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ABSTRACT

Temperature and substrate availability are among the key factors controlling microbial metabolism. The relative importance of these two drivers on soil organic matter turnover is, however, hotly debated. In this study, we investigated the effect of temperature changes on the potential enzyme activities involved in C (phenol-oxidase) and N (protease and amidase) cycling by incubating peat soils collected in winter and summer at the two typical temperatures recorded in the field during these two distinct periods (4 and 19 °C, respectively). In addition, to evaluate the effect of substrate limitations, we also compared the respiration rates of the thermally adapted soils with and without plant litter additions. Results showed that both collection season and incubation temperature had a significant effect on the two enzymes involved in N-cycling, with summer and increasing temperatures having detrimental effects on the potential activities of protease and amidase, whereas none of these factors affected phenol-oxidase activity. Furthermore, while adding readily decomposable substrate accelerated decomposition rates, CO_2 flux rates were similar for all temperature conditions. Interestingly, the greatest contribution of litter to CO_2 emissions occurred in the summer samples incubated at the lower temperature, whereas for the winter samples the stimulating effect on soil respiration was observed under warmer conditions. These findings suggest that the responses of soil microbial communities to temperature and substrate availability seem to strongly depend on the long-term temperature conditions and its interaction with substrate availability.

1. Introduction

Temperature changes drive microbial activities (Waldrop and Firestone, 2004); however, the effect of warming on soil organic matter (SOM) decomposition appears to be mitigated when soils become 'acclimated' to the new warmer conditions. This has been the main explanation for the observed declines in soil respiration after warming mineral soils over time (e.g. Luo et al., 2001; Eliasson et al., 2005; Bradford et al., 2008). Soil communities can quickly acclimate to new thermal conditions by shifting their species composition (Bárcenas-Moreno et al., 2009) or through phenotypic physiological changes (Pettersson and Bååth, 2003), although it is usually difficult to distinguish between the two processes (Wu et al., 2010).

Another explanation for these decreases in the respiration after long-term warming is the substrate depletion as a result of increased microbial activities (Knorr et al., 2005; Hartley et al., 2007; Bradford et al., 2008; Curiel Yuste et al., 2010). However, because substrate supply also changes seasonally, temperature effects on soil respiration could be hindered at those times when substrate is less abundant (Bengtson and Bengtsson, 2007).

The dynamics in carbon-rich soils such as peats deserve special attention because they store about one third of the global soil organic C pool (Yu et al., 2011). Therefore, in contrast to mineral soils where the fresh C supply is restricted, these large pools can show temperature stimulation for several months without showing any significant reduction in soil respiration rates over time (Hartley et al., 2008; Vicca et al., 2009). Furthermore, cooling these soils has

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led to a gradual decline in soil heterotrophic respiration, which was not reversed once temperature rose again. Extended periods of warming exposure were, therefore, necessary for respiration rates to recover to their pre-cooling levels (Hartley et al., 2008). This lack of evidence for thermal acclimation in C-rich soils suggests that the observed reductions in CO_2 production over time may be mainly caused by substrate depletion. However, substrate availability alone cannot fully explain the observed responses (Wetterstedt et al., 2010; Bradford, 2013) and different substrates and/or different combinations of temperatures could lead to different microbial responses.

A final complication to interpreting and predicting soil microbial responses to warming is whether historical climate regimes can affect contemporary microbial responses through the selection of a better adapted microbial community to the seasonal changes in precipitation and temperature occurring in a particular region. Likewise, soil microbial communities previously exposed to extreme rainfall patterns were more drought tolerant and showed fewer changes when subjected to drying–rewetting cycles in the laboratory, although the differences with field treatments did not persist over time (Evans and Wallenstein, 2012). The latter effect again suggests an interaction of responses with substrate availability, which might have been altered due to changes in plant growth or tissue chemistry affecting the quantity and quality of the substrates entering the soils (Evans and Wallenstein, 2012). Thus, the relative roles of direct temperature effects (moisture availability), and substrate availability and their interactions in explaining soil microbial activity responses to warming need further elucidation.

Extracellular activities can be used to evaluate functional responses of microorganisms to changes in their environment (Carreiro et al., 2000), but also as indicators of substrate availability (Caldwell, 2005; Weintraub and Schimel, 2005; Wallenstein et al., 2009). It has been suggested that extracellular enzyme activities involved in SOM decomposition are thermally adapted (Bradford et al., 2010), but their response to increasing temperatures will determine the magnitude of ecosystem warming responses only if they provide a rate-limiting step for the acquisition of substrate by the soil microbial community (Bradford, 2013).

In addition, because enzyme activity and soil respiration do not always follow the same pattern (Bonnett et al., 2006; Allison and Treseder, 2008), such complementary measurements are especially useful when trying to understand the dynamics of soil microbial responses and acclimation processes (Alef and Nannipieri, 1995).

Moreover, since the temperature sensitivity of the potential enzyme activities involved in C and N cycles can also show different patterns (Luxhoi et al., 2002; Koch et al., 2007), they might also display different responses to climate change. In the case of peatland ecosystems, phenol oxidase activity has been recognised as an important regulator of C storage since it is involved in the initial breakdown of phenolic compounds which tend to accumulate in these acid C rich soils and which are known to inhibit microbially mediated decomposition processes (Freeman et al., 2001; Fenner et al., 2007). Further, proteolytic enzymes, such as proteases (which hydrolyzes proteins to polypeptides and aminoacids) and amidases (releasing ammonium using amides as substrate), also play a key role in these N-limited ecosystems where N availability is the main factor controlling soil microbial activities and vegetation dynamics (Currey et al., 2010).

Therefore, in this study, we investigated the direct effects of seasonal *vs.* short-term temperature changes on potential enzyme activities involved in C and N mineralisation by incubating intact peat soils, collected in winter and in summer, at the typical winter (4 °C) and summer temperatures (19 °C) observed in the field. We hypothesised that microbial communities in these systems are well adapted to prevailing seasonal temperatures (Fenner et al., 2005) and that by switching the ambient temperatures, we could evaluate whether pre-existing seasonal differences in soil communities or rapid changes in ambient temperature are the main factors controlling microbial thermal adaptation. In addition, because indirect effects resulting from climate-driven changes in plant productivity can alter the supply of carbon to soil and the structure and activity of microbial communities involved in decomposition processes and carbon release from soils (Bardgett et al., 2008), we also tested the impacts of litter additions on heterotrophic soil respiration rates to determine if potential temperature-induced changes in microbial activities or in substrate availability are the main controlling factors of SOM turnover in these systems. We anticipated that substrate depletion will be faster in warmed soils and that new litter inputs would increase the differences in soil respiration rates between warmed and cooled soils (Hartley et al., 2007).

2. Materials and methods

2.1. Experimental set-up

Intact soil cores (PVC cylinders, 6 cm diameter × 10 cm deep) were collected in winter (February 2007) and summer (June 2007) from an upland blanket active bog (EU Habitat code 7130; 960 m a.s.l.) located within the Serra do Xistral (Lugo, NW Spain; 43°27′ 36″ N, 7° 34′ 12″ W). The term 'active' refers to supporting a significant area of vegetation that is normally peat-forming (EUR, 2013). The peat soil is acid (pH = 4.26 ± 0.04) with high organic matter content (top 10 cm: %C = 45.1 ± 0.43 and %N = 2.4 ± 0.03). The plant community at this site is dominated by *Molinia caerulea* L., with *Carex durieui* Steud. and *Deschampsia flexuosa* L. also being present (Rodríguez Guitián et al., 2009). Climatic records from the nearest meteorological station of Fragavella (43°46′ N, 7°44′ W; Meteogalicia, Xunta de Galicia: http://www.meteogalicia.es/) indicate that, for the period 2000–2006, the mean annual temperature was 10.7 °C, ranging from 3.5 °C in winter to 19.8 °C in summer and that the average annual rainfall for the same period was 1445 mm.

Twenty soil cores (10 'winter cores' and 10 'summer cores') were brought back to the laboratory where they were incubated in two temperature controlled chambers set at either 'winter temperatures' (4 °C) or 'summer temperatures' (19 °C) in a full factorial design with five replicates. Other environmental conditions were kept constant, such as the day–night cycle (8:16 h light:dark), the relative humidity (75%) and the original soil moisture content at the time of sampling (approximately 77%;winter cores = 77.5% ± 0.6 and summer cores = 77.8% ± 0.5), by regular weighing and additions of distilled water. The cores were incubated for 75 days with a final destructive sampling.

2.2. Enzyme assays

Phenol oxidase potential activity was determined for each soil core following the method by Pind et al. (1994) but modified by van Bodegom et al. (2005). This consisted of measuring the amount of diqc (2,3-dihydroindole-5,6-quinone-2-carboxilate) produced after oxidation of a phenolic-rich compound (model substrate: L-DOPA (L-3,4-dihydroxyphenylalanine)). Briefly, 2 mL of 5 mM L-DOPA (Sigma–Aldrich) were added to 4 aliquots (2 mL) of the soil suspension (prepared by mixing 10 g of fresh soil with 40 mL of 50 mM acetate buffered at pH 6). Two additional aliquots of the soil suspension, but without the model substrate being added, were used as blanks. Thereafter, all six soil solutions were incubated at 20 °C for 1 h, followed by centrifugation for 3 min (25,000 rpm) and finally, the absorbance of the supernatants measured at 460 nm.

Because for any specific enzymatic reaction, the optimal pH for the oxidation reaction is a function of both the pH optimum of the enzyme and the response of substrate redox potential to pH (Bach et al., 2013), the pH values of the suspensions, before and after the incubation, were also measured. The micromolar extinction coefficient was determined for the oxidation products of L-DOPA by mixing 1 mL of 1 mM L-DOPA (in acetate buffer) with 3 mL of horseradish peroxidase (0.1 mg mL^{-1} also in acetate buffer), followed by the measurement of their optical density (OD) at 460 nm (van Bodegom et al., 2005). The phenol oxidase activity was calculated as μmol diqc g^{-1} dw soil h^{-1}.

Protease potential activity was determined from all incubated cores according to the methods proposed by Ladd and Butler (1972) and Alef and Nannipieri (1995) which are based on the quantification of the amount of amino acid released after incubating fresh soil samples with sodium caseinate added as protein source. In brief, 1 g of soil (fresh weight) was mixed with 5 mL of 50 mM Tris-HCl buffer (pH 8.1) and 5 mL of 2% sodium caseinate and then incubated at 50 °C for 2 h. Thereafter, 5 mL of 15% trichloroacetic acid (TCA) was added to the protein-enriched samples and the suspensions centrifuged at 10,000 rpm for 10 min. Next, the supernatant (1 mL) was mixed with 1.5 mL of an alkaline reagent and 1 mL of Folin-Ciocalteau 33% and after a second centrifugation at 10,000 rpm for 3 min, the mixtures were left to decant for 1 h. Finally, the concentration of tyrosine in the supernatant was measured spectrophotometrically at 700 nm. The same procedures were followed for the control samples but, in this case, sodium caseinate was added after the incubation and immediately before adding the TCA solution. The absorbance values were obtained by means of a calibration curve using different volumes (0, 1, 2, 3, 4 and 5 mL) of a standard buffered tyrosine solution in Tris-HCl mixed with 5 mL of sodium caseinate and the whole volume was brought up to 10 mL using the buffer solution. Then, 5 mL of TCA solution was added and the measurements were performed in the same way as described above. The protease activity was expressed as μg tyrosine g^{-1} dw soil h^{-1}.

Amidase potential activity was determined by measuring the amount of NH_4^+ produced after hydrolysis of a known concentration of amide (Frankenberger and Tabatabai, 1980; Alef and Nannipieri, 1995). In this case, the soil suspension was prepared by mixing 1 g of fresh soil, 40 μL of toluene and 1.8 mL of 0.1 M Tris-H_2SO_4 buffer (pH 8.5), followed by the addition of 0.2 mL of 0.5 M acetamide solution. This mixture was incubated at 37 °C for 24 h and the reaction was stopped by adding 8 mL of 1 M KCl. After centrifuging for 3 min at 3500 rpm, the supernatants (2 mL) were transferred to glass vials and acidified with approx. 200 μL of 6 M HCl to precipitate the coloured substrates. After a second centrifugation at 13,000 rpm for 1 min, the concentration of NH_4^+ in the supernatant was measured using a Skalar Autoanalyser. Blanks were obtained following the same procedure described above, with the exception that, in this case, acetamide was added after the KCl solution. Finally, in order to avoid any possible overestimation of the NH_4^+ concentrations (due to the close proximity of the maximum absorbance wavelengths for NH_4^+ (580 nm) and humic substances (450 nm)), all soil solution samples were acidified with HCl. Amidase activity was calculated as μg NH_4^+ g^{-1} dw soil h^{-1}.

2.3. Leaf litter degradability

Degradability rates of dominant plant species at the site (*M. caerulea* L.) were measured to provide an integrative estimate of microbial enzymatic activities following incubation. This was achieved by taking, at the end of the incubation period, two soil subsamples of 10 g (fresh weight) from each incubated core. After careful removal of the coarse roots and any plant remains by hand, each of the soil subsamples were introduced into 40 glass jars (80 cm^3). By removing plant roots we discarded the autotrophic component of soil respiration (estimated to contribute ≈50% to belowground respiration; Hanson et al., 2000) which has its own temperature sensitivity and potential time-lag effects (Heinemeyer et al., 2011). Half of the jars received 0.1 g of air dried *Molinia* litter collected at the site (equivalent to approximately the average litter input commonly recorded for ombrotrophic peatlands; i.e. 400 g m^{-2} y^{-1} according to Moore et al., 2002), whereas the other half acted as controls. The litter of this vascular plant has a high C:N ratio (C:N = 73.5) but low contents of secondary metabolites (Table 1).

All experimental units were incubated under dark conditions inside a temperature controlled chamber set at 20 °C for one week. At the end of this incubation period and immediately after the glass jars had been air-tight sealed, gas samples were taken by removing 50–100 μL (CO_2) and 500 μL (CH_4) headspace gas through a silicon septum using a syringe (t_0). A second gas sample was taken after incubating the jars for 24 h (t_{24}) to enable determination of flux rates. CO_2 and CH_4 concentrations were determined using a gas chromatograph (HP 5890, Hewlett Packard) equipped with a Porapak N column (80/100 mesh) and dual TCD/FID detectors for CO_2 and CH_4 determination, respectively. *M. caerulea* decomposability rates were calculated as the difference between CO_2 production from jars with and without litter being added and expressed as μg C g^{-1} dw soil day^{-1}.

2.4. Statistical analyses

The effects of season (winter and summer) and incubation temperature (4 °C and 19 °C) on the three potential enzymatic activities were evaluated using a factorial ANOVA. Potential protease activity was the only variable showing homogeneous variance (Levene test) and amidase values also failed to fit a normal distribution (Shapiro–Wilk test). Due to the small number of observations (20), data transformation was unsuccessful in meeting the normality and homoscedasticity criteria and therefore, we followed the standard statistical recommendation (McDonald, 2014) and used the most typical transformation used in this research field (base-10 Log transformation). A factorial ANOVA was also used to test the effect of season and incubation temperature on gas fluxes but without transforming the data since this variable met the homoscedasticity criterion. Prior to the statistical analyses, Grubb's test was used to exclude any outlier in the data set. ANOVAs were followed by a Tukey's Studentized range (HSD) test ($\alpha = 0.05$) as posterior analysis.

Finally, Pearson's correlation coefficients were calculated to investigate potential relationships between enzymatic activities and respiration rates.

All the statistical analyses were performed using SAS system v9.3 (SAS Institute, Cary, NC, USA, 2004).

3. Results

3.1. Effects of seasonality and temperature changes on potential enzymatic activities

Potential phenol oxidase activity was the only enzymatic activity which was not affected by any of the investigated factors (Table 2; Fig. 1a). In contrast, the season in which the cores were collected significantly affected protease and amidase activities, with winter samples releasing 73 and 75% more tyrosine and ammonium, respectively, than those collected during summer ($p < 0.05$).

Furthermore, incubation temperature had a significant influence on these two soil enzymatic activities measured in these peat

Table 1
Chemical characteristics of *Molinia caerulea* litter. Values are means (±SE).

%C	%N	C/N	%Lignin	Lignin/N	%Phenolics	%Tannins
44.44 (0.45)	0.60 (0.01)	73.50 (1.49)	12.36 (0.18)	20.59 (0.56)	1.05 (0.02)	0.51 (0.02)

soils (Table 2) and thus, colder temperatures resulted in a 68% increase in potential protease activity and 87% in potential amidase activity ($p < 0.05$).

The interaction of season with temperature was also significant for these two enzymatic activities ($p < 0.05$; Table 2). Samples collected in summer and incubated at the warmest temperature treatment (19 °C) showed the lowest protease activity (336.9 ± 45 μg tyrosine g^{-1} dw soil h^{-1}) when compared with the rest of the treatments (on average 1016 ± 104.7 μg tyrosine g^{-1} dw soil h^{-1}; Fig. 1b). In contrast, potential amidase activity showed the highest enzymatic activity in the winter samples kept at cold temperatures (on average 297.2 ± 26.8 μg NH_4^+ g^{-1} dw soil h^{-1}; Fig. 1c). All the other samples exhibited similar (lower) activities (Fig. 1c).

3.2. Effects of temperature-induced changes in microbial activities on C fluxes

Measurements of CH_4 fluxes during the incubation period showed that these peat soils hardly emitted any methane and measured gas concentrations were always below 0.02 μg CH_4–C g^{-1} dw soil day^{-1}, with very little variation across treatments (data not shown).

With respect to CO_2 production, results showed that there was no significant effect of either season, incubation temperature or the interaction between the two factors on the amounts of CO_2 emitted by those peat soils without litter inputs (Table 3; Fig. 2a). Although the addition of *Molinia* litter increased decomposition rates by 40% across all treatments, none of the investigated factors had a significant influence on the amounts of CO_2 emitted from amended soils (Table 3 and Fig. 2b).

Nonetheless, the contribution of litter to CO_2 emissions from these peat soils was significantly affected by season and by its interaction with incubation temperatures (Table 3). In particular, the contribution of litter additions to soil respiration rates was on average 37% greater in summer than in winter samples ($p < 0.05$). Furthermore, the greatest effect of adding *Molinia* litter was observed when the summer samples were incubated at lower temperatures (Fig. 2c). This contrasted with the winter cores which showed the opposite response to incubation temperature, with a greater acceleration of litter decomposition rates when they were incubated at those temperatures typically occurring in the summer, which resulted in the amounts of CO_2 released being increased by two-fold as a result of warming ($p < 0.05$).

3.3. Correlations between potential enzymatic activities and respiration rates

Amidase activities were positively related to unammended soil respiration ($R^2 = 0.5$, $p = 0.026$), but negatively related to the amount of CO_2 attributed to litter additions ($R^2 = -0.82$, $p = 0.0001$). These two relationships were also observed in analysing only the soils collected during the winter period ($R^2 = 0.74$, $p = 0.037$ and $R^2 = -0.87$, $p = 0.01173$, respectively), but not for those ones taken in summer. In addition, under cold incubation temperatures, CO_2 production from unamended soils was also positively related to increased protease activities ($R^2 = 0.78$, $p = 0.012$), whereas when the peat soils were incubated at 19 °C the addition of the *Molinia* litter resulted in a negative relationship between phenol oxidase and CO_2 emissions from these amended soils ($R^2 = -0.85$, $p = 0.002$).

4. Discussion

4.1. Decoupled temperature responses of C and N cycling enzymes

Our enzymatic assays partly confirm previous observations indicating that there is a thermal optimum for decomposition processes in peatlands (Fenner et al., 2005). But, in contrast to this previous study, the enzyme involved in C-cycling processes and investigated here (phenol oxidase) did not appear to be strongly influenced by temperature. Moreover, in contrast to previous observations (Fenner et al., 2005), no hysteresis in temperature optima of phenol oxidase activities were found. Such discrepancies may be attributed to a much shorter incubation period (2 weeks) or to site-specific differences since optimal conditions for the activity of this enzyme also vary depending on microbial community composition (Kirk and Farrell, 1987; Sinsabaugh, 2010). In addition, phenol-oxidase activities are also known to be limited by oxygen and consequently, peat aeration has been considered the main critical mechanism by which the release of the vast amounts of C stored in these soils is being currently restricted (Freeman et al., 2001). The investigated site is an upland system (ca. 1000 m.a.s.l.), where the soils do not often get waterlogged (also corroborated by the low methane emissions observed in this study) and have an active burrowing mesofaunal populations (enchytraeids) which positively contribute to increased peat aeration (Carrera and Briones, 2013) and C transformations (Carrera et al., 2009, 2011).

Interestingly, the temperature response of the two N-cycling enzymes (protease and amidase) to temperature was completely different. In this case, warming the winter peat soils significantly decreased potential amidase activities while warming summer cores resulted in a significant decrease in protease activity. This contrasts to previous results which indicated that potential activities of protease are positively related to temperature and that amidase only responds to temperature when C is available (Fraser et al., 2013). Thus, the enzyme pool may depend on product demands and substrate availability. Peatlands are typically considered to be N-limited (e.g. Hobbie et al., 2002) and crucially, the N content of the dominant plant species at our investigated site (*M. caerulea*) is low when compared to other vascular plants (Breeuwer et al.,

Table 2
Results from the factorial ANOVAs for log-transformed data of the three soil enzymatic activities.

Source of variation	df	Phenol oxidase (μmol diqc g^{-1} dw soil h^{-1})		Protease (μg tyrosine g^{-1} dw soil h^{-1})		Amidase (μg NH_4^+ g^{-1} dw soil h^{-1})	
		F	P	F	P	F	p
Season	1	1.55	0.232	6.62	0.022	22.22	0.000
Temperature	1	0.76	0.397	6.21	0.026	5.09	0.041
Season × Temperature	1	4.52	0.129	9.12	0.009	25.16	0.000

2010). This is due to allocation of little N to the photosynthetic apparatus in *Molinia* (Berendse and Elberse, 1990) and suggests that microorganisms living in these peatlands might need access to exogenous N (Parton et al., 2007). The negative correlation between potential amidase activity and *Molinia* degradation observed in our litter decomposition study could indicate a downregulation and consequently, N availability could also be crucial in controlling these N-cycling enzymatic activities.

In the field, changes in nutrient concentration of *M. caerulea* senescent leaves starts in the autumn (Morton, 1977) and imply N translocation from leaves to the basal internodes and roots over winter (Aerts and Berendse, 1989), which is then mobilised in spring to enable shoot growth (Thornton and Bausenwein, 2000). Therefore, increased potential protease (and amidase) activity and

Table 3
Results from the factorial ANOVAs for CO_2 production (μg CO_2–C g^{-1} dw soil day^{-1}) from peat soils with and without *Molinia* litter being added as well as the litter contribution to CO_2 emissions (calculated as the difference between CO_2–C production from soil + litter and that from soil without litter additions).

Source of variation	Soil respiration without litter			Soil respiration with litter			Litter contribution		
	df	F	P	df	F	P	df	F	P
Season	1	0.59	0.453	1	0.52	0.482	1	11.08	0.005
Temperature	1	1.95	0.183	1	0.04	0.836	1	0.05	0.824
Season × Temperature	1	3.58	0.078	1	1.62	0.222	1	16.23	0.001

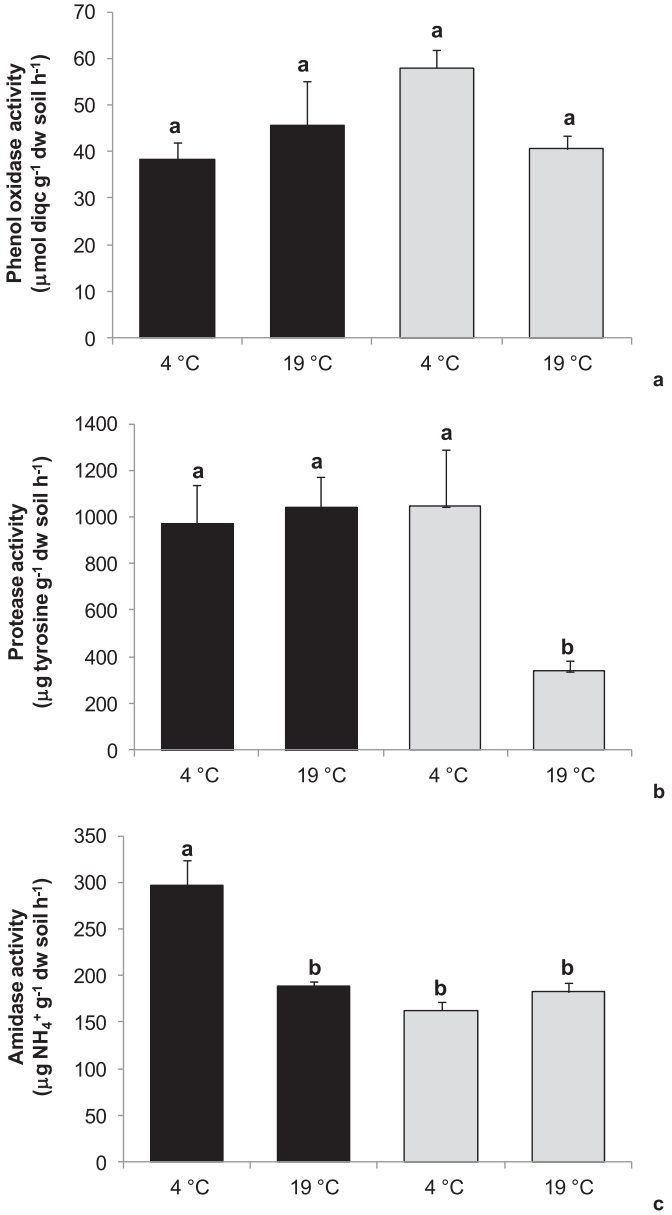

Fig. 1. Enzymatic activities of: a) phenol oxidase (μmol diqc g^{-1} dw soil h^{-1}), b) protease (μg tyrosine g^{-1} dw soil h^{-1}) and c) amidase (μg NH_4^+ g^{-1} dw soil h^{-1}). Means and standard errors are shown for each winter (black columns) and summer samples (grey columns) incubated at either 4 °C or 19 °C. Different letters indicate significant differences between treatments ($p < 0.05$).

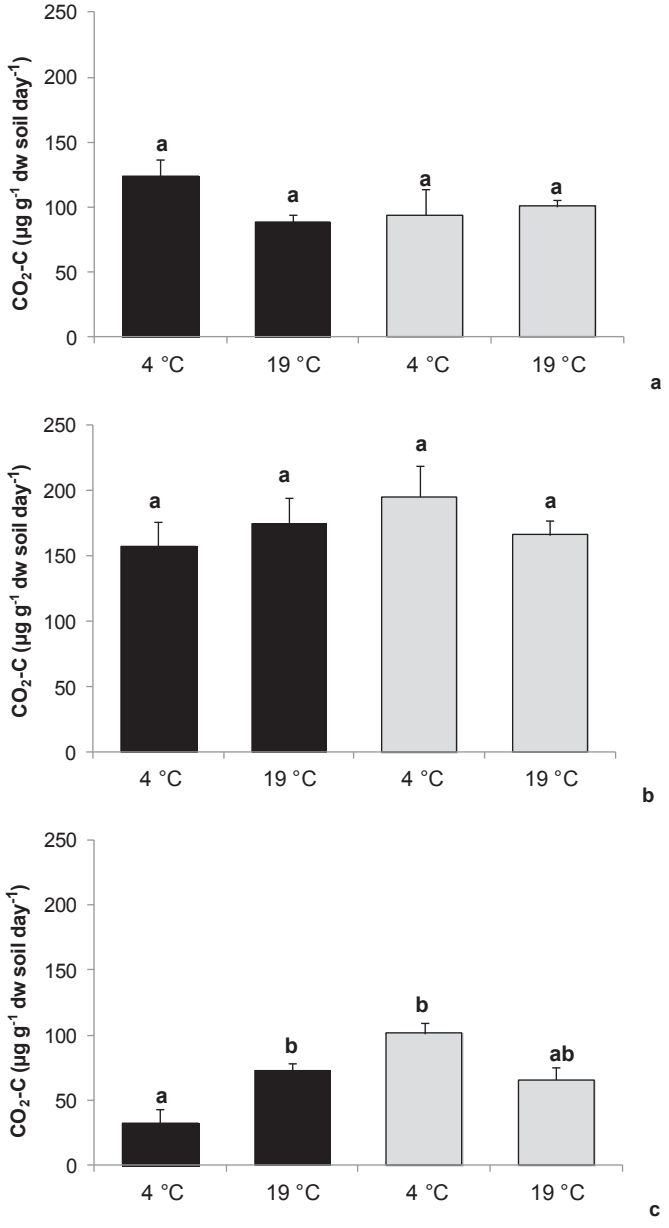

Fig. 2. CO_2 production (μg CO_2–C g^{-1} dw soil day^{-1}) (a) from peat soils in the absence of *Molinia* litter, (b) from peat soils after *Molinia* litter has been added and (c) contribution of litter to CO_2 emissions (calculated as the difference between CO_2–C production from soil + litter and that from soil without litter additions). Means and standard errors are shown for each winter (black columns) and summer samples (grey columns) incubated at either 4 °C or 19 °C. Different letters indicate significant differences between treatments ($p < 0.05$).

mobilization of N prior to tissue death are probably dominant processes in the winter soils, whereas during summer plants compete with microorganisms for N, creating depletion zones around the rhizosphere (Kuzyakov and Xu, 2013).

4.2. No temperature acclimation in C cycling processes in peatlands

The prevailing dogma states that microbes spend most of their time in the soil in a dormant stage until environmental conditions become favourable for their growth or they are stimulated by soil invertebrates and plant roots ('sleeping beauty paradox' *sensu* Lavelle et al., 1995). Indeed, our results from our litter decomposition study indicated that, in the absence of plant inputs, both summer and winter cores incubated at either temperature (4 or 19 °C) produced similar CO_2 flux rates. If acclimation causes the reduction in soil respiration after experimental warming, we anticipated that cooling should subsequently lead to an increase in respiration rates, whereas sustained warming will result in a further decrease (Hartley et al., 2008). Therefore, our findings indicate that the soil microbial communities do not seem to exhibit thermal acclimation in these peat soils.

Although litter additions rapidly accelerated decomposition, respiration rates were again similar across treatments. Like before, if microbial activities had acclimated to soil warming, increased substrate availability would have decreased the differences between warmed and cooled soils (Hartley et al., 2008). The absence of such effects is another clear indication that the amounts of substrate available for microorganisms play the most determinant role in the temperature sensitivity of SOM decomposition processes.

Interestingly, though, the contribution of litter additions to soil respiration differed radically between experimental treatments, with the summer cores producing the greatest CO_2 emissions at the lowest incubation temperatures and conversely, the winter cores showing a stimulating effect of soil respiration under warmer conditions. These results could also indicate some kind of 'soil microbial legacy' where seasonal adapted soil microbial populations could adjust their metabolic rates to compensate for temperature changes (Atkin et al., 2000).

4.3. Implications of historical seasonal legacies and substrate availability for predicting microbial responses to temperature

Our results are in agreement previous observations indicating that warming effects on soil enzymes are small compared to seasonal differences (Weedon et al., 2014) and therefore, more frequent extreme hot temperatures and prolonged warming during the summer periods might prevent further C losses from these soils if these new temperature regimes increase beyond the thermal optima of the extracellular enzymes involved in SOM decomposition. Indeed, recent studies (Briones et al., 2014) have shown that, under reduced moisture contents (60%), warmer (>15 °C) peat soils tend to respire less C. Conversely, the positive effect of increasing temperatures on soil respiration rates from winter soils could represent an important C flux. Since the highest temperature increases are predicted to occur during winter in the northern hemisphere as a result of the weakening of summer circulation (Coumou et al., 2015) warmer winters are, therefore, very likely to greatly amplify CO_2 production rates in these vulnerable ecosystems.

In addition, the contrasting responses of C and N cycling enzymes to temperature also confirm that these two processes are indirectly affected by nutrient availability (Weedon et al., 2014). Our peat soil is N-limited but has a high pool of labile C of about 1 mg C g^{-1} dw soil (extracted in K_2SO_4; unpublished data) which is about two orders higher when compared to that measured in mineral soils (e.g. Lagomarsino et al., 2006; Llorente and Turrión, 2010). In addition, the alkyl-C to O-alkyl-C ratio calculated from the ^{13}C NMR spectroscopy data from a nearby bog (Pontevedra-Pombal, 2002) is very low (<10%) compared to other soils (Webster et al., 2001; Ussiri and Johnson, 2003) and indicates that no greater accumulation of recalcitrant compounds occurs at this site. This suggests that differences in the availability of C and N sources, which could also change seasonally, are responsible the divergent enzymatic responses at this site and consequently, future model predictions should distinguish between the responses of these two types of enzymes (Weedon et al., 2014).

5. Conclusions

The results of this incubation study suggest that microbial enzymatic activities involved in N cycling are sensitive to temperature and that the thermal microbial optima for biogechemical processes vary seasonally. In contrast, substrate availability seems to play the most dominant role in regulating C cycling processes than temperature, in line with previous findings showing that availability of C is an important regulator of enzyme activities in wetlands (Shackle et al., 2000). Our data agree with recently proposed theories that soil microbial populations can exhibit physiological adjustments to changes in both temperature and substrate availability (Bradford, 2013) and that environmental legacies could determine the magnitude and the duration of these effects (Evans and Wallenstein, 2012). This has important implications for biogeochemical processes in peatlands, where changes in climatic conditions and nutrient availability are currently altering plant species composition, with an increasing dominance of ericaceous dwarf shrubs currently being observed under warmer conditions (e.g. Breeuwer et al., 2009) or towards gramineous species such as *Molinia* in response to greater N deposition (e.g. Tomassen et al., 2004). Therefore, the balance between microbial thermal adaptation and the quality and quantity of plant inputs will determine the overall dominance of either fast growing grass species with high litter decomposability which will result in greater C emissions or that of those longer life-span woody species with lower decomposition rates which will promote a greater increase in the C sink function of these systems (Ward et al., 2013). Since microbial activities are strongly linked to above-ground communities, a better understanding of the feedbacks occurring between aboveground and belowground communities will improve our future predictions of the response of the peatland C balance to environmental and climate change (Bardgett et al., 2008; Ward et al., 2015) and will help to identify possible ecological thresholds in C dynamics that might reduce the vulnerability of these C stocks (Limpens et al., 2008).

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