IMPACT OF ENVIRONMENTAL CHANGE ON PRIMARY PRODUCTION IN MODEL MARINE COASTAL ECOSYSTEMS

Natalie Ruth Hicks

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews

2011

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May 2011

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Acknowledgements

A huge number of people have helped me at various stages throughout my PhD. I'm not going to remember all the names, so will keep it brief.

Firstly huge thanks to my supervisors! Dave Paterson, for his constant support through all the ups and downs of the entire process, for reading everything as many times as I could convince him, for his helpful insightful comments and suggestions, for allowing me a bit of free reign to carry out the practical side but also being my fall back for when things needed greater interpretation or just a bit of 'Dave' tweaking. Also Martin Solan, for his consistent support particularly through the highs and lows of practical set ups, the enthusiasm that kept me going even when things weren't going to plan - 'it's looking great' is a definite Solan cover-up when it is looking far from great! Thank you both, I guess the fact we're still working (indirectly) together is a good sign.

Massive thanks goes to Mark Bulling, he shared all the good and bad times for the duration of this work, all the fieldwork collections, set-ups and take downs, for all the statistical advice when needed and for providing me with entertainment during the very many very long days and frustrations when it was just the two of us. You are the only person I can do an entire 17hr experimental dismantle with, without speaking from start to finish!

Thanks to both research groups I have had the pleasure of working with. SERG at St Andrews: Irvine, thank you for the light relief and help with dismantles, you are the LN_2 expert and you still scare me with that! Also thanks to Emma and Beccy for their helpful and timely advice and support when needed. Thanks for letting me claim desk space on my occasional visits. At Oceanlab at Aberdeen, and Cruickshank, thank you to the many people

that have assisted in all the fieldwork: Lorna, Val, John, Thom, Leigh, Nikki, Gema, Jas, Dot, Ken and not forgetting Owen.

Huge thanks also to my 'inner circle' (spread across the world) that kept me sane and gave me opportunities to escape for a bit! Petter (för filmer, kaffe och kramar - tack så mycket!), Lorna (proofreading and general rant listener), Neil (sympathetic flatmate), Marcie and Lukas (děkuji), Laney and Fin (permanent background supporters) – you have no idea how much support you've given me the last couple of years, particularly emotional, oh and the food helped too. I promise to be more sociable and less geeky now!

The biggest thanks of all go to my family, who have consistently supported me through this PhD. To my Mum and Dad, Ben, Bisky and Dave - I hope you can understand a bit more of this now! Thank you for your unwavering support from start to finish, for listening to me talk about my work in greater detail than you probably cared to know. I will try and come home a bit more often, and have got a 'real' job now. Without your support none of this would have been possible – happy reading, this is for you!

Contents

Hicks, N, Bulling, M, Solan, M, Raffaelli, D, White, P & Paterson, DM (2011) 'Impact of biodiversity-climate futures on primary production and metabolism in a model benthic estuarine system', *BMC Ecology*, vol 11, no. 7

Bulling, MT , **Hicks, N,** Murray, L , Paterson, DM , Raffaelli, D, White, PCL & Solan, M (2010) 'Marine biodiversity-ecosystem functions under uncertain environmental futures', *Philosophical Transactions of the Royal Society of London Series B* , vol 365, no. 1549, pp. 2107-2116

Abstract

Coastal ecosystems, including estuaries, provide a range of services to humans, mediated by the species within these ecosystems. Microphytobenthos (MPB) play a vital role in many key processes within estuarine ecosystems, and provide a food source for higher trophic levels. Anthropogenic activity is already causing changes to ecosystems, through pollution, overexploitation and, more recently, climate change. Increasing temperature and carbon dioxide levels, and altered biodiversity, are likely to affect species, and their interactions, within these ecosystems. Much ecological research has focused on the effects of a single stressor on specific species or ecosystems, with relatively little work examining the effects of multiple stressors. The research in this thesis investigates the effects of altered environmental variables (light, tidal regime, temperature and carbon dioxide) and different macrofaunal diversity on primary production (MPB biomass) through a series of manipulative lab-based mesocosm experiments. This work also examines the temporal variability of environmental stressors on species across two trophic levels. Results demonstrate how multiple environmental stressors interact in a complex and non-additive way to determine an ecosystem response (MPB biomass, nutrient concentration), and the effects of altered biodiversity were underpinned by strong species effects. Temporal variation of stressors had a strong effect on ecosystem response. In marine coastal ecosystems, environmental changes through ocean acidification will have economic and social repercussions, directly impacting the human services and livelihoods that these systems provide. As such, future research should be focused on identifying and mitigating the inevitable multiple effects that future global change may have on coastal ecosystems.

1. Introduction

1.1 Coastal ecosystems

Marine coastal ecosystems are among the most ecologically important environments on earth (Cardoso *et al.,* 2008; Kroeker *et al.,* 2010) and one of the most valuable natural systems (Forster *et al.,* 2006). Almost 50% of the world's human population live within 100 km of the coast, and this proportion continues to increase (Paerl *et al.,* 2006). Benthic ecosystems harbour particularly high levels of diversity, provide a large portion of global productivity (Solan *et al.,* 2006) and play a vital role in the storage and recycling of nutrients (Covich *et al.,* 2004). Estuarine ecosystems provide a variety of services such as tourism, recreation, food production, nutrient cycling and primary production (Defew *et al.,* 2002; Harley *et al.,* 2006; Paterson *et al.,* 2009). The net carbon status and metabolism of coastal systems has received increasing scientific interest (Migne *et al.,* 2009), particularly with the current concerns over environmental change and exploitation (Engelson *et al.,* 2008). Small environmental perturbations may shift the balance between autotrophy and heterotrophy (Porubsky *et al.,* 2008) and affect the organisms within these ecosystems, and the services they provide.

1.1.1 Anthropogenic changes to coastal ecosystems

Anthropogenic activity has increased over the last century, and as a result almost all ecosystems (marine and terrestrial) have been altered by man (Ronnback *et al.,* 2007) on both a global and local scale (Hooper *et al.,* 2005). Coastal ecosystems, in particular estuaries, are highly vulnerable to a variety of anthropogenic stressors, such as pollution, over-exploitation and habitat destruction (Adams, 2005). This is due to high population densities and rapid economic growth (Ronnback *et al.,* 2007) in coastal regions. Coastal ecosystems are also threatened by changes in land-use, increasing development of roads and buildings and excess

nutrient release (eutrophication) through changes in agricultural practices (Adams *et al.,* 2005; Cardoso *et al.,* 2008). These activities can lead to habitat modification, and directly affect the species within these ecosystems and their many interactions (Ronnback *et al.,* 2007, Cardoso *et al.,* 2008). These important ecosystems provide a variety of services and goods to humans, but are now threatened by global climate change (Cardoso *et al.,* 2008), which is currently considered one of the most important drivers for the decline in global biodiversity (Sala *et al.,* 2000; Duffy *et al.,* 2007; Schweiger *et al.,* 2008). Consequently, despite their high biodiversity, marine ecosystems are currently estimated to have some of the highest rates of species loss (Covich *et al.,* 2004).

1.2 Biodiversity and Ecosystem Function (BEF)

1.2.1. Early BEF research

Concerns about declining biodiversity on both local and global scales have led to increased research into how biodiversity may affect function and stability of ecosystems (Caliman *et al.,* 2007). Ecosystem function is a recent term that encompasses all of the properties and processes provided by an ecosystem (Hooper *et al.,* 2005). Ecosystem goods are often defined as those properties that can be given a market or monetary value, such as tourism or recreation, and ecosystem services are a product of the ecosystem processes which directly or indirectly benefit humans, such as the maintenance of water quality (Hooper *et al.,* 2005).

Early terrestrial studies demonstrated how the species identity and species richness (often used interchangeably with 'biodiversity') of plants can affect primary production, nutrient use and ecosystem stability from climate variability and human disturbance (Tilman, 1999; Loreau *et al.,* 2001). Initial studies of species diversity and ecosystem processes, such as the

Naeem (Naeem *et al.,* 1994; Naeem *et al.,* 1995) and Tilman diversity studies (Tilman and Downing, 1994; Tilman, 1999) focused on terrestrial communities such as grasslands (see Loreau *et al.,* 2002), and assumed that different plant species differ in their use of resources (Forster *et al.,* 2006). Similar studies have been carried out in freshwater and marine ecosystems to establish the relationship between diversity and ecosystem function (Covich *et al.,* 2004). Experimental studies in model mesocosm systems show changes in biodiversity can impact the ecosystem (Petchey and Gaston, 2002). Recent studies have demonstrated how high diversity within an ecosystem can increase its resilience to natural and anthropogenic stressors (Adams, 2005; Levin and Lubchenco, 2008), which may play a role in buffering an ecosystem against the effects of future climate change (Yachi and Loreau, 1999). Current species extinction rates are estimated to be at least 1000 times greater than any previous extinction rates, including the mass extinction events (Sala *et al.,* 2000). As ecological research in the last decade has focused on biodiversity and its effect on ecosystems, it has become apparent that declining species diversity can alter the ecological processes that occur within an ecosystem (Covich *et al.,* 2004; Hooper *et al.,* 2005; Ieno *et al.,* 2006; Duffy *et al.,* 2007).

1.2.2. Species diversity and functional traits

Further experimental studies in marine and terrestrial ecosystems demonstrate how important biodiversity and species richness is for many ecosystem processes, such as bioturbation, and functions, such as primary productivity (Petchey, 2000; Loreau *et al.,* 2001; Covich *et al.,* 2004; Schweiger *et al.,* 2008; Blake and Duffy, 2010; Brown *et al.,* 2010; Traill *et al.,* 2010). As a result of this research, community structure and species diversity are known to be important for ecosystem function (Ieno *et al.,* 2006; O'Connor and Crowe, 2005), and high

biodiversity can enhance resource use, productivity and stability within an ecosystem (Duffy *et al.,* 2007). Numerous studies on diversity demonstrate that despite the complexity of the relationship between ecosystem function and species diversity, simple curves can be fitted to this relationship, but the shape of these curves vary from study to study (Cardinale *et al.,* 2006; Thrush *et al.,* 2006). In addition, it has become clear that the impact of biodiversity on a community or ecosystem is influenced by the functional traits of the species found within the ecosystem (Lohrer, 2004). Functional traits can be broadly defined as behavioural, morphological or biological traits that affect ecosystem properties or the response of a species to certain environmental conditions (Hooper *et al.,* 2005). In many studies, species are grouped together according to their functional traits to form functional groups or types (Hooper *et al.,* 2005). In theory, functional groups consist of species that have either similar effects on ecosystem processes or properties, or respond in the same way to environmental changes (Hooper *et al.,* 2005). It is extremely difficult to classify species into distinct functional groups, as functional traits of how an individual species influences ecosystem processes may differ in how it responds to environmental changes (Hooper *et al.,* 2005), and this will differ between biomes. In marine benthic ecosystems, bioturbation is one of the main activities that influences sediment properties (Solan *et al.,* 2004), and as a result many benthic fauna are grouped according to their mode of bioturbation (as in Francois *et al.,* 1997; Mermillod-Blondin *et al.,* 2004).

Since both species and functional diversity can influence ecosystem processes, therefore the loss of one or more species is likely to affect the functioning of an ecosystem, or the loss of certain processes or services (Covich *et al.,* 2004). However, this may be dependent on the ecosystem in question (Covich *et al.,* 2004). In an ecosystem with high species diversity, the loss of one species will have less effect on ecosystem function than the same loss in an

ecosystem with low species diversity (Yachi and Loreau, 1999; Petchey, 2000; Covich *et al.,* 2004; Petchey and Gaston, 2006). The 'insurance' theory assumes that an ecosystem with many species will continue to function even if some of the species stop contributing (Yachi and Loreau, 1999). However, species' functional characteristics, or traits (e.g. bioturbation or primary production), will influence ecosystem properties more than the abundance of a species, suggesting functional diversity within an ecosystem is more important than species diversity (Hooper *et al.,* 2005). In addition, the impact of the extinction of one species within an ecosystem is extremely hard to predict as some scenarios do not result in loss of function until many species are lost (Solan *et al.,* 2004).

Many theories have been put forward to explain the relationship between biodiversity and ecosystem function (Naeem *et al.,* 2002; Naeem *et al.,* 2009). The three main concepts are that species may be redundant; species are singular; and species effects are context dependant therefore their impacts are idiosyncratic (Naeem *et al.,* 2002 in Loreau *et al.,* 2002). The 'redundancy' hypothesis assumes that species are redundant to a certain point, i.e. the loss of species at high diversity levels is compensated by other species. The 'singular' hypothesis implies that each species contributes uniquely to an ecosystem (often keystone species) and their loss will affect ecosystem processes (Naeem *et al.,* 2002). The 'idiosyncratic' hypothesis suggests that ecosystem function is influenced by the response or behaviour of species in that community, which may change under different environmental conditions, thus the impact of species loss may be unpredictable (Naeem *et al.,* 2002). Many different complexities underlie these different relationships, and a large proportion current ecological research focuses on understanding and identifying the mechanisms or causality that drives these relationships (Naeem *et al.,* 2009).

1.3 Biodiversity and Ecosystem Function in the Marine Benthos

Marine benthic systems provide an ideal model with which to examine the effects of biodiversity on ecosystem processes (Solan *et al.,* 2004; Bulling *et al.,* 2006; Ieno *et al.,* 2006; Bulling *et al.,* 2010). Full factorial mesocosm experiments (such as Raffaelli *et al.,* 2003; Ieno *et al.,* 2006; Bulling *et al.,* 2010) have shown the effect of biodiversity on ecosystem function was idiosyncratic at low diversity, and support the idea that functional diversity may be more important than species richness (Mermillod-Blondin *et al.,* 2005). The goods and services provided by estuarine ecosystems, such as nutrient cycling and primary productivity, are mediated by the infauna (bioturbating and deposit feeding invertebrates) and microphytobenthos (diatoms, euglenids and cyanobacteria) that inhabit the sediment (Forster *et al.,* 2006; Paterson *et al.,* 2009; Fitch and Crowe, 2011). Both the microphytobenthos (MPB) and the fauna affect total benthic metabolism through their interaction, activity and abundance (Tang and Kristensen, 2007). The MPB are extremely important for primary production (Kromkamp *et al.,* 2006) and hence support secondary production within the estuarine ecosystem (Hagerthey *et al.,* 2002). The macrofauna enhance benthic-pelagic processes and alter rates of nutrient fluxes (Karlson *et al.,* 2007).

1.3.1 Microphytobenthos

Microphytobenthos (MPB) are the main primary producers in estuarine mudflats, dominated by diatoms, cyanobacteria and euglenids, and can contribute up to 50% of the total estuarine primary production (Jesus *et al.,* 2006). The abundance and species composition of microphytobenthos is influenced by a variety of factors, such as nutrient availability, light and tidal cycles, and grazing by infaunal invertebrates (Guarini *et al.,* 2000; Hillebrand *et al.,* 2000; Hagerthey *et al.,* 2002; Jesus *et al.,* 2009). MPB form biofilms on the surface sediment during emersion (Consalvey *et al.,* 2004), and these biofilms enhance sediment stability

(Paterson, 1989; Jesus *et al.,* 2006; Tolhurst *et al.,* 2006) through secretion of extracellular polymeric substance (EPS) (Paterson, 1989; Middelburg *et al.,* 2000). EPS also enables diatoms to move through the sediment for photosynthesis, and as such diatoms can be characterised by their motility. Epipelic diatoms are highly motile and move freely through the sediment using this EPS, whilst in contrast episammic diatoms are attached to the sediment and are not motile (Consalvey *et al.,* 2004). The vertical migratory behaviour of epipelic diatoms is influenced by a variety of factors, such as the presence of light and tidal cover (Paterson, 1989; Guarini *et al.,* 2000; Consalvey *et al.,* 2004; Jesus *et al.,* 2009).

As well as providing sediment stability, MPB is an organic carbon source for bacteria (Tang and Kristensen, 2007) and the dominant estuarine invertebrates, such as *Hediste (Nereis) diversicolor*, *Hydrobia ulvae* and *Corophium volutator* (Defew *et al.,* 2002; Ieno *et al.,* 2006; Dyson *et al.,* 2007). In addition, MPB play an important role in coastal morphology (Kromkamp *et al.,* 2006; Paterson *et al.,* 2009). For these reasons, MPB are functionally important in estuarine systems. MPB biofilms are dynamic and can grow and develop rapidly (Tolhurst *et al.,* 2008), and estimating the biomass, distribution and primary production of MPB can help to understand estuarine ecosystems (Kromkamp *et al.,* 2006). Measurements of MPB biomass can be used as a proxy for primary production (Honeywill *et al.,* 2002; Consalvey *et al.,* 2005; Jesus *et al.,* 2006; Kromkamp *et al.,* 2006)

1.3.2 Macrofauna effects

The presence and density of benthic invertebrates influences a variety of ecosystem processes such as nutrient fluxes (Mermillod-Blondin *et al.,* 2005) and oxygen penetration into the sediment surface. The dominant species found in estuarine mudflats (such as *H.diversicolor, C. volutator* and *H. ulvae*) provide a wide range of functional traits (Mermillod-Blondin *et*

al., 2005; Ieno *et al.,* 2006). The feeding behaviour and activity of these invertebrates can result in sediment particle reworking and movement (bioturbation) which can enhance the depth of oxygen penetration and alter the rate of nutrient fluxes (Karlson *et al.,* 2007). The behaviour and movement of each invertebrate alters the sediment in a variety of ways, from burrowing and creating tunnels, to feeding on the surface algae, to irrigating the burrows by creating a current through undulating movements (Tang and Kristensen, 2007). The diversity of functional traits of these species is important for sediment-based biogeochemical processes (Karlson *et al.,* 2007). The interaction of fauna and microphytobenthos influences benthic metabolism by altering fluxes of CO_2 and oxygen within the sediment and the overlying water (Tang and Kristensen, 2007) and influencing nutrient dynamics.

Bioturbation is one of the main processes in which invertebrates influence the structure of the sediment, and is defined as the reworking of sediment particles and irrigation of pore water within the sediment (Gilbert *et al.,* 2007). Macrofauna that bioturbate are usually classified on their mode of sediment mixing (Francois *et al.,* 1997). Bioturbatory activities range from construction and maintenance of burrows, to feeding behaviour and bioirrigation (Nogaro *et al.,* 2008). Although a dominant species in estuarine mudflats, *H. ulvae* is not an extensive bioturbator as it predominantly grazes on and within the surficial sediment, thus only mixing the sediment horizontally (Hagerthey *et al.,* 2002). In contrast, both *H. diversicolor* and *C. volutator* construct burrows within the sediment (Mermillod-Blondin *et al.,* 2004). *H. diversicolor* constructs deep mucus-lined burrows which extend 6 – 20 cm depth, and then ventilates (bioirrigates) them intermittently (Tang and Kristensen, 2007). This bioirrigation activity decreases in frequency during darkness (Tang and Kristensen, 2007). *C. volutator* constructs U-shaped burrows in the top 2-3 cm of the sediment (Mermillod-Blondin *et al.,* 2005).

These burrows increase the depth of oxygen penetration and stimulate microbial activity on the surface area of the burrows (Biles *et al.,* 2002; Mermillod-Blondin *et al.,* 2005). In addition, the construction of these burrows doubles the solute exchange between the sediment and the overlying water column and releases nutrients trapped in porewater (Mermillod-Blondin *et al.,* 2004). Bioturbating benthic organisms are functionally classified according to their bioturbating method and their feeding strategy, thus *C. volutator* is classified as a biodiffuser (moves individual sediment particles in a random manner) (François *et al.,* 2002; Mermillod-Blondin *et al.,* 2005), while *H. diversicolor* is considered a gallery-diffuser, as it stimulates non-local transport of materials to the depth of the burrow, and induces diffusive mixing at the surface (François *et al.,* 2002; Nogaro *et al.,* 2008). Through extensive bioturbation, these two species increase the depth of oxygen penetration by up to 20 cm, and enhance the release of nutrients, thus reducing numbers of sulphate reducing bacteria (Mermillod-Blondin *et al.,* 2004).

Benthic mesocosm studies (e.g. Mermillod-Blondin *et al.,* 2004 and Ieno *et al.,* 2006) demonstrate how the presence of one species can influence nutrient fluxes. In these studies, single species treatments with *H. diversicolor* showed a higher flux of ammonium from the sediment to the overlying water column, but this flux did not increase when other bioturbating species were added (Mermillod-Blondin *et al.,* 2005). Mesocosm studies have also demonstrated how the presence of invertebrates reduces MPB biomass and how one species (*C. volutator*) has a greater impact than other species through its mode of bioturbation (Dyson *et al.,* 2007). These studies clearly demonstrate how bioturbation by infaunal invertebrates can influence the biogeochemistry of the sediment and the overlying water (Gilbert *et al.,* 2007). The interactions between these benthic organisms (invertebrates and microphytobenthos) is important for ecosystem function (Caliman *et al.,* 2004), and may

have negative or positive effects on ecosystem processes, such as nutrient cycling (Biles *et al.,* 2002) .

1.4 Climate Change and Ocean Acidification

Marine, terrestrial and freshwater ecosystems are constantly under pressure from anthropogenic activities (Ronnback *et al.,* 2007). Climate change is one of the major threats to global biodiversity (Sala *et al.,* 2000; Schweiger *et al.,* 2010). Current observations of global average air and sea surface temperatures and rising sea levels are evidence of climate change, particularly global warming (IPCC, 2007). Many ecosystems have been, and continue to be, affected by local and regional climate changes, in particular rises in temperature (IPCC, 2007).

Climate change is expected to have huge ecological consequences on terrestrial, marine and freshwater ecosystems (Sala *et al.,* 2000; IPCC, 2007; Brown *et al.,* 2010; Drinkwater *et al*., 2010). The predicted changes in atmospheric carbon dioxide (CO_2) and temperature are likely to cause independent and interactive measurable changes to a variety of ecosystem processes within the next 100 years (Vitousek *et al.,* 1997; Sala *et al.,* 2000; Drinkwater *et al.,* 2010). Atmospheric concentrations of CO_2 have risen dramatically from pre-industrial levels of 275 ppmv to the current level of 370 ppmv, and annual emissions increased by 80% between 1970 and 2004 (IPCC, 2007). IPCC projections suggest that atmospheric CO_2 concentrations may reach 1000 ppmv by 2100.

Rising atmospheric CO_2 levels have already lowered the ocean pH (ocean acidification) by 0.1 units in the last 200 years (Caldeira and Wicket, 2003), as the oceans act as a sink for CO_2 (Feely *et al.,* 2004). If CO_2 levels continue to rise, then the ocean pH may become the lowest

recorded in the past 300 million years (Caldeira and Wicket, 2003; Feely *et al.,* 2004; Kroeker *et al.,* 2010; Orr *et al.,* 2010). Increasing ocean acidification is likely to severely impact biological ecosystems within the oceans, affecting both individual species and entire communities in benthic and planktonic environments (Feely *et al.,* 2004). Ocean acidification is one of the greatest concerns in environmental marine research, and is often referred to as 'the other CO_2 problem' in conjunction with global warming (Doney *et al.,* 2009). Effects of ocean acidification have already been documented (Caldeira and Wickett, 2003; Orr *et al.,* 2005; Pörtner, 2008; Brown *et al.,* 2010; Kroeker *et al.,* 2010; Joint *et al.,* 2011). There is now intense research investigating the potential consequences of ocean acidification on marine ecosystems (Connell and Russell, 2010; Kroeker *et al.,* 2010).

As a side effect of rising carbon dioxide levels, the global average surface temperature has increased by 0.74ºC in the last century, and sea levels continue to rise at 3.1mm per year as a result of this warming (IPCC, 2007). There are fears that this global warming may cause irreversible or abrupt changes, depending on the scale and speed that climate change occurs (IPCC, 2007). In the last fifty years, the rate of warming has doubled that of the preceding 100 years (IPCC, 2007). As well as changes in temperature and CO_2 levels, the last few decades have seen an increase in frequency of extreme weather conditions (such as droughts) and changes in precipitation patterns (IPCC, 2007). Whilst there is no doubt that individual drivers of climate change will affect ecosystems (Long *et al.*, 2004; Hargrave *et al.*, 2009), the combined effects of multiple drivers is likely to influence ecosystem function interactively and/or cumulatively (Halpern *et al.,* 2008).

14.1 Climate change in marine ecosystems

To date, most ecological research on climate change has focused on the effect of temperature at an individual species level (Harley *et al.,* 2006; Cardoso *et al.,* 2008). These studies suggest that species abundance and distribution will change according to species ability to adapt to and tolerate different temperatures (Harley *et al.,* 2006; Firth *et al*., 2009). However, more recent studies have suggested that this relationship is not as simple as first perceived, and that interaction between species is likely to have some effect (Harley *et al.,* 2006). In addition, rising temperature is not the only climate variable that will influence species' distribution and abundance (Cardoso *et al.,* 2008). Changing climatic variables are likely to have different effects on different species, but may cause changes within a community by affecting a few key species (Harley *et al.,* 2006).

1.4.2 Future Research and Concerns

Global concerns about climate change impacts are on such a large scale that it is extremely difficult to research this in the field (Benton *et al.,* 2007). Most climate change studies focus on terrestrial ecosystems and few examine the combined effect of rising temperature and carbon dioxide levels on different trophic levels (Benton *et al.,* 2007). In terrestrial systems there are a few large scale in-situ studies examining effects of elevated CO_2 concentrations, such as the ongoing FACE experiments on forest vegetation (Hendrey *et al.,* 1999; Calfapietra *et al.,* 2010). To date, in-situ studies are still rare in the marine sector (with the exception of EPOCA's Svalbard 2010 campaign – www.epoca-project.eu), and the uncertainty on definite future CO_2 levels makes it uncertain as to the possible impacts on ecosystems (Benton *et al.,* 2007). The concerns over climate changes and declining biodiversity, combined with pressure from the scientific community, has resulted in UNEP announcing the formation of an IPCC-type panel for addressing loss of biodiversity and

ecosystem function (Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) www.ipbes.net) at the end of 2010. Whilst this is a definite step forward, anthropogenic climate change continues to accelerate, and marine coastal ecosystems are at the forefront of these changes, thus it is even more imperative that research focuses on identifying immediate threats to counter the changes that will be seen on a local and global scale.

1.5 Aims of this thesis

This thesis aims to address some of the uncertainty surrounding the effects of ocean acidification and environmental change in estuarine systems, using MPB biomass (proxy for primary production) as a measured ecosystem response. Using a full factorial experimental approach, each data chapter will address a specific question (see Fig. 1.1 for schematic).

All research within this thesis is from a series of lab-based mesocosm experiments, and each chapter adds a level of complexity to the preceding chapter. The first set of experiments (Chapter 3) identifies the role of microphytobenthos in nutrient cycling within the benthic model system, under different macrofaunal diversity levels. This chapter provides a basis for understanding the model system, and allows further manipulation. The second set of experiments (Chapter 4) build on this by manipulating the temperature and carbon dioxide levels within the experiment, and Chapter 5 introduces variability of temperature (fluctuating temperature instead of constant). The final set of experiments examine the effects of a tidal regime on MPB biomass under the same CO_2 and mean temperature regimes used in the preceding chapters.

Main thesis questions to be addressed:

1. *Identify the role of MPB in nutrient cycling and investigate interactions with an additional trophic level (macrofauna) in benthic model system (Chapter 3)*

Hypothesis: the presence of MPB alters the levels of nutrients in the overlying water

Hypothesis: the diversity of the macrofauna negatively affects MPB biomass

2. *Determine how MPB biomass changes under predicted future climate scenarios (CO_2, temperature) and altered macrofaunal biodiversity (Chapter 4)*

Hypothesis: MPB biomass changes under different CO_2, temperature and biodiversity regimes

3. *Identify how MPB biomass changes under different CO_2 regimes with varying degrees of temperature fluctuation around three constant mean temperatures and with altered macrofaunal biodiversity (Chapter 5)*

Hypothesis: MPB biomass changes under different CO_2, temperature, fluctuation and biodiversity regimes

4. *Examine how the presence of a tidal cycle affects MPB biomass under different future climate scenarios (CO_2 and temperature) (Chapter 6)*

Hypothesis: The presence of a tidal cycle positively affects MPB biomass under different climatic scenarios

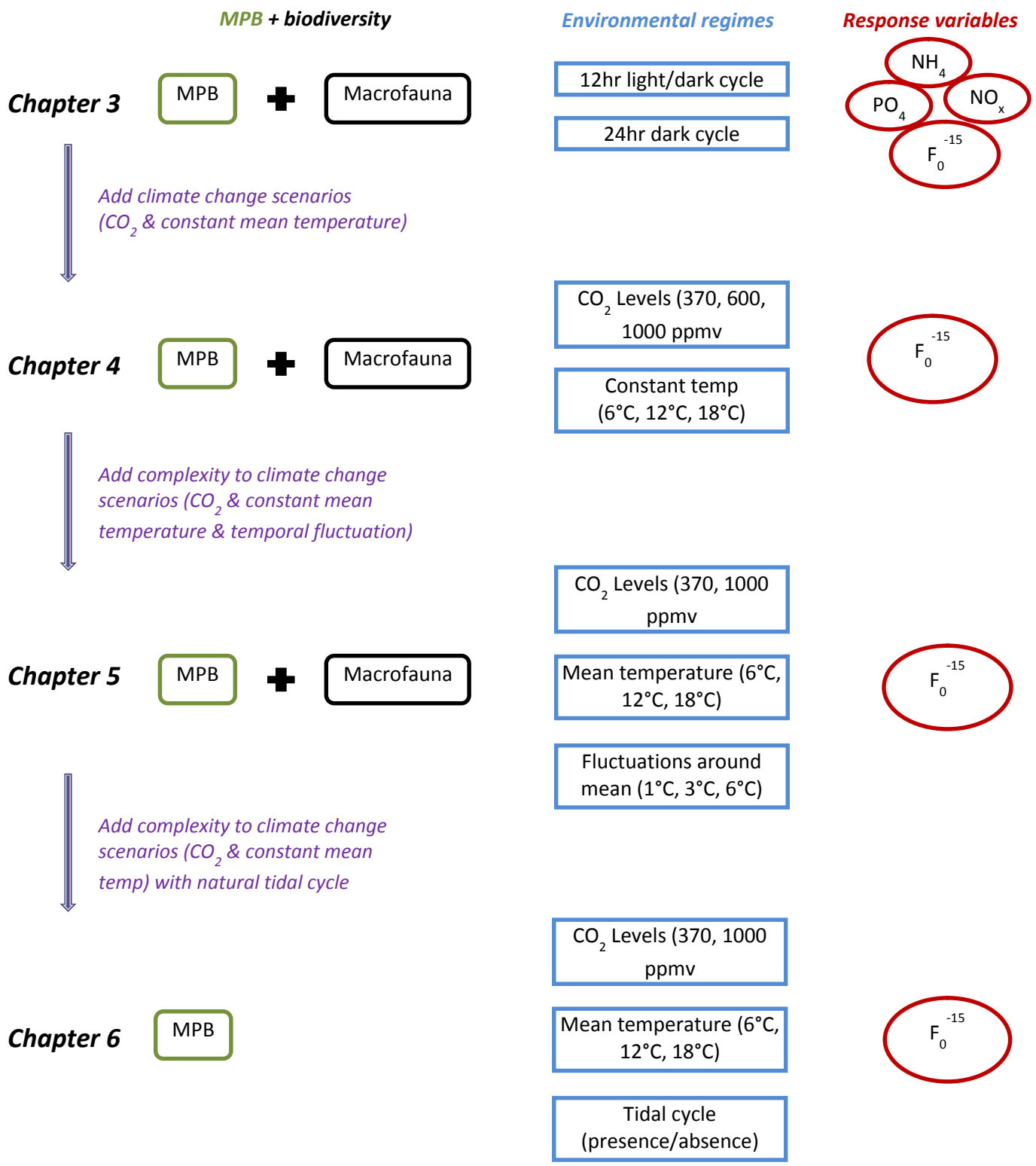


Figure 1.1 Flowchart outlining the main data chapters of this thesis, illustrating how each chapter builds on the work from the previous chapters. F_0^{-15} stands for the minimum fluorescence measurement (F_0) taken after 15 minutes of darkness ($^{-15}$), NH_4 – ammonium; NO_x – nitrate and nitrite; PO_4 – phosphate.

2. Methods and Materials

This chapter details the study site and species used within this thesis, and outlines the generic techniques and procedures used in the experiments. The general details on the mesocosms and tanks used are also included with a summary of the implemented environmental regimes.

2.1 Study site

The Ythan Estuary is a small estuary located in Newburgh, Aberdeenshire (Fig. 2.1) on the North East Coast of Scotland (57° 20.085'N, 02° 0.206' W). The Ythan forms part of a SSSI with the nearby Forvie National Nature Reserve, and this is managed by Scottish National Heritage. The estuary supports a wide variety of important wading bird populations, and is also designated a Special Protection Area (SPA) under the EU Directive on the Conservation of Wild Birds due to the diverse wildlife found in the habitats. Overwintering waterfowl populations regularly number over 20 000 visiting birds on the mudflats. The estuary itself is approximately 8 km in length.

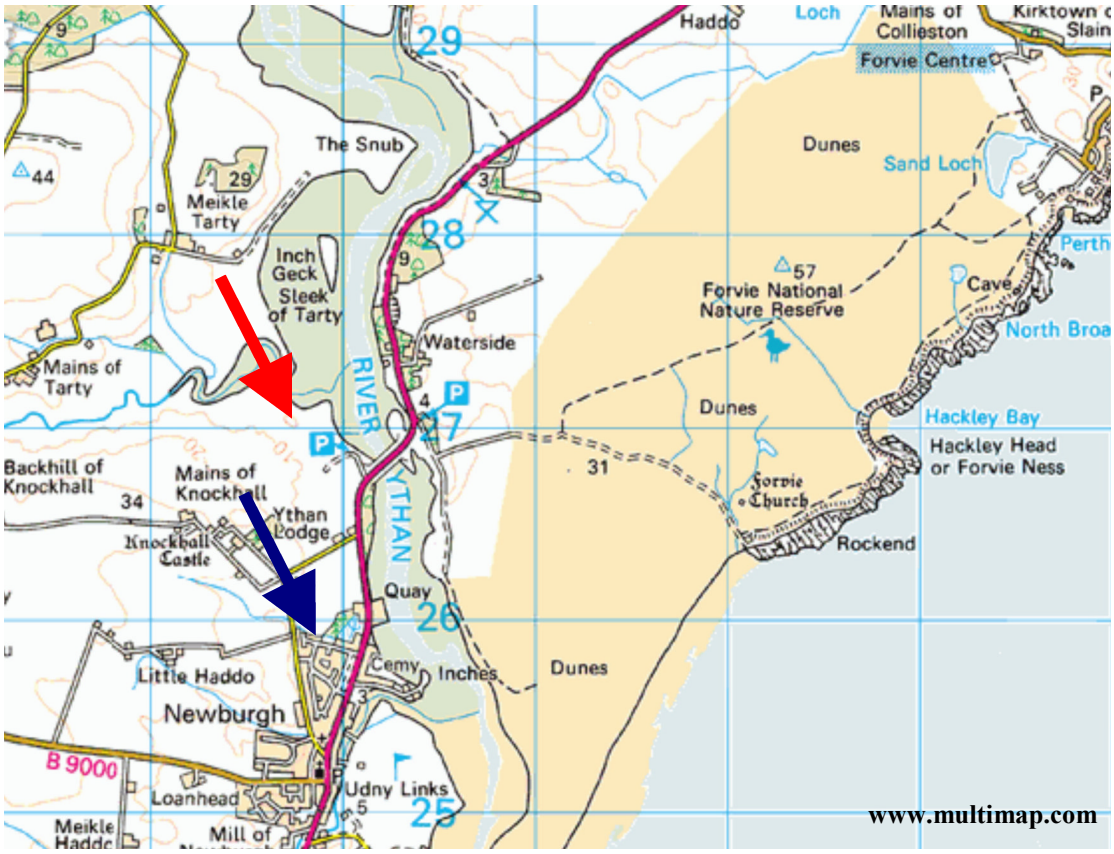


Figure 2.1 Map of Ythan estuary showing sample site (red arrow) in Sleek of Tarty and Oceanlab, University of Aberdeen (blue arrow), Aberdeenshire (image courtesy of multimap.com)

The many different habitats within this site have been studied intensively for over 20 years by staff at the University of Aberdeen. The diversity and abundance of the biota make it a suitable study site for intensive research from species level to ecosystems, and the high biomass of macrofauna and the proximity of Oceanlab (University of Aberdeen) make it ideal for estuarine research. The sediment collection site for the muddy sediment and the macrofauna forms an extensive mudflat at low tide conditions, and the cohesive sediment is anoxic from approximately 2-3 cm depth, hence only the surficial sediment was collected for experimental set up. The macrofauna in this site consist predominantly of the polychaete *Hediste (Nereis) diversicolor*, the amphipod *Corophium volutator*, the gastropod *Hydrobia ulvae* and the small bivalve *Macoma balthica*..

2.2 Sediment collection

The top two centimetres (20 mm) of sediment was collected by hand from the Ythan estuary on the Sleek of Tarty mudflat (as indicated in Fig. 2.1). The sediment was brought back to Oceanlab and sieved through a 500µm mesh in a seawater (UV sterilised, 10 µm filtered, ~ salinity 33) bath to remove all macrofauna and macroalgae. After sieving, the sediment was left to settle for 48 h to ensure retention of the fine sediment particles. The supernatant was then removed, and the sediment homogenised into a slurry ready for distribution into the experimental mescosms.

2.3 Microphytobenthos (MPB) collection

MPB-rich sediment, easily identified by the dark surface pigmentation on the mud, was collected from the surface sediment of the Ythan estuary. This sediment was sieved through a 500µm mesh to remove macrofauna, stones and macroalge, and was spread onto a shallow

tray and placed in natural daylight during the day, and under constant light at night, for 48 h. It was then homogenised to a slurry ready for addition to the mesocosms.

2.4 Macrofaunal collection

Three macrofaunal species were selected for this work - the polychaete *Hediste (Nereis) diversicolor* (HD), the gastropod *Hydrobia ulvae* (HU) and the amphipod *Corophium volutator* (CV) (Fig. 2.2). Not only are these the dominant species found on the study site, they are also known grazers of MPB (Hagerthey *et al.,* 2002), and have different bioturbation activities which influence nutrient release from the sediment to the overlying water (Emmerson *et al.,* 2002; Bulling *et al.,* 2010). All three species were collected from the same site as the sediment.

Figure 2.2 The three macrofaunal invertebrates used: (a) polychaete *Hediste (Nereis) diversicolor* (HD), (b) the gastropod *Hydrobia ulvae* (HU) and (c) the amphipod *Corophium volutator* (CV)

C. volutator and *H. ulvae* were removed from the sediment during the sieving process. *H. diversicolor* was manually collected by hand, digging into the sediment to a depth of appoximately 20 cm (200 mm) and turning the sediment over. This made it easy to find the worm burrows, and the burrows were gently pulled apart and the worm removed and placed into a bucket of seawater. All invertebrates were placed in tanks of seawater and aerated for no more than 48 h before being used in the experiments.

2.5 Mesocosms

2.5.1 Static Mesocosms (Chapters 3, 4, 5)

The mesocosms used in the biodiversity-climate mesocosm experiments were made from Perspex cores (330 mm high, 100 mm internal diameter) with a plastic base sealed with a rubber O-ring (custom made by BEC plastics). The use of static mesocosms makes it easy to determine the effects of macrofauna on ecosystem functioning (Raffaelli *et al.,* 2003; Ieno *et al.,* 2006) under different climatic (carbon dioxide and temperature) regimes.

2.5.1.1 Mesocosm set-up

During experimental set up, the mesocosms were filled with the sieved and homogenised sediment to a depth of 10 cm (785 cm^3). The initial fill of seawater (10µm filtered, UV-sterilised, ~ salinity of 33) was replaced (20 cm of overlying seawater, 2.35 l) after 24 h to remove the nutrient pulse associated with mesocosm assembly (Ieno *et al.,* 2006). MPB-rich sediment (125 cm^3) was added to each mesocosm after the initial fill of water and left to settle for 24 h (Fig. 2.3). All mesocosms were individually aerated throughout the experiment from the air within the environmental chamber where the experiments were run, and covered with a transparent film to allow light penetration and prevent water loss. Each chamber contained 32 mesocosms for the first experiment (Chapter 3), and then 24 mesocosms for the subsequent experimental runs (Chapters 4 and 5). Each mesocosm was fully contained and the water was not able to move between the individual mesocosms.

2.5.1.2 Macrofaunal communities

Replicate (Chapter 3: n=4; Chapters 4 & 5: n=3) macrofaunal communities were assembled in single and multispecies treatments (control, HD, HU, CV, HDHU, HDCV, HUCV, HDHUCV). These unique species permutations were used to eliminate any hidden

treatments. Control mesocosms (Chapter 3: n=4; Chapters 4 & 5: n=3) contained microphytobenthos without macrofauna. Macrofaunal biomass was fixed at 2 g wet weight per mesocosm, equivalent to the natural biomass of 255 g m^2 as found at the study site (Biles *et al.,* 2003). This constant biomass was maintained irrespective of the number of species within the community, so for single species treatments the biomass would be 2 g of that species; for two species treatments the biomass would be 1 g of each species to give a total biomass of 2 g; and for three species each species would contribute 0.66 g of the total biomass.

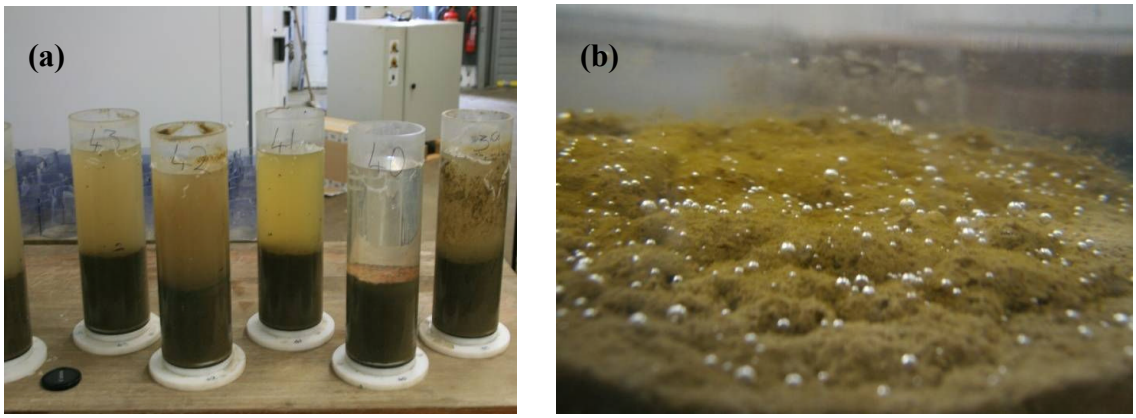


Figure 2.3 (a) The mesocosms at the end of the experiment and (b) a microphybenthic biofilm on the sediment surface within one of the static mesocosms

2.5.1.3 Climatic Regimes

Two separate sets of experiments were carried out using three mean temperatures (6 ºC, 12 ºC and 18 ºC, reflecting the annual variation at the study site – see Appendix 1). The first set of experiments kept these mean temperatures at a constant level (Chapter 4) and the second set of experiments used fluctuating temperature regimes (Chapter 5) around the same three means. In both chapters, atmospheric CO_2 concentrations were maintained using a CO_2 monitor attached to an external CO_2 gass cylinder (BOC gases Ltd, UK) with a digital

controller (Technics horticultural carbon dioxide controller). An Infra-Red Gas Analyser (IRGA, ADC LCA3) was used to calibrate and validate the CO_2 regulation (± 30 ppm).

Constant Temperature Regimes (Chapter 4)

Nine unique climate regimes were used to simulate future climate scenarios, using the three constant temperatures and three carbon dioxide levels (380 ppmv (present day), 600 ppmv, 1000 ppmv) in an orthogonal design. The carbon concentrations of 600 ppmv and 1000 ppmv were based on IPCC projections for future carbon dioxide levels in 50 and 100 years time respectively (TAR, IPCC, 2001 and 4th Assessment Report, IPCC, 2007).

Fluctuating Temperature Regimes (FTR, Chapter 5)

Eighteen unique climate regimes were used to simulate future climate scenarios, using the three mean temperatures and two carbon dioxide levels (380 ppmv and 1000 ppmv). In natural environments the temperature over a 24 h period is not constant, so this series of experiments incorporated temperature variation. The temperature fluctuations were 1 ºC, 3 ºC and 6 ºC around the mean (illustrated in Fig. 2.4), reaching the highest and lowest temperature of this cycle once in every 24 h.

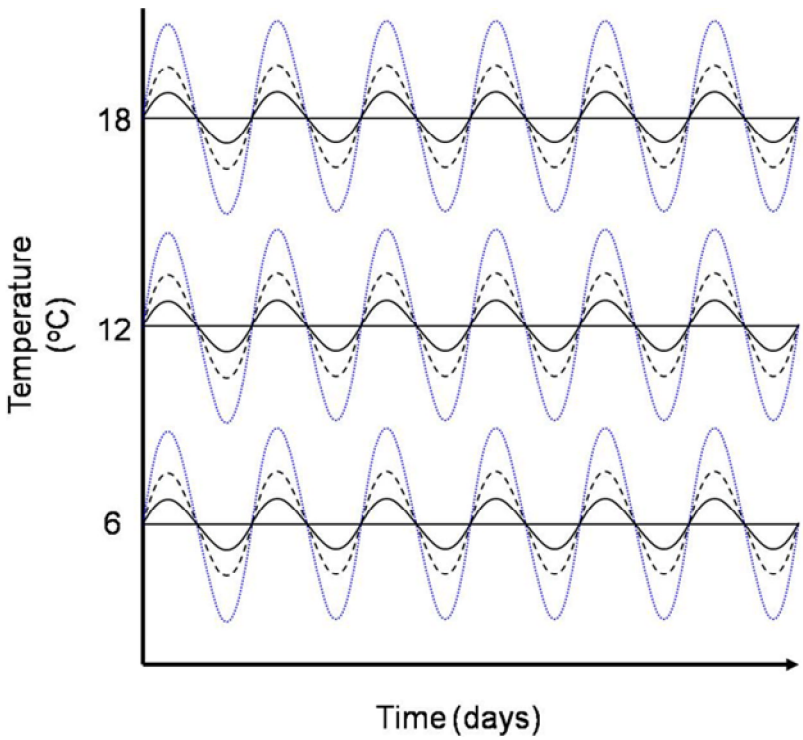


Figure 2.4 The three fluctuations of 1°C, 3°C and 6 °C around the constant mean temperatures of 6 °C, 1 2°C and 1 8°C

2.5.2 Tidal Tanks

Three types of tanks (mesocosms) were designed to fit within the environmental chambers for the tidal experiment. All were custom built by BEC Plastics out of clear perspex. Two reservoir tanks, four tidal tanks and 96 small mesocosms or 'cores' (Fig. 2.5) were specially designed to compare MPB biomass in a tidal regime versus constant immersion under three constant temperature (6 ºC, 12 ºC and 18 ºC) and two CO_2 regimes (370 ppmv and 1000 ppmv).

2.5.2.1 Tank set-up

Each chamber had 1 reservoir tank, 2 tidal tanks and 48 small cores. The reservoir tank at the bottom of the chamber collected and supplied the water to the tidal tanks situated on a shelf directly above it. Each 'tidal' tank contained 24 small cores filled with MPB-rich sediment. The tidal tanks were filled with sieved (500 µm) sand, collected from the Ythan beach, to a depth of 4 cm. The cores were placed in the tidal tanks in three rows of eight and filled with MPB-rich sediment (collected as previously described) on top of the sand to a depth of 1cm. The tidal tanks were then placed in the chamber and filled with overlying seawater to a depth of 22 cm. The seawater was able to move between the small cores, and to avoid any pseudoreplication issues, no measurements (e.g. nutrient analysis) were taken from the overlying seawater.

2.5.2.2 Tidal cycle

The innudation cycle for the tidal tanks (always the front tank in the chamber) was controlled using a persistaltic pump (model 323Du) from Watson-Marlow and 6.4 mm bore platinum cured silicon tubing. The cycle was set and controlled from a computer connected to the pump within the chamber through an RS232 cable. The tidal cycle was set to replicate the natural low tide and high tide cycle in the estuary, and this time was kept consistent for all runs, as set at the initial sample date, to eliminate any interacting effect of light and tide on MPB biomass. During the tidal cycle, seawater was pumped from the tidal tank into the reservoir tank below, and then back again. The experiment was run for one week.

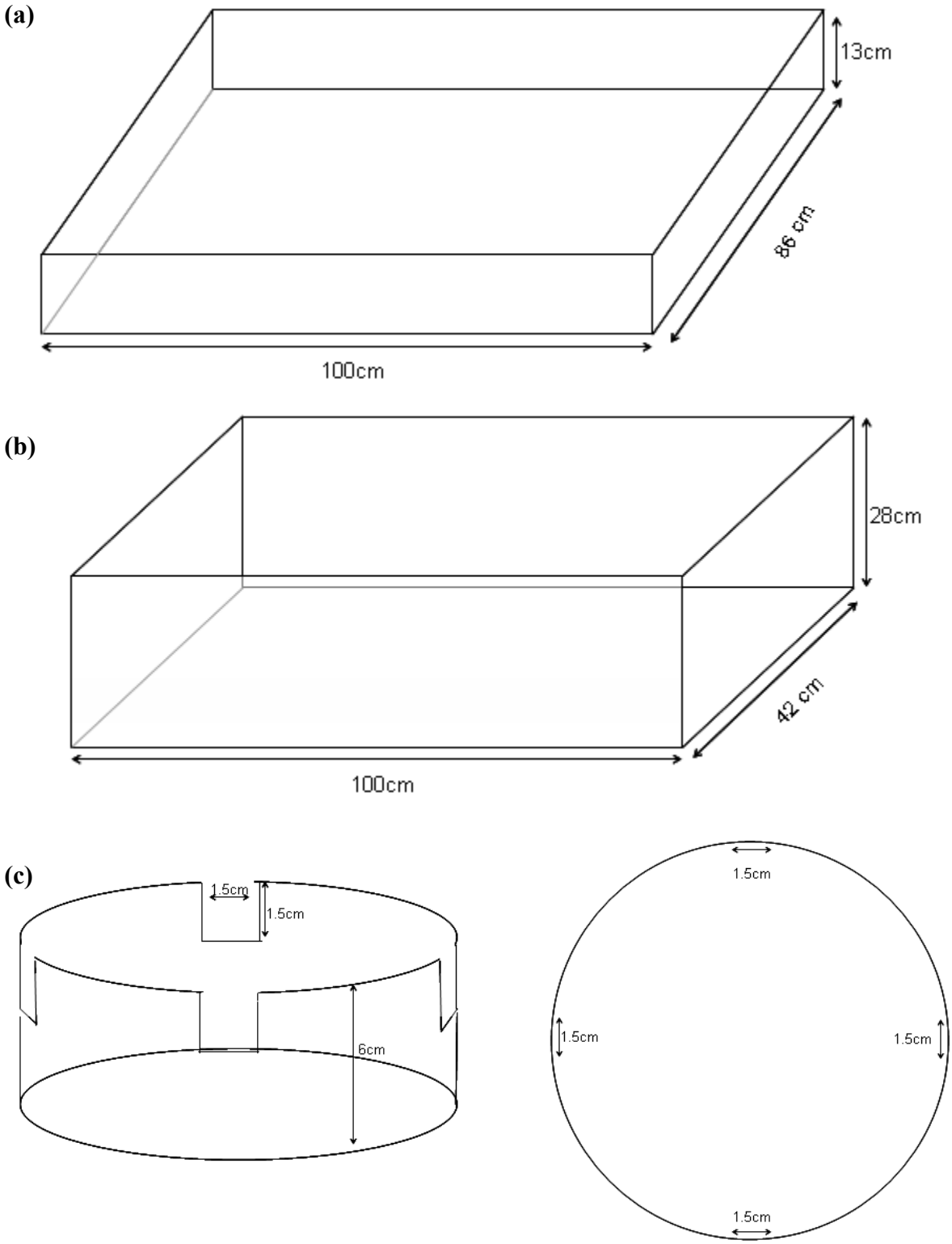


Figure 2.5 The visualisations of the tanks used in the tidal experiments (a) reservoir tank; (b) tidal tanks and (c) individual mesocosm 'cores' for MPB. All were custom made out of Perspex by BEC Plastics.

2.5.2.3 Climate regimes

The tidal experiments were carried out under six climatic regimes, using the three constant temperatures (6 ºC, 12 ºC and 18 ºC) and two CO_2 levels (370 ppmv and 1000 ppmv) in an full factorial design.

2.6 Environmental chambers

Mesocosms and tanks were placed in two environmental chambers (V 4100, Vötsch Industrietechnik) capable of controlling temperature to the nearest 0.1 ºC. The experiments were run under a 12 h light- 12 h dark (L/D) cycle (high intensity discharge sodium lamps, model GE11678, 400w x2, average 300 µmoles m^{-2} sec^{-1}). The lights were suspended from the chamber roof with the base of the light facing the left for the front light and facing the right for the back light, to ensure maximum and even light cover for the mesocosms with minimal shading. Each experiment was run for a week, based on previous experiments indicating this is the optimal time for MPB biomass and species activity (Defew *et al.,* 2002; Dyson *et al.,* 2007). The cores were placed into the chamber randomly for each run to ensure there was no chamber effect and no positional effect within the chamber on the response variables.

2.7 Response Variables

The main aim of this thesis was to examine how different environmental variables (CO_2, temperature, tidal cycle) and biodiversity affect MPB biomass. However, a variety of response variables were measured to give a varied measure of 'ecosystem' response. These variables are detailed below and will be discussed in the individual chapters.

2.7.1 MPB biomass

Traditional methods of measuring MPB biomass, such as sediment removal for chlorophyll *a* (Chl *a*) extraction, and the lens tissue method, are destructive to the sample site (Honeywill *et al.,* 2002). Fluorescence measurements provide an alternative non-destructive and non-intrusive method to estimate surficial algal biomass (Honeywill *et al.,* 2002; Jesus *et al.,* 2006). This technique measures light energy emitted from the pigments that are used during photosynthesis (Consalvey *et al.,* 2005). Using pulse amplitude modulated (PAM) fluorometers, the microphytobenthos are exposed to short pulses of light which induces fluorescence, but does not contribute to photosynthesis (Consalvey *et al.,* 2005). PAM fluorometry can be used in microphytobenthic studies to indicate biomass and primary productivity (Consalvey *et al.,* 2005). This is a widely accepted proxy method for measuring the surficial chlorophyll *a* and algala biomass, and thus fluorescence is a useful method for estimating primary productivity (Honeywill *et al.,* 2002; Consalvey *et al.,*2005; Kromkamp *et al.,* 2006) . Previous studies (Honeywill *et al.,* 2002, Jesus *et al.,* 2006) have illustrated how the minimum fluorescence measured (F_o) by the PAM correlates positively with chlorophyll a content after 15 minutes of dark adaptation (Kromkamp *et al.,* 2006). A dark adaptation period is used to 'reset' the photosynthetic apparatus prior to fluorescent measurements being taken, and this technique produces comparable results (Fig 2.6). The period used for dark adaptation has been debated (see Jesus *et al.,* 2006), but 15 minutes is commonly employed for microphytobenthos in muddy sediments (as in:Honeywill *et al.,* 2002; Jesus *et al.,* 2005; Jesus *et al.,* 2006).

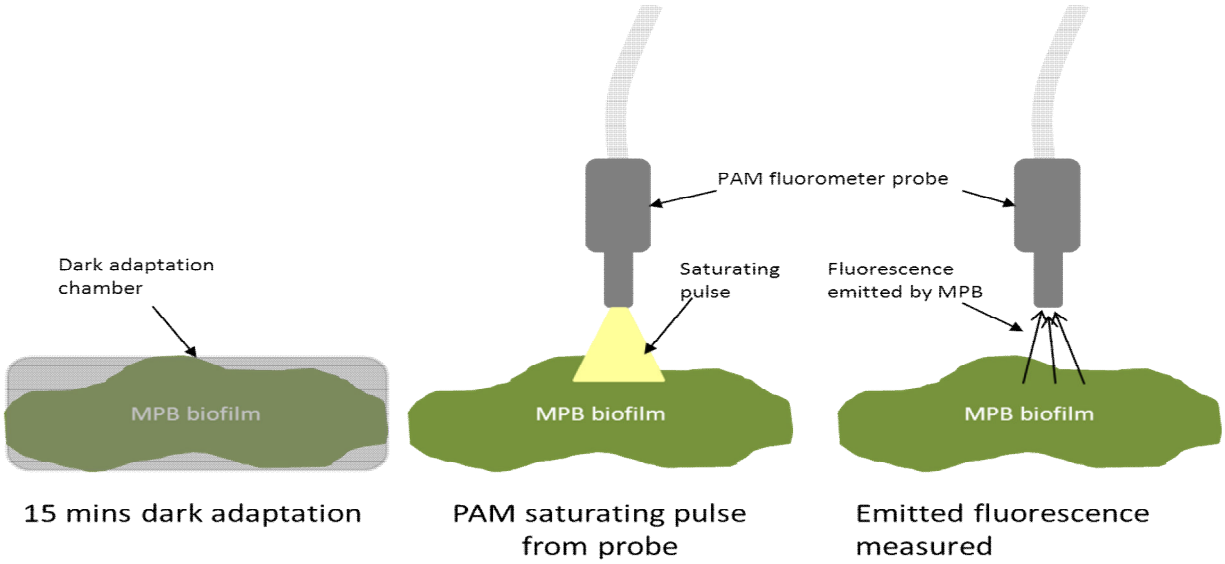


Figure 2.6 The use of PAM fluorometry to measure MPB biomass. The sediment is dark adapted for 15 minutes before measuring minimum fluorescence, which indicates the chlorophyll *a* content in the sediment surface.

For all climate experiments, the biomass of the microphytobenthos was measured using a PAM fluorometer (DIVING-PAM, Heinz-Walz GmbH, figure 2.7). The mesocosms were dark adapted for 15 min before F_o^{15} PAM readings were takenThe measuring probe with the saturation beam was always 4mm from the sediment surface, and the settings and calibrations for PAM were as advised by Perkins (Honeywill *et al.,* 2001).

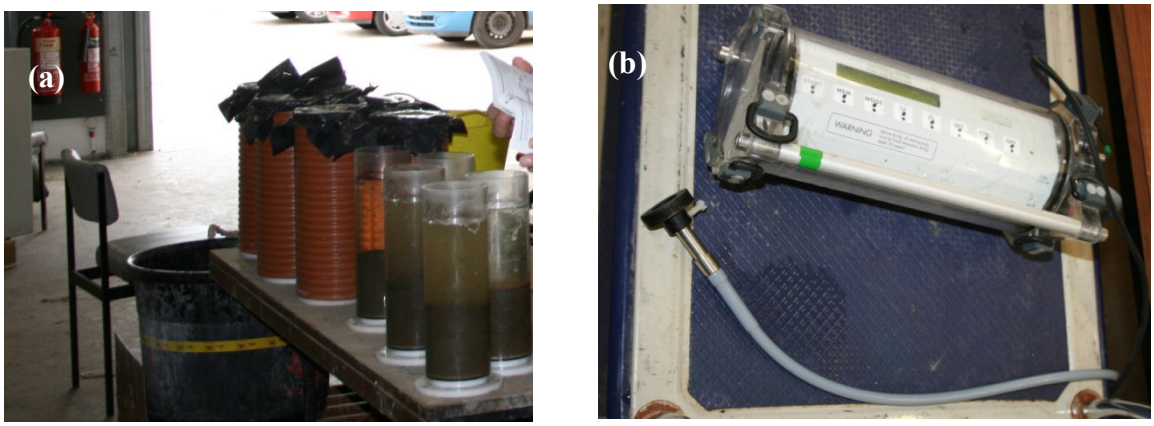


Figure 2.7 (a) the cores being dark adapted for 15 minutes before fluorescence readings are taken with the (b) PAM fluorometer used for the experiments

2.7.1.1 Chlorophyll a

A small sample of sediment was frozen in each mesocosm using the contact core method (Honeywill *et al.,* 2002), wrapped in foil and stored in a -80 °C freezer until removed, freeze-dried and homogenised for spectrophotometric pigment analysis (as in HIMOM, 2003). . In each experiment, contact cores (frozen discs of surface sediment: 56 mm diameter; 2-3 mm depth, as described in Honeywill *et al.,* 2002) of the sediment surface were also taken for further chlorophyll *a* analysis as a safety measure in case of any uncertainty with the PAM readings.

2.7.2 Nutrient analysis

At the end of each experiment, water samples were taken using 0.45µm Nalgene pre-filters and were analysed for three nutrients (NH_4-N, NO_3-N and PO_4-P). Nutrient concentration was determined using a modular Flow Injection Auto-Analyser (FIA Star 5010 series) with an artificial seawater carrier solution (2.5% NaCl).

2.7.3 pH and Oxygen concentration

The pH and oxygen concentration of the overlying water was measured at the start and end of each experiment (Fig. 2.8a). The pH readings were taken using a portable pH meter and the oxygen readings were measured using an optode (Seatronics).

2.7.4 Bioturbation

Luminophore particles are tracer particles that often used to quantify sediment reworking (bioturbation), and this provides an index of macrofaunal bioturbatory activity (Gilbert *et al.,* 2003; Solan *et al.,* 2004). Luminophores (2 g per mesocosm) were evenly distributed on the

sediment surface 24 hours after the macrofauna were added (Fig. 2.8b) . A sediment core (10 cm deep, 3.5 cm diameter) was taken from each mescosm at the end of the experiment and sliced (0.5mm for the top 2 cm, and 1 cm for the remainder). Each slice was individually bagged and frozen, before being homogenised, dried and analysed for total count of luminophore particles using standard image analysis techniques to provide the bioturbation index (as in Solan *et al.,* 2004; Bulling *et al.,* 2010).

Figure 2.8 (a) oxygen readings from the overlying water using the optode (b) the cores lined up ready for the pre-weighted luminophores (in the vials in front of each core) to be added

2.8 Data Analysis

All analyses were performed using the statistical programming software 'R' (R Development Core Team 2005), and the additional 'nlme' (linear and non-linear mixed effects models) package (Pinheiro *et al.,* 2006).

2.8.1 Static Mesocosm Experiments

All response variables were regarded as a measure of ecosystem functioning, with macrofaunal species richness (or species combination), CO_2 concentration, temperature, light regime and temperature fluctuation as nominal explanatory variables. Initially, a linear regression model was fitted and assessed for normality (Q-Q plots), homogeneity of variance,

and outlying values (Cook's distance) (as in Ieno *et al.,* 2006; Dyson *et al.,* 2007; Bulling *et al.,* 2008; Bulling *et al.,* 2010).

Due to the gradient of species richness increasing within a finite species pool, variation across treatments was unequal (Ieno *et al.,* 2006), and this was reflected in the plots of the model residuals. To account for this heterogeneity of variance, a generalised least squares (GLS; Pinheiro and Bates, 2001; West *et al.,* 2007; Zuur *et al.,* 2007) mixed modelling approach was used in preference to a linear regression of transformed data (as in Dyson *et al.,* 2007; Bulling *et al.,* 2008). The most appropriate variance-covariate structure for each model was determined using a combination of AIC scores and examination of plots of fitted values versus residuals based on a full model using restricted maximum likelihood (REML, West et al., 2007; Bulling *et al.,* 2010). The minimum adequate model was then determined using manual backwards stepwise selection, using maximum likelihood methods. Only the highest relevant interaction terms were assessed at each stage, nested terms within the interactions not being tested, following Underwood (1997). The importance of each independent term within the final models was tested using a likelihood ratio test between the full and reduced models (with the tested independent term removed, including interactions).

2.8.2 Tidal Experiments

To account for the slight differences in light levels across the tidal tanks, position within the tank where the readings were taken was added as a random effect in a linear mixed effects model. This allowed the model to account for variation across the tidal tanks in relation to position. CO_2 concentration, temperature and tidal regime were treated as nominal explanatory variables and formed the 'fixed' component of the model. All interaction terms for the explanatory variables were included in the base model. Initially, a linear regression

model was fitted and assessed for normality (Q-Q plots), homogeneity of variance, and outlying values (Cook's distance) (as above).

The most appropriate variance-covariate structure for the model was determined using a combination of AIC scores and examination of plots of fitted values versus residuals based on a full model using restricted maximum likelihood (REML; West et al., 2007; Bulling *et al.,* 2010). The minimum adequate model was then determined using manual backwards stepwise selection, using maximum likelihood methods. As before, only the relevant highest interaction terms were assessed at each stage (Underwood, 1998).

3. Interactions between trophic levels mediate biodiversity-ecosystem functioning in a marine system under two different light regimes

3.1 Introduction

Marine ecosystems are currently under threat from a range of anthropogenic activities, such as overfishing, habitat destruction, pollution, and climate change (Sala *et al.,* 2000; Halpern *et al.,* 2008). These activities affect the biodiversity of ecosystems across all trophic levels (Pauly *et al.,* 1998; Halpern *et al.,* 2008). Despite the wealth of research conducted over the last twenty years on defining the relationship between biodiversity and ecosystem function, very few studies have addressed the role of trophic complexity (Raffaelli, 2006; Cardinale *et al.,* 2006; Duffy *et al.,* 2007; Reiss *et al.,* 2009).

The majority of biodiversity and ecosystem studies based in marine ecosystems have only considered one trophic level, such as the role of primary producers (Loreau *et al.,* 2001; Hooper *et al.,* 2005; Balvanera *et al.,* 2006; Long *et al.,* 2007), despite calls for research to incorporate multi-trophic levels (Balvanera *et al.,* 2006; Raffaelli, 2006; Duffy, 2007). In natural communities, the interaction between species in different trophic levels influences a variety of ecosystem processes (Loreau *et al.,* 2001; Duffy and Stachowicz, 2006; Duffy, 2007). It is well-documented that the interactive effects of MPB and invertebrates influence ecosystem processes in marine systems (Huxham *et al.,* 2006; Dyson *et al.,* 2007; Tang and Kristensen, 2007). However, studies that have incorporated trophic levels have been predominantly observational (Huxham *et al.,* 2006), or have been carried out in darkness (Cibic *et al.,* 2007), making it impossible to determine the role of photosynthetic organisms and how diurnal activity of macrofauna may affect benthic metabolism (Wenzhofer and Glud, 2004). In addition, few studies have examined how the benthic trophic status (the

balance between heterotrophic and autotrophic organisms) influences nutrient fluxes in marine coastal sediments (Engelsen *et al.,* 2008).

Microphytobenthos (MPB) play an important role in estuarine primary productivity and nutrient recycling (Underwood and Kromkamp, 1999; Forster *et al.,* 2006) and enhance sediment stability by forming biofilms on the sediment surface (Hagerthey *et al.,* 2002). These benthic microalgae form the trophic base of estuarine food chains (Huxham *et al.,* 2006) as a substantial food source for the macrobenthos (Huxham *et al.,* 2006; Cibic *et al.,* 2007). Estuarine sediments are vital sites of biogeochemical activity (Engelson *et al.,* 2008) and this constant nutrient cycling contributes significantly to global nutrient budgets.

In estuarine systems, the interaction between the deposit feeders and grazers (infauna) and the primary producers (MPB) mediates nutrient flux at the sediment-water interface (Huxham *et al.,* 2006). Manipulative biodiversity and ecosystem function studies have identified the role of MPB in nutrient cycling (Underwood and Kromkamp, 1999; Sundback *et al.,* 2000; Forster *et al.,* 2006; Cibic *et al.,* 2007), and the separate impact of infauna, through bioturbation, on biogeochemical processes (Mermillod-Blondin *et al.,* 2005; Ieno *et al.,* 2006; Karlson *et al.,* 2007). The presence of MPB is known to directly alter nutrient fluxes through uptake and assimilation of nutrients, such as NH_4, and indirectly alters the rate of nutrient transfer across the sediment-water interface through photosynthesis (Thornton *et al.,* 2007), acting as a 'filter' between the sediment and overlying water (Sundback *et al.,* 2000; Cibic *et al.,* 2007). Since photosynthesis occurs in the light, the flux of nutrients, particularly ammonia, from the MPB-rich sediment into the overlying water is reduced in light (Thornton *et al.,* 2007). In darkness when photosynthesis is not taking place, there is a net flux of ammonium from the sediment into the overlying seawater (Sundback *et al.*, 2004; Thornton

et al., 2007). The impact of microphytobenthos on sediment biogeochemical processes is mediated by the presence and activity (grazing, excretion, bioturbation) of macrofauna (Tang and Kristensen, 2007). These activities alter sediment-water nutrient fluxes by altering surficial MPB biomass and increasing oxygen concentration depth within the sediment, (Sundback *et al.,* 2000; Dyson *et al.,* 2007). Thus the interaction between MPB and macrofauna directly affects benthic metabolism (Huxham *et al.,* 2006; Tang and Kristensen, 2007).

This study examines the interaction between MPB (primary producers) and macrofauna (consumers) and its influence on nutrient concentrations under both dark and light treatments in order to identify the role of photosynthesis by MPB (Sundback *et al.,* 2004; Cibic *et al.,* 2007) in nutrient cycling, and to examine the relationship between MPB and macrofauna.

3.2 Materials and Methods

3.2.1 Sediment, MPB and macrofaunal collection

The collection and treatment of sediment, MPB and macrofauna are described in Chapter 2 (Methods and Materials).

3.2.2 Experimental Design

The experimental design included 64 static mesocosms used to determine the effects of light, macrofaunal species richness and species assemblage composition on MPB biomass and overlying water nutrient concentrations. The mesocosms were divided equally between two environmental chambers, and one chamber was set to run in constant darkness, whilst the other chamber was set to run on a 12 h light-dark cycle to facilitate photosynthesis by the MPB. All mesocosms were randomly placed into the chamber, individually aerated for the duration of the experiment (6 days), and maintained at a constant temperature of 12º C (±0.1ºC). To ensure that the MPB biofilms were not nutrient limited during the experimental period, seawater was replaced after 72 h (Defew *et al.,* 2002).

3.2.2 Chlorophyll a

As a proxy for MPB biomass, chlorophyll *a* content was determined spectrophotometrically from surficial sediment (less than 3mm depth), and expressed as an absolute value calculated against known standards using spinach for chlorophyll content. Sediment (~6 cm^3) was frozen and collected at the end of the experiment using the contact core method (Honeywill *et al.,* 2002), freeze-dried and homogenised.

3.2.3 Nutrient concentration

Water samples were taken from each mesocosm at the end of the experiment using a 0.45µm Nalgene filter to remove any large particles. The samples were immediately frozen (-4 °C) and then removed prior to nutrient analysis. The concentration of nutrients (NH_4-N, NO_3-N and PO_4-P) in the overlying seawater was used as an indicator of ecosystem functioning and determined using a modular Flow Injection Auto-Analyser (FIA Star 5010 series) with an artificial seawater carrier solution (2.5% NaCl).

3.2.4 Data Analysis

The data (chlorophyll *a* (MPB biomass) and nutrients) were analysed using a linear regression with a GLS extension (as described in Chapter 2), with nutrient concentration and MPB biomass treated as dependent variables. Species richness, species identity and light regime were treated as nominal explanatory variables, and chlorophyll *a* was treated as an explanatory variable for the nutrient models. The final models were checked using the manual backwards stepwise selection method (West *et al.,* 2007).

3.3 Results

3.3.1 MPB biomass

The minimum adequate models for chlorophyll *a* (MPB biomass) contained species richness or species identity as the single independent variable. Light was not significant to the model. MPB biomass decreased with increasing macrofaunal species richness (Fig. 3.1: *L-ratio = 12.75, df = 3, p = 0.0052*). Chlorophyll *a* content was highest in the absence of macrofauna (in the controls), but did not necessarily reduce incrementally with each level of species richness.

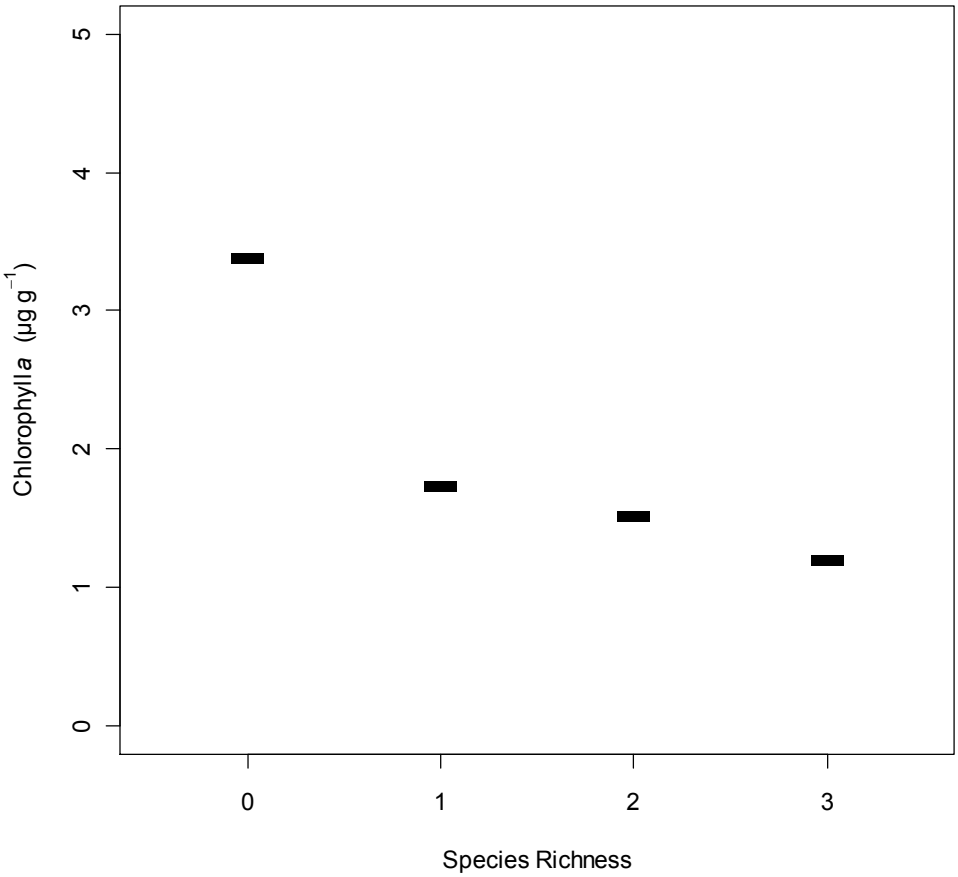


Figure 3.1 Model visualisation of the predicted chlorophyll *a* concentrations (MPB biomass) with an increasing species richness gradient

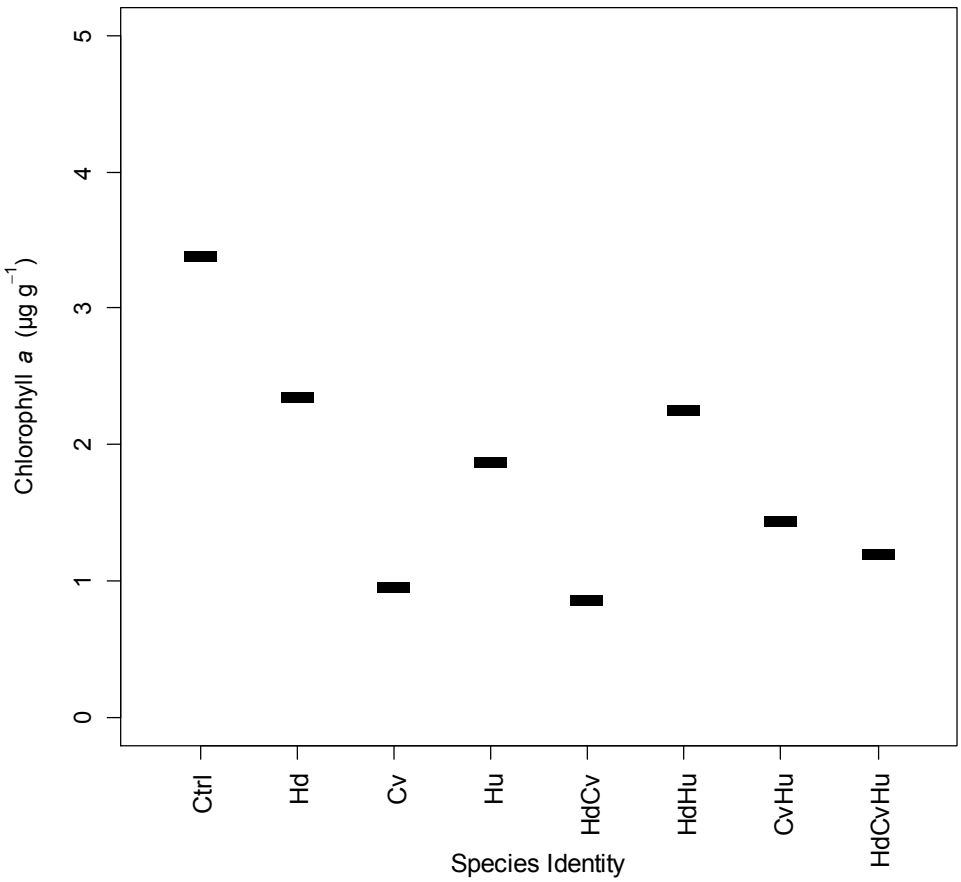
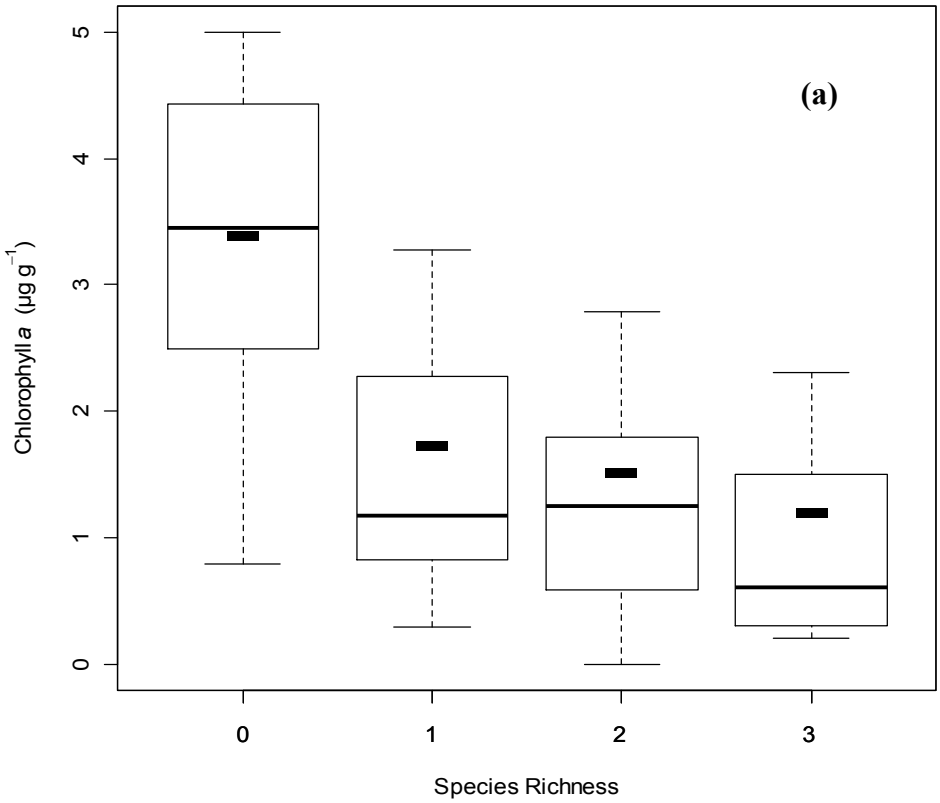


Figure 3.2 Model visualisation of the predicted chlorophyll *a* concentrations (MPB biomass) with different species assemblage compositions

The species richness effects were dependent on the composition of the macrofaunal assemblage (Fig. 3.2: *L-ratio = 52.75, df = 7, p <0.0001*). *C. volutator* (coefficient = -2.42 ±0.46, t=-5.33, p<0.0001) had the greatest negative impact on chlorophyll *a* content, followed by *H. ulvae* (coefficient = -1.51 ±0.48, t=-3.13, p<0.01) and *H. diversicolor* (coefficient = -1.03 ±0.51, t=-1.99, p=0.05), in the single species treatments. The effects of *C. volutator* were also expressed in the multispecies mixtures. With the exception of the HdCv treatment, the final model visualisations for species richness and species identity accurately reflect the trends seen in the raw data (Fig. 3.3).



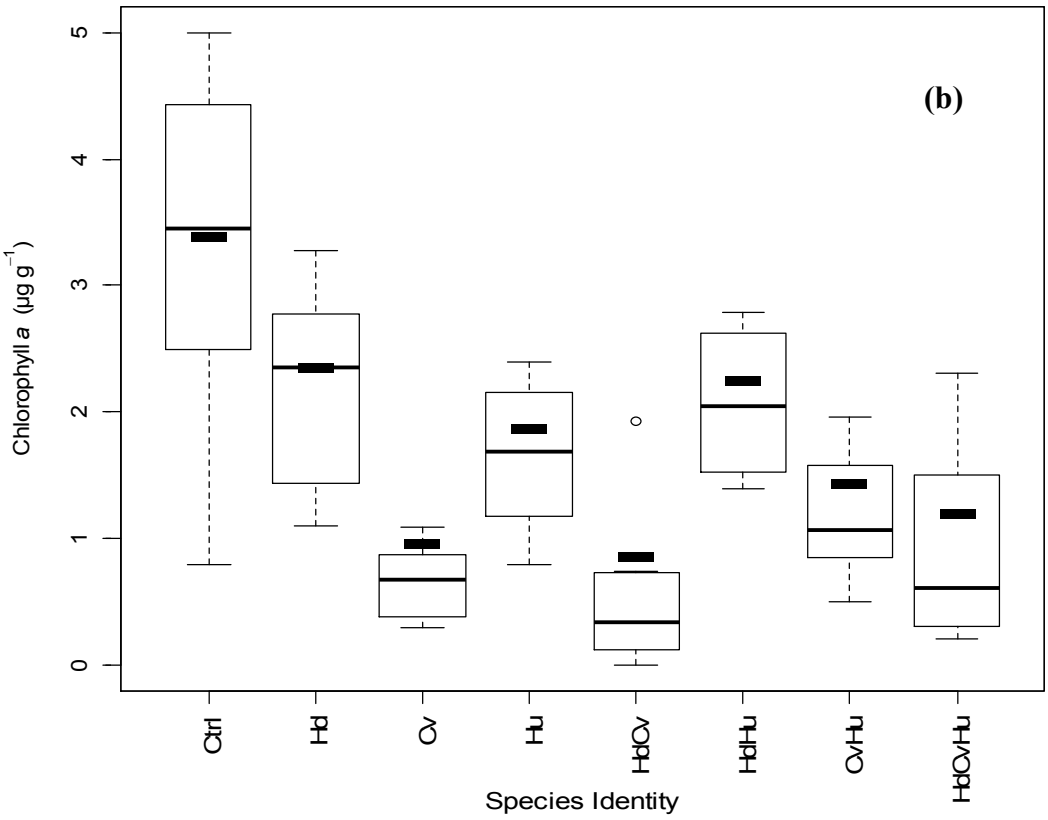


Figure 3.3 Box plots of raw data showing chlorophyll *a* concentration (MPB biomass) with increasing species richness (a) a differing species assemblage composition (b). Both graphs show the model visualisations (solid bars) on top of the raw data boxplot. The narrow line within the box represents the median of the raw data, and the upper and lower edges of the box represent the upper and lower quartile respectively, with the 'whiskers' representing the spread of data.

3.3.2 Nutrient concentrations

The minimum adequate models for NH_4-N and PO_4-P contained a three-way interaction with the three independent variables (species richness/identity × light regime × chlorophyll *a*). The raw data boxplots can be found in Appendix 1.

3.3.2.1 Species Richness

In the NH_4-N model (Fig. 3.4), light regime had the greatest effect, followed by species richness and lastly by chlorophyll *a* (L-ratio = 9.827126, df = 17, p = 0.0201). In contrast, in the PO_4-P model (Fig. 3.5), species richness had the greatest influence, followed by light and a marginal influence of chlorophyll *a*.

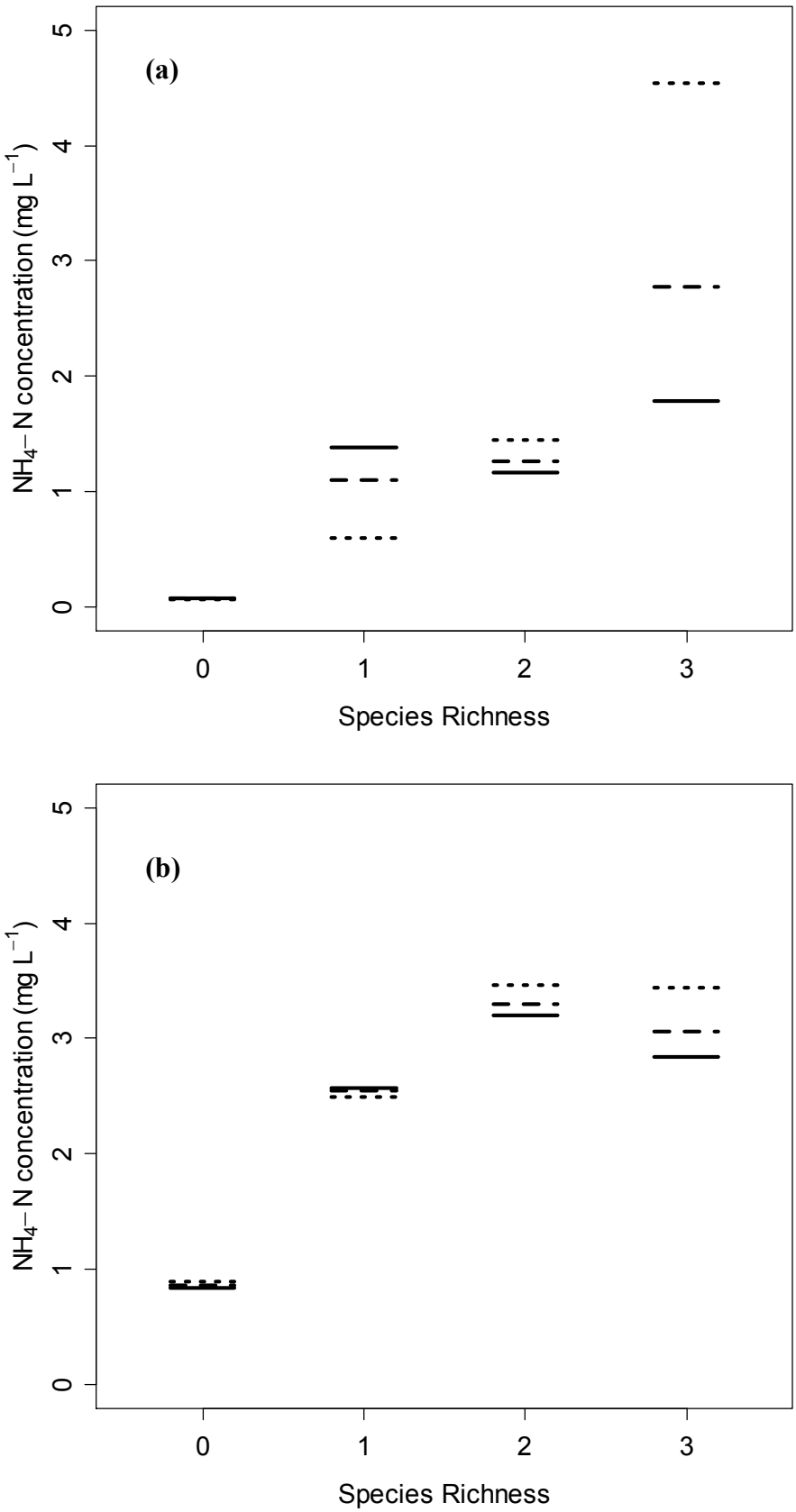


Figure 3.4 NH_4-N model visualisations of the three-way interaction species richness × light × chlorophyll *a* on nutrient concentration under a 12hr Light/Dark cycle (a) and under constant darkness (b) with increasing species richness. Chlorophyll *a* concentrations are fixed to lower quartile (solid line), median (dashed line) and upper quartile (dotted line) of measured chlorophyll values

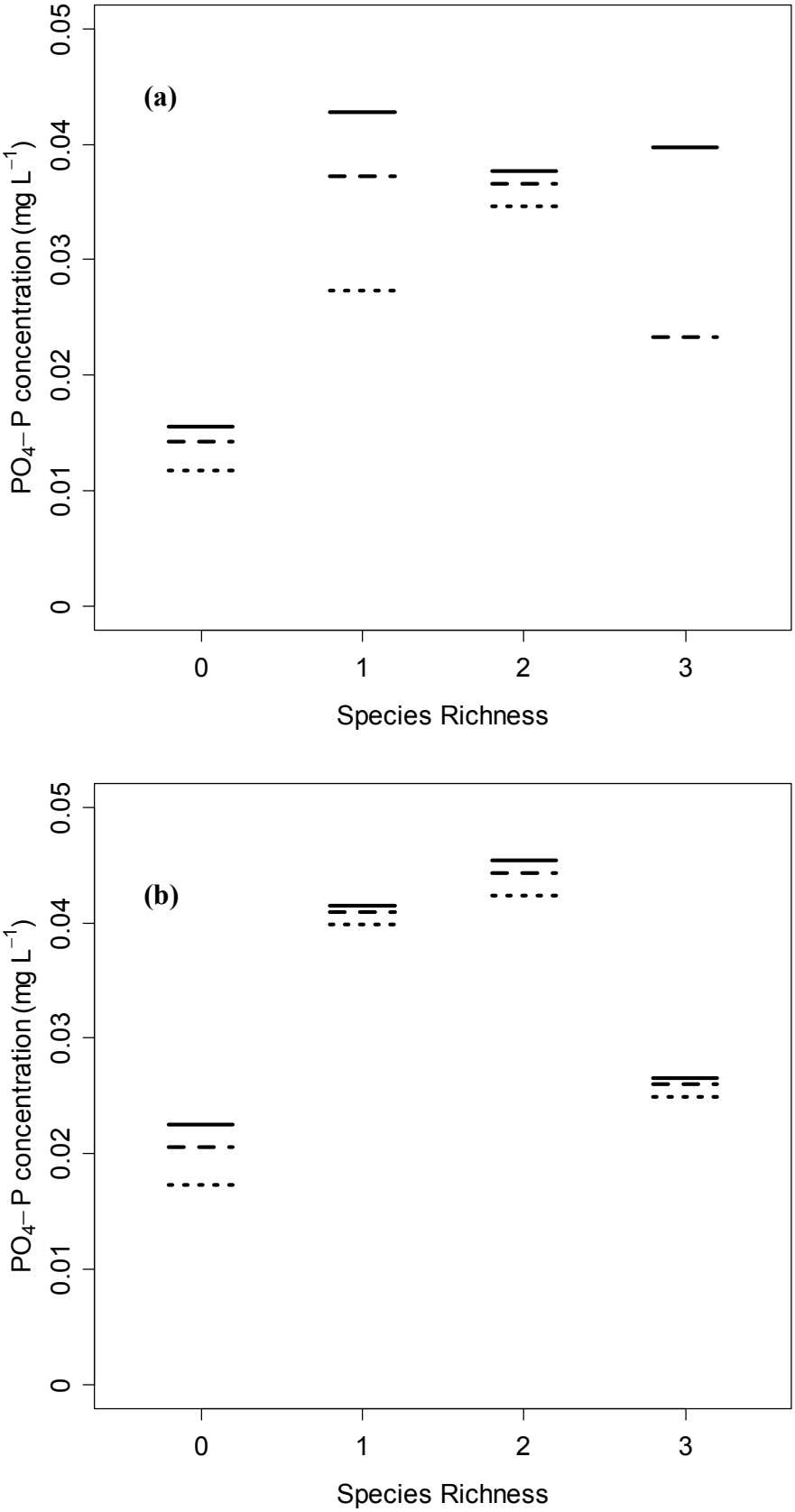


Figure 3.5 PO_4-P model visualisations of the three-way interaction species richness × light × chlorophyll *a* on nutrient concentration under a 12hr Light/Dark cycle (a) and under constant darkness (b) with increasing species richness. Chlorophyll *a* concentrations are fixed to lower quartile (solid line), median (dashed line) and upper quartile (dotted line) of measured chlorophyll values

Concentrations of NH_4-N tended to be higher in the dark regime relative to the L/D regime, irrespective of species richness level, but this effect was less noticeable in the PO_4-P levels. Levels of NH_4-N and PO_4-P were lowest in the control treatments, but in the presence of macrofauna the effect of chlorophyll a content was variable, with higher chlorophyll a leading to higher NH_4-N but lower PO_4-P concentrations. Interestingly, the form of the species richness – NH_4-N concentration relationship was modified by light regime, changing from an asymptotic curve in the dark to a linear to exponential curve in the L/D regime (Fig. 3.4).

3.3.2.2 Species Identity

A further GLS regression analysis was performed, replacing the explanatory variable 'species richness' with species identity, to determine whether species compositional effects underpinned the observed biodiversity effects. The minimal adequate models for both nutrients (NH_4-N*: L-ratio = 21.84782, df = 33, p = 0.0027* and PO_4-P: *L-ratio = 19.72946, df = 34, p =0.0062*) included a three-way interaction (species identity × light regime × chlorophyll *a*). As in the species richness model, light was the most influential variable and chlorophyll *a* was the least important for NH_4-N, whilst for PO_4-P species composition had the greatest effect on nutrient concentration.

The higher levels of NH_4-N seen in the dark regime appeared to be influenced by species composition, most noticeably in the *H. diversicolor* (Hd) single species treatment and multi species treatments (Fig. 3.6). Whilst *H. diversicolor* drives the interaction influencing nutrient concentration in the dark regime, in the L/D regime *C. volutator* (in single and multispecies treatments) has the greatest effect.

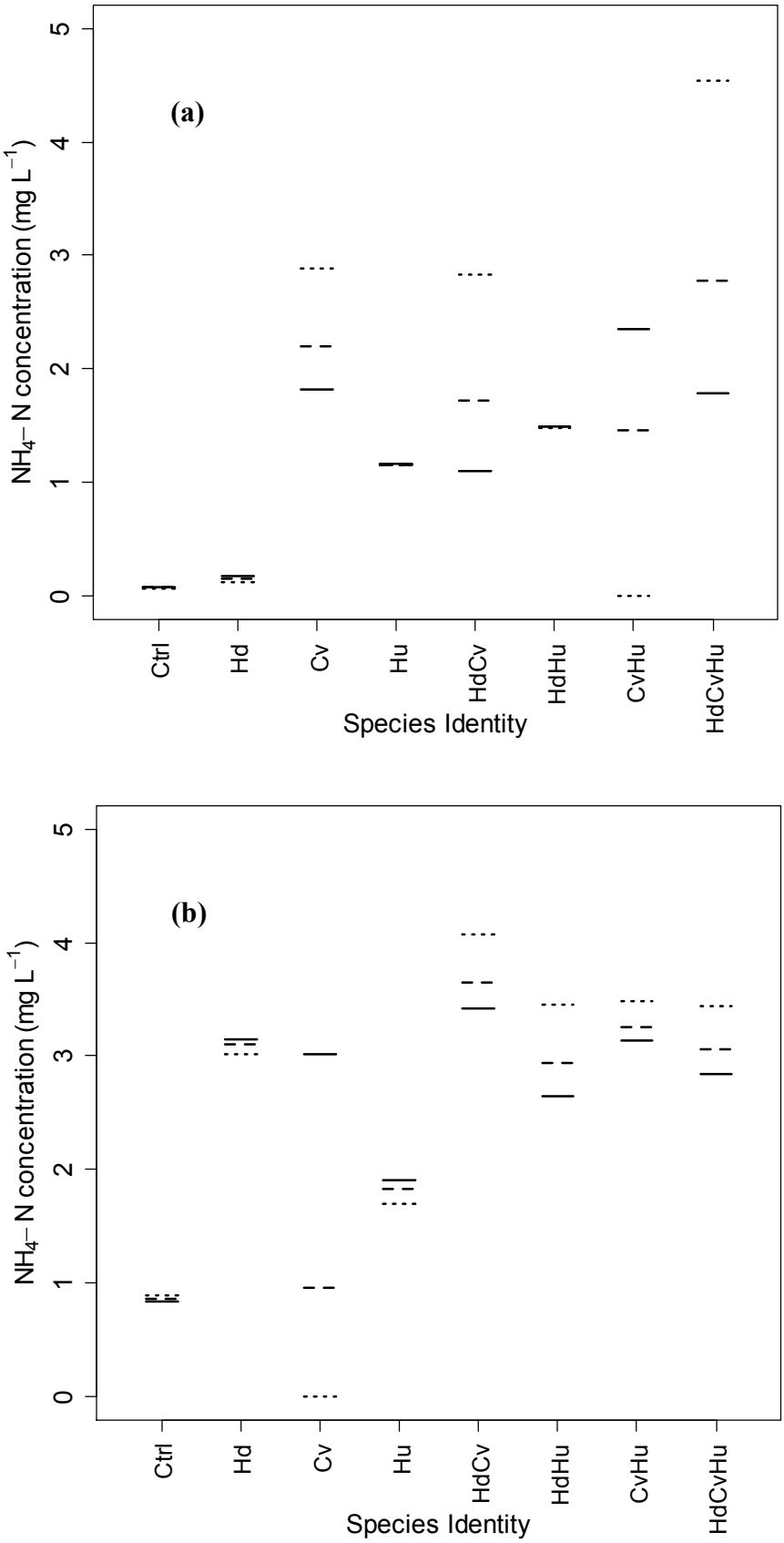


Figure 3.6 NH_4-N model visualisations of the three-way interaction species identity × light × chlorophyll *a* on nutrient concentration under a 12hr Light/Dark cycle (a) and under constant darkness (b) with increasing species richness. Chlorophyll *a* concentrations are fixed to lower quartile (solid line), median (dashed line) and upper quartile (dotted line) of measured chlorophyll values

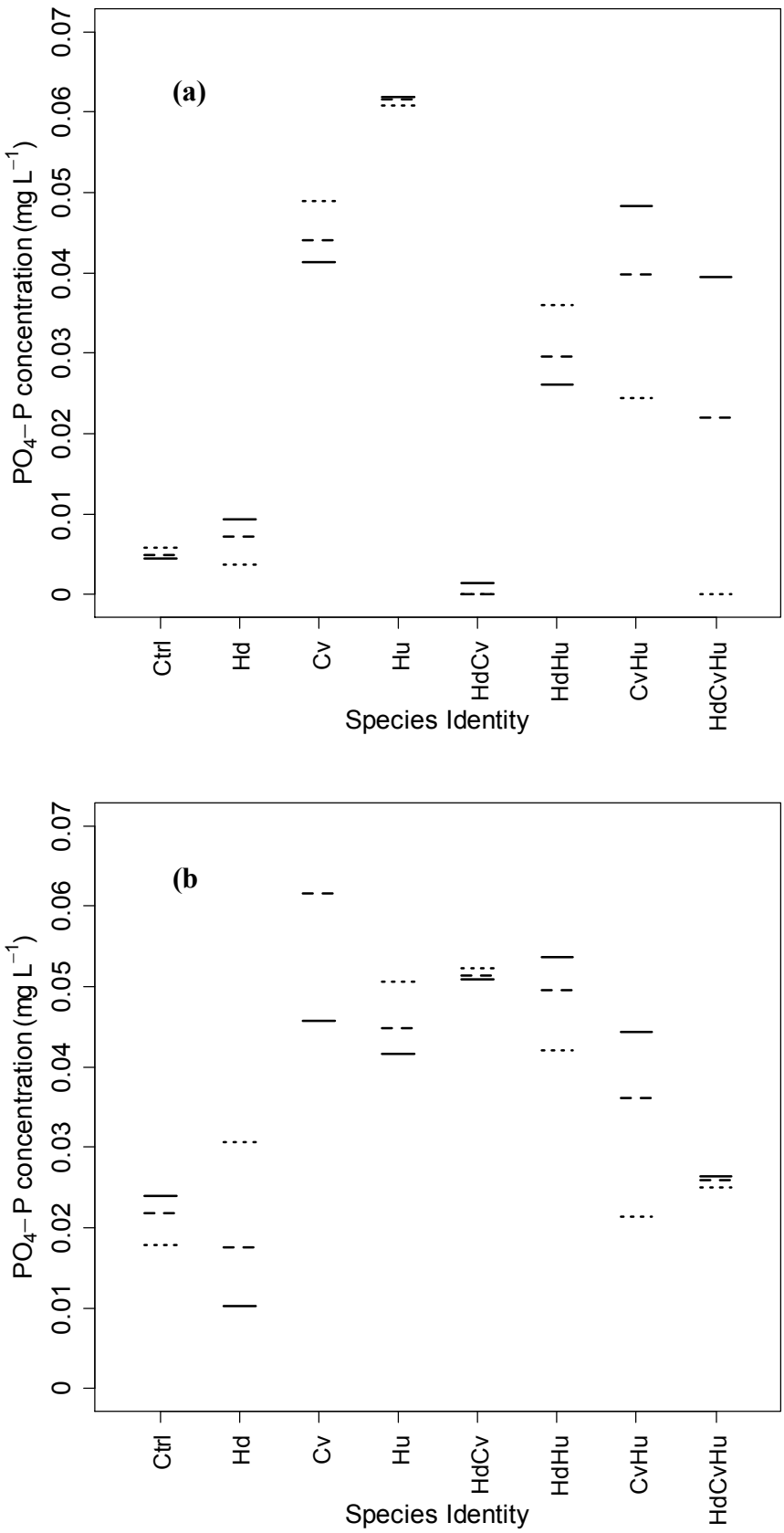


Figure 3.7 PO_4-P model visualisations of the three-way interaction species identity × light × chlorophyll *a* on nutrient concentration under a 12hr Light/Dark cycle (a) and under constant darkness (b) with increasing species richness. Chlorophyll *a* concentrations are fixed to lower quartile (solid line), median (dashed line) and upper quartile (dotted line) of measured chlorophyll values

For PO_4-P, *H. ulvae* was influential in determining the nutrient levels in the L/D regime, which was matched by *C. volutator* in the dark regime (Fig. 3.7). As in the L/D regime for NH_4-N, the single species treatment of *H. diversicolor* produced similar nutrient levels as seen in the controls under the same light regime. Notably, the interaction between the two species mixture *H. diversicolor* and *C. volutator* (HdCv) and chlorophyll *a* in the L/D regime resulted in extremely low levels of PO_4-P.

3.3.2.3 NO_x-N concentration

The minimal adequate model for NO_xN consisted of two two-way interactions for both the species richness model (Fig 3.8) and the species identity model (Fig 3.9). Species richness effects were variable across the richness gradient, but this was most influential variable on nutrient concentration, as reflected in the model visualisations. There was a notable difference in NO_x-N concentrations between the light regimes, particularly in the control treatment (Fig. 3.8a). Levels of NO_x-N varied between species richness levels and with chlorophyll *a* concentration (Fig. 3.8b).

The biodiversity effects were driven by dominant species identity effects. In the NO_x-N models, the single species treatment of *C. volutator* was dominant, as reflected in the model visualisations of the two-way interactions (Fig. 3.9) and in the raw data boxplots (Appendix 1). These effects were not as pronounced in the multispecies treatments. With the exception of *C. volutator* in monoculture, increased concentrations of chlorophyll *a* had either no effect (Hd and Hu) or significantly decreased NO_x-N concentration (all of the multispecies treatments – Fig. 3.9b).

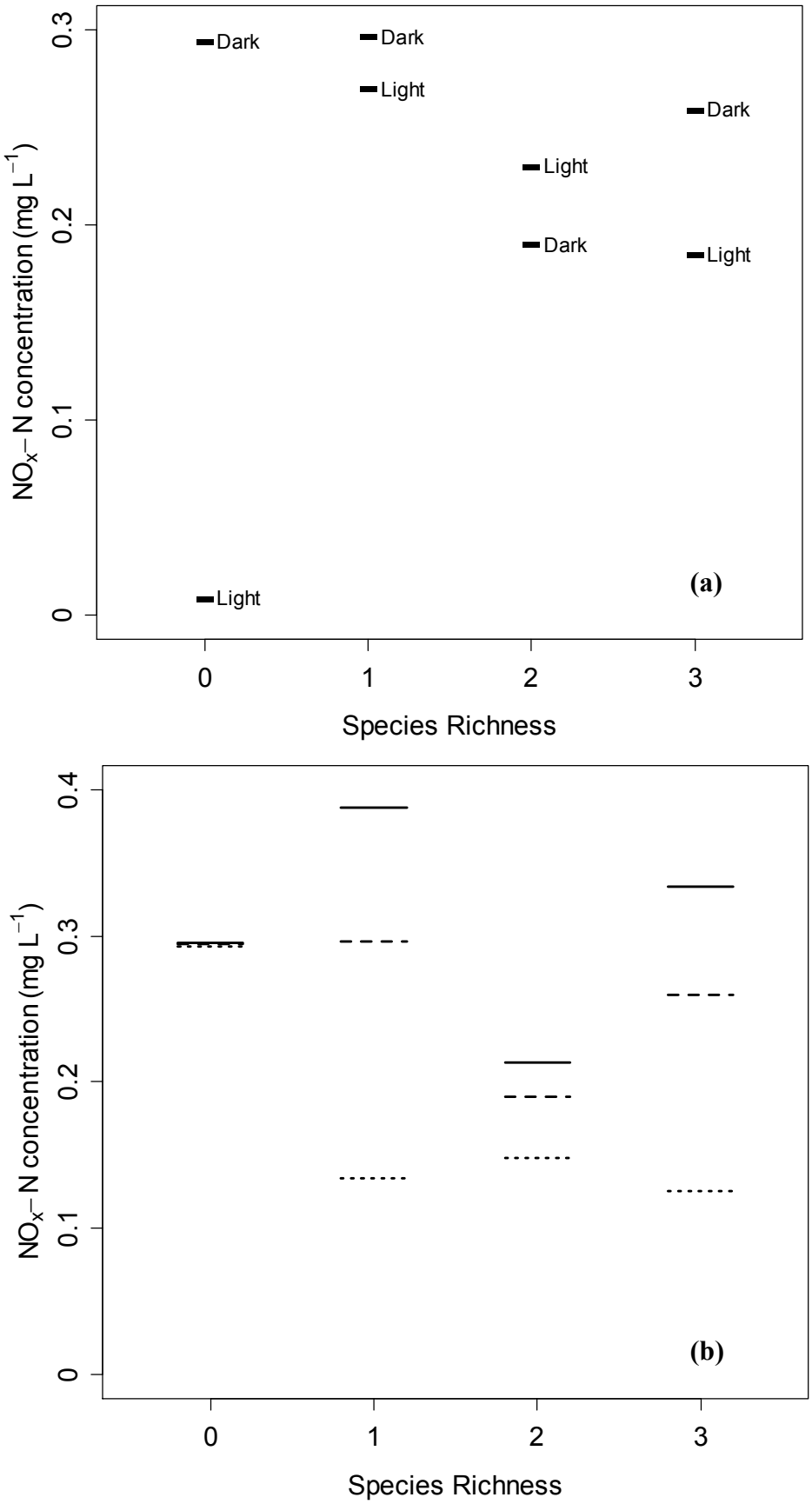


Figure 3.8 NO_x-N model visualisations of the two-way interactions (a) species richness × light and (b) species richness × chlorophyll *a* on nutrient concentration under a 12hr Light/Dark cycle. Chlorophyll *a* concentrations are fixed to lower quartile (solid line), median (dashed line) and upper quartile (dotted line) of measured chlorophyll values

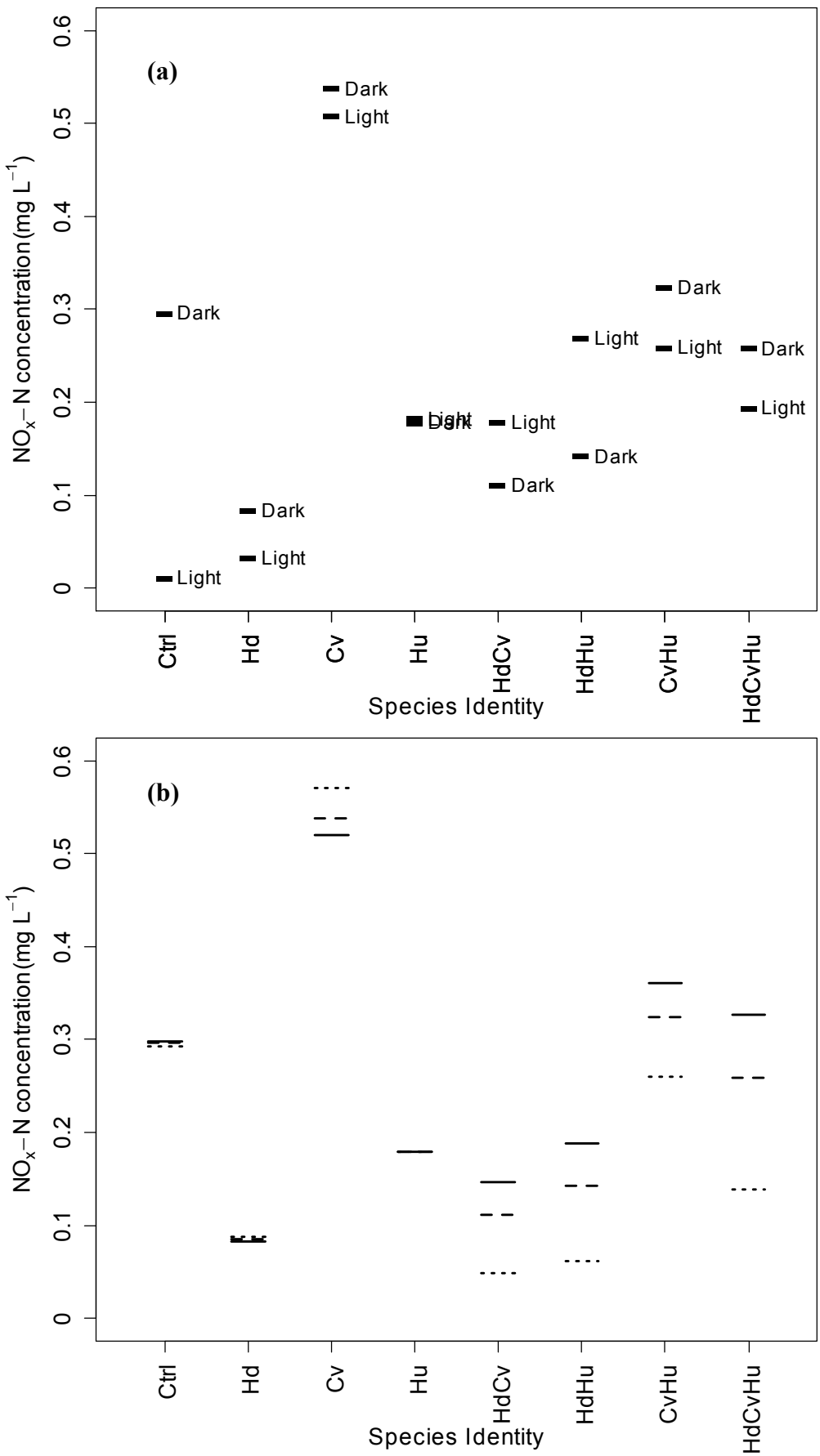


Figure 3.9 NO_x-N model visualisations of the two way interactions (a) species identity × light and (b) species identity × chlorophyll *a* on nutrient concentration under a 12hr Light/Dark cycle. Chlorophyll *a* concentrations are fixed to lower quartile (solid line), median (dashed line) and upper quartile (dotted line) of measured chlorophyll values

3.4 Discussion

The metabolic activity of MPB and the burrowing and respiration of invertebrates alters nutrient fluxes at the sediment water interface (Thornton *et al.,* 2007). Although there was no significant difference in MPB biomass between light regimes, the difference in nutrient concentrations, particularly NH_4-N, between the light regimes clearly illustrates the role that photosynthetic microalgae play in mediating nutrient exchange between the sediment and the overlying water. The lower ammonium concentrations in the light regime can be explained by the MPB preferentially utilising NH_4, rather than nitrate and nitrite, as a nitrogen source (Sundback *et al.,* 2000; Welker *et al.,* 2002). Previous studies have shown that NH_4 levels can decrease by up to 100% in the light due to MPB activity (Sundback *et al.,* 2000; Thornton *et al.,* 2007), and this is reflected in our results with the contrasting NH_4-N concentrations between the L/D and dark regime.

In the absence of light, MPB did not take up the nutrients in the overlying water as they were unable to photosynthesise, and this directly impacts on the rate of nutrient transfer between the sediment-water interface (Cibic *et al.,* 2007) as reflected by the concentrations in the dark regime. Lower levels of ammonium in the cores experiencing the L/D regime may also have been due to decreased denitrification and increased nitrification (Dong *et al.,* 2000), which occurs in the top oxic layer of the sediment (Thornton *et al.,* 2007). These processes are directly mediated by elevated oxygen concentrations through MPB photosynthesis (Risgaard-Petersen, 2003). Whilst the presence of light appears to have directly impacted MPB photosynthetic activity through nutrient flux, macrofauna are also known to be influenced by light/dark cycles (Wenzhofer and Glud, 2004; Tang and Kristensen, 2007). Thus the role of macrofaunal activity on nutrient release into the water column cannot be excluded.

Increased surficial oxygenation increases phosphate adsorption to the sediment (Jin *et al.,* 2006), reducing the levels of PO_4-P in the overlying water (Sundback and Graneli, 1988). Higher levels of PO_4-P were found in the dark treatment where photosynthesis was not occurring and oxygen concentration would have been lower (Jiang *et al.,* 2008). With both NH_4-N and PO_4-P, there was a positive relationship between MPB biomass and nutrient concentration and increasing species richness. Previous studies have suggested that MPB biomass is affected by both nutrient concentrations (bottom up control) and invertebrate grazing (top down control) (Hagerthey *et al.,* 2002; Welker *et al.,* 2002). NH_4-N and PO_4-P concentrations were lowest in the treatment without macrofauna, with the MPB assimilating the nutrients from the water column (Engelsen *et al.,* 2008). As MPB biomass decreased and macrofaunal species richness increased, the removal of nutrients from the water column decreased, indicating a combination of both bottom up and top down control.

In contrast, there was no clear relationship between MPB biomass and NO_x-N. In the control treatments, the dark regime showed higher levels of NO_x-N, possibly due to increased denitrification as the MPB would not have been driving nitrification levels through oxygenic photosynthesis, and light inhibits denitrification (Sundback *et al.,* 2000). However, in the treatments with macrofauna, higher NO_x-N concentrations were found than in the controls, in both the light and dark regimes.

The behaviour and activity of benthic invertebrates also affects nutrient transfer between the sediment and the overlying water (Cibic *et al.,* 2007; Engelson *et al.,* 2008). The role of MPB in nutrient cycling is identifiable in the controls (no macrofauna), but the activity of the invertebrates in the remaining treatments demonstrates how infauna mediate the nutrient levels through bioturbation, bioirrigation, excretion and grazing (Hagerthey *et al.,* 2002;

Cibic *et al.,* 2007). Invertebrate activity had a varied effect on each nutrient between the light regimes, possibly due to ingrained tidal and diurnal behavioural cycles (Wenzhofer and Glud, 2004). Each species altered the MPB biomass and the nutrient flux through differing bioturbation modes and feeding behaviours (Sundback *et al.,* 2000; Hagerthey *et al.,* 2002; Dyson *et al.,* 2007).

The distinct roles played by the invertebrates and the MPB in nutrient cycling were clearly illustrated. Active photosynthesis by MPB results in nutrient retention in the sediment and the removal of nutrients from the overlying water (Sundback and McGlathery, 2005; Engelson *et al.,* 2008). In contrast, the bioturbatory activity of the macrofauna increases the flux of nutrients from the sediment into the water column (Karlson *et al.,* 2007; Norling *et al.,* 2007; Engelson *et al.,* 2008). Thus, in benthic nutrient cycling, MPB can be classified as 'net users', and the macrofauna can be termed as 'net exporters'. The interaction between the users and the exporters determines the trophic status of the benthos (Engelson *et al.,* 2008).

The species richness effects reflected in both the MPB biomass and nutrient concentrations were strongly underpinned by species identity effects. In NH_4-N and PO_4-P, increasing species richness caused increased nutrient concentration (as in Ieno *et al.,* 2006). In high species richness treatments, it is likely that there was greater activity (possibly through bioturbation and grazing) which would have reduced MPB biomass and aided the flux of nutrients from the sediment to the overlying water (Mermillod-Blondin *et al.,* 2005). The single species treatments highlighted how the different bioturbation and grazing activities of the macrofauna affected MPB biomass and nutrient concentration. *C. volutator* had the strongest effect on MPB biomass in all species treatments, which is supported by previous studies (Mermillod-Blondin *et al.,* 2005; Hagerthey *et al.,* 2002; Dyson *et al.,* 2007). This is

due to the grazing and bioturbatory activity of *C. volutator*, which constantly resuspends sediment (Dyson *et al.,* 2007), thus inhibiting photosynthesis and preventing a stable MPB biofilm from forming on the sediment surface (Middelburg *et al.,* 2000). The individual effect of *C. volutator* on MPB biomass was visible in the mixed species treatments, but less apparent for nutrient concentration. *H. ulvae* had a direct negative effect on MPB biomass through grazing (Hagerthey *et al.,* 2002).

In contrast, *H. diversicolor* had the least negative effect on MPB biomass and nutrient concentrations, which supports findings by previous studies (Nizzoli *et al.,* 2007; Dyson *et al.,* 2007; Ieno *et al.,* 2006). *H. diversicolor* constructs extensive burrows in the sediment and, through irrigation of these burrows, increases solute exchange between the sediment and water, mediating microbial activity (Nizzoli *et al.,* 2007; Tang and Kristensen, 2007). However, the activity of the macrofauna is well documented to be strongly influenced by light/dark cycles (Wenzhofer and Glud, 2004; Tang and Kristensen, 2007). *H. diversicolor* ventilates more frequently in light regimes and is less active during darkness (Wenzhofer and Glud, 2004; Deschenes *et al.,* 2005), which alters benthic metabolism (Tang and Kristensen, 2007), and this was reflected in *H. diversicolor* treatments for nutrients under the two light regimes. Although there were clear species identity effects, these were not as apparent in the multi-species treatments, supporting the theory that although species diversity is important for biogeochemical processes, the presence of certain 'functional' species may play a greater role in enhancing benthic-pelagic coupling (Raffaelli *et al.,* 2003; Mermillod-Blondin *et al.,* 2005).

This study highlights the roles of MPB and species richness (and species assemblage composition) on nutrient concentration, and whilst both microphytobenthos and macrofauna

are important in influencing nutrient flux, their effects appear to be linked, illustrating the importance of examining more than one trophic level within an ecosystem (Long *et al.,* 2007). These results clearly demonstrate that in-depth knowledge of the interactions between macrofauna and MPB is important in understanding ecosystem processes in marine benthic sediments. However, in order to identify the independent role of MPB from macrofaunal species effects, it would be worthwhile manipulating MPB biomass in future studies.

Diversity effects at one trophic level are likely to affect other trophic levels within an ecosystem (Duffy *et al.,* 2007). In the same way, diversity should be incorporated both vertically (between trophic levels) and horizontally (within trophic levels) for results to reflect the true mechanisms behind ecosystem functioning in ecology (Duffy *et al.,* 2007). Very little attention has been given to including inter-trophic and intra-trophic diversity in experimental and theoretical studies (Thébault and Loreau, 2003). The trophic interactions that occur within an ecosystem affect the relationship between biodiversity and ecosystem functioning, and, unlike in single trophic level systems, ecosystem responses to changes in diversity, and environmental changes, are likely to be complex and unpredictable (Thébault and Loreau, 2003). To date, over a decade of research on biodiversity and ecosystem functioning has led to a better understanding of how species loss may affect an ecosystem in model systems with single trophic levels (Thébault and Loreau, 2003; Cardinale *et al.,* 2006; Duffy *et al.,* 2007). A key challenge for future research is to understand the complex relationship between biodiversity and ecosystem processes in multi-trophic systems, both experimentally and theoretically, in order to comprehend how natural communities may respond to anthropogenically-driven environmental changes over the next few decades.

4. Effects of constant mean temperature and elevated carbon dioxide on primary production in an intertidal marine model system

4.1 Introduction

Rising global temperatures and increasing atmospheric CO_2 concentrations are causing changes to a wide range of ecosystems (Parmesan and Yohe, 2003). The influence of these changing conditions on ocean chemistry and the distribution of species in marine systems (Caldeira and Wickett, 2003; Harley *et al.*, 2006; Primack *et al.*, 2009) is of great interest to scientists. Atmospheric concentrations of CO_2 have risen from pre-industrial levels (275 ppmv) to 370 ppmv and continue to increase by ~1.5 ppmv yr^{-1} (Hoffert *et al.*, 2002). Up to 50% of the global increase in carbon dioxide has been absorbed by the oceans (Sabine *et al.* 2004) and the pH of the sea is predicted to fall by up to 0.5 pH units by the end of the century (Caldeira and Wickett, 2003). Concerns over the likely consequences are now widespread (IPCC, 2007; Brown *et al.*, 2010). Studies of elevated carbon dioxide concentrations have demonstrated potential impacts on nutrient availability, primary productivity and decomposition (Parmesan and Yohe, 2003; Hargrave *et al.*, 2009; Bulling *et al.*, 2010). This directly influences the functionality of ecosystems (Hargrave *et al.*, 2009) across multiple trophic levels (Walther *et al.*, 2002) and effects are difficult to anticipate.

Empirical research to-date has concentrated on the responses of a variety of ecosystems to individual anthropogenic drivers of change (terrestrial - Long *et al.*, 2004; marine - McWilliams *et al.*, 2005; terrestrial soil - Lou *et al.*, 2006; freshwater - Hargrave *et al.*, 2009), but few studies have considered the combined effects of multiple drivers (Halpern *et al.*, 2008). This is of particular importance because cumulative and/or interactive effects between drivers are very likely to be influential in determining levels of ecosystem

functioning (Folt *et al*., 1999; Christensen *et al.*, 2006; Duffy *et al*., 2007; Crain *et al.*, 2008; Przeslawski *et al.*, 2008; Beveridge *et al*., 2010; Bulling *et al.*, 2010; Gaedke *et al*., 2010). It is well-recognised that services derived from ecosystems are essential to human welfare (Dobson *et al.*, 2006; Millennium Ecosystem Assessment Report, 2006; Ronnback *et al*., 2007; Halpern *et al.*, 2008; Paterson *et al*., 2009) and could be critically affected through climate change (Menzel *et al*., 2008). Consequently, research that examines the effects of multiple climate change factors, such as temperature and carbon dioxide, and altered levels of biodiversity on ecosystem functioning is essential and timely.

In the face of growing concern about climate change, the net carbon status of many coastal and estuarine systems has received increasing attention (Migne *et al*., 2009). Net allochthonous systems rely on an external carbon supply for the majority of their carbon metabolism (heterotrophic) while autochthonous systems are dominated by *in situ* carbon fixation (autotrophic). This distinction has been widely applied in the study of lotic systems (e.g. the river continuum hypothesis, Vannote *et al.,* 1980) and has recently been applied to the metabolic status of coastal systems to understand their potential to respond to exploitation (Gatusso *et al*., 1998; Engelson *et al.,* 2008). Relatively subtle environmental perturbations may alter the balance between autotrophy and heterotrophy (Porubsky *et al*., 2008) having profound effects on these ecosystems and the organisms that exploit them. MPB are the main primary producers in many intertidal and shallow subtidal depositional environments (Tang and Kristensen, 2007), and enhance benthic-pelagic coupling through the formation of biofilms (Hagerthey *et al.*, 2002; Forster *et al.*, 2006). Our hypothesis was that interactions between climate change variables and biodiversity would inhibit autochthonous productivity by MPB (Huxham *et al.*, 2006; Cibic *et al.*, 2007; Beardall *et al.*, 2009) and hence would affect the net trophic status of these vulnerable coastal systems. In this paper, we examine the

effects of two climate change variables (temperature and atmospheric CO_2 concentration) combined with a range of biodiversity levels (macrofaunal species richness) on the biomass of MPB within the context of multiple trophic levels (Lohrer *et al*., 2004) using a model experimental mesocosm system.

4.2 Materials and Methods

4.2.1 Sediment

Surface sediment (< 2 cm depth) was collected from tidal mud flats on the Ythan Estuary, Aberdeenshire, Scotland, UK (57° 20.085'N, 02° 0.206' W) and sieved (500 µm) in a seawater bath (UV sterilised, 10 µm filtered, salinity 33) to remove macrofauna. The sediment was left to settle for 48 h before the supernatant was removed, the sediment homogenised and placed in the mesocosms to a depth of 10 cm (785 cm^3) .

4.2.2 Microphytobenthos

To standardize the biomass of the MPB, MPB-rich surface sediment was collected from the Ythan estuary, spread onto a shallow tray (<1 cm depth) and left under constant light for 48 h. This material was then homogenised and distributed (125 cm^3) between mesocosms prior to the addition of seawater.

4.2.3 Macrofauna

The polychaete *H. diversicolor* (HD), the gastropod *H. ulvae (HU)* and the amphipod *C. volutator* (CV) were collected from the study site. These species represent a range of functional types in the way that they bioturbate sediments, and hence drive nutrient flux (Ieno *et al* 2006, Solan et al. 2008), and their grazing pressure on MPB (Dyson *et al*., 2007).

Replicate (n=3) macrofaunal communities were assembled in single and multispecies treatments (HD, HU, CV, HDHU, HDCV, HUCV, HDHUCV). These unique species permutations eliminate pseudoreplication (Huston, 1994) and allow the generic effects of altered biodiversity to be examined. Macrofaunal biomass was set at 2 g wet weight per mesocosm (divided equally between the species present), similar to the natural biomass found

at the study site (Biles *et al.*, 2003). Control mesocosms (n=3) containing the standard MPB biomass but without any macrofauna were also established.

4.2.4 Mesocosm assembly

Mesocosms were Perspex cores 33 cm high with an internal diameter of 10 cm. Following the addition of sediment (10 cm deep) to each mesocosm, MPB and 2.35 l of seawater (UV-sterilized, 10 µm pre-filtered, salinity ≈ 33) were added to give an overlying depth of 20 cm. This intial fill of water was replaced after 24 h to remove the nutrient pulse associated with assembly (Ieno *et al.,* 2006) and macrofauna were then added. All mesocosms were non-tidal and were aerated individually throughout the experiment.

4.2.5 Environmental regimes

Mesocosms were placed in two environmental chambers (V 4100, Vötsch Industrietechnik) capable of controlling temperature (±0.1 ºC). The experiments were run with a 12 h light-12 h dark (L/D) cycle using high intensity discharge sodium lamps (model GE11678, 400w x2, average 300 µmoles m^{-2} s^{-1}). Nine environmental regimes were employed, using three constant temperatures (6 ºC, 12 ºC and 18 ºC, reflecting the annual variation at the study site (see Appendix 1) and three atmospheric carbon dioxide concentrations (370 ppmv (present day), 600 ppmv, 1000 ppmv) in an orthogonal design. Concentrations of 600 ppmv and 1000 ppmv were based on IPCC projections for approximately 50 and 100 years time respectively (TAR, IPCC, 2001 and 4th Assessment Report, IPCC, 2007) and reflect the accepted view that CO_2 levels will rise over the long-term. Atmospheric CO_2 concentrations within the environmental chamberwere maintained using a CO_2 monitor attached to an external CO_2 gas cylinder (BOC gases Ltd, UK) with a digital controller (Technics horticultural carbon dioxide controller), and the mesocosms were individually aerated with the CO_2 enriched air within

the chamber. An Infra-Red Gas Analyser (IRGA, ADC LCA3) was used to calibrate and validate the CO_2 regulation (± 30 ppm). Mesocosms within an environmental chamber were randomly assigned positions to factor out any effects of spatial heterogenity. Each experiment was run for 7 days.

4.2.6 PAM fluorescence

The biomass of the MPB was measured at the end of the experiment using a PAM fluorometer (DIVING-PAM, Heinz-Walz GmbH). This is a widely accepted proxy method for measuring the surficial biomass (chlorophyll *a*) of MPB (Consalvey *et al*., 2004; Consalvey *et al.,* 2005). Fluorescence measurements were made after 15 minutes of dark-adaptation (after Honeywill *et al.*, 2002; Jesus *et al.*, 2005; Jesus *et al.,* 2006).

4.2.7 Data Analysis

MPB biomass was treated as a response variable, with macrofaunal species richness (or species combination), CO_2 concentration and temperature as nominal explanatory variables. Initially, a linear regression model was fitted and assessed for normality (Q-Q plots), homogeneity of variance, and outlying values (Cook's distance) (Pinheiro and Bates, 2001; Zuur *et al.*, 2007). As our experimental design established a gradient of species richness that increased within a finite species pool, variation across treatments was likely to be unequal (Ieno *et al.*, 2006), and this was confirmed by plots of the model residuals. To account for this heterogeneity of variance, a generalised least squares (GLS; Pinheiro and Bates 2001; West *et al.*, 2007; Zuur *et al.*, 2007) mixed modelling approach was used in preference to a linear regression of transformed data (Dyson *et al*., 2007; Bulling *et al.*, 2008; Bulling *et al.,* 2010). The most appropriate variance-covariate structure for each model was determined using a combination of AIC scores and the examination of plots of fitted values versus

residuals based on a full model specification using restricted maximum likelihood (REML, West *et al.*, 2007). The minimum adequate model was then determined through manual backwards stepwise selection, using maximum likelihood methods. The significance of the relevant highest order interaction terms was assessed at each stage, terms nested within these not being tested, following Underwood (1998). The influence of each independent term within the minimum adequate model was assessed using a likelihood ratio test between the minimum adequate model and reduced models (with all terms involving the relevant independent factor removed, including interactions). All analyses were performed using the 'nlme' package (ver. 3.1, Pinheiro *et al.,* 2006) in the 'R' statistical and programming environment (R Development Core Team 2010).

4.3 Results

4.3.1 Microphytobenthos response to climatic variables

The minimal adequate model for the controls (containing no macrofauna), with MPB biomass as the dependent variable, included a two-way interaction (CO_2 × temperature; *L*-ratio = 18.23, d.f. = 12, $p = 0.0011$). Of the two climatic variables, temperature (*L*-ratio = 37.71, d.f. = 6, $p < 0.0001$) was more influential than CO_2 (*L*-ratio = 24.51, d.f. = 6, $p < 0.0001$). There was an apparent decline in MPB biomass with from 6ºC to the two higher temperatures (Fig. 4.1), whilst the CO_2 concentration of 600 ppmv was associated with the higher MPB biomass levels at 6 °C and 18 °C. This trend was reflected in the model visualisation (Fig. 4.2), with MPB biomass highest at 6 °C across all CO_2 levels.

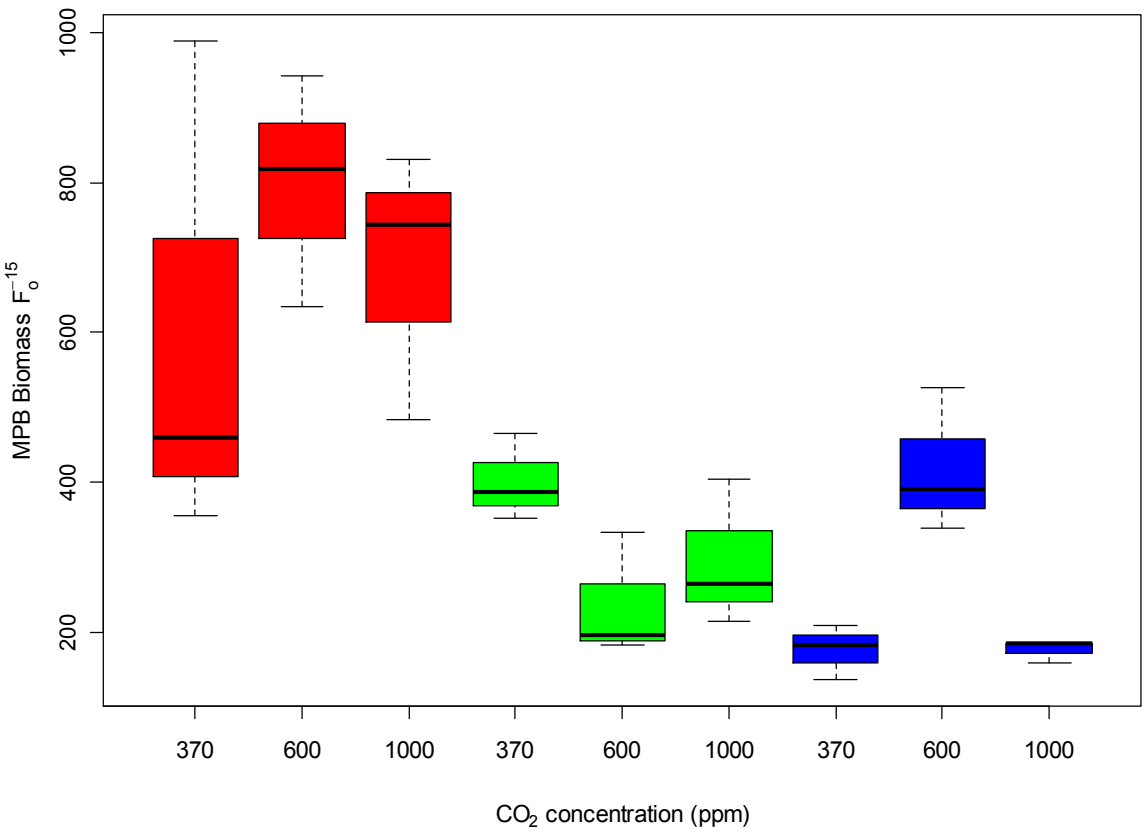


Figure 4.1 Boxplot of raw data showing MPB Biomass across the three CO_2 concentrations and three mean temperatures. The temperatures are colour coded with red (6 °C), green (12 °C) and blue (18 °C) for each CO_2 concentration along the x-axis.

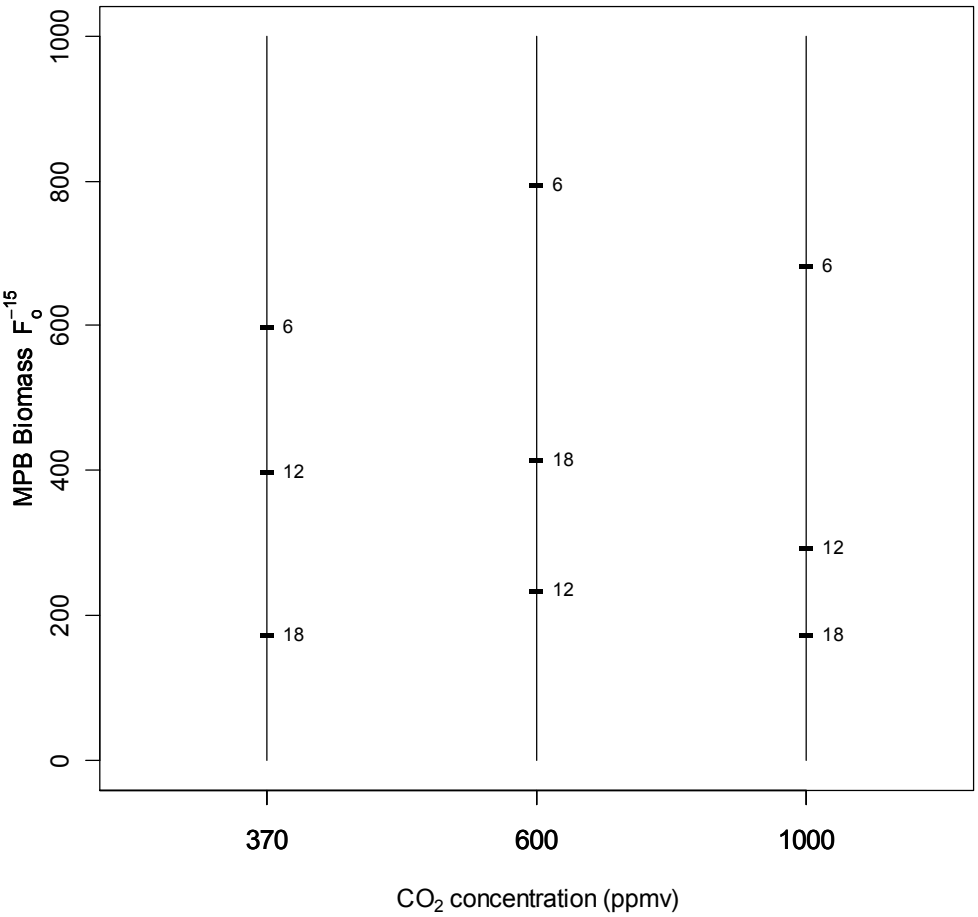


Figure 4.2 Model visualisation of the two-way interaction CO_2 × temperature on MPB biomass (y-axis) across the three mean temperature regimes for each CO_2 concentration. The visualisation shows the CO_2 concentration along the x-axis, and at each concentration the different temperatures are labelled next to the model prediction bars.

4.3.2 Microphytobenthos response to climatic variables and macrofaunal species richness

A regression analysis was performed, treating MPB biomass as the dependent variable with the climate variables and macrofaunal species richness as independent variables. The minimal adequate model comprised a three-way interaction (species richness × temperature × CO_2; *L*-ratio = 23.37, d.f. = 48, $p = 0.02$). With CO_2 concentrations set at either present day (370 ppmv) or 600 ppmv, the decrease in MPB biomass was consistent with increasing temperature (Fig. 4.3, and see raw data boxplots in Appendix 1). Species richness was the most influential variable (*L*-ratio = 95.81, d.f. = 27, $p < 0.0001$), followed by temperature (*L*-ratio = 79.18, d.f. = 24, $p = 0.0051$), and CO_2 concentration (*L*-ratio = 45.48, d.f. = 24, $p < 0.0001$). The effect of species richness on MPB biomass was most apparent at 6 ºC (Fig 4.3).

Biomass was highest in the absence of macrofauna, and the greatest biomass levels in all three carbon dioxide treatments occurred at the lowest temperature treatment of 6 ºC.

For the elevated carbon dioxide levels at 6 ºC, there was an increase in MPB biomass across all species richness levels. This general effect of elevated CO_2 was not found at the higher temperatures, and overall MPB biomass decreased with rising temperature (Fig 4.3).

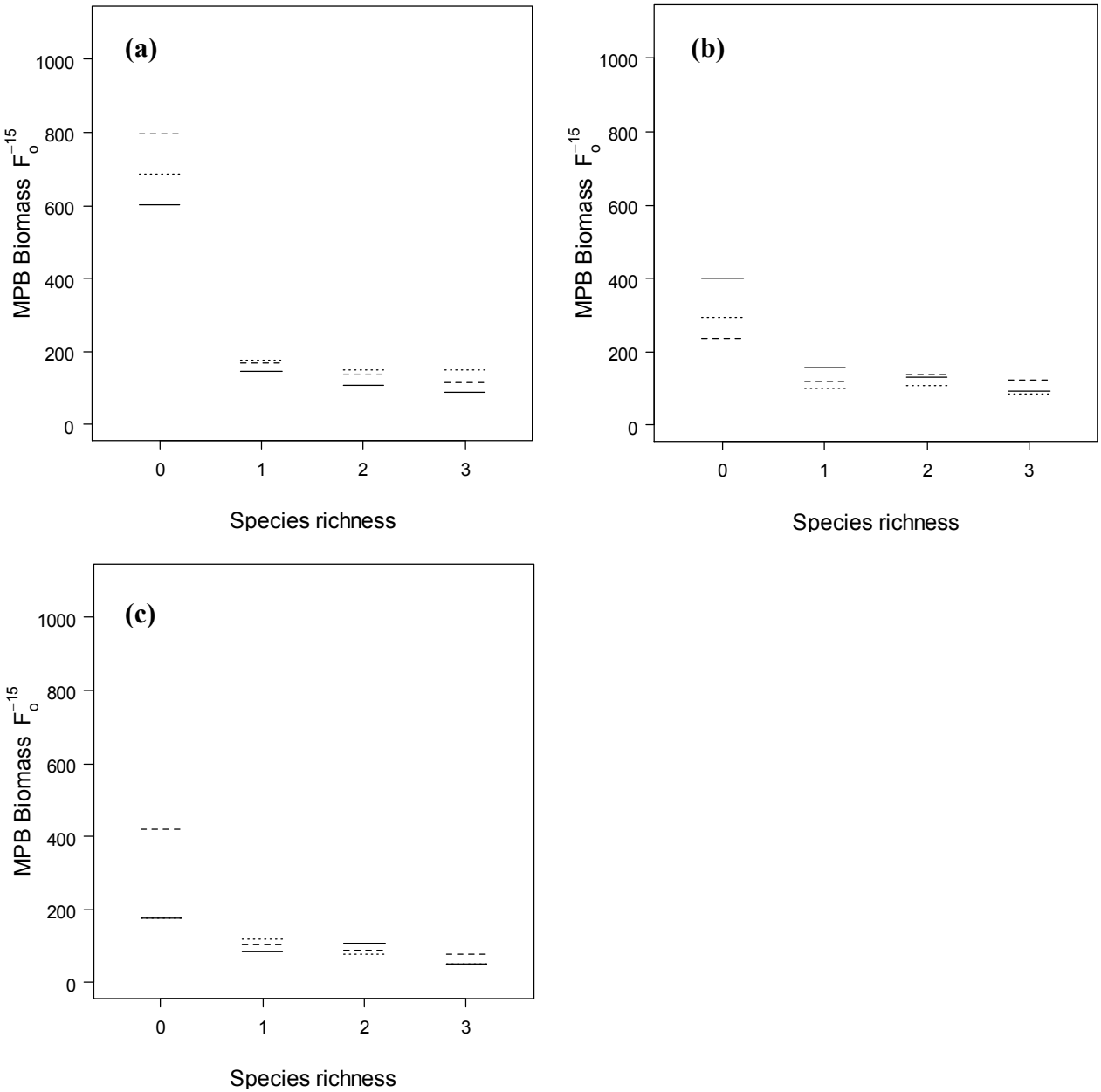


Figure 4.3 Model visualisation of the three way interaction CO_2 × temperature × Species Richness on MPB biomass across the three CO_2 regimes for (a) 6 ºC; (b) 12 ºC; and (c) 18 ºC. Species richness is along the x-axis, MPB biomass along the y-axis, and the different lines represent CO_2 concentration: 370 ppmv (solid line), 600 ppmv (dashed line) and 1000 ppmv (dotted line)

4.3.3 Microphytobenthos response to climatic variables and macrofaunal species composition

A further regression analysis was performed, replacing the explanatory variable species richness with species composition, to determine whether compositional effects underpinned the observed effects of biodiversity. The minimal adequate model comprised a three-way interaction (species composition × temperature × CO_2; *L*-ratio = 65.52, d.f. = 96, $p < 0.0001$). Species composition was the most influential variable (*L*-ratio = 314.52, d.f. = 63, $p < 0.0001$), followed by temperature (*L*-ratio = 177.46, d.f. = 48, $p < 0.0001$), and CO_2 (*L*-ratio = 101.26, d.f. = 48, $p < 0.0001$). Increased species diversity had a detrimental effect on MPB biomass, but the magnitude of these effects was dependent on the composition of the invertebrate assemblage within each treatment (Fig. 4.4; and raw data boxplots in Appendix 1). *C. volutator* had a greater negative effect on MPB biomass than the other macrofaunal species in single species treatments, and the presence of *C. volutator* was a dominant component in the effects of the multispecies treatments (Fig. 4.4). This dominant effect of *C. volutator* decreasing MPB biomass was consistent under all environmental regimes. Importantly, the interaction of the climate drivers mediated the relationship between MPB biomass and species composition, although this mediation was less pronounced in treatments with C. *volutator*, and appeared to have no consistent pattern.

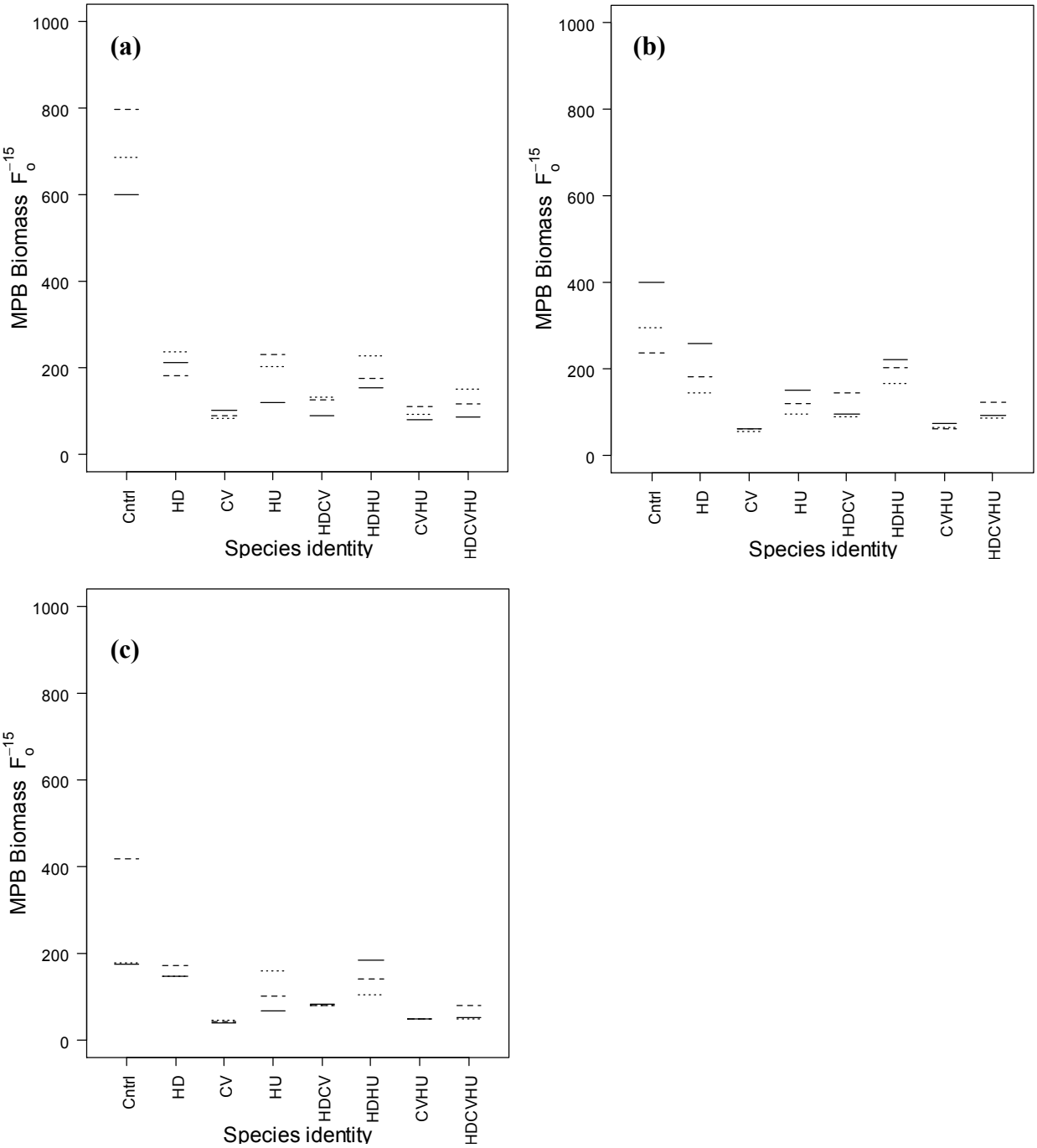


Figure 4.4 Model visualisation of the three way interaction CO_2 × temperature × Species Richness on MPB biomass across the three CO_2 regimes for (a) 6 ºC; (b) 12 ºC; and (c) 18 ºC. Species identity is along the x-axis, MPB biomass along the y-axis, and the different lines represent CO_2 concentration: 370 ppmv (solid line), 600 ppmv (dashed line) and 1000 ppmv (dotted line)

4.4 Discussion

Few ecological studies have examined the effect of multiple simultaneous stressors on individual species and ecosystems (Crain *et al.*, 2008; Fitch and Crowe, 2011). The statistical approach used here does not allow the separation of individual stressor effects, although some inference in terms of the most critical factors can be made from the model metrics (L-ratios) and from the model visualisations. Complex interactions were found between the effects of temperature, CO_2 concentration and macrofaunal species richness / species assemblage composition on ecosystem response, measured as MPB biomass. The MPB biomass provides a proxy estimate of the productive potential of mudflat systems (Guarini *et al.*, 2002) and has been used to model net system productivity. Thus, changes in MPB biomass are a crucial element of system performance and will have reverberating effects through the higher trophic levels (Beardall *et al.*, 2009; Brown *et al.*, 2010), just as changes in infaunal diversity will affect resource utilisation (Levinton and Kelaher, 2004).

In the present study, MPB biomass was higher at low temperature across all CO_2 regimes, and did not increase as CO_2 increased, indicating that CO_2 was not limiting to MPB under these conditions. The models suggested that species richness and temperature were more influential on MPB than CO_2, but the interactions between the explanatory variables were significant. An important implication of this study is that MPB performance is likely to decline as the environment is altered due to global climate change, and that this effect is likely to drive systems more firmly towards heterotrophy. A shift from autotrophic to heterotrophic conditions, or a shift to more extreme heterotrophy, is likely to have significant, but as yet undetermined, implications for ecosystem goods and services. Unless the net import of allochthonous carbon changes, then overall productivity may be expected to decline with potential effects on resource utilisation. Variation in temperature has already been

shown to affect the carbon metabolism of coastal systems (Muren *et al*., 2005) and this supports our hypothesis that autochthonous productivity may be altered through interaction between the climate change variables and species diversity.

In this study, the presence of macrofaunal species resulted in substantial decreases in MPB biomass, and there appeared to be a general decline in MPB with increasing macrofaunal species richness, specifically at the lowest temperature. This general trend is partly to be expected as all three macrofaunal species are known consumers of MPB (Hagerthey *et al.*, 2002; Defew *et al.*, 2003). However, the presence of *C. volutator* had a disproportionately strong effect in reducing MPB biomass, consistent with previous research (Hagerthey *et al.*, 2002; Mermillod-Blondin *et al.*, 2005; Dyson *et al.*, 2007). Rather than being attributed to consumption, the mechanism for this dominant effect is likely to be due to the constant resuspension of sediment (Biles *et al*., 2002; Dyson *et al.*, 2007) during grazing and bioturbation (Fig. S5), leading to inhibition of photosynthesis by MPB, and also the prevention of MPB biofilm formation on the sediment surface (Middelburg *et al.*, 2000).

Individual species responses to climate change are often highly uncertain (Drinkwater *et al.*, 2010), and environmental change could alter the balance between the functional groups present (through extinction, invasion or changes in abundance or behaviour), as well as the number and identity of species present in an ecosystem (Hillebrand *et al.*, 2010). This makes it very difficult to predict how an ecosystem may respond based on diversity alone (Thébault and Loreau, 2003). MPB utilised nutrients from the water column whilst bioturbation by invertebrates increased the concentrations (NH_4-N, PO_4-P) available (Bulling *et al.*, 2010). Therefore, species-specific responses to climate change will affect more than one trophic level, and the nature of interactions between species will change as a consequence (Walther

et al., 2002; Pzreslawski *et al.,* 2008; Brown *et al.*, 2010). In the present study, the decline in MPB was driven by complex interactions between environmental variables and diversity effects, and mediated through infaunal grazing activity. In this case, there was no apparent compensatory increase in MPB biomass through an increase in nutrients caused by bioturbatory activity. The functional importance of species is also context-dependent, and functional impact may alter as conditions change. Thus, while functional diversity is important, it may be overshadowed by species identity as different species take up more prominent roles under changing scenarios (O'Connor and Crowe, 2005; Stachowicz *et al.*, 2008). In this experiment, the dominant effect of one species (*C. volutator*) illustrates how the extinction of influential (rare or common) species may have profound effects on the ecosystem –and that these effects may be direct or indirect (Wootton, 2002; Downing, 2005).

Many studies focusing on the ecological consequences of altered biodiversity within the context of specific drivers of environmental change have concentrated on single variables and few species (Walther *et al.*, 2002; Byrne *et al.*, 2009; Drinkwater *et al.*, 2010; Hillebrand *et al.*, 2010). Whilst these studies are informative in understanding the mechanisms behind ecosystem response, care must be taken in making predictions based on simplistic assumptions, such as additive and linear relationships. Studying the next level of complexity is problematic, and while a mesocosm approach may help provide conceptual advances, we recognise the limitations of any artificial system in providing realistic interpretations of natural ecosystem response (Benton *et al*., 2007). However, it was shown that interactive effects can have a fundamental influence on MPB biomass and since the balance between autotrophic and heterotrophic status in transitional systems may be delicate (Migne *et al.,* 2009), then there is a real possibility that climate change may force an overall change in metabolism in coastal systems. Given that coastal systems will be at the forefront of climate

change effects, they may undergo profound changes in the near future with associated implications for ecosystem services.

5. Impacts of fluctuating temperature and elevated carbon dioxide on primary production in an intertidal marine model system

5.1 Introduction

The recent increase in climate change based marine research, with a strong focus on ocean acidification, demonstrates compelling evidence of how changing global environmental factors will impact a variety of ecosystems (Jiang and Kulczycki, 2004; Menzel *et al.,* 2008; Crain *et al.,* 2009; Drinkwater *et al.,* 2010; Kroeker *et al.,* 2010; Pörtner and Peck, 2010). Marine ecosystems already show evidence of changes as a direct result of rising carbon dioxide levels (Orr *et al.,* 2005; Drinkwater *et al.,* 2010). These effects range from impacts at the individual species level (Harley *et al.,* 2006), such as changes in calcification rates, larval development or growth (Martin and Gattuso, 2009; Parker *et al.,* 2010) to changing ecosystem processes such as nutrient cycling and primary production (Jiang and Morin, 2004; Cardoso *et al.,* 2008; Guinotte and Fabry, 2008; Brown *et al.,* 2010; Kroeker *et al.,* 2010). A large proportion of these studies have focused either on single stressors (Vinebrooke *et al.,* 2004; Cardoso *et al.,* 2008; Darling and Côté, 2008) or on the response of a specific species (Harley *et al.,* 2006; Dupont *et al.,* 2008).

However, ecosystems are simultaneously subject to a number of natural and anthropogenic stressors (Vinebrooke *et al.,* 2004; Crain *et al.,* 2008; Darling and Côté, 2008; Halpern *et al.,* 2008), thus studies that focus on a single stressor may be inadequate in providing an accurate prediction on organismal or ecological responses to climate change (Darling and Côté,, 2008). Species respond to direct environmental changes (e.g. changes in temperature and carbon dioxide) and indirect effects as a result of these changes (such as food availability) (Adams, 2005; Beveridge *et al.,* 2010; Pörtner and Peck, 2010). Whilst species diversity is

often negatively affected by biotic or abiotic stressors (Hooper *et al.,* 2005; Blake and Duffy, 2010), in general the species rich ecosystems are more resilient to environmental change and provide resistance stability (Thebault and Loreau, 2005; Blake and Duffy, 2010). In addition, very little attention has been given to the interactive effects of multiple stressors over more than one trophic level (Crain *et al.,* 2008; Blake and Duffy, 2010; Traill *et al.,* 2010). Thus it is vital, but challenging, to identify the role of species diversity in ecosystem stability and resistance to multiple environmental stressors, particularly in the context of ocean acidification.

It is widely accepted that multiple environmental stressors will alter ecosystem processes in unpredictable ways, and this will depend on the specific stressors (Blake and Duffy, 2010). However, these effects will be mediated by the temporal patterns of these stressors, which in turn will be influenced by the frequency and intensity of these patterns (Molinos and Donohue, 2010). To-date, many studies focus on the mean effect of stressors, such as average temperature, or the frequency of perturbations (Molinos and Donohue, 2010). However, variability around the mean, or temporal variability, can affect how an ecosystem responds to stressors (Benedetti-Cecchi 2005; Benedetti-Cecchi *et al.,* 2006; Bertocci *et al.,* 2005), particularly as temporal variability is common in natural systems, such as intertidal ecosystems. This adds further uncertainty to how ecosystems may respond to changing environmental variables.

Coastal benthic marine ecosystems, particularly estuaries, play an important role in global nutrient cycling and primary production (Harley *et al.,* 2006; Crain *et al.,* 2009). Primary production, dominated by the MPB in estuarine systems, is likely to be affected by changes in the climate, which in turn will affect the higher trophic levels within an ecosystem (Beardall *et al.,* 2009; Brown *et al.,* 2010). Using a model benthic system, this study combines

multiple environmental stressors (elevated carbon dioxide and temperature) and the temporal diurnal variation of temperature under differing biodiversity regimes, and determines how the interaction of these factors impacts benthic primary production.

5.2 Methods and Materials

5.2.1 Sediment

Sediment collected and treated as described in Chapter 2 (Methods and Materials).

5.2.2 Microphytobenthos

MPB-rich surface sediment was collected and treated as described in Chapter 2, before it was homogenised and distributed (125 cm^3) between mesocosms prior to the addition of seawater.

5.2.3 Macrofauna

Three macrofaunal species, as described in the Methods and Materials (Chapter 2), were collected from the study site. The invertebrates were established into replicate (n=3) macrofaunal communities as described in previous chapters, with constant biomass of 2 g wet weight per mesocosm. Control mesocosms (n=3) contained no macrofauna.

5.2.4 Mesocosm assembly

Mesocosms were static perspex cores, 33 cm high filled with sediment (10 cm deep), MPB and 2.35 l of seawater (UV-sterilized, 10 µm pre-filtered, salinity ≈ 33) were added to give an overlying depth of 20 cm. The mesocosm set up is detailed fully in the Methods and Materials section (Chapter 2) of this thesis.

5.2.5 Environmental regimes

Mesocosms were placed in two environmental chambers (V 4100, Vötsch Industrietechnik, temperature control ±0.1 ºC). The experiments were run with a 12 h light-12 h dark (L/D) cycle using high intensity discharge sodium lamps (model GE11678, 400w x2, average 300 µmoles m^{-2} s^{-1}). Eighteen environmental regimes were employed, using three mean

temperatures (6 ºC, 12 ºC and 18 ºC), two atmospheric carbon dioxide concentrations (370 ppmv (present day) and 1000 ppmv), and three fluctuating temperature regimes (FTR = 1 ºC, 3 ºC and 6 ºC) around the mean.

Atmospheric CO_2 concentrations were maintained using a CO_2 monitor attached to an external CO_2 gas cylinder (BOC gases Ltd, UK) with a digital controller (Technics horticultural carbon dioxide controller) (see Chapter 2 for details). Mesocosms were randomly positioned within an environmental chamber to factor out any spatial heterogenity effects. Each experiment was run for 7 days.

5.2.6 PAM fluorescence

The biomass of the MPB was measured at the end of the experiment using a PAM fluorometer, after 15 minutes of dark-adaptation per mesocosm (after Honeywill *et al.*, 2002; Jesus *et al.*, 2005; Jesus *et al.,* 2006).

5.2.7 Data Analysis

MPB biomass was treated as a response variable, with macrofaunal species richness (or species combination), CO_2 concentration, fluctuation and temperature as nominal explanatory variables. After applying a basic linear regression model to the data, this model was then assessed for normality (using Q-Q plots), homogeneity of variance, and any outlying values (Cook's distance) (Pinheiro and Bates, 2001; Zuur *et al.*, 2007). Due to the experimental design, variation across the species richness treatments was unequal (Ieno *et al.*, 2006), as confirmed by plots of the model residuals. Following the analysis used in the previous chapters, and to counter the heterogeneity, a generalised least squares (GLS; Pinheiro and Bates 2001; West *et al.*, 2007; Zuur *et al.*, 2007) mixed modelling approach was used (Dyson

et al., 2007; Bulling *et al.*, 2008, 2010). The best variance-covariate structure for each model was determined using AIC scores, and was confirmed with plots of the fitted model values versus residuals using restricted maximum likelihood (REML, West *et al.*, 2007). A manual backwards stepwise selection with maximum likelihood methods was used to produce the minimum adequate model. Each of the highest interaction terms was assessed for significance as detailed in Underwood (1998). In the final model, the influence of each independent term was assessed, using a likelihood ratio test,. All analyses were performed using the 'nlme' package (ver. 3.1, Pinheiro *et al.,* 2006) in the 'R' statistical and programming environment (R Development Core Team 2010).

5.3 Results

The data analysis consisted of three models, the first examined only the MPB response and excluded treatments containing macrofauna. The second model defined the effects of the climatic variables under an increasing species richness gradient, whilst the third model examined the species assemblage effect underpinning the biodiversity effects.

5.3.1 Microphytobenthos response to climatic variables

The minimal adequate model for the controls (containing no macrofauna), with MPB biomass as the dependent variable comprised a three-way interaction (CO_2 × temperature × fluctuation; *L*-ratio = 37.47, d.f. = 27, p = <0.0001).

Fluctuating temperature regime (FTR) was the most influential variable (*L*-ratio = 83.73, d.f. = 12, p = <0.0001), followed by CO_2 concentration (*L*-ratio = 76.14, d.f. = 9, p = <0.0001), with temperature having the least effect in this model (*L*-ratio = 67.29, d.f. = 12, p = <0.0001).

The highest readings of MPB biomass were found in the ambient CO_2 treatment (Fig. 5.1), but there was no clear overall trend of increasing MPB biomass with temperature or fluctuation. The model predictions accurately reflect the trends found in the raw data (Fig. 5.2).

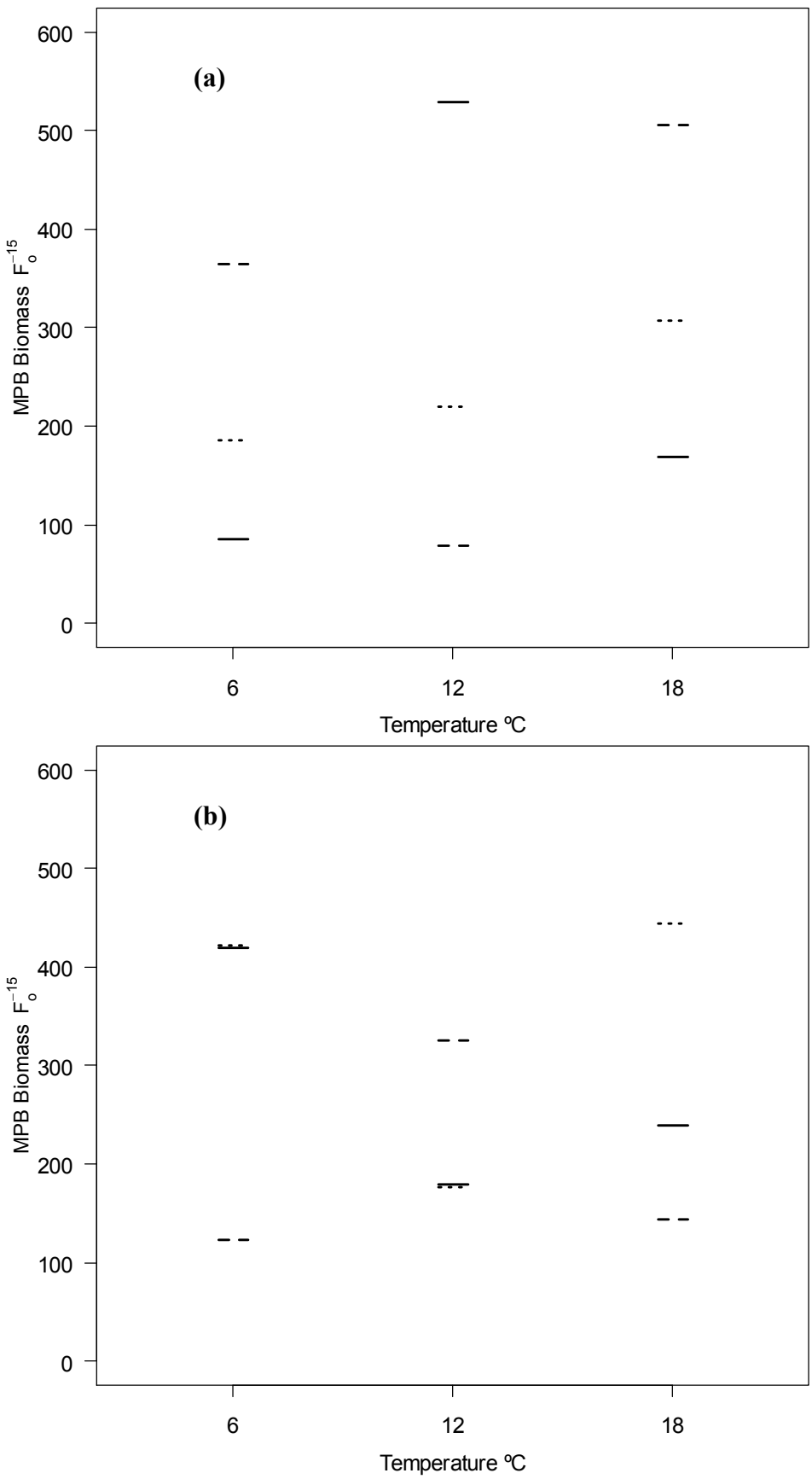


Figure 5.1 Model visualisations of the three-way interaction CO_2 × temperature × fluctuation on MPB biomass(y-axis) with increasing mean temperature along the x-axis under (a) ambient CO_2 (370 ppmv) and (b) elevated CO_2 (1000 ppmv). The lines indicate the fluctuation regimes: 1ºC - solid line; 3ºC – dashed line; 6ºC – dotted line.

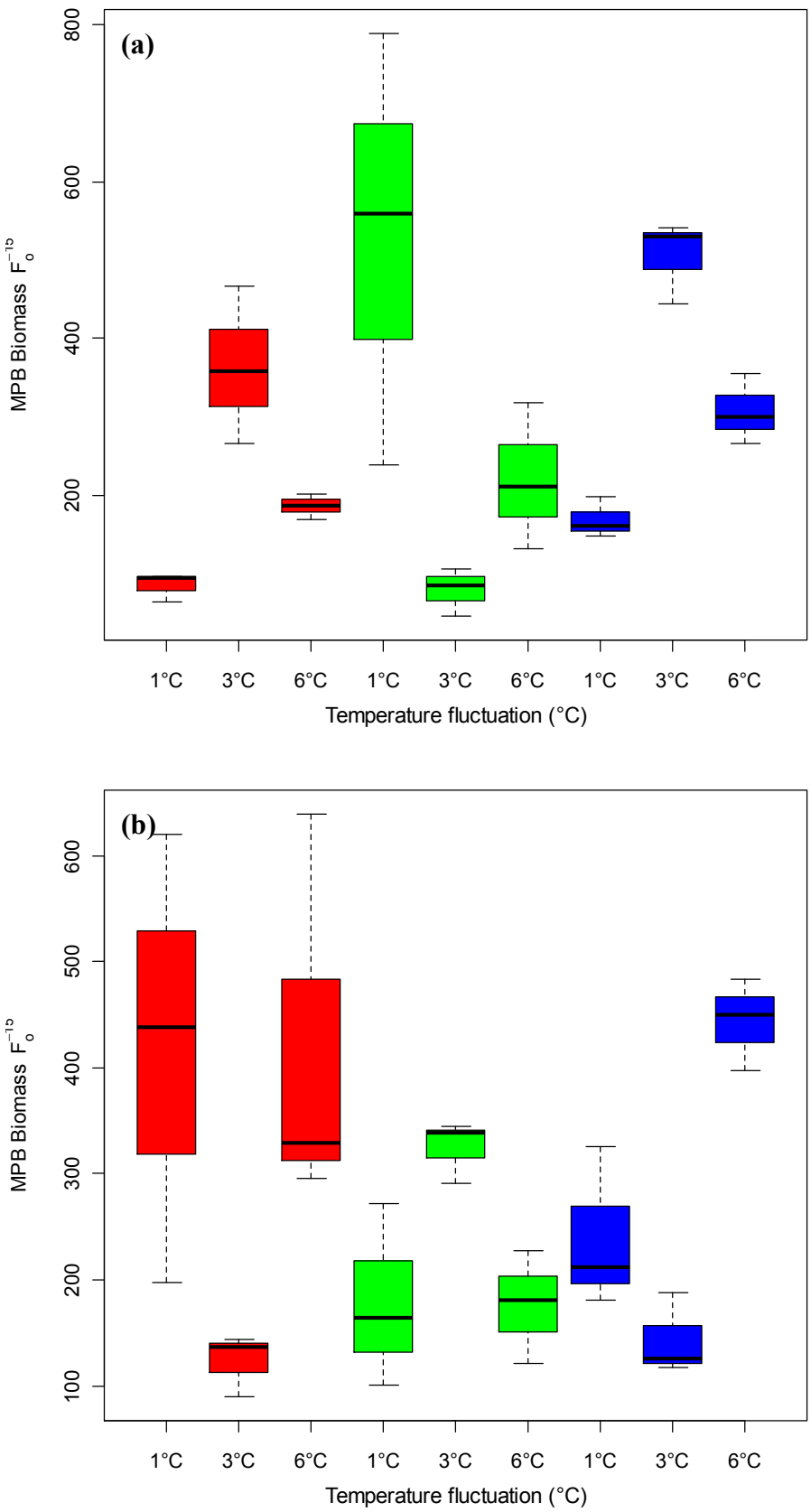


Figure 5.2 Boxplots of the raw data showing changing MPB biomass with increasing fluctuation under (a) ambient CO_2 (370 ppmv) and (b) elevated CO_2 (1000 ppmv). The three mean temperatures are colour coded with red = 6ºC, green =12ºC and blue = 18ºC.

5.3.2 Microphytobenthos response to climatic variables and macrofaunal species richness

MPB biomass was treated as the dependent variable with the climate variables and macrofaunal species richness as independent variables. The minimal adequate model comprised a four-way interaction (species richness × CO_2 × temperature × fluctuation; *L*-ratio = 39.19, d.f. = 84, p = <0.0001).

With CO_2 concentrations set at either present day (370 ppmv) or 1000 ppmv, MPB biomass decreased with species richness, which was most apparent at 18 ºC (Figs. 5.3 and 5.4). FTR was the most influential variable (*L*-ratio = 207.90, d.f. = 48, $p < 0.0001$), closely followed by species richness (*L*-ratio = 205.43, d.f. = 54, $p < 0.0001$). CO_2 concentration (*L*-ratio = 184.43, d.f. = 36, p = <0.0001) was the next influential variable, with temperature being the least influential variable (*L*-ratio = 173.95, d.f. = 48, $p < 0.0001$). Biomass was highest in the absence of macrofauna across all climatic regimes. FTR, temperature and CO_2 concentration had an inconsistent and idiosyncratic effect on MPB biomass.

In general, the higher MPB biomass readings were found in the 3 ºC FTR under both CO_2 concentrations for all temperatures, with the exception of 12 ºC mean temperature at ambient CO_2 (Fig. 5.3b).

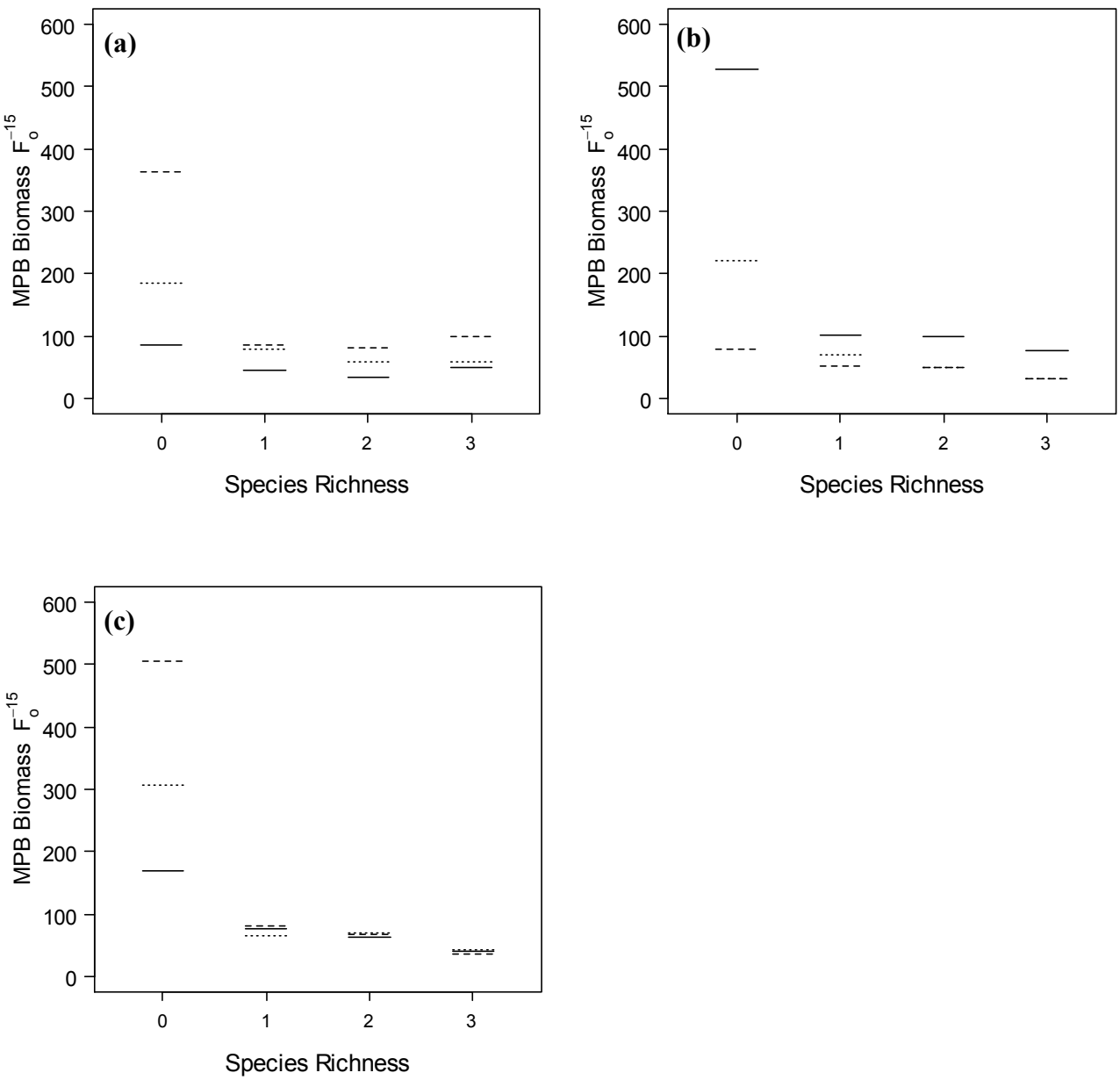


Figure 5.3 Model visualisations of the interaction between temperature, fluctuating temperature regime and species richness on MPB biomass (y-axis) under ambient CO_2 concentrations (370 ppmv) at the three mean temperatures of (a) 6 ºC; (b) 12 ºC; and (c) 18 ºC. The lines indicate the fluctuating regime: 1 ºC - solid line; 3 ºC – dashed line; 6 ºC – dotted line.

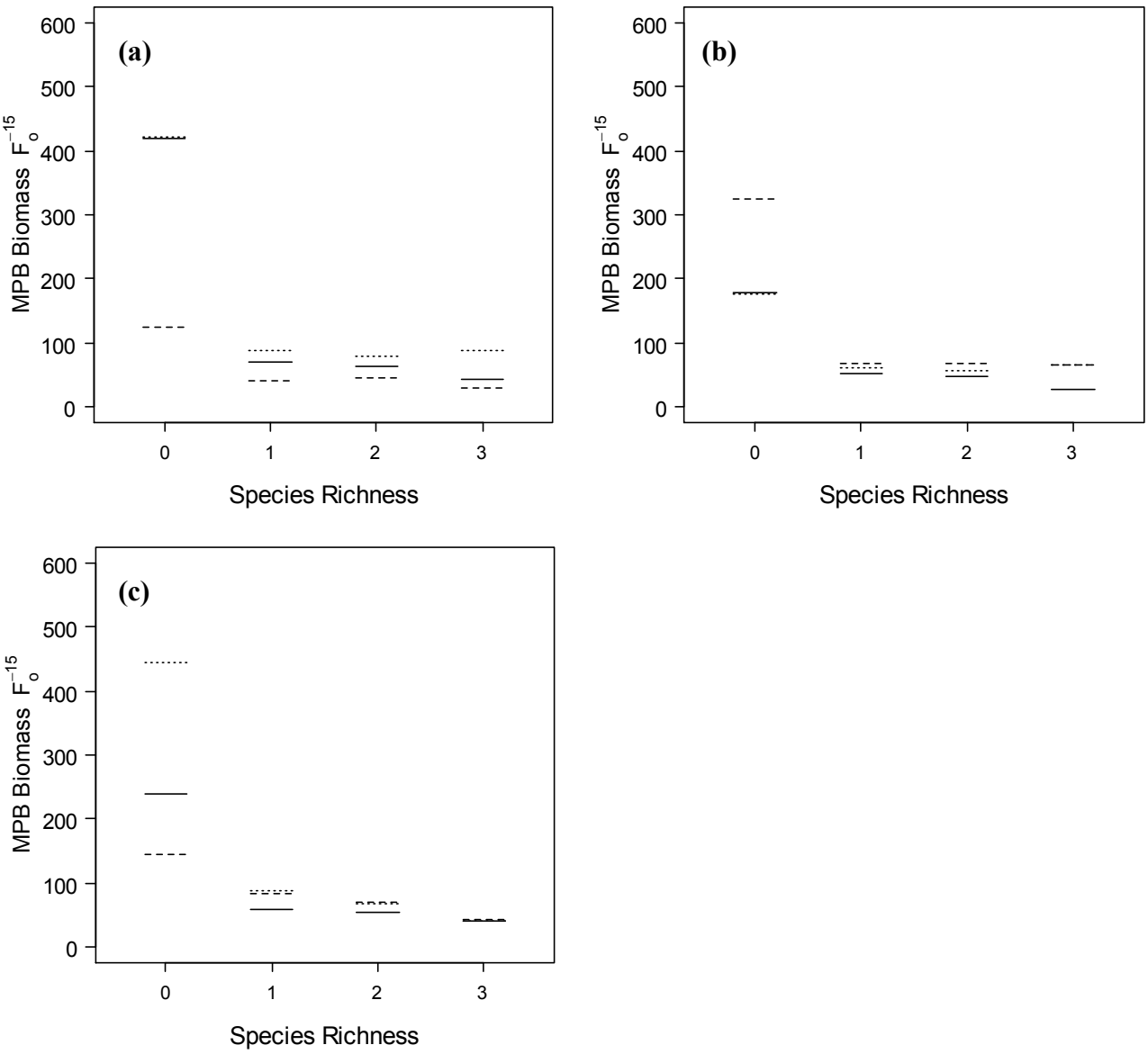


Figure 5.4 Model visualisations of the interaction between temperature, fluctuating temperature regime and species richness on MPB biomass under elevated CO_2 concentrations (1000 ppmv) at the three mean temperatures of (a) 6 ºC; (b) 12 ºC; and (c) 18 ºC. The lines indicate the fluctuating regime: 1 ºC - solid line; 3 ºC – dashed line; 6 ºC – dotted line.

5.3.3 Microphytobenthos response to climatic variables and macrofaunal species composition

To determine whether the effects of species assemblage underpinned the diversity effects, a further regression analysis was performed, replacing the explanatory variable 'species richness' with species composition ('identity'). The minimal adequate model comprised a four-way interaction (species composition × CO_2 × temperature × fluctuation; *L*-ratio = 65.52, d.f. = 96, $p < 0.0001$).

Species composition was the most influential variable (*L*-ratio = 638.42, d.f. = 126, $p < 0.0001$), followed by temperature (*L*-ratio = 430.73, d.f. = 96, $p < 0.0001$), and FTR (*L*-ratio = 409.04, d.f. = 96, $p = <0.0001$). CO_2 concentration was the least influential variable (*L*-ratio = 355.78, d.f. = 72, $p < 0.0001$).

Increased species diversity had a negative effect on MPB biomass, but this was mediated by the composition of the invertebrate assemblage within each treatment. *C. volutator* had a more detrimental effect on MPB biomass than the other macrofaunal species in single species treatments, and the presence of *C. volutator* was a dominant component in the effects of the multispecies treatments (Figs. 5.5 & 5.6). This dominant effect of *C. volutator* decreasing MPB biomass was consistent under all environmental regimes, but was most apparent at the highest mean temperature 18ºC. Whilst MPB biomass was negatively affected by the presence of all invertebrates, the effects of *H. diversicolor* and *H. ulvae* in single species treatments was less pronounced than the *C. volutator* treatments, and varied within each climatic regime. Overall, the interaction of the three climatic variables (temperature, fluctuation and CO_2) was mediated by the species assemblage composition.

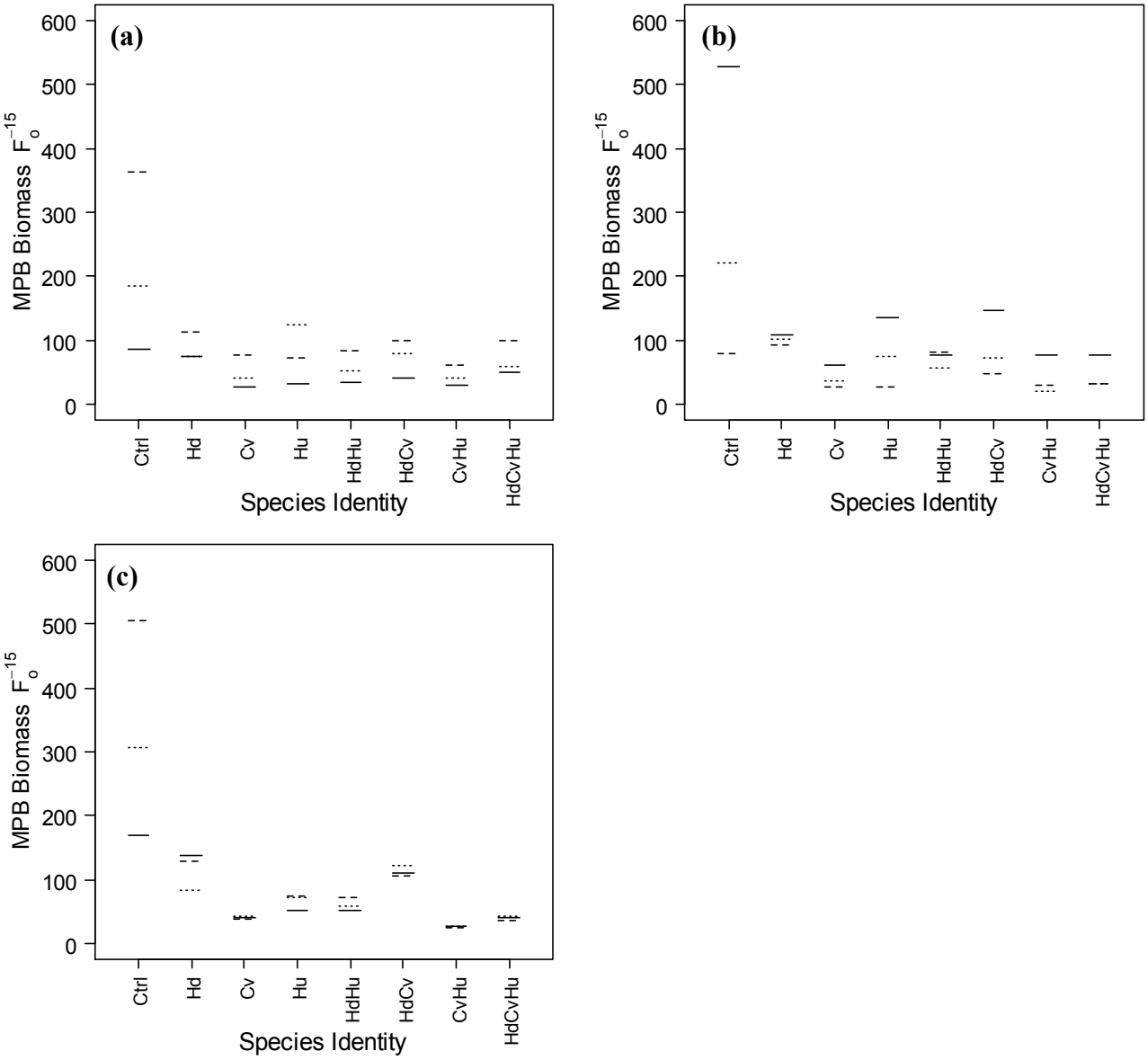


Figure 5.5 Model visualisations of the interaction between temperature, fluctuation and species assemblage composition on MPB biomass under ambient CO_2 concentrations (370 ppmv) at the three mean temperatures of (a) 6 ºC; (b) 12 ºC; and (c) 18 ºC. The lines indicate the different fluctuating regime: 1 ºC - solid line; 3 ºC – dashed line; 6 ºC – dotted line. Larger versions of these graphs can be found in Appendix 1.

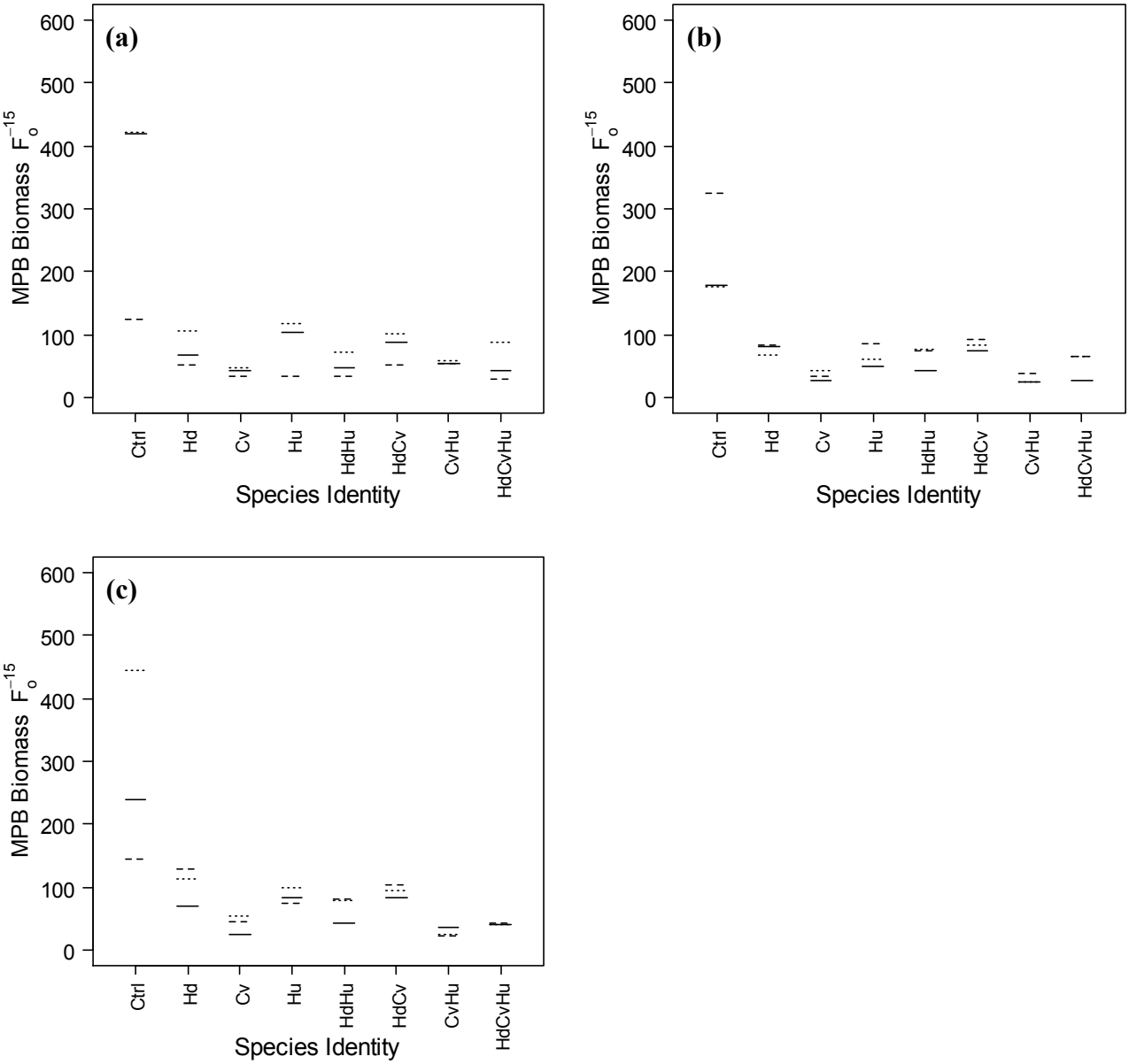


Figure 5.6 Model visualisations of the interaction between temperature, fluctuation and species assemblage composition on MPB biomass under elevated CO_2 concentrations (1000 ppmv) at the three mean temperatures of (a) 6 ºC; (b) 12 ºC; and (c) 18 ºC. The lines indicate the different fluctuation regime: 1ºC - solid line; 3 ºC – dashed line; 6 ºC – dotted line. Larger versions of these graphs can be found in Appendix 1.

5.4 Discussion

Few ecological studies have examined the interaction of temporal patterns of multiple simultaneous stressors on individual species and ecosystems (Crain *et al.,* 2008; Molinos and Donohue, 2010). Complex interactions were found between the effects of the climate variables (temperature and CO_2 concentration), the intensity of temperature fluctuation, and macrofaunal biodiversity on MPB biomass. This study supports previous studies illustrating that the effects of multiple stressors on ecosystem process do not necessarily follow a simple additive model (Crain *et al.,* 2008; Blake and Duffy, 2010; Bulling *et al.,* 2010).

However, in the context of ecosystem response to changing climatic variables, it is apparent that species richness will play a vital role (Hooper *et al.,* 2005). In this study, whilst MPB biomass (chl *a*) declined with macrofaunal species richness, the variation within the richness treatments decreased compared to the variation found in the treatments without macrofauna. This suggests support for the theory that biodiversity provides stability (Thebault and Loreau, 2005; Blake and Duffy, 2010; Traill *et al.,* 2010). Highly diverse ecosystems may be more resistant to effects from multiple stressors, through the different responses of species to the changing environmental conditions (Traill *et al.,* 2010). Since declining diversity is often a side effect of many anthropogenic stressors, it is imperative that the role of species diversity in ecosystem stability under future climate changes is thoroughly understood (Hooper *et al.,* 2005).

In the context of climate change, species may be functionally diverse in their response to stressors (Blake and Duffy, 2010). This 'response diversity' is likely to play a greater role in ecosystem stability under varying climate scenarios than might be predicted from the functional roles that species play in contributing to current ecosystem processes. In this

study, the negative impact of MPB biomass under differing species assemblages was driven by the dominance of *C. volutator*, and was consistent across the different climatic treatments and diversity levels, suggesting little response diversity in this community.

Studies examining the interaction of multiple stressors on ecosystem processes have, for simplicity, focused mainly on the mean effect of the perturbations (Molinos and Donohue, 2010). However, empirical studies have demonstrated that temporal variability around the mean can produce different effects to those found when only changing the mean (Bertocci *et al.,* 2005; Benedetti-Cecchi *et al.,* 2006). In this study, the same mean temperature was used but with three different levels of fluctuation around the mean value over the same diurnal period, and this temporal variability produced differing results within the same mean.

Natural and anthropogenic-driven disturbances are temporally variable in their occurrence and therefore effects on ecosystems. For example, in tidal ecosystems, the temperature and salinity is temporally variable on a daily, weekly and annual basis. Spatial or temporal variation of biotic and abiotic processes can affect species under mean constant conditions (Cardinale *et al.,* 2002; Bertocci *et al.,* 2005; Benedetti-Cecchi, 2005). This demonstrates the difficulty in predicting the impacts of multiple stressors when it is not simply a cumulative effect of the combined individual stressors (Blake and Duffy, 2010). This interpretation is further complicated when incorporating temporal variability. The results of this study and others (Bertocci *et al.,* 2005; Benedetti-Cecchi 2005) illustrate strong effects of temporal variation, and since natural disturbances are inherently temporally variable, future research to predict the effects of environmental stressors should account for this variation. To date, this has been largely neglected, with the exception of a few studies (Bertocci *et al.,* 2005; Benedetti-Cecchi *et al.,* 2005, 2006; Molinos and Donohue, 2010).

The direct and indirect effects of multiple stressors on ecosystem processes will also be mediated through trophic interactions (Beveridge *et al.,* 2010; Brown *et al.,* 2010; Triall *et al.,* 2010). Changes in primary production in marine ecosystems, as a result of multiple stressors, will have implications for higher trophic levels in benthic and pelagic systems and will affect many of the services these ecosystems provide to humans (such as sustainable fisheries, Brown *et al.,* 2010). Short-term studies like this are important in identifying individual species and ecosystem responses to elevated CO_2 levels. However, longer term experiments, combined with effective observational studies, may identify potential acclimation by the macrofauna within a more realistic timeframe with regards to natural ocean acidification rates.

Despite the ongoing and intense climate change research, there are still areas in ecology that need to be addressed before we can effectively mitigate and adapt to global environmental change. An integrated approach of field studies, laboratory observations and modelling is needed to identify the direct and indirect effects of multiple stressors on ecosystem processes. The temporal variability of environmental stressors should also be accounted for in future ecological research. The predicted changes in climatic variables will affect ecosystems simultaneously with existing anthropogenic pressures, such as pollution, overexploitation and habitat destruction, causing profound implications for marine and terrestrial ecosystems. This in turn will have economic and social effects for the human services that many of these ecosystems provide. There are many uncertainties surrounding current predictions and understanding of these effects, and the complexity of natural ecosystems makes the task of identifying and predicting future changes seem daunting. However, this can be achieved by focusing research effort into understanding the interaction between multiple environmental stressors and how this is mediated by trophic interactions across different ecosystems.

6. Tidal cycle vs immersion effect: changes in primary production under different carbon dioxide, temperature and tidal regimes

6.1 Introduction

Microphytobenthos play an important role in estuarine carbon cycling and can enhance or inhibit benthic-pelagic coupling. MPB form the link between the meiofauna and the higher trophic levels with the food chain, providing the main carbon food source for invertebrates in tidal mudflats, and contributing to up to 50% of the total primary production within an estuarine ecosystem (Underwood and Kromkamp, 1999). MPB biomass is influenced by a variety of environmental factors, such as light, temperature, tidal cycle and grazing pressure (Blanchard *et al.,* 2001; Hagerthey *et al.,* 2001). Epipelic diatoms, which are dominant in estuarine MPB biofilms, exhibit endogenous vertical migration rhythms, which are closely synchronised to tidal cycle (Serôdio *et al.,* 1997; Guarini *et al.,* 2000; Jesus *et al.,* 2009). The tidal cycle influences benthic primary production through altered light and temperature (Serôdio and Catarino, 1999) as the water height changes during immersion through to emersion. The spatial and temporal variation of MPB biomass is also strongly influenced by tidal exposure (Jesus *et al.,* 2009), and the process of primary production is usually restricted to these emersion periods (Guarini *et al.,* 2000).

Few experimental studies have examined how tidal cycle, together with other environmental variables, such as temperature or CO_2, may impact MPB biomass. Understanding how abiotic factors affect MPB biomass is vital to allow accurate prediction and mediation of climate change effects on primary production within estuarine and coastal ecosystems (van der Wal *et al.,* 2010).

The coastal ecosystems that the microphytobenthos inhabit are at the forefront of global change. These systems are well-recognised for their contribution to human welfare, providing a variety of services ranging from nutrient cycling, food and water production to recreation. Their vulnerability to anthropogenic activity, such as pollution, overexploitation and habitat defragmentation, causes concern on the potential damage caused by climate change. Recent studies have illustrated how climatic variables such as elevated carbon dioxide and temperature will impact primary production in marine systems (Beardall *et al.,* 2009; Brown *et al*., 2010; Hicks *et al.*, 2011), but these are based on mesocosm studies with 24 hr immersion. Since MPB are tidal organisms, with an endogenous rhythm synchronised to the tidal regime, it is likely that tidal cycle will also affect MPB biomass. This study aims to ascertain the effects of tidal regime on MPB biomass under elevated carbon dioxide and temperature regimes, and to determine if the effect of these variables is additive, interactive or synergistic.

6.2 Materials and Methods

6.2.1 Sediment and MPB collection

Muddy surface sediment and microphytobenthos was collected as described in Chapter 2. Sand was collected from the sand dunes of the Ythan Estuary, slightly further downstream from the mudflats, and sieved (1 mm) to remove larger particles, macrofauna and algae. The sand was placed into the four tidal tanks to a depth of 4cm. The muddy sediment was left to settle for 48 h before the supernatant was removed.

The MPB-rich sediment was mixed with the muddy sediment (50% MPB-rich sediment, 50% sediment), homogenised and distributed between the small mesocosms within the tidal tanks to a depth of 2cm prior to the seawater.

6.2.2 Experimental design

The experiment was designed to ascertain the effect of tidal cycle on MPB biomass under elevated CO_2 and constant mean temperature regimes. The experimental design included two reservoir tanks (L100cm, W86cm, H13cm), four tidal tanks (L100cm, W42cm, H28cm) and 96 small mesocosms or 'cores' (H6cm, ID10cm) made out of perspex. Within each tidal tank there were 24 small cores laid in rows of 8 (Fig. 6.1). Following the addition of sediment and MPB, seawater (UV-sterilized, 10 µm pre-filtered, salinity ≈ 33) was added to give an overlying depth of 22 cm. The reservoir and tidal tanks were placed in two environmental chambers (V 4100, Vötsch Industrietechnik) capable of controlling temperature (±0.1 ºC). The reservoir tanks were placed on the bottom of the chambers, with the tidal tanks resting on a shelf above them (Fig. 6.2). Two environmental chamber were used, with one set to a tidal cycle and the other set for constant immersion.

Figure 6.1 The 24 small cores in three rows of eight in the tidal tank in the environment chamber. The water was low tide in this image, with the water below the level of the MPB-rich sediment in the small

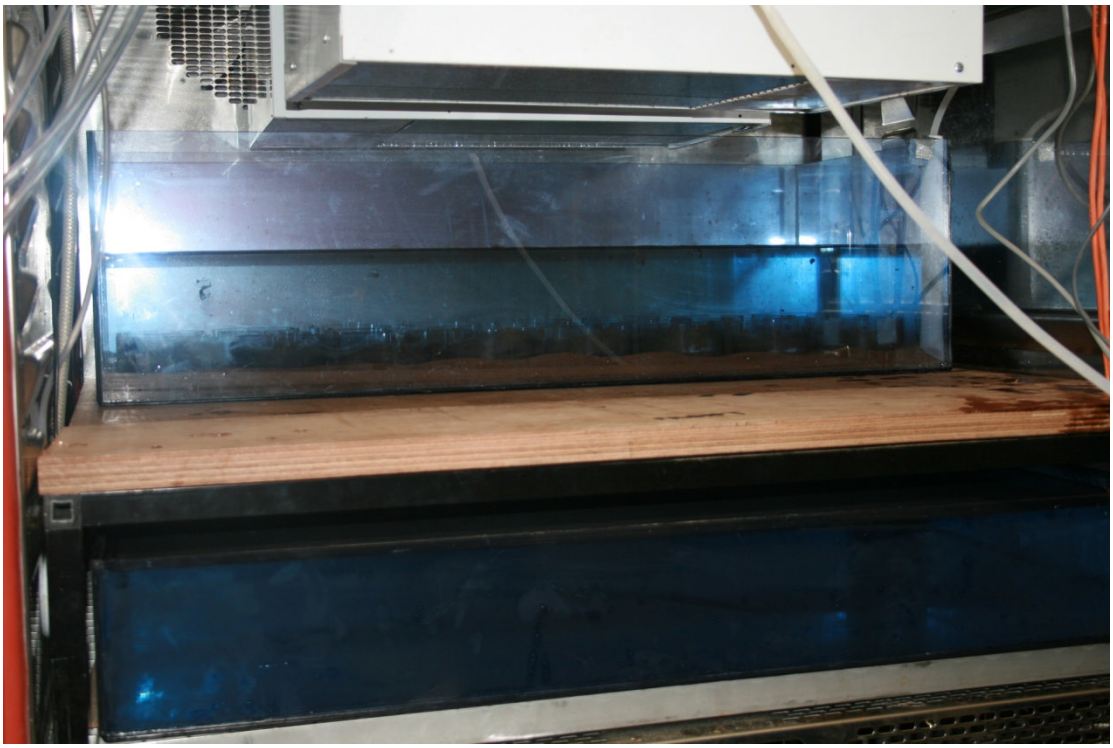


Figure 6.2 The reservoir tank was placed on the bottom reinforced shelf of the chamber, with the tidal tanks positioned lengthways on a shelf directly above the reservoir tanks. During the tidal cycle, water was pumped between the reservoir tank and the front tidal tank.

The experiments were run on a 12 h light-12 h dark (L/D) cycle using high intensity discharge sodium lamps (model GE11678, 400w x2, average 300 µmoles m^{-2} s^{-1}) suspended above the tidal tanks. Six environmental regimes were employed, using three constant temperatures (6 ºC, 12 ºC and 18 ºC, reflecting the annual variation at the study site and two atmospheric carbon dioxide concentrations (370 ppmv (present day) and 1000 ppmv). The high concentrations of 1000 ppmv was based on IPCC projections for approximately 100 years time (TAR, IPCC, 2001 and 4th Assessment Report, IPCC, 2007) and reflect the accepted view that CO_2 levels will rise over the long-term. Atmospheric CO_2 concentrations were maintained using a CO_2 monitor attached to an external CO_2 gas cylinder (BOC gases Ltd, UK) with a digital controller (Technics horticultural carbon dioxide controller). An Infra-Red Gas Analyser (IRGA, ADC LCA3) was used to calibrate and validate the CO_2 regulation (± 30ppm).

The tidal cycle was controlled using a peristaltic pump (model 323Du) from Watson-Marlow and 6.4 mm bore platinum tubing. The cycle was set and controlled from a computer connected to the pump within the chamber through an RS232 cable. The tidal cycle was set to replicate the natural low tide and high tide cycle in the estuary on the sampling day, and this time was kept consistent for all runs to eliminate any interacting effect of light and tide on MPB biomass. During the tidal cycle, seawater was pumped from the tidal tank (one tank per chamber) into the reservoir tank below, and then back again. The experiment was run for one week.

6.2.3 PAM fluorescence measurements (response variable)

The biomass of the MPB was measured at the end of the experiment using a PAM fluorometer (DIVING-PAM, Heinz-Walz GmbH) at emmersion period of the tidal cycle. Fluorescence measurements were made after 15 minutes of dark-adaptation (after Honeywill *et al.*, 2002; Jesus *et al.*, 2005; Jesus *et al.,* 2006), with five readings taken per small mesocosm.

6.2.4 Data Analysis

MPB biomass was treated as a response variable, with CO_2 concentration, temperature and tidal regime as nominal explanatory variables. Initially, a linear regression model was fitted and assessed for normality (Q-Q plots), homogeneity of variance, and outlying values (Cook's distance) (Pinheiro and Bates, 2001; Zuur *et al.*, 2007). To account for the difference in light levels within the tidal tanks, a random effects mixed modelling approach (Zuur *et al.*, 2007) was used in preference to transforming the data. This allowed incorporation of position in the tidal tank in relation to light. The most appropriate variance-covariate structure for each model was determined using a combination of AIC scores and the examination of plots of fitted values versus residuals based on a full model specification using restricted maximum likelihood (REML, West *et al.*, 2007). The minimum adequate model was then determined through manual backwards stepwise selection, using maximum likelihood methods. The significance of the relevant highest order interaction terms was assessed at each stage, terms nested within these not being tested, following Underwood (1998). The influence of each independent term within the minimum adequate model was assessed using a likelihood ratio test between the minimum adequate model and reduced models (with all terms involving the relevant independent factor removed, including interactions). All

analyses were performed using the 'nlme' package (ver. 3.1, Pinheiro *et al.,* 2006) in the 'R' statistical and programming environment (R Development Core Team 2010).

6.3 Results

The final model incorporated a three-way interaction between CO_2 concentration, temperature and tidal regime (*L*-ratio = 37.65065, p <.0001), with position to the light as a random factor in the fixed structure of the model.

There was a strong tidal effect on MPB biomass, as shown by the model visualisation (Fig. 6.3), with the highest MPB biomass found in the tidal tanks across both CO_2 treatments and all three temperatures, and this is reflected in the raw data (see boxplots, Fig 6.4).

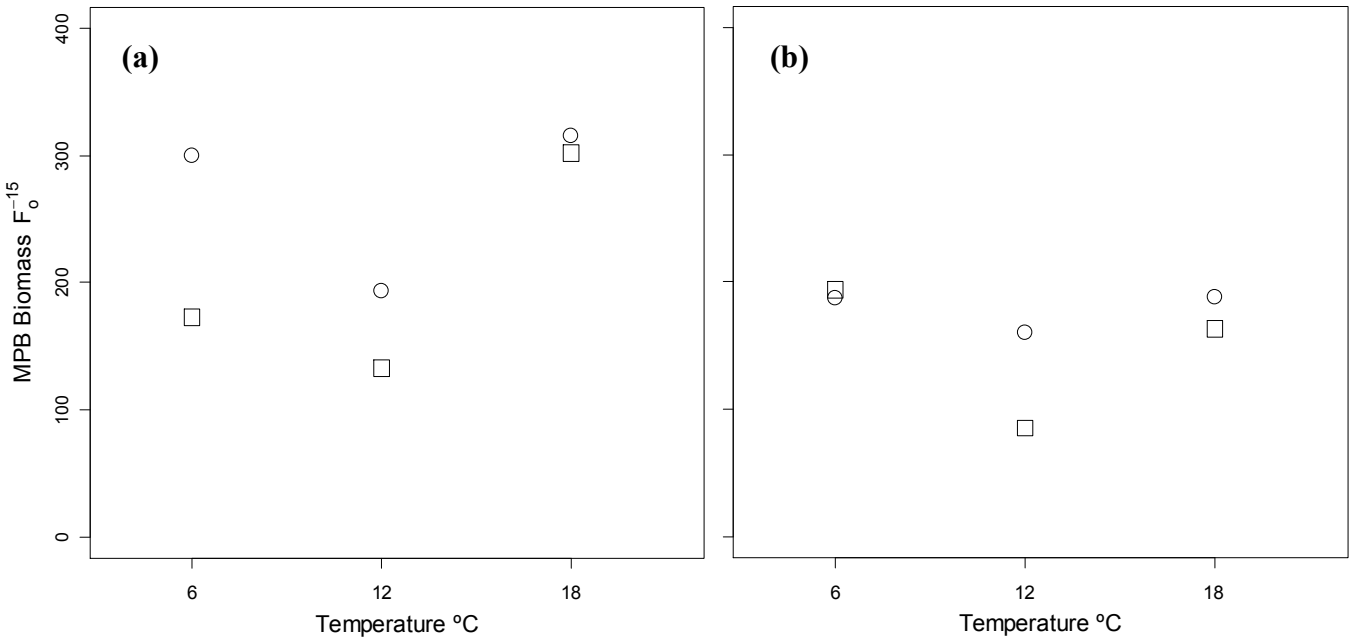


Figure 6.3 Model visualisation showing MPB biomass (F_o^{-15} - y-axis) changes under (a) ambient CO_2 concentration (370 ppmv) and (b) elevated CO_2 concentration (1000 ppmv). The open circles represent the tidal regime and the open squares represent constant immersion.

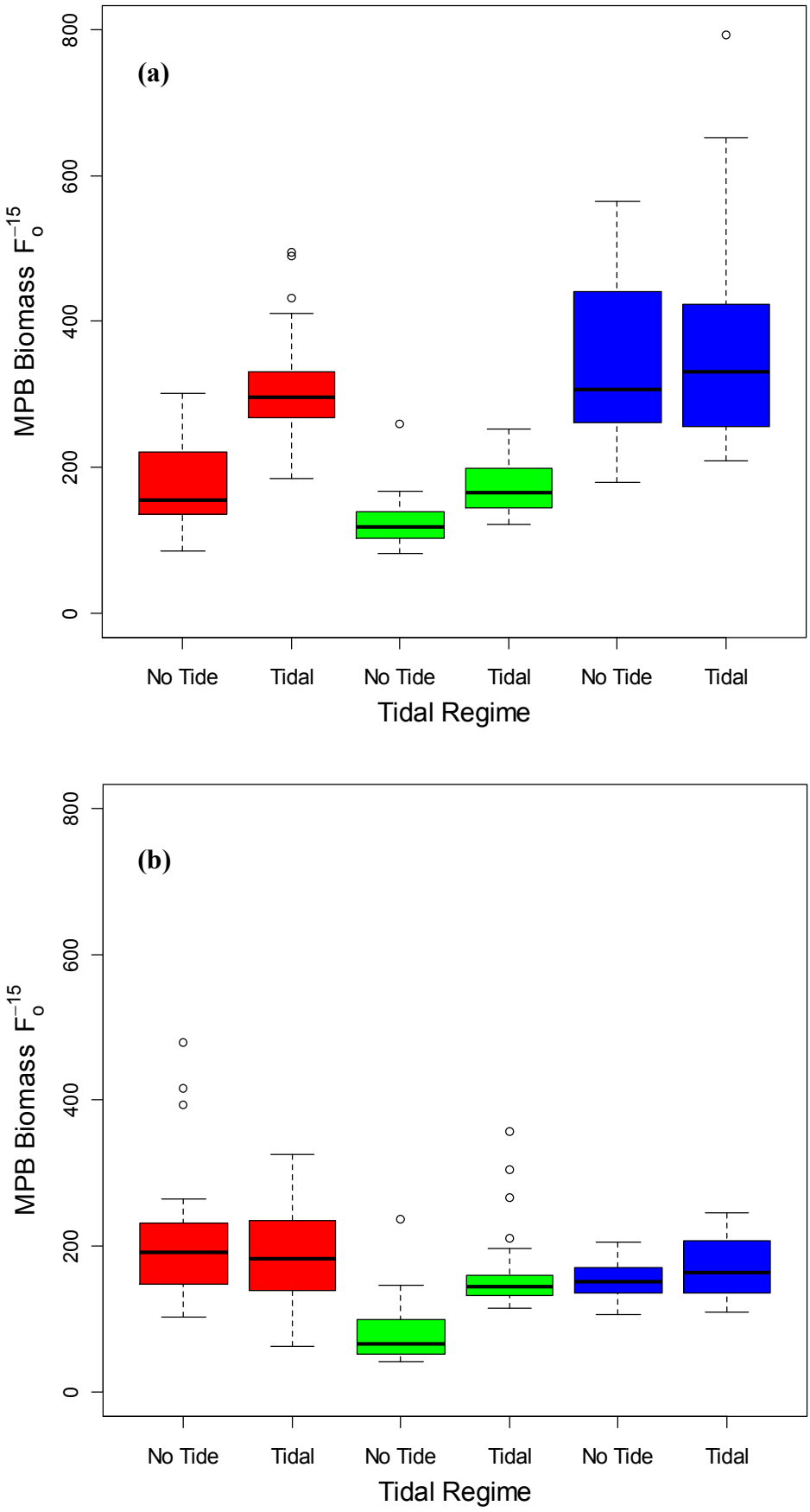


Figure 6.4 Boxplots of the raw data showing how MPB biomass (F_o^{-15}) changes under (a) ambient CO_2 concentration (370ppmv) and (b) elevated CO_2 concentration (1000ppmv). The colours indicate the temperature regime with red = 6 °C, green = 12 °C; blue = 18 °C.

Of the three environmental factors, temperature was the most influential (*L*-ratio = 251.4156, d.f. = 8, $p<0.0001$) followed by CO_2 concentration (*L*-ratio = 175.4460, d.f. = 6, $p<0.0001$), with tidal regime being the least influential variable on MPB biomass within the minimum adequate model. However, all three factors are highly significant to the model as single terms, and as interactive terms within the three-way and two-way interactions.

MPB biomass was higher in the tidal regime across all temperature levels and CO_2 concentrations, and this trend is apparent in the raw data plots and the model visualisation. In general, MPB biomass was lower in the high CO_2 concentrations in comparison to the ambient CO_2 regime.

6.4 Discussion

MPB biomass is influenced by many environmental variables, and this study illustrates how tidal cycle, temperature and CO_2 concentration can interactively alter MPB biomass. Whilst many mesocosm studies are carried out on static, fully immersed systems, this is the first study to combine the effects of climate change variables with tidal effects on MPB assemblages, and places the results into context within a tidal system. The emersion period of a tidal cycle is when the MPB migrate to the sediment surface, form a biofilm and actively photosynthesise (Serôdio *et al.,* 1997; Guarini *et al.,* 2000; Jesus *et al.,* 2009). In the absence of a tidal cycle, the endogenous rhythm of this migration persists under laboratory conditions (Guarini *et al.,* 2000). In the experimental design, the set tidal regime mimics the sample day, and is kept consistent for the ensuing runs. Whilst it would be ideal to have the correct simulation of tides, this leads to further problems with syncing the light cycle, so for simplicity in this experiment the tidal cycles were kept consistent with the first run.

The MPB biomass readings were consistently higher in the tidal tanks than in the fully immersed treatments. However, the effect of tidal regime was mediated by the environmental factors of CO_2 concentration and temperature. As evidenced in previous chapters of this thesis, higher CO_2 concentration did not result in higher MPB biomass, and the highest biomass was found in the ambient CO_2 treatments. In relation to temperature regimes, MPB biomass was lowest at 12ºC under all tidal and CO_2 regimes, which is consistent with results in the preceding chapters. All three environmental factors significantly affect MPB biomass, but these affects are interactive.

Many algal and fauna species inhabiting intertidal sediments have endogenous circatidal or diurnal rhythms. These include metabolic and behavioural responses to photoperiod or tidal

immersion (Biles *et al.,* 2003; Tang and Kristensen, 2007; Last *et al.,* 2009; Vieira *et al.,* 2010).

Whilst mesocosm studies are by their very nature highly controlled (Biles *et al.,* 2003; Benton *et al.,* 2007), this study adds an extra level of complexity, by having a tidal regime, to increase the similarity to the natural study system. This research illustrates how abiotic factors such as light availability and tidal cycle, together with the effects of global change (altered CO_2 concentrations, elevated temperature) are likely to have an interactive effect on measured ecosystem processes. As dominant primary producers, MPB form the base of the food chain in estuarine and coastal ecosystems, and through trophic interactions, the effects of climate change will both directly and indirectly affect different trophic levels.

Since ecosystems consist of a series of complex interactions between chemical, physical and biological components (Fabry *et al.,* 2008; Gaedke *et al.,* 2010), mesocosm studies play a vital role in understanding the ecological processes that may be affected, or driven, by global climate change. However, it is important to note that model systems, as used in this research, do not aim to simulate the natural environment, thus results must be interpreted carefully before they can be translated and applied to real-world natural systems. Despite, experimental mesocosm systems can be used to detect biotic interactions that may be less noticeable in field experiments where abiotic or physical variables may be more influential (Crowe *et al.* in press). These results demonstrate that implementing tidal cycle into the experimental design helps to accurately ascertain how potential changes in climatic variables, and their interactions, may influence intertidal systems, and the organisms that inhabit them. The tidal cycle affects MPB migration through the sediment, and thus primary productivity over the tidal cycle (Serodio et al, 1997) and thus, natural behavioural patterns of other intertidal

organisms is likely to be influenced by laboratory conditions imposed by mesocosm studies, However, experimental manipulation can provide a sound understanding of the mechanisms underlying a system's response, and this in turn can improve model predictions (Crowe *et al.,* in press). It is therefore advisable that future mesocosm studies are run alongside field experiments testing similar hypotheses, so that the results of the two can be compared to assess the relevance of laboratory studies in real-world applications (O'Connor and Bruno, 2009).

7. General Discussion

Summary of the main thesis questions:

1. *Identify the role of MPB in nutrient cycling and investigate interactions with an additional trophic level (macrofauna) in benthic model system (Chapter 3)*

Hypothesis: the presence of MPB alters the levels of nutrients in the overlying water

In chapter 3, it was shown that MPB negatively affects the levels of nutrients in the overlying water. MPB assimilate nutrients from the water column during photosynthesis, thus depleting the overall concentration.

Hypothesis: the diversity of the macrofauna affects MPB biomass

Results showed MPB was negatively affected by the presence of macrofauna, and magnitude of effect was species specific. Macrofaunal effects on MPB directly (through resuspension) and indirectly (grazing on MPB) caused higher nutrient levels in the overlying water.

2. *Determine how MPB biomass changes under predicted future climate scenarios (CO_2, temperature) and altered macrofaunal biodiversity (Chapter 4)*

Hypothesis: MPB biomass changes under different CO_2, temperature and biodiversity regimes

MPB biomass is affected by environmental changes (temperature and carbon dioxide) and altered biodiversity, with the three variables interacting to determine MPB biomass. This effect tended to be negative, with the highest MPB biomass found in the control treatments.

There were strong macrofaunal species specific effects within the different diversity treatments.

3. *Identify how MPB biomass changes under different CO_2 regimes with varying degrees of temperature fluctuation around three constant mean temperatures and with altered macrofaunal biodiversity (Chapter 5)*

Hypothesis: MPB biomass changes under different CO_2, temperature, fluctuation and biodiversity regimes

As seen in chapter 4, the carbon dioxide, mean temperature and biodiversity had an interactive negative effect on MPB biomass, but this was influenced by the degree of fluctuation around the mean temperature. This illustrates how temporal variation may be as important as changes in mean conditions (e.g. temperature).

4. *Examine how the presence of a tidal cycle affects MPB biomass under different future climate scenarios (CO_2 and temperature) (Chapter 6)*

Hypothesis: The presence of a tidal cycle positively affects MPB biomass under different climatic scenarios

Tidal cycle plays an important role in determining MPB biomass, with higher biomass found in the treatments with a tidal cycle. However, this interacted with the temperature and carbon dioxide levels to influence overall MPB biomass.

Trophic interaction of MPB and macrofauna

It is recognised that coastal ecosystems, particularly estuaries, provide a variety of essential services to humans. A significant proportion of global nutrient cycling and primary production occurs within estuaries providing many harvestable resources. These goods and processes could be critically affected by elevated temperatures and carbon dioxide, in combination with the better known anthropogenic stressors such as pollution, over-exploitation and habitat destruction (Adams 2005; Rönnback *et al.,* 2007; Paterson *et al.,* 2009). In order to assess these potential impacts, it is necessary to select some measure of ecosystem change. One of the main providers in benthic estuarine systems are the MPB. The MPB play a variety of important roles within these ecosystems: they are the main primary producers; the net importers of nutrients; and the metabolic engines mediating the nutrient flux between the sediment and the overlying water (Chapter 3) (Sundbäck *et al.,* 2000; Thornton *et al.,* 2007). While not the subject of this thesis, the microbial contribution to nutrient cycling is acknowledged (Loreau *et al.,* 2001; Hulot *et al.,* 2001; Pinckney *et al.,* 2003) and the sum total of the fluxes or change recorded incorporate the microbial activity inherent in the mesocosm systems.

However, this activity is altered by the presence of macrofauna, the primary consumers of MPB. The bioturbatory activity of the macrofauna increases the supply of nutrients from the sediment into the water column which enhances benthic-pelagic coupling and MPB growth. In turn, the MPB take up the nutrients released by the macrofaunal activity, and thus the net nutrient flux is determined by the interaction between these two trophic levels - the producers and the consumers. Macrofauna reduce MPB biomass through grazing and sediment resuspension, but this effect appears to be species specific to the infauna, demonstrating that species identity is important for the trophic linkages between MPB and macrofauna. In this

thesis, the activity of one species (*C. volutator*) dominates the macrofaunal effect on MPB through constant sediment resuspension (Dyson *et al.,* 2007; Hicks *et al.,* 2011). However, results from manipulative experiments should be interpreted carefully, as species effects may be more dominant in lab-based studies than is reflected in natural systems. Often lab conditions are favourable to one or more species, perhaps through exclusion of predators or natural environmental variation (Crowe *et al,* in press). Thus strong species effects detected in mesocosms may not be as important as abiotic or physical processes found in natural systems (Romanuk *et al.*, 2009; Crowe *et al*., in press).

System complexity

The research detailed in this thesis is unique as it addresses a range of ecological questions concerning the effects of climate change, incorporating different trophic levels, multiple environmental stressors (based on future climate scenarios) and variability of stressors and environmental factors (temperature fluctuation and tidal regime) in benthic systems.

The work covered in the light/dark experiment (Chapter 3) builds on existing knowledge of MPB in estuarine systems, and places it in the context of a wider ecosystem by integrating an extra trophic level with the addition of the macrofauna. This study is in line with previous research identifying the role of photosynthetic algae in nutrient exchange (Sundbäck *et al.,* 2000; Tang and Kristensen, 2007; Thornton *et al.,* 2007) but also determines how this activity is affected by the grazing and bioturbation of the consumers (macrofauna) under differing diversity levels. With increasing concerns over the changing climate, it is now important to not only be able to identify the role of species and species interactions within ecosystems, but also to determine how these may be affected by a changing environment. It is generally accepted that high species richness means ecosystems are more ‘stable’ and are buffered from

changes forced through altered environmental variables or anthropogenic stressors (Hooper *et al.,* 2005; Thebault and Loreau, 2005; Grman *et al.,* 2010). Declining biodiversity will cause decreased stability and reduce the scale of essential services, such as primary production and nutrient cycling (France and Duffy, 2006). However, individual species are likely to respond to changes in an unpredictable and complex way, and this response will be confounded by species interactions, and the addition of multiple stressors (Crain *et al.,* 2008; Brown *et al.,* 2010). The direct and indirect effects of multiple stressors on ecosystems will be mediated through trophic interactions, and interactions between the stressors (Beveridge *et al.,* 2010; Traill *et al.,* 2010).

Ocean acidification

Ocean acidification is a major marine threat causing changes to many marine species. Most research has focused on the effects on calcification and calcifying organisms (Vezina and Hoegh-Guldberg, 2008) and there has been markedly less emphasis on non-calcifying and photosynthetic species (Connell and Russell, 2010). It has been suggested that photosynthetic organisms will benefit from the increasing CO_2 concentrations by an increase in primary production (Kroeker *et al.,* 2010). However, the results in these experiments suggest that elevated CO_2 does not result in higher primary production or increased MPB biomass, supporting the theory that at present CO_2 levels, MPB are saturated with regard to CO_2 for photosynthesis (Connell and Russell, 2010). To-date, results from research examining the effects of elevated CO_2 and temperature on marine algae have been mixed, with some species responding positively with increased growth and abundance and others unaffected (Anderson and Anderson, 2006; Russell *et al.,* 2009).

Multiple drivers

The current surge of interest in ocean acidification has produced a wide range of studies examining the biological responses of species to warming and elevated pH (Hillebrand *et al.,* 2010; Kroeker *et al.,* 2010). Variation in temperature may have profound effects and has the potential to alter the carbon metabolism of estuarine systems (Muren *et al.,* 2005; Hicks *et al.,* 2011). Altered primary production will affect the higher trophic levels within the ecosystem (Beardall *et al.,* 2009; Brown *et al.,* 2010). For simplicity, the majority of research has assessed either the impact of one altered environmental variable or focused on individual organisms (Walther *et al.,* 2002; Long *et al.,* 2004; Hargrave *et al.,* 2009; Drinkwater *et al.,* 2010; Hillebrand *et al.,* 2010), and few authors have considered the simultaneous effects of multiple drivers (Halpern *et al.,* 2007). However, ecological interactions between species mean the combined effects of multiple drivers will have complex direct and indirect effects on many trophic levels (Crain *et al.,* 2008; Brown *et al.,* 2010). An important effect on coastal ecosystem goods and services would be the potential impact of altered primary productivity on the sustainability of commercial fisheries (Brown *et al.,* 2010).

In this work, the interactive effects of elevated CO_2 and temperature, and biodiversity, had a predominantly negative effect on MPB biomass. Despite suggestions that increased CO_2 would benefit photosynthetic organisms (Kroeker *et al.,* 2010), elevated carbon dioxide did not appear to have a positive or dominant effect on MPB biomass. The interacting biotic factors had an unpredictable and non-additive effect on primary production, which was consistent in the tidal cycle study, and both the constant and fluctuating temperature experiments. Cumulative and/or interactive effects between multiple drivers will influence different levels of ecosystem functioning (Christensen *et al.,* 2006; Duffy *et al.,* 2007;

Przeslawski *et al.,* 2008; Bulling *et al.,* 2010; Gaedke *et al.,* 2010) and directly affect human welfare by critically changing the services derived from these ecosystems (Dobson *et al.,* 2006; Halpern *et al.,* 2008; Menzel *et al.,* 2009; Paterson *et al.,* 2009). In addition, the natural complexity of predicted climate change variables, such as variation of temperature (as seen in Chapter 5) needs to be considered in future research to allow accurate interpretation of the results for real world application and prediction. Multiple environmental stressors will affect ecosystem processes in many unpredictable ways (Blake and Duffy, 2010), and this will be mediated by the specific stressors, and the temporal variability of these stressors (Benedetti-Cecchi, 2005; Molinos and Donohue, 2010). Another complexity that is often overlooked is that these anthropogenically-induced environmental changes will affect ecosystems simultaneously with existing pressures such as pollution and habitat destruction. Both natural and anthropogenic-driven stressors are likely to be temporally variable, and thus intrinsically unpredictable.. Consequently, research that can directly address these concerns is essential and urgent.

Imbedded biodiversity effects

In addition to the manipulated environmental variables, this work also examined the effects of biodiversity, and of dominant species with the study ecosystems. Climate change is one of the most important factors currently affecting biodiversity (Schweiger *et al.,* 2008), so understanding how altered biodiversity influences ecosystem response is vital for comprehending and mediating future climate changes. Intense ecological research has demonstrated that species richness plays a vital role in ecosystem stability (Hooper *et al.,* 2005; Thebault and Loreau, 2005; Blake and Duffy, 2010; Grman *et al.,* 2010; Traill *et al.,* 2010), and this will determine how an ecosystem responds to environmental changes.

However, there is still a large amount of uncertainty surrounding how different individual species may respond to changing environmental conditions.

Species respond to stressors in different and unpredictable ways, and in the context of climate change, species are likely to be functionally diverse in their response, regardless of whether they are dominant species (numerically or through biomass) (Blake and Duffy, 2010). In fact, this 'response diversity' may determine ecosystem stability under climate change rather than the better known concept of 'functional diversity' (see Petchey and Gaston, 2006). However, the negative response of MPB biomass under different species assemblages across all climatic treatments was underpinned by the consistent dominance of one species, *C. volutator*, suggesting there is low response diversity in this system. Although the focus of this research was predominantly on MPB biomass, a proxy for primary production, it is important to acknowledge that changes in infaunal diversity will affect resource utilisation (Levinton and Kelaher, 2004), and this may be reflected in other ecosystem processes, e.g. nutrient cycling, that were not reported here. Thus the decline in MPB biomass was driven by complex interactions between the environmental variables and biodiversity / species assemblage composition.

Complexity of variation in ecological studies

Aspects of environmental change that have often been overlooked in ecological studies include aspects of variance, frequency, intensity and temporal change of potential impacts. In this case, the following apply:

- Variance = range around mean of the effect (e.g. temperature). In this thesis, although the mean temperatures in the fluctuating chapter were the same as those in the constant temperature chapter, the results were strongly influenced by the variance around the mean, from as little as 1ºC up to 6ºC

- Frequency = re-occurrence of event, such as the frequency of storms, flooding or drought

- Intensity = magnitude of event, like the rating of hurricanes or tornadoes, or flood warnings

- Temporal variation: gradual change with time (diurnal/seasonal/annual). Natural cycles in temperature occur daily in intertidal habitats, and this in turn changes on a larger timescale, such as annually.

Temporal variability of anthropogenic stressors, such as pollution or disturbance, will affect ecosystem processes, and the effects of variance in the context of climate change are understudied (Benedetti-Cecchi *et al.,* 2006; Bertocci *et al.,* 2010; Molinos and Donohue, 2010). So whilst the interaction of multiple stressors will unpredictably alter ecosystem processes (Blake and Duffy, 2010; Shears and Ross, 2010), these effects in turn will be mediated by the temporal variance, frequency, and intensity of these stressors (Molinos and Donohue, 2010). It is imperative, therefore, that future research accounts for the natural variability of environmental stressors whenever possible. To-date, few ecological studies have examined the interaction of temporal patterns of multiple stressors on individual species, and across trophic levels (Crain *et al.,* 2008; Molinos and Donohue, 2010; Shears and Ross, 2010).

Relevance of mesocosm studies for real world application

There has been a recent shift of focus in ecosystem-function research moving from experimental lab-based approaches to in situ field studies, but due to the complexity of climate research (manipulated carbon dioxide and temperature levels), experiments still form a useful tool to identify the driving factors behind an ecosystem response (Benton *et al.,* 2007; Gaedke *et al.,* 2010) and allow the generation of models and theories. It is vital to ensure that the natural study system is simulated as closely as possible to enable correct interpretation of the results (Benton *et al.,* 2007), as these studies will enable us to comprehend the mechanisms behind ecosystem response. Experimental manipulative studies are often criticised for being unrealistic and irrelevant (Benton *et al*., 2007). Some ecosystem processes and natural behavioural patterns may be compromised within laboratory settings (Thompson *et al.,* 1998; Crowe *et al*., in press) or not reflected in natural ecosystems (Carpenter, 1996). Intertidal animals are adapted to rapidly changing their behaviour under different environmental factors, thus trait-dependent interactions may be masked or exaggerated in laboratory experiments where the environment is strictly controlled (Chapman, 2000; Crowe *et al.,* in press). Few studies have compared results from lab-based experiments to similar field experiments, and those that have show varied results (Crowe *et al*., in press). Thompson *et al.* (1998) found inconsistent results between the laboratory and field results, with barnacle settlement influenced by biotic factors in the field. However, similar effects were seen in the field findings, albeit at a smaller scale than observed under manipulated laboratory conditions, in Farrell and Crowe's study on mussels (Farrell and Crowe, 2007). Despite this, mesocosm studies have helped develop understanding of the mechanisms underlying the processes affected by diversity (France and Duffy, 2006), and these studies are well suited for directly testing ecological theory (Bulling *et al*., 2006). One

of the greatest strengths of manipulative mesocosm experiments is the ability to easily replicate treatments and to closely control parameters, such as carbon dioxide levels and temperature as seen in this thesis, allowing identification of processes and mechanisms that may be present in natural systems (Bulling *et al.,* 2006; Naeem *et al*., 2009). Future experimental research would benefit from a combined approach of laboratory based studies run alongside field experiments of a similar nature, to test whether effects seen in the controlled experiments are still present under natural variability (Crowe *et al.,* in press).

This work is currently unique as it combines multiple stressors, two trophic levels (one with altered biodiversity), and temporal variability of environmental stressors, thus addressing many of the current ecological questions concerning climate change and ocean acidification. Due to the interactive effects between climatic drivers and other anthropogenic stressors, such as overfishing, habitat destruction and pollution (Solan *et al.,* 2004; Byrne *et al.,* 2009), interpreting the results from ecological studies can be complex and difficult. This work has detailed how environmental changes and temporal variables of climatic drivers may affect ecosystem processes across different trophic levels. In marine coastal ecosystems, these effects will have economic and social repercussions for the human services and livelihoods that they provide. A fully integrated approach incorporating high quality experimental research with long-term observational data and environmental modelling will be vital to ensure effective mitigation and adaptation to future environmental change.

Final conclusion

Human induced changing environmental variables continue to have a direct impact on marine and terrestrial ecosystems, on the species and processes within these ecosystems. The stressors of elevated temperature and carbon dioxide will interact with current existing anthropogenic stressors, such as pollution, habitat destruction and overexploitation to cause

varied and unpredictable effects within many ecosystems. These effects, in turn, will have socio-economic consequences for the human services derived from these ecosystems, and the current uncertainty surrounding predictions makes it extremely difficult to effectively mediate future changes. Climate change is not a purely ecological problem, the potential social and economic repercussions of environmental changes make it imperative that a multi-disciplinary approach is used to focus research effort into understanding and identifying how ecosystems will respond to future changes.

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APPENDIX 1

This appendix contains additional information that is not in the main thesis body:

- Graph showing variation of seawater temperature at the study site
- Raw data boxplots for light/dark data in Chapter 3
- Raw data boxplots for constant CO_2 data in Chapter 4
- Enlarged plots of Figures 5.5 and 5.6 in Chapter 5

Chapter 2

Daily water temperature readings from the Ythan Estuary (study site) in Newburgh, Aberdeenshire provided a guide for the temperatures used in the mesocosm experiments.

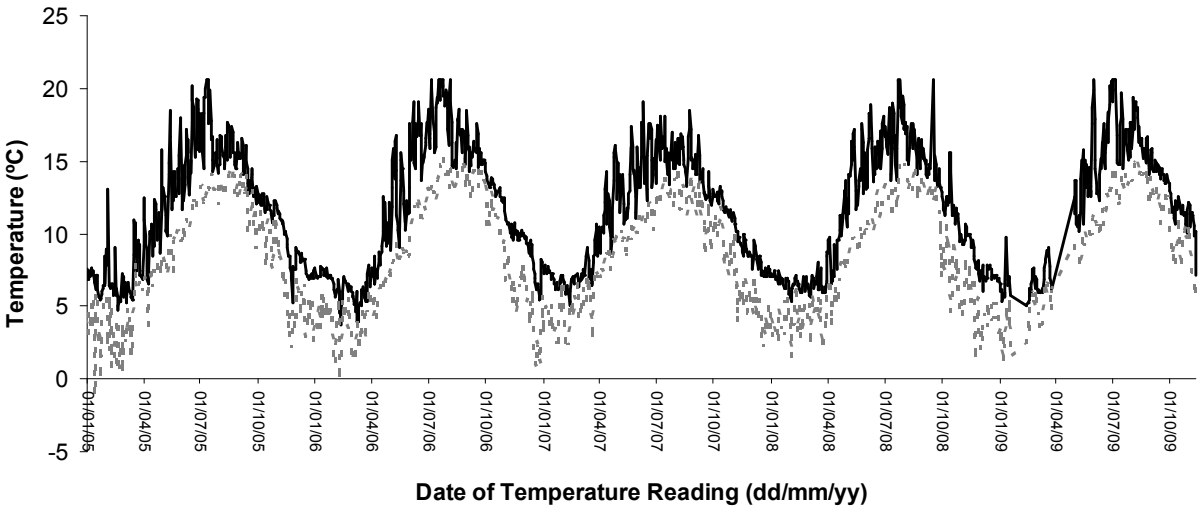


Figure A1 The annual variation of water temperature at the study site on the Ythan estuary, showing the minimum (dotted line) and maximum (solid line) daily water temperature.

Chapter 3

Raw data boxplots for MPB biomass under the constant CO_2 regimes with increasing species richness and different species assemblage regimes

Species Richness Plots for Nutrient Concentrations

(i) NH_4-N raw data boxplot

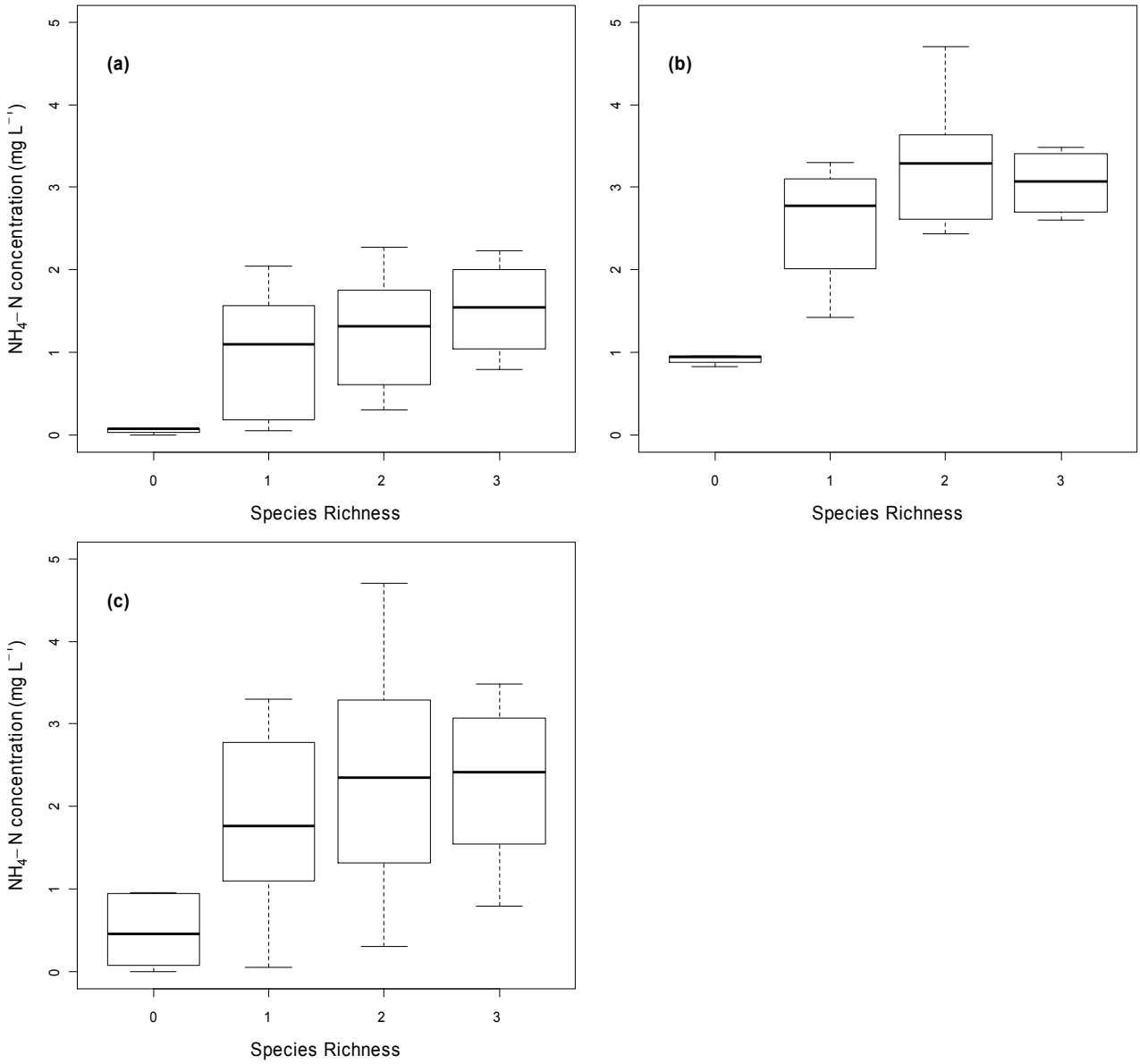


Figure A2 Boxplots of the raw data for NH_4-N across species richness treatments in the (a) L/D regime; (b) dark regime (c) both regimes combined. NH_4-N is along the y-axis, with increasing species richness along the x-axis.

(ii) PO_4-P raw data boxplot

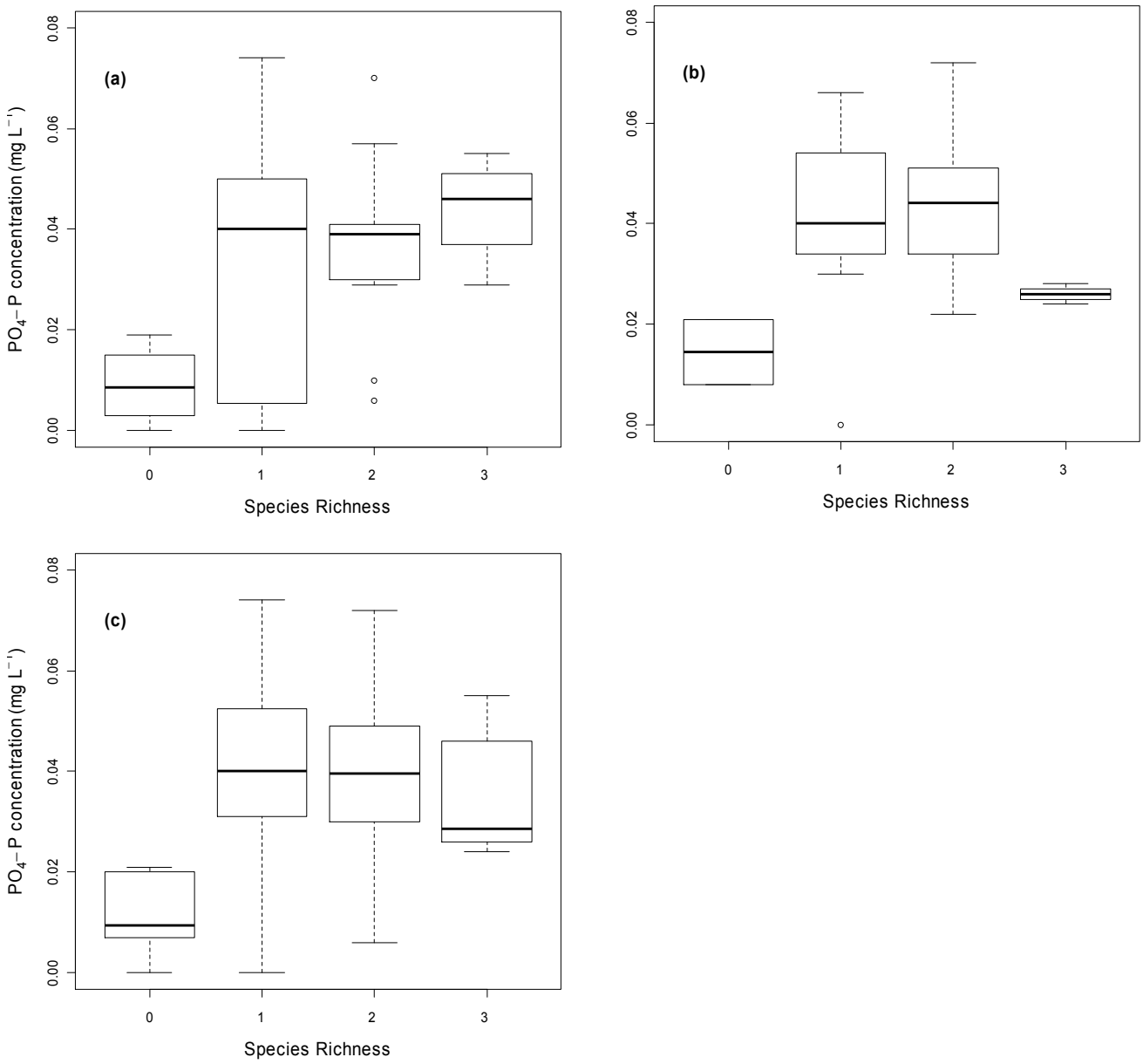


Figure A3 Boxplots of the raw data for PO_4-P across species richness treatments in the (a) L/D regime; (b) dark regime (c) both regimes combined. PO_4-P is along the y-axis, with increasing species richness along the x-axis.

(iii) NO_x-N raw data boxplot

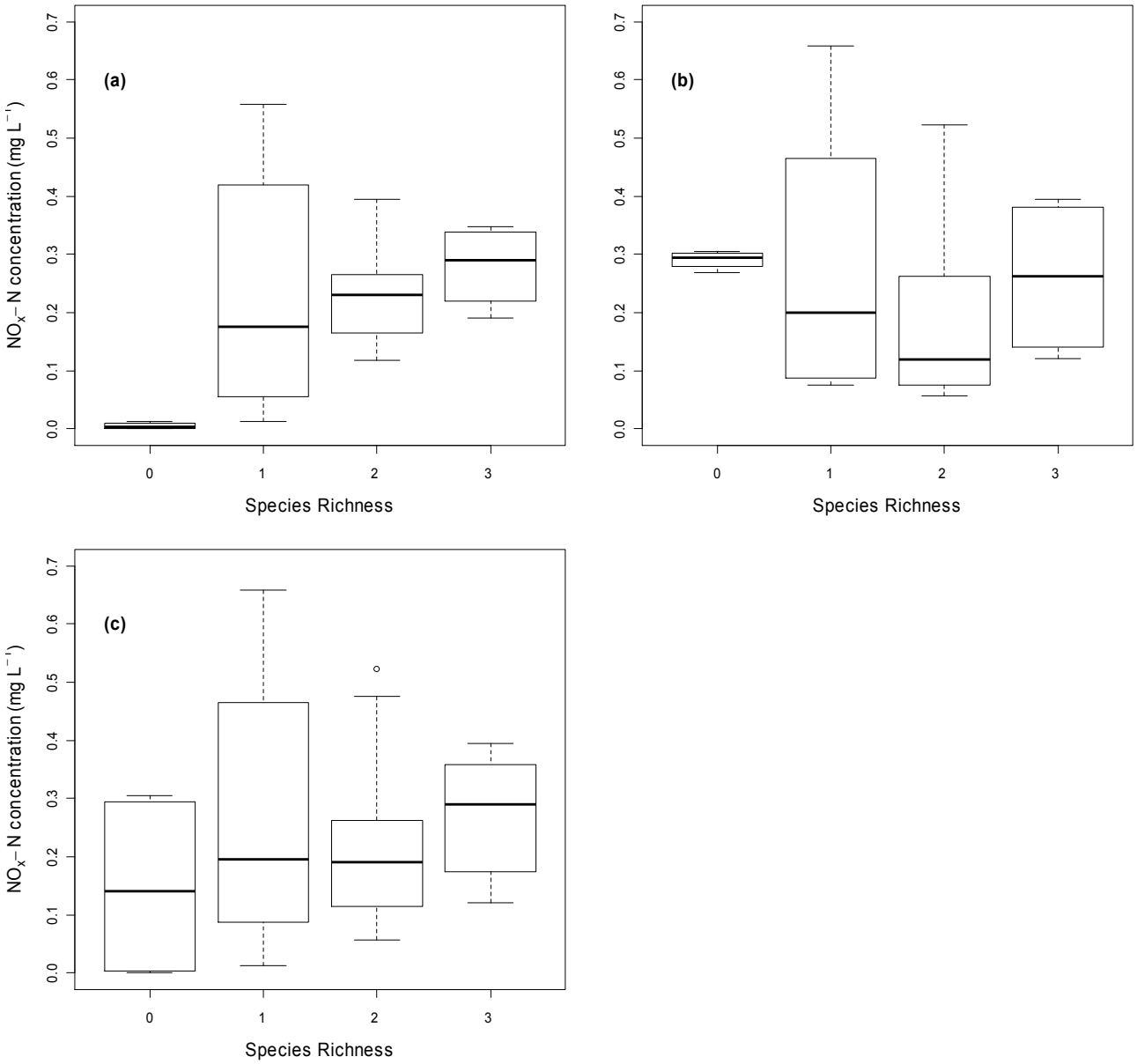


Figure A4 Boxplots of the raw data for NO_x-N across species richness treatments in the (a) L/D regime; (b) dark regime (c) both regimes combined. NO_x-N is along the y-axis, with increasing species richness along the x-axis.

Species Identity Plots for Nutrient Concentrations

(i) NH_4-N raw data boxplot

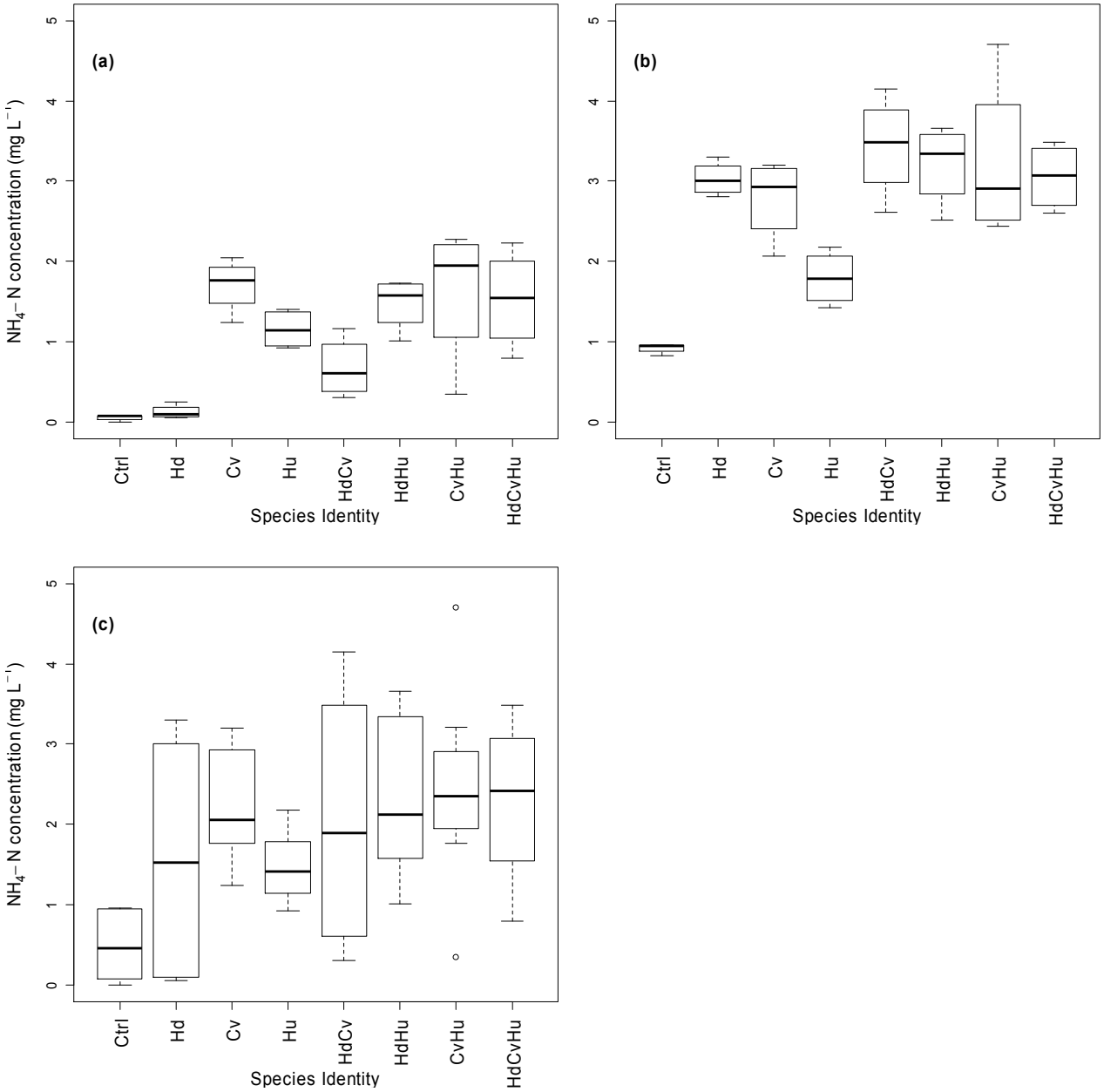


Figure A5 Boxplots of the raw data for NH_4-N across different species identity treatments in the (a) L/D regime; (b) dark regime (c) both regimes combined. NH_4-N is along the y-axis, with increasing species richness along the x-axis. Ctrl = Control, Hd = *H. diversicolor,* Cv = *C. volutator,* Hu = *H. ulvae.*

(ii) PO_4-P raw data boxplot

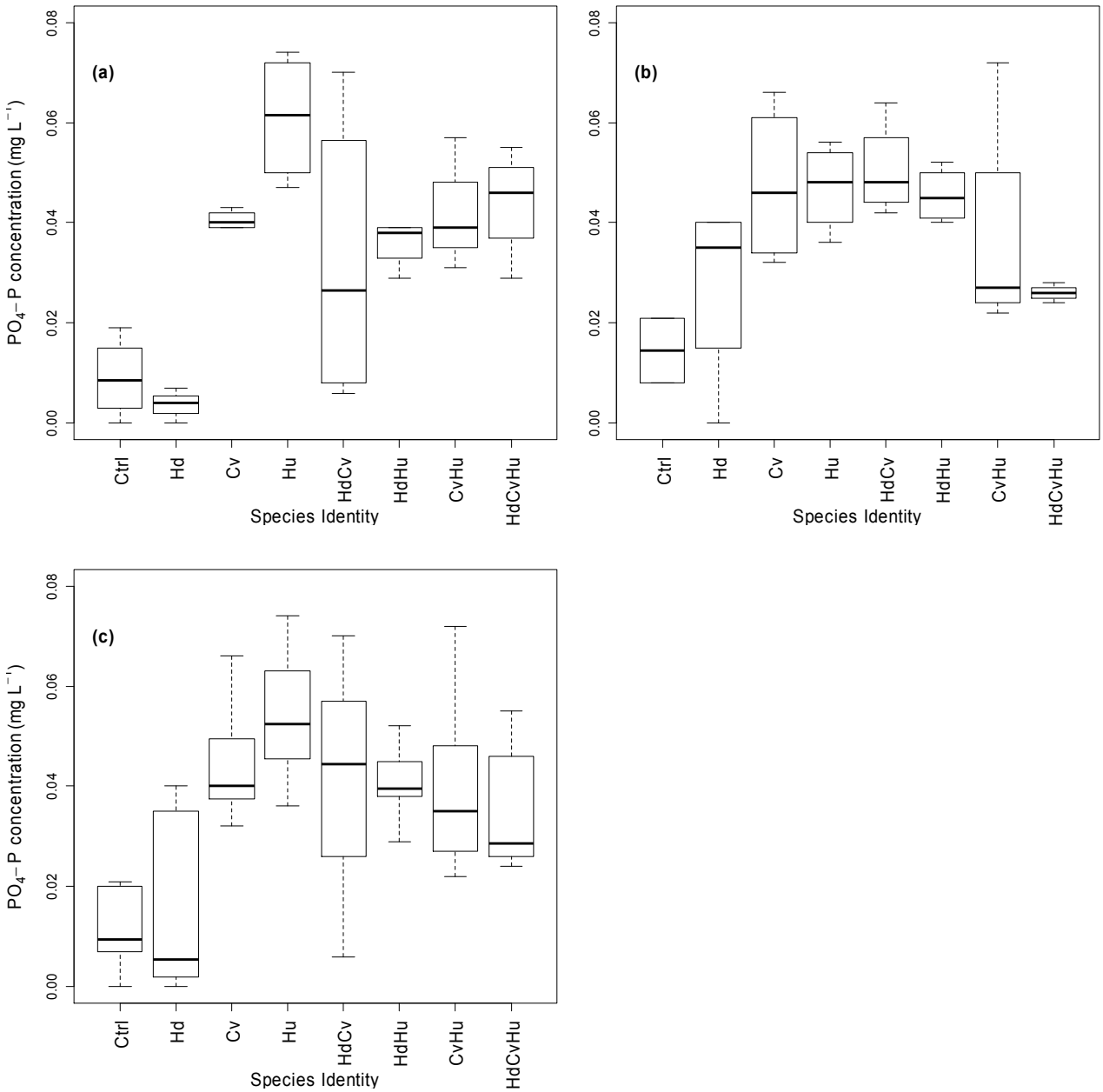


Figure A6 Boxplots of the raw data for PO_4-P across different species identity treatments in the (a) L/D regime; (b) dark regime (c) both regimes combined. PO_4-P is along the y-axis, with increasing species richness along the x-axis. Ctrl = Control, Hd = *H. diversicolor,* Cv = *C. volutator,* Hu = *H. ulvae.*

(iii) NO_x-N raw data boxplot

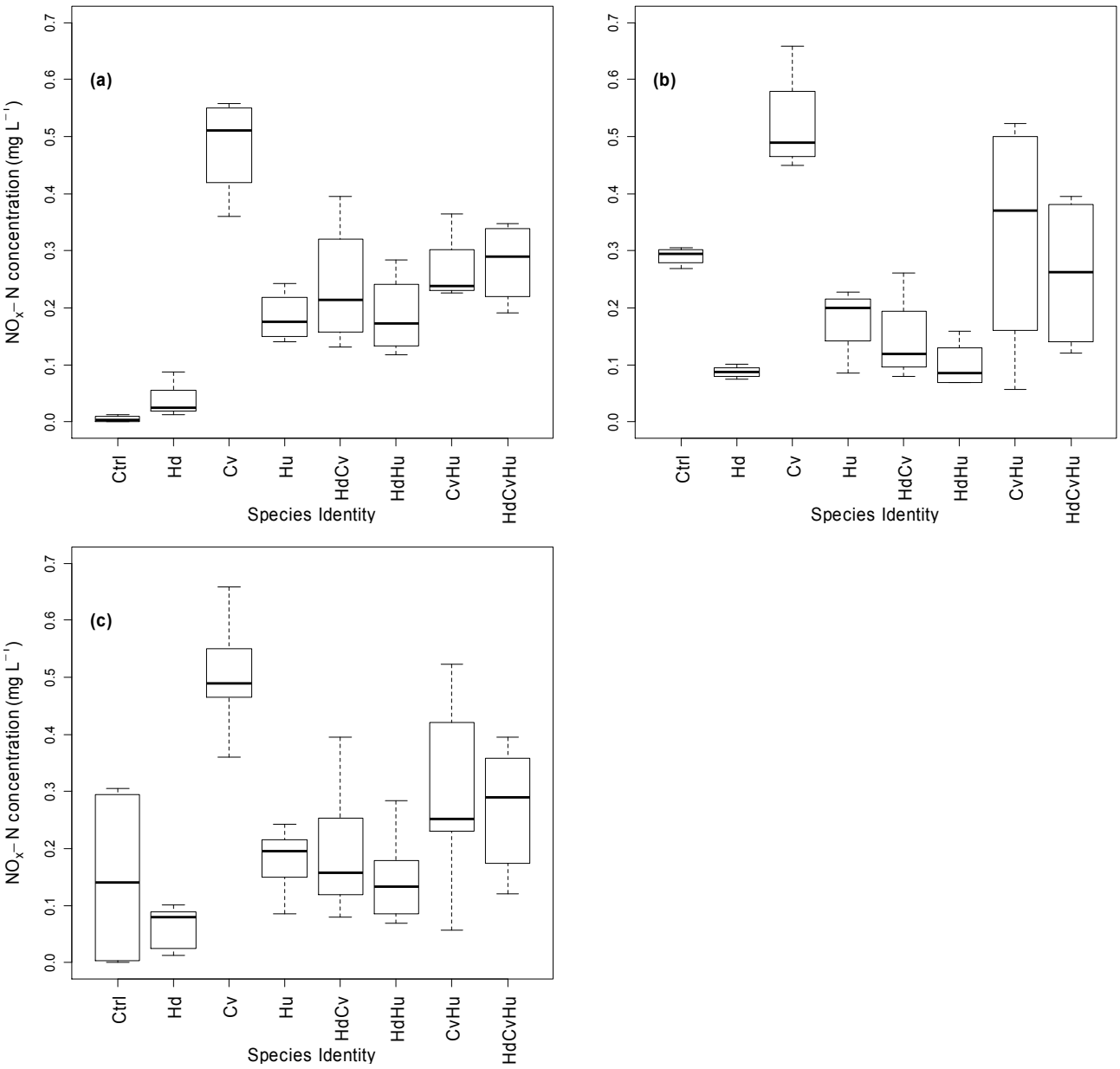


Figure A7 Boxplots of the raw data for NO_x-N across different species identity treatments in the (a) L/D regime; (b) dark regime (c) both regimes combined. NO_x-N is along the y-axis, with species identity along the x-axis. Ctrl = Control, Hd = *H. diversicolor,* Cv = *C. volutator,* Hu = *H. ulvae.*

Chapter 4

Raw data boxplots for MPB biomass under the constant CO_2 regimes with increasing species richness

Species Richness

Boxplots showing the raw data for MPB biomass with increasing Species Richness at 6°C (left column), 12°C (middle column) and 18°C (right column) for each CO_2 concentration (370ppmv, top row; 600ppmv, middle row; 1000ppmv, bottom row).

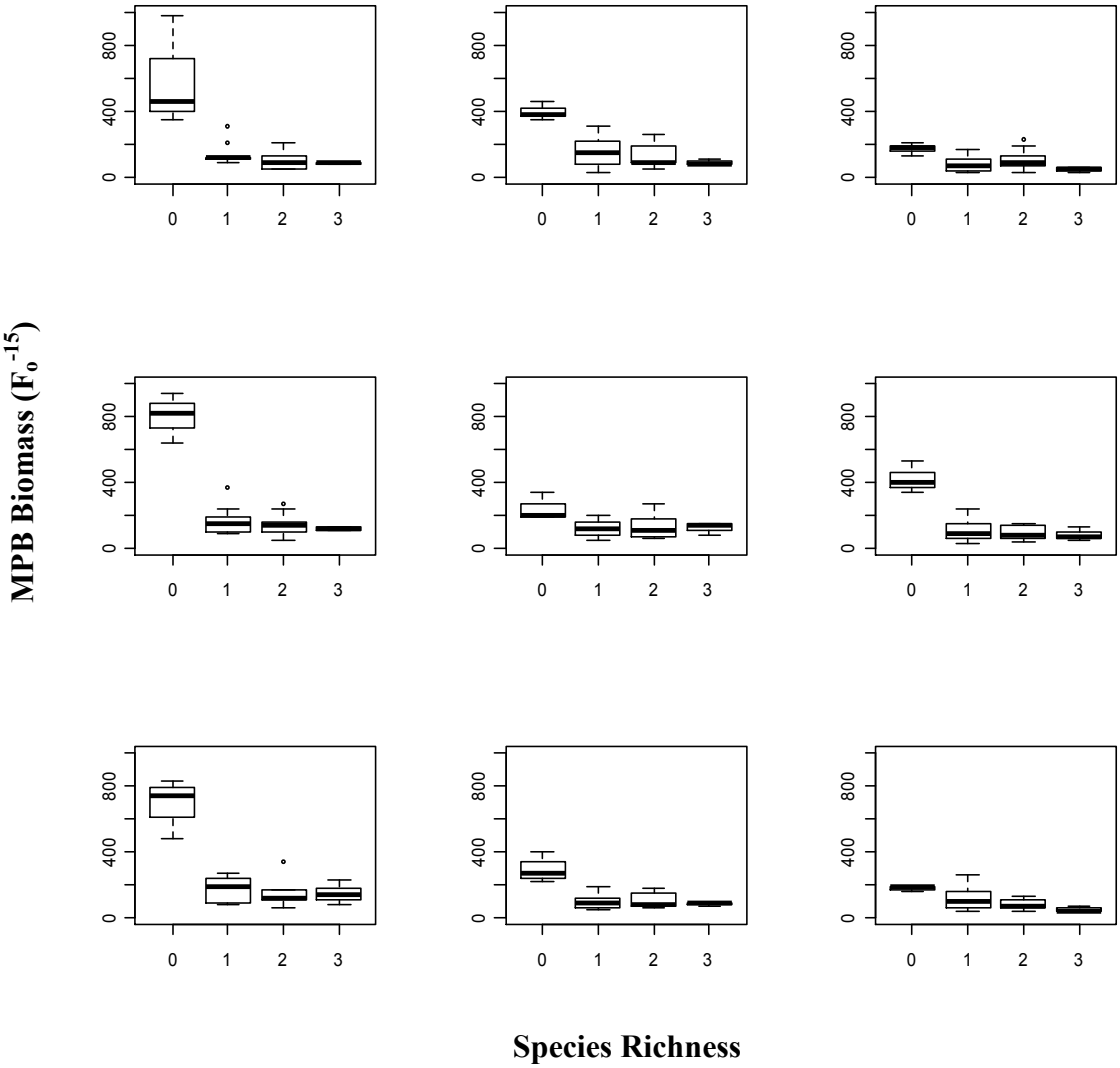


Figure A8 Raw data for MPB across an increasing species richness gradient at 6 °C (left column), 12 °C (middle column) and 18 °C (right column) for each CO_2 concentration (370 ppmv, top row; 600 ppmv, middle row; 1000 ppmv, bottom row).

Species Identity

Raw data boxplots for MPB biomass under the constant CO_2 regimes: the species identity codes stand for: 0-control; 1-*H. diversicolor (Hd)*; 2-*C. volutator (Cv);* 3-*H. ulvae (Hu)*; 4- HdCv; 5- HdHu; 6-CvHu; 7-HdCvHu

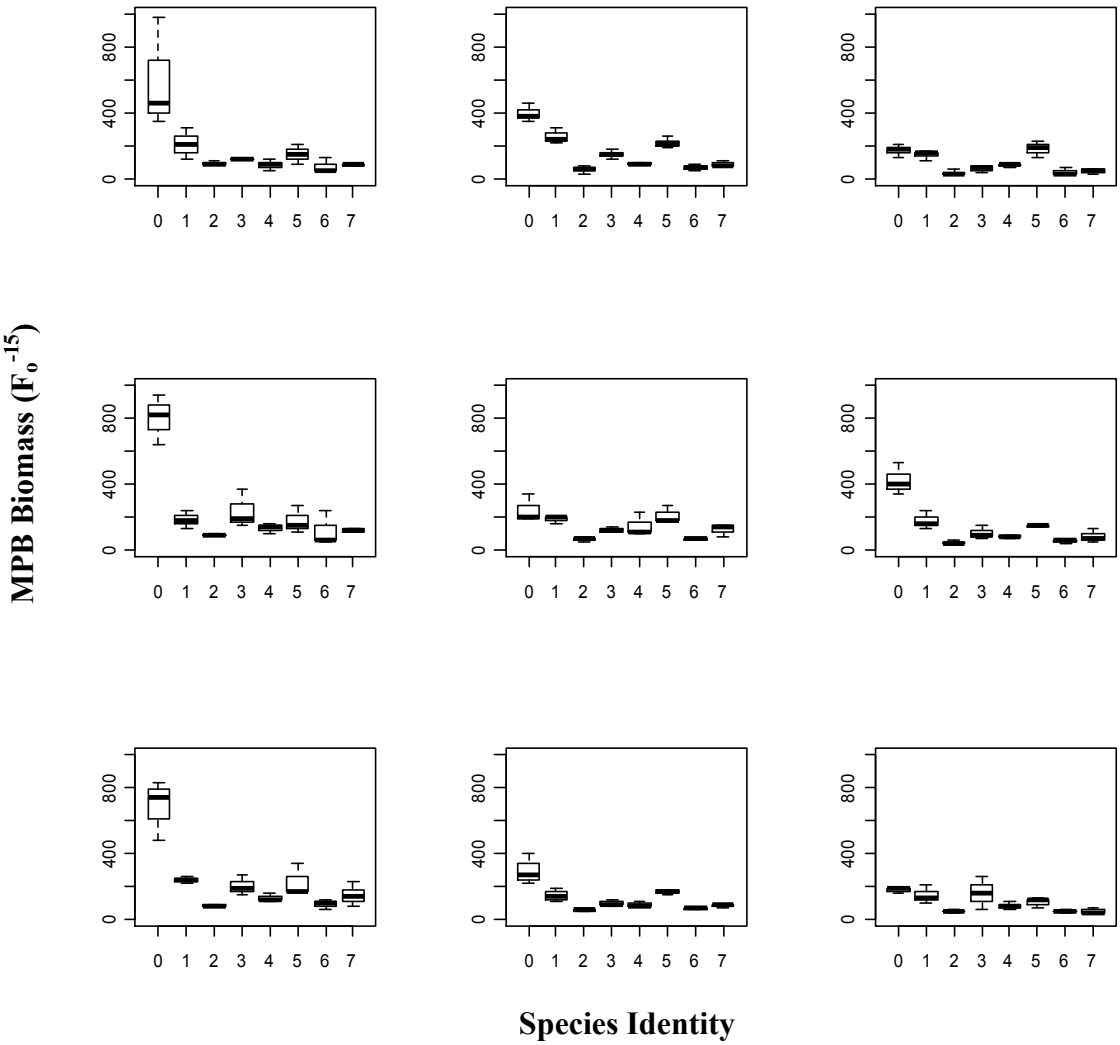


Figure A9 Raw data for MPB biomass for each Species Identity at 6 °C (left column), 12 °C (middle column) and 18 °C (right column) for each CO_2 concentration (370 ppmv, top row; 600 ppmv, middle row; 1000 ppmv, bottom row).

Chapter 5

Enlarged figures from Chapter 5 – Fluctuating temperature regimes and elevated carbon dioxide

Figure 5.5 shows the model visualisations of the interaction between temperature, fluctuation and species assemblage composition on MPB biomass under ambient CO_2 concentrations (370 ppmv)

Figure 5.5 a) Visualisation of the interaction between temperature, fluctuation and species assemblage composition on MPB biomass under ambient CO_2 concentrations (370 ppmv) at the mean temperature of 6 ºC

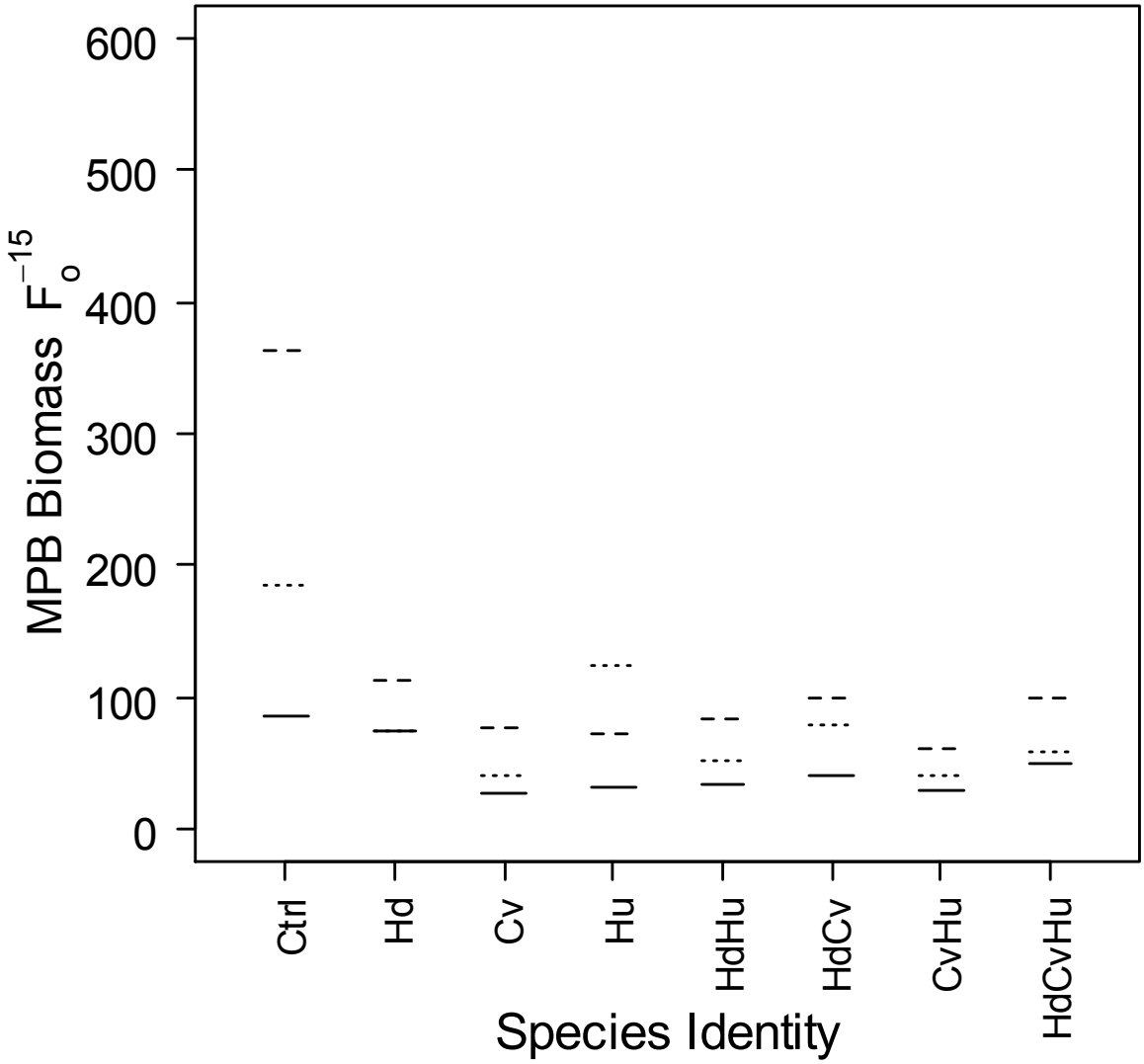


Figure 5.5 b) Visualisation of the interaction between temperature, fluctuation and species assemblage composition on MPB biomass under ambient CO_2 concentrations (370 ppmv) at the mean temperature of 12 ºC

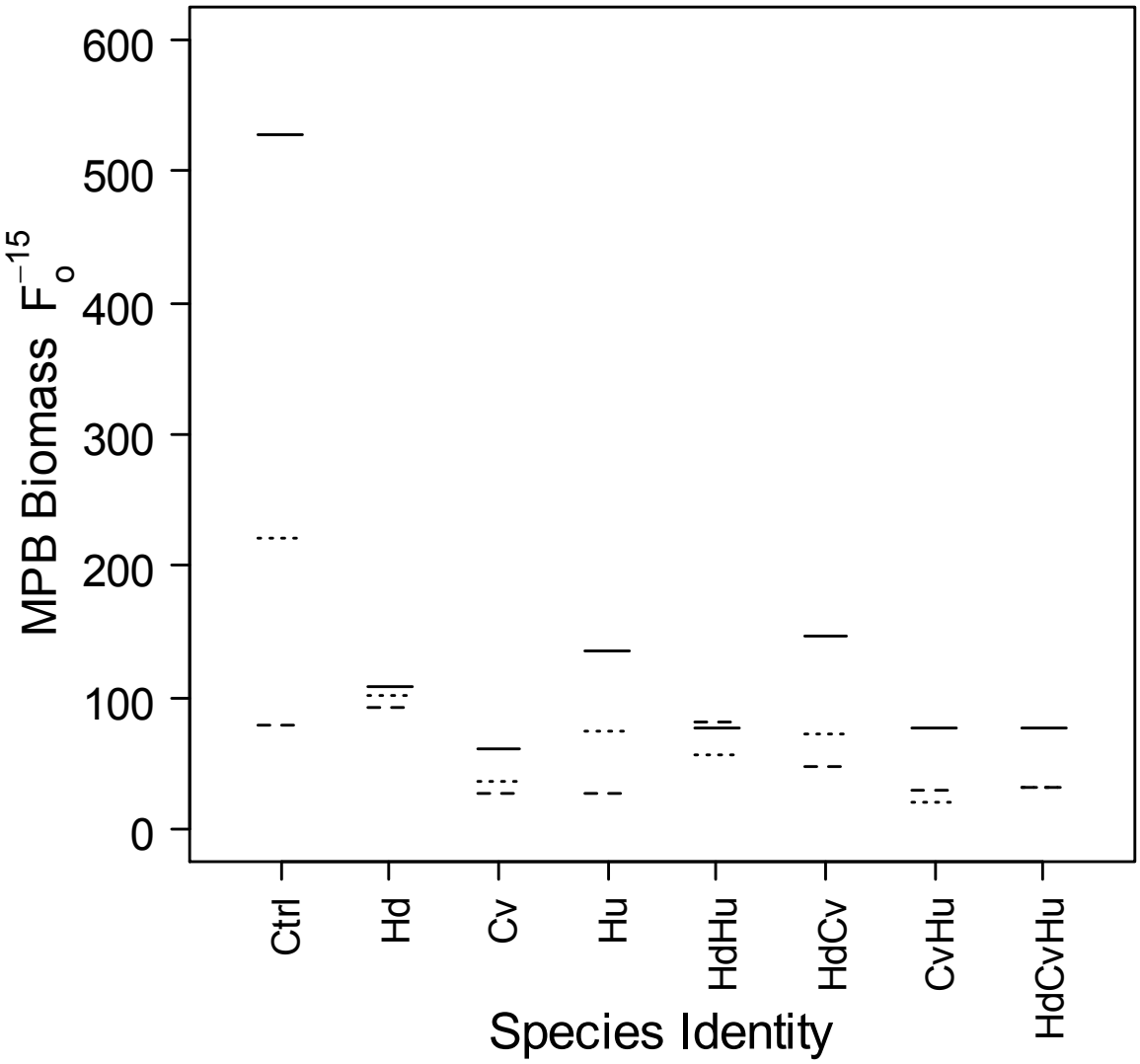


Figure 5.5 c) Visualisation of the interaction between temperature, fluctuation and species assemblage composition on MPB biomass under ambient CO_2 concentrations (370 ppmv) at the mean temperature of 18 ºC

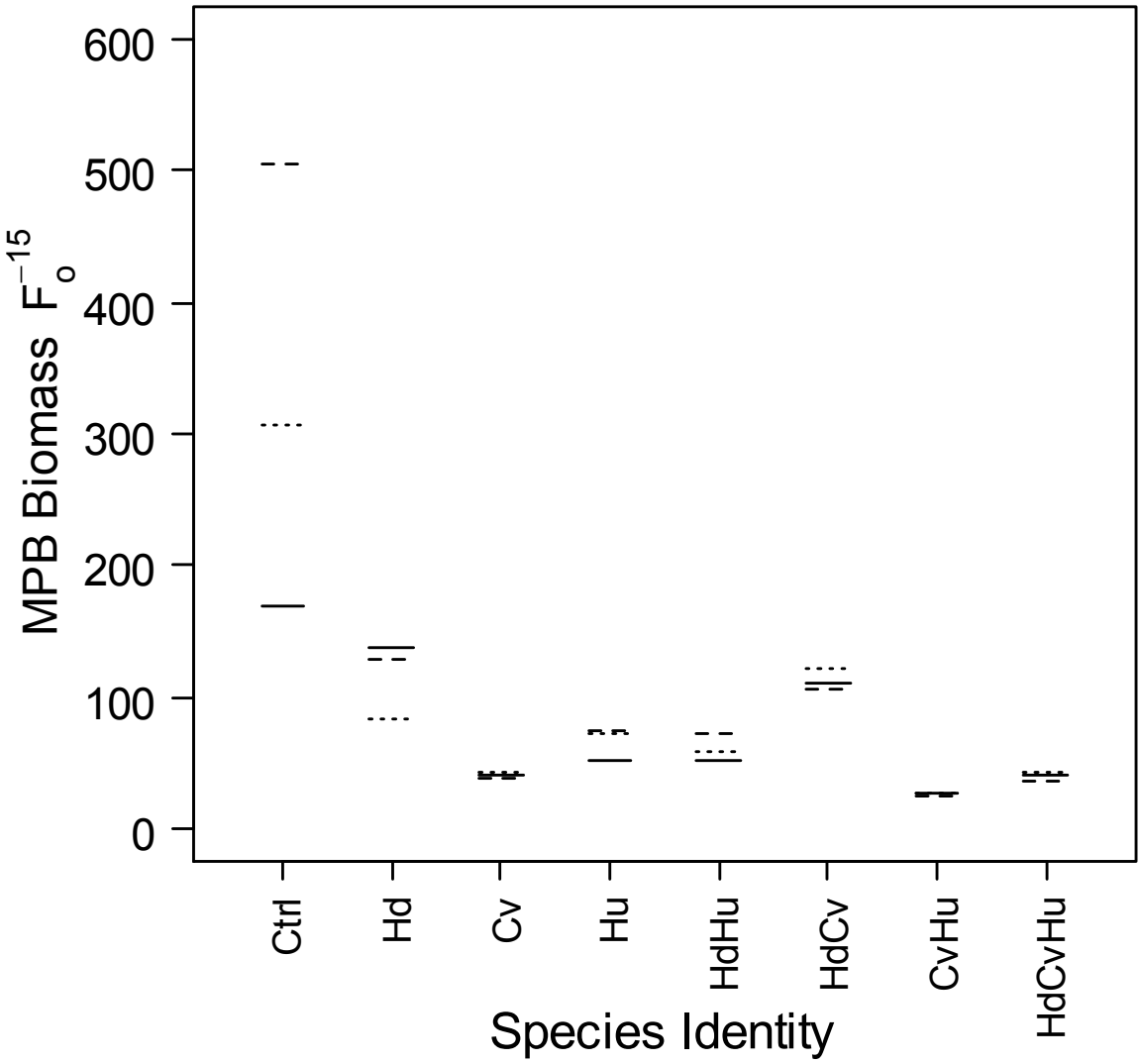


Figure 5.6 shows the model visualisations of the interaction between temperature, fluctuation and species assemblage composition on MPB biomass under ambient CO_2 concentrations (1000 ppmv)

Figure 5.6 a) Visualisation of the interaction between temperature, fluctuation and species assemblage composition on MPB biomass under ambient CO_2 concentrations (1000 ppmv) at the mean temperature of 6 ºC

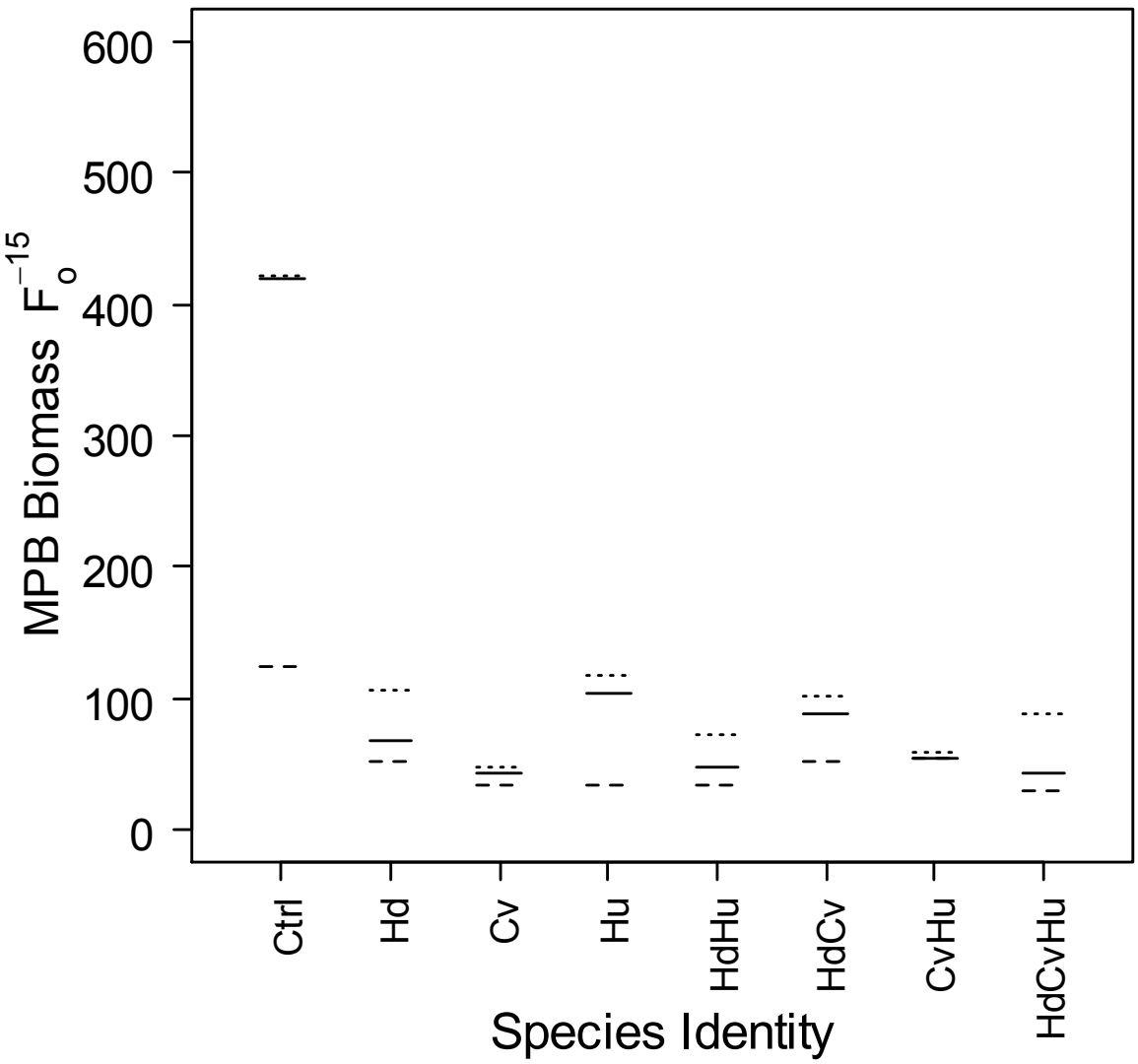


Figure 5.6 b) Visualisation of the interaction between temperature, fluctuation and species assemblage composition on MPB biomass under ambient CO_2 concentrations (1000 ppmv) at the mean temperature of 12 ºC

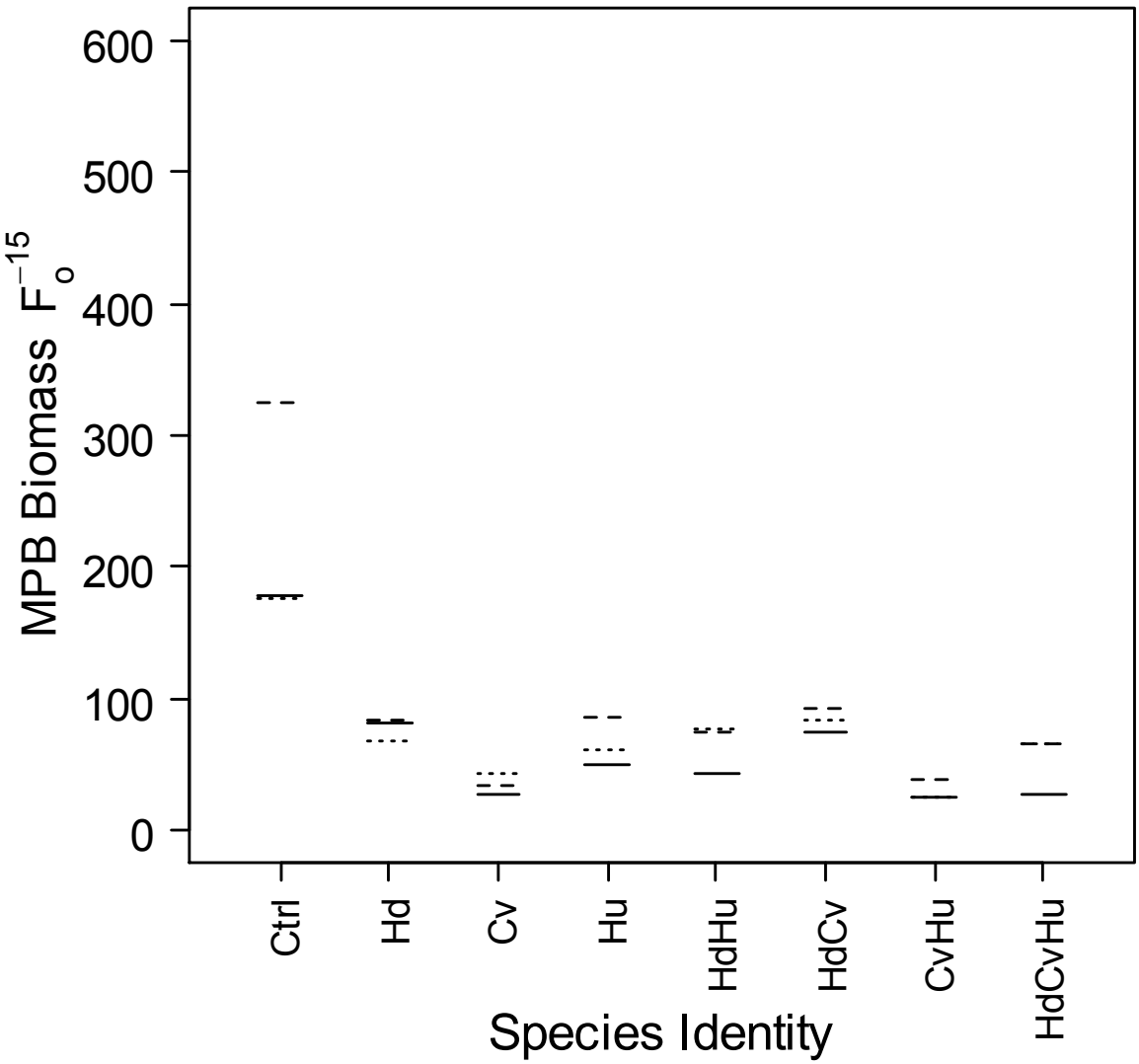
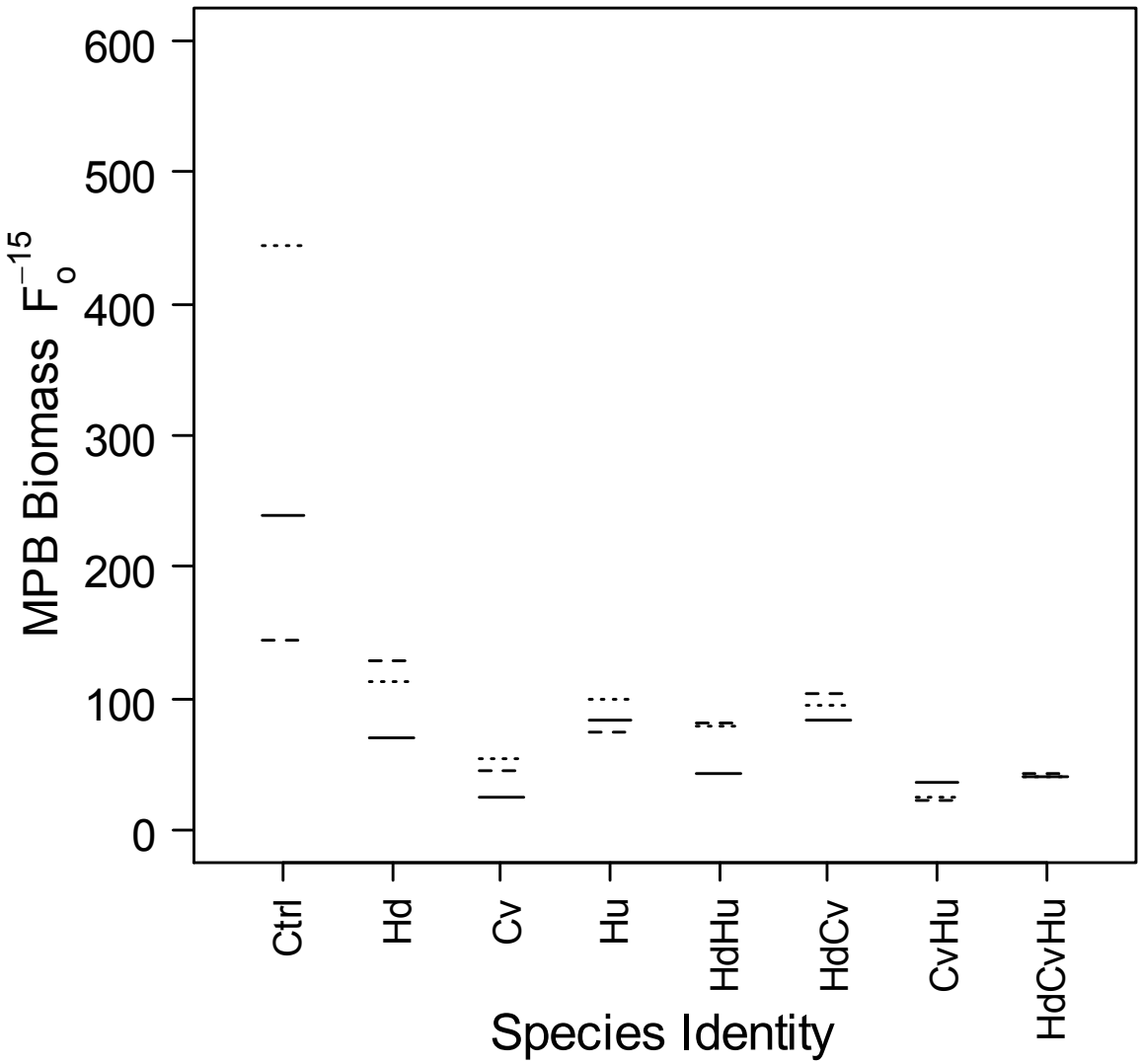


Figure 5.6 c) Visualisation of the interaction between temperature, fluctuation and species assemblage composition on MPB biomass under ambient CO_2 concentrations (1000 ppmv) at the mean temperature of 18 ºC



APPENDIX 2

This appendix contains two peer reviewed papers, from the work detailed in this thesis, that have been published.

Hicks, N, Bulling, M, Solan, M, Raffaelli, D, White, P & Paterson, DM (2011) 'Impact of biodiversity-climate futures on primary production and metabolism in a model benthic estuarine system', *BMC Ecology*, vol 11, no. 7

Plus additional information

Bulling, MT , **Hicks, N,** Murray, L , Paterson, DM , Raffaelli, D, White, PCL & Solan, M (2010) 'Marine biodiversity-ecosystem functions under uncertain environmental futures', *Philosophical Transactions of the Royal Society of London Series B* , vol 365, no. 1549, pp. 2107-2116

Plus additional information

Impact of biodiversity-climate futures on primary production and metabolism in a model benthic estuarine system

Natalie Hicks, Mark T. Bulling, Martin Solan, Dave Raffaelli, Piran C. L. White and David M. Paterson

BMC Ecology Volume 11, No 7, 2011

doi: 10.1186/1472-6785-11-7

http://www.biomedcentral.com/1472-6785/11/7

Impact of biodiversity-climate futures on primary production and metabolism in a model benthic estuarine system

Natalie Hicks*[1,2], Mark T. Bulling[2,3], Martin Solan[2], Dave Raffaelli[3], Piran C. L. White[3] and David M. Paterson[1]

[1] *Sediment Ecology Research Group, Scottish Oceans Institute , University of St Andrews, East Sands, St. Andrews, Fife, KY16 8LB, United Kingdom*

[2] *Oceanlab, University of Aberdeen, Main Street, Newburgh, Aberdeenshire, AB41 6AA, United Kingdom*

[3] *Environment Department, University of York, Heslington, York, YO10 5DD, United Kingdom*

* Corresponding author

Email addresses:

NH: natalie.hicks.50@aberdeen.ac.uk

MTB: M.Bulling@derby.ac.uk

MS: m.solan@abdn.ac.uk

DR: dr3@york.ac.uk

PCLW: pclw1@york.ac.uk

DP: dp1@st-andrews.ac.uk

Abstract

Background

Understanding the effects of anthropogenically-driven changes in global temperature, atmospheric carbon dioxide and biodiversity on the functionality of marine ecosystems is crucial for predicting and managing the associated impacts. Coastal ecosystems are important sources of carbon (primary production) to shelf waters and play a vital role in global nutrient cycling. These systems are especially vulnerable to the effects of human activities and will be the first areas impacted by rising sea levels. Within these coastal ecosystems, microalgal assemblages (microphytobenthos: MPB) are vital for autochthonous carbon fixation. The level of *in situ* production by MPB mediates the net carbon cycling of transitional ecosystems between net heterotrophic or autotrophic metabolism. In this study, we examine the interactive effects of elevated atmospheric CO_2 concentrations (370, 600, and 1000 ppmv), temperature (6°C, 12°C, and 18°C) and invertebrate biodiversity on MPB biomass in experimental systems. We assembled communities of three common grazing invertebrates (*Hydrobia ulvae*, *Corophium volutator* and *Hediste diversicolor)* in monoculture and in all possible multispecies combinations. This experimental design specifically addresses interactions between the selected climate change variables and any ecological consequences caused by changes in species composition or richness.

Results

The effects of elevated CO_2 concentration, temperature and invertebrate diversity were not additive, rather they interacted to determine MPB biomass, and overall this effect was negative. Diversity effects were underpinned by strong species composition effects, illustrating the importance of individual species identity.

Conclusions

Overall, our findings suggest that in natural systems, the complex interactions between changing environmental conditions and any associated changes in invertebrate assemblage structure are likely to reduce MPB biomass. Furthermore, these effects would be sufficient to affect the net metabolic balance of the coastal ecosystem, with important implications for system ecology and sustainable exploitation.

Background

Rising global temperatures and increasing atmospheric CO_2 concentrations are causing changes to a wide range of ecosystems [1]. The influence of these changing conditions on ocean chemistry and the distribution of species in marine systems [2,3, 4] is of particular concern. Atmospheric concentrations of CO_2 have risen from pre-industrial levels (275 ppmv) to 370 ppmv and continue to increase by ~1.5 ppmv yr^{-1} [5]. Up to 50% of the global increase in carbon dioxide has been absorbed by the oceans [6] and the pH of the sea is predicted to fall by up to 0.5 pH units by the end of the century [2]. Concerns over the likely consequences are now widespread [7,8]. Studies of elevated carbon dioxide concentrations have demonstrated potential impacts on nutrient availability, primary productivity and decomposition [1,9,10]. This directly influences the functionality of ecosystems [9] across multiple trophic levels [11] and effects are difficult to anticipate.

Empirical research to date has concentrated on the responses of a variety of ecosystems to individual anthropogenic drivers of change (terrestrial [12]; marine [13]; terrestrial soil [14]; freshwater [9]), and few studies have considered the combined effects of multiple drivers [15]. This is of particular importance because cumulative and/or interactive effects between drivers are very likely to be influential in determining levels of ecosystem functioning [10,16-

22,]. It is well recognised that services derived from ecosystems are essential to human welfare [15,23-26] and could be critically affected through climate change [26,27]. Consequently, research that examines the effects of multiple climate change factors, such as temperature and carbon dioxide, and altered levels of biodiversity on ecosystem functioning is essential and timely.

In the face of growing concern about climate change, the net carbon status of many coastal and estuarine systems has received increasing attention [28]. Net allochthonous systems rely on an external carbon supply for the majority of their carbon metabolism (heterotrophic) while autochthonous systems are dominated by *in situ* carbon fixation (autotrophic). This distinction has been widely applied in the study of lotic systems (e.g. the river continuum concept [29]) and has recently been applied to the metabolic status of coastal systems to understand their potential to respond to exploitation [30,31]. Relatively subtle environmental perturbations may alter the balance between autotrophy and heterotrophy [32] having profound effects on these ecosystems and the organisms that exploit them. MPB are the main primary producers in many intertidal and shallow subtidal depositional environments [33], and enhance benthic-pelagic coupling through the formation of biofilms [34,35]. Our hypothesis was that interactions between climate change variables and biodiversity would inhibit autochthonous productivity by MPB [36,37,38] and hence would affect the net trophic status of these vulnerable coastal systems. This is because CO_2 levels *per se* do not appear to enhance MPB photosynthesis, while acidification and grazing activity both have a negative influence. In this paper, we examine the effects of two climate change variables (temperature and atmospheric CO_2 concentration) within the context of a range of biodiversity levels (macrofaunal species richness) on the biomass of MPB using a model multi-trophic level [39] experimental mesocosm system.

Methods

Sediment

Surface sediment (< 2 cm depth) was collected from tidal mud flats on the Ythan Estuary, Aberdeenshire, Scotland, UK (57° 20.085'N, 02° 0.206' W) and sieved (500 µm) in a seawater bath (UV sterilised, 10 µm filtered, salinity 33 psu) to remove macrofauna. The sediment was left to settle for 48 h before the supernatant was removed, the sediment homogenised and placed in the mesocosms to a depth of 10 cm (785 cm^3) .

Microphytobenthos

To standardize the biomass of the MPB, MPB-rich surface sediment was collected from the Ythan estuary, spread onto a shallow tray (<1 cm depth) and left under constant light for 48 h. This material was then homogenised and distributed (125 cm^3 aliquots) between mesocosms prior to the addition of seawater.

Macrofauna

The polychaete *Hediste (Nereis) diversicolor* (HD), the gastropod *Hydrobia ulvae (HU)* and the amphipod *Corophium volutator* (CV) were collected from the study site. These species represent a range of functional types in the way that they bioturbate sediments, and hence drive nutrient flux [40,41], and their mode of grazing on MPB [42].

Replicate (n=3) macrofaunal communities were assembled in single and multispecies treatments (HD, HU, CV, HDHU, HDCV, HUCV, HDHUCV). These unique species permutations eliminate pseudoreplication [43] and allow the generic effects of altered

biodiversity to be examined. Macrofaunal biomass was set at 2 g wet weight per mesocosm (divided equally between the species present), similar to the natural biomass found at the study site [44]. Control mesocosms (n=3) containing the standard MPB biomass but without any macrofauna were also established. There were a total of 24 mesocosms per environmental chamber (see supporting information in Additional file 1).

Mesocosm structure and assembly

Mesocosms were Perspex cores 33 cm high with an internal diameter of 10 cm. Following the addition of sediment (10 cm deep) to each mesocosm, 125 cm^3 of MPB- rich sediment and 2.35 l of seawater (UV-sterilized, 10 µm pre-filtered, salinity ≈ 33) were added to give an overlying depth of 20 cm. This initial fill of water was replaced after 24 h to remove the nutrient pulse associated with assembly [40] and macrofauna were then added. All mesocosms were non-tidal and were aerated individually throughout the experiment with the defined CO_2 atmospheric level within the chamber. A total of 216 mesocosms were required.

Environmental regimes

Mesocosms were placed in two environmental chambers (24 per chamber per run, V 4100, Vötsch Industrietechnik) with temperature control (±0.1ºC). The experiments were run with a 12 h light - 12 h dark (L/D) cycle using high intensity discharge sodium lamps (model GE11678, 400w x2, average 300 µmoles m^{-2} s^{-1}). Nine environmental regimes were employed, using three constant temperatures (6 ºC, 12 ºC, and 18 ºC, reflecting the annual variation at the study site (Additional file 1, Figures S1 – S4)) and three atmospheric carbon dioxide concentrations (370 ppmv (present day), 600 ppmv, 1000 ppmv) in an orthogonal design (see supporting information in Additional file 1) . Concentrations of 600 ppmv and 1000 ppmv were based on IPCC projections for approximately 50 and 100 years time

respectively [7] and reflect the accepted view that CO_2 levels will rise over the long-term. Atmospheric CO_2 concentrations were maintained using a CO_2 monitor attached to an external CO_2 gas cylinder (BOC gases Ltd, UK) with a digital controller (Technics horticultural carbon dioxide controller). An Infra-Red Gas Analyser (IRGA, ADC LCA3) was used to calibrate and validate the CO_2 regulation (± 30 ppm). Mesocosms within an environmental chamber were randomly assigned positions to factor out any effects of spatial heterogenity. Each experiment was run for 7 days.

PAM fluorescence

The biomass of the MPB was measured at the end of the experiment using a PAM fluorometer (DIVING-PAM, Heinz-Walz GmbH). This is a widely accepted proxy method for measuring the surfice biomass (chlorophyll *a*) of MPB [45,46]. Fluorescence measurements were reported as F_0^{-15}. This indicates that the minimum fluorescence was determined after 15 minutes of dark adaptation [47,48,49]. This time period is a compromise between the time required in MPB to stabilise ubiquinone oxidation, but not so long that surface biomass is altered [45] by cell migration. Three measurements were taken per mesocosm.

Data Analysis

MPB biomass was treated as a response variable, with macrofaunal species richness (or species combination), CO_2 concentration and temperature as nominal explanatory variables. Initially, a linear regression model was fitted and assessed for normality (Q-Q plots), homogeneity of variance, and outlying values (Cook's distance) [50,51]. As our experimental design established a gradient of species richness that increased within a finite species pool, variation across treatments was likely to be unequal [40], and this was confirmed by plots of

the model residuals. To account for this heterogeneity of variance, a generalised least squares (GLS) [50,51,52] mixed modelling approach was used in preference to a linear regression of transformed data [10,42,53]. The most appropriate variance-covariate structure for each model was determined using a combination of AIC scores and the examination of plots of fitted values versus residuals based on a full model specification using restricted maximum likelihood (REML) [52]. The minimum adequate model was then determined through manual backwards stepwise selection, using maximum likelihood methods. The significance of the relevant highest order interaction terms was assessed at each stage, terms nested within these not being tested, following Underwood [54]. The influence of each independent term within the minimum adequate model was assessed using a likelihood ratio test (L-ratio) between the minimum adequate model and reduced models (with all terms involving the relevant independent factor removed, including interactions). The L-ratio can be used to assess the order of importance of the independent terms. All analyses were performed using the 'nlme' package (ver. 3.1) [55] in the 'R' statistical and programming environment [56].

Results

Microphytobenthos response to climatic variables

The minimal adequate model for the controls (containing no macrofauna), with MPB biomass as the dependent variable, included a two-way interaction ($CO_2 \times$ temperature; *L*-ratio = 18.23, d.f. = 12, $p = 0.0011$). Of the two climatic variables, temperature (*L*-ratio = 37.71, d.f. = 6, $p < 0.0001$) was more influential than CO_2 (*L*-ratio = 24.51, d.f. = 6, $p < 0.0001$). There was an apparent decline in MPB biomass with increasing temperature (Figure 1 and Additional file 1, Figure S5), whilst the CO_2 concentration of 600 ppmv was associated with

the higher MPB biomass levels, particularly at 6°C and 18°C. This trend was reflected in the model visualisation (Figure 1), with MPB biomass highest at 6°C across all CO_2 levels.

Microphytobenthos response to climatic variables and macrofaunal species richness

A regression analysis was performed, treating MPB biomass as the dependent variable with the climate variables and macrofaunal species richness as independent variables. The minimal adequate model comprised a three-way interaction (species richness × temperature × CO_2; *L*-ratio = 23.37, d.f. = 48, $p = 0.02$). Species richness was the most influential variable (*L*-ratio = 95.81, d.f. = 27, $p < 0.0001$), followed by temperature (*L*-ratio = 79.18, d.f. = 24, $p = 0.0051$), and CO_2 concentration (*L*-ratio = 45.48, d.f. = 24, $p < 0.0001$). The effect of species richness on MPB biomass was most apparent at 6ºC (Figure 2 and Additional file 1, Figure S6). Biomass was highest in the absence of macrofauna, and the greatest biomass levels in all three carbon dioxide treatments occurred at the lowest temperature treatment of 6ºC.

For both elevated carbon dioxide levels at 6ºC, there was an increase in MPB biomass across all species richness levels. This general effect of elevated CO_2 was not found at the higher temperatures, and overall MPB biomass decreased with rising temperature (Figure 2b,c).

Microphytobenthos response to climatic variables and macrofaunal species composition

A further regression analysis was performed, replacing the explanatory variable species richness with species composition, to determine whether compositional effects underpinned the observed effects of biodiversity. The minimal adequate model comprised a three-way interaction (species composition × temperature × CO_2; *L*-ratio = 65.52, d.f. = 96, $p < 0.0001$). Species composition was the most influential variable (*L*-ratio = 314.52, d.f. = 63, $p <$

0.0001), followed by temperature (*L*-ratio = 177.46, d.f. = 48, $p < 0.0001$), and CO_2 (*L*-ratio = 101.26, d.f. = 48, $p < 0.0001$). Increased species diversity had a detrimental effect on MPB biomass, but the magnitude of these effects was dependent on the composition of the invertebrate assemblage within each treatment (Figure 3 and Additional file 1, Figure S7). *C. volutator* had a greater negative effect on MPB biomass than the other macrofaunal species in single species treatments, and the presence of *C. volutator* was a dominant component in the effects of the multispecies treatments (Figure 3). This dominant effect of *C. volutator* decreasing MPB biomass was consistent under all environmental regimes. Importantly, the interaction of the climate drivers mediated the relationship between MPB biomass and species composition, although this mediation was less pronounced in treatments with C. *volutator*, and appeared to have no consistent pattern.

Discussion

Few ecological studies have examined the effect of multiple simultaneous stressors on individual species and ecosystems [19]. The statistical approach used here does not allow the separation of individual stressor effects, although some inference in terms of the most critical factors can be made from the model metrics (L-ratios) and from the model visualisations. We found complex interactions between the effects of temperature, CO_2 concentration and macrofaunal species richness / species assemblage composition on ecosystem response, measured as MPB biomass. The MPB biomass provides a proxy estimate of the productive potential of mudflat systems [57] and has been used to model net system productivity. Thus, changes in MPB biomass are a crucial element of system performance and will have reverberating effects through the higher trophic levels [8,38], just as changes in infaunal diversity will affect resource utilisation [58].

In the present study, MPB biomass was higher at low temperature across all CO_2 regimes, and did not increase as CO_2 increased, indicating that CO_2 was not limiting to MPB under these conditions and that increasing temperature was detrimental. The models suggested that species richness/composition and temperature were more influential on MPB than CO_2, but the interactions between the explanatory variables were significant. The interactive effects of species composition and temperature led to a significant reduction in MPB biomass. The implication of this for temperate estuaries may be quite profound. The overall metabolic balance of estuarine systems between net autotrophy or heterotrophy is under debate [28,**Error! Reference source not found.**] but the role of autotrophic production by MPB is clear. Reduction of this contribution to the carbon balance will push the system toward a more heterotrophic condition. A shift from autotrophic to heterotrophic conditions, or a shift to more extreme heterotrophy, is likely to have significant, but as yet undetermined, implications for ecosystem goods and services. Unless the net import of allochthonous carbon changes, then overall productivity may be expected to decline with potential effects on resource utilisation. Variation in temperature has already been shown to affect the carbon metabolism of coastal systems [59] and this supports our hypothesis that autochthonous productivity may be reduced through interaction between the climate change variables and species diversity.

In this study, the presence of macrofaunal species resulted in substantial decreases in MBP biomass, and there appeared to be a general decline in MPB with increasing macrofaunal species richness, specifically at the lowest temperature. This general trend is partly to be expected as all three macrofaunal species are known consumers of MPB [34,60]. However, the presence of *C. volutator* had a disproportionately strong effect in reducing MPB biomass, consistent with previous research [34,42,61]. Rather than being attributed to consumption, the

mechanism for this dominant effect is likely to be due to the constant resuspension of sediment [42,62] during grazing and bioturbation (Additional file 1, Figure S8), leading to inhibition of photosynthesis by MPB, and also the prevention of MPB biofilm formation on the sediment surface [63].

Individual species responses to climate change are often highly uncertain [64], and environmental change could alter the balance between the functional groups present (through extinction, invasion or changes in abundance or behaviour), as well as the number and identity of species present in an ecosystem [65]. This makes it very difficult to predict how an ecosystem may respond based on diversity alone [66]. MPB utilise nutrients from the water column and pore waters whilst bioturbation by invertebrates is known to increase the flux rates and concentrations (NH_4-N, PO_4-P) available [10]. Therefore, species-specific responses to climate change will affect more than one trophic level, and the nature of interactions between species will change as a consequence [8,11,20]. In the present study, the decline in MPB was driven by complex interactions between environmental variables and diversity effects, and mediated through infaunal grazing activity. In this case, there was no apparent compensation through an increase in nutrients caused by bioturbatory activity. The functional importance of species is also context-dependent, and functional impact may alter as conditions change. Thus, while functional diversity is important, it may be overshadowed by species identity as different species take up more prominent roles under changing scenarios. In our experiment, the dominant effect of one species (*C. volutator*) illustrates how the extinction of influential (rare or common) species may have profound effects on the ecosystem –and that these effects may be direct or indirect [67,68].

Many studies focusing on the ecological consequences of altered biodiversity within the context of specific drivers of environmental change have concentrated on single variables and few species [11,64,65,69]. Whilst these studies are informative in understanding the mechanisms behind ecosystem response, care must be taken in making predictions based on simplistic assumptions, such as additive and linear relationships. Studying the next level of complexity is problematic, and while a mesocosm approach may help provide conceptual advances, we recognise the limitations of any artificial system in providing realistic interpretations of natural ecosystem response [70]. However, we have shown that interactive effects can have a fundamental influence on MPB biomass and since the balance between autotrophic and heterotrophic status in transitional systems may be delicate [27], then there is a real possibility that climate change may force an overall change metabolism in coastal systems. Given that coastal systems will be at the forefront of climate change effects, they may undergo profound changes in the near future with associated implications for ecosystem services.

Conclusions

The interactive effects between climate drivers and other anthropogenic factors, such as pollution, habitat destruction and overfishing [20,69,71] add further difficulty to any interpretation of climate change effects. However, there is an urgent need to provide relevant scientific results as guidance for effective action in response to climate change. This requires recognition of the complex interactions between the chemical, physical and biological components of an ecosystem [22,72-74] through a combination of empirical studies, long-term observational data and ecological modelling [64,72,**Error! Reference source not found.**]. Such data will allow us to gain a greater understanding of the link between ecosystems and the services we obtain from them, levels of variation in ecosystem structure

and functioning, and data on how these systems are changing within the context of multiple anthropogenic drivers.

Authors' contributions

NH carried out the experimental procedures, coordinated the study, performed the statistical analysis and drafted the manuscript. MTB assisted in all experimental procedures, provided statistical support and helped draft the manuscript. MS and DMP participated in the design and coordination of the study, and assisted interpretation of statistical analysis. DR and PCLW conceived initial ideas for the study. All authors read, edited and approved the final manuscript.

Acknowledgements

We thank D. Mackinnon, K. Cruickshank and O. McPherson for technical assistance, and I. Davidson, T. Fujii, V. Johnston, T. Linley, L.M. Murray, J. Polanski, and L. Teal for support in the field. All work was supported by NERC grant NE/E006795/1.

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Figures

Figure 1 - Model visualisation of temperature and CO_2 on MPB biomass using only controls (no macrofauna). The interaction of temperature (increasing along the x-axis) and CO_2 (the horizontal bars on each temperature level) on MPB biomass (F_0^{-15}).

Figure 2 - Model visualisation of the three-way interaction (species richness x temperature x CO_2) on MPB biomass. The interaction of temperature, CO_2 and species richness on MPB biomass (F_0^{-15}) at three constant temperatures (a) 6 ºC, (b) 12 ºC, (c) 18 ºC. In the visualisation, the CO_2 levels are represented as present-day 370 ppmv (solid line), 600 ppmv (dashed line) and 1000 ppmv (dotted line).

Figure 3 - Model visualisation of the three-way interaction (species identity x temperature x CO_2) on MPB biomass. The interaction of temperature, CO_2 and species richness on MPB biomass (F_0^{-15}) at three constant temperatures (a) 6 ºC, (b) 12 ºC, (c) 18 ºC. In the visualisation, the CO_2 levels are represented as present-day 370 ppmv (solid line), 600 ppmv (dashed line) and 1000 ppmv (dotted line).

Additional file 1

Additional file – Supporting Information

- Additional graphs of raw data, summary of study site temperatures, final models in full, figures referred to in the text as Figures S, schematics of experimental set up and design.
- Word Document

- Supporting material for the data presented in the main body of the paper, including annual temperature graph of study site, supplementary figures referred to in the text, box plots of raw data, bootstrapping of models, and full final models.

Figure 1

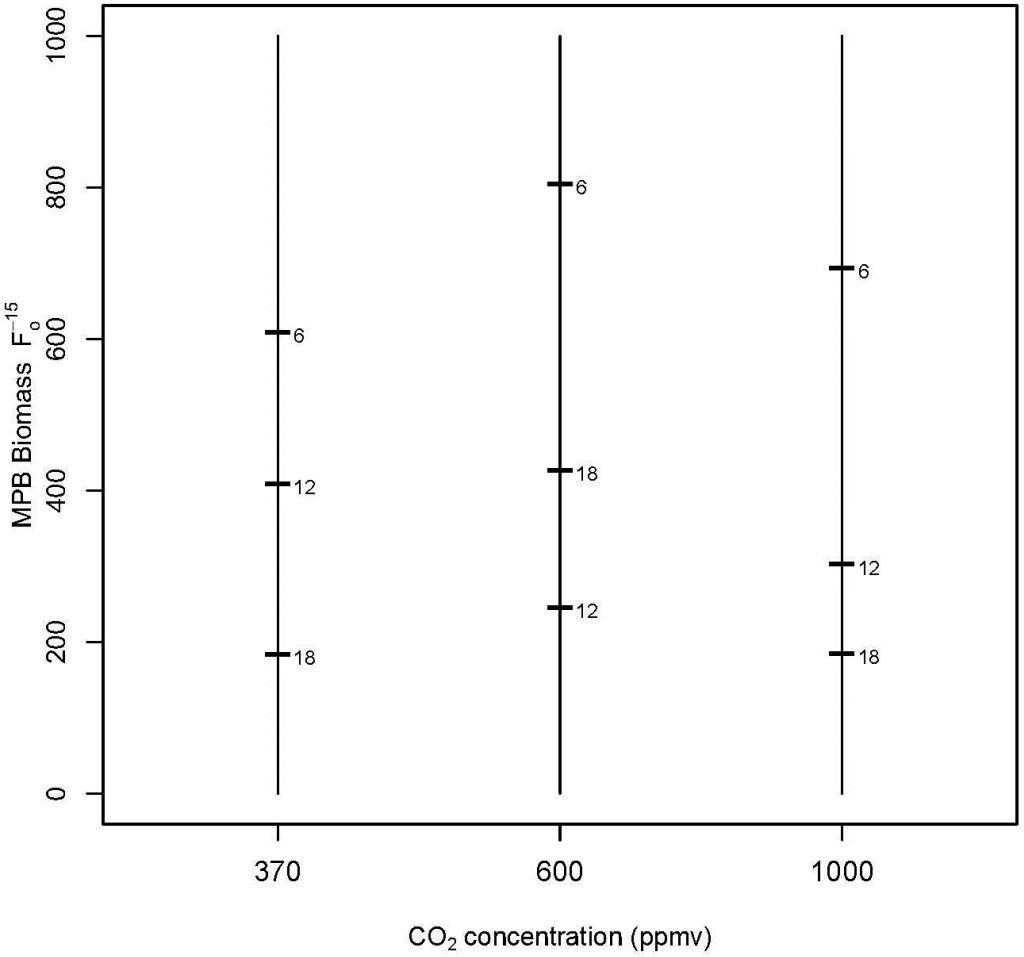

MPB Biomass F_o^{-15}
0
200
400
600
800
1000
6
12
18
6
18
12
6
12
18
370
600
1000
CO_2 concentration (ppmv)

Figure 2 (a)

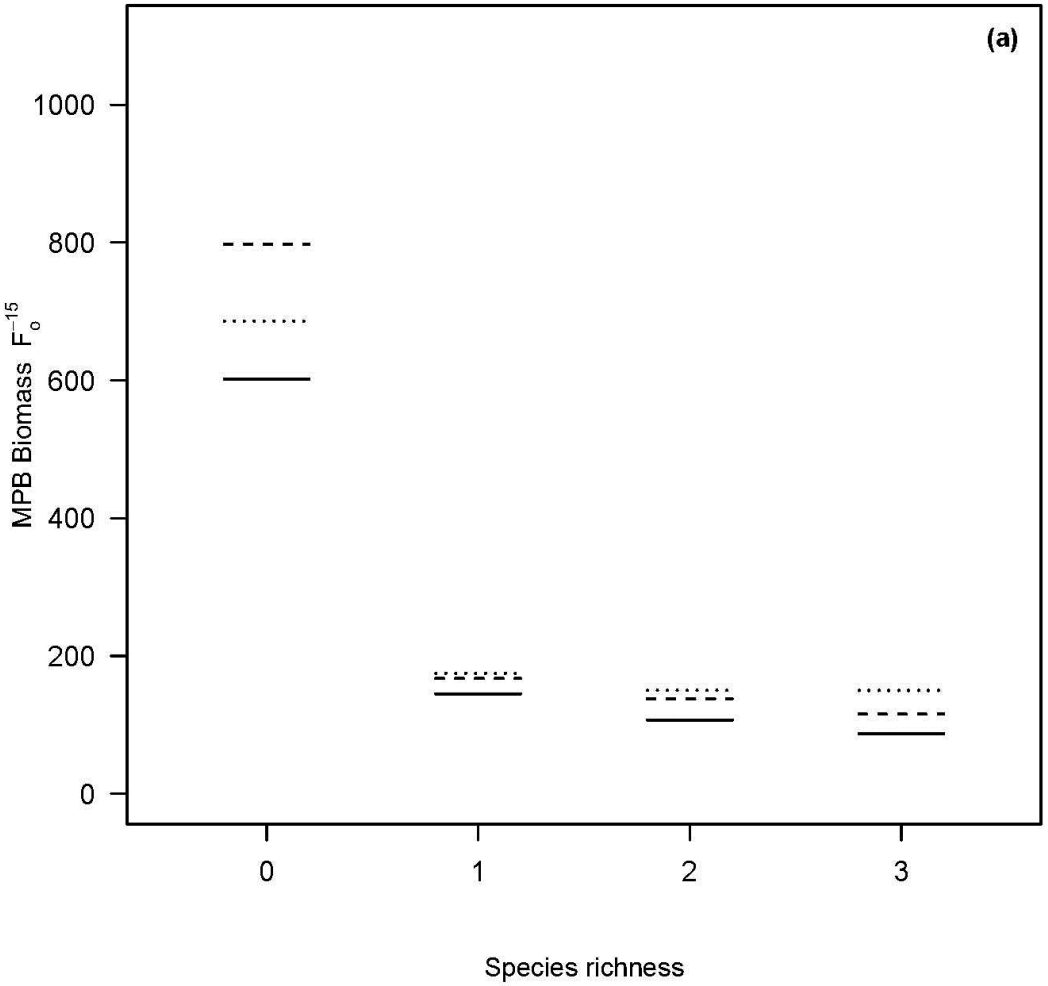

(a)
1000
800
600
400
200
0
MPB Biomass F_o^{-15}
0
1
2
3
Species richness

Figure 2 (b)

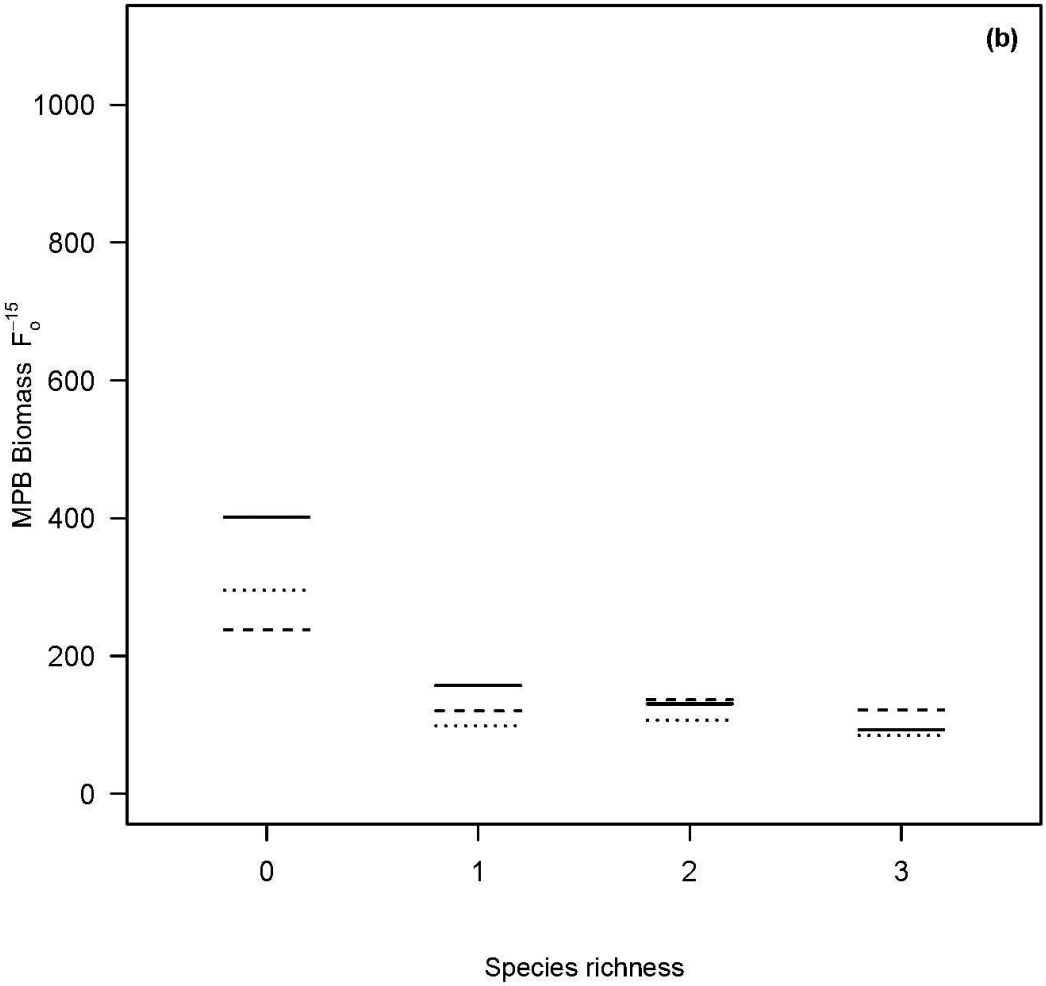

(b)
1000
800
600
400
200
0
MPB Biomass F_o^{-15}
0
1
2
3
Species richness

Figure 2 (c)

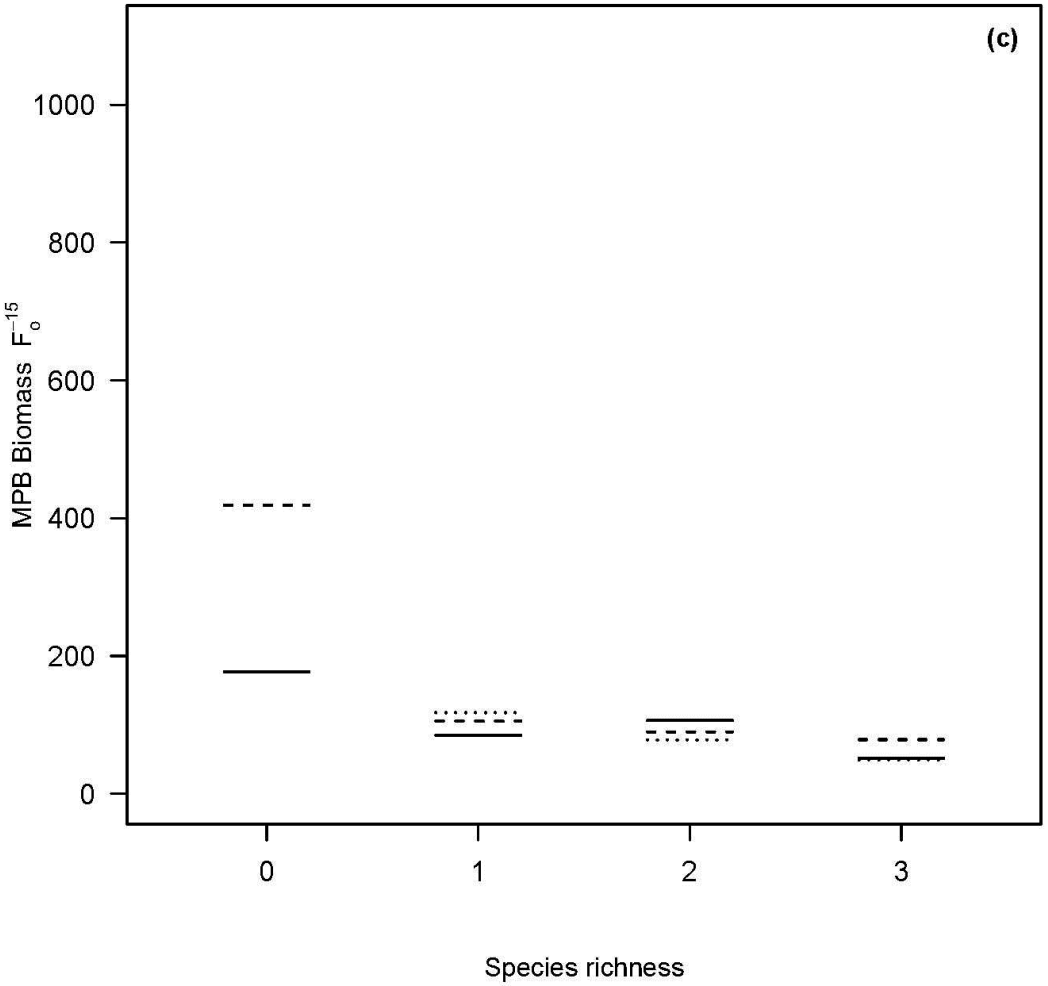

(c)
1000
800
600
400
200
0
MPB Biomass F_o^{-15}
0
1
2
3
Species richness

Figure 3 (a)

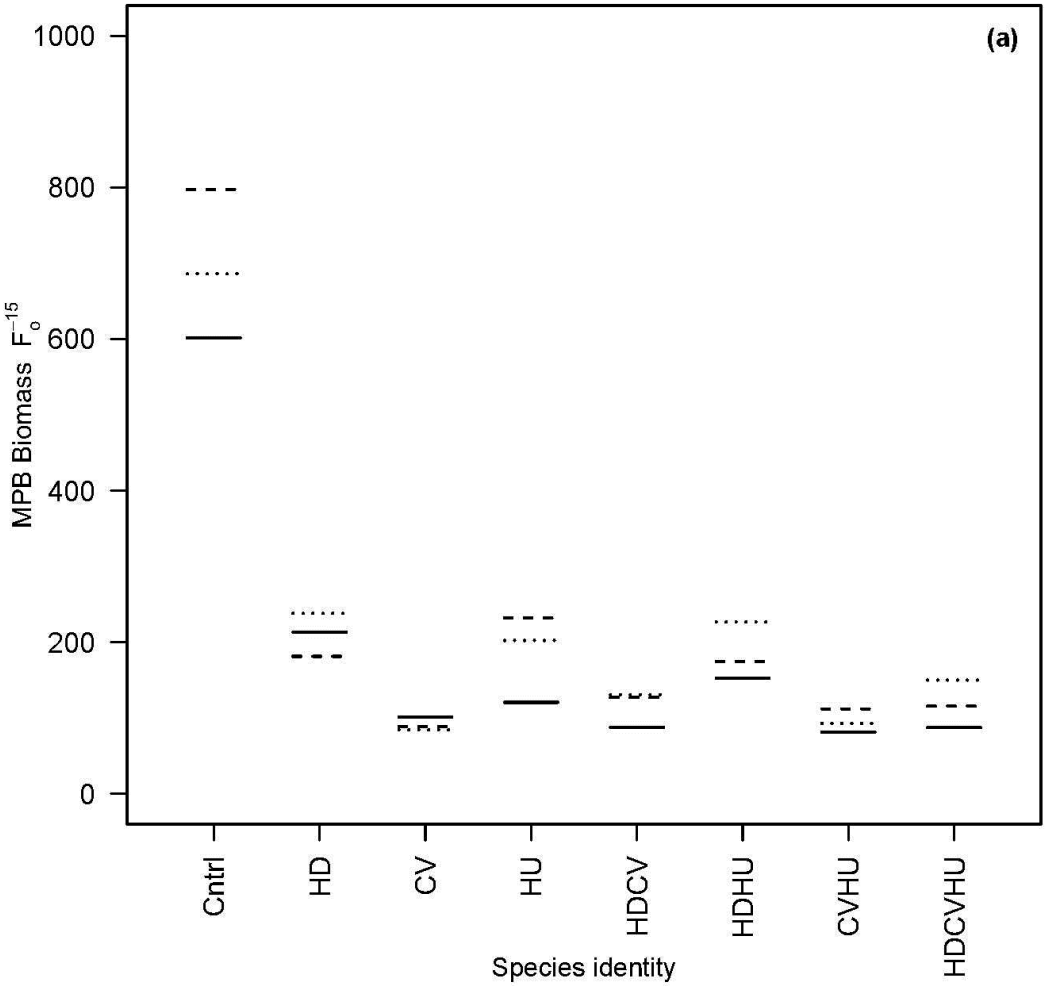

(a)
1000
800
600
400
200
0
MPB Biomass F_o^{-15}
Cntrl
HD
CV
HU
HDCV
HDHU
CVHU
HDCVHU
Species identity

Figure 3 (b)

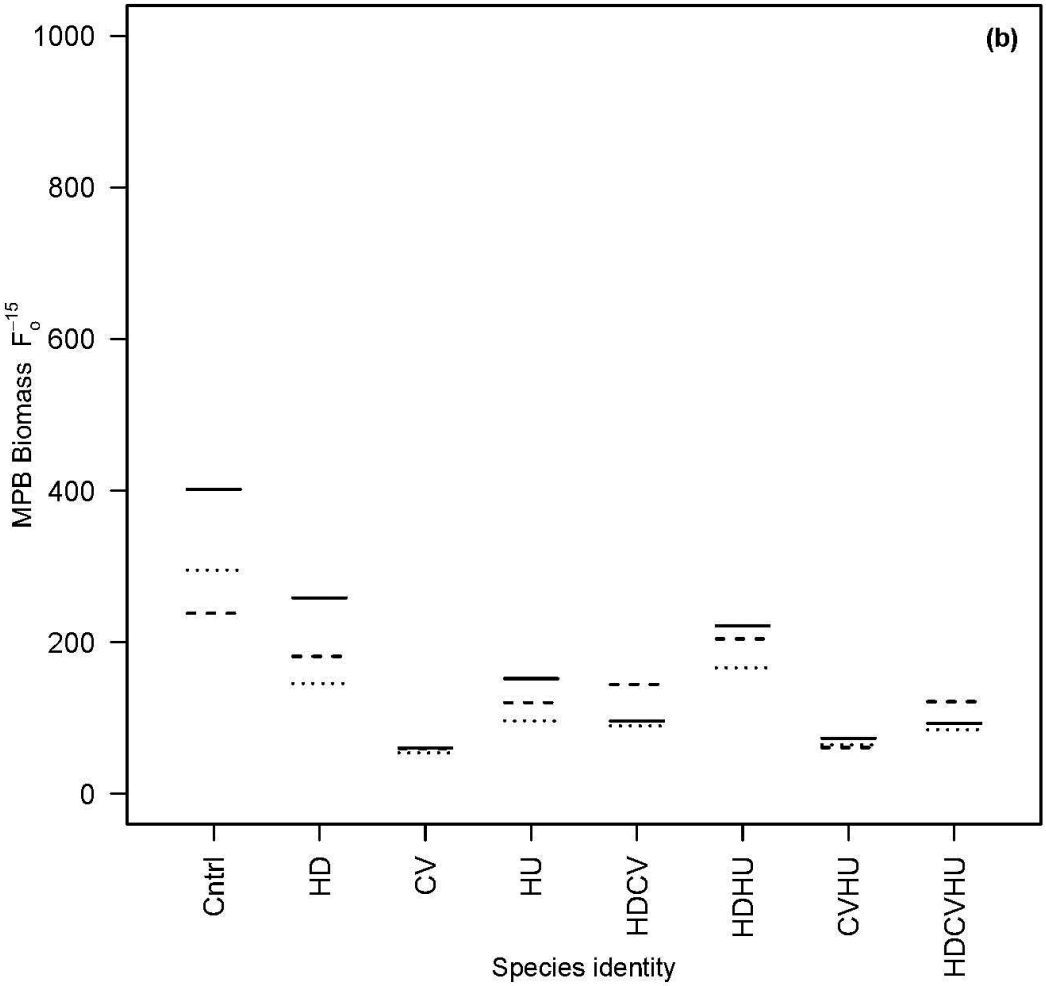
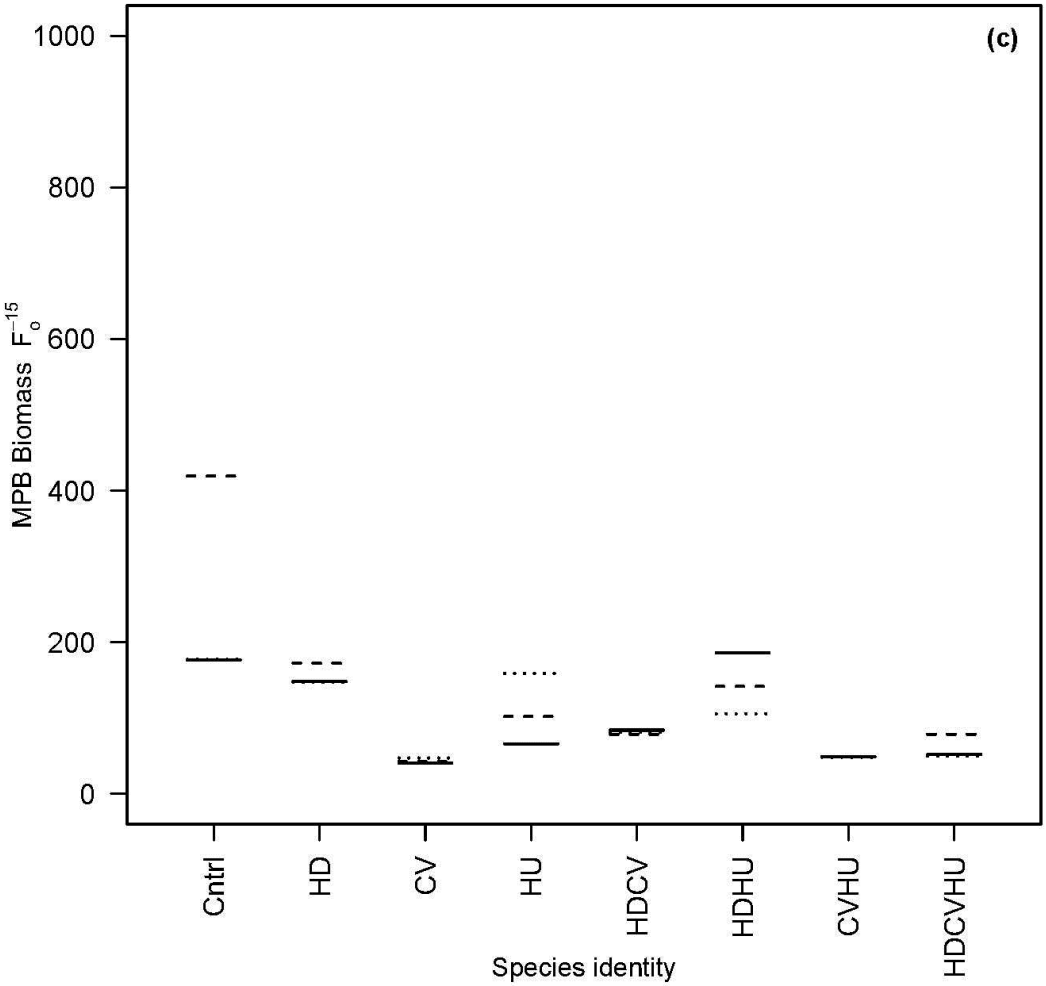

(b)
1000
800
600
400
200
0
MPB Biomass F_o^{-15}
Cntrl
HD
CV
HU
HDCV
HDHU
CVHU
HDCVHU
Species identity

Figure 3 (c)


(c)
1000
800
600
400
200
0
MPB Biomass F_o^{-15}
Cntrl
HD
CV
HU
HDCV
HDHU
CVHU
HDCVHU
Species identity

Supplementary Material

Impact of biodiversity-climate futures on primary production and metabolism in a model benthic estuarine system

Natalie Hicks*[1,2], Mark T. Bulling[2,3], Martin Solan[2], Dave Raffaelli[3], Piran C. L. White[3] and David M. Paterson[1]

[1] *Sediment Ecology Research Group, Scottish Oceans Institute , University of St Andrews, East Sands, St. Andrews, Fife, KY16 8LB, United Kingdom*

[2] *Oceanlab, University of Aberdeen, Main Street, Newburgh, Aberdeenshire, AB41 6AA, United Kingdom*

[3] *Environment Department, University of York, Heslington, York YO10 5DD, United Kingdom*

**Correspondence author: Tel: + 44 1224 274401; Fax: + 44 1224 274402; email: natalie.hicks.50@aberdeen.ac.uk*

Figure S1: Set up of environmental chambers

Mesocosms were placed in an environmental chamber (VC 4100, Vötsch Industrietechnik) which maintained a constant temperature environment (± 0.1 ˚C). The atmosphere within the chamber was maintained (± 30 ppm) using a CO_2 monitor and controller linked to the gas regulator. Regulation of atmospheric CO_2 was achieved using an infra red gas analyser (ADC, LCA3) and the CO_2 rich air was bubbled into each individual mesocosm. This follows the experimental set up of Bulling *et al*. 2010.

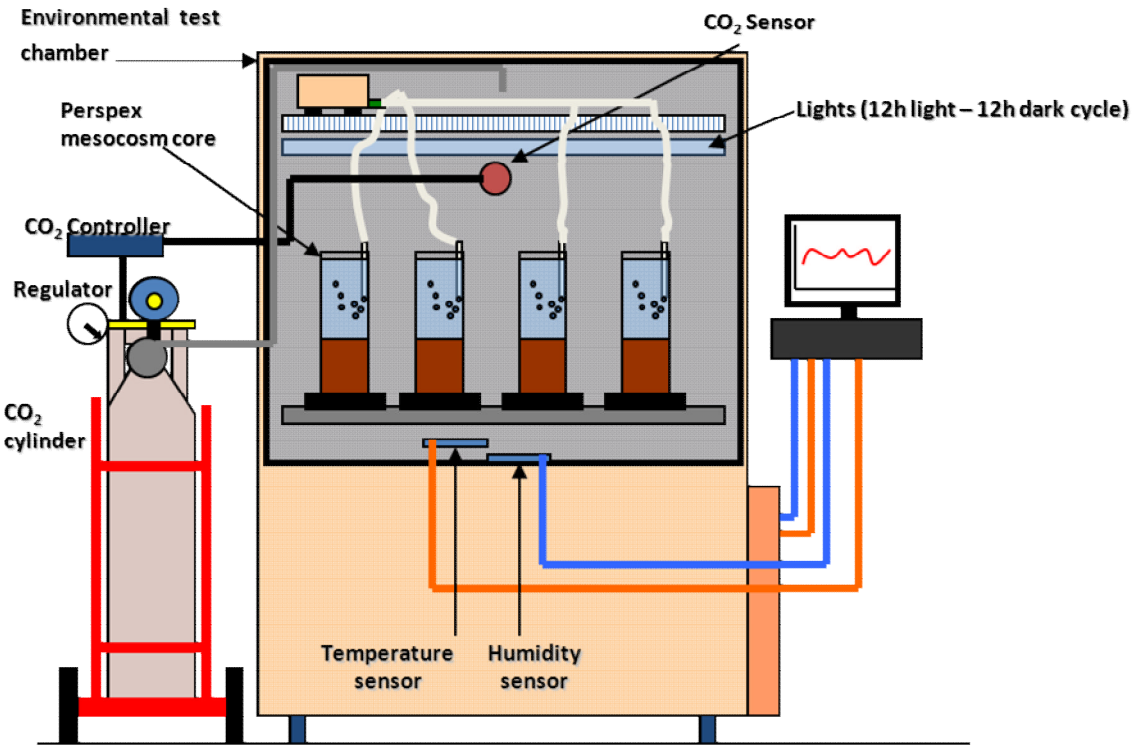
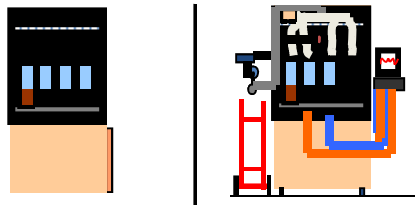


Figure S2: Experimental design and treatments

The experiment was run during the late spring and early summer months of 2008, with each successive temperature treatment applied at appropriate times (sequence listed below, upper panel) to match the ambient seasonal water temperature at the study site (see below, lower panel).



	Chamber 1		Chamber 2	
	Temp °C	CO_2 ppmv	Temp °C	CO_2 ppmv
BLOCK 1	6	370	6	600
BLOCK 2	6	1000	12	370
BLOCK 3	12	600	12	1000
BLOCK 4	18	370	18	600
BLOCK 5	18	1000		

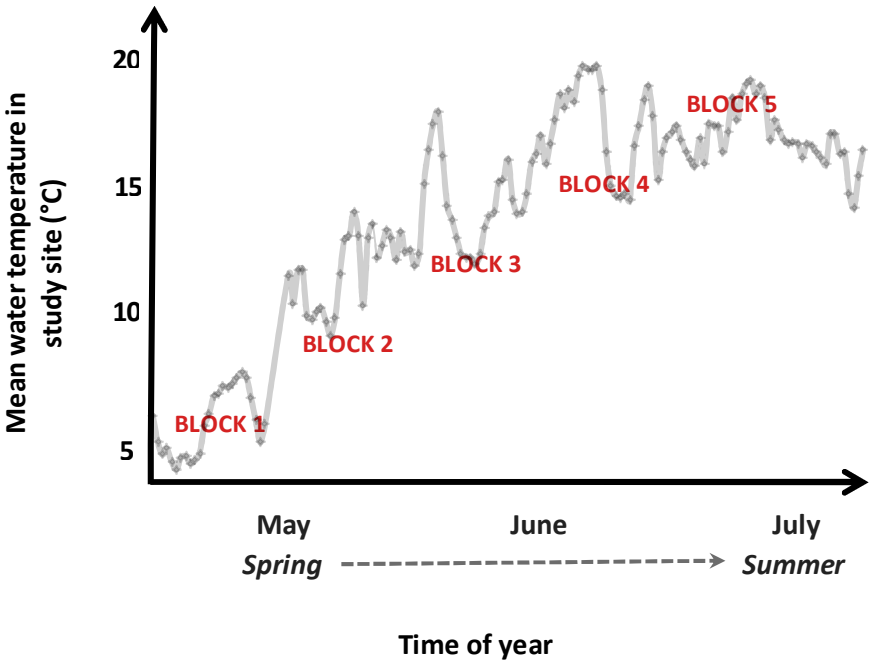


Figure S3: Macrofaunal Species Richness and Composition

For each experimental run there were three replicates of eight treatments (i.e. n = 24 mesocosms per chamber, depicted below). The controls contained only MPB with no macrofauna.

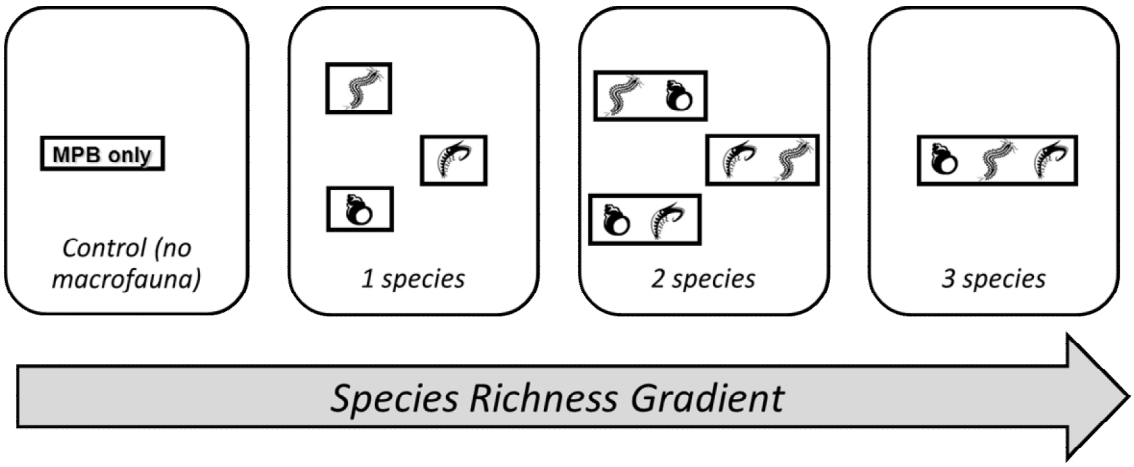


Figure S4: Annual Variation of Water Temperature from Ythan Estuary

The maximum (solid line) and minimum (dotted line) daily water temperatures from 01/01/2005 to 11/11/2009 recorded in the Ythan estuary, Newburgh, Aberdeenshire

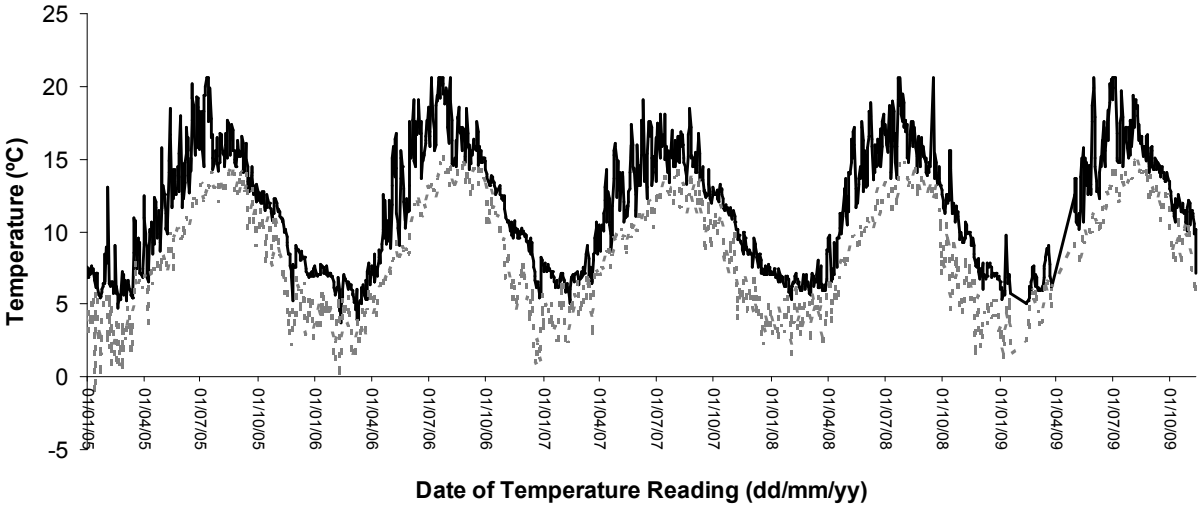


Figure S5: Boxplots of Raw Data

Boxplot showing the raw data for mean MPB biomass at 6°C (red), 12°C (green) and 18°C (blue) for each CO_2 concentration

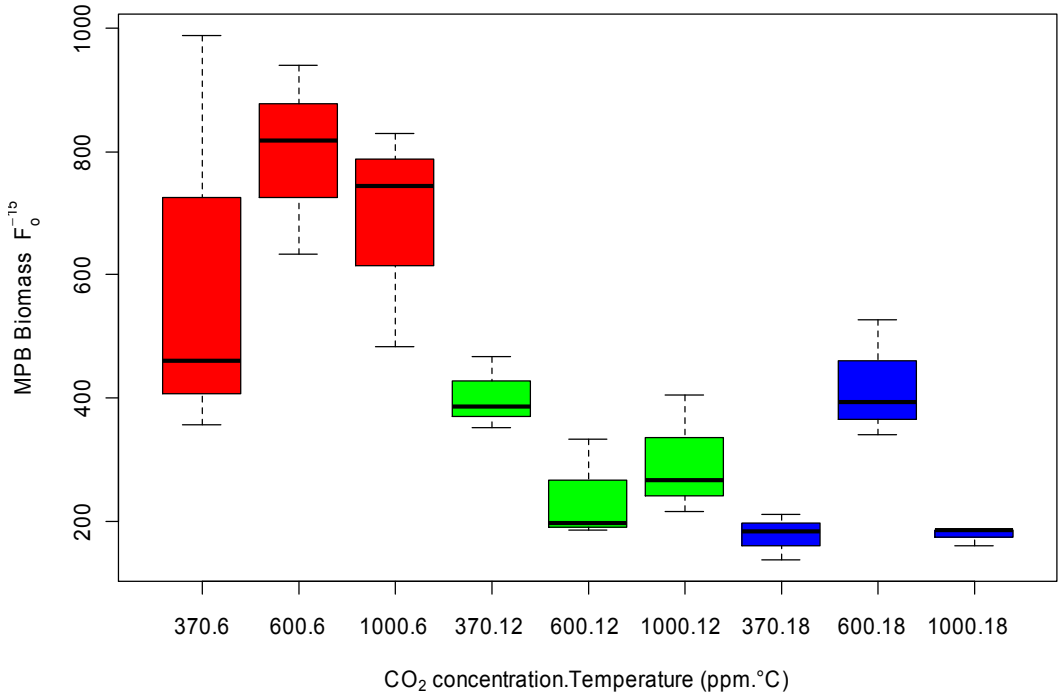


Figure S6a: Bootstrapped models with confidence intervals

Confidence intervals (the error bars) around model predictions were estimated using a bootstrapping methodology (Efron & Tibshirani, 1993). Model residuals were randomly reordered to create an adjusted dataset and the statistical model parameters were recalculated using this adjusted dataset. Model predictions were made for every possible combination of independent variable factor levels. This whole process was repeated 1000 times, to allow generation of bootstrapped distributions around the original model predictions. Bootstrapped models of MPB biomass with increasing Species Richness at 6°C (left column), 12°C (middle column) and 18°C (right column) for each CO_2 concentration (370 ppmv, top row; 600 ppmv, middle row; 1000 ppmv, bottom row).

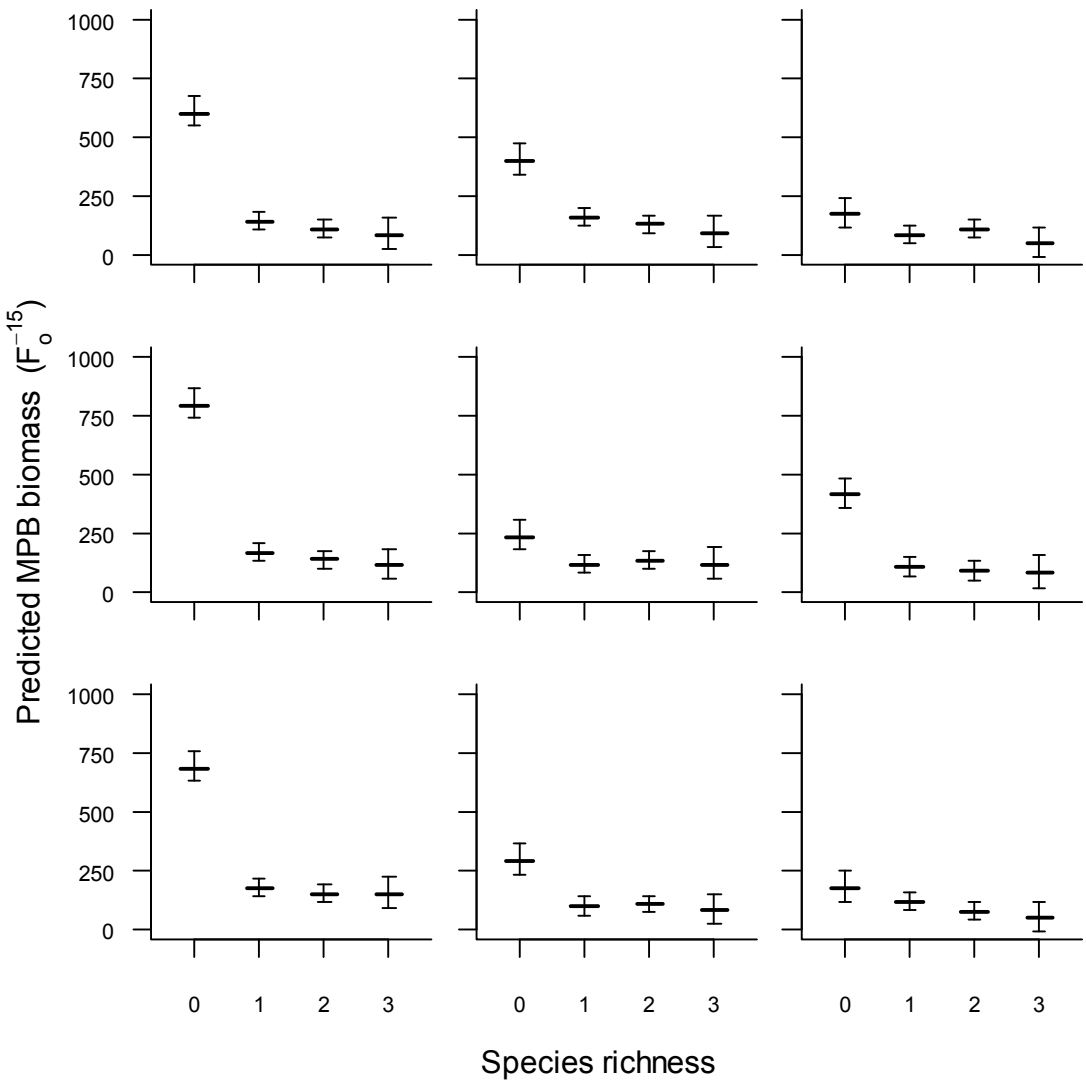


Figure S6b: Boxplots of Raw Data for Species Richness

Boxplots showing the raw data for MPB biomass with increasing Species Richness at 6°C (left column), 12°C (middle column) and 18°C (right column) for each CO_2 concentration (370 ppmv, top row; 600 ppmv, middle row; 1000 ppmv, bottom row).

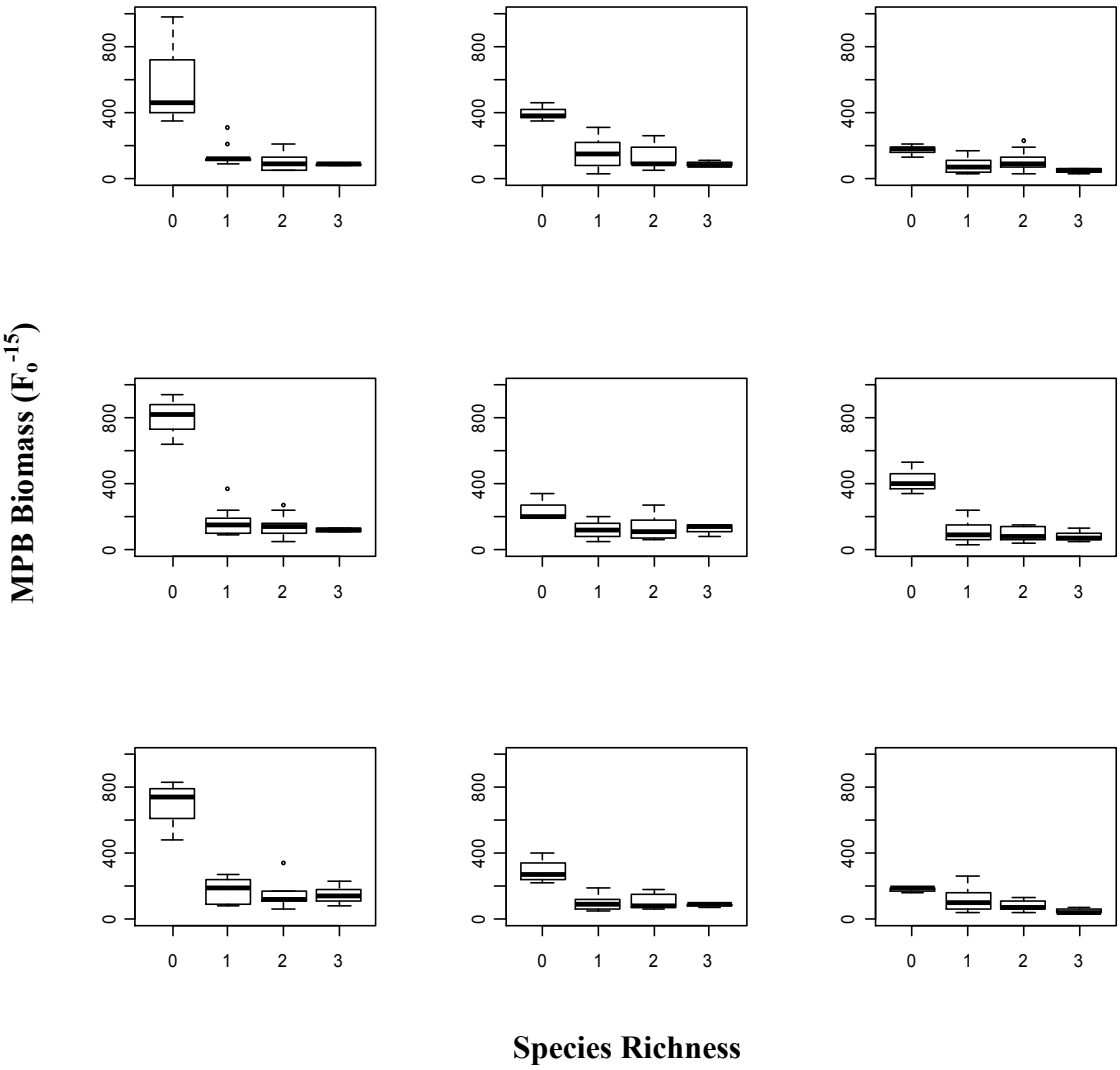


Figure S7a: Bootstrapped models with confidence intervals

Confidence intervals around model predictions were estimated using the bootstrapping methodology as described in Fig. S3a. Bootstrapped models of MPB biomass for each Species Identity at 6°C (left column), 12°C (middle column) and 18°C (right column) for each CO_2 concentration (370 ppmv, top row; 600 ppmv, middle row; 1000 ppmv, bottom row).

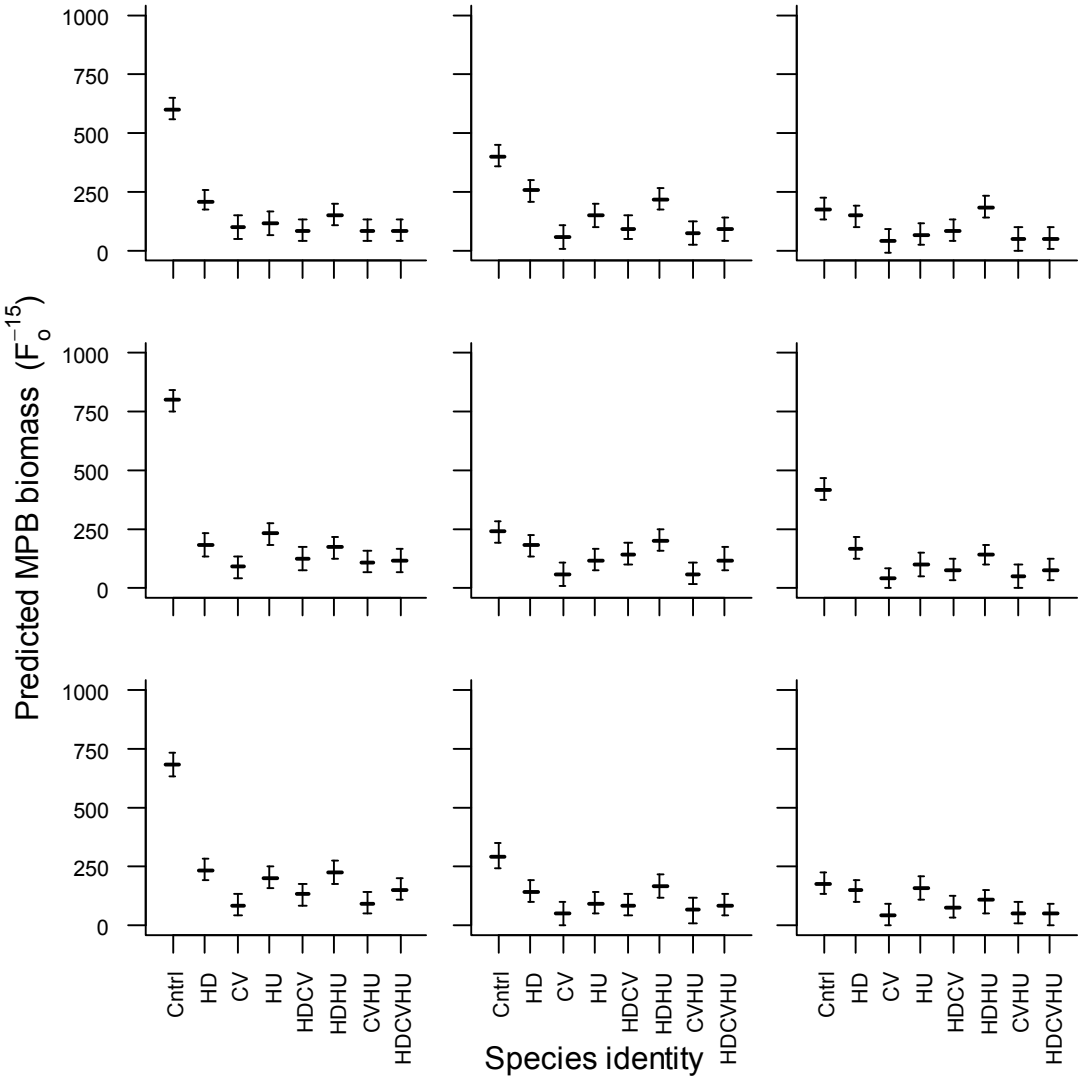


Figure S7b: Boxplots of Raw Data for Species Identity

Boxplots showing the raw data for MPB biomass for each Species Identity at 6°C (left column), 12°C (middle column) and 18°C (right column) for each CO_2 concentration (370 ppmv, top row; 600 ppmv, middle row; 1000 ppmv, bottom row).

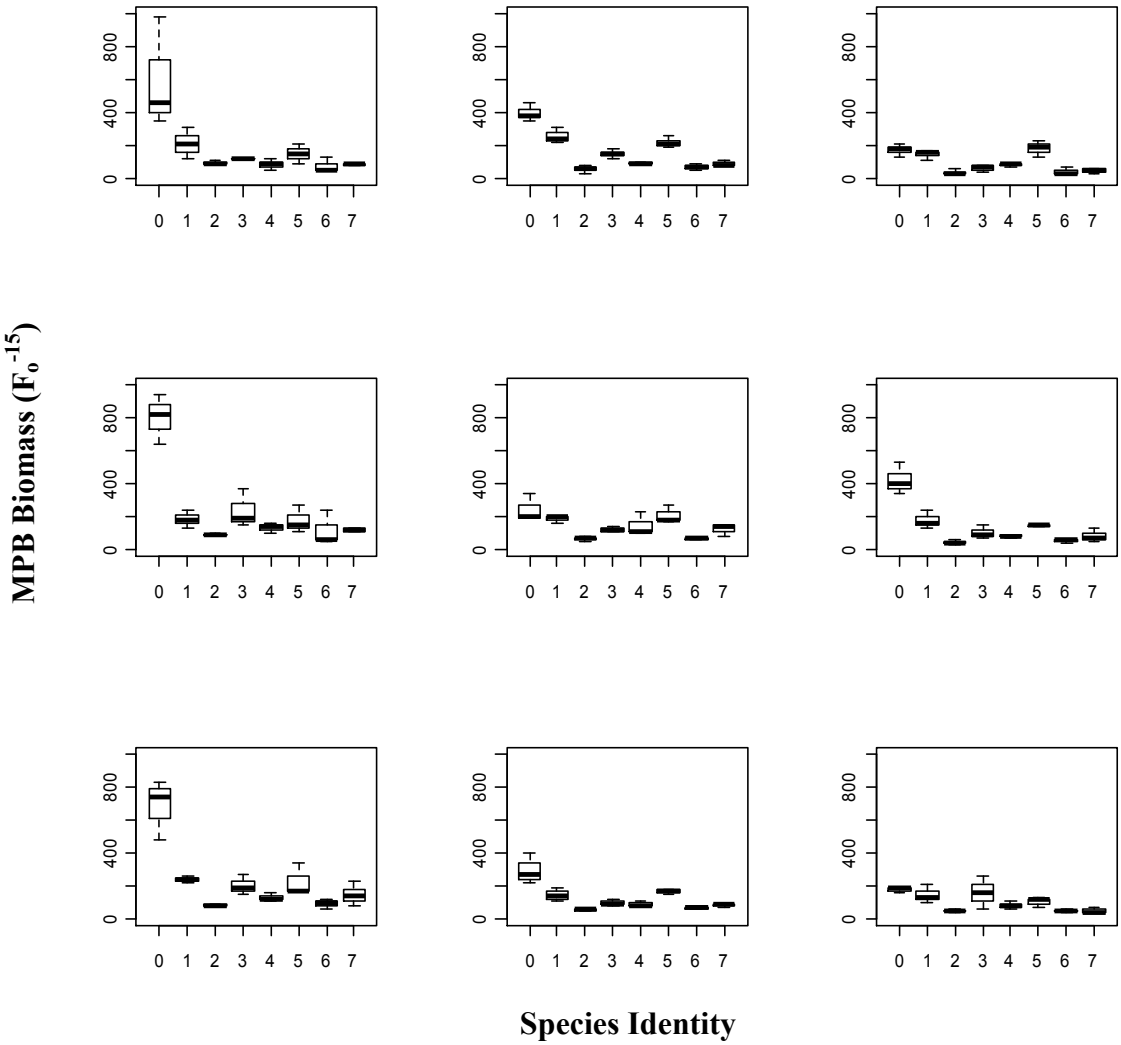
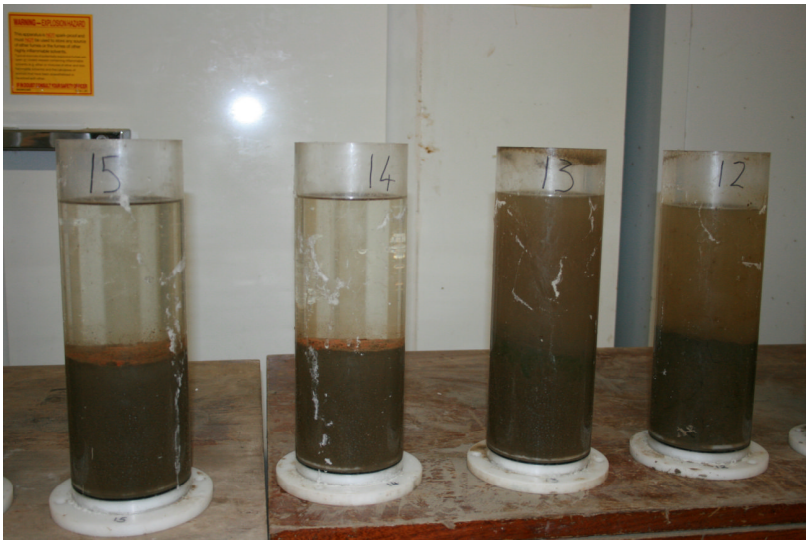


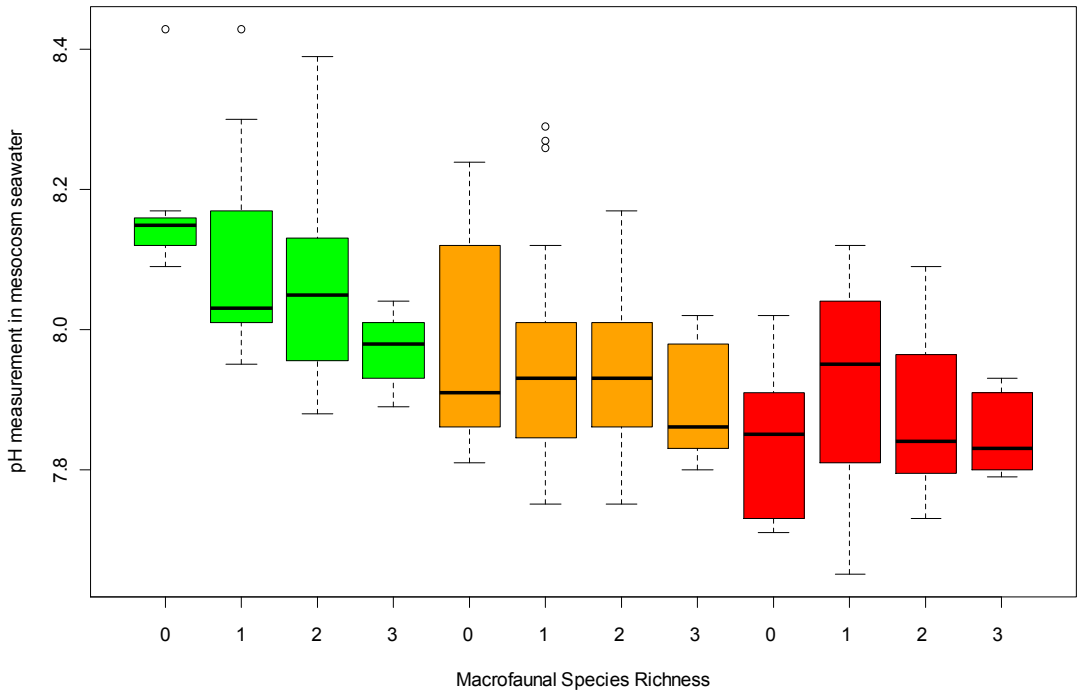
Figure S8: Image of Sediment Resuspension

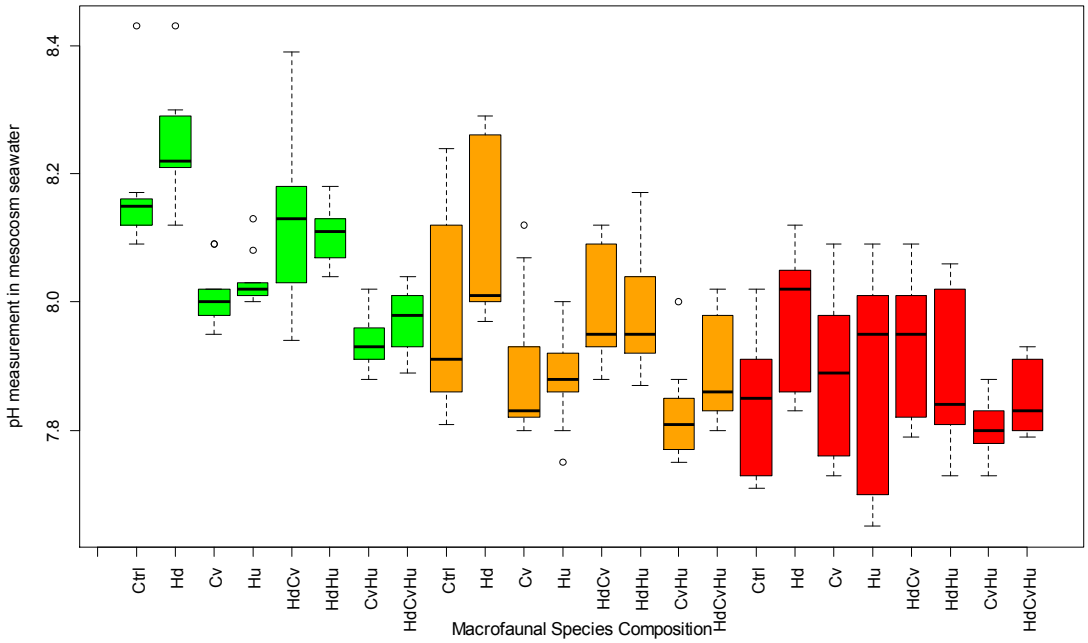
Four sample cores at the end of an experimental run illustrating the effect of activity by *Corophium volutator* in resuspending sediment into the water column. The two on the right contain *C. volutator* whilst the two on the left with clear water contain no *C. volutator*.



Boxplots of raw data of pH measurements within mesocosms

Boxplots showing the raw data of pH measured within the mesocosms for each of the CO_2 regimes across an increasing species richness gradient (top graph) and species assemblage composition (bottom graph). The colour of the boxes indicates the CO_2 regime: green (370 ppmv); orange (600 ppmv); red (1000 ppmv). This shows the pH decreasing with increased atmospheric CO_2.





Structure of the minimal adequate models

a) Model for MPB biomass with carbon dioxide and temperature as independent variables

MPB Biomass = f(CO_2 conc. + Temperature
+ CO_2 conc. × Temperature)

The model was a linear regression model with a gls extension (Temperature variance-covariate).

b) Model for MPB biomass with carbon dioxide, temperature and species richness as independent variables

MPB Biomass = f(Species richness + CO_2 conc. + Temperature
+ Species richness × CO_2 conc.
+ Species richness × Temperature
+ CO_2 conc. × Temperature
+ Species richness × CO_2 conc. × Temperature)

The model was a linear regression model with a gls extension (Temperature and Species richness variance-covariates).

c) Model for PO_4-P concentrations with species richness as an independent variable.

MPB Biomass = f(Species identity + CO_2 conc. + Temperature
+ Species identity × CO_2 conc.
+ Species identity × Temperature
+ CO_2 conc. × Temperature
+ Species identity × CO_2 conc. × Temperature)

The model was a linear regression model with a gls extension (Temperature and Species identity variance-covariates).

References

Bulling MT, Hicks N, Murray LM, Solan M, Raffaelli D, White PCL, Paterson DM: **Marine biodiversity-ecosystem functions under uncertain environmental futures.** *Philosophical Transactions of the Royal Society B* 2010*,* **365**, 2107-2116.

Efron B, Tibshirani RJ: **An Introduction to the Bootstrap.** Chapman and Hall, New York, 1993.

END OF SUPPLEMENTARY MATERIAL

Impact of biodiversity-climate futures on primary production and metabolism in a model benthic estuarine system

Mark T. Bulling, Natalie Hicks, Leigh Murray, David M. Paterson, Dave Raffaelli, Piran C. L. White and Martin Solan

Philosophical Transactions of the Royal Society: Biological Sciences

Volume 165, pp 2107-2116, 2010

doi: 10.1098/rstb.2010.0022

Marine biodiversity-ecosystem functions under uncertain environmental futures

Mark T. Bulling[1], Natalie Hicks[2], Leigh Murray[1], David M. Paterson[2], Dave Raffaelli[3], Piran C. L. White[3] and Martin Solan[1]

[1]*Oceanlab, University of Aberdeen, Main Street, Newburgh, Aberdeenshire, AB416AA, UK*

[2]*Gatty Marine Laboratory, University of St. Andrews, East Sands, St. Andrews, Fife, KY16 8LB, UK*

[3]*Environment Department, University of York, Heslington, York, YO10 5DD, UK*

Anthropogenic activity is currently leading to dramatic transformations of ecosystems and losses of biodiversity. The recognition that these ecosystems provide services that are essential for human well-being has led to a major interest in the forms of the biodiversity-ecosystem functioning relationship. However, there is a lack of studies examining the impact of climate change on these relationships and it remains unclear how multiple climatic drivers may affect levels of ecosystem functioning. Here we examine the roles of two important climate change variables, temperature and concentration of atmospheric carbon dioxide, on the relationship between invertebrate species richness and nutrient release in a model benthic estuarine system. We found a positive relationship between invertebrate species richness and the levels of release of NH_4-N into the water column, but no effect of species richness on the release of PO_4-P. Higher temperatures and greater concentrations of atmospheric carbon dioxide had a negative impact on nutrient release. Importantly, we found significant interactions between the climate variables, indicating that reliably predicting the effects of future

climate change will not be straightforward as multiple drivers are unlikely to have purely additive effects, resulting in increased levels of uncertainty.

Keywords: Benthic, carbon dioxide, climate change, bioturbation, mesocosm, temperature, ocean acidification, uncertainty

1. INTRODUCTION

Anthropogenic activities continue to cause significant global climate change (IPCC 2007). Partly as a consequence of climate change, and also as a direct result of human activities (e.g. harvesting, habitat destruction and pollution), there has been a significant and rapid decline in global biodiversity (Vitousek *et al*. 1997, Sala *et al.* 2000). The speed of this decline has been estimated to be over 1000 times greater than rates of extinction calculated from the fossil record (Lawton & May 1995, Pimm *et al.* 1995, Millenium Ecosystem Assessment 2005). The recognition that ecosystems provide humans with goods and services that are vital for our survival and wellbeing has led to substantial concern over how such ecosystem services may be affected by both climate change and the decline in biodiversity (Naeem *et al*. 1994, Tilman *et al*. 1997, Kinzig *et al.* 2002, Hooper *et al.* 2005, Millenium Ecosystem Assessment 2005, Balvanera *et al.* 2006, IPCC 2007).

Two of the potentially most important effects of climate change on ocean ecosystems are increasing temperatures (Hughes 2000, IPCC 2007) and rising atmospheric CO_2 concentrations (Crowley & Berner 2001, Caldeira & Wickett 2003, Freely et al. 2004, IPCC 2007). The oceans act as a significant temperature sink (Levitus *et al*. 2005), absorbing an estimated 80% of the heat added due to global warming to date (IPCC 2007). This has led to an increase of 0.1 ^{o}C in the average

temperature of the upper 700 m of the oceans over the last 40 years (IPCC 2007). The rate of sea temperature rise is predicted to increase over the next decades, with the estimated rise in the mean surface temperature for the end of the 21st century lying between 1.1 ^{o}C and 6.4 ^{o}C (IPCC 2007).

The current concentration of atmospheric CO_2 is approximately 380 ppm, having increased from preindustrial levels of 280 ppm (IPCC 2007). By the year 2100, assuming a 'business-as-usual' scenario, atmospheric CO_2 concentration is predicted to be between 450 and-1000 ppm (IPCC 2007),. The world's oceans act as a CO_2 sink, with dissolved CO_2 forming carbonic acid which dissociates into protons and bicarbonate ions, causing a decrease in ocean pH, a process known as 'ocean acidification' (Caldeira & Wickett 2003, Freely *et al*. 2004). Surface ocean pH is currently 0.1 unit lower than the preindustrial value, and by the end of the century is likely to be a further 0.14 – 0.35 units lower, leading to changes greater than any experienced in the past 300 Myr (Caldeira & Wickett 2003, Freely *et al*. 2004, IPCC 2007).

Impacts of climate change on ecosystems, however, are unlikely to occur as isolated single drivers. With multiple impacts, the question then arises as to whether they are likely to affect ecosystems in an additive way or whether there will be significant interactions between the drivers (Fabry *et al*. 2008, Przeslawski *et al*. 2008, Byrne *et al*. 2009). Understanding these relationships has important implications for our ability to predict the interactions between climate change, biodiversity loss and ecosystem services. To date, research on the effects of increasing temperature and ocean acidification on marine ecosystems has focused on single drivers in isolation, and there has been a particular emphasis on specific effects such as coral calcification (for overviews see Fabry *et al*. 2008, Przeslawski *et al*. 2008). Thus there is currently a disconnect between

the literature examining the biodiversity – ecosystem functioning (BEF) relationship and the literature examining the effects of climate change on ocean ecosystems (Schmitz et al. 2003) , as well as a general lack of studies examining interactive effects of climate change on ecosystem functioning.

Here we test for interactive effects of macrofaunal species richness, temperature and atmospheric CO_2 concentration on levels of nutrient release from the sediment to the water column in a model marine benthic system. An important mechanism underpinning nutrient release is the process of bioturbation, the mixing of porewater solutes and sediment particles by the movement and activities of the benthic organisms (Richter 1952). Bioturbation affects sediment permeability, breaks down chemical gradients in pore waters and subducts organic matter, thus influencing rates of remineralization and inorganic nutrient efflux (Gray 1974, Rhoads 1974, Kristensen & Blackburn 1987). Benthic habitats process and recycle a significant proportion of nutrients that are necessary for primary production in coastal waters and hence underpin the delivery of ecosystem services from these systems, with ammonium being of particular importance in nitrogen-limited marine waters (Logan *et al.* 1995). Quantifying the impact of temperature and carbon dioxide, and their interactions, on the relationship between benthic macrofaunal biodiversity and ecosystem functioning is therefore of considerable importance for predicting the effect of climate change on these systems (Thrush & Dayton 2002, Lohrer *et al*. 2004).

2. MATERIALS AND METHODS

We used a mesocosm approach to investigate the effects of changes in temperature and CO_2 on ecosystem functioning in a model three-species estuarine benthic system. Our experimental design incorporated all possible combinations of three invertebrate macrofauanal species exposed to different levels of atmospheric CO_2 and temperature.

Three levels of atmospheric CO_2 were used, ranging from present day levels (380ppm) to predicted future levels (600ppm and 1000ppm) that represent low-middle and upper values in the range of the IPCC (2007) estimates for the year 2100. These levels of atmospheric CO_2 were arranged in an orthogonal design with three temperature levels (6, 12 and 18˚C), temperatures that are within the range experienced by these species throughout the annual cycle. All combinations of species and environmental variables were replicated three times. Ecosystem functioning was assessed by measuring levels of NH_4-N and PO_4-P in the water column at the end of the experiment (7 days). In order to assess the mechanistic effects of different climate scenarios we also measured levels of bioturbation activity using fluorescent tracer particles (luminophores; Mahaut & Graf 1987), following the methodology described in Solan *et al*. (2004).

(a) Mesocosms

Sediment was collected from the Ythan estuary, Aberdeenshire, Scotland, UK (57˚20.085' N, 02˚0.206' W), sieved (500 µm) in seawater to remove unwanted macrofauna, and left to settle for 24 hours, allowing us to also retain the fine fraction (less than 63 µm). Excess water was removed, the sediment slurry homogenized and distributed between mesocosms (perspex cores 33 cm high with an internal diameter of 10 cm) to a depth of 10 cm (equivalent to 785cm^3), thus minimising any effects of sediment heterogeneity between mesocosms and ensuring the absence of macrofauna.

Seawater (UV-sterilized, 10 µm pre-filtered, salinity ≈ 33) was initially added to the mesocosms to a depth of 20 cm, left for 24 hours and then refilled with seawater to eliminate nutrient pulses associated with assembly (Ieno et al. 2006) and to ensure that changes in nutrients could be attributable to activity during the

experimental period. Mesocosms were aerated throughout the experimental period (7 days).

(b) Regulation of atmospheric CO_2, temperature and light

Mesocosms were placed in environmental chambers (VC 4100, Vötsch Industrietechnik) which maintained a constant temperature environment (± 0.1˚C). Levels of atmospheric CO_2 within the chamber were maintained (± 30ppm) using a CO_2 monitor in the chamber connected to a valve system on a standard CO_2 cylinder (BOC gases Ltd., UK) via a digital controller (Technics horticultural carbon dioxide controller). The regulation of atmospheric CO_2 was calibrated and validated using an Infra-red gas analyser (ADC LCA3). Light was supplied by two 400 W metal halide bulbs (Newlec) in each chamber, on a 12 h light – 12 h dark cycle. Mesocosms were arranged randomly within a chamber to minimise the effect of any spatial heterogeneity in light levels.

(c) Macrofauna

Three species of macrofauna were used: the gallery-forming *Hediste diversicolor* (Polychaeta), the surficial modifier *Hydrobia ulvae* (Gastropoda) and the regenerator *Corophium volutator* (Crustacea). These species represent a broad range of taxonomic, functional effect and functional response groups and they fulfil key structural and functional roles within benthic communities. They are numeric and biomass dominants but have contrasting mobility, modes of bioturbation and bioirrigation, and effects on ecosystem functioning, particularly with respect to setting redox depth and controlling carbon and nutrient cycling. Macrofauna were collected

from the Ythan estuary and stored in aerated seawater tanks for 24 h before they were placed in the mesocosms (day 0).

(d) Measures of ecosystem functioning

Pre-filtered (Nalgene, 0.45 µm) water samples were taken on the final day of the experiment. NH_4–N and PO_4–P concentrations were determined using standard protocols with a modular flow injection auto-analyser (FIA Star 5010 series) using an artificial seawater carrier solution.

(e) Measures of ecosystem process

We distributed 2 g of 90-125µm luminophore particles evenly across the sediment surface 24 h after the introduction of the macrofauna. At the end of the experiment, a sediment core (3.5 cm diameter and 10cm deep) was taken from each mesocosm and sliced (0.5 cm thick for the uppermost 2cm, and 1 cm thick from 2 – 10 cm depth). The number of luminophore particles within each slice was determined using standard image analysis techniques based on thresholding (Solan *et* al. 2004). These counts provided a vertical luminophore profile for each mesocosm which could be used to determine a bioturbation index of activity (Db; Diaz & Cutter 2001; Gilbert *et al*. 2003; Solan *et al*. 2004). Here, Db is an index of the rate of redistribution of the luminophores due to bioturbatory activity and is expressed as an estimate of the increase in the variance of depths to which the luminophores would have moved over the time scale of a year.

(f) Species richness treatments

Within each environmental combination there were eight species richness treatments comprising all species richness combinations; a control (no macrofauna), three monocultures (*Hediste diversicolor*, Hd; *Hydrobia ulvae*, Hu; *Corophium volutator*, Cv), three two-species treatments (Hd + Hu, Hd + Cv, Hu + Cv) and one three-species treatment (Hd + Hu + Cv). This design minimises hidden treatment effects (*sensu* Huston 1997) and eliminates pseudo-replication. The repetition (n = 3) of each permutation allows the generality of any diversity effects to be evaluated. A complementary analysis could also be performed where species combination replaces species richness as an independent factor, allowing 'species combination' effects on ecosystem function or process to be detected. To ensure that any observed changes in ecosystem process were directly attributable to treatment manipulations and not to species density, total biomass was held constant at 2.0 g per mesocosm (equivalent to 255 gm^{-2}), a level consistent with that found at the study site (e.g. Biles et al. 2003). As nutrient cycling is primarily a microbial process that is mediated by macrofaunal bioturbation, replicate cores in the absence of macrofauna were required so that the contribution of other components of the sediment (microbial and meiofaunal communities) could be distinguished from those of the macrofauna. There were three replicates for each species – environment (temperature × CO_2) treatment combination, giving a total of 216 mesocoms.

(g) Analysis

A generalized least squares (GLS; Pinheiro & Bates 2000, Zuur *et al*. 2007) statistical mixed modelling approach was used, treating NH_4-N, PO_4-P and Db as dependent variables, and levels of CO_2, temperature and species richness (or species combination) as independent fixed factors. A GLS framework was preferred over

linear regression using transformed data because it retains the structure of the data while accounting for unequal variance in the variance–covariate terms. In each case, as a first step, a linear regression model was fitted. Model validation showed no evidence of nonlinearity but there was evidence of unequal variance among the explanatory variables. The GLS framework was then adopted in order to model this heterogeneity of variance. The most appropriate random structure was found by examination of AIC scores in conjunction with plots of fitted values versus residuals for models with different variance-covariate terms relating to the independent variables, using restricted maximum likelihood (REML, West et al., 2007). The fixed component of the GLS model was refined by manual backwards stepwise selection using maximum likelihood (ML) to remove insignificant terms, and the final model was presented using REML. Following Underwood (1998), the highest potential level of interaction that was assessed was the three-way interaction, and nested levels within these were not examined. To assess the importance of individual independent variables, a likelihood ratio test was used to compare the full minimal adequate model with models in which the independent variable and all the interaction terms including it were omitted. Analyses were performed using the 'R' statistical and programming environment (R Development Core Team 2005) and the 'nlme' package (Linear and nonlinear mixed effects models; Pinheiro et al. 2006).

3. RESULTS

(a) NH_4-N

The minimal adequate model with species richness as an independent term was a linear regression model with a GLS extension (see electronic supplementary material), and incorporated all three 2-way interaction terms: species richness ×

temperature, CO_2 × temperature and species richness × CO_2 (table 1). Species richness was the most influential variable (*L*-ratio = 150.26, d.f. = 15, $p < 0.0001$), followed by temperature (*L*-ratio = 65.08, d.f. = 12, $p < 0.0001$), and CO_2 (*L*-ratio = 45.71, d.f. = 12, $p < 0.0001$).

There was a general increase in NH_4-N concentration with increasing species richness, and this was most rapid at the intermediate temperature of 12˚C (figure 1). At present day concentrations of CO_2, the highest levels of NH_4-N were obtained at 18°C. However at the higher CO_2 concentrations, this peak in NH_4-N concentrations generally occurred at 12˚C.

Within the pattern of increasing NH_4-N concentration with increasing species richness, there was a decrease in concentration with increasing level of CO_2. This effect was greatest at the higher species richness levels. At 18˚C there was a marked decrease between NH_4-N levels under present day CO_2 concentrations and those under the two possible future CO_2 concentrations. This coincided with a general flattening of the relationship between species richness and NH_4-N concentration at 18˚C.

The minimal adequate model treating the particular combinations of species as an independent variable (species identity) was also a linear regression model with a GLS extension (see electronic supplementary material) incorporating all three 2-way interaction terms: species identity × temperature, species identity × CO_2 and CO_2 × temperature (table 1). Species identity was the most influential variable (*L*-ratio = 204.16, d.f. = 35, $p < 0.0001$), followed by temperature (*L*-ratio = 108.68, d.f. = 20, $p < 0.0001$), and CO_2 (*L*-ratio = 73.14, d.f. = 20, $p < 0.0001$).

The overall trend of increasing NH_4-N concentration with increasing species richness could be discerned in this model (figure 2). However, within species

richness levels there was a marked variation in NH_4-N concentrations, particularly at the two lower temperatures. Higher concentrations of NH_4-N were found when *C. volutator*, *H. ulvae* or both were present. However, this pattern was absent at 18˚C where marked species identity effects were absent.

(b) PO_4-P

The analysis of levels of PO_4-P with species richness as an independent variable resulted in a linear model with a GLS extension (SM) with only single additive terms for temperature and CO_2 (table 1).

Estimated coefficients for this model (figure 3) indicated that there was no significant difference between the concentrations of PO_4-P at 6 and 12˚C, but concentrations at these temperatures were significantly different to those at 18˚C. Estimated coefficients for the effect of CO_2 (figure 3) indicated significantly different concentrations of PO_4-P at each level of CO_2 concentration, with the greatest PO_4-P concentration at 600 ppm and the lowest at 1000 ppm. However, the actual differences in PO_4-P concentrations across these CO_2 concentrations were very small (< 0.025 mgL^{-1}).

The resulting model for the dependent variable PO_4-P with species identity as an independent variable (SM) contained the 2-way interaction term species identity × temperature, and the single term CO_2 (table 1). The most influential independent variable was temperature (L-ratio = 161.15, d.f. = 13, $p < 0.0001$), followed by species identity (L-ratio = 52.57, d.f. = 21, $p < 0.001$) and CO_2 (L-ratio = 30.64, d.f. = 2, $p < 0.0001$).

There were marked species identity effects on the levels of PO_4-P within the 2-way interaction term species identity × temperature (figure 4a). Patterns in these

effects were generally similar between temperatures of 12 or 18˚C, but these were different to patterns in concentration observed at 6˚C. At the higher temperatures, *C. volutator* and combinations of *C. volutator* and *H. ulvae* tended to be associated with higher concentrations of PO_4-P, whilst combinations of *C. volutator* and *H. diversicolor* were associated with low concentrations of PO_4-P. However, at the lowest temperature, treatments containing *C. volutator* were associated with lower concentrations of PO_4-P, whilst the highest PO_4-P concentrations were linked to the *H. ulvae* monoculture treatment. Concentrations of PO_4-P were similar at 6 and 12 ˚C, although the data showed a high degree of variation, and these PO_4-P concentrations were generally significantly above those observed at 18˚C. Coefficient estimates for the effect of CO_2 (figure 4b) reveal that the highest concentrations of PO_4-P were observed where CO_2 was 600 ppm, followed by 380 ppm, and then 1000 ppm.

(c) Particle bioturbation (Db)

The minimal adequate model for Db as a dependent variable and with species richness as an independent variable simply consisted of the 2-way interaction term species richness × temperature (table 1). The most influential variable was species richness (L-ratio = 185.73, d.f. = 9, $p < 0.0001$) followed by temperature (L-ratio = 37.56, d.f. = 8, $p < 0.0001$).

There was a general decline in Db with increasing species richness (figure 5). However, the Db values for monocultures were markedly higher at 6 and 12˚C than at 18˚C, making the declines in Db with species richness much greater at these temperatures.

The minimal adequate model with species identity as an independent variable (SM), contained the 3-way interaction term CO_2 × species identity × temperature (table 1). Species identity was the most influential variable (*L*-ratio = 327.11, d.f. = 63, $p < 0.0001$), followed by temperature (*L*-ratio = 148.14, d.f. = 48, $p < 0.0001$) and CO_2 (*L*-ratio = 77.94, d.f. = 48, $p < 0.01$).

In the monoculture treatments with a CO_2 concentration of 380 ppm and a temperature of 6˚C, the greatest Db values were associated with *H. diversicolor* (figure 6), with values for the other two species being markedly lower. However, when temperature or CO_2 levels increased, these Db values decreased dramatically, and at the highest CO_2 concentrations, all Db values for *H. diversicolor* monocultures were on a par with those for the other species. Even under environmental conditions where Db was high for *H. diversicolor* monoculture treatments, treatments involving *H. diversicolor* in combination with other species tended to result in much lower Db values.

4. DISCUSSION

When data from BEF experiments are integrated (Balvanera et al. 2006, Cardinale et al. 2006), the relationships between biodiversity and ecosystem functioning are mostly positive. We found a positive relationship between species richness and NH_4-N concentration incorporating strong species identity effects particularly *H. diversicolor*, but no relationship between species richness and PO_4-P concentration, although species identity effects were found. These results generally agree with previous studies on this particular benthic estuarine system (Emmerson et al. 2001, Dyson et al. 2007, Bulling et al. 2008) and the results for NH_4-N are broadly in line with findings elsewhere (e.g. Hansen and Kristensen 1997, Mermillod-Blondin et al.

2005). However, Calimen et al (2007) did find a positive relationship between PO_4-P concentrations and species richness in freshwater mesocosm experiments.

Significant species composition effects were found for both measures of nutrient release (NH4-N and PO4-P) and for bioturbation (Db), and significant species richness effects were found for NH4-N and Db, although not for PO4-P. A cursory consideration of the differences between the two models for PO4-P may be interpreted as contradictory conclusions on the relative importance of biodiversity. However, the ability to incorporate species composition in the modelling process (achieved here because of the complete experimental design) has revealed mechanistic complexities that would otherwise remain hidden, including which species disproportionately affects functioning.

The general negative relationship between species richness and bioturbation (Db) was modified by temperature. However, the model incorporating species composition as an independent variable revealed that this relationship was primarily driven by *H. diversicolor*. Monocultures of *H. diversicolor* generally resulted in markedly higher levels of bioturbation but this effect decreased in the presence of other species and with increasing temperature and, generally, with increasing CO_2 concentration. This dominance of *H. diversicolor* is consistent with previous results (Ieno et al. 2006), and is a consequence of *H. diversicolor's* ability to deeply burrow into the sediment. As Db is strongly influenced by the depth of the luminophore profile obtained, the decline in Db at higher levels of species richness is likely to be due to a change in behaviour of *H. diversicolor*, reducing the extent of its movement into deeper sediment and altering its behaviour (bioirrigation > particle displacement) when other species were present and in relation to temperature levels and CO_2 concentrations (Ouelette et al. 2004). Experimental work examining the effect of

seawater acidification on the polychaete worm *Nereis virens* (Widdicombe & Needham 2007) found the reverse effect of increasing NH_4-N concentrations with decreasing pH. Burrow characteristics were unchanging between pH treatment levels and it was concluded that the impact of seawater pH on nutrient flux was probably due to changes in the microbial community. The shorter duration of our experiment (7 days) relative to that for *N. virens* (5 weeks), however, means that changes in microbial community composition is unlikely to fully explain the patterns observed here. A more parsimonious explanation is a combination of context dependent switching behaviour in *N. diversicolor* (Riisgård & Kamermans 2001) and biogeochemical effects to lowered pH levels. Context-dependent changes in behaviour have been shown in experiments investigating the effect of temperature on the polychaetes *Nereis virens* (Deschênes et al. 2005) and *Neanthes virens* (Ouellette et al. 2004), with a general increase in activity levels with increasing temperature. Ouellette et al. (2004) found the highest Db value at the intermediate temperature (13 ^{o}C), but they also found increasing biotransport levels with increasing temperature. Deschênes et al. (2005) found changes in behaviour with temperature, with locomotion being strongly inversely correlated with temperature.

The change in behaviour of *H. diversicolor* and the resultant consequences for ecosystem process and function in our experiment has important implications for the understanding of biodiversity-functioning relationships under future climate scenarios. This was a change in functional response, indicating that functional groups (Petchey & Gaston 2002; Naeem & Wright 2003) may be fluid in nature, with some species changing their behaviour in response to shifting environmental conditions. Functional groupings defined under current conditions may not be a reflection of functional groups in the future, suggesting that current functional redundancy may not

be a reliable representation of redundancy in the future, a problem that is compounded when multiple functions are considered. This fluidity has the potential to significantly alter biodiversity-function relationships, and suggests that the precautionary principle (Myers 1993) may have an increased relevance when managing such systems for the delivery of ecosystem services for the future under changing climatic conditions.

In similar marine systems, Emmerson et al. (2001) found variability in the contributions of particular species to ecosystem function resulting in a consistent, but idiosyncratic, increase in function with increasing richness. Results here suggest that this concept can be extended to incorporate extra dimensions describing climate change variables. Overall patterns between species richness and function were consistent despite strong species composition effects, but differences in temperature and / or CO_2 concentration could elicit idiosyncratic changes in functioning levels due to the variability in the behavioural responses of the species to these changes in climatic variables.

To date, the majority of research on the effects of global climate change has been focussed on single drivers (Fabry *et al*. 2008, Przeslawski *et al*. 2008, Byrne *et al*. 2009), despite recognition that multiple drivers may act in non-additive ways (Christensen *et al.* 2006; Byrne *et al*. 2009; Laurance & Useche 2009). Reich *et al.* (2001) found interactive effects between atmospheric CO2 and biodiversity, and between nitrogen deposition and biodiversity on biomass levels in a terrestrial plant system. However, they did not find significant interactions involving both variables CO2 and nitrogen. Our findings strongly suggest that there will be significant interaction effects between climate change variables and biodiversity on levels of future ecosystem functioning and services. We found significant interactions between temperature, atmospheric CO2 concentration and species richness and/or species composition determining concentrations of NH4-N. There was a

general trend of increasing NH4-N concentration (ecosystem functioning) with increasing species richness but this tendency was modified by the two climate change variables. Our results also suggest that the effects of climate change variables on the BEF relationship may be nonlinear for certain ecosystem functions. We found a distinct peak in the steepness of the species richness—NH4-N concentration relationship at the intermediate temperature. It is notable that there was variability in the complexity of the models developed. Those for NH4-N concentrations involved three two-way interaction terms. Of the two models for Db, only the model incorporating species identity contained a three-way interaction term, and PO4-P concentrations were driven by the climatic variables in an additive manner or via a single two-way interaction.

Given the already high uncertainty associated with predictions of future climatic conditions and the expected regional variations (IPCC, 2007), the possible presence of interactive effects between climatic drivers and biodiversity, as well as contrasting and non-linear effects on ecosystem functioning, must be major concerns in terms of prediction and management of the consequences of climate change. In assessing the probable ecological consequences of anthropogenic and environmental change, we need to embrace the concept that multiple drivers, and their interactions and feedbacks, are likely to modify the natural environment in ways that are difficult to predict. There is therefore a requirement for a more holistic framework that aims to understand how multiple drivers, alone and in concert, affect multiple functions (Elmqvist *et al.* 2003; Hector & Bagchi 2007; Gamfeldt *et al.* 2008).

The orders of overall influence of independent variables within a model were consistent across all models for NH_4-N and Db, with species richness or species composition being markedly the most influential, followed by temperature, and finally CO_2 concentration. However, for the species richness model for PO_4-P, temperature

was most influential followed by CO_2 concentration, with species richness not having a significant influence. The corresponding model with species combination as an independent variable, however, incorporated both climatic drivers and biodiversity (temperature being most influential, followed by species composition and CO_2 concentration), highlighting the importance of the roles of individual species. Differences in the order and strength of influence of the climatic and species variables for the different ecosystem functions and process considered here, also emphasise the need for a more holistic approach for developing future management strategies in the light of specific mitigation priorities.

It is important to note that under two levels of CO2 concentration the fauna was exposed to conditions that are likely to be found 100 years into the future. This longer time scale would allow other mechanisms to play an influential role, in particular changes in species' distributions and evolutionary adaptation processes. The study was also run for a short time (7 days) which limits the available time for species to acclimate. In this context it is important to recognize that this experiment was not designed to examine and predict specific physiological and behavioural responses of organisms to climate change and the resulting changes in ecosystem functioning. Rather, our model system is a simplification of a natural community and is therefore amenable to manipulation (Benton et al. 2007), allowing us to explicitly test the sensitivity of the BEF relationship to climate change variables and potential interaction effects.

Our results, based on benthic marine systems, have shown that the relationships between ecosystem function, biodiversity and climate change are not straightforward, and that they are mediated by interactions between the different drivers within the system. Future BEF research needs to focus on the mechanistic

interactions of multiple drivers and multiple functions, and on the development of more holistic approaches to underpin future management strategies.

Acknowledgements

The authors would like to thank O. McPherson for technical assistance, and T. Fujii for help in the field. They would also like to thank two anonymous reviewers for helpful and constructive comments. This work was supported by NERC grant NE/E006795/1.

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Table 1. Summary of significant terms found in the linear regression models with a generalised least squares extension, treating levels of NH_4-N, PO_4-P and particle bioturbation (Db) as dependent variables, and species richness (or species identity), atmospheric CO_2 concentration and temperature as fixed explanatory variables.

SPECIES RICHNESS MODELS

Dependent variable	**Significant terms**	**L-Ratio**	**d.f.**	***p***
NH_4-N				
	species richness × temperature	23.96	6	< 0.001
	CO_2 × temperature	19.40	4	< 0.001
	species richness × CO_2	12.39	6	0.054
PO_4-P				
	temperature	116.14	2	< 0.0001
	CO_2	23.25	2	< 0.0001
Db				
	species richness × temperature	30.25	6	< 0.0001

SPECIES IDENTITY MODELS

Dependent variable	**Significant terms**	**L-Ratio**	**d.f.**	***p***
NH_4-N				
	species identity × temperature	67.14	14	< 0.0001
	species identity × CO_2	32.70	14	< 0.01
	CO_2 × temperature	26.10	4	< 0.0001
PO_4-P				
	species identity × temperature	26.72	14	0.021
	CO_2	30.64	2	< 0.0001
Db				
	CO_2 × species identity × temperature	60.11	28	< 0.001

Figure legends

Figure 1. Predicted NH_4-N concentrations from the minimal adequate regression model for varying species richness levels at three atmospheric CO_2 concentrations (380 (solid line), 600 (dashed line) and 1000 ppm (dotted line)) and the three temperatures *a)* 6 ^oC, *b)* 12 ^oC and *c)* 18 ^oC.

Figure 2. Predicted NH_4-N concentrations from the minimal adequate regression model for each combination of species (*Hd* = *Hediste diversicolor*, *Hu* = *Hydrobia ulvae*, *Cv* = *Corophium volutator*) at three atmospheric CO_2 concentrations (380 (solid line), 600 (dashed line) and 1000 ppm (dotted line)) and the three temperatures *a)* 6 ^oC, *b)* 12 ^oC and *c)* 18 ^oC.

Figure 3. Predicted PO_4-P concentrations from the minimal adequate regression model at three atmospheric CO_2 concentrations (380, 600 and 1000 ppm) and the three temperatures 6 ^oC (solid line), 12 ^oC (dashed line) and 18 ^oC (dotted line). This minimal adequate model resulted from the full model which included species richness in the full model. Effects of temperature and CO_2 were additive with no significant interaction term.

Figure 4. a) Predicted PO_4-P concentrations from the minimal adequate regression model (with species identity rather than species richness as an independent variable) for each combination of species (*Hd* = *Hediste diversicolor*, *Hu* = *Hydrobia ulvae*, *Cv* = *Corophium volutator*) at the three temperatures 6 ^oC (solid line), 12 ^oC (dashed line)

and 18 ^{o}C (dotted line). Atmospheric CO_2 concentration is held constant. b) Estimates from the minimal adequate regression model for PO_4-P concentrations of coefficients associated with the effects of the higher atmospheric CO_2 concentrations relative to the effect at 380 ppm. Error bars represent standard errors.

Figure 5. Predicted Db (cm^2 y^{-1}) levels from the minimal adequate regression model for varying species richness levels at the three temperatures 6 ^{o}C (solid line), 12 ^{o}C (dashed line) and 18 ^{o}C (dotted line).

Figure 6. Predicted Db levels from the minimal adequate regression model for each combination of species (*Hd* = *Hediste diversicolor*, *Hu* = *Hydrobia ulvae*, *Cv* = *Corophium volutator*) at three atmospheric CO_2 concentrations (380 (solid line), 600 (dashed line) and 1000 ppm (dotted line)) and the three temperatures *a)* 6 ^{o}C, *b)* 12 ^{o}C and *c)* 18 ^{o}C.

Figure 1

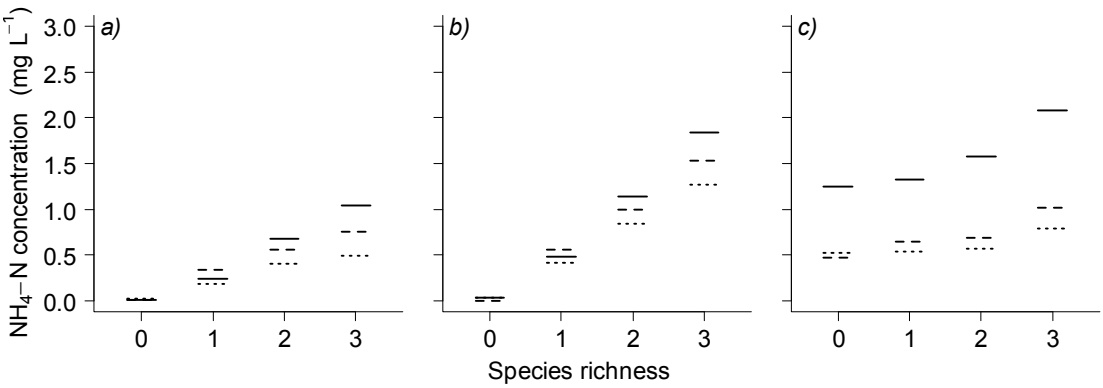


Figure 2

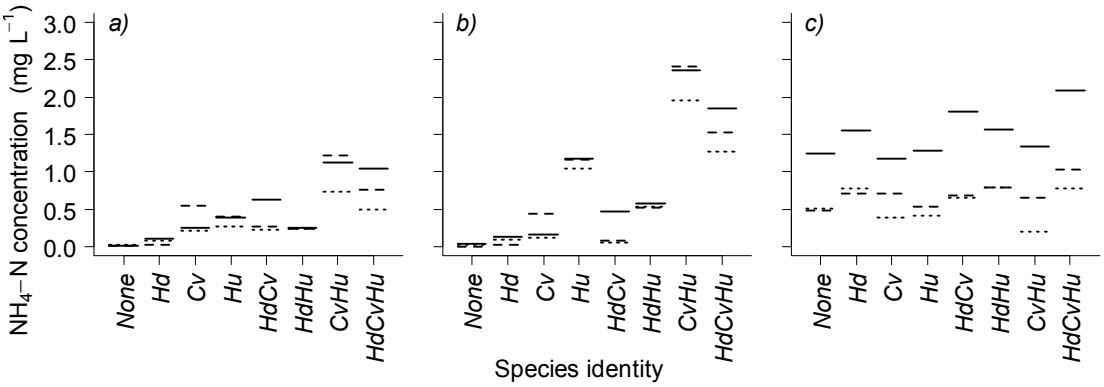


Figure 3

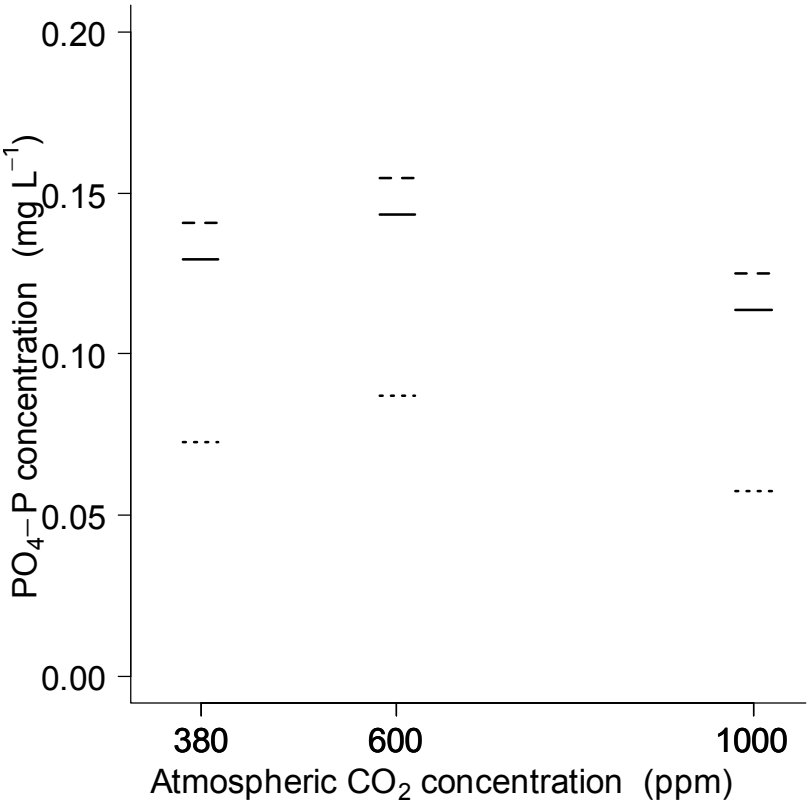

0.20
0.15
0.10
0.05
0.00
PO_4–P concentration (mg L^{-1})
380
600
1000
Atmospheric CO_2 concentration (ppm)

Figure 4

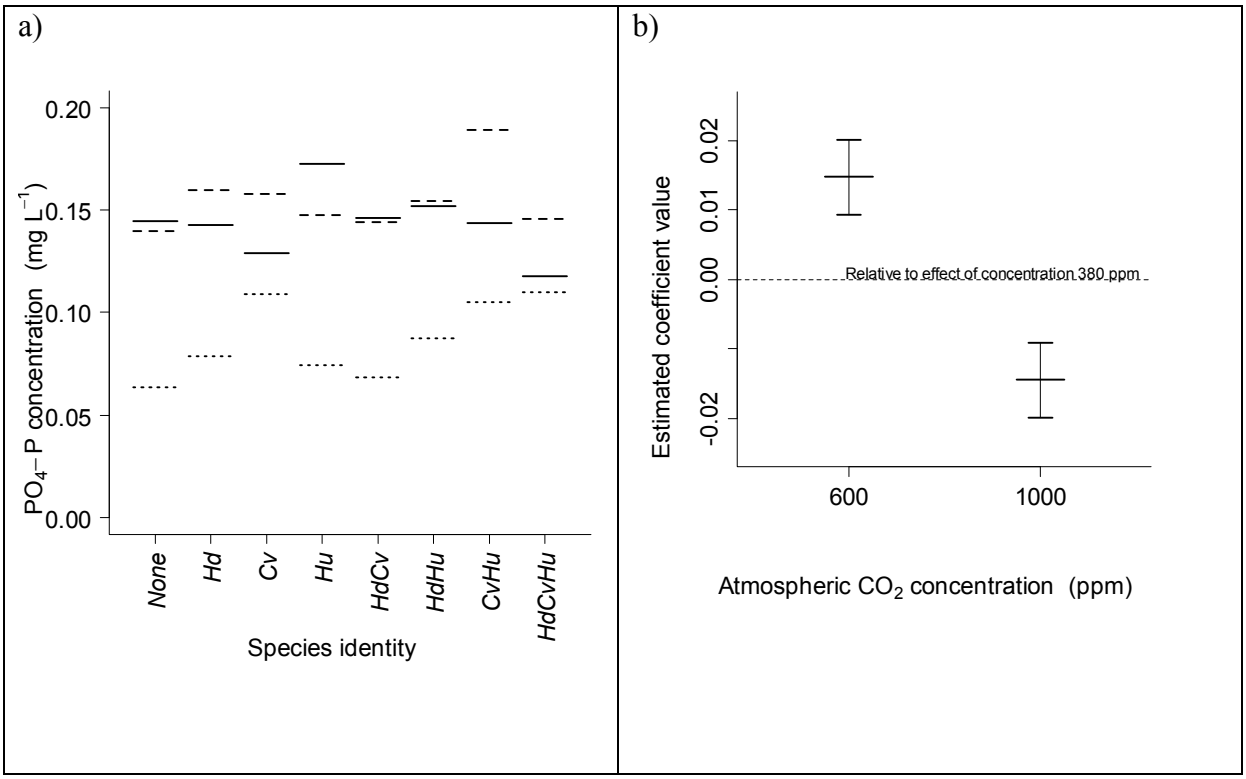

a)
0.20
0.15
0.10
0.05
0.00
PO_4– P concentration (mg L^{-1})
None
Hd
Cv
Hu
HdCv
HdHu
CvHu
HdCvHu
Species identity
b)
0.02
0.01
0.00
-0.02
Estimated coefficient value
Relative to effect of concentration 380 ppm
600
1000
Atmospheric CO_2 concentration (ppm)

Figure 5

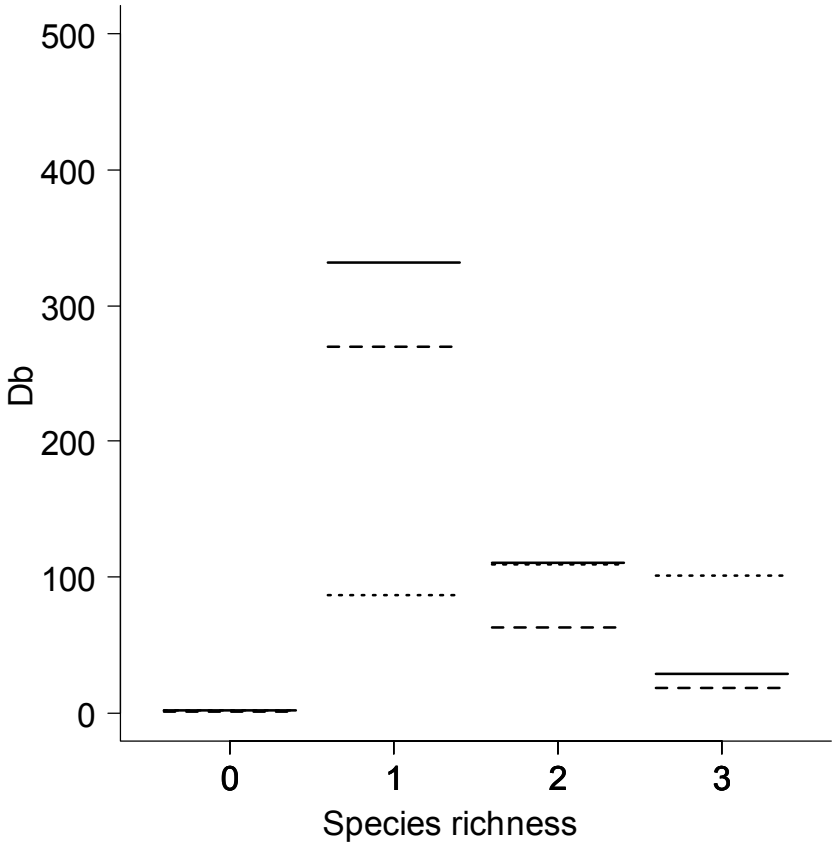
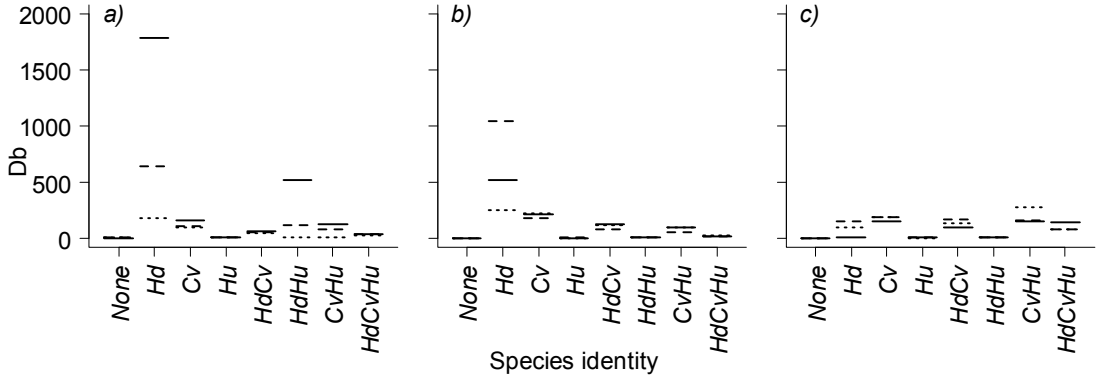


Figure 6


a)
b)
c)
Db
2000
1500
1000
500
0
None
Hd
Cv
Hu
HdCv
HdHu
CvHu
HdCvHu
Species identity

Supplementary Material

Marine biodiversity-ecosystem processes under uncertain environmental futures

Mark T. Bulling[1], Natalie Hicks[2], Leigh Murray[1], David M. Paterson[2], Dave Raffaelli[3], Piran C. L. White[3] and Martin Solan[1]

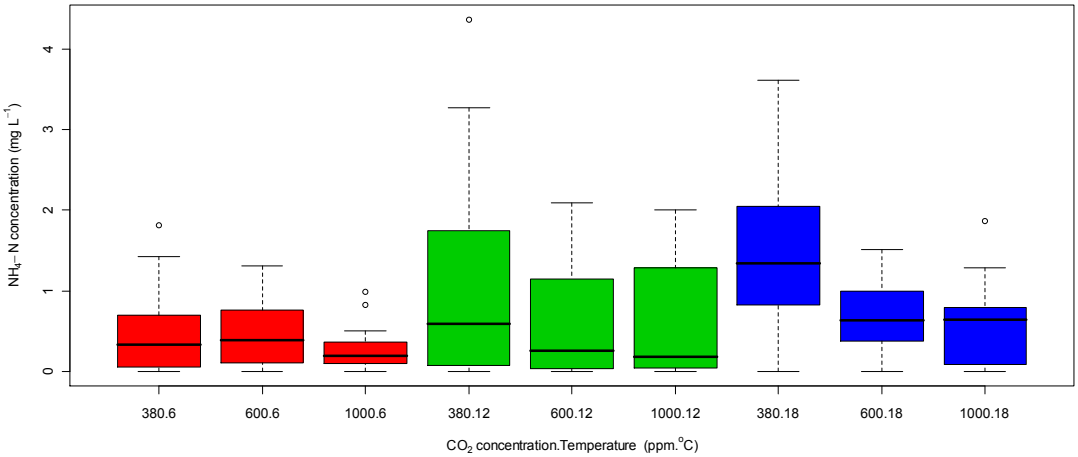
[1]*Oceanlab, University of Aberdeen, Main Street, Newburgh, Aberdeenshire, AB416AA, UK*

[2]*Gatty Marine Laboratory, University of St. Andrews, East Sands, St. Andrews, Fife, KY16 8LB, UK*

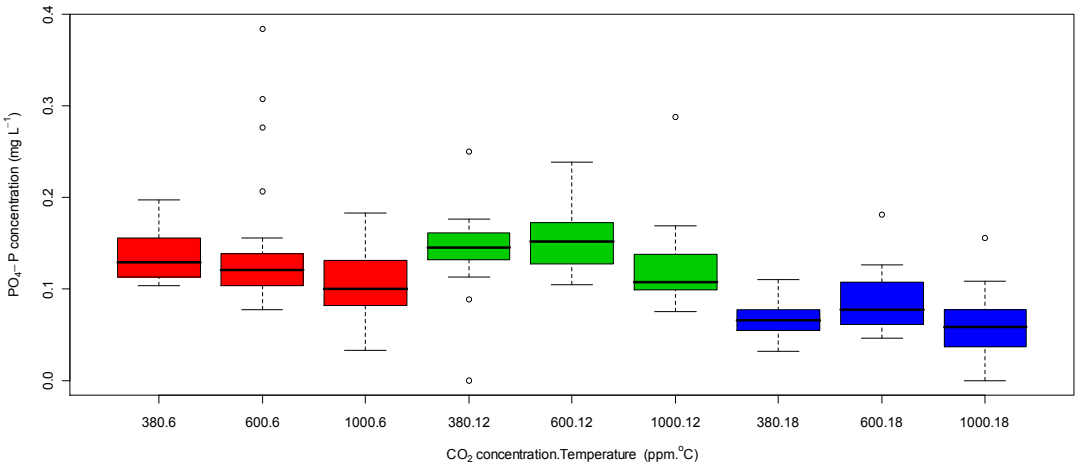
[3]*Environment Department, University of York, Heslington, York, YO10 5DD, UK*

1) Plots of raw data

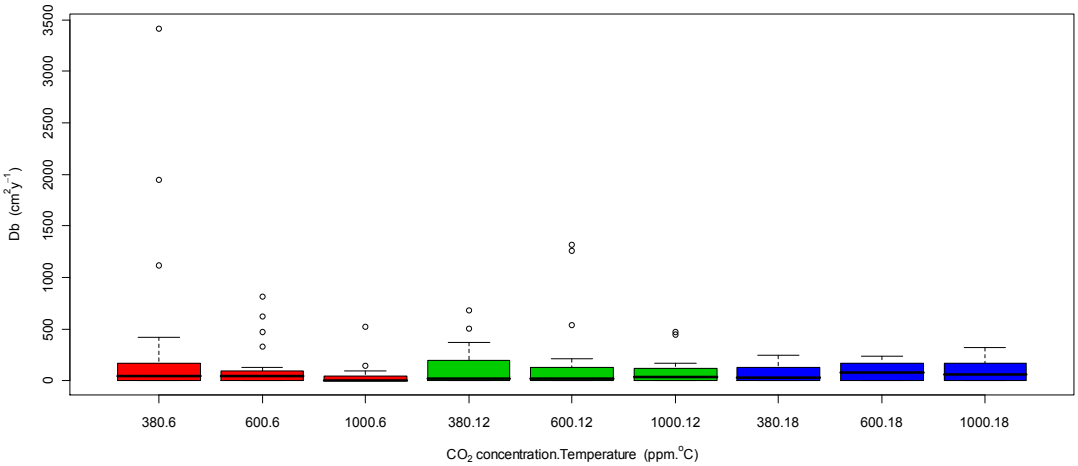
a) Boxplot of NH_4-N concentrations under all CO_2 concentration × temperature combinations (indicated on the x-axis). In each case, the median is indicated at the midpoint, the upper and lower quartiles are indicated by the hinges, whiskers represent the spread (extending to the most extreme data point which is no more than 1.5 times the interquartile range from the box), and open circles indicate outliers.



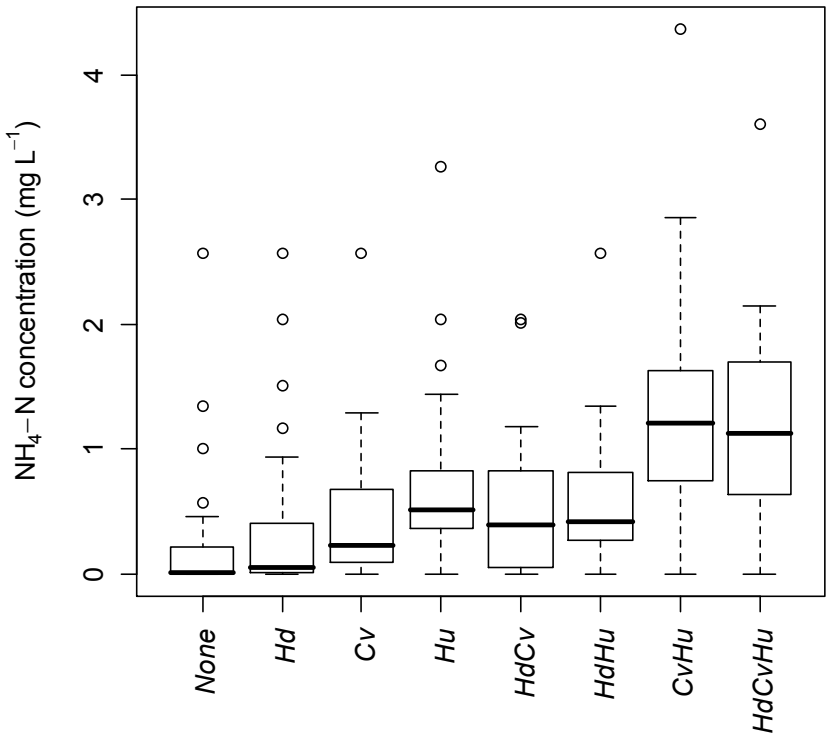
b) Boxplot of PO_4-P concentrations under all CO_2 concentration × temperature combinations (indicated on the x-axis). In each case, the median is indicated at the midpoint, the upper and lower quartiles are indicated by the hinges, whiskers represent the spread (extending to the most extreme data point which is no more than 1.5 times the interquartile range from the box), and open circles indicate outliers.



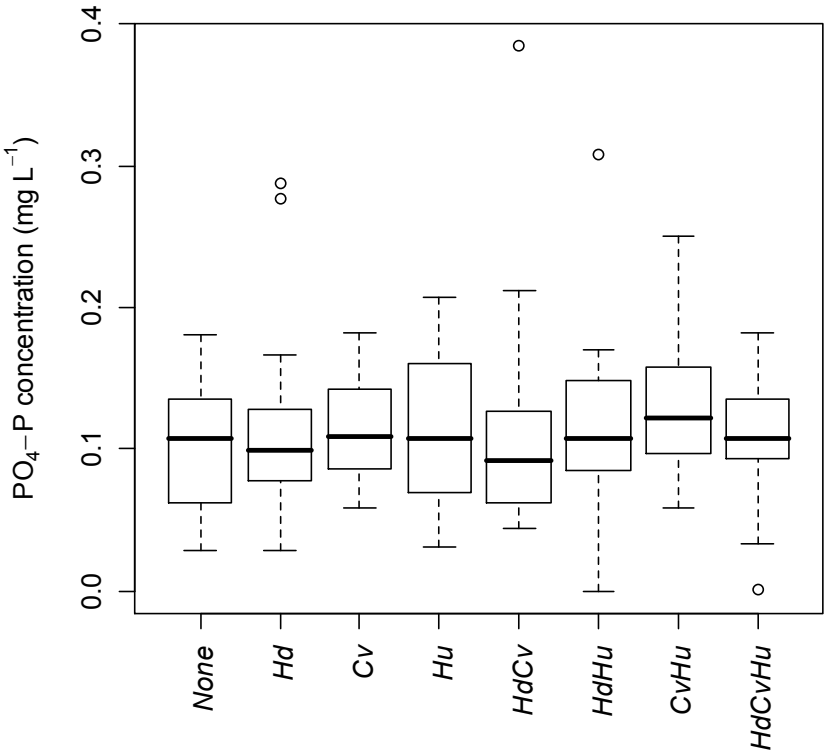
c) Boxplot of the bioturbation coefficient, Db, under all CO_2 concentration × temperature combinations (indicated on the x-axis). In each case, the median is indicated at the midpoint, the upper and lower quartiles are indicated by the hinges, whiskers represent the spread (extending to the most extreme data point which is no more than 1.5 times the interquartile range from the box), and open circles indicate outliers.



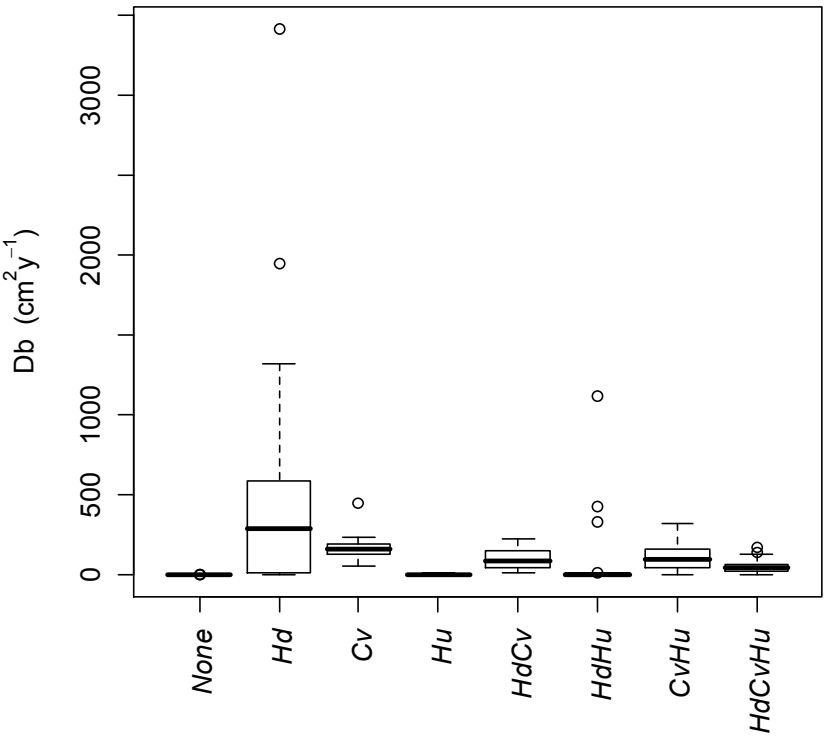
d) Boxplot of NH_4-N concentrations for each species identity treatment. In each case, the median is indicated at the midpoint, the upper and lower quartiles are indicated by the hinges, whiskers represent the spread (extending to the most extreme data point which is no more than 1.5 times the interquartile range from the box), and open circles indicate outliers. The species composition of each mixture is indicated on the x-axis, where species identity corresponds to: no macrofauna (None); *Hediste diversicolor* (Hd), *Corophium volutator* (Cv) and *Hydrobia ulvae* (Hu)



e) Boxplot of PO_4-P concentrations for each species identity treatment. In each case, the median is indicated at the midpoint, the upper and lower quartiles are indicated by the hinges, whiskers represent the spread (extending to the most extreme data point which is no more than 1.5 times the interquartile range from the box), and open circles indicate outliers. The species composition of each mixture is indicated on the x-axis, where species identity corresponds to: no macrofauna (None); *Hediste diversicolor* (Hd), *Corophium volutator* (Cv) and *Hydrobia ulvae* (Hu)



f) Boxplot of the bioturbation coefficient, Db, for each species identity treatment. In each case, the median is indicated at the midpoint, the upper and lower quartiles are indicated by the hinges, whiskers represent the spread (extending to the most extreme data point which is no more than 1.5 times the interquartile range from the box), and open circles indicate outliers. The species composition of each mixture is indicated on the x-axis, where species identity corresponds to: no macrofauna (None); *Hediste diversicolor* (Hd), *Corophium volutator* (Cv) and *Hydrobia ulvae* (Hu)



2. *Structure of the minimal adequate models*

a) Model for NH_4-N concentrations with species richness as an independent variable.

```
NH4-N concentration = f(Species richness + CO2 conc. + Temperature
+ Species richness × CO2 conc.
+ Species richness × Temperature
+ CO2 conc. × Temperature)
```

The model was a linear regression model with a gls extension (Temperature and Species richness variance-covariates).

b) Model for NH_4-N concentrations with species identity as an independent variable.

```
NH4-N concentration = f(Species identity + CO2 conc. + Temperature
+ Species identity × CO2 conc.
+ Species identity × Temperature
+ CO2 conc. × Temperature)
```

The model was a linear regression model with a gls extension (Temperature and Species identity variance-covariates).

c) Model for PO_4-P concentrations with species richness as an independent variable.

```
PO4-P concentration = f(CO2 conc. + Temperature)
```

The model was a linear regression model with a gls extension (Temperature variance-covariate).

d) Model for PO_4-P concentrations with species identity as an independent variable.

```
PO4-P concentration = f(Species identity + CO2 conc. + Temperature
+ Species identity × Temperature)
```

The model was a linear regression model with a gls extension (Temperature variance-covariate).

e) Model for Db levels with species richness as an independent variable.

```
Db = f(Species richness + Temperature
+ Species richness × Temperature)
```

The model was a linear regression model with a gls extension (Temperature and Species richness variance-covariates).

f) Model for Db levels with species identity as an independent variable.

```
Db = f(Species identity + CO2 conc. + Temperature
+ Species identity × CO2 conc.
+ Species identity × Temperature
+ CO2 conc. × Temperature
+ Species identity × CO2 conc. × Temperature)
```

The model was a linear regression model with a gls extension (Temperature and Species identity variance-covariates).

END OF SUPPLEMENTARY MATERIAL