Title:

Thermal adaptation of microbial communities enhances soil C loss under global warming

Authors:

Kristiina Karhu[1,2], Marc Auffret[3,4], Jennifer A.J. Dungait[5], David W. Hopkins[6], James I. Prosser[3], Brajesh K. Singh[7], Jens-Arne Subke[8], Philip A. Wookey[6], Göran I. Ågren[9], Teresa Sebastià[10], Fabrice Gouriveau[10], Göran Bergkvist[9], Patrick Meir[11,12], Andrew T. Nottingham[11] and Iain P. Hartley[1]

[1]College of Life and Environmental Sciences, Geography, University of Exeter, Exeter EX4 4RJ, UK. [2]Department of Forest Sciences, University of Helsinki, 00014 Helsinki, Finland. (current address) [3]Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, Scotland, AB24 3UU, UK. [4]Dairy and swine research and development centre, Agriculture and Agri-Food Canada, Sherbrooke, Quebec, J1M 0C8, Canada. [5]Rothamsted Research – North Wyke, Okehampton, Devon, EX20 2SB, UK. [6]School of Life Sciences, Heriot-Watt University, Edinburgh, Scotland, EH14 4AS, UK. [7]Hawkesbury Institute for the Environment, University of Western Sydney, Penrith 2751 NSW Australia. [8]Biological and Environmental Sciences, University of Stirling, Stirling FK9 4LA, UK. [9]Swedish University of Agricultural Sciences (SLU), 750 07 Uppsala, Sweden. [10]Laboratory of Functional Ecology and Global Change, Forest Technology Centre of Catalonia (CTFC), 25280 Solsona, Spain. [11]School of Geosciences, University of Edinburgh, Edinburgh EH8 9XP, UK. [12]Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia.

Soils store at least twice as much carbon (C) as plant biomass[1], and, each year, soil microbial respiration releases ~60 Pg of C to the atmosphere as carbon dioxide (CO_2)[2]. In the short term, soil microbial respiration increases exponentially with temperature[3], and thus models predict that warming-induced increases in CO_2 release from soils could represent an important positive feedback to 21st century climate change[4]. However, the magnitude of this feedback remains uncertain, not least because the adaptation of soil microbial communities to changing temperatures has the potential to either substantially decrease ('compensatory adaptation'[5-7]) or substantially increase ('enhancing adaptation'[8,9]) warming-induced C losses. By collecting contrasting soils along a climatic gradient from the Arctic to the Amazon, we undertook the first global analysis of the role microbial thermal adaptation plays in controlling rates of CO_2 release from soils. Here we show that, enhancing adaptation was between three and ten times more common than compensatory adaptation. Furthermore, the strongest enhancing responses were observed in soils with high C contents and from cold climates; enhancing thermal adaptation increased the temperature sensitivity of respiration in these soils by a factor of 1.4. This suggests that the substantial stores of C present in organic and high-latitude soils may be more vulnerable to climate warming than currently predicted.

Text:

Short-term experiments have demonstrated that the rate of microbial respiration in soil increases exponentially with temperature, and this general relationship has been used in parameterising 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 change on plant respiration in the medium- to long-term, with major consequences for modelling C-cycle feedbacks to climate change[12]. In soil there is growing evidence of the potential for a similar compensatory effect through microbial adaptation to temperature[13] ('compensatory adaptation': defined here to include the potential for physiological acclimation, adaptation within populations, and changes in microbial community size and structure). However, it is unclear if microbial community-level responses should always be compensatory. In fact, responses that enhance the direct and instantaneous effect of temperature changes on soil respiration ('enhancing adaptation') have also been observed[8,9,14]. To date there has been no large-scale evaluation of the role of microbial adaptation in controlling the temperature sensitivity of soil respiration. This lack of understanding adds considerable uncertainty to predictions of the magnitude and direction of carbon-cycle feedbacks to climate change[15].

When soil is warmed, 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 extremely difficult to determine if this is caused by the loss of the readily decomposable C or by a compensatory adaptation response within the microbial community, as both would reduce activity[16-19]. To differentiate between these two mechanisms, we established a new approach[8] that involves soil cooling in the laboratory. In contrast to a soil warming study, compensatory adaptation and substrate loss should have opposite effects on microbial activity under cooling. In the absence of plant C inputs, soil C losses do still occur in cooled soils, thus reducing activity, albeit at a slower rate than in the controls. However, compensatory adaptation should result in a gradual increase in respiration rate as adaptation compensates for the effects of the temperature change; this is exactly what is observed for thermal

acclimation of plant respiration[11]. Furthermore, because we quantified rates of soil C loss, we can also identify enhancing adaptation 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 the effects of microbial thermal adaptation on soil respiration rates, collecting soil from sites representing a range of ecosystem types (deciduous and evergreen broadleaf forest, coniferous forest, heathland, grassland and arable agricultural sites) across a gradient of mean annual temperature (MAT) from -6°C to 26°C (Fig. 1, Extended Data Table 1). Fifteen samples of each soil were pre-incubated at a temperature 3°C above the MAT of their collection site (see Fig. 2a). After respiration rates stabilised, ten samples were cooled by 6°C (MAT-3°C), and five were maintained at the control temperature of 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[20]. 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.

Our approach establishes two clear criteria for quantifying the magnitude of either compensatory or enhancing adaptation (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 (for cooled samples), was plotted against cumulative C loss (see Methods), and a linear slope was fitted to the data. This enabled comparison of relative respiration rates of cooled vs. control treatments for a given amount of C loss (see Methods and Extended data Fig. 1), with the ratio of these slopes ($Ratio_{slope}$) being used to calculate the magnitude of adaptation. $Ratio_{slope}$ was calculated as cooled treatment slope divided by control treatment slope, where

ratios <1 indicate compensatory adaptation (i.e. normalised respiration rates became greater at a given level of soil C loss in the cooled treatment), and ratios >1 indicate enhancing adaptation (i.e. normalised respiration rates became 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. This CO_2 flux ratio ($Ratio_{CO_2}$) was calculated as the control respiration rate divided by re-warmed respiration rate and, again, a ratio <1 indicates compensatory adaptation, and a ratio >1 indicates enhancing adaptation.

All three possible responses were observed: compensatory adaptation (Fig. 2c), no-response (Fig. 2b), and enhancing adaptation (Fig. 2d). However, for the 22 soils analysed, many more statistically significant cases of enhancing adaptation were observed, than compensatory adaptation ($Ratio_{slope}$: 11 versus 1; $Ratio_{CO_2}$: 10 versus 3). By taking all soils together, overall, we observed significant enhancing adaptation using both the $Ratio_{slope}$ and $Ratio_{CO_2}$ approaches (Fig. 3). Arable/'managed', and low C content soils, were the only soils to show average $Ratio_{slope}$ values close to or below 1 (Fig. 3a). Statistically significant adaptation was not identified for individual ecosystem types, perhaps due to the lower statistical power, but 'natural ecosystems' and forest soils showed significant enhancing adaptation. Enhancing adaptation was strongest in soils with high C contents, high C:N ratios and low pHs (Fig. 3a). Soils sampled from cold regions showed the strongest enhancing adaptation, but significant enhancing adaptation was also observed in the +14°C group. The lack of an effect in the 7-14°C group may be related to high number of arable and low C soils in this group, rather than the temperature range *per se*. Similar patterns were observed for $Ratio_{CO_2}$ (Fig. 3b), but with fewer statistically significant differences; of the sub-groups, only 'natural ecosystems' and C content 4-7%, showed statistically significant enhancing adaptation. Finally, in all cases of

strong enhancing or compensatory adaptation, responses were clearly reversible after re-warming, with respiration subsequently approaching control rates (e.g. Figs 2c & d). This indicates that these $Ratio_{CO_2}$ differences could not have been caused by cooling altering the quality of the remaining C.

Compensatory thermal adaptation has previously been observed in ectomycorrhizal fungi grown on agar[20], and also in monocultures of heterotrophic fungi[7]. The dominance of enhancing adaptation identified in our study is probably related to the fact that we measured responses at the community level. Although the development of warm-adapted enzymes could lead to compensatory adaptation[21], competition between different microbial groups for energy sources may be more important in determining the overall respiration response of the community, than adapted enzymes. We hypothesise that species that are unable to maintain high rates of activity at higher temperatures may be outcompeted, especially as thermal constraints on the decomposition of more recalcitrant compounds are relaxed. This could explain why compensatory thermal adaptation at the community-level was rare. It has been argued that all fluxes should be expressed per unit biomass to quantify the magnitude of adaptation[13]. In the current study, changes in biomass do not explain a substantial proportion of our observed responses; the magnitude of enhancing or compensatory adaptation response = was not significantly related to differences in biomass between cooled and control treatments ($Ratio_{slope}$, $P = 0.097$, $R^2 = 0.146$; $Ratio_{CO_2}$: $P = 0.342$, $R^2 = 0.050$). Thus, to demonstrate the importance of adaptation for the overall rate of CO_2 release, and because it is not possible to monitor biomass continuously, we present the raw fluxes. The predominance of enhancing adaptation implies that decreased soil respiration rates in response to long-term ecosystem warming[22,23] are probably related to the loss of readily decomposable C, rather than to compensatory microbial adaptation, and thus are part of the overall C loss process.

In our study, compensatory adaptation was most important in arable soil and soils with low C contents (Fig. 3a) thus limiting the potential importance of compensatory thermal adaptation in controlling rates of climate change-induced C losses. In contrast, enhancing adaptation was most common in soils with high organic matter content and from cold regions. Enhancing adaptation may be especially common in cold climates because shutting down metabolism in response to cooling allows for survival at low temperature, with rapid recovery of activity in response to re-warming bringing a competitive advantage. For the boreal and arctic soils in our study (sites of MAT <7°C, n=7, Fig. 3.), enhancing adaptation increased the temperature sensitivity of C release by a factor ~1.4 during the 90 days of cooling; the temperature sensitivity, expressed as a Q_{10} value (proportional change in respiration for a 10^{o}C change in temperature), increased from 4.6 at the time of cooling to 6.3 at the end of the incubation. Given that boreal and arctic regions contain more than 50% of the global soil C stock[24], the enhancing adaptation observed in these soils could have significant consequences for the global C budget, although potential losses must be placed into the context of new C inputs[25]. In summary, adaptation of soil microbial respiration to temperature is likely to increase, rather than decrease, the potential for C loss under climate change, especially from organic soils and cold ecosystems. We argue that it is a key process that should be represented in Earth system models.

Methods Summary

Soil sampling and properties

Soil cores (20 - 30 per site) were coarsely sieved (5.6 mm) and mixed to produce a composite sample and soil properties (C, N, pH, particle size) were analysed.

Incubation

The composite sample was set to the optimal moisture content of 60% of water holding capacity (WHC) and divided into 15 parts, which were placed inside 0.5-l rectangular plastic containers. These soil containers were placed inside incubators with temperature adjusted to MAT +3°C, and maintained at the optimum moisture content. The 15 replicates were randomly assigned to three treatments (n = 5): control, cooled, re-warmed. Microbial biomass for these treatments was measured at the end of incubation using the fumigation extraction method[26].

CO_2 flux measurement

Soil respiration was measured by placing each soil sample into a 1.8-l air-tight plastic container. Respiration rates were measured by connecting each container to an infrared gas analyser in a closed loop configuration, and then repeating the measurement ~18 hours later.

Statistical analysis

For each individual soil, statistically significant differences in the slopes of the relative respiration rates in cooled vs. control treatments, plotted against cumulative C loss, were tested using an independent samples t-test ($P < 0.05$). We also tested whether the absolute CO_2 production rate after re-warming differed from the control treatment, at the corresponding percentage C loss, using one-sample t-test ($P < 0.05$).

For the full dataset, and different soil groups (see Fig. 3), we determined whether there was statistically significant evidence of compensatory or enhancing adaptation. $Ratio_{slope}$ or $Ratio_{CO_2}$ values were natural log transformed and mean values and 95% confidence intervals were calculated. Following established approaches, after taking anti logs, we considered there to be statistically significant adaptation where the 95% confidence intervals did not cross 1[27].

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 3 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 (Fischer Scientific). 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 Fisherbrand FB59103 filter papers 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[28] and dividing it into 15 parts: approximately 180 - 490 g of soil fresh weight, depending on the soil type, was placed inside 0.5-l 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 15 replicates were randomly assigned to three treatments (n=5): 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 for these treatments was measured using the fumigation extraction method[26] on day 174.

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 l rectangular soil container (without the lid) was placed inside a larger 1.8-l rectangular plastic container (Lock & Lock^R). This incubation chamber was connected to an infrared gas analyser (EMG-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 adaptation 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 thermal adaptation (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) leads 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 'apparent compensatory adaptation' of CO_2 fluxes.

In order to identify and quantify microbial adaptation responses, 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). If there is no adaptation, when fluxes are 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 allows any statistically significant differences between re-warmed and control CO_2 fluxes to be used as evidence of microbial adaptation ($Ratio_{CO_2}$), again, as long as fluxes are plotted against cumulative C loss.

However, even when there was no adaptation, 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 slopes of control and cooled soils are now identical (Extended Data Fig. 1d). Thus, any significant difference in the slopes of normalised respiration rates plotted against cumulative C loss (Extended Data Fig. 1d) allows compensatory or enhancing adaptation to be detected, and the magnitude of the adaptation quantified by calculating $Ratio_{slope}$.

Statistical analysis

All statistical analyses were conducted using IMB SPSS statistics 21. For each individual soil, statistically significant differences in the slopes of linear regression lines fitted to cooled and control treatment relative respiration rates plotted against cumulative C loss were tested using an independent samples t-test ($P < 0.05$). One soil, 3D, was not included in this analysis. For this soil, the slope and R^2 of the linear regression were both very close to zero, so there was considerable uncertainty in the calculation of $Ratio_{slope}$. Had 3D been included it would have provided a very high enhancing adaptation value. 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).

For the full dataset, and different soil groups (see Fig. 3), we determined whether there was statistically significant evidence of compensatory or enhancing adaptation, by following an established natural log transformation approach[27]. $Ratio_{slope}$ or $Ratio_{CO_2}$ values were natural log transformed and mean values and 95% confidence intervals were calculated. After taking antilogs, we considered there to be statistically significant adaptation were the 95% confidence intervals did not cross 1. For $Ratio_{slope}$, ln(x+1) transformation was used, as the dataset contained one negative value. This negative value was only present in one group in which statistically significant adaptation was detected (*Overall*, Fig. 3a). Thus, it was possible to check if ln(x+1) transformation had any effect on the patterns. All cases of significant adaptation remained statistically significant, irrespective of whether (ln) or (ln(x+1)), transformations were used. For $Ratio_{CO_2}$, ln transformation was used, as all values were close to 1.

Finally, the relationship between the differences in microbial biomass between treatments (cooled biomass / control biomass) and the magnitude of adaptation, was analysed by linear regression.

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. Eliasson, P.E., McMurtrie, R.E., Pepper, D.A., Strömgren, M., Linder, S. & Ågren G.I. The response of heterotrophic CO_2 flux to soil warming. *Glob. Change Biol.* **11**, 167–181 (2005).

19. Knorr, W., Prentice, I.C., House, J.I., Holland, E.A. Long-term sensitivity of soil carbon turnover to warming *Nature* **433**, 298–301 (2005).

20. 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).

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

22. 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).

23. Rustad, L.E. et al. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming *Oecologia* **126**, 543–562 (2001).

24. 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).

25. Sistla, S.A. et al. Long-term warming restructures Arctic tundra without changing net soil carbon storage. *Nature* **497**, 615-618 (2013).

26. 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).

27. 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).

28. 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).

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

Acknowledgements

We thank Alice Holt, Forestry Commission, T. Taylor from RSPB Aylesbeare Common Reserve, J. Harris from the Cranfield University, C. Moscatelli and S. Marinari from Tuscia University, J. A. Carreira de la Fuente from the University of Jaén, and R. Giesler from the Umeå University for help with site selection and soil sampling. We thank N. England for technical assistance with constructing the incubation system, A. Elliot for conducting the particle size analyses, and S. Rouillard for assistance with graphics. This work was carried out with Natural Environment Research Council (NERC) funding (grant code NE/H022333/1). K.K. was supported by Academy of Finland post-doctoral research grant, while finalising this manuscript. P.M was supported by ARC FT110100457 and NERC NE/G018278/1.

Author contributions

K.K. conducted the CO_2 measurements and statistical analyses. K.K. and M.A. led the data analysis and interpretation. I.P.H., 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.

Author information

Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to K.K. (kristiina.karhu@helsinki.fi).

Figure legends

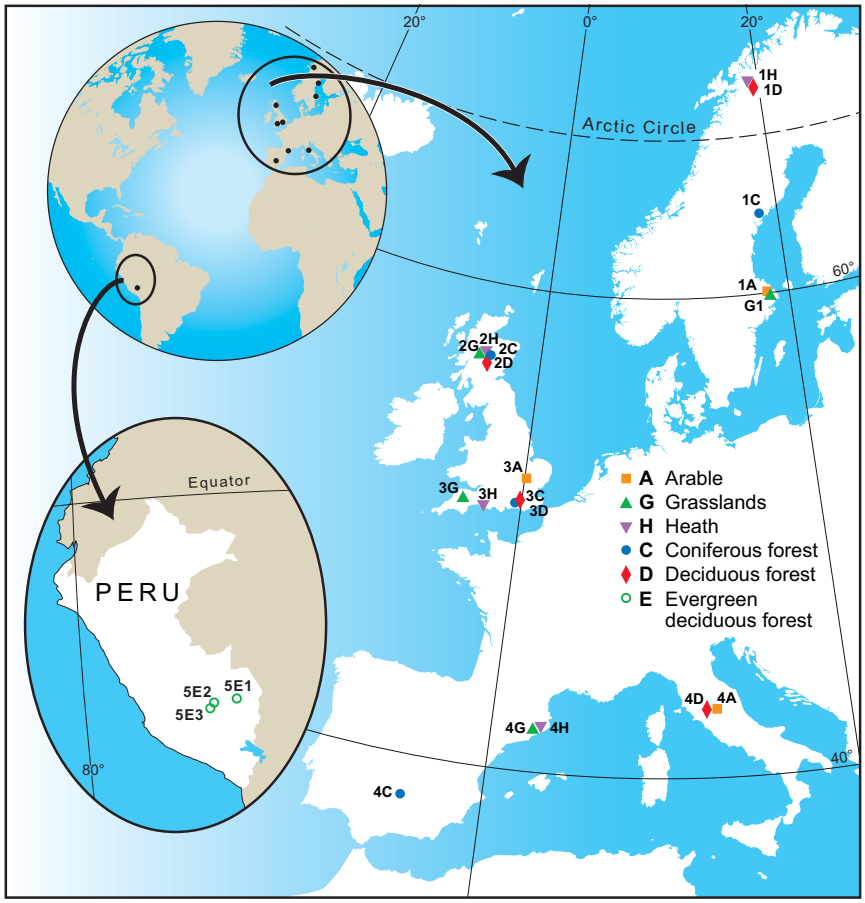
Figure 1 Soil was sampled from Boreal and Arctic, Temperate, Mediterranean and Tropical climates. Agricultural, grassland, heath, coniferous forest and deciduous forest sites were sampled in each climatic region (except the Tropics, where broadleaved evergreen forest sites were sampled along an altitudinal 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.

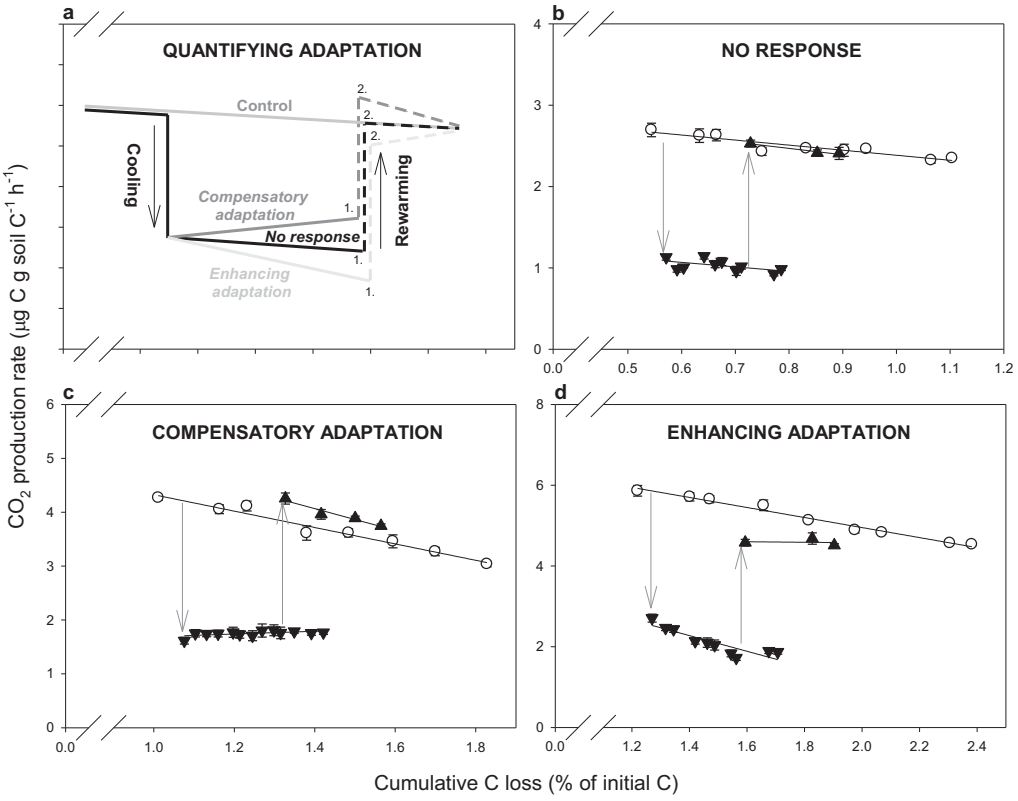
Figure 2 Schematic diagram (a) and examples of results (b, c, d) illustrating no response (soil 2C), compensatory adaptation (soil 3A) and enhancing adaptation (soil 1C), respectively. A break in the x-axis scale (II) denotes that pre-incubation data are not shown. The schematic diagram **(a)**, indicates how a gradual increase in soil respiration rate after cooling provides support for compensatory adaptation, while a more rapid decline in cooled soils indicates enhancing adaptation (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 adaptation (see also Extended Data Fig. 1). In panels (b-d), mean respiration rates, ±1SE, are presented.

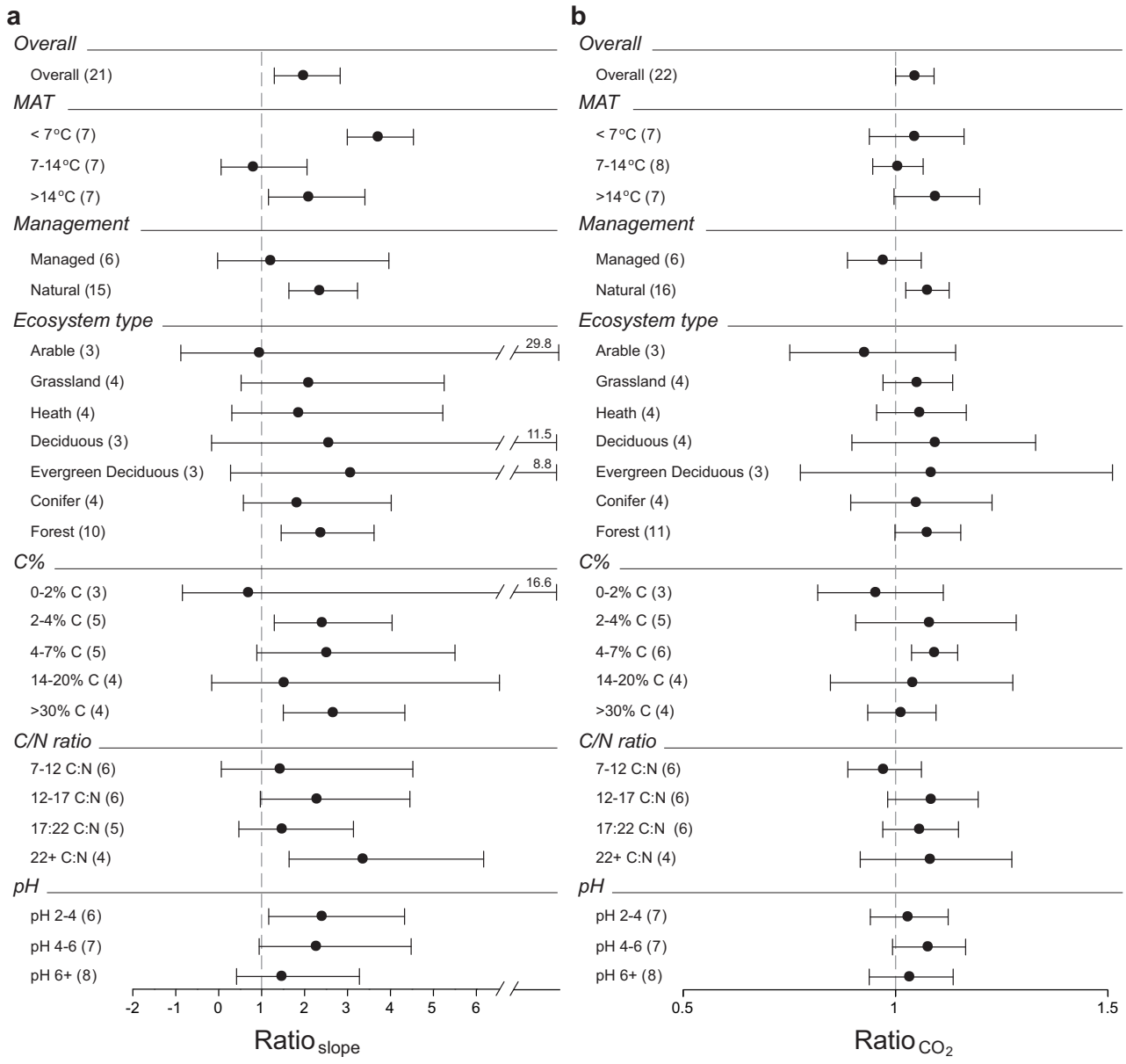
Figure 3 The mean ± 95% confidence intervals of $Ratio_{slope}$ (a) and $Ratio_{CO_2}$ values (b) overall (i.e. including all data), and for different soil groups, based on ecosystem type, management, climate and various soil properties. Values > 1 indicate enhancing adaptation, and values < 1 indicate compensatory adaptation.

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 = broadleaf evergreen 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 thermal adaptation. 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 much 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.


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


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


a
Overall
Overall (21)
MAT
< 7°C (7)
7-14°C (7)
>14°C (7)
Management
Managed (6)
Natural (15)
Ecosystem type
Arable (3)
Grassland (4)
Heath (4)
Deciduous (3)
Evergreen Deciduous (3)
Conifer (4)
Forest (10)
C%
0-2% C (3)
2-4% C (5)
4-7% C (5)
14-20% C (4)
>30% C (4)
C/N ratio
7-12 C:N (6)
12-17 C:N (6)
17:22 C:N (5)
22+ C:N (4)
pH
pH 2-4 (6)
pH 4-6 (7)
pH 6+ (8)
29.8
11.5
8.8
16.6
-2
-1
0
1
2
3
4
5
6
Ratio slope
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 Deciduous (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 (6)
17:22 C:N (6)
22+ C:N (4)
pH
pH 2-4 (7)
pH 4-6 (7)
pH 6+ (8)
0.5
1
1.5
Ratio CO2