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RESEARCH ARTICLE

A positive feedback to climate change: The effect of temperature on the respiration of key wood-decomposing fungi does not decline with time

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Abstract

Heterotrophic soil microorganisms are responsible for ~50% of the carbon dioxide released by respiration from the terrestrial biosphere each year. The respiratory response of soil microbial communities to warming, and the control mechanisms, remains uncertain, yet is critical to understanding the future land carbon (C)-climate feedback. Individuals of nine species of fungi decomposing wood were exposed to 90 days of cooling to evaluate the medium-term effect of temperature on respiration. Overall, the effect of temperature on respiration increased in the medium term, with no evidence of compensation. However, the increasing effect of temperature on respiration was lost after correcting for changes in biomass. These results indicate that C loss through respiration of wood-decomposing fungi will increase beyond the direct effects of temperature on respiration, potentially promoting greater C losses from terrestrial ecosystems and a positive feedback to climate change.

KEYWORDS
basidiomycetes, carbon cycle feedbacks, climate change, CO_2, fungi, respiration, respiratory thermal response, temperature, wood decomposition

1 | INTRODUCTION

Heterotrophic soil microorganisms decompose organic matter and are responsible for emitting ~60 Pg of C per year to the atmosphere as carbon dioxide (CO_2) via respiration. This accounts for ~50% of C release by respiration from the terrestrial biosphere each year (Singh et al., 2010). The instantaneous effect of temperature causes an increase in heterotrophic soil microbial respiration (Davidson & Janssens, 2006; Kirschbaum, 2006), but, in field warming experiments, this initial positive response of heterotrophic soil microbial respiration to warming often declines in the long term (years-decades). This phenomenon could be caused by direct (acclimation, evolution and species sorting) or indirect (e.g. substrate availability, moisture) effects of temperature (Bárcenas-Moreno et al., 2009; Bradford, 2013; Davidson & Janssens, 2006). If direct effects of warming reduce respiration rates, this would help to maintain C sequestration in terrestrial ecosystems and weaken the positive C-climate feedback, but if the response is associated with substrate depletion it would represent a symptom of the gradual loss of soil C. Thus, distinguishing between these two competing hypotheses is essential. It has previously, been demonstrated that a cooling and rewarming approach can account for changes in substrate availability, thus making it possible to quantify the direct effects of temperature on microbial activity over weeks to months (Hartley et al., 2008; Karhu et al., 2014).

To determine the mechanisms underlying the decline in heterotrophic soil microbial respiration with warming in the longer term, focus has been predominantly on the response of whole soil microbial communities to temperature (Bradford et al., 2008, 2010, 2019; Dacal et al., 2019; Hartley et al., 2007, 2008; Karhu et al., 2014). However, when studying whole communities, the mechanisms underlying observed responses have been challenging to identify and, thus, considerable uncertainty in how heterotrophic soil microbial communities will respond to warming remains (Auffret et al., 2016). To increase mechanistic understanding, investigations of individual species of heterotrophic soil microorganisms offer opportunities for isolating physiological responses from evolutionary and ecological responses.

Previous studies have begun to investigate the effect of temperature on the respiration of individual fungal species, however contrasting responses have been observed. Arbuscular mycorrhizal fungi in soil (Heinemeyer et al., 2006), ectomycorrhizal fungi on agar (Malcolm et al., 2008) and cord-forming wood decay basidiomycetes on agar (Crowther & Bradford, 2013), reduced respiration rates with warming. However, a saprotrophic ascomycete fungus grown on agar (Romero-Olivares et al., 2015) and sucrose or lignin (Allison et al., 2018) increased respiration rates with warming. Many of these studies have measured the effect of temperature on fungal respiration using unnatural substrates over the short-term (days), and therefore may not be relevant in explaining the reduction in warming responses observed over months to years in field experiments. Further study of the respiratory thermal responses of individual microorganism species decomposing natural substrates over an extended time (months) is required to aid understanding of responses taking place in natural systems.

Wood decay fungi are the primary decomposers of dead wood in temperate forest ecosystems (Baldrian & Lindahl, 2011; Boddy & Watkinson, 1995; Rayner & Boddy, 1988). Temperate forests account for 25% of forest globally (Martin et al., 2001) and store 14% of global C (Pan et al., 2011). Therefore, wood decay fungi have a key role in the C cycle in a changing climate. White rot basidiomycetes account for over 90% of all wood decay fungi (Janusz et al., 2017), and their unique ability to rapidly decompose lignocellulose and to penetrate bulky wood resources allows them to dominate wood decomposition (Eichlerová et al., 2015). Consequently, white rot basidiomycetes represent an important group of microorganisms involved in decomposition and their dominant role in decomposing a specific substrate (wood) makes them ideal model organisms for respiratory thermal response studies.

To gain a mechanistic understanding of heterotrophic soil microorganism responses to warming, this study investigated the respiratory thermal response of individual cultures of nine species of white rot basidiomycetes decomposing beech wood (*Fagus sylvatica*). The chosen species have different ecological roles in wood decomposition (primary, early and late secondary colonisers) in temperate woodlands (Boddy & Hiscox, 2016). Primary colonisers obtain initial access to uncolonised resources and early and late secondary colonisers are involved in later stages of community development (Cooke & Rayner, 1984). Early secondary colonisers typically show antagonistic/combative characteristics or stress-tolerance (Boddy & Heilmann-Clausen, 2008), whereas late secondary colonisers tend to be more competitive and some form mycelial cords which allow them to forage for new resources by growth of mycelia from wood into soil, rather than relying on spreading by spores (Boddy, 1993). We used an established cooling and rewarming approach to determine whether extended exposure (60–90 days) to a new temperature resulted in the effects on fungal respiration: (1) decreasing (compensatory thermal response), (2) increasing (enhancing thermal response) or (3) remaining unchanged (no thermal response). Ergosterol content, as an estimate of living fungal biomass, was measured so respiration rates could also be expressed per unit biomass (mass-specific respiration; R_{mass}) to improve our mechanistic understanding of the thermal responses. We tested the key hypothesis that single species of basidiomycetes decomposing wood would show compensatory thermal responses and decrease the effect of temperature on respiration in the medium term.

2 | MATERIALS AND METHODS

2.1 | Pre-colonisation of wood blocks

Nine species of beech (*F. sylvatica*) wood-inhabiting basidiomycetes (Table 1), dominant at different stages of decay, were used to colonise 2 × 2 × 2 cm beech wood blocks. Blocks were sterilised

TABLE 1 Fungal species used to colonise wood blocks.

Ecological role	Species	Strain
Primary coloniser (P)	*Vuilleminia comedens*	VcWVJH1
	Fomes fomentarius	JHC 1676
	Chondrostereum purpureum	F599 P844
Early secondary coloniser (ES)	*Trametes versicolor*	TvCCJH1
	Stereum hirsutum	ShSS1
	Bjerkandera adusta	BaSS1
Late secondary coloniser (LS)/cord former	*Hypholoma fasciculare*	HfGTWVZ
	Phanerochaete velutina	Pv29
	Resinicium bicolor	Rb1

Note: All fungi are white rot wood decay basidiomycetes. Cultures were obtained through isolation from wood or fruit bodies, from the Cardiff University Culture Collection.

by autoclaving three times over 72 h, then placed onto cultures of single species growing on 0.5% malt agar (0.5% MA: 5 g L^{-1} malt extract, 15 g L^{-1} agar; Lab M, UK) and incubated at 20°C in the dark for 108 days (Figure S1). Wood block pre-colonisation was confirmed by the re-isolation of fungi from a sample of wood blocks ($n = 10$). Individual wood blocks were split in half along the grain using a surface-sterilised chisel, and pieces of wood (2 mm^3) were excised approximately 2, 7, 12 and 17 mm from the wood block edge, placed onto 2% malt agar (2% MA: 20 g L^{-1} malt extract, 15 g L^{-1} agar; Lab M, UK) and incubated at 20°C until mycelia had emerged and could be identified morphologically. The mean density of uncolonised wood blocks (0 day) was 0.542 (mg mm^{-3}; 10 replicates), determined as oven dry weight (80°C for 72 h) per fresh volume (mm^{-3}), measured using digital callipers.

2.2 | Wood block microcosm set-up

Pre-colonised wood blocks were scraped free of adhering mycelium and agar using a sterile scalpel, 3 days prior to set up. Each wood block was placed directly on to perlite (20 mL; siliceous rock that does not absorb carbon dioxide [CO_2]; Homebase, UK) moistened with 2 mL sterile distilled water (dH_2O) to achieve a water potential of −0.012 kPa (determined by the method of Fawcett & Collis-George, 1967), in a plastic 100 mL lidded deli pot (Cater4you, UK). Each microcosm was weighed and dH_2O added to the perlite every 14 days to maintain moisture. Holes (4 × 1 mm diameter) in each pot covered by microporous surgical tape (3M, Bracknell, UK) allowed aeration but prevented contamination with other species.

2.3 | Wood block microcosm incubation

Wood block microcosms were incubated (Sanyo Electric/Panasonic Cooled Incubator, MIR-154) at 20°C for a 43 days pre-incubation period (Figure S1). The pre-incubation period allowed respiration rates to stabilise. Four respiration measurements were taken from each wood block microcosm so that microcosms could be assigned to temperature treatments to establish similar mean respiration rates and trajectories across temperature treatments prior to cooling. Wood block microcosms of each species were assigned to one of four temperature treatments ($n = 5$): pre-cooling (destructively sampled at 151 days, prior to cooling), cooled (incubated at 12°C at 151 days for 90 days), rewarmed (incubated at 12°C at 151 days for 60 days and then rewarmed to 20°C for 30 days) and control (incubated at 20°C for a further 90 days; Figure S1). Wood blocks from cooled, rewarmed and control treatments were destructively sampled at 241 days. Destructive sampling involved each individual wood block being split into quarters along the grain using a surface-sterilised chisel. The quarters were flash frozen in liquid nitrogen and stored at −80°C for quantification of ergosterol as an indicator of fungal biomass. The incubation temperatures chosen are common in temperate woodlands and within the range experienced by basidiomycetes during the main decomposition season (Boddy, 1983; Magan, 2008). Cooling for 90 days provides sufficient time for thermal compensation and is a time period relevant to seasonal changes in temperature, that have been hypothesised to cause thermal compensation (Karhu et al., 2014; Malcolm et al., 2008). The rewarmed treatment was chosen to investigate the reversibility of any response observed with cooling.

2.4 | Respiration measurements

Respiration was measured by placing each wood block microcosm with a closed lid inside a larger airtight 700 mL plastic container (Lock & Lock® container, Hana Cobi Plastic Co. Ltd, Seoul, Korea), which was connected to an infrared gas analyser (IRGA; EGM-4, PP systems, version 1.47, Hitchen, UK) in a closed-loop configuration, following Hartley et al. (2008). The CO_2 concentration in the headspace of the incubation chamber was measured immediately after closure, and then again after 18 h. Respiration was calculated assuming that CO_2 accumulation within containers was linear (tests confirmed that this assumption was appropriate over this time by Karhu et al., 2014). Respiration rates were expressed as μg C g^{-1} wood h^{-1}. Respiration was measured weekly, and the first respiration measurements after cooling and rewarming were made 24 h after the temperature change.

2.5 | Ergosterol as an indicator of fungal biomass

Ergosterol is a dominant membrane lipid found almost exclusively in fungi, including basidiomycetes (Weete et al., 2010), and is frequently assayed as an indicator of living fungal biomass, based on the assumption that it is unstable and therefore rapidly degraded upon death of fungal hyphae (Mille-Lindblom et al., 2004).

Wood blocks ($n = 5$) from pre-cooling (151 days), cooled, rewarmed and control treatments (241 days) were removed from storage at −80°C and freeze dried for 48 h (ScanVac CoolSafe, UK), then ground to sawdust using a spice grinder (Wahl James Martin, UK). Total ergosterol was extracted from 0.5 g samples following established methods (Bååth, 2001; Šnajdr et al., 2008), and analysed by a diode-array detector coupled to a 1200 series Rapid Resolution HPLC system (Agilent Technologies, Palo Alto, USA) using a ACE Equivalence 5 C18, 4.6 × 250 mm analytical column (Advanced Chromatography Technologies Limited, Aberdeen, Scotland, UK). Ergosterol concentrations (μg mL^{-1}) were expressed as μg ergosterol g^{-1} wood.

2.6 | Quantifying respiratory responses

In the absence of C inputs, C losses will occur due to decomposition and associated microbial respiration, with greater C losses at warmer temperatures due to greater fungal activity. To account for

differences in C availability, the respiration rate (μg C gdw^{-1} h^{-1}) of control, cooled and rewarmed treatments were plotted against the cumulative respiration (mg C gdw^{-1}), and comparisons between the temperature treatments were made at the same cumulative respiration (Table S1, Figure S2).

The species could show three possible thermal responses following cooling: compensatory, enhancing or no response (Figure 1). Two methods were used to quantify either compensatory or enhancing responses, following Karhu et al. (2014). For the first quantitative method, control and cooled treatment respiration rates were normalised to their first measurement of respiration taken after cooling and plotted against cumulative respiration (Figure 1a). The control and cooled treatment relative respiration rates (normalised to the time of cooling) were compared at a corresponding cumulative respiration producing a response ratio for each species (RR_{CC}: response ratio, control versus cooled; Figure 1a, Figure S2):

$$RR_{CC} = \frac{T2_{control} / T1_{control}}{T2_{cooled} / T1_{cooled}}, \quad (1)$$

where $T1_{control}$ and $T1_{cooled}$ are the respiration rates in the control and cooled treatments, respectively, shortly after cooling, $T2_{cooled}$ is the cooled respiration rate at the end of incubation, and $T2_{control}$ is the control respiration rate at the same cumulative respiration as cooled samples at the end of incubation. In *Hypholoma fasciculare*, *Phanerochaete velutina*, *Resinicium bicolor*, a large reduction in respiration rates was observed between 1 and 3 days after cooling (Figure S3). Thus, the respiration rates at 3 days after cooling was used in the calculation to exclude the short-term responses to cooling and hence, focus on responses that would be observed in the medium term and are relevant to understanding respiration declines with warming in field experiments.

The second quantitative method produced response ratios comparing control and rewarmed treatment respiration rates at a corresponding cumulative respiration (RR_{CR}: response ratio, control versus rewarmed; Figure 1b, Figure S2):

$$RR_{CR} = \frac{T_{control}}{T_{rewarmed}}, \quad (2)$$

where $T_{control}$ is the control treatment respiration rate at a corresponding level of cumulative respiration as rewarmed samples at 1, 5 or 9 days after rewarming, and $T_{rewarmed}$ is the rewarmed treatment respiration rate at 1, 5 or 9 days after rewarming. Response ratios were produced at 1, 5 and 9 days after rewarming to investigate whether any compensatory or enhancing responses increase or decrease over time, with decreases expected as the cooling responses were predicted to be reversible.

Respiration rates were also expressed per unit fungal biomass (mass-specific respiration; R_{mass}: μg C g^{-1} ergosterol h^{-1}) and new response ratios produced using the equations above. Fungal biomass was only measured before cooling and at the end of the experiment. Therefore, the ergosterol content of control samples, at a corresponding level of cumulative respiration as cooled samples at end

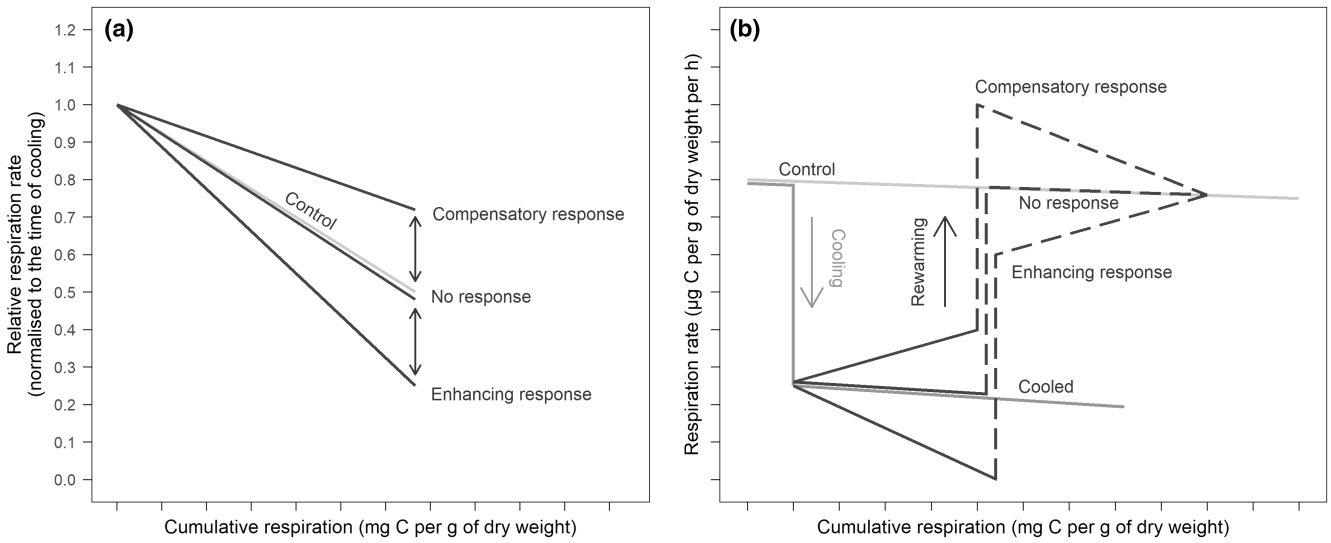

FIGURE 1 Quantification of respiratory responses by comparing the (a) relative respiration rates of cooled and control treatments and (b) absolute respiration rates of rewarmed and control treatments, at a corresponding cumulative respiration. Panel (b) shows a secondary quantification method used to support the primary quantification method shown in panel (a). Panel (b) demonstrates the expected trajectory of the respiratory responses to cooling after rewarming. (a) The same relative respiration rates of control and cooled treatments indicate a no response following cooling. A slower relative rate of decline or gradual increase in respiration rate after cooling provides support for a compensatory response, whereas a greater relative rate of decline in respiration rate following cooling indicates an enhancing response. (b) A no response occurs when the absolute respiration rate of the rewarmed treatment is equal to the absolute respiration rate in the control treatment at the same cumulative respiration. An increase in absolute respiration rate of rewarmed treatment above that of the control treatment shows evidence for a compensatory response, however an increase in absolute respiration rate of rewarmed treatment to below that of the control treatment supports an enhancing response. In addition, the difference between the absolute respiration rates of the cooled and rewarmed treatments declines with time if the response is compensatory, and increases with time if the response is enhancing.

of incubation, had to be estimated by linearly interpolating between the ergosterol content of pre-cooling samples and control samples at the end of incubation. Response ratios comparing control and rewarmed treatment R_{mass} were not calculated, as the ergosterol at the time of rewarming was uncertain.

For respiration rates expressed per unit wood mass and per unit fungal biomass, response ratio values <1 indicate a compensatory response and values >1 indicate an enhancing response. Response ratios for the individual species were natural-log-transformed, means calculated and an exponent taken, to produce a mean response ratio and 95% confidence intervals for all species overall and each ecological role. Natural-log-transformed response ratios of replicates ($n=5$) of each species were used to produce 95% confidence intervals for each individual species (Karhu et al., 2014).

Temperature responses of respiration (proportional changes in respiration per 10°C change in temperature; Q_{10}) were calculated at 1 day after cooling (3 days for *H. fasciculare*, *P. velutina* and *R. bicolor*) and at 90 days after cooling to determine the short- and medium-term effects of cooling, respectively:

$$Q_{10} = \left(\frac{R_{control}}{R_{cooled}}\right)^{\frac{10}{(Temp_{control} - Temp_{cooled})}}, \quad (3)$$

where $R_{control}$ and R_{cooled} are the respiration rates in the control and cooled treatments, respectively, at 1 or 90 days after cooling. For the 90 days calculation, cooled respiration rates were compared with control respiration rates at the same cumulative respiration (see Figure S2a for how this comparison is made). $Temp_{control}$ and $Temp_{cooled}$ are the control and cooled treatment temperatures, respectively.

A Q_{10} was also calculated to express the differences in respiration rates between rewarmed and cooled treatments at 1, 5 and 9 days after rewarming:

$$Q_{10} = \left(\frac{R_{rewarmed}}{R_{cooled}}\right)^{\frac{10}{(Temp_{rewarmed} - Temp_{cooled})}}, \quad (4)$$

where $R_{rewarmed}$ is the rewarmed treatment respiration rate at 1, 5 or 9 days after rewarming, and R_{cooled} is the cooled treatment respiration rate at a corresponding level of cumulative respiration as rewarmed samples at 1, 5 or 9 days after rewarming. $Temp_{rewarmed}$ and $Temp_{cooled}$ are the rewarmed and cooled treatment temperatures, respectively.

2.7 | Statistical analysis

All statistical analyses were conducted using R statistical software (R version 3.6.3, R Core Team, 2020). One-way analysis of variance (ANOVA) models were used to compare the respiration rates of control, cooled and rewarmed treatments at the final measurement of pre-incubation (143 days), prior to cooling (151 days), for each species. Differences in ergosterol (μg g $wood^{-1}$) were analysed using two-way ANOVA and Tukey's pairwise comparisons, with temperature treatment and species as main effects, and an interaction effect included. In addition, the effect of ecological role on ergosterol content was analysed using two-way ANOVA, with temperature treatment and ecological role as main effects. The difference in ergosterol content between temperature treatments of each species was determined using one-way ANOVA models and Tukey's pairwise comparisons. To support the response ratio method (RR_{CC}), a statistical comparison of the slopes using the *F* ratio method was conducted. The relationships between relative respiration rate and cumulative respiration for control and cooled treatments were compared for each species, with respiration rates at the two temperatures standardised to the respiration rate measured at the time of cooling. This was necessary to ensure that relative changes in respiration were being compared due to the greater absolute respiration rates at the higher temperature. Using the known *F* distribution, a *p* value was calculated from the *F* ratio and two degrees of freedom values. To test for statistically significant responses of species overall, each ecological role and each individual species, Paired *t*-tests were used to compare the cooled treatment relative respiration rates at the end of incubation to control treatment relative respiration rates, at the cumulative respiration of the cooled treatment samples at end of incubation (when control and cooled treatment relative respiration rates were expressed per unit wood or per unit fungal biomass). Paired *t*-tests were also used to compare the rewarmed treatment respiration rates at 1, 5 and 9 days after rewarming to control treatment respiration rates at a corresponding level of cumulative respiration as rewarmed treatment samples at 1, 5 and 9 days after rewarming, respectively.

3 | RESULTS

3.1 | Overall respiration rates

For each of the species, there were no significant differences in respiration rates between wood blocks allocated to the different temperature treatments before cooling ($p>.05$; Table S2, Figure 2). The respiration rates, and as a result the cumulative respiration for all treatments, were greatest for *Trametes versicolor* (ES), followed by *P. velutina* (LS) and *Vuilleminia comedens* (P) (Figure 2). *Fomes fomentarius* (P) and *H. fasciculare* (LS) had much lower respiration rates and cumulative respiration, while *Chondrostereum purpureum* (P) had the lowest respiration rates and cumulative respiration for all treatments (Figure 2).

3.2 | Respiratory response to cooling

In response to the cooling treatment, individual species of basidiomycetes decomposing wood showed an overall enhancing response and increased the effect of temperature on respiration in the medium term (Figure 3, Table S3, Figure 4a; $RR_{CC}=1.19$, $p<.05$). No

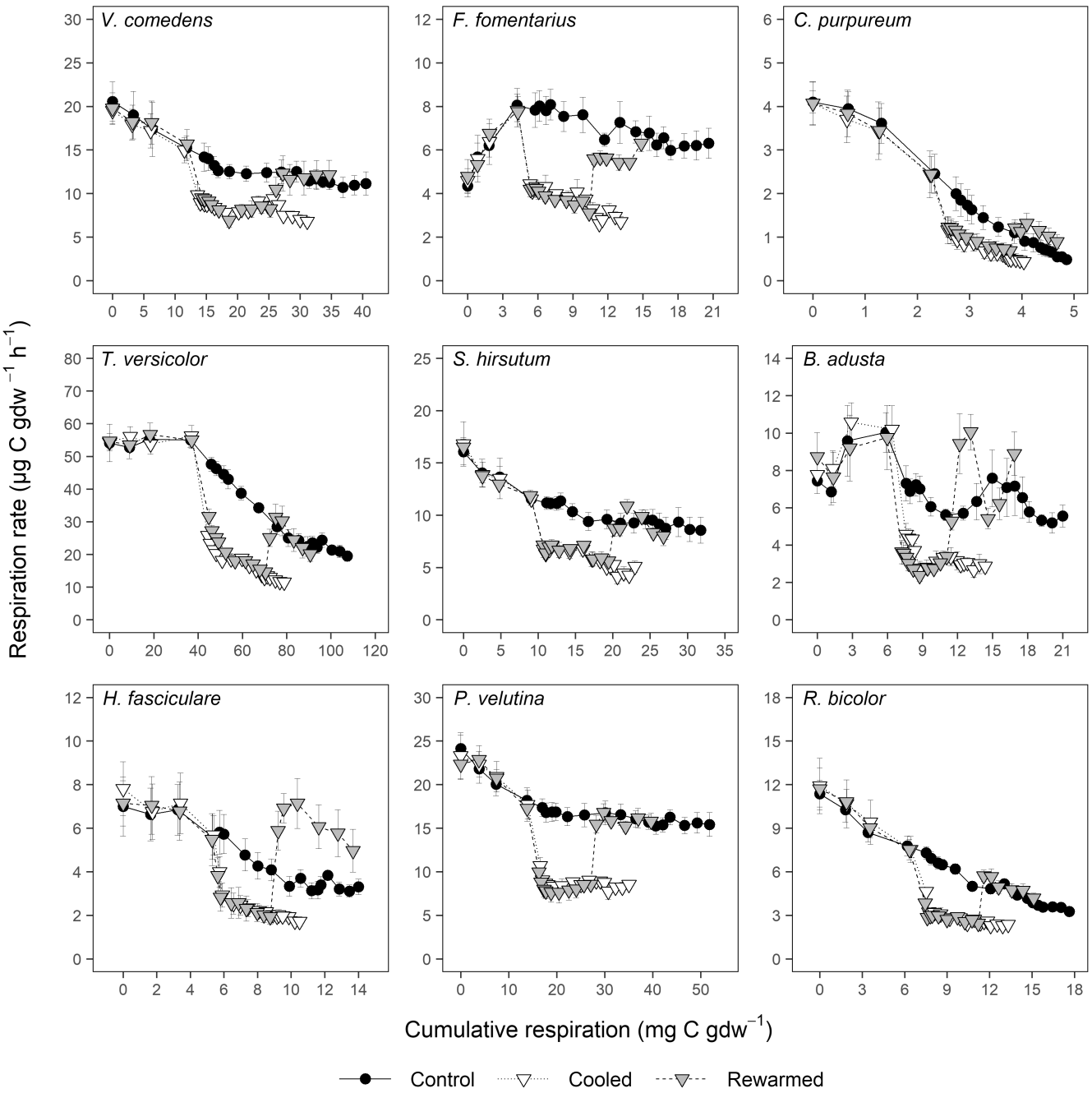

FIGURE 2 Respiration rate of three temperature treatments (control, cooled and rewarmed) during 43 days pre-incubation period prior to cooling and 90 days incubation following cooling, of each species (mean ± SE of the mean, $n = 5$). Cumulative respiration was calculated from the start of pre-incubation (108 days).

statistically significant compensatory responses that decreased the effect of temperature on respiration were observed (Figure 3, Table S3, Figure 4a). Early secondary colonisers showed a marginally significant enhancing response overall ($RR_{CC} = 1.29$, $p < .1$), whereas primary and late secondary colonisers showed no responses ($p > .05$; Table S3, Figure 4a). Three species (*F. fomentarius* [P], *T. versicolor* [ES], *Bjerkandera adusta* [ES]) showed enhancing responses ($RR_{CC} > 1$, $p < .01$; Table S3, Figure 4a). Six species (*V. comedens* [P], *C. purpureum* [P], *Stereum hirsutum* [ES], *H. fasciculare* [LS], *P. velutina* [LS], *R. bicolor* [LS]) showed no responses ($p > .05$; Table S3, Figure 4a). These thermal responses using the quantitative method RR_{CC} were confirmed by the statistical comparison of control and cooled relative respiration rate fitted lines (Table S4), however, *S. hirsutum* (ES) also showed an enhancing response by the fitted line method ($p < .05$), but not the RR_{CC} calculation. The effects of these thermal responses on the temperature sensitivity of respiration are illustrated in Table 2. *R. bicolor* (LS) was the only species to show a reduction in the temperature sensitivity of respiration in the medium term, but even here the respiratory Q_{10} comparing control and cooled treatments remained above 2 (Table 2).

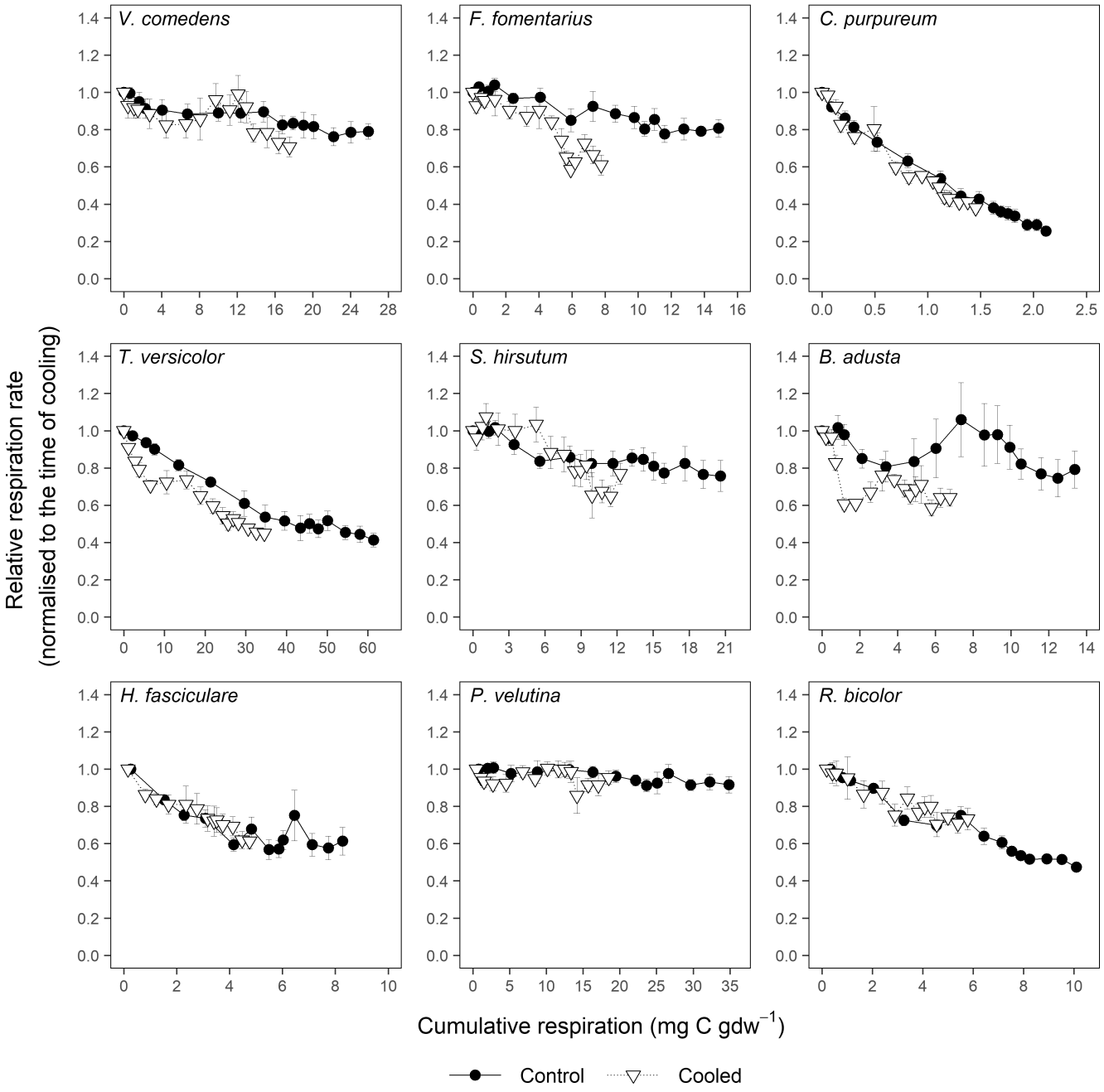

FIGURE 3 Relative respiration rate (normalised to the time of cooling) of control and cooled treatments during 90 days of incubation following cooling, of each species (mean ± SE of the mean, $n = 5$). Cumulative respiration was calculated from the time of cooling (151 days), at the start of 90 days incubation.

3.3 | Respiratory response to rewarming

In response to the rewarming treatment, individual species of basidiomycetes and each of the three ecological roles showed no response overall and therefore no change in the effect of temperature on respiration ($p > .05$; Table S5, Figure S4, Figure 4b). One day after rewarming, six species (*V. comedens* [P], *C. purpureum* [P], *S. hirsutum* [ES], *H. fasciculare* [LS], *P. velutina* [LS], *R. bicolor* [LS]) showed no responses ($p > .05$), however *F. fomentarius* (P) showed an enhancing response ($p < .01$), and *T. versicolor* (ES) and *B. adusta* (ES) showed marginally significant enhancing responses ($p < .1$; Table S5, Figure S4, Figure 4b). The enhancing responses to rewarming were generally lost over time as the effects of cooling were reversed, but this varied between 5 days (*T. versicolor* [ES]) to 31 days (*F. fomentarius* [P]). The effects of these thermal responses on the temperature sensitivity of respiration are shown in Table 2. The differences in respiration between cooled and rewarmed treatments tended to increase after rewarming, as respiration rates recovered to or above the control levels. This resulted in the temperature sensitivity of respiration increasing in

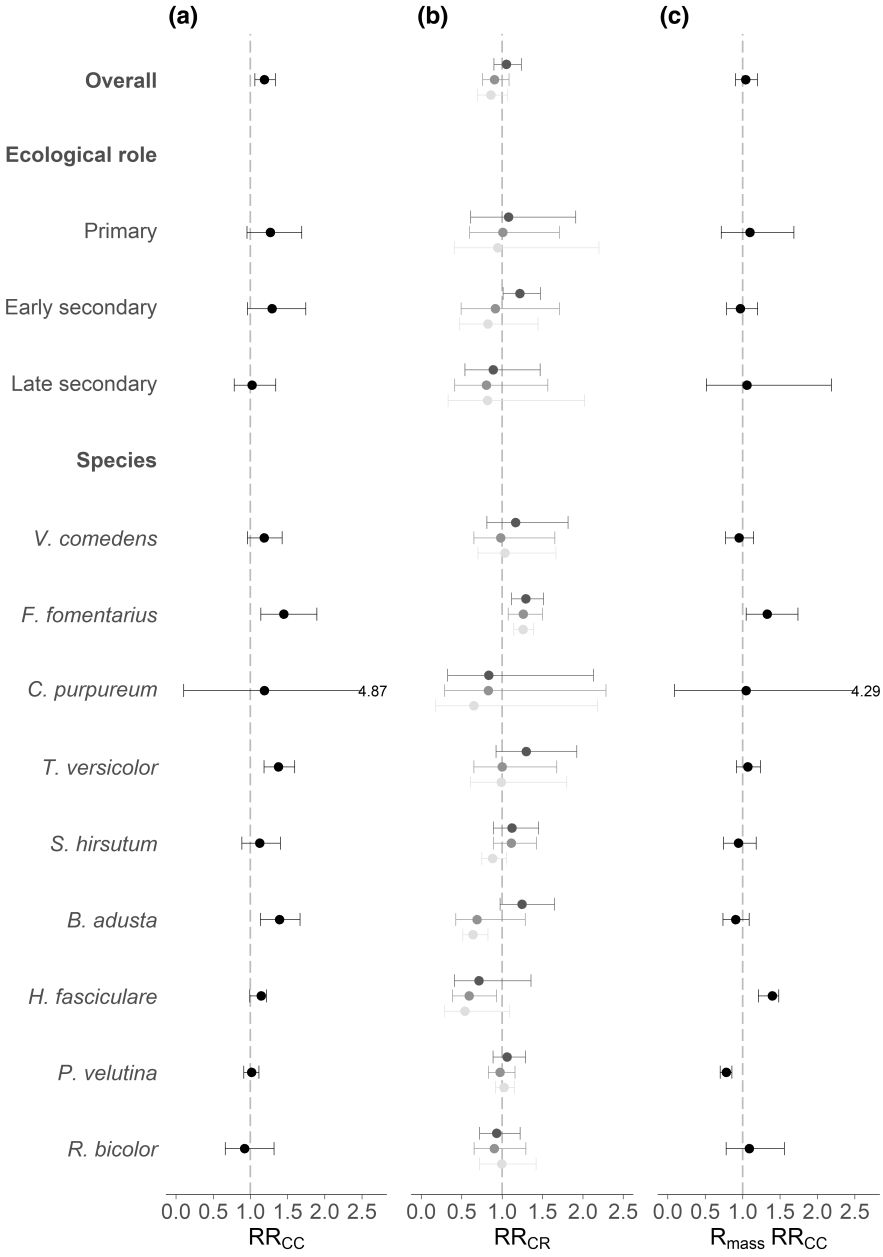

FIGURE 4 The mean and 95% confidence intervals ($n = 5$) of (a) RR_{CC}, (b) RR_{CR} for 1 (black), 5 (dark grey) and 9 (light grey) days after rewarming, and (c) R_{mass} RR_{CC}, for all species overall, each ecological role and each individual species. RR_{CC}: response ratio, control versus cooled; control treatment relative respiration rate divided by the cooled treatment relative respiration rate, at the cumulative respiration of the cooled treatment at the end of incubation. RR_{CR}: response ratio, control versus rewarmed; control treatment respiration rate at a corresponding level of cumulative respiration as rewarmed samples at 1, 5 and 9 days after rewarming divided by the rewarmed treatment respiration rate at 1, 5 and 9 days after rewarming. R_{mass} RR_{CC}: Mass-specific respiration response ratio, control versus cooled; control treatment relative R_{mass} divided by the cooled treatment relative R_{mass}, at a corresponding level of cumulative respiration as cooled treatment samples at end of incubation. Values <1 indicate a compensatory response and values >1 indicate an enhancing response. Effects are significant ($p < .05$) where confidence intervals do not cross one.

the days following rewarming in every species except *R. bicolor* (LS) (Table 2).

3.4 | Mass-specific respiratory response to cooling

When accounting for changes in fungal biomass by using ergosterol as an indicator (Figure S5), the enhancing response was lost with no response to the cooling being observed overall (R_{mass} $RR_{CC} = 1.04$, $p > .05$; Table S6, Figure 4c). Primary, early secondary and late secondary colonisers each showed no responses when considering the relative mass-specific respiration ($p > .05$; Table S6, Figure 4c). However, two species (*F. fomentarius* [P], $p < .05$; *H. fasciculare* [LS], $p < .01$) showed mass-specific enhancing responses, and one species (*P. velutina* [LS]) showed a mass-specific compensatory response ($p < .01$; Table S6, Figure 4c). Six species (*V. comedens* [P], *C. purpureum* [P], *T. versicolor* [ES], *S. hirsutum* [ES], *B. adusta* [ES], *R. bicolor* [LS]) showed no responses when comparing the relative mass-specific respiration ($p > .05$; Table S6, Figure 4c).

4 | DISCUSSION

Our study is the first to investigate the medium term respiratory thermal response of individual species of fungi using a natural substrate. Following cooling, single species of basidiomycetes showed an overall enhancing response that increased the effect of temperature on respiration in the medium term, with no evidence of compensatory responses. In response to rewarming, individual basidiomycete species produced no response overall and therefore no change in the

TABLE 2 A comparison of the instantaneous and medium-term effects of temperature on wood decomposition in the nine single species cultures. Firstly, the temperature sensitivity (Q_{10}) of respiration based on differences between cooled and control treatments, is compared at 1 day after cooling (short-term) (3 days after cooling for *Hypholoma fasciculare*, *Phanerochaete velutina* and *Resinicium bicolor*) with 90 days after cooling (medium-term). Secondly, the temperature sensitivity (Q_{10}) of respiration based on differences between rewarmed and cooled treatments is compared at 1, 5 and 9 days after rewarming.

	Q_{10} comparing cooled and control treatments		Q_{10} comparing rewarmed and cooled treatments		
Species	1 or 3 days	90 days	1 days	5 days	9 days
Vuilleminia comedens	1.58	1.95	1.40	1.74	1.64
Fomes fomentarius	2.03	3.22	1.96	2.09	2.18
Chondrostereum purpureum	1.96	2.43	3.19	3.37	4.86
Trametes versicolor	2.15	3.21	2.23	3.23	3.46
Stereum hirsutum	1.88	2.18	1.99	2.07	2.87
Bjerkandera adusta	1.80	2.72	1.92	4.19	4.89
Hypholoma fasciculare	2.32	2.74	3.95	5.21	6.59
Phanerochaete velutina	2.17	2.21	2.09	2.34	2.20
Resinicium bicolor	2.61	2.36	2.80	2.95	2.66
Overall	2.05	2.56	2.39	3.02	3.48

effect of temperature on respiration. When accounting for changes in fungal biomass, individual species of basidiomycetes showed no response overall, however some enhancing responses that increased the effect of temperature on respiration in the medium term were still identified. The overall lack of evidence for compensatory thermal responses suggests that respiration rates of basidiomycetes are unlikely to decline with warming until the availability of woody substrates is reduced.

4.1 | Respiratory response to cooling

By using a cooling approach to control the substrate availability, basidiomycete species growing alone in wood showed an overall enhancing response. This is a further decrease in the rate of respiration, lowering the respiration rate beyond the instantaneous response to cooling, which represents an increase in the effect of temperature on respiration. This overall enhancing response was driven by two early secondary colonisers (*T. versicolor* and *B. adusta*) and one primary coloniser (*F. fomentarius*). The other six species showed no response, with the effect of cooling on respiration rates not increasing or decreasing over time. In terms of the implications of this finding, an enhancing response to warming would cause any initial increase in respiration to increase further in the medium term, while a no response means that the initial increase in respiration caused by warming is maintained. Overall, we reject our key hypothesis that single species of basidiomycetes decomposing wood show compensatory thermal responses and decrease the effect of temperature on respiration in the medium term.

Our finding of an overall enhancing response contrasts with cord-forming basidiomycetes grown on agar that acclimated to temperature within days, with warm-acclimated individuals having lower mass-specific respiration rates at intermediate temperatures than cold-acclimated isolates (Crowther & Bradford, 2013). We instead found no compensatory responses, in agreement with studies growing *Neurospora discreta*, an ascomycete fungus, on agar (Romero-Olivares et al., 2015) and on sucrose and lignin (Allison et al., 2018). However, these studies growing fungi on agar, sucrose or lignin do not imitate well the structural or chemical heterogeneity of most natural resources (Crowther et al., 2018). Our study, used a natural substrate over a 90-day manipulation, and thus, the results may be more representative of natural systems and of timescales relevant to seasonal cycles. The lack of compensatory responses across the species, and the overall enhancing response observed, suggest that wood decomposition will remain highly sensitive to temperature. Reflecting this, the medium-term effect of temperature on the respiration of wood-decomposing basidiomycete species varied between a Q_{10} of 1.95 and 3.22 across the nine species (Table 2). Thus, our results suggest that climate warming retains the potential to promote substantial C losses from terrestrial ecosystems.

4.2 | Respiratory response to rewarming

In all cases of enhancing responses, respiration rates after rewarming subsequently approached rates of the control. This reversibility of the response indicates that the observations were not caused by cooling altering the decomposability of the remaining C. There was evidence of a faster response of the species of basidiomycetes to the rewarming than the cooling treatment. Of the three species showing an enhancing response, *T. versicolor* (ES) and *B. adusta* (ES) rapidly reversed the increased effect of temperature on respiration within 5 days of rewarming. In *F. fomentarius* (P), however, recovery of respiration to the control level respiration rate took 31 days after rewarming, but this recovery was still quicker than the full cooling

effect took to occur. The faster response to a temperature increase indicates that even a short period of warming may influence the effect of temperature on respiration and increase C losses.

Following rewarming, respiration rates of *B. adusta* (ES) and *H. fasciculare* (LS) increased to substantially above the rates observed for controls. In response to cooling, *B. adusta* (ES) showed an enhancing response and *H. fasciculare* (LS) produced no response. Thus, it is not clear if the rewarming response is evidence for the species compensating for the cooling, but, for *H. fasciculare* (LS) in particular, rewarmed and control treatments did ultimately have similar cumulative respiration at the end of the experiment. It is important to emphasise, however, that the cumulative respiration of *B. adusta* (ES) and *H. fasciculare* (LS) were two of the lowest. The species with the lowest cumulative respiration, *C. purpureum* (P), also showed a trend towards greater respiration in the rewarmed samples than the control. Therefore, it is possible that these responses were related to these species not being able to decompose key substrates at low temperatures, but the substrates then becoming available again as thermal constraints on decomposition were reduced after rewarming. Perhaps supporting this explanation, the highest respiration rates observed following rewarming in both species were very similar to those observed in the period before the cooling treatments were imposed (Figure 2).

4.3 | Mass-specific respiratory response to cooling

When respiration rates were normalised for fungal biomass the overall enhancing responses were lost across the full dataset and for the different ecological groups. Therefore, enhancing responses, when not normalised for fungal biomass, may have been driven by the inhibition of growth and biomass production at the lower temperature. This is supported by lower ergosterol content in the cooled samples than in the control samples for several of the species (Figure S5). However, *F. fomentarius* (P) showed an enhancing response even after normalising for fungal biomass and *H. fasciculare* (LS) showed an enhancing response after normalising for fungal biomass (previously no response). This demonstrates that enhancement was not entirely driven by the effects of temperature on growth and biomass production. After this normalisation, *P. velutina* (LS) revealed a compensatory response (previously no response), the only evidence of thermal compensation detected in this study.

4.4 | Respiratory thermal response of basidiomycetes with different ecological roles

With warming, it may be advantageous for primary and early secondary colonisers to show an enhancing response, rather than a compensatory response, as they need to utilise the resources rapidly prior to the arrival of more combative species. This would increase their respiration and decomposition rates, allowing them to gain and establish their territory before they are outcompeted by the later secondary colonisers. Conversely, later secondary colonisers have more control over the resource because they are generally, but not always, more combative and accordingly eventually outcompete the primary and early secondary colonisers (Boddy, 2000). Consequently, the compensatory response shown by *P. velutina* (LS) when normalising for biomass may be advantageous, increasing C-use efficiency and allowing more C to be allocated to mycelium to search and compete for already colonised territory. However, as this was the only compensatory response observed in this study, care should be taken not to over-interpret this single result, as later secondary colonisers also need to decompose resources to utilise the nutrients within, in order to fund energetically expensive antagonistic mechanisms to outcompete and obtain territory from early secondary colonisers (Hiscox & Boddy, 2017).

4.5 | Implications for fungal communities and long-term field-based soil warming experiments

Ninety days represents the approximate length of different seasons in temperate ecosystems, between which the largest fluctuations in temperature occur (Boddy, 1983). Therefore, the fact that wood decay basidiomycetes did not show compensatory responses to a temperature change over this seasonal timescale suggests basidiomycetes are unlikely to show compensatory responses in the longer term. Basidiomycetes found on the surface of dead wood, fine woody debris and leaf litter layer experience greater temperature fluctuations over diurnal and seasonal timescales than other soil microorganisms that exist in deeper soil horizons with more consistent thermal environments (Boddy, 1983; Rayner & Boddy, 1988). Therefore, if compensatory respiratory responses to temperature are important for microbial function, we would have expected basidiomycetes to display them. The limited evidence of compensatory responses of wood decay basidiomycetes that are present in temperate ecosystems and experience a wide temperature regime suggests that similar results will be observed from fungi and other soil microorganisms experiencing more constant temperature regimes, including those found within deeper soil layers. Our findings suggest that substrate depletion is likely to be the key mechanism underlying the decline in soil microbial respiration observed in long-term field-based soil warming experiments.

5 | CONCLUSION

We demonstrate that single species of basidiomycetes decomposing wood do not show compensatory thermal responses, and hence will not reduce the effect of temperature on respiration. Rather, some show enhancing thermal responses that could increase the effect of temperature on respiration, and others show no thermal responses that could maintain the effect of temperature on respiration. Consequently, with increasing global temperatures, wood decay basidiomycetes may increase their activity, reducing the role

of terrestrial ecosystems as C sinks and potentially contributing to a positive land C-climate feedback to climate change.

AUTHOR CONTRIBUTIONS

Katie L. Journeaux: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; validation; visualization; writing – original draft; writing – review and editing. **Lynne Boddy:** Conceptualization; funding acquisition; methodology; supervision; writing – review and editing. **Lucy Rowland:** Conceptualization; funding acquisition; supervision; writing – review and editing. **Iain P. Hartley:** Conceptualization; funding acquisition; methodology; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at 10.5061/dryad.t1g1jwt7f.

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