

RESEARCH ARTICLE

Shifts in microbial trophic strategy explain different temperature sensitivity of CO₂ flux under constant and diurnally varying temperature regimes

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One sentence summary: Copiotrophy vs oligotrophy strategists determine thermal sensitivity of CO₂ flux.

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ABSTRACT

Understanding soil CO₂ flux temperature sensitivity (Q_{10}) is critical for predicting ecosystem-level responses to climate change. Yet, the effects of warming on microbial CO₂ respiration still remain poorly understood under current Earth system models, partly as a result of thermal acclimation of organic matter decomposition. We conducted a 117-day incubation experiment under constant and diurnally varying temperature treatments based on four forest soils varying in vegetation stand and soil horizon. Our results showed that Q_{10} was greater under varying than constant temperature regimes. This distinction was most likely attributed to differences in the depletion of available carbon between constant high and varying high-temperature treatments, resulting in significantly higher rates of heterotrophic respiration in the varying high-temperature regime. Based on 16S rRNA gene sequencing data using Illumina, the varying high-temperature regime harbored higher prokaryotic alpha-diversity, was more dominated by the copiotrophic strategists and sustained a distinct community composition, in comparison to the constant-high treatment. We found a tightly coupled relationship between Q_{10} and microbial trophic guilds: the copiotrophic prokaryotes responded positively with high Q_{10} values, while the oligotrophs showed a negative response. Effects of vegetation stand and soil horizon consistently supported that the copiotrophic vs oligotrophic strategists determine the thermal sensitivity of CO₂ flux. Our observations suggest that incorporating prokaryotic functional traits, such as shifts between copiotrophy and oligotrophy, is fundamental to our understanding of thermal acclimation of microbially mediated soil organic carbon cycling. Inclusion of microbial functional shifts may provide the potential to improve our projections of responses in microbial community and CO₂ efflux to a changing environment in forest ecosystems.

Keywords: microbial trophic strategy; temperature sensitivity (Q_{10}); constant vs varying temperature regimes; decomposition; 16S rRNA gene sequencing

INTRODUCTION

Forest covers 27.6% of terrestrial land and contributes to 39.1% of soil carbon (C) stock on Earth (Janzen 2004). As minor changes in C allocation belowground have profound impacts on atmospheric CO₂ (Dixon et al. 1994; Schindlbacher et al. 2011; Ziegler et al. 2013), studies on warming-induced soil C dynamics in forest ecosystems have gained increasing attentions (Monson et al. 2006; Ziegler et al. 2013; DeAngelis et al. 2015). The magnitude of warming-induced increases in soil C release through respiration can result from the change of C availability or thermal adaptation of microbial activity (Bradford et al. 2008). However, well-established adaptive tradeoffs in microbial heterotrophic decomposition of discrete C pools and their temperature dependencies are still elusive.

Temperature quotient (Q₁₀), an exponential index of temperature sensitivity, measures the additional CO₂ efflux for every 10°C increase and is widely used to describe the thermal response of soil organic matter (SOM) decomposition (Kirschbaum 1995; Luo et al. 2001; Kirschbaum 2006). The Q₁₀ value can be highly constrained by substrate quantity and quality (Fierer et al. 2005; Zhou et al. 2011; Frey et al. 2013). For example, the CO₂ efflux Q₁₀ declines with the warming-induced substrate depletion (Knorr et al. 2005) but rises with the increasing molecular complexity of substrates (Conant et al. 2011). Further, empirical studies have shown that the thermal acclimation of SOM decomposition is significantly related to microbial community-level responses (Karhu et al. 2014; Wei et al. 2014), especially to the responses mediated by prokaryotes (Rousk, Frey and Bååth 2012; Creamer et al. 2015). Alterations in the microbial community associated with distinct temperature dependency consequently result in differential metabolic responses in CO₂ efflux, with negative or positive effects (Bradford et al. 2008; Nie et al. 2013). These contrasting observations indicate the need to identify new microbially mediated mechanisms relating to more consistent responses of SOM decomposition.

Microbial community composition and structure are sensitive to alterations of soil properties, such as substrate availability (Fierer and Jackson 2006; Castro et al. 2010; Maestre et al. 2015). With respect to their adaptation to nutrient acquisition, microbes are historically classified into two main guilds: copiotrophs and oligotrophs (Koch 2001). Highly available C sources favor fast-growing copiotrophic lifestyles, while slow-growing oligotrophic strategists are more tolerant to substrate depletion (Fierer, Bradford and Jackson 2007; Bastida et al. 2015). Specifically, total C concentrations show negative relationships with the relative abundances of oligotrophic taxa, such as *Acidobacteria*, *Firmicutes*, *Verrucomicrobia* and *Chloroflexi* (Rui, Peng and Lu 2009; Schmidt et al. 2015; Zhalnina et al. 2015), while fast-growing *Proteobacteria* and *Bacteroidetes* taxa are positively related to the increased soil C availability and CO₂ flux rates (Fierer, Bradford and Jackson 2007; Thomson et al. 2010; Vuono et al. 2015). The *Proteobacteria* and *Bacteroidetes* are believed to remain active and abundant with decreasing temperature (Rui, Peng and Lu 2009; Lin et al. 2015; Mateos-Rivera et al. 2016), while warming enriches the abundances of *Acidobacteria* and *Firmicutes* (Hayden et al. 2012; DeAngelis et al. 2015; Mateos-Rivera et al. 2016). Improved understanding of the temperature regime responses of different microbial groups, such as trophic strategists like copiotrophs and oligotrophs, may be critical in merging the knowledge gap between climate warming and thermal acclimation of SOM decomposition.

The warming effects on CO₂ flux differ not only in the essential edaphic traits (e.g. C availability, microbial community struc-

ture) but also in external environmental variables. For instance, the thermal acclimation of microbially mediated CO₂ flux can vary upon temperature regimes (constant vs varying) (Hartley et al. 2008; Zhu and Cheng 2011). Moreover, distinct C-cycle feedbacks coupled with the changes of microbial composition and diversity may be determined by vegetation stand (Sampson et al. 2007), soil horizon (Schütt et al. 2014) or their interactions with thermal acclimation (Chen et al. 2010). The analysis of 16S rRNA gene amplicons has also revealed that the differentiation in prokaryotic community composition can be driven by vegetation type (Banning et al. 2011; Russo et al. 2012; Lin et al. 2014) or soil horizon (Hayden et al. 2012; Zhalnina et al. 2015). The inclusion of fluctuating temperatures in comparison with constant temperatures across a range of environmental variables (e.g. vegetation stand, soil horizon) will help elucidate whether environmentally distinct microbial communities will more clearly exhibit distinct responses during the thermal acclimation of C processes.

In this study, we hypothesized that (i) constant versus varying temperature regimes would result in preferential selection of microbial groups and differences in CO₂ efflux Q₁₀ values; and furthermore, (ii) acclimation in microbial community to vegetation stand and horizon will interactively influence the CO₂ flux Q₁₀. To elucidate the extent to which the above abiotic and biotic variables influence the thermal acclimation of CO₂ flux, we carried out a 117-day incubation study using the soils from 2 × vegetation stands, 2 × soil horizons and 2 × temperatures under varying and constant settings, respectively. We measured temporal dynamics of CO₂ efflux rates during the incubation, and applied high-throughput sequencing of 16S rRNA gene amplicons for prokaryotic community composition at the end of the incubation.

MATERIALS AND METHODS

Site description and sample collection

In September 2013, soils were randomly collected from horizon O (S_O, ca. 0–5 cm) and A (S_A, ca. 5–10 cm) separately in the coniferous forest (F_C) (42° 04'N, 128° 14'E, altitude 1400 m) and the deciduous forest (F_D) (42° 23'N, 128° 05'E, altitude 800 m) in Changbai Mountain reservoir in Jilin, China. Fresh soils were transported on ice and sieved < 5 mm and stored at 4°C until use. F_D-S_A, F_D-S_O, F_C-S_A and F_C-S_O soils had different features as explained by (i) C contents (%): 15.76, 36.23, 9.67 and 35.54; (ii) N contents (%): 1.06, 1.61, 0.40 and 1.33; (iii) C/N ratios: 14.87, 22.50, 24.18 and 26.72; (iv) pH values (soil: water = 1: 2.5): 5.35, 5.66, 4.46 and 4.80. The F_D and F_C soils were recognized as Albi-Boric Argosols and Bori-Udic Cambosols, respectively (Tian et al. 2014).

Microcosm setup

The aboveground maize (stalk and leaf) were collected from fields in Jilin, China (43° 19'N, 124° 14'E) in 2013. Maize residues (C% = 42.16; N% = 0.80) were sterilized and dried at 60°C to a constant weight over 1 week, cut into small pieces, ground (< 5 mm) and stored at room temperature until used. The residues were then mixed thoroughly with soils and added at the rate of 0.5 g C residue/g C soil (dry weight). A high level of residue addition can ensure a relatively constant supply of substrate for microbial growth throughout the incubation (Hartley et al. 2008; Bárcenas-Moreno et al. 2009). The maize residue was used as a standard organic material for the investigation of microbially mediated C processes in the forest ecosystems, as was previously conducted by Coûteaux et al. (2002). After measuring

and adjusting the bulk density each soil, the soil mixture was maintained ca. 60% water holding capacity and was packed into bottom-sealed polyvinyl chloride columns (inner diameter = 5 cm; height = 16 cm), and each column contained 6.5 g C residue and 12.8 g C soil (dry mass). Sterilized ultrapure water was added into soil mixture every 2–3 days to maintain the constant weight each column throughout the incubation period. Temperature regimes included constant low temperature at 10°C (CL_T), constant high temperature at 30°C (CH_T) and varying temperature between 10°C and 30°C with four programming stages each day to mimic diurnal temperature change outdoors: (i) 10°C for 6 h, varying low (VL_T), (ii) smoothly increasing from 10°C to 30°C for 6 h, (iii) 30°C for 6 h, varying high (VH_T) and (iv) smoothly decreasing from 30°C to 10°C for 6 h. The soil mixture was aerated at the flow rate of 30–50 mL/min using a pump automatically controlled by a timer, which periodically switching the pump on for 2-min and then off for 1-min intervals of time. To maintain moisture levels of the soil mixture, the air flow was initially pumped into a closed water tank and then separately propelled into each soil column. All soil mixtures were incubated in triplicate (36 total microcosms).

CO₂ flux rates and Q₁₀ values

CO₂ flux rates were measured separately at 0, 1, 2, 4, 8, 16, 22, 40, 67 and 117 days. Before C flux detection, the timer was bypassed and the pump was switched on to continuously circulate CO₂-free air through a soda lime column to remove atmospheric CO₂ inside the soil microcosms over 1 h. Thereafter, the timer was returned to its original status and CO₂ flux per column was collected in 0.5 M NaOH solution traps and C concentrations were subsequently measured through the TOC/TN 2000 Analyzer (Jena, Germany) (Zhu and Cheng 2011). The CO₂ samples under varying temperature regimes were collected separately at each temperature stage.

As Fang and Moncrieff (2001) suggested, Q₁₀ values were derived as:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10/(T_2-T_1)} \quad (1)$$

where R₁ and R₂ indicated respiration rates separately measured at temperature T₁ and T₂. R₁ and R₂ were derived either from constant or from varying temperature setting, by pairwise comparison between constant low (CL_T) and constant high (CH_T), or varying high (VH_T) and varying low (VL_T) temperature regimes, respectively.

Soil DNA extraction, 16S rRNA gene amplification and purification

At the end of the incubation (day 117), soil samples were collected and stored at -80°C for DNA analysis. The VL_T and VH_T samples were separately collected at the end of each temperature stage. Soil genomic DNA was extracted from 0.5 g soil with the PowerSoil DNA Isolation Kit (MoBio Laboratories, USA), and its concentration and A260/280 ratio were detected through a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., USA). The universal primers 515F (5'-GTGYCAGCMGCCGCGTA-3') and 909R (5'-CCCCGYCAATTCMTTTRAGT-3') were used for 16S rRNA gene amplification (Tao et al. 2014). A 12 base-paired length barcode was incorporated between the 515F primer and the adaptor. PCR reactions were performed with 50 μL reactions containing

1 × PCR reaction buffer, 0.4 μM dNTPs, 1.5 mM MgCl₂, 1.0 μM each primer, 1 U Ex Taq polymerase (TaKaRa Bio Inc, Japan) and 20 ng template DNA. The temperature programming was as follows: 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 40 s, annealation at 56°C for 60 s, extension at 72°C for 60 s and a final extension at 72°C (Li et al. 2014). All DNA templates were independently amplified twice and pooled together to form a composite PCR product and purified by MiniBEST Agarose Gel DNA Extraction Kit (TaKaRa Bio Inc, Japan) after electrophoresis with 1.5% agarose gel.

16S rRNA gene-based sequencing and data processing

Amplicon sequencing was performed on an Illumina MiSeq system (Illumina, Inc., USA). The sequence data were processed using QIIME Pipeline-Version 1.7.0 (<http://qiime.org/tutorials/tutorial.html>). All sequence reads were trimmed and assigned to each sample based on their barcodes with Ribosomal Database Project (RDP) Pipeline Initial Process (<http://pyro.cme.msu.edu/>). Multiple steps were required to remove the sequences with low quality, such as length < 150 bp, ambiguous base 'N' and average base quality score < 20. Sequences were clustered into operational taxonomic units (OTUs) and taxonomy was assigned using the open-reference OTU picking method in QIIME at a 97% identity threshold according to greengenes taxonomy via the RDP classifier (Wang et al. 2007; Li et al. 2014). The aligned 16S rRNA gene sequences were used for chimera check using the Uchime algorithm (Edgar et al. 2011). The sequences observed only once (singletons) were removed. The minimum number of high-quality reads in a sample was 7759. Therefore, a total of 7759 reads were randomly resampled from each sample. Such a sequencing depth, according to Lundin et al. (2012), could accurately describe global patterns in prokaryotic alpha- and beta-diversity. Rarefaction curves of different treatments from barcoded sequencing results were given in Fig. SI-1 (Supporting Information) and heatmap clustering in Fig. SI-2 (Supporting Information). We calculated alpha-diversity (phylogenetic distance whole tree (PD), chao1 estimator of richness, observed species and Shannon's diversity index) and beta-diversity (principal coordinate analysis (PCoA), heatmap clustering). Sequences were deposited in the European Nucleotide Archive (<http://www.ebi.ac.uk/ena>) under accession number 410658.

Statistical analyses

We used a factorial analysis of variance (ANOVA (Bonferroni post hoc test) to assess the effects of temperature regime, vegetation stand, soil horizon and their interactions on prokaryotic 16S rRNA gene sequence datasets. A repeated measures ANOVA (Bonferroni post hoc test) was applied for comparison on CO₂ rates and their Q₁₀ values derived from the varying environmental variables and their interactions across gas sampling time. Marked correlations between the temperature sensitivity of CO₂ and different prokaryotic phylotypes were tested with correlation matrices. Before ANOVA, the data were tested for the assumption of normality, and thus data for the CO₂ flux rate, Q₁₀ of CO₂ flux rate, alpha-diversity of prokaryotic community and temperature sensitivity of prokaryotic alpha diversity were log₁₀-transformed to meet requirements. All statistics were performed with Statistica 10.0 (StatSoft, Inc., USA). Differences at P < 0.05 were considered statistically significant. In order to analyze the distinction in prokaryote community, (i) PCoA from pairwise distances (unweighted and weighted

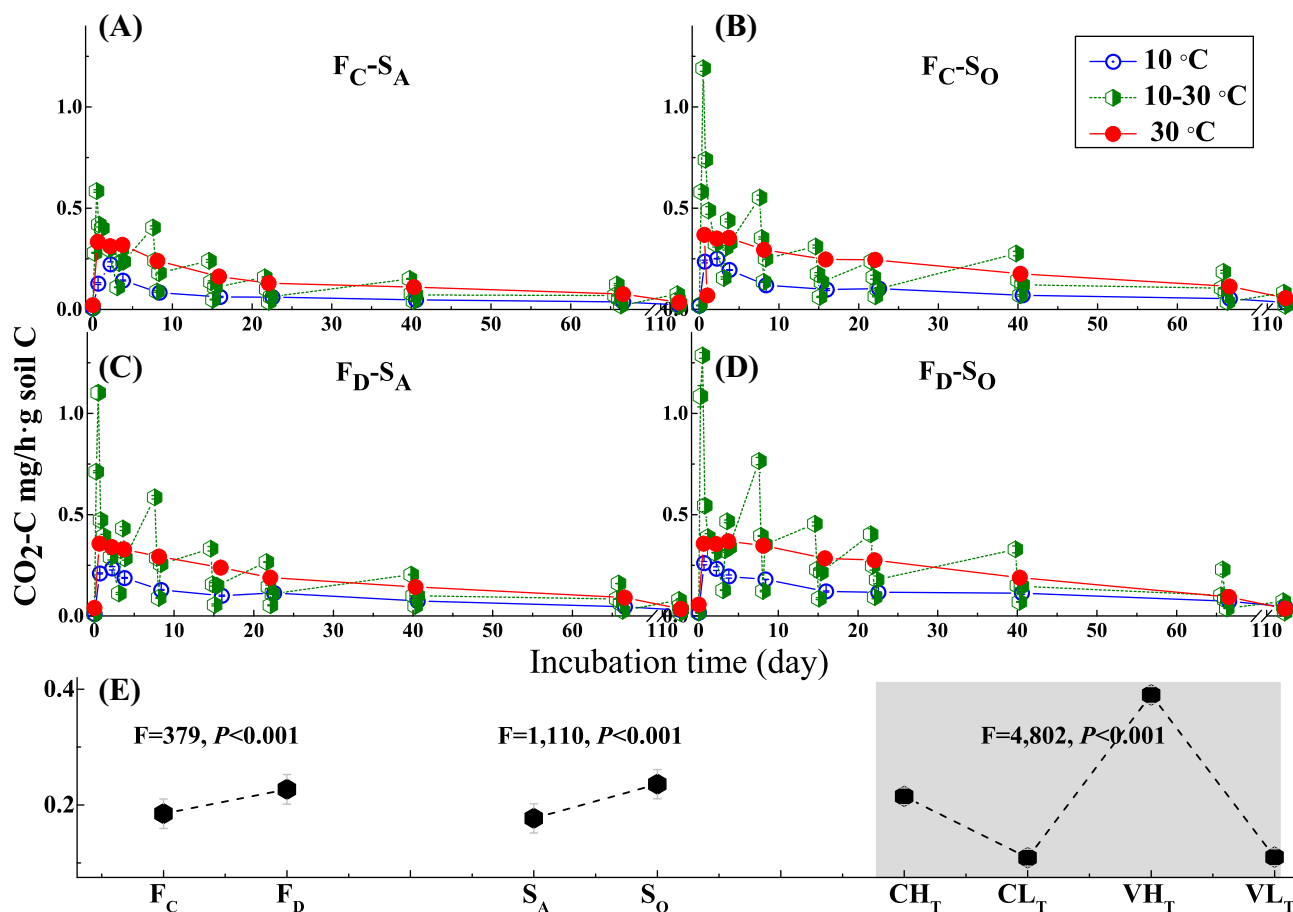


Figure 1. CO₂ flux rates as affected by different variables. Temperature regimes include constant low temperature at 10°C (CL_T) (empty circles), constant high temperature at 30°C (CH_T) (solid circles) and 10°C–30°C varying temperature regime (half-filled circles) including low at 10°C for 6 h (VL_T) and high at 30°C for 6 h (VH_T). Panels A, B, C and D separately indicate horizon A of coniferous forest (F_C-S_A), horizon O of coniferous forest (F_C-S_O), horizon A of deciduous forest (F_D-S_A) and horizon O of deciduous forest (F_D-S_O). Panel E shows the repeated measures ANOVA on the effects of different environmental variables for CO₂ rates including cross-site (F_C vs F_D, N = 24), soil horizon (S_A vs S_O, N = 24) or temperature regimes (CH_T, CL_T, VH_T and VL_T, N = 12). Error bars represent 1 × SE.

UniFrac distances for the 16S rRNA data) was applied with randomly selected 7759 sequences per sample (rarefaction), (ii) and a correlation matrix was formed with data relative abundance higher than 1% using the function heatmap.2 of the gplots package in R 3.1.3. Figures were plotted by Origin 9.0 (OriginLab Corp., USA).

RESULTS

CO₂ rates and Q₁₀ values

A repeated measures ANOVA showed that the instantaneous CO₂ flux rates (mg CO₂-C/g soil_{dw}·h) were lowest under CL_T (0.109) and VL_T (0.110), intermediate at CH_T (0.215) and highest with VH_T (0.390) ($P < 0.001$; Fig. 1E). The Q₁₀ values of CO₂ efflux on average increased from the constant (i.e. CH_T vs CL_T; 1.46) to the varying (i.e. VH_T vs VL_T; 2.07) temperature regime ($P < 0.001$; Fig. 2E). Such an increase was impacted by the interaction of temperature × vegetation stand and temperature × soil horizon ($P < 0.05$; Table SI-3, Supporting Information). As to the effect of vegetation stand ($P < 0.001$; Table SI-3, Supporting Information), in F_C, the Q₁₀ increased 28.9% from the constant (1.52) to the varying (1.96) temperature regime; in F_D, the Q₁₀ increased 55.0% from the constant (1.40) to the varying (2.17) temperature regime.

Alpha- and beta-diversity of prokaryotic community

With the randomly selected 7759 reads from each sample, it was confirmed that the number of observed prokaryotic OTUs on average ranked as CH_T (3966) < VL_T (4450) < CL_T (4724) ≈ VH_T (4761) ($P < 0.001$, Fig. 3E), which being consistent with Chao1, Shannon and PD index in general (Fig. 3E). The temperature dependency of prokaryotic alpha-diversity was distinguished between the varying and constant temperature regimes (i.e. CH_T/CL_T < 1 ≤ VH_T/VL_T) to a greater extent under F_C than F_D (e.g. observed OTUs and Shannon index), and under horizon O than A (e.g. Shannon index) ($P < 0.05$; Table SI-3, Supporting Information). The principal coordinate analysis (PCoA) (Fig. 4; Table SI-4, Supporting Information) and heatmap clustering (Fig. SI-2, Supporting Information) showed that soil microcosms with different prokaryotic community composition could be segregated by temperature setting (varying vs constant), vegetation stand (F_C vs F_D), and soil horizon (S_O vs S_A).

Specific temperature dependency of different prokaryotic phylotypes

The 16S rRNA gene amplicons were mainly affiliated with 12 phyla (Fig. 5) and roughly distributed into three major types of temperature dependency (Table 1). Temperature dependency

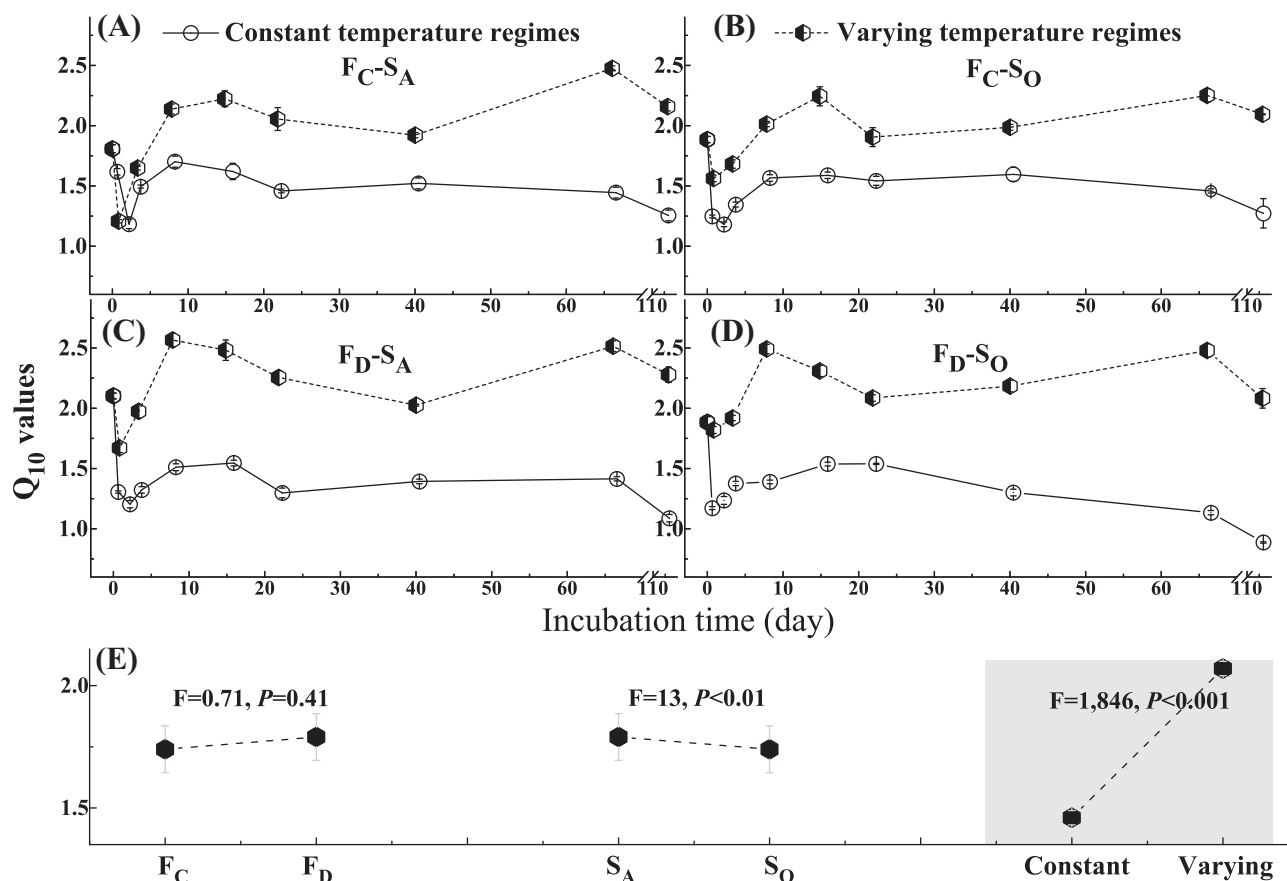


Figure 2. Q_{10} values of CO_2 flux rates as affected by different variables. Q_{10} values were either derived from constant temperature regimes (empty circles) or varying temperature regimes (half-filled circles), by pairwise comparison between constant low and constant high, or varying high and varying low, respectively. Panels A, B, C and D separately indicate horizon A of coniferous forest (F_C-S_A), horizon O of coniferous forest (F_C-S_O), horizon A of deciduous forest (F_D-S_A) and horizon O of deciduous forest (F_D-S_O). Panel E shows the repeated measures ANOVA on the effects of different environmental variables for Q_{10} values including cross-site (F_C vs F_D , $N = 12$), soil horizon (S_A vs S_O , $N = 12$) or temperature regimes (constant vs variation, $N = 12$). Error bars represent $1 \times SE$.

type I accounted for 21.7%–48.3% of total prokaryotic OTUs from each sample (Fig. 5), and was characterized by higher ratios of CH_T/CL_T (1.06–5.34) than VH_T/VL_T (0.921–1.32) (Table 1). Such a character especially occurred to a greater extent at S_A and F_C (e.g. *Acidobacteria*) compared to their counterparts S_O and F_D (Table 1), respectively. The temperature dependency type II (35.4%–57.0% of all prokaryotic OTUs from each sample), on the contrary, decreased from the ratios of VH_T/VL_T (0.956–1.50) to CH_T/CL_T (0.374–0.831). Additionally, the *Proteobacteria/Acidobacteria* ratio of CL_T significantly increased with F_C , while the *Firmicutes/Bacteroidetes* ratio of CH_T was greatest with horizon A (Table 1).

Tracking species of interest

The 50 OTUs (each >1% of all prokaryotic OTUs from each sample), used in the heatmap clustering (Fig. SI-2, Tables SI-5 and SI-6, Supporting Information), were mainly affiliated with *Proteobacteria* (17/50, i.e. 17 OTUs out of the 50 most abundant OTUs), *Acidobacteria* (10/50), *Bacteroidetes* (9/50), *Chloroflexi* (5/50), respectively. The OTUs of temperature dependency type I followed a consistent tendency of CH_T/CL_T vs VH_T/VL_T under F_C and horizon A, e.g. OTU60 (*Candidatus Koribacter*) (6.05 vs 0.754 in F_C ; 3.48 vs 0.726 in horizon A, respectively), OTU48 (*Acidobacteriaceae*) and OTU49 (*Acidobacteriaceae*) (1.56–2.23 vs 0.852–0.900 in F_C ; 1.58–1.75 vs 0.866–0.952 in A, respectively) (Table SI-6,

Supporting Information). Temperature dependency type II OTUs showed consistent temperature sensitivity between CH_T/CL_T and VH_T/VL_T (Table SI-6, Supporting Information) (except for OTU649 (*Alphaproteobacteria*) in Table SI-5, Supporting Information).

DISCUSSION

Constant and varying temperature regimes favor different microbial strategists

More pronounced thermal responses in CO_2 flux under the varying temperature regime were mainly attributed to significantly higher CO_2 efflux of varying high (VH_T) than that of constant high (CH_T) treatments (Figs 1 and 2). As the microbially mediated CO_2 flux is a proxy for the readily decomposable C substrates (Fierer, Bradford and Jackson 2007; Sampson et al. 2007; Schütt et al. 2014), greater C availability to the microbial community under VH_T than CH_T is reasonable. Furthermore, as increased prokaryotic diversity is always positively related to increased substrate C availability (Leff et al. 2012; Maestre et al. 2015), the fact that the alpha-diversity was highest with VH_T but lowest with CH_T (Fig. 3) supported our expectation that VH_T had greater substrate C availability and thus higher heterotrophic respiration.

The distinct CO_2 flux Q_{10} between the varying and the constant settings could also be reflected by proportional differences

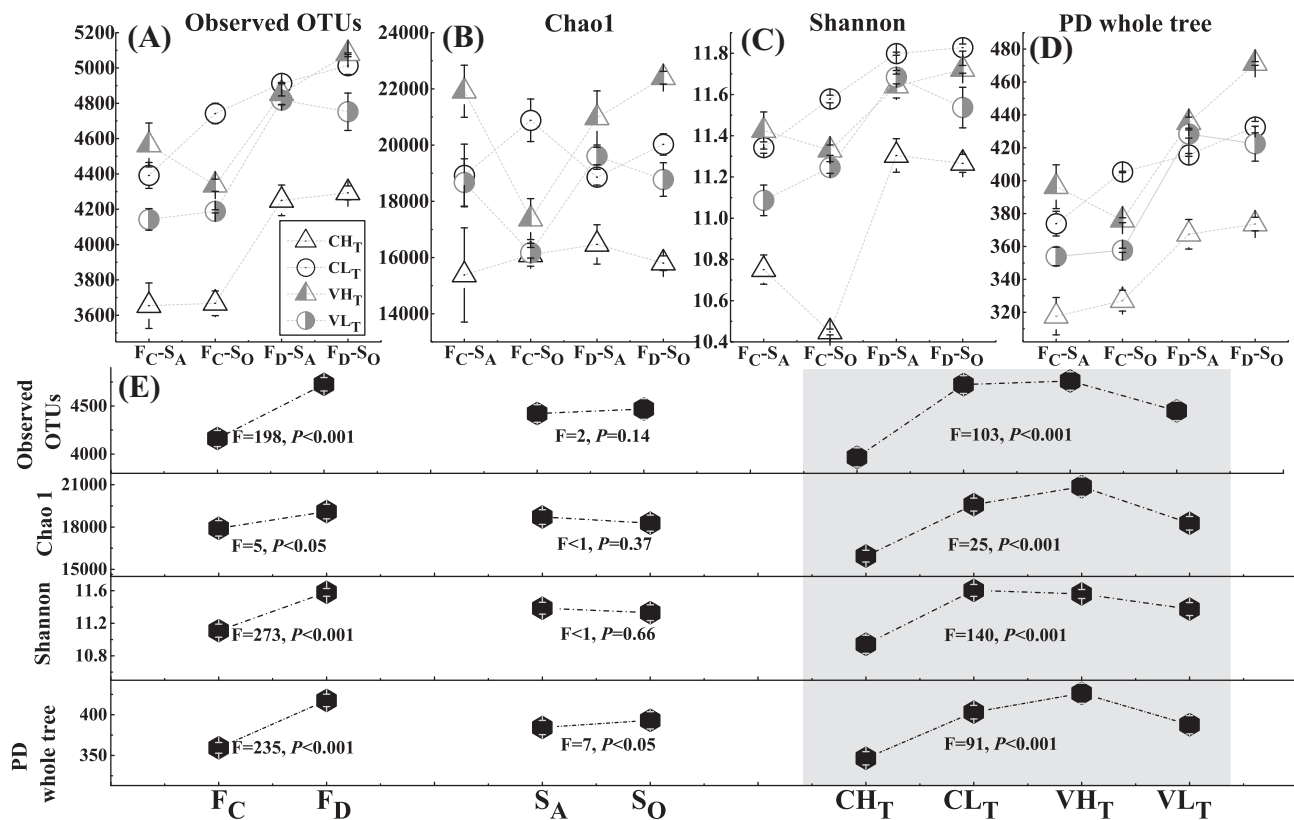


Figure 3. Alpha-diversity of prokaryotic community as affected by different variables. Temperature regimes include constant high temperature at 30°C (CH_T) (empty triangles), constant low temperature at 10°C (CL_T) (empty circles) and 10°C–30°C varying temperature regime including high at 30°C for 6 h (VH_T) (half-filled triangles) and low at 10°C for 6 h (VL_T) (half-filled circles). Vegetation stands include coniferous forest (F_C) and deciduous forest (F_D). Soil horizons include horizon A (S_A) and horizon O (S_O). Panels A, B, C and D separately indicate alpha-diversity of observed OTUs, Chao1, Shannon and PD indexes. Panel E separately plots ANOVA comparisons of observed OTUs, Chao1, Shannon and PD indexes between F_C and F_D, S_A and S_O or different temperature regimes i.e. CH_T, CL_T, VH_T and VL_T. F_C (N = 21), F_D (N = 21); S_A (N = 22), S_O (N = 20); CH_T (N = 12), CL_T (N = 9), VH_T (N = 10), VL_T (N = 11). Error bars represent 1 × SE.

in prokaryotic phyla: the relative abundances of prokaryotes of temperature dependency type I were characteristic of a negative feedback (i.e. VH_T/VL_T < CH_T/CL_T), but those of temperature dependency type II responded positively (i.e. VH_T/VL_T > CH_T/CL_T) (Tables 1, SI-5, and SI-6, Supporting Information). This distinction suggests that type I prokaryotes can overcome greater C depletion conditions of CH_T, but fail to compete under the more highly available C status found in VH_T. The opposite condition was true for type II. Therefore, the type I and II groups were appropriately categorized as oligotrophs and copiotrophs, respectively. This is in agreement with previous studies (Cleveland et al. 2006; Sun et al. 2013; Vuono et al. 2015), in which *Acidobacteria* and *Firmicutes* (type I) were reported as oligotrophs and were more resistant to C deficiency, whereas elevated C availability was positively correlated with copiotrophs that were comprised of *Proteobacteria* and *Bacteroidetes* (type II). Due to a large amount of substrate addition (0.5g C residue/g C soil, dry weight), we are not surprised to find that the copiotrophic lifestyle generally dominated over oligotrophs, either at the phylum level (Fig. 5) or genus level (Table SI-6, Supporting Information).

In the context of thermal acclimation, different prokaryotic trophic groups can respond differently to changes in temperature (Maestre et al. 2015). Studies have shown that *Acidobacteria* (Castro et al. 2010; Wu et al. 2013; Mateos-Rivera et al. 2016) and *Firmicutes* (Hayden et al. 2012; Ikeda et al. 2015) are more abundant as temperature increases, while lower temperatures enrich *Bacteroidetes* (Castro et al. 2010; Pankratov et al. 2011) and *Proteobacteria* (Wu et al. 2013; Lin et al. 2015; Mateos-Rivera et al.

2016). Altogether, taking into account C availability, the increase in the *Proteobacteria*/*Acidobacteria* ratio is a proxy for increasing C availability and may be brought on by decreasing temperatures, while the *Firmicutes*/*Bacteroidetes* ratio positively relates to C depletion and increasing temperatures (Cirne et al. 2007; Yergeau et al. 2012; Xiong et al. 2014). The scenario was exhibited in this study in regard to the above temperature responses of the prokaryotic phylotypes: (i) CL_T (10°C) should maintain larger amounts of available C compounds due to the least C depletion, and was associated with the highest *Proteobacteria*/*Acidobacteria* ratio; (ii) CH_T (30°C) definitely caused the greatest depletion of available C compounds, and was associated with the highest *Firmicutes*/*Bacteroidetes* ratio (Fig. 1, Table 1).

The results that different temperature regimes favor different microbial trophic strategists and higher CO₂ flux Q₁₀ under varying than constant temperature regimes clearly validate our first hypothesis. It is known that the varying substrate availability due to repeated labile C inputs can lead to more stable and even distributions of the prokaryotic community compared with constant environmental conditions (Mau et al. 2014). In addition, microbial communities with greater phylogenetic evenness may function more efficiently (Werner et al. 2011). Thus, the 10°C–30°C varying temperature regime can induce more versatile C metabolic processes and avoid exclusively decomposing easily available C compounds. Over the 4-month adaptation to the diurnally varying temperature regimes, a larger set of fast-growing copiotrophic prokaryotes can promptly respond to the changes in the easily decomposable C pool and then quickly prevail

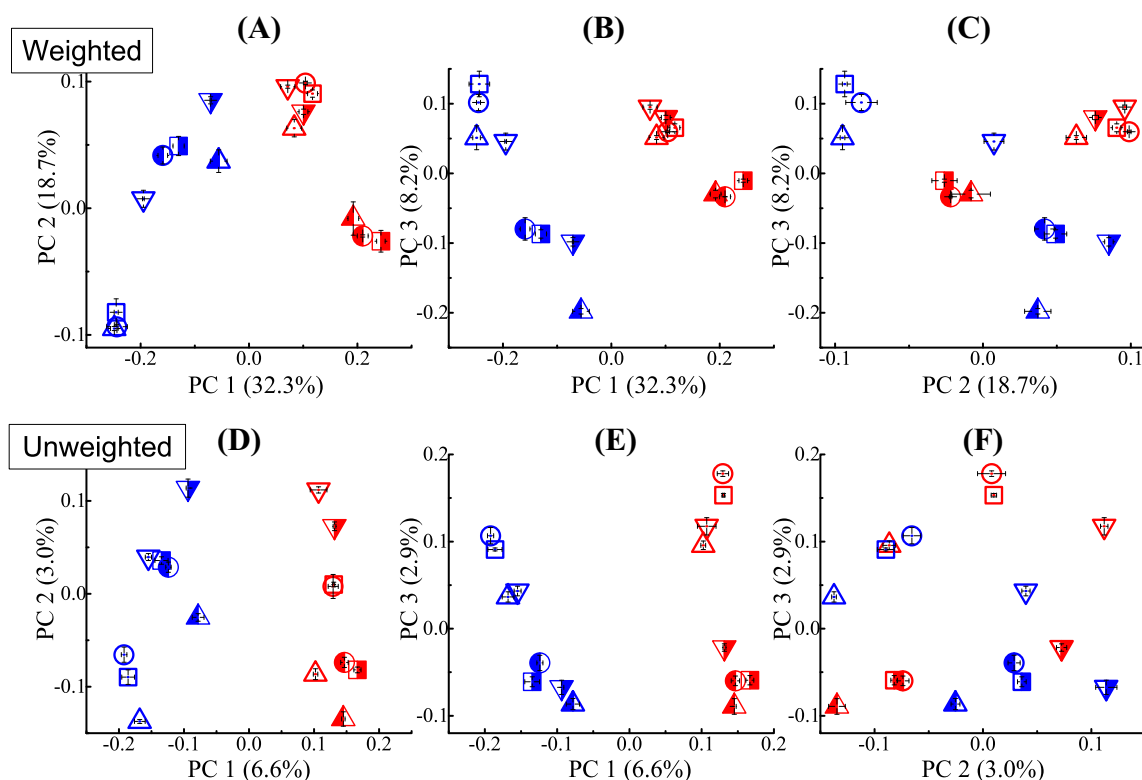


Figure 4. Beta-diversity of prokaryotic community by PCoA. Temperature regimes include constant high temperature at 30°C (CH_T) (regular triangles), constant low temperature at 10°C (CL_T) (inverse triangles) and 10°C–30°C varying temperature regime including high at 30°C for 6 h (VH_T) (circles) and low at 10°C for 6 h (VL_T) (squares). Vegetation stands include coniferous forest (F_C) in red and deciduous forest (F_D) in blue. Soil horizons include horizon A (S_A) (half-filled) and horizon O (S_O) (empty). PC1, PC2 and PC3 are separately significantly correlated with vegetation stand (VS), temperature (Tem) and soil horizon (SH) (Tables S1–3). Error bars represent 1 × SE. All soil mixtures were incubated in triplicate.

under more optimal temperature levels, as revealed by a significant increase in the observed OTU numbers from 4450 at VL_T to 4761 at VH_T (both being greater than 3966 at CH_T) ($P < 0.05$, Fig. 1). On the contrary, according to the previous studies (Raneklev and Bååth 2001; Bárcenas-Moreno et al. 2009; van Gestel, Reischke and Bååth 2013), the relatively long-term constant 30°C regime (CH_T) may inhibit a pool of bacteria that are less adapted, resulting in an irreversible species sorting where microbial individuals already better adapted can outcompete other less-adapted species and further maintain their dominance. Moreover, the pre-exposure to fast prokaryotic growth enhanced by constant warming leads to a greater loss of readily decomposable C (Rousk, Frey and Bååth 2012; Karhu et al. 2014) and a reduction of copiotrophic prokaryotic abundance and diversity (Han et al. 2013; Heeg et al. 2014), finally resulting in the superiority of the oligotrophic lifestyle in CH_T, as well as significantly lower alpha-diversity under CH_T (Fig. 3) and alpha-diversity CH_T/CL_T ratios being significantly less than 1 (Table SI-3, Supporting Information).

Thermal acclimation as affected by vegetation stand and soil horizon

As previously reported (Davidson et al. 2006; Chen et al. 2010), we found that the top soil horizon (S_O) had greater CO₂ flux rates than greater depth (S_A) (Fig. 1E). We postulated that this difference is a result of the upper horizon being comprised of larger amounts of available C and N than at depth. As Hayden et al. (2012) and Kim et al. (2012) suggested, fresh litter inputs comprising high-quality C, at sufficient levels,

can accelerate microbially mediated C decomposition in the surface soil. In contrast, the deeper horizons mainly contain more sieved 'labile C' and more recalcitrant compounds allocated from the top organic horizon. The greater recalcitrance of substrates in the subsurface has been reported to lead to a higher thermal dependency compared to the surface soils (Fierer et al. 2003), which was also found in our study (Fig. 2E, Table SI-3, Supporting Information).

As to vegetation stand effects, the significantly greater organic C decomposition rate in the deciduous stand (F_D) compared to the coniferous stand (F_C) (Fig. 1E) is also mainly due to the different C and N pools, especially much lower C/N ratios associated with the F_D soils. This result is supported by Raich and Tufekcioglu (2000) and Wunderlich et al. (2012), in which they suggested that greater substrate availability for deciduous ecosystems can sustain faster C cycles and reduce other nutrient acquisition costs. In addition, higher Q₁₀ values in F_D than F_C under varying temperature settings in this study (Table SI-3, Supporting Information) were also detected by Sampson et al. (2007), who suggested that the sufficient belowground supply of labile C (high C availability) in deciduous systems may cause higher Q₁₀ values compared to coniferous systems.

Interestingly, the tendencies of CO₂ efflux rates and Q₁₀ values, as affected by horizon or vegetation stand, are both uniformly and closely coupled to specific shifts in microbial copiotrophic and oligotrophic fractions.

As for horizon effects, CH_T had a greater *Firmicutes/Bacteroidetes* ratio (C depletion indicator) in horizon A than O (Table 1). The result confirms that the subsurface soil horizon is exposed to greater C deficiency compared to surface

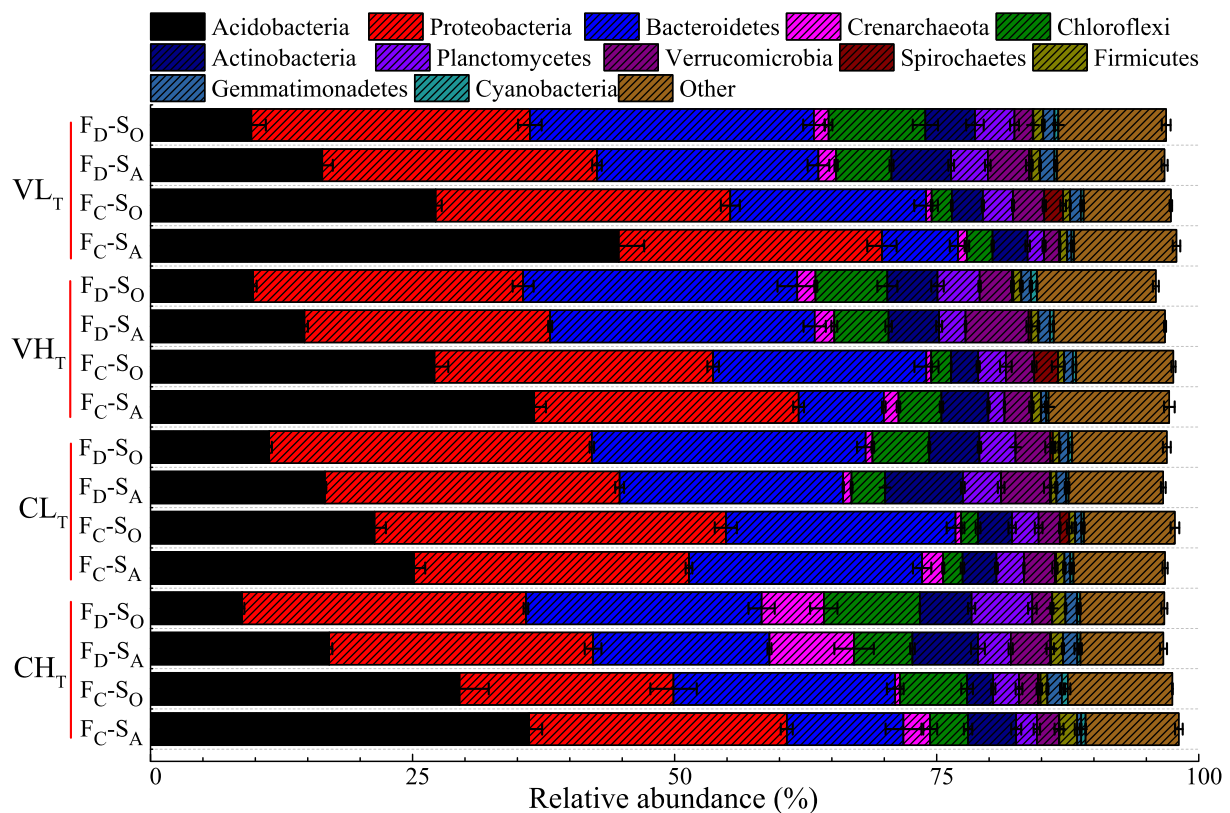


Figure 5. Relative abundances of major prokaryotic phylotypes. Temperature regimes include constant low temperature at 10°C (CL_T), constant high temperature at 30°C (CH_T) and 10°C–30°C varying temperature regime including low at 10°C for 6 h (VL_T) and high at 30°C for 6 h (VH_T). Vegetation stands include coniferous forest (F_C) and deciduous forest (F_D). Soil horizons include horizon A (S_A) and horizon O (S_O). Error bars represent 1 × SE. All soil mixtures were incubated in triplicate.

soil (Table 1). This is also supported by our detections of greater oligotrophic dominance in A (rather than O) by *Acidobacteria* as well as the OTU48, OTU49 and OTU60 (Tables 1, SI-5 and SI-6, Supporting Information), as the *Acidobacteria* and their members OTU60 (*Koribacteraceae*), OTU48 (*Acidobacteriaceae*) and OTU49 (*Acidobacteriaceae*) prefer degrading cellulose-derived C and adapting to the recalcitrance of complex compounds in SOM (Rawat et al. 2012; Koyama et al. 2014; López-Mondéjar et al. 2015; Schmidt et al. 2015). In addition, compared to top horizon O, root inputs through plant growth induce greater seasonal and diurnal changes of labile C availability in the deeper horizon A (Davidson et al. 2006). Altogether, the pulse dynamic available C in horizon A (compared to O) can only be perceived by and allocated into specialized copiotrophic groups, who are able to react to and utilize the newly introduced labile C, and subsequently quickly die or become dormant through the rapid turnover of labile C. Consequently, we detected some copiotrophic groups (temperature type II, e.g. *Bacteroidetes*, Table 1) and species (OTU290 (*Cytophaga*), Tables SI-5 and SI-6, Supporting Information) exhibiting greater temperature sensitivity in horizon A than in horizon O, which partially supporting larger Q₁₀ of CO₂ flux in horizon A than O ($P < 0.05$, Fig. 2E, Table SI-3, Supporting Information).

As for vegetation stand effects, the copiotrophs in F_C appeared to be less able to overcome C limitation than F_D, since F_C had a more significant difference between VH_T/VL_T and CH_T/CL_T ratios for the copiotrophic *Proteobacteria* (Table 1) and the OTU633 (*Proteobacteria*), OTU635 (*Alphaproteobacteria*), OTU750 (*Betaproteobacteria*), OTU763 (*Burkholderia*) and OTU926 (*Xanthomonadaceae*), as well as *Bacteroidetes* OTU269 (*Bacteroidetes*)

(Tables SI-5 and SI-6, Supporting Information) compared to their F_D counterparts. In fact, such pronounced detrimental effects of C depletion on the microbial community in F_C (rather than F_D) can also be revealed by greater declines in observed OTUs, the Shannon index ($P < 0.05$; Table SI-3, Supporting Information) and the *Proteobacteria*/*Acidobacteria* ratio (a proxy for high C availability) under more C exhausted temperature regimes (i.e. CH_T) (Table 1). Particularly, the C depletion under CH_T caused more significant increases in the oligotrophic *Acidobacteria* (Table 1) as well as their members OTU48 (*Acidobacteriaceae*), OTU49 (*Acidobacteriaceae*) and OTU60 (*Koribacteraceae*) under F_C compared to F_D (Tables SI-5 and SI-6, Supporting Information). Additionally, lower pH values evidently occurred with F_C compared to F_D, which suggests a higher maintenance C demand (i.e. stress status) of bacteria under F_C than under F_D (Blagodatskaya et al. 1998). Given that the fast-growing copiotrophic strategists were more inclined to respond to labile C than the oligotrophic types, it is reasonable that lower Q₁₀ values are observed in F_C than F_D under varying temperature settings (Table SI-3, Supporting Information).

In summary, the observations that shifts in microbial trophic groups explain microbial thermal C acclimation in forest ecosystems validate our second hypothesis about a common microbially mediated mechanisms on CO₂ efflux Q₁₀.

Unifying mechanism—copiotrophs versus oligotrophs control

To our knowledge, we show for the first time an experimental proof to substantiate a strongly coupled relationship between

Table 1. ANOVA of the effects of environmental variables for the temperature sensitivity of the major prokaryotic phyla and the ratios between specific prokaryotic phyla.

T-types	Taxonomic affiliation	Independent variables and their ranks		
		T	T × VS	T × SH
I	Acidobacteria	CH _T /CL _T (1.15)>VH _T /VL _T (0.939)***	F _C : CH _T /CL _T (1.41)>VH _T /VL _T (0.912)***; F _D : CH _T /CL _T (0.898)≈VH _T /VL _T (0.966)	S _A : CH _T /CL _T (1.23)>VH _T /VL _T (0.864)***; S _O : CH _T /CL _T (1.07)≈VH _T /VL _T (1.01)
	Chloroflexi	CH _T /CL _T (2.38)>VH _T /VL _T (1.10)***	Nd	S _A : CH _T /CL _T (1.85)>VH _T /VL _T (1.33)**; S _O : CH _T /CL _T (2.91)>VH _T /VL _T (0.865)***
	Crenarchaeota	CH _T /CL _T (5.34)>VH _T /VL _T (1.32)***	F _C : CH _T /CL _T (1.04)≈VH _T /VL _T (1.42); F _D : CH _T /CL _T (9.63)>VH _T /VL _T (1.22)***	Nd
	Gemmatimonadetes	CH _T /CL _T (1.45)>VH _T /VL _T (0.949)**	Nd	S _A : CH _T /CL _T (1.11)≈VH _T /VL _T (1.05); S _O : CH _T /CL _T (1.78)>VH _T /VL _T (0.845)***
	Firmicutes	CH _T /CL _T (1.90)>VH _T /VL _T (0.936)***	F _C : CH _T /CL _T (1.69)>VH _T /VL _T (1.06)*; F _D : CH _T /CL _T (2.11)>VH _T /VL _T (0.807)***	Nd
	Planctomycetes	CH _T /CL _T (1.06)≈VH _T /VL _T (0.921)	F _C : CH _T /CL _T (0.860)≈VH _T /VL _T (0.946); F _D : CH _T /CL _T (1.27)>VH _T /VL _T (0.896)*	S _A : CH _T /CL _T (0.807)≈VH _T /VL _T (0.854); S _O : CH _T /CL _T (1.32)>VH _T /VL _T (0.989)*
II	Proteobacteria	CH _T /CL _T (0.831)<VH _T /VL _T (0.956)**	F _C : CH _T /CL _T (0.774)<VH _T /VL _T (0.978)**; F _D : CH _T /CL _T (0.888)≈VH _T /VL _T (0.933)	S _A : CH _T /CL _T (0.916)≈VH _T /VL _T (0.953); S _O : CH _T /CL _T (0.746)<VH _T /VL _T (0.959)***
	Bacteroidetes	CH _T /CL _T (0.779)<VH _T /VL _T (1.10)***	Nd	S _A : CH _T /CL _T (0.645)<VH _T /VL _T (1.17)***; S _O : CH _T /CL _T (0.914)≈VH _T /VL _T (1.03)
	Verrucomicrobia	CH _T /CL _T (0.751)<VH _T /VL _T (1.50)***	F _C : CH _T /CL _T (0.803)<VH _T /VL _T (1.34)***; F _D : CH _T /CL _T (0.699)<VH _T /VL _T (1.66)***	Nd
	Spirochaetes	CH _T /CL _T (0.374)<VH _T /VL _T (1.49)***	Nd	S _A : CH _T /CL _T (0.522)<VH _T /VL _T (1.304)*; S _O : CH _T /CL _T (0.226)<VH _T /VL _T (1.672)***
III	Actinobacteria	CH _T /CL _T (1.02)≈VH _T /VL _T (1.02)	Nd	Nd
	Cyanobacteria	CH _T /CL _T (1.60)≈VH _T /VL _T (1.34)	Nd	Nd
	Proteobacteria/Acidobacteria ratio	VL _T (1.39)<VH _T (1.60)**; CH _T (1.49)<CL _T (1.68)*	F _C : CH _T (0.700)≈VH _T (0.837)≈VL _T (0.801)<CL _T (1.26)**; F _D : CH _T (2.29)≈CL _T (2.20)≈VH _T (2.11)≈VL _T (2.09)	Nd
	Firmicutes/Bacteroidetes ratio	CL _T (0.0282)≈VH _T (0.0427)≈VL _T (0.0521)<CH _T (0.0776)***	Nd	S _A : CL _T (0.0311)≈VH _T (0.0573)≈VL _T (0.0655)<CH _T (0.111)***; S _O : CH _T (0.0437)≈CL _T (0.0245)≈VH _T (0.0282)≈VL _T (0.0360)

Prokaryotic phyla were affiliated with three types of temperature dependency (T-type), i.e. type I, II and III. Environmental variables include temperature (T), vegetation stand (VS) and soil horizon (SH). Temperature regimes include constant high (CH_T), constant low (CL_T), high temperature in varying temperature regime (VH_T) and low temperature in varying temperature regime (VL_T). F_C, coniferous forest; F_D, deciduous forest. S_A, soil horizon A; S_O, soil horizon O. Nd, no detected. Differences between factor levels were tested by Bonferroni post hoc test. ***, P < 0.001; **, P < 0.01; *, P < 0.05.

CO₂ flux Q₁₀ and prokaryotic phylotypes (copiotrophy and oligotrophy) (Fig. 6). We argue that innate microbial property, such as r-K selection, may serve as distinguishing traits in describing these driving forces. The copiotrophic species have a positive relationship with the CO₂ efflux Q₁₀, due to their susceptibility to the easily decomposable C. Conversely, the oligotrophs prefer low-quality C, but appear to be more 'sluggish' to highly available C. The negative response by the oligotrophs is probably due to more complicated and diversified metabolic pathways of insoluble recalcitrant C (Blagodatskaya et al. 2007), which offsets the enhanced temperature response of CO₂ emissions from the copiotrophs. To strengthen the underlying mechanism, we suggest the use of a new term—'copiotrophs versus oligotrophs

control'—to describe the effect of copiotrophic dominance in a community that facilitates heterotrophic respiration of C emission into the atmosphere, and that the oligotrophic dominance will counterbalance such effects.

The copiotrophs versus oligotrophs control term is fundamental and quite beneficial for our conceptual understanding of microbial interactions with organic C cycling in soils, which is invaluable to help interpret microbially mediated ecological processes. For example, the Q₁₀ in our study showed inconsistent trends between constant and diurnally varying temperature regimes compared with those reported by Zhu and Cheng (2011): we detected that the CO₂ efflux Q₁₀ was larger under varying than constant temperature regime, which being in line with

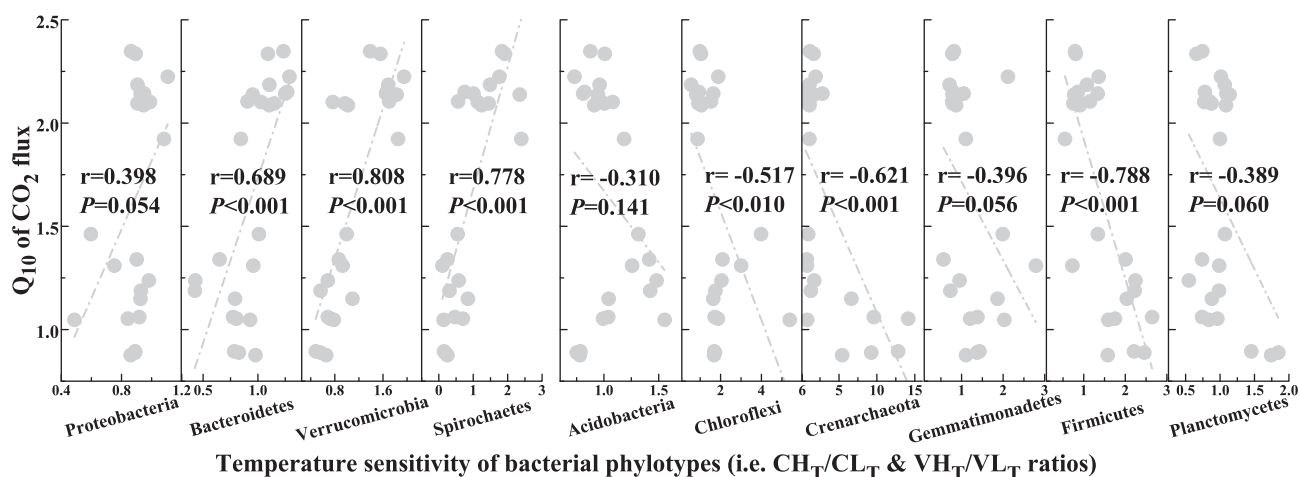


Figure 6. Relationship between temperature dependency of prokaryotic phylotypes and CO₂ flux rates. Marked correlations are significant at $P < 0.05$ ($N = 24$). Y-axis, Q_{10} values derived by pairwise comparison of CO₂ rates between constant low (CL_T) and constant high (CH_T), or varying high (HL_T) and varying low (VL_T), respectively. X-axis, the temperature sensitivity of prokaryotic phylotypes indicated as the VH_T/VL_T or CH_T/CL_T ratios of their relative abundances.

Thiessen et al. (2013); however, the opposite was found by Zhu and Cheng (2011).

Such inconsistencies could be better explained by relating back to the copiotrophs versus oligotrophs control mechanism. Due to a sufficient supply of C substrate (high C availability) in our soils, the fast-growing copiotrophic organisms in the varying temperature regime might be expected to maintain dominant growth and outgrow the oligotrophs throughout the entire period of the incubation. Hence, the Q_{10} values of CO₂ efflux are greater under the varying than constant temperature regimes in our experiment. In contrast, as for the study of Zhu and Cheng (2011), a 30-day pre-incubation can reduce substrate resource, cause a stress status of low C availability and result in an oligotroph-dominated microbial community. In their study, although the whole microbial community may be more active to a greater extent under a varying rather than a constant temperature regime, such an oligotroph-dominated community is less responsive to C availability and thus results in lower C efflux Q_{10} values under varying than constant temperature regime. These two studies are not controversial and contradictory; instead, they together indicate that the CO₂ efflux Q_{10} may not be due to substrate availability, but can be more likely controlled by the shift between copiotrophs and oligotrophs. To illustrate this, we frame a simplistic conceptual model to show how microbial trophic guilds may have an impact on respiratory rates across a substrate quality gradient (Fig. 7).

CONCLUSIONS AND IMPLICATIONS

Shifts in microbial trophic strategy, such as copiotrophy and oligotrophy, are critical microbial adaptations to an environment under constant and varying temperature changes. Our analysis demonstrates that the responses of complex natural prokaryotic communities to different temperature regimes can be categorized and quantified into simple classification schemes. The categorization of microbial populations into a simple trophic guild could be particularly valuable for modeling efforts, serving as a novel way for incorporating microbial parameters into Earth System Models, where the vast diversity of microbes are rarely explicitly considered despite the importance of microbial roles in biogeochemical cycling and ecosystem functioning. Our results in this study indicated that whether microbially mediated

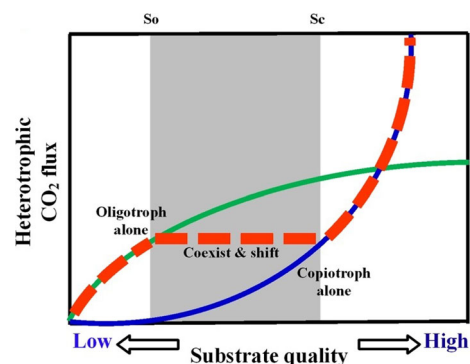


Figure 7. A simplistic conceptual model to illustrate the mechanism showing that the sensitivity of CO₂ flux can be influenced by dominance shift between oligotrophic and copiotrophic groups in a given ecosystem. The dynamics of the curve's derivatives depict the changes in CO₂ efflux sensitivities. The lines of solid green, solid blue and dotted red correspond to systems with only oligotrophs, only copiotrophs and both oligotrophs and copiotrophs, respectively. We assume oligotrophs completely outgrow their competitors when substrate quality is notably low ($< S_o$) and copiotrophs overwhelm when substrate quality is adequately high ($> S_c$). In the gray region with coexisting oligotrophic and copiotrophic group, it displays gradual shift from one group to another along the gradient of substrate quality.

CO₂ efflux Q_{10} increases or decreases depends on which microbial trophic guilds are dominantly present and to what magnitude they respond to a changing temperature. Thus, to improve the prediction of ecosystem-level responses to global change factors driven by natural and anthropogenic forces, it is important to have a sufficient mechanistic understanding of microbial phylogenetic responses to temperature change.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSEC](http://FEMSEC.org) online.

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Conflict of interest. None declared.

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