COMPARATIVE ESTUARINE DYNAMICS : TROPHIC LINKAGES AND ECOSYSTEM FUNCTION / JOSEPH KENWORTHY

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Comparative estuarine dynamics: trophic linkages and ecosystem function

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This thesis is submitted in partial fulfilment for the degree of PhD
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University of St Andrews

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Abstract

Estuarine systems are of crucial importance to the provision of goods and services on a global scale. High human population densities in coastal systems have caused an increasing input of pollutants, of which nutrient pollution is of major concern. Increasingly, these areas are also impacted by physical disturbance, which can originate from anthropogenic sources (e.g. bait digging, shipping) or climate change causing increasingly frequent and intense storms. The individual impacts of such stressors on ecosystems have been investigated however their combined impacts have received less attention. Cumulative impacts of multiple stressors are unpredictable and will likely result in non-additive effects. Further, the effect of local environmental context on multiple stressors is a relatively understudied topic.

Work in this thesis compared the combined impact of nutrient enrichment and physical disturbance in Scotland and Australia, using a series of manipulative field experiments. Results demonstrate that response to stressors is highly context dependent, varying between and within geographic locations. While the background levels of stress may vary, by comparing these two locations it is possible to comment on the adaptations and response that communities within different parts of the world display when subjected to additional stress. This study demonstrates that environmental context must be considered when implementing future management practices.

Further work demonstrated that the impact of multiple stressors varies depending on how the stress is applied –whether stressors are applied simultaneously or whether there is a delay between two stressors. This study was among the first of its kind, assessing the implications of how multiple stressors react with each other given the order and intensity in which stressors were applied. Results demonstrated that systems can become sensitised to stress making them increasingly vulnerable to additional stress. Future research should be focussed on incorporating ecologically relevant scenarios of how stressors will impact estuaries while considering how environmental context will mediate impacts.

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1 General introduction

Estuaries are geologically recent and ephemeral features of the coast (McLusky, 1999; Russell, 1967) which have been used by humans for centuries, providing a livelihood, water, and a means of transportation (Sala et al., 2000). Most of the major cities across the world are located along estuaries and rivers, equating to approximately 75% of the world's population being supported by estuarine systems and their coastal watersheds (Paerl, 2006). The impact of these high population densities has led to rising pollution levels brought about by the industrial revolution, farming techniques, and sewage disposal (Stauffer, 1998). Estuarine systems are important for upkeep of numerous services that are important to humanity. These services can become detrimentally altered through both climatic and anthropogenic impacts placing increasing pressures on the estuarine ecosystems and the functions they support (Hoegh-Guldberg & Bruno, 2010; Lotze et al., 2006; Sala et al., 2000).

The services derived from estuarine systems are driven by the organisms that inhabit these areas and that are specialised for life in these transitional systems between freshwater and marine environments. While there are many types of estuarine ecosystems to explore, the purpose of this thesis is to focus on benthic intertidal sedimentary habitats. The major primary producers in these systems, termed microphytobenthos (MPB) and consisting mainly of benthic diatoms, are functionally important in estuarine depositional habitats. The organisms that make up the MPB contribute significantly on both global and local scales to multiple functions and services. The most important of these include their contribution to the global carbon budget (Cahoon, 1999; Guarini et al., 2008), biostabilisation (Spears et al., 2008) and as a food source for macrofauna (Castenholz, 1961).

Estuarine systems are found globally, however there are few studies that directly compare their functional capacity across continents (Elliott & Whitfield, 2011). This has led to generalisations and implementations of management procedures designed for European systems, for example, on Australian estuaries with very different characteristics (Hutchings, 1999). Estuaries in Australia have not been studied to the extent of those in Europe, and few direct comparisons have been made (Barnes, 2014; Eyre & Balls, 1999; Eyre, 1998), although in recent years an increasing number of studies have been undertaken, arising from a need for assessment and monitoring of Australia's rivers (see Digby et al., 1999).

The rationale behind this review is to effectively define an estuary, to outline the differences between European and Australian estuaries, and to understand their importance in terms of ecosystem functioning. It will serve as a foundation for a comparative study between estuaries in the northern and southern hemisphere. Through studying estuaries in different parts of the world, an insight into the functional consequences of ecosystem variation on the resilience of these systems to climate change and coastal development can be achieved. The experimental approach used in this thesis has been designed to compare and contrast the effects of disturbance regimes on intertidal estuarine ecosystem functioning, exploring the changes to trophic interactions in each system.

1.1 Defining an estuary

In the early stages of estuarine research it became apparent that an accurate and universally understood definition of an estuary was needed. This led to the Venice symposium in 1958 which defined the zones of estuarine and brackish water systems. Zones were based on salinity gradients (Elliott & McLusky, 2002). This eventually led to one of the most widely adopted definitions of an estuary, as defined by Pritchard (1967). This definition states that an estuary is:

"A semi-enclosed coastal body of water, which has a free connection with the open sea, and within which sea water is measurably diluted with fresh water derived from land drainage."

It should be noted that Pritchard dismissed the classification of brackish water seas (i.e. the Baltic Sea) as estuaries due to the lack of tides. This leads to an understanding that although estuaries are termed as being brackish, not all brackish waters are estuaries (Elliott & McLusky, 2002). Further definitions were made in the following years to include the influence of biotic zones (Elliott & McLusky, 2002), tides (Fairbridge, 1980) and upper and lower boundaries (Urban Waste Water Treatment (England and Wales) Regulations, 1994). The UK, through its urban waste-water regulations (1994), defined the lower boundary of an estuary as being of a salinity less than 95% of the adjacent seawater for 95% of the time (Urban Waste Water Treatment (England and Wales) Regulations, 1994). The most widely accepted definitions of estuaries are now influenced by Fairbridge (1980) incorporating the influence of tides on the estuarine system, therefore defining an estuary as an inlet of the sea, reaching as far as the upper limit of the tidal rise while still encompassing the fact that an estuary is a semi-enclosed body of water, or a formation of the coast.

In terms of system ecology and how it changes along an estuarine gradient, the most important factors to include in the definition are those relating the hydrography to ecological transformations (Telesh & Khlebovich, 2010). Salinity has a large effect on the organisms living within an estuary, with species range, diversity and community composition all influenced by salinity tolerances (Attrill, 2002; Currie & Small, 2006; Hirst, 2004). Only a relatively small number of species are considered truly estuarine (Hutchings, 1999), living exclusively in brackish water (Attrill, 2002; Remane & Schlieper, 1971). For example, surveys of the Hawkesbury River in Australia found 47 mollusc species of which 15 were considered truly estuarine (Jones et al., 1986), and 127 species of polychaetes of which only 7 were able to survive in salinities less than 25 (Hutchings & Murray, 1984).

These previous definitions do not incorporate the dynamics of many Australian and South African estuaries (among others) which can periodically become cut off from the sea and become hypersaline (Elliott & Whitfield, 2011; Potter et al., 2010). Some define these as “ephemeral estuaries,” or seasonally closed, becoming similar in characteristics to a coastal lagoon (Kench, 1999). Taking these factors into account, the definition of an estuary for the purposes of this review will be as follows:

An estuary is a semi-enclosed formation of the coast where freshwater and seawater mix creating a salinity gradient. The estuary may periodically be cut-off from the sea allowing the formation of hypersaline conditions in times of high evaporation and low fluvial input. There is a tidal influence whereby water enters and becomes diluted with freshwater when the estuary is not cut off from the sea.

1.2 Estuarine characteristics

1.2.1 Salinity and freshwater influxes

In European and many northern hemisphere river systems there is generally more rainfall in winter months compared to summer months, although this may also vary from week to week to a greater degree than between seasons (Elliott & McLusky, 2002). European estuaries remain open at all times to the influence of tides and therefore the salinity of the estuary will always be fluctuating (Elliott & McLusky, 2002). While the majority of the literature is dominated with European examples, these do not represent estuarine systems worldwide, for example, salinity regimes in Australian estuaries are generated mainly by runoff and precipitation. While these factors also effect European systems, Australian estuaries have less of a tidal influence affecting salinity, with seasonality and patterns of rainfall having a greater effect in these areas (Heggie & Skyring, 1999); for example, freshwater flow can cease during the summer months due to drought (Hastie & Smith, 2006). Long-shore drift and decreased fluvial input can cause certain types of estuary to become closed off from the sea during these periods of drought (Elliott & McLusky, 2002). This closure allows for the stabilisation of salinity over prolonged periods of drought due to the lack of tidal exchange (Elliott & McLusky, 2002), and the more constant input of insolation (Eyre & Balls, 1999) though in times of non-drought fluctuations can occur rapidly due to rainfall and runoff becoming trapped. Due to lack of tidal exchange, long residence times and high evaporation, the estuary, or parts of it, may become hypersaline (for example, Port Courtis; Currie & Small, 2006). Where hypersaline conditions occur in estuarine systems they are referred to as inverse or negative systems, so termed due to higher salinities further upstream (Pritchard, 1967).

Many Australian estuaries are marine throughout the tidal cycle, fluctuating less than their northern hemisphere counterpart (Hutchings, 1999). For example the Hawkesbury river (NSW, Australia) can remain fully saline up to 15 km from its mouth (Jones et al., 1986), whereas the Tay estuary (Scotland, UK) undergoes greater fluctuations varying with the tidal cycle (McManus, 2005). Salinities are altered in Australia through sporadic flooding events (Currie & Small, 2006), though these are more often associated with the more tropical areas. These cause changes in salinity, and the sediments they bring as opposed to the tidal variations observed in European or temperate estuaries (McManus, 2005). Many tropical Australian estuaries are also subjected to periodic flooding due to episodic events (e.g. cyclones) and the influence of the Southern Oscillation (Kuhnel et al., 1990), whereas European estuaries are regulated seasonally (Elliott & McLusky, 2002). These periodic events cause freshwater influxes into Australian estuaries, washing sediments from catchment areas into the estuarine system, leading to a higher percentage of fine sediments within the estuary (Currie & Small, 2006).

Most major Australian rivers are used for water storage or irrigation (Hutchings, 1999). This limits the amount of freshwater input into estuaries and to the sea. Many are also used for ports and marinas (Hutchings, 1999), similarly to European estuaries which have been used for land claim, flood protection and weir construction (McLusky et al., 1992). These constructions limit freshwater/seawater exchange, influencing salinity regimes by impacting the tidal influence within estuaries.

1.2.2 Sediments

Estuaries are sites of deposition of fine particles (< 63 µm) creating extensive mudflats of cohesive sediments (McCave, 1984; Stal, 2010). The sedimentology of the deposit plays an important role in the distribution, abundance and community composition of organisms in estuaries (Currie & Small, 2006; Hutchings, 1999). Altered sediment compositions brought about through biological or physical means can cause changes in community composition (Borja et al., 2000). For example, cyanobacterial mats can cause shifts in sediment type as they trap sediment particles, thus enabling the growth of other organisms such as diatoms. Particles will continue to become trapped by the biota, potentially causing an increase in bed level (Stal, 2010).

The characteristics of suspended matter have been shown to be caused by numerous factors, including season, tidal phase and anthropogenic impacts (Chen et al., 2005). Suspended sediments in European estuaries are more likely related to local re-suspension as opposed to flow related to seasonal patterns (Eyre & Balls, 1999), whereas in subtropical and tropical Australian estuaries, it is flooding events and seasonal patterns that cause large increases in suspended sediments brought from upriver (Currie & Small, 2006; Eyre & Balls, 1999; Eyre, 1998; Hutchings, 1999).

1.2.3 Nutrients and chlorophyll

Many factors play an important role in nutrient cycling. In an estuary there is a complex interplay between hydrologic discharge, stratification (vertical and horizontal; thermal and haline), mixing by the tide and wind, and storm event frequency and magnitude (Paerl, 2006). This means that varying patterns of nutrient cycling are likely to be found even between estuaries in the same region as well as between those in Europe and Australia (Eyre & Balls, 1999).

Abril et al. (2002) compared different European estuaries in terms of suspended particulate matter (SPM), dissolved organic carbon (DOC) and particulate organic carbon (POC). They found that rivers were the major source of carbon in the estuaries studied, deriving their nutrients upstream, for example through terrestrial carbon inputs (Abril et al., 2002; Augley et al., 2007; Jordan et al., 1991). Similarly in many Australian estuaries, upstream and terrestrial soils are a major source for carbon particulates (Ford et al., 2005; Olley, 2002). Other findings suggested that the upper estuarine systems (salinities < 15) were found to be as much as ten times higher in SPM and DOC than the lower estuary. The lower estuary (salinities > 15) had the greater algal POC, especially during spring and summer. In order to fully comprehend DOC turnover, long term bio-assays, isotopic studies and/or elemental analysis of the dissolved organic matter needs to be undertaken (Abril et al., 2002; Augley et al., 2007).

The nutrient loading is generally found to be much lower in temperate Australian estuaries than those in the northern hemisphere (compare Monbet (1992) with Scanes et al. (2007)). In a comparative study between northern (temperate) and southern (tropical) hemisphere estuaries, Eyre and Balls (1999) found that dissolved inorganic phosphorus and nitrate concentrations were lower in the Australian estuaries. While few studies have directly compared northern hemisphere and Australian temperate estuaries, many have noted lower nitrogen loading levels in Australia (e.g. Hauxwell & Valiela, 2004; Kelly, 2008; Scanes et al., 2007; Young et al., 1996). The lower nutrient loading in Australian estuaries is caused by rapid denitrification and nutrient limitations influenced by the biogeography and low rainfall in the estuarine catchments (Harris, 2001; Young et al., 1996), Australia is a very old weathered continent which has led to very low phosphorus values in its terrestrial sediments (Beadle, 1962).

Wave-dominated Australian estuaries have lower chlorophyll and dissolved inorganic nitrogen (DIN) concentrations than estuaries in the northern hemisphere (Scanes et al., 2007). In the northern hemisphere, there are stronger relationships between catchment disturbance and chlorophyll (for example compare Monbet (1992) with Scanes et al. (2007)). Chlorophyll and DIN concentrations tend to correlate, equating to approximately 0.7-0.8 µg of chlorophyll per µM of DIN (Kelly, 2008) (Kelly 2008), and have been used to compare different estuaries (e.g. Monbet, 1992). Significant human wastewater can influence the nutrient loading within estuaries (Abril et al., 2002; Stauffer, 1998) and therefore have the ability to alter this dynamic. Coastal development has occurred much more recently in Australia compared to many places in the northern hemisphere. This has led to these areas becoming increasingly under pressure from nutrient stress and it is these nutrient levels that are important in determining the community structure (NRC, 2000). This highlights that when comparing estuarine nutrient levels and community structures, it is important to assess the level of pollution and human impacts to understand the biological diversity and how the provision of goods and services are affected.

1.3 Ecosystem function and services

The natural environment is under increasing pressure due to the anthropogenic impacts that are modifying the structure and function of the earth's biota (Sala, 2003; Sala et al., 2000; Vitousek et al., 1997b; Wang et al., 2007), this includes a large proportion of marine systems becoming subjected to multiple anthropogenic stressors (Halpern et al., 2008). Through changes such as the loss or alteration of biodiversity, the functioning of an ecosystem can become significantly altered (Balvanera et al., 2006; Bracken et al., 2008; Cardinale et al., 2006; Solan et al., 2004). Ecosystem function has become a term with multiple definitions which can change depending on author, discipline/speciality or experiment. However at the route of most definitions it can be simplified as the interactions between biota (either through inter- or intra-specific interactions) and/or the physical environment which pertain to a measurable process (e.g. carbon cycling) or ecological compartment (e.g. production of biomass), see Paterson et al. (2012) for full definitions. Further, the ecosystem services provided by biodiversity (e.g. nutrient recycling) are derived from the functions that those species perform (Perrings et al., 2010). The relationship between biodiversity and ecosystem function can be very different depending upon the community involved (Levin et al., 2001) and changes in community structure may become detrimental to the ecosystem services provided (Balvanera et al., 2005).

The Earth's biodiversity is undergoing dramatic changes with regard to species distribution and abundance (Naeem et al., 2002). These changes can have detrimental consequences towards ecosystem functioning and the prediction of these consequences has become a critical issue (e.g. Chapin et al., 2000; Larsen et al., 2005; Solan et al., 2004). Anthropogenic disturbances causing extinctions and changing community structures disrupt processes that help to maintain the ecosystem functions and their integrity (Larsen et al., 2005). The effect of human activities and the consequences to the environment and ecosystem function can be schematically represented (Figure 1.1).

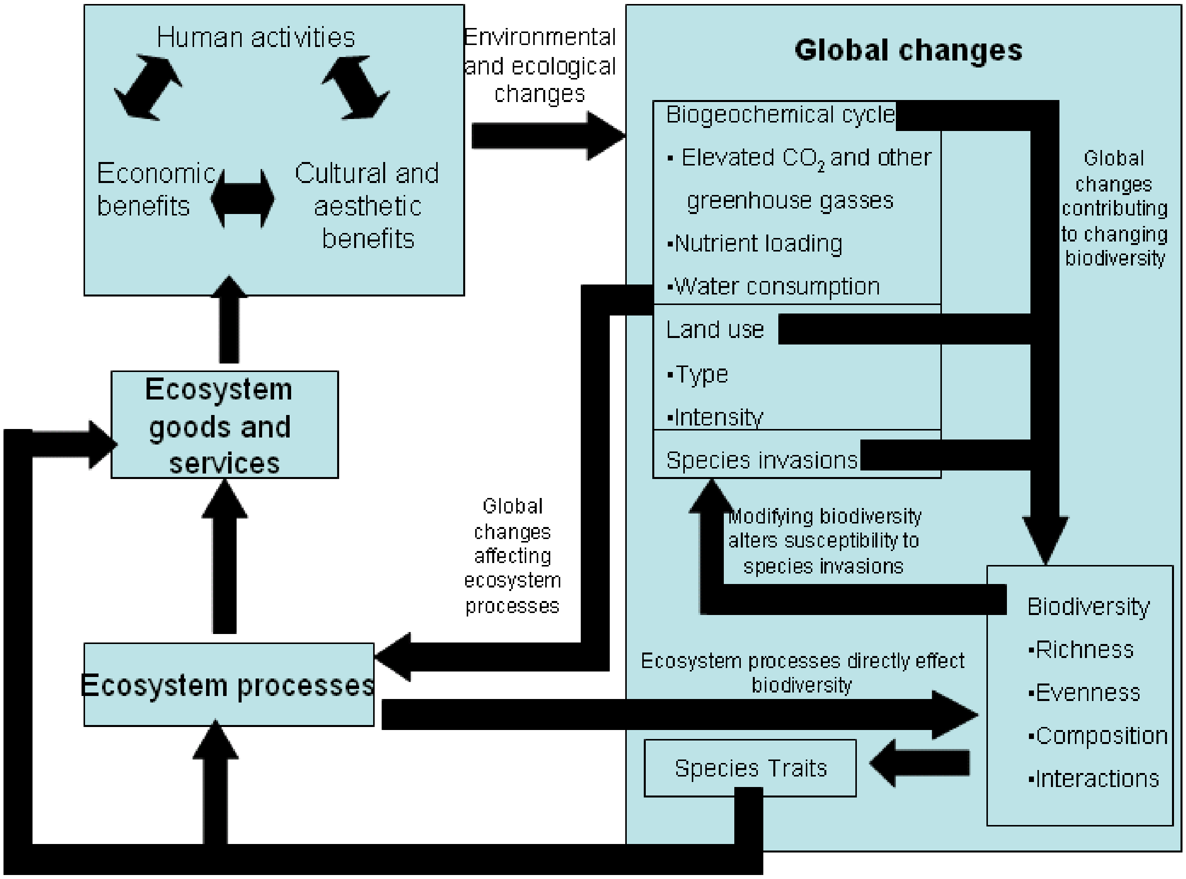


Figure 1.1: Anthropogenic activities create feedback loops and environmental changes that affect biodiversity and ecosystem function. Arrows show direction of change. Adapted from Chapin et al. (2000).

1.4 Estuarine ecology and ecosystem function

Estuaries are areas of high primary and secondary production and have been shown to have a higher biomass than adjacent coastal systems, whereas they are relatively less biologically diverse (Levin et al., 2001). Estuaries are extremely important in terms of the goods and services they provide to humanity on both local and global scales. In the literature, although almost 15 years old and subject to criticisms, one of the most widely cited valuations of ecosystem services is Costanza et al. (1997, revised in 2014). It is useful as a starting point to understand the valuation of the world's ecosystem services. For estuaries, they attributed a value of US\$ 28,196 ha^{-1} yr^{-1} based on the value of the US dollar in 2007. Starting with the factors of greatest importance, this was measured in terms of nutrient cycling, disturbance regulation, food production, recreation, habitat, biological control, culture and raw materials. A review from Barbier et al. (2011) evaluates estuaries and coastal ecosystems in terms of their various components (salt marsh, mangrove, sea grasses). It is argued that how these components interact has important implications on the services provided. This section gives an overview of the importance of estuarine habitats in terms of the various functions and ecological processes that occur within.

1.4.1 Microphytobenthos

Microphytobenthos (MPB) is an important component of the functions, goods and services obtained from an estuarine environment. MPB is an assemblage of photosynthetic organisms that inhabit the sediment. It comprises both prokaryotes and eukaryotes (Underwood & Kromkamp, 1999). MPB consists mainly of protists, dominated by diatoms (Paterson & Hagerthey, 2001), euglenoids (Paterson et al., 1998) and cyanobacteria (Stal, 1995). The composition of the MPB may vary seasonally (Barranguet et al., 1997) or due to the nature of the substratum, for example cyanobacteria dominate on coarser sediments (Stal, 2010). MPB are spatially (Guarini et al., 1998; Jesus et al., 2005) and temporally variable (Jesus et al., 2009; Paterson et al., 1998) over small and large scales, ranging from cm-km or minutes-seasons-years (Spilmont et al., 2011). In terms of succession of an MPB assemblage, different species can become incorporated into the matrix, including pelagic diatoms (MacIntyre et al., 1996). This matrix can become multi-layered as it grows, consisting of epipelic diatoms overlaying chain forming species (Tolhurst et al., 2008a).

Daily rhythms of vertical migration of diatoms in the sediment has been observed as early as the 1960's through simplistic observational experiments darkening areas of sediment (Perkins, 1960), although these migrations were attributed as being daily rather than tidally influenced as is now understood (Consalvey et al., 2004). Epipelic diatoms are motile and able to migrate to the surface during the day at low tide, migrating downwards before immersion (Round & Eaton, 1966; Stal & Defarge, 2005; Tolhurst et al., 2003). Epipsammic diatoms do not move through the sediment and live attached to sand grains preferring coarser sediments (Moss, 1977; Round, 1965).

Due to the movements of the epipelic diatoms, the MPB biomass of the surface sediment can vary over an emersion period (Jesus et al., 2005). This mosaic community therefore influences the bulk density and water content within the sediments. The distribution and community composition of the MPB depends upon the granulometric composition of the sediments, and upon the factors that influence the size and density of the particles (Paterson & Hagerthey, 2001; Underwood & Barnett, 2006). Cyanobacteria have a preference for coarser sandy habitats as opposed to finer, muddy sediments (Stal, 2010); in both environments, however, they are able to facilitate the binding of sediments (Yallop et al., 2000).

The majority of MPB biofilms occur within the top 200 µm of the sediment (Tolhurst et al., 2008a). These biofilms may also contain non-photosynthetic organisms such as chemo-organotrophs or chemo-autotrophs. These can play an important role in the functionality of the sediments (e.g. sulphate reduction) and the biofilm composition may vary according to salinities and exposure levels (Bolhuis & Stal, 2011). Both the photosynthetic and non-photosynthetic communities contribute to the production of extracellular polymeric substances (EPS; Bolhuis & Stal, 2011; Stal, 2010; Wotton, 2005). The functions of EPS produced by MPB and bacteria include attachment to the substrata and formation of biofilms; stabilisation; locomotion within sediments; production of polymer gels; food for many organisms; desiccation protection; a shield against solar radiation; acting as buffers against changes in the physico-chemical conditions; and as a predation deterrent (Flemming & Wingender, 2010; Gerbersdorf & Wieprecht, 2014; Gerbersdorf et al., 2008; Grabowski et al., 2011; Middleburg et al., 2000; Raszka et al., 2006; Tolhurst et al., 2008a; Wotton, 2004, 2005).

1.4.2 Sediment stabilisation

Many studies show a strong correlation between sediment erodibility, MPB biomass (recorded in terms of chlorophyll *a*) and the amount of EPS (recorded as colloidal carbohydrates) present in intertidal sediments (Austen et al., 1999; Gerbersdorf et al., 2008; Grabowski et al., 2011; Taylor & Paterson, 1998). Through the production of EPS, microphytobenthic assemblages act to stabilise the sediment and therefore reduce its erosion potential (Austen et al., 1999; Black et al., 2002; Grabowski et al., 2011; Hubas et al., 2011; Tolhurst et al., 2002, 2008a; Wotton, 2004). EPS act to bind sediments through adhesion (Paterson & Hagerthey, 2001; Stal & Defarge, 2005; Underwood et al., 1995), also trapping and absorbing water within micro-pores(Grabowski et al., 2011). These processes aid to stabilise sediments, protecting them from erosion.

While the stability of sediments can depend upon physico-chemical properties – including water content, mineralogy and salinity (Grabowski et al., 2011) – it can be as much a factor of the biota present influencing system dynamics (biostabilisation; Black et al., 2002). Both factors influence sediment stability (Black et al., 2002; Hubas et al., 2011; Paterson, 1989; Paterson et al., 2000; Tolhurst et al., 2002). For example, Spears et al. (2008) illustrated that sediment stability was greater in estuarine habitats compared to freshwater riverine habitats, even though EPS concentrations were higher in the freshwater. This emphasises the combined effect of the salinity, the different types of organisms present and the degree of EPS production have on sediment stability.

1.4.3 Primary productivity

Estuaries are sites of intense primary production compared to other environments (MacIntyre et al., 1996; Underwood & Kromkamp, 1999). Benthic microalgae can account for up to 50% of an estuary's total primary production (Underwood & Kromkamp, 1999). Figures of primary production can range between 7 and 875 g C m^{-2} yr^{-1} for estuarine phytoplankton and between 29 and 234 g C m^{-2} yr^{-1} for MPB (Underwood & Kromkamp, 1999). Estimates of world primary production from MPB have been as high as approximately 500 million tons of carbon annually (Cahoon, 1999) and are seen as a possible "missing sink" in the annual carbon cycle (Guarini et al., 2008). Put into perspective, the contribution of all diatoms to global photosynthetic production is estimated to be equivalent to that of all the tropical rainforests combined (Bowler et al., 2010).

Measurements on both cultured and natural MPB communities have shown that 40-80% of total primary production is secreted into the environment mainly as EPS (Goto et al., 1999; Wolfstein et al., 2002), becoming available as a food source. These values are much lower in phytoplankton: 1.5-22% (Goto et al., 1999). MPB is an important food source for benthic macro-invertebrates on tidal flats, with isotopic studies showing that they preferentially feed upon benthic microalgae as opposed to phytoplankton, or adjacent salt marsh or riverine detritus (Kang et al., 2003). This relationship between primary consumers and their feeding interactions has been shown to differ dependent upon the hydrogeomorphology of the estuary (Garcia et al., 2011). Estuaries can also act as an important carbon sink due to the burial of organic carbon (Abril et al., 2002; Wollast, 1991).

1.4.4 Vegetated habitats

Vegetated habitats are important globally and those found within estuaries are similar to coastal environments. Salt marshes, mangroves and seagrasses are important globally in terms of their importance to carbon storage, nutrient cycling, water filtration, shoreline protection enhancing the productivity of fisheries (Costanza et al., 2014; Duarte, 2002; Duarte et al., 2005). While there are similarities between the types of vegetation in European and Australian estuaries, European estuaries may contain salt marsh habitats, whereas Australian estuaries may in addition include mangroves. Mangroves and salt marshes have many similarities but they also have much dissimilarity, particularly with reference to export of organic matter (Table 1.1). Both are an important component of an estuarine habitat. Intertidal salt marshes and mangroves are some of the most productive habitats on the planet, producing on average 1275 g C m^{-2} yr^{-1} (Cai, 2011). An extensive examination by Bouillon et al. (2008) concluded that mangrove net primary productivity (NPP) was 0.22 Pg C yr^{-1}, with estimates of global NPP of both salt marshes and mangroves as much as 0.50 Pg C yr^{-1} (Cai, 2011). Therefore both salt marsh and mangrove habitats within estuaries are an extremely important area of carbon sequestration (Barbier et al., 2011; Bouillon et al., 2008; Cai, 2011; Duarte et al., 2005). Mangroves are also sites of extremely high carbon storage when compared to other forest types in terms of both above and below ground carbon storage (Donato et al., 2011). Costanza et al. (2014) estimated their combined value at US$ 193843 ha^{-1} yr^{-1}.

Table 1.1: Differences in factors affecting the export in mangroves and salt marshes. Adapted from Lee (1995)

	Mangrove	**Salt Marshes**
Senescent plant biomass	Most likely exported	Retained, decomposed within marsh
Turnover of which is exported	High amount of export	Low degree of export
Tidal regime	Strong tidal energy (usually) leading to greater amount of export	Most often weak tidal energy, meaning lower export
Litter quality	Low utilisation by detritivores	Easy utilisation by detritivores (species dependant)

The leaf litter and detrital inputs form these vegetated habitats are an important base of estuarine and coastal food webs (Gladstone-Gallagher et al., 2014; Lee, 2008). Assemblages within these habitats are dominated by specialist species, for example by possessing the specialist ability to break down and assimilate litter (Neilson et al., 1986). Particulate organic matter (POM) is processed by organisms able to shred leaf litter, e.g. grapsid crabs (Sheaves & Molony, 2000). This creates finer POM which can be exported out of the system by the tide or utilised by organisms on adjacent mudflat systems (Dittmann, 2000; Lee, 2008).

Salt marshes, seagrasses and mangroves can affect the sedimentation within an estuary, acting to trap sediments (Barbier et al., 2011; Kathiresan, 2003; Stumpf, 1983). Through trapping and binding sediments, salt marshes and mangroves can reduce an areas erosion potential and aid in coastal protection (Van Santen et al., 2007). Salt marshes in some areas have been documented to keep up with sea level rise through the trapping of sediments and organic matter (Cai, 2011; Craft, 2007; Maynard et al., 2011). Other factors could tip this balance in the future leading to net erosion.

1.4.5 Nursery habitats and fisheries productivity

Nursery habitats are defined as areas within or outside of an adult's range and are areas of significantly higher juvenile and larval forms (Beck et al., 2001), many of which are commercially important species (Beck et al., 2001; Dahlgren et al., 2006; Layman et al., 2006). Within estuaries, mangroves, salt marshes and seagrasses are found to have higher densities of larvae/juveniles than adjacent unvegetated mud flats (Beck et al., 2001; Duarte et al., 2005; Nagelkerken et al., 2008), these places offer a structural refuge from predators (Beck et al., 2001; Laegdsgaard & Johnson, 2001). Estuarine nurseries may be a place of reduced predation and competition for many species, providing benefits unavailable in the open ocean (Heupel & Simpfendorfer, 2011). Species that utilise estuaries as nursery habitat are not always seen as strong osmoregulators and therefore may undergo behavioural adaptations to respond to the varying stress factors, such as changing salinities. For example juvenile *Cancer edwardsii*, a commercially fished species, has been shown to burrow within sediments in response to prolonged periods of hyposalinity (Pardo et al., 2011). Some species are critically dependent upon estuaries, for example; black bream fisheries in Australia, but estuaries are also important to opportunistic and recreational fisheries (Robinson, 2001).

1.4.6 Carbon dynamics within estuaries

Carbon transported by rivers makes an important contribution to the global carbon cycle (Abril et al., 2002; Ludwig et al., 1996; Meybeck, 1993). Rivers and estuaries transport significant amounts of carbon each year. In England and Wales alone, carbon export has been estimated that 10.34 Mg C km^{-2} yr^{-2}. Of this proportion exported out of the riverine/estuarine system, 4.19 Mg C km^{-2} yr^{-2} is transferred into the atmosphere (Worrall et al., 2007). The cohesive sediments within estuaries provide an important site for carbon sequestration (Grabowski et al., 2011). Many estuarine systems have been shown to be net sinks of carbon acting as areas of storage (Worrall et al., 2007).

1.4.7 Pollutant amelioration

It has been said that "*Effluents are an important by-product of human civilisation*" (Hynes, 1960). Humans have been polluting estuaries for hundreds of years due to agricultural practices, sewage, industrial wastes, etc. From this waste, nutrients are released and have been shown to adversely affect estuarine environments (Figure 1.2), causing eutrophication and ultimately a loss of biodiversity and ecosystem functioning (Vitousek et al., 1997a and references therein).

Figure 1.2 *Ulva* spp. blooming in the Eden estuary, a sign of local eutrophication. This image was kindly supplied by Royal Air Force, Leuchars, Crown copyright.

One of the major pathways of pollutant amelioration within estuaries is through the removal of wastewater and toxicants through the adsorption of organic pollutants (Raszka et al., 2006; Sheng et al., 2008). Sediments can also act to bind pollutants, making them biologically-unavailable and reducing the threat to the environment but they are retained within the estuary until they are released or exported out of the system (Kennish, 2002; Mead & Moores, 2004). There is the potential for these contaminants to be released through both natural (storms) and anthropogenic (dredging, shipping) activities (Kennish, 2002).

If nutrients enter rivers or estuaries at a rate faster than they can be removed they can cause the system to collapse. This is evident by eutrophication events in many rivers over the last few decades (Stauffer, 1998). Slight changes in nitrogen levels have been shown to switch systems between autotrophy and heterotrophy (Porubsky et al., 2008). Economic pressures of the past 200 years has led to many rivers now being termed as "dead" due to increased effluent discharge

(Davidson et al., 1991). Without sufficient legislation and improved management many more will "die," adversely affecting the ecosystem services provided. Increased nutrient input can lead to increased hypoxic or anoxic events through an increase in biomass and primary productivity (NRC, 2000). This can lead to changes in the phytoplankton and benthic community structures due to increased nutrients and anoxic conditions respectively (NRC, 2000).

1.5 Stressors on estuarine systems

A disturbance or stressor has been defined by Pickett and White(1985) as "*any discrete event in time that disrupts ecosystem, community or population structure and changes resources, substrate availability or the physical environment.*" Both natural and anthropogenic events fall under this category, with any perturbations or external sources having the ability to cause a change. Climate change is a significant driver of alterations to biodiversity, changing community composition and consequently altering the ecosystem services provided (Balvanera et al., 2006; Larsen et al., 2005; Worm et al., 2006). The physical impacts of climate change that may modify coastal ecosystems include increasing temperatures, potentially leading to the melting of ice caps and the thermal expansion of water leading to subsequent rising sea levels; increased frequency of influxes of freshwater into coastal areas; and the exacerbation of existing stress factors, such as eutrophication and storm events (Crain et al., 2008; Hoegh-Guldberg & Bruno, 2010).

Climate change and other anthropogenic impacts are likely to have a measurable detrimental impact on ecosystem processes within the next 100 years, brought about through changing community compositions and species losses (Sala et al., 2000; Vitousek et al., 1997b). Depending on the community composition, individual species or functional groups may have a more significant impact on the ecosystem functions (Bracken et al., 2008; Solan et al., 2004). Therefore, if these keystone species are lost due to climate change, the provisions of services from the ecosystem will alter. Due to the unique role each functional group (e.g. detritivore, suspension feeder) has in the community, a change in species could alter both the magnitude and direction of the change in ecosystem function (Gaston & Spicer, 2004; Naeem et al., 2002; Petrology et al., 2001).

Many studies have focussed on the impact of stressors on estuarine systems. These systems are the terminus of large catchment areas and so are major repositories for contaminants, becoming concentrated as they wash in from the large densely populated catchment areas (Heip et al., 1995). Contaminants such as trace metals and nutrient pollution can accumulate in an estuary, impacting water quality (Birch et al., 2010; MacFarlane & Booth, 2001; Roy et al., 2001). Particularly within urbanised estuaries, water quality can become poor due to the overloading of nutrients. Excess nutrients from point sources (e.g. sewage treatment) and diffuse runoffs from the catchment area can cause progressive enrichment and eutrophication events (Bricker et al., 2008).

The combined effect of multiple stressors can be difficult to predict, as they may interact additively, synergistically or antagonistically, depending on environmental conditions (Crain et al., 2008; Fitch & Crowe, 2011). Although the number of studies assessing the combined effects of multiple stressors are presently few (see Halpern et al., 2008), an increasing number are now simultaneously manipulating multiple stressors, in order to examine interactive effects on ecosystem functioning, instead of inferring impacts from separate tests of single factors. For example, Hicks et al. (2011) subjected estuarine communities to multiple effects of climate change – elevated temperatures, CO_2 and altered community composition. They found that the impacts of multiple stressors of climate change cannot be predicted by adding the effects together and can alter different portions (e.g. filter feeders, MPB, phytoplankton) of an ecosystem in different ways (Porter et al., 2013).

1.6 Aims of thesis

This thesis aims to bridge a gap in estuarine research by directly comparing temperate estuaries in both the northern and southern hemispheres. While many studies have looked at single estuaries or multiple estuaries in a single hemisphere, few have directly compared experiments in the northern and southern hemisphere making continental comparisons. The objective of this work is to replicate experiments in two estuaries in Australia and two in Scotland to compare and contrast the functional differences through manipulative field experiments. Recent trends in manipulative ecological experiments have seen the application of multiple stressors on environments to determine the effect on ecosystem function. Experiments designed here manipulate two stressors, nutrient enrichment and physical stress, on benthic intertidal estuarine habitats in a fully orthogonal design. To date the preferred method of analysing multiple stressors is in a controlled laboratory setting. While this is helpful for understanding effects of stressors, this does not explain how they will interact given local environmental context as this study aims to address.

1.7 Main thesis questions to be addressed

1. Determine the response of Australian estuarine mudflat communities to physical and nutrient disturbances through manipulative field experiments using a fully orthogonal design controlling multiple stressors on estuarine communities to simulate eutrophication (nutrient enrichment) and storm damage (physical disturbance). Each experiment measures chlorophyll and collects benthic diatoms and benthic macro-invertebrates to analyse trophic linkages.
2. Examine the response of Scottish mudflat communities to physical and nutrient disturbances. A repeat of orthogonal design implemented in Australian estuaries, focussing on the change in ecosystem function.
3. Compare estuarine dynamics of estuarine mudflat communities in Scotland and Australia. Compare and contrast results found in Scotland and Australia. Explore what this means in terms of wider issues such as climate change and ecosystem management.
4. Examine the effect of interactions of multiple stressors, does order of stress matter? Examine whether the addition of a second stressor (either nutrient enrichment or physical disturbance) has a significant impact on an estuarine system. The rationale behind this is that multiple stressors do not always naturally occur simultaneously.

2 Methodology

2.1 Study Sites

Sites were selected in Australia and Scotland to represent different geographic regions within the temperate zone. These areas allow a comparative study of the nature and resilience of estuarine benthic systems across continent. Two estuaries were selected in each country. In Australia, Botany Bay and the Lane Cove estuaries located close to Sydney in New South Wales were selected. In Scotland, the Eden and Tay estuaries were selected (Figure 2.1). Sites were selected on the seaward side of estuaries to nullify the confounding effect of salinities during comparison of sites.

The site in Botany Bay was located at its confluence with the Georges River – a catchment area of approximately 960 km^2 in urbanised south western Sydney. Georges River is approximately 8 km in length with a water area of 27 km^2 and Botany Bay is another 8 km, with a water area of approximately 40km^2. Lane Cove, in comparison, is a much smaller estuarine system (a catchment area of approximately 94 km^2) which drains into the Parramatta River and is approximately 14 km from the seaward entrance of Port Jackson (Sydney Harbour). It has an area of approximately 3 km^2. Both estuaries are tide- dominated drowned-river estuaries. The average tidal ranges around Sydney are approximately 1.5 m.

The Tay and Eden estuaries are very similar to each other in terms of physical parameters (i.e. tidal range, slope, exposure; see Dyer et al., 2000). The river Eden is approximately 30 km long, with a catchment of 400 km^2 with approximately 76% of the major land use within the area used for agriculture (Url 1). The Eden estuary has an area of 10.41 km^2 and an intertidal mudflat area of 9.37 km^2. Tay has an area of 122 km^2, an intertidal area of 57 km^2. Both are in close proximity to each other and have an average tidal range of 3.7 m (Davidson & Buck, 1997). The River Tay is the longest in Scotland (188 km) and, with many major tributaries, incorporates a large catchment area of approximately 4587 km^2 producing the largest freshwater outflow of any UK river system.

In contrast to the Australian systems, the Tay and the Eden Estuary are less urbanised and are designated special areas of conservation (SAC) within the UK representing high quality estuarine habitats (Url 1). They are both combined in one SAC which incorporates the estuarine outflows and surrounding marine environments. According to the Joint Nature Conservation Committee (JNCC), the combined SAC incorporates 55.1% marine areas and sea inlets, 27.7% estuary, mudflat and lagoons, 1.2% salt marsh and salt pastures, 8% sand dunes and sandy beaches, 2% shingle and sea cliffs, and 6% inland water bodies. With 97% of the total area being classified as an estuarine habitat, almost half of this area is classified as an intertidal sand or mudflat. The sites in Australia show different habitat types, incorporating smaller mudflat areas, but also incorporating large areas of mangrove habitat.

The Australian sites were investigated between May and October 2012, and the Scottish sites between April and September 2013. These times of year were chosen to represent times of minimal changes in community structure and environmental conditions over the 4-5 month experimental period. While comparing an Australian summer with summer in Scotland would have been preferred, this was not possible given the time constraints of the project. It is common to conduct field experiments in winter in Australia due to ease of access to sites, minimal temperature and environmental variation, and low recruitment. It should also be noted that previous research has shown that chlorophyll can reach a maximum in Australian NSW intertidal habitats in winter months, the opposite to the normal pattern observed in the northern hemisphere (Murphy et al., 2009). Comparing winter in Australia to winter in Scotland was not a possibility given the unpredictability of the weather in Scotland, given that temperatures commonly drop well below freezing during this period making access to sites difficult. This would also have caused drastically different environmental conditions to those observed in Australia. By comparing summer in Scotland to winter in Australia, similar temperature regimes were observed. Due to different environmental conditions in each location – e.g. day length, amount of light, rainfall, etc. – regardless of time of year, it would be impossible to totally control all variables. Given that experiments comparing multiple systems are rare, with global and local generalisations being made from single studies, conducting this type of study is an important step towards our understanding of estuarine ecosystems.

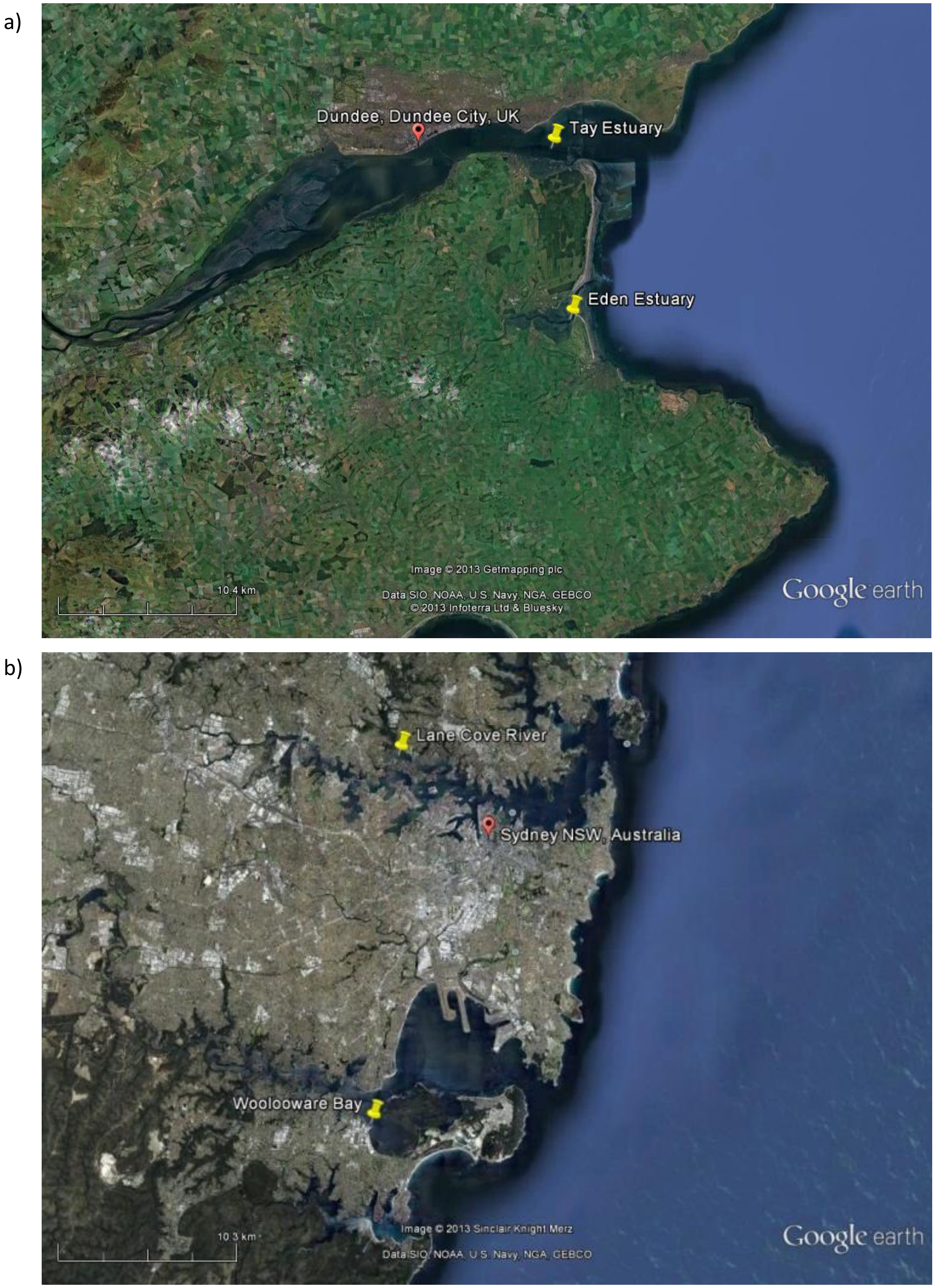


Figure 2.1: Location of study sites in Scotland (a) and Australia (b) in relation to nearby cities.

2.2 Experimental approaches

A single site was selected in each estuary. While sites were selected to be comparable in terms of grain size, salinity and other environmental variables, it was not always possible to completely regulate these factors. Sites needed to be of a large enough uniform conditions in an area of un-vegetated habitat and of similar tidal elevation. Other factors considered were the ease of access to the site and public influence, which needed to be minimal so as not to disrupt experiments. In all estuaries, experiments plots were 50 x 50 cm and separated by a buffer zone of 2-3 m from adjacent plots. The size of the plot was based on previous research (see below). The buffer zone was to prevent any interaction between treatments within the plots and allow unfettered access to each for taking measurements and applying manipulations without cross disturbance. Plots were located around the upper shore, below the high tide mark, in areas inundated twice daily by the tide. Due to the smaller tidal ranges and smaller horizontal distances between high and low tide in Australia, plots encroached further down the shore.

2.2.1 Disturbance regimes

Experimental plots were subjected to one of three levels of each of two stressors (hereafter referred to as multiple stressors). These were nutrient enrichment and physical disturbance, applied in a crossed design that gave nine experimental treatments. Stressor 1- Physical disturbance – which is normally caused by storms, bait digging, boat propellers or anchor contact with mudflats – was manipulated by hand raking. Stressor 2: - Nutrient enrichment – which is often anthropogenic through waste-water treatment and agriculture – was manipulated by adding slow release fertilizer. Plots were randomly assigned to either high, low or zero levels of either type of stress, with 7 plots of each of the nine experimental treatments, left for a 4 month period. Due to the low pressure storm event coinciding with the first sampling time point at the Lane Cove site, certain plots were inundated at time of sampling as tidal heights did not drop sufficiently to allow access. Measurements were taken where possible at this time point.

Plots assigned to the low or high physical disturbance treatment were raked to a depth of approximately 2-4 cm. Plots were disturbed using a garden rake that was approximately 50 cm in width and were raked beyond the 50 x 50 cm plot area, equating to approximately 1 m^2 being raked centred on the experimental plot. Plots were raked in a cross hatched pattern whereby a second stroke of the rake was conducted perpendicular to a first. The low treatment consisted of two strokes of the rake. The high treatment consisted of 6 strokes, each stroke perpendicular to the previous. This disturbance continued throughout the experimental period, with plots being raked monthly. Physical disturbance due to storm events is difficult to simulate although successful applications have been done previously utilising rakes or hoes to facilitate the mechanical disturbance brought about through storm damage (Oliveira et al., 2014; Whomersley et al., 2010). Monthly rakings have been documented to have an effect on macrofaunal communities (Whomersley et al., 2010), for this reason this level of frequency was chosen to represent the physical disturbance patterns that may occur on these types of shores, simulating the physical disruption of sediments that may occur due to storm damage or anthropogenic disturbance.

Nutrient plots were enriched using Scotts Osmocote Pro 8-9 month coated fertiliser pellets (NPK ratio:16:4.8:8.3). These pellets were recommended as being a controlled method of testing the effects of gradual nutrient release in soft sediment benthic environments (Worm et al., 2000), providing a continuous and steady release of nutrients without need for replenishment. Plots were given 0 g (zero), 500 g (low) or 1000 g (high) of fertiliser, representing the three levels of this factor. The high nutrient treatment was based on the level of nutrient loading that might be experienced in the vicinity of the discharge point of a moderately sized sewage treatment plant (Morris & Keough, 2002, 2003a; O'Brien et al., 2010). High and low treatments were designed based on previous research to promote a response from the MPB and macrofauna (Morris & Keough, 2003b; O'Brien et al., 2010). Each of the high and low treatments have been observed to cause impacts to microphytobenthos (MPB) and macrofauna in previous studies conducted in other temperate Australian estuaries (Morris & Keough, 2003b; O'Brien et al., 2010). For each of the high and low nutrient treatments, nutrients were dispensed via five bags made from nylon (adapted panty-hose) and were buried approximately 2-4 cm below the surface of the sediment. The nylon provided a semi-permeable membrane through which nutrients could leach out into the plots. A procedural control (sediment bags) was used to control for the disturbance associated with burying the fertiliser bags. Once plots had been enriched, no further enrichment was required.

2.2.2 Sediment sampling

Sampling for all selected variables was conducted prior to the experiments and any further disturbances, i.e. immediately before the application of experimental treatments. Due to the extensive processing time involved with the macrofauna, samples were restricted to the beginning, middle (2 months) and end (4 months) of the experimental period. All other variables were sampled monthly.

2.3 Contact coring

Sediment properties were assessed from contact cores (Figure 2.2) whereby the top 2 mm (area 2463 mm^2) of the sediment surface was flash-frozen with liquid nitrogen (Ford & Honeywill, 2002). This method allowed the almost instantaneous freezing of the sediment at time of sampling, preventing degradation of the sample prior to storage and analysis.

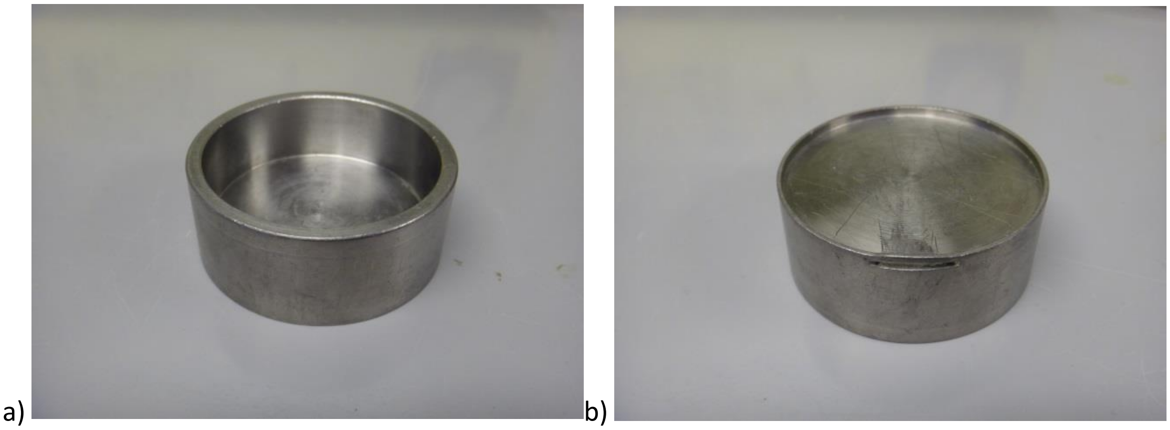

a) b)

Figure 2.2: Contact core used to take surface sediment samples through flash freezing with liquid Nitrogen. a) Surface well for filling with liquid nitrogen. b) Bottom well to take 2mm core

The contact corer was laid onto the sediment surface and liquid nitrogen was poured into its surface reservoir (Figure 2.2a) to cause the sediment in the lower section (Figure 2.2b) to freeze. Once frozen, sediment around the collar and base of the corer was scraped off using a knife, leaving a flat 2 mm core. This sample core was then wrapped in labelled foil and stored in liquid nitrogen prior to laboratory processing. Sediment was freeze dried in the dark for use in some of the following procedures to avoid photochemical breakdown. After freeze drying, samples were stored in a -80°C freezer to prevent any further pigment breakdown.

2.3.1 Organic content

The organic content of the sediment was determined through the loss-on-ignition method.

A known weight of freeze dried sediment, ground to a fine powder, was placed in a crucible for use in a muffle furnace. Sediment was heated at 450 °C for 4 hours then removed and left to cool to room temperature in a desiccator before reweighing, minimising exposure to atmospheric moisture. The following equation was used to determine organic content:

$$\% \text{ organic content} = 100 - \left(100\left(\frac{sediment\ wt\ after\ ignition\ (g)}{sediment\ wt\ before\ ignition\ (g)}\right)\right) \qquad \text{Equation 1}$$

2.4 Sediment stability

2.4.1 Cohesive Strength Meter (CSM)

The Cohesive Strength Meter (CSM); Figure 2.3) is used to measure sediment stability or the erodibility of the sediment. The CSM is able to measure small scale spatial variations in the erosion threshold (Tolhurst et al., 1999; Vardy et al., 2007). A water-filled chamber is placed onto the sediment in which the infra-red transmission is measured, the device then fires a vertical jet of seawater at the sediment surface. Pressure is increased incrementally until enough sediment becomes suspended within the chamber, reducing transmission to 90% of the initial value. This 10% drop in transmission has been shown to be sufficient in representing when the surface starts to erode (Vardy et al., 2007). The integral computer records the jet pressure and the transmission value. This was then retrieved onto a PC whereby the pressure at which the transmission drops below 90% of the starting value can be extracted and placed into calibration equations to equate to the erosion threshold of the sediment.

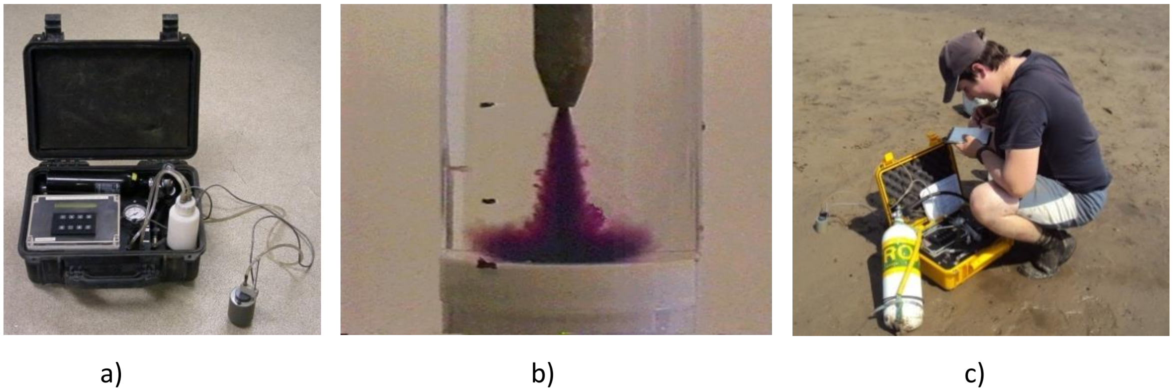

a) b) c)

Figure 2.3: Cohesive strength meter (CSM). a) All components. b) Jet utilised by CSM to blast sediment surface. c) CSM in use in the field

2.4.2 Shear vane

The shear vane (Figure 2.4) was used to determine bed shear strength and measures the amount of torque required to shear the sediment. This can be used as a rudimentary proxy for sediment stability. The shear vane was pushed into the sediment to a known depth and rotated steadily with one hand. At the failure point of the sediment bed, the dial reaches a maximum which is used to translate to a measure of sediment shear strength (Nm).

Figure 2.4: Shear Vane: for use in measuring the shear strength of sediment

2.4.3 Water content

Sediment water content was determined using the contact cores (Equation 2). Sediment was weighed before (Wt_{wet}) and after (Wt_{dry}) freeze drying.

$$\% \text{ water content} = ((Wt_{wet} - Wt_{dry}) / Wt_{wet}) \times 100 \qquad \text{Equation 2}$$

2.4.4 Wet bulk density

Wet bulk density was determined as the weight of wet sediment per cubic cm (Equation 3). This measure is used to determine the consolidation of the sediments and is negatively correlated with erodibility of the sediments, whereby denser sediments are more solid and compacted and less erodible (Grabowski et al., 2011).

$$\text{Bulk density (g cm}^{-3}\text{)} = Wt_{wet}\text{ (g)} / \text{volume (cm}^{3}\text{)} \qquad \text{Equation 3}$$

2.5 Macrofauna collection

Large cores (10 cm diameter, 10 cm depth) were taken in each plot to allow the quantification of macrofaunal biodiversity. Sediment core samples were sieved through a 500 µm diameter mesh sieve and material retained on the sieve was fixed in buffered 10% formalin. Retained material was subsequently, transferred into 70% ethanol and examined under a dissecting microscope to separate fauna from other remaining material. Fauna were identified to species level where possible. Certain species of amphipod were difficult to differentiate due to small differences between species (e.g. Urohausteriidae and Gammaridae) and so were identified to genus level. Certain phyla are particularly difficult to identify with limited time and equipment, such as oligochaetes. There is limited knowledge of these species in Australia and identifications were made based upon morphotypes, limited attempt was made to separate oligochaetes other than common and visually distinct species.

2.6 Microphytobenthos measurements

2.6.1 Spectrophotometer calibration

Spectrophotometry was used to calculate the biomass of the MPB. Chlorophyll concentration measurements were used as a proxy for microphytobenthic biomass (Tolhurst et al., 2005). To ascertain the accuracy of the spectrophotometer it was calibrated against known concentrations of chlorophyll *a*. A stock solution of chlorophyll *a* was prepared by dissolving 1 mg of spinach (Sigma^{TM}) in 250 ml of 90% acetone. Serial dilutions of the stock provided a range of concentrations (4, 2, 1, 0.5 and 0.25 mg l^{-1}). To prevent pigment degradation, serial dilutions and the stock sample were immediately wrapped in tinfoil and stored at 4 °C. Using equation 4, chlorophyll *a* concentration was calculated using the spectrometer to measure absorbance at 662 nm and 750 nm, where E is the extinction coefficient of chlorophyll *a*.

$$Chlorophyll\ concentration\ (mg\ l^{-1}\ =\ \left(\frac{[A_{662}]-[A_{750}]}{E}\right) \times 1000 \qquad \text{Equation 4}$$

2.6.2 Extraction of pigments

Approximately 200 mg of freeze dried sediment was pre-weighed and placed in an Eppendorf tube with 1.5 ml of 90% acetone allowing the calculation of chlorophyll a, b and c using equations established by Jeffrey & Humphrey (1975). The ratio of sediment to acetone was kept constant to minimise errors involved with using different ratios. Extraction of pigments took place over a 48 hour period in a -80 °C freezer. Samples were agitated after 24 hours for 10 seconds by using a thumb vortex.

2.6.3 Pigment analysis

Following the extraction period, samples were centrifuged at 1300 rpm for 3 minutes and the extractant used for pigment analysis in the spectrometer. Analyses were carried out under dim light, keeping the samples cold prior to analysis. Absorbances were read at 630, 647, 664 and 750 nm, respectively. These values were used in Equation 5 to calculate Chlorophyll *a* content (where Ve is the volume extractant – 1.5 ml) which was further used to calculate the concentration of chlorophyll (Equation 6), where A = the dry weight of the entire contact core and B = the surface area of the contact core.

$$Chl\ a(\mu g\ g^{-1}) = \frac{(11.85[E_{664} - E_{750}] - 1.54[E_{667} - E_{750}] - 0.08[E_{630} - E_{750}) - Ve}{weight\ of\ sample}$$

Equation 5

$$Chl\ a\left(mg\ m^{-2}\right) = a\left(ug\ g^{-1}\right) \times A \div B \div 1000$$

Equation 6

2.6.4 Spectroradiometry

An Ocean Optics USB2000 spectroradiometer was used to measure the spectral reflectance from the sediment surface. This was used as an alternative measure of the MPB biomass. This is a handheld device attached to a laptop computer and used out in the field. It is quicker than spectrophotometric analysis and so can be used to measure over a wider area in a short amount of time. It can also be used to differentiate different forms of MPB – diatoms, cyanobacteria, euglenoids. Comparing the visible and infra-red parts of the spectra (Figure 2.5) various metrics can be calculated including: Normalised Difference Vegetation Index (NDVI – Equation 7), a value used to calculate photosynthetically-related biomass focusing on reflectance of chlorophyll *a* (Méléder et al., 2003; Serodio et al., 2009). The following equation was utilised to calculate NDVI where R is the reflectance value at a specific wavelength:

NDVI = (R750 –R675) / (R750 +R 675)

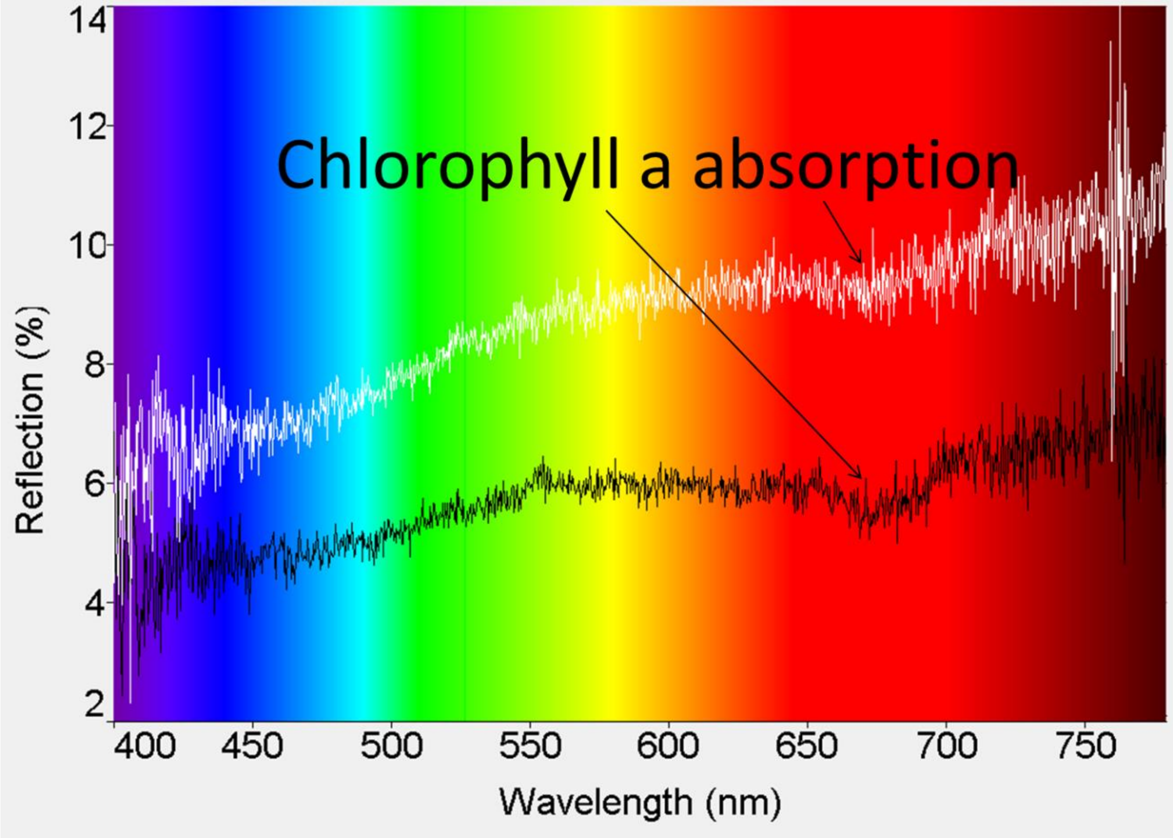


Figure 2.5: The spectral reflectance signal of 2 sediment samples. Certain troughs (highlighted on graph) correspond to the absorption of photosynthetically active pigments (e.g. chlorophyll *a*). The ratio between these troughs and the infra-red reflectance can be used to calculate biomass of MPB. White line is representative of a lower MPB biomass than the black line.

2.7 Statistics

Univariate analyses were done separately for each site using 2- or 3- factor analysis of variance, with nutrients, physical disturbance and month used as fixed factors. Analyses were conducted using PERMANOVA+ (Anderson et al., 2008) and Minitab (Minitab 17 Statistical Software, 2010) after appropriately checking for heterogeneity of variance and normality of the data. PRIMER v6 software (Clarke & Gorley, 2006) was also used to calculate diversity indices. Multivariate analyses were used to examine the community structure using PERMANOVA to differentiate the different treatments in a 2- factor analysis and using SIMPER analysis to identify species causing dissimilarity between treatments (Anderson, 2001; Clarke, 1993).

3 Interacting effects of nutrient enrichment and physical disturbance on Australian estuarine benthic communities

3.1 Introduction

3.1.1 Multiple stressors

A stressor is an external factor that disrupts an ecosystem or community, decreasing the overall fitness of a population (Barrett et al., 1976; Parker et al., 1999). Some stressors result from natural occurrences (e.g. El Niño / La Niña), while others are brought about through anthropogenic activities, such as agriculture, industrialisation and urbanisation or climate change. Stressors may be physical, chemical or biological in origin – for example, contaminants; physical disturbance, including habitat destruction and modification; non-native species; and climate change (Lotze et al., 2006). Understanding the consequence of these stressors for individuals, their populations, communities and ecosystems is critical to managing their impact (Barrett et al., 1976). How organisms respond, linearly or nonlinearly to increasing stressor intensities is hence, a topic that has dominated the ecological literature for many years (Bulling et al., 2008, 2010; Power, 1997; Solan et al., 2004) but has still not been fully resolved. The majority of studies on stressors have also considered their effects independently from one another (Crain et al., 2008; Harley et al., 2012; Hoffman et al., 2003; Whomersley et al., 2010). For example, the ecotoxicological literature is dominated by studies exposing individual species to increasing doses of individual contaminants and assessing lethal concentrations (Bat & Raffaelli, 1998; Mayer-Pinto & Ignacio, 2015; Mayer-Pinto et al., 2011). Yet, stressors to ecological systems rarely occur singularly (Crain et al., 2008; Halpern et al., 2008) and may overlap in time and space synchronously or asynchronously. For example, climate change modifies not only temperature, but also $p\mathrm{CO_2}$, rainfall, and storm intensity and frequencies (Bijma et al., 2013; Hoegh-Guldberg & Bruno, 2010). A single climatic event can facilitate multiple forms of stress. For example, rainfall events not only erode intertidal flats and mobilise sediments (Pilditch et al., 2008; Tolhurst et al., 2006, 2008b) but can deliver a cocktail of contaminants such as nutrients, polyaromatic hydrocarbons and litter to estuaries through deposition and runoff (Birch et al., 2010; DeLorenzo et al., 2012; Kennish, 2002). Previous research has shown that effects of multiple stressors cannot easily be predicted from the effects of singular stressors because, when combined, they can have additive, subtractive or synergistic effects (Bijma et al., 2013; Bulling et al., 2010; Crain et al., 2008; Przeslawski et al., 2014; Sundback et al., 2010).

Many studies examining the impacts of multiple stressors on organisms use laboratory experiments to isolate them from their natural habitats and communities (Godbold et al., 2011; Hicks et al., 2011; Sousa et al., 2011; Sundback et al., 2010). However, the impact of stressors on specific organisms is likely to be mediated by environmental and biological context. For example, organisms may be able to make use of microhabitats to minimise exposure to stressors (Allen & Baltz, 1997; Jones & Boulding, 1999) and ecological interactions such as competition, facilitation and predation may dampen or exacerbate stressor impacts (Christensen et al., 2006; Hicks et al., 2011). Furthermore, whether individuals, populations and ecosystems have been exposed to similar or other stressors previously may influence their response (e.g. local adaptation of marine invertebrates reviewed in Sanford & Kelly, 2011). *In situ* mesocosms have been used effectively to add an element of realism to assessments of stressor impacts (e.g. Christensen et al., 2006; Dzialowski et al., 2014) and reviewed in Crane et al. (Crane et al., 2007). Nevertheless, while studies such as these offer interesting insights and important relationships between changes in ecosystem function and biodiversity under scenarios of climate change, they do not represent what is happening under natural conditions. Field studies are still needed.

3.1.2 Stressors on estuarine systems

Estuarine systems are economically valuable (Barbier et al., 2011), and as a consequence have been heavily exploited (Kennish, 2002; Lotze et al., 2006).They are often the hubs of human population growth due to their immense value as sources of food and transport. It has been estimated that up to 75% of the world's population live near to estuarine and coastal watersheds (Paerl, 2006). Within Australia, due to the arid and uninhabitable centre, this proportion is even larger. Most major cities in Australia are located close to or on large estuarine watersheds, with government estimates of up to 80-85% of the total population living in these areas (Australian Bureau of Statistics, 2002).

The rapid development and increasing population of Australia has caused these estuarine systems to become increasingly threatened. Development can cause direct and intentional impacts – for example, dams and irrigation systems alter flow (e.g. Hawkesbury River; Jones et al., 1986). Coastal armouring also impacts environments by changing landscapes and physically altering dynamics of a system (Firth et al., 2013). Anthropogenic physical disturbance can also originate through less intentional sources, such as recreation – e.g. boat wakes, anchorage, coastal walking (Bishop, 2005; Bowles & A, 1982; Rossi et al., 2007) – and livelihood – e.g. bait digging, fishing, dredging (Brown & Herbert Wilson, 1997; Ellis et al., 2000; Somerfield et al., 1995; Wynberg & Branch, 1994).

Numerous studies have focussed on the effects of these types of stressor on sediment habitats and have been discussed in depth in Hall (1994). There can be direct effects on the community structure caused by intentional collection of commercial species or bait digging, although disturbance effect of collection can result in mortal effects on non-target species as well as the intended organisms (Brown & Herbert Wilson, 1997; Contessa & Bird, 2004; Hall & Harding, 1997; Wynberg & Branch, 1994). Physical disturbance has the potential to alter the distribution, abundance and diversity of macrofaunal assemblages (Fraterrigo & Rusak, 2008). While sediment grain size can mediate the effect of disturbance on communities (Lindegarth & Hoskin, 2001), grain size itself can also become altered by intense disturbance effects (Bishop, 2005) thereby facilitating a change in community structure. Disturbing the sediment can also alter the characteristics of the mudflat, causing compactions of the sediments and creating overlying pools of water. Compactions of sediments reduces the depth to which oxygen can penetrate sediment (Contessa & Bird, 2004) as well as altering nitrogen cycling (Rossi et al., 2007). Pools of water are likely to disrupt the atmospheric gas exchange at low tide (Rossi et al., 2007). Additionally compaction or physical vibrations caused by the disturbance is likely to collapse burrows and disturb burrowing organisms (Tolhurst et al., 2000).

Physical disturbance could also originate through human induced climate change altering the intensity and frequency of storm events (Bijma et al., 2013). Future scenarios of climate change are predicted to increase the frequency and severity of storm events (IPCC, 2014). The wind from storm events are likely to promote tidal and wave action on mudflats (Allen & Duffy, 1998; Austen & Widdicombe, 2006; Cowie et al., 2000). Surface sediment can be scoured from the mudflats causing mortalities in benthic organisms (Yeo & Risk, 1979). Additionally, these storm events promote rainfall which will decrease the erosion threshold of these sediments (Tolhurst et al., 2006) and also flush nutrients and pollutants into the estuarine environment (Drapper et al., 2000).

The high population densities make these areas particularly susceptible to pollutants. Nutrient enrichment has been identified as one of the most severe forms of pollution within estuarine systems (Kennish, 2002; Windom, 1992). While nutrient pollution can come from a variety of sources including runoff, atmospheric deposition, sewage, food production, and decomposition

(Baron et al., 2012; Nixon, 1995; O'Brien et al., 2010), fertilisers are among the key sources, introducing excess nitrogen and phosphorus to these systems (Bricker et al., 2008; Nixon, 1995; Vitousek et al., 1997a). Worldwide, up to 50% of the fertiliser applied to farmlands has the potential to reach estuarine environments, through leaching into the watersheds and streams (McLusky & Elliott, 2011). In Australia, estuaries are built around major cities and nutrient pollution is more likely to be derived from fertilisers applied to golf courses and gardens, cleaning products, and waste-water discharge.

The communities within an individual estuarine system are adapted to its natural background level of nutrients and over time Australian systems have become phosphorus limited (Beadle, 1962; Cloern, 2001; Heip et al., 1995). Additional anthropogenic nutrient loading has the potential to raise nutrient levels, therefore altering the resource limitations (Cloern, 2001; Vitousek et al., 1997a), local environmental context has the potential to mediate such additions due to local adaptations and background nutrient levels (Bishop & Kelaher, 2013). In comparison to North American and European systems, nutrient enrichment is relatively moderate in Australia, given the smaller population and relatively recent developmental history (Eyre & Balls, 1999; Hauxwell & Valiela, 2004; Kelly, 2008). The combined nutrient limitations and limited background nutrient loadings means that Australian systems have a tendency to be oligotrophic (Jeffrey, 1974; Nicastro & Bishop, 2013) and therefore when additional nutrients are added they are rapidly taken up by biological systems with any traces of excess nutrients disappearing rapidly from time of impact (Scanes et al., 2007).

In benthic habitats, the growth of small, fast growing primary producers such as macroalgae and microphytobenthos (MPB) can be stimulated. This growth may lead to cascading positive effects up the food web (Bishop et al., 2006; York et al., 2012). In oligotrophic systems moderate nutrient additions can become beneficial to these habitats enhancing productivity (Verhoeven et al., 2012). However, excessive nutrient addition has the potential to cause a collapse in the system with prolonged exposure leading to an increase in grazing pressure before a major shift and alteration in community structure and function (Pascal et al., 2013). Additionally, the longer-term effects of anthropogenic nutrient enrichment could lead to toxicity caused by ammonia-based fertilisers (Gray et al., 2002); facilitation of toxic algal growth (Cloern, 2001; Devlin et al., 2011); or smothering by benthic algae leading to the death of benthic organisms (Kennish, 2002).

Another complication of excessive autotrophic growth is the increasing frequency of algal blooms which can potentially be toxic, cause hypoxic and anoxic conditions, kill fish and alter community structure (Kennish, 2002; Vitousek et al., 1997b). This condition is central to many European and northern hemisphere estuarine systems where increasing the nutrient loading causes eutrophication and high productivity leading to large amounts of organic matter. This in turn over-stimulates bacterial growth and respiration, depleting sediments of oxygen and eventually causing collapse (Gray et al., 2002; Nixon, 1995). While similar occurrences are common in Australian systems, these blooms are more likely to be caused by favourable conditions in stratification and light availability than by excessive nutrient loading (Davis & Koop, 2006). Regardless of the cause, prolonged exposure to increasing algal blooms could detrimentally alter the resilience of these systems to further environmental change (Chapin et al., 2000).

Nutrient enrichment has the tendency to be linked to other stressors, often occurring simultaneously with them (Cloern, 2001). Nutrient enrichment is significantly influenced by the climate (Baron et al., 2012; Cloern, 2001), for example, storm and rainwater events flushing nutrient pollution into estuarine and coastal areas (Drapper et al., 2000). While many studies focus on the mechanical damage caused by anthropogenic physical disturbances, the persistent effects of longer-term physical disturbance that could be derived from tidal and current movements is rarely addressed (e.g. Austen & Widdicombe, 2006; Cowie et al., 2000) and few actually assess the combined effects of physical disturbance and nutrient enrichment. Those that addressed these interactions have shown that while the increases in biodiversity, due to nutrient enrichment were subdued by the interaction with physical disturbance (Austen & Widdicombe, 2006), there were non-additive interacting effects (Austen & Widdicombe, 2006; Widdicombe & Austen, 2001). While these studies fail to address the impact of these stressors on the MPB, being laboratory-based mesocosm studies; there is also a lack of empirical field research into the combined effects of these stressors. The present study examines the interacting effects of nutrient enrichment and physical disturbance on the macrobenthic community structure and MPB in a natural estuarine environment.

3.2 Hypotheses

The following hypotheses will be addressed in this chapter:

H1 – Nutrient enrichment positively influences primary production.

H2 – Bottom up effects from increased primary production will lead to increased biodiversity.

H3 – Physical disturbance will negatively affect macrofaunal biodiversity.

H4 – Physical disturbance will subdue the effect of nutrient enrichment on both primary productivity and macrofauna.

3.3 Materials and Methods

3.3.1 Study sites

Two sites were selected to analyse the comparative effects of multiple stressors on Australian estuarine benthic systems (Chapter 2, Botany Bay and Lane Cove River).

3.3.2 Experimental design

Plots were manipulated in a two factor crossed design investigating the impacts of physical disturbance (through raking sediment) and nutrient enrichment as previously described (Chapter 2). Each factor had three associated levels of intensity, zero, low or high (Figure 3.1). A procedural control was devised to recreate the physical effects of burying the fertiliser bags. The experiment lasted 5 months with samples being taken monthly, from May to September 2012.

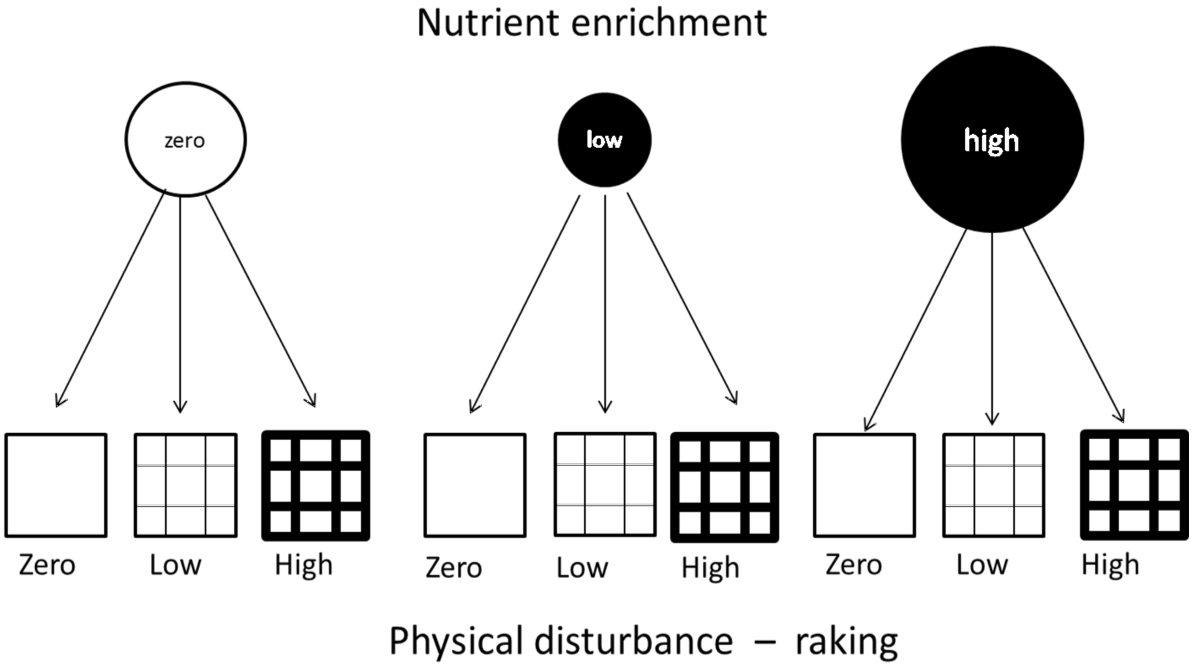


Figure 3.1: Schematic outlining the two-factor experimental design manipulating nutrient loading (through input of fertiliser) and physical disturbance (in the form of raking). Each factor had three levels – zero, low and high. For nutrient enrichment, low and high correspond to 0.5 kg and 1 kg fertiliser per plot. For physical disturbance, low and high correspond to 1 and 3 sets of cross hatched raking. Plot size – 0.25 m^2

3.3.3 Sampling

Sediment was collected and treated (described in Chapter 2) for use in quantifying the organic content (Chapter 2.4.2), macrofauna (Chapter 2.6), and microphytobenthos biomass through spectrophotometric and handheld remote sensing techniques (Chapter 2.7). Samples were taken from different areas within the plot at each time point to minimise interference between repeated sampling.

Due to time constraints, contact cores were not taken at the start of the experiment for all of the plots. Subsamples were taken randomly to characterise the site in terms of sediment chlorophyll and organic content and data used to describe the background levels. The month 1 sampling time point coincided with a low pressure storm event which meant at time of sampling the tide did not drop low enough for long enough to be able to collect contact cores from all plots despite them being located well above the low tide mark. All other variables were measured. For the spectroradiometer, the measure was hampered by surface water for certain plots but enough measurements were taken to be able to utilise an unbalanced design for this time point where n>3 for all treatments.

3.3.4 Statistics

Variables measured are detailed above. Univariate and multivariate analyses were conducted separately for each site using 3- factor analysis of variance, with nutrients, physical disturbance and month used as fixed factors. Analyses were conducted using PERMANOVA and Minitab after appropriately checking for heterogeneity of variance (PERMDISP – multivariate; Bartletts test – univariate) and normality of the data (Shapiro–Wilk test). 2- factor analyses were conducted to assess pre –existing differences between treatments and sites were compared using t-tests. Macrofauna composition data was square root transformed for PERMANOVA in order to down-weigh the effect of species abundance. The effect of rare species did not affect results therefore square root transformation was deemed an appropriate transformation. Due to the large variation of the data and dissimilarities between months in the macrofauna, a two-way analysis was conducted for these time points, analysing each month separately.

PRIMER software was also used to calculate diversity indices. Multivariate univariate analyses were conducted to examine the community structure using PERMANOVA and diversity indices. Diversity was calculated using PRIMER and included species richness, total abundance, species richness (Margalef index), Pielou’s evenness, Shannon diversity and Simpson Index. Following these analyses pairwise *post hoc* tests were conducted to differentiate significant differences between factors. Further to this SIMPER analysis was used to identify species causing dissimilarity in community structure between treatments. Species were chosen based on those having a dissimilarity/standard deviation ratio > 1.

3.4 Results

3.4.1 Site background

At the beginning of the experiment there was no significant difference between sites in terms of organic content and chlorophyll *a* showing there were no pre-existing differences between them (Figures 3.2 and 3.3).

3.4.2 Organic content

Organic content at each site was found to differ between months (Table 3.1). At Botany Bay no treatment effects were found. At Lane Cove treatment effects were identified after 3 months (Table 3.1, Figure 3.2; D x M interaction). After 4 months of manipulation, physical disturbance significantly increased organic content. No effects of nutrient enrichment on organic content were identified.

Table 3.1: PERMANOVA output of three-way interaction between Nutrients (N), Disturbance (D) and Month (M) analysing their effects on sediment organic content at Botany Bay (based on 4 months of data) and Lane Cove (based on 3 months of data). n=3-7 for all treatments levels.

	Botany Bay			**Lane Cove**		
	DF	Pseudo-F	P (perm)	DF	Pseudo-F	P (perm)
Nutrient Enrichment (N)	2	0.64	0.529	2	0.30	0.743
Disturbance (D)	2	0.08	0.919	**2**	**3.75**	**0.026**
Month (M)	**4**	**3.01**	**0.031**	**2**	**5.32**	**0.006**
NxD interaction	4	1.37	0.247	4	1.83	0.126
NxM interaction	6	0.67	0.670	4	0.16	0.956
DxM interaction	6	1.11	0.357	**4**	**2.61**	**0.038**
NxDxM interaction	12	0.49	0.922	8	1.52	0.155

Key: **Bold** = significant effect at $p < 0.05$

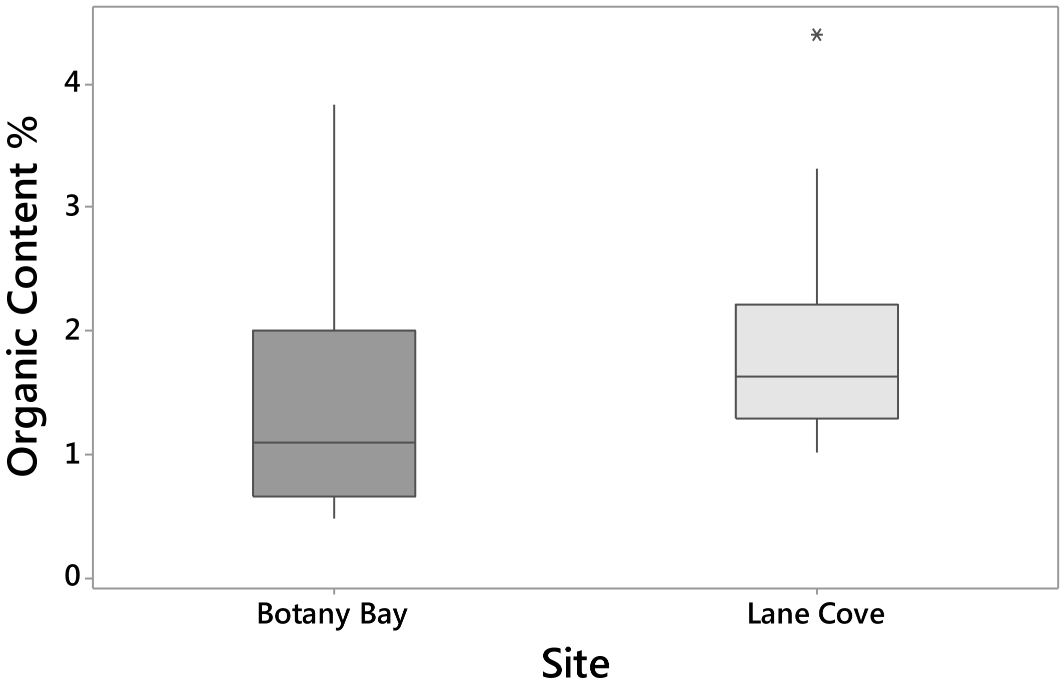


Figure 3.2: **Organic content (%) within the sediment at start of experiment at sites in Botany Bay (mean = 1.49, median = 1.08, SD = 1.01, IQR = 0.65-2.00, n = 20) and Lane Cove (mean = 1.82, median = 1.62, SD = 0.79, IQR = 1.28-2.21, n = 25).**

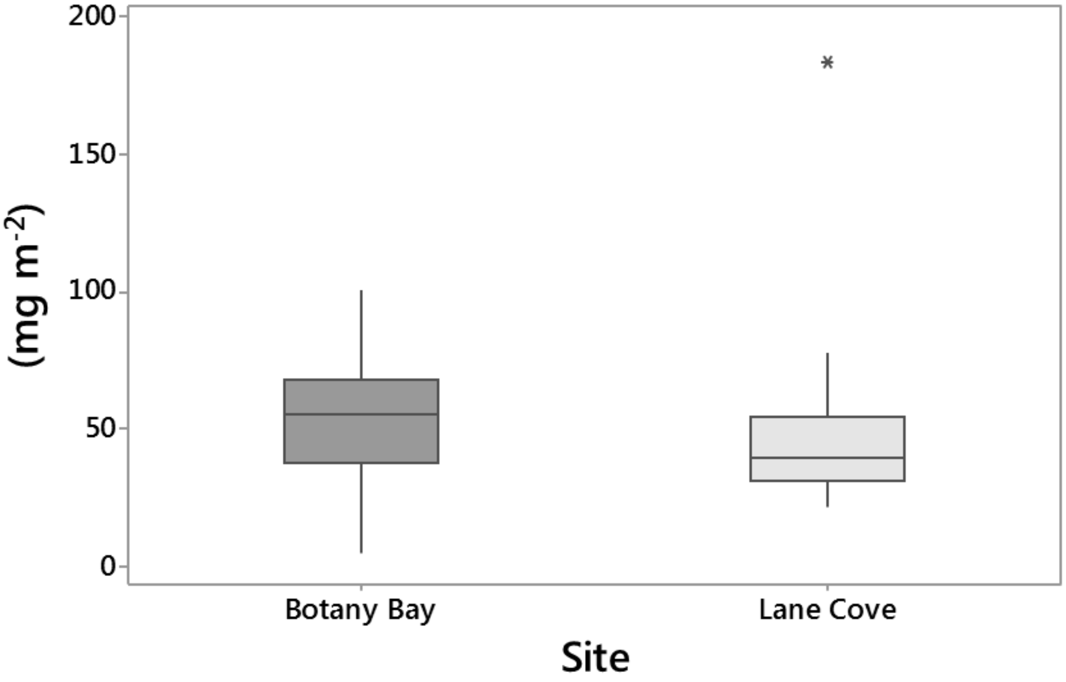


Figure 3.3: **Chlorophyll content (mg m^{-2}) within the sediment at start of experiment at sites in Botany Bay (mean = 51.96, median = 55.10, SD = 22.44, IQR = 37.44-67.44, n = 23) and Lane Cove (mean = 47.12, median = 39.07, SD = 31.48, IQR = 31.07-54.07, n = 25).**

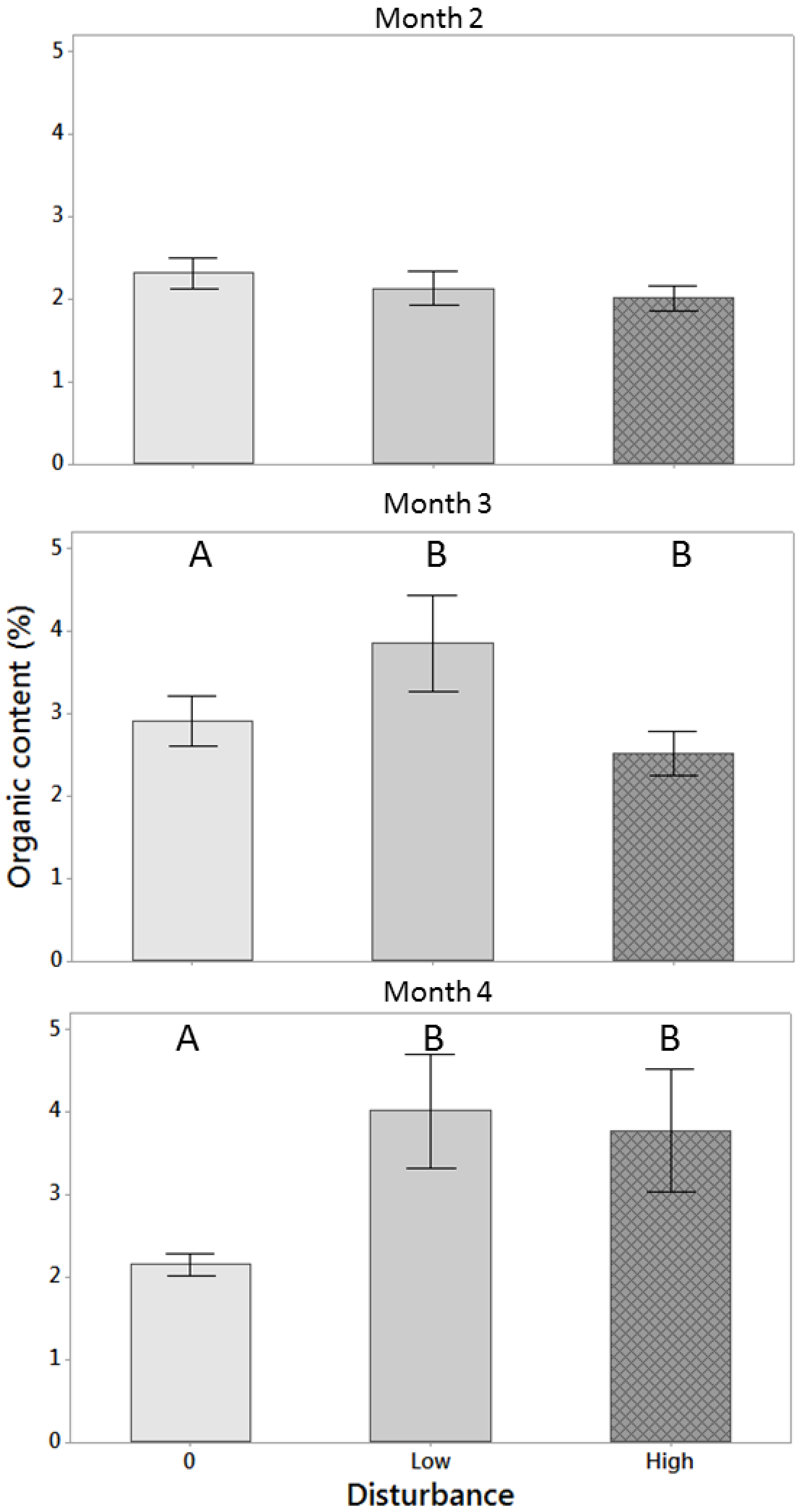


Figure 3.4: Organic content (%) in sediments comparing the final 3 months of experimental manipulations at Lane Cove. Data pooled to show physical disturbance effects only. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

3.4.3 Microphytobenthic biomass

3.4.3.1 Chlorophyll a

Sediment chlorophyll *a* at both sites displayed significant variation over time (Table 3.2; Month: $p < 0.05$). A three-way analysis showed that at Botany Bay the effect of disturbance on chlorophyll concentration also varied through time (Table, 2.2; Figure 3.5, D x M interaction). One month following physical disturbance (Figure 3.5a), chlorophyll content was greater in the high disturbance region than the control ($t = 2.90$, $p = 0.006$) or low disturbance ($t = 2.57$, $p = 0.014$) treatments. The direction of this relationship reversed after 3 months (Figure 3.5b), chlorophyll concentration was significantly less in the high disturbance treatment compared to plots with no nutrient additions ($t = 2.13$, $p = 0.042$) and this relationship was maintained after 4 months. Low disturbance had no effect. There was a significant main effect of nutrient enrichment at Botany Bay (Table 3.2; Figure 3.6). The two nutrient enriched treatments (low and high) did not differ significantly but both the high ($t = 4.25$, $p < 0.001$) and low ($t = 3.85$, $p < 0.001$) nutrient enrichments significantly increased chlorophyll *a* concentration above the zero nutrient treatment.

At Lane Cove disturbance was the only main effect (Table 3; Figure 3.7). At this site while high disturbance significantly decreased the level of chlorophyll ($t = 1.97$, $p = 0.042$), the low disturbance treatment did not ($t = 1.74$, $p = 0.065$). Chlorophyll concentration was significantly lower in the highly disturbed treatment compared to the low treatment ($t = 3.12$, $p = 0.001$).

There was a significant positive correlation between organic content and chlorophyll concentration (Figure 3.8) at both Botany Bay ($r_s = 0.247$, $p = 0.003$, $n = 170$) and Lane Cove ($r_s = 0.48$, $p < 0.001$, $n = 237$).

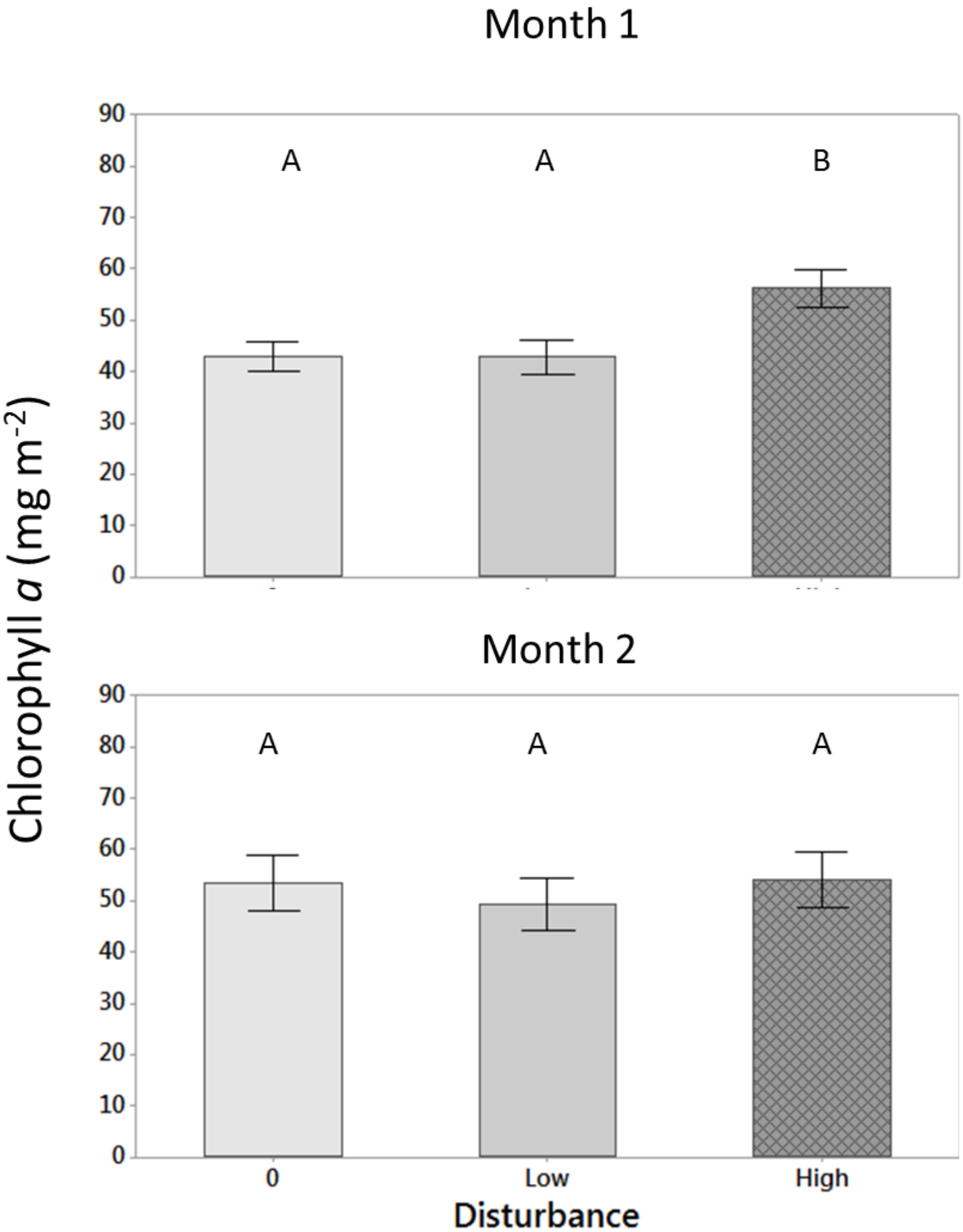


Figure 3.5a Change in chlorophyll content (mg m^{-2}) over four month experiment at Botany Bay comparing levels of physical disturbance. No interacting effects of nutrient enrichment seen at this site so data were pooled across nutrient enrichment treatments. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

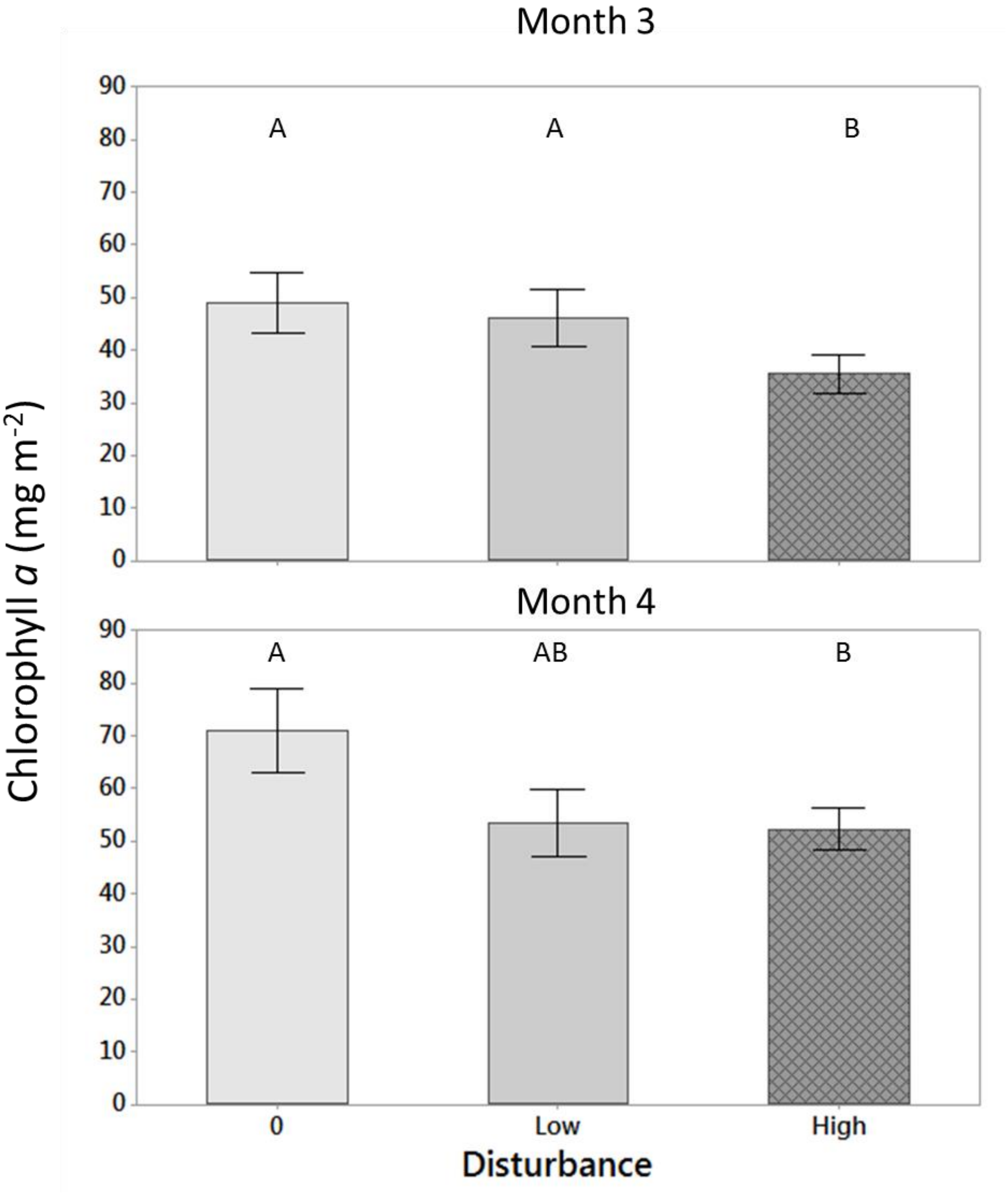


Figure 3.5b Change in chlorophyll content (mg m^{-2}) over four month experiment at Botany Bay comparing levels of physical disturbance. No interacting effects of nutrient enrichment seen at this site so data were pooled across nutrient enrichment treatments. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

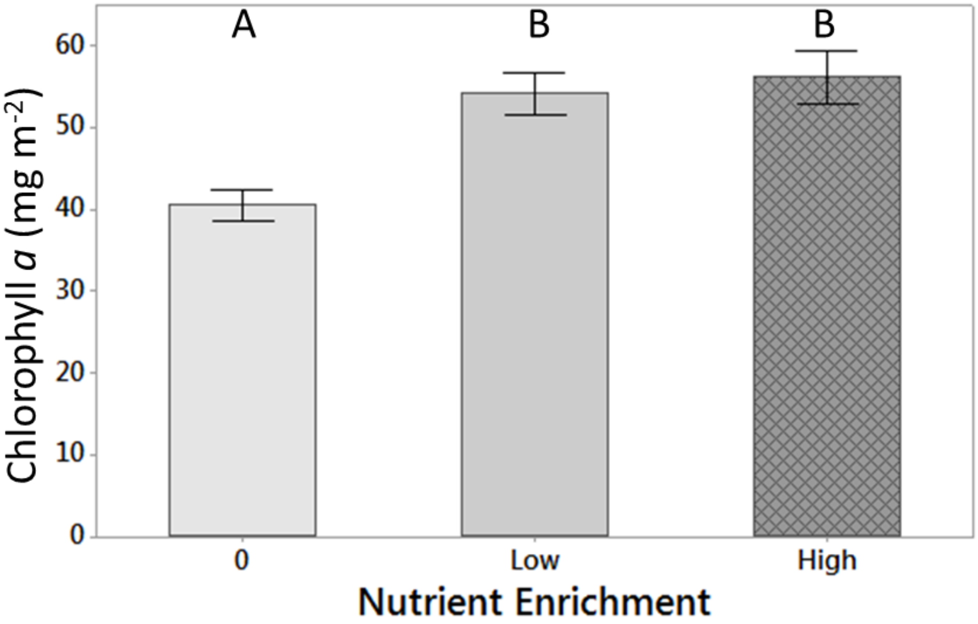


Figure 3.6 Change in chlorophyll content (mg m^{-2}) over four month experiment at Botany Bay comparing levels of nutrient enrichment. No interacting effects of disturbance or between months were found at this site so these data were combined. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

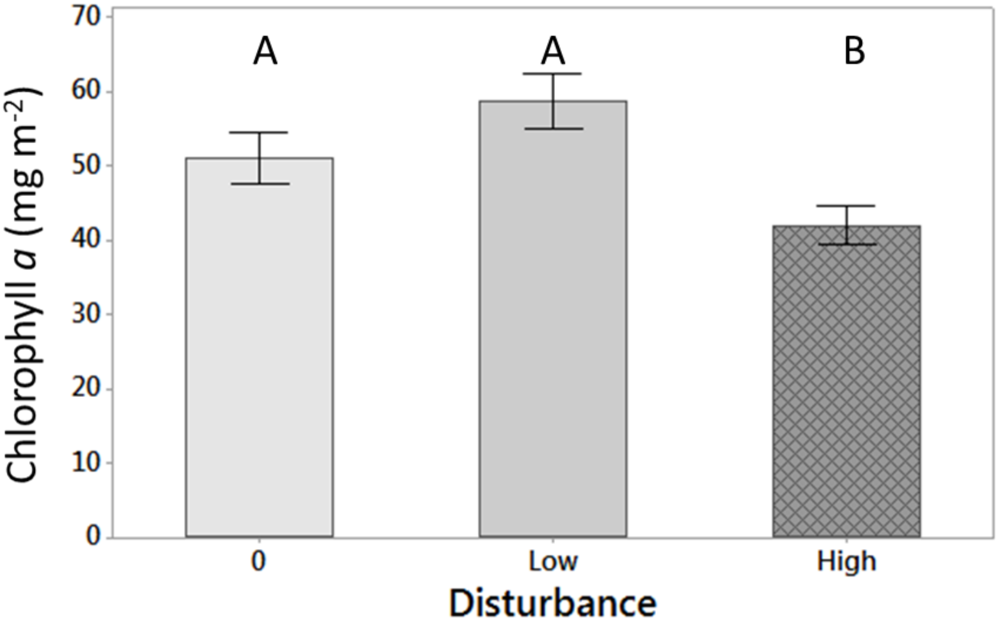


Figure 3.7: Change in chlorophyll content (mg m^{-2}) after four months of physical disturbance at Lane Cove. No interacting effects of nutrient enrichment or between months were seen at this site so these data were pooled across months. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

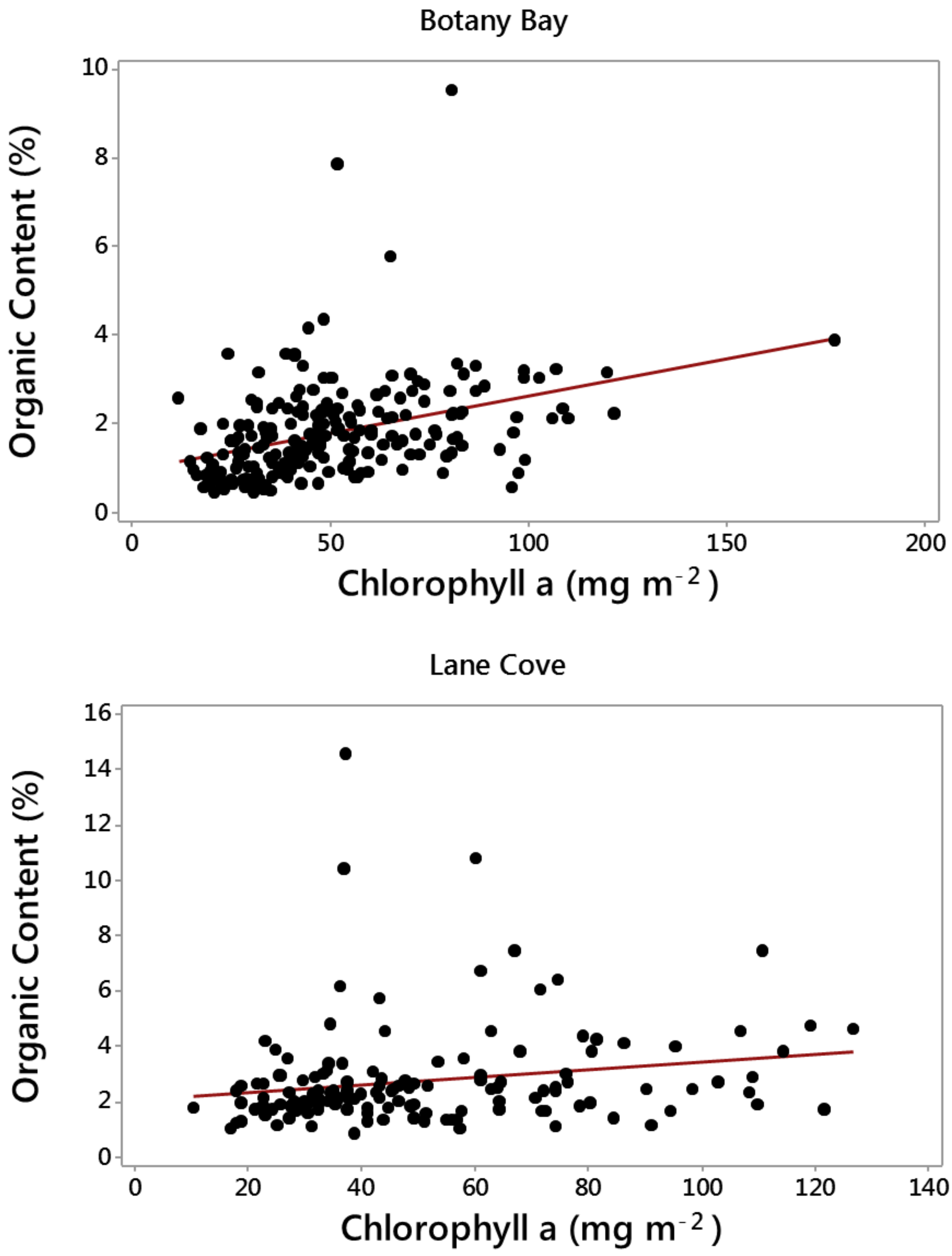


Figure 3.8: Correlation between organic content (%) and chlorophyll concentration (mg m^{-2}) found in contact cores. Data pooled across all months of experiment. Line of best fit represented.

Table 3.2: PERMANOVA output of three way interaction between Nutrients (N), Disturbance (D) and Month (M) analysing the effects on chlorophyll a content in Botany Bay (based on 4 months of data) and Lane Cove (based on 3 months of data) n=3-7 for all treatments

	Botany Bay			Lane Cove		
	DF	Pseudo-F	P(perm)	DF	Pseudo-F	P(perm)
Nutrient Enrichment (N)	2	**9.74**	**0.001**	2	1.76	0.181
Disturbance (D)	2	1.06	0.34	2	**6.65**	**0.003**
Month (M)	3	**5.71**	**0.002**	2	**10.95**	**0.001**
NxD interaction	4	1.53	0.207	4	0.24	0.922
NxM interaction	6	0.56	0.784	4	0.63	0.643
DxM interaction	6	**2.27**	**0.034**	4	0.73	0.567
NxDxM interaction	12	0.73	0.703	8	1.74	0.097

Key: **Bold** = significant effect at p < 0.05

3.4.3.2 NDVI

At the start of the experiment, there were no pre-existing differences between treatments in terms of NDVI (Table 3.3). Descriptive statistics are shown in Table 3.4 emphasising mean values for the different treatments. At the start of the experiment, the NDVI was significantly higher at the Lane Cove site than at Botany Bay (t = 5.71, df = 95, p <0.001).

Table 3.3: PERMANOVA output of two-way interaction between Nutrients and Disturbance examining pre-existing differences between treatments at the start of the experiments at Botany Bay and Lane Cove, n =5- 7 for all treatments, 3 levels per factor

		Botany Bay		Lane Cove	
	df	**Pseudo-F**	**P(perm)**	**Pseudo-F**	**P(perm)**
Nutrient enrichment	2	0.14	0.873	0.48	0.623
Disturbance	2	2.31	0.111	0.76	0.471
Interaction	4	0.61	0.661	2.45	0.057

Key: **Bold** = significant effect at p < 0.05

Table 3.4: Descriptive statistics examining pre-existing mean and standard errors (SE) in Normalised Difference Vegetation Index (NDVI) for treatments at the start of the experiments at Botany Bay and Lane Cove, n = 5-7 for all treatments. C = Control, L = Low, H = High, N = Nutrient enriched treatment, D = Disturbance treatment.

	Botany Bay			Lane Cove		
Treatment	**n**	**Mean**	**SE**	**n**	**Mean**	**SE**
C	5	0.073	0.009	7	0.102	0.010
LD	6	0.064	0.002	7	0.081	0.009
HD	6	0.082	0.010	6	0.132	0.015
LN	5	0.069	0.005	6	0.094	0.010
LNLD	6	0.073	0.008	7	0.099	0.009
LNHD	6	0.087	0.007	6	0.095	0.010
HN	7	0.074	0.006	7	0.113	0.013
HNLD	5	0.073	0.007	7	0.106	0.015
HNHD	7	0.075	0.007	7	0.093	0.012
Site	**53**	**0.075**	**0.002**	**61**	**0.101**	**0.004**

At Lane Cove there were no significant differences due to the main effects of nutrient enrichment or physical disturbance. However, there was an interaction of the two stressors (Table 3.5; Figure 3.10). When subjected to low physical disturbance both low ($t = 3.33$, $p = 0.002$) and high ($t = 2.56$, $p = 0.016$) nutrient enrichment significantly increase the NDVI compared to the zero treatment. Additionally when there was no nutrient enrichment high disturbance caused NDVI to increase in comparison to low disturbance ($t = 2.45$, $p = 0.014$) and while there was a slight increase in comparison to the undisturbed plot this was not significant ($t = 1.88$, $p = 0.062$). There was also an interaction when plots were subjected to low nutrient enrichment where low physical disturbance significantly increased the NDVI in comparison to the control ($t = 2.89$, $p = 0.008$) and high treatments ($t = 2.15$, $p = 0.037$).

At both Botany Bay and Lane Cove NDVI within the sediment showed significant variation over time (Table 3.5; Month $p < 0.05$). At Botany Bay there was a main effect of the physical disturbance (Pseudo-$F_{(2,207)} = 3.16$, p(perm) = 0.048; Figure 3.9) whereby under high physical disturbance the NDVI significantly decreased in comparison to the control treatment ($t = 2.31$, $p = 0.025$). A three-way analysis showed that at Botany Bay the effect of nutrient enrichment on NDVI also varied through time (Table 3.5; Figure 3.11, N x M interaction). It took two months for the nutrient enrichment treatments to have a measurable effect on the NDVI where both low ($t = 4.38$, $p = 0.002$) and high ($t = 4.00$, $p = 0.002$) enriched treatments have a higher value of NDVI than the un-enriched treatment (Figure 3.11a). This response is maintained until the fourth and final month when NDVI is only significantly affected by the high nutrient treatment compared to the unenriched treatments (Figure 3.11b; t = 2.43, p = 0.014).

Table 3.5: PERMANOVA output of three-way interaction between Nutrients (N), Disturbance (D) and Month (M) analysing the effects on NDVI in Botany Bay and Lane Cove over 4 months of constant nutrient enrichment and physical disturbance manipulations at zero, low and high intensities. n=5-7 for all treatments.

		Botany Bay		Lane Cove	
	df	**Pseudo-F**	**P(perm)**	**Pseudo-F**	**P(perm)**
Nutrient Enrichment (N)	2	**14.37**	**0.001**	0.82	0.443
Disturbance (D)	2	**3.16**	**0.048**	2.37	0.085
Month (M)	3	**9.99**	**0.001**	**26.56**	**0.001**
NxD interaction	4	1.36	0.321	**3.17**	**0.021**
NxM interaction	6	**2.36**	**0.031**	0.25	0.952
DxM interaction	6	1.27	0.321	1.09	0.367
NxDxM interaction	12	1.15	0.300	1.37	0.202

Key: **Bold** = significant effect at $p < 0.05$

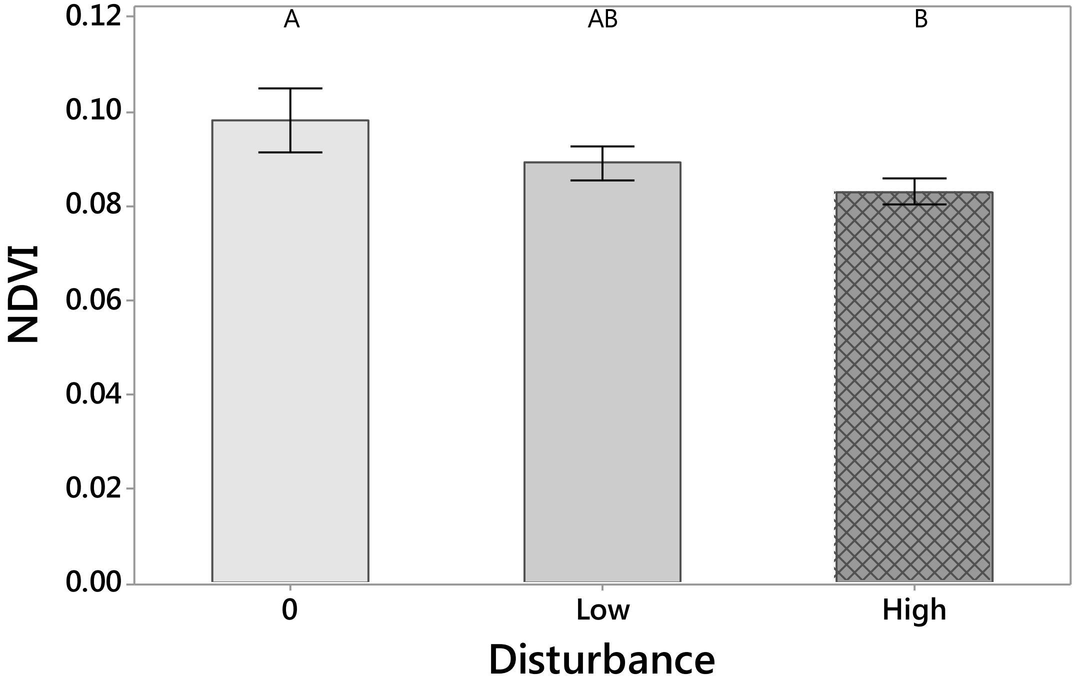


Figure 3.9: Change in Normalised Difference Vegetation Index (NDVI) after four months of physical disturbance at Botany Bay. No interacting effects with Nutrient enrichment or month were observed at this site so these data were pooled. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

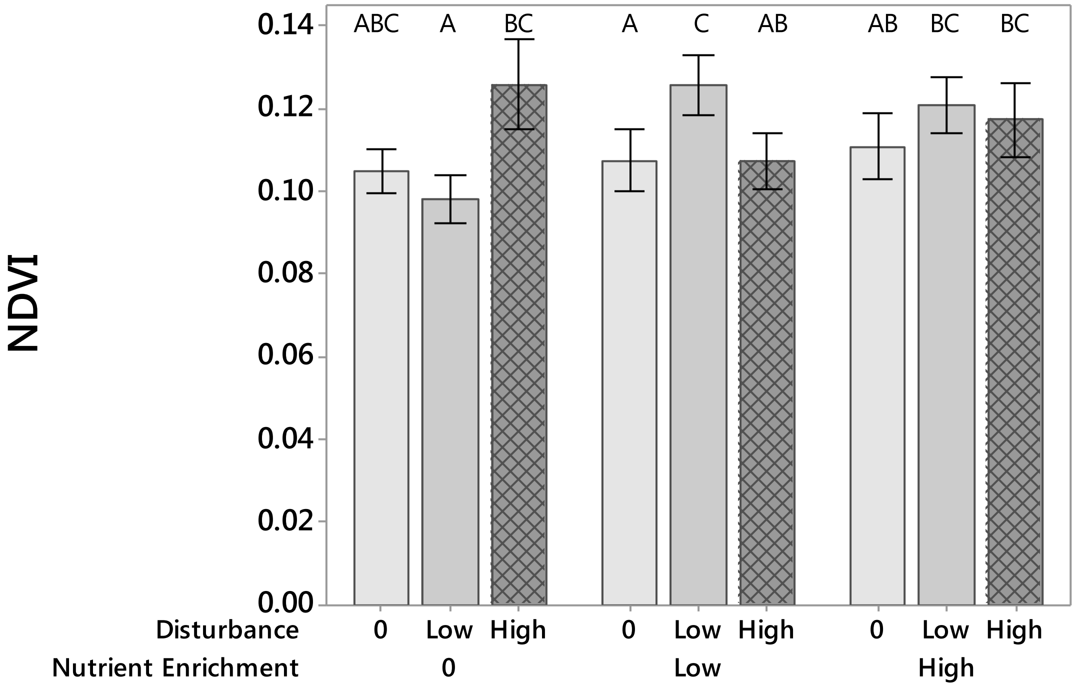


Figure 3.10: Change in Normalised Difference Vegetation Index (NDVI) over four month experiment at Lane Cove comparing 3 levels (0 Low and High) of nutrient enrichment and disturbance. No interacting effects were observed between months so data were pooled across treatments. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

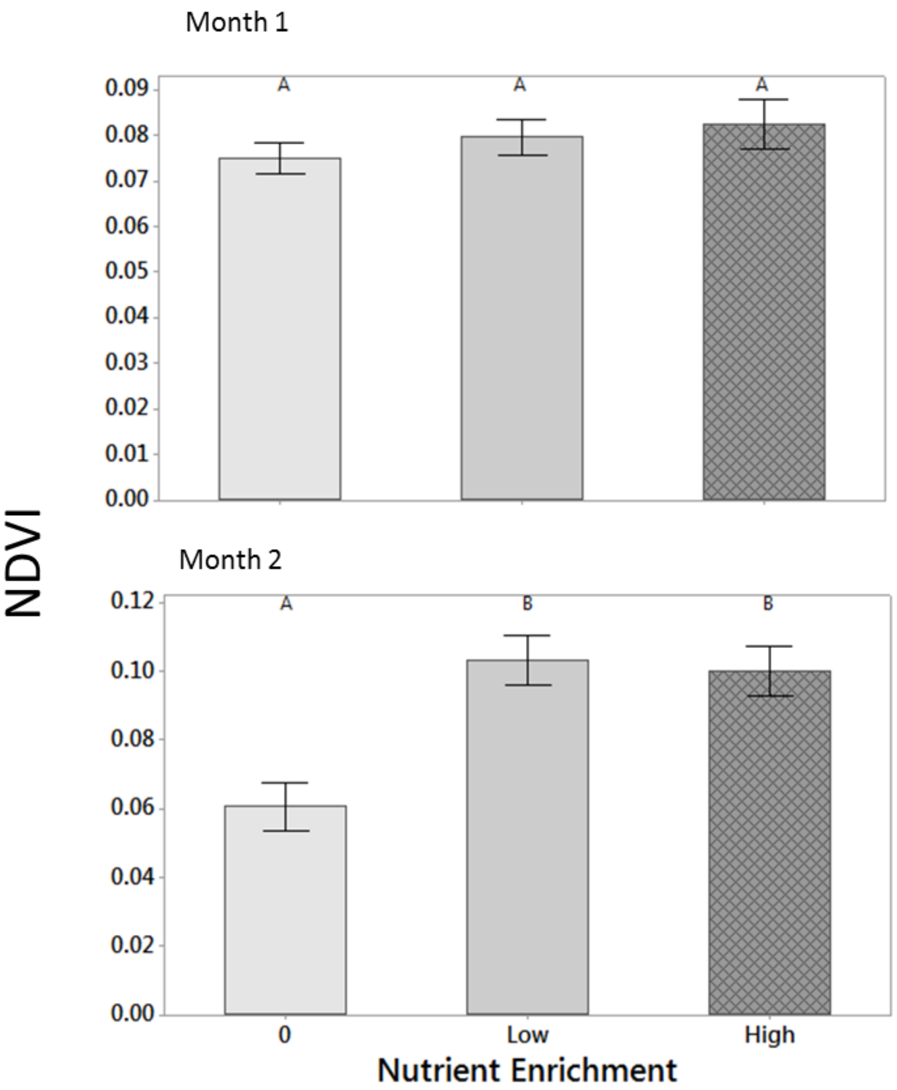


Figure 3.11a: Change in Normalised Difference Vegetation Index (NDVI) over four month experiment at Botany Bay comparing levels of nutrient enrichment. No interacting effects with disturbance were observed at this site so data were pooled across nutrient enrichment treatments. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

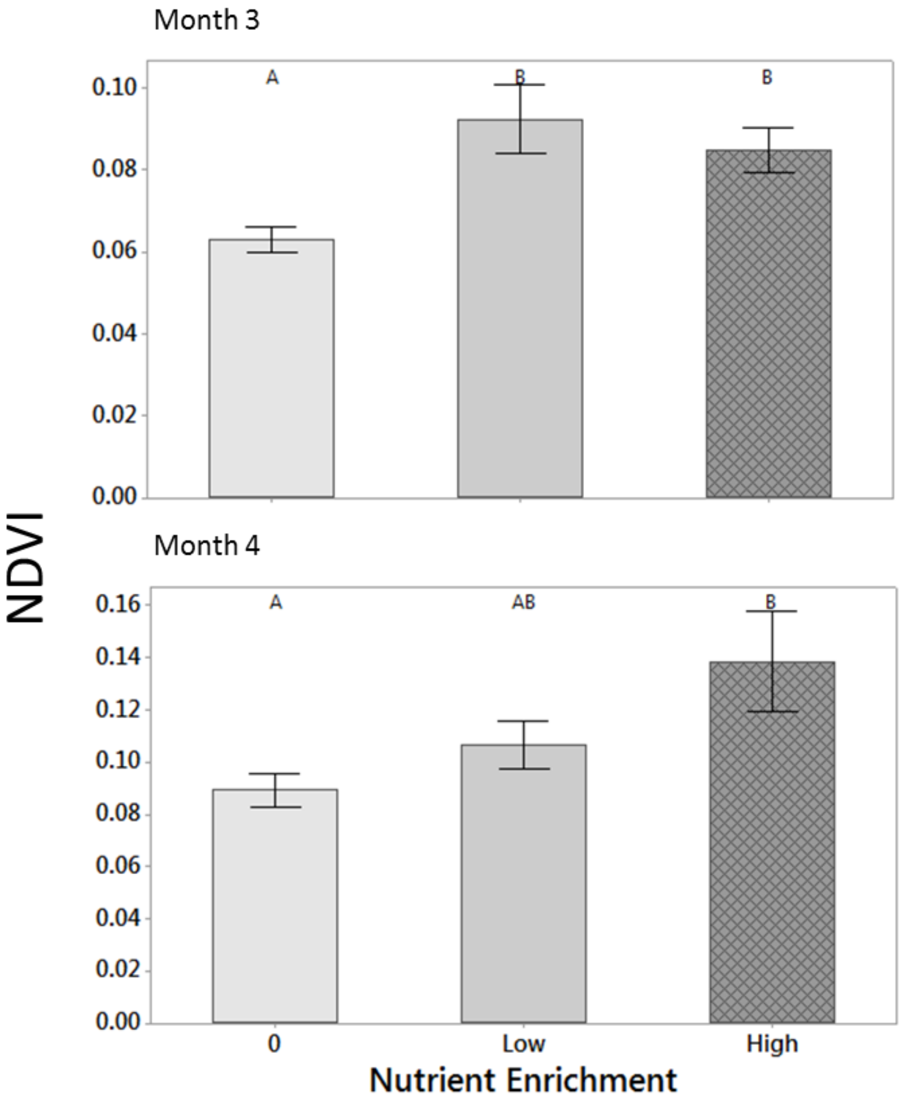


Figure 3.11 b: Change in Normalised Difference Vegetation Index (NDVI) over four month experiment at Botany Bay comparing levels of nutrient enrichment. No interacting effects with disturbance were observed at this site so data were pooled across nutrient enrichment treatments. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

3.4.4 Macrofauna biodiversity

There were no pre-existing differences between treatments due to macrofauna community composition or derived diversity indices at Botany Bay. At Lane Cove there were no significant pre-existing differences between treatments based upon community structure or most diversity indices, however, the species richness appeared to naturally differ between treatments (Figure 3.12, Interaction: $F_{(4,52)}$ = 0.82, p(perm) = 0.517; Nutrient enrichment $F_{(2,52)}$ = 5.47, p(perm) = 0.007; Disturbance: $F_{(2,52)}$ = 0.32, p(perm) = 0.730). There was only a response from the macrofauna after four months of disturbance and no treatment effects were identified after 2 months.

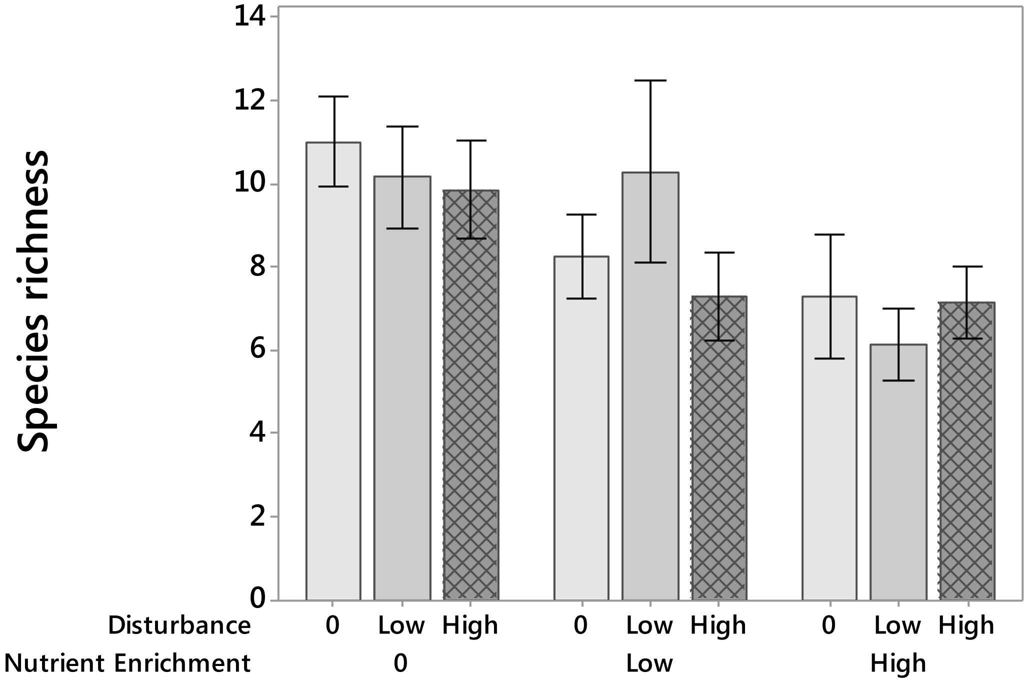


Figure 3.12: ***A priori*** **differences in species richness (total number of species per treatment) at Lane Cove between 3 levels (0 Low and High) of nutrient enrichment and disturbance. n = 7. Points indicate mean +/- SE.**

Following four months of stress, there was a main effect of nutrient enrichment on community composition at Botany Bay while at Lane Cove there was a minimal effect (Figure 3.13; square root transformed Botany Bay: Pseudo- $F_{(2,52)}$ = 1.92, p(perm) = 0.035; Lane Cove: Pseudo-$F_{(2,52)}$ = 1.48, p(perm) = 0.059). There were no effects of physical disturbance or interactions between stressors in either Botany Bay or Lane Cove on the community composition.

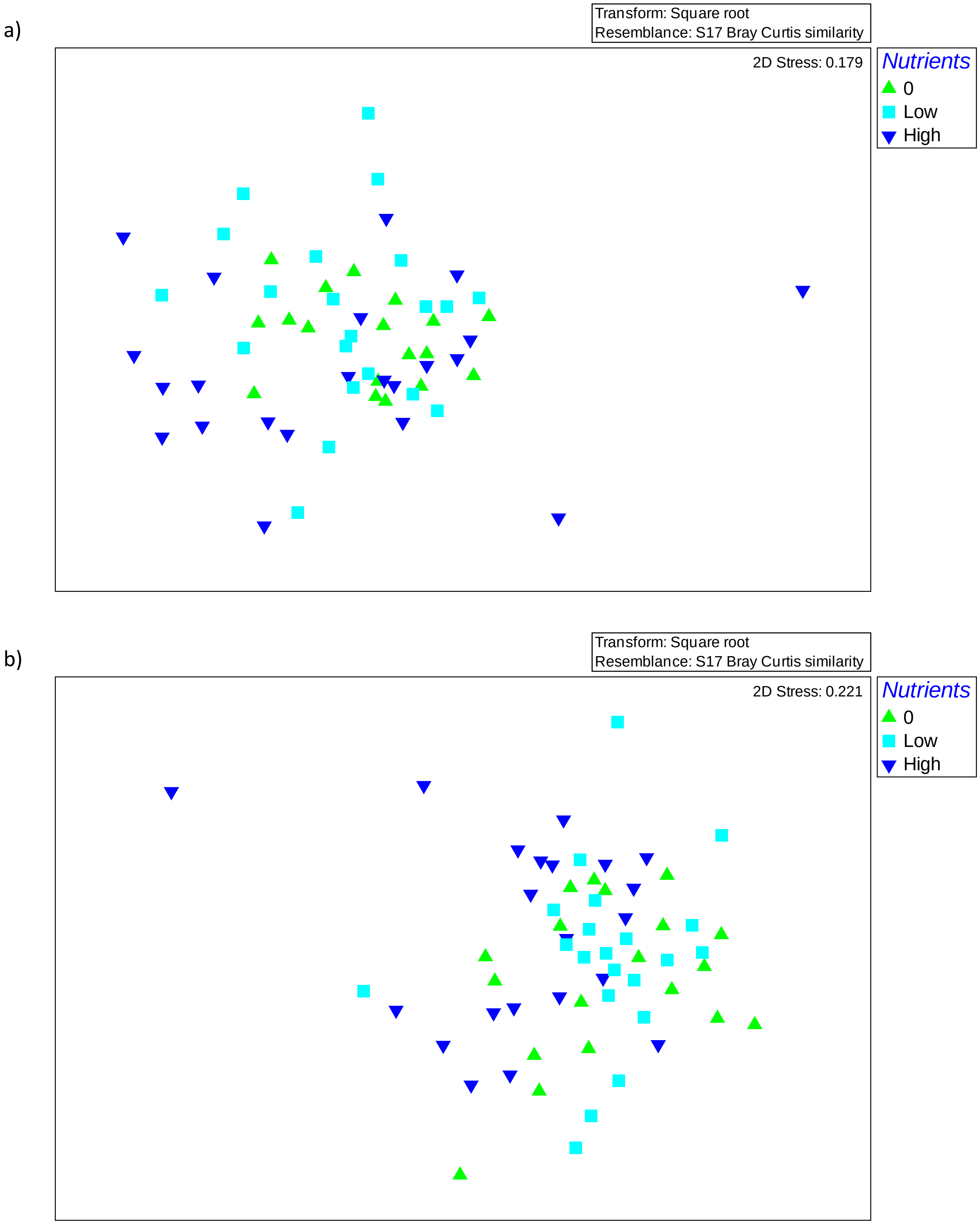


Figure 3.13: nMDS plot showing macrofaunal composition Botany Bay (a) and Lane Cove (b) following 4 months of zero low and high nutrient enrichment.

There were no treatment effects on diversity indices for either Botany Bay or Lane Cove. However, in Lane Cove, there was a treatment effect of the nutrient enrichment on the total abundance where increasing enrichment caused a decrease in total abundance (Figure 3.14; Pseudo-$F_{(2,52)}$ = 3.66, p(perm) = 0.028).

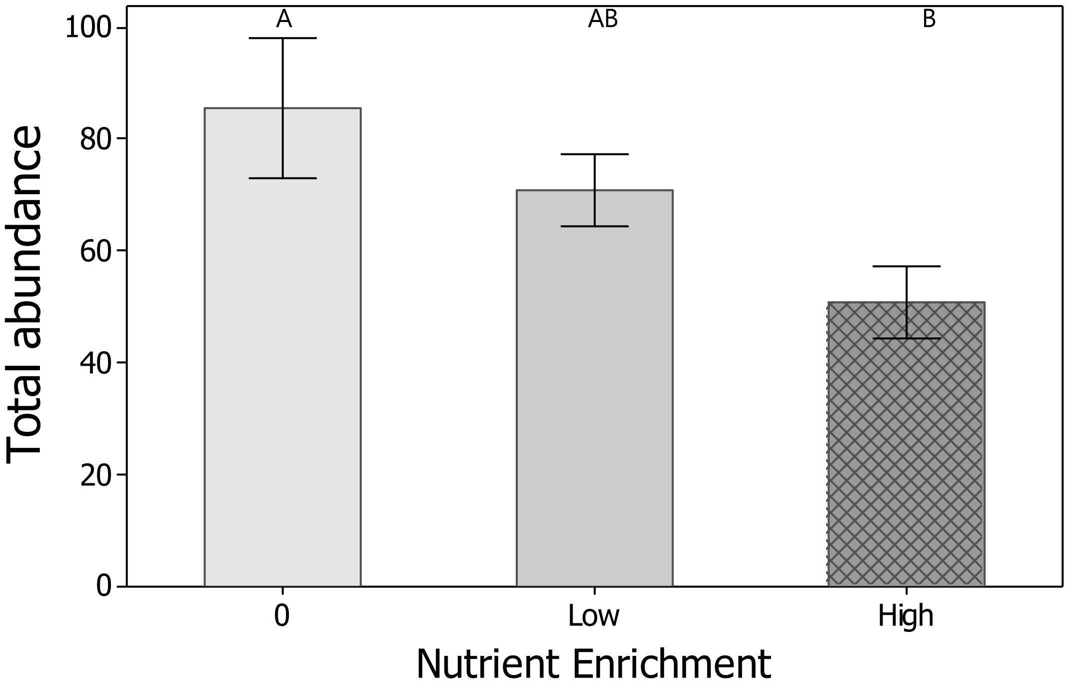


Figure 3.14: Differences in total abundance at Lane Cove between 3 levels of nutrient enrichment (Low and High). No interacting effects were observed between months so data were pooled across treatments. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

3.4.5 Key species

SIMPER analysis was used to identify the species causing most dissimilarity between treatments. Of the 6 species that caused most dissimilarity in Botany Bay, 4 coincided with those found in Lane Cove (Figure 3.15). These were *Mysella* sp., *Mediomastus australiensis*., *Prionospio* sp., and *Nephtys australiensis.* The additional species were *Salinator fragilis* and *Owenia australis*. Lane Cove had a total of 12 discriminating species which also included two species of *Gammarus* amphipods, *Oedicerotidae* amphipods, large nematodes, *Scoloplos* sp., *Laternula sp., Platynereis uniseris* and *Australonereis ehlersi.* Of the four species common to each site (Figure 3.15) only the abundance of *Prionospio sp.* was significantly altered by the treatments showing interactive effects at Botany Bay (Table 3.6; Figure 3.15). Their abundance was lower in plots receiving high nutrients compared to both the zero and low treatments. When subjected to zero and low nutrients, the plots receiving high physical disturbance had significantly lower *Prionospio sp.* abundances than those subjected to zero and low disturbance. There was no difference between treatments when subjected to high nutrient enrichment. At Botany Bay, no other species displayed species specific effects. At Lane Cove, *Scoloplos sp.* displayed a significant interaction between treatments (Table 3.6; Figure 3.16) where increased nutrient enrichment caused a sequential decrease in abundance. Additionally, increased physical disturbance significantly lowered abundance in comparison to the control. Additionally nutrient enrichment caused a significant sequential decrease in abundance of nematodes (Figure 3.16).

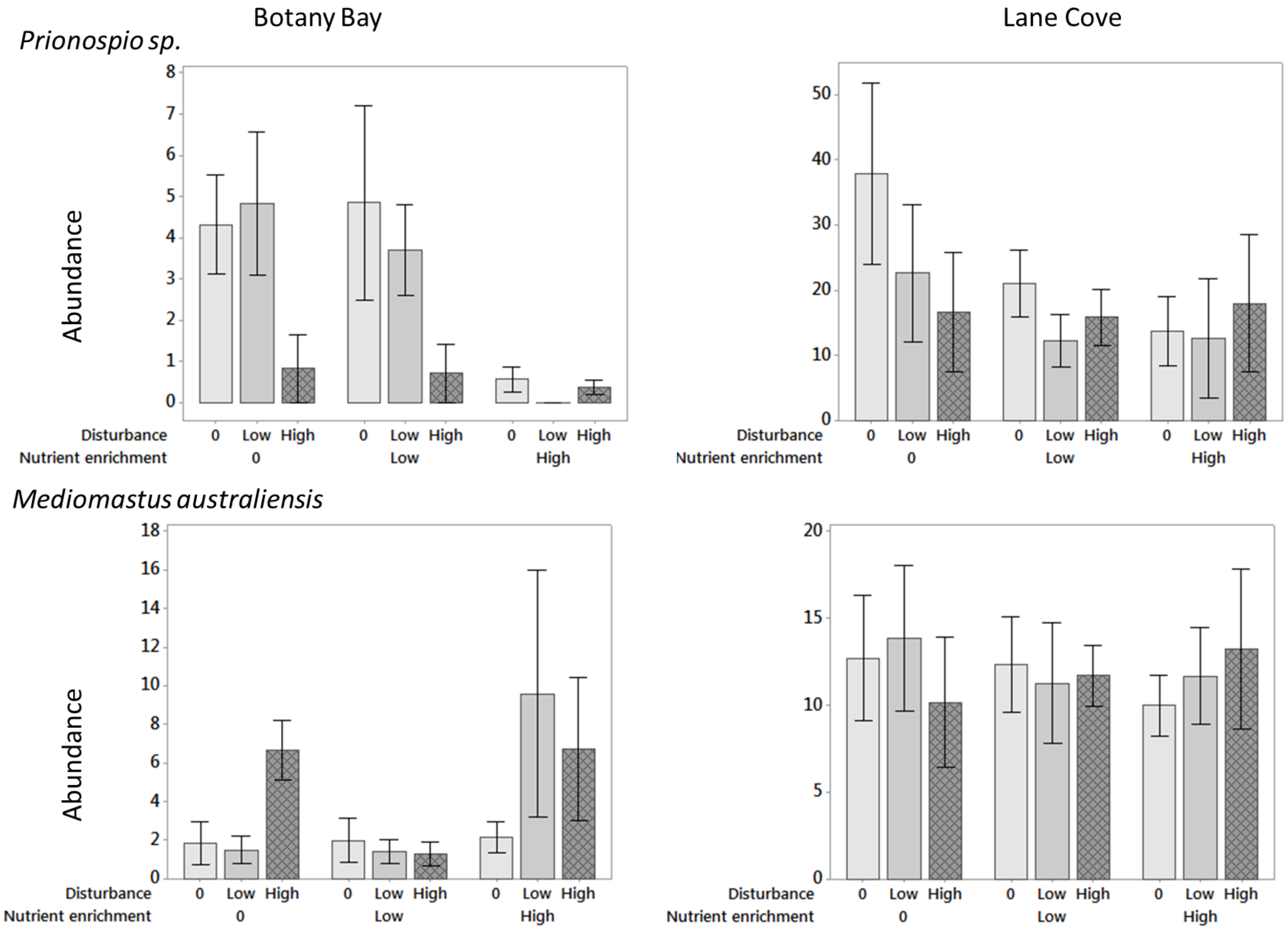
Table 3.6: PERMANOVA output of two-way interaction (I) between Nutrients (N) and Disturbance (D) analysing the effects of square root transformed abundances per plot in Botany Bay and Lane Cove after 4 months of constant nutrient enrichment and physical disturbance at zero, low and high intensities in a fully orthogonal crossed design. N=7 for all treatments.

		Botany Bay		Lane Cove	
Species	**Factor**	**F**	**P**	**F**	**P**
Mysella sp.	**N**	0.71	0.499	2.52	0.080
	D	0.06	0.943	0.48	0.626
	I	0.63	0.636	1.22	0.306
Mediomastus australiensis	**N**	2.09	0.121	0.02	0.970
	D	1.16	0.317	0.05	0.959
	I	1.40	0.231	0.15	0.953
Nephtys australiensis	**N**	0.42	0.656	0.12	0.886
	D	0.08	0.915	0.49	0.607
	I	0.45	0.775	0.40	0.811
Prionospio sp.	**N**	**10.70**	**0.001**	0.97	0.383
	D	**8.60**	**0.006**	0.54	0.568
	I	**3.14**	**0.028**	0.25	0.915
Salinator fragilis	**N**	0.71	0.494	-	-
	D	1.03	0.363	-	-
	I	0.87	0.485	-	-
Owenia australis	**N**	0.08	0.926	-	-
	D	0.08	0.911	-	-
	I	0.94	0.436	-	-
Scoloplos sp.	**N**	-	-	**5.25**	**0.006**
	D	-	-	1.13	0.329
	I	-	-	**2.91**	**0.039**
Platynereis uniseris	**N**	-	-	0.53	0.567
	D	-	-	0.03	0.980
	I	-	-	0.98	0.406
Oedicerotidae	**N**	-	-	2.32	0.094
	D	-	-	0.99	0.385
	I	-	-	1.33	0.249
Large nematodes	**N**	-	-	**3.25**	**0.045**
	D	-	-	0.31	0.751
	I	-	-	1.17	0.331
Australonereis ehlersi	**N**	-	-	1.31	0.265
	D	-	-	1.65	0.201
	I	-	-	1.09	0.385

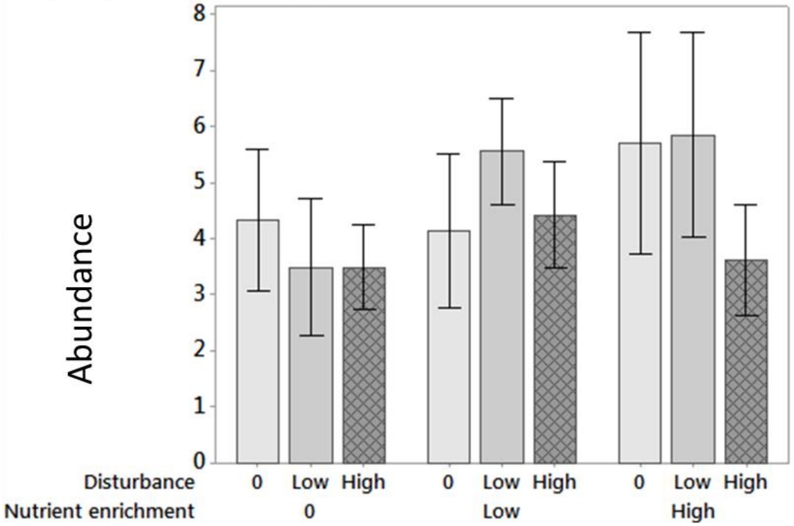
Table 3.6 continued:		**Botany Bay**		**Lane Cove**	
Species	**Factor**	**F**	**P**	**Species**	**Factor**
Gammarus sp. 1	**N**	-	-	1.18	0.301
	D	-	-	0.21	0.823
	I	-	-	1.68	0.177
Gammarus sp. 2	**N**	-	-	0.21	0.809
	D	-	-	0.99	0.384
	I	-	-	2.11	0.094
Laternula sp.	**N**	-	-	0.97	0.405
	D	-	-	0.31	0.725
	I	-	-	1.61	0.174

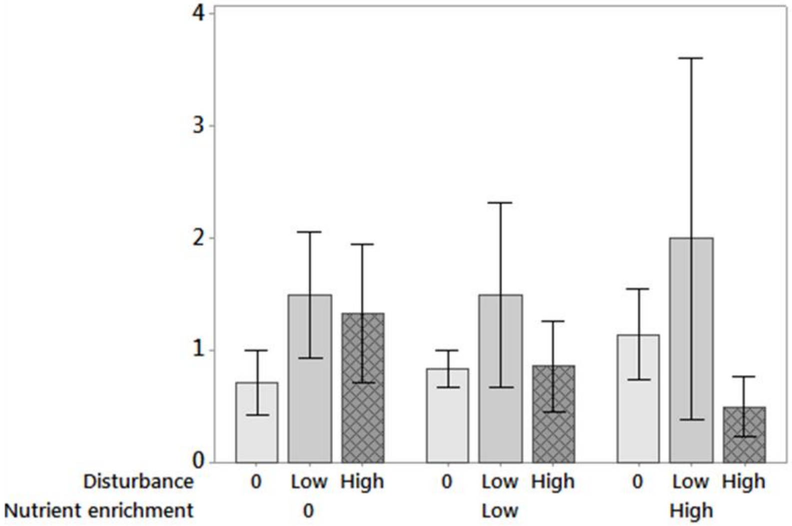
Key: **Bold** = significant effect at $p < 0.05$

Figure 3.15 following pages: Bar graph showing change in average core abundance for 4 common species found in Botany Bay and Lane Cove following 4 months of nutrient enrichment and physical disturbance crossed at 3 levels (0 Low and High) Points indicate mean +/- SE. N = 7.

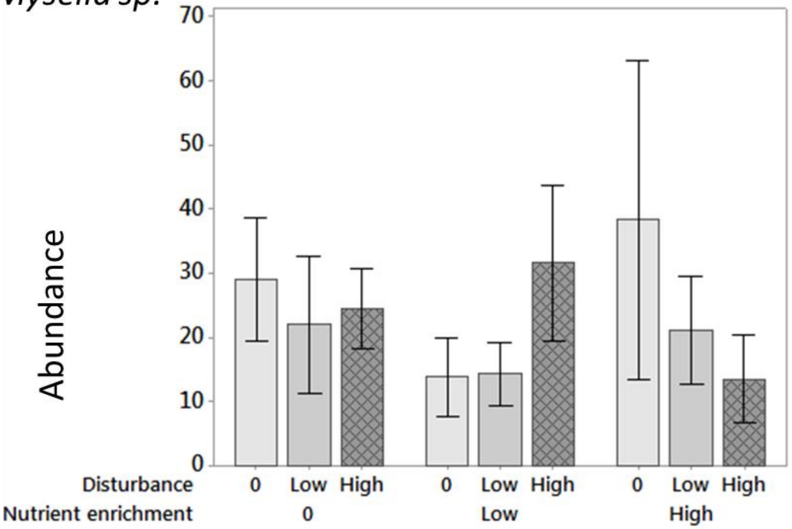

Botany Bay
Lane Cove
Prionospio sp.
Mediomastus australiensis
Abundance
Abundance
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
0
2
4
6
8
10
12
14
16
18
0
5
10
15
20
Disturbance
Nutrient enrichment
0
Low
High
0
Low
High
0
Low
High
0
Low
High

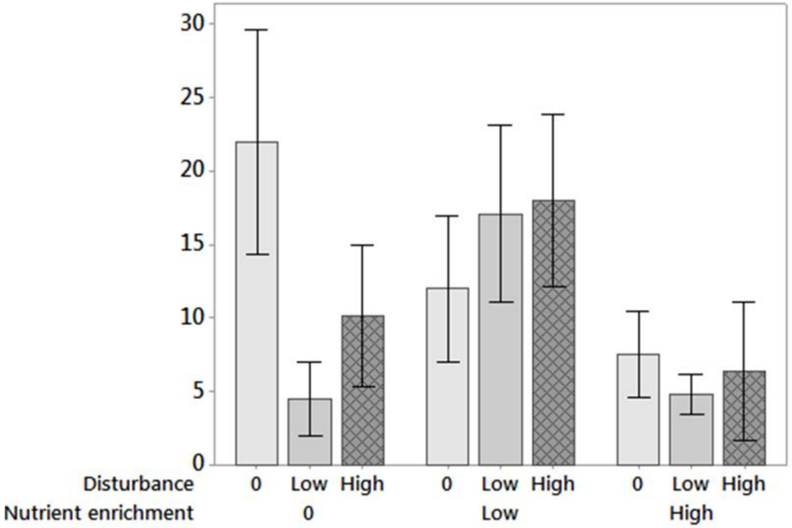
Nephtys australiensis





Mysella sp.





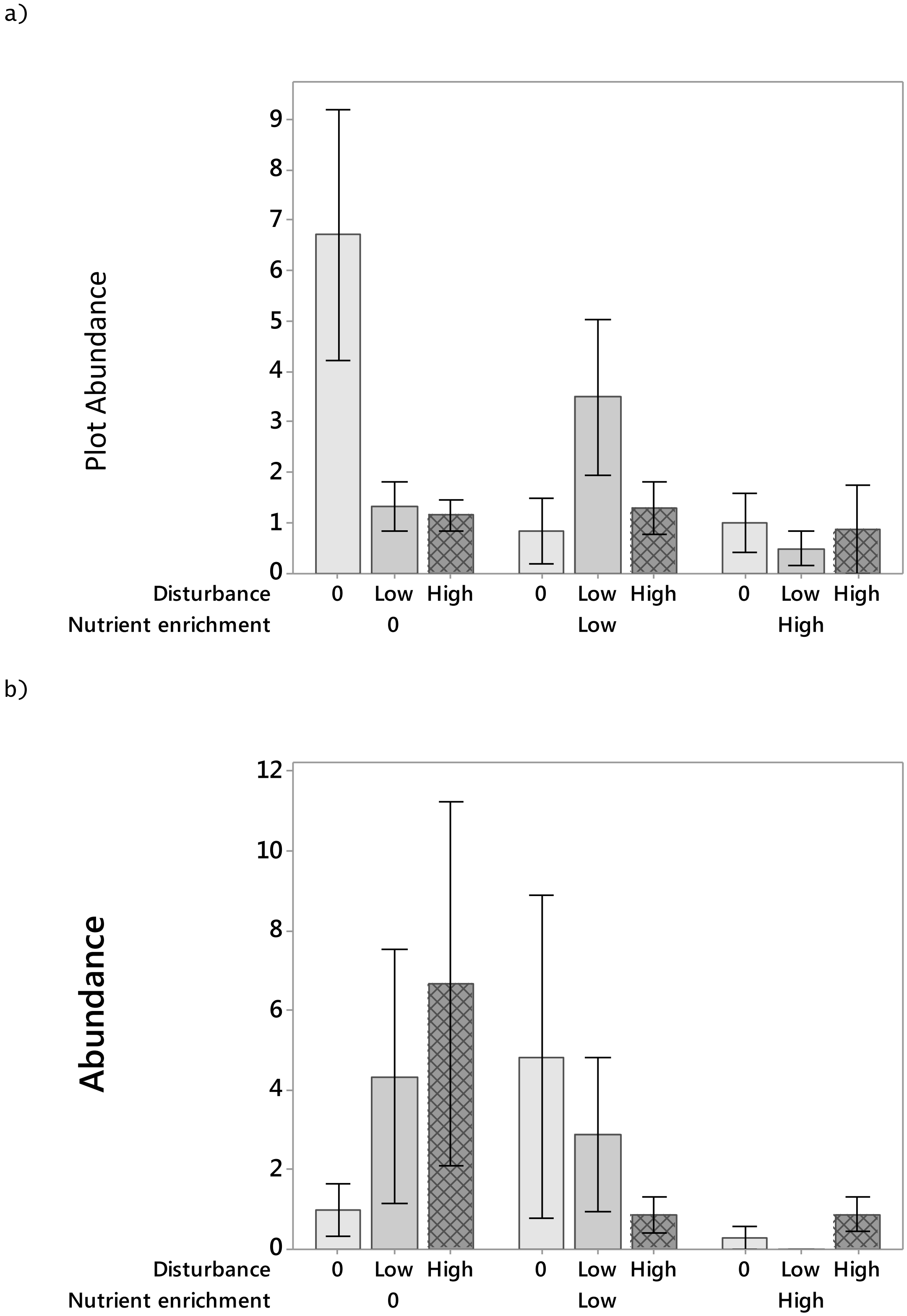


Figure 3.16: Bar graph showing change in average species abundance for *Scoloplos* sp.(a) and nematodes (b)found in Lane Cove following 4 months of nutrient enrichment and physical disturbance crossed at 3 levels (0 Low and High) Points indicate mean +/- SE. N = 7

At Lane Cove there was no correlation between any single environmental variable and macrofauna community composition, however at Botany Bay community composition showed a weak positive correlation with sediment organic content (Rho = 0.128, p = 0.001; Table 3.7). When environmental variables NDVI, sediment chlorophyll and organic content were grouped into a multivariate matrix, at both sites there was a weak positive correlation with macrofauna community structure and environmental variables (Table 3.7).

Table 3.7: multivariate Spearman's rank correlations (Rho) between macrofauna community composition and environmental variables at Botany Bay and Lane Cove. Data taken from month 4, all n>60

	Lane Cove		**Botany Bay**	
	Rho	p	Rho	p
NDVI	0.079	0.094	0.071	0.149
Sediment chlorophyll	0.093	0.102	0.05	0.2
Organics	0.049	0.256	**0.128**	**0.001**
All variables	**0.163**	**0.026**	**0.123**	**0.051**

Key: **Bold** = significant effect at p < 0.05

3.5 Discussion

This study has demonstrated that nutrient enrichment and physical disturbance can have significant impacts on the macrofauna and MPB in intertidal sediments. Previous research has emphasised the singular impacts of nutrient addition and disturbance on benthic systems, however few have examined these in a multi stressor scenario under field conditions. It is generally assumed that results from stressors could be scaled up for field conditions in multiple locations but the insight gained from this study is that results are highly context dependant. While only two sites in close proximity were examined in Australia, each showed a unique response to stressors. There is a growing trend in the literature emphasising the context dependency of results, even across geographically proximate and/or environmentally similar locations (e.g. Bishop & Kelaher, 2013; Gladstone-Gallagher et al., 2014; Whomersley et al., 2010).

Examining the effects of multiple stressors is very important given that in the real world stressors very rarely occur alone. Previous studies have examined how nutrient enrichment interacts with predation stress (Morris & Keough, 2003a), light (Stutes et al., 2006), shelter (Forehead et al., 2012), bioturbation (O'Brien et al., 2009), organic enrichment (Fitch & Crowe, 2011) and temperature (Alsterberg et al., 2012; Fitch & Crowe, 2011). However, the interaction of nutrient enrichment and physical disturbance has received little attention and previously conducted under laboratory conditions (e.g. Austen & Widdicombe, 2006). Despite clear hypotheses about how physical disturbance may influence the effects of nutrient enrichment (see Forehead et al., 2012), this study failed to display a clear or simple interaction between nutrient enrichment and physical disturbance, consistent with the failure of other *in situ* studies to demonstrate spatially consistent interactions between other stressors under field conditions (Alsterberg et al., 2014; Crain et al., 2008; O'Connor & Donohue, 2013). In the present study, interactions were not found within the MPB or macrofaunal communities, however, species specific responses were observed. Additionally, species which were ubiquitous across both sites showed site specific reactions to stress, this being most profound in *Prionospio sp.* where an increase in both physical disturbance and nutrient enrichment caused a decrease in abundances at Botany Bay but there were no significant effects at Lane Cove, thought there was a marked decrease in abundances due to stressors compared to the control.

Interactions under field conditions are difficult to identify. Organisms switching behaviour has been identified as a mechanism for which negates the effects of multiple stressors (Bulling et al., 2010; Fitch & Crowe, 2011). Unlike many small scale mesocosm experiments, natural habitats are heterogeneous in terms of environmental variables or community composition (Dyson et al., 2007). For example, MPB has been shown to be extremely patchy (Murphy et al., 2008; Spilmont et al., 2011; Tolhurst & Chapman, 2005). Under natural conditions, habitat heterogeneity can cause an important influence on interpretation of results, potentially buffering against treatment effects (Godbold et al., 2011) or the natural spatial heterogeneity is negating patterns due to treatment effects (Bulling et al., 2008). In this regard field experiments offer limited opportunity to discover the effects of stressors on populations that could be identified in a controlled environment (Crane et al., 2007). However, as they are representative of natural conditions, field experiments offer an insight into how a system would react given a local environmental context and heterogeneity of a habitat, and are therefore, arguably, more beneficial.

3.5.1 MPB

The majority of studies demonstrate a bottom-up response of the MPB to nutrient additions (Huang et al., 2013; O'Brien et al., 2010; Pascal et al., 2013). However, in the present study, this trend was only found at Botany Bay. This was recorded in both the sediment cores and the remotely sensed NDVI. At the start of the experiment there were no treatment effects, implying these results are genuine treatment effects. This is corroborated by the changing patterns between months, with treatment effects only seen after two months. Conversely, at Lane Cove there were no main effects of nutrient enrichment on MPB. There was an interaction between the two stressors recorded in the NDVI; however this can be attributed to an artefact of pre-existing differences between treatments observed at this site. Unexpectedly at this site, sediment chlorophyll seemed to be influenced by disturbance. While neither treatment was significantly different from the controls, sediment chlorophyll content was significantly lower in the high disturbance treatment compared to the low. As not enough data was taken from the start of the experiment to get appropriate baseline readings, it is possible this is an artefact of pre-existing spatial variation across the site.

Physical disturbance on sedimentary habitats can have significant effects on biogeochemical and physical variables on a small scale (Rossi et al., 2007) therefore having bottom up effects on the MPB. For example, habitat complexity can be modified by raking the sediment, which in turn can lead to pools of water to form. Overlying water changes the dynamics in the disturbed patch, lowering availability of atmospheric CO_2 (Rossi et al., 2007). Raking also loosens sediment, making it more susceptible to the tides and erosion, stimulating scour which will damage organisms (Yeo & Risk, 1979).

While at Botany Bay, the effect of physical disturbance was to significantly decrease the chlorophyll content in the sediment, moderate disturbance causes an increase in Lane Cove. The intermediate disturbance hypothesis suggests that moderate disturbance would promote growth of a system before ultimate collapse with further increases. The results for Lane Cove support this in that the low disturbance treatment seemed to be promoting MPB biomass. Previous research has shown that moderate disturbance may support the intermediate disturbance hypothesis in benthic systems while further increases cause a crash in the system (Lee et al., 2011). Severe disturbance has been documented to have a negative impact on MPB (Rossi et al., 2007). Similarly to the effect of multiple stressors, the intensity of a disturbance does not always offer predictable, non-linear responses (Vye et al., 2015; Whomersley et al., 2010). Additionally, given that organic content is made up of MPB as well as other sources of organic material (Dubois et al., 2012; Hardison et al., 2013) it was expected that there be a relationship between organic content and MPB. While there was limited correlation between the two variables at Botany Bay, Lane Cove showed no relationship between the two while still showing treatment effects on organic content. At this site however organic content seemed to increase due to disturbance whereas chlorophyll decreased. As previously mentioned organic matter is not solely made up of the MPB but is also derived from decayed materials and detrital material not necessarily containing chlorophyll. Through raking the sediment, some of this deeper material could become caught up in the sediment and not washed away, therefore being incorporated in the results.

3.5.2 Macrofauna

At both sites nutrient enrichment had an influence on the macrofaunal community structure but effects of physical disturbance were minimal. Previous studies have shown how physical disturbance can have negative effects on benthic macrofauna (Rossi et al., 2007). Softer bodied organisms are sensitive to disturbance and are likely to become damaged (Brown & Herbert Wilson, 1997). The lack of a main effect of physical disturbance can be attributed to the local environmental context buffering treatment effects. The openness of the plots could also have led to outside recruitment and movement of organisms, preventing effects accumulating. Previous research has highlighted the difference between *in situ* and laboratory mesocosm experiments (Cowie et al., 2000). Through a comparison of field and mesocosms, Cowie et al (2000) observed a negative effect on the macrofauna in both experimental mesocosms and *in situ* treatments; however, the strength of the decline of species was greater in mesocosm experiments. This again indicates the importance of scale and environmental variability when using mesocosm experiments to extrapolate relationships to field conditions.

Nutrient enrichment impacted sites in different ways. In the literature, nutrient pollution has been linked to both decreased (Botter-Carvalho et al., 2014; Fitch & Crowe, 2010) and increased (Morris & Keough, 2003a, 2003b) macrofaunal abundances and species richness. At both Botany Bay and Lane Cove, nutrients altered community composition but did not affect species richness or diversity indices. At Lane Cove, total abundance was significantly lowered due to increased nutrients. At Lane Cove, there were pre-existing differences in species richness among plots assigned to different nutrient treatments, even prior to experimental intervention. This difference had disappeared after 4 months, with no other treatment effects, indicating that nutrient additions had a potential positive influence on species richness.

In previous research, the effect of stressors has been shown to impact community structure of dominant, common and rare species in similar ways (e.g. Austen & Widdicombe, 2006). In Botany Bay, assessed through presence absence transformations, rarer species were identified to have no effect in differentiating between treatments, therefore square root transformations were deemed sufficient to analyse. Comparatively at Lane Cove, it was noted that the effect of rare species caused a significant main effect due to nutrient enrichment. Rarer species at this site were an important aspect of the community. While in many communities the rarer species may help to maintain the functions derived from the system (Tilman et al., 2014), they can have minimal effect in comparison to more dominant species (Davies et al., 2011; Solan et al., 2004). However the presence of rare species allows a system to be more stable in the face of fluctuating environmental variables. Stressors causing a change in the composition of rare species have the potential to drastically alter the system dynamics of that community, hindering its ability to recover or maintain important functions (Mykrä et al., 2011; Tilman et al., 2014). This highlights the importance of context when interpreting the impact of multiple stressors which have the potential to alter community stability in certain circumstances, thereby leading to a degradation of a systems overall functionality.

3.5.3 Bottom-up / top-down effects of primary production

There were stronger treatment effects observed in the MPB than the macrofauna and no correlations were found with regards to the macrofaunal community composition and the MPB proxy measurements (sediment chlorophyll and NDVI) indicating that there was a limited bottom up effect of the MPB on the macrofaunal community. Nutrient enrichment was found to have an effect on the community composition at both sites. There were also species specific effects. This indicates a mix of top down and bottom up effects on the macrofaunal community. At Botany Bay there was a weak correlation between the macrofauna and the organic content within the sediment. As there were no treatment effects on the organic content at this site, it indicates there is potentially some form of top down effects from the macrofauna.

3.5.4 Physical disturbance subdues effects of nutrient enrichment on MPB and Macrofauna

In this study minimal interactions were identified and it was difficult to interpret given the high variability observed between individual plots. Additionally the effects of stressors such as nutrient enrichment can easily complicate interactions as bottom up effects can be replaced by top down effects (Pascal et al., 2013).

3.5.5 Context dependency of results

Comparative studies in Australian estuarine systems have shown that nutrient pollution is only a weak driver of change, with community composition more strongly related to other environmental variables such as grain size (Nicastro & Bishop, 2013), again highlighting the importance of environmental context. Additionally, background nutrient levels play an important role in determining how a system will react to nutrient additions (Pearson & Rosenberg, 1978). The effects of raking have previously found to be highly context dependent (Whomersley et al., 2010).

Environmental context is very important in interpreting results. It would be expected that communities that undergo frequent disturbance are inhabited by species that are capable of surviving such conditions. Further stress that typifies these environments would be unlikely to provoke change due to the resilience of organisms adapted to the prevailing conditions. Conversely, further stress of this nature could be enough to push a system over the edge, where a threshold is reached, resulting in system collapse or change to another state. Whomersley et al. (2010) found that given high levels of carbon at certain sites, further organic enrichment caused a collapse indicating a threshold. Additionally, the speed of recovery can be mediated by habitat characteristics such as grain size, organic content and water content (Dernie et al., 2003). Short-term studies can be useful to interpret immediate impacts however the importance of outside recruitment should not be overlooked in longer term studies, potentially negating the effect of a single disturbance event (Lee et al., 2011).

Given that results are highly context dependant it is not possible to make broad scale conclusions about all benthic intertidal estuarine systems, and, given that only two sites were studied, it is difficult to ascertain what the causes of the differing responses are. Further, it is unlikely that major generalities can be inferred given the context dependency of systems demonstrated here and in other studies. Not only are there confounding variables in the physical and environmental variables but also with the prevailing community compositions and prevalent levels of stress. For example, systems that undergo greater stress may have enhanced community resistance and therefore the ability to withstand further disturbance (Loreau, 2000).

Although care was taken to choose sites of a similar nature for comparison, it was not possible to perfectly match sites and all variables. This is analogous of natural systems as estuaries are influenced by local surroundings and dependent upon prevailing conditions. No two sites would be exactly the same and so this study puts that into perspective as results were highly context specific and there was no distinct pattern that governs all sites universally.

3.6 Conclusions

The effects of nutrient enrichment and physical disturbance both influence MPB, macrofaunal community structure and specific species; however limited non-additive interactions between the two stressors were identified. The interactive effects of stressors in experimental systems can be difficult to assess given high spatial variability of MPB and macrofauna in sites. While laboratory studies can clearly define the effects of multiple stressors and indicate how they influence communities, these results cannot always be translated into natural systems. *In situ* studies such as those conducted in this experiment highlight the importance of environmental context which could mediate the effects of multiple stressors. Laboratory studies do not allow recruitment from outside impacted sites, and the heterogeneity of a habitat can mediate stressor impacts either buffering against treatment effects or through high spatial variability negating patterns. Further studies are required to assess multiple stressors in natural field conditions including applying stressors in multiple locations both within a local system and further afield. Environmental context is likely to play an important role in mediating stressors; understanding how the effects of multiple stressors affect similar environments in different global regimes remains an important problem to be addressed in future ecological studies.

Table 3.8: Verification of hypotheses set at the beginning of the chapter

Hypotheses	Accept ✓ or reject X
H1 – Nutrient enrichment positively influences primary production	✓
H2 – Bottom up effects from increased primary production will lead to increased biodiversity	**X**
H3 – Physical disturbance will negatively affect macrofaunal biodiversity	**X**
H4 – Physical disturbance will subdue the effect of nutrient enrichment on both primary productivity and macrofauna	**X**

4 The interacting effects of nutrient enrichment and physical disturbance on the structure and function of the benthic communities of UK estuarine mudflats.

4.1 Introduction

Estuarine mudflats are highly productive coastal systems important in terms of the functions and services, such as primary and secondary production and sediment biostabilisation, that they provide to humanity (McLusky & Elliott, 2011). They are areas of high primary productivity derived primarily from the microphytobenthos (MPB) (MacIntyre et al., 1996; Underwood & Kromkamp, 1999). MPB can account for up to 50% of an estuaries total productivity (Underwood & Kromkamp, 1999) and this combined with their relative palatability to consumers (Kang et al., 2003; Oakes et al., 2012) allows estuarine mudflats to support an extremely high biomass of macro-invertebrates in comparison to other coastal ecosystems (Levin et al., 2001). Disturbance of these systems has the potential to alter productivity, which in turn will alter the composition of higher trophic levels (Beardall et al., 2009; Brown et al., 2010). The functionality of the environment will change through a loss of species or change in community structure (Lefcheck et al., 2015; Yamanaka et al., 2013). Additionally, top down processes, such as grazing, also have the potential regulate MPB productivity, and may be modified by disturbances (Hicks et al., 2011).

Altering the community structure of benthic sedimentary ecosystems can dramatically change the erosion thresholds of these environments. MPB produce mucilage in the form of extra-cellular polymeric substances (EPS) that help to stabilise the sediment, thereby reducing coastal erosion (Blanchard et al., 2000; Cahoon, 1999; Lubarsky et al., 2010; Tolhurst et al., 2002). Through movement and grazing on MPB, macrofaunal species typically destabilise sediments (Kristensen et al., 2013; Pilditch et al., 2008). Exceptions to the generally positive effect of MPB and the negative effect of macrofauna on sediment stability (Underwood & Paterson, 2003) may, however, occur. Oxygen bubbles can form at certain growth stages of an MPB biofilm, these can “blister” the surface promoting erosion during tidal flow (de Jonge & van den Bergs, 1987; Tolhurst et al., 2008a). Certain species of macrofauna such as filter feeders can remove fine erodible particles or entrain sediments in consolidated mucus-rich faecal pellets, thereby stabilising sediments (Andersen et al., 2010; Nowell et al., 1981). Additionally, tube forming species act to bind sediments aiding sediment stabilisation (Fager, 1964). Biotic interactions can therefore alter the stability and erosion potential of a benthic system in positive and negative ways (Austen et al., 1999; Harris et al., 2015).

Ecosystems are simultaneously exposed to multiple stressors of natural and anthropogenic origin, which in combination can alter benthic community structure and function (Crain et al., 2008; Halpern et al., 2008). The influence of these stressors is increasing due to coastal development and climate change (Bijma et al., 2013; Fitch & Crowe, 2011; Harley et al., 2006; IPCC, 2014). While multiple stressor studies are becoming increasingly common (Darling & Côté, 2008; Przeslawski et al., 2014) the importance of field studies that have a realistic environmental context is still often undervalued (Bishop & Kelaher, 2013; Parker et al., 1999; Whomersley et al., 2010). Additionally, predicting the effects of cumulative stressors can prove difficult as they often interact, generating non-additive effects (Bulling et al., 2010; Crain et al., 2008; Sundback et al., 2010) on the performance of multiple ecosystem functions (Alsterberg et al., 2014; Bulling et al., 2010; Byrnes et al., 2014; Crain et al., 2008; Sundback et al., 2010). While the effects of multiple stressors on communities and particular trophic levels have received greater attention in recent years (Alsterberg et al., 2014), assessing multiple stressor effects on various ecosystem functions is a relatively understudied topic.

As previously discussed in Chapter 3, two of the most prevalent stressors in estuarine mudflat systems originate from anthropogenically-induced nutrient enrichment and physical disturbance and both can result in significant changes to macrofaunal community structure and MPB (Austen & Widdicombe, 2006; Cowie et al., 2000; Tiegs et al., 2009; Verhoeven et al., 2012; Whomersley et al., 2010). In many estuarine systems, nutrient enrichment is a consequence of farmland fertilisation of estuarine catchments (Bricker et al., 2008; Nixon, 1995; Vitousek et al., 1997a) with up to 50% of the applied fertiliser reaching estuarine environments (McLusky & Elliott, 2011). Additional sources of nutrient enrichment can be derived from runoff, waste products and sewage being flushed into estuarine systems (Nixon, 1995; Souza et al., 2013). The intensity and frequency of storm events are increasing as a consequence of climate change (Bijma et al., 2013). These events cause physical disturbance and damage environments (Allen & Duffy, 1998; Austen & Widdicombe, 2006; Cowie et al., 2000). Further, boat wakes and bait digging cause small scale significant detrimental impacts to communities (Bishop, 2005; Contessa & Bird, 2004).

Each disturbance type has associated impacts. For example, where nutrients are a limiting resource, their addition to estuarine systems may enhance primary production, and consequently, secondary production (Morris & Keough, 2003b). Physical disturbance on the other hand may act on dominant organisms, inhibiting their ability to out-compete other species leading to a shift in community composition and an increase in biodiversity (Cowie et al., 2000) showing an “intermediate disturbance hypothesis” type response (Connell, 1978). While the effect of each stressor on benthic community structure has been studied singularly (Cowie et al., 2000; Dernie et al., 2003; Hall & Harding, 1997; Morris & Keough, 2003a; O’Brien et al., 2010; Whomersley et al., 2010; Worm et al., 2000; York et al., 2012), their combined effects are understudied (but see Austen & Widdicombe, 2006; Tiegs et al., 2009; Widdicombe & Austen, 2001), and field studies are rare. Additionally, the singular and combined effects of these stressors on various ecosystem functions such as sediment stability have received little attention.

The complex interacting effects of MPB and macrofauna on sediment stability necessitate that any evaluation of ecosystem service in response to multiple stressors can only meaningfully be done in a natural field setting. The imperative of addressing this question in a natural setting is compounded by the important role that physical factors play in determining sediment erosion potential, in some instances outweighing any relationship between sediment stability and the biota (Defew et al., 2002; Paterson et al., 2000; Tolhurst et al., 2003). This study aims to assess the impacts of nutrient enrichment and physical disturbance on MPB biomass and macrofaunal assemblages and how changes to the complex interactions between the biota and the environment produces changes in ecosystem functions, such as primary production potential, biodiversity and sediment stability.

4.2 Hypotheses

The following hypotheses expand upon work completed in Chapter 3:

H1 – Nutrient enrichment will positively influence primary production in terms of MPB biomass and alter macrofaunal community composition.

H2 – Physical disturbance will negatively affect both the abundance of MPB and macrofaunal biodiversity.

H3 – By changing the abundance of MPB and altering macrofaunal community structure each stressor will modify the productivity and sediment stability of the ecosystem.

H4 – Context specific effects of multiple stressors will be observed.

4.3 Methods

4.3.1 Study sites

Two sites were selected to examine the comparative effects of multiple stressors on the community structure and ecosystem function of UK estuarine benthic systems. As outlined in chapter 2, these were the Eden and Tay estuaries. At the site in the Eden, the ephemeral green alga *Ulva* sp. became washed up, smothering the entire site after 2 months. This was a naturally occurring event and *Ulva* sp. could not be removed without damaging plots. The coverage was universal over the site and so was left untouched. The algae lingered for the remainder of the experiment. Sediment samples and measurements were collected by carefully removing loose algae.

4.3.2 Experimental design

Plots were manipulated in a two factor crossed design investigating the impacts of physical disturbance (through raking sediment) and nutrient enrichment as described in the chapters two and three.

4.3.3 Sampling

Sediment was collected and treated, as described in Chapter 2, for use in quantifying the organic content (Chapter 2.4.2), water content (Chapter 2.5.3), wet bulk density (Chapter 2.5.4), macrofauna (Chapter 2.6), MPB biomass through spectrophotometric and handheld remote sensing techniques (Chapter 2.7) and two measurements of sediment stability: through use of a cohesive strength meter (CSM; Chapter 2.5.1) and shear vane (Chapter 2.5.2). Samples were taken from different areas within the plot at each time point to minimise the effect of repeated sampling. The experiment lasted 4 months with samples being taken monthly, from April to August 2013. Sediment samples, MPB and sediment stability measurements were taken monthly at four time points as well as prior to the stressor manipulations. The macrofauna were sampled prior to manipulations and after two and four months of stress.

4.3.4 Statistics

PRIMER software was used to calculate the diversity indices, Pielou's evenness, Shannon diversity and Simpson Index, for fauna. The total abundance and species richness of fauna in each sample was also determined.

Firstly, for all variables, a two- factor analyses, with the factors nutrients and physical disturbance, were conducted to assess the pre –existing differences among plots prior to the application of disturbance treatments. Where these were not significant, variables were compared between the Eden and Tay estuaries. Variables were checked for normativity using Shapeiro-Wilk test and for homogeneity of variance using the Bartletts test. Following these pre-checks, site differences were tested using 2 samples unpaired t-tests for normal data (assuming homogeneity of variances accordingly) or their non-parametric equivalent – the Mann-Whitney U test.

Secondly, separate 3-way PERMANOVA analyses based on Euclidean distance matrices, with nutrients, physical disturbance and month as fixed factors, were conducted for each site to assess treatment effects on the variables organic content, chlorophyll content and concentration, NDVI, and the CSM and shear vane measures of sediment stability. Two way PERMANOVA analyses, with the factors nutrients and physical disturbance, were conducted on Bray Curtis dissimilarity matrices calculated from the multivariate matrix of macroinvertebrate community structure. Due to the large temporal variation in macrofaunal communities among months, separate two-way PERMANOVAs were conducted for each month and site. Prior to each analysis, heterogeneity of variance (PERMDISP – multivariate) was assessed. Macrofauna composition data analysed using PERMANOVA was square root transformed prior to analysis to down-weight the effect of species abundance. Following analyses, pairwise post hoc tests were conducted to examine sources of significant treatment effects.

Thirdly, following multivariate analysis of macrofaunal data, SIMPER analysis was used to identify species causing dissimilarity in community structure between treatments. Species were chosen based on those having a dissimilarity/standard deviation ratio > 1. Individual species and measures of diversity (species richness, total abundance, Pielou’s evenness, Shannon diversity and Simpson Index) were analysed using univariate two-way PERMANOVAs conducted for each month and site using Euclidean distance matrices.

4.4 Results

4.4.1 Site background

At both sites and for all variables, there were no pre-existing differences between experimental plots prior to the stressor manipulations ($p > 0.05$ for each variable analysed).

Instead, the Eden and Tay were different in terms of their environmental variables. The Eden had significantly higher sediment chlorophyll concentration than the Tay (Figure 4.1a; $t = 9.74$, $df = 138$, $p < 0.001$), whereas the Tay had a significantly greater NDVI than the Eden (Figure 4.1b; $t = 4.56$, $df = 126$, $p < 0.001$). The sediment within the Eden contained a greater percentage of organic material than the Tay (Figure 4.2a; $W = 7183$, $p < 0.001$) as well as a higher average bulk density of the sediment (Figure 4.2b; $W = 7274$, $p < 0.001$).

4.4.2 Environmental variables

4.4.2.1 Organic Content

Over the four months following application of disturbances, and across all of the monthly sampling times, interacting effects of nutrient enrichment and physical disturbance on sediment organic content were evident in the Eden (Table 4.1; Figure 4.3; sig. N x D interaction: $\text{Pseudo-F}_{(4,216)} = 3.16$, p(perm) = 0.017), but there was no effect of either stressor in the Tay (Table 4.1). In the Eden, within plots subjected to zero nutrient enrichment, the organic content of sediments was lower in plots subjected to high physical disturbance than to zero ($t = 2.41$, $p = 0.008$) or low disturbance ($t = 2.18$, $p = 0.019$), and the latter two did not differ significantly. In plots receiving low nutrient disturbance, there was no significant difference in organic content between the high physical disturbance treatment and the controls, and the high and low physical disturbance treatments, but the plots receiving low physical disturbance had a significantly lower organic content than controls ($t = 2.46$, $p = 0.010$). Plots subjected to high nutrient enrichment did not vary in organic content according to the level of physical disturbance.

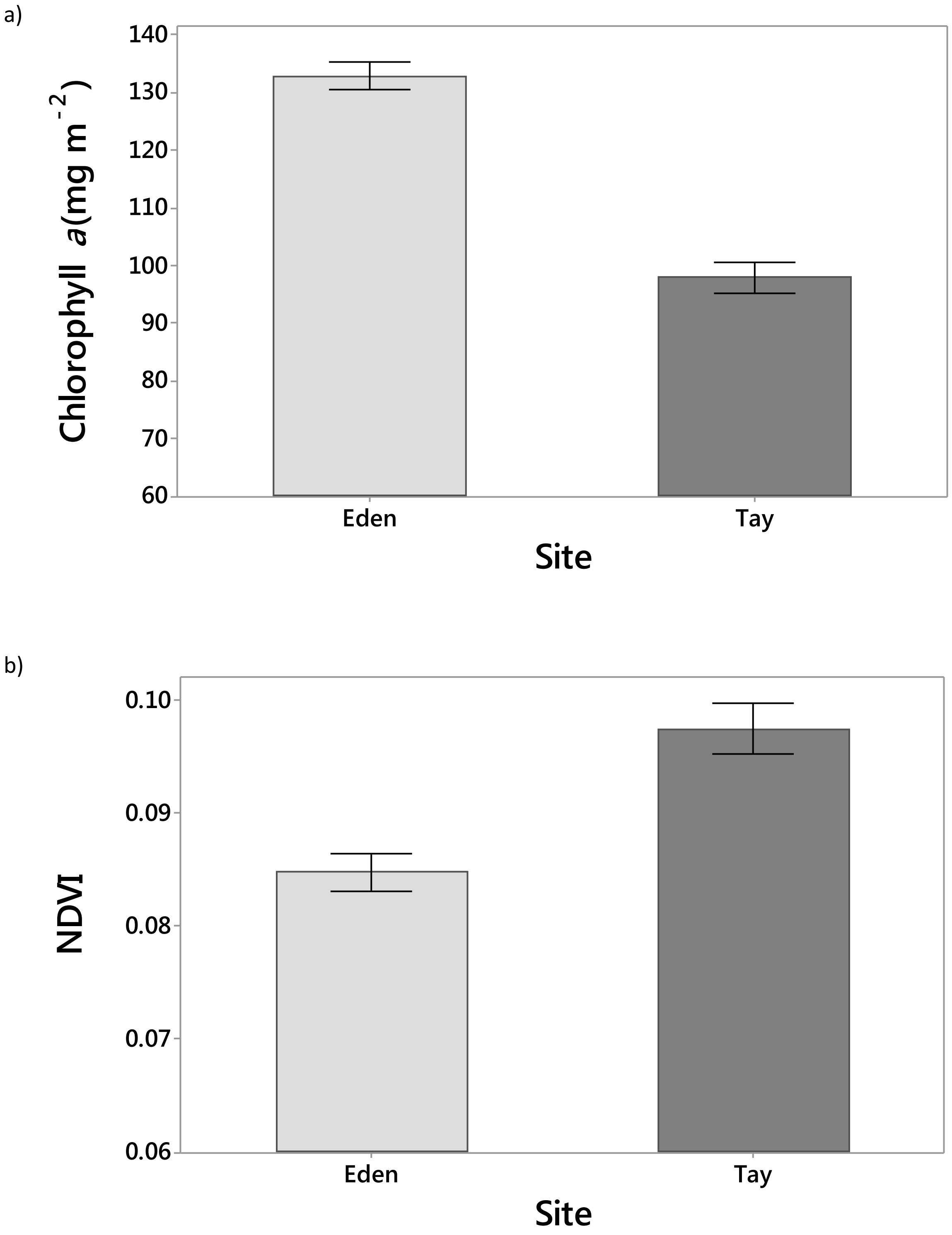
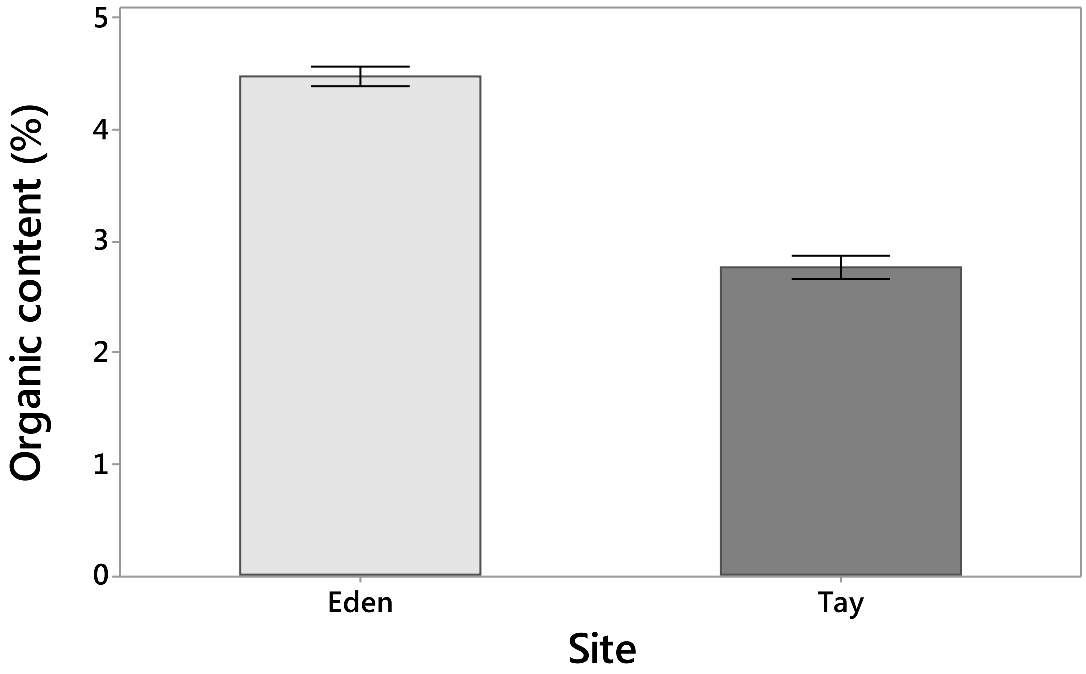


Figure 4.1 Site differences in terms of a) Chlorophyll content (mg m^{-2}) and b) Normalised Difference Vegetation Index (NDVI) within the sediment at start of experiment at sites in the Eden and Tay estuaries. N = 70. Points indicate mean +/- SE.

a)



b)

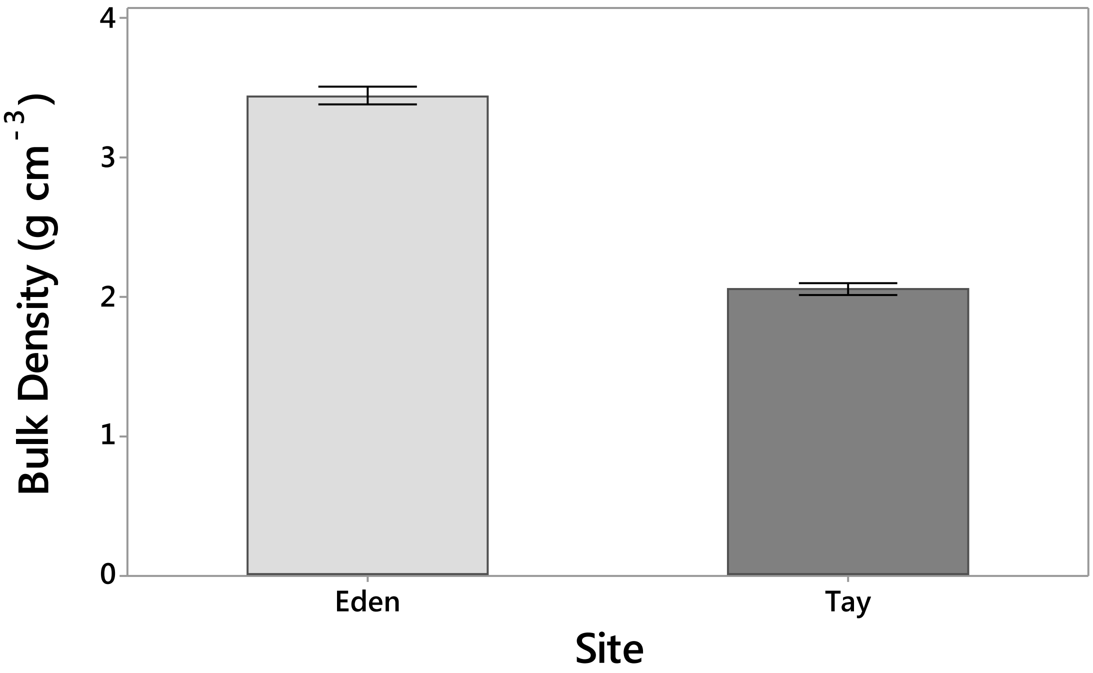


Figure 4.2: Site differences in terms of a) sediment organic content (%) and b) bulk density of sediment (g cm^{-3}) within the sediment at start of experiment at sites in the Eden and Tay estuaries. N = 70. Points indicate mean +/- SE.

Within plots subjected to zero physical disturbance there was a significant difference between the low and high nutrient enriched treatments where the higher enrichment level corresponded with lower organic content (t = 2.08, p =0.027). There were, however, no differences between either enrichment treatment and the control. Where plots were subjected to low physical disturbance, those also subjected to low nutrient enrichment had a lower organic content than those subjected to either zero (t = 1.88, p = 0.053) or high enrichment (t = 2.41, p = 0.013). There were no differences between the highly enriched and the control treatment. Within the highly physically disturbed treatments, plots that were subjected to both low (t = 2.12, p =0.017) and high (t = 2.65, p = 0.014) nutrient enrichment contained significantly greater organic material than those subjected to disturbance alone. There were no differences between either low or high nutrient enriched treatment.

Table 4.1: Three-way PERMANOVA analysis examining the interacting effects of Nutrients (N), Disturbance (D) and Month (M) on sediment organic content in the Eden and Tay estuaries. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, Low and High), month contained 4 levels. n=7 for all treatments levels

	Eden			**Tay**	
	DF	Pseudo-F	P (perm)	Pseudo-F	P (perm)
Nutrient Enrichment (N)	2	0.31	0.752	1.14	0.379
Disturbance (D)	2	1.86	0.161	0.27	0.875
Month (M)	3	**11.66**	**0.001**	1.73	0.122
NxD	4	**3.16**	**0.017**	0.43	0.886
NxM	6	0.27	0.939	0.70	0.757
DxM	6	0.99	0.451	1.42	0.167
NxDxM	12	1.21	0.278	0.96	0.527
Residuals	216				

Key: **Bold** = significant effect at $p < 0.05$

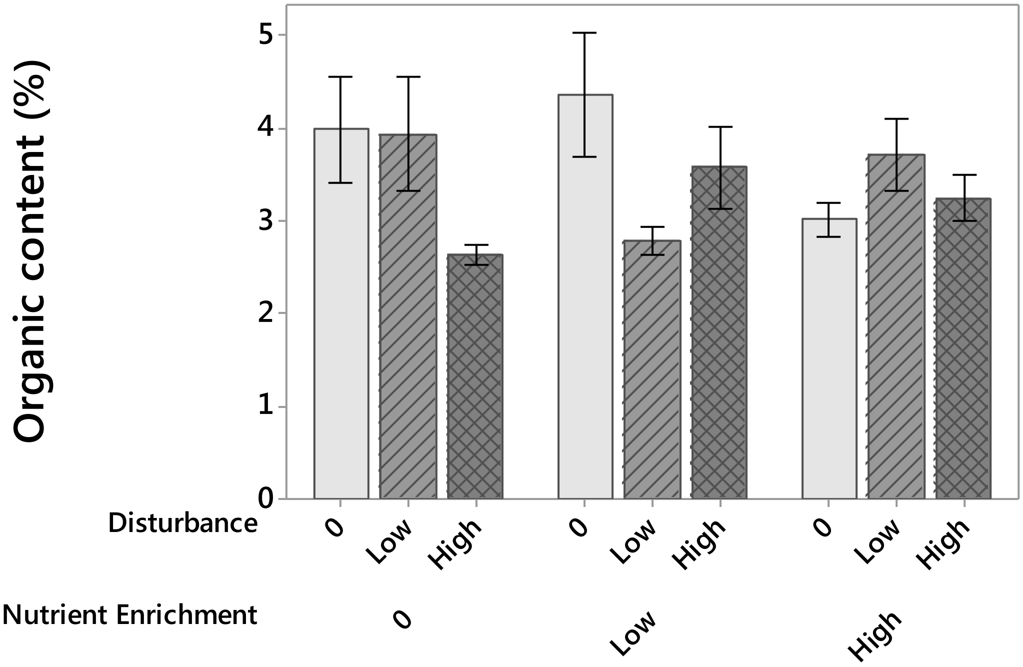


Figure 4.3: Organic content (%) within sediments in the Eden estuary. Data pooled from 4 months of data to display interaction between physical disturbance and nutrient enrichment at zero low and high levels. Points indicate mean +/- SE. N = 7

4.4.2.2 Sediment chlorophyll

The chlorophyll concentration within the sediment varied between months but only responded to stressors in the Eden where a 3-way interaction between nutrient enrichment, physical disturbance and month was observed (Table 4.2; N x D x M interaction: Pseudo-$F_{(12,216)}$ = 3.36, p = 0.001). At this site data were split and each month was analysed separately (Figure 4.4). After one month of stressor application (Figure 4.4) there were no differences between treatments. Treatment effects were observed only after 2 months of experimental manipulations where there was an interaction between the stressors (Figure 4.4; sig N x D interaction: Pseudo-$F_{(2, 54)}$ = 2.49, p = 0.036). Within the nutrient treatments only the unenriched plots responded to physical disturbance, with the low disturbance treatment containing higher chlorophyll concentrations than either the control (t = 2.84, p = 0.017) or the highly disturbed plots (t = 3.96, p = 0.003), the latter of which did not significantly differ. Within the physical disturbance treatments, only the plots subjected to high physical disturbance responded to nutrient enrichment where plots subjected to high nutrient enrichment contained greater chlorophyll than those subject to the zero enrichment (t = 2.80, p =0.021). There was no difference between the control and the low nutrient treatment, or the low and high level of enriched treatments ($p > 0.05$).

Following 3 months of disturbance there were no interactions between stressors and instead only a main effect of disturbance was found (Figure 4.4; Pseudo-$F_{(2,54)}$ = 3.45, p = 0.031). Plots subjected to high physical disturbance contained lower sediment chlorophyll than plots subjected to low disturbance (t = 2.92, p = 0.010). There were no differences between the controls and treatments subjected to either high or low physical disturbance.

By the end of the experiment, after 4 months of continuous exposure to stressors, there was a significant interaction between nutrient enrichment and physical disturbance (Figure 4.4; Pseudo-$F_{(4,54)}$ = 4.61, p(perm) = 0.003). Within each nutrient enrichment treatment, the added effect of high physical disturbance always resulted in a trend for lower chlorophyll concentrations than in low physical disturbance or control plots. Only in plots subjected to low nutrient enrichment, however, did the added influence of low (t = 3.91, p = 0.004) or high (t = 5.07, p = 0.002) physical disturbance result in a significantly lower chlorophyll concentration. Neither the low or high physical disturbance treatment differed from each other. *Post hoc* comparisons revealed that within plots subjected to zero physical disturbance, the low nutrient enriched treatment contained significantly greater chlorophyll concentrations than plots subjected to high nutrient enrichment (t = 3.08, p = 0.018) or the controls (t =3.68, p = 0.011). There were no differences among nutrient enrichment treatments in plots receiving either the low or no physically disturbance.

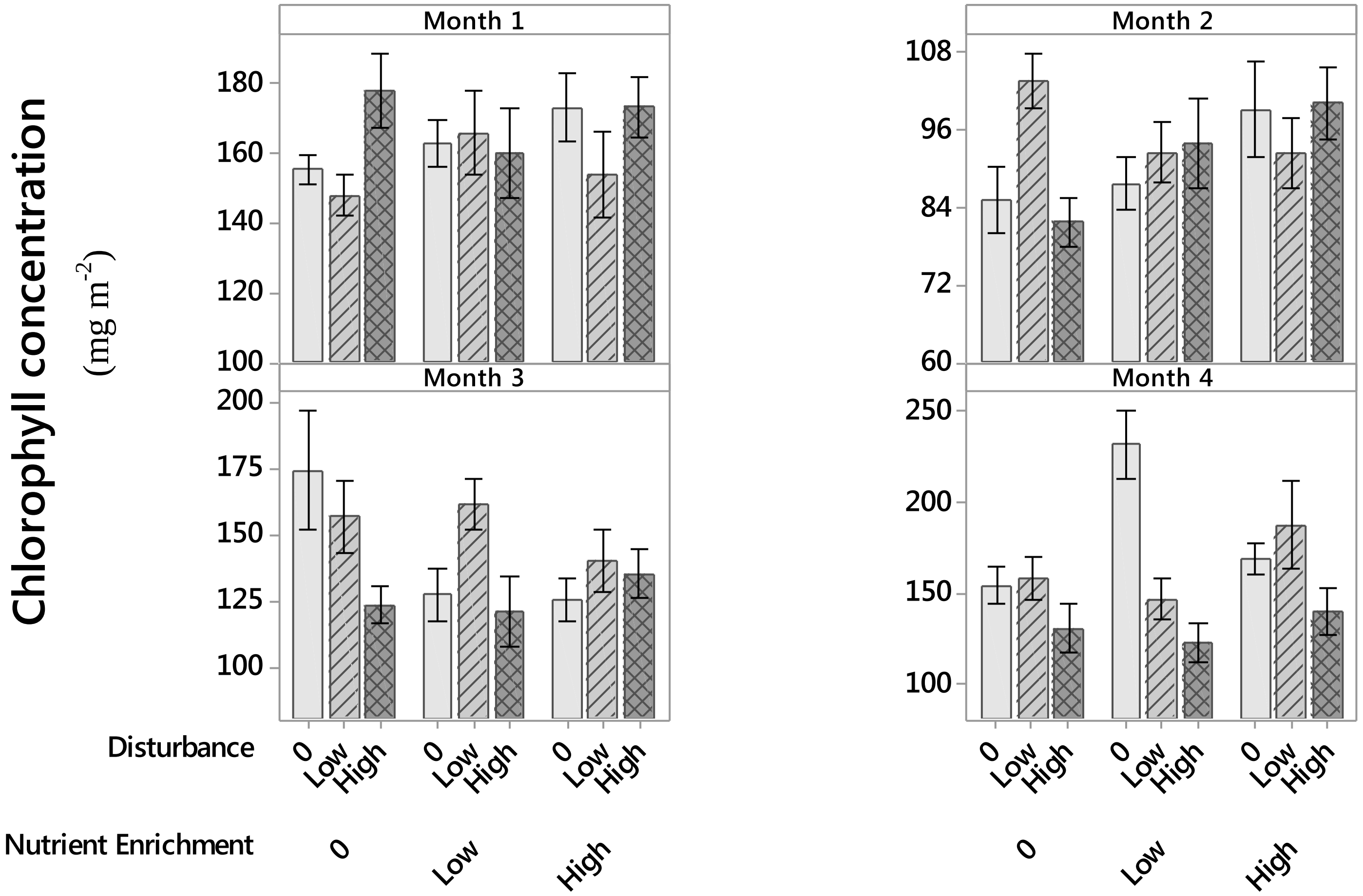


Figure 4.4: Sediment chlorophyll concentration (mg m^{-2}) in the Eden estuary each month following the addition of nutrient enrichment and physical disturbance at zero low and high levels. Points indicate mean +/- SE. N = 7

Table 4.2: PERMANOVA output of three-way interaction between Nutrients (N), Disturbance (D) and Month (M) analysing their effects on sediment chlorophyll concentration in the Eden and Tay estuaries. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, Low and High), month contained 4 levels. n=7 for all treatments levels

		Eden		**Tay**	
	DF	Pseudo-F	P (perm)	Pseudo-F	P (perm)
Nutrient Enrichment (N)	2	0.63	0.516	0.54	0.575
Disturbance (D)	2	**6.15**	**0.003**	0.05	0.942
Month (M)	3	**81.39**	**0.001**	**34.19**	**0.001**
NxD interaction	4	1.39	0.240	0.25	0.895
NxM interaction	6	1.45	0.175	0.74	0.630
DxM interaction	6	**6.40**	**0.001**	1.86	0.082
NxDxM interaction	12	**3.36**	**0.001**	0.59	0.854
Residuals	216				

Key: **Bold** = significant effect at $p < 0.05$

Results observed for the chlorophyll content (Table 4.3) within the sediment (chlorophyll per gram of sediment) differed from results observed within chlorophyll concentration (chlorophyll per m^2). The chlorophyll content at both sites varied among months. In the Eden there was a main effect of physical disturbance (Figure 4.5; Table 4.3) where the highly physically disturbed plots had a significantly lower chlorophyll content than the controls (t = 3.58, p = 0.001) or the plots receiving low physical disturbance (t = 2.68, p = 0.003). Within the Tay, there was an interaction between month and physical disturbance (Figure 4.6; Pseudo-$F_{(6.216)}$ = 2.43, p = 0.026), as well as between nutrient enrichment and disturbance (Figure 4.7; Table 4.3). One month following the application of stressors (Figure 4.6), the highly physically disturbed plots had a higher chlorophyll content than the controls (t = 2.07, p = 0.034). There were no differences between the controls and the low physical disturbance treatment or between the low and high physical disturbance treatments. Following 2 months of exposure to stressors, there were no differences between physical disturbance treatments (Figure 4.6). After 3 months, both the low (t = 2.62, p = 0.009) and high (t = 3.13, p = 0.003) physical disturbance treatments resulted in a lower chlorophyll content than found in control plots. While the high disturbance treatment had the lowest chlorophyll content it was not significantly different from the low physical disturbance treatment (t = 1.91, p =0.059). By the end of the experiment, while the lowest chlorophyll concentrations were found in the high disturbed plots there were no significant differences between treatments (Figure 4.6).

Exploring the interaction between nutrient enrichment and physical disturbance in the Tay (Figure 4.7), physical disturbance had a significant effect when applied to plots subjected to low nutrient enrichment, but not when applied to plots receiving high or no enrichment. In the low nutrient enrichment plots, chlorophyll content was less in plots receiving the low (t = 1.80, p = 0.045) or high (t = 1.71, p = 0.062) physical disturbance treatment than in the control plots, with no difference between the two disturbed treatments. Nutrient enrichment had a significant effect on chlorophyll content in plots receive no physical disturbance, but had no effect in plots receiving low or high physical disturbance. Within plots receiving no physical disturbance, chlorophyll contents were significantly greater in plots receiving the low than no nutrient enrichment (t = 2.54, p = 0.003), but no differences among other treatments were found.

Table 4.3: PERMANOVA output of three-way interaction between Nutrients (N), Disturbance (D) and Month (M) analysing their effects on sediment chlorophyll content in the Eden and Tay estuaries. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, Low and High), month contained 4 levels. n=7 for all treatments levels

		Eden		**Tay**	
	DF	Pseudo-F	P (perm)	Pseudo-F	P (perm)
Nutrient Enrichment (N)	2	0.56	0.591	1.068	0.373
Disturbance (D)	2	**4.88**	**0.011**	2.33	0.092
Month (M)	3	**8.24**	**0.001**	**22.23**	**0.001**
NxD interaction	4	1.33	0.256	**2.54**	**0.044**
NxM interaction	6	1.53	0.168	0.96	0.449
DxM interaction	6	1.82	0.084	**2.43**	**0.026**
NxDxM interaction	12	0.94	0.499	0.68	0.794
res	216				

Key: **Bold** = significant effect at $p < 0.05$

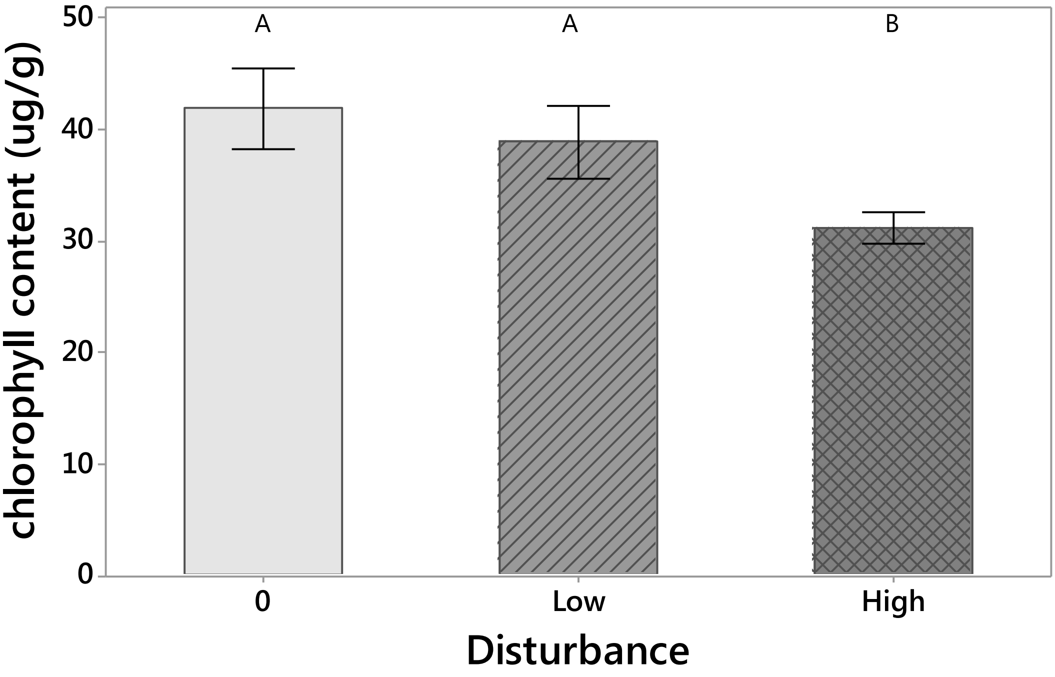


Figure 4.5: Sediment chlorophyll content (μg g^{-1}) of experimental plots in the Eden estuary receiving zero, low or high levels of physical disturbance for 4 months. Data pooled across months and levels of nutrient enrichment as these factors did not have any significant effects. Points indicate mean +/- SE. N = 7 for all treatment levels

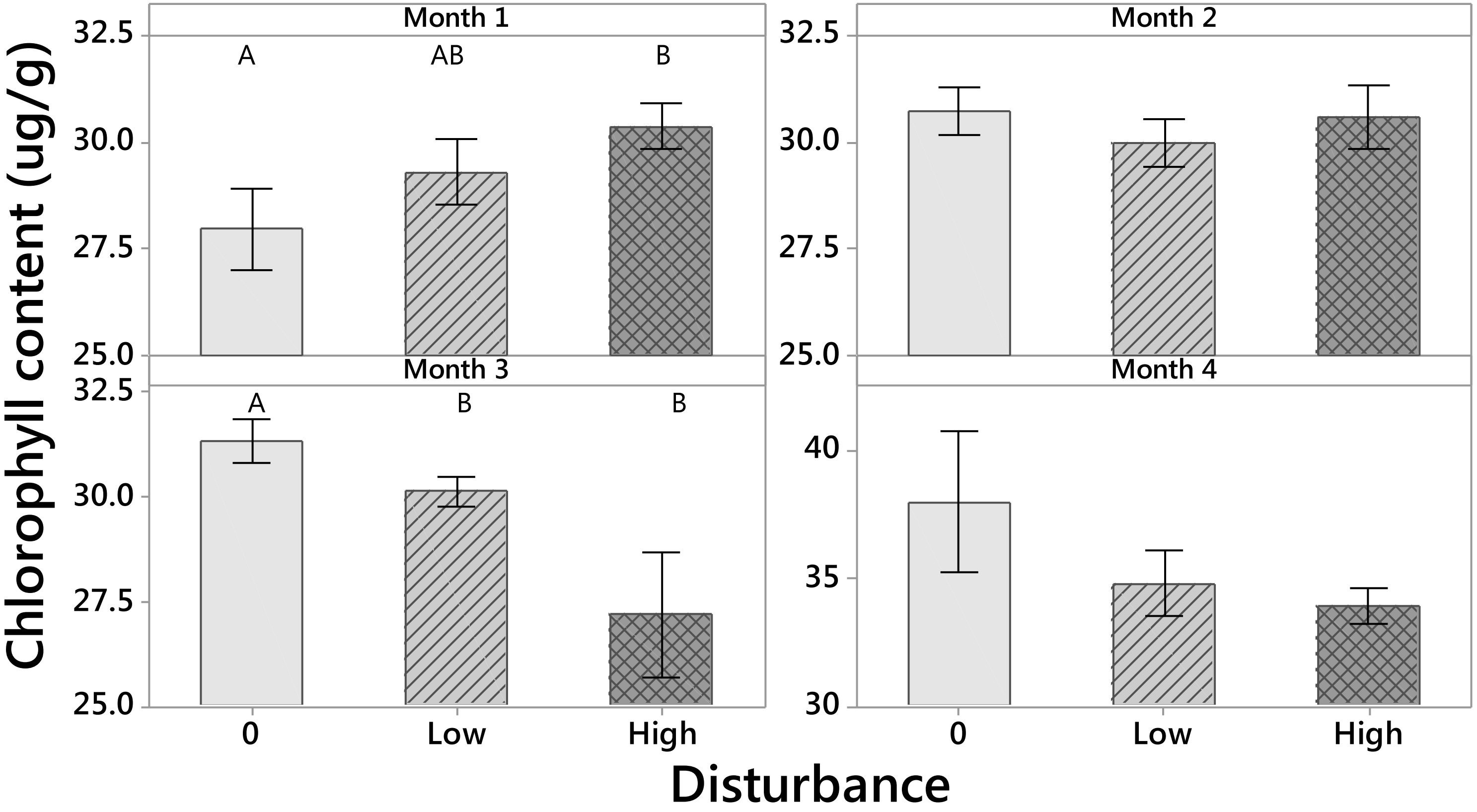


Figure 4.6: Sediment chlorophyll content (µg g^{-1}) of experimental plots in the Tay estuary each month following the additions of nutrient enrichment and physical disturbance stressors receiving zero, low or high levels. Data pooled across nutrient levels this factor did not have any significant effects. Points indicate mean +/- SE. N = 7 for all treatment levels

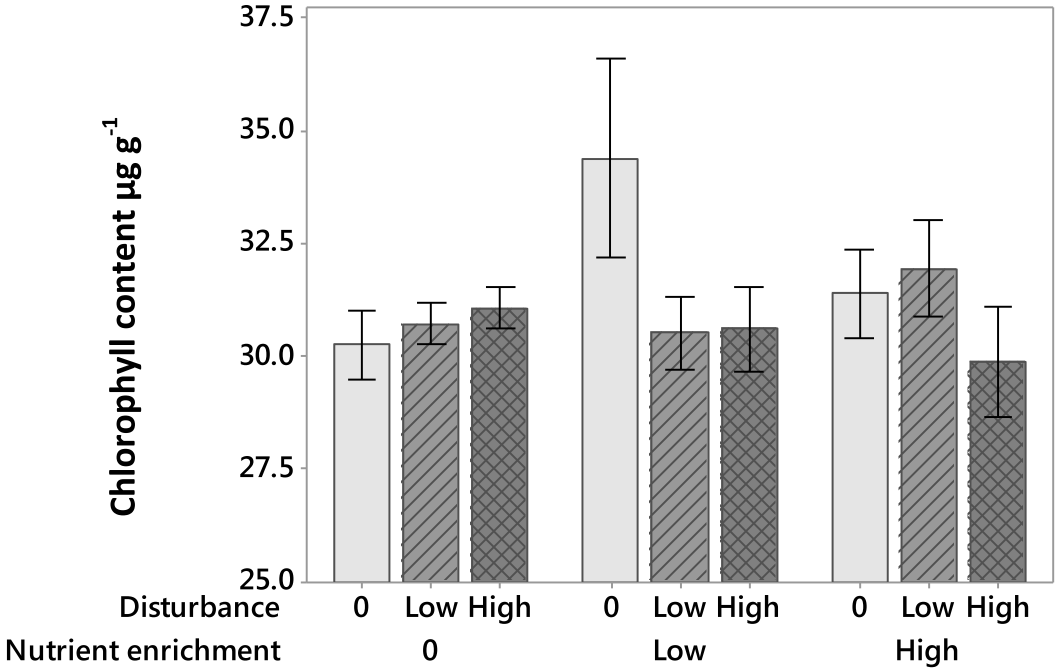


Figure 4.7: Sediment chlorophyll content (µg g^{-1}) of experimental plots in the Tay estuary receiving zero, low or high levels of physical disturbance for 4 months. Data pooled across months this factor did not have any significant effects. Points indicate mean +/- SE. N = 7 for all treatment levels

4.4.2.3 NDVI

Conversely to the results identified in sediment chlorophyll analyses, there were no effects of stressors on the NDVI in the Eden but there were 3 sets of significant two way interactions identified in the Tay (Table 4.4): between nutrients and disturbance (Figure 4.8; Pseudo-$F_{(4,216)}$ = 2.89, p = 0.024); nutrients and month (Figure 4.9; Pseudo-$F_{(6,216)}$ = 2.30, p = 0.049); and disturbance and month (Figure 4.10; Pseudo-$F_{(6,216)}$ = 2.25, p = 0.050).

When the interaction between nutrient enrichment and physical disturbance was examined, there was no significant effect of physical disturbance in plots subjected to zero nutrient enrichment ($p > 0.05$), although there was a weak and non-significant trend for plots subjected to high physical disturbance to have a lower NDVI than the other two treatments. Of the plots subjected to low nutrient enrichment, the high and low physically disturbed plots significantly differed from each other (HD > LD; t = 2.21, p = 0.031) but not from the controls. Within the plots subjected to high nutrient enrichment, both levels of physical disturbance displayed a trend for higher NDVI than the controls, though these differences were not statistically significant ($p > 0.05$). Within the physically disturbed treatments, plots that were subjected to zero physical disturbance displayed an effect of nutrient enrichment with the plots receiving low nutrient enrichment having a significantly higher NDVI compared to the controls (t = 2.07, p = 0.043), but NDVI not differing between other treatments. Among plots subjected to low disturbance, plots receiving the high nutrient enrichment contained a significantly greater NDVI than the controls (t = 3.15, p =0.002), but other differences among treatments were not significant. Finally in the plots receiving high physical disturbance, NDVI was greater in plots receiving low (t 4.23, p = 0.001) or high (t = 5.63, p = 0.002) nutrient enrichment than in controls, with the two nutrient enrichment treatments not differing.

At each of the 4 months of sampling, the control treatment had a lower NDVI than either of the nutrient enrichment treatments, although this pattern was not always statistically significant (Figure 4.9). One month following the addition of nutrients (Figure 4.9), plots subjected to low nutrients had a significantly greater NDVI than the controls (t = 1.99, p = 0.051), but other differences among nutrient enrichment treatments were not apparent. Two months following addition (Figure 4.9), both the low (t = 2.56, p = 0.017) and high (t = 2.45, p = 0.022) enrichment treatments had a significantly greater NDVI than the controls, but did not significantly differ from one other. The third month following nutrient additions there were no differences between treatments (Figure 4.9). By the end of the experiment (Figure 4.9) both the low (t = 3.35, p = 0.002) and high (t = 5.41, p = 0.001) enrichment treatments had a greater NDVI than in the controls and did not significantly differ from one another. At this final sampling time, the highest NDVI reading of the experiment was recorded, with greater relative differences between the manipulated treatments and the controls than in other months.

The relationship between the level of physical disturbance and NDVI varied among months (Figure 4.10). One month following commencement of disturbance, there were no differences between levels of physical disturbance (Figure 4.10). After two months (Figure 4.10), there was a trend for increasing NDVI with increasing physical disturbance, but only the high physical disturbance and the control treatments were significantly different (t = 2.56, p = 0.018). This relationship been NDVI and physical disturbance had reversed by the third month, at which a trend for decreasing NDVI with increasing physical disturbance was apparent, but with, again, only the control and the highly disturbed treatment significantly differing (t = 2.23, p = 0.027). By the end of the experiment (4 months) there were no differences among disturbance treatments (Figure 4.10).

Table 4.4: Three-way PERMANOVA analysis examining the interacting effects of Nutrients (N), Disturbance (D) and Month (M) on Normalised difference vegetation index (NDVI) in the Eden and Tay estuaries. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, Low and High), month contained 4 levels. n=5-7 for all treatments levels

	Eden			Tay		
	DF	Pseudo-F	P (perm)	DF	Pseudo-F	P (perm)
Nutrient Enrichment (N)	2	0.67	0.517	2	**13.78**	**0.001**
Disturbance (D)	2	1.23	0.260	2	0.68	0.519
Month (M)	3	**70.34**	**0.001**	3	**64.71**	**0.001**
NxD interaction	4	0.76	0.584	4	**2.89**	**0.024**
NxM interaction	6	0.06	0.997	6	**2.30**	**0.049**
DxM interaction	6	0.42	0.882	6	**2.25**	**0.050**
NxDxM interaction	12	1.24	0.272	12	0.63	0.792
res	212			216		

Key: **Bold** = significant effect at $p < 0.05$

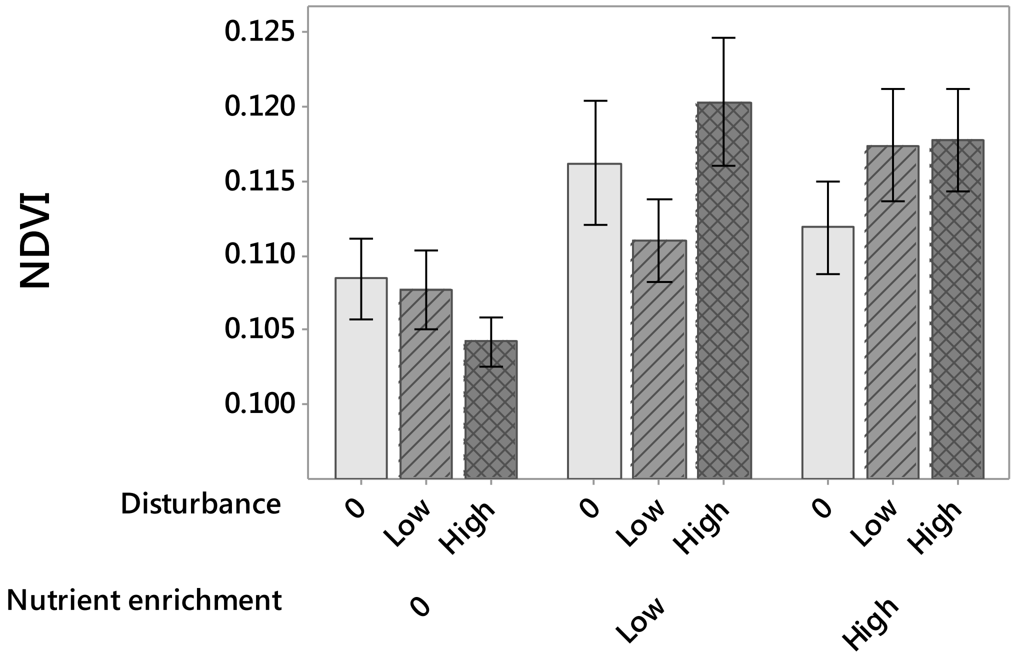


Figure 4.8: Normalised difference vegetation index (NDVI) in the Tay estuary following the addition of nutrient enrichment and physical disturbance stressors at zero low and high levels. Data pooled across 4 months of stress to display interaction between stressors. Points indicate mean +/- SE. N = 7

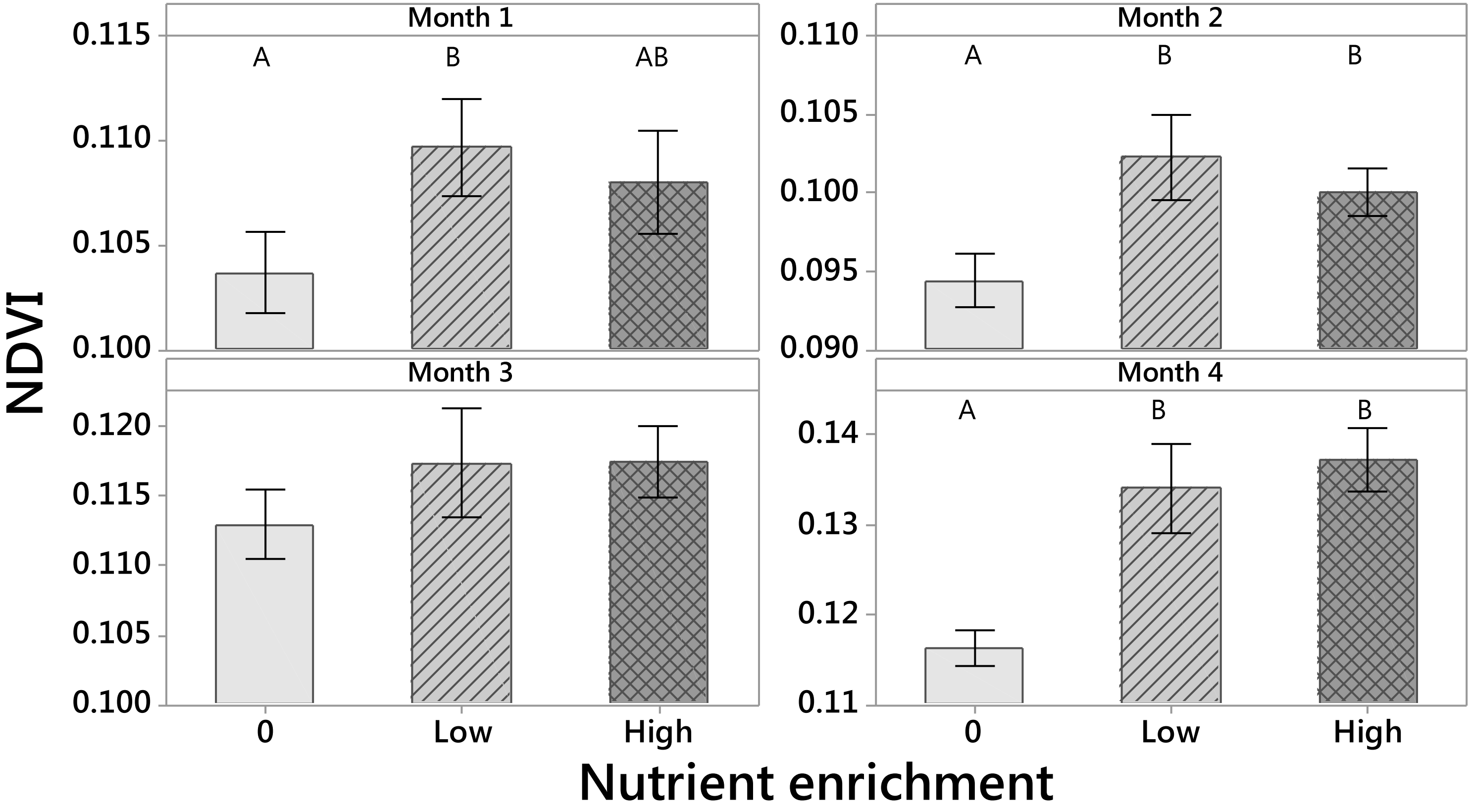


Figure 4.9: Normalised difference vegetation index (NDVI) among nutrient enrichment treatments 1-4 months after commencement of application to experimental plots in the Tay estuary. Data pooled across disturbance treatments, which did not significantly differ. Points indicate mean +/- SE. N = 7

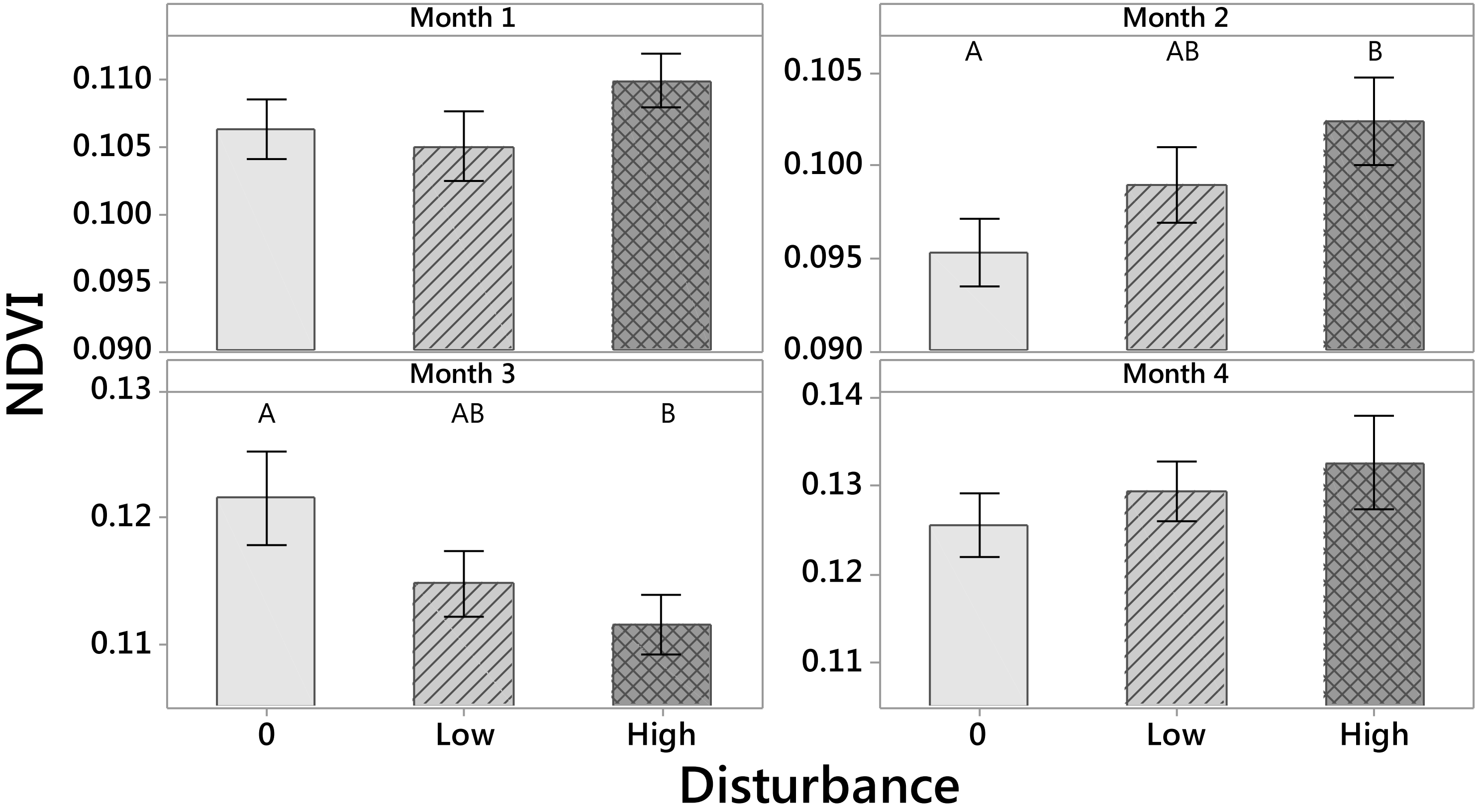


Figure 4.10: Normalised difference vegetation index (NDVI) among disturbance treatments 1-4 months after the commencement of application to experimental plots in the Tay estuary. Data pooled across nutrient enrichment treatments which did not significantly differ. Points indicate mean +/- SE. N = 7

4.4.3 Macrofauna

4.4.3.1 Community structure

There were no treatment effects on the macrofaunal community composition or biodiversity indices at either site until the fourth month. Following four months of disturbance, there was a main effect of physical disturbance only on macrofauna community composition at both sites (Table 4.5; Figure 4.11). In the Eden, *post hoc* comparisons indicate that community composition differed between each level of physical disturbance ($p < 0.05$), while in the Tay, only the controls and the high disturbance treatments differed ($t = 2.10$, $p = 0.001$).

Table 4.5: Two-way PERMANOVA analysis examining the interacting effects of Nutrients (N) and Disturbance (D) analysing their effects on macrofauna community composition in the Eden and Tay estuaries. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, Low and High), n=7 for all treatments levels

		Eden		Tay	
	DF	Pseudo-F	P (perm)	Pseudo-F	P (perm)
Nutrient Enrichment	2	0.56	0.854	1.34	0.195
Disturbance	2	**3.50**	**0.003**	**2.49**	**0.005**
Interaction	4	0.77	0.748	1.03	0.384
Residuals	54				

Key: **Bold** = significant effect at $p < 0.05$

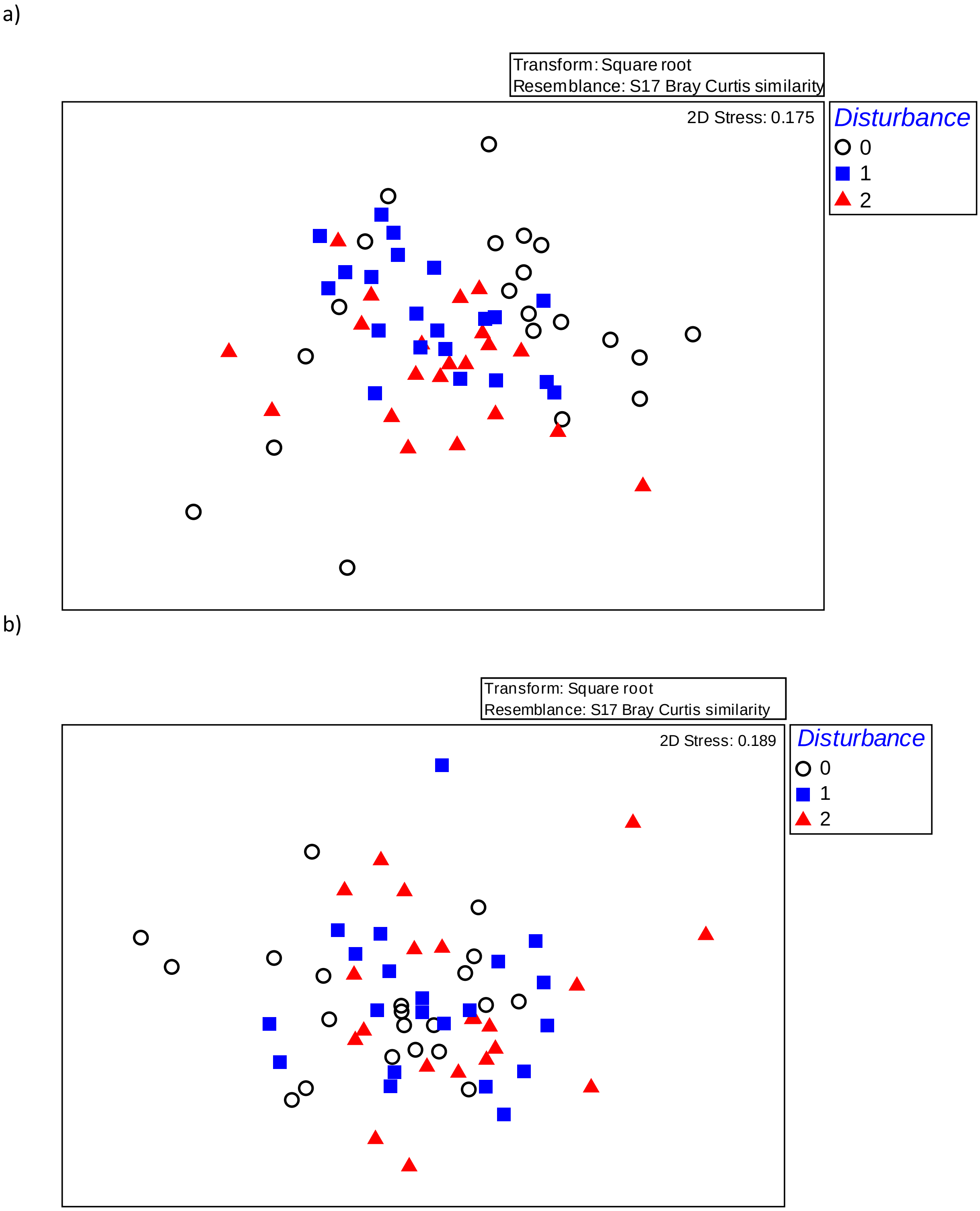


Figure 4.11: nMDS plot showing macrofaunal composition in the Eden (a) and Tay (b) estuaries following 4 months of zero (0, white circles), low (1, blue squares) and high (2, red triangles) physical disturbance. Each symbol represents a single replicate. Nutrient enrichment treatments are not shown as these did not significantly differ. N = 7

4.4.3.2 Biodiversity

There were no treatment effects of either nutrient enrichment or physical disturbance on the species richness or total abundance of invertebrates at either site. There were main effects of disturbance on biodiversity indices, but not nutrient enrichment, or an interaction of the two stressors. In the Eden only the Shannon index varied with physical disturbance (Table 4.6), with the index significantly lower in the high disturbance treatment compared to the controls ($t = 2.65$, $p = 0.008$; Figure 4.12a), but not significantly differing between other pairs of treatments. In the Tay, the evenness, Shannon and Simpson indices (Table 4.6) each displayed a significant main effect of physical disturbance. All 3 indices displayed a similar pattern where increasing physical disturbance caused a decreasing biodiversity (Figure 4.12b).

4.4.3.3 Key Species

SIMPER analysis was used to identify the species causing most dissimilarity between treatments. In total there were 12 species in samples from the Eden and 11 from the Tay. These were: *Corophium volutator, Hydrobia ulvae, Macoma balthica, Hediste diversicolor, Pygospio elegans, Eteone longa, Fabriciola baltica, Tubificoides* sp., an ostracod, an unidentified oligochaete and nematodes (considered as a group and not separated into species) . The additional species found in the Eden was *Tubificoides benedii.*

In the Eden *Hydrobia ulvae, Pygospio elegans, Eteone longa, Fabriciola baltica,* large nematodes and the unidentified oligochaete species were significantly affected by the disturbance stressor ($p < 0.05$), where plot abundances decreased due to the increase in disturbance (Figure 4.13). There were no effects of nutrient enrichment or interactions between the two stressors on any species.

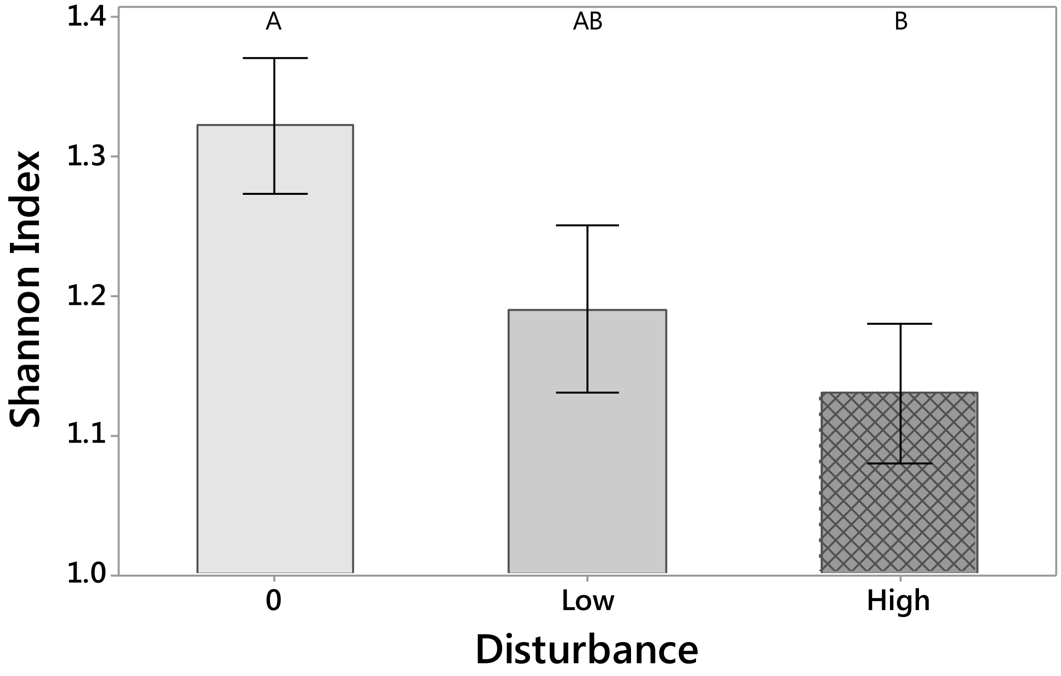
In the Tay, there were main effects of physical disturbance on *Pygospio elegans* where increased disturbance caused a stepwise decrease in abundance (Figure 4.14). At this site there was a significant main effect of nutrient enrichment on the unknown oligochaete species where high enrichment decreased abundances in comparison to the control only, but with no difference between the two nutrient enrichment treatments (Figure 4.14). Further, nematodes displayed interactive effects of the two stressors where abundances were less in the low nutrient enrichment treatment than in the controls or the highly enriched treatment, but only when subjected to high physical disturbance. Additionally high physical disturbance resulted in significantly lower abundances compared to the controls in the treatments also subjected to low physical disturbance.

Table 4.6: Two-way PERMANOVA analysis examining the interacting effects of Nutrients (N) and Disturbance (D) analysing their effects on macrofauna diversity in the Eden and Tay estuaries. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, Low and High), n=7 for all treatments levels

			Species Richness		**Abundance**		**Evenness**		**Shannon**		**Simpson**	
	Source	df	Pseudo-F	P(perm)	Pseudo-F	P(perm)	Pseudo-F	P(perm)	Pseudo-F	P(perm)	Pseudo-F	P(perm)
Eden	N	2	0.65	0.557	0.65	0.526	2.15	0.129	2.81	0.067	1.90	0.171
	D	2	1.94	0.152	2.72	0.078	2.40	0.106	**3.64**	**0.024**	2.69	0.078
	NxD	4	0.87	0.502	1.50	0.197	0.98	0.410	1.08	0.396	0.62	0.658
	Res	54										
Tay	N	2	1.08	0.342	0.23	0.806	0.66	0.531	0.34	0.705	0.61	0.543
	D	2	2.34	0.110	1.48	0.239	**7.04**	**0.001**	**8.70**	**0.002**	**8.52**	**0.001**
	NxD	4	0.70	0.588	1.53	0.216	0.66	0.642	0.54	0.693	0.44	0.758
	Res	54										

Key: **Bold** = significant effect at $p < 0.05$

a)



b)

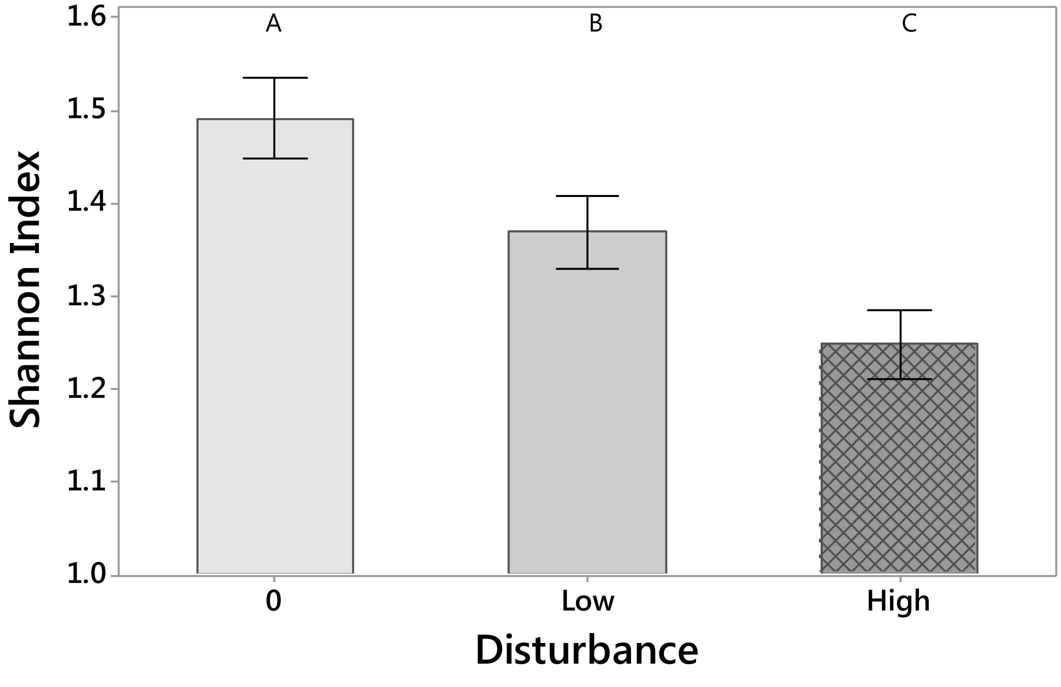


Figure 4.12: Change in biodiversity biodiversity (Shannon Index) in the a) Eden and B) Tay estuaries following the addition of nutrient enrichment and physical disturbance stressors at zero low and high levels after 4 months of stress. Data pooled to display the main effects of physical disturbance only. Points indicate mean +/- SE. N = 7 for all treatment levels

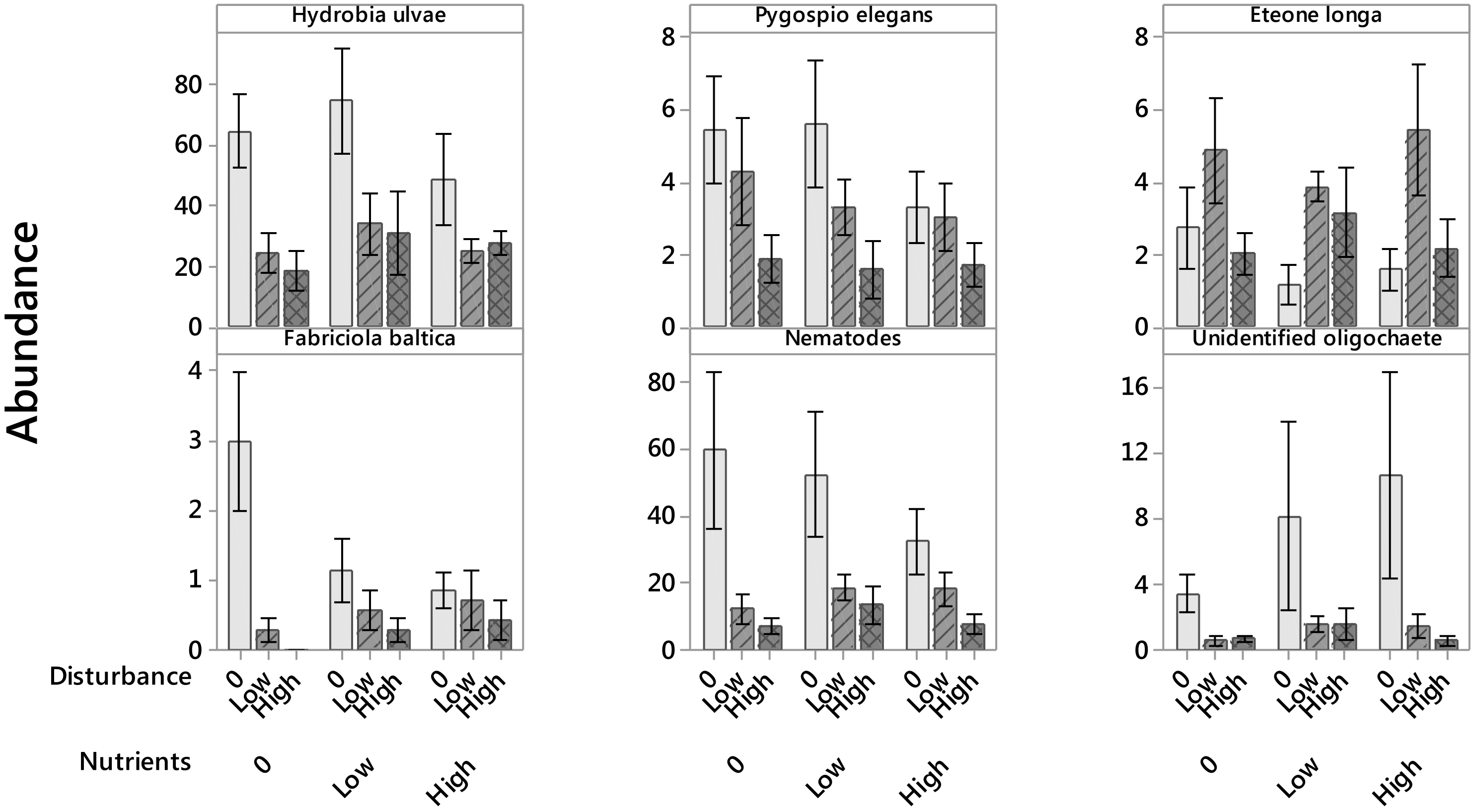


Figure 4.13: Abundances (individuals per core) of the key species displaying change due to stressors in the Eden following the addition of nutrient enrichment and physical disturbance stressors at zero low and high levels after 4 months of stress. Points indicate mean +/- SE. N = 7 for all treatment levels

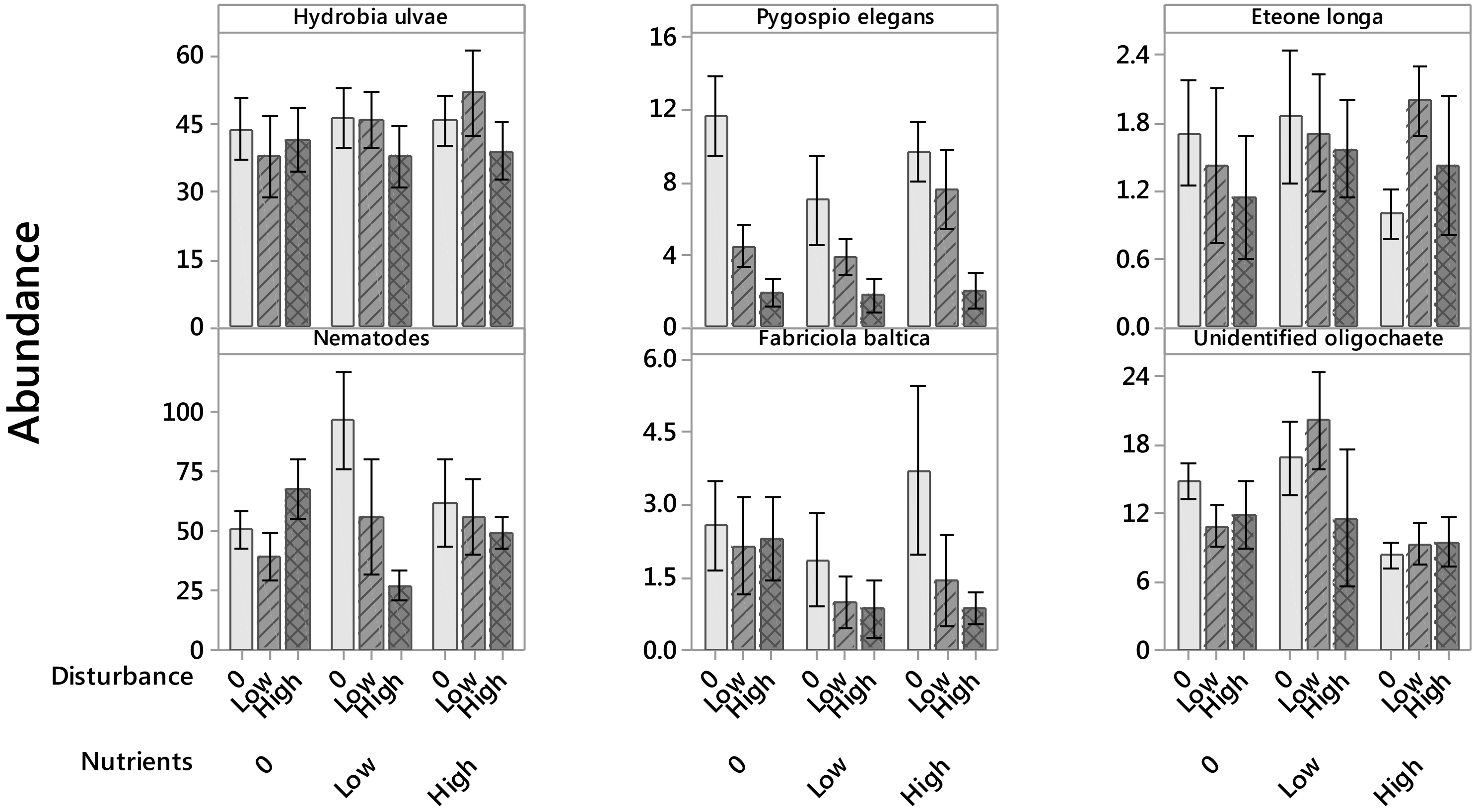


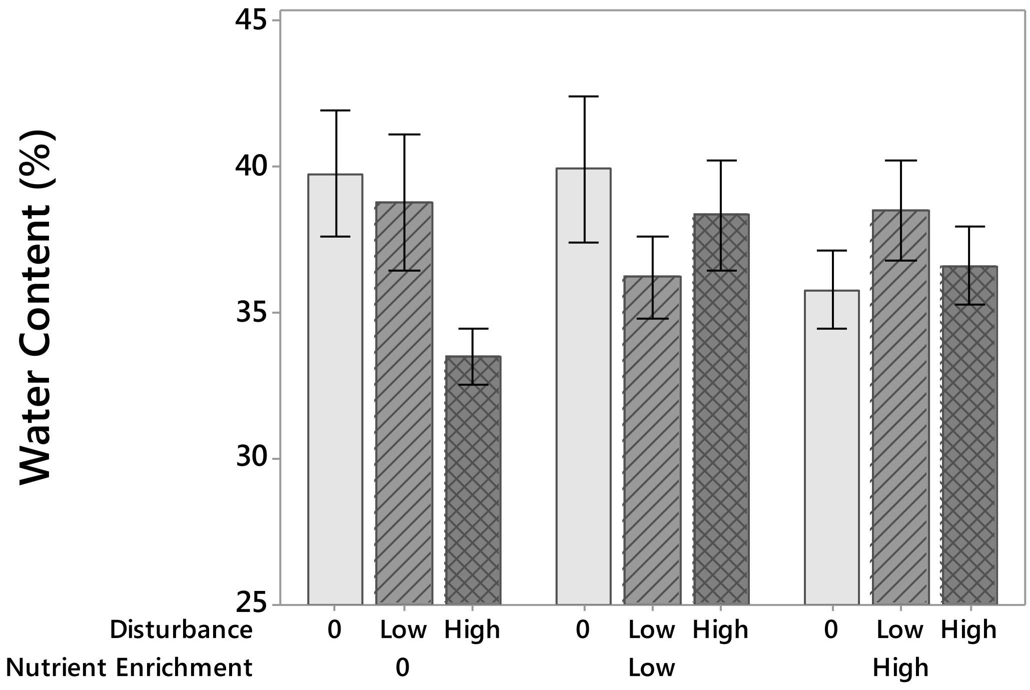
Figure 4.14: Abundances (individuals per core) of the key species displaying change due to stressors in the Tay following the addition of nutrient enrichment and physical disturbance stressors at zero low and high levels after 4 months of stress. Points indicate mean +/- SE. N = 7 for all treatment levels

4.4.4 Sediment stability

At the start of the experiment there were no pre-existing differences between treatments. Following the addition of stressors, each of the variables examined (bulk density of sediments, water content, shear vane measure of stability and CSM measure of erosion potential) displayed significant differences among months at each site. The bulk density was not altered by the experimental treatments. By contrast the water content of sediments at each site was affected by the interaction between nutrient enrichment and physical disturbance (Table 4.7; sig. N x D interaction). In the Eden, among plots subjected to high physical disturbance, those also subjected to either high (t = 2.52, p = 0.015) or low (t = 2.60, p = 0.006) nutrient enrichment contained higher water contents than the controls (Figure 4.15a). Conversely in the Tay, among plots subjected to high physical disturbance, plots also subjected to high (t = 1.92, p = 0.062) or low (t = 4.49, p = 0.001) nutrient enrichment contained lower water content compared to the controls (Figure 4.15b), with the low nutrient enrichment treatment containing lower water contents than the high (t = 2.04, p = 0.049). There were, however, no effects of nutrient enrichment on plots receiving the low or no physical disturbance, at either site.

Additionally, in the Eden, among plots subjected to zero nutrient enrichment, those subjected to high physical disturbance contained significantly lower water contents compared to the control (t = 3.07, p = 0.004) or low physical disturbance treatment (t = 2.34, p = 0.024). There were no further differences between treatments at this site (Figure 4.15a). In the Tay, the opposite effect occurred where the high physical disturbance treatment contained a greater water content than the controls (t = 2.33, p = 0.034), with no significant differences between low and high disturbance or low and undisturbed treatments (Figure 4.15b). Further, among plots that were subjected to low nutrient enrichment, plots that were also subjected to high physical disturbance contained lower water contents than either the plots receiving low (t = 3.58, p = 0.001) or no (t = 3.22, p = 0.002) physical disturbance.

a)



b)

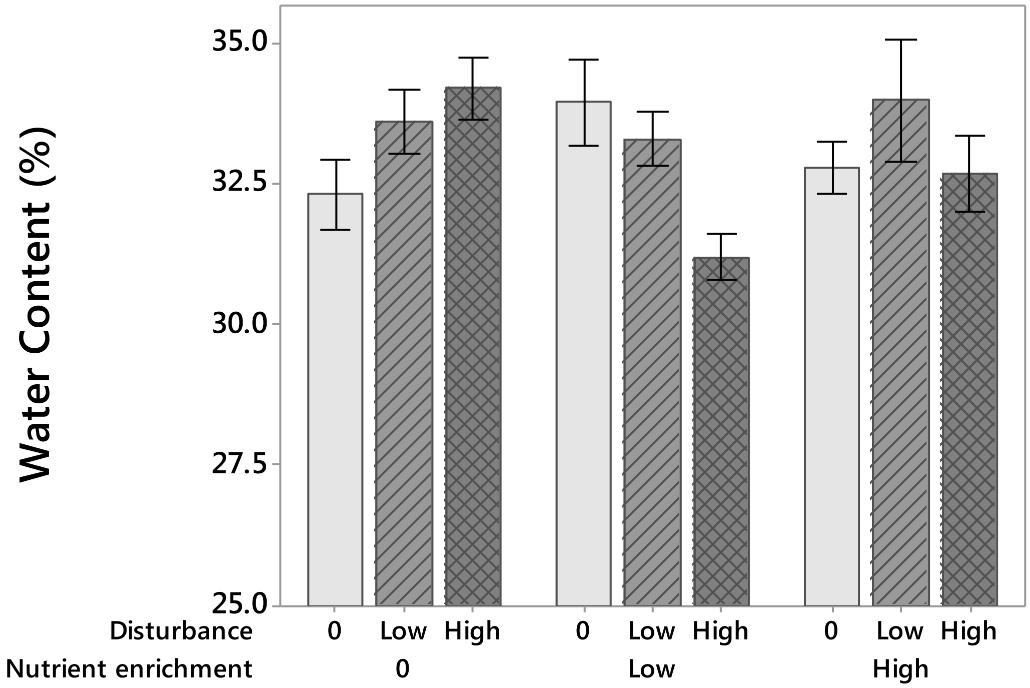


Figure 4.15: Water content (%) in the a) Eden and b) Tay estuaries following the addition of nutrient enrichment and physical disturbance stressors at zero low and high levels. Data pooled across 4 months of stress to display interaction between stressors. Points indicate mean +/- SE. N = 7

The cohesive strength meter (CSM) and shear vane take direct measurements with regards to the erodibility of the sediments. Neither CSM nor shear vane measurements displayed an interacting effect of nutrient enrichment and physical disturbance, at either site. While the CSM measurement displayed no significant effects of either stressor on erodibility in the full analysis, the shear vane measurement displayed a main effect of physical disturbance at each site (Table 4.8) and a main effect of nutrient enrichment in the Eden. In the Eden, erodibility, as measured by CSM, was greater in the control and high physical disturbance treatments, which did not significantly differ from one another, than in the low physical disturbance treatment (Figure 4.16a). In the Tay, increasing levels of physical disturbance decreased erodibility, therefore increased the sediment stability (Figure 4.16b). In the Eden increasing levels of nutrient enrichment caused a decrease in stability (Figure 4.17).

While there were no significant effects of stressors on the CSM measure of sediment erodibility in the full design, there were large and significant differences between months that dominated analyses. When each month was analysed separately, some treatment effects on critical shear stress were apparent in the Tay whereby increased physical disturbance generally increased the erosion threshold (Figure 4.18), but there were no main effects of nutrient enrichment ($p > 0.05$). The main effect of physical disturbance was significant at the month 4 time point only (Pseudo-$F_{(2,54)} = 3.19$, p(perm) = 0.039). At this time point the erosion threshold was greater in the low physical disturbance treatment than the control ($t = 2.53$, $p = 0.016$), but there were no differences between the high treatment and either of the control or low treatments. There were no treatment effects in any of the months in the Eden.

Table 4.7: Three-way PERMANOVA analysis of the interaction between Nutrients (N), Disturbance (D) and Month (M) on sediment water content (%) in the Eden and Tay estuaries. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, Low and High), month contained 4 levels. n=7 for all treatments levels

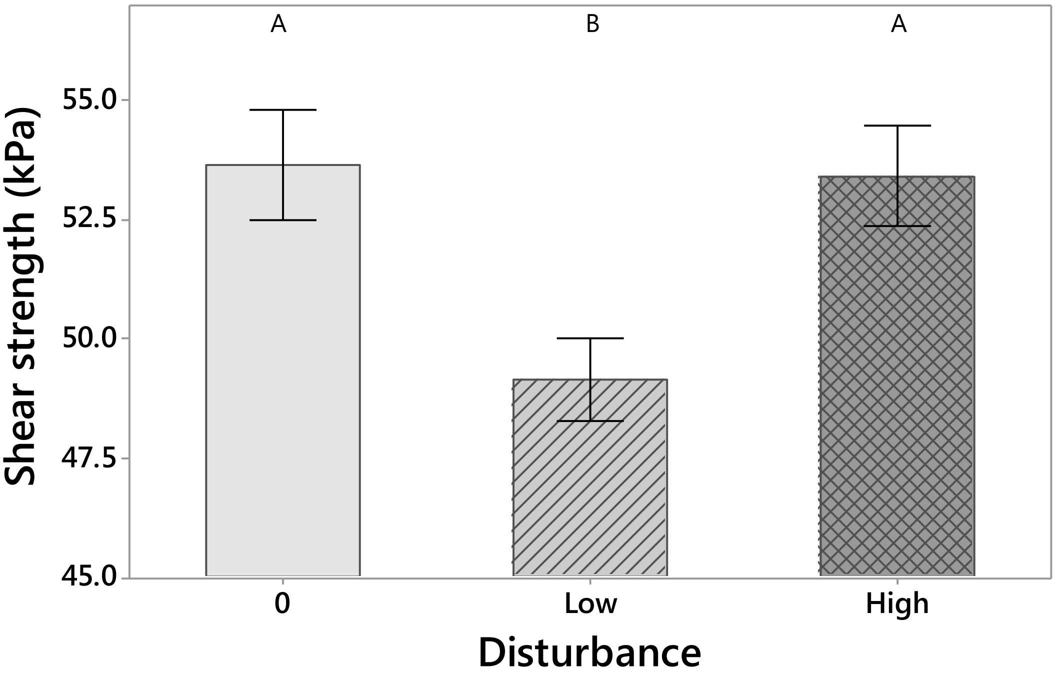
		Eden		Tay	
	DF	Pseudo-F	P (perm)	Pseudo-F	P (perm)
Nutrient Enrichment (N)	2	0.43	0.668	0.60	0.577
Disturbance (D)	2	1.77	0.180	**1.74**	**0.180**
Month (M)	3	**26.03**	**0.001**	**12.54**	**0.001**
NxD interaction	4	**2.47**	**0.052**	**3.63**	**0.006**
NxM interaction	6	0.32	0.922	0.14	0.990
DxM interaction	6	0.90	0.511	0.48	0.841
NxDxM interaction	12	0.81	0.643	0.49	0.906
res	216				

Key: **Bold** = significant effect at $p < 0.05$

Table 4.8: PERMANOVA output of three-way interaction between Nutrients (N), Disturbance (D) and Month (M) analysing their effects on shear vane measure of sediment stability in the Eden and Tay estuaries. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, Low and High), month contained 4 levels. n=5-7 for all treatments levels

		Eden		Tay	
	DF	Pseudo-F	P (perm)	Pseudo-F	P (perm)
Nutrient Enrichment (N)	2	**4.86**	**0.006**	2.17	0.124
Disturbance (D)	2	**7.26**	**0.002**	**5.26**	**0.009**
Month (M)	3	**13.19**	**0.001**	**228.17**	**0.001**
NxD interaction	4	1.81	0.142	0.45	0.782
NxM interaction	6	1.21	0.298	1.00	0.440
DxM interaction	6	0.85	0.543	0.91	0.494
NxDxM interaction	12	0.65	0.796	0.89	0.550
res	216				

Key: **Bold** = significant effect at $p < 0.05$



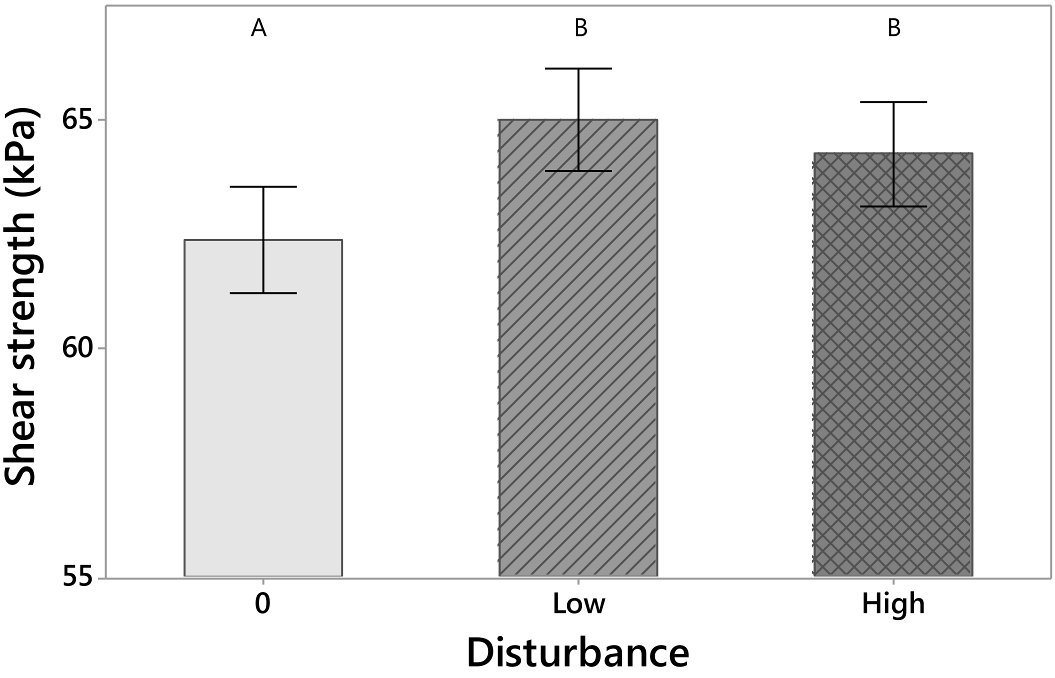


Figure 4.16: Sediment stability (Shear strength (kPa) measured by the shear vane) in the a) Eden and B) Tay estuaries following the addition of nutrient enrichment and physical disturbance stressors at zero low and high levels after 4 months of stress. Data pooled to display the main effects of physical disturbance only. Points indicate mean +/- SE. N = 7 for all treatment levels

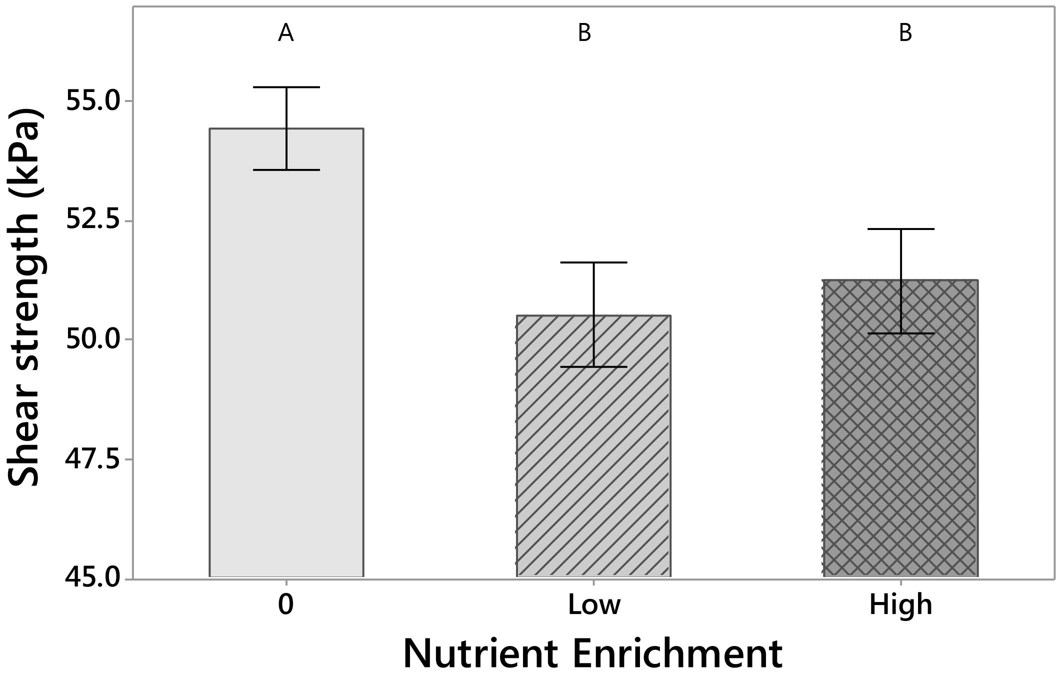


Figure 4.17: Sediment stability (Shear strength (kPa) measured by the shear vane) in plots of the Eden receiving zero, low or high levels of nutrient enrichment after 4 months of stress. Data pooled across physical disturbance treatments that did not significantly differ. Points indicate mean +/- SE. N =7

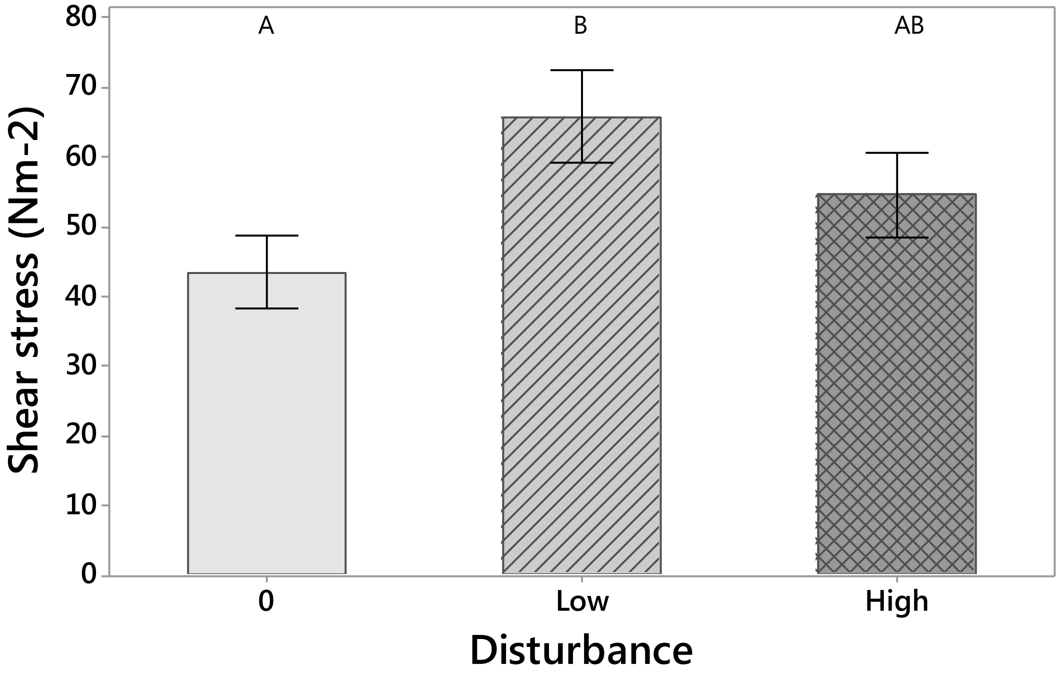


Figure 4.18: Cohesive Strength Meter (CSM) derived erosion thresholds in plots of the Tay receiving zero, low or high levels of physical disturbance after 4 months of stress. Data pooled across nutrient enrichment treatments that did not significantly differ. Points indicate mean +/- SE. N= 7

There were few correlations between the sediment stability variables and macrofaunal community composition at either site. In the Eden, the values derived from the shear vane were weakly positively correlated with the Bray Curtis dissimilarities analysing macrofaunal community structure ($r_s = 0.125$, $p = 0.021$), while in the Tay the macrofauna Bray Curtis dissimilarities only correlated with the water content ($r_s = 0.341$, $p = 0.001$). The abundance of 3 species displayed correlation with the sediment stability variables. In both the Eden and the Tay, *Macoma balthica* was negatively correlated with erosion threshold measured by the shear vane in each of the estuaries (Eden: $r_s = 0.294$, $p = 0.015$; Tay: $r_s = 0.379$, $p = 0.001$). Additionally *Macoma balthica* was negative correlated with the CSM measurement in the Eden ($r_s = 0.393$, $p = 0.001$) but not the Tay. The CSM variable was also positively correlated with *Hediste diversicolor* in the Eden ($r_s = 0.278$, $p = 0.022$) and negatively with ostracods in the Tay ($r_s = 0.241$, $p = 0.045$). There were no further correlations with individual species.

Sediment stability variables were also correlated with the MPB component of the sediment (Table 4.8). Chlorophyll content correlated positively with water content and negatively with bulk density at each site. The opposite effect occurred with chlorophyll concentration though there were no correlations in the Tay with respect to water content. The water content responded positively to NDVI in the Eden but negatively in the Tay whereas there were no correlations with bulk density. At both sites the organic content was moderately positively correlated with water content, and negatively correlated with bulk density of the sediments in the Eden only. The measured erosion threshold variables (CSM and shear vane) displayed no relationships with sediment chlorophyll or organic contents. At both sites there were positive relationships between the NDVI and the Shear vane measurements (Table 4.8). However in the Eden, there was a weak negative relationship between the CSM measurement and NDVI.

Table 4.9: Spearman's rank correlations (r_s) between sediment stability variables – Water content (%), Bulk density (g cm^{-3}) Shear vane and CSM derived measures of sediment erosion threshold (Nm^{-2}) – and environmental variables – Chlorophyll *a* content (µg g^{-1}) and concentration (mg m^{-2}) NDVI and organic content (%) in the Eden and Tay estuaries. Correlation coefficient (Rho) value = top, p-value = below.

	Water Content		**Bulk Density**		**Shear Vane**		**CSM**	
	Eden	**Tay**	**Eden**	**Tay**	**Eden**	**Tay**	**Eden**	**Tay**
Chlorophyll *a* concentration	**-0.325**	-0.070	**0.635**	**0.743**	-0.035	-0.860	-0.021	0.079
	<0.001	0.266	**<0.001**	**0.004**	0.576	0.171	0.741	0.234
Chlorophyll *a* content	**0.733**	**0.182**	**-0.588**	**-0.183**	0.024	-0.076	0.090	-0.018
	<0.001	**0.004**	**<0.001**	**0.004**	0.709	0.226	0.154	0.786
NDVI	**0.189**	**-0.235**	0.075	0.111	**0.215**	**0.345**	**-0.148**	0.049
	0.003	**<0.001**	0.242	0.079	**0.001**	**<0.001**	**0.020**	0.443
Organic content	**0.799**	**0.581**	**-0.614**	-0.064	0.065	-0.099	-0.066	0.053
	<0.001	**<0.001**	**<0.001**	0.315	0.302	0.117	0.346	0.404

4.5 Discussion

This chapter replicated experiments undertaken in Australia in Scotland and examined multiple stressor effects. While the stressors analysed were the same, the composition of local communities and environmental conditions were very different between Scotland and Australia and, consequently, differences in community responses to stressors were expected. The effects of multiple stressors can be highly context specific (Bishop & Kelaher, 2013; Gladstone-Gallagher et al., 2014; Whomersley et al., 2010), and in the previous chapter effects of stressors varied between the two Australian sites, situated within 50 km of one another. Here, it was also expected that strong site differences would be identified between the two Scottish sites, especially given the effect of an *Ulva sp.* bloom blanketing the Eden but not the Tay site.

The experiments in Scotland also extended the previous research in Australia by considering how multiple stressors influence not only the structure but also the function derived from communities, which is a relatively understudied topic (Alsterberg et al., 2014). Along with macrofaunal biodiversity, the functions considered were sediment stability and primary production (using MPB biomass as a proxy). Both stressors were identified to have singular effects on the function of the benthic communities at the two Scottish sites, with the stressor of overriding effect being physical disturbance. While nutrient enrichment was a significant driver of change for the primary producers, the macrofauna and the sediment stability responded more strongly to the influence of physical disturbance.

4.5.1 Stressor effects on the MPB

It was originally hypothesised that nutrient enrichment would have a positive effect on the MPB. From the observed results, it can be inferred that nutrient enrichment did indeed have a positive impact on MPB at both sites, but the effect was stronger and more persistent in the Tay than the Eden estuary. One explanation for the site difference may be that in the Eden, the site was affected by an *Ulva* sp. bloom that presumably reduced light reaching the sediment surface for MPB photosynthesis. Additionally there was homogeneous coverage of these algae over the site meaning that sediment chlorophyll was perhaps derived from buried algae rather than due to solely the MPB. These *Ulva sp.* blooms are naturally occurring events that may be enhanced by increased nutrient loadings (Raffaelli, 2000) and have the potential to change community compositions and environmental conditions within a site (Defew et al., 2002). This bloom did not appear to have been caused by the experimental nutrient addition as it extended over a much larger stretch of the estuary than the single study site. The act of removing the algae to get an NDVI reading could have caused some change to chlorophyll concentrations. Once removed, there was a thick black anoxic sediment layer on the surface so it is unsurprising there were no difference in the NDVI remote sensing measure among nutrient treatments. Laboratory analyses revealed that the chlorophyll concentrations within sediment in the Eden generally increased with nutrient enrichment, although they were also susceptible to an interactive effect of disturbance. In the Tay, the chlorophyll concentration within the sediments only increased with nutrient enrichment in plots that were unaffected by physical disturbance

The second hypothesis stated that disturbance would decrease MPB biomass. This was corroborated by the findings in this study where at both sites the overriding effect of disturbance was to decrease MPB biomass recorded as NDVI or sediment chlorophyll. However, at each site an interaction was observed between stressors, where positive effects of disturbance were found at certain levels of nutrient enrichment. Where an effect of physical disturbance was observed, it could be explained by a number of potential mechanisms. First, where the availability of nutrients is limiting to MPB, physical disturbance could promote algal growth by releasing nutrients trapped in sediments (Porter et al., 2006). Second, the act of raking to physically disturb sediments could trap detritus in sediments which could decay over time releasing nutrients and enhancing production of MPB (Dizon & Yap, 1999; Rublee, 1982). Third, the effect may have been indirect effect arising from effects of physical disturbance on macrofaunal bioturbation. In this study, disturbance altered species compositions in the macrofauna. Bioturbation is a well-studied mechanism known to increase the availability of nutrients and cause oxygenation to a system and therefore increase MPB biomass (Donadi et al., 2013; Ieno et al., 2006; Solan et al., 2004).

While at each site, main effects and interactions were observed due to the effects of the stressors, the patterns observed were not necessarily the same for sediment chlorophyll measurements and NDVI measurements. While the NDVI is generally a good indicator of sediment chlorophyll at the surface and hence MPB biomass (Barille et al., 2011; Murphy et al., 2005; Serodio et al., 2009) the relationship does weaken under certain environmental conditions (Barille et al., 2011; Jesus et al., 2006) and consequently varies among locations (Kromkamp et al., 2006). Indeed in this study, the background levels of chlorophyll at each site revealed that the Eden contained higher sediment chlorophyll than the Tay but also a lower NDVI. There are several potential reasons. First, many studies show that the relationship between NDVI and sediment chlorophyll is site specific

(Kromkamp et al., 2006) meaning that each site the NDVI will vary based upon environmental factors such as grain size (Barille et al., 2011), water content (Jesus et al., 2006) or prevailing light conditions (Serodio et al., 2009). Attempts were made to standardise environmental variables between sites (i.e. same grain size, sampling at the same time of day and tidal cycle), however factors such as the water content of sediments could not be controlled. Another potential explanation for the differences between sediment chlorophyll and NDVI is that the MPB can vary in relation to where it is found within the sediments (Consalvey et al., 2004; Jesus et al., 2006). While the contact cores measure the top 2 mm of the sediment, the NDVI measurement records the very surface only. There can be a gradient in chlorophyll *a* between the surface and 2 mm below (Taylor & Paterson, 1998) with the MPB varying their location in sediments dependant on time of day, environmental variables or sediment grain size (Consalvey et al., 2004; Jesus et al., 2009; Taylor & Paterson, 1998).

While chlorophyll concentrations are a more accurate measure of chlorophyll within the sediments compared to content (Tolhurst et al., 2005), content is still a widely used measure of chlorophyll. This is especially true in areas where there are no confounding variables such as differences in grain size or water content which decrease the accuracy of calculating content. Further at the Tay site, chlorophyll content displayed stressor effects whereas concentration did not. There are many reasons as to why there could be a difference between content (% or chlorophyll per unit of weight of sediment) and concentration (chlorophyll over an area; summarised in Tolhurst et al., 2005). In these experiments, the difference it is likely to be due to the standardisation of the contact coring technique. While efforts were made to collect contact cores 2 mm in diameter, there can be some human error in filing down to the correct size, especially under cold field conditions. This would create variable contact core sizes leading to inaccurate calculation of chlorophyll concentrations.

4.5.2 Stressor effects on the macrofauna

Contrary to the hypothesis that the macrofauna would respond negatively to nutrient enrichment, causing a decrease in biodiversity and a change in community structure, there were no effects of nutrient enrichment at either site that affected community structure, biodiversity or total abundances. The only significant effects of nutrients on macrofauna were their negative effect on oligochaete abundance and an interactive effect of nutrients and physical disturbance on nematodes. These effects were only observed in the Tay, consistent with previous observations that species such as oligochaetes tolerate enrichment at some sites (e.g. Méndez, 2002) but not others (Whomersley et al., 2010).

Previous research has shown that enrichment can increase (Morris & Keough, 2003a, 2003b) or decrease (Botter-Carvalho et al., 2014; Fitch & Crowe, 2010) biodiversity and abundances, depending upon environmental context and ecosystem studied. While stressor effects can sometimes be observed in manipulative experiments, the duration of the experiment is important in determining whether a response can be identified. Short-term experiments are unlikely to be able to identify a response of nutrient enrichment (e.g. Cebrian et al., 2012; Pascal et al., 2013) whereas longer term applications of nutrient are more likely to alter community structure (Davis et al., 2010). A particular example in the Ythan in Scotland revealed that it took 15 weeks of nutrient enrichment for any differences in experimental treatments to be observed (Raffaelli & Emmerson, 2001) whereas an American example states effects may only become observed after years of chronic enrichment (Pascal et al., 2013).

As hypothesised, physical disturbance had a much stronger negative impact on macrofaunal community composition, biodiversity indices and individual species responses than nutrient enrichment. Some negative effects of physical disturbance were apparent at both sites, although the range of nutrient conditions across which they were found varied, perhaps due to the *Ulva sp.* bloom in the Eden. Species impacted by physical disturbance included 3 species of polychaete, the gastropod *Hydrobia ulvae*, nematodes and oligochaetes, all of which displayed negative responses to the disturbance. This is consistent with similar studies which have found that physical disturbances such as raking generally cause decreases in the abundances of these species (Cowie et al., 2000; Hall & Harding, 1997; Hall, 1994; Whomersley et al., 2010), though effects may be context dependent with abundances increasing due to disturbances in some instances (Hall & Harding, 1997; Whomersley et al., 2010)

One mechanism by which physical disturbances, such as raking, might influence macrofaunal communities is through changing sediment surface properties which in turn might alter biogeochemical processes in the sediments (Rossi et al., 2007). Direct effects of raking, in causing damage to soft bodied organisms, damaging burrows and removing large organisms, are more like to be the drivers of changes in community composition (Hall & Harding, 1997). The absence of a longer-term effect of raking on many of the species such as *Corophium volutator* may be because these species are so numerous that they can recolonise plots from outside the raked area. This could indicate that a combination of the small spatial scale of the disturbance combined with the relatively infrequent raking and sampling regimes may not have been great enough to illicit an observed response (Whomersley et al., 2010).

4.5.3 Sediment stability

It was hypothesised that the two stressors would have negative additive effects on sediment stability. Yet, while there was an additive response of stressors on erosion threshold of sediments at each site, in the Eden the direction of the response was negative whereas in the Tay it was positive. The site specificity could be due to the effect of the algal bloom affecting the stability in the Eden site (Defew et al., 2002). *Ulva* blooms have been known to increase water content which decreases stability by decreasing the solidation and compaction of sediments (Bolam et al., 2000; Defew et al., 2002). While there was an interactive effect of stressors on the water content at each site, this variable is dependent on the time of sampling, weather conditions and algae present on the sediment surface. A positive relationship was identified between water content and macrofauna in the Tay, perhaps due to the influence of burrow forming species (De Deckere et al., 2001). It is unsurprising there were no links between water content and macrofaunal composition in the Eden given this variable was clearly a result of the overlying algae rather than due to a change in macrofaunal composition.

The CSM and shear vane techniques used for analysing sediment stability were affected by the manipulated stressors. Increased physical disturbance increased the erosion threshold measured by CSM and increased the resistance to torque measured by the shear vane in the Tay, whereas a decrease in resistance was observed by the shear vane in the Eden due to low disturbance only. The macrofauna can have a large negative impact on sediment stability (Underwood & Paterson, 2003), although there was limited evidence for this in the present study. While under controlled conditions macrofauna can have a large effect on the sediment stability, under natural field conditions the complex environmental processes that occur can mediate these effects (Defew et al., 2002; Paterson et al., 2000; Tolhurst et al., 2003). *Macoma baltica* were the only species that displayed any correlation with measured erodibility, but at each site many burrowing species decreased in abundance with increasing physical disturbance. The removal of these burrowing species may have increased stability.

The MPB component was identified to have a stronger impact on sediment stability than the macrofauna. Although the sediment chlorophyll (either content or concentration) did not display correlations with the sediment stability measures, the NDVI at each site was positively correlated with the shear vane measurement. NDVI also displayed a weak negative correlation with the CSM measure in the Eden which is counterintuitive to what is known about the stabilising effect of a biofilm. This can be explained through the process by which the CSM and the NDVI measurements are derived. Because the NDVI measure of chlorophyll records the very surface of the sediment, the negative relationship between CSM and NDVI could be due to the influence of the *Ulva sp.* bloom which would cause a decrease in sediment stability yet increase the amount of chlorophyll recorded by the device mistakenly reading the buried algae instead of the MPB (Bolam et al., 2000; Defew et al., 2002). Additionally the CSM is a surficial measure meaning that any sediment could have been loosely trapped in the algae and easily eroded by the device. The effects of sediment disturbance on the MPB could have had knock on effects to the sediment stability. Additionally through reductions of MPB content and reductions in macrofaunal abundances, the net effect of sediment stabilisation could have led to these process cancelling each other out leading to under-represented changes in sediment stability (Huxham et al., 2006).

The measures of sediment stability displayed weak correlations to each of the environmental variables and to the macrofauna. While the decrease in the macrofauna is possibly the most effective explanation for why the sediment stability increased in the Tay there are other possible explanations that were unexplored by this study. Extracellular polymeric substances are produced by MPB and bacterial components of sediments and are widely known to bind sediments, aiding in sediment stabilisation (Tolhurst et al., 2002). The act of raking the sediments causes a change in bed roughness, which could promote MPB growth but also promote bacterial growth (Dizon & Yap, 1999; Murray et al., 2002; Rublee, 1982). Additionally disturbances have been shown to promote the production of mucus in both the meio- and micro-benthos as an response to stabilise their burrows (Probert, 1984).

The reason the CSM could have displayed a minimal response to stressors compared with the shear vane measure could have arisen due of the type of stress each puts onto the sediment. While both measurements allow us to interpret the stability of the sediments, the CSM erodes the sediment at the very surface of the sediments and is a vertical measurement whereas the shear vane pushes deeper into the sediment to record the horizontal shear strength of the sediments. The differing responses of these measurements identified in the Eden can be explained by the act of removing the algae disrupting the surface sediments making it more susceptible to vertical erosion by the CSM but resulting in no change to the sub surface shear vane measurement.

The negative effect of nutrient enrichment on sediment stability in the Eden is unexplained by this study. Increased enrichment did nothing to the macrofauna and so there were no knock on effects. Increased enrichment caused an increase in MPB and so should have facilitated more binding of the sediments by the MPB and therefore an increase in sediment stability. A positive effect of enrichment on sediment stability may not have been observed in this study due to complex interacting effects between the nutrient stressor and environmental conditions (Huxham et al., 2006). In particular, the bloom of *Ulva* could have modulated the relationship between nutrient enrichment and sediment stability, if nutrient enrichment caused an increase in algae which further disrupted sediments in these plots. Changes in microphytobenthic or bacterial species assemblages due to nutrient enrichment may also result in a decrease in sediment stability (Gerbersdorf et al., 2009).

4.6 Conclusions

Multiple stressors nutrient enrichment and physical disturbance were assessed for their impacts on sediment communities in the UK. Each stressor displayed additive response and minimal interactive effects on the macrofauna or MPB components. Nutrient enrichment was the most important stressor affecting the MPB whereas the macrofauna were affected by physical disturbance. This study furthered multiple stressor research by including the effects of stressors on ecosystem functioning recorded as sediment stability. In estuarine intertidal systems sediment stability is kept in balance by the MPB and macrofauna increasing and decreasing stability, respectively. Though MPB biomass increased due to nutrient enrichment there were no bottom-up effects on sediment stability in these environments. Additionally while disturbance negatively affected the macrofauna, the sediment stability also respectively increased at the site in the Tay. The interactive ability of multiple stressors to alter these systems under natural field conditions seems mediated by the local environmental context and local environmental processes.

Table 4.10: Verification of hypotheses set at the beginning of the chapter

Hypotheses	Accept ✓ or reject X
H1 – Nutrient enrichment will positively influence primary production in terms of MPB biomass and alter macrofaunal community composition.	**✓for MPB only**
H2 – Physical disturbance will negatively affect both the abundance of MPB and macrofaunal biodiversity.	✓
H3 – By changing the abundance of MPB and altering macrofaunal community structure each stressor will modify the productivity and sediment stability of the ecosystem.	✓
H4 – Context specific effects of multiple stressors will be observed.	✓

5 Comparing the effects of multiple stressors between UK and Australian estuarine systems

5.1 Introduction

Estuarine and coastal systems are found globally and are important and economically valuable ecosystems (Barbier et al., 2011). Historically, they have served as focal points of human settlement due to their location between ocean and river transportation routes, and due to their high productivity and proximity to freshwater. Today, up to 75% of the world's population live within estuarine and coastal watersheds (Paerl, 2006). Within Australia the proportion of the population living in the coastal zone is much higher, around 80-85% (Australian Bureau of Statistics, 2002), while in the UK and in particular Scotland, this figure is close to 100% (Eurostat, 2015). The high human population densities surrounding estuaries make them among the most heavily impacted ecosystems on earth (Kennish, 2002; Lotze et al., 2006). Given the importance of estuaries, the ways in which they respond to human modification needs to be understood, so that appropriate management strategies may be developed to protect them.

Responses to stressors are likely to be highly context specific (Bishop & Kelaher, 2013) and can greatly vary depending on geographic location (Raffaelli & Emmerson, 2001). Australian systems have a relatively recent history of human colonisation in comparison to estuaries in the UK. Nevertheless, management of Australian estuarine systems is often based on the assumption that they function and respond to stressors in similar ways as better-known European and North American systems (Hutchings, 1999). In recent years this assumption has been challenged by studies showing that northern hemisphere paradigms such as the Remane model may not necessarily apply to Australian systems (Elliott & Whitfield, 2011; Whitfield et al., 2012). Understanding when and where northern hemisphere models of ecosystem structure and function can be applied to Australian systems is particularly important given rapid population growth in Australia (Kennish, 2002). It is unrealistic to assume that pressures on estuarine systems will be uniform across the world. Locally appropriate management strategies are needed.

UK and Australian estuarine systems are widely different in terms of prevailing stress, environmental conditions and types of fauna present. The Australian systems are microtidal (less than 2 m) whereas in the UK they are macrotidal. The Australian estuarine systems tend to have less of a tidal influence than their UK counterparts (Heggie & Skyring, 1999). This affects salinity regimes and leads to Australian systems being marine throughout the tidal cycle, whereas in the

UK there are large daily fluctuations in salinity (Hutchings, 1999; McManus, 2005). Hence, in Australian estuaries, marine flora and fauna that are adapted to fairly stable salinity regimes can persist, whereas in UK estuaries, euryhaline taxa – those able to withstand variable salinity – dominate leading to typically lower diversity than in adjacent marine environments (Attrill, 2002; Remane & Schlieper, 1971; Whitfield et al., 2012).

In comparison to European systems, nutrient enrichment is relatively moderate in Australia, given the smaller population and relatively recent developmental history (Eyre & Balls, 1999; Hauxwell & Valiela, 2004; Kelly, 2008). Further, Australia is an old continent and as such is very nutrient poor in comparison to nutrient rich landscapes around European systems. While nitrogen tends to be the limiting factor for autotrophic growth in UK estuaries (Heip et al., 1995), in Australia, because the landscape is nutrient poor, phosphorus tends to limit growth (Beadle, 1962). Hence, Australian and Scottish estuaries may be expected to respond differently to nutrient inputs that alter N:P ratios (Cloern, 2001; Vitousek et al., 1997a). It might be expected that Australian systems, which tend towards oligotrophy, may be more sensitive to nutrient loading than more eutrophic systems (Jeffrey, 1974; Nicastro & Bishop, 2013), with increases in nutrients likely to be rapidly taken up by the primary producers (Scanes et al., 2007).

While ecological systems are often compared within a geographic region (e.g. Nicastro & Bishop, 2013), there are limited studies that directly compare estuarine structure and function across continents (Elliott & Whitfield, 2011), and, in particular, how estuarine ecosystems respond differently to stressors. Addressing continental differences can be difficult due to major differences in species or physical processes, in addition to practical and monetary complications of large scale experiments. There are many ways to address the impracticalities involved in studying multiple geographic regions, for example through making broad ecological comparisons such as analysing abundances, diversity, phyla or functional groups present in each location (e.g. Dittmann & Vargas, 2001). Additional tools include meta-analyses (e.g. Strain et al., 2014) however there are many pros and cons of this approach (see Stewart, 2010) and empirical experiments need to form the basis for this research.

Empirically, there are limited examples of first hand experimentation manipulating stressors in different geographic locations. One such example was conducted by Raffaelli and Emmerson (2001) where experiments conducted in Scotland were compared to those conducted in Australia by Hall et al. (2000). In this scenario experimenters differed, perhaps resulting in discrepancies in the implementation of the experimental procedure. The advantages of experiments in this chapter are that they are conducted by the same experimenter using consistent methods and protocols. The previous chapters have addressed the effects of the multiple stressors nutrient enrichment and physical disturbance on locations in Australia (Chapter 3) and Scotland (Chapter 4). This chapter draws together results from both chapters and discusses their importance.

5.2 Hypotheses

H1 –The two UK systems will have greater microphytobenthos (MPB) biomass than the two Australian systems

H2 – While species will differ, diversity will be greater in Australian estuaries than UK estuaries due to the lower salinity variation

H3 – The response of the MPB and macrofauna to stressors will differ between continents due to prevailing nutrient loadings

H4 – MPB will be limited by nutrients in Australian systems and so respond more strongly to nutrient enrichment than MPB in UK systems

H5 – Physical disturbance will be detrimental to macrofauna in both systems, particularly with respect to nutrient limiting sites in Australia

H6 – Sediment stability will show region-specific responses to stressors due to the varied responses of the organisms present.

5.3 Methodology

5.3.1 Study sites

Two sites were selected in both the UK and Australia to analyse the comparative effects of multiple stressors on Australian estuarine benthic systems. These were Botany Bay and Lane Cove River in Australia, and the Eden and Tay estuaries in Scotland (see Chapter 2 for full site descriptions).

5.3.2 Experimental design

Plots were manipulated in a two factor crossed design investigating the impacts of physical disturbance (through raking sediment) and nutrient enrichment as described in the previous chapters. At each site the experiment lasted 4 months.

5.3.3 Sampling

Sediment was collected and treated, as described in Chapter 2, for use in quantifying the organic content (Chapter 2.4.2), water content (Chapter 2.5.3), wet bulk density (Chapter 2.5.4), macrofauna (Chapter 2.6), microphytobenthos through spectrophotometric and handheld remote sensing techniques (Chapter 2.7) and the cohesive strength meter (CSM) measure of sediment stability (Chapter 2.5) . Samples were taken from different areas within the plot to maintain independence of samples among sampling times. Sediment for quantifying organic content and MPB as well as the remote sensing measure of the normalised difference vegetation index (NDVI) were taken monthly. Macrofaunal cores were collected at 3 time points – prior to experimental manipulations, two months following manipulations, and 4 months following manipulations.

A low pressure storm event prevented access to plots at a single time point at the Lane Cove site, 1 month following manipulations and prevented samples being taken (see Chapter 3). A green algal bloom smothered the site in the Eden halfway through the experiment just prior to the 2 month measurement (see Chapter 4 for discussion of effects of bloom). Measurements were taken at this site by carefully removing loose algae. Due to equipment failure in Australia, the CSM measurement could only be taken in Botany Bay at the end of the experiment following four months of stressor addition . There were no measurements of sediment stability prior to experimental manipulations at this site.

5.3.4 Statistics

Pre-existing differences in abiotic and biotic variables between locations (Scotland, Australia) and sites within locations were assessed using two-way nested PERMANOVAs. These were conducted on organic matter content, chlorophyll a content of surface sediments, NDVI, and, for macrofauna, multivariate community structure based on the species and the phyla composition, Pielou's evenness index, Shannon diversity index, total within core abundance and species richness. Univariate PERMANOVAs were run on Euclidean distance matrices, while multivariate PERMANOVAs used Bray Curtis dissimilarity matrices. PRIMER software was used to calculate the diversity indices, Pielou's evenness and Shannon diversity, for fauna. Prior to each analysis, variables were tested for heterogeneity of variance (PERMDISP – multivariate; Bartletts test – univariate) and normality of the data (Shapiro–Wilk test). Macrofauna composition data analysed using PERMANOVA were square root transformed prior to analysis to down-weight the effect of species abundance. Where the number of possible permutations was lower than 100, Monte Carlo testing was run to generate p-values from constructed asymptotic permutation distributions for the pseudo-F statistic (Anderson et al., 2008).

Taxa in common between sites were counted and Sørensen Index (Sørensen, 1948) calculated for each taxonomic level to calculate between-site measures of beta diversity using the equation:

QS = ((2 X C) / (A + B)

Where A and B are the taxa in each location, and C is the number of taxa in common. The index is limited between 0 and 1 where 1 indicates sites are taxonomically identical.

Four way PERMANOVA analyses, with the factors -nutrients and physical disturbance (each at three levels – 0 low and high), and with site (Botany Bay, Lane Cove, Eden and Tay) nested within location (Australia, UK) were run on the variables listed above, using the methods previously described, as well as univariate analyses on individual species or phyla abundances. For the environmental variables NDVI, chlorophyll concentration and organic content, month was used as a fifth factor. This factor contained 3 levels; 2, 3 and 4 months after the application of stressors. The 1st month after stressor application was removed from analysis due to lack of replication in the Australian sites. The macrofauna analysed were taken from the fourth month of the experiment only. Following analyses, pairwise post hoc tests were conducted to examine sources of significant treatment effects.

5.4 Results

5.4.1 Site differences

There were environmental and biological differences between sites in Scotland and in Australia. Organic contents within sediments were greater at each of the two sites in the UK sites than the two sites in Australia but significant differences between the sites within locations (Pseudo-$F_{(2,177)}$ = 76.38 , p(perm) = 0.001) prevented a location effect being evident (Figure 5. 1: Pseudo-$F_{(1,177)}$ = 3.30 , p(MC) = 0.209). Similarly, total chlorophyll concentration displayed differences in sites within each location (Pseudo-$F_{(2,185)}$ =40.62, p(perm) = 0.001), over and above which a significant location effect could not be detected (Figure 5. 2; Pseudo-$F_{(1,185)}$ =9.41, p(MC) = 0.113) despite higher concentration at each of the sites in the UK than the two sites in Australia. The surface measurement of chlorophyll (NDVI: Figure 5. 3) displayed no differences between locations

($\text{Pseudo-F}_{(1,250)} = 0.05$, p(MC) = 0.838) however there were clear site differences within each location ($\text{Pseudo-F}_{(2,250)} = 28.72$, p(perm) = 0.001).

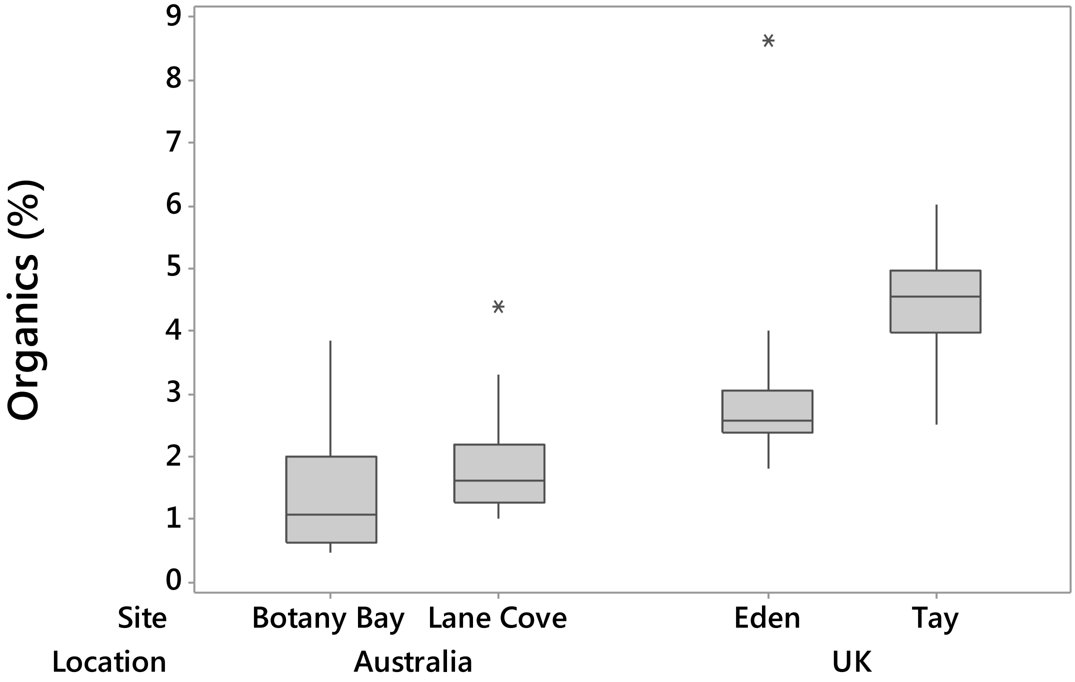


Figure 5. 1: Sediment organic content (%) at sites in Botany Bay (N=20) and Lane Cove (N = 25) Australia and the Eden (N = 70) and the Tay (N = 70) estuaries in the UK prior to experimental manipulation. Boxplot shows median values (middle line), 50th percentile values (box) and 90th percentile values (vertical line). * denotes outliers

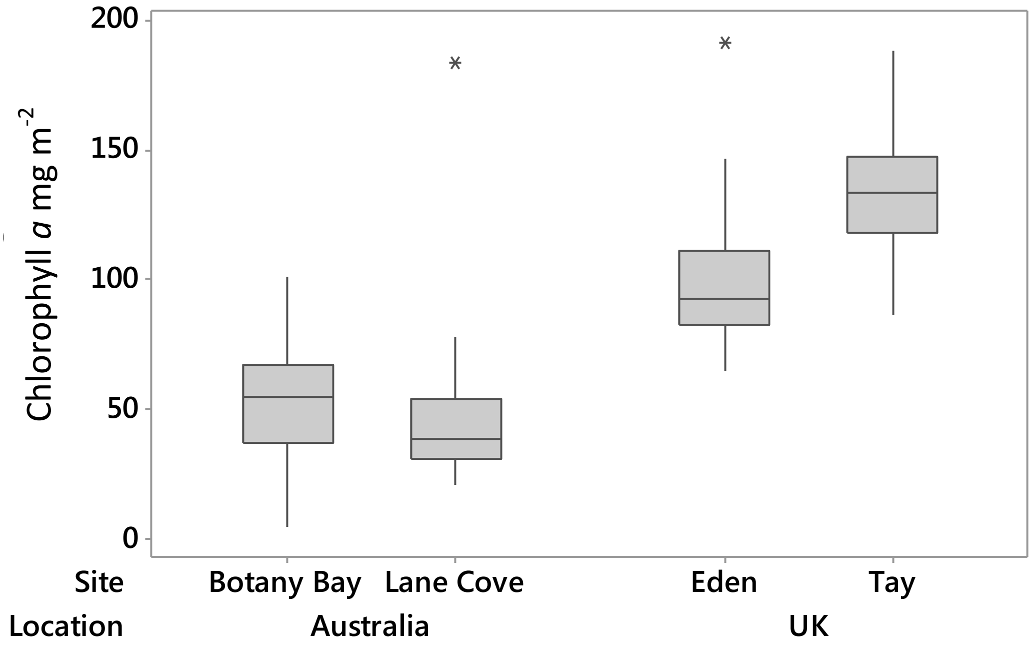


Figure 5. 2: Chlorophyll concentration (mg m^{-2}) of sediments at sites in Botany Bay (N=23) and Lane Cove (N = 25) and the Eden (N = 70) and Tay (N=70) estuaries in the UK prior to experimental manipulation. Boxplot shows median values (middle line), 50th percentile values (box) and 90th percentile values (vertical line). * denotes outliers

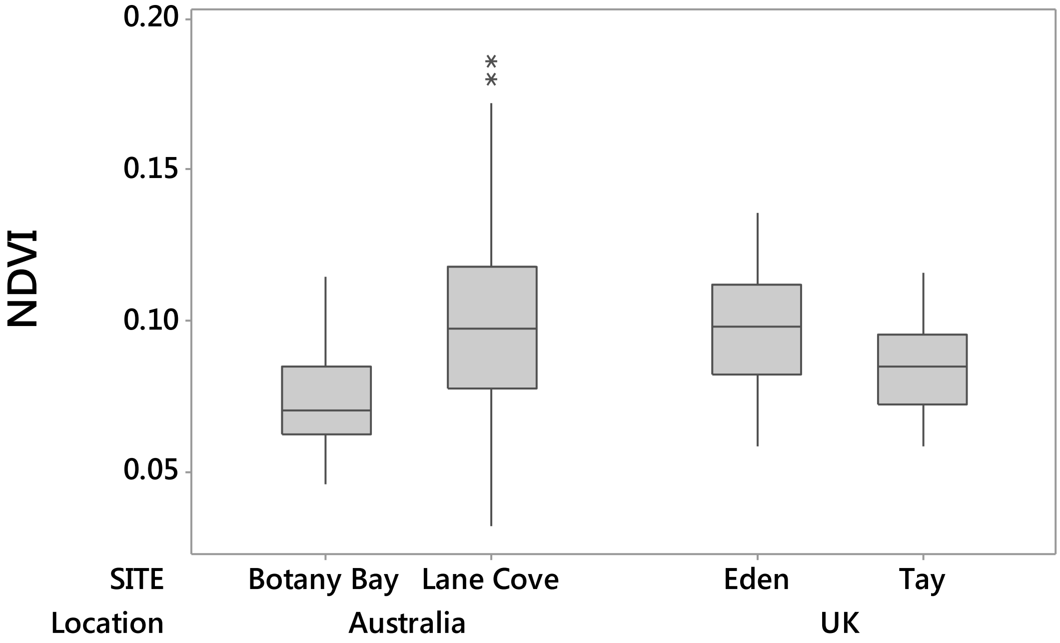


Figure 5. 3: Normalised difference vegetation index (NDV) of sediment in sites in Botany Bay and Lane Cove sites in Australia and the Eden and Tay estuaries in the UK prior to experimental manipulation. N = 70 Boxplot shows median values (middle line), 50th percentile values (box) and 90th percentile values (vertical line). * denotes outliers

The species richness of invertebrates was greater in the UK than Australia (Figure 5.4 main effect of location; Pseudo-$F_{(1,275)}$ = 27.1, p(MC) = 0.026). There was greater variation in species richness between plots in Australia (Figure 5. 4) than between plots in the UK reflecting the greater species richness at the site level of Australian than UK estuaries – total of 47 taxa in Botany Bay, 46 in Lane Cove, 19 in the Eden and 15 in the Tay. There was no between site variation (Pseudo-$F_{(2,275)}$ = 0.61, p(perm) = 0.534). The greatest abundances were found in the Eden, followed by the Tay. The sites in Australia had much lower abundances of macrofauna than those in the UK with plots in Botany Bay containing the lowest abundances of all, although there were no significant differences between locations (Pseudo-$F_{(1,275)}$ = 18.99, p(MC) = 0.052). There were, however, differences between sites (Pseudo-$F_{(2,275)}$ = 22.63, p(perm) = 0.001). There were no differences in Evenness (Pseudo-$F_{(1,275)}$ = 0.83, p(MC) = 0.430) or Shannon Indexes between the two locations (Pseudo-$F_{(1,275)}$ =0.06, p(MC) = 0.828) however, both displayed site specific differences (Evenness: Pseudo-$F_{(2,275)}$ = 37.36, p(perm) = 0.001; Shannon: Pseudo-$F_{(2,275)}$ = 25.21, p(perm) = 0.001.

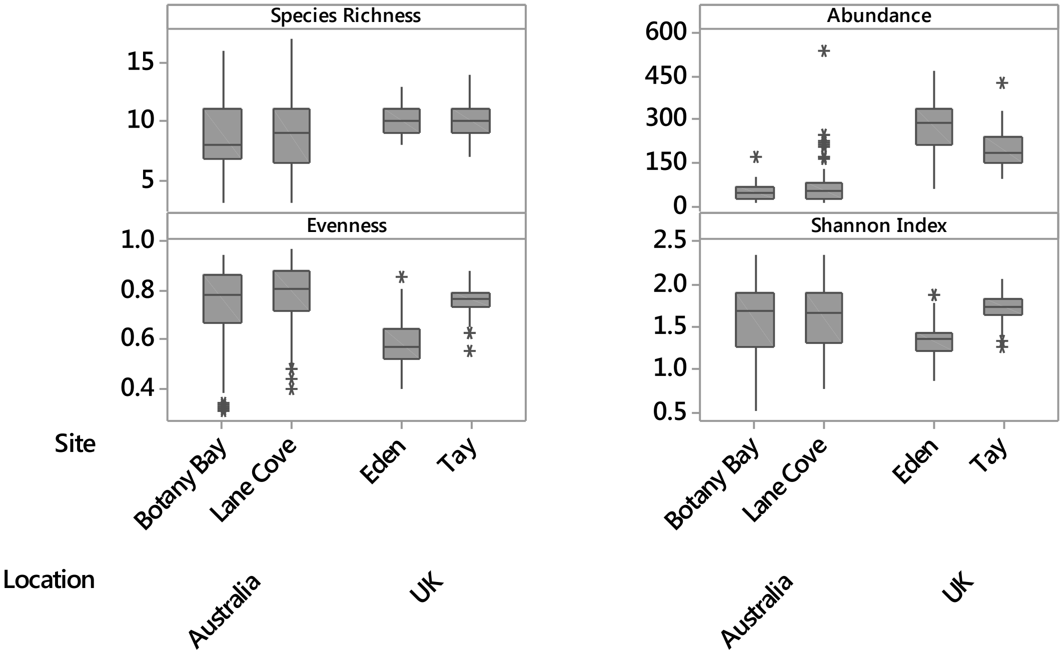


Figure 5. 4: Species richness, total abundance, evenness and Shannon index of the macrofaunal community in sites in Botany Bay and Lane Cove sites in Australia and the Eden and Tay estuaries in the UK prior to experimental manipulation. N = 70 Boxplot shows median values (middle line), 50th percentile values (box) and 90th percentile values (vertical line). * denotes outliers

There were large differences in macrofaunal community composition between Australia and the UK at the level of Class and at finer levels of taxonomic resolution. At the level of Phylum, macrofaunal communities were dominated by annelid, crustacean and mollusc species at each location (Figure 5. 5) but some location -specific differences were apparent (Figure 5. 5; Pseudo-$F_{(1,275)}$ = 7.98, p(MC) = 0.011), and sites within locations also significantly differed (Figure 5. 5; Pseudo-$F_{(2,275)}$ = 27.341, p(perm) = 0.001). There was greater variation within the sites in Australia than in the UK (PERMDISP: $F_{(3,275)}$ = 81.06, p(perm) = 0.001). The variability was greatest in Lane Cove followed by Botany Bay, while both UK sites had similar variability.

The finest level at which differences between sites occurred was at the level of species in Australia (QS = 0.60) while in the UK species taxonomic similarity was still high (QS = 0.82). At the level of Class, similarity was high between the UK and Australia (QS = 0.78) although there were significant differences between the two locations in terms of class community composition (Figure 5. 5; Pseudo-$F_{(1,275)}$ =10.82, p(MC) = 0.002) along with differences between sites within location (Pseudo-$F_{(2,275)}$ = 31.25, p(perm) = 0.001). Sites in the UK had a large proportion of oligochaetes (Figure 5. 5), a group that were not recorded in sites sampled in Australia. The majority of the annelids in the Eden were *Tubificoides* oligochaetes, and approximately half of the annelids present in the Tay were oligochaetes. Additionally, multivariate dispersion of class community composition was greater for the Australian sites than the UK (PERMDISP: $F_{(3,275)}$ = 126.36, p(perm) = 0.001), and all sites were significantly different ($P < 0.05$.)

The finest taxonomic resolution at which major differences in taxonomy between Australia and Scotland began was at the level of Order (QS = 0.54). The two locations displayed no genera or species in common. Annelids were the most diverse group at each site (Figure 5. 5). The Arthropods were dominated by a single species in the UK sites (*Corophium volutator*) whereas in Australia there were high abundances of calanoid copepods as well as numerous *Gammarid* amphipods, other amphipods and crabs (Figure 5. 6). The molluscs in the UK were mainly comprised a single gastropod (*Hydrobia ulvae)* and a bivalve species (*Macoma balthica*). A large proportion of the molluscs in the Australian sites were bivalves, mainly *Mysella vitrea* – with a small proportion of Amphibolidae gastropods (Figure 5. 6).

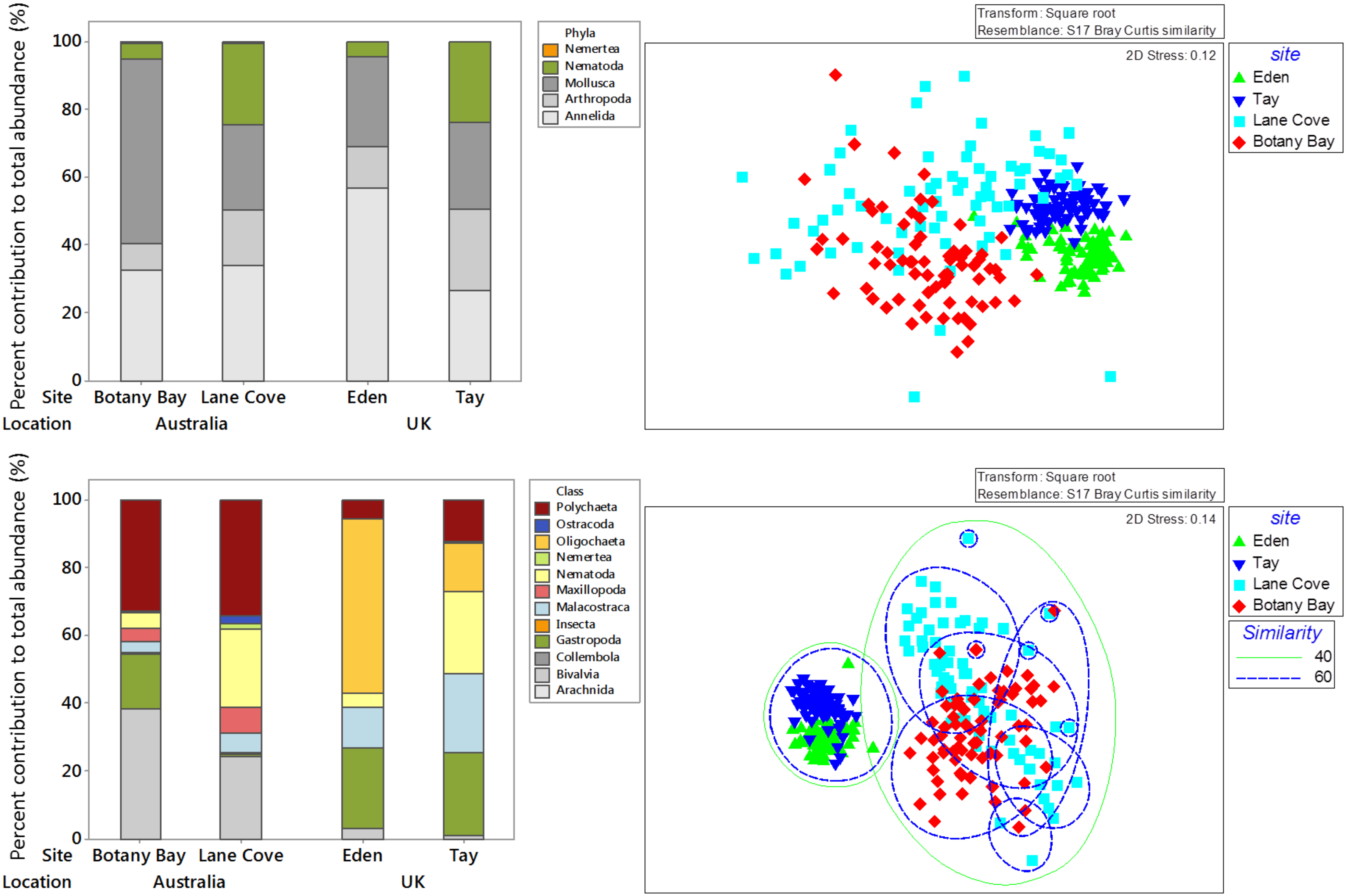


Figure 5. 5: Community composition of macrofauna in sites in Botany Bay (red diamonds) and Lane Cove (Light blue squares) in Australia and the Eden (green upward pointing triangles),and Tay (blue down pointing triangles) in the UK. Multi – panel: top left: The composition, at the level of Phyla; bottom left: The composition at the level of Class; Top right: nMDS plot showing macrofaunal phyla composition; bottom right: nMDS plot showing macrofaunal class composition. N = 70.

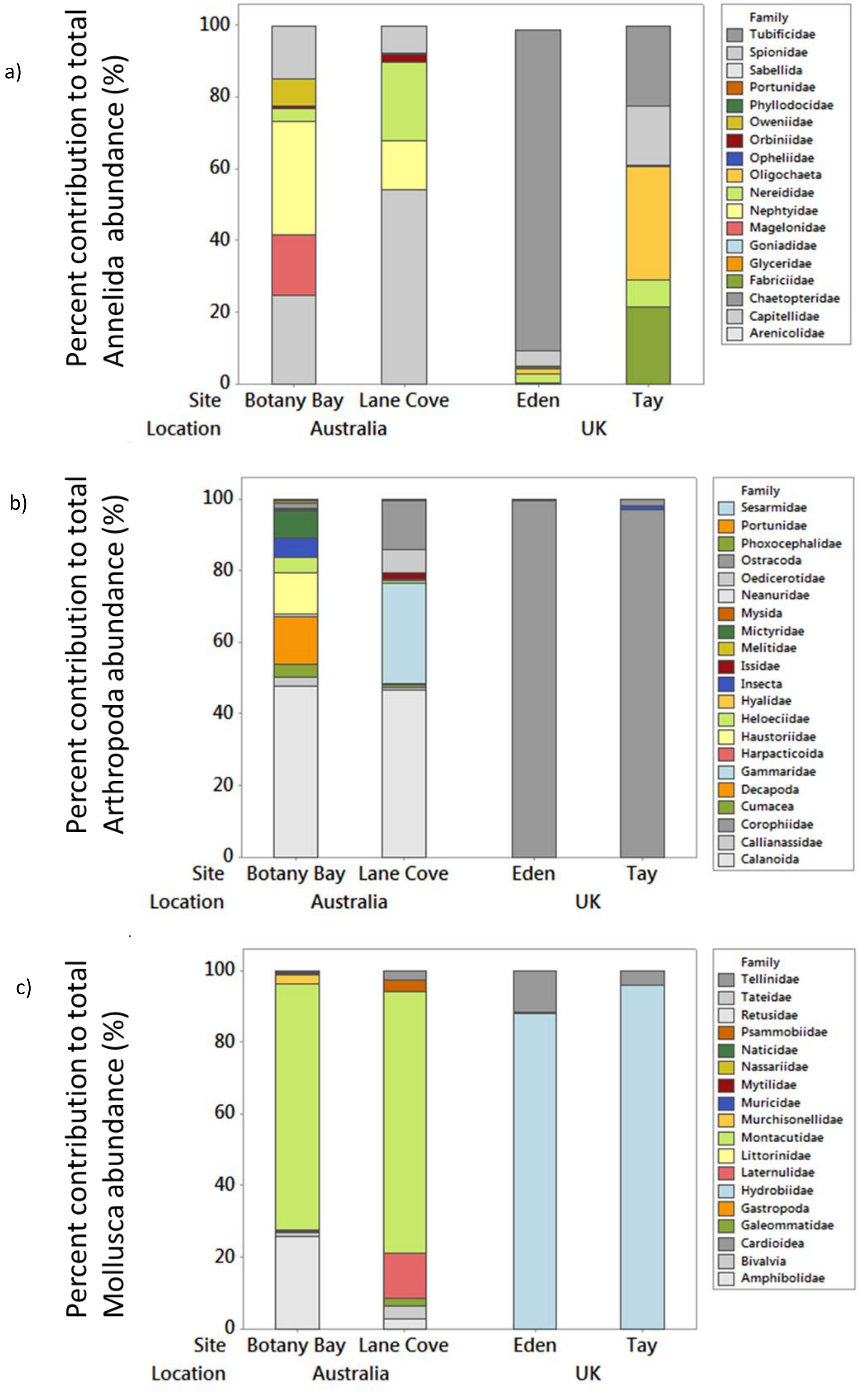


Figure 5. 6: Taxonomic composition of different families of a) Annelids, b) Arthropods and c) Molluscs associated with the macrobenthos from Botany Bay and Lane Cove sites in Austral and Eden and Tay Sites in the UK.

Table 5. 1: Taxonomic similarity of macrobenthos from A) Botany Bay and Lane Cove sites in Australia and B) the Eden and Tay Sites in the UK with further comparisons of Australia and the UK (C). QS = Sørensen Index. Values are based on present knowledge of taxonomic revisions and could change due to future taxonomic revisions.

A)	Botany Bay	Lane Cove	Taxa in common	QS
Phyla	5	5	5	1.00
Class	9	10	9	0.95
Order	23	21	20	0.91
Family	40	33	32	0.88
Genus	42	37	32	0.81
Species	47	46	28	0.60

B)	Eden	Tay	Taxa in common	QS
Phyla	4	4	4	1.00
Class	7	8	7	0.93
Order	12	11	10	0.87
Family	14	12	11	0.85
Genus	15	13	12	0.86
Species	19	15	14	0.82

C)	Australia	UK	Taxa in common	QS
Phyla	5	4	4	0.89
Class	10	8	7	0.78
Order	24	13	10	0.54
Family	41	15	8	0.29
Genus	47	16	0	0.00
Species	65	20	0	0.00

5.4.2 Effects of stressors

The effects of stressors on environmental variables and macrofauna were discussed in detail in previous chapters for both Australia (Chapter 3) and Scotland (Chapter 4). Site effects were summarised (Table 5. 2). In a combined analysis comparing the interactive effects of nutrient enrichment and physical disturbance, between sites and locations, the effect of nutrient enrichment on organic content varied between geographic locations (Table 5. 3; Figure 5. 7). There were no effects of nutrient enrichment in Australia whereas in the UK, plots receiving low nutrient enrichment had significantly greater organic content than plots receiving high enrichment (t = 17.75, p(MC) = 0.039). There were no differences between the un-enriched plots and the low (t = 3.06, p(MC) = 0.192) or high nutrient enrichment plots (t = 2.06, p(MC) = 0.309). Chlorophyll concentration displayed site-specific responses to nutrient enrichment and physical disturbance that were not consistent between locations (sig. NxDxMxS(L) interaction; Table 5. 3). Over and above all, nutrient enrichment had a greater effect than physical disturbance and enhanced chlorophyll *a* concentration. Physical disturbance caused site specific positive or negative changes. The interactions between the two stressors are summarised (Table 5. 2) and explained in full in Chapters 3 and 4.

Table 5. 2: Summary of the response of variables of interest to nutrient enrichment and physical disturbance at sites in the UK (E = Eden, T = Tay) and in Australia (BB = Botany Bay, LC = Lane Cove). Y denotes a significant change for variables that have no directional change or the presence of an interactive effect between stressors. + = positive change. - = negative change. +/- = a positive or negative change depending on month following application of stressors. No sign indicates there was no effect of stressors.

	Nutrient enrichment				Disturbance				Interaction			
	E	T	BB	LC	E	T	BB	LC	E	T	BB	LC
Organic content								+	Y			
Chlorophyll		+	+		-	+	-			Y		
NDVI		+	+	+		+/-	-	+		Y		Y
Macrofauna community			Y	Y	Y	Y						
Macrofauna diversity					-	-						
Total abundance				-								

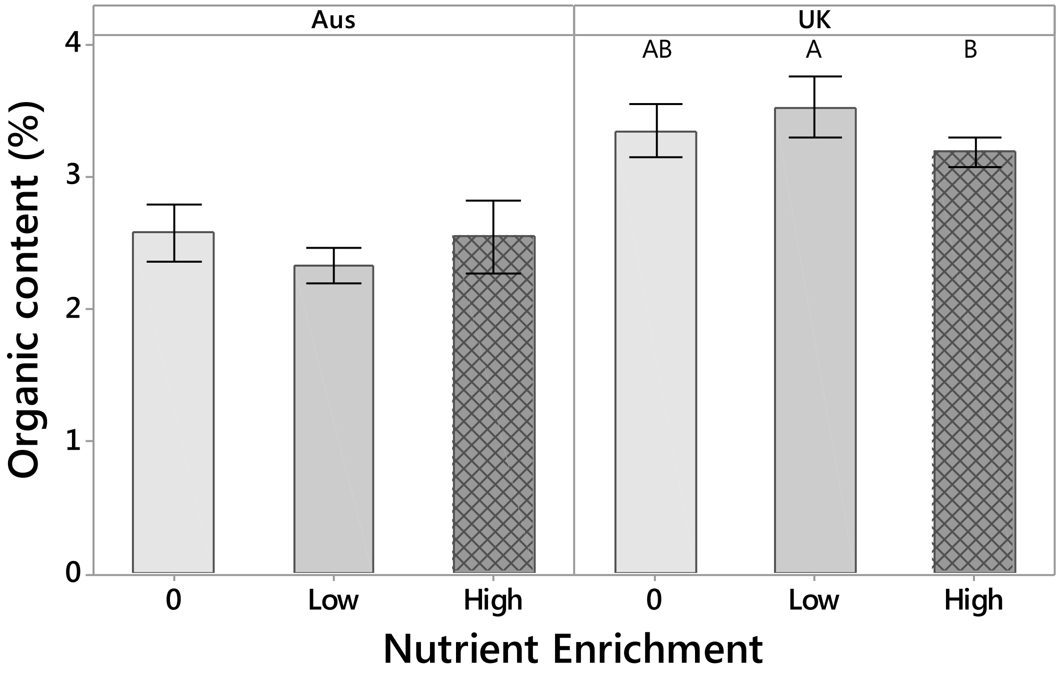


Figure 5. 7 Change in organic content (%) within sediments in two sites in Australia and two sites in the UK due to the influence of nutrient enrichment at 0, low and high levels. Data pooled across sites within country, months and physical disturbance treatments due to these factors not having any confounding effects. Points indicate mean +/- SE. N = 5-7 for all treatment levels. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

NDVI displayed an interaction between nutrient enrichment and physical disturbance that varied between locations (Table 5. 3, Figure 5. 8). In the UK, in plots that were subjected to high disturbance, those that also received high enrichment had greater NDVI than those that were un-enriched (t = 27.79, p(MC) = 0.021), but other effects of nutrient enrichment were not observed at this location or in Australia. Although other patterns were apparent, these were not statistically significant. In the UK, there was a trend for increasing physical disturbance to negatively affect NDVI in plots that were not enriched but to have no effect on those receiving nutrients. In Australia there was a trend for plots that were un-enriched to respond positively to physical disturbance and plots that were enriched to respond negatively.

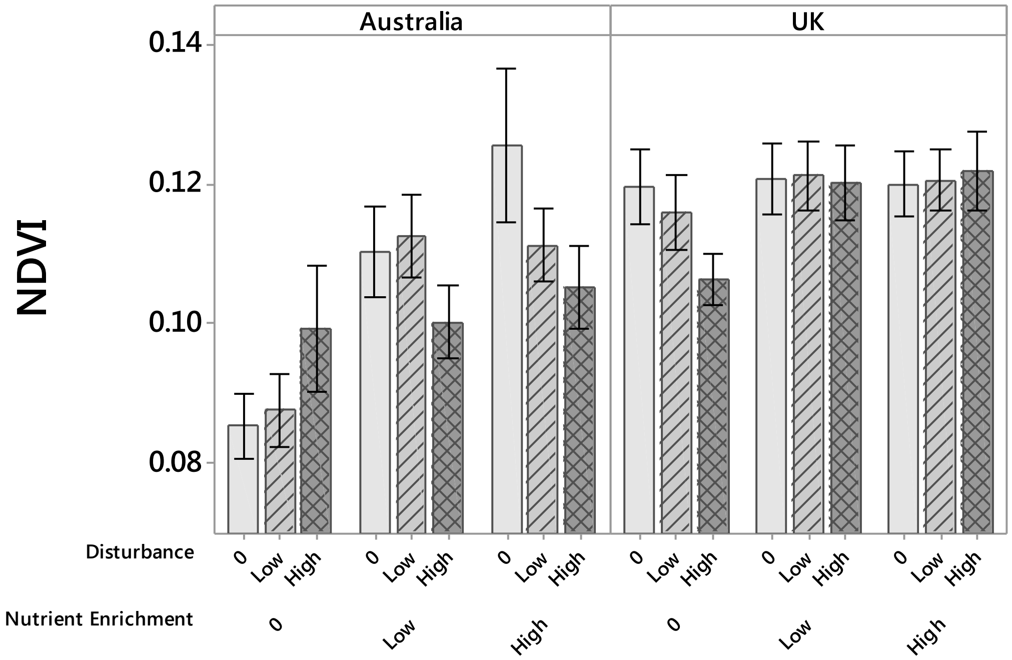


Figure 5. 8: Change in Normalised difference vegetation index (NDVI) within sediments in two sites in Australia and two sites in the UK due to the influence of nutrient enrichment and physical disturbance at 0, low and high levels. Data pooled across sites within country and within factor month due to these factors not having any confounding effects. Points indicate mean +/- SE. N = 5-7 for all treatment levels.

Table 5. 3: Five factor PERMANOVA analysis of the interaction between Nutrients (N), Disturbance (D) , Month (M) and site (S) nested within location (L – Australia or UK) on sediment water content (%), chlorophyll concentration and NDVI in Botany Bay and Lane Cove in Australia and the Eden and Tay estuaries in the UK. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, Low and High), month contained 3 levels. n=5-7 for all treatments levels

	Organic content			Chlorophyll concentration			NDVI		
	df	**Pseudo-F**	**P(MC)**	**df**	**Pseudo-F**	**P(MC)**	**df**	**Pseudo-F**	**P(MC)**
N	2	0.39	0.707	**2**	**8.52**	**0.047**	2	4.22	0.112
D	2	0.16	0.855	2	3.79	0.127	2	0.51	0.631
M	2	2.35	0.207	2	3.62	0.121	2	0.83	0.504
L	1	1.85	0.329	1	272.76	**0.006**	1	1.68	0.353
S(L)	**2**	**16.65**	**0.001**	**2**	**6.33**	**0.002**	**2**	**24.71**	**0.001**
NxD	4	0.82	0.54	4	0.67	0.645	4	0.62	0.656
NxM	4	0.17	0.948	4	0.58	0.692	4	1.00	0.45
NxL	**2**	**9.06**	**0.037**	2	3.05	0.162	2	1.12	0.412
DxM	4	0.29	0.898	**4**	**4.60**	**0.027**	4	0.97	0.463
DxL	2	1.04	0.434	2	0.36	0.737	2	0.03	0.96
MxL	2	0.20	0.831	2	4.97	0.077	2	2.86	0.171
NxS(L)	4	0.14	0.96	4	0.54	0.723	**4**	**3.79**	**0.003**
DxS(L)	4	1.83	0.129	**4**	**3.18**	**0.020**	**4**	**3.24**	**0.017**
MxS(L)	**4**	**2.97**	**0.029**	**4**	**15.02**	**0.001**	**4**	**24.70**	**0.001**
NxDxM	8	1.32	0.279	8	0.68	0.730	8	0.72	0.691
NxDxL	4	0.65	0.633	4	2.67	0.114	**4**	**3.91**	**0.050**
NxMxL	4	0.90	0.507	**4**	**4.54**	**0.031**	4	0.72	0.586
DxMxL	4	0.97	0.475	**4**	**5.60**	**0.024**	4	0.97	0.485
NxDxS(L)	8	1.84	0.067	8	0.70	0.695	8	0.84	0.541
NxMxS(L)	8	0.57	0.805	8	0.74	0.656	8	0.98	0.431
DxMxS(L)	8	1.76	0.075	8	0.69	0.708	8	0.98	0.467
NxDxMxL	8	0.97	0.52	8	1.07	0.413	8	0.61	0.762
NxDxMxS(L)	16	1.18	0.244	**16**	**1.79**	**0.028**	16	1.39	0.130
Residuals	607			620			635		

Key: **Bold** = significant effect at $p < 0.05$

There were differences in the way that macrofaunal diversity of the two locations responded to stressors (Table 5. 4). Interacting effects between location and nutrient enrichment on species richness were apparent (Figure 5. 9), whereby in Australia plots receiving low enrichment had significantly greater numbers of species than either the un-enriched (t = 15.67, p(MC) = 0.049) or highly enriched plots (t = 10.50, p(MC) = 0.046). Additionally in the UK, highly enriched plots had greater numbers of species than the low enriched plots (t = 29.00, p(MC) = 0.022) but not the un-enriched plots (t = 0.86, p(MC) = 0.554). There was no difference between the un-enriched and highly enriched plots (t = 1.31, p(MC) = 0.438). Additionally, there was an interaction between location and physical disturbance (Figure 5. 10). There was no overarching effect of physical disturbance in Australia. In the UK, high physical disturbance resulted in lower species richness in comparison to the undisturbed treatment (t = 25.00, p(MC) = 0.022), but there was no difference between the low disturbed treatment and the control (t = 3.40, p (MC) = 0.181) or the high treatment (t = 4.71, p(MC) = 0.141).

Total abundance displayed site specific responses to nutrient enrichment and physical disturbance that were not consistent between locations (sig. NxDxS(L) interaction; Table 5. 4). These interactions are explained in full in Chapters 3 and 4 and summarised (Table 5. 2).

There was an interaction effect between physical disturbance and location on the Evenness Index (Table 5. 4; Figure 5. 11). Physical disturbance had no effect in the UK whereas in Australia, low physical disturbance resulted in higher evenness than in undisturbed treatments (t = 20.47, p(MC) = 0.034), but with no other treatment effects apparent.

There was an interaction effect between nutrient enrichment and location on the Shannon Index (Table 5. 4; Figure 5. 12). Pairwise comparisons reveal there were no significant differences between levels of nutrient treatments although the low treatment in Australia resulted in higher diversity than either the high or zero treatments. There were negligible differences between nutrient treatments in the UK.

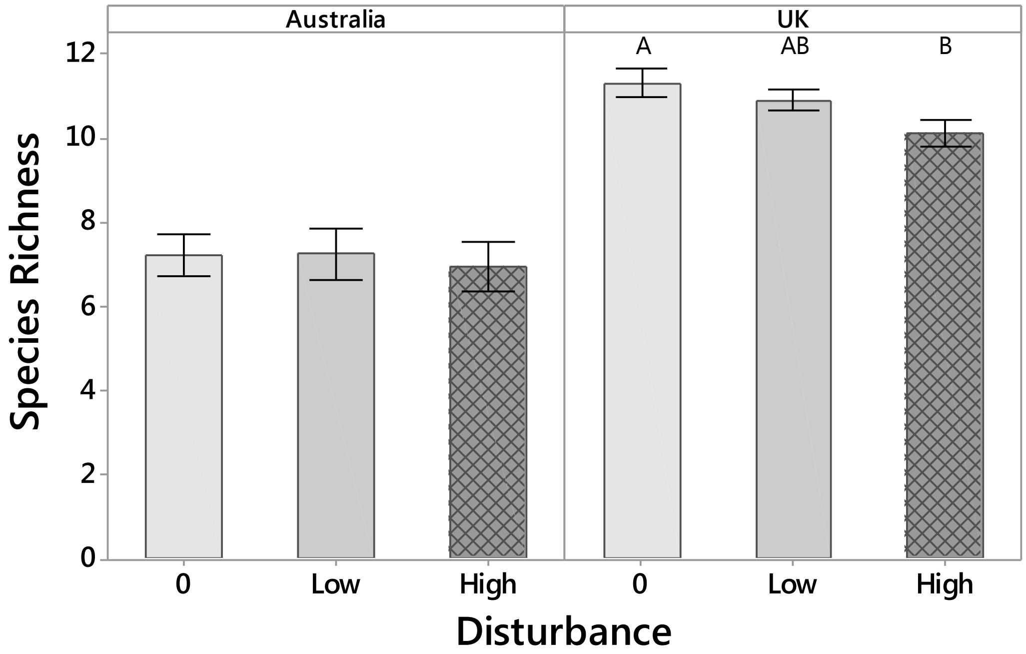


Figure 5. 9: Change in Species richness of the macrofaunal community within sediments in two sites in Australia and two sites in the UK due to the influence of nutrient enrichment at 0, low and high levels. Data pooled across sites within country and physical disturbance treatments due to these factors not having any confounding effects. Points indicate mean +/- SE. N = 5-7 for all treatment levels. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

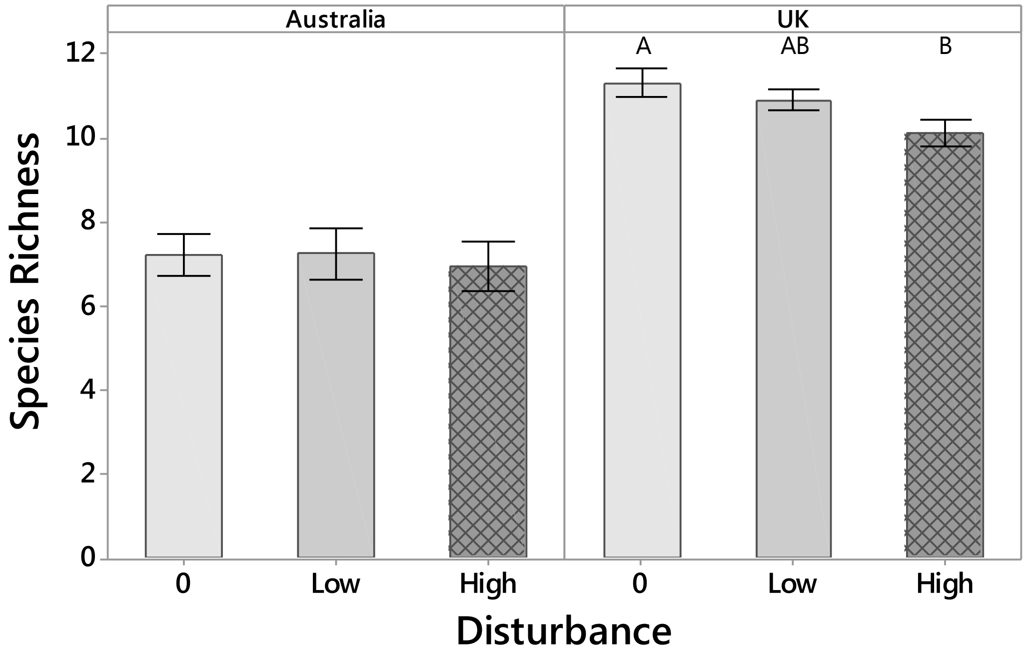


Figure 5. 10: Change in Species richness of the macrofaunal community within sediments in two sites in Australia and two sites in the UK due to the influence of physical disturbance at 0, low and high levels. Data pooled across sites within country and nutrient enrichment treatments due to these factors not having any confounding effects. Points indicate mean +/- SE. N = 5-7 for all treatment levels. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

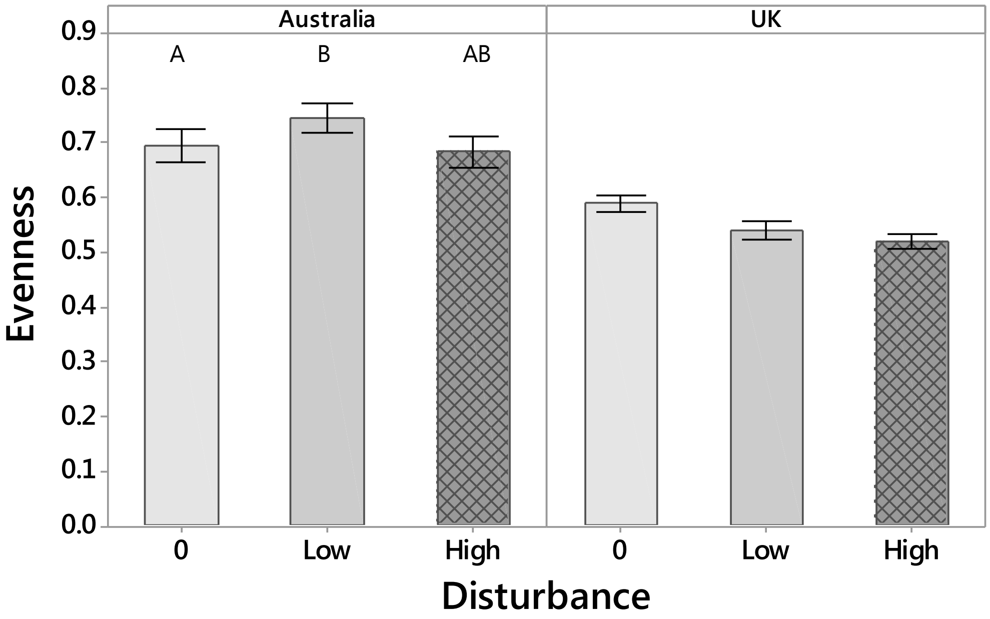


Figure 5. 11: Change in evenness of the macrofaunal community within sediments in two sites in Australia and two sites in the UK due to the influence of physical disturbance at 0, low and high levels. Data pooled across sites within country and nutrient enrichment treatments due to these factors not having any confounding effects. Points indicate mean +/- SE. N = 5-7 for all treatment levels. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

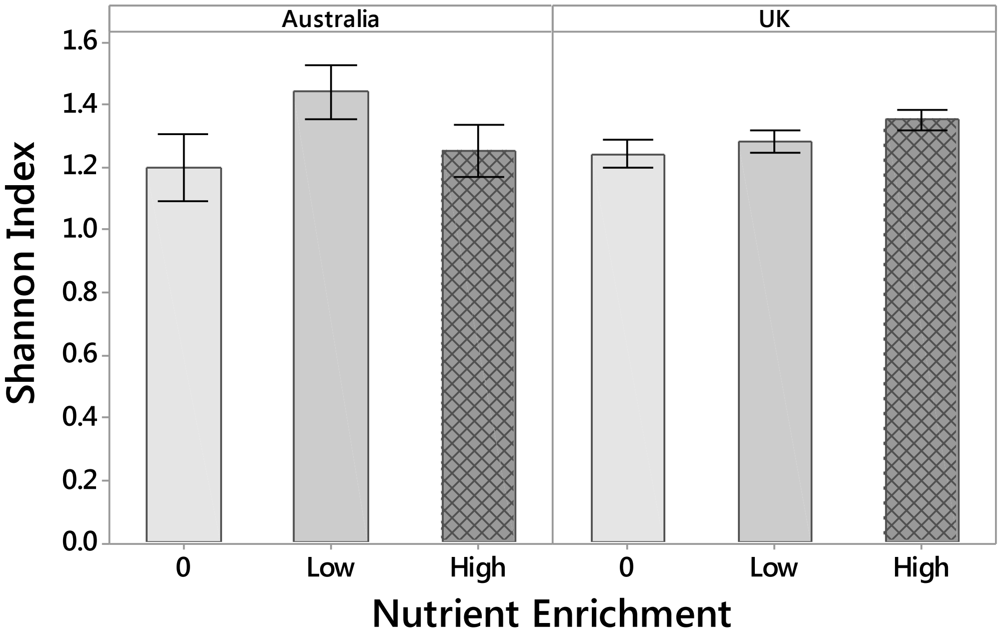


Figure 5. 12: Change in Shannon diversity of the macrofaunal community within sediments in two sites in Australia and two sites in the UK due to the influence of nutrient enrichment at 0, low and high levels. Data pooled across sites within country and physical disturbance treatments due to these factors not having any confounding effects. Points indicate mean +/- SE. N = 5-7 for all treatment levels. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

Table 5. 4 – Four way PERMANOVA analysis of the interaction between Nutrients (N), Disturbance (D) , and site (S) nested within location (L – Australia or UK) on univariate macrofaunal species richness, plot abundance, evenness and Shannon diversity, and multivariate analysis of community structure in Botany Bay and Lane Cove in Australia and the Eden and Tay estuaries in the UK. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, Low and High). n=7 for all treatments levels

		Species richness		**Abundance**		**Evenness**		**Shannon diversity**		**Community structure**	
Source	**df**	**Pseudo-F**	**P(perm)**	**Pseudo-F**	**P(perm)**	**Pseudo-F**	**P(perm)**	**Pseudo-F**	**P(perm)**	**Pseudo-F**	**P(perm)**
N	2	4.27	0.103	0.68	0.548	1.87	0.249	2.12	0.251	1.15	0.366
D	2	**23.91**	**0.007**	**5.91**	**0.050**	5.26	0.067	5.16	0.085	1.09	0.406
L	1	3.42	0.188	**241.59**	**0.008**	8.39	0.100	0.14	0.726	**5.53**	**0.026**
S(L)	2	**35.68**	**0.001**	2.39	0.095	**8.90**	**0.001**	**46.64**	**0.001**	**68.72**	**0.001**
NxD	4	1.26	0.336	0.35	0.851	0.78	0.573	1.95	0.193	1.21	0.379
NxL	2	**19.7**	**0.004**	0.30	0.778	1.08	0.397	**9.29**	**0.031**	1.21	0.379
DxL	2	**8.34**	**0.037**	3.14	0.155	**6.51**	**0.048**	3.93	0.108	1.04	0.460
NxS(L)	4	0.13	0.979	0.84	0.501	0.52	0.730	0.32	0.865	**1.36**	**0.022**
DxS(L)	4	0.08	0.991	0.86	0.494	0.37	0.825	0.72	0.567	**1.50**	**0.007**
NxDxL	4	2.01	0.181	0.72	0.594	0.51	0.721	0.57	0.678	1.18	0.304
NxDxS(L)	8	0.74	0.633	**1.95**	**0.047**	0.85	0.589	0.67	0.705	0.86	0.866
Residuals	216										

Key: **Bold** = significant effect at $p < 0.05$

The four factor analysis on macrofaunal species community structure revealed significant interactions between sites and stressors that varied among sites (Table 5. 4; Figures 5. 13 and 5.14). At the UK sites, there were no significant effects of nutrients on macrofaunal community structure (Figure 5. 13). In Lane Cove the high nutrient treatment was significantly different to the low treatment (Figure 5. 13; t = 1.36, p = 0.038), but other pairwise differences between nutrient treatments were not significant. In Botany Bay, the high nutrient treatment was significantly different to the control (t = 1.53, p = 0.037), but the other nutrient treatments did not differ.

Effects of physical disturbance on macrofaunal communities were identified in three of the four estuaries (Figure 5. 14). In the Eden, both the high (t = 2.08, p = 0.002) and low (t = 1.89, p = 0.002) physical disturbance treatment significantly differed in their macrofaunal communities to the controls, but the low and high physical disturbance treatments did not differ (t = 1.47, p = 0.065). In the Tay, the community structure in the high disturbance treatment was significantly different to that of the controls (t = 2.10, p = 0.002) whereas there was no difference between the low physical disturbance treatment and the controls (t = 1.18, p = 0.176) or the low and high disturbance treatments (t = 1.28, p = 0.146). In Botany Bay, the community structure in the high disturbance treatment was significantly different to that of the controls (t = 1.49, p = 0.048) and the low treatment (t = 1.45, p = 0.033), but there was no difference between the undisturbed and the low disturbance treatments (t = 0.68, p = 0.849). There was no effect of physical disturbance in Lane Cove (p> 0.05).

Taking into account the taxonomically similarity of the sites at higher taxonomic levels, the four factor multivariate nutrient and physical disturbance design was applied to the phyla community structure. The results suggest that there was an interaction between stressors that was dependent on location (Sig NxDxL Pseudo-$F_{(4,216)}$ = 2.10, p(MC) = 0.042). There were 5 Phyla of macrofauna common to UK and Australian systems, but as the nemerteans were found in limited numbers across the sites no comparisons could be made. Arthropods displayed no differences in abundance among nutrient or physical disturbance treatments or their interaction (Table 5. 5; Figure 5. 15).

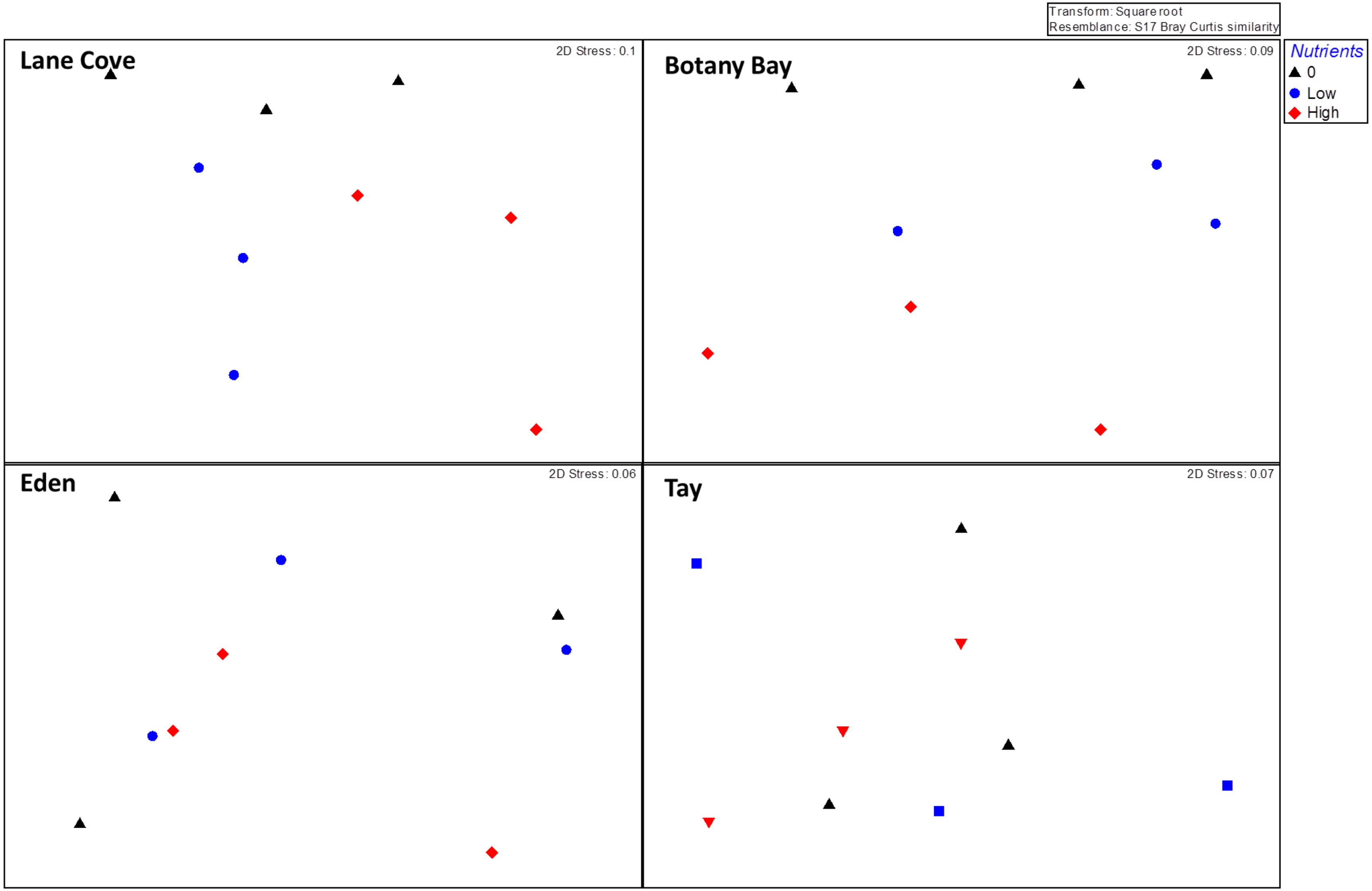


Figure 5.13: nMDS plot displaying macrofaunal composition in the Eden and Tay estuaries in the UK and Botany Bay and Lane Cove River in Australia following 4 months of zero low and high nutrient enrichment and physical disturbance. Each symbol represents central median value for each treatment. Colour and symbol represents level of nutrient enrichment: 0 = black triangle, low = blue circle, high = red diamond.

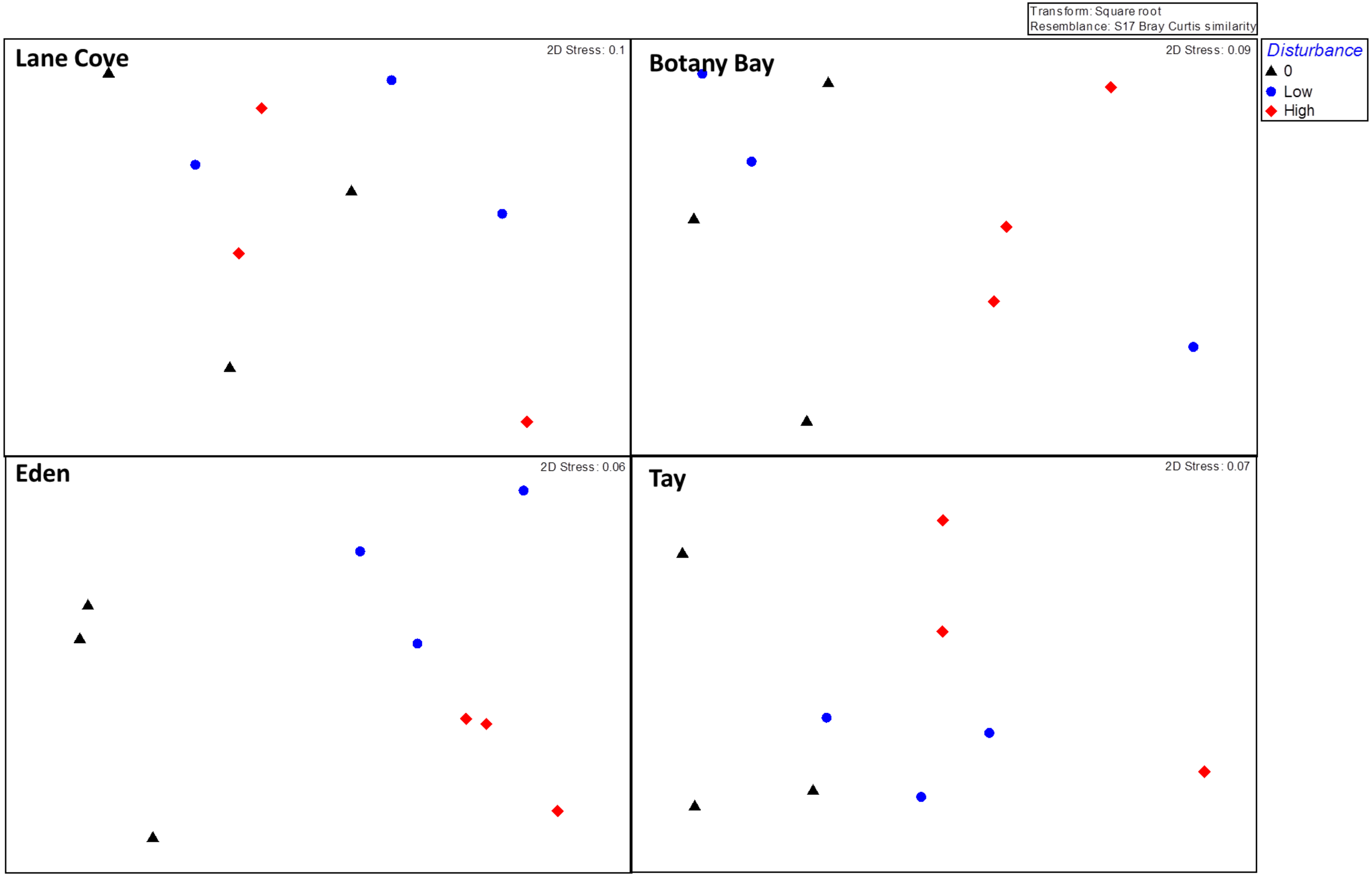


Figure 5.14: nMDS plot displaying macrofaunal composition in the Eden and Tay estuaries in the UK and Botany Bay and Lane Cove River in Australia following 4 months of zero low and high nutrient enrichment and physical disturbance. Each symbol represents central median value for each treatment. Colour and symbol represents level of physical disturbance: 0 = black triangle, low = blue circle, high = red diamond.

Molluscs displayed site-specific responses to physical disturbance that were not consistent within locations (Table 5. 5; Figure 5. 16). Pairwise tests reveal that in the Eden both the highly disturbed (t = 3.70, p(perm) = 0.003) and low disturbance (t = 3.49, p(perm) = 0.002) plots contained significantly fewer molluscs than the undisturbed plots, but there was no difference in mollusc abundance between the two disturbed treatments (t = 0.57, p = 0.578). At none of the other three sites was an effect of physical disturbance observed.

Annelids also displayed site-specific responses to the stressors (Figure 5. 17). Pairwise tests reveal that in the Eden, within plots that were subjected to low physical disturbance, un-enriched plots had significantly greater abundances of annelids than the low enriched (t = 2.83, p(perm) = 0.020) or the highly enriched plots (t = 3.68, p(perm) = 0.009) which, in turn, did not significantly differ (t = 0.32, p(perm) = 0.795). There was no effect of nutrient enrichment under any other level of physical disturbance. Also within the Eden estuary, among plots subjected to zero nutrients, the high disturbance treatment had significantly lower annelid abundances than plots receiving low (t = 4.75, p(perm) = 0.001) or no (t = 1.83, p(perm) = 0.094) physical disturbance, but there was no difference between low or undisturbed plots (t = 0.49, p = 0.631). In the Tay, within plots subjected to no nutrient addition, high physical disturbance caused a significant decrease in annelids in comparison to the undisturbed plots (t = 2.01, p(perm) = 0.046). There was no difference between the low physical disturbance treatment with either the high (t = 1.49, p(perm) = 0.172) or the undisturbed treatments (t = 0.67, p(perm) = 0.526). In the Tay when plots were subjected to high nutrient enrichment, plots that were also subjected to high physical disturbance contained lower abundances than those also subjected to zero physical disturbance (t = 2.27, p(perm) = 0.015), with pairwise comparisons indicating no other differences between disturbance treatments. No effects of the stressors on annelids were found in Australia.

Nematodes displayed a location specific response due to disturbance (Table 5. 5; Figure 5. 18). Although pairwise tests revealed no significant difference, there was a non-significant trend for increasing levels of physical disturbance treatments to decrease nematode abundance in the UK but not in Australia.

Table 5. 5: Four way PERMANOVA analysis of the interaction between Nutrients (N), Disturbance (D) , and site (S) nested within location (L – Australia or UK) on abundance of Arthropods, Molluscs, Annelids and nematodes per plot in Botany Bay and Lane Cove in Australia and the Eden and Tay estuaries in the UK. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, Low and High). n=7 for all treatments levels

		Arthropods		**Molluscs**		**Annelids**		**Nematodes**	
Source	**df**	**Pseudo-F**	**P(MC)**	**Pseudo-F**	**P(MC)**	**Pseudo-F**	**P(MC)**	**Pseudo-F**	**P(MC)**
N	2	0.04	0.963	0.50	0.651	1.08	0.407	2.11	0.227
D	2	0.86	0.533	2.45	0.188	3.77	0.135	**13.40**	**0.018**
L	1	**34.28**	**0.025**	18.98	0.053	1.71	0.332	6.24	0.135
S(Lo)	2	**8.98**	**0.001**	**4.39**	**0.017**	**84.59**	**0.001**	**25.54**	**0.001**
NxD	4	0.54	0.722	1.02	0.467	1.00	0.438	0.99	0.443
NxL	2	0.25	0.823	0.11	0.902	0.48	0.676	1.27	0.383
DxL	2	0.60	0.593	0.96	0.489	2.15	0.249	**13.74**	**0.017**
NxS(L)	4	0.39	0.830	1.20	0.301	2.30	0.056	0.23	0.934
DxS(L)	4	0.30	0.903	**3.38**	**0.013**	0.84	0.505	0.67	0.595
NxDxL	4	0.78	0.559	2.51	0.131	0.49	0.740	0.80	0.579
NxDxS(L)	8	0.92	0.493	0.62	0.746	**2.25**	**0.032**	1.84	0.064
Residuals	216								

Key: **Bold** = significant effect at $p < 0.05$

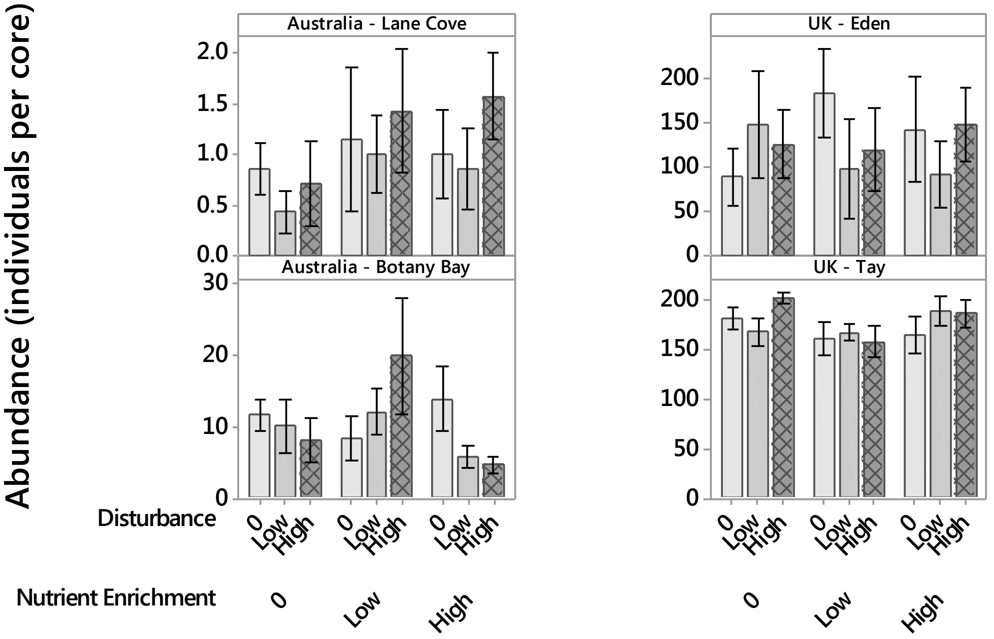


Figure 5. 15: Abundance of arthropods among plots receiving no, low or high nutrient enrichment, and physical disturbance at the end of a 4 month experiment, replicated in Australian and UK estuaries Points indicate mean +/- SE. N = 7

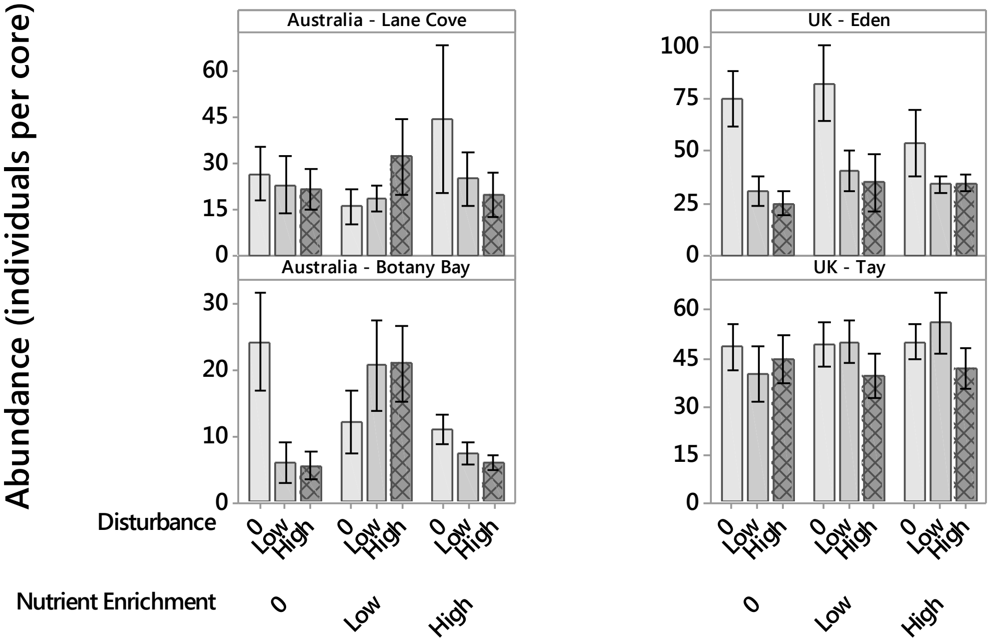


Figure 5. 16: Abundance of molluscs among plots receiving no, low or high nutrient enrichment, and physical disturbance at the end of a 4 month experiment, replicated in Australian and UK estuaries Points indicate mean +/- SE. N = 7

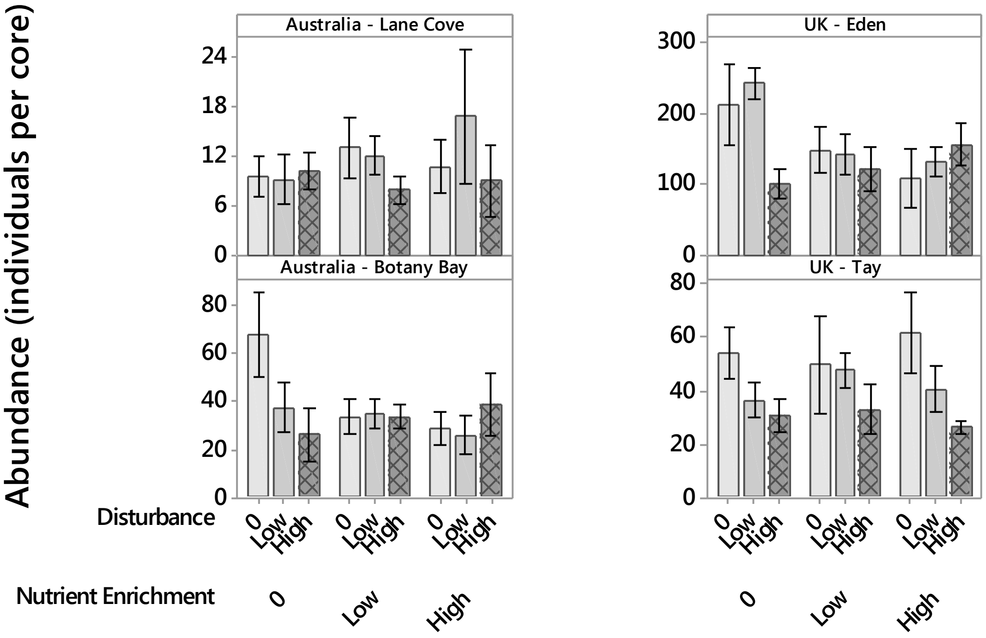


Figure 5. 17: Abundance of annelids among plots receiving no, low or high nutrient enrichment, and physical disturbance at the end of a 4 month experiment, replicated in Australian and UK estuaries Points indicate mean +/- SE. N = 7

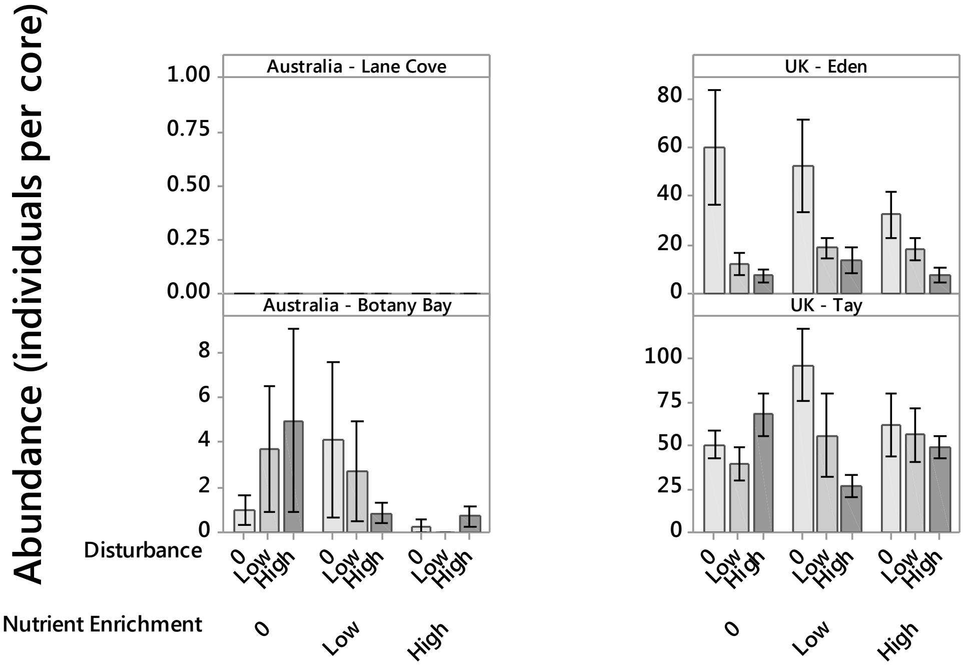


Figure 5. 18: Abundance of nematodes among plots receiving no, low or high nutrient enrichment, and physical disturbance at the end of a 4 month experiment, replicated in Australian and UK estuaries Points indicate mean +/- SE. N = 7

5.4.3 Sediment stability

Changes In sediment stability due to treatment effects was compared in Botany Bay in Australia and the Tay in the UK (Figure 5. 19). At each site there was a main effect of physical disturbance only (Botany Bay: Pseudo-$F_{(2,48)}$ = 3.18, p = 0.047; Tay: Pseudo-$F_{(2,48)}$ = 3.19, p = 0.040). There were no effects of nutrient enrichment (Botany Bay: Pseudo-$F_{(2,48)}$ = 1.25, p = 0.281; Tay: Pseudo-$F_{(2,48)}$ = 0.05, p = 0.943) or an interaction between stressors at either site (Botany Bay: Pseudo-$F_{(4,48)}$ =0.69, p = 0.599; Tay: Pseudo-$F_{(4,48)}$ =0.30, p = 0.857). In Botany Bay (Figure 5. 18a) disturbance caused a decrease in sediment stability in comparison to undisturbed plots. While there was no differences between the controls and the low disturbance treatment (t = 0.78, p = 0.436), sediment stability in the high disturbance treatment was lower than both the control (t = 2.35, p = 0.025) and the low treatment (t = 2.01, p = 0.052). In the Tay, the opposite effect was observed where increased physical disturbance caused an increase in sediment stability (Figure 5. 19b). While there were no significant differences between the control treatment and the high disturbance treatment (t = 1.35, p = 0.169) there were also no differences between the low treatment and the high treatment (t = 1.19, p = 0.247) yet plots subjected to low physical disturbance had significantly greater sediment stability that the controls (t = 2.53, p = 0.018).

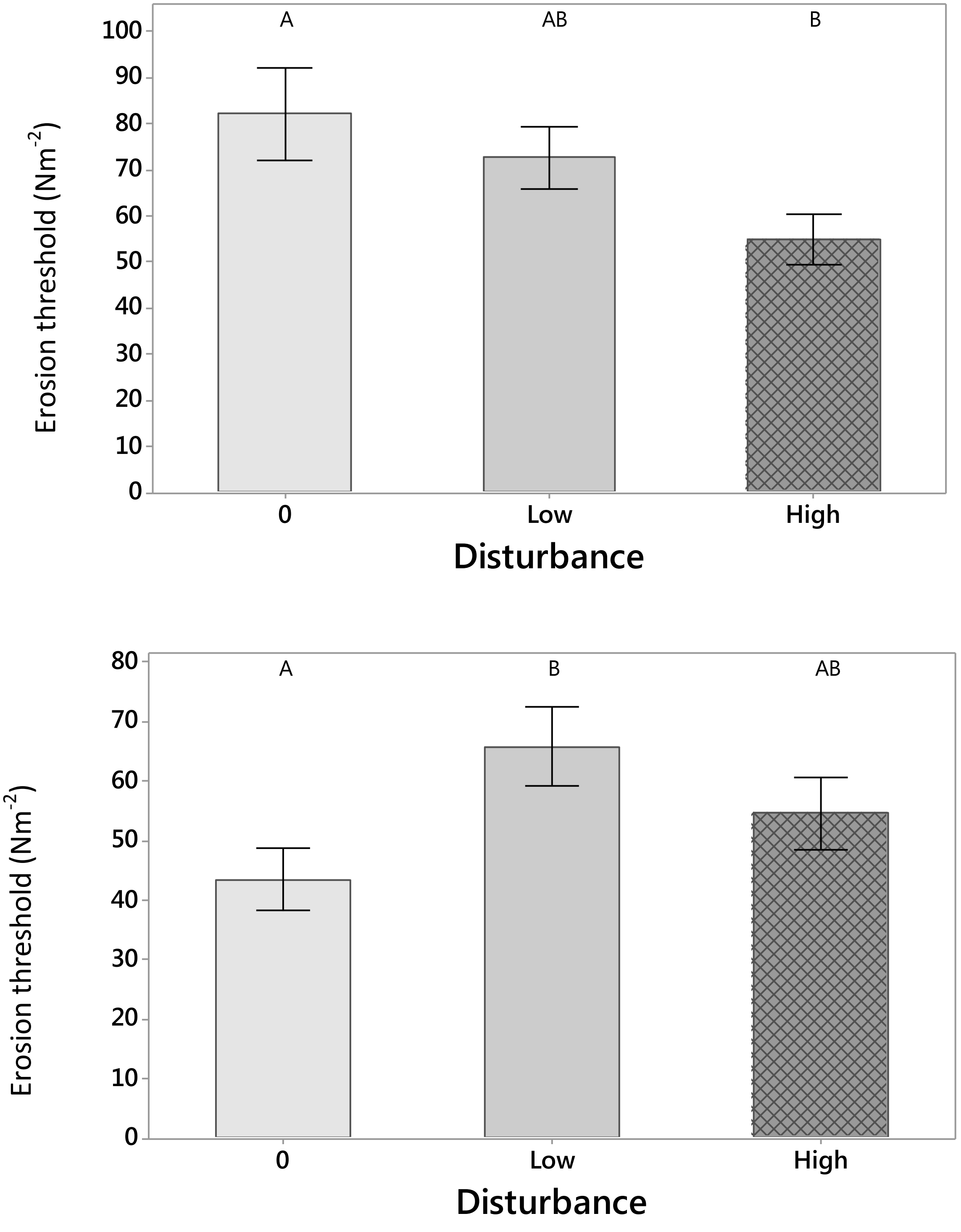


Figure 5. 19 Cohesive Strength Meter (CSM) derived erosion thresholds in plots of a) Botany Bay in Australia and b) Tay in the UK receiving zero, low or high levels of physical disturbance after 4 months of stress. Data pooled across nutrient enrichment treatments that did not significantly differ. Points indicate mean +/- SE. N= 6-7

5.5 Discussion

This chapter draws together experiments replicated in Scotland and Australia that examined the multiple effects of nutrient enrichment and physical disturbance on benthic intertidal communities. At each of the two locations, the composition and prevailing environmental conditions were very different, and consequently differences in responses to stressors were expected. The context specificity of community response to multiple stressors has been documented in numerous studies (Bishop & Kelaher, 2013; Gladstone-Gallagher et al., 2014; Whomersley et al., 2010), and chapters in this thesis have also highlighted the importance of local environmental context in determining a community response. Multiple stressor effects have not before been analysed in very different parts of the world as part of a comparative study. Through comparing estuaries within each location and across geographic regions the question of whether the overarching response to stressors was greater between regions or due to localised environmental context was examined.

While the MPB displayed site-specific responses to stressors, the macrofaunal community displayed differing responses according to their location, either in Australia or Scotland. Australian macrofaunal communities were more susceptible to the effects of nutrient enrichment while in Scotland, the effects of physical disturbance were of greater importance in determining community structure. When effects of the stressors on community structure were considered at the level of phyla, thereby removing the effect of differing species being present at each location, there were location-specific non-additive effects of physical disturbance and nutrient enrichment. Further analysis revealed location specific interactive effects of the two stressors on nematodes whereas other phyla followed site specific responses. Additionally the combined effects of stressors on sediment stability varied depending on location, however only one site at each location was compared.

5.5.1 Geographic differences in environmental conditions

It was hypothesised that due to the more oligotrophic status of east Australian estuaries, their greater marine, reduced riverine influence and the climatic differences between the locations substantial differences in environmental conditions would be found between Australia and the UK. Consistent with expectations that Australian estuaries might be less productive than UK estuaries due to the greater nutrient limitation, the sediments within the two Australian estuaries had a much lower organic content than sediments in the UK estuaries.

The two methods used to assess microphytobenthos (MPB) in the four estuaries revealed differing spatial patterns. Overall, contact coring indicated higher chlorophyll *a* concentrations in surface sediments in Scottish than Australian estuaries, although large differences between sites dominated analyses. By contrast, the normalised difference vegetation index (NDVI) measurement which assesses vegetation cover over larger areas and is generally considered more representative, did not provide evidence of differences in MPB among locations. The lack of location effects exhibited in the NDVI measurements were unexpected given previous observations that east Australian estuarine systems have lower water column chlorophyll concentrations (Scanes et al., 2007), and lower numbers of sediment diatoms than European systems (Inglis, 1996), consistent with their more oligotrophic status. MPB are known to be spatially and temporally variable (Murphy et al., 2008; Orvain et al., 2012) and so comparing two sites per geographic location at a single time point does not give a complete picture. Due to logistical reasons the time of sampling differed between the two locations – winter in Australia and summer in the UK. However these times coincide with maxima in annual MPB biomass in the respective locations (Murphy et al., 2009) so was a valid comparison.

According to the Remane model, estuaries are species-poor systems (Remane & Schlieper, 1971; Whitfield et al., 2012) but of high productivity (Levin et al., 2001). In Australian estuaries, however, the species richness of macrofauna can be much higher than the global average due to the more stable salinity regimes associated with their relatively small freshwater inputs and marine-dominated conditions (Dye & Barros, 2005; Powis & Robinson, 1980; Whitfield et al., 2012). In this study, upwards of 50 species were identified in Australia but fewer than 20 in the UK. The high numbers of species are not unique to the two Australian sites observed here with such high values observed in many temperate Australian systems (see Hutchings, 1999).

Diversity, as measured using Shannon Index and Evenness measures, did not differ between the UK and Australia. In the UK, species richness was greater than in Australia even though the number of species was being drawn from a smaller pool at the site-scale. In the Australian systems the majority of species identified within plots were represented by a few individuals only. This indicates a greater number of rarer species in Australian sites. These patterns of uniform biodiversity but non uniform faunal assemblages have been noted in other Australian studies (e.g. Barnes, 2014).

Within-location similarity was greater at all taxonomic levels than between-location similarity although there was high similarity associated with the phyla and class of organisms present between each location. Taxonomically all sites were similar at the level of phylum although multivariate analysis of the phyla community structure revealed differences between sites. Among the many factors that determine biodiversity is the supply of energy and resource availability (Currie, 1991; Hall et al., 2000). While resource availability is a more limiting factor in Australian estuarine systems in terms of nutrient inputs (Beadle, 1962; Heip et al., 1995), the sites located in this study were fringing mangroves areas which introduce a source of organic enrichment. These areas are known to be more diverse than bare sediments (Hutchings, 1999).

5.5.2 Response to nutrient enrichment and physical disturbance

Estuarine benthic systems in the UK and Australia were subjected to nutrient enrichment and physical disturbance resulting in context specific responses. It was hypothesised that nutrient enrichment would increase MPB biomass in each location, but that the multiple effects of this and the physical disturbance stressor would be non-additive as nutrient enrichment has been noted to react synergistically with other stressors (Strain et al., 2014). As in other studies, this study found that effects of nutrient enrichment were modified by the effect of disturbance. While in the previous chapters there were interactions between the two stressors, and the effect of nutrient enrichment dominated the MPB response, in this combined study the response of the MPB varied between sites and locations. Generally nutrient enrichment caused an increase in MPB biomass. This effect was less prevalent in UK systems where nutrient enrichment appeared to negate the negative effects of physical disturbance only. The Australian systems are oligotrophic therefore these results concur with known paradigms that MPB productivity is enhanced where nutrient resources are limiting (Hall et al., 2000; Morris & Keough, 2003b; Verhoeven et al., 2012).

Increased nutrient additions may also influence higher trophic levels with increased exposure to higher nutrient conditions leading to increased grazing pressure and alteration of community structure and function (Pascal et al., 2013). In this study, nutrient enrichment only affected the macrofauna in Australia, where an increase in abundances and diversity was observed. Nutrient enrichment had limited effects on any phyla although there were site specific responses on the annelids in the UK sites. Nutrient additions are documented to cause an increase in biodiversity at sites that are nutrient limited whereas at sites that have abundant background nutrient loadings there has been documented a significant decrease in biodiversity (Morris & Keough, 2003b).

One mechanism that has been proposed to explain these patterns is based on the resource heterogeneity hypothesis (RHH) (Tilman, 1987). The RHH states that a landscape with low uniform resource availability will be able to sustain only a few species. As habitat quality increases so too does the spatial variability and resource diversity, meaning these systems are able to support higher diversities due to a greater patchiness of the environment. Further increases in habitat quality flip this relationship and the heterogeneity of the environment decreases causing a decrease in overall biodiversity. This reduction in heterogeneity is due to a reduction in patchiness of the environment caused by the increase in uniform favourable habitat characteristics. This lowered heterogeneity facilitates competitively dominant species to take over, lowering diversity. With respect to nutrient enrichment studies, the RHH predicts that further enrichment will only increase diversity at sites that with low initial productivities, while enrichment will result in lower diversity at sites that are already rich in resources. The predictions of the hypothesis are consistent with the observed response identified in Raffaelli and Emmerson (2001) and Hall et al. (2000) where plots were manipulated with fertiliser in nutrient poor sites in Australia and a nutrient rich site in the UK. Further, the predictions are consistent with the response identified in the Australian sites in this study. By contrast, nutrients had very little effect in the UK sites. Previous studies have found that the response of macrofauna to nutrient enrichment is highly context specific (Whomersley et al., 2010) and may take at least 4 months to become apparent in nutrient rich systems (Cebrian et al., 2012; Raffaelli & Emmerson, 2001).

Physical disturbance negatively affected community structure in UK sites and had a small effect in Botany Bay. While in the UK there was a decline in diversity due to this stressor there was no such effect in Australia. This could be due to the greater number of total species present within each Australian site. Disturbance has a negative effect on burrowing species (Cowie et al., 2000; Hall & Harding, 1997; Hall, 1994; Whomersley et al., 2010) and the act of raking sediment causes the damage or exclusion of larger bodied organisms (Brown & Herbert Wilson, 1997; Hall & Harding, 1997). In the UK, there was a much smaller pool of species present at each site which could replace ones that were excluded from disturbed plots, and for this reason we could be observing declines in species richness and diversity in the UK but not in Australia.

Analysis of macrofaunal community structure at the taxonomic resolution of phyla, the identity of which did not vary between locations, revealed that there was an interaction between stressors affecting the community structure. As with analyses conducted at finer taxonomic resolution, the analysis at coarser resolution revealed that physical disturbance was the more important stressor in the UK and enrichment in Australia. Many impacts on the phyla were site specific. While disturbance reduced abundances of annelids and molluscs, the magnitude of change varied between sites. Environmental context is an important factor in determining how organisms will respond to stress. The response of individual species can depend upon the prevailing environmental conditions (Hall & Harding, 1997; Whomersley et al., 2010)

Sediment stability was affected by physical disturbance only, both in the Tay and in Botany Bay, however the direction of change was not the same. In the Australian site, the sediment stability decreased with increasing disturbance whereas in the UK the opposite effect occurred. The positive effect of physical disturbance on sediment stability in the UK was discussed in detail in Chapter 4 and was attributed to the removal of macrofauna and destruction of burrows which destabilise undisturbed sediments (Underwood & Paterson, 2003). The opposite effect observed in the Australian site could be attributed to the species present. Again the physical disturbance at this site acted to change the community structure and as there was a greater number of species at this site, others could have moved in and taken over creating new burrows, facilitating a decrease in sediment stability.

5.5.3 Importance of study

How systems in different parts of the world respond to stressors has implications on management practices around the world. Australian estuaries have typically been managed based on practices derived from northern hemisphere estuaries (Hutchings, 1999). It is apparent from this study that there are profound differences in the way these systems will react to stress and local management practices should take precedence over those that have been borrowed from elsewhere. Multiple stressors may have interacting effects that are not easy to predict or interpret. Potential synergies between stressors have profound implications for the type of management strategies that would be needed at the local scale (Brown et al., 2013). While not all harmful stressors interact to cause negative impacts, for example UV-B alleviates harmful effects brought about due to a reduction in pH and oil pollution in marine systems (Coelho et al., 2015), unpredictable negative effects are more likely to occur (Brown et al., 2013; Crain et al., 2008; Darling & Côté, 2008). While this study has interpreted large scale geographic differences based upon a limited number of study sites there is precedence to upscale manipulative experiments of analysing changes to ecosystem functioning (Lohrer et al., 2015).

5.6 Conclusions

The combined effects of the multiple stressors nutrient enrichment and physical disturbance were compared in sites in Australia and Scotland. While the MPB displayed site specific responses to stressors over and above distinct regional patterns, the macrofaunal community displayed regional specific responses due to the main effects of stressors, this, therefore, highlights the need for location specific management practices. At all taxonomic levels nutrient enrichment had a greater effect on the macrofauna in Australia whereas the act of physically disturbing sediments disrupted the macrofaunal community in the UK. These location specific effects are important to understand in the wider context of multiple stressor studies as many generalities are inferred from studies in single locations or multiple sites within a region. While the context specificity of responses to stressors is becoming a prevailing concept in multiple stressor studies, this current study emphasises that the responses to stressors can alter between regions to a greater degree than due to localised environmental context.

Table 5.6: Verification of hypotheses set at the beginning of the chapter

Hypotheses	**Accept ✓ or reject X**
H1 –The two UK systems will have greater microphytobenthos (MPB) biomass than the two Australian systems	✓
H2 – While species will differ, diversity will be greater in Australian estuaries than UK estuaries due to the lower salinity variation	✓
H3 – The response of the MPB and macrofauna to stressors will differ between continents due to prevailing nutrient loadings	✓
H4 – MPB will be limited by nutrients in Australian systems and so respond more strongly to nutrient enrichment than MPB in UK systems	**X – site specific responses were observed**
H5 – Physical disturbance will be detrimental to macrofauna in both systems, particularly with respect to nutrient limiting sites in Australia	**X**
H6 – Sediment stability will show region-specific responses to stressors due to the varied responses of the organisms present.	✓

6 The sequence and timing of multiple stressors and their effects on sediment communities

6.1 Introduction

Stressors rarely occur singularly in nature (Halpern et al., 2008) and there is growing realisation that their combined effect is not as simple as interpreting additive responses (Crain et al., 2008; Przeslawski et al., 2014; Sundback et al., 2010). Stressors may interact in synergistic or antagonistic ways, with the nature of the interaction dependant on the intensity and/or type of the stressors (Vye et al., 2015). Earlier in this thesis, the nature of interactions when selected stressors occurred simultaneously was considered in detail. Although simultaneously occurring stressors are common, for example rainfall influencing nutrients (Birch et al., 2010) or runoff causing heavy metals to wash into estuaries (DeLorenzo et al., 2012; Drapper et al., 2000), there is not always synchrony between co-occurring stressors (e.g. Ferrier et al., 2001). For example, a system could become physically disturbed by anthropogenic activity and a later rainfall event could flush additional nutrients into the system, thereby causing a second stressor at a different point in time.

While the asynchrony of stressors is a relatively new topic in ecological studies, the medical literature is rife with work examining the effects of subsequent stressors, drugs or psychological disorders following an initial stimulus (Antelman, 1988; Antelman et al., 2000; van der Sijs et al., 2009). The time dependent co-administration of drug interactions has been discussed in detail in van der Sijs et al. (2009) whereby the effects of a second drug was assessed following the prior application of an initial drug. Additionally, stressor-induced sensitisation to subsequent stress has also been documented due to certain forms of medications (e.g. antidepressants), psychological disorders or trauma (Antelman et al., 2000; Barr et al., 2002; Yui & Ikemoto, 2004). The mechanism behind this is a form of remembered response to the initial stressor (Antelman, 1988; Antelman et al., 2000), whereby the organism or pathway (e.g. neurological, chemical) can become sensitised to a subsequent stress. This has been termed time-dependant sensitisation. In these scenarios a stressful stimulus is taken as one that causes a reaction creating a behavioural or neurological response from an organism (Antelman, 1988). These studies detail the response at the individual or cellular level, whereas ecological studies typically consider an entire community or population. In ecology, these types of physiological responses at the individual level can be scaled up, affecting the community as a whole (e.g. Hanson & Stark, 2011). However care should be taken as within a community, the population dynamics may dampen or enhance individual level effects.

The concept behind these experiments can be used to generate hypotheses analysing the temporal inconsistancies in the application of multiple stressors in ecological systems. It has been previously identified that certain environmental stressors modify the sensitivity of a system to further stress. For example warming can modify a systems response to further toxicant or nutrient pollution (Alsterberg & Sundbäck, 2013; Sundbäck et al., 2007). Another example is hydrodynamic stress armouring sediments to subsequent stress that would otherwise lead to erosion (Gomez, 1994; Parker & Sutherland, 1990; Reed et al., 1999). The majority of studies examining the effects of multiple stressors employ experiments where the application of stressors was simultaneous; therefore an unstressed state becomes challenged immediately by two stressors. Realistically, a sequential application of stressors would be more likely, any delay between stressors would allow for temporary adaptations to occur (e.g. Clavier et al., 2005). Through these adaptations, an ecosystem could reach a new stable state (Thrush et al., 2012). Through addition of a second type of stress, the already stressed system could be pushed over a threshold of stress resistance, resulting in ecosystem collapse.

García Molinos and Donohue (2010) were among the first to demonstrate that the temporal patterns of stressors determined how they interacted. Their findings suggest that synchrony between stressors does not always lead to the greatest impacts, highlighting the importance of studying asynchronous as well as synchronous disturbances. Many empirical studies have focussed on controlling the total amount of applied disturbances over an experimental period while fluctuating the temporal pattern in which stressors impact the environment (Figure 6.1; Berga et al., 2012; García Molinos & Donohue, 2010, 2011; Maggi et al., 2012; Oliveira et al., 2014; Pincebourde et al., 2012). The drawback of these experiments is that they alter the temporal variability over which stressors are applied; they do not address the sequential impact of multiple stressors. While experimenters make a contrived attempt to standardise the total number of disturbance events, in natural systems (as with the medical literature discussed above) the relative frequencies of multiple stressors are not always the same. In nature both single (Hillebrand et al., 2010; Murray et al., 2013; Whomersley et al., 2010) and multiple stressors (Fitch & Crowe, 2011; Rodil et al., 2013; Sundback et al., 2010) are known to have a major impact on community structure. What is not known is whether the order or sequence in which stressors are applied influences their impact on the dynamics of the system. Further, the combined impacts of multiple stressors when applied at different temporal scales are likely to display non-additive interactions due to the variation in temporal application of stressors (García Molinos and Donohue, 2010).

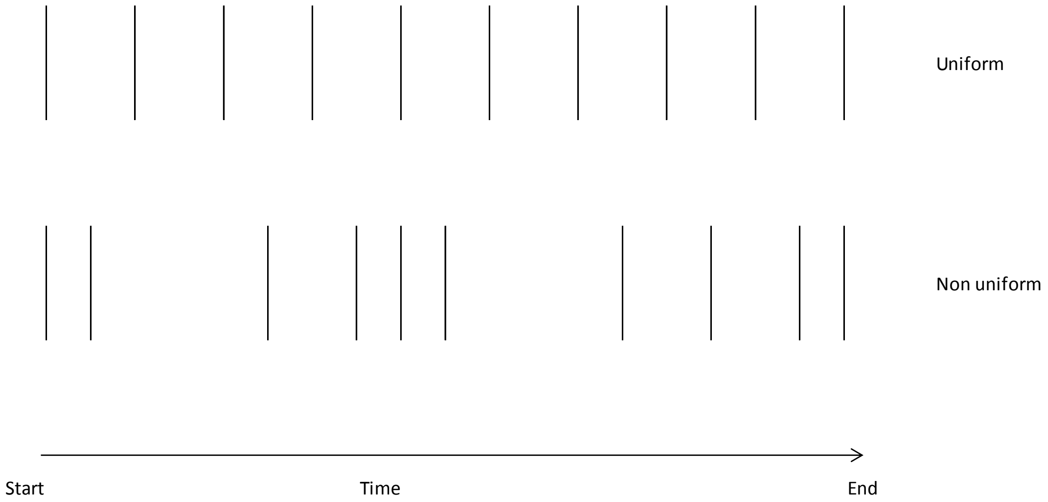


Figure 6.1: Example of manipulations of temporal patterns of multiple stressors applied asynchronously. This design maintains the total number of disturbance events for each stressor (vertical line, n = 10 over experimental period) and time from first and last application of each stressor (uniform and non-uniform application of stressor) over the experiment. Each vertical line represents the application of a stressor. In this example two stressors were applied – a uniform stressor applied at regular intervals and a non-uniform stressor applied at irregular intervals.

Urbanised estuaries are subjected to multiple stressors throughout the year. These areas are continuously modified by both anthropogenic and climatic forcing's, although all forces do not necessarily act upon them continuously or simultaneously. Nutrient enrichment and physical disturbance of sediments are two main forms of stressor that may act on estuarine benthic communities. Storms and runoff deliver nutrients from urbanised catchments to estuarine systems (Baron et al., 2012; Cloern, 2001), in some instances leading to eutrophication (Cloern, 2001; Nixon, 1995; Paerl, 2006). Propeller scars, boat wakes, anchor drag or bait digging physically disturb estuarine sediments (Bell et al., 2002; Bishop, 2005; Wynberg & Branch, 1994). These two types of disturbance co-occur but because they are not directly linked may occur simultaneously or asynchronously.

While recent research has shown that unpredictable, non-additive effects are likely to occur when the application of multiple stressors is asynchronous (García Molinos & Donohue, 2010, 2011), there has been little attention given to understanding what happens if the order in which stressors are applied is altered. The aims of this study are to assess the order in which stressors are applied, with the application of a second stressor being applied after a two month delay. The following questions are to be assessed:

1 – *What effect does adding a second stressor have to the system experiencing an initial stressor?*

2 – *Does the order in which stressors are applied affect the final effect on community structure?*

3 –*Is there a difference between the synchronous and asynchronous application of multiple stressors?*

6.2 Hypotheses

The following null hypotheses will be examined:

H1 – A second stressor will have no effect on the system due to the overriding effect of the first stressor.

H2 – The order in which stressors are applied will have a significant effect on the community, the effect of stressors will be less important than the order in which they are applied.

H3 – There will be a significant difference between subjecting the system to stressors simultaneously or at different times (synchronous vs asynchronous application).

6.3 Methods

6.3.1 Experimental design

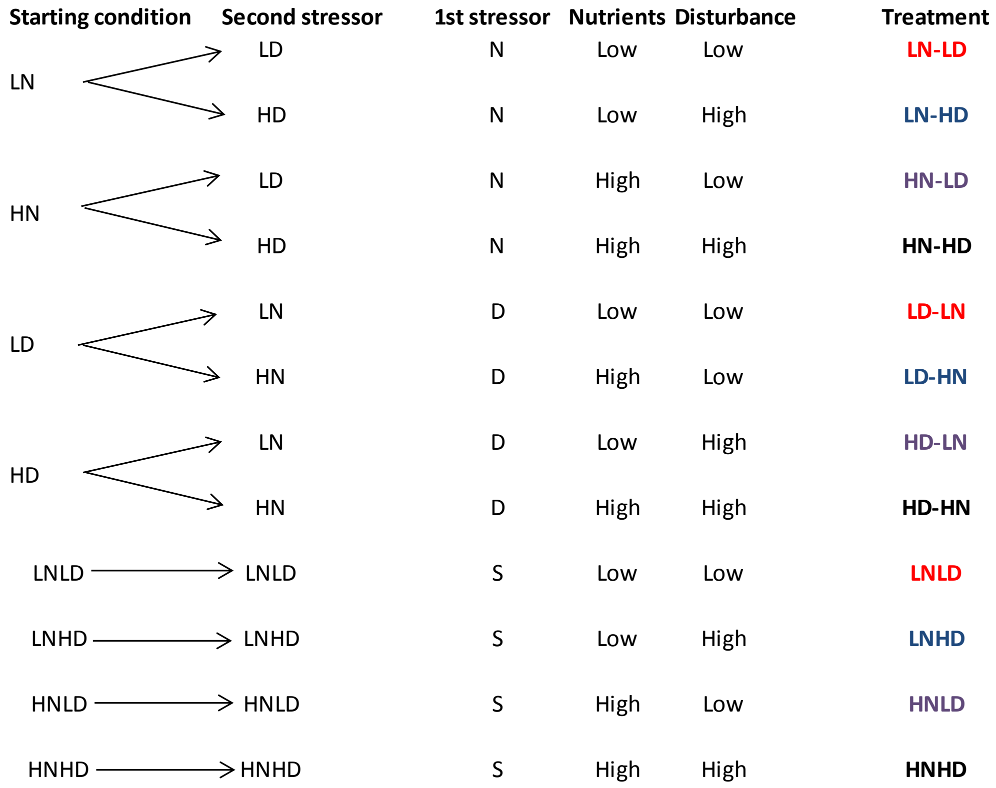
The experiments, run between May and September 2012, were replicated at two sites; one in Botany Bay and the other in the Lane Cove River, New South Wales, Australia (see Chapter 2 for a full site description).

First, a two-factor orthogonal experiment assessed how pre-exposure to one environmental stressor influences the impact of a second. Eighty four experimental plots, each 2-3 meters apart were established at each site, and were randomly assigned to one of four initial disturbances, to give 21 plots to each condition : (1) low nutrient addition; (2) high nutrient addition; (3) low physical disturbance; or (4) high physical disturbance (Figure 6.2). After 2 months, each plot either received a second disturbance of a second type, or was not further disturbed (control). In other words, plots that had initially received low or high nutrient addition received either zero, low or high physical disturbance, and plots that had initially received low or high physical disturbance received either zero, low or high physical treatments (Figure 6.2). This design gave a total of 7 replicates for each of the 12 resulting experimental treatments (Figure 6.2). Plots were sampled prior to the first disturbance, so as to assess any pre-existing differences in community structure among the plots, and also after two months (i.e. after the first but before the second disturbance) to test for effects of the initial stressor (as compared to control, undisturbed plots), and after four months to assess how the effect of the second stressor varied according to pre-exposure to another stressor. Plots initially receiving nutrient enrichment were analysed separately to those initially receiving physical disturbance due to lack of replication at the zero level which would have created an unbalanced 3-factor design.

	Treatment	Starting condition	Second stressor	Nutrients	Disturbance
Nutrient analysis					
	LN-		0D	Low	0
	LN-LD	LN	LD	Low	Low
	LN-HD		HD	Low	High
	HN-		0D	High	0
	HN-LD	HN	LD	High	Low
	HN-HD		HD	High	High
Disturbance analysis					
	LD-		0N	0	Low
	LD-LN	LD	LN	Low	Low
	LD-HN		HN	High	Low
	HD-		0N	0	High
	HD-LN	HD	LN	Low	High
	HD-HN		HN	High	High

Figure 6.2: Schematic diagram representing experimental design and analyses to determine how the application of an additional stressor after being subjected to a single stressor for two months affects the community structure. 0 = Control, L = Low, H = High, N = nutrient additions, D = Disturbance. 2 way interaction between Nutrients and Disturbance

Second, to assess whether the order in which stressors were applied, whether applied together or following a delay, influenced their cumulative impact, plots receiving both nutrient and physical disturbance at different times (as described above and hereby referred to as the asynchronous treatments) were compared to those receiving the two disturbances synchronously (experiments were ran concurrently with those in Chapter 3). There were three factors in the experimental design: (1) the Order in which two stressors were applied (simultaneously S; nutrient first, N; or physical disturbance first, D); (2) the magnitude of the nutrient stressor (low, LN; high, HN); and (3) the magnitude of the physical disturbance stressor (low, LD; high, HD; Figure 6.3). The macrofaunal communities and MPB establishing within plots were compared after 4 months.



Starting condition	Second stressor	1st stressor	Nutrients	Disturbance	Treatment
LN	LD	N	Low	Low	**LN-LD**
	HD	N	Low	High	**LN-HD**
HN	LD	N	High	Low	**HN-LD**
	HD	N	High	High	**HN-HD**
LD	LN	D	Low	Low	**LD-LN**
	HN	D	High	Low	**LD-HN**
HD	LN	D	Low	High	**HD-LN**
	HN	D	High	High	**HD-HN**
LNLD	LNLD	S	Low	Low	**LNLD**
LNHD	LNHD	S	Low	High	**LNHD**
HNLD	HNLD	S	High	Low	**HNLD**
HNHD	HNHD	S	High	High	**HNHD**

Figure 6.3: Schematic diagram representing experimental design and analysis to determine how the order of applied stressors (whether asynchronous or simultaneous) affects the final community structure. L = Low, H = High, N = nutrient additions, D = Disturbance, S = Simultaneous application. Colour represents the same end point LNLD = Red, LNHD = Blue, HNLD = Purple, HNHD = Black. 3 way interaction between 1st stressor, Nutrients and Disturbance.

Third, to assess how the impacts of particular stressors compared between treatments when they were offered together, either synchronously or asynchronously, versus singularly, a series of four separate contrasts were made (Figure 6.4). In each, the effects of two single stressors (e.g. low nutrients, high disturbance) were compared between treatments where they were offered to together, synchronously or asynchronously and individually, as well as against a control where there were no manipulations. The communities establishing within plots were compared after 4 months.

Low Nutrients Low Disturbance

Start		End point
LD	→	LD-LN
LN	→	LN-LD
LNLD	→	LNLD
LN	→	LN
LD	→	LD
C	→	C

Low Nutrients High Disturbance

Start		End point
HD	→	HD-LN
LN	→	LN-HD
LNHD	→	LNHD
LN	→	LN
HD	→	HD
C	→	C

High Nutrients Low Disturbance

Start		End point
HN	→	HN-LD
LD	→	LD-HN
HNLD	→	HNLD
HN	→	HN
LD	→	LD
C	→	C

High Nutrients High Disturbance

Start		End point
HD	→	HD-HN
HN	→	HN-HD
HNHD	→	HNHD
HN	→	HN
HD	→	HD
C	→	C

Figure 6.4: Schematic diagram representing experimental design and analyses to determine how the order of applied stressors affects the final community structure. Design compares end point where plots were subjected to nutrient and disturbance stressors singularly, simultaneously or after a 2 month delay between 1st and 2nd stressor. C = Control, L = Low, H = High, N = nutrient additions, D = Disturbance.

6.3.2 Sampling methods

The stressors of nutrient enrichment and physical disturbance were applied and maintained for the experimental duration in 0, low or high intensities. Nutrients were released via the application of slow release fertiliser pellets and disturbance was maintained monthly by raking the sediments (Chapter 2.3). Sampling of plots was conducted prior to the start of the experiment (to assess any pre-existing differences that may confound results), and at additional time points as indicated above. At each time, a single core of sediment was collected from each plot to assess macrofauna community structure and biodiversity (Chapter 2.6), and microphytobenthic biomass through handheld remote sensing techniques (Chapter 2.7).

6.3.3 Statistical analyses

For each of the three experiments detailed above, PERMANOVAs were initially run to assess differences in NDVI and macrofaunal community structure among the factors of interest. With regards to the NDVI analyses, there was high variability within single months, negating treatment effects. The main effect of month was added to the PERMANOVA design to factor differences between the 3rd and 4th months of the experiment to analyse the effects following disturbance. This type of analysis could not be run for the macrofauna as there were only samples from the start, middle and end of the experiment.

For the analyses of the macrofaunal community structure, data were square-root transformed to down-weight the effect of species abundance, and visualised using nMDS plots that use replicates as points. Diversity was calculated using PRIMER and included species richness, total abundance, species richness (Margalef index), Pielou's evenness, Shannon diversity and Simpson Index. Following these analyses pairwise *post hoc* tests were conducted to differentiate significant differences between factors. SIMPER analyses assessed which taxa facilitated highest contributions to multivariate dissimilarity among treatments. Univariate ANOVAs, or analogous design to the PERMANOVAs, were run on these taxa.

6.4 Results

6.4.1 What effect does adding a second stressor have to the system experiencing an initial stressor?

At time 0 for Botany Bay, prior to the application of the initial experimental manipulations, there were no pre-existing differences in microphytobenthic biomass among experimental plots that confounded experiments (Table 6.1). At Lane Cove there were pre-existing differences between experimental plots: an interaction between nutrient enrichment and physical disturbance in the analysis where disturbance was the first stressor applied prior to the manipulation of stressors (Table 6.1; Pseudo-$F_{(2, 31)}$ = 4.16, p(perm) = 0.016) where in plots without nutrient enrichment, the NDVI in the high physical disturbance treatment was significantly higher than the low disturbance treatment (t = 3.29, p = 0.004). There were no further significant pairwise differences. Additionally there were no pre-existing differences among experimental plots in the analysis examining the impact of nutrients as the initial stressor (Table 6.1). Further, there were no differences between treatments prior to the addition of the second stressor at either Botany Bay or Lane Cove (Table 6.2); the pre-existing effects had been cancelled out by the manipulations.

Following the addition of the second stressor, the NDVI varied between treatment types at both sites in each analysis (Table 6.3). When nutrients were applied first, there was a main effect of physical disturbance on the microphytobenthic biomass at Botany Bay (Pseudo-$F_{(2,65)}$ = 3.27, p(perm) = 0.043) where increased disturbance lowered the NDVI (Figure 6.5). *Post hoc* tests revealed a significant difference between the control and low treatment (t = 2.29, p = 0.036) but not between the low and high treatments or the high and control treatments. At Lane Cove there was an interaction between month and the effect of physical disturbance (Pseudo-$F_{(2,70)}$ = 4.59, p(perm) = 0.010) where one month following the addition of the physical disturbance, the high disturbance treatment had greater NDVI than the low disturbance treatment (Figure 6.7a; t = 2.31, p = 0.024) with no other differences, and by the end of the experiment, the high physical disturbance treatment had lower NDVI than either the control (Figure 6.7b; t = 1.85, p = 0.079) or the low disturbance treatment (Figure 6.7b; t = 2.26, p = 0.025).

When physical disturbance was applied first, further nutrient enrichment caused an interaction between the two stressors at Botany Bay (Table 6.3; N x D interaction: Pseudo-$F_{(2,67)}$ = 3.50, p(perm) = 0.026) and an interaction with month at Lane Cove (Table 6.3; N x D x m interaction: Pseudo-$F_{(2,63)}$ = 2.91, p(perm) = 0.045). At Botany Bay, the high nutrient treatment had a significantly greater NDVI than either the low (t = 2.56, p = 0.015) or control (t = 2.59, p = 0.019) treatments when they were first subjected to low disturbance (Figure 6.6). Additionally, when they were initially subjected to high disturbance, both nutrient treatments increased the NDVI, however only the low nutrient enriched plots had a significantly greater NDVI (Figure 6.6; t = 2.46, p = 0.021) and there were no differences between the enriched treatments.

At Lane Cove, one month following the second disturbance (Figure 6.8a) only the high physically disturbed plots displayed any effect due to the effects of nutrient enrichment whereby low nutrients had a lower NDVI than found in the control plots (t = 2.82, p = 0.014). Two months following the second disturbance (Figure 6.8b), only the low physically disturbed plots displayed any effect due to the effects of nutrient enrichment whereby low nutrients contained a lower NDVI than found in the high enriched plots (t = 3.45, p = 0.006). Neither was significantly different from the plots subjected to zero nutrients.

Table 6.1: PERMANOVA output of two way interactions between Nutrient enrichment (N) and physical Disturbance (D) analysing their effects on microphytobenthic biomass, recorded as NDVI (Normalised Difference Vegetation Index) at Botany Bay and Lane Cove prior to experimental manipulations. Starting condition indicates whether nutrients or disturbance were applied first prior to a second stressor. n=5-7 for all treatments levels.

Starting condition:	**Nutrient enrichment**						**Disturbance**					
Site:	**Botany Bay**			**Lane Cove**			**Botany Bay**			**Lane Cove**		
	df	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**
Nutrients	1	0.03	0.870	1	3.43	0.073	2	1.28	0.324	2	1.19	0.312
Disturbance	2	0.83	0.470	2	1.12	0.354	1	0.04	0.820	1	0.20	0.660
Interaction	2	1.24	0.793	2	0.01	0.982	2	2.25	0.130	2	4.16	0.016
Residuals	28			35			29			35		

Key: **Bold** = significant effect at $p < 0.05$

Table 6.2: PERMANOVA output of three way interactions between Nutrient enrichment (N) physical Disturbance (D) and Month (M) analysing their effects on microphytobenthic biomass, recorded as NDVI (Normalised Difference Vegetation Index) at Botany Bay and Lane Cove prior to experimental manipulations after 2 months of being subjected to a single stressor. Starting condition indicates whether nutrients or disturbance were applied first prior to a second stressor. n=5-7 for all treatments levels.

Starting condition:	**Nutrient enrichment**						**Disturbance**					
Site:	**Botany Bay**			**Lane Cove**			**Botany Bay**			**Lane Cove**		
	df	Pseudo-F	P(perm)	df	Pseudo-F	P(perm)	df	Pseudo-F	P(perm)	df	Pseudo-F	P(perm)
Nutrients (N)	1	0.19	0.660	1	1.25	0.278	2	2.76	0.072	2	0.40	0.707
Disturbance (D)	2	1.32	0.265	2	1.20	0.316	1	0.10	0.749	1	0.65	0.440
Month (M)	1	2.71	0.092	1	**32.70**	**0.001**	1	**6.42**	**0.009**	1	**14.20**	**0.001**
N X D	2	2.03	0.125	2	1.15	0.335	2	1.51	0.249	2	2.36	0.115
N x M	1	0.02	0.906	1	2.68	0.109	2	0.39	0.706	2	0.18	0.836
D x M	2	0.63	0.539	2	1.43	0.221	1	0.00	0.969	1	0.64	0.420
N x D x M	2	0.31	0.729	2	0.85	0.436	2	1.54	0.214	2	0.97	0.410
Residuals	69			70			65			57		

Key: **Bold** = significant effect at $p < 0.05$

Table 6.3: PERMANOVA output of three way interactions between Nutrient enrichment (N), physical Disturbance (D) and Month (M) analysing their effects on microphytobenthic biomass, recorded as NDVI (Normalised Difference Vegetation Index) at Botany Bay and Lane after 4 months of experimental manipulations. Starting condition indicates whether nutrients or disturbance were applied first. n=5-7 for all treatments levels.

Starting condition:	**Nutrient enrichment**						**Disturbance**					
Site:	**Botany Bay**			**Lane Cove**			**Botany Bay**			**Lane Cove**		
	df	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**
Nutrients (N)	1	0.50	0.488	1	1.74	0.193	2	4.06	0.029	2	3.20	0.054
Disturbance (D)	2	**3.27**	**0.043**	2	0.28	0.750	1	0.01	0.939	1	1.66	0.193
Month (M)	1	**4.92**	**0.023**	1	0.51	0.467	1	**23.08**	**0.001**	1	0.34	0.559
N X D	2	1.70	0.211	2	0.06	0.940	2	**3.50**	**0.026**	2	0.35	0.698
N x M	1	1.69	0.212	1	0.94	0.354	2	0.33	0.707	2	1.07	0.360
D x M	2	0.59	0.574	2	**4.59**	**0.010**	1	0.34	0.557	1	0.03	0.856
N x D x M	2	1.95	0.153	2	0.15	0.851	2	1.13	0.293	2	**2.91**	**0.045**
Residuals	65			70			67			63		

Key: **Bold** = significant effect at $p < 0.05$

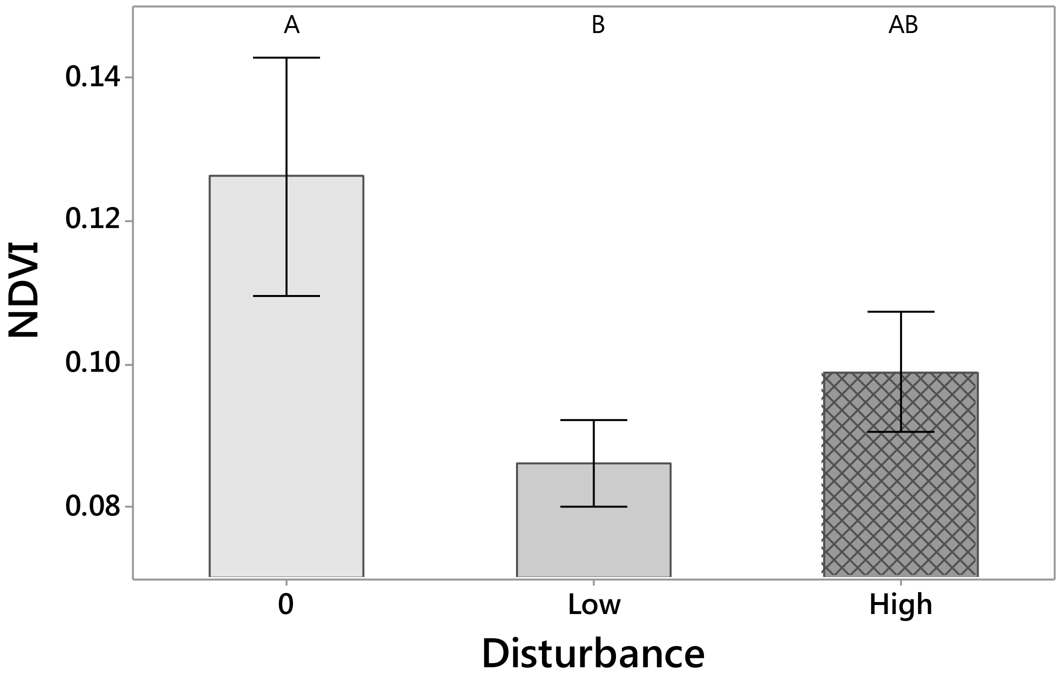


Figure 6.5: Change in NDVI (Normalised Difference Vegetation Index) at Botany Bay due to the influence of physical disturbance which was applied after two months of prior nutrient stress. Data pooled across the 3rd and 4th months of data collection and all intensities of nutrient enrichment (low and high). Plots were subjected to high and low stressors. Points indicate mean +/- SE. N = 5-7 for each treatment. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

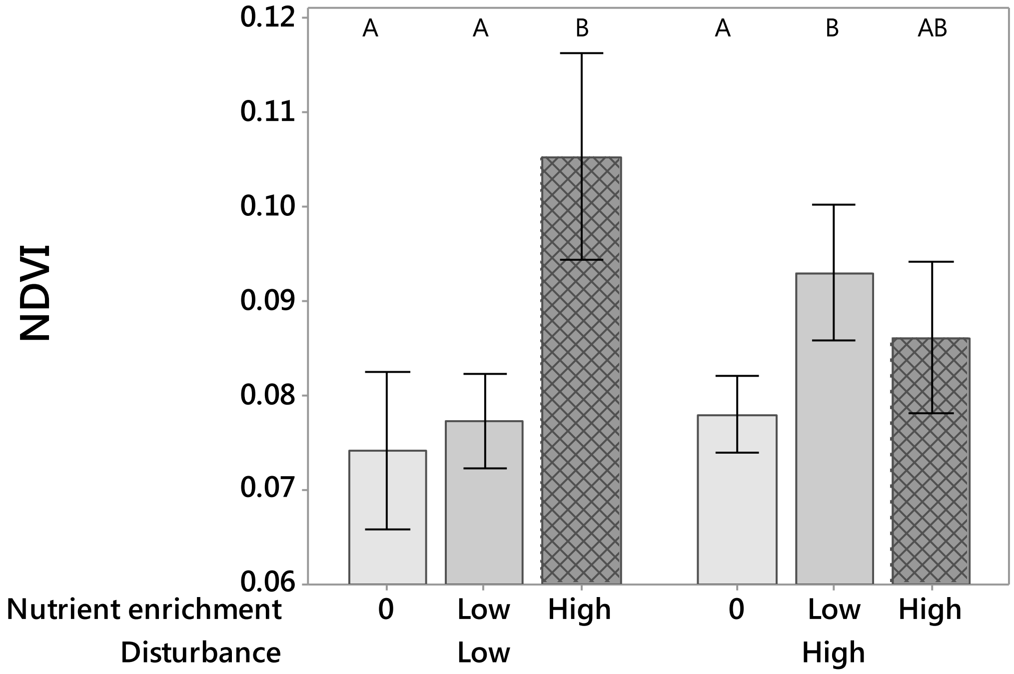
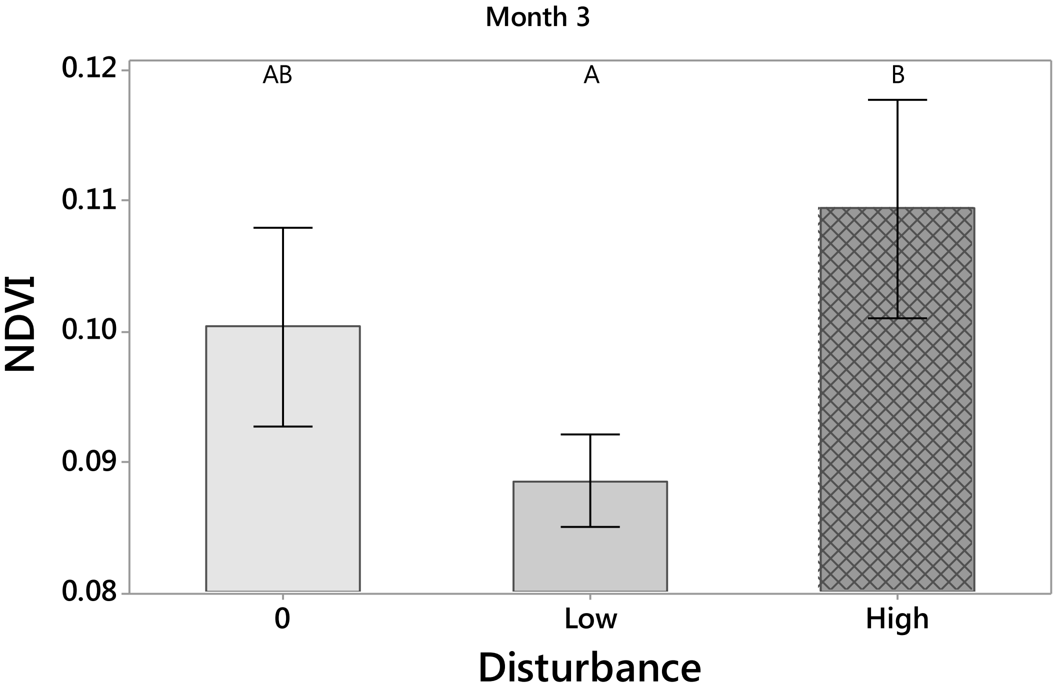


Figure 6.6: NDVI (Normalised Difference Vegetation Index) at Botany Bay due to the influence of nutrient enrichment which was applied after two months of prior physical disturbance stress. Data pooled across the 3rd and 4th months of data collection. Plots were subjected to high and low stressors. Points indicate mean +/- SE. N = 5-7 for each treatment. Different letters indicate statistically significant differences between treatments within each level of Disturbance (PERMANOVA *post hoc* tests).

a)



b)

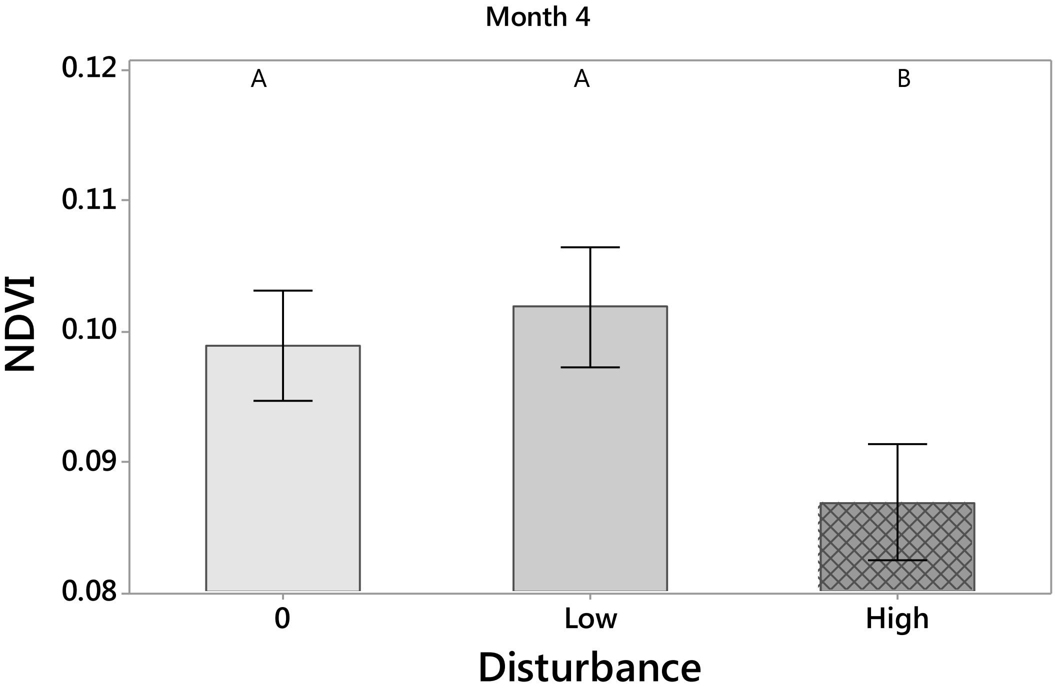


Figure 6.7: NDVI (Normalised Difference Vegetation Index) at Lane Cove due to the influence of physical disturbance which was applied after two months of prior nutrient stress, after 3 and 4 months of the total experimental duration. Data pooled all intensities of nutrient enrichment (low and high). Plots were subjected to high and low stressors. Points indicate mean +/- SE. N = 5-7 for each treatment. Different letters indicate statistically significant differences between treatments (PERMANOVA *post hoc* tests).

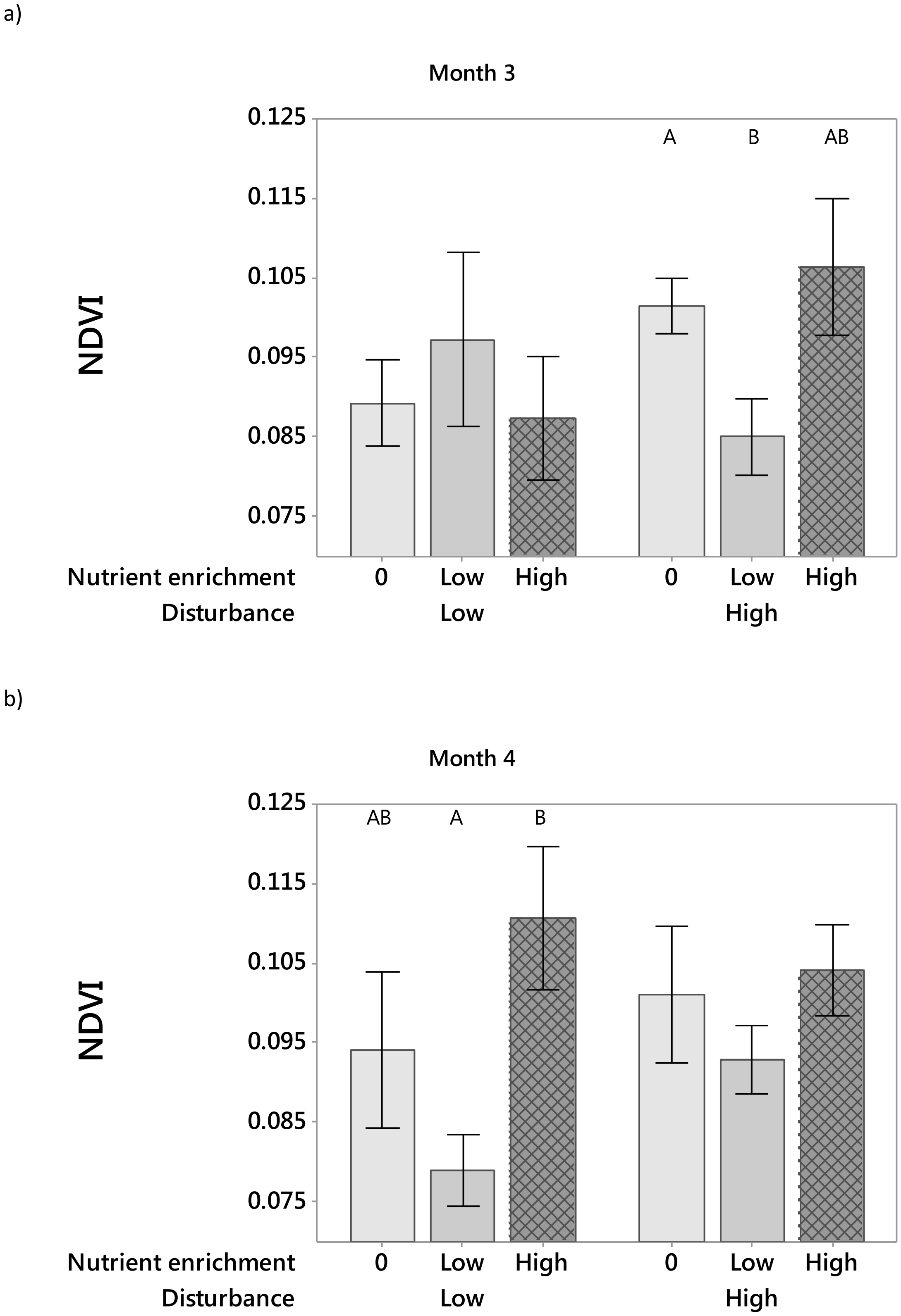


Figure 6.8: NDVI (Normalised Difference Vegetation Index) at Botany Bay due to the influence of nutrient enrichment which was applied after two months of prior physical disturbance, after a) 3 and b) 4 months of the total experimental duration. Plots were subjected to high and low stressors. Points indicate mean +/- SE. N = 5-7 for each treatment. Different letters indicate statistically significant differences between treatments within each level of Disturbance (PERMANOVA *post hoc* tests).

Regarding the macrofaunal component, at time 0, prior to the application of the initial experimental treatments, there were no pre-existing differences in the infaunal community structure among experimental plots, at either Botany Bay or Lane Cove that confounded treatment assignments (Table 6.4a). After two months of being subjected to a single stressor, benthic invertebrate communities did not differ significantly among treatments at Botany Bay (Table 6.4b), but at Lane Cove, macrofaunal communities in plots that had received low nutrient disturbance as their initial stressor differed between plots that were also subjected to the high and the low physical disturbance ($t = 1.80$, $p = 0.004$) and the high physical disturbance treatment and the controls ($t = 1.94$, $p = 0.005$). These unexpected differences were attributed to the key species, identified using SIMPER analysis. These species were *Prionosio sp.*, *Mediomastus australiensis*, *Mysella vitrea* and *Gammarus sp. Gammarus sp.* and *Prionosio sp.* were found in greater abundances at the start of the experiment in the low nutrient, high disturbance treatment. While this was not significantly different from the others at the start of the experiment, these pre-existing levels became enhanced after 2 months, leading to the observed significant differences.

By the end of the experiment, the addition of a second stressor significantly impacted infaunal communities only when nutrients were the stressor applied first (Table 6.4c). In Botany Bay, the effects of physical disturbance were dependent on the level to which plots had been nutrient enriched (sig. interaction: Pseudo-$F_{(2,35)} = 1.69$, p(perm) = 0.046; Table 6.4c; Figure 6.9). Among plots initially receiving the low nutrient additions macrofaunal communities differed between plots subsequently receiving the high physical disturbance and no physical disturbance ($t = 1.52$, $p = 0.028$; Figure 6.9). At Lane Cove, an effect of physical disturbance was observed, irrespective of the level of previous nutrient enrichment to plots (main effect of disturbance: Table 6.4c; $p < 0.05$; Figure 6.10). Plots receiving high physical disturbance differed in community composition to those receiving low physical disturbance and to those receiving no physical disturbance. The level of nutrient enrichment of plots also influenced community composition, displaying an additive effect to physical disturbance (main effects of nutrient enrichment, Table 6.4c; $p < 0.05$; Figure 6.10).

Table 6.4: PERMANOVA output of two way interactions between Nutrient enrichment (N) and physical Disturbance (D) analysing their effects on macrofauna community composition at Botany Bay and Lane Cove a) prior to experimental manipulations, b) prior to the addition of a second stressor, and c) after 4 months of experimental conditions. Starting condition indicates whether nutrients or disturbance were applied first prior to a second stressor. n=5-7 for all treatments levels.

	Starting condition:	**Nutrient enrichment**						**Disturbance**					
	Site:	**Botany Bay**			**Lane Cove**			**Botany Bay**			**Lane Cove**		
		df	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**	**df**	**Pseudo-F**	**P(perm)**
a)	**Nutrients**	1	0.45	0.887	1	1.06	0.357	2	0.44	0.979	2	0.32	0.999
	Disturbance	2	0.84	0.641	2	1.38	0.168	1	0.42	0.929	1	0.55	0.842
	Interaction	2	1.56	0.095	2	0.98	0.457	2	1.29	0.177	2	1.37	0.177
	Residuals	33			36			36			34		
b)	**Nutrients**	1	0.45	0.861	1	1.01	0.430	2	1.53	0.091	2	1.59	0.072
	Disturbance	2	1.07	0.402	2	1.75	0.027	1	0.85	0.533	1	0.57	0.849
	Interaction	2	0.72	0.748	2	1.65	0.048	2	0.42	0.978	2	1.00	0.487
	Residuals	32			33			38			31		
c)	**Nutrients**	1	0.97	0.462	1	1.80	0.035	2	1.80	0.353	2	1.36	0.147
	Disturbance	2	1.14	0.336	2	2.06	0.006	1	1.31	0.233	1	0.41	0.942
	Interaction	2	1.69	0.046	2	1.16	0.236	2	1.61	0.083	2	1.03	0.420
	Residuals	35			33			32			29		

Key: **Bold** = significant effect at $p < 0.05$

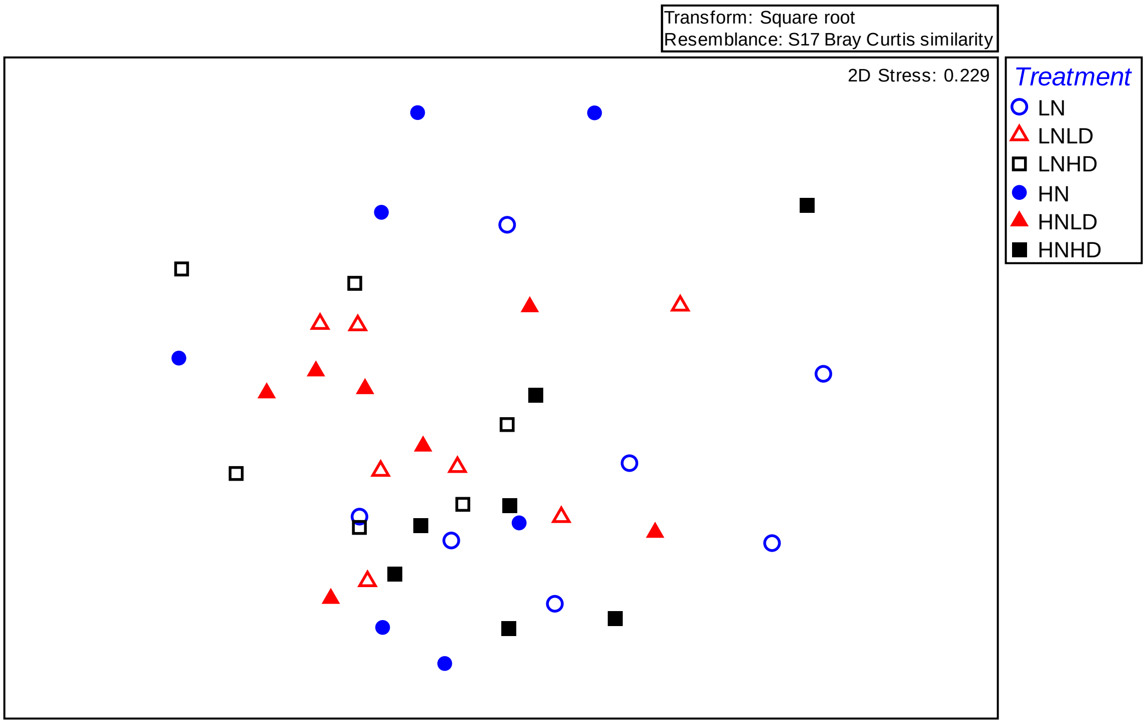


Figure 6.9: nMDS plot showing macrofaunal composition at Botany Bay following two months of low (LN) and high (HN) nutrient enrichment. Plots were then subjected to physical disturbance at zero, low (LD) and high (HD) levels for a further two months. N = 5-7

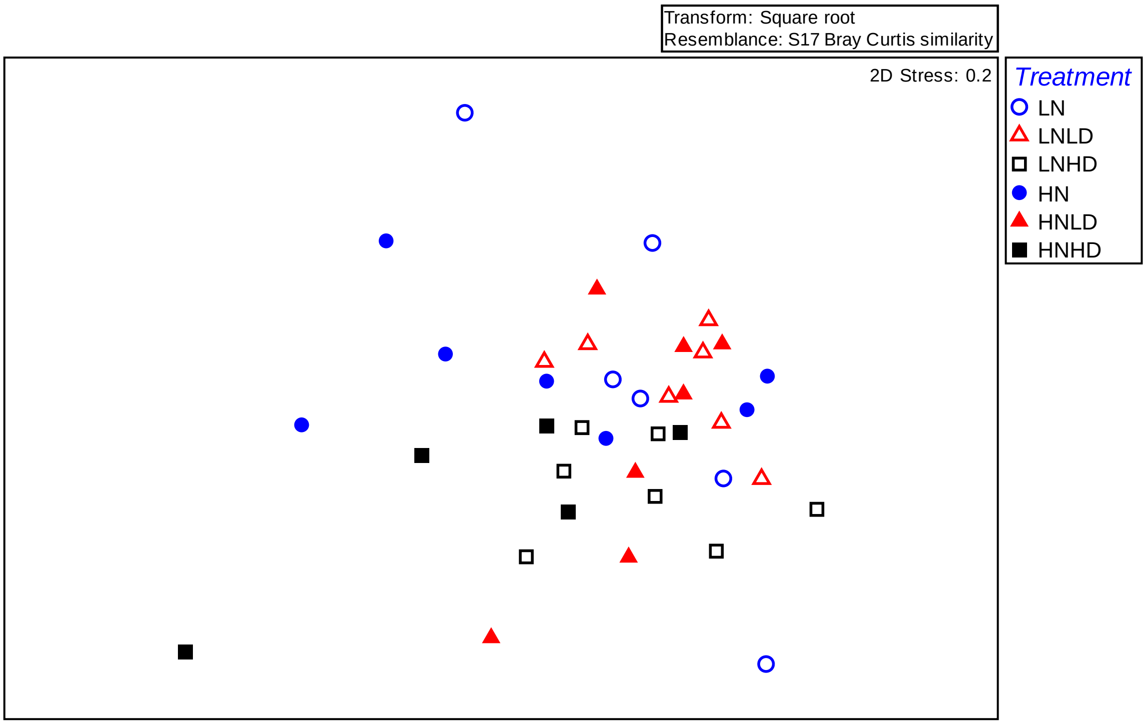


Figure 6.10: square root transformed nMDS plot showing macrofaunal composition at Lane Cove following two months of low (LN) and high (HN) nutrient enrichment. Plots were then subjected to physical disturbance at zero, low (LD) and high (HD) levels for a further two months. N = 5-7

SIMPER analysis revealed that after 4 months the same species were responsible for the dissimilarity between treatments in both analyses regardless of which stressor was initially applied. There were 6 species responsible for the dissimilarity between treatments at Botany Bay and 9 at Lane Cove, of which 4 were ubiquitous between sites: *Prionosio sp.*, *Mediomastus australiensis*, *Nephtys australiensis* and *Mysella vitrea* . Additional species at Botany Bay were *Salinator fragilis* and *Owenia fusiformis*. Additional species at Lane Cove were *Gammarus* sp., an unidentified *Oedicerotidae* amphipod, nematodes, *Australonereis ehlersi* and *Scoloplos sp.*

Following the addition of physical disturbance after two months of prior nutrient enrichment, the effect of the second stressor caused a significant impact to certain species at both sites. The abundance of *Prionosio sp.* decreased significantly with increasing nutrient enrichment (i.e. LN > HN; Figure 6.11; Botany Bay: $F_{(1,35)}$ = 11.21, p = 0.002; Lane Cove: $F_{(1,35)}$ = 8.73, p = 0.009). Additionally, increasing disturbance significantly altered abundances at Botany Bay (Figure 6.11a; $F_{(2,35)}$ = 3.94, p = 0.023) where abundances were lower in the low disturbance treatment compared to the control (t = 2.85, p = 0.008) and at Lane Cove (Figure 6.11b; $F_{(2,35)}$ = 5.06, p = 0.014) where abundance was greater in the low disturbance treatment compared to the control (t = 2.84, p = 0.013) and the high treatments (t = 2.36, p = 0.028). There was no interaction between stressors. Additionally at Botany Bay, there was a significant interaction between stressors for (Interaction: $F_{(2,35)}$ = 5.30, p = 0.009). In plots first exposed to low nutrient enrichment, increasing physical disturbance led to an increase in the abundance of this taxon (LN: C < LD < HD), but in plots first exposed to high nutrient enrichment, the abundances were greater in plots exposed to low disturbance compared to the control (t = 2.07, p = 0.057) and the high disturbance treatment (t = 2.70, p = 0.025). There was no difference between the control and high treatments. No other species were significantly affected by the stressors however the majority displayed a weak trend where increased disturbance stress caused a decrease in abundance, particularly with respect to Lane Cove (Figure 6.11b).

Similarly, when nutrients were added as the second stressor there was a general trend for many species to show a negative response to increasing nutrients, however in a univariate analysis, few of these responses were significant (Figure 6.12). At Botany Bay there was an interaction between stressors with regard to *Prionosio sp.* abundance (Interaction: $F_{(2,33)}$ = 3.31, p(perm) = 0.044). In the plots first receiving low physical disturbance, there were higher abundances of *Prionosio sp.* in the controls when compared to the low (t = 2.74, p = 0.022) and the high (t = 2.03, p = 0.082) nutrient enrichment treatments. When subjected to high disturbance, there were no differences between nutrient enrichment treatments (HD: C = LN = HN). Furthermore, the undisturbed treatments varied, with the abundance within the low disturbance treatment greater having greater abundances compared to the high ($t = 3.21$, $p = 0.010$).

At Lane Cove there was a significant interaction between stressors for both *Australonereis ehlersi* (Interaction: $F_{(2,29)} = 3.47$, $p = 0.039$) and *Scoloplos sp.* ($F_{(2,29)} = 4.08$, $p = 0.030$) when physical disturbance was the initial stressor. For both species there was no difference between any of the high disturbance treatments (HD: C = LN =HN). Under the low disturbance scenarios, *Australonereis ehlersi* displayed higher abundances in those subjected to low ($t = 3.13$, $p = 0.013$) and high ($t = 2.96$, $p = 0.049$). There were no differences between the enriched treatments (i.e. HD: C < LN = HN). For *Scoloplos sp.*, there were no differences between zero and low nutrient treatments however high nutrient enrichment caused greater abundances compared to the control ($t = 2.19$, $p = 0.073$) and low treatments ($t = 4.24$, $p = 0.006$).

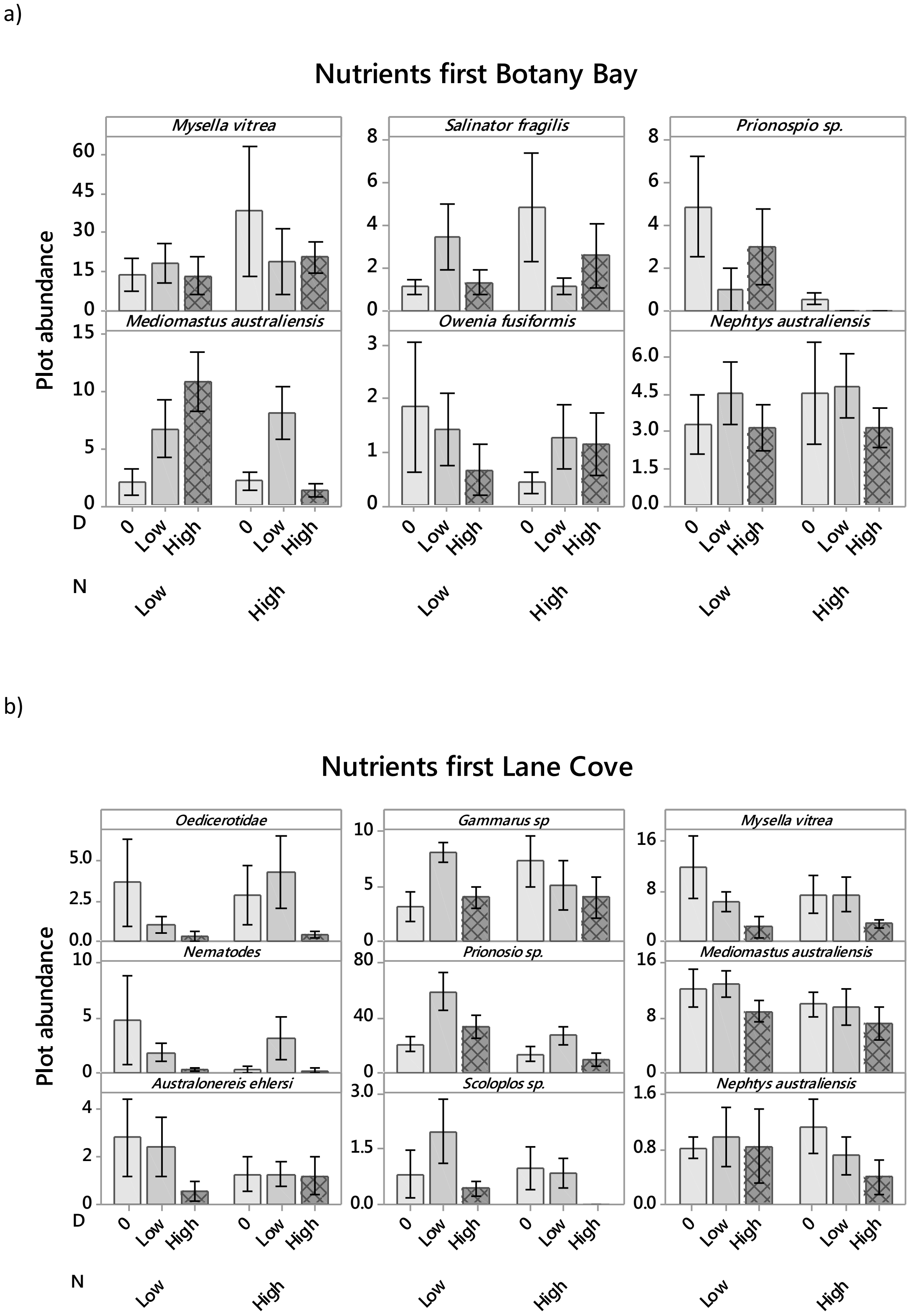


Figure 6.11: Change in total abundance of macrofauna species per plot at a) Botany Bay and b) Lane Cove after four months of plots being subjected to nutrient enrichment (N) for 2 months prior to being subjected to physical disturbance (D). Plots were subjected to high and low stressors. Points indicate mean +/- SE. N = 7

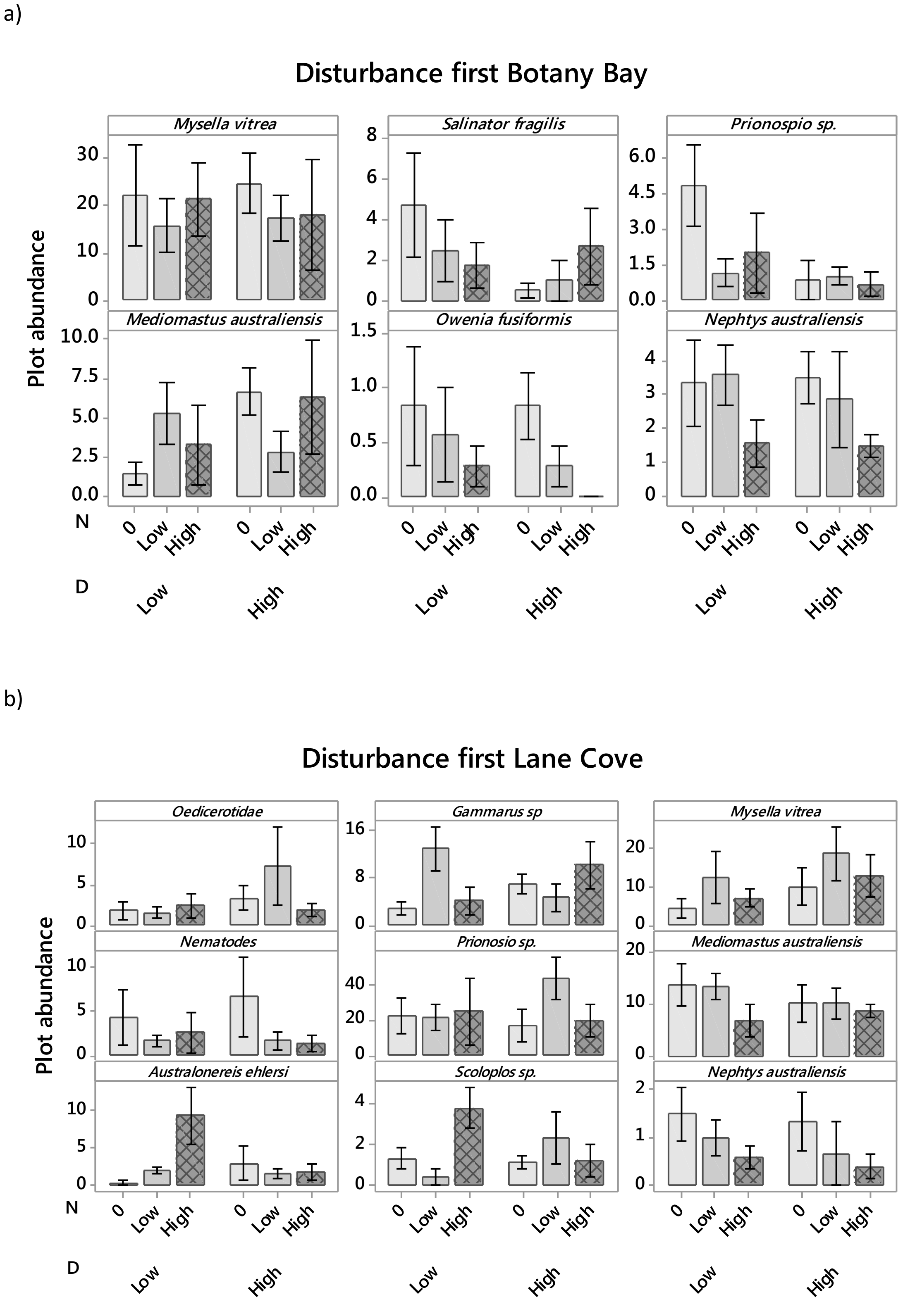


Figure 6.12: Change in total abundance of macrofauna species per plot at a) Botany Bay and b) Lane Cove after four months of plots being subjected to physical disturbance for 2 months prior to being subjected to nutrient enrichment. Plots were subjected to high and low stressors. Points indicate mean +/- SE. N = 7

6.4.2 Does the order in which stressors were applied affect the final community?

Prior to experimental manipulations there were no pre-existing effects of treatments on NDVI that would confound results (Table 6.5a). After 2 months of being subjected to a single stressor, prior to the addition of a second stressor in the asynchronous treatments, the order of application had a significant impact on the NDVI in Botany Bay only (Table 6.5b; Pseudo-$F_{(2,72)} = 4.11$, p = 0.014). Both the plots subjected to simultaneous stressors (t = 3.49, p =0.002) and plots subjected to nutrients initially (t = 2.02, p = 0.050) had a significantly greater NDVI than those subjected to physical disturbance alone. After 4 months, there were no differences in terms of NDVI between the different orders of application of stressors (synchronous or asynchronous; Table 6.5c). At Lane Cove there was a main effect of both stressors where nutrient enrichment increased NDVI and physical disturbance decreased NDVI (Table 6.5c).

When the data from the months following physical disturbance were analysed together (Table 6.6; Figure 6.13), there was a 3 way interaction between nutrients, disturbance and order of application at Botany Bay (Pseudo-$F_{(2,133)} = 3.67$, p = 0.031). With regards to differences between the order of application of stressors, among plots that were subjected to low physical disturbance and high nutrient enrichment, plots that were subjected to nutrient enrichment initially had a significantly lower NDVI than ether the synchronous treatment (t = 2.28, p = 0.027) or when physical disturbance was the initial stressor (t = 2.67, p = 0.018). There were no further significant differences between the order of application of stressors at other levels of nutrient enrichment or physical disturbance (p > 0.05). There were no significant effects of the order of application at Lane Cove (p > 0.05).

Table 6.5: Three-way PERMANOVAs examining effects on microphytobenthic biomass (recorded as NDVI) of Nutrient enrichment (N; 2 levels: low and high nutrient), physical Disturbance (D; 2 levels: low and high nutrient) and the order in which stressors were applied (O; 3 levels: nutrient first, disturbance first, simultaneous disturbances) at a) the start of the experiment (Month 0) and b) after 2 months (prior to the addition of a second stressor) and c) after 4 months. Manipulations were replicated at two sites, Botany Bay and Lane Cove. n=5-7 for all treatments levels.

		Botany Bay			**Lane Cove**		
a)		df	Pseudo-F	P(perm)	df	Pseudo-F	P(perm)
	Nutrient enrichment (N)	1	0.04	0.840	1	0.10	0.765
	Disturbance (D)	1	0.12	0.728	1	1.13	0.317
	Order (O)	2	0.25	0.776	2	0.63	0.541
	NxD	1	1.72	0.213	1	3.42	0.076
	NxO	2	0.92	0.382	2	1.43	0.271
	DxO	2	0.97	0.389	2	1.65	0.201
	NxDxO	2	0.08	0.908	2	2.38	0.107
	Residuals	57			68		
b)							
	Nutrient enrichment (N)	1	0.32	0.602	1	0.72	0.387
	Disturbance (D)	1	0.45	0.487	1	0.00	0.973
	Order (O)	2	**4.11**	**0.014**	2	0.49	0.633
	NxD	1	1.94	0.176	1	0.65	0.403
	NxO	2	0.10	0.896	2	1.29	0.304
	DxO	2	2.92	0.066	2	0.00	0.995
	NxDxO	2	1.33	0.271	2	1.31	0.262
	Residuals	72			72		
c)							
	Nutrient enrichment (N)	1	0.26	0.588	1	**5.72**	**0.018**
	Disturbance (D)	1	0.01	0.925	1	**5.96**	**0.015**
	Order (O)	2	0.27	0.769	2	0.32	0.750
	NxD	1	0.82	0.382	1	1.03	0.319
	NxO	2	0.51	0.622	2	2.87	0.077
	DxO	2	0.45	0.650	2	2.82	0.076
	NxDxO	2	2.16	0.129	2	0.77	0.485
	Residuals	66			70		

Key: **Bold** = significant effect at $p < 0.05$

Table 6.6: Four-way PERMANOVA output examining effects on microphytobenthic biomass (recorded as NDVI) of Nutrient enrichment (N; 2 levels: low and high nutrient), physical Disturbance (D; 2 levels: low and high nutrient), the order in which stressors were applied (O; 3 levels: nutrient first, disturbance first, simultaneous disturbances) and months following the addition of a second stressor (two months). Manipulations were replicated at two sites, Botany Bay and Lane Cove. n=5-7 for all treatments levels.

	Botany Bay			Lane Cove		
	df	Pseudo-F	P(perm)	df	Pseudo-F	P(perm)
Nutrient enrichment (N)	1	0.14	0.692	1	**8.43**	**0.005**
Disturbance (D)	1	0.25	0.597	1	0.38	0.533
Order (O)	2	0.34	0.715	2	1.62	0.213
Month (M)	1	**27.15**	**0.001**	1	0.99	0.323
N x D	1	0.28	0.617	1	0.02	0.893
N x O	2	0.80	0.448	2	0.54	0.595
N x M	1	0.22	0.626	1	0.00	0.979
D x O	2	1.01	0.331	2	2.74	0.065
D x M	1	0.41	0.547	1	**5.35**	**0.027**
O x M	2	0.43	0.656	2	0.93	0.395
N x D x O	2	**3.67**	**0.031**	2	0.37	0.711
N x D x M	1	0.97	0.307	1	1.80	0.172
N x O x M	2	0.11	0.882	2	1.76	0.158
D x O x M	2	0.03	0.971	2	3.04	0.057
N x D x O x M	2	0.33	0.721	2	1.98	0.149
Residuals	133			137		

Key: **Bold** = significant effect at $p < 0.05$

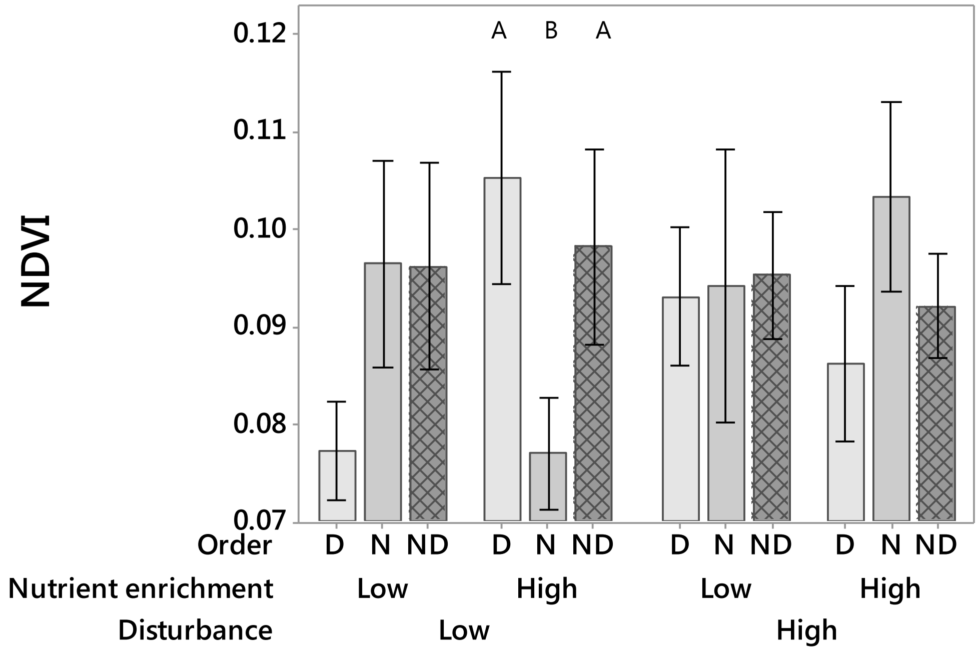


Figure 6.13: Change in NDVI (Normalised Difference Vegetation Index) at Botany Bay due to the influence of the order of application of stressors (synchronously: ND, asynchronously nutrients first – N; or disturbance first – D) when subjected to physical disturbance and nutrient enrichment (and low and high levels). Data pooled for two months following the implementation of the second stressor. Points indicate mean +/- SE. N = 5-7 for each treatment level. Different letters indicate statistically significant differences between treatments within each level of Disturbance (PERMANOVA *post hoc* tests).

Prior to experimental manipulations there was a significant 3-way interaction between order, nutrients and disturbance at Botany Bay (Table 6.7). *Post hoc* tests reveal that among plots assigned to receive the nutrient treatment first and the high disturbance treatment second, communities differed between plots assigned to the high and low nutrient treatments (t = 1.44, p = 0.023). At Lane Cove there were no pre-existing differences.

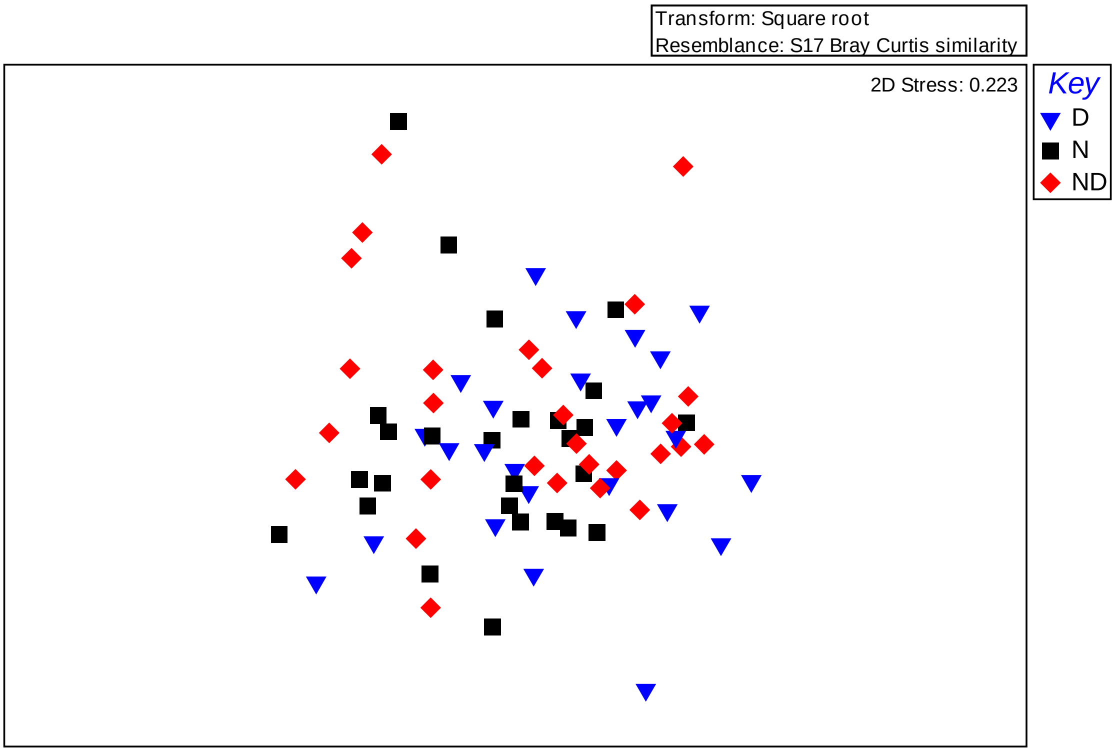
After 4 months (i.e. 2 months after the application of the second stressor), the order in which stressors were applied had a significant effect on the macrofaunal community structure at both sites (Table 6.7; Figure 6.14). Pairwise tests indicated that at Botany Bay macrofaunal communities differed between plots receiving the physical disturbance first, and plots receiving either nutrients first (t = 1.45, p = 0.036) or the two stressors simultaneously (t = 1.50, p = 0.04). By contrast, the macrofaunal communities did not differ between plots receiving the nutrient disturbance first, or the two stressors together (t = 1.25, p = 0.081). At Lane Cove, all treatment levels of the factor Order were significantly different to one another (P < 0.05). Further, at this site there was a significant main effect of nutrients indicating that irrespective of the level of physical disturbance different levels of nutrient enrichment can alter the community structure.

Table 6.7: Three-way PERMANOVAs examining effects on macrofaunal communities of Nutrient enrichment (N; 2 levels: low and high nutrient), physical Disturbance (D; 2 levels: low and high nutrient) and the order in which stressors were applied (O; 3 levels: nutrient first, disturbance first, simultaneous disturbances) at a) the start of the experiment (Month 0) and b) after 4 months. Manipulations were replicated at two sites, Botany Bay and Lane Cove. n=5-7 for all treatments levels.

		Botany Bay			**Lane Cove**	
a)	df	Pseudo-F	P(perm)	df	Pseudo-F	P(perm)
Nutrient enrichment (N)	1	1.22	0.253	1	0.95	0.459
Disturbance (D)	1	0.82	0.600	1	0.91	0.509
Order (O)	2	0.58	0.926	2	0.86	0.636
NxD	1	0.76	0.648	1	0.43	0.935
NxO	2	0.66	0.853	2	0.41	0.993
DxO	2	1.20	0.237	2	1.39	0.135
NxDxO	2	**1.82**	**0.026**	2	1.29	0.167
Residuals	70			72		
b)						
Nutrient enrichment (N)	1	1.01	0.422	1	**2.05**	**0.021**
Disturbance (D)	1	0.43	0.920	1	1.55	0.115
Order (O)	2	**2.04**	**0.016**	2	**2.57**	**0.001**
NxD	1	1.42	0.185	1	1.17	0.287
NxO	2	1.15	0.293	2	1.30	0.134
DxO	2	0.66	0.856	2	0.81	0.721
NxDxO	2	1.63	0.079	2	1.14	0.276
Residuals	71			72		

Key: **Bold** = significant effect at p < 0.05

a)



b)

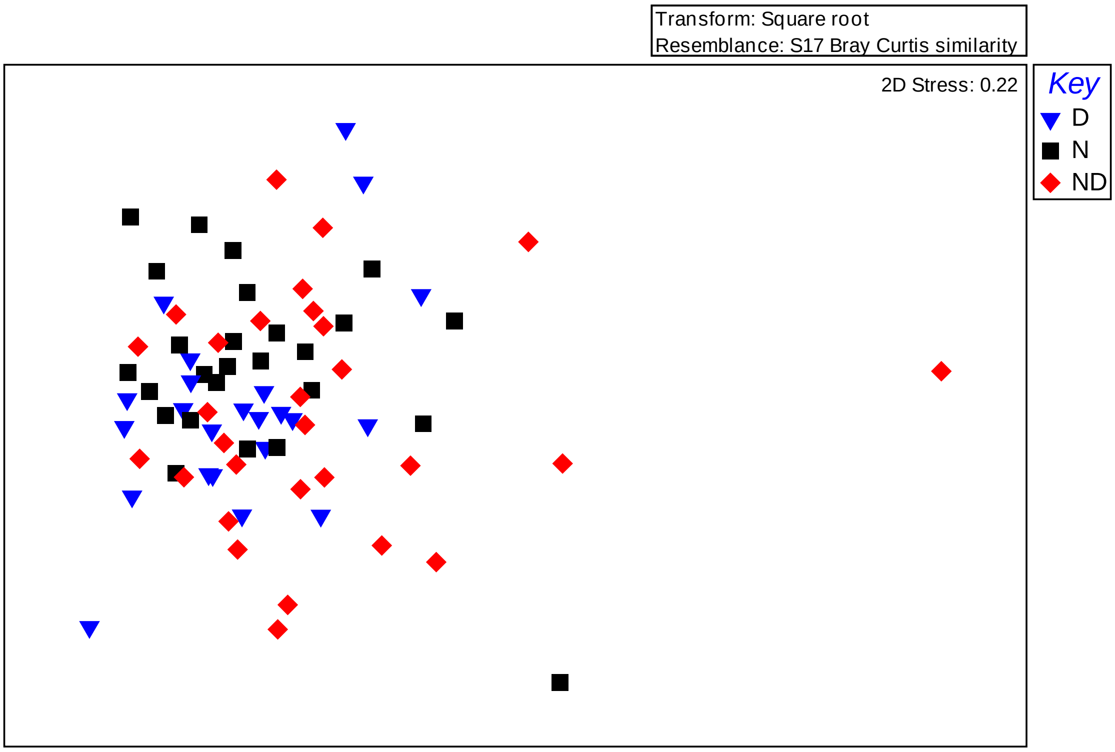


Figure 6.14: nMDS plot comparing macrofaunal community composition among plots at Botany Bay (top) and Lane Cove (bottom) following addition of nutrient and physical disturbances together (ND) or asynchronously, with the nutrient (N) or the physical disturbance (D) first. Points represent individual plots, with data square-root transformed prior to analysis. N = 5-7

There were no effects of the order of application on macrofaunal diversity indices at Botany Bay after 4 months. At Lane Cove after 4 months of stressors, the order in which they were applied altered total abundance, as well as the Shannon and Simpson biodiversity indices. The total abundance of species altered due to an interaction between the order of stressor application and disturbance (sig. D X O interaction: $F_{(2,66)} = 4.12$, p(perm) = 0.02; Figure 6.15). When subjected to low disturbance the plots subjected to simultaneous stress had a significantly lower abundance than those initially subjected to nutrients alone (t = 2.16, p = 0.040). The plots subjected to physical disturbance initially did not differ from the other treatments. When plots were subjected to high physical disturbance, those that were initially subjected to physical disturbance contained greater abundances than those initially subjected to nutrients (t = 2.64, p = 0.014). Neither treatment differed from the plots subjected to simultaneous stress. Additionally, increasing nutrient enrichment decreased the total abundance (LN > HN; Main effect nutrient enrichment: $F_{(1,66)} = 9.82$, p(perm) = 0.003).

The Shannon and Simpson biodiversity indices at Lane Cove varied due to an interaction between order of stressor application and nutrient enrichment (Figure 6.16; sig. D X O interaction Shannon: Pseudo-$F_{(2,66)} = 5.18$, p = 0.009; Simpson: Pseudo-$F_{(2,66)} = 6.63$, p = 0.005). Both indices displayed similar patterns whereby only plots subjected to low nutrient enrichment displayed differences due to the order of the application of stressors (i.e. no differences between high treatments). Plots subjected to both stressors simultaneously contained greater diversity than those subjected to nutrients or disturbance initially. There were no differences between treatments subjected to an initial stressor. Additionally the Shannon index displayed a significant decrease in biodiversity due to increasing physical disturbance (Shannon: Pseudo-$F_{(1,66)} = 6.81$, p = 0.011).

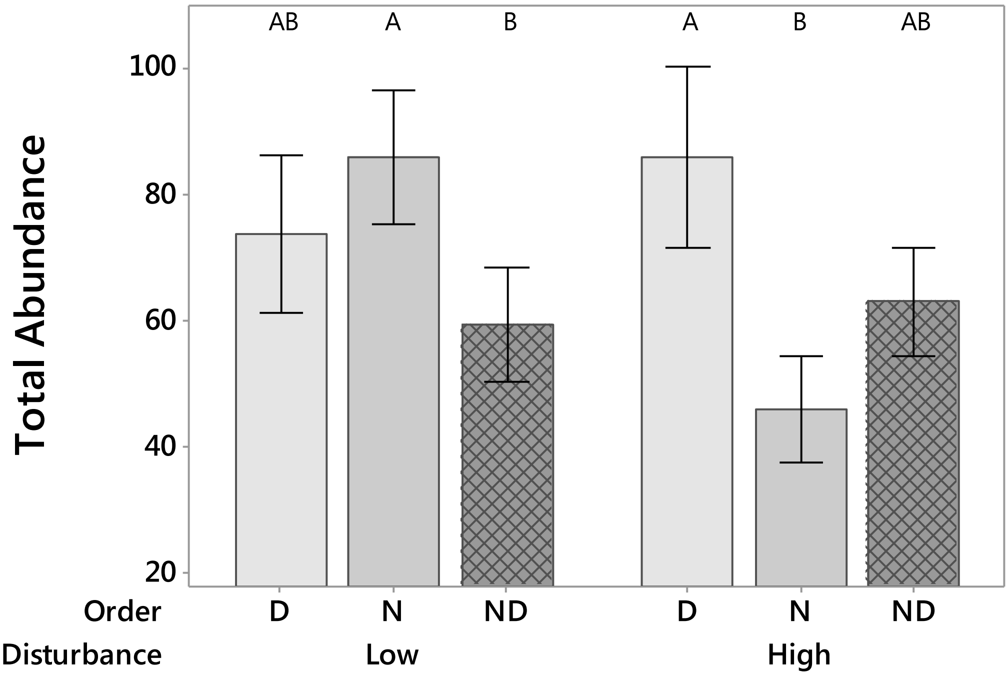
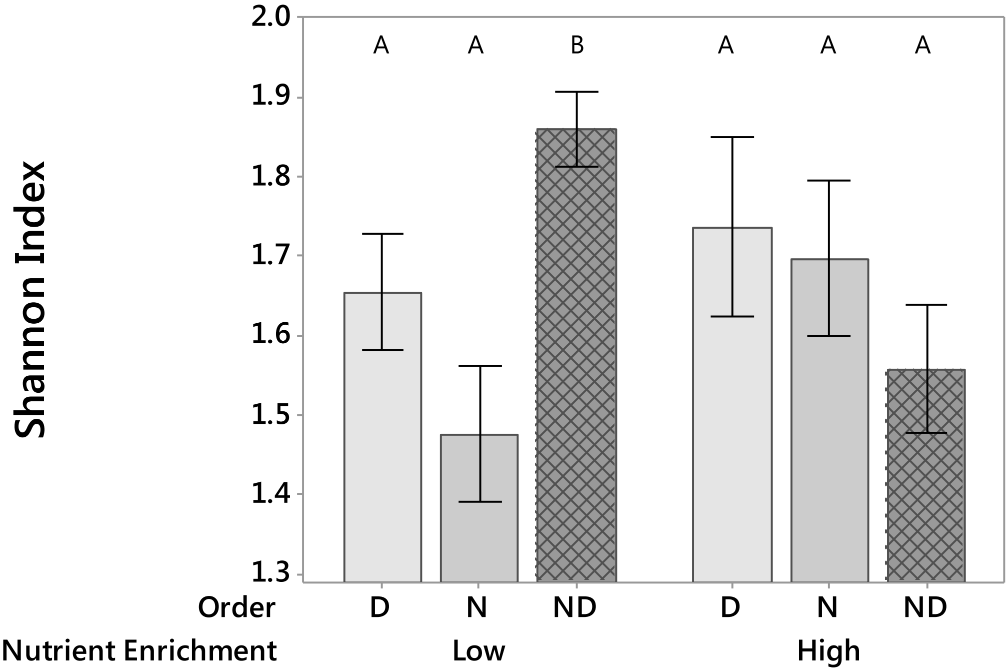


Figure 6.15: Change in total abundance of macrofauna per plot at Lane Cove after four months of plots being subjected to Nutrient enrichment and physical disturbance either simultaneously (ND) or asynchronously where plots were subjected to an initial stressor of physical disturbance (D) or nutrient enrichment (N). Plots were subjected to high and low stressors; the nutrient treatments have been pooled to display interactive effects of the order of stressor application and disturbance. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments within each level of Disturbance (PERMANOVA *post hoc* tests).

a)



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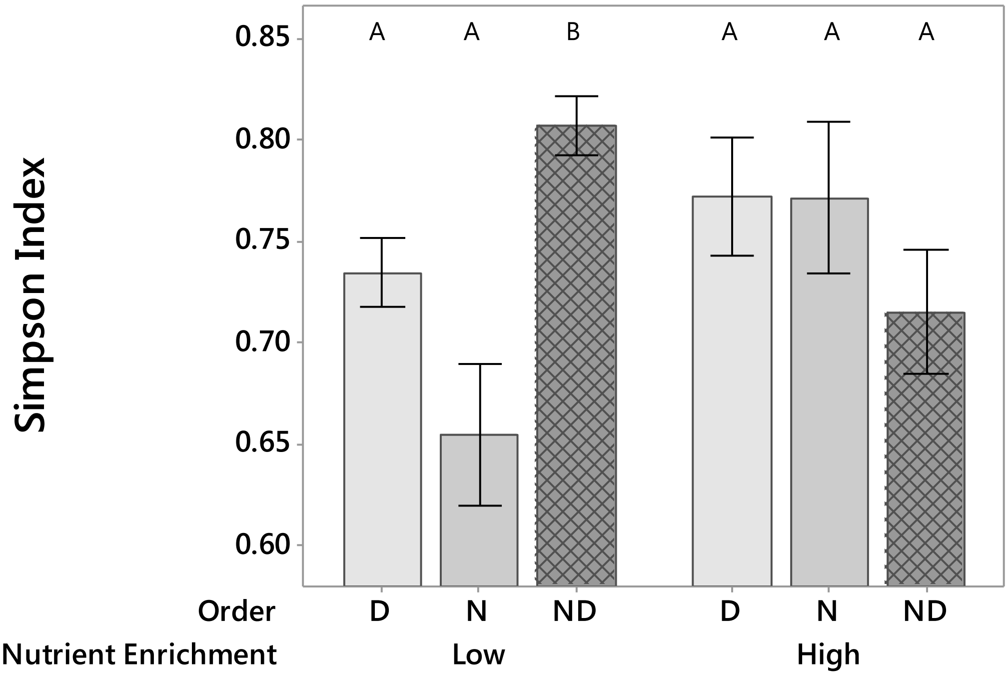


Figure 6.16 Change in a) Shannon index and b) Simpson index at Lane Cove after four months of plots being subjected to nutrient enrichment and physical disturbance either simultaneously (ND) or asynchronously where plots were subjected to an initial stressor of physical disturbance (D) or nutrient enrichment (N). Plots were subjected to high and low stressors; the disturbance treatments have been pooled to display interactive effects of the order of stressor application and nutrient enrichment. Points indicate mean +/- SE. Different letters indicate statistically significant differences between treatments within each level of Disturbance (PERMANOVA *post hoc* tests).

SIMPER analysis revealed that there were 6 species responsible for the dissimilarity between order treatments at Botany Bay and 12 at Lane Cove, of which 4 were ubiquitous between sites: *Prionosio sp.*, *Mediomastus australiensis*, *Nephtys australiensis* and *Mysella vitrea* . At Botany Bay, the abundances of 4 out of the 6 species responded significantly to the order in which stressors were applied whereas at Lane Cove, 6 of the 12 responded significantly to this factor (Table 6.8; Figure 6.17). Of the 4 discriminating species common to both sites, only *Prionosio sp.* responded significantly to stressors at both sites. At Botany Bay there was a 3 way interaction between the order of stressor application, the level of physical disturbance and nutrient disturbance (Interaction: $F_{2,71} = 5.03$, $p = 0.012$). Within plots receiving low nutrients and low disturbance, the abundance of *Prionosio sp.* was greater in the plots that received stressors simultaneously compared with those receiving nutrients ($t = 2.49$, $p = 0.043$) or physical disturbance ($t = 2.02$, $p = 0.068$) initially. There were no differences between asynchronous treatments. There were no differences between order treatments within any other levels of stressor. At Lane Cove, the abundance of *Prionosio sp.* responded to the interacting effects of physical disturbance and order of stressor application (Interaction: Pseudo- $F_{(2,66)} = 3.04$, p(perm) = 0.050). When subjected to low physical disturbance, plots that were simultaneously disturbed contained lower abundances than in either asynchronous treatment (nutrients first: $t = 3.77$, $p = 0.001$; disturbance first: $t = 2.01$, $p = 0.061$). Neither asynchronous treatment differed. There were also no differences between treatments subjected to high disturbance. Additionally, increasing nutrients caused a significant decrease in abundance (sig. main effect of nutrients: pseudo-$F_{(1,66)} = 7.06$, p(perm) = 0.013).

Other species specific responses to stress at Botany Bay (Figure 6.17a) included a 3 way interaction between the order of application of stressors, the level of nutrient enrichment and physical disturbance for Mediomastus australiensis (Interaction: $F_{(2,71)} = 3.35$, p(perm) = 0.041). Where plots were subjected to low nutrient enrichment and high physical disturbance, plots subjected to nutrient enrichment as the initial stressor contained significantly greater abundances than those subjected first to physical disturbance ($t = 2.97$, $p = 0.011$) or subjected simultaneously ($t = 4.39$, $p = 0.003$). There were no other significant differences between order of application within levels of nutrient enrichment or disturbance. Additionally where nutrients were added first, *Mediomastus australiensis* abundance was affected by level of physical disturbance in the high nutrient, but not the low nutrient treatment, and affected by level of nutrient enrichment in the high but not the low physical disturbance treatment. When physical disturbance occurred first, or the two stressors were applied simultaneously, Mediomastus australiensis did not respond to either the level of nutrient enrichment, the level of physical disturbance, or their interaction. Additionally at Botany Bay, the response of both *Owenia fusiformis* ($F_{(2,71)} = 3.25$, p = 0.047) and *Nephtys australiensis* ($F_{(2,71)} = 4.22$, p = 0.0.018) to stressors depended on the order in which they were added, with greater abundances in plots receiving nutrients first (*Owenia fusiformis*: t = 2.44, p = 0.013; *Nephtys australiensis*: t = 2.14, p = 0.034) or simultaneously (*Owenia fusiformis*: t = 2.21, p = 0.025; *Nephtys australiensis*: t = 2.79, p = 0.010) than in plots receiving physical disturbance first (D < N = ND). There were no interactions or other main effects for these or the other two discriminating species.

Of the species responding significantly at Lane Cove, the Psammobiidae responded to the order of application of stressors (Order: $F_{2,66} = 5.52$, P =0.006), fewer individuals were found in plots where stressors were applied simultaneously than in plots where disturbance was applied first (t = 3.41, p = 0.002). There were no other differences among the three orders of application. For this there was an interaction between nutrient enrichment and physical disturbance ($F_{(2,66)} = 3.90$, p = 0.041) whereby the plots subjected to low nutrients displayed a negative effect due to increased disturbance, whereas the high nutrient treatments did not display any treatment effects. Additionally the response of *Australonereis ehlersi* to stressors depended on the order in which they were added ($F_{(2,66)} = 4.55$, p = 0.018), with greater abundances in plots receiving nutrients first (t = 2.26, p = 0.031) or simultaneously (t = 2.77, p = 0.009) than in plots receiving physical disturbance first (D < N = ND). Additionally for this species, increased physical disturbance led to lower abundances (sig. main effect of disturbance: $F_{(1,66)} = 4.54$, p = 0.036).

At Lane Cove, the abundance of *Oedicerotidae* displayed an interaction between order of stressor application and nutrient enrichment (Interaction: $F_{(2,66)} = 3.42$, p = 0.043). Where plots were subjected to low nutrient enrichment, the plots that were initially subjected to nutrient enrichment had a significantly lower abundance than those plots initially subjected to disturbance (t = 2.44, p = 0.021) or where both were applied synchronously (t = 3.32, p = 0.002). There was no difference between the synchronous treatment and those subjected to physical disturbance as the first stressor. There were also no differences between the orders of application of stressors when they were subjected to high nutrient enrichment. Additionally for this species there was an interaction between nutrient enrichment and physical disturbance (Interaction: $F_{2,66} = 6.18$, p = 0.009) whereby increasing nutrient enrichment decreased abundances only when subjected to high physical disturbance (t = 2.28, p = 0.041), there were no differences between levels of physical disturbance.

There was a 3-way interaction between order of stressor application and the level of each of the two stressors for *Scoloplos sp.* (Interaction: $F_{(2,66)} = 3.51$, p = 0.033). Where plots were subjected to low nutrient enrichment and low physical disturbance, plots subjected to physical disturbance as the initial stressor contained significantly lower abundances than those subjected to synchronous stressors (t = 2.31, p = 0.033), there were no other differences between orders under these levels of stress. Where plots were subjected to high nutrient enrichment and low physical disturbance, plots subjected to physical disturbance as the initial stressor contained significantly greater abundances than those subjected to synchronous stressors (t = 3.77, p = 0.013) or plots subjected to nutrient enrichment initially (t = 3.04, p = 0.023). There were no differences in abundance found in the plots initially subjected to nutrients or synchronous with disturbance. Additionally, there were no differences between the order of application found in the additional stressor level treatments (low nutrients / high disturbance; high nutrients / high disturbance). Additionally, where physical disturbance was added first, abundance was affected by level of nutrient enrichment in plots that were subjected to low disturbance. There were no further significant differences between levels of treatment within orders of application.

Table 6.8: PERMANOVA output of three-way interaction between Nutrients level (Low High), Disturbance level (Low High) and the order in which stressors were applied (simultaneously or nutrients / disturbance first) analysing the effects of square root transformed abundances per plot in Botany Bay and Lane Cove after 4 months. N=5-7 for all treatments, Botany Bay total N = 83, Lane Cove total N = 78. Continued over page.

			Botany Bay		Lane Cove	
Species	**Treatment**	**df**	**Pseudo-F**	**P(perm)**	**Pseudo-F**	**P(perm)**
Mysella vitrea	**N**	1	0.01	0.915	2.61	0.098
	D	1	0.05	0.830	0.06	0.812
	O	2	0.06	0.947	3.03	0.068
	N X D	1	1.12	0.290	0.03	0.888
	N X O	2	0.25	0.771	2.65	0.084
	D X O	2	0.01	0.993	1.81	0.166
	N X D X O	2	1.53	0.211	0.17	0.845
Prionosio sp.	**N**	1	**10.26**	**0.005**	**7.06**	**0.013**
	D	1	0.15	0.700	0.02	0.890
	O	2	0.97	0.388	**5.44**	**0.011**
	N X D	1	0.23	0.628	0.64	0.416
	N X O	2	1.49	0.245	1.29	0.269
	D X O	2	2.09	0.149	**3.04**	**0.050**
	N X D X O	2	**5.03**	**0.012**	0.35	0.735
Mediomastus australiensis	**N**	1	0.01	0.918	2.22	0.126
	D	1	0.21	0.637	0.38	0.527
	O	2	1.80	0.160	0.35	0.713
	N X D	1	0.88	0.347	0.30	0.583
	N X O	2	**3.38**	**0.034**	0.39	0.657
	D X O	2	0.28	0.772	0.41	0.670
	N X D X O	2	**3.35**	**0.041**	0.79	0.438
Nephtys australiensis	**N**	1	1.02	0.350	0.36	0.525
	D	1	1.38	0.230	2.50	0.116
	O	2	**4.22**	**0.018**	0.43	0.654
	N X D	1	0.34	0.588	0.01	0.948
	N X O	2	0.60	0.547	0.03	0.964
	D X O	2	0.23	0.800	0.05	0.956
	N X D X O	2	0.13	0.878	0.14	0.881
Owenia australis	**N**	1	0.08	0.783		
	D	1	2.18	0.156		
	O	2	**3.25**	**0.047**		
	N X D	1	0.28	0.616		
	N X O	2	0.64	0.519		
	D X O	2	0.02	0.982		
	N X D X O	2	0.32	0.750		

Table 6.8 continued

Species	Treatment	df	Pseudo-F	P(perm)	Pseudo-F	P(perm)
Salinator fragilis	**N**	1	0.19	0.672		
	D	1	0.42	0.494		
	O	2	0.3	0.727		
	N X D	1	3.41	0.079		
	N X O	2	0.37	0.695		
	D X O	2	0.06	0.930		
	N X D X O	2	0.22	0.816		
Oedicerotidae	**N**	1			1.20	0.268
	D	1			0.05	0.825
	O	2			2.58	0.067
	N X D	1			**6.18**	**0.009**
	N X O	2			**3.42**	**0.043**
	D X O	2			2.57	0.088
	N X D X O	2			0.44	0.646
Gammarus sp.	**N**	1			3.02	0.083
	D	1			0.73	0.370
	O	2			0.48	0.618
	N X D	1			2.48	0.116
	N X O	2			0.12	0.885
	D X O	2			0.48	0.612
	N X D X O	2			1.95	0.150
Large nematodes	**N**	1			1.25	0.244
	D	1			1.91	0.160
	O	2			0.77	0.485
	N X D	1			0.42	0.539
	N X O	2			0.60	0.544
	D X O	2			1.30	0.298
	N X D X O	2			0.86	0.452
Australonereis ehlersi	**N**	1			0.02	0.903
	D	1			**4.54**	**0.036**
	O	2			**4.55**	**0.018**
	N X D	1			0.01	0.932
	N X O	2			1.91	0.167
	D X O	2			1.87	0.141
	N X D X O	2			2.93	0.065
Scoloplos sp.	**N**	1			0.84	0.325
	D	1			3.90	0.055
	O	2			1.44	0.252
	N X D	1			1.57	0.199
	N X O	2			**4.34**	**0.012**
	D X O	2			0.41	0.694
	N X D X O	2			**3.51**	**0.033**

Table 6.8 continued

Species	Treatment	df	Pseudo-F	P(perm)	Pseudo-F	P(perm)
Psammobiidae	**N**	1			0.16	0.694
	D	1			**5.02**	**0.033**
	O	2			**5.52**	**0.008**
	N X D	1			**3.90**	**0.041**
	N X O	2			1.45	0.229
	D X O	2			1.08	0.336
	N X D X O	2			0.35	0.718
Tellina deltoidalis	**N**	1			1.99	0.164
	D	1			0.00	0.970
	O	2			1.72	0.177
	N X D	1			1.09	0.277
	N X O	2			1.79	0.168
	D X O	2			0.42	0.675
	N X D X O	2			1.78	0.171
Platynereis uniseris	**N**	1			0.05	0.847
	D	1			0.70	0.420
	O	2			1.13	0.338
	N X D	1			1.42	0.214
	N X O	2			0.13	0.874
	D X O	2			0.34	0.695
	N X D X O	2			0.73	0.474

Key: **Bold** = significant effect at $p < 0.05$

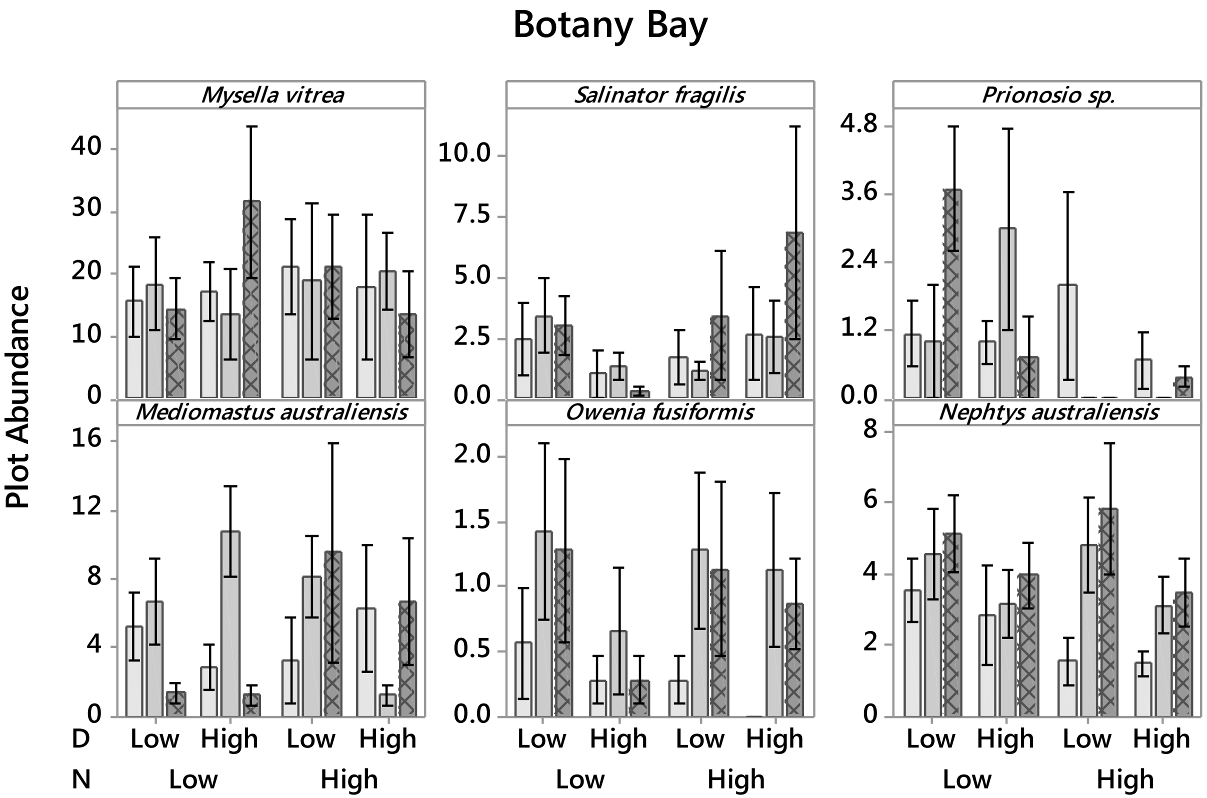


Figure 6.17a: Bar graph to show change in total abundance of macrofauna species per plot at Botany Bay and Lane Cove after four months of plots being subjected to Nutrient enrichment (N) and physical disturbance (D) either simultaneously (Grey hatched) or asynchronously where plots were subjected to an initial stressor of physical disturbance (light grey) or nutrient enrichment (Dark grey). Plots were subjected to high and low stressors. Points indicate mean +/- SE. N = 7

Lane Cove

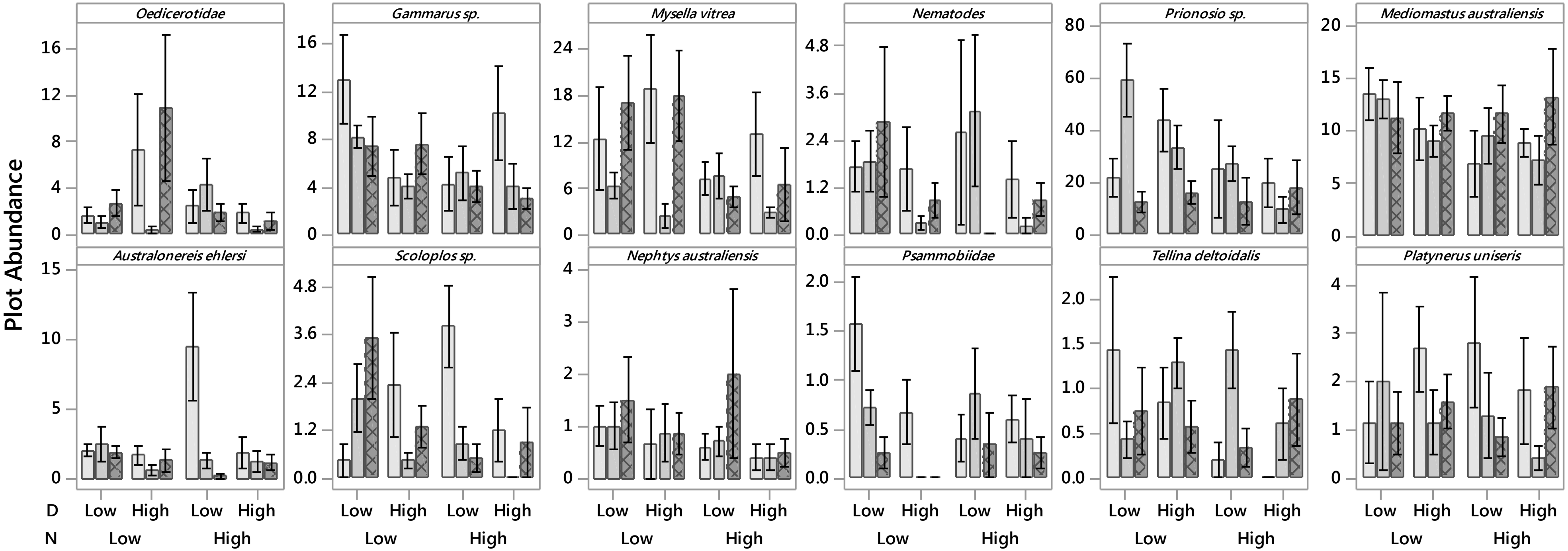
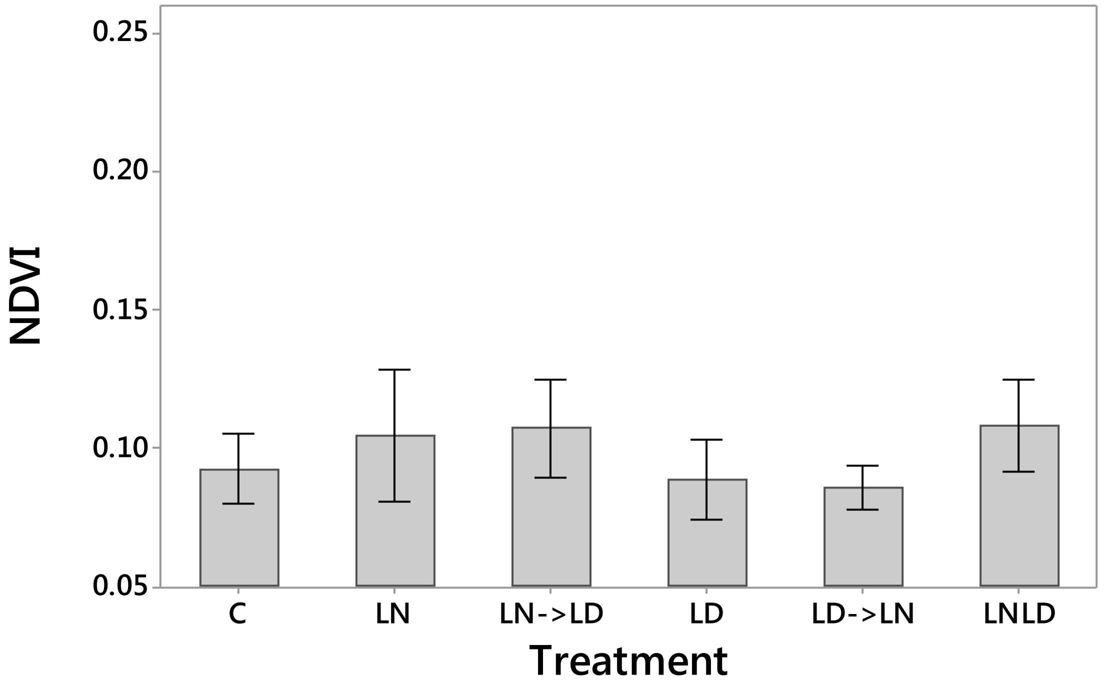


Figure 6.17b: Bar graph to show change in total abundance of macrofauna species per plot at Botany Bay and Lane Cove after four months of plots being subjected to Nutrient enrichment (N) and physical disturbance (D) either simultaneously (Grey hatched) or asynchronously where plots were subjected to an initial stressor of physical disturbance (light grey) or nutrient enrichment (Dark grey). Plots were subjected to high and low stressors. Points indicate mean +/- SE. N = 7

6.4.3 How does the order of application compare with those subjected to single stress?

When split into their constituent parts (LNLD, LNHD, HNLD and HNHD) and compared to controls, after 4 months of experimental manipulations the only differences in NDVI between treatments were found in the HNLD (Pseudo-$F_{(5,33)}$ = 2.59, p(perm) = 0.025) and HNHD (Pseudo-$F_{(5,33)}$ = 2.74, p(perm) = 0.026) analyses at Botany Bay (Figure 6.18c and d). There were no pre-existing differences between treatments at the start of the experiment. In both analyses the singular nutrient treatment had a greater NDVI that the physical disturbance treatment. In the HNLD condition, the high nutrient treatment also had a greater NDVI than the asynchronous treatment where nutrients were the initial stressor. In addition the control or the low disturbance treatment did not differ from any of the multiple stressor treatments. Finally, the asynchronous treatments differed from each other where the plots first subjected to high nutrient enrichment had a greater NDVI. In the HNHD condition (Figure 6.18 d) there was a significant difference between the plots subjected to the singular effect of high nutrients and plots that were subjected to simultaneous multiple stressors. In addition, the plots subjected to the singular effect of high disturbances had a lower NDVI than those plots that were subjected to simultaneous multiple stressors whereas the singular disturbance treatment did not differ from either of the asynchronous treatments. The control treatment did not differ from any treatment subjected to multiple stressors. Finally there were no differences between any of the treatments subjecting plots to multiple stressors. There were also no differences in NDVI found in these analyses at Lane Cove.

a)



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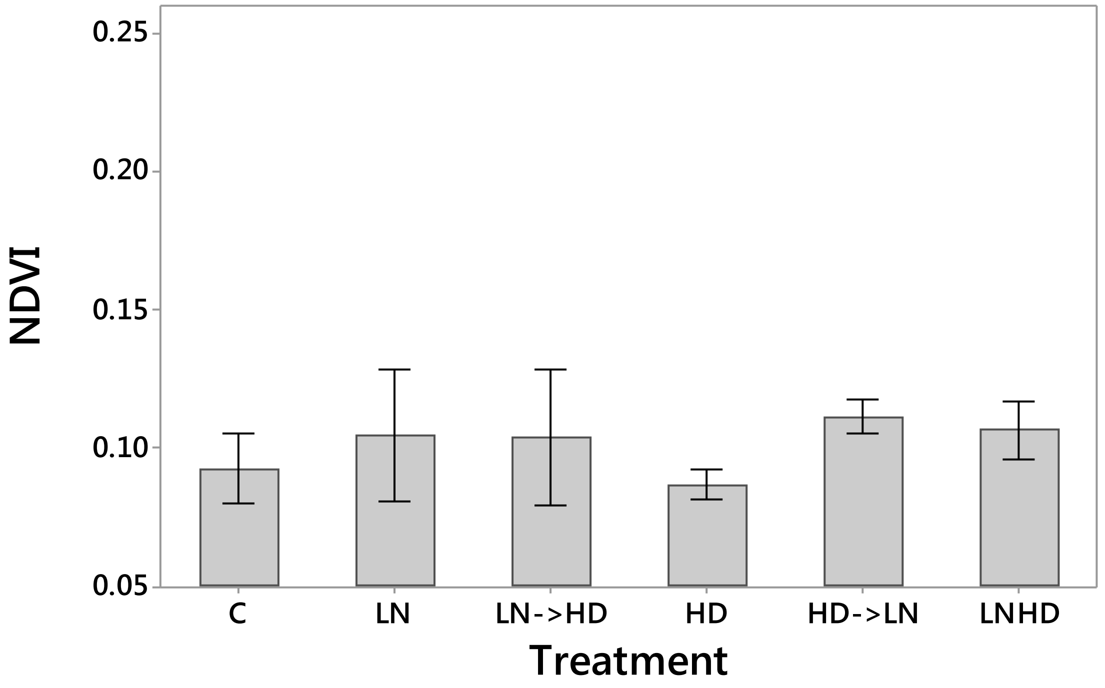
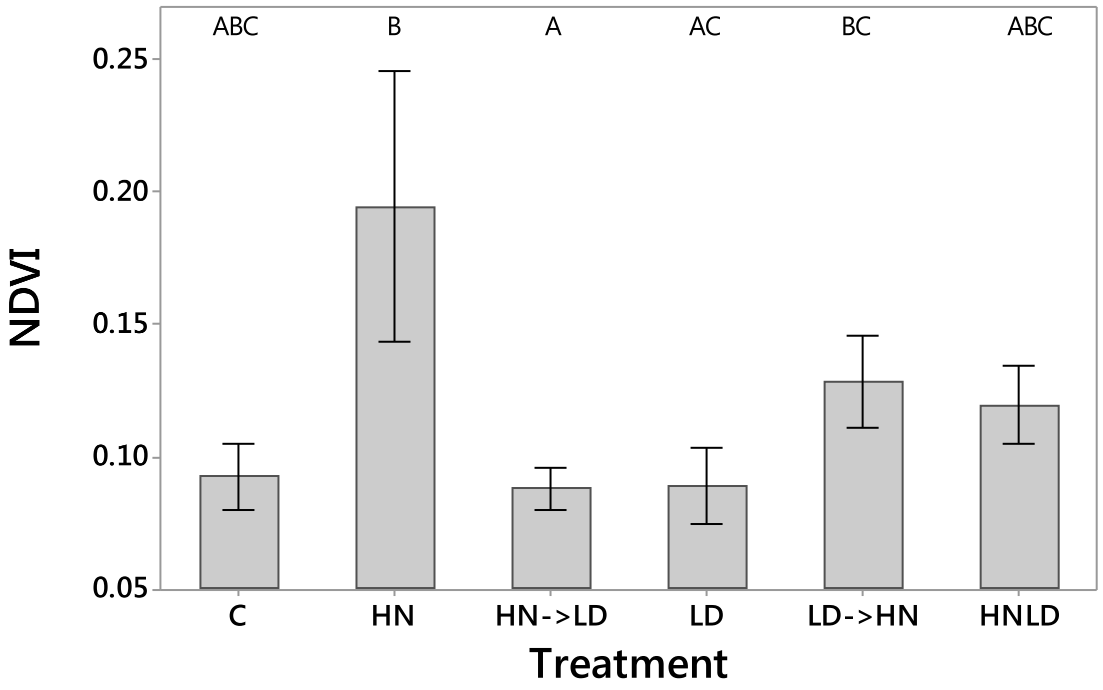


Figure 6.18: Bar graph comparing microphytobenthic biomass (recorded as NDVI) among plots in Botany Bay for 4 separate analyses representing a final combination of stressors: a) LNLD, b) LNHD, c) HNLD and d) HNHD. Plots received nutrients (N) and disturbance (D) stressors in high (H) or low (L) intensities either singularly, simultaneously (LNHD) or sequentially (indicated by -> symbol) where nutrients (e.g. HN->HD) or disturbance (e.g. HD->HN) were added first, 2 months prior to the second stressor. C = Control. N = 5-7. Within an analysis where significant differences due to treatments occurred, letters are used to indicate statistically significant differences between treatments, a shared letter indicates no significant difference (PERMANOVA *post hoc* tests).

c)



d)

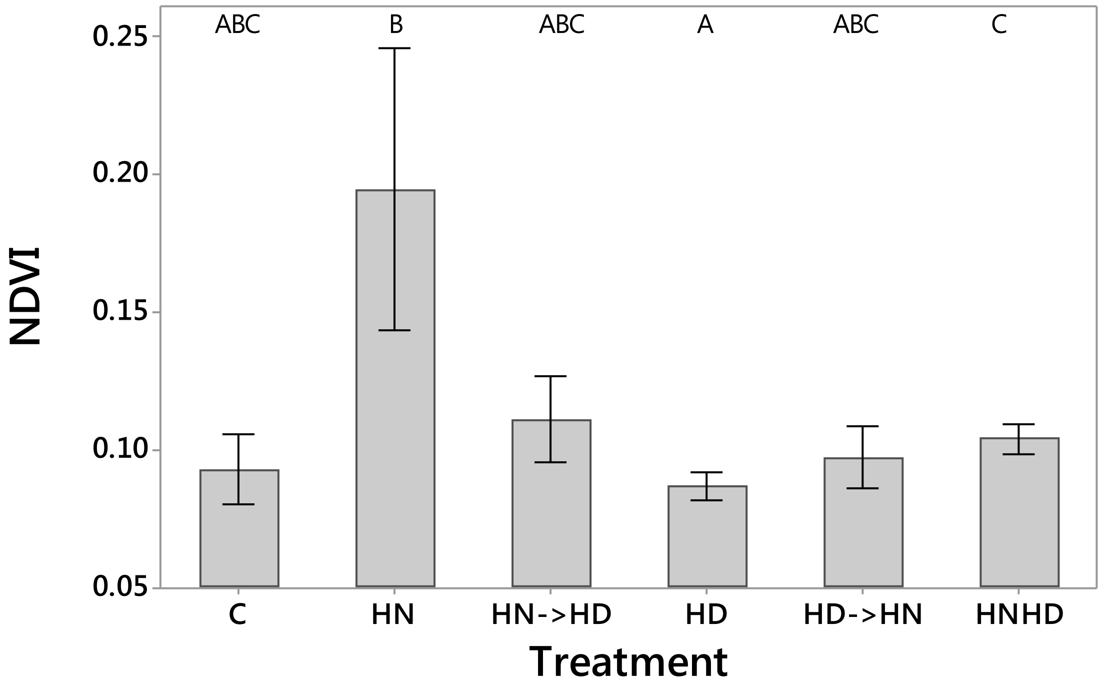


Figure 6.18 continued: Bar graph comparing microphytobenthic biomass (recorded as NDVI) among plots in Botany Bay for 4 separate analyses representing a final combination of stressors: a) LNLD, b) LNHD, c) HNLD and d) HNHD. Plots received nutrients (N) and disturbance (D) stressors in high (H) or low (L) intensities either singularly, simultaneously (LNHD) or sequentially (indicated by -> symbol) where nutrients (e.g. HN->HD) or disturbance (e.g. HD->HN) were added first, 2 months prior to the second stressor. C = Control. N = 5-7. Within an analysis where significant differences due to treatments occurred, letters are used to indicate statistically significant differences between treatments, a shared letter indicates no significant difference (PERMANOVA *post hoc* tests).

When treatments were split into their constituent parts (LNLD, LNHD, HNLD and HNHD) and compared to controls, at the start of the experiment, there were no pre-existing differences between the macrofaunal community structure within treatments ($p > 0.05$). After 4 months of experimental manipulations, community composition significantly differed only in LNHD (Pseudo-$F_{5,33}$ = 1.86, p = 0.002; Figure 6.19) and HNLD (Pseudo-$F_{5,33}$ = 1.63, p = 0.027; Figure 6.20) at Botany Bay and LNHD at Lane Cove (Pseudo-$F_{5,33}$ = 1.66, p = 0.005; Figure 6.21). Pairwise comparisons for all treatments can be found in Table 6.9.

In the LNHD analyses at Botany Bay, the community structure within the control treatment only differed significantly from the plots that were subjected to initially subjected to nutrient enrichment (Table 6.9; t = 1.68, p = 0.032). There was no difference between plots subjected to the singular effects of physical disturbance and those subjected to the two stressors. Only in plots subjected to nutrient enrichment initially did the community composition differ from those subjected to nutrient enrichment alone (t = 1.52, p = 0.035). Finally, community composition varied between plots subjected to nutrients initially and those subjected to disturbance initially (t = 1.66, p = 0.029) as well as those subjected synchronously (t = 1.51, p = 0.036).

Conversely in the LNHD analysis at Lane Cove, the community structure within the control treatment only differed significantly from the plots that were initially subjected to nutrient enrichment (Table 6.9; t = 1.93, p = 0.004). There was no difference between plots subjected to the singular effects of nutrient enrichment and those subjected to the two stressors. Only in plots initially subjected to nutrient enrichment did the community composition differ from those subjected to physical disturbance alone (t = 1.70, p = 0.006). Finally, similarly to Botany Bay, community composition varied between plots initially subjected to nutrients and those initially subjected to disturbance (t = 1.73, p = 0.009) as well as those synchronously affected (t = 2.05, p = 0.001).

In the HNLD analyses at Botany Bay, the community structure within the control treatment differed significantly from the plots that were initially subjected to nutrient enrichment (Table 6.9; t = 1.66, p = 0.016). There was also a slight difference between the control and synchronously affected plots (t = 1.40, p = 0.051). There was no difference between plots subjected to the singular effects of nutrient enrichment and those subjected to the two stressors. Plots initially subjected to nutrient enrichment (t = 1.70, p = 0.021) and those synchronously affected with physical disturbance (t = 1.48, p = 0.041) with physical disturbance showed significant differences in community structure compared to physical disturbance alone. Finally, there were no significant pairwise differences in community composition between any of the 3 treatments subjected to two stressors (synchronously or asynchronously).

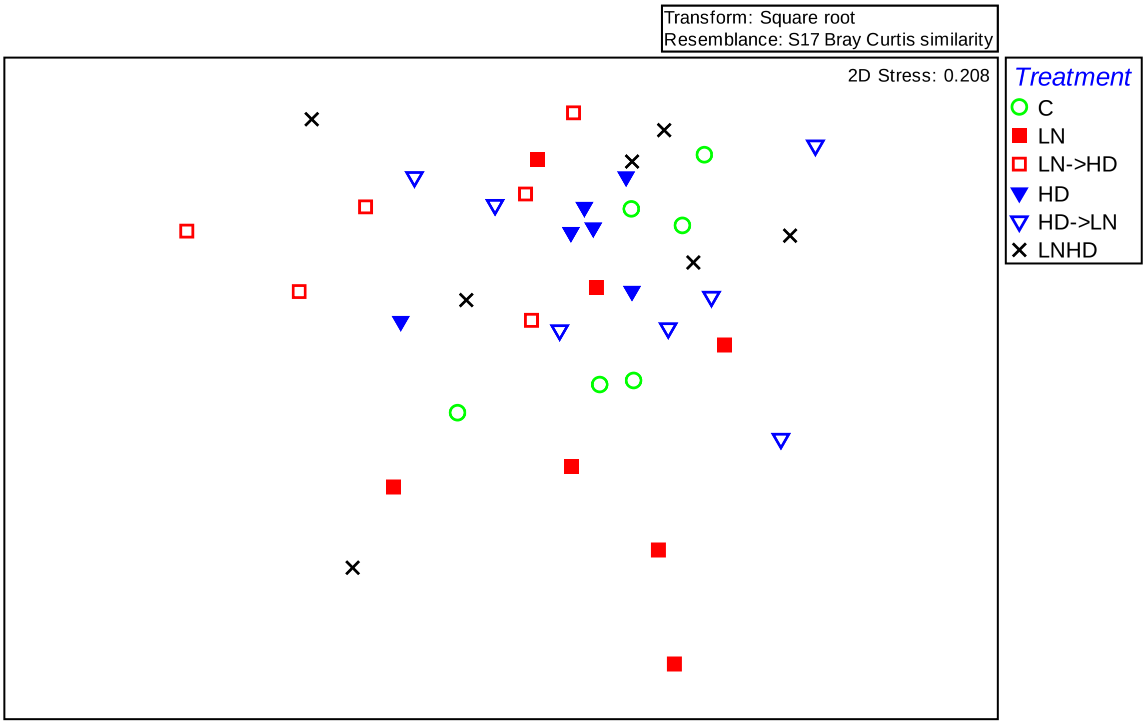


Figure 6.19: nMDS plot comparing macrofaunal community composition among sediment plots in Botany Bay receiving low nutrients (LN) and high disturbance (HD) stressors singularly, simultaneously (LNHD) or sequentially where nutrients (LN->HD) or disturbance (HD->LN) were added first, 2 months prior to the second stressor. C = Control. Points represent replicate plots, with data square-root transformed prior to analysis. N = 5-7

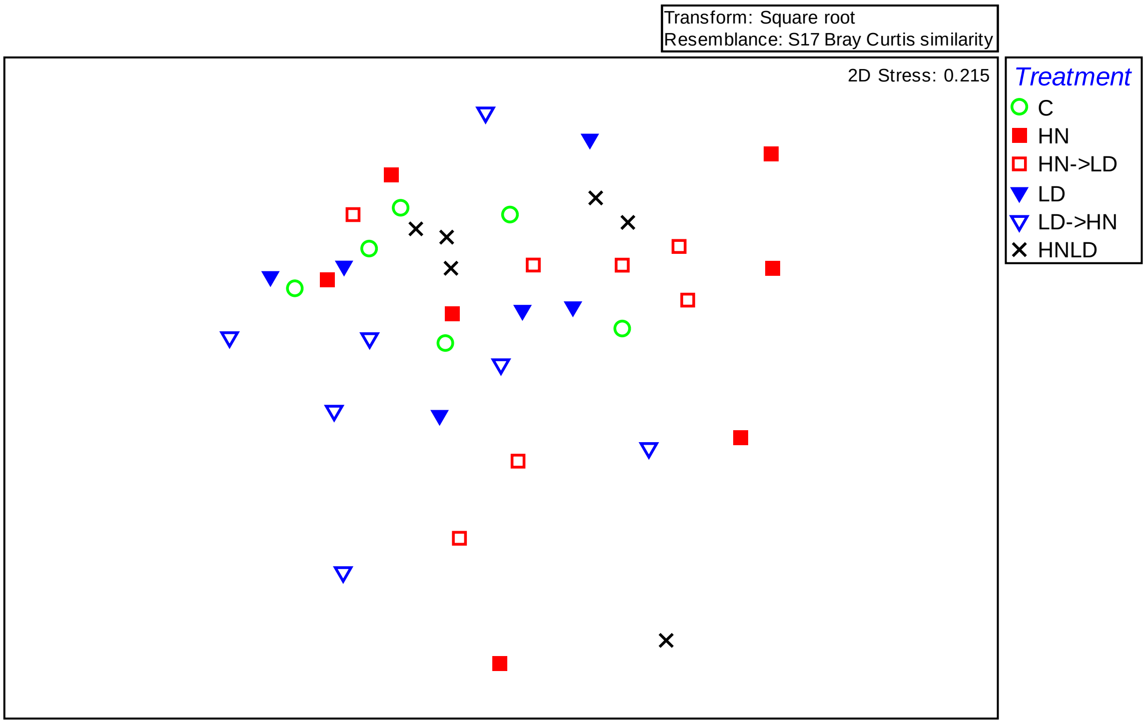


Figure 6.20: nMDS plot comparing macrofaunal community composition among sediment plots in Botany Bay receiving high nutrients (HN) and low disturbance (LD) stressors singularly, simultaneously (HNLD) or sequentially where nutrients (HN->LD) or disturbance (LD->HN) were added first, 2 months prior to the second stressor. C = Control. Points represent replicate plots, with data square-root transformed prior to analysis. N = 5-7

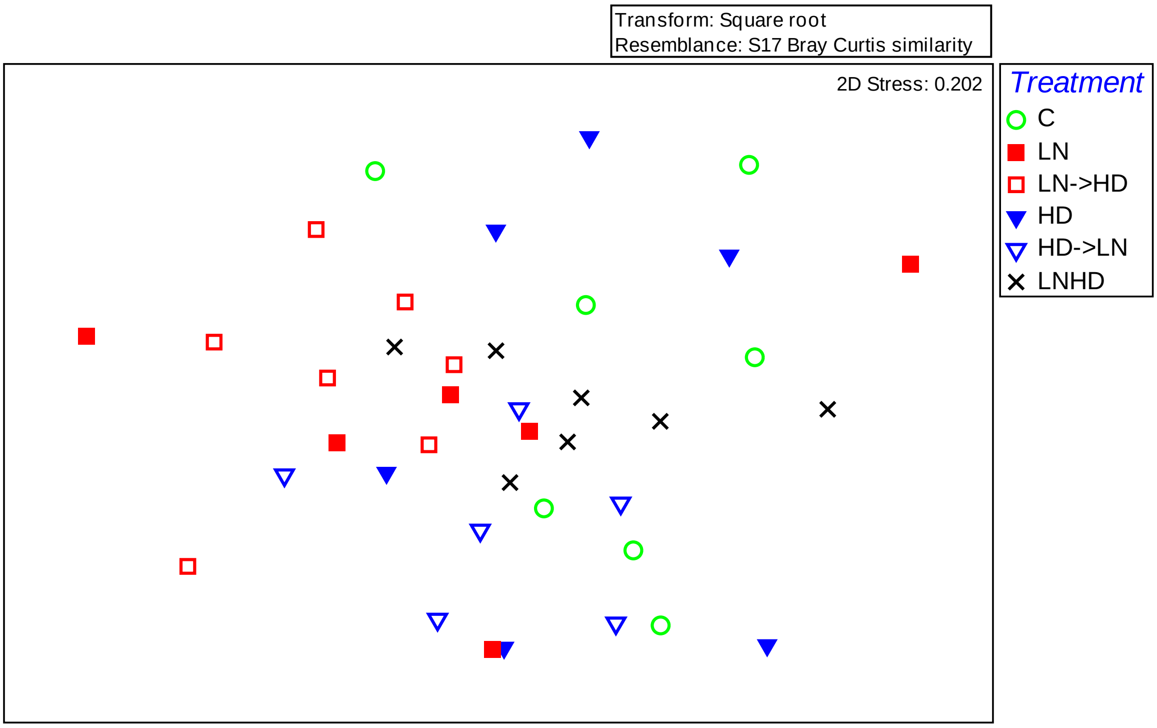


Figure 6.21: MDS plot comparing macrofaunal community composition among sediment plots in Lane Cove receiving low nutrients (LN) and high disturbance (HD) stressors singularly, simultaneously (LNHD) or sequentially where nutrients (LN->HD) or disturbance (HD->LN) were added first, 2 months prior to the second stressor. C = Control. Points represent replicate plots, with data square-root transformed prior to analysis. N = 5-7

Table 6.9: PERMANOVA pairwise comparisons output following 1-way ANOVA comparing macrofaunal community composition among sediment plots in Botany Bay and Lane Cove receiving nutrients (LN / HN) and disturbance (LD / HD) stressors singularly, simultaneously (LNHD / HNLD) or sequentially where nutrients (LN->HD / HN->LD) or disturbance (HD->LN / LD->HN) were added first, 2 months prior to the second stressor. C = Control. N = 5-7.

Botany Bay - LNHD				Lane Cove - LNHD				Botany Bay - HNLD			
Treatment 1	Treatment 2	t	P(perm)	Treatment 1	Treatment 2	t	P(perm)	Treatment 1	Treatment 2	t	P(perm)
C	HD	1.50	0.068	C	HD	0.73	0.885	C	HN	1.22	0.181
C	HD->LN	1.30	0.124	C	HD->LN	1.35	0.073	**C**	**HN->LD**	**1.66**	**0.016**
C	LN	1.01	0.430	C	LN	1.00	0.449	**C**	**HNLD**	**1.40**	**0.051**
C	**LN->HD**	**1.67**	**0.032**	**C**	**LN->HD**	**1.93**	**0.004**	C	LD	0.57	0.883
C	LNHD	1.07	0.361	C	LNHD	0.95	0.539	C	LD->HN	1.35	0.104
HD	HD->LN	1.24	0.171	HD	HD->LN	1.24	0.119	HN	HN->LD	1.04	0.381
HD	**LN**	**1.57**	**0.033**	HD	LN	0.98	0.484	HN	HNLD	1.08	0.309
HD	LN->HD	1.47	0.061	**HD**	**LN->HD**	**1.70**	**0.006**	HN	LD	1.24	0.157
HD	LNHD	0.98	0.486	HD	LNHD	0.92	0.615	HN	LD->HN	1.20	0.194
HD->LN	LN	1.32	0.080	HD->LN	LN	1.05	0.370	HN->LD	HNLD	0.83	0.724
HD->LN	**LN->HD**	**1.66**	**0.029**	**HD->LN**	**LN->HD**	**1.73**	**0.009**	**HN->LD**	**LD**	**1.70**	**0.021**
HD->LN	LNHD	1.25	0.131	HD->LN	LNHD	1.22	0.171	HN->LD	HN->LD	1.46	0.064
LN	**LN->HD**	**1.52**	**0.035**	LN	LN->HD	1.20	0.153	**HNLD**	**LD**	**1.48**	**0.041**
LN	LNHD	1.25	0.125	LN	LNHD	0.92	0.585	HNLD	LD->HN	1.35	0.114
LN->HD	**LNHD**	**1.51**	**0.036**	**LN->HD**	**LNHD**	**2.05**	**0.001**	LD	LD->HN	1.28	0.162

Key: **Bold** = significant effect at p < 0.05

SIMPER analysis identifying the key contributors to dissimilarity among treatments identified the same species as in the previous analysis – 6 in Botany Bay, and 12 in Lane Cove. At Botany Bay, many of these species responded subtly to the effect of the treatments, while only *Prionosio sp.* showed a significant response in all 4 analyses (Figure 6.22). Pairwise tests in the LNLD analysis (Figure 6.22a) revealed that there were no differences between the control and the single stressor treatments. Where in combination, the abundance was lower in both of the delayed treatments whereas plots subjected to simultaneous stress showed no change in comparison to the controls. In the LNHD condition (Figure 6.22b), the combined treatments had lower abundances than the control although only the simultaneous treatment was significantly different from the control. The high disturbance treatment was significantly different to the control but not any of the combined treatments while the low nutrient treatments only differed from the simultaneous treatment which contained significantly lower abundances. The analysis of the HNLD treatments (Figure 6.22c) revealed that the high nutrient treatment had significantly lower abundances than the control, however there were no differences between this and any of the combined treatments. The low disturbance treatment contained greater abundances than all the combined treatments however when low disturbance was applied initially there was no significant differences. In the HNHD analysis (Figure 6.22 d), pairwise comparisons indicated there were no differences between *Prionosio sp.* abundances in any of the stressed treatments, while all were significantly different from the control ($p < 0.05$).

Mediomastus australiensis showed a significant response to stressors only under the LNHD scenario (Figure 6.23; Pseudo-$F_{5,35} = 5.52$, $p = 0.004$). Their abundance was greater in the plots receiving the low nutrient and then the high physical disturbance treatments sequentially than in any of the other treatments. The plots receiving high disturbance alone contained the next most *Mediomastus australiensis*. The other treatments, including those receiving only low nutrient disturbance, the two stressors together, or the high physical disturbance before the nutrient addition did not significantly differ from the control.

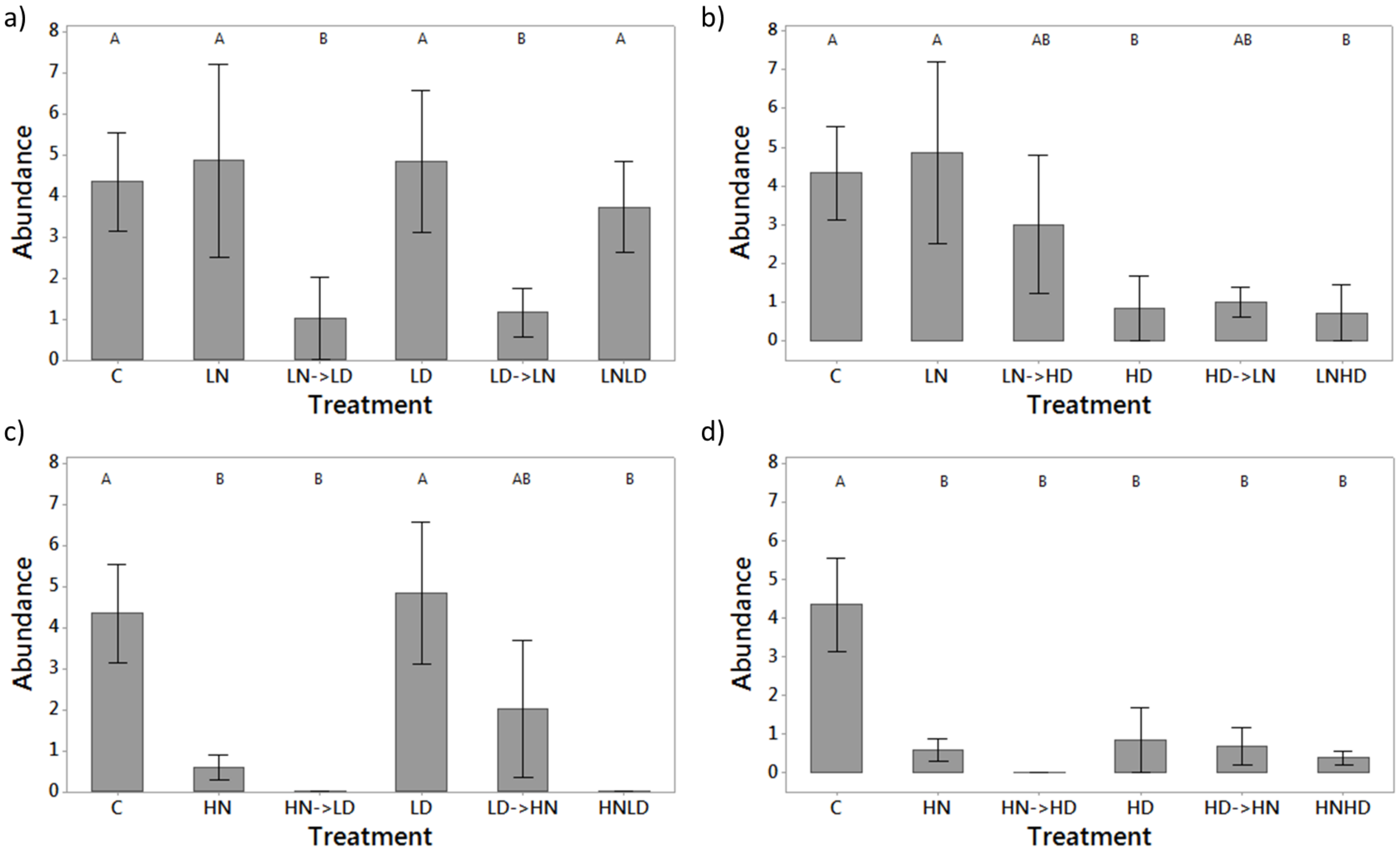


Figure 6.22: Bar graph comparing *Prionosio sp.* abundance per core among plots in Botany Bay for 4 separate analyses representing a final combination of stressors: a) LNLD, b) LNHD c) HNLD and d) HNHD. Plots received nutrients (N) and disturbance (D) stressors in high (H) or low (L) intensities either singularly, simultaneously (LNHD) or sequentially (indicated by -> symbol) where nutrients (e.g. HN->HD) or disturbance (e.g. HD->HN) were added first, 2 months prior to the second stressor. C = Control. N = 5-7. Within an analysis where significant differences due to treatments occurred, letters are used to indicate statistically significant differences between treatments, a shared letter indicates no significant difference (PERMANOVA *post hoc* tests).

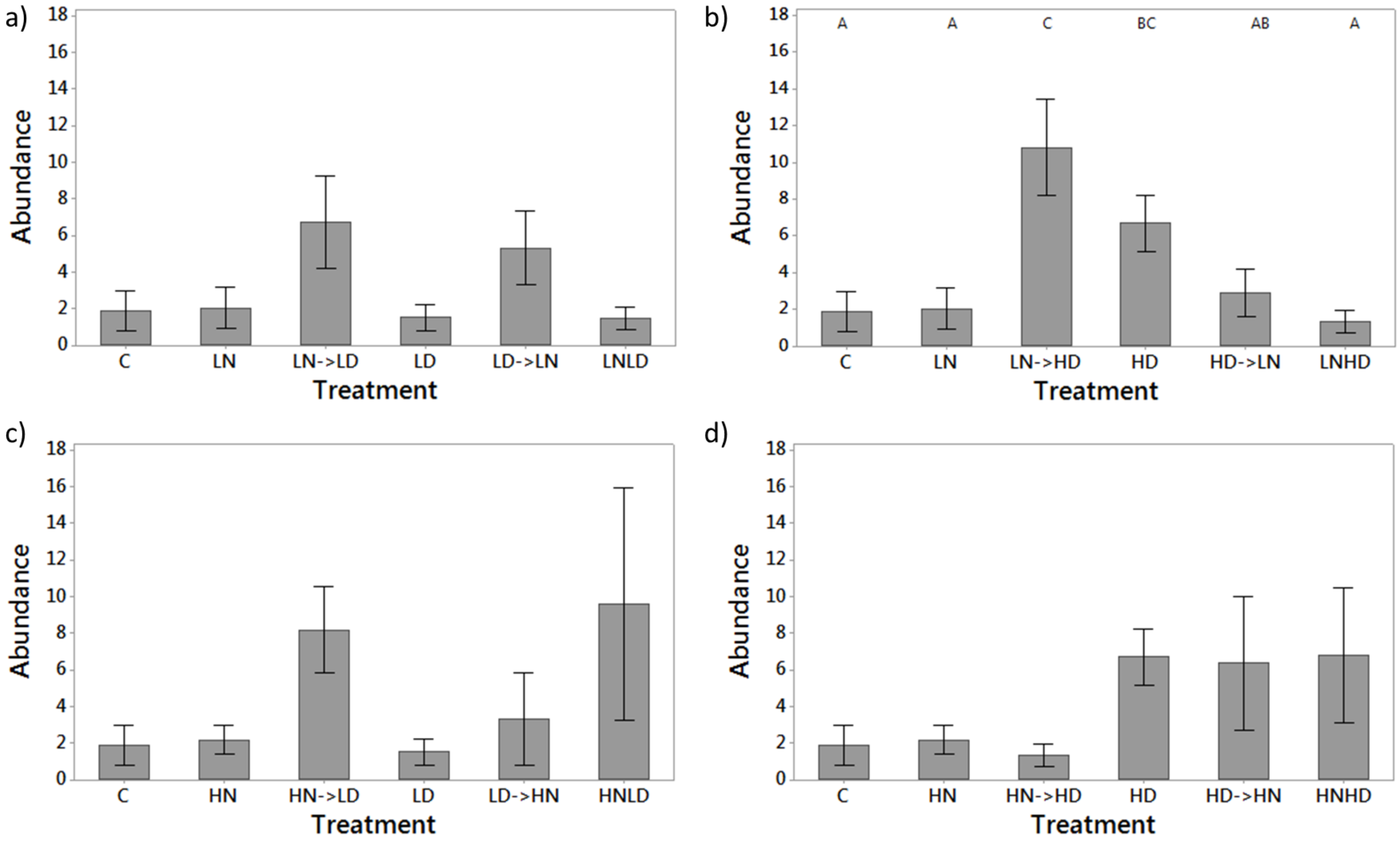


Figure 6.24: ***Mediomastus australiensis*** **abundance per core among plots in Botany Bay for 4 separate analyses representing a final combination of stressors: a) LNLD, b) LNHD c) HNLD and d) HNHD. Plots received nutrients (N) and disturbance (D) stressors in high (H) or low (L) intensities either singularly, simultaneously (e.g. LNHD) or sequentially (indicated by -> symbol) where nutrients (e.g. HN->HD) or disturbance (e.g. HD->HN) were added first, 2 months prior to the second stressor. C = Control. N = 5-7. Within an analysis where significant differences due to treatments occurred, letters are used to indicate statistically significant differences between treatments, a shared letter indicates no significant difference (PERMANOVA** ***post hoc*** **tests).**

The dissimilarity between treatments found in the only significant multivariate analyses comparing LNHD treatments at Lane Cove(Figure21) was caused by a significant change in abundances of *Oedicerotidae* (Pseudo-$F_{(5,33)} = 2.53$, $p = 0.048$), *Scoloplos sp.* (Pseudo- $F_{(5,33)} = 2.65$, $p = 0.033$), the Psammobiidae (Pseudo- $F_{(5,33)} = 2.97$, $p = 0.021$) and *Tellina deltoidalis* (Pseudo- $F_{(5,33)} = 3.03$, $p = 0.021$).

At Lane Cove, *Scoloplos sp.* responded significantly to treatments in all 4 analyses, in each analysis, the abundance of this taxon was significantly lower in each of the disturbance treatments in comparison to the control (Figure 6.24). The only difference between disturbed treatments occurred in the HNLD analysis (Figure 6.24c) whereby the abundance of *Scoloplos sp.* was greater in the asynchronous treatment where disturbance was added initially in comparison to when nutrients were added singularly, initially or simultaneously with disturbance.

For *Oedicerotidae* there were no differences between treatments in any analysis apart from among the LNHD treatments (Figure 6.25b). The abundance of this species was greatest in plots receiving a mix of both stressors. Stressors applied simultaneously caused the highest abundances and plots receiving these treatments were significantly different to the controls and those receiving nutrients initially. Additionally plots that received physical disturbance as the initial stressor had a significantly greater abundance of individuals than in the control treatments. Treatments receiving singular disturbance did not differ from the control.

For unidentified Psammobiidae bivalve, the abundance varied significantly between treatments in the LNLD (Pseudo- $F_{(5,33)} = 8.01$, $p = 0.001$) and LNHD analysis but neither high nutrient analysis (Figure 6.26). In both analyses the highest abundances were found in the asynchronous plots initially subjected to physical disturbance. While in neither analysis was this treatment significantly different from the singular disturbance treatment it was from the singular treatment which was subjected to nutrient enrichment, further, in the LNHD analysis, this treatment level contained significantly greater abundances than the other asynchronous treatment (nutrients first) and the simultaneous treatment.

For *Tellina deltoidalis* the only differences in abundance were found in the LNHD treatment (Figure 6.27). While all treatments subjected to nutrient enrichment contained greater abundances than the control or the singular disturbance treatment, only the asynchronous treatment first subjected to nutrients contained significantly greater abundances. Further all treatments that were subjected to nutrients did not significantly differ in terms of abundance.

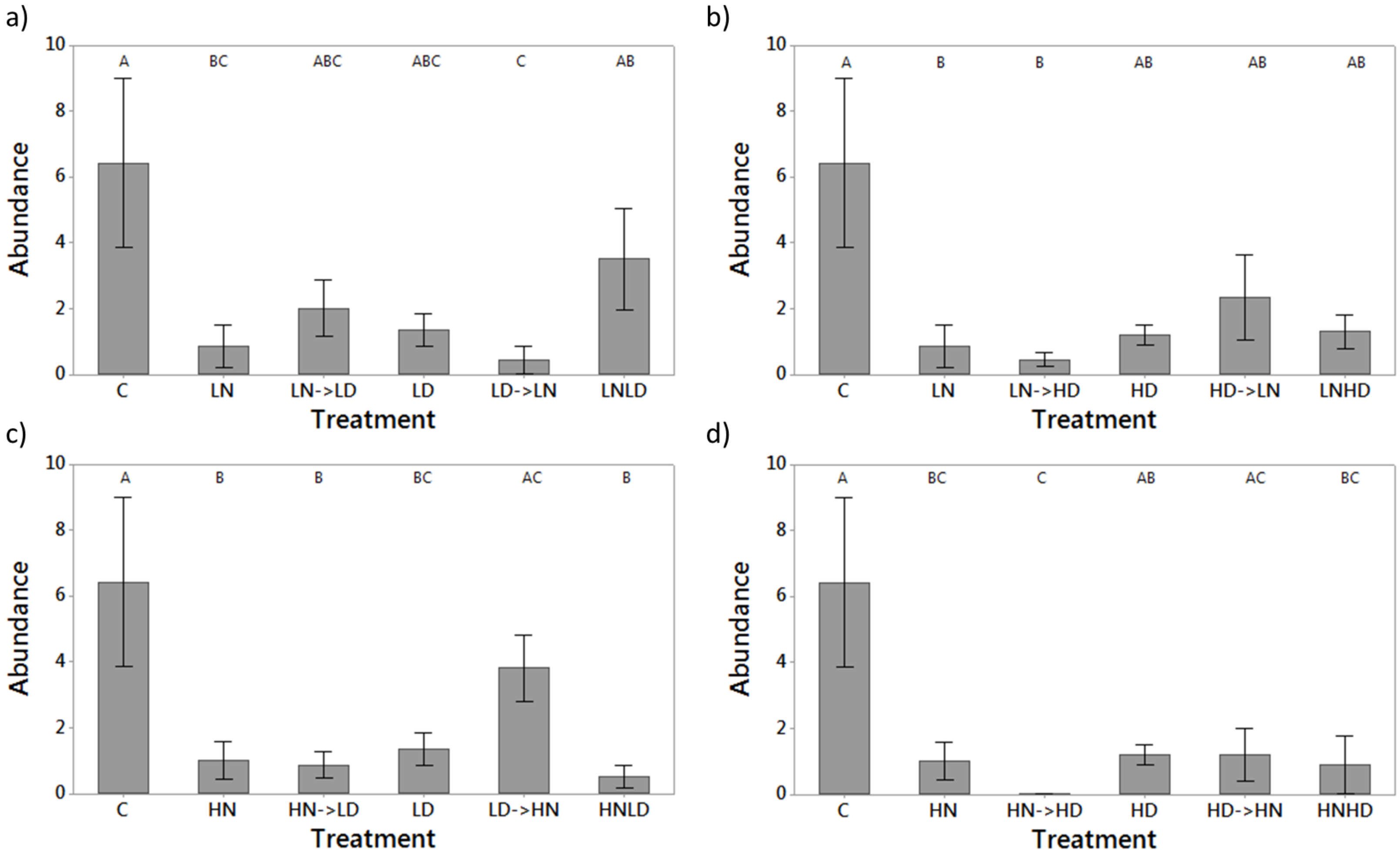


Figure 6.24: ***Scoloplos sp.*** **abundance per core among plots in Lane Cove for 4 separate analyses representing a final combination of stressors: a) LNLD, b) LNHD c) HNLD and d) HNHD. Plots received nutrients (N) and disturbance (D) stressors in high (H) or low (L) intensities either singularly, simultaneously (LNHD) or sequentially (indicated by -> symbol) where nutrients (e.g. HN->HD) or disturbance (e.g. HD->HN) were added first, 2 months prior to the second stressor. C = Control. N = 5-7. Within an analysis where significant differences due to treatments occurred, letters are used to indicate statistically significant differences between treatments, a shared letter indicates no significant difference (PERMANOVA *post hoc* tests).**

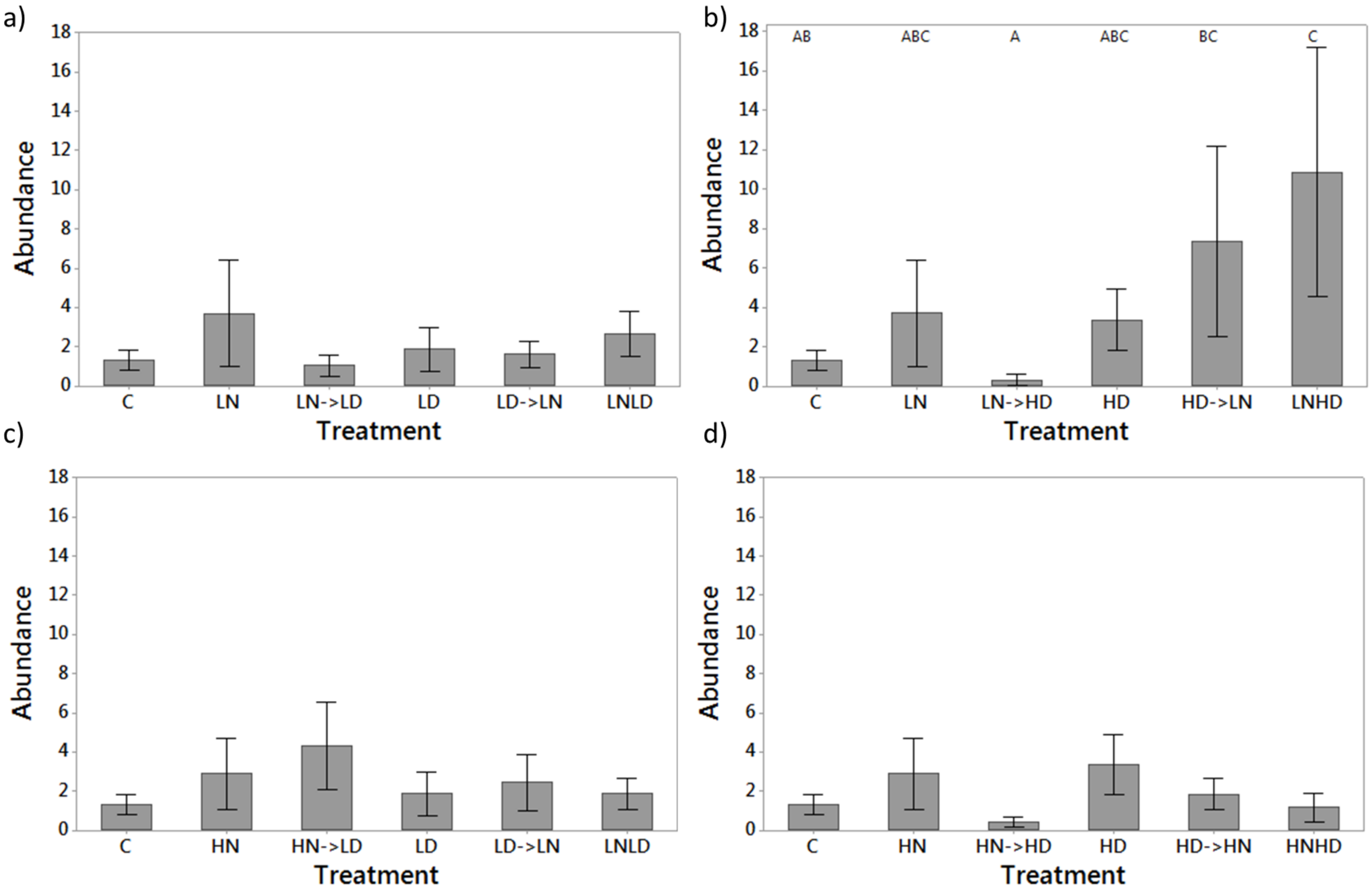


Figure 6.25: ***Oedicerotidae*** **abundance per core among plots in Lane Cove for 4 separate analyses representing a final combination of stressors: a) LNLD, b) LNHD c) HNLD and d) HNHD. Plots received nutrients (N) and disturbance (D) stressors in high (H) or low (L) intensities either singularly, simultaneously (LNHD) or sequentially (indicated by -> symbol) where nutrients (e.g. HN->HD) or disturbance (e.g. HD->HN) were added first, 2 months prior to the second stressor. C = Control. N = 5-7. Within an analysis where significant differences due to treatments occurred, letters are used to indicate statistically significant differences between treatments, a shared letter indicates no significant difference (PERMANOVA** ***post hoc*** **tests).**

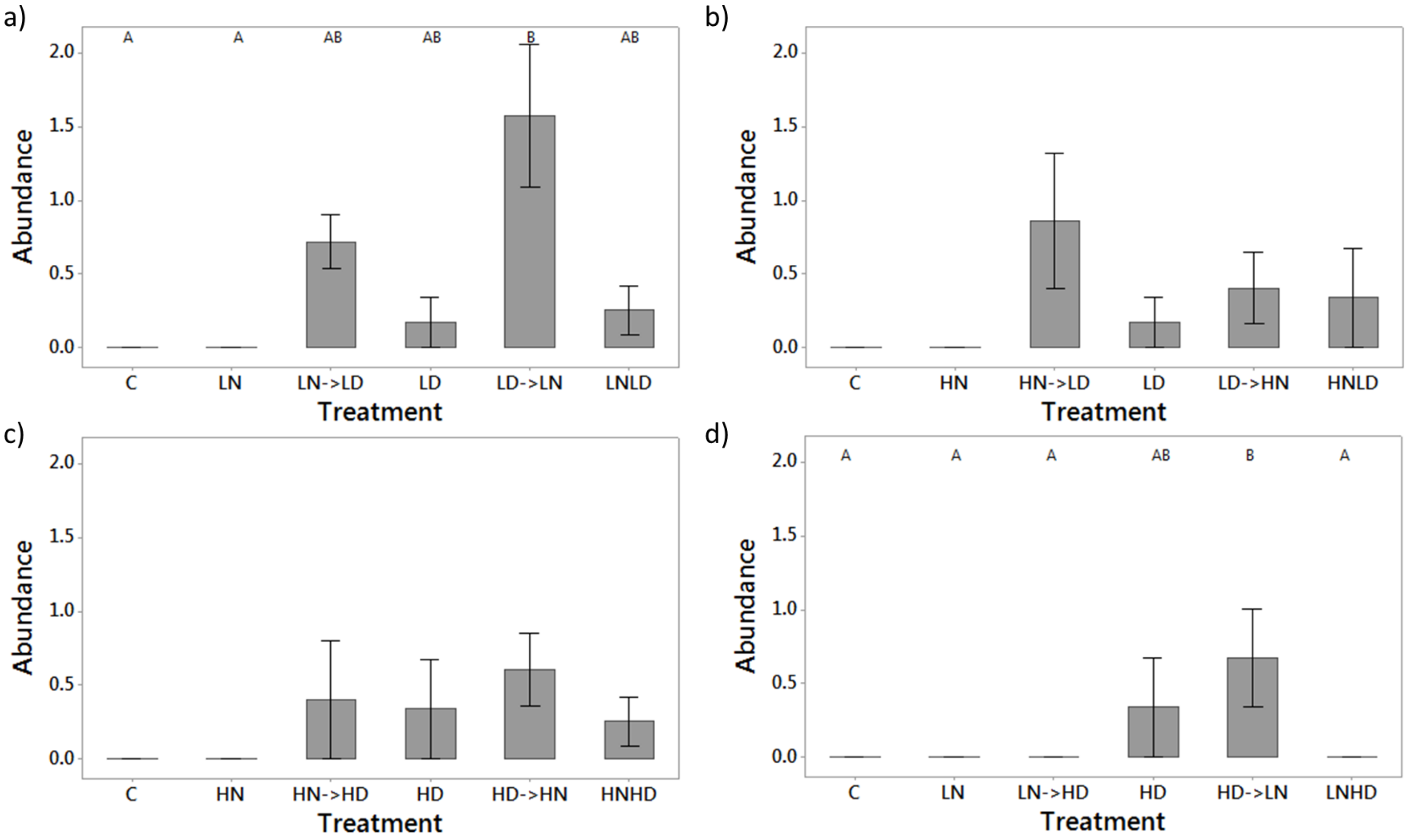


Figure 6.26: Psammobiidae abundance per core among plots in Lane Cove for 4 separate analyses representing a final combination of stressors: a) LNLD, b) LNHD c) HNLD and d) HNHD. Plots received nutrients (N) and disturbance (D) stressors in high (H) or low (L) intensities either singularly, simultaneously (LNHD) or sequentially (indicated by -> symbol) where nutrients (e.g. HN->HD) or disturbance (e.g. HD->HN) were added first, 2 months prior to the second stressor. C = Control. N = 5-7. Within an analysis where significant differences due to treatments occurred, letters are used to indicate statistically significant differences between treatments, a shared letter indicates no significant difference (PERMANOVA *post hoc* tests).

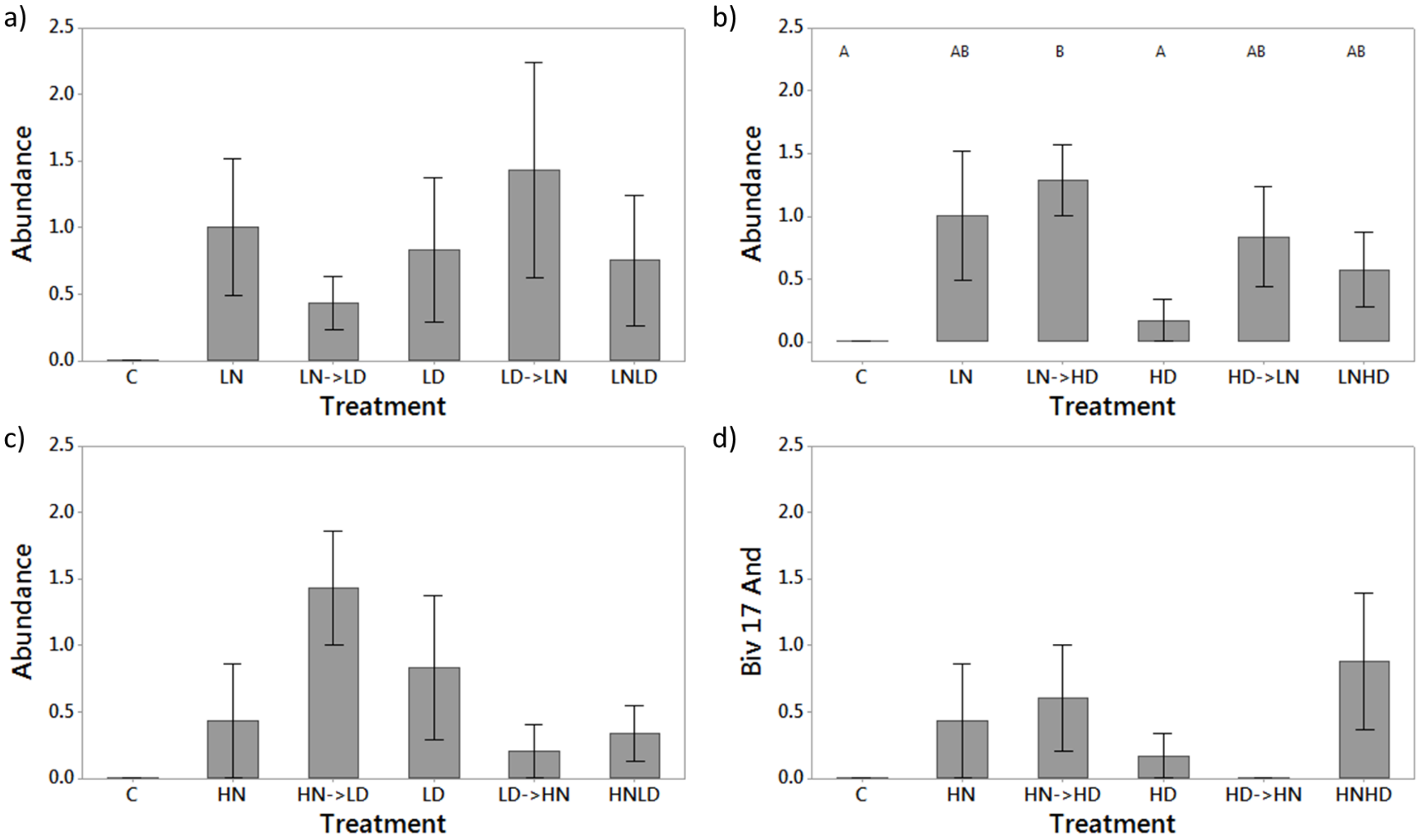


Figure 6.27: *Tellina deltoidalis* abundance per core among plots in Lane Cove for 4 separate analyses representing a final combination of stressors: a) LNLD, b) LNHD, c) HNLD and d) HNHD. Plots received nutrients (N) and disturbance (D) stressors in high (H) or low (L) intensities either singularly, simultaneously (LNHD) or sequentially (indicated by -> symbol) where nutrients (e.g. HN->HD) or disturbance (e.g. HD->HN) were added first, 2 months prior to the second stressor. C = Control. N = 5-7. Within an analysis where significant differences due to treatments occurred, letters are used to indicate statistically significant differences between treatments, a shared letter indicates no significant difference (PERMANOVA *post hoc* tests).

6.5 Discussion

At each site, physical disturbance had an overriding effect on altering the macrofaunal community structure. When nutrient enrichment was the first stressor applied, physical disturbance acted to alter community structure, conversely, when physical disturbance was the initial stressor, the added impact of nutrient enrichment had no further effect. At Lane Cove this meant that there were no significant differences between communities that were subjected to the physical disturbance stressor from the start of the experiment (whether through simultaneous application with nutrient enrichment or following delayed nutrient enrichment). This differed from Botany Bay, where all treatment types resulted in significantly different community structures. Similarly the microphytobenthic component was significantly altered by the order of stressors with non-additive interactions between stressors identified only when disturbance was applied as the initial stressor, however care should be taken interpreting the MPB response as results suggest that the MPB did not vary over and above the natural background variability observed at these sites.

This study furthers the exploration of effects of multiple stressors on ecosystems by addressing the topic of temporal coincidence – the possibility of multiple stressors to co-occur in space and time (Pincebourde et al., 2012) – exploring the nature of this coincidence, asking if and how the order and timing of multiple stressors might alter the community. This work differs from other studies on multiple stressors where research is dominated by experiments that manipulate stressors that go from an unstressed state to one in which they are subjected to two stressors simultaneously (Darling & Côté, 2008). While multiple stressors are more likely to occur in nature than single stressors, it is not correct to assume that stressors will impact a system simultaneously, or their effects will be felt immediately after an impact. In this study, the community's response to the multiple stressors nutrient enrichment and physical disturbance varied according to how stressors were applied – whether stressor A was applied before, after, or together with stressor B. Findings suggest that the response varies not only according to the intensity of stress, but also due to environmental context.

Limited effects were observed on the MPB however they were generally found to be affected more by the nutrient enrichment than the physical disturbance, as demonstrated earlier in this thesis. While there were individual stressor effects, the MPB did not seem to vary according to the order in which stressors were applied, indicating the end point was the same regardless of starting conditions. These organisms are fast growing and in Australia are nutrient limited therefore nutrients are a stronger driver of change (Cloern, 2001; Davis & Koop, 2006).

The macrofauna community structure changed depending on the nature of the temporal coincidence of stressors. Though nutrient enrichment may have a delayed effect on the macrofaunal community structure (e.g. Bishop & Kelaher, 2007; Morris & Keough, 2003b), they are likely to react more quickly to the effects of physical disturbance which could detrimentally alter the physical environment (Bishop, 2005). The direct effect of raking include causing damage to soft bodied organisms; destruction of burrows; and the removal of larger bodied organisms (Hall & Harding, 1997). The experimental duration was long enough that the exclusion and removal of certain species could have promoted the growth of more opportunistic species or led to outside of plot recruitment of more resilient species. Mechanisms for differences between treatments are explored below.

6.5.1 What effect does adding a second stressor have to the system experiencing an initial stressor?

In this study there was a little change in community structure after two months of experimental manipulations. It has been shown previously (in this thesis and otherwise) that it can sometimes take longer than 2 months for treatment effects in macrofaunal communities to become apparent (Morris & Keough, 2003b). While in some studies macrofauna rapidly respond to stressors (e.g. Morris & Keough, 2002), effects can vary depending on the type of stress (O'Brien et al., 2010), the specific location (Worm et al., 1999), heterogeneity of environment (Dyson et al., 2007), seasonal variability (Conde et al., 2013) or the species present (Rossi, 2003).

By contrast, after 4 months effects of both the original and the second stressor were apparent. Community structure was significantly altered by further disturbance only when subjected to nutrient enrichment in the first instance. Although there were pre-existing differences in the abundance of several taxa among plots at Lane Cove prior to the addition of stressors, treatment effects were apparent over and above this background variation, indicating effects of experimental intervention.

The significant effect of disturbance following months of nutrient stress indicates sensitivity to these prior nutrient additions. Aquatic systems have long been identified as being sensitive to nutrient stress (Cloern, 1999), and when derived from anthropogenic sources such as sewage or fertilisers, not only can it have ecological impacts, altering resource availability and food quality

(Evans-White et al., 2009), but nutrient stress may also cause eco-toxicological impacts (Camargo & Alonso, 2006). The possible toxicity and resultant stress from nutrient enrichment could cause additional stress above that which communities are previously adapted. The addition of the first stressor could push the system to a threshold whereby a further stressor impact causes a significant alteration of community structure resulting from reduced resilience (Evans-White et al., 2009; Folke et al., 2004).

While the effect of physical disturbance following prior nutrient enrichment was observed to cause significant changes, the opposite effect was unobserved – there were no effects of nutrients following prior physical disturbance. This could be because disturbance has an overriding effect on the community or it could be attributed to nutrient enrichment not having enough time to facilitate a change in community structure given that it was identified that it could take 4 months for treatment effects to be observed. This, however, is representative of short term nutrient enrichment following a physical disturbance stress. In the literature previous studies analysing short term responses of nutrient enrichment are not always able to identify responses (Cebrian et al., 2012; Pascal et al., 2013) whereas longer term applications of nutrient enrichment are more likely to alter community structure in the long term (Davis et al., 2010).

6.5.2 Does the order in which stressors were applied affect the final community?

At both sites the order in which stressors were applied (whether simultaneously or asynchronously) significantly altered the community. Species specific reactions to the stressors and the order of application were identified at each site. Additionally species common to each displayed site specific reactions. For many species the simultaneous application of stressors resulted in higher abundances compared to the asynchronous treatments. Additionally, for a number of species at both sites the treatments where nutrients were applied first resulted in higher abundances. Mechanisms behind higher abundances seen in simultaneous application of stressors could be due to acclimation to stressor effects over the full four month period. The organisms in the asynchronous treatments in this experiment had the potential to become physiologically weakened by the initial stressor prior to the addition of the second stressor (Bijma et al., 2013; Heip et al., 1995; Macreadie et al., 2011), thus resulting in a change in community structure through reduced fitness of the community (Culumber & Monks, 2014; Visram & Douglas, 2007).

Only at Lane Cove did diversity display a main effect due to treatment type, independent of order of application. Both nutrients and physical disturbance caused a decrease in biodiversity, irrespective of whether the stressors were added together or asynchronously. Only under the low enriched treatment were the effects of order able to be differentiated from each other, indicating that the main effects of the stressors were more important for altering diversity than the order in which they were applied. Nutrient levels have been documented to control the stable state of a system and further disturbances can trigger a switch in stable states (Gunderson, 2001). Diversity of the system could be linked to the interactions between stressors and the organisms present as opposed to the order in which stressors were applied, with one or more stressors having an overriding effect on the biodiversity (Worm et al., 1999).

While these results are unsurprising, care should be taken in their interpretation. Three scenarios were compared: stressor A applied before, after or simultaneously with stressor B. This means that in the first scenario stressor A was applied for 4 months and stressor B for 2 months. The second scenario was the inverse: stressor A for 2 months and stressor B for 4 months. The final scenario meant stressors A and B were both applied for 4 months each. In nature, the durations of these stressors would naturally alter depending on which scenario the stressors are applied, care needs to be taken in interpreting whether these effects are brought about due to the total length of time each stressor was applied or solely due to the order in which they were applied. As stressors were maintained over the same period of time, the changes in community structure could indicate a sensitivity or a decrease in resilience to the application of additional stress (Folke et al., 2004), any effect of the length of time would be analogous to a natural variation in how stressors would be applied. While controlling these factors is preferential, it would not give an accurate interpretation on what is happening in nature.

6.5.3 How does the order of application compare with those subjected to single stress?

While the order in which stress was applied had subtle effects on community structure, there is limited evidence to suggest that the order significantly altered the community from its natural background variability or from the impact of single stressors alone. There could be various mechanisms behind this but the most likely explanation is inferred from the variability observed in the controls. The natural variability of the shores are likely to vary at very small scales, from metres to even centimetres (Chapman et al., 2010; Chapman & Tolhurst, 2007) making any interpretation from these types of studies difficult.

Additionally both sites displayed species specific reactions to stressors. *Prionosio sp.* were a key species at both sites however, while the abundance of *Prionosio sp.* showed a response across all treatments at Botany Bay, there were no significant effects of the stressors at Lane Cove. Furthermore, there were species specific responses due to the type of order treatment (single, multiple, delayed, simultaneous) that varied due to an interaction between level and type of stress at either site. Given these treatment specific responses, particular levels of stressors applied in these experiments could work in combination with the local environmental conditions, causing the system to be pushed over the edge, altering individual abundances and overall community structure (Bishop & Kelaher, 2013; Hiddink et al., 2009; O'Connor & Donohue, 2013; Whomersley et al., 2010). The resilience of these systems can be related to the specific magnitude, frequency or duration of any particular type disturbance (Folke et al., 2004; Gunderson, 2000).

6.5.4 Implications of study and future directions

Natural systems are under stress from numerous temporally variable sources. Previous research has made an effort to control the temporal occurrences of manipulated stressors, thereby altering the temporal variability of stressor application while maintaining the relative frequencies of occurrence of a particular stressor (e.g. Berga et al., 2012; García Molinos & Donohue, 2010, 2011; Maggi et al., 2012; Oliveira et al., 2014). While these studies have been extremely important in interpreting the effects of particular stressors and the temporal relationships that asynchrony between multiple stressors can contribute, these studies are unable to fully replicate and interpret the additional effects of adding a second stressor, or addressing whether a reversed order of stressors will affect the community.

While the effect of altering the order of stressors is subtle, a delayed second stressor has a clear impact on the macrofaunal community structure. Further, the impact of that second stressor on community structure can be dependent upon the type of stressor that acts first. These combined factors are troubling when taking into account future environmental changes as any change in community structure will alter the functions that a system provides (Chapin et al., 2000; Vitousek et al., 1997b). Modifications such as physical disturbance and nutrient enrichment are likely stressors to increase in the future, therefore too is their rate of coincidence. By understanding how stressors such as these interact in space and time makes this study important for determining how stressors could sensitise an environment to further stress. By identifying the times of year environments are particularly susceptible to a certain type of stressor, the application of management procedures could be implemented to mediate and protect against a second type of stress that would be detrimental to an ecosystem. Through this and further study of additional types and combinations of stressors it will be possible to predict how these systems may become altered by exposure to multiple stressors that act asynchronously.

Further experimental analysis in this area needs to occur. To better this experimental design experiments would control durations of stressors, the number of times each stressor is applied, maintaining the total amount of stress applied to a system. Additionally having control plots that were subjected to a second disturbance after the two month delay would allow us to interpret whether effects are solely due to the order in which stressors are applied or if there is an effect caused by the total duration of stress to the environment.

6.6 Conclusions

This study expands on previous research on the effects of nutrient enrichment and physical disturbance stressors on estuarine benthic communities, and is among the first of its kind to offer empirical evidence that the nature of temporal coincidences governs the effect on the final community structure. These types of studies will be a valuable tool in managing present impacts; in this study it was highlighted that a benthic system presently subjected to nutrient stress may be more at risk from physical disturbance, whereas an already physically disturbed system may not be as sensitive to further stress. While there is a plethora of evidence in the medical literature underlining the effects of stress induced sensitivity to multiple stressors on physiological reactions in individuals, it has yet to be taken to community scaled research or adapted to ecological systems. This new evidence highlights flaws of previous multiple stressor studies, most of which subject systems to stressors simultaneously, therefore incorrectly simulating a natural response to multiple stressors were stressors may occur simultaneously in nature but not necessarily going from an undisturbed to a totally disturbed state.

Table 5.6: Verification of hypotheses set at the beginning of the chapter

Hypotheses	Accept ✓ or reject X
H1 – A second stressor will have no effect on the system due to the overriding effect of the first stressor.	**X – starting stressor dependent**
H2 – The order in which stressors are applied will have a significant effect on the community, the effect of stressors will be less important than the order in which they are applied.	✓
H3 – There will be a significant difference between subjecting the system to stressors simultaneously or at different times (synchronous vs asynchronous application).	✓

7 General Discussion

This thesis investigated the effects of multiple stressors in selected benthic estuarine intertidal environments of Australia and the UK. Nutrient enrichment and physical disturbance were identified as potential interacting stressors in these environments. Individually, these stressors have been observed to impact ecosystem structure and function (De Backer et al., 2014; Dizon & Yap, 1999; Morris & Keough, 2003b; Rossi & Underwood, 2002; Russell & Connell, 2007; Whomersley et al., 2010), can arise from multiple sources but each may have their own unique impact. In terms of disturbance, raking was utilised as a mechanical proxy that could simulate the act of bait digging (Brown & Herbert Wilson, 1997) but can also be used to simulate the effects of storm damage (Oliveira et al., 2014). Further types of mechanical disturbance can come from trampling (Rossi et al., 2007) or boat wakes (Bishop, 2005), all of which subject systems to subtly different variants of this mechanical stress.

Nutrient enrichment can come from many sources. In this study, local nutrient enrichment was manipulated using slow release fertiliser replicating the effects of commercial farming (Morris & Keough, 2003a; Worm et al., 2000) but additional nutrient stress can come from sewage or run off (DeLorenzo et al., 2012; Drapper et al., 2000; Kennish, 2002). These sources typically introduce inorganic nutrients to an estuary (Pearson & Rosenberg, 1978) but further enrichment can come from organic compounds (Bishop & Kelaher, 2007, 2008; Gladstone-Gallagher et al., 2014; Kelaher et al., 2013), the effects of which can be dramatically different from inorganic fertiliser enrichment. The microphytobenthos (MPB) are important primary producers and while the MPB can obtain nutrients from organic (nucleic acids, proteins, urea) and inorganic sources (phosphate, nitrate, ammonium) the uptake of each is through different physiological mechanisms (Bowler et al., 2010). Additionally, detrital sources are an important food source for certain species (e.g. Bui & Lee, 2014; Gladstone-Gallagher et al., 2014) which means organic enrichment would influence multiple trophic levels in different ways compared to fertiliser enrichment.

Of the two stressors investigated, the interacting effects of nutrient enrichment with other stressors are far better understood, nutrients having been shown to interact with predation stress (Morris & Keough, 2003a), light (Stutes et al., 2006), shelter (Forehead et al., 2012), bioturbation (O'Brien et al., 2009), organic enrichment (Fitch & Crowe, 2011) and temperature (Alsterberg et al., 2012; Fitch & Crowe, 2011). Globally, nutrient enrichment has been shown to display synergistic interactions more frequently than other types of stressor (Strain et al., 2014). Studies of physical disturbance are less numerous (see McCabe & Gotelli, 2000), although have been documented interacting with other types of physical disturbance (e.g. natural vs. anthropogenic: Hughes & Connell, 1997) or climate stressors.

Few studies have examined stressor interactions through manipulative experiments. Additionally, the combined effects of enrichment and disturbance have received little attention (but see Austen & Widdicombe, 2006; Tiegs et al., 2009; Widdicombe & Austen, 2001). Most previous studies on the effects of multiple stressors have been conducted in laboratory settings that cannot adequately represent all components of an ecosystem, and hence may give misleading results on the effects of multiple stressors that cannot be extrapolated to the real world. This study investigated the multiple effects of nutrient enrichment and physical disturbance in a field setting, thereby giving a more realistic interpretation of their impact than previous research.

7.1 Summary of main thesis topics

7.1.1 The effects of nutrient enrichment and physical disturbance on Australian estuarine benthic communities

Chapter 3 discusses the singular effects of nutrient enrichment and physical disturbance and how their combined impact will affect Australian benthic estuarine systems. Hypotheses outlining the bottom up positive effects of nutrient enrichment on primary production were postulated. Nutrient enrichment had a positive effect on the microphytobenthos (MPB) but there was limited evidence to suggest a bottom up effect on the macrofauna. Further, an effect of nutrient enrichment on MPB only occurred at one site out of the two sites studied. This result was surprising given many previous observations of nutrient enrichment increasing MPB biomass (Hall et al., 2000; Huang et al., 2013; O'Brien et al., 2010; Pascal et al., 2013), particularly in nutrient poor systems like the oligotrophic estuaries of eastern Australia (Hall et al., 2000; Raffaelli & Emmerson, 2001). However, in oligotrophic systems such as in Australia, when nutrients are added to a system they are rapidly taken up by biological systems (Scanes et al., 2007) and, as MPB are fast growing ephemeral communities (Bowler et al., 2010; MacIntyre et al., 1996), responses may be of short duration and not detected by monthly sampling. This fast growth may be mediated by rapid top down control by grazers. This suggests that in these systems top down control of the MPB by the macrofauna may be of greater influence than bottom up effects of nutrient enrichment.

It was also postulated that physical disturbance would have a negative impact on the macrofaunal community. Physical disturbances, caused by raking or bait digging in sediments, have been linked to reduced abundances of larger bodied organisms, destruction of sediments, burrows and damage to softer bodied species (Brown & Herbert Wilson, 1997; Dernie et al., 2003; Hall & Harding, 1997; Hall, 1994; Rossi et al., 2007). There was limited evidence in this system to suggest that the macrofauna were affected by the level of physical disturbances applied by this study. While there were negative effects of disturbance on some species of polychaetes, the impact of physical disturbance on the overall community structure was negligible. Indeed, physical disturbance is a stressor that has been noted to be highly context specific in terms of the effects to communities, or even individual species (Whomersley et al., 2010). In comparison to other field studies (e.g. Brown & Herbert Wilson, 1997; Whomersley et al., 2010), the size of plots in these experiments were smaller which might have allowed outside plot recruitment, negating any observed disturbance effects (Lee et al., 2011). The implications are such that the scale of disturbance will ultimately govern how a system responds.

Nutrient enrichment had a much greater effect on the macrofaunal community than physical disturbance, although effects were site specific. Where effects of nutrient enrichment occurred, they were on largely effective in terms of community structure with the abundance of some species negatively and others positively influenced. There were limited effects on the species diversity at either site. This is opposite to other Australian studies which have observed an increase in biodiversity in response to nutrient enrichment (Hall et al., 2000; Morris & Keough, 2003a, 2003b). The variability in these environments is extremely high and could contribute to lack of treatment effects observed in univariate analyses (see Hall et al., 2000; Morris & Keough, 2003b).

7.1.2 The effects of nutrient enrichment and physical disturbance on the structure and function of the benthic communities of UK estuarine mudflats

Chapter 4 replicated experiments of the type that were undertaken in Australia in Scotland. Additionally Chapter 4 analysed the effects of stressors on a key ecosystem function, sediment stability. The macrofaunal community was altered due to the effects of physical disturbance, whereas in Australia, there was only a main effect of nutrient enrichment. The small scale of plot size was inferred to have allowed recruitment from outside the affected area in Australia. While the raked area was the same in both countries, a possible explanation for an observed effect of physical disturbance in the UK but not Australia could therefore be that out of plot recruitment was less effective in the UK. Similar effects of the stressors on MPB were observed in the UK study sites as in Australia, with nutrient enrichment having site-specific effects which, where they occurred, were greater than the effects of physical disturbance. Whereas in the Australian studies there was a slight negative effect of physical disturbance on the MPB irrespective of nutrient enrichment, in the UK the effect of physical disturbance was dependent on nutrient enrichment. Where effects of physical disturbance did occur, they caused an increase in MPB biomass. Possible mechanisms for this are discussed in Chapter 4 and range from trapped nutrients being released through raking the sediments (Porter et al., 2006); organic detritus getting trapped within sediments by the act of raking, thus causing additional organic enrichment (Dizon & Yap, 1999; Rublee, 1982); or through the alteration of macrofaunal species, implying there is a top down mechanism where the MPB increases due to the act of raking causing a relaxation of pressure caused by the consumers.

Only a main effect of physical disturbance on sediment stability was detected, and only at one site. While it is difficult to ascertain the causes of the site-specific effects of physical disturbance, they may be related to an algal bloom at one of the sites. Algal blooms have been linked to decreased sediment stability (Bolam et al., 2000; Defew et al., 2002). Burial of algal material during raking of the physically disturbed sites may have caused an instability in the sediments leading to a decrease in erosion threshold. Such burial of algae during raking could be analagous to the effects of algal burial during storm events.

7.1.3 Comparing the effects of multiple stressors between the UK and Australia

Differences in the benthic community structure and environmental conditions in UK and Australian estuaries probably contributed to differences in their response to stressors. The concentration of MPB in surface sediments was generally greater at UK than Australian sites and the macrofaunal community differed between these locations. In Australia, the dominant taxa were bivalves and polychaetes whereas in the UK, the community contained a higher overall proportion of gastropods and amphipods. Additionally there were large numbers of oligochaetes found in the UK whereas in Australia oligochaetes were rare.

There are 3 potential hypotheses to explain these differences between locations. Firstly, context dependency of stressors has already been attributed as a factor for the within region variation between response to stress. There is much greater variation between environmental conditions between geographic regions ultimately resulting in different responses (Strain et al., 2014). Secondly, the oligotrophic conditions in Australian systems were postulated to alter the process by which organisms would respond to nutrient stress. The resource heterogeneity hypothesis (Tilman, 1987) was utilised to speculate that in nutrient poor systems the response of communities to further enrichment would be beneficial, whereas in nutrient rich systems a further increase would do nothing or cause harm to the system. Thirdly, the community structure was completely different between the geographic regions. Response to stressors depends on a species resistance to stress (Christensen et al., 2006; Vinebrooke et al., 2004) hence a variation in response.

7.1.4 The sequence and timing of multiple stressors and their effects on sediment communities

Previous multiple stressor studies tend to subject systems to stressors simultaneously. However, in many instances the timing of stressors may be asynchronous (Darling & Côté, 2008; Pincebourde et al., 2012). Chapter 6 explored how the synchrony of multiple stressors influences their cumulative impact. It was identified that while multiple stressors cause a change in the macrofaunal community, a change in the temporal coincidence of stressors can alter the way the community reacts. This is among the first studies of its kind to address asynchrony of stressor application in benthic marine environments and furthers present research by highlighting the varied community response due to the order in which stressors are applied. This is a problematic result since the action of multiple stressors may be difficult to predict in terms of their timing and duration but warns against simplistic predictions of effects.

7.2 Context dependency of stressor effects

One of the key results of this study was the context specificity of stressor effects, at site and continental scales. Spatially inconsistent patterns due to multiple stressors have been documented in numerous studies indicating environmental context is an extremely important determinant of how communities respond to stressors (Alsterberg et al., 2014; Bishop & Kelaher, 2013; Crain et al., 2008; Gladstone-Gallagher et al., 2014; O'Connor & Donohue, 2013; Whomersley et al., 2010). Context specificity may arise from environmental differences among sites that mediate stressor effects, or differences in community structure that determine ecological sensitivity.

Stressor effects on individual species can display site specific responses, for example, increasing in abundances in response to stress in one location but not others (Whomersley et al., 2010). This indicates either environmental mediation of stressor effects or a community level response whereby certain species can fill a void left by a stressor impacting another species (Bishop & Kelaher, 2013; O'Connor & Donohue, 2013). This switching behaviour of the fauna can cause difficulties in determining stressor effects under natural conditions if a pool of species is large enough or there are other species to compensate (Bulling et al., 2010; Fitch & Crowe, 2011). Further, functional redundancy is likely prevalent in a system where there are many species (Hooper et al., 2005; Loreau, 2004). The longer the duration of stressors, or through increases in the number stressors affecting a system, the functional redundancy of the system will likely become lowered, therefore further species losses will eventually result in loss of functionality (Reich et al., 2012).

Changes in community structure is strongly related to environmental variables such as grain size (Nicastro & Bishop, 2013), the influence of prevailing conditions means that localised environmental context will be important in mediating responses to stressors. Additionally, the speed at which a system can recover can also be due to these environmental characteristics (Dernie et al., 2003). Background levels of prevailing stress often mediate how a system will respond to further stress which can facilitate one of two responses. Either it will do nothing as localised adaptations prevail and organisms have enhanced resistance and so are able to withstand the further disturbance (Loreau, 2000; Sanford & Kelly, 2011); or the application of additional stress can push the system over a threshold value, resulting in a system collapse (Pearson & Rosenberg, 1978; Whomersley et al., 2010). How far away systems are from tipping points is related to the conditions to which fauna are adapted and the trade-offs they have made either through evolutionary or local adaptations (Sanford & Kelly, 2011).

Estuarine systems are naturally stressed environments, undergoing daily changes in physico-chemical characteristics such as temperature, salinity, tides and nutrients (Elliott & McLusky, 2002; McLusky & Elliott, 2007). In each location, the biota will be adapted to multiple natural stressors; however certain characteristics of the environment may mean that any single location will be under greater stress than another. For example, one could argue that Australian systems experience greater thermal stress than UK systems and so are naturally closer to a tipping point. Conversely, an alternative can be hypothesised on the basis that UK systems experience greater salinity stress than Australian systems due to the influence of tides, and lack thereof in Australian estuaries creating stable rather than fluctuating environments (Heggie & Skyring, 1999). While it is reasonable to assume that one location may be more naturally stressed than others due to any particular environmental characteristic, the fact that the communities within these areas are adapted to localised conditions means that estuaries in either location are highly resilient ecosystems (Elliott & Whitfield, 2011). Taking into account these varying environmental characteristics it would be reasonable to assume different stressors would push either system over a tipping point. Within the estuaries analysed in this study there was no evidence that a tipping point or threshold had been breached. Instead, each system displayed context specific changes and adaptations to stressors resulting in subtle alterations to the community structure or MPB. These results highlight how resilient estuarine systems can be when subjected to additional stress (see Elliott & Quintino, 2007), and that it may take extreme external pressure to result in a collapse of these resilient ecosystems.

Laboratory and mesocosm studies are the preferred technique for analysing multiple stressors due the ability to control conditions, analysing systems in isolation (Godbold et al., 2011; Hicks et al., 2011; Sousa et al., 2011; Sundback et al., 2010). Field experiments are better for examining how a natural system reacts to stressors, however they lack the utility to isolate the mechanism by which particular stressor impacts occur (Crane et al., 2007; Daehler & Strong, 1996) so both approaches are needed. Natural environments tend to be heterogeneous whereas mesocosm or laboratory studies tend to be controlled homogeneous environments (Dyson et al., 2007). This heterogeneity can buffer against treatment effects (Godbold et al., 2011) or negate patterns that would otherwise be observed due to treatment effects under laboratory conditions (Bulling et al., 2008).

However, field experiments should not be disregarded: the environmental context plays an important role in mediating stressor effects. Field experiments give us an insight into how natural systems will react to stress, a feat that cannot be synthesised in mesocosm experiments. While the effects of stressors may be dampened and reduced in a natural environment (Cowie et al., 2000), there are some factors that cannot be interpreted from a mesocosm study. Microhabitats can be utilised by organisms to avoid stressor effects (Allen & Baltz, 1997; Jones & Boulding, 1999), ecological interactions (e.g. predation) may dampen or enhance stressor effects (Christensen et al., 2006; Hicks et al., 2011), or climatic conditions can interact with anthropogenic stressors (e.g. Daufresne & Boët, 2007). This last example cannot be replicated fully in a laboratory setting.

Findings from this study allow us to hypothesise that the effect of physical disturbance can in some situations cause burial of organic material which can facilitate the MPB growth or cause change in macrofauna (Dizon & Yap, 1999; Rublee, 1982). This type of mechanical disturbance could be analogous to a storm event or replicate the effect of tides on an exposed shore (Oliveira et al., 2014). This stressor may not be the focus of a particular study but under natural conditions becomes a serendipitous part of the system, interacting with other stressors which may be of influence in the study. Understanding the effects of stressors is just as important as understanding the effects on natural systems without which complete interpretation of stressor effects would be flawed. Field experiments should be conducted in tandem with laboratory studies to understand the implications of stressors and how they may be remediated with a localised context.

Manipulation of stressors in this study was conducted at the plot scale, with the effects of nutrient enrichment and physical disturbance analogous to eutrophication and storm damage respectively. These stressors would affect the whole system, not just small plot areas. The small scale of plots in this experiment allowed for recruitment from outside affected areas which could have mediated responses to stressors (Lee et al., 2011). Additionally, the species likely to display treatment effects were the ones that were sedentary, such as tube forming polychaetes. At the scale of entire site disturbances, movement of species between affected areas would not be possible. Treatment effects at this level are likely to facilitate the selection for organisms that can tolerate stressors (Sanford & Kelly, 2011).

7.3 Multiple stressor effects - additive or non-additive

The aim of this thesis was to analyse multiple stressor effects on benthic estuarine systems. While many studies have analysed stressors separately, these systems are at risk from numerous sources. The stressors analysed in this study are just two of many types that these systems are subjected to year round. Estuaries are the terminus of large catchment areas and are therefore subjected to a wide array of stressors and act as a focus point for them and impact upon water quality (Birch et al., 2010; MacFarlane & Booth, 2001; Roy et al., 2001). Future climate change may elevate temperatures, bring about changes in $p\mathrm{CO}_2$, rainfall, and storm intensity and frequencies (Bijma et al., 2013; Hoegh-Guldberg & Bruno, 2010). Anthropogenic stressors often co-occur with climate change, causing further impact (Halpern et al., 2008). For example, future urbanisation and industrialisation around estuaries and coastal environments will cause increases in the amounts of sewage, heavy metals and polyaromatic hydrocarbons (Kennish, 2002; Pascual et al., 2012).

While there are very few environments that are unstressed (Halpern et al., 2008), the prevalence of disturbance is increasing due to anthropogenic impacts and climate change (Bijma et al., 2013; Fitch & Crowe, 2011; Harley et al., 2006; IPCC, 2014). This increases the number of potential stressor interactions causing detrimental changes to community structure and function (Crain et al., 2008; Halpern et al., 2008). These interactions can result in additive or non-additive effects, which, as has been highlighted in this study, can be highly context dependent (Bulling et al., 2010; Crain et al., 2008; Fitch & Crowe, 2011; Przeslawski et al., 2014; Sundback et al., 2010). One of the most widely cited papers assessing multiple stressors in marine systems was a review by Crain and others (2008) which analysed 171 studies that manipulated two or more stressors. With only 26% of studies displaying additive effects, their findings have highlighted the unpredictability in interpreting multiple stressor effects based on their singular impacts. Another meta-analysis by Darling and Côté (2008) synthesising 112 experiments in aquatic and terrestrial environments similarly revealed that only a quarter of studies displayed truly additive effects, with synergies and antagonisms being more prevalent.

In recent years, the importance in multifactorial analysis in assessing stressors has become increasingly recognised among ecologists as an important concept. While much progress has occurred in analysing stressors together, between 2000-2009, only 35% of climate change experiments involved two or more stressors (Wernberg et al., 2012). Further, most present research has focussed on pulling apart the interactions of two stressors, yet ecosystems are almost always subjected to more than two co-occurring stressors simultaneously (Crain et al., 2008). Future research needs to investigate these interactions as synergies become more likely when analysing a greater number of stressors (Crain et al., 2008). More needs to be done, the majority of research still focusses on single stressor studies (Harvey et al., 2013; Kroeker et al., 2013; Przeslawski et al., 2014; Wernberg et al., 2012). In order to create more ecologically realistic research, there is a distinct need to move away from studying stressors singularly in order to be able to fully understand how future change will impact environments.

The nature of stressor interactions is determined by multiple factors. While the type of stressor is an important determinant of how it interacts with others (Strain et al., 2014), so too is the intensity of the stressor (Vye et al., 2015). Additionally the timing of stressors can alter the way in which stressors interact (García Molinos & Donohue, 2011). Again, this temporal aspect has been explored earlier in this thesis. Additionally, stressors in natural environments can be grouped into either press (chronic or long term) or pulse (acute or intermittent) disturbances. The stability of the environment will be related to the ability of organisms to recover in between disturbance events with synergisms between separate disturbance events likely (Underwood, 1989). Further to the above statement about incorporating realism into ecological research, ecological research should also incorporate interactions between press and pulse disturbance events and how these will influence interacting stressors. These combined issues make unravelling multiple stressor interactions an extremely complex topic, however dealing with complexity is a core concept in ecology (e.g. Polis, 1998).

7.4 Effects of stressors on ecosystem functions and service provision

Both natural and anthropogenic stressors are known to affect ecosystem functioning (Bulling et al., 2010; Larsen et al., 2005; Naeem et al., 2002; Solan et al., 2004). How biodiversity affects ecosystem functioning has been debated in recent years however most results conclude that greater diversity increases the functionality of a system (Lefcheck et al., 2015; Tilman et al., 2014) and therefore its resilience to environmental change (Allison, 2004; Gunderson, 2000). With this in mind a stressor impact on an ecosystem that causes alterations of community structure or loss of less resistant species would result in a loss of ecosystem function (Davies et al., 2011; Duffy, 2003; Solan et al., 2004). Stressors are therefore detrimental to upholding the functioning of an ecosystem, environments with few species are at greater risk due to the lack of functional redundancy (Balvanera et al., 2014; Cardinale et al., 2012; Davies et al., 2011; Tilman et al., 2014).

The non-additive effects of multiple stressors can alter the functionality of a system, the complexity of multiple stressor effects on individuals or entire communities will alter the mechanisms for which ecosystem functioning will change (Alsterberg et al., 2014; Bulling et al., 2010; Byrnes et al., 2014; Crain et al., 2008; Sundback et al., 2010; Vinebrooke et al., 2004). In this study, a loss of sediment stability was observed due to the effects of physical disturbance alone through alterations of the community structure, meaning no single species maintained the functioning as has been observed in other systems (Davies et al., 2011). Where stressors will impact a key species or there is no functional redundancy, loss of function will be attributed to how a particular stressor will impact certain species (Solan et al., 2004).

Further to this, it is only in the past few years that research is evolving to undertake experiments analysing how these interactions will change the functions derived from different environments (Alsterberg et al., 2014). A multifunctionality approach, i.e. the simultaneous performance of multiple ecosystem functions, would be beneficial when analysing the effects of multiple stressors on communities and ecosystem functions (Alsterberg et al., 2014; Byrnes et al., 2014; Lefcheck et al., 2015). The bulk of current multiple stressor studies only analyse their impact on single functions. To date the only study to take such an approach was conducted by Alsterberg et al. (2014). The authors combined data from multiple studies experiments, analysing the response of 6 functions to 10 stressors in sediment ecosystems. While the singular studies detected impacts on individual functions due to stressors, the multifunctionality approach detected very little change due to stress. This approach highlights the resilience of these systems to stress that cannot be ascertained when analysing response variables singularly

7.5 Relevance of results for management

Understanding the consequence of multiple stressors for individuals, their populations, communities and ecosystems is critical to managing their impact (Barrett et al., 1976). Many rivers and estuaries are hugely impacted compared to historical values (Davidson et al., 1991). The stresses that have been outlined above are examples of what these systems are subjected to on a daily basis, with additional stress and disturbances predicted in the future (Halpern et al., 2008; Sala et al., 2000). These changes require action in the form of management to systems and, as has been continually demonstrated in this thesis, local environmental context is the principal factor in which to base management processes.

Appropriate management strategies need to be developed to protect ecosystem values in these, and other, environments. Consideration of multiple stressors is crucial for determining how systems should be managed in the future, with multiple stressors affecting the upkeep of a systems functions and services. For example, in fisheries and aquaculture, multiple stressor impacts need to be considered to optimise conditions for production (Munari et al., 2011). Additionally, consideration at multiple trophic levels is important in sustaining fisheries; multiple stressor effects on primary production is as likely to harm a fishery as much as multiple stressor effects on a fished species (Brown et al., 2010).

Putting aside the argument for context specific management strategies for the moment, reactions to multiple stressors can have, broadly speaking, 3 types of impact: additive, synergistic or antagonistic. If stressors are additive management strategies should be implemented to remove or reduce stress to cut their impacts. Understanding when a system reaches a threshold can inform managers at what point stress is too high and a collapse is imminent (Thrush et al., 2012). Non additive effects are more difficult to manage although not impossible. Brown et al. (2013) formulate procedures to follow to reduce the impact from non-additive stressor impacts. In the case of synergies, mitigating the impact of local stressors, ones which could easily be controlled, would have the greatest positive impact. For antagonistic stressors, management of local impacts only worked in areas that were not also subjected to climate change stressors. Brown *et al* (2013) go on to emphasise that antagonistic interactions need particular focus and further investigation into how the stressors should be reduced prior to management procedures because a reduction of one stressor may in actuality cause greater detrimental impacts. For example, there are documented cases where interacting stressors may cause a benefit rather than a detriment (Coelho et al., 2015), removal of one facilitates a negative impact of a second. Further management strategies should be utilised to focus control on stressors such as nutrient enrichment which can cause unpredictable synergistic impacts (Strain et al., 2014). The results from this study highlight the importance of localised management plans to protect from the effects of multiple stressors. Localised management of stressors known to cause non additive effects should be the focus of management procedures or investigation.

7.6 Future research needs

These experiments have highlighted the importance that context dependency has on modulating the effects of multiple stressors. The mechanisms behind this – outlined above as varying benthic community structures, background nutrient loadings, and sediment grain sizes – should be further investigated to ascertain the nature of this site specificity. To analyse the role of environmental factors, manipulative field experiments should be replicated across a wide range of clearly defined gradients, for example in multiple estuaries with varied nutrient loadings. Additionally manipulating various environmental and biological factors should tell us which have a greater impact in mediating stress responses – this could be in the form of species removals or through mesocosm studies.

Further analysis should be conducted to ascertain the nature of the interaction between nutrient enrichment and physical disturbance, and the mechanism for which these stressors affect the study system. The level of nutrient enrichment and the raking regime were chosen to represent levels in which these systems could realistically be subjected to and was based upon previous research (Austen & Widdicombe, 2006; Morris & Keough, 2003b; Whomersley et al., 2010; Worm et al., 2000). Further, the length of time taken for stressor effects to illicit a response can vary greatly in benthic habitats (Cebrian et al., 2012; Pascal et al., 2013; Raffaelli & Emmerson, 2001). Future studies could investigate non linearities using a greater range of stressor levels, and perhaps a response-surface approach. Longer application of stressors would be more likely to result in alterations of community structure (Davis et al., 2010), and investigating other intensities may have allowed us to observe responses to stress where there were none, potentially allowing us to push the system over a threshold allowing us to identify the level at which stress would cause an impact, informing future management procedures.

Future study should analyse other combinations of stressors in these environments. Nutrient enrichment and physical disturbance were two of many that were identified. Ideally, using a fully orthogonal design, further research should incorporate numerous types of stressor. Of particular importance, organic enrichment and heavy metals or other contaminants should be analysed due to their prevalence in estuaries. These are consequences of increases in anthropogenic stress such as through increased sewage (Kennish, 2002). Further, the number of variables should be increased. Diatoms and meiofauna should be analysed as their response to stress does not always relate to macrofauna changes (Austen & Widdicombe, 2006). Despite the challenges involved in setting up experiments with three or more stressors, the results would provide more ecologically useful information to understand responses of fauna and flora to future environmental and anthropogenic stress.

7.7 Conclusion

Multiple stressors have interactive effects that are not always predictable from analysing their individual impacts. Estuarine environments are areas that are subjected to multiple stressors daily, understanding how they respond to interacting stressors is important in being able to predict their response to future anthropogenic stress and climate change, allowing appropriate management practises of these areas. Further, the impacts of these interacting stressors are likely to be highly context dependent. Environmental context plays an important role in mediating stressor effects.

Responses can vary between locations within and between geographic regions, as has been demonstrated by this study. The response of a system is further governed by its resilience to stress, and how a system responds to stress varies depending upon environmental conditions, background stress levels and community structure. Future research should unravel how environmental context specifically affects communities to enable better future predictions to protect against future impacts.

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