Global Change Biology

Global Change Biology (2009) **15**, 2950–2957, doi: 10.1111/j.1365-2486.2009.01882.x

Adaptation of soil microbial communities to temperature: comparison of fungi and bacteria in a laboratory experiment

GEMA BÁRCENAS-MORENO*, MARIA GÓMEZ-BRANDÓN†, JOHANNES ROUSK‡ and ERLAND BÅÅTH‡

*Departamento de Agroquímica y Medio Ambiente, Universidad Miguel Hernandez, E-03202 Alicante, Spain, †Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, E-36310 Vigo, Spain, ‡Department of Microbial Ecology, Lund University, Ecology Building, SE-223 62 Lund, Sweden

Abstract

Temperature not only has direct effects on microbial activity, but can also affect activity indirectly by changing the temperature dependency of the community. This would result in communities performing better over time in response to increased temperatures. We have for the first time studied the effect of soil temperature (5–50 °C) on the community adaptation of both bacterial (leucine incorporation) and fungal growth (acetate-in-ergosterol incorporation). Growth at different temperatures was estimated after about a month using a short-term assay to avoid confounding the effects of temperature on substrate availability. Before the experiment started, fungal and bacterial growth was optimal around 30 °C. Increasing soil temperature above this resulted in an increase in the optimum for bacterial growth, correlated to soil temperature, with parallel shifts in the total response curve. Below the optimum, soil temperature had only minor effects, although lower temperatures selected for communities growing better at the lowest temperature. Fungi were affected in the same way as bacteria, with large shifts in temperature tolerance at soil temperatures above that of optimum for growth. A simplified technique, only comparing growth at two contrasting temperatures, gave similar results as using a complete temperature curve, allowing for large scale measurements also in field situations with small differences in temperature.

Keywords: bacterial growth, community adaptation, fungal growth, soil, temperature, temperature response

Received 19 November 2008 and accepted 6 January 2009

Introduction

Thermal acclimation is a common phenomenon in plant ecophysiology, where a reduction in respiration, together with an improvement in the efficiency of carbon use, is seen after exposure to a higher temperature over a prolonged period of time (Atkin & Tjoelker, 2003). Similar results, seen in soil, where the magnitude of the initial respiration response declined over time when a soil was exposed to increased temperature, were initially also interpreted as thermal acclimation of the soil microorganisms (e.g. Luo *et al*., 2001). However, the situation has been shown to be confounded by a more rapid decrease in easily available substrate at higher temperatures than at lower ones. When this was taken into account, the results was more likely caused by substrate depletion rather than thermal acclimation (Ågren & Bosatta, 2002; Kirschbaum, 2004; Eliasson *et al*., 2005; Hartley *et al*., 2007). Acclimation of microorganisms to temperature is also unlikely, bearing in mind that altering the temperature within the normal physiological temperature range of a bacterium will result in an immediate change in the growth rate to that characteristic of the new temperature (Neidhardt *et al*., 1990).

A lack of evidence of compensatory thermal acclimation of microbial respiration was also reported in a recent experimental study by Hartley *et al*. (2008), where cooling was studied instead of heating. However,

Correspondence: Erland Bååth, tel. + 46 46 222 42 64, e-mail: erland.baath@mbioekol.lu.se

rather than acclimation, exposure to lower temperatures for extended times resulted in a decrease in respiration rate, while a subsequent increase in temperature resulted in an increase in respiration rate greater than the instantaneous temperature response. Hartley *et al*. (2008) interpreted the latter effect as a change in the microbial community, so as to become better adapted to the new temperature conditions, although this was not explicitly determined. This was also suggested as one explanation of the lag phase observed when wheat straw was added to soil and decomposition was monitored at temperatures between 5 and 45 °C (Bauer *et al*., 2008). However, there is little knowledge on the extent to which the soil microbial community adapts to changes in temperature, although temperature adaptation of bacterial growth and respiration in aquatic habitats due to seasonal changes in water temperature has been reported (Li & Dickie, 1987; Thamdrup *et al*., 1998). Seasonal variations in carbon-cycling processes in soil have also been found (Fenner *et al*., 2005; Monson *et al*., 2006). However, seasonal temperature changes do not always appear to result in a change in the temperature response of the microbial community (Sand-Jensen *et al*., 2007).

The response of the soil microbial community will change if the temperature is changed. This can easily be shown by determining the instantaneous bacterial growth rate before and after a change in soil temperature, using, for example, the thymidine or leucine incorporation technique to indicate soil bacterial growth (Díaz-Raviña *et al*., 1994; Pietikäinen *et al*., 2005). We have previously reported that extreme changes in temperature in peat soil (up to 55 °C to imitate self-heating) shifted the optimum of the bacterial community from around 25 to 55 °C within 3 days (Ranneklev & Bååth, 2001), while a shift from 5 to 30 °C in an agricultural soil only induced a minor, but significant, shift in temperature response of the bacterial community after about a month (Pettersson & Bååth, 2003). However, no systematic studies have yet been performed on the effect of soil temperature on the thermal response of soil microorganisms over a wide temperature range. Neither has the effect of soil temperature on the thermal response of the other important group of soil microorganisms, fungi, been studied, although the acetate-in-ergosterol technique has been used to estimate fungal growth in order to determine the temperature response curve of the soil fungal community in two soils (Pietikäinen *et al*., 2005).

We therefore decided to study how changing the temperature in a soil affected the thermal response of microorganisms over a period of time; a similar time frame to that in the study by Hartley *et al*. (2008). We first determined the temperature response of the soil microorganisms. We then incubated the soil at different temperatures (5–50 °C) for approximately a month, including temperatures below and above the optimum for soil microbial growth, and determined the temperature response of the relative bacterial growth once again. We then compared the effect of soil incubation temperature on the adaptation of bacterial and fungal growth. We found that soil temperatures above the optimum for microbial growth (about 30 °C) profoundly altered the temperature response, shifting the optimum to that of the soil incubation temperature, while lower temperatures had only minor, but significant, effects on temperature relationships.

Materials and methods

Soil and incubation conditions

An arable soil from southern Sweden, with an organic matter content of 14% and a pH (H_2O) of 5.4, was used. The climate is maritime with occasional frost periods. Mean soil temperature would be approximately 10 °C. The soil was sampled in September 2007 when the air temperature was about 15 °C. After sieving (2 mm), 200 g fresh weight of soil (at 50% water holding capacity) was placed in plastic pots with lids. Samples were taken for the determination of the initial temperature dependency for both fungal and bacterial growth before incubation. Duplicate samples were then kept at 5, 15, 25, 30, 35, 40, 45 and 50 °C. The lids were removed to aerate the pots every other day. After 31 days, samples were removed for the measurement of the temperature response of bacterial growth, while fungal growth was measured after 44 days (due to logistic problems).

Temperature response of bacterial and fungal growth

The temperature dependency of microbial growth was essentially measured as described by Díaz-Raviña *et al*. (1994) and Pietikäinen *et al*. (2005). Bacterial growth was estimated using the leucine incorporation technique on bacteria extracted from soil using homogenization–centrifugation (Bååth, 1994; Bååth *et al*., 2001) with some modifications. Two grams of soil was placed in a 50 mL centrifuge tube and 20 mL distilled water at the same temperature as the soil incubation temperature was added. After 3 min at full speed on a multi-vortex shaker and 10 min low-speed centrifugation (1000 *g*), 1.5 mL of the bacterial suspension was distributed between eight 2 mL micro-centrifugation vials. These were then placed in a water bath for 30 min at 3, 15, 25, 30, 35, 40, 45 and 50 °C to achieve the correct temperature before L-[4,5-^{3}H]leucine (171 Ci mmol^{-1}, 1.0 mCi mL^{-1}, Amersham, Buckinghamshire, UK) and nonradioactive

L-leucine were added, resulting in a final concentration of 270 nM leucine. Incubation times were 24 h at 3 °C, 6 h at 15 °C and 2 h for the other temperatures. The bacterial incorporation of leucine was terminated by adding trichloroacetic acid. Washing and measurement of the incorporated ^{3}H-leucine was then performed according to Bååth *et al*. (2001). The amount of leucine incorporated into the extracted bacterial suspension per hour and per gram of soil was used as a measure of bacterial growth.

Fungal growth was estimated with the acetate-in-ergosterol incorporation technique (Newell & Fallon, 1991) adapted for soil (Bååth, 2001). Fungal growth before incubation was estimated over the whole temperature interval (5–50 °C) on duplicate samples, while fungal growth from soils incubated at different temperatures was only measured at two incubation temperatures, 5 and 45 °C. Briefly, 1 g of soil was transferred to test tubes to which 1.5 mL distilled water, preheated to the incubation temperature, and 480 μL 1 mM unlabelled acetate (pH = 6) were added. These were then placed in a water bath for 30 min and 20 μL 1-[^{14}C]acetic acid (sodium salt, 7.4 MBq mL^{-1}, 2.04 GBq mmol^{-1}, Amersham) was added, resulting in a final acetate concentration of 220 μM. The soil slurry was incubated for 8 h (45 °C) or 72 h (5 °C), after which 1 mL 5% formalin was added to terminate growth. Shorter incubation times were used in the initial determination of the fungal temperature response curve (23 h at 5 °C, 11 h at 15 °C and 6 h at other temperatures). Ergosterol was then extracted, separated and quantified using HPLC and a UV detector (282 nm) according to Rousk & Bååth (2007). The ergosterol peak was collected. The amount of incorporated radioactivity was determined using a scintillator, and the amount of acetate incorporated into ergosterol per hour per gram of soil was used as a measure of fungal growth.

Calculations

The temperature response of the relative growth rate of the bacterial community was calculated in three ways. (i) The data were normalized by dividing each value by the bacterial growth rate at optimum temperature to take into account the differences in growth rates induced during the incubation of the soils at different temperatures. (ii) To be able to analyze the data statistically, bacterial growth was first normalized to one temperature (by dividing the data at all temperatures by the growth rate at 30 °C), and then logarithmically transformed to adjust for unequal variance. A two-factor analysis of variance (ANOVA) was then applied, with soil incubation temperature and temperature for bacterial growth as the two fixed factors. A significant interaction between these factors would indicate that the soil incubation temperature had an effect on the bacterial community, resulting in community temperature adaptation. (iii) A simplified estimate was calculated, where only the logarithmically transformed ratio of bacterial growth at two temperatures was used in a one-way ANOVA. This final analysis was applied to the fungal growth data.

Results

The temperature response of the soil bacterial and fungal communities before the experiment started was very similar (Fig. 1a and b, respectively). Both groups of organisms showed optimal growth rates around 30 °C, which decreased rapidly with increasing temperature, with no significant fungal growth at 45 °C and above,

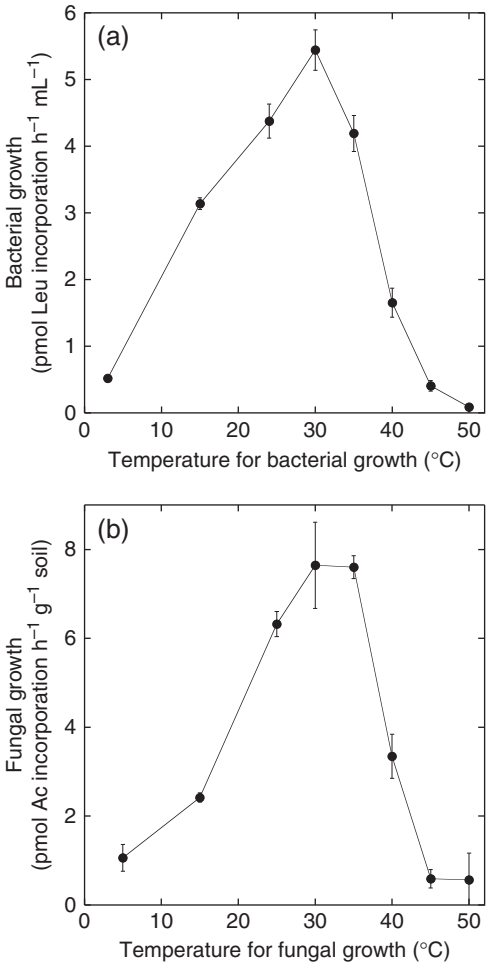

Fig. 1 Initial temperature dependence of the soil microbial community. (a) Bacterial growth at different temperatures, estimated using leucine incorporation. (b) Fungal growth at different temperatures estimated using acetate-in-ergosterol incorporation. Bars indicate SEs.

and no bacterial growth at 50 °C. The decrease was less rapid at lower temperatures; the bacterial growth being around 10 times lower at the lowest temperature studied compared with the optimal growth rate, while for fungi the value was eight times lower. Thus, temperatures of 30 °C and below were at, or below, the optimal temperature for microbial growth, and at 35 °C and above, the temperature was above the optimum temperature for microbial growth in the soil.

Incubating the soil at different temperatures had profound effects on the response of the bacterial community (Fig. 2a), especially at temperatures of 35 °C and above. At these temperatures the optimum shifted to temperatures above 30 °C, being 35 °C at a soil incubation temperature of 35 °C, 40 °C at 40 °C and 45 °C at both 45 and 50 °C. The whole temperature curves were also shifted to higher temperatures in a similar way to the optimum temperature.

Only small changes were seen in the bacterial temperature relationships at soil incubation temperatures of 30 °C and below. To be able to detect such differences, the data were normalized to one growth temperature (30 °C) and logarithmically transformed (Fig. 2b). The considerable effect of soil temperatures of 35 °C and above can easily be seen, but it also became evident that lower temperatures affected the growth. Thus, at 3 °C, the bacteria from soils incubated at 5 °C had the highest relative growth rates, followed by those at 15 °C, with relative growth rates in soils at the other temperatures decreasing with increasing soil incubation temperature, even for bacterial communities from soils incubated at 25 and 30 °C. The relative bacterial growth rate at 40 and 45 °C showed the opposite behavior; the lowest bacterial growth rates were found for the bacterial communities from soil incubated at the lowest temperatures. The different effects of soil incubation temperature on the bacterial growth at low and high temperatures is emphasized by the significant interaction term using all temperatures (soil incubation temperature × temperature for bacterial growth: $F_{49,64} = 88.6$, $P < 0.0001$) or using only soil incubation temperatures of 30 °C and below ($F_{21,32} = 2.17$, $P < 0.05$).

A simplified way of estimating changes in soil bacterial temperature relationships was introduced by Pettersson & Bååth (2003), who used the logarithm of the ratio of the growth rate at two extreme temperatures; a higher ratio indicating a bacterial community more adapted to higher temperatures. Calculating such a ratio using the most extreme temperatures (45 and 3 °C, Fig. 2c) also showed that a soil incubation temperature of 30 °C and below only had a minor effect on the temperature relationship of bacterial growth. However, above this soil incubation temperature the bacterial community changed dramatically ($F_{7,8} = 250$,

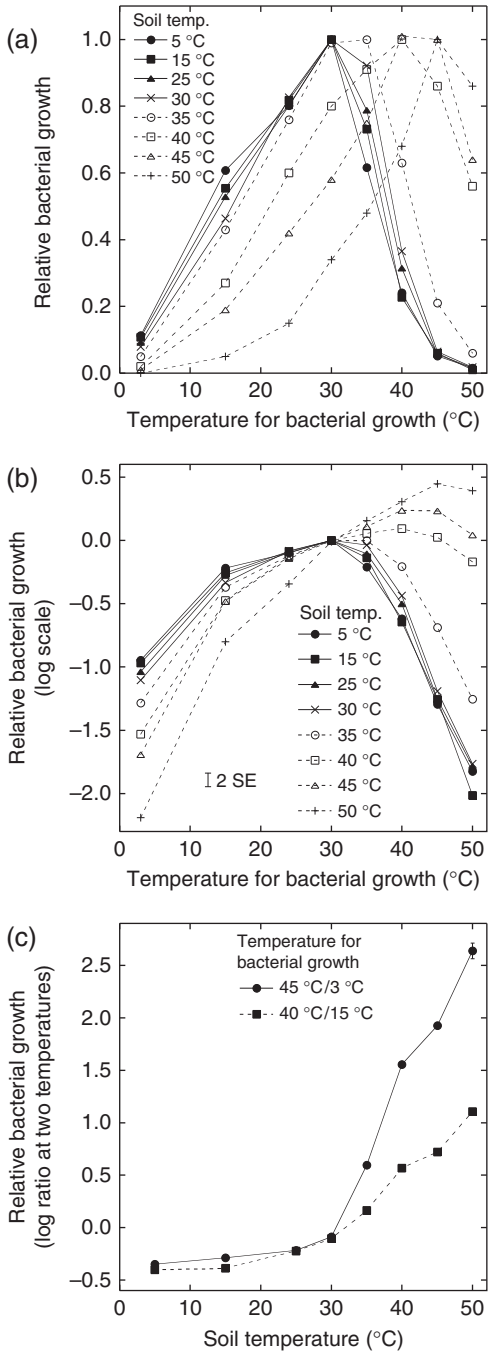

Fig. 2 Temperature dependence of the bacterial community in soils incubated at 5–50 °C for a month, estimated using leucine incorporation. (a) Bacterial growth normalized to that at the optimum temperature in each soil. Each point is the mean of measurements on two separate samples. (b) The log of the ratio of bacterial growth relative to that at 30 °C. The bar indicates 2 SE (from ANOVA). (c) The log of the ratio of bacterial growth at 45 °C/3 °C and 40 °C/15 °C where a higher ratio indicates a community more adapted to higher temperatures. Bars indicating SE from ANOVA are shown for the highest temperature point (smaller than the symbol for the 40 °C/15 °C treatment).

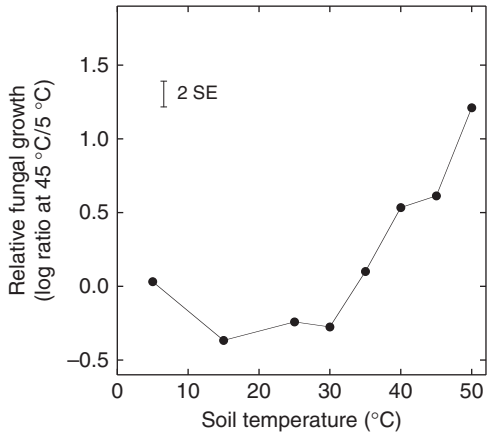

Fig. 3 Temperature dependence of the fungal community in soils incubated at 5–50 °C estimated using acetate-in-ergosterol incorporation. The log of the ratio of fungal growth at 45 °C/5 °C is shown; a higher ratio indicating a community more adapted to higher temperatures. The bar indicates 2 SE (from ANOVA).

$P < 0.0001$). The same pattern was found for bacterial growth using less extreme temperatures (40 and 15 °C) ($F_{7,8} = 1670$, $P < 0.0001$). Because of the smaller variation between replicates using this ratio, there were significant differences between all soil incubation temperatures ($P < 0.05$, Tukey's HSD) except between 5 and 15 °C.

The effect of soil incubation temperature on the temperature dependence of fungal growth was only studied using the last method comparing two extreme temperatures (45 and 5 °C, Fig. 3). Soil incubation temperature was found to have a significant effect ($F_{7,8} = 44.4$, $P < 0.0001$). Similar to the bacterial growth rate, the change was most evident above a soil incubation temperature of 35 °C. There was no significant difference between the temperature dependence of fungal growth in soil incubated at 35 °C and below (Tukey's HSD).

Discussion

The temperature dependences of bacterial and fungal growth before the experiment started (Fig. 1) were similar to those reported previously for temperate soils (Díaz-Raviña *et al*., 1994; Pietikäinen *et al*., 2005), with optimum growth well above normal *in situ* soil temperature. This is also frequently found in aquatic environments (Li & Dickie, 1987; Sand-Jensen *et al*., 2007), and appears to be a common characteristic in environments with fluctuating temperatures. Enteric bacteria isolated from sea turtles, which are ectothermic and thus encounter changes in water temperature, also had optima well above those found in their host, especially during the winter period (Bronikowski *et al*., 2001).

The soil incubation temperature affected the temperature dependence of the bacterial and fungal communities, especially at temperatures above that for optimum growth. This was expected, because these temperatures will kill many of the original organisms, enabling colonization by other organisms adapted to growth at higher temperatures. There was also evidence of community adaptation to the soil temperature regime at temperatures lower than the optimum, however, because communities grew better closer to the temperature regime to which they had been exposed. Although we did not use the same temperature regimes as Hartley *et al*. (2008), it is likely that the increase in activity above the immediate response to increasing the temperature from 2 to 10 °C found by them could be explained by a similar shift in the microbial community, as also suggested by them. Thus, our results are consistent with the findings of Hartley *et al*. (2008) that temperature adaptation of the microbial community may accelerate decomposition rates after a temperature increase.

Three mechanisms can explain the change in community temperature response: (1) acclimation, where growth at a certain temperature gives a phenotypic advantage without any genotypic change, (2) genotypic adaptation within a species (evolution) and (3) species sorting, where species already genetically better adapted to a certain temperature regime will outcompete other less well-adapted species. Although the present study was not designed to differentiate between these three mechanisms, it is likely that the last, species sorting, is the most important one within the time frame studied here. This is certainly the case for the dramatic shift in temperature response in soils maintained at 35 °C and higher, because these temperatures will be lethal to the original community. Furthermore, even after several thousand generations of growth of *Escherichia coli* at extreme temperatures, the boundaries of the thermal niche were only shifted 1–2 °C (Mongold *et al*., 1996), indicating that no dramatic changes in the temperature response of a species due to genotypic adaptation will be found, even after a very long time. Acclimation is also an unlikely explanation, because it can only induce minor shifts in the temperature response of a bacterium (Leroi *et al*., 1994).

It is also likely that species sorting is the main cause of the change in temperature response at lower soil temperatures (below 30 °C), because even small genotypic changes appear to take several hundred generations to emerge (Bennett *et al*., 1990). This would take much longer than the 1 month studied here, considering that earlier studies indicate that soil bacteria have mean

generation times of the order of days at 20 °C (Bååth, 1998). Acclimation, i.e. phenotypic changes, cannot be ruled out as a mechanism, but it is likely that this will mainly affect the duration of the lag phase. Furthermore, it has been shown that the lag phase will only be affected when the temperature is outside the normal physiological range of growth (Mellefont & Ross, 2003), and temperatures of 5 to 30 °C are within this range for mesophilic bacteria, which should be predominant in our soil. It is also likely that physiological processes, such as acclimation, will only regulate the short-term response of soil communities, while shifts in community composition will be more important over longer periods (Schimel *et al*., 2007).

The duration of the lag phase for bacterial growth when adapting to new environmental conditions has been described in terms of the amount of work required to adjust to a new environment and the rate at which that work can be done (Robinson *et al*., 1998; Mellefont & Ross, 2003). Community adaptation to temperature could be described in a similar way: work has to be done (a certain alteration in the community to adjust to the new conditions) and it takes a certain time to perform the work (the time taken to alter the community by competition between species more or less adapted to the new conditions). If it is assumed that the 'work' required for the community to adapt to a certain temperature is only dependent on the temperature difference, this work would be the same for the same increase or decrease in temperature. However, the time required to do this work would not be the same, as it is dependent on the growth rates of the competing organisms, and these are higher at higher temperatures. Thus, it should take longer for a community to adapt to a decrease in temperature than to an increase in temperature. This was also found by Pettersson & Bååth (2003), who reported a change in the growth of bacteria after increasing the temperature from 5 to 30 °C within 1 month, while no change was seen after the subsequent decrease back to 5 °C. Similar results were recently reported by Hartley *et al*. (2008), who found the respiration response of the microbial community to soil warming to be faster than that to cooling.

The time required for the temperature response to change will be shorter at soil temperatures above the optimum than below. Apart from the fact that the adjustment of the community to the new conditions will be greater at the higher temperature (the amount of 'work' will be greater), the effects will also be categorically different. For instance, killing the original community by exceeding their upper limit for growth would allow the very rapid growth of a new community already adapted to high temperatures, due to lack of competition and large amounts of easily available food (dead microorganisms). Thus, although we only measured the temperature relationship of the bacterial community after 1 month, it is likely that changes in the temperature relationship would be found much earlier at high temperatures. Such changes have previously been found after 3 days when heating peat soil to 55 °C (Ranneklev & Bååth, 2001).

The less time-consuming way of comparing temperature relationships using the ratio of growth at two very different temperatures appeared to be no less appropriate than using the whole temperature curve to describe community adaptation. This simplification will allow measurements to be made on a large number of samples, making it possible to study community adaptation to small shifts in temperature regimes, i.e. those used in most experiments on soil warming and which are highly relevant in global climate change scenarios. Although it is preferable to measure growth at two very different temperatures, because this would result in the greatest effects, this is not necessarily the most efficient strategy. Using very different temperatures may introduce large errors into the measurements. This is due to difficulties in estimating very low growth rates with sufficient precision using the methodologies presently available. This can be illustrated by comparing the effect of the soil temperature regimes on the bacterial growth ratio at 45 °C/3 °C and 40 °C/15 °C, where the latter had a smaller effect, but nevertheless had better statistical significance due to less variation. The best choice is probably a low temperature and one slightly above the optimum.

This is the first time the effect of different soil temperature regimes on the temperature relationship of both fungal and bacterial growth has been measured, allowing a comparison. The technique used to estimate fungal growth (acetate-in-ergosterol incorporation) is currently more laborious than that used for bacteria. Therefore, only the simplified methodology, using two temperatures, was used. The results also showed greater variation. However, the main result, that fungi and bacteria reacted similarly to the changes in temperature regimes, with most of the changes being seen at soil temperatures above 35 °C, was still easily shown. Consequently, temperature alone does not seem to selectively affect one microbial group more than the other, and thus will not cause a shift in their relative importance. However, more studies in this respect are needed, especially to compare the effects of changes in temperature regimes in the lower temperature range, bearing in mind that earlier studies have indicated that fungi are favored at low temperatures (Ley & Schmidt, 2002; Pietikäinen *et al*., 2005).

Compared with the temperature regimes studied here, the expected mean temperature changes induced

by global climate change are of course much smaller. One must bear in mind, however, that the time frame studied here is short. Nonetheless, our study showed that the use of instantaneous growth rates of bacteria and fungi could provide a valuable tool for determining whether the temperature increases expected in global climate change scenarios would induce changes in microbial community tolerance to temperature. The use of only two temperatures will also help in that one can easily process a large number of samples, enabling the detection of subtle differences. Furthermore, our study has shown that environmental temperatures above the optimum have the greatest effect on the temperature response. Although such large changes in temperature will only occasionally occur under natural conditions, with only a few degrees change in mean temperatures, such events will have drastic and rapid effects on the microbial community. In view of the apparently faster response to a temperature increase than to a temperature decrease, considering both soil respiration (Hartley *et al.*, 2008) and bacterial growth rates (Pettersson & Bååth, 2003), even a short period of considerable warming might affect the temperature response of microbial communities over a long period of time. Also, the probability, and consequently the frequency in the long term, of warming spells may increase even with small increases in mean temperature. Last, altered temperature relationships of the microbial community due changing temperatures are only one way that the microbial community is affected by altered temperatures. Direct effects on activity and changes in substrate availability will of course also be of utmost importance. However, we have suggested one way of differentiating between these different temperature responses using measurement of instantaneous growth rates of the microbial community.

Acknowledgements

This study was supported by grants from the Swedish Research Council (VR) to E. B., G. B. M. and M. G.-B. acknowledge the support of an FPU fellowship from the Spanish Ministerio de Educacíon.

References

Ågren GI, Bosatta E (2002) Reconciling differences in predictions of temperature response of soil organic matter. *Soil Biology and Biochemistry*, **34**, 129–132.

Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Sciences*, **8**, 343–351.

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 (1998) Growth rates of bacterial communities in soils at varying pH: a comparison of the thymidine and leucine incorporation techniques. *Microbial Ecology*, **36**, 316–327.

Bååth E (2001) Estimation of fungal growth rates in soil using ^{14}C-acetate incorporation into ergosterol. *Soil Biology and Biochemistry*, **33**, 2011–2018.

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 and Biochemistry*, **33**, 1571–1574.

Bauer J, Kirschbaum MUF, Weihermüller L, Huisman JA, Herbst M, Vereecken H (2008) Temperature response of wheat decomposition is more complex than the common approaches of most multi-pool models. *Soil Biology and Biochemistry*, **40**, 2780–2786.

Bennett AF, Dao KM, Lenski RE (1990) Rapid evolution in response to high-temperature selection. *Nature*, **346**, 79–81.

Bronikowski AM, Bennett AF, Lenski RE (2001) Evolutionary adaptation to temperature. VIII. Effects of temperature on growth rate in natural isolates of *Escherichia coli* and *Salmonella enterica* from different thermal environments. *Evolution*, **55**, 33–40.

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

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.

Fenner N, Freeman C, Reynolds B (2005) Observations of a seasonally shifting thermal optimum in peatland carbon-cycling processes; implications for the global carbon cycle and soil enzyme methodologies. *Soil Biology and Biochemistry*, **37**, 1814–1821.

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.

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

Leroi AM, Bennett AF, Lenski RE (1994) Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proceedings of the National Academy of Sciences USA*, **91**, 1917–1921.

Ley RE, Schmidt SK (2002) Fungal and bacterial responses to phenolic compounds and amino acids in high altitude barren soils. *Soil Biology and Biochemistry*, **34**, 989–995.

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

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

Mellefont LA, Ross T (2003) The effect of abrupt shifts in temperature on the lag phase duration of *Escherichia coli* and

Klebsiella oxytoca. *International Journal of Food Microbiology*, **83**, 295–305.

Mongold JA, Bennett AF, Lenski RE (1996) Evolutionary adaptation to temperature. 4. Adaptation of *Escherichia coli* at a niche boundary. *Evolution*, **50**, 35–43.

Monson RK, Lipson DL, Burns SP, Turnipseed AA, Delany AC, Williams MW, Schmidt SK (2006) Winter forest soil respiration controlled by climate and microbial community composition. *Nature*, **439**, 711–714.

Neidhardt FC, Ingraham JL, Schaechter M (1990) *Physiology of the Bacterial Cell: A Molecular Approach*. Sinauer Associates, Sunderland, MA, USA.

Newell SY, Fallon RD (1991) Toward a method for measuring instantaneous fungal growth-rates in field samples. *Ecology*, **72**, 1547–1559.

Pettersson M, Bååth E (2003) Temperature-dependent changes in the soil bacterial community in limed and unlimed soil. *FEMS Microbiology Ecology*, **45**, 13–21.

Pietikäinen J, Pettersson M, Bååth E (2005) Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiology Ecology*, **52**, 49–58.

Ranneklev SB, Bååth E (2001) Temperature-driven adaptation of the bacterial community in peat measured by thymidine and leucine incorporation. *Applied and Environmental Microbiology*, **67**, 1116–1122.

Robinson TP, Ocio MJ, Kaloti A, Mackey BM (1998) The effect of growth environment on the lag phase of *Listeria monocytogenes*. *International Journal of Food Microbiology*, **44**, 83–92.

Rousk J, Bååth E (2007) Fungal and bacterial growth in soil with plant materials of different C/N ratios. *FEMS Microbiology Ecology*, **62**, 258–267.

Sand-Jensen K, Lagergaard Pedersen N, Søndergaard M (2007) Bacterial metabolism in small temperate streams under contemporary and future climates. *Freshwater Biology*, **52**, 2340–2353.

Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology*, **88**, 1386–1394.

Thamdrup B, Hansen JW, Jørgensen BB (1998) Temperature dependence of aerobic respiration in a coastal sediment. *FEMS Microbiology Ecology*, **25**, 189–200.