Fungal interactions reduce carbon use efficiency

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Should the manuscript be accepted, the data supporting the results will be archived in Dryad and the data DOI will be included at the end of the article.

Abstract

The efficiency by which fungi decompose organic matter contributes to the amount of carbon that is retained in biomass versus 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 are 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.

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 versus 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 carbon 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 behavior 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 in order 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 (~6 mo), fungal efficiency has been observed to decline with increasing richness (Toljander *et al.* 2006). Yet, at the 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.

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 across 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 parameterize 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 versus 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) versus 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_2 since time of collection without serial transfer. Fungi were selected from multiple environments rather than from a single environment in order to minimize 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 until 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

127 capture a wide range of natural variation while also ensuring that species occur in overlapping habitats
128 and have functionally equivalent ecological roles.

129 Fungi were grown separately and in three-species mixtures at each of the six conditions for 10
130 days. Preliminary trials showed that by 20 days communities could be dominated by a single fungus,
131 thereby overwhelming initial induced changes in CUE; 10 d was thus selected in order to isolate induced
132 changes in functioning from community turnover. These trials likewise showed that a period of 10 days
133 ensured that C and N remained in excess of supply, thereby minimizing the role of exploitative
134 competition for limiting nutrients. Furthermore, by using three species per community, each species
135 directly neighbored both other species, ensuring all species directly interacted with one another (Fig. S1).
136 Such direct pairwise interactions cannot be guaranteed with four species, for example, because the species
137 diagonal to one another may not meet.

138 Each of the ten species was grown individually at each of the six treatment conditions, replicated
139 three times each (10 species x 6 treatments each x 3 replicates = 180 total monoculture microcosms). For
140 the communities, 60 unique communities were selected at random from among the 120 possible three-
141 species communities, with 10 communities randomly assigned to each of the six treatment levels,
142 replicated three times each (10 unique communities for each of 6 treatments = 60 unique communities x
143 3 replicates = 180 total community microcosms). Following classical diversity-function study designs
144 (Reich *et al.* 2001; Tilman *et al.* 2006), we chose to use a random set of communities for each treatment
145 level rather than assay the same set of communities across all treatments. This approach was selected due
146 to high variability in the monoculture outcomes, which suggested that between-community variation was
147 likely to be substantial relative to between-treatment variation. Thus, with only 10 unique communities
148 assayed at all treatment conditions, the results would be highly contingent on the random subset of
149 communities we selected, potentially introducing systemic bias. By instead selecting 10 random
150 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 tradeoff 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-colonized 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 center 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_2-free air to remove CO_2 from the headspace.

Respiration carbon

Gas sampling was conducted on days 5 and 10. Headspace CO_2 concentrations were measured using infra-red gas analysis (Li-COR model LI-7000, Lincoln, NE, USA) following established methods (Keiser *et*

176 *al.* 2011). After taking gas samples on day 5, the chambers were again flushed with CO_2-free air to ensure
177 CO_2 concentrations in the headspace remained below those inhibitory to fungal growth. Total respiration
178 C (henceforth 'respiration') for each isolate over the 10-d experiment was calculated by multiplying the
179 two headspace C concentrations (days 5 and 10) by the total chamber volume (500 mL), and adding these
180 two values.
181
182 *Biomass carbon and nitrogen*
183 Biomass production was estimated following Crowther & Bradford (2013). After 10 d, the fungal biomass
184 was recovered by heating the agar at 121°C for 5 min. The melted solution was filtered through a 53-μm
185 sieve and rinsed with 1 L of 90°C deionized-water, agitating slightly to remove residual agar, and
186 subsequently rinsed for 2 min with 20°C deionized-water. The resulting biomass was dried at 65°C to
187 constant mass and weighed. Note that the combined biomass of the multispecies mixtures was measured
188 at the community level, such that the individual biomass of each species in each community was not
189 quantified. The three replicate samples for each treatment were combined to obtain sufficient biomass for
190 elemental analysis, milled with a mortar and pestle into a fine powder, and analyzed for C and N content
191 (ESC4010, Costech Analytical Technologies Inc., Valencia, CA, USA). Total biomass C (henceforth
192 'biomass') and biomass N were estimated by multiplying the %C and percent %N values by total dry-
193 weight biomass in each sample.
194
195 *Estimating carbon use efficiency*
196 The definition of CUE can vary widely, depending on the timescales of interest and the relevant C pools
197 measured (Geyer *et al.* 2016). Here, we calculated CUE as the net amount of C in biomass (B), relative to
198 the total amount of C that was either mineralized (i.e., respired; R) or incorporated into biomass. That is:
199 $$CUE = \frac{B}{B + R}$$

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 modeling 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 Supplemental 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

224 the equal-weighting (simple average) approach is used throughout so that any deviations from additivity
225 can be directly attributed to species interactions (Kirwan *et al.* 2009; Connolly *et al.* 2013).

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

228 $$predicted\ CUE_k = \frac{predicted\ Biomass_k}{predicted\ Respiration_k + predicted\ Biomass_k}$$

229 The proportional changes in biomass, respiration, and CUE attributable to interspecific interactions were
230 subsequently calculated by comparing the predicted to observed values, e.g., proportional change in CUE
231 = (observed CUE – predicted CUE) · (predicted CUE)$^{-1}$ (see Supplemental Methods for detailed
232 calculations). These proportional change calculations (Fig. 2g-i) were made on a community-by-
233 community basis in order to account for baseline differences in performance across communities.
234
235 *Statistical analysis*

236 Mixed-effect models were used to quantify how temperature, N, and interspecific interactions affect
237 community performance. Separate models were run for individual vs. community results, and all analyses
238 were conducted using the statistical software *R*, version 3.2.3 (R Core Team 2017). For each model and
239 each outcome, the fixed effects included temperature, N, and the temperature-by-N interaction. For the
240 individual-level analyses, the outcome variable was respiration, biomass, or CUE. For the community-
241 level analyses, the outcome variable was proportional change ([observed – predicted] / predicted) in
242 biomass, respiration, or CUE. A random effect for 'replicate' was included to account for correlation
243 across the three replicate samples of each individual/community combination. For each outcome variable,
244 two models were fit: one accounting for species-specific effects by including a species indicator variable
245 (presence/absence) as a random effect; and a second set of models with no species indicator (Tables S2-
246 S11). The most parsimonious of these two models for each outcome was selected as the one with the

smallest Bayesian-information-criterion (BIC) 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 Benjamin & Hochberg (1995).

Results

Carbon and nitrogen in biomass

Total biomass varied substantially across treatments, with a mean of 47±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 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±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±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 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 mg-C·°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 mg·°C^{-1}, $p=0.24$; Fig. 2a, Table S2). Mean respiration was not statistically different at 16°C between N treatments ($p=0.12$), but was significantly higher at 28°C under High-N (48.0 vs. 9.0 mg, respectively; $p<0.001$). Patterns in biomass production rates largely mirrored those of respiration (Fig. 2b; Table S3). Biomass C increased 2.3 mg·°C^{-1} under High-N ($p<0.001$) and 1.0 mg·°C^{-1} under Low-N ($p=0.06$). At 16°C, biomass was 14.6 mg higher under High-N than Low-N ($p=0.01$) and 30.2 mg higher at 28°C ($p<0.001$).

Under Low-N, CUE remained essentially constant at ~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

294 equal rates. Conversely, under High-N, respiration increased proportionately faster than biomass, leading
295 to a decrease in CUE of 1% (in absolute terms) per degree increase in temperature (β=0.01; $p<0.02$).
296 Indeed, at High-N, CUE exhibited a 25% decrease across the temperature range, dropping from 0.61 at
297 16°C to 0.46 at 28°C.
298
299 *Biotic responses across communities*
300 The overall effects of N and temperature on community-level respiration, biomass, and CUE were
301 qualitatively similar to those among individuals (Fig. 2d-f; Tables S5-S7). However, when directly
302 compared to the individual values, key differences emerged (Fig. 2g-i). Respiration was significantly
303 increased in communities, relative to community-specific predicted values (Fig. 2g, Table S8; n=161 after
304 removing missing values). For Low-N, the change in respiration (Δ-respiration) was 46% and 44%
305 elevated at 16°C and 28°C, respectively ($p<0.001$ for both), with this difference constant across
306 temperatures (β=-0.002; p=0.87). For High-N, Δ-respiration showed a significant negative temperature
307 response (p=0.04), ranging from a 101% increase in respiration at 16°C down to a 61% increase at 28°C.
308 In contrast to expectations, biomass production was significantly higher in communities than
309 predicted based on individual performance (Fig. 2h, Table S9). Δ-biomass was significantly elevated at
310 16°C, exhibiting a 43% increase ($p<0.001$) regardless of N conditions. This increase in biomass production
311 attenuated under increasing temperatures, leading to a non-significant decrease at 28°C of 1% for High-
312 N (p=0.87) and 15% for Low-N (p=0.14).
313 Despite the increase in biomass in communities, the proportionately larger Δ-respiration values
314 led to significant reductions in CUE under most treatment conditions (Fig, 2i, Table S10). The exception
315 was Low-N at 16°C, where CUE was essentially unchanged relative to monoculture performance (2.1%
316 decrease; p=0.48). Conversely, even though Δ-biomass was significantly elevated at High-N at 16°C, the
317 resulting CUE was ~12% lower ($p<0.001$), driven by the fact that Δ-respiration was approximately twice

as large as Δ-biomass. The average ΔCUE under both Low-N and High-N communities showed marked negative temperature responses (β=-0.016 and β=-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±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±0.019 and 0.05±0.012, respectively, with their combined effects yielding a 0.06±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 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

342 shown to respond to continual increases in diversity, with this effect often mediated by environmental
343 conditions (Tiunov & Scheu 2005; Hiscox *et al.* 2015; Matulich & Martiny 2015). Yet, as our research
344 shows, respiration or biomass alone are not sufficient for inferring differences in C-allocation strategies,
345 since respiration and CUE (and thus presumably decomposition rates) need not be correlated.
346 Furthermore, the effects of increasing diversity on fungal activity are potentially mediated through
347 community turnover, community assembly history, or environmental fluctuations (Toljander *et al.* 2006;
348 Fukami *et al.* 2010; Hagerty *et al.* 2014), such that the links between fungal efficiency and diversity (e.g.,
349 Toljander *et al.* 2006) cannot be directly attributed to greater investment in combative activities. Thus, an
350 outstanding question is whether individual-level CUE will continue to decline with increasing levels of
351 diversity, or if these induced biotic costs are essentially fixed, regardless of the number of unique
352 competitors in the community.

353 Despite consistent trends across treatments, there was large variation in CUE, with the community-
354 specific random effects explaining 68% of the overall variation (Fig. 3a). Some of this variation may be
355 due to the fact that we only assayed two C pools (biomass and respiration), and we did not measure, for
356 example, differences in extracellular enzyme C allocation, which remains challenging due to
357 methodological limitations (Geyer *et al.* 2016). Nonetheless, our results show that – regardless of
358 allocation to these other C pools – the efficiency by which fungi retain C in biomass vs. respire C into the
359 atmosphere differs in the presence of interspecific interactions, with large variation across all treatment
360 levels. Alternate explanations for this variability may be differing levels of biodiversity (e.g., phylogenetic
361 or functional) or trait expression within communities, or potentially different competitive dynamics within
362 each community. In communities typified by allelopathy and interference competition for space, the
363 structure of the competitive network (e.g., whether it is intransitive versus hierarchical) has been shown
364 to be a critical component of species behavior and survival (Jackson & Buss 1975; Maynard *et al.* 2017).
365 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; Fig. 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 that 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 in order 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, analyzed the data, and wrote the initial manuscript. TWC and MAB assisted in designing the experiments, analyzing the data, and preparing the manuscript.

Supporting Information Files

Appendix S1: Figure S1-S6, Tables S1-S8, Supplemental Methods

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Figure Captions:

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. Despite these significant trends, %C was highly consistent across all samples – 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). **(b)** 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.

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 competitive 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 patters 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 competitive 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 ~100% increase at 16°C down to a ~60% increase at 28°C above predicted values. **(h)** Competition led to significant increases in biomass-carbon at low temperature, but showed negligible change at 28°C. **(c)** CUE was significantly reduced under competition in all conditions except for 16°C, Low-N, with as much as a 25% reduction at 28°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.

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.

661 **Figure 1**

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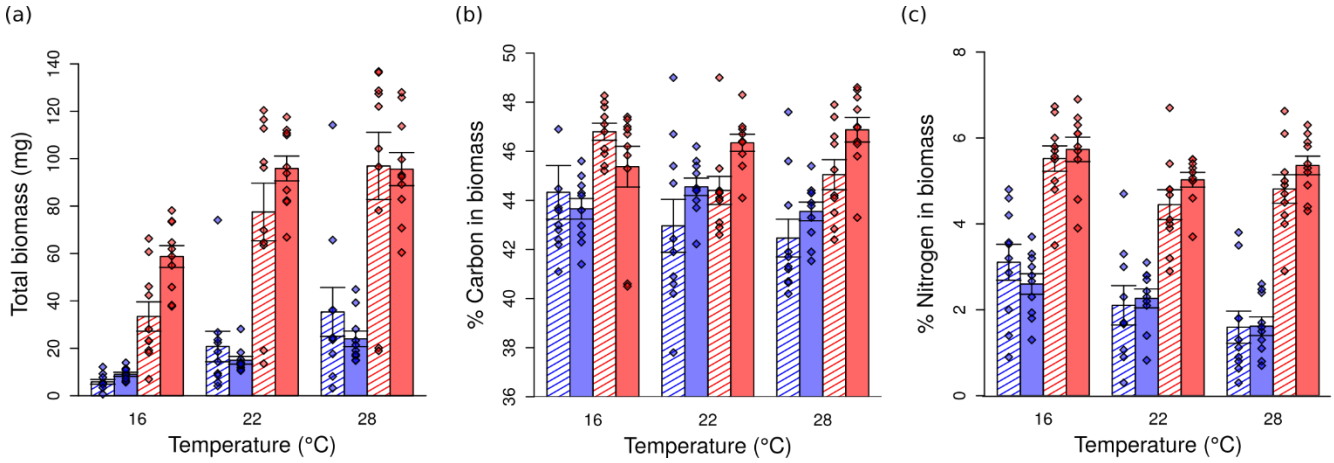

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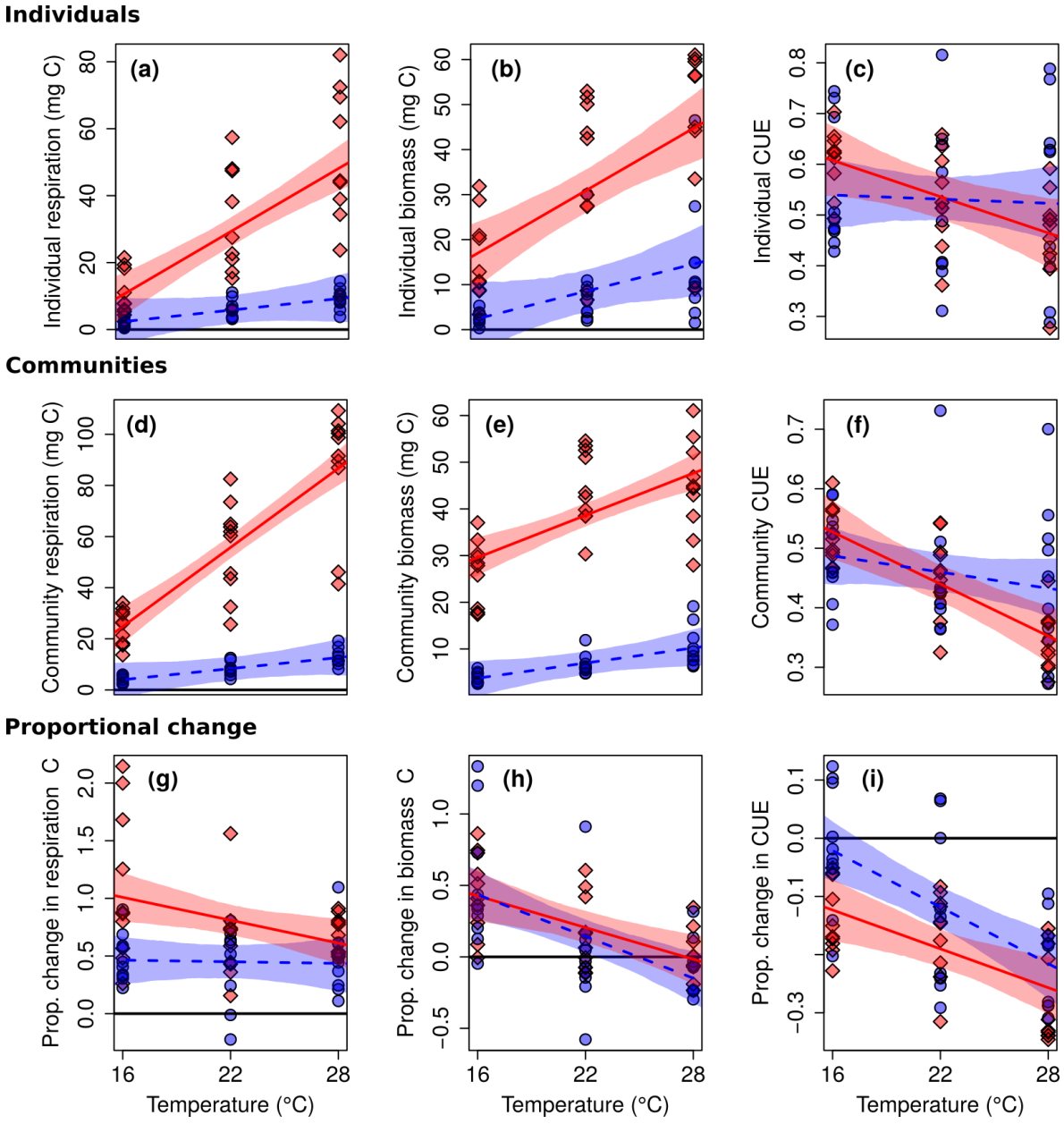
Figure 2


Individuals
(a)
Individual respiration (mg C)
(b)
Individual biomass (mg C)
(c)
Individual CUE
Communities
(d)
Community respiration (mg C)
(e)
Community biomass (mg C)
(f)
Community CUE
Proportional change
(g)
Prop. change in respiration C
(h)
Prop. change in biomass C
(i)
Prop. change in CUE
16
22
28
Temperature (°C)

671 **Figure 3**

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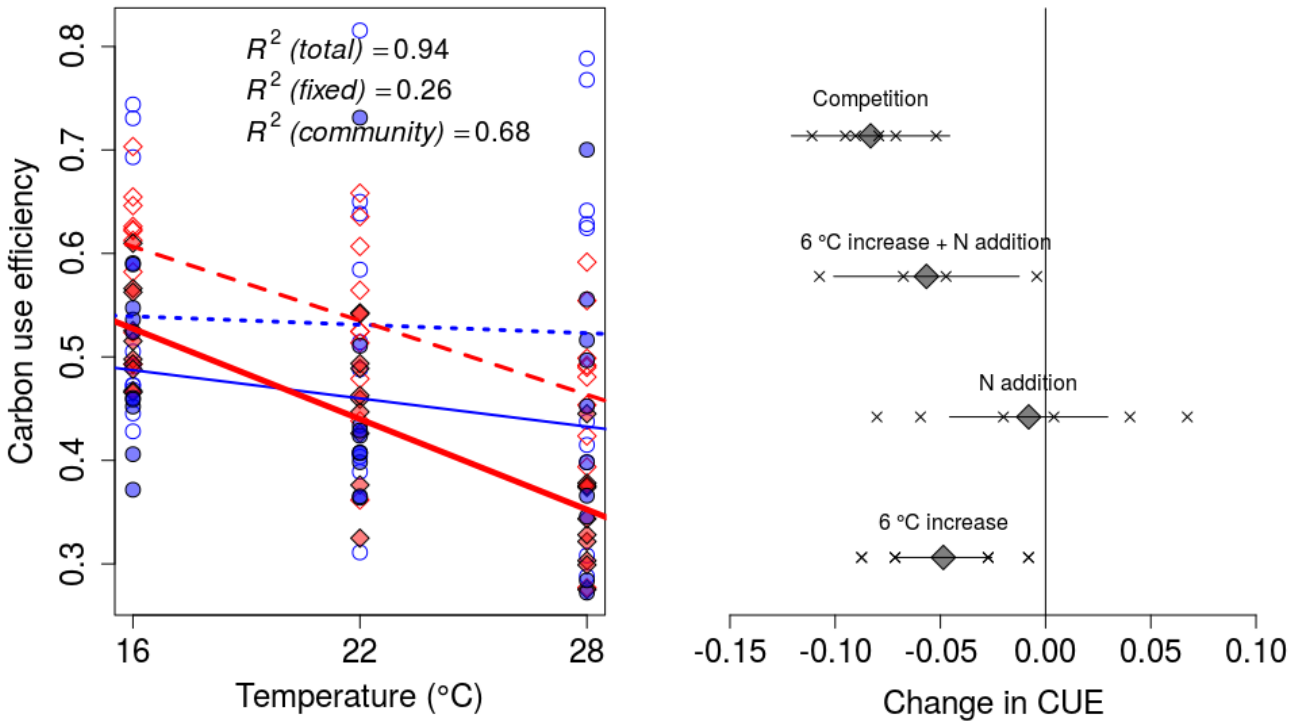

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