

# Microbial diversity decline and community response are decoupled from increased respiration in warmed tropical forest soil

## Extended Data

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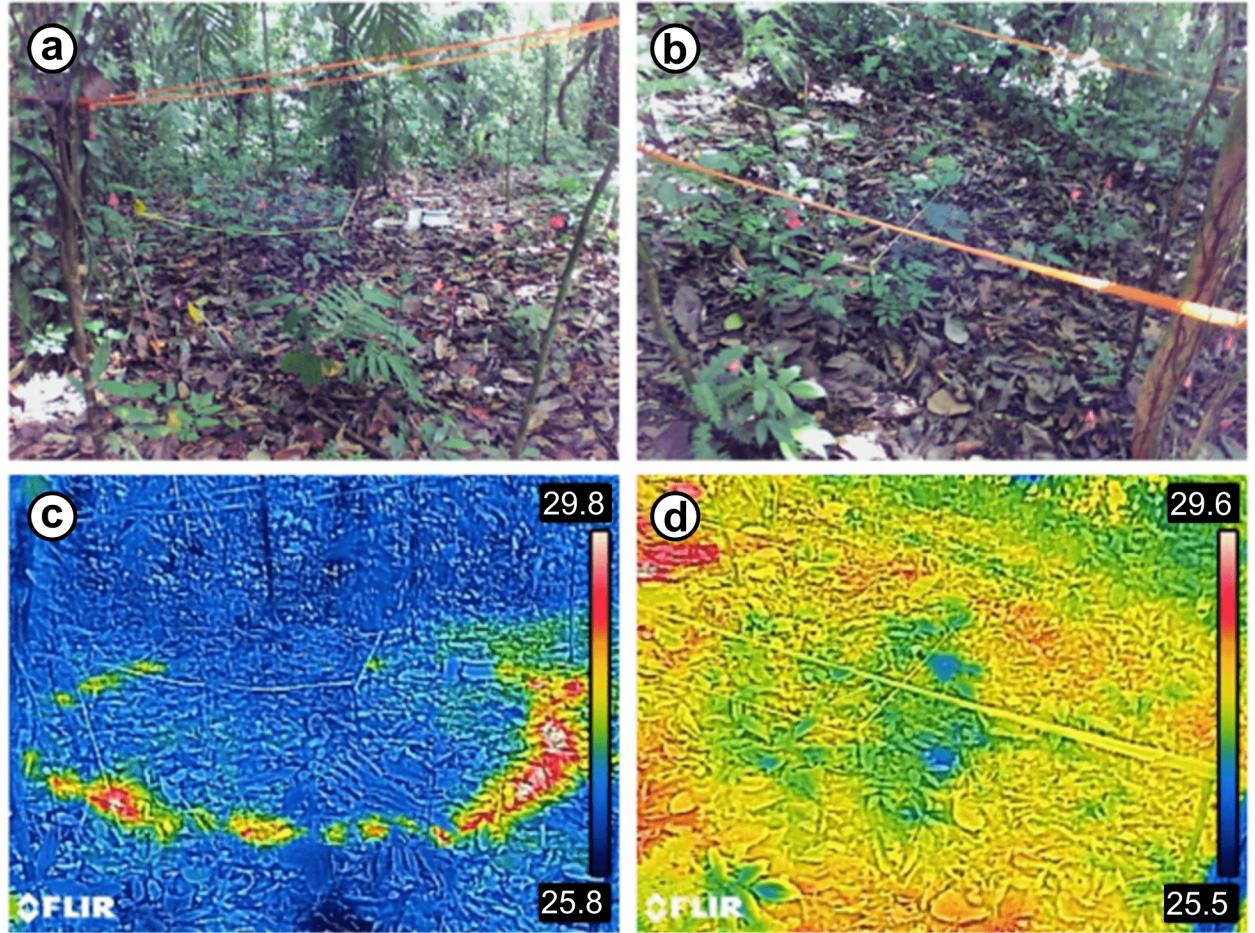
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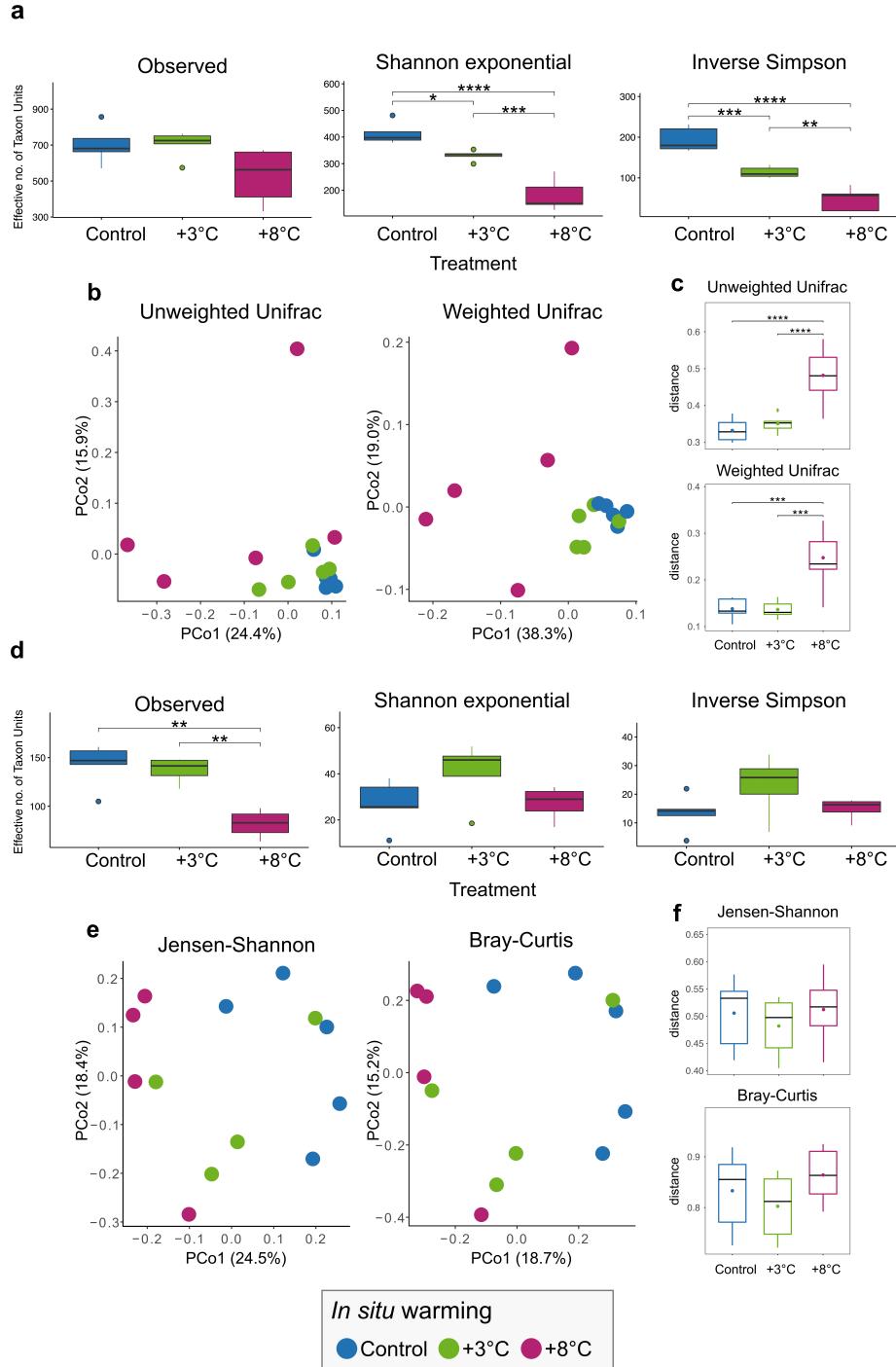
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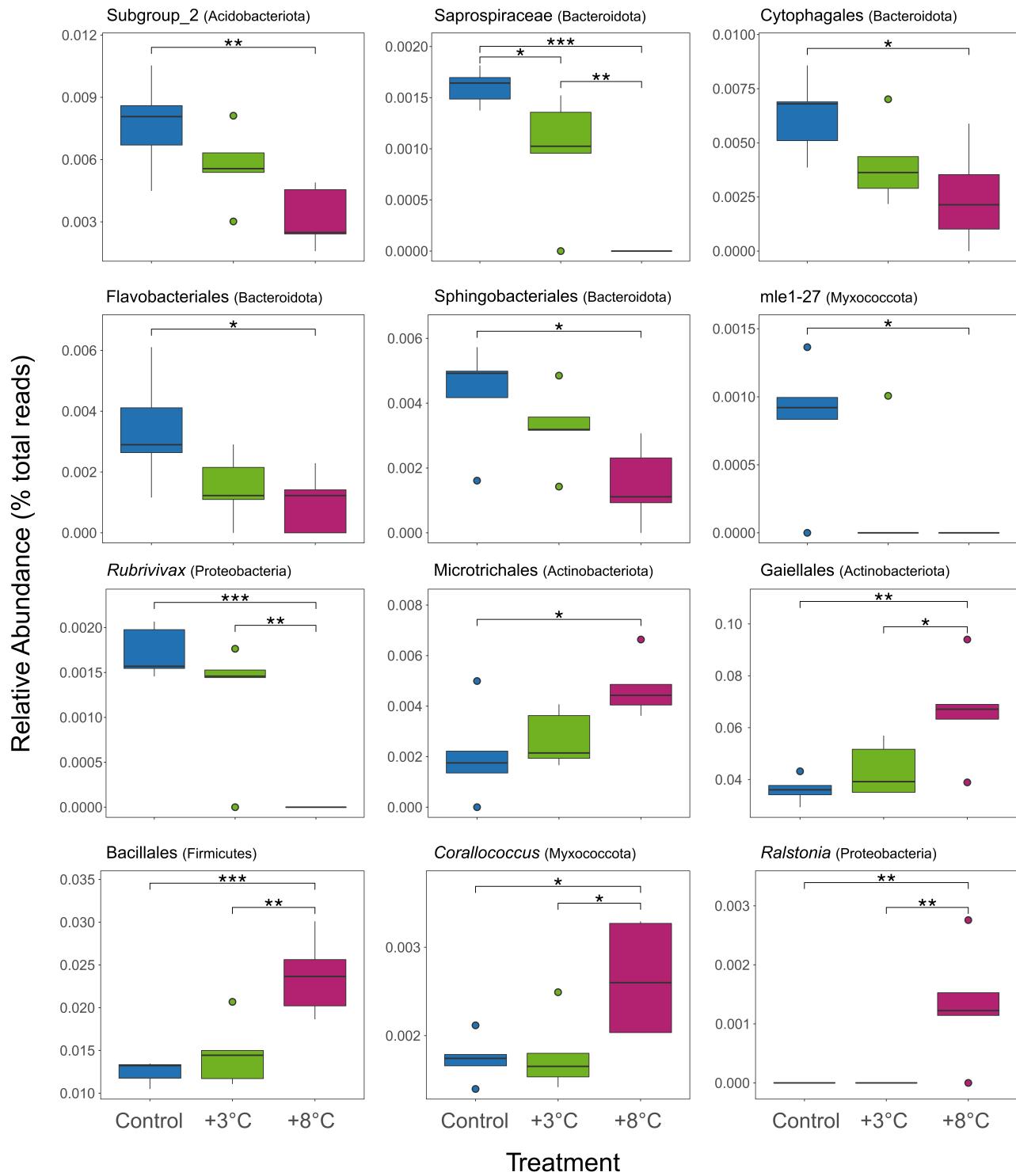
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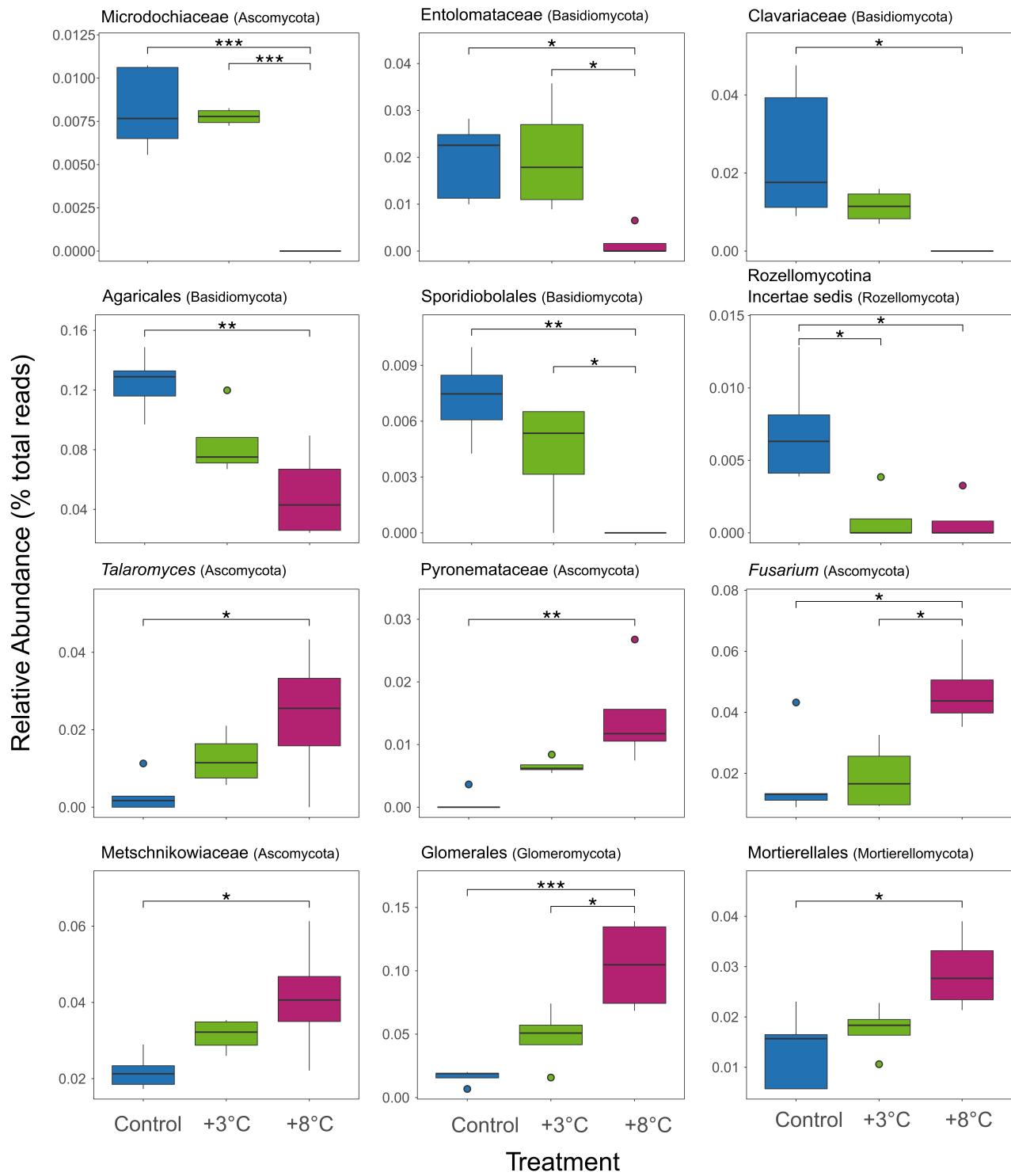
**Extended Data Figure 1 | One of five warmed plots at SWELTR.** The images show the soil surface temperature shortly after the warming structure was switched on (**a** and **c**) and after a period of thermal equilibration<sup>c</sup> (**b** and **d**). The circular heating structure was 3.5 m in diameter and extended to 1.2 m depth, which resulted in an effective heated plot of approximately 5 m diameter  $\times$   $> 1.5$  m depth (i.e. to the bedrock, situated at around 1.5–2.0 m across the study site). The experiment consisted of five warmed and control plot-pairs in total.



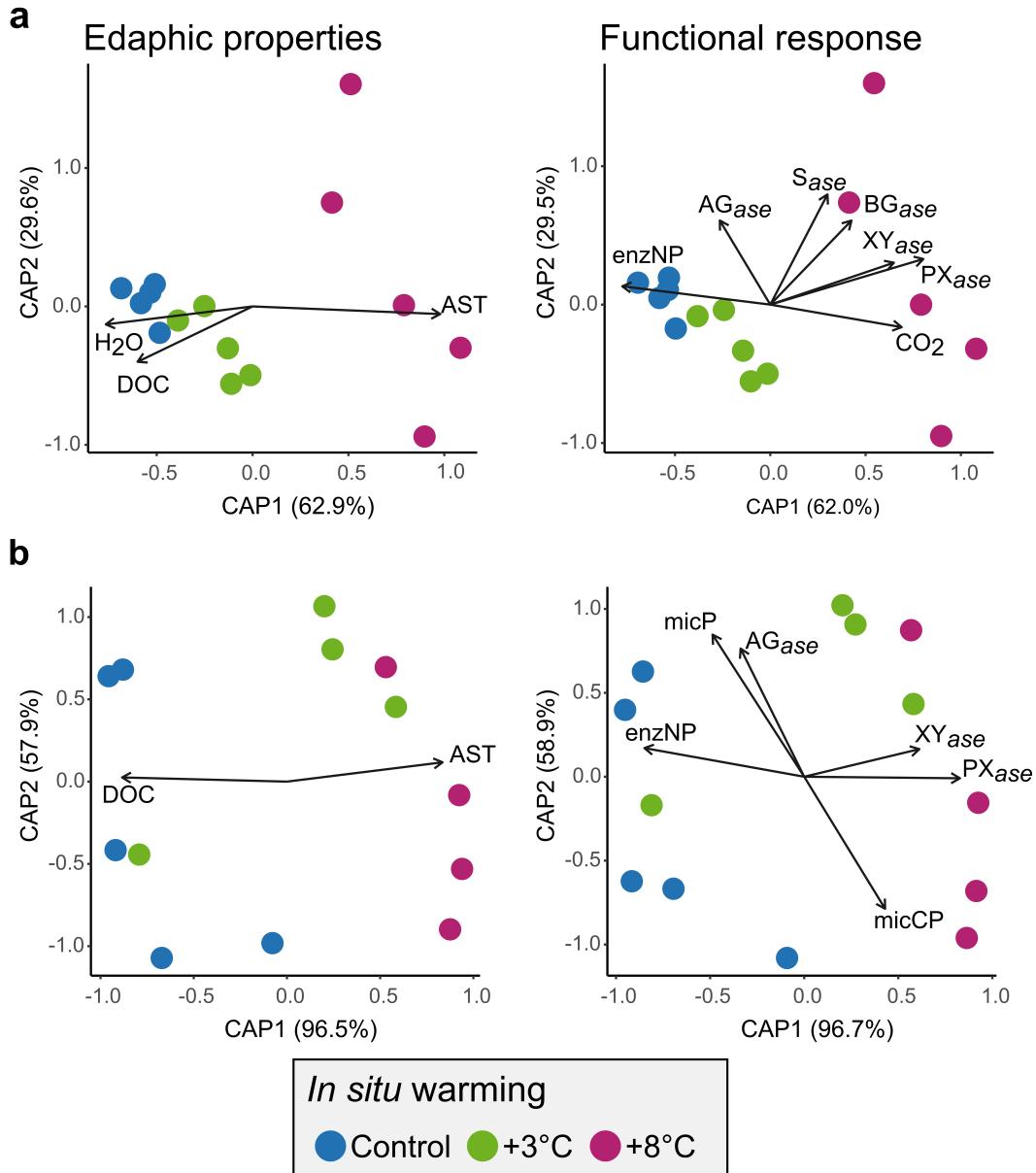
**Extended Data Figure 2 | Diversity response of soil bacteria (a–c) and fungi (d–f) to two years of warming by +3°C and +8°C.** Shapiro-Wilk Normality and Bartlett tests indicated all alpha diversity estimates (following PERfect filtering) were normally distributed and differences were assessed for (a) bacteria and (d) fungi using analysis of variance (ANOVA) followed by Tukey HSD post hoc tests. Compositional similarity of microbial communities (beta-diversity) represented as PCoA ordination plots of PERfect filtered data for (b) bacteria—estimated using Unweighted (left) and Weighted Unifrac (right) distance matrices; and (e) fungi estimated—using Jensen–Shannon divergence (left) and Bray–Curtis (right) distance matrices. Within group distances for the (c) bacteria and (f) fungi datasets. The centre line of each box plot represents the median, the lower and upper hinges represent the first and third quartiles and whiskers represent + 1.5 the interquartile range. Significant differences denoted by asterisks (\*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , \*\*\*\*  $p \leq 0.0001$ ).



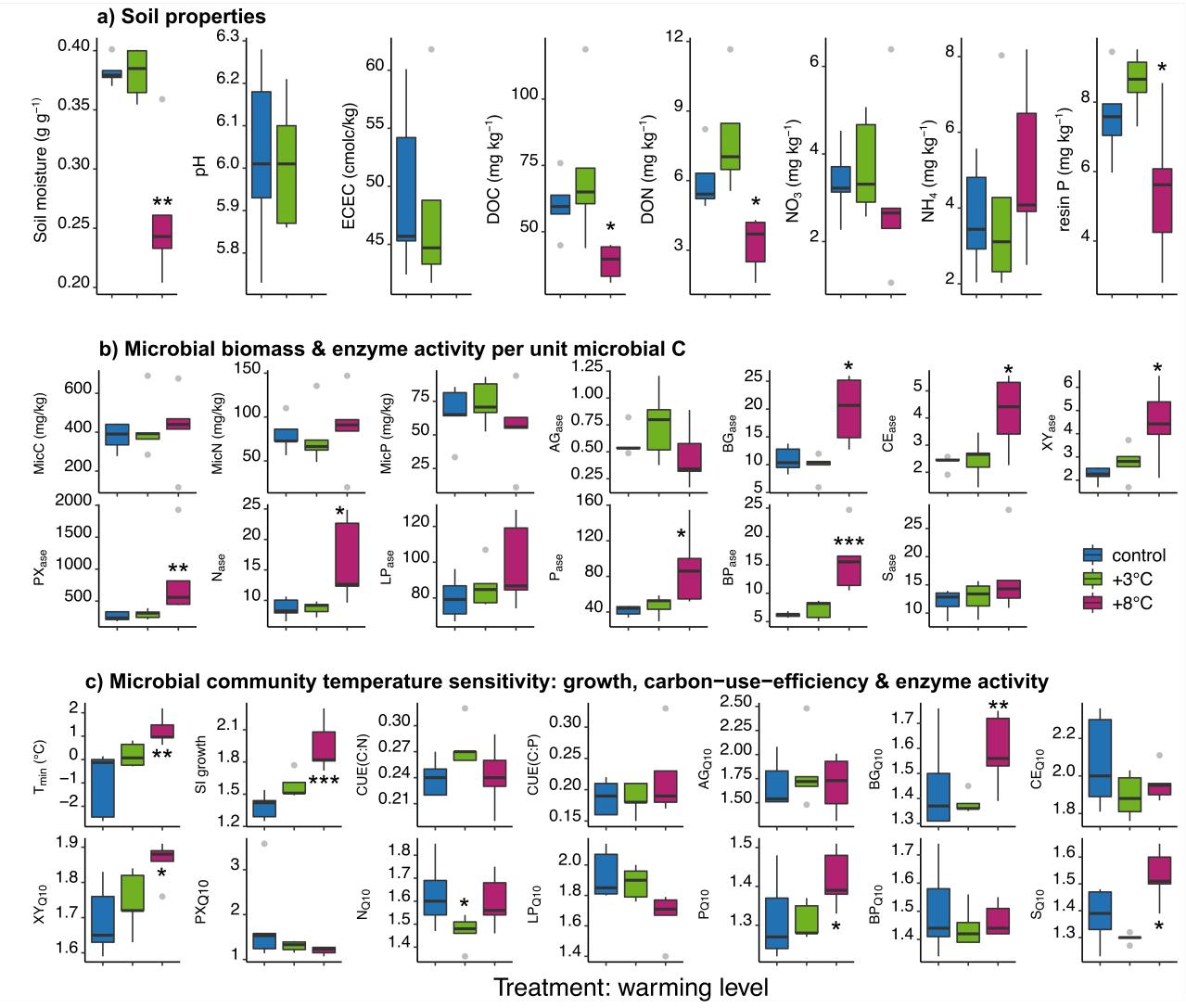
**Extended Data Figure 3 | The response of select soil bacteria taxa to two years of warming by +3°C and +8°C.** Differences assessed for multiple-group pair-wise comparisons using ANOVA followed by Tukey HSD post hoc tests. PERfect filtered read count data was  $\log_{10}$  transformed and normalized using total sum scaling (TSS). The centre line of each box plot represents the median, the lower and upper hinges represent the first and third quartiles and whiskers represent  $+ 1.5$  the interquartile range. Significant differences denoted by asterisks (\*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , \*\*\*\*  $p \leq 0.0001$ ).



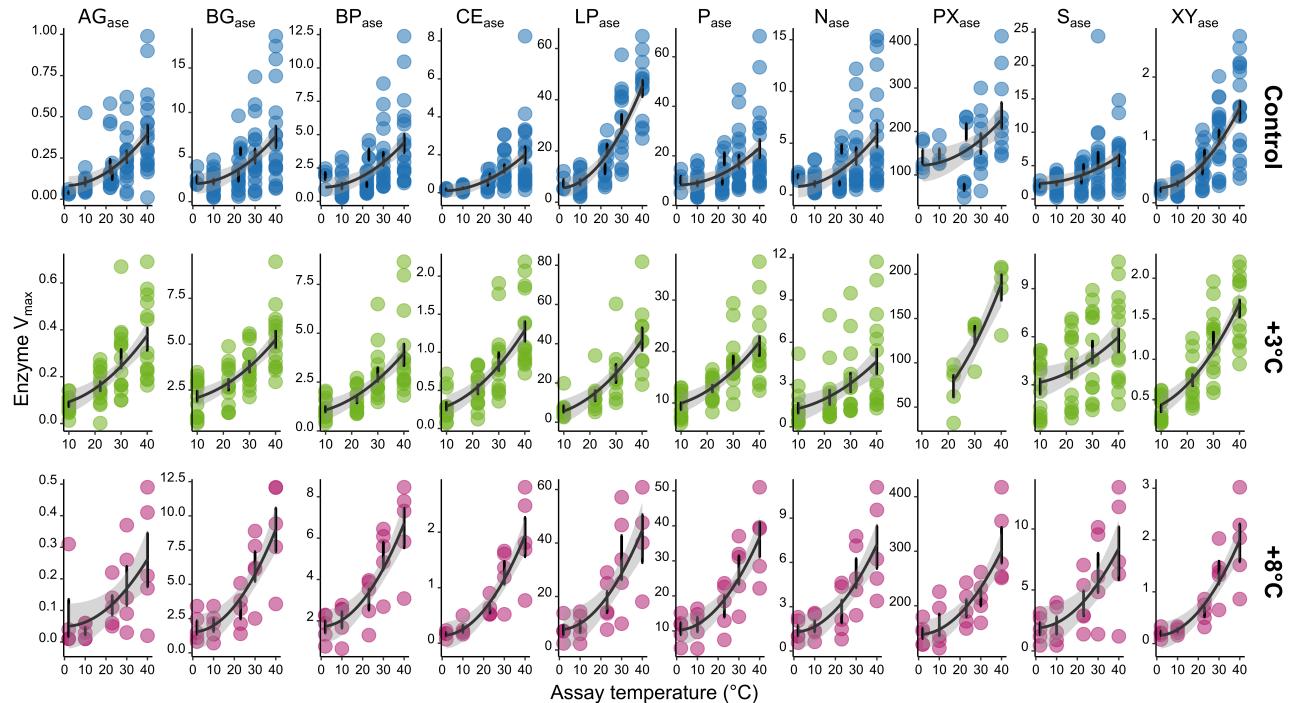
**Extended Data Figure 4 | The response of select soil fungal taxa to two years of warming by +3°C and +8°C.** Differences assessed for multiple-group pair-wise comparisons using ANOVA followed by Tukey HSD post hoc tests. PERfect filtered read count data was  $\log_{10}$  transformed and normalized using total sum scaling (TSS). The centre line of each box plot represents the median, the lower and upper hinges represent the first and third quartiles and whiskers represent +1.5 the interquartile range. Significant differences denoted by asterisks (\*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , \*\*\*\*  $p \leq 0.0001$ ).



**Extended Data Figure 5 | Distance-based Redundancy Analysis (db-RDA)** of PIME filtered data based on Bray-Curtis dissimilarity showing the relationships between community composition change for (a) bacteria and (b) fungi versus edaphic properties (left) and microbial functional response (right).



**Extended Data Figure 6 | Soil, enzyme, and microbial responses to +3°C and +8°C *in situ* soil warming.** Data are grouped by (a) soil properties, (b) microbial functional responses, and (c) microbial temperature adaptive responses; we used the same grouping to test three hypotheses on how each of these responses were correlated to changes in microbial diversity and community composition (Fig. 2; Extended data: Table 2, Fig. 5). All properties were determined for soil samples collected during the 2018 wet season (June and November); see methods. Units for enzyme  $V_{\max}$  are  $\text{nmol MU g}^{-1} \text{ min}^{-1}$ , except Phenol oxidase in  $\mu\text{mol g}^{-1} \text{ h}^{-1}$  and Leucine aminopeptidase in  $\text{nmol AMC g}^{-1} \text{ min}^{-1}$ . The centre line of each box plot represents the median, the lower and upper hinges represent the first and third quartiles and whiskers represent  $\pm 1.5$  the interquartile range. Significant differences between treatments and controls are highlighted by asterisks (ANOVA; \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ ). For  $n = 5$  plots.



**Extended Data Figure 7 | Soil enzyme activities in response to incubation temperature (i.e. instantaneous temperature response determined in laboratory assays).** Data are maximum potential enzyme activity ( $V_{\text{max}}$ ), determined by activity under saturating substrate conditions. Enzymes are:  $\alpha$ -glucosidase ( $\text{AG}_{\text{ase}}$ ),  $\beta$ -glucosidase ( $\text{BG}_{\text{ase}}$ ), phospho-diesterase ( $\text{BP}_{\text{ase}}$ ), celllobiohydrolase ( $\text{CE}_{\text{ase}}$ ), leucine aminopeptidase ( $\text{LP}_{\text{ase}}$ ), phosphomonoesterase ( $\text{P}_{\text{ase}}$ ),  $N$ -acetyl  $\beta$ -glucosaminidase ( $\text{N}_{\text{ase}}$ ), phenol oxidase ( $\text{PX}_{\text{ase}}$ ), sulfatase ( $\text{S}_{\text{ase}}$ ) and  $\beta$ -xylosidase ( $\text{XY}_{\text{ase}}$ ). Units for enzyme  $V_{\text{max}}$  are nmol MU  $\text{g}^{-1} \text{ min}^{-1}$ , except Phenol oxidase in  $\mu\text{mol g}^{-1} \text{ h}^{-1}$  and Leucine aminopeptidase in nmol AMC  $\text{g}^{-1} \text{ min}^{-1}$ . All data are for  $n = 10$  plots, determined during the wet season 2018. Controls include 4 sampling periods (June, Sept, Oct, Dec 2018);  $+3^\circ\text{C}$  include 3 sampling periods (June, Sept, Dec 2018);  $+8^\circ\text{C}$  include 1 sampling period (Sept 2018).

**Extended Data Table 1 | Relationship of bacterial and fungal richness with (a) environmental drivers, (b) microbial functional responses and (c) microbial temperature adaptive responses.** For environmental drivers of richness (a), we included temperature, moisture and key edaphic properties: models included fixed effects of Environmental drivers: treatment level (warming by +3°C and +8°C), soil moisture, soil properties (pH, N, resin P and ECEC), treatment:moisture interaction. For microbial functional correlates of richness (b), we included CO<sub>2</sub> efflux, microbial C and activity of four enzymes ( $V_{max}$  for phosphomonoesterase,  $\beta$ -glucosidase,  $\beta$ -xytanase and N-acetyl  $\beta$ -glucosaminidase). For microbial temperature adaptation correlates of richness (c), we included CUE (determined by C:N and C:P ratios of enzymatic activity), the temperature sensitivity ( $Q_{10}$ ) of four enzymes ( $Q_{10}$  of  $V_{max}$  for phosphomonoesterase,  $\beta$ -glucosidase,  $\beta$ -xytanase and N-acetyl  $\beta$ -glucosaminidase), the minimum temperature for microbial growth ( $T_{min}$ ) and the sensitivity index for microbial growth (SI = log 40/4 growth). For all models we included a random effect of plot pair (i.e. space).

<b>a) Environmental drivers of richness</b>					
Bacteria					
<i>Fixed effects</i>	<i>Estimate</i>	<i>SE</i>	<i>d.f.</i>	<i>t value</i>	<i>P value</i>
+3°C warming	-43.2	36.5	11	-1.2	0.26
+8°C warming	-350.9	56.4	11	-6.2	<0.001 ***
Soil moisture	-216	370.3	11	-0.6	0.57
Random effect (space: plot pair)	663.3	142	11	4.67	<0.001 ***
Fungi					
<i>Fixed effects</i>	<i>Estimate</i>	<i>SE</i>	<i>d.f.</i>	<i>t value</i>	<i>P value</i>
+3°C warming	-37.8	10.4	6	-3.6	0.0105 *
+8°C warming	-66.2	23	9	-2.9	0.0187 *
Soil moisture	75.9	146.1	9	0.5	0.6163
Random effect (space: plot pair)	122.6	55.5	9	2.2	0.0559
<b>b) Microbial functional correlates of richness</b>					
Bacteria					
<i>Fixed effects</i>	<i>Estimate</i>	<i>SE</i>	<i>d.f.</i>	<i>t value</i>	<i>P value</i>
CO <sub>2</sub> efflux	-10.3	2.1	12	-4.9	<0.001 ***
micC	-0.6	0.1	12	-5.0	<0.001 ***
Random effect (space: plot pair)	714	42	12	17	<0.001 ***
Fungi					
<i>Fixed effects</i>	<i>Estimate</i>	<i>SE</i>	<i>d.f.</i>	<i>t value</i>	<i>P value</i>
CO <sub>2</sub> efflux	-1.8	0.3	7	-5.7	<0.001 ***
micC	-0.2	0.02	7	-8.9	<0.001 ***
Random effect (space: plot pair)	178	7.6	10	23	<0.001 ***
<b>c) Microbial temperature adaptation correlates of richness</b>					
Bacteria					
<i>Fixed effects</i>	<i>Estimate</i>	<i>SE</i>	<i>d.f.</i>	<i>t value</i>	<i>P value</i>
CUE <sub>cn</sub>	2.5	0.91	8	6.0	0.55
T <sub>min</sub> of growth	-0.2	0.08	12	-2.2	0.05 *
Random effect (space: plot pair)	5.5	0.9	8	6.0	<0.001 ***
Fungi					
<i>Fixed effects</i>	<i>Estimate</i>	<i>SE</i>	<i>d.f.</i>	<i>t value</i>	<i>P value</i>
CUE <sub>cp</sub>	1.1	1.8	8	5.8	0.55
BG <sub>Q10</sub>	2.3	1.2	8	1.9	0.09
XY <sub>Q10</sub>	-7.6	2.1	8	-3.6	<0.01 **
T <sub>min</sub> of growth	-0.1	0.05	8	-2.3	<0.05 *
Random effect (space: plot pair)	10.1	1.7	8	5.8	<0.001 ***

**Extended Data Table 2 | The relationship between (a) bacterial and (b) fungal beta-diversity and edaphic environment (i), soil process rates (ii) and microbial temperature adaptive responses (iii) following 2 years of soil warming by +3°C to +8°C.** We used two independent methods, bioenv and envfit (vegan package), to determine significant multivariate correlations between meta-data and Bray-Curtis dissimilarity matrices for community data. Tests were performed for separate meta-data subsets to address specific hypotheses on how microbial community correlated with (a) drivers from the edaphic environment, (b) functional responses/soil process rates, (c) temperature adaptive physiological change in the community. Significant parameters are: for (a) average soil surface temperature (AST), soil gravimetric moisture ( $H_2O$ ), dissolved organic carbon (DOC); for (b) microbial P (micP),  $\alpha$ -glucosidase  $V_{max}$  ( $AG_{ase}$ ),  $\beta$ -glucosidase  $V_{max}$  ( $BG_{ase}$ ), sulfatase  $V_{max}$  ( $S_{ase}$ ),  $\beta$ -xytanase  $V_{max}$  ( $XY_{ase}$ ), leucine aminopeptidase  $V_{max}$  ( $LP_{ase}$ ), N-acetyl  $\beta$ -glucosaminidase  $V_{max}$  ( $N_{ase}$ ), phenol oxidase  $V_{max}$  ( $PX_{ase}$ ), average soil  $CO_2$  efflux ( $CO_2$ ), enzymatic N:P ratio (enzNP); and for (c) carbon-use efficiency ( $CUE_{cp}$ ), the minimum temperature for microbial growth ( $T_{min}$ ) and the temperature sensitivity index of microbial growth (SI); the  $Q_{10}$  of  $V_{max}$  for respective enzymes, denoted by subscript  $Q_{10}$ . Refer to methods for details on how  $T_{min}$ , SI and CUE were calculated.

a) Bacteria		parameter	envfit		bioenv	
Metadata set			$r^2$	P-value	$r^2$	P-value
i. Edaphic properties	AST		0.829	0.001	1.000	0.001
	$H_2O$		0.519	0.006		
	DOC		0.446	0.037		
ii. Microbial functional response	$AG_{ase}$		0.444	0.026	0.559	0.001
	$BG_{ase}$		0.560	0.007		
	$S_{ase}$		0.737	0.002	0.614	0.001
	$XY_{ase}$		0.519	0.009	0.456	0.002
	$PX_{ase}$		0.764	0.001	0.612	0.001
	$CO_2$		0.504	0.013		
	enzNP		0.624	0.004	0.462	0.006
iii. Temperature adaptive response	$S_{Q10}$		0.496	0.015	0.439	0.001
	$LP_{Q10}$		0.413	0.041	0.377	0.005
	$T_{min}$ for growth		0.446	0.030	0.404	0.005
	$CUE_{cp}$				0.325	0.013
	$P_{Q10}$				0.518	0.001

b) Fungi		parameter	envfit		bioenv	
Metadata set			$r^2$	P-value	$r^2$	P-value
i. Edaphic properties	AST		0.485	0.037	1.000	0.001
	DOC		0.535	0.028		
ii. Microbial functional response (process rates)	micP		0.692	0.002		
	micCP		0.583	0.016		
	$AG_{ase}$		0.506	0.037		
	$PX_{ase}$		0.500	0.035	0.685	0.001
	enzNP		0.547	0.014	0.553	0.001
	$XY_{ase}$				0.505	0.002
iii. Temperature adaptive response	$XY_{Q10}$		0.617	0.010	0.726	0.001
	$CUE_{cp}$		0.479	0.035		
	$T_{min}$ for growth		0.475	0.028	0.616	0.001

**Extended Data Table 3 | The influence of soil abiotic environment on soil CO<sub>2</sub> efflux (a), and the effect of in situ warming levels (by +3°C and +8°C) on soil CO<sub>2</sub> efflux (b) and soil moisture (c).** Results are from repeated measures ANOVA fitted by maximum likelihood, where time is a random effect. Data were log-transformed prior to analyses. (n = 5 subplots).

**a) Abiotic effects on soil CO<sub>2</sub> efflux**

	Parameter	SE	DF	P-value
<b>Fixed effects</b>				
Temperature	2.238	0.134	95.168	<2e-16 ***
Moisture	-3.659	9.193	96.702	0.691
<b>Random effects</b>				
Intercept (time)	-58.03	5.545	96.728	<2e-16 ***

**b) Effect of warming levels on soil CO<sub>2</sub> efflux**

	Parameter	SE	DF	P-value
<b>Fixed effects</b>				
Warming (+3°C)	3.692	1.271	326	0.00392 **
Warming (+8°C)	11.249	11.249	326	6.36e-16 ***
<b>Random effects</b>				
Intercept (time)	4.736	0.899	326	2.47e-07 ***

**c) Effect of warming levels on soil moisture**

	Parameter	SE	DF	P-value
<b>Fixed effects</b>				
Warming (+3°C)	-0.074	0.027	78.264	0.00799 **
Warming (+8°C)	-0.120	0.025	17.686	0.00015 ***
<b>Random effects</b>				
Intercept (time)	0.386	0.023	18.364	1.22e-12 ***