INTRASPECIFIC VARIATION IN THE LEAF TRAITS AND DECOMPOSITION OF ESTUARINE PRIMARY PRODUCERS ACROSS SPATIAL GRADIENTS

Lara Beth Ainley

B. Sc. (Hons) Marine Science

M. Sc. Marine Biodiversity and Conservation

Department of Biological Sciences

Macquarie University

North Ryde 2109, Sydney, Australia

Supervisor: Dr Melanie Bishop

Associate Supervisors: Dr Adriana Vergés, Dr Joshua Madin

Submitted June 2014 for the degree of Doctor of Philosophy in Biological Sciences

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

SUMMARY

Decomposition is a key process for carbon and nutrient cycles. Hence, understanding how biotic and abiotic factors interact to control decomposition is an important avenue of ecological research. This thesis investigated how climatic setting and nutrient enrichment directly and, via effects on leaf traits, indirectly influence the decomposition of two key estuarine primary producers, the seagrass *Zostera muelleri* and the mangrove *Avicennia marina*. It then considered how differences in decomposition processes among climatic settings influence the structure of benthic communities. Sampling within 16 estuaries spanning 7° of latitude, and varying in anthropogenic nutrient loading, revealed that the seagrass and mangrove displayed latitudinal variation in leaf traits, which in many instances differed between the two species. Nutrient statuses of estuaries were, by contrast, a poor predictor of leaf traits and the relationship between leaf traits and nutrient loading was highly temporally variable. Nevertheless, latitude and nutrient status of estuaries interacted to influence the decomposition rate of litter from a common source, perhaps due to spatial variation in decomposer communities. Further, at a common site, litter sources from high and low latitudes differed in decomposition rate, although relationships differed between the two species of macrophyte. Spatial variation in mineralization of detritus, resulting from differences in climate and unidentified factors, lead to spatially variable effects of organic enrichment on benthic communities. Overall, this study found that climatic setting and nutrient enrichment can act synergistically to influence decomposition processes via a combination of direct and indirect effects. Nevertheless, the relationship between decomposition processes and environmental factors differed markedly between the two co-occurring estuarine primary producers considered by this study. Hence, intraspecific variability in decomposition processes cannot be reliably predicted by broad-scale interspecific patterns.

DECLARATION

I declare that this thesis titled 'Intraspecific variation in the leaf traits and decomposition of estuarine primary producers across spatial gradients' is my own work and has not been submitted in any form for another degree or at any other University or institution. This thesis contains original material. Any additional help received during the preparation of this work has been indicated in the 'Contributions' section.

Lara Beth Ainley

42234433

June 2014

CONTRIBUTIONS

This thesis contains material that was presented at an invited seminar at The National Wetlands Research Centre (NWRC-USGS), Lafayette, Louisiana, USA, April 2013; and has been prepared for publication as follows:

Chapter 1: General introduction

I performed the literature review and writing of this chapter with constructive feedback and suggestions from my supervisor, Melanie Bishop.

Chapter 2: Large-scale intraspecific patterns of variability in leaf traits differ between two co-occurring estuarine angiosperms.

Authors: Lara Ainley, Adriana Vergés, Melanie Bishop

This chapter has been prepared for submission to *Journal of Ecology*. My contribution to this research: Concept = 50%, Data collection = 100%, Data analysis = 100%, Writing = 80%, Total = 82.5%. I received constructive feedback and suggestions from my supervisors, Melanie Bishop, Adriana Vergés and Joshua Madin.

This research was presented at The Australian Marine Science Association (AMSA) Annual Conference, Gold Coast, Queensland, Australia. July 2013.

Chapter 3: The effect of coastal development on litter decomposition varies between climatic settings.

Authors: Lara Ainley, Melanie Bishop

This chapter has been prepared for submission to *Oikos*. My contribution to this research: Concept = 60%, Data collection = 100%, Data analysis = 100%, Writing = 80%, Total = 85%. I received constructive feedback and suggestions from my supervisor, Melanie Bishop.

This research was presented at two conferences:

The Australian Marine Science Association (AMSA) Annual Conference, Hobart, Tasmania, Australia. July 2012.

The 42nd Annual Benthic Ecology Meeting, Savannah, Georgia, USA. March 2013.

Chapter 4: Relationships between intraspecific variation in leaf traits and decomposition vary between species.

Authors: Lara Ainley, Magali Raybaud, Adriana Vergés, Melanie Bishop

This chapter has been prepared for submission to *Oecologia*. My contribution to this research: Concept = 65%, Data collection = 55%, Data analysis = 100%, Writing = 80%, Total = 75%. I received constructive feedback and suggestions from my supervisors, Melanie Bishop and Adriana Vergés. I also received help in the data collection from Magali Raybaud.

Chapter 5: Tipping points: context-dependent effects of organic enrichment on sediment invertebrate communities.

Authors: Lara Ainley, Jennifer Rowland, Melanie Bishop

This chapter has been prepared for submission to *Oikos*. My contribution to this research: Concept = 70%, Data collection = 60%, Data analysis = 100%, Writing = 80%, Total = 77.5%. I received constructive feedback and suggestions from my supervisor, Melanie Bishop. I also received help in taxonomic identification and suggestions from Jennifer Rowland.

Chapter 6: General discussion

I performed the literature review and writing of this chapter with constructive feedback and suggestions from my supervisor, Melanie Bishop.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor, Dr Melanie Bishop. You have been an incredible teacher and mentor, and I would not have come to this point without your support. This PhD is a testament to you and the dedication you give to every one of your students. I am truly grateful for the time and effort you have given to fostering my fledgling academic career.

I would also like to take this opportunity to thank my two associate supervisors, Adriana and Josh. You have helped me in so many ways throughout the course of this degree. Your knowledge and support have been invaluable.

To my colleagues, old and new, at the Benthic Ecology Lab: Andrea, Andrés, Belinda, Brad, Cliff, Daisy, Dan, Dom, Emma, Jen, Joe, Ka-Man, Leanne, Leigh, Lincoln, Mirella, Peter, Ramila and Valter. We're all in the same boat and it has been such a pleasure working with and learning from all of you. Mirella and Jen, you have been such great friends throughout my time at Macquarie and I hope to give you both as much support as you have shown me. I have learnt a lot from each of you and coffee breaks will never be the same again. To Joe, Dom, and everyone I have introduced to dumplings and Carcassonne, thanks for all the laughs.

For all the people I have had the pleasure of teaching with: Kath, Lesley, Dom, Suead, Tarun, Alex, Priscilla and Matt. It has been a wonderful experience teaching with you.

To my friends at Macquarie: Adam, Alex, Diego, Emma, James, Jake, Julietta, Kath, Linda, Marcella, Matt, Pete, Priscilla, Siobhan, Suead, Toni and Wander. This is a very easy thank you to make as you are all going, or have gone through, the same PhD experience. You are some of the only people who can actually relate to this experience and I thank you for your kindness and friendship. Linda, I'll meet you at the bar for schnapps!

To all my amazing friends: there are too many of you to name. Many of you won't have understood the decisions I have made whilst doing this PhD, the sacrifice of personal life in the pursuit of something 'greater'. However, you have still given me all the support you could without judgment, especially during my notable absences from social events and on the netball court where I often channeled my frustrations. I can now return your phone calls.

A very special thank you to my family: my dad Russell, Laetitia, Sam, Lachlan, Sue, Ron, Emily, Em, Panna, Josh and my extended family. You probably aren't aware of how much you have helped me over the past 3.5 years. This PhD was certainly a rollercoaster and you were with me for every 'up' and every 'down'. I cannot ever express how important you are to me and I am forever grateful to have such care and kindness in my life.

Finally I would like to acknowledge my inspiration, my mother Leigh. Your love, support and success throughout your short life gave me inspiration and determination to always follow my dreams. I hope to one day be as great as you were.

1. GENERAL INTRODUCTION

Detritus and the importance of decomposition as an ecological process

Detritus is non-living organic matter and may result from the damage, senescence or death of whole organisms, or parts of these, as well as their production of fecal material. Detrital material may be broken down by bacteria, fungi and detritivorous invertebrates, or accumulated as recalcitrant carbon (Schlesinger 1977, Mann 1988, Cebrián 1999). Detritus is consequently a central component of energy and nutrient flow through ecosystems (Lindeman 1942, Cebrián and Duarte 1994, Duarte and Cebrián 1996) and a key determinant of the structure and function of food webs (Teal 1962, Odum 1969). It is also an important global carbon store (Cebrián and Duarte 1995).

In terrestrial and aquatic ecosystems, primary producers are a major source of detritus. In many ecosystems, most primary production is not consumed by herbivores but instead enters detrital pathways (Wetzel 1983, Odum and Biever 1984, Moore and Hunt 1988, Moore et al. 2004). The production of detritus by plants is determined by the productivity of the living photosynthesisers themselves, the rate at which their biomass is removed by herbivores and the rate of mobilization of detritus through damage or senescence (Duarte and Cebrián 1996). Detritus may be retained by the ecosystem in which it is produced (termed autochthonous detritus) or exported into adjacent ecosystems by wind or water (termed allochthonous detritus, Pace et al. 2004). Ecosystems where organic material is produced are considered ‘carbon sources’ (Cebrián and Duarte 1994). Ecosystems in which large amounts of recalcitrant carbon accumulate are considered ‘carbon sinks’ (Cebrián 1999).

Decomposition processes are often limited by the break-down by detritivores of large fragments of dead plant material, releasing dissolved organic carbon (DOC) and nutrients.

Following an initial period of rapid leaching, detritus undergoes fragmentation by abiotic processes and invertebrate 'shredders', resulting particulate organic matter, POM, (Cummins and Klug 1979, Webster and Benfield 1986, Wallace and Webster 1996). Microorganisms then colonise the POM, first by bacteria and then by fungi, breaking down the material and further releasing DOC and nutrients. As nitrogen is remineralised, the C:N of remaining material increases, slowing the decomposition process. Much of the labile organic carbon is used by decomposers as a substrate for respiration, a process that consumes oxygen and produces the by-product, CO_2. Hence, where decomposition occurs in low oxygen environments, such as below the sediment surface, anoxia can be induced. The humic material remaining after mobilization of DOC and nutrients is highly refractory and can persist over long periods, with minimal decomposition.

Given the importance of detritus to carbon and nutrient cycles, elucidating the controls on detrital dynamics and how they relate to variability in food web models is an essential direction for ecological research (Cebrián 2002, Moore et al. 2004).

Factors affecting decomposition

Whether plant detritus is rapidly remineralized, buried or stored in recalcitrant forms influences carbon and nutrient cycling. Consequently, many studies have sought to trace the fate of plant detritus (Cebrián and Duarte 1994, Duarte and Cebrián 1996), and those factors that influence its decomposition (e.g. Swift et al. 1979, Valiela et al. 1985, Zhang et al. 2008). These studies have found that the rate of decomposition is determined by three primary factors: (1) the physical and chemical characteristics of detrital material; (2) the abiotic environment; and (3) the structure and function of the decomposer community (Swift et al. 1979, Coûteaux et al. 1995). At large spatial scales dominant controls of decomposition are typically abiotic (Aerts 1997, Silver and Miya 2001). At smaller spatial scales the

interplay between leaf traits, herbivory and the decomposer community may be more important for decompositon (Pérez-Harguindeguy et al. 2000, Wardle 2006).

Litter traits are major drivers of decomposition and ultimately determine: (1) the palatability of detritus to heterotrophic consumers, and (2) their susceptibility to mechanical breakdown. Of particular importance are the nitrogen content, the carbon to nitrogen ratio (C:N) and the lignin content (Melillo et al. 1982, Enríquez et al. 1993). Rates of detrital decomposition generally increase with nitrogen content and decrease with lignin (Melillo et al. 1982). Consequently, slow decomposing detritus of high C:N is often referred to as refractory detritus and rapidly decomposing detritus of low C:N as labile detritus. Palatability to consumers is also influenced by the concentration of chemical defenses (Cronin and Hay 1996), specifically the phenol-based secondary metabolites that may deter detritivores as well as herbivores, impeding the progress of decomposition.

Climate, latitude, mean annual temperature and mean annual rainfall have been described as key correlates of decomposition (Zhang et al. 2008), as have hydrodynamic conditions (Leroy and Marks 2006) and water quality (Jenkins and Suberkropp 1995, Dangles et al. 2001). Among these environmental drivers influencing decomposition, temperature is the most widely discussed. Decomposition rates generally increase with temperature, although there is considerable variability in the rate at which it increases among latitudes and among litter types (Kirschbaum 1995). The effect of temperature appears to be greatest in colder climates (Kirschbaum 2006) and where the organic material is more recalcitrant (Knorr et al. 2005). The temperature dependence of organic matter decomposition is of considerable ecological importance (Kirschbaum 1995, Kirschbaum 2006) and has important implications for carbon storage and re-release to the atmosphere (Davidson and Janssens 2006).

In addition to temperature, in aquatic environments water quality, specifically nutrient concentration, is another widely discussed driver of variability in detrital dynamics.

Diffuse nutrient enrichment is of particular concern (Carpenter et al. 1998, Gulis and Suberkropp 2003), with many coastal waterways classified as moderately to severely degraded by nutrient pollution (Howarth et al. 2002). Generally, where nutrients such as nitrogen and phosphorus are limited, primary production, and hence detrital production, is limited and decomposition is slow (Vitousek and Howarth 1991, Feller et al. 1999). Nutrient enrichment stimulates primary production and increases the supply of nitrogen rich, labile detritus (Feller et al. 1999, Robinson and Gessner 2000, Gulis and Suberkropp 2003, Smetacek and Cloern 2008). However, the influence of nutrient enrichment on organic matter decomposition extends beyond the supply and traits of detritus to the decomposer community. Nutrient enrichment can often stimulate the activity of heterotrophic micro-organisms such as fungi and bacteria, increasing the rate of microbial metabolism of detritus and subsequent decomposition (Suberkropp and Chauvet 1995, Gulis et al. 2004).

The structure and function of the decomposer community, comprised of microorganisms and macroinvertebrates, also has influence over the decomposition process (Anderson and Sedell 1979, Gessner and Chauvet 1994). The role of the decomposer community in dictating the rate of detrital decomposition is particularly important in low energy environments, where there is little fragmentation of detritus by abiotic processes (Webster and Benfield 1986). In streams, studies have found that changes in decomposer community can modify rates of litter decomposition (Hieber and Gessner 2002, Srivastava et al. 2009). Decomposer community composition can also be determined by environmental setting as well as leaf traits (Gessner et al. 2010).

Our understanding of the relative contribution of leaf traits, environmental characteristics and decomposer communities to decomposition processes has primarily been driven from correlative and observational studies (Aerts 1997, Cornwell et al. 2008). Although some clever manipulative studies in freshwater and terrestrial environments have disentangled effects of leaf traits, environment and decomposer communities (e.g.

Kominoski et al. 2007, Schindler and Gessner 2009, Ferreira et al. 2012, Pettit et al. 2012), our knowledge of how multiple factors interact to determine decomposition is poor, particularly in coastal and marine environments.

Detrital processes in estuarine ecosystems

Estuaries are coastal bodies of seawater that are at least intermittently affected by the influence of freshwater flows and the action of tides (Elliott and McLusky 2002). They are transition zones between the land, freshwater rivers and marine environments. Estuaries are highly productive ecosystems and provide valuable ecosystem services, such as carbon sequestration and storage (Barbier et al. 2011). Much of the productivity of estuaries is underpinned by detrital pathways (Mann 1972, Odum et al. 1973).

As low points in the environment, estuaries accumulate organic matter from terrestrial, freshwater and marine environments, as well as the estuary itself. Detritus is mobilized and transported into estuaries by wind, rainfall events, river flows, tides and currents (Polis et al. 1997). Detritus may be present in estuaries as dissolved organic matter, or particulate organic matter (Pinckney et al. 2001). Larger pieces of detritus, mobilized by tides can also accumulate at the high water mark of intertidal shorelines, forming accumulations known as ‘wrack’. In modifying characteristics of the physical environment, such as moisture, light, temperature, flow velocity and the availability of carbon and nutrients, wrack provides habitat and food to communities of terrestrial and aquatic invertebrates (Moore et al. 2004).

On estuarine tidal flats that lack macrophytic primary producers, the deposition of allochthonous detritus is particularly important in fuelling invertebrate communities and the fish and shorebird populations they sustain (Polis et al. 1997, Doropoulos et al. 2009, Bishop et al. 2010). Yet, whereas moderate detrital loading supports estuarine productivity, the rapid bacterial decomposition of large accumulations of labile detritus stimulated by nutrient

enrichment can reduce benthic productivity by depleting sediment oxygen, in some instances, to the point of anoxia (Pearson and Rosenberg 1978, de Jonge et al. 2002).

In some cases, for example during macroalgal blooms (Valiela et al. 1997), the rate of primary productivity exceeds the rate of grazing. Since the biological oxygen demand is already at a peak due to maximum rates of grazing and metabolic activity, oxygen concentrations in the envrionment are low. Excess material that does not immediately move through microbial or consumer food webs begin to decay and oxidise, further depleting oxygen and resulting in hypoxic or anoxic conditions (Valiela et al. 1997). Excess burial of higher quality material and a switch from aerobic to anaerobic decomposition pathways can also occur due to the onset of hypoxic conditions (Middleburg and Levin 2009).

Pearson and Rosenberg (1978) propose a model for the response of sediment dwelling organisms to organic enrichment. Initially, as enrichment increases, the abundance and richness of benthic invertebrates increase as food sources become non-limiting. As enrichment increases further, however, physico-chemical conditions begin to degrade as a consequence of the bacterial decomposition of organic matter causing oxygen depletion. Stress-tolerant, opportunistic species proliferate causing a secondary peak in abundance. Eventually, with continued enrichment, the sediment becomes anoxic and an 'afaunal threshold' is reached, beyond which environmental conditions become too stressful for even the most tolerant species.

Impacts of environmental change on detrital pathways in estuaries

Human activities have degraded estuarine ecosystems worldwide in a multitude of ways (Alongi 2002, Crain et al. 2008), and stressors to estuarine ecosystems continue to increase at an unparalleled rate (Kennish 2002). By 2025 it is suggested that estuaries will be the most impacted of all ecosystems by anthropogenic stressors (Kennish 2002). Due to the demands of a growing human population and agricultural expansion, an increase in the

diversity and intensity of anthropogenic stressors will cause large-scale ecosystem simplification, species extinctions and subsequent losses of ecosystem services (Gray et al. 2002, Barbier et al. 2011).

Nutrient enrichment, and resulting organic loading, are serious threats affecting the ecological stability of estuaries (Bricker et al. 2008). Furthermore, global temperatures are expected to rise another 2-4°C by 2100 (IPCC 2013) and the rate of climate warming has doubled over the past 100 years, now greater than any other time in the past 1000 years (Walther et al. 2002), causing changes across a multitude of ecosystems and environments. The effects of climate warming, combined with increasing anthropogenic pressure on our coastal environments, are likely to exacerbate the impact of local stressors, particularly nutrient enrichment and eutrophication (Diaz and Rosenberg 2008). Together, increasing temperature and nutrient enrichment are key drivers of change in estuaries, resulting in complex changes to detrital dynamics at a variety of scales.

Individually, we know a lot about the impact of temperature (Aerts 1997, Silver and Miya 2001) and nutrient enrichment (Udy and Dennison 1997, Lovelock et al. 2004) on detrital dynamics in estuarine ecosystems. However, there is a lack of empirical data that investigates the cumulative and potentially synergistic effects of multiple stressors on ecosystems at multiple scales (Davis and Koop 2006, Crain et al. 2008). Furthermore, much of the research on impacts of nutrients and temperature on decomposition has been in terrestrial environments or the northern hemisphere (Aerts 1997, Hobbie and Vitousek 2000, Mack et al. 2004). The estuaries of south-east Australia (the subject of this thesis) are oligotrophic compared to many estuaries of North America and Europe, due to the low nutrient content of Australian soils and highly variable freshwater flow (Harris 2001, Scanes et al. 2007), the absence of a major system of upwelling along the east Australian coastline (Roughan and Middleton 2002), and the smaller human population than in many other parts of the world. Hence, Australian estuaries may differ markedly from northern hemisphere

estuaries in their response to environmental change. While we can draw upon terrestrial and northern hemisphere studies for insight, empirical studies investigating interactive effects of climate and nutrient enrichment on detrital pathways of Australian estuaries are needed.

Temperature and nutrient enrichment have the potential to modify detrital dynamics by altering leaf traits and the decomposition environment. Climatic setting, due to its influence on leaf traits, has been identified as a key contributor to spatial variation in decomposition at large spatial scales (Conn and Dighton 2000, Moore et al. 2004, Wright et al. 2004, Gallagher and Leishman 2012). However, at regional scales, decomposition is strongly influenced by the interplay between litter quality (Pérez-Harguindeguy et al. 2000) and the structure and function of the decomposer community (Couteaux 1995, Wardle 2006) that are both strongly controlled by nutrient availability (Cronin and Lodge 2003, Hättenschwiler et al. 2005).

Seagrass and mangrove decomposition in estuaries of New South Wales, Australia

The overall aim of this thesis is to investigate how climatic setting and anthropogenic nutrient enrichment of estuaries interact to influence the leaf traits and decomposition of two broadly distributed species of aquatic macrophyte, the seagrass *Zostera muelleri* and the mangrove *Avicennia marina*, along the coastline of New South Wales, Australia. *Zostera muelleri* (syn. Z. capricornii, Les et al. 2002) is an intertidal seagrass that readily colonizes soft sediments in low energy, coastal systems and provides habitat food and shelter for many invertebrate species (Carruthers et al. 2007, Garthwin et al. 2013). It is a dominant primary producer (Kerr and Strother 1990, Nicastro and Bishop 2013) and widely distributed in temperate and tropical waters of eastern Australia. This species ranges from Daru, Papua New Guinea (9.077°S, 143.208°E) as the northern limit to the Auckland Islands, New Zealand (50.767°S, 166.138°E) as the southern limit (Short et al. 2010, IUCN 2013).

Avicennia marina is a widely distributed mangrove and is tolerant to large variations in environmental conditions. This species is often found in the intermediate to downstream reaches of estuaries where the salinity range is 0-32‰ and frequent tidal inundation is available for successful seed propagation (Robertson and Alongi 1992, Duke et al. 1998). *Avicennia marina* can be found as far north as the Red Sea (27.565°N, 34.416°E) or northern Taiwan (27.733°N, 120.911°E), and is noted for being the most southerly distributed mangrove species, as far south as Merimbula (36.887°S, 149.906°E) in Australia (Duke et al. 2010, IUCN 2013).

To test hypotheses about how: (1) leaf traits of primary producers; (2) rates of detrital decomposition; and (3) effects of detrital decomposition on sediment communities differ according to climate and nutrient enrichment, I conducted a combination of mensurative sampling and experimental manipulations across environmental gradients in climate and nutrient enrichment. Study sites spanned 1000 km of coastline, 7° of latitude and a 4°C difference in temperature. They were situated in estuaries with nutrient inputs that have been significantly enhanced following European settlement, and estuaries that have nutrient regimes that have undergone minimal anthropogenic change (Roper et al. 2011).

In **chapter 2**, I describe sampling in 16 NSW estuaries, at four sampling times, to examine how leaf traits of *Z. muelleri* and *A. marina* vary spatially according to climatic setting and nutrient enrichment, and whether spatial patterns are consistent through time. Given the importance of leaf traits to decomposition processes, it is important to understand the sources of intraspecific spatial and temporal variation in the physico-chemical and morphological properties of leaves. Although ecologists have made significant advances in understanding interspecific leaf trait variability on a global scale (Reich and Oleksyn 2004, Wright et al. 2004), intraspecific variability is of considerable ecological importance, but is often overlooked (but see Boege and Dirzo 2004, Lecerf and Chauvet 2008, Albert et al. 2010, Borer et al. 2013). From numerous large-scale studies across many species (e.g. Reich

and Oleksyn 2004), we know that interspecific leaf trait variability has a strong relationship to temperature, latitude and nutrient enrichment. I expect that I will see similar patterns in intraspecific variation in leaf traits.

Chapters 3 and 4 investigate the relative importance, on the decomposition of leaf litter, of direct effects of environmental setting and indirect effects of environmental setting, arising from effects on leaf traits. In **chapter 3** the decomposition rate of leaf litter from a common source is compared across estuaries differing in climate and in nutrient enrichment using a fully orthogonal design. Differences in rates of decomposition are related both to abiotic differences among the estuaries, and differences in their detritivore communities. I expect that the rate of decomposition will be fastest in estuaries with a warm climate and that are subject to significant anthropogenic nutrient loading than those that are cooler or less nutrient enriched. **Chapter 4** considers how spatial variation in leaf traits, explored in **chapter 2**, contributes to variation in decomposition rates of leaf litter. Specifically, a common garden experiment is conducted where leaf litter of a common species, but from different source sites (and hence with different leaf traits), is transplanted into a common site, and decomposition rates are compared. I expect that leaves from cooler climates will be smaller and tougher, leading to slower decomposition rates. By independently manipulating leaf traits and environmental setting, I am able to tease apart their relative importance in contributing to variation in decomposition rates.

Finally, **chapter 5** investigates whether the effect of detrital enrichment on estuarine sediment communities varies as a function of climatic setting. Although I expect that in all climatic settings, sediment communities will display a response to increasing detrital enrichment that is consistent with Pearson and Rosenberg (1978), I expect that in warmer climates, where rates of biological processes are accelerated, the amount of detrital loading needed to induce anoxia of surface sediments, and hence the induction of an afaunal state, will be lower than in cooler climates.

As our coastal environments continue to be modified by climatic change and coastal development, an understanding of how climate and nutrient regime interact to influence detrital dynamics is essential for predicting the consequences for the structure and function of estuarine ecosystems. Only with an understanding of the mechanisms by which global change modifies ecological systems will we be able to develop appropriate management strategies for allowing their functions to adapt to this change.

References

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. Oikos **79**:439-449.

Albert, C. H., W. Thuiller, N. G. Yoccoz, R. Douzet, S. Aubert, and S. Lavorel. 2010. A multi‐trait approach reveals the structure and the relative importance of intra‐vs. interspecific variability in plant traits. Functional Ecology **24**:1192-1201.

Alongi, D. M. 2002. Present state and future of the world's mangrove forests. Environmental conservation **29**:331-349.

Anderson, N. H., and J. R. Sedell. 1979. Detritus processing by macroinvertebrates in stream ecosystems. Annual review of entomology **24**:351-377.

Barbier, E. B., S. D. Hacker, C. Kennedy, E. W. Koch, A. C. Stier, and B. R. Silliman. 2011. The value of estuarine and coastal ecosystem services. Ecological Monographs **81**:169-193.

Bishop, M. J., M. A. Coleman, and B. P. Kelaher. 2010. Cross-habitat impacts of species decline: response of estuarine sediment communities to changing detrital resources. Oecologia **163**:517-525.

Boege, K., and R. Dirzo. 2004. Intraspecific variation in growth, defense and herbivory in Dialium guianense (Caesalpiniaceae) mediated by edaphic heterogeneity. Plant Ecology **175**:59-69.

Borer, E. T., M. E. Bracken, E. W. Seabloom, J. E. Smith, J. Cebrián, E. E. Cleland, J. J. Elser, W. F. Fagan, D. S. Gruner, and W. S. Harpole. 2013. Global biogeography of autotroph chemistry: is insolation a driving force? Oikos **122**:1121-1130.

Bricker, S. B., B. Longstaf, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2008. Effects of nutrient enrichment in the nation's estuaries: A decade of change. Harmful Algae **8**:21-32.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications **8**:559-568.

Carruthers, T. J. B., W. C. Dennison, G. A. Kendrick, M. Waycott, D. I. Walker, and M. L. Cambridge. 2007. Seagrasses of south–west Australia: A conceptual synthesis of the world's most diverse and extensive seagrass meadows. Journal of Experimental Marine Biology and Ecology **350**:21-45.

Cebrián, J. 1999. Patterns in the Fate of Production in Plant Communities. The American Naturalist **154**:449-468.

Cebrián, J. 2002. Variability and control of carbon consumption, export, and accumulation in marine communities. Limnology and Oceanography **47**:11-22.

Cebrián, J., and C. M. Duarte. 1994. The dependence of herbivory on growth rate in natural plant communities. Functional Ecology:518-525.

Cebrián, J., and C. M. Duarte. 1995. Plant growth-rate dependence of detrital carbon storage in ecosystems. Science **268**:1606-1608.

Conn, C., and J. Dighton. 2000. Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. Soil Biology & Biochemistry **32**:489-496.

Cornwell, W. K., J. H. Cornelissen, K. Amatangelo, E. Dorrepaal, V. T. Eviner, O. Godoy, S. E. Hobbie, B. Hoorens, H. Kurokawa, and N. Pérez‐Harguindeguy. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters **11**:1065-1071.

Coûteaux, M. M., P. Bottner, and B. Berg. 1995. Litter decomposition, climate and litter quality. Trends in ecology & evolution **10**:63-66.

Crain, C. M., K. Kroeker, and B. S. Halpern. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecol Letters **11**:1304-1315.

Cronin, G., and M. E. Hay. 1996. Induction of seaweed chemical defenses by amphipod grazing. Ecology **77**:2287-2301.

Cronin, G., and D. M. Lodge. 2003. Effects of light and nutrient availability on the growth, allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. Oecologia **137**:32-41.

Cummins, K. W., and M. J. Klug. 1979. Feeding ecology of stream invertebrates. Annual Review of Ecology and Systematics:147-172.

Dangles, O., F. Guerold, and P. Usseglio-Polatera. 2001. Role of transported particulate organic matter in the macroinvertebrate colonization of litter bags in streams. Freshwater Biology **46**:575-586.

Davidson, E. A., and I. A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature **440**:165-173.

Davis, J. R., and K. Koop. 2006. Eutrophication in Australian rivers, reservoirs and estuaries–a southern hemisphere perspective on the science and its implications. Hydrobiologia **559**:23-76.

de Jonge, V. N., M. Elliott, and E. Orive. 2002. Causes, historical development, effects and future challenges of a common environmental problem: eutrophication. Hydrobiologia **475**:1-19.

Diaz, R. J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science **321**:926-929.

Doropoulos, C., G. A. Hyndes, P. S. Lavery, and F. Tuya. 2009. Dietary preferences of two seagrass inhabiting gastropods: Allochthonous vs autochthonous resources. Estuarine, Coastal and Shelf Science **83**:13-18.

Duarte, C. M., and J. Cebrián. 1996. The fate of marine autotrophic production. Limnology and Oceanography **41**:1758-1766.

Duke, N. C., M. C. Ball, and J. C. Ellison. 1998. Factors influencing biodiversity and distributional gradients in mangroves. Global Ecology and Biogeography Letters **7**:27-47.

Duke, N. C., K. Kathiresan, S. G. Salmo III, E. S. Fernando, J. R. Peras, S. Sukardjo, T. Miyagi, J. C. Ellison, N. E. Koedam, Y. Wang, J. Primavera, O. Jin Eong, J. Wan-Hong Yong, and V. Ngoc Nam. 2010. *Avicennia marina.in* I. 2012, editor. IUCN Red List of Threatened Species.

Elliott, M., and D. S. McLusky. 2002. The need for definitions in understanding estuaries. Estuarine, Coastal and Shelf Science **55**:815-827.

Enríquez, S., C. M. Duarte, and K. Sand-Jensen. 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia **94**:457-471.

Feller, I. C., D. F. Whigham, J. P. O'Neill, and K. L. McKee. 1999. Effects of nutrient enrichment on within-stand cycling in a mangrove forest. Ecology **80**:2193-2205.

Ferreira, V., A. C. Encalada, and M. A. S. Graça. 2012. Effects of litter diversity on decomposition and biological colonization of submerged litter in temperate and tropical streams. Freshwater Science **31**:945-962.

Gallagher, R. V., and M. R. Leishman. 2012. A global analysis of trait variation and evolution in climbing plants. Journal of Biogeography **39**:1757-1771.

Garthwin, R. G., A. G. Poore, and A. Vergés. 2013. Seagrass tolerance to herbivory under increased ocean temperatures. Marine Pollution Bulletin.

Gessner, M. O., and E. Chauvet. 1994. Importance of Stream Microfungi in Controlling Breakdown Rates of Leaf-Litter. Ecology **75**:1807-1817.

Gessner, M. O., C. M. Swan, C. K. Dang, B. G. McKie, R. D. Bardgett, D. H. Wall, and S. Hättenschwiler. 2010. Diversity meets decomposition. Trends in ecology & evolution **25**:372-380.

Gray, J. S., R. S. S. Wu, and Y. Y. Or. 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. Marine Ecology Progress Series **238**:249-279.

Gulis, V., A. D. Rosemond, K. Suberkropp, H. S. Weyers, and J. P. Benstead. 2004. Effects of nutrient enrichment on the decomposition of wood and associated microbial activity in streams. Freshwater Biology **49**:1437-1447.

Gulis, V., and K. Suberkropp. 2003. Leaf litter decomposition and microbial activity in nutrient‐enriched and unaltered reaches of a headwater stream. Freshwater Biology **48**:123-134.

Harris, G. 2001. A nutrient dynamics model for Australian waterways: land use, catchment biogeochemistry and water quality in Australian rivers, lakes and estuaries. Department of the Environment and Heritage.

Hättenschwiler, S., A. V. Tiunov, and S. Scheu. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology Evolution and Systematics. **36**:191-218.

Hieber, M., and M. O. Gessner. 2002. Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology **83**:1026-1038.

Hobbie, S. E., and P. M. Vitousek. 2000. Nutrient limitation of decomposition in Hawaiian forests. Ecology **81**:1867-1877.

Howarth, R. W., A. Sharpley, and D. Walker. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. Estuaries **25**:656-676.

IPCC. 2013. Summary for Policymakers. *in* Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge, United Kingdom and New York, NY, USA.

IUCN. 2013. IUCN Red List of Threatened Species. Version 2013.2.

Jenkins, C. C., and K. Suberkropp. 1995. The Influence of Water Chemistry on the Enzymatic Degradation of Leaves in Streams. Freshwater Biology **33**:245-253.

Kennish, M. J. 2002. Environmental threats and environmental future of estuaries. Environmental conservation **29**:78-107.

Kerr, E. A., and S. Strother. 1990. Seasonal changes in standing crop of Zostera muelleri in south-eastern Australia. Aquatic Botany **38**:369-376.

Kirschbaum, M. U. F. 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biology & Biochemistry **27**:753-760.

Kirschbaum, M. U. F. 2006. The temperature dependence of organic-matter decomposition—still a topic of debate. Soil Biology and Biochemistry **38**:2510-2518.

Knorr, W., I. C. Prentice, J. I. House, and E. A. Holland. 2005. Long-term sensitivity of soil carbon turnover to warming. Nature **433**:298-301.

Kominoski, J. S., C. M. Pringle, B. A. Ball, M. A. Bradford, D. C. Coleman, D. B. Hall, and M. D. Hunter. 2007. Nonadditive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream. Ecology **88**:1167-1176.

Lecerf, A., and E. Chauvet. 2008. Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. Basic and Applied Ecology **9**:598-605.

Leroy, C. J., and J. C. Marks. 2006. Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. Freshwater Biology **51**:605-617.

Les, D. H., M. L. Moody, S. W. L. Jacobs, and R. J. Bayer. 2002. Systematics of seagrasses (Zosteraceae) in Australia and New Zealand. Systematic Botany **27**:468-484.

Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. Ecology **23**:399-417.

Lovelock, C. E., I. C. Feller, K. L. Mckee, B. M. J. Engelbrecht, and M. C. Ball. 2004. The effect of nutrient enrichment on growth, photosynthesis and hydraulic conductance of dwarf mangroves in Panamá. Functional Ecology **18**:25-33.

Mack, M. C., E. A. Schuur, M. S. Bret-Harte, G. R. Shaver, and F. S. Chapin. 2004. Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. Nature **431**:440-443.

Mann, K. H. 1972. Macrophyte production and detritus food chains in coastal waters. Memorie dell' Istituto Italiano di Idrobiologia **29**:353-383.

Mann, K. H. 1988. Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. Limnology and Oceanography **33**:910-930.

Melillo, J. M., J. D. Aber, and J. F. Muratore. 1982. Nitrogen and Lignin Control of Hardwood Leaf Litter Decomposition Dynamics. Ecology **63**:621-626.

Middleburg, J. and L. Levin. 2009. Coastal hypoxia and sediment biogeochemistry. Biogeosciences Discussions **6**:3655-3706.

Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. de Ruiter, Q. Dong, A. Hastings, N. C. Johnson, K. S. McCann, K. Melville, P. J. Morin, K. Nadelhoffer, A. D. Rosemond, D. M. Post, J. L. Sabo, K. M. Scow, M. J. Vanni, and D. H. Wall. 2004. Detritus, trophic dynamics and biodiversity. Ecology Letters **7**:584-600.

Moore, J. C., and H. W. Hunt. 1988. Resource Compartmentation and the Stability of Real Ecosystems. Nature **333**:261-263.

Nicastro, A., and M. J. Bishop. 2013. Effects of tidal inundation on benthic macrofauna associated with the eelgrass Zostera muelleri. Estuarine, Coastal and Shelf Science **117**:238-247.

Odum, E. P. 1969. The strategy of ecosystem development. Science **164**:262-270.

Odum, E. P., and L. J. Biever. 1984. Resource quality, mutualism, and energy partitioning in food chains. American Naturalist **124**:360-376.

Odum, W. E., J. C. Zieman, and E. J. Heald. 1973. The importance of vascular plant detritus to estuaries. Pages 91-114 *in* Proceedings, second coastal marsh and estuary management symposium. Baton Rouge, Louisiana.

Pace, M. L., J. J. Cole, S. R. Carpenter, J. F. Kitchell, J. R. Hodgson, M. C. Van De Bogert, D. L. Bade, E. S. Kritzberg, and D. Bastviken. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature **427**:240-243.

Pearson, T. H., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environemnt. Oceanography and Marine Biology: Annual Review. **16**:229-311.

Pérez-Harguindeguy, N., S. Díaz, J. H. C. Cornelissen, F. Vendramini, M. Cabido, and A. Castellanos. 2000. Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. Plant and Soil **218/2**:21-30.

Pettit, N. E., T. Davies, J. B. Fellman, P. F. Grierson, D. M. Warfe, and P. M. Davies. 2012. Leaf litter chemistry, decomposition and assimilation by macroinvertebrates in two tropical streams. Hydrobiologia **680**:63-77.

Pinckney, J. L., H. W. Paerl, P. Tester, and T. L. Richardson. 2001. The role of nutrient loading and eutrophication in estuarine ecology. Environmental Health Perspectives **109**:699.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**:289-316.

Reich, P. B., and J. Oleksyn. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proceedings of the National Academy of Sciences of the United States of America **101**:11001-11006.

Robertson, A. I., and D. M. Alongi. 1992. Tropical mangrove ecosystems. American Geophysical Union, Washington, USA.

Robinson, C. T., and M. O. Gessner. 2000. Nutrient addition accelerates leaf breakdown in an alpine springbrook. Oecologia **122**:258-263.

Roper, T., B. Creese, P. Scanes, K. Stephens, R. Williams, J. Dela-Cruz, G. Coade, B. Coates, and M. Fraser. 2011. Assessing the condition of estuaries and coastal lake ecosystems in NSW. Office of Environment and Heritage, Sydney.

Roughan, M., and J. H. Middleton. 2002. A comparison of observed upwelling mechanisms off the east coast of Australia. Continental Shelf Research **22**:2551-2572.

Scanes, P., G. Coade, M. Doherty, and R. Hill. 2007. Evaluation of the utility of water quality based indicators of estuarine lagoon condition in NSW, Australia. Estuarine, Coastal and Shelf Science **74**:306-319.

Schindler, M. H., and M. O. Gessner. 2009. Functional leaf traits and biodiversity effects on litter decomposition in a stream. Ecology **90**:1641-1649.

Schlesinger, W. H. 1977. Carbon balance in terrestrial detritus. Annual Review of Ecology and Systematics **8**:51-81.

Short, F. T., S. L. Williams, T. J. B. Carruthers, M. Waycott, G. A. Kendrick, J. W. Fourqurean, A. Callabine, W. J. Kenworthy, and W. C. Dennison. 2010. *Zostera muelleri*.*in* IUCN, editor. IUCN Red List of Threatened Species.

Silver, W. L., and R. K. Miya. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia **129**:407-419.

Smetacek, V., and J. E. Cloern. 2008. On phytoplankton trends. Science **319**:1346-1348.

Srivastava, D. S., B. J. Cardinale, A. L. Downing, J. E. Duffy, C. Jouseau, M. Sankaran, and J. P. Wright. 2009. Diversity has stronger top-down than bottom-up effects on decomposition. Ecology **90**:1073-1083.

Suberkropp, K., and E. Chauvet. 1995. Regulation of Leaf Breakdown by Fungi in Streams - Influences of Water Chemistry. Ecology **76**:1433-1445.

Swift, M. J., O. W. Heal, and J. M. Anderson. 1979. Decomposition in Terrestrial Ecosystems. University of California Press, Berkeley, CA.

Teal, J. M. 1962. Energy flow in the salt marsh ecosystem of Georgia. Ecology. **43**:614-649.

Udy, J. W., and W. C. Dennison. 1997. Physiological responses of seagrasses used to identify anthropogenic nutrient inputs. Marine and freshwater research **48**:605-614.

Valiela, I., J. McClelland, J. Hauxwell, P.J. Behr, D. Hersh and K. Foreman. 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnology and Oceanography **42**:1105-1118.

Valiela, I., J. M. Teal, S. D. Allen, R. Vanetten, D. Goehringer, and S. Volkmann. 1985. Decomposition in salt marsh ecosystems: the phases and major factors affecting disappearance of above-ground organic matter. Journal of Experimental Marine Biology and Ecology **89**:29-54.

Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry **13**:87-115.

Wallace, J. B., and J. R. Webster. 1996. The role of macroinvertebrates in stream ecosystem function. Annual review of entomology **41**:115-139.

Walther, G. R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. Beebee, J. M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. Nature **416**:389-395.

Wardle, D. A. 2006. The influence of biotic interactions on soil biodiversity. Ecology Letters **9**:870-886.

Webster, J. R., and E. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology and Systematics **17**:567-594.

Wetzel, R. G. 1983. Limnology, 2nd edition. Saunders College Publishing., Philadelphia, PA.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M. L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature **428**:821-827.

Zhang, D. Q., D. F. Hui, Y. Q. Luo, and G. Y. Zhou. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. Journal of Plant Ecology **1**:85-93.

2. LARGE-SCALE INTRASPECIFIC PATTERNS OF VARIABILITY IN LEAF TRAITS DIFFER BETWEEN TWO CO-OCCURRING ESTUARINE ANGIOSPERMS.

Lara Ainley[1]*, *Adriana Verges*[2], *Melanie Bishop*[1]

1 Department of Biological Sciences, Macquarie University, North Ryde NSW, Australia

2 School of Biological, Earth and Environmental Sciences, The University of New South Wales, UNSW Sydney NSW, Australia

* Corresponding Author: Lara Ainley, Ph: +612 9850 6285

Email addresses: lara.ainley@students.mq.edu.au (L. Ainley),

a.verges@unsw.edu.au (A. Vergés),

melanie.bishop@mq.edu.au (M. Bishop)

Running headline: Intraspecific vs. interspecific leaf traits

Summary

1. Studies seeking to identify sources of variability and trade-offs in leaf traits have done so by assembling large databases of traits, across species and time points. It is unclear to what extent spatial patterns derived in such a manner apply to individual species, and the extent to which spatial patterns vary temporally.
2. We assessed whether the leaf traits of two species, the mangrove *Avicennia marina* and the eelgrass *Zostera muelleri*, display similar patterns of intraspecific variability across gradients of latitude and nutrient enrichment, and the persistence of these spatial patterns through time.
3. Of five leaf traits examined, only carbon to nitrogen ratio and mechanical elasticity displayed latitudinal patterns that were consistent between the two plant species, and with previously reported interspecific patterns of variation. Specific leaf area, leaf toughness and total phenolics, by contrast, displayed species-specific patterns that varied markedly through time.
4. Nutrient effects on leaf traits were highly temporally variable, and also displayed markedly different patterns of intraspecific variability between the two species.
5. *Synthesis:* Overall, spatio-temporal patterns of intraspecific variability in leaf traits differed markedly between the two co-occurring estuarine angiosperms considered. Hence interspecific patterns in leaf traits, derived from amalgamated data sets cannot be assumed to reflect underlying intraspecific patterns of variation, and may not have ecologically straightforward interpretation.

Keywords: seagrass; mangrove; estuary; Australia; environmental gradients

Introduction

Ecologists have for many years recognized the role of leaf traits in mediating ecosystem functioning (Díaz and Cabido, 2001, Lavorel and Garnier, 2002). Leaves are the key sites of carbon fixation for plants and, as considerable repositories of carbon and nitrogen, play a critical role in the biogeochemical cycling of a variety of macronutrients through grazing and detrital pathways (Chapin, 2003, Doney et al., 2012). Physico-chemical traits, such as the concentration of unpalatable phenolics, leaf toughness and carbon and nitrogen contents, influence decomposition processes and the palatability of leaves to consumers (Cornelissen and Thompson, 1997, Enríquez et al., 1993, Pérez-Harguindeguy et al., 2000, Hättenschwiler and Vitousek, 2000). Structural attributes such as leaf area, density and shape can influence how leaves modify the abiotic environment, for example through attenuation of light, wind or currents (Ellsworth and Reich, 1993, Fonseca and Cahalan, 1992, Peterson et al., 2004). Consequently, an understanding of those factors that determine and constrain functional leaf traits is of fundamental importance to ecology.

The morphological, chemical and physical traits of leaves vary both within and among species (Ackerly and Cornwell, 2007, Reich et al., 1999, Albert et al., 2010a). The variability in leaf traits may reflect genetic diversity among individuals, evolutionary constraints of species or phenotypic responses to the abiotic and biotic environment (Albert et al., 2010a, Díaz and Cabido, 1997). Responses of leaf traits to the environment may be due to direct or indirect consequences of allocation trade-offs imposed by resource availability (Lavorel and Garnier, 2002). Studies seeking to identify sources of variability and trade-offs in leaf traits have primarily done so by assembling large databases of traits. These are often based on means acquired across multiple studies and using approaches such as multiple regressions to look for inter-relationships (e.g. TRY, Kattge et al., 2011). Although this cross-species approach has greatly enhanced our understanding of how leaf traits vary on a global scale (e.g. Reich and Oleksyn, 2004, Wright et al., 2004, Moles et al., 2011), it gives

an incomplete description of intraspecific variation in leaf traits at smaller scales (Albert et al., 2010b). Pooling species together may mask patterns at the intraspecific level and even reverse the direction of relationships (Shea and Chesson, 2002). Moreover, meta-analyses that combine data collected over many years may produce outcomes that are ecologically very difficult to interpret (Körner, 2007). Given that intraspecific, as well as interspecific, variation in leaf traits can have large effects on ecosystem function (e.g. Boege and Dirzo, 2004, Lecerf and Chauvet, 2008), there is need to reconcile the relationship of intraspecific to interspecific variation in traits.

In terrestrial environments, temperature and soil nutrient status have been identified among key correlates of leaf traits (Ordoñez et al., 2009). Temperature influences the rate at which metabolic processes such as photosynthesis and respiration proceed (Berry and Raison, 1981), such that plants growing in cooler climates display reduced investment in shoots and leaves, and have thicker leaves with less leaf area per unit mass, than plants growing in warmer climates (Potter and Jones, 1977, Loveys et al., 2002). In warmer climates, leaf carbon to nitrogen ratio (C:N) is generally greater than in cooler climates, possibly because cool temperatures limit carbon uptake more than they limit nitrogen uptake, or because in tropical climates, soils are generally older with lower nitrogen contents (Aerts, 1997, Reich and Oleksyn, 2004). Consequently, interspecific gradients of decreasing specific leaf area and decreasing C:N ratio with increasing latitude are often apparent (Hulshof et al., 2013, Reich and Oleksyn, 2004).

Plant species that grow in nutrient-rich environments produce large amounts of nutrient-rich litter, whereas species that occupy nutrient-poor environments produce less litter of lower nutrient content and higher defensive chemical concentration, as a strategy that enables the nutrients to be conserved in long-lived leaves (Melillo et al., 1982, Hobbie, 1992, Berendse, 1994, Crews et al., 1995, Aerts and Chapin, 2000). Land clearing, the production and application of fertilizer, discharge of human waste and combustion of fossil fuels are

producing ongoing nutrient enrichment of coastal waterways (Nixon, 1995, Cloern, 2001). These relationships have, however, primarily been derived from biogeographic comparisons among species or from manipulative experiments at small scales (Feller, 1995, Ordoñez et al., 2009). It is unclear how temperature and nutrient enrichment interact to influence intraspecific variation in leaf traits at large scales, particularly among aquatic angiosperms that have received far less research attention than their terrestrial counterparts (but see Duarte, 1990, Feller, 1995, Lovelock et al., 2004, Udy and Dennison, 1997).

Here, we assessed whether two widely distributed and co-occurring species, the eelgrass *Zostera muelleri* and the grey mangrove *Avicennia marina*, displayed similar patterns of intraspecific variability in key functional leaf traits across a climatic gradient of 7° of latitude on the temperate east Australian coast and between estuaries varying in the degree that they have been modified by anthropogenic nutrient input. Both temperature and nutrient loading are presently undergoing major anthropogenic modification and manipulative experiments have demonstrated a causative influence on leaf traits. Specifically, we tested the null hypotheses that (1) the two species would display similar patterns of spatio-temporal variation in leaf traits to one another, (2) intraspecific variation in leaf traits would display the same pattern with respect to climate and nutrient enrichment as interspecific variation in leaf traits, described by other studies (see Reich and Oleksyn, 2004, Wright et al., 2004), and (3) the key environmental correlates of leaf traits would be similar for the two species. Our study focused on five leaf traits – specific leaf area, C:N, toughness, elasticity and total phenolics – that are important in determining the palatability of leaves to herbivores and detritivores (Enríquez et al., 1993, Cornelissen and Thompson, 1997, Pérez-Harguindeguy et al., 2000, Hättenschwiler and Vitousek, 2000).

Materials and methods

EXPERIMENTAL DESIGN

To investigate sources of spatial variability in the leaf traits of the seagrass *Zostera muelleri* and the mangrove *Avicennia marina*, and to assess whether these vary seasonally, a field survey was conducted every six months, in the summer and winter of 2011 and 2012. During each survey, leaf traits and environmental variables were sampled in sixteen estuaries along the eastern coastline of Australia, spanning approximately 7° of latitude, and at roughly equal distances apart within this range (Fig. 1). Estuaries were classified based on a ratio of present day (measured) to pre-European settlement (modeled) total nitrogen loading (TN; Roper et al., 2011) that is an indicator of the level of human impact/modification influencing the estuaries since European settlement. Eight of the 16 estuaries were classified as being largely unmodified by humans, on the basis of their low ratio (TN < 1.5) and the other eight estuaries were classified as being highly modified by human impacts based on higher ratios (TN > 2.5). All were classified as intermediate to mature, wave-dominated estuaries or embayments (Roy et al., 2001), with an average tidal range of 1.5 m, predominantly open entrance conditions and with known populations of *Z. muelleri* and *A. marina* (Roper et al., 2011).

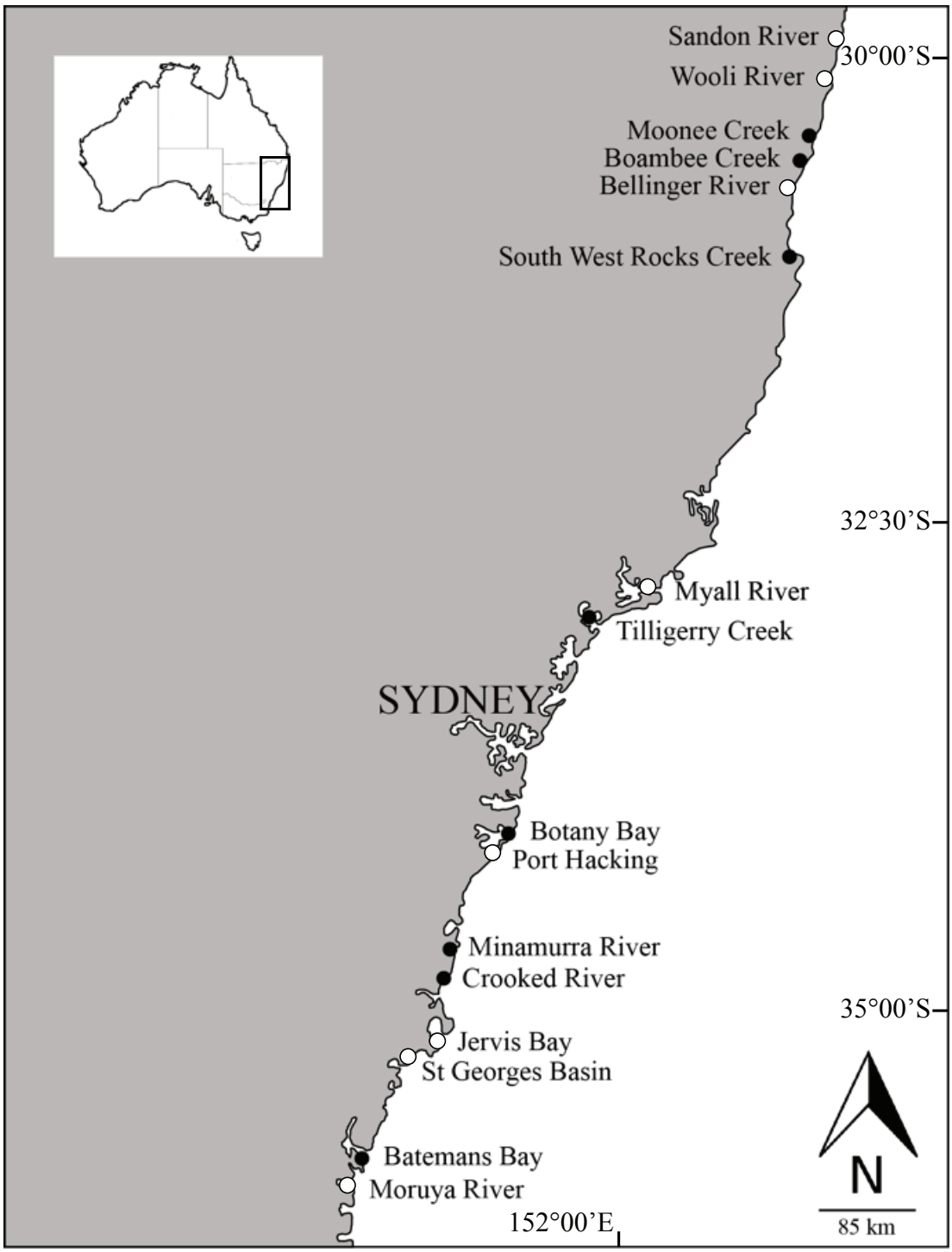

Fig. 1 Distribution of highly modified (black circles) and largely unmodified (white circles) field sites (n = 16) across the east coast of Australia.

DATA COLLECTION

Within each estuary, sampling of leaf traits and environmental variables was done in each of ten replicate sites. Each site was approximately 25 m^2 in area and separated from other sites by at least 10 m. At each site, five of the outer blades of *Z. muelleri* shoots were collected from an intertidal elevation of 0 – 0.3 m relative to Mean Spring Low Water (MSLW), and five leaves of *A. marina* were collected from an elevation of 0.6 – 1 m above MSLW. For each species, the samples came from different randomly selected plants, and comprised older and mature leaves. At each site we also quantified *Z. muelleri* shoot density by counting the number of shoots present within a single randomly positioned 10 cm diameter core (area = 78.5 cm^2) and *A. marina* density by counting the number of trunks present within a 5 x 5 m area. At each site and at each sampling date, measurements of temperature (°C), salinity (ppt) and pH were taken from the top 25 cm of 10-50 cm deep water using a hand-held water quality probe (CyberScan PC 10, Eutech Instruments, Singapore). To obtain continuous measurements of temperature (°C), a single data logger programmed to collect readings every 2 h was deployed in each estuary at a single location, attached to a fixed substrate/object, close to the group of sites for 12 months from August 2011 – August 2012 in water 0.1 – 0.5 m below MSLW. Other environmental variables for each estuary were available from Roper et al. (2011; Table 1).

(Table 1)
[1] From published data (Roper et al. 2011).
[2] Estuaries were classified as either highly modified or largely unmodified on the basis of their present day (measured) to pre-European settlement (modeled) total nitrogen loading (Highly modified: TN > 2.5; largely unmodified: TN < 1.5).

Table 1. Environmental conditions within each of the sixteen estuaries sampled. Where available (indicated with *) temperature (Temp. °C) was obtained from data loggers measuring continuously, otherwise temperature, salinity and pH were measured *in situ* and averaged over all summer or winter months. Shoot densities for *Zostera muelleri* (ZM) and trunks densities for *Avicennia marina* (AM) are presented. Day-length calculations were based on latitude and mean Julien day during summer and winter months.

			Estuarine Characteristics[1]				Mean (± SE) summer conditions (Dec/Jan/Feb)						Mean (± SE) winter conditions (Jun/Jul/Aug)					
Estuary	**Latitude**	**Estuarine[2] Condition**	**TN[2] ratio**	**Area (km²)**	**Flushing Time (d)**	**Dilution Factor**	**Temp. (°C)**	**pH**	**Salinity (ppt)**	**Day Length (h)**	**ZM Shoot Density**	**AM Trunk Density**	**Temp. (°C)**	**pH**	**Salinity (ppt)**	**Day Length (h)**	**ZM Shoot Density**	**AM Trunk Density**
Sandon River	-29.673	Largely Unmodified	1.02	2.62	2.34	0.83	* 24.72 ± 0.07	8.01 ± 0.07	15.82 ± 3.01	11.65	12.26 ± 1.27	3.58 ± 0.37	* 17.22 ± 0.06	7.92 ± 0.18	33.21 ± 0.40	11.50	16.20 ± 3.22	3.84 ± 0.46
Wooli Wooli River	-29.888	Largely Unmodified	1.06	3.75	6.15	0.92	* 24.37 ± 0.07	6.45 ± 0.08	18.87 ± 3.84	11.64	9.05 ± 1.27	2.09 ± 0.17	* 17.92 ± 0.05	8.13 ± 0.14	34.75 ± 1.10	11.49	11.65 ± 1.31	2.45 ± 0.37
Moonee Moonee Creek	-30.212	Highly Modified	4.30	0.41	5.02	0.33	* 24.84 ± 0.06		17.10 ± 1.64	11.63	6.35 ± 1.08	3.79 ± 0.29	* 18.16 ± 0.11	8.23 ± 0.17	34.00 ± 0.54	11.48	9.40 ± 1.47	3.25 ± 0.36
Boambee Creek	-30.355	Highly Modified	10.54	0.99	2.39	0.22	* 24.44 ± 0.09	7.65 ± 0.06	28.40 ± 8.41	11.63	9.65 ± 1.46	6.50 ± 0.51	* 18.11 ± 0.21	8.12 ± 0.04	38.82 ± 1.13	11.47	13.45 ± 1.90	6.70 ± 0.80
Bellinger River	-30.502	Largely Unmodified	1.32	8.16	3.73	0.16	22.55 ± 0.35		15.40 ± 2.91	11.62	10.20 ± 0.88	4.85 ± 0.48	15.3 ± 0.21	7.50 ± 0.08	15.50 ± 1.01	11.47	11.45 ± 2.09	5.75 ± 1.01
South West Rocks Creek	-30.883	Highly Modified	2.61	0.94	1.09	0.91	* 24.88 ± 0.10	8.13 ± 0.03	21.14 ± 3.18	11.61	6.90 ± 1.38	5.00 ± 0.56	* 17.47 ± 0.12	8.12 ± 0.01	38.00 ± 0.58	11.45	8.95 ± 1.73	4.45 ± 0.39
Myall River	-32.671	Largely Unmodified	1.42	115.20	15.97	15.23	22.83 ± 0.28	8.15 ± 0.03	24.20 ± 3.63	11.55	11.75 ± 1.26	6.00 ± 0.37	13.88 ± 0.19	7.15 ± 0.11	8.70 ± 0.10	11.36	12.20 ± 1.24	3.35 ± 0.41
Tilligerry Creek	-32.728	Highly Modified	3.81	134	36.15	25.79	* 23.90 ± 0.05	7.86 ± 0.23	23.80 ± 3.32	11.54	17.21 ± 4.53	5.55 ± 0.33	* 14.99 ± 0.05			11.35	14.67 ± 1.03	5.50 ± 0.82
Botany Bay	-34.001	Highly Modified	6.24	39.55	35.27	21.07	* 23.24 ± 0.06	8.02 ± 0.05	21.59 ± 3.24	11.50	43.65 ± 1.13	5.20 ± 0.43	* 14.99 ± 0.04	6.87 ± 0.36	29.99 ± 0.60	11.28	44.35 ± 6.48	6.65 ± 1.38
Port Hacking	-34.073	Largely Unmodified	1.47	11.70	29.08	34.83	21.67 ± 0.63	7.43 ± 0.02	21.90 ± 3.65	11.49	9.60 ± 1.13	3.55 ± 0.31	14.17 ± 0.17	5.84 ± 0.41	33.58 ± 0.47	11.28	14.65 ± 1.47	4.20 ± 0.41
Minnamurra River	-34.628	Highly Modified	2.58	1.86	1.04	0.08	22.40 ± 0.09	7.72 ± 0.06	23.13 ± 3.20	11.47	10.65 ± 1.90	8.15 ± 0.34	12.63 ± 0.13	7.41 ± 0.21	21.71 ± 3.36	11.25	10.20 ± 1.38	7.15 ± 0.84
Crooked River	-34.773	Highly Modified	3.19	0.28	2.17	0.12	22.88 ± 0.30	7.45 ± 0.07	18.10 ± 3.80	11.47	8.30 ± 1.43	6.75 ± 0.22	12.44 ± 0.05	7.85 ± 0.04		11.24	8.00 ± 2.47	7.20 ± 0.83
Jervis Bay	-35.104	Largely Unmodified	1.44	123.89	54.38	217.03	* 22.50 ± 0.06	8.12 ± 0.08	22.83 ± 3.25	11.45	11.70 ± 1.48	4.85 ± 0.31	* 14.68 ± 0.04	7.71 ± 0.27	22.40 ± 3.50	11.22	11.40 ± 0.96	8.10 ± 1.82
St Georges Basin	-35.185	Largely Unmodified	1.40	40.91	288.33	19.80	23.12 ± 0.35	8.00 ± 0.12	20.27 ± 3.26	11.45	16.90 ± 4.46	3.70 ± 0.23	11.98 ± 0.36	7.53 ± 0.22	14.59 ± 2.28	11.22	12.00 ± 1.83	3.90 ± 0.61
Batemans Bay	-35.757	Highly Modified	2.68	34.48	37.81	7.27	* 22.83 ± 0.08	7.86 ± 0.05	21.07 ± 2.62	11.43	10.00 ± 2.17	3.53 ± 0.21	* 13.00 ± 0.08	7.47 ± 0.23	21.74 ± 3.07	11.18	6.25 ± 1.07	3.05 ± 0.32
Moruya River	-35.906	Largely Unmodified	1.40	6.14	4.41	0.26	20.42 ± 0.32	8.34 ± 0.03	20.95 ± 2.69	11.42	17.60 ± 5.04	3.35 ± 0.29	13.83 ± 0.18	7.45 ± 0.24	22.59 ± 3.19	11.17	8.90 ± 1.11	3.55 ± 0.78

MEASURING LEAF TRAITS

The biomechanical, morphological, and chemical traits of each leaf were measured in the laboratory. To quantify the size and shape of leaves, we measured the maximal width and thickness of each leaf (to the nearest 0.01 mm) using digital calipers and the length (to the nearest 1 mm) of each leaf, from shoot base (defined by a growth scar) to apex for *Z. muelleri* and from petiole to leaf tip for *A. marina* (Cornelissen et al., 2003), using a ruler. The total area of the five leaves per site was measured (to the nearest 0.001 cm^2) using a leaf area meter (LI-3100C, Li-Cor Inc., Nebraska, USA). Specific Leaf Area (SLA) was calculated on the groups of five leaves per site by dividing their total leaf area by their dry weight (see methods below).

The maximum load (N) that could be sustained by each leaf prior to failure, and the corresponding extension (mm) was assessed using an Instron Universal Testing System (5542, INSTRON, Massachusetts, USA). To enable calculation of these metrics, leaves were placed lengthways between two pneumatic clamps that gradually separated at a rate of 0.17 $mm.s^{-1}$ until the leaf broke. The distance between the pneumatic clamps was adjusted to be appropriate for all leaves within each site. Force and extension were recorded every 0.1 s during each trial. The load (*N*) and leaf extension (*ex*) at failure were used to calculate the ultimate tensile stress (UTS, eqn. 1, where *t* is the leaf thickness and *w* width), elasticity (E, eqn. 2, where *gl* is the gauge length between the two pneumatic clamps) and toughness (the energy that the leaf can absorb before breakage, calculated as the area under the force by extension curve using the trapezoidal rule, "trapz" function, within the package "pracma"; Borchers, 2013):

$$UTS = N/t.w \quad \text{eqn. 1}$$

$$E = UTS.(gl/ex) \quad \text{eqn. 2}$$

All calculations were conducted using R (R Core Team, 2013).

In order to assess the chemical traits of leaves, leaf parts were collected from the biomechanical testing, pooled across the five leaves per site to produce 10 samples per estuary, and dried at 60°C for 3-4 days until constant weight, and then crushed and ground into a fine powder using a planetary ball mill (MM400, RETSCH, Haan, Germany). The carbon and nitrogen content was assessed for five 2.5-3.5 mg sub-samples per estuary, obtained by pooling leaves across pairs of sites, using an elemental analyser (CHN-900, Leco, Michigan, USA). The concentration of phenolic compounds in the leaf material for each of the ten samples per estuary was determined using a Folin-Ciocalteu colormetric assay followed by spectrophotometry (Ainsworth and Gillespie, 2007, Singleton et al., 1999). Specifically, sub-samples of 4-4.5 mg of ground material were added to 1 mL of methanol (50% CH_3OH:H_2O) and incubated at 4°C for 24 h. Next, 0.5 mL Folin-Ciocalteu phenol reagent and 1.5 mL sodium carbonate (20% Na_2CO_3) were added to 0.1 mL of extract and incubated for a further 2 h at room temperature. Absorbance at 765 nm was measured using a spectrophotometer. The total phenolic content of the samples was calculated using eqn. 3 (where abs^{765} is the spectral absorbance at 765 nm, *int* and *sl* are the y-intercept and slope of the standard linear relationship between phenol concentration and absorbance, and *w* is the sample mass in mg):

$$\text{Total phenolics} = (10.abs^{765} - int)/sl.w \qquad \text{eqn. 3}$$

STATISTICAL ANALYSES

Sources of spatio-temporal variability in individual leaf traits were examined using permutational analyses of variance (PERMANOVA, PRIMER v6; Anderson et al., 2007). PERMANOVAs use permutations to partition the variation among individual and interaction terms using a dissimilarity matrix as the data input (in this instance Euclidean distance matrices). In contrast to ANOVAs, PERMANOVAs make no assumptions about the underlying distribution of the variables (Anderson, 2005). The PERMANOVAs included the

factors: year (random, two levels: 2011, 2012), season (fixed, two levels: summer, winter), estuarine condition (fixed, two levels: highly modified, largely unmodified) and the co-variate latitude. Separate analyses were run on elasticity, toughness, SLA, carbon to nitrogen ratio (C:N) and total phenolic concentration with ten replicates per estuary, except for C:N samples for which there were five replicates per estuary. Where insufficient unique permutations were generated, p-values were calculated using the Monte Carlo method (Anderson et al., 2008). Where factors were statistically significant ($\alpha < 0.05$), the PERMANOVAs were followed by pairwise *a posteriori* tests to identify sources of difference.

To assess the contribution of the environmental variables to the variation observed in the multivariate data set comprising the five leaf traits (i.e. elasticity, toughness, SLA, C:N and total phenolics), we ran a multivariate multiple regression (DistLM, Anderson et al., 2008) and distance-based redundancy analysis (db-RDA, McArdle and Anderson, 2001). The multivariate multiple regression identified significant relationships by fitting linear models between Euclidean distance matrices for individual environmental variables and for the full set of leaf traits. Db-RDA was used to visually represent the relationship between the subset of environmental predictors that were identified using AIC selection criteria (Burnham and Anderson, 2004) in the BEST procedure of PRIMER (Clarke and Warwick, 2001) to be most tightly correlated with the leaf traits, and the leaf traits themselves. Analyses were run on estuary averages of leaf traits and of environmental variables (n = 16). Log_{10} transformations were applied to flushing time and dilution factor to reduce skewness.

To evaluate relationships among leaf traits across the study sites, we ran Pearson's correlations between pairs of traits. Due to the differing levels of replication among variables, these analyses used average values of each trait for a given estuary at each sampling time (n = 64). *Zostera muelleri* leaves were not collected from Tilligerry Creek and Minnamurra River, and leaves of both species were not collected from Crooked River, during

the winter 2012 collection due to inaccessibility to sites and extreme weather conditions, resulting in imbalances of the data for statistical analyses.

Results

SOURCES OF SPATIO-TEMPORAL VARIATION IN LEAF TRAITS

All of the five leaf traits displayed spatial variability with respect to latitude in one or both of the species considered (Table 2, Fig. 2). Both *Z. muelleri* and *A. marina* leaves increased in mechanical elasticity and C:N as latitude decreased (Fig. 2a-b, g-h). This relationship was evident irrespective of estuarine condition (Table 2). Similarly, the toughness of *Z. muelleri* leaves, increased towards lower latitudes (Fig. 2c), in both modified and unmodified estuaries. By contrast, no relationship between toughness and latitude was found for *A. marina* leaves (Fig. 2d). Specific leaf area decreased towards lower latitudes for *A. marina* leaves in 2011 but not in 2012 (Fig. 2f) and displayed no relationship with latitude for *Z. muelleri* blades. Total phenolic concentration in *A. marina* leaves decreased weakly towards lower latitudes (Fig. 2j) but displayed no relationship with latitude for *Z. muelleri* blades.

Table 2. Permuted analysis of co-variance (PERM-ANCOVA) for *Zostera muelleri* and *Avicennia marina* leaf traits. Model includes four factors: 1) Latitude (the covariate, random); 2) Year (random, two levels: 2011, 2012); 3) Season (fixed, two levels: summer, winter); and 4) Estuary Condition (fixed, two levels: highly modified, largely unmodified). Where there were insufficient unique permutations (*), Monte-Carlo p-values were interpreted. Due to lost/missing data, this dataset was unbalanced resulting in variation among residual degrees of freedom.

Zostera muelleri		**Elasticity (MPa)**			**Toughness (N.mm)**			**SLA ($cm^2.g^{-1}$)**			**C:N**			**Total Phenolics (%)**		
Source	**df**	**MS**	**p-F**	**p-value**	**MS**	**p-F**	**p-value**	**MS**	**p-F**	**p-value**	**MS**	**p-F**	**p-value**	**MS**	**p-F**	**p-value**
Latitude	1	129740	63.02	**0.001**	1388	148.6	**0.001**	960	0.5	0.451	1325	170.19	**0.001**	0.27	0.13	0.718
Year (ye)	1	< 1	< 0.01	0.991	92	9.9	**0.003**	239600	135.2	**0.001**	< 1	< 0.01	0.959	43.37	22.00	**0.001**
Season (se) *	1	11703	1.07	0.486	37	0.1	0.851	214200	5.9	0.257	885	98.00	0.064	44.59	17.93	0.160
Estuarine Condition (ec) *	1	54585	2.71	0.381	78	5.3	0.260	11373	0.9	0.502	21	0.28	0.666	0.01	< 0.01	0.997
ye x se	1	10872	5.28	**0.024**	762	82.4	**0.001**	36217	20.4	**0.001**	9	1.16	0.280	2.49	1.26	0.286
ye x ec	1	20008	9.71	**0.004**	15	1.6	0.191	12820	7.2	**0.008**	76	9.79	**0.004**	4.01	2.03	0.143
se x ec	1	12265	3.03	0.313	20	29.7	0.187	931	1.6	0.435	12	46.70	0.214	6.56	163.38	0.125
ye x se x ec	1	4036	1.96	0.154	1	0.1	0.782	582	0.3	0.566	< 1	0.03	0.861	0.04	0.02	0.883
		1211900	Residual df = 588		5432	Residual df = 588		1771	Residual df = 593		8	Residual df = 279		1.97	Residual df = 553	

Avicennia marina		**Elasticity (MPa)**			**Toughness (N.mm)**			**SLA ($cm^2.g^{-1}$)**			**C:N**			**Total Phenolics (%)**		
Source	**df**	**MS**	**p-F**	**p-value**	**MS**	**p-F**	**p-value**	**MS**	**p-F**	**p-value**	**MS**	**p-F**	**p-value**	**MS**	**p-F**	**p-value**
Latitude	1	650	83.34	**0.001**	8	0.01	0.928	6625	87.3	**0.001**	779	102.1	**0.001**	56.8	22.4	**0.001**
Year (ye)	1	551	71.05	**0.001**	2804	2.83	0.102	7475	99.6	**0.001**	219	29.3	**0.001**	57.7	22.9	**0.001**
Season (se) *	1	20	1.17	0.502	1291	4.21	0.300	15998	0.9	0.531	105	133.7	0.055	68.4	16.1	0.164
Estuarine Condition (ec) *	1	26	16.04	0.154	2362	21.44	0.141	633	835.2	**0.031**	2	21.4	0.146	4.8	0.4	0.666
ye x se	1	17	2.22	0.137	306	0.31	0.583	18534	247.0	**0.001**	1	0.1	0.759	4.2	1.7	0.205
ye x ec	1	2	0.21	0.633	110	0.11	0.754	1	< 0.1	0.925	< 1	< 0.1	0.910	11.8	4.7	**0.028**
se x ec	1	< 1	0.01	0.845	3934	0.51	0.635	6	0.2	0.746	< 1	< 0.1	0.808	0.3	< 0.1	0.724
ye x se x ec	1	46	5.99	**0.014**	7742	7.83	**0.010**	43	0.6	0.462	14	1.9	0.153	11.4	4.5	**0.033**
		8	Residual df = 613		989	Residual df = 613		75	Residual df = 612		7	Residual df = 303		2.5	Residual df = 592	

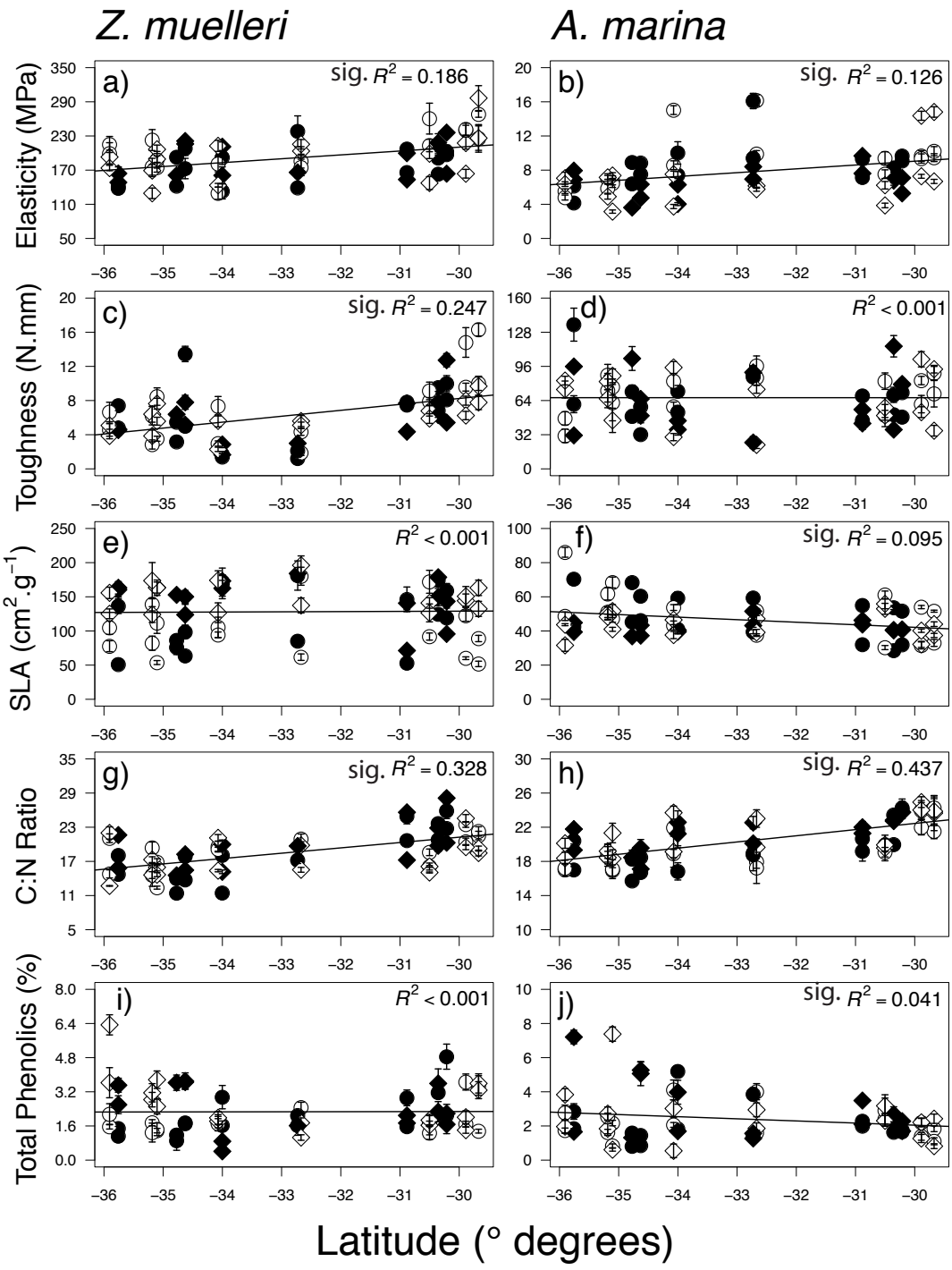

Fig. 2 Latitudinal variation in the leaf traits of *Zostera muelleri* (a, c, e, g, i) and *Avicennia marina* (b, d, f, h, j) within highly modified (black symbols) and largely unmodified (white symbols) estuaries of New South Wales, Australia. Points represent mean (± SE) trait values per estuary (n=10) at each of the four sampling times (giving a total of n = 64). Samples collected during 2011 are represented by a circle and samples collected during 2012 are represented by a diamond. 'Sig.' denotes instances where the R2 value for the relationship between latitude and the variable of interest was significant at $\alpha = 0.05$.

Few differences in leaf traits were apparent between highly modified and largely unmodified estuaries, and where these did occur, they were generally ephemeral (Table 2). Mangrove SLA was the only leaf trait, of either species, to display a main effect of estuarine condition, with a significantly smaller SLA in highly modified than largely unmodified estuaries (Table 2). Seagrass elasticity, SLA and C:N showed significant interactions between year and estuarine condition (see 'yexec', Table 2). In 2011, seagrass leaves were less elastic and had greater SLA in highly modified than largely unmodified estuaries, but in 2012 no significant difference in these traits was evident between estuarine conditions. By contrast, C:N was greater in highly modified than largely unmodified estuaries in 2012, but displayed no significant difference in 2011. Elasticity, toughness and total phenolics of *A. marina* leaves showed significant interactions between year, season and estuarine condition (see 'yexsexec', Table 2). In 2011, when leaves were more elastic, tougher and contained lower concentrations of phenolics than in 2012, elasticity was significantly less in highly modified than largely unmodified estuaries in the winter, and concentration of phenolics were similarly significantly lower in highly modified than largely unmodified estuaries in the summer, the season when concentrations were generally lowest. During 2012, patterns were reversed with greater elasticity in highly modified than largely unmodified estuaries in both seasons, and greater concentrations of phenolics in highly modified than largely unmodified estuaries in summer. Differences between estuarine conditions in the toughness of mangrove leaves were only detected in 2012, with weaker leaves in highly modified than largely unmodified estuaries during winter months and greater leaf toughness in highly modified estuaries during summer.

KEY ENVIRONMENTAL CORRELATES OF LEAF TRAITS

Two of nine environmental variables were significantly correlated to *Z. muelleri* leaf traits (Table 3a-b). Flushing time (*Pseudo-F* = 3.95; *P* = 0.033) explained 22%, and day-

length (*Pseudo-F* = 4.46; *P* = 0.025) explained 24% of spatio-temporal variability in *Z. muelleri* leaf traits (Table 3a). Flushing time was also marginally correlated with *A. marina* leaf traits (*Pseudo-F* = 2.89; *P* = 0.083), explaining 17% of the variability (Table 3b).

The overall combination of environmental variables that was most closely correlated to *Z. muelleri* leaf traits collectively explained 65.4% of the variability (R^2 = 0.654, AIC = 102.84, BEST procedure, PRIMER) and included the TN ratio (the ratio of measured to modeled total nutrient loading), flushing time, day-length and *Z. muelleri* shoot density (Fig. 3a). The overall combination of environmental variables that was most closely correlated to *A. marina* leaf traits collectively explained 31.9% of the variability (R^2 = 0.319, AIC = 74.29, BEST procedure, PRIMER) and included the flushing time and temperature (Fig. 3b).

Table 3. Results of multivariate multiple regression analyses (DistLM) determining relationships between environmental variables and leaf traits of (a) the seagrass *Zostera muelleri*, and (b) the mangrove *Avicennia marina*. Prop = the proportion of variability explained by each trait.

a) *Zostera muelleri*			
Variable	**Pseudo-F**	**P-value**	**Prop.**
TN ratio[1]	2.755	0.084	0.164
Estuary Area (km^2)	1.271	0.279	0.083
log (flushing time)	3.950	**0.033**	0.220
log (dilution factor)	2.303	0.118	0.141
Temperature (°C)	2.255	0.123	0.139
pH	1.106	0.324	0.073
Salinity (ppt)	0.210	0.816	0.015
Daylength (h)	4.464	**0.025**	0.242
Shoot Density	2.511	0.095	0.152

b) *Avicennia marina*			
Variable	**Pseudo-F**	**P-value**	**Prop.**
TN ratio	0.540	0.600	0.037
Estuary Area (km^2)	0.832	0.429	0.056
log (flushing time)	2.890	0.083	0.171
log (dilution factor)	0.811	0.423	0.055
Temperature (°C)	2.453	0.115	0.149
pH	0.186	0.853	0.013
Salinity (ppt)	1.863	0.173	0.117
Daylength (h)	2.334	0.120	0.143
Trunk Density	2.434	0.111	0.148

[1] TN ratio = ratio of present day (measured) to pre-European settlement (modeled) total nitrogen loading.

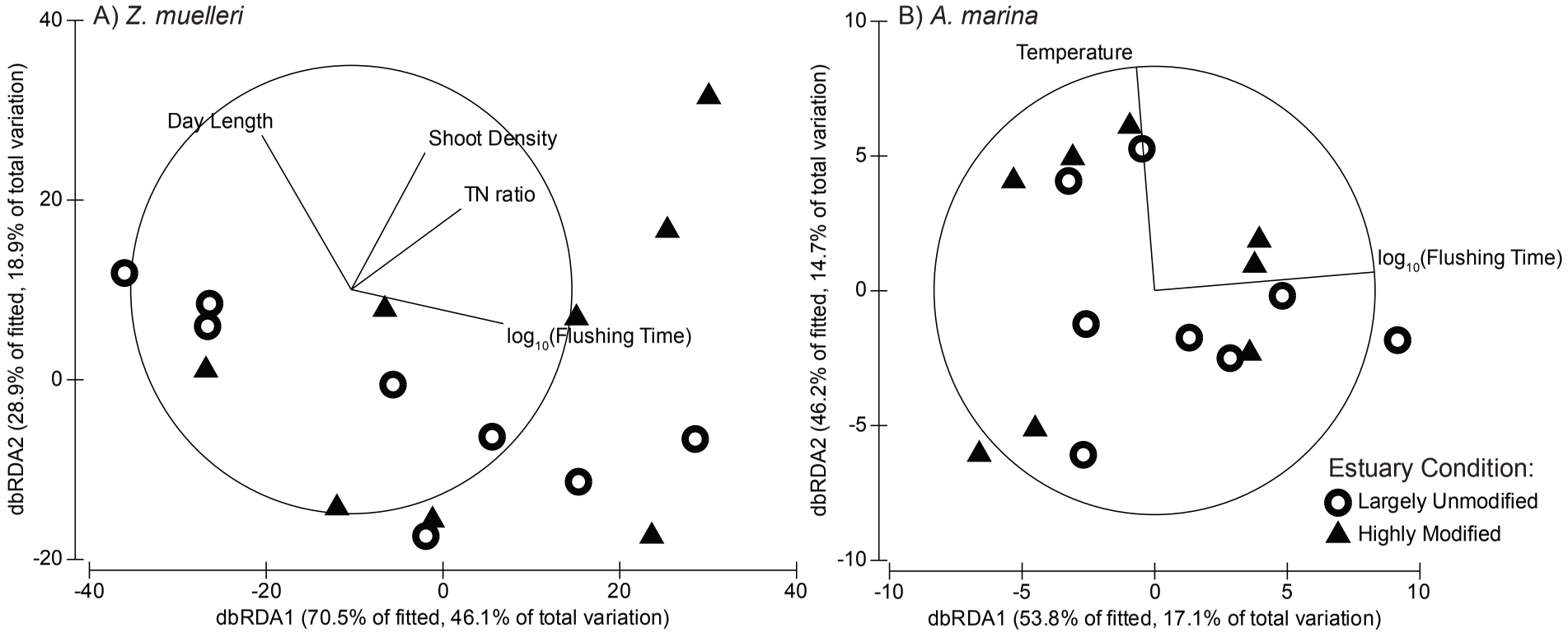

Fig. 3 Distance based redundancy analysis (db-RDA) plots representing the reduced model of environmental variables, identified using AIC selection criteria and the BEST procedure of PRIMER, as the key explanatory variables of spatial variation in leaf traits of (a) the seagrass *Zostera muelleri*, and (b) the mangrove *Avicennia marina.*

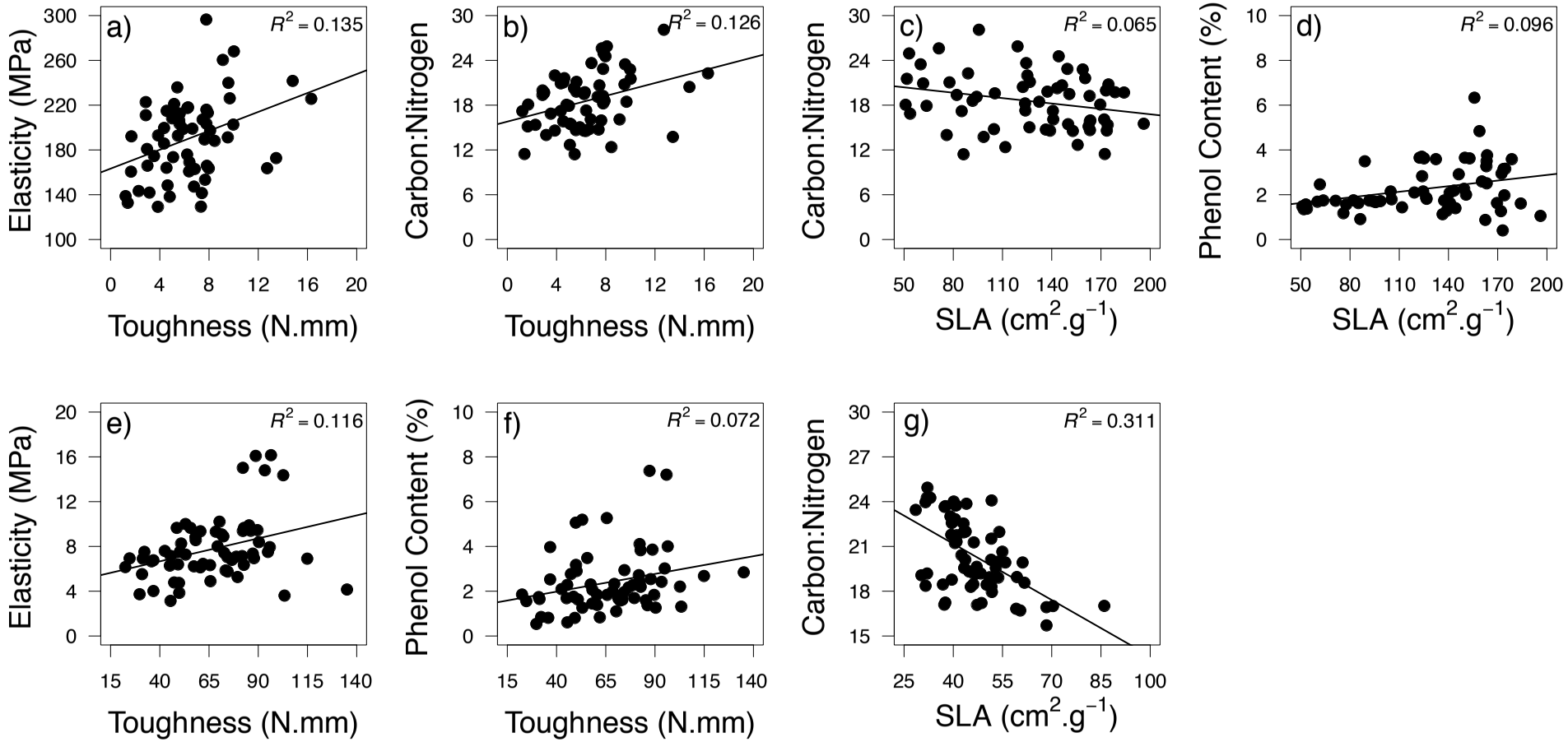

Fig. 4 Significant correlations between leaf traits of the seagrass *Zostera muelleri* (a-d) and of the mangrove *Avicennia marina* (e-g). Points represent mean trait values for each estuary (n = 16) at each sampling period (n = 4), to give a total of n = 64.

Table 4. Pearson's Product Moment Correlations between leaf traits of seagrass (upper triangle) and mangrove (lower triangle), where PCC = Pearson's correlation coefficient. Significant correlations are indicated in bold. Seagrass has 58 df and mangrove has 61 df due to loss of replicates at various times. Data based on means for each site, season and year (n=64)

		Elasticity (MPa)	Toughness (N.mm)	SLA ($cm^2.g^{-1}$)	C:N	Phenol Content (%)
Elasticity (MPa)	*PCC*		0.367	0.021	0.202	0.122
	R^2		0.135	< 0.01	0.041	0.015
	t-value		3.01	0.16	1.57	0.94
	p-value		**0.004**	0.875	0.122	0.352
Toughness (N.mm)	*PCC*	0.341		-0.185	0.355	0.217
	R^2	0.116		0.034	0.126	0.047
	t-value	2.83		-1.43	2.9	1.7
	p-value	**0.006**		0.158	**0.005**	0.095
SLA ($cm2.g-^{1}$)	*PCC*	-0.171	-0.128		-0.254	0.31
	R^2	0.029	0.016		0.065	0.096
	t-value	-1.36	-1.01		-2.00	2.48
	p-value	0.18	0.317		**0.05**	**0.016**
C:N	*PCC*	0.231	0.155	-0.558		-0.03
	R^2	0.053	0.024	0.311		< 0.01
	t-value	1.85	1.22	-5.25		-0.23
	p-value	0.069	0.226	**< 0.001**		0.818
Phenol Content (%)	*PCC*	0.161	0.268	-0.228	0.059	
	R^2	0.026	0.072	0.052	< 0.01	
	t-value	1.27	2.17	-1.83	0.46	
	p-value	0.207	**0.034**	0.072	0.645	

INTRASPECIFIC LEAF TRAIT CORRELATIONS

For each of *Z. muelleri* and *A. marina* leaves, there were positive correlations between elasticity and toughness (Fig. 4a; Fig. 4e), and a negative correlation between C:N and specific leaf area (Fig. 4c; Fig. 4g). Toughness was also positively correlated to C:N for *Z. muelleri* blades (Fig. 4b), and to total phenolic concentration for *A. marina* leaves (Fig. 4f). There was a positive correlation between specific leaf area and total phenolic concentration for *Z. muelleri* blades (Fig. 4d). All other correlations between variables were non-significant (Table 4).

Discussion

Previous studies identifying environmental correlates of leaf traits have primarily done so by assembling global databases that span multiple species, and include asynchronously collected data points (e.g. Wright et al., 2005, Moles et al., 2011). Our study sought to determine whether the relationships derived from such studies also apply to intraspecific variability in leaf traits across smaller gradients, and whether these relationships are temporally persistent. Our study examined intraspecific variation in five leaf traits (specific leaf area, carbon to nitrogen ratio, toughness, elasticity and total phenolics), within two aquatic angiosperms (*Zostera muelleri* and *Avicennia marina*), across gradients of latitude and nutrient enrichment. We found that of the leaf traits examined, only two (mechanical elasticity and carbon to nitrogen ratio) displayed consistent spatial patterns across the two species. We also found that in several instances, intraspecific variation in leaf traits across the environmental gradients were in the opposite direction to the patterns of interspecific variation, reported by previous studies. Overall, latitude was a better predictor of leaf traits than nutrient enrichment, for which spatial patterns were highly variable between years and seasons.

Interspecific comparisons have revealed that, the carbon to nitrogen ratio (C:N) of leaves generally increases with decreasing latitude and increasing temperature (Reich and Oleksyn, 2004). Our examination of seagrass and mangrove leaves found that within species, C:N also increased with decreasing latitude. In contrast to interspecific comparisons which typically span tropical through to temperate latitudes, our study spanned only 7° and represented sub-tropical and temperate climates. Previous studies have suggested that the relationship between latitude and C:N of leaves may in part be explained by the age and lower nitrogen content of tropical soils (Reich and Oleksyn, 2004), but our finding that the relationship is strong even over much smaller gradients, over which geology does not vary markedly, suggests that physiology also plays an important role in determining this relationship (see also Lovelock et al., 2007).

The other leaf trait for which the two species displayed similar patterns of intraspecific variation across the latitudinal gradient was mechanical elasticity. Leaf elasticity, like C:N, increased with decreasing latitude. Despite this, elasticity and C:N were not significantly correlated. Although the underlying mechanism for this gradient in elasticity was not determined, greater elasticity would enable plants to better tolerate physical stressors (such as wind and wave action) and that may have greater prevalence at lower latitudes. For both species, elasticity was generally (and particularly in 2012) greater during the winter months during which physical stressors, for example strong winds and waves, may be more prevalent (eg. Yang et al. 2005).

Patterns of latitudinal variation in the other leaf traits differed between the two species. Whereas the SLA of seagrass blades displayed a weak, but significant, negative relationship with increasing latitude, mangrove leaves displayed a significant positive relationship. The pattern in mangrove leaves is consistent with previous findings that within species SLA often (but not always De Frenne et al., 2010) increases with latitude (Clevering et al., 2001, Etterson, 2004, Miyazawa and Lechowicz, 2004). Leaf toughness increased with

decreasing latitude for seagrass, and was positively correlated to C:N within this species, but no relationship between latitude and leaf toughness of C:N and leaf toughness was found for the mangrove. Although previous studies have found that production of phenolics increases with excess carbon availability, and hence C:N, we did not find any relationship between the two variables, for either species in this study, nor did we find a latitudinal pattern in total phenolics.

Despite significant latitudinal trends, correlation coefficients between leaf traits were weak to moderate (Fowler et al., 1998), indicating that latitude only explained a moderate proportion of leaf trait variability of seagrass and mangrove in estuarine environments. For both species, elasticity was significantly correlated with toughness and the specific leaf area was significantly correlated with C:N. Further, total phenolic concentration varied between 2011 and 2012 for both species, suggesting that this is an inducible defense that is temporally variable in activation. Although spanning close to 1000 km, our study was limited in latitudinal range compared to the natural distributions of *Z. muelleri* and *A. marina* (Duke et al., 2010, Short et al., 2010). Hence, our data likely did not encompass the full possible range of variability in the leaf traits of the two species, perhaps weakening latitudinal effects.

Small-scale manipulative studies have found the leaf traits of seagrass and mangrove respond markedly to nutrient addition (e.g. Lovelock et al., 2004, Kelaher et al., 2013). Fertilization generally results in enhanced nutrient concentrations of leaves, an increase in their surface area, and a decrease in their toughness (Feller, 1995). However, in the present study, leaf traits did not consistently differ between estuaries with anthropogenically enhanced nutrient loadings and estuaries with relatively unmodified nutrient regimes. Where differences did occur, their direction and magnitude varied among sampling times and wee rarely consistent between the two species, mangrove and eelgrass. Temporal variation in patterns, between highly modified and largely unmodified estuaries may reflect temporal variation of rainfall patterns that are a key driver of diffuse nutrient loading.

Of the environmental variables considered by this study, we found that the best predictors of leaf trait variability were day-length and temperature for seagrass blades and flushing time and day-length for mangrove leaves. As both day-length and temperature vary with latitude and climate, strengthening our suggestion for the dependence of leaf traits on latitude, this is consistent with our observation of strong latitudinal gradients in many of our leaf traits. This is also in line with recent evidence that suggests the duration of light available and growing season are the primary drivers latitudinal gradients in leaf tissue chemistry (Borer et al., 2013). Hydrological characteristics such as flushing time would affect the fate, dispersion and ultimately the utilization of nutrients in the system (Davis and Koop, 2006).

Our study is important in showing that two co-occurring species of aquatic macrophyte display markedly different intraspecific patterns of variability in many of their leaf traits and that these patterns are highly temporally variable, at the scale of months and years. This study highlights that intraspecific leaf traits cannot be reliably predicted by broad-scale interspecific patterns alone. Hence, meta-analyses that combine data from multiple species and over many years may produce outcomes that are ecologically very difficult to interpret (Körner, 2007). Further studies are now needed to disentangle why certain species display different environmental correlates of leaf traits and inter-relationships among traits to others.

Acknowledgements

This research was funded by an ARC Discovery Grant (DP1093444) to M. Bishop and Macquarie University Higher Degree Research funding to L. Ainley. We thank L. A. E. Ainley, G. Carrol, B. Coates, B. Cooke, D. Duursmaa, R. Furtado, A. Harmer, T. Hogg, M. Masood, S. Oyston, R. Perez, L. Szlachta, W. Tozer, M. Verhoeven, R. Walsh, E. Wilkie and L. Williams for assistance with field and lab-work. This manuscript benefited from helpful discussion with R. Gallagher and A. Nicastro. J. Madin provided helpful reviews on earlier versions of this manuscript.

References

Ackerly, D. D. & Cornwell, W. (2007) A trait‐based approach to community assembly: partitioning of species trait values into within‐and among‐community components. *Ecology letters,* **10,** 135-145.

Aerts, R. (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. *Oikos,* **79,** 439-449.

Aerts, R. & Chapin, F. S. (2000) The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. *Advances in Ecological Research,* **30,** 1-67.

Ainsworth, E. A. & Gillespie, K. M. (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols,* **2,** 875-877.

Albert, C. H., Thuiller, W., Yoccoz, N. G., Douzet, R., Aubert, S. & Lavorel, S. (2010a) A multi‐trait approach reveals the structure and the relative importance of intra‐vs. interspecific variability in plant traits. *Functional Ecology,* **24,** 1192-1201.

Albert, C. H., Thuiller, W., Yoccoz, N. G., Soudant, A., Boucher, F., Saccone, P. & Lavorel, S. (2010b) Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology,* **98,** 604-613.

Anderson, M. J. (2005) Permutational multivariate analysis of variance. *PERMANOVA Department of Statistics, University of Auckland, Auckland*.

Anderson, M. J., Gorley, R. N. & Clarke, K. R. (2007) *PERMANOVA+ for PRIMER: guide to software and statistical methods.* PRIMER-E, Plymouth.

Anderson, M. J., Gorley, R. N. & Clarke, K. R. (2008) PERMANOVA+ for PRIMER: Guide to software and statisitcal methods. PRIMER-E Ltd., Plymouth UK.

Berendse, F. (1994) Competition between Plant-Populations at Low and High Nutrient Supplies. *Oikos,* **71,** 253-260.

Berry, J. & Raison, J. (1981) Responses of macrophytes to temperature. *Physiological Plant Ecology I*pp. 277-338. Springer.

Boege, K. & Dirzo, R. (2004) Intraspecific variation in growth, defense and herbivory in Dialium guianense (Caesalpiniaceae) mediated by edaphic heterogeneity. *Plant Ecology,* **175,** 59-69.

Borchers, H. W. 2013. Practical Numerical Math Functions (pracma). v1.3.3 ed.

Borer, E. T., Bracken, M. E., Seabloom, E. W., Smith, J. E., Cebrián, J., Cleland, E. E., Elser, J. J., Fagan, W. F., Gruner, D. S. & Harpole, W. S. (2013) Global biogeography of autotroph chemistry: is insolation a driving force? *Oikos,* **122,** 1121-1130.

Burnham, K. P. & Anderson, D. R. (2004) Multimodel inference - understanding AIC and BIC in model selection. *Sociological Methods & Research,* **33,** 261-304.

Chapin, F. S. (2003) Effects of plant traits on ecosystem and regional processes: a conceptual framework for predicting the consequences of global change. *Annals of Botany,* **91,** 455-463.

Clarke, K. R. & Warwick, R. M. (2001) A further biodiversity index applicable to species lists: variation in taxonomic distinctness. *Marine Ecology Progress Series,* **216,** 265-278.

Clevering, O. A., Brix, H. & Lukavská, J. (2001) Geographic variation in growth responses in *Phragmites australis*. *Aquatic Botany,* **69,** 89-108.

Cloern, J. E. (2001) Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series,* **210,** 223-253.

Cornelissen, J. H. C., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D. E., Reich, P. B., ter Steege, H., Morgan, H. D., van der Heijden, M. G. A., Pausas, J. G. & Poorter, H. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany,* **51,** 335-380.

Cornelissen, J. H. C. & Thompson, K. (1997) Functional leaf attributes predict litter decomposition rate in herbaceous plants. *New Phytologist,* **135,** 109-114.

Crews, T. E., Kitayama, K., Fownes, J. H., Riley, R. H., Herbert, D. A., Muellerdombois, D. & Vitousek, P. M. (1995) Changes in Soil-Phosphorus Fractions and Ecosystem Dynamics across a Long Chronosequence in Hawaii. *Ecology,* **76,** 1407-1424.

Davis, J. R. & Koop, K. (2006) Eutrophication in Australian rivers, reservoirs and estuaries–a southern hemisphere perspective on the science and its implications. *Hydrobiologia,* **559,** 23-76.

De Frenne, P., De Schrijver, A., Graae, B. J., Gruwez, R., Tack, W., Vandelook, F., Hermy, M. & Verheyen, K. (2010) The use of open-top chambers in forests for evaluating warming effects on herbaceous understorey plants. *Ecological research,* **25,** 163-171.

Díaz, S. & Cabido, M. (1997) Plant functional types and ecosystem function in relation to global change. *Journal of vegetation science,* **8,** 463-474.

Díaz, S. & Cabido, M. (2001) Vive la difference: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution,* **16,** 646-655.

Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., Galindo, H. M., Grebmeier, J. M., Hollowed, A. B., Knowlton, N., Polovina, J., Rabalais, N. N., Sydeman, W. J. & Talley, L. D. (2012) Climate change impacts on marine ecosystems. *Annual Review of Marine Science,* **4,** 11-37.

Duarte, C. M. (1990) Seagrass Nutrient Content. *Marine Ecology Progress Series,* **67,** 201-207.

Duke, N. C., Kathiresan, K., Salmo III, S. G., Fernando, E. S., Peras, J. R., Sukardjo, S., Miyagi, T., Ellison, J. C., Koedam, N. E., Wang, Y., Primavera, J., Jin Eong, O., Wan-Hong Yong, J. & Ngoc Nam, V. (2010) *Avicennia marina*. *IUCN Red List of Threatened Species* (ed I. 2012).

Ellsworth, D. S. & Reich, P. B. (1993) Canopy Structure and Vertical Patterns of Photosynthesis and Related Leaf Traits in a Deciduous Forest. *Oecologia,* **96,** 169-178.

Enríquez, S., Duarte, C. M. & Sand-Jensen, K. (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. *Oecologia,* **94,** 457-471.

Etterson, J. R. (2004) Evolutionary potential of Chamaecrista fasciculata in relation to climate change. I. Clinal patterns of selection along an environmental gradient in the great plains. *Evolution,* **58,** 1446-58.

Feller, I. C. (1995) Effects of Nutrient Enrichment on Growth and Herbivory of Dwarf Red Mangrove (*Rhizophora mangle*). *Ecological Monographs,* **65,** 477-505.

Fonseca, M. S. & Cahalan, J. A. (1992) A preliminary evaluation of wave attenuation by four species of seagrass. *Estuarine, Coastal and Shelf Science,* **35,** 565-576.

Fowler, J., Cohen, L., Jarvis, P. & Wiley, J. (1998) *Practical statistics for field biology.* Wiley Chichester.

Hättenschwiler, S. & Vitousek, P. M. (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology & Evolution,* **15,** 238-243.

Hobbie, S. E. (1992) Effects of plant species on nutrient cycling. *Trends in Ecology & Evolution,* **7,** 336-9.

Hulshof, C. M., Violle, C., Spasojevic, M. J., McGill, B., Damschen, E., Harrison, S. & Enquist, B. J. (2013) Intra‐specific and inter‐specific variation in specific leaf area reveal the importance of abiotic and biotic drivers of species diversity across elevation and latitude. *Journal of Vegetation Science,* **24,** 921-931.

Kattge, J., Díaz, S., Lavorel, S., Prentice, C., Leadley, P., Bönisch, G., Garnier, E., Westoby, M., Reich, P. B., Wright, I. J., Cornelissen, J. H. C., Violle, C., Harrison, S. P., van Bodegom, P. M., Reichstein, M., Enquist, B. J., Soudzilovskaia, N. A., Ackerly, D. D., Anand, M., Atkin, O., Bahn, M., Baker, T. R., Baldocchi, D., Bekker, R., Blanco, C. C., Blonder, B., Bond, W. J., Bradstock, R., Bunker, D. E., Casanoves, F., Cavender-Bares, J., Chambers, J. Q., Chapin, F. S., Chave, J., Coomes, D., Cornwell,

W. K., Craine, J. M., Dobrin, B. H., Duarte, L., Durka, W., Elser, J., Esser, G., Estiarte, M., Fagan, W. F., Fang, J., Fernández-Méndez, F., Fidelis, A., Finegan, B., Flores, O., Ford, H., Frank, D., Freschet, G. T., Fyllas, N. M., Gallagher, R. V., Green, W. A., Gutierrez, A. G., Hickler, T., Higgins, S. I., Hodgson, J. G., Jalili, A., Jansen, S., Joly, C. A., Kerkhoff, A. J., Kirkup, D., Kitajima, K., Kleyer, M., Klotz, S., Knops, J. M. H., Kramer, K., Kühn, I., Kurokawa, H., Laughlin, D., Lee, T. D., Leishman, M., Lens, F., Lenz, T., Lewis, S. L., Lloyd, J., Llusia, J., Louault, F., Ma, S., Mahecha, M. D., Manning, P., Massad, T., Medlyn, B. E., Messier, J., Moles, A. T., Müller, S. C., Nadrowski, K., Naeem, S., Niinemets, Ü., Nöllert, S., Nüske, A., Ogaya, R., Oleksyn, J., Onipchenko, V. G., Onoda, Y., Ordoñez, J., Overbeck, G., Ozinga, W. A., et al. (2011) TRY - a global database of plant traits. *Global Change Biology,* **17,** 2905-2935.

Kelaher, B. P., Van Den Broek, J., York, P. H., Bishop, M. J. & Booth, D. J. (2013) Positive responses of a seagrass ecosystem to experimental nutrient enrichment. *Marine Ecology Progress Series,* **487,** 15-25.

Körner, C. (2007) The use of 'altitude' in ecological research. *Trends in Ecology & Evolution,* **22,** 569-574.

Lavorel, S. & Garnier, E. (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology,* **16,** 545-556.

Lecerf, A. & Chauvet, E. (2008) Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. *Basic and Applied Ecology,* **9,** 598-605.

Lovelock, C. E., Feller, I. C., Ball, M. C., Ellis, J. & Sorrell, B. (2007) Testing the growth rate vs. geochemical hypothesis for latitudinal variation in plant nutrients. *Ecology Letters,* **10,** 1154-63.

Lovelock, C. E., Feller, I. C., Mckee, K. L., Engelbrecht, B. M. J. & Ball, M. C. (2004) The effect of nutrient enrichment on growth, photosynthesis and hydraulic conductance of dwarf mangroves in Panamá. *Functional Ecology,* **18,** 25-33.

Loveys, B., Scheurwater, I., Pons, T., Fitter, A. & Atkin, O. (2002) Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast‐and slow‐growing plant species. *Plant, Cell & Environment,* **25,** 975-988.

McArdle, B. H. & Anderson, M. J. (2001) Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology,* **82,** 290-297.

Melillo, J. M., Aber, J. D. & Muratore, J. F. (1982) Nitrogen and Lignin Control of Hardwood Leaf Litter Decomposition Dynamics. *Ecology,* **63,** 621-626.

Miyazawa, K. & Lechowicz, M. J. (2004) Comparative seedling ecology of eight north American spruce (Picea) species in relation to their geographic ranges. *Annals of Botany,* **94,** 635-644.

Moles, A. T., Bonser, S. P., Poore, A. G. B., Wallis, I. R. & Foley, W. J. (2011) Assessing the evidence for latitudinal gradients in plant defence and herbivory. *Functional Ecology,* **25,** 380-388.

Nixon, S. W. (1995) Coastal Marine Eutrophication - a Definition, Social Causes, and Future Concerns. *Ophelia,* **41,** 199-219.

Ordoñez, J. C., Van Bodegom, P. M., Witte, J. P. M., Wright, I. J., Reich, P. B. & Aerts, R. (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography,* **18,** 137-149.

Pérez-Harguindeguy, N., Díaz, S., Cornelissen, J. H. C., Vendramini, F., Cabido, M. & Castellanos, A. (2000) Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. *Plant and Soil,* **218/2,** 21-30.

Peterson, C. H., Luettich, R. A., Micheli, F. & Skilleter, G. A. (2004) Attenuation of water flow inside seagrass canopies of differing structure. *Marine Ecology Progress Series,* **268,** 81-92.

Potter, J. R. & Jones, J. W. (1977) Leaf area partitioning as an important factor in growth. *Plant Physiology,* **59,** 10-14.

Reich, P. B., Ellsworth, D. S., Walters, M. B., Vose, J. M., Gresham, C., Volin, J. C. & Bowman, W. D. (1999) Generality of leaf trait relationships: A test across six biomes. *Ecology,* **80,** 1955-1969.

Reich, P. B. & Oleksyn, J. (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America,* **101,** 11001-6.

Roper, T., Creese, B., Scanes, P., Stephens, K., Williams, R., Dela-Cruz, J., Coade, G., Coates, B. & Fraser, M. (2011) Assessing the condition of estuaries and coastal lake ecosystems in NSW. *Monitoring, evaluation and reporting program, Technical report series*. Office of Environment and Heritage, Sydney.

Roy, P. S., Williams, R. J., Jones, A. R., Yassini, I., Gibbs, P. J., Coates, B., West, R. J., Scanes, P. R., Hudson, J. P. & Nichol, S. (2001) Structure and function of south-east Australian estuaries. *Estuarine, Coastal and Shelf Science,* **53,** 351-384.

Shea, K. & Chesson, P. (2002) Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution,* **17,** 170-176.

Short, F. T., Williams, S. L., Carruthers, T. J. B., Waycott, M., Kendrick, G. A., Fourqurean, J. W., Callabine, A., Kenworthy, W. J. & Dennison, W. C. (2010) *Zostera muelleri*. *IUCN Red List of Threatened Species* (ed IUCN).

Singleton, V. L., Orthofer, R. & Lamuela-Raventos, R. M. (1999) [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology* (ed P. Lester), pp. 152-178. Academic Press.

Team, R. C. (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Udy, J. W. & Dennison, W. C. (1997) Physiological responses of seagrasses used to identify anthropogenic nutrient inputs. *Marine and Freshwater Research,* **48,** 605-614.

Wright, I. J., Reich, P. B., Cornelissen, J. H., Falster, D. S., Garnier, E., Hikosaka, K., Lamont, B. B., Lee, W., Oleksyn, J., Osada, N., Poorter, H., Villar, R., Warton, D. I. & Westoby, M. (2005) Assessing the generality of global leaf trait relationships. *New Phytologist,* **166,** 485-96.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W., Lusk, C., Midgley, J. J., Navas, M. L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V. I., Roumet, C., Thomas, S. C., Tjoelker, M. G., Veneklaas, E. J. & Villar, R. (2004) The worldwide leaf economics spectrum. *Nature,* **428,** 821-7.

Yang, B., R. Dalrymple, and S. Chun. 2005. Sedimentation on a wave-dominated, open-coast tidal flat, south-western Korea: summer tidal flat–winter shoreface. *Sedimentology* **52**:235-252.

3. THE EFFECT OF COASTAL DEVELOPMENT ON LITTER DECOMPOSITION VARIES BETWEEN CLIMATIC SETTINGS.

Lara B. Ainley, Melanie J. Bishop*

Department of Biological Sciences, Macquarie University, Sydney New South Wales 2109, Australia

* Corresponding Author: Lara Ainley, Ph: +612 9850 6285

Email addresses: lara.ainley@students.mq.edu.au (L. Ainley),

melanie.bishop@mq.edu.au (M. Bishop)

Abstract

Decomposition of organic material is an important ecological process for carbon and nutrient cycling and has a major role in fueling coastal food webs. Anthropogenic modification of coasts has the potential to modify decomposition rates by altering the physical environment in which decomposition occurs, the structure of decomposer communities and leaf litter traits. We assessed: (1) how decomposition processes differ between estuaries with high and low anthropogenic nutrient loads; and (2) whether the effect of nutrient loading is greater in warmer than in cooler climates. At each of two temperate latitudes of New South Wales, Australia, separated by 1000 km and differing in mean water temperature by 4.5°C, we established subtidal litterbags of the seagrass *Zostera muelleri*, the mangrove *Avicennia marina*, and mixtures. Within each latitude, litterbags were deployed within two estuaries with an anthropogenically enhanced nutrient loading and two with a largely unmodified nutrient loading. Litter decomposition and colonization was tracked over 65 days. For each detrital source, there was evidence of an interactive effect of latitude and estuarine nutrient status on decomposition rate. Initially, seagrass decomposed more rapidly at the higher latitudes, but by the end of the experiment, decomposition was greater in highly modified estuaries of the lower latitudes, than elsewhere. Throughout the experiment, decomposition of mangrove leaves was slower in the largely unmodified estuaries of the higher latitudes than the lower latitudes or in highly modified estuaries. Environmental variables explained some but not all the variation in litter decay rates, with macrofaunal community structure, and in particular amphipod abundance, also contributing. Hence, spatial variation in decomposition was at least partially mediated by variation in decomposer communities. Overall, our results suggest that climate warming and coastal development may act synergistically to influence rates of decomposition in estuarine environments, in part by modifying decomposer communities. Therefore to minimize the risk of climate warming on

accelerated decomposition rates and subsequent sediment hypoxia, management strategies need to focus on limiting the nutrient loading of susceptible coastal environments.

Key Words: Coastal Development; Decomposition; Leaf Litter; Detritus; Estuary; *Zostera*; *Avicennia*; Latitude; Nutrient Enrichment; Macroinvertebrates; Seagrass; Mangrove

Introduction

In both terrestrial and aquatic ecosystems, primary producers are key carbon sinks, fixing CO_2 from the atmosphere during photosynthesis. Some of this carbon is lost to herbivores through consumption, but most persists in the environment as detritus (Cebrián 1999, Moore et al. 2004, Wetzel 1995). Detritus may remain at the site of production, or be transported by wind or water across habitat boundaries to spatially subsidise other ecosystems (see Polis et al. 1997). Over time detritivores and microbial communities break down some of the detritus, liberating carbon as well as nutrients to the environment (Aerts 1997, Swift et al. 1979, Webster and Benfield 1986). Recalcitrant detritus may, however, store carbon for prolonged periods (Cebrián 1999). Consequently, understanding the factors that influence decomposition is of critical importance to global carbon cycles (Enríquez et al. 1993, Hättenschwiler et al. 2005, Loreau et al. 2001) and food web dynamics (Moore et al. 2004).

The rate at which detritus decomposes is driven by three primary factors – the abiotic environment, chemical and physical traits of the litter and the structure and function of decomposer communities (Coûteaux et al. 1995, Swift et al. 1979). Of these, the abiotic environment is generally considered the dominant control on decomposition at large spatial scales (Aerts 1997, Silver and Miya 2001). In particular, latitude, mean annual temperature and mean annual rainfall are key correlates of decomposition in terrestrial environments (Zhang et al. 2008), with rates generally increasing with temperatures and precipitation

(Conant et al. 2011, Conant et al. 2008, Davidson and Janssens 2006, Kirschbaum 1995). Abiotic conditions may directly determine decomposition by influencing rates of biological reactions, or indirectly through influences on leaf traits and/or consumer communities (Hemminga and Buth 1991).

At regional scales, decomposition rates are strongly influenced by the interplay between litter quality (Pérez-Harguindeguy et al. 2000) and the structure and function of decomposer communities (Coûteaux et al. 1995, Wardle 2006). Among leaf traits, nitrogen content is generally considered one of the most important factors in determining rates of litter decomposition and palatability to consumers (Conn and Dighton 2000, Moore et al. 2004) As the nitrogen content of litter increases, so does the rate of breakdown. High concentrations of secondary metabolites such as lignin, cellulose and tannins can deter consumers (Gessner et al. 2010), and slow microbial decay (Canfield 1994, Fenchel et al. 1998, Snelgrove et al. 2000). As litter material becomes more recalcitrant during the later stages of decay, decomposition is limited by the inability of microbial organisms to metabolize the complex molecules (Kristensen et al. 1995). More labile material is typically colonized by bacteria that quickly break down the detritus, while fungi tends to colonize the more recalcitrant material that decays at a slower rate (Moore et al. 2004).

In many instances, detrital pools contain a mixture of species, that display very different rates of decomposition when together than when alone (Bishop and Kelaher 2008). For example, where labile and refractory sources of detritus decompose together, the decomposer communities associated with labile sources may spill over to the refractory sources to accelerate their decomposition (Moore and Fairweather 2006, Smith and Bradford 2003). Blair et al. (1990) describes non-additive effects of litter mixing on the nitrogen flux in mixed-species litter compared to single-species litter.

In combination, coastal development and climate warming have the potential to produce major changes in patterns of carbon cycling in coastal marine environments directly,

by modifying abiotic conditions, and indirectly, by modifying the structure and function of consumer communities, and traits of primary producers. Increasing temperatures, projected to warm by at least 2°C and as much as 4°C between 2013 and 2100 (IPCC 2013), may accelerate rates of decomposition by speeding up chemical reactions and enhancing biological activity (Davidson and Janssens 2006, Kirschbaum 1995). Where stored carbon is released from soils to the atmosphere as CO_2, it may create a positive feedback system that accelerates rates of warming (Davidson and Janssens 2006). Simultaneously, the enhancement of nitrogen and phosphorous input to coastal environments by agricultural run-off and urban activities may influence litter quality and the decomposer community. Enhanced nutrient input to coastal systems can decrease the carbon to nitrogen ratio (C:N) of producers, such as seagrasses (Duarte 1990, Lee et al. 2004). Furthermore, in stream and estuarine ecosystems, nutrient enrichment generally stimulates the activity and biomass of heterotrophic organisms such as bacteria and fungi (Suberkropp et al. 2010, Van Ryckegem et al. 2007), but can decrease shredder abundance (Pérez et al. 2013). Yet, it is unclear how these two co-occurring stressors will interact to influence decomposition rates, and hence future carbon cycling in estuarine environments. If large synergistic effects of temperature and nutrient enrichment are seen, adaptation of estuarine ecosystems to climate change may be contingent on reducing nutrient loading.

Here, we make use of a spatial gradient along the east coast of Australia, in a "space-for-time-substitution", to assess how ongoing warming and coastal development might interact to influence carbon cycling in estuarine ecosystems. We test how the decomposition of the leaves of two key aquatic primary producers, the seagrass *Zostera muelleri* and the mangrove *Avicennia marina*, vary with latitude, and with anthropogenic enhancement of nutrient supply to estuaries, when in isolation of one another and when mixed. We expect that with decreasing latitude and increasing nutrient enrichment, the rate of decomposition will increase. Further, we expect that the difference in the rate of decomposition between

more and less enriched estuaries will be greater at the warmer temperatures of the lower latitudes, than the cooler temperatures of higher latitudes, because rates of metabolism generally increase with temperature (Schmitz 2013). We hypothesize that differences in decomposition rates among latitudes will, at least in part, be mediated by variation in decomposer communities.

Materials and Methods

STUDY SITES

To test the hypothesis that decomposition of macrophyte litter would be greater (1) within a warmer than a cooler climate, and (2) in estuaries in which nutrient loading has been anthropogenically enhanced versus those that have had largely unmodified nutrient inputs, experimental litterbags were deployed in eight estuaries along the coast of New South Wales (NSW), Australia. Four of the estuaries were located on the north coast of NSW, between latitudes of -29.6 and -30.4 degrees south and with average water temperature of 23.2°C during the study (northerly latitude [N]; Fig. 1). The remaining four estuaries were located on the south coast of NSW between -34.6 and -35.2 degrees south with water temperatures 4.5° cooler, averaging 18.8°C during the study (southerly latitude [S]; Fig. 1). Within each latitudinal zone, we randomly selected two replicate estuaries with nutrient loadings that have been largely unmodified since European settlement (largely unmodified [L]; ratio of pre-European settlement loading to present total nitrogen loading (T:N) < 1.5; Roper et al. 2011); and two replicate estuaries with anthropogenically enhanced nutrient loadings (highly modified [H]; ratio of pre-European settlement to present total nitrogen loading (T:N) > 2.5; Roper et al. 2011). All estuaries were classified as intermediate to mature, wave-dominated estuaries or embayments (Roy et al. 2001), with predominantly open entrance conditions (Roper et al. 2011) and with known populations of *Zostera muelleri* and *Avicennia marina* (NSWDPI 2011). Within each estuary, litterbags were deployed at a single shallow subtidal

site (Mean Spring Low Water 0.1-0.3 m) during September 2012. Litterbags were interspersed on an un-vegetated intertidal flat, 10-20 m from populations of *Z. muelleri* and *A. marina*.

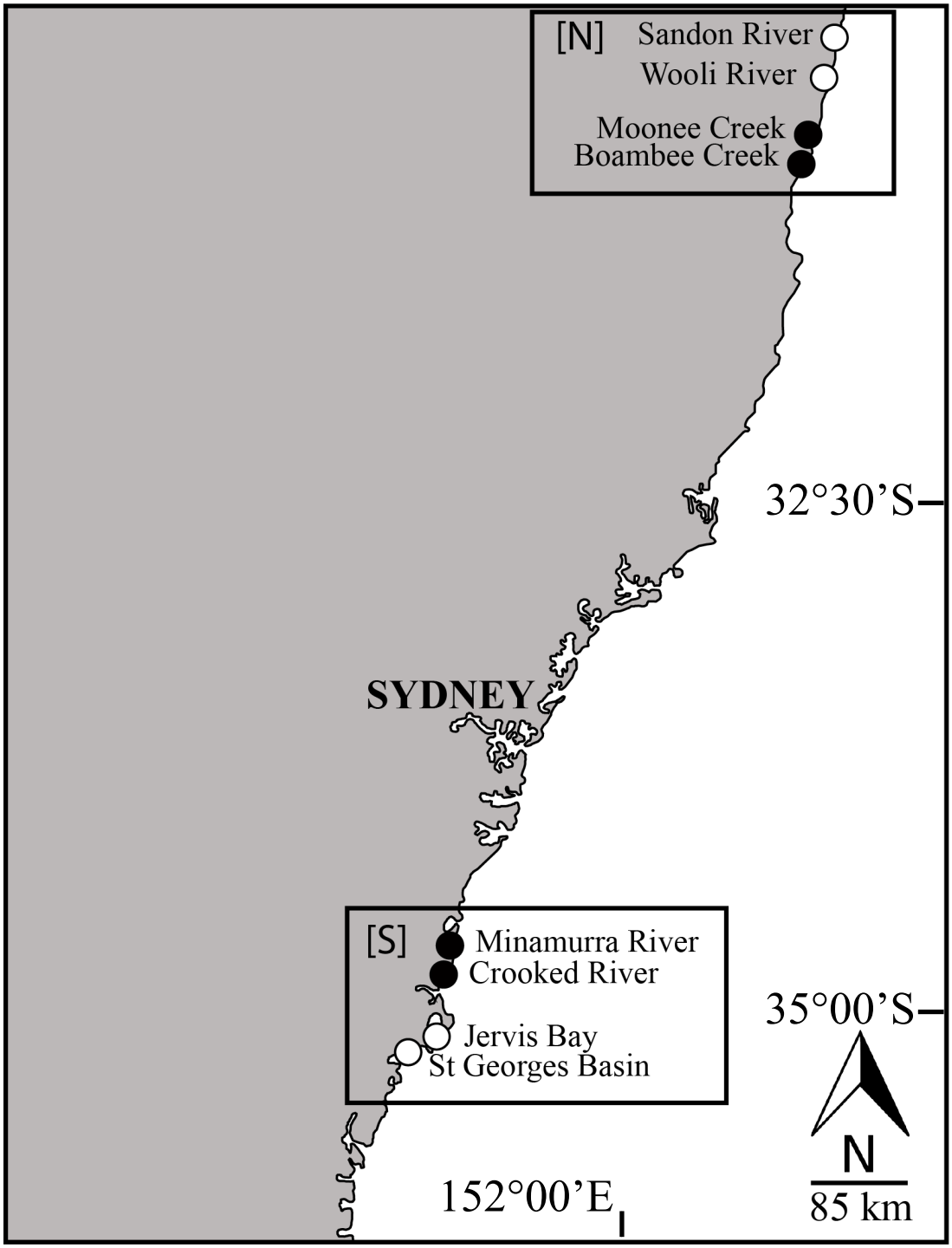

Fig. 1. The estuaries sampled at northern [N] and southern [S] latitudes of New South Wales, Australia. Estuaries with nutrient loadings that have been highly modified estuaries by humans are indicated by filled black circles and those with largely unmodified nutrient loadings are indicated by open white circles.

Table 1. The environmental conditions present at each of the eight field sites, including total rainfall (mm) and mean water temp (°C; ± 1 SE) for each decomposition period from date of deployment. N = northern (low latitude), S = southern (high latitude), L = largely unmodified, H = highly modified.

					Decomposition period 1 (0-13 days)		Decomposition period 2 (0-27 days)		Decomposition period 3 (0-65 days)	
Estuary	**Latitude (°S)[1]**	**T:N ratio[1]**	**Area (km^2)[1]**	**Flushing Time (d)[1]**	**Total Rainfall (mm)[2]**	**Mean Water Temp. (°C)[3]**	**Total Rainfall (mm)[2]**	**Mean Water Temp. (°C)[3]**	**Total Rainfall (mm)[2]**	**Mean Water Temp. (°C)[3]**
NL1	-29.6728	1.02	2.62	2.34	15.4	18.22 ± 0.11	80.0	18.17 ± 0.11	148.9	19.04 ± 0.12
NL2	-29.8878	1.06	3.75	6.15	15.4	18.27 ± 0.14	80.0	18.19 ± 0.14	148.9	19.09 ± 0.13
NH1	-30.2122	4.30	0.41	5.02	28.0	18.51 ± 0.28	156.4	18.11 ± 0.28	225.6	18.42 ± 0.16
NH2	-30.3546	10.54	0.99	2.39	28.0	18.51 ± 0.28	156.4	18.11 ± 0.28	225.6	18.42 ± 0.16
SH1	-34.6280	2.58	1.86	1.04	13.0	16.37 ± 0.12	23.8	16.43 ± 0.08	137.0	17.02 ± 0.15
SH2	-34.7728	3.19	0.28	2.17	13.0	16.37 ± 0.12	23.8	16.43 ± 0.08	137.0	17.02 ± 0.15
SL1	-35.1039	1.44	123.89	54.38	19.4	16.37 ± 0.12	33.0	16.43 ± 0.08	145.4	17.02 ± 0.15
SL2	-35.1852	1.40	40.91	288.33	19.4	16.37 ± 0.12	33.0	16.43 ± 0.08	145.4	17.02 ± 0.15

[1] Source: Roper et al. (2011)

[2] Source: Bureau of Meterology (2012)

[3] Source: HOBO (UA-002-08, Onset, Massachusetts, USA) temperature data loggers

DATA COLLECTION

At each site, three litterbag treatments were established using an additive design: (1) monospecific seagrass (i.e. *Z. muelleri* only); (2) monospecific mangrove (i.e. *A. marina* only); and (3) a mixture of seagrass and mangrove. The mixed litter treatment was included because in many estuaries these species often decompose together on the shore, and may decompose at a different rate when together than when alone. Litterbags were constructed from 1 mm nylon mesh material (Allied Filter Fabrics) and were 100 x 150 mm in size. The mesh size (1 mm) was selected to allow a portion of the benthic community (those individuals < 1 mm diameter) to moderate decomposition while minimizing the loss of litter fragments through the mesh (Bedford 2004, Bradford et al. 2002, Dye 2006). Each of the two litter types were collected from Careel Bay, Pittwater (-33.618°S, 151.327°E), situated in Sydney, NSW, approximately midway between the northern and southern latitudes where the material was deployed. From randomly selected plants, we collected the oldest leaves, next likely to senesce. Care was taken to sample the outer blades of the seagrass shoot and mature mangrove leaves that showed signs of discolouration (yellowing). By deploying leaves from a common source across the eight estuaries, we could assess how environmental setting directly, and indirectly by changing decomposer communities, influences the decomposition of the two different litter sources. Litterbags (n = 21 per treatment per site) assigned to one of the monospecific treatments received 10 g of the litter source (Nicastro et al. 2012). Litterbags assigned to the mixed species treatment received 10 g of each species to give a total of 20 g of material. 10 g of each species was chosen to allow for direct comparison with monospecific litter of the same weight. For each litter species, ten samples of 10 g were dried to constant weight at 60°C to determine the wet to dry conversion factor.

Sixty-three litterbags (n = 21 for each of the three litter treatments) were deployed at each site in August 2011, within 48 h of litter collection. Litterbags were anchored with a peg to the sediment surface. Replicate bags (n = 7, except where losses prevented this) of each

treatment were retrieved 13, 27 and 65 days later. Following retrieval, litterbags were emptied and rinsed carefully over a 0.5 mm sieve to remove sediment. Macroinvertebrates, seagrass and mangrove litter retained on the sieve were separated. Litter material of each species was dried separately at 60°C to constant weight (approximately 3-4 days) and weighed. The percentage of mass remaining in each litterbag was calculated relative to the sub-samples of litter dried and weighed at the start of the experiment. Macrofaunal samples were fixed in 5% formalin in seawater then later transferred to 70% ethanol for identification and enumeration under a dissecting microscope. Subsets of macroinvertebrate samples (monospecific treatments collected at 13 and 65 days only) were identified to species, or where this was not possible, morphospecies (see Barnard and Karaman, 1991, Beesley et al., 1998, Beesley et al., 2000, Jones and Morgan, 2002).

STATISTICAL ANALYSES

Decay constants and half-lives were calculated for each litter source, in each estuary, by fitting mass loss data for the four time points (0, 13, 27 and 65 days) to an exponential decay model (Olson 1963). First, a two-factor analysis of variance (ANOVA) was used to determine whether across all treatments, the half-lives of litter displayed similar spatio-temporal patterns between monocultures and mixtures of seagrass and mangrove litter. Second, to assess whether at individual time points, individual litter sources displayed spatial differences in the percent mass remaining, separate three-way ANOVAs were then run. These had the factors latitude (2 levels, fixed: north, south), nutrient status (2 levels, fixed: highly modified, largely unmodified) and estuary (two levels, random: nested within latitude and nutrient status). A separate ANOVA was run for each treatment (seagrass and mangrove) and time (13, 27 or 65 days) because these two factors otherwise dominated analyses, reducing our ability to detect the spatial differences of interest. Prior to the analyses, Cochran's test was run to test the homogeneity of variance and the Shapiro-Wilk Test was

run to test for normality. Unless otherwise indicated, these assumptions were met. Where significant treatment effects were detected at $\alpha = 0.05$, ANOVAs were followed by Tukey HSD tests to examine the sources of the differences.

Multivariate methods in the PRIMER package (Clarke and Warwick 1994) assessed sources of spatio-temporal variability in macroinvertebrate communities among litterbag treatments. First, three-way permutational multivariate analyses of variance (PERMANOVAs; Anderson 2001), of analogous design to the ANOVAs described above, tested the hypothesis that for each litter source, macroinvertebrate communities would differ spatially according to latitude and nutrient status. The analyses, run separately for each litter source at each time, used Bray-Curtis dissimilarity measures, calculated from raw data. Next, SIMPER analyses identified taxa that contributed most (Diss/SD > 1.3) to the multivariate differences among spatial treatments at each time point. Finally, separate three-way ANOVAs for each treatment (seagrass and mangrove) and time (13 and 65 days; samples at 27 days were not analysed due to time and resource constraints) assessed sources of spatial variation in the total abundance, total richness, abundance of shredders/detritivores (as these are most likely to control the breakdown of litter material) and the abundance of discriminating taxa groups (identified by SIMPER).

To test the hypotheses that decomposition would be correlated to (1) environmental variables (at the scale of estuaries), and (2) macroinvertebrate assemblage structure (at the scale of litterbags), Spearman's rank correlations were conducted using the RELATE procedure of PRIMER. Multivariate data set was used in the correlation of decomposition against environmental variables including latitude (°S), size (km^2), flushing time (days), T:N, total rainfall (mm), mean daily water temperature (°C; Table 1). Where significant correlations occurred, the BIOENV procedure was used to determine the subset of (1) variables or (2) species that best account for the variability in the decay data.

Results

LITTER DECOMPOSITION

Overall, across all treatments, *Zostera muelleri* decomposed at 222 ± 32% (mean ± 1 SE) of the rate of *Avicennia marina* (Table 2). *Zostera muelleri* displayed half-lives of 32-44 days, whereas *A. marina* had half-lives of 46-165 days (Table 2). Whereas *Z. muelleri* displayed the greatest rate of mass loss early in the experiment, the rate of mass loss of *A. marina* was initially slow and increased through time (Fig. 2). Main effects showed the half-life, and hence decay rate, of neither *Z. muelleri* or *A. marina* litter to differ significantly between monocultures and mixtures (ANOVA: $F_{1,16} = 0.160$, $p = 0.694$). Hence, only the spatial variation at each time point in the remaining mass of monocultures is presented and statistically analysed.

Table 2. The mean (± SE) decay constant (k d^{-1}) and half life ($t_{½}$ days) of *Zostera muelleri* and *Avicennia marina* litter, decomposing in isolation (monoculture) or in the presence of the other species (mixture), at each of eight study sites. Study sites were situated within estuaries of northern (N) or southern (S) coasts of New South Wales that were substantially nutrient-enriched by humans since European settlement (H) or that had largely unmodified nutrient loadings (L). Estimates of k and $t_{½}$ were derived separately for each study site and litter source using linear regression analysis between time and $\ln(x_t/x_0)$, where x_0 is the initial amount of material and x_t is the amount remaining after time t in days. The y-intercept was forced through 100%, the initial amount of litter.

Estuary	*Zostera muelleri* monoculture					*Zostera muelleri* in mixture					*Avicennia marina* monoculture					*Avicennia marina* in mixture				
	r^2	k (d^{-1})	SE	$t_{½}$	p value	r^2	k (d^{-1})	SE	$t_{½}$	p value	r^2	k (d^{-1})	SE	$t_{½}$	p value	r^2	k (d^{-1})	SE	$t_{½}$	p value
NH1	0.88	0.021	0.005	33	0.019	0.75	0.022	0.007	32	0.059	0.95	0.009	0.001	76	0.005	1.00	0.013	<0.001	52	<0.001
NH2	0.82	0.022	0.006	32	0.034	0.83	0.020	0.005	35	0.033	0.94	0.011	0.002	66	0.006	0.93	0.012	0.002	58	0.007
NL1	0.67	0.017	0.007	42	0.088	0.79	0.019	0.006	37	0.043	1.00	0.012	<0.001	56	<0.001	0.99	0.013	0.001	52	0.001
NL2	0.80	0.016	0.005	44	0.039	0.87	0.020	0.005	34	0.020	0.98	0.011	0.001	63	0.001	0.99	0.015	0.001	46	<0.001
SH1	0.77	0.020	0.006	35	0.051	0.74	0.018	0.006	38	0.062	1.00	0.011	<0.001	61	<0.001	0.99	0.013	0.001	52	<0.001
SH2	0.73	0.019	0.007	36	0.067	0.72	0.016	0.006	43	0.071	0.99	0.01	0.001	71	0.001	0.99	0.010	0.001	71	0.001
SL1	0.83	0.023	0.006	31	0.032	0.8	0.021	0.006	34	0.040	0.71	0.009	0.003	74	0.072	0.73	0.008	0.003	87	0.064
SL2	0.68	0.017	0.007	41	0.087	0.8	0.020	0.006	35	0.040	0.65	0.004	0.002	157	0.098	0.69	0.004	0.002	165	0.083

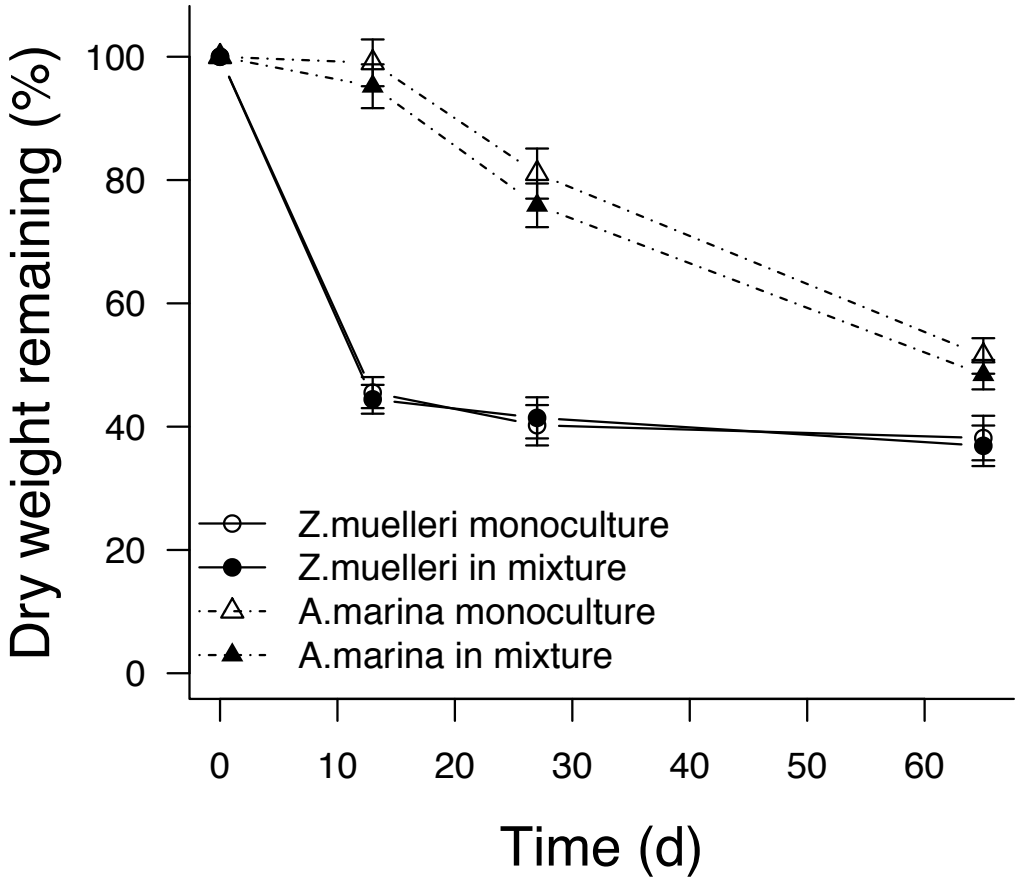

Fig. 2. Mean (± 1SE) percentage of the initial litter weight remaining after 13, 27 and 65 days for monocultures (open shapes) and mixtures (closed shapes) of *Zostera muelleri* (circles) and *Avicennia marina* (triangles) from litter bags deployed during August-October 2011 on the NSW coast. Values are averaged across all spatial treatments.

With the exception of *Z. muelleri* litter at 65 days, the mass of litter remaining in bags varied at the scale of estuaries (Fig. 3). Nevertheless, over and above this background variability, effects of latitude and nutrient status were seen (Table 3). After 13 days, significantly less *Z. muelleri* remained within estuaries from the cooler, southern latitudes (mean ± 1 SE: 43 ± 2%) than the mass remaining at the warmer, northern latitudes (48 ± 3%), but litter mass was not affected by nutrient status (Table 3). After 27 days, there was no effect of either latitude or nutrient status on *Z. muelleri* litter mass, but by day 65 there was less mass remaining in highly modified than in largely unmodified estuaries of the warmer, northern estuaries, but no difference between nutrient treatments at the southern latitude (sig. Latitude x Nutrient Status interaction, Table 3).

Avicennia marina litter mass was determined by the interacting effects of latitude and nutrient status at each time of sampling (Fig. 3d-f). At each time, the mass of litter remaining was greater in largely unmodified estuaries of the southern latitude, than in any of the other treatments (Fig. 3, Table 3).

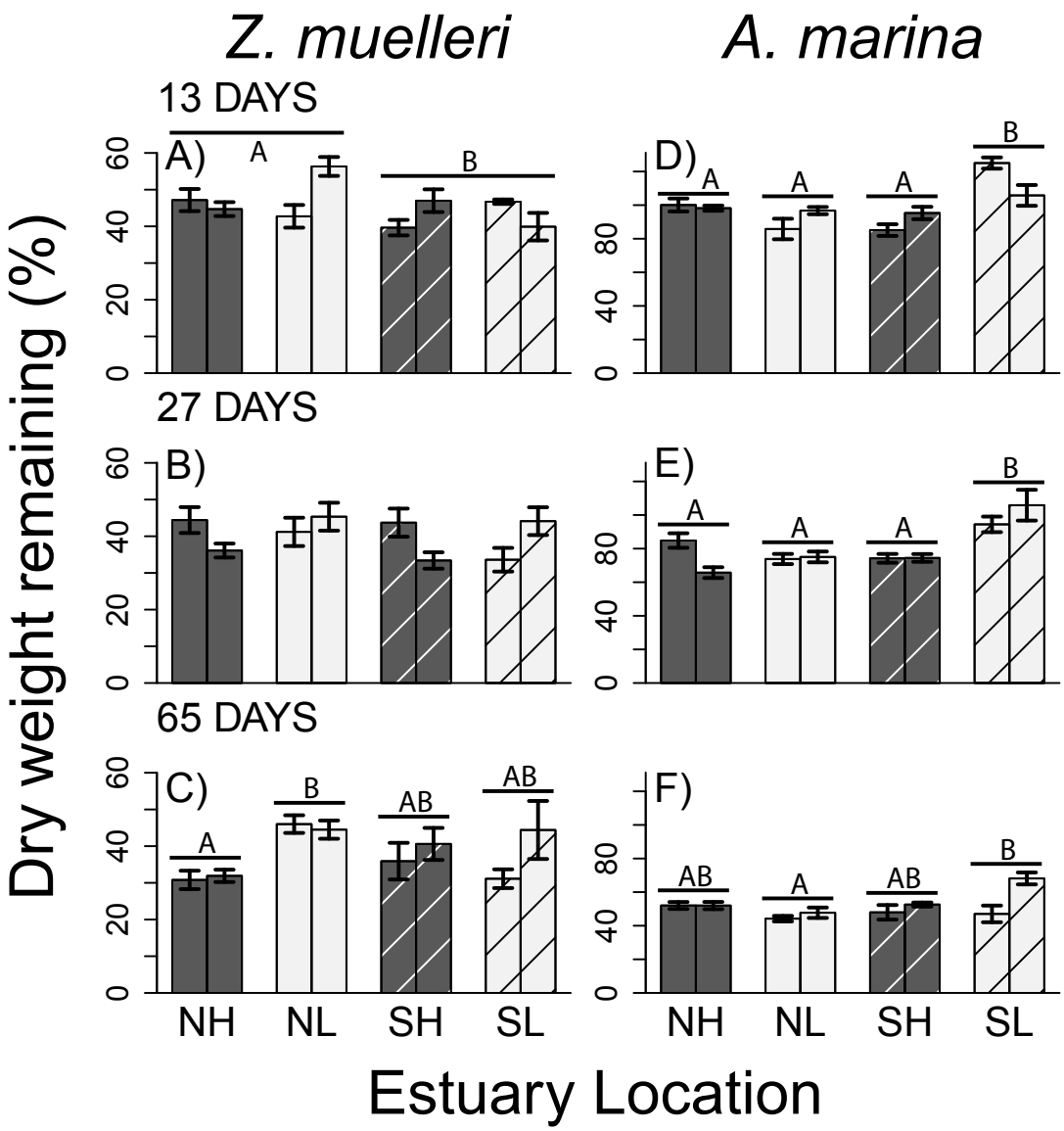

Fig. 3. Mean (± 1 SE) percentage of the initial litter weight remaining in monospecific litterbags of (A-C) *Zostera muelleri* and (D-F) *Avicennia marina* after 13 (A, D), 27 (B, E) and 65 (C, F) days. Bags were deployed in replicate estuaries (n = 2) of northern (N) and southern (S) New South Wales, Australia, with nutrient statuses that have been highly modified (H) and largely unmodified (L) by humans. Letters denote significant (at $\alpha = 0.05$) differences among treatments (Tukey HSD tests for significant ANOVAs).

Table 3. Analyses of variance assessing sources of spatial variation in the percentage of the initial dry weight of *Zostera muelleri* and *Avicennia marina* litter remaining after 13, 27 and 65 days. There were 3 factors specified within the model: Latitude (2 levels, fixed: north, south); Nutrient status (2 levels, fixed: highly modified, largely unmodified); and Estuary (2 levels, random, nested within Latitude and Estuary condition). Data were analyzed untransformed. Due to lost/erroneous data, this dataset was unbalanced resulting in variation among residual degrees of freedom.

	Zostera muelleri											
	13 days				**27 days**				**65 days**			
	df	**MS**	***F***	**P**	**df**	**MS**	***F***	**P**	**df**	**MS**	***F***	**P**
Latitude	1	236.8	5.027	**0.030**	1	135.3	1.880	0.178	1	0.4	0.005	0.946
Nutrient Status	1	37.7	0.799	0.376	1	50.9	0.707	0.405	1	795.2	9.678	**0.003**
Latitude x Nutrient status	1	45.8	0.972	0.330	1	15.7	0.218	0.643	1	558.2	6.793	**0.013**
Estuary (Lat x Nut)	4	238.2	5.056	**0.002**	4	230.7	3.206	**0.022**	4	116.1	1.413	0.247
Residual	42	47.1			43	72.0			40	82.2		
	Avicennia marina											
	13 days				**27 days**				**65 days**			
	df	**MS**	***F***	**P**	**df**	**MS**	***F***	**P**	**df**	**MS**	***F***	**P**
Latitude	1	752	6.982	**0.011**	1	1986	15.68	**<0.001**	1	314.9	5.281	**0.026**
Nutrient Status	1	709	6.578	**0.014**	1	1708	13.49	**0.001**	1	0.1	0.002	0.962
Latitude x Nutrient status	1	3530	32.77	**<0.001**	1	2291	18.10	**<0.001**	1	576.4	9.666	**0.003**
Estuary (Lat x Nut)	4	464	4.304	**0.006**	4	418.4	3.304	**0.019**	4	361.4	6.060	**0.001**
Residual	44	108			44	126.6			44	59.6		

MACROINVERTEBRATE LITTER COMMUNITIES

A total of 21,084 individuals from 60 different taxa were collected from litterbags. Of these, the ten amphipod taxa were collectively the most abundant, comprising approximately 90% of animals.

Macroinvertebrate communities differed significantly among estuaries, but over and above this variation, spatial variation from latitude and nutrient status was not detected (Table 4). SIMPER analysis revealed that the Isaeidae amphipod family contributed most to multivariate dissimilarity among estuaries.

Despite the non-significant differences between latitudes and nutrient statuses in the multivariate community data, the total abundance of invertebrates responded to the interacting effects of latitude and nutrient status in each of the *Z. muelleri* and *A. marina* litterbags, after 13 and 65 days (Table 5, Table 6). After 13 days, in both the *Z. muelleri* and *A. marina* bags, the total abundance of invertebrates was greater in the highly modified estuaries of the southern latitude than in any of the other treatments that, in turn, did not significantly differ (Fig. 4a, Fig. 5a). By 65 days, the total abundance of invertebrates in the highly modified estuaries of the southern latitude had becomes statistically indistinguishable to those of three of the other treatments and, instead, the bags deployed in the largely unmodified estuaries of the higher latitude contained fewer invertebrates than the bags deployed in other locations (Fig. 4b, Fig. 5b). Spatial patterns in the abundance of invertebrates were largely driven by differences in the abundance of leaf shredders, and in particular Isaeidae, (Fig. 4, Fig. 5). Separate analyses of each of these groups revealed the same spatial patterns in abundance as seen for total abundance (Table 5, Table 6).

Table 4. PERMANOVAs assessing sources of spatial variation in the macrofaunal community structure of *Zostera muelleri* and *Avicennia marina* litter deployed for 13 and 65 days. There were 3 factors specified within the model: Latitude (2 levels, fixed: north, south); Nutrient status (2 levels, fixed: highly modified, largely unmodified); and Estuary (2 levels, random, nested within Latitude and Nutrient status). Data were analyzed untransformed.

	Zostera muelleri							
	13 days				**65 days**			
	df	**MS**	**Pseudo-*F***	**P (perm)**	**df**	**MS**	**Pseudo-*F***	**P (perm)**
Latitude	1	7582	0.57	0.705	1	3841	0.52	0.660
Nutrient status	1	4275	0.32	0.983	1	14844	1.99	0.095
Latitude x Nutrient status	1	7805	0.59	0.725	1	11315	1.52	0.243
Estuary (Lat. X Nut.)	4	13369	8.33	**0.001**	4	7669	4.17	**0.001**
Residual	43	1606			40	1837		
	Avicennia marina							
	13 days				**65 days**			
	df	**MS**	**Pseudo-*F***	**P (perm)**	**df**	**MS**	**Pseudo-*F***	**P (perm)**
Latitude	1	9887	0.95	0.489	1	11393	0.89	0.381
Nutrient status	1	9374	0.90	0.521	1	9181	0.71	0.564
Latitude x Nutrient status	1	10344	1.00	0.440	1	8330	0.35	0.670
Estuary (Lat. X Nut.)	4	10440	7.25	**0.001**	4	12947	8.78	**0.001**
Residual	44	1440			44	1475		

Table 5. Analyses of variance assessing sources of spatial variation in the total abundance and richness of macrofauna, and the abundance of leaf shredders and of Isaeidae amphipods in *Zostera muelleri* litterbags after 13 and 65 days. There were 3 factors specified within the model: Latitude (2 levels, fixed: north, south); Nutrient status (2 levels, fixed: highly modified, largely unmodified); and Estuary (2 levels, random, nested within Latitude and Nutrient status). Data were analyzed untransformed. Due to lost/erroneous data, this dataset was unbalanced resulting in variation among residual degrees of freedom.

		***Zostera muelleri* at 13 days**											
		Total Abundance			**Total Richness**			**Shredders**			**Isaeidae**		
	df	**MS**	***F***	**P**	**MS**	***F***	**P**	**MS**	***F***	**P**	**MS**	***F***	**P**
Latitude	1	52919	4.87	**0.033**	2.93	0.57	0.454	21066	1.93	0.172	20509	1.89	0.177
Nutrient status	1	41976	3.86	0.056	4.60	0.90	0.349	50562	4.63	**0.037**	51890	4.78	**0.034**
Latitude x Nutrient status	1	128596	11.83	**0.001**	0.01	0.00	0.960	128669	11.79	**0.001**	135943	12.51	**<0.001**
Estuary (Lat. x Nut.)	4	152343	14.02	**<0.001**	33.11	6.46	**<0.001**	121562	11.14	**<0.001**	118162	10.88	**<0.001**
Residual	43	10868			5.13			10916			10863		
		***Zostera muelleri* at 65 days**											
		Total Abundance			**Total Richness**			**Shredders**			**Isaeidae**		
	df	**MS**	***F***	**P**	**MS**	***F***	**P**	**MS**	***F***	**P**	**MS**	***F***	**P**
Latitude	1	16139	3.81	0.058	0.09	0.01	0.913	3038	1.45	0.236	35	0.03	0.859
Nutrient status	1	474	0.11	0.740	64.67	9.15	**0.004**	298	0.14	0.708	4531	4.13	**0.049**
Latitude x Nutrient status	1	90977	21.47	**<0.001**	64.88	9.18	**0.004**	50078	23.91	**<0.001**	19731	18.00	**<0.001**
Estuary (Lat. x Nut.)	4	37919	8.95	**<0.001**	65.69	9.30	**<0.001**	16499	7.88	**<0.001**	4444	4.05	**0.007**
Residual	40	4238			282.61			2094			1096		

Table 6. Analyses of variance assessing sources of spatial variation in the total abundance and richness of macrofauna, and the abundance of leaf shredders and of Isaeidae amphipods in *Avicennia marina* litterbags after 13 and 65 days. There were 3 factors specified within the model: Latitude (2 levels, fixed: north, south); Nutrient status (2 levels, fixed: highly modified, largely unmodified); and Estuary (2 levels, random, nested within Latitude and Nutrient status). Data were analyzed untransformed.

		***Avicennia marina* at 13 days**											
		Total Abundance			**Total Richness**			**Shredders**			**Isaeidae**		
	df	**MS**	***F***	**P**	**MS**	***F***	**P**	**MS**	***F***	**P**	**MS**	***F***	**P**
Latitude	1	160903	11.06	**0.002**	2.31	0.56	0.460	117377	8.45	**0.006**	108562	7.83	**0.008**
Nutrient status	1	254660	17.50	**<0.001**	0.17	0.04	0.839	260997	18.79	**<0.001**	249369	18.00	**<0.001**
Latitude x Nutrient status	1	257575	17.70	**<0.001**	16.01	3.85	0.056	235544	16.96	**<0.001**	222055	16.02	**<0.001**
Estuary (Lat. x Nut.)	4	134235	9.23	**<0.001**	50.84	12.22	**<0.001**	102202	7.36	**<0.001**	94016	6.78	**<0.001**
Residual	44	14549			4.16			13888			13858		
		***Avicennia marina* at 65 days**											
		Total Abundance			**Total Richness**			**Shredders**			**Isaeidae**		
	df	**MS**	***F***	**P**	**MS**	***F***	**P**	**MS**	***F***	**P**	**MS**	***F***	**P**
Latitude	1	23264	13.87	**<0.001**	15.50	2.89	0.096	7083	6.51	**0.014**	4251	3.99	0.052
Nutrient status	1	1108	0.66	0.421	83.77	15.61	**<0.001**	4284	3.94	0.054	4963	4.66	**0.036**
Latitude x Nutrient status	1	24160	14.40	**<0.001**	2.37	0.44	0.509	10300	9.46	**0.004**	664	6.26	**0.016**
Estuary (Lat. x Nut.)	4	42495	25.34	**<0.001**	94.73	17.65	**<0.001**	20640	18.96	**<0.001**	14968	14.06	**<0.001**
Residual	44	1677			5.37			1089			1065		

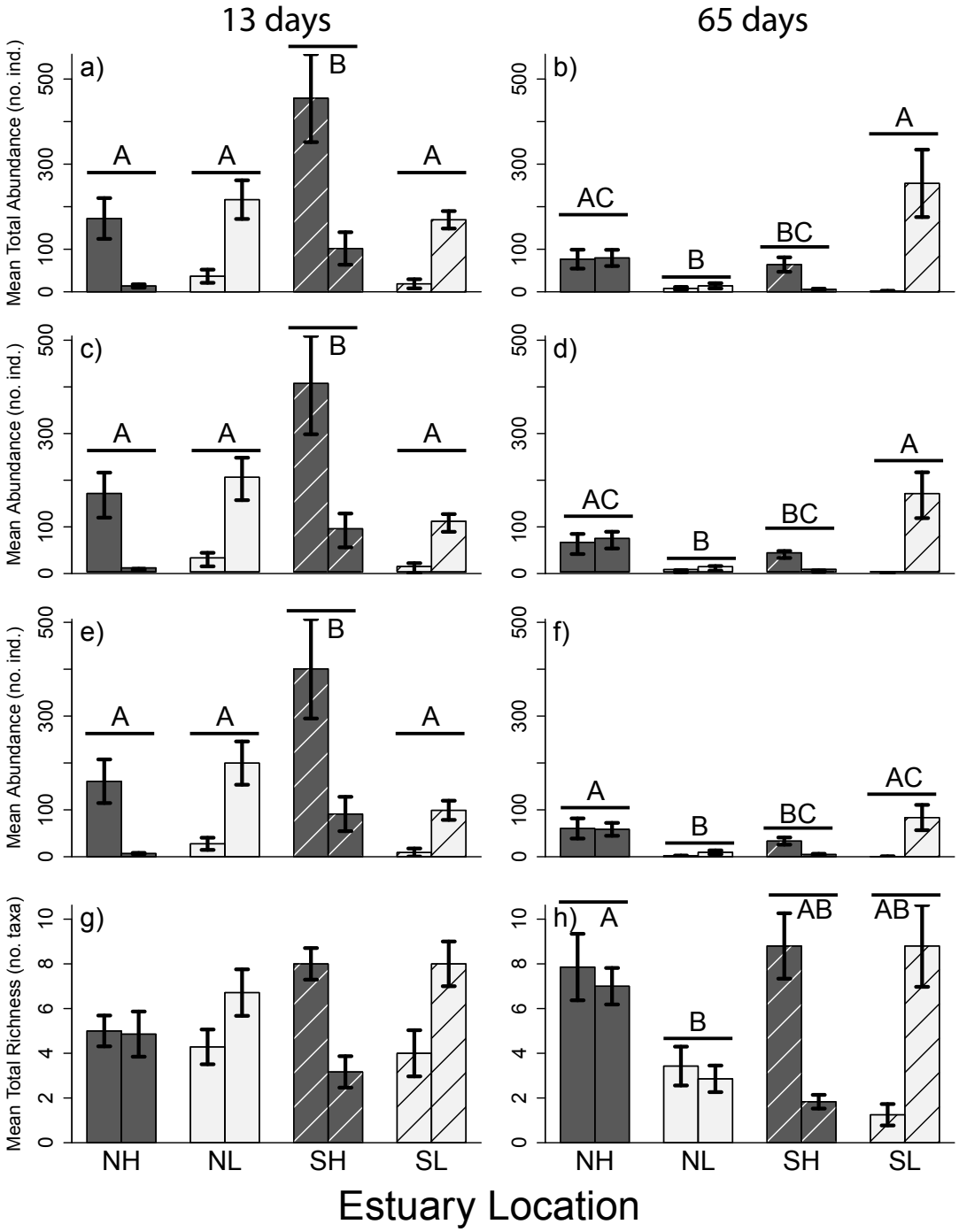

Fig. 4. Mean (± 1 SE) abundance of total invertebrates (A-B), shredders (C-D), Isaeidae amphipods (E-F) and total richness (G-H) in *Zostera muelleri* litterbags after 13 (A, C, E, G) and 65 (B, D, F, H) days. Bags were deployed in replicate estuaries (n = 2) of northern (N) and southern (S) New South Wales, Australia, with nutrient statuses that have been highly modified (H) and largely unmodified (L) by humans. Letters denote significant (at $\alpha = 0.05$) differences among treatments (Tukey HSD tests for significant ANOVAs).

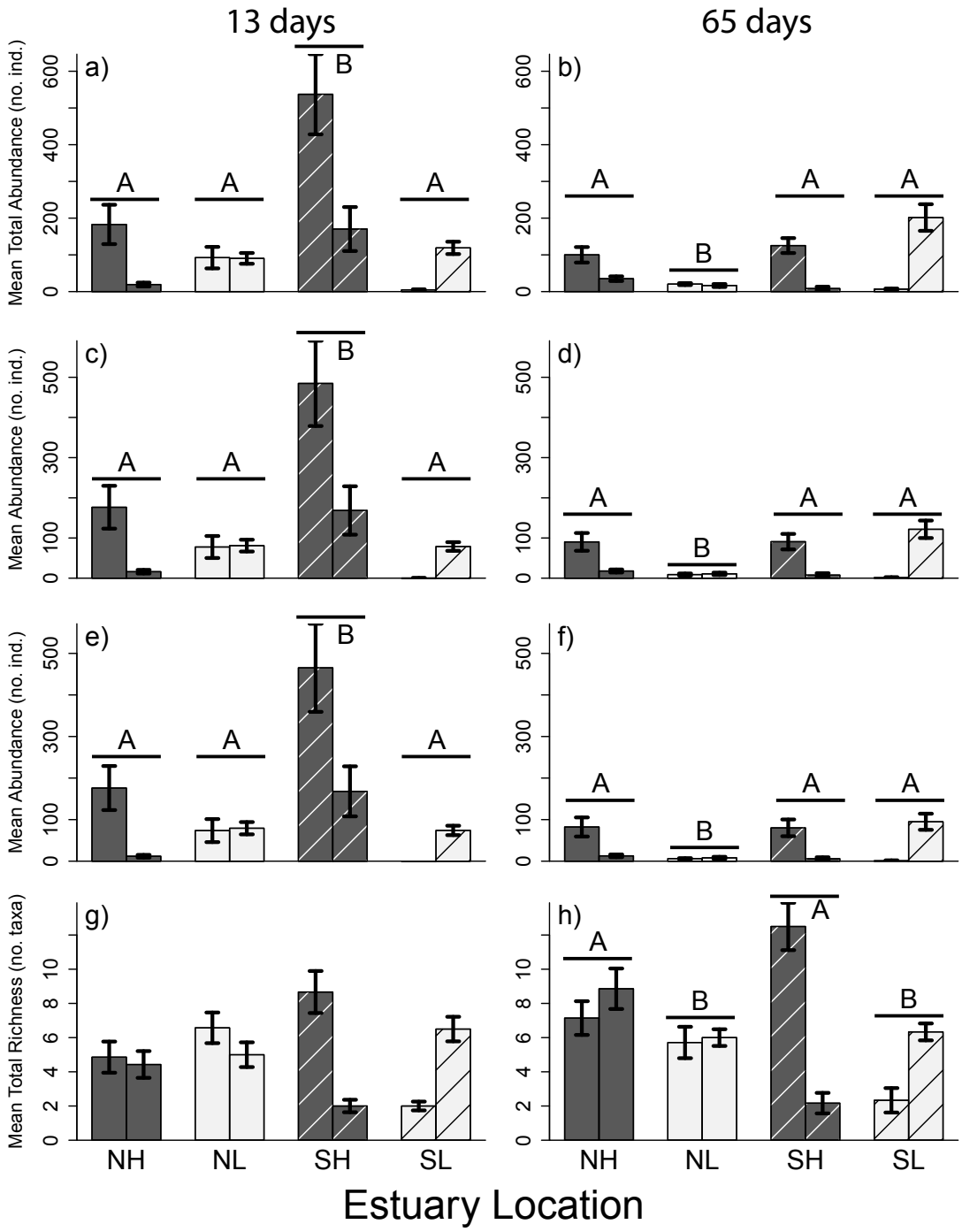

Fig. 5. Mean (± 1 SE) abundance of total invertebrates (A-B), shredders (C-D), Isaeidae amphipods (E-F) and total richness (G-H) in *Avicennia marina* litterbags after 13 (A, C, E, G) and 65 (B, D, F, H) days. Bags were deployed in replicate estuaries (n = 2) of northern (N) and southern (S) New South Wales, Australia, with nutrient statuses that have been highly modified (H) and largely unmodified (L) by humans. Letters denote significant (at $\alpha = 0.05$) differences among treatments (Tukey HSD tests for significant ANOVAs).

The taxon richness of macroinvertebrates, in contrast to their abundance, only differed significantly among latitudes and estuary conditions after 65 days (Table 5, Table 6). In each of the *Z. muelleri* and *A. marina* litterbags, richness was less in largely unmodified estuaries of the lower latitude than in the highly modified estuaries, at either latitude (Fig. 4h, Fig. 5h). In the case of *Z. muelleri* litter, the richness of macroinvertebrates was also lower in the largely unmodified estuaries of the lower latitudes than in the largely unmodified estuaries of the higher latitudes. In the *A. marina* litterbags, richness was statistically indistinguishable between these two treatments (Fig. 4h, Fig. 5h).

RELATIONSHIP BETWEEN LITTER DECOMPOSITION AND BIOTIC AND ABIOTIC VARIABLES

There was no significant correlation between the remaining dry weight of *Z. muelleri* litter and the multivariate matrix of environmental variables at 13, 27 or 65 days (RELATE; 13 days: ρ = -0.253, p = 0.795; 27 days: ρ = -0.109, p = 0.698; 65 days: ρ = -0.042, p = 0.569). Similarly, there was no significant correlation between the dry weight of *A. marina* litter and the environmental matrix at 13 days (RELATE: ρ = 0.381, p=0.167). At 27 days, however, a significant correlation between the dry weight of *A. marina* litter and the environmental variables was detected (RELATE: ρ = 0.868, p=0.003) with approximately 86.8% of the spatial variability in dry weight of the mangrove litter explained by the T:N, area, flushing time and total rainfall of each estuary, during the second period (0-27 days). This correlation was still evident at day 65 (RELATE: ρ = 0.626, p=0.030) with approximately 68.7% of the spatial variability in litter dry weight explained by the area and flushing time of each estuary, during the third period (0-65 days).

The remaining dry weight of *Z. muelleri* litter at 13 days did not correlate with macroinvertebrate community structure (RELATE: ρ = -0.110, p > 0.05). By day 65, a weak correlation between the dry weight of *Z. muelleri* litter and macroinvertebrate communities

was, however, evident (RELATE: ρ = 0.109, p = 0.018) where the amphipods *Corophium* sp., Isaeidae, and *Tickalerus* sp.; and the annelids *Capitellidae* sp. and *Nereis (Hediste) diversicolor* were the five taxa that collectively explained the most variation in *Z. muelleri* litter, although cumulatively only 8.6%. A significant relationship between *A. marina* litter mass and macroinvertebrate community structure was evident after 13 and 65 days (RELATE; 13 days: ρ = 0.294, p = 0.001; 65 days: ρ = 0.149, p = 0.005). At 13 days the amphipods *Corophium* sp., Isaeidae, Oedicerotidae and *Tickalerus* sp. as well as the gastropod *Rissonia rhyllensis* accounted for approximately 33% of the variability in the dry weight of *A. marina* litter. At 65 days the cumacean *Dimorphostylis* sp., the decapod *Sesarma* sp., the gastropod *Trochidae* sp. and the isopod *Anthuridae* sp. accounted for approximately 20.8% of the variability in the dry weight of *A. marina* litter.

Discussion

We found interacting effects of latitude and human modification of estuarine nutrient statuses on the decomposition of detrital material. Over a 65-day period, leaf litter from the eelgrass *Z. muelleri* displayed a greater rate of decomposition in highly modified estuaries of the lower latitudes, than in higher latitudes or largely unmodified estuaries. Litter from the mangrove *A. marina*, by contrast decomposed more slowly in largely unmodified higher-latitude estuaries than in lower-latitude or highly modified estuaries. Hence, overall litter decomposition was enhanced by a warmer climate, and by human modification of estuarine nutrient statuses, sometimes interactively. Our experiments, which utilized leaf litter from a common source, suggested that this pattern was at least partially mediated by spatial differences in communities of macroinvertebrates, mainly leaf shredders and detritivores.

In contrast to previous studies (eg. Blair et al. 1990, Moore and Fairweather 2006, Wardle et al. 1997), we did not detect non-additive effects of litter mixing on decomposition rates. On the contrary, we found no significant difference in the rate of decomposition of

either *Z. muelleri* or *A. marina* when decomposing on its own versus in a mixture. Previous studies investigating effects of litter mixing sourced all litter resources from the same community (e.g. seagrasses; Moore and Fairweather 2006). Our study, by contrast, mixed two litter resources sourced from different ecological communities (a seagrass bed and a mangrove stand). It is possible that these litter resources were sufficiently different that microbial spillover, which is often implicated as the driver of non-additive effects (Moore and Fairweather 2006, Smith and Bradford 2003), could not occur.

As expected, based on differences in their chemistry (Pérez-Harguindeguy et al. 2000), *Z. muelleri* and *A. marina* litter displayed very different patterns of decomposition. *Zostera muelleri* litter, which has a lower carbon to nitrogen ratio (10.0-24.9; L. Ainley unpublished data) than *A. marina* (15.7-28.1), decomposed considerably faster than the latter. Differences between the two species in chemical properties not tested here, such as the lignin content and/or the proportion of refractory material, also likely contributed to differences in decomposition. *Zostera muelleri* decomposition followed an exponential decay trend (Olson 1963) consisting of a period of rapid mass loss, to which leaching likely contributed, followed by a slowing of decomposition as the litter becomes more recalcitrant (Berg 2000). The *A. marina* litter by contrast, displayed a more consistent rate of decomposition through time. Overall, the mean decay constants calculated for each litter source during this study (mean ± SE; *Z. muelleri* = 0.0194 ± 0.0009; *A. marina* = 0.0096 ± 0.0009) fell within the range of previously published values (Z. muelleri = 0.0115; A. marina = 0.007-0.012, Enríquez et al. 1993, Tam et al. 1998).

Whereas the pattern displayed by *A. marina* litter of least mass in the higher-latitude, largely unmodified estuaries was established by the first sampling time (13 days) and persisted throughout the study, *Z. muelleri* displayed different spatial patterns of mass loss at each of the sampling times. Initially *Z. muelleri* displayed greater mass loss at the higher latitudes, irrespective of estuarine modification, but by the end of the experiment, after 65

days of decomposition, the low latitude highly modified estuaries had less litter remaining than any of the other treatments. Temporal variation in the spatial patterns displayed by *Z. muelleri* but not *A. marina* decomposition may be explained by the differing contribution of leaching to the mass loss of each. Whereas *Z. muelleri* appeared to display significant leaching of soluble compounds early in its decay, this was not the case with *A. marina*. Hence, whereas the dominant control on the decomposition of *Z. muelleri* likely shifted from physical, during initial leaching, to biotic during later breakdown of recalcitrant material (see Chapin et al. 2002), breakdown of the more refractory *A. marina* presumably relied on biotic processing, even early on. Furthermore, *Z. muelleri* decomposition was driven by latitudinal differences at the start of the experiment and, by the end of the experiment, by both latitude and estuarine modification. This suggests that climatic variables may have a greater influence, compared to estuarine modification, during the initial phases of decomposition and that the role of nutrient enrichment may strengthen during the decomposition of more refractory material at later stages, either directly or by influencing/enhancing biotic communities. Consistent with this hypothesis, macroinvertebrate community structure was correlated to *A. marina* litter decomposition at both 13 and 65 days, but to *Z. muelleri* litter decomposition only at 65 days. By contrast, the relationship between environmental variables and *Z. muelleri* litter mass, though non-significant at each sampling time, was strongest early in the study and weakened through time.

Across all sites, the decomposer community was dominated by amphipods, especially of the family Isaeidae, which accounted for 90% of total faunal abundance. Many genera within the Isaeidae have enlarged gnathopods with a tooth on the end and well developed mandibles (Lowry 1972, Myers 1995) which enables them to shred detrital material. At the first sampling time, 13 days into the study, the greatest abundances of this taxon were observed among litterbags deployed within the highly modified estuaries of the higher latitudes, a pattern that was evident irrespective of litter source. The greater abundance of

this taxon in highly modified estuaries of the higher latitudes may explain the faster decomposition rates of the mangrove litter in these estuaries – a pattern that was established early in our study. By the second sampling time, at 65 days, this taxon no longer differed in abundance between highly modified and largely unmodified estuaries at higher latitudes, but instead displayed a pattern of greater abundance in the highly modified estuaries of the lower latitudes. This pattern was the converse to that displayed by the mass of *Z. muelleri* remaining at this latitude. Hence, it is likely that spatial differences in the decomposition of litter were partially explained by spatial differences in the decomposer community, and in particular, the abundance of the shredders of the family Isaeidae.

The design of our mensurative study did not allow us to disentangle effects of temperature from those of other co-varying climatic variables or the effects of nutrient enrichment from other co-occuring anthropogenic stressors. Based on the results of previous studies (eg: Mckee 1995, Hobbie 1996, Reichstein et al. 2000, Feller et al. 2003, Lovelock et al. 2004), however, we expect that the 4.5°C difference in water temperature between low and high latitude estuaries, and the difference in nutrient loading between highly modified and largely unmodified estuaries likely contributed. The previous studies have shown nutrient enrichment to accelerate the rate of decomposition of both seagrass (Duarte 1990, Lee et al. 2004) and mangrove leaves. Temperature accelerates decomposition rates in both terrestrial and aquatic systems (Hobbie 1996, Reichstein et al. 2000). Consistent with interacting effects of these two factors, we found that the most rapid decomposition of the seagrass litter was in the warmest estuaries, most influenced by human development (low latitude, highly modified) and the slowest decomposition of mangrove leaves was in the coolest estuaries, least influenced by human development (high latitude, largely unmodified). Hence, to our knowledge, our study is the first to cross latitude and human development, and the associated temperatures and nutrient statuses, at an ecologically realistic scale. It is unclear why, at the lower latitude, estuarine modification was associated with accelerated

decomposition of seagrass litter, but at the higher latitude decomposition of mangrove litter was slower in the less modified estuaries. This might be because in the warmer latitudinal zone, *A. marina* decomposition is already occurring at its maximal rate, independent to the effect of nutrient enrichment. This idea of a decomposition limit is not entirely new and has been assessed in the terrestrial literature (Berg et al. 1996, Dalias et al. 2001, Reichstein et al. 2000), but has not been previously hypothesized for other systems.

Our finding that decomposition rates vary with latitude and human modification of estuaries raises the possibility that ongoing climate change and coastal development could have large-scale impacts on carbon cycling in estuaries. If the effects of latitude are indeed driven by temperature, our results suggest that as temperatures rise, the impact of anthropogenic pressures on our coastal systems could accelerate rates of decomposition. Accelerated rates of detrital decomposition will release stored carbon to the atmosphere and may deplete sediment oxygen resources (Davidson and Janssens 2006). In extreme cases, this may lead to the development of hypoxic conditions and a reduction in biodiversity, fisheries resources and ecosystem functioning, which could also be exacerbated by increased primary productivity and detrital load (Diaz and Rosenberg 2008). Hence, adaptation of estuarine ecosystems to climate warming may be contingent to reducing or capping anthropogenic nutrient loads to a level below which strong synergistic interactions occur. Knowledge of synergistic effects of temperature and nutrient enrichment on decomposition processes will be crucial for coastal managers to understand potential changes to ecosystem functioning and avoid or mitigate potential effects of accelerated decomposition and reduced sediment oxygen conditions.

Acknowledgements

This research was funded by an ARC Discovery Grant (DP1093444) to M. Bishop and Macquarie University Higher Degree Research funding to L. Ainley. We thank J.

Rowland, B. Coates, E. Wilkie, M. Verhoeven, K. Dormer, S. Towler and A. Nicastro for assistance with field and lab work.

References

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. - Oikos 79: 439-449.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. - Austral Ecology 26: 32-46.

Barnard, J. L. and Karaman, G. S. 1991. The Families and Genera of Marine Gammaridean Amphipoda (Except Marine Gammaroids) Part 1. Records of the Australian Museum, Supplement, 13.

Bedford, A. P. 2004. A modified litter bag design for use in lentic habitats. - Hydrobiologia 529: 187-193.

Berg, B. 2000. Litter decomposition and organic matter turnover in northern forest soils. - Forest Ecology and Management 133: 13-22.

Berg, B., et al. 1996. Maximum decomposition limits of forest litter types: A synthesis. - Canadian Journal of Botany-Revue Canadienne De Botanique 74: 659-672.

Beesley, P. L., et al., 2000. Polychaetes & Allies: The Southern Synthesis, CSIRO Publishing.

Beesley, P. L., et al., 1998. Mollusca: The Southern Synthesis, Australian Government Publishing Service.

Bishop, M. J. and Kelaher, B. P. 2008. Non-additive, identity-dependent effects of detrital species mixing on soft-sediment communities - Oikos 117:531-542.

Blair, J. M., et al. 1990. Decay rates, nitrogen fluxes, and decomposer communiies of single- and mixed-species foliar litter. - Ecology 71: 1976-1985.

Bradford, M. A., et al. 2002. Microbiota, fauna, and mesh size interactions in litter decomposition. - Oikos 99: 317-323.

Bureau of Meteorology. 2012. Climate Data Online. - Bureau of Meteorology.

Canfield, D. E. 1994. Factors influencing organic carbon preservation in marine sediments. - Chemical Geology 114: 315-329.

Cebrián, J. 1999. Patterns in the Fate of Production in Plant Communities. - The American Naturalist 154: 449-468.

Chapin, F. S., et al. 2002. Terrestrial decomposition. - In: Chapin, F. S. and Matson, P. A. (eds.), Principles of Terrestrial Ecosystem Ecology. Springer-Verlag, pp. 151-175.

Clarke, K. R. and Warwick, R. M. 1994. Change in marine communities: an approach to statistical analysis and interpretation. - Natural Environment Research Council Plymouth.

Conant, R. T., et al. 2011. Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. - Global Change Biology 17: 3392-3404.

Conant, R. T., et al. 2008. Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. - Ecology 89: 2384-2391.

Conn, C. and Dighton, J. 2000. Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. - Soil Biology & Biochemistry 32: 489-496.

Coûteaux, M. M., et al. 1995. Litter decomposition, climate and litter quality. - Trends in Ecology & Evolution 10: 63-66.

Dalias, P., et al. 2001. Long-term effects of temperature on carbon mineralisation processes. - Soil Biology and Biochemistry 33: 1049-1057.

Davidson, E. A. and Janssens, I. A. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. - Nature 440: 165-173.

Diaz, R. J. and Rosenberg, R. 2008. Spreading dead zones and consequences for marine ecosystems. - Science 321: 926-929.

Duarte, C. M. 1990. Seagrass Nutrient Content. - Marine Ecology Progress Series 67: 201-207.

Dye, A. H. 2006. Inhibition of the Decomposition of *Zostera capricorni* Litter by Macrobenthos and Meiobenthos in a Brackish Coastal Lake System. - Estuaries and Coasts 29: 802-809.

Enríquez, S., et al. 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. - Oecologia 94: 457-471.

Feller, I. C., et al. 2003. Nitrogen limitation of growth and nutrient dynamics in a disturbed mangrove forest, Indian River Lagoon, Florida. - Oecologia 134: 405-414.

Fenchel, T., et al. 1998. Bacterial Biogeochemistry: the Ecophysiology of Mineral Cycling. - Academic Press.

Gessner, M. O., et al. 2010. Diversity meets decomposition. - Trends in Ecology & Evolution 25: 372-380.

Hättenschwiler, S., et al. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. - Annual Review of Ecology Evolution and Systematics. 36: 191-218.

Hemminga, M. A. and Buth, G. J. C. 1991. Decomposition in salt marsh ecosystems of the S.W. Netherlands: the effects of biotic and abiotic factors. - Vegetatio 92: 73-83.

Hobbie, S. E. 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. - Ecological Monographs 66: 503-522.

IPCC. 2013. Summary for Policymakers. - In: Stocker, T. F., et al. (eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change

Jones, D. S. and Morgan, G. J. 2002. A Field Guide to Crustaceans of Australian Waters, Reed New Holland.

Kirschbaum, M. U. F. 1995. The Temperature-Dependence of Soil Organic-Matter Decomposition, and the Effect of Global Warming on Soil Organic-C Storage. - Soil Biology & Biochemistry 27: 753-760.

Kristensen, E., et al. 1995. Aerobic and Anaerobic Decomposition of Organic Matter in Marine Sediment: Which is Fastest? - Limnology and Oceanography 40: 1430-1437.

Lee, K. S., et al. 2004. Development of a nutrient pollution indicator using the seagrass, Zostera marina, along nutrient gradients in three New England estuaries. - Aquatic Botany 78: 197-216.

Loreau, M., et al. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. - Science 294: 804-808.

Lovelock, C. E., et al. 2004. The effect of nutrient enrichment on growth, photosynthesis and hydraulic conductance of dwarf mangroves in Panamá. - Functional Ecology 18: 25-33.

Lowry, J. K. 1972. Taxonomy and Distribution of Microprotopus along the East Coast of the United States (Amphipoda, Isaeidae). - Crustaceana. Supplement: 277-286.

Mckee, K. L. 1995. Interspecific Variation in Growth, Biomass Partitioning, and Defensive Characteristics of Neotropical Mangrove Seedlings - Response to Light and Nutrient Availability. - American Journal of Botany 82: 299-307.

Moore, J. C., et al. 2004. Detritus, trophic dynamics and biodiversity. - Ecology Letters 7: 584-600.

Moore, T. N. and Fairweather, P. G. 2006. Decay of multiple species of seagrass detritus is dominated by species identity, with an important influence of mixing litters. - Oikos 114: 329-337.

Myers, A. A. 1995. Amphipoda (Crustacea) of Madang Lagoon: Aoridae, Isaeidae, Ischyroceridae and Neomegamphopidae. - Records of the Australian Museum, Supplement 22.

Nicastro, A., et al. 2012. Direct and indirect effects of tidal elevation on eelgrass decomposition. - Marine Ecology Progress Series 456: 53-62.

NSWDPI. 2011. Estuarine Habitats. - NSW Department of Primary Industries.

Olson, J. S. 1963. Energy Storage and the Balance of Producers and Decomposers in Ecological Systems. - Ecology 44: 322.

Pérez, J., et al. 2013. Leaf-litter processing in headwater streams of northern Iberian Peninsula: moderate levels of eutrophication do not explain breakdown rates. - Hydrobiologia 718: 41-57.

Pérez-Harguindeguy, N., et al. 2000. Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. - Plant and Soil 218/2: 21-30.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. - Annual Review of Ecology and Systematics 28:289-316.

Reichstein, M., et al. 2000. Temperature dependence of carbon mineralisation: conclusions from a long-term incubation of subalpine soil samples. - Soil Biology & Biochemistry 32: 947-958.

Roper, T., et al. 2011. Assessing the condition of estuaries and coastal lake ecosystems in NSW. Monitoring, evaluation and reporting program, Technical report series. - Office of Environment and Heritage.

Roy, P. S., et al. 2001. Structure and function of south-east Australian estuaries. - Estuarine, Coastal and Shelf Science 53: 351-384.

Schmitz, O. J. 2013. Global climate change and the evolutionary ecology of ecosystem functioning. - Annals of the New York Academy of Sciences 1297: 61-72.

Silver, W. L. and Miya, R. K. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. - Oecologia 129: 407-419.

Smith, V. C. and Bradford, M. A. 2003. Do non-additive effects on decomposition in litter-mix experiments result from differences in resource quality between litters? - Oikos 102: 235-242.

Snelgrove, P. V. R., et al. 2000. Linking biodiversity above and below the marine sediment-water interface. - Bioscience 50: 1076-1088.

Suberkropp, K., et al. 2010. Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream: Results of a 5-year continuous enrichment. - Limnology and Oceanography 55: 149-160.

Swift, M. J., et al. 1979. Decomposition in Terrestrial Ecosystems. - University of California Press.

Tam, N. F. Y., et al. 1998. Litter production and decomposition in a subtropical mangrove swamp receiving wastewater. - Journal of Experimental Marine Biology and Ecology 226: 1-18.

Van Ryckegem, G., et al. 2007. Fungi on leaf blades of *Phragmites australis* in a brackish tidal marsh: diversity, succession, and leaf decomposition. - Microbial Ecology 53: 600-611.

Wardle, D. A. 2006. The influence of biotic interactions on soil biodiversity. - Ecology Letters 9: 870-886.

Wardle, D. A., et al. 1997. Biodiversity and Plant Litter: Experimental Evidence Which Does Not Support the View That Enhanced Species Richness Improves Ecosystem Function. - Oikos 79: 247-258.

Webster, J. R. and Benfield, E. F. 1986. Vascular Plant Breakdown in Freshwater Ecosystems. - Annual Review of Ecology and Systematics 17: 567-594.

Wetzel, R. G. 1995. Death, Detritus, and Energy-Flow in Aquatic Ecosystems. - Freshwater Biology 33: 83-89.

Zhang, D. Q., et al. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. - Journal of Plant Ecology 1: 85-93.

4. RELATIONSHIPS BETWEEN INTRASPECIFIC VARIATION IN LEAF TRAITS AND DECOMPOSITION VARY BETWEEN SPECIES.

Lara B. Ainley[1], Magali Raybaud[2], Adriana Verges[3], Melanie J. Bishop[1]*

[1] Department of Biological Sciences, Macquarie University, North Ryde NSW 2109, Australia

[2] Agro Campus Ouest, 65 Rue de Saint Brieuc CS 84215, 305042 Rennes Cedex, France

[3] Department of Biological, Earth and Environmental Sciences, The University of New South Wales, Kensington NSW 2052, Australia

* Corresponding Author: Lara Ainley, Ph: +612 9850 6285

Email addresses: lara.ainley@students.mq.edu.au (L. Ainley),

magali06.r@gmail.com (M. Raybaud),

a.verges@unsw.edu.au (A. Vergés),

melanie.bishop@mq.edu.au (M. Bishop)

Abstract

Climatic setting may influence decomposition processes directly by determining the biotic environment for decomposition, and indirectly by determining leaf traits. Previous studies indicate that, across species, there are strong relationships between latitude and leaf traits that mediate decomposition rates, but whether within-species spatial variation in leaf traits also mediates decomposition rates is less well understood. Here, we examined intraspecific variability in the leaf traits of seagrass (*Zostera muelleri*) and mangrove (*Avicennia marina*) across a gradient of 5° latitude in eastern Australia. We assessed how this spatial variation contributed to differences in the decomposition rate of litter in a common garden experiment. Both seagrass and mangrove leaves from lower latitudes were tougher, had lower nitrogen contents and greater carbon to nitrogen ratios than litter from higher latitudes. Despite their similar spatial patterns of intraspecific variation in leaf traits, in the common garden experiment, the two species displayed differing relationships between leaf traits and decomposition. Seagrass litter from the higher latitude decomposed more quickly than the lower-nitrogen and tougher litter from the lower latitude. By contrast, the lower-nitrogen and tougher mangrove litter from the lower latitude decomposed faster than the mangrove litter from the higher latitude. Hence, in addition to directly influencing decomposition, climate may also indirectly influence decomposition by influencing intraspecific variation in leaf traits. Nevertheless, our finding that the relationship between leaf traits and decomposition rate varies among species suggests that intraspecific relationships between climate, leaf traits and decomposition cannot necessarily be inferred from interspecific relationships.

Keywords: decomposition; seagrass; mangrove; latitude; interspecific variation

Introduction

The decomposition of plant material is a key process in the carbon cycle (Swift et al. 1979). In many ecosystems, only a small proportion of carbon, fixed by primary producers, is grazed and most enters detrital pathways (Moore et al. 2004; Wetzel 1995). Some detritus is mobilized by consumers such as bacteria, fungi and detritivores (Enríquez et al. 1993). The carbon is then either transferred to higher trophic levels or released back to the atmosphere through respiration (Persson et al. 1980). Recalcitrant components, however, accumulate, storing carbon (Cebrián 1999). Understanding those processes that influence whether detritus is decomposed or stored is therefore important for understanding carbon cycling (Kirschbaum 2006; Rice and Tenore 1981; Schimel et al. 1994).

Together, litter quality, climatic conditions and decomposer communities regulate rates of detrital decomposition (Aerts 1997; Melillo et al. 1982; Pérez-Harguindeguy et al. 2000). Among abiotic variables, latitude, mean annual temperature and mean annual rainfall have been identified as important correlates of decomposition in terrestrial environments (Zhang et al. 2008). Hydrodynamic conditions and water quality are also important for decomposition in aquatic environments (Jenkins and Suberkropp 1995; Leroy and Marks 2006). The effects of abiotic variables on decomposition may arise indirectly through their influence on decomposer communities (Feld and Hering 2007), or alternatively through their influence on leaf traits (Cornwell et al. 2008). Leaves display predictable variation in key traits such as size, carbon to nitrogen ratio (C:N) and toughness across latitudes and climatic regimes (Ordoñez et al. 2009; Reich and Oleksyn 2004; Wright et al. 2004). Leaf traits such as carbon, nitrogen and lignin contents, toughness, morphology, and the concentration of secondary metabolites are in turn important in determining palatability to decomposers, and hence, decomposition rates (Cornelissen et al. 1999; Cornwell et al. 2008; Melillo et al. 1982). Among these, the C:N is considered the most important trait influencing

decomposition, with lower ratios correlating to faster decomposition (Enríquez et al. 1993; Pérez-Harguindeguy et al. 2000; Zhang et al. 2008).

Our knowledge of the relationship between climate, leaf traits and decomposition rates has primarily arisen from studies comparing mean trait values among species (Cornwell et al. 2008; Wright et al. 2004). By contrast, the contribution of intraspecific variability to patterns of decomposition has received little attention (Garnier et al. 2001; Roche et al. 2004). Leaf traits are highly variable, both within and among species (Albert et al. 2010), and typically reflect the plant's environmental setting (Ackerly et al. 2000; Lee et al. 2007). Although, historically it was assumed that the consequences of intraspecific variation were much smaller than interspecific variation, a growing number of studies have suggested that intraspecific variability can also have large influences on plant communities (e.g. Boege and Dirzo 2004; Lecerf and Chauvet 2008). Consequently, there is need for greater quantification of the patterns and ecological consequences of intraspecific variation in leaf traits (Albert et al. 2010).

Here, we consider: (1) whether the leaves of two key, estuarine macrophytes display intraspecific variation in traits between two latitudes and (2) how intraspecific variability translates to differences in decomposition rate. The seagrass *Zostera muelleri* and the mangrove *Avicennia marina* are two broadly distributed aquatic macrophytes along the east coast of Australia that are major contributors to detrital pools of estuaries. Each displays considerable intraspecific variability in leaf traits across their ranges (see chapter 2). Based on interspecific variation in leaf traits across latitudinal gradients (Makkonen et al. 2012), we hypothesize that, for each species, leaf size, toughness and C:N will be smaller, and nitrogen content greater at higher than lower latitude. Consequently, we expect that when deployed at a common site, the litter from the higher latitude will decompose faster than the litter from the lower latitude.

Materials and methods

LITTER SOURCES

Leaf litter of the seagrass *Zostera muelleri* and the mangrove *Avicennia marina* was collected from each of two estuaries on the north (hereafter 'lower latitude' [L]; Sandon River: 29.673°S, 153.333°E and Wooli River: 29.888°S, 153.268°E) and two on the south (hereafter 'higher latitude' [H]; Jervis Bay: 35.104°S, 150.787°E and St Georges Basin: 35.185°S, 150.594°E) coast of New South Wales (NSW), Australia in September 2013 (Fig. 1). All four estuaries were classified as largely unmodified by human activities (Roper et al. 2011) and each was an intermediate to mature, wave-dominated estuary or embayment with predominantly open entrance conditions (Roy et al. 2001). The lower and higher latitude estuaries differed in mean air temperature by approximately 5.4°C during summer months (Dec, Jan, Feb) and 4.6°C during winter months (Jun, Jul, Aug; Bureau of Meteorology 2014). Within each estuary, leaves were collected fresh from randomly selected plants at a single site. Seagrass leaves were collected from a tidal elevation of mean spring low water (MSLW) -0.4-0.2 m and mangrove leaves from a tidal elevation of MSLW 0.8-1.2 m.

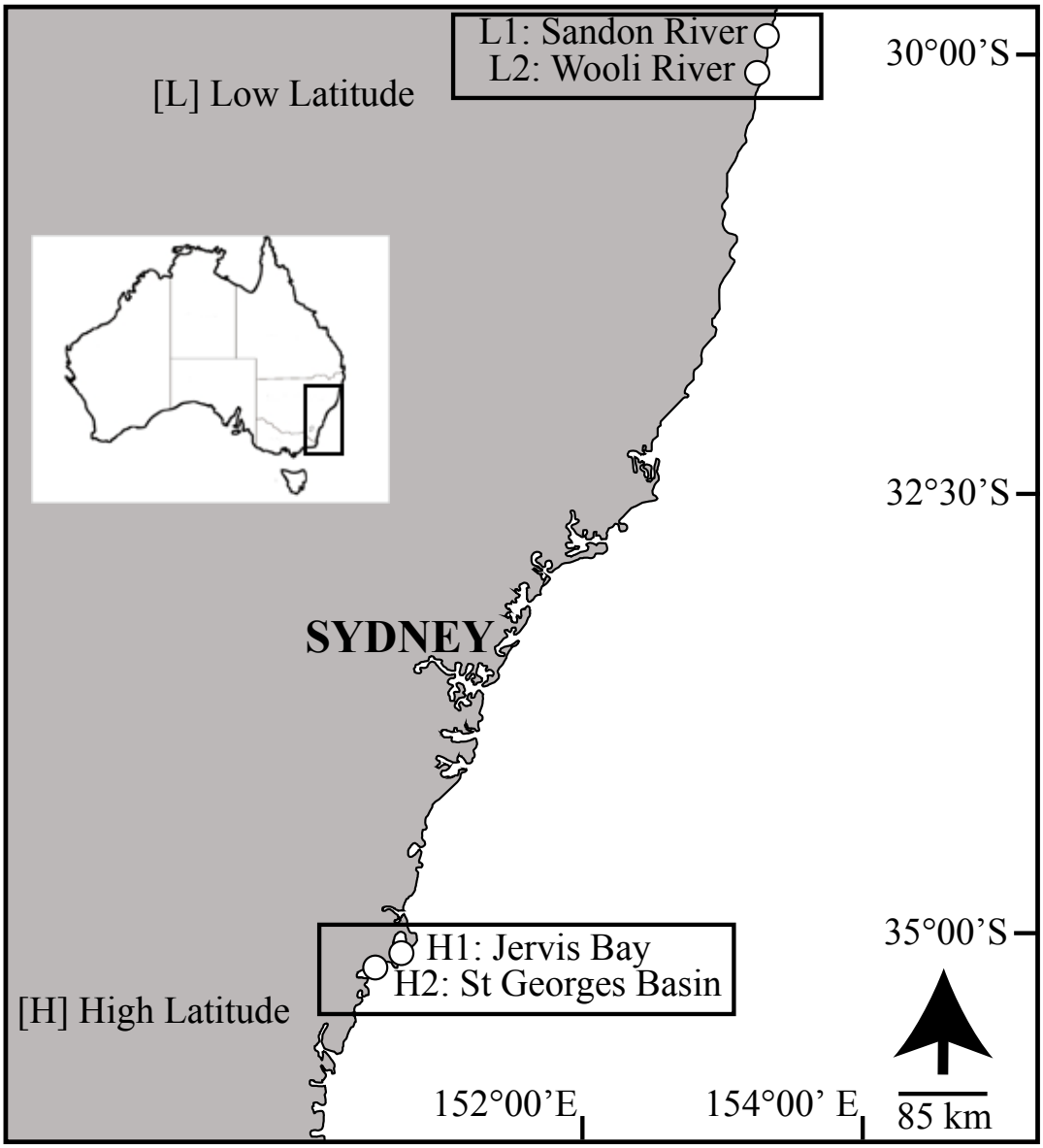

Fig. 1 Locations of estuaries at the lower [L] and higher [H] latitudes of NSW, Australia, where seagrass and mangrove leaves were collected.

We estimated the leaf traits of each litter source using a sub-sample of fresh material from each site. We quantified the morphology of five randomly selected mangrove leaves and seagrass blades per site by measuring the maximal width and thickness of each (to the nearest 0.01 mm) using digital calipers, and the length (to the nearest 1 mm) from shoot base (defined by a growth scar) to apex for seagrass and from petiole to leaf tip for mangrove (Cornelissen et al. 2003) using a ruler. The maximum load (N) that could be sustained by each leaf prior to failure, and the corresponding extension (mm), was assessed using an Instron Universal Testing System (5542, INSTRON, Massachusetts, USA). To enable calculation of these metrics, leaves were placed lengthways between two pneumatic clamps that gradually separated at a rate of 0.17 $mm.s^{-1}$ until the leaf broke. Force and extension were recorded every 0.1 s during each trial. The load (*N*) and extension (*ex*) at failure were used to calculate the toughness (the energy that the leaf can absorb before breakage) as the area under the force by extension curve. Leaves were then weighed, dried at 60°C to constant weight and re-weighed to determine the wet-weight to dry-weight conversion factor and ground into a fine powder. Sub-samples (2.5-3.5 mg; n = 5 per estuary) of ground leaf material were then used to assess the C:N using an elemental analyser (CHN-900, Leco, Michigan, USA).

FIELD EXPERIMENT

A common garden experiment, examining effects of litter source on decomposition rate, was conducted during September to December 2013 at Careel Bay, Pittwater (-33.618°S, 151.327°E), just to the north of Sydney, and approximately midway between the two latitudes at which litter was collected. Pittwater is a tide-dominated, drowned river valley with a tidal range of 1.4 m, a catchment size of approximately 10 km^2, and with abundant populations of *Zostera muelleri* and *Avicennia marina* (Roy et al. 2001; Wilton 2010). The estuary experiences a temperate climate with mean annual temperatures ranging from 14.5-

22.5°C and mean annual rainfall of 1223 mm over the past three decades (BOM, 2013). Seagrass litter was deployed at a tidal elevation of MSLW -0.2-0.3 m and the mangrove litter at 0.6-0.9 m (MSLW). Decomposition rates were assessed at different tidal elevations for each species because mangrove forests are situated from the mid to high intertidal while seagrass beds are situated in the low intertidal to shallow subtidal and, following senescence, much of the litter material from these macrophytes is retained in these areas.

Litter from each of the four sites was deployed in litterbags at Careel Bay on September 17th 2013, within 48 h of litter collection. The litterbags were 100 x 120 mm in size and constructed from 1 mm nylon mesh (Allied Filter Fabrics). The mesh size of the litterbags allowed a portion of the decomposer community to moderate decomposition while minimizing the loss of litter fragments through the bag (Dye 2006). Each seagrass litterbag (n = 30 per source site, to give 120 total) received 10 g, wet weight, of the assigned litter source. Each mangrove litterbag (n = 18 per source site, to give 72 total) received four whole leaves, equating to a wet-weight of 3.89 ± 0.06 g (mean ± 1 S.E.). Litterbags were anchored to the sediment surface with a peg at the assigned tidal elevation. Seagrass litterbags were placed in an un-vegetated area and mangrove litterbags were placed amongst the pneumatophores on the sea-ward side of the mangrove stand. Replicate bags (n = 6) of each litter source were retrieved 5, 13, 24, 49 and 72 days later for the seagrass, and 5, 24 and 49 days later for the mangrove. The schedules for sampling the two litter sources differed because these two data sets were originally intended to be part of two separate studies.

Following retrieval, litter material was rinsed to remove sediments and fauna and dried at 60°C to constant weight (approximately 3-4 days) and weighed. The percentage mass remaining per litterbag was calculated relative to the sub-samples of litter from each site that were dried and weighed at the start of the experiment.

STATISTICAL ANALYSES

Two-way nested analyses of variance (ANOVAs) with the factors latitude (low vs. high) and estuary (2 levels, random nested within latitude) assessed sources of spatial variation in the length, width, thickness, toughness and C:N of the litter at the start of the experiment.

Following the decomposition experiment, decay constants were calculated for the decomposition of each litter source by regressing ln-transformed proportionate mass loss data against time (see Olson 1963). Then, for each litter type (i.e. seagrass and mangrove), nested ANOVAs as described above, but with time as a covariate, assessed whether litter decomposition differed between latitudes. Prior to analyses, Cochran's test for homogeneity of variance and the Shapiro-Wilk test for normality were run. Unless otherwise stated, these assumptions were met. Where significant differences in factors of interest were detected (at $\alpha = 0.05$), Tukey HSD tests were run to determine the source of the differences. Where differences between estuaries were non-significant (at $\alpha > 0.25$) these were pooled and the analyses re-run.

Results

LEAF TRAITS

Prior to decomposition, the length and width of neither the seagrass nor the mangrove leaves significantly differed between latitudes, although significant variability at the scale of sites was evident in the width of mangrove leaves (Table 1, Table 2). Instead, mangrove leaves were 1.3 times thicker at the higher than the lower latitude (Table 1), although the thickness of seagrass blades did not vary spatially (Table 2).

For both litter types, leaf toughness was significantly greater in material from the lower than the higher latitude, for seagrass by a factor of 2.5 and mangroves by a factor of 2 (Table 2, Fig. 2a-b). For the seagrass litter, the nitrogen content of higher latitude leaves was

significantly greater, by approximately 1.8 times, than the lower latitude leaves (Table 2, Fig. 2c) and consequently, the C:N was approximately 1.7 times greater in the lower than higher latitude seagrass litter (Fig. 2e). Mangrove litter was similarly greater in nitrogen at higher than lower latitudes (Table 2, Fig. 2d), but there was no significant difference in the C:N of mangrove leaves between lower and higher latitudes (Table 2, Fig. 2f).

Table 1. Mean (± 1 S.E.) width, thickness, length, elasticity, toughness, nitrogen content and carbon to nitrogen ratio, of the seagrass *Zostera muelleri* and the mangrove *Avicennia marina*. Samples were collected at each of two estuaries at lower (L1, L2) and higher (H1, H2) latitudes of New South Wales, Australia (n = 5 per estuary).

Zostera muelleri

Estuary	Width (mm)	Thickness (mm)	Length (mm)	Elasticity (MPa)	Toughness (N.mm)	Nitrogen Content (%)	Carbon: Nitrogen
L1	3.12 ± 0.27	0.16 ± 0.02	231 ± 30	284 ± 30	24.8 ± 7.4	1.47 ± 0.03	19.6 ± 0.2
L2	3.16 ± 0.17	0.19 ± 0.01	231 ± 20	129 ± 8	20.2 ± 5.0	1.23 ± 0.01	22.6 ± 0.3
H1	2.56 ± 0.11	0.12 ± 0.02	217 ± 8	186 ± 18	13.6 ± 1.2	2.41 ± 0.06	12.3 ± 0.0
H2	2.97 ± 0.23	0.16 ± 0.03	254 ± 26	192 ± 64	4.3 ± 0.8	2.34 ± 0.02	12.1 ± 0.5

Avicennia marina

Estuary	Width (mm)	Thickness (mm)	Length (mm)	Elasticity (MPa)	Toughness (N.mm)	Nitrogen Content (%)	Carbon: Nitrogen
L1	37.4 ± 0.6	0.67 ± 0.03	96.4 ± 1.3	7.27 ± 0.79	127.9 ± 12.2	1.73 ± 0.05	22.6 ± 1.0
L2	37.9 ± 0.8	0.59 ± 0.06	94.2 ± 4.5	6.34 ± 1.04	79.4 ± 7.2	2.17 ± 0.05	18.4 ± 0.4
H1	40.7 ± 1.8	0.90 ± 0.03	92.6 ± 2.5	8.16 ± 1.03	55.4 ± 5.5	2.10 ± 0.03	20.7 ± 0.1
H2	34.8 ± 2.2	0.69 ± 0.04	89.0 ± 2.0	8.33 ± 0.99	46.9 ± 14.3	2.21 ± 0.05	19.3 ± 0.6

Table 2. Analyses of variance (ANOVA) assessing sources of spatial variation in the width, thickness, length, toughness, nitrogen content and carbon to nitrogen ratio of leaves from the seagrass *Zostera muelleri* and the mangrove *Avicennia marina*. There were two factors in the model: Latitude (2 levels, fixed: lower, higher) and Estuary (2 levels, random: nested within Latitude). Significant results where $P < 0.05$ are highlighted in bold. Estuaries were pooled within latitudes where they did not significantly differ at $P = 0.25$. The nitrogen content of seagrass and the carbon to nitrogen ratio of mangrove were arsine transformed, and the seagrass toughness was ln-transformed, to meet assumptions of ANOVA (Underwood 1997). All other data were analysed untransformed.

	Seagrass				Mangrove			
Source	**df**	**MS**	**F**	**P**	**df**	**MS**	**F**	**P**
WIDTH								
Latitude	1	0.70	3.35	0.084	1	0.02	< 0.01	0.970
Estuary (La)	pooled				2	44.25	3.84	**0.044**
Residuals	18	0.21			16	11.60		
THICKNESS								
Latitude	1	0.01	3.06	0.100	1	0.14	16.47	**0.001**
Estuary (La)	2	< 0.01	1.63	0.227	2	0.06	7.59	**0.005**
Residuals	16	0.01			16	0.01		
LENGTH								
Latitude	1	92.5	0.04	0.847	1	101.3	2.67	0.120
Estuary (La)	pooled				pooled			
Residuals	18	2418.4			18	38.0		
TOUGHNESS								
Latitude	1	4.49	15.20	**0.001**	1	13818	25.44	**< 0.001**
Estuary (La)	2	1.90	6.44	**0.009**	2	3034	5.59	**0.014**
Residuals	16	0.30			16	543		
N %								
Latitude	1	0.01	840.8	**< 0.001**	1	< 0.01	23.29	**< 0.001**
Estuary (La)	2	< 0.01	16.6	**< 0.001**	2	< 0.01	28.22	**< 0.001**
Residuals	16	< 0.01			16	< 0.01		
C:N								
Latitude	1	393.7	784.7	**< 0.001**	1	< 0.01	0.35	0.564
Estuary (La)	2	11.3	22.5	**< 0.001**	2	0.06	14.94	**< 0.001**
Residuals	16	0.5			16	< 0.01		

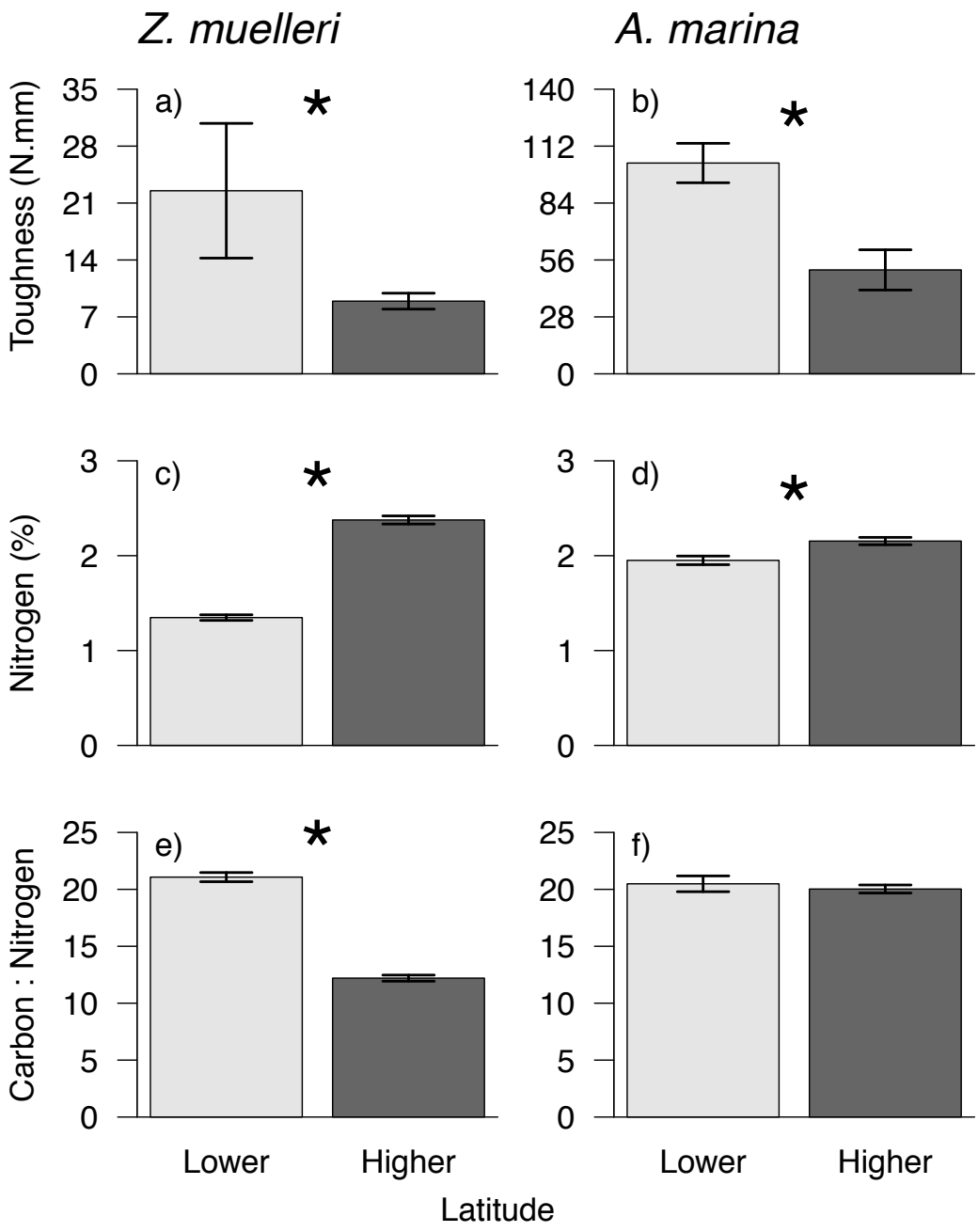

Fig. 2 Mean (± 1 S.E.) toughness, nitrogen content and carbon to nitrogen ratio of *Zostera muelleri* (a, c, e) and *Avicennia marina* (b, d, f). Samples were collected from a lower (grey bars) and higher (black bars) latitude of New South Wales, Australia (n = 5). The asterix (*) indicates significant differences between latitudes.

LITTER DECOMPOSITION

The mass loss of seagrass and mangrove litter followed an exponential decay curve, with the regression of ln-mass loss against time significant for each litter type from each source site (Table 3). Within each latitude, the rate of mass loss of seagrass litter differed between estuaries. Nevertheless, over and above this spatial variation, effects of latitude on seagrass decomposition were detected (Table 4). Overall, seagrass litter from the higher latitudes decayed at twice the rate of litter from the lower latitudes (Table 3, Fig. 3).

Within latitudes, there was no significant difference in the rate of mangrove litter mass loss between estuaries. Between latitudes, a significant difference in the rate of mass loss of mangrove litter was detected (Table 4), where mangrove litter from the lower latitudes decomposed faster than litter from the higher latitudes (Table 3, Fig. 4).

Table 3. The mean (± S.E.) decay constant (k d^{-1}) and half-life ($t_{1/2}$ d) of seagrass *Zostera muelleri* and mangrove *Avicennia marina* litter. Litter came from each of two estuaries at lower (L1, L2) and higher (H1, H2) latitudes of New South Wales, Australia and were decomposed at a common site (there were n = 5 litter bags per estuary). Estimates of k and $t_{1/2}$ were derived separately for each estuary and litter source using linear regression analysis between time and $\ln(x_t/x_0)$, where x_0 is the initial amount of material and x_t is the amount remaining after time t in days. The y-intercept was forced through 100%, the initial amount of material. R^2 and P values indicate the strength and significance of the regression. Estuaries within latitudes were pooled where they did not significantly differ at P = 0.25.

Estuary	k (d^{-1})	S.E.	$t_{1/2}$ (d)	R^2	P-value
Zostera muelleri					
NORTH					
N1	0.0103	0.001	67	0.95	<0.001
N2	0.0156	0.004	44	0.79	0.008
SOUTH					
S1	0.0270	0.005	26	0.85	0.003
S2	0.0307	0.006	23	0.84	0.004
Avicennia marina					
NORTH	0.0401	0.008	17	0.89	0.016
SOUTH	0.0356	0.008	19	0.88	0.019

Table 4. Analyses of variance (ANOVA) assessing sources of spatial variation in the decomposition of the seagrass *Zostera muelleri* and the mangrove *Avicennia marina*. There were two factors in the model: the Latitude (2 levels, fixed: lower, higher) and Estuary (2 levels, random: nested within Latitude) from which litter was sourced, with time as the co-variate. Significant results where $P < 0.05$ are highlighted in bold. Tukey HSD post-hoc tests show sources of the differences. Estuaries were pooled within latitudes where they did not significantly differ at $P = 0.25$.

Zostera muelleri				
Source	**DF**	**MS**	**F-value**	**P-value**
Time	4	2335	30.5	**< 0.001**
Latitude	1	24918	325.5	**< 0.001**
Estuary (Latitude)	2	3170	41.1	**< 0.001**
Residuals	112	77		
Tukey HSD	Latitude: Higher > Lower			
	Estuary (Latitude): L1 > L2 > H1 = H2			

Avicennia marina				
Source	**DF**	**MS**	**F-value**	**P-value**
Time	2	3209	90.0	**< 0.001**
Latitude	1	215	6.1	**0.017**
Residuals	68	36		
Tukey HSD	Latitude: Lower > Higher			

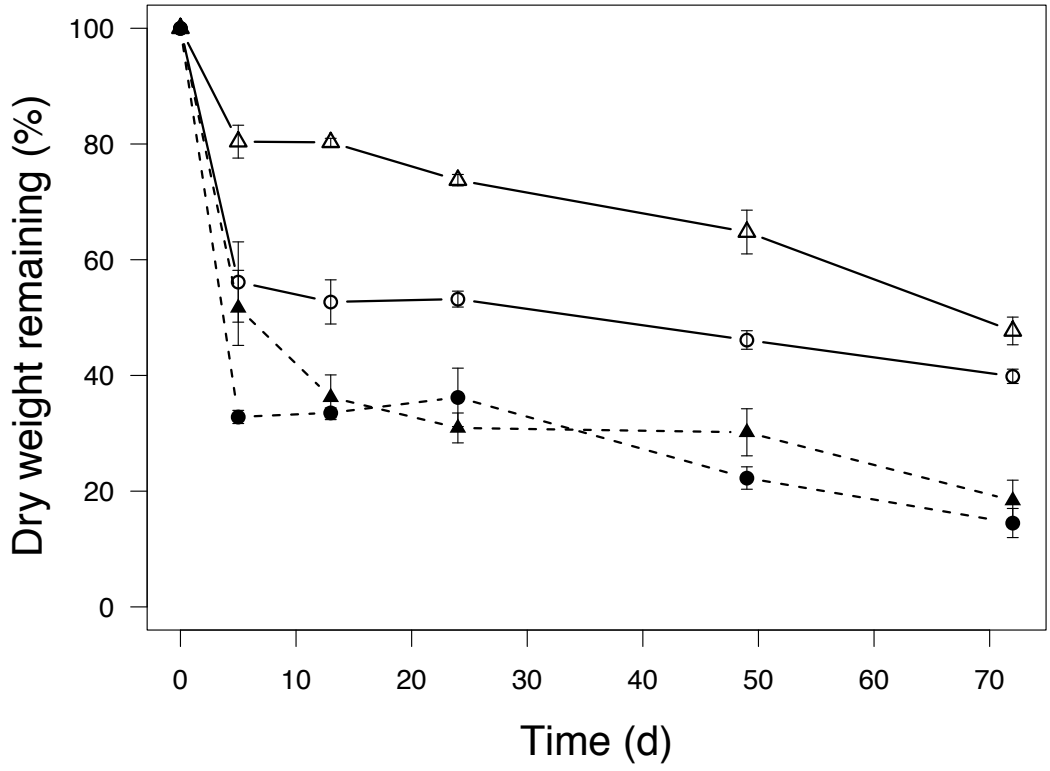

Fig. 3 Mean (± 1 S.E.) percentage of the initial litter mass of the seagrass *Zostera muelleri* remaining after 5, 13, 24, 49 and 72 days following deployment at a common site. Litter was collected from each of two estuaries at lower (L1 △, L2 ○; solid lines) and higher (H1 ▲, H2 ●; dashed lines) latitudes of New South Wales, Australia (n = 6 litter bags per treatment and time).

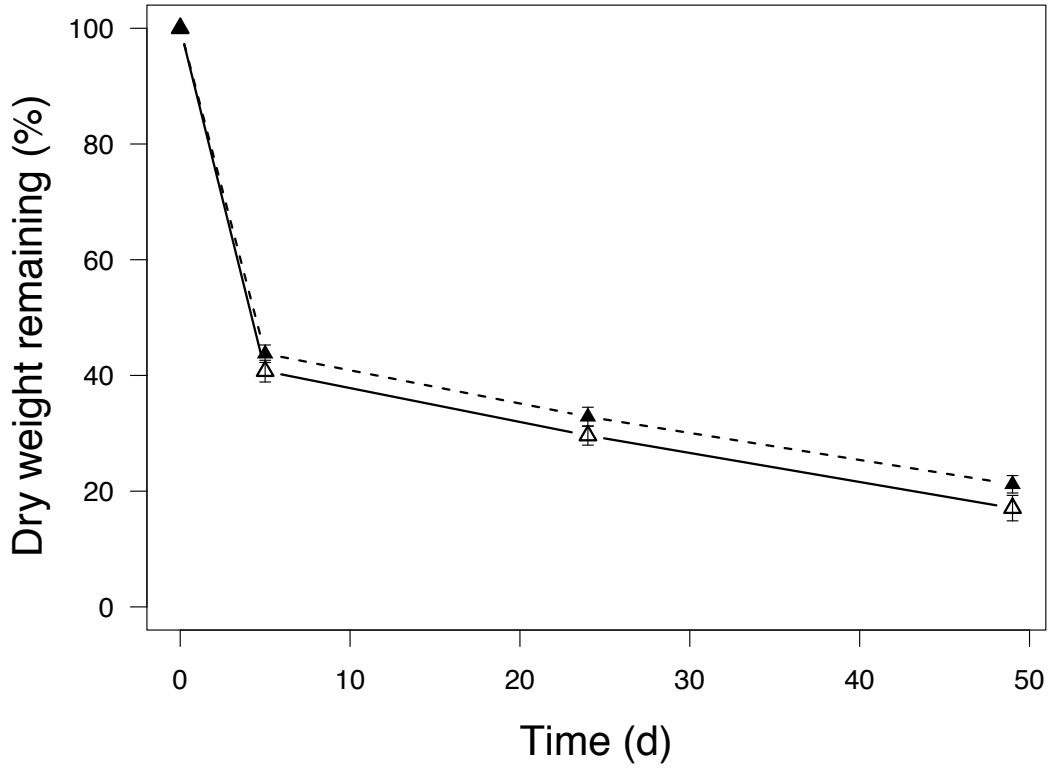

Fig. 4 Mean (± 1 S.E.) percentage of the initial litter mass of the mangrove *Avicennia marina* remaining after 5, 24 and 72 days following deployment at a common site. Litter was collected from lower (△, solid lines) and higher (▲, dashed lines) latitudes of New South Wales, Australia. Points represent site averages (n = 2 per latitude) because, within latitudes, these did not significantly differ.

Discussion

As predicted, based on studies examining interspecific variation in leaf traits across latitudinal gradients (Ordoñez et al. 2009; Reich and Oleksyn 2004; Wright et al. 2004), mangrove (*Avicennia marina*) and seagrass (*Zostera muelleri*) leaves collected from a lower latitude of NSW Australia were tougher and had lower nitrogen content than the leaves of conspecifics collected from a higher latitude (Fig. 2). Consistent with their greater nitrogen content and lower toughness, the seagrass litter from the higher latitude displayed a greater rate of decomposition than litter from the lower latitude when placed at a common site (Fig. 3). Mangrove litter, by contrast, displayed the reverse pattern (Fig. 4) indicating that nitrogen content and toughness alone cannot predict intraspecific variation in rates of decomposition.

The intraspecific patterns of variation in seagrass and mangrove leaf traits across a gradient of 5° of latitude were consistent with those described by previous studies examining interspecific variation of terrestrial plant leaves over much larger spatial gradients (e.g. Reich and Oleksyn 2004; Wright et al. 2004). Latitudinal variation in leaf toughness has been linked to longer leaf life-spans of lower latitude plants (Wright et al. 2004), and identified as an important contributor to latitudinal variation of herbivory (Salgado and Pennings 2005; Siska et al. 2002) and decomposition (Pérez-Harguindeguy et al. 2000). Latitudinal variation in leaf nitrogen content also contributes to palatability and is thought to arise via two mechanisms (Siska et al. 2002): (1) the shorter growing season of higher latitude plants means that they need to acquire higher nitrogen contents to allow for rapid growth (see also Borer et al. 2013); and (2) the lower nitrogen content of lower latitude plants may be an adaptive response to greater pressure from herbivores (Valentine and Heck 2001), although this is still under debate (Moles et al. 2011).

Based on intraspecific differences in the traits of leaves collected from higher and lower latitudes of NSW, we expected that their rates of decomposition at a common site would also differ. Along with decomposer community and climate, leaf traits are a key factor

influencing decomposition rates (Aerts 1997; Melillo et al. 1982; Pérez-Harguindeguy et al. 2000). Nitrogen content, in particular, has been found to be a key correlate of decomposition rate (Enríquez et al. 1993; Pérez-Harguindeguy et al. 2000; Zhang et al. 2008). Thus we expected that for each species, nitrogen-rich litter from the high latitude would decompose at a faster rate than litter from the lower latitude, which had lower nitrogen content. Faster decomposition was observed for the seagrass sourced from the higher than the lower latitude, consistent with this hypothesis, and its greater nitrogen content. However, the decomposition rate of mangrove litter, by contrast, followed the reverse pattern. While significant, the effect size of the difference in mangrove nitrogen content between latitudes was very small, and it is likely that other factors, not explicitly examined in the current study, such as lignin or tannin content, may have greater influences on mangrove decomposition.

Despite having similar patterns of intraspecific leaf trait variability, mangrove decomposition was faster for the lower latitude material, contradicting our hypothesis. Hence, the widely accepted relationship between leaf traits and decomposition does not apply to all species. Mackey and Smail (1996) showed that decomposition of mangrove litter was faster at lower latitudes due to a stronger influence of temperature, than other controls. But this does not explain the results of our common garden, where the environmental setting in which decomposition occurred was the same for all litter sources. Instead, the slower decomposition of the mangrove leaves from the higher than lower latitude in our common garden may be explained by spatial variation in the concentration of phenolics, such as tannins. Tannins can reduce nutrient cycling by impeding decomposition rates, binding proteins, causing mortality of microbial populations and inhibiting enzyme activities (Kraus et al. 2003). Although we did not quantify concentrations of tannins in the present study, our previous quantification of mangrove leaf traits at 16 sites along the NSW coast revealed that the total phenolic content of mangrove leaves increased with latitude (Ainley et al. unpublished data; chapter 2). Further, across species, chemical defenses including tannins

have been found to increase with latitude (Moles et al. 2011). Plants in less productive, higher latitude sites may invest more heavily in secondary metabolites to allow them to hang on to leaves for longer (Moles et al. 2011), resulting in slower decomposition.

For each of the litter sources, differences in the decomposition of the litter from lower and higher latitude estuaries were established during the first five days of the experiment, and persisted throughout the study. During the initial phase of decomposition, water-soluble and labile fractions are rapidly leached from the litter, leaving slow-decaying recalcitrant fractions (Berg 2000). The greater content of water-soluble nitrogen in higher than lower latitude seagrass litter is consistent with the greater mass loss of the higher latitude seagrass during this period. Although the higher latitude mangrove litter also contained greater nitrogen contents than the lower latitude litter, this difference was smaller than for the seagrass, and the mobilization of other water-soluble compounds (e.g. polyphenols; Hättenschwiler and Vitousek 2000; Northup et al. 1998), not measured by the present study and that were more abundant in the lower latitude litter, may instead explain this pattern.

Beyond this initial leaching period, the rate of mass loss was remarkably similar between litter sources from higher and lower latitude sites. Following leaching, biotic processes play a major role in influencing rates of decomposition (Bray et al. 2012; Gessner et al. 2010). In deploying litter at a common study site, it was, presumably, subject to the same decomposer community. Our results suggest that within species, this consumer community did not differentially act on litter of differing traits. Nevertheless, even at the end of our 70 day experiment, more than 60% of the initial litter mass of experimental treatments, in some cases, remained. Extension of the experiment for longer periods would be required to confirm that beyond the initial leaching phase, intraspecific variation in leaf traits has a negligible impact on decomposition rates.

Although our study found intraspecific differences in the traits of seagrass blades and mangrove leaves between latitudes, we did not assess whether these are genetically

underpinned or solely represent phenotypic responses to the differing environments (see Ackerly et al. 2000). For example, it is possible that differences in the rate of decomposition of the litter from the two latitudes was a function of the leaves being at different stages of their phenology. Experiments comparing the leaf traits of genetically similar plants grown in each of the environments would be required to disentangle this. Nevertheless, our results suggest that in addition to the direct effects of climate on decomposition, climate can indirectly influence decomposition rates by influencing leaf traits that are important mediators of decomposition rate (Cornwell et al. 2008). Reciprocal transplant experiments are now required to ascertain how the direct and indirect effects (e.g. on leaf traits and decomposer communities) of climate interact to determine the decomposition rates of leaf litter.

Previous work has focused on how interspecific variation in leaf traits mediates decomposition processes (Coq et al. 2010; Cornelissen et al. 1999; Pérez-Harguindeguy et al. 2000). Our work adds to the growing evidence that intraspecific leaf trait variability is also an important determinant of decomposition rate (e.g. Lecerf and Chauvet 2008; Wardle et al. 2003). Interestingly, our research shows that the effects of intraspecific variation in leaf traits may vary among species. Consequently, approaches that examine only interspecific patterns of variation may mask intraspecific effects and even reverse the direction of relationships (Shea and Chesson 2002).

Acknowledgements

This research was funded by Macquarie University Higher Degree Research funding to L. Ainley and an Australian Research Council Discovery Grant (DP1093444) to M. Bishop. We thank J. Rowland for assistance with field and lab-work, and L. Critchley and J. Kenworthy for helpful reviews on earlier versions of this manuscript.

References

Ackerly DD et al. (2000) The Evolution of Plant Ecophysiological Traits: Recent Advances and Future Directions: New research addresses natural selection, genetic constraints, and the adaptive evolution of plant ecophysiological traits. Bioscience 50:979-995

Aerts R (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. Oikos 79:439-449. doi: Doi 10.2307/3546886

Albert CH et al. (2010) Intraspecific functional variability: extent, structure and sources of variation. Journal of Ecology 98:604-613. doi: Doi 10.1111/J.1365-2745.2010.01651.X

Berg B (2000) Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management 133:13-22. doi: Doi 10.1016/S0378-1127(99)00294-7

Boege K, Dirzo R (2004) Intraspecific variation in growth, defense and herbivory in *Dialium guianense* (Caesalpiniaceae) mediated by edaphic heterogeneity. Plant Ecology 175:59-69. doi: Doi 10.1023/B:Vege.0000048092.82296.9a

Borer ET et al. (2013) Global biogeography of autotroph chemistry: is insolation a driving force? Oikos 122:1121-1130

Bray SR, Kitajima K, Mack MC (2012) Temporal dynamics of microbial communities on decomposing leaf litter of 10 plant species in relation to decomposition rate. Soil Biology & Biochemistry 49:30-37. doi: Doi 10.1016/J.Soilbio.2012.02.009

Bureau of Meteorology (2014) Climate Data Online. Bureau of Meteorology

Cebrián J (1999) Patterns in the Fate of Production in Plant Communities. The American Naturalist 154:449-468. doi: 10.1086/303244

Coq S, Souquet JM, Meudec E, Cheynier V, Hättenschwiler S (2010) Interspecific variation in leaf litter tannins drives decomposition in a tropical rain forest of French Guiana. Ecology 91:2080-2091

Cornelissen JHC et al. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Australian Journal of Botany 51:335-380. doi: Doi 10.1071/Bt02124

Cornelissen JHC et al. (1999) Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. New Phytologist 143:191-200. doi: Doi 10.1046/J.1469-8137.1999.00430.X

Cornwell WK et al. (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11:1065-1071. doi: 10.1111/j.1461-0248.2008.01219.x

Dye AH (2006) Inhibition of the Decomposition of *Zostera capricorni* Litter by Macrobenthos and Meiobenthos in a Brackish Coastal Lake System. Estuaries and Coasts 29:802-809

Enríquez S, Duarte CM, Sand-Jensen K (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94:457-471

Feld CK, Hering D (2007) Community structure or function: Effects of environmental stress on benthic macroinvertebrates at different spatial scales. Freshwater Biology 52:1380-1399. doi: Doi 10.1111/J.1365-2427.2007.01749.X

Garnier E et al. (2001) Consistency of species ranking based on functional leaf traits. New Phytologist 152:69-83. doi: Doi 10.1046/J.0028-646x.2001.00239.X

Gessner MO et al. (2010) Diversity meets decomposition. Trends in Ecology & Evolution 25:372-380. doi: 10.1016/j.tree.2010.01.010

Hättenschwiler S, Vitousek PM (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends in Ecology & Evolution 15:238-243

Jenkins CC, Suberkropp K (1995) The Influence of Water Chemistry on the Enzymatic Degradation of Leaves in Streams. Freshwater Biology 33:245-253. doi: Doi 10.1111/J.1365-2427.1995.Tb01165.X

Kirschbaum MUF (2006) The temperature dependence of organic-matter decomposition—still a topic of debate. Soil Biology and Biochemistry 38:2510-2518

Kraus TEC, Dahlgren RA, Zasoski RJ (2003) Tannins in nutrient dynamics of forest ecosystems - a review. Plant and Soil 256:41-66. doi: Doi 10.1023/A:1026206511084

Lecerf A, Chauvet E (2008) Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. Basic and Applied Ecology 9:598-605. doi: Doi 10.1016/J.Baae.2007.11.003

Lee KS, Park SR, Kim YK (2007) Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. Journal of Experimental Marine Biology and Ecology 350:144-175. doi: Doi 10.1016/J.Jembe.2007.06.016

Leroy CJ, Marks JC (2006) Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. Freshwater Biology 51:605-617. doi: Doi 10.1111/J.1365-2427.2006.01512.X

Mackey AP, Smail G (1996) The decomposition of mangrove litter in a subtropical mangrove forest. Hydrobiologia 332:93-98. doi: Doi 10.1007/Bf00016688

Makkonen M et al. (2012) Highly consistent effects of plant litter identity and functional traits on decomposition across a latitudinal gradient. Ecology Letters 15:1033-1041. doi: 10.1111/j.1461-0248.2012.01826.x

Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and Lignin Control of Hardwood Leaf Litter Decomposition Dynamics. Ecology 63:621-626. doi: Doi 10.2307/1936780

Moles AT, Bonser SP, Poore AGB, Wallis IR, Foley WJ (2011) Assessing the evidence for latitudinal gradients in plant defence and herbivory. Functional Ecology 25:380-388. doi: Doi 10.1111/J.1365-2435.2010.01814.X

Moore JC et al. (2004) Detritus, trophic dynamics and biodiversity. Ecology Letters 7:584-600. doi: Doi 10.1111/J.1461-0248.2004.00606.X

Northup RR, Dahlgren RA, McColl JG (1998) Polyphenols as regulators of plant-litter-soil interactions in northern California's pygmy forest: a positive feedback? Biogeochemistry 42:189-220

Olson JS (1963) Energy Storage and the Balance of Producers and Decomposers in Ecological Systems. Ecology 44:322. doi: 10.2307/1932179

Ordoñez JC, Van Bodegom PM, Witte JPM, Wright IJ, Reich PB, Aerts R (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Global Ecology and Biogeography 18:137-149

Pérez-Harguindeguy N, Díaz S, Cornelissen JHC, Vendramini F, Cabido M, Castellanos A (2000) Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. Plant and Soil 218/2:21-30. doi: 10.1023/a:1014981715532

Persson T, Bååth E, Clarholm M, Lundkvist H, Söderström B, Sohlenius B (1980) Trophic structure, biomass dynamics and carbon metabolism of soil organisms in a Scots pine forest. Ecological Bulletins:419-459

Reich PB, Oleksyn J (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. Proceedings of the National Academy of Sciences of the United States of America 101:11001-11006. doi: 10.1073/pnas.0403588101

Rice DL, Tenore KR (1981) Dynamics of Carbon and Nitrogen during the Decomposition of Detritus Derived from Estuarine Macrophytes. Estuarine, Coastal and Shelf Science 13:681-690. doi: Doi 10.1016/S0302-3524(81)80049-7

Roche P, Díaz-Burlinson N, Gachet S (2004) Congruency analysis of species ranking based on leaf traits: which traits are the more reliable? Plant Ecology 174:37-48

Roper T et al. (2011) Assessing the condition of estuaries and coastal lake ecosystems in NSW Monitoring, evaluation and reporting program, Technical report series. Office of Environment and Heritage, Sydney

Roy PS et al. (2001) Structure and function of south-east Australian estuaries. Estuarine, Coastal and Shelf Science 53:351-384. doi: Doi 10.1006/Ecss.2001.0796

Salgado CS, Pennings SC (2005) Latitudinal variation in palatability of salt-marsh plants: Are differences constitutive? Ecology 86:1571-1579

Schimel DS et al. (1994) Climatic, Edaphic, and Biotic Controls over Storage and Turnover of Carbon in Soils. Global Biogeochemical Cycles 8:279-293. doi: Doi 10.1029/94gb00993

Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. Trends in Ecology & Evolution 17:170-176. doi: Doi 10.1016/S0169-5347(02)02495-3

Siska EL, Pennings SC, Buck TL, Hanisak MD (2002) Latitudinal variation in palatability of salt-marsh plants: which traits are responsible? Ecology 83:3369-3381

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in Terrestrial Ecosystems. University of California Press, Berkeley, CA

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press

Valentine JF, Heck KL (2001) The role of leaf nitrogen content in determining turtlegrass (*Thalassia testudinum*) grazing by a generalized herbivore in the northeasternGulf of Mexico. Journal of Experimental Marine Biology and Ecology 258:65-86

Wardle DA, Nilsson MC, Zackrisson O, Gallet C (2003) Determinants of litter mixing effects in a Swedish boreal forest. Soil Biology & Biochemistry 35:827-835. doi: Doi 10.1016/S0038-0717(03)00118-4

Wetzel RG (1995) Death, Detritus, and Energy-Flow in Aquatic Ecosystems. Freshwater Biology 33:83-89. doi: Doi 10.1111/J.1365-2427.1995.Tb00388.X

Wilton K (2010) Changes in coastal wetland habitats in Careel Bay, Pittwater, NSW, from 1940 to 1996. Wetlands (Australia) 19:72-86

Wright IJ et al. (2004) The worldwide leaf economics spectrum. Nature 428:821-827. doi: 10.1038/nature02403

Zhang DQ, Hui DF, Luo YQ, Zhou GY (2008) Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. Journal of Plant Ecology 1:85-93. doi: Doi 10.1093/Jpe/Rtn002

5. TIPPING POINTS: CONTEXT-DEPENDENT EFFECTS OF ORGANIC ENRICHMENT ON SEDIMENT INVERTEBRATE COMMUNITIES.

Lara Ainley, Jennifer Rowland, Melanie Bishop*

Department of Biological Sciences, Macquarie University, North Ryde NSW 2109, Australia

* Corresponding Author: Lara Ainley, Ph: +612 9850 6285

Email addresses: lara.ainley@students.mq.edu.au (L. Ainley),

Jennifer.rowland@mq.edu.au (J. Rowland),

melanie.bishop@mq.edu.au (M. Bishop)

Keywords: estuarine, eutrophication, Australia, threshold, latitude

Abstract

Coastal development has lead to increases in the frequency, duration and intensity of eutrophication events on a global scale. Although the effects of eutrophication are well documented, less is known about how environmental context influences the magnitude of its effects. To assess how climatic setting influences the impact to sediment communities of organic enrichment, we conducted parallel field manipulations on the north and south coast of New South Wales (NSW), Australia, at intertidal sites differing in air temperature by 3°C. We hypothesized that effects of organic enrichment would be more severe in the warmer climate of northern NSW than the cooler climate of southern NSW. At each latitude, experimental enrichments of plots with 0 g, 50 g or 100 g of dried and shredded *Ulva* sp. were performed at two replicate sites and physico-chemical variables and macroinvertebrate communities were sampled before and 1, 7, 30 and 60 days after enrichment. At all sites, environmental degradation occurred in plots enriched with either moderate (50 g) or high (100 g) organic loading, indicating that moderate enrichment was sufficient to trigger environmental change. By contrast, faunal impacts were often greater in plots receiving the high than the moderate loading or only physical disturbance (0 g), indicating that the thresholds for environmental and ecological change can differ. In general, the biological responses included a reduction in the abundance of sub-surface bivalve taxa, and, consequently a reduction in species richness at two of the four study sites, for at least part of the study. In several instances, opportunistic taxa also responded positively to organic enrichment. The magnitude of change in both environmental and biological variables varied markedly among sites, and often did not display consistent differences among latitudes. These results highlight the context-dependency of ecosystem responses to local stressors. Studies are now needed to establish the mechanisms by which environmental setting modifies the effects of organic enrichment.

Introduction

Globally, eutrophication is a key driver of change to coastal ecosystems (Diaz and Rosenberg 2008, Smith et al. 1999). Human populations are rapidly expanding in the coastal environment (Small and Nicholls 2003). Agriculture and land clearing, application of fertilizers and fossil fuel combustion (via atmospheric nitrogen deposition) mobilizes nitrogen and phosphorus and increases its supply to coastal waters (Cloern 2001, Nixon 1995, Tilman et al. 2001). In coastal environments where nitrogen and/or phosphorus are typically limiting resources, excess nutrient input stimulates primary production, particularly that of fast growing algae (Cloern 2001). Where grazing pressure is insufficient to offset this enhanced primary production, organic matter inputs to the ecosystem increase (Cloern 2001). Moderate increases in organic loading may enhance benthic productivity (e.g. Pearson and Rosenberg 1978). Rapid decomposition of large amounts of detritus, however, depletes oxygen in sediments and bottom waters (de Jonge et al. 2002). The resulting hypoxia or anoxia can result in the mortality of benthic species of limited mobility, reducing biodiversity and modifying ecosystem functioning (Baird et al. 2004, Folke et al. 2004). It has been estimated that economic losses due to the effects of coastal eutrophication exceed billions of dollars each year (Smith and Schindler 2009).

Although the effects of hypoxia and anoxia are often most apparent in bottom waters of deep estuaries and bays, where organic matter tends to accumulate, and in which mixing with oxygenated surface waters is reduced (e.g. Bishop et al. 2006, Diaz and Rosenberg 2008, Rabalais et al. 1994), they can also be apparent on intertidal mudflats (e.g. Majeed 1987, McLusky 1982). The Pearson and Rosenberg (1978) model, elaborated upon by Gray et al (2002), describes the sequence of changes in sedimentary communities that typically occurs along gradients of organic enrichment. Moderate enrichment can increase the abundance and richness of sediment-dwelling invertebrates by increasing the resource base (Elliott 1994). As organic loading increases, the black anoxic layer moves closer to the

sediment surface, the total organic carbon to nitrogen ratio (TOC:N) drops below 10, and the macrofaunal community becomes increasingly dominated by high abundances of opportunistic surface-dwelling taxa such as hydrobiidae gastropods and capitellid polychaetes (Gray et al. 2002, Pearson and Rosenberg 1978). Under scenarios of severe enrichment, the sediment anoxic layer can reach the surface, causing the sediment to become defaunated (Gray et al. 2002).

The susceptibility of sedimentary communities to effects of sediment anoxia and hypoxia is, however, not only dependent on organic matter supply, but also on those environmental factors that determine its rate of breakdown. The breakdown of organic matter is the net result of a bacterial food chain, in which fermenting microorganisms hydrolyse the detritus and produce low molecular weight species, which are then used by anaerobic organisms or sulphate-reducing bacteria (Jørgensen 1982, Westrich 1983). Bacterial respiration is known to increase with temperature (Apple et al. 2006). Hence, in warmer climates, there may be a greater susceptibility of sediments to hypoxia or anoxia following organic enrichment.

Here, we replicate a detrital enrichment experiment at two study sites in the north and two in the south of New South Wales, Australia to test how the effects of organic enrichment vary according to environmental context. The northern and southern study sites were separated by 5° of latitude and differed in water temperature by 2°C and air temperature by 3°C. We expected that at each study site, faunal communities would experience non-linear effects of organic enrichment corresponding to the predictions of the Pearson-Rosenberg (1978) model. Further, we expected that at the warmer study sites, the threshold organic matter loading at which sediment anoxia is induced, and sediments are defaunated would be reduced.

Methodology

EXPERIMENTAL DESIGN

To test hypotheses about effects of organic enrichment on sediment oxygen and on macrofaunal communities, we conducted a detrital enrichment experiment that was replicated at four sites in January 2013 (the Austral summer; Fig. 1). Two sites were in northern NSW (hereafter 'low' [L]: latitude: approximately 29.78°S) and two were in southern NSW (hereafter 'high' [H]: latitude: approximately 35.14°S). The northern and southern NSW sites were separated by approximately 650 km and 5° of latitude. Each site was situated in a separate wave-dominated estuary or embayment with predominantly open entrance conditions (Roy et al. 2001). So as to ensure the magnitude of experimental enrichments was large relative to background organic loading, all estuaries were classified as largely unmodified by human activities (Roper et al. 2011). Sites were selected on a shallow, intertidal (0.2-0.4 m above mean spring low water; MSLW), unvegetated tidal flat with an average sediment grain size of 0.26 ± 0.01 mm and tidal range of ~ 1.5 m during the study period.

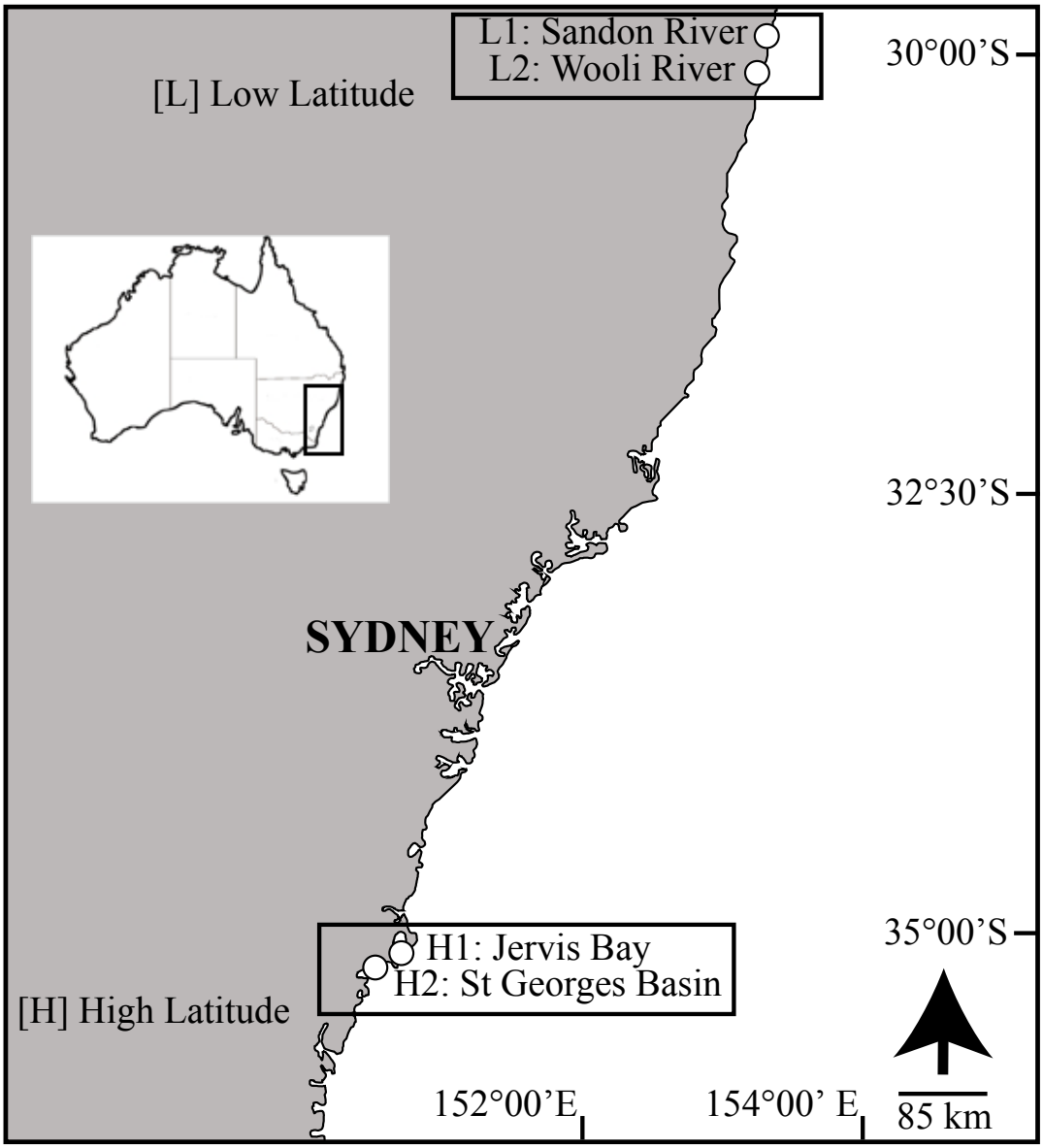

Fig. 1. Locations of estuaries at the lower [L] and higher [H] latitudes of NSW, Australia, where experimental manipulations were carried out.

At each field site, 21 square experimental plots, each 0.25 m^2 in area were established. Plots were separated by at least 2 m and marked with a single PVC stake such that they were open to migration of mobile taxa. Each plot received one of three enrichment treatments (with n = 7 of each): (1) procedural control with 0 g of organic material added; (2) moderate loading with 50 g (dry weight) of the green algae *Ulva* sp. added; and (3) high loading with 100 g of dried *Ulva* sp. added. *Ulva* sp. was used in experimental enrichments because it is an opportunistic species that responds rapidly in biomass production to nutrient enrichment (Rosenberg and Ramus 1982). Blooms of the species are frequently found on the shores of eutrophic estuaries (Martins et al. 1999). The high loading was chosen to represent the amount of organic matter that might enter sediments after a eutrophic event (see Rossi 2006), and the moderate loading was set at half of this.

The *Ulva* sp. used in detrital manipulations was collected fresh from field locations in Sydney and dried at 60°C to represent the natural desiccation of wrack on intertidal shores at low tide. Dried material was ground into pieces (< 2 mm diameter) to represent the particulate form in which most detritus enters sediments. The particulate detritus was added to designated plots by evenly hand-churning it into the top 2-3 cm of sediment at low tide when plots were out of water. This method of detrital addition has previously been used to successfully enrich sediments with a variety of organic matter resources, with over 80 % of material retained by sediments following periods of inundation (e.g. Bishop and Kelaher 2008, Bishop and Kelaher 2013). Plots designated as procedural controls were hand-churned, without addition of detritus. Our experiment compared enriched plots to procedural controls, but not to undisturbed sediments. Previous research along the NSW coast utilizing similar methods found no difference in macroinvertebrate communities between procedural controls and undisturbed plots, in the absence of detrital addition (e.g. Bishop and Kelaher 2007).

FIELD SAMPLING

The oxygen-reduction potential of sediments in experimental plots, their chlorophyll *a* concentration and depth of oxygenation and benthic macroinvertebrate communities were sampled immediately prior (0 d) to experimental manipulation and one day (1 d), one week (7 d), one month (30 d) and two months (60 d) after. Due to threat of bushfires, one of the replicate estuaries at the low latitude (L2) could not be accessed 1 d after enrichment. The sampling intervals were chosen based on previously reported half-lives of *Ulva* sp. of 8-10 days (see Rossi 2006). So as to maintain independence of sampling times, sediments were collected from a different position within each plot on each sampling date. All samples were stored at 4°C until analysis.

Measurements of the oxygen reduction potential (*Eh*) of sediments at a depth of approximately 4.5 cm were recorded *in situ* from each plot using a platinum electrode (Pt-electrode; IJ64; IONODE, Tennyson, Australia) with an Ag/AgCl/sat KCl reference solution of known mV (+198 mV at 25 °C). The electrode was attached to a pH/mV meter and recorded *Eh* to the nearest 1 mV and temperature (*T*) to the nearest 0.1°C. Triplicate *Eh* measurements were taken from each experimental plot, averaged to give one value per plot and corrected to the standard hydrogen electrode at 25°C by adding +198 mV to the direct reading obtained in the field (Pearson and Stanley 1979).

Sediment for chlorophyll *a* analysis was collected from each plot using a single 2 cm diameter core depressed to a depth of approximately 0.5 cm in each plot. To measure the chlorophyll *a*, a 30-50 mg sub-sample from each core of homogenized sediment was added to a standard volume (1 mL) of 90% acetone for 48 h at -20 °C for pigment extraction. Absorbances of the extract were read at 630, 647, 664 and 750 nm using a spectrophotometer under dim light. Chlorophyll *a* ($\mu g.g^{-1}$) was then calculated following Jeffrey and Humphrey (1975).

We determined the depth of oxygen-depletion in sediments by collecting a 3 cm diameter and 9 cm deep core of sediment using a clear PVC tube. The depth at which sediment was depleted of oxygen was ascertained visually by assessing the shallowest depth at which blackened sediment was observed. Following measurement of this oxygen-depletion depth, the sediment within each core was retained for organic content analysis. To measure the total organic content, sediment samples were first dried at 60 °C to constant weight. Then, following the calcination, or loss on ignition, protocol, 2 g sub-samples of dried sediment were placed in a muffle furnace at 450 °C for 4 h. Sample weight was recorded before and after calcination to determine the proportion of weight lost as an estimate of organic matter content.

Benthic invertebrate communities were sampled using 10 cm diameter push cores depressed to a depth of 5 cm. This sampling depth was chosen for the macroinvertebrate community because organic material was churned into the top 1-2 cm of sediment, such that decomposition of organic matter, and hence sediment hypoxia, was expected to primarily occur in surface sediments. Benthic community samples were rinsed through a 0.5 mm sieve and the macrofauna retained were fixed in a buffered 5% formalin/seawater solution, then transferred to 70% ethanol. The taxa present in each sample were enumerated by species or, where this was not possible, morphospecies under a stereo dissecting microscope.

DATA ANALYSIS

Four-way mixed-model permutational analyses of variance (PERMANOVAs, using the PRIMER package, Clarke and Warwick 1994) assessed sources of spatio-temporal variation in environmental variables and in macroinvertebrate community structure. PERMANOVA analyses were chosen over ANOVAs because, unlike ANOVAs, they are robust to violations of normality (Anderson 2001, Anderson et al. 2008). Each analysis had the factors: loading (3 levels, fixed: 0 g, 50 g and 100 g); time (5 levels, fixed: 0, 1, 7, 30 and

60 d); latitude (2 levels, fixed: low and high); and site (2 levels, random: nested within latitude). Times were considered independent as care was taken to sample from different positions in experimental plots on each date. Separate univariate analyses were run on: sediment oxygen-reduction potential; depth of sediment oxygen-depletion; sediment organic matter content; chlorophyll *a* concentration; total abundance of invertebrates; the abundance of infauna; the abundance of epifauna; and total taxon richness. Species were categorised as either infauna or epifauna, following identification, based on their published habitats. The analyses used Euclidean distance matrices calculated from untransformed data. Prior to each analysis, outlying data points were identified visually using boxplots and removed, and the number of replicates within each treatment was re-balanced to n = 7 by averaging the remaining replicates in that treatment, following Underwood (1997).

Separate two-way multivariate PERMANOVAs, examining effects of time and organic loading on macroinvertebrate community structure, were run for each site. This approach was used for analyses of community structure because large differences in the identity of taxa present at each site otherwise dominated four-way analyses (sig. Site effect: pseudo-$F_{2,342}$ = 45.02, $p < 0.001$), preventing factors of interest from being appropriately examined. These multivariate PERMANOVAs used Bray Curtis measures of dissimilarity calculated from untransformed data and were followed by SIMPER (similarities percentages) analyses (in PRIMER) to identify discriminating taxa that contributed most (Dissimilarity/SD > 1) to multivariate differences among treatments. Two factor (time, loading) univariate analyses were run on the abundance of these 'discriminating' taxa.

Results

ENVIRONMENTAL VARIABLES

None of the environmental variables significantly differed among plots assigned to the control, moderate or high enrichment treatments prior to experimental interventions. Pre-

existing differences in environmental variables were, however, apparent among the four sites (a posteriori tests, sig. Ti x Si(La) interactions; Table 1). H2 had lower sediment oxygen-reduction potential (ORP), and greater sediment organic content and chlorophyll *a* concentration than the other three sites. L2 had higher sediment ORP and lower concentration of chlorophyll *a* than the other sites. The depth at which sediment became oxygen-depleted did not, however, differ among estuaries prior to organic enrichment.

The response of sediment ORP to organic enrichment varied among the four study sites (sig. Lo x Si(La) x Ti interaction; Table 1). One day following enrichment, ORP at L1 and H1 was significantly lower in the two enriched treatments than the control treatment, with the two enriched treatments not significantly differing (Fig. 2a, c). At L1 this pattern had disappeared by 7 d following enrichment, but at H1, the pattern persisted for at least 7 d. At the other two sites, L2 and H2, there was no effect of enrichment on ORP at any of the sampling times (Fig. 2).

Table 1. Univariate PERMANOVAs examining sources of spatio-temporal variation in abiotic variables. Each analysis had four factors: loading of organic matter (Lo: fixed, 3 levels: 0 g, 50 g and 100 g of *Ulva* sp. per 0.25 m^2); latitude (La: fixed, 2 levels: low, high); time (Ti: fixed, 5 levels: before organic enrichment, 1, 7, 30 and 60 days after enrichment); and site (Si: random nested within latitude, 2 levels: L1/L2, H1/H2). Terms significant at $\alpha = 0.05$ are highlighted in bold. n = 7 experimental plots per treatment and site; p-*F* = pseudo-*F* ratio.

		Oxygen-reduction potential (ORP)			**Min. depth of O_2-depleted sediment**			**Organic content (%)**			**Chlorophyll *a* concentration**		
Source	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**
Loading-Lo	2	9554	3.08	0.161	31.09	16.05	**0.011**	0.05	0.33	0.720	1.47	0.33	0.733
Latitude-La	1	163270	3.20	0.080	176.16	7.50	**0.024**	1.49	2.52	0.137	16.81	2.43	0.146
Time-Ti	4	1281400	11.22	0.075	467.75	5.76	0.145	18.54	0.86	0.441	430.40	1.61	0.318
Site-Si(La)	2	115610	86.73	**0.001**	82.10	18.22	**0.001**	21.89	227.03	**0.001**	269.84	151.09	**0.001**
Lo x La	2	7029	2.52	0.064	12.27	1.71	0.179	0.16	1.22	0.353	7.67	2.07	0.114
Lo x Ti	8	2013	0.65	0.558	2.15	1.11	0.432	0.38	2.36	0.217	1.56	0.35	0.744
La x Ti	4	77851	1.53	0.310	85.24	3.63	0.087	0.64	1.08	0.416	5.64	0.82	0.534
Lo x Si(La)	4	3129	2.35	0.060	1.91	0.42	0.789	0.16	1.66	0.173	4.55	2.55	0.045
Si(La) x Ti	7	50987	38.25	**0.001**	23.47	5.21	**0.001**	0.59	6.15	**0.001**	6.91	3.87	**0.003**
Lo x La x Ti	8	1900	0.68	0.684	5.34	0.74	0.670	0.03	0.25	0.969	6.35	1.71	0.179
Lo x Si(La) x Ti	14	2788	2.09	**0.018**	7.19	1.60	0.085	0.13	1.32	0.185	3.70	2.07	**0.016**
Residuals	342	1333			4.51			0.10			1.79		

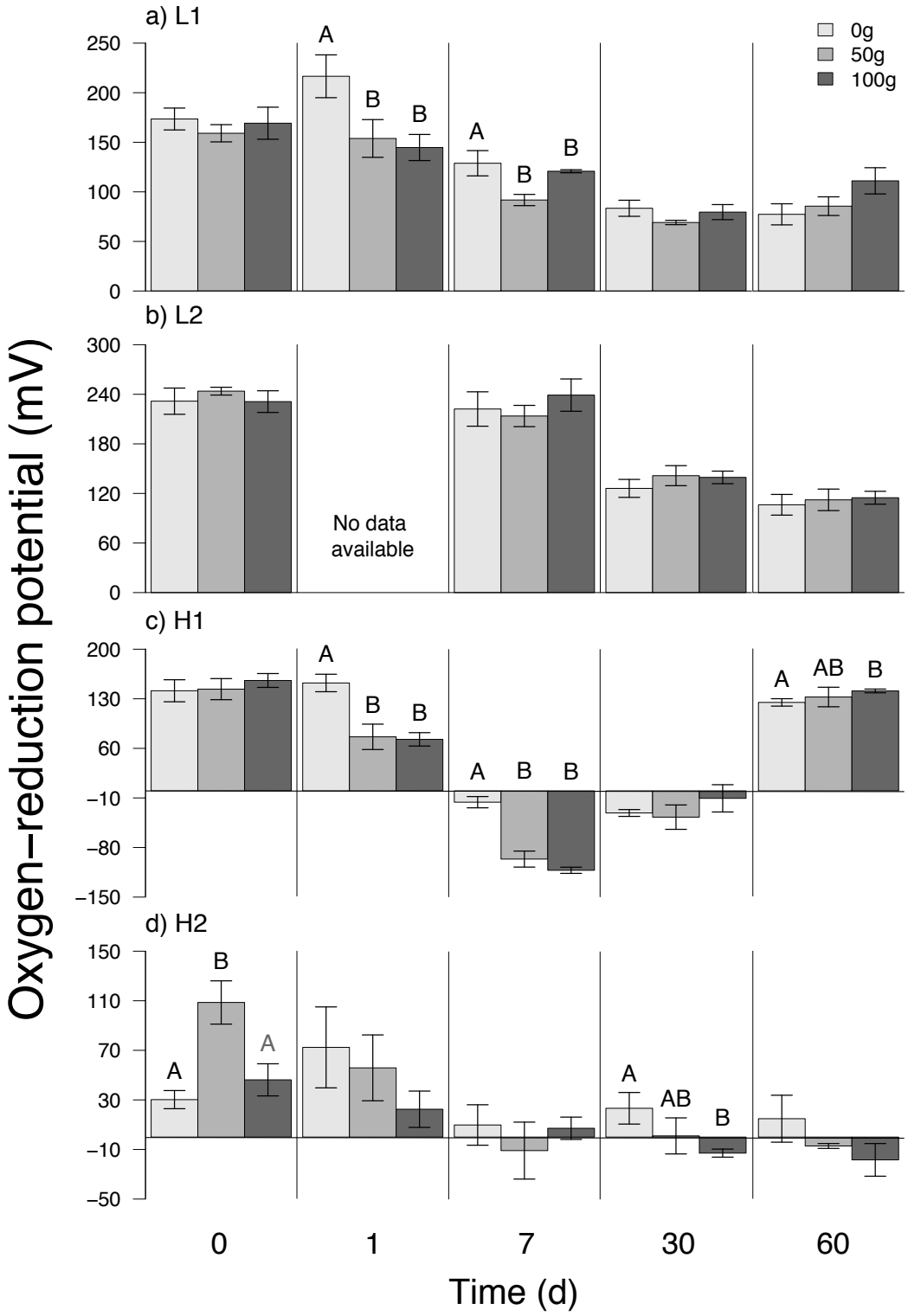

Fig. 2. Mean (± 1 S.E.) oxygen-reduction potential of sediment at 4.5 cm depth immediately before (0 d) and 1, 7, 30 and 60 d following physical disturbance only (0 g) or moderate (50 g) or high (100 g) loading with *Ulva* sp. organic matter (n = 7, 0.25 m^2 plots per treatment). Measurements were collected in two low latitude (L1 and L2) and two high latitude (H1 and H2) estuaries of New South Wales Australia during summer 2013.

By contrast, the depth at which sediment became oxygen-depleted (i.e. blackened) differed according to latitude and loading, independent of time and site (Table 1). Across all sites, times and both latitudes, oxygen-depletion occurred at a shallower depth in treatments that were organically loaded than the control treatment, irrespective of whether the organic loading was moderate or high (Fig. 3a). Furthermore, oxygen-depletion occurred at a significantly shallower depth in the cooler, high latitude sites (H1 and H2) than the warmer, low latitude sites (L1 and L2; Fig. 3b).

Although sediment organic matter content did not display a significant response to organic loading, the concentration of chlorophyll *a* (Chl-a) in surface sediments displayed site-specific responses to this manipulation (Table 1). One day following organic enrichment, differences in Chl-a were detected among organic loading treatments at L1, where significantly less Chl-a was detected in plots receiving high than no loading (Fig. 4a), and at H2 where there was significantly less Chl-a in the moderately loaded plots than the controls (Fig. 4d). At H1, by contrast, no significant effect of loading was seen after 1 d (Fig. 4c; and L2 was not sampled at this time). Instead, 7 d after organic enrichment, Chl-a at L2 was significantly less in the moderate loading treatment than in the control, but did not differ among treatments (Fig. 4b), and at H1 Chl-a was significantly greater in both enriched treatments compared to the control treatment (Fig. 4c). The effect at L1 of organic loading on Chl-a had disappeared by 7 d after enrichment, and the effects at L2 and H1 were not evident at 30 or 60 d. Only at H2 were effects of organic loading on Chl-a still apparent at 30 and 60 d after enrichment (Fig. 4).

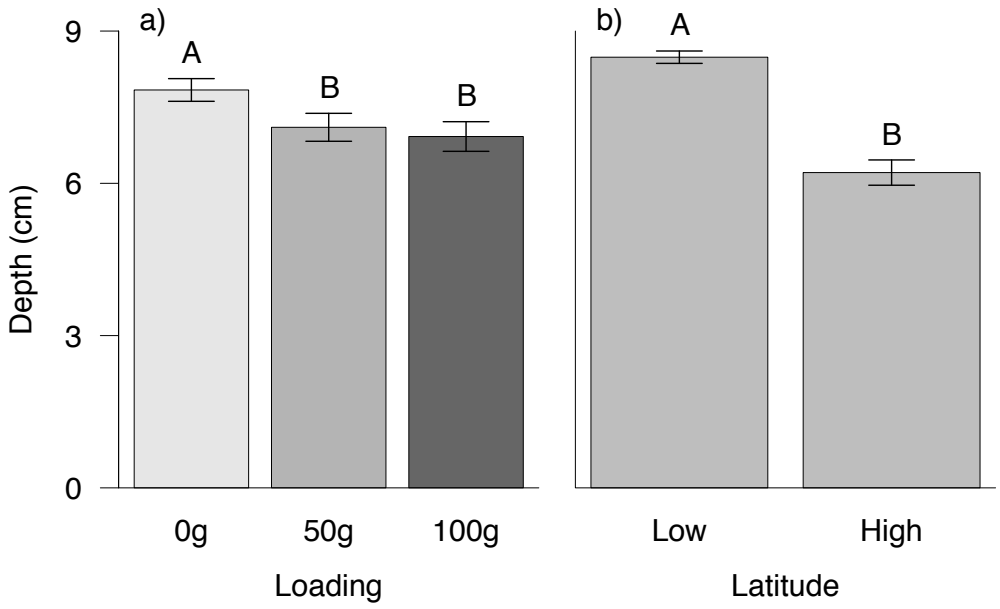

Fig. 3. Mean (± 1 S.E.) minimum depth at which sediments became oxygen-depleted (MDO) in: a) plots receiving physical disturbance only (0 g) or moderate (50 g) or high (100 g) loading with, sp. organic matter, averaged across all sites and times; and b) plots at low and high latitudes of New South Wales, Australia, averaged across sites and times.

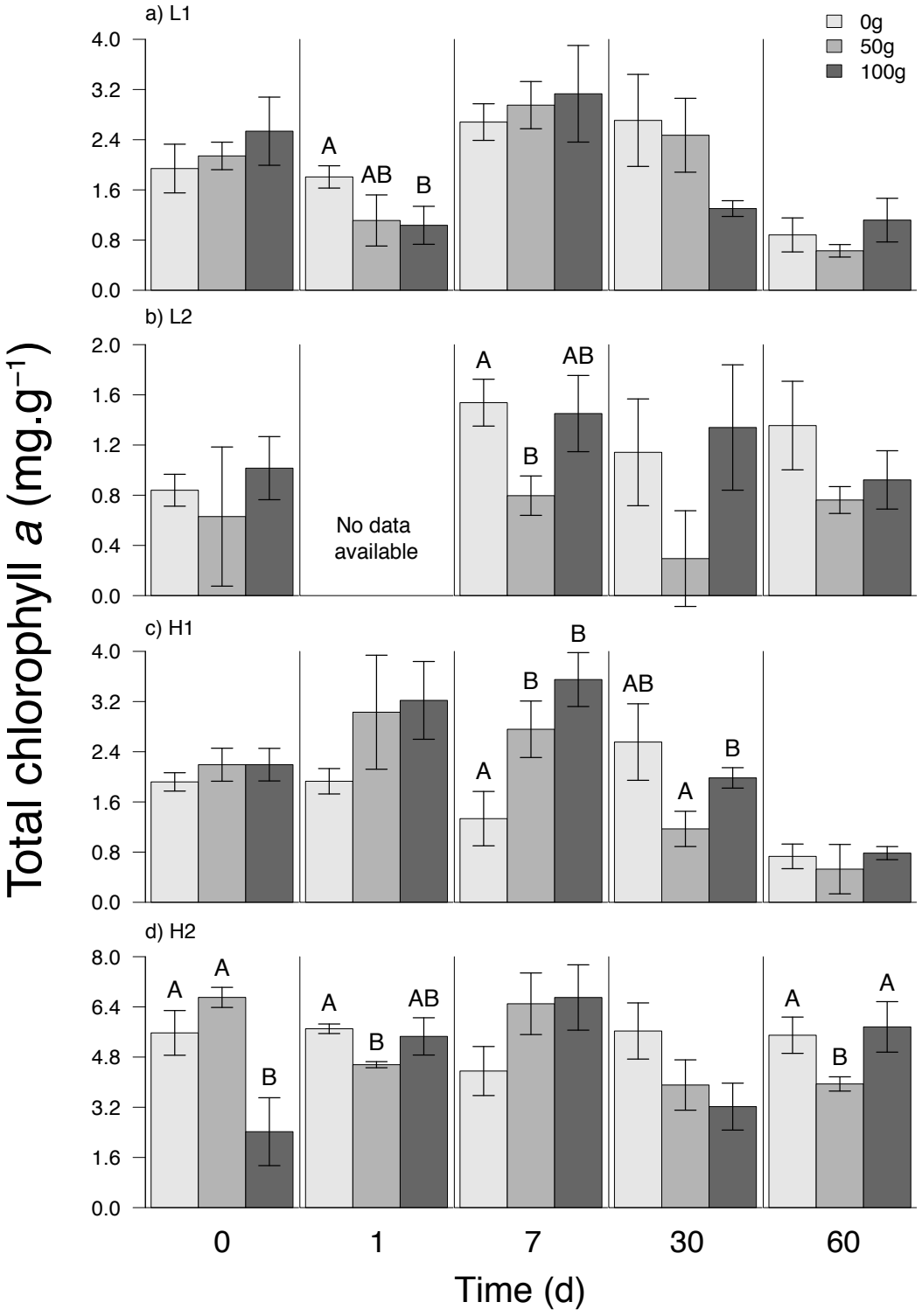

Fig. 4. Mean (± 1 S.E.) chlorophyll *a* concentration of sediment immediately before (0 d) and 1, 7, 30 and 60 d following physical disturbance only (0 g) or moderate (50 g) or high (100 g) loading with *Ulva* sp. organic matter (n = 7, 0.25 m^2 plots per treatment). Measurements were collected in two low latitude (L1 and L2) and two high latitude (H1 and H2) estuaries of New South Wales, Australia.

FAUNAL COMMUNITIES

Collectively, 7 376 individuals from 61 different taxa were enumerated from sediment samples and approximately 90% of these represented infauna, or sediment-dwelling, taxa. Values of total abundance ranged from 0 to 2070, and total richness ranged from 0 to 10, per replicate (per 0.25 m^2). Bivalves were major contributors to lower latitude assemblages (L1 = 65%; L2 = 30% of total abundance), and polychaetes were major contributors to higher latitude assemblages (H1 = 32%; H2 = 87% of total abundance). Assemblages at L2 also contained large numbers of amphipods (54%), and assemblages at H1, bivalves (39%).

The total abundance of macrofauna displayed a site-specific response to organic loading (sig. Lo x Si(La) x Ti interaction, Table 2; Fig. 5). At three of the four sites, L1, H1 and H2, the abundance of fauna differed between control and organically enriched plots following manipulation. At the fourth site, L2, which was not sampled 1 d after enrichment, no significant differences in the abundances of macrofauna were evident at any of the other sampling times. At L1, faunal abundance was initially less in the highly enriched than the control plots, although not significantly different among the other treatments, and the difference between the high loading and control treatments had disappeared by 7 d following enrichment (Fig. 5a). At H1, abundances were significantly less in the high compared to the moderate loading treatment 30 d after enrichment (Fig. 5c). At H2, abundances were initially, after 1 d, significantly greater in the high loading treatment. By 30 d, however, faunal abundances were lower in the high loading treatment compared to both moderate loading and control treatments, a pattern that was still evident at the final sampling at 60 d (Fig. 5d).

Table 2. Univariate PERMANOVAs examining sources of spatio-temporal variation in biotic variables. Each analysis had four factors: loading of organic matter (Lo: fixed, 3 levels: 0 g, 50 g and 100 g of *Ulva* sp. per 0.25 m^2); latitude (La: fixed, 2 levels: low, high); time (Ti: fixed, 5 levels: before organic enrichment, 1, 7, 30 and 60 days after enrichment); and site (Si: random nested within latitude, 2 levels: L1/L2, H1/H2). Terms significant at $\alpha = 0.05$ are highlighted in bold. n = 7 experimental plots per treatment and site; p-*F* = pseudo-*F* ratio.

		Total abundance			**Infaunal abundance**			**Epifaunal Abundance**			**Total richness**		
Source	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**
Loading-Lo	2	235820	4.37	0.107	210700	8.78	**0.049**	8285	0.95	0.520	9.06	3.78	0.122
Latitude-La	1	443830	2.39	0.142	333540	1.24	0.395	18227	0.67	0.640	17.28	6.90	**0.021**
Time-Ti	4	364860	0.14	0.749	65396	0.01	0.925	127650	0.28	0.626	207.87	6.62	0.130
Site-Si(La)	2	2620300	24.74	**0.001**	4807700	48.79	**0.001**	469850	93.49	**0.001**	31.77	10.94	**0.001**
Lo x La	2	322890	1.13	0.388	284920	0.80	0.596	8645	0.67	0.700	6.16	2.45	0.068
Lo x Ti	8	317720	5.88	0.080	317660	13.24	**0.025**	2305	0.26	0.780	2.22	0.93	0.462
La x Ti	4	726900	3.91	0.058	552060	2.06	0.200	20894	0.77	0.566	8.68	3.46	0.074
Lo x Si(La)	4	53365	0.50	0.752	23066	0.23	0.921	8777	1.75	0.139	2.39	0.82	0.538
Si(La) x Ti	7	185800	1.75	0.091	268260	2.72	**0.007**	27172	5.41	**0.001**	2.50	0.86	0.559
Lo x La x Ti	8	711820	2.50	0.068	632360	1.77	0.159	15486	1.20	0.345	2.78	1.11	0.429
Lo x Si(La) x Ti	14	285200	2.69	**0.001**	356980	3.62	**0.001**	12913	2.57	**0.001**	2.51	0.87	0.597
Residuals	342	105930			98544			5026			2.90		

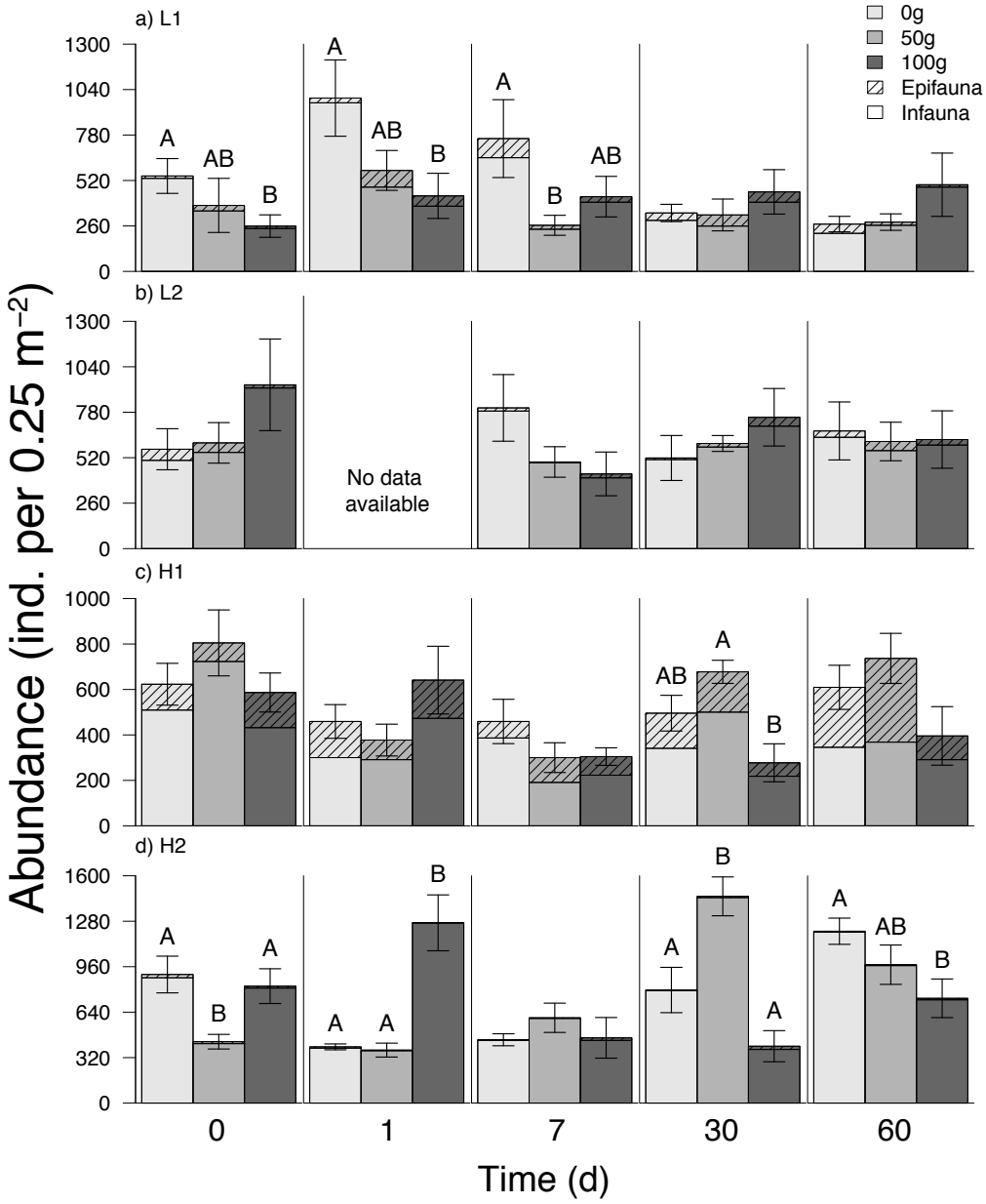

Fig. 5. Mean (± 1 S.E.) total abundance of invertebrates in plots immediately before (0 d) and 1, 7, 30 and 60 d following physical disturbance (0 g) or moderate (50 g) or high (100 g) loading with *Ulva* sp. organic matter (n = 7, 0.25 m^2 plots per treatment). Abundances are divided into epifauna (patterned section of bars) and infauna (unpatterned sections). Measurements were collected in two low latitude (L1, L2; a-b); and two high latitude (H1, H2; c-d) estuaries of New South Wales, Australia.

These patterns in faunal abundance were primarily driven by the infaunal taxa that displayed almost identical patterns to total faunal abundance (Fig. 5). Differences in epifaunal abundance among organic enrichment treatments, detected at L1, L2 and H1, generally appeared late in the experiment. At L1, epifauna were significantly less abundant in the moderate loading treatment, than the control, at 7 and 60 d, but at other times there was no significant difference among treatments. At L2, a significant difference in the epifaunal abundance among treatments was only detected at 30 d, with abundances greater in the high loading treatment compared to the control. Finally, at H2, a significant difference in epifuana among loading treatments was only apparent at 60 d, with abundance in the high loading treatment greater than in either the moderate loading or the control treatment.

Total taxon richness responded to organic enrichment at the high latitude sites H1 and H2 only. At H1, richness was across all sampling times significantly less in plots receiving the high loading than in control plots, but differences were not apparent among the other treatments (Fig. 6a). At H2, a significant interaction between loading and time was detected, yet, *a posterori* tests failed to identify the source of this interaction. There was, however, a non-significant trend for a lower richness of invertebrates in each of the enriched treatments as compared to the controls at 1 d following organic enrichment, followed by some recovery in richness in the plots receiving the moderate loading (Fig. 6b).

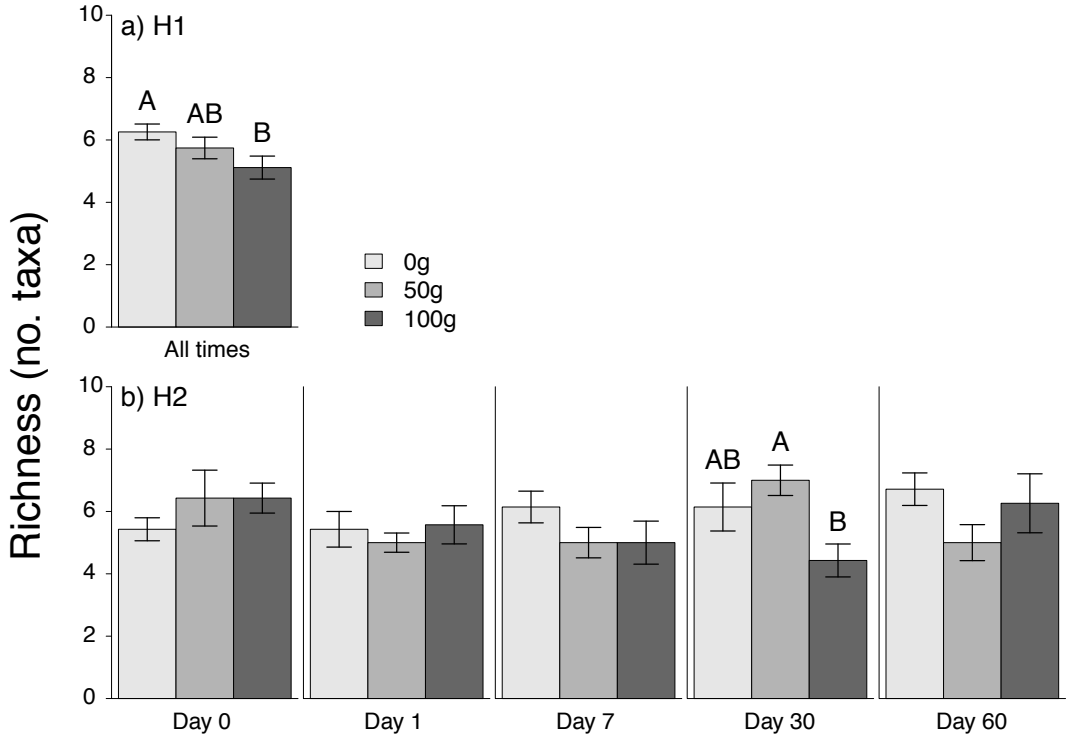

Fig. 6. Mean (± 1 S.E.) total richness of invertebrates at: a) H1 and b) H2. There was no significant interaction between time and loading at H1, so main effects of loading, average across all times are shown. At H2, there was a significant interaction between loading and time, so effects of loading are shown separately for times immediately before (0 d) and 1, 7, 30 and 60 d following physical disturbance (0 g) or moderate (50 g) or high (100 g) loading with *Ulva* sp. organic matter (n = 7, 0.25 m^2 plots per treatment).

Multivariate PERMANOVAs examining effects of time and organic loading on macroinvertebrate community structure showed no significant change in community structure in response to organic loading at any of the four sites (Table 3). Instead, significant site-scale temporal variation was detected at all sites. By contrast, univariate PERMANOVAs, using the same factors (loading and time), detected significant variation in response to organic loading for 'discriminating' taxa at each site (Table 4).

SIMPER analysis revealed that at L1, L2 and H1, the bivalve *Mysella vitrea* was the taxon that discriminated most among detrital enrichment treatments (Table 5). At H2, the polychaetes *Mediomastus australiensis* and *Nereis* sp. were the best discriminators, with other key discriminators including *Urohaustorius gunni* and *Gammarus* sp. at L2; Galeomatidae sp. and *Salinator fragilis* at H1; and *Perineris* sp. at H2 (Table 5).

Table 3. Two-way PERMANOVA examining sources of spatio-temporal variation in the multivariate community structure of macroinvertebrates at each of four study sites. The analyses had two factors: loading (Lo: fixed, 3 levels: 0 g, 50 g and 100 g of *Ulva* sp. per 0.25 m^2) and time (Ti: fixed, 5 levels: before organic enrichment, 1, 7, 30 and 60 days after enrichment). The four study sites were distributed across two latitudes of New South Wales, Australia (low: L1 and L2; high: H1 and H2). Terms significant at $\alpha = 0.05$ are highlighted in bold. n = 7 experimental plots per treatment and site; p-*F* = pseudo-*F* ratio.

		Community assemblage		
Source	**df**	**MS**	**p-*F***	**P**
a) L1				
Lo	2	2860	1.78	0.062
Ti	4	15485	9.62	**0.001**
Lo x Ti	8	1678	1.04	0.399
Residual	90	1609		
b) L2				
Lo	2	1864	1.03	0.417
Ti	3	14220	7.88	**0.001**
Lo x Ti	6	1101	0.61	0.979
Residual	72	1804		
c) H1				
Lo	2	2384	1.39	0.124
Ti	4	12572	7.34	**0.001**
Lo x Ti	8	2108	1.23	0.090
Residual	90	1713		
d) H2				
Lo	2	1250	0.99	0.452
Ti	4	13462	10.65	**0.001**
Lo x Ti	8	1539	1.22	0.170
Residual	90	1265		

Table 4. P-values for univariate PERMANOVAs examining sources of spatio-temporal variation in discriminating and key taxa for individual estuaries at low latitudes: a) L1, b) L2, and high latitudes: c) H1, d) H2, of New South Wales, Australia. Each analysis had two factors: loading (Lo: fixed, 3 levels: 0 g, 50 g and 100 g of *Ulva* sp. per 0.25 m^2; n = 7 per treatment) and time (Ti: fixed, 5 levels: before organic enrichment, 1, 7, 30 and 60 days after enrichment). Terms significant at $\alpha = 0.05$ are highlighted in bold. For full statistical details see supplementary material.

a) L1		***Mysella vitrea***		
Source	**df**	**MS**	**p-*F***	**P**
Lo	2	260851	4.34	**0.015**
Ti	4	866370	14.4	**0.001**
Lo x Ti	8	95606	1.59	0.143
Residual	90	60138		

b) L2		***Mysella vitrea***			***Urohaustorius gunni***			***Gammarus* sp.**		
Source	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**
Lo	2	4517	0.22	0.844	23173	1.27	0.280	139844	3.42	**0.031**
Ti	3	289203	14.0	**0.001**	36994	2.03	0.101	373587	9.15	**0.001**
Lo x Ti	6	9929	0.48	0.824	18691	1.02	0.430	29716	0.73	0.643
Residual	72	20714			18238			40847		

c) H1		***Mysella vitrea***			**Galeomatidae sp.**			***Salinator fragilis***		
Source	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**
Lo	2	41194	2.10	0.105	5819	2.03	0.152	5819	1.70	0.205
Ti	4	38463	1.96	0.089	24645	8.62	**0.001**	18156	5.31	**0.003**
Lo x Ti	8	11353	0.58	0.794	6241	2.18	**0.028**	9232	2.70	**0.015**
Residual	90	19631			2860			3422		

d) H2		***Mediomastus australiensis***			***Nereis* sp.**			***Perinereis* sp.**		
Source	**df**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**	**MS**	**p-*F***	**P**
Lo	2	1790	0.31	0.750	40113	1.57	0.228	13551	0.71	0.504
Ti	4	71134	12.2	**0.001**	617687	24.1	**0.001**	789660	41.2	**0.001**
Lo x Ti	8	7882	1.35	0.212	56607	2.21	0.032	55163	2.88	**0.015**
Residual	90	5846			25611			19176		

Table 5. Taxa identified by SIMPER as best discriminating (standard deviation to dissimilarity ratio > 1) among enrichment treatments at each of four study sites along the New South Wales coast, Australia (low latitude: L1 and L2; and high latitude: H1 and H2).

Species	Dissimilary/SD
Estuary: L1	
Mysella vitrea	1.22
Estuary: L2	
Mysella vitrea	1.05
Urohaustorias gunni	1.04
Gammarus sp.	1.01
Estuary: H1	
Mysella vitrea	1.10
Galeomatidae sp.	1.07
Salinator fragilis	1.01
Estuary: H2	
Mediomastus australiensis	1.31
Nereis sp.	1.30
Perinereis sp.	1.06

Of the three sites at which *M. vitrea* was identified as a key discriminator among organic enrichment treatments, it only displayed a significant difference in abundance among treatments at one (Table 4). At L1, the bivalve was more abundant in the control than both the organically enriched plots, irrespective of time (Table 4b, Fig. 7a). At L2, the abundance of *Gammarus* sp. also displayed a main effect of loading, its abundance less in plots receiving a moderate loading of detritus as compared to controls, but with all other pairwise analyses of treatments statistically non-significant (Table 4b, Fig. 7b). *Urohaustorius gunni*, despite being a key contributor to multivariate dissimilarity at L2, did not significantly differ among treatments (Table 4b).

At H1, Galeomatidae sp. and *S. fragilis* displayed effects of loading that were only apparent at 60 d (Table 4c; Fig. 8a-b). At this time, Galeomatidae sp. was significantly less abundant in plots receiving the high loading than in control plots and *S. fragilis* was less abundant in plots receiving the high than the moderate loading, but all other pairwise comparisons were non-significant.

At H2, *Perinereis* sp. and *Nereis* sp. all displayed significant interactions between loading and time (Table 4d). Abundances in all treatments increased after 7 d, and by 30 d, abundances of *Perinereis* sp. were significantly less in the high loading treatment compared to the moderate loading treatment, but did not differ between enriched treatments and controls (Fig. 8c). *Nereis* sp. differed in response to organic enrichment at 60 d, and was significantly more abundant in the high loading treatment compared to the control only (Fig. 8d). *Mediomastus australiensis* displayed a main effect of time, being more abundance in experimental plots at 30 or 60 d than shorter periods of time (Table 4d).

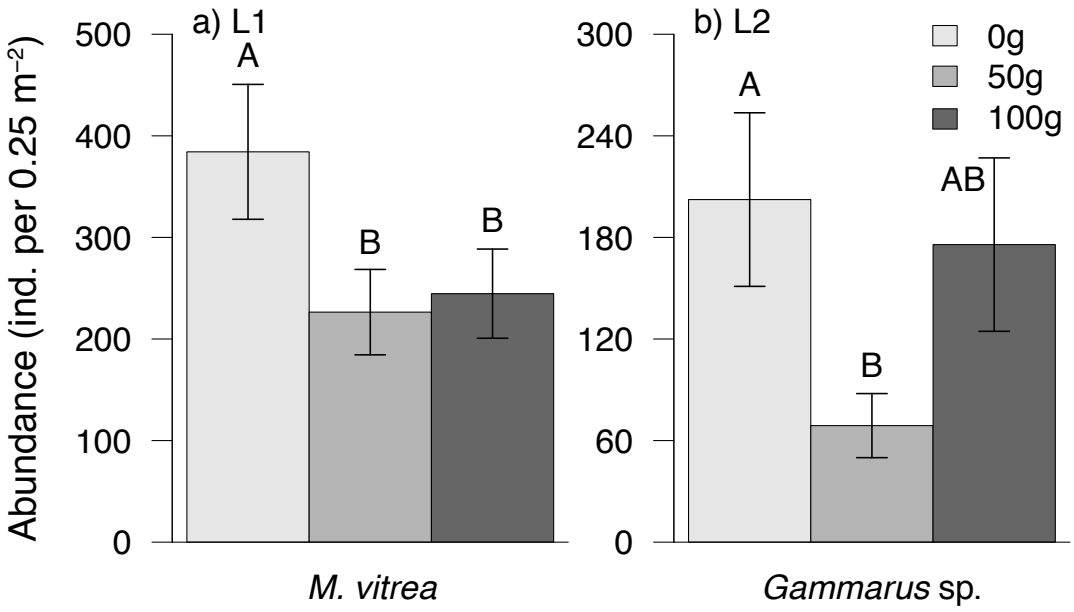

Fig. 7. Mean (± 1 S.E.) abundance of a) *Mysella vitrea* and b) *Gammarus* sp. in plots receiving physical disturbance only (0 g) or moderate (50 g) or high (100 g) loading with *Ulva* sp. organic matter (n = 7, 0.25 m^2 plots per treatment). Measurements were collected from estuaries at low latitude estuary, L1, of the New South Wales coast, Australia.

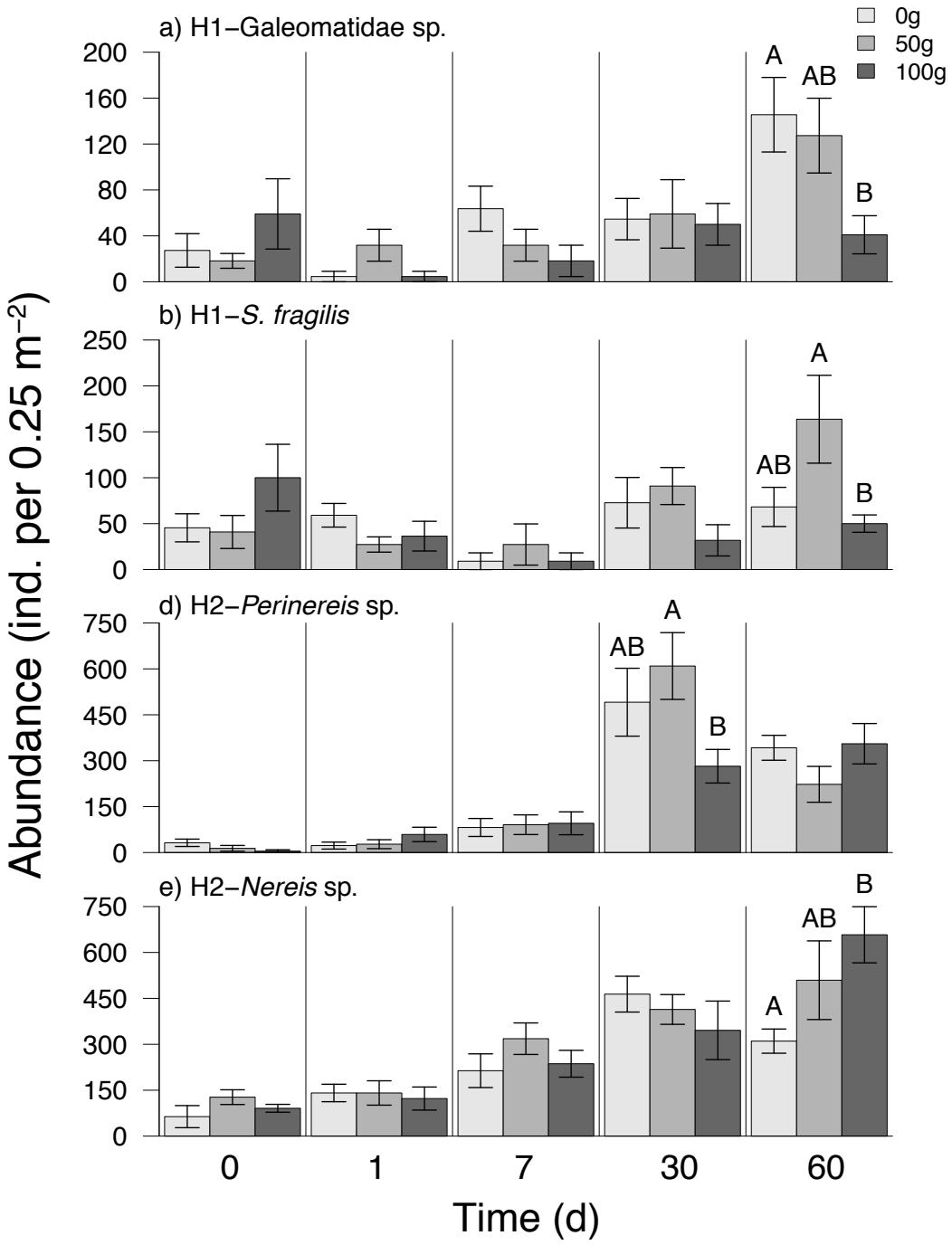

Fig. 8. Mean (± 1 S.E) abundance of a) Galeomatidae sp. at site H1, b) *Salinator fragilis* at H1, c) *Perinereis* sp. at H2, and d) *Nereis* sp. at H2 immediately before and 1, 7, 30 and 60 days following physical disturbance only (0 g) or moderate (50 g) or high (100 g) loading with *Ulva* sp. organic matter (n = 7, 0.25 m^2 plots per treatment).

Discussion

Overall, we found that enriching sediments with either moderate or high loadings of *Ulva* sp. organic matter was sufficient to cause degradation of environmental conditions, and produce changes in benthic faunal communities. In many instances, the response of fauna to enrichment was independent of loading. This non-linear, threshold-like response indicates that even a moderate organic loading is sufficient to trigger changes in community structure (Conley et al. 2009, Pearson and Rosenberg 1978). Contrary to our hypothesis, changes in environmental conditions and faunal communities following loading were not greater at low than high latitude. Instead, site-specific differences in the effects of organic loading were seen, suggesting that local scale processes largely control responses in these systems, particularly physical factors (Conley et al. 2009).

Consistent with predictions of the Pearson-Rosenberg (1978) model of organic enrichment, the addition of *Ulva* sp. to estuarine sediments resulted in a reduction in the oxygen reduction potential of sediments and a decrease in the depth to which sediment was oxygenated. These effects, although spatially variable in magnitude, were evident at all sites, immediately following organic enrichment, and were of similar magnitude between the moderate and high organic loadings. Hence, even our moderate level of organic enrichment appeared to be above the threshold required to induce significant environmental changes. Although, as in other studies, the effect of enrichment on oxygen-reduction potential was generally short-lived (Rossi 2006), the reduction in sediment oxygenation depth persisted throughout the 60 d experiment, with no recovery apparent. Contrary to expectation, the depth to which sediment was oxygenated was less at high than low latitudes. Rather than reflecting differences in rates of bacterial breakdown of organic matter, due to differences in temperature between the latitudes (Arnosti et al. 1998, Conant et al. 2011), this may reflect differences in sediment properties (Van Veen and Kuikman 1990). The more southerly, high

latitude sites, had more muddy sediments with a higher organic content than the lower latitude sites, even prior to enrichment (see also Nicastro and Bishop 2013).

Whereas the direction of change in abiotic conditions following organic enrichment was consistent among sites, responses of chlorophyll *a* were more variable. At two of the three sites sampled 1 d following enrichment, the concentration of chlorophyll *a* initially decreased in sediments organically enriched, perhaps reflecting an artifact of disturbance, whereas at the third site it increased. Over the longer time frame of 7 d, however, chlorophyll *a* generally increased in response to organic enrichment, although this effect had disappeared by 30 d, and was replaced with idiosyncratic site-specific patterns. The generally positive response of chlorophyll *a* to enrichment is consistent with the rapid leaching of nitrogen from highly labile *Ulva* sp. following addition to sediments causing enhancement of overall productivity (Kelaher et al. 2003, Rossi 2006). Australian estuaries are highly oligotrophic and primary production is often nutrient-limited (Scanes et al. 2007). The similar response of chlorophyll *a* concentration to moderate and high organic enrichment suggests that even the smaller amount of *Ulva* sp. was sufficient to release the system from nutrient limitation. The short-term response of this enhancement in chlorophyll *a* by organic enrichment is consistent with the previously reported half-life of *Ulva* sp. of 8-10 d (Rossi 2006). Over the longer term, top-down effects of grazing may be more important in determining spatial patterns of chlorophyll *a* concentration (Bishop et al. 2007).

Whether faunal abundance responded positively or negatively to environmental changes induced by organic enrichment was highly site-specific, and reflected marked differences in the species assemblage present at each of our study sites, determined by local scale processes and physical conditions (de Jonge et al. 2002). Even prior to experimental enrichment, large differences in the composition of macrofaunal communities were seen between study sites. At two of the three sites sampled 1 d after organic enrichment (each at the higher latitude), a marked enhancement in faunal abundance was apparent in plots

receiving the high but not the low loading of *Ulva* sp. Analyses identifying discriminating taxa did not identify any particular taxa driving this pattern. Instead, the pattern may reflect a general immigration of mobile fauna into plots to capitalize on the enhanced resource availability (Hall 1994, Pearson and Rosenberg 1978). Over longer periods of time, this pattern at the high latitude sites reversed, with greater abundances of invertebrates in the control or moderately enriched plots than in the highly enriched plots. This pattern was driven largely by lesser abundances in the highly enriched plots of Galeomatidae sp. bivalves at H1 and *Salinator fragilis* at H2. Galeomatidae sp. bivalves can be found up to 10 cm below the surface of the sediments (Gofas 1991). As many species of this family are sensitive to changes in sediment nutrients, including oxygen, their low abundance in highly enriched plots may have been a response to the reduction in the depth to which sediments were oxygenated (Diaz and Rosenberg 1995, King et al. 2004). *Salinator,* however, is a surface dwelling herbivorous gastropod (Raffaelli et al. 2003), so it is unlikely that this was a response to declining sediment oxygen. Abundances of this taxon are often high in seagrass habitats and generally do not respond directly to organic enrichment (Poore 1982), but may respond indirectly through interactions with other species (Taylor et al. 2010).

At other sites, the species responding most strongly to organic enrichment were the bivalve *Mysella vitrea* (at L1) and the amphipod *Gammarus* sp. (at L2). The abundance of *M. vitrea,* which can be found at depths of up to 10 cm below the surface of the sediment (Kerr and Corfield 1998), displayed a temporally persistent pattern of lower abundance in each of the enriched treatments than the control plots. This pattern bore a strong relationship to differences among treatments in the depth to which sediment was oxygenated. In other studies, the abundance of *Mysella* sp. has been positively correlated with redox potential (Kerr and Corfield 1998, Nilsson and Rosenberg 1994) and has been notably absent from organic-rich seagrass sediments (Nicastro and Bishop 2013). Hence, it appears it may have directly responded to the declining sediment conditions. The surface dwelling amphipod,

Gammarus sp. (Hervant et al. 1999, Slattery 1985), was significantly less abundant at L2 in moderately than either highly enriched or control sediments. This pattern was almost identical to that displayed by chlorophyll *a* at this site suggesting that this detritivore may have been responding to a reduced availability of this putative food supply (Bundschuh and Schulz 2011).

The Pearson-Rosenberg model (1978) predicts that as organic enrichment progresses, species richness will decline and opportunistic species will dominate. Consistent with the model, species richness was lower in highly enriched than moderately enriched or control plots for the duration of our experiment at one high latitude sites, and for part of the experiment at the other. At one of the sites, H2, the opportunistic polychaetes *Perinereis* sp. and *Nereis* sp. displayed increased abundances in enriched versus control plots at 60 d after enrichment. Over the duration of the experiment, the capitellid *M. australiensis* increased in abundance in all experimental plots at one of the high latitude sites, presumably responding to disturbance rather than nutrient addition per se. It is possible that our method of adding detritus to sediments, which also physically disturbed sediments, reduced our capacity to detect difference in the magnitude of the effects of organic enrichment.

These results highlight the context-dependency of ecosystem responses to local stressors (Conley et al. 2009, de Jonge et al. 2002). In this study it is likely that differences in sediment properties (see Nicastro and Bishop 2013) and benthic community structure among study sites modulated effects of organic enrichment, with climatic context, by contrast, having relatively little influence. Nevertheless, our high and low latitude sites differed by only 5° of latitude and 3°C in temperature, and it is possible that, had sites of greater climatic difference been compared, a stronger effect of latitude may have been apparent. In other studies, the warmer climates of lower latitudes accelerate rates of detrital remineralization (Kätterer et al. 1998, Kirschbaum 1995), potentially exacerbating the degradation of environmental conditions through over-stimulating bacterial respiration, increased frequency

and severity of eutrophication, and faunal communities reaching hypoxia thresholds sooner (Conley et al. 2009, Diaz and Rosenberg 1995, Vaquer-Sunyer and Duarte 2008). Additional studies are now needed to confirm the mechanisms by which environmental context modifies the effects of organic enrichment. This will enable strategies for managing eutrophication to be appropriately tailored to individual sites.

Acknowledgements

This research was funded by Macquarie University Higher Degree Research funding to L. Ainley and an Australian Research Council Discovery Grant (DP1093444) to M. Bishop. We thank D. McAfee, R. Furtado M. Raybaud and K. Spruce for assistance with field and lab-work.

References

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. - Austral Ecology 26: 32-46.

Anderson, M. J., et al. 2008. Permanova+ for primer: Guide to software and statisitcal methods. - PRIMER-E Ltd.

Apple, J. K., et al. 2006. Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. - Aquatic Microbial Ecology 43: 243-254.

Arnosti, C., et al. 1998. Temperature dependence of microbial degradation of organic matter in marine sediments: Polysaccharide hydrolysis, oxygen consumption, and sulfate reduction. - Marine Ecology Progress Series 165: 59-70.

Baird, D., et al. 2004. Consequences of hypoxia on estuarine ecosystem function: Energy diversion from consumers to microbes. - Ecological Applications 14: 805-822.

Bishop, M. J. and Kelaher, B. P. 2007. Impacts of detrital enrichment on estuarine assemblages: Disentangling effects of frequency and intensity of disturbance. - Marine Ecology Progress Series 341: 25-36.

Bishop, M. J. and Kelaher, B. P. 2008. Non-additive, identity-dependent effects of detrital species mixing on soft-sediment communities. - Oikos 117: 531-542.

Bishop, M. J. and Kelaher, B. P. 2013. Replacement of native seagrass with invasive algal detritus: Impacts to estuarine sediment communities. - Biological Invasions 15: 45-59.

Bishop, M. J., et al. 2007. Trophic cul-de-sac, *pyrazus ebeninus*, limitis trophic transfer through an estuarine detritus based food web. - Oikos 116: 427-438.

Bishop, M. J., et al. 2006. Benthic biological effects of seasonal hypoxia in a eutrophic estuary predate rapid coastal development. - Estuarine, Coastal and Shelf Science 70: 415-422.

Bundschuh, M. and Schulz, R. 2011. Population response to ozone application in wastewater: An on-site microcosm study with *gammarus fossarum* (crustacea: Amphipoda). - Ecotoxicology 20: 466-473.

Clarke, K. R. and Warwick, R. M. 1994. Changes in marine communties: An approach to statistical analysis and interpretation. - PRIMER-E Ltd.

Cloern, J. E. 2001. Our evolving conceptual model of the coastal eutrophication problem. - Marine Ecology Progress Series 210: 223-253.

Conant, R. T., et al. 2011. Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. - Global Change Biology 17: 3392-3404.

Conley, D. J., et al. 2009. Ecosystem thresholds with hypoxia. Eutrophication in coastal ecosystems. Springer, pp. 21-29.

de Jonge, V. N., et al. 2002. Causes, historical development, effects and future challenges of a common environmental problem: Eutrophication. - Hydrobiologia 475: 1-19.

Diaz, R. J. and Rosenberg, R. 1995. Marine benthic hypoxia: A review of its ecological effects and the behavioural responses of benthic macrofauna. - Oceanography and marine biology. An annual review 33: 245-203.

Diaz, R. J. and Rosenberg, R. 2008. Spreading dead zones and consequences for marine ecosystems. - Science 321: 926-929.

Elliott, M. 1994. The analysis of macrobenthic community data. - Marine Pollution Bulletin 28: 62-64.

Folke, C., et al. 2004. Regime shifts, resilience, and biodiversity in ecosystem management. - Annual Review of Ecology Evolution and Systematics 35: 557-581.

Gofas, S. 1991. The family galeommatidae (bivalvia, leptonacea) in the eastern atlantic. - Veliger 34: 344-353.

Gray, J. S., et al. 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. - Marine Ecology Progress Series 238: 249-279.

Hall, S. J. 1994. Physical disturbance and marine benthic communities: Life in unconsolidated sediment. - Oceanography and Marine Biology. 32: 179-239.

Hervant, F., et al. 1999. Comparative study on the metabolic responses of subterranean and surface-dwelling amphipods to long-term starvation and subsequent refeeding. - Journal of Experimental Biology 202 Pt 24: 3587-3595.

Jeffrey, S. and Humphrey, G. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. - Biochem Physiol Pflanz BPP 1975.

Jørgensen, B. B. 1982. Mineralization of organic matter in the sea bed—the role of sulphate reduction. -.

Kätterer, T., et al. 1998. Temperature dependence of organic matter decomposition: A critical review using literature data analyzed with different models. - Biology and fertility of soils 27: 258-262.

Kelaher, B. P., et al. 2003. Foraging by the mud snail, *ilyanassa obsoleta* (say), modulates spatial variation in benthic community structure. - Journal of Experimental Marine Biology and Ecology 292: 139-157.

Kerr, G. and Corfield, J. 1998. Association between the ghost shrimp *trypaea australiensis* dana 1852 (crustacea : Decapoda) and a small deposit-feeding bivalve *mysella vitrea* laserson 1956 (mollusca : Leptonidae). - Marine and Freshwater Research 49: 801-806.

King, C. K., et al. 2004. An assessment of five australian polychaetes and bivalves for use in whole-sediment toxicity tests: Toxicity and accumulation of copper and zinc from water and sediment. - Archives of Environmental Contamination and Toxicology 47: 314-323.

Kirschbaum, M. U. F. 1995. The temperature-dependence of soil organic-matter decomposition, and the effect of global warming on soil organic-c storage. - Soil Biology & Biochemistry 27: 753-760.

Majeed, S. A. 1987. Organic matter and biotic indexes on the beaches of north brittany. - Marine Pollution Bulletin 18: 490-495.

Martins, I., et al. 1999. The effect of salinity on the growth rate of the macroalgae *enteromorpha intestinalis* (chlorophyta) in the mondego estuary (west portugal). - Acta Oecologica 20: 259-265.

McLusky, D. 1982. The impact of petrochemical effluent on the fauna of an intertidal estuarine mudflat. - Estuarine, Coastal and Shelf Science 14: 489-499.

Nicastro, A. and Bishop, M. J. 2013. Effects of tidal inundation on benthic macrofauna associated with the eelgrass *zostera muelleri*. - Estuarine, Coastal and Shelf Science 117: 238-247.

Nicastro, A. and Bishop, M. J. 2013. Weak and habitat-dependent effects of nutrient pollution on macrofaunal communities of southeast australian estuaries. - PLoS One 8: e65706.

Nilsson, H. C. and Rosenberg, R. 1994. Hypoxic response of two marine benthic communities. - Marine ecology progress series. Oldendorf 115: 209-217.

Nixon, S. W. 1995. Coastal marine eutrophication - a definition, social causes, and future concerns. - Ophelia 41: 199-219.

Pearson, T. and Stanley, S. 1979. Comparative measurement of the redox potential of marine sediments as a rapid means of assessing the effect of organic pollution. - Marine Biology 53: 371-379.

Pearson, T. H. and Rosenberg, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environemnt. - Oceanography and Marine Biology: Annual Review. 16: 229-311.

Poore, G. C. B. 1982. Benthic communities of the gippsland lakes, victoria. - Australian Journal of Marine and Freshwater Research 33: 901-915.

Rabalais, N. N., et al. 1994. Comparison of continuous records of near-bottom dissolved-oxygen from the hypoxia zone along the louisiana coast. - Estuaries 17: 850-861.

Raffaelli, D., et al. 2003. Biodiversity and ecosystem processes in shallow coastal waters: An experimental approach. - Journal of Sea Research 49: 133-141.

Roper, T., et al. 2011. Assessing the condition of estuaries and coastal lake ecosystems in nsw. Monitoring, evaluation and reporting program, Technical report series. - Office of Environment and Heritage.

Rosenberg, G. and Ramus, J. 1982. Ecological growth strategies in the seaweeds *gracilaria foliifera* (rhodophyceae) and *ulva* sp (chlorophyceae) - soluble nitrogen and reserve carbohydrates. - Marine Biology 66: 251-259.

Rossi, F. 2006. Small-scale burial of macroalgal detritus in marine sediments: Effects of *ulva* spp. On the spatial distribution of macrofauna assemblages. - Journal of Experimental Marine Biology and Ecology 332: 84-95.

Roy, P. S., et al. 2001. Structure and function of south-east australian estuaries. - Estuarine, Coastal and Shelf Science 53: 351-384.

Scanes, P., et al. 2007. Evaluation of the utility of water quality based indicators of estuarine lagoon condition in nsw, australia. - Estuarine, Coastal and Shelf Science 74: 306-319.

Slattery, P. N. 1985. Life histories of infaunal amphipods from subtidal sands of monterey bay, california. - Journal of Crustacean Biology 5: 635-649.

Small, C. and Nicholls, R. J. 2003. A global analysis of human settlement in coastal zones. - Journal of Coastal Research 19: 584-599.

Smith, V. H. and Schindler, D. W. 2009. Eutrophication science: Where do we go from here? - Trends in Ecology & Evolution 24: 201-207.

Smith, V. H., et al. 1999. Eutrophication: Impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. - Environmental pollution 100: 179-196.

Taylor, S. L., et al. 2010. Impacts of detritus from the invasive alga caulerpa taxifolia on a soft sediment community. - Marine Ecology Progress Series 420: 73-81.

Tilman, D., et al. 2001. Forecasting agriculturally driven global environmental change. - Science 292: 281-284.

Underwood, A. J. 1997. Experiments in ecology: Their logical design and interpretation using analysis of variance. - Cambridge University Press.

Van Veen, J. A. and Kuikman, P. J. 1990. Soil structural aspects of decomposition of organic matter by micro-organisms. - Biogeochemistry 11: 213-233.

Vaquer-Sunyer, R. and Duarte, C. M. 2008. Thresholds of hypoxia for marine biodiversity. - Proceedings of the National Academy of Sciences 105: 15452-15457.

Westrich, J. T. 1983. Consequences and controls of bacterial sulfate reduction in marine sediments. P. D. Dissert. Yale Univ, New Haven, CT. p. 530

6. GENERAL DISCUSSION

Intraspecific leaf trait variation as a major driver of decomposition

This thesis examined how decomposition processes in estuaries vary in accordance with climatic setting and anthropogenic nutrient enrichment, both as a consequence of intraspecific variation in leaf traits and direct effects of environmental setting on decomposition. Ongoing climate warming and nutrient enrichment have the potential to modify decomposition processes by altering both detrital leaf traits and the physical environment. There is a lack of empirical data that investigates the influence of environmental change on important ecological processes, such as decomposition, at ecologically relevant scales. I used existing gradients of latitude and nutrient enrichment to test interactive effects of these factors on intraspecific leaf trait variability and decomposition using mensurative, observational and manipulative field experiments. In making use of large-scale spatial gradients, I utilized a "space-for-time" substitution to help identify what the ongoing ramifications of warming and nutrient enrichment might be.

Following a state-wide classification of estuaries in New South Wales (NSW), Australia by Roper et al. (2011), a series of 8 highly enriched and 8 relatively unenriched estuaries along the same latitudinal gradient were selected as field sites to be used for these experiments. Although not all experiments were carried out using all 16 estuaries, with this large-scale approach, sources of spatio-temporal variation in leaf traits and decomposition could be tested. Further, by conducting enrichment experiments in relatively unmodified estuaries, I could simulate forecasted changes to detrital regimes under scenarios of eutrophication.

This thesis found that decomposition processes varied across climatic and nutrient gradients, both as a consequence of the direct and indirect effect of the abiotic environment

and an indirect effect of spatial variation in leaf traits, within species. More specifically, I found that:

1. Intraspecific variation in leaf traits is important in contributing to spatial variation in decomposition, even over relatively small spatial scales. Hence, studies examining key controls of decomposition should not only focus on interspecific variation in leaf traits, but also intraspecific variation.
2. Climatic and nutrient gradients can have non-additive influences on decomposition processes, arising from direct and indirect effects.
3. There is considerable variation in decomposition processes at the local scale, arising from differences in the abiotic environment and community assembly among sites. Hence, local environmental context plays an important role in mediating the ecosystem response to changes in the traits and supply of detrital material.

Intraspecific variation in leaf traits

Studies have long recognized the importance of variation in leaf traits in contributing to decomposition processes (Díaz and Cabido 2001, Lavorel and Garnier 2002, Chapin 2003). Across species, leaf traits display predictable spatial patterns (Reich et al. 1999), and variation in particular traits, in turn, has a strong influence on decomposition (Cornwell et al. 2008). Many studies, however, assume that in contrast to interspecific variation in leaf traits, intraspecific variation is small, and hence relatively unimportant in determining decomposition rates (Albert et al. 2010a, Auger and Shipley 2013, but see Lecerf and Chauvet 2008, Albert et al. 2010b for counter examples).

I examined (**chapter 2**) spatio-temporal variation in the traits of the seagrass *Zostera muelleri* and the mangrove *Avicennia marina*, which are dominant primary producers along the southeast coast of Australia (Kerr and Strother 1990, Duke et al. 2010, Nicastro and

Bishop 2013), and key contributors to detrital pools. Sampling of spatial gradients in nutrients and climate revealed considerable intraspecific variation in key leaf traits implicated in controlling decomposition. Even across a relatively modest latitudinal gradient of 7°, a strong gradient in several key traits including the carbon to nitrogen ratio (C:N) was found. Patterns of spatio-temporal variation differed between seagrass and mangrove leaves, and did not necessarily follow the same gradients of interspecific variation in leaf traits reported by previous studies (e.g.Reich and Oleksyn 2004, Wright et al. 2004). Furthermore, spatial patterns in the leaf traits showed significant temporal variation. Only the C:N showed a temporally persistent, that was similar between seagrass and mangrove. Since leaf traits are a key predictor for decomposition (Cornelissen and Thompson 1997, Cornelissen et al. 1999, Pérez-Harguindeguy et al. 2000), it was posited that their spatial variability would lead to large-scale variation in decomposition processes.

Direct and indirect effects of environmental gradients on decomposition

Along with leaf traits, (Aerts 1997, Díaz and Cabido 2001, Moore et al. 2004) I hypothesized that rates of leaf litter decomposition would vary across environmental gradients both as a consequence of direct effects of the abiotic environment, and also as a result of indirect effects of the abiotic environment on leaf traits and on decomposer communities.

Experiments in which seagrass and mangrove leaves from a common source were transplanted into different abiotic environments revealed spatial differences in their decomposition rate (**chapter 3**). Overall, litter decomposition was enhanced in warmer climates, and in nutrient enriched estuaries, but non-additively. The nature of the interaction differed for seagrass and mangrove litter. The effects, which occurred independent of

variation in leaf traits, may have reflected both direct effects of the abiotic environment and indirect effects arising from spatial variation in decomposer communities.

Indirect effects of environment, arising from intraspecific variation in leaf traits also contributed to spatial variation in decomposition. Common garden experiments, where leaves from different source populations, and hence with different traits, were transplanted into a common site, supported the important role of intraspecific variation in leaf traits in contributing to decomposition processes (**chapter 4**). In several instances, the two species displayed differing relationships between leaf traits and decomposition rates. These results highlight that patterns of intraspecific variability in leaf traits may not necessarily reflect patterns of interspecific variation, and relationships between leaf traits and decomposition processes may vary among species such that they cannot necessarily be predicted from interspecific patterns (Albert et al. 2010a).

The direct and indirect effects of climatic setting on decomposition rates acted in opposite directions. Where as litter of a common source decomposed faster in warmer than cooler climates, the high C:N litter obtained from warmer climates decomposed more slowly than the lower C:N litter from cooler climates, when decomposed side by side. In real systems, where these two processes occur simultaneously, it is unclear how the direct and indirect effects would interact or would cancel one another out. Gessner et al. (2010) discusses the effects of changes in diversity of both litter supply and decomposer communities on carbon and nutrient cycling and confirms that the relative contribution of controlling factors shows considerable variation. However, studies are still needed that can unravel the mechanism of interactions between direct and indirect effects on decomposition, and that incorporate greater ecological realism in their design (Hector et al. 2009, Hillebrand and Matthiessen 2009).

Effects of environmental setting on the community-level impacts of detrital remineralisation

In mediating decomposition rates and determining leaf traits, environment may determine whether detrital decomposition fuels or inhibits benthic communities. Whereas small loadings of slow decomposing, refractory detritus, can enhance the resource base of benthic consumers (Pearson and Rosenberg 1978), large loadings of fast decomposing labile detritus may lead to environmental degradation and community collapse, as a consequence of over-stimulation of bacterial respiration during detrital decomposition (Pearson and Rosenberg 1978, Diaz and Rosenberg 2008, Vaquer-Sunyer and Duarte 2008).

The final data chapter of this thesis (**chapter 5**) asked the question of how environmental setting influences the ultimate impact of detritus in estuarine ecosystems. In contrast to the previous chapters, this chapter made use of the ephemeral green alga, *Ulva* sp. that is symptomatic of eutrophication. Parallel enrichment experiments in two estuaries of northern and two in southern NSW revealed significant site-specific effects of detrital addition on benthic community structure that did not necessarily correspond to climatic setting. Differences among sites in background organic loading, properties of sediments that influence organic matter retention, and in benthic community structure likely contributed to this result. Studies independently manipulating sediment properties, background organic loading and metacommunities of fauna are now needed to assess the causes of differences among sites in the effects of detrital loading. Nevertheless, the highly site-specific effects of detrital loading again point to the important interplay between abiotic environment, litter traits and biotic assemblage composition in mediating decomposition processes, that in turn influence community structure and energy and nutrient cycling.

Addressing the heterogeneity of detritus and its impacts in estuarine ecosystems

Our understanding of the relative contribution of leaf traits, environmental characteristics and decomposer communities to decomposition process has primarily been driven from correlative and observation studies in terrestrial environments (Aerts 1997, Cornwell et al. 2008). Our knowledge of how multiple factors interact to determine decomposition is poor, particularly in coastal and marine environments. Consequently in models of food web dynamics in aquatic ecosystems, it is often assumed that detritus is a static and homogenous resource. My work has begun to identify the nature and variability of direct and indirect controls on decomposition in estuarine environments. Further identifying and acknowledging the scales at which these controls act, will enable appropriate interpretations to be made.

Manipulative studies that disentangle the effects of multiple factors are needed to generate a comprehensive and mechanistic understanding of how the environment, leaf traits and the decomposer community interact and influence decomposition. In many instances, factors act non-additively, enhancing or inhibiting their individual effects (Crain et al. 2008). Understanding the relationships among factors enables us to determine the relative contributions of each factor, and the scales at which these factors may dominate over others.

I used a primarily descriptive approach to assess the effects of climatic setting and nutrient enrichment, rather than controlled manipulations of temperature and nutrients. This limited my ability to draw causative conclusions. Nevertheless, I detected clear relationships between biotic and abiotic variables across ecologically relevant gradients of nutrient enrichment and climate, which can serve as the building blocks for future studies.

Although it was hypothesized that broad scale environmental gradients in aspects of climate, such as temperature and rainfall, would be most important in influencing decomposition process, I also found that local scale factors were of importance. This study

did not, however, directly examine the spatial scales of variation in detrital processes. Studies with a nested design that examines decomposition at a hierarchy of scales would be required.

A major outcome of this work was that the decomposition of seagrass and mangrove, and how it was affected by the same controlling factors, was vastly different between these two fundamentally different species. This warrants further attention and suggests that our current base of knowledge, primarily based on interspecific variation in traits, does not apply to all species. Understanding why each species displays a different relationship between intraspecific variation in leaf traits and its decomposition is essential.

Finally, within the scope of this project, I was unable to assess some important components of the decomposition process. Decomposition by microbial organisms was not examined in this thesis. However, I acknowledge that this group may be an important contributor to underlying local scale processes and the observed differences between mangrove and seagrass decomposition processes.

The imperative to understand the controls on decomposition at all scales

Human activities continue to degrade ecosystems worldwide (Alongi 2002, Kennish 2002, Crain et al. 2008). Estuaries are considered among the most impacted environments from anthropogenic stressors (Kennish 2002). Due to the increasing demands of human populations and agricultural expansion in our coastal environments, the frequency, duration and intensity of environmental changes and degradation are likely to increase, resulting in important losses of ecosystem structure and functioning (Gray et al. 2002, Barbier et al. 2011). The results of this work provide important insights for understanding the consequences of environmental change to decomposition processes, driven by large-scale processes such as warming temperatures and anthropogenic stressors.

The consequences of changes to decomposition processes have important implications for carbon sequestration and storage and ecosystem dynamics (Davidson and Janssens 2006). The interacting effect of temperature and nutrient enrichment has the potential to accelerate the rate of decomposition, as well as modify environmental conditions (Bardgett et al. 2008), potentially reducing resilience to future change. Accelerated rates of detrital decomposition will release stored carbon to the atmosphere and deplete sediment oxygen resources (Davidson and Janssens 2006). In extreme cases, this leads to the development of hypoxic conditions and a reduction in biodiversity, fisheries resources and ecosystem functioning. Increasing temperature and nutrient enrichment, also stimulates coastal eutrophication due to increased primary productivity and detrital load, and can further exacerbate changes to decomposition processes (Diaz and Rosenberg 2008).

Knowledge of the interactive effects, which can be non-additive, of multiple stressors on decomposition processes in estuarine environments is crucial for the management of coastal ecosystems. Adaptation of estuarine environments to ongoing climate warming and anthropogenic pressure may be contingent on reducing or capping anthropogenic nutrient loads to levels below known thresholds that trigger abrupt changes in decomposition processes and ecosystem structure and functioning. Further, to develop the tools necessary for effective management, and allow us to respond to change, we must first understand the mechanisms of change driven by multiple factors and interface the disparate scales that such factors are studied (Levin 1992, Chave 2013).

References

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. Oikos **79**:439-449.

Albert, C. H., W. Thuiller, N. G. Yoccoz, R. Douzet, S. Aubert, and S. Lavorel. 2010a. A multi‐trait approach reveals the structure and the relative importance of intra‐vs. interspecific variability in plant traits. Functional Ecology **24**:1192-1201.

Albert, C. H., W. Thuiller, N. G. Yoccoz, A. Soudant, F. Boucher, P. Saccone, and S. Lavorel. 2010b. Intraspecific functional variability: extent, structure and sources of variation. Journal of Ecology **98**:604-613.

Alongi, D. M. 2002. Present state and future of the world's mangrove forests. Environmental conservation **29**:331-349.

Auger, S., and B. Shipley. 2013. Inter-specific and intra-specific trait variation along short environmental gradients in an old-growth temperate forest. Journal of Vegetation Science **24**:419-428.

Barbier, E. B., S. D. Hacker, C. Kennedy, E. W. Koch, A. C. Stier, and B. R. Silliman. 2011. The value of estuarine and coastal ecosystem services. Ecological Monographs **81**:169-193.

Bardgett, R. D., C. Freeman, and N. J. Ostle. 2008. Microbial contributions to climate change through carbon cycle feedbacks. Isme Journal **2**:805-814.

Chapin, F. S. 2003. Effects of plant traits on ecosystem and regional processes: a conceptual framework for predicting the consequences of global change. Annals of Botany **91**:455-463.

Chave, J. 2013. The problem of pattern and scale in ecology: what have we learned in 20 years? Ecology Letters **16**:4-16.

Cornelissen, J. H. C., N. Pérez-Harguindeguy, S. Díaz, J. P. Grime, B. Marzano, M. Cabido, F. Vendramini, and B. Cerabolini. 1999. Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. New Phytologist **143**:191-200.

Cornelissen, J. H. C., and K. Thompson. 1997. Functional leaf attributes predict litter decomposition rate in herbaceous plants. New Phytologist **135**:109-114.

Cornwell, W. K., J. H. Cornelissen, K. Amatangelo, E. Dorrepaal, V. T. Eviner, O. Godoy, S. E. Hobbie, B. Hoorens, H. Kurokawa, N. Pérez-Harguindeguy, H. M. Quested, L. S. Santiago, D. A. Wardle, I. J. Wright, R. Aerts, S. D. Allison, P. van Bodegom, V. Brovkin, A. Chatain, T. V. Callaghan, S. Díaz, E. Garnier, D. E. Gurvich, E. Kazakou, J. A. Klein, J. Read, P. B. Reich, N. A. Soudzilovskaia, M. V. Vaieretti, and M. Westoby. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters **11**:1065-1071.

Crain, C. M., K. Kroeker, and B. S. Halpern. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters **11**:1304-1315.

Davidson, E. A., and I. A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature **440**:165-173.

Diaz, R. J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science **321**:926-929.

Díaz, S., and M. Cabido. 2001. Vive la difference: plant functional diversity matters to ecosystem processes. Trends in ecology & evolution **16**:646-655.

Duke, N. C., K. Kathiresan, S. G. Salmo III, E. S. Fernando, J. R. Peras, S. Sukardjo, T. Miyagi, J. C. Ellison, N. E. Koedam, Y. Wang, J. Primavera, O. Jin Eong, J. Wan-Hong Yong, and V. Ngoc Nam. 2010. *Avicennia marina*. IUCN Red List of Threatened Species.

Gessner, M. O., C. M. Swan, C. K. Dang, B. G. McKie, R. D. Bardgett, D. H. Wall, and S. Hättenschwiler. 2010. Diversity meets decomposition. Trends in ecology & evolution **25**:372-380.

Gray, J. S., R. S. S. Wu, and Y. Y. Or. 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. Marine Ecology Progress Series **238**:249-279.

Hector, A., T. Bell, J. Connolly, J. Finn, J. Fox, L. Kirwan, M. Loreau, J. McLaren, B. Schmid, and A. Weigelt. 2009. The analysis of biodiversity experiments: from pattern toward mechanism. Biodiversity, ecosystem functioning, and human wellbeing: an ecological and economic perspective. Oxford University Press, Oxford, UK:3-13.

Hillebrand, H., and B. Matthiessen. 2009. Biodiversity in a complex world: consolidation and progress in functional biodiversity research. Ecology Letters **12**:1405-1419.

Kennish, M. J. 2002. Environmental threats and environmental future of estuaries. Environmental conservation **29**:78-107.

Kerr, E. A., and S. Strother. 1990. Seasonal changes in standing crop of *Zostera muelleri* in south-eastern Australia. Aquatic Botany **38**:369-376.

Lavorel, S., and E. Garnier. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Functional Ecology **16**:545-556.

Lecerf, A., and E. Chauvet. 2008. Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. Basic and Applied Ecology **9**:598-605.

Levin, S. A. 1992. The problem of pattern and scale in ecology: the Robert H. MacArthur award lecture. Ecology **73**:1943-1967.

Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. de Ruiter, Q. Dong, A. Hastings, N. C. Johnson, K. S. McCann, K. Melville, P. J. Morin, K. Nadelhoffer, A. D. Rosemond, D. M. Post, J. L. Sabo, K. M. Scow, M. J. Vanni, and D. H. Wall. 2004. Detritus, trophic dynamics and biodiversity. Ecology Letters **7**:584-600.

Nicastro, A., and M. J. Bishop. 2013. Effects of tidal inundation on benthic macrofauna associated with the eelgrass *Zostera muelleri*. Estuarine, Coastal and Shelf Science **117**:238-247.

Pearson, T. H., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environemnt. Oceanography and Marine Biology: Annual Review. **16**:229-311.

Pérez-Harguindeguy, N., S. Díaz, J. H. C. Cornelissen, F. Vendramini, M. Cabido, and A. Castellanos. 2000. Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. Plant and Soil **218/2**:21-30.

Reich, P. B., D. S. Ellsworth, M. B. Walters, J. M. Vose, C. Gresham, J. C. Volin, and W. D. Bowman. 1999. Generality of leaf trait relationships: A test across six biomes. Ecology **80**:1955-1969.

Reich, P. B., and J. Oleksyn. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proceedings of the National Academy of Sciences of the United States of America **101**:11001-11006.

Roper, T., B. Creese, P. Scanes, K. Stephens, R. Williams, J. Dela-Cruz, G. Coade, B. Coates, and M. Fraser. 2011. Assessing the condition of estuaries and coastal lake ecosystems in NSW. Office of Environment and Heritage, Sydney.

Vaquer-Sunyer, R., and C. M. Duarte. 2008. Thresholds of hypoxia for marine biodiversity. Proceedings of the National Academy of Sciences **105**:15452-15457.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M. L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature **428**:821-827.