Title: Microbial community responses weaken soil carbon loss in Tibetan alpine grasslands under short-term warming

Running Head: Carbon loss under warming less than predicted

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Abstract

Changes in labile carbon pools (LC) and microbial communities are the main factors controlling soil heterotrophic respiration (R_h) in warming experiments. Warming is expected to increase R_h but this increase is not continuous. Currently, the proportional contribution of LC and soil microbiome in attenuating effect of extended warming on R_h is under debate. This gap in our knowledge is leading to considerable uncertainty in the prediction of carbon cycle feedbacks to climate change. Here we used a two-step incubation approach to reveal the relative contribution of labile carbon limitation and soil microbial community responses in attenuating effect of extended warming on R_h. Soil samples from three Tibetan ecosystems - an alpine meadow, and alpine steppe and a desert steppe - were exposed to a temperature gradient of 5-25°C. After an initial incubation period, soils were either sterilized then inoculated with parent soil microbes to assess the LC limitation effects while controlling for microbial community responses or soil microbes from the incubations were used to inoculate sterilized parent soils to assess the microbial community effects while controlling for LC limitation. We found both LC limitation and microbial community responses lead to significant declines in soil respiration by 37% and 30%, respectively, but their relative contribution are ecosystem specific. LC limitation caused a greater R_h decrease for desert steppe soils. Our study shows that soil carbon loss due to R_h in Tibetan alpine soils - especially in copiotrophic soils - will be weakened by microbial community responses under short term warming.

Key words

50 soil incubation, soil heterotrophic respiration, soil respiration acclimation, labile
51 carbon limitation, microbial community response
52
53

1 INTRODUCTION

There are large uncertainties in the extent to which climate warming will accelerate losses from soil carbon to atmospheric CO_2 through soil respiration (Cox *et al.*, 2000, Dufresne *et al.* 2002, Friedlingstein *et al.*, 2003). Many studies show that positive responses of soil respiration to warming declines over time due to LC limitation and/or microbial community responses to temperature (*i.e.*, thermal adaptation) (Luo *et al.*, 2001, Oechel *et al.*, 2000, Rustad, 2001). We use the term 'microbial community response' here *sensu* Kahu *et al* (2014), which includes microbial acclimation (physiological responses of individuals) and ecological responses (for example, competition altering species composition), because measurements of R_h are made at the level of the whole microbial community. These community responses can be either compensatory or enhancing (that is, reducing or increasing the effect of a temperature change on respiration rates) (Bradford *et al.*, 2010, Kahu *et al*. 2014). However, the relative importance of LC limitation and microbial community response in controlling R_h decline remains unclear and highly controversial, especially for microbial community response (Bradford *et al.*, 2010, Hartley *et al.*, 2007). A study spanning ecosystems from the Arctic to the Amazon found that the dominant response of microbial communities to temperature was to enhance the temperature sensitivity of R_h (*i.e.*, enhancement response) (Karhu *et al.*, 2014). In contrast, Dacal *et al*. (2019) and Bradford *et al*. (2019) found that microbial community responses to temperature reduced the temperature sensitivity of R_h (*i.e.*, compensatory response) in laboratory R_h assays with excess carbon substrate using globally collected soils. Further

complicating these contrasting results, the climate-sensitive alpine ecosystems were underrepresented in previous studies (Cannone *et al.*, 2007, Peng *et al.*, 2009). These different results lead to large uncertainties and contrasting consequences in terms of soil carbon-climate feedbacks (Hartley *et al.*, 2007). The Tibetan plateau hosts the world's largest pastoral alpine ecosystem and the soil there contains about 2.5% of the word's pool of soil carbon, which is predicted to experience considerable losses under future warming scenario (Crowther *et al.*, 2016, Peng *et al.*, 2009). In last fifty years, the surface temperatures on the Tibetan plateau rose 1.5-1.8^{o}C, approximately three times the global warming rate (Qiu, 2008, Bin Wang *et al*. 2008). Thus, addressing the thermal adaptation of R_h on the Tibetan plateau is important for evaluating climate-carbon feedbacks in alpine biome.

As Bradford *et al*. (2019) found, the major challenge in assessing the effects of labile carbon (LC) limitation and microbial community responses to temperature on R_h is their strong coupling. To address this challenge, we designed a two-step laboratory incubation study using soils collected from Tibetan plateau grasslands (Fig. 1a). The first step of the incubations was performed under controlled temperature and moisture for 56 days to investigate the changes in R_h during incubation (Experiment 1). An initial R_h increase was expected due to the increased temperature and moisture. After soil was incubated for an extended period, the R_h should decrease due to LC limitation and/or microbial community responses to temperature (Fig 1b). However, we were unable to differentiate these two mechanisms in Experiment 1. To investigate the effects of these two factors on the declined R_h, Experiment 1 was followed by a

second step where Experiment 1 soils were sterilized and then inoculated with the microbes extracted from parent soils to test the effects of LC limitation on R_h while controlling for microbiota (Experiment 2). Finally, microbes extracted from Experiment 1 were used to inoculate sterilized parent soils to test the effects of microbial community responses while controlling for LC limitation (Experiment 3). Sterilized parent soils were inoculated with microbes extracted from initial parent soils to serve as controls for Experiments 2 and 3. The only difference between Experiment 2 and control is soil substrate. Thus, we can assess the effects of LC limitation on R_h based on the R_h difference between Experiment 2 and the control (Fig. 1b). Similarly, the effects of microbial community response on R_h can be assessed by the R_h difference between Experiment 3 and the control (Fig. 1b). We hypothesized that microbial compensatory and enhancing responses would be confirmed by respectively lower and higher R_h in Experiment 3 relative to the control (Fig. 1b). Using this two-step incubation, we investigate the relative contributions of LC limitation and microbial community responses to R_h decline in soils from Tibetan alpine grasslands.

2 MATERIALS AND METHODS

2.1 *Soil sampling and properties analysis*

Soil samples (0-10 cm depth) were collected within a 100m × 100m plot (Supplementary Fig. S1) using a 7cm diameter soil corer during the 2014 growing season (July) from three dominant Tibetan vegetation types: alpine meadow (AM), alpine steppe (AS), and desert steppe (DS). Twenty soil samples were taken along the

diagonals of each plot, sieved to 2 mm to remove roots and stones, and gently mixed to produce a homogeneous composite sample. Soils were sieved because variable root respiration and decomposition of fine roots in intact cores would complicate the interpretation of the results with respect to the R_h. Root accounted for more than 50% of intact cores for AM, while 10-20% for AS. Within one week, soils were stored at 4^{o}C for subsequent incubation.

Temperature and precipitation data for each site was obtained from the National Meteorological Bureau of China database (http://data.cma.cn/) during the period from 1981-2010 (Table 1). Original soil properties were measured with four analytical replicates according to standard procedures (Karhu *et al.*, 2014). Briefly, total organic carbon (TOC) and inorganic carbon (IC) were measured from the sieved composite sample by a Shimadzu TOC-5000A Total Organic Carbon Analyzer (Shimadzu Corporation, Kyoto, Japan). LC pools were extracted by acid hydrolysis as described previously (Belay-Tedla *et al.*, 2009) and quantified by the TOC-5000A Total Organic Carbon Analyzer (Shimadzu). The LC here predominantly contains polysaccharides which are of both plant origin (such as hemi-cellulose and starch) and microbial origin (mostly microbial cell walls) (Belay-Tedla *et al.*, 2009). Soil pH was measured with a 1:2.5 soil to deionized water ratio (by volume) soil slurry using an Accumet AB 15 pH meter (Fisher Scientific). Soil water content was determined by drying subsamples at 105 ^{o}C for 24 h. The soil water holding capacity (WHC) was determined by wetting soil for 2 h, followed by draining through filter papers (Fisherbrand FB59103) for 2 h. The water content of soil at 100% water holding

capacity was then measured gravimetrically by drying a subsample at 105 ºC for 24h.

2.2 *Microbial biomass, activity, and species composition analysis*

Microbial biomass of the initial soil samples was estimated using chloroform fumigation-extraction (Vance *et al.*, 1987) and extractable DNA concentration methods. Community DNA was extracted from 0.5 g dry-weight soil using a PowerSoil DNA isolation kit (MoBio, USA) according to the manufacturer's instructions and quantified by NanoDrop (Thermo Scientific, Wilmington, MA). Excellent agreement was found among extractable DNA (dsDNA), substrate-induced respiration, and chloroform-labile carbon based microbial biomass for soils with pH lower than 8 (Marstorp et al. 2000, Semenov et al. 2018). The pH of our studying samples were less than 7 and the concentrations of dsDNA and chloroform-labile carbon were well correlated (r=0.94, p<0.01, n=5). Therefore, we used the extractable DNA to measure microbial biomass in the following analysis because less sample was consumed using this method. The substrate utilization profile patterns and physiological activity of the initial soil microbial communities were analyzed by the rate of average well color development (AWCD) at 15 ºC by ECO MICROPLATE™ (BIOLOG, CA, USA) after 48 h of incubation as described by Zhou *et al*. (2011). The activity of bacteria that were able to grow on the substrates provided in the plates under culture conditions was measured. Microbial species composition was measured using Illumina pyrosequencing of PCR-amplified V4-V5 hypervariable regions of the 16S rRNA according to established methods (Caporaso *et al.*, 2012, Wu *et al.*, 2015,

163 Xue *et al.*, 2016). Sequencing data were processed using QIIME 2 (Bolyen *et al.*
164 2018). Detailed protocols for microbial biomass, activity, and species composition
165 analysis are provided in the Supplementary Information.

166 2.3 *Soil incubation and the measurement of soil heterotrophic respiration (R_h)*

167 The oxygen content of the Tibetan air is only about 50-60% of those at sea level.
168 To avoid confounding effects with differences in oxygen content, all incubations were
169 conducted at the Naqu Ecological and Environmental Observation and Research
170 Station, China (31°17' N, 92 ° 06' E; 4501 m a.s.l.) which is located in our AM
171 sampling area. The initial soils were divided to two parts, one part was used for
172 Experiment 1, the other part was stored at 4°C until the end of Experiment 1 and then
173 used for cross incubations in Experiments 2 and 3. We stored the soil at 4°C to
174 prevent dramatic changes in microbial biomass and activity as recommended by ISO
175 (ISO, 1993). For Experiment 1, the soil moisture content of the initial soil samples
176 was adjusted to 30% and 60% WHC by adding sterile deionized water. Soil water
177 content significantly affects R_h by changing substrate mobility and accessibility by
178 microorganisms and can be a confounding effect on R_h (Davidson *et al.*, 1998).
179 Generally, 60% WHC is optimal for microbial activity (Fierer & Schimel, 2002,
180 Rey *et al.*, 2005), thus, 30% WHC induces stress on microbial community. Fifty
181 grams (dry-weight) of soil was placed inside 0.6-litre glass bottles with pierced
182 rubber stoppers. These pierced stoppers enabled gas exchange, but minimized
183 evaporation and soil drying. Soil bottles were pre-incubated at 4 °C for 48 hours to
184 allow short-term equilibration after manipulating the soil and then placed inside three

185 incubators at three temperatures (5, 15 and 25^{o}C) (Yichun cooled incubator,
186 BPH-9270D, Shanghai, China) for another 56 days (Experiment 1). The optimum
187 temperature for microbial growth in these alpine ecosystems is well above the field
188 temperatures (Rousk & Bååth, 2011), so 5^{o}C and 15^{o}C were chosen because they
189 are about 5^{o}C higher than the average mean annual air temperature and the mean
190 growing season monthly air temperature for the sampling sites, respectively. The soil
191 temperature at the research site can reach 20^{o}C at the surface, where a few centimeters
192 of soils is exposed to direct sunlight during the growing season. Thus, incubation at
193 25^{o}C (5^{o}C higher than the maximum seasonal temperature) provided an optimum
194 growth temperature. In addition, the temperatures used here are well established in R_h
195 thermal acclimation studies and the resulted Q_{10} values were used in climate-carbon
196 models (Liang *et al.* 2015). In total, 72 incubations were performed, including 3 soil
197 types (*i.e.*, AM, AS, and DS) × 3 incubation temperatures × 2 soil moisture treatments
198 × 4 replicates. During incubation, treatment WHC was maintained by weighing the
199 bottles and then adding the correct amount of sterile deionized water on a weekly
200 basis. Deionized water reservoirs were maintained in each incubator to ensure that the
201 added water was at the correct temperature. R_h was measured during the incubation
202 period at days 0, 2, 5, 9, 13, 18, 23, 30, 44, and 56 as described in detail previously
203 (Chang *et al.*, 2012). In brief, the headspace was sampled initially and again after 1 h
204 of incubation by removing 10 cm^3 of headspace gas into stoppered syringes. CO_2
205 concentration was measured by Agilent GC 7890 gas chromatograph
206 (Agilent Technologies, Palo Alto, USA) immediately after gas sampling. R_h was

207 expressed as μg C per g of dry soil per h. CO_2 production was calculated by the
208 integral method using the 'pracma' package in R (Borchers, 2015). In addition,
209 subsamples of soils were collected at day 0, 18, and 56 during Experiment 1 to
210 measure LC content and microbial biomass, activity and community composition
211 using the above methods. The contribution of LC to R_h during Experiment 1 was
212 calculated using Equation 1:

213 Contribution of LC to R_h= (C in decreased LC) / (respired C as CO_2) [Eq1]

214 Following Experiment 1, each incubated soil sample was divided into two parts (Fig.
215 1a): (1) one part (10g dry weight) was sterilized and incubated with microbiota that
216 has been extracted from 10g dry weight of corresponding original soils stored at 4°C,
217 controlling for the microbial community responses to estimate effects of LC changes
218 on R_h (Experiment 2); (2) the other part (10g dry weight) was used for microbiota
219 extraction, where the extracted microbiota was used to inoculate 10g dry weight of the
220 corresponding sterilized original soil, controlling for the LC limitation to estimate
221 effects of microbial community responses on R_h (Experiment 3). To remove any
222 effect of cross-incubation, we also inoculated the microbiota extracted from 10g dry
223 weight of original soils to corresponding sterilized original soils (10g dry weight) to
224 serve as a control.

225 The soils were continuously incubated for another 18 days under the same conditions
226 as those in Experiment 1 and R_h was measured on days 1, 5, 9, 14, and 18. Details of
227 soil sterilization and microbial extraction procedures are provided in the

Supplementary Information. To assess the soil sterilization effects on soil LC content, LC content was measured before and after sterilization for soils used in Experiments 2 and 3.

2.4 *Statistical analyse*

All the data analyses were performed using R software (version 3.2.2). Multiple comparisons testing using Turkey's HSD was conducted for all measured variables. The relationships between changes in R_h and measured carbon and microbial variables were evaluated using partial-correlation analyses, controlling for temperature and moisture. Partial Mantel test was employed to detect the correlations between R_h changes and microbial composition. Data from days 18 and 56 were used for the partial-correlation and partial Mantel analyses.

3 RESULTS

3.1 *Changes in LC content and microbial biomass showed significant associations with R_h reduction*

Most measured properties significantly changed during incubation in Experiment 1 (Fig. 2, Table 2). CO_2 production during Experiment 1 was positively correlated with soil type (DS 9±0.7, AS 16±1.4, AM 23±2.3 mg CO_2/g soil), temperature (5°C 10.8±0.9, 15°C 15±1.2, 25°C 22±2.6 mg CO_2/g soil), and moisture (30%WHC 12.4±0.9, 60%WHC 19.3±2 mgCO_2/g) (Supplementary Fig. S2a). R_h increased initially and peaked between days 10 and 25 during Experiment 1 (Fig. 2, Supplementary Fig. S3). The day at which R_h began to decline (*i.e.*, R_h peak) was

249 significantly affected by temperature (5ºC 33±2.6, 15ºC 18±1.8, 25ºC 14±1.2 day)
250 (Table 2, Supplementary Fig. S2b). Additionally, substantial decreases in LC were
251 detected at the end of Experiment 1 in all soils (DS 42.6±1.9, AS 40.4±3.1, and AM
252 46.1±1.9% decreases) (Fig. 2), but no significant effects of temperature and moisture
253 on LC were found (Table 2, Supplementary Fig. S2c). LC accounted for 30±3.6,
254 130±15.4, and 125±10.1% of the carbon released by R_h during the initial 18 days, and
255 28±1.3, 38±3.8, and 37±3.2% for the following 38 days for DS, AS, and AM,
256 respectively (Supplementary Fig. S4). For the following 38 days, the contribution of
257 LC to R_h carbon was negatively correlated with temperature and moisture
258 (Supplementary Fig. S4).

259 Microbial characteristics also changed significantly during Experiment 1. Microbial
260 biomass followed R_h patterns in AM and AS soils (Fig. 2), while no significant
261 changes were observed in DS soils (Fig. 2). Temperature (5ºC 7.6±3, 15ºC 11.8±2.6,
262 25ºC 0.02±3.9% decreases) and moisture (30%WHC 2.1±2.9, 60%WHC 10.9±2.4%
263 decreases) significantly affected microbial biomass (Table 2, Supplementary Fig.
264 S2d). Initial microbial activity, as assessed by BIOLOG, was significantly different
265 among different soil types (AWCD: DS 0.01±0.004, AS 0.21±0.03, AM 0.30±
266 0.02). We detected significant decreases in microbial activity at the end of
267 Experiment 1 in all the soils (Fig. 2). Soil type, temperature, and moisture had
268 significant effects on microbial activity decreases (Table 2, Supplementary Fig. S2e).
269 Similarly, microbial mass-specific activity, expressed as microbial activity per unit of
270 microbial biomass, was highest for AM microbes, followed by AS and DS microbes

(Fig. 3c). The microbial mass-specific activity decreased at the end of Experiment 1, especially for AS and AM soils under higher incubation temperatures (Fig. 3c). No significant effects of moisture on microbial mass-specific activity were found. Microbial affinity to carbohydrate substrates decreased in all three soils where polymer degradation increased (Fig. 3d). Significant changes in microbial composition with incubation time were found for all treatments (Fig. 3a, Supplementary Table S1). The microbial community similarity to day 0 samples decreased during the incubation in all the three soils (Supplementary Fig. S2f), and soil type and temperature significantly affected the community similarity decreases (Table 2). The archaeal phyla *Thaumarchaeota* was abundant in all soils (Fig. 3b), and was mostly composed of members of the ammonia oxidizing *Nitrososphaeraceae* species. Significantly more *Firmicutes* but fewer *Proteobacteria* were found in DS soils relative to AS and AM soils (Fig. 3b). *Thaumarchaeota*, *Acidobacteria*, *Delta- and Gamma-proteobacteria*, increased and *Bacteroidetes* decreased after DS soil incubation in Experiment 1 (Fig. 3b). *Firmicutes*, *Chloroflexi*, and *Cyanobacteria* significantly decreased after AS soil incubation. *Actinobacteria* increased but *Bacteroidetes* decreased after AM soil incubation.

Partial-correlation analysis showed that the R_h decline from day 18 to the end of Experiment 1 was correlated with LC or microbial biomass decreases depending on the soil type. For example, R_h decline in DS soils was mainly correlated to decreased LC (Table 3). On the other hand, both LC and microbial biomass decreases were significantly correlated to R_h decrease in AS soils. In AM soils, the R_h decline was

293 significantly correlated to the decrease in LC, microbial biomass, and microbial
294 activity (Table 3).

295 3.2 *Both LC limitation and microbial community responses decrease R_h*

296 Experiment 2 showed that R_h generally decreased by 37±3% due to LC limitation
297 (Fig. 4a, c). We inoculated sterilized parent soils with the microbes extracted from
298 parent soils to serve as a control for Experiments 2 and 3. DS, AS, and AM soils
299 produced 2.0±0.07, 4.6±0.89, 10.0±1.41 mg CO_2/g soil, respectively, in the control.
300 In Experiment 2, the effects of LC limitation were assessed by measuring the R_h
301 difference between Experiment 2 and the control. The CO_2 produced in Experiment 2
302 was 1.18±0.07, 2.32±0.37, 5.01±0.91 mg CO_2/g soil for DS, AS, and AM soils,
303 respectively, which is significantly less than the control, indicating significant effects
304 of LC limitation. The LC limitation on R_h was significantly affected by temperature
305 and soil type (Fig. 4a, c, Supplementary Table S2). R_h decreases relative to control
306 were greatest in the soils with high incubation temperatures (5ºC 29±5.2, 15ºC
307 32±4.8, 25ºC 50±4.2%) and high carbon content (DS 27±5.2, AS 41±5, AM
308 44±4.4%) (Fig. 4a and Supplementary Fig. S5). However, moisture did not show
309 significant effects on R_h decreases, but significant interactions between temperature
310 and moisture were found (Supplementary Table S2). High moisture caused less R_h
311 differences between Experiment 2 and control in AS and AM soils under15ºC (Fig. 4a
312 and Supplementary Fig. S5).

313 In Experiment 3, a reduction of approximately 30±3.1% R_h was found to be due to

microbial community responses (Fig. 4b). The effects of microbial community responses were assessed by the R_h differences between Experiment 3 and control. The CO_2 produced in Experiment 3 was 1.53±0.09, 2.56±0.29, 4.84±0.98 mg CO_2/g soil for DS, AS, and AM soils, respectively, indicating significant effects of microbial community response. The R_h difference between Experiment 3 and the control were significantly affected by temperature, moisture, and soil type (Fig. 4b, Supplementary Table S2 and Fig. S5). Similarly to Experiment 2, temperature (5ºC 24±4.9, 15ºC 24±4, 25ºC 44±6.3%) and carbon content (DS 12±5.2, AS 33±5, AM 47±3.8%) were positively correlated to R_h decrease (Fig. 4b, Supplementary Fig. S5). Additionally, soil moisture negatively correlated with the R_h decreases in Experiment 3 relative to control (30% WHC 38±4.2%, 60% WHC 23±4.4%). Significant interactions between temperature and soil type were also detected (Supplementary Table S2).

The decreases in R_h attributable to LC limitation was great than that due to microbial community responses under most treatments, especially for DS soils (Fig. 4c). LC limitation generally resulted in 27±5.2% decrease in R_h for DS soils, which was significant greater than the R_h decrease caused by microbial community responses, 12±5.2%.

Tables:

Table 1 Summary of site characteristics, soil and microbial characteristics, and plants in this study. Different letters indicate significant difference at $p<0.05$ level. DS, desert steppe; AS, alpine steppe; AM, alpine meadow; MAT, mean annual air temperature; MAP, mean annual precipitation; IC, inorganic carbon; TOC, total organic carbon; AWCD, average well color development.

	DS	AS	AM
Location	33º24' N, 79º 42' E	31º26' N, 90º 2' E	31º17' N, 92º 06' E
Elevation (m)	4264	4678	4501
MAT (ºC)	0.1 [a]	-0.83 [b]	-1.13 [c]
Mean monthly air temp. –high (ºC)	16.3 [a]	8.7 [b]	9 [b]
Mean monthly air temp. –low (ºC)	-15.8 [c]	-10.9 [a]	-12.6 [b]
MAP (mm/yr)	73.4 [c]	321.96 [b]	430.2 [a]
Mean monthly precipitation. –high (mm)	56.8 [c]	84.6 [b]	103.1 [a]
Mean monthly precipitation –low (mm)	0 [c]	1.4 [b]	2.6 [a]
Dominant plant spp.	*Stipa tianschanica Roshev. var. gobica (Roshev.) P. C. Kuo*	*Stipa purpurea Griseb.*	*Kobresia pygmaea*
IC	0.01% [a]	0 [b]	0 [b]
TOC	0.87% [c]	1.58% [b]	3.02% [a]
Labile carbon (mg/g dry soil)	1.58 [c]	4.28 [b]	6.62 [a]
Microbial carbon (μg/g dry soil)	32.75 [c]	55.24 [b]	88.82 [a]
Extractable DNA (μg/g dry soil)	17.14 [c]	26.69 [b]	32.35 [a]
AWCD	0.01 [c]	0.21 [b]	0.3 [a]
Cabohydrate/polymer activity	0.87 [c]	1.16 [b]	3.27 [a]
pH	7.08 [a]	6.88 [b]	6.48 [c]
Soil moisture (W/W%)	5.46 [b]	4.10 [b]	8.34 [a]

340 Table 2 Significance tests of temperature (T), moisture (M), soil type (S), and incubation day effects (D) and their interaction on changes in soil

341 heterotrophic respiration (R_h), labile carbon content (LC), microbial biomass, microbial physiological activity, community similarity, and R_h

342 acclimation time (expressed as time point for maximum R_h). Significant differences are indicated in bold.

Source	R_h changes		LC changes		Microbial biomass changes		Metabolic activity changes		Community similarity changes		R_h acclimation time	
	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
T	127.1	**<0.01**	1.55	0.22	9.65	**<0.01**	13.01	**<0.01**	3.06	0.08	36.23	**<0.01**
M	25.37	**<0.01**	13.5	0.29	43.26	**<0.01**	4.24	**0.04**	0.76	0.38	0.51	0.48
S	20.51	**<0.01**	82.87	**<0.01**	31.07	**<0.01**	52.93	**<0.01**	7.67	**<0.01**	1.28	0.29
T:M	11.49	**<0.01**	11.14	**<0.01**	1.41	0.25	1.93	0.15	3.53	0.06	1.69	0.19
T:S	8.11	0.14	4.88	**<0.01**	2.1	0.09	0.47	0.76	0.53	0.59	4.30	**<0.01**
M:S	8.41	0.48	3.86	**0.03**	3.64	**0.03**	0.32	0.72	5.57	**<0.01**	3.80	**0.03**
T:M:S	0.93	**0.46**	3.76	**0.01**	5.05	**<0.01**	2.81	**0.03**	1.74	0.18	2.85	**0.03**
D	103.92	**<0.01**	24.07	**<0.01**	143.48	**<0.01**	21.54	**<0.01**	8.16	**0.01**		
D:T	55.74	**<0.01**	3.21	**0.05**	1.24	0.29	3.31	**0.04**	1.49	0.23		
D:M	23.15	**<0.01**	32.99	**<0.01**	2.06	0.15	4.78	**0.03**	2.50	0.12		
D:S	15.19	**<0.01**	68.66	**<0.01**	52.19	**<0.01**	6.41	**<0.01**	11.66	**<0.01**		
D:T:M	25.71	**<0.01**	2.86	0.06	2.98	**0.05**	3.9	**0.02**	0.28	0.60		
D:T:S	15.16	**<0.01**	9.95	**<0.01**	6.52	**<0.01**	2.46	**0.05**	3.67	**0.03**		
D:M:S	19.27	**<0.01**	32.54	**<0.01**	8.4	**<0.01**	12.97	**<0.01**	0.56	0.57		
D:T:M:S	23.91	**<0.01**	1.03	0.4	3.12	**0.02**	0.97	0.42	0.56	0.46		

Table 3 Partial correlation coefficients between changes in soil heterotrophic respiration (R_h) and soil labile carbon, microbial biomass, and microbial physiological activity (AWCD), and microbial composition evaluated using partial-correlation analysis, controlling for temperature and moisture. Significant differences are indicated in bold. **P<0.01, *P<0.05. Data from days 18 and 56 were used for this analysis.

		Labile Carbon	Microbial b Miomass	AWCD	Microbial Composition
Overall		**0.17***	**0.31****	0.05	**-0.19***
DS		**0.5****	0.06	0.07	-0.02
AS		**0.24***	**0.36****	0.01	0.09
AM		**0.33***	**0.31***	**0.5****	-0.25
30% WHC		0.11	**0.29****	**0.48****	**-0.32****
60% WHC		**0.36****	**0.43****	0.09	-0.18
5ºC		0.22	-0.01	0.07	-0.13
15 ºC		0.01	**0.44****	0.1	-0.04
25 ºC		**0.39****	**0.44****	0.08	**-0.34***
	30% WHC	0.41	-0.16	0.12	**-0.56****
	60% WHC	**0.61****	0.36	0.03	0.22
DS	5 ºC	**0.76****	0.2	**-0.66****	0.23
	15 ºC	0.22	0.28	0.1	0.04
	25 ºC	**0.64***	-0.01	0.45	-0.04
	30% WHC	**0.45***	0.3	0.29	0.22
	60% WHC	**0.83****	**0.58****	0.06	0.06
AS	5 ºC	**0.69****	0.18	-0.2	0.20
	15 ºC	0.42	**0.66***	-0.07	0.51
	25 ºC	0.44	**0.59***	0.26	0.06
	30% WHC	**0.46***	**0.76****	0.33	-0.38
	60% WHC	0.31	0.09	**0.65****	-0.28
AM	5 ºC	0.01	-0.03	0.21	0.12
	15 ºC	0.21	0.42	0.49	-0.44
	25 ºC	**0.79****	0.33	**0.84****	0.32

Figures and Figure Legends:

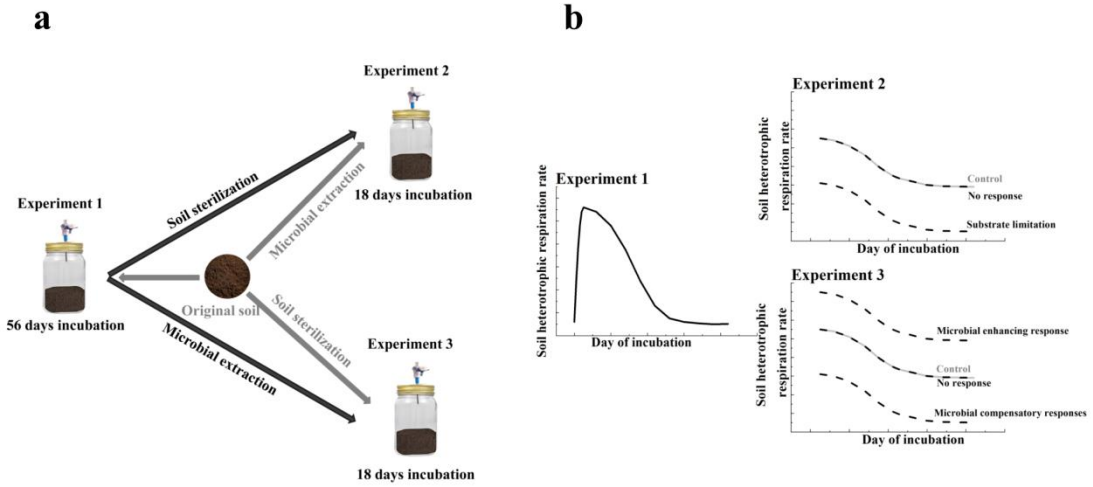
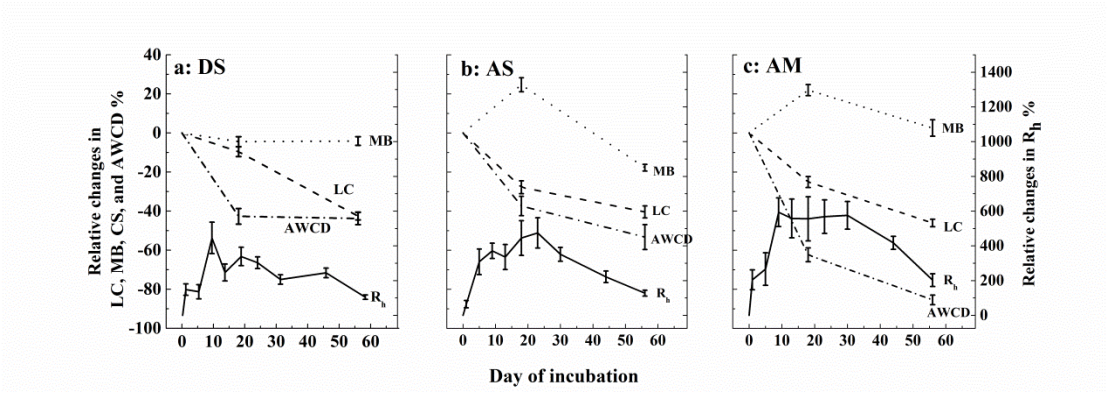


Figure 1 Schematic diagram of the experimental design (a) and the patterns of soil heterotrophic respiration rate that would be observed in the case of substrate limitation and non-limitation in Experiment 2 and in the case of microbial community compensatory response, no response, and enhanced response in Experiment 3 (b).



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363 Figure 2 Relative changes in soil heterotrophic respiration rate (R_h), labile carbon
364 content (LC), microbial biomass (MB), and physiological activity (as measured by
365 normalized average well color development, AWCD) with increasing incubation time in
366 desert steppe (DS, a), alpine steppe (AS, b), and alpine meadow (AM, c) soils to
367 starting point (day 0). Error bars show standard errors of the means.

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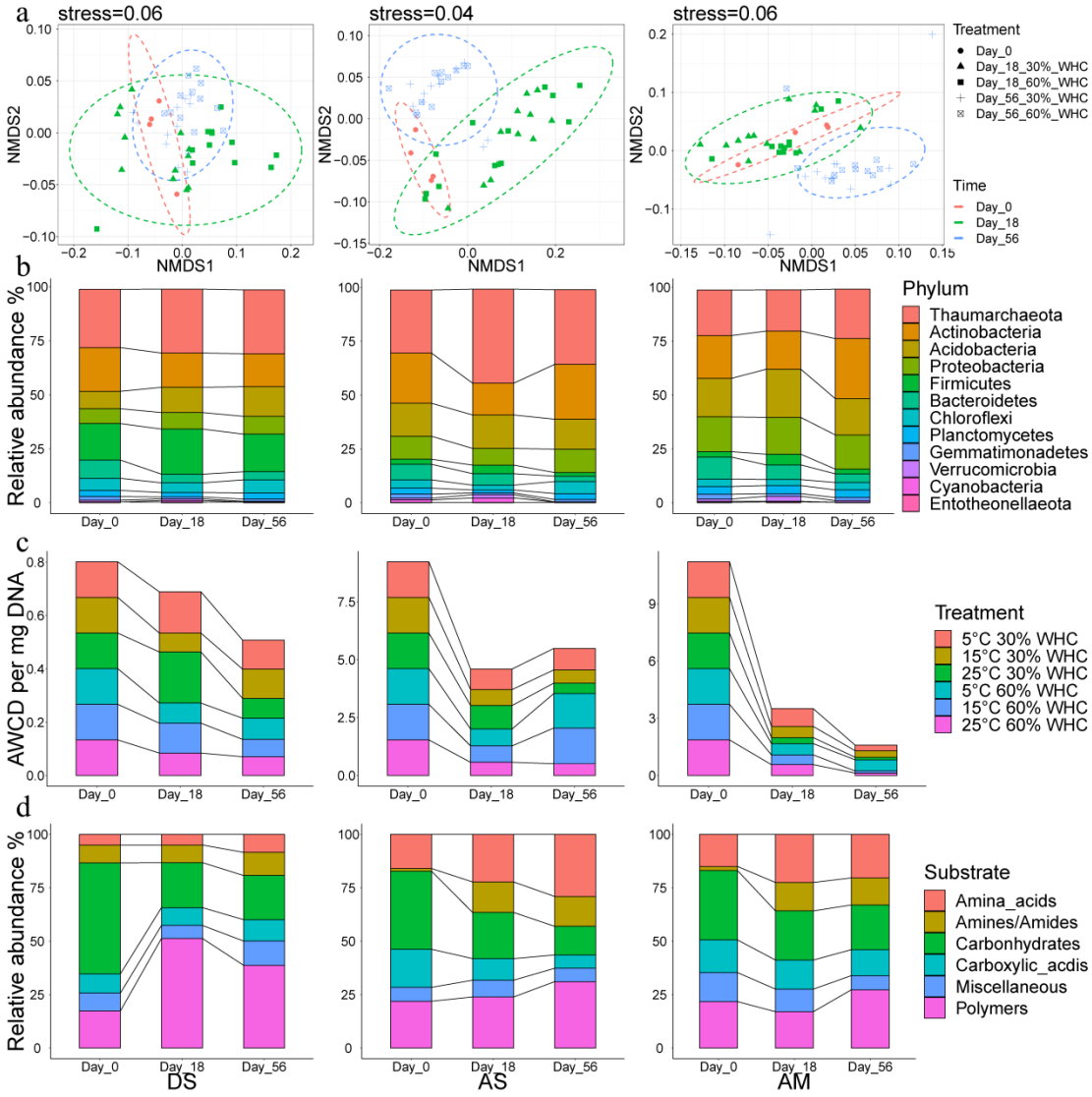

Figure 3. Bacterial characteristics change along time in Experiment 1. a: Nonmetric multidimensional scaling (NMDS) plots of community composition using weighted Unifrac dissimilarity; b: the relative abundance of bacterial phyla; c: bacterial mass-specific activity; d: substrate utilization profile patterns. DS: desert steppe; AS: alpine steppe; AM: alpine meadow.

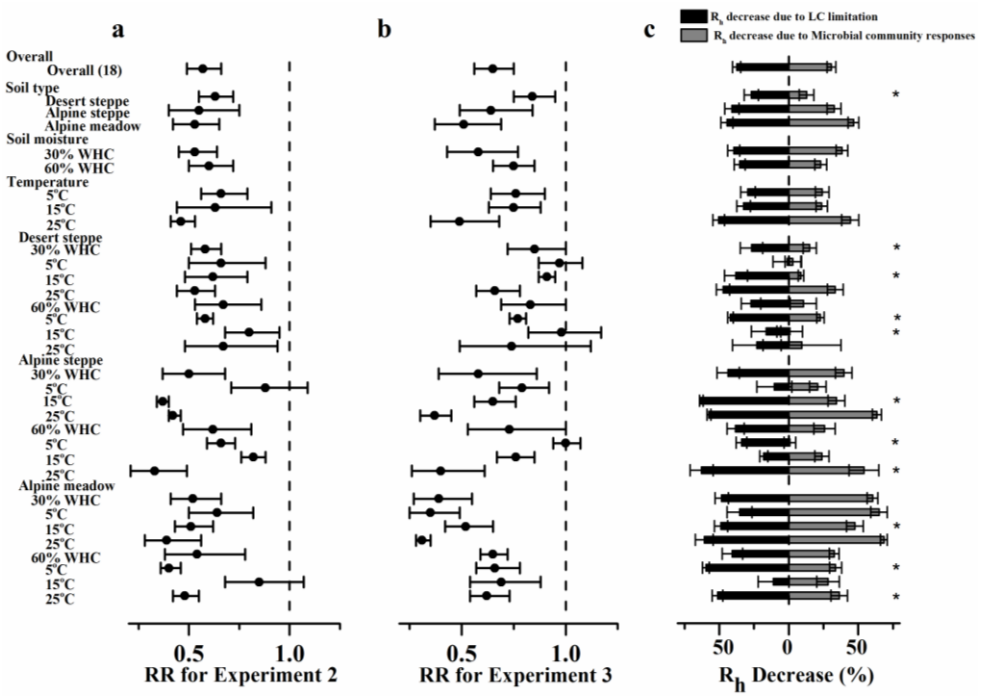

Figure 4. The impacts of the labile carbon limitation (a) and microbial community responses (b) on the response of soil heterotrophic respiration, and statistic analysis on their difference (c). The mean 95% confidence intervals of response ratio for R_h are presented for all soil groups (*i.e.*, including all 72 cases), and for different soil groups, based on ecosystem type, soil moisture, soil temperature and each treatment. Values>1 indicate an enhanced response, and values<1 indicate a compensatory response. Asterisks denote treatments when the R_h decrease caused by labile carbon limitation and microbial community responses are significantly different (paired-sample t tests).

4 DISCUSSION

The significant associations between LC and R_h changes indicated that LC limitation might be an important cause of R_h reduction; this strongly supported by Experiment 2 (Fig. 4a). The relative abundance of different microorganisms is an indicator for carbon availability (Fierer *et al.*, 2007). For example, the decrease in relative abundance of copiotrophic *Bacteroidetes* during Experiment 1 supports LC limitation (Fig. 2b). The important role of LC limitation in thermal adaptation of R_h has been reported previously (Eliasson *et al.*, 2005, Hartley *et al.*, 2007, Kirschbaum, 2004, Knorr *et al.*, 2005, Melillo *et al.*, 2017, Walker *et al.*, 2018). In mineral soils, physical access to occluded or sorbed substrates is critical for R_h (Bradford, 2013, Dungait *et al.*, 2012). Increased temperature and moisture can increase LC desorption, diffusion, and LC microbial availability (Conant *et al.*, 2011, Schimel & Schaeffer, 2012), which may be one of the explanations of initial increases in R_h (Fig. 2). Gradually, the LC decreased over time, causing LC limitation, and followed by a R_h decline (Fig. 2). Soil LC was consumed faster under higher temperatures, and caused earlier R_h acclimation time (*i.e.*, peak P_h point) (Fig. 2). More LC consumed under higher incubation temperatures caused more LC limitation, which was supported in the Experiment 2 (Fig. 4a). High moisture can increase LC availability and decreased LC limitation, which likely decreased the effects of LC limitation for AS and AM soils incubated at 15^{o}C (Fig. 4a).

Although, autoclaving the soil for Experiment 2 significantly increased the LC content, the incubated soils from Experiment 1 still showed significantly lower LC

content compared with the un-incubated soils (Supplementary Fig. S6). Thus, the LC effects on R_h were able to be directly tested in Experiment 2.

Soil bacterial communities are the most abundant fraction, and grow fast, making them critical to labile carbon mineralization. Fungi are also important soil microbial community members, especially for cycling carbon in forest ecosystem (Clemmensen *et al.*, 2013). However, the fungal community grows slower, predominantly controlling decomposition of recalcitrant organic matter (Boer *et al.*, 2005) and were found to be resistant to short-term warming in Tibetan grassland soils (Xiong *et al.*, 2014). For these reason, we only studied the bacterial community in our cross incubations. Our results showed that microbial community responses caused an approximately 30% decrease in R_h (Fig. 4b). The importance of microbial community responses in controlling R_h has been well observed (Allison *et al.*, 2010, Bradford *et al.*, 2019, Dacal *et al.*, 2019, Karhu *et al.*, 2014). Microbial species composition, biomass, and activity dramatically changed during Experiment 1 (Fig. 2, 3), and showed significant correlations with R_h (Table 3). This indicates that they may be important causes of the temporal changes in R_h. Due to the LC limitation late in Experiment 1, non-labile carbon was utilized to a greater extent by microorganisms (Supplementary Fig. S4), which requires greater energy input relative to LC utilization and therefore may reduce microbial carbon use efficiency (CUE) (Allison *et al.*, 2010, Sugai & Schimel, 1993). Thus, the decreased microbial biomass late in Experiment 1 is likely due to LC limitation and lower CUE. Significantly decreased microbial physiological activity was found for all soils (Fig. 2) and was significantly

441 correlated with R_h in AM soils (Table 3). The decreased physiological activity might
442 be attributable to microbial community changes and thermal acclimation. Decreased
443 relative abundance of *Bacteroidetes* after Experiment 1 (Fig. 3b) was found to be
444 correlated with lower soil carbon mineralization rates (Fierer *et al*. 2007).
445 Additionally, the lower mass-specific activity under higher temperatures indicates
446 microbial thermal adaptation (Fig. 3c), which may also decrease microbial activity
447 (Bradford, 2013). In addition, LC limitation might also contribute to the activity
448 decrease. It has been found that carbon limitation may shift enzyme expression
449 toward higher affinity enzymes, where the trade-off is a reduction in potential
450 catalytic rates (Bradford, 2013, Steinweg *et al.*, 2008). Moreover, we found that
451 highest effect of microbial community responses in AM soils under low moisture and
452 high temperature, which should be due to decreased microbial activity (Fig. 2 and 3c).
453 Significant correlations between microbial species composition and R_h changes were
454 only found for soils under 30% WHC and 25 ^oC (Table 3), though microbial
455 composition significantly changed after Experiment 1 (Supplementary Table S1). This
456 might be due to the substantial functional redundancy of microbial species in soil
457 carbon turnover processes (Allison & Martiny, 2008, Prosser, 2012, Rousk *et al.*,
458 2009). Therefore, the changes in microbial biomass and mass-specific activity may
459 contribute to the thermal acclimation of R_h.

460 The thermal adaptation of R_h for DS soils was mainly caused by LC limitation, while
461 both LC limitation and microbial community responses contribute to the thermal
462 adaptation of R_h for AS and AM soils (Fig. 4). The different contribution of microbial

community response to R_h decline might be due to the different LC concentration and microbial communities among the soils (Fig. 3b). Firstly, based on the Michaelis-Menten kinetics (MMK), the R_h should be more controlled by V_{max} under high substrate concentration environments. In contrast, the importance of LC increases with their decreasing concentration. Secondly, due to the soil nutrient condition, more copiotrophic microbes should colonize AS and AM soils relative to DS soils, such as *Proteobacteria* (Fig. 3b). It has been found that copiotrophic microbes have bigger genomes and can rapidly and tightly regulate metabolism according to environment change (Lauro *et al.*, 2009). When temperature rises, copiotrophic microbes may better adapt to warmer conditions by their ability to express more stable but lower activity isoenzymes relative to oligotrophic microbes, that is lower V_{max} in MMK (Bradford, 2013). However, the oligotrophic microbes have lower but relatively stable activity, that is stable V_{max} in MMK (Morita 1997, Koch, 2001). Therefore, the changes in LC should be more important for R_h changes in DS relative to AS and AM. The role of microbial community responses in R_h acclimation is debated due to a lack of empirical evidence which is needed to clearly assess the effects of microbial community response while controlling for differences in carbon substrate (Bradford, 2013, Hartley *et al.*, 2009, Nie *et al.*, 2013). Our results support the hypothesis that microbial community responses, such as decreases in microbial biomass and mass-specific activity, are as important a cause of R_h reduction as LC limitation.

In summary, this study shows that in the short term, the stimulation effects of warming

485 on R_h in Tibetan alpine soils is time dependent. Increased R_h may occur in the initial
486 stages of warming and subsequently declin. This R_h decline is likely due to LC
487 limitation as well as microbial community responses (*i.e.*, decreases in microbial
488 biomass and activity). Additionally, the relative contributions of LC limitation and
489 microbial community responses to R_h decline are ecosystem-specific. LC limitation is
490 the main reason for the R_h decline of DS soils, while both LC limitation and microbial
491 community contributes to R_h decline of AS and AM soils. Overall, the microbial
492 community response under short term warming is expected to reduce soil organic
493 matter decomposition.

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References:

Allison SD, Martiny JBH (2008) Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 11512-11519.

Allison SD, Wallenstein MD, Bradford MA (2010) Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience*, 3, 336-340.

Belay-Tedla A, Zhou X, Su B, Wan S, Luo Y (2009) Labile, recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the US Great Plains subjected to experimental warming and clipping. *Soil Biology and Biochemistry*, 41, 110-116.

Boer Wd, Folman LB, Summerbell RC, Boddy L (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews*, 29, 795-811.

Bolyen E, Rideout Jr, Dillon Mr et al. (2018) QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. *PeerJ Preprints*, 6, e27295v1

Borchers HW (2015) Pracma: practical numerical math functions. R package version 1.8. 3.

Bradford MA (2013) Thermal adaptation of decomposer communities in warming soils. *Frontiers in microbiology*, 4 doi:10.3389/fmicb.2013.00333.

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

Bradford MA, Mcculley RL, Crowther TW, Oldfield EE, Wood SA, Fierer N (2019) Cross-biome patterns in soil microbial respiration predictable from evolutionary theory on thermal adaptation. Nature Ecology & Evolution, 3, 223-231

Bradford MA, Watts BW, Davies CA (2010) Thermal adaptation of heterotrophic soil respiration

525 in laboratory microcosms. *Global change biology,* 16, 1576-1588.
526 Cannone N, Sgorbati S, Guglielmin M (2007) Unexpected impacts of climate change on alpine
527 vegetation. Frontiers in Ecology and the Environment, 5, 360-364.
528 Caporaso JG, Lauber CI, Walters WA *et al.* (2012) Ultra-high-throughput microbial community
529 analysis on the Illumina HiSeq and MiSeq platforms. *The ISME journal,* 6, 1621-1624.
530 Chang X, Zhu X, Wang S *et al.* (2012) Temperature and moisture effects on soil respiration in
531 alpine grasslands. *Soil Science,* 177, 554-560.
532 Clemmensen K, Bahr A, Ovaskainen O *et al.* (2013) Roots and associated fungi drive long-term
533 carbon sequestration in boreal forest. *Science,* 339, 1615-1618.
534 Conant RT, Ryan MG, Agren GI *et al.* (2011) Temperature and soil organic matter decomposition
535 rates–synthesis of current knowledge and a way forward. *Global change biology,* 17,
536 3392-3404.
537 Cox PM, Betts RA, Jones CD, Spall SA, Totterdell IJ (2000) Acceleration of global warming due
538 to carbon-cycle feedbacks in a coupled climate model. *Nature,* 408, 184-187.
539 Crowther TW, Todd-Brown KE, Rowe CW *et al.* (2016) Quantifying global soil carbon losses in
540 response to warming. *Nature,* 540, 104-108.
541 Dacal M, Bradford Ma, Plaza C, Maestre Ft, García-Palacios P (2019) Soil microbial respiration
542 adapts to ambient temperature in global drylands. *Nature Ecology & Evolution*
543 3, 232-238 .
544 Davidson EA, Belk E, Boone RD (1998) Soil water content and temperature as independent or
545 confounded factors controlling soil respiration in a temperate mixed hardwood forest.
546 *Global change biology,* 4, 217-227.

547 Dufresne Jl, Fairhead L, Le Treut H *et al.* (2002) On the magnitude of positive feedback between
548 future climate change and the carbon cycle. *Geophysical Research Letters*, 29,
549 4341-4344.
550 Dungait JA, Hopkins DW, Gregory AS, Whitmore AP (2012) Soil organic matter turnover is
551 governed by accessibility not recalcitrance. *Global change biology,* 18, 1781-1796.
552 Eliasson PE, Mcmurtrie RE, Pepper DA, Strömgren M, Linder S, Agren GI (2005) The response
553 of heterotrophic CO2 flux to soil warming. *Global change biology,* 11, 167-181.
554 Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria.
555 *Ecology,* 88, 1354-1364.
556 Fierer N, Schimel JP (2002) Effects of drying–rewetting frequency on soil carbon and nitrogen
557 transformations. *Soil Biology and Biochemistry,* 34, 777-787.
558 Friedlingstein P, Dufresne JL, Cox P, Rayner P (2003) How positive is the feedback between
559 climate change and the carbon cycle? *Tellus B: Chemical and Physical Meteorology*, 55,
560 692-700.
561 Hartley IP, Heinemeyer A, Ineson P (2007) Effects of three years of soil warming and shading on
562 the rate of soil respiration: substrate availability and not thermal acclimation mediates
563 observed response. *Global change biology,* 13, 1761-1770.
564 Hartley IP, Hopkins DW, Garnett MH, Sommerkorn M, Wookey PA (2009) No evidence for
565 compensatory thermal adaptation of soil microbial respiration in the study of Bradford et
566 al.(2008). *Ecology letters,* 12, E12-E14.
567 ISO (1993) Soil Quality-Sampling Part 6: Guidance on Collection, Handling and Storage of Soil
568 for Assessment of Aerobic Microbial Processes in the Laboratory. 1st edn, Geneva,
569 10381-10386.

570 Karhu K, Auffret MD, Dungait JAJ *et al.* (2014) Temperature sensitivity of soil respiration rates
571 enhanced by microbial community response. *Nature*, 513, 81-84.
572 Kirschbaum Mu (2004) Soil respiration under prolonged soil warming: are rate reductions caused
573 by acclimation or substrate loss? *Global Change Biology*, 10, 1870-1877.
574 Knorr W, Prentice I, House J, Holland E (2005) Long-term sensitivity of soil carbon turnover to
575 warming. *Nature*, 433, 298-301.
576 Koch Al (2001) Oligotrophs versus copiotrophs. *Bioessays*, 23, 657-661.
577 Lauro FM, Mcdougald D, Thomas T *et al.* (2009) The genomic basis of trophic strategy in marine
578 bacteria. *Proceedings of the National Academy of Sciences of the United States of*
579 *America*, 106, 15527-15533
580 Liang J, Li D, Shi Z et al. (2015) Methods for estimating temperature sensitivity of soil organic
581 matter based on incubation data: a comparative evaluation. Soil Biology and
582 Biochemistry, 80, 127-135.
583 Luo Y, Wan S, Hui D, Wallace Ll (2001) Acclimatization of soil respiration to warming in a tall
584 grass prairie. *Nature*, 413, 622-625.
585 Marstorp H, Guan X, Gong P (2000) Relationship between dsDNA, chloroform labile C and
586 ergosterol in soils of different organic matter contents and pH. *Soil Biology &*
587 *Biochemistry*, 32, 879-882.
588 Melillo JM, Frey SD, Deangelis KM *et al.* (2017) Long-term pattern and magnitude of soil carbon
589 feedback to the climate system in a warming world. *Science*, 358, 101-105.
590 Morita Ry, Morita R (1997) Bacteria in oligotrophic environments: starvation-survival lifestyle,
591 Chapman & Hall New York

592 Nie M, Pendall E, Bell C, Gasch Ck, Raut S, Tamang S, Wallenstein MD (2013) Positive climate
593 feedbacks of soil microbial communities in a semi-arid grassland. *Ecology letters*, 16,
594 234-241.
595 Oechel WC, Vourlitis GI, Hastings SJ, Zulueta RC, Hinzman L, Kane D (2000) Acclimation of
596 ecosystem CO2 exchange in the Alaskan Arctic in response to decadal climate warming.
597 *Nature*, 406, 978-981.
598 Peng S, Piao S, Wang T, Sun J, Shen Z (2009) Temperature sensitivity of soil respiration in
599 different ecosystems in China. *Soil Biology and Biochemistry*, 41, 1008-1014.
600 Prosser JI (2012) Ecosystem processes and interactions in a morass of diversity. *FEMS*
601 *microbiology ecology*, 81, 507-519.
602 Qiu J (2008) China: The third pole. *Nature News*, 454, 393-396.
603 Rey A, Petsikos C, Jarvis P, Grace J (2005) Effect of temperature and moisture on rates of carbon
604 mineralization in a Mediterranean oak forest soil under controlled and field conditions.
605 *European Journal of Soil Science*, 56, 589-599.
606 Rousk J, Bååth E (2011) Growth of saprotrophic fungi and bacteria in soil. *FEMS microbiology*
607 *ecology*, 78, 17-30.
608 Rousk J, Brookes Pc, Bååth E (2009) Contrasting soil pH effects on fungal and bacterial growth
609 suggest functional redundancy in carbon mineralization. *Applied and Environmental*
610 *Microbiology*, 75, 1589-1596.
611 Rustad L (2001) Global change - Matter of time on the prairie. *Nature*, 413, 578-579.
612 Rustad L, Campbell J, Marion G *et al.* (2001) A meta-analysis of the response of soil respiration,
613 net nitrogen mineralization, and aboveground plant growth to experimental ecosystem

warming. *Oecologia*, 126, 543-562.

Schimel J, Schaeffer S (2012) Microbial control over carbon cycling in soil. *Frontiers in microbiology,* 3 doi: 10.3389/fmicb.2012.00348.

Semenov M, Blagodatskaya E, Stepanov A, Kuzyakov Y (2018) DNA-based determination of soil microbial biomass in alkaline and carbonaceous soils of semi-arid climate. *Journal of Arid Environments*, 150, 54-61

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

Sugai SF, Schimel JP (1993) Decomposition and biomass incorporation of 14C-labeled glucose and phenolics in taiga forest floor: effect of substrate quality, successional state, and season. *Soil Biology and Biochemistry*, 25, 1379-1389.

Tucker Cl, Bell J, Pendall E, Ogle K (2013) Does declining carbon-use efficiency explain thermal acclimation of soil respiration with warming? *Global change biology,* 19, 252-263.

Vance E, Brookes P, Jenkinson D (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, 19, 703-707.

Walker TW, Kaiser C, Strasser F *et al.* (2018) Microbial temperature sensitivity and biomass change explain soil carbon loss with warming. *Nature climate change*, 8, 885-889.

Wang B, Bao Q, Hoskins B, Wu G, & Liu Y (2008) Tibetan Plateau warming and precipitation changes in East Asia. Geophysical Research Letters 35(14).

Wu LY, Wen CQ, Qin YJ *et al.* (2015) Phasing amplicon sequencing on Illumina Miseq for robust environmental microbial community analysis. *BMC Microbiology,* 15 doi:

636 10.1186/s12866-015-0450-4.

637 Xiong J, Peng F, Sun H, Xue X, Chu H (2014) Divergent Responses of Soil Fungi Functional
638 Groups to Short-term Warming. *Microbial Ecology*, 1-8.

639 Xue K, M. Yuan M, J. Shi Z *et al.* (2016) Tundra soil carbon is vulnerable to rapid microbial
640 decomposition under climate warming. *Nature Clim. Change*, 6:595-600.

641 Zhou J, Xue K, Xie J *et al.* (2011) Microbial mediation of carbon-cycle feedbacks to climate
642 warming. *Nature climate change*, 2, 106-110.

643
644