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The trade-off between growth rate and yield in microbial communities and the consequences for under-snow soil respiration in a high elevation coniferous forest

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Abstract Soil microbial respiration is a critical component of the global carbon cycle, but it is uncertain how properties of microbes affect this process. Previous studies have noted a thermodynamic trade-off between the rate and efficiency of growth in heterotrophic organisms. Growth rate and yield determine the biomass-specific respiration rate of growing microbial populations, but these traits have not previously been used to scale from microbial communities to ecosystems. Here we report seasonal variation in microbial growth kinetics and temperature responses (Q_{10}) in a coniferous forest soil, relate these properties to cultured and uncultured soil microbes, and model the effects of shifting growth kinetics on soil heterotrophic respiration (R_h). Soil microbial communities from under-snow had higher growth rates and lower growth yields than the summer and fall communities from exposed soils, causing higher biomass-specific respiration rates. Growth rate and yield were strongly negatively correlated. Based on experiments using specific growth inhibitors, bacteria had higher growth rates and lower yields than fungi, overall, suggesting a more important role for bacteria in determining R_h. The dominant bacteria from laboratory-incubated soil differed seasonally: faster-growing, cold-adapted *Janthinobacterium* species dominated in winter and slower-growing, mesophilic *Burkholderia* and *Variovorax* species dominated in summer. Modeled R_h was sensitive to microbial kinetics and Q_{10}: a sixfold lower annual R_h resulted from using kinetic parameters from summer versus winter communities. Under the most realistic scenario using seasonally changing communities, the model estimated R_h at 22.67 mol m^{-2} $year^{-1}$, or 47.0% of annual total ecosystem respiration (R_e) for this forest.

Keywords *Abies lasiocarpa* · *Burkholderia* · *Janthinobacterium* · *Pinus contorta* · *Picea engelmanii* · *Variovorax*

Abbreviations

SIGR	Substrate induced growth response
SIR	Substrate induced respiration
R_h	Heterotrophic respiration
R_s	Soil respiration
R_e	Ecosystem respiration

Introduction

A major current scientific challenge is scaling from the functional properties of organisms to processes at

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the ecosystem and global levels (Enquist et al. 2003; Torsvik and Ovreas 2002; Zak et al. 2006). Microbial respiration is a process that has particular importance at the ecosystem and global scales, representing about half of total CO_2 flux from soils (Hanson et al. 2000). Furthermore, effects of human-induced climate change on soil microbial communities and their metabolic activities could create potentially devastating feedbacks to the Earth's biosphere (Meir et al. 2006).

It is likely that soil microbial respiration is highly sensitive to the unique physiological characteristics of soil microbial communities. In particular, the rate and efficiency of growth determine how much CO_2 is produced during microbial growth. Biomass made up of fast-growing species respires faster than an equal amount of biomass made up of slow-growing species. Microbes with low growth yields (biomass produced per unit substrate consumed) convert a larger fraction of substrate into CO_2 during growth, and so respire faster than more efficiently growing organisms. It has been observed that there is an inevitable thermodynamic trade-off between growth rate and yield among heterotrophic organisms (Pfeiffer et al. 2001). Past authors have proposed that two opposing ecological strategies exist at either end of this spectrum: a fast-growing, low yield competitive strategy and a slow-growing, high yield cooperative strategy (Kreft and Bonhoeffer 2005; Pfeiffer et al. 2001). For microbes, the cooperative, slow, efficient growth strategy is more successful in spatially structured environments such as biofilms (Kreft 2004; Kreft and Bonhoeffer 2005; MacLean and Gudelj 2006; Pfeiffer et al. 2001).

These previous studies have focused on the trade-off between growth rate and yield in the context of evolutionary issues such as altruism and the origin of multicellularity. However, the potentially profound ecological and biogeochemical consequences of this trade-off have not been investigated, nor has this relationship been investigated in complex microbial communities. The theoretical relationship between biomass-specific respiration rates and growth kinetics suggests a principle for understanding how physiological properties of microbial communities can scale up to shape ecosystem respiration. Previous work at our subalpine coniferous forest site (Colorado Rocky Mountains, USA) has shown that soil respiration is a dominant control over the ecosystem C balance (Monson et al. 2002), that soil respiration is strongly correlated with soil microbial biomass (Scott-Denton et al. 2003), and that seasonal changes in microbial community composition contribute to unexpectedly high rates and temperature responses of soil respiration beneath the snow pack in late winter and early spring (Lipson 2007; Monson et al. 2006b). The current study combines soil respiration experiments, molecular culture-independent descriptions of soil microbial communities, physiological studies of bacterial and fungal isolates in pure culture, and a mathematical model that predicts soil respiration in a forest ecosystem from these fundamental functional properties. The purpose of the soil respiration experiments is to test whether growth kinetics of the microbial community vary seasonally, particularly between snow-covered versus summer conditions, and whether there is a consistent negative relationship between growth rate and yield. The molecular and physiological experiments are designed to link the observed seasonal changes to specific components of the microbial community. The model is used to quantify the potential effects of seasonal changes in microbial kinetics on soil respiration. Also the modeled results are compared with observations and past biogeochemical models of CO_2 flux from the same ecosystem to test whether soil respiration can be predicted from simple kinetic properties of the microbial community.

Materials and methods

Site description and sample collection

This study was conducted at the Niwot Ridge AmeriFlux site located in a subalpine forest near Nederland, Colorado (40°1′58″ N; 105°32′47″ W, 3,050 m a.s.l.). The forest is dominated by *Abies lasiocarpa* (subalpine fir), *Pinus contorta* (lodgepole pine), and *Picea engelmanii* (Engelmann spruce). The site is generally snow-covered from December to May. The soils have a distinct organic layer (up to 10 cm depth) overlying a sandy mineral layer derived from granitic moraine (Monson et al. 2005, 2002; Scott-Denton et al. 2003). The soil measurements were made on the organic layer, which accounts for the majority of microbial activity (Scott-Denton et al. 2003). On each sampling date, a minimum of five soil samples were collected, placed in plastic bags, and kept cold until analysis. The primary samples used in this study were

collected between July 2004 and April 2005. Table 2 also includes data from samples collected in July 2003.

Soil microbial biomass and growth kinetics measurements

The growth kinetics of soil microbial communities were measured with the substrate induced growth response (SIGR) method using potassium glutamate as substrate (Colores et al. 1996; Lipson et al. 1999; Schmidt 1992). Soils were incubated in side-arm flasks with ^{14}C-labeled substrate (4 mg C g^{-1} soil, ~0.1 μCi), CO_2 was trapped in 1.0 M NaOH in the side-arm, which was removed periodically and counted by liquid scintillation. For an exponentially growing population, it can be shown that:

$$dC/dt = ((1 - Y_c)/Y_c)\ \mu_{max} X(t) \quad (1)$$

where dC/dt is the respiration rate, Y_c the growth yield (biomass C produced per unit substrate C consumed), μ_{max} the maximum exponential growth rate, and X(t) is the microbial biomass C at time, t (Colores et al. 1996). Hence the biomass-specific respiration rate is related positively to growth rate, and negatively to growth yield. The maximum exponential growth rate was estimated by non-linear regression of respiration rate versus time, and growth yield was calculated by recovery of $^{14}CO_2$ after the rate returned to basal levels. SIGR biomass was calculated from these parameters and the initial respiration rate. To infer the relative contributions of bacteria and fungi in SIGR experiments, inhibitors of bacteria (ampicillin, 100 μg g^{-1} soil, and chloramphenicol, 50 μg g^{-1} soil) or fungi (cycloheximide, 1,000 μg g^{-1} soil) were added to soils (Lipson et al. 2002). Soils were incubated overnight at 4°C with half the final dose of inhibitor (or the equivalent amount of H_2O for controls), and then received a second dose of inhibitors along with the substrate at the beginning of the growth experiment. These three inhibitor treatments (antibacterial, antifungal, control) were incubated at two temperatures (4, 14°C) on each date. Additional incubations were carried out at 0°C in April 2005 and at 22°C in July 2004, in order to more closely match ambient temperatures. At the end of the July 2004 and Jan 2005 inhibitor experiments, soils were frozen for later DNA extraction. These SIGR experiments used potassium glutamate as the substrate (4 mg glutamate-C g^{-1} soil). Substrate-induced respiration (SIR) was measured using glutamate or glucose as substrate (Anderson and Domsch 1978). Glutamate SIR was taken from the initial rate of $^{14}CO_2$ production in SIGR measurements. For the calculation of metabolic quotient, glucose SIR was measured at 22°C using 4 mg glucose-C g^{-1} soil, after basal respiration rates were measured in the laboratory at 22°C using a portable infra-red gas analyzer (PP systems, EGM-1 with SRC-1 soil chamber). Glutamate and glucose were chosen as substrates because they produced the largest response of any substrate tested, they are central in metabolism for all heterotrophs, they and their breakdown products can act as catabolic repressors of less preferred substrates (Stulke and Wolfgang 1999), and their SIR correlate well with other measures of total microbial biomass (Lipson et al. 1999). Microbial biomass *C* was estimated by chloroform fumigation-extraction (Brookes et al. 1985).

Derivation of relationship between growth kinetics and respiration rate

During exponential growth, microbial biomass carbon at time t is given by

$$X(t) = X_0\ e^{\mu t} \quad (2)$$

where X_0 is the initial biomass and μ is the exponential growth rate. The amount of carbon assimilated into microbial biomass during growth is given by

$$X(t) - X_0 = Y\ S(t) \quad (3)$$

where S(t) is quantity of substrate carbon used and Y is the growth yield (biomass C produced per unit substrate C consumed). The amount of CO_2-carbon formed during growth, C(t), is the difference of substrate carbon used and carbon assimilated into biomass:

$$C(t) = S(t) - [X(t) - X_0] \quad (4)$$

Solving Eq. 3 for S(t), substituting this expression into Eq. 4, and simplifying:

$$C(t) = [{}^1/_Y - 1][X(t) - X_0] \quad (5)$$

Substituting from Eq. 2, we have

$$C(t) = [{}^1/_Y - 1][X_0\ e^{\mu t} - X_0] \quad (6)$$

Taking the derivative with respect to time gives

$$\frac{dC}{dt} = \left[{}^{1}/_{Y} - 1\right]\mu\, X_0\, e^{\mu t} \quad (7)$$

and again substituting Eq. 2 gives

$$\frac{dC}{dt} = \left[{}^{1}/_{Y} - 1\right]\mu\, X(t) \quad (8)$$

which is equivalent to Eq. 1. Note that dC/dt increases with increasing μ, and decreases with increasing Y.

Descriptions of microbial communities in post-SIGR soils

Clone libraries of 16S rRNA genes were constructed from soils in control treatments of the SIGR experiments in summer (July 2004) and winter (Jan 2005). To extract DNA from soils, ~5 g soil, 1 g silicon–zirconium beads, and 10 ml lysis buffer were vortexed at high speed for 2 min, followed by a standard alkaline lysis protocol (Ausebel 1994). DNA was purified by gel electrophoresis, using Qiaex II resin (Qiagen). Bacterial 16S rRNA genes were amplified by polymerase chain reaction (PCR) from purified soil DNA using the universal bacterial primers, F8 (5′-AGAGTTTGATCCTGGCTCAG) and R1510 (5′-GGTTACCTTGTTACGACTT). Each PCR reaction contained one unit taq polymerase (Fisher Biosciences) with the vendor-supplied buffer, 3.0 mM $MgCl_2$, 1.25 μM of each primer, 200 μM of each nucleotide triphosphate, and 20 mg l^{-1} bovine serum albumin. The PCR reaction consisted of an initial 2 min denaturation step at 94°C, followed by 32 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min, and a final extension step at 72°C for 10 min. Separate PCR were run for each of the three replicates of soil DNA from the winter and summer SIGR experiments, and then the replicates from the same season were pooled before cloning. PCR products were purified by gel electrophoresis, and then ligated into the PCR 2.1-TOPO vector, using the TOPO TA cloning kit with Top10 chemically competent *E. coli* cells (Invitrogen). Transformed cells were plated on selective media and screened according to the manufacturers instructions. Cloned 16S rRNA genes were partially sequenced by capillary electrophoresis, using the ABI Prism 3100 DNA sequencer at the CSUPERB MicroChemical Core Facility at San Diego State University. The sequencing primer used was the universal bacterial primer, R1111 (5′-TTGCGCTCGT TGCGGGACT-3′). In general, each sequencing reaction produced reads at least 600 bp in length. Bacterial isolates were similarly identified by PCR amplification and sequencing of 16S rRNA genes. Fungal isolates were identified by sequencing a portion of the internally transcribed spacer (ITS) region, using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG) and ITS4 (5′-TCCTCCGCTTATTGATATGC) (Millner et al. 1998). These sequences, and others from GenBank (www.ncbi.nlm.nih.gov) and from a previous study of these soils (Lipson 2007), were aligned using the greengenes alignment tool (greengenes.lbl.gov). Neighbor joining and maximum likelihood trees were produced, and the winter and summer communities were compared using the PTP function of PAUP (Lipson and Schmidt 2004; Martin 2002). This method tests the null hypothesis that the two samples are random samples of the same community by comparing the original tree to a probability distribution made from 10,000 random permutations of the tree (Martin 2002). Sequences are available in GenBank, as accession numbers DQ835014-DQ835069.

Isolation and growth of bacteria and fungi from soils

Bacterial isolates were obtained from soils incubated in summer (July 2004) and winter (Jan 2005) SIGR experiments by plating soil dilutions onto solid media containing (per l): 1 g potassium glutamate, 1 g $MgSO_4 \cdot 7H_2O$, 10 mM K_2HPO_4 (pH 7.0), 1 mM NH_4NO_3, 0.1 mM $CaCl_2$, 15 g agar, and 1 ml soil extract (10:1). Plates were incubated at 14°C, and colonies were restreaked for purification. Additionally, fungal and bacterial isolates were obtained from pre-sterilized resin bags placed in soils during the summer. Nylon bags filled with 10 g anion exchange resin were sterilized in an autoclave and soaked in a variety of phosphate-buffered substrates, including glutamate, salicylate, carboxymethylcellulose, citrate, acetate and a C-free control with phosphate buffer. The resin bags were incubated in the field from 17 July to 27 August, 2003. The resins were collected, aseptically dissected, and isolates were obtained on the media described above, except with the previously mentioned C sources. Growth of bacterial isolates was measured in liquid media (as above, without agar), in 96-well plates, using optical

density (595 nm) measured on a plate reader (SpectraMax 190, Molecular Devices). To begin these experiments, log-phase cultures were diluted to the same initial cell density for all strains. In a separate experiment, bacterial and fungal growth in liquid culture was monitored by production of $^{14}CO_2$. Growth curves were fit with a logistic growth model, and were also compared by analysis of covariance, using slopes of the initial, linear phases of growth.

Modeling soil respiration from microbial parameters

To demonstrate the effect of seasonal variations in microbial growth kinetics on soil respiration, a model was constructed to simulate soil microbial respiration based on seasonal variations in soil temperature and growth characteristics of the microbial community (SIGR, μ_{max}, Y_c, and Q_{10}), measured in the laboratory. The daily soil temperature regime was simulated separately for each month. For each month, mean, minimum and maximum soil temperatures were assumed, and the hourly soil temperature on each day was simulated using the following periodic function:

$$T = (\text{Amplitude}/2) * \text{COS}(\pi * (\text{hour}/12) + \pi) + T_{mean} \quad (9)$$

Amplitude is the difference between daily maximum and minimum temperatures assumed for the month, hour is the time of day, varying between 0 and 23, and T_{mean} is the daily average soil temperature assumed for that month. January to May temperatures were based on measured soil temperatures (Monson et al. 2006a). June to October temperatures were based on minimum, maximum and mean daily air temperatures from the CULTER climate database (http://culter.colorado.edu), except that the daily amplitude was reduced by 50% to account for the thermal diffusivity of the soil based on our previous observations of diurnal fluctuations in soil temperature (Campbell and Norman 1998; Lipson and Monson 1998). During the snow-covered months for which no soil temperature data existed, November and December, the temperature regime was assumed to be similar to January.

Heterotrophic respiration at 14°C (R_{h14}) was calculated from SIGR biomass, μ_{max}, and Y_c according to Eq. 1, using linear interpolations of the values in Table 1 and Figs. 1 and 2. The kinetic parameters, μ_{max} and Y_c, and microbial biomass were derived from SIGR experiments performed at 14°C (except for the 4/21/05 date, for which the 4°C SIGR biomass estimate was used). The simulated soil temperature (T) and the Q_{10} (Fig. 1) were used to calculate the temperature-adjusted rate (R_{hT}) using the following relationship:

$$R_{hT} = R_{h14} * \exp((T - 14) * \ln(Q_{10})/10) \quad (10)$$

Respiration was calculated on an hourly basis from simulated soil temperatures, and summed to produce a daily value. This value was then multiplied to produce a monthly value. The Q_{10} values used for these calculations were measured between 4 and 14°C. Soil temperatures extended below this range during the winter months. However in a previous study at the same location, SIGR measurements were carried out on soils collected from beneath the snowpack using temperatures ranging from 0 to 14°C, and there was no significant difference between substrate-responsive microbial biomass measured at 0 and 4°C (Monson et al. 2006b). Therefore it is unlikely that there is a major discontinuity in winter microbial activity between 0 and 4°C.

The model was run in three ways to demonstrate the importance of the microbial community: (1) using winter parameters for the entire year, (2) using summer parameters for the entire year, and (3) shifting between the two extremes using linear interpolation between measured data points. The relative effects of the temperature response, Q_{10}, versus the growth parameters, μ_{max} and Y_c, were investigated by allowing all the parameters to vary, or holding one set constant at the yearly average while varying the others.

Table 1 Substrate induced growth response estimates of microbial biomass C (μg C g^{-1})

Temperature (°C)	6/23/2004	11/17/2004	1/11/2005	4/21/2005
14	948.8 (8.6)	988.7 (259.8)	220.5 (1.7)	209.2 (21.5)
4	No growth	No growth	No growth	866.1 (177.6)

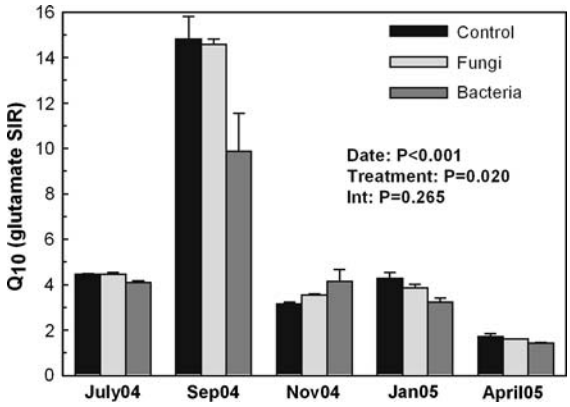

Fig. 1 Seasonal changes in Q_{10} of glutamate SIR in which bacterial and fungal components were estimated using specific inhibitors. *P* values are for the 2-way ANOVA, with date and inhibitor treatment coded as categorical variables

Results

Temperature responses of the soil microbial community varied substantially between summer and winter. Microbial biomass active at 14°C was highest in summer and fall and lower during the snow-covered months of winter and spring. No growth response was observed at 4°C until early spring, when biomass beneath the snowpack reached levels equivalent to those active at 14°C in summer (Table 1). The temperature response (Q_{10}) of SIR varied by season and by functional group (Fig. 1). The fungal inhibitor, cycloheximide, significantly lowered Q_{10} overall (though the trend was reversed on one date), indicating that for most dates bacteria were more active at low temperatures than fungi. In the summer, inhibitors of bacteria had no effect on biomass estimates, but caused an 8–16% decrease in the other seasons, indicating an increased contribution of bacterial activity in SIGR measurements in the colder seasons (Fig. 2a). Conversely, the fungal inhibitor had the lowest effect in winter. The different seasonal patterns of bacterial and fungal biomass lead to a significant date by treatment interaction (Fig. 2a).

Microbial growth-related parameters also varied seasonally and by functional group. Maximum exponential growth rates (μ_{max}) of soil microbial biomass growing on glutamate were higher during the snow-covered periods of winter and spring than summer or fall (Fig. 2b). The microbial biomass of soils treated with the fungal inhibitor consistently grew faster than the microbial biomass of controls or soils treated with antibacterial compounds, indicating that SIGR-responsive bacteria had higher growth rates than fungi, overall. Growth yields were lower in the winter

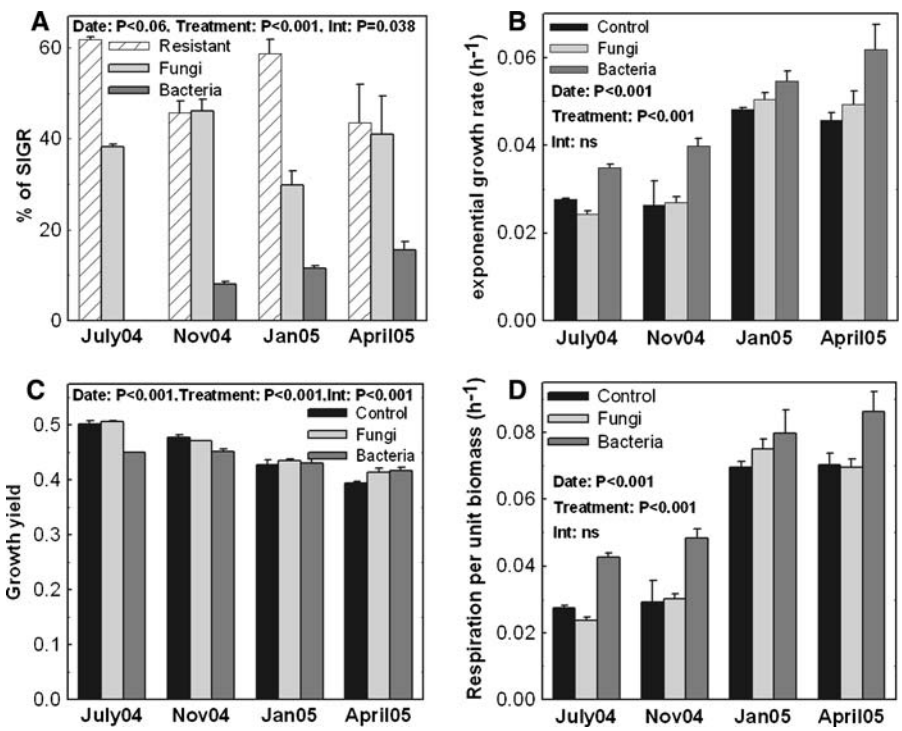

Fig. 2 Microbial growth kinetics in soils collected in different seasons, measured using SIGR experiments performed at 14°C in the presence of antifungal and antibacterial compounds: **a** bacterial, fungal and inhibitor-resistant biomass, expressed as a fraction of control treatments, **b** exponential growth rates, **c** growth yields, **d** ratio of respiration rate to active microbial biomass C. *P*-values are for the 2-way ANOVA, with date and inhibitor treatment coded as categorical variables

and spring than in summer or fall (Fig. 2c). Inhibitors of fungi and bacteria had significant effects on growth yield that varied by season, as shown by the significant treatment × date interaction (Fig. 2c). During the summer and fall, cycloheximide lowered growth yield, indicating that bacteria active at these times had lower yields than fungi.

The two parameters, growth rate and yield, collectively determine the quantity of CO_2 produced over time by a growing population of microbes. The increased growth rate and decreased growth yield in under-snow microbial communities combine to produce an especially high respiration rate during growth, when compared to summer communities growing at the same temperature (Fig. 2d). The antifungal treatment consistently had the highest such ratio, indicating a higher specific respiration rate for bacteria in these experiments. For a given temperature range there was always a negative relationship between growth rate and yield (Fig. 3), accentuating seasonal changes in respiration per unit biomass as the two parameters change in opposite directions (Fig. 2d). Independent measurements of soil respiration per unit microbial biomass show significant increases in winter relative to summer (Table 2).

To determine whether these seasonal changes in growth and respiratory kinetics were associated with changes in the bacterial community, clone libraries of 16S rRNA were constructed for the winter and summer communities that grew during the SIGR incubations. The most abundant bacteria identified in

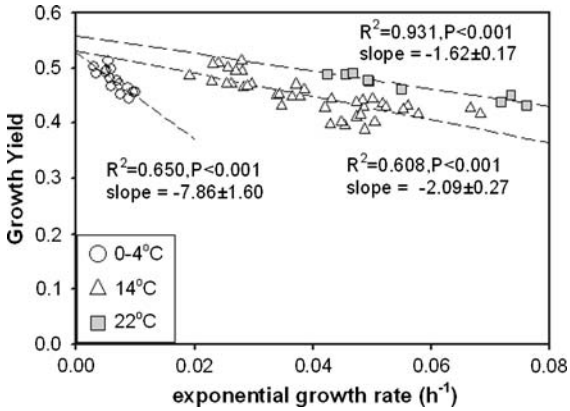

Fig. 3 Relationship between exponential growth rate and growth yield in SIGR experiments performed on soils at temperatures ranging from 0 to 22°C. Regression slopes ± standard errors are shown

Table 2 Two indices of the level of metabolic activity in winter (Jan 2005) and summer soils (July 2003, July to Aug 2004)

	Summer	Winter	*P*(ANOVA)
Metabolic quotient[a]	0.87 ± 0.08	1.99 ± 0.33	<0.0001
Specific respiration[b]	0.20 ± 0.02	0.34 ± 0.05	0.03

[a] Ratio of soil respiration, measured in the lab at 22°C, to glucose substrate-induced respiration (unitless)

[b] Ratio of soil respiration, measured in the lab at 22°C, to microbial biomass C, measured by fumigation-extraction (units of d^{-1})

the clone libraries were also isolated in pure culture. The cultured and cloned bacteria from the SIGR incubations were closely related or identical to native bacteria found in un-incubated subalpine soils (Fig. 4). Based on the permutation tail probability (PTP) test (Martin 2002), the winter and summer post-SIGR communities are phylogenetically distinct ($P = 0.006$). The winter library was dominated by sequences of *Janthinobacterium*. The summer library was dominated by sequences of *Burkholderia,* and a cluster of sequences that includes the genus *Variovorax* (Table 3). *Pseudomonas* species were cultured from both soils, but were found at relatively low frequencies in the summer library. Fungal isolates were obtained during summer, and sequencing of the internal transcribed spacer (ITS) region identified two isolates as Ascomycetes, related to *Cladosporium cladosporoides* and *Phoma* sp., and a third isolate as a Zygomycete, related to *Mortierella hyalina*. The fourth fungal isolate is currently unidentified.

Growth kinetics of bacterial isolates were characterized in liquid culture by turbidity, and bacterial and fungal growth kinetics were also characterized in separate experiments using CO_2 production. Growth rates and Q_{10} of isolates mirrored those observed at the whole soil level (Table 4). The winter bacterial isolates grew faster than the summer bacterial isolates (with the exception of the *Pseudomonas* isolates which were cultured from both winter and summer soils). All growth rates between isolates differed significantly from each other, except for *Sphingomonas* and *Burkholderia* at 4°C, and *Sphingomonas* and *Variovorax* in the accumulated CO_2 experiment. The summer bacterial isolates grew especially slowly at 4°C, resulting in higher Q_{10} values. All differences between temperature responses were significant.

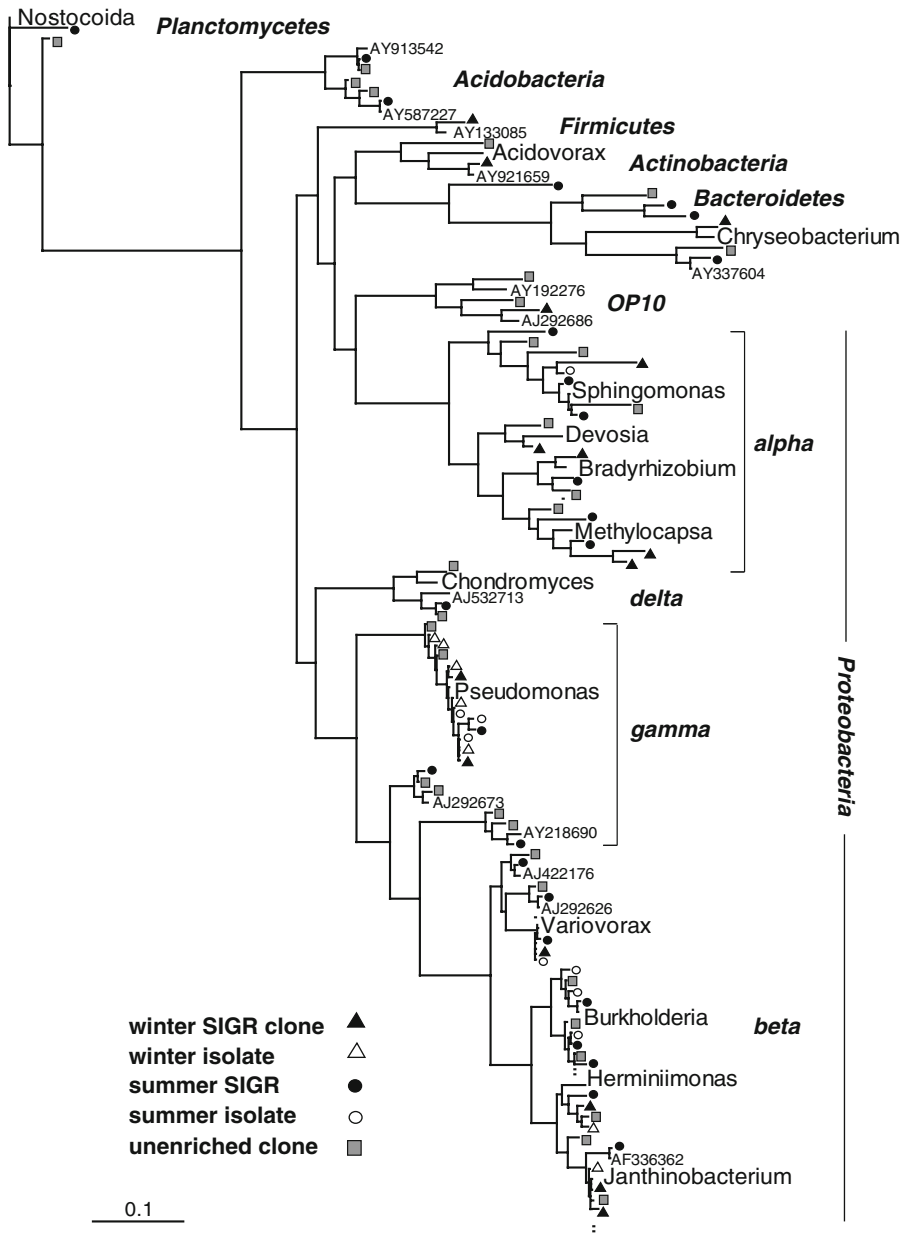

Fig. 4 Neighbor joining phylogenetic tree of 16S rRNA sequences from cultured isolates and clone libraries obtained from winter and summer SIGR experiments and from unenriched subalpine soils (Lipson 2007). Accession numbers and genus names are guide sequences from GenBank

Seasonal patterns in growth yields of isolates did not match whole soil patterns as clearly as did rate and Q_{10}, though the high growth yields of summer fungal isolates were consistent with the inhibitor SIGR experiments (Fig. 2c). *Pseudomonas* isolates had both high growth rates and yields. When *Pseudomonas* was excluded, yield was significantly negatively related to rate (Fig. 5).

We constructed a model to test whether the large observed seasonal variation in microbial growth kinetics could significantly influence heterotrophic respiration (R_h). The simulated soil temperature regime is shown in Fig. 6. The most realistic scenario, made by interpolating between seasonally measured kinetic values, produced an annual R_h of 22.67 mol CO_2 m^{-2} $year^{-1}$. This model agrees well with SIPNET ecosystem process models conditioned on eddy flux data from the site (Sacks et al. 2007; Zobitz et al. 2008), and with chamber and gradient measurements of soil respiration (R_s) (Monson et al.

Table 3 Dominant bacteria in SIGR clone libraries (%)

	Winter	Summer
Proteobacteria (total)	81.0	75.9
Burkholderia	0	17.2
Janthinobacterium	38.0	0
Pseudomonas	9.5	3.4
Sphingomonas	4.8	10.3
Variovorax	4.8	17.2
Bacteroidetes	4.8	13.8
Acidobacteria	0	6.9

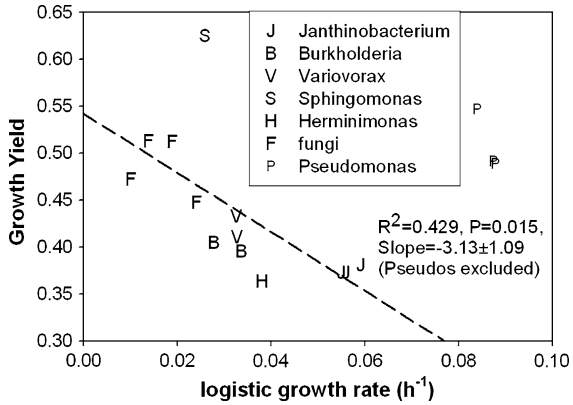

Fig. 5 Relationship between growth rate and growth yield for bacterial and fungal isolates measured in liquid cultures at 14°C. The regression results shown exclude the *Pseudomonas* isolates

2006a; Scott-Denton et al. 2006) (Fig. 7). A sensitivity analysis revealed that, because of the winter community's high specific respiration rates and ability to grow at low temperatures, annual R_h would be 6.02 times higher if the winter microbial community persisted all year (66.59 mol CO_2 m^{-2} $year^{-1}$) compared to a summer community persisting all year (11.07 mol CO_2 m^{-2} $year^{-1}$). The relative importance of growth kinetics (Y_c and μ_{max}) and Q_{10} in determining R_h were explored by holding one set constant while varying the other. Winter Q_{10} values increased annual R_h by a factor of 2.35 over those obtained using summer values, while growth kinetics produced a factor of 2.56. Multiplied together, these account for the factor of 6.02 cited above.

Discussion

Although microbial communities dominate most biogeochemical processes on earth, we know little about how ecophysiological traits of individual microbes scale up to the ecosystem and global levels (Enquist et al. 2003; Torsvik and Ovreas 2002; Zak et al. 2006). Such a linkage is needed if we are to develop truly mechanistic models of how changing climate and other disturbances will affect global biogeochemical fluxes. The link between the physiology of individual microbial isolates and biogeochemical processes is generally uncertain, given the complexity and low culturability of microbial communities (Hugenholtz et al. 1998; Torsvik and Ovreas 2002). The present study is one of the first to our knowledge to successfully account for seasonal variations in a major biogeochemical function based on underlying kinetics of microbial growth and respiration. Seasonal variations in microbial growth kinetics and Q_{10} were linked to changes in the composition of the microbial community. The inhibitor studies showed that of those microbes active in the SIGR experiments, bacteria had higher specific respiration rates than fungi as a result

Table 4 Growth kinetics in liquid culture for isolates obtained in summer (s), winter (w) or both (s/w)

Isolates	Season	R_{OD} (14°C)[a]	R_{OD} (4°C)[a]	R_{co_2} (14°C)[a]	Yield	Q_{10}
Janthinobacterium	w	0.069	0.032	0.057	0.375	2.14
Herminiimona	w	0.049	0.028	0.038	0.363	1.76
Pseudomonas	s/w	0.115	0.041	0.087	0.509	2.83
Burkholderia	s	0.042	0.011	0.032	0.400	3.71
Variovorax	s	0.025	0.008	0.033	0.421	3.21
Sphingomonas	s	0.033	0.012	0.026	0.624	2.76
Fungi[b]	s	nd	nd	0.018	0.486	nd

[a] Logistic growth rates (R, h^{-1}) measured at 4 and 14°C by optical density (OD) or by accumulated CO_2

[b] Means of four fungal isolates

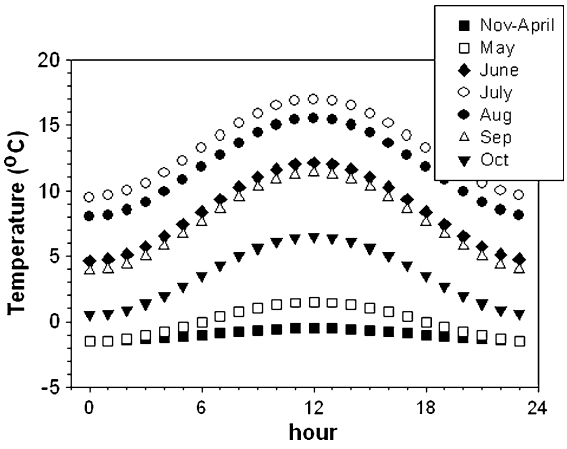

Fig. 6 Simulated hourly temperatures for each month used in the model of R_h

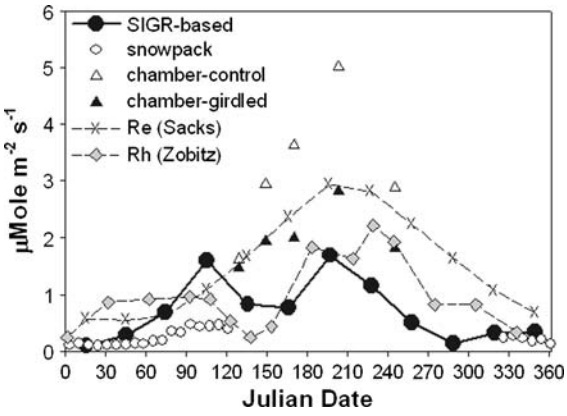

Fig. 7 Comparison of R_h predicted from the SIGR-based model with various models (*symbols with lines*) (Sacks et al. 2007; Zobitz et al. 2008) and measurements (*symbols only*) (Monson et al. 2006a; Scott-Denton et al. 2006) of R_h, R_s and R_e. Girdled measurements estimated R_h by eliminating plant respiration from R_s (Scott-Denton et al. 2006)

of a combination of higher growth rates and lower growth yields. Variations in fungal:bacterial ratios have been linked to altered specific respiration rate in previous studies (Lipson et al. 2005; Sakamoto and Oba 1994). The increased proportion of active bacterial biomass under the snowpack contributes to the higher specific respiration rates observed in soils in winter and spring. The molecular and pure culture studies further demonstrated that the communities that gave rise to the contrasting respiratory kinetics were composed of distinct species. Moreover, the growth kinetics and Q_{10} of bacterial and fungal cultures generally reflected the properties of the whole soil in the season in which they were isolated. Given the complexity of microbial communities, it is surprising that whole soil properties can be partly explained by the physiology of a small number of representative isolates. That the bacteria in this study are actually representative is shown by their close relationships with native bacteria from subalpine soils, and that *Burkholderia*, which dominated the summer SIGR libraries, were also the most abundant sequences in natural subalpine soils in the summer (Lipson 2007). It is unclear why *Pseudomonas* did not follow the negative relationship between growth rate and yield. Perhaps glutamate is the preferred substrate for *Pseudomonas*, alone, allowing simultaneously rapid and efficient growth compared to the other isolates.

One of the most fundamental aspects of our findings is that the trade-off between microbial growth rate and cellular yield at the individual species level can scale up to greatly affect ecosystem CO_2 fluxes. A consistent inverse relationship between growth rate and yield was observed across gradients of season and of bacterial:fungal ratio. This relationship accentuated the effects of seasonal variations on soil respiration by producing two distinct community types: a fast-growing, low yield, under-snow community with high specific respiration rates and a slow-growing, high yield, snow-free community with lower specific respiration rates.

The model of soil microbial respiration produced from measured growth kinetics and Q_{10} showed that changes in microbial community had marked impacts on the ecosystem-level process, R_h. The model predicted a sixfold difference in annual R_h between the two extreme community types. The main purpose of the model was to explore the importance of microbial growth kinetics to soil respiration. However, the full version of the model, in which community parameters shift seasonally between measured values, compared well with previously measured and modeled values of R_h and R_s, both in seasonal patterns and magnitude. The bimodal seasonal pattern of R_h was predicted in another modeling study (Zobitz et al. 2008), and a decline in soil respiration that we predict for the end of the spring was also observed in studies conducted at the end of the snow-covered period (Monson et al. 2006a). Annual R_h from our model represents 47.0% of the mean R_e estimated for this forest (Sacks et al. 2007). Based on estimates of the contribution of R_s to R_e in

this forest (Monson et al. 2006a), and the range of R_h/R_s reported in the literature (Hanson et al. 2000; Subke et al. 2006), this value should (and does) fall in the range of 6.3–56.3%. The annual R_h predicted from the SIGR-based model is likely an overestimate, as effects of C- or H_2O-limitation were not included.

The trade-off between growth rate and yield has been noted in several other ecological contexts. In a study of soda lakes, high yield, low growth-rate *Thioalkalimicrobium* species were more tolerant of starvation than the low yield, high growth-rate *Thioalkalivibrio* species (Sorokin et al. 2003). The yield-rate concept has also been applied to understanding cross-feeding in microbial populations (Costa et al. 2006; Pfeiffer and Bonhoeffer 2004). In the present study, the winter microbial community apparently employs a relatively wasteful, but competitive, strategy to exploit the higher resource environments below snow packs, while the summer community is more adapted to a low resource, cooperative strategy. The cooperative strategy succeeds in spatially structured environments, like biofilms (Kreft 2004; Kreft and Bonhoeffer 2005). It is possible that the summer community is largely associated with root biofilms. A recent study also found that cooperative and competitive strategies can co-exist in well-mixed environments provided there are seasonal variations in the concentrations of substrate or toxic metabolic intermediates (MacLean and Gudelj 2006). A mechanism by which microorganisms can increase growth rate at the expense of yield is to carry out fermentation in addition to respiration (Kreft and Bonhoeffer 2005; Pasteur 1861; Pfeiffer et al. 2001). A fermentative metabolism would thrive in saturated, O_2-deprived soils during snowmelt. More C may be available in the late winter or early spring due to dead and damaged roots, freeze-thaw lysis of cells, and high water content. High levels of sucrose were observed during the winter in this ecosystem, possibly from lysis of plant roots (Scott-Denton et al. 2006). In the summer, C limitation may be more severe, especially in dry years (Scott-Denton et al. 2003). A similar seasonal trend in substrate availability was observed in a nearby Colorado alpine ecosystem (Lipson et al. 1999, 2000). Future studies should investigate whether substrate affinity also shifts seasonally, as one would predict. However, tests of the hypothesized trade-off between bacterial growth rates and their ability to compete at low resource availability have produced mixed results (Velicer and Lenski 1999).

A major implication of this study is that the composition of the soil microbial community strongly impacts the soil respiration rate, and that a disruption of microbial communities could alter ecosystem respiration. In the coniferous forest studied here, plant species changes could lead to different root-associated biofilms, changing summer microbial respiration. Changes in the depth and duration of spring snow pack could alter the balance between the winter and summer community types. Mountain snow packs have been declining recently (Mote et al. 2005), and the C balance of forest ecosystems is sensitive to changes in snow patterns (Black et al. 2000; Goulden et al. 1998; Monson et al. 2002, 2005). A disturbance that increases the resource availability to soil microbes, for example tree mortality, could exacerbate the effects of the disturbance on the C cycle by selecting for a more wasteful, fast-growing community with high specific respiration rate. On the other hand, the temperature adaptations of the winter and summer communities provide stability to this system; our model showed that a seasonally shifting community produced a more moderate respiratory response to temperature fluctuations. Microbial communities are generally well adapted to their ambient temperature regimes. A comparative study of decomposition rates across a wide range of ecosystem types found little influence of mean annual temperature (Giardina and Ryan 2000), and microbial activity has been observed to acclimate quickly to experimental warming (Luo et al. 2001). While changes in substrate availability can explain much of these effects (Eliasson et al. 2005), the present study provides evidence that such temperature adaptations could also result from changes in microbial community structure. Understanding the growth kinetics and temperature responses of microbial communities could provide the key to predicting ecosystem responses to global change.

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