Decreased mass specific respiration under experimental warming is robust to the microbial biomass method employed

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Keywords: Acclimation, adaptation, soil respiration, thermal biology, temperature, carbon cycling, climate change, climate warming, microbial community, CO_2

Type of article: Technical comment

Abstract 50 words; manuscript 788 words; 15 references; 2 figures; **mailing and e-mail address of Mark A. Bradford is as above; tel. +1 203 436 9148, fax. +1 203 432 3929*

Abstract

Hartley *et al.* question whether reduction in R_{mass}, under experimental warming, arises because of the biomass method. We show the method they treat as independent yields the same result. We describe why the substrate-depletion hypothesis cannot alone explain observed responses, and urge caution in the interpretation of the seasonal data.

Keywords

Acclimation, adaptation, soil respiration, thermal biology, temperature, carbon cycling, climate change, climate warming, microbial biomass, CO_2

Hartley *et al.* (2009) make two important observations on our work (Bradford *et al.* 2008) and re-analyse our seasonal data. We respond to each observation and then discuss the re-analysis.

The first observation is that we calculated R_{mass} as a ratio between two respiration-based measures. The positive relationship between these two variables, and importantly the negative intercept, means that as SIR biomass increases R_{mass} follows a positive hyperbolic function. Specifically, across higher biomass values (in the organic horizon) there is little change in R_{mass} but at lower biomass values (in the mineral horizon) R_{mass} co-varies markedly. Had the intercept between sucrose respiration and SIR biomass been zero then R_{mass} would have been constant; had it been positive then R_{mass} would have decreased as biomass increased. Hartley *et al.* (2009) present their seasonal re-analysis (see below) using CFE microbial biomass; they consider it a more independent measure. If we calculate R_{mass} using CFE then we observe that under experimental warming R_{mass} is reduced (Fig. 1). That is, our observation that prolonged experimental warming decreases R_{mass} is robust to the microbial biomass method employed.

The second observation is that if our method to calculate R_{mass} is appropriate, the lower R_{mass} is more likely due to a depletion in labile carbon, rather than thermal adaptation. From this Hartley *et al.* (2009) conclude that the substrate-depletion hypothesis most likely explains the ephemeral augmentation of respiration in warming experiments. We agree that substrate-depletion likely contributes to this augmentation and present the first field evidence that labile carbon pools decline in response to experimental warming (see Bradford *et al.* 2008). However, the substrate-depletion hypothesis does not make explicit predictions about microbial biomass or R_{mass} (Kirschbaum 2004; Eliasson *et al.* 2005; Knorr *et al.* 2005); no change in microbial metabolism or carbon supply is invoked to explain respiration dynamics (see Kirschbaum 2004). This makes inferences from the hypothesis about microbial biomass and activity responses

speculative. For example, the depletion of labile carbon pools does not imply that microbial biomass should decline due to carbon limitation, since the substrate-depletion hypothesis assumes equal carbon supply in control and heated soils at equilibrium. This led us (Bradford *et al.* 2008) to speculate that decreased root-carbon supply could explain the microbial biomass decreases we observed under experimental warming. Decreases could also have arisen through reduced carbon-use efficiencies (Steinweg *et al.* 2008), altered growth rates (Bárcenas-Moreno *et al.* 2009), and/or shifts in microbial community composition in the plots (Frey *et al.* 2008). Whether depletion of labile carbon pools drives any of these changes is currently unclear. Nonetheless, the substrate-depletion hypothesis cannot solely explain observed responses of soil microbes and their respiration to warming; nor was it presented as a panacea (see Kirschbaum 2004). The soil and global change communities need to focus more attention on microbial and plant responses when explaining soil respiration responses to warming.

In their re-analysis of our seasonal data, Hartley *et al.* (2009) suggest there is evidence for thermal adaptation enhancing the response of soil microbial respiration to warming. We acknowledge their conclusion but suggest that perhaps we and Hartley *et al.* (2009) over-stepped what could be concluded about R_{mass} responses to seasonal temperature change using the SIR and CFE methods, respectively. Although CFE and SIR share a common origin (Anderson & Domsch 1978; Vance *et al.* 1987; Jenkinson *et al.* 2004), and yield biomass estimates that are correlated (Wardle & Parkinson 1991; Anderson & Joergensen 1997), they both have limitations. First, they provide ‘estimates’ of biomass. We relied on SIR because it is more effective at resolving active biomass differences at plot-scales (Wardle & Ghani 1995); CFE is often poor for detecting fine-scale variation. After finding approximately equivalent experimental-warming responses using both methods (Fig. 1 and Bradford *et al.* 2008), we proceeded to the seasonal

analysis using only SIR. Yet, Hartley *et al.*'s (2009) re-analysis highlights how this affects our interpretation of the seasonal data (Fig. 2). There is clearly a need for development of methodology to provide robust, fine-scale, independent measures of microbial biomass. In the absence of these, we emphasize the seasonal patterns that are independent of the biomass method, and even biomass correction. Particularly pronounced is the seasonal shift in the shape of the temperature response, suggesting the optimum is shifted to the right in the warm season (Fig. 2a-c). In addition, sucrose respiration rates for each season diverge markedly across the temperature range (Fig. 2), highlighting the importance of considering biomass changes. These patterns are obscured for soil respiration (Fig. 2). This may mean that soil respiration responses to warming can mask marked shifts in microbial biomass and temperature response of microbial respiration. We conclude that the relative roles and interactions of substrate-depletion versus microbial responses remain unresolved in warming soils.

ACKNOWLEDGEMENTS

This research was supported by the Office of Science (BER), U.S. Department of Energy, the Andrew W. Mellon Foundation and U.S. National Science Foundation grants to the Coweeta LTER program. We thank four anonymous reviewers, the editors and Iain Hartley and Phil Wookey for constructive comments.

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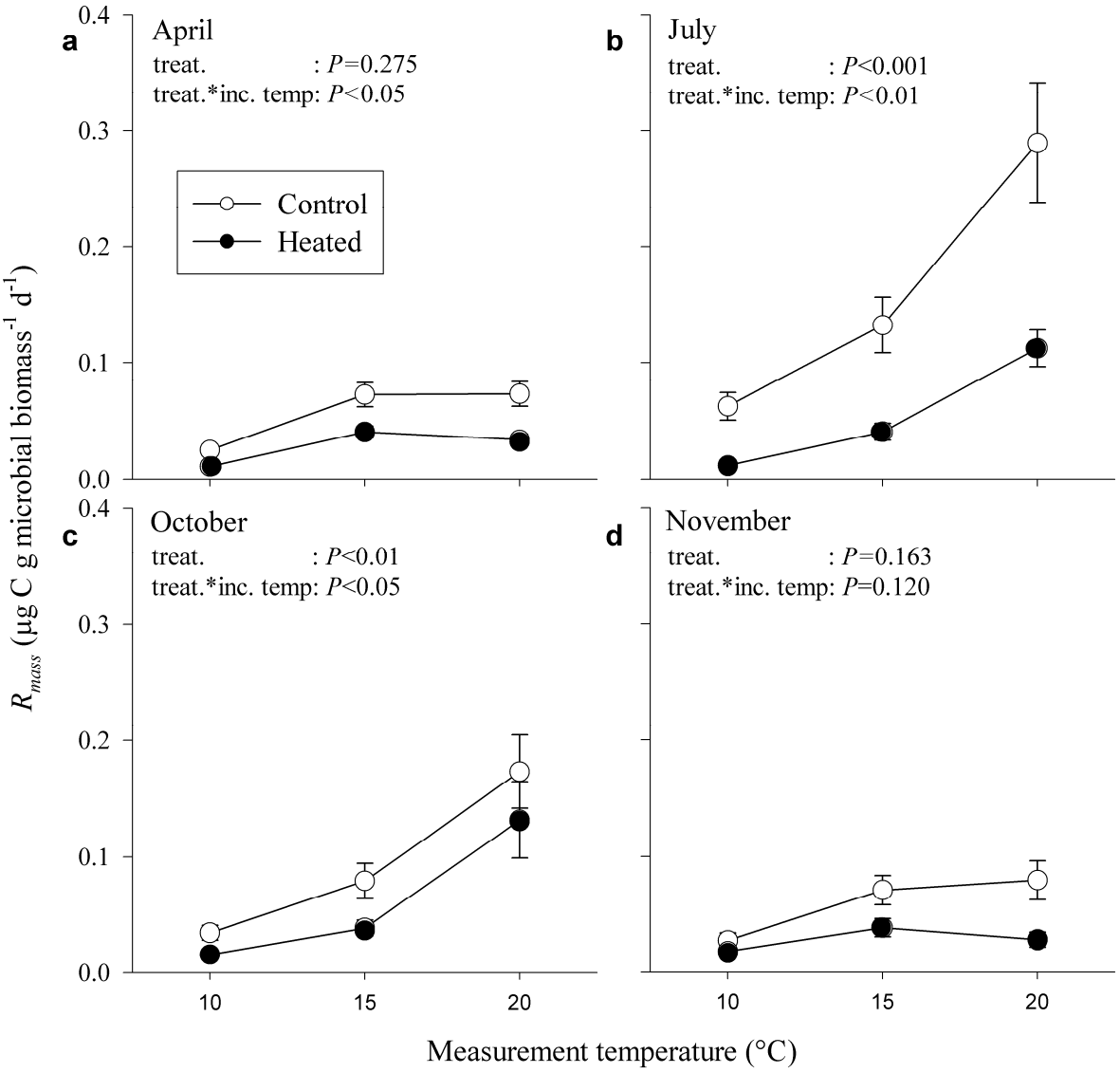
Figure 1 Rates of soil microbial respiration of sucrose, expressed per unit CFE microbial biomass, in control and heated soils at three measurement temperatures. These plots are equivalent to Fig. S4 in Bradford *et al.* (2008) excepting that in the original figure rates of sucrose respiration are expressed per unit SIR microbial biomass. Field soils were sampled from control (closed circles) and heated (open circles) plots (n = 6) and then assayed to assess sucrose mineralization rates across a temperature range from 10 to 20°C, and biomass using the CFE method (for details see Bradford *et al.* 2008). Shown are data from assays performed for the upper mineral soil horizon across early spring (April) to late fall (November). The observed pattern is that R_{mass} is generally lower, at a specific measurement temperature, following long-term, experimental warming. Values are means ± 1 s.e.m., n = 6. Given that R_{mass} is essentially a ratio, note that standard errors were propagated from the errors in the microbial biomass and sucrose respiration data. This same pattern was observed with the SIR biomass corrected data (see Bradford *et al.* 2008).

Figure 2 Respiration rates of soils sampled in the cool and warm seasons at three measurement temperatures, following the approach of Hartley *et al.* (2009). Note that this approach pools across the experimental treatments and soil horizons. Therefore the patterns observed in Fig. 1 do not relate to what is shown in this figure. In their re-analysis of our seasonal data using CFE microbial biomass, Hartley *et al.* (2009) conclude that the large increase in R_{mass} rates at measurement temperatures of 20°C, for soils sampled in the warm season (a), implies that thermal adaptation will enhance the response of soil microbial respiration to persistent warming. A different interpretation is obtained if one uses SIR estimates of biomass to calculate R_{mass} rates (b). There are potentially issues with both of these approaches. Indeed, mean daily temperature

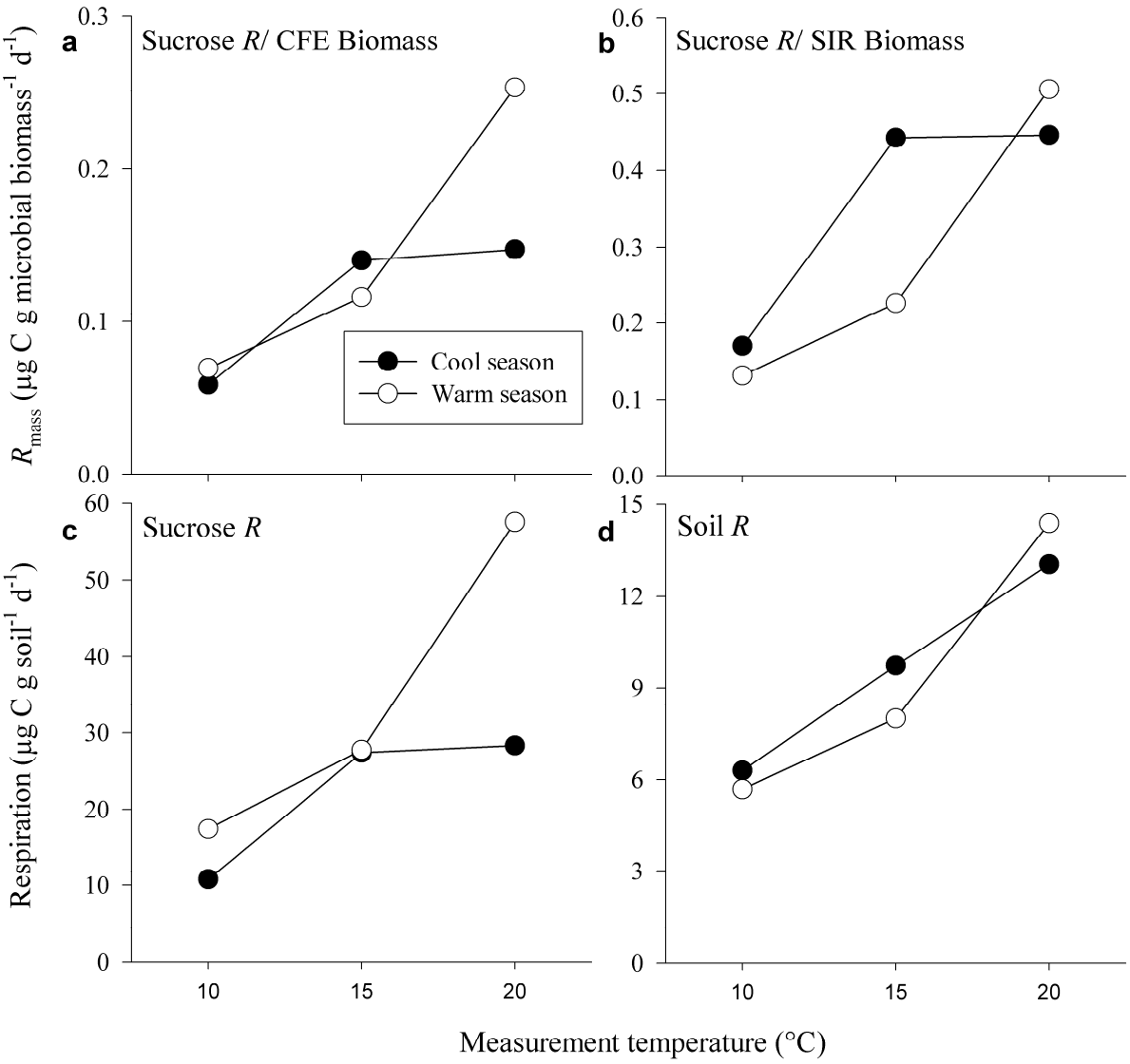
across the preceding 9 or 11 weeks explained 64 and 75% of the seasonal variation in R_{mass} (based on SIR) for the organic and mineral horizons, respectively (see Bradford *et al.* 2008). However, the same analysis using CFE biomass to calculate R_{mass} explained no significant variation (r^2 values <0.01; showing less than 1% of variance explained). This may be because CFE biomass values are highly variable at fine-spatial scales compared to SIR biomass estimates (see text for additional discussion). However, the apparent seasonal shift in the thermal optimum for R_{mass} appears independent of the biomass method employed (a,b), and is also observed if sucrose respiration data are not corrected for biomass (c). That is, that rates in cool season soils increase markedly between measurement temperatures of 10 and 15°C, and little between 15 and 20°C, whereas the opposite pattern is observed for warm season soils (a-c). That thermal optima for R_{mass} rates track seasonal temperature corresponds with similar tracking of other microbial activities involving carbon degradation (Fenner *et al.* 2005) and is a consistent pattern in our seasonal dataset. Notably, the pattern is not observed for soil respiration, expressed where substrate-limitation has not been alleviated, and without correction for biomass (d and see text).

176 **Fig. 1**


a
April
treat. : P=0.275
treat.*inc. temp: P<0.05
Control
Heated
b
July
treat. : P<0.001
treat.*inc. temp: P<0.01
c
October
treat. : P<0.01
treat.*inc. temp: P<0.05
d
November
treat. : P=0.163
treat.*inc. temp: P=0.120
R_{mass} (µg C g microbial biomass^{-1} d^{-1})
0.0
0.1
0.2
0.3
0.4
10
15
20
Measurement temperature (°C)

177

178 **Fig. 2**


a
Sucrose R/ CFE Biomass
b
Sucrose R/ SIR Biomass
c
Sucrose R
d
Soil R
Cool season
Warm season
R_{mass} (μg C g microbial biomass^{-1} d^{-1})
Respiration (μg C g soil^{-1} d^{-1})
Measurement temperature (°C)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
10
20
30
40
50
60
3
6
9
12
15
10
15
20

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