LETTER

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Temperature sensitivity of soil respiration rates enhanced by microbial community response

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Soils store about four times as much carbon as plant biomass[1], and soil microbial respiration releases about 60 petagrams of carbon per year to the atmosphere as carbon dioxide[2]. Short-term experiments have shown that soil microbial respiration increases exponentially with temperature[3]. This information has been incorporated into soil carbon and Earth-system models, which suggest that warming-induced increases in carbon dioxide release from soils represent an important positive feedback loop that could influence twenty-first-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 carbon losses substantially. Here we collect soils from different ecosystems along a climate gradient from the Arctic to the Amazon and investigate how microbial community-level responses control the temperature sensitivity of soil respiration. We find that the microbial community-level response more often enhances than reduces the mid- to long-term (90 days) temperature sensitivity of respiration. 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 carbon stores in Arctic and boreal soils could be more vulnerable to climate warming than currently predicted.

Short-term experiments have demonstrated that the rate of soil microbial respiration increases exponentially with temperature, and this general relationship has been used to parameterize soil carbon (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 may be a response in microbial communities that is analogous to thermal acclimation in plants, given that these communities adapt to changes in temperature[13]. However, it is unclear whether microbial community responses always reduce the effect of a temperature change on respiration rates. Responses that enhance the instantaneous effect of temperature changes on soil respiration have also been observed[8,9,14]. Until now there has been no large-scale evaluation of the role of microbial community responses in controlling the temperature sensitivity of soil respiration, adding 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 responses (for example, competition altering species composition), all of which 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 or enhancing (that is, reducing or increasing the effect of a temperature change on respiration rates in the longer term). We investigated how microbial community-level responses affect the temperature sensitivity of soil respiration.

When soil is warmed for an extended period, the initial increase in biological activity leads to a loss of readily decomposable C (ref. 5). Microbial activity then tends to decline in the longer term, but it is often impossible to determine whether this is caused by the loss of the readily decomposable C or by a compensatory response of the microbial community, given that both would reduce activity[16,17]. To differentiate between these two mechanisms, we established an approach[8] that involves cooling soil in the laboratory. Compensatory community responses and substrate loss should have opposite effects on microbial activity under cooling conditions. 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 a 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 and 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 stabilize. On day 84, five samples were destructively sampled for microbial biomass determination, ten samples were cooled

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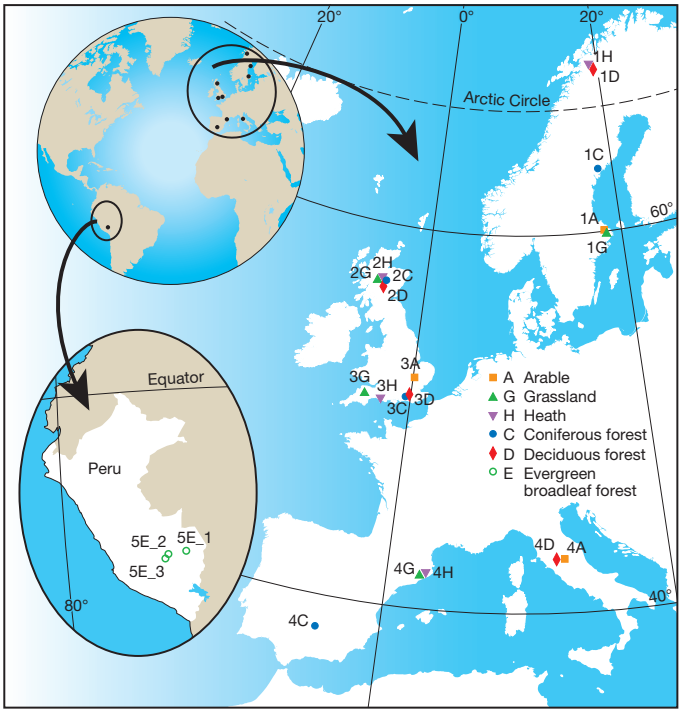

Figure 1 | Soil was sampled from boreal and Arctic, temperate, Mediterranean and tropical climates. Arable, grassland, heath, coniferous forest and deciduous forest (A, G, H, C and D) sites were sampled in each climatic region (except the tropics, where evergreen (E) broadleaf forest sites were sampled along an altitudinal gradient in the Peruvian Andes), and within each ecosystem type, sites are numbered from 1 to 5 in order of increasing MAT. Details of sampling sites (vegetation and soil characteristics) are presented in Extended Data Table 1.

by 6 °C (MAT minus 3 °C), and five controls were maintained at MAT plus 3 °C for the remaining 90 days of the experiment. Five of the cooled samples were incubated at MAT minus 3 °C for 90 days, a time period relevant to seasonal changes in temperature, which have been hypothesized to cause thermal adaptation[18]. The other five cooled samples were rewarmed to MAT plus 3 °C after 60 days at MAT minus 3 °C, and incubated at MAT plus 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 and Extended Data Fig. 1). First, the carbon dioxide (CO_2) flux, normalized to the flux at the time of cooling (control samples) or immediately after cooling (cooled samples), was plotted against cumulative C loss (see Methods and Extended Data Figs 1–7). The impact of community responses on respiration rates at the measurement temperatures (RR_{MT}, where RR represents the response ratio) was calculated as the normalized control respiration rate, at the percentage C loss corresponding to the total percentage C loss in the cooled soils (see Supplementary Information), divided by the normalized cooled respiration rate at the end of the incubation. Ratios <1 indicate a compensatory response (that is, normalized respiration rates were greater at a given level of soil C loss in the cooled treatment), and ratios >1 indicate an enhancing response (that is, normalized 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 rewarmed after 60 days of cooling with control sample respiration rates at the same C loss (see Supplementary Information). This ratio at a common temperature[19] (RR_{CT}) was calculated as the control respiration rate divided by rewarmed 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 RR_{MT} on a microbial-biomass-specific basis (see Methods).

All three possible community-level responses were observed: compensatory responses (Fig. 2c), enhancing responses (Fig. 2d) and no response (Fig. 2b). However, for the 22 soils analysed, many more statistically significant cases of enhancing responses were observed (see Supplementary Information). Overall average response ratios ($n = 22$ soils) were significantly above 1 ($P < 0.01$ for RR_{MT}, Fig. 3a; $P < 0.05$ for RR_{CT}, Fig. 3b). In all cases of clear enhancing or compensatory responses, respiration rates after rewarming subsequently approached control rates (see Fig. 2c, d). This reversibility of the response indicates that the patterns were not caused by cooling altering the decomposability of the remaining C, and emphasizes the comparability, in terms of effects on rates of respiration, of microbial community responses to cooling and warming.

The average RR_{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 or 'managed', low-C-content, and low carbon-to-nitrogen ratio (C:N) soils were the only soils to show average RR_{MT} values close to or below 1 (Fig. 3a). Enhancing responses were generally more common in soils with high C content, high C:N ratios and low pH values (Fig. 3a); forest and 'natural ecosystem' groups also showed enhancing responses. C:N ratio was the only soil or site variable (Fig. 3a) that was significantly correlated with the RR_{MT} responses across all data ($\ln[RR_{MT}] = 0.188 \times \ln[C:N] - 0.406$, $R^2 = 0.335$, $P = 0.005$), and the low RR_{MT} for the MAT 7 °C–14 °C group may have been related to the greater

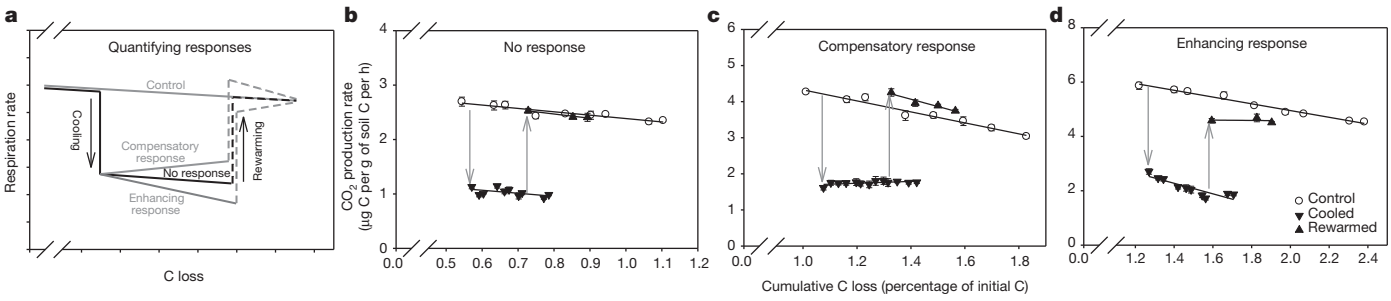

Figure 2 | The patterns of CO_2 flux that would be observed in the case of no response, compensatory and enhancing community-level responses. The schematic diagram (**a**), indicates how a gradual increase in soil respiration rate after cooling provides support for a compensatory response, while a more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

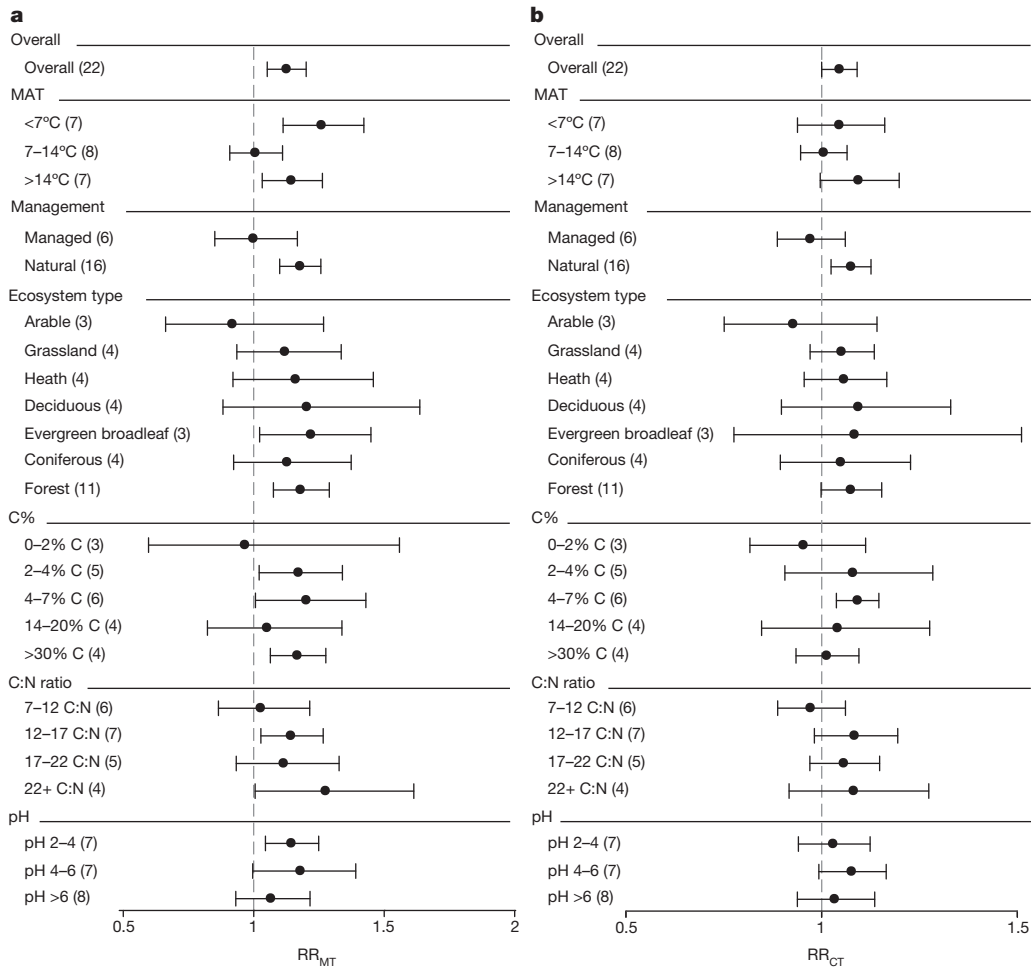

Figure 3 | The impact of the microbial community responses on the response of soil respiration to changes in temperature. The mean ± 95% confidence intervals of RR_{MT} (**a**) and RR_{CT} values (**b**) are presented overall (that is, including all data), and for different soil groups, based on ecosystem type, management, climate and various soil properties (*n* is given in parentheses). Values >1 indicate an enhancing response, and values <1 indicate a compensatory response.

number of managed, low C:N ratio soils in this group rather than the temperature range itself.

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 much between cooled and control soils (whether measured by chloroform-fumigation extraction or quantitative polymerase chain reaction, qPCR), and thus mass-specific patterns did not differ substantially from the raw responses; mass-specific responses (RR_{MT_MS}) had slightly greater average values ($RR_{MT\_MS} > RR_{MT}$) but also showed greater variability (Extended Data Fig. 8 and Supplementary Information). Overall, changes in microbial biomass could not explain the observed microbial community responses.

The greater enhancing responses in cold soils and in soils with high 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 upregulation of respiration rates at lower temperatures[11], but our data demonstrate that adaptation to cooling by microbial communities in 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 was the only variable that was positively correlated with RR_{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 high C:N values. 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 to 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 content (Fig. 3), 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 downregulating microbial respiration rates. Finally, given that boreal and arctic regions contain more than half of the global soil C stock[26], the strong enhancing responses observed in these soils could have important consequences for the global C budget.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions K.K. conducted the CO_2 measurements and statistical analyses. K.K. and M.D.A. conducted the chloroform-fumigation extraction 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.J.D., D.W.H., J.-A.S., P.A.W., M.-T.S., F.G., G.B., P.M., A.T.N. and N.S. 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. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to K.K. (kristiina.karhu@helsinki.fi).

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 minimize 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 was measured with an Accumet AB 15/15+ pH meter (Fisher Scientific) from a soil slurry with 1:2.5 ratio by volume of soil to deionized water. Particle size was measured using a Saturn digitizer, and the soil texture class was defined according to the UK-ADAS classification. Soil water content was determined by drying subsamples at 105 °C for 24 h. The soil water holding capacity 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 °C for 24 h.

Incubation. Soil for incubation studies was prepared by setting the composite sample to the optimal moisture content of 60% of water holding capacity [27] and dividing it into 20 parts. Approximately 180–490 g (fresh weight) of soil, depending on the soil type, was placed inside 0.5-litre rectangular plastic containers. These containers had pierced lids that enabled gas exchange, but minimized evaporation and soil drying. Soil containers were placed inside incubators (Sanyo Electric/Panasonic cooled incubator, MIR-154) with temperature adjusted to MAT plus 3 °C. For sites with a 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. Temperatures inside incubators were 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 water holding capacity by regularly weighing the soil containers and adding deionized water to compensate for moisture loss.

The 20 replicates were randomly assigned to four treatments ($n = 5$): pre-cooling (incubated at MAT plus 3 °C, destructively sampled at the end of the pre-incubation period on day 84), control (incubated at MAT plus 3 °C for 174 days), cooled (incubated at MAT plus 3 °C for 84 days, then cooled to MAT minus 3 °C for 90 days), and rewarmed (incubated at MAT plus 3 °C for 84 days, then cooled to MAT minus 3 °C for 60 days and rewarmed to MAT plus 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 quantitative PCR (qPCR). This allowed RR_{MT} also to be expressed per unit CFE biomass ($RR_{MT\_MS\_CFE}$) and per unit qPCR biomass ($RR_{MT\_MS\_qPCR}$). Total microbial biomass was estimated based on qPCR results as the sum of relative gene abundance (expressed per gram of soil dry weight) of the bacterial *16S* ribosomal RNA gene, the archaeal *16S* rRNA gene and the fungal *ITS1* gene.

CO_2 flux measurement. Soil respiration was initially measured weekly, and later biweekly. After cooling and rewarming, the first respiration measurement was started 24 h after the temperature change, and weekly CO_2 measurements were made during these periods. To measure soil respiration, each 0.5-litre rectangular soil container (without the lid) was placed inside a larger airtight 1.8-litre rectangular plastic container. This incubation chamber was connected to an infrared gas analyser (EGM-4, PP systems, version 4.17) 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. The 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 per g of soil C per h).

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 normalized 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 were no compensatory or enhancing community-level response (Extended Data Fig. 1). First, 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 rewarmed samples compared to the control soils (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 minimizes differences in C availability between the control and cooled treatments (see Supplementary Information), we still needed to account for these small differences, to ensure that C availability did not affect the patterns observed. To do this, we first had to account for differences in C availability in cooled versus control soils by plotting respiration rates against cumulative C loss (Extended Data Figs 1c and 2–7). 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 rewarmed samples are now equal to control-treatment respiration rates (Extended Data Fig. 1c). This allowed any statistically significant differences between rewarmed and control CO_2 fluxes to be used as evidence of microbial community-level responses affecting CO_2 flux (RR_{CT}), again, as long as fluxes are plotted against cumulative C loss (see Extended Data Figs 2–7). RR_{CT} was calculated as control-treatment respiration rate (regression line value at similar C loss as the rewarmed samples, see Supplementary Information) divided by rewarmed-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 normalized 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 normalized rates are plotted against cumulative C loss the relative respiration rates of control and cooled soils are identical (Extended Data Fig. 1d). Thus, any significant difference in the normalized respiration rates plotted against cumulative C loss (Extended Data Figs 1d and 2–7) 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 at 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 values for the corresponding percentage of C loss (see Supplementary Information) to account for any effects of different C availability (Extended Data Figs 2–7). RR_{MT} was calculated as control-treatment relative respiration rate divided by cooled-treatment (average) relative respiration rate.

We also calculated mass-specific RR_{MT} values, using relative respiration rates at the maximum C loss for cooled samples, divided by 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 percentage C loss which corresponded to the maximum C loss in the cooled samples. To do this, we interpolated between the pre-cooling biomass and the biomass measured at the end of the experiment, on the basis of 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 °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 a 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.

Assuming that respiration rates (R) increase exponentially with temperature (T), respiration can be modelled as $R(T) = ae^{bT}$, where a and b are fitted parameters, and the equation gives $Q_{10} = e^{10b}$. Q_{10} is also defined as $Q_{10} = R(T + 10)/R(T)$, and we calculated Q_{10} values on the basis of respiration measurements at two different measurement temperatures T_1 and T_2 as:

$$Q_{10} = \left(\frac{R(T_2)}{R(T_1)}\right)^{\frac{10}{(T_2 - T_1)}}$$

where $R(T_2)$ and $R(T_1)$ are respiration rates in the two incubation temperatures (MAT plus 3 °C and MAT minus 3 °C, respectively).

Statistical analysis. All statistical analyses were conducted using IMB SPSS statistics 21 http://www-01.ibm.com/software/analytics/spss/products/statistics/. 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 C loss similar to that in the cooled soil. We used one-sample Student's t-tests 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 at the end of incubation, was interpolated on the basis of the control biomass at the time of cooling and at the end of incubation. We tested whether the absolute CO_2 production rates after rewarming differed from the control, using one-sample *t*-tests ($P < 0.05$ was considered significant) comparing the first rewarming 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 Information).

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

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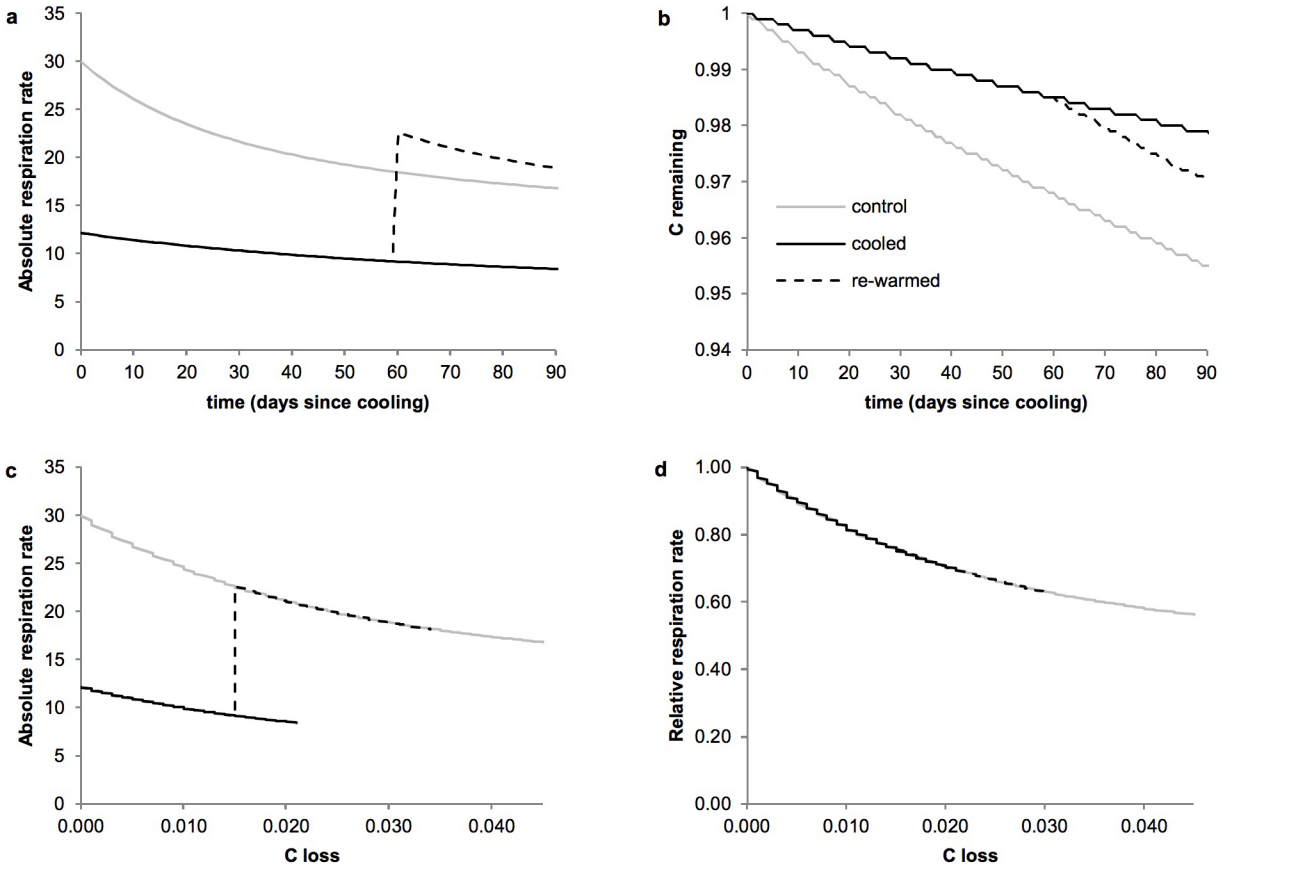
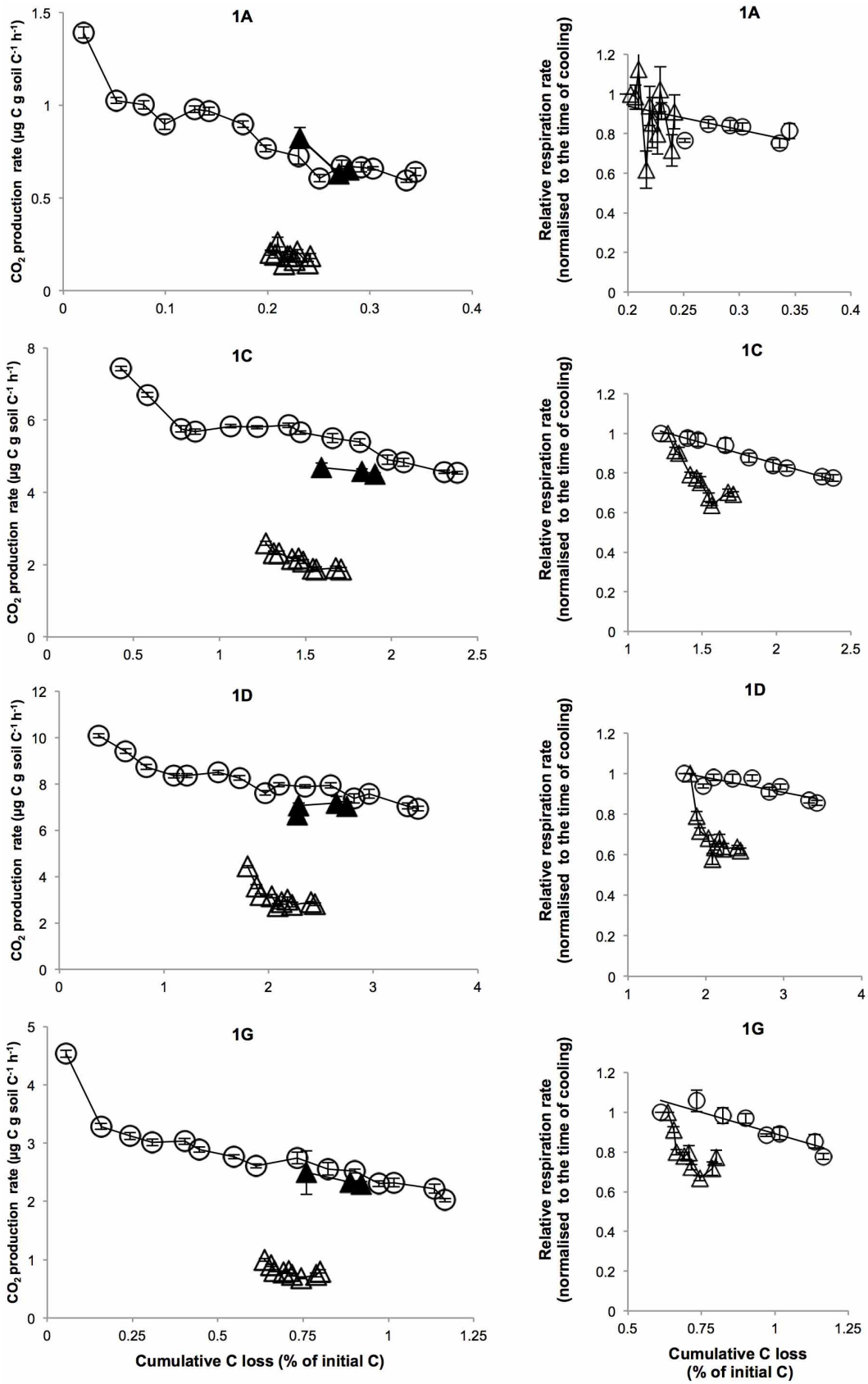
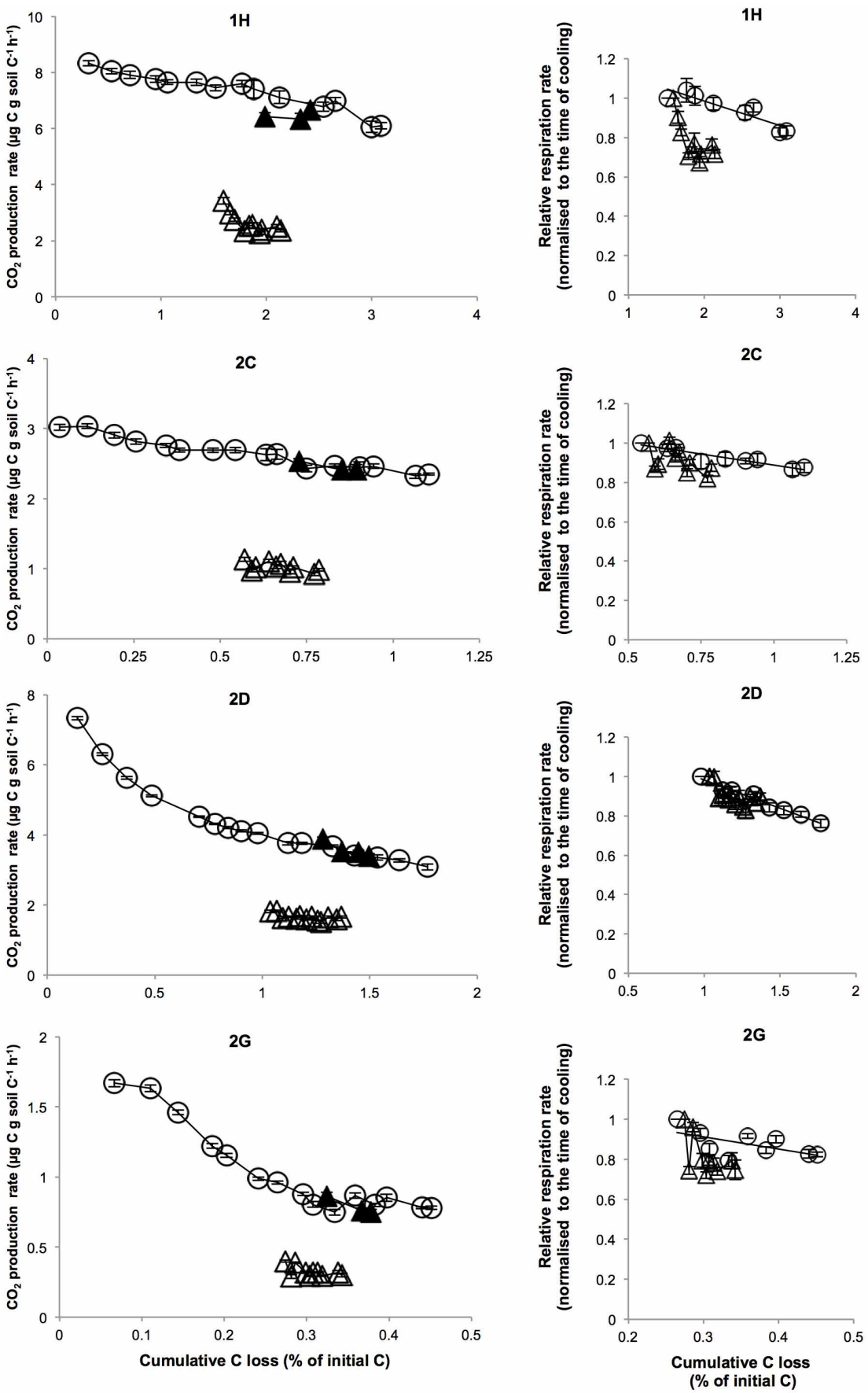


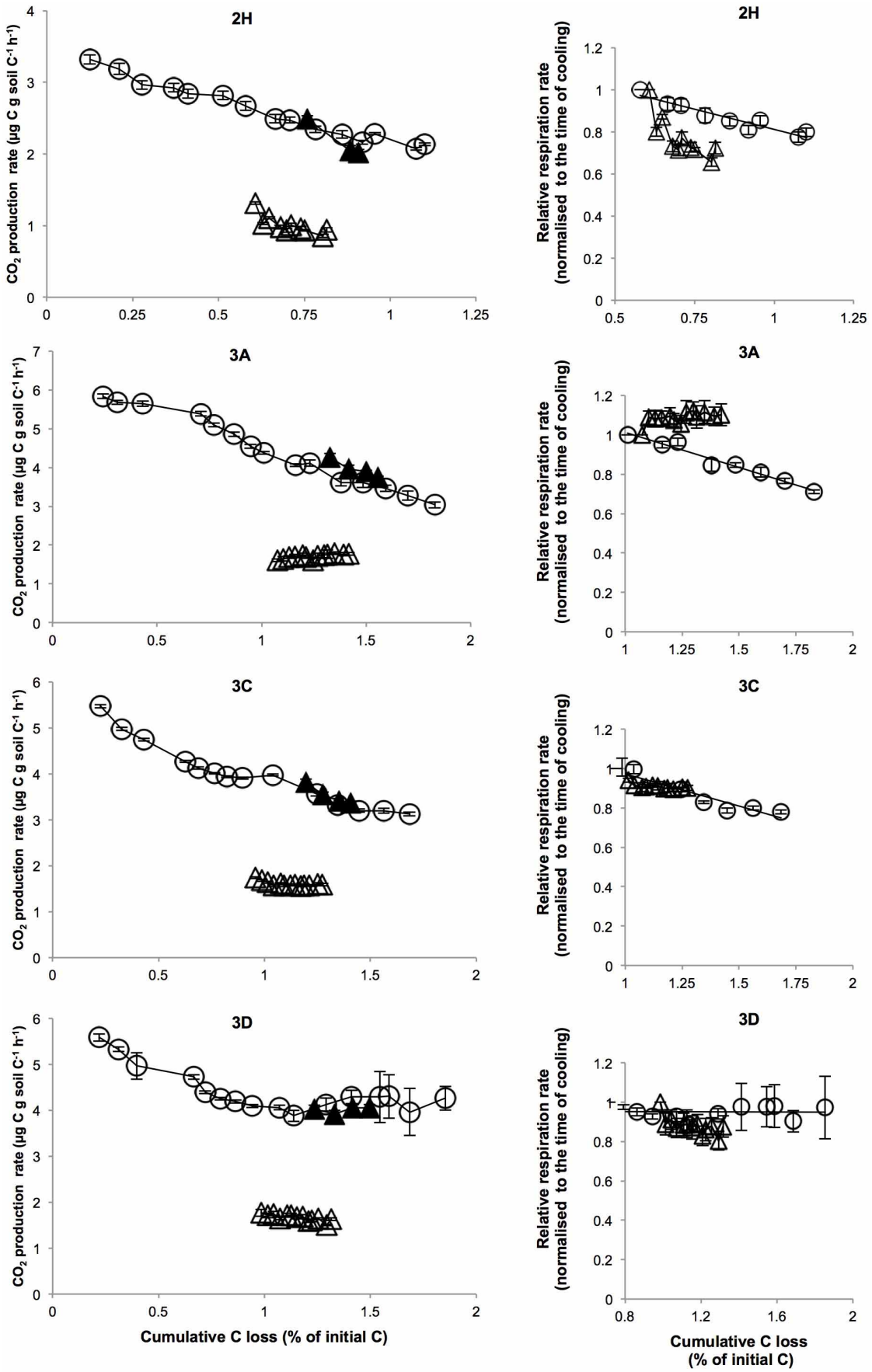
Extended Data Figure 1 | The results of the Q model, presenting the patterns that would be observed if there were no compensatory or enhancing microbial community responses. **a**, Absolute respiration rates in the three treatments (control, cooled and rewarmed) are plotted against time. **b**, Changes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



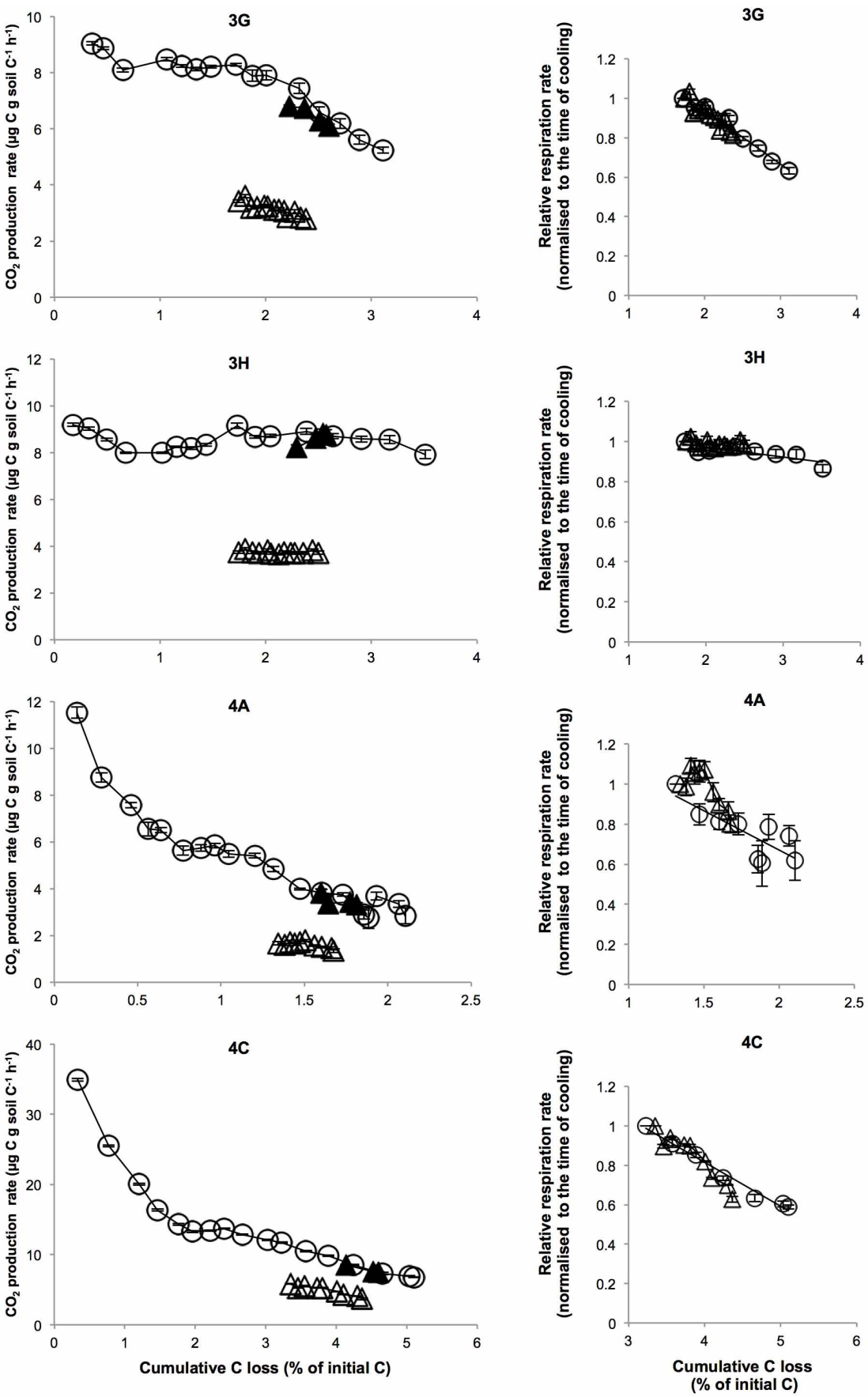
Extended Data Figure 2 | Respiration rates of all treatments (control, cooled and re-warmed) for the individual soils 1A, 1C, 1D and 1G, including the 84-day pre-incubation period. RR_{CT} was calculated as control (open circles) respiration rate divided by rewarmed (black uptriangles) respiration rate based on the CO_2 fluxes presented in the left panels (mean and standard error, $n=5$, technical replicates). In the right panels relative respiration rates normalized for the time of cooling are shown for the control (open circles) and cooled treatments (open uptriangles). The final cooled treatment measurements were compared to the control treatment regression line at a similar C loss to calculate RR_{MT} (control/cooled). Error bars represent standard error.



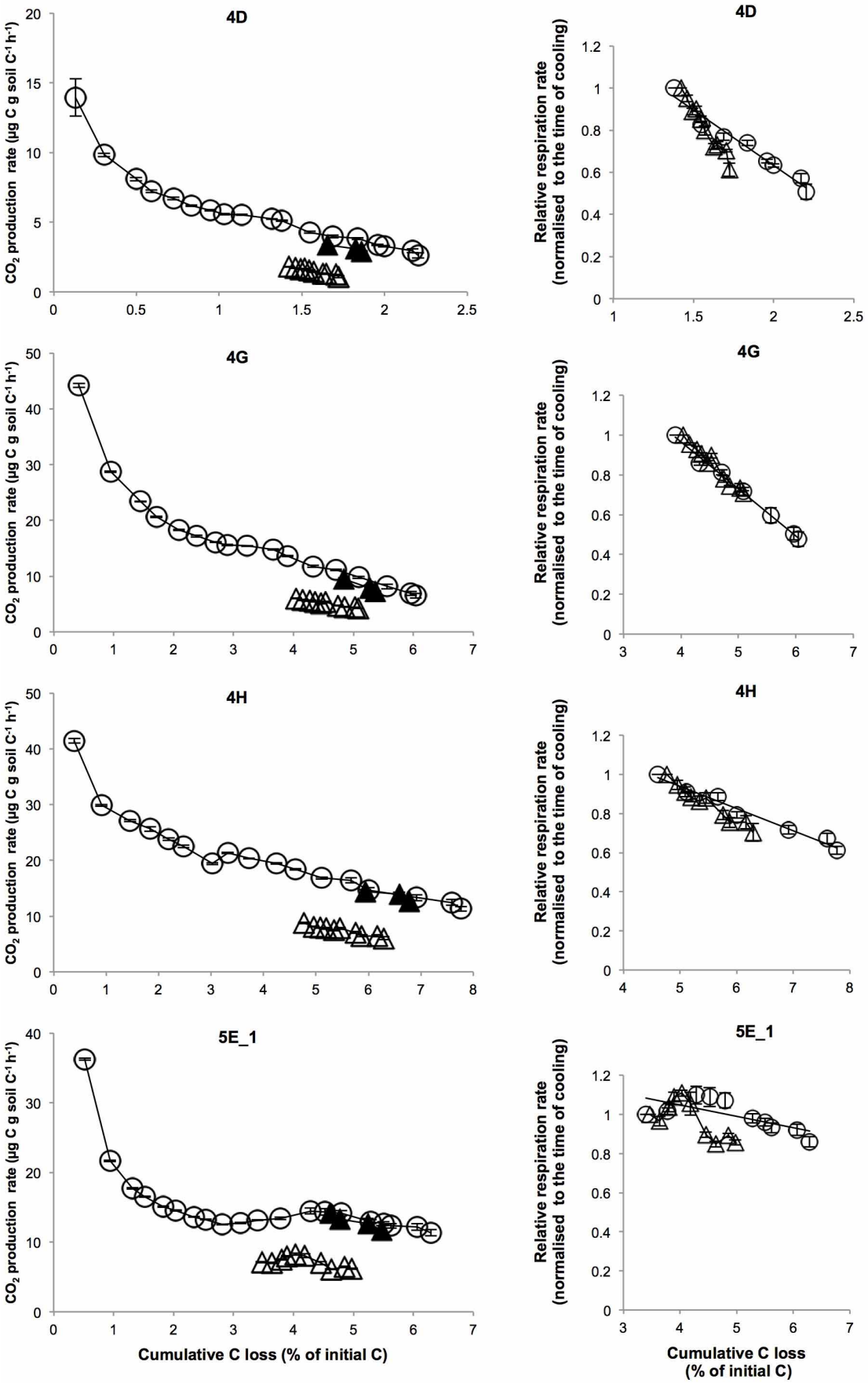
Extended Data Figure 3 | Respiration rates of all treatments (control, cooled and re-warmed) for the individual soils 1H, 2C, 2D and 2G, including the 84-day pre-incubation period. As for Extended Data Fig. 2.



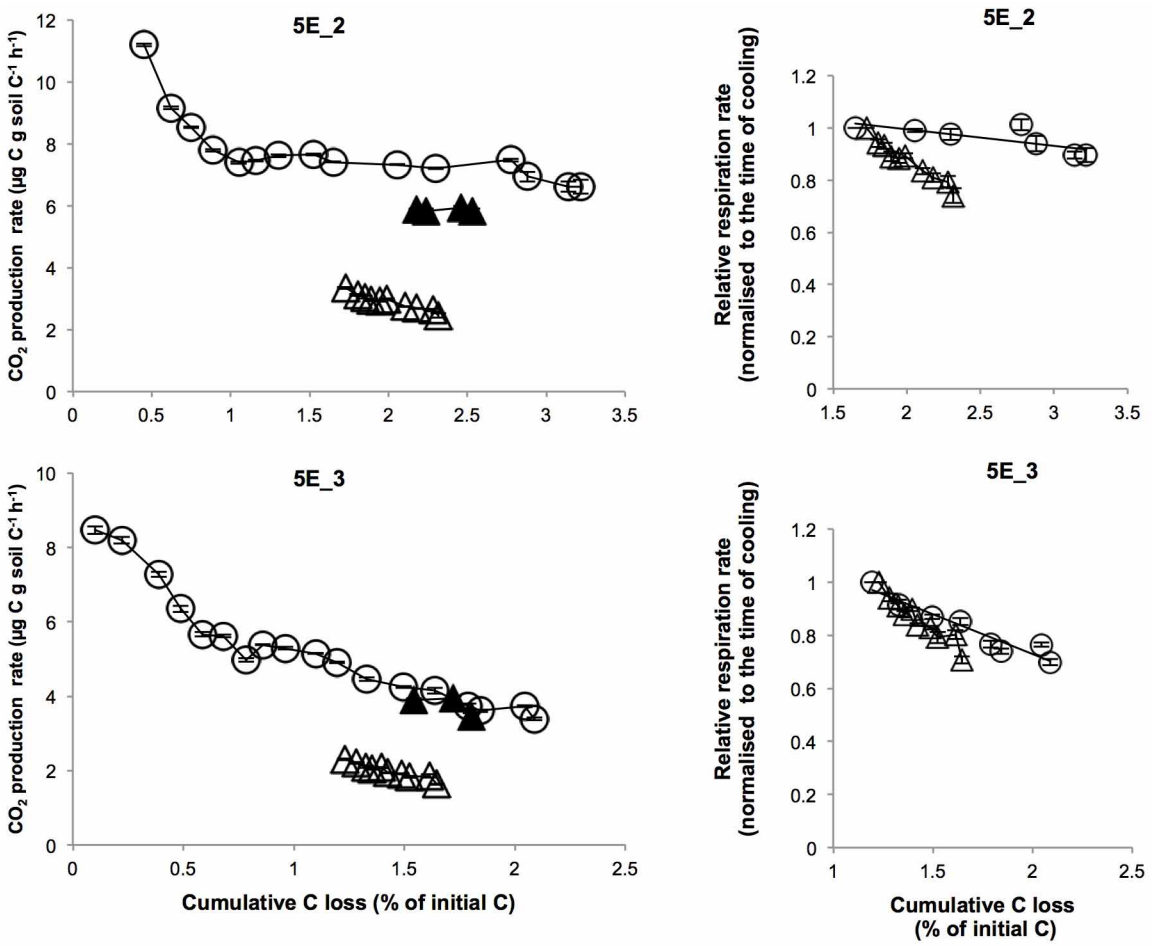
Extended Data Figure 4 | Respiration rates of all treatments (control, cooled and re-warmed) for the individual soils 2H, 3A, 3C and 3D, including the 84-day pre-incubation period. As for Extended Data Fig. 2.



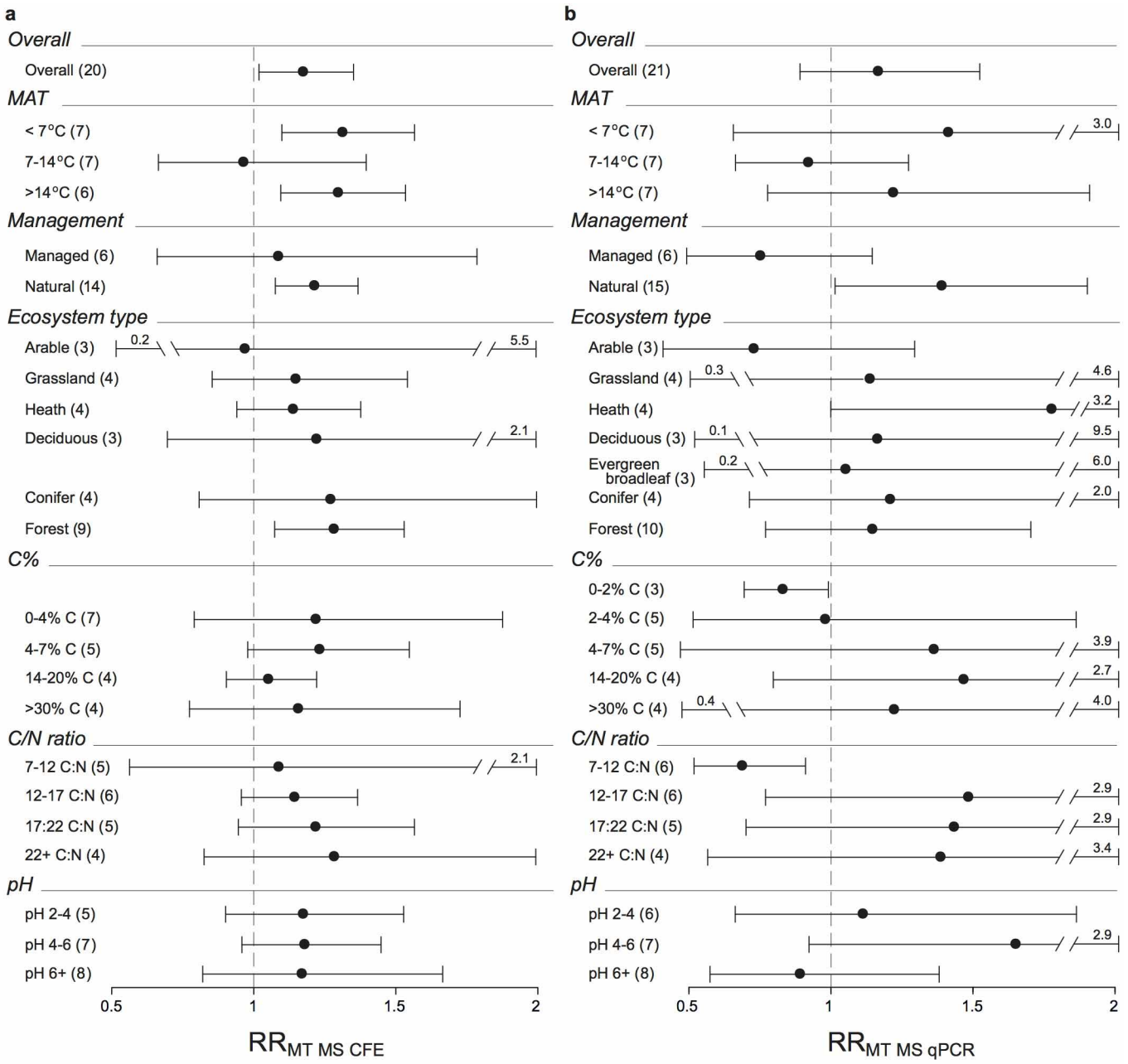
Extended Data Figure 5 | Respiration rates of all treatments (control, cooled and re-warmed) for the individual soils 3G, 3H, 4A and 4C, including the 84-day pre-incubation period. As for Extended Data Fig. 2.



Extended Data Figure 6 | Respiration rates of all treatments (control, cooled and re-warmed) for the individual soils 4D, 4G, 4H and 5E_1, including the 84-day pre-incubation period. As for Extended Data Fig. 2.



Extended Data Figure 7 | Respiration rates of all treatments (control, cooled and re-warmed) for the individual soils 5E_2 and 5E_3, including the 84-day pre-incubation period. As for Extended Data Fig. 2.



Extended Data Figure 8 | The mean ± 95% confidence intervals of mass-specific RR_{MT} values, calculated per CFE biomass (a) and per qPCR biomass (b). Overall values (that is, including all data) and values for the different soil groups, based on ecosystem type, management, climate and the various soil properties, are presented (*n* is given in parentheses; bars are cut if they extend beyond 2.0 or 0.5, numbers on broken lines represent the final *x*-axis value rounded to 1 decimal place). One evergreen broadleaved forest soil (5E_1) had a biomass too low to be measured using the CFE method. Therefore in **a**, we cannot present confidence intervals for evergreen broadleaf forests because there are now 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 the average for soils with 0–4% C). Values >1 and <1 indicate enhancing and compensatory responses, respectively. The patterns are very similar to RR_{MT} calculated per gram of soil C (Fig. 3a).

Extended Data Table 1 | Sampling site and soil characteristics

Sampling site					Soil characteristics				
Site	Location	MAT	Eco-system	Vegetation	pH	Soil texture	C (%)	N (%)	C/N ratio
1A	Sweden, Uppsala, Ekhaga 59°49'54"N, 17°48'25"E, (10 m asl)	5.1	A (m)	*Cereals*	6.69	silty clay	3.70	0.36	10.31
1C	Sweden, Umeå, Vindeln 64°13'47"N, 19°47'07"E, (190 m asl)	2.8	C (n)	*Pinus sylvestris, Vaccinium vitis-idaea, Pleurozium schreberi*	4.21	sandy silt loam	3.88	0.09	40.84
1D	Sweden, Abisko , ABACUS-site 68°19'30"N, 18°49'47"E, (520 m asl)	-2.0	D (n)	*Betula pubescens Ehrh. ssp. czerepanovii, Empetrum hermaphroditum , Vaccinium uliginosum, V. vitis-idea*	4.16	sandy silt loam	5.75	0.16	37.00
1G	Sweden, Uppsala, Ekhaga 59°49'54"N, 17°48'25"E, (10 m asl)	5.1	G (m)	*Festuca spp., Ranunculus acris, Achillea millefolium, Taraxacum officinale*	6.6	silty clay	4.56	0.44	10.39
1H	Sweden, Abisko, Njulla 68°22'11"N, 18°41'37"E, (1100 m asl)	-6.1	H (n)	*Vaccinium vitis-idaea, Empetrum nigrum*	4.52	n.a.	6.30	0.33	18.84
2C	UK, Scotland , Glen Cononish, 56°25'3"N, 4°42'15"W, (212 m asl)	8.4	C (n)	*Pinus sylvestris, Hylocomium splendens, V. myrtillus, Pteridium aquilinum*	3.85	n.a.	53.47	1.87	28.58
2D	UK, Stirling, Hermitage Wood: 56° 9'13"N, 3°54'44"W, (129 m asl)	8.4	D (n)	*Acer pseudoplatanus, Fagus sylvatica, Tilia x europaea, Mercurialis perennis*	4.05	silt loam	14.37	1.08	13.34
2G	UK, Scotland, Meall Ghaordaidh 56°31'30"N, 4°24'58"W, (1007 m asl)	3.1	G (n)	*Nardus stricta – Carex bigelowii*	4.39	n.a.	32.93	2.10	15.72
2H	UK, Scotland, Meall Ghaordaidh 56°31'7"N, 4°24'18"W, (757 m asl)	4.6	H (n)	*Calluna vulgaris, V. myrtillus, V. vitis-idaea, Empetrum nigrum ssp. nigrum, Rubus chamaemorus*	3.65	n.a.	53.72	1.47	36.49
3A	UK, Cranfield University, Silsoe 52°00'34"N, 0°26'04"W, (70 m asl)	10.2	A (m)	*Cereals*	6.77	clay loam	1.76	0.26	6.81
3C	UK, Alice Holt 51°11' N, 0°51' W, (100 m asl)	10.7	C (m)	*Pinus sylvestris, Pteridium aquilinum*	3.67	n.a.	15.85	0.87	18.26
3D	UK, Alice Holt 51°9'N, 0°51'W, (80 m asl)	10.7	D (n)	*Quercus robur, Fraxinus excelsior*	3.96	silt loam	5.09	0.35	14.43
3G	UK, North Wyke 50°46'48"N, 3°55'13"W, (213 m asl)	9.9	G (m)	*Lolium perenne,Juncus effusus,Holcus lanatus*	5.12	silty clay loam	6.17	0.53	11.65
3H	UK, Aylesbeare common 50°42'04"N, 3°20'15"W, (150 m asl)	10.3	H (n)	*Ulex europaeus, Calluna vulgaris*	4.02	n.a.	18.72	1.01	18.48
4A	Italy, Tuscia Uni, Viterbo 42°25'30"N, 12°05'09" E, (310 m asl)	14.3	A (m)	*Organic crop rotation*	6.67	silty clay loam	1.02	0.10	10.13
4C	Spain, Sierra Morena 38°10'43" N, 3°47'27" W, (379 m asl)	18.5	C (n)	*Pinus pinaster*	6.33	sandy silt loam	3.54	0.25	14.33
4D	Italy, Roccarespampani, Viterbo 42°23'25"N, 11°55'15"E, (160m asl)	15.4	D (n)	*Quercus cerris L.*	6.48	silt loam	3.92	0.30	13.22
4G	Spain, Barcelona, Collserola 41°24'21"N, 2°6'30"E, (293 m asl)	16	G (n)	*Hyparrhenia hirta, Spartium junceum, Foeniculum vulgare, Brachypodium retusum*	6.71	sandy silt loam	4.28	0.33	13.04
4H	Spain, Barcelona, Premià de Dalt 41°30'48"N, 2°19'25"E, (428 m asl)	16	H (n)	*Erica arborea, Erica scorpius, Cistus salvifolius*	6.60	sandy silt loam	3.97	0.21	18.55
5E_1	Peru, Explorer's Inn plot 3 (TAM-05) 12°50'11"S, 69°16'45"W, (210 m asl)	24.4	E (n)	*Elaeocarpaceae, Moraceae Fabaceae*	3.56	sandy silt loam	1.55	0.15	10.60
5E_2	Peru, San Pedro 2 (SPD-2) 13°2'56"S, 71°32'13"W, (1500 m asl)	17.4	E (n)	*Clethraceae*	3.52	n.a.	19.36	1.33	14.59
5E_3	Peru, Wayqecha (WAY-01) 13°11'24"S, 71°35'13"W, (3025 m asl)	11.1	E (n)	*Clusiaceae, Cunoniceae*	3.54	n.a.	48.39	2.61	18.53

List of sites (site abbreviations correspond to Fig. 1), 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 parentheses: 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 content.