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REGULAR ARTICLE

Snow cover manipulation effects on microbial community structure and soil chemistry in a mountain bog

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Abstract
Background and Aims Alterations in snow cover driven by climate change may impact ecosystem functioning, including biogeochemistry and soil (microbial) processes. We elucidated the effects of snow cover manipulation (SCM) on above-and belowground processes in a temperate peatland.
Methods In a Swiss mountain-peatland we manipulated snow cover (addition, removal and control), and assessed the effects on *Andromeda polifolia* root enzyme activity, soil microbial community structure, and leaf tissue and soil biogeochemistry.
Results Reduced snow cover produced warmer soils in our experiment while increased snow cover kept soil temperatures close-to-freezing. SCM had a major influence on the microbial community, and prolonged 'close-to-freezing' temperatures caused a shift in microbial communities toward fungal dominance. Soil temperature largely explained soil microbial structure, while other descriptors such as root enzyme activity and pore-water chemistry interacted less with the soil microbial communities.
Conclusions We envisage that SCM-driven changes in the microbial community composition could lead

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to substantial changes in trophic fluxes and associated ecosystem processes. Hence, we need to improve our understanding on the impact of frost and freeze-thaw cycles on the microbial food web and its implications for peatland ecosystem processes in a changing climate; in particular for the fate of the sequestered carbon.

Keywords Soil bacterial and fungal communities · Peatland · Phosphatase activity · Phospholipid fatty acids (PLFA) · Snow cover manipulation · Winter Ecology

Introduction

Over the last century, mean annual temperatures in Europe have increased, and it is expected that warming will continue during the 21th century with a 2.2−5.1 °C rise in mean annual temperature for Southern Europe (IPCC 2007). As a result, Northern hemisphere snow cover area declined (Groisman and Davies 2001; IPCC 2007) with an estimated average loss of $3.1 \times 10^3 km^2 year^{-1}$, which seems to be associated with spring warming (Brown 2000). Spring warming has caused the snow free period (i.e. earlier spring melt) to advance by 3–5 days per decade (Dye 2002; Tedesco et al. 2009). As snow has an insulating effect on soil and vegetation, reducing the occurrence of sub-zero temperatures (Edwards et al. 2007), changes in the timing of snowmelt can impact ecosystem and soil (microbial) processes (Kreyling et al. 2012; Templer 2012). Although the effects of snow on vascular plant community dynamics are relatively well known (Wipf and Rixen 2010), less is known about interactions between belowground and aboveground processes.

Snow cover varies inter-annually, but a recent decline in the number of snow days at altitudes below 1,300 m a.s.l. has been related to increased temperatures (Scherrer et al. 2004). Winter or spring warming in peatlands may result in near complete snow-thaw, leaving the vegetation and soil unprotected to recurring sub-zero temperatures, thereby increasing the potential for soil frost and the frequency of freeze-thaw cycles (FTCs); a process indicated as "colder soils in a warmer world" (Groffman et al. 2001). This can, concurrently, both directly and indirectly impact ecosystems (Wipf and Rixen 2010) by altering plant growth, microbial activity, and soil organic matter turnover, and thus the annual carbon (C), nitrogen (N) and phosphorus (P) dynamics (Freppaz et al. 2008; Matzner and Borken 2008; Reinmann et al. 2012). In boreal and (sub-) arctic ecosystems, increased FTCs have been related to warmer winter conditions and the associated early disappearance of an insulating snow cover (Henry 2008). There is however, no consensus on the existence of such a relationship, and spatial analysis in temperate ecosystems even showed the number of FTCs to decrease with winter warming (Kreyling and Henry 2011). This is interesting, as European peatlands near the southern distributional limit of peat formation can be seen as particularly threatened by environmental changes such as winter warming, yet the effects may be different from earlier reported peatland studies in the boreal and (sub-) arctic zone.

Increased FTCs favoured the release of inorganic N by degradation of soil organic matter in Alpine soils (Freppaz et al. 2008), or by root damage causing hampered N-uptake by plants (Kreyling et al. 2012). For subarctic fens it has been shown that spring warming-induced snowmelt benefits peatland C-balance (Aurela et al. 2004). Hence, attention has been paid to the physico-chemical impacts of snow removal in peatlands, but little is known about the impact of FTCs on: 1) the rhizoplane, and 2) the size and composition of microbial assemblages. Recently, Weedon et al. (2011) showed that although summer warming strongly increased N fluxes in a sub-arctic peatland, microbial assemblages were highly stable. Spring warming (in combination with winter snow addition), however, caused two out of six microbial groups to decrease as a consequence of changes in the frequency and duration of soil FTCs. Indeed, the microbial community is highly susceptible to soil frost (Larsen et al. 2002). Moreover, the composition of winter and summer microbial assemblages differ significantly (Lipson et al. 2002; Monson et al. 2006). For example, the fungal:bacterial ratio in microbial communities during winter is higher than during summer, mainly as fungi are more adapted to cold temperatures. Microbial communities in winter are consequently thought to be fuelled by fungal decomposition of organic polymers and phenolic compounds (Lipson et al. 2002; Schadt et al. 2003). Microbial community turnover can be fast with increasing temperatures (Mackelprang

et al. 2011), thereby releasing nutrients potentially available to plants in spring (Lipson and Schmidt 2004). The timing of snowmelt plays an important role in this process and therefore it is likely that snow cover manipulation (SCM) influences the composition of microbial assemblages.

In this study, we examine the effects of SCM (removal, control, addition) on the soil chemical and physical environment, the biomass and composition of the microbial communities in the rhizoplane of *Andromeda polifolia* L., and on the root enzyme (phosphatase) activity of this ericaceous peatland species in a mountain peatland situated close to the southern limit of peat formation in Europe. We hypothesized (1) that SCM causes changes in soil edaphics and temperature. Given that large quantities of available nutrients are contained within the microbial biomass, which may become available by frost-induced microbial mortality, we further hypothesize (2) that prolonged colder soils will cause P to increase, which will result into decreased root phosphatase activity. Additionally, (3) a prolonged period of *close-to-zero* soil temperatures will increase microbial mortality, and will therefore cause a decline in microbial biomass. This effect will be largest for bacteria, and so we expect that SCM induces a shift in the structure of microbial soil communities.

Material and methods

Study site and species

The Praz Rodet peatland (46°33′57″ N, 06°10′23″ E; 1,036 m.a.s.l.; 5 ha) is located in the Vallée de Joux (Switzerland) in the Jura mountains. Climatic conditions are characterised by a mean annual precipitation of 1,500 mm and mean annual temperature <5 °C (Mitchell et al. 2001). The first snow of the winter season 2010/2011 fell during the second half of November 2010, and although amounts of snowfall were relatively low (see Fig. S1, for a comparison with a 5-years average for snow depths at the La Cure meteorological station; 46°28″ N, 06°05″ E; 1,185 m a.s.l), snow cover was almost continuous and varied between 5 and 20 cm until early March 2011.

As the upper layer of the peat is most affected by snow cover manipulation, a maximum effect of snow manipulation is expected for the rhizoplane of shallow-rooted species. Therefore, we chose *Andromeda polifolia* L. as a target species since it is a common and widespread species in European bogs, and has 98 % of the total biomass belowground, concentrated in the 0–15 cm peat layer with the bulk at 2.5–7.5 cm (Wallén 1986).

Experimental design

In October 2010, fifteen square plots were laid out in the central, treeless part of the bog. Selected plots were characterized by a dominance of *Sphagnum magellanicum* Brid. and *S. rubellum* Wilson. In order to ensure homogeneity in the vegetation composition, plots had a size of 1 m^2. The vascular plant layer was dominated by *Andromeda polifolia* L., *Calluna vulgaris* (L.) Hull., *Carex pauciflora* Lightf., *Eriophorum vaginatum* L., and *Trichophorum cespitosum* (L.) Hartm. Plots were distributed over five blocks, each plot within a block representing one of three different snow manipulation treatments (control, snow removal and snow addition; Fig. 1), which were allocated randomly. Snow manipulations were carried out fortnightly (or 3 weeks), starting from February until complete snowmelt, or blanket removal at the snow addition plots (see below, Table 1). Snow in the removal plots was removed during this period and added to the snow addition plots. Aluminium shovels were used to either add or remove snow. The bottom snow layer (*c*. 2 cm) was removed by hand to ensure minimal disturbance to the vegetation. No snow manipulations were carried out for the control plots (Fig. 1). Between visits, we could not avoid periodic snow cover on the removal plots. To prevent early snow-thaw on the addition plots, remaining snow was covered by reflecting blankets (80 %; Luminax 80 Estero, Argitenux, Eboli, Italia; Fig. 1) from April 1st. As the albedo of a 1 cm snow pack can already be 0.7 (i.e. 70 % of the light is reflected; (Perovich 2007), the effect of the blankets on light transmission through the snow layer to the plant community can be considered minimal. Blankets were removed on April 15, 2011; all snow had disappeared at that date.

Soil temperatures were measured every 4 h in each plot from November 2010 to May 2011, using SL51T-Button Temperature Loggers (Signatrol, UK; 0.5 °C resolution), which were calibrated in iced water. Two loggers were placed in the centre of each plot at two

Fig. 1 (**a**) Photo of the experimental site with one block of treatments: snow removal (−Snow), control, and snow addition (+ Snow). (**b**) Snow cover was prolonged at the snow addition site, and was later protected using blankets (**c**) to prevent snowmelt

depths, i.e., 2 and 12 cm. Mean daily temperature and daily temperature fluctuations in control and manipulated plots were calculated; the latter was determined as the difference between daily minimum and daily maximum temperature. Freeze thaw cycles were calculated as the number of occurrences when the soil temperature (at 2 cm depth) crosses the 0 °C isotherm and then returns to above-zero temperature.

Pore water chemistry and dissolved organic carbon (DOC)

Pore water samples were collected fortnightly in the first 10 cm below the peat surface, starting on 25 March 2011 and using Rhizon soil moisture samplers (type MOM, pore size 0.2 μm, Eijkelkamp, Giesbeek, NL). Samples were taken from the plot centre, stored in glass bottles and transported to the lab. Water samples were collected five times. Samples were directly filtered after sampling using Nalgene® 0.2 μm syringe filters and stored dark at −20 °C prior to analysis. All samples were analysed spectrophotometrically for DOC, NO_3-N, NH_4-N and PO_4-P concentrations using a Skalar SAN^{PLUS} segmented flow analyser (Skalar analytical, Breda, NL). Because samples were missing in some plots due to soil frost, nutrient concentrations in pore-water data were averaged over the experimental period. Concomitantly, we define these averaged data as winter soil chemistry.

Tissue chemistry and root enzyme activity in *Andromeda polifolia*

After full melt-out, five *A. polifolia* individuals were randomly selected in each plot, marked, and leaf length was measured. Early May (May 5th), leaf length of these individuals was measured again and tissue growth was calculated as the difference between both measures. Above- and belowground biomass of *Andromeda polifolia* was sampled on May 18th, 2011. In each plot, five plants were randomly harvested from a 20 cm×20 cm square in the centre of the plots. Above-and belowground biomass was separated, after which aboveground biomass was dried for at least 48 h at 70 °C. N and P concentrations in the leaf tissue per plot was measured by digesting 250 mg milled leaf

Table 1 Values (mean ± SEM) of pore water chemistry and *Andromeda polifolia* leaf chemistry in the different manipulation treatments (n=5). Pore water values represent mean values from samples taken between March 25 and May 18. ¶ indicates non-significant difference ($0.05 \leq P \leq 0.1$)

		Winter soil chemistry					*Andromeda polifolia* leaf tissue		
Treatment	Main effect on soil temperatures	DOC ($mg l^{-1}$)	PO_4-P ($mmol\ l^{-1}$)	NH_4-N ($mmol\ l^{-1}$)	NO_3-N ($mmol\ l^{-1}$)	NH_4-N: NO_3-N	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	N : P
Snow removal	Reduced period Tsoil ≅ 0 °C	39.8±6.8	2.3±0.5	52.9±13.5	5.6±1.3	11.3±1.0	9.3±0.2	0.59±0.01	15.7±0.4
Control	-	42.4±4.7	1.9±0.4	39.0±8.4	6.8±1.4	8.3±1.3	9.2±0.3	0.57±0.02	16.0±0.2
Snow addition	Prolonged period Tsoil ≅ 0 °C	33.5±3.33	3.9±0.9¶	41.5±13.5	6.0±1.5	8.6±1.2	9.6±0.3	0.64±0.04	15.3±0.7

material with H_2SO_4, salicylic acid, Se and H_2O following a modified Kjeldahl destruction (Moore and Chapman 1976), followed by spectrophotometrical analyses using a Skalar SAN^{PLUS} segmented flow analyser (Skalar analytical, Breda, NL). From two randomly selected *Andromeda polifolia* individuals, the upper 10 cm of the root system was separated, and washed with deionized water. Root-phosphatase activity was measured using a modified ρ-nitrophenyl phosphate assay method (Johnson et al. 1999; Robroek et al. 2009). To do so, *c*. 0.05 g fresh root was placed in a glass test-tube containing 8 ml tris (hydroxymethyl)aminomethane/maleic acid buffer with 0.005 M 4-nitrophenyl phosphate. Test-tubes were placed at 37 °C for 120 min, and every 30 min 500 μl sample was extracted and added to a test-tube containing 4 ml 2 M NaOH. The concentration of para-nitrophenol was used as a proxy for phosphatase enzyme productivity, and determined spectrophotometrically at 410 nm (Shimadzu, UV-120-01).

Soil microbial biomass

Five days after snow manipulation ended (i.e. April 20th, 2011), peat (soil) samples were collected from the centre of all plots. First, soil volumetric water content (ML2x-ThetaProbe Soil Moisture Sensor, Delta-T Devices) was measured, after which the living *Sphagnum* surface layer (± 4 cm) was removed, and a 25 cm^2 (5 cm deep) sample was collected. Total microbial biomass, fungal and bacterial biomass were determined using lipid biomarkers (phospholipid fatty acids: PLFAs). Phospholipid fatty acids were extracted from the peat using an adapted protocol, following White et al. (1979) and Andersen et al. (2010). Briefly, PLFAs were extracted from approximately 5 g of fresh peat. A third of the extracted lipids were suspended in 900 μl of hexane and 300 μl of this hexane solution was transferred to a conical vial and nitrogen evaporated. 100 μl 0.1 mg/l nonadecanoate internal standard (19:0) was added, after which the samples were analysed by gas chromatography. Peaks were quantified automatically using the computing integrator and compared to the Supelco 37-component FAME mix (47885-U) and the BAME mix (47080-U). We used the PLFAs i15:0, 15:0, i16:0, 16:1ω7c, 17:0 and cy19:0 as markers of bacteria, while the 18:2ω6c was used as a marker for fungi (Frostegård and Bååth 1996). Other PLFAs were common membrane lipids (16:0 and 18:0), or not detected (i.e. below detection limit: a15:0, i17:0, cy17:0, 18:3ω6c, 18:9) in our samples.

Statistical analysis

All data were tested for normality prior to analysis using Shapiro-Wilcoxon normality tests and for homogeneity of variances using Bartlett tests, both in the *stats* package in R (R Development Core Team 2011), and log-transformed if necessary. The effects of snow manipulation on pore-water DOC, PO_4-P, NH_4-N, NO_3-N, NH_4-N:NO_3-N, target plant tissue N and P concentrations, root phosphatase activity, and soil microbial PLFA were tested using a randomized block ANOVA. Block was initially included in the models as a fixed factor, but as it was not significant, subsequent analyses were performed without block in the model. Tukey's *post-hoc* tests were used to analyse differences between treatments. One of the snow addition plots was omitted from the analyses because of missing data for all soil edaphic variables.

Redundancy analyses (RDA) were applied to soil microbial PLFAs to test the effects 1) of snow manipulation treatment (coded as classes with three levels: control, snow removal and snow addition) and 2) soil descriptors (pore water chemistry, DOC, soil temperatures and volumetric water content, and root enzyme activity) on their community structure. The significance of the models and of each explanatory variable included in the models was tested using 1,000 permutations (Gillet et al. 2010). Additionally, variation partitioning using RDA and adjusted R^2 was applied to compare the respective effect of snow cover manipulation and each environmental variable alone (Peres-Neto et al. 2006). We further used Multiple Factor Analysis (MFA) to symmetrically link five groups of descriptors split in five sub-matrices: the soil microbial PLFAs, the soil chemical environment (pore water chemistry and DOC), soil temperatures (−2 and −12 cm), volumetric water content (VWC), *Andromeda polifolia* enzyme (phosphatase) activity, and the data set describing snow manipulation treatment (control, snow removal and snow addition, each coded as binary variables). MFA was chosen because it allows simultaneous coupling of several groups or subsets of variables defined on the same objects and to assess the general structure of the data (Escofier and Pagès 1994). Briefly, MFA is basically a PCA applied to the whole set of variables in which each subset is

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

All multivariate analyses were performed with the software R 2.12.1 (R Development Core Team 2011) using the *vegan* (Oksanen et al. 2012) and *FactoMineR* (Lê et al. 2008) packages.

Results

Temperature dynamics

Snow depths in 2010/2011 winter were rather anomalous when compared to the average (5 years) snow depth in the Swiss Jura, with a thicker initial snowpack late November/early December, but a much thinner snowpack from January through April (Fig. S1; MeteoSwiss). Nevertheless, snow cover in the Praz Rodet peatland was almost continuous throughout the experimental season. Before snow cover manipulation (SCM), soil temperatures at 2 and 12 cm depth were more or less comparable between the plots (Fig. 2). From mid-December, temperature loggers recorded zero or sub-zero temperatures, and personal observations showed that all plots were frozen at the surface, while temperatures at 12 cm depth were close to freezing only from February (Fig. 2). SCM clearly resulted in differences in the temperature regime between the treatments, especially at 2 cm depth in the peat soil. Due to air temperatures well above 0 °C for most of the manipulation period, snow removal resulted in an earlier (*c*. 2 weeks) onset of soil thaw as compared to the control plots. Snow addition plots experienced temperatures close to 0 °C about 1 month longer, and at both soil depths (Fig. 2).

SCM not only affected the timing of soil thawing, but it also affected the mean daily soil temperature fluctuations. Subsurface soil temperatures in the snow removal plots fluctuated much more than in snow addition plots (0.78±0.11 °C and 0.22±0.03 °C, respectively), while temperature fluctuations in the control plots (0.49± 0.08 °C) were intermediate compared to the two manipulation treatments ($P \leq 0.001$). Such trends were also observed at 12 cm depth ($P \leq 0.001$). SCM did affect the number of freeze-thaw cycles between treatments (−Snow: 15±3.6; Control: 14±0.5; +Snow: 9±1.1), however, when tested using ANOVA these differences were not statistically significant ($F_{2,11}=1.7$, $P=0.22$).

Winter soil chemistry, *Andromeda polifolia* leaf tissue chemistry and root enzymatic activity

Neither snow removal nor snow addition caused a change in soil volumetric water content ($F_{2,11}=0.73$, $P=0.502$). Overall, the effect of snow manipulation on dissolved organic carbon concentration (DOC: $F_{2,11}=0.67$, $P=0.533$) or on any of the major nutrient concentrations in the pore-water (PO_4-P: $F_{2,11}=3.07$, $P=0.087$; NH_4-N: $F_{2,11}=0.52$, $P=0.609$; NO_3-N: $F_{2,11}=2.07$, $P=0.839$) was not significant (Table 1), although snow addition slightly increased pore-water PO_4-P (Tukey *post hoc* Control < Addition, $P=0.085$; Table 1).

Snow cover manipulation did not affect leaf tissue N and P of *A. polifolia* (N: $F_{2,9}=1.04$, $P=0.40$; P: $F_{2,8}=1.38$, $P=0.30$; Table 1), nor N:P ratio ($F_{2,9}=0.67$, $P=0.54$; Table 1). Overall, root phosphatase activity was affected by SCM ($F_{2,11}=4.26$, $P=0.04$), with increased activity in the snow addition plots (Tukey *post hoc* Control < Addition, $P \leq 0.05$). Root phosphatase activity thus increased with prolonged snow cover, which means that prolonged temperatures around freezing resulted into a higher enzymatic activity (Fig. 3).

Soil microbial biomass and community shifts

Phospholipid fatty acid (PLFA) analysis indicated that total soil microbial biomass was not affected by snow manipulation ($F_{2,11}=0.6$, $P=0.57$). In particular, fungal biomass was not significantly affected by snow manipulation (Fig. 4; $F_{2,11}=2.3$, $P=0.15$), although snow removal (i.e., reduced period where temperatures are around freezing) seemed to induce fungal growth. Bacterial biomass was marginally affected by snow manipulation (Fig. 4; $F_{2,11}=3.4$, $P=0.07$); prolonged low temperatures due to snow addition (see Fig. 2) caused a small, though non-significant, reduction in bacterial biomass. As a result of these small responses, the ratio between fungal and bacterial PLFA biomass shifted

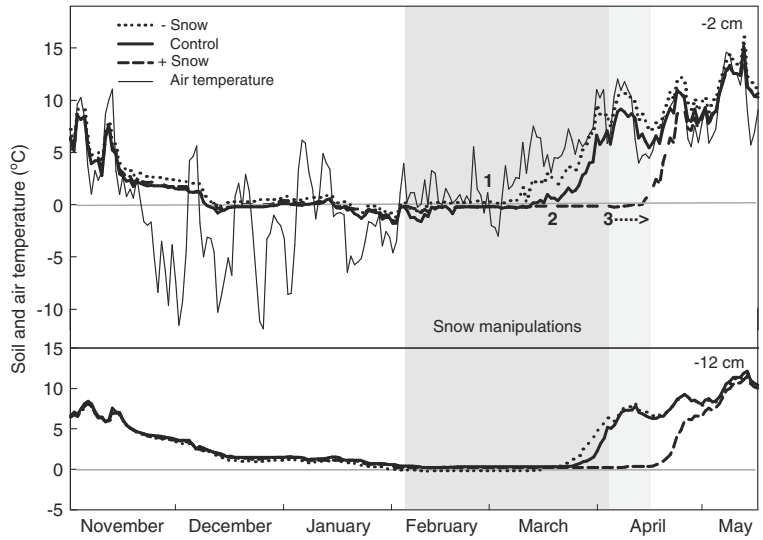

Fig. 2 Soil mean daily temperature at 2 and 12 cm depth, and mean daily air temperature (50 cm above the peat surface), throughout the experimental period. Snow manipulation treatments (*grey box*) were carried from February until snowmelt/disappearance. Snow melt/disappearance dates are indicated by numbers: 1 snow disappearance at snow removal plots, 2 full melt-out at control plots, 3 full melt-out at the addition plots. The dotted arrow in the lighter shaded grey box indicates the period where snow cover was 'prolonged' using reflecting blankets

toward a fungal dominated community when snow cover was prolonged, and thus soil temperatures close to freezing were prolonged ($F_{2,11}$=6.5, P=0.01).

RDA analysis on soil microbial PLFAs showed that snow manipulation treatment had a major influence on the microbial community structure, with snow manipulation explaining 17 % ($P \leq 0.05$) of the variation of PLFAs (Fig. 5a, Table 2). This analysis shows that snow addition plots were mostly separated from snow removal and control plots in the ordination space (Fig. 5a), while the split between snow removal and control plots was less clear. Detailed examination of the individual PLFAs showed that PLFAs from bacterial origin (16:1ω7c, 17:0 and cy19:0) did not significantly respond to snow manipulation (ANOVA; $F_{2,11}$=1.0, P=0.406; $F_{2,11}$=0.7, P=0.532 and $F_{2,11}$=2.6, P=0.122, respectively), while the PLFA abundance from fungal origin (18:2ω6c) increased, though only marginally ($F_{2,11}$=3.7, P=0.06), in snow removal treatment (Fig. 4). General membrane PLFAs (18:0, 16:0) either increased, again only marginally (18:0, $F_{2,11}$=3.3, P=0.07), with snow addition or remained unaffected (16:0, $F_{2,11}$=2.6, P=0.121).

The relationships between soil microbial PLFAs and environmental factors showed that changes in soil temperatures at −2 cm and −12 cm mainly explained the patterns of the PLFAs community structure during snow manipulation (Fig. 5b, Table 2). The soil microbial PLFAs in the snow addition plots were generally negatively correlated to soil temperatures, while those in the snow removal and control plots were mostly positively correlated to temperature. Variance partitioning showed that soil temperature and moisture explained *ca*. 14 % (P=0.14) of all variation, while individually, soil temperature at −2 cm and −12 cm explained 11 % (P=0.06) and 12 % ($P \leq 0.05$) of PLFAs variations, respectively. Other descriptors such as root enzyme activity and pore water chemistry interacted less with PLFAs (Table 2).

Taken together in the Multi Factor Analysis, the

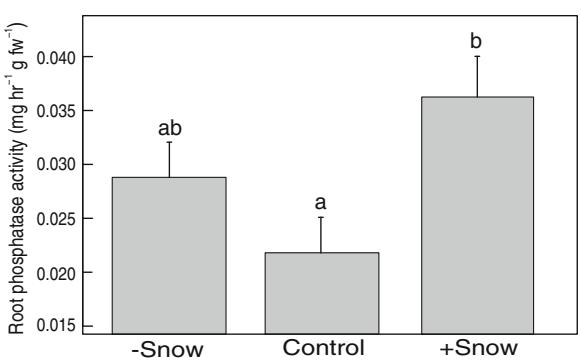

Fig. 3 Root surface phosphatase activity among the snow manipulation treatments. Snow removal (−Snow), control and snow addition (+Snow). Different letters indicate significant differences ($P \leq 0.05$)

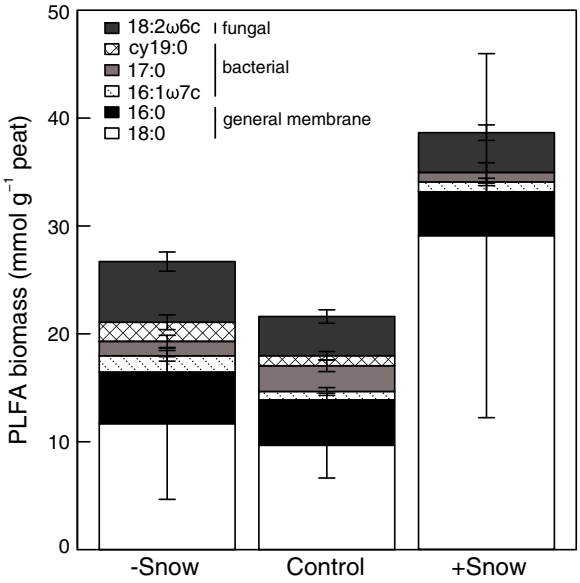

Fig. 4 Soil microbial biomass structured by the individual Phospholipid Fatty Acids among the snow manipulation treatments (mean ± SEM). Snow removal (−Snow), control and snow addition (+Snow)

five data sets also suggest a split of plots into three groups corresponding to snow manipulation treatment, i.e. snow removal, control and snow addition plots (Fig. 5c). The correlation circle showed that increases of the general membrane PLFAs 18:0, root phosphatase activity and PO_4-P concentration were positively correlated with the snow addition treatment, while soil temperatures were related to control plots and main bacterial and fungal PLFAs (18:2w6c, 17:0, 16:0 and cy19:0) (Fig. 5d). Multi Factor Analysis also shows that SVW, DOC and NH_4-N concentrations were rather linked to the snow removal treatment. The patterns of these 'species-environment' relationships are further illustrated by the *RV*-coefficients. Soil microbial PLFAs and root phosphatase activity were linked ($P \leq 0.1$) to snow manipulation, but not to the soil chemical environment. Among abiotic variables, the soil chemical environment was significantly correlated to soil temperature and moisture content (Table 3).

Discussion

Soil temperature effects on root enzymatic activity

Opposite to the general 'consensus' that reduced snow covers cause colder soils (e.g. Groffman et al. 2001), in our experiment soils were warmer by reducing snow cover. Snow has a thermal insulating effect on soil and vegetation so that a snow cover generally restricts soil sub-zero temperatures and reduces the frequency of freeze–thaw cycles (Groffman et al. 2001; Benoy et al. 2007; Hentschel et al. 2009). The insulating properties of snow, however, strongly depend on snow depth and snow density (Stieglitz et al. 2001). During the study period, the snow cover at Praz Rodet bog was thin (only a few centimetres) when first sub-zero air temperatures were recorded. Full decoupling between air and soil temperatures due to the thin snow cover may not, at that stage, have happened (Edwards et al. 2007), so that after several days with air temperatures close to −10 °C the peat soil temperature dropped and caused the peat surface to freeze. Subsequent snowpack development insulated the soil, therefore retaining temperature close to zero, and keeping the soil frozen (pers. observation). Differences in subsurface (−2 cm) soil temperatures between the treatments were most apparent between March and April, when early snow removal resulted into a shortened period of 'close-to-zero' temperatures (Fig. 2), but did not result in an increase in freeze-thaw cycles as reported by other authors (Groffman et al. 2001; Edwards et al. 2007). As also described in Kreyling and Henry (2011), in our study air temperatures did not fall below zero after snow removal, which explains the gradual warming of the soil from that point onward. On the other hand, prolonged snow cover prevented the soil from warming so that soil temperature in the snow addition plots remained close to zero throughout the manipulation period (Fig. 2). The thermal insulating effect of snow also became apparent in the mean daily temperature fluctuations, which were significantly smaller in plots with a prolonged snow cover. In the control and the snow removal plots, snow depths were likely not sufficient to fully decouple air and soil temperatures (Edwards et al. 2007), explaining the greater fluctuations of the temperature in these treatments.

As hypothesized, prolonged colder soil temperatures (snow addition) caused winter pore water P to slightly increase, but like Fitzhugh et al. (2001), who found similar results, we cannot explain these findings by envisaged microbial leaching as microbial biomass increased with snow addition. Fitzhugh et al. (2001) found increased nutrient (N and P) availability upon freezing, which they report to be the result of root

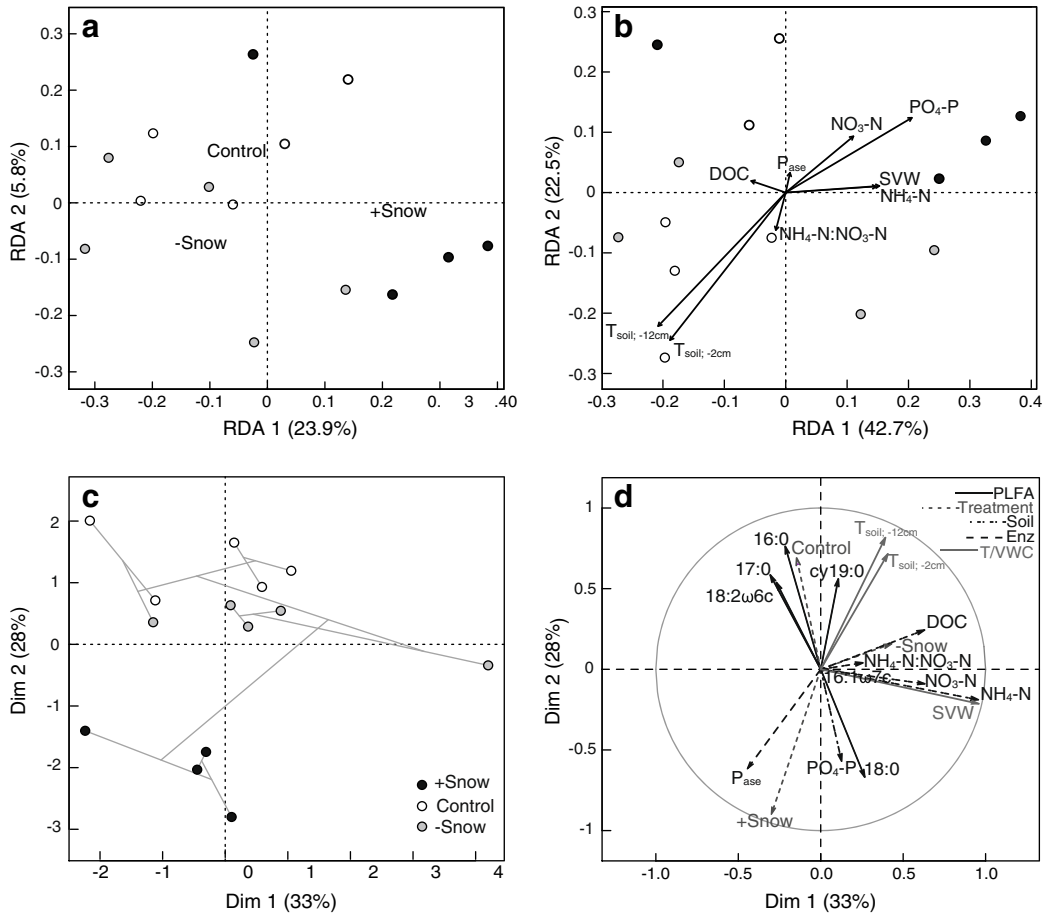

Fig. 5 (**a**) Redundancy analyses (RDA; axes 1 and 2) biplot of microbial PLFA data, presenting PLFA site scores and snow manipulation sites scores. (**b**) RDA triplot (axes 1 and 2) of the Hellinger-transformed microbial PLFA data constrained by the soil chemical environment, soil physical environment, and enzyme activity data. For abbreviations, see Table 2. (**c**) Multi Factor Analysis (MFA) of the PLFA biomass and the 'environmental' data-sets (soil chemical environment, soil physical environment, and root enzyme activity). Biplot axes 1 and 2 (both significant at $P \leq 0.05$ level) are shown together with the results of a hierarchical agglomerative clustering (*grey lines*) obtained by the 'Ward' method on the Euclidean distance matrix and the MFA site scores for the three treatments (+Snow, snow addition; Control; -Snow; snow removal). (**d**) Correlation between the variables of each data-set (PLFA = microbial PLFA biomass; Treatment = snow manipulate on treatment; Soil = soil chemical environment; Enz = root phosphatase activity; T/VWC = soil temperature and volumetric water content) and the MFA site scores on both axes. The radius of grey circle represents the maximum length of a partial standardized axis

mortality and consequent decreased plant uptake. Indeed, physiological stress due to prolonged frost, or increased FTCs, can provoke root damage (Kreyling et al. 2012) and disrupt plant nutrient uptake (Tierney et al. 2001). Opposite to the above-mentioned studies, we did not record extreme frost and differences in freeze-thaw cycles were only marginal, so that frost-induced root mortality seems unlikely. In our study, neither leaf tissue N and P, nor their stoichiometry, were affected by snow cover manipulation (SCM; Table 1). Additionally, in our experiment, only winter P concentration, but not N, was slightly enhanced in the pore water. This would apparently rule out differences in phenology (i.e. start of the growing season), or root damage, as an explanatory mechanism for the increased pore water phosphorus concentration in the snow addition plots. Indeed, SCM did not significantly affect leaf growth of *Andromeda polifolia* ($F_{2,12}$= 2.13, P=0.16). Alternatively, and more likely, the increased pore water P concentration was due to increased phosphatase enzyme activity in the snow addition plots (Fig. 3). Phosphatase has been shown

Table 2 Summary of redundancy analyses (RDA) on soil microbial PLFAs and soil chemical and physical explanatory variables. Data show fraction of variance explained by the RDA model and the corresponding *P*-value

	Abbreviation	RDA	
		%[§]	*P*-value
Snow manipulation treatment[φ]		**16.9**	**0.05***
Soil chemical environment		**7.7**	**0.68**
Dissolved Organic Carbon (DOC, ml l^{-1})	DOC	7.0	0.96
Nitrate-N (mmol l^{-1})	NO_3-N	2.5	0.58
Ammonium-N (mmol l^{-1})	NH_4-N	1.3	0.49
Phosphate-P (mmol l^{-1})	PO_4-P	6.5	0.12
Ammonium-N:Nitrate-N	NH_4-N:NO_3-N	6.4	0.92
Root phosphatase activity (mg hr^{-1} g fw^{-1})	**Pase**	**7.4**	**0.97**
Soil temperature and moisture content		**13.9**	**0.14**
Soil volumetric water content (%)	SVW	0.8	0.48
Soil temperature at 2 cm depth (°C)	$T_{soil;\ -2cm}$	11.1	0.06[•]
Soil temperature at 12 cm depth (°C)	$T_{soil;\ -12cm}$	12.1	0.05*

[§]Percentage of variance explained (adjusted R^2). [φ]RDAs constrained by treatment. Significant *P*-values are indicated with *, while *P*-values<0.1 are indicated by[•]. The four overall data-sets are indicated in bold, while the individual factors whintin these groups are written below

to be an effective enzyme in mineralizing organically-bound phosphate (Robroek et al. 2009; Fujita et al. 2010; van Dijk et al. 2012). Soil microorganisms and plants are both able to produce phosphatase, but we cannot estimate their relative contributions in our experimental set-up. Yet, as the total microbial biomass increased with snow addition (Fig. 4), it can be expected that also the microbial biomass in the rhizosphere increased. Extracellular enzymes produced by these rhizosphere-associated microorganisms may thus most likely have resulted in the increase of P-ase activity.

Kang et al. (1998) noted that there is a link between root phosphatase activity and pore water dissolved organic C (DOC) concentration, as phosphatase is an enzyme involved in the process of rhizodeposition, resulting in C loss from the roots. In our case, we did not find any effect of snow manipulation on DOC concentration. Contradictory, labile DOC in a riparian forest soil increased when snow was removed, causing the soil to be exposed to deeper frost intensity (Haei et al. 2012). Although we cannot be conclusive about the mechanism, the transfer of labile C into pore water may have stimulated the fungal community. As a result, mineralization was enhanced, but pore water DOC concentration did not increase because of microbial C uptake.

Effects of snow cover manipulation on microbial community

Opposite to our hypothesis (i.e. prolonged sub-zero temperatures reduce microbial biomass) we did not find a reduction in the microbial biomass due to prolonged temperatures close to freezing. Microbial biomass even seemed to increase in the snow addition

Table 3 RV coefficients (RV) and corresponding *P*-values among the five groups of variables used in the multiple factor analysis (MFA) of the entire dataset split into five groups of variables describing the PLFA composition, snow manipulation treatment, soil chemical and physical environmental conditions, and root phosphatase activity

	PLFA		Treatment		Soil chemistry		P-ase		Temp. + VWC	
	RV	*P*-value	RV	*P*-value	RV	*P*-value	RV	*P*-value	RV	*P*-value
Polylipid Fatty Acids (PLFA)	1.00	1.00								
Snow manipulation treatment (Treatment)	*0.31*	*0.08*	1.00	1.00						
Soil chemical environment (Soil chemistry)	0.10	0.63	0.08	0.67	1.00	1.00				
Enzyme activity (P-ase)	0.01	0.96	**0.30**	**0.05**	0.09	0.34	1.00	1.00		
Soil temperature and moisture content	0.18	0.29	*0.25*	*0.10*	**0.92**	**0.00**	0.11	0.32	1.00	1.00

Significant coefficients are in bold. Coefficients in italic indicate significance at $P \leq 0.1$ level

plots (Fig. 4), which was mainly caused by an increase of the general membrane PLFA 18:0. Microbial biomass and activity were shown to decline with increased freeze-thaw cycles (Larsen et al. 2002) or lower soil minimum temperature (Brooks et al. 1998). The increased microbial biomass with prolonged low but continuous temperatures as found in our study could thus be due to a longer period in which a winter community is able to build up. Indeed, the fungal:bacterial ratios increased by snow addition, indicating that the microbial community was still in a winter state (Lipson et al. 2002; Monson et al. 2006). The observed small shift in the ratio between fungi and bacteria can have important consequences for C and nutrient cycling in peatlands. Fungi play an important role in decomposition processes because of their ability to degrade complex, polymeric C compounds, their extensive hyphal growth habit, fast growth rates, and the ability to translocate nutrients through their hyphal network (Thormann 2006).

Differences in microbial biomass after snow manipulation are most probably caused by differences in microbial growth after melt-out, induced by the use of microbial necromass. Indeed, the slight but significant decrease of bacterial biomass with snow addition (Fig. 4), i.e. with increased soil frost, indicates increased bacterial mortality (Brooks et al. 1998), which stimulated the growth of other microbial groups. The absence of microbial growth in the warmer snow removal plots in this study may be caused by the time delay between soil thaw and sampling, by which the substratum (i.e. the necromass) may have been depleted. Zinger et al. (2009), however, found differences in the bacterial and fungal communities between Alpine tundra soils with different dates of snow melt, and these differences were independent of season. This indicates that snow cover dynamics strongly influence the microbial community, but sampling time is of minor importance as an explanatory factor for the microbial community structure.

Although the effects of snow manipulation were only marginal regarding individual PLFAs, our results clearly show an effect of the snow manipulation treatment on the microbial community structure (Fig. 5a). This difference in community structure was largely driven by treatment differences in soil temperature (Fig. 5b), and may indicate that snow addition delayed the switch from winter to spring microbial communities. Such delay can be expected to affect nutrient availability to plants, as the turnover involves the release of nutrients for plants (Lipson and Schmidt 2004). Recently, Jassey et al. (2012) also demonstrated a large effect of temperature on the structure of microbial communities in a peatland in the French Jura. They postulate that changes in microbial assemblages influence the plant-soil-microbial interactions, and thus the biogeochemical cycling in peatlands. In our study, however, no clear differences in soil and plant tissue nutrient concentrations were observed (Table 1). Several studies, however, reported a significant effect of snow cover/temperature on biochemical processes (see Edwards et al. 2007 for an overview). Buckeridge and Grogan (2010) indicated that the transition from winter to spring in snow-covered ecosystems consists of four distinct phases which all represent unique environmental conditions and biogeochemical pools, and especially the period between late winter and early spring, largely influences biochemical conditions. Additionally, Lipson et al. (2002) explain the shifts in the microbial communities by the sudden change in temperature and substrate availability after melt-out. Freeze-thaw cycles are generally believed to be the key mechanism controlling biogeochemical conditions, and the absence of distinct freeze-thaw cycles in our study may explain the absence of biochemical responses to our treatment. Nevertheless, snow removal (i.e. when soil frost was reduced) was associated to DOC and NH_4-N and NO_3-N (Fig. 5d). Reinmann et al. (2012) also found such effects, and especially an increase in the NH_4:NO_3 ratios in leachate water, in three forest soils when soil frost was reduced. These results are particularly interesting because Weedon et al. (2011) showed that spring warming effects on N transformation could not be explained by changes in the microbial community structure as assessed with our method (PLFAs), but seemed to be related to seasonal dynamics of the microbial biomass as a whole. Our results indicate a strong relationship between soil chemical conditions and soil temperature and moisture content, which are both influenced by snow manipulation. Soil microbial communities were also largely influenced by snow manipulation, with a dominating effect of soil temperatures. Based on the PLFAs, the structure of the microbial community was, however, not related to soil chemistry, indicating that the soil temperature and moisture content separately affect the soil microbial structure and geochemistry of the peat soil.

Conclusions

In the atypical 2010/2011 winter, where snow cover was rather thin and sub-zero air temperatures proceeded the establishment of an insulating snowpack, our snow manipulation led to a divergence from the general observation (see for example, Groffman et al. 2001) that snow cover protects the soil against frost. The removal of snow under the conditions of this atypical winter resulted in warmer soils in early spring. Oppositely, snow addition prolonged soil temperatures close to freezing, due to the absence of a snow cover at time of arrival of first sub-zero air temperatures. In the light of the inter-annual variability in snow cover, long-term manipulation experiments are necessary to better understand biogeochemical responses to changing winter conditions. In our short-term experiment, the effects of snow manipulation on the individual PLFAs were rather weak. Nevertheless, we show that snow manipulation altered the structure of microbial communities by increasing the fungal/bacterial ratio in colder soils. Changes in the decomposer community can be envisaged to cascade to higher trophic levels, such as ciliates and small testate amoebae, by favouring fungivores rather than bacterivores. Such modifications of the trophic links in the microbial food web could lead to a substantial reconfiguration of trophic fluxes (Ledger et al. 2012; Jassey et al. 2012) and their associated ecosystem processes. Hence, we need to improve our understanding on the impact of frost and freeze-thaw cycles on the microbial food webs and the implications for the robustness of peatland ecosystem processes in a changing climate, and in particular for the fate of the sequestered carbon.

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