

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

3 **Mark A. Bradford,^{1*} Matthew D. Wallenstein,² Steven D. Allison,³ Kathleen K. Treseder,³**
4 **Serita D. Frey,⁴ Brian W. Watts,⁵ Christian A. Davies,⁵ Thomas R. Maddox,⁵ Jerry M.**
5 **Melillo,⁶ Jacqueline E. Mohan⁵ and James F. Reynolds⁷**

6 *¹School of Forestry and Environmental Studies, Yale University, New Haven, CT 06511, USA*

7 *²Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523,*
8 *USA*

9 *³Ecology & Evolutionary Biology, University of California, Irvine, CA 92697, USA*

10 *⁴Department of Natural Resources, University of New Hampshire, Durham, NH 03824, USA*

11 *⁵Odum School of Ecology, University of Georgia, Athens, GA 30602, USA*

12 *⁶The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543, USA*

13 *⁷Nicholas School of the Environment and Earth Sciences, and Department of Biology, Duke*
14 *University, Durham, NC 27708, USA*

15 Author e-mails: mark.bradford@yale.edu, mawallen@nrel.colostate.edu, allisons@uci.edu,
16 treseder@uci.edu, serita.frey@unh.edu, booralun@uga.edu, drcadavies@gmail.com,
17 trmaddox@uga.edu, jmelillo@mbl.edu, jmohan@uga.edu, james.f.reynolds@duke.edu

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22 *of Mark A. Bradford is as above; tel. +1 203 436 9148, fax. +1 203 432 3929*

23 Abstract

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

28

29 Keywords

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31 change, climate warming, microbial biomass, CO₂

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

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

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

57 speculative. For example, the depletion of labile carbon pools does not imply that microbial
58 biomass should decline due to carbon limitation, since the substrate-depletion hypothesis
59 assumes equal carbon supply in control and heated soils at equilibrium. This led us (Bradford *et*
60 *al.* 2008) to speculate that decreased root-carbon supply could explain the microbial biomass
61 decreases we observed under experimental warming. Decreases could also have arisen through
62 reduced carbon-use efficiencies (Steinweg *et al.* 2008), altered growth rates (Bárcenas-Moreno *et*
63 *al.* 2009), and/or shifts in microbial community composition in the plots (Frey *et al.* 2008).
64 Whether depletion of labile carbon pools drives any of these changes is currently unclear.
65 Nonetheless, the substrate-depletion hypothesis cannot solely explain observed responses of soil
66 microbes and their respiration to warming; nor was it presented as a panacea (see Kirschbaum
67 2004). The soil and global change communities need to focus more attention on microbial and
68 plant responses when explaining soil respiration responses to warming.

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

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

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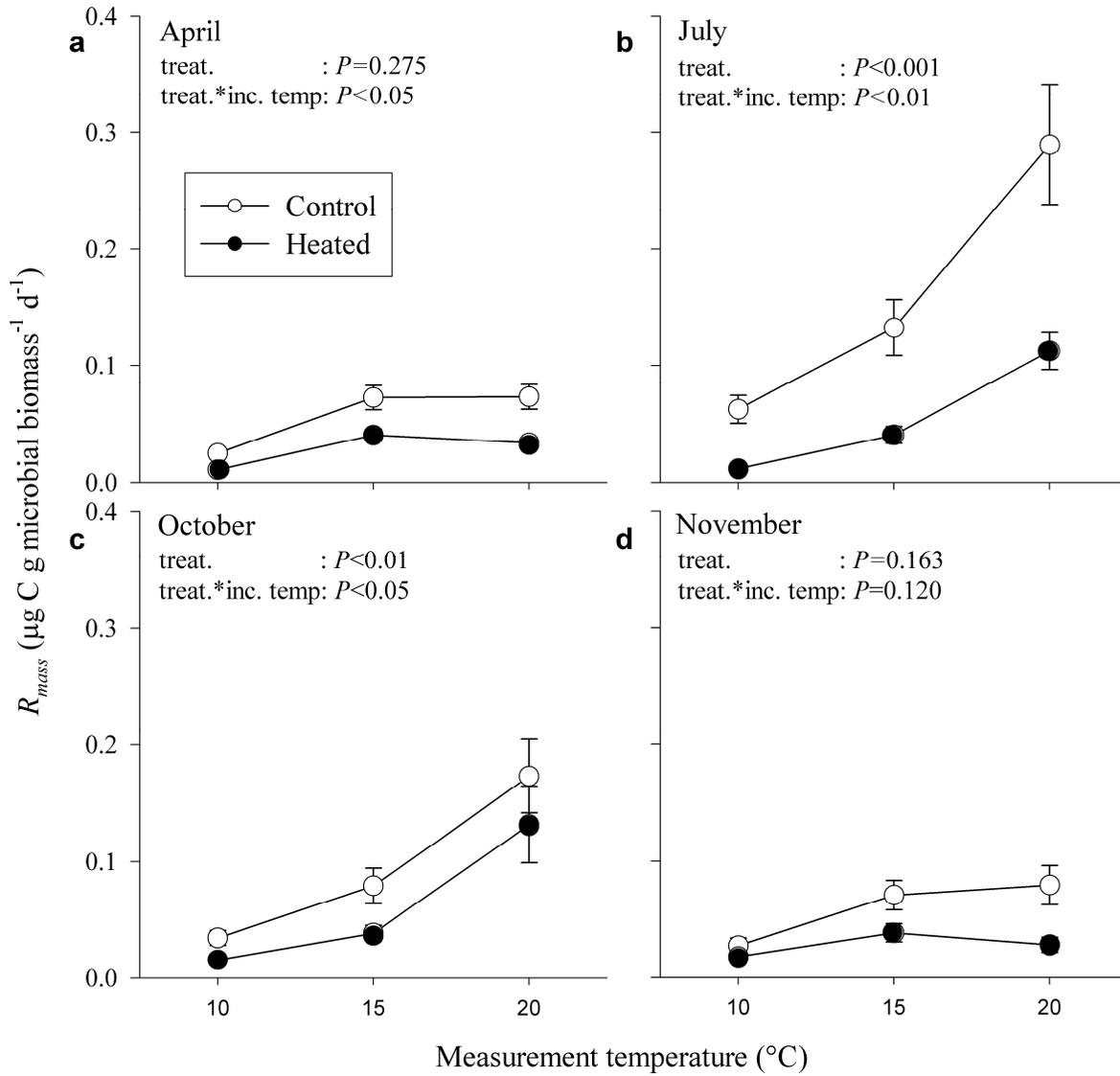
139 **Figure 1** Rates of soil microbial respiration of sucrose, expressed per unit CFE microbial
140 biomass, in control and heated soils at three measurement temperatures. These plots are
141 equivalent to Fig. S4 in Bradford *et al.* (2008) excepting that in the original figure rates of
142 sucrose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

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

162 across the preceding 9 or 11 weeks explained 64 and 75% of the seasonal variation in R_{mass}
163 (based on SIR) for the organic and mineral horizons, respectively (see Bradford *et al.* 2008).
164 However, the same analysis using CFE biomass to calculate R_{mass} explained no significant
165 variation (r^2 values <0.01 ; showing less than 1% of variance explained). This may be because
166 CFE biomass values are highly variable at fine-spatial scales compared to SIR biomass estimates
167 (see text for additional discussion). However, the apparent seasonal shift in the thermal optimum
168 for R_{mass} appears independent of the biomass method employed (a,b), and is also observed if
169 sucrose respiration data are not corrected for biomass (c). That is, that rates in cool season soils
170 increase markedly between measurement temperatures of 10 and 15°C, and little between 15 and
171 20°C, whereas the opposite pattern is observed for warm season soils (a-c). That thermal optima
172 for R_{mass} rates track seasonal temperature corresponds with similar tracking of other microbial
173 activities involving carbon degradation (Fenner *et al.* 2005) and is a consistent pattern in our
174 seasonal dataset. Notably, the pattern is not observed for soil respiration, expressed where
175 substrate-limitation has not been alleviated, and without correction for biomass (d and see text).

176 **Fig. 1**



178 **Fig. 2**