Respiratory thermal response of wood decay basidiomycetes

Submitted by **Katie Louise Journeaux**, to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Geography, December 2021.

I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

Signature……Katie Journeaux……

Cite as:

Journeaux, K. L., 2021. Respiratory thermal response of wood decay basidiomycetes. PhD thesis, University of Exeter, Exeter, UK.

Abstract

Terrestrial ecosystems absorb over one-quarter of anthropogenic carbon dioxide (CO_2) released into the atmosphere each year. Heterotrophic soil microbial respiration, associated with the decomposition of organic matter, contributes to approximately half of the CO_2 released from the terrestrial biosphere to the atmosphere per year. However, with increasing global temperatures, there is the potential for soil microbial respiration to increase, resulting in a substantial release of CO_2 into the atmosphere, therefore contributing extensively to the positive land C-climate feedback and accelerating climate change. In the short-term (days-years), heterotrophic soil microbial respiration is strongly and positively related to temperature. In the long-term (years-decades), however, the positive response of soil microbial respiration to warming declines, which could be caused by direct (acclimation, evolution and species sorting) or indirect (e.g. substrate availability, moisture) effects of warming. The primary focus on whole soil microbial community responses has made it difficult to identify potential mechanisms involved in controlling long-term warming responses, thus the response of respiration to warming in the long term remains controversial. To address this uncertainty, it is necessary to study individual species and gradually more diverse and complex decomposer communities. To this end, the respiratory thermal response of wood decay basidiomycetes, the dominant decomposers of wood, were investigated. Respiration rates of individual species and two- and three-species assemblages of basidiomycetes and semi-natural wood decay communities, decomposing beech wood (*Fagus sylvatica*), were measured during a 90-day cooling approach. In addition, a warming approach was applied to the two- and three-species assemblages and semi-natural wood decay communities. The direction of any thermal response (decreased temperature sensitivity of respiration (compensatory), increased temperature sensitivity of respiration (enhancing), and no change in the temperature sensitivity of respiration (no response)) was determined. To increase our understanding of the respiratory thermal responses, the growth response of the basidiomycete species to temperature was also measured.

Following cooling, individual species of basidiomycetes showed an overall enhancing response, with no compensatory responses identified. Two-species

assemblages and the three-species assemblage showed no thermal responses overall to cooling, but with some evidence of compensatory and enhancing responses. Semi-natural wood decomposing communities showed no thermal response overall to cooling, with more enhancing than compensatory responses detected. With warming, two-species assemblages showed no thermal response overall, with more compensatory than enhancing responses detected, whereas the three-species assemblage that was dominated by one species towards the end of incubation demonstrated a compensatory response overall. The compensatory responses from the two- and three-species assemblages were likely caused by exceeding the optimum temperature for growth for some species or by the differences in the progression of the species interactions. Therefore, there was little evidence of compensatory responses that would decrease the temperature sensitivity of respiration. Semi-natural wood decay communities showed no thermal response overall to warming, but some enhancing responses were identified.

The findings showed that the temperature sensitivity of wood decomposition was increased when basidiomycetes were grown alone, however, this was reduced during competitive interspecific interactions between basidiomycetes and species in wood decomposing communities. With increasing global temperatures, individual species, growing alone during the early colonisation and decomposition of wood and in decay columns in stable wood communities, may increase their respiration, but simple communities of interacting basidiomycetes and more diverse wood decomposing communities in natural systems, will more often cause no change in the temperature sensitivity of respiration overall. However, the species present in communities will most likely determine the direction and strength of respiratory responses to temperature, and thus the overall temperature sensitivity of respiration of wood decay communities. With limited evidence for compensatory responses and more evidence of enhancing responses detected, it is, thus, considered unlikely that the temperature sensitivity of wood decomposition will decline as global temperatures rise. Therefore, there remains the potential for a positive feedback to climate change through increased wood decomposition with warming.

Acknowledgements

I would like to take the opportunity to say thank you to my amazing supervisory team: Professor Iain Hartley, Professor Lynne Boddy and Associate Professor Lucy Rowland. I feel extremely privileged to have worked with you all; it has made my PhD an incredibly positive and rewarding experience. Your enthusiasm, exceptional scientific knowledge and continuous support have motivated and informed my progression throughout. Iain, thank you for your infinite scientific guidance and feedback, reassurance and patience when answering my long emails and constant questions! Lynne, thank you for allowing me to continue my interest in fungi that was initiated during my undergraduate degree, all of your incredible fungal knowledge and advice, and interesting fungi lunch talks. Lucy, thank you for all your feedback, guidance and kind words. I would like to thank Natural Environment Research Council GW4+ Doctoral Training Partnership for funding this research and training courses.

Furthermore, I would like to thank the Geography Technical Team: Joana Zaragoza-Castells, Angela Elliott, Karen Leslie, Rebecca Nicholas and Mandy Lee, for making the Geography department a welcoming and enjoyable place to work. Particular thanks to Joana and Ang for all of your support, guidance and positivity. You always went above and beyond to help me, in and out of lab hours, and I will always appreciate it. Particular gratitude to Ang for coming into labs early and leaving late on many occasions so I was able to complete my lab work. Ang, thank you for your amazing technical support with the fungal biomass assays and for keeping me calm!

I truly appreciated the support of the Biosciences Teaching Technical Team: Heather Ford, Claire Courtney-Baker, Laura Biddle, Cameron Clark, Emily Wong and Charlotte Lopez. Thank you for allowing me to constantly use your autoclaves, Class 2 cabinets and -80 °C freezer, and spread all of my plastic deli pots, Petri dishes, agar and fungal cultures everywhere in your small prep room! Thank you for always making me feel welcome, for the friendly chats and scientific advice. Thank you to Debbie Salmon for your exceptional support with the fungal biomass assays. I would not have been able to obtain these results

without your extensive HPLC and general scientific knowledge. Thank you to Jade O'Leary for your fungal knowledge and advice over the years.

Thank you to my Exeter friends, particularly Gen Hinde, Alex Whittle and David Bartholomew, for all the fun times, chats, support and encouragement. Thank you to Exeter City Korfball Club that kept me active and occupied playing Korfball matches, and for the many new friends I made, particularly Mike Gilpin and Jenna Atkinson, for the enjoyable and memorable walks and days out. Thank you to everyone at 29 Clifton Hill for your friendships over the last few years. To my Cardiff friends, I am eternally grateful for your special friendships, particularly Kirsty Franklin, Louise Burgess, Zoe Melvin, Jenna Murray and Tarryn Maynard. Your support from our undergraduate days to present day have carried me along this journey. A big thank you to the best Mum and Dad, for all of your love, support and encouragement throughout my whole life! Finally, to my siblings, Lucy, Tim, Emma and Toby (and of course our dogs Wilson and George), I thank you for your wisdom, kindness and constant fungal jokes.

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List of Abbreviations

ANOVA	Analysis of variance
Ba	*Bjerkandera adusta*
C	Carbon
CH_4	Methane
CO_2	Carbon dioxide
Cp	*Chondrostereum purpureum*
DAD	Diode-array detector
dH_2O	Distilled water
ESMs	Earth system models
Ff	*Fomes fomentarius*
Hf	*Hypholoma fasciculare*
H_2O	Water vapour
HPLC	High-performance liquid chromatography
IRGA	Infrared gas analyser
KOH	Potassium hydroxide
MA	Malt agar
N	Nitrogen
N_2O	Nitrous oxide
O_3	Ozone
Pv	*Phanerochaete velutina*
QCs	Quality controls
Rb	*Resinicium bicolor*
RCPs	Representative Concentration Pathways
RR_{CC}	Response Ratio for control versus cooled
RR_{CR}	Response Ratio for control versus rewarmed
RR_{CW}	Response Ratio for control versus warmed
R_{mass}	Mass-specific respiration
R_{mass} RR_{CC}	Mass-specific respiration Response Ratio for control versus cooled
SE	Standard error
Sh	*Stereum hirsutum*
Tv	*Trametes versicolor*
Vc	*Vuilleminia comedens*

VMM	Vogel’s minimum medium
UV	Ultraviolet

Chapter 1. General introduction and literature review: Climate change, respiratory thermal response of heterotrophic soil microorganisms and wood decay fungi

1.1 Climate change

Greenhouse gases in the atmosphere absorb and emit solar radiation in the thermal infrared range, warming the Earth's surface causing a natural greenhouse effect (Cubasch *et al.*, 2013). The greenhouse effect occurs in the troposphere and is crucial for life on Earth (Cubasch *et al.*, 2013). The primary greenhouse gases in the Earth's atmosphere are water vapour (H_2O), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and ozone (O_3) (Cubasch *et al.*, 2013). Human activities, however, are substantially increasing the atmospheric concentrations of the greenhouse gases CO_2, CH_4 and N_2O, enhancing the greenhouse effect and hence increasing global temperatures (Cubasch *et al.*, 2013). The increase in atmospheric CO_2, CH_4 and N_2O concentrations since pre-industrial times is the main driving cause of current climate change (Ciais *et al.*, 2013). In 2020, the global annual mean concentrations of atmospheric CO_2, CH_4 and N_2O were 412 parts per million (ppm), 1879 parts per billion (ppb), and 333 ppb (Dlugokencky and Tans, 2021), respectively, representing increases of ~48%, ~160% and ~23% since 1750, the beginning of the Industrial Era (Joos and Spahni, 2008). The rise in CO_2, CH_4 and N_2O is a result of anthropogenic emissions from fossil fuel combustion and land use change (Tans, 2009; Ciais *et al.*, 2013). Of these anthropogenic greenhouse gases, it is the increase in the atmospheric concentration of CO_2 that has contributed the most to the positive radiative forcing, that is, uptake of energy by the climate system, and is, therefore, the major driving force of increasing temperature and contributes the most to overall climate change (IPCC, 2013). Consequently, from 1850-1900 to 2017, global surface temperatures have warmed by approximately 1 °C (0.8 °C – 1.2 °C), increasing at 0.2 °C (0.1 °C – 0.3 °C) per decade (Allen *et al.*, 2018). Four scenarios, known as Representative Concentration Pathways (RCPs), have been developed to predict greenhouse gas emissions and climate warming by 2100: RCP2.6 (indicating a 2.6 watts per metre squared – W/m^2 – forcing increase relative to pre-industrial conditions), RCP4.5, RCP6.0, and RCP8.5 (IPCC, 2013). Increasing global mean surface

temperatures for 2081–2100 relative to 1986–2005 are projected to be in the ranges derived from the concentration-driven CMIP5 model simulations, that is, 0.3 °C to 1.7 °C (RCP2.6), 1.1 °C to 2.6 °C (RCP4.5), 1.4 °C to 3.1 °C (RCP6.0), 2.6 °C to 4.8 °C (RCP8.5) (IPCC, 2013). We are currently closest to following RCP8.5, the most serious of the RCPs, projecting the largest increase in global mean surface temperatures by 2100 (IPCC, 2013).

Rising global temperatures are influencing other key environmental variables, including changes in precipitation patterns, increases in humidity and the intensity and frequency of extreme weather events (IPCC, 2013). Although all of these environmental variables are predicted to increase with warming, there is much less certainty and confidence in their magnitude of change and long-term predictions (IPCC, 2013). Continuing to refine our knowledge on atmospheric CO_2 concentration and the consequent increases in global temperatures will improve future predictions of these other environmental variables. Future changes in atmospheric CO_2 concentrations depend strongly on anthropogenic emissions, but also on the proportion of emissions absorbed by the oceans and land surface, which currently absorb more than half of anthropogenic emissions (IPCC, 2013). However, there are concerns that climate change may affect the abilities of marine and terrestrial ecosystems to absorb CO_2. Thus, the future role of terrestrial ecosystems in reducing rates of warming is uncertain, hence, is critically important to understand.

1.2 The role of terrestrial ecosystems in regulating the carbon cycle and climate

The carbon (C) cycle and climate are tightly coupled; an increase in atmospheric CO_2 concentration leads to climate change, and climate change in turn affects the atmospheric CO_2 concentration, therefore the climate, atmospheric CO_2, and the C cycle form a feedback loop (Friedlingstein, 2015). The terrestrial biosphere plays a major role in regulating atmospheric composition and climate, and thus this feedback loop (Lovelock and Margulis, 1974). Globally, the terrestrial biosphere reservoir contains ~600 Pg C in organic compounds in vegetation living biomass (Ciais *et al.*, 2013) and ~2,500 Pg C in dead organic matter, in litter and the top 2m of soil (Köchy *et al.*, 2015). In comparison, only 828 Pg C is stored in

the atmosphere (Ciais *et al.*, 2013). Between 1750 and 2011, only ~50% of anthropogenic emissions (240 Pg C) was stored in the atmosphere due to the removal and storage of anthropogenic CO_2 into natural C cycle sinks (Ciais *et al.*, 2013). Terrestrial ecosystems accumulated 160 Pg of anthropogenic C between 1750 and 2011, therefore absorbing ~28% of the total anthropogenic CO_2 emissions (Ciais *et al.*, 2013). Between 1959 and 2019, 32 % of the total anthropogenic CO_2 emissions were stored in the land (Friedlingstein *et al.*, 2020). This uptake of atmospheric CO_2 into the terrestrial ecosystem is a result of plant photosynthesis exceeding the release of C back into the atmosphere through respiration and disturbance, consequently acting as a C sink (Friedlingstein *et al.*, 2020). This results in a negative CO_2-C cycle feedback, mitigating the atmospheric growth rate of CO_2 and reducing global warming (Friedlingstein, 2015; Friedlingstein *et al.*, 2020). The current capacity of terrestrial ecosystems to absorb atmospheric CO_2 and act as C sinks, and subsequently reduce global warming, however, may not continue in the long term. With global warming, respiration has the potential to be more temperature-sensitive than photosynthesis, which could stimulate the loss of C from terrestrial ecosystems to the atmosphere, forming a positive land C–climate feedback that could accelerate climate change (Heimann and Reichstein, 2008; Friedlingstein, 2015; Crowther *et al.*, 2016). Observations are evidencing a positive C-climate feedback, where warming is causing the release of C to the atmosphere, partially offsetting increases in terrestrial C sinks caused by rising atmospheric CO_2 concentrations (Friedlingstein, 2015). Earth system models (ESMs) simulate positive C-climate feedbacks over the 21st century, although with substantial uncertainty (Friedlingstein *et al.*, 2014). The interaction between global warming and soil respiration, and the strength of the land C–climate feedback, remains one of the greatest sources of uncertainty in future climate projections (Friedlingstein *et al.*, 2014). Observations are required to constrain the land C cycle sensitivity to warming and reduce model uncertainties. Determining the extent to which heterotrophic soil microbial respiration will increase with warming is critical to establishing the potential for terrestrial ecosystems to continue to act as C sinks, and in determining the magnitude of this positive land C-climate feedback, in order to predict future climate change.

Heterotrophic soil microorganisms, including fungi, bacteria and archaea, are

responsible for the decomposition of soil organic matter (Baldrian, 2017a). Heterotrophic soil microbial respiration, associated with the decomposition of organic matter, releases ~60 Pg of C per year to the atmosphere as CO_2, contributing to ~50% of C released from the terrestrial biosphere per year (Singh *et al.*, 2010). However, with increasing global temperatures, there is the potential for heterotrophic microbial metabolism, and therefore respiration through organic matter decomposition, to increase in soils, resulting in a substantial release of CO_2 into the atmosphere (Davidson and Janssens, 2006). Heterotrophic soil microorganisms have the potential to contribute extensively to the positive land C-climate feedback; hence, further understanding of their temperature-respiration relationship is required.

1.3 Temperature-respiration relationship of heterotrophic soil microorganisms

Respiration has a strong temperature dependence (Raich and Schlesinger, 1992). In the short-term (days-years), rates of soil organic matter decomposition, and therefore heterotrophic soil microbial respiration, is strongly and positively related to temperature (Kirschbaum, 1995, 2006; Davidson and Janssens, 2006), as seen in numerous field studies (Rustad *et al.*, 2001; Melillo *et al.*, 2002, 2017; Eliasson *et al.*, 2005; Wu *et al.*, 2011; Lu *et al.*, 2013; Hicks Pries *et al.*, 2017; Romero-Olivares *et al.*, 2017; Nottingham *et al.*, 2020). In the long-term (years-decades), however, the positive response of respiration to warming has declined after 2 or more years in past field studies (Rustad *et al.*, 2001; Eliasson *et al.*, 2005), and in some cases returned to pre-warming levels after 1 (Luo *et al.*, 2001), 5 (Jarvis and Linder, 2000; Eliasson *et al.*, 2005), or greater than 10 years (Oechel *et al.*, 2000; Melillo *et al.*, 2002, 2017; Romero-Olivares *et al.*, 2017). The mechanisms contributing to this decline in the positive response of heterotrophic soil microbial respiration with warming in the long term is uncertain and remain under scientific debate.

The direct effect of temperature on heterotrophic soil microorganisms can be explained by three mechanisms: (1) acclimation: physiological adjustments of individuals without any genetic change; (2) evolution: genetic changes within populations or species; and (3) species sorting: species already genetically better

adapted to a certain temperature regime outcompete less well-adapted species (Bárcenas-Moreno *et al.*, 2009; Bradford, 2013). Temperature, therefore, acts on the structure and function of cells and populations, and can produce both individual (acclimation) and community (evolution and species sorting) responses simultaneously. However, indirect effects of temperature, such as substrate availability and moisture, can also influence heterotrophic soil microorganisms (Davidson and Janssens, 2006; Bradford, 2013). Determining whether it is direct or indirect effects of temperature that are responsible for the decline in the positive response of heterotrophic soil microbial respiration with warming in the long term is crucial, as they have opposing consequences for climate change. If it is the direct effects of temperature on heterotrophic soil microorganisms that are responsible for the reduction in soil microbial respiration with warming, then this will maintain C sequestration in terrestrial ecosystems and weaken the positive C-climate feedback (Bradford, 2013). However, if it is indirect effects of temperature causing the reduction in soil microbial respiration with warming, which is predominantly attributed to substrate limitation due to the depletion of labile organic C pools by increased microbial activity (Bradford, 2013), then there will be diminished sequestration of recently plant-derived C, resulting in C losses from terrestrial ecosystems and the acceleration of climate change (Kirschbaum, 2004; Knorr *et al.*, 2005). Currently, most models ascribe the ephemeral response of heterotrophic soil respiration to elevated temperature to indirect mechanisms, specifically substrate depletion (Kirschbaum, 2004; Eliasson *et al.*, 2005; Knorr *et al.*, 2005), based on experimental (Hartley *et al.*, 2007) and observational support (Bengtson and Bengtsson, 2007). Other studies have argued, however, that the direct effect of temperature on soil microorganisms and substrate depletion are both responsible for the short-lived augmentation in heterotrophic soil microbial respiration in response to warming (Bradford *et al.*, 2008), and therefore that the potential for warming-induced C losses from soils is overestimated. The mechanisms behind the decline in heterotrophic soil microbial respiration with warming in the long term remain under intense scientific debate. Determining whether soil microbial responses to long-term warming compensate for the direct effects of the temperature change before substantial amounts of C are lost is critically important in understanding future rates of decomposition and C losses from terrestrial ecosystems, and ultimately reducing uncertainties regarding the feedback response with warming.

1.4 Respiratory thermal response of heterotrophic soil microorganisms

1.4.1 Terminology and definitions used for direct and indirect effects of temperature on heterotrophic soil microbial respiration

Terminology and definitions used for direct and indirect effects of environmental factors on soil microorganisms, specifically temperature on soil microbial respiration, vary greatly within soil biology (Table 1.1). The term 'acclimation' refers to the physiological adjustment of organisms in response to an environmental factor (Hochachka and Somero, 2002; Kirschbaum, 2004; Bradford *et al.*, 2008) (Table 1.1). The term 'thermal acclimation' was more widely used in the field of plant biology. Traditional studies on thermal acclimation of plants focused on the effect of temperature on individual growth rates (Hochachka and Somero, 2002), before exploring the effect of temperature on photosynthesis and respiration. Thermal acclimation studies of soil microorganisms, however, have primarily focused on the response of respiration to temperature. In plant biology, the term 'thermal acclimation' refers to strictly physiological changes, and is generally defined as the adjustment in the rate of a reaction to compensate for a change in temperature (Atkin and Tjoelker, 2003). In soil biology, 'thermal acclimation' and many other associated terms are used to represent the direct and indirect effects of temperature on soil microbial respiration (Table 1.1). The terminology, definitions, and the ecological-level at which terms are applied remain diverse and complicated in this research field.

In soil biology, 'thermal acclimation' most often refers to the subsequent adjustment in respiration rate to compensate for a change in temperature (Hartley *et al.*, 2008; Crowther and Bradford, 2013), following Atkin and Tjoelker (2003) (Table 1.1). 'Thermal acclimation' refers to physiological adjustments within generations, involving no genetic component (Bárcenas-Moreno *et al.*, 2009; Bradford, 2013). Therefore, the use of the term 'thermal acclimation' has been criticised when measuring the long-term response of microbial respiration to changes in temperature at the community-level, due to the potential for genetic changes to contribute to adjustments in respiration rates (e.g. Hartley *et al.* (2008)). 'Thermal acclimation' should only be used when studying physiological changes at the individual-level within a generation. Further, 'thermal

acclimatisation', often used when not referring to the mechanisms underlying the response (Table 1.1), is an inappropriate term to use at the community-level (Luo *et al.*, 2001). Responses of soil microorganisms to temperature at the community-level should be termed thermal adaptation to incorporate any genetic component.

The term 'thermal adaptation' refers to evolutionary change over multiple generations (species/genetic turnover) or beneficial *de novo* mutations, that manifest as physiological change (Hochachka and Somero, 2002) (Table 1.1). However, it is common for the term 'thermal adaptation' to be defined as an adjustment in respiration rate to compensate for a change in temperature (definition for 'thermal acclimation'), without stating the precise mechanisms underlying the adaptive response (Bradford, 2013). 'Thermal adaptation' should only be used when referring to changes taking place over generations, thus involving a change in genetic material (Hochachka and Somero, 2002). More recently, the term 'community-level response' has been applied when studying the respiration of soil microbial communities to temperature (Karhu *et al.*, 2014) (Table 1.1). This 'community-level response' can be either compensatory or enhancing, which refers to decreasing or increasing the effect of a temperature change (temperature sensitivity) on respiration rates respectively (Karhu *et al.*, 2014). A compensatory 'community-level response', therefore, is in the same direction as thermal acclimation, but may be driven by multiple mechanisms due to investigating at the level of the whole community. 'Thermal adaptation' or 'community-level response' are, therefore, more appropriate terms than 'thermal acclimation' when studying at the community-level.

The term 'apparent thermal acclimation' has been used when referring to reduced respiration rates by the indirect effects of temperature (Bradford, 2013), or a combination of indirect and direct effects of temperature (Tucker *et al.*, 2013) (Table 1.1). The indirect effects of temperature, therefore, are also important to acknowledge and must be controlled for when investigating the response of soil microbial respiration with warming.

Table 1.1 Terminology and definitions used for direct and indirect effects of environmental factors on soil microorganisms, specifically temperature on respiration.

Terminology	Definitions
Acclimation	Alteration of biochemical systems due to changes in the synthesis of cellular machinery that manifest as physiological change (Hochachka and Somero, 2002). Physiological adjustment by organisms to their altered environmental condition (Kirschbaum, 2004). Physiological adjustments of individuals (or organs) in response to a single environmental factor (Bradford *et al.*, 2008).
Acclimatisation	Approximately equivalent to the acclimation definition, however, is used in field studies, therefore may include non-physiological responses. The specific mechanism(s) underlying the response is not specified (Luo *et al.*, 2001).
Thermal acclimation	The adjustment in the rate of a reaction/respiration to compensate for a change in temperature (Atkin and Tjoelker, 2003), followed by Hartley *et al.* (2008) and Crowther and Bradford (2013). Intrinsic physiological changes to temperature (Tucker *et al.*, 2013).
Thermal adaptation	Direct organism response to temperature across multi-generational timescales that manifest as physiological change. Evolutionary change over generations (species/genetic turnover - temperature acts on existing genetic variation among organisms to select those best adapted to grow at the new environmental temperature) or beneficial *de novo* mutations (Hochachka and Somero, 2002). Includes genetic changes within populations or species (Bradford, 2013).
Community-level response	A compensatory or enhancing response, which refers to decreasing or increasing the effect of a temperature change (temperature sensitivity) on respiration rates respectively (Karhu *et al.*, 2014). May include physiological adjustments of individuals (acclimation), genetic changes within populations or species (adaptation) and shifts in species composition (ecological) (Karhu *et al.*, 2014).
Apparent thermal acclimation	Indirect effects of temperature (e.g. reductions in soil organic matter or moisture availability) on respiration rates (Bradford, 2013). The combined effects of substrate depletion and changes in physiological properties on respiration rates (Tucker *et al.*, 2013).

As acclimation and adaptation have strict definitions (Bárcenas-Moreno *et al.*, 2009; Bradford, 2013), and the terminology and definitions used for the direct effects of temperature on heterotrophic soil microorganisms remain complicated in this research field, this thesis will use the term 'thermal response', which can be compensatory, enhancing or no response, and refers to a decrease, increase or no change in the effect of a temperature change (temperature sensitivity) on respiration rates respectively, following Karhu *et al.* (2014).

1.4.2 The importance of the respiratory thermal response of heterotrophic soil microorganisms with climate change

The major contributors to terrestrial respiration are plants, mycorrhizal fungi and decomposer microorganisms (Raich *et al.*, 2014). Compensatory thermal responses of these organisms could reduce the temperature sensitivity of respiration in the long term, and potentially ameliorate the predicted soil C losses with climate change (Luo *et al.*, 2001; Melillo *et al.*, 2002). True thermal acclimation of respiration, measured within individuals and expressed on a biomass basis, has been well documented in plants (Atkin and Tjoelker, 2003; Loveys *et al.*, 2003), and has been shown in mycorrhizal fungi (Heinemeyer *et al.*, 2006; Malcolm *et al.*, 2008), and in the fungal component of lichens (Lange and Green, 2005). Thermal acclimation of free-living heterotrophic soil microorganisms is much harder to study and even compensatory thermal responses (Table 1.1) remain controversial. Understanding the temperature sensitivity of all the major contributors to terrestrial respiration is required to predict the strength of the positive land C-climate feedback and enhance the robustness of ESMs to project future climate (Reich, 2010; Bonan and Doney, 2018; Arora *et al.*, 2020). Specifically, the wide range of microbial feedbacks hypothesised in models reflects the limited understanding of this important climate response and has confounded attempts to model the change in soil C under warming, producing contrasting modelling outcomes (Wieder *et al.*, 2013; Hagerty *et al.*, 2018). Accordingly, further empirical studies to determine with confidence whether the respiration of heterotrophic soil microorganisms show compensatory responses to warming, and the underlying mechanisms, is an essential research priority. Understanding the magnitude, timescales and mechanisms of potential respiratory thermal responses of heterotrophic soil

microorganisms are critical to include in ESMs to reduce uncertainty in model projections of terrestrial feedback to climate change (Wieder *et al.*, 2013).

1.4.3 Determining the respiratory thermal response of heterotrophic soil microorganisms

When increasing the temperature, for example in long-term field warming experiments, it is challenging to distinguish between the direct and indirect effects of temperature on heterotrophic soil microorganisms. That is, whether it is a compensatory thermal response or substrate depletion that is the cause of the decline in soil microbial respiration with warming in the long term, as both mechanisms will lead to reduced respiration. The direct effect of temperature on heterotrophic soil microorganisms can be determined when measured independently of any indirect effects of temperature, which can be achieved in controlled incubation experiments (Kirschbaum, 2006).

Past empirical studies have utilised different methods to control for substrate availability to identify thermal responses of soil microbial respiration following warming. For example, soil samples from a >15 year soil-warming experiment in a mid-latitude deciduous forest were supplied with ^{13}C-labelled sucrose to ensure non-limiting substrate conditions when incubated at three constant temperatures (10, 15 and 20 °C) (Bradford *et al.*, 2008). A different method involved soil types being provided with weekly additions of glucose as a source of labile C substrate, enough to ensure non-limiting substrate conditions without substantial accumulation, when exposed to three constant thermal regimes (10, 20 and 30 °C) (Bradford *et al.*, 2010). Thermal adaptation of the respiration of soil microbial communities in response to experimental warming was found, when sucrose (Bradford *et al.*, 2008) and glucose (Bradford *et al.*, 2010) provided non-limiting substrate availability. However, adding labile substrate can cause fluctuations in substrate availability and can select for microbial communities that favour labile substrate over complex organic matter. Alternatively, soil samples from a 3-year temperate grassland soil-warming experiment have been sieved to release physically protected soil organic matter to reduce differences in substrate availability between warmed and control soil samples (Hartley *et al.*, 2007). Sieving the soil reduced the differences in rates of respiration between warmed

and control soil samples, and prolonged incubation at a common temperature did not result in differences in respiration rates declining over time, suggesting it was not a compensatory thermal response that explained the lower rates of respiration in the warmed soil samples (Hartley *et al.*, 2007). Given the limitations and contrasting findings of previous studies, new approaches are needed to separate soil microbial respiratory thermal responses and substrate depletion.

An alternative method that has been applied to distinguish between a compensatory thermal response and substrate depletion, that avoids the issue of higher temperatures increasing the rate that readily-decomposable organic matter is lost and consequently controls substrate availability, is a cooling approach (Hartley *et al.*, 2008; Karhu *et al.*, 2014). The cooling approach determining a compensatory thermal response of respiration, however, also relies on thermal compensation being a reversible process. For example, at higher temperatures, thermal compensation results in a down-regulation of respiration, whereas at cooler temperatures, thermal compensation results in an up-regulation of respiration. Therefore, an up-regulation in respiration after applying the cooling approach identifies an organism's ability to down-regulate its respiration in response to warming. Two past studies have applied this cooling approach to study compensatory thermal responses (Hartley *et al.*, 2008; Karhu *et al.*, 2014). Firstly, soils were isolated from the field to eliminate C input. A pre-incubation period at the mean annual temperature experienced by the soil in the natural environment was conducted, where CO_2 fluxes were measured to determine the loss of the readily decomposable C, and therefore to ensure stable respiration rates, and consequently the control of substrate availability. The cooling approach involved four temperature treatments to determine a compensatory thermal response: pre-cooling (destructively sampled at the end of pre-incubation before cooling), cooled (*high-low* treatment), rewarmed (*high-low-high* treatment) and control (*constant high* treatment). Three potential responses could be observed: compensatory (decreased temperature sensitivity of respiration), enhancing (increased temperature sensitivity of respiration) and no response (Fig. 1.1). Compensatory responses decreased the effects of temperature change on respiration rates after cooling and enhancing responses increased the effects of temperature change on respiration rates after cooling. In the first study using subarctic soils only enhancing responses were observed,

where cooling further reduced respiration that was only reversed by re-exposure to warmer temperatures, therefore thermal compensation of microbial respiration in subarctic soils was not detected (Hartley *et al.*, 2008). In the second study, however, using 22 soils along a climate gradient from the Arctic to the Amazon, all three possible community-level responses were observed, including evidence of thermal compensation (Karhu *et al.*, 2014). Nevertheless, more statistically significant enhancing responses were observed, thus the temperature sensitivity of respiration was increased for the majority of soil microbial communities (Karhu *et al.*, 2014). The limited evidence of compensatory thermal responses suggests it is substrate depletion, and not soil microbial communities down-regulating their respiration rates, that is responsible for the decline in the initial positive response of soil respiration to increased temperatures in long-term warming studies (Hartley *et al.*, 2008; Karhu *et al.*, 2014). The detection of compensatory thermal responses, however, indicates that soil microbial communities have the ability to reduce their respiration rates in response to warming. Overall, the detection of all three responses (compensatory, enhancing and no response) continues to cause considerable uncertainty in how heterotrophic soil microbial communities will respond to warming, particularly as the mechanisms underlying these responses have proven challenging to identify.

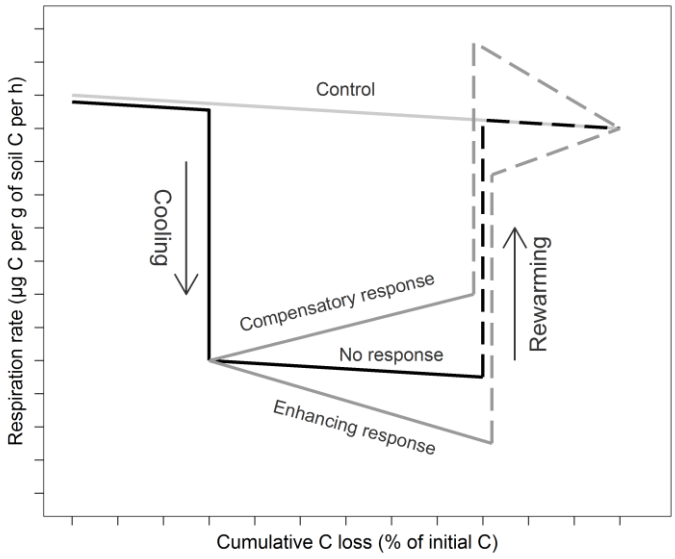


Figure 1.1 Schematic diagram showing the logic behind the cooling approach to determine thermal compensation and the three potential responses (compensatory, enhancing and no response) that could be observed, as in Hartley *et al.* (2008) and Karhu *et al.* (2014). A compensatory response decreases the temperature sensitivity of respiration and an enhancing response increases the temperature sensitivity of respiration.

Plants can thermally acclimate in response to temperature change (Atkin and Tjoelker, 2003), however, heterotrophic soil microorganisms are fundamentally different. Plants thermally acclimate to maintain a positive C balance (Atkin and Tjoelker, 2003), crucial for growth, tissue repair and acquisition of essential nutrients. Heterotrophic soil microorganisms do not have the physiological requirement to balance C fixation with utilisation. Therefore, heterotrophic soil microorganism growth not being constrained by C fixation, but by the availability of soil organic matter, suggests that it may not be advantageous for heterotrophic soil microorganisms to down-regulate their respiration rates (Hartley and Singh, 2018). Moreover, plant genome size and numerous gene copies provide a mechanism for physiological adjustment (Wendel *et al.*, 2016), whereas small genomes and low gene copies of microorganisms would constrain physiological adjustment (Land *et al.*, 2015). Currently, it is unclear as to what advantage heterotrophic soil microorganisms would gain from actively down-regulating their metabolism at high temperatures when thermal constraints on organic matter breakdown have been relaxed (Hartley *et al.*, 2008).

1.4.4 Respiratory thermal response of individual species of heterotrophic soil microorganisms need to be prioritised

Past research, mainly focusing on the respiratory response of whole soil microbial communities to temperature, has made any potential mechanisms involved difficult to identify. Studying at the community-level allows the respiration responses to arise through multiple mechanisms, i.e., ranging from the individual- to the community-level (as discussed previously) (Hochachka and Somero, 2002; Bárcenas-Moreno *et al.*, 2009; Bradford, 2013). Consequently, this has led to a limited mechanistic understanding of heterotrophic soil microbial respiratory responses to temperature, causing major uncertainty in the research field. To overcome this, future research would benefit from targeting particular components of the soil microbial community. Specifically, individual species of heterotrophic soil microorganisms that have dominant roles in the decomposition of soil organic matter could be investigated, to develop a mechanistic understanding of soil microbial respiratory responses to temperature that is not achieved from studying at the community-level. Species of heterotrophic soil microorganisms could be studied individually and in gradually more diverse

assemblages and communities, to gain a mechanistic understanding of their respiration response to temperature as the ecological-level builds. Studying individual species and more diverse assemblages and communities, whilst controlling substrate availability to detect compensatory thermal responses, will require the use of substrate and axenic cultures in the laboratory. Although this may limit the ability to make real-world predictions, it would enable the exploration of physiological mechanisms that cannot be isolated *in situ*, and may underpin important ecological patterns. Studying respiratory thermal responses from the individual-level to increasingly diverse assemblages and communities in the laboratory would identify the ecological importance of compensatory thermal responses. Identifying the temperature-respiration response of particular species of heterotrophic soil microorganisms could help to identify potential keystone species that dominate the whole soil microbial community respiratory response to temperature. Understanding the respiratory response of a variety of species of heterotrophic soil microorganisms could in turn help to identify ecosystems and thus C stocks that are more vulnerable or resistant to future warming.

1.5 Temperate forest ecosystems, wood decomposition and climate change: Wood decay fungi as a model organism to explore the respiratory thermal response of heterotrophic soil microorganisms

1.5.1 Temperate forest ecosystems and the accumulation of dead wood

Forests are the most extensive terrestrial ecosystem, covering approximately one third of the total land area (FAO, 2010) and storing 861 Pg C (Pan *et al.*, 2011). Temperate forests account for 25% of forest globally (Martin *et al.*, 2001) and store 14% of global C (Pan *et al.*, 2011). Present in North America, South America, Europe, Asia, and Australia–New Zealand, temperate forests include mixed deciduous, mixed coniferous, and temperate rainforests (Currie and Bergen, 2008), characterised by a pronounced seasonality of warm wet summers and mild winters (Martin *et al.*, 2001). Temperate forests are highly productive ecosystems, which contain a large diversity of woody plants and support a rich array of biota (Currie and Bergen, 2008). The climate in temperate regions, such as North America, Europe and Asia, is expected to warm, however, temperature changes at a regional scale remain uncertain (Bindoff *et al.*, 2013). The major

contribution of temperate forests to the terrestrial biosphere and C storage makes them an important ecosystem to understand in a changing climate.

Beech (*Fagus*), a genus comprising deciduous broadleaved tree species in the family Fagaceae, is widely distributed across the temperate areas of Europe, Asia, and North America (Denk, 2003). European beech (*Fagus sylvatica*), for example, is one of the most widespread deciduous broadleaved trees in Europe, forming extensive woodlands and dominating over a large altitudinal range (Packham *et al.*, 2012). European beech has expanded its range to cover almost the whole of the British Isles and southern, central and western Europe (Packham *et al.*, 2012; Caudullo *et al.,* 2017). The extensive range of European beech, and the subsequent accumulation and decay of dead wood, makes this tree species an ecologically important model to interpret responses to global warming.

Dead wood is a key component in natural forests, and globally stores 73 Pg C, 8% of the global forest C stock (Pan *et al.*, 2011). The accumulation of dead wood varies largely among different forests, with volumes in natural forests reaching up to 1200 m^3 ha^{-1}, which can exceed the biomass of living trees (Baldrian, 2017b). Dead wood comprises fine woody debris (diameter 1-10 cm), including tree and shrub branches and roots, and coarse woody debris (diameter > 10 cm), which in turn includes fallen trees and large branches (Rayner and Boddy, 1988). The latter represents the majority of dead wood (Baldrian, 2017b). The impermeability, high lignin content and low nitrogen (N) concentration of dead wood make it resistant to rapid penetration by microorganisms (Rayner and Boddy, 1988). Dead wood forms major structural features with essential ecological functions in energy flow, nutrient cycling and sequestration, and in the enhancement of forest biodiversity by forming habitats for saproxylic organisms such as invertebrates, fungi, bryophytes and lichens, together with birds and mammals (Harmon *et al.*, 1986). Dead wood influences forest microclimate, stores water that sustains vegetation during dry periods, and provides a continuous supply of organic material to the soil organic layer (Harmon *et al.*, 1986), overall enhancing forest productivity. The effect of increasing temperatures on dead wood decomposition, and therefore the release of CO_2 via respiration of dead wood decomposers, could have a major influence on dead wood accumulation and its role as a C store.

1.5.2 Wood decomposition in a changing climate

Wood decomposition is an important component of global C cycling, releasing 2.1-11 Pg C as CO_2 each year in forest ecosystems (Bani *et al.*, 2018). The decomposition of wood is regulated by abiotic conditions, particularly temperature and moisture, and by the biotic communities that mediate decomposition (Rayner and Boddy, 1988; Baldrian *et al.*, 2013a). Warming and altered precipitation regime expected with climate change will have major influences on wood decomposition (Boddy, 1983a; Davidson and Janssens, 2006). Temperature and moisture effects on wood decomposition are complex (Boddy, 1983a; A'Bear *et al.*, 2014). While a reduction in moisture has been observed to decrease wood decomposition rate, an increase in temperature has been observed to compensate for the negative effect of drying and increase wood decomposition rate (A'Bear *et al.*, 2014). Additionally, warming has been observed to increase wood decomposition in other studies (Rubenstein *et al.*, 2017; Edman *et al.*, 2021), making the effect of temperature, in particular, on wood decomposition important to understand in a changing climate. Warming has both direct and indirect effects on decomposition, through increasing the metabolism of microbial decomposers (Schimel and Schaeffer, 2012; Baldrian *et al.*, 2013a), and altering the microbial community composition (Allison and Martiny, 2008; de Vries *et al.*, 2012; Keiser and Bradford, 2017), respectively. Wood decay fungi are the dominant decomposers of wood (Rayner and Boddy, 1988), and there is emerging evidence that the fungal community composition, instead of climate, is more important in governing wood decomposition (A'Bear *et al.*, 2012; Bradford *et al.*, 2014; van der Wal *et al.*, 2015; Hiscox *et al.*, 2016a; Venugopal *et al.*, 2016, 2017; Maynard *et al.*, 2018; O'Leary *et al.*, 2019; Edman *et al.*, 2021). Therefore, the response of the dominant decomposers of wood, wood decay fungi, to temperature, and the potential effect this could have on fungal community composition, is key to determining wood decomposition rates and the role that dead wood has on C storage in a changing climate.

1.5.3 The role of wood decay fungi in the C cycle

Wood decay fungi are primary decomposers of dead wood in temperate forest ecosystems (Rayner and Boddy, 1988; Boddy and Watkinson, 1995; Baldrian

and Lindahl, 2011). White rot basidiomycetes account for over 90% of all wood decay fungi (Janusz *et al.*, 2017), and can decompose the major wood polymers (cellulose, hemicellulose and lignin) (Rayner and Boddy, 1988; Blanchette, 1995). Cellulose microfibrils are embedded in a lignin matrix, forming a lignocellulose complex that is physically and chemically resistant to degradation (Rayner and Boddy, 1988; Blanchette, 1995). Although it is the decomposition of cellulose, hemicellulose and the nutrient reserve polysaccharide starch that provides C and energy for growth, access to these substrates by the majority of wood decay fungi requires lignin decomposition (Baldrian, 2008). The unique ability of white rot basidiomycetes to efficiently decompose lignocellulose and to penetrate bulky wood resources allows them to dominate wood decomposition (Eichlerová *et al.*, 2015). White rot basidiomycetes, therefore, as the only organisms known to extensively mineralise lignin and thus wood completely (van der Wal *et al.*, 2015), are ecologically important.

Hyphae, long branching filaments that make up the mycelium, the main body of wood decay fungi, interact with the wood resource quality (physical: e.g. hardness, toughness, porosity, size; chemical: e.g. nutrients, vitamins, extractives) and microclimate (temperature, moisture, gaseous regime and pH), causing physical and chemical modifications, and varying rates of decay (Rayner and Boddy, 1988; Freschet *et al.*, 2012). The mycelium acts as both a reservoir and distribution system of essential nutrients (Watkinson *et al.*, 2006). Many wood decay fungi are only able to spread to new resources as spores (resource-unit-restricted), however, some can grow out of wood in search of new resources in the form of mycelial cords (non-resource-unit-restricted) (Boddy and Heilmann-Clausen, 2008), which are aggregations of predominantly parallel, longitudinally aligned hyphae (Boddy, 1993). These cords form extensive, long-lived systems which interconnect discontinuous woody resources across the soil-litter interface, having an essential role in C sequestration, as well as nutrient cycling, in ecosystems (Boddy, 1999). The major role of wood decay fungi, specifically white rot basidiomycetes, in C sequestration, in particular, requires greater understanding of their respiratory thermal response, and therefore wood decomposition, under climate change to better predict C distribution, and ultimately any climate-ecosystem C feedback.

1.5.4 Wood decay fungi as a model organism to explore the respiratory thermal response of heterotrophic soil microorganisms

As the primary decomposers of wood, wood decay fungi contribute considerably to the ~60 Pg C emitted by heterotrophic soil microorganisms each year (Reay *et al.*, 2007; Singh *et al.*, 2010). The respiratory response of wood decay fungi to warming, therefore, will represent a major component of the total respiratory response of heterotrophic soil microorganisms to warming. Wood decay fungi are dominant on the floor of deciduous woodlands, occupy large volumes of discrete woody substrata, grow out of wood through the soil and have the ability to decompose wood completely (Rayner and Boddy, 1988; Boddy, 1993). This, together with their major functional significance in wood decomposition (Boddy, 1993), make wood decay basidiomycetes a biologically significant model to explore the respiratory thermal response of heterotrophic soil microbial species, assemblages and communities.

Past studies have begun to investigate whether individual fungal species can acclimate (physiological adjustment of an individual without any genetic change; Table 1.1) to temperature. The fungal component of lichens under near-natural conditions has shown acclimation to temperature by the adjustment of respiration to seasonal changes in temperature (Lange and Green, 2005). Arbuscular mycorrhizal fungi in soil were observed to acclimate to temperature by reduced respiration rates after warming (Heinemeyer *et al.*, 2006). Some species of free-living ectomycorrhizal fungi on potato dextrose agar exhibited the ability to acclimate to temperature as evidenced by reduced respiration rates for warmer-incubated fungi compared with cooler-incubated fungi (Malcolm *et al.*, 2008). Intact ectomycorrhizal sporocarps showed evidence of acclimation, where mass-specific respiration at 10 °C declined with increasing temperature (Lilleskov, 2017). Nevertheless, *Neurospora discreta*, a globally distributed saprotrophic ascomycete fungus present in soils, was grown on Vogel's minimum medium (VMM) agar at 16 °C and 28 °C, both greater than the mean annual temperatures experienced by *Neurospora* strains (Romero-Olivares *et al.*, 2015). At both temperatures, although a reduction in mycelial growth rate and biomass was observed, an increase in mass-specific respiration was found for adapted strains on VMM agar compared to parental strains, consequently, mass-specific

respiration did not acclimate to temperature (Romero-Olivares *et al.*, 2015). Moreover, *Neurospora discreta* was grown on VMM agar at 16 °C and 22 °C and then inoculated onto sucrose or lignin, but no evidence of acclimation was found as respiration increased at the higher temperature (Allison *et al.*, 2018). However, individual cord-forming wood decay basidiomycetes grown on 2% malt agar (MA) at 12 °C, 20 °C and 28 °C, temperatures commonly experienced in temperate woodlands, acclimated to temperature (Crowther and Bradford, 2013). In almost all cases, warm-acclimated individuals had lower growth and mass-specific respiration rates at intermediate temperatures than cold-acclimated isolates (Crowther and Bradford, 2013). Many of the studies, particularly those investigating free-living heterotrophic soil microorganisms, used agar, an artificial medium which, although useful for producing a simplified model system to perform manipulative experiments, provide homogeneous, well-mixed conditions that inherently fail to imitate the structural or chemical heterogeneity of most natural resources (Crowther *et al.*, 2018). For this reason, it is crucially important to investigate the response of individual fungal species decomposing a biologically meaningful substrate to temperature, rather than when grown on agar. The respiratory thermal response of wood decay fungi decomposing natural substrate has not been investigated and is, therefore, required to more closely replicate natural systems.

1.6 Wood decay basidiomycetes

Fungal species vary in their life history (ecological) strategy (ruderal, combative, stress-tolerant; Table 1.2), which they have evolved to cope with the environment they inhabit (Boddy and Hiscox, 2016). Fungal species may have characteristics associated with one or multiple ecological strategies (Table 1.2), which may vary during different life cycle stages, or between physiologically different regions of the same mycelia (Boddy and Hiscox, 2016). The differences in ecological strategies largely determine the ability of fungal species to obtain initial access to uncolonised resources (primary resource capture), compete with resource colonisers (secondary resource capture) and retain captured territory within the resource (Boddy and Hiscox, 2016). The ecological strategies of fungal species determine the stage of community development at which they are present, and thus fungal species can be categorised into three non-mutually exclusive groups:

primary, early secondary, and late secondary colonisers (Boddy and Hiscox, 2016). Primary colonisers are involved in the pioneer stage of community development and obtain initial access to uncolonised resources (Cooke and Rayner, 1984). They commonly show ruderal and/or stress-tolerant characteristics (Table 1.2) and colonise standing dead wood from propagules that are latently present in functional sapwood (Boddy *et al.*, 2017). Those that colonise felled wood have predominantly ruderal characteristics (Boddy and Heilmann-Clausen, 2008) (Table 1.2). Their effective dispersal, rapid spore germination, and ability to exploit organic compounds in uncolonised resources allow their successful colonisation (Boddy and Heilmann-Clausen, 2008). Primary colonisers remaining within the resource are dependent on their combative ability and abiotic regimes continuing to favour their growth. Secondary colonisers are involved in later stages of community development and typically show combative characteristics (Boddy and Heilmann-Clausen, 2008) (Table 1.2). The success of early and late secondary colonisers is usually dependent on combative antagonistic mechanisms during interactions to gain territory from primary and/or secondary colonisers (Boddy, 2000). Late secondary colonisers often form mycelial cords (Boddy, 1993). Very late secondary colonisers typically show stress-tolerant characteristics (Boddy and Heilmann-Clausen, 2008) (Table 1.2).

Although during the early stages of colonisation and in stable communities, where decay columns of individual wood decay basidiomycetes occur in a resource (Boddy *et al.*, 2017), wood decay basidiomycetes also experience many types of interactions, with the most common interaction being competition with other wood decay fungal species for territory and the resources within (Boddy, 2000). Within fungal communities, hierarchies of combative ability occur where late secondary colonisers > early secondary colonisers > primary colonisers (Boddy, 2000). However these relationships are not always transitive, where species A is more combative than species B, and both are more combative than species C (A>B>C) (Boddy, 2000). Intransitive hierarchies can also occur where species A is more combative than species B, species B is more combative than species C, but species C is more combative than species A (A>B, B>C, C>A) (Boddy, 2000). This is because species operate different attack and defence mechanisms in different combinations (Hiscox and Boddy, 2017). It is these interspecific fungal

interactions that largely determine fungal community structure and development (Boddy and Heilmann-Clausen, 2008), which affect decay rate and C turnover in woodland ecosystems (Owens *et al.*, 1994; Boddy, 2000).

Table 1.2 Characteristics defining the life history strategies of ruderal, combative and stress-tolerant fungal species. Adapted from Boddy and Hiscox (2016).

Characteristics	Ruderal	Combative	Stress-tolerant
Characteristic features	Rapid growth; primary resource capture	Antagonistic ability against competitors	Tolerance of specific stresses (e.g. temperature extremes, low water availability, pH extremes, allelopathic chemicals)
Growth rate	Rapid spore germination and growth	Growth not particularly slow	Growth sometimes slow
Enzymatic ability	Relatively narrow ability	Wide ability	Wide ability
Substrates utilised	Easily assimilable	More recalcitrant	More recalcitrant
Commitment of biomass to reproduction	Rapid and substantial	Relatively low	Relatively low
Persistence within the resource	Low; easily replaced	Persistence depends on ability to capture and defend territory	Persistent while specific stress remains

1.7 Conclusions

The terrestrial biosphere is an important C store and has a major influence on the atmospheric composition and climate. An increase in global temperature has the potential to increase rates of heterotrophic soil microbial respiration through increased decomposition of soil organic matter. The redistribution of C from the terrestrial ecosystem into CO_2 in the atmosphere has the potential to generate a positive land C-climate feedback and accelerate climate change. In the short-

term, heterotrophic soil microbial respiration is strongly and positively related to temperature, however, the response of respiration to warming in the long term is uncertain. Whether heterotrophic soil microorganisms will show compensatory or enhancing thermal responses in the long term remains a major uncertainty, and has been very hard to determine in complex communities, stimulating focus towards the response of individual heterotrophic soil microorganisms. The respiratory response of wood decay basidiomycetes to temperature will act as a biologically significant model to develop a mechanistic understanding and to better predict the respiratory response of heterotrophic soil microorganisms to warming, an essential requirement to project feedbacks between soil C stocks, atmospheric CO_2, and climate change.

1.8 Thesis aims

This thesis describes a set of studies that aims to determine whether wood decay basidiomycetes, a particular component of the soil microbial community, that decomposes beech wood (*Fagus sylvatica*), a single type of organic matter, produce compensatory respiratory thermal responses. This thesis sets out to develop a mechanistic understanding of respiration responses to temperature by studying: (1) individual species and (2) two- and three-species assemblages of basidiomycetes decomposing wood, and (3) semi-natural wood decomposing communities (Fig. 1.2). The respiration responses of wood decay basidiomycetes to temperature can be used to better predict global warming effects on CO_2 release from terrestrial ecosystems. A general hypothesis is proposed: individual species and two- and three-species assemblages of basidiomycetes decomposing wood, and semi-natural wood decomposing communities, show compensatory thermal responses overall and decrease the temperature sensitivity of respiration in the medium to long term. This is explored in the following experimental chapters.

Chapter 2 investigates the growth response of nine basidiomycete species on agar to temperature, by measuring the radial extension. The nine species studied are used in the experimental chapters that follow.

Chapter 3 explores the ability of nine species of basidiomycetes colonising beech wood, that represent those present in communities at different stages of decay, to compensate for the direct effects of a temperature change, by measuring the CO_2 production from respiration under constant thermal regimes. The ability of individual species and each ecological role (primary colonisers, early secondary colonisers and late secondary colonisers) to show thermal compensation are assessed. The fungal biomass (estimated as ergosterol content) response to temperature and thus mass-specific respiration (R_{mass}) are determined.

Chapter 4 follows by investigating the ability of basidiomycetes in two- and three-species assemblages, colonising beech wood in 2- and 3-dimensional competitive systems respectively, to compensate for the direct effects of a temperature change, by measuring the CO_2 production from respiration under constant thermal regimes.

Chapter 5 explores the capacity of beech wood blocks that are originally uncolonised or pre-colonised by a single basidiomycete species, and then exposed to naturally occurring decomposer communities in a temperate woodland, to show compensatory thermal responses of respiration. The CO_2 production from respiration under constant thermal regimes is assessed.

Chapter 6 synthesises the findings of this thesis and discusses ergosterol as a biomarker for fungal biomass. The implications of the respiratory thermal responses of basidiomycetes decomposing wood and wood decomposing communities in the long term and for long-term field-based soil warming experiments, as well as for modelling microbial processes to improve global soil C projections, are discussed. Furthermore, future research priorities and the implications and conclusions of this research are stated.

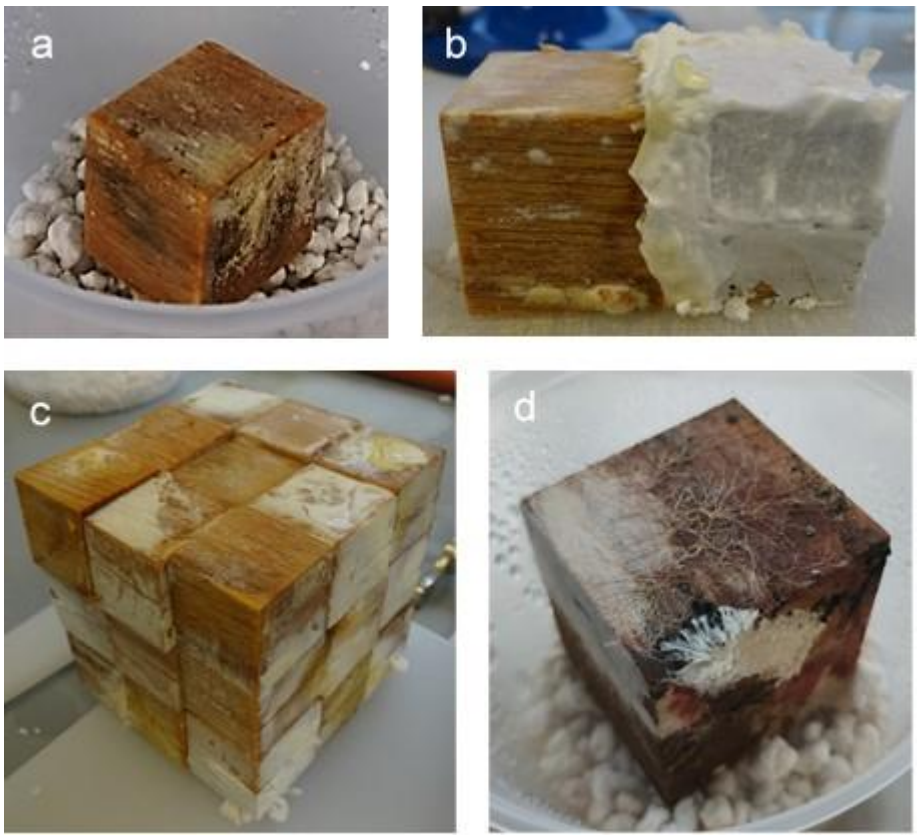


Figure 1.2 Photographs of experimental designs used in this thesis. (a) Individual basidiomycete species colonising beech wood (*Fagus sylvatica*) blocks, used in Chapter 3. (b) Two species of basidiomycetes interacting in a 2-dimensional wood block system, used in Chapter 4. (c) Three species of basidiomycetes competing in a 3-dimensional wood block system, used in Chapter 4. (d) Semi-natural wood decomposing communities, used in Chapter 5.

Chapter 2. Growth response of wood decay basidiomycetes to temperature

2.1 Abstract

The growth response of wood decay basidiomycetes to temperature has a crucial impact on their function and distribution, thus their ecological role in temperate woodlands. Understanding the growth response of wood decay basidiomycetes to temperature will aid our interpretation of the respiratory thermal responses observed in the succeeding experimental chapters. The extension rate of nine species of wood decay basidiomycetes on agar was measured at eight temperatures (10-35 °C). The extension rate and optimum temperature for growth varied between species, with extension rates ranging between 0.005 to 12.82 mm d^{-1} across all temperatures and species, and optimum temperature for species ranging between 25 and 30 °C. The growth responses on agar may not represent those in wood, however, they provide insights into the respiratory thermal responses of wood decay basidiomycetes in the following experimental chapters.

2.2 Introduction

Wood decomposition is determined by many abiotic factors, specifically temperature, and the wood decay fungi present (Rayner and Boddy, 1988; Baldrian *et al.*, 2013a). Temperature has a prominent role in the ecological niche of species of wood decay basidiomycetes: influencing growth rate, extracellular enzyme production and activity, and competitive ability, and consequently the rate of wood decomposition (Rayner and Boddy, 1988; Magan, 2008). The growth response of basidiomycetes to temperature vary between species, although most are mesothermic with minimum, optimum and maximum temperatures for growth around 5, 25 and 40 °C, respectively (Cartwright and Findlay, 1958; Magan, 2008). Furthermore, the optimum temperature for growth on agar for most temperate species of basidiomycetes is between 24 and 30 °C (Cartwright and Findlay, 1934; Boddy, 1983b). Determining the direct effects of temperature on the growth of the species of wood decay basidiomycetes that will be further studied in the subsequent chapters is important for understanding the thermal

responses of their respiration and associated wood decomposition in the medium to long term, which is the main aim of this PhD.

The aim of this study was to investigate the growth response of nine species of wood decay basidiomycetes on agar to temperature. Mycelial extension was measured over a range of temperatures that the species would experience in temperate woodlands (Boddy, 1983a; Magan, 2008), allowing extension rates at different temperatures and therefore the optimum temperature for growth to be obtained for each species. The opaque nature of wood makes investigating the growth response of fungi colonising their natural resource challenging. However, agar, an artificial medium, provided a transparent resource and consistent growing conditions which allowed multiple replicates and the standardised comparison of growth responses between temperatures and species. The growth response of basidiomycetes on agar, however, may not represent that in wood, but will provide insights into the thermal responses of respiration seen in the experimental chapters that follow.

2.3 Methods

2.3.1 Species of wood decay basidiomycetes

Nine species of native, beech (*Fagus sylvatica*)-inhabiting basidiomycetes (Table 2.1) from different stages of decay, that will be studied in the following experimental chapters, were used.

Table 2.1 Fungal species used.

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

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

2.3.2 Extension rates

Plugs (7 mm in diameter) were cut from the actively growing margin of 7 d old fungal cultures (Table 2.1) and individually inoculated onto 2% malt agar (2% MA: 20 g l^{-1} malt extract, 15 g l^{-1} agar; Lab M, UK) in the centre of 9 cm diameter Petri dishes (Sarstedt, Germany). Petri dishes were incubated at 10, 12, 15, 20, 25, 28, 30 and 35 °C for 1 d, then mycelial extension (mm) was measured along four radii extending from the edge of the inoculum plug over time until mycelia had reached the Petri dish edge. Five replicates at each temperature of each species were used.

2.3.3 Data analysis

Mycelial extension measurements taken within the first 10 mm from the inoculum plug and the last 10 mm from the Petri dish edge were removed, so only data during linear growth were analysed. The mean extension rate (mm d^{-1}) of each replicate (n = 5, per temperature per species) was estimated by linear regression, for each temperature and species. The relative extension rate (normalised to 10 °C) was calculated by dividing the mean extension rate (mm d^{-1}) of each replicate by the mean extension rate (mm d^{-1}) at 10 °C, for each temperature and species. The temperature producing the fastest extension rate (optimum temperature for extension rate) of each species was obtained and ratios of extension rate at 20 °C/12 °C, 28 °C/20 °C and 28 °C/12 °C of each species were calculated.

2.4 Results

2.4.1 Extension rates

Rates of extension varied across all temperatures and species, ranging between 0.005 to 12.82 mm d^{-1} (Fig. 2.1). *B. adusta*, an early secondary coloniser, had the fastest extension rate at all temperatures, and *H. fasciculare*, a late secondary coloniser, was consistently slower, with the slowest extension rate at four temperatures (15, 20, 25 and 28 °C) (Fig. 2.1). *R. bicolor*, *F. fomentarius*, *P. velutina* and *V. comedens* had the slowest extension rate at 10, 12, 30 and 35 °C, respectively (Fig. 2.1).

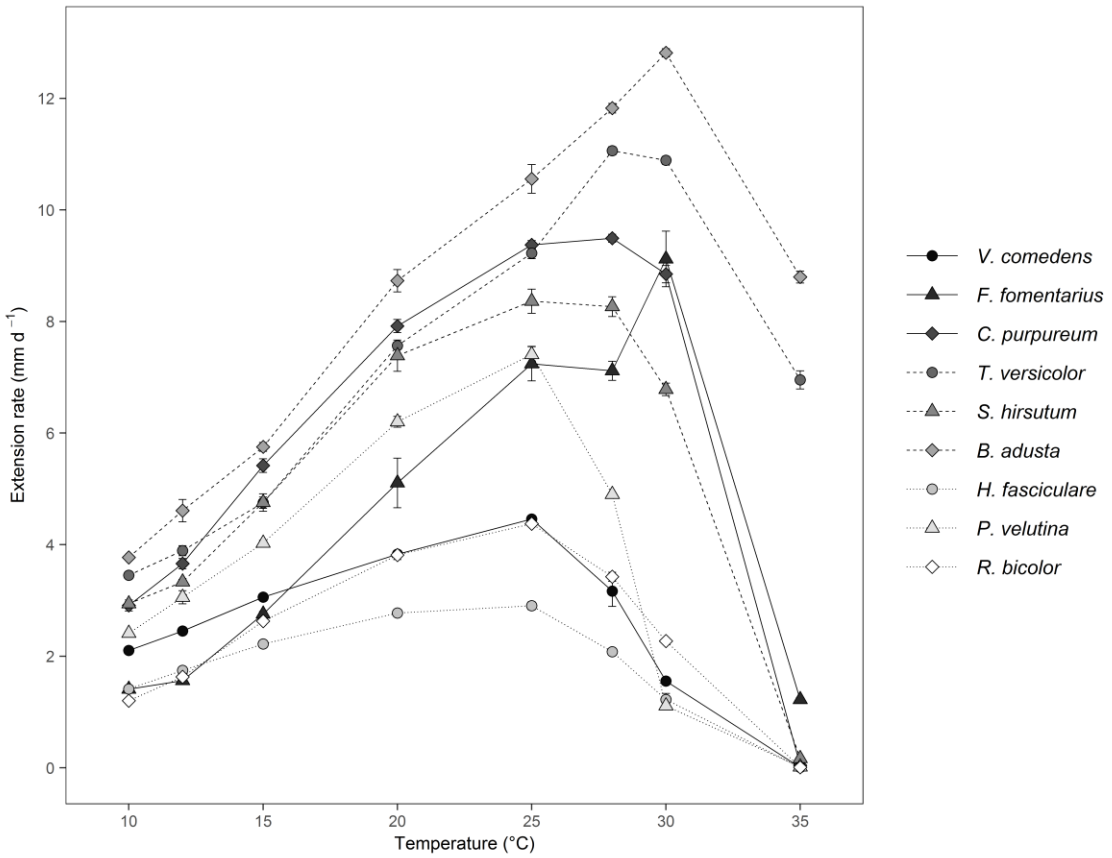


Figure 2.1 Effect of temperature on extension rate of nine species of wood decay basidiomycetes on agar. The mean (n = 5) ± SE of the mean is shown, error bars that are not visible are too small to be seen.

The relative extension rate (normalised to 10 °C) showed that the change in extension rate as the temperature increased varied between species (Fig. 2.2). Between 10 and 25 °C, *F. fomentarius* had the greatest relative increase in extension rate by a factor of 5.14, and *H. fasciculare* had the smallest relative increase in extension rate by a factor of 2.06 (Fig. 2.2). The relative extension rate decreased below 1 only at 30 and 35 °C (Fig. 2.2). *P. velutina*, *V. comedens* and *H. fasciculare* had relative extension rates lower than 1 at 30 °C, and *V. comedens*, *H. fasciculare*, *P. velutina*, *R. bicolor*, *C. purpureum*, *S. hirsutum* and *F. fomentarius* had relative extension rates lower than 1 at 35 °C (Fig. 2.2).

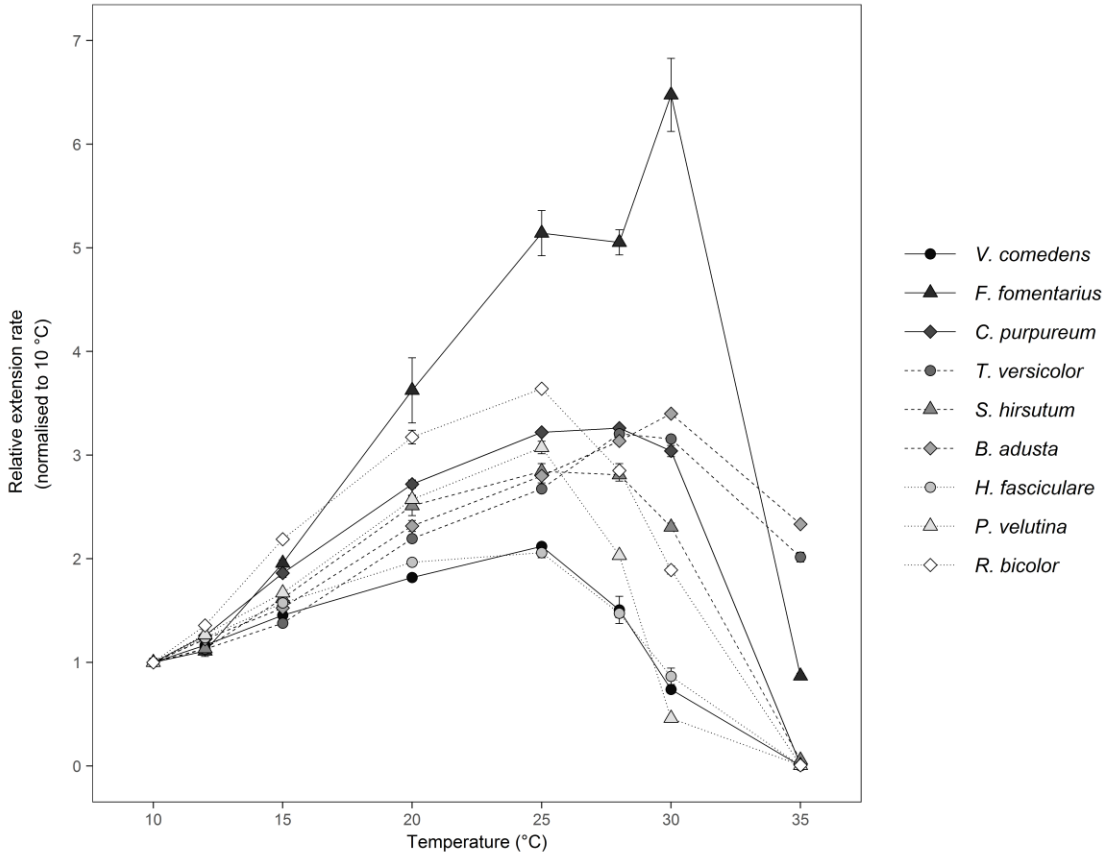


Figure 2.2 Effect of temperature on relative extension rate (normalised to 10 °C) of nine species of wood decay basidiomycetes on agar. The mean (n = 5) ± SE of the mean is shown, error bars that are not visible are too small to be seen.

The optimum temperature for extension rate was highest for *F. fomentarius* and *B. adusta* at 30 °C, followed by *C. purpureum* and *T. versicolor* at 28 °C, and then lowest for *V. comedens*, *S. hirsutum*, *H. fasciculare*, *P. velutina* and *R. bicolor* at 25 °C (Table 2.2). The extension rates at 20 °C were faster than at 12 °C for all species (Table 2.2). The extension rates at 28 °C were faster than at 20 °C for *F. fomentarius* and *C. purpureum*, which are primary colonisers, and for *T. versicolor*, *S. hirsutum* and *B. adusta*, which are early secondary colonisers, however, were slower for *V. comedens*, which is a primary coloniser, and *H. fasciculare*, *P. velutina* and *R. bicolor*, which are late secondary colonisers (Table 2.2). The extension rates at 28 °C were faster than at 12 °C for all species (Table 2.2).

Table 2.2 Optimum temperature for extension rate, and ratio of extension rate at 20 °C/12 °C, 28 °C/20 °C and 28 °C/12 °C, of each species.

Species	Optimum temperature for extension rate (°C)	Ratio of extension rate at 20 °C/12 °C	Ratio of extension rate at 28 °C/20 °C	Ratio of extension rate at 28 °C/12 °C
V. comedens	25	1.56	0.83	1.29
F. fomentarius	30	3.27	1.39	4.55
C. purpureum	28	2.16	1.20	2.59
T. versicolor	28	1.94	1.46	2.84
S. hirsutum	25	2.22	1.12	2.48
B. adusta	30	1.89	1.35	2.56
H. fasciculare	25	1.59	0.75	1.19
P. velutina	25	2.03	0.79	1.60
R. bicolor	25	2.34	0.90	2.10

The extension rate was expressed as mm per d.

2.5 Discussion

The optimum temperature for growth of the nine species of wood decay basidiomycetes was between 25 and 30 °C, as in previous studies (Cartwright and Findlay, 1934; Boddy, 1983b). A past study that investigated the extension rate of species of basidiomycetes on 2% MA found the same optimum temperature for growth for *V. comedens* (25 °C), *B. adusta* (30 °C) and *P. velutina* (25 °C), although different strains were used (Boddy, 1983b). The present study found a similar optimum temperature for growth for *T. versicolor* (28 °C, compared to 30 °C) but a higher optimum temperature for growth for *S. hirsutum* (25 °C, compared to 20 °C) than a previous study (Boddy, 1983b). The extension rates at the optimum temperature for growth in the present study were similar to those of the previous study for *V. comedens* (4.46 mm d^{-1}, compared to ~4 mm d^{-1}), *B. adusta* (12.82 mm d^{-1}, compared to ~12 mm d^{-1}) and *P. velutina* (7.41 mm d^{-1}, compared to ~5 mm d^{-1}) (Boddy, 1983b). The fastest extension rate in the present study compared to the previous study for *T. versicolor* (11.06 mm d^{-1}, compared to ~8 mm d^{-1}) varied slightly even though similar optimum temperatures for growth were found (Boddy, 1983b). Whereas the fastest extension rate for *S. hirsutum* (8.36 mm d^{-1}, compared to ~8.5 mm d^{-1}) was similar to the previous study even though different optimum temperatures for growth were observed (Boddy, 1983b). Additionally, the optimum temperature for growth for *F. fomentarius* (30 °C) was the same as observed by Fritz (1924), although a different strain was used. Moreover, the optimum temperature of *S. hirsutum*

(25 °C) in the present study was the same as another strain of the species used in a past study (Cartwright and Findlay, 1934), however the fastest extension rate of 8.36 mm d^{-1} in the current study was lower than the fastest extension rate of ~20 mm d^{-1} reported in the past study (Cartwright and Findlay, 1934). It is unclear why the extension rates of *S. hirsutum* differed at 25 °C, it is perhaps due to the different strains used or the extension rate of ~20 mm d^{-1} was not accurately measured, as it also differs from the fastest extension rate of *S. hirsutum* reported by Boddy (1983b).

The effect of temperature on extension rates and optimum temperature for growth varied between species of basidiomycetes, and this will be discussed in the context of the results presented in the following experimental chapters, where appropriate.

Chapter 3. Respiratory thermal response of individual species of wood decay basidiomycetes

3.1 Abstract

Heterotrophic soil microorganisms are responsible for ~50% of carbon (C) produced by respiration from the terrestrial biosphere each year. Elucidating their response to warming is, therefore, critical to understanding the role of terrestrial ecosystems in the future, which could shift from a C sink to a C source and increase the positive land C-climate feedback. The positive response of heterotrophic soil microbial respiration to warming declines in the long term (years-decades), which may be caused by thermal compensation or substrate depletion. Focus on complex soil microbial communities has made attributing mechanisms involved in controlling warming responses difficult. Therefore, understanding and predicting responses to warming remains challenging. To address this uncertainty, the respiratory thermal response of nine individual species of wood decay basidiomycetes colonising wood was investigated. Respiration responses to 90-days of cooling, that avoided the confounding factor of warming-induced substrate depletion, were determined. Thermal responses (compensatory, enhancing, no response), which indicated a decrease, increase and no change in temperature sensitivity of respiration respectively, were quantified. A rewarming treatment investigated the reversibility of responses and the comparability of responses to cooling and warming. Ergosterol content – an indicator of living fungal biomass – was measured to allow respiration rates to be expressed per unit biomass (mass-specific respiration; R_{mass}). Overall, in response to cooling, individual species of basidiomycetes showed an enhancing response, and no compensatory responses were observed. Therefore, the temperature sensitivity of the respiration increased over time. With rewarming there was overall no response, but two species were able to significantly recover their respiration rates to above control levels. However, these species did not originally show compensatory responses to cooling. When respiration was expressed per unit biomass the enhancing responses were largely lost, suggesting the enhancing responses were caused by the impacts of temperature on fungal growth and biomass production. Our results suggest that the respiration of wood decay basidiomycetes is highly temperature-sensitive and greater fungal

growth rates at higher temperatures will increase activity levels beyond the direct effects of temperature on respiration, potentially promoting greater C losses from terrestrial ecosystems and positive feedbacks to climate change.

3.2 Introduction

Heterotrophic soil microorganisms decompose organic matter and are already responsible for emitting ~60 Pg of C per year to the atmosphere as carbon dioxide (CO_2) via respiration, which accounts for ~50% of C produced by respiration from the terrestrial biosphere each year (Singh *et al.*, 2010). An initial positive response of heterotrophic soil microbial respiration to warming declines in the long term (years-decades), which could be caused by direct (acclimation, evolution and species sorting) or indirect (e.g. substrate availability, moisture) effects of temperature (Davidson and Janssens, 2006; Bárcenas-Moreno *et al.*, 2009; Bradford, 2013). 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. To determine the mechanism underlying the decline in heterotrophic soil microbial respiration with warming in the long term, focus has been predominantly on the response of whole soil microbial communities to temperature (Hartley *et al.*, 2007, 2008; Bradford *et al.*, 2008, 2010, 2019; Karhu *et al.*, 2014; Dacal *et al.*, 2019). But considerable uncertainty in how heterotrophic soil microbial communities will respond to warming remains, particularly as the mechanisms underlying these responses have proven challenging to identify (Auffret *et al.*, 2016). To increase mechanistic understanding, investigations of individual species of heterotrophic soil microorganisms offer opportunities for isolating physiological responses separate from evolutionary and ecological responses.

Past studies have begun to investigate whether individual mycorrhizal and decomposer fungal species can acclimate (physiological adjustment of an individual without any genetic change) to temperature (Bárcenas-Moreno *et al.*, 2009; Bradford, 2013). Arbuscular mycorrhizal fungi in soil (Heinemeyer *et al.*, 2006), free-living ectomycorrhizal fungi on agar (Malcolm *et al.*, 2008) and cord-forming wood decay basidiomycetes on agar (Crowther and Bradford, 2013), acclimated to warming by reducing respiration rates. However, the saprotrophic

ascomycete fungus *Neurospora discreta* grown on agar (Romero-Olivares *et al.*, 2015) and sucrose or lignin (Allison *et al.*, 2018) did not acclimate to warming, instead respiration increased at the higher temperature. Many of the studies investigating free-living heterotrophic soil microorganisms have used agar, an artificial medium, which inherently fails to imitate the structural or chemical heterogeneity of most natural resources (Crowther *et al.*, 2018). Furthermore, short-term acclimation responses (days) observed on agar are unlikely to 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 understand responses taking place in natural systems. Wood decay fungi, particularly white rot basidiomycetes, are the primary decomposers of dead wood, which contribute considerably to the total respiratory response of heterotrophic soil microorganisms to warming (Rayner and Boddy, 1988; Boddy and Watkinson, 1995; Baldrian and Lindahl, 2011). Accordingly, the respiratory thermal response of white rot basidiomycetes decomposing their natural substrate over a timescale of months is essential.

To gain a mechanistic understanding of heterotrophic soil microorganism responses to warming, this study investigates the respiratory thermal response of individual species of white rot basidiomycetes decomposing wood. The chosen species have different ecological roles in wood decomposition (primary, early and late secondary colonisers) in temperate woodlands. To identify a thermal response of respiration, and avoid the confounding factor of warming-induced substrate depletion, a cooling approach was applied (following Hartley *et al.* (2008) and Karhu *et al.* (2014)). The fungal species were grown in natural substrate (beech (*Fagus sylvatica*) wood blocks) in microcosms under controlled laboratory conditions. The respiratory thermal response can be either compensatory, enhancing or no response, that is a decrease, increase or no change in the temperature sensitivity of respiration rates, respectively. The cooling approach relies on compensatory and enhancing responses being reversible. For example, at warmer temperatures, if thermal compensation results in a down-regulation of respiration, at cooler temperatures, thermal compensation must result in an up-regulation of respiration. Similarly, at warmer temperatures, if thermal enhancement causes an up-regulation of respiration, at cooler

temperatures, thermal enhancement must cause a down-regulation of respiration. By avoiding issues associated with substrate depletion, an up-regulation in respiration after cooling definitively demonstrates that the population of a specific species can compensate for initial effects of the change in temperature on its activity. A rewarmed treatment was applied to investigate the reversibility of any response observed to cooling. Moreover, ergosterol content as an estimate of living fungal biomass was measured and respiration rates were expressed per unit biomass (mass-specific respiration; R_{mass}) to improve our mechanistic understanding of the thermal responses. Based on previous studies with single-species fungal cultures (Heinemeyer *et al.*, 2006; Malcolm *et al.*, 2008; Crowther and Bradford, 2013), it was hypothesised that individual species of basidiomycetes decomposing wood show compensatory thermal responses and decrease the temperature sensitivity of respiration in the medium to long term.

3.3 Methods

3.3.1 Colonisation of wood blocks

Nine species of native, beech (*Fagus sylvatica*)-inhabiting basidiomycetes (Table 3.1) dominant at different stages of decay were used to colonise 2 x 2 x 2 cm beech wood blocks. Blocks were sterilised by autoclaving 3 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 d.

Table 3.1 Fungal species used to colonise wood blocks.

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

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

After pre-colonisation of wood blocks for 108 d, 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. These wood blocks were then removed from the experiment.

3.3.2 Wood block microcosm set up

Pre-colonised wood blocks were scraped free of adhering mycelium and agar using a sterile scalpel, 3 d prior to set up. Each colonised wood block was placed directly on to perlite (20 ml; siliceous rock that does not absorb 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 and Collis-George, 1967), in a plastic 100 ml lidded deli pot (Cater4you, UK). 20 wood block microcosms were set up per species. Each microcosm was weighed and dH_2O added to the perlite every 14 d to maintain moisture. Holes (4 x 1 mm diameter) in each pot covered by microporous surgical tape (3M, Bracknell, UK) allowed for aeration but prevented contamination with other species.

3.3.3 Wood block microcosm incubation

Wood block microcosms were incubated (Sanyo Electric/Panasonic Cooled Incubator, MIR-154) at 20 °C for a 43 d pre-incubation period. The pre-incubation period allowed respiration rates to stabilise and four respiration measurements to be taken from each wood block microcosm, so that wood block microcosms could be assigned to temperature treatments based on establishing similar mean respiration rates and trajectories across temperature treatments prior to cooling. The 20 wood block microcosms per species were assigned to one of four temperature treatments (n = 5): pre-cooling (destructively sampled at 151 d, prior to cooling), cooled (incubated at 12 °C at 151 d for 90 d), rewarmed (incubated at 12 °C at 151 d for 60 d and then rewarmed to 20 °C for 30 d) and control (incubated at 20 °C for a further 90 d) (Fig. 3.1). The incubation temperatures

chosen are common in temperate woodlands and within the range experienced by basidiomycetes during the main decomposition season (Boddy, 1983a; Magan, 2008). Cooling for 90 d provides sufficient time for thermal compensation (Malcolm *et al.*, 2008; Crowther and Bradford, 2013) and is a time period relevant to seasonal changes in temperature, that have been hypothesised to cause thermal compensation (Malcolm *et al.*, 2008; Karhu *et al.*, 2014). The rewarmed treatment was chosen to investigate the reversibility of any response observed with cooling.

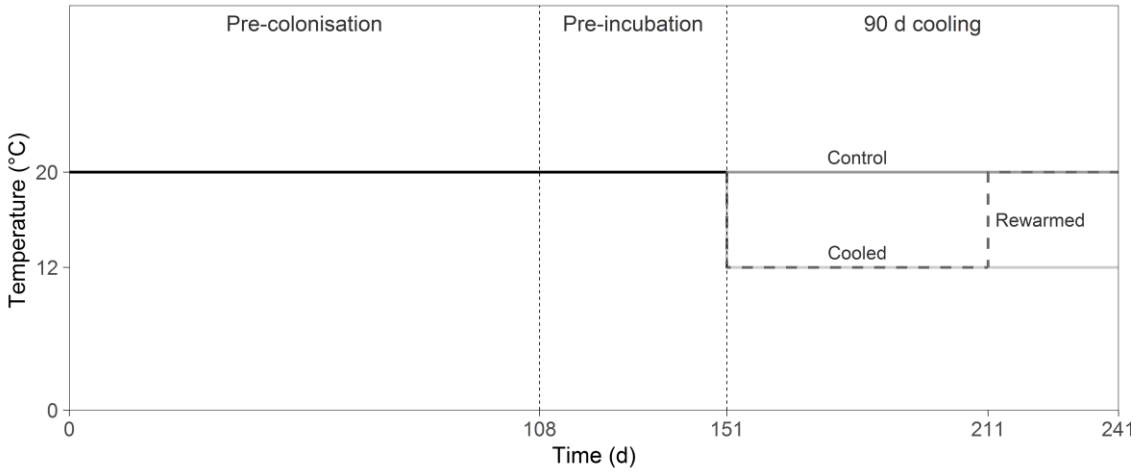


Figure 3.1 Wood block pre-colonisation and wood block microcosm incubation shown as a timeline. Wood blocks were pre-colonised and then wood block microcosms were set up and pre-incubated. Wood block microcosms were assigned to one of four temperature treatments: pre-cooling, cooled, rewarmed and control. Pre-cooling replicates were removed at 151 d, before cooling.

3.3.4 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). Holes in the pot of each wood block microcosm covered by microporous surgical tape allowed CO_2 to accumulate in the larger 700 ml plastic container whilst maintaining a sterile environment. The CO_2 concentration in the headspace of the incubation chamber was measured immediately after closure, and then again after 18 h. The CO_2 concentration was logged on a computer connected to the IRGA every 1.6 s for 90 s to allow the

CO_2 concentration to stabilise. 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)). The difference in initial and end CO_2 concentration was divided by the incubation time, and then divided by 1000000. This was multiplied by the headspace volume divided by 22.4 L, and then multiplied by 12. Respiration was multiplied by 1000000 to be expressed as µg C per h, and then finally divided by the dry weight of wood to be expressed as µg C per g of dry weight per h. Respiration was measured weekly, and the first respiration measurements after cooling and rewarming were made 24 h after the temperature change.

3.3.5 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). However, ergosterol concentration within mycelium changes with age, growth rate, environmental conditions and developmental stage of the fungus (Martin *et al.,* 1990). Nonetheless, it is considered a better proxy for fungal biomass estimation than phospholipid fatty acids and glucosamine, the other commonly used methods (Baldrian *et al.*, 2013b).

Wood blocks (n = 5) from pre-cooling (151 d), cooled, rewarmed and control treatments (241 d) 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 and analysed as described previously (Bååth, 2001; Šnajdr *et al.*, 2008). 0.5 g of sawdust was weighed into 15 ml glass vial, 3 ml of 10% potassium hydroxide (KOH) in methanol and 1 ml of cyclohexane were added, then vortexed for 30 s and sonicated at 70 °C for 90 min (sonicated for 2x 20 min). 1 ml of dH_2O and 2 ml of cyclohexane was added, vortexed for 30 s, centrifuged for 5 min at 3500 rpm (2465 g) and top phase (cyclohexane) removed. The remaining solution was washed 2x with 2 ml of cyclohexane (as in previous step) and the combined cyclohexane fractions evaporated at ambient temperature (maximum 30 °C)

(Genevac EZ-2 evaporator, SP Scientific, Ipswich, UK). 1 ml of methanol was added to each sample, which was redissolved by being vortexed for 30 s and heated at 40 °C for 20 min. Each sample was vortexed again for 30 s, and 1 ml of liquid transferred to 1.5 ml plastic Eppendorf tube. Eppendorf tubes were centrifuged for 3 min at 6000 rpm (1952 g) and 700 µl of liquid transferred to 1.5 ml amber glass high-performance liquid chromatography (HPLC) vials.

Ergosterol analysis was performed using a diode-array detector (DAD) coupled to a 1200 series Rapid Resolution HPLC system (Agilent Technologies, Palo Alto, USA). 10 µl of sample extract was loaded onto an ACE Equivalence 5 C18, 4.6 x 250 mm analytical column (Advanced Chromatography Technologies Limited, Aberdeen, Scotland, UK). The isocratic mobile phase consisted of methanol (HPLC grade) at a flow rate of 1.2 ml min^{-1}, and the column temperature was held at 40 °C for the duration of the 17 min run. Ergosterol was detected using ultraviolet (UV) light at 282 nm. Data were analysed using Agilent OpenLab software. Ergosterol (PHR1512-200MG, Millipore Sigma, USA) dissolved in cyclohexane was diluted to provide ergosterol standard concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 µg ml^{-1}. The ergosterol standards ranging from 100 µg ml^{-1} to 0.78 µg ml^{-1} were run through the extraction protocol, and a calibration curve ($y = 10.358x-1.5151$, $r^2 = 0.9996$) was produced to calculate ergosterol concentrations (µg ml^{-1}) (Supplementary Fig. 3.1). Experimental ergosterol standards were re-injected over the length of the analysis as quality controls (QCs). Additionally, ergosterol was dissolved in methanol and diluted to the same concentrations as above (100 – 0.78 µg ml^{-1}), to produce methanol standards. Experimental ergosterol standard QCs and methanol standards were run every 10 samples to ensure that there was not any carryover, that instrument sensitivity was consistent, and that the standards did not degrade over the length of the analysis. The experimental ergosterol standard QCs increased slightly over time but not significantly (for 6.25 µg ml^{-1} the SE was 0.86, and for 50 µg ml^{-1} the SE was 6.3, over 5 d). To check the robustness of the extraction protocol, methanol standards were compared to the experimental ergosterol standards that were treated as the samples and run through the extraction protocol. In the range of 6.25-50 µg ml^{-1} the average difference (experimental loss) was 20.87% indicating that the extraction protocol is ~80% efficient. The experimental ergosterol standards, which had been through the extraction protocol were used

to calculate the concentrations, so that this loss was accounted for. Ergosterol concentrations (µg ml^{-1}) were converted to µg ergosterol per g of wood taking into account the mass of sawdust used.

3.3.6 Estimation of decay rate

The density of wood blocks can be used to estimate the decay rate (Boddy, 1983a). After pre-colonisation of wood blocks with each species (108 d), the mean initial density of wood blocks was determined (mg mm^{-3}; 8 replicates) as oven dry weight (80 °C for 72 h) per fresh volume (mm^{-3}), measured using digital callipers. After the 43 d pre-incubation period (151 d), pre-cooling treatment wood blocks for each species were destructively sampled and the mean density determined (mg mm^{-3}; 5 replicates), prior to any temperature change. Wood blocks from cooled, rewarmed and control treatments for each species were destructively sampled and density determined (mg mm^{-3}; 15 replicates) at the end of the experiment (241 d). Destructive sampling involved each individual wood block being split along the grain into quarters using a surface-sterilised chisel. Three of the quarters were foil wrapped, flash frozen in liquid nitrogen and stored at -80 °C for quantification of ergosterol as an indicator of fungal biomass. The remaining quarter was used to determine wood block density.

3.3.7 Quantifying respiratory responses

In the absence of C inputs, C losses will occur and thus reduce respiration rates. At the higher temperature, the control treatments will have greater respiration rates, consequently leading to greater C losses than the cooled treatments. C losses will still occur in the cooled treatments and therefore reduce respiration rates, however, at a slower rate than in the control treatments. To account for these 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}), for each species.

The species could show three possible thermal responses following cooling: compensatory, enhancing or no response (Fig. 3.2). A gradual increase in respiration rate after cooling provides clear evidence for a compensatory

response. A more rapid decline in respiration rate after cooling than in the control treatment provides support for an enhancing response. To quantify different rates of decline in respiration in the different treatments, control and cooled treatment respiration rates were normalised to their first measurement of respiration taken after cooling and plotted against cumulative respiration. Hence, the relative respiration rates (normalised to the time of cooling) of control and cooled treatments, plotted against cumulative respiration, would be identical when no response following cooling has occurred. In turn, any significant difference in the relative respiration rates (normalised to the time of cooling) plotted against cumulative respiration allows the detection of compensatory or enhancing responses (Fig. 3.2b).

Furthermore, rewarmed and control treatment respiration rates can be compared and used as evidence of fungal responses affecting the respiration rate after cooling, when the absolute respiration rate is plotted against cumulative respiration. When a no response occurs, the absolute respiration rate of the rewarmed treatment will be equal to the absolute respiration rate in the control treatment at the same cumulative respiration. Therefore, any statistically significant differences between rewarmed and control treatment respiration rates also allows the detection of compensatory or enhancing responses, when respiration rate is plotted against cumulative respiration (Fig. 3.2a).

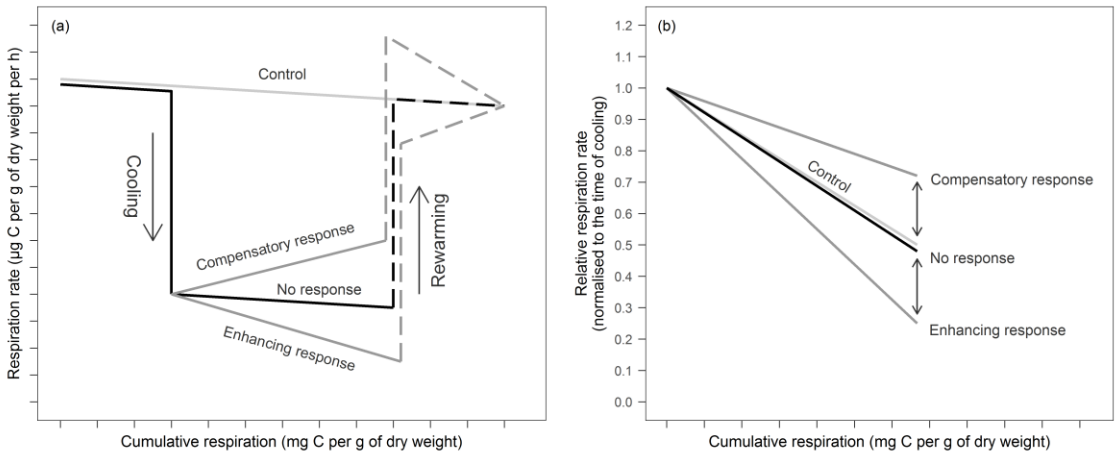


Figure 3.2 Schematic diagram showing the rationale of the cooling approach and quantification of responses. (a) Logic behind the cooling approach and the respiration rate that would be observed for a compensatory, enhancing and no response following cooling. A gradual increase in respiration rate after cooling provides support for a compensatory response, whereas a rapid reduction in respiration rate following cooling would be indicative of an enhancing response. Differences in rates of respiration in rewarmed versus control samples at the same cumulative respiration can also be used to quantify responses. More subtle compensatory and enhancing responses can be detected using the method outlined in panel b. A greater relative rate of decline in respiration rate after cooling, compared with the control, provides support for an enhancing response, or, even when there is not an absolute recovery in respiration rates, a slower relative decline in activity in the cooled treatment is evidence of a compensatory response.

To make comparisons between the temperature treatments at the same cumulative respiration, to account for any effects of different C availability, we needed to calculate the control treatment respiration rates at a corresponding level of cumulative respiration, as the cooled treatment samples at the end of incubation (total cumulative respiration since the time of cooling for cooled samples), and as the rewarmed treatment samples at 1, 5 and 9 d after rewarming (cumulative respiration since the time of cooling), for each species. This was done using linear regressions (Table 3.2, Fig. 3.3).

Table 3.2 Total cumulative respiration since time of cooling for cooled treatment and cumulative respiration since time of cooling at 1, 5 and 9 d after rewarming for rewarmed treatment (mean ± SE of the mean). These were used in a linear regression of control treatment respiration rate after cooling, to calculate control treatment respiration rate at a corresponding level of cumulative respiration as cooled treatment samples at end of incubation and rewarmed treatment samples at 1, 5 and 9 d after rewarming, for each species.

Species	**Total cumulative respiration since time of cooling for cooled treatment (mg C gdw^{-1})**	**Cumulative respiration since time of cooling at 1, 5 and 9 d after rewarming for rewarmed treatment (mg C gdw^{-1})**			**Linear regression of control treatment respiration rate after cooling (µg C gdw^{-1} h^{-1})**	**R^2**
		1 d	**5 d**	**9 d**		
V. comedens	17.54 ± 0.91	11.85 ± 0.85	12.94 ± 0.99	14.08 ± 1.16	y = -0.10640x + 13.48918	0.8236
F. fomentarius	7.75 ± 0.46	5.44 ± 0.32	5.97 ± 0.34	6.51 ± 0.36	y = -0.13425x + 7.94801	0.8483
C. purpureum	1.45 ± 0.35	1.27 ± 0.23	1.38 ± 0.25	1.50 ± 0.27	y = -0.67933x + 1.87360	0.9882
T. versicolor	34.52 ± 1.54	27.52 ± 0.96	30.20 ± 1.25	33.15 ± 1.62	y = -0.47161x + 45.58432	0.9465
S. hirsutum	12.25 ± 1.02	9.44 ± 0.52	10.27 ± 0.58	11.21 ± 0.63	y = -0.12290x + 10.97708	0.8094
B. adusta	6.77 ± 0.85	4.25 ± 0.30	4.95 ± 0.39	5.89 ± 0.43	y = -0.08592x + 6.94512	0.1965
H. fasciculare	4.76 ± 0.67	3.58 ± 0.78	3.92 ± 0.81	4.74 ± 0.91	y = -0.30795x + 5.30509	0.7905
P. velutina	18.48 ± 1.11	11.94 ± 1.23	13.47 ± 1.29	15.04 ± 1.34	y = -0.048905x + 16.99017	0.7496
R. bicolor	5.79 ± 0.63	4.14 ± 0.34	4.68 ± 0.37	5.19 ± 0.41	y = -0.3838x + 6.9142	0.9577

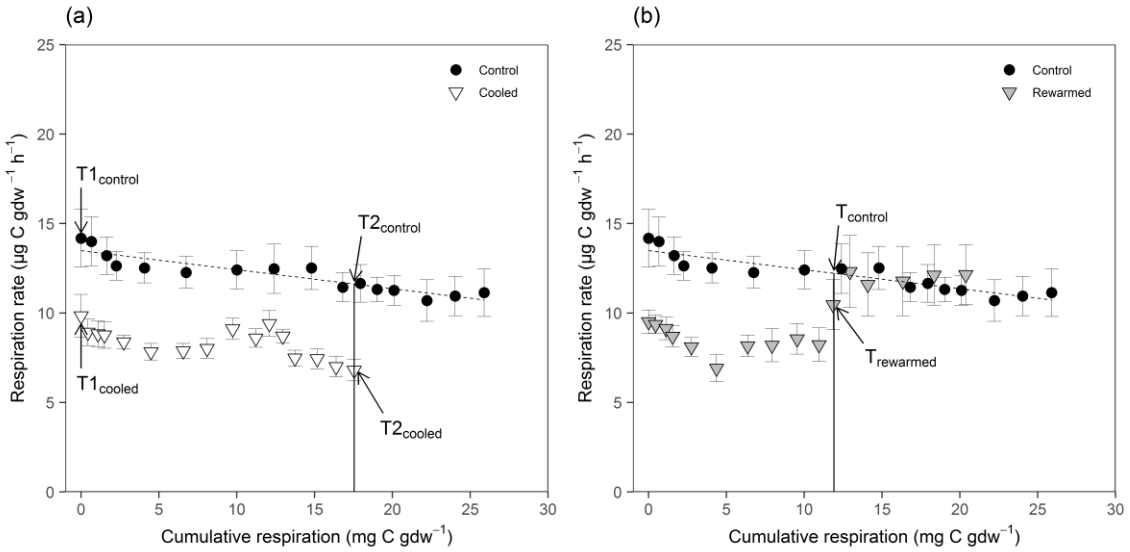


Figure 3.3 Example of linear regression through control treatment to calculate control treatment respiration rates at a corresponding level of cumulative respiration, as (a) cooled treatment samples at the end of incubation and (b) rewarmed treatment samples at the time after rewarming (1 d after rewarming shown). *V. comedens* has been used in panels (a) and (b). Labels show the values used in the RR_{CC} and RR_{CR} equations. Cumulative respiration was calculated from the time of cooling (151 d), at the start of 90 d incubation.

Two methods were used to quantify either compensatory or enhancing responses (Fig. 3.2), following Karhu *et al.* (2014). The first quantitative method produced response ratios comparing control and cooled treatment relative respiration rates (RR_{CC}: Response Ratio, control versus cooled; Fig. 3.2). The total cumulative respiration of cooled treatment samples was inputted into the linear regression equation for the control treatment respiration rate after cooling (Table 3.2), to calculate the control treatment respiration rate at a corresponding level of cumulative respiration as cooled treatment samples at end of incubation (Fig. 3.3a). The relative respiration rates normalised to the time of cooling were calculated for control and cooled treatments. The control treatment relative respiration rate was calculated by dividing the control respiration rate at a corresponding level of cumulative respiration as cooled samples at the end of incubation ($T2_{control}$) by the control respiration rate at 1 d (*V. comedens*, *F. fomentarius*, *C. purpureum*, *T. versicolor*, *S. hirsutum*, *B. adusta*) or 3 d (*H. fasciculare*, *P. velutina*, *R. bicolor*) after cooling ($T1_{control}$) (Fig. 3.3a). The cooled treatment relative respiration rate was calculated by dividing the cooled respiration rate at the end of incubation ($T2_{cooled}$) by the cooled respiration rate at 1 d (*V. comedens*, *F. fomentarius*, *C. purpureum*, *T. versicolor*, *S. hirsutum*, *B.*

adusta) or 3 d (*H. fasciculare*, *P. velutina*, *R. bicolor*) after cooling ($T1_{cooled}$) (Fig. 3.3a). The control treatment relative respiration rate was divided by the cooled treatment relative respiration rate to produce a response ratio (RR_{CC}) for each species.

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

where $T2_{control}$, $T1_{control}$, $T2_{cooled}$ and $T1_{cooled}$ are defined as above.

The control and cooled treatment relative respiration rates at the total cumulative respiration for cooled samples at the end of the incubation was used to incorporate the long-term effect that 90 days' cooling had on respiration rates. In *H. fasciculare*, *P. velutina*, *R. bicolor*, a further large reduction in respiration rates was observed between 1 and 3 d after cooling (Supplementary Fig. 3.2). Thus, the respiration rates at 3 d after cooling was used in the calculation to exclude the short-term instantaneous responses to cooling that would be lost in the short-term (days) temperature sensitivity measurements, hence, focusing on responses that would be observed in the medium to long term and are more relevant to understanding long-term reductions in responses to warming in field experiments.

The second quantitative method produced response ratios comparing control and rewarmed treatment respiration rates (RR_{CR}: Response Ratio, control versus rewarmed). The cumulative respiration of rewarmed samples at 1, 5 and 9 d after rewarming was inputted into the control treatment linear regression equation (Table 3.2), to calculate the control treatment respiration rate after cooling at a corresponding level of cumulative respiration as rewarmed samples at 1, 5 and 9 d after rewarming (Fig. 3.3b). The control treatment respiration rate at a corresponding level of cumulative respiration as rewarmed samples at 1, 5 and 9 d after rewarming ($T_{control}$) was divided by the rewarmed treatment respiration rate at 1, 5 and 9 d after rewarming ($T_{rewarmed}$) (Fig. 3.3b), to produce a response ratio (RR_{CR}) for each species.

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

where $T_{control}$ and $T_{rewarmed}$ are defined as above.

Response ratios were produced at 1, 5 and 9 d 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.

To quantify either compensatory or enhancing responses when correcting for fungal biomass, using ergosterol as a biomarker for living fungal biomass, respiration rates were expressed per unit fungal biomass and referred to as mass-specific respiration (R_{mass}). The ergosterol content of control samples, at a corresponding level of cumulative respiration as cooled samples at end of incubation, was interpolated on the basis of the control ergosterol content at the time of cooling (pre-cooling) and at the end of incubation. R_{mass} were expressed as µg C per g of ergosterol per h. Control R_{mass} at time of cooling ($T1_{controlRmass}$) was calculated by dividing the control respiration rate at 1 d (*V. comedens, F. fomentarius, C. purpureum, T. versicolor, S. hirsutum, B. adusta*) or 3 d (*H. fasciculare, P. velutina, R. bicolor*) after cooling ($T1_{control}$) by the mean ergosterol per g of wood of pre-cooling samples ($E_{pre\text{-}cooling}$). Cooled R_{mass} at time of cooling ($T1_{cooledRmass}$) was calculated by dividing the cooled respiration rate at 1 d (*V. comedens, F. fomentarius, C. purpureum, T. versicolor, S. hirsutum, B. adusta*) or 3 d (*H. fasciculare, P. velutina, R. bicolor*) after cooling ($T1_{cooled}$) by the mean ergosterol per g of wood of pre-cooling samples ($E_{pre\text{-}cooling}$). Control R_{mass} at the corresponding level of cumulative respiration as cooled samples at end of incubation was calculated ($T2_{controlRmass}$). The control respiration rate, at a corresponding level of cumulative respiration as cooled samples at end of incubation ($T2_{control}$), was divided by the mean ergosterol per g of wood of control samples at a corresponding level of cumulative respiration as cooled samples at end of incubation ($E_{control}$) (obtained by interpolating between the ergosterol per g of wood of pre-cooling samples and of control samples at the end of incubation. Control samples did not experience any temperature change during the incubation, so it was assumed that any changes took place gradually during the

90 d period and thus that linear interpolation was appropriate). Cooled R_{mass} at end of incubation ($T2_{cooledRmass}$) was calculated by dividing the cooled respiration rate at end of incubation ($T2_{cooled}$) by the mean ergosterol per g of wood of cooled samples at end of incubation (E_{cooled}). The relative R_{mass} normalised to the time of cooling were calculated for control ($T2_{controlRmass}$ / $T1_{controlRmass}$) and cooled ($T2_{cooledRmass}$ / $T1_{cooledRmass}$) treatments, using the same method as the respiration rates expressed per unit wood mass. Response ratios comparing control and cooled treatment relative R_{mass} expressed per unit fungal biomass (R_{mass} RR_{CC}: Mass-specific respiration Response Ratio, control versus cooled) were calculated using the same method as the response ratios of the relative respiration rates expressed per unit wood mass.

$$R_{mass}\ RR_{CC} = \frac{T2_{controlRmass}/T1_{controlRmass}}{T2_{cooledRmass}/T1_{cooledRmass}} \quad (3)$$

where $T2_{controlRmass}$, $T1_{controlRmass}$, $T2_{cooledRmass}$ and $T1_{cooledRmass}$ are defined as above.

Response ratios comparing control and rewarmed treatment R_{mass} were not calculated, as the ergosterol at the time of rewarming was not known.

For respiration rates expressed per unit wood mass, response ratio values < 1 indicate a compensatory response (cooled treatment relative respiration rates were greater than control treatment relative respiration rates, at a given cumulative respiration; rewarmed treatment respiration rates were greater than control treatment respiration rates, at a given cumulative respiration) and values > 1 indicate an enhancing response (cooled treatment relative respiration rates were lower than control treatment relative respiration rates, at a given cumulative respiration; rewarmed treatment respiration rates were lower than control treatment respiration rates, at a given cumulative respiration). Response ratio values < 1 indicating a compensatory response and values > 1 indicating an enhancing response also applies to respiration rates expressed per unit fungal biomass. For each quantitative method, the response ratios for the individual species were natural-log-transformed, mean calculated and 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).

3.3.8 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 d), prior to cooling (151 d), for each species.

Ergosterol (µg g wood^{-1}) of samples was 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 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 d after rewarming to control treatment respiration rates at a corresponding level of cumulative respiration as rewarmed treatment samples at 1, 5 and 9 d after rewarming, respectively. Additionally, to further support the response ratio method (RR_{CC}), F ratio was used for statistical comparison of control and cooled treatment relative respiration rate (normalised to the time of cooling) fitted lines for each species. Using the known F distribution, a *P* value was calculated from the F ratio and two degrees of freedom values.

One-way ANOVA models and Tukey's pairwise comparisons were used to compare wood block densities between species after pre-colonisation of wood blocks with each species (108 d), and wood block densities between species of samples in control treatment at the end of incubation (241 d).

3.4 Results

3.4.1 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 ($P > 0.05$; Supplementary Table 3.1, Fig. 3.4). The respiration rates, and as a result the cumulative respiration for all treatments, were greatest for *T. versicolor*, followed by *P. velutina* and *V. comedens* (Fig. 3.4). *F. fomentarius* and *H. fasciculare* had much lower respiration rates and cumulative respiration, while *C. purpureum* had the lowest respiration rates and cumulative respiration for all treatments (Fig. 3.4).

3.4.2 Ergosterol as an indicator of fungal biomass

The influence of temperature treatment and species on ergosterol was compared using two-way ANOVA. Temperature treatment ($F_{3, 144} = 4.362$, $P = 0.006$) had a significant effect on ergosterol content (Supplementary Table 3.2). Ergosterol was greater in the control treatment (38.58 ± 2.48 µg g wood^{-1}), compared with pre-cooling (33.97 ± 2.11 µg g wood^{-1}, $P = 0.025$), cooled (33.60 ± 2.34 µg g wood^{-1}, $P = 0.013$) and rewarmed (36.90 ± 2.42 µg g wood^{-1}, $P = 0.724$) treatments (Supplementary Table 3.2). Ergosterol of cooled ($P = 0.996$) and rewarmed ($P = 0.274$) treatments at the end of incubation did not significantly differ from that at pre-cooling or to each other ($P = 0.180$). Species ($F_{8, 144} = 68.986$, $P < 0.001$) had a significant effect on ergosterol (Supplementary Table 3.2). *B. adusta* (18.78 ± 1.48 µg g wood^{-1}) had the lowest ergosterol and *S. hirsutum* (62.13 ± 2.95 µg g wood^{-1}) had the highest ergosterol. Importantly, a significant interaction between temperature treatment and species for ergosterol was found, showing the response of ergosterol to temperature treatments was dependent on the species ($F_{24, 144} = 1.710$, $P = 0.029$) (Supplementary Table 3.2).

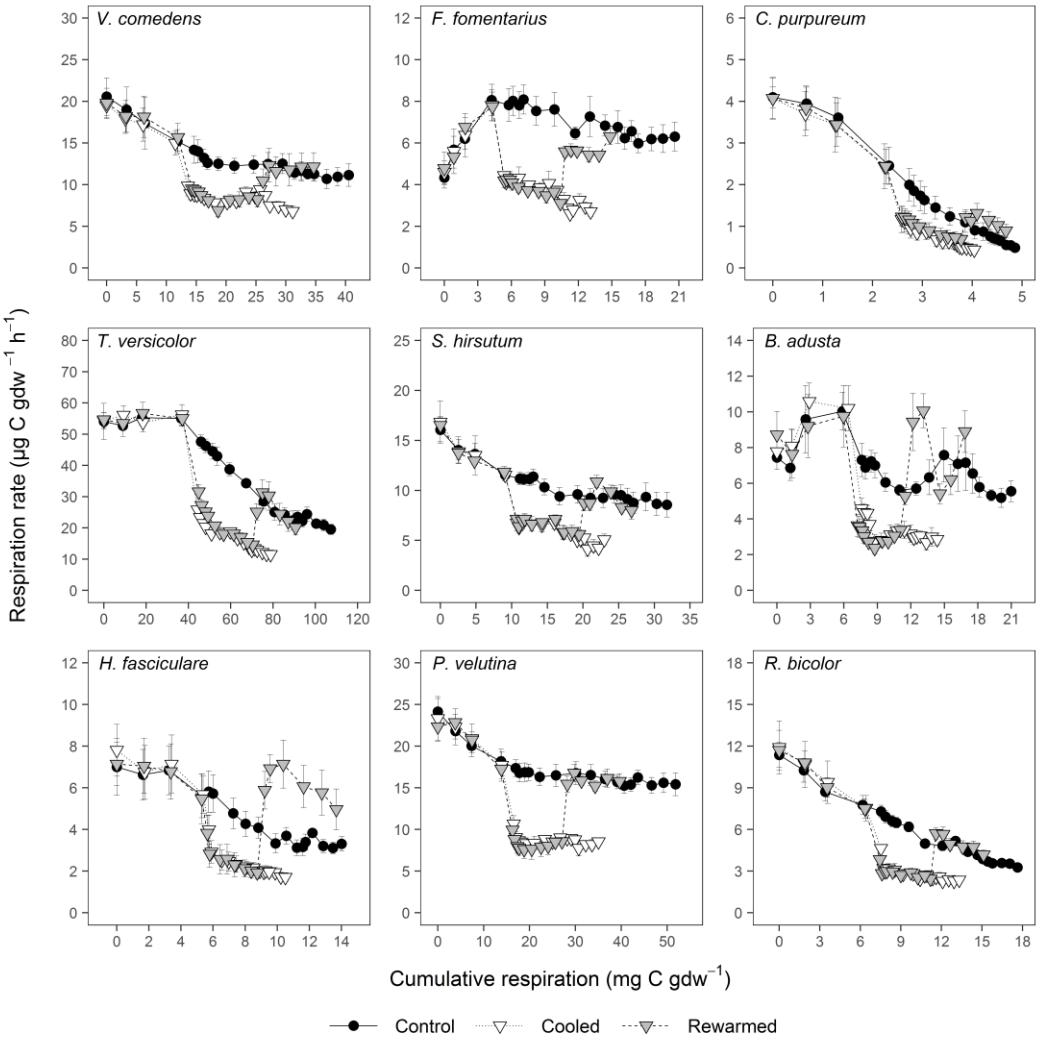


Figure 3.4 Respiration rate of three temperature treatments (control, cooled and rewarmed) during 43 d pre-incubation period prior to cooling and 90 d incubation following cooling, of each species (mean ± SE of the mean, n = 5). Cumulative respiration was calculated from the start of pre-incubation (108 d).

The difference in ergosterol between temperature treatments of each species was determined using one-way ANOVA models. Ergosterol content significantly differed between temperature treatments in *T. versicolor* ($F_{3, 16}$ = 4.077, *P* = 0.025) and marginally in *B. adusta* ($F_{3, 16}$ = 3.221, *P* = 0.051) (Fig. 3.5, Supplementary Table 3.3). In *T. versicolor*, the control treatment (25.96 ± 3.95 µg g wood^{-1}) had significantly greater ergosterol than pre-cooling treatment (14.99 ± 1.85 µg g wood^{-1}, *P* = 0.026), and marginally significantly greater ergosterol than cooled treatment (16.41 ± 1.02 µg g wood^{-1}, *P* = 0.059). In *B.adusta*, the pre-cooling treatment (24.83 ± 3.16 µg g wood^{-1}) had significantly greater ergosterol than the cooled treatment (14.47 ± 1.76 µg g wood^{-1}, *P* = 0.049). The temperature treatments had no significant influence on ergosterol in *V. comedens* ($F_{3, 16}$ =

2.640, *P* = 0.085), *F. fomentarius* ($F_{3, 16}$ = 0.760, *P* = 0.533), *C. purpureum* ($F_{3, 16}$ = 2.51 , *P* = 0.096), *S. hirsutum* ($F_{3, 16}$ = 1.006, *P* = 0.416), *H. fasciculare* ($F_{3, 16}$ = 1.137, *P* = 0.364), *P. velutina* ($F_{3, 16}$ = 3.151, *P* = 0.054) or *R. bicolor* ($F_{3, 16}$ = 2.602, *P* = 0.088) (Fig. 3.5, Supplementary Table 3.3). Furthermore, the ecological role did not influence the ergosterol content ($F_{2, 174}$ = 1.271, *P* = 0.283).

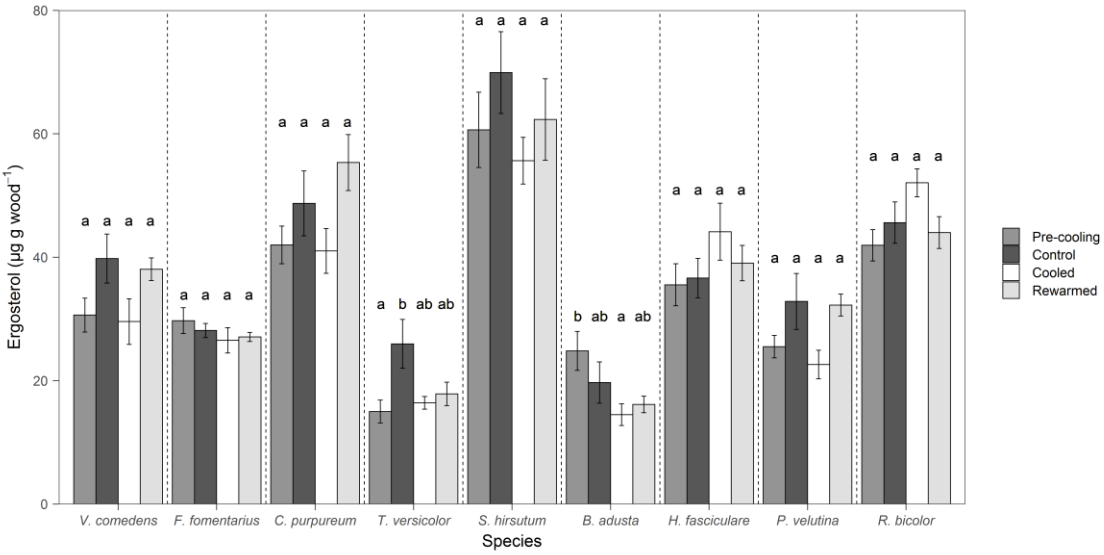


Figure 3.5 Ergosterol content of samples in pre-cooling, control, cooled and rewarmed treatments of each species (mean ± SE of the mean, n = 5). Different letters indicate significant (*P* < 0.05) differences in ergosterol between temperature treatments of each species from Tukey's pairwise comparisons.

3.4.3 Respiratory response to cooling and rewarming

Individual species of basidiomycetes decomposing wood showed enhancing responses, but showed no compensatory responses (Fig. 3.6). Overall, individual species of basidiomycetes decomposing wood showed an enhancing response (RR_{CC} = 1.19, *P* < 0.05; Table 3.3, Fig. 3.7a) and increased the temperature sensitivity of respiration in the medium to long term. When considering the ecological role, early secondary colonisers showed a marginally significant enhancing response overall (RR_{CC} = 1.29, *P* < 0.1), whereas primary and late secondary colonisers showed no responses (*P* > 0.05; Table 3.3, Fig. 3.7a). Three species (*F. fomentarius, T. versicolor, B. adusta*) showed enhancing responses (RR_{CC} > 1, *P* < 0.01; Table 3.3, Fig. 3.7a), with *F. fomentarius* being a primary coloniser and *T. versicolor* and *B. adusta* being early secondary colonisers. Six species (*V. comedens*, *C. purpureum*, *S. hirsutum*, *H. fasciculare*,

P. velutina, *R. bicolor*) showed no responses ($P > 0.05$; Table 3.3, Fig. 3.7a). 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 3.4), however, *S. hirsutum* also showed an enhancing response by the fitted line method ($P < 0.05$), but not the RR_{CC} calculation.

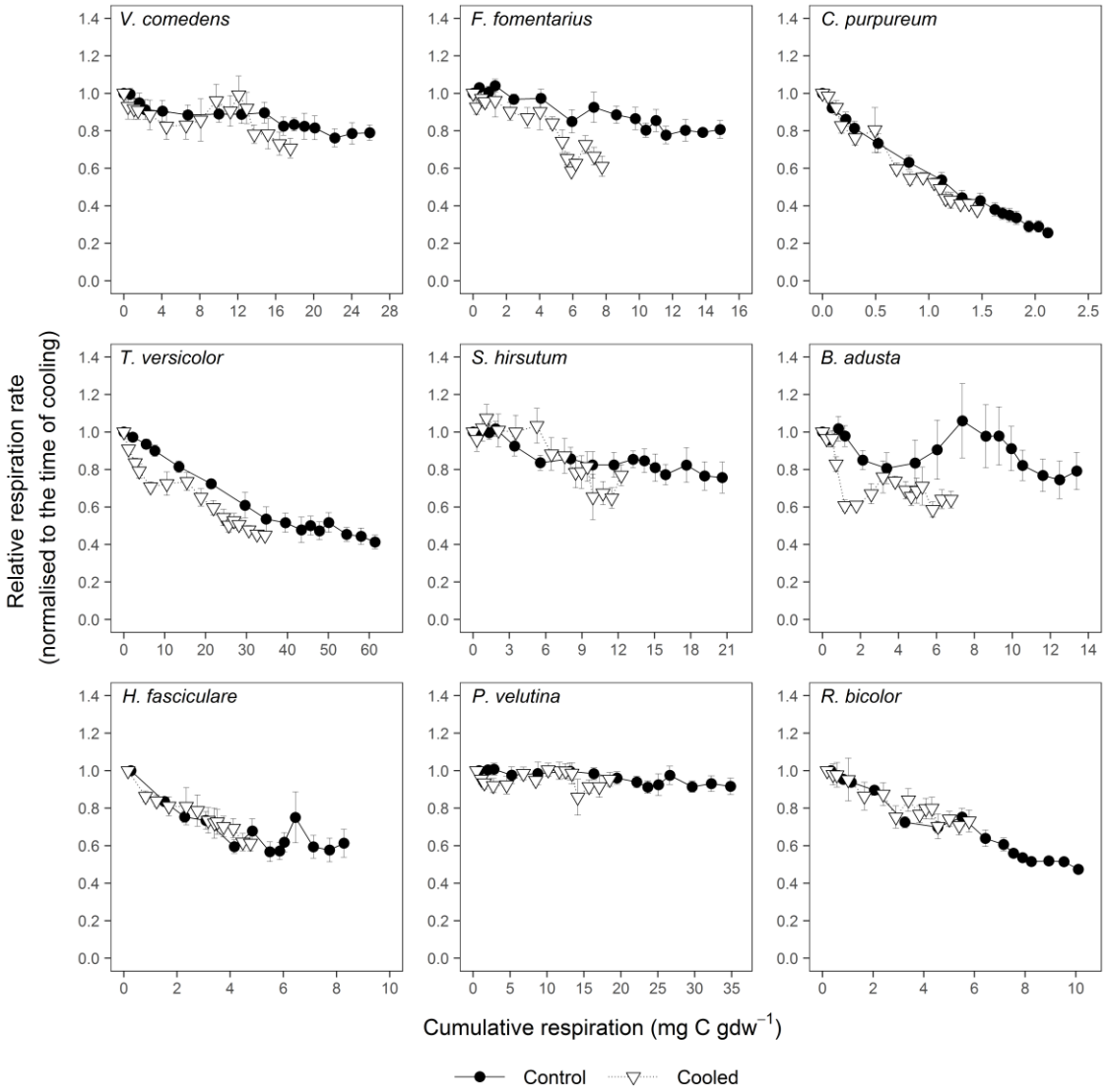


Figure 3.6 Relative respiration rate (normalised to the time of cooling) of control and cooled treatments during 90 d of incubation following cooling, of each species (mean ± SE of the mean, n = 5). Cumulative respiration was calculated from the time of cooling (151 d), at the start of 90 d incubation.

Table 3.3 Respiration responses (RR_{CC}) quantified by comparing control and cooled treatment relative respiration rates, at a corresponding level of cumulative respiration as cooled treatment samples at end of incubation, of species overall, each ecological role and each species.

	Control vs. cooled treatment relative respiration rates			
	RR_{CC}	***t***	***P***	**Response**
Overall	1.19	3.239*	0.012	Enhancing
Ecological role				
Primary	1.27	2.604	0.121	
Early secondary	1.29	3.869 ·	0.061	
Late secondary	1.02	0.336	0.769	
Species				
V. comedens	1.19	2.181 ·	0.095	
F. fomentarius	1.45	4.805**	0.009	Enhancing
C. purpureum	1.19	0.542	0.617	
T. versicolor	1.38	6.190**	0.003	Enhancing
S. hirsutum	1.12	1.339	0.252	
B. adusta	1.39	5.430**	0.006	Enhancing
H. fasciculare	1.14	2.295 ·	0.083	
P. velutina	1.01	0.099	0.926	
R. bicolor	0.92	-0.616	0.571	

*· indicates a significant difference at P < 0.1; * at P < 0.05; and ** at P < 0.01.*

Table 3.4 F ratio for statistical comparison of control and cooled treatment relative respiration rate (normalised to the time of cooling) fitted lines of each species.

Species	**df**	**F**	***P***
V. comedens	2, 23	0.919	0.413
F. fomentarius	2, 21	25.951***	<0.001
C. purpureum	2, 21	2.847 ·	0.081
T. versicolor	2, 20	25.113***	<0.001
S. hirsutum	2, 22	3.802*	0.038
B. adusta	2, 21	10.538***	<0.001
H. fasciculare	2, 15	0.298	0.747
P. velutina	2, 19	2.356	0.122
R. bicolor	2, 19	0.226	0.800

*· indicates a significant difference at P < 0.1; * at P < 0.05; and *** at P < 0.001.*

Overall, individual species of basidiomycetes showed a no response after rewarming ($P > 0.05$; Table 3.5, Fig. 3.7b). When considering the ecological role, primary, early secondary and late secondary colonisers each showed no responses after rewarming ($P > 0.05$; Table 3.5, Fig. 3.7b). 1 d after rewarming, six species (*V. comedens*, *C. purpureum*, *S. hirsutum*, *H. fasciculare*, *P. velutina*, *R. bicolor*) showed no responses ($P > 0.05$), however one species (*F. fomentarius*) showed an enhancing response ($P < 0.01$), and two species (*T.*

versicolor, *B. adusta*) showed marginally significant enhancing responses ($P < 0.1$; Table 3.5, Fig. 3.7b). *F. fomentarius* experienced a slow recovery of respiration rate back up to the control level respiration rate after rewarming, showing prolonged enhancing responses 5 and 9 d after rewarming (Table 3.5, Fig. 3.7b). The respiration rate of *F. fomentarius* remained below the control level respiration rate 24 d after rewarming, with recovery 31 d after rewarming. *T. versicolor* showed a no response 5 d after rewarming (Table 3.5, Fig. 3.7b), as the rewarmed respiration rate recovered to the control level respiration rate. The rewarmed respiration rate of *B. adusta*, however, increased above the control level respiration rate 5 ($P > 0.05$) and 9 ($P < 0.05$) d after rewarming (Table 3.5, Fig. 3.7b). Additionally, the rewarmed respiration rate of *H. fasciculare* increased above the control level respiration rate 1 d ($P > 0.05$), 5 d ($P < 0.05$) and 9 d ($P < 0.1$) after rewarming (Table 3.5, Fig. 3.7b), and maintained this greater respiration rate to the end of incubation. This increase in rewarmed respiration rate by *B. adusta* and *H. fasciculare* above the control level respiration rate after rewarming, may not, however, be clear evidence of compensatory responses as these species did not compensate for the effect of cooling.

Table 3.5 Respiration responses (RR_{CR}) quantified by comparing control and rewarmed treatment respiration rates at 1, 5 and 9 d after rewarming, at a corresponding level of cumulative respiration as rewarmed treatment samples at 1, 5 and 9 d after rewarming, respectively, of species overall, each ecological role and each species.

	1 d after rewarming				**5 d after rewarming**				**9 d after rewarming**			
	RR_{CR}	***t***	***P***	**Response**	**RR_{CR}**	***t***	***P***	**Response**	**RR_{CR}**	***t***	***P***	**Response**
Overall	1.05	1.547	0.160		0.91	-1.027	0.334		0.86	-1.309	0.227	
Ecological role												
Primary	1.08	1.680	0.235		1.01	0.647	0.584		0.95	0.839	0.490	
Early secondary	1.22	1.560	0.259		0.92	-0.534	0.647		0.82	-1.752	0.222	
Late secondary	0.89	-0.490	0.673		0.80	-1.637	0.243		0.82	-0.829	0.494	
Species												
V. comedens	1.17	1.196	0.298		0.98	-0.097	0.928		1.03	0.212	0.842	
F. fomentarius	1.29	5.317**	0.006	Enhancing	1.26	4.444*	0.011	Enhancing	1.26	7.093**	0.002	Enhancing
C. purpureum	0.83	-0.571	0.598		0.83	-0.536	0.620		0.65	-1.106	0.331	
T. versicolor	1.30	2.458 ·	0.070		1.00	0.008	0.994		0.99	-0.056	0.958	
S. hirsutum	1.12	1.306	0.262		1.11	1.329	0.255		0.88	-1.845	0.139	
B. adusta	1.25	2.598 ·	0.060		0.69	-1.783	0.149		0.64	-3.874*	0.018	Compensatory
H. fasciculare	0.71	-1.502	0.208		0.59	-3.062*	0.038	Compensatory	0.54	-2.333 ·	0.080	
P. velutina	1.06	0.902	0.418		0.97	-0.414	0.700		1.03	0.645	0.554	
R. bicolor	0.93	-0.752	0.494		0.90	-0.839	0.449		1.00	-0.035	0.974	

*· indicates a significant difference at P < 0.1; * at P < 0.05; and ** at P < 0.01.*

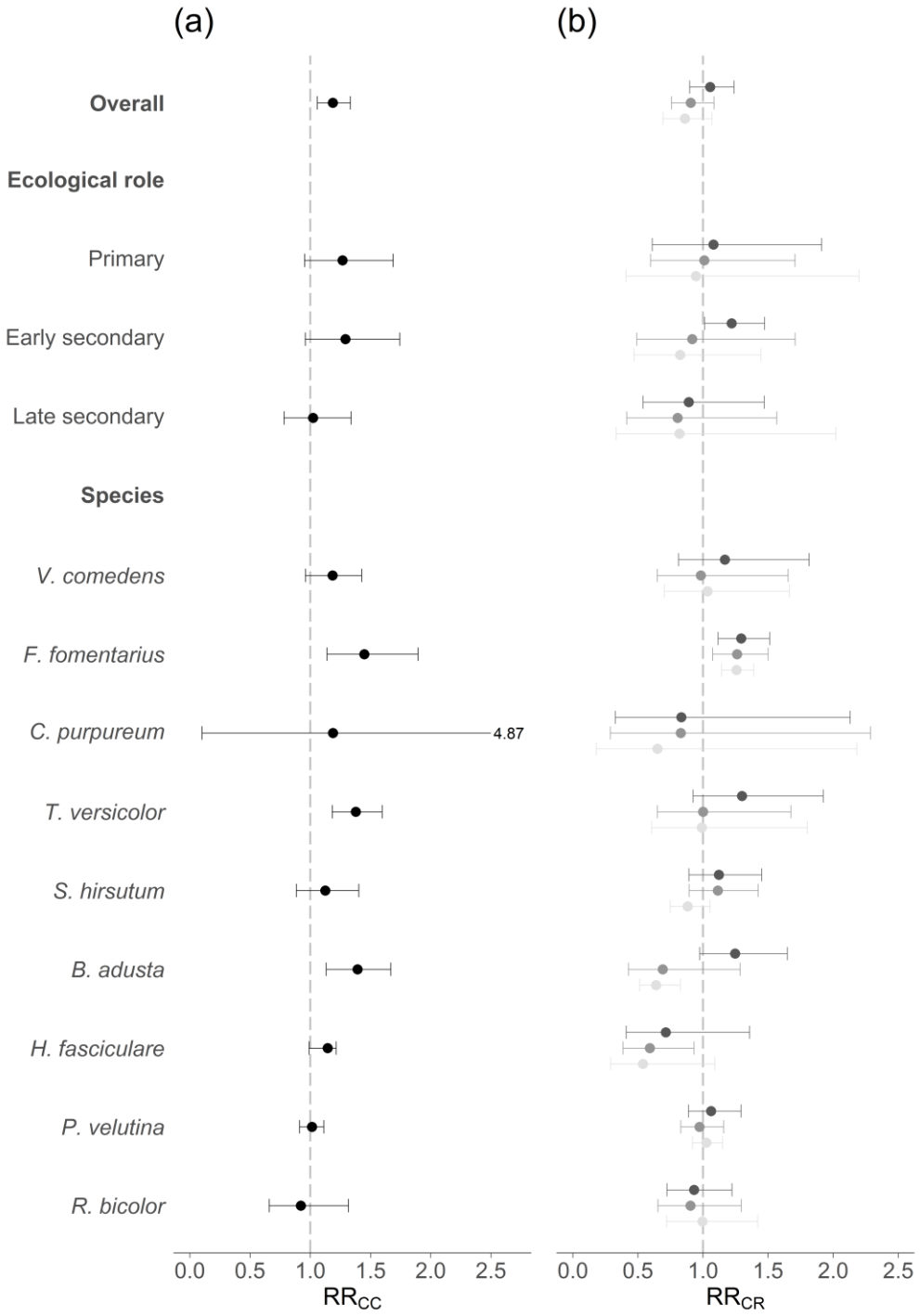


Figure 3.7 Response of respiration rate of species overall, each ecological role and each species to changes in temperature. The mean and 95% confidence intervals (n = 5) of (a) RR_{CC} and (b) RR_{CR} for 1 (black), 5 (dark grey) and 9 (light grey) d after rewarming. RR_{CC}: Response Ratio, control 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 d after rewarming divided by the rewarmed treatment respiration rate at 1, 5 and 9 d after rewarming. Values < 1 indicate a compensatory response and values > 1 indicate an enhancing response. Effects are significant ($P < 0.05$) where confidence intervals do not cross one.

3.4.4 Mass-specific respiratory response to cooling

When accounting for living fungal biomass by using ergosterol as an indicator, comparing the control and cooled treatment relative R_{mass} resulted in individual species overall showing a no response (R_{mass} RR_{CC} = 1.04, $P > 0.05$; Table 3.6, Fig. 3.8). Primary, early secondary and late secondary colonisers each showed no responses when considering the relative R_{mass} ($P > 0.05$; Table 3.6, Fig. 3.8). However, two species (*F. fomentarius*, $P < 0.05$*; H. fasciculare,* $P < 0.01$) showed mass-specific enhancing responses, and one species (*P. velutina*) showed a mass-specific compensatory response ($P < 0.01$; Table 3.6, Fig. 3.8). Six species (*V. comedens, C. purpureum, T. versicolor, S. hirsutum, B. adusta, R. bicolor*) showed no responses when comparing the relative R_{mass} ($P > 0.05$; Table 3.6, Fig. 3.8). It is, however, important to consider the ergosterol as an indicator of living fungal biomass with caution, as discussed previously.

Table 3.6 Mass-specific respiration responses (R_{mass} RR_{CC}) quantified by comparing control and cooled treatment relative mass-specific respiration (R_{mass}), at a corresponding level of cumulative respiration as cooled treatment samples at end of incubation, of species overall, each ecological role and each species.

	R_{mass} RR_{CC}	***t***	***P***	**Response**
Overall	1.04	0.235	0.820	
Ecological role				
Primary	1.10	0.875	0.474	
Early secondary	0.97	-1.050	0.404	
Late secondary	1.06	0.019	0.987	
Species				
V. comedens	0.95	-0.974	0.385	
F. fomentarius	1.33	3.550*	0.024	Enhancing
C. purpureum	1.05	0.099	0.926	
T. versicolor	1.07	1.169	0.307	
S. hirsutum	0.95	-0.995	0.376	
B. adusta	0.91	-1.687	0.167	
H. fasciculare	1.40	7.681**	0.002	Enhancing
P. velutina	0.78	-6.321**	0.003	Compensatory
R. bicolor	1.09	0.741	0.500	

** indicates a significant difference at P < 0.05; and ** at P < 0.01.*

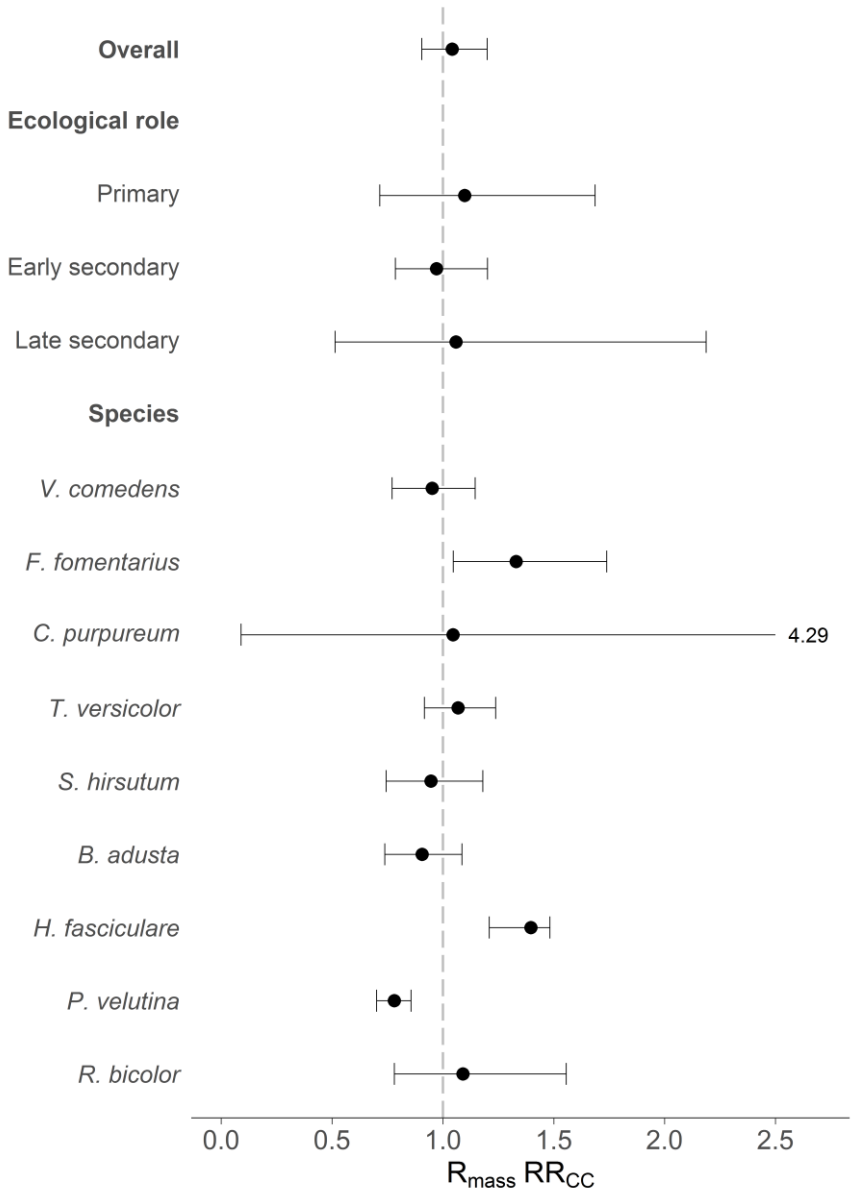


Figure 3.8 Response of relative mass-specific respiration (R_{mass}) of species overall, each ecological role and each species to change in temperature. The mean and 95% confidence intervals (n = 5) of R_{mass} RR_{CC}. R_{mass} RR_{CC}: Mass-specific respiration Response Ratio, control versus cooled; 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 ($P < 0.05$) where confidence intervals do not cross one.

3.4.5 Wood decay rate

After pre-colonisation, the density (i.e. the state of decay) was significantly different between the species ($F_{8, 62} = 10.581$, $P < 0.001$). In particular, *T. versicolor* (0.391 ± 0.012 mg mm^{-3}) had a significantly lower density (i.e. was

significantly more decayed) than the other species ($P < 0.05$) (Supplementary Fig. 3.3, Supplementary Table 3.4). At the end of incubation, the wood block density of control treatments was significantly different between species ($F_{8, 36} = 13.63$, $P < 0.001$) (Supplementary Fig. 3.4, Supplementary Table 3.5). However, as a result of the variability in the wood block densities of samples in the temperature treatments, the respiration measurements act as a more sensitive and accurate measure of the decay rate.

3.5 Discussion

3.5.1 Respiratory response to cooling and rewarming

By using a cooling approach to control the substrate availability, we demonstrate that, overall, individual species of basidiomycetes colonising wood show an enhancing response, owing to a further decrease in the rate of respiration, lowering the respiration rate beyond the instantaneous response to cooling, and therefore increasing the temperature sensitivity of respiration. This overall enhancing response was driven by the early secondary colonisers, with three species producing enhancing responses (RR_{CC}: *T. versicolor* and *B. adusta*; F ratio: *T. versicolor*, *S. hirsutum*, *B. adusta*). However, primary and late secondary colonisers showed no responses, with only one primary coloniser (*F. fomentarius*) producing an enhancing response, and all late secondary colonisers showing no responses. As such, individual species of basidiomycetes colonising wood show no compensatory responses, thus do not decrease the temperature sensitivity of respiration. Our findings are in agreement with a previous study that found no evidence of thermal compensation when investigating the respiratory response of *Neurospora discreta*, an ascomycete fungus, to temperature when grown on sucrose and lignin (Allison *et al.*, 2018). Instead, strains of *Neurospora discreta* that evolved at the higher temperature had moderately greater respiration rates (Allison *et al.*, 2018), which supports the enhancing responses (*F. fomentarius, T. versicolor*, *S. hirsutum*, *B. adusta*) identified in the present study. Following cooling, rewarmed samples confirmed the enhanced temperature sensitivity of respiration in one species (*F. fomentarius*), where the negative impact of cooling resulted in a slow recovery of respiration rate, whereas three species (*T. versicolor*, *S. hirsutum*, *B. adusta*) were able to rapidly reverse the enhanced

temperature sensitivity of respiration with rewarming. Altogether, we demonstrate that individual species of basidiomycetes colonising wood overall do not show a compensatory thermal response, hence will not reduce the temperature sensitivity of respiration. Rather, individual species of basidiomycetes will enhance the temperature sensitivity of respiration, and will increase their respiration rate associated with wood decomposition with warming in the medium to long term.

Enhancing responses, where exposure to cooling further reduced respiration rates, could be a result of physiological and/or biochemical changes to avoid or mitigate cellular injuries after the decrease in temperature, that promote survival but reduce metabolic activity (Hochachka and Somero, 2002; Schimel *et al.*, 2007), as suggested by Hartley *et al.* (2008) that used the cooling approach. However, the temperature reduction from 20 °C to 12 °C in the current study is unlikely to be low enough to induce such changes, but the rapid nature of the cooling may have affected species metabolism. Additionally, cooling took longer to reduce the respiration rates of *F. fomentarius* and *S. hirsutum* compared to *T. versicolor* and *B. adusta*. The reduction in respiration of *F. fomentarius* and *S. hirsutum* happening towards the end of incubation could be associated with depletion of more readily decomposable C and the challenges of breaking down recalcitrant lignocellulose whilst at the lower temperature (Blanchette, 1995). This highlights the importance of the temperature sensitivity of respiration of different species occurring over various timescales, which will have implications on the rate of C loss, and indicates the need to investigate thermal responses over an extended time period.

The respiration rate of *B. adusta* was unstable throughout the whole incubation in all temperature treatments. The increase in respiration rate in the control treatment may be due to the eventual breakdown of recalcitrant lignocellulose and the subsequent available C (Blanchette, 1995), followed by a decrease in respiration rate towards the end of incubation as the C was diminished. Again, this may apply to the sudden increase in respiration rate in rewarmed samples after exposure to the warmer temperature, which also occurred for *H. fasciculare*. In response to cooling, *B. adusta* showed an enhancing response and *H. fasciculare* produced no response. Thus, it is not clear if the increase in

respiration rates in rewarmed samples relative to the control samples is evidence for the species compensating for the cooling. However, the increase in activity in the rewarmed samples did lead to samples in the rewarmed and control treatments, for *H. fasciculare* in particular, having similar cumulative respiration at the end of the experiment. It is though important to emphasise that the cumulative respiration of *B. adusta* and *H. fasciculare* were two of the lowest (the species with the lowest cumulative respiration, *C. purpureum*, 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 degrade key substrates at low temperatures, but then substrates becoming available again at the warmer temperature after 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, and emphasises the comparability, in terms of effects on respiration rates, of fungal responses to cooling and warming. There was evidence of a faster response of the species of basidiomycetes to the rewarming than the cooling treatment. Of the four species showing an enhancing response, three species (*T. versicolor, S. hirsutum, B. adusta*) rapidly reversed the enhanced temperature sensitivity of respiration within 5 d of rewarming. In *F. fomentarius*, however, recovery of respiration to the control level respiration rate took 31 d 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 will affect the temperature sensitivity of respiration and increase C losses.

3.5.2 Mass-specific respiratory response to cooling

When respiration rates were normalised for fungal biomass, individual species of basidiomycetes showed no thermal response overall, therefore the overall enhancing response when not accounting for fungal biomass was lost. All three ecological roles - primary, early secondary and late secondary colonisers - showed no thermal responses after accounting for fungal biomass. The one primary coloniser (*F. fomentarius*) continued to produce an enhancing response,

whereas the three early secondary colonisers (*T. versicolor*, *S. hirsutum*, *B. adusta*) produced no responses when correcting for fungal biomass. Therefore, the enhancing responses in *T. versicolor*, *S. hirsutum* and *B. adusta*, when not normalising for fungal biomass, may have been driven by the inhibition of growth and biomass production at the lower temperature. This is reflected in the lower ergosterol content in the cooled samples than in the control samples for these species, however this reduced ergosterol content was only marginally significantly lower for *T. versicolor*. However, with *F. fomentarius* showing an enhancing response even after correcting for fungal biomass, and *H. fasciculare* now showing an enhancing response (previously no response), demonstrates that enhancement was not entirely driven by the effects of temperature on growth and biomass production. After normalising for biomass, *P. velutina* now revealing a compensatory response (previously no response) is the only evidence of thermal compensation detected in the present study. As such, the potential for some basidiomycete species to down-regulate their metabolism with warming exists. It is, however, important to note that no relationship between species respiration rate and ergosterol content was found, that is, the species that had the highest and lowest respiration rates did not have the highest and lowest ergosterol contents respectively. In fact, the species with the highest (*S. hirsutum*) and lowest (*B. adusta*) ergosterol both had intermediate respiration rates compared to the other species. As a consequence, ergosterol as a biomarker for fungal biomass and the mass-specific respiration responses should be interpreted with caution, despite ergosterol being considered more appropriate than other biomarker approaches (Baldrian *et al.*, 2013b).

Crowther and Bradford (2013) showed that cord-forming basidiomycetes grown on agar can acclimate to temperature within days, with in almost all cases, warm-acclimated individuals having lower mass-specific respiration rates at intermediate temperatures than cold-acclimated isolates. However, contrary to expectations, the present study using the same species (but different strains) of cord-forming basidiomycetes growing in wood found no thermal response overall of mass-specific respiration. The mass-specific respiration of *P. velutina* showing a compensatory response in the present study would agree with the acclimation response of *P. velutina* (different strain) in Crowther and Bradford (2013). However, the mass-specific respiration of *H. fasciculare* showing an enhancing

response in the present study contrasts with two different strains of *H. fasciculare* showing acclimation responses and one different strain of *H. fasciculare* not showing an acclimation response in Crowther and Bradford (2013). No thermal response overall of mass-specific respiration of individual species of cord-forming basidiomycetes growing in wood in the current study differs from the dominant acclimation response of mass-specific respiration of cord-forming basidiomycete species growing in agar reported previously (Crowther and Bradford, 2013). The fundamental difference in mass-specific respiratory thermal responses in Crowther and Bradford (2013) and the present study could be due to the different substrates (agar vs semi-natural wood) used or timescale (days vs months) over which measurements were taken, respectively. In contrast, adapted strains of *Neurospora discreta* on agar increased their mass-specific respiration rate with warming compared to parental strains (Romero-Olivares *et al.*, 2015). Therefore, Romero-Olivares *et al.* (2015) observed no evidence of thermal compensation, which would be more in align with the limited evidence of mass-specific compensatory responses (only *P. velutina*) identified in the current study. The increase in mass-specific respiration detected with warming in Romero-Olivares *et al.* (2015) would agree with the mass-specific enhancing responses (*F. fomentarius, H. fasciculare*) detected in the present study. Crowther and Bradford (2013) and Romero-Olivares *et al.* (2015) have both used agar, an artificial medium which, although useful for producing a simplified model system to perform manipulative experiments, provide homogeneous, well-mixed conditions that inherently fail to imitate the structural or chemical heterogeneity of most natural resources (Crowther *et al.*, 2018). Our study, however, is the first to investigate the respiratory thermal response of individual species of fungi using a natural substrate in the medium to long term. The present study provides mass-specific respiratory thermal responses of individual species of basidiomycetes that are more informative of those present in decomposing wood in natural systems and over a timescale that better represents the main period of wood decomposition.

3.5.3 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 behave in a tragedy of the commons situation, and utilise the available resources rapidly. This, in turn, would increase their respiration and decomposition rates, allowing them to gain and establish their territory before they are outcompeted by the later secondary colonisers. On the other hand, later secondary colonisers have more control over the resources, hence it is less of a tragedy of the commons situation. Later secondary colonisers are generally, but not always, more combative and accordingly outcompete the primary and early secondary colonisers eventually (Boddy, 2000). Consequently, a compensatory response might occur, as shown by *P. velutina* when normalising for biomass, and may be an advantage, for example a greater C-use efficiency allowing more C to be allocated to mycelium to search and compete for already colonised territory. However, as 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 and Boddy, 2017).

3.5.4 Respiratory thermal response of soil microorganisms may depend on their thermal environment

Broadly distributed generalists and species from highly variable thermal environments are more likely to be capable of compensatory responses than scarcely distributed specialists and species from more stable, limited thermal environments. Basidiomycetes found on the surface of dead wood, fine woody debris and leaf litter layer experience a range of temperature fluctuations over diurnal and seasonal timescales (Boddy, 1983a; Rayner and Boddy, 1988). Therefore, we may have expected them to be more likely to show thermal compensation responses than other soil microorganisms that exist in deeper soil horizons with more consistent thermal environments. The limited evidence of compensatory responses of wood decay basidiomycetes that are present in temperate ecosystems with a wide temperature regime suggests that similar results will be observed from fungi with narrow temperature regimes, including those found within deeper soil layers.

3.5.5 Implications of the respiratory thermal response of individual basidiomycete species for fungal communities, whole soil communities and long-term warming experiments

The present study used a reductionist approach to understand the respiratory thermal response of individual species of basidiomycetes. The responses of individual populations of basidiomycete species with warming may influence the outcomes of competitive interactions between species, thus the species present within fungal communities. The thermal response of respiration of dominant species in communities with warming will thus be important. Furthermore, respiratory thermal responses may differ when species are engaged in competitive interspecific fungal interactions, which may affect the overall respiratory response of fungal communities with warming. Therefore, further studies exploring the respiration response to temperature of more diverse species assemblages and communities when interactions are occurring is important to increase our mechanistic knowledge of wood decomposition rates with warming.

In a previous study that used the cooling approach to investigate the respiration rate of whole soil microbial communities to temperature, using 22 soils along a climate gradient from the Arctic to the Amazon, one soil exhibited a compensatory thermal response (Karhu *et al.*, 2014). The soil that showed a compensatory thermal response was dominated by a limited number of *Basidiomycota*, mostly saprotrophic fungi, while the other soils were dominated by ectomycorrhizal fungi, however the potential importance of saprotrophic *Basidiomycota* for controlling this compensatory thermal response could not be confirmed (Auffret *et al.*, 2016). The results of our study, however, indicate that saprotrophic basidiomycetes were likely not the mechanism behind the compensatory thermal response detected.

Many long-term (years-decades) warming experiments in the field observe a decline in soil microbial respiration with warming (Jarvis and Linder, 2000; Oechel *et al.*, 2000; Luo *et al.*, 2001; Melillo *et al.*, 2002, 2017; Romero-Olivares *et al.*, 2017; García-Palacios *et al.*, 2018). The detection of an overall enhancing thermal response of respiration, and no thermal response when normalising for

biomass, indicates that the mechanism underlying this decline in respiration in soil communities with warming is more likely a result of indirect effects of temperature, such as substrate depletion, as opposed to a compensatory thermal response of soil microorganisms down-regulating respiration rates.

3.6 Conclusion

The cooling approach, that minimised the confounding factor of warming-induced substrate depletion, allowed thermal responses of fungal respiration to be detected. Individual species of basidiomycetes colonising wood did not show compensatory thermal responses that decrease the temperature sensitivity of respiration. Instead, basidiomycete species decomposing wood showed an overall enhancing thermal response that increased the temperature sensitivity of respiration in the medium to long term. Consequently, species of basidiomycetes will increase their respiration rate associated with wood decomposition overall with warming. When respiration rates were normalised for fungal biomass, basidiomycete species showed no thermal response overall, which suggests that some enhancing responses were caused by the inhibition of growth and biomass production. However, with some enhancing responses still identified after normalising for biomass, shows that enhancement was not entirely driven by the effects of temperature on growth and biomass production. The reductionist approach of studying individual populations of species allowed the capture of physiological responses that would otherwise be concealed in natural complex systems, providing a valuable mechanistic understanding of respiratory responses that contribute to fungal and whole soil communities. The use of natural substrate, rather than artificial media, has improved our mechanistic insights and consequently inferences of real world responses. Exploring the response of individual fungal species is the first step; the species present need to be gradually increased to determine whether these respiratory thermal responses are maintained in more complex fungal assemblages and communities. Overall, with increasing global temperatures, species of basidiomycetes decaying wood will enhance the temperature sensitivity of respiration in the medium to long term, reduce the role of terrestrial ecosystems to act as C sinks and contribute extensively to the positive land C-climate feedback and accelerate climate change.

Chapter 4. Respiratory thermal response of multispecies assemblages of wood decay basidiomycetes

4.1 Abstract

Chapter 3 demonstrated that the temperature sensitivity of the respiration of species of basidiomycetes decomposing wood increases with warming. Wood decay basidiomycetes are, however, often involved in competitive interspecific interactions within communities. To address the respiratory thermal response of basidiomycete interactions in wood, two-species assemblages and a three-species assemblage of basidiomycetes colonising beech wood (*Fagus sylvatica*) were set up in 2- and 3-dimensional competitive systems, respectively. A 90-day cooling and warming approach was applied, where respiration rates were measured and progression and outcomes of interactions determined. Thermal responses (compensatory, enhancing, no response), which indicated a decrease, increase and no change in temperature sensitivity of respiration respectively, were quantified. Overall, two-species assemblages and the three-species assemblage showed no thermal responses to cooling. With warming, two-species assemblages showed no thermal response overall, whereas the three-species assemblage demonstrated a compensatory response. Compensatory responses may have been related to the differences in the progression of the species interactions or the warmed treatment exceeding the optimum temperature for growth for some of the species. No strong evidence for compensatory responses that would gradually reduce the temperature sensitivity of respiration was produced. The results suggest that with increasing global temperatures, simple communities of interacting wood decay basidiomycetes will more often cause no change in the temperature sensitivity of respiration overall in the medium to long term. The limited evidence for compensatory responses indicates that there remains the potential for a positive feedback to climate change associated with the effect of temperature on wood decomposition. However, the overall loss of the enhancing responses observed in single species cultures suggests that competitive interactions may reduce the overall temperature sensitivity of wood decomposition in the medium to long term.

4.2 Introduction

Individual species of basidiomycetes colonising wood show an overall enhancing thermal response that increases the temperature sensitivity of respiration associated with wood decomposition in the medium to long term (Chapter 3). With rising global temperatures, species of wood decay basidiomycetes will increase their respiration rate, subsequently contributing to the positive land C-climate feedback and climate change. The response of individual basidiomycete species is relevant in stable communities in wood (Boddy *et al.*, 2017), however, species in communities frequently compete with each other for territory and the resources within (Boddy, 2000). As such, understanding the respiratory thermal responses of multiple fungal species that are engaged in interspecific interactions within a community is required.

Outcomes of fungal interactions can be: (1) deadlock, where neither species gain territory from the other; (2) complete replacement, where one species gains territory from the other; (3) partial replacement, where one species captures some but not all of the others territory; or (4) reciprocal replacement, where one species takes some of the territory previously occupied by the other and *vice versa* (Boddy, 2000). The success of species in interspecific interactions is determined by combative and/or defensive antagonistic mechanisms (Boddy and Hiscox, 2016). Within fungal communities, hierarchies of combative ability occur where late secondary colonisers > early secondary colonisers > primary colonisers (Boddy, 2000). In addition, outcomes of combative interactions change under different abiotic and biotic regimes (Griffith and Boddy, 1991; Crowther *et al.*, 2014; Hiscox *et al.*, 2016a). The complexity of fungal interactions, therefore, makes fungal community change difficult to predict. Further knowledge of the respiratory thermal response of wood decay basidiomycetes during competitive interspecific interactions is required to advance our understanding of their response, and contribution to the whole soil microbial community response, to warming.

This study explores the respiratory thermal response of two-species assemblages and a three species assemblage of basidiomycetes colonising beech wood (*Fagus sylvatica*), set up in 2- and 3-dimensional competitive

systems, respectively. Using the cooling approach as in Chapter 3, and adding a warming approach, changes in respiration rates were measured to gain a mechanistic understanding of the response of multispecies assemblages of wood decay basidiomycetes to warming. Individual species of basidiomycetes that showed enhancing and no responses following cooling (Chapter 3), that would compete for territory and likely coexist in the long term based on a previous study (Hiscox *et al.*, 2015a), were selected to form basidiomycete assemblages, and in turn, determine whether responses were maintained during interactions. It was hypothesised that competing species of basidiomycetes decomposing wood in increasingly complex assemblages would show overall enhancing thermal responses and increase the temperature sensitivity of respiration in the medium to long term.

4.3 Methods

4.3.1 Species of wood decay basidiomycetes and colonisation of wood blocks

When growing alone in wood, *F. fomentarius* and *S. hirsutum* showed enhancing responses after cooling (Chapter 3). To determine whether enhancing responses continue when species coexist in the same territory, interactions involving *F. fomentarius* and *S. hirsutum* with *V. comedens* and *P. velutina* were performed. *V. comedens* and *P. velutina*, that showed clear no responses following cooling (Chapter 3), were selected based on their interactions with *F. fomentarius* and *S. hirsutum* in natural communities, and the likelihood of the species interacting in the community in the long term. *S. hirsutum* was expected to deadlock with *V. comedens* and *P. velutina* (Hiscox *et al.*, 2015a), however knowledge of interactions involving *F. fomentarius* was uncertain.

The four species of basidiomycetes (Table 4.1) were used to separately colonise 2 x 2 x 2 cm beech wood blocks, which were incubated at 20 °C in the dark for 108 d, as in Chapter 3 (Section 3.3.1). After pre-colonisation of wood blocks, colonisation was confirmed by re-isolation of the fungi from a sample of wood blocks (n = 10) (Section 3.3.1). As an estimate of the initial decay state (108 d), the density was calculated as the oven dry weight (80 °C for 72 h) per fresh volume (mm^{-3}) (Section 3.3.6), of a sample of colonised wood blocks for each

species (mg mm^{-3}, 10 replicates) used for two-species interactions and three-species interactions (Supplementary Fig. 4.1). The density of wood blocks pre-colonised with species used for the two-species interactions were not significantly different ($F_{3, 36}$ = 0.756, P = 0.526) (Supplementary Fig. 4.1a), however, the density of wood blocks pre-colonised with species used for the three-species interaction were significantly different ($F_{2, 27}$ = 3.408, P < 0.05) (Supplementary Fig. 4.1b). Wood blocks pre-colonised with *S. hirsutum* (0.593 ± 0.029 mg mm^{-3}) were significantly less decayed than those with *F. fomentarius* (P < 0.05). Wood block densities of *S. hirsutum* and *F. fomentarius* did not significantly differ from *V. comedens* (P > 0.05) (Supplementary Fig. 4.1b).

Table 4.1 Fungal species used to colonise wood blocks.

Ecological role	Species	Strain
Primary coloniser	*Vuilleminia comedens*	VcWVJH1
	Fomes fomentarius	JHC 1676
Early secondary coloniser	*Stereum hirsutum*	ShSS1
Late secondary coloniser/cord former	*Phanerochaete velutina*	Pv29

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

4.3.2 Wood block preparation

Before the two-species assemblage set up, pre-colonised wood blocks were scraped free of adhering mycelium and agar using a sterile scalpel. Individual wood blocks were then placed directly on to 20 ml perlite (Homebase, UK) moistened with 2 ml sterile distilled water (dH_2O) to achieve a water potential of -0.012 kPa, in a plastic 100 ml lidded deli pot (Cater4you, UK), as described in Chapter 3 (Section 3.3.2). 60 wood block microcosms were set up for *F. fomentarius* and *S. hirsutum*, and 40 wood block microcosms were set up for *V. comedens* and *P. velutina*. Moisture was maintained in each microcosm and holes in each pot covered by microporous surgical tape (3M, Bracknell, UK) allowed for aeration but prevented contamination with other species, as explained in Chapter 3 (Section 3.3.2). Individual wood block microcosms were incubated at 20 °C and three respiration measurements were taken as detailed in Chapter 3 (Section 3.3.4), over 32 d of the pre-incubation period before temperature change. Wood blocks colonised by *F. fomentarius* and *S. hirsutum* were sorted into three groups of 20 blocks, and *V. comedens* and *P. velutina* into two groups

of 20 blocks. Individual wood blocks were then assigned to one of four temperature treatments (n = 5: pre-treatment, control, cooled, warmed; explained below), based on establishing similar mean respiration rates and trajectories across temperature treatments prior to temperature change (Supplementary Fig. 4.2). Preceding the three-species assemblage set up, due to the greater number of wood blocks involved, pre-colonised wood blocks remained on agar at 20 °C for 32 d of the pre-incubation period. Therefore, respiration measurements during 32 d of the pre-incubation period and assignment of individual wood blocks to temperature treatments did not occur for the three-species assemblage.

4.3.3 Establishing model fungal assemblages

Pre-colonised wood blocks for two-species assemblages were scraped free of adhering mycelium and agar using a sterile scalpel, at 33 d of the pre-incubation period, 3 d prior to set up. At 37 d of the pre-incubation period, using all four species, 5 types of two-species interactions were produced (Fig. 4.1a). Pre-colonised wood blocks of one species were randomly combined with pre-colonised wood blocks of the other species that were assigned to the same temperature treatment. Two-species assemblages were formed by combining 2 pre-colonised wood blocks (one species per block) with cut xylem vessel ends touching (Fig. 4.1a). Wood blocks were secured with an elastic band (removed after 7 d) and placed in 100 ml plastic lidded deli pot (Cater4You, UK) containing 20 ml perlite (Homebase, UK) and 2 ml of sterile dH_2O. For each of the two-species interactions, 20 replicates were set up for respiration measurements, and 36 replicates were assembled for destructive sampling during incubation to determine the progression of interaction.

Pre-colonised wood blocks for the three-species assemblage were scraped free of adhering mycelium and agar using a sterile scalpel, at 33 d of the pre-incubation period, 3 d before set up. At 37 d of the pre-incubation period, using three species, 1 type of three-species interaction was produced (Fig. 4.1b). The three-species assemblage was created by combining 9 blocks colonised by each of the three species (27 wood blocks in total, one species per block) in a 3 x 3 x 3 matrix design such that species were evenly distributed and blocks containing the same species were not adjacent to one another (Fig. 4.1b). Wood blocks were

joined with an elastic band around each layer (removed after 7 d) and placed in 614 ml plastic lidded deli pot (Cater4You, UK) containing 85 ml perlite (Homebase, UK) and 12 ml of sterile dH_2O. For the three-species interaction, 20 replicates were set up for respiration measurements, and 18 replicates were assembled for destructive sampling during incubation to determine the progression of interaction.

For the two- and three-species assemblages, each wood block interaction microcosm was weighed and sterile dH_2O added to the perlite every 14 d to maintain moisture. Holes (4 x 1 mm diameter) in each pot covered by microporous surgical tape (3M, Bracknell, UK) allowed for aeration but prevented contamination with other species (Section 3.3.2). Wood block interaction microcosms were incubated (Sanyo Electric/Panasonic Cooled Incubator, MIR-154) at 20 °C for 6 d until the end of the 43 d pre-incubation period (151 d). During this time, one respiration measurement from each wood block interaction microcosm was taken (40 d of pre-incubation period; 148 d) before the temperature change (151 d). Based on the re-isolation of fungi from sacrificed samples (n = 3) (Section 3.3.1), territory occupied by species at temperature change (151 d) was assumed to be the same as when interactions were assembled.

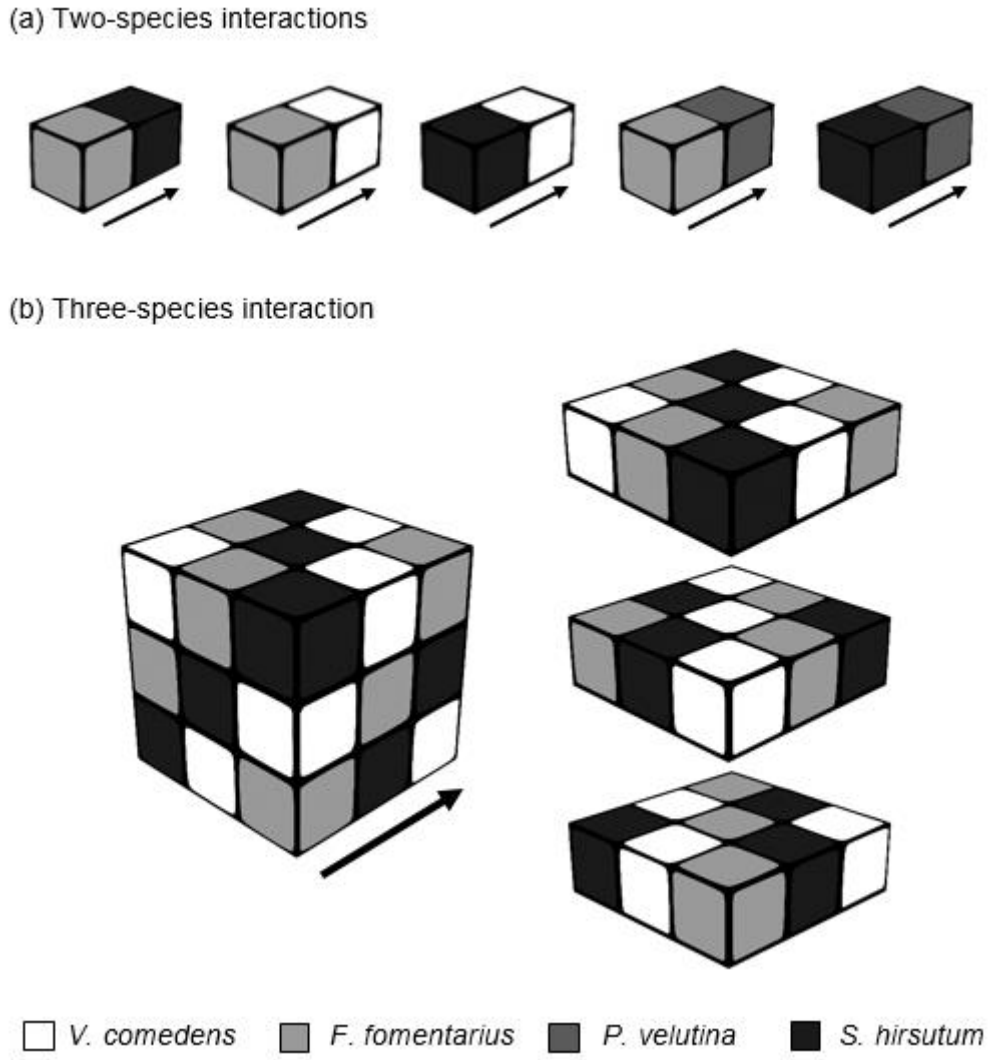


Figure 4.1 Experimental set up of (a) two-species interactions and (b) a three-species interaction. Two-species interactions involved pairings of wood blocks colonised by *V. comedens* (white), *F. fomentarius* (light grey), *P. velutina* (dark grey) or *S. hirsutum* (black), forming a 2-block 2-dimensional community. Three-species interactions involved nine wood blocks colonised by *V. comedens*, *F. fomentarius* and *S. hirsutum*, arranged such that same species blocks were not adjacent, forming a 27-block 3-dimensional community. Arrows indicate xylem directional flow.

4.3.4 Wood block interaction microcosm incubation

As briefly mentioned previously (Section 4.3.2), the 20 wood block interaction microcosms of each two- and three-species assemblages were assigned to one of four temperature treatments (n = 5): pre-treatment (destructively sampled at 151 d before temperature change), cooled (incubated at 12 °C at 151 d for 90 d), warmed (incubated at 28 °C at 151 d for 90 d) and control (incubated at 20 °C for a further 90 d) (Fig. 4.2). The pre-treatment replicates were destructively sampled

and density measurements (mg mm^{-3}; 5 replicates) were taken prior to any temperature change. A change in temperature for 90 d and the cooled and control incubation temperatures are justified in Chapter 3 (Section 3.3.3). The warmed incubation temperature matches the magnitude of change of the cooled treatment.

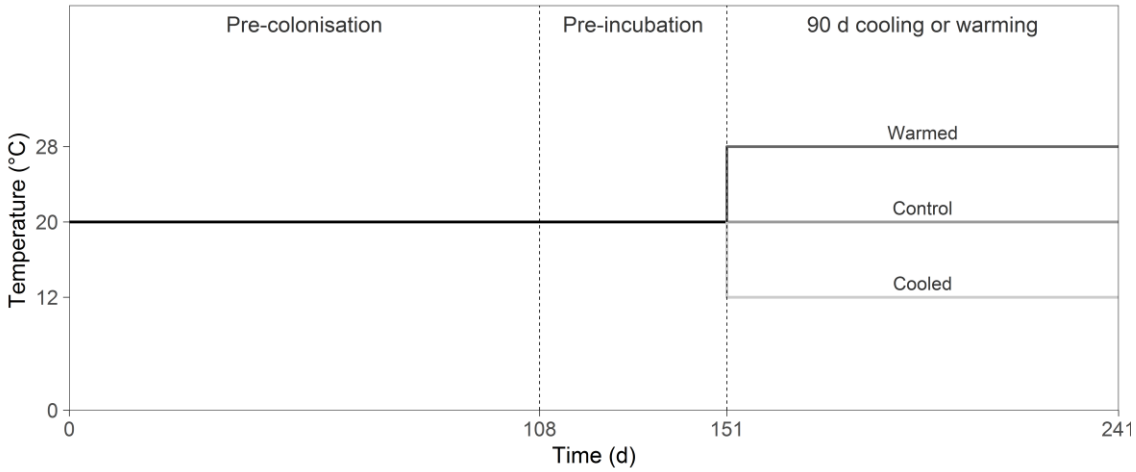


Figure 4.2 Wood block pre-colonisation and wood block interaction microcosm incubation shown as a timeline. Wood blocks were pre-colonised and then wood block interaction microcosms were set up during pre-incubation. Wood block interaction microcosms were assigned to one of four temperature treatments: pre-treatment, cooled, warmed and control. Pre-treatment replicates were removed at 151 d, before temperature change.

4.3.5 Respiration measurements

Respiration was measured by placing each wood block interaction microcosm with a closed lid inside a larger airtight 700 ml and 2.6 L plastic container (Lock & Lock® container, Hana Cobi Plastic Co Ltd, Seoul, Korea), for two- and three-species assemblages respectively, which was connected to an infrared gas analyser (IRGA; EGM-4, PP systems, version 1.47, Hitchen, UK) in a closed-loop configuration, as in Chapter 3 (Section 3.3.4). Holes in the pot of each wood block interaction microcosm covered by microporous surgical tape allowed CO_2 to accumulate in the larger 700 ml or 2.6 L plastic container whilst maintaining a sterile environment. For two-species assemblages, the CO_2 concentration in the headspace of the incubation chamber was measured immediately after closure, and then again after 18 h for the control and cooled treatments, and after 6 h for the warmed treatment. The warmed treatment was incubated for 6 h to prevent a

high CO_2 concentration that could be harmful to the species. For the three-species assemblage, the CO_2 concentration in the headspace of the incubation chamber was measured immediately after closure, and then again after 4 h for all temperature treatments. Respiration measurements of the assemblages were made one day after the temperature change, and then weekly thereafter. Respiration rates were expressed as µg C per g of dry weight per h. The respiration measurements acted as the most sensitive and accurate measure of the decay rate.

4.3.6 Determination of progression and outcome of interactions

The two- and three-species assemblages in the cooled, warmed and control treatments that had respiration measurements taken were then destructively sampled at the end of 90 d incubation (241 d). This involved each individual wood block being split in half along the grain using a surface-sterilised (flamed in 70% ethanol) chisel. Pieces of wood (2 mm^3) were excised from the middle of the inside-face 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.

To determine the progression of interactions during 90 d incubation, other wood block interaction microcosms that were exposed to the temperature treatments but respiration not measured, were destructively sampled at four (8, 22, 50 and 78 d after temperature change) and two (7 and 45 d after temperature change) time points over the 90 d incubation, for two- and three-species interactions respectively. For each interaction type, three replicates for each temperature treatment at each time point were destructively sampled. Again, individual wood blocks were split in half along the grain, pieces of wood taken and placed onto 2% MA and outgrowing mycelium was identified morphologically, as above.

Species identification from the pieces of wood taken allowed the territory occupied by each species to be calculated, as a percentage of the whole block system, for each replicate. The mean percentage territory occupied by each species for each temperature treatment for each interaction was determined from

the wood blocks at the destructive sampling time points during the 90 d incubation (when respiration was not measured), and at the end of the 90 d incubation (241 d) from wood blocks used for respiration measurements. The outcome of interactions (mean of 5 replicates) at the end of 90 d incubation was determined, and was either: deadlock, where neither species was replaced by the other; partial replacement, where one species was partially replaced by the other; or complete replacement, where one species was completely replaced by the other. For each interaction and temperature treatment, the time (d) at which 75% and 100% of territory was occupied by the replacing species was estimated using a linear regression of the mean percentage territory occupied.

4.3.7 Quantifying respiratory responses

As explained in Chapter 3 (Section 3.3.7), higher temperatures will cause greater respiration rates, hence leading to greater C losses than lower temperatures. Differences in C availability can be accounted for by plotting the respiration rate (µg C gdw^{-1} h^{-1}) of control, cooled and warmed treatments against the cumulative respiration (mg C gdw^{-1}) for each interaction.

The multispecies assemblages could show three possible thermal responses following cooling and warming: compensatory, enhancing or no response (Fig. 4.3). After cooling, the respiration rate and subsequent thermal response (Fig. 4.3a), and the relative respiration rate (normalised to the time of cooling) of control and cooled samples compared to detect more subtle compensatory and enhancing responses (Fig. 4.3b), are explained in Chapter 3 (Section 3.3.7). The approach used to identify thermal responses after warming was analogous to the cooling approach, but with the following differences. Following warming, a more rapid decrease in the respiration rates expressed per unit wood mass and the relative respiration rates (normalised to the time of warming) of the warmed treatment compared to the control treatment provides evidence for a compensatory response (Fig. 4.3c,d). An increase in respiration rates and relative respiration rates (normalised to the time of warming) after warming, or a slower decline, of the warmed treatment than in the control treatment provides support for an enhancing response (Fig 4.3c,d). Hence, after warming, the direction of respiration that indicates a compensatory or enhancing response is the opposite

of that after cooling. However, by changing the way the response ratios that involved the warmed treatment were calculated, it was possible to ensure that compensatory responses were still represented by values < 1, and enhancing responses by values > 1 (see below).

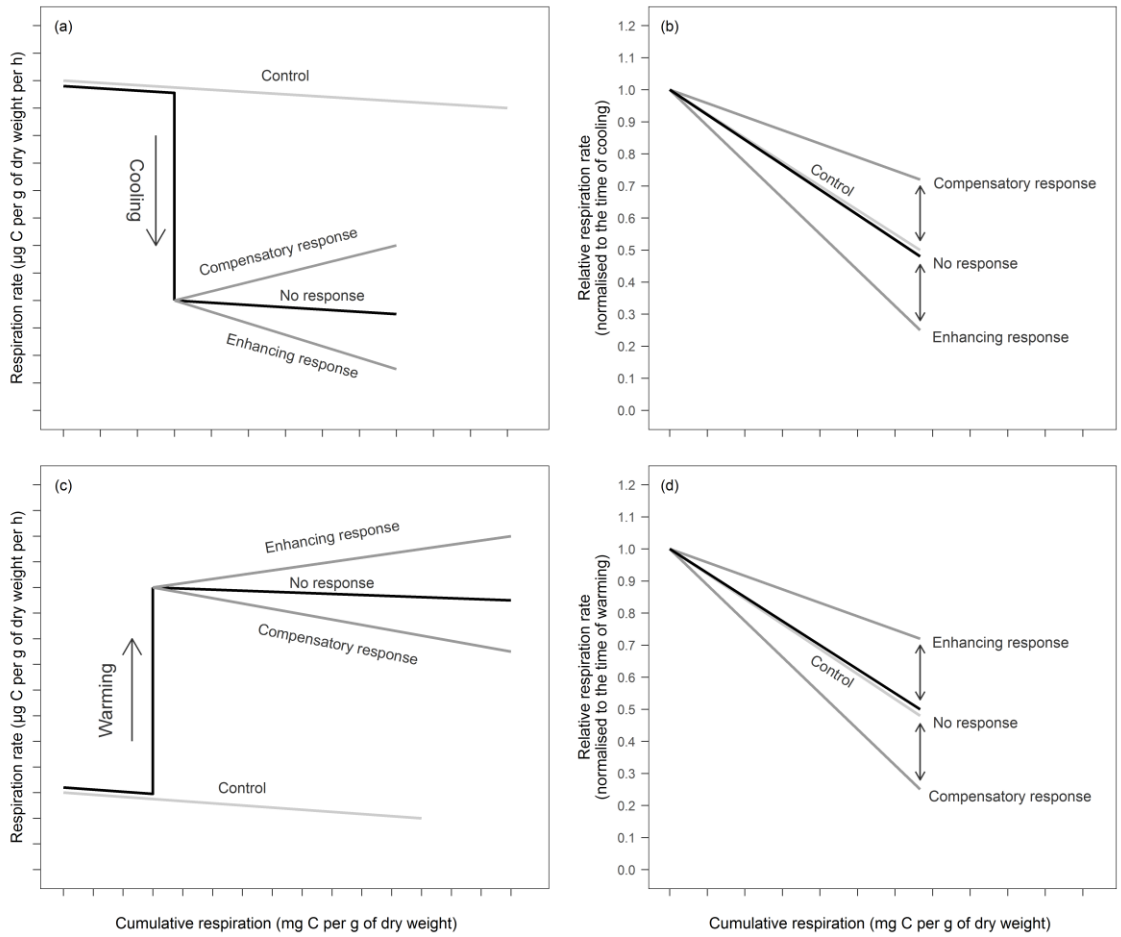


Figure 4.3 Schematic diagram showing the rationale of the (a) cooling and (c) warming approach, and the respiration rates that would be observed for a compensatory, enhancing and no response following temperature change. (b) Relative respiration rate (normalised to the time of cooling) of control and cooled samples, and (d) relative respiration rate (normalised to the time of warming) of control and warmed samples, can be used to detect more subtle compensatory and enhancing responses.

The respiration rates of control and cooled temperature treatments were compared at the same cumulative respiration, to account for any effects of different C availability, by calculating the control treatment respiration rate at the total cumulative respiration of cooled treatment samples at the end of incubation, using linear regressions (Table 4.2), as in Chapter 3 (Section 3.3.7). For control and warmed temperature treatments, the warmed treatment respiration rate was calculated at the total cumulative respiration of control treatment samples at the

end of incubation, using linear regressions, for four of the assemblages (Table 4.3, Fig. 4.4a). The negative effect of warming on two assemblages caused respiration rates and thus cumulative respiration of warmed temperature treatment to be lower than that of the control treatment. In these cases (indicated in bold in Table 4.3), the total cumulative respiration of the warmed treatment rather than the control treatment was used in the linear regressions to avoid extrapolating beyond the dataset, and thus allowing treatments to be compared at the same cumulative respiration (Table 4.3, Fig. 4.4b).

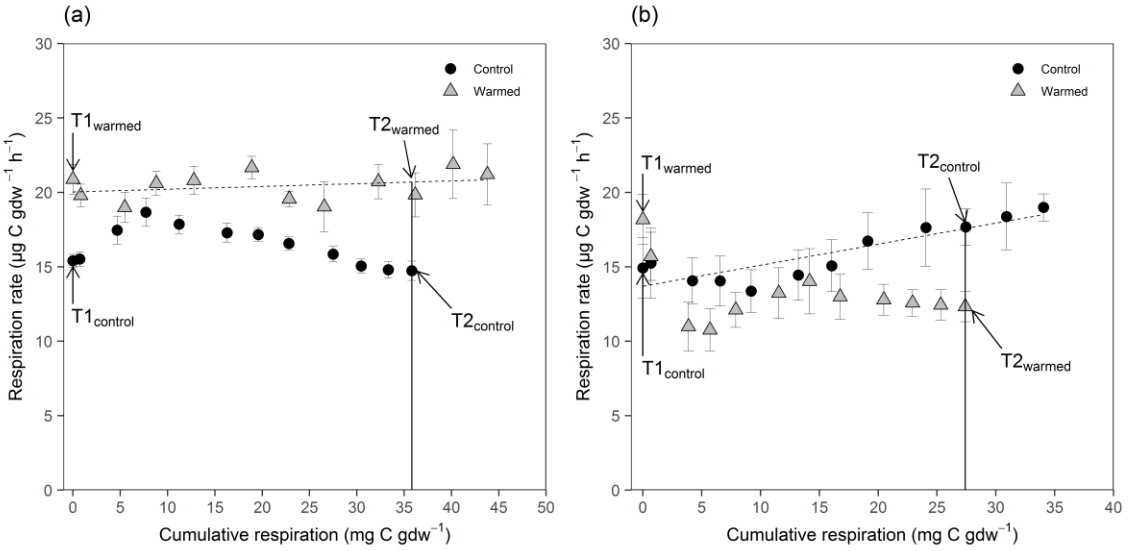


Figure 4.4 Example of linear regression through (a) warmed treatment, to calculate warmed treatment respiration rate at the total cumulative respiration of control treatment samples at the end of incubation and (b) control treatment, to calculate control treatment respiration rate at the total cumulative respiration of warmed treatment samples at the end of incubation. *S. hirsutum* vs. *P. velutina* has been used in panel (a) and *F. fomentarius* vs. *V. comedens* has been used in panel (b). Labels show the values used in the RR_{CW} equation. Cumulative respiration was calculated from the time of temperature change (151 d), at the start of 90 d incubation.

Table 4.2 Total cumulative respiration since time of cooling for cooled treatment (mean ± SE of the mean), used in a linear regression of control treatment respiration rate after temperature change, to calculate control treatment respiration rate at a corresponding level of cumulative respiration as cooled treatment samples at end of incubation.

Interaction	**Total cumulative respiration since time of cooling for cooled treatment (mg C gdw^{-1})**	**Linear regression of control treatment respiration rate after temperature change (µg C gdw^{-1} h^{-1})**	**R^2**
Two-species			
F. fomentarius vs. *S. hirsutum*	15.98 ± 0.32	y = -0.30894x + 19.68404	0.9301
F. fomentarius vs. *V. comedens*	16.09 ± 1.72	y = 0.14150x + 13.69472	0.7391
S. hirsutum vs. *V. comedens*	18.77 ± 0.51	y = 0.11228x + 14.85751	0.9107
F. fomentarius vs. *P. velutina*	23.73 ± 1.11	y = -0.07996x + 22.06872	0.7583
S. hirsutum vs. *P. velutina*	19.72 ± 1.01	y = -0.142808x + 19.691766	0.9795
Three-species			
V. comedens vs. *F. fomentarius* vs. *S. hirsutum*	18.35 ± 0.52	y = -0.37720x + 22.21532	0.9794

Table 4.3 Total cumulative respiration since time of temperature change for control or warmed treatments (mean ± SE of the mean), used in linear regressions to calculate respiration rates at a corresponding level of cumulative respiration. In contrast to the cooling experiment in which cooling always reduced respiration rates (Table 4.2), warming sometimes increased and sometimes decreased respiration rates compared to that of the control treatment. Therefore, for the interactions in bold, the linear regression was applied to the control treatment to calculate the respiration rate at a corresponding level of cumulative respiration as warmed treatment samples at the end of incubation, whereas for all other interactions, the linear regression was applied to the warmed treatment to calculate the respiration rate at a corresponding level of cumulative respiration as control treatment samples at the end of the incubation.

Interaction	**Total cumulative respiration since temperature change for control or warmed treatments (mg C gdw^{-1})**	**Linear regression of warmed or control treatment respiration rates after temperature change (µg C gdw^{-1} h^{-1})**	**R^2**
Two-species			
F. fomentarius vs. *S. hirsutum*	28.92 ± 1.82	y = -0.46560x + 23.13588	0.8687
F. fomentarius* vs. *V. comedens	**27.39 ± 2.80**	**y = 0.14150x + 13.69472**	**0.7391**
S. hirsutum* vs. *V. comedens	**31.76 ± 3.38**	**y = 0.11228x + 14.85751**	**0.9107**
F. fomentarius vs. *P. velutina*	43.66 ± 3.58	y = -0.06966x + 29.80043	0.0816
S. hirsutum vs. *P. velutina*	35.81 ± 1.09	y = 0.01864x + 20.02991	-0.0035
Three-species			
V. comedens vs. *F. fomentarius* vs. *S. hirsutum*	31.19 ± 1.03	y = -0.701x + 30.944	0.8267

The method applied to quantify either compensatory or enhancing responses after cooling (Fig. 4.3) was the same as Chapter 3 (Section 3.3.7). The control treatment and cooled treatment relative respiration rates were calculated as in Section 3.3.7, but for the two-species interactions and three-species interaction, the respiration rates were divided by the respiration rates at 3 d and 1 d after cooling, respectively, to focus on responses that would be observed in the long term (Section 3.3.7). See Supplementary Fig. 4.3 for the relative respiration rates of control and cooled treatments normalised to 1 d after cooling for the two-species interactions. Response ratios comparing control and cooled treatment relative respiration rates (RR_{CC}: Response Ratio, control versus cooled; Fig. 4.3b) were produced (Section 3.3.7). Response ratio values < 1 indicate a compensatory response (cooled treatment relative respiration rates were greater than control treatment relative respiration rates, at a given cumulative respiration) and values > 1 indicate an enhancing response (cooled treatment relative respiration rates were lower than control treatment relative respiration rates, at a given cumulative respiration), as outlined in Chapter 3 (Section 3.3.7). The response ratios were treated the same as described previously, to produce a mean response ratio and 95% confidence intervals for two-species interaction overall, and each two-species interaction and three-species interaction (Section 3.3.7).

The method applied to quantify either compensatory or enhancing responses after warming (Fig. 4.3) was the same as cooling, but with the following differences. The control treatment relative respiration rate ($T2_{control}$ / $T1_{control}$) and warmed treatment relative respiration rate ($T2_{warmed}$ / $T1_{warmed}$) were calculated, with the respiration rates of two-species interactions and three-species interaction being divided by the respiration rates at 1 d after warming (3 d for *S. hirsutum* vs. *V. comedens* to focus on long-term responses) (Section 3.3.7). See Supplementary Fig. 4.3 for the relative respiration rates of control and warmed treatments normalised to 1 d after warming for *S. hirsutum* vs. *V. comedens*. Response ratios comparing control and warmed treatment relative respiration rates (RR_{CW}: Response Ratio, control versus warmed; Fig. 4.3d) were calculated for each interaction, however the warmed treatment relative respiration rate was divided by the control treatment relative respiration rate.

$$RR_{CW} = \frac{T2_{warmed}/T1_{warmed}}{T2_{control}/T1_{control}} \quad (1)$$

where $T2_{warmed}$, $T1_{warmed}$, $T2_{control}$ and $T1_{control}$ are shown in Fig. 4.4. $T1_{warmed}$ and $T1_{control}$ are the warmed and control respiration rates at 1 d after warming respectively, and $T2_{warmed}$ and $T2_{control}$ are the warmed and control respiration rates, respectively, at the same cumulative respiration.

This ensured that response ratio values < 1 indicate a compensatory response (warmed treatment respiration rates were lower than control treatment respiration rates, at a given cumulative respiration) and values > 1 indicate an enhancing response (warmed treatment respiration rates were greater than control treatment respiration rates, at a given cumulative respiration). Mean response ratio and 95% confidence intervals for two-species interaction overall, and each two-species interaction and three-species interaction were produced (Section 3.3.7).

4.3.8 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 (148 d) of control, cooled and warmed treatments after interaction set up, during the pre-incubation period prior to temperature change (151 d), of each two-species interaction and three-species interaction.

To test for statistically significant responses of two-species interaction overall, and each two-species interaction and three-species interaction, 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. Paired t-tests were also used to compare the warmed and control treatment relative respiration rates, at the lowest total cumulative respiration of the warmed or control treatments. Moreover, to further support the response ratio method (RR_{CC} and RR_{CW}), F ratio was used for statistical comparison of control and cooled treatment relative respiration rate and of control and warmed treatment relative respiration

rate (normalised to the time of cooling and warming respectively) fitted lines for each interaction. Using the known F distribution, a *P* value was calculated from the F ratio and two degrees of freedom values.

One-way ANOVA models and Tukey's pairwise comparisons were used to compare wood block densities after pre-colonisation of wood blocks with each species (108 d).

4.4 Results

4.4.1 Progression and outcomes of interactions

The outcomes of interactions after 90 d incubation varied between species assemblages and temperature treatments (Table 4.4, Fig. 4.5). Across the 5 replicates for each of the 18 assemblages (six types exposed to three temperature treatments), 8 assemblages showed a consistent interaction outcome, whereas 10 showed variability in the interaction outcome (Table 4.4). Evidence of deadlock, partial and complete replacement of species in interactions was observed at the end of the 90 d experiment (Table 4.4, Fig. 4.5). The interaction outcome was only deadlock in all 5 replicates for *S. hirsutum* vs. *V. comedens* in the cooled treatment. Deadlock predominantly occurred in *S. hirsutum* vs. *V. comedens* in the control treatment and in *F. fomentarius* vs. *V. comedens* in the cooled and warmed treatments, but the mean territory change lead to some partial replacement by *S. hirsutum* and *V. comedens* in these interactions, respectively (Table 4.4). Therefore, deadlock or partial replacement, where both species continued to be present and interact for the whole 90 d incubation, occurred in four of the two-species assemblages (Table 4.4, Fig. 4.5). Only a single species was present at the end of the 90 d experiment in all temperature treatments in one (*F. fomentarius vs. P. velutina*) of the two-species assemblages (Table 4.4, Fig. 4.5). In the three-species assemblage, only one species remained in the control and warmed treatment, and two species in the cooled treatment (Table 4.4, Fig. 4.5).

In the two-species interactions over 90 d, *V. comedens* partially replaced *F. fomentarius* in all temperature treatments, *S. hirsutum* partially replaced *V.*

comedens (excluding in the cooled treatment where it deadlocked) and partially or completely replaced *F. fomentarius,* and *P. velutina* completely replaced *F. fomentarius* and partially replaced *S. hirsutum* (Table 4.4, Fig. 4.5). In the three-species interaction over 90 d, *S. hirsutum* partially or completely replaced *V. comedens* and *F. fomentarius* (Table 4.4, Fig. 4.5). In general, primary colonisers (*V. comedens, F. fomentarius*) were replaced by the early secondary coloniser (*S. hirsutum*), which in turn was replaced by the late secondary coloniser (*P. velutina*).

The progression of interactions and time for territory to be obtained by the replacing species varied across the assemblages and temperature treatments (Table 4.4, Fig. 4.6). Replacement was slowest for the cooled treatment (except *F. fomentarius* vs. *P. velutina*) and quickest for the control or warmed treatment depending on the interaction (Table 4.4, Fig. 4.6). The replacement was quicker in the control treatment for interactions where species were replaced by *V. comedens* and *P. velutina*, and quicker in the warmed treatment where species were replaced by *S. hirsutum* (Table 4.4, Fig. 4.6).

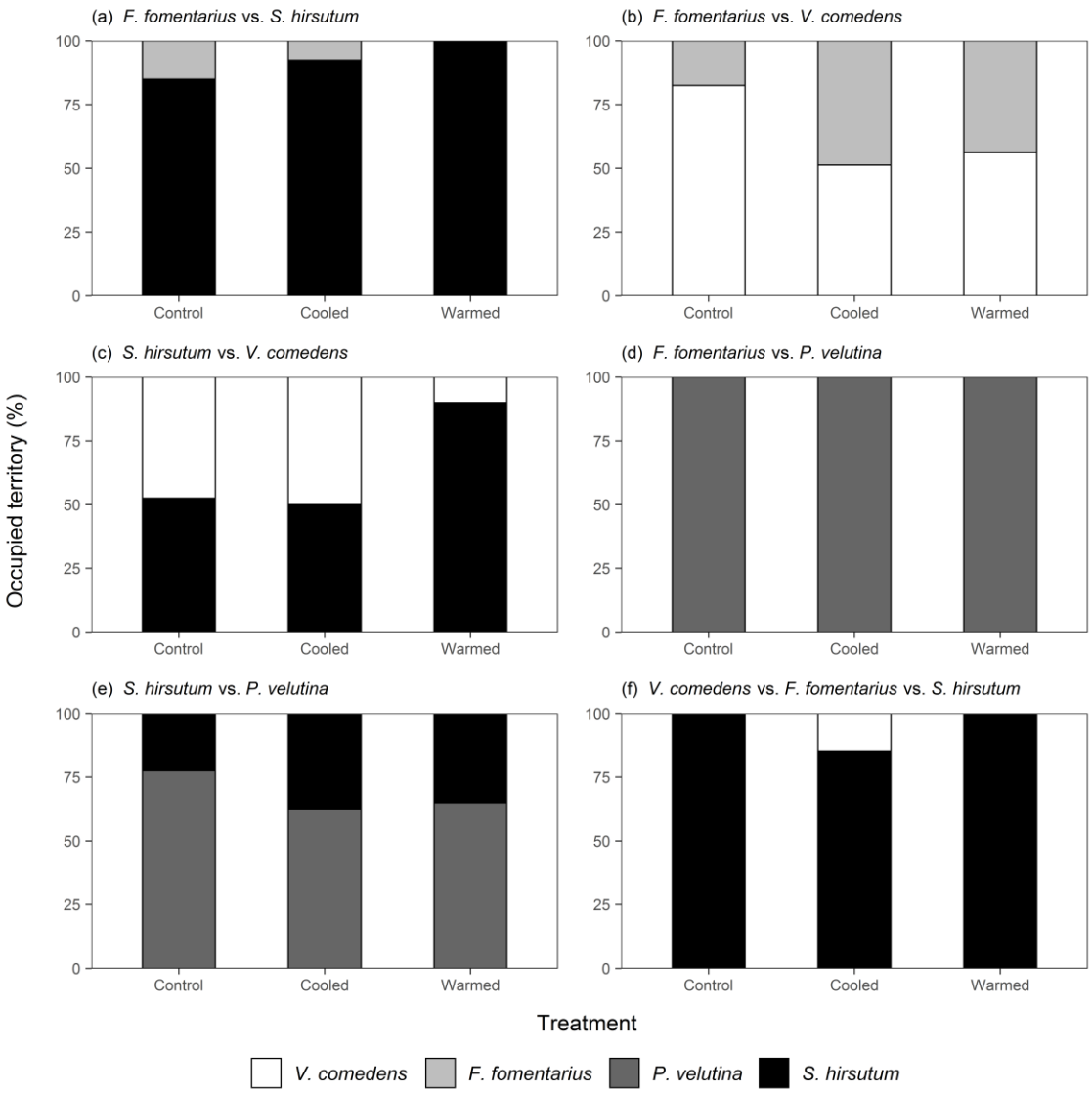


Figure 4.5 Occupied territory (%) by species in control, cooled and warmed treatment samples at the end of 90 d incubation following temperature change of (a-e) two-species interactions and (f) three-species interaction.

Table 4.4 Outcomes of interactions (mean of 5 replicates) at end of 90 d incubation and estimated time (d) until 75% and 100% territory was occupied by replacing species during the 90 d incubation. Interaction outcomes are defined as: D, deadlock where neither species is replaced by the other; PR, partial replacement of one species by the other; R, complete replacement of one species by the other. (-) indicates when the time until 75% and 100% territory occupied by replacing species was estimated beyond the 90 d incubation.

Interaction	**Outcome at 90 d**			**Estimated time until 75% and 100% territory occupied by replacing species (d)**					
	Control	**Cooled**	**Warmed**	**Control**		**Cooled**		**Warmed**	
				75%	**100%**	**75%**	**100%**	**75%**	**100%**
Two-species									
F. fomentarius vs. *S. hirsutum*	PR by *S. hirsutum* (3 R by *S. hirsutum*, 2 PR by *S. hirsutum*)	PR by *S. hirsutum* (4 R by *S. hirsutum,* 1 PR by *S. hirsutum*)	R by *S. hirsutum* (5)	34	-	49	-	27	73
F. fomentarius vs. *V. comedens*	PR by *V. comedens* (3 R by *V. comedens,* 1 PR by *V. comedens,* 1 D)	PR by *V. comedens* (4 D, 1 PR by *V. comedens*)	PR by *V. comedens* (4 D, 1 PR by *V. comedens*)	64	-	-	-	-	-
S. hirsutum vs. *V. comedens*	PR by *S. hirsutum* (4 D, 1 PR by *S. hirsutum*)	D (5)	PR by *S. hirsutum* (4 R by *S. hirsutum*, 1 D)	-	-	-	-	46	-
F. fomentarius vs. *P. velutina*	R by *P. velutina* (5)	R by *P. velutina* (5)	R by *P. velutina* (5)	16	72	29	75	11	79
S. hirsutum vs. *P. velutina*	PR by *P. velutina* (2 R by *P. velutina*, 2 D, 1 PR by *P. velutina*)	PR by *P. velutina* (3 D, 1 PR by *P. velutina*, 1 R by *P. velutina*)	PR by *P. velutina* (3 D, 1 PR by *P. velutina*, 1 R by *P. velutina*)	50	-	-	-	76	-
Three-species									
V. comedens vs. *F. fomentarius* vs. *S. hirsutum*	R by *S. hirsutum* (5)	PR by *S. hirsutum* (5)	R by *S. hirsutum* (5)	48	81	71	-	44	77

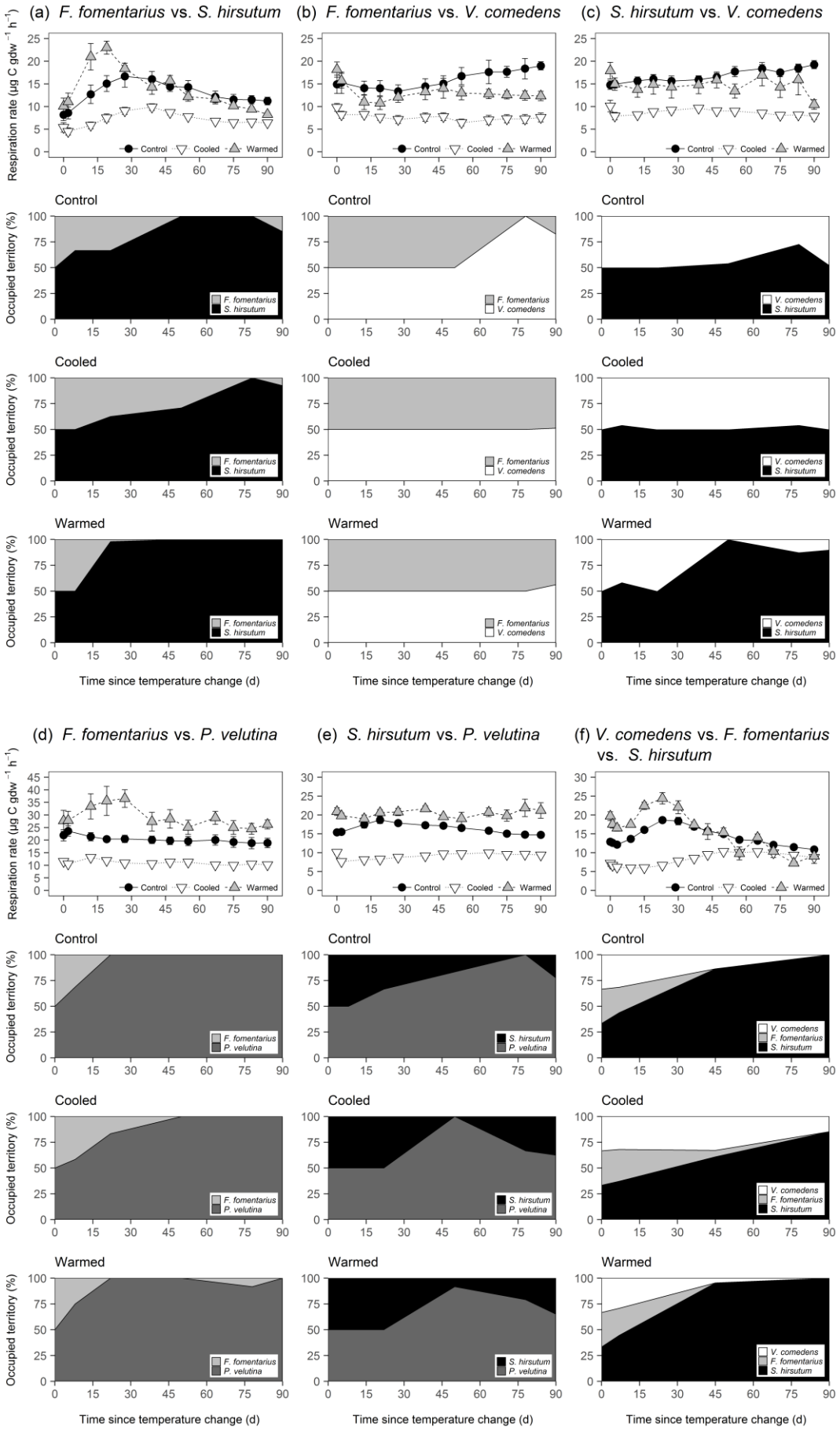


Figure 4.6 Respiration rate over 90 d since temperature change and occupied territory (%) by species in control, cooled and warmed treatment samples of (a-e) two-species interactions and (f) three-species interaction.

4.4.2 Overall respiration rates

For each of the assemblages, there were no significant differences in respiration rates between interactions allocated to the different temperature treatments before temperature change ($P > 0.05$; Supplementary Table 4.1, Fig. 4.7). Respiration rates and cumulative respiration were similar across interactions, with only *F. fomentarius* vs. *P. velutina* having a particularly higher respiration rate and cumulative respiration (Fig. 4.7). Generally, respiration rates of interactions that were dominated by *S. hirsutum* tended to increase and then gradually decline (Fig. 4.7a,c,f). Respiration rates of interactions that included *P. velutina* increased and/or remained constant (Fig. 4.7b,d,e).

4.4.3 Respiratory response to cooling

The relative respiration rate (normalised to the time of cooling) of the control and cooled treatment generally increased and then decreased (Fig. 4.8). Overall, two-species assemblages of basidiomycetes showed no thermal response to cooling ($RR_{CC} = 1.04$, $P = 0.497$; Table 4.5, Fig. 4.10a), therefore had no overall effect on the temperature sensitivity of respiration in the medium to long term. Of the two-species interaction combinations, four showed no responses, but *S. hirsutum* vs. *P. velutina* showed a compensatory response ($RR_{CC} = 0.89$, $P < 0.001$; Table 4.5, Fig. 4.10a). The three-species interaction showed no thermal response ($RR_{CC} = 0.98$, $P = 0.548$; Table 4.5, Fig. 4.10a). In addition, the thermal response of the two- and three-species interactions was determined by the statistical comparison of control and cooled relative respiration rate fitted lines (Table 4.6). Of the two-species interaction combinations, two displayed no responses, however *F. fomentarius vs. V. comedens* indicated an enhancing response and *F. fomentarius* vs. *P. velutina* and *S. hirsutum* vs. *P. velutina* showed compensatory responses (Table 4.6). The three-species interaction showed no thermal response (Table 4.6).

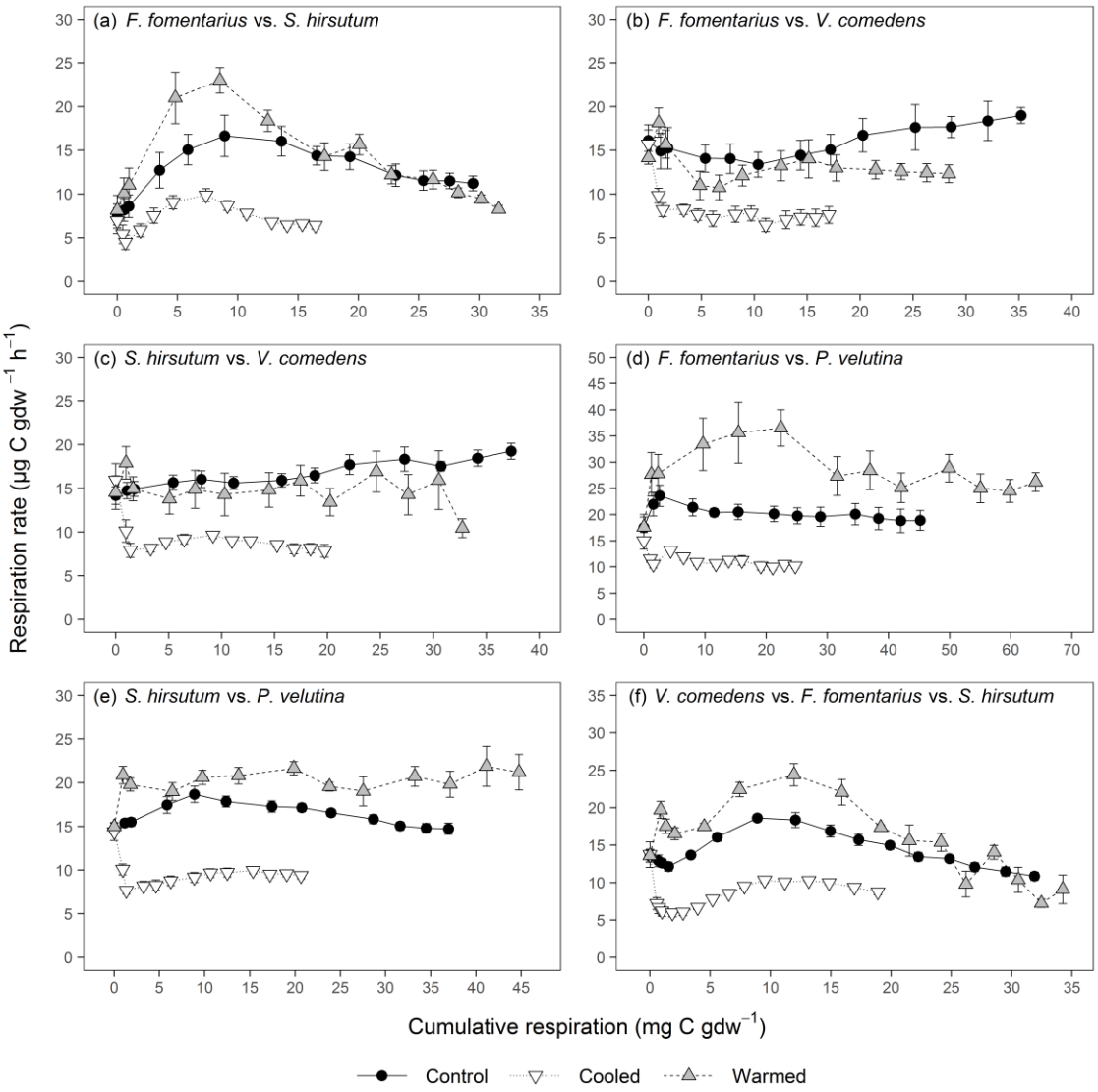


Figure 4.7 Respiration rate of three temperature treatments (control, cooled and warmed) after interaction set up (148 d) and during 90 d incubation following cooling and warming (151 d), of (a-e) two-species interactions and (f) three-species interaction (mean ± SE of the mean, n = 5). Cumulative respiration was calculated from interaction set up (148 d).

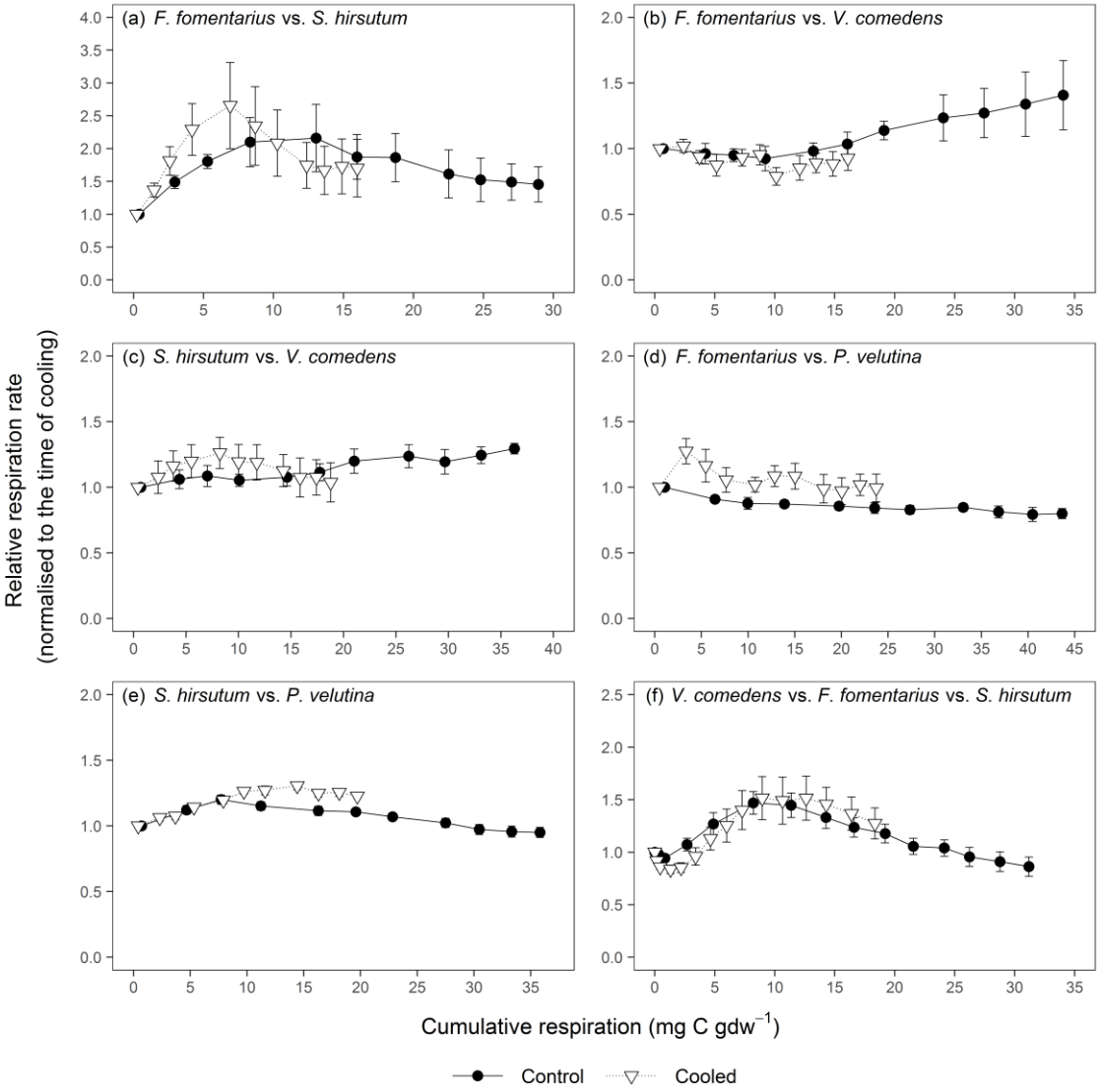


Figure 4.8 Relative respiration rate (normalised to the time of cooling) of control and cooled treatments during 90 d of incubation following cooling, of (a-e) two-species interactions and (f) three-species interaction (mean ± SE of the mean, n = 5). Cumulative respiration was calculated from the time of cooling (151 d), at the start of 90 d incubation.

Table 4.5 Respiration responses (RR_{CC}) quantified by comparing control and cooled treatment relative respiration rates, at a corresponding level of cumulative respiration as cooled treatment samples at end of incubation, of two-species assemblage overall, each two-species assemblage and three-species assemblage.

	Control vs. cooled treatment relative respiration rates			
	RR_{CC}	***t***	***P***	**Response**
Two-species interaction overall	1.04	0.746	0.497	
Two-species interaction				
F. fomentarius vs. *S. hirsutum*	1.20	0.028	0.979	
F. fomentarius vs. *V. comedens*	1.13	1.428	0.227	
S. hirsutum vs. *V. comedens*	1.14	0.658	0.546	
F. fomentarius vs. *P. velutina*	0.88	-1.325	0.256	
S. hirsutum vs. *P. velutina*	0.89	-13.600***	0.0002	Compensatory
Three-species interaction				
V. comedens vs. *F. fomentarius* vs. *S. hirsutum*	0.98	-0.655	0.548	

*** *indicates a significant difference at P < 0.001.*

Table 4.6 F ratio for statistical comparison of control and cooled treatment relative respiration rate (normalised to the time of cooling) fitted lines of each two-species assemblage and three-species assemblage.

Interaction	**df**	**F**	***P***
Two-species			
F. fomentarius vs. *S. hirsutum*	2, 14	0.454	0.644
F. fomentarius vs. *V. comedens*	2, 14	6.813**	0.009
S. hirsutum vs. *V. comedens*	2, 14	2.062	0.164
F. fomentarius vs. *P. velutina*	2, 14	13.348***	<0.001
S. hirsutum vs. *P. velutina*	2, 14	7.260**	0.007
Three-species			
V. comedens vs. *F. fomentarius* vs. *S. hirsutum*	2, 21	1.360	0.278

** *indicates a significant difference at P < 0.01; and *** at P < 0.001.*

4.4.4 Respiratory response to warming

The relative respiration rate (normalised to the time of warming) of the warmed treatment varied between assemblages, as it increased to above and below the relative respiration rate of the control treatment (Fig. 4.9). In response to warming, two-species assemblages of basidiomycetes showed no thermal response overall (RR_{CW} = 0.77, P = 0.129; Table 4.7, Fig. 4.10b), accordingly having no overall effect on the temperature sensitivity of respiration in the medium to long term. Of the two-species interaction combinations, three displayed no responses, but *F. fomentarius* vs. *V. comedens* and *S. hirsutum* vs. *V. comedens* showed compensatory responses (RR_{CW} < 1, P < 0.01, Table 4.7, Fig. 4.10b). The three-species interaction showed a compensatory response (RR_{CW} = 0.55, P < 0.05; Table 4.7, Fig. 4.10b). The statistical comparison of control and warmed relative respiration rate fitted lines showed that, of the two-species interactions, one showed no response, but *F. fomentarius* vs. *V. comedens, S. hirsutum* vs. *V. comedens* and *S. hirsutum* vs. *P. velutina* exhibited compensatory responses and *F. fomentarius* vs. *P. velutina* displayed an enhancing response (Table 4.8). The three-species interaction showed a compensatory response (Table 4.8).

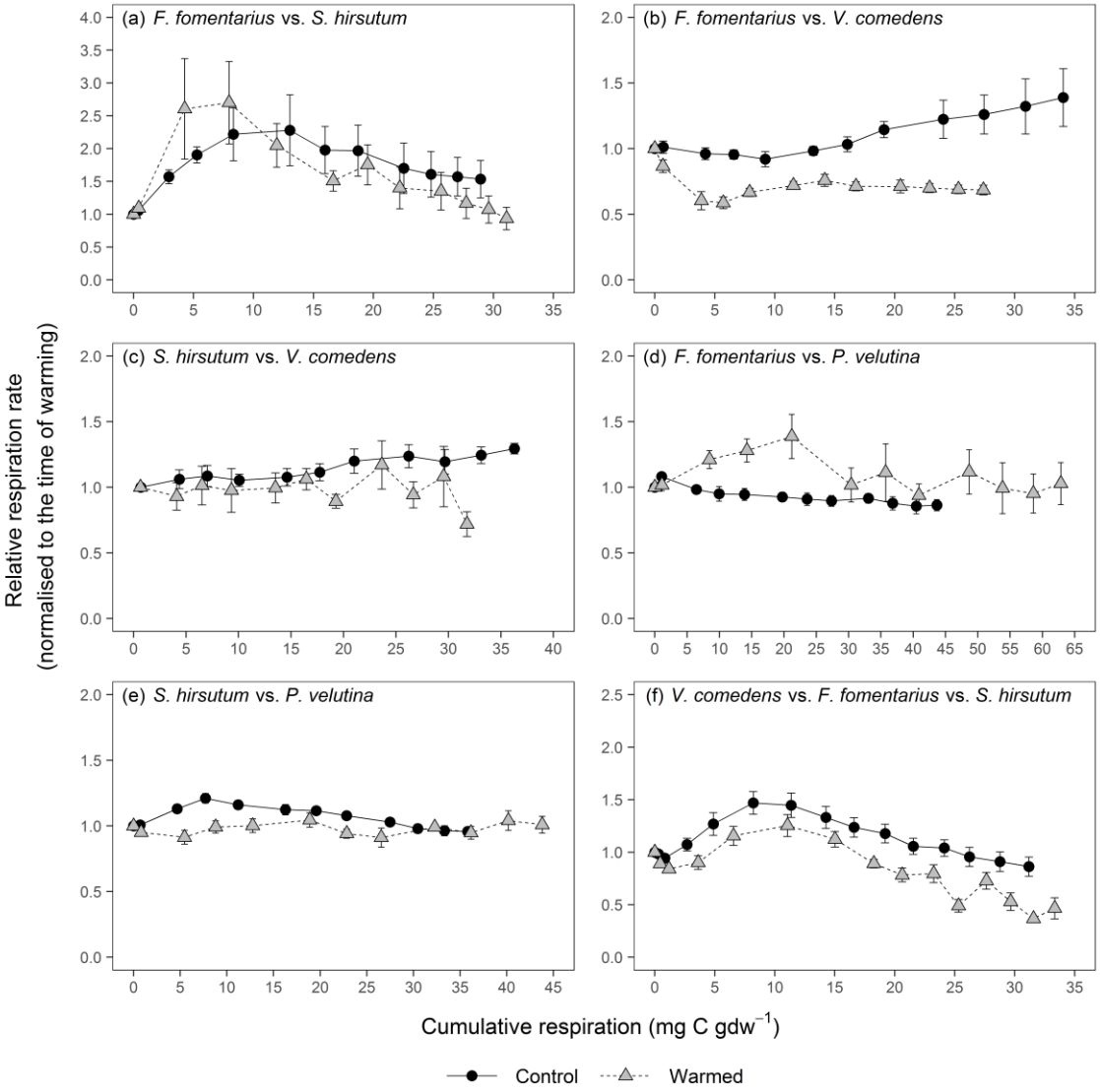


Figure 4.9 Relative respiration rate (normalised to the time of warming) of control and warmed treatments during 90 d of incubation following warming, of (a-e) two-species interactions and (f) three-species interaction (mean ± SE of the mean, n = 5). Cumulative respiration was calculated from the time of warming (151 d), at the start of 90 d incubation.

Table 4.7 Respiration responses (RR_{CW}) quantified by comparing control and warmed treatment relative respiration rates, at a corresponding level of cumulative respiration, of two-species assemblage overall, each two-species assemblage and three-species assemblage.

	Control vs. warmed treatment relative respiration rates			
	RR_{CW}	***t***	***P***	**Response**
Two-species interaction overall	0.77	-1.905	0.129	
Two-species interaction				
F. fomentarius vs. *S. hirsutum*	0.70	-1.897	0.131	
F. fomentarius vs. *V. comedens*	0.58	-9.926***	0.0006	Compensatory
S. hirsutum vs. *V. comedens*	0.57	-5.593**	0.005	Compensatory
F. fomentarius vs. *P. velutina*	1.12	2.311·	0.082	
S. hirsutum vs. *P. velutina*	1.04	0.983	0.381	
Three-species interaction				
V. comedens vs. *F. fomentarius* vs. *S. hirsutum*	0.55	-3.641*	0.022	Compensatory

*· indicates a significant difference at P < 0.1; * at P < 0.05; ** at P < 0.01; and *** at P < 0.001.*

Table 4.8 F ratio for statistical comparison of control and warmed treatment relative respiration rate (normalised to the time of warming) fitted lines of each two-species assemblage and three-species assemblage.

Interaction	**df**	**F**	***P***
Two-species			
F. fomentarius vs. *S. hirsutum*	2, 19	0.974	0.396
F. fomentarius vs. *V. comedens*	2, 18	36.417***	<0.001
S. hirsutum vs. *V. comedens*	2, 17	9.505**	0.002
F. fomentarius vs. *P. velutina*	2, 17	6.873**	0.006
S. hirsutum vs. *P. velutina*	2, 18	6.358**	0.008
Three-species			
V. comedens vs. *F. fomentarius* vs. *S. hirsutum*	2, 25	7.760**	0.002

******** *indicates a significant difference at P < 0.01; and *** at P < 0.001.*

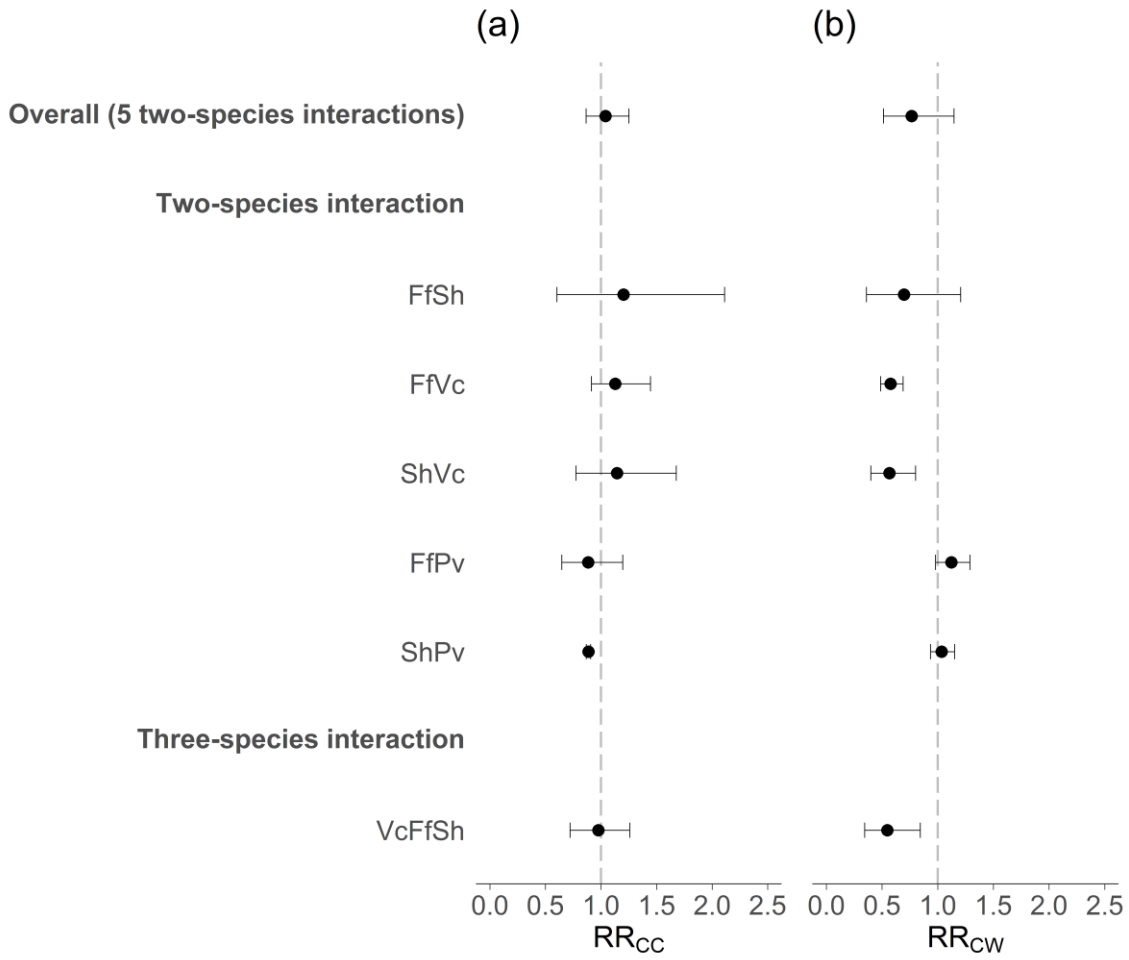


Figure 4.10 Response of respiration rate of two-species interaction overall, each two-species interaction and three-species interaction to changes in temperature. The mean and 95% confidence intervals (n = 5) of (a) RR_{CC} and (b) RR_{CW}. RR_{CC} is the Response Ratio for control versus cooled; control treatment relative respiration rate divided by the cooled treatment relative respiration rate, at the cumulative respiration of the cooled treatment at the end of the experiment. RR_{CW} is the Response Ratio for control versus warmed; warmed treatment relative respiration rate divided by the control treatment relative respiration rate, at the lowest cumulative respiration of the control or warmed treatment at the end of the experiment. Values < 1 indicate a compensatory response and values > 1 indicate an enhancing response. Effects are significant (P < 0.05) where confidence intervals do not cross one. Ff: *F. fomentarius*, Sh: *S. hirsutum*, Vc: *V. comedens*, Pv: *P. velutina*.

4.5 Discussion

This study is the first to investigate the temperature sensitivity of the respiration of multispecies assemblages of basidiomycetes decomposing wood in the medium to long term. It discovered that simple assemblages of basidiomycetes more often produced no respiratory thermal response overall, consequently did not decrease or increase the sensitivity of respiration following cooling or

warming. Some assemblages showed compensatory or enhancing responses, but compensatory responses were more frequent than enhancing responses. The compensatory responses, however, should be considered with caution as some may have occurred because of differences in the progression of the species interactions or the warmed treatment exceeding the optimum temperature for growth for some species (Chapter 2).

4.5.1 Progression and outcomes of interactions

Overall, this study was successful in measuring the respiratory thermal response of multiple fungal species interacting during a 90 d incubation after cooling and warming. In four of the two-species assemblages, both species remained involved in competitive interactions, either in deadlock or partial replacement, in all temperature treatments until the end of incubation (excluding *F. fomentarius* vs. *S. hirsutum* in the warmed treatment). However, in one of the two-species assemblages, *P. velutina* completely replaced *F. fomentarius* in all temperature treatments and thus only one species was present at the end of incubation. The three species assembly was not maintained throughout the experiment; the presence of *F. fomentarius* and *V. comedens* rapidly declined, with only *V. comedens* and *S. hirsutum* remaining in the cooled treatment and *S. hirsutum* in the control and warmed treatments at the end of the experiment. Nevertheless, the respiratory response of interacting basidiomycetes to cooling and warming was able to be determined.

The frequency of replicates where *S. hirsutum* and *V. comedens* deadlocked was expected based on this outcome occurring at 20 °C in a previous study (Hiscox *et al.*, 2015a). The partial replacement of *V. comedens* by *S. hirsutum* in the warmed treatment is supported by *S. hirsutum* having a greater extension rate and thus overall functioning than *V. comedens* at 28 °C, although this warmer temperature is above the optimum temperature for growth for both species (Chapter 2). Further, *S. hirsutum* was expected to deadlock with *P. velutina* at 20 °C (Hiscox *et al.*, 2015a), which is partly supported by the present study that observed evidence of deadlocking in some replicates. Outcomes of interactions involving the primary coloniser *F. fomentarius* were uncertain. The present study showed that *F. fomentarius* was the least combative and consequently was

partially outcompeted by the primary coloniser *V. comedens* and partially and/or completely replaced by the later stage colonisers *S. hirsutum* and *P. velutina*. Although more evidence of replacement of *F. fomentarius* by *V. comedens* was observed in the control treatment, deadlock was more common in the cooled and warmed treatment, perhaps due to the cooled treatment being further below the optimum temperature for both species and the warmed treatment being above the optimum temperature for *V. comedens* (Chapter 2). Generally, primary colonisers (*V. comedens, F. fomentarius*) being replaced by the early secondary coloniser (*S. hirsutum*), which in turn was replaced by the late secondary coloniser (*P. velutina*) aligns with the transitive hierarchy of combative ability, where primary colonisers < early secondary colonisers < late secondary colonisers (Boddy, 2000).

4.5.2 Respiratory response to cooling

Two-species assemblages overall and the three-species assemblage showed no thermal response of respiration to cooling. This contrasts to the overall enhancing response of respiration from individual basidiomycete species to cooling (Chapter 3). Interspecific competitive interactions seems to reduce the overall temperature sensitivity of respiration, likely due to energetically expensive antagonistic mechanisms maintaining respiration rates. This highlights the importance of exploring the respiratory thermal responses of increasingly diverse fungal assemblages.

S. hirsutum and *V. comedens* was the only example of comparing interactions in deadlock (there was only some minor replacement by *S. hirsutum* in the control treatment) in the control and cooled treatment, and no thermal response was observed. Although this is only one example of species in deadlock, it suggests that at the initial stages of competition where limited change in territory has occurred and antagonistic mechanisms are greater (Boddy and Hiscox, 2016), species will not alter their temperature sensitivity of respiration. *F. fomentarius* vs. *V. comedens* were often deadlocked in the cooled treatment (only some minor replacement by *V. comedens*), and the relative respiration rate showed only a slight gradual decline over time. This minimal reduction in relative respiration rate in the cooled treatment and no thermal response at the end of the experiment

from this assemblage supports species in deadlock not changing their sensitivity of respiration with temperature.

No thermal response from *S. hirsutum* vs. *V. comedens* would be expected based on *V. comedens* and *S. hirsutum* producing no thermal response to cooling after 90 d when grown alone (Chapter 3). However, in the previous study, *S. hirsutum* also showed some evidence of enhancement as the cooled treatment relative respiration rate declined in the later stage of the experiment (Chapter 3). In the present study, the relative respiration rate of the cooled treatment showed a declining trend in the latter part of the experiment, which with more time may have led to an enhancing response. This may have been caused by the negative impact of cooling on *S. hirsutum* as detected in Chapter 3. In the current study it does, however, seem more likely that *S. hirsutum* during interactions produced no thermal response to cooling, as when dominating in the two-species assemblage *F. fomentarius* vs. *S. hirsutum* and in the three-species assemblage, consistent no thermal responses were detected.

In the two-species assemblage *S. hirsutum* vs. *P. velutina*, *P. velutina* partially replaced *S. hirsutum* in the control and cooled treatments, occupying 75% of the territory by 50 d and at the end of experiment respectively. Comparing the control and cooled treatment relative respiration rates revealed a consistent compensatory response. This compensatory response was unexpected, as *S. hirsutum* seemed to show no thermal responses in the other two-species assemblages, and *P. velutina,* as the dominant species, demonstrated a clear no response after cooling when studied as an individual species (Chapter 3). Nevertheless, the compensatory response would be supported by *P. velutina* having the ability to produce a compensatory response to cooling when normalising for fungal biomass (Chapter 3). However, the compensatory response may have been caused by reduced CO_2 production from antagonistic interactions in the control treatment due to the interaction progressing faster in the warmer wood blocks, compared to the cooled treatment. Additionally, in the two-species assemblage *F. fomentarius* vs. *P. velutina*, where *P. velutina* completely replaced *F. fomentarius* by 72 d and 75 d in the control and cooled treatments respectively, a compensatory response also occurred during incubation. This is further evidence of *P. velutina* showing thermal compensation

when dominating in interactions, however a no response is detected at the end of incubation when territory is only occupied by *P. velutina*. However, the respiratory response of *F. fomentarius* vs. *P. velutina* should be considered with caution, as the compensatory response occurred immediately and declined in magnitude with time. This is not consistent with a compensatory response that can gradually reduce the temperature sensitivity of respiration.

F. fomentarius vs. *V. comedens* was the only assemblage to produce some evidence of enhancement with cooling, however this appears to be driven by the control relative respiration rate increasing as *V. comedens* becomes dominant, which has a greater respiration rate than *F. fomentarius* (Chapter 3), and not by *F. fomentarius* and *V. comedens* whilst in deadlock down-regulating their respiration rate with cooling. Therefore, during species interactions after cooling, evidence for enhancement that increases the direct effect of temperature on respiration rates is limited in the current study, and contrasts to the more frequent enhancing responses observed from individual species decomposing wood (Chapter 3).

4.5.3 Respiratory response to warming

Two-species communities showed no thermal response overall to warming, although compensatory and enhancing responses occurred, and the three-species community showed a compensatory response to warming. However, these compensatory responses and an enhancing response produced are likely not strong evidence of responses that would decrease and increase the temperature sensitivity of respiration respectively.

The compensatory responses may have been caused by species being replaced quicker in the warmed treatment which led to reduced antagonistic mechanisms and subsequently reduced CO_2 production in the warmed than control treatment, or by the warmed treatment exceeding the optimum temperature for growth for some species. This was likely the case for *S. hirsutum* vs. *V. comedens* and *V. comedens* vs. *F. fomentarius* vs. *S. hirsutum. S. hirsutum* partially or completely replaced the other species in these interactions faster in the warmed than the control treatment, and the warmed treatment (28 °C) was higher than the thermal

optimum for *S. hirsutum* (25 °C) (Chapter 2), the dominant species. This may have led to lower respiration rates in the warmed than control treatments, thus producing compensatory responses. Additionally, although *F. fomentarius* vs. *S. hirsutum* produced a no response, with more time, a compensatory response would likely have occurred and these explanations may also apply to this interaction. However, as *S. hirsutum* replaced *V. comedens* and *F. fomentarius* quicker in the warmed treatment compared to the control treatment, another alternative explanation could be that *S. hirsutum* had an increased C-use efficiency at 28 °C. Therefore, more C was converted into biomass rather than respired, hence *S. hirsutum* was able to replace species quicker. Moreover, for *F. fomentarius* vs. *V. comedens*, the territory occupied remained approximately equal in the warmed treatment. It is possible that the warmed treatment being past the thermal optimum for *V. comedens* (25 °C) but not for *F. fomentarius* (30 °C) (Chapter 2), reduced the respiration of *V. comedens*, which has the higher respiration rate of the two species (Chapter 3), and thus the respiration of the interaction overall, leading to a compensatory response. For *S. hirsutum* vs *P. velutina*, the compensatory response was detected due to a change in the respiration of the control treatment, rather than a decrease in the respiration of the warmed treatment. *P. velutina* partially replaced *S. hirsutum* in the control and warmed treatments, but the relative respiration rate of the warmed treatment remained stable throughout the incubation. It was the slight increase in control treatment relative respiration rate that led to some evidence of compensation, which was lost by the end of the experiment. The warmed treatment was also higher than the optimum temperature for growth for *P. velutina* (25 °C) (Chapter 2), therefore no evidence of a decline in the respiration of the warmed treatment where *P. velutina* dominated does show that this species can at least maintain performance when beyond its optimal thermal conditions for growth.

The one enhancing response produced with warming, by *F. fomentarius* vs. *P. velutina*, the respiration rate of the warmed treatment remained above the rate of the control, but declined during the latter part of the incubation. *P. velutina* consistently replaced species quicker in the control rather than the warmed treatment, which provides evidence of the negative impact this warmer temperature had on its growth. This enhancing response may have been caused by the interaction taking longer to resolve in the warmed than control treatment,

leading to increased CO_2 production from antagonistic mechanisms in the warmed treatment. *P. velutina* may have had a decreased C-use efficiency in the warmed treatment, where more C was respired rather than allocated to biomass, causing the higher respiration rate and slower ability to replace species in the warmed treatment. Alternatively, it is possible that *F. fomentarius,* that had the higher thermal optima of 30 °C, contributed more to this increase in respiration at the beginning of the interaction before being outcompeted, which would be expected with warming, based on its response to cooling when growing alone (Chapter 3). Then as *P. velutina* completely replaced *F. fomentarius*, the positive impact of warming on respiration declined. However, when *P. velutina* existed alone after completely replacing *F. fomentarius* in control and warmed treatments, a marginally significant enhancing response still occurred at the end of incubation. This suggests that *P. velutina* may increase the sensitivity of its respiration with warming, even when beyond its optimum temperature for growth.

Therefore, a number of explanations could be driving the compensatory and enhancing responses identified following warming. No strong evidence for compensatory responses that would decrease the temperature sensitivity of respiration was produced. It should be noted that the moisture in wood block microcosms and the respiration rates did not decline during the 14 d between watering, therefore limiting moisture conditions did not influence the respiratory responses observed following warming (Supplementary Fig. 4.4 and 4.5).

4.5.4 Cooling versus warming approach

Using the cooling and warming approach had the potential to maximise our understanding of the respiratory response of fungal assemblages to temperature. However, the respiration response to the warming treatment was more difficult to interpret, due to the temperature applied being higher than the optimum temperature for growth for some species (Chapter 2), which was discovered after conducting the current study. Hence, investigating the respiratory response to cooling and warming across a similar temperature range would make for a fairer comparison, and further consolidate our understanding. Consequently, the respiratory thermal responses ascertained from cooling are considered more accurate.

4.5.5 Implications of the respiratory thermal response of multispecies basidiomycete assemblages for fungal communities, whole soil communities and long-term warming experiments

The respiratory thermal response of multispecies basidiomycete assemblages would suggest that more diverse and complex communities of interacting wood decay basidiomycetes are more likely to demonstrate limited change in the temperature sensitivity of their respiration. The thermal responses observed will likely be community-specific and dependent on the frequency of interspecific interactions. As fungal interactions resolve, the thermal response of the dominant species will be increasingly important. Although compensatory responses were more common than enhancing responses in the present study, which was the opposite to what was shown by individual basidiomycete species after cooling (Chapter 3), limited evidence of clear compensatory responses by interacting basidiomycetes that would gradually reduce the temperature sensitivity of respiration was observed. This was because the compensatory responses were likely caused by differences in the progression of the species interactions or exposing some basidiomycetes to temperatures beyond their thermal optima, or the responses occurred in the short-term that declined in magnitude during the incubation.

In a previous study that used the cooling approach to investigate the respiration rate of whole soil microbial communities to temperature in 22 soils along a climate gradient from the Arctic to the Amazon, one soil exhibited a compensatory thermal response (Karhu *et al.*, 2014). The respiratory thermal response of individual species of basidiomycetes as mentioned previously (Chapter 3), and now also multispecies assemblages of basidiomycetes, both suggest that saprotrophic basidiomycetes were likely not the mechanism behind this compensatory thermal response detected after cooling. Furthermore, the respiration declines over time in many long-term soil warming experiments in the field (Jarvis and Linder, 2000; Oechel *et al.*, 2000; Luo *et al.*, 2001; Melillo *et al.*, 2002, 2017; Romero-Olivares *et al.*, 2017; García-Palacios *et al.*, 2018). In agreement with Chapter 3, because the results of the present study show limited evidence of clear compensatory responses, this supports the conclusion that the dominant mechanism of indirect effects of temperature such as substrate

depletion, rather than thermal compensation, underlies the decline in the initial positive response of soil microbial respiration to warming in long-term field experiments.

4.6 Conclusion

A cooling and warming approach allowed respiratory thermal responses of two- and three-species basidiomycete assemblages decomposing wood to be determined. Two-species assemblages overall and the three-species assemblage showed no thermal response to cooling, consequently no change in the temperature sensitivity of respiration in the medium to long term. With warming, two-species assemblages showed no thermal response overall, whereas the three-species assemblage that was dominated by one species displayed a compensatory response. However, the compensatory responses observed should be interpreted with caution, as they may have been caused by differences in the progression of the species interactions or the warmed treatment being greater than the optimum temperature for growth for some species. Competitive interspecific interactions in multispecies basidiomycete assemblages seem to reduce the overall temperature sensitivity of wood decomposition. However, the highly variable respiratory responses to temperature observed show how the responses may be interaction specific. As such, this suggests that understanding the response of more complex and diverse communities indicative of those that occur naturally in temperate woodlands are required. This will make it possible to determine whether the enhancing responses that were generally observed in single-species populations or the greater evidence for compensatory responses observed in simple multispecies assemblages, apply in increasingly complex natural fungal communities. Overall, with increasing global temperatures, low diversity assemblages of interacting basidiomycetes decomposing wood more often cause no change in the temperature sensitivity of respiration in the medium to long term.

Chapter 5. Respiratory thermal response of semi-natural wood decomposing communities

5.1 Abstract

The direction and strength of respiratory responses to temperature, most likely dependent on the species present in communities, will determine the overall temperature sensitivity of respiration of natural wood decay communities. Previously, it has been shown that the temperature sensitivity of the respiration of individual species of basidiomycetes decomposing wood increases with warming, yet in simple two- and three-species assemblages of interacting basidiomycetes there was more often no change in the temperature sensitivity of respiration with warming in the medium to long term. The respiratory response of more diverse wood decomposing communities to increasing temperatures is unknown. To this end, semi-natural communities decomposing beech wood (*Fagus sylvatica*) were investigated. Beech wood blocks that were uncolonised or pre-colonised with the white rot basidiomycete species *V. comedens* or *T. versicolor* for 154 d, were placed at the soil-litter layer interface in a temperate woodland for 100 d, and then exposed to a 107-day cooling (20 to 12 °C) and warming (12 to 20 °C) experiments in the laboratory. Respiration rates from the three semi-natural wood decomposing communities were measured and the direction of any thermal responses (decreased temperature sensitivity of respiration (compensatory), increased temperature sensitivity of respiration (enhancing), and no change in the temperature sensitivity of respiration (no response)) determined. Overall, the three semi-natural wood decay communities showed no thermal response of respiration to cooling or warming. Compensatory and enhancing responses were both detected, but more enhancing than compensatory responses were identified. The direction and strength of respiratory responses to temperature, most likely dependent on the species present in communities, will determine the overall temperature sensitivity of respiration of natural wood decay communities. With more evidence of enhancing responses detected, it seems unlikely that compensatory responses will weaken positive feedbacks between warming and wood carbon loss. Rather, it is more likely that wood decomposing communities will produce more enhancing responses with warming, consequently causing an overall increase in the

temperature sensitivity of respiration in the medium to long term. Therefore, there remains the potential for a positive feedback to climate change through increased wood decomposition with warming.

5.2 Introduction

Individual species of wood decay basidiomycetes colonising wood showed enhancing thermal responses that increase the temperature sensitivity of respiration in the medium to long term (Chapter 3). Two- and three-species assemblages of wood decay basidiomycetes colonising wood, however, predominantly showed no thermal responses and therefore no change in the temperature sensitivity of respiration in the medium to long term (Chapter 4). Thus, increasingly diverse basidiomycete assemblages, and the inclusion of competitive interspecific interactions, seems to reduce the overall temperature sensitivity of wood decomposition. However, to further our understanding of wood decomposition with increasing global temperatures, the temperature sensitivity of respiration of more natural and diverse basidiomycete communities colonising wood needs to be explored.

This study investigates the temperature sensitivity of respiration of semi-natural wood decomposing communities within beech wood (*Fagus sylvatica*). Beech wood blocks that were uncolonised or pre-colonised with single species of white rot basidiomycetes (*V. comedens* or *T. versicolor*) were placed at the soil-litter interface in a temperate woodland to allow some natural colonisation and decomposition in natural environmental conditions. Wood blocks were then exposed to the cooling (Chapters 3 and 4) and warming (Chapter 4) approach in the laboratory as used previously. The cooling and warming approach was applied across the same temperature range and not beyond the expected thermal optima of the basidiomycete species used for wood block pre-colonisation or those present in temperate woodlands. Thus, any fundamental differences in the respiration responses to cooling and warming across the same temperature range could be determined. Given the potential for interspecific competition to reduce the temperature sensitivity of decomposition, it was hypothesised that both the pre-colonised wood blocks and those uncolonised when placed on the

forest floor would show no thermal responses and as such no change in the temperature sensitivity of respiration in the medium to long term.

5.3 Methods

5.3.1 Colonisation of wood blocks

Beech (*Fagus sylvatica*) wood blocks that were 3 x 3 x 3 cm in dimension were either: (1) uncolonised; or (2) pre-colonised with one of two species of wood decay basidiomycetes (Table 5.1) by incubating blocks at 20 °C in the dark for 154 d, as described in Chapter 3 (Section 3.3.1). Pre-colonisation of wood blocks was confirmed by the re-isolation of fungi from a sample of wood blocks (n = 10), as in Chapter 3 (Section 3.3.1). The density as an estimate of the initial decay state (154 d) of a sample of uncolonised and pre-colonised wood blocks was determined (mg mm^{-3}, 10 replicates; Supplementary Fig. 5.1a), as explained in Chapter 3 (Section 3.3.6).

Table 5.1 Fungal species used to colonise wood blocks.

Ecological role	Species	Strain
Primary coloniser	*Vuilleminia comedens*	VcWVJH1
Early secondary coloniser	*Trametes versicolor*	TvCCJH1

Both species are white-rot wood decay basidiomycetes. Cultures were obtained through isolation from wood or fruit bodies, from the Cardiff University Culture Collection.

5.3.2 Natural colonisation of wood blocks

Pre-colonised wood blocks were scraped free of adhering mycelium and agar using a sterile scalpel (Section 3.3.2). Uncolonised and pre-colonised wood blocks were marked using a pyrography iron to indicate the wood block type. Plastic coated garden wire (Toolzone, UK) was tied around each block, with a loop in the wire for a tent peg to secure it to the ground. Wood blocks were placed in a location dominated by *F. sylvatica* in a mixed conifer and deciduous temperate woodland in Stoke Woods, Exeter (latitude 50.751269, longitude -3.527733), and positioned at the soil-litter interface for 100 d (2nd Aug 2020 – 10th Nov 2020). The leaf litter and soil pH was 5.74 and 5.53, respectively. Two data loggers (TinyTag, UK) placed in the soil-litter layer measured the temperature

every 1 h (Supplementary Fig. 5.2). The minimum and maximum temperature recorded was 5.66 °C and 24.13 °C, respectively.

5.3.3 Wood block microcosm set up

Following collection from the field, the density of a sample of non-pre-colonised and pre-colonised wood blocks was determined (mg mm^{-3}, 10 replicates; Supplementary Fig. 5.1b) (Section 3.3.6). The remaining wood blocks were scraped free of adhering soil and mycelium using a scalpel. Each wood block was placed directly on to perlite (20 ml; Homebase, UK) moistened with 2 ml sterile distilled water (dH_2O) to achieve a water potential of -0.012 kPa, in a plastic 100 ml lidded deli pot (Cater4you, UK), as described in Chapter 3 (Section 3.3.2). 56 wood block microcosms were set up for each pre-coloniser species (*V. comedens, T. versicolor*) and for those with no pre-coloniser. Moisture was maintained in each microcosm and holes in each pot covered by microporous surgical tape (3M, UK) allowed for aeration but prevented contamination with other species, as explained in Chapter 3 (Section 3.3.2).

5.3.4 Wood block microcosm incubation

For the cooling approach, 32 microcosms of each wood block type were incubated (Sanyo Electric/Panasonic Cooled Incubator, MIR-154, UK) at 20 °C for a 96 d pre-incubation period before cooling. Three respiration measurements from each wood block microcosm were taken during this pre-incubation period. For each wood block type, the 32 microcosms were then assigned to one of four temperature treatments (n = 8): pre-cooling (destructively sampled at 350 d before cooling), cooled (incubated at 12 °C at 350 d for 107 d), rewarmed (incubated at 12 °C at 350 d for 63 d and then rewarmed to 20 °C for 44 d) and control (incubated at 20 °C for a further 107 d) (Fig. 5.1a), as described and justified in Chapter 3 (Section 3.3.3). The pre-cooling replicates were destructively sampled before cooling and density measurements (mg mm^{-3}; 8 replicates) were taken.

For the warming approach, 24 microcosms of each wood block type were incubated (Sanyo Electric/Panasonic Cooled Incubator, MIR-154, UK) at 12 °C

for a 96 d pre-incubation period before warming. Three respiration measurements from each wood block microcosm were taken during this pre-incubation period. For each wood block type, the 24 microcosms were then assigned to one of three temperature treatments (n = 8): pre-warming (destructively sampled at 350 d before warming), warmed (incubated at 20 °C at 350 d for 107 d) and control (incubated at 12 °C for a further 107 d) (Fig. 5.1b). The pre-warming replicates were destructively sampled prior to warming and density measurements (mg mm^{-3}; 8 replicates) were taken. In Chapter 4, warming resulted in a reduction in activity in some species assemblages, as 28 °C was above the optimum temperature for growth for some of the study species. Thus, the present study used the same temperature ranges for the cooling and warming experiments, and 12 to 20 °C is below the optimum temperature for growth for the basidiomycete pre-coloniser species and likely below the optimum temperature for the species present in the temperate woodland used for the natural colonisation.

5.3.5 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, as in Chapter 3 (Section 3.3.4). Holes in the pot of each wood block microcosm covered by microporous surgical tape allowed CO_2 to accumulate in the larger 700 ml plastic container whilst maintaining a sterile environment. The CO_2 concentration in the headspace of the incubation chamber was measured immediately after closure, and then again after 19 h. The CO_2 concentration was logged on a computer connected to the IRGA every 1.6 s for 90 s to allow the CO_2 concentration to stabilise. Respiration was measured 1 d after cooling and rewarming, and 1 d after warming. Three respiration measurements were taken over 8 d following these temperature changes, and then biweekly from then on. Respiration rates were expressed as µg C per g of dry weight per h.

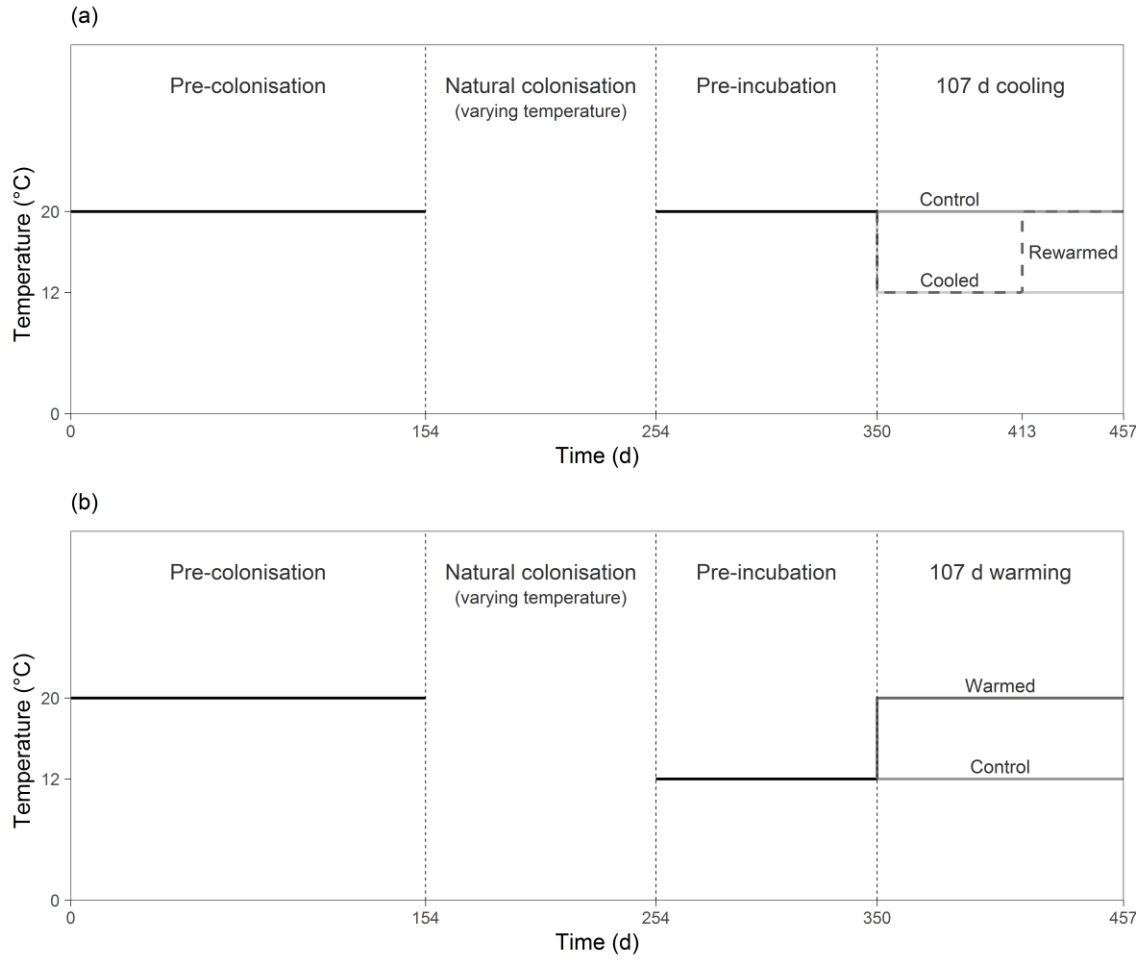


Figure 5.1 Wood block pre-colonisation, natural colonisation, pre-incubation and wood block microcosm incubation shown as a timeline. Wood blocks were pre-colonised, naturally colonised in a temperate woodland and pre-incubated. Wood block microcosms for (a) cooling approach, were assigned to one of four temperature treatments: pre-cooling, cooled, rewarmed and control, and (b) warming approach, were assigned to one of three temperature treatments: pre-warming, warmed and control. Pre-cooling and pre-warming replicates were removed at 350 d, before temperature change.

5.3.6 Territory occupied by pre-coloniser species

The territory occupied within wood blocks pre-colonised by *V. comedens* and *T. versicolor* was determined by destructive sampling, as detailed in Chapter 4 (Section 4.3.6), but pieces of wood were excised approximately 2, 10, 18 and 27 mm from the wood block edge. The territory occupied by each pre-coloniser species (n = 10) was determined as the mean percentage of the whole block, at the end of pre-colonisation (154 d) and end of natural colonisation (254 d). In addition, the percentage territory occupied by the pre-coloniser species in each wood block at the end of pre-incubation before temperature change (350 d) and

the end of 107 d incubation after temperature change (457 d), was calculated, and then the mean percentage territory occupied was determined for each temperature treatment.

5.3.7 Quantifying respiratory responses

The cooling approach method and justifications, and calculations used to quantify respiration responses, were analogous to Chapter 3 (Section 3.3.7). As done previously, the total cumulative respiration of cooled samples was put into the control treatment linear regression equation to calculate the control treatment respiration rate at the equivalent cumulative respiration of cooled samples at the end of incubation (Table 5.2). The cumulative respiration of rewarmed samples at 1, 8 and 44 d after rewarming was put into the control treatment linear regression equation to calculate the control treatment respiration rate at the same cumulative respiration as rewarmed samples at 1, 8 and 44 d after rewarming (Table 5.2). Control and cooled relative respiration rates were calculated by dividing by the respiration rate at 1 d after cooling (Section 3.3.7). Response ratios comparing control and cooled treatment relative respiration rates (RR_{CC}: Response Ratio, control versus cooled) and response ratios comparing control and rewarmed treatment respiration rates (RR_{CR}: Response Ratio, control versus rewarmed) at 1, 8 and 44 d after rewarming were produced (Section 3.3.7). The warming approach method (except for the temperatures applied) and rationale, and the calculations used to determine respiration responses, were the same as Chapter 4 (Section 4.3.7). However, the total cumulative respiration of control samples was put into the warmed treatment linear regression equation to calculate the warmed treatment respiration rate at the equivalent cumulative respiration of control samples at the end of incubation (Table 5.3). Control and warmed relative respiration rates were calculated by dividing by the respiration rate at 1 d after warming (Section 4.3.7). Response ratios comparing the control and warmed treatment relative respiration rates (RR_{CW}: Response Ratio, control versus warmed) were calculated (Section 4.3.7). Respiration could show three possible thermal responses following cooling and warming: compensatory, enhancing or no response, as detailed in Chapter 4 (Section 4.3.7; Fig. 4.2). Response ratio values < 1 indicate a compensatory response and values > 1

indicate an enhancing response, as explained in Chapters 3 and 4 (Sections 3.3.7 and 4.3.7).

5.3.8 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 (336 d) of control, cooled and rewarmed treatments before cooling, and of control and warmed treatments before warming, during the pre-incubation period prior to temperature change (350 d), of each pre-coloniser species.

Paired t-tests to determine statistically significant responses of pre-coloniser species overall and each pre-coloniser species to the cooling approach were tested in the same way as Chapter 3 (Section 3.3.8), and to the warming approach were tested analogously to Chapter 4 (Section 4.3.8) but at the total cumulative respiration of the control treatments.

One-way ANOVA models and Tukey's pairwise comparisons were used to compare the densities of a sample of wood blocks at the end of pre-colonisation in the laboratory (154 d) and the end of natural colonisation in the temperate woodland (254 d).

Table 5.2 Total cumulative respiration since time of cooling for cooled treatment and cumulative respiration since time of cooling at 1, 8 and 44 d after rewarming for rewarmed treatment (mean ± SE of the mean). These were used in a linear regression of control treatment respiration rate after cooling, to calculate control treatment respiration rate at a corresponding level of cumulative respiration as cooled treatment samples at end of incubation and rewarmed treatment samples at 1, 8 and 44 d after rewarming, for each pre-coloniser species.

Pre-coloniser species	**Total cumulative respiration since time of cooling for cooled treatment (mg C gdw^{-1})**	**Cumulative respiration since time of cooling at 1, 8 and 44 d after rewarming for rewarmed treatment (mg C gdw^{-1})**			**Linear regression of control treatment respiration rate after cooling (µg C gdw^{-1} h^{-1})**	**R^2**
		1 d	**8 d**	**44 d**		
None	52.51 ± 16.22	27.45 ± 11.13	33.14 ± 13.12	62.46 ± 22.33	y = -0.28405x + 45.77259	0.9438
V. comedens	19.78 ± 2.90	13.29 ± 2.14	15.82 ± 2.53	27.35 ± 4.22	y = -0.13231x + 17.24948	0.8335
					y = 0.463441x + 5.370947	0.9983
T. versicolor	84.50 ± 4.32	43.45 ± 3.33	54.54 ± 3.84	106.21 ± 6.41	y = -0.06606x + 69.91109	0.0856

V. comedens required two linear regressions to represent the control treatment respiration rate.

Table 5.3 Total cumulative respiration since time of warming for control treatment (mean ± SE of the mean). This was used in a linear regression of warmed treatment respiration rate after warming, to calculate warmed treatment respiration rate at a corresponding level of cumulative respiration as control treatment samples at end of incubation, for each pre-coloniser species.

Pre-coloniser species	**Total cumulative respiration since time of warming for control treatment (mg C gdw^{-1})**	**Linear regression of warmed treatment respiration rate after warming (µg C gdw^{-1} h^{-1})**	**R^2**
None	27.77 ± 4.48	y = -0.05990x + 24.89387	0.3785
V. comedens	28.14 ± 4.05	y = -0.33380x + 26.16320	0.9726
T. versicolor	59.92 ± 2.05	y = -0.18055x + 56.65313	0.9183

5.4 Results

5.4.1 Territory occupied by pre-coloniser species

Wood blocks were successfully pre-colonised by *V. comedens* and *T. versicolor* in the laboratory (Table 5.4). During wood block decomposition in the woodland, *T. versicolor* maintained its presence with no change in occupancy, but *V. comedens* was partially replaced from some of its territory, most likely by other wood decay basidiomycete species from the soil-litter layer of the woodland (Table 5.4). During the subsequent laboratory pre-incubation, before cooling or warming treatments were applied, *V. comedens* maintained the majority of its territory and *T. versicolor* continued to occupy all territory (Table 5.4). At the end of 107 d incubation following cooling and warming, *V. comedens* had been partially replaced in all temperature treatments, with greatest replacement in the rewarming treatment, however, *T. versicolor* sustained total occupancy of resources in all temperature treatments (Table 5.4).

5.4.2 Overall respiration rates

For each of the three pre-coloniser species (none, *V. comedens, T. versicolor*), there were no significant differences in respiration rates between wood blocks allocated to the different temperature treatments before cooling ($P > 0.05$; Fig. 5.2a, Supplementary Table 5.1) or warming ($P > 0.05$; Fig. 5.2b, Supplementary Table 5.2). The respiration rates, and thus the total cumulative respiration at the end of incubation, were greatest for *T. versicolor* pre-colonised blocks for all temperature treatments, and were generally lowest for *V. comedens* pre-colonised blocks, with the exception of the similar respiration rates and cumulative respiration observed for non-pre-colonised blocks and *V. comedens* pre-colonised blocks in the 12 °C control treatment in the warming experiment (Fig. 5.2).

Table 5.4 Territory occupied (%) by pre-coloniser species at each stage of experiment.

Pre-coloniser species	Day	Stage of experiment	Duration (d)	Temperature change	Treatment	Occupied territory (%) (Mean ± SE)
V. comedens	154	End of pre-colonisation	154	-	-	100 ± 0
	254	End of natural colonisation	100	-	-	97.50 ± 2.50
	350	End of pre-incubation	96	Cooled	Pre-cooling	87.50 ± 12.50
		(prior to temperature change)		Warmed	Pre-warming	87.50 ± 6.68
	457	End of 107 d incubation	107	Cooled	Control	62.50 ± 18.30
		(after temperature change)			Cooled	71.88 ± 15.98
					Rewarmed	43.75 ± 13.15
				Warmed	Control	96.88 ± 3.13
					Warmed	93.75 ± 4.09
T. versicolor	154	End of pre-colonisation	154	-	-	100 ± 0
	254	End of natural colonisation	100	-	-	100 ± 0
	350	End of pre-incubation	96	Cooled	Pre-cooling	100 ± 0
		(prior to temperature change)		Warmed	Pre-warming	100 ± 0
	457	End of 107 d incubation	107	Cooled	Control	100 ± 0
		(after temperature change)			Cooled	100 ± 0
					Rewarmed	100 ± 0
				Warmed	Control	100 ± 0
					Warmed	100 ± 0

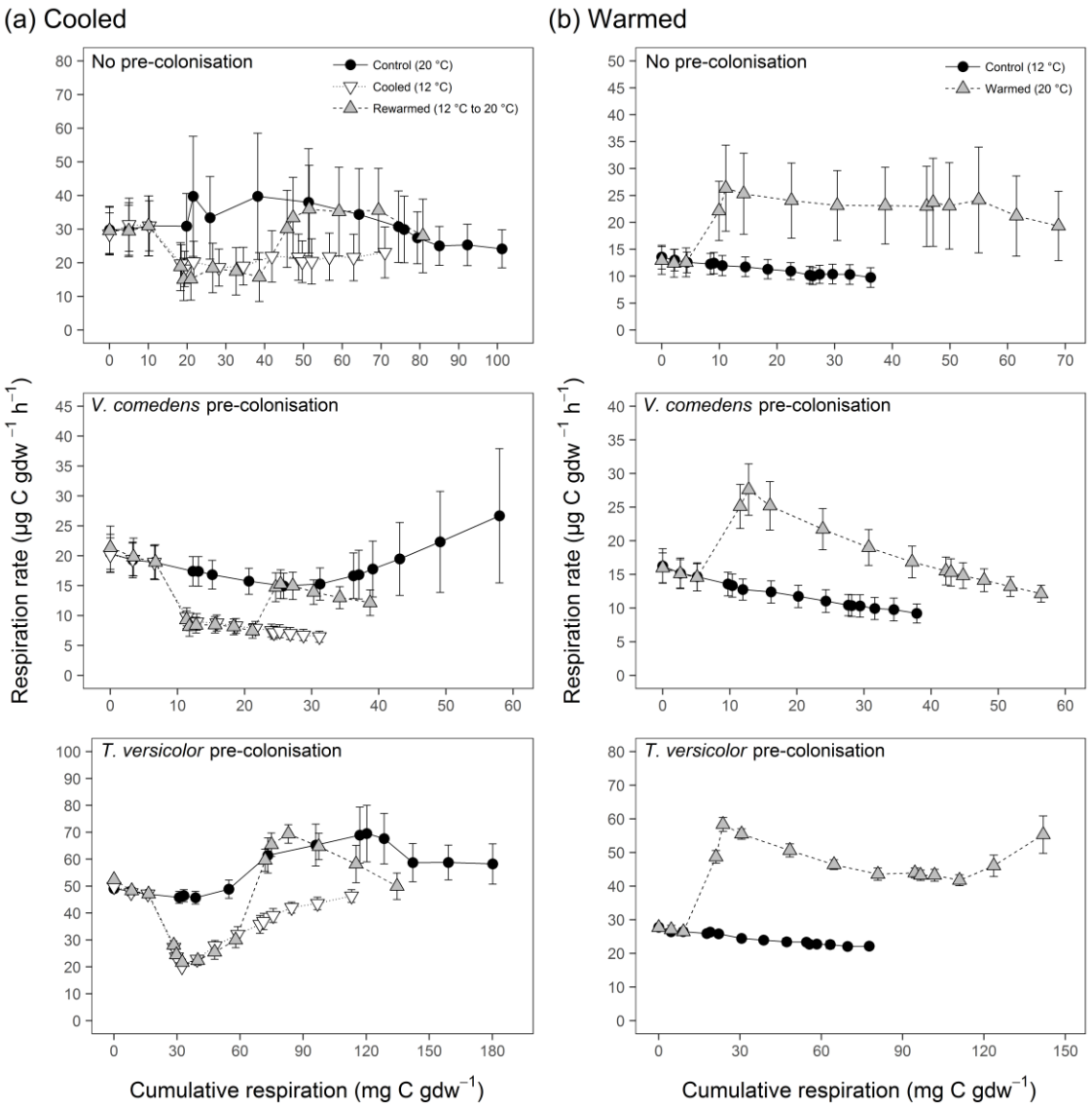


Figure 5.2 Respiration rate during 96 d pre-incubation period before temperature change and 107 d incubation after (a) cooling and (b) warming of wood blocks with no pre-colonisation, and samples pre-colonised with *V. comedens* and *T. versicolor* (mean ± SE of the mean, n = 8). Cumulative respiration was calculated from the start of pre-incubation (254 d).

5.4.3 Respiratory response to cooling and rewarming

The relative respiration rate (normalised to the time of cooling) from the non-pre-colonised blocks and *T. versicolor* pre-colonised blocks in the cooled treatment increased during the experiment (Fig. 5.3a). However, the relative respiration rate from the *V. comedens* pre-colonised blocks in the cooled treatment declined during the experiment (Fig. 5.3a). The non-pre-colonised blocks showed no thermal response (RR_{CC} = 0.85, P = 0.811; Table 5.5, Fig. 5.4a), whereas *T. versicolor* pre-colonised blocks exhibited a compensatory response (RR_{CC} =

0.83, *P* = 0.013; Table 5.5, Fig. 5.4a) and *V. comedens* pre-colonised blocks showed an enhancing response (RR_{CC} = 1.27, *P* = 0.008; Table 5.5, Fig. 5.4a) to cooling. The mean respiration response of the three pre-coloniser species, however, showed no overall thermal response of respiration to cooling (RR_{CC} = 0.96, *P* = 0.564; Table 5.5, Fig. 5.4a).

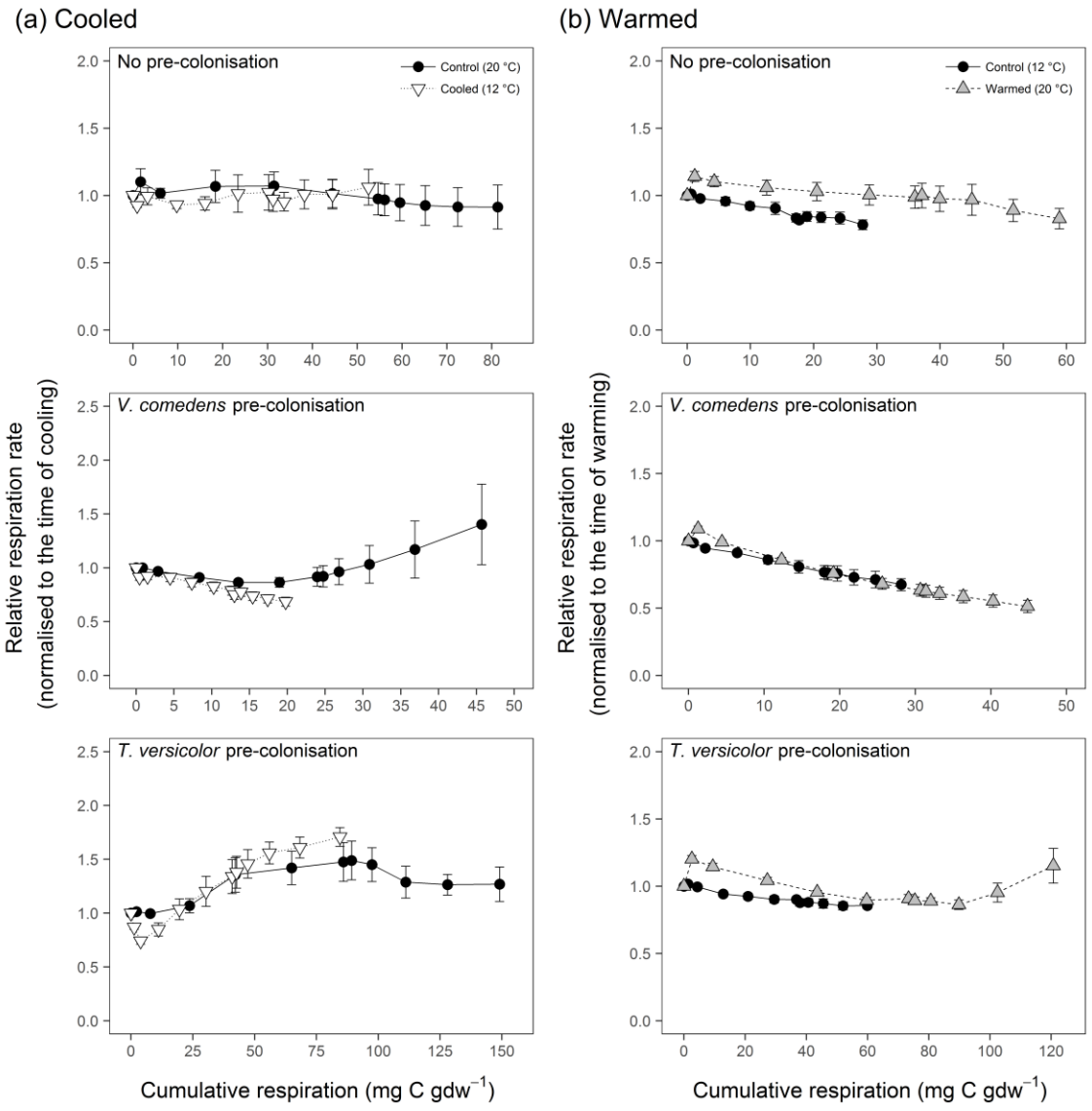


Figure 5.3 Relative respiration rate normalised to the time of (a) cooling, of control and cooled treatments and (b) warming, of control and warmed treatments, of samples with no pre-colonisation, and samples pre-colonised with *V. comedens* and *T. versicolor*, during 107 d of incubation following temperature change (mean ± SE of the mean, n = 8). Cumulative respiration was calculated from the time of cooling and warming (350 d), at the start of 107 d incubation.

Table 5.5 Respiration responses (RR_{CC}) quantified by comparing control and cooled treatment relative respiration rates, at a corresponding level of cumulative respiration as cooled treatment samples at end of incubation. Results for the three pre-coloniser species overall and individually are shown.

	Control vs. cooled treatment relative respiration rates			
	RR_{CC}	***t***	***P***	**Response**
Overall	0.96	-0.686	0.564	
Pre-coloniser species				
None	0.85	-0.248	0.811	
V. comedens	1.27	3.677**	0.008	Enhancing
T. versicolor	0.83	-3.319*	0.013	Compensatory

***** *indicates a significant difference at P < 0.05 and ** at P < 0.01.*

For rewarming, the mean respiration response across the three pre-colonisation treatments showed no thermal response ($P > 0.05$; Table 5.6, Fig. 5.4b). An enhancing response was observed initially for communities of the non-pre-colonised blocks after rewarming (RR_{CR} = 1.26, P = 0.020), but then a no response ($P > 0.05$) rapidly occurred (Table 5.6, Fig. 5.4b). There were minimal differences in the respiration rates of the rewarmed and control treatments for *V. comedens* and *T. versicolor* during the 17 d after rewarming, but the respiration rates of the rewarmed treatments then continued to decline below the rate of the control treatment for the remaining 27 d, leading to *V. comedens* (RR_{CR} = 1.48, $P < 0.001$) and *T. versicolor* (RR_{CR} = 1.26, P = 0.045) pre-colonised blocks showing enhancing responses at 44 d after rewarming (Table 5.6, Fig. 5.4b). No clear evidence of compensatory responses was detected.

Table 5.6 Respiration responses (RR_{CR}) quantified by comparing control and rewarmed treatment respiration rates at 1, 8 and 44 d after rewarming, at a corresponding level of cumulative respiration as rewarmed treatment samples at 1, 8 and 44 d after rewarming, respectively. Results for the three pre-coloniser species overall and individually are shown.

	1 d after rewarming				**8 d after rewarming**				**44 d after rewarming**			
	RR_{CR}	***t***	***P***	**Response**	**RR_{CR}**	***t***	***P***	**Response**	**RR_{CR}**	***t***	***P***	**Response**
Overall	1.14	2.325	0.146		0.99	-0.726	0.543		1.23	1.690	0.233	
Pre-coloniser species												
None	1.26	3.147*	0.020	Enhancing	1.01	1.245	0.260		1.00	0.372	0.722	
V. comedens	1.05	0.279	0.788		1.01	0.070	0.946		1.48	7.069***	0.0002	Enhancing
T. versicolor	1.12	1.716	0.130		0.96	-0.856	0.420		1.26	2.435*	0.045	Enhancing

***** *indicates a significant difference at P < 0.05 and *** at P < 0.001.*

5.4.4 Respiratory response to warming

The relative respiration rate (normalised to the time of warming) from the non-pre-colonised blocks, *V. comedens* and *T. versicolor* pre-colonised blocks in the warmed treatment increased and then decreased during the experiment (Fig. 5.3b). The non-pre-colonised blocks (RR_{CW} = 1.32, $P < 0.001$) and *T. versicolor* pre-colonised blocks (RR_{CW} = 1.10, $P = 0.014$) showed enhancing responses, whereas those pre-colonised with *V. comedens* (RR_{CW} = 0.99, $P = 0.933$) showed no response to warming (Table 5.7, Fig. 5.4c). The mean respiration response of the three pre-coloniser species, however, showed no thermal response of respiration to warming (RR_{CW} = 1.13, $P = 0.291$, Table 5.7, Fig. 5.4c).

Table 5.7 Respiration responses (RR_{CW}) quantified by comparing control and warmed treatment relative respiration rates, at a corresponding level of cumulative respiration as control treatment samples at end of incubation. Results for the three pre-coloniser species overall and individually are shown.

	Control vs. warmed treatment relative respiration rates			
	RR_{CW}	***t***	***P***	**Response**
Overall	1.13	1.423	0.291	
Pre-coloniser species				
None	1.32	6.189***	0.0005	Enhancing
V. comedens	0.99	-0.087	0.933	
T. versicolor	1.10	3.231*	0.014	Enhancing

***** *indicates a significant difference at P < 0.05 and *** at P < 0.001.*

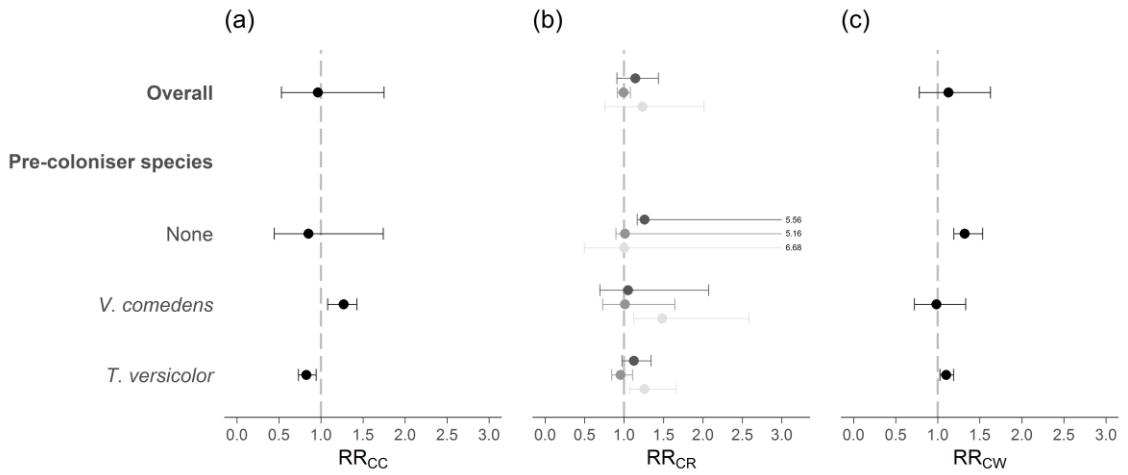


Figure 5.4 Respiration responses for the three pre-coloniser species overall and individually. The mean and 95% confidence intervals (n = 8) of (a) RR_{CC}, (b) RR_{CR} for 1 (black), 8 (dark grey) and 44 (light grey) d after rewarming and (c) RR_{CW}. RR_{CC} is the Response Ratio for control versus 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 the experiment. RR_{CR} is the Response Ratio for control versus rewarmed; control treatment respiration rate at a corresponding level of cumulative respiration as rewarmed samples at 1, 8 and 44 d after rewarming divided by the rewarmed treatment respiration rate at 1, 8 and 44 d after rewarming. RR_{CW} is the Response Ratio for control versus warmed; warmed treatment relative respiration rate divided by the control treatment relative respiration rate, at the cumulative respiration of the control treatment at the end of the experiment. Values < 1 indicate a compensatory response and values > 1 indicate an enhancing response. Effects are significant ($P < 0.05$) where confidence intervals do not cross one.

5.5 Discussion

This study is novel in investigating the temperature sensitivity of the respiration of semi-natural wood decomposing communities to cooling and warming. It revealed that semi-natural wood decay communities overall produced no respiratory thermal response that would have increased or decreased the medium- to long-term response to cooling or warming. However, compensatory and enhancing responses were shown by some of the wood decomposing communities, but enhancing responses that increased the effect of a temperature change on the respiration rates were more common than compensatory responses that decreased the effect of a temperature change on respiration rates.

5.5.1 Occupied territory in wood blocks

During decomposition in the temperate woodland, *T. versicolor* maintained its territory and *V. comedens* retained the majority of its territory. This is supported by a previous study that placed *T. versicolor* and *V. comedens* pre-colonised wood disks (2 x 10 cm) at the soil-litter interface in a mixed deciduous woodland dominated by *F. sylvatica* in September for 6 months, and found no change in territory of *T. versicolor*, and *V. comedens* remained present in 81.8% of disks (Hiscox *et al.*, 2015b). This was expected as the early secondary coloniser *T. versicolor* is generally more combative and hence can maintain territory for longer than the primary coloniser *V. comedens* (Boddy, 2000). During the laboratory incubation in the current study, it can be assumed that *T. versicolor* was responsible for the majority of the respiratory thermal response from *T. versicolor* pre-colonised blocks. However, ascomycetes that are supposedly incapable of decomposing lignocellulose and instead live opportunistically, may have been present, as identified in *T. versicolor* pre-colonised blocks after 12 months decomposition in a woodland in a previous study (Hiscox *et al.*, 2015b). For *V. comedens* pre-colonised blocks, the partial replacement suggests that ascomycetes and other basidiomycetes are contributing to the respiratory response, which is also supported by the same previous study (Hiscox *et al.*, 2015b). It is not clear why *V. comedens* retained more territory in blocks that underwent the warming experiment compared to the cooling experiment, but it suggests that *V. comedens* had better defensive abilities against invaders during the 12 °C laboratory pre-incubation, that consequently enabled *V. comedens* to maintain more of its territory until the end of incubation.

The fungal community composition and territory occupied in the wood blocks that were not pre-colonised prior to placement in the field is unknown, however the respiration rates would suggest an established wood decomposing community was present. They would likely have been initially colonised by fungi with ruderal characteristics, most likely ascomycetes, and then by basidiomycetes (Chapela and Boddy, 1988; Boddy and Heilmann-Clausen, 2008; Boddy *et al.*, 2017). Uncolonised beech wood disks left on the floor of a *F. sylvatica* dominated woodland for 12 months were predominantly colonised by ascomycetes, but basidiomycetes comprised approximately a third of the species present (Hiscox *et al.*, 2015b). Basidiomycete decay columns within larger wood volumes of beech in a previous study became clearly resolved from 24 weeks (Chapela and

Boddy, 1988), which suggests that they would not have been well established at the time of collection from the field. This would suggest that both ascomycete and basidiomycete species were likely present within the non-pre-colonised wood blocks in the current study, but ascomycetes were more likely the dominant taxa.

5.5.2 Respiratory response to cooling and rewarming compared to Chapter 3

The overall respiration response of the three wood decomposing communities (no pre-colonisation, *V. comedens, T. versicolor*) showed no thermal response to cooling. The overall respiratory response observed was likely dependent on the specific microbial community decomposing the wood, as all three thermal responses of respiration were detected: non-pre-colonised blocks showed no response, *T. versicolor* pre-colonised blocks exhibited a compensatory response and *V. comedens* pre-colonised blocks demonstrated an enhancing response, to cooling.

The *T. versicolor* pre-colonised blocks that had been decayed in the field in the present study exhibited a different response to cooling than *T. versicolor* as an individual species colonising beech wood (Chapter 3), with a compensatory response in the former and an enhancing response in the latter. Although *T. versicolor* pre-colonised blocks had an extended pre-colonisation period and the natural colonisation in the temperate woodland, the densities were similar prior to measurements in the laboratory during the pre-incubation period before cooling in both studies. Further, the respiration rates during pre-incubation in the laboratory were also similar prior to cooling. The respiration pattern and rates over the duration of the two experiments did vary, with the respiration of *T. versicolor* pre-colonised blocks being unstable but generally increasing over time in this study, whilst the respiration of *T. versicolor* as the only species present being consistently stable and declining over time in Chapter 3. In turn, the respiration rates and thus cumulative respiration were substantially different by the end of incubation, with *T. versicolor* pre-colonised blocks having higher cumulative respiration in all temperature treatments in the current study than *T. versicolor* blocks in Chapter 3. Although it should be noted that *T. versicolor* pre-colonised blocks in the present study were incubated for an additional 17 d following cooling. While *T. versicolor* was dominant in the *T. versicolor* pre-

colonised blocks in the current study, the unstable respiration rates suggests that the decomposition on the forest floor affected the response of *T. versicolor* to temperature, perhaps a result of other microbial species being present.

V. comedens pre-colonised blocks showing an enhancing response to cooling in the current study contrasts from *V. comedens* as an individual species colonising beech wood consistently showing no response to cooling in a previous study that used the same method (Chapter 3). The densities and respiration rates of *V. comedens* blocks were similar prior to measurements in the laboratory during the pre-incubation period before cooling in both studies. The respiration rates of *V. comedens* pre-colonised blocks in the control treatment, however, unexpectedly increased in the present study producing a higher cumulative respiration, than that observed for blocks solely colonised by *V. comedens* in Chapter 3. The respiration rates and cumulative respiration of cooled treatments, however, were similar in the current study and in Chapter 3. The partial replacement of *V. comedens* by other microbial taxa was most likely responsible for the enhancing response detected in the present study, thus altering the no response that occurred when *V. comedens* occupied territory alone (Chapter 3).

The respiration of non-pre-colonised blocks showed no response to cooling. As expected, the non-pre-colonised blocks had a higher density prior to pre-incubation in the laboratory, hence were at an earlier stage of decomposition, than the pre-colonised wood blocks before cooling. The respiratory response of the non-pre-colonised blocks likely represents that of initial wood decomposing communities at the earliest stage of decomposition.

The overall respiration response of the three wood decomposing communities also showed no thermal response after rewarming, which aligns with the overall thermal response after cooling. *T. versicolor* and *V. comedens* pre-colonised blocks in the present study showed no thermal responses for the majority of the incubation after rewarming, which aligned with the consistent no responses in respiration of *T. versicolor* and *V. comedens* blocks after rewarming in Chapter 3, however evidence of enhancement occurred in *T. versicolor* and *V. comedens* pre-colonised blocks at the end of incubation in this current study. It was the unexpectedly high respiration rates of the control treatments for *T. versicolor* and

V. comedens pre-colonised blocks that did not follow the predicted decline in respiration rates over time that produced the enhancing responses at the end of the rewarming period. This increase in respiration rate of control treatments was perhaps due to a change in the microbial community structure, where different species used alternative mechanisms to degrade lignocellulose and access more resources (Blanchette, 1995). For example, some white rot basidiomycete species will decompose lignin selectively (selective delignification), whereas other species will degrade lignin simultaneously with cellulose (simultaneous delignification), potentially leading to different rates of decomposition (Martínez *et al.*, 2005). Further investigation into the species present and relative degradation of the key wood polymers (cellulose, hemicellulose, lignin) would improve our understanding. Overall, *T. versicolor* and *V. comedens* pre-colonised blocks generally showed no responses to rewarming, which is consistent with the lack of response by *T. versicolor* and *V. comedens* to rewarming in Chapter 3. The non-pre-colonised blocks showed an initial enhancing response after rewarming but this resolved into no response within a few days thus supporting the no response observed with cooling. The rewarming therefore provides no clear evidence of compensation.

5.5.3 Comparison of respiratory response to cooling and warming

The three wood decomposing communities showed contrasting respiratory thermal responses to cooling and warming in this study. The communities responding to the cooling to 12 °C and warming to 20 °C may have changed differently during the experiment as temperature affects the combative ability of basidiomycetes and alters interaction outcomes and thus the dominating species (Hiscox *et al.*, 2016a). This impact of temperature on competition and species presence may also apply to ascomycetes. Several studies have found that the fungal community composition, through species-specific fungal traits, rather than climate, is more important in governing wood decomposition (Bradford *et al.*, 2014; van der Wal *et al.*, 2015; Venugopal *et al.*, 2016, 2017; Maynard *et al.*, 2018). Therefore, the presence of particular species may have contributed to the variety of thermal responses detected, and ultimately the rate of wood decay. Although respiratory responses were not the same across both cooling and

warming, there was limited evidence for compensatory responses that would reduce the effect of temperature on wood decomposition.

5.5.4 Mechanisms responsible for respiratory responses

The mechanisms responsible for the adjustments in respiration rates in the current study are unknown. The lack of strong evidence for thermal compensation in more complex and diverse wood decay communities in the current study, is in broad agreement with the fact that no clear compensatory responses were observed for individual basidiomycete species decomposing wood (Chapter 3), and no responses were the most common response in simple assemblages of interacting basidiomycetes decomposing wood (compensatory responses detected were thought to be a result of differences in the progression of the species interactions or the warmed treatment passing the optimum temperature for growth for some species) (Chapter 4). Enhancing responses were produced by some basidiomycete species growing alone (Chapter 3), thus the enhancing responses may have been a sum of physiological responses of many species in the present study. In the current study, microbial biomass or potential enzyme activities were not measured and thus their control on the thermal response of respiration is unknown. However a previous study, that measured respiration from soils from different ecosystems along a climate gradient from the Arctic to the Amazon, detected more enhancing than compensatory responses and found no changes in microbial biomass or potential enzyme activities that could explain the overall patterns of the respiration responses observed, and the pattern of responses persisted when expressed per unit biomass (Karhu *et al.*, 2014; Auffret *et al.*, 2016). Further, a meta-analysis of 25 field experiments found no significant declines in fungal biomass after 10 years of warming (Romero-Olivares *et al.*, 2017) and the fungal biomass could not explain the respiration responses to temperature shown by individual basidiomycete species in Chapter 3. This suggests that microbial biomass, and potential enzyme activities, may not be the key factors controlling the respiratory responses to temperature. Enhancing responses were generally more common in soils with high C content, high C:N ratios and low pH values in the previous study (Karhu *et al.*, 2014). The non-pre-colonised blocks were less decomposed than the pre-colonised blocks prior to measurements in the laboratory and produced the strongest enhancing

response, therefore, the higher C content and higher C:N ratio could have allowed this stronger enhancing response. Generally, ascomycetes prefer more alkaline conditions whereas basidiomycetes prefer an acidic environment (Tudor *et al.*, 2013), so wood tends to decline in pH as decomposition progresses with basidiomycetes becoming more dominant. *V. comedens* decreases the pH of colonised wood more than *T. versicolor* (Hiscox *et al.*, 2015b), which may have contributed to the second strongest enhancing response detected. Further research into the influence of microbial biomass, enzyme activity, C content, C:N ratios and biochemical changes in wood such as pH on respiration responses to temperature would further improve our mechanistic understanding. As well as the physiological response of individuals, measuring respiration rates at the community-level in the present study may have encompassed evolutionary changes in microbial function and shifts in the species composition (Bárcenas-Moreno *et al.*, 2009; Bradford, 2013), with the latter being influenced by priority effects in fungal communities (Fukami *et al.*, 2010; Fukami, 2015; Hiscox *et al.*, 2015b; Hiscox *et al.*, 2016b).

Priority effects, where the identity and abundance of species that first colonise a resource affects the colonisation success of later arriving species and thus the assembly history, has been widely demonstrated in wood decay fungal communities (Fukami *et al.*, 2010; Dickie *et al.*, 2012; Ottosson *et al.*, 2014; Fukami, 2015; Hiscox *et al.*, 2015b; Hiscox *et al.*, 2016b). Priority effects are partly determined through biochemical alteration of the resource through enzyme activity, mycelial growth and deposition of secondary metabolites (Allison, 2012). In a previous study, distinctly different communities developed in uncolonised disks compared with disks pre-colonised by *V. comedens* and *T. versicolor* after 12 months of decomposition on the forest floor of *F. sylvatica* dominated woodland (Hiscox *et al.*, 2016b). However, after 24 months of decomposition, the three disk types did not lead to significantly different successor communities, which suggests that priority effects become weaker as decomposition progresses (Hiscox *et al.*, 2016b). Therefore, priority effects influencing the species present are likely to drive the species-specific respiratory thermal responses and subsequent decay rate during the early stages of succession. This is further supported by a past study that found the magnitude of respiration responses to temperature to be correlated with the initial microbial community structure (Auffret

et al., 2016), likely a consequence of the strength of responses from particular species. However, as decomposition progresses, the community composition becomes less influenced by priority effects, resulting in more randomly assembled communities (van der Wal *et al.*, 2015; Hiscox *et al.*, 2016b). The respiration response and rate of decay of more stochastic communities during later successional stages will depend on the dominant decomposers. Further, it is the reductions in competitive interactions that likely allow the formation of more randomly assembled communities (van der Wal *et al.*, 2015), thus an increase in the number and variety of species present will likely occur, which can enhance rates of wood decomposition (Maynard *et al.*, 2017). Wood decay communities with less competitive interactions are likely to be more stable with individuals colonising resources as if they exist alone, thus more enhancing responses may occur, as seen in Chapter 3. As decomposition progresses, respiration responses may become more varied and less predictable. Overall, the species present within a community and the stage of wood decomposition are likely to influence the respiratory responses observed.

5.5.5 The respiratory thermal response of semi-natural communities compared to individual fungal species decomposing wood and long-term warming experiments

Overall, the limited evidence of fungal dominated communities showing compensatory responses and decreasing the direct effect of temperature on respiration, in the current study, contrasts to this ability shown by individual arbuscular mycorrhizal fungi in soil (Heinemeyer *et al.*, 2006), free-living ectomycorrhizal fungi on agar (Malcolm *et al.*, 2008) and individual cord-forming wood decay basidiomycetes grown on agar (Crowther and Bradford, 2013). Nevertheless, no thermal compensation of respiration by the saprotrophic ascomycete fungus *Neurospora discreta* growing on agar, sucrose or lignin supports our results (Romero-Olivares *et al.*, 2015; Allison *et al.*, 2018). The ability of fungal species to compensate their respiration to a temperature change seems to vary, however the use of artificial media where labile substrate is constantly available can make inferring responses that occur in the real world challenging. The present study, instead, improves our understanding of the

respiratory thermal response of wood decomposing communities dominated by saprotrophic fungi when colonising their natural substrate.

The overall no response in respiration with temperature and hence temperature sensitivity in the present study is supported by no significant differences in the temperature sensitivity of soil respiration between control and warmed plots found in temperate forests (Carey *et al.*, 2016). Although our study did not show overall enhancing responses to temperature, detecting more enhancing than compensatory responses aligns with the findings of past studies on soil respiration responses to temperature (Hartley *et al.*, 2008; Karhu *et al.*, 2014).

The current study finding no overall ability of semi-natural wood decomposing communities to decrease the direct effect of temperature contrasted with recent studies that found compensatory adaptation of soil microbial respiration to the ambient thermal regime (Bradford *et al.*, 2019; Dacal *et al.*, 2019). When measured at a common assay temperature, soil microbial respiration rates were lower for soils sampled from higher mean annual temperature regimes after controlling for differences in microbial biomass and substrate depletion (Bradford *et al.*, 2019; Dacal *et al.*, 2019). Overall compensatory adaptation of soil microbial respiration may have been found in these past studies due to respiration being measured on a biomass-specific basis or under idealised conditions with excess labile substrate.

Many long-term (years-decades) warming experiments found declines in soil respiration with warming (Jarvis and Linder, 2000; Oechel *et al.*, 2000; Luo *et al.*, 2001; Melillo *et al.*, 2002, 2017; Romero-Olivares *et al.*, 2017; García-Palacios *et al.*, 2018). The present study showing no consistently strong compensatory responses of respiration to temperature suggests that decomposer fungi are not the dominant mechanism downregulating the soil microbial respiration rates with warming in these long-term experiments. Instead, the depletion of accessible C, reductions in microbial biomass or changes in microbial community composition remain the most likely explanations for the decline in soil respiration in long-term soil warming experiments.

The current study has strengthened our mechanistic understanding of respiratory thermal responses of fungal-dominated wood decay communities to temperature. However, it has only involved three wood decomposer communities obtained from one location in one *F. sylvatica* dominated temperate woodland, that represents only early- to mid-stages of decomposition. Further knowledge of the respiratory responses of a wider variety of wood decomposing communities at varying successional and decomposition stages is required. Future work should consider the relationship between the thermal response of respiration and the specific species comprising the community, as well as the stage of wood decomposition. The fungal species composition and decomposition state within a location could then provide a tangible link between respiration and temperature, and ultimately the ecosystem function.

5.6 Conclusion

Until now, there has been no evaluation of the role of wood decomposing community responses in controlling the temperature sensitivity of respiration. The respiration of semi-natural wood decay communities in beech wood, overall, showed no response to cooling or warming, which would lead to no change in the temperature sensitivity of respiration in the medium to long term. When considering the response of each community, however, the present study found minimal evidence of strong compensatory responses that could reduce the effects of warming on wood decomposition, and instead detected more evidence of enhancing responses that could increase the effects of warming on wood decomposition. Therefore, the respiratory response of wood decomposing communities to temperature vary, and are likely dependent on the species comprising the community and the wood decomposition stage. The overall respiratory response of wood decay communities to warming will depend on the direction and magnitude of responses produced by the varying decomposer communities combined. It seems unlikely that compensatory responses will weaken positive feedbacks between warming and carbon loss from the decomposition of woody debris. Rather, it is more likely that wood decomposing communities will produce more enhancing responses with warming, consequently causing an increase in the temperature sensitivity of respiration in the medium to long term.

Chapter 6. Synthesis and Conclusions

The work presented in this thesis adds to the current knowledge of the respiratory response of wood decomposing fungi to temperature, and has advanced our understanding of the respiration response of whole soil microbial communities to warming in the long term. This is the first time that the thermal response of the respiration of basidiomycetes decomposing wood has been investigated in the medium to long term, and elucidates the importance of using natural substrate to represent the responses in natural systems. This work has progressed the mechanistic understanding of respiratory thermal responses by gradually increasing the complexity of systems studied in microcosms under controlled laboratory conditions: from individual species (Chapter 3) and simple assemblages of basidiomycetes (Chapter 4) to semi-natural wood decomposing communities (Chapter 5). Furthermore, the use of the cooling approach has provided the most robust method of identifying any possible compensatory responses, as an increase in respiration rate could only be caused by thermal compensation. The rewarming treatment within the cooling approach showed the reversibility of the responses and further developed our understanding. In addition, the use of the warming approach on wood block microcosms supported the responses observed after cooling. The complementary approaches applied has maximised our understanding of the physiological response of basidiomycetes and wood decomposing communities to temperature. This work has revealed that individual species increase the temperature sensitivity of respiration, but fungal assemblages and wood decomposing communities cause no change in the temperature sensitivity of respiration overall, although, more enhancing than compensatory responses were identified.

6.1 Respiratory responses to cooling

Following cooling, individual species of basidiomycetes showed an overall enhancing response that increased the temperature sensitivity of respiration, with no evidence of compensatory responses (Chapter 3). When respiration rates were normalised for fungal biomass (estimated as ergosterol content), the individual basidiomycete species showed no thermal response overall, which implies that some enhancing responses were caused by the inhibition of growth

and biomass production at the lower temperature (Chapter 3). However, some enhancing responses were still identified after normalising for biomass, therefore enhancing responses not driven by biomass changes can occur (Chapter 3). Two-species assemblages and the three-species assemblage showed no thermal responses of respiration overall to cooling, thus no change in the temperature sensitivity of respiration (Chapter 4). Evidence of both compensatory and enhancing responses were shown by multispecies assemblages (Chapter 4). In general, the compensatory responses detected were either a short-term response with the magnitude of compensation declining during the incubation, or may have been caused by reduced CO_2 production from antagonistic interactions at the higher temperature treatment due to the interaction progressing faster in warmer wood blocks and one species gradually replacing the other (Chapter 4). Therefore, strong evidence for compensatory responses that would gradually reduce the temperature sensitivity of respiration was not generated in the multispecies assemblage experiments (Chapter 4). Furthermore, the one enhancing response by a two-species assemblage was driven by the increase in control respiration rather than a decline in the cooled respiration, thus was not clear evidence of enhancement (Chapter 4). Consequently, multispecies assemblages predominantly showed no change in the temperature sensitivity of respiration. Semi-natural wood decomposing communities showed no thermal response overall to cooling and thus no change in the temperature sensitivity of respiration (Chapter 5). However, the semi-natural wood decay communities did show more enhancing than compensatory responses with cooling (Chapter 5).

6.2 Respiratory responses to warming

With warming, two-species assemblages showed no thermal response overall that caused no change in the temperature sensitivity of respiration, but more compensatory than enhancing responses were identified (Chapter 4). The three-species assemblage demonstrated a compensatory response with warming (Chapter 4). However, these compensatory responses were likely to be caused by either the warmed treatment exceeding the optimum temperature for growth for some species, or species being replaced quicker in the warmed treatment which led to reduced CO_2 production from less antagonistic interactions in the warmed than control treatment (Chapter 4). One of the compensatory responses

was due to a change in the respiration of the control treatment, rather than a decrease in the respiration of the warmed treatment (Chapter 4). Therefore, these compensatory responses by the multispecies assemblages were not consistent with compensatory responses that would decrease the temperature sensitivity of respiration. Additionally, the one enhancing response produced by a two-species assemblage was likely due to the interaction taking longer to resolve in the warmed than control treatment, leading to increased CO_2 production from antagonistic mechanisms in the warmed treatment (Chapter 4). Overall, the compensatory and enhancing responses shown by the multispecies assemblages are most likely not responses to the direct effect of warming, but rather the effects of temperature on competition between different species (Chapter 4). Semi-natural wood decay communities showed no thermal response overall to warming, therefore no change in the temperature sensitivity of respiration (Chapter 5). However, these semi-natural wood decomposing communities did produce some enhancing responses, but no compensatory responses (Chapter 5).

6.3 Summary of respiratory thermal responses

Overall, the work has revealed that as the fungal wood decomposing system increased in complexity and species diversity, no change in the temperature sensitivity of respiration occurred. Individual species of basidiomycetes increased the effect of a temperature change on rates of respiration, however, simple multispecies basidiomycete assemblages and semi-natural wood decay communities caused no change in the effect of a temperature change on respiration rates. Some evidence of clear enhancing responses were identified, but there was limited evidence for clear compensatory responses. As such, the occurrence of interspecific interactions in the more diverse systems seemed to reduce the overall temperature sensitivity of wood decomposition. The general hypothesis proposed in Chapter 1, which was individual species and two- and three-species assemblages of basidiomycetes decomposing wood, and semi-natural wood decomposing communities, will show compensatory thermal responses overall and decrease the temperature sensitivity of respiration in the medium to long term, was not supported.

6.4 Further investigation of respiratory thermal responses

To further explore the findings, the respiratory thermal responses of the individual species of basidiomycetes (Chapters 3) can be correlated with their growth responses to temperature on agar (Chapter 2). In addition, the relationship between the respiratory thermal responses and absolute respiration rates of the fungal systems in Chapters 3, 4 and 5 in this thesis can be explored, although, with limited numbers of data points, these analyses must be considered speculative at this stage.

6.4.1 The relationship between respiratory thermal responses and growth responses to temperature of individual species of basidiomycetes

Species with a higher optimum temperature for growth and a higher extension rate ratio at 20 °C versus 12 °C (greater decrease in extension rate when cooled to 12 °C from 20 °C), would be expected to be the most negatively impacted by the cooling, and consequently more likely to produce enhancing responses. Although only nine basidiomycetes were studied, there is some evidence of species with a higher optimum temperature for growth producing higher RR_{CC} values ($P < 0.01$) and therefore stronger enhancing responses, due to 12 °C being further from their optimal growth temperature (Fig. 6.1a). However, no relationship between R_{mass} RR_{CC} and optimum temperature for growth occurred ($P = 0.66$) (Fig. 6.1b). The extent of decrease in extension rate at 12 °C compared to 20 °C did not predict the RR_{CC} ($P = 0.48$) (Fig. 6.1c) or R_{mass} RR_{CC} ($P = 0.42$) (Fig. 6.1d). Generally, the temperature sensitivity of the individual basidiomycete species growth does not show a clear relationship with the temperature sensitivity of their respiration (Fig. 6.1). Further investigation would be required to determine whether a species' thermal optimum for growth is an accurate predictor of its respiratory thermal response.

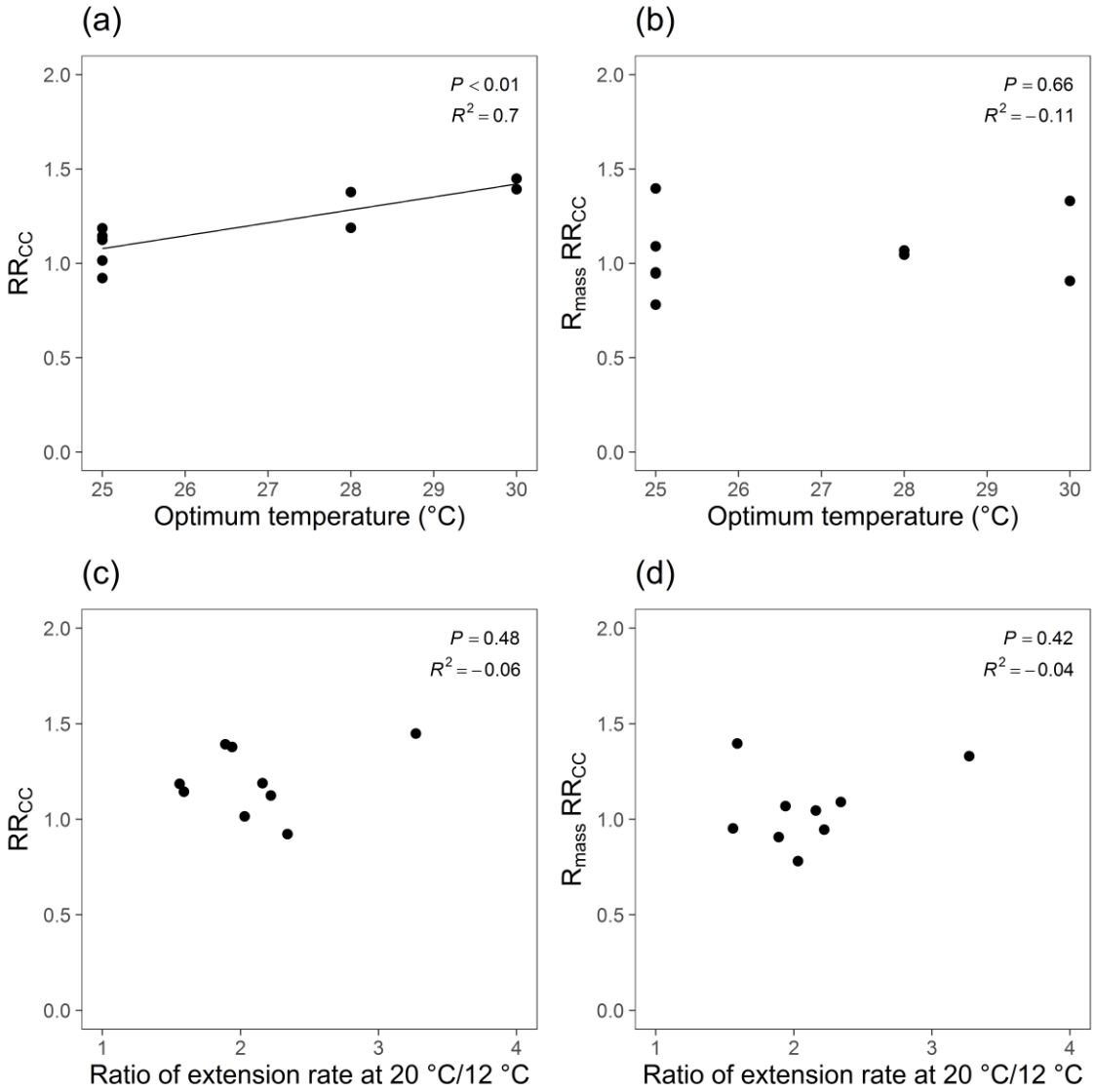


Figure 6.1 The relationship between (a) RR_{CC} and (b) R_{mass} RR_{CC}, and the optimum growth temperature for the individual species. The relationship between (c) RR_{CC} and (d) R_{mass} RR_{CC}, and the ratio of extension rate at 20 °C/12 °C for the individual species. Linear regressions were used to test for statistically significant relationships; a solid line indicates a significant difference at $P < 0.05$. RR_{CC} is the Response Ratio for 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 the experiment. R_{mass} RR_{CC} is the mass-specific respiration Response Ratio for control versus cooled; control treatment relative mass-specific respiration rate divided by the cooled treatment relative mass-specific respiration rate, at the cumulative respiration of the cooled treatment at the end of the experiment. Values < 1 indicate a compensatory response and values > 1 indicate an enhancing response.

6.4.2 The relationship between respiratory thermal responses and absolute respiration rates

There may be a relationship between the species absolute metabolic rate and the respiratory thermal response. Individual species of basidiomycetes with control respiration rates in the medium to higher range generally produced greater RR_{CC} values, thus greater enhancing responses, but no significant relationship was found (P = 0.47) (Fig. 6.2a). The species with higher metabolisms potentially have different and more complex functional strategies that could not be performed at the lower temperature, compared to strategies used by those with lower metabolic rates. Additionally, individual basidiomycete species with higher respiration rates at the time of cooling also produced greater RR_{CR} values after rewarming (P = 0.15), indicative of greater enhancing responses (Fig. 6.3a). The fact that species with the higher absolute respiration rates did not produce compensatory responses may explain the limited evidence of compensatory responses in the semi-natural wood decomposing communities. Individual basidiomycete species with higher absolute respiration rates showing enhancing responses may cause wood decomposing communities overall to show an increase in temperature sensitivity of respiration, thus having major ecological impacts.

However, two-species assemblages (P = 0.13) and semi-natural wood decomposing communities (P = 0.32) with higher control respiration rates at the time of cooling produced lower RR_{CC} values, indicating towards compensatory responses (Fig. 6.2b,c). Yet the two-species assemblages with the higher control respiration rates at the time of warming produced greater RR_{CW} values (P = 0.28), which would indicate enhancement (Fig. 6.4a). No relationship between the control respiration rate and RR_{CR} (P = 0.80) (Fig. 6.3b) or RR_{CW} (P = 0.83) (Fig. 6.4b) occurred for the semi-natural communities, but there were only three different communities investigated so preventing firm conclusions from being made. The relationship between the respiratory thermal response and species metabolic rate is challenging to interpret using these data, particularly with some compensatory responses not likely caused by the direct effect of the temperature change, as discussed previously. However, the respiratory thermal responses of species, assemblages and communities with higher metabolic rates will have more ecological significance than those with lower metabolic rates, thus, it requires further investigation.

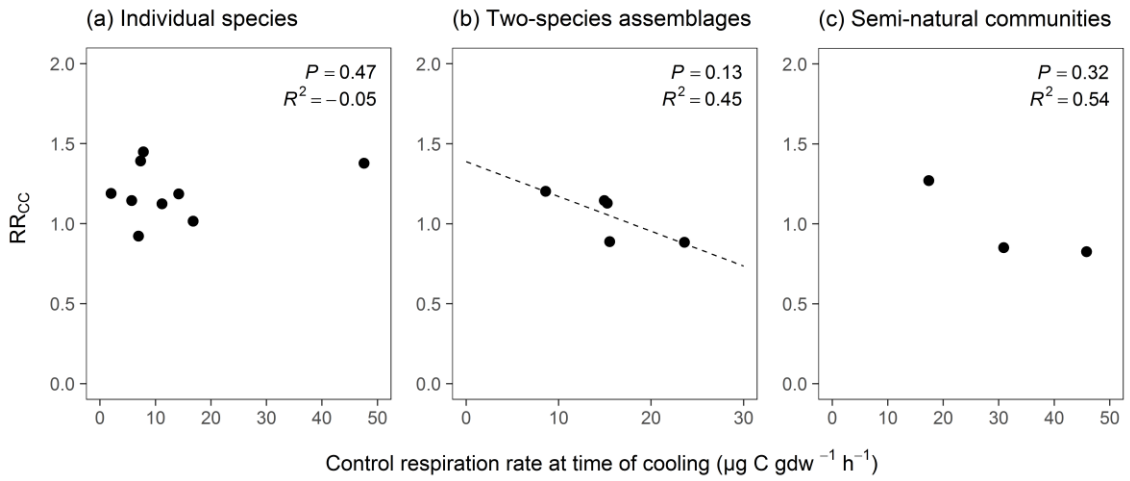


Figure 6.2 The relationship between RR_{CC} and control respiration rate at the time of cooling for (a) individual species, (b) two-species assemblages and (c) semi-natural communities. Linear regressions were used to test for statistically significant relationships; a dashed line indicates a significant difference at $P < 0.20$. RR_{CC} is the Response Ratio for 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 the experiment. Values < 1 indicate a compensatory response and values > 1 indicate an enhancing response.

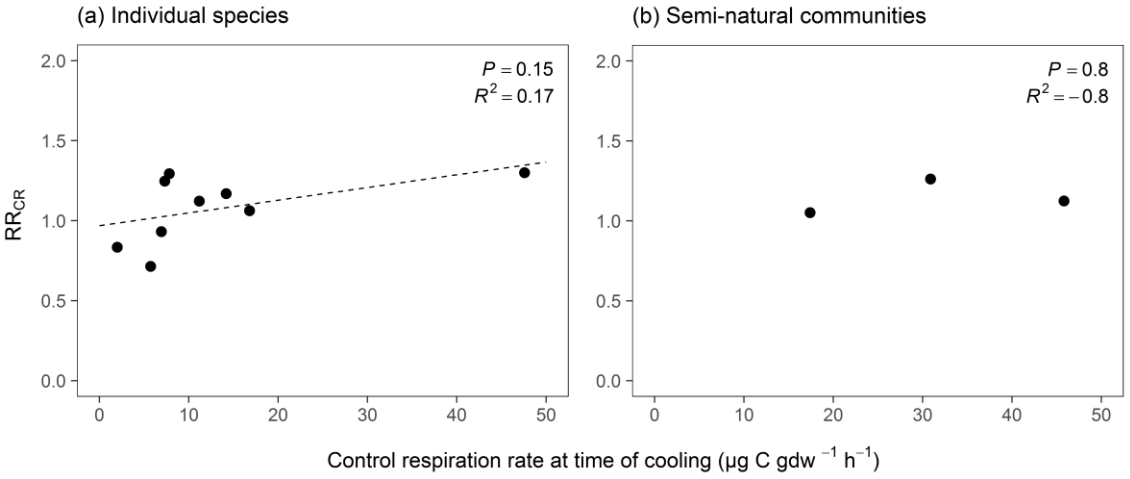


Figure 6.3 The relationship between RR_{CR} and control respiration rate at the time of cooling for (a) individual species and (b) semi-natural communities. Linear regressions were used to test for statistically significant relationships; a dashed line indicates a significant difference at $P < 0.20$. RR_{CR} is the Response Ratio for control versus rewarmed; control treatment respiration rate at a corresponding level of cumulative respiration as rewarmed samples at 1 d after rewarming divided by the rewarmed treatment respiration rate at 1 d after rewarming. Values < 1 indicate a compensatory response and values > 1 indicate an enhancing response.

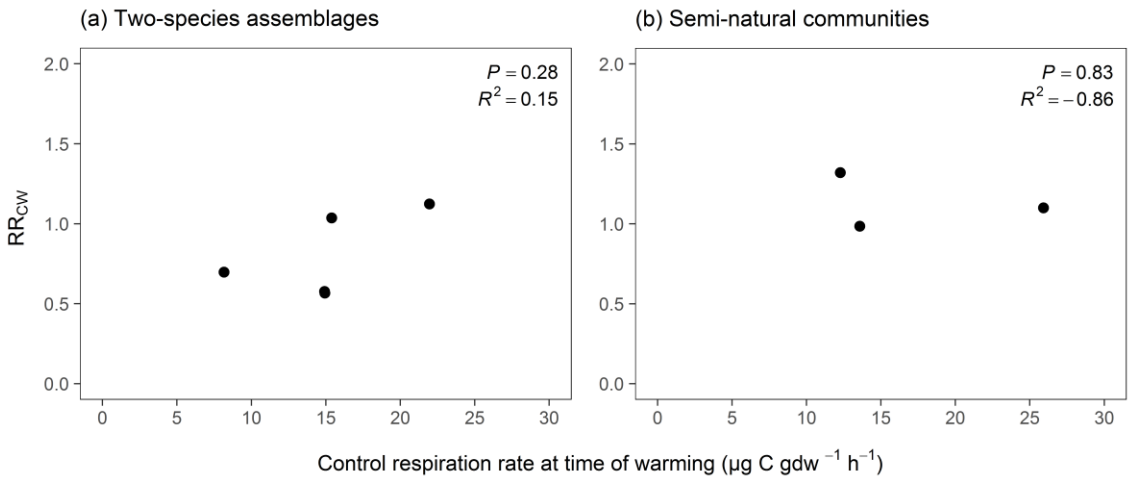


Figure 6.4 The relationship between RR_{CW} and control respiration rate at the time of warming for (a) two-species assemblages and (b) semi-natural communities. Linear regressions were used to test for statistically significant relationships. RR_{CW} is the Response Ratio for control versus warmed; warmed treatment relative respiration rate divided by the control treatment relative respiration rate, at the lowest total cumulative respiration of the warmed or control treatment. Values < 1 indicate a compensatory response and values > 1 indicate an enhancing response.

The individual species of basidiomycetes generally produced stable, declining respiration rates (Chapter 3), which would be expected with decreasing substrate availability as wood was decomposed over time. The species with stable respiration rates in control and cooled treatments, which then remained stable after rewarming, were those that produced no thermal responses (Chapter 3). However, it was the species with unstable respiration rates in both control and cooled treatments, which also led to unstable respiration rates after rewarming, that showed enhancing or unexpected responses (Chapter 3). *H. fasciculare*, although did not compensate for the effect of cooling, showed an unexpectedly high increase in respiration after rewarming. This may be due to the temperature change affecting the availability and use of substrate. Cooling may have affected the ability of *H. fasciculare* to breakdown lignin, the most complex wood polymer, which could have in turn reduced the availability of cellulose and hemicellulose. This limit in substrate availability may have been lost following rewarming, allowing respiration rates to increase. Investigations into the relative changes of the major wood polymers at different temperatures involving different species would improve our understanding of the relationship between the breakdown of wood and respiration rates. However, at present, the extent to which changes in substrate chemistry may have played a role in the thermal responses is unknown.

The multispecies assemblages and semi-natural wood decomposing communities generally showed less stable, often increasing respiration rates, rather than the gradual and steeper declines shown by the species growing alone. The unstable and increased respiration rates were expected due to the interspecific interactions (Hiscox *et al.*, 2015a). Therefore, the strategies employed by species when decomposing wood, the mechanisms used and wood polymers targeted, may influence their respiratory thermal response.

6.5 Ergosterol as a biomarker for fungal biomass

Ergosterol is considered a better proxy for fungal biomass estimation than phospholipid fatty acids and glucosamine, the other commonly used methods, but it is acknowledged to have many inadequacies (Baldrian *et al.*, 2013b). For the nine basidiomycete species grown alone in wood (Chapter 3), ergosterol content at the end of incubation was a poor predictor of the respiration in the control ($P = 0.48$) and cooled ($P = 0.18$) treatments at the end of incubation (Fig. 6.5a,b). The respiration rate varied per unit ergosterol, therefore it does not show a clear relationship between fungal biomass and respiration (Fig. 6.5a,b). A positive relationship was expected between ergosterol per unit wood and respiration rate, but instead a negative relationship occurred (Fig. 6.5a,b). It is possible that the species membranes vary in ergosterol content or the species differ metabolically, thus contributing to this unexpected relationship. The species having different metabolic rates is supported by the species showing varied mass-specific respiration rates at the end of incubation in control ($P < 0.001$) and cooled ($P < 0.001$) treatments (Fig. 6.5c,d). The species differed in their respiration rate per unit ergosterol, but this does not seem to be related to their ecological role (Fig. 6.5c,d). The ergosterol-respiration relationship appears to be species-specific, which has implications for the use of ergosterol as a biomarker for fungal biomass. The use of ergosterol as an indicator for fungal biomass should be considered with caution, and may not be useful in diverse communities in particular as only a minor change in community composition would alter the biomass-respiration relationship.

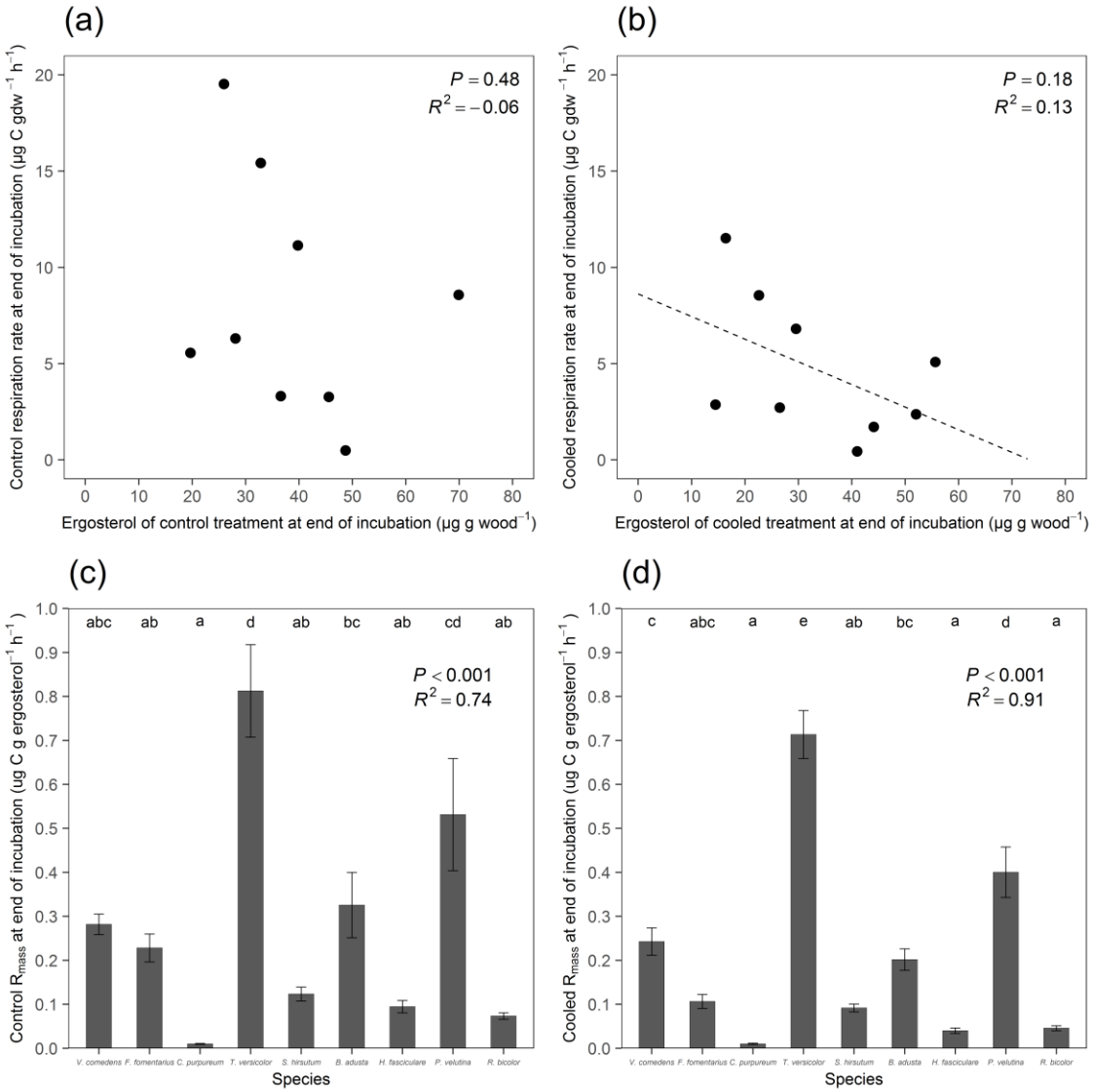


Figure 6.5 The relationship between (a) control respiration rate and ergosterol of control treatment, at the end of 90 d incubation following cooling for the individual species, and (b) cooled respiration rate and ergosterol of cooled treatment, at the end of 90 d incubation after cooling for the individual species. (a-b) Linear regressions were used to test for statistically significant relationships; a dashed line indicates a significant difference at $P < 0.20$. The relationship between the (c) control R_{mass} and (d) cooled R_{mass}, at the end of 90 d incubation following cooling (mean ± SE of the mean, n = 5), and the individual species. (c-d) One-way ANOVA models were used to test for statistically significant relationships; different letters indicate significant ($P < 0.05$) differences from Tukey's pairwise comparisons. R_{mass} is the mass-specific respiration.

6.6 Implications of the respiratory thermal responses of basidiomycetes decomposing wood and wood decomposing communities in the long term and for long-term field-based soil warming experiments

Investigating the respiratory thermal responses of basidiomycetes decomposing wood and semi-natural wood decomposing communities over a 90- or 107-day timescale informs our understanding of their responses in the long term and of findings from long-term soil warming experiments in the field. Wood decomposing fungi responding to a temperature change over at least 90 days represents the length of seasonal cycles, a time period where wood decay fungi experience the largest changes in temperature (Boddy 1983a). Therefore, if wood decay fungi are unable to show clear compensatory responses to a temperature change over this seasonal timescale, during which the largest fluctuations in temperature occur in nature (Boddy 1983a), then wood decay fungi are unlikely to show strong evidence for compensatory responses in the long-term (years-decades). As such, wood decomposing fungi showing limited evidence for compensatory responses over at least 90 days in this thesis suggests compensatory responses are unlikely to occur in the long term. This is supported by the positive response of soil microbial respiration with warming in long-term field-based experiments after 2 years (Melillo *et al.,* 2002, 2017; Hicks Pries *et al.,* 2017; Nottingham *et al.,* 2020). However, the positive response of respiration to warming has declined after 2 or more years in past field studies (Rustad *et al.,* 2001; Eliasson *et al.,* 2005), and in some cases returned to pre-warming levels after 1 (Luo *et al.,* 2001), 5 (Jarvis and Linder, 2000; Eliasson *et al.,* 2005), or greater than 10 years (Oechel *et al.,* 2000; Melillo *et al.,* 2002, 2017; Romero-Olivares *et al.,* 2017). Nevertheless, the limited evidence for clear compensatory responses to a temperature change over the relevant timescale of 90- or 107-days in this thesis indicates that the mechanism underlying this decline in respiration in soil communities with warming is more likely a result of indirect effects of temperature, such as substrate depletion, as opposed to a compensatory thermal response of soil microorganisms down-regulating respiration rates.

6.7 Implications of the respiratory thermal responses of basidiomycetes decomposing wood and wood decomposing communities for modelling microbial processes to improve global soil C projections

Had there been strong evidence of basidiomycetes decomposing wood and wood decomposing communities showing clear compensatory or enhancing responses, then these respiratory responses would need to be included in ESMs

to more accurately project climate change feedbacks (Wieder *et al.*, 2013). However, this thesis showed limited evidence for clear compensatory responses, and the strong evidence for enhancing responses by the individual species of basidiomycetes was reduced in the more complex and diverse systems. Therefore, investigating the respiratory thermal response of wood decomposing fungi has not identified new fundamental processes that should be included in ESMs. Nonetheless, responses of various aspects of microbial physiology to temperature should continue to be studied and better represented in ESMs to improve global soil C predictions (Wieder *et al.,* 2013).

6.8 Future research priorities

Future research priorities to develop our understanding of the findings reported in this thesis include determining the growth rate and optimum temperature of basidiomycetes growing in beech wood (instead of agar, as in Chapter 2). Individual basidiomycete species would need to be inoculated at the end of long pieces of wood and incubated at different temperatures (10-35 °C). Small pieces of wood, removed from sections that span the entire length of the resource, would need to be placed onto 2% malt agar (MA) to allow mycelia to grow and species to be identified morphologically. A large number of replicates and time points would be required to monitor the species growth over time. Although this would be challenging, it would develop our ecological understanding of the respiratory thermal responses. Furthermore, the use of ergosterol as an indicator for fungal biomass could be further investigated. The ergosterol per unit dry weight of mycelium of each species grown at a range of temperatures could be measured to determine whether ergosterol content is species and temperature-dependent. This would advance our understanding of the mass-specific respiratory thermal responses (Chapter 3). Moreover, the activities of cellulolytic and ligninolytic extracellular enzymes, and those involved in nutrient acquisition, in the samples in the temperature treatments could be measured to assess their role as mechanisms underpinning the respiratory thermal responses identified (Chapters 3, 4 and 5). In addition, DNA could be extracted to identify the species present in the semi-natural wood decay communities, to determine any relationship between the community composition, species diversity and the respiratory thermal response (Chapter 5).

Future research should continue to focus on semi-natural wood decomposing communities to improve our understanding of whether the no change in temperature sensitivity of respiration overall continues to apply in a wider range of complex and diverse fungal systems. Wood decay communities from different woodland ecosystems and at different stages of decomposition could be explored. Gene categories or genomic attributes could be investigated to see if they can predict the temperature sensitivities of wood decay fungal species and communities. Furthermore, the respiratory thermal responses could be measured under changing temperature conditions to represent the widely fluctuating temperatures fungi experience over diurnal cycles in temperate woodlands. Our understanding of the physiology of basidiomycetes and wood decomposing communities should be incorporated into microbial models that help to predict the changes in respiration of whole soil microbial communities with warming, and overall global C-cycle feedbacks.

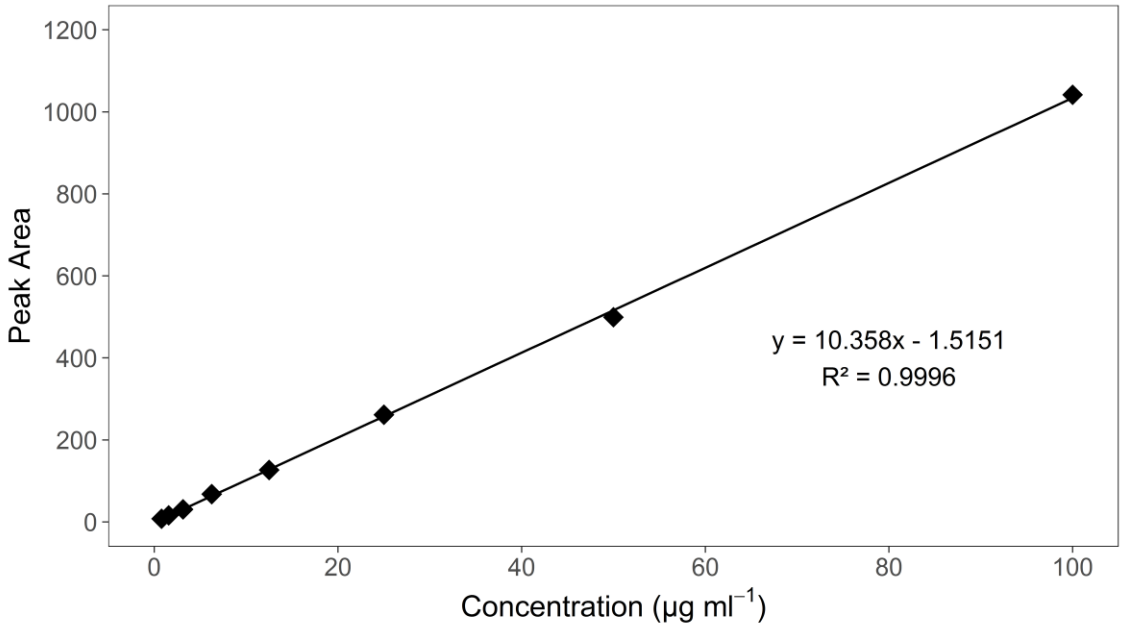
6.9 Implications and Conclusions

Investigating the respiratory thermal response of basidiomycetes decomposing wood and more diverse wood decomposing communities has improved our inferences of respiratory responses in natural systems with warming. Basidiomycete species, particularly early secondary colonisers, during the early stages of colonisation and decomposition when often growing alone, and in stable communities when occupying distinct territories or decay columns in wood (Boddy *et al.*, 2017), are likely to produce enhancing responses that increase their respiration rate with warming. Greater respiration rates may occur through increased mass-specific respiration, fungal growth and biomass production. As earlier colonising species affect the colonisation success of later arriving species, the respiratory thermal response of species will influence the community development (Boddy *et al.*, 2017). Species that show enhancing responses in particular will alter the timings and trajectory of the species that comprise the community. Within wood decomposing communities, competitive interspecific interactions will more often cause no change in the temperature sensitivity of respiration. However, the highly variable respiratory responses to temperature shown by interacting wood decay fungi indicate how community-specific responses will be, and with some clear evidence of enhancing responses, some

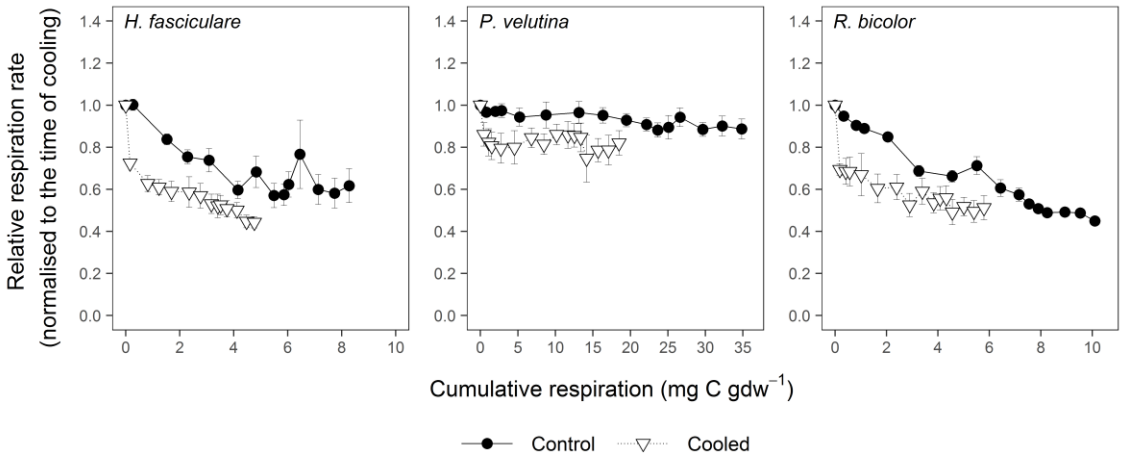
increases in respiration during wood decomposition will occur with warming. Furthermore, the combative ability of wood decay fungi, interaction progression and outcomes change with temperature (Hiscox *et al.*, 2016a). At higher temperatures, early secondary colonisers (*T. versicolor, B. adusta*) are able to outcompete usually later colonising cord-forming species (*H. fasciculare, P. velutina*) (Hiscox *et al.*, 2016a). Therefore, early secondary colonisers are likely to become more combative and dominant in communities, increasing the time and volume of wood they decompose, with warming. This, in turn, will likely increase the frequency of enhancing responses and thus respiration rates with rising global temperatures. Additionally, as interspecific interactions resolve quicker with warming (Hiscox *et al.*, 2016a), enhancing responses of individual species in stable communities will likely become more important. Overall, the species present in communities will most likely determine the direction and strength of respiratory responses to temperature, and thus the overall temperature sensitivity of respiration of wood decay communities.

There was minimal evidence for compensatory responses that could reduce the effects of warming on wood decomposition, instead, there was more evidence of enhancing responses that could increase the effects of warming on wood decomposition. As basidiomycetes and wood decay communities on the forest floor experience highly variable thermal environments, their limited ability to show compensatory responses suggests other saprotrophic fungi in deeper soil layers and in more stable thermal regimes are not likely to produce compensatory responses. The mechanism underlying the decline in respiration of soil microbial communities in long-term field warming experiments is unlikely to be driven by saprotrophic fungi down-regulating their respiration rates, and rather, is more likely a result of indirect effects of temperature, such as substrate depletion. Overall, enhancing responses that will increase respiration rates will reduce the role of temperate woodlands to act as C sinks. Rather than compensatory thermal responses weakening positive feedbacks between warming and wood carbon loss, there remains the potential for a greater positive feedback to climate change through increased wood decomposition with warming.

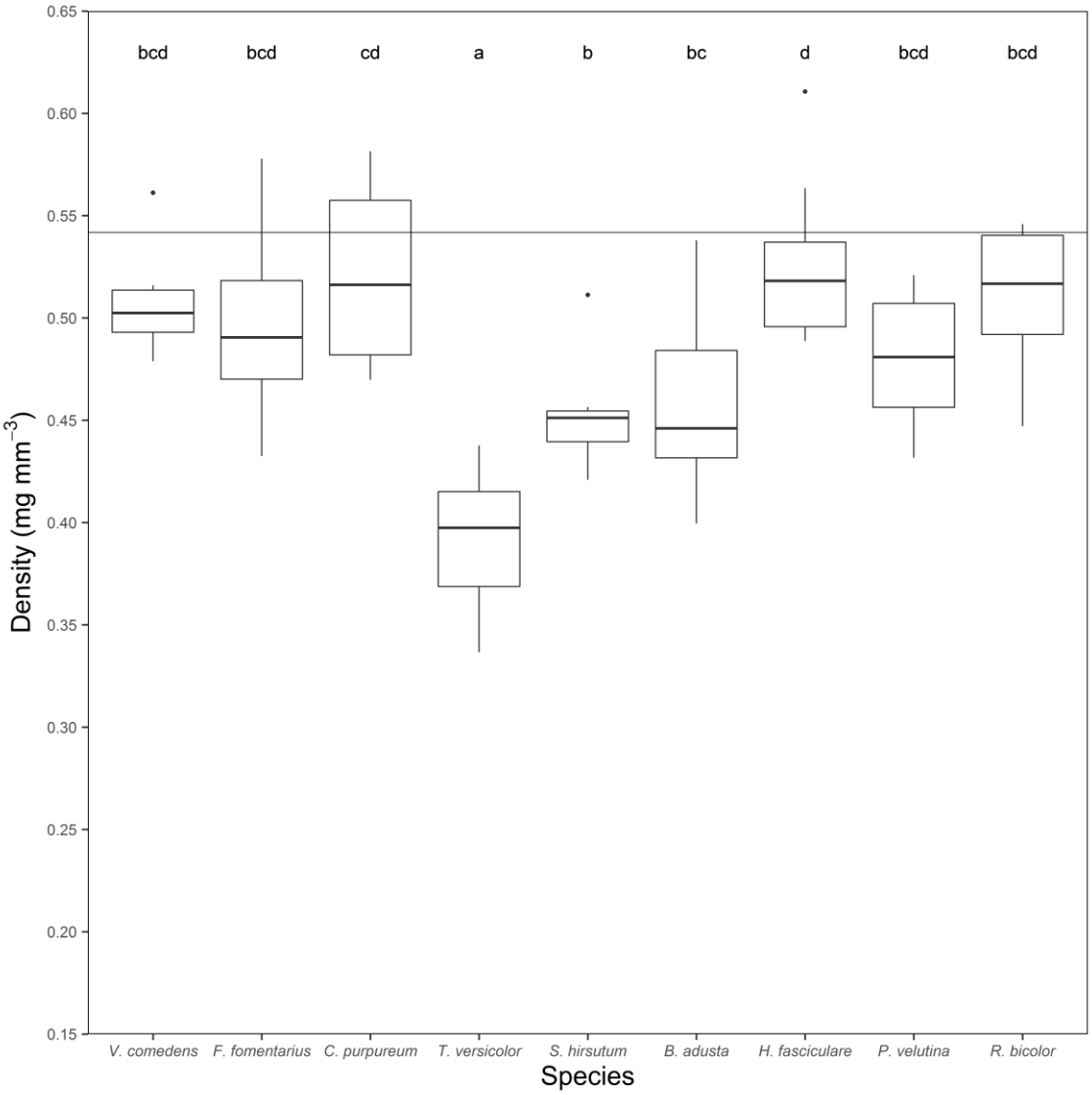
Appendix 1: Chapter 3 supplementary material



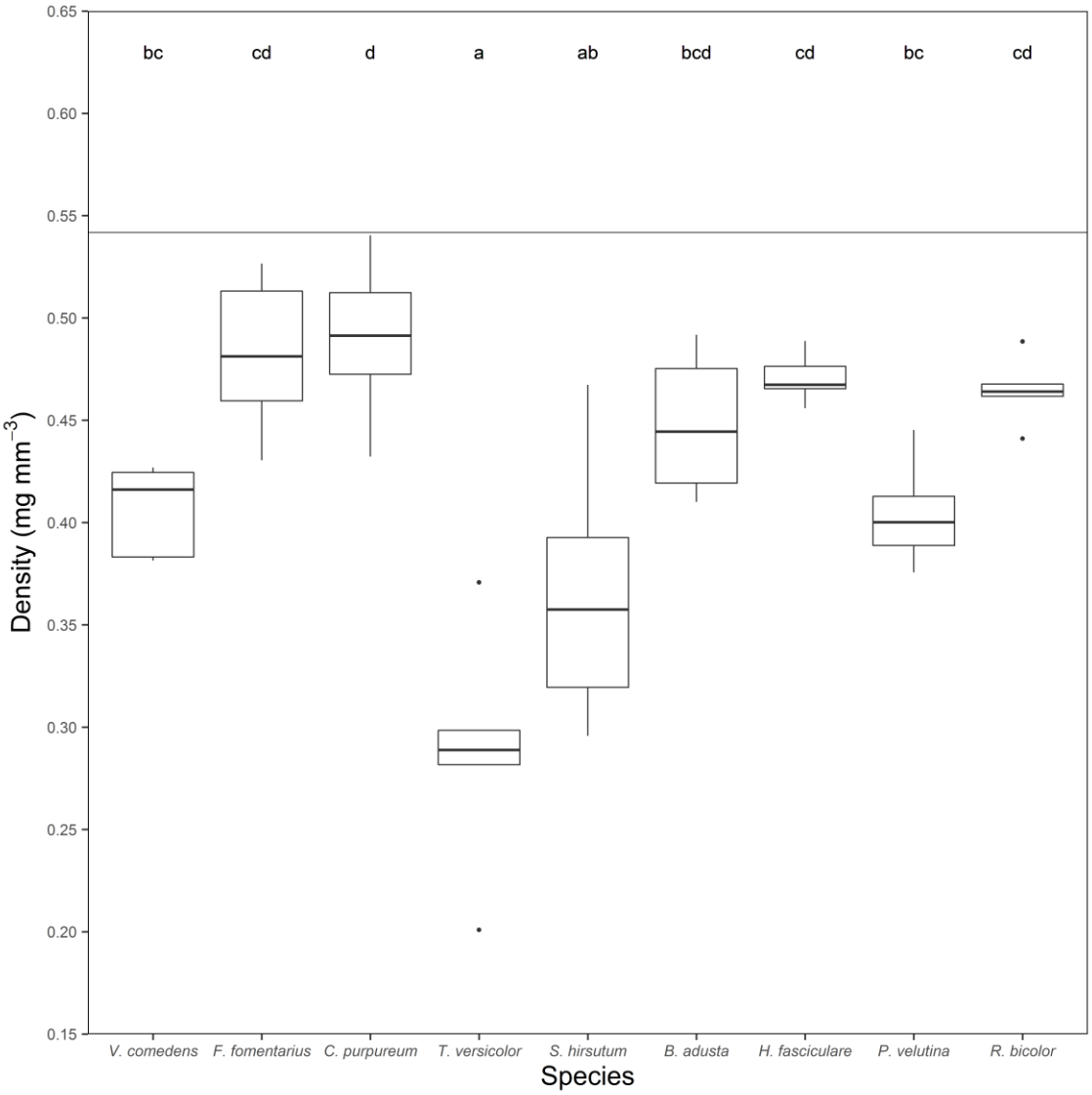
Supplementary Figure 3.1 Calibration curve produced to calculate ergosterol concentrations (µg ml^{-1}), using ergosterol standards ranging from 100 µg ml^{-1} to 0.78 µg ml^{-1} that were run through the extraction protocol.



Supplementary Figure 3.2 Relative respiration rate of control and cooled treatments during 90 d of incubation following cooling, of *H. fasciculare*, *P. velutina* and *R. bicolor* (mean ± SE of the mean, n = 5), normalised to 1 d after cooling. Cumulative respiration was calculated from the time of cooling (151 d), at the start of 90 d incubation.



Supplementary Figure 3.3 Wood block density after pre-colonisation of wood blocks with each species (108 d). Horizontal line shows the mean density of uncolonised wood blocks (n = 10) at the start of the experiment (0 d). Different letters indicate significant (P < 0.05) differences in wood block density from Tukey's pairwise comparisons. Boxes represent the interquartile range with a horizontal line for the median and the whiskers represent 1.5*interquartile range or the maximum and minimum point. Dots represent points outside the extent of 1.5*interquartile range.



Supplementary Figure 3.4 Wood block density of samples in the control treatment at the end of incubation (241 d) of each species. Horizontal line shows the mean density of uncolonised wood blocks (n = 10) at the start of the experiment (0 d). Different letters indicate significant (P < 0.05) differences in wood block density from Tukey's pairwise comparisons. Boxes represent the interquartile range with a horizontal line for the median and the whiskers represent 1.5*interquartile range or the maximum and minimum point. Dots represent points outside the extent of 1.5*interquartile range.

Supplementary Table 3.1 Overall effect (one-way ANOVA: $F_{\text{degrees of freedom}}$ and *P* value) of control, cooled and rewarmed treatments on respiration rates at the final measurement of pre-incubation (143 d), prior to cooling (151 d), of each species.

Species	**$F_{2, 12}$**	***P***
V. comedens	0.055	0.947
F. fomentarius	0.049	0.952
C. purpureum	<0.001	1.000
T. versicolor	0.069	0.934
S. hirsutum	0.100	0.906
B. adusta	0.027	0.974
H. fasciculare	0.010	0.991
P. velutina	0.090	0.914
R. bicolor	0.034	0.967

P > 0.05 indicates a non-significant difference.

Supplementary Table 3.2 Pairwise comparisons of ergosterol content between species and temperature treatment overall, and between the same temperature treatment across species.

Pairwise comparison	Difference	Lower 95% confidence interval	Upper 95% confidence interval	*P*
Species				
Ff-Vc	-6.645	-14.285	0.995	0.144
Cp-Vc	**12.262**	**4.622**	**19.902**	**<0.001**
Tv-Vc	**-15.701**	**-23.341**	**-8.061**	**<0.001**
Sh-Vc	**27.619**	**19.979**	**35.259**	**<0.001**
Ba-Vc	**-15.724**	**-23.364**	**-8.084**	**<0.001**
Hf-Vc	4.325	-3.315	11.965	0.693
Pv-Vc	-6.208	-13.848	1.432	0.213
Rb-Vc	**11.385**	**3.745**	**19.025**	**<0.001**
Cp-Ff	**18.907**	**11.267**	**26.547**	**<0.001**
Tv-Ff	**-9.056**	**-16.696**	**-1.416**	**0.008**
Sh-Ff	**34.264**	**26.624**	**41.904**	**<0.001**
Ba-Ff	**-9.079**	**-16.719**	**-1.439**	**0.008**
Hf-Ff	**10.970**	**3.330**	**18.610**	**<0.001**
Pv-Ff	0.437	-7.203	8.077	1.000
Rb-Ff	**18.031**	**10.390**	**25.671**	**<0.001**
Tv-Cp	**-27.963**	**-35.603**	**-20.323**	**<0.001**
Sh-Cp	**15.357**	**7.717**	**22.997**	**<0.001**
Ba-Cp	**-27.986**	**-35.626**	**-20.346**	**<0.001**
Hf-Cp	**-7.937**	**-15.577**	**-0.297**	**0.035**
Pv-Cp	**-18.470**	**-26.110**	**-10.830**	**<0.001**
Rb-Cp	-0.877	-8.517	6.763	1.000
Sh-Tv	**43.320**	**35.680**	**50.960**	**<0.001**
Ba-Tv	-0.023	-7.663	7.617	1.000
Hf-Tv	**20.026**	**12.386**	**27.666**	**<0.001**
Pv-Tv	9.493	1.853	17.133	0.004
Rb-Tv	**27.086**	**19.446**	**34.726**	**<0.001**
Ba-Sh	**-43.343**	**-50.983**	**-35.703**	**<0.001**
Hf-Sh	**-23.294**	**-30.934**	**-15.654**	**<0.001**
Pv-Sh	**-33.827**	**-41.467**	**-26.187**	**<0.001**
Rb-Sh	**-16.234**	**-23.874**	**-8.594**	**<0.001**
Hf-Ba	**20.049**	**12.409**	**27.689**	**<0.001**
Pv-Ba	**9.516**	**1.876**	**17.156**	**0.004**
Rb-Ba	**27.109**	**19.469**	**34.750**	**<0.001**
Pv-Hf	**-10.533**	**-18.173**	**-2.893**	**0.001**
Rb-Hf	7.060	-0.580	14.700	0.095
Rb-Pv	**17.593**	**9.953**	**25.233**	**<0.001**
Temperature treatment				
Control-Pre-cooling	**4.612**	**0.409**	**8.816**	**0.025**
Cooled-Pre-cooling	-0.369	-4.572	3.835	0.996
Rewarmed-Pre-cooling	2.923	-1.281	7.126	0.274
Cooled-Control	**-4.981**	**-9.184**	**-0.777**	**0.013**

Rewarmed-Control	-1.689	-5.893	2.514	0.724
Rewarmed-Cooled	3.291	-0.912	7.495	0.180
Species:Temperature treatment				
Ff:Pre-cooling-Vc:Pre-cooling	-0.896	-19.915	18.123	1.000
Cp:Pre-cooling-Vc:Pre-cooling	11.377	-7.642	30.395	0.916
Tv:Pre-cooling-Vc:Pre-cooling	-15.625	-34.643	3.394	0.317
Sh:Pre-cooling-Vc:Pre-cooling	**30.014**	**10.995**	**49.032**	**<0.001**
Ba:Pre-cooling-Vc:Pre-cooling	-5.793	-24.811	13.226	1.000
Hf:Pre-cooling-Vc:Pre-cooling	4.916	-14.103	23.935	1.000
Pv:Pre-cooling-Vc:Pre-cooling	-5.121	-24.139	13.898	1.000
Rb:Pre-cooling-Vc:Pre-cooling	11.307	-7.712	30.326	0.921
Cp:Pre-cooling-Ff:Pre-cooling	12.273	-6.746	31.291	0.827
Tv:Pre-cooling-Ff:Pre-cooling	-14.729	-33.747	4.290	0.449
Sh:Pre-cooling-Ff:Pre-cooling	**30.909**	**11.891**	**49.928**	**<0.001**
Ba:Pre-cooling-Ff:Pre-cooling	-4.897	-23.915	14.122	1.000
Hf:Pre-cooling-Ff:Pre-cooling	5.812	-13.207	24.831	1.000
Pv:Pre-cooling-Ff:Pre-cooling	-4.225	-23.244	14.794	1.000
Rb:Pre-cooling-Ff:Pre-cooling	12.203	-6.816	31.222	0.835
Tv:Pre-cooling-Cp:Pre-cooling	**-27.001**	**-46.020**	**-7.983**	**<0.001**
Sh:Pre-cooling-Cp:Pre-cooling	18.637	-0.382	37.656	0.064
Ba:Pre-cooling-Cp:Pre-cooling	-17.169	-36.188	1.849	0.151
Hf:Pre-cooling-Cp:Pre-cooling	-6.461	-25.479	12.558	1.000
Pv:Pre-cooling-Cp:Pre-cooling	-16.497	-35.516	2.521	0.213
Rb:Pre-cooling-Cp:Pre-cooling	-0.070	-19.088	18.949	1.000
Sh:Pre-cooling-Tv:Pre-cooling	**45.638**	**26.619**	**64.657**	**<0.001**
Ba:Pre-cooling-Tv:Pre-cooling	9.832	-9.187	28.851	0.987
Hf:Pre-cooling-Tv:Pre-cooling	**20.541**	**1.522**	**39.559**	**0.017**
Pv:Pre-cooling-Tv:Pre-cooling	10.504	-8.515	29.523	0.967
Rb:Pre-cooling-Tv:Pre-cooling	**26.932**	**7.913**	**45.951**	**<0.001**
Ba:Pre-cooling-Sh:Pre-cooling	**-35.806**	**-54.825**	**-16.787**	**<0.001**
Hf:Pre-cooling-Sh:Pre-cooling	**-25.098**	**-44.116**	**-6.079**	**<0.001**
Pv:Pre-cooling-Sh:Pre-cooling	**-35.134**	**-54.153**	**-16.115**	**<0.001**
Rb:Pre-cooling-Sh:Pre-cooling	-18.706	-37.725	0.312	0.061
Hf:Pre-cooling-Ba:Pre-cooling	10.709	-8.310	29.727	0.958
Pv:Pre-cooling-Ba:Pre-cooling	0.672	-18.347	19.691	1.000
Rb:Pre-cooling-Ba:Pre-cooling	17.100	-1.919	36.119	0.156
Pv:Pre-cooling-Hf:Pre-cooling	-10.037	-29.055	8.982	0.982
Rb:Pre-cooling-Hf:Pre-cooling	6.391	-12.628	25.410	1.000
Rb:Pre-cooling-Pv:Pre-cooling	16.428	-2.591	35.447	0.220
Ff:Control-Vc:Control	-11.666	-30.685	7.352	0.891
Cp:Control-Vc:Control	8.936	-10.083	27.955	0.997
Tv:Control-Vc:Control	-13.825	-32.844	5.194	0.597
Sh:Control-Vc:Control	**30.133**	**11.114**	**49.152**	**<0.001**
Ba:Control-Vc:Control	**-20.093**	**-39.111**	**-1.074**	**0.024**
Hf:Control-Vc:Control	-3.170	-22.189	15.848	1.000
Pv:Control-Vc:Control	-6.947	-25.966	12.071	1.000
Rb:Control-Vc:Control	5.812	-13.207	24.831	1.000
Cp:Control-Ff:Control	**20.602**	**1.584**	**39.621**	**0.017**
Tv:Control-Ff:Control	-2.159	-21.177	16.860	1.000
Sh:Control-Ff:Control	**41.800**	**22.781**	**60.818**	**<0.001**

Ba:Control-Ff:Control	-8.426	-27.445	10.592	0.999
Hf:Control-Ff:Control	8.496	-10.523	27.515	0.999
Pv:Control-Ff:Control	4.719	-14.300	23.738	1.000
Rb:Control-Ff:Control	17.478	-1.540	36.497	0.127
Tv:Control-Cp:Control	**-22.761**	**-41.780**	**-3.742**	**0.003**
Sh:Control-Cp:Control	**21.197**	**2.178**	**40.216**	**0.011**
Ba:Control-Cp:Control	**-29.029**	**-48.048**	**-10.010**	**<0.001**
Hf:Control-Cp:Control	-12.107	-31.125	6.912	0.846
Pv:Control-Cp:Control	-15.883	-34.902	3.135	0.283
Rb:Control-Cp:Control	-3.124	-22.143	15.895	1.000
Sh:Control-Tv:Control	**43.958**	**24.940**	**62.977**	**<0.001**
Ba:Control-Tv:Control	-6.268	-25.286	12.751	1.000
Hf:Control-Tv:Control	10.655	-8.364	29.673	0.960
Pv:Control-Tv:Control	6.878	-12.141	25.897	1.000
Rb:Control-Tv:Control	**19.637**	**0.618**	**38.656**	**0.033**
Ba:Control-Sh:Control	**-50.226**	**-69.245**	**-31.207**	**<0.001**
Hf:Control-Sh:Control	**-33.304**	**-52.323**	**-14.285**	**<0.001**
Pv:Control-Sh:Control	**-37.081**	**-56.099**	**-18.062**	**<0.001**
Rb:Control-Sh:Control	**-24.321**	**-43.340**	**-5.303**	**0.001**
Hf:Control-Ba:Control	16.922	-2.097	35.941	0.172
Pv:Control-Ba:Control	13.145	-5.873	32.164	0.706
Rb:Control-Ba:Control	**25.905**	**6.886**	**44.923**	**<0.001**
Pv:Control-Hf:Control	-3.777	-22.796	15.242	1.000
Rb:Control-Hf:Control	8.982	-10.036	28.001	0.997
Rb:Control-Pv:Control	12.759	-6.260	31.778	0.763
Ff:Cooled-Vc:Cooled	-3.047	-22.066	15.972	1.000
Cp:Cooled-Vc:Cooled	11.446	-7.573	30.465	0.911
Tv:Cooled-Vc:Cooled	-13.165	-32.183	5.854	0.703
Sh:Cooled-Vc:Cooled	**26.071**	**7.052**	**45.089**	**<0.001**
Ba:Cooled-Vc:Cooled	-15.099	-34.118	3.919	0.392
Hf:Cooled-Vc:Cooled	14.559	-4.460	33.578	0.477
Pv:Cooled-Vc:Cooled	-6.963	-25.982	12.056	1.000
Rb:Cooled-Vc:Cooled	**22.479**	**3.460**	**41.498**	**0.004**
Cp:Cooled-Ff:Cooled	14.493	-4.526	33.512	0.487
Tv:Cooled-Ff:Cooled	-10.118	-29.137	8.901	0.980
Sh:Cooled-Ff:Cooled	**29.118**	**10.099**	**48.136**	**<0.001**
Ba:Cooled-Ff:Cooled	-12.053	-31.071	6.966	0.852
Hf:Cooled-Ff:Cooled	17.606	-1.413	36.624	0.118
Pv:Cooled-Ff:Cooled	-3.916	-22.935	15.103	1.000
Rb:Cooled-Ff:Cooled	**25.526**	**6.507**	**44.545**	**<0.001**
Tv:Cooled-Cp:Cooled	**-24.611**	**-43.630**	**-5.592**	**0.001**
Sh:Cooled-Cp:Cooled	14.624	-4.394	33.643	0.466
Ba:Cooled-Cp:Cooled	**-26.546**	**-45.564**	**-7.527**	**<0.001**
Hf:Cooled-Cp:Cooled	3.113	-15.906	22.131	1.000
Pv:Cooled-Cp:Cooled	-18.409	-37.428	0.610	0.074
Rb:Cooled-Cp:Cooled	11.033	-7.986	30.052	0.940
Sh:Cooled-Tv:Cooled	**39.235**	**20.217**	**58.254**	**<0.001**
Ba:Cooled-Tv:Cooled	-1.935	-20.954	17.084	1.000
Hf:Cooled-Tv:Cooled	**27.723**	**8.705**	**46.742**	**<0.001**
Pv:Cooled-Tv:Cooled	6.202	-12.817	25.221	1.000
Rb:Cooled-Tv:Cooled	**35.644**	**16.625**	**54.663**	**<0.001**
Ba:Cooled-Sh:Cooled	**-41.170**	**-60.189**	**-22.151**	**<0.001**

Hf:Cooled-Sh:Cooled	-11.512	-30.531	7.507	0.905
Pv:Cooled-Sh:Cooled	**-33.033**	**-52.052**	**-14.015**	**<0.001**
Rb:Cooled-Sh:Cooled	-3.591	-22.610	15.427	1.000
Hf:Cooled-Ba:Cooled	**29.658**	**10.639**	**48.677**	**<0.001**
Pv:Cooled-Ba:Cooled	8.137	-10.882	27.155	0.999
Rb:Cooled-Ba:Cooled	**37.579**	**18.560**	**56.597**	**<0.001**
Pv:Cooled-Hf:Cooled	**-21.522**	**-40.540**	**-2.503**	**0.008**
Rb:Cooled-Hf:Cooled	7.920	-11.098	26.939	1.000
Rb:Cooled-Pv:Cooled	**29.442**	**10.423**	**48.461**	**<0.001**
Ff:Rewarmed-Vc:Rewarmed	-10.971	-29.990	8.048	0.944
Cp:Rewarmed-Vc:Rewarmed	17.289	-1.730	36.308	0.141
Tv:Rewarmed-Vc:Rewarmed	**-20.189**	**-39.208**	**-1.170**	**0.023**
Sh:Rewarmed-Vc:Rewarmed	**24.260**	**5.241**	**43.278**	**0.001**
Ba:Rewarmed-Vc:Rewarmed	**-21.912**	**-40.930**	**-2.893**	**0.006**
Hf:Rewarmed-Vc:Rewarmed	0.996	-18.022	20.015	1.000
Pv:Rewarmed-Vc:Rewarmed	-5.800	-24.819	13.218	1.000
Rb:Rewarmed-Vc:Rewarmed	5.943	-13.076	24.962	1.000
Cp:Rewarmed-Ff:Rewarmed	**28.260**	**9.242**	**47.279**	**<0.001**
Tv:Rewarmed-Ff:Rewarmed	-9.218	-28.237	9.801	0.995
Sh:Rewarmed-Ff:Rewarmed	**35.231**	**16.212**	**54.250**	**<0.001**
Ba:Rewarmed-Ff:Rewarmed	-10.940	-29.959	8.078	0.946
Hf:Rewarmed-Ff:Rewarmed	11.968	-7.051	30.986	0.861
Pv:Rewarmed-Ff:Rewarmed	5.171	-13.848	24.190	1.000
Rb:Rewarmed-Ff:Rewarmed	16.914	-2.104	35.933	0.172
Tv:Rewarmed-Cp:Rewarmed	**-37.478**	**-56.497**	**-18.460**	**<0.001**
Sh:Rewarmed-Cp:Rewarmed	6.970	-12.048	25.989	1.000
Ba:Rewarmed-Cp:Rewarmed	**-39.201**	**-58.219**	**-20.182**	**<0.001**
Hf:Rewarmed-Cp:Rewarmed	-16.293	-35.311	2.726	0.235
Pv:Rewarmed-Cp:Rewarmed	**-23.089**	**-42.108**	**-4.071**	**0.002**
Rb:Rewarmed-Cp:Rewarmed	-11.346	-30.365	7.673	0.919
Sh:Rewarmed-Tv:Rewarmed	**44.449**	**25.430**	**63.468**	**<0.001**
Ba:Rewarmed-Tv:Rewarmed	-1.722	-20.741	17.296	1.000
Hf:Rewarmed-Tv:Rewarmed	**21.186**	**2.167**	**40.204**	**0.011**
Pv:Rewarmed-Tv:Rewarmed	14.389	-4.630	33.408	0.504
Rb:Rewarmed-Tv:Rewarmed	**26.132**	**7.114**	**45.151**	**<0.001**
Ba:Rewarmed-Sh:Rewarmed	**-46.171**	**-65.190**	**-27.152**	**<0.001**
Hf:Rewarmed-Sh:Rewarmed	**-23.263**	**-42.282**	**-4.244**	**0.002**
Pv:Rewarmed-Sh:Rewarmed	**-30.060**	**-49.079**	**-11.041**	**<0.001**
Rb:Rewarmed-Sh:Rewarmed	-18.316	-37.335	0.702	0.078
Hf:Rewarmed-Ba:Rewarmed	**22.908**	**3.889**	**41.927**	**0.003**
Pv:Rewarmed-Ba:Rewarmed	16.111	-2.908	35.130	0.256
Rb:Rewarmed-Ba:Rewarmed	**27.855**	**8.836**	**46.874**	**<0.001**
Pv:Rewarmed-Hf:Rewarmed	-6.797	-25.815	12.222	1.000
Rb:Rewarmed-Hf:Rewarmed	4.947	-14.072	23.966	1.000
Rb:Rewarmed-Pv:Rewarmed	11.744	-7.275	30.762	0.884

Comparisons are derived from two-way ANOVA. P value adjustment: Tukey method for comparing a family of 9 estimates for species, 4 estimates for temperature treatment and 36 estimates for temperature treatment across species (only comparisons of the same temperature treatment across species are shown). Significant differences (P < 0.05) are shown in bold. Vc: V. comedens, Ff: F. fomentarius, Cp: C. purpureum, Tv: T. versicolor, Sh: S. hirsutum, Ba: B. adusta, Hf: H. fasciculare, Pv: P. velutina, Rb: R. bicolor.

Supplementary Table 3.3 Pairwise comparisons of ergosterol content between temperature treatments of each species.

Species	Pairwise comparison	Difference	Lower 95% confidence interval	Upper 95% confidence interval	*P*
Vc	Control-Pre-cooling	9.168	-3.683	22.018	0.214
	Cooled-Pre-cooling	-1.047	-13.897	11.804	0.995
	Rewarmed-Pre-cooling	7.430	-5.421	20.281	0.378
	Cooled-Control	-10.214	-23.065	2.636	0.146
	Rewarmed-Control	-1.738	-14.588	11.113	0.980
	Rewarmed-Cooled	8.477	-4.374	21.327	0.272
Ff	Control-Pre-cooling	-1.603	-8.128	4.923	0.895
	Cooled-Pre-cooling	-3.198	-9.723	3.328	0.516
	Rewarmed-Pre-cooling	-2.645	-9.171	3.880	0.659
	Cooled-Control	-1.595	-8.120	4.931	0.896
	Rewarmed-Control	-1.043	-7.568	5.483	0.967
	Rewarmed-Cooled	0.552	-5.973	7.078	0.995
Cp	Control-Pre-cooling	6.727	-10.283	23.738	0.676
	Cooled-Pre-cooling	-0.977	-17.988	16.034	0.998
	Rewarmed-Pre-cooling	13.342	-3.668	30.353	0.154
	Cooled-Control	-7.704	-24.715	9.306	0.579
	Rewarmed-Control	6.615	-10.395	23.626	0.687
	Rewarmed-Cooled	14.319	-2.691	31.330	0.116
Tv	**Control-Pre-cooling**	**10.967**	**1.125**	**20.810**	**0.026**
	Cooled-Pre-cooling	1.413	-8.429	11.256	0.976
	Rewarmed-Pre-cooling	2.865	-6.977	12.708	0.838
	Cooled-Control	-9.554	-19.397	0.289	0.059
	Rewarmed-Control	-8.102	-17.945	1.741	0.127
	Rewarmed-Cooled	1.452	-8.391	11.295	0.974
Sh	Control-Pre-cooling	9.288	-14.586	33.161	0.687
	Cooled-Pre-cooling	-4.989	-28.863	18.884	0.931
	Rewarmed-Pre-cooling	1.676	-22.197	25.549	0.997
	Cooled-Control	-14.277	-38.150	9.596	0.350
	Rewarmed-Control	-7.612	-31.485	16.262	0.799
	Rewarmed-Cooled	6.665	-17.208	30.539	0.854
Ba	Control-Pre-cooling	-5.132	-15.457	5.193	0.504
	Cooled-Pre-cooling	**-10.353**	**-20.678**	**-0.029**	**0.049**
	Rewarmed-Pre-cooling	-8.689	-19.014	1.636	0.116
	Cooled-Control	-5.221	-15.546	5.104	0.490
	Rewarmed-Control	-3.557	-13.881	6.768	0.760
	Rewarmed-Cooled	1.664	-8.660	11.989	0.966
Hf	Control-Pre-cooling	1.081	-13.438	15.601	0.996
	Cooled-Pre-cooling	8.596	-5.923	23.116	0.359
	Rewarmed-Pre-cooling	3.510	-11.009	18.030	0.899
	Cooled-Control	7.515	-7.005	22.035	0.471
	Rewarmed-Control	2.429	-12.091	16.949	0.963
	Rewarmed-Cooled	-5.086	-19.606	9.434	0.750
Pv	Control-Pre-cooling	7.341	-4.164	18.846	0.298
	Cooled-Pre-cooling	-2.889	-14.394	8.617	0.888
	Rewarmed-Pre-cooling	6.750	-4.755	18.256	0.366
	Cooled-Control	-10.230	-21.735	1.275	0.091

	Rewarmed-Control	-0.591	-12.096	10.914	0.999
	Rewarmed-Cooled	9.639	-1.866	21.144	0.118
Rb	Control-Pre-cooling	3.673	-7.295	14.640	0.775
	Cooled-Pre-cooling	10.126	-0.842	21.093	0.076
	Rewarmed-Pre-cooling	2.066	-8.902	13.034	0.948
	Cooled-Control	6.453	-4.515	17.421	0.364
	Rewarmed-Control	-1.606	-12.574	9.361	0.974
	Rewarmed-Cooled	-8.059	-19.027	2.908	0.194

Comparisons are derived from one-way ANOVA models for each species. P value adjustment: Tukey method for comparing a family of 4 estimates. Significant differences (P < 0.05) are shown in bold. Vc: V. comedens, Ff: F. fomentarius, Cp: C. purpureum, Tv: T. versicolor, Sh: S. hirsutum, Ba: B. adusta, Hf: H. fasciculare, Pv: P. velutina, Rb: R. bicolor.

Supplementary Table 3.4 Pairwise comparisons of wood block densities between species, after pre-colonisation of wood blocks with each species (108 d).

Pairwise comparison	Difference	Lower 95% confidence interval	Upper 95% confidence interval	*P*
Ff-Vc	-0.012	-0.072	0.049	0.999
Cp-Vc	0.013	-0.049	0.076	0.999
Tv-Vc	**-0.117**	**-0.177**	**-0.056**	**<0.001**
Sh-Vc	-0.055	-0.115	0.006	0.105
Ba-Vc	-0.049	-0.114	0.017	0.307
Hf-Vc	0.018	-0.039	0.075	0.983
Pv-Vc	-0.027	-0.087	0.033	0.879
Rb-Vc	0.004	-0.057	0.064	1.000
Cp-Ff	0.025	-0.037	0.088	0.930
Tv-Ff	**-0.105**	**-0.165**	**-0.045**	**<0.001**
Sh-Ff	-0.043	-0.104	0.017	0.360
Ba-Ff	-0.037	-0.102	0.028	0.669
Hf-Ff	0.030	-0.028	0.087	0.765
Pv-Ff	-0.015	-0.076	0.045	0.996
Rb-Ff	0.015	-0.045	0.076	0.996
Tv-Cp	**-0.130**	**-0.193**	**-0.068**	**<0.001**
Sh-Cp	**-0.068**	**-0.131**	**-0.006**	**0.022**
Ba-Cp	-0.062	-0.129	0.005	0.093
Hf-Cp	0.005	-0.055	0.064	1.000
Pv-Cp	-0.041	-0.103	0.022	0.494
Rb-Cp	-0.010	-0.072	0.053	1.000
Sh-Tv	**0.062**	**0.002**	**0.122**	**0.040**
Ba-Tv	**0.068**	**0.003**	**0.133**	**0.034**
Hf-Tv	**0.135**	**0.078**	**0.192**	**<0.001**
Pv-Tv	**0.090**	**0.029**	**0.150**	**<0.001**
Rb-Tv	**0.120**	**0.060**	**0.181**	**<0.001**
Ba-Sh	0.006	-0.059	0.071	1.000
Hf-Sh	**0.073**	**0.016**	**0.130**	**0.004**
Pv-Sh	0.028	-0.033	0.088	0.861
Rb-Sh	0.058	-0.002	0.119	0.066
Hf-Ba	**0.067**	**0.004**	**0.129**	**0.028**
Pv-Ba	0.022	-0.044	0.087	0.978
Rb-Ba	0.052	-0.013	0.117	0.221
Pv-Hf	-0.045	-0.102	0.012	0.238
Rb-Hf	-0.014	-0.072	0.043	0.996
Rb-Pv	0.031	-0.030	0.091	0.785

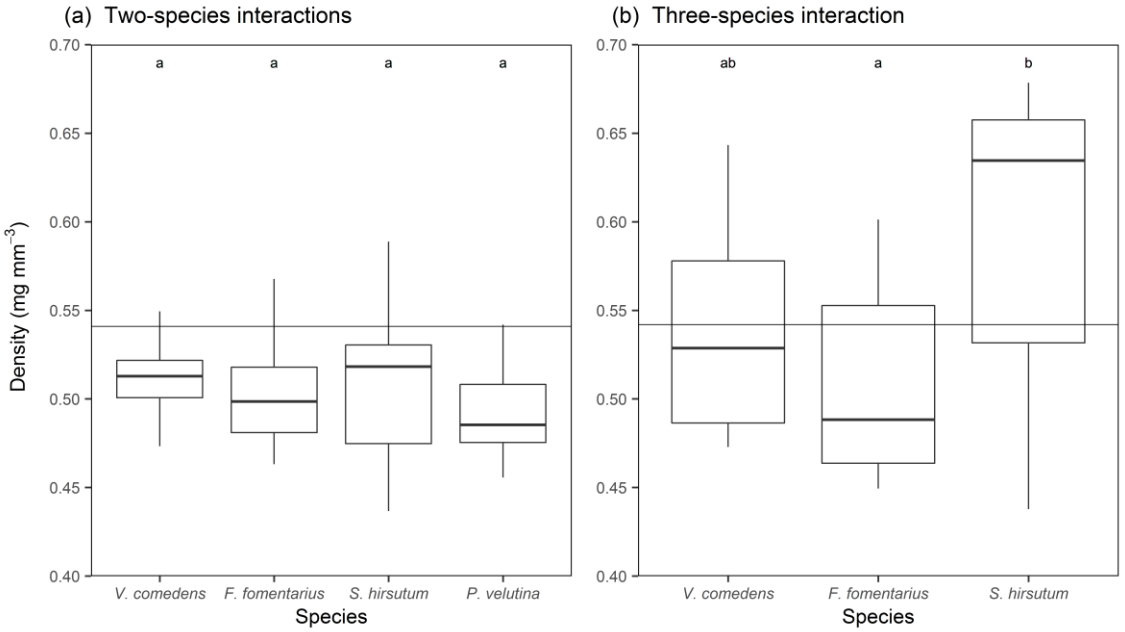
Comparisons are derived from one-way ANOVA. P value adjustment: Tukey method for comparing a family of 9 estimates. Significant differences (P < 0.05) are shown in bold. Vc: V. comedens, Ff: F. fomentarius, Cp: C. purpureum, Tv: T. versicolor, Sh: S. hirsutum, Ba: B. adusta, Hf: H. fasciculare, Pv: P. velutina, Rb: R. bicolor.

Supplementary Table 3.5 Pairwise comparisons of wood block densities between species, of samples in control treatment at the end of incubation (241 d).

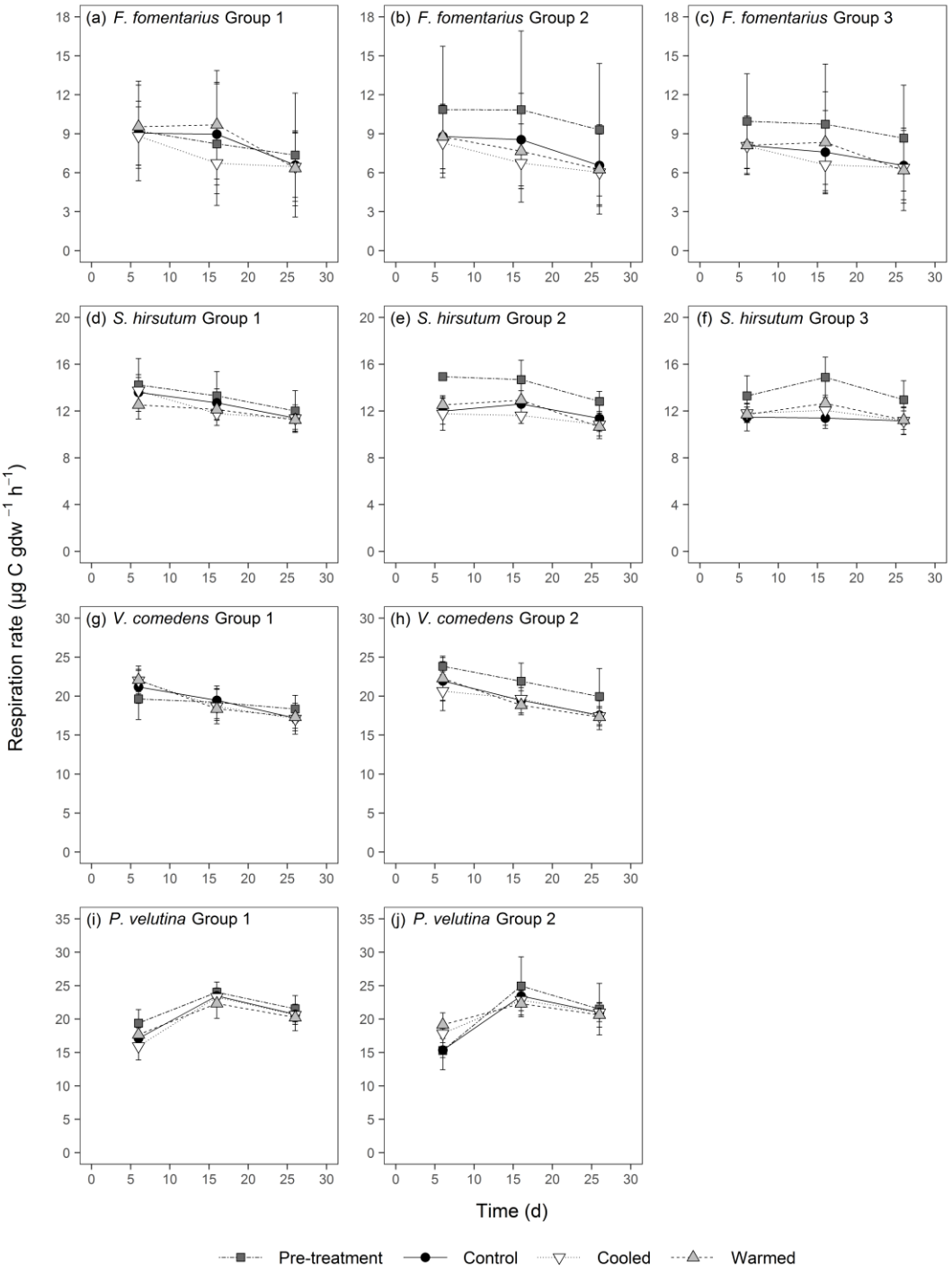
Pairwise comparison	Difference	Lower 95% confidence interval	Upper 95% confidence interval	*P*
Ff-Vc	0.076	-0.007	0.159	0.097
Cp-Vc	**0.083**	**0.000**	**0.166**	**0.049**
Tv-Vc	**-0.118**	**-0.201**	**-0.035**	**0.001**
Sh-Vc	-0.040	-0.123	0.043	0.807
Ba-Vc	0.042	-0.041	0.125	0.766
Hf-Vc	0.064	-0.019	0.147	0.241
Pv-Vc	-0.002	-0.085	0.081	1.000
Rb-Vc	0.058	-0.025	0.141	0.363
Cp-Ff	0.008	-0.075	0.091	1.000
Tv-Ff	**-0.194**	**-0.277**	**-0.111**	**<0.001**
Sh-Ff	**-0.116**	**-0.199**	**-0.033**	**0.002**
Ba-Ff	-0.034	-0.117	0.049	0.909
Hf-Ff	-0.011	-0.094	0.072	1.000
Pv-Ff	-0.078	-0.161	0.005	0.082
Rb-Ff	-0.018	-0.101	0.065	0.999
Tv-Cp	**-0.202**	**-0.285**	**-0.119**	**<0.001**
Sh-Cp	**-0.123**	**-0.206**	**-0.040**	**0.001**
Ba-Cp	-0.042	-0.125	0.041	0.771
Hf-Cp	-0.019	-0.102	0.064	0.997
Pv-Cp	**-0.085**	**-0.168**	**-0.002**	**0.040**
Rb-Cp	-0.025	-0.108	0.058	0.984
Sh-Tv	0.078	-0.005	0.161	0.077
Ba-Tv	**0.160**	**0.077**	**0.243**	**<0.001**
Hf-Tv	**0.183**	**0.100**	**0.266**	**<0.001**
Pv-Tv	**0.116**	**0.033**	**0.199**	**0.001**
Rb-Tv	**0.176**	**0.093**	**0.259**	**<0.001**
Ba-Sh	0.082	-0.001	0.165	0.057
Hf-Sh	**0.104**	**0.021**	**0.187**	**0.006**
Pv-Sh	0.038	-0.045	0.121	0.844
Rb-Sh	**0.098**	**0.015**	**0.181**	**0.011**
Hf-Ba	0.023	-0.060	0.106	0.992
Pv-Ba	-0.044	-0.127	0.039	0.723
Rb-Ba	0.016	-0.067	0.099	0.999
Pv-Hf	-0.066	-0.149	0.017	0.210
Rb-Hf	-0.006	-0.089	0.077	1.000
Rb-Pv	0.060	-0.023	0.143	0.322

Comparisons are derived from one-way ANOVA. P value adjustment: Tukey method for comparing a family of 9 estimates. Significant differences (P < 0.05) are shown in bold. Vc: V. comedens, Ff: F. fomentarius, Cp: C. purpureum, Tv: T. versicolor, Sh: S. hirsutum, Ba: B. adusta, Hf: H. fasciculare, Pv: P. velutina, Rb: R. bicolor.

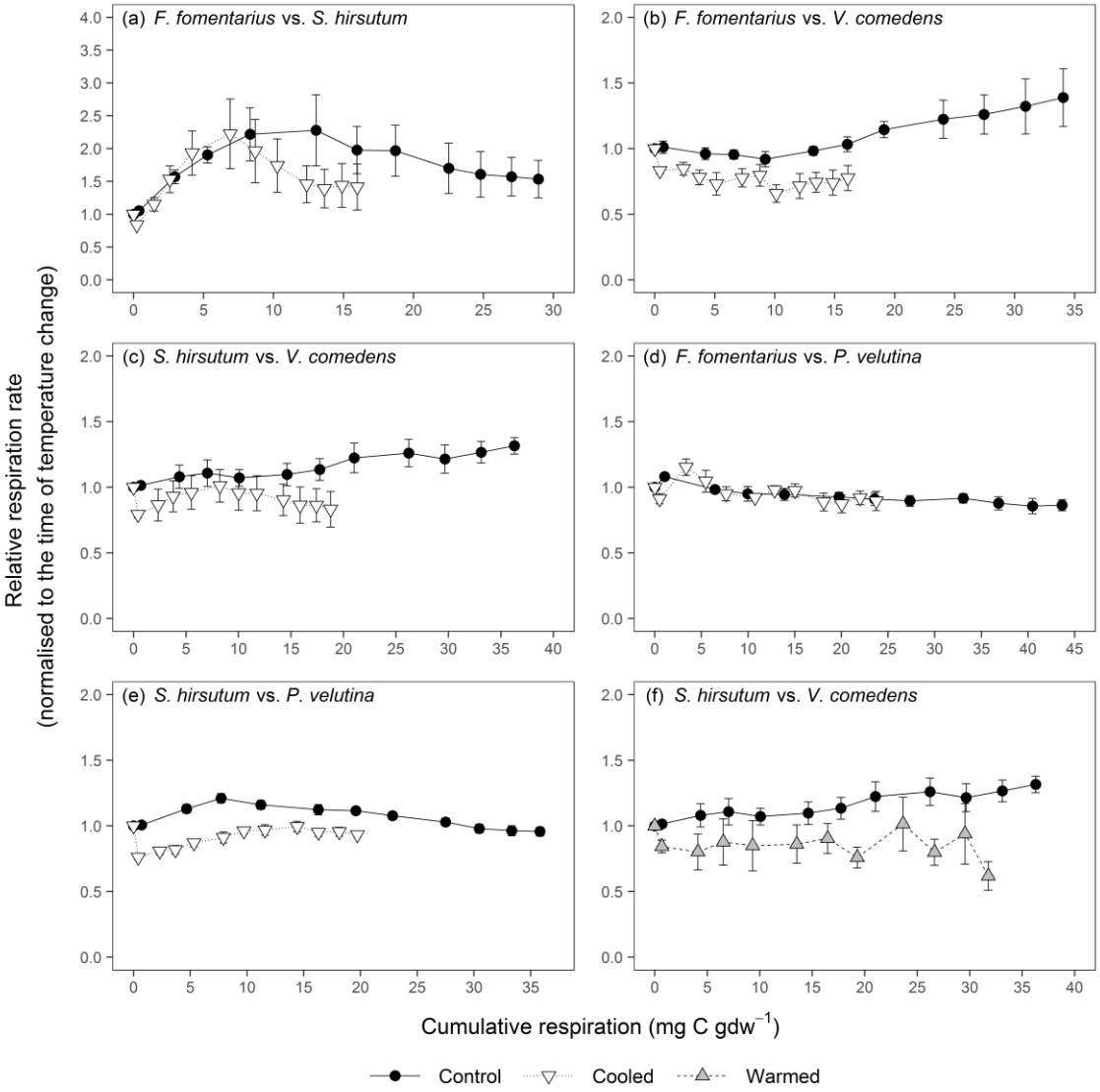
Appendix 2: Chapter 4 supplementary material



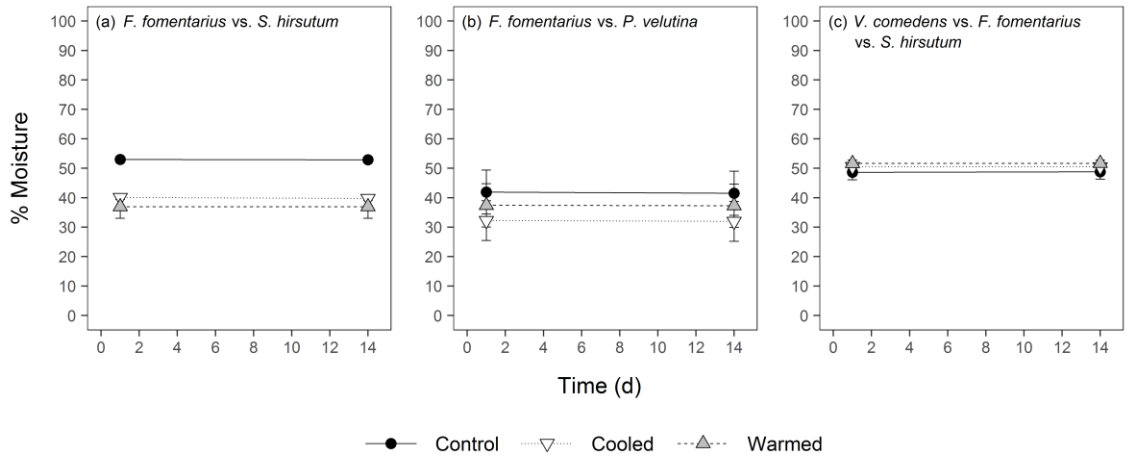
Supplementary Figure 4.1 Density of a sample of wood blocks (n = 10) colonised with each species (108 d) of those to be used for (a) two-species interactions and (b) three-species interaction. Horizontal line shows the mean density of uncolonised wood blocks (n = 10) (0 d). Different letters indicate significant (P < 0.05) differences in wood block density from Tukey's pairwise comparisons. Boxes represent the interquartile range with a horizontal line for the median and the whiskers represent 1.5*interquartile range or the maximum and minimum point.



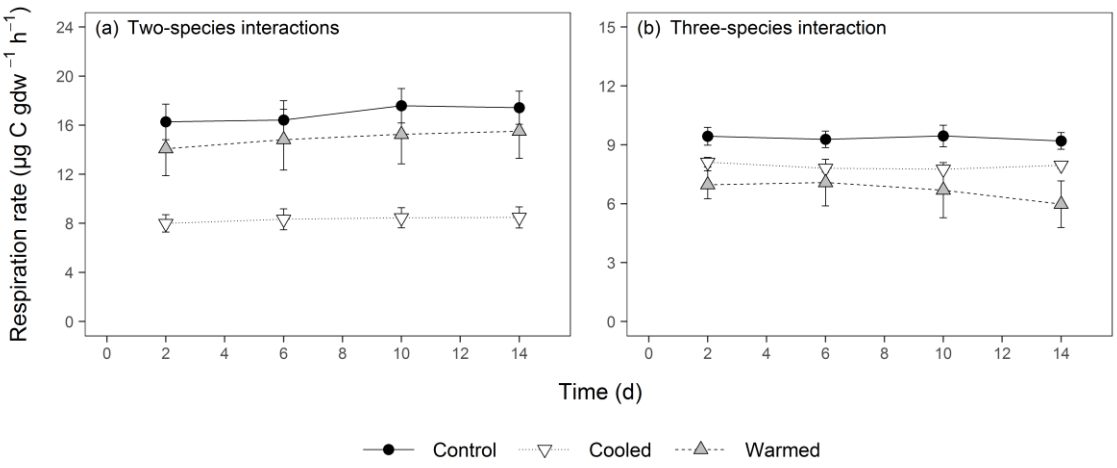
Supplementary Figure 4.2 Respiration rate of individual wood blocks pre-colonised with each species during pre-incubation, prior to two-species interaction set up (mean ± SE of the mean, n = 5). (a-c) Three groups of *F. fomentarius* wood blocks were used for three of the two-species interactions. (d-f) Three groups of *S. hirsutum* wood blocks were used for three of the two-species interactions. (g-h) Two groups of *V. comedens* wood blocks were used for two of the two-species interactions. (i-j) Two groups of *P. velutina* were used for two of the two-species interactions. Individual wood blocks were assigned to one of four temperature treatments (pre-treatment, control, cooled, warmed) based on establishing similar mean respiration rates and trajectories across temperature treatments prior to temperature change.



Supplementary Figure 4.3 (a-e) Relative respiration rate of control and cooled treatments during 90 d of incubation following cooling, of each two-species interaction (mean ± SE of the mean, n = 5), normalised to 1 d after cooling. (f) Relative respiration rate of control and warmed treatments during 90 d of incubation following warming, of *S. hirsutum vs. V. comedens* two-species interaction (mean ± SE of the mean, n = 5), normalised to 1 d after warming. Cumulative respiration was calculated from the time of temperature change (151 d), at the start of 90 d incubation.



Supplementary Figure 4.4 Moisture (%) over 14 d of control, cooled and warmed treatments of three interaction combinations: (a) *F. fomentarius* vs. *S. hirsutum*; (b) *F. fomentarius* vs. *P. velutina* and; (c) *V. comedens* vs. *F. fomentarius* vs. *S. hirsutum* (mean ± SE of the mean, n = 3).



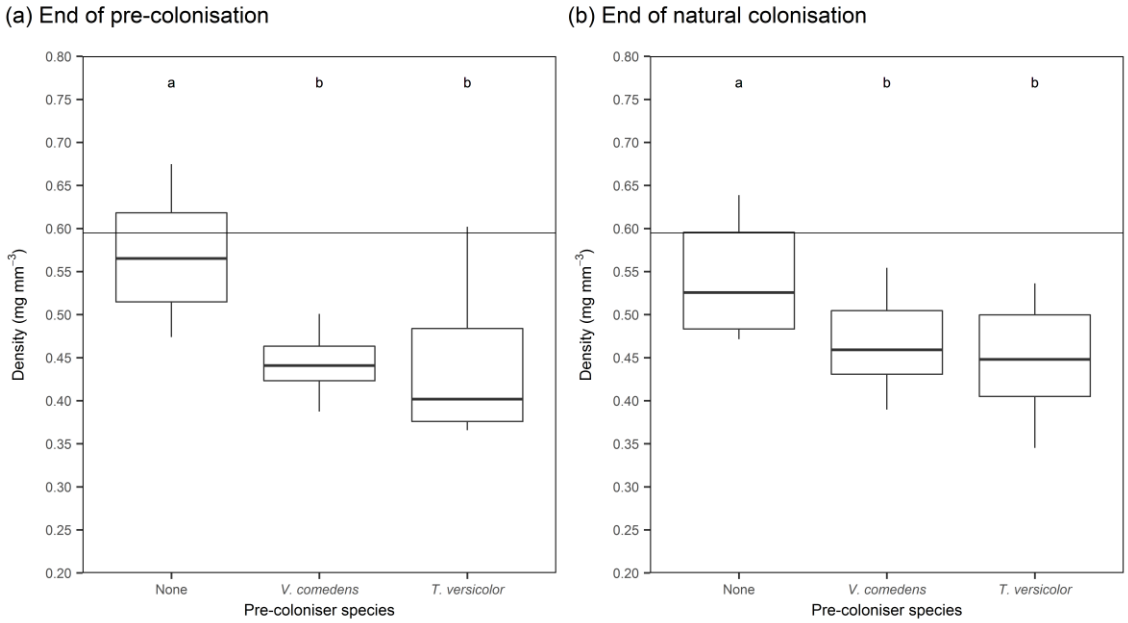
Supplementary Figure 4.5 Respiration rate over 14 d of control, cooled and warmed treatments of (a) two-species interactions and (b) three-species interaction (mean ± SE of the mean, n = 9).

Supplementary Table 4.1 Overall effect (one-way ANOVA: $F_{\text{degrees of freedom}}$ and *P* value) of control, cooled and warmed treatments on respiration rates after interaction set up (148 d), prior to temperature change (151 d), of each interaction.

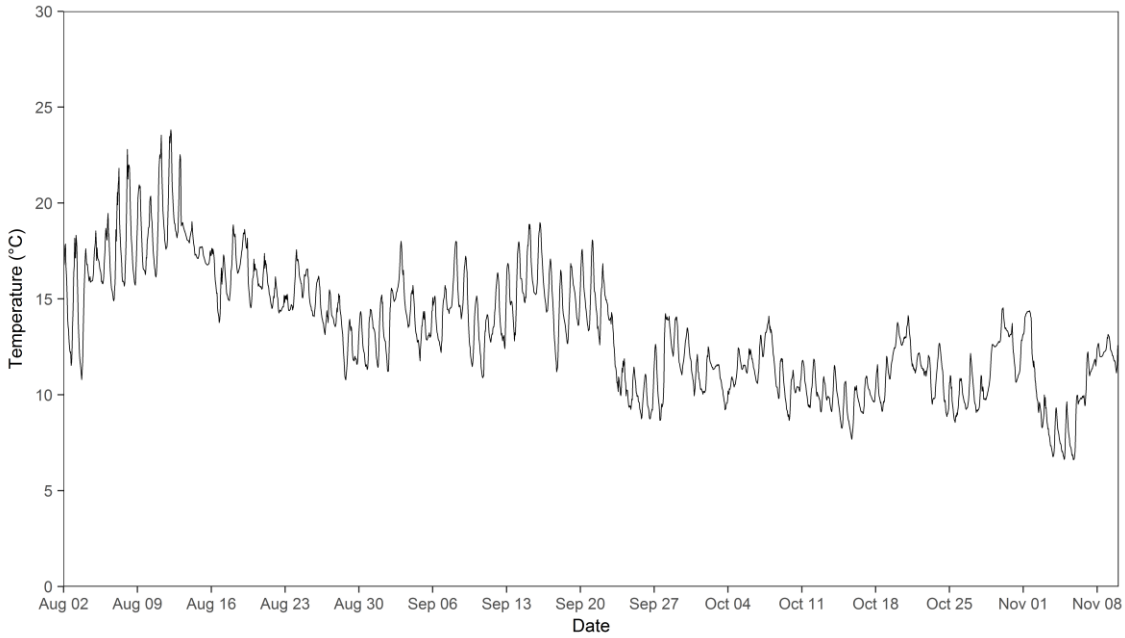
Interaction	$F_{2, 12}$	***P***
Two-species		
F. fomentarius vs. *S. hirsutum*	0.1594	0.8544
F. fomentarius vs. *V. comedens*	0.5055	0.6155
S. hirsutum vs. *V. comedens*	0.3186	0.7331
F. fomentarius vs. *P. velutina*	0.5370	0.5979
S. hirsutum vs. *P. velutina*	0.1828	0.8352
Three-species		
V. comedens vs. *F. fomentarius* vs. *S. hirsutum*	0.0074	0.9926

P > 0.05 indicates a non-significant difference.

Appendix 3: Chapter 5 supplementary material



Supplementary Figure 5.1 Density of a sample of wood blocks (n = 10) at (a) end of pre-colonisation in the laboratory (154 d) and (b) end of natural colonisation in the temperate woodland (254 d). Horizontal line shows the mean density of uncolonised wood blocks (n = 10) at the start of the experiment (0 d). Different letters indicate significant (P < 0.05) differences in wood block density from Tukey's pairwise comparisons. Boxes represent the interquartile range with a horizontal line for the median and the whiskers represent 1.5*interquartile range or the maximum and minimum point.



Supplementary Figure 5.2 Mean temperature (°C) of the soil-litter interface recorded in the temperate woodland by data loggers (average from two measurement locations; TinyTag, UK). Data were recorded every 1 h for 100 d (2nd Aug 2020 – 10th Nov 2020).

Supplementary Table 5.1 Overall effect (one-way ANOVA: $F_{degrees\ of\ freedom}$ and *P* value) of control, cooled and rewarmed treatments on respiration rates at the final measurement of pre-incubation (336 d), prior to cooling (350 d), of each pre-coloniser species.

Pre-coloniser species	$\mathbf{F_{2, 21}}$	***P***
None	<0.001	1.00
V. comedens	<0.001	1.00
T. versicolor	<0.001	1.00

P > 0.05 indicates a non-significant difference.

Supplementary Table 5.2 Overall effect (one-way ANOVA: $F_{degrees\ of\ freedom}$ and *P* value) of control and warmed treatments on respiration rates at the final measurement of pre-incubation (336 d), prior to warming (350 d), of each pre-coloniser species.

Pre-coloniser species	$\mathbf{F_{1, 14}}$	***P***
None	<0.001	1.00
V. comedens	<0.001	0.98
T. versicolor	<0.001	0.98

P > 0.05 indicates a non-significant difference.

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