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Temperature adaptation of bacterial communities in experimentally warmed forest soils

JOHANNES ROUSK*, SERITA D. FREY† and ERLAND BÅÅTH*

*Section of Microbial Ecology, Department of Biology, Lund University, Lund, Sweden, †Department of Natural Resources and the Environment, University of New Hampshire, Durham, NH 03824, USA

Abstract

A detailed understanding of the influence of temperature on soil microbial activity is critical to predict future atmospheric CO_2 concentrations and feedbacks to anthropogenic warming. We investigated soils exposed to 3–4 years of continuous 5 °C-warming in a field experiment in a temperate forest. We found that an index for the temperature adaptation of the microbial community, T_{min} for bacterial growth, increased by 0.19 °C per 1 °C rise in temperature, showing a community shift towards one adapted to higher temperature with a higher temperature sensitivity ($Q_{10(5–15\ °C)}$ increased by 0.08 units per 1 °C). Using continuously measured temperature data from the field experiment we modelled *in situ* bacterial growth. Assuming that warming did not affect resource availability, bacterial growth was modelled to become 60% higher in warmed compared to the control plots, with the effect of temperature adaptation of the community only having a small effect on overall bacterial growth (<5%). However, 3 years of warming decreased bacterial growth, most likely due to substrate depletion because of the initially higher growth in warmed plots. When this was factored in, the result was similar rates of modelled *in situ* bacterial growth in warmed and control plots after 3 years, despite the temperature difference. We conclude that although temperature adaptation for bacterial growth to higher temperatures was detectable, its influence on annual bacterial growth was minor, and overshadowed by the direct temperature effect on growth rates.

Keywords: bacterial growth, leucine incorporation, minimum temperature, Q_{10}, soil warming, temperature adaptation

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Introduction

About 60 Pg C per annum, or about 40% of the natural carbon dioxide (CO_2) emissions to the atmosphere, are the product of microbial decomposition of soil organic matter (SOM) (Schimel, 1995). Consequently, even small changes in soil microbial activity can have important impacts on the global C cycle. One of the most important factors controlling the activity of the soil microbial community is temperature (Waksman & Gerretsen, 1931; Lloyd & Taylor, 1994), and thus a detailed understanding of the temperature sensitivity of soil microorganisms is a critical prerequisite to predict future atmospheric CO_2 concentration and feedbacks to anthropogenic warming (Ågren, 2010; Conant *et al*., 2011).

In addition to the direct effect of temperature on microbial activity and growth and its indirect effect on, for example, the quality of SOM (Davidson & Janssens, 2006; Hartley *et al*., 2007; Conant *et al*., 2008a,b; Craine *et al*., 2010), the environmental temperature regime may also select for a microbial community with different temperature sensitivities. There is currently a debate about how influential such a microbial community temperature adaptation would be for SOM mineralization (Kirschbaum, 2004; Bradford *et al*., 2008, 2010; Hartley *et al*., 2009, 2010; Allison *et al*., 2010). Since mineralization and the growth of the microbial decomposer community are linked (Rousk & Bååth, 2011), the temperature adaptation of microbial growth may be used to infer changes in SOM mineralization. Previous studies have shown that the bacterial growth relationship with temperature closely tracks *in situ* seasonal temperature dynamics in marine systems (Li & Dickie, 1987), and that soils experimentally exposed to temperatures ranging between 0 and 50 °C quickly developed (within a month) microbial communities with growth-temperature relationships matching the incubation temperature (Bárcenas-Moreno *et al*., 2009). Antarctic soils collected along a natural temperature gradient were similarly shown to harbour bacterial communities with growth-temperature relationships that tracked the average yearly *in situ* temperature, and it was predicted that the anticipated warming of 2.6 °C of the Antarctic Peninsula (Christensen *et al*., 2007) would increase the

Correspondence: Johannes Rousk, tel. + 46 70 750 7522, fax + 46 46 222 3800, e-mail: johannes.rousk@biol.lu.se

temperature sensitivity ($Q_{10\ (0–10\ °C)}$) of bacterial growth by about 0.5 units (Rinnan *et al*., 2009).

In the present work, we investigated how experimental warming of a temperate forest soil affected bacterial growth and its temperature relationship, that is, the temperature adaptation of the bacterial community. To do this, we assessed soil samples collected from the Soil Warming × Nitrogen Addition Study at the Harvard Forest Long-Term Ecological Research (LTER) site. We investigated how 3–4 years of continuous warming (~5 °C above ambient) affected the actively growing bacterial community. Specifically, we determined if soil warming affects the bacterial growth-temperature relationship. We predicted that 5 °C-warming would shift the temperature sensitivity of growth of the microorganisms to one adapted to higher temperatures as indicated by increasing the T_{min} (the apparent minimum temperature for growth; see Materials and methods section) by about 1.2 °C (or 0.24 °C per 1 °C; Rinnan *et al*., 2009). Furthermore, we predicted that this shift in temperature adaptation would result in increased temperature sensitivity (higher Q_{10}) for bacterial growth. Lastly, we modelled the variation of *in situ* growth rates of bacteria, by combining the determined temperature relationships for bacterial growth with continuous measurements of *in situ* soil temperatures, to distinguish direct effects of the temperature increase on bacterial growth from those caused by a temperature adaptation of the community.

Materials and methods

Soils, field experiment and sampling

Soils were collected from the Soil Warming × Nitrogen Addition Study located on the Prospect Hill Tract at the Harvard Forest Long-Term Ecological Research (LTER) site, Massachusetts, USA, which has been described in detail elsewhere (Contosta *et al*., 2011). Briefly, the forest at the site is even aged, mixed hardwoods, and the soils are of the Gloucester series (fine loamy, mixed, mesic, Typic Dystrochrepts, USDA). Mean annual air temperature is 7 °C and average total annual precipitation is 1100 mm. Using a completely randomized design, 24 3 × 3 m plots were assigned one of four experimental treatments with six replicates per treatment: control (C), warming (W), nitrogen addition (N), and warming × nitrogen (WN). Warming of the soils was applied using buried heating cables located at approximately 10 cm depth and spaced every 20 cm (installed 10 months before initiation of the warming treatment). The warmed soils are continuously warmed to 5 °C above ambient, and the N additions are applied in equal doses during the May – October growing season as an aqueous solution of NH_4NO_3 at a rate of 50 kg N ha^{-1} yr^{-1}. The experiment was activated in early August 2006, and soil samples for this study were collected on two separate occasions, in late September 2009 and mid April 2010, that is, after 3.1 and 3.8 years of warming respectively.

The average soil temperature, measured at ~5 cm depth (i.e. at the interface between the organic and mineral soil horizons) during the experimental period increased by 4.6 °C, from 10.3 °C (with a range of −2.8 °C to 25.3 °C) in the control soils to 14.9 °C (with a range of 1.4–29.1 °C) in the warmed. This resulted in only a minor reduction in soil moisture (e.g., 9% lower soil moisture in warmed compared with control soils in the O-horizon; Contosta *et al*., 2011). Bacterial growth and temperature sensitivity (using two temperatures; see below) were analysed in 2009, and a more detailed bacterial temperature sensitivity analysis was conducted in 2010 (using 4 replicates and 9 different temperatures from 0.5 to 36 °C). Two 8 cm wide and 10 cm deep cores were collected from each replicate at each sampling time and separated into organic (excluding the litter layer; i.e. the humus layer was sampled) and mineral horizons. The cores were sieved (<2 mm) and stored and transported on ice until analyses were performed 4–5 days after sampling. We also analysed the 2009 soil samples for organic C and total N by dry combustion using a CHN elemental analyser. Soil pH was measured using 0.1 M KCl (1 : 5, w:v, glass electrode) (Table 1).

Microbial analyses

Bacterial growth was estimated using leucine (Leu; Kirchman *et al*., 1985) incorporation in bacteria extracted from soil using the homogenization/centrifugation technique (Bååth, 1994) with modifications (Bååth *et al*., 2001; Rousk & Bååth, 2011). This method estimates the rate of bacterial biomass production (growth) through tracking incorporation of trace concentrations of isotopically labelled leucine into bacterial macromolecules. This is a standard and well-established method to measure bacterial growth in aquatic habitats (Kirchman, 2001). Briefly, 2 μl of radiolabelled Leu ([^{3}H]Leu, 37 MBq ml^{-1}, 5.74 TBq $mmol^{-1}$, Perkin Elmer, UK) combined with non labelled Leu was added to each tube, resulting in 275 nM Leu in the bacterial suspensions. The amount of Leu incorporated into extracted bacteria per h and g soil was used as a measure of bacterial growth.

Microbial temperature relationships

The temperature dependency of the soil bacterial community was measured using leucine incorporation, as described above, at different temperatures during the incubation step with radioactive leucine (Bárcenas Moreno *et al*., 2009; Rinnan *et al*., 2009). Importantly, the exposure to different temperatures during the incubation step was always kept short, to a time corresponding to approximately 2 h at 22 °C, adapting the duration of the incubation period for the other temperatures (i.e. 1 h at 32 °C, 4 h at 12 °C, etc.). Within these time periods no change in growth rates due to altered growth conditions occurs (Rousk & Bååth, 2011), with the exception of the direct temperature effect on rates. Thus, the microbial communities did not have sufficient time to adapt to any temperature change during the measurements.

Table 1 Soil properties measured in mineral (M) and organic (O) horizons of the soils from the 2009 sampling

	Control		Warmed		Nitrogen (N)		Warmed + N	
	O-horizon	M-horizon	O-horizon	M-horizon	O-horizon	M-horizon	O-horizon	M-horizon
Organic carbon (mg C g^{-1} soil)	270 (40)	80 (11)	310 (37)	73 (7)	250 (32)	76 (9)	300 (36)	75 (7)
Total nitrogen (mg N g^{-1} soil)	11 (1.7)	4 (0.5)	13 (1.5)	4 (0.3)	10 (1.2)	4 (0.4)	12 (1.5)	4 (0.3)
Soil pH (0.1 M KCl)	3.4 (0.4)	4.1 (0.2)	3.3 (0.1)	3.9 (0.3)	3.5 (0.2)	4.2 (0.1)	3.4 (0.2)	4.1 (0.2)

Values are mean values (n = 6) with SE within brackets.

The observed data were modelled using the 'square-root relationship' used for growth of bacteria in pure culture at temperatures below the optimal growth temperature (Ratkowsky *et al*., 1982, 1983; Ross *et al*., 2010). This relationship was found to accurately model temperature relationships of bacterial growth in both water (Li & Dickie, 1987) and soil (Díaz-Raviña *et al*., 1994; Rinnan *et al*., 2009). A version of the square root model valid below optimum temperature for growth is as follows

$$Leu^{1/2} = a(T - T_{min})$$

where *Leu* is the leucine incorporation (relative bacterial growth normalized to 16 °C) measured at the temperature T (°C), T_{min} is the apparent minimum temperature for leucine incorporation, and a is a slope parameter related to total leucine incorporation. This model, using data from below the temperature optimum, was used to calculate T_{min} in the data set from 2010. For the 2009 data set, we assumed a linear relationship between $Leu^{½}$ and T and used the two incubation temperatures (16 and 4 °C) to estimate T_{min} and subsequently $Q_{10\ (5–15\ °C)}$ (Rinnan *et al*., 2009). It has previously been shown that the parameters T_{min} and the optimal temperature for growth (T_{opt}) both can be used as indices for temperature adaptations (Li & Dickie, 1987; Rinnan *et al*., 2009), where higher values indicate a community adapted to higher temperatures, and vice versa.

Modelling in situ bacterial growth

To model *in situ* bacterial growth during the course of the field experiment, we used soil temperatures continuously measured at the field site in the unheated control and in the warmed soils (at hourly resolution; Contosta *et al*., 2011) and the observed relationship for bacterial growth and temperatures. First, we combined the temperature relationship from the unheated control soils with the *in situ* temperatures of the same treatment (using data from Fig. 1 to initiate the modelling) to estimate the bacterial growth over the year (Fig. 4, data in blue). As an initial comparison, we calculated bacterial growth in the warmed soils using the same temperature relationship as that for the unheated control, that is, assuming no temperature adaptation or other indirect effects of temperature on for example quality of SOM or altered moisture conditions (Fig. 4, data in red). We also calculated bacterial growth assuming a temperature-adapted community (Figs. 2 and 3). This did not differ much from the calculation without temperature adaptation (Fig. 4, data in red), and thus is not shown. However, after 3 years there were both an adaptation and a decreased bacterial growth in the warmed soils. We could substantiate that the changes of bacterial temperature relationships and growth conditions had occurred by the first sampling, September 2009, and that they remained virtually the same until the second sampling, April 2010 (see Results section). To be conservative, therefore, we estimated the *in situ* growth rates of the warmed bacterial communities including both the effects of adaptation and decreased bacterial growth only for this period (Fig. 4, data in green).

Statistics

Two-way analyses of variance (ANOVA), using warming and nitrogen addition as fixed factors and blocked on horizon (O and M; treated as a fixed factor without interaction terms), were used to compare treatment effects. The data were logarithmically transformed prior to statistical analysis since variance scaled with the mean.

Results

Bacterial growth rates

Bacterial growth was reduced by warming, both in the organic and mineral horizon ($P < 0.0001$; Fig. 1a and b), while being unaffected by N addition ($P = 0.13$) without interaction between the treatment factors ($P = 0.53$). Warming decreased bacterial growth (compared at a standardized temperature of 16 °C) by 35% in the organic and by 45% in the mineral soil horizon.

Temperature dependence of bacterial growth

A square-root function (Ratkowsky *et al*., 1982, 1983; Ross *et al*., 2010) was used to describe the temperature dependence of bacterial growth (Fig. 2; R^2=0.997 and 0.996 for the control and warmed soils respectively) for temperatures below 30 °C, the approximate optimum temperature for growth. Although differences were

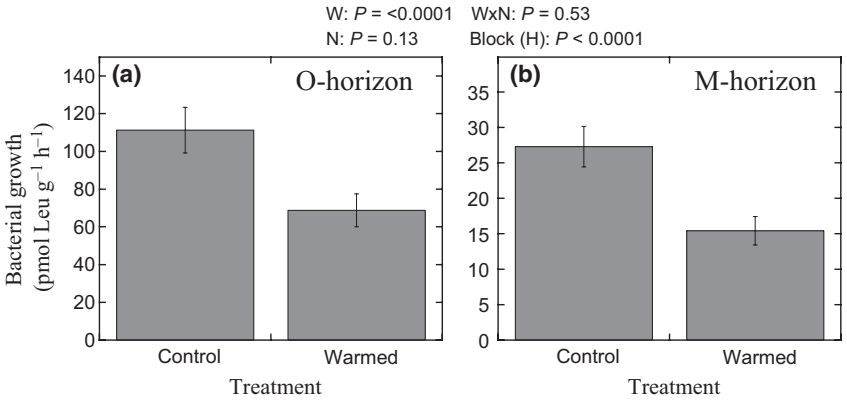

Fig. 1 The effect of ~ 5 °C-warming for 3 years on bacterial growth estimated using leucine incorporation at 16 °C in the organic (panel a) and mineral (panel b) horizons. Results from a 2-way ANOVA factoring in soil warming (W) and nitrogen addition (N) and blocked (fixed factor without interaction terms) on soil horizon (H) is reported above the panels. Bars represent the mean ± 1 standard error (n = 12).

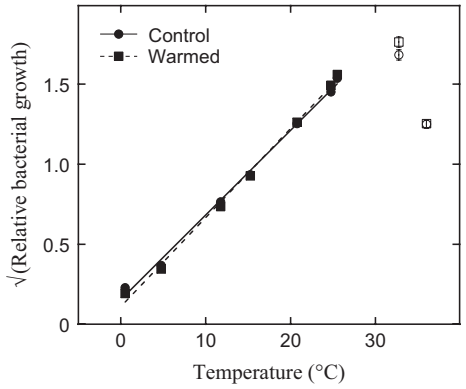

Fig. 2 The temperature relationship for bacterial growth. A 'square-root' function is used to describe the temperature dependence of relative bacterial growth for temperatures below 30 °C, the optimum temperature for growth (closed symbols). The y-axis is square root transformed, and the values on the x-axis indicate the incubation temperature of the short-term leucine incorporation assay. Values standardized to unity at 16 °C. Values represent the mean ± 1 standard error (n = 4).

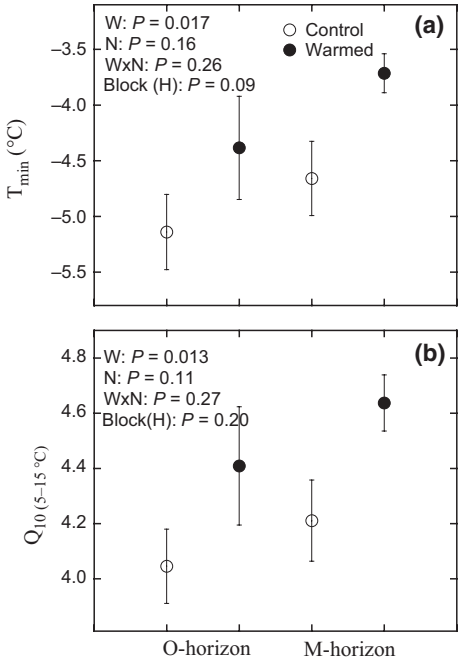

Fig. 3 The effect of ~5 °C-warming on the temperature sensitivity of bacterial growth as indicated by two indices (see Materials and methods section): (a) the minimum temperature for growth (T_{min}) and (b) $Q_{10\ (5–15\ °C)}$ (the ratio between the rate at the 15 °C and 5 °C incubation temperature). Results from a two-way ANOVA factoring in soil warming (W) and nitrogen addition (N) and blocked (fixed factor without interaction term) on soil horizon (H), is reported in the top left of each panel. Bars represent the mean ± 1 standard error (n = 12).

relatively subtle, soil warming increased T_{min} significantly by 0.92 °C (P = 0.002).

A similar shift was found in the earlier (2009) sampling when both the O- and M-horizon was studied, but with only two temperatures to calculate T_{min} (Fig. 3). Warming increased T_{min} by around 0.9 °C (P = 0.017; Fig. 3a), whereas no influence by N addition (P = 0.16), interaction between warming and N addition (P = 0.26), or soil horizons (P = 0.09) could be detected. This shift in the bacterial community's temperature relationship resulted in shifts in the temperature sensitivity of growth, $Q_{10\ (5–15\ °C)}$, which increased in warmed soils (P = 0.013; Fig. 3b) and was unaffected by N or soil horizon and without interaction between warming and N (all P > 0.10). The increase in these indices for the bacterial temperature relationship was thus robust between horizons, and also between the two sampling dates (i.e. the shift in T_{min} and Q_{10} between control and warmed soils did not change between September 2009 and April 2010).

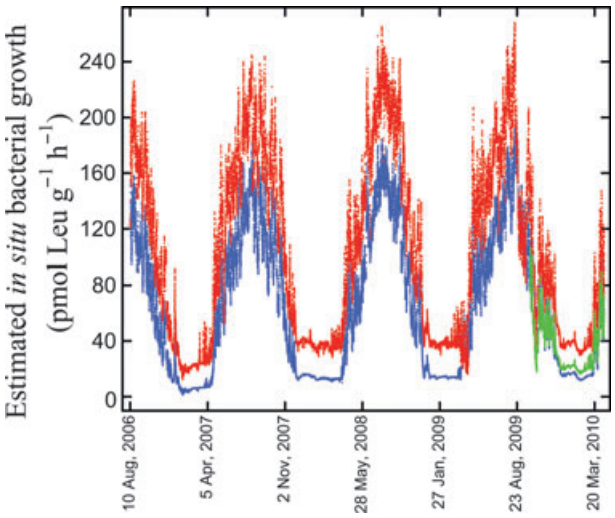

Fig. 4 Estimated *in situ* bacterial growth in the O-horizon over the 3 years of the warming study using temperature relationships for bacterial growth (Figs 2 and 3) combined with *in situ* continuous measurements of soil temperature (Contosta *et al.*, 2011). Data in blue indicate bacterial growth for the control soils using the temperature relationship obtained for unheated control soils; data in red indicate bacterial growth in warmed soil assuming no change in bacterial temperature adaptation or in the quality of available resources; data in green indicate bacterial growth using the determined temperature relationship for growth of the warm-adapted bacterial communities under the growth conditions in the warmed soils. Since we could only substantiate that the shift to warm-adapted bacteria had occurred by August 2009, and had not changed to April 2010, we constrain our estimates to this interval (see Materials and methods section).

Modelling in situ bacterial growth

We used continuously measured field soil temperature data (Contosta *et al.*, 2011) in conjunction with the determined temperature-growth relationships to model *in situ* bacterial growth rates over the years of the warming study (Fig. 4). Soil temperature was measured at the interface between O- and M-horizons, and therefore the same dependent data (*in situ* temperature) were used to model the *in situ* bacterial growth in both. Consequently, the temporal pattern for bacterial growth was the same in O- and M-horizons and thus only the O-horizon is shown (i.e. the M-horizon looked identical, except for different *y*-axis scaling). For the interval between the initiation of the experiment, August 2006, and until our final sampling for the present analysis, April 2010, we estimated *in situ* bacterial growth for the control soils (Fig. 4, data in blue) using the relative temperature relationship obtained for control soils (Figs. 2, 3) and the growth rates estimated at a temperature of 16 °C (Fig. 1). We then used soil temperature data collected from the warmed plots to estimate the direct influence of temperature on growth rates, i.e. how warming would have influenced the growth of the bacterial community over the same period assuming no change in the bacterial temperature adaptation or changes in growth conditions, such as the availability of resources (Fig. 4, data in red). With these assumptions the average *in situ* bacterial growth rate was predicted to increase from 69.6 and 17.1 pmol Leu g^{-1} h^{-1} in O- and M-horizons, respectively, to 109.6 and 27.2 pmol Leu g^{-1} h^{-1}, or by about 60%. We also compared the predicted bacterial growth in the warmed soils using the temperature adaptation for the warmed bacterial community (from Fig. 2), but this only affected the calculated average growth rates to a minor degree (<5%).

The warming treatments also resulted in indirect effects that reduced bacterial growth (Fig. 1). We are unable to determine precisely when during the experiment this decrease as well as the temperature adaptation of the bacterial community (Fig. 2) occurred. However, we could conclude that these temperature-induced changes in the bacterial temperature relationship and temperature-modulated growth conditions of the bacterial community was present by the first sampling date (Sept 2009, after about 3 years of warming) and was not further changed by the second sampling (April 2010, after almost 4 years of warming). Since it is possible that longer exposure would alter the growth conditions for bacteria, we only estimated bacterial growth during the period Sept 2009 and April 2010 (Fig. 4, data in green). This data would thus factor in both the temperature adaptation of the bacterial community and changes in conditions for bacterial growth including e.g., substrate depletion effects. Bacterial growth rates estimated under these conditions yielded mean estimates of 66.6 and 15.1 pmol Leu g^{-1} h^{-1} in O and M-horizons, respectively, i.e. similar rates as in the unheated soil despite the ~5 °C increase in temperature.

Discussion

Our measurements suggest that 3 years of experimental ~5 °C-warming resulted in a significant temperature adaptation of the bacterial community, increasing T_{min} by 0.87 ± 0.06 °C (mean ± se of measurements in both horizons for the 2009 and 2010 samplings), i.e. an increase in T_{min} for bacterial growth of 0.19 °C per 1 °C rise in temperature. This shift in the temperature relationship of bacterial growth closely corresponds to that previously documented for soils collected along a natural and thus long-term temperature gradient in Antarctica of about 0.24 °C per 1 °C rise in mean annual

temperature (Rinnan *et al*., 2009). This shift translates into an increased temperature sensitivity of bacterial growth, with $Q_{10\ (5–15\ °C)}$ increasing by 0.40 ± 0.03 units. That is, $Q_{10\ (5–15\ °C)}$ increased by 0.08 units per 1 °C increase in temperature. Again, this value is similar to a previous assessment of a long-term temperature gradient in Antarctic soils where $Q_{10\ (0–10\ °C)}$ was reported to increase by 0.18 units and $Q_{10\ (10–20\ °C)}$ by 0.03 units per 1 °C temperature increase (Rinnan *et al*., 2009). Thus, we can extend the previous observation of differences across a natural inter-ecosystem latitudinal temperature gradient (Rinnan *et al*., 2009) with that of a field experiment where soil temperatures are experimentally manipulated.

Experimentally warming forest soil for 3–4 years resulted in reduced bacterial growth (at a standardized temperature, Fig. 1). This finding is consistent with measurements of both respiration rates (at standardized temperatures) and microbial biomass measurements (Melillo *et al*., 2002; Eliasson *et al*., 2005; Hartley *et al*., 2007; Rinnan *et al*., 2007; Bradford *et al*., 2008; Frey *et al*., 2008), as well as bacterial growth measurements (Rinnan *et al*., 2011). Various explanations have been forwarded for the reductions in biomass and activity of the microbial community. For instance, it has been proposed to reflect a reduction in the quality of available resources for the decomposer microbial community due to the higher microbial activity in warmed soils resulting in loss of labile substrate (Kirschbaum, 2004; Eliasson *et al*., 2005; Hartley *et al*., 2007; Conant *et al*., 2011) or to be related to shifts in microbial physiology that modulated the growth efficiency of the soil microbial community (Bradford *et al*., 2008, 2010; Allison *et al*., 2010). Irrespective of the precise mechanism, that microbial process rates and biomass concentrations often decline as a consequence of prolonged experimentally elevated temperatures in natural ecosystems is well-established (Conant *et al*., 2011), and our results on this are of a confirmatory nature.

There has been a long-standing debate regarding how rapid and pronounced a temperature adaptation of the microbial community in soil is (Luo *et al*., 2001; Kirschbaum, 2004; Eliasson *et al*., 2005; Hartley *et al*., 2007, 2008, 2009; Bradford *et al*., 2008, 2010; Balser & Wixon, 2009; Allison *et al*., 2010). We demonstrate that an adaptation of the bacterial community is evident after 3–4 years experimental warming (see above). Since the bacterial temperature sensitivity in the soils warmed for only 3 years closely matched that predicted from a natural inter-ecosystem latitudinal (i.e. long term) temperature gradient (Rinnan *et al*., 2009), it seems that further change of the bacterial growth temperature relationship after a longer heat exposure is unlikely. More important is how we can use the obtained data to relate different types of temperature-related effects on the microbial community: the altered temperature sensitivity of the bacterial community was small (<5% effect) compared with the large direct effect of temperature on bacterial growth (Fig. 4, increase from data in blue to data in red), and the subsequent reduction in growth due to indirect effects of temperature that modulated the growth conditions of the bacterial community (reduction from data in red to data in green). We thus predict that warming effects mediated through altered temperature sensitivity (i.e. a warm-adapted microbial community) will have only minor consequences for microbial process rates, as indicated by bacterial growth, compared to e.g., temperature-related reduction of soil C quality (Conant *et al*., 2011), even from a long-term perspective.

Although the obtained results have bearing on microbial process rates overall, more information is needed to explicitly translate microbial growth rates to ecosystem C exchange. Most pressingly, microbial growth efficiency, the partitioning of SOC into microbial growth and respiration, could be influential for the soil C response to warming (Ågren, 2010; Allison *et al*., 2010). The temperature sensitivity of this parameter has yet to be systematically assessed, and early observations report both suggestions for (Steinweg *et al*., 2008; S.D. Frey, A.R. Contosta & A.B. Cooper, unpublished) and against (López-Urrutia & Morán, 2007; Dijkstra *et al*., 2011) a temperature dependency for growth efficiencies within physiologically meaningful temperature intervals.

In conclusion, we confirm that climate warming will influence actively growing bacterial communities in soil directly, increasing growth rates, and indirectly, by modulating the microbial growth conditions by for e.g., substrate depletion effects. Moreover, we show that the temperature sensitivity of bacterial growth will increase due to adaptation to a warmer temperature regime. However, these changes in the temperature sensitivity (adaptation) of the decomposer microbial community will have relatively subtle effects on microbial processes, as indicated by bacterial growth rates; effects that will be overshadowed by the direct temperature effects and subsequent effects on substrate availability. To include temperature adaptation therefore appears not to be a priority in efforts to model bacterial processes.

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References

Ågren GI (2010) Climate change: microbial mitigation. *Nature Geoscience*, **3**, 303–301.

Allison SD, Wallenstein MD, Bradford MA (2010) Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience*, **3**, 336–240.

Bååth E (1994) Measurement of protein-synthesis by soil bacterial assemblages with the leucine incorporation technique. *Biology and Fertility of Soils*, **17**, 147–153.

Bååth E, Pettersson M, Söderberg KH (2001) Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. *Soil Biology & Biochemistry*, **33**, 1571–1574.

Balser TC, Wixon DL (2009) Investigating biological control over soil carbon temperature sensitivity. *Global Change Biology*, **15**, 2935–2949.

Bárcenas-Moreno G, Gómez-Brandón M, Rousk J, Bååth E (2009) Adaption of soil microbial communities to temperature: comparison of fungi and bacteria in a laboratory experiment. *Global Change Biology*, **15**, 2950–2957.

Bradford MA, Davies CA, Frey SD *et al.* (2008) Thermal adaptation of soil microbial respiration to elevated temperature. *Ecology Letters*, **11**, 1316–1327.

Bradford MA, Watts BW, Davies CA (2010) Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. *Global Change Biology*, **16**, 1576–1588.

Christensen JH, Hewitson B, Busuioc A *et al.* (2007) Regional climate projections. In: *Climate Change 207: The physical science basis. Contributions of working group I to the fourth assessment report of the intergovernmental panel on climate change* (eds Solomon S, *et al.*), pp. 847–940. Cambridge University Press, Cambridge, UK.

Conant RT, Drijber RA, Haddix ML, Paul EA, Plante AF, Six J (2008a) Sensitivity of organic matter decomposition to warming varies with its quality. *Global Change Biology*, **14**, 868–877.

Conant RT, Steinweg JM, Haddix ML *et al.* (2008b) Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. *Ecology*, **89**, 2384–2391.

Conant RT, Ryan MG, Ågren GI *et al.* (2011) Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. *Global Change Biology*, **17**, 3392–3404.

Contosta AR, Frey SD, Cooper AB (2011) Seasonal dynamics of soil respiration and N mineralization in chronically warmed and fertilized soils. *Ecosphere*, **2**, art36, doi:10.1890/ES10-00133.1.

Craine JM, Spurr R, McLauchlan KK, Fierer N (2010) Landscape-level variation in temperature sensitivity of soil organic carbon decomposition. *Soil Biology & Biochemistry*, **42**, 373–375.

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

Díaz-Raviña M, Frostegård Å, Bååth E (1994) Thymidine, leucine and acetate incorporation into soil bacterial assemblages at different temperatures. *FEMS Microbiology Ecology*, **14**, 221–231.

Dijkstra P, Thomas SC, Heinrich PL, Koch GW, Schwartz E, Hungate BA (2011) Effect of temperature on metabolic activity of intact communities: evidence for altered metabolic pathway activity but not for increased maintenance respiration and reduced carbon use efficiency. *Soil Biology & Biochemistry*, **43**, 2023–2031.

Eliasson PE, McMurtrie RE, Pepper DA, Strömgren M, Linder S, Ågren GI (2005) The response of heterotrophic CO_2 flux to soil warming. *Global Change Biology*, **11**, 167–181.

Frey SD, Drijber R, Smith H, Melillo J (2008) Microbial biomass, functional capacity, and community structure after 12 years of soil warming. *Soil Biology & Biochemistry*, **40**, 2904–2907.

Hartley IP, Heinemeyer A, Ineson P (2007) Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. *Global Change Biology*, **13**, 1761–1770.

Hartley IP, Hopkins DW, Garnett MH, Sommerkorn M, Wookey PA (2008) Soil microbial respiration in arctic soil does not acclimate to temperature. *Ecology Letters*, **11**, 1092–1100.

Hartley IP, Hopkins DW, Garnett MG, Sommerkorn M, Wookey PA (2009) No evidence for compensatory thermal adaptation of soil microbial respiration in the study of Bradford *et al.* (2008). *Ecology Letters*, **12**, E12–E14.

Hartley IP, Hopkins DW, Sommerkorn M, Wookey PA (2010) The response of organic matter mineralisation to nutrient and substrate additions in sub-arctic soils. *Soil Biology & Biochemistry*, **42**, 92–100.

Kirchman D (2001) Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. *Methods in Microbiology*, **30**, 227–237.

Kirchman D, K'nees E, Hodson H (1985) Leucine incorporation and its potential as a measure of protein-synthesis by bacteria in natural aquatic systems. *Applied and Environmental Microbiology*, **49**, 599–607.

Kirschbaum MUF (2004) Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? *Global Change Biology*, **10**, 1870–1877.

Li WKW, Dickie PM (1987) Temperature characteristics of photosynthetic and heterotrophic activities: seasonal variations in temperate microbial plankton. *Applied and Environmental Microbiology*, **53**, 2282–2295.

Lloyd J, Taylor JA (1994) On the temperature dependence of soil respiration. *Functional Ecology*, **8**, 315–323.

López-Urrutia A, Morán XAG (2007) Resource limitation of bacterial production distorts the temperature dependence of oceanic carbon cycling. *Ecology*, **88**, 817–822.

Luo Y, Wan S, Hui D, Wallace LL (2001) Acclimatization of soil respiration to warming in a tall grass prairie. *Nature*, **413**, 622–625.

Melillo JM, Steudler PA, Aber JD *et al.* (2002) Soil warming and carbon-cycle feedbacks to the climate system. *Science*, **298**, 2173–2176.

Ratkowsky DA, Olley J, McMeekin TA, Ball A (1982) Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology*, **149**, 1–5.

Ratkowsky DA, Lowry RK, McMeekin TA, Stokes AN, Chandler RE (1983) Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *Journal of Bacteriology*, **154**, 1222–1226.

Rinnan R, Michelsen A, Bååth E, Jonasson S (2007) Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. *Global Change Biology*, **13**, 28–39.

Rinnan R, Rousk J, Yergeau E, Kowalchuk GA, Bååth E (2009) Temperature adaptation of soil bacterial communities along an Antarctic climate gradient: predicting responses to climate warming. *Global Change Biology*, **15**, 2616–2625.

Rinnan R, Michelsen A, Bååth E (2011) Long-term warming of a subarctic heath decreases soil bacterial community growth but has no effects on its temperature adaptation. *Applied Soil Ecology*, **47**, 217–220.

Ross T, Olley J, McMeekin TA, Ratkowsky DA (2010) Some comments on Huang, L. (2010) Growth kinetics of Escherichia coli 0157: H7 in mechanically tenderized beef. *International Journal of Food Microbiology*, **147**, 78–80.

Rousk J, Bååth E (2011) Growth of saprotrophic fungi and bacterial in soil. *FEMS Microbiology Ecology*, **78**, 17–30.

Schimel DS (1995) Terrestrial ecosystems and the carbon cycle. *Global Change Biology*, **1**, 77–91.

Steinweg JM, Plante AF, Conant RT, Paul EA, Tanaka DL (2008) Patterns of substrate utilization during long-term incubations at different temperatures. *Soil Biology & Biochemistry*, **40**, 2722–2728.

Waksman SA, Gerretsen FC (1931) Influence of temperature and moisture upon the nature and extent of decomposition of plant residues by microorganisms. *Ecology*, **12**, 33–60.