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LETTER

Thermal acclimation in widespread heterotrophic soil microbes

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Abstract

Respiration by plants and microorganisms is primarily responsible for mediating carbon exchanges between the biosphere and atmosphere. Climate warming has the potential to influence the activity of these organisms, regulating exchanges between carbon pools. Physiological 'down-regulation' of warm-adapted species (acclimation) could ameliorate the predicted respiratory losses of soil carbon under climate change scenarios, but unlike plants and symbiotic microbes, the existence of this phenomenon in heterotrophic soil microbes remains controversial. Previous studies using complex soil microbial communities are unable to distinguish physiological acclimation from other community-scale adjustments. We explored the temperature-sensitivity of individual saprotrophic basidiomycete fungi growing in agar, showing definitively that these widespread heterotrophic fungi can acclimate to temperature. In almost all cases, the warm-acclimated individuals had lower growth and respiration rates at intermediate temperatures than cold-acclimated isolates. Inclusion of such microbial physiological responses to warming is essential to enhance the robustness of global climate-ecosystem carbon models.

Keywords

Carbon cycle feedbacks, climate warming, evolutionary trade-offs, soil carbon storage, soil respiration, thermal acclimation, thermal acclimatisation.

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INTRODUCTION

Temperature regulates biogeochemical cycling by changing the rates at which organisms process carbon and nutrients. Understanding the biological mechanisms regulating carbon exchanges between aquatic, terrestrial and atmospheric carbon pools, and how carbon fluxes will respond to and influence climate change, are among the most urgent scientific and political challenges for ecosystem ecologists (Bardgett *et al.* 2008). Traditionally, the respiratory release of CO_2 into the atmosphere is thought to be more temperature-sensitive than photosynthesis (carbon fixation), generating a positive climate-ecosystem carbon feedback (Davidson & Janssens 2006) with the potential to accelerate climate warming by up to 1.4 times (Cox *et al.* 2000). More recently the temperature-sensitivity (Q_{10}) of ecosystem-scale respiration has been shown to be lower than initial expectations (Mahecha *et al.* 2010), and long-term warming studies show that terrestrial ecosystem respiration rates consistently return to pre-warming levels within a few years (e.g. Melillo *et al.* 2002; Eliasson *et al.* 2005). Along with decreases in labile organic carbon availability (Kirschbaum 2004; Eliasson *et al.* 2005; Hartley *et al.* 2007), theoretical models partially attribute this ephemeral respiration response to biological 'thermal acclimatisation' (Oechel *et al.* 2000; Luo *et al.* 2001; Davidson & Janssens 2006). That is, changes in species composition and/or physiological acclimation may weaken the positive effect of warming on respiration. The major contributors to ecosystem respiration are plants, mycorrhizal fungi and decomposer microorganisms, but the temperature-sensitivities of these components are not equivalent (Boone *et al.* 1998; Malcolm *et al.* 2008). Thus, understanding and characterising the responsiveness of these separate contributors in isolation is highlighted as an essential research priority, to help predict accurately how warming will affect carbon efflux across different ecosystems (Heinemeyer *et al.* 2012).

Respiratory 'thermal acclimation' (the subsequent adjustment in the rate of respiration to compensate for an initial change in temperature) is well documented for plants (e.g. Loveys *et al.* 2003). Warm-acclimated plants exhibit lower mass-specific respiration rates than cool-acclimated individuals when grown at a common temperature. Acclimation has also been identified in their mycorrhizal symbionts [arbuscular mycorrhizal fungi in association with plants (Heinemeyer *et al.* 2006) and free-living ectomycorrhizal fungi grown in agar (Malcolm *et al.* 2008)], although the activity of mycorrhizae *in situ* is strongly influenced by the host plant physiology, a fact used to argue that they are essentially autotrophic (Heinemeyer *et al.* 2006). Including plant and mycorrhizal acclimation has recently been shown to substantially improve the robustness of global-scale carbon models (Smith & Dukes 2013), but the acclimation responses of heterotrophic soil microbes, the third major group contributing to terrestrial respiration, remain uncertain (Mahecha *et al.* 2010; Reich 2010). It is believed that, unlike their autotrophic counterparts, heterotrophic species are unlikely to acclimate to warmer temperatures because they gain no evolutionary advantage from such physiological 'down-regulation' (Hartley *et al.* 2007, 2008). Resolving this uncertainty is essential if we are to understand ecosystem-scale carbon exchange responses to warming (Reich 2010).

Several biochemical trade-offs suggest that thermal acclimation should be a common property of all organisms (Hochachka & Somero 2002; Tjoelker *et al.* 2008). For example, enzymes involved in respiratory metabolism are conserved across all three domains of life. Like all enzymes, the functioning of respiratory enzymes requires both an initial conformation that allows binding of substrates, and a conformational change that facilitates the reaction. Although the increased stability of enzymes selected for at high temperatures ensures the maintenance of binding structures, the highly flexible nature of cold-adapted enzymes enables them to efficiently change conformation and facilitate interactions (Hochachka & Somero 2002). Consequently, when compared at intermediate temperatures, catabolic rates (k_{cat}; the rate at which substrate is converted to product per active site per unit time) are generally

higher for cold- than warm-adapted enzymes (Hochachka & Somero 2002). Similar changes in the permeability of cell membranes mean that rates of passive regulation, active transport and enzyme activity are also higher in cold, than warm-acclimated organisms (Hazal & Williams 1990). Such biochemical trade-offs have been proposed to partially explain the levelling of mass-specific respiration rates (R_{mass}) of microbial communities following prolonged warming (Bradford *et al.* 2008, 2010). Evidence for this is, however, confounded by the fact that warming can also drive changes in community composition, which may simply favour less metabolically active species (Hall *et al.* 2010; Treseder *et al.* 2012). It therefore remains unclear if individual heterotrophic soil microbes can acclimate to temperature, and to what extent any physiological responses may influence ecosystem-scale respiration responses to warming. Incorporating such physiological responses of heterotrophic soil microbial communities into carbon cycling models has been identified as essential for accurately predicting future climate-ecosystem carbon feedbacks (Högberg & Read 2006; Allison *et al.* 2010; Reich 2010; Conant *et al.* 2011; Treseder *et al.* 2012). A mechanistic understanding of thermal acclimation has been precluded by the lack of empirical studies exploring the potential for these physiological responses in individual microbes.

Lignocellulytic basidiomycete fungi are the dominant decomposers in woodland ecosystems (Boddy 2000; Crowther *et al.* 2012a). Some species can form cords (aggregations of mycelia) that stretch tens of metres across the soil-litter interface (Cairney 2005). As well as contributing extensively to soil enzyme activity and respiration, these cord-formers are among a limited group of organisms capable of decomposing the lignin and cellulose within woody litter. Their large individual size and functional significance make cord-forming fungi useful model organisms to explore the potential for heterotrophic thermal acclimation as the responses of individuals can be easily monitored over time, and may represent an important component of the community-scale response across a range of woodland ecosystems. Whereas most studies exploring potential thermal acclimation in soil microbial communities focus on respiration (e.g. Bradford *et al.* 2008; Hartley *et al.* 2008), acclimation studies with plants and animals traditionally focus on individual growth rates (Hochachka & Somero 2002). Mycelial growth may, in fact, provide more valuable insights into the effect of temperature on microbial functioning, as extension rates directly influence the capacity of fungi to encounter and decompose organic resources (Boddy 2000). The significance of estimating growth over respiration as a measure of microbial activity has been emphasised in recent studies investigating thermal adaptation of terrestrial and aquatic communities (Balser & Wixon 2009; Barcenas-Moreno *et al.* 2009; Rinnan *et al.* 2009), because changes in growth can determine total microbial biomass and hence carbon cycling (Allison *et al.* 2010). However, a key variable influencing microbial growth rate is carbon-use efficiency, and estimating this requires measures of both microbial growth and respiration. Measurement of both, as opposed to only one, of these variables will provide the fullest understanding of how physiological temperature responses of heterotrophs will influence soil respiration and organic matter decay (Allison *et al.* 2010).

We explored the potential for individual saprotrophic basidiomycete fungi, isolated from temperate woodland soil, to acclimate to temperature. Mycelial extension and respiration rates were compared for cold-, intermediate- and warm-incubated fungi across a range of temperatures experienced in the field. Following traditional approaches in thermal biology (Hochachka & Somero 2002), assays took place in non-limiting substrate conditions to control for reductions in carbon availability following warming. Differences in substrate availability can affect an organism's ability to acclimate, or obscure detection of an acclimation response (Atkin & Tjoelker 2003; Hartley *et al.* 2008). The heterogeneous nature of soil carbon, and species-specific growth efficiencies, led us to use fungi growing in uniform axenic cultures. Although we appreciate that this limits our ability to make real-world predictions, it enables us to explore physiological mechanisms that could not be isolated *in situ* (Malcolm *et al.* 2008). Indeed, there is renewed emphasis on the need for culturing to comprehend the complexities of soil microbial communities, including the physiological mechanisms controlling community-scale responses to temperature (Lennon & Jones 2011; A'Bear *et al.* 2012). We tested the hypotheses that: (1) given fundamental evolutionary trade-offs in enzyme and cell membrane structure (Hochachka & Somero 2002), all fungi will show thermal acclimation, where cold-acclimated fungi will grow and respire more rapidly than warm-acclimated fungi at intermediate assay temperatures and (2) given that temperature influences competitive outcomes between lignocellulytic basidiomycete fungi (Crowther *et al.* 2012b), the extent of acclimation and rates at which they acclimate to new temperatures will be species-specific. We also expected growth efficiencies to decline linearly with increasing temperature, given the expectation that maintenance energy costs are proportionally higher as temperature increases (Allison *et al.* 2010).

MATERIALS AND METHODS

Overview of study design

Five cord-forming basidiomycete fungi were incubated on agar plates for 9 weeks at 12 °C, 20 °C and 28 °C (low, intermediate and high temperatures commonly experienced in temperate woodlands). Fungi from each treatment temperature were then re-plated and assayed for growth and respiration immediately and after 10 days at 12 °C, 20 °C and 28 °C. The definitive test for thermal acclimation involves higher growth and/or mass-specific respiration (i.e. respiration per unit biomass; R_{mass}) rates for organisms incubated at cooler as opposed to warmer treatment temperatures when compared at intermediate assay temperatures after a short time period (i.e. before organisms have time to adjust to assay temperatures; Hochachka & Somero 2002). Two types of acclimation are described for individuals (Atkin & Tjoelker 2003). Type I acclimation exhibits as no difference in activity (e.g. growth) between cold- and warm-incubated organisms at cooler assay temperatures, but lower activity of warm- than cold-incubated organisms at intermediate and higher assay temperatures (see Bradford *et al.* 2008). Type II acclimation exhibits as lower activity of warm- than cold-adapted organisms, where the relative difference is constant across all assay temperatures. Significant temperature treatment × assay interactions (with higher activity for cold- than warm-adapted individuals) indicate type I acclimation, where incubation temperature effects vary between assay temperatures. Inclusion of mycelial growth rates allowed us to explore rates of acclimation to assay temperatures. It is important to discern the *rate* of acclimation because microbial community adjustments are

thought to manifest over several weeks (Bradford *et al.* 2008), but acclimation in individual plants, animals and symbiotic fungi generally occurs over a few days.

Fungal culturing and incubations

Five cord-forming basidiomycetes, *Resinicium bicolor* (*Rb*), *Phanerochaete velutina* (*Pv*) and three strains of *Hypholoma fasciculare* (*Hf* DD2, *Hf* DD3 and *Hf* JH) from the Cardiff University Collection were used. These species are common across old- and new-world temperate forest. They were subcultured on 2% malt extract agar (MEA) in 9-cm dia. Petri dishes, sealed with Parafilm®. Each fungus was subcultured onto 15 separate dishes, using three fungal plugs per dish to accelerate colonisation rates. Five dishes of each taxon were incubated at 12, 20 or 28 °C for 9 weeks, providing ample time for thermal acclimation (Malcolm *et al.* 2008).

Assay conditions

Nine fungal plugs were taken from each Petri dish after 9 weeks. One plug was placed into each of three Petri dishes containing fresh MEA. One each of these three dishes were placed at 12, 20 and 28 °C across 10 days to assay growth. This gave 225 dishes, comprising 3 incubation temperatures × 3 assay temperatures × 5 replicates × 5 fungi. The remaining 6 fungal plugs were added individually to 50-mL centrifuge tubes containing 10-mL agar slopes (i.e. slants) to assay respiration at 1 and 10 days. This gave 450 slopes, comprising 3 treatment temperatures × 5 fungi × 5 replicates × 2 respiration assays (days 1 and 10). For the 1-day respiration assays, slopes were first incubated for 9 days at the treatment temperatures to allow fungi to colonise the agar, and then placed at the assay temperatures for 1 day. For 10-day respiration assays, slopes were incubated for 9 days at the assay temperatures before measurement of respiration rates across 1 day at the same temperatures.

Mycelial growth rates

Digital images of fungi growing in Petri dishes were photographed at a height of 15 cm, mycelial extent was calculated using IMAGEJ (National Institutes of Health, USA) following Crowther *et al.* (2011a). Extension rate (mm day^{-1}) was recorded every 2 days across the 10-day assay. Measures were not continued beyond 10 days because fungal mycelia reached plate edges. We report the extension rate across the first 2 days as the *de facto* test of thermal acclimation in growth rate, because such a short-term assay ensures that there is limited time for the organisms to acclimate to the assay temperature (Atkin & Tjoelker 2003). We report the final extension measurement as an indicator of how rapidly the organisms acclimate to the new temperature regime of the assay.

Respiration measurements

As with growth, we tested for respiratory thermal acclimation to the incubation temperatures, and then potentially to the assay conditions, using immediate (1 day) and longer term (10 day) assays respectively. Respiration was measured following Bradford *et al.* (2010), where centrifuge tubes were fitted with caps modified for gas analysis, flushed with CO_2-free air, and then incubated for 16 h, before headspace CO_2 concentrations were determined using an infrared gas analyser (IRGA; LI-7000, LI-COR, Lincoln, NE, USA). Fungal mycelia were then separated from the agar by melting it (autoclaving at 121 °C for 10 min) and filtering through Whatman #1 filter paper. Biomass was determined after drying (60 °C) to constant mass, enabling us to estimate mass-specific respiration (R_{mass}) expressed as CO_2 produced per dry mass of fungus.

Growth efficiency estimates

The efficiency with which microbes assimilate carbon into biomass has been termed microbial growth efficiency, substrate-use efficiency or carbon-use efficiency. Several methods for calculating microbial carbon-use efficiencies involve measuring carbon uptake, along with losses through enzyme production and exudation (Manzoni *et al.* 2012). As we neither measured carbon uptake nor losses other than respiration, we estimated growth efficiency (GE) using growth and respiration rates following Eiler *et al.* (2003). Here, $GE = \Delta growth/(\Delta growth + R_{mass})$, where Δgrowth is fungal extension rate and R_{mass} is the mass-specific respiration rate. Mycelial extent was strongly correlated with biomass for each fungus ($R^2 > 0.9$ in all fungi except *Rb* where $R^2 = 0.73$) with a ~1 mm:1 mg ratio for each fungus. We therefore used extension rate over biomass because this provides the most direct estimate of the amount of carbon allocated to foraging structure of the mycelium.

Statistical analysis

To test for effects of incubation temperature (treatment) and assay temperature on mycelial extension rates, we used Generalised Linear Models with time as a covariate. Species-specific extension rates (Crowther *et al.* 2011a) and growth responses to temperature (Crowther *et al.* 2012b) have previously been recorded in these fungal strains, so models were constructed separately for each fungus (with treatment, assay and time as factors). To account for non-normality (Shapiro–Wilk normality test) and/or homoscedasticity (Flinger test), individual family and link functions were used to fit each models to its residual error distributions (see SI for details). Significant ($P < 0.05$) 3-way interaction terms required construction of individual models to explore treatment (Type II acclimation) and treatment × assay (Type I acclimation) effects at each time point. Planned comparisons (contrast function) were used to test for treatment effects at the intermediate (i.e. 20 °C) assay temperature.

A Generalised Linear Model was used to explore differences in R_{mass} with fungus, time, treatment and assay as factors. A strongly significant ($P < 0.001$) 4-way interaction suggested that the treatment × assay interaction varied by fungal species and time. Individual models were, therefore, constructed for each fungus at the 1 and 10-day assay, and planned comparisons to evaluate treatment temperature effects at the intermediate assay temperature (see SI).

A Generalised Linear Model (See SI) was used to test for differences in growth efficiencies, with fungus, treatment and assay as factors. Planned comparisons were used to compare specific effects of assay temperature for each taxon. All analyses were performed using R (R Development Core Team 2010).

RESULTS

Growth rates

Extension rates of all fungi increased throughout the 10-day assays, confirming that fungi were not limited in terms of nutrients or space. For each fungus, growth rates acclimated to incubation temperature and, within the 10-day assays, also exhibited rapid acclimation to the assay temperature. The acclimation to the assay temperatures served to reduce the initial treatment (i.e. incubation temperature) differences in growth over 10-days. These dynamics were confirmed by significant 3-way interactions between incubation temperature, assay temperature and time (*Pv:* $F_{4,162} = 69$, $P < 0.001$; *Rb:* $F_{4,162} = 3.9$, $P = 0.004$; *Hf DD2:* $F_{4,162} = 3.0$, $P = 0.02$; *Hf DD3:* $F_{4,162} = 96$, $P < 0.001$; *Hf JH:* $F_{4,162} = 48$, $P < 0.001$). These 3-way interactions precluded model simplification and so we used planned comparisons to explore treatment effects at each time point.

All fungi exhibited type I acclimation at 2 days. That is, cold-acclimated fungi grew faster than intermediate- and warm-incubated isolates, but differences were greater at 20 °C and 28 °C than at 12 °C (treatment × assay interactions: $P < 0.001$ for all fungi). Treatment effects were most pronounced at intermediate assay temperatures (20 °C), where extension rates of warm-acclimated *P. velutina, R. bicolor, Hf DD2, Hf DD3 and Hf JH* were 87%, 3.9%, 0%, 1.6% and 4.6% those of the cold-acclimated isolates respectively (Fig. 1).

Extension rates of cold- and warm-incubated *P. velutina* isolates were significantly ($P \leq 0.05$) different until day 6, when differences between treatments notably diminished (Fig. 1; incubation temperature: $F_{2,36} = 1.3$, $P = 0.275$; interaction: $F_{4,36} = 2.4$, $P = 0.067$). From this time onward the *P. velutina* isolates at the same assay temperature all extended at the same rate until mycelia reached tray edges (Fig. 1). *Resinicium bicolor* and *H. fasciculate JH* took longer to acclimate to the assay temperatures. Extension rates of both varied consistently between treatments until day 10, when treatment (*Rb:* $F_{2,36} = 0.43$, $P = 0.655$; *Hf JH:* $F_{2,36} = 0.59$; $P = 0.561$) and interaction (*Rb*: $F_{4,36} = 0.118$, $P = 0.975$; *Hf JH:* $F_{4,36} = 0.158$; $P = 0.945$) effects were all non-significant. In contrast to the other taxa, *H. fasciculare DD2 and DD3* did not fully acclimate by the time mycelia reached tray edges. Specifically, although there were no significant ($P > 0.05$) differences between treatments at 12 °C and 20 °C, extension rates of the cold-acclimated isolates were still significantly (*Hf DD2*: $P = 0.012$; *Hf DD3*: $P = 0.002$) greater than the warm-acclimated isolates at the 28 °C assay (Fig. 1).

Respiration rates at 1-day assays

Respiratory thermal acclimation was less consistent across fungi than growth acclimation. *Phanerochaete velutina* showed Type II acclimation (treatment: $F_{2,36} = 15$, $P < 0.001$), where cold-acclimated isolates respired more, and to a similar relative extent (treatment × assay: $F_{4,36} = 0.788$, $P = 0.547$), than warm-acclimated isolates at all assay temperatures (Fig. 2). At intermediate assay temperatures, R_{mass} rates of warm-acclimated isolates were ~61% lower than those of cold-acclimated fungi.

Resinicium bicolor, H. fasciculare DD3 and *H. fasciculare JH* all displayed type I acclimation (*Rb* – treatment × assay: $F_{4,36} = 3.5$, $P = 0.018$; *Hf DD3* – treatment × assay: $F_{4,36} = 3.7$, $P = 0.013$; *Hf JH* – treatment × assay: $F_{4,36} = 4.1$, $P = 0.008$). R_{mass} rates were significantly ($P < 0.05$) higher for cold-, than intermediate- or warm-acclimated isolates, with the greatest differences at the highest assay temperature (Fig. 2). At the 20 °C assay, R_{mass} rates of warm-acclimated *R. bicolor, H. fasciculare DD3* and *H. fasciculare JH* were 39%, 43% and 60% lower than those of cold-acclimated isolates respectively.

R_{mass} responses of *H. fasciculare DD2* did not fit type I or II patterns. Significant treatment ($F_{2,36} = 25$, $P < 0.001$) and interaction ($F_{4,36} = 6.6$, $P < 0.001$) terms did, however, suggest that incubation temperature modified R_{mass} rates at the assay temperatures. Notably, warm-acclimated fungi had higher rates than fungi incubated at lower temperatures at the intermediate and high assay temperatures. At 20 °C, R_{mass} rates of cold-acclimated isolates were significantly ($P < 0.001$) greater than those acclimated to intermediate temperatures, but significantly ($P < 0.001$) lower than warm-acclimated isolates (Fig. 2).

Respiration rates at 10-day assays

The 10-day respiration assays were included to explore whether observed acclimation of growth rates to the assay temperatures were mirrored by respiration responses (i.e. if differences diminished over 10 days). Generally, there was substantially less variation in R_{mass} rates between isolates incubated across different treatments at the 10- compared to 1-day assays (Fig. 2). *Phanerochaete velutina* and *R. bicolor* both adjusted completely to the assay temperatures, showing no significant treatment (*Pv:* $F_{2,36} = 1.4$, $P = 0.26$; *Rb:* $F_{2,36} = 0.86$, $P = 0.434$) or interaction (*Pv:* $F_{4,36} = 0.15$, $P = 0.960$; *Rb:* $F_{4,36} = 0.25$, $P = 0.908$) effects. In contrast, all three strains of *H. fasciculare* failed to fully acclimate to assay temperatures: R_{mass} rates of cold-acclimated isolates were still significantly (*Hf DD2:* $F_{2,36} = 12$, $P < 0.001$; *Hf DD3:* $F_{2,36} = 26$, $P < 0.001$; *Hf JH:* $F_{2,36} = 12$, $P = 0.247$) higher than those of warm-acclimated isolates at all three temperatures (Fig. 2).

Growth efficiencies

Following the 10-day assays, growth efficiencies were unaffected by the temperature of the incubation treatment ($F_{2,180} = 1.3$, $P = 0.281$). Assay temperature, however, significantly ($F_{2,210} = 36$, $P < 0.001$) affected growth efficiencies, which were consistently greatest for fungi assayed at 20 °C and lowest for those at 28 °C (Fig. 3). Growth efficiencies were not significantly ($P > 0.05$) different for isolates of *P. velutina* and *R. bicolor* at assay temperatures of 12 °C and 28 °C (*Pv*: $P = 0.89$; *Rb*: $P = 0.98$), but all three *H. fasciculare* strains had higher growth efficiencies at 12 than 28 °C ($P < 0.001$ in all cases).

DISCUSSION

Contrary to the expectation that heterotrophic soil microbes do not acclimate to temperature (Hartley *et al.* 2008), we demonstrate thermal acclimation in not only the respiration but also growth of individual cord-forming, basidiomycete fungi. Also contrary to expectations for soil microbes (Allison *et al.* 2010), fungal growth efficiency responded in a unimodal pattern, with the greatest efficiencies at intermediate assay temperatures. Growth has been argued to be a better indicator of microbial activity than respiration (Balser & Wixon 2009; Barcenas-Moreno *et al.* 2009; Rinnan *et al.* 2009),

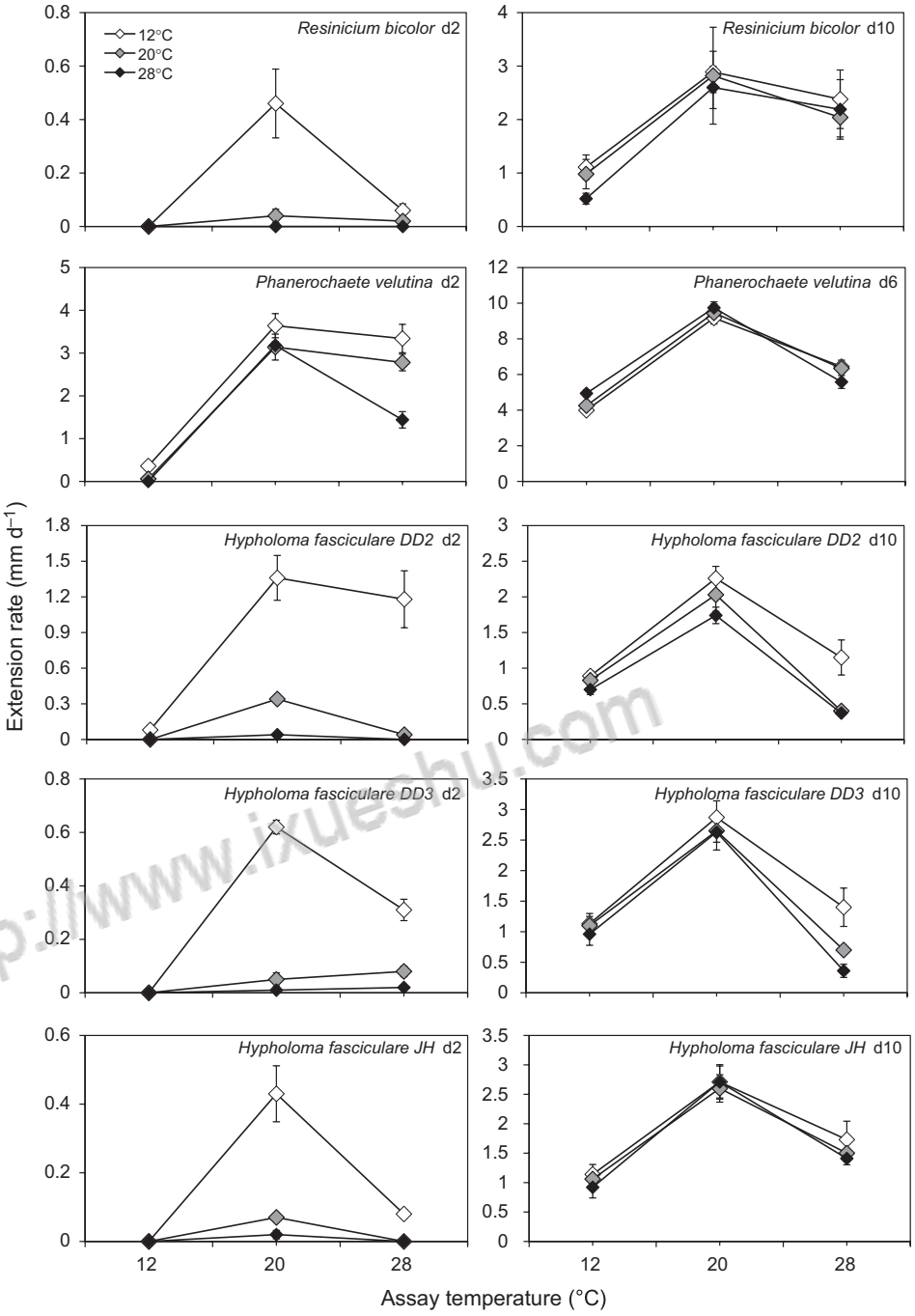

Figure 1 Mycelial extension rates of cord-forming basidiomycetes, *Resinicium bicolor, Phanerochaete velutina,* and three strains of *Hypholoma fasciculare* (*DD2, DD3* and *JH*) incubated at 12 °C (white), 20 °C (grey) and 28 °C (black). Values represent mean (± SE) extension rates (mm 2 days^{-1}) of fungi measured at three assay temperatures (*x*-axis: 12 °C, 20 °C and 28 °C). Measurements taken 2 days (first column) and 10 days (second column; only 6 days for *P. velutina* as this species had acclimated to assay temperatures by this time) from the start of the assays.

and certainly this is likely true for fungi because mycelial extension rates directly regulate their capacity to forage for and decompose organic matter (Boddy 2000). Conant *et al.* (2011) highlighted that accurately predicting ecosystem responses to warming requires a detailed understanding of the physiological responses of saprotrophic microbes to warming. By using individual cultured fungi we controlled for the community-scale changes in microbial composition that mask detection of physiological responses (Bradford *et al.* 2008) and provide, to our knowledge, the first empirical evidence of thermal acclimation in any group of soil microbial heterotrophs. Such physiological responses of heterotrophic microbes to temperature could ameliorate the predicted effects of warming on ecosystem carbon efflux directly, via reductions in heterotrophic respiration (Bradford *et al.* 2008; Reich 2010), and indirectly as lower growth efficiency of warm-adapted fungi limits their capacity to access and decompose organic matter (Allison *et al.* 2010).

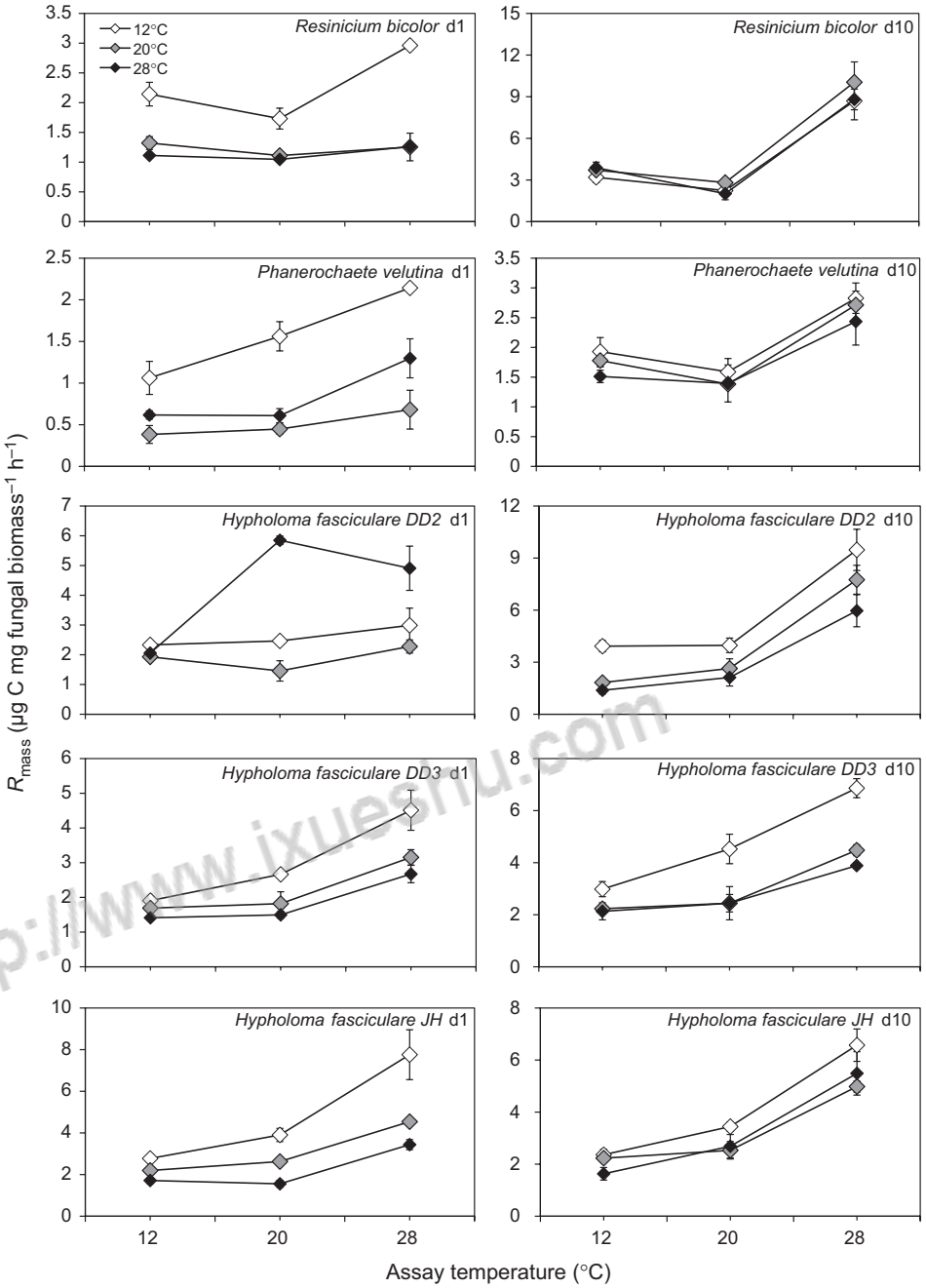

Figure 2 Mass-specific respiration rates (R_{mass}) of cord-forming basidiomycetes, *Resinicium bicolor, Phanerochaete velutina,* and three strains of *Hypholoma fasciculare* (*DD2, DD3* and *JH*) incubated at 12 °C (white), 20 °C (grey) and 28 °C (black). Values represent mean (± SE) R_{mass} rates of fungi measured at three assay temperatures (*x*-axis: 12 °C, 20 °C and 28 °C). Measurements taken 1 day (first column) and 10 days (second column) following the start of the assays.

The higher growth (all fungi) and respiration (all except *H. fasciculare DD2*) rates of cold-acclimated fungi at intermediate and high assay temperatures were expected, given evolutionary trade-offs in enzyme and cell membrane structure associated with biochemical adaptation to temperature (Hochachka & Somero 2002). However, thermal acclimation in the activities of individual, free-living soil saprotrophs had not previously been demonstrated, and remained a matter of extensive debate (e.g. Bradford *et al.* 2008; Hartley *et al.* 2008; Reich 2010). The magnitude and consistency of acclimation in our fungi was therefore striking, and contrasted with work on individual mycorrhizal fungi. Malcolm *et al.* (2008) suggested that ectomycorrhizal basidiomycetes vary substantially in their capacity to acclimate; although some species adjusted to incubation temperatures, the majority of species showed no acclimation response. Malcolm *et al.* (2008) suggested that their 7-day incubations could have been insufficient to allow respiratory acclimation, but our analyses suggest that 7-day incubations may have permitted the fungi to acclimate to new assay temperatures before respiration measurements were taken. In our study, measures of growth over time allowed us to estimate the rates at which fungi acclimated to assay

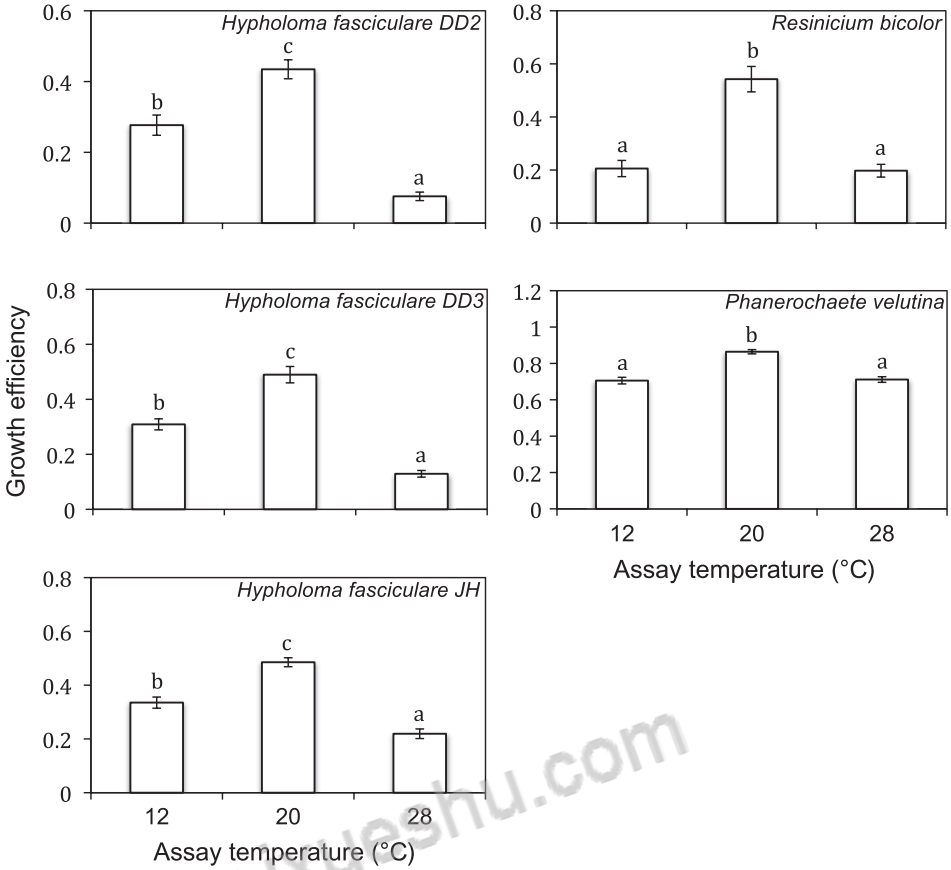

Figure 3 Growth efficiencies ($\Delta growth/(\Delta growth + R_{mass})$) of cord-forming basidiomycetes, *Resinicium bicolor, Phanerochaete velutina,* and three strains of *Hypholoma fasciculare* (*DD2, DD3* and *JH*) after 10 days incubation at 12 °C, 20 °C and 28 °C. Growth efficiency is given as a proportion of 1, representing the proportion of carbon allocated to growth as opposed to respiration. Values represent mean (± SE) growth efficiencies and letters indicate significant ($P < 0.05$) differences between temperatures.

temperatures. These measures show that, as with plants and animals (Atkin & Tjoelker 2003; Tjoelker *et al.* 2008), fungal adjustment rates manifest over a couple of days to weeks. Growth of *P. velutina* had, for example, completely acclimated after 6 days, and so it is likely that no differences between treatments would have been detected over a 7-day assay. We propose that most microbial species have the capacity to thermally acclimate, but that they are species-specific (and potentially environment-specific) in the rates at which they adjust to temperature.

Fungi varied in not only timing but also the extent to which they acclimated. After 2 days and at intermediate temperature assays, growth rates of warm-acclimated *R. bicolor* isolates were 96% slower than cold-acclimated isolates. In contrast, under the same conditions, growth rates of warm-acclimated *P. velutina* were only 12% less than those cold-acclimated. This variation in extent of acclimation was also apparent for respiration suggesting that, at a community-scale, the short- (diurnal and seasonal) and long-term (climate warming) responses to temperature will depend on fungal species composition. Species/communities that acclimate quickly and efficiently to warm temperatures will, for example, require and therefore mineralise less organic carbon than those less responsive (Malcolm *et al.* 2008) and so the contribution of heterotrophic respiration to the total ecosystem temperature response is likely to vary between communities (Heinemeyer *et al.* 2012). Such variation is also likely to exert selective pressures on fungal communities (Hall *et al.* 2010). For example, the slow and inefficient responses of *H. fasciculare DD2* and *DD3* (neither fully acclimated to warm assay temperatures and had lowest carbon-use efficiencies at 28 °C) may explain their poor competitive abilities observed during interspecific interactions at high temperatures (Crowther *et al.* 2012b). Given that it is the outcomes of these competitive fungal interactions that determine microbial community composition and functioning in woodland ecosystems (Crowther *et al.* 2011b), species-specific acclimation responses to temperature may partially regulate nutrient cycling rates.

We did not estimate growth efficiencies initially as respiration was measured using 1-day assays and slow extension rates meant that growth was only reliably measured after 2 days. At day-10, growth and respiration were measured on the same time frame providing no evidence that growth efficiency was influenced by the incubation temperature treatment. Efficiency was, however, strongly dependent on assay temperature, being higher at the intermediate temperatures, and lowest at high temperatures. This unimodal response contrasts with assumptions that soil microbial communities become increasingly inefficient with rising temperature, a supposition that directly determines the extent of soil carbon losses in current climate-ecosystem models (Allison *et al.* 2010). In addition, the activities of soil and aquatic microbial communities are thought to increase until ~30–40 °C (Balser & Wixon 2009; Barcenas-Moreno *et al.* 2009; Hall *et al.* 2010; Rinnan *et al.* 2009), but we observed higher mycelial

growth rates and efficiencies at 20 °C than at 28 °C. The linear response of microbial communities may be the sum of many individual unimodal responses, emphasising that community growth rates are not necessarily suitable proxies for growth responses of individual microbial taxa. Our data do at least support theoretical considerations that warming can reduce microbial carbon-use efficiency (Manzoni *et al.* 2012). Although this is generally assumed to lead to increased ecosystem carbon efflux through respiration (Manzoni *et al.* 2012), low growth efficiencies at high temperatures could feedback to slow ecosystem carbon efflux by reducing the capacity of microbes to forage for, and decompose organic resources (Allison *et al.* 2010). Reduced growth efficiency may help explain the increased fungal mortality observed in experimentally warmed soils (Heinemeyer *et al.* 2006), and help explain reductions in soil carbon efflux recorded under long-term warming (Saleska *et al.* 1999; Melillo *et al.* 2002).

The question as to whether heterotrophic microbes respond physiologically to temperature challenges our understanding of the linkages between community and ecosystem ecology. Our work suggests that thermal acclimation has the potential to reduce the growth and R_{mass} of heterotrophic soil fungi following warming (Bradford *et al.* 2010), and that reduced growth efficiencies at warmer temperatures may retard organic matter decomposition. As primary decomposing agents in woodland ecosystems, contributing up to 90% of total heterotrophic respiration (Boddy 2000), the responses of saprotrophic basidiomycete fungi to warming are likely to represent a significant proportion of the total heterotrophic microbial response. However, we stress that the responses of individual culture organisms cannot fully reflect the entire community response. Microbial communities *in situ* are highly complex, and subject to variable microenvironments, substrate availabilities and biotic interactions that are likely to influence the growth and respiration dynamics we report. Instead, we highlight mechanisms that are likely to occur in heterotrophic microbes across a range of ecosystems, the effects of which are likely to vary in extent and magnitude between communities. Indeed, broadly distributed generalists and/or species from variable temperature environments (i.e. the fungi used in the present study) are likely to be more capable of acclimating than specialists or species experiencing a limited thermal range (potentially the sub-arctic soil communities used in Hartley *et al.* 2008). As with soil animals (van Dooremalen *et al.* 2012), such differences may also exist between microbes from different soil depths, as surface or litter-dwelling species that experience a range of fluctuating temperatures may be more adept at acclimating than species from the less variable, deeper soil horizons. The rapid rates of acclimation we observed also contrasted with the time taken (up to 3 years) for ecosystem-level responses to manifest in field warming studies (Jarvis & Linder 2000; Oechel *et al.* 2000; Eliasson *et al.* 2005). This further emphasises the difficulties in extrapolating from individual- to community-scale responses and suggests that heterotrophic microbial acclimation may occur on a faster time-scale than the other components of the terrestrial ecosystem. Future research should address the potential for thermal acclimation in a broader selection of heterotrophic soil microbial taxa from different environments, and assess the consequences for ecosystem-scale respiration in the field. What remains clear from our data is that, despite current uncertainty (e.g. Reich 2010), at least some soil microbial heterotrophs have the capacity to acclimate to warmer temperatures.

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AUTHORSHIP

TWC and MAB conceived the study and wrote the manuscript. TWC conducted the study.

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