

## LETTER

## Fungal interactions reduce carbon use efficiency

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### Abstract

The efficiency by which fungi decompose organic matter contributes to the amount of carbon that is retained in biomass vs. lost to the atmosphere as respiration. This carbon use efficiency (CUE) is affected by various abiotic conditions, including temperature and nutrient availability. Theoretically, the physiological costs of interspecific interactions should likewise alter CUE, yet the magnitude of these costs is untested. Here we conduct a microcosm experiment to quantify how interactions among wood-decay basidiomycete fungi alter growth, respiration and CUE across a temperature and nitrogen gradient. We show that species interactions induced consistent declines in CUE, regardless of abiotic conditions. Multispecies communities exhibited reductions in CUE of up to 25% relative to individual CUE, with this biotic effect being greater than the observed variation attributable to abiotic conditions. Our results suggest that the extent to which fungal-mediated carbon fluxes respond to environmental change may be influenced strongly by species interactions.

### Keywords

Biogeochemical, biotic interactions, carbon cycle, diversity function, interference.

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### INTRODUCTION

Terrestrial microbes mediate the balance of carbon (C) between the biosphere and atmosphere (Schimel & Schaeffer 2012). The efficiency by which microbes use C for biomass vs. respiration – termed carbon use efficiency (CUE) – has emerged as a lynchpin variable in soil biogeochemical models, with different CUE assumptions yielding widely different soil C stocks under climate change (Allison *et al.* 2010; Frey *et al.* 2013; Wieder *et al.* 2013). Abiotic factors (primarily temperature and substrate quality) are known to exert strong controls on microbial physiology, such that the links between environmental conditions and CUE have received considerable attention in recent years (Sinsabaugh *et al.* 2013; Geyer *et al.* 2016). In contrast, despite an appreciation that biotic interactions play a fundamental role in governing microbial functioning (Wardle 2006; Crowther *et al.* 2015a,b), the extent to which biotic interactions affect microbial CUE is largely unexplored. The omission of biotic interactions from biogeochemical and Earth system models has been highlighted as a critical uncertainty for projecting the magnitude of C feedbacks between the biosphere and atmosphere (Milcu *et al.* 2012; Davidson *et al.* 2014; Crowther *et al.* 2016).

Basidiomycete fungi comprise a core component of the terrestrial C cycle, being the dominant decomposers of recalcitrant organic matter in forested ecosystems (Boddy 2000; Osono 2007; Crowther & Bradford 2013; Baldrian 2016). Biotic interactions among these fungi can alter community-level function indirectly, by influencing community composition (Toljander *et al.* 2006; Fukami *et al.* 2010), and directly, by altering individual behaviour and physiology (Crowther *et al.* 2011, 2015a). Several biological processes suggest that fungal

CUE should be particularly sensitive to direct interspecific interactions. Fungi alter their physiology, trait expression and metabolism in order to kill, inhibit growth and displace their competitors; a set of interactions collectively referred to as ‘interference competition’ (Boddy 2000; Hiscox *et al.* 2015). Individuals likewise alter their morphology, stoichiometry and biomass to ‘wall off’ territory and prevent against overgrowth (Boddy 2000; Hiscox *et al.* 2010). A key feature of these interactions – with implications for C cycling – is that they often force organisms to engage in activities that are otherwise counterproductive (i.e. inefficient) in the absence of competitors (Case & Gilpin 1974).

Our inability to discern the effects of direct interactions on fungal CUE stems partially from methodological limitations. When measured over long time scales (*c.* 6 months), fungal efficiency has been observed to decline with increasing richness (Toljander *et al.* 2006). Yet, at these timescales, simultaneous shifts in diversity and community turnover can lead to the appearance of altered growth efficiencies, when in fact efficiencies at the level of the individual remain constant (Hagerty *et al.* 2014). Nevertheless, rates of fungal respiration, decomposition and enzyme production have all been shown to differ in the presence of interspecific interactions (Baldrian 2004; Tiunov & Scheu 2005; Song *et al.* 2012; Hiscox *et al.* 2015), suggesting that fungal CUE should likewise differ. As with non-microbial systems, disentangling the mechanisms underpinning community-level functioning therefore requires precise knowledge of how individuals perform in mono- and polyculture (Loreau & Hector 2001; Tiunov & Scheu 2005; Cadotte 2013). Methodologically, this approach has been challenging in microbial systems due to the difficulty isolating, culturing or measuring individual microbes growing in isolation.

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We use a saprotrophic basidiomycete fungal system to explore how direct interactions alter fungal CUE, relative to the effects of abiotic variation. Using 10 unique fungal species grown under a range of temperature and nitrogen conditions, we quantify how fungal biomass and respiration vary across abiotic conditions, and among individuals vs. three-species communities. We focus on two questions: (1) how do interactions in fungal communities alter respiration, biomass and subsequent CUE, relative to individual performance? And (2) how strong is the influence of biotic interactions relative to abiotic conditions in governing fungal CUE? Since basidiomycete fungi are only one of the many microbial groups involved in C cycling, our aim is not to generate precise estimates of CUE that can be used to parameterise biogeochemical models. Rather, the goal is to address fundamental uncertainty about how biotic interactions affect fungal respiration, biomass and CUE, so as to generate basic knowledge about how microbial processes are represented in biogeochemical models (Bradford *et al.* 2014).

## METHODS

### Overview

We compared the CUE of individuals vs. communities grown under six treatment conditions, consisting of three temperatures (16, 22, 28 °C) and two resource conditions (low vs. high nitrogen availability), in a fully factorial design. Nitrogen availability was selected to span a broad gradient in resource stoichiometry, reflecting the C:N of dead wood ('Low-N', C:N = 145:1) vs. high-quality leaf-litter and microbial tissues ('High-N', C:N = 10:1). The temperature range was selected to ensure that all species exhibited sufficient growth rates, while also capturing a reasonable range of temperatures (low, intermediate and high) experienced in temperate forests over the course of a growing season (Crowther & Bradford 2013).

The study species were 10 basidiomycete wood-decay fungi (Table S1), obtained from the US Forest Service culture collection at the Center for Forest Mycology Research, Madison, WI, USA. All isolates were collected from fruiting bodies on dead wood, and were stored in liquid N<sub>2</sub> since time of collection without serial transfer. Fungi were selected from multiple environments rather than from a single environment to minimise local-adaptation or habitat filtering from constraining the range of competitive strategies exhibited by the fungi. However, these specific species were also selected on the basis of being endemic to mixed temperate forests in North America – with most having widespread global distributions (Table S1) – and with all species commonly occurring in early-to-mid stages of wood decay (i.e. ranging from freshly fallen dead wood up to when labile C compounds and cellulose are largely depleted and wood has started to lose its structural integrity; Laiho & Prescott 2004; Rajala *et al.* 2012). Thus, by *a priori* selecting species with known phylogenetic variation and taxonomic identities, our goal was to capture a wide range of natural variation while also ensuring that species occur in overlapping habitats and have functionally equivalent ecological roles.

Fungi were grown separately and in three-species mixtures at each of the six conditions for 10 days. Preliminary trials showed that by 20 days communities could be dominated by

a single fungus, thereby overwhelming initial induced changes in CUE; 10 days was thus selected to isolate induced changes in functioning from community turnover. These trials likewise showed that a period of 10 days ensured that C and N remained in excess of supply, thereby minimising the role of exploitative competition for limiting nutrients. Furthermore, using three species per community, each species directly neighbored both other species, ensuring all species directly interacted with one another (Fig. S1). Such direct pairwise interactions cannot be guaranteed with four species, for example because the species diagonal to one another may not meet.

Each of the 10 species was grown individually at each of the six treatment conditions, replicated three times each (10 species × 6 treatments each × 3 replicates = 180 total monoculture microcosms). For the communities, 60 unique communities were selected at random from among the 120 possible three-species communities, with 10 communities randomly assigned to each of the six treatment levels, replicated three times each (10 unique communities for each of 6 treatments = 60 unique communities × 3 replicates = 180 total community microcosms). Following classical diversity-function study designs (Reich *et al.* 2001; Tilman *et al.* 2006), we chose to use a random set of communities for each treatment level rather than assay the same set of communities across all treatments. This approach was selected due to high variability in the monoculture outcomes, which suggested that between-community variation was likely to be substantial relative to between-treatment variation. Thus, if we had used only 10 unique communities assayed at all treatment conditions, the results would be highly contingent on the random subset of communities we selected, potentially introducing systemic bias. By instead selecting 10 random communities at each of the six treatment levels (thereby capturing 60 of 120 possible communities) our results better reflect the average response across all communities, with the potential trade-off of reduced statistical power because the communities are not 'matched' across treatment levels.

### Microcosm design

Microcosms consisted of 10-cm dia. deep-well Petri dishes containing 25 mL of 2% (w/v) malt extract agar (C:N of 145:1), with the High-N treatment amended with 5 g peptone (C:N of 1:25). This design resulted in 248 mg C and 1.7 mg N in each Low-N microcosm (C:N = 145:1), and 250 mg C and 23.7 mg N in each High-N microcosm (C:N = 10:1). Liquid media was pH-adjusted after autoclaving to obtain a uniform pH of 5.7 across all treatments. Following traditional diversity-function study designs (Reich *et al.* 2001; Tilman *et al.* 2006; Godoy *et al.* 2014), the monoculture and mixed-species microcosms were 'seeded' with the same initial number of colonies (i.e. 'plugs') to ensure that any observed differences were not due to initial differences in colony counts. Intraspecific competitive costs were controlled for because the monocultures were inoculated with genetically identical starter colonies taken from a single colony.

Three pre-colonised agar plugs (5-mm dia.), comprised of either the same individual (monocultures) or three different species (communities), were placed onto the agar surface in a triangular pattern, each 2 cm from the centre of the dish

(Fig. S1). The dishes were then placed in a sterile, 500-mL polypropylene container with screw-top lid. Lids were modified with a 5-mm hole covered with a 3.2-mm-thick butyl-rubber adhesive to allow for gas sampling. Each inoculated plate was placed in a separate container, and the lid was closed and sealed with silicone caulk. The containers were subsequently flushed with CO<sub>2</sub>-free air to remove CO<sub>2</sub> from the headspace.

### Respiration carbon

Gas sampling was conducted on days 5 and 10. Headspace CO<sub>2</sub> concentrations were measured using infrared gas analysis (model LI-7000; Li-COR, Lincoln, NE, USA) following established methods (Keiser *et al.* 2011). After taking gas samples on day 5, the chambers were again flushed with CO<sub>2</sub>-free air to ensure CO<sub>2</sub> concentrations in the headspace remained below those inhibitory to fungal growth. Total respiration C (henceforth 'respiration') for each isolate over the 10-day experiment was calculated by multiplying the two headspace C concentrations (days 5 and 10) by the total chamber volume (500 mL), and adding these two values.

### Biomass carbon and nitrogen

Biomass production was estimated following Crowther & Bradford (2013). After 10 days, the fungal biomass was recovered by heating the agar at 121 °C for 5 min. The melted solution was filtered through a 53-µm sieve and rinsed with 1 L of 90 °C deionised-water, agitating slightly to remove residual agar, and subsequently rinsed for 2 min with 20 °C deionised-water. The resulting biomass was dried at 65 °C to constant mass and weighed. Note that the combined biomass of the multispecies mixtures was measured at the community level, such that the individual biomass of each species in each community was not quantified. The three replicate samples for each treatment were combined to obtain sufficient biomass for elemental analysis, milled with a mortar and pestle into a fine powder, and analysed for C and N content (ESC4010; Costech Analytical Technologies Inc., Valencia, CA, USA). Total biomass C (henceforth 'biomass') and biomass N were estimated by multiplying the %C and percent %N values by total dry-weight biomass in each sample.

### Estimating CUE

The definition of CUE can vary widely, depending on the timescales of interest and the relevant C pools measured (Geyer *et al.* 2016). Here, we calculated CUE as the net amount of C in biomass (B), relative to the total amount of C that was either mineralised (i.e. respired; R) or incorporated into biomass. That is:

$$\text{CUE} = \frac{B}{B + R}$$

This value ranges between one (completely efficient) and zero (completely inefficient). The formulation does not capture C invested in extracellular enzymes, which is challenging to measure due to a lack of methods for disentangling enzymatic C from unassimilated substrate C or biomass C (Geyer

*et al.* 2016). Although quantifying the potential activity of extracellular enzymes is straightforward (Baldrian 2009; Crowther *et al.* 2011), it is not obvious how to translate this measure into an estimate of the total mass of C in the enzyme pool. Hence, microbial efficiency is commonly defined by the ratio of growth to respiration (Del Giorgio & Cole 1998; Frey *et al.* 2013). The benefit to our approach is that it does not rely on derived variables (e.g. via modelling metabolic pathways) or upon tracking a single type of C molecule through the system (e.g. glucose), which can complicate inference since different C compounds are associated with different growth efficiencies (Frey *et al.* 2013). This CUE calculation thus provides a simple metric to explore how fungal C allocation differs in the presence and absence of interspecific interactions.

### Quantifying the cost of interspecific interactions

The change in respiration, biomass and CUE attributable to interspecific interactions was quantified by comparing observed to predicted values, with predicted values calculated under the null hypothesis that species' biomass and respiration rates do not vary between mixtures and monocultures. Specifically, the predicted respiration and biomass values for each community were calculated by taking the simple average of the three monoculture values for each of the three species in that community (see Supporting information Methods for an example), thereby reflecting the null hypothesis that community performance is additive with respect to monoculture performance, and that species' biomass and respiration rates are proportional to their initial relative abundances. An alternate approach was considered where this average was weighted by the proportion of the area occupied by each individual when growing in monoculture, thereby 'overweighting' faster growing fungi (Fig. S3). However, this approach did not differ appreciably, and so the equal-weighting (simple average) approach is used throughout so that any deviations from additivity can be directly attributed to species interactions (Kirwan *et al.* 2009; Connolly *et al.* 2013).

Predicted CUE for each community was subsequently calculated using the predicted respiration and biomass values for that community:

$$\text{predicted CUE}_k = \frac{\text{predicted Biomass}_k}{\text{predicted Respiration}_k + \text{predicted Biomass}_k}$$

The proportional changes in biomass, respiration and CUE attributable to interspecific interactions were calculated by comparing the predicted to observed values, e.g. proportional change in CUE = (observed CUE – predicted CUE) × (predicted CUE)<sup>-1</sup> (see Supporting information Methods for detailed calculations). These proportional change calculations (Fig. 2g–i) were made on a community-by-community basis to account for baseline differences in performance across communities and treatments.

### Statistical analysis

Mixed-effect models were used to quantify how temperature, N and interspecific interactions affect community

performance. Separate models were run for individual vs. community results, and all analyses were conducted using the statistical software *R*, version 3.2.3 (R Core Team 2017). For each model and each outcome, the fixed effects included temperature, N and the temperature-by-N interaction. For the individual-level analyses, the outcome variable was respiration, biomass or CUE. For the community-level analyses, the outcome variable was proportional change [(observed – predicted)/predicted] in biomass, respiration or CUE. A random effect for ‘replicate’ was included to account for correlation across the three replicate samples of each individual/community combination. For each outcome variable, two models were fit: one accounting for species-specific effects by including a species indicator variable (presence/absence) as a random effect; and a second set of models with no species indicator (Tables S2–S11). The most parsimonious of these two models for each outcome was selected as the one with the smallest Bayesian-information-criterion score. The 95% confidence bands for the fixed effects (Fig. 2) were calculated by taking the marginal responses across the random effects.

To directly compare average effect sizes for CUE between the individual and community analyses (Fig. 3a), a second mixed model was fit using the combined individual and community data, which made no assumptions about additivity. The outcome variable in this model was the ‘observed CUE’ in the monoculture and mixed-species microcosms, with the independent variables being temperature, N, community richness (1 or 3 species) and the pairwise interactions among these three variables. As with the first model, species-specific effects were accounted for by including species indicator variables as random effects. A temperature step of 6 °C was chosen to compare the effects of temperature to those of N and interspecific interactions (Fig. 3b); this step size prevented the need to extrapolate the results to temperatures not directly used in the experiment (i.e. temperatures other than 16, 22 and 28 °C)

and, more importantly, because this 6 °C step is ecologically relevant, falling within the range of regional temperature increases expected over the next century (IPCC 2013). Post hoc contrasts were used to compare the relative importance of species interactions, temperature and N. All *P*-values were adjusted for multiple comparisons following Benjamini & Hochberg (1995).

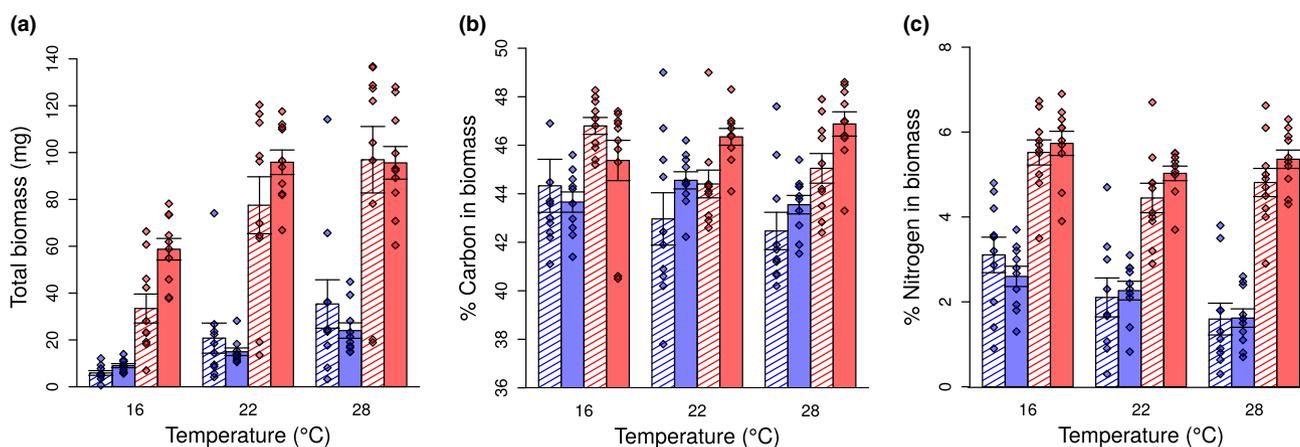
## RESULTS

### Carbon and nitrogen in biomass

Total biomass varied substantially across treatments, with a mean of  $47 \pm 40.8$  mg across all microcosms (Fig. 1a). Biomass was significantly higher in High-N than Low-N conditions (76.3 vs. 18.3 mg,  $P < 0.001$ ), and biomass showed a significant positive temperature response (3.0 mg increase per unit increase in temperature;  $P < 0.001$ ). At High-N, total biomass was significantly higher in communities than in monoculture (18.7 mg difference,  $P = 0.04$ ), but was not significantly different at Low-N ( $P = 0.10$ ).

The mean %C in biomass was  $44.7 \pm 0.02\%$  across all microcosms, and varied less than 5%, in relative terms, across all treatments (Fig. 1b). %C was significantly higher under High-N conditions (2.2% increase across treatments,  $P < 0.001$ ) and marginally higher in communities than monoculture (0.7% increase,  $P = 0.07$ ), but did not significantly differ across temperatures. An average of 18% of the substrate C was respired or incorporated into biomass over the course of the experiment, with a maximum of 66% and a minimum of 1% across all microcosms.

The mean %N in biomass was  $3.7 \pm 1.8\%$ , with this value being significantly higher under High-N conditions than Low-N conditions (5.2% vs. 2.2%,  $P < 0.001$  for the difference; Fig. 1c). %N showed a significant negative temperature



**Figure 1** Total fungal biomass, %C in biomass and %N in biomass across all treatments. (a) Total biomass varied substantially across treatments, with a > 150-fold difference in biomass between the highest and lowest values. Biomass was significantly higher in High-N than Low-N conditions, and increased with increasing temperature. At High-N, total biomass was significantly higher in communities than in monoculture, but showed no overall difference between monoculture and mixtures at Low-N. (b) Mean %C was significantly higher under High-N conditions and marginally higher in communities than monoculture, but was not significantly affected by temperature. Overall, %C was highly consistent across all samples (note the truncated y-axis range) – particularly in relation to the enormous variation in biomass – such that variation in %C explained less than 1% of the variation in CUE (see Fig. S5). (c) The mean %N in biomass was predominantly determined by the C:N ratio of the substrate. %N also showed a slight negative temperature response, but did not differ significantly between monocultures and mixtures.

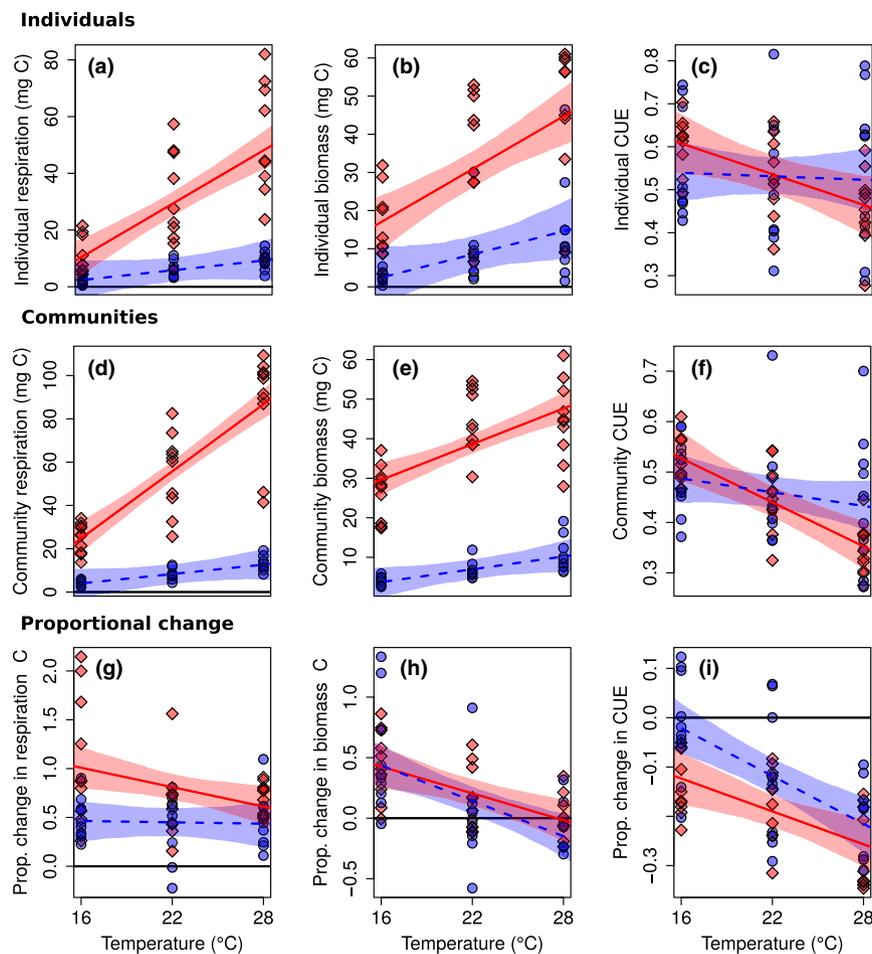
response of  $-0.12\%$  per degree increase in temperature ( $P < 0.001$ ), and did not otherwise differ significantly between monocultures and mixtures. An average of 16.0 and 16.1% of the substrate N was incorporated into biomass in the Low-N and High-N microcosms, respectively, with a maximum of 34% and a minimum of 2% across all microcosms.

### Abiotic responses among individuals

In the absence of biotic interactions, total respiration showed a strong positive temperature response under High-N (increase of  $3.1 \text{ mg}\cdot\text{C}^{-1}$ ;  $P < 0.001$ ;  $n = 164$  after removing samples with missing respiration C and/or biomass C) and a slight positive response under Low-N (increase of  $0.6 \text{ mg}\cdot\text{C}^{-1}$ ,  $P = 0.24$ ;

Fig. 2a, Table S2). Mean respiration was not statistically different at  $16^\circ\text{C}$  between N treatments ( $P = 0.12$ ), but was significantly higher at  $28^\circ\text{C}$  under High-N ( $48.0$  vs.  $9.0 \text{ mg}$  respectively;  $P < 0.001$ ). Patterns in biomass production rates largely mirrored those of respiration (Fig. 2b; Table S3). Biomass C increased  $2.3 \text{ mg}\cdot\text{C}^{-1}$  under High-N ( $P < 0.001$ ) and  $1.0 \text{ mg}\cdot\text{C}^{-1}$  under Low-N ( $P = 0.06$ ). At  $16^\circ\text{C}$ , biomass was  $14.6 \text{ mg}$  higher under High-N than Low-N ( $P = 0.01$ ) and  $30.2 \text{ mg}$  higher at  $28^\circ\text{C}$  ( $P < 0.001$ ).

Under Low-N, CUE remained essentially constant at  $c. 0.53$ , regardless of temperature ( $\beta = 0.001$ ;  $P = 0.81$ ; Fig. 2c, Table S4), driven by the fact that biomass and respiration rates increased at approximately equal rates. Conversely, under High-N, respiration increased proportionately faster



**Figure 2** The effects of temperature, nitrogen and interspecific interactions on fungal CUE. Blue lines and points show Low-N conditions; red lines and points show High-N conditions. (a–c) The effects of abiotic conditions on individual CUE in the absence of interspecific interactions. Respiration (a) and biomass (b) increased across temperatures and under High-N relative to Low-N conditions. (c) Carbon use efficiency was essentially unchanged across temperatures under Low-N, yet showed a negative temperature response under High-N. (d–f) Community-level patterns largely mirrored the monoculture results, though communities exhibited (d) higher overall respiration, (e) increased biomass production and (f) a steeper negative temperature response at High-N. (g–i) The proportional change in respiration, biomass and CUE due to interspecific interactions. The solid black zero line represents no change from predicted performance (adjusted for baseline differences in community-specific values), whereas a value of 1.0 for example would denote a 100% increase from expected performance. (g) At Low-N, respiration was 50% higher than predicted across the temperature range, whereas at High-N, communities showed a strong negative temperature response, ranging from a  $c. 60\%$  increase at  $16^\circ\text{C}$  down to a  $c. 60\%$  increase at  $28^\circ\text{C}$  above predicted values. (h) Interspecific interactions led to significant increases in biomass-carbon at low temperature, but showed negligible change at  $28^\circ\text{C}$ . (i) CUE was significantly reduced in communities under all conditions except for  $16^\circ\text{C}$ , Low-N, with as much as a 25% reduction at  $28^\circ\text{C}$  compared to predicted values. Note that  $n = 164$  for the individual analysis and  $n = 161$  for the community analysis after removing samples with missing respiration and/or biomass C.

than biomass, leading to a decrease in CUE of 1% (in absolute terms) per degree increase in temperature ( $\beta = 0.01$ ;  $P < 0.02$ ). Indeed, at High-N, CUE exhibited a 25% decrease across the temperature range, dropping from 0.61 at 16 to 0.46 at 28 °C.

### Biotic responses across communities

The overall effects of N and temperature on community-level respiration, biomass and CUE were qualitatively similar to those among individuals (Fig. 2d–f; Tables S5–S7). However, when directly compared to the individual values, key differences emerged (Fig. 2g–i). Respiration was significantly increased in communities, relative to community-specific predicted values (Fig. 2g, Table S8;  $n = 161$  after removing missing values). For Low-N, the change in respiration ( $\Delta$ -respiration) was 46 and 44% elevated at 16 and 28 °C, respectively ( $P < 0.001$  for both), with this difference constant across temperatures ( $\beta = -0.002$ ;  $P = 0.87$ ). For High-N,  $\Delta$ -respiration showed a significant negative temperature response ( $P = 0.04$ ), ranging from a 101% increase in respiration at 16 °C down to a 61% increase at 28 °C.

In contrast to expectations, biomass production was significantly higher in communities than predicted based on individual performance (Fig. 2h, Table S9).  $\Delta$ -biomass was significantly elevated at 16 °C, exhibiting a 43% increase ( $P < 0.001$ ) regardless of N conditions. This increase in biomass production attenuated under increasing temperatures, leading to a non-significant decrease at 28 °C of 1% for High-N ( $P = 0.87$ ) and 15% for Low-N ( $P = 0.14$ ).

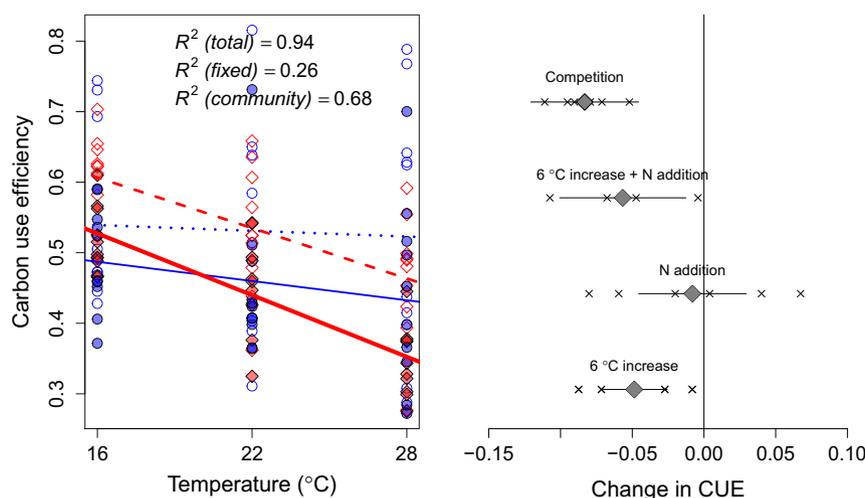
Despite the increase in biomass in communities, the proportionately larger  $\Delta$ -respiration values led to significant reductions in CUE under most treatment conditions (Fig. 2i, Table S10). The exception was Low-N at 16 °C, where CUE

was essentially unchanged relative to monoculture performance (2.1% decrease;  $P = 0.48$ ). Conversely, even though  $\Delta$ -biomass was significantly elevated at High-N at 16 °C, the resulting CUE was *c.* 12% lower ( $P < 0.001$ ), driven by the fact that  $\Delta$ -respiration was approximately twice as large as  $\Delta$ -biomass. The average  $\Delta$ CUE under both Low-N and High-N communities showed marked negative temperature responses ( $\beta = -0.016$  and  $\beta = -0.011$  for Low- and High-N respectively), such that at 28 °C these values converged (21.5 vs. 26.6% decrease under Low- and High-N respectively;  $P = 0.35$  for difference).

When combining the individual and community results (Fig. 3a, Table S11), temperature, N and interspecific interactions explained only 26% of the total variability in CUE due to high overall variation among both individuals and communities. Interactions and temperature led to consistent declines in CUE regardless of N availability, whereas N addition yielded non-significant increases at 16 °C (0.05 increase;  $P = 0.10$ ) and marginally significant declines at 28 °C (0.07 decline;  $P = 0.04$ ). In absolute terms, species interactions decreased CUE by, on average,  $0.09 \pm 0.019$  across the whole range of abiotic conditions (Fig. 3b). By comparison, N addition or a 6 °C increase in temperature led to decreases in CUE of  $0.008 \pm 0.019$  and  $0.05 \pm 0.012$ , respectively, with their combined effects yielding a  $0.06 \pm 0.023$  decrease.

### DISCUSSION

Our study reveals that direct interactions among fungi significantly alter community-level CUE. By comparing individual-level CUE to community-level CUE, we show that interspecific interactions led to consistent declines in CUE across all abiotic conditions tested, with these costs being equal to or greater than the change in CUE attributable to shifts in



**Figure 3** The relative importance of biotic and abiotic factors as drivers of fungal CUE. Broken lines and open points represent individual CUE, and solid lines and filled points represent community CUE. Blue circles and blue lines show Low-N conditions; red diamonds and red line show High-N conditions. (a) Despite consistent trends, competition, nitrogen and temperature explained only 26% of the variation in CUE, with community-specific random effects explaining an additional 68% (total  $R^2=0.95$ ). Competition (broken vs. solid lines) and temperature led to consistent declines in CUE, whereas the nitrogen effect was positive at 16 °C and negative at 28 °C among both individuals (dashed lines) and communities (solid lines). (b) When holding all other variables constant, a 6 °C increase in temperature (e.g. 16–22 °C or 22–28 °C) led to consistent declines in CUE, with an average decrease of 0.05 across all conditions. Nitrogen addition, on average, led to a decline of 0.008 across all treatments, driven by offsetting positive and negative responses. Competition consistently reduced CUE in all scenarios [solid vs. broken lines in (a)], with an average reduction of 0.08 across conditions.

abiotic conditions (Fig. 3b). At the community level, the largest biotic-induced declines, whether measured in absolute (Fig. 3a) or relative terms (Fig. 2i), were observed at 28 °C under high-N conditions, suggesting the costs of these biotic interactions are highest under optimal conditions due to respiration outpacing biomass production.

Our experiment was designed to test if the presence vs. absence of interspecific interactions alter fungal CUE, and, as such, it remains unclear if these biotic costs will continue to accumulate under increasing levels of diversity. Fungal decomposition rates, respiration rates and growth rates have all been shown to respond to continual increases in diversity, with this effect often mediated by environmental conditions (Tiunov & Scheu 2005; Hiscox *et al.* 2015; Matulich & Martiny 2015). Yet, as our research shows, respiration or biomass alone are not sufficient for inferring differences in C-allocation strategies, since respiration and CUE (and thus presumably decomposition rates) need not be correlated. Furthermore, the effects of increasing diversity on fungal activity are potentially mediated through community turnover, community assembly history or environmental fluctuations (Toljander *et al.* 2006; Fukami *et al.* 2010; Hagerty *et al.* 2014), such that the links between fungal efficiency and diversity (e.g. Toljander *et al.* 2006) cannot be directly attributed to greater investment in combative activities. Thus, an outstanding question is whether individual-level CUE will continue to decline with increasing levels of diversity, or if these induced biotic costs are essentially fixed, regardless of the number of unique competitors in the community.

Despite consistent trends across treatments, there was large variation in CUE, with the community-specific random effects explaining 68% of the overall variation (Fig. 3a). Some of this variation may be due to the fact that we only assayed two C pools (biomass and respiration), and we did not measure, for example, differences in extracellular enzyme C allocation, which remains challenging due to methodological limitations (Geyer *et al.* 2016). Nonetheless, our results show that – regardless of allocation to these other C pools – the efficiency by which fungi retain C in biomass vs. respire C into the atmosphere differs in the presence of interspecific interactions, with large variation across all treatment levels. Alternate explanations for this variability may be differing levels of biodiversity (e.g. phylogenetic or functional) or trait expression within communities, or potentially different competitive dynamics within each community. In communities typified by allelopathy and interference competition for space, the structure of the competitive network (e.g. whether it is intransitive vs. hierarchical) has been shown to be a critical component of species behaviour and survival (Jackson & Buss 1975; Maynard *et al.* 2017). Whether or not network structure likewise serves as a regulator of ecosystem function is unknown, but seems plausible given its role in shaping the biotic milieu in which organisms exist. We suggest that an understanding of how biodiversity (species, functional and phylogenetic) and competitive network structure interactively alter fungal activity is an important next step for identifying the mechanistic drivers behind fungal-dominated ecosystem process rates.

Although CUE was reduced in multispecies communities, total biomass C in communities was slightly greater than

predicted based on individual performance (Fig. 2h). Across all communities, there was no correlation between overgrowth ability and monoculture biomass production, nor between hyphal extension rate and biomass production (Fig. S6), such that a ‘selection effect’ (where species with higher-than-average biomass production rates dominate the community) is unlikely to explain this observed increase in biomass. Rather, these results support previous inference that fungi increase their biomass %C allocation and/or their total biomass production in the presence of external stressors. Indeed, when exposed to drought and grazing stress, basidiomycetes produce calcium oxalate crystals on their hyphae, leading to higher %C content (Crowther *et al.* 2015a); and they often alter their morphology during interspecific competition to produce thick, dense bands of mycelia to prevent overgrowth and to ‘wall-off’ territory from competitors (Boddy 2000; see Fig. S1). Here, total C in biomass was much more strongly correlated with overall biomass production ( $r = 0.99$ ,  $P < 0.001$ ) than with %C ( $r = 0.21$ ,  $P = 0.05$ ), such that variation in %C explained < 1% of the total variation in total biomass C. Thus, these induced changes in biomass C (and ultimately CUE; Figs 2i, S5) appear to be largely the result of compensatory changes in biomass production rather than induced changes in biomass stoichiometry.

Microbial CUE is highly sensitive to a myriad of environmental factors beyond temperature and N (Sinsabaugh *et al.* 2013), such that the patterns observed here are likely to vary under different abiotic gradients and across different microbial guilds. For example the C substrate we used was near-optimal and non-limiting, whereas more recalcitrant C compounds, such as lignin, would be expected to reduce CUE (Moorhead *et al.* 2013). Indeed, in fungal systems where survival is dictated by stress tolerance or by exploitative competition for limiting resources (e.g. among ruderal or stress-tolerant wood-decay fungi; Boddy 2001), the induced costs of interspecific interactions are likely to be less important than traditional ecological processes such as community turnover, differential resource use or facilitation (Allison 2012; Buchkowski *et al.* 2015; Maynard *et al.* 2015). The study system used here (wood-decay basidiomycete fungi) was selected because these organisms are generally known to be antagonistic (Boddy 2000), and so our results may not extend to microbial systems where resource competition – rather than interference competition – is the dominant structuring force. Conversely, it likewise cannot be discounted that some degree of indirect competition for limiting nutrients, or that non-competitive effects (e.g. facilitative interactions; Tiunov & Scheu 2005) took place in the microcosms, thereby partially explaining our results. However, the microcosms were constructed using a labile C substrate, with an average of 18% of the substrate C and 16% of the substrate N used over the 10 days, suggesting that the observed declines in CUE were due to direct antagonistic interactions rather than resource-mediated exploitative competition. Nevertheless, an important next step is to explore how these biotic costs differ across abiotic contexts, and among microbial communities that engage in differing levels of interference vs. exploitative competition.

Our results support previous inference that direct interspecific interactions among wood-decay basidiomycete fungi can force individuals to alter their activity in ways that are

otherwise inefficient in the absence of competitors (Toljander *et al.* 2006; Hiscox *et al.* 2015). Direct competition of this sort is not only found in wood-decay fungi, but it is also ubiquitous in leaf-decay fungi, soil fungi and mycorrhizal fungi (Boer *et al.* 2005; Schneider *et al.* 2010; Bödeker *et al.* 2016), suggesting that these results may be particularly relevant to fungal-dominated terrestrial communities. Yet basidiomycete fungi engage in specific activities that are not otherwise observed in many microbial systems (e.g. the phenomenon of 'walling off' territory along the interaction zone), such that the relevance of our findings to other fungal guilds and microbial systems remains an outstanding question. Thus, our findings reveal a potential mechanism by which direct interactions can exert strong controls on the CUE of fungal-dominated communities. The next steps will be to explore the importance of this mechanism across diverse microbial systems to gain a unified understanding of how biotic and abiotic factors interactively determine C storage in terrestrial ecosystems.

## CONCLUSION

Disentangling the biotic and abiotic drivers of ecosystem function is particularly challenging in fungal communities due to the hyper-complex and opaque nature of the system. Here, by comparing individual performance to community performance, we isolated the relative contribution of fungal interactions as drivers of community-level fungal CUE. These interspecific interactions led to strong, consistent declines in CUE, with the magnitude of this decline approximately equal to the combined effects of temperature and substrate quality. Our data suggest that the extent to which fungal-mediated C fluxes respond to environmental change may be strongly influenced by biotic interactions within these communities.

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## AUTHOR CONTRIBUTIONS

DSM and MAB conceived of the study. DSM conducted the experiments, analysed the data and wrote the initial manuscript. TWC and MAB assisted in designing the experiments, analysing the data and preparing the manuscript.

## DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.p20n8>.

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