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Soils store about four times as much carbon (C) as plant biomass[1], and soil microbial respiration releases about 60 Pg of C per year to the atmosphere as carbon dioxide (CO_2)[2]. Short-term experiments have shown that soil microbial respiration increases exponentially with temperature[3]. This information has been incorporated into soil C and Earth system models, which predict that warming-induced increases in CO_2 release from soils could represent an important positive feedback to 21st century climate change[4]. The magnitude of this feedback remains uncertain, however, not least because the response of soil microbial communities to changing temperatures has the potential to either decrease[5-7] or increase[8-9] warming-induced C losses substantially. Here we collect soils from different ecosystems along a climate gradient from the Arctic to the Amazon and investigate the role microbial community-level responses play in controlling the temperature sensitivity of soil respiration. We find that the microbial community level response enhances the mid- to long-term (90 days) temperature sensitivity of respiration more often than reducing it. Furthermore, the strongest enhancing responses were observed in soils with high carbon to nitrogen ratios and in soils from cold climatic regions. After 90 days, microbial community responses increased the temperature sensitivity of respiration in high-latitude soils by a factor of 1.4 compared to the instantaneous temperature response. This suggests that the substantial C stores in Arctic and Boreal soils could be more vulnerable to climate warming than currently predicted.

Text:

Short-term experiments have demonstrated that the rate of soil microbial respiration increases exponentially with temperature, and this general relationship has been used to parameterise

soil C and Earth System models[4,10]. However, plant physiologists have demonstrated that short-term measurements are inadequate for representing the dynamic response of plant respiration to changes in temperature. In plants, thermal acclimation, defined as the "subsequent adjustment in the rate of respiration to compensate for an initial change in temperature"[11] greatly reduces the impact of temperature changes on respiration in the medium- to long-term, and incorporating this acclimation into models alters predicted rates of terrestrial C uptake[12]. In soil, there is growing interest in the potential for a response analogous to thermal acclimation in plants, as microbial communities adapt to changes in temperature[13]. However, it is unclear if microbial community responses should always reduce the effect of a temperature change on respiration rates. In fact, responses that enhance the instantaneous effect of temperature changes on soil respiration have also been observed[8,9,14]. To date there has been no large-scale evaluation of the role of microbial community responses in controlling the temperature sensitivity of soil respiration. This lack of understanding adds considerable uncertainty to predictions of the magnitude and direction of C-cycle feedbacks to climate change[15].

Despite several attempts at clarification, the use of terminology remains quite confused in this research field[13]. Because measurements of soil microbial respiration are made at the level of the whole community, they encompass acclimation (physiological responses of individuals), adaptation (genetic changes within species), and ecological (e.g. competition altering species composition) responses that can lead to adjustments in respiration rates following a sustained change in temperature[13]. For this reason, rather than acclimation or adaptation, which have strict definitions, we have chosen to use the term "community-level response". These community-level responses can be either compensatory (reducing the effect of a temperature change on respiration rates in the longer term) or enhancing (enhancing the effect of a

temperature change on respiration rates in the longer term). The critical research question remains: how do microbial community-level responses affect the temperature sensitivity of soil respiration?

When soil is warmed for an extended time period, the initial increase in biological activity leads to a loss of readily decomposable C[5]. Microbial activity then tends to decline in the longer term, but it is often impossible to determine if this is caused by the loss of the readily decomposable C or by a compensatory response by the microbial community, as both would reduce activity[16-17]. To differentiate between these two mechanisms, we established a new approach[8] that involves soil cooling in the laboratory. Compensatory community responses and substrate loss should have opposite effects on microbial activity under cooling. In the absence of C inputs, soil C losses still occur in cooled soils, thus reducing activity, albeit at a slower rate than in the controls. However, a compensatory response of the microbial community should result in a gradual increase in respiration rate as the community compensates for the effects of the cooling; this is analogous to what is observed for thermal acclimation of plant respiration[11]. Furthermore, because we can quantify rates of soil C loss, we can also identify enhancing responses if respiration rates decline more rapidly in the cooled soil than in the control.

Using our cooling approach, we carried out the first global investigation of how microbial community responses to temperature changes affect soil respiration rates, collecting soil from sites representing a range of ecosystem types (arable, grassland, deciduous and evergreen broadleaf forest, coniferous forest, and heath) across a gradient of mean annual temperature (MAT) from -6°C to 24°C (Fig. 1, Extended Data Table 1). Twenty samples of each soil were pre-incubated at 3°C above the MAT of their collection site (see Fig. 2a) for 84 days to allow

respiration rates to stabilise. On day 84, five samples were destructively sampled for microbial biomass determination, ten samples were cooled by 6°C (MAT-3°C), and five controls were maintained at MAT+3°C for the remaining 90 days of the experiment. Five of the cooled samples were incubated at MAT-3°C for 90 days, a time period relevant to seasonal changes in temperature, which have been hypothesised to cause thermal adaptation[18]. The other five cooled samples were re-warmed to MAT+3°C after 60 days at MAT-3°C, and incubated at MAT+3°C for the remaining 30 days of the experiment, allowing the reversibility of any response to be determined.

Our approach establishes two clear criteria for quantifying either compensatory or enhancing community-level responses (Fig. 2a, Extended Data Fig. 1). First, the CO_2 flux, normalised to the flux at the time of cooling (control samples) or immediately after cooling (cooled samples), was plotted against cumulative C loss (see Methods; Extended Data Figs. 1 to 7). The impact of community responses on respiration rates at the measurement temperatures ($Ratio_{MT}$) was calculated as the normalised control respiration rate, at the %C loss corresponding to the total %C loss in the cooled soils (see Supplementary), divided by the normalised cooled respiration rate at the end of the incubation. Ratios <1 indicate a compensatory response (i.e. normalised respiration rates were greater at a given level of soil C loss in the cooled treatment), and ratios >1 indicate an enhancing response (i.e. normalised respiration rates were lower at a given level of soil C loss in the cooled treatment). A second quantitative measure was obtained by comparing the respiration rates of samples re-warmed after 60 days of cooling with control sample respiration rates at the same C loss (see Supplementary). This ratio at a common temperature[19] ($Ratio_{CT}$) was calculated as the control respiration rate divided by re-warmed respiration rate and, again, ratios <1 and >1 indicate compensatory and enhancing responses, respectively. Given that changes in biomass have

been considered to be important in previous studies[8], we also calculated $Ratio_{MT}$ on a microbial biomass-specific basis (see Methods).

All three possible community-level responses were observed: compensatory responses (Fig. 2c), no-response (Fig. 2b), and enhancing responses (Fig. 2d). However, for the 22 soils analysed, many more statistically significant cases of enhancing responses were observed (See Supplementary). Overall average response ratios (n=22 soils) were significantly above 1 ($P < 0.01$ for $Ratio_{MT}$; $P < 0.05$ for $Ratio_{CT}$). In all cases of clear enhancing or compensatory responses, respiration rates after re-warming subsequently approached control rates (e.g. Figs 2c & d). This reversibility of the response indicates that patterns were not caused by cooling altering the quality of the remaining C, and emphasises the comparability, in terms of effects on rates of respiration, of microbial community responses to cooling and warming.

The average $Ratio_{MT}$ values were greatest for boreal and Arctic soils (MAT<7 °C group, Fig. 3a), but also significantly above 1 for the MAT>14 °C group. For the MAT<7 °C group, the microbial community response increased the temperature sensitivity of soil respiration by a factor of 1.4 during the 90 days of cooling; the temperature sensitivity, expressed as a Q_{10} value (proportional change in respiration for a 10 °C change in temperature), increased from 4.6 at the time of cooling to 6.3 at the end of the incubation (See Methods).

Arable/'managed', low C content, and low carbon to nitrogen (C:N) ratio soils, were the only soils to show average $Ratio_{MT}$ values close to or below 1 (Fig. 3a). Enhancing responses were generally more common in soils with high C contents, high C:N ratios and low pHs (Fig. 3a), and forest and “natural ecosystem” groups also showed enhancing responses. C:N ratio was the only soil or site variable (Fig. 3) that was significantly correlated with the $Ratio_{MT}$

responses across all data ($lnRatio_{MT}$ = 0.188*lnCNratio - 0.406, R^2=0.335, P = 0.005), and the low $Ratio_{MT}$ for the MAT 7-14 °C group, may have been related to the greater number of managed, low C:N ratio soils in this group rather than the temperature range *per se*.

Overall, our results demonstrate that microbial community-level responses enhance the impacts of temperature changes on soil respiration rates. To improve mechanistic understanding, and for modelling dynamics[15,20], it has been argued that changes in biomass must be accounted for explicitly in quantifying microbial community responses[13]. In our study, for a given C loss, biomass did not differ significantly between cooled and control soils (whether measured by chloroform fumigation extraction or qPCR), and thus mass-specific patterns did not differ substantially from the raw responses; mass-specific responses had slightly greater average values ($Ratio_{MT\_MS} > Ratio_{MT}$) but also showed greater variability (Extended Data Fig. 8, Supplementary). Overall, changes in microbial biomass could not explain the observed microbial community responses.

The greater enhancing responses in cold soils and soils with wide C:N ratios require further consideration. The requirements for surviving at low temperatures are known to present strong selection pressures that induce fundamental changes at the cellular level[21,22]. In plants, cold acclimation results in an up-regulation of respiration rates at lower temperatures[11], but our data demonstrate that adaptation to cooling by microbial communities from high-latitude soils, reduces respiration rates, which may be consistent with strategies that promote survival but reduce metabolic activity[21,22]. However, strong enhancing responses were also observed in some tropical and Mediterranean soils so the development of cold tolerance cannot be the full explanation for the observed responses. C:N ratio was the only variable that was positively correlated with $Ratio_{MT}$ across all data. If the temperature sensitivities of key N

cycle processes are greater than some C cycle processes[23,24], then it is possible that N availability may limit microbial activity following cooling, especially in soils with wide C:N ratios. This could potentially induce adaptive changes in allocation to N versus C acquisition to meet stoichiometric requirements[23], which could in turn be reversed on rewarming. Links between C and N cycling may also help explain why our results differ from some previous studies. Compensatory thermal adaptation has previously been observed in ectomycorrhizal fungi grown on agar[18], and also in monocultures of heterotrophic fungi[7]. The dominance of enhancing adaptation responses identified in our study could be related to the fact that community-level competition for C and N sources is important for determining the overall response to warming.

In conclusion, enhancing community-level responses were much more common than compensatory responses, with the latter mainly limited to arable soils and soils with low C contents (Fig. 3a), thus limiting the potential importance of compensatory responses for rates of climate change-induced C losses. The predominance of enhancing responses implies that decreased soil respiration rates in response to long-term ecosystem warming in the field[25] are probably related to the loss of readily decomposable C, rather than to any community-level response down-regulating microbial respiration rates. Finally, given that boreal and arctic regions contain more than 50% of the global soil C stock[26], the strong enhancing responses observed in these soils could have significant consequences for the global C budget.

References

1. IPCC. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp. (2013).

2. Shao, P., Zeng, Z, Moore, D.J.P. & Zeng, X. Soil microbial respiration from observations and Earth System Models. *Environ. Res. Lett.* **8**, 034034 (2013).

3. Davidson, E.A. & Janssens, I.A. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **440**, 165-173 (2006).

4. Friedlingstein, P., et al. Climate-carbon cycle feedback analysis: Results from the C4MIP model intercomparison. *J. Climate* **19**, 3337–3353 (2006).

5. Bradford et al. Thermal adaptation of soil microbial respiration to elevated temperature. *Ecol. Letts.***11**, 1316-1327 (2008).

6. Bradford, M.A., Watts, B.W. & Davies, C.A. Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. *Glob. Change Biol.* **16**, 1576-1588 (2010).

7. Crowther, T.W & Bradford, M.A. Thermal acclimation in widespread heterotrophic soil microbes. *Ecol. Letts.* **16**, 469-477 (2013).

8. Hartley, I.P. et al. Soil microbial respiration in arctic soil does not acclimate to temperature. *Ecol. Letts.* **11**, 1092-1100 (2008).

9. Nie, M. et al Positive climate feedbacks of soil microbial communities in a semi-arid grassland. *Ecol. Letts.* **16**, 234-241 (2013).

10. Kirschbaum, M.U.F. The temperature dependence of organic-matter decomposition—still a topic of debate. *Soil Biol. Biochem.* **38**, 2510-2518 (2006).

11. Atkin, O.K. & Tjoelker, M.G. Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends Plant Sci.* **8**, 343–351 (2003).

12. Galbraith, D. et al. Multiple mechanisms of Amazonian forest biomass losses in three dynamic global vegetation models under climate change. *New Phytol.* **187**, 647-665 (2010).

13. Bradford, M.A. Thermal adaptation of decomposer communities in warming soils. *Frontiers of Microbiology* **4**, 00333 (2013).

14. Hartley, I.P., Heinemayer, A., Evans, S.P. & Ineson, P. The effect of soil warming on bulk soil vs. rhizosphere respiration. *Glob. Change Biol.* **13**, 2654–2667 (2007).

15. Wieder, W.R., Bonan, G.B. & Allison, S.D. Global soil carbon projections are improved by modelling microbial processes. *Nature Climate Change* **3**, 909-912 (2013).

16. Ågren, G.I. & Bosatta, E. Reconciling differences in predictions of temperature response of soil organic matter. *Soil Biol. Biochem.* **34**, 129–132 (2002).

17. Kirschbaum, M.U.F. Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? *Glob. Change Biol.* **10**, 1870–1877 (2004).

18. Malcolm, G.M., López-Gutiérrez, J.C, Eissenstat, D.M & Koide, R.T. Acclimation to temperature and temperature sensitivity of metabolism by ectomycorrhizal fungi. *Glob. Change Biol.* 14, 1169-1180 (2008).

19. Loveys, B.R., Atkinson, L.J., Sherlock, D. J., Roberts, R.L., Fitter A.H. & Atkin, O.K. Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast- and slow-growing plant species. *Glob. Change Biol*. 9, 895-910 (2003).

20. Allison, S.D., Wallenstein, M.D. & Bradford, M.A. Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience* 3, 336-340 (2010).

21. Hochachka, P.W & Somero, G.N. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press, New York, 466 pp. (2002).

22. Schimel, J., Balser, T.C. & Wallenstein, M. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* **88**, 1386-1394 (2007).

23. Billings, S.A. & Ballantyne IV, F. How interactions between microbial resource demands, soil organic matter stoichiometry, and substrate reactivity determine the direction and magnitude of soil respiratory responses to warming. *Glob. Change Biol.* **19**, 90-102 (2013).

24. Lehmeier, C.A., Min, K., Niehues, N.D., Ballantyne IV, F. & Billings, S.A. Temperature-mediated changes of exoenzyme-substrate reaction rates and their consequences for carbon to nitrogen flow ratio of liberated resources. *Soil Biol. Biochem.* **57**, 374-382 (2013).

25. Luo, Y., Wan, S., Hui, D. & Wallace, L.L. Acclimatization of soil respiration to warming in a tall grass prairie. *Nature* **413**, 622–625 (2001).

26. FAO, IIASA, ISRIC, ISSCAS and JRC. Harmonized World Soil Database (version 1.1), Food and Agriculture Organization of the United Nations (FAO), International Institute for Applied Systems Analysis (IIASA), ISRIC - World Soil Information, Institute of Soil Science - Chinese Academy of Sciences (ISSCAS), Joint Research Centre of the European Commission (JRC), Laxenburg, Austria. (http://www.iiasa.ac.at/Research/LUC/External-World-soil-database/HTML/). (2009).

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Author contributions

K.K. conducted the CO_2 measurements and statistical analyses. K.K. and M.A. conducted the CFE and qPCR analyses, respectively, and led the data analysis and interpretation. I.P.H. (lead investigator), P.A.W., D.W.H., B.K.S. and J.I.P designed the study. G.I.Å. and K.K. were responsible for the modelling presented in the methods. K.K., I.P.H., J.A.D., D.W.H., J-A.S., P.A.W., T.S., F.G., G.B., P.M., and A.T.N. were involved in planning site selection and soil sampling. All authors were involved in interpreting the results and contributed to writing the manuscript.

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Figure legends

Figure 1 Soil was sampled from Boreal and Arctic, Temperate, Mediterranean and Tropical climates. Arable, grassland, heath, coniferous forest and deciduous forest sites were sampled in each climatic region (except the Tropics, where evergreen broadleaf forest sites were sampled along an altitudinal gradient in the Peruvian Andes), and within each ecosystem type, sites are numbered in order of increasing MAT. Details of sampling sites (vegetation and soil characteristics) are presented in Extended Data Table 1.

Figure 2 The patterns of CO_2 flux that would be observed in the case of no response, compensatory and enhancing community level response. The schematic diagram (a), indicates how a gradual increase in soil respiration rate after cooling provides support for compensatory response, while a more rapid decline in cooled soils indicates enhancing response (1.), as well as how differences in rates of respiration in re-warmed versus control samples (2.) can be used to quantify the magnitude and direction of the community level response (see also Extended Data Fig. 1). Examples of measured CO_2 fluxes illustrating no response (panel b, soil 2C), compensatory response (panel c, soil 3A) and enhancing response (panel d, soil 1C). In panels (b-d), mean respiration rates ±1SE (n=5), are presented. A break in the x-axis scale (II) denotes that pre-incubation data are not shown.

Figure 3 The impacts of the microbial community responses on the response of soil respiration to changes in temperature. The mean ± 95% confidence intervals of $Ratio_{MT}$ (a) and $Ratio_{CT}$ values (b) are presented overall (i.e. including all data), and for different soil

groups, based on ecosystem type, management, climate and various soil properties. Values > 1 indicate an enhancing response, and values < 1 indicate a compensatory response.

Methods

Soil sampling and properties

Soil samples were taken using a soil corer (10 cm diameter and 10 cm depth). 20 to 30 soil cores were sampled per site to obtain a representative sample. Soils were coarsely sieved to 5.6 mm to minimise disturbance, and gently mixed to produce a homogeneous composite sample.

Initial soil C and N contents were measured from the sieved composite sample with three analytical replicates using a Flash 2000 organic elemental analyser (Thermo Scientific). Soil pH(H_2O) was measured from a soil slurry with 1:2.5 v:v ratio of soil to deionised water with an Accumet AB 15/15+ pH meter (Fisher Scientific Ltd). Particle size was measured using a Saturn digitiser, and soil texture class defined according to UK-ADAS classification. Soil water content was determined by drying sub samples at 105°C for 24 h. 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% WHC was then measured gravimetrically by drying a sub sample at 105 °C for 24 h.

Incubation

Soil for incubation studies was prepared by setting the composite sample to the optimal moisture content of 60% of WHC[27] and dividing it into 20 parts. Approximately 180 - 490 g of soil fresh weight, depending on the soil type, was placed inside 0.5-litre rectangular plastic containers. These containers had pierced lids that enabled gas exchange, but minimised evaporation and soil drying. Soil containers were placed inside incubators (Sanyo Electric Co., Ltd./Panasonic cooled Incubator, MIR-154) with temperature adjusted to MAT+3°C. For sites with MAT close to or below 0°C, the control incubation temperature was 7°C. Soil temperature was not reduced below 0°C to avoid freeze-thaw effects. Temperature inside incubators was monitored using Tinytag External temperature loggers (Tinytag Plus 2, model TGP- 4020; Gemini Data Loggers) connected to thermistor probes (PB-5001-1M5). Soil moisture was maintained at the optimum 60% of WHC by regularly weighing the soil containers and adding deionised water to compensate for moisture loss.

The 20 replicates were randomly assigned to four treatments (n=5): pre-cooling (incubated at MAT +3°C, destructively sampled at the end of the pre-incubation period on day 84), control (incubated at MAT+3°C for 174 days), cooled (incubated at MAT+3°C for 84 days, then cooled to MAT-3°C for 90 days), and re-warmed (incubated at MAT+3°C for 84 days, then cooled to MAT-3°C for 60 days and re-warmed to MAT+3°C for 30 days). Microbial biomass was measured for the pre-cooling treatment on day 84, and for the three other treatments on day 174 using the chloroform fumigation extraction method (CFE)[28], and qPCR. This allowed $Ratio_{MT}$ to also be expressed per unit CFE biomass ($Ratio_{MT\_MS\_CFE}$) and per unit qPCR biomass ($Ratio_{MT\_MS\_qPCR}$). Total microbial biomass was estimated based on qPCR results as the sum of relative gene abundance (expressed per g soil d.w.) of bacterial 16S rRNA gene, archaeal 16S rRNA gene, and fungal ITS1 gene.

CO_2 flux measurement

Soil respiration was initially measured weekly, and later biweekly. After cooling and re-warming the first respiration measurement was started 24 h after temperature change, and weekly CO_2 measurements were made during these key periods. To measure soil respiration, each 0.5-litre rectangular soil container (without the lid) was placed inside a larger 1.8-litre rectangular plastic container (Lock & Lock^R). This incubation chamber was connected to an infrared gas analyser (EGM-4, PP systems, version 4.17, Hitchen, UK) in a closed loop configuration. The first CO_2 measurement (time 0) was taken 1 h after closing containers. CO_2 concentration inside containers was recorded again after 18 h. Soil CO_2 production rate was calculated assuming that CO_2 accumulation within containers was linear (tests confirmed that this assumption was appropriate over this time period), and fluxes were expressed per gram of initial soil C (μg C g soil C^{-1} h^{-1}).

Quantifying the magnitude of the community level respiration responses

To compare changes in activity in the cooled and control soils, it was essential to plot normalised respiration rates against cumulative C loss. Modelling the experiment using the Q-model[29] explains why this is necessary, with modelled CO_2 fluxes presenting the patterns that would be observed if there was no compensatory or enhancing community level-response (Extended Data Fig. 1). Firstly, the modelling demonstrates that greater respiration rates in the warmer control soils compared to the cooled soils (Extended Data Fig. 1a) lead to a faster rate of C loss (Extended Data Fig. 1b). Thus, when fluxes are plotted against time, there is a more rapid decline in control respiration rates (steeper slope), compared to cooled soils, and a greater respiration rate in the re-warmed samples compared to the control (Extended Data

Fig. 1a). In other words, plotting the absolute respiration rates against time can cause an 'apparent compensatory community response' in terms of CO_2 fluxes. Although our approach minimises differences in C availability between the control and cooled treatments (see Supplementary), we still needed to account for these small differences, to ensure that C availability did not affect the patterns observed. To do this, we had to first to account for differences in C availability in cooled vs. control soils by plotting respiration rates against cumulative C loss (Extended Data Fig. 1c, Extended Data Figs. 2 to 7). If there is no microbial community level mechanism affecting the CO_2 flux, when fluxes were plotted against cumulative C loss, the absolute respiration rates in the re-warmed samples are now equal to control treatment respiration rates (Extended Data Fig. 1c). This allowed any statistically significant differences between re-warmed and control CO_2 fluxes to be used as evidence of microbial community level responses affecting CO_2 flux ($Ratio_{CT}$), again, as long as fluxes are plotted against cumulative C loss (see Extended Data Figs. 2 to 7). $Ratio_{CT}$ was calculated as control treatment respiration rate (regression line value at similar C loss as the re-warmed samples, see Supplementary) divided by re-warmed treatment (average of n=5 replicates) respiration rate.

However, even when there is no response, because the absolute activity is lower in the cooled soils, this still results in a smaller absolute reduction in activity than in the controls, and thus a less steep slope, when absolute respiration rates are plotted against cumulative C loss (Extended Data Fig. 1c); the proportional reduction in activity is identical but the absolute reduction in activity is smaller in the cooled soils. To overcome this issue, respiration rates were normalised to the rate measured at the time of cooling (control samples) and to the rate measured immediately after cooling (cooled samples). The modelling demonstrates that when these normalised rates are plotted against cumulative C loss the relative respiration rates of

control and cooled soils are now identical (Extended Data Fig. 1d). Thus, any significant difference in the normalised respiration rates plotted against cumulative C loss (Extended Data Fig. 1d, Extended Data Figs. 2 to 7) allows detection of compensatory or enhancing community level responses. These values were compared at the maximum C loss for cooled samples (last measurement for cooled treatment in the end of the incubation) to incorporate the full effect that 90 days cooling had on respiration rates. Cooled sample respiration rates were compared to control regression line value for the corresponding percentage of C loss (See Supplementary) to account for any effects of different C availability (See Extended Data Figs. 2 to 7). $Ratio_{MT}$ was calculated as control treatment relative respiration rate/cooled treatment (average) relative respiration rate.

We also calculated mass specific $Ratio_{MT}$ values, using relative respiration rates at the maximum C loss for cooled samples, divided by chloroform-fumigation extraction (CFE) and qPCR biomass. For cooled samples this was the biomass measured at the end of the incubation, but we had to calculate biomass in the control soils at the %C loss which corresponded to the maximum C loss in the cooled samples. To do this, we interpolated between pre-cooling biomass and biomass measured at the end of the experiment, based on the amount of C that was lost over this period. Control samples did not experience any temperature change during the incubation, so we can assume that any change in microbial biomass after day 84 was due to slowly decreasing C availability.

To determine the extent to which the microbial community response modified the temperature sensitivity of respiration, we calculated Q_{10} values for the soils in the MAT<7^{o}C group. The Q_{10} value at the time of cooling was calculated using the respiration rate of control treatment samples immediately before and cooled treatment samples immediately

after cooling (the samples were allowed to equilibrate at the colder temperature for 24 h before starting the measurement, which is a typical way of determining short-term Q_{10} values for soil respiration). This was compared to a "long-term" Q_{10} value affected by the prolonged cooling. The Q_{10} value was calculated at a similar C loss using the cooled sample respiration rate at the end of the experiment (maximum C loss for cooled samples) and comparing this to control treatment respiration at a similar C loss (earlier measurement point for control samples, at corresponding C loss to the cooled sample at the end of the experiment). This describes the full extent that 90 days of cooling had on the Q_{10} values, compared to the short-term temperature sensitivity measured at the time of cooling.

Q_{10} =R(T+10)/R(T), and assuming that respiration rates increase exponentially with temperature $R(T) = ae^{bT}$, $Q_{10} = e^{(10*b)}$, Q_{10} values were calculated based on measurements at two different measurement temperatures as:

$$Q_{10} = \left(\frac{RT2}{RT1}\right)^{10/(T2-T1)},$$

where RT2 and RT1 are respiration rates in the two incubation temperatures (MAT± 3°C).

Statistical analysis

All statistical analyses were conducted using IMB SPSS statistics 21. We tested whether the relative respiration rate of cooled samples differed from the relative respiration rate of control samples at similar C loss. We used the last measurement of the cooled treatment at the end of the incubation and compared this to the regression line of the control treatment at a similar C loss than in the cooled soil. We used one-sample t-test comparing the cooled samples (n=5) to the control line. Differences between biomass-specific relative respiration rates of cooled

and control samples (at the maximum C loss of the cooled samples) were tested in the same way. The biomass of control samples, at similar C loss as cooled samples in the end, was interpolated based on the control biomass at the time of cooling and at the end of incubation. We tested whether the absolute CO_2 production rates after re-warming differed from the control, using one-sample t-tests ($P < 0.05$) comparing the first re-warming measurement to the control respiration for the corresponding percentage C loss (calculated from the regression line equation). The *P* values were also Bonferroni corrected to counteract the problem of multiple comparisons (see Supplementary).

For the full dataset, and different soil groups (see Fig. 3, Extended Data Fig. 8), we calculated 95% confidence intervals for the different ratios, by following an established natural log transformation approach[30]. $Ratio_{MT,}$ $Ratio_{MT\_MS\_CFE,}$ $Ratio_{MT\_MS\_qPCR,}$ and $Ratio_{CT}$ values were natural log transformed and mean values and 95% confidence intervals were calculated. After taking antilogs, we could present mean ±95% confidence intervals for each ratio (Fig. 3, Extended Data Fig. 8).

27. Howard, D.M. & Howard, P.J.A. Relationships between CO_2 evolution, moisture content and temperature for a range of soil types. *Soil Biol. Biochem.* **25**, 1537–1546 (1993).

28. Vance, E.D., Brookes, P.C., Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **19**, 703-707 (1987).

29. Ågren, G.I. & Bosatta, E. Quality: A bridge between theory and experiment in soil organic matter studies. *Oikos* **76**, 522-528 (1996).

30. Liu, L. & Greaver, T.L. A review of nitrogen enrichment effects on three biogenic GHGs: the CO2 sink may be largely offset by stimulated N2O and CH4 emission. *Ecol. Letts.* **12**, 1103–1117 (2009).

Extended Data Figure legends

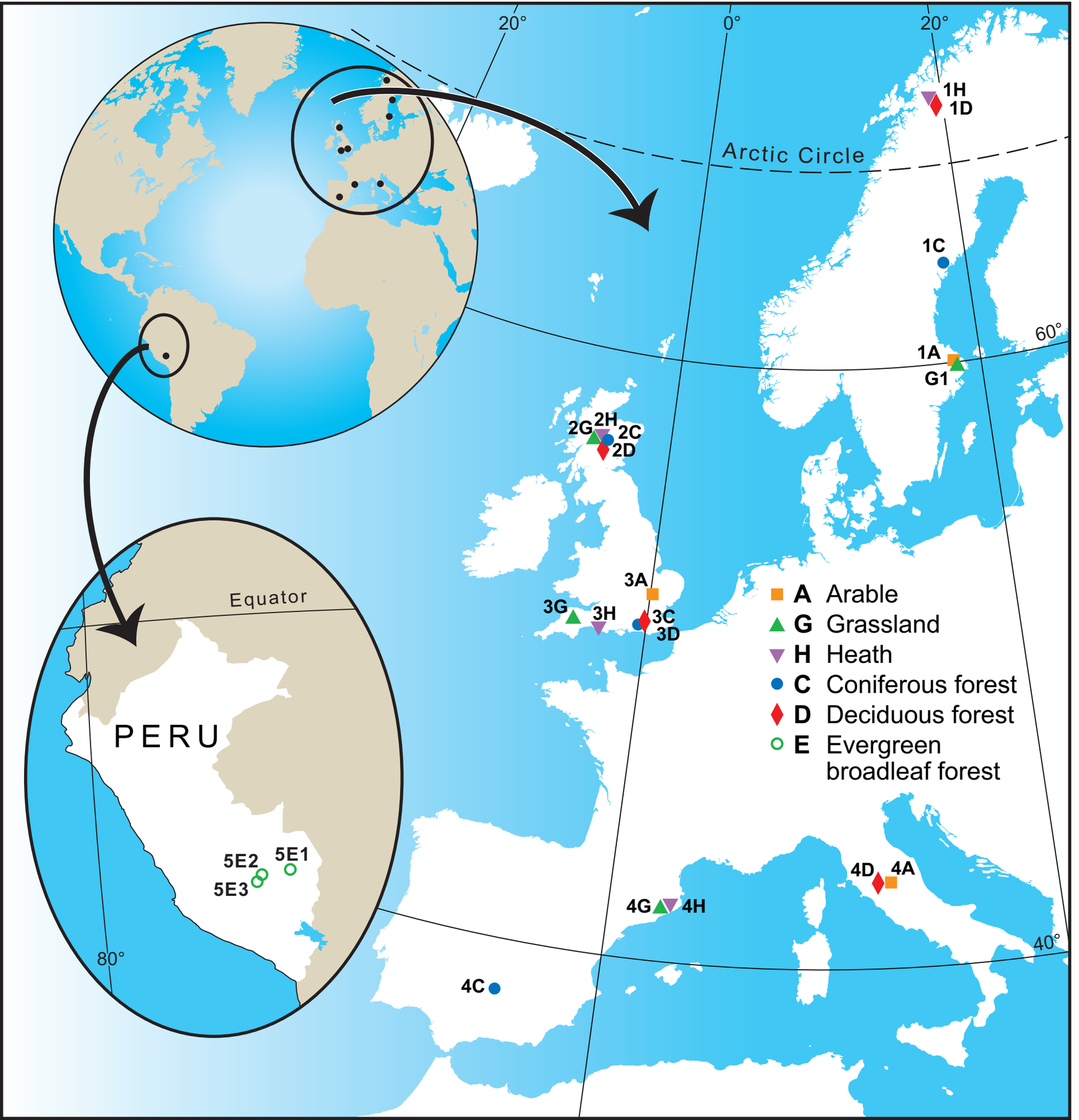
Extended Data Table 1. Sampling site and soil characteristics. List of sites (site abbreviations correspond to Fig. 1), mean annual temperatures (MAT), ecosystem types, vegetation, and physico-chemical soil properties. Abbreviations used for each ecosystem type: A = arable, C =coniferous evergreen forest, D = deciduous broadleaf forest, G = grassland, H = ericaceous heath, E = evergreen broadleaf forest. Management is indicated in parenthesis: n = natural ecosystem, m = managed ecosystem. This classification was used in Fig. 3 to divide sites into managed and natural ecosystems. Soil characteristics in this table were used to classify soils into groups based on pH, and soil C and N contents.

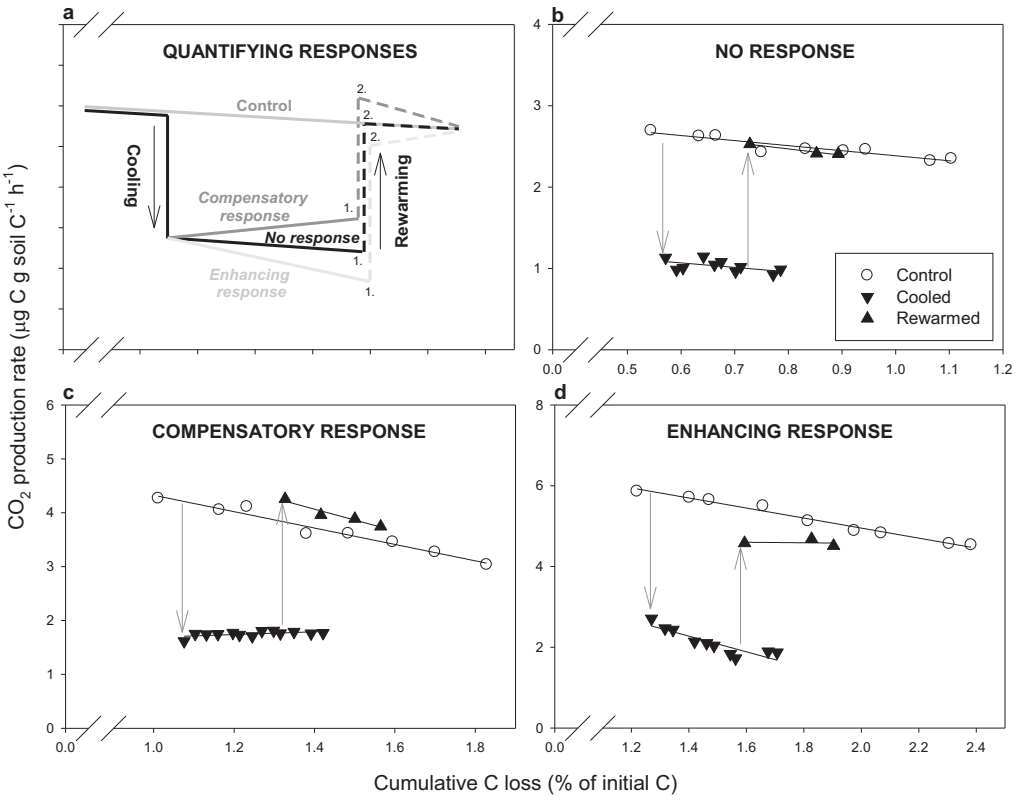
Extended Data Figure 1 The results of the Q model, presenting the patterns that would be observed if there was no compensatory or enhancing microbial community responses. In panel **(a)** absolute respiration rates in the three treatments (control, cooled and re-warmed) are plotted against time. In panel **(b)**, changes in C availability over time are presented, indicating that rates of C loss are greater in the control soils. In panel **(c)**, respiration rates are plotted against C loss, resulting in the differences between re-warmed and control soil respiration rates being eliminated. In panel **(d)**, respiration rates are normalised to rates immediately after cooling, and cooled and control treatments now show identical relationship between respiration rate and C loss.

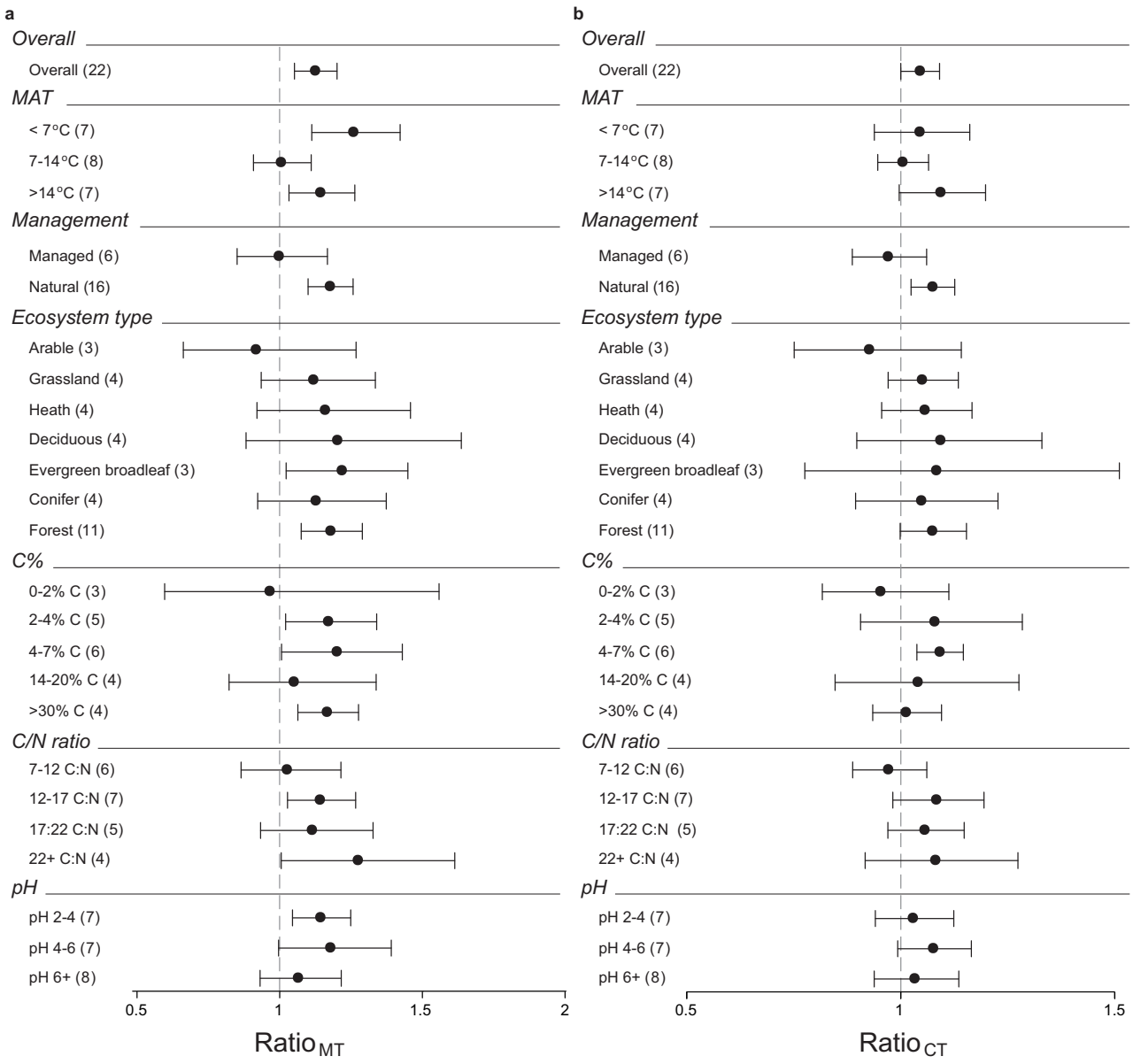
Extended Data Figures 2 to 7. Respiration rates of all treatments (control-cooled-re-warmed) for each individual soil, including the 84 day pre-incubation period. $Ratio_{CT}$ was calculated as control (open circles) respiration rate/re-warmed (black up-pointing

triangles) respiration rate based on the CO_2 fluxes presented in the left-hand panel. In the right-hand panel relative respiration rates normalised for the time of cooling, are shown for the control (open circles) and cooled treatments (open up-pointing triangles). The final cooled treatment measurements were compared to the control treatment regression line at a similar C loss to calculate $Ratio_{MT}$ (control/cooled).

Extended Data Figure 8 The mean ± 95% confidence intervals of mass specific $Ratio_{MT}$ values, calculated per CFE biomass (panel a) and per qPCR biomass (panel b). Overall values (i.e. including all data), and values for the different soil groups, based on ecosystem type, management, climate and the various soil properties, are presented. One evergreen broadleaved forest soil (5E_1) had too low biomass to be measured with the CFE method. Therefore in panel a, we cannot present confidence intervals for Evergreen broadleaf forests due to there now being only two replicates. Similarly, only two soils remained in the 0-2 %C group, so these were combined with the 2-4 %C group (we show average for soils with 0-4 %C). Values > 1 and <1 indicate enhancing, and compensatory responses, respectively. The patterns are extremely similar to $Ratio_{MT}$ calculated per g of soil C (Fig. 3a).


20°
0°
20°
Arctic Circle
60°
40°
1H
1D
1C
1A
G1
2G
2H
2C
2D
3A
3G
3H
3C
3D
4D
4A
4G
4H
4C
A Arable
G Grassland
H Heath
C Coniferous forest
D Deciduous forest
E Evergreen broadleaf forest
Equator
PERU
80°
5E2
5E1
5E3


a
QUANTIFYING RESPONSES
Control
Cooling
Compensatory response
No response
Enhancing response
Rewarming
2.
1.
b
NO RESPONSE
Control
Cooled
Rewarmed
c
COMPENSATORY RESPONSE
d
ENHANCING RESPONSE
CO2 production rate (µg C g soil C-1 h-1)
Cumulative C loss (% of initial C)


a
Overall
Overall (22)
MAT
< 7°C (7)
7-14°C (8)
>14°C (7)
Management
Managed (6)
Natural (16)
Ecosystem type
Arable (3)
Grassland (4)
Heath (4)
Deciduous (4)
Evergreen broadleaf (3)
Conifer (4)
Forest (11)
C%
0-2% C (3)
2-4% C (5)
4-7% C (6)
14-20% C (4)
>30% C (4)
C/N ratio
7-12 C:N (6)
12-17 C:N (7)
17:22 C:N (5)
22+ C:N (4)
pH
pH 2-4 (7)
pH 4-6 (7)
pH 6+ (8)
0.5
1
1.5
2
Ratio MT
b
Overall
Overall (22)
MAT
< 7°C (7)
7-14°C (8)
>14°C (7)
Management
Managed (6)
Natural (16)
Ecosystem type
Arable (3)
Grassland (4)
Heath (4)
Deciduous (4)
Evergreen broadleaf (3)
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pH
pH 2-4 (7)
pH 4-6 (7)
pH 6+ (8)
0.5
1
1.5
Ratio CT