Seasonal variation in temperature sensitivity of bacterial growth in a temperate soil and lake

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Abstract

Faster bacterial biomass turnover is expected in water compared to soil, which would result in more rapid community adaption to changing environmental conditions, including temperature. Bacterial community adaptation for growth is therefore predicted to have larger seasonal amplitudes in lakes than in soil. To test this prediction, we compared the seasonal variation in temperature adaptation of bacterial community growth in a soil and lake in Southern Sweden ($T_{in\ situ}$ 0–20°C, mean 10°C) during 1.5 years, based on monthly samplings including two winters and summers. An indicator of community adaptation, minimum temperature for growth (T_{min}), was calculated from bacterial growth measurements (Leu incorporation) using the Ratkowsky model. The seasonal variation in T_{min} (sinusoidal function, $R^2 = 0.71$) was most pronounced for the lake bacterial community, with an amplitude for T_{min} of 3.0°C (-4.5 to -10.5°C) compared to 0.6°C (-7 to -8°C) for the soil. Thus, T_{min} in water increased by 0.32°C/degree change of $T_{in\ situ}$. Similar differences were also found when comparing four lakes and soils in the winter and summer (amplitudes 2.9°C and 0.9°C for lakes and soils, respectively). Thus, seasonal variation in temperature adaptation has to be taken into account in lakes, while for soils a constant T_{min} can be used.

Keywords: bacterial growth, community adaptation, leucine incorporation, seasonality, T_{min}, temperature

Introduction

Temperature is one of the most important environmental factors affecting microbial growth and activity in both water and soil, and predicting effects of increasing temperatures on microbial performance in the future is therefore of prime interest in assessing global change effects (Davidson and Janssens 2006, López-Urrutia et al. 2006, Daufresne et al. 2009, Kirchman et al. 2009, Sierra et al. 2015). Temperature effects will be important in understanding the balance of primary production and microbial degradation in a long-term perspective and on a global scale, but also in a short-term perspective and on a local scale, like seasonal effects.

Seasonal temperature fluctuations will co-vary with changes in soil carbon quality, moisture content, or seasonal effects on the plant community in soil (Moyano et al. 2012, Kirschbaum 2013) or nutrient concentrations and primary productivity in water (Sommer et al. 1986). These factors may confound direct temperature effects on microbial growth. Davidson and Janssens (2006), therefore, differentiated between apparent and intrinsic temperature dependency, where the latter referred to conditions with only temperature affecting the result. As stated by Kirschbaum (1995), only the intrinsic temperature relationship provides a general and constant relationship between growth and temperature. Intrinsic temperature effects on bacterial growth can be assessed in short-term incubation experiments, which thus will allow comparisons across biomes as different as soil and water. Temperature changes will have two direct effects on the intrinsic temperature dependency of bacterial growth. An immediate effect is on growth rate, which increase with increasing temperature up to an optimum temperature, while decreasing again at higher temperatures (Pomeroy and Wiebe 2001, Bååth 2018). Temperature will also in a longer perspective act as a selection pressure, selecting for a microbial community better adapted to the *in situ* temperature conditions (Bååth 2018).

Microbial community adaptation to temperature can be by different mechanisms (Bárcenas-Moreno et al. 2009, Bradford 2013). (1) There could be physiological acclimation, with rapid changes within existing taxa; (2) there could be an evolutionary genetic adaptation, more slowly resulting in new genotypes of existing taxa; (3) there could be species sorting, when species better adapted to the emerging conditions replace species less well-adapted. On a temporal scale of months to years, it has been argued that species sorting is the most likely main mechanism for community adaptation in soil (Bárcenas-Moreno et al. 2009, Donhauser et al. 2020, Nottingham et al. 2021). Irrespective of the mechanism, the consequence will be that increasing temperatures will promote microbial communities that grow better at higher temperatures and *vice versa* with decreasing temperatures. The term 'community adaptation' will be used here, as elsewhere (Li and Dickie 1987, Simon and Wünsch 1998, Bradford 2013, Bååth 2018, Li et al. 2021, Nottingham et al. 2021), to describe this enhanced performance of the community after a shift in temperature.

The intrinsic temperature response of bacterial growth, i.e. the immediate effect of an altered temperature, follows the square root (Ratkowsky) model in pure cultures (Ratkowsky et al. 1982). This model predicts a linear increase in the square root of growth

with increasing temperature from a minimum temperature (T_{min}, determined by the *x*-axis intercept of a plot of temperature vs. square root of growth) almost until optimum growth (T_{opt}). The square root model also applies to temperature effects on bacterial community growth. This relationship has been found in both water and soil (Bell and Ahlgren 1987, Li and Dickie 1987, Pietikäinen et al. 2005, Bååth 2018), from cold habitats in the Antarctic (Rinnan et al. 2009, van Gestel et al. 2020) to warm areas like tropical rain forest and desert soil, as well as in warm summer water (Shiah and Ducklow 1994, van Gestel et al. 2013, Nottingham et al. 2019). T_{min} is denoted apparent, since in most climate zones it will be below the freezing point of water. T_{min} will, however, allow for direct estimation of the temperature effect in nonfrozen habitats using the square root equation and thus determine the immediate effect of temperature on bacterial growth (Bååth 2018).

T_{min} will also indicate the extent of temperature adaptation of the bacterial community, with T_{min} being lower in cold than in warm habitats. A global envelope for T_{min} of bacterial growth in soil from around $-15°C$ in Arctic conditions to around $0°C$ in tropical climate has been suggested (Bååth 2018), and a similar range in water has also been put forward ($-20–0°C$; van Gestel et al. 2020). Thus, long-term changes in T_{min} due to climate changes are expected. Short-term changes in T_{min} (within few years) has been found in field size global change experiments in soil (Rousk et al. 2012, Nottingham et al. 2021, 2022). Similar studies have not been performed in water. Li and Dickie (1987) found, however, seasonal changes in T_{min} for bacterial growth in the Atlantic Ocean with *in situ* temperature varying between 0 and 20°C. In soil, no effect of seasonality has been found, neither in a temperate climate (soil temperature amplitude around 10°C; Birgander et al. 2018) nor even in a desert soil with > 20°C soil surface amplitude over the year (van Gestel et al. 2013). However, both studies only had a single sampling during the cold period of the year, making firm conclusions problematic. Still, there are some indications for a larger effect of seasonality on temperature adaptation of bacterial growth in aquatic habitats than in soil.

Since changes in community adaption based on species sorting will depend on more sensitive species being outcompeted by more tolerant ones, one would expect that a faster turnover of the bacterial cells will result in faster community adaptation to changing conditions. The bacterial concentration in lake water is typically around 10^6 cells/ml (Kirchman 2018), while in soil around 10^9 bacteria/g of moist soil are found (Bååth 1998), which implies around 3 magnitudes higher bacterial concentration in soil water than in lake water. With similar bacterial growth in both habitats, bacterial turnover will be faster in water than in soil (Bååth 1998, Hobbie and Hobbie 2013). Due to the possibility for soil bacteria in small soil pores to evade predation by nematodes and protozoa (Heynen et al. 1988), which is not possible for bacteria in water (Sherr and Sherr 2002), a faster turnover of the bacterial biomass in water is also expected. Thus, seasonal changes, e.g. in temperature adaptation, are expected to be faster in water than in soil habitats.

The objective of the present study was to compare seasonal temperature adaptation of bacterial community growth in lakes and soils. We performed a seasonal study, with monthly samplings during 1.5 years (encompassing two winter and two summer seasons), on the effect of *in situ* temperature on the variation in bacterial growth adaptation to temperature, comparing a soil and lake with similar climate (temperate climate zone, approximately varying between 0 and 20°C during the year). We also compared temperature adaptation in four additional lakes and soils in winter and summer. We hypothesized that seasonal variation of *in situ* temperatures would affect the temperature adaptation of

bacterial growth, with correlation between T_{min} and *in situ* temperature. Furthermore, we expected the magnitude of variation in T_{min} to be larger in water than in soil, due to a faster turnover of bacteria in water compared to soil.

Materials and methods
Sampling

A soil and a lake, situated in or near Lund, Southern Sweden, were sampled approximately monthly during 1.5 years, in total 16 times. Sampling started late summer (September) 2016 and ended during winter 2018 (January). This encompassed two seasons with high summer temperatures (around 20°C) and two seasons with low winter temperatures (almost 0°C). The sampling sites are situated around 10 km apart and have the same temperate, seasonal climate.

The soil was a grassland soil, with pH around 8.1 and organic matter around 11%. The lake sampled—Lake Krankesjön—is a shallow, calcareous, and nutrient-rich lake with high production of both macrophytes and phytoplankton and pH varying between approximately 7.5 and 8.5 (Blindow et al. 1993).

At two occasions, four additional lakes and soils adjacent to them, were sampled. All sites are situated in Southern Sweden within 80 km from the main study lake. The lakes were Lake Ivösjön, Lake Oppmannasjön, Lake Råbelövsjön, and Lake Hammarsjön. Sampling was made once in the winter and once in the summer to capture the extreme *in situ* temperatures: the 22nd of February 2017 (water temperature 1.2–3.9°C, soil temperature 3.6–4.2°C) and the 23rd of August 2017 (water temperature 20.0–21.5°C, soil temperature 17.5–20.0°C). Mean values of the four lakes and soils were included as one data point in time in further calculations.

Lake water was collected near the shore from just below the surface into a 1-l plastic bottle. Soil was taken from the top 5 cm into a 50-ml Falcon tube; both samplings were performed in the morning. At the same time, the *in situ* temperature was measured at around a depth of 5 cm both in soil and water. The samples were transported to the laboratory and bacterial growth at different temperatures was measured using the leucine (Leu) incorporation method, starting within 1.5 h of sampling.

Bacterial growth measurements

Bacterial growth was estimated with the Leu incorporation method (Kirchman et al. 1985, Smith and Azam 1992, Bååth et al. 2001). For the soil samples, 1 g of soil was mixed with 20 ml of distilled water (with approximately *in situ* temperature) on a multivortex for 3 min, followed by a low speed centrifugation for 10 min at 1000 × *g*. The resulting supernatant was used as the bacterial community sample (Bååth et al. 2001). The water samples were used unfiltered. A volume of 1.5 ml of the soil and water sample were transferred to microcentrifugation vials in duplicate for each incubation temperature. The vials were preincubated at seven different temperatures in water baths: 2 h for 0°C, 1 h for 4, 10, 16, and 25°C, and 30 min for 30 and 35°C. A volume of 2 μl of 1-[4,5-^{3}H]-Leucine (5.7 TBq mmol^{-1}, PerkinElmer, USA) and unlabeled Leu were then added, resulting in a final concentration of 275 nM. The incubation time with Leu was varied with incubation temperature in order to achieve more similar total incorporation of Leu irrespective of temperature, as used earlier (Pietikäinen et al. 2005). At 0°C, incubation time with Leu was around 22 h, at 4°C 6–7 h, at 10°C 4 h, at 16°C 2 h, and at 25, 30, and 35°C 1 h. After incubation, growth was terminated by adding 75 μl of 100%

trichloroacetic acid (TCA) resulting in a final concentration of 5% TCA. Nonincorporated Leu was removed following the washing steps described by Bååth et al. (2001). Finally, 1 ml of scintillation cocktail (Ultima Gold; PerkinElmer) was added to the samples and radioactivity was measured using a liquid scintillation counter (PerkinElmer Liquid Scintillation Analyzer, Tri-Carb 2910 TR). Obtained values are presented as relative biomass production, i.e. Leu incorporation as disintegrations per minute (dpm) incorporated into the extracted bacterial solution/g wet soil/h or dpm/ml water/h.

Calculations and statistics

At each sampling occasion, two different indices of temperature adaptation of the bacterial community was calculated. T_{min}, i.e. the apparent minimum temperature for growth, was calculated using the square root model (Ratkowsky model; Ratkowsky et al. 1982), where the square root of bacterial growth is linearly related to the incubation temperature below optimum temperature for growth.

$$\sqrt{Growth} = a^* \left(T - T_{min} \right), \tag{1}$$

where a is a slope variable, T the incubation temperature, and T_{min} the apparent minimum temperature for growth. T_{min} is determined by linear extrapolation as the crossing with the x-axis. To avoid introducing nonlinear parts around optimum temperature for growth (T_{opt}), in winter time (December–March) only incubation temperatures $\leq 16°C$ was used for this calculation, while in the remaining year also 25°C was included, since a higher T_{opt} was expected in summer (Figure S1, Supporting Information). T_{min} and T_{opt} is expected to increase if the bacterial community adapts to higher temperature (Bååth 2018). A temperature sensitivity index (SI) was also calculated as Log(growth at 35°C/growth at 4°C). An SI has previously been shown to be a sensitive and robust index to indicate community adaptation to temperature correlating well with T_{min} (Nottingham et al. 2019). Increasing SI indicates adaptation to higher temperatures of the bacterial community and *vice versa*. T_{opt} was not explicitly determined, since T_{opt} was expected to be around 30°C, and thus higher than *in situ* temperatures, even in the summertime.

We used the data for the three variables (*in situ* temperature, T_{min}, and SI), plotted over time, to evaluate seasonal effects on temperature adaptation of bacterial growth. We fitted a sinusoidal equation to the data to capture reoccurring variations, with the frequency period set to 1 year.

$$Y = a + b^* \sin \left(c + X^* \left(2^* \pi / 365 \right) \right), \tag{2}$$

where a is the mean over one period, b is the amplitude above or below the mean value, and c indicates the starting point in time. Y is one of the three variables and X is number of days since starting the measurement series.

We also linearly regressed T_{min} and SI vs. *in situ* temperatures as an additional way of expressing seasonal effects of temperature on temperature adaptation of the bacterial communities for the soil and water samples. Finally, we calculated relative bacterial growth at a fixed temperature (10°C) at each sampling time and compared with growth at *in situ* temperatures to isolate direct effects of temperature from effects of other seasonally variable environmental factors on bacterial growth.

Results

The effect of temperature on bacterial growth in soil and water

Incubation temperature affected the growth rate of the bacterial communities from soil and lake water in a similar way at all sampling occasions. This is exemplified by the results from two sampling occasions, one in winter (*in situ* temperature 2.8°C and 2.3°C in soil and water, respectively) and one in late summer (*in situ* temperature 15.1°C and 16.5°C; Fig. 1). Bacterial growth rates were very low at 0°C, increased with temperature up to a T_{opt} around 30°C, and then decreased at higher temperatures (Fig. 1A and B).

The relationship between bacterial activity and temperature from 0°C to T_{opt} is often modelled with an exponential model, with a constant Q_{10} over the whole temperature interval (Portner et al. 2010, Sierra et al. 2015). However, the results here did not correspond to a constant Q_{10}, since a log transformation of growth rates resulted in a curvature, with the temperature effect being steeper at lower temperatures (Fig. 1C and D). Thus, Q_{10} was higher at lower temperatures, in both soil and water.

The bacterial response at temperatures below T_{opt} was well-modelled by the square root model, with R^2 values > 0.98 in all cases (Fig. 1E and F). T_{min} estimated for bacterial growth in soil was similar in winter and summer, −7.6 and −7.2°C, respectively, while in water T_{min} was lower in winter than in summer, −13.5 and −4.0°C, respectively (Fig. 1E and F).

Seasonal effects on temperature adaptation of bacterial community growth

The mean *in situ* temperature in the soil and lake was similar, i.e. 9.5 ± 0.45°C and 10.0 ± 0.60°C, respectively, calculated from the sinusoidal function (Fig. 2A). The temperature was slightly above 0°C in both winter periods and slightly below 20°C in the summers. Seasonal variation of *in situ* temperature was, however, slightly more evident in the lake than in soil with an amplitude of *in situ* temperature above and below the mean of 9.4°C and 7.4°C, respectively.

The square root model to calculate T_{min}, applied to all soil samples, had a mean R^2 of 0.989 (SD = 0.0075). Water sample R^2 had a mean of 0.982 (SD = 0.0164). Thus, data was well-modelled by the square root equation (Figure S1, Supporting Information). Mean T_{min} for all samples calculated from the sinusoidal function was the same for bacterial growth in the soil and lake, −7.3 ± 0.16°C and −7.6 ± 0.36°C (Fig. 2B). This is equivalent to a Q_{10} between 0 and 10°C of 5.4 and between 10 and 20°C of 2.5. In the lake T_{min} varied between −4 and −5°C in the summer time and −10 and −11°C in the winter time, with seasonal changes in T_{min} being well-modelled by a sinusoidal function ($R^2 = 0.71$). The amplitude was 3.0 ± 0.50°C above and below the mean, i.e. T_{min} changed 0.32°C for every degree change of *in situ* temperature. In soil, T_{min} was more stable over the year, around −8°C in the winter and −7°C in the summer, with an amplitude of 0.62 ± 0.22°C ($R^2 = 0.35$). Thus, in soil T_{min} of bacterial growth only changed with 0.083°C for every degree change of *in situ* temperature.

Similar results for seasonal effects as for T_{min} were found using the temperature SI (Fig. 2C). In water, SI was around 0.7–0.9 in winter and 0.1–0.2 in summer (amplitude 0.38 ± 0.056), while in soil hardly any seasonal effect was seen (amplitude only 0.086 ± 0.031). However, the magnitude of SI was different, being constantly higher in soil (mean 0.90 ± 0.023) than in water (mean 0.43 ± 0.041). This was due to faster decline in growth with increasing temperature above T_{opt} in water compared to soil (cf Fig. 1C and D; Figure S1, Supporting Information).
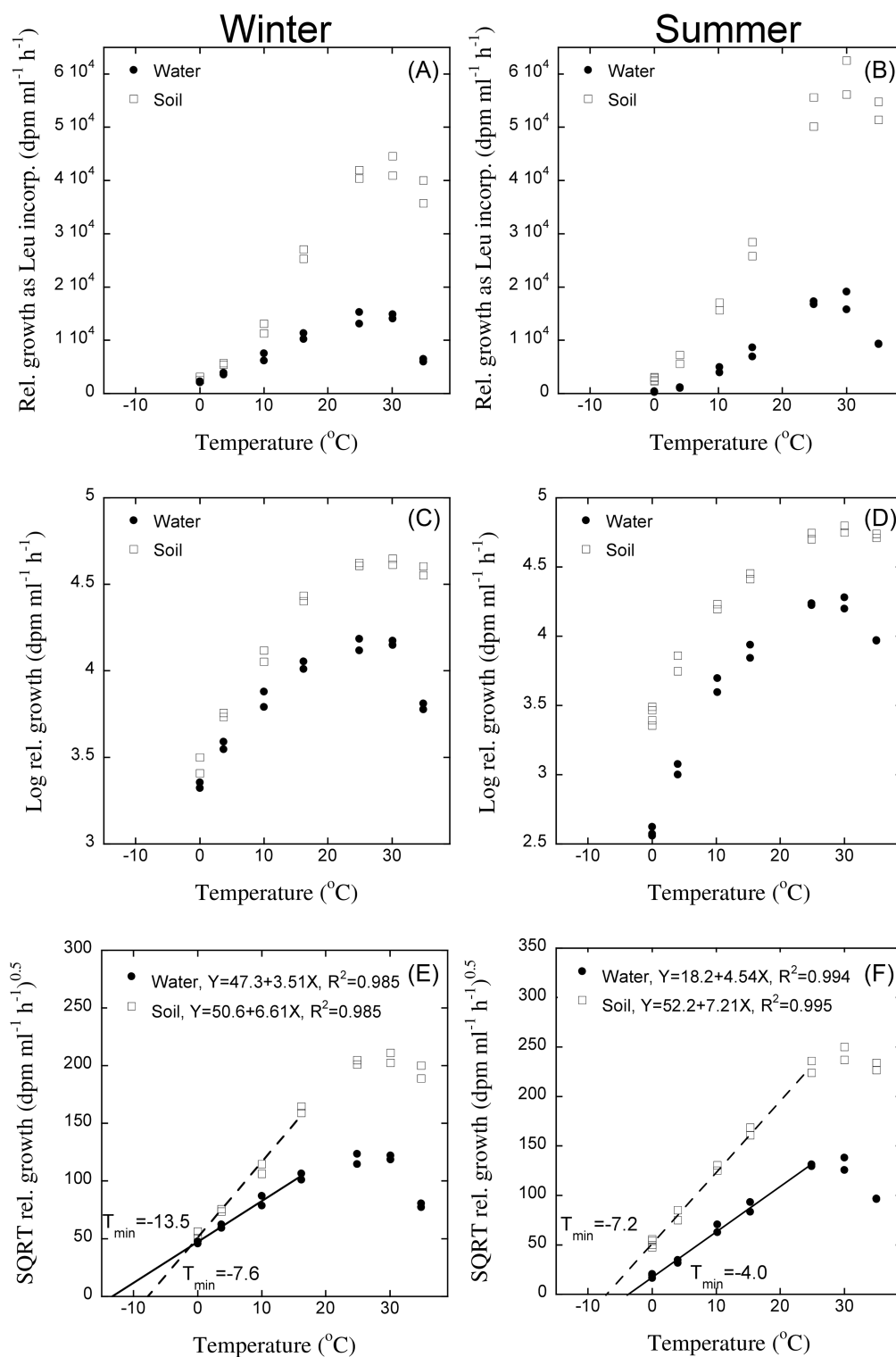
Fig. 1

Figure 1. Bacterial growth (Leu incorporation) at different temperatures for a lake and soil sampled in winter (19 January 2017; A, C, and E), water and soil temperature 2.3°C and 2.8°C, respectively, and summer (14 August 2017; B, D, and E), water and soil temperature 16.5°C and 15.1°C, respectively. Bacterial growth was expressed as a relative measure, as dpm Leu incorporated into bacteria per h and ml lake water and into bacteria extracted into water per h and g soil. (A) and (B) untransformed, (C) and (D) log transformed, and (E) and (F) square root transformed. T_{min} was calculated from the square root (Ratkowsky) equation on data below T_{opt} (filled symbols) in (E) and (F).
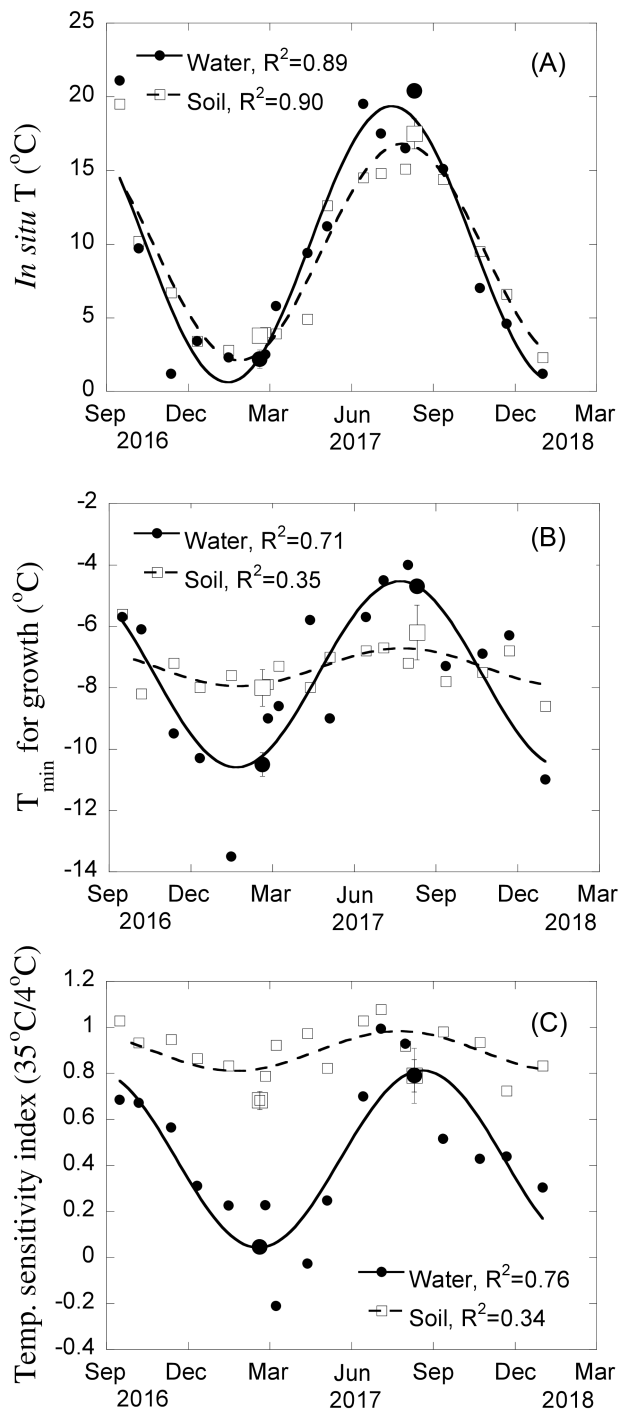
Figure 2. Seasonal variation during 1.5 years in a lake and soil for (A) *in situ* temperature (5 cm depth), (B) T_{min} for bacterial growth, and (C) the temperature SI for bacterial growth (log growth at 35°C/4°C). Data were modelled using a sinusoidal function with a 1-year frequency. Larger symbols with bars indicate mean ± SE for four lakes and soils from the same region.
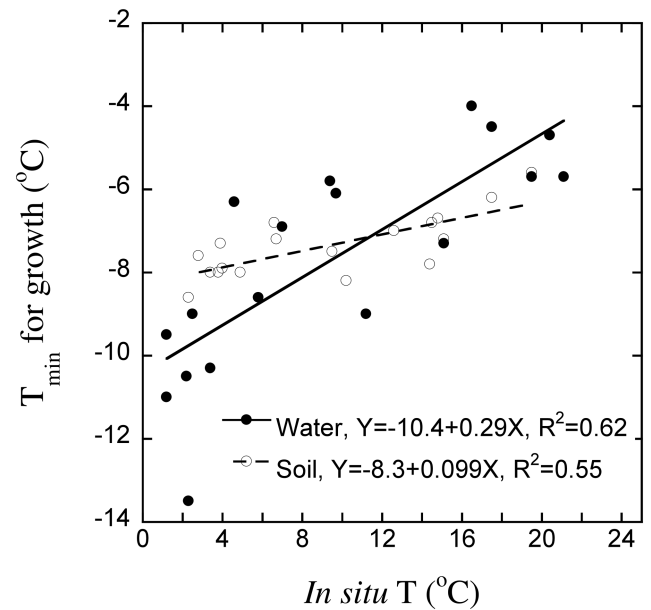

Figure 3. Correlation between *in situ* temperature and T_{min} for bacterial growth in a lake and soil with approximately monthly sampling occasions during 1.5 years.

The seasonal temperature adaptation differed between the four lakes and soils sampled in winter and summer (Fig. 2B, large data points with SE bars). In the summer, T_{min} for soil and lake water was -6.2 ± 0.89°C and -4.7 ± 0.19°C, respectively. In winter, T_{min} was lower than in summer, especially in the water, i.e. -8.0 ± 0.60°C and -10.5 ± 0.39°C in soil and water, respectively. Thus, the difference between community adaptation to tempera-

ture (estimated as mean of individual differences in T_{min} of each site) in summer and winter, was 5.7 ± 0.5°C for the four lakes and only 1.8 ± 1.4°C for the soils, resulting in a seasonal amplitude of 2.9°C for the lakes and 0.9°C for the soils. This was similar to seasonal amplitude observed using monthly samples from one lake and one soil (3.0°C and 0.6°C, respectively; Fig. 2B).

Summarizing the seasonal effect of temperature on the temperature adaptation bacterial community growth, T_{min} from water showed a clear correlation with *in situ* temperature ($R^2 = 0.62$, $P < .001$), with a slope of 0.29 ± 0.056 (Fig. 3). For soil this relationship was less evident ($R^2 = 0.55$, $P < .01$) with a slope of only 0.099 ± 0.022.

Accounting for direct effects of temperature to isolate other seasonal changing environmental factors

Modelling relative bacterial growth (as dpm Leu incorporation) at *in situ* temperatures resulted in similar seasonal patterns in water (sinusoidal-function $R^2 = 0.57$; Fig. 4A) and soil ($R^2 = 0.38$; Fig. 4B) with rapid growth in the summer and slow in the winter. This resulted in linear positive correlations both for water (Fig. 4C; $R^2 = 0.77$) and soil (Fig. 4D; $R^2 = 0.41$), when regressing bacterial growth at *in situ* temperatures during the year against *in situ* temperature. Bacterial growth was around four to five times higher in the summer with high temperatures compared to the winter with low temperatures. However, in addition to *in situ* temperature, any other environmental factor co-varying with temperature during the year may affect this relationship. Using individual regressions for each sampling occasion (cf the square root model in Fig. 1E and F; Figure S1, Supporting Information), the bacterial growth at a standard temperature (10°C) was calculated, to factor out the direct effect of temperature on bacterial growth. Both in water and soil the correlation between bacterial growth and *in situ* temperature disappeared (Fig. 4C and D; $R^2 = 0.08$ and 0.03 for lake water and soil, respectively), and instead there was a tendency for lower standardized growth in the summer than in the winter (Fig. 4A and B).
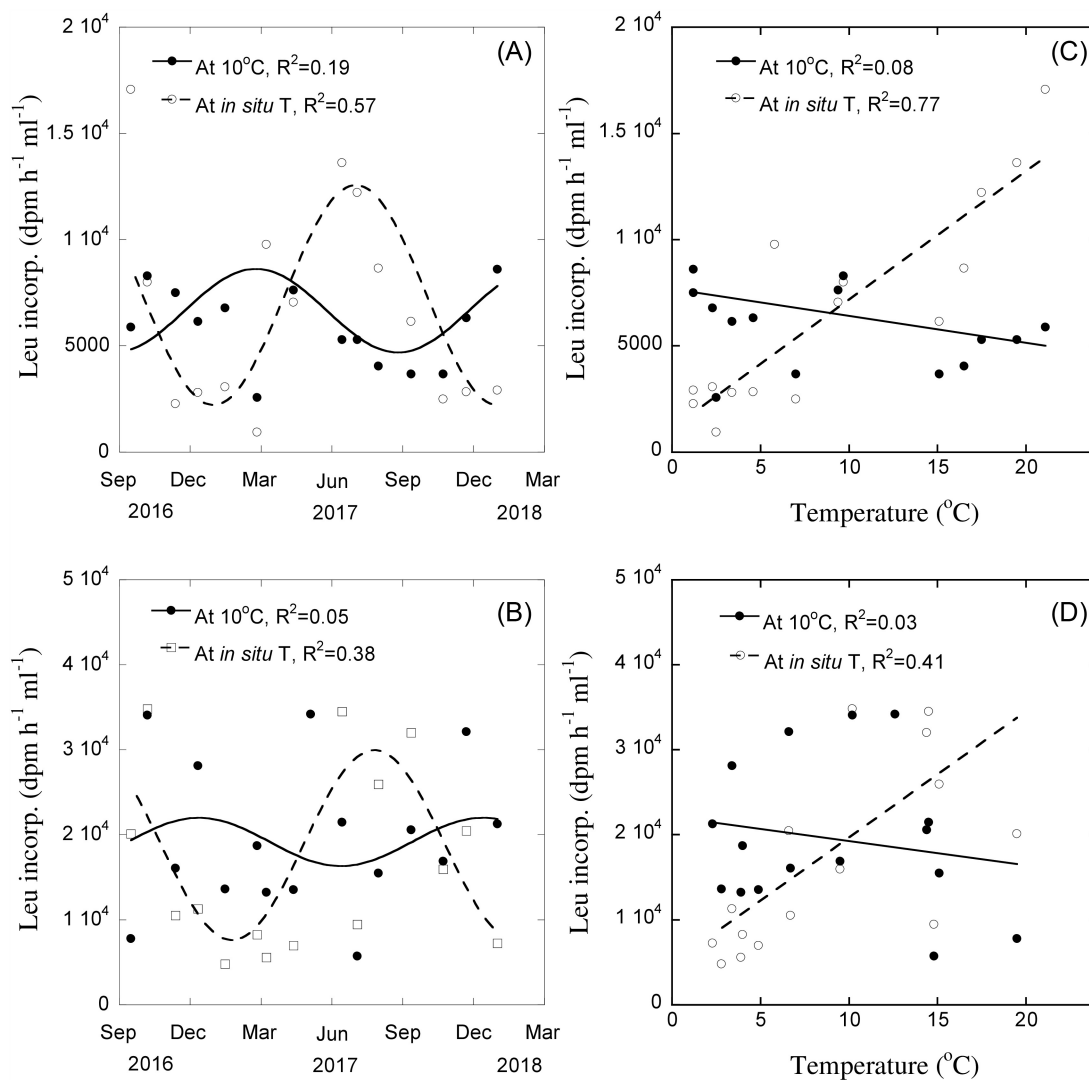
Figure 4. Bacterial growth (Leu incorporation) at *in situ* temperatures and at a standardized temperature (10°C) for approximately monthly samplings during 1.5 years. (A) and (C) lake water and (B) and (D) soil. (A) and (B) are plotted as seasonal changes modelled using a sinus function with a 1-year frequency, (C) and (D) are plotted against *in situ* temperature.

Discussion

Seasonal effects on temperature adaptation in lake water and soil

Temperature adaptation of the bacterial community growth (calculated as T_{min} or SI) varied seasonally both in soil and lake water, with summer communities adapted to higher temperatures and winter communities adapted to lower temperatures, in accordance with our hypothesis. Although this was only studied in depth for one lake and one soil, data from the additional four lakes and soils supported that this is a general phenomenon, at least in the studied climatic zone.

We also found that community temperature adaptation due to the seasonal changes was much more pronounced in lake water than in soil, in line with our hypothesis, both in the frequently sampled lake and soil, as well as in the additional four lakes and soils. For water, this is in accordance with earlier studies finding clear seasonal adaptations in aquatic habitats (Li and Dickie 1987, Shia and Ducklow 1994, as recalculated by van Gestel et al. 2020). In soil, a study based on few samples have suggested no season-

ality (Birgander et al. 2018), but the present study indicates that a small seasonal change in T_{min} and SI takes place also in soil, at least in a temperate climate zone with seasonal variation of *in situ* temperatures between 0 and 20°C.

Since the temperature regime was similar in both soil and water, we suggest that the difference in seasonality of temperature adaptation was due to differences in turnover time of the biomass of communities in lakes and soils, a difference already suggested by Bååth (1998). We did not explicitly measure turnover times in the present study. However, bacterial biomass growth per ml lake water was only two to three times slower compared to that per ml extracted bacteria from soil (compare Fig. 4C and D). Bacterial numbers in lake water usually are around 10^6/ml (Kirchman 2018), while extracted bacteria from soil with the present methodology can be more than 10 times higher, e.g. $1.5-10 \times 10^7$ cells/ml bacterial suspension (Bååth 1998), suggesting that bacterial turnover (growth per biomass) indeed was slower in soil than in lake water. Bacterial community adaptation to other factors than temperature has earlier been found to be faster during conditions of more rapid growth and turnover of bacteria, e.g. toler-

ance to heavy metals and pH (Díaz-Raviña and Bååth 1996, Pettersson and Bååth 2003), emphasizing the importance of turnover of biomass on the rate of community adaptation.

Earlier studies in soil have indicated that T_{min} for bacterial community growth vary with mean annual temperature (MAT) across sites with geographical differences in MAT (Bååth 2018, Nottingham et al. 2019). However, there was never a one-to-one relationship, but always less variation in T_{min} than in MAT, varying between 0.2 and 0.55°C change in T_{min}/degree change in MAT. A provisional value for temperature adaptation of 0.3°C change in T_{min} with every degree of change in MAT was also suggested for soil (Bååth 2018, Nottingham et al. 2019) A slightly larger value, although uncertain given the limited amount of data, was found for a compilation of studies from aquatic systems, 0.55°C/degree change in water temperature (Van Gestel et al. 2020). This number is similar to that found for seasonal changes in the North Atlantic (0.35–0.55°C/degree; Li and Dickie 1987). The seasonal variation in lake water of 0.32°C/degree of *in situ* temperature found here was therefore only slightly lower than the predicted span, suggesting that during the year the bacterial community became fully or close to fully adapted to the expected temperature adaptation both in the winter and summer season. This was not the case in the soil, where the seasonal change was only 0.086°C/degree of *in situ* temperature.

Preferentially, one should model T_{min} over the year against *in situ* temperature some time before sampling, allowing for community adaptation to occur. Since there is no obvious time frame to select, a very frequent sampling scheme is needed to determine such an optimum time interval, which was not the purpose of the present study. Still, since seasonal changes of *in situ* temperatures are gradual and slow, the temperature at a sampling occasion to some extent reflects *in situ* temperatures several days and even weeks before. Thus, the use of the temperature at the sampling occasion have to a large extent already incorporated presampling temperatures.

Bacterial growth follows the square root model both in lake water and soil

The square root model, only using data at temperatures well below T_{opt}, adequately described the temperature effect on bacterial community growth, as shown before both for water and soil habitats (see references in the 'Introduction'; Figures S1 and S2, Supporting Information). Importantly, the use of the same temperature sensitivity model both for water and soil allowed for a valid comparison of the temperature adaptation of the bacterial community in these very different habitats, as indicated by the same yearly mean T_{min} in soil and water from the same climate zone. Mean T_{min} over the year (-7.3 to -7.6°C for soil and water, respectively) was in the expected range previously found in the temperate climate zone with a MAT around 10°C [cf compilations for soil (Bååth 2018) and water habitats (Van Gestel et al. 2020)]. This suggests that despite very different environmental conditions in water and soil, temperature was the major factor determining temperature adaptation of the bacterial communities both in water and soil.

We did not explicitly determine T_{opt} for growth, but comparing growth at the two incubation temperatures with fastest growth (usually at 25 and 30°C) suggested T_{opt} to be between these temperatures (cf Fig. 1). However, T_{opt} will be of little practical consequence, since a T_{opt} between 25 and 30°C will be much higher than the *in situ* temperature, even during high temperature summer conditions (around 20°C in both habitats). Thus, the bacterial

community can be considered to be within the 'normal physiological range' of the temperature relationship (*sensu* Neidhardt et al. 1990), indicating that there was never any explicit temperature stress, as found at extreme temperatures (Yura et al. 1993, Panoff et al. 1998). T_{opt} is normally higher than *in situ* temperatures (Li and Dickie 1987, Bååth 2018), especially in cold (Joint and Smale 2017, van Gestel et al. 2020), but also in warm habitats (Shiah and Ducklow 1994, Nottingham et al. 2019), showing that under most natural conditions, T_{opt} is not important in determining effects of temperature. Thus, the present study concentrated on determining T_{min}, since this characteristic, when applied with the square root model, will determine the temperature response under *in situ* conditions (Bååth 2018).

The use of a SI (log growth at 35°C/4°C) has been suggested as a rapid way to elucidate temperature adaptation for bacterial communities instead of using T_{min}, since only two measurement temperatures are needed (Ranneklev and Bååth 2001, Nottingham et al. 2019). In accordance with this, the use of SI gave very similar results as the use of T_{min} within habitats, showing seasonal variations in temperature adaptation with a larger amplitude in water than in soil (Fig. 2). At temperatures above T_{opt} bacterial growth decreased both for water and soil communities. This decrease was, however, faster for lake than for soil communities resulting in constantly lower SI (Fig. 2C; Figure S1, Supporting Information), which was not related to any difference in temperature adaptation (compare similar mean T_{min} over the year). Thus, SI could not be used to compare temperature adaptation in water and soil, and we therefore caution against using SI to compare between different habitats. We do not know why increasing temperature above T_{opt} result in more prominent decrease in growth in water compared to soil, although water communities having a faster turnover than soil communities may be implicated. During winter in a desert system with temperatures sometime < 0°C, T_{opt} was > 40°C, reflecting bacteria adapted to the high summer temperatures still being present (van Gestel et al. 2013), presumably due to low bacterial biomass turnover. A faster bacterial turnover, like in water habitats, would not allow bacteria, adapted to previous conditions half-a-year earlier, to remain that long within the community. Thus, high temperature adapted species, thriving under summer conditions, would be out-competed more rapidly in water than in soil, possibly giving rise to the different patterns.

Relevance for modelling bacterial growth in short- and long-term

There were no differences in annual T_{min} of bacterial growth between soil and lake water. Thus, when predicting changes in temperature sensitivity over longer time periods, e.g. due to global change effects, it may be possible to use the same T_{min} for both habitats, where T_{min} is determined by MAT as suggested by Bååth (2018) and van Gestel et al. (2020). A constant T_{min} can also be used for soil when seasonal effects are studied, since the seasonal variation in T_{min} will be small and negligible. For aquatic systems seasonal variations in T_{min} need to be taken into consideration, especially in climatic regions with large seasonal amplitudes of *in situ* temperatures. However, by using data on seasonal variation of $T_{in\ situ}$ and the relationship between $T_{in\ situ}$ and T_{min} determined here, this can easily be accomplished. Besides seasonal variations in *in situ* temperatures, there will also be diurnal temperature variations, especially in the top centimetres of soils (Parton and Logan 1981). This will affect the direct growth of bacteria, which can be easily modelled using the square root equation with the proper T_{min} (Bååth 2018). However, our results suggest that T_{min} will not

be affected by diurnal variations, since during 1 day there will not be enough time for any substantial change in T_{min}, particularly not in soil.

Studying how *in situ* temperature affects temperature adaptation of bacterial growth, by assessing T_{min}, Q_{10} or E_a [the latter two can be calculated from T_{min}; see Bååth (2018)], could result in a correlation between *in situ* temperature and temperature adaptation. It is, however, not self-evident which *in situ* temperature to use, e.g. mean annual, monthly, daily, or even hourly temperature can be used. For soil, MAT will be adequate when comparing different soils, since seasonal changes in T_{min} appear to be minor. The use of monthly or daily temperatures will, however, obscure the relation between soil temperature and T_{min}, since the soil temperature will vary much more rapidly than T_{min}. For water, it will be the other way around. Using MAT to predict T_{min} will underestimate T_{min} in the summer and overestimate T_{min} in winter. Even monthly temperatures may be inadequate, and we recommend using weekly or daily mean *in situ* temperatures.

Concluding remarks

For soil a substantial amount of data already exists on the relationship between MAT and T_{min} (Bååth 2018), while for water such a relationship is less constrained due to few data points (van Gestel et al. 2020). More data from aquatic habitats are still needed. Even now, the relationships that were presented here would allow for an approximate value of T_{min} also in water habitats. This will permit temperature effects to be factored out, and effects of other seasonally variable factors can be targeted (cf Fig. 4), instead of having to rely on correlations of growth and temperature with little control of confounding factors (e.g. Staroscik and Smith 2004, Kim and Ducklow 2016).

The present study on lakes and soils in a temperate climate revealed a faster temperature adaptation of the aquatic community, which displayed a larger amplitude of T_{min} compared to soil communities. There are, however, soil and water habitats with both smaller and larger seasonal temperature variations than those studied here, e.g. on the one hand Antarctic marine habitats with constantly low temperatures (van Gestel et al. 2020) and tropical soils with constantly high temperatures (Nottingham et al. 2020, 2022), and on the other hand desert soils with > 40°C difference between summer and winter temperatures (van Gestel et al. 2013). Small ponds can even exhibit large temperature fluctuations variations on a daily basis (Idso and Foster 1974). The extent to which other habitats exhibit seasonal variation in bacterial growth adaptation will thus be an interesting aspect to explore, as would the temperature adaptation of other organism groups, like fungi and archaea.

Lastly, the more rapid adaption of bacterial communities in water than in soil suggest that while bacterial communities in water are well-adapted to temperature conditions all year round, soil bacterial communities are not. The deficient temperature adaption, especially in winter time, was also mentioned by van Gestel et al. (2013) in their study of a desert ecosystem. To what extent this mismatch in function in soil influences ecosystem function is an interesting aspect for further studies.

Supplementary data

Supplementary data are available at *FEMSEC* online.

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