

**Effect of *Theora lubrica* on the response of coastal soft sediment nitrogen cycling to ocean acidification**

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## Attestation of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Sam Wakefield

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## Abstract

Ocean acidification could influence nitrogen cycling in coastal soft sediments, which are moderated by bioturbating macrofauna. The functioning of coastal ecosystems has a strong connection with nitrogen fluxes that occur at the sediment–seawater interface; the disturbance of the sediment matrix via bioturbation can significantly alter these fluxes. To investigate how decreasing seawater pH affects the fluxes of  $O_2$ ,  $NH_4^+$ ,  $NO_2^-$  and  $NO_3^-$ , I incubated sediment core samples of intact coastal subtidal silt in four seawater recirculating systems and injected  $CO_2$  to adjust their pH to 8.0, 7.8, 7.6 and 7.4. I also incorporated bioturbation via a Bivalve treatment by adding 10 *Theora lubrica* (introduced infaunal bivalve) to a sediment core. Furthermore, the experiment was done in full darkness to eliminate photosynthesis, and salinity and temperature were controlled variables.

Initial measurements at in situ  $pCO_2$  indicated, that the Bivalve treatment significantly increased  $NH_4^+$  and  $NO_3^-$  effluxes, and  $O_2$  influxes, but had no effect on  $NO_2^-$  fluxes. After a 20-day incubation, the final measurements revealed, that seawater acidification significantly increased  $NH_4^+$  and  $NO_2^-$  effluxes, but had no effect on the fluxes of  $NO_3^-$  and  $O_2$ . Furthermore, I detected no significant effects on nitrogen fluxes by the interaction between the pH and Bivalve treatments; however, the interaction significantly decreased  $O_2$  influxes.

I hypothesise that the addition of *T. lubrica* stimulated ammonification and nitrification at in situ  $pCO_2$  during the initial measurements. I also suspect that seawater acidification decreased coupled nitrification-denitrification during the final measurements. Furthermore, I suggest that *T. lubrica* caused both direct and indirect effects on the sediment matrix, leading to the significant decrease in  $O_2$  influxes during lower seawater pH within the Bivalve treatment cores.

Overall, my study was conclusive because I was able to prove that *T. lubrica* had no influence on coastal soft sediment nitrogen cycling during seawater acidification. Furthermore, I demonstrated that seawater acidification significantly affected sediment nitrogen cycling, which means ocean acidification could have a profound impact on coastal ecosystem functioning in the future.

## Introduction

### Global change CO<sub>2</sub>

It is a consensus in the scientific community that carbon is the most important chemical element found on Earth because all living organisms require it for growth and reproduction. Moreover, carbon is a finite resource that exists both organically and inorganically, forming the ever-complex carbon cycle (Keeling 1973). A key molecule involved in the carbon cycle is carbon dioxide (CO<sub>2</sub>) because it drives primary production (e.g., photosynthesis in algae and plants), which is a fundamental process that initiates food chains and webs in terrestrial and marine ecosystems (Michaels & Silver 1988). Furthermore, CO<sub>2</sub> significantly impacts Earth's climate (Ramanathan & Feng 2009) and the carbonate chemistry of the ocean (Doney et al. 2009).

CO<sub>2</sub> makes a small contribution to the overall composition of gases within the Earth's atmosphere. Nitrogen gas (N<sub>2</sub>) is the most abundant at 78.08%, followed by oxygen (O<sub>2</sub>, 20.95%), argon (Ar, 0.93%) and CO<sub>2</sub> (0.03%). Other trace gases exist, but are less abundant than CO<sub>2</sub>; examples of minor trace gases include: neon (Ne), helium (He), methane (CH<sub>4</sub>), krypton (Kr), carbon monoxide (CO), nitrous oxide (N<sub>2</sub>O) and xenon (Xe, Pilson 2012). Even though CO<sub>2</sub> is considered as a trace gas within the Earth's atmosphere, massive influxes of CO<sub>2</sub> released by human civilisation within recent years have caused significant climate change (Labelle & Murray 1999).

The Earth has chemical, biological and physical mechanisms that work synergistically to account for natural fluctuations in atmospheric CO<sub>2</sub>. A suitable analogy for the Earth is that it acts like a massive thermostat to retain balance between aquatic and terrestrial ecosystems. However, mankind is testing the performance of this thermostat by pumping large quantities of CO<sub>2</sub> into the atmosphere within a short space of time. Two pioneering scientists within the field of anthropogenic global warming, Roger Revelle & Hans Suess, founded an expression during a study that they conducted in 1957 being "the great geophysical experiment", which perfectly describes the Earth's ongoing climate situation. The aim of their study was to estimate the fate of anthropogenic CO<sub>2</sub> emissions within the environment by utilizing

carbon isotope techniques; they concluded that the ocean had absorbed most of these emissions, where the remainder was partitioned into the atmosphere, and terrestrial ecosystems (Revelle & Suess 1957).

Anthropogenic CO<sub>2</sub> emissions weren't considered as a genuine environmental threat during the mid 20th century. However, Revelle & Suess's (1957) study provided a solid baseline for Earth's CO<sub>2</sub> situation, which raised awareness that dramatic changes in the Earth's climate and carbonate chemistry of the ocean could occur within decades. With particular reference to the ocean and its role in absorbing CO<sub>2</sub>, marine scientists were the first to acknowledge studies like the former and began to explore the potential effects of CO<sub>2</sub> emissions on marine ecosystems. According to Brewer (2013) it was only near the end of the 20<sup>th</sup> century when the wider scientific community started to appreciate CO<sub>2</sub> emissions as a legitimate global issue because adverse environmental impacts started to become apparent. Before exploring the undesired phenomenon that is caused by a high CO<sub>2</sub> ocean, the following will pinpoint the main sources of CO<sub>2</sub> emissions and provide approximations of the total amount released since the beginning of the industrial revolution to current times.

Over the course of the Anthropocene it was only until the mid 18<sup>th</sup> century when CO<sub>2</sub> emissions started to skyrocket, which marked the start of the industrial revolution. Le Quéré et al. (2009) highlights that fossil fuel combustion from motor vehicles is the primary source of CO<sub>2</sub>, with small contributions from gas flaring and cement production. Further, they claim that land use changes such as deforestation, logging and intensive cropland soil cultivation are the second largest source of CO<sub>2</sub>. The latest report conducted by the Intergovernmental Panel on Climate Change (IPCC) estimated that atmospheric CO<sub>2</sub> concentrations increased by 40% in 2011 compared to preindustrial levels, which is the equivalent increase from 279 to 391 ppm. To quantify this change in mass, an estimated 555 Gt of CO<sub>2</sub> was emitted into the atmosphere from 1750 to 2011. Fossil fuel combustion contributed 68% (375 Gt) of these emissions, leaving the remaining 32% (180 Gt) owing to land use change (IPCC 2013).

Since the mid 20<sup>th</sup> century, the popularity of studying CO<sub>2</sub> emissions increased and advancing technology provided greater accuracy for measuring CO<sub>2</sub> concentrations in

the environment. Following Revelle & Suess's (1957) study, the scientific community's understanding of CO<sub>2</sub> emission distribution has changed. It was initially perceived that the ocean absorbed most of the CO<sub>2</sub>, however, the bearing of current knowledge has established a significant shift in the CO<sub>2</sub> situation. The literature indicates that approximately 40 to 45% (220 to 240 Gt) of CO<sub>2</sub> emissions remain in the atmosphere, leaving 55 to 60% (315 to 335 Gt) being sequestered by the ocean and terrestrial ecosystems (Ballantyne et al. 2012, Canadell et al. 2007, Knorr 2009). The IPCC (2013) claims that the ocean absorbed 155 Gt (49%) of CO<sub>2</sub> emissions, where the remaining 165 Gt (51%) were up taken by terrestrial ecosystems since the beginning of the industrial revolution.

Aquatic and terrestrial ecosystems are natural sinks and sources of CO<sub>2</sub>, which are helping to buffer the constantly high anthropogenic CO<sub>2</sub> emission rates. Sabine et al. (2004) estimated that the ocean has only reached a third of its long-term potential to absorb CO<sub>2</sub>. Furthermore, they quantified that the ocean dominated the uptake of CO<sub>2</sub> emissions since the beginning of the 20<sup>th</sup> century. The ocean has a large capacity to absorb CO<sub>2</sub> because of its sheer volume. Charette & Smith (2010) estimated that the surface area coverage of Earth by the ocean is close to 70%, which creates a large air-sea gas exchange interface. However, the exchange of CO<sub>2</sub> from the atmosphere into the ocean is a slow and delayed process. If humans decided to completely stop emitting CO<sub>2</sub>, it would take the ocean centuries to thousands of years to perform a complete CO<sub>2</sub> recovery (Archer & Brovkin 2008).

### **Ocean acidification**

The following sections focus on the undesired phenomenon that is caused by a high CO<sub>2</sub> ocean being ocean acidification (OA). The main symptom of OA is decreasing seawater pH (Fabry et al. 2008). The average pH of seawater has decreased by 0.11 units (from 8.25 to 8.14) since the beginning of the industrial revolution, which is an equivalent 30% increase in hydronium ions (H<sub>3</sub>O<sup>+</sup>, Mackie, McGraw & Hunter 2011). Raven et al. (2005) predicted that average ocean pH is on track to decrease by another 0.4 units (equivalent 150% increase of H<sub>3</sub>O<sup>+</sup> ions) by 2100, which would fall somewhere between 7.7 and 7.8.

It seems counterintuitive that CO<sub>2</sub> has the ability to lower seawater pH because it lacks hydrogen ions (H<sup>+</sup>) to donate. However, CO<sub>2</sub> becomes acidic as it diffuses into seawater because it undergoes significant chemical changes. Jacobson (2005) summarised that two laws of physics drive the diffusion of atmospheric CO<sub>2</sub> into the ocean: Dalton's law of partial pressures and Henry's law of gas solubility. Therefore, OA isn't just a function of humans over exploiting carbon-based resources, but it is also the chemical/physical principles of how CO<sub>2</sub> interacts with aqueous solutions.

The initial reaction between CO<sub>2</sub> and H<sub>2</sub>O causes the onset of reactions that increase the concentration of H<sub>3</sub>O<sup>+</sup> ions because carbonic acid (H<sub>2</sub>CO<sub>3</sub>) is produced (Equation 1). H<sub>2</sub>CO<sub>3</sub> has the capability of reacting with H<sub>2</sub>O twice, forming a bicarbonate ion (HCO<sub>3</sub><sup>-</sup>) first (Equation 2) and a carbonate ion (CO<sub>3</sub><sup>2-</sup>) second (Equation 3); in both instances H<sub>3</sub>O<sup>+</sup> ions are the by-product. These reactions are as follows:



In addition to pH reduction, OA decreases CO<sub>3</sub><sup>2-</sup> ion concentrations; these ions are the primary species found in seawater for neutralising acid. H<sub>2</sub>CO<sub>3</sub> has the ability to form CO<sub>3</sub><sup>2-</sup> ions whilst performing its second ongoing reaction with H<sub>2</sub>O (Equation 3). However, the principle of chemical equilibrium limits the production of CO<sub>3</sub><sup>2-</sup> ions in these reactions. Chemical equilibrium is a continual process that helps the ocean to retain ionic balance and stability (Mackie, McGraw & Hunter 2011, Zeebe 2012, Zeebe & Wolf-Gladrow 2001).

With reference to Equations 2 and 3, the pK values are 6 and 9.19 respectively, in seawater at 10 °C. These values can be used to calculate chemical equilibrium ratios for each reaction, which is the ratio between reactants and products. Preindustrial H<sub>3</sub>O<sup>+</sup> ion concentrations are used to standardise these ratios. The following represents the chemical equilibrium ratios for Equations 2 - 3:

$$10^{-6.00} : 10^{-8.25} = 1 : 177.8 \quad (4)$$

$$10^{-9.19} : 10^{-8.25} = 8.7 : 1 \quad (5)$$

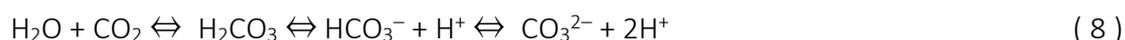
Equation 2 favours its products ( $\text{HCO}_3^-$  and  $\text{H}_3\text{O}^+$  ions); therefore, if an amount of the reactants were added, 177.8 parts would react to form its products and 1 part would remain as reactants (Equation 4). Conversely, Equation 3 favours its reactants ( $\text{HCO}_3^-$  ions and  $\text{H}_2\text{O}$ ); therefore, if  $\text{H}_3\text{O}^+$  ions were added and reacted with  $\text{CO}_3^{2-}$  ions, 8.7 parts would form reactants and 1 part would remain as products (Equation 5). Equation 6 represents another reaction that is relevant to  $\text{CO}_3^{2-}$  ion reduction.



$$10^4 : 1 \quad (7)$$

When Equation 6 occurs by adding  $\text{CO}_2$ ,  $10^4$  parts react with its products ( $\text{CO}_3^{2-}$  ions and  $\text{H}_2\text{O}$ ) to form  $\text{HCO}_3^-$  ions and 1 part remains as products (Equation 7). A variety of other reactions can occur in seawater with  $\text{CO}_2$ . However, by referring to Equations 1 - 3 & 6,  $\text{CO}_2$  perturbation favours the removal of  $\text{CO}_3^{2-}$  ions to produce  $\text{HCO}_3^-$  ions, which are the most abundant carbon species in the ocean (Mackie, McGraw & Hunter 2011, Raven et al. 2005).

$\text{HCO}_3^-$  ions have acidic and basic properties, acting like a mediator in the ocean's carbonate buffer system. Because of chemical equilibrium, most reactions containing  $\text{HCO}_3^-$  ions favour their production. The following is a modified version of Equations 1 – 3:



The carbonate buffer system (Equation 8) shows that  $\text{HCO}_3^-$  ions are at the centre of equilibrium.  $\text{HCO}_3^-$  ionic reservoirs in the ocean allows for the storage of excess carbon during times of intensive  $\text{CO}_2$  influxes.  $\text{HCO}_3^-$  ions are also an important chemical for a large amount of sea life. For example, they are critical for animals that generate shells or skeletons (e.g., tropical reef-building corals, cold-water corals, coralline algae, benthic molluscs, echinoderms, coccolithophores, foraminifera, pteropods, etc.). Other animals such as halimeda, jellyfishes and fishes secrete calcium carbonate ( $\text{CaCO}_3$ ) for internal structure and support. If the process of OA promotes the growth of the  $\text{HCO}_3^-$  ionic reservoir, then a speculation could be that it has the potential to boost calcification rates. However, this is not the case because OA causes the reduction of  $\text{CO}_3^{2-}$  which consequently lowers the  $\text{CaCO}_3$  saturation

state ( $\Omega$ , Guinotte & Fabry 2008, Kurihara 2008, Mackie, McGraw & Hunter 2011, Zeebe 2012, Zeebe & Wolf-Gladrow 2001).

Calcification is a process used by animals to draw in  $\text{Ca}^{2+}$  (calcium ions) and  $\text{HCO}_3^-$  ions to form  $\text{CaCO}_3$ . The following is the general reaction involved with calcification:



Equation 9 shows that animals assimilate  $\text{HCO}_3^-$  ions to form  $\text{CO}_3^{2-}$  ions, which allow them to produce  $\text{CaCO}_3$ . Equation 10 explains how OA reduces an organism's ability to secrete  $\text{CaCO}_3$ :

$$\Omega = Q_{\text{sp}} / K_{\text{sp}} \quad (10)$$

$Q_{\text{sp}}$  describes the present state of a solution that is not at ionic equilibrium, whereas,  $K_{\text{sp}}$  describes a solution that is at a state of saturated ionic equilibrium. If a solution is under-saturated ( $\Omega < 1$ ), then  $\text{CaCO}_3$  dissolution occurs to restore ionic equilibrium. By contrast, if a solution becomes saturated or super-saturated ( $\Omega \geq 1$ ),  $\text{CaCO}_3$  dissolution rates decrease. The average  $\Omega$  of the ocean's surface is currently above 5, however, by the end of this century it's predicted to fall below 5. This subtle change in  $\Omega$  increases the energy cost of calcifying organisms to secrete  $\text{CaCO}_3$ . Furthermore, a reduction in  $\text{CO}_3^{2-}$  ions and  $\Omega$  is indicative of  $\text{H}^+$  ion production, which changes the proton gradient between an organism's internal cellular reservoir and the surrounding seawater; this can hinder a calcifying organism's ability to maintain pH homeostasis (Cyronak, Schulz & Jokiel 2015, Mackie, McGraw & Hunter 2011, Zeebe 2012, Zeebe & Wolf-Gladrow 2001).

There are two polymorphs of  $\text{CaCO}_3$  that exist in the ocean: calcite and aragonite. Some animals secrete these polymorphs on their own or have the ability of producing both at the same time. Examples of animals that strictly use calcite are: coccolithophores and foraminifera. An example of an animal that strictly uses aragonite is a pteropod. Examples of animals that can utilize both polymorphs are: corals (cold water & tropical) and molluscs. Aragonite is more soluble than calcite, which means that less energy is required for it to dissolve. Therefore, calcite is preserved at greater depths because aragonite has a significantly shallower saturation depth or horizon (the depth at which a polymorph dissolves). The current saturation

horizon for aragonite is approximately 1000 and 2500 m for the Pacific and Atlantic Oceans, respectively. Calcite's saturation horizon is much deeper at 3000 and 4500 m in the Pacific and Atlantic Oceans, respectively. However, as OA intensifies these horizons become shallower; consequently, a large proportion of  $\text{CaCO}_3$  assumed to be permanently buried in the great depths of the ocean will start to dissolve (Mackie, McGraw & Hunter 2011). Feely, Doney and Cooley (2009) predicted that by the end of this century vast areas of the ocean could become under-saturated with respect to both aragonite and calcite.

In Earth's history, OA has occurred numerous times long before human civilisation because of high atmospheric  $\text{CO}_2$  levels caused by the eruption of volcanoes (terrestrial and marine) and carbon reservoirs underneath the seafloor (Kump, Bralower & Ridgwell 2009). A significant event that occurred 251 million years ago called the EPE ("End-Permian Extinction") was predicted to have caused the death of up to 95% of all marine life (Benton & Twitchett 2003). The eruption of the Siberian Traps (groups of Russian volcanoes) during this event caused a gradual release of  $\text{CO}_2$  over 10,000 years. Scientists have been able to quantify the amount of  $\text{CO}_2$  released into the atmosphere during events similar to the EPE by analysing air bubbles trapped inside preserved ice fields in Antarctica (Lüthi et al. 2008, Raynaud et al. 1993). Royer et al. (2004) estimated, that atmospheric  $\text{CO}_2$  concentrations have been much higher in the past ( $\text{pCO}_2 > 6000$  ppm) and according to Ridgwell (2005) these levels caused significant OA events ( $\text{pH} < 7.5$ ).

Current  $\text{CO}_2$  emission rates are exponential compared to previous natural events as pointed out in a model drawn by Ridgwell et al. (2009). They predicted that modern ocean pH would drop rapidly in a short space of time (hundreds of years), compared to previous OA events, which were caused by the gradual release of  $\text{CO}_2$  over longer periods (tens of thousands of years). Therefore, the rate of  $\text{CO}_2$  release is the greatest concern, rather than the overall amount of  $\text{CO}_2$  being released. Wittmann & Pörtner (2013) conducted a study that measured the response of 5 animal taxa: corals, echinoderms, fishes, crustaceans and molluscs to OA using experimental mesocosms. They suggested that future wholesale shifts in seawater carbonate chemistry would

cause the extinction of corals, echinoderms and molluscs because they are sensitive to change and might not retain the physiological capacity to adapt.

The Earth utilises the weathering of terrestrial rocks as a primary mechanism to remove excess atmospheric CO<sub>2</sub> (Gaillardet et al. 1999). Terrestrial weathering rates increase in a high CO<sub>2</sub> world because global warming promotes rainfall. Knutson et al. (2010) claimed on a global scale that by 2100 the intensity of tropical cyclones would increase by 2–11% because of climate change. Rainwater is mildly acidic because the diffusion of atmospheric CO<sub>2</sub> into clouds causes the production of H<sub>2</sub>CO<sub>3</sub>, which means that calcium-based minerals gradually dissolve during rainfalls (Carroll 1962). Equations 1 - 3 can be used to explain the interaction of CO<sub>2</sub> with rainwater.

Terrestrial weathering produces HCO<sub>3</sub><sup>-</sup> ions, which feed into rivers. Therefore, rivers have higher alkalinity during times of elevated atmospheric CO<sub>2</sub>. Kump, Bralower & Ridgwell (2009) suggested that alkalinity surges by rivers into the coastal zone allowed ocean surface waters to remain supersaturated with respect to CaCO<sub>3</sub> during volcanic eruptions in the past. This was conducive for the precipitation and accumulation of limestone. Kump, Bralower & Ridgwell's (2009) article also provided an overview of the two main types of terrestrial weathering that exist: carbonate and silicate. The equations for each type are as follows:



As well as explaining the basic chemical principles of calcification, Equation 9 can also be applied to CaCO<sub>3</sub> precipitation. Both types of weathering produce a single mole of carbonate (which eventually buries) and CO<sub>2</sub> (Equations 11–12). The main difference between the two types of weathering is that silicate requires two moles of atmospheric CO<sub>2</sub> to produce two HCO<sub>3</sub><sup>-</sup> ions (Equation 12), whereas carbonate only requires one mole (Equation 11). As reinforced by Berner & Caldeira (1997), it is silicate weathering that is going to help recover Earth's CO<sub>2</sub> problem because it causes a net removal of CO<sub>2</sub> following CaCO<sub>3</sub> precipitation. Carbonate weathering has no effect on Earth's CO<sub>2</sub> situation because the amount of CO<sub>2</sub> it removes equals that produced during CaCO<sub>3</sub> precipitation. Mackie, McGraw & Hunter (2011) predicted

that the oceans carbonate buffer system would restore to a new unknown steady state as fossil fuel resources become exhausted.

### **Coastal ocean acidification**

Coastal waters are some of the most biologically productive ecosystems on Earth (Martin et al. 1987, Platt & Subba Rao 1975). Brunner et al. (2008) reinforces that they sustain a prolific number of fisheries and provide ideal conditions for aquatic farming, which supports the global demand for protein. Furthermore, they provide a pathway for transport, support tourism and offer a platform for a wide range of recreational opportunities (Martínez et al. 2007). Therefore, monitoring the health and longevity of the coastal setting is of high priority.

Duarte et al. (2013) explained that the carbonate chemistry of seawater in coastal areas is more complex than in the open ocean because of the biogeochemical processes that fluctuate at the land–sea interface. Furthermore, the diversity of coastlines can cause variations in seawater carbonate chemistry to occur within different locations of the same relative area (Manzello et al. 2012). The carbonate weather of coastal areas is habitat specific because a variety of factors other than anthropogenic CO<sub>2</sub> can alter the chemical properties of seawater. Examples of these factors include: salinity, terrestrial runoff, riverine input, diffusion of bioactive constituents at the air–sea interface, primary production, respiration, etc. (Waldbusser & Salisbury 2014).

Coastal processes often act synergistically and promote one another; for example, terrestrial runoff can cause coastal waters to become eutrophic because of nutrient loading caused by high nitrogen, carbon and phosphorus (NCP) concentrations. NCP can directly affect water chemistry, but also indirectly because these excess nutrients enhance primary production. Common symptoms of eutrophic waters include: algae blooms and increased microbial degradation of organic matter (OM), which can lower local pH (Wallace et al. 2014). Furthermore, most algal species yield higher photosynthetic rates during warmer and longer photoperiods associated with seasonal change (King & Schramm 1976). It is difficult to understand the dynamics of coastal carbonate systems because they are intricate. Efforts to conduct high resolution monitoring within coastal settings have been made (e.g., Dupont et al.

2008, Jokić et al. 2008, Murray et al. 2014), but will need to continue in order to reveal long-term patterns and trends in the carbonate climate of coastal waters.

### **Soft sediments**

A very important component of coastal ecosystems are sediments, which can range in composition from coarse sand to fine muddy silts (Henriksen, Hansen & Blackburn 1981). I chose to study silty sediments because they are very common and make a large contribution to ecosystem functioning. This sediment type is home to macrofauna (e.g., bivalves, polychaetes, echinoderms, crabs, shrimps, brittle stars, etc.), microalgae and microbes, which all contribute to the decomposition of OM and cycling of nutrients (Bouma et al. 2009, Nixon 1981). In coastal areas, the majority of suspended organic matter (SOM) sinks and accumulates on the surface layer of sediments because shallow depths limit the amount of SOM broken down by free-floating microbes. Therefore, sediment microbial communities have a stronger influence on the availability of inorganic nutrients for primary production (Welsh 2003).

The opposite happens within the open ocean because most SOM in these areas completely breaks down and recycles long before it reaches the bottom; the sheer depth of the open ocean and the slow sinking rate of SOM provides free floating microbes sufficient time to decompose and recycle organic matter. Karl (2002) indicated that the majority of SOM decomposition and nutrient cycling in deep seas occurs within the first 150 m from the surface. Therefore, open ocean sediments play a minor role in the nutrient levels of local water columns. By contrast, coastal soft sediments have a strong influence on the nutrient concentrations within the overlying seawater; therefore, a strong connection exists between the seafloor and coastal water columns. Benthic–pelagic coupling is the technical term used to describe this connection (Waldbusser & Salisbury 2014) and is the area of focus within this study.

Benthic-pelagic coupling is an important and diverse concept that occurs in coastal areas. The processes that occur in sediments and the water column have a great dependence on each other where continual solute exchange happens (Raffaelli et al. 2003, Soetaert et al. 2000). Kemp et al. (1999) provided examples of key events that are involved with benthic-pelagic coupling: OM - deposition and decomposition,

benthic - respiration and nutrient cycling, nutrient rich sediment re-suspension (usually by winds or storms) and primary production (benthic and pelagic). Benthic-pelagic coupling is fundamental to food chains/webs because it converts organic nutrients into inorganic nutrients that primary producers require for photosynthesis. Algae are at the base of all food webs and require solutes just as much as light to thrive. Therefore, benthic-pelagic coupling is critical for the recycling of nutrients and transfer of energy throughout different trophic levels existing in food webs (Marcus & Boero 1998).

The rate of microbial activity in coastal sediments is high because they receive large amounts of OM. As a result, productive coastal sediments become anoxic about one to three mm below the sediment surface because of intensive biogeochemical reactions (Aller 1994, Revsbech, Madsen & Jørgensen 1986). The O<sub>2</sub> penetration depth of sediments is predominantly a function of the amount of OM received by sediments and sediment porosity. Aerobic decomposition is the most common pathway for sediments to breakdown OM, therefore, sediments that receive high OM loading will have a shallower O<sub>2</sub> penetration depth. Further, cohesive sediments composed of silt and/or fine grains lack porewater advection, compared to permeable sediments like sandy beaches, which obtain high porosity and allow fresh oxygenated seawater to enter the pore spaces (Glud 2008).

The O<sub>2</sub> penetration depth of deep-sea sediments is much greater than coastal sediments because pelagic microbial communities oxidise most SOM before it reaches the bottom. A study by Sayles, Smith & Goudreau (1996) measured *in situ* sediment porewater O<sub>2</sub> profiles in the Northwest Atlantic Ocean off New Jersey and found the following results: the shallower site (2551 ± 1 m) had an O<sub>2</sub> penetration depth of approximately 10 cm, whereas the slightly deeper site (2629 ± 1 m) had an O<sub>2</sub> penetration depth >20 cm. Due to the limited O<sub>2</sub> availability in coastal sediments, animals that populate these habitats have specifically adapted to overcome this hardship.

In addition to O<sub>2</sub>, nutrients and OM are exchanged between the sediment porewater and overlying seawater via two methods: active and passive. The most dominant type of active solute exchange is advection. Ahmed, Elhassan, & Bashir (2012) described

that advection is driven by wind and tidal interactions. This leads to the delivery of nutrients, SOM and particulates into the open seawater. When advection is powerful and fast intense re-suspension can happen. As a result, primary production can significantly reduce during re-suspension events because suspended particles limit the penetration of light available for photosynthesis. Sloth et al. (1996) demonstrated this by using mesocosms containing sediment from Danish coastal waters. They induced sediment re-suspension events via advection to measure the photosynthetic response of pelagic and benthic algal communities. They found no significant effects on pelagic primary production, however, re-suspension significantly reduced benthic primary production rates for approximately a week until the particulates settled.

The other method of solute exchange is passive, which is driven by molecular diffusion. This is a common process that happens between the sediment-seawater interface in burrows and holes created by macrofauna, which will be discussed (Okubo et al. 2001).

### **Microbial nutrient cycling**

Nutrients that are released via benthic-pelagic coupling vary in chemical structure and each serve their own specific purpose in marine ecosystems. The three main nutrient types that exist in seawater are ones that contain nitrogen, phosphorous and/or carbon as their central element. Unique nutrient cycles exist for each type, which are driven by a diversity of microbial phyla (Nixon 1981). Arrigo's (2004) review highlighted that the two primary domains of microbes that exist and cycle nutrients in sediments are bacteria and archaea, which inhabit the sediment porewater and substrate. Azam & Malfatti (2007) discussed that these microbes convert OM into inorganic matter (IM) via redox reactions, which drive biogeochemical processes in sediments. Microbes oxidise OM by consuming the following electron acceptors in this particular order: O<sub>2</sub>, nitrate (NO<sub>3</sub><sup>-</sup>), manganese oxide (MnO), iron oxides (e.g., Fe<sub>2</sub>O<sub>3</sub>, Fe<sub>3</sub>O<sub>4</sub>, etc.) and sulphate (SO<sub>4</sub><sup>2-</sup>). There is a high microbial demand for O<sub>2</sub> because it produces the greatest amount of energy for OM decomposition. Microbes always use the strongest electron acceptor available, with the primary goal to completely re-mineralise the OM they are targeting at the time (Chen & Strous 2013, Froelich et al. 1979).

From the perspective of microbial redox reactions, Archer, Morford & Emerson (2002) and Van Cappellen & Wang (1996) distinguished that sediments have three zones: oxic, suboxic and anoxic. Since O<sub>2</sub> is the most powerful electron acceptor that microbes can utilise, aerobic respiration yields the greatest efficiency to re-mineralise OM (Hansen & Blackburn 1991). Ebenhöh & Heinrich (2001) highlighted that aerobic respiration produces approximately 32 moles of adenosine triphosphate (ATP - biological energy currency) when one mole of glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) is oxidised. This is more than that of fermentation, which is a very common anaerobic pathway that only produces two ATP molecules. The following reaction represents the overall aerobic remineralisation of OM carried out by microbes in marine sediments:



Equation 13 represents the Redfield ratio, where the ratio of carbon:nitrogen:phosphorous is 106:16:1. The organic derivatives of plankton and seawater follow this ratio (Redfield 1934). Furthermore, aerobic remineralisation is the initial phase in nitrification because ammonia (NH<sub>3</sub>) is oxidised (Gruber 2008). The nitrogen cycle is the most important biogeochemical process that occurs within sediments because nitrogen is the building block of all proteins and nucleic acids. Carbon and phosphorous cycles are important as well, however, the nitrogen cycle is the focus in most marine sediment studies with regard to benthic–pelagic coupling (Bonaglia et al. 2014, Fanjul et al. 2011, Foshtomi et al. 2015, Rysgaard, Christensen & Nielsen 1995, etc.). The nitrogen cycle was the area of focus within the context of this study.

Before the nitrogen cycle is covered in depth, a brief overview of four other common microbial processes that occur in the biogeochemical layers of the sediment will be discussed: manganese (Mn) reduction, iron (Fe) reduction, sulphate (SO<sub>4</sub><sup>2-</sup>) reduction and methanogenesis. Mn reduction happens in the suboxic layer and can occur simultaneously with denitrification (Myers & Nealson 1988). A study conducted by Post (1999) focuses on the compound manganese oxide (MnO), which is the sole electron acceptor for Mn reduction. Examples of bacteria that are capable of carrying out Mn reduction are: *Shewanella putrefaciens* (Fredrickson et al. 2008), *Bacillus infernus* (Nicholson 2002), genera *Geobacter*, *Desulfovibrio*, *Desulfuromus*,

*Arcobacter* and *Pelobacter* (Burdige & Nealson 1986, Roden & Lovely 1993, Lovely et al. 1993, Vandieken et al. 2012).

Fe reduction is another important biogeochemical process that occurs in sediments. A review by Weber et al. (2006) highlighted that the primary electron acceptor for Fe reduction is iron III hydroxide ( $\text{Fe}(\text{OH})_3$ ), which has a very similar molecular structure to MnO. The first microbial strain to be isolated and capable of Fe reduction was a genera of *Pseudomonas*. This bacteria couples Fe reduction with growth derived from respiration to break down rigid molecules. It is common for sediment bacteria to be multifunctional. For example, some Mn reducing microbes have the ability to reduce Fe (Arnold et al. 1986).

Fe reduction happens deep in the suboxic zone, which is significantly lower than for  $\text{NO}_3^-$  and Mn reduction; according to Lovely & Phillips (1988), this separation exists because  $\text{NO}_3^-$  and MnO inhibits Fe reduction by re-oxidising its final products (Fe reductants). If one were to look at a standard cross section of marine sediment, the layer in which ferric ions ( $\text{Fe}^{3+}$ ) are reduced to ferrous ions ( $\text{Fe}^{2+}$ ) is characterised by a mixed brown/green coloured boundary. This was pointed out in Lyle's (1983) article, which focused on this particular biogeochemical zone.

The next biogeochemical process that will be covered is  $\text{SO}_4^{2-}$  reduction. Bowles et al. (2014) stated that this is an important and ubiquitous set of reactions because it has strong implications in carbon fixation and redox cycling. Furthermore, they described that  $\text{SO}_4^{2-}$  reduction occurs when higher energy yielding electron acceptors become exhausted. Sulphide ( $\text{S}^{2-}$ ) is the end product of  $\text{SO}_4^{2-}$  reduction, which further reacts with Fe monosulphides. The product of this reaction causes the sediment to turn a dark black and is very distinct when viewing marine sediment cross sections (Love, 1967). Postgate (1979) provided some examples of anaerobes that are commonly involved with  $\text{SO}_4^{2-}$  reduction: *Desulfobacteraceae* and *Desulfovibrionaceae*. According to Jørgensen & Revsbech (1985)  $\text{SO}_4^{2-}$  reduction is on average the most dominant microbial process out of all the nutrient re-mineralisation pathways that occur in marine sediments. However, in some cases, Mn reduction has been proven to outcompete  $\text{SO}_4^{2-}$  reduction because particular coastal areas can have unusually high levels of MnO (Canfield 1994, Thamdrup 2000). Therefore, the dominant microbial

process of a coastal area is defined significantly by the geochemical composition of marine sediments.

The last biogeochemical process being covered before the nitrogen cycle is methanogenesis, which is a microbial process that produces methane (CH<sub>4</sub>) by reducing acetate (C<sub>2</sub>H<sub>3</sub>O<sub>2</sub><sup>-</sup>), hydrogenated acetate (C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>H), hydrogen (H) and/or CO<sub>2</sub> (Nealson & Stahl 1997). According to Oremland & Polcin (1982) methanogenesis is inhibited and outcompeted by SO<sub>4</sub><sup>2-</sup> reduction if they occur in the same zone. This is because both processes use some of the same initial compounds to conduct specific redox reactions. However, SO<sub>4</sub><sup>2-</sup> reducers are unable to assimilate compounds such as methanol, trimethylamine and/or methionine, which are critical agents for methanogenesis to happen. Furthermore, they explain that the subtle differences between these two processes allow them to occur simultaneously, but in separate zones.

Methanogenesis occurs deep in the anoxic zone of sediments below the region of SO<sub>4</sub><sup>2-</sup> reduction, and utilises anaerobes that come from a group of archaeon's called methanogens. Examples of methanogens are as follows: *Methanobacterium bryantii*, *Methanococcus voltae*, *Methanosarcina barkeri*, *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales* and *Methanopyrales* (Hedderich & Whitman 2013, Kadam et al. 1989). King (1984) reinforced the importance of methanogenesis to marine sediments because it's the final step in the decomposition of OM.

I chose to investigate the nitrogen cycle in my project because it has significant influences on coastal ocean productivity as pointed out by Ryther & Dunstan (1979) and is a prevalent process that occurs in marine sediments (Galloway et al. 2004). Galloway et al. (2008) described that nitrogenous-based compounds such as NO<sub>3</sub><sup>-</sup> feed autotrophs, which initiate food chains and webs. They elaborate that it progresses through trophic levels, which starts right from small organisms (e.g., microbes and algae) and finishes at apex predators (e.g., sharks and killer whales).

A complete nitrogen cycle starts when atmospheric N<sub>2</sub> is fixed and incorporated into OM, and ends when NO<sub>3</sub><sup>-</sup> is denitrified into N<sub>2</sub>, which is released back into the atmosphere (Gayon & Dupetit 1886). The nitrogen cycle is a synergistic process that

has many stages, where nitrogenous compounds go through multiple chemical transformations via microbial reactions. Further, these compounds transition between oceanic and atmospheric realms via the air–sea gas exchange interface (Zehr & Ward 2002). The following is a list of the separate stages involved in the nitrogen cycle, which scientists have conceptualised since its discovery: biological N<sub>2</sub> fixation (BNF), assimilation of dissolved organic nitrogen (DON), ammonification, NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> (ammonium) assimilation, anaerobic ammonia oxidation (anammox), nitrification, denitrification, assimilatory (ANRA) and dissimilatory (DNRA) NO<sub>3</sub><sup>-</sup> reduction to NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> (Zehr & Kudela 2011).

Atmospheric N<sub>2</sub> is the final product of the nitrogen cycle, however, the production of atmospheric N<sub>2</sub> can also be considered the first step in the cycle; Zehr & Kudela (2011) indicated, that BNF creates a looping effect by reintroducing atmospheric N<sub>2</sub> back into the ocean. BNF is a process by which diazotrophs (nitrogen-fixing microbes) draw in and assimilate N<sub>2</sub> with a reducing enzyme called nitrogenase (Kim & Rees 1994), and produce aqueous NH<sub>3</sub> (Burriss 1966). The bond binding the N<sub>2</sub> molecule is a strong triple covalent bond, which is why a specialised enzyme is critical for breaking it down. Shanmugam et al. (1978) provided some examples of the microbial groups that diazotrophs belong to: heterotrophs, diatoms and cyanobacteria.

Organic nitrogen is a pivotal component of OM and can be processed via two pathways: assimilation of DON (Goeyens et al. 1987) or ammonification (Middelburg & Nieuwenhuize 2000). Regarding the assimilation of DON, autotrophs (e.g., phytoplankton, macroalgae) and heterotrophs (e.g., zooplankton) have the ability to directly consume DON from seawater such as: urea and/or amino acids (Kirchman 1994, Tyler et al. 2005). According to Ramaiah (2004), in the scenario that these organisms are too slow to find and uptake this resource via assimilation, *Heterobacter* are close behind to re-mineralise nitrogen existing in all forms of OM. These microbes source OM and decompose it via a process called deamination, which releases NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup> into the water column. Overall, this process is referred to as ammonification. Further, Ramaiah (2004) elaborated, that *Heterobacter* target particular structures within OM. Examples include: chitin, peptides, proteins, ribonucleic acids (RNA), amino acids and/or urea.

There are three different pathways to which  $\text{NH}_3/\text{NH}_4^+$  can be processed. The first pathway is the direct consumption of  $\text{NH}_3/\text{NH}_4^+$ , which converts inorganic nitrogen into living tissue. This process is the direct opposite to ammonification and is commonly referred to as  $\text{NH}_3/\text{NH}_4^+$  assimilation (Zehr & Kudela 2011).  $\text{NH}_3/\text{NH}_4^+$  assimilation is driven and catalysed by specialised enzymes such as glutamine synthetase, glutamate synthase and glutamate dehydrogenase; these exist in the metabolic organs of ammonia consuming organisms (Muro-Pastor et al. 2005).

The second pathway that  $\text{NH}_3/\text{NH}_4^+$  can be processed is via anammox, which was conceptualised by Mulder et al. (1995); this pathway converts  $\text{NH}_4^+$  into  $\text{N}_2$  gas. According to Dalsgaard et al. (2005) the anaerobic bacteria that conduct anammox belong to the phylum Planctomycetes, which are usually from the genus *Candidatus*. The electron acceptors that these bacteria use to complete this pathway can be nitrite ( $\text{NO}_2^-$ , Engström et al. 2005),  $\text{Fe}^{2+}$  (Oshiki et al. 2013) or manganese II ion ( $\text{Mn}^{2+}$ , Kuypers et al. 2003).

Lastly, the third pathway that  $\text{NH}_3/\text{NH}_4^+$  can be processed is via nitrification, which is a two-staged oxidation reaction that occurs within the sediment; firstly,  $\text{NH}_3/\text{NH}_4^+$  is oxidised to produce  $\text{NO}_2^-$  and then oxidation occurs again to produce  $\text{NO}_3^-$  (Mortimer et al. 2004). Different bacterial groups drive each stage of nitrification, however, there is one exception that will be discussed. The first stage of oxidation can be conducted by lineages of *Proteobacter* (e.g., *Nitrosococcus*, *Nitrosomonas*, *Nitrosospira*, *Nitrosolobus* and *Nitrosovibrio*) or archaea (e.g., phylum *Thaumarcheota*, Francis et al. 2007, Suzuki et al. 1974). A study conducted by Wobus et al. (2003) on the microbiological diversity of marine sediments with varied nutrient loads suggested that *Proteobacter* are dominant in mesotrophic (intermediate nutrients levels) or eutrophic (high nutrient levels) conditions, whereas *Thaumarcheota* archaeon's dominate in oligotrophic conditions (low nutrients levels).

$\text{NO}_2^-$  oxidising bacteria conduct the second stage of nitrification. Examples of these include: *Nitrobacter*, *Nitrospina* and *Nitrospira* (Jetten et al. 2003). The ability of these bacterial groups to conduct each stage of oxidation depends on their genetic traits responsible for producing the specialised enzymes that catalyse reactions. The first stage of oxidation is catalysed by the enzyme  $\text{NH}_3$  monooxygenase and the second

stage is by  $\text{NO}_2^-$  oxidoreductase (Lücker et al. 2010, Lücker et al. 2013, Sundermeyer-Klinger et al. 1984). According to Daims et al. (2015), *Nitrospira* has the ability of conducting both stages of oxidation; this complete  $\text{NH}_3/\text{NH}_4^+$  oxidation process is referred to as comammox (complete  $\text{NH}_3/\text{NH}_4^+$  oxidation) and has been a very popular topic among microbiologists since its discovery (Pinto et al. 2016, Pjevac et al. 2017, Santoro 2016).

The last two pathways of the nitrogen cycle explain what happens to the end product of nitrification ( $\text{NO}_3^-$ ), other than denitrification. The two other pathways are ANRA and DNRA. According to Zehr & Kudela (2011), ANRA is similar to BNF because both processes produce the same end product ( $\text{NH}_3/\text{NH}_4^+$ ), which is directly assimilated by organisms for growth and reproduction. Flores et al. (2005) revealed that particular species of bacteria and cyanobacteria are the microbes, which possess the acquired genes to conduct ANRA. These genes obtain specific traits, which allow these microbes to produce specialised  $\text{NO}_2^-$  and  $\text{NO}_3^-$  assimilatory enzymes; they catalyse the following set of reactions:  $\text{NO}_3^-$  is assimilated to produce  $\text{NO}_2^-$ , and then  $\text{NO}_2^-$  is assimilated to produce  $\text{NH}_3/\text{NH}_4^+$ . By contrast, DNRA produces  $\text{NH}_3/\text{NH}_4^+$ , which isn't directly assimilated for biological development (Sørensen 1978). Zehr & Kudela (2011) suggested, that  $\text{NH}_3/\text{NH}_4^+$  is fed back into the nitrogen cycle post DNRA. From there, it can be processed by any of the following microbial pathways: ammonia assimilation, nitrification and/or anammox.

### **Benthic primary production and the role of microphytobenthos**

MacIntyre et al. (1996) described that microphytobenthos is a specific group of microalgae (e.g., unicellular eukaryotes, cyanobacteria, etc.), which inhabit the first few millimetres of the sediment surface. A slight green/brown tinge to the sediment surface indicates their presence, where they will only exist on illuminated sediments to meet their photosynthetic requirements. Bertics & Ziebis (2009) elaborated that chemical (e.g., microbial activity) and physical (e.g., bioturbation) processes within sediments significantly stimulates benthic primary production. It is common for benthic micro-algal communities to exceed the biomass of pelagic microalgae in overlying water columns (Underwood & Kromkamp 1999).

Measuring the benthic primary production of coastal environments poses great challenges, due to some of the following reasons: quantifying benthic micro-algal biomass via measurements of chlorophyll-*a* concentrations can have up to 40% error unless high-performance liquid chromatography technology is used. Benthic microalgae have patchy distribution patterns, both vertically and horizontally within the sediment surface and benthic primary production is sensitive to seasonal change and geographic variation (Atkinson et al. 2008, Attard et al. 2014, Bruno et al. 2006, Charpy-Roubaud & Sournia 1990, Hartig et al. 1998, Staehr et al. 2012). Grant (1986) and Pinckney & Zingmark (1993) summarised that benthic primary production depends on light, inorganic nutrients and organic carbon.

Glas et al. (2012) mentioned, that benthic microalgae (e.g., foraminifera) are capable of driving local pH values very high during the day by consuming CO<sub>2</sub> via photosynthesis within estuaries and/or other shallow water coastal settings. However, they elaborated, that benthic - microalgae and microbes have the opposite effect in darkness because respiration dominates and local pH values are driven down due to CO<sub>2</sub> production. Therefore, benthic microalgae play a significant role in the carbonate chemistry of the sediment porewater because of their influence on dissolved CO<sub>2</sub> levels.

Two pivotal elements that benthic microalgae require are nitrogen and phosphorous. In conjunction with photosynthesis and respiration, benthic microalgae assimilate NO<sub>3</sub><sup>-</sup> and phosphate (PO<sub>4</sub><sup>3-</sup>) to synthesise OM. Therefore, benthic microalgae are key players in the regulation of nutrients within coastal ecosystems (Burkepile & Hay 2006). Cahoon (2014) suggested, that microalgae are very robust because they live in neritic zones all around the world and therefore, have a high tolerance to fluctuating water parameters (e.g., pH, nitrogen, phosphorous, temperature, salinity, etc.)

Benthic microalgae have a short life expectancy (Fenchel 1968), and according to Pinnegar et al. (2000) and Posey et al. (2002), they are subject to constant predation by larger animals (e.g., fish, stingrays, starfish, etc.) and sediment inhabiting macrofauna (e.g., polychaetes, crabs, urchins, shrimp, etc.). Therefore, they are important not only for alleviating pollution, but for coastal food chains and webs (Paine 1980).

The most common species of benthic microalgae that exist in coastal settings are diatoms (they can also exist in the water column). Werner (1977) argued that diatoms are the most prolific organism on Earth and the most important. This is because diatoms are at the base of most aquatic food webs, have extensive contributions to CO<sub>2</sub>/O<sub>2</sub> levels on Earth and are closely connected to coastal sediments. Round et al. (1990) described that diatoms are unicellular and exhibit a diverse range of morphological forms due to the structure of their cell walls integrating silica (no longer than 2 mm in length).

Diatoms appear as a thin brown layer on most shallow water surfaces because they utilise pigments such as carotenoid fucoxanthin (Grossart et al. 2005). Smol & Stoermer's (2010) study on environmental monitoring indicated that diatom communities are widely used as indicators of water quality in marine and freshwater ecosystems because they are able to provide evidence of past and present aquatic conditions. Furthermore, diatoms are a key food source for macrofauna that live within coastal sediments (MacIntyre et al. 1996).

### **Macrofaunal bioturbation**

Animals that inhabit coastal sediments are referred to as bioturbating macrofauna because their activity significantly impacts the biogeochemistry of sediments (Aller 1982). The morphology and behaviour of these animals are diverse; each species impacts the structure and porewater chemistry of sediments according to their specific niche (Bertics & Zebis 2009). Kristensen et al. (2012) developed an efficient term for bioturbation: "all transport processes carried out by animals that directly or indirectly affect sediment matrices. These processes include both particle reworking and burrow ventilation". Alternative definitions for bioturbation have been established, however, those terms are too specific and focus on certain areas of bioturbation. An example of this can be seen in Rhoad's (1967) and Winston & Anderson's (1971) definition: "The effects that animal particle reworking and biogenic structures have on the biological, ecological and biogeochemical properties of modern sediments". This definition dismisses burrow ventilation, which is a critical component of bioturbation.

Sediment bioturbators have been classified as ecosystem engineers because they fundamentally change the structure and functioning of sediments; as a consequence, significant shifts in sediment nutrient cycling can occur (Mermillod-Blondin & Rosenberg, 2006). Therefore, the activity of these organisms plays a strong role in coastal food - chains and webs. According to Kristensen et al. (2012), bioturbation has two categories: particle reworking and ventilation. Particle reworking encapsulates all faunal disturbances that directly shift and/or turnover sediment particles, both horizontally and vertically. François et al. (1997) and Solan & Wigham (2005) conceptualized particle reworking into four subcategories, which are as follows: biodiffuser, upward conveyor, downward conveyor and regenerator. Biodiffusers are macrofauna, which randomly and constantly rework sediment surfaces over short distances (Kristensen et al. 2012).

Biodiffusers cause local effects to the biogeochemistry of sediments, where this process has similar effects to that of molecular and/or eddy diffusion. Biodiffusion occurs close to the sediment surface and is carried out by animals such as worms, crabs, sand dollars and urchins (e.g., Gilbert et al. 2007, Lohrer et al. 2005, Penhalopes et al. 2009, Quintana et al. 2007). Kristensen et al. (2012) described that upward conveyors are macrofauna, which create burrows much deeper than biodiffusers below the sediment surface. These animals are vertically orientated and feed with their head facing down. Unlike biodiffusers, upward conveyors cause non-local effects because they expel particles out the opening of their burrows. The expulsion of particles is caused by waste release or by the subsidence of sediment material from digging. Post expulsion, burrow openings tend to backfill with new particles via gravity. Upward conveyors are predominantly species of worms and shrimp (e.g., Cadée 1976, Dobbs & Whitlatch 1982).

Downward conveyors create burrows very similar to that of upward conveyors; however, they are orientated with their head facing towards the burrow opening (Kristensen et al. 2012). These animals draw in particles and defecate within their own burrows, where new material and waste accumulates at the burrow ending. Therefore, their burrows become clogged and require constant irrigation/construction for maintenance (Shull 2001). Downward conveyors cause

local and non-local effects because their retention of particles from burrow openings is non-selective; downward conveyors are predominantly worms (e.g., Shull & Yasuda 2001).

The last subcategory of particle reworking is regenerators, which are macrofauna that perform rigorous and continual excavation to maintain burrows (Kristensen et al.'s 2012). These burrows tend to be much wider and deeper than upward and downward – conveyor burrows. Therefore, burrow wall collapsing and infilling is very common; species of crab fall into this category (e.g., Huang et al. 2007).

The second major category within bioturbation is burrow ventilation; Shull et al. (2009) highlighted that this is a critical process, which macrofauna perform for a continual supply of O<sub>2</sub> and food. Living in coastal soft sediments provides challenges for macrofauna because most of these sediments become anoxic a few mm below the sediment surface (Aller 1994, Revsbech et al. 1986). Macrofauna continuously and/or intermittently ventilate their burrows to ensure O<sub>2</sub> concentration is optimal. Not only does burrow ventilation resupply O<sub>2</sub>, but it also cleans out any form of unwanted detritus (Kristensen 1988). Kristensen et al. (2012) breaks down burrow ventilation into three subcategories: open-ended, blind-ended in permeable sediments and blind-ended in impermeable sediments. The following will describe these three subcategories.

Open-ended ventilation occurs when macrofauna produce burrows with two or more openings at the sediment surface. In the absence of the burrow inhabitant, radial diffusion occurs across the burrow wall–seawater interface. However, when the burrow is inhabited, the burrow seawater is advected (e.g., worms through peristaltic movements), which enhances the solute exchange between the burrow and the surrounding sediment. Blind-ended ventilation occurs within impermeable sediment burrows, which have a single opening. This process results in bidirectional water movement and radial diffusion across the burrow wall-seawater interface. Lastly, blind-ended burrow ventilation in permeable sediments occurs in burrows with one opening. It is similar to blind-ended ventilation in impermeable sediments; however, they differ because this form of ventilation produces unidirectional water movement do to the lack of sediment porosity. Furthermore, bioirrigation occurs when water

exits burrows via the advective percolation of pore water towards the sediment surface–seawater interface (Kristensen et al. 2012).

Particle reworking and burrow ventilation cause complex and indirect effects on the biogeochemistry of sediments, as discussed by Laverock et al. (2011). For example, when macrofauna rework sediments to create burrows or for feeding purposes, this increases the sediment–seawater exchange surface area (Kristensen 1988). Quintana et al. (2007) solidified that particle reworking alters the physical structure of sediments, which affects the chemical zones of sediments. According to Aller & Aller (1998), when macrofauna ventilate burrows for oxygenation and cleansing purposes, the introduction of fresh seawater surrounding their burrow openings enhances the sediment-seawater solute exchange. Since burrows increase the solute exchange surface of sediments, ventilation further enhances the exchange of inorganic nutrients into burrow walls, which then leach into the surrounding sediment (Kristensen 2000). Burrows that are properly maintained significantly increase in the penetration of O<sub>2</sub> into sediments (Krantzberg 1985).

Bioturbation is crucial for sediment functioning and plays a significant role in coastal ecosystems (Bertics et al. 2010, Laverock et al. 2010, Mermillod-Blondin & Rosenberg 2006). A basic concept of understanding the importance of bioturbation is defined in a study conducted by Laverock et al. (2011). They highlighted, that macrofaunal structures within sediments make significant alterations to the physiochemical properties of sediments, which ultimately stimulates microbial activity. Further, macrofaunal structures provide a channel for deeper subsurface penetration of overlying seawater. In essence, macrofauna enhance the coupling of the sediment with the overlying water column. Bioturbated areas of the sediment provide a unique environment for nitrogen cycling, but also promote microbial abundance and diversity. It has been established that the lining of macrofaunal burrow walls can have higher microbial diversity and abundance than the surrounding sediment, both surface and subsurface (Laverock et al. 2010, Parnell et al. 2009).

## Response of coastal soft sediment nutrient cycling to ocean acidification

The nutrient fluxes of seawater within shallow semi-enclosed bays are predominantly a function of the microbes and macrofauna, which inhabit sediments. Coleman & Williams (2002) indicated that the commercial fishing of the benthic environment encourages coastal ecosystem cascading effects. Bottom trawling through sediments would not only remove key bioturbating species, but also compromise macrofaunal structures. Therefore, the anthropogenic disturbance of sediments can create an unbalanced coastal ecosystem, which highlights their importance. Sediments were a pivotal component in my experiment because I wanted to investigate the effects that OA had on the sediment-seawater nutrient exchange within coastal areas.

Widdicombe et al. (2008) noted that a lack of empirical data existed regarding how benthic ecosystems would respond to OA; since their study the amount of research on this has significantly grown. Investigating the sediment response of nutrients to OA is difficult because sediments obtain high biological diversity. According to Snelgrove (1999), close to 98% of all marine life resides on (epifauna) or within (infauna) sediments. Furthermore, the chemistry and biology of sediments are not only diverse worldwide, but also on local scales (Gray 2002).

An investigation by Wood et al. (2009) provided conclusive evidence that *Amphiura filiformis* (brittlestar) stimulated two aspects of sediment nutrient cycling under acidified conditions (pH 7.7, 7.3 and 6.8). It was found that *A. filiformis* significantly increased the sediment uptake of  $\text{PO}_4^{3-}$  and the sediment release of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  into the overlying seawater. Further, *A. filiformis* had no effect on  $\text{NH}_3/\text{NH}_4^+$  fluxes regardless of the induced treatments (animal density and seawater acidification). Moreover, seawater acidification had no effect on the bioturbation performance of *A. filiformis*.

A study conducted by Nilsson & Sköld (1996) on the same species of brittlestar indicated that they were capable of carrying out appendage regeneration and sexual reproduction during seawater acidification. This indicates, that *A. filiformis* has the ability to tolerate seawater acidification over the short-term duration of an experiment, but not necessarily in the long term.

*Nereis virens* is a species of polychaete that borrows into soft sediments, and is a popular candidate for this line of research because their unique mode of bioturbation significantly stimulates sediment nutrient cycling (Hutchings 1998). An experiment conducted by Widdicombe & Needham (2007) had two treatment types: the addition of *N. virens* and decreasing pH (control: 7.9, 7.3, 6.5 and 5.6). Nutrient results from the control pH conditions indicated that *N. virens* significantly increased the sediment uptake of  $\text{NH}_3/\text{NH}_4^+$ ,  $\text{NO}_2^-$  and silicate ( $\text{SiO}_4^{3-}$ ), but had no effects on  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  fluxes.  $\text{SiO}_4^{3-}$  fluxes were unaffected by seawater acidification. However, seawater acidification did increase the sediment uptake of  $\text{NO}_3^-$  and release of  $\text{NH}_3/\text{NH}_4^+$ . Furthermore, it caused a decrease in the sediment release of  $\text{NO}_2^-$  and a decrease in the sediment uptake of  $\text{PO}_4^{3-}$ . The treatment of pH had no effect on the structure and size of polychaete burrows, which led to the conclusion that nutrient fluxes during seawater acidification were isolated as a microbial response to the stress of decreasing pH. Therefore, experiments do reveal that some infaunal species are still able to function during hypercapnia for a short period of time.

### **New Zealand *Theora lubrica* population: demography and biology**

The following section provides an overview on the demography and biology of an introduced infaunal bivalve species to New Zealand, *Theora lubrica*. This species was abundant at my study site, which made it an accessible resource for my experiment. Moreover, their proliferation provided a motive to investigate if they had any effects on the nutrient response of sediments to OA.

*T. lubrica* is an exotic species to New Zealand coastal waters, originating from Japan (Powell 1976). Hayward et al. (1999) and Morely (1995) indicated that these species thrive in fine, subtidal sediment subjected to constant terrigenous depositions. Populations of *T. lubrica* within New Zealand have been monitored since the 1970's (Powell 1976), where they have been classified as invasive to New Zealand coastal waters (James & Hayden 2000).

*T. lubrica* populations continue to spread throughout New Zealand coastal subtidal sediments and appear to have cemented themselves as a permanent resident in our waterways (Morely 1995, Powell 1976). Because *T. lubrica* thrives in eutrophicated

areas, they can be used as biological indicators of organically rich or polluted sediments (Imabayashi and Tsukuda 1984). It has been recorded that *T. lubrica* can dominate close to 70% of a macrobenthic community within nutrient rich muddy sediments (Imabayashi & Wakabayashi 1992). Yokoyama & Ishihi (2003) described that *T. lubrica* is a small deposit-feeding bivalve, which feeds on detritus, microphytobenthos and phytoplankton. Further, *T. lubrica* dig twice their shell length into the sediment and project a siphon to the sediment surface for feeding, respiration and waste excretion.

Yokoyama & Ishihi (2003) mentioned that *T. lubrica* rapidly grow and become sexually mature in just three months given the right conditions. Lohrer et al. (2010) highlighted that these species have to maintain a constant connection with the sediment-seawater interface to live in sediments. They suggested that bioturbative disturbances of the sediment surface by *Echinocardium cordatum* (small infaunal sea urchin) could limit the invasive success of *T. lubrica*. Lohrer et al. (2008) found that heavily bioturbated sediment plots by *E. cordatum* resulted in a lower abundance of *T. lubrica*, compared to undisturbed plots. Similar results were found in Lohrer et al.'s (2010) study; increasing *E. cordatum* density caused a decrease in the abundance of *T. lubrica*.

Lohrer et al. (2010) found that *T. lubrica* populations experience seasonal variability in abundance at the mouth of the Mahurangi Harbour; they were significantly less abundant in this area during the colder sampling periods. This suggests that *T. lubrica* recruits at the mouth of harbours during summer after spawning, but cannot survive in these areas long term. In light of this information, *T. lubrica* predominantly live a sedentary lifestyle, but can shift to a more ideal habitat post recruitment. Their mobility and broadcast spawning techniques have allowed them to spread throughout the country. Townsend et al. (2014) suggested that *T. lubrica* migrated to New Zealand Harbours via the hulls and/or inside the water ballast containers of ships arriving to various ports in the North and South Island. It is from these harbours that they were introduced, which have enabled them to expand their populations in coastal sediments throughout the country.

*T. lubrica* has also invaded harbours in Australia, Russia and America (Konstantin et al. 2004, Ranasinghe et al. 2005, Wilson et al. 1998). Within the context of New Zealand, research has focused on the interaction of *T. lubrica* with indigenous fauna and, whether or not, they impose deleterious effects on native species. Dodd (2009) found *T. lubrica* in gut of the indigenous New Zealand snapper (*Pagrus auratus*), therefore, the introduction of *T. lubrica* has expanded the feeding opportunities for snapper. Moreover, empirical data indicates a positive association between *T. lubrica* and the native horse mussel (*Atrina zelandica*). The abundance of *T. lubrica* can significantly increase within a 10 cm radius of *A. zelandica* because their faecal deposits are a key food source targeted by *T. lubrica* (Townsend et al. 2014).

In the same study, Townsend et al. (2014) provided an example of a negative association between *T. lubrica* and another native macrofaunal species aside from *E. cordatum*, being the indigenous cockle, *Austrovenus stutchburyi*. *T. lubrica* abundance significantly decreased when in the presence of *A. stutchburyi*. It is believed that the tubes and/or dense mats formed by *A. stutchburyi* inhibit the settlement of *T. lubrica* larvae and juveniles. Therefore, it appears that the introduction of *T. lubrica* hasn't hindered the livelihood of many native macrofauna species because most indigenous species are bigger and are able to outcompete them. Here, I suggest that the influence of *T. lubrica* on the environment is not only a function of how it interacts with other infaunal species, but also how it affects sediment nutrient cycling within the context of OA.

## Hypothesis

Considering that *T. lubrica* is a well-established invasive species in New Zealand coastal waters, it is important to predict if and how they will influence the biogeochemical response of soft sediments to OA via CO<sub>2</sub> perturbations; in particular, I wish to focus on the effects of *T. lubrica* on sediment DIN (dissolved inorganic nitrogen) fluxes during seawater acidification (O<sub>2</sub> fluxes were also measured). Previous studies on *T. lubrica* in New Zealand have focused more on the demography of this species and how they interact with native fauna. Therefore, my research is novel because it will be contributing to a current gap in knowledge. The following is my overall research question:

“How does *T. lubrica* influence the response of nitrogen cycling in coastal soft sediments to OA?”

Within this question I have drawn three null hypotheses, which are the following:

H<sub>01</sub>: *T. lubrica* has no significant effect on DIN fluxes within coastal soft sediments.

H<sub>02</sub>: OA has no significant effects on DIN fluxes within coastal soft sediments.

H<sub>03</sub>: There is no significant interaction between OA and *T. lubrica*, which influences the biogeochemical response of coastal soft sediments.

I established that my predictor variables were the following: (I) *T. lubrica* treatment, (II) pH treatment, and (III) *T. lubrica* dry weight (mg). Furthermore, my response variables were the following: (I) TOU (total oxygen uptake) and fluxes of (II)  $\text{NH}_4^+$ , (III)  $\text{NO}_2^-$ , and (IV)  $\text{NO}_3^-$ .

## Materials and methods

### Experimental design

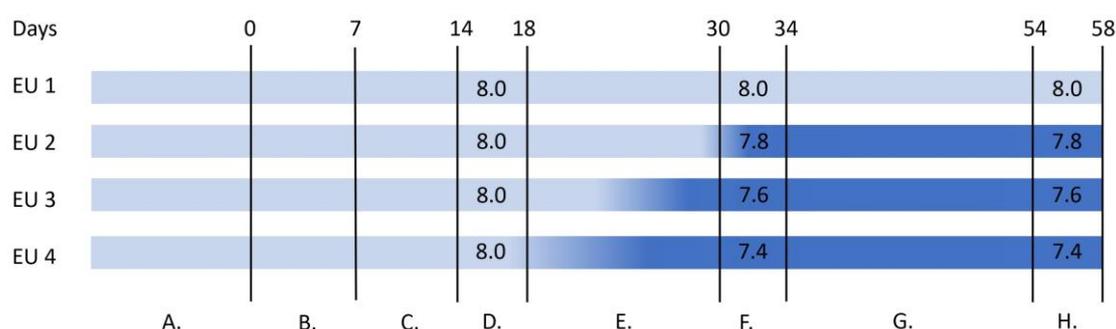
I designed an experiment that focused on measuring the sediment exchange of O<sub>2</sub> and DIN fluxes (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>). I induced the following treatments: seawater acidification and the addition of infaunal bivalve *T. lubrica*. I collected all sediment cores and *T. lubrica* specimens from my established study site in Man O' War Bay, Waiheke Island. I conducted my experiment in full darkness to eliminate photosynthesis because I wanted to specifically measure sediment microbial responses.

I collected 32 sediment cores of subtidal silt and submerged 8 cores into each of the four experimental units (EU, seawater re-circulating systems) back at the AUT OA laboratory, Auckland City. Within each EU I made two rows: four Control cores and four Bivalve treatment cores (10 added *T. lubrica* specimens). In situ *p*CO<sub>2</sub> (partial pressure of CO<sub>2</sub>, pH 8.0) was maintained in all EU for close to three weeks after adding the sediment cores. *T. lubrica* specimens were added to the Bivalve treatment cores a week following the introduction of the sediment cores, which allowed them to settle in. Following the acclimatisation period of the added *T. lubrica*, I performed an initial set of measurements on the sediment-seawater exchange of O<sub>2</sub> and DIN fluxes for each core.

After the initial measurements, I lowered seawater pH by 0.05 per day<sup>-1</sup> until EU 2–4 reached PH levels of 7.8, 7.6, and 7.4, respectively I established EU 1 as the control, which I maintained at in situ *p*CO<sub>2</sub>. I performed a final set of measurements after the sediments cores were subjected to pH treatments for 20 days. The differences between the initial/final set of measurements provided information on how the sediment exchange of O<sub>2</sub> and DIN fluxes changed over time because of the effects caused by the pH and Bivalve treatments (Table 1).

Table 1.

Timeline of the key experimental procedures: (A) Natural seawater introduction and equipment calibration, (B) Sediment introduction and acclimatisation, (C) *Theora lubrica* introduction (Bivalve treatment) and acclimatisation, (D) Initial incubations at in situ  $p\text{CO}_2$  (partial pressure of  $\text{CO}_2$ ), (E) initiation of the seawater pH treatments (stepwise decrease:  $0.05 \text{ units day}^{-1}$ ), (F) Target pH treatments reached, (G) Acclimatisation of the sediment cores to the pH treatments and (H) final incubation.



## Study site

Waiheke Island is a 35-minute boat trip from Auckland City and is situated in the Hauraki Gulf, New Zealand (Fig. 1). Manighetti & Carter (1999) described the Hauraki Gulf as a shallow semi-enclosed bay, which can reach depths close to 50 m and has surface area coverage of approximately  $4000 \text{ km}^2$ . Sikes et al. (2009) reported that the gulf contains a variety of sediment types, which range from permeable sands to cohesive mud's composed of terrigenous silt and clay.

Wong & O'Shea (2010) classified three sediment types within Man O' War Bay, Waiheke Island: gravel, muddy gravel, and mud. These authors performed a sediment grain size analysis and found the following: the gravel was poorly sorted, the muddy gravel was very poorly sorted and the mud was moderately to moderately well sorted. Furthermore, they reported that the macrofaunal species richness and abundance was highest in the gravel and lowest in the mud. The most common macrofaunal species that they found in the muddy sediment were (Descending average abundance): *T. lubrica*, Ostracoda Gen. spp (crustacean), *Prionospio* sp. (polychaete), *Sthenelais* sp. (polychaete), *Paraphoxus* sp. (arthropod), *Cossura consimilis* (polychaete) and *Echinocardium cordatum* (echinoderm).

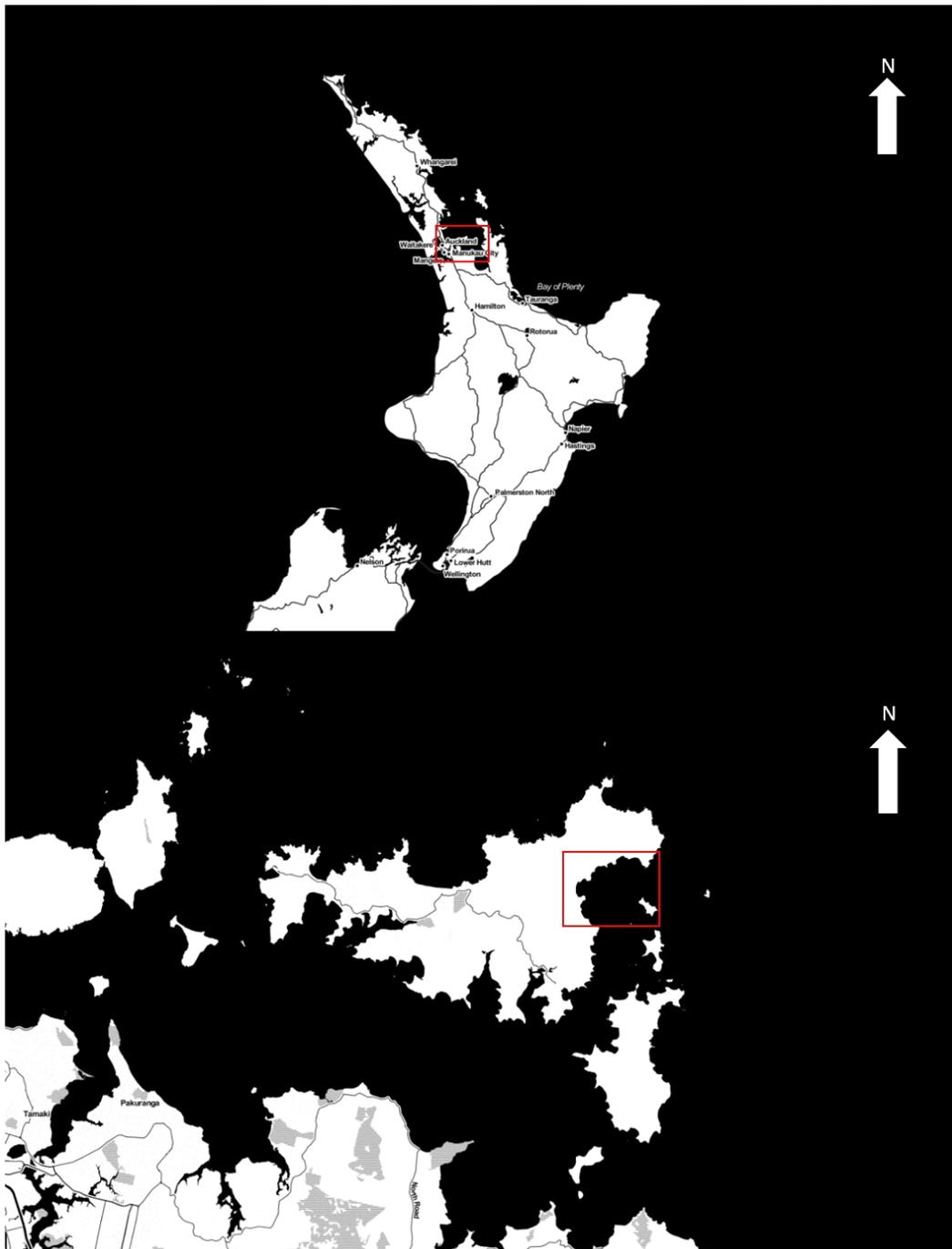


Figure 1.

Map showing the location of Man O' War Bay (red line), Waiheke Island. Retrieved from <http://maps.stamen.com/>.

A separate study conducted by Wilson & Vopel (2012) focused on characterising soft sediments within the same bay. They determined that the water content within the top 9 cm layer of the sediment surface decreased from 75% to 65% after being dried for 24 h at 90 °C. OM content in this layer was calculated by the weight loss of

sediments via combustion in a furnace for 6 h at 400 °C; OM content of the sediment was determined to be  $6.3 \pm 0.9\%$  (dry weight, mean  $\pm$  SD,  $n = 54$ ). They also analysed particle size distribution (% volume) in the same layer of the sediment using a laser-based particle analyser (Malvern Master-sizer 2000) and found that the sediment was composed of 73% silt, 17% sand and 9% clay. Moreover, using AVS (acid volatile sulfides) profiling techniques they indicated that this sediment type had areas concentrated with iron sulfides (seen as black regions in sediment cross sections). Sediments with high concentrations of these minerals reflect intensive  $\text{SO}_4^{2-}$  reduction, indicating an enriched organic load (Sorokin and Zakusina 2012).

### **Sediment and bivalve collection**

A total of 32 sediment cores were collected from Man O' War Bay ( $36^\circ 47.205' \text{ S}$ ,  $175^\circ 10.235' \text{ E}$ , Fig. 2) 9 to 10.5 m's deep on 20 February 2018. To collect a sediment core, a SCUBA diver pushed a clear acrylic tube (height: 300 mm, internal diameter: 90 mm) approximately two-thirds of the way down into the sediment. The diver sealed these tubes with top and bottom lids fitted with rubber O-rings. The distance between the sediment surface and lower end of the tube lid ranged from 75 to 144 mm.

Sediment cores were collected haphazardly, where care was taken by divers to avoid sampling sediments with large macrofauna. The sediment at the study site was structured by two key species of macrofauna: *E. cordatum* and an unidentified species of mud shrimp. *E. cordatum* ploughs through the sediment surface, whereas, the mud shrimp creates large burrows (Anecdotal: data not supplied). Divers placed the cores where the sediment surface was smooth and undisturbed. Each set of sediment cores were collected within a 20 m radius from a fixed position on the seafloor.

Upon divers surfacing, I placed all sediment cores into insulated bins containing crushed ice to cool the samples. The sediment cores were transported back to the AUT OA laboratory within four hours and left overnight with their lids off. This allowed for any suspended particles to settle within the cores before they were submerged into the experimental units the following day.

Previous fieldwork indicated that *T. lubrica* is an abundant infaunal bivalve in Man O' War Bay. I collected *T. lubrica* specimens for the experiment on 7 March 2018 from Man O' War Bay ( $36^{\circ}47.279' S$ ,  $175^{\circ}9.770' E$ , Fig. 2) 6 to 7.5 m deep using a Van Veen sediment grab sampler. I passed all the sediment grabs through a 0.5 mm sieve to isolate the macrofauna and repeated this process until I gathered approximately 600 specimens. From this amount, I allocated 160 individuals for the main experiment and another 100 individuals for gathering demographical information.

I stored all *T. lubrica* specimens in a bucket half filled with seawater and transported them back to the laboratory within four hours. I water changed this bucket with fresh seawater from the laboratory and added a small aquarium aerator (Precision 2500, Aqua One) to oxygenate the seawater. The following day I used these specimens and added 10 *T. lubrica* to each sediment core within the Bivalve treatment row.



Figure 2.

Map showing the locations of the animal collection (yellow line) and sediment sampling (red line) sites in Man O' War Bay, Waiheke Island. The coordinates for the centre of the animal collection and sediment sampling site areas are  $36^{\circ}47.279' S$ ,  $175^{\circ}9.770' E$  and  $36^{\circ}47.205' S$ ,  $175^{\circ}10.235' E$ , respectively. Retrieved from <https://earth.google.com/>.

## Natural seawater collection

I collected natural seawater for the experimental units on 7 February 2018 from a public boat ramp, South End Orewa Beach (36° 47.175' S, 175° 10.466' E; Fig. 3). Seawater was extracted on the incoming tide (1–2 m water depth) to avoid potential nutrient contamination by riverine inlets and/or mangroves catchments. I drained the seawater into AUT's aquaculture reservoir system, which has a closed loop that mechanically filters (0.2 mm) and UV sterilizes seawater. I treated the seawater for 24 hours in the system before adding it to the experimental units. Seawater was mixed between experimental units with a separate pump (2400, Eheim) to create consistent chemical conditions.



Figure 3.

Map showing the location of the natural seawater collection site (red line) in Orewa, Auckland. The coordinates for the centre of the seawater sampling site area are 36° 47.175' S, 175° 10.466' E. Retrieved from <https://earth.google.com/>.

## Experimental setup

Each experimental unit had a plastic incubation bin as its base (length: 1.12 m, width: 0.72 m, height: 0.6 m), a header mixing barrel (diameter: 0.6 m, height: 0.9 m), an aquarium chiller (HC Chiller 300A, Hailea), a five Watt UV sterilizer (ClearTec, Pond One), a pressurized canister filter (Aquis Canister Filter 1200, Aqua One or a 4E, Eheim), a variable speed wave maker (WP-25, Jebao) and a return pump feeding the mixing barrel from the incubation bin (3260, Eheim).

Each experimental unit circulated 560 L of natural seawater (351 L in the incubation bin and 209 L in the mixing barrel), which flowed 24/7 at a rate of approximately 540 L h<sup>-1</sup>. The seawater flow within each experimental unit started from the incubation bin by the return pump, which passed the seawater through the aquarium chillers and UV sterilizers. The outflow of this return line filled the mixing barrels, causing the seawater to be gravity fed back into the incubation bins via overflow pipes. The gravitational flow from the mixing barrel, plus the flow generated by the pressurized canister filters and wave-makers produced sufficient advection within the incubation bins. This prevented stagnation in the overlying seawater directly above the sediment cores and provided sufficient mixing for consistent pH conditions. The total area of the visible sediment surface was 628 cm<sup>2</sup> per experimental unit. This excludes the exchange surfaces such as the lining of polychaete burrows. Refer to Figure 4 for a visual representation of the experimental setup.

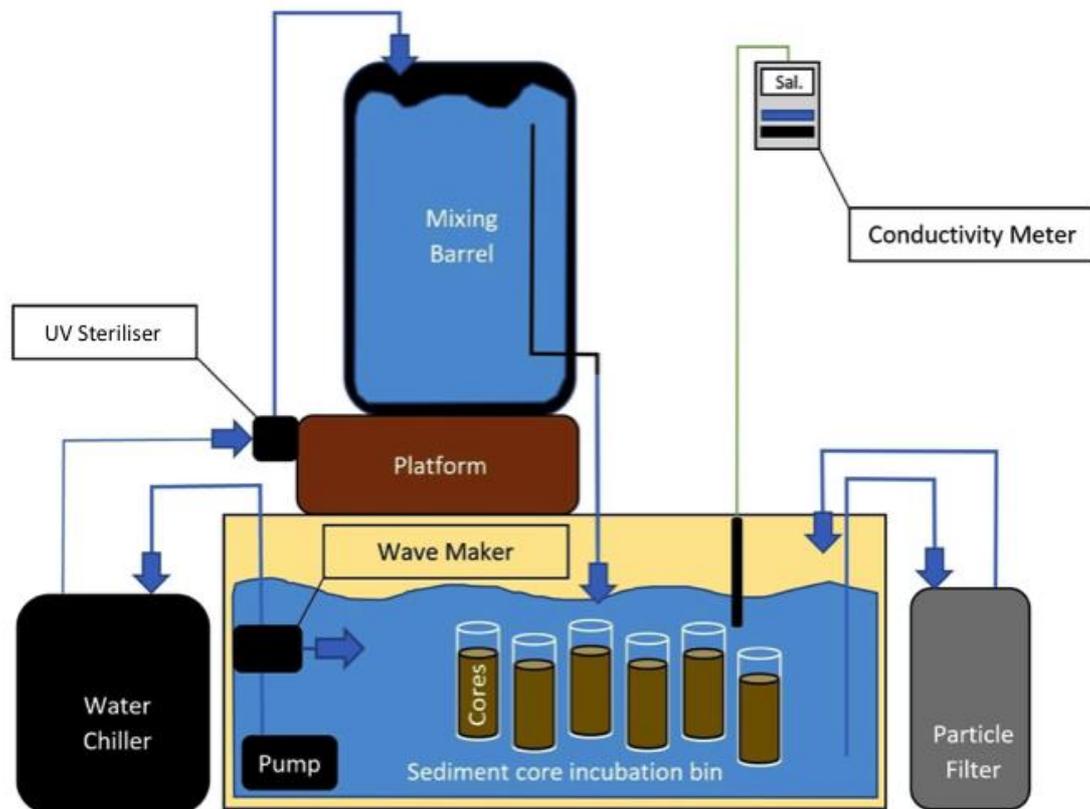


Figure 4.

Diagram representing the flow of natural seawater in the experimental units. Each unit circulated 560 L of seawater between a mixing barrel and an incubation bin. A pump within the incubation bins pushed seawater through an aquarium chiller and UV steriliser into the mixing barrels. Seawater returned via gravity from the mixing barrels, adding to the turbulence created by the particle filters and wave makers in the incubation bins. Seawater temperature and salinity in each unit were measured by a conductivity meter.

### Environmental control

The main seawater properties that I had to control were temperature, pH and salinity. I monitored seawater salinity on a daily basis with a portable Knick Portamess 913 meter, where target values were between 33.5 and 34.0. I added small amounts of reverse osmosis de-ionized water (PRF-RO, Pentair) to the experimental units, which counteracted evaporation. Temperature was maintained between 16 and 17 °C using an air conditioning unit (Super Wave, Fujitsu) set to 17 °C. This was operated in

conjunction with the aquarium chillers used for each experimental unit, which were set to 16 °C.

Seawater pH was recorded and controlled by injecting CO<sub>2</sub>-enriched air (20% carbon dioxide and 21% oxygen in nitrogen) into the mixing barrels. The system consisted of the following components: a DAQ-M instrument (Loligo Systems), a laptop (Lenovo) installed with CapCTRL software (version 1.3.0, Loligo Systems), a gas control set (including: solenoid valves, air stones, tubing, etc.), pH meters (pH 3310, WTW) and pH electrodes (SenTix HWD, WTW).

The pH electrodes were installed just below the surface of the seawater in the mixing barrels, and their readings were transmitted to the CapCTRL software. This software recorded all measurements and sent this information to the DAQ-M instrument (connected to the solenoid valves). The solenoid valves opened when seawater pH of the experimental units rose above their set points. This released CO<sub>2</sub>-enriched air from the bottom of the mixing barrels through air stones. The solenoid valves would shut off when the pH of the experimental units restored to their original set points. This cycle was continual because the experiment was an open re-circulating system, meaning that the seawater was in direct contact with the atmosphere. This caused the seawater to always lose CO<sub>2</sub> to the atmosphere because of their partial pressure differences. Refer to Figure 5 for a visual representation of the environmental control. Within each experimental unit, the difference in seawater pH between the incubation bins and mixing barrels were monitored closely because of the degassing effects. I used a portable pH meter to regularly measure seawater pH and temperature within the incubation bins. If these measurements were different to the target pH set points of each experimental unit, I adjusted the CapCTRL software to achieve valid seawater pH.

I performed a standard three-point calibration on the pH meters using the following pH buffer solutions: 4.01, 7.00 & 9.18. I then measured the pH of certified seawater reference material 'TRIS buffer (batch #26)' to determine the initial readings of the pH meters post calibration. I obtained the TRIS buffer from A. Dickson, Marine Physical Laboratory, Scripps Institution of Oceanography (Dickson et al. 2007d). I then used the "DelValls & Dickson pH calc" excel file

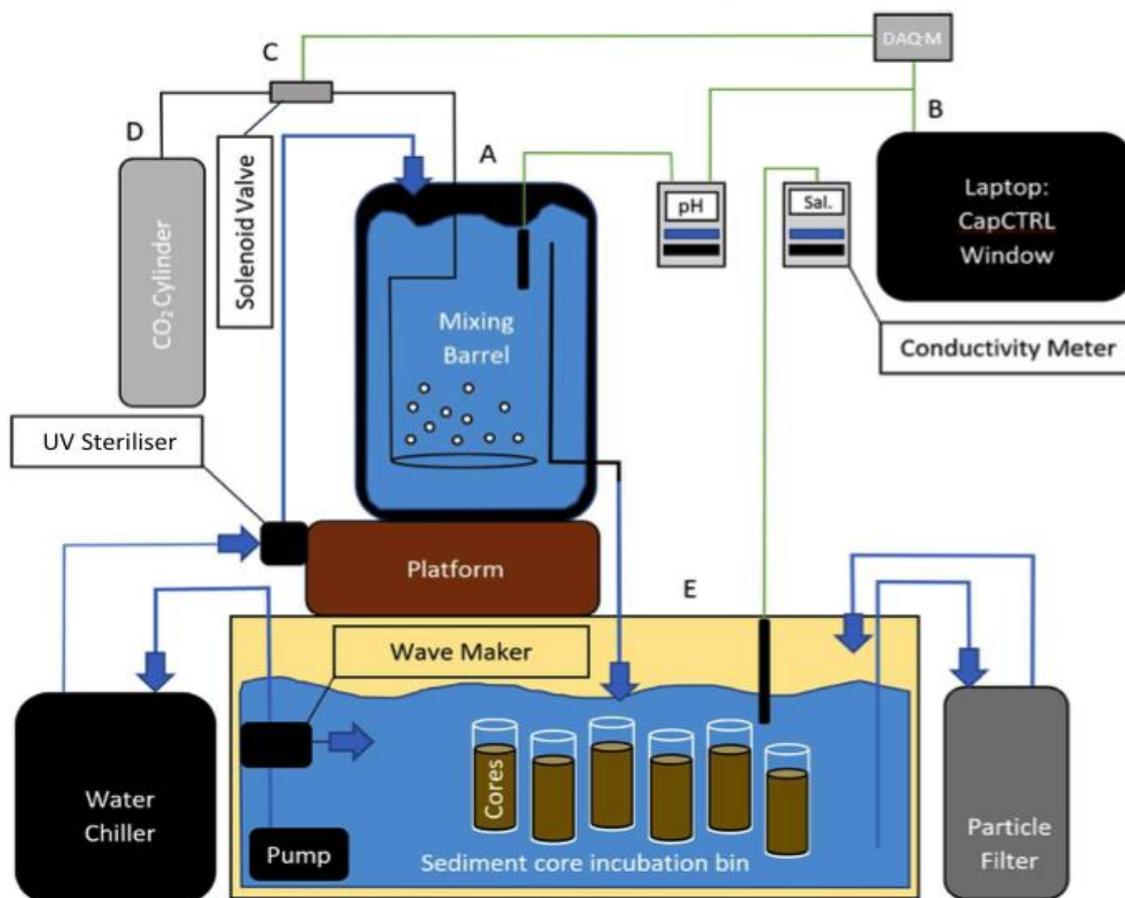


Figure 5.

Diagram representing the pH control system. (A) The electrode in the mixing barrels transmitted pH and temperature recordings to the CapCTRL software on the laptop. (B) CapCTRL sent signals to the DAQ-M instrument, (C) which regulated the solenoid valves. (D) The solenoid valves controlled gas flow from the CO<sub>2</sub> cylinders into the mixing barrels. The solenoid valves would shut off when the pH of the experimental units reached their target set points. System degassing restored pH overtime; therefore, the cycle would repeat itself if the pH increased beyond the target set points. (E) A conductivity meter measured seawater temperature and salinity.

(data available upon request) to determine the theoretical pH of TRIS buffer. I expected the measured pH to differ from the theoretical pH of TRIS buffer because seawater has greater ionic strength compared to pH buffer solutions (Riebesell et al. 2010). I calculated offset values for these differences and repeated it for each pH

electrode. These offset values were incorporated into the CapCTRL software, which finalised the calibration of the pH electrodes.

### **Sediment core incubations**

During incubations, sediment cores were sealed from their surrounding environment. To avoid seawater stagnation overlying the sediment, I incorporated a peristaltic pump to create mild advection. The incubation equipment consisted of four sediment core lids (each had a ball valve and two small tube fittings: inflow and outflow), a four-channel O<sub>2</sub> meter (Witrox-4 oxygen meter, Loligo systems) and a custom peristaltic pump unit. Fibre optic cabling connected the O<sub>2</sub> meter to optodes fitted into the tubing lines of the peristaltic pump unit, which were connected to the core lids via the inflow and outflow fittings. Seawater flowed passed the optodes during incubations, which allowed the O<sub>2</sub> meter to send signals through the fibre optic cabling every second to measure seawater O<sub>2</sub> concentrations.

I could only incubate four sediment cores per set. Therefore, I created a sampling strategy to work around this restriction. During each incubation period, I incubated EU 4 first, followed by EU 3, then EU 2 and finished with EU 1. The Control cores were incubated first, followed by the Bivalve cores and then I finished with the blank (sediment exclusive core). Before each incubation set commenced, I extracted and filtered (0.45 µm) an initial 20 mL seawater sample (T<sub>0</sub>) from the incubation bin of the EU and stored it at -20 °C. I then fitted and closed all the lids of the cores. I also started a new log file in the Witrox 4 software, which recorded real-time O<sub>2</sub> concentrations (µmol L<sup>-1</sup>). Before leaving the cores to incubate for approximately four hours, I recorded the initial O<sub>2</sub> concentrations of each core.

Before taking post-incubation seawater samples (T<sub>F</sub>), I recorded the final O<sub>2</sub> concentrations of each core and ended O<sub>2</sub> profiling. I opened valves one core at a time and carefully extracted 20 mL of seawater from the top of each core to avoid sediment disturbance. After all T<sub>F</sub> were extracted, filtered and stored, incubation lids were carefully removed from the cores to avoid sediment suspension.

## **Inorganic nitrogen flux analyses**

I submitted all seawater samples to be measured for  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  concentrations with a micro-segmented flow analyser (Astoria Pacific). This was conducted in the scientific analytical laboratory based out of the AUT Sciences building, Auckland City. These concentrations were used to calculate DIN fluxes.

## **Seawater and sediment analyses**

I took weekly seawater samples from each experimental unit for the determination of dissolved inorganic carbon (DIC) and total alkalinity (TA) concentrations. I extracted these samples in accordance to the instructions provided by SOP 1 (Dickson et al. 2007a). I carefully filled 1 L glass bottles (Schott Duran) with seawater from each experimental unit and left a 10 mL headspace, which provided a safe volume to add 200  $\mu\text{L}$  of saturated mercuric chloride ( $\text{MgCl}_2$ ). I then sealed the samples, inverted them to mix and stored them in darkness. At the conclusion of the experiment, I sent all the DIC and TA seawater samples to the Department of Chemistry at the University of Otago, Dunedin for analyses. The SOP 2 (Dickson et al. 2007b) analytical method was used for the determination of DIC by coulometry. A separate closed titration system operated under the SOP 3a procedure (Dickson et al. 2007c) was used for the determination of TA.

At the conclusion of the experiment, I did an initial sieve to each sediment core (0.25 mm) to capture any macrofauna in the sediment and preserved them in 10% formalin. Prior to analysing the macrofaunal abundance of the sediment, I sieved each sample again (same mesh size as the initial sieve) to further remove unwanted sediment and formalin. Macrofauna were counted and identified to the following taxonomic level: Polychaeta, Ostracoda, Malacostraca, Bivalvia, Ophiuroidea, Echinoidea. Furthermore, I measured the length of *T. lubrica* specimens found in each core. I used the counts of *T. lubrica* individuals within the Control cores to represent the natural abundance of this species in Man O' War Bay.

## ***T. lubrica* demographic measurements**

I measured the length (mm), wet weight (mg) and dry weight (mg) of 100 *T. lubrica* specimens in a separate sieve sample to the sediment analysis conducted post

experiment. Length was measured with callipers, taking the longest possible measurement from end to end, where length values were rounded to the nearest whole number. To measure wet weight, each specimen was dabbed dry with a hand towel removing superficial moisture and then weighed on an analytical balance scale. The dry weight was measured after dehydrating the specimens in an oven for 12 hours at 80 °C.

### Data analyses

With regard to the DIN fluxes, I calculated resultant concentrations ( $\mu\text{mol L}^{-1}$ ) of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  for the initial and final incubations. I then converted these concentrations into fluxes ( $\mu\text{mol m}^{-2} \text{h}^{-1}$ ) by factoring in the visible surface area of the sediment in each core, the distance between the sediment surface and bottom of the lid in each core, and the exact duration each core was incubated for. Furthermore, I used the difference between the initial and final fluxes to calculate delta DIN fluxes (delta DIN flux =  $\text{DIN flux}_{\text{final}} - \text{DIN flux}_{\text{init}}$ ). If a DIN flux was positive, this indicated the sediment release of DIN into the overlying seawater. A negative DIN flux indicated DIN uptake. Further, positive delta DIN fluxes indicated a decrease in uptake or an increase in release of DIN by the sediment. A negative delta DIN flux indicated an increase in uptake or a decrease in release of DIN by the sediment.

TOU was calculated similarly to DIN flux, except the resultant concentrations of  $\text{O}_2$  for the initial and final incubations were used instead. I used the difference between initial and final TOU to calculate delta TOU (delta TOU =  $\text{TOU}_{\text{final}} - \text{TOU}_{\text{init}}$ ). If TOU was positive, this indicated the sediment release of  $\text{O}_2$  into the overlying seawater. Negative TOU indicated sediment  $\text{O}_2$  uptake. Further, if delta TOU was positive, this indicated a decrease in uptake or an increase in release of  $\text{O}_2$  by the sediment. A negative delta TOU indicated an increase in uptake or a decrease in release of  $\text{O}_2$  by the sediment.

*T. lubrica* dry weight (mg) was calculated with a linear model I created from the demographic data (Fig. 9). In conjunction with the macrofaunal counts I performed at the conclusion of the experiment, I also measured the lengths of all the *T. lubrica* specimens in each core. I summed up all of these length measurements, which

provided a complete total length value of *T. lubrica* within each sediment core. I then input these values into the linear model, which gave an output of the total dry weight of *T. lubrica* found in each core. These dry weight values were important in the analysis because I used them to create delta TOU and DIN flux figures.

### **Statistical analyses**

I used RStudio to calculate the mean, mode and range for the length, wet weight and dry weight from the *T. lubrica* demographic data. I used the same software to create the linear model for calculating *T. lubrica* dry weight based off the demographic data I measured from the *T. lubrica* specimens I collected with a 0.5 mm sieve. I produced a scatter plot of dry weight as a function of length, which provided data points for modelling. I generated a line of best fit through the data points using a standard linear model function. I used the measured lengths of individual *T. lubrica* post experiment and input these values into the linear model equation to estimate their dry weight. Furthermore, I conducted a linear model summary in RStudio to report the significance of the slope and y-intercept of the linear model.

I calculated the averages of sediment TOU and DIN fluxes ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$ ) for the Control and Bivalve cores during initial seawater conditions (standard deviations were incorporated). I also conducted paired *t*-tests between the averages of the Control and Bivalve cores to detect if they were significantly different from each other. Prior to acidifying the seawater within the experimental units, it was important to establish if the Bivalve treatment had significant effects on the sediment exchange of  $\text{O}_2$  and DIN fluxes.

Further analyses were conducted during the initial seawater conditions to detect if significant TOU and/or DIN flux differences existed between the experimental units. I achieved this by using Tukey HSD (honest significant difference) tests, which determined if TOU and/or DIN fluxes were significantly higher or lower between experimental units. Separate tests were done for the Bivalve and Control cores. These tests were important because I wanted to investigate the chemical consistencies across the experimental units. If an experimental unit had significantly higher fluxes than other bins, I had to consider this during the analysis to be accurate.

I used modelling in RStudio to produce trends for the TOU and DIN flux data. Further, I used ANOVA tests to determine if the trends between two models were significantly different from each other. I conducted ANOVAs based on models having one response variable (i.e. delta DIN fluxes or delta TOU rates) and two predictor variables (i.e. pH and Bivalve treatments, or *T. lubrica* dry weight and pH treatments [7.4 and 8.0]). For the figures containing *T. lubrica* dry weight as a predictor variable, I excluded modelling pH treatments 7.6 and 7.8. This improved the visual clarity of figures and put the focus towards the extremes of the pH treatments I employed. Each ANOVA produced p-values for the two-predictor variables to detect their individual effects on the response variable and another p-value to determine an interaction between the two-predictor variables. Interaction p-values were used to detect if the trends between two models were significantly different from each other. Lastly, if any cores were contaminated with large macrofauna (e.g., *E. cordatum*, crabs, shrimps, etc.), the data from them were deemed to be outliers. Also, inaccurate solute flux data due to equipment error were removed from the analysis.

## Results

### Seawater temperature, salinity and carbonate chemistry

The average seawater temperature was  $16.9 \pm 0.2$  °C in EU 1 and  $16.7 \pm 0.2$ ,  $0.2$  &  $0.1$  °C in EU 2–4 with a minimum and maximum of  $16.0$  and  $17.1$  °C, respectively, across all experimental units (Fig. 6, Table 2). Each experimental unit evaporated approximately one to two liters of freshwater each day, which I replaced with ultrapure water to maintain seawater salinity within the range of  $33.0$  and  $34.2$  (Fig. 7, Table 2).

Seawater DIC and TA during initial conditions were  $2.0$  and  $2.2$  mmol kg SW<sup>-1</sup>, respectively, across all experimental units (Table 3). Initial seawater  $p\text{CO}_2$  ranged from  $435$  to  $476$   $\mu\text{atm}$ , which equated to all experimental units having a seawater  $\text{pH}_T$  of  $8.0$ .  $\text{CO}_2$  perturbation in seawater decreased the  $\text{pH}_T$  of EU 2–4 from  $8.0$  to  $7.9$ ,  $7.4$  and  $7.1$ , respectively (Table 3). These final seawater  $\text{pH}_T$  values were different from the target pH set points in the experimental design; I address this issue in the discussion.

Table 2.

Temperature (°C) and salinity of the natural seawater in experimental units (EU) 1–4. (A) EU 1, (B) EU 2, (C) EU 3 & (D) EU 4. SD, standard deviation.

EU	1	2	3	4
Temperature				
Mean $\pm$ SD	$16.9 \pm 0.2$	$16.7 \pm 0.2$	$16.7 \pm 0.2$	$16.7 \pm 0.2$
Maximum	17.1	17.0	17.0	17.0
Minimum	16.3	16.0	16.4	16.5
Salinity				
Mean $\pm$ SD	$33.8 \pm 0.2$	$33.7 \pm 0.2$	$33.7 \pm 0.2$	$33.6 \pm 0.2$
Maximum	34.2	34.1	34.2	34.0
Minimum	33.1	33.2	33.0	33.1

Table 3.

Initial/final seawater carbonate chemistry in experimental units (EU) 1–4. (A) EU 1, (B) EU 2, (C) EU 3 & (D) EU 4. DIC, dissolved inorganic carbon; TA, total alkalinity;  $p\text{CO}_2$ , partial pressure of  $\text{CO}_2$ .

EU		DIC (mmol kg SW <sup>-1</sup> )	TA	pH <sub>T</sub>	$p\text{CO}_2$ ( $\mu\text{atm}$ )
1	Initial	2.0	2.2	8.0	476
	Final	2.0	2.2	8.0	486
2	Initial	2.0	2.2	8.0	459
	Final	2.1	2.2	7.9	588
3	Initial	2.0	2.2	8.0	454
	Final	2.3	2.3	7.4	1852
4	Initial	2.0	2.2	8.0	435
	Final	2.4	2.3	7.1	4571

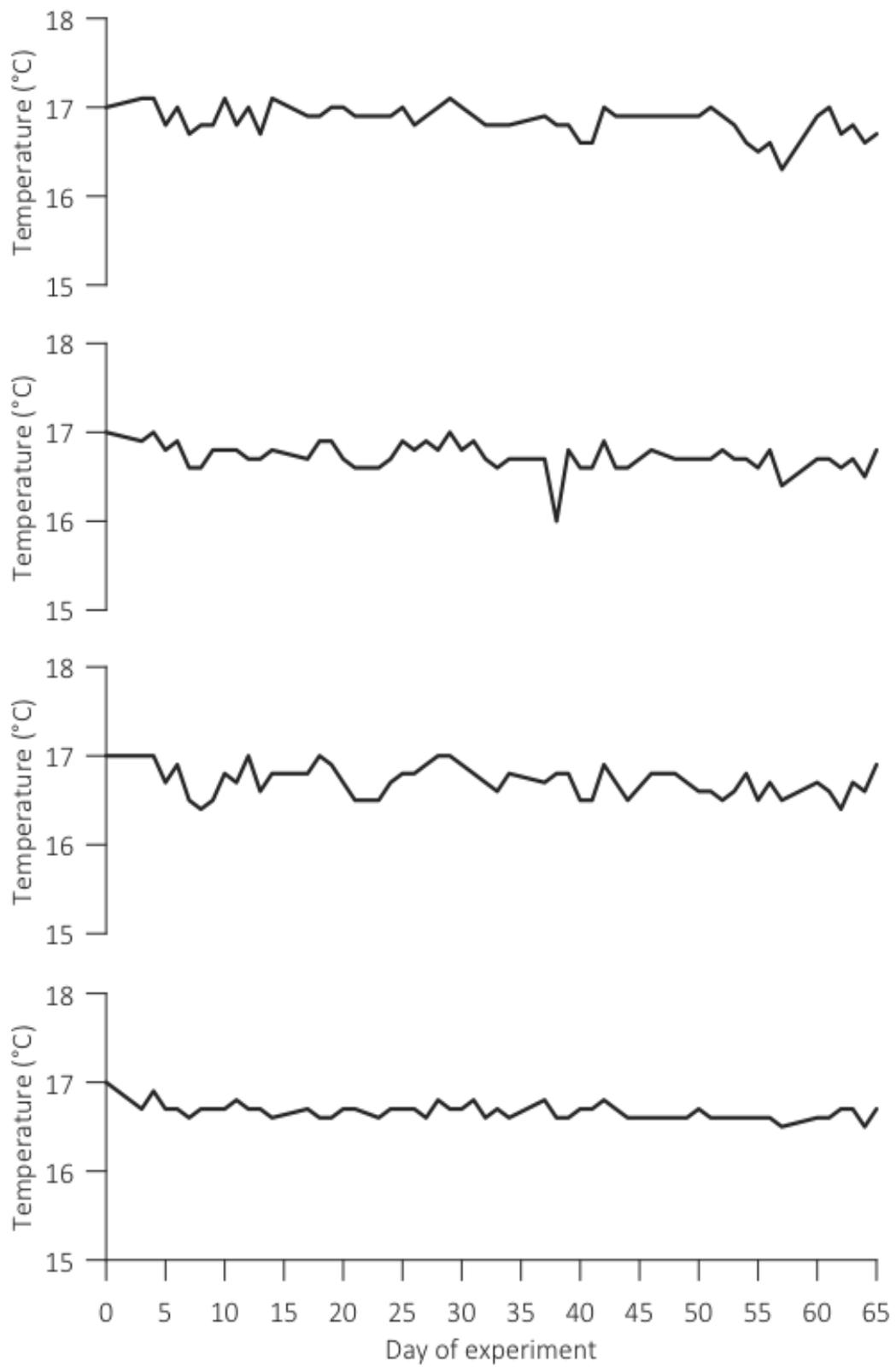


Figure 6.

Temperature of natural seawater in the experimental units (EU) 1–4. (A) EU 1, (B) EU 2, (C) EU 3 & (D) EU 4.

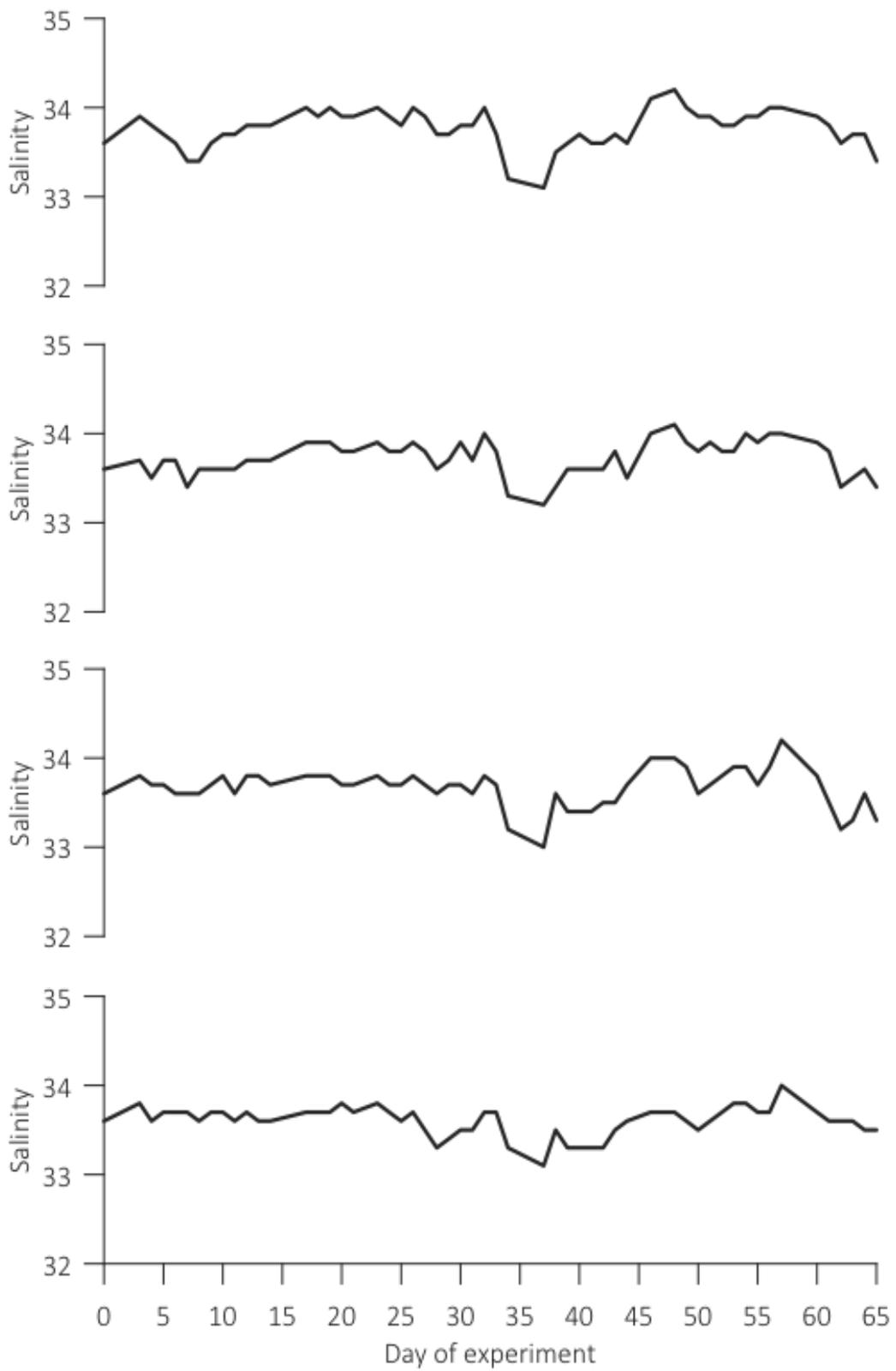


Figure 7.  
 Salinity of natural seawater in the experimental units (EU) 1–4. (A) EU 1, (B) EU 2, (C) EU 3 & (D) EU 4.

## Abundance of macrofauna in Man O' War Bay

I used the counts of macrofauna within the Control cores to represent the natural population of the study site within Man O' War Bay. 6 different classes of macrofauna were identified from the samples, where the abundance of macrofauna in each core ranged from zero to 18; the average macrofaunal abundance was  $10 \pm 5$  (Mean  $\pm$  SD,  $n = 16$ ) with a mode of 11. Across all of the control cores bivalves were the most abundant (125), followed by Malacostraca (18) and Polychaeta (10, data available upon request).

## Abundance and demographics of *Theora lubrica* in Man O' War Bay

On average, I found  $8 \pm 4$  *T. lubrica* individuals per Control core (Mean  $\pm$  SD,  $n = 16$ ), which translated to an average abundance of  $314 \pm 157$  ind.  $m^{-2}$  based of the visible sediment surface in the cores. Further, the abundance ranged from zero to 15 (Table 4). *T. lubrica* specimens in the separate sieve sample showed a normalized shell length distribution (Fig. 8A). Shell length ranged from 7 to 15 mm, where the average length was  $11.0 \pm 1.6$  mm (Mean  $\pm$  SD,  $n = 100$ ) with a mode of 12 mm (data available upon request).

Characteristics of a normalized distribution were also found in the dry weight data from the same sieve sample, and to a lesser extent in the wet weight data (Fig. 8B, C). The wet weight of *T. lubrica* specimens was found between 8 and 42 mg with an average and mode of  $26.0 \pm 8.4$  and 21 mg, respectively (data available upon request), and the dry weight was about 25% of their wet weight, ranging from 2 to 12 mg with an average of  $6.6 \pm 2.3$  mg and a mode of 7.0 mg (data available upon request).

I used the data from the same sieve sample to produce a linear fit of *T. lubrica* shell length as a function dry weight (Fig. 9). The trend shows that the individuals in the sample gained approximately 0.86 mg in dry weight per mm increase in length. The slope (t-value: 7.747, p-value:  $<0.0001$ ) and y-intercept: -2.8034 mg (t-value: -2.379, p-value:  $<0.05$ ) were both significant.

Table 4.

*Theora lubrica*. Abundance (Ab), shell length (SL, mm) and dry weight (DW, mg) in Control (CTR, no added *T. lubrica*) and Bivalve (BIV, added *T. lubrica*) sediment cores post experiment. EU, experimental unit; SD, standard deviation.

EU	Core ID	CTR			Core ID	BIV		
		Ab	SL	DW		Ab	SL	DW
1	52	10	6.3	2.6	47	12	6.9	3.1
	43	7	6.3	2.5	10	16	7.8	3.8
	7	7	5.7	2.1	30	17	8.1	4.1
	1	6	11.0	6.6	17	14	7.6	3.7
2	12	10	5.6	2.0	45	30	11.0	6.6
	15	10	5.6	2.0	9	19	7.6	3.7
	54	15	7.9	3.9	42	24	7.9	3.9
	20	7	5.7	2.1	35	12	7.1	3.2
3	16	1	5	1.4	5	-	-	-
	50	7	5.3	1.7	3	20	7.5	3.5
	40	0	-	-	44	18	6.9	3.1
	28	11	5.9	2.2	38	12	7.8	3.8
4	21	11	6.9	3.1	25	19	7.1	3.2
	2	11	5.1	1.5	14	25	7.3	3.4
	31	8	5.4	1.8	41	20	7.3	3.4
	6	4	6	2.3	8	11	7.4	3.5
Mean		8	6.3	2.6	-	18	7.8	3.9
SD	-	4	2.0	1.8	-	5	2.2	1.9
Max	-	15	11	6.6	-	30	11	6.6
Min	-	0	5	1.4	-	11	6.9	3.1

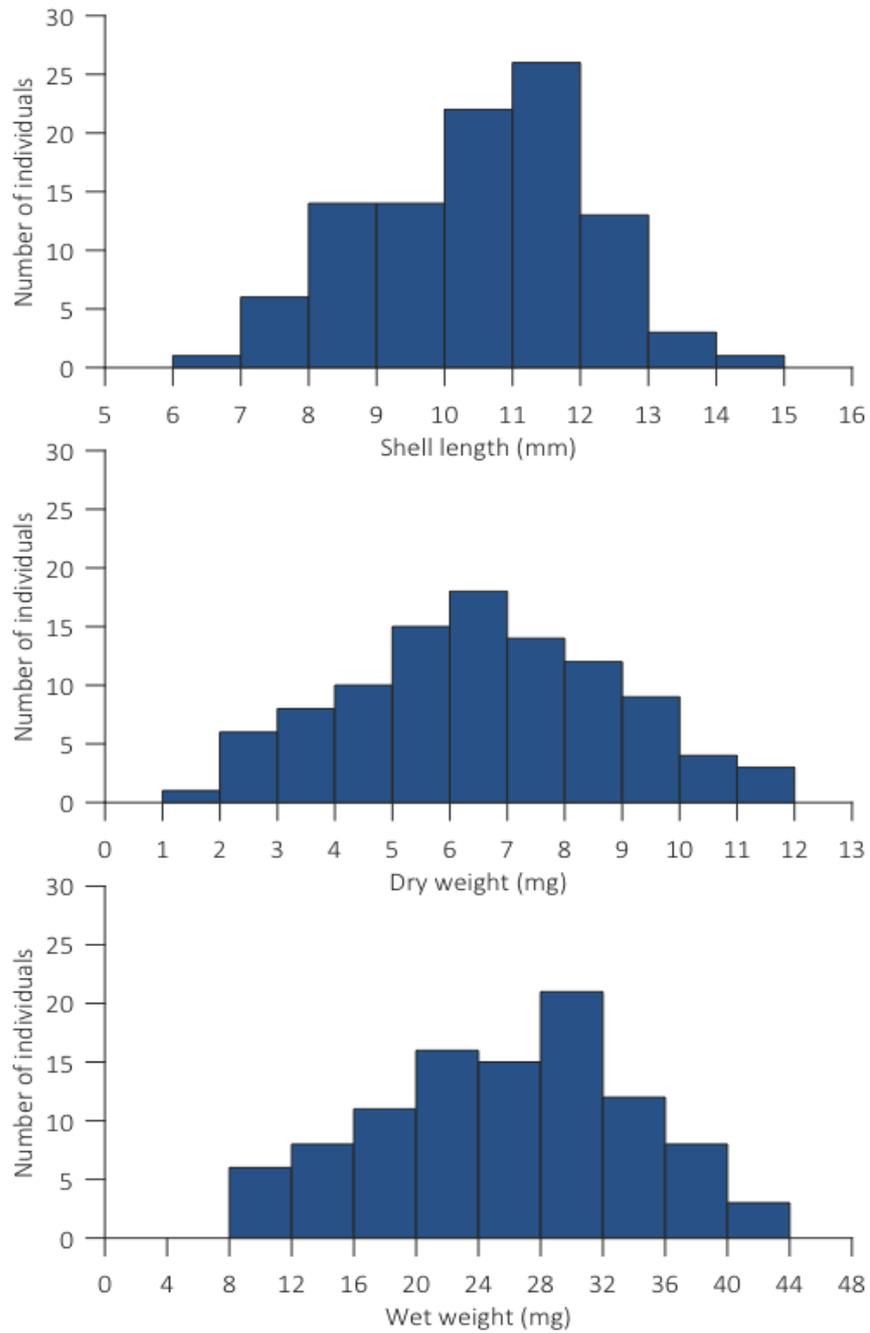


Figure 8.

*Theora lubrica*. Distributions of (A) shell length, (B) dry weight and (C) wet weight from 100 individuals found in sieved samples (0.5 mm mesh size) from the Man O'War Bay population, Waiheke Island.

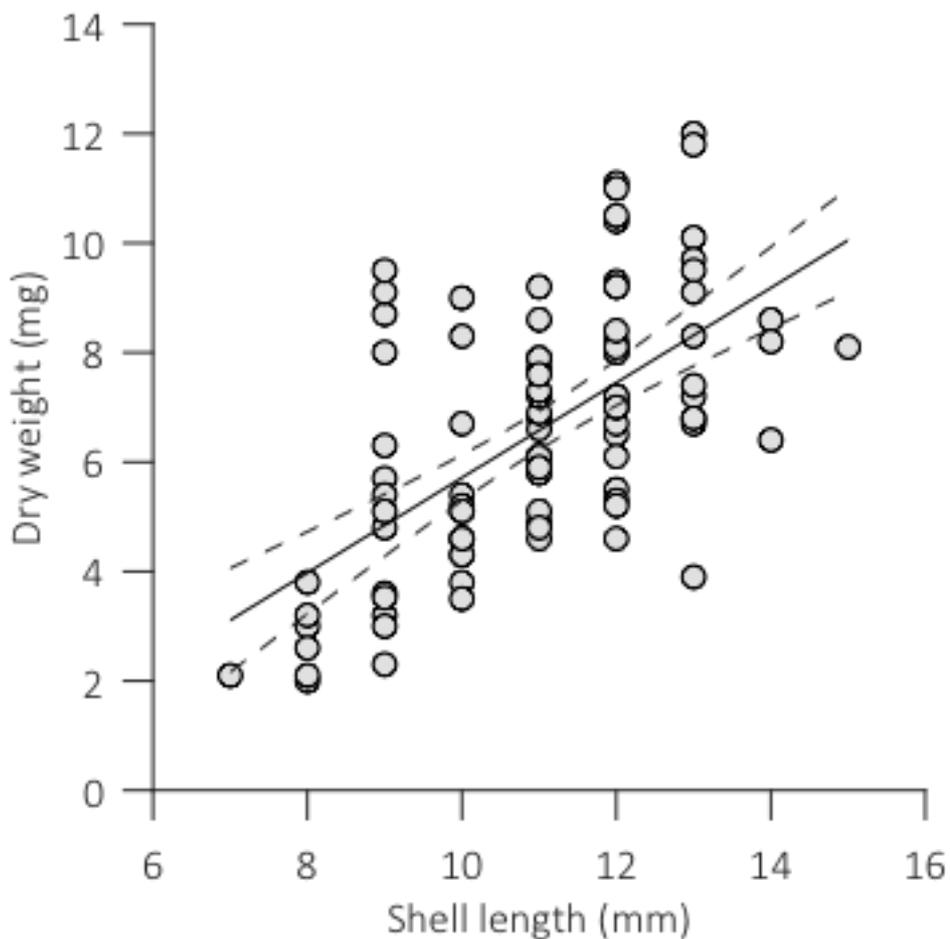


Figure 9.

*Theora lubrica*. Dry weight as a function of shell length from 100 individuals found in sieved samples (0.5 mm mesh size) from the Man O'War Bay population, Waiheke Island. The solid line indicates the linear fit of the data ( $y = 0.8559x - 2.8034$ ,  $r^2 = 0.3708$ ).

### Abundance of *Theora lubrica* within the Bivalve cores

The addition of 10 *T. lubrica* individuals to an intact sediment core made up a Bivalve core. Inspection of the cores post experiment revealed, that this addition on average just over doubled the natural average abundance of *T. lubrica*  $18 \pm 5$  (Mean  $\pm$  SD,  $n = 15$ , Table 4). The abundance of *T. lubrica* in the Bivalve cores ranged from 11 to 30. Core 5 was excluded from the analysis because zero *T. lubrica* were counted. If a successful Bivalve treatment occurred, then the lowest *T. lubrica* count should have

been 10 in any Bivalve core by the end of the experiment. Excluding data from core 5 will be explained in the discussion.

### Initial sediment O<sub>2</sub> consumption and inorganic nitrogen flux

I found that TOU was significantly higher in EU 1 than in EU 3 for the Bivalve cores (Fig. 10A, Table 5). On average, the Control cores removed significantly less O<sub>2</sub> from their overlying seawater (TOU =  $-401 \pm 60 \mu\text{mol m}^{-2} \text{h}^{-1}$ ; Mean  $\pm$  SD, n = 12) than the Bivalve cores (TOU =  $-591 \pm 78 \mu\text{mol m}^{-2} \text{h}^{-1}$ ; Mean  $\pm$  SD, n = 9, Fig. 10A, Table 5).

On average, all of the DIN flux data for the Control and Bivalve cores indicated a release of NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> into the overlying seawater by the sediment (positive average flux). The Control cores had a significantly lower average release of NH<sub>4</sub><sup>+</sup> ( $0.11 \pm 0.42 \mu\text{mol m}^{-2} \text{h}^{-1}$ ) compared to the Bivalve cores ( $0.86 \pm 1.28 \mu\text{mol m}^{-2} \text{h}^{-1}$ , Fig. 10B, Table 5). The average fluxes of NO<sub>2</sub><sup>-</sup> from the Control and Bivalve cores did not significantly differ,  $0.15 \pm 0.04$  and  $0.12 \pm 0.03 \mu\text{mol m}^{-2} \text{h}^{-1}$ , respectively (Fig. 10C, Table 5). Similar to the NH<sub>4</sub><sup>+</sup> flux results, the average release of NO<sub>3</sub><sup>-</sup> into the overlying seawater by the Control cores ( $0.45 \pm 0.48 \mu\text{mol m}^{-2} \text{h}^{-1}$ ) was significantly less than the Bivalve cores ( $1.04 \pm 0.96 \mu\text{mol m}^{-2} \text{h}^{-1}$ , Fig. 10D, Table 5).

Table 5.

Statistical analyses for the initial solute fluxes (TOU; total oxygen uptake, NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> & NO<sub>3</sub><sup>-</sup>) by the sediment cores at in situ pCO<sub>2</sub> (partial pressure of CO<sub>2</sub>). Data from the Control (CTR, no added *Theora lubrica*) and Bivalve (BIV, added *T. lubrica*) cores are displayed. ANOVA, *t*-test, Tukey HSD test. RV, response variable; EU, experimental unit; TR, treatment; Int, interaction; NS, not significant.

RV	EU	TR	Int	<i>t</i> -test or Tukey HSD test
TOU	NS	<0.0001	<0.05	CTR < BIV; BIV (EU 1 > 3)
NH <sub>4</sub> <sup>+</sup>	<0.0001	<0.01	<0.01	CTR < BIV; BIV (EU 1 > 3 & 4)
NO <sub>2</sub> <sup>-</sup>	NS	NS	NS	
NO <sub>3</sub> <sup>-</sup>	<0.001	<0.01	<0.001	CTR < BIV; CTR (EU 3 > 2), BIV (EU 1 > 3 & 4)

Significant differences between experimental units were detected in the DIN fluxes of NH<sub>4</sub><sup>+</sup> (Bivalve cores only) and NO<sub>3</sub><sup>-</sup> (Control and Bivalve cores, Table 5). For NH<sub>4</sub><sup>+</sup>, the

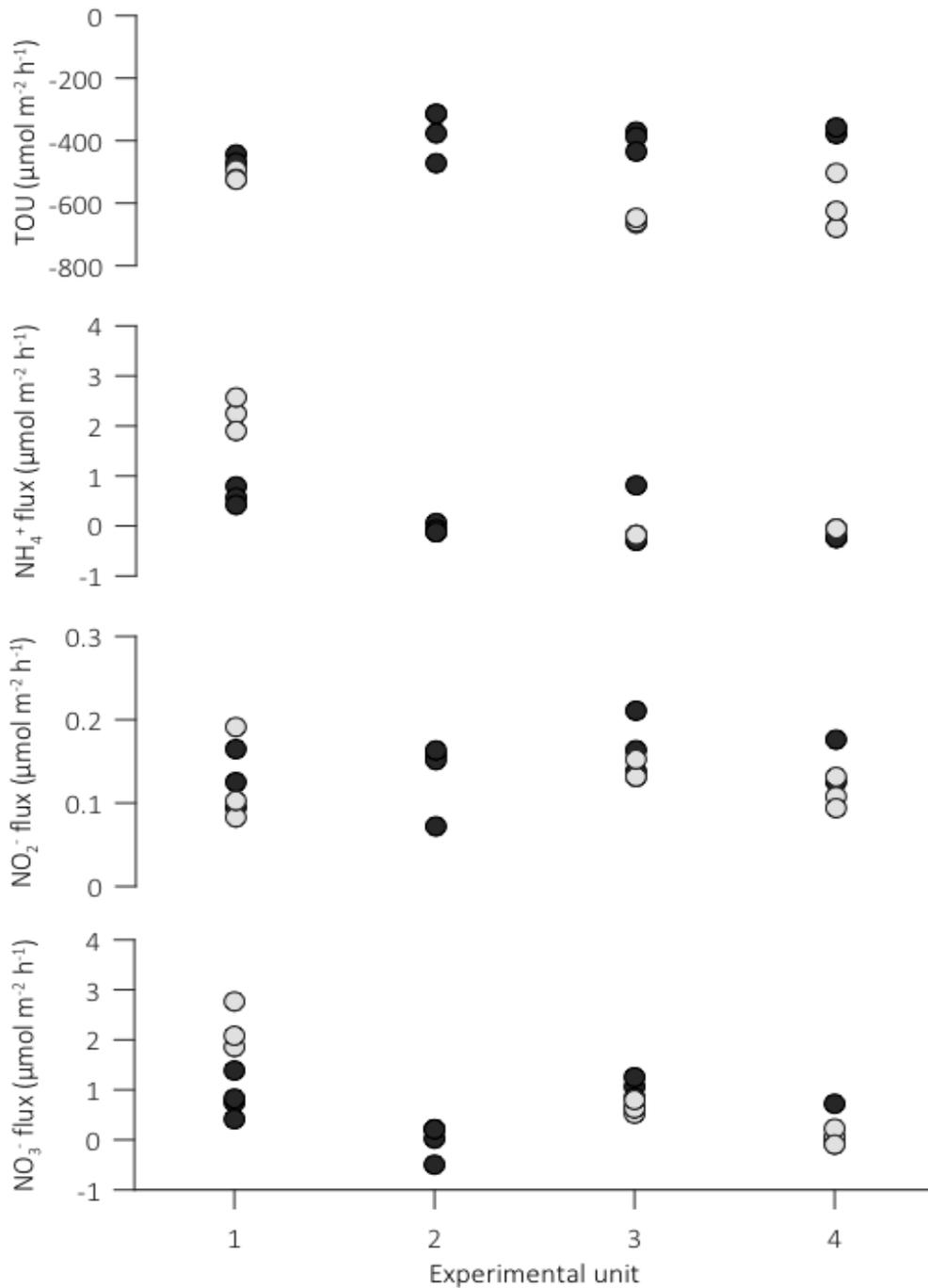


Figure 10.

Initial solute fluxes by the sediment cores without (Control treatment, filled circles) and with added individuals of the bivalve *Theora lubrica* (Bivalve treatment, open circles). (A) TOU (total oxygen uptake), (B)  $\text{NH}_4^+$ , (C)  $\text{NO}_2^-$  & (D)  $\text{NO}_3^-$ . Four cores were incubated per treatment in four experimental units at in situ  $p\text{CO}_2$  (partial pressure of  $\text{CO}_2$ ). Positive and negative fluxes indicate sediment solute release and uptake, respectively.

flux measured in the Bivalve cores from EU 1 were significantly greater than in EU 3 & 4, which were similar (Fig. 10B, Table 5). The experimental unit effect was highest in the flux of  $\text{NO}_3^-$ ; the flux in the Control cores were significantly greater in EU 3 than in EU 2, and for the Bivalve cores the flux in EU 1 was significantly greater than in EU 3 & 4, which were similar (Fig. 10D, Table 5).

I determined that 11 of the sediment cores produced solute flux outliers during the initial measurements because they were contaminated by large macrofauna or equipment error caused inaccurate values. The outliers were produced by sediment cores from EU 1 (one each from the Control and Bivalve treatment rows), EU 2 (all the Bivalve cores), EU 3 (one each from the Control and Bivalve treatment rows) and EU 4 (one from the Control and two from the Bivalve treatment rows); these cores were removed from the solute flux analysis, but were counted for macrofauna (data available upon request).

### **Effect of seawater pH on sediment TOU**

I represented the solute flux data for the final conditions of the sediment by using the differences between initial and final flux ( $\text{delta solute flux} = \text{solute flux}_{\text{final}} - \text{solute flux}_{\text{init}}$ ). This was more appropriate than directly comparing the absolute solute flux data between the initial and final incubations because the experimental unit effects detected in initial TOU, and  $\text{NH}_4^+$  and  $\text{NO}_3^-$  fluxes would have caused statistical bias (Fig. 10A, B & D, Table 5).

Regarding sediment  $\text{O}_2$  consumption during the final conditions of the sediment, both the Control and Bivalve cores produced similar positive delta TOU values within EU 1, which was free of  $\text{CO}_2$  perturbation (pH 8.0, Fig. 11A & 13). This indicated, that the TOU by the Control and Bivalve cores increased over the course of the experiment; therefore, a decrease in sediment  $\text{O}_2$  consumption.

Lower seawater pH in EU 2–4 caused the Control cores to produce negative delta TOU (filled symbols in Fig. 11A). That is, the TOU of the Control cores in the pH treatment units decreased over the course of the experiment. By contrast, delta TOU of the Bivalve cores increased with decreasing pH.

ANOVA supported the difference in delta TOU trends between the Control and Bivalve cores because a significant interaction was detected between the pH and Bivalve treatments. Furthermore, I found that the effects of the Bivalve treatment alone on delta TOU was significant, but not significant for the pH treatment (Table 6).

### Effect of seawater pH on sediment inorganic nitrogen flux

The negative delta DIN fluxes of  $\text{NH}_4^+$  (Bivalve cores) and  $\text{NO}_2^-$  (Control and Bivalve cores) produced in EU 1 indicated, that the initial release of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  by the sediment into the overlying seawater decreased over the course of the experiment (Fig. 11B, C & 13). Figures 11B & C indicate that decreased seawater pH increased delta  $\text{NH}_4^+$  and  $\text{NO}_2^-$  fluxes in both core types (Control and Bivalve). That is, the initial release of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  by the sediment into the overlying seawater increased over the course of the experiment with decreasing seawater pH.

The trends produced by the Control and Bivalve cores for the delta  $\text{NH}_4^+$  and  $\text{NO}_2^-$  fluxes were similar, which was supported by ANOVA with a non-significant interaction between pH and Bivalve treatments. The independent effects of pH on delta  $\text{NH}_4^+$  and  $\text{NO}_2^-$  fluxes were significant, but the effect of Bivalve treatment alone was not significant (Table 6).

Table 6.

Statistical analyses for the difference between initial/final solute fluxes (delta: TOU; total oxygen uptake,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  &  $\text{NO}_3^-$ ) of the Control (CTR, no added *Theora lubrica*) and Bivalve (BIV, added *T. lubrica*) sediment cores as a function of the pH treatments. ANOVA. RV, response variable; TR, treatment (BIV); Int, interaction; NS, not significant.

RV	TR	p-value		Adjusted $R^2$ (trends)	
		pH	Int	BIV	CTR
Delta TOU	<0.001	NS	<0.01	0.32	0.49
Delta $\text{NH}_4^+$	NS	<0.0001	NS	0.91	0.66
Delta $\text{NO}_2^-$	NS	<0.01	NS	0.46	0.53
Delta $\text{NO}_3^-$	NS	NS	NS	0.57	0.01

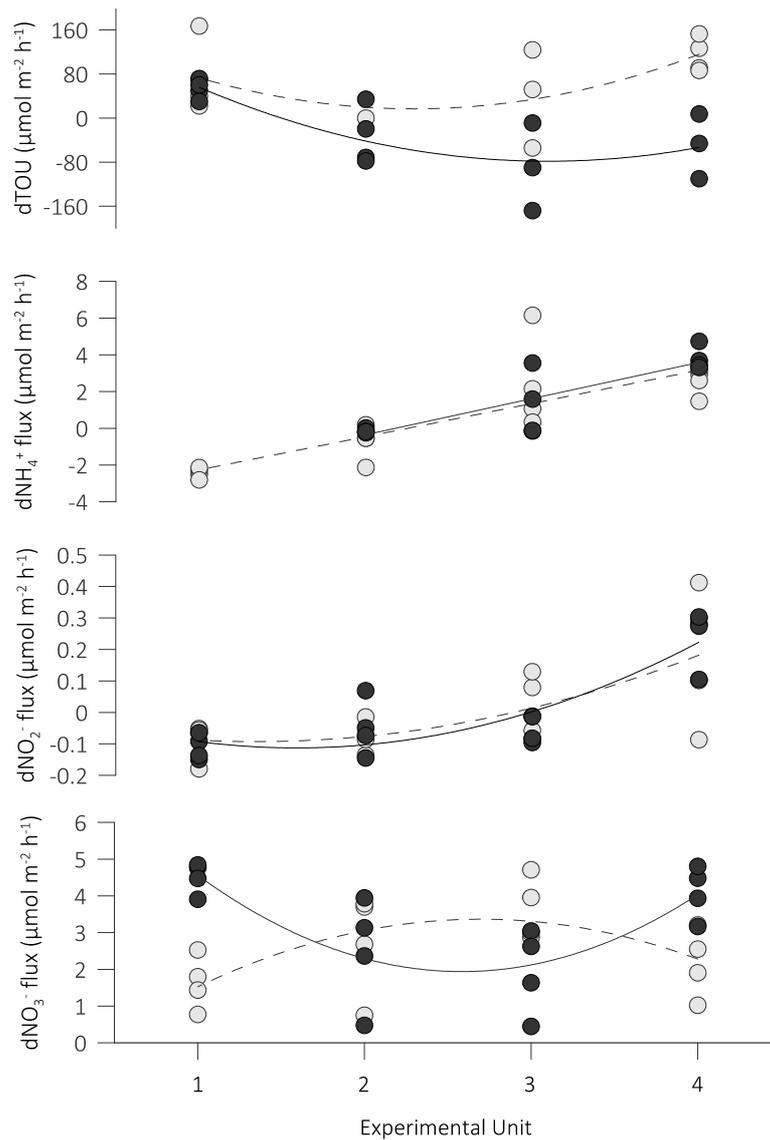


Figure 11.

Difference between initial/final solute fluxes by the sediment cores as a function of the pH treatments. Delta: (A) TOU (total oxygen uptake), (B) NH<sub>4</sub><sup>+</sup>, (C) NO<sub>2</sub><sup>-</sup> & (D) NO<sub>3</sub><sup>-</sup>. Data from the Control (no added *Theora lubrica*, filled circles) and Bivalve (added *T. lubrica*, open circles) cores are displayed. Solid and dashed lines indicate the linear fits for the Control and Bivalve treatments, respectively. Four cores were incubated per treatment in four experimental units at pH: 8.0 (EU 1), 7.9 (EU 2), 7.4 (EU 3) and 7.1 (EU 4). Positive delta fluxes indicate a decrease in sediment solute uptake or an increase in solute release, and negative delta fluxes indicate an increase in sediment solute uptake or a decrease in solute release.

Delta  $\text{NO}_3^-$  fluxes produced by the Control and Bivalve cores were positive in EU 1, indicating that the sediment release of  $\text{NO}_3^-$  into the overlying seawater increased over the course of the experiment (Fig. 11D & 13). This was opposite to the delta  $\text{NH}_4^+$  and  $\text{NO}_2^-$  fluxes, which were negative. *t*-test indicated, that the delta  $\text{NO}_3^-$  fluxes in EU 1 from the Control cores, were significantly greater than the Bivalve cores (<0.001). ANOVA detected no significant effects of pH and Bivalve treatments on the difference between initial/final  $\text{NO}_3^-$  flux between the Control and Bivalve cores. Furthermore, there was no significant interaction between the pH and Bivalve treatments. No obvious trends were detected for the delta  $\text{NO}_3^-$  fluxes as a function of pH (Fig. 11D, Table 6).

### **The effect of *Theora lubrica* dry weight on delta solute flux**

I presented the difference between initial and final solute flux as a function of *T. lubrica* dry weight because I wanted to detect any effect of *T. lubrica* dry weight and pH on delta solute fluxes. I excluded pH treatments 7.9 and 7.4 from the data that produced Figure 12 to reduce cluttering within the graphs, which left the two opposing pH treatments of 8.0 (EU 1) and 7.1 (EU 4) to compare. I excluded the data for initial solute flux as a function of *T. lubrica* dry weight because my main focus was to support the results for the final conditions of the sediment.

Regarding delta TOU as a function of *T. lubrica* dry weight, ANOVA indicated a significant interaction between *T. lubrica* dry weight and the pH treatments (Table 7). The independent effects of *T. lubrica* dry weight on delta TOU were significant, but not significant for pH. The trend produced under pH 8.0 conditions indicated, that increasing *T. lubrica* dry weight had no effect on delta TOU. However, the pH 7.1 trend shows that increasing *T. lubrica* dry weight caused an increase in delta TOU (Fig. 12A).

The dry weight of *T. lubrica* had no significant effects on the difference between the initial/final fluxes of  $\text{NH}_4^+$ . ANOVA indicated, a non-significant interaction between *T. lubrica* dry weight and pH, however, the independent effects of pH on delta  $\text{NH}_4^+$  flux was significant. Also, the trends for the pH 8.0 and 7.1 treatments were similar, where delta  $\text{NH}_4^+$  fluxes weren't a function of *T. lubrica* dry weight (Fig. 12B, Table 7).

Table 7.

Statistical analyses for the difference between initial/final solute fluxes (delta: TOU; total oxygen uptake,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  &  $\text{NO}_3^-$ ) of the sediment cores as a function of *Theora lubrica* dry weight (mg). Data from the pH treatments: 8.0 and 7.1 are displayed. ANOVA. RV, response variable; Int, interaction; NS, not significant.

RV	p-value (treatment)			Adjusted $R^2$ (trends)	
	<i>T. lubrica</i> dry weight	pH	Int	pH 8.0	pH 7.1
Delta TOU	<0.05	NS	<0.05	0.19	0.81
Delta $\text{NH}_4^+$	NS	<0.0001	NS	0.92	0.05
Delta $\text{NO}_2^-$	NS	<0.01	NS	0.04	0.08
Delta $\text{NO}_3^-$	NS	NS	NS	0.64	0.09

Similar to the initial/final difference in  $\text{NH}_4^+$  flux, ANOVA indicated, a non-significant interaction between *T. lubrica* dry weight and pH within the delta  $\text{NO}_2^-$  flux data (Table 7). The independent effects of pH on delta  $\text{NO}_2^-$  flux were significant, but not significant for *T. lubrica* dry weight. The trends for pH treatments 8.0 and 7.1 were similar. Just like the delta  $\text{NH}_4^+$  flux data, delta  $\text{NO}_2^-$  flux wasn't a function of *T. lubrica* dry weight (Fig. 12C, Table 7).

Lastly, no significant interactions were detected between *T. lubrica* dry weight and pH for the difference between initial/final  $\text{NO}_3^-$  flux. ANOVA also indicated, no significance in the independent effects of *T. lubrica* dry weight and pH on delta  $\text{NO}_3^-$  flux. This contrasted to the ANOVA results for the delta  $\text{NH}_4^+$  and  $\text{NO}_2^-$  fluxes because the independent effects of pH were significant for both of those delta DIN fluxes. The trends for pH treatments 8.0 and 7.1 were similar, where delta  $\text{NO}_3^-$  fluxes weren't a function of *T. lubrica* dry weight (Fig. 12D, Table 7).

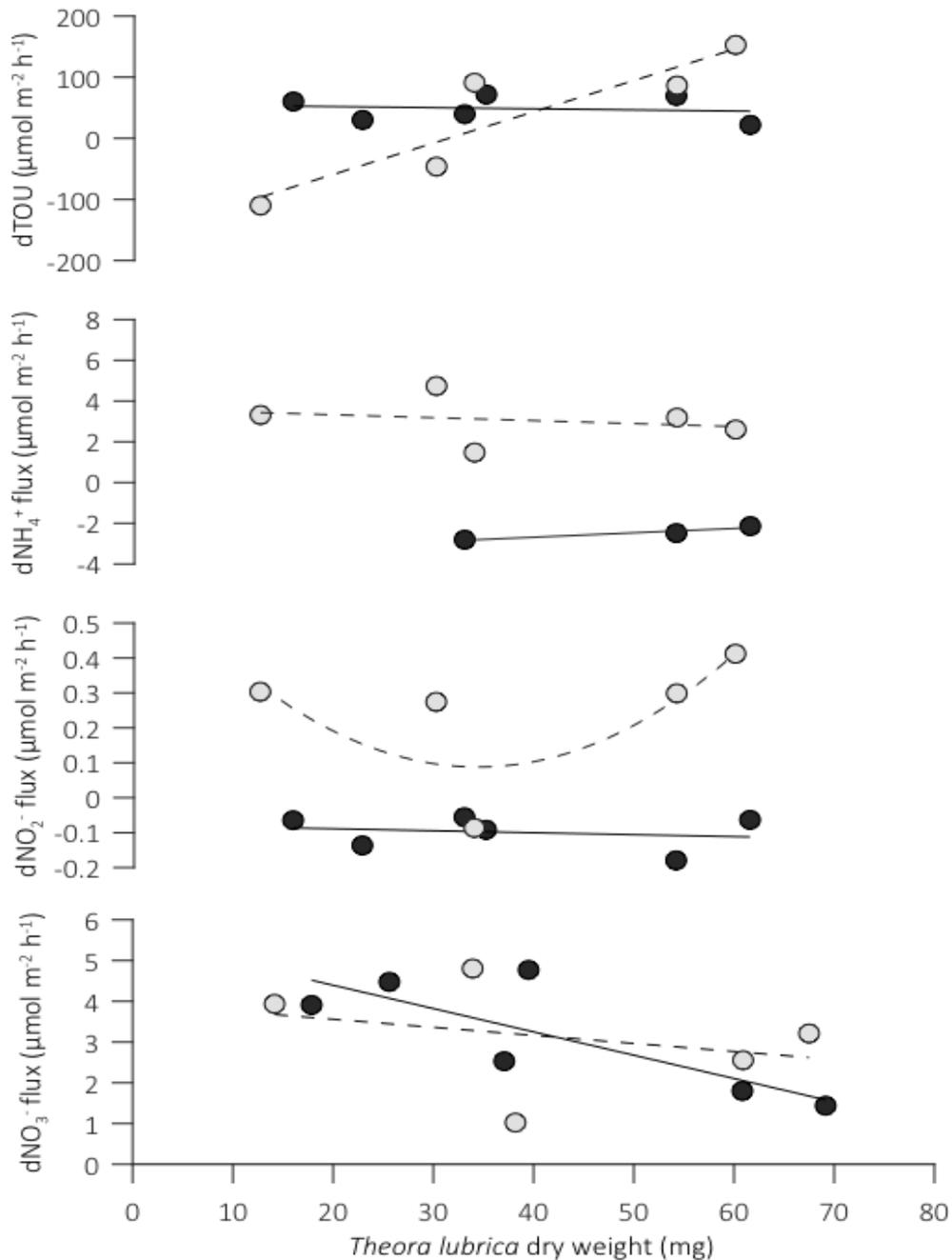


Figure 12.

Difference between initial/final solute fluxes by the sediment cores as a function of *Theora lubrica* dry weight. Delta: (A) TOU (total oxygen uptake), (B) NH<sub>4</sub><sup>+</sup>, (C) NO<sub>2</sub><sup>-</sup> & (D) NO<sub>3</sub><sup>-</sup>. Data are displayed from two pH treatments: 8.0 (filled circles) and 7.1 (Open circles). Solid and dashed lines indicate the linear fits for the pH treatments 8.0 and 7.1, respectively. Positive delta fluxes indicate a decrease in sediment solute uptake or an increase in solute release, and negative delta fluxes indicate an increase in sediment solute uptake or a decrease in solute release.

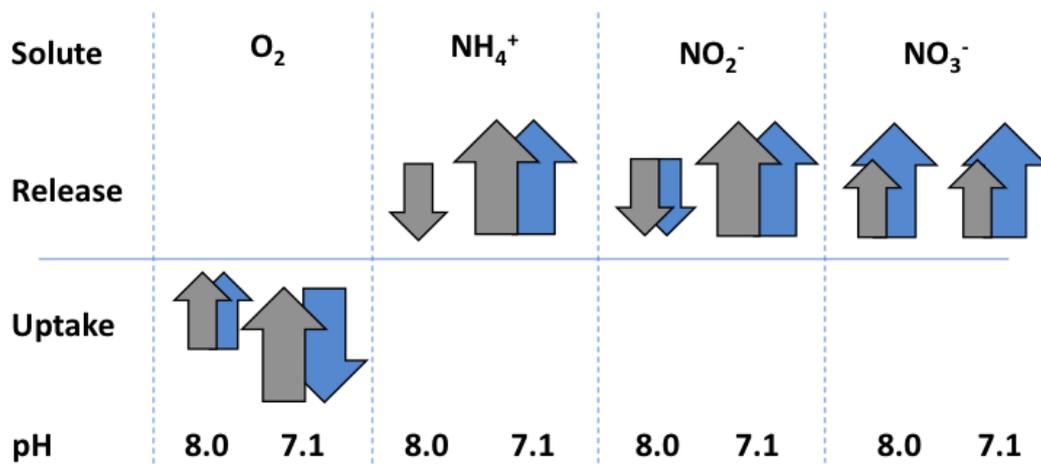


Figure 13.

Relative change in sediment – seawater solute fluxes (delta:  $O_2$ ,  $NH_4^+$ ,  $NO_2^-$  &  $NO_3^-$ ) of the Control (blue arrows, no added *Theora lubrica*) and Bivalve (grey arrows, added *T. lubrica*) sediment cores as a function of the pH treatments. Arrows pointing up or down indicate positive or negative delta solute fluxes, respectively. Arrows below the horizontal line indicate the relative change in sediment solute uptake and arrows above the line indicate the relative change in sediment solute release.

All raw data is available upon request

## Discussion

### Effect of *T. lubrica* on coastal soft sediment nitrogen cycling

I detected significant increases in  $\text{NH}_4^+$  and  $\text{NO}_3^-$  effluxes by the Bivalve cores during in situ  $p\text{CO}_2$  conditions. The addition of *T. lubrica* to intact sediment cores had no significant effects on  $\text{NO}_2^-$  fluxes during the same conditions (Fig. 10B, C & D, Table 5).

I can reject  $H_0$  because the addition of *T. lubrica* to sediment cores significantly stimulated  $\text{NH}_4^+$  and  $\text{NO}_3^-$  fluxes by the sediment during the initial measurements.

I suggest that the significant increase in  $\text{NH}_4^+$  and  $\text{NO}_3^-$  effluxes within the Bivalve cores during the initial measurements was caused by the stimulation of benthic ammonification and nitrification, respectively. It has been established, that macrofauna can enhance ammonification via bioturbation and/or directly through their excretions (Andersen 1991). I consider *T. lubrica* to be a mild bioturbator because their activity within the sediment appears to be minimal (anecdotal: observation during the experiment). I hypothesize that the significantly greater  $\text{NH}_4^+$  effluxes within the Bivalve cores during the initial measurements were caused more by the waste production from the addition of *T. lubrica*, compared to their contributions via bioturbation. Deposit-feeding bivalves such as *T. lubrica* excrete waste in the form of faeces and pseudofaeces (Shumway et al. 1985), which fuel ammonification.

My hypothesis for explaining the significant increase in  $\text{NH}_4^+$  effluxes within the Bivalve cores from the current study is supported by Yamada & Kayama's (1987) experiment. They found that the addition of *Theora lata* (small infaunal bivalve) to undisturbed sediment microcosms at in situ  $p\text{CO}_2$  conditions caused significant  $\text{NH}_4^+$  effluxes. The excretions of *T. lata* were proven to have a greater effect on  $\text{NH}_4^+$  porewater production compared to their bioturbation component; therefore, the excretions of *T. lata* would have provided food for ammonification. Some of the  $\text{NH}_4^+$  effluxes produced by the microcosms treated with *T. lata* were estimated to be 7 times greater than that of the control microcosms with no additional *T. lata*.

Yamada & Kayama (1987) found, that the addition of *T. lata* to undisturbed sediment microcosms had no effect on  $\text{NO}_3^-$  fluxes, which didn't support the results to that of

*T. lubrica* from the current study. A concept established by Laverock et al. (2011) described that the effects which macrofauna have on sediment biogeochemistry is predominantly a function of species morphology and how they behave within sediments. Therefore, I expected *T. lubrica* and *T. lata* to have a similar effect on  $\text{NO}_3^-$  fluxes because they have the following parallels: genus, morphology, shallow sediment position, sedentary lifestyle and deposit feeding mode (Kutkuchi 1981, Yokoyama & Ishihi 2003). Yamada & Kayama's (1987) study was the most relevant because *T. lata* occupies a very similar niche to *T. lubrica*.

Similar to *T. lubrica* in the current study, Michaud et al. (2006) found that the addition of *Macoma balthica* (clam) to sediment microcosms at in situ  $p\text{CO}_2$  conditions significantly increased  $\text{NH}_4^+$  and  $\text{NO}_3^-$  effluxes. They elaborated that *M. balthica* resides close to the sediment surface in the nitrification zone, where relative concentrations of  $\text{NO}_3^-$  are higher there than in the seawater directly overlying the sediment. The presence of *M. balthica* in the nitrification zone stimulates the degradation of OM into  $\text{NH}_4^+$  and the oxidation of  $\text{NH}_3$  to  $\text{NO}_3^-$ . Therefore, significant  $\text{NO}_3^-$  effluxes occur when *M. balthica* flushes out its burrow, as well as causing the sediment release of  $\text{NH}_4^+$ . *T. lubrica* buries itself near the sediment surface like *M. balthica*, and therefore, would also reside in the nitrification zone. I suggest that *T. lubrica* uses similar flushing mechanisms to *M. balthica* within the same sediment zone, which could explain why they cause similar patterns in DIN fluxes when added to sediment microcosms. *M. balthica* grow a bit longer (max length: 22–25 mm) than *T. lubrica*, but are still classified as a small infaunal bivalve (Gilbert 1973). In light of this, the effects of *M. balthica* on sediment biogeochemistry are still relevant to that of *T. lubrica* from the current study.

A previous experiment using *Mya arenaria* (larger infaunal clam) supports the significant increase in  $\text{NH}_4^+$  efflux caused by the addition of *T. lubrica* to undisturbed sediment cores at in situ  $p\text{CO}_2$  conditions from the current study. However, the addition of *M. arenaria* to sediment cores significantly increased  $\text{NO}_3^-$  influxes (Pegri & Blackburn 1995). These results don't support the  $\text{NO}_3^-$  fluxes from the current study because the addition of *T. lubrica* to sediment cores at in situ  $p\text{CO}_2$  conditions significantly increased  $\text{NO}_3^-$  effluxes. *M. arenaria* buries itself deeper into

the sediment than *T. lubrica*, which causes their burrows to penetrate into the zone of denitrification, as well as nitrification. This behavioural mechanism connects areas of sediment where  $\text{NO}_3^-$  concentrations are lower than that of the seawater overlying the sediment. Therefore when *M. arenaria* flushes out its burrow, a net loss of  $\text{NO}_3^-$  occurs from the seawater overlying the sediment because  $\text{NO}_3^-$  deficient porewater within the denitrification zone is replenished (Michaud et al. 2006).

It is evident that infaunal bivalves cause default  $\text{NH}_4^+$  effluxes to occur despite their size (results from the current study, Michaud et al. 2006, Pelegri & Blackburn 1995, Yamada & Kayama 1987). However, the same concept doesn't apply for sediment  $\text{NO}_3^-$  fluxes because infaunal bivalve size is proportional to burrowing depth, which has a significant effect on the porewater supply of  $\text{NO}_3^-$ . Small infaunal bivalves bury themselves close to the sediment surface in the nitrification zone, which causes significant  $\text{NO}_3^-$  effluxes to occur when they flush out their burrows (current study, Michaud et al. 2006). By contrast, larger bivalves bury themselves deeper into the sediment where they penetrate into the nitrification and denitrification zones, which causes significant  $\text{NO}_3^-$  influxes to occur when they flush out their burrows (Michaud et al. 2006, Pelegri & Blackburn 1995). Therefore, comparing the effect of larger bivalves on the sediment biogeochemistry to that of *T. lubrica* from the current study starts become irrelevant because they have significant morphological differences.

I expected  $\text{NH}_4^+$  and  $\text{NO}_2^-$  effluxes to decrease, and  $\text{NO}_3^-$  effluxes to increase overtime at in situ  $p\text{CO}_2$  conditions (Fig. 13) because I didn't resupply OM to the EU. Therefore, the available nutrients for sediment nitrogen cycling would have gradually become exhausted during the experiment leading to a decrease in  $\text{NH}_4^+$  and  $\text{NO}_2^-$  flux, and an increase in  $\text{NO}_3^-$  flux being the end product of nitrification. Furthermore the results indicated, that  $\text{NO}_3^-$  effluxes were greater in the Control cores compared to the Bivalve cores overtime at in situ  $p\text{CO}_2$  ( $t$ -test:  $<0.001$ , Figs 11D, 13). The opposite occurred during the initial measurements because the addition of *T. lubrica* to undisturbed sediment cores demonstrated significantly greater  $\text{NO}_3^-$  effluxes than the Control cores. A possible explanation for the lower  $\text{NO}_3^-$  effluxes by the Bivalves cores during the final measurements could be, that the additional *T. lubrica* within the Bivalve cores starved overtime; starvation would have decreased the bioturbative

activity and waste excretion by *T. lubrica*, leading to a decrease in sediment biogeochemical stimulation.

### **Effect of decreasing seawater pH on coastal soft sediment nitrogen cycling**

I found that decreasing seawater pH significantly increased the efflux of  $\text{NH}_4^+$  and  $\text{NO}_2^-$ , but had no significant effects on  $\text{NO}_3^-$  fluxes (Fig. 11B–D, 12B–D, 13, Table 6 & 7). Therefore,  $\text{H}_2\text{O}_2$  can be rejected because decreasing seawater pH significantly stimulated the sediment release of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  into the overlying seawater.

Some studies support the significant sediment release of  $\text{NH}_4^+$  during seawater acidification found in the current study. Braeckman et al. (2014) found that seawater acidification caused significant  $\text{NH}_4^+$  effluxes to occur from two sediment types (fine sandy and permeable) because nitrification rates decreased. They suggested that seawater acidification altered the pH within the sediment matrix, leading to the reduction of  $\text{NH}_3$  in the sediment porewater being the precursor of nitrification (Suzuki et al. 1974, Ward 1987); seawater can buffer decreasing pH by shifting the balance of  $\text{NH}_4^+/\text{NH}_3$  towards  $\text{NH}_4^+$ , which is the proposed sediment porewater mechanism that reduced benthic nitrification rates in Braeckman et al.'s (2014) study. The same seawater buffering mechanism is well documented as an explanation for decreasing pelagic nitrification rates during seawater acidification (Beman et al. 2011, Huesemann et al. 2002, Kitidis et al. 2011). This mechanism could explain the significant  $\text{NH}_4^+$  effluxes in the sediment cores from the current study. However, the mechanism's role in decreasing benthic nitrification rates requires further investigation because the effects of seawater acidification on the sediment matrix differs between sediment types (Braeckman et al. 2014).

Widdicombe & Needham (2007) and Widdicombe et al. (2009) observed similar significant  $\text{NH}_4^+$  effluxes by muddy sediment cores during seawater acidification compared to the current study. Based on the DIN fluxes being complementary between both studies Widdicombe et al. (2009) suggested that there was strong supporting evidence of reduced benthic nitrification rates to seawater acidification; to support this hypothesis the Authors assumed that the microbial communities responsible for benthic nitrification were negatively affected by seawater

acidification. The inhibition of benthic nitrifying bacteria during seawater acidification supports the efflux of  $\text{NH}_4^+$  because the aerobic oxidation of  $\text{NH}_3$  would have become hindered; thus, promoting the porewater production of  $\text{NH}_4^+$ . Widdicombe et al.'s (2009) explanation for significant  $\text{NH}_4^+$  effluxes by sediment cores during seawater acidification suggested that decreasing pH directly compromises the functioning of sediment microbes involved with nitrification. Thus, providing an alternative explanation for the sediment release of  $\text{NH}_4^+$  during seawater acidification from the current study.

Other studies such as Kitidis et al. (2011) and Laverock et al. (2013) investigated the effects of seawater acidification on benthic  $\text{NH}_3$  oxidation rates (aerobic). The results from both studies were inconclusive because seawater acidification had no significant effects on sediment  $\text{NH}_4^+$  fluxes. This indicates that benthic nitrification within some sediments are tolerant to decreasing seawater pH. I suggest that the effects of seawater acidification on sediment DIN fluxes differ between studies depending on the following: sediment type, intensity of pH treatment, the duration of pH treatment, structure of sediment microbial communities, etc. I was successful in revealing some mechanisms to explain the significant  $\text{NH}_4^+$  effluxes found in the current study. However, the significant efflux of  $\text{NO}_2^-$  found in the current study appears to be an anomaly.

For sediment  $\text{NO}_2^-$  fluxes, the literature indicates that seawater acidification can cause the following: no effect (Kitidis et al. 2011, Laverock et al. 2013, Widdicombe et al. 2009, Wood et al. 2009), significantly decrease  $\text{NO}_2^-$  effluxes (Gazeau et al. 2014, Widdicombe & Needham 2007) or significantly increase  $\text{NO}_2^-$  influxes (Braeckman et al. 2014, Widdicombe et al. 2013). Therefore, no supporting evidence exists to explain the significant increase in  $\text{NO}_2^-$  effluxes during seawater acidification from the current study. The interpretation of the significant  $\text{NO}_2^-$  flux results from the current study is difficult because if benthic  $\text{NH}_3$  oxidation rates decreased during seawater acidification, I would have expected  $\text{NO}_2^-$  effluxes to significantly decrease. Therefore, I hypothesize that the significant  $\text{NO}_2^-$  effluxes observed during seawater acidification from the current study were caused by reduced benthic  $\text{NO}_2^-$  oxidation rates. A reduction in the benthic oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  could explain an increase in the

porewater supply of  $\text{NO}_2^-$ , leading to the sediment release of  $\text{NO}_2^-$ . Within this hypothesis, I also suggest that benthic denitrification was reduced; this could explain how sediment  $\text{NO}_3^-$  fluxes were unaffected during seawater acidification. If these results were repeatable in future studies using the same sediment, my hypothesis could hold.

### **Interactive effects of seawater acidification and *T. lubrica* on coastal soft sediment functioning**

Significant interactive effects were detected between decreasing seawater pH and the addition of *T. lubrica* for the difference between initial/final TOU, but not for the difference between initial/final DIN fluxes (Figs 11–13, Tables 6 & 7). I can reject  $H_03$  because the interactive effects I detected between decreasing seawater pH and the addition of *T. lubrica* significantly increased delta TOU (decrease in sediment  $\text{O}_2$  uptake). I suggest that the significant interaction between the pH and Bivalve treatments caused both direct and indirect effects on TOU during the final measurements.

A possible contributor to the decrease in sediment  $\text{O}_2$  uptake during seawater acidification within the Bivalve cores from the current study could be that decreasing seawater pH causes physiological stress in *T. lubrica*. Metabolic depression during acidified seawater conditions is an adaptive mechanism utilised by some bivalves to conserve  $\text{O}_2$  and energy; this mechanism is time limited and becomes compromising if overexploited during extended periods of seawater acidification (Pörtner et al. 2004). *Ruditapes decussatus* (Fernández-Reiriz et al. 2011) and *Chlamys nobilis* (Liu and He 2012) are examples of subtidal bivalves that utilise metabolic depression as a physiological stress response to OA.

If *T. lubrica* utilized metabolic depression during seawater acidification, their respiration rates would have decreased; thus, decreasing overall sediment  $\text{O}_2$  consumption rates. It is difficult to estimate the proportion that the Bivalve treatment contributed to TOU because *T. lubrica* is small, where benthic respiration via algae and microbes would heavily outweigh sediment  $\text{O}_2$  consumption via animal respiration. Comparing the respiration rates of *T. lubrica* during control seawater pH

and acidified seawater conditions using cores exclusive of sediment should be considered for future research; this would indicate if the direct effects of seawater acidification on *T. lubrica* had any significant effects on TOU during the final measurements in the current or future studies.

Other than seawater acidification potentially causing direct effects on the metabolism of *T. lubrica* in the current study, the interaction detected between the pH and Bivalve treatments might have caused significant indirect effects on TOU during the final measurements; In other words, the interaction could have caused modifications to the sediment environment overtime, contributing to the overall decrease in sediment O<sub>2</sub> consumption.

A potential modification that *T. lubrica* could have made to the sediment environment in the study is by consuming the benthic microalgae that are able to live under dark/minimal light conditions for long periods of time. *T. lubrica* are deposit feeders that feed on algae and OM from the sediment surface (Kutkuchi 1981, Yokoyama & Ishihi 2003). Therefore, I hypothesize that fewer algae were present in the Bivalve cores compared to the Control cores. Based on this hypothesis, *T. lubrica* would have reduced the algae mediated O<sub>2</sub> demand of the sediment in the Bivalve cores overtime. A common response in many species of benthic microalgae to OA is a significant increase in respiration (Hurd et al. 2009, Wu, Gao & Riebesell 2010, Yang & Gao 2012). Therefore, I suggest that the Control cores produced lower delta TOU than the Bivalve cores as pH decreased (Figs 11A & 13) because the greater amount of algae in the Control cores could have increased their respiration rates during seawater acidification.

The Bivalve treatment could have also modified the sediment environment in the current study by causing significant indirect effects on the functioning of benthic microbes. A competition between *T. lubrica* and the microbes could have started during the experiment because they both target OM as a food source; if this happened, I would have expected the amount of OM in the Bivalve cores to be lower than that within the Control cores during the final measurements because OM wasn't replenished in the EU during the investigation. Deposited OM enhances the metabolism of benthic microbes (Middelburg et al. 2005). Therefore, the potential

difference in the supply of OM for microbial decomposition could help to explain why the sediment consumption of O<sub>2</sub> was significantly greater in the Control cores compared to the Bivalve cores during seawater acidification. It is unlikely that microbial respiration was significantly altered by seawater acidification during the experiment because it is common for microbial respiration to outcompete photosynthesis within the coastal zone, causing temporary localized OA events (Joint et al. 2011). However in addition to the supply of OM, the pH treatment could have further enhanced the metabolism of microbes.

The last indirect effect I suggest that could have caused a significant decrease in sediment O<sub>2</sub> consumption within the Bivalve cores during the final measurements in the current study could be that seawater acidification reduced the bioturbation of *T. lubrica*. Decreased seawater pH is proven to increase shell dissolution, lesions and mortality within many bivalve species (Clements & Hunt 2017); therefore, I would expect a relative decrease in bioturbation because of the stress associated with these symptoms during seawater acidification. Infaunal bivalves flush out their burrows because of the following: to prevent themselves from being smothered by sediment backfilling, to expel waste and for ventilation/feeding purposes. These burrows provide a unique environment for benthic solute exchange because they extend the sediment-seawater interface and the O<sub>2</sub> penetration depth of sediments (Norkko & Shumway 2011).

The microbes that inhabit the linings of macrofaunal burrows have a strong dependence on bioturbation to supply fresh oxygenated seawater for respiration, and metabolic processes (Glud 2008). Therefore, if seawater acidification decreased the burrow flushing (mode of bioturbation) of *T. lubrica*, I would have expected a proportional decrease in the sediment O<sub>2</sub> consumption by the microbes within the burrows; this effect wouldn't be large because *T. lubrica* are small, however, a decrease in bioturbation by *T. lubrica* could have contributed to the overall significant increase in delta TOU within the Bivalve cores during seawater acidification.

## Strategies to improve methodology for future research

The manipulation of the *T. lubrica* abundance in the Bivalve cores during the experiment was successful. The average abundance of *T. lubrica* found in the Control and Bivalve cores were  $8 \pm 4$  and  $18 \pm 5$ , respectively (Table 4). The Bivalve treatment consisted of adding 10 *T. lubrica* individuals to an undisturbed sediment core, which matched the difference between the average abundance of *T. lubrica* in the Control and Bivalve cores ( $18 - 8 = 10$ ).

Core 5 (Bivalve core) had a *T. lubrica* abundance of zero (Table 4), which was unexpected because I subjected it to the Bivalve treatment. Assuming that none of the *T. lubrica* died during the experiment, I expected all of the Bivalve cores to have a minimum *T. lubrica* abundance of 10. The anomaly I found in core 5 (no *T. lubrica*) is unlikely to have been caused by the pH treatment because the minimum abundance of the other Bivalve cores was 11 (Table 4). If decreasing seawater pH were fatal to *T. lubrica*, then all the other Bivalve cores would show similar *T. lubrica* abundances found in core 5. Furthermore, there was no evidence of *T. lubrica* swimming out of the sediment cores during the experiment because I found no *T. lubrica* at the bottom of the incubation bins at the end of the experiment. Therefore, I suggest that an animal within the sediment could have predated the *T. lubrica* in core 5 or this anomaly came about due to human error.

Overall, I thought that *T. lubrica* was a great candidate for this experiment because they were easy to collect and acclimatized well to laboratory conditions. However, I recommend for future studies to increase the amount of *T. lubrica* individuals added to the Bivalve cores. For example, instead of adding 10 *T. lubrica* individuals to an undisturbed sediment core to make a Bivalve core, I would add 30 or more. *T. lubrica* are small, where by Amplifying the Bivalve treatment could help reveal a potential significant response from the sediment that would otherwise be washed out by noise created by the experiment.

I successfully maintained control  $\text{pH}_T$  conditions (8.0) in all the EU during the initial incubations (Table 3). Only slight variations were found in initial  $\text{pCO}_2$ , but initial DIC and TA were consistent in all the EU at 2.0 and 2.2  $\text{mmol kg SW}^{-1}$ , respectively.

According to Table 3, EU 1 was the only system that held target  $\text{pH}_T$  (8.0) during the final incubations. The Final  $\text{pH}_T$  values for EU 2–4 were 7.9, 7.4 and 7.1, respectively. My target final  $\text{pH}_T$  values for EU 2 - 4 were 7.8, 7.6 and 7.4, respectively. Therefore, final  $\text{pH}_T$  was too high in EU 2 and too low in EU 3 & 4. The final DIC and  $\text{pCO}_2$  in EU 2–4 increased, which was expected because I injected  $\text{CO}_2$  into these units. However, the final TA increased from 2.2 to 2.3 in EU 3 & 4, which was unexpected because both initial and final TA in all of the EU should have remained unchanged.

Table 3 indicates that I was successful in administering a pH treatment that incrementally decreased seawater pH across the EU from 1–4, despite target  $\text{pH}_T$  not being achieved in EU 2–4 during the final incubations. My analyses for the final incubations were based on the EU holding their target  $\text{pH}_T$  because the DIC and TA data in Table 3 was based off single seawater sampling events, which I received late in the project. I recommend that future studies increase the amount of replicates per sampling event to improve the legitimacy of the DIC and TA analyses; single sampling events have no redundancy, where seawater collecting errors and/or inaccurate analysis would compromise the calculations for  $\text{pCO}_2$  and  $\text{pH}_T$ .

The sediment cores were exposed to the final pH treatment for 20 days, which was enough time to detect sediment solute flux responses to decreasing seawater pH. However, I would be interested to see how the sediment solute flux responses change over a longer pH treatment period, considering that OA is an intensifying phenomenon that is predicted to continue past this century (Raven et al. 2005). The pH treatment from the current study would be considered a short-term exposure compared to other studies, which predominantly use medium to longer pH treatment periods. Examples include: 35 days (Widdicombe & Needham 2007), 40 days (Wood, Widdicombe & Spicer 2009), 7 weeks (Dashfield et al. 2008), 20 weeks (Widdicombe et al. 2009). Therefore, I recommend that future studies strive for the longest possible pH treatment period (within their financial and time budgets) to see if the sediment solute flux responses change overtime.

Salinity was a variable that I maintained well considering that I had to manually add fresh purified water to restore correct levels. My goal was to keep salinity between 33.5 and 34.0, which I achieved for the most part. However, I caused a few deviations

from this desired range (Fig. 7). I recommend the use of float switches for future experiments because this would improve the consistency of salinity and make the EU more independent.

The seawater advection within the EU was sufficient to maintain consistent chemical conditions, and prevent stagnation in the sediment cores and other areas within the incubation bins during the current study. However, I suspect that the turbulence of the wave maker in the incubation bins was a bit strong because I noticed a slight amount of suspension from the top terrigenous layer from some of the sediment cores during the experiment. This could have altered the sediment solute flux responses of some of the sediment cores during the incubations. Therefore, I recommend that future studies take time to make sure that the positioning and setting of the wave makers don't cause suspension from the sediment cores.

## Conclusion

The phenomenon of OA has raised concern about how marine ecosystems could respond within the near future. The aim of my study was to investigate how *T. lubrica* moderated the biogeochemical response of coastal soft sediments to decreasing seawater pH. Studying soft sediments is of high importance because they are responsible for the majority of OM remineralisation and nutrient cycling within coastal ecosystems (Nixon 1981).

The results from my study indicated, that the addition of *T. lubrica* to sediment cores during the initial measurements significantly increased TOU and effluxes of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , but had no effect on  $\text{NO}_2^-$  fluxes at in situ  $p\text{CO}_2$  conditions. I suggest that these results could be demonstrating a stimulation of ammonification and nitrification. Furthermore, I found that decreasing pH during the final measurements significantly increased effluxes of  $\text{NH}_4^+$  and  $\text{NO}_2^-$ , but had no effect on delta  $\text{NO}_3^-$  fluxes or TOU. I suggest that these results could be demonstrating a decrease in coupled nitrification and denitrification. Lastly, I detected an interaction between the addition of *T. lubrica* and decreasing pH, which caused sediment  $\text{O}_2$  consumption to significantly decrease; delta DIN fluxes were unaffected by the interaction. I suggest that the Bivalve treatment caused both direct and indirect effects, which led to the significant decrease in delta TOU during the final measurements.

By referring to my original research question, “How does *T. Lubrica* influence the response of nitrogen cycling in coastal soft sediments to OA?” I can conclude that *T. lubrica* had no significant effects on sediment DIN fluxes during decreased seawater pH conditions. I hope that my work encourages future research on the same species, to further validate the results from the current study or to provide new significant findings.

My research was successful because it has filled a gap in current knowledge within the context of OA research on marine sediments in New Zealand. I provided new demographical information on the population of *T. lubrica* at my study site in Man O' War Bay, Waiheke Island, and also provided insight on how they could influence the biogeochemical response of soft sediments during decreased seawater pH conditions.

Furthermore, I established an OA facility and maintained the variables with reasonable success. This facility allowed me to investigate the potential effects that a non-native bivalve species could have on coastal nutrient cycling during predicted OA events within this century.

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