson & Wardle, 2010; Eisenhauer *et al*., 2012). Adequate model fits are indicated by nonsignificant χ^2 tests ($P > 0.05$), high GFI ($0.8 < GFI < 1$), low AIC, low RMSEA (< 0.05), and low RMR ($RMR < 0.05$) (Grace *et al*., 2010). Models were built and separately tested for ambient and warmed plots based on the biomass of individual microbial groups in surface and litter, abundances of mosses and vascular plants, polyphenol content, and a subset of four variables (DOC, NH_4^+, NO_3^-, and PO_4^{3-}) retained by stepwise selection from the water chemistry matrix. We used RV-coefficients from multiple factor analysis (MFA) to construct our correlation matrices used in SEM (Grace *et al*., 2010). MFA symmetrically linked the six groups of descriptors described above. MFA was selected because it allowed the simultaneous coupling of several groups or subsets of variables defined on the same objects and assess the general structure of the data (Escofier & Pages, 1994). RV-coefficients (Pearson correlation coefficient) were used to measure the similarities between two data matrices (Josse *et al*., 2008). SEM was performed using Amos 5 (Amos Development Corporation, Crawfordville, FL, USA). For each analysis, R^2 values were obtained for each dependent matrix, showing the amount of the variance explained by the model (Grace *et al*., 2010; Jonsson & Wardle, 2010).

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

Results

Belowground subsystem

Microbial network structure. The structure of microbial communities clearly responded to warming as showed by PRCs (Fig. 1 a and b). Microbial community structure in warmed plots significantly deviated from ambient plots at the surface layers ($F = 4.6$, $P = 0.02$) whereas the same, but weaker warming effect was recorded at the litter layers ($F = 3.1$, $P = 0.05$). The response of warmed plots clearly appeared in 2009 in both sampling depth, one year after the beginning of the warming experiment (Fig. 1 a and b). The respective effects of microtopography, time, and temperature increase on the variation of microbial communities living at the surface layers were of 13.7%, 6.6%, and 10.5% respectively (Table 1), while their respective effects at the litter layers were of 0.6%, 24.7%, and 14.3% respectively. Only some microbial groups were affected by microtopography variations (e.g. microalgae and testate amoebae) along *Sphagnum* shoots (Table S1). Furthermore, variance partitioning revealed that the interaction between microtopography and warming was not significant along *Sphagnum* shoots (Table 1), highlighting a similar warming effect among lawns and hummocks.

The detailed examination of the time-dependent warming effect on individual microbial groups revealed a strong negative effect on testate amoebae, especially at the surface layers (Fig. 1 a; Table S1). This effect was largely due to a decline of the biomass of the testate amoebae with high trophic level ($F = 5.6$, $P = 0.002$) of 40% and 75%, after one and two warming years respectively. The total biomass of this category of species decreased from 440 $\mu gC\ g^{-1}$ DM to 131 $\mu gC\ g^{-1}$ DM in warmed conditions over two years (Table S1). Although the decrease in the biomass of testate amoebae with high trophic level was not significant at the litter layers ($F = 3.9$, $P = 0.063$), the trend of their

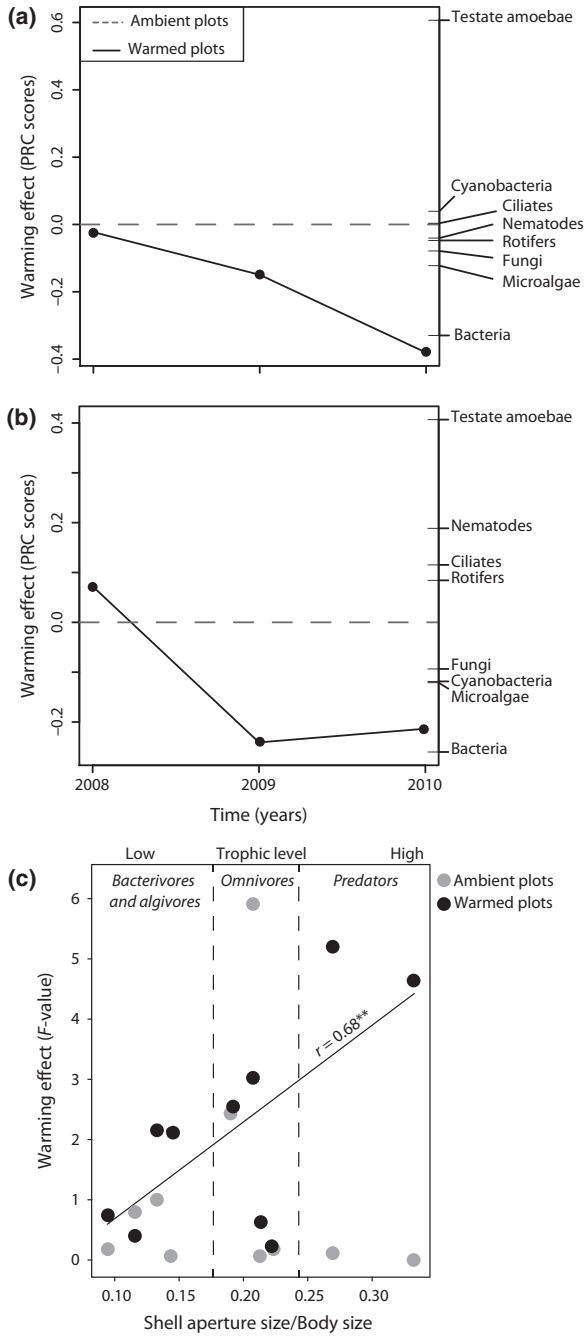

Fig. 1 Microbial community response to climate warming in a *Sphagnum*-dominated peatland. Principal response curve diagrams with scores for microbial functional groups living in the surface (a) ($P = 0.02$) and the litter (b) ($P = 0.05$) layers of the *Sphagnum* carpet. (c) Warming effect on testate amoeba species in relation to the size of their shell-aperture over their body size. The warming effect was determined using the *F*-value from ANOVA tests. Testate amoebae that contributed to less than 3% of maximum abundance in all samples were removed from the data set to reduce the influence of rare taxa in this analysis. Line is regression line significant at $^{**}P < 0.01$ level (ANOVA test).

response followed that of testate amoebae in surface layers with a decrease in 28% and 41% of their biomass after one and two warming years respectively (Table S1). Moreover, we recorded that the warming effect on the abundance of main testate amoeba species was positively correlated to the ratio shell-aperture size over body size at the surface layers ($r = 0.68$, $F = 4.25$, $P < 0.04$; Fig. 1 c). Here again, such trend was nonsignificant in the litter ($r = 0.36$, $F = 2.3$, $P = 0.17$). We further observed that the microbial network from surface and litter became increasingly enriched in bacteria and depleted in top predators with warming. The relative biomass of bacteria in the surface layers significantly increased from 31% in ambient plots to 45% in warmed plots, and from 29% to 36% in the litter layers (Fig. 2, Table S1). In parallel, the relative biomass of testate amoebae with high trophic level decreased from 31% in ambient plots to 12% in warmed plots in the surface layers and from 21% to 11% in the litter layers respectively. Consequently, the decrease in testate amoebae with high trophic level explained 10% ($F = 4.86$, $P = 0.03$) and 16% ($F = 7.91$, $P < 0.01$) of the rise of the relative bacterial biomass in warmed plots from surface and litter respectively.

Water chemistry composition. The PRC analysis showed that water chemistry composition was not affected by warming ($F = 2.1$, $P = 0.11$). Time, mainly explained the variance in water chemistry composition with 19.8% of the total variation. However, variance partitioning revealed a slight, but significant, interaction between time and temperature increase on water chemistry composition with 21.9% of the variance (Table 1). Warming slightly affected water chemistry composition over two years. Elevated temperatures modified the annual dynamic of some chemical components such as DOC, and NO_3^- (Table 2), even if these variations remained very low. Most importantly, we found in warmed plots that nitrates ($r = -0.64$, $F = 8.4$, $P < 0.01$) and DOC ($r = -0.66$, $F = 3.8$, $P = 0.05$) were negatively correlated to the decrease in the biomass of testate amoebae with high trophic levels, whereas no such relationships were found in ambient plots (Fig. 3 a–c). In the same way, no such relationships were found for the biomass of the testate amoebae with low trophic levels, both in ambient and warmed plots.

Aboveground subsystem

Mosses and vascular plants abundances. Plant species composition was stable across years and did not respond to warming over the course of the two years, as showed by the PRC ($F = 0.61$, $P = 0.96$). Redundancy analysis showed that vegetation species composition

Table 1 Summary of RDA on microbial, plant and water chemistry data sets and environmental factors: fraction of variance explained and significance of individual variables taken alone or in interaction

	Microbial communities (top)		Microbial communities (inter)		Plant communities		Water chemistry composition	
	%	*F*-value (*P*-value)	%	*F*-value (*P*-value)	%	*F*-value (*P*-value)	%	*F*-value (*P*-value)
Microtopography (Alt)	13.7	8.6 ($P = 0.001$)	0.6	1.2 ($P = 0.35$)	26.0	12.8 ($P = 0.001$)	3.6	3.0 ($P = 0.04$)
Time	6.6	3.7 ($P = 0.007$)	24.7	8.7 ($P = 0.001$)	0.6	1.2 ($P = 0.30$)	19.8	8.3 ($P = 0.001$)
delta T °C	10.5	6.1 ($P = 0.001$)	14.3	7.9 ($P = 0.001$)	2.0	0.3 ($P = 0.89$)	1.7	2.3 ($P = 0.096$)
delta T °C*Year	16.5	2.1 ($P = 0.06$)	33.8	4.1 ($P = 0.002$)	5.4	0.6 ($P = 0.63$)	21.9	2.9 ($P = 0.04$)
delta T °C*Alt	22.5	0.44 ($P = 0.83$)	12.6	0.71 ($P = 0.63$)	24.5	1.1 ($P = 0.30$)	4.2	0.4 ($P = 0.82$)

*Percentage of variance explained (adjusted *R*2).

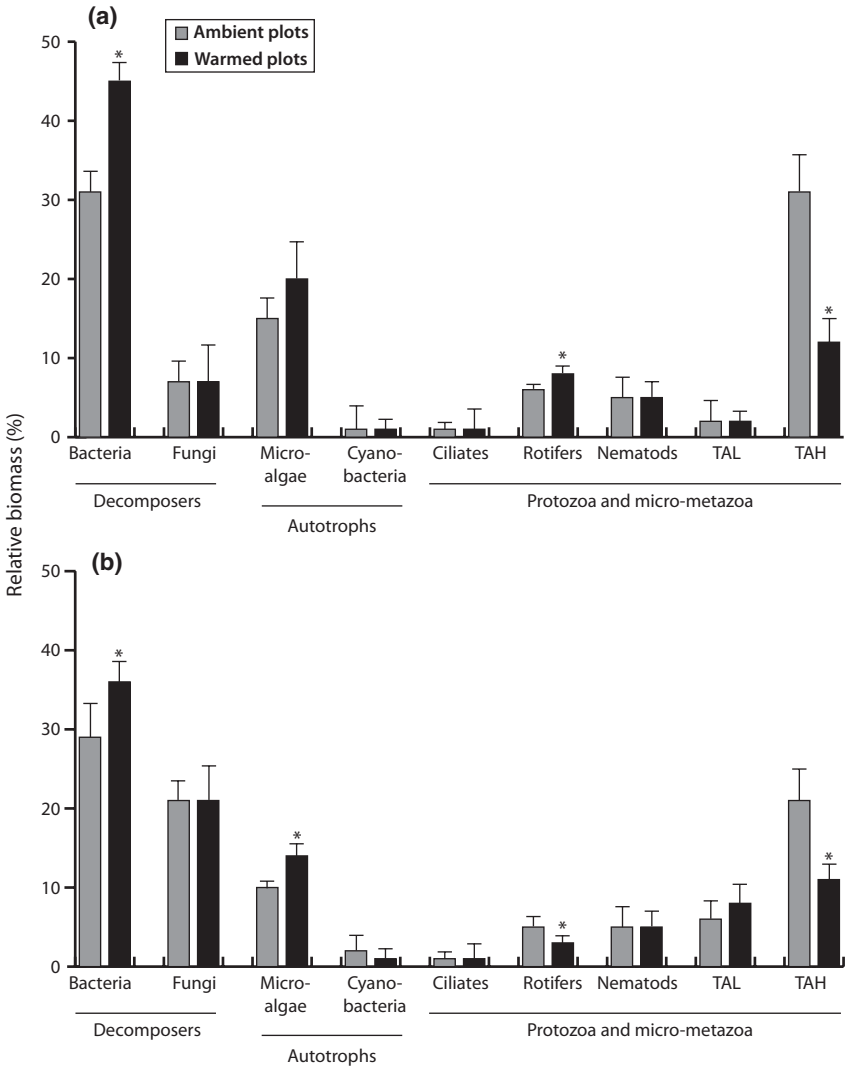

Fig. 2 Barplot of the relative contribution of microbial groups to the total microbial community (% of total biomass) living at the surface (a) and litter (b) layers in ambient and warmed conditions. Asterisks indicate a warming effect (ANOVA tests). TAL = testate amoebae with a low trophic level; TAH = testate amoebae with a high trophic level.

strongly responded to microtopography variations ($F = 12.8$, $P = 0.001$; Table 1). The detailed examination of the warming effect on vegetation structure in lawns and hummocks showed that the total abundance of vascular plants significantly increased with warming in both micro-types ($F = 5.1$, $P = 0.032$, ANOVA) and that of

Table 2 Mean (± SE) of water chemical variables measured in a *Sphagnum*-dominated peatland subjected to experimental warming (WAR) or ambient conditions (AMB) in 2008, 2009 and 2010. Significant warming effects are shown in bold (ANOVA tests)

		2008		2009		2010		*F*-value (*P* -value)		
		AMB	WAR	AMB	WAR	AMB	WAR	warming	time	microtopography
pH	Mean	3.88	3.41	4.54	4.23	3.96	3.91	1.97 (P = 0.17)	0.76 (P = 0.39)	4.2 (P = 0.07)
	SE	0.03	0.17	0.30	0.26	0.05	0.07			
DOC (mg L^{-1})	Mean	42.0	40.2	40.7	40.5	47.3	**48.5**	4.2 (P = 0.001)	87.2 (P = 0.001)	3.2 (P = 0.11)
	SE	4.3	0.8	1.1	0.7	1.0	0.8			
DN (mg L^{-1})	Mean	1.00	0.7	0.74	0.63	0.7	0.71	0.88 (P = 0.35)	1.8 (P = 0.19)	0.2 (P = 0.90)
	SE	0.20	0.03	0.11	0.01	0.01	0.02			
PO_4^{3-} (mg L^{-1})	Mean	1.9	2.3	11.5	**10.5**	10.1	9.7	8.93 (P = 0.001)	28.2 (P = 0.001)	0.17 (P = 0.69)
	SE	0.2	0.7	2.3	0.2	0.3	0.3			
Ptot (mg L^{-1})	Mean	12.4	13.4	20.3	19.6	11.7	13.5	0.24 (P = 0.78)	0.25 (P = 0.61)	0.55 (P = 0.47)
	SE	0.4	0.8	2.2	2.2	0.5	2.0			
NH_4^+ (mg L^{-1})	Mean	55.0	50.2	43.4	51.5	43.2	40.2	1.1 (P = 0.31)	5.23 (P = 0.03)	4.32 (P = 0.07)
	SE	6.2	1.7	2.6	2.8	2.2	9.3			
NO_3^- (mg L^{-1})	Mean	8.7	7.7	10.3	**4.4**	25.7	**28.0**	6.23 (P = 0.02)	21.2 (P = 0.001)	0.29 (P = 0.59)
	SE	2.0	1.5	4.4	2.6	1.6	1.8			
Phenol (mg g^{-1} DM)	Mean	1.20	1.11	1.00	0.95	1.49	2.31	0.03 (P = *0.87*)	5.49 (P = 0.03)	10.4 (P = 0.001)
	SE	0.06	0.07	0.09	0.13	0.20	0.22			

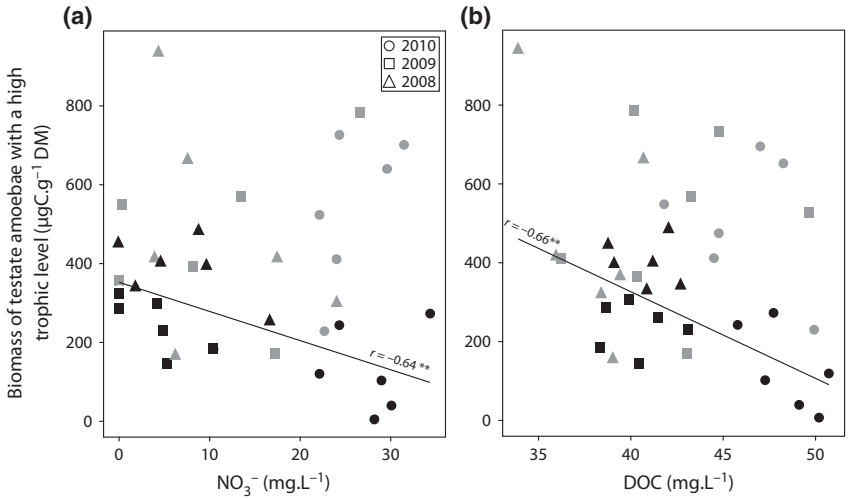

Fig. 3 (a) Nitrates (NO_3^-) and (b) labile C (DOC) concentrations plotted against the biomass of testate amoebae with high trophic levels (i.e., species with high shell-aperture size/body size ratio) in ambient and warmed plots. Lines are regression line significant at $^{**}P < 0.01$ level (ANOVA test).

mosses decreased in lawns (F = 7.15, P = 0.019, ANOVA). Total vascular plant abundance increased from 29% of mean number of hits to 31% in lawns and from 37% of mean number of hits to 41% in patterns of hummocks and hollows (Fig. 4 a). Such an increase was due to a rise in the abundance of the evergreen low shrub *Andromeda polifolia* (F = 8.37, $P < 0.01$; Fig. 4 c). The abundance of other evergreen vascular plants (*Eriophorum vaginatum*, *Vaccinium oxycoccus*), graminoids (*Carex limosa*, *C. rostrata*, *C. pauciflora*) and forbs (*Drosera rotundifolia*) did not respond to climate warming. Total moss abundance decreased in lawns from 55% to 45% after two warming years (Fig. 4 b), especially *Sphagnum fallax*, which significantly decreased from 49.0% to 42.9% (F = 4.1, P = 0.03; Fig. 4 d). Other moss species such as *S. magellanicum* and *S. palustris* did not respond to warming.

Sphagnum-polyphenols. Total water-soluble polyphenol content ranged between 0.5 mg g^{-1} DM and 2.5 mg g^{-1} DM (Table 2). Phenolic content was not

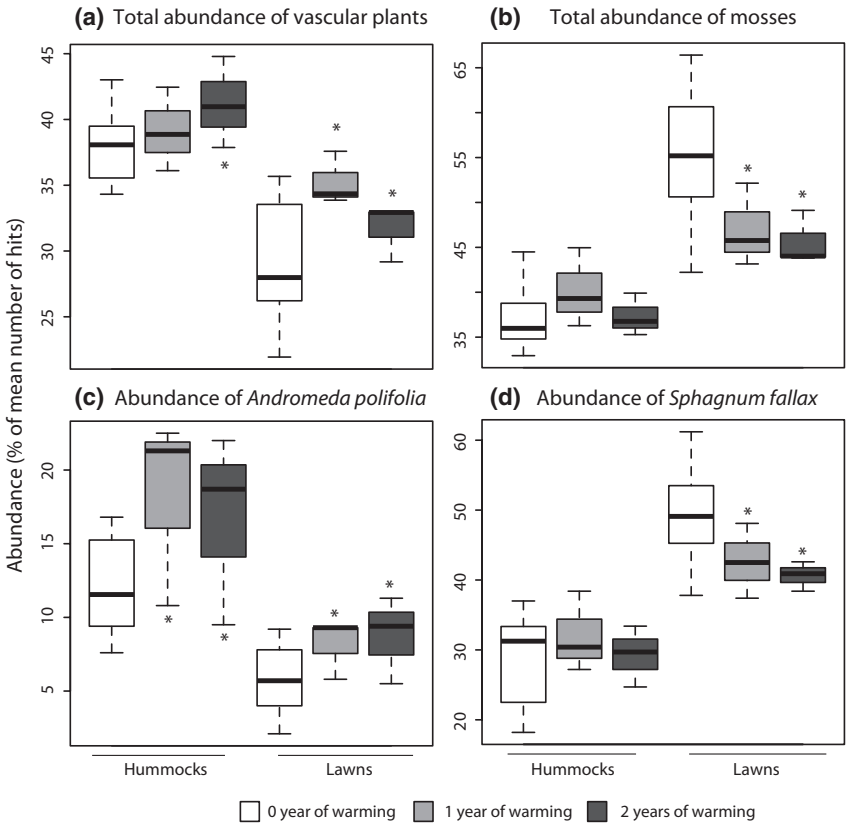

Fig. 4 Total abundance of (a) vascular plants and (b) mosses (expressed as % of mean number of hits). Abundance of (c) *Andromeda polifolia* and (d) *Sphagnum fallax* (expressed as% of mean number of hits). *Asterisks* indicate significant differences between treatments ($P < 0.01$, ANOVA tests).

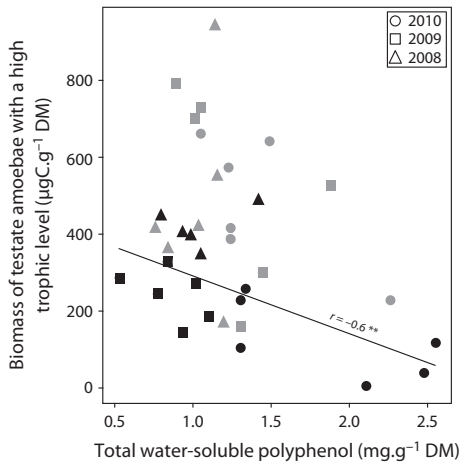

Fig. 5 Total water-soluble polyphenol content (mg.g^{-1} DM) plotted against the biomass of testate amoebae with high trophic levels (i.e. species with high shell-aperture size/body size ratio). Line is regression line significant at $^{**}P < 0.01$ level (ANOVA tests).

affected by warming over the 2 years, but a significant negative link between phenolics and air temperatures was found ($r = -0.38$, $F = 4.3$, $P = 0.034$). Furthermore, we found a negative relationship between water-soluble polyphenol and the biomass of testate amoebae with high trophic levels in warmed plots ($r = -0.6$, $F = 9.9$, $P < 0.001$) whereas the same relationship in ambient plots was not significant ($r = -0.2$, $F = 1.7$, $P = 0.21$) (Fig. 5). Moreover, variance partitioning revealed that water-soluble polyphenols and temperature increase explained 13% ($F = 6.2$, $P = 0.02$) and 19% ($F = 8.9$, $P = 0.005$) of the decrease in the biomass of testate amoebae with high trophic levels at the surface layers respectively. Such a relationship was neither found for testate amoebae with low trophic levels, nor in the litter layers.

Effect of warming on above- and belowground linkages. The use of a path-relation network showed that warming affected above- and belowground interactions (Fig. 6). The final models adequately fitted the data under ambient ($\chi^2_7 = 6.51$, $P = 0.51$; AIC = 34.19; GFI = 0.88; RMSEA < 0.001; RMR = 0.14) and warmed conditions ($\chi^2_7 = 1.00$, $P = 0.99$; AIC = 29.03; GFI = 0.98; RMSEA < 0.001; RMR = 0.034). Under ambient conditions, the model showed that microbial community structure in surface layers had significant links to microbial community structure in litter ($P < 0.01$), and that *Sphagnum*-polyphenols were neg-

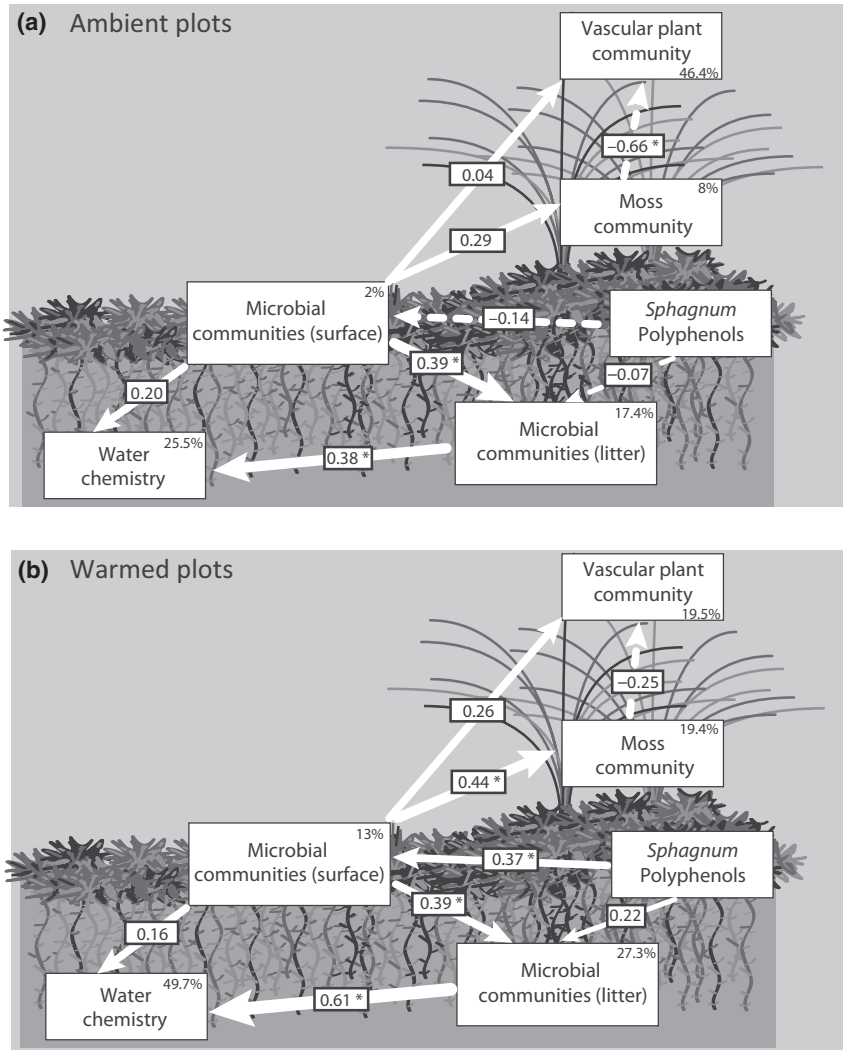

Fig. 6 Structural equation models of above- and belowground components in a *Sphagnum*-dominated peatland subjected to (a) ambient or (b) warmed conditions. Biomass of individual microbial groups living in surface and litter layers of *Sphagnum* carpet, percentage cover of moss and vascular plant species, polyphenols produced by *Sphagnum*, and water chemistry matrices were used to build the path-relation network. Dashed arrows indicate negative relationships. Numerals near each path indicate the weight of the path relationship; *$P < 0.01$. Numerals (%; adjusted R-squared) near each components indicate the percentage of variance explained by the model.

atively linked to microbial community structure in surface and litter ($P = 0.09$) (Fig. 6 a). Significant associations were further found between microbial communities living in litter and water chemistry composition ($P < 0.01$; Fig. 6 a). The model also showed a strong negative association between moss community and vascular plant community ($P < 0.01$). The ambient model explained a low proportion of the variance in mosses (8%) and microbial community structure at the surface (2%), whereas it explained 17.5%, 25.5%, and 46.4% of microbial community structure in litter, water chemistry, and vascular plant community respectively (Fig. 6 a).

In warmed plots, treatment effect on testate amoebae with high trophic levels significantly increased the link of microbial communities living in surface with moss community structure ($P < 0.01$; Fig. 6 b). The link between *Sphagnum*-polyphenols and microbial communities also increased and shifted from a negative association in ambient plots to a positive association in warmed plots, especially on microorganisms living in surface layers. This analysis also revealed a decrease in the negative association between mosses on vascular plants community structure in warmed conditions. Moreover, the model showed that the relationships between water chemistry and microbial communities increased with warming, especially in litter layers ($P < 0.01$). The SEM model on warmed plots explained a larger proportion of the variance in moss community structure

(19.4%), water chemistry (49.7%), microorganisms in surface (13%), and litter (27.3%) layers, whereas that of vascular plants diminished (19.5%) (Fig. 6 b).

Discussion

Our results from a field manipulation experiment in a *Sphagnum* peatland showed that warming affected the structure and functioning of soil microbial communities, as well as total vascular plant and moss abundances so resulting in a modification of linkages between above- and belowground subsystems.

Warming effect on microbial communities resulted in rapid trophic alteration in the microbial food web. The impact of warming on microbial structure decreased with depth; the response of microbial communities was highly significant in the surface layers, similar but weaker in the litter layers. Such a result is in line with previous observations of a weak warming effect on *Sphagnum*-polyphenol, enzymatic activities and water-extractable organic matter at this depth (Delarue *et al*., 2011; Jassey *et al*., 2011a, 2012b). Warmed communities disproportionately lost top predators and omnivores, and became increasingly dominated by decomposers and autotrophs (Fig. 2). These results suggested that the stability of the microbial food web was affected by warming, as it was demonstrated in other studies in different contexts (Petchey *et al*., 1999; Dossena *et al*., 2012; Heckmann *et al*., 2012). More specifically, warming impacted testate amoebae with a large body size and shell-aperture size, key organisms of the structure and functioning of microbial network due to their position at the top of the food web (Mitchell *et al*., 2003; Wilkinson & Mitchell, 2010; Jassey *et al*., 2012a). This decrease was linked and coincided with an increase in the relative biomass of bacteria, both in the surface and litter layers. Such changes in the relative distribution of microbial group among trophically defined levels may potentially alter ecosystem functioning beyond (Petchey *et al*., 1999; Dossena *et al*., 2012). For example and although it was not explored here, warming has been recognized to alter the structure of bacterial communities by favouring bacterial communities over methanogens, which may result changes in balance between CO_2 and CH_4 fluxes from peatlands (Kim *et al*., 2012). Furthermore, the strong impact of climate warming on testate amoebae is of particular interest because they link mosses and microbial heterotrophic producers (i.e. fungi and bacteria) via the microbial loop. This loop describes a trophic pathway in microbial food web where dead organic matter is returned to higher trophic levels via its incorporation into bacterial biomass coupled with the microbial food chains (e.g. bacteria → ciliate → testate amoebae; Gilbert *et al*., 1998). This recycles C and nutrients from dead organic matter to plant communities (Mitchell *et al*., 2003). The decline of testate amoebae with high trophic levels probably led to the loss of a trophic level (top-predators) in the microbial network leading to a shortening of microbial food chains, as showed in aquatic systems in another context (Hansson *et al*., 2012). Such food chain shortening should greatly affect the microbial food web stability and structure, which further could destabilize ecosystem processes they control (Petchey *et al*., 1999; Mitchell *et al*., 2003; Wilkinson & Mitchell, 2010; Heckmann *et al*., 2012). Indeed, testate amoebae with large shell-aperture consume a wide range of prey such as small testate amoebae, ciliates, rotifers, and small nematodes, typically bacterivores and algivores species (Gilbert *et al*., 2003; Wilkinson & Mitchell, 2010; Jassey *et al*., 2012a). Their decrease could accelerate the turnover of microbial biomass due to the diminution of their top-down control (predation), speeding up carbon and nutrient recycling via the microbial loop in long term (Petchey *et al*., 1999; Mitchell *et al*., 2003; Dossena *et al*., 2012). Such modifications of microbial structure and stability suggest that climate warming will impact belowground subsystem functioning, as suggested by Allison & Martiny (2008).

Interestingly, in other *Sphagnum*-dominated peatlands, summer warming was shown to accelerate N cycling due to changes in the belowground subsystem (Weedon *et al*., 2012). In our study, the largest differences in water chemistry composition were found among sampling years. Annual variations in soil moisture, evapotranspiration, and oxido-reduction processes associated with the water table position probably explained these variations (Proctor, 1994; Andersen *et al*., 2010; Macrae *et al*., 2012). Although water chemical response to warming was inconsistent across years–probably due to soil moisture variations (Macrae *et al*., 2012), our results highlighted that water chemistry composition responded to warming in interaction with annual variations by slightly increasing nutrient (as NO_3^-) and C-exports (as DOC) levels in the soil solution after 2 years. Reasons of such slight warming effect may be explained by the fact that microorganisms living in surface layers are exposed to the chemistry of capillary water rather than water deeper in the peat (i.e. water table) (Lamentowicz *et al*., 2010). As a consequence of this vertical gradient, the relationships between bryophyte and water deeper in the peat are not optimal (Mitchell *et al*., 2000b; Hájková & Hájek, 2004). Nevertheless, increasing amount of DOC and NO_3^- in warmed plots, even varying annually, may be interpreted as an indication of a slight destabilization of the belowground functioning of peatland (Evans *et al*., 2005; Carrera *et al*., 2009; Weedon *et al*., 2012). Moreover,

the actual amount of C and nutrient fluxes from the microbial loop is dependent of the length of food chains in microbial food webs (Pomeroy, 2000). Thus, the negative correlations between NO_3^-/DOC and the biomass of testate amoebae with high trophic levels suggest that the trophic alteration of the microbial structure due to a shortening of microbial food chains affected these biogeochemical fluxes.

We clearly showed that warming affected the belowground subsystem, however, this phenomenon is often indirectly linked to changes in aboveground communities (Wardle *et al*., 2004). A growing number of studies have showed how changes in plant communities affect soil communities (Wardle *et al*., 1999; Kardol & Wardle, 2010) and how the soil biota in turn affects the plant community structure, leading to feedbacks between the plant and soil subsystems (Kardol *et al*., 2006; De Deyn *et al*., 2008; Wardle *et al*., 2012). The increase in vascular plants and the decrease in *Sphagnum* in response to warming probably modified the quantity and/or quality of plant-derived organic matter, which in turn affected microbial communities such as decomposers (Delarue *et al*., 2011). In addition, we revealed significant relationships between *Sphagnum*-polyphenols and microbial communities in warmed plots, suggesting that these aboveground factors interact in driving belowground subsystem (Fig. 6). Polyphenols released by plants have been recognized as playing a fundamental ecological role in the regulation of soil microbiota and hence of microbial processes by way of chemical interactions (Hattenschwiler & Vitousek, 2000; DA Inderjit *et al*., 2011; Jassey *et al*., 2011a). For the first time, we found that warming greatly impacted polyphenol-microbial community interactions by a shift from a negative to a positive effect in warmed conditions (Fig. 6), and that the amount of *Sphagnum*-polyphenols have a similar influence than warming on key microorganisms such as testate amoebae. The strong negative correlation between polyphenols and testate amoebae with high trophic levels in warmed conditions revealed a potential inhibitory effect of phenols on this category of microorganisms in context of warming (Jassey *et al*., 2011b). Even if warming did not directly impact the total amount of *Sphagnum*-polyphenols, warming effects on qualitative production are quite possible, as previously showed in dwarf shrubs ecosystems (Hansen *et al*., 2006). Such findings strongly suggest that polyphenols produced by *Sphagnum* play a key role in the regulation of testate amoeba community structure, as well as in the microbial network stability in ways that can positively affect decomposition (Inderjit *et al*., 2011; Jassey *et al*., 2011b). Indeed, by their inhibitory effect on top predators, *Sphagnum*-polyphenols may indirectly enhance C and nutrient mineralization, thus favouring *Sphagnum* nutrient uptake. Bacterial growth and microbial OM breakdown could be stimulated by such inhibition of top-predators, leading to an increase in nutrient levels and C release in peatlands, which further would destabilize the productivity of plant communities and peatland carbon stock (Fenner & Freeman, 2011; Jassey *et al*., 2011a).

Reciprocally, we also assume that direct effects of climate warming on the microbial food web modify the linkages between above- and belowground communities. Warming effect on microbial food webs could greatly influence aboveground productivity and plant community structure through the stimulation of nutrient dynamics, which indirectly influence nutrient uptake of aboveground consumers such as vascular plants (Mitchell *et al*., 2003; Kardol & Wardle, 2010). The rise of temperature involved a decrease in the negative link between mosses and vascular plants, highlighting a reduction of *Sphagnum* mosses repression on vascular plant life (Fig. 6), as shown in response to other perturbations such as fertilization and drainage (Berendse *et al*., 2001; Lang *et al*., 2009). Even if vegetation species composition did not change overall with warming, we recorded significant changes in total vegetation structure in course of two years of warming (Fig. 4). The observed slight increases in nutrient and C levels in soil solution are likely to alter the competition between mosses and vascular plants (Berendse *et al*., 2001; Keuper *et al*., 2011). *Sphagnum* mosses are known to be efficient at absorbing nitrogen, preventing deep-rooted vascular plants growing in a dense *Sphagnum* carpet (van Breemen, 1995; Turetsky, 2003), but excessive tissue nitrogen accumulation due to warming effect on the microbial food web should exacerbate the decline of *Sphagnum* and the increase in vascular plants (Limpens & Berendse, 2003; Breeuwer *et al*., 2008, 2009). Such a warming-induced increase in vascular plant cover would further contribute to diminishing carbon sequestration in *Sphagnum* peatlands due to a plant litter more easily degradable (Verhoeven & Toth, 1995).

We conclude that climate warming greatly destabilizes peatland functioning by changing the interactions between plant and microbial communities. These results imply that warming changes peatlands directly by modifying the structure of microbial communities with the loss of top predators and the increase in bacteria and indirectly by changing ecosystem functions via modifications of plant-soil-microbial interactions through the increase in nutrients cycling and the positive and/or negative effect of *Sphagnum*-polyphenol on the microbial food web. Our observations support the hypothesis that microbial food web

associated with mosses positively contribute to global warming by controlling ecosystem feedbacks (Lindo & Gonzalez, 2010; Singh *et al*., 2010; Jassey *et al*., 2011b). Consequently the moss-microbiota system exerts a strong control over the structure and dynamics of peatland ecosystems. Furthermore, the interplay between *Sphagnum*-polyphenols and microbial communities adds another crucial new contributor to the list of mechanisms by which mosses, as ecosystem engineers, may tightly regulate biogeochemical cycling and climate feedback in peatlands (van Breemen, 1995; Cornelissen *et al*., 2007; Gornall *et al*., 2007; Keuper *et al*., 2011).

Acknowledgements

This research is a contribution of the ANR PEATWARM project (Effect of moderate warming on the functioning of *Sphagnum* peatlands and their function as carbon sink). PEATWARM is supported by the French National Agency for Research under the 'Vulnerability: Environment- Climate' Program (ANR-07-VUL-010). Further funding was provided to VEJ.J by the Franche-Comté Region, to A.B by EPFL and WSL, and to EAD.M by WSL, EPFL and University of Neuchâtel. The authors would like to thank T. Sime-Ngando and J. Colombet (University of Clermont-Ferrand, FR) for their help with the flow cytometry, and G. Bernard for field assistance. R.D. Bardgett (University of Lancaster, UK) R. Payne (Manchester Metropolitan University, UK) and R.T.E. Mills (Swiss Federal Research Institute WSL, CH) are kindly acknowledged for their fruitful comments on the manuscript and English edits.

References

Allison SD, Martiny JBH (2008) Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 11 513 – 11 519.

Andersen R, Rocherfort L, Poulin M (2010) Peat, water and plant tissue chemistry monitoring: a seven-year case-study in a restored peatland. *Wetlands*, **30**, 159–170.

Bardgett RD, Wardle DA (2010) *Aboveground -Belowground Linkages, Biotic interactions, Ecosystem processes, and Global Change*. Oxford University Press, New York, Oxford Series In Ecology and Evolution.

Bardgett RD, Freeman C, Ostle NJ (2008) Microbial contributions to climate change through carbon cycle feedbacks. *ISME Journal*, **2**, 805–814.

Berendse F, Van Breemen N, Rydin H *et al*. (2001) Raised atmospheric CO_2 levels and increased N deposition cause shifts in plant species composition and production in *Sphagnum* bogs. *Global Change Biology*, **7**, 591–598.

van Breemen N (1995) How *Sphagnum* bogs down other plants. *Tree*, **10**, 270–275.

Breeuwer A, Heijmans M, Robroek BJM, Berendse F (2008) The effect of temperature on growth and competition between Sphagnum species. *Oecologia*, **156**, 155–167.

Breeuwer A, Heijmans M, Gleichman M, Robroek BJM, Berendse F (2009) Response of *Sphagnum* species mixtures to increased temperature and nitrogen availability. *Plant Ecology*, **204**, 97–111.

van den Brink PJ, ter Braak CJF (1999) Principal response curves: analysis of time-dependent multivariate responses of a biological community to stress. *Environmental Toxicology and Chemistry*, **18**, 138–148.

Buttler A (1992) Permanent plot research in wet meadows and cutting experiment. *Vegetatio*, **103**, 113–124.

Carrera N, Barreal ME, Gallego PP, Briones MJI (2009) Soil invertebrates control peatland C fluxes in response to warming. *Functional Ecology*, **23**, 637–648.

Clymo RS, Turunen J, Tolonen K (1998) Carbon accumulation in peatland. *Oikos*, **81**, 368–388.

Cornelissen JHC, Lang SI, Soudzilovskaia NA, During HJ (2007) Comparative cryptogam ecology: a review of bryophyte and lichen traits that drive biogeochemistry. *Annals of Botany*, **99**, 987–1001.

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, **440**, 165–173.

De Deyn GB, Cornelissen JHC, Bardgett RD (2008) Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters*, **11**, 516–531.

Delarue F, Laggoun-Défarge F, Buttler A, Gogo S, Jassey VEJ, Disnar JR (2011) Effects of short-term ecosystem experimental warming on water-extractable organic matter in an ombrotrophic *Sphagnum* peatland (Le Forbonnet, France). *Organic Geochemistry*, **42**, 1016–1024.

Dise NB (2009) Peatland response to global change. *Science*, **326**, 810–811.

Dorrepaal E, Toet S, van Logtestijn RSP, Swart E, van de Weg MJ, Callaghan TV, Aerts R (2009) Carbon respiration from subsurface peat accelerated by climate warming in the subarctic. *Nature*, **460**, 616–679.

Dossena M, Yvon-Durocher G, Grey J, Montoya JM, Perkins DM, Trimmer M, Woodward G (2012) Warming alters community size structure and ecosystem functioning. *Proceedings of the Royal Society B*, **279**, 3011–3019. doi: 10.1098/rspb.2012.0394.

Eisenhauer N, Cesarz S, Koller R, Worm K, Reich PB (2012) Global change belowground: impacts of elevated CO2, nitrogen, and summer drought on soil food webs and biodiversity. *Global Change Biology*, **18**, 435–447.

Escofier B, Pages J (1994) Multiple factor-analysis (afmult package). *Computational Statistics & Data Analysis*, **18**, 121–140.

Evans CD, Monteith DT, Cooper DM (2005) Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts. *Environmental Pollution*, **137**, 55–71.

Fenner N, Freeman C (2011) Drought-induced carbon loss in peatlands. *Nature Geoscience*, **4**, 895–900.

Fenner N, Ostle NJ, McNamara N, Sparks T, Harmens H, Reynolds B, Freeman C (2007) Elevated CO2 effects on peatland plant community carbon dynamics and DOC production. *Ecosystems*, **10**, 635–647.

Gilbert D, Amblard C, Bourdier G, Francez AJ (1998) The microbial loop at the surface of a peatland: Structure, function, and impact of nutrient input. *Microbial Ecology*, **35**, 83–93.

Gilbert D, Mitchell EAD, Amblard C, Bourdier G, Francez AJ (2003) Population dynamics and food preferences of the testate amoeba Nebela tincta major-bohemica-collaris complex (Protozoa) in a Sphagnum peatland. *Acta Protozoologica*, **42**, 99–104.

Gornall JL, Jonsdottir IS, Woodin SJ, Van der Wal R (2007) Artic mosses govern belowground environment and ecosystem processes. *Oecologia*, **153**, 931–941.

Grace JB, Anderson TM, Olff H, Scheiner SM (2010) On the specification of structural equation models for ecological systems. *Ecological Monographs*, **80**, 67–87.

Hájková P, Hájek M (2004) Bryophyte and vascular plants responses to base-richness and water level gradients in western carpathian *Sphagnum*-rich mires. *Folia Geobotanica*, **39**, 335–351.

Hansen AH, Jonasson S, Michelsen A, Julkunen-Tiitto R (2006) Long-term experimental warming, shading and nutrient addition affect the concentration of phenolic compounds in artic-alpine deciduous and evergreen dwarf shrubs. *Oecologia*, **147**, 1–11.

Hansson LA, Nicolle A, Granéli W *et al*. (2012) Food-chain length alters community responses to global change in aquatic systems. *Nature Climate Change* (in press).

Hattenschwiler S, Vitousek PM (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology and Evolution*, **15**, 238–243.

Heckmann L, Drossel B, Brose U, Guill C (2012) Interactive effects of body-size structure and adaptive foraging on food-web stability. *Ecology Letters*, **15**, 243–250.

Husson F, Josse J, Lê S, Mazet J (2009) FactoMineR: Factor Analysis and Data Mining with R. In. R package, version 1.12. Available at: http://CRAN.R-project.org/package=FactoMineR. (Accessed 10 June 2010).

Inderjit, Wardle DA, Karban R, Callaway RM (2011) The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology and Evolution*, **26**, 655–662.

IPCC (2007) *Climate change 2007: the physical science basis summary for policymakers*. In, Contribution of Working Group I to the Fourth Assement report of the IPCC.

Jassey VEJ, Chiapusio G, Gilbert D, Buttler A, Toussaint ML, Binet P (2011a) Experimental climate effect on seasonal variability of polyphenol/phenoloxidase interplay along a narrow fen-bog ecological gradient in *Sphagnum fallax*. *Global Change Biology*, **17**, 2945–2957.

Jassey VEJ, Gilbert D, Binet P, Toussaint ML, Chiapusio G (2011b) Effect of a temperature gradient on *Sphagnum fallax* and its associated living microbial communities: a study under controlled conditions. *Canadian Journal of Microbiology*, **57**, 226–235.

Jassey VEJ, Shimano S, Dupuy C, Toussaint ML, Gilbert D (2012a) Characterizing the feeding habits of the testate amoebae *Hyalosphenia papilio* and *Nebela tincta* along a narrow "fen-bog" gradient using digestive vacuole content and ^{13}C and ^{15}N isotopic analyses. *Protist*, **163**, 451–464.

Jassey VEJ, Chiapusio G, Gilbert D, Toussaint M-L, Binet P (2012b) Peroxidase and phenoloxidase activities in *Sphagnum*-dominated peatlands in a warming climate. *Soil Biology and Biochemistry*, **46**, 49–52.

Jonsson M, Wardle DA (2010) Structural equation modelling reveals plant-community drivers of carbon storage in boreal forest ecosystems. *Biology Letters*, **6**, 116–119.

Josse J, Pages J, Husson F (2008) Testing the significance of the RV coefficient. *Computational Statistics & Data Analysis*, **53**, 82–91.

Kardol P, Wardle DA (2010) How understanding aboveground-belowground linkages can assist restoration ecology. *Trends in Ecology and Evolution*, **25**, 670–679.

Kardol P, Martijn Bezemer T, van der Putten WH (2006) Temporal variation in plant-soil feedback controls succession. *Ecology Letters*, **9**, 1080–1088.

Keuper F, Dorrepaal E, Van Bodegom PM, Aerts R, Van Logtestijn RSP, Callaghan TV, Cornelissen JHC (2011) A Race for Space? How *Sphagnum fuscum* stabilizes vegetation composition during long-term climate manipulations. *Global Change Biology*, **17**, 2162–2171.

Kim S-Y, Freeman C, Fenner N, Kang H (2012) Funtional and structural responses of bacterial and methanogen communities to 3-year warming incubation in different depths of peat mire. *Applied Soil Ecology*, **57**, 23–30.

Lamentowicz M, Lamentowicz L, van der Knaap WO, Gabka M, Mitchell EAD (2010) Contrasting Species-Environment Relationships in Communities of Testate Amoebae, Bryophytes and Vascular Plants Along the Fen-Bog Gradient. *Microbial Ecology*, **59**, 499–510.

Lang SI, Cornelissen JHC, Holzer A, ter Braak CJF, Ahrens M, Callaghan TV, Aerts R (2009) Determinants of cryptogam composition and diversity in *Sphagnum*-dominated peatlands: the importance of temporal, spatial and functional scales. *Journal of Ecology*, **97**, 299–310.

Limpens J, Berendse F (2003) How litter quality affects mass loss and N loss from decomposing *Sphagnum*. *Oikos*, **103**, 537–547.

Lindo Z, Gonzalez A (2010) The Bryosphere: an integral and influential component of the earth's biosphere. *Ecosystems*, **13**, 612–627.

Macrae ML, Devito KJ, Strack M, Waddington JM (2012) Effect of water table drawdown on peatland nutrient dynamics: implications for climate change. *Biogeochemistry*, in press.

Mitchell EAD, Borcard D, Buttler AJ, Grosvernier P, Gilbert D, Gobat JM (2000a) Horizontal distribution patterns of testate amoebae (Protozoa) in a Sphagnum magellanicum carpet. *Microbial Ecology*, **39**, 290–300.

Mitchell EAD, Buttler A, Grosvernier P *et al.* (2000b) Relationships among testate amoebae (Protozoa), vegetation and water chemistry in five *Sphagnum*-dominated peatlands in Europe. *New Phytologist*, **145**, 95–106.

Mitchell EAD, Gilbert D, Buttler A, Amblard C, Grosvernier P, Gobat JM (2003) Structure of microbial communities in Sphagnum peatlands and effect of atmospheric carbon dioxide enrichment. *Microbial Ecology*, **46**, 187–199.

Oksanen J, Blanchet G, Kindt R *et al.* (2010) vegan: Community Ecology Package. R package version 1.17-1. Available at: http://CRAN.R-project.org/package=vegan. (Accessed 10 June 2010).

Petchey OL, McPhearson PT, Casey TM, Morin PJ (1999) Environmental warming alters food-web structure and ecosystem function. *Nature*, **402**, 69–72.

Pinheiro JC, Bates DM (2000) *Mixed-Effects Models in S and S-PLUS*. Springer, NY, USA, 530 p.

Pomeroy L.R (2000) Food web connections: links and sinks. In: *Microbial systems: new frontiers. Proc. 8th Int. Symp. on Microbial Ecology*, (Eds. Bell C.R, Brylinsky M, Johnson-Green P) pp. 81–87. Atlantic Canada Society for Microbial Ecology, Halifax, Canada.

Proctor MCF (1994) Seasonal and shorter-term changes in surface- water chemistry on four English ombrogenous bogs. *Journal of Ecology*, **82**, 597–610.

R Development Core Team (2010) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Singh BK, Bardgett RD, Smith P, Reay DS (2010) Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature Review Microbiology*, **8**, 779–790.

Tsyganov AN, Aerts R, Nijs I, Cornelissen JHC, Beyens L (2012) *Sphagnum*-dwelling Testate Amoebae in Subarctic Bogs are More Sensitive to Soil Warming in the Growing Season than in Winter: the Results of Eight-year Field Climate Manipulations. *Protist*, **163**, 400–414.

Turetsky MR (2003) The role of bryophytes in carbon and nitrogen cycling. *Bryologist*, **106**, 395–409.

Verhoeven JTA, Toth E (1995) Decomposition of carex and sphagnum litter in fens - effect of litter quality and inhibition by living tissue-homogenates. *Soil Biology and Biochemistry*, **27**, 271–275.

Veteli TO, Mattson WJ, Niemela P, Julkunen-Tiitto R, Kellomaki S, Kuokkanen K, Lavola A (2007) Do elevated temperature and CO_2 generally have counteracting effects on phenolic phytochemistry of boreal trees? *Journal of Chemical Ecology*, **33**, 287–296.

Ward SE, Bardgett RD, McNamara NP, Adamson JK, Ostle NJ (2007) Long-term consequences of grazing and burning on northern peatland carbon dynamics. *Ecosystems*, **10**, 1069–1083.

Wardle DA (2006) The influence of biotic interactions on soil biodiversity. *Ecology Letters*, **9**, 870–886.

Wardle DA, Bonner KI, Barker GM *et al.* (1999) Plant removals in perennial grassland: Vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. *Ecological Monograph*, **69**, 535–568.

Wardle DA, Bardgett RD, Van der Putten WH, Wall DH (2004) Linking between aboveground and belowground biota. *Science*, **304**, 1629–1633.

Wardle DA, Jonsson M, Bansal S, Bardgett RD, Gundale MJ, Metcalfe DB (2012) Linking vegetation change, carbon sequestration and biodiversity: insights from island ecosystems in a long-term natural experiment. *Journal of Ecology*, **100**, 16–30.

Weedon JT, Kowalchuck GA, Aerts R *et al.* (2012) Summer warming accelerates subartic peatland nitrogen cycling without changing enzyme pools or microbial community structure. *Global Change Biology*, **18**, 138–150.

Wilkinson DM, Mitchell EAD (2010) Testate Amoebae and Nutrient Cycling with Particular Reference to Soils. *Geomicrobiology Journal*, **27**, 520–533.

Yeates GW, Foissner W (1995) Testate amoebae as predators of nematodes. *Biology and Fertility of Soils*, **20**, 1–7.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Average biomass (± SE) and average relative abundance (%) of microbial groups in a *Sphagnum*-dominated peatland subjected to experimental warming (WAR) or ambient conditions (AMB) in 2008, 2009, and 2010. Significant warming effect appears in bold (ANOVA tests).