Soil microbial respiration in arctic soil does not acclimate to temperature

Authors:
Iain P. Hartley[1,*], David W. Hopkins[1,2], Mark H. Garnett[3], Martin Sommerkorn[4] and Philip A. Wookey[1]

Affiliations:
[1] School of Biological and Environmental Sciences, University of Stirling, Stirling, FK9 4LA, UK

[2] Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK

[3] NERC Radiocarbon Laboratory, Scottish Enterprise Technology Park, Rankine Avenue, East Kilbride, Glasgow G75 0QF

[4] Macaulay Institute, Craigiebuckler, Aberdeen, AB15 8QH, UK

E-mails:
Iain P. Hartley: i.p.hartley@stir.ac.uk
David W. Hopkins: d.w.hopkins@scri.ac.uk
Mark H. Garnett: M.Garnett@nercrcl.gla.ac.uk
Martin Sommerkorn: M.Sommerkorn@macaulay.ac.uk
Philip A. Wookey: philip.wookey@stir.ac.uk

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Correspondence author (*):
Iain P. Hartley, School of Biological and Environmental Sciences, University of Stirling, Stirling, FK9 4LA, UK; E-mail: i.p.hartley@stir.ac.uk
Tel: +44 1786 467757; Fax: +44 1786 467843

Abstract

Warming-induced release of CO_2 from the large carbon (C) stores present in arctic soils could accelerate climate change. However, declines in the response of soil respiration to warming in long-term experiments suggest that microbial activity acclimates to temperature, greatly reducing the potential for enhanced C losses. As reduced respiration rates could be equally caused by substrate depletion, evidence for thermal acclimation remains controversial. To overcome this problem, we carried out a cooling experiment with soils from arctic Sweden. If acclimation causes the reduction in respiration observed in warming experiments, then it must also subsequently increase rates post cooling. We demonstrate that thermal acclimation did not occur. Rather, over the following 90 days, cooling resulted in a further reduction in respiration which was only reversed by extended re-exposure to warmer temperatures. We conclude that, over the time scale of a few weeks to months, warming-induced changes in the microbial community in arctic soils will amplify the instantaneous increase in the rates of CO_2 production.

Key words: Adaptation, acclimation, arctic, carbon cycling, climate change, CO_2, respiration, microbial community, soil, temperature

INTRODUCTION

Rising global temperatures are likely to increase the rate of soil organic matter decomposition resulting in a substantial release of CO_2 (Raich & Schlesinger 1992; Kirschbaum 1995), and this phenomenon has the potential to accelerate climate change by up to 40% (Cox *et al.* 2000). In fact, the importance of soil C-cycling is recognized in the updated IPCC scenarios (IPCC 2007). However, increasingly, ecologists are recognizing that in order to predict long-term trends in ecosystem C fluxes and biological feedbacks, greater emphasis needs to be placed on measuring potential acclimation and adaptation responses (Oechel *et al.* 2000; Enquist 2007). Critically, acclimation has the potential to reduce the projected soil-C losses associated with global warming (Luo *et al.* 2001).

Respiratory thermal acclimation has been defined as "the subsequent adjustment in the rate of respiration to compensate for an initial change in temperature" (Atkin & Tjoelker 2003). When many plant species are exposed to higher temperatures for a prolonged period of time, physiological acclimation results in a reduction in respiration rates allowing for the maintenance of a positive C balance (Atkin & Tjoelker 2003). Similarly, thermal acclimation of respiration has been demonstrated for both ectomycorrhizal (Malcolm *et al.* 2008) and arbuscular mycorrhizal fungi in soils (Heinemeyer *et al.* 2006), and the fungal symbiont in lichens (Lange & Green 2005). Further, although cooling reduces respiration rates, prolonged exposure often results in a subsequent increase in plant respiration rates, allowing for the maintenance of critical metabolic processes (Armstrong *et al.* 2006). Many physiological modifications have been observed in microbial communities present at low temperatures which allow for continued growth (D'Amico *et al.* 2006),

and this may suggest that there is potential for up-regulation of activity following extended exposure to the cold.

In soils, although increased rates of respiration have been observed in many warming experiments (Rustad *et al.* 2001), the magnitude of the initial positive response to temperature often declines over time (Rustad *et al.* 2001; Eliasson *et al.* 2005). Because alterations in microbial community structure accompany soil warming in both the field (Zhang *et al.* 2005) and the laboratory (Zogg *et al*. 1997; Andrews *et al.* 2000; Pettersson & Bååth 2003; Pietikäinen *et al.* 2005), as well as in response to seasonal changes in temperature (Schadt *et al.* 2003; Lipson & Schmidt 2004; Wallenstein *et al.* 2007), the reduction in the initial positive response of soil respiration to warming may be the result of acclimation[1] of microbial respiration (Luo *et al.* 2001; Balser *et al.* 2006; Luo 2007; Wan *et al.* 2007).

Investigating temperature responses of soil respiration and microbial activity is complicated by the fact that the effect of experimental soil warming is confounded by the depletion of the most readily-decomposable soil C fractions. This could equally explain the reduction in respiration rates observed in long-term studies (Rustad *et al.* 2001; Eliasson *et al.* 2005). Consequently, the main evidence for thermal acclimation of soil microbial respiration remains questionable (Kirschbaum 2004; Eliasson *et al.* 2005; Knorr *et al.* 2005; Hartley *et al*., 2007b).

Identifying the potential for thermal acclimation of microbial respiration in arctic regions is particularly important due to the high rates of global warming already being experienced at high latitudes (ACIA 2005), the general sensitivity of communities close to environmental extremes to changing conditions, and the large amounts of C stored in these systems (Post *et al.* 1982). In addition, substantial

[1]As the long-term response of microbial respiration to changes in temperature almost certainly involves a genetic component, acclimation is probably an inappropriate term for this response. We will return the issue of terminology in the discussion section.

changes in microbial communities have been observed between seasons in tundra soils (Schadt *et al.* 2003; Lipson & Schmidt 2004; Wallenstein *et al.* 2007) raising the possibility of acclimation of microbial respiration in these systems. Accurate predictions of the long-term rates of C and nitrogen cycling in arctic soils, which in turn may determine total ecosystem C storage (Hobbie *et al*. 2000), plant productivity (van Wijk *et al*. 2005) and species composition (Weintraub & Schimel 2005), require a much greater understanding of microbial acclimation responses.

Here we present the results from one of the first studies to investigate the effect of an extended period of cooling on microbial respiration, utilizing organic soils taken from a sub-arctic tundra heath system in northern Sweden. If thermal acclimation is responsible for the down-regulation of microbial activity observed at high temperatures, then microbial activity must be gradually up-regulated when temperatures are reduced. This is because, as a compensatory response, acclimation must be reversible; otherwise temporary exposure to higher temperatures would result in a permanent down-regulation of respiration, preventing the recovery of rates even when temperature have declined, for example between summer and winter. In support of this logic, changes in soil microbial community structure have been observed both when soil temperatures increase (Andrews *et al.* 2000; Lipson & Schmidt 2004) and decrease (Schadt *et al.* 2003; Monson *et al.* 2006), and the thermal optimum for the activity of key C-cycling enzymes has been to shown increase and decrease with seasonal changes in temperature (Fenner *et al*. 2005). Furthermore, thermal acclimation of plant respiration, in response to seasonal and experimental changes in temperature, is dynamic and reversible, occurring both in response to warming and cooling (Atkin & Tjoelker 2003; Atkin *et al.* 2005; Zaragoza-Castells *et al.* 2008).

Therefore, the use of experimental cooling allowed us to minimize the confounding factor of warming-induced substrate depletion (substrate depletion will occur at a slightly faster rate in the control soils, but total carbon losses should be sufficiently small to avoid confounding the results) whilst still determining whether soil microbial respiration acclimates to temperature. We demonstrate that (i) soil microbial respiration does not acclimate to temperature, (ii) the short-term temperature sensitivity of respiration is unaltered by the prevailing temperature regime, and (iii) when soil temperatures were reduced for an extended period of time, changes in the microbial community resulted in a further decrease in the baseline rate of respiration, lowering rates of CO_2 production beyond the instantaneous response to temperature.

METHODS

Soil sampling and incubation

On 13th September 2006, twenty-six soil cores (68 mm diameter and 100 mm deep) were removed from an area of tundra heath above the tree-line (at an altitude of approximately 750 m), about 200 km north of the Arctic Circle, near Abisko, northern Sweden (68°18'07''N, 18°51'16''E). The mean annual temperature at this site is -1°C with mean January and July temperatures of -12 and 11°C, respectively (van Wijk *et al.* 2005). The dominant plant species are ericaceous shrubs, mainly of the genera *Vaccinum* and *Empetrum*, with some dwarf birch (*Betula nana* L.) also present. The soils have an organic horizon of between approximately 5 and 20 cm deep (mean depth = 11 cm), overlying well-drained medium to coarse-grained till deposits with

some large boulders and intermittent pockets of mineral soil. In this study, only the organic horizon was sampled. This soil is well-suited for investigating the long-term response of soil microbial respiration to changing temperatures because it contains a large amount of C, but does not experience waterlogging (except briefly during spring melt), and field conditions can thus be well replicated in the laboratory. Further, issues such as the mineral protection of SOM changing with temperature are avoided (Rasmussen *et al.* 2006).

The soils were transported to the University of Stirling using cooled air cargo. The water content of the soil was raised to water holding capacity (WHC) and samples were placed in an incubator (MIR-153, SANYO, Loughborough, UK) at 10^{o}C (±1^{o}C) for 110 days to allow respiration rates to stabilize as the most labile C pool was depleted and for the microbial community to adjust to this temperature. Sixteen cores were then transferred to a separate incubator (same make and model) set at 2^{o}C (±1^{o}C). Of these 16 cores, 10 were then maintained at 2^{o}C for 90 days (*high-low* treatment), and the other 6 cores were returned to the 10^{o}C incubator after 60 days at 2^{o}C (the *high-low-high* treatment). The remaining 10 cores were maintained at 10^{o}C for the whole 200-day incubation (*constant high* treatment). Soil samples were maintained at WHC throughout by frequent addition of distilled water. Data loggers (Tinytag® Plus, Gemini Data Loggers Ltd., Chichester, UK) connected to thermistor probes (PB-5001, Gemini Data Loggers Ltd., Chichester, UK) confirmed that the temperatures in the incubators remained stable. The incubation temperatures used are within the range regularly experienced by the soil during the growing season, and soil temperatures were not reduced below 0^{o}C to avoid changes in substrate availability caused by the alterations in the proportion of liquid water present (Mikan *et al.* 2002; Monson *et al.* 2006) and freeze-thaw effects.

Respiration measurements

Respiration measurements were carried out using an infra-red gas analyzer (EGM-4, PP Systems, Hitchen, UK) connected to an incubation chamber (700 ml Lock & Lock® container, Hana Cobi Plastic Co Ltd., Seoul, Korea) in a closed loop configuration. The rate of CO_2 accumulation in the headspace was logged every 1.6 seconds until a 35 ppm increase in CO_2 concentration had occurred. Therefore, measurements were made close to ambient CO_2 concentrations. Respiration rates were expressed as $\mu g\ C\ g\ C^{-1}\ h^{-1}$.

Finally, at the end of the incubation, the short-term temperature sensitivity of respiration (between 2 and 10^{o}C) in six replicates taken from the *high-low* and *constant high* treatments was measured. The samples were transferred to an incubator at 2^{o}C, and one day later respiration rates were measured. The incubator temperature was then raised to 6^{o}C and subsequently 10^{o}C, before being reduced back to 6^{o}C and then 2^{o}C. The soils were maintained at each new temperature for approximately 24 hours. Mean respiration rates were calculated at each temperature to allow changes in baseline rates of respiration over the five-day experiment to be included in the Q_{10} calculation (Fang *et al.* 2005). Changes in baseline rates of respiration could have been caused by changes in soil moisture (although samples were watered each day), or growth of microbial biomass in the previously cooled soils (Monson *et al*. 2006). The aim of this temperature manipulation was to determine whether the direct or instantaneous response of respiration to temperature had been altered by the cooling treatment and, therefore, we wanted to account for any changes in baseline rates. Respiration rates were natural log transformed and plotted against temperature. Linear

regressions were then used to calculate the slope (K) of the relationship and Q_{10} values calculated using Equation 1.

$$Q_{10} = e^{10K} \qquad \text{Equation 1}$$

Substrate-induced respiration

At the end of the experiment, soil from all 26 samples was sieved through a 2 mm mesh, large root fragments were removed and sub-samples dried for moisture and C content (loss on ignition) determination. After all samples had been incubated at 10^{o}C over-night, a solution containing 15 mg of glucose per gram of soil C was added to a 5 g (fresh wt.) sub-sample of each soil, with the corresponding volume (1 cm^3) of distilled water added to a further 5 g sub-sample. Total CO_2 production after 24 hours at 10^{o}C was measured using gas chromatography (Model 90-P, Varian Aerograph, Palo Alto, CA, USA). The difference between the two treatments was considered to represent substrate-induced respiration (SIR), which is considered to be proportional to the size of microbial biomass (Anderson & Domsch 1978).

Statistics

Statistical analyses were carried out using SPSS (SPSS Science, version 15, Birmingham, UK). Before cooling, one-way ANOVAs were used to determine whether there were any significant differences between the respiration rates of the soils in the different temperature treatment groups. Post-cooling, for the *high-low* and *high-low-high* samples, linear regressions were used to determine whether the

respiration rates changed significantly over the following 60 days. After the *high-low-high* samples were returned to 10°C, repeated measures ANOVAs and paired *t*-tests were used to determine whether there were significant differences between dates, both immediately before and after the cooling treatment was applied, and between the *high-low-high* and *constant high* treatments. At the end of the incubation, independent samples *t*-tests were used to determine whether the short-term temperature sensitivity of respiration differed significantly between the *high-low* and *constant high* soils, and paired *t*-tests were used to determine whether respiration rates differed between the increasing and decreasing phase of the manipulation. An independent samples *t*-test was used to determine whether the rate of SIR differed between samples that were at 10°C at the end of the experiment (as there was no significant difference between the two treatments, *constant high* and *high-low-high* soils were grouped together) compared with the soils that were at 2°C at the end of the incubation (the *high-low* soils).

RESULTS

Respiration rates

Before cooling, there were no significant differences in respiration rates measured at 10°C between the soils in the three temperature treatments ($P = 0.622$; Fig. 1a). On day 110, the *high-low* and *high-low-high* cores were cooled from 10°C to 2°C and the following day the respiration rates had declined by about 67%. Over the following 60 days, rather than an increase in the rate of respiration indicative of acclimation, respiration rates declined significantly by on average 28% (Fig. 1b). The effect of

temperature manipulation on the rate of respiration can be expressed using Q_{10} functions (Equation 1):

$$R_T = R_0 * Q_{10}^{(T/10)} \quad \text{Equation 1}$$

Where R_T is the respiration rate at temperature (T), R_0 is the respiration rate at 0^oC and Q_{10} is the proportion change in the rate of respiration given a 10^oC change in temperature. The equations corresponding to the mean effect of cooling for 1 and 60 days across both the *high-low* and *high-low-high* soils are as follows:

$$R_T = 2.18 * 4.01^{(T/10)} \quad \text{Day 1}$$

$$R_T = 1.44 * 6.06^{(T/10)} \quad \text{Day 60}$$

The reduction in the baseline rate of respiration caused by the cooling treatment has increased the apparent temperature sensitivity of respiration by ~50% (i.e. Q_{10} values have increased from 4.01 to 6.06).

However, in the *high-low* treatment, about 50 days after cooling, respiration rates stabilized with there being no significant subsequent change in rates between days 157 and 200 (linear regression: $P = 0.404$; Fig. 1). In contrast, over the entire incubation period, the respiration rate of the *constant high* cores did not change significantly (linear regression: $P = 0.359$) indicating that the gradual reduction in respiration rates only occurred when soil temperatures were reduced. These results demonstrate that sustained exposure to low temperatures amplified the negative effect of cooling on soil respiration rates.

On day 171, the *high-low-high* cores were returned to 10°C and respiration rates increased by approximately 72%. However, this rate was significantly less than that measured on day 109, immediately before the temperature reduction (paired *t*-test: P = 0.037; Fig. 1c). This indicated that the reduction in respiration rates observed at 2°C was still apparent when samples were returned to 10°C. Over the following 28 days (i.e. days 172-200) the respiration rate increased by approximately 22% with the rate measured on day 193 differing significantly from the rate measured on day 172 (P = 0.028; Fig. 1c). Further, the increase in respiration rates during this period only occurred in the *high-low-high* samples and not in the *constant high* samples (P = 0.026; Fig. 1c). Thus, extended exposure to 10°C was required for the respiration rates to recover to their pre-cooling levels.

Temperature sensitivity of respiration

At the end of the 200-day incubation period, the response of the *constant high* and *high-low* samples to short-term changes in temperature was investigated. Overall, respiration rates were highly temperature sensitive, but there was no significant difference between treatments (Fig. 2; P = 0.149) suggesting that extended exposure to 2°C had not resulted in microbial respiration becoming more (or less) temperature sensitive.

However, the response of respiration to the increasing phase of the temperature manipulation was significantly higher in the *high-low* soils than in the *constant high* soils (*high-low*: Q_{10} = 4.736±0.248; *constant high*: Q_{10} = 3.959±0.189; P = 0.032). This appeared to have been caused by a significant increase in the baseline rate of respiration in the *high-low* soils as demonstrated by significantly (or

marginally significantly) higher rates of respiration on the declining phase of the temperature manipulation (Fig. 2; 6^{o}C: P = 0.053, 2^{o}C: P = 0.001). No corresponding significant increase in the rate of respiration was observed in the *constant-high* treatment. The Q_{10} values calculated for the declining phase of the manipulation were similar and not significantly different (*high-low*: Q_{10} = 3.859±0.214; *constant high*: Q_{10} = 3.655±0.197; P = 0.497).

Substrate-induced respiration

A significantly greater rate of SIR (measured at 10^{o}C in all cases) was observed in the soil samples that were at 10^{o}C at the end of the experiment compared to those that were at 2^{o}C (*t*-test: P = 0.027; 75.3 vs. 66.7 μg C g^{-1} soil C h^{-1}).

DISCUSSION

Thermal acclimation

Our soil-cooling experiment produced no evidence that microbial respiration acclimates to temperature. The length of incubation carried out in our experiment should have allowed for thermal acclimation of microbial respiration to occur given that changes in microbial communities have been observed between seasons in tundra soils (Schadt *et al.* 2003; Lipson & Schmidt 2004; Wallenstein *et al.* 2007), and in response to temperature changes in laboratory experiments of a similar duration (Pettersson & Bååth 2003). Therefore, our results provide support for the modeling studies (Kirschbaum 2004; Eliasson *et al.* 2005; Knorr *et al.* 2005) that have proposed

that the decline in the initial positive response of soil respiration to increased temperatures in long-term warming studies is due to substrate depletion and not acclimation of microbial respiration.

Unlike plants it appears that the respiration of free-living, heterotrophic soil microbes does not acclimate to temperature. This is perhaps not surprising given the fundamental differences that exist between autotrophic and heterotrophic organisms. Whilst physiological acclimation serves to maintain a positive C balance in plants when shifted to a higher growth temperature (Atkin & Tjoelker 2003), it is unclear what advantage microbes would gain from reduced activity once temperature constraints have been relaxed. Thermal acclimation has been observed in mycorrhizal fungi (Heinemeyer *et al.* 2006; Malcolm *et al.* 2008) and the fungal component of lichens (Lange & Green 2005), but the activity of these microbes is tightly linked to, and controlled by (Heinemeyer *et al.* 2006), the rate of photosynthesis in their symbiotic partners. As such, these organisms are not representative of free-living heterotrophic microbes in soils.

Previously, it has been shown that the temperature sensitivity of microbial activity may increase in microbial communities adapted to low temperatures (Monson *et al.* 2006), and that it may be the temperature response rather than the baseline rate of respiration that changes when systems acclimate to temperature (Luo *et al.* 2001; Wan *et al.* 2007). However, we found little evidence for the microbial respiration being more temperature sensitive in the cooled soils. The apparent down-regulation of the temperature response, that was observed in previous studies (Luo *et al.* 2001; Wan *et al.* 2007), was based on changes in seasonal Q_{10}s in intact plant-soil systems. These results could have been caused by seasonal changes in the contributions of roots versus soil microbes to total belowground respiration. Hartley et al. (2007a)

demonstrated that rhizosphere respiration responded less to soil warming than microbial respiration in bare soil. As the contribution of the more temperature insensitive flux, rhizosphere respiration, is likely to be greatest during mid season, a time when soil temperatures are likely to be highest, this could explain the apparent reduction in the temperature sensitivity of respiration in warmed plots (i.e. differences between warmed and ambient plots are expected to be lowest during the time of year when rhizosphere respiration contributes the most to belowground respiration). Our results indicate that it is unlikely that the development of a microbial community which responds little to changes in temperature can explain the lower seasonal Q_{10}s measured in the warmed plots in previous studies (Luo *et al.* 2001; Wan *et al.* 2007). In our study, by carrying out our measurements in the absence of a rhizosphere, we avoided the possibility of microbial responses being mediated through changes in plant activity.

Adaptation enhancing a positive feedback

Our study goes further than demonstrating that thermal acclimation does not occur in these sub-arctic soils. Exposure to low temperatures for an extended period reduced the rate of respiration beyond the initial short-term response (Fig. 1b) and, similarly, extended exposure to moderate temperatures resulted in an increase in activity beyond the instantaneous response to temperature (Fig. 1c). Further, as the rate of SIR (measured at 10^{o}C in all cases) was significantly lower in the cooled soils, it appears the microbial community had been affected. Whether the lower SIR rate in the cooled soil was due to a reduction in microbial biomass *per se* or reflected a shift in microbial community structure is debatable. However, the results from our study

suggest that the microbial community was altered by the cooling and that this resulted in a further reduction in respiration rates. Therefore, at the low to moderate temperatures experienced in many soils, such as the arctic soil investigated here, when global warming increases soil temperatures it seems probable that C losses will be enhanced by changes in microbial community functioning.

In support of this suggestion, a soil-warming study demonstrated that, during winter months, microbial activity in warmed plots was higher than in control plots even when measurements were made at a common temperature; it was concluded that warming had produced a more active microbial community (Hartley *et al.* 2007a). Further, it has been demonstrated that the temperature optimum for the activity of key microbial enzymes in organic soils may shift with time of year (Fenner *et al.* 2005), and that thermal tolerances of bacterial community activity gradually change in response to temperature manipulations (Pettersson & Bååth 2003). Rather than a compensatory response, it appears that, in the longer term, changes in the microbial community may result in a further increase in activity as temperatures rise. Therefore, soil-C losses from cold environments, and during winter periods, are likely to be enhanced by climate change due to changes in soil microbial communities amplifying the instantaneous response to temperature.

Here we return to the issue of terminology; the changes in the microbial community which resulted in the decreasing rate of respiration for the 60-day period after cooling, and the increase in the rate of respiration following warming of the *high-low-high* soils, should be termed adaptation as it almost certainly contains a genetic component. We reiterate that the term acclimation is probably never appropriate when referring to a change occurring at the level of the whole community.

If a compensatory response is observed then perhaps the term "compensatory adaptation" would be more appropriate.

Previously, studies which have modeled mineralization kinetics based on the results of incubation studies have suggested that substrate pool sizes may increase at higher temperatures (MacDonald et al. 1995; Waldrop & Firestone 2004; Rasmussen *et al*. 2006). Molecules that decompose in reactions with large activation energies are likely to decompose especially slowly at low temperatures (Davidson & Janssens 2006; Hartley & Ineson 2008), but may become more available at increased temperatures, potentially explaining the increased pool sizes and shifts in substrate utilization patterns observed in these studies (e.g. Waldrop & Firestone 2004). Within this context, in the study presented here, the gradual reduction in respiration rates post-cooling may reflect a loss of the most labile pool of substrates which are most available to microbes at low temperatures. This may in turn have induced the changes in the microbial community that occurred (reflected by the reduction in SIR). On return to the warmer temperature, thermal constraints on substrate availability may have been relaxed and the microbes again adapted to their prevailing environment.

This is just one potential explanation for the reduction in respiration rates that occurred post-cooling and the changes in the microbial community. However, it is clear that thermal acclimation of microbial respiration did not occur, and adaptive responses of soil microbes to increasing temperatures may accelerate decomposition rates, at least at the low to moderate temperatures experienced in many soils.

Timescale of the response of microbial respiration to warming

In light of the findings of this study we can perhaps consider three separate processes which may determine the rate of soil C losses from arctic soils over different timescales. Firstly, in agreement with the study of Mikan *et al.* (2002), we found a strong instantaneous response of microbial respiration to changes in temperature (Fig. 2). When changes in the baseline rate of respiration were accounted for it appeared that the temperature sensitivity of respiration was not affected by the thermal regime the microbes had experienced.

Secondly, cooling reduced the baseline rate of respiration as the microbial community was altered by the new temperature, and this medium-term response to the temperature manipulation was reversible. It should be mentioned that there was some evidence of a faster response of the microbial community to the warming than the cooling treatment. It took almost 60 days for the full cooling effect to occur whilst rates had fully recovered within 30 days of warming in the *high-low-high* samples. In addition, there was some evidence of an almost immediate, partial up-regulation of the baseline rate of respiration in the *high-low* soils during the short-term temperature manipulation. Therefore, at a timescale of about 1 month, respiration rates are likely to increase in warmer arctic soils as changes in the microbial community result in an increase in the baseline rate.

Thirdly, at the decadal time scale, there may be a change in both total SOM stocks as warming stimulates C loss, and also a change in the composition of SOM as substrate pools with shorter turnover times are preferentially lost (Ågren & Bosatta, 2002; Kirschbaum 2004; Eliasson *et al.* 2005; Knorr *et al.* 2005). These changes will result in a subsequent decline in the rates of microbial respiration.

Finally, *in situ*, if higher decomposition rates increase soil nutrient availability (Schmidt *et al*. 2002; Pregitzer *et al*. 2008), increased plant productivity may partly or fully offset these C losses, and so determine the extent to which rates of microbial respiration decline. However, further research is required to estimate the importance of this potential feedback.

CONCLUSION

Compensatory thermal acclimation of soil microbial respiration did not occur in our experiment. Rather, the effect of temperature on microbial community functioning increased respiration rates beyond the instantaneous effect of temperature. This response may enhance substantially soil-C losses, at least at low to moderate temperatures. Taking into account the rapid rate of climate change predicted for high-latitude ecosystems, and the high temperature sensitivity of decomposition measured at low temperatures, the large C stores in arctic and alpine soils may be especially vulnerable. Given that they contain over 20% of soil C, increased decomposition in these ecosystems has the potential to accelerate climate change. Finally, our study highlights the need to consider not only the instantaneous responses of processes to changes in abiotic factors, but also any adaptive responses that may subsequently occur at the community or ecosystem level. This remains a major challenge for understanding and predicting ecological responses and biological feedbacks to climate change.

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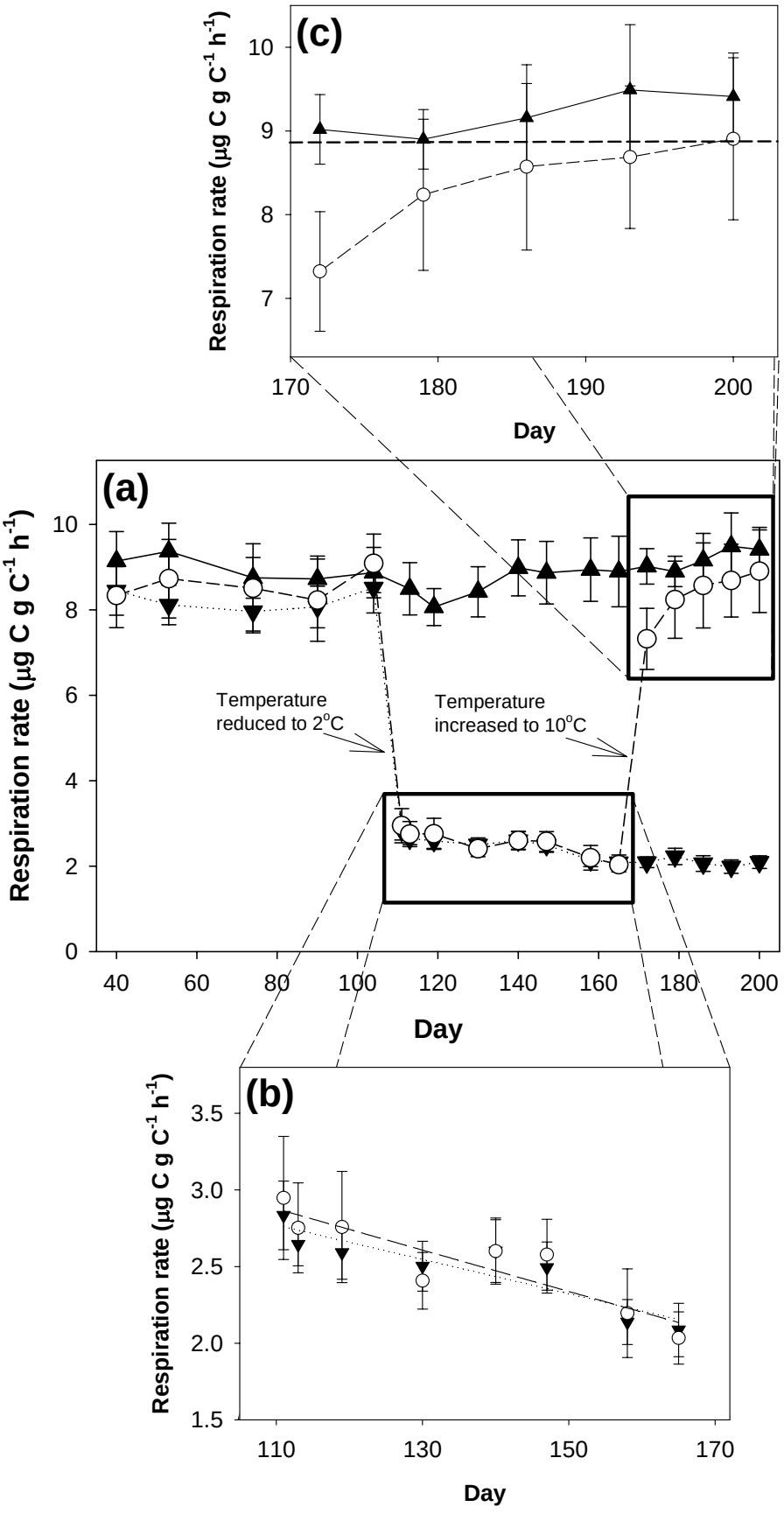
FIGURE LEGENDS

Figure 1 The mean soil respiration rates in the three different temperature treatments (*constant high* —▲—, *high-low* •••▼•••, *high-low-high* – –○– –). Error bars represent ±1SE (*constant high* and *high-low*: n = 10; *high-low-high*: n = 6). The main panel (a) shows the whole of the incubation period during which respiration measurements were made. The timing of the reduction in temperature from 10^{o}C to 2^{o}C in the *high-low* and *high-low-high* treatments is indicated as is the subsequent return to 10^{o}C in the *high-low-high* treatment. Panels (b) and (c) highlight the periods of key interest. Panel (b) shows the decline in the rate of respiration at 2^{o}C over the first 60 days at the lower incubation temperature in the *high-low* and *high-low-high* treatments. Linear regressions are fitted to each temperature treatment separately although there is no significant difference between the two fitted lines (*high-low* (dotted line): $y = -0.0112x + 4.00$, $R^2 = 0.817$; *high-low-high* (dashed line): $y = -0.0135x + 4.36$, $R^2 = 0.815$). Panel (c) shows the rate of respiration at 10^{o}C in the *high-low-high* and *constant high* samples immediately after the *high-low-high* samples were returned to 10^{o}C. The horizontal dashed line indicates the mean rate of respiration in the *high-low-high* samples on day 109 immediately before the *high-low-high* samples were transferred to 2^{o}C. Initially the rate of respiration in the *high-low-high* samples was significantly less than on day 109 (paired t-test: P = 0.037) and significantly lower than in the *constant high* treatment (t-test: P = 0.044), but these differences were subsequently lost as the respiration rates in the *high-low-high* samples increased. A significant interaction term between time and temperature treatment (repeated measures ANOVA; P = 0.026) indicated that the increase in respiration rates only occurred in the *high-low-high* samples.

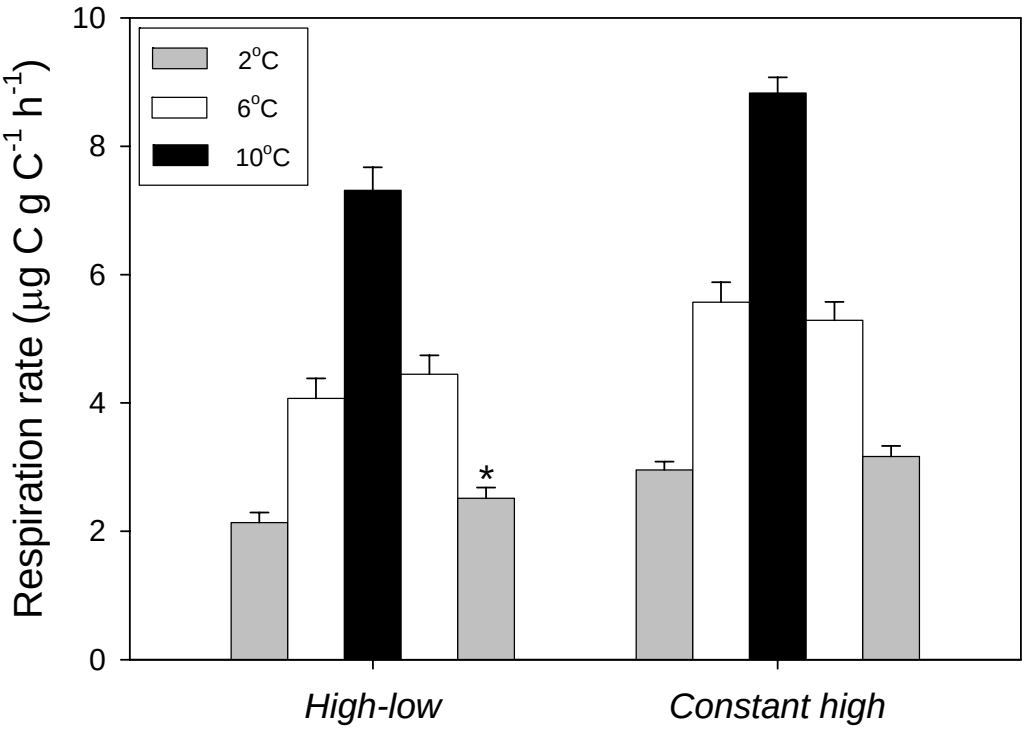
Figure 2 The response of respiration to the short-term changes in temperature in the *high-low* and *constant high* samples. Mean respiration rates on both the increasing and decreasing phase of the temperature manipulation are shown. Error bars represent +1SE (n = 6). In the *high-low* samples, there was a significant increase in the rate of respiration measured at 2^{o}C on the declining phase of the manipulation relative to the rate measured on the increasing phase (labeled "*")..The mean Q_{10} values (proportional change in the rate of respiration given a 10^{o}C change in temperature), calculated from mean respiration rates at each temperature, were 4.25±0.224 for the *high-low* treatment and 3.80±0.186 for the *constant high* treatment. There was no significant difference between these two Q_{10} values (*t*-test: P = 0.149).

1 Figure 1


(c)
Respiration rate (µg C g C-1 h-1)
10
9
8
7
170
180
190
200
Day
(a)
Respiration rate (µg C g C-1 h-1)
10
8
6
4
2
0
Temperature reduced to 2°C
Temperature increased to 10°C
40
60
80
100
120
140
160
180
200
Day
(b)
Respiration rate (µg C g C-1 h-1)
3.5
3.0
2.5
2.0
1.5
110
130
150
170
Day

2

Figure 2


Respiration rate (µg C g C-1 h-1)
10
8
6
4
2
0
2°C
6°C
10°C
*
High-low
Constant high