No evidence for compensatory thermal adaptation of soil microbial respiration in the study of Bradford *et al.* (*Ecol. Lett*., 2008, 11, 1316-1327).

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Abstract

Bradford *et al.* (2008) conclude that thermal adaptation will reduce the response of soil microbial respiration to rising global temperatures. However, we question both the methods used to calculate mass-specific respiration rates and the interpretation of the results. No clear evidence of thermal adaptation reducing soil microbial activity was produced.

Bradford *et al.* (2008) demonstrate that the rate of mass-specific microbial respiration in soils (R_{mass}) declines in response to both experimental warming and seasonal increases in temperature, potentially reducing the long-term response of soil respiration to global warming. However, there are issues with the method used to measure R_{mass}.

R_{mass} was calculated as a ratio between the rate of substrate-induced respiration (SIR) measured after 4 hours (yeast extract added at 20^oC) and 24 hours (sucrose added at 10, 15 and 20^oC). Fig. 1 illustrates how closely correlated these two assays are. The authors consider the former to measure microbial biomass, and the sucrose assay to measure microbial activity. These two SIR assays are close analogs and, in calculating R_{mass}, the authors are effectively dividing something by itself.

Furthermore, through isotopic (^{13}C) separation, the sucrose-induced respiration assay did not include CO_2 from soil organic matter decomposition. This may explain why the intercept of the relationship between the two SIR assays is not zero (Fig. 1). Alternatively, differences in the range of compounds added in the yeast extract, including amino acids, could have resulted in some differences in the

physiological processing of the added substrates. Whatever the cause of the non-zero intercept, the overall result is that R_{mass} varies systematically with the underlying value of the x-axis variable (yeast-extract induced respiration in Fig. 1), especially when "x" is low. Reflecting this, due to the low microbial biomass, R_{mass} in the mineral soils increased significantly with the underlying rate of yeast-extract induced respiration (y = 0.00625x + 0.162, R^2 = 0.701, P = 0.0264). In terms of the results from the Harvard forest long-term experiment, as soil warming reduced microbial biomass, this inevitably led to a reduction in R_{mass}, at least partially explaining the lower R_{mass} calculated for the warmed mineral soils. In contrast, the higher rates of SIR in the organic soils resulted in the calculation of R_{mass} being affected less by changes in the underlying rate of yeast-extract induced respiration, potentially explaining why R_{mass} was not reduced in the warmed organic soils.

It is extremely important to emphasise a point made by Bradford *et al.* (2008); when temperatures are increased it is impossible to distinguish between direct thermal adaptation and microbial community responses mediated through warming-induced changes in substrate availability (labile substrate availability was shown to have been reduced substantially by soil warming in this study). Even if the method for calculating R_{mass} had been appropriate, given that no reduction in R_{mass} was observed in the organic soils, the lower R_{mass} in the warmed mineral soils would more likely have been explained by the response of the microbial community to the indirect effect of warming; the loss of labile C. Steinweg *et al*. (2008) demonstrated that microbial communities in soils which are depleted in readily-decomposable carbon respond more slowly to the addition of labile substrates.

Therefore, in the original paper, the seasonal trends represented the only real evidence for compensatory thermal adaptation. Given the issues associated with the

SIR method of calculating R_{mass}, we have recalculated R_{mass} this time expressing it per unit chloroform fumigation-extraction (CFE) biomass (a more independent assay of microbial biomass). Rather than correlating R_{mass} with the mean soil temperature over an arbitrary time-period prior to each sampling date (63 or 77 days in the original paper), we simply present R_{mass} at 10, 15 and 20°C for the two warmer vs. the two cooler months (Fig. 2). Although there may be some evidence of a small down-regulation in R_{mass} measured at 15°C during the warmer months, this is overshadowed by the large increase in R_{mass} when measured at 20°C. This result suggests that exposure to warmer temperatures has increased mass-specific respiration at higher temperatures. In contrast to the conclusions of the original paper, and in agreement with previous work (Hartley *et al*. 2007 & 2008), these results raise the possibility of thermal adaptation enhancing the response of soil microbial respiration to a persistent warming trend.

In summary, we question the novel methodological approach adopted by Bradford *et al*. (2008), and suggest that their conclusions are not supported by the data. We conclude that substrate depletion remains the factor most likely to explain the subsequent decline in respiration rates that follows the initial stimulation of activity in long-term, soil-warming experiments.

References

Bradford, M.A., Davies, C.A., Frey, S.D., Maddox, T.R., Melillo, J.M., Mohan, J.E., Reynolds, J.F., Treseder, K.K. & Wallenstein, M.D. (2008) Thermal adaptation of soil microbial respiration to elevated temperature. *Ecol. Lett.*, 11, 1316-1327.

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

Hartley, I.P., Hopkins, D.W., Garnett, M.H., Sommerkorn, M. & Wookey, P.A. (2008) Soil microbial respiration in arctic soil does not acclimate to temperature. *Ecol. Lett.*, 11, 1092-1100.

Steinweg, J.M., Plante, A.F., Conant, R.T., Paul, E.A. & Tanaka, D.L. (2008) Patterns of substrate utilization during long-term incubations at different temperatures. *Soil Biol. Biochem.*, 40, 2722-2728.

Figure legends

Figure 1 The relationship between yeast extract-induced respiration (considered to represent microbial biomass) and the rate of sucrose respiration (considered to represent microbial activity) when both measured at 20°C. The values are taken from Table 1 (SIR data) and Figure S3 (20°C data) in Bradford *et al*. (2008). Our graph plots the monthly mean values for organic (circles) and mineral soils (triangles), from warmed (closed symbols) and control plots (open symbols). An overall linear regression between the two variables is shown (hashed line). It should be noted that the intercept of the regression line (-7.69) differs significantly from zero (P < 0.001).

Figure 2 R_{mass} expressed per unit chloroform fumigation extraction (CFE) biomass (*i.e.* rate of microbial respiration of sucrose divided by CFE biomass). The data are presented as an average for the two cool (April and November) and two warm (July and October) months. Based on the argument developed in the original paper, October is considered to be a warm month due to its recent thermal history. The data are taken from Table 1 (CFE data) and Figure S3 (sucrose respiration). Each data point represents the overall average R_{mass} value calculated from the warmed and control, organic and mineral soils.
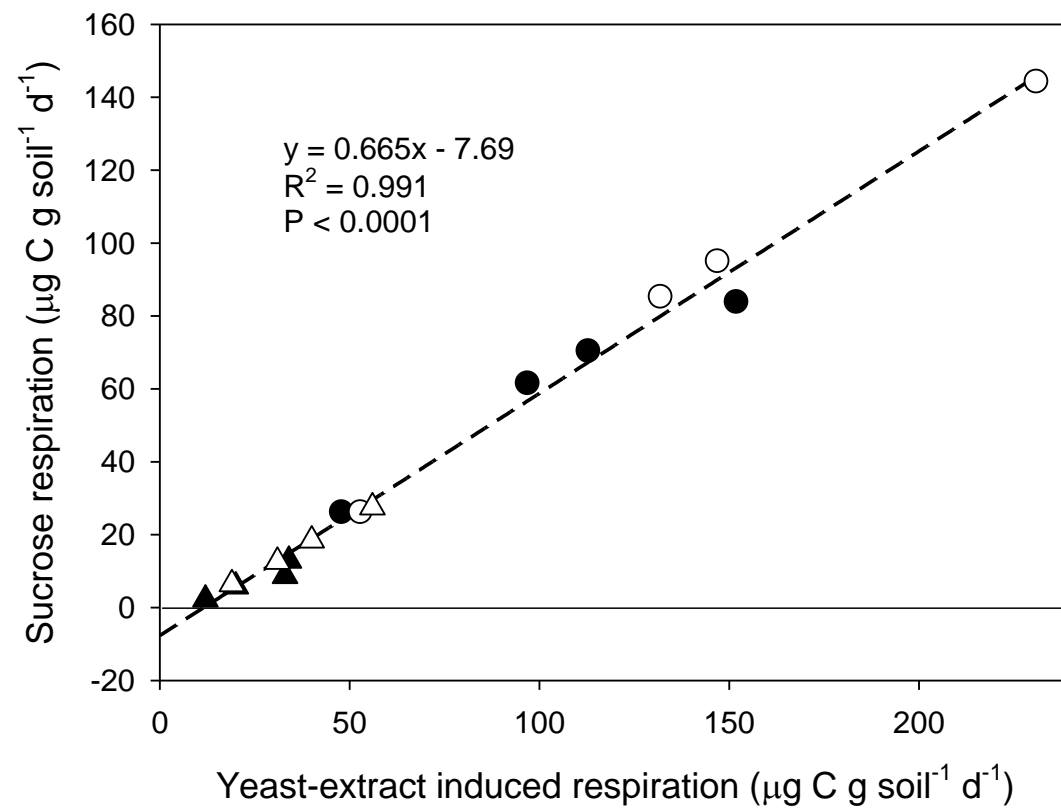
Figure 1

Figure 2