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Abstract

Invasive species have been identified as one of the major threats to biodiversity and ecosystem functioning, but the nature and magnitude of their effects depends on the environmental context and on the abundance of the invader. The Pacific oyster, *Crassostrea gigas*, is a globally invasive ecosystem engineer which can monopolise shores and alter native biodiversity. Less is known, however, about its effects on ecosystem functioning or whether its effects differ in different habitats or at different abundances. This research used an interdisciplinary approach to characterise the impact of invasive oysters on biodiversity and ecosystem functioning and to determine how these impacts would vary in different habitats and at increasing abundances.

In Chapter II, the effects of increasing cover of *C. gigas* on biodiversity in intertidal boulder-fields was assessed. Furthermore, the effects of the physical structure and biological activity were separated using dead and living oysters. *C. gigas* increased diversity on boulders, but effects were non-linear with regards to the cover of *C. gigas*. When present at low levels of cover, *C. gigas* increased biodiversity on boulders, but at higher levels there was no further increase in biodiversity and boulders became heavily dominated by macroalgae, *Fucus Vesiculosus*, and a key grazer, *Littorina Littorea*, which possibly indirectly affected the establishment of other species. Either directly or indirectly, the establishment of a protected biogenic habitat built by the honeycomb worm, *Sabellaria alveolata*, on the undersides of boulders was reduced with increasing cover of *C. gigas* on their upper surfaces. The effects of *C. gigas* on the establishment of other species were found to be mostly attributable to the physical structure rather than their biological activities.

In Chapter III, plots with increasing cover of *C. gigas* were set-up in mussel-beds and

mud-flats within two estuaries and were sampled after 4 and 15 months. The effects on biodiversity were mostly context-dependent: biodiversity increased with increasing cover of *C. gigas* in mud-flats, but was unaffected or reached a threshold and decreased with the highest level of cover in mussel-beds, depending on the estuary. Some species, such as *L. littorea* and an invasive barnacle, *Elminius modestus*, were facilitated by *C. gigas* regardless of location or habitat.

Ecosystem functioning in mussel-beds and mud-flats in one of the estuaries in Chapter III was also affected by *C. gigas* (Chapter IV). Several biogeochemical properties and processes were altered, but responses were non-linear with regards to cover and some differed between habitats. Sediment-water fluxes and benthic turnover rates of NH_4^+ were greatest at medium cover of *C. gigas* in both habitats, but for $Si(OH)_4$ they increased with increasing cover of oysters in mud-flats but decreased at the greatest cover of oysters in mussel-beds. Community respiration was only affected at the highest cover of *C. gigas*.

The increase in community respiration was further investigated in Chapter V where the effects of increasing cover of *C. gigas* in mud-flats on ecosystem processes and associated microbial assemblages were tested. The increase in community respiration was at least party attributable to an increase in microbial activity with high covers of *C. gigas*. Ecosystem processes and microbial assemblage structure responded non-linearly with regards to the cover of *C. gigas*. The assemblage composition of methanogens and ammonia-oxidising microbes in anoxic sediments were only altered by low covers of *C. gigas* while ammonia-oxidisers in oxic sediments were only altered by high covers of *C. gigas*. At any level of cover, *C. gigas* increased gaseous carbon emission from sediments. NH_4^+ flux reached a threshold at medium cover of *C. gigas* as it did in Chapter IV and indirect mediation from algae facilitated by high covers of *C.*

gigas is suggested as a mechanism.

This research has shown that *C. gigas* can significantly alter biodiversity and several ecosystem processes. The nature and magnitude of many of these effects differed depending on the type of habitat, the location and on the level of cover of *C. gigas*. At a larger scale, high covers of *C. gigas* may impact the conservation status and alter the capacity of estuaries to provide ecosystem services, such as commercial shellfish production.

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Ode to a microbe

Earth's invisible engineer,
From mountains high, to oceans clear
Your infinitesimal army,
Working to maintain harmony,
Whether oxic or anoxic,
Whether toxic or non-toxic,
You fix, take up and nitrify,
Mineralise, denitrify
Our capricious key element,
Unlocking us from discontent.

You spread into the atmosphere,
The geo and the biosphere,
But what of ever-growing heat,
Does this not force you to compete?
Do microbes sweat or just respire?
Will gases force us to expire?
What of filthy sewerage slurry,
You amass this in a hurry?
Although polluted mud and soil,
You plunge into your thankless toil.

Substrates flooded with organic matter
Are set upon by non-existent lips,
Through the frenzy, I hear pitter patter,
Reinforcements from non-existent ships!
Flagella whip, reporting for duty,
Peptidoglycan all shimmering bright,
Shadowed by the ostentatious beauty
Of a world anchored on their silent plight.

Chapter I - General introduction

1.1 Ecosystem services

As human beings, we may feel we are buffered against the effects of environmental change by modern culture and technology. We are, however, fundamentally dependent on the many services provided by ecosystems. Ecosystem services were defined by the Millennium Ecosystem Assessment (MEA, 2005) as the processes and conditions of natural ecosystems that support human activity and sustain human life. According to the MEA (2005) they can be grouped into four categories: “supporting” (major ecosystem resources, energy and nutrient cycles), “provisioning” (production of goods), “regulating” (maintenance of ecosystem processes) and “cultural” (non-material benefits). These broad groupings, however, can lead to some ecosystem services being counted more than once (Ojea, 2010), thus potentially leading to miscalculations when assessing the economic valuation of ecosystems. The concept is further complicated by the fact that the terms “ecosystem services”, “ecosystem functioning” and “ecosystem processes” are often used interchangeably or with alternate meanings within the literature (Fischer et al., 2009). The success of applied ecology depends on its scientific excellence and on its relevance to management (Ormerod et al., 2002). The latter of which is underpinned by the translation of science into recommendations and eventually policy (Webb and Raffaelli, 2008). It is, therefore, of the utmost importance to carefully and unambiguously define terms such as ecosystem services, functioning and processes. There is still much debate regarding such definitions and classifications (Costanza, 2008; Wallace, 2008; Fisher et al., 2009; Haines-Young and Potschin, 2009) and perhaps there are no completely unambiguous definitions for such complex systems, nonetheless, it is still important have to be clear about the meaning of the terms used. In this thesis, ecosystem services are regarded as conceptualisations of the useful direct and indirect contributions of ecosystems to human welfare. It should be noted

that what is regarded as “useful” may change over time as societal needs change. Ecosystem services are produced and maintained by the structure (composition and biological or physical organisation) and functioning of ecosystems. And so we need to conserve natural ecosystems, not only on moral grounds, but also in order to maintain the ecosystem services that sustain us and enrich our lives (Convention on Biological Diversity (CBD, 2012)). The delivery of ecosystem services depends on the functioning of ecosystems. Ecosystem functions can be defined as the interactions between ecosystem structure and processes that underpin the capacity of an ecosystem to provide services (Hooper et al., 2005). Ecosystem functioning, in broad terms, is “the flow of energy and materials through biotic and abiotic components of the ecosystem” (Diaz and Cabido, 2001) and is quantified and qualified by measuring the magnitudes and dynamics of ecosystem processes (Loreau et al., 2002). Ecosystem processes are a measure of the rates of change within ecosystems either physical, chemical or biological for example, primary and secondary production, community respiration and nutrient and energy fluxes (Kinzig et al., 2001; Hiscock et al., 2006).

Global biodiversity is rapidly declining and much of this loss can be attributed to human activities (Sala and Knowlton, 2006; Butchart et al., 2010). Biodiversity can be defined as the variety of life, including ecosystem diversity, species diversity and genetic diversity within species (CBD, 2012). Loss of biodiversity can significantly affect the functioning of ecosystems (Hooper et al., 2005; Balvanera et al., 2006; Cardinale et al., 2006; Stachowicz et al., 2007; Naeem et al., 2009), which in turn affects humans both directly and indirectly, through the provision of ecosystem services (Costanza et al., 1997; Worm et al., 2006). In fact, the link between biodiversity, ecosystem functioning and service provision has been recognised in the new Strategic Plan of the Convention on Biological Diversity (CBD, 2012). Although widely accepted, the relationship between biodiversity and ecosystem functioning is still not entirely understood (Gamfeldt and

Bracken, 2009). It is important for researchers to measure and understand a number of processes (Gamfeldt et al., 2008), since the loss of a particular species or functional group may impact upon some processes but not others, therefore results may vary depending on which processes are measured (Duffy et al., 2001; Biles et al., 2003; Solan et al., 2004; Matthiessen et al., 2007; Vaughn et al., 2007).

Micro-organisms play the major role in maintaining ecosystem processes, particularly in nutrient cycling, decomposition and mineralisation or remineralisation of organic matter (Pomeroy, 1974; Schulz and Zabel, 2000). In fact, they are the primary means by which organic matter is recycled and made available to primary producers, and as such, they provide many ecosystem services that sustain life (Bell et al., 2005; Ortego-Morales et al., 2010). However, the relationship between microbial biodiversity and functioning and the environmental controls on microbial community composition remain poorly understood (Oremland et al., 2005; Gutknecht et al., 2006; Hallin et al., 2009). There is growing awareness that the impact of macrobiota on ecosystem functioning may arise from, or be mediated by, their impacts on microbial communities and the biogeochemical processes they drive (Windham, 2001; van der Putten et al., 2007).

1.2 Invasive species – one of the main threats to ecosystems

Invasive species have been recognised as one of the main direct drivers of change to biodiversity and ecosystems (MEA, 2005; Simberloff, 2005; Charles and Dukes, 2007; McGeoch et al., 2010; Pyŝek and Richardson, 2010; Thomsen et al., 2011a). Although, biological invasions have been commonplace throughout evolutionary time, as species ranges are often dynamic and may extend due to geographic and climatic variation, anthropogenic pathways of introduction are allowing a much wider and faster proliferation to new habitats (Vitousek et al., 1997; Nentwig, 2007; Butchart et al.,

2010)). For example, in the marine realm, aquaculture and cargo shipping have greatly increased the speed, frequency, magnitude and spatial extent of the spread of non-indigenous species (Lodge, 1993; Galil et al., 2007; Gollasch, 2007).

Non-indigenous is used throughout pseudonymously with “non-native”, “alien” and “exotic” to indicate an organism found living beyond its historical native range. Despite being transported beyond their native range, not many of these species become successfully established and spread rapidly in the new environment and hence can be deemed as “invasive” (Ricciardi and Cohen, 2007; Falk-Petersen et al., 2006). The definition of what makes a species “invasive” has been subject to debate for some time (Colautti and MacIsaac, 2004). One definition is “an organism that is non-indigenous to the ecosystem under consideration and is likely to cause harm to native ecosystems, habitats, species and/or the economy that outweighs any beneficial effects” (ISAC, 2006; Perrings et al., 2010; CBD, 2012).

Defining what is meant by “harmful” or “beneficial” is subjective and ecological researchers should not make “positive” or “negative” value judgments, but rather provide empirical evidence, detailing the nature and magnitude of effects, which helps inform management decisions regarding invasions (Rosenzweig, 2001; Hagman and Shine, 2007). From a policy perspective, however, making these distinctions is important and, in general, a loss of native biodiversity or a decrease in the quality or quantity of ecosystem services will be considered a “negative” response, whilst the opposite will be considered “positive” (Beck et al., 2008). On the contrary, changes to ecosystem functioning should not be defined as positive or negative from society’s point of view, but resultant changes to ecosystem services can be, since these are subjective and complex (Duffy, 2009). For example, a diversity-mediated increase in primary production which increases commercial shellfish production would be considered

positive, while the eutrophication of a water body would be negative. It is important to remember that not all non-indigenous species cause negative environmental impacts (Colautti and MacIsaac, 2004; Ricciardi and Cohen, 2007). In fact, there are many documented cases of positive effects (Sagoff, 2005), for example, some non-indigenous plants increase the production of commercial honey (Charles and Dukes, 2007). Those invasive species which do cause negative effects, however, can cause irreparable damage to species, ecosystems and economies. For instance, about half of the species listed as threatened or endangered under the Endangered Species Act (ESA, 2001) are considered to be at risk primarily because of competition with or predation by, invasive species (Wilcove et al., 1998).

As recognition of the potential impacts of invasive species has grown, so has the economic literature concerning costs to terrestrial (Olson, 2006) and aquatic (Lovell et al., 2006) ecosystems. Damage caused by invasive species worldwide is estimated at more than $1.4 trillion per year, representing nearly 5 % of the world economy (Pimentel et al., 2001). Many of these costs are due to a deterioration of ecosystem services induced by invaders (Pejchar and Mooney, 2009; Vilá et al., 2010). Of course, the costs of prevention, management and mitigation can also be substantial (Olson, 2006). A prolific example is the invasive Zebra mussel (*Dreissena polymorpha*), which causes severe damage to the power industry in North America due to biofouling, and costs approximately $5 billion each year to control (New York Sea Grant, 1994). Such expenditure must be justified and strategically targeted by understanding the potential impacts of invasive species through research producing robust empirical evidence (Pyšek and Richardson, 2010).

1.3 Potential threats of invasive species

Invasive species occur in terrestrial, freshwater and marine environments and many can alter native biodiversity and ecosystem processes (Ehrenfeld, 2010; Molnar et al., 2008) through a range of direct and indirect mechanisms (Crooks, 2002). Invasive species can reduce biodiversity in invaded habitats (Sala et al., 2000; Grosholz, 2005; Molnar, 2008; Kimbro et al., 2009). In other cases, they can increase or have no effect on biodiversity (Molnar et al., 2008). Although the effects on alpha diversity have been well studied, invasive species can also affect beta diversity (Wright, 2011). In fact, many invasive species have been implicated in causing homogenisation of the Earth's biota (Lodge, 1993; Vitousek et al., 1997; Rahel, 2002; McKinney and Sorte, 2007). Homogenisation most commonly occurs via exclusion and local extinction of native species (Olden et al., 2008) and can occur in terms of genetic, taxonomic or functional groups (Olden and Rooney, 2006). Homogenisation of communities can cause simplifications of food-web structures at multiple trophic levels and may increase the susceptibility of communities to future invasions (Olden et al., 2004).

Predicting the effects of invasive species on populations and communities requires an understanding of the mechanisms by which invaders interact with native species. Direct interactions may involve competition (Byers, 2000; Seabloom et al., 2003), parasitism (Griffen, 2009) or predation (Pitt and Witmer, 2007). Some invasive plants, for example, out-compete natives for light, nutrients and space (Bennett et al., 2011), while many bird extinctions are attributed to predation from invasive rats and cats (King, 1985; Griffin et al., 1989). Invasive species can also interbreed and "hybridize" with related native species, potentially causing genetic extinction of rare or endemic species (Rhymer and Simberloff, 1996; Largiadèr, 2007).

Indirect effects of invasive species are often found to be as important or more important

than direct effects (Wootton, 1994; Russell et al., 2007). Facilitation of native or other non-native organisms by invasive species is common (Rodriguez, 2006) and can have further indirect effects on other species. For example, invasive plants may provide refuge for herbivores which then consume other native plant species. This is known as “apparent competition” (Dangremond et al., 2010). Additionally, when invasive species facilitate other invaders, this can lead to “invasional melt-down”, which is a term coined by Simberloff (2006) relating to a community level phenomenon in which the rate of invasion and overall impacts of invaders are accelerated as one invasive species facilitates another and so forth.

Either indirectly by affecting biodiversity, or directly by chemical or physical alterations to the receiving environment, invasive species can alter ecosystem processes and functioning, thereby affecting the provision of ecosystem services (Charles and Dukes, 2007; Pejchar and Mooney, 2009; Ehrenfeld, 2010; Eviner et al., 2012). Invasive species can alter primary or secondary productivity (Dukes and Mooney, 2004; Bruschetti et al., 2011), decomposition rates (Giles et al., 2006; Karberg and Lilleskov, 2009), nutrient or energy (Ehrenfeld, 2003; Gomez-Aparicio and Canham, 2008; Vila et al., 2011) cycles. Despite this, there have been relatively few studies assessing effects of invasive species on ecosystem functioning compared with those assessing effects on individual species, populations or communities (Ehrenfeld, 2010; Sousa et al., 2011).

1.4 Context-dependency of impacts of invaders

Few generalizations have stemmed from research on invasive species (Thomsen et al., 2011a). This lack of generality arises from the fact that the nature and magnitude of the effects of invasive species may vary depending on the environmental context (i.e. the associated abiotic and biotic factors of the receiving environment) and on the unique

(e.g. identity) and universal (e.g. abundance) attributes of the invader (Thomsen et al., 2011a). Indeed, an invasive species may have a negative impact in one location but have no effect or a beneficial one elsewhere (Colautti and MacIsaac, 2004; Somaweera and Shine, 2012). Recently, several researchers (Lockwood et al., 2007; Olenin et al., 2007; Thiele et al., 2011; Thomsen et al., 2011a and b) have presented "frameworks" that aim to organise impact studies identifying research gaps and eventually strengthening predictions of the impacts of invasive species. Economic resources are not available to control all invasive species, so some frameworks aim to rank the impacts of invaders in order to assist in prioritising management decisions (Thiele et al., 2011). Such rankings can be based, for example, on the magnitude of alterations to native species and communities, habitats and ecosystem functioning and on how widespread the invader is (Olenin et al., 2007). The credibility of these ranks, however, is limited by some key knowledge gaps, including how the effects of invaders on biodiversity and ecosystem functioning differ (i) at a range of different abundances (percentage covers, densities or biomasses) and (ii) in different habitats (Thomsen et al., 2011a).

Typically studies on the impacts of invasive species compare presence or absence of invaders (Thomsen et al., 2011a). In order to improve predictions of their impacts, it is advantageous to relate invader effects directly to their abundance, which may vary through time, as populations expand or decline, or differ spatially through patchy distribution. Impacts may form continuous gradients with abundance (Parker et al., 1999). Alternatively, the relationship between effect and invader abundance may be non-linear, thus complicating predictions (Yokomizo et al., 2009). If responses are non-linear, then small abundances of an invader may have different or disproportionate effects on native ecosystems than larger abundances. For instance, if small populations of an invader have positive or neutral impacts on native species, then a threshold value

may exist, above which effects become negative (Groffman et al., 2006).

In general, no description in ecology makes sense without reference to particular temporal and spatial scales (Levin, 1992) since biotic (Underwood and Chapman, 1996) and abiotic properties (Chapman and Tolhurst, 2007) of ecosystems are inherently patchy. As such, relationships between invader abundance and their effects may be modified by variation in the biological, physical and chemical characteristics of habitats at a range of scales (Thomsen et al., 2011b). The characteristics of the invaded habitat, including the composition of invaded communities, structural complexity and heterogeneity and type and mineralogical composition of substrata, can alter species interactions (Kneitel and Chase, 2004) and regulate the impacts of invaders on biodiversity and ecosystem functioning (Boyer et al., 2009). Therefore, the development of accurate predictions of the impacts of invasive species requires their effects to be assessed in the full environmental gradient of invaded habitats (Thiele et al., 2011).

Relationships between biodiversity and ecosystem functioning are also context dependent, varying in relation to factors such as environmental conditions (Yachi and Loreau, 1999; O'Connor and Crowe, 2005; Vaughn et al., 2007), assemblage composition (Worm and Duffy, 2003) and density of organisms (Griffin et al., 2008), thus further complicating predictions of the effects of invaders. Furthermore, the functional distinctiveness between the invader and the native biota can alter its overall impact, but not in a unidirectional manner. For example, if the invader is functionally similar to native biota, effects such as competition (Byers, 2009) and hybridisation (Mallet, 2005) may be exacerbated. Alternatively, if a habitat forming invader is functionally distinct from native biota, its effects may be exacerbated because it alters the native environment more radically than functionally similar species (Ricciardi and Atkinson, 2004). Ultimately, the complex interactions of species coupled with the inherent

complexity of ecosystems, makes predicting the ecological or economic impacts of invasive species very difficult (Williamson, 1999), as it does finding any general law in ecology (Lawton, 1999).

1.5 Impacts of invasive ecosystem engineers

Ecosystem engineers, as defined by Jones et al. (1997), are organisms which control resource availability through physical alterations to biotic or abiotic materials, including the alteration or provision of habitat (Bruno and Bertness, 2001). Organisms can be "autogenic engineers" when they themselves are part of the engineered habitat or "allogenic engineers" when they transform biotic or abiotic materials from one physical state to another through their biological activities (Jones et al., 1994; Crooks, 2002). One reason that invasive ecosystem engineers are of particular interest is because they can change the abundance or diversity of structural elements in habitats, thus altering habitat complexity or heterogeneity (McCoy and Bell, 1991). In turn, this can alter biodiversity by affecting processes such as recruitment (Benedetti-Cecchi and Cinelli, 1992; McQuaid and Lindsay, 2005), mortality (Menge, 1978), dispersal (Raffaelli and Hughes, 1978) and response to disturbance (Lohse, 1993). In short, invasive ecosystem engineers can exert control over ecosystem processes by mediating the flow, availability or quality of nutrient, trophic and physical resources (Crooks, 2002). It is important to note that the impacts of invasive ecosystem engineers are not only modulated by the species composition of invaded communities and the characteristics of invaded habitats, but also by the presence of other functionally similar ecosystem engineers (Padilla, 2010; Queirós et al., 2011).

1.6 Crassostrea gigas *as an invasive ecosystem engineer: potential impacts on biodiversity and ecosystem functioning*

The Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), originally from Japan, is the leading species in global shellfish aquaculture (Miossec et al., 2009) and as a consequence has become extensively invasive (Chew, 1990), with wild populations inhabiting many coasts and estuaries worldwide, including Australasia (Ayres 1991; Dinamani 1991), Europe (Grizel and Héral, 1991; Reise, 1998; Drinkwaard 1999), North America (Span, 1978; Quayle, 1988) and South Africa (Robinson et al., 2005). Wild populations of *C. gigas* have been found in a variety of different habitats, including mudflats and mussel beds (Reise et al., 2006; Ruesink, 2007; Buttger et al., 2008; Markert et al., 2010), marshes (Escapa et al., 2004), polychaete reefs (Dubois et al., 2006a) and rocky shores (Krassoi et al., 2008; Trimble et al., 2009). *C. gigas* is an ecosystem engineer and its success is partly due to its ability to modify habitats in a way that enhances its own further colonisation (Diederich, 2005). It does this by constructing complex reefs that facilitate the settlement of conspecifics and increase the chance of their survival (Bartol et al., 1999; Gutiérrez at el., 2003; Grabowski and Powers, 2004). As such, it can form very dense populations covering extensive areas (Wrange et al., 2010).

The name *Crassostrea gigas*, translated from Greek, means “thick” (*Crass*), “giant” (*gigas*) “oyster” (*ostrea*). True to its name, *C. gigas* develops thick shells and can grow very large, sometimes up to 40 cm in length (Nehring, 2006). Often they are also more tolerant to environmental stress (Piano et al., 2002) and have greater fecundity (Soletchnik et al., 2002), growth (Askew, 1972) and clearance rates (Honkoop et al., 2003) than native bivalves. It is, therefore, not surprising that in some places where it is invasive, *C. gigas* can outcompete native bivalves such as cockles, mussels (Diederich et al., 2005) and native oysters (Grizel and Héral, 1991). The reduction of cockles can

indirectly harm migratory birds by reducing food availability (Smaal et al., 2005; Van den Berg et al., 2005). At a community level, *C. gigas* has been found associated with a greater abundance and diversity of organisms (Gutierrez et al., 2003; Peterson et al., 2003; Dubois et al., 2006a; Markert et al., 2010; Lejart and Hily, 2011), or with changes to the composition or structure of assemblages (Kochmann et al., 2008; Markert et al., 2010), or with assemblages that do not differ from those associated with indigenous habitats (Görlitz, 2005).

Despite extensive research on the effects of invasive populations of *C. gigas* on biodiversity, very little is known about its effects on ecosystem functioning, or about the mechanisms by which it affects either biodiversity or functioning. The influence of *C. gigas* on biodiversity and ecosystem processes may be due to its physical structure or its biological activities. The shell of *C. gigas* adds hard substrata to the receiving habitat and may be colonized by other native and non-native organisms (Diederich et al., 2005). Because the shell of *C. gigas* is structurally complex, it may increase biodiversity (Lejart and Hily, 2011) by enhancing foraging efficiency of predators or providing refuge and ameliorating physical stress (Grabowski and Powers, 2004; Jackson et al., 2008). The shell can also alter small-scale hydrodynamics (Moulin et al., 2007), thus altering settlement and sedimentation patterns of other organisms and particulate matter respectively (Butman et al., 1988; Eckman et al., 1994). By filter-feeding, *C. gigas* can compete with other filter-feeders for food such as cockles (Smaal, 2005; Van den berg et al., 2005), polychaetes (Dubois et al., 2006b), native oysters (Bourne, 1979; Krassoi et al., 2008) and blue mussels (Cognie et al., 2006; Diederich, 2006), or can alter the diversity or structure of assemblages in the water column by consuming phytoplankton, zooplankton (Pietros and Rice, 2003) and larvae (Pechenik et al., 2004). Furthermore, filtration by *C. gigas* may increase water clarity, enhancing light penetration, thereby increasing the growth of algae (Sousa et al., 2009).

The deposition of faeces and pseudofaeces by *C. gigas* can organically enrich sediments, potentially altering redox potentials at the sediment–water interface, grain size distributions which can affect the distribution of benthic fauna (Rhoads, 1974; Cruz Motta et al., 2003) and biogeochemical processes in the sediment, potentially altering nutrient cycling. Nutrient cycling, in broad terms, is the movement and exchange of organic and inorganic matter back into the production of living matter. Sediments of marine coastal ecosystems are important for nutrient recycling and micro-organisms play the dominant role in the recycling, decomposition and mineralisation or remineralisation of organic matter (Jørgensen, 2000, see Figure 1). Organic matter is typically provided to marine sediments by benthic photosynthesis or by the deposition of dissolved and particulate organic matter from the water column. The breakdown of organic matter in surface sediments releases inorganic nutrients to the overlying water and thereby supports primary productivity (del Georgio and Williams, 2005). Decomposition, the breakdown of organic matter by catabolic metabolism results in (Re)mineralisation where organic matter is converted into inorganic components which may be used directly by plants. As a result of (re)mineralisation, organic matter is incorporated into microbial and faunal biomass and inorganic nutrients, such as NH_4^+, phosphate and CO_2 are released into the environment (Figure 1). The decomposition of organic matter is controlled by micro-organisms through aerobic and anaerobic processes (Kaiser et al., 2005). The most efficient process to obtain energy is oxic respiration of organic matter, where the oxygen molecule is used as an electron acceptor and organic matter as an electron donor. This can only take place where free oxygen is present, which is usually in the first few millimeters or centimeters of marine sediment. The depth of the oxic layer is determined by geophysical components of the sediment such as porosity and permeability and can be influenced by activities such as bioturbation of organisms living in the sediment (Mermillod-Blondin, 2011). In general,

following redox gradients, aerobic respiration is followed by nitrate reduction, reduction of manganese, then iron, sulphate and finally methane (Froelich et al., 1979, Figure 1). The succession of the different electron acceptors is based on the energy yield of the reactions and on the types of micro-organisms present (Jørgensen, 2000). The energy yield of aerobic respiration is highest compared to all other pathways, but not all micro-organisms can respire aerobically. When a favourable electron acceptor is depleted, the next favourable will be used, sometimes with vertical overlap (Schulz and Zabel, 2000).

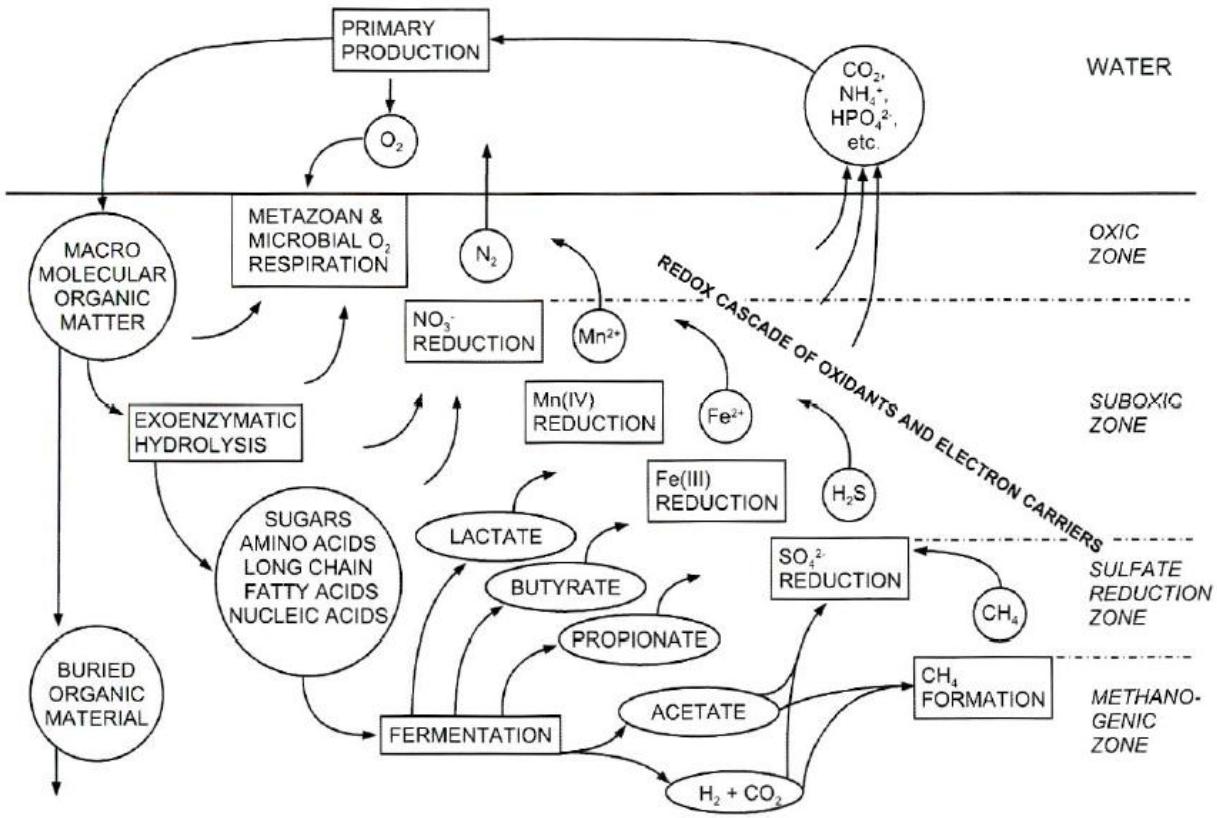

Figure 1. Conceptual model of organic matter decomposition pathways and the geochemical zonation in marine sediments. Figure reproduced from Jørgensen (2000).

In marine sediments, organic matter constitutes an important food source for benthic fauna and micro-organisms. Carbon and nitrogen cycling are of the utmost importance as these elements are of primary importance to the growth of all organisms (Schulz and Zabel, 2000). Heterotrophic organisms use organic carbon as an electron donor to obtain energy and as a carbon source to build up biomass. Carbon fixation underpins the cycling of other essential elements through complex food webs at various trophic levels. Although the supply of inorganic carbon for photosynthesis is seldom limiting in marine systems, the role of the ocean in the global carbon cycle has received intense attention in recent decades as a result of anthropogenic disturbance (Kaiser et al., 2005). The majority of carbon is stored in marine sediments and rocks (Wisniewski and Lugo, 1992). Nitrogen is the element that most frequently limits primary production in the oceans (Kaiser et al., 2005). In addition to serving as a source of inorganic nitrogen to the water column through (re)mineralisation, sediments may also act as a source of atmospheric nitrogen via denitrification. Through changes to the quantity and quality of organic matter, *C. gigas* could alter biogeochemical processes such as decomposition and (re)mineralisation of organic matter.

In fact, possibly from such alterations, dense populations of farmed *C. gigas* have been found to increase concentrations of ammonia and sulphur to toxic levels in the sediment and subsequently reduce the abundance of many benthic organisms (Bouchet et al., 2007). Despite this, no studies have explicitly investigated the effects of different abundances of wild *C. gigas* on biodiversity and ecosystem functioning. One particular concern of invasive *C. gigas* is their potential to control and limit the standing stock of phytoplankton in the water column, thus altering the carrying capacity of estuaries (Gibbs et al., 2005).

Recently, populations of wild *C. gigas* have been found in Ireland with the greatest

occurrence in Lough Foyle and Lough Swilly in County Donegal (Kochmann et al., unpublished). Each of these loughs is designated as a Special Protection Area (SPA) and a Special Area of Conservation (SAC), important for protected habitats and several internationally and nationally listed bird species (NPWS, 2011). The loughs are also important for Ireland economically in terms of tourism and aquaculture. The growth and spread of invasive populations of *C. gigas* around Ireland may threaten important cultural, provisioning and supporting ecosystem services.

1.7 Aims of this thesis

The aims of this research were to assess (i) the potential impacts of *C. gigas* on biodiversity and ecosystem functioning and to characterise how effects vary with respect to (ii) different environmental contexts, such as in different habitats and at (iii) different abundances of *C. gigas*. These aims were achieved using manipulative field experiments which are presented as different chapters in this thesis. Chapter II examines the effect of increasing cover of *C. gigas* on biodiversity in intertidal boulder-fields and attempts to separate the effects of the physical structure and biological activities of *C. gigas*. Chapter III addresses the effect of increasing cover of *C. gigas* on biodiversity within mussel-beds and mud-flats and further examines how these effects vary between locations and after different lengths of time. Chapter IV examines the functional consequences of increasing cover of *C. gigas* at one of the locations described in Chapter III. Chapter V provides a detailed analysis of the effects of increasing cover of *C. gigas* on the diversity, composition and activity of microbial assemblages coupled with simultaneous alterations to ecosystem functioning. Potential causal links between changes to microbial assemblages and alterations to ecosystem processes are discussed. Finally, Chapter VI draws together the elements of the research described above, placing them into a general context of current and proposed future research.

Chapter II: Impacts of non-indigenous oysters on biodiversity and a protected biogenic habitat in an intertidal boulder-field

2.1 Introduction

Invasions of introduced species have become increasingly common due to the influence of anthropogenic activities (Chapin et al., 2000; Naylor et al., 2001; Butchart et al., 2010). Where they become established, invasive species can have serious impacts on native biodiversity and ecosystem functioning (Simberloff, 2005; Molnar et al., 2008). Organisms which create biogenic habitat, also referred to as ecosystem engineers (Jones et al., 1997) or foundation species (Dayton, 1975), are a functionally important group which may increase diversity of other organisms (Bruno et al., 2003). Invasive ecosystem engineers can have far reaching impacts on the structure of assemblages over space and time (Jones et al., 1994) through multiple direct and indirect mechanisms (Crooks, 2002). Understanding the complex mechanisms by which invasive species alter assemblages is crucial in order to appreciate the full extent of their potential impacts and to improve strategies for their reduction (Watling et al., 2011).

The Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), an ecosystem engineer originally from Japan and South-east Asia, has become globally invasive due to its extensive use in aquaculture since the 1900s (Bourne, 1979). *C. gigas* is of particular concern as an invasive species because of its ability to form dense populations, in some cases over 400 individuals per m^2 (Wrange et al., 2010), and to dominate entire shores (Diederich et al., 2005; Ruesink et al., 2005). *C. gigas* has been found to increase biodiversity, alter assemblage structure and change the abundance and distribution of native species in a number of habitats worldwide, including mudflats and mussel beds (Reise et al., 2006; Ruesink, 2007; Buttger et al., 2008; Markert et al., 2010), marshes (Escapa et al., 2004), polychaete reefs (Dubois et al., 2006a) and rocky platforms (Krassoi et al., 2008; Trimble

et al., 2009). Many of these studies have, however, been observational rather than experimental (but see Kochmann et al., 2008), so causal links remain largely unconfirmed. As the density of *C. gigas* increases, the effect it has on the physical, chemical and biological properties of the environment may change in nature or magnitude (Sousa et al., 2009) but so far no studies have explicitly addressed the effect of increasing densities of *C. gigas* on biodiversity. Indeed variation in impacts with density has been identified as a key gap in empirical research into biological invasions (Thomsen et al., 2011a).

Despite the extensive research on impacts of *C. gigas*, there has been no work to date addressing the effects of *C. gigas* on the establishment of assemblages in intertidal boulder fields. Boulder fields are unique habitats, often inhabited by a range of rare or endemic species (Kangas and Shepard, 1984; Chapman, 2005) including biogenic habitat forming species, which are a particularly important component of biodiversity (Bruno et al., 2003) and often considered a priority for conservation. *C. gigas* exists in wild populations in a number of habitats globally and coexists with protected and important biogenic habitat forming species, such as the Honey-comb worm, *Sabellaria alveolata* (Linnaeus, 1767) (Cognie et al., 2006) a reef-building polychaete which creates habitats protected under Annex I of the EU Habitats Directive. Despite the importance of this biogenic habitat, the impacts of *C. gigas* on the establishment of *S. alveolata* reefs have received little attention (Dubois et al., 2006a).

The influence of *C. gigas* on other biota may either be due to its physical structure or its biological activity or a combination of both. The structure of *C. gigas* can displace or facilitate other organisms. Under certain environmental conditions, *C. gigas* can out-compete other sessile organisms, such as eelgrass (Tallis et al., 2009), cockles (Smaal, 2005; Van den berg et al., 2005), native oysters (Bourne, 1979; Krassoi et al., 2008) and blue mussels (Cognie et al., 2006; Diederich, 2006). Their shells also provide novel

habitat, however, which can facilitate other native and non-indigenous organisms. The shell of *C. gigas* is structurally complex and has been found to increase the abundance and diversity of organisms in a variety of habitats (Gutierrez et al., 2003; Peterson et al., 2003; Dubois et al., 2006a; Markert et al., 2010; Lejart and Hily, 2011). Alternatively, diversity can remain unchanged, but the structure of assemblages may differ to those of indigenous biogenic habitats (Kochmann et al., 2008; Markert et al., 2010). Because the structure of *C. gigas* is so complex it can greatly alter small-scale hydrodynamics (Moulin et al., 2007) and subsequently affect the establishment of other taxa, since the hydrodynamic properties of the benthic boundary layer (in terms of flow velocity and sediment transport) are important in determining the settlement of particulate matter and larvae (Butman et al., 1988; Eckman et al., 1994).

The biological activity of *C. gigas* can also affect the settlement of particulate matter and larvae by increasing turbulence in the water column through its filter-feeding activities (Troost et al., 2009). At the same time, by filtering particulate matter in the water column, *C. gigas* can compete for food with other filter-feeders, (such as polychaetes: Ropert and Goulletquer, 2000; Dubois et al., 2009) and decrease their survival and growth rates, or in some cases, can directly filter the larvae of other organisms (Pechenik et al., 2004) thereby decreasing the number of larvae that settle. Furthermore, filtration by *C. gigas* may increase water clarity, enhancing light penetration, thereby increasing the growth of algae (Sousa et al., 2009). The deposition of faeces and pseudo-faeces by *C. gigas* can alter the physical and chemical properties of the environment, in terms of increased sedimentation and nutrient enrichment, in extreme cases this process can lead to toxic levels of ammonium and hydrogen sulphide for other organisms, such as eelgrass (Kelly and Volpe, 2007). In some circumstances, however, bio-deposition can facilitate organisms. For example, ammonia excreted by *C. gigas* can increase the growth of algae (Reusch et al., 1994).

The aim of this study was, therefore, to test the effects of increasing cover of *C. gigas* on biodiversity and the establishment of *S. alveolata* on intertidal boulders and to distinguish between the influence of the physical structure of the oysters and their biological activity. An initial survey to assess differences in the abundance of *S. alveolata* on boulders with and without *C. gigas* was followed by an experimental manipulation to test the following hypotheses: i) biodiversity and the establishment of *S. alveolot*a on boulders will be increasingly modified with increasing densities of oysters; ii) If the effects of *C. gigas* are due to its physical structure alone, the same result would be expected regardless of whether the oysters were living or dead iii) If the effects are due to the biological activity of *C. gigas* only, the result would only occur when the oysters were living and there would be no effects of dead oysters. iv) The effects could also be due to a combination of the physical structure and biological activity of *C. gigas*, in which case both living and dead oysters would have an effect, but the influence of living oysters would be different from that of dead oysters.

2.2 Materials and methods

2.2.1 Study site

The present study was done in the mid to low shore area of an intertidal boulder-field at Lough Swilly (Ballylin Point, County Donegal, Ireland: 55° 2' 36.12", -7° 33' 36.09") with recently established populations of wild *C. gigas* oysters. The boulder-field extends approximately five kilometres along the coast and is situated in a sheltered estuary that is rarely visited by people. Most of the boulders on the shore are made of sandstone, although granite and shale boulders are also present. The diameter of boulders on the shore ranged from 10 to 200 cm, although the majority were between 18 and 24 cm and the average (± S.E.) was 22.3 (±0.5) cm. The density of boulders was approximately 5 per m^2 and boulders were resting either on bedrock, sediment, small pebbles or other

boulders. Most boulders were colonised by a mixture of algae, barnacles, oysters, and gastropods on the topside and barnacles, bryozoans, sponges, polychaetes, gastropods and crustaceans on the underside.

2.2.2 Cover of *S. alveolata* on boulders with and without *C. gigas*

On a 50 m section parallel to the shore, 40 boulders with oysters and 40 boulders without oysters were identified, numbered and their positions marked on a map. Boulders chosen were of a similar size and rock-type and occurred at the mid to low tide level. Out of these, 20 boulders with oysters and 20 boulders without oysters were randomly selected. The percentage cover of *S. alveolata* on these boulders was estimated by point-intercept sampling using a 10 cm side grid subdivided into 2 cm side quadrats (i.e. 25 intersections) which was randomly placed twice on each of the topside and underside of the boulder to obtain a measure out of 100 for each boulder.

2.2.3 Experimental addition of *C. gigas* onto boulders

The experiment involved two fixed and orthogonal factors; 1: 'state of oysters' (2 levels; living or dead) and 2: 'cover of oysters' (4 levels; 0, 5, 50 and 100 % cover) which equated to approximately 0, 1, 4 and 8 individual oysters. Seven replicate boulders were allocated randomly to each treatment, giving a total of 56. All boulders used in the study were similar in shape, made of sandstone and were approximately 25 x 20 x 10 cm in size and weighed approximately 12 kg. All oysters (living and dead) used in the experiment were collected from nearby mussel beds in Lough Swilly. Oysters in the range 40 - 100 mm maximal length were collected, cleaned of any flora or fauna on their shells, and randomly allocated to treatments so that any differences in size and shape would be randomized among treatments. The left and right valves of the dead oysters were glued together so that their physical structure did not differ from that of the living oysters. Oysters were then attached

to the tops of boulders using a two-part epoxy resin (ARALDITE rapid; Huntsman Advanced Materials, USA) as in Jackson (2009) and allowed to dry for 12 h before the boulders were deployed at the site. To account for any possible effects of the glue, a procedural control was included, in which only glue was added to 7 boulders. The amount of glue that was added was similar to that of the boulders with 50 % cover of oysters. All the boulders used in this study were collected from the upper shore, because they were free of an existing marine assemblage, but they were also scraped, and cleaned with a blowtorch to ensure that there was no remaining biofilm. In order to account for possible differences between upper and lower shore boulders, another procedural control was included in which 7 boulders from the lower shore were also scraped and cleaned with a blowtorch. Boulders were deployed in mid April 2010 and were sampled after 4, 9 and 14 months. The first and last sampling periods were chosen because the reproductive peak for *S. alveolata* is between June and September and the experiment was run for long enough to allow settlement and establishment of this species (Culloty et al. 2010). The length of time between sampling periods was sufficient to allow assemblages disturbed by the non-descructive sampling to fully recover (Chapman & Underwood 1996).

2.2.4 Sampling of experimental boulders

The surface area of each boulder was approximated to that of a sphere and was calculated using the average diameter. The total individual *S. alveolata* tubes on the topside and underside of the boulders were recorded and combined to obtain a measurement for the whole boulder which was converted to a measure of density per m^2. All other organisms on the boulders were also identified and counted, but were not removed from the boulders so as to minimise disturbance. Organisms which could not be counted individually in the field, such as algae, bryozoans and barnacles were recorded as percentage cover using the point-intercept method described in Section 2.2.2.

2.2.5 Statistical analysis

To compare the percentage cover of *S. alveolata* on boulders with and without oysters in the initial sampling programme, a two-tailed t-test was used.

Variation in assemblage structure among treatments at each sampling time was compared using two factor PERMANOVA (Anderson, 2001) based on the design described in Section 2.2.3 and using Bray-Curtis dissimilarities (Bray and Curtis, 1957) of square root transformed data with 9999 permutations under the reduced model. Where differences in assemblage structure were found, the data were ordinated on a 2-dimensional non-metric multidimensional scaling (nMDS) diagram, with the stress values representing the level of distortion of the actual rank order of distance among samples (Clarke, 1993). Where significant differences were found SIMPER (Clarke, 1993) analyses were also done of square root transformed data and were used to assess the contribution of different taxa to dissimilarities between treatments.

Univariate analyses were also done of selected variables using the same design described above. These included the following diversity indices; species richness (SR), Shannon-Wiener index (H') and Pielou's evenness (J'). Differences in the density of *S. alveolata* and the density or percentage cover of some other taxa thought to be susceptible to the impacts of oysters, or found as dominant space occupiers on the shore, were also compared. Specifically, these were bladder-wrack algae, *Fucus vesiculosus* (Linnaeus, 1767), solitary ascidians, *Ascidia conchilega* (Müller, 1776), and common periwinkles, *Littorina littorea* (Linnaeus, 1758).

The potential influence of artefacts due to the experimental procedures on multivariate assemblage structure and the density of *S. alveolata* was tested using two analyses: firstly

to test for the effects of glue one-way PERMANOVAs and one-way ANOVAs were done with the factor; 'type of boulder' with 4 levels (blank control, glue control, 50 % living oyster or 50 % dead oyster boulders). The glue control was only done for the 50 % cover treatment due to practical limitations. Secondly, to test for possible effects of using upper shore boulders in the mid-low shore, a one-way PERMANOVA and a two-tailed t-test were done comparing upper shore blank controls with mid-low shore blank controls.

All multivariate analyses were done using the PRIMER package (PRIMER-e, 2009). All univariate analyses were done with Analysis of Variance (ANOVA) on untransformed data using the software Win-GMAV (Underwood and Chapman, 1998). Heterogeneity of variance was tested using Cochran's *C*-test. When this was significant data were square root transformed to decrease the probability of inflated Type I error rates, i.e. incorrectly rejecting a true null hypothesis (Cochran, 1947). Furthermore, it should be noted that heterogeneity of variance only causes serious problems for the use of ANOVA when the variance of one group is significantly larger than the others or when the design is unbalanced (Underwood, 1997). This did not occur in the current experiment, nor in the subsequent experiments. When significant differences were detected by ANOVA ($P < 0.05$), Student-Newman Keuls (SNK) tests were done to identify patterns of difference.

2.3 Results

2.3.1 Amount of *S. alveolata* on boulders with or without *C. gigas*

The percentage cover of *S. alveolata* on boulders ranged from 0 to 100% and there was significantly less cover on boulders with oysters than on boulders without oysters (Figure 2, t-test: $P = 0.04$).

2.3.2 Experimental addition of *C. gigas*

After 4 months, 6 replicate boulders were found for each treatment. After 9 months, only 4 were found. This sampling period occurred in January during particularly harsh weather which included snow and hail, making it difficult to locate the boulders. After 14 months, 6 replicates were found in all except the treatment with 5 % cover of living oysters, for which 5 replicates were found. In this case, analyses were done with n=6. The mean of the 5 available replicates was used as the 6^{th} replicate for this treatment and the residual degrees of freedom were reduced by 1 (Sokal and Rohlf, 1981; Underwood, 1997).

2.3.3 Effects of *C. gigas* on biodiversity

A total of 38 taxa were found during this experiment (Appendix 1). Species richness (SR) did not differ among treatments after 4 and 9 months, but after 14 months SR was affected by the cover of oysters regardless of their state, where boulders with 5 % cover of oysters had more taxa than boulders with no oysters (Figure 3a, Table 1, SNK procedure).

Shannon-Wiener diversity (H') did not differ after 4 months, but after 9 months was affected by the cover of oysters (Table 1), although no differences could be resolved by SNK procedure. After 14 months, H' was affected by the interaction between cover and state of oysters: boulders with 5 % cover of living oysters had a greater diversity than boulders with 50, 100 % or no living oysters whereas diversity was greater on boulders with 5, 50 or 100 % cover of dead oysters than on boulders with no oysters (Figure 3b, Table 1, SNK procedure).

Pielou's evenness (J') was affected by the cover of oysters at all times, regardless of their state (Table 1). After 4 months, J' on boulders with 100 % cover of oysters was less than that of the other treatments. After 9 months no differences could be resolved by SNK

procedure and after 14 months J' on boulders with no oysters was less than on boulders with 5, 50 or 100 % cover of oysters (Figure 3d, Table 1, SNK procedure).

2.3.4 Effects of *C. gigas* on community structure

After 4 and 9 months, assemblage structure did not differ among treatments (Table 2). After 14 months, however, it differed between boulders with living and dead oysters and among different levels of cover of oysters on boulders (Table 2). Boulders with 5 % or no cover of oysters had assemblages with different structure from those on boulders with 50 or 100 % cover of oysters. These differences are apparent in the nMDS plot which shows boulders with 100 and 50 % cover of oysters separated from boulders with 5 % and no cover of oysters (Figure 4). Overall, boulders with living oysters had a greater density of the gastropods *L. littorea* and *Gibbula umbilicalis,* the common chiton *Lepidochitona cinerea* (Linnaeus, 1767), juvenile *C. gigas* oysters, polychaete worms of the family Spirorbidae and greater cover of *F. vesiculosus* than boulders with dead oysters which had a greater density of *S. alveolata*, the gastropods *Nucella lapillus* (Linneaus, 1758) and *Patella vulgata* (Linneaus, 1758), and the common Keel worm, *Pomertoceros triqueter* (Linneaus, 1758) (SIMPER analysis). In addition, there was a greater density of *S. alveolata*, *P. vulgata*, *G. umbilicalis* and *P. triqueter* on boulders with 5 or 0 % cover of oysters than on boulders with 50 or 100 % cover of oysters. On the contrary, there was a greater density of *L. littorea*, juvenile *C. gigas* oysters and greater cover of *F. vesiculosus* on boulders with 50 or 100 % cover of oysters than on boulders with 5 or 0 % cover of oysters (SIMPER analysis). Interestingly, boulders with 5 % cover of living oysters had the greatest density of several organisms including *L. cinerea*, *P. lamarcki*, *G. umbilicalis*, *P. vulgata*, the anenome *Actinia equina* (Linneaus, 1758) and polychaete scale worms of the family Polynoidae.

2.3.5. Effects of *C. gigas* on the establishment of *S. alveolata*

After 4 months, the recruitment of *S. alveolata* was very patchy and there were no differences among treatments (Figure 5a, Table 3). After 9 months, there was an increasing density of *S. alveolata* with a decreasing cover of oysters, regardless of whether the oysters were living or dead, specifically, there was significantly more *S. alveolata* on boulders without any oysters than on boulders with 5, 50 or 100 % cover of oysters (Figure 5b, Table 3, SNK procedure). This pattern was more pronounced on boulders with living oysters, but was significant for the factor 'cover' alone, indicating no major influence of the state of the oysters. By 14 months, although the density of *S. alveolata* had decreased everywhere, the previous pattern was retained with more *S. alveolata* on boulders without *C. gigas* regardless of whether the oysters were living or dead (Figure 5c, Table 3, SNK procedure). *S. alveolata* was primarily found on the bottom surfaces of boulders, in 84 of the 120 times it occurred throughout the experiment (χ^2 = 19.2, *d.f.* = 1, P < 0.0001).

2.3.6 Effects of *C. gigas* on the establishment of *F. vesiculosus*, *A. conghilega* and *L. littorea*

After 4 months, there was an insufficient cover of *F. vesiculosus* to analyse the data. After 9 months, there was significantly greater cover of *F. vesiculosus* on boulders with 100 % cover of oysters, regardless of whether they were living or not, than on boulders with less or no oysters (Figure 6a, Table 3, SNK procedure). After 14 months, there was significantly greater cover of *F. vesiculosus* on boulders with 100 % and 50 % cover of oysters than on boulders with 5 % or no oysters (Figure 6b, Table 3, SNK procedure). There was also a significantly greater cover of *F. vesiculosus* on boulders with living oysters than on boulders with dead oysters (Figure 6b, Table 3, SNK procedure).

After 4 and 9 months there was a significantly greater density of *A. conchilega* on boulders with dead oysters than on boulders with living oysters (Figure 7a and b, Table 3), but after 14 months the density of *A. conchilega* had greatly decreased on all boulders and was insufficient for a formal analysis.

After 4 months there were no differences in the density of *L. littorea*. After 9 months, there was a greater density of *L. littorea* on boulders with 100 % cover of oysters, although this was not significant (Table 3, Figure 8b). After 14 months there was a significantly greater density of *L. littorea* on boulders with 100 % cover of oysters than on boulders with no oysters (Figure 8c, Table 3, SNK procedure).

2.3.7 Effects of the experimental procedure

No artefacts of the experimental procedure in terms of adding glue or using upper shore boulders were found at any time during this experiment either for assemblage structure or for the density of *S. alveolata*. Assemblage structure was similar on blank control boulders, glue control boulders and boulders with a 50 % cover of living or dead oysters after 4 (PERMANOVA: Pseudo-F = 0.703, *d.f.* = 3, P = 0.834) and 9 months (PERMANOVA: Pseudo-F = 0.822, *d.f.* = 3, P = 0.662). But after 14 months, assemblage structure on blank control boulders was the same as that on glue control boulders, but each differed from that on boulders with 50 % cover of living or dead oysters (PERMANOVA: Pseudo-F = 2.285, *d.f.* = 3, P = 0.005, SNK procedure). After 4 months, the density of *S. alveolata* did not differ among blank control, glue control or 50 % living oyster boulders (ANOVA: F = 0.45, *d.f.* = 3, P = 0.717). After 9 and 14 months, the density of *S. alveolata* on blank control and glue control boulders were both greater than on boulders with 50 % cover of living or dead oysters (ANOVA: F = 4.3, *d.f.* = 3, P = 0.028 and F = 3.66, *d.f.* = 3, P = 0.029 respectively). These results mirrored those from the main experiment and indicate that

there were no effects of adding glue but there were effects of adding oysters to boulders.

The structure of assemblages on blank upper shore boulders was not different from that of assemblages on blank lower shore boulders at 4 (PERMANOVA: Pseudo-F = 0.885, *d.f.* = 1, P = 0.528), 9 (PERMANOVA: Pseudo-F = 0.418, *d.f.* = 1, P = 0.889) or 14 months (PERMANOVA: Pseudo-F = 0.661, *d.f.* = 1, P = 0.703). Furthermore, the density of *S. alveolata* on blank upper shore boulders did not differ from that on blank lower shore boulders at 4 (T-test: P = 0.720), 9 (T-test: P = 0.863) or 14 months (T-test: P = 0.761). This indicates that there were no effects of using boulders from the upper shore in the experiment.

Table 1. ANOVA for species richness, Shannon-Weiner diversity and Pielou's evenness on boulders with increasing cover of living and dead oysters after 4, 9 and 14 months.

	4 months			9 months			14 months			
Source variation	*d.f.*	MS	F	*d.f.*	MS	F	*d.f.*	MS	F	
Species										
State of oysters	1	16.33	2.11	1	2.53	0.43	1	2.52	0.21	
Cover of oysters	3	10.72	1.38	3	3.11	0.53	3	34.58	2.93	*
S x C	3	1.17	0.15	3	13.61	2.34	3	29.58	2.50	
Residual	40	7.75		24	5.82		40	11.82		
Shannon-										
State of oysters	1	5977.00	2.34	1	0.13	0.94	1	0.06	0.51	
Cover of oysters	3	0.18	0.71	3	0.47	3.39 *	3	1.06	9.47	***
S x C	3	0.02	0.09	3	0.14	1.04	3	0.35	3.13	*
Residual	40	0.26		24	0.14		40	0.11		
Pielou's										
State of oysters	1	0.01	0.83	1	0.03	1.12	1	0.00	0.20	
Cover of oysters	3	0.03	3.41 *	3	0.08	3.12 *	3	0.08	3.72	*
S x C	3	0.01	0.92	3	0.01	0.42	3	0.01	0.38	
Residual	40	0.01		24	0.03		40	0.02		

Significant results are indicated, * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Table 2. PERMANOVA of assemblage structure on square root transformed data of boulders with increasing cover of living and dead oysters after 4, 9 and 14 months with 9999 permutations of residuals under the reduced model.

	4 months			9 months			14 months			
Source of variation	*d.f.*	MS	Pseudo F	*d.f.*	MS	Pseudo F	*d.f.*	MS	Pseudo F	
State of oysters (=S)	1	2461.80	1.06	1	1812.30	1.11	1	3463.50	2.18	*
Cover of oysters (=C)	3	2094.80	0.90	3	2372.30	1.46	3	7215.70	4.55	**
S x C	3	450.67	0.19	3	1123.60	0.69	3	2243.90	1.41	
Res	40	2329.80		24	1626.30		40	1587.10		
Total	47			31			47			

Significant results are indicated, * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Table 3. ANOVA for density or cover of *S. alveolata*, *F. vesiculosus*, *A. conchilega* and *L. littorea* on boulders with increasing cover of living and dead oysters after 4, 9 and 14 months. After 4 months, there was insufficient cover of *F. vesiculosus* and after 14 months there was insufficient cover of *A. conchilega* to permit analysis.

	4 months				9 months				14 months			
Source of variation	*d.f.*	MS	F		*d.f.*	MS	F		*d.f.*	MS	F	
Density of *S. alveolata*												
State of oysters (=S)	1	74.11	1.17		1	830.28	0.67		1	4722.91	1.53	
Cover of oysters (=C)	3	21.71	0.34		3	5173.61	4.21	*	3	12951.13	4.21	*
S x C	3	98.99	1.57		3	98.61	0.08		3	800.17	0.26	
Residual	40	63.15			24	1230.09			40	3078.19		
Cover of *F. vesiculosus*												
State of oysters (=S)	1	-	-		1	913.78	1.82		1	3816.33	8.07	**
Cover of oysters (=C)	3	-	-		3	1866.78	3.72	*	3	6847.14	14.49	***
S x C	3	-	-		3	130.61	0.26		3	1227.50	2.60	
Residual	40	-			24	501.82			40	472.63		
Density of *A. conchilega*												
State of oysters (=S)	1	26.16	8.39	**	1	7.39	4.48	*	1	-	-	
Cover of oysters (=C)	3	2.96	0.95		3	0.66	0.40		3	-	-	
S x C	3	3.81	1.22		3	3.27	1.98		3	-	-	
Residual	40	3.12			24	1.65			40	-		
Density of *L. littorea*												
State of oysters (=S)	1	97.66	2.15		1	50.77	0.09		1	1.46	0.50	
Cover of oysters (=C)	3	28.86	0.64		3	1157.28	2.01		3	10.52	3.58	*
S x C	3	19.79	0.44		3	55.97	0.10		3	1.85	0.63	
Residual	40	45.42			24	574.40			40	2.94		

Significant results are indicated, * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

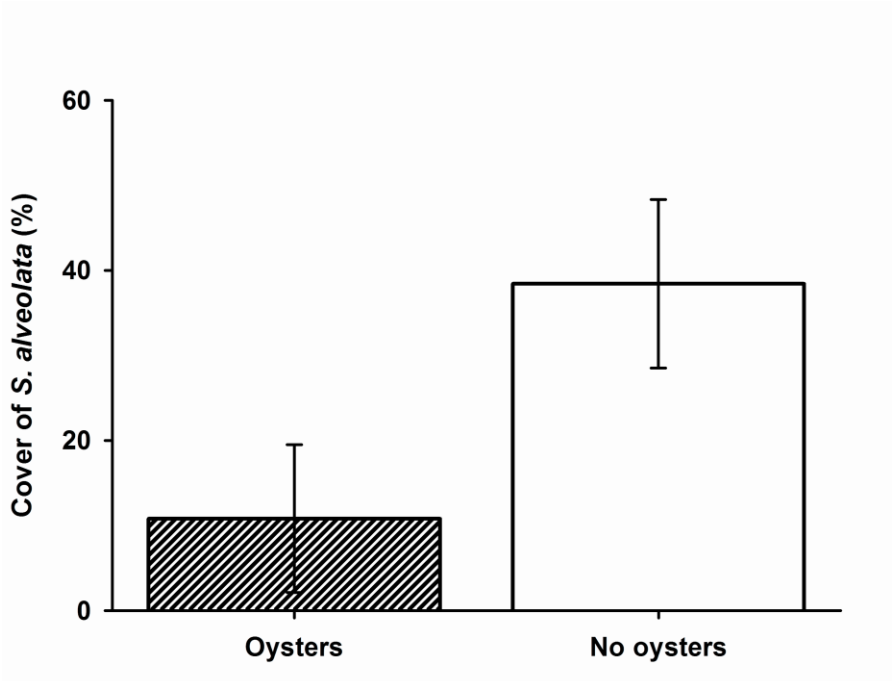

Figure 2. Mean % cover (+/- S.E.) of *S. alveolata* on boulders with (hashed lines) and without (clear) *C. gigas*. n = 20.

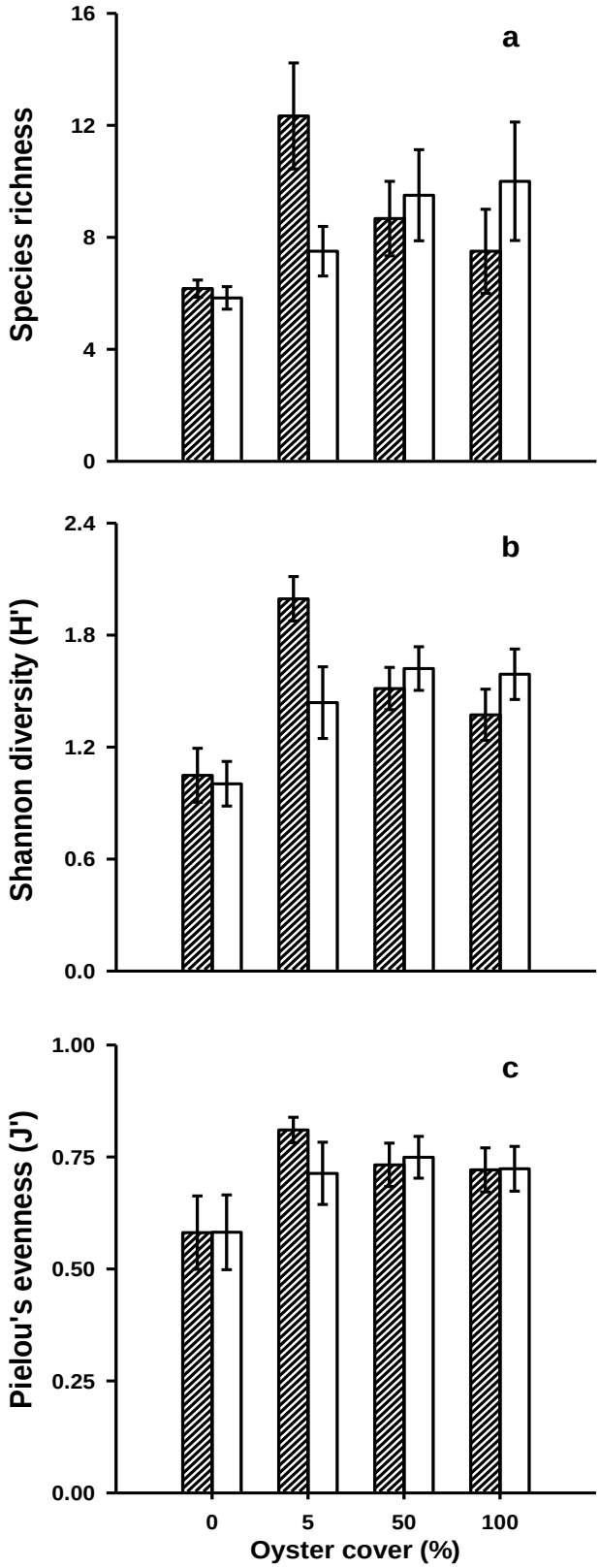

Figure 3. Mean (+/- S.E.) values of diversity indices per boulder (a) species richness, (b) Shannon-Weiner diversity and (c) Pielou's evenness for assemblages on boulders with living (hashed lines) and dead (clear) oysters at 0, 5, 50 and 100 % cover after 14 months.

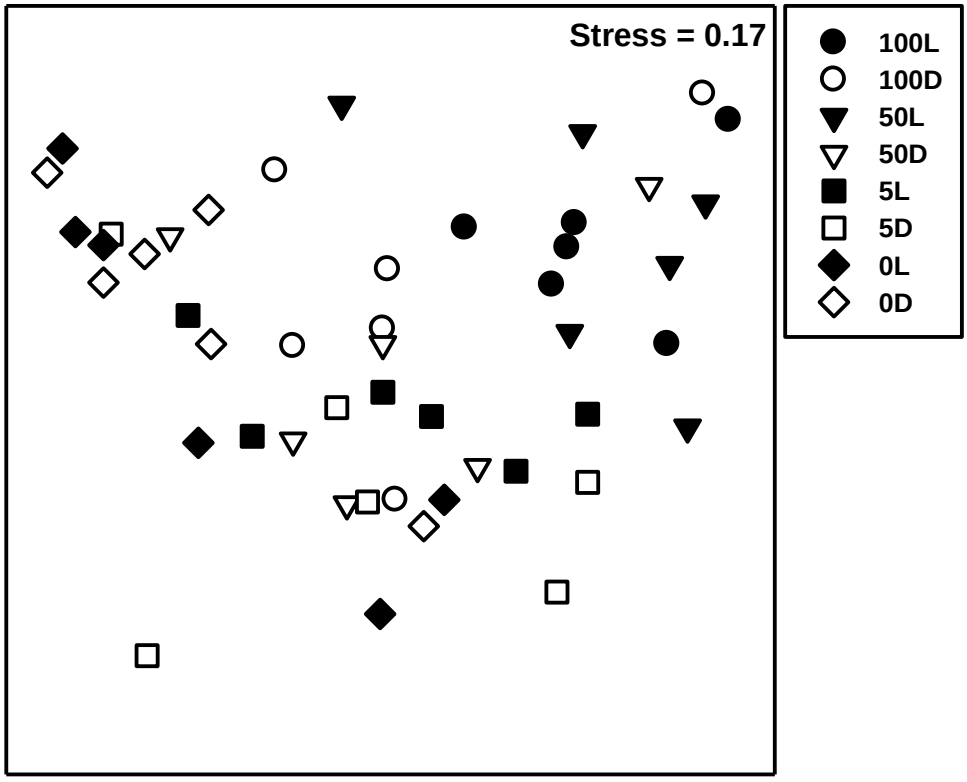

Figure 4. nMDS plot of square root transformed data of assemblages on experimental boulders after 14 months. Black shapes represent boulders with living oysters added on them and white shapes represent boulders with dead oysters added on them. Boulders have 100 (circles), 50 (triangles), 5 (squares) and 0 % (diamonds) cover of oysters. In the legend, the letter 'L' denotes living oysters and the letter 'D' denotes dead oysters.

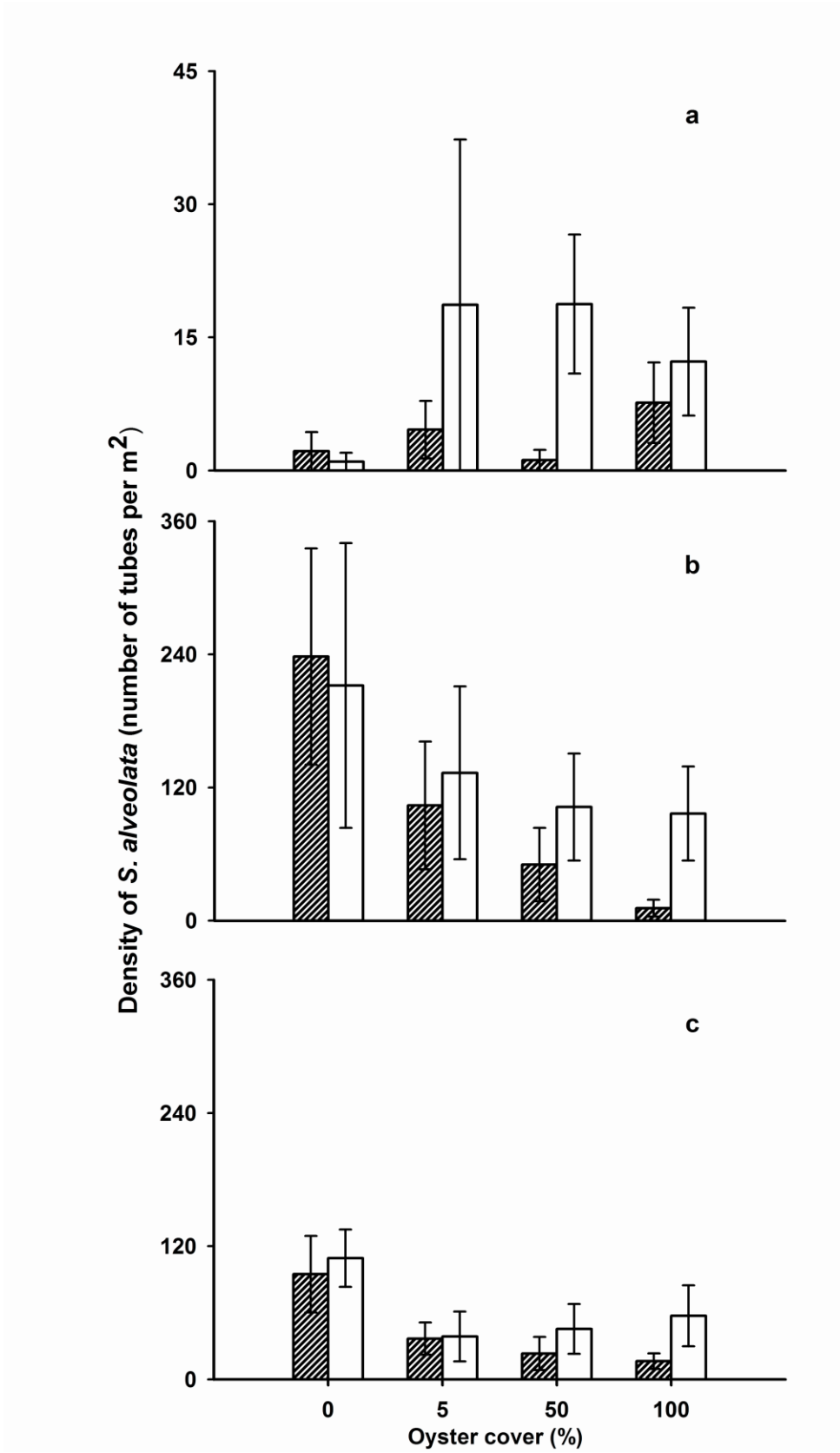

Figure 5. Mean density (+/- S.E.) of *S. alveolata* (number of tubes per m^2) on boulders with experimentally added living (hashed lines) or dead (clear) oysters at 0, 5, 50 and 100 % cover after 4 (a), 9 (b) and 14 (c) months.

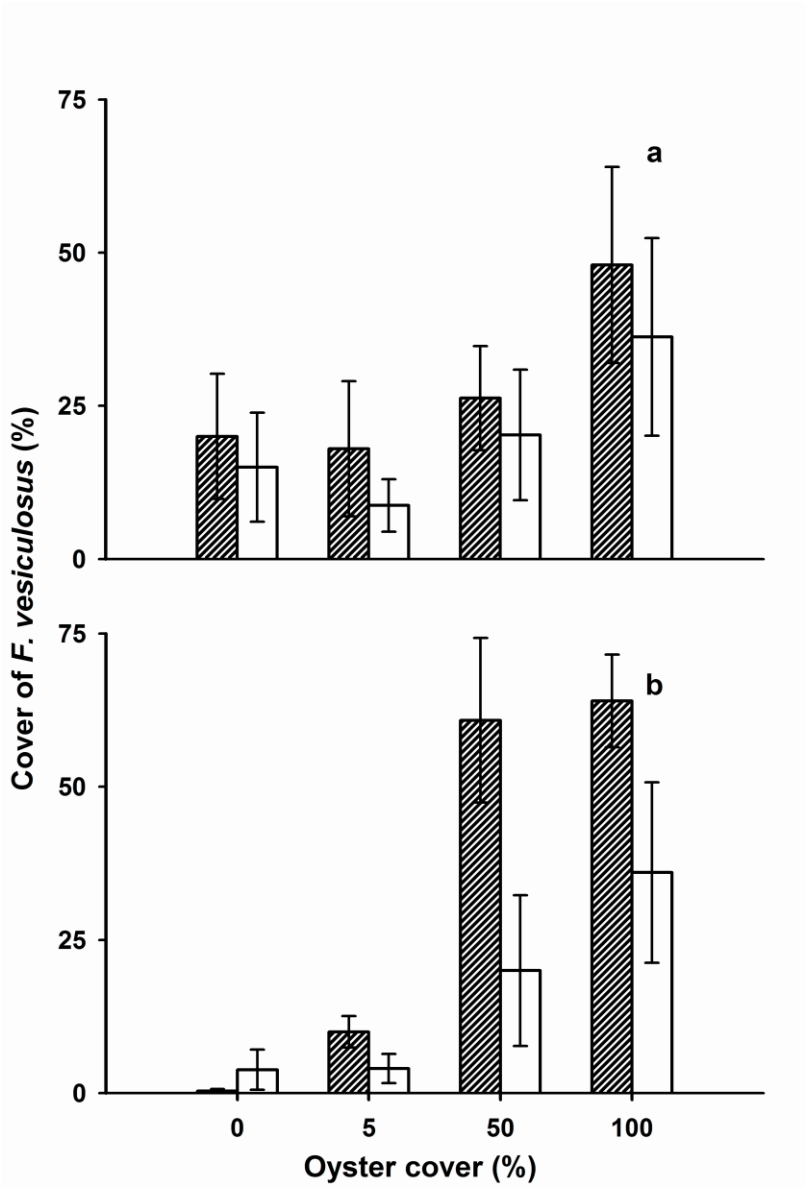

Figure 6. Mean percent cover (+/- S.E.) of *F. vesiculosus* on boulders with experimentally added living (hashed lines) or dead (clear) oysters in cover of 0, 5, 50 and 100 % after 9 (a) and 14 (b) months.

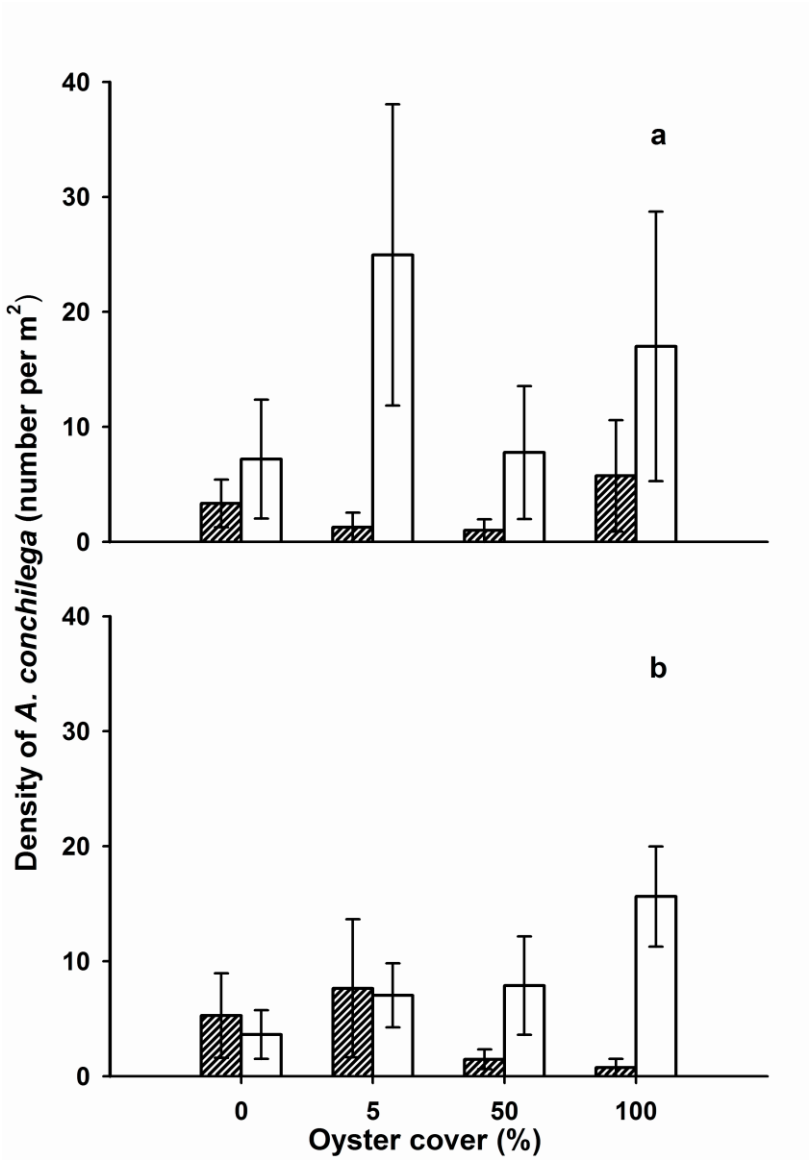

Figure 7. Mean density (+/- S.E.) of *A. conchilega* (number per m^2) on boulders with experimentally added living (hashed lines) or dead (clear) oysters at 0, 5, 50 and 100 % cover after 4 (a) and 9 (b) months.

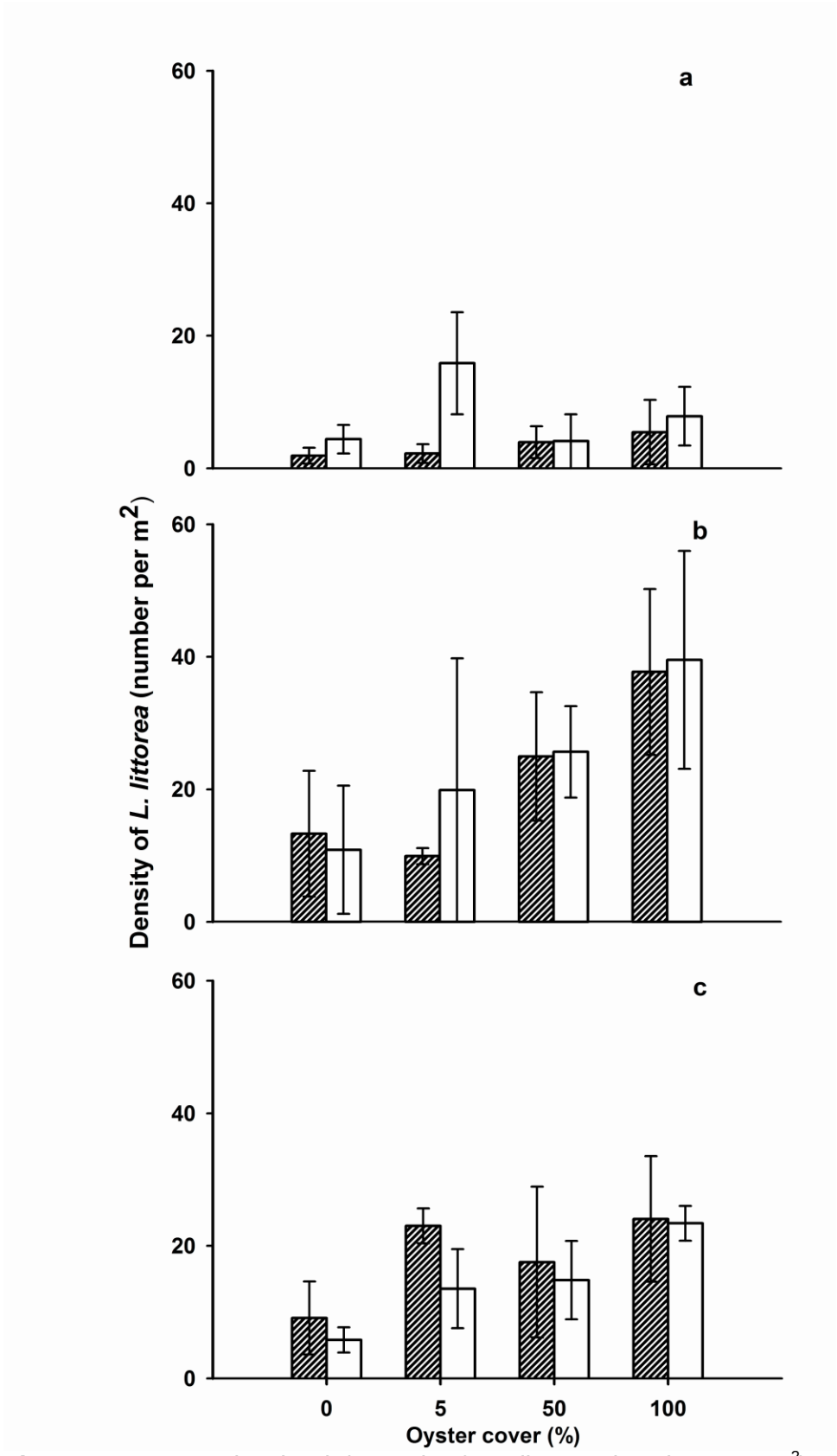

Figure 8. Mean density (+/- S.E.) of *L. littorea* (number per m^2) on boulders with experimentally added living (hashed lines) or dead (clear) oysters at 0, 5, 50 and 100 % cover after 4 (a), 9 (b) and 14 (c) months.

2.4 Discussion

The addition of *C. gigas* to the tops of boulders altered biodiversity, evenness, assemblage structure, and the establishment of several species. The nature and magnitude of most of these effects differed according to the cover of oysters alone, indicating that effects were mainly due to the physical structure of *C. gigas*, although some species also responded to their biological activity. Overall, boulders with oysters, regardless of their state or cover, supported more diverse and more even assemblages than boulders without oysters. This is probably due to an increase in habitat complexity (McCoy and Bell, 1991) provided by the physical structure of oyster shells, but effects differed depending on the cover of oysters. On boulders with a low cover of oysters (5 %), biodiversity and the establishment of several species was facilitated by the physical structure and the biological activities of *C. gigas*, as boulders with the least cover of living oysters had the greatest species richness and Shannon-Wiener diversity, the most even assemblages and supported a greater density of several taxa. With greater cover of oysters (50 and 100 %), however, these patterns seemed to reach a threshold or start to decline depending on the state of oysters. On boulders with dead oysters (physical structure only), biodiversity increased with increasing cover of oysters up until the second greatest cover (50 %) where it levelled out. This may be due to a lack of rocky habitat available for colonisation by other organisms due to the space being occupied by oysters. In contrast, the addition of living oysters (physical structure and biological activity) increased biodiversity with the least cover of oysters (5 %) but there were declines in biodiversity relative to this, with greater cover. It is possible that the biological activities of *C. gigas* at greater cover can inhibit other organisms from becoming established, for example, through competition for food. Alternatively, in both living and dead states, *C. gigas* facilitated the establishment of a few dominant species which may have indirectly prevented others from becoming established. For example, boulders with the greatest cover (50 and 100 %) of *C. gigas* were dominated by algae (*F. vesiculosus*) and grazing gastropods (*L. littorea*), resulting in less available

space for colonisation of other organisms compared to boulders with less cover of oysters (5 %), which still provided a more complex habitat than boulders with no oysters. Perhaps over a longer time period (> 14 months), the diversity on boulders with the greatest cover of living oysters would continue to decrease, although this could not be ascertained from the current study.

Despite the fact that biodiversity was increased by the least cover of living oysters, the establishment of *S. alveolata* on these boulders was greatly reduced (by approximately half) compared to boulders without any oysters. In fact, the establishment of *S. alveolata* was decreased even at the lowest cover of dead oysters, indicating that the physical structure of oysters can have strong effects. This could be due to either direct impacts of the oyster shells themselves because they occupy space or alter small scale hydrodynamics, or it could be due to indirect effects of the oysters' interactions with other organisms. Direct competition for space is unlikely since in this experiment *S. alveolata* was mostly found on the underside of boulders (even on boulders without *C. gigas*) and *C. gigas* were only attached to the topside. Therefore, hydrodynamics are a more likely cause. Modification of flow regimes caused by the physical structure of oysters (Lenihan, 1999; Soniat et al., 2004) can affect the arrival of particulate matter and settlement of larvae, thus affecting assemblage structure (Butman et al., 1988; Eckman et al., 1994; Commito et al., 2005). For many marine organisms, passive settlement patterns are more important than active ones and are dependent on the flow speed of the water (Butman, 1987 and 1989; Snelgrove and Butman, 1994; Boxshall, 2000). Rough surfaces, such as oyster shells, decrease flow speed, thereby increasing the potential for larvae to reach the substratum and settle (Wright and Boxshall, 1999). But the increased cover of *C. gigas* may decrease the amount of food available for other suspension feeders such as *S. alveolata*. Moulin et al. (2007) found that dense accumulations of invasive slipper limpets decreased local flow velocities, decreasing the resuspension of particulate matter and thus

decreasing the availability of food for other filter feeders. It is, therefore, possible that settlement of *S. alveolata* increased with increasing cover of *C. gigas*, but that post-settlement processes causing increased mortality and/or reduced growth (e.g. due to modifications in the flow of food particles) were responsible for the decrease in its establishment. Effects on the establishment of *S. alveolata* could also have been due to indirect effects of *C. gigas* due to its provision of habitat for other organisms. *F. vesiculosus*, for example, which was facilitated by *C. gigas*, can reduce the recruitment of other algae (Kiirikki, 1996) and invertebrates (Lewis and Bowman, 1975; Grant, 1977) by its "whiplash effect". It is also possible that damage may have been caused to *S. alveolata* by *F. vesiculosus* via mechanical abrasion (Leonard, 1999) or that smothering of tube ends by algal fronds may have inhibited their ability to feed and grow. The increase in density of *L. littorea* associated with *C. gigas* may also have contributed to the reduction in the establishment of *S. alveolata*. Grazing periwinkles, such as these, can cause mortality in the newly settled larvae of invertebrates through physical dislodgement or "bulldozing" effects (Miller and Carefoot, 1989; Hidalgo et al., 2008).

Unlike *S. alveolata*, *L. littorea* increased in density with increasing cover of *C. gigas*. Similar results have previously been found in regards to oysters on mussel beds (Diederich, 2005; Markert et al., 2010), likely because the shell of *C. gigas* is very structurally complex, providing additional refuge and biofilm for grazing compared to adjacent rocky surfaces (Kochmann et al., 2008). The cover of *F. vesiculosus* also increased with increasing density of oysters, which is contrary to other studies on mussel beds, which have found a decrease of *F. vesiculosus* with the occurrence of *C. gigas* (Diederich, 2005; Buttger et al., 2008; Padilla, 2010). This could be because of differences in the type of *F. vesiculosus* that is present in different habitats. In mussel beds of the Wadden Sea, there is an asexual form of *F. vesiculosus* referred to as "mussel *Fucus*" or *F. vesiculosus* forma *mytili* (Nienburg). Mussel *Fucus* never has a holdfast and is associated

with *Mytilus edulis* because the bysall threads of the mussel help to secure it to the substratum (Albrecht, 1998). On rocky shores, however, the sexual form of *F. vesiculosus* is more likely to occur and can secure itself to the substratum (in this case the shell of *C. gigas*) by a holdfast.

Aside from structural effects, *F. vesiculosus* was also affected by the biological functioning of the oysters, as it had a much greater cover on boulders with living oysters after 14 months. This may be due to nutrient enrichment from the deposition of faeces and pseudofaeces from oysters (Reusch et al., 1994). The opposite pattern was found for the density of *A. conchilega* which decreased on boulders with living oysters, possibly due to competition for food, as *A. conchilega* is also a filter-feeder. There may also be some effects of the biological functioning of *C. gigas* on the establishment of *S. alveolata*, because although not significant, the patterns were more pronounced on boulders with living than with dead *C. gigas*. Both of these filter feeders ingest food particles in the same size range (Dubois et al., 2003), so there is a possibility that they may compete for food. *C. gigas* can also reduce the settlement of other organisms by decreasing the abundance of larvae in the water column through larviphagy (Troost et al., 2008), although whether or not this applies to *S. alveolata* or *A. conchilega* is unknown. More profound effects, however, may result from the deposition of faeces and pseudofaeces from oysters which can increase the concentration of fine particles in the water column, clogging the tubes of *S. alveolata* and decreasing their filtration rates (Dubois et al., 2009).

Although the potential impacts of invasive marine organisms which form durable structures, such as shells, have been identified as a priority for management and conservation initiatives (Sousa et al., 2009), little has been done to assess their impact on protected habitats. *C. gigas* is already a globally invasive species and it is still spreading (Troost, 2010). It is clear from the results of this study that, even with limited cover and by

its physical structure alone, it can alter assemblage structure and increase biodiversity but markedly reduce the extent of protected biogenic habitats. Notably, *C. gigas* reduced the establishment of *S. alveolata*, a polychaete which creates some of the most extensive intertidal biogenic reefs in Europe (Desroy et al., 2011), which are key components of marine communities and have a great diversity of associated species (Holt et al., 1998; Frost et al. 2004).

C. gigas is one of a number of invasive habitat-forming species or ecosystem engineers (Jones et al., 1997) that have been shown to have profound direct and indirect effects on other organisms due to their physical structure alone (Crooks, 2002). For example, an experiment by Peterson and Andre (1980) found that the growth rates of some bivalves can be reduced by the presence of the dead shells of other dominant bivalve species (Branch and Steffani, 2004; Ward and Ricciardi, 2007). Understanding the mechanisms by which invasive species may alter receiving ecosystems is paramount to developing effective management strategies. The fact that *C. gigas* can have strong effects by virtue of its physical structure alone highlights the importance of assessing the total cover (dead and living) of invasive species which form habitats that can persist in the environment long after death and requires dead as well as living oysters to be taken into account when planning mitigation and restoration.

In the current study, assemblage structure on boulders was altered by the physical structure and the biological activities of oysters, and these impacts differed depending on the cover of oysters. Few studies (Aldridge, 2009; Pawson et al., 2010) have explicitly compared the effects of invasive species at different densities or cover (Thomsen et al., 2011a), but knowing the nature and magnitude of impacts as their populations increase can help to make management decisions that avoid detrimental economic or ecological consequences. This is particularly important for species which have great impacts even at

small densities or cover (Yokomizo et al., 2009), which the current study suggests may be the case for *C. gigas*.

Chapter III - Effects of non-indigenous oysters on biodiversity vary with increasing cover and environmental context

3.1 Introduction

Non-indigenous invasive species can profoundly affect ecosystems and economies worldwide (Chapin et al., 2000; Mack et al., 2000) through their alterations to native biodiversity (Bax et al., 2003; Molnar et al., 2008; Wright, 2011) and ecosystem processes (Ehrenfeld, 2010). As the density of an invasive species increases, the effect it has on the physical, chemical and biological properties of the environment may change in nature or magnitude (Sousa et al., 2009). Although there has been extensive research into impacts of invasive species, few studies have made comparisons of impacts at different abundances (Thomsen et al., 2010). This is a significant shortcoming in invasion biology because it precludes the ability to make generalisations about abundance-dependent impacts (Thomsen et al., 2010). The effects of invasive species are also likely to be context-dependent, differing depending on the properties of the receiving environment (Padilla, 2010; Queiros et al., 2011). Hence there is increasing recognition of the need to explore the effects of invasive species in different habitats in order to be able to understand mechanistic interactions between invaders and abiotic conditions (Sousa et al., 2009; Thomsen et al., 2011b).

Among the most influential invasive species are those which alter existing habitats and/or create new habitats that persist in the environment for a long time. Such species have been referred to as “ecosystem engineers” (Jones et al., 1994) or “foundation species” (Dayton, 1975). Invasive ecosystem engineers can have far reaching impacts on the structure of assemblages over space and time (Jones et al., 1994) through multiple direct and indirect mechanisms (Crooks, 2002). Many bivalves are ecosystem engineers and can physically alter the environment, affecting the availability of resources to other

organisms either through their physical structure (shell) or their biological activities (filter-feeding and biodeposition). Due to their potential to alter the structure and functioning of receiving ecosystems, invasive bivalves have been identified as a priority for conservation and management initiatives (Sousa et al., 2009) as they can have severe cascading economic impacts (Aldridge et al., 2004). For example, invasive bivalves having strong impacts on native ecosystems include the Zebra mussel, *Dreissena polymorpha* (Pallas, 1771) (Bially and MacIsaac, 2000), the Mediterranean blue mussel, *Mytilus galloprovincialis* (Lamarck, 1819) (Robinson et al., 2007) and the Pacific oyster, *Crassostrea gigas* (Linnaeus, 1767) (Troost, 2010).

C. gigas is a commercially important species (accounting for 98% of global commercial oyster production) and, as a consequence of deliberate introduction, has become globally invasive. *C. gigas* is able to modify habitats in a way that enhances its own further spread (Diederich, 2005) and as such, it can form very dense populations (over 400 individuals per m^2; Wrange et al., 2010). It is an ecosystem engineer which modifies the physical properties of the receiving environment and provides a complex three dimensional habitat for other organisms to colonise (Markert et al., 2010).

Invasive populations of *C. gigas* have been found to alter the abundance and distribution of native species in other parts of the world including the United Kingdom, Canada, Australia, New Zealand and Western Europe (Troost, 2010). Effects have been documented in a variety of different habitats, including mudflats and mussel beds (Reise et al., 2006; Ruesink, 2007; Buttger et al., 2008; Markert et al., 2010), marshes (Escapa et al., 2004), polychaete reefs (Dubois et al., 2006a) and rocky platforms (Krassoi et al., 2008; Trimble et al., 2009). In some cases, *C. gigas* was associated with an increase in the abundance and diversity of organisms (Gutierrez et al., 2003; Peterson et al., 2003; Dubois et al., 2006a; Markert et al., 2010; Lejart and Hily, 2011). In other cases, diversity

was unchanged, but the structure of assemblages associated with *C. gigas* differed from those associated with indigenous habitats (Kochmann et al., 2008; Markert et al., 2010). It is difficult to make generalisations about the impact of *C. gigas* on biodiversity since the effects of invasive species, as with many anthropogenic impacts, are context-dependent, varying in nature and magnitude depending on the receiving environment (Hewitt et al., 2008; Padilla, 2010; Thomsen et al., 2010; Queiros et al., 2011).

The majority of studies of impacts of *C. gigas* have been mensurative (Ruesink et al., 2005) rather than experimental (Escapa et al., 2004; Kochmann et al., 2008) and therefore causal links between increases in density of *C. gigas* and changes in biodiversity are not fully understood. The aims of this study were therefore to test the following hypotheses 1.) Biodiversity, assemblage structure and the abundance of individual taxa will be altered by *C. gigas* 2.) These impacts vary with increasing cover of *C. gigas*, 3.) Impacts will vary in different habitats, 4.) Impacts will vary in different locations and 5.) Impacts will vary after different lengths of time since establishment.

3.2 Materials and methods

3.2.1 Study sites

This study was done at two locations in Ireland, Lough Foyle (Quigley's Point, County Donegal: 55° 7' 14.87", -7° 11' 53.59) and Lough Swilly (Ballylin Point, County Donegal, Ireland: 55° 2' 36.12", -7° 33' 36.09"). At each location experimental plots were set-up on two different types of habitat, both in lower intertidal areas: mussel-beds and mud-flats. Mussel-bed habitats consisted of dense populations of blue mussels, *Mytilus edulis* (Linneaus, 1758). Mud-flat habitats were patches of mud interspersed between the mussel-beds which were not dominated by any other biogenic habitat building organism or by hard substratum. Sediment within mussel-beds was a mixture of fine sand, silt with

large shell fragments throughout. Sediment within mud-flats was a mixture of very fine sand, silt and clay. Plots were spaced at least 5 m apart and were spread across a section of shore that was approximately 100 m in length.

3.2.2 Experimental set-up

The experiment was set up during late April 2009 and had 2 factors: Habitat (fixed and orthogonal with 2 levels, mussel-bed or mud-flat) and cover (fixed and orthogonal with 4 levels, 0, 5, 50 or 100 % cover of oysters). These four levels of cover were arranged into 50 x 50 cm plots in mussel bed and muddy sediment habitat and equated to the following densities: 0, 16 +/- 0.5, 120 +/- 8 and 240 +/- 12 individuals per m^2. These four densities equated to 0, 26.06 +/- 2.13, 390.88 +/- 31.95 and 781.77 +/- 63.90 g per m^2 ash free dry weight. Mixtures of different sizes of oysters were used, ranging from 40 – 100 mm maximal length. All oysters used in this experiment were found in situ and were not moved between locations. Oysters were rinsed with seawater and cleaned of any epibionts prior to use and then simply inserted upright into the mud and mussel-bed habitats to mimic the positions in which they are found in natural populations. Oysters were inserted to simulate an overgrowth, rather than a complete replacement of mussels (Figure 9). Because sampling was destructive, 8 replicate plots of each treatment were set up and 4 replicates were sampled at each of 2 times. Plots were spaced at least 5 m apart and were spread across a section of shore that was approximately 25 m in length and 100 m in width.

3.2.3 Sampling methods

The experiment was sampled after 4 and 15 months, during late July, 2009 and late June, 2010 respectively. To measure epifauna and flora, a 25 x 25 cm quadrat was randomly placed onto each plot and a 2 minute search was conducted in which large mobile macrofauna, including gastropods and crabs, were counted and percentage covers of algae were estimated visually. After this, a core with 10 cm diameter was taken down to 10

cm depth in the centre of the plot. The top section, which included epifauna and the first 1-2 cm of sediment, was removed and placed into a container and preserved in 5 % formalin. Any epifauna or flora on the surface of the bivalves was later identified and counted and the sediment was sieved through a 125µm mesh, sorted and identified. Sessile epifauna including bryozoans, barnacles and polychaetes were counted as individuals not as colonies. The remainder of the core (from 2 to 10 cm depth) was sieved through a 500 µm mesh to retain macrofauna then placed into a container and topped up with 5 % formalin stained with rose bengal to preserve fauna for later sorting and identification in the laboratory. Organisms in quadrats and cores were scaled up to numbers per m^2.

3.2.4 Statistical analysis

Univariate analyses were done using 2-factor analysis of variance (ANOVA) on the same design described in 3.2.2. Analyses were done on the total number of individuals (N), species richness (SR) and Shannon-Wiener diversity (H'). Variation in assemblage structure among treatments at each sampling time and within each location was compared using two-factor PERMANOVA (Anderson, 2001) based on the design described above and using Bray-Curtis dissimilarities (Bray and Curtis, 1957) of square root transformed data with 9999 permutations under the reduced model. Assemblage data were ordinated on a 2-dimensional non-metric multidimensional scaling (nMDS) diagram, with the stress values representing the level of distortion of the actual rank order of dissimilarity among samples (Clarke, 1993). Where significant differences in assemblage structure were found, SIMPER (Clarke, 1993) analyses were done on square root transformed data and were used to assess the contribution of different taxa to dissimilarities between treatments. Tests for homogeneity of multivariate dispersions (PERMDISP: Anderson, 2004; Anderson, 2006) were done to identify heterogenous variability among experimental groups and were also used as a measure of β-diversity (Anderson et al., 2006), which can be defined as the

variation in community structure among samples, sites or experimental units. Differences in the abundance of taxa thought to be susceptible to the impacts of oysters based on previous studies, or found as dominant space occupiers on the shore were also analysed. These included common periwinkles, *Littorina littorea* (Linnaeus, 1758), green shore crabs, *Carcinus maenas* (Linnaeus, 1758), invasive barnacles, *Elminius modestus* (Darwin, 1854), and the total abundance of polychaetes.

Analyses of Variance were done using the software Win-GMAV (Underwood and Chapman, 1998). Prior to univariate ANOVAs, homogeneity of variance was tested and corrected for by the same method as detailed in Chapter II. When significant differences were detected by ANOVA ($P < 0.05$), post-hoc Student-Newman Keuls (SNK) tests were used to identify patterns of difference. All multivariate analyses were done using the PRIMER package (PRIMER-e, 2009).

3.3 Results

A total of 60 taxa were found throughout the duration of the experiment (Appendix 2). The abundance and identity of these varied between locations and over time but included 9 gastropod species, 6 bivalve species, 4 amphipod species, 3 algal species and 16 different families of polychaetes. At Lough Foyle after 15 months several plots were missing or damaged so only 3 replicate plots for each treatment were used in the analyses. In addition, by 15 months, the “100 %” plots at Lough Foyle were no longer completely intact and more closely represented 75 % cover.

3.3.1 Effects of *C. gigas* on the total number of individuals

After 3 months, N was unchanged within mussel-bed habitats but increased with increasing cover of oysters within mud-flat habitats at both locations (Figure 10a). Specifically, at Lough Foyle the 50 and 100 % plots had greater N than the 0 and 5 % and

the 5 had more N than the 0 %. At Lough Swilly the 100 was greater than the 50, 5 and 0 % (Table 4, SNK procedure).

After 15 months at Lough Foyle, N differed between habitats, with greater N in mussel-bed than in mud-flat habitats (Figure 10b). At Lough Swilly N was altered by the interaction between habitat and cover (Table 4). N increased with oyster cover within mud-flat habitats, but within mussel-bed habitats the 50 % was greater than the 0 and the 100 % plots which were equal (Figure 10b, SNK procedure).

3.3.2 Effects on biodiversity after 4 months

At Lough Foyle, neither SR nor H' differed among different covers of oysters in mussel-bed habitats, but within mud-flat habitats, the plots with 100 % cover of oysters had greater SR and H' than the plots with 0 and 5 % cover (Figure 10c and e, Table 4). In mud-flat habitats, plots with 50 % cover of oysters also had greater H' than plots without oysters (Table 4, SNK procedure). At Lough Swilly, neither SR nor H' differed among different covers of oysters in mussel-bed habitats, but within mud-flat habitats, SR and H' were all greater within plots with 5, 50 and 100 % cover of oysters than with plots with no oysters (Figure 10c and e, Table 4, SNK procedure).

3.3.3 Effects on biodiversity after 15 months

At Lough Foyle, SR and H' were affected by the cover of oysters regardless of the type of habitat (Table 4). H' was greater in plots with 50 % cover of oysters than in plots with no oysters, but no differences could be detected for SR (SNK procedure). At Lough Swilly, within mussel-bed habitats, SR and H' plots with 5 and 50 % cover of oysters had greater SR than those with 100 % cover of oysters and plots with 0, 5 and 50 % cover of oysters had greater H' than plots with 100 % cover of oysters (Figure 10d and f, Table 4, SNK procedure). Within mud-flat habitats, plots with 50 and 100 % cover of oysters had greater

SR and H' than plots with 0 and 5 % cover of oysters and plots with 5 % cover of oysters also had greater H' than plots with no oysters.

3.3.4 Effects on assemblage structure after 4 months

At Lough Foyle, assemblage structure did not vary with cover of oysters on mussel-bed habitats. In mud-flat habitats, plots with 100 % cover of oysters differed from those with 0, 5 and 50 % cover of oysters and plots with 5 or 50 % cover of oysters differed from those with no oysters (Table 5, Post-hoc procedure). This was also indicated by the clear separation of the 0 and 100 % plots on the nMDS plot (Figure 11a). In mud-flat habitats, differences in assemblage structure on plots with 100 % compared to plots with 0, 5 or 50 % cover of oysters were driven by variations in the density of *E. modestus*, bryozoans *Conopeum seurati* (Canu, 1928), *L. littorea*, and Spionidae worms, which all occurred in greater density in plots with 100 % cover of oysters and nematodes which occurred less in plots with 100 % cover (Table 6). Similarly, differences in assemblage structure in plots with 5 or 50 % cover from plots with no oysters, were driven by *E. modestus*, *C. seurati*, *Melita palmata* (Montagu, 1804), nematodes and Spionidae worms which occurred in greater density in plots 0 than on plots with 5 % cover of oysters and *C. seurati*, *L. littorea*, *E. modestus*, Oligochaetes and Spionidae worms all occurred in greater density in plots with 50 % cover than in plots with no oysters (Table 6).

At Lough Swilly, assemblage structure did not vary with cover of oysters in mussel-bed habitats. In mud-flat habitats, however, plots with 100 % cover of oysters differed from those with 0 and 50 % cover and plots with 5 and the 50 % cover of oysters differed from those with no oysters (Table 5, Post-hoc procedure). The nMDS plot for the mud-flat habitat clearly shows the close grouping of the plots with 50 and 100 % cover which are separated from the plots with 0 and 5 % cover of oysters (Figure 11b). In mud-flat habitats, differences in assemblage structure between plots with different covers of oysters were

driven by greater densities of *E. modestus*, *C. seurati*, *L. littorea* and copepods but less density of nematodes in plots with 100 % cover than in plots with 0 or 50 % cover of oysters (Table 7). Similarly, plots with 5 or 50 % cover of oysters had greater densities of *E. modestus*, *C. seurati*, Spionidae worms and copepods but less nematodes than in plots with no oysters (Table 7).

3.3.5 Effects on assemblage structure after 15 months

At Lough Foyle, assemblage structure did not vary with cover of oysters on mussel-beds, but in mud-flat habitats plots with 50 and 100 % cover of oysters differed from those with no oysters (Table 5, SNK procedure). Plots with 50 or 100 % cover of oysters can be seen on the nMDS plot grouped close together, separated from the 0 % plots (Figure 11c). In mud-flat habitats, differences in assemblage structure were driven by a greater density of *E. modestus*, *C. seurati*, *L. littorea* and Spionidae worms in plots with 50 or 100 % cover of oysters but less density of copepods and oligochaetes than in plots with no oysters (Table 8).

At Lough Swilly, assemblage structure of plots with 100 % cover of oysters in mussel-bed habitats differed from those with no oysters (Table 5, SNK procedure). In mud-flat habitats, plots with 100 % cover of oysters differed from those with 0 or 5 % cover and plots with 50 % also differed from those with no oysters (Table 5, SNK procedure). This is particularly evident from the clear grouping of the assemblages on plots with 100 % cover (Figure 11d). In mussel-bed habitats, differences in assemblage structure were driven by a greater cover of *F. vesiculosus* and density of *E. modestus* in plots with 100 % cover of oysters and less density of copepods than in plots with no oysters (Table 9). In mud-flat habitats, differences in assemblage structure were driven by a greater density of Phyllodocidae worms, oligochaetes, *L. littorea*, *E. modestus* and a greater cover of *F. vesiculosus* in plots with 50 or 100 % cover than plots with 0 or 5 % cover of oysters and less density of

copepods in plots with 100 % cover than in plots with no oysters (Table 9).

3.3.6 Effects on β-diversity

β-diversity (multivariate dispersion indices) significantly varied among covers of oysters in mud-flat habitats at Lough Swilly after 15 months, where plots with 100 % cover of oysters were less dispersed than those with 0, 5 or 50 % cover (PERMDISP: F = 10, df1 = 7, df2 = 24, P = 0.001).

3.3.7 Effects on individual taxa after 4 months

At Lough Foyle the density of *L. littorea* and Polychaete worms were greater in mussel-bed than in mud-flat habitats and, in both habitats were greater in plots with 100 % cover of oysters than on plots with 0 or 5 % cover of oysters (Table 10, Figure 12a and g, SNK procedure). *E modestus* had a greater density on plots with 50 or 100 % than on those with 0 or 5 % cover of oysters on mussel-beds and a greater density on plots with 100 % than those with 0, 5 or 50 % cover of oysters on mud-flat habitats (Table 6, Figure 12c). *C. maenas* had a greater density on plots with 100 % than those with 0, 5, or 50 % cover of oysters on mussel-beds, but in mud-flat habitats had a greater density on plots with 50 % than those with 0, 5 or 100 % cover of oysters (SNK procedure). Although there were no significant effects on the density of polychaetes (Table 10), their density within mud-flat habitats did increase with increasing cover of oysters (Figure 12g).

At Lough Swilly the density of *L. littorea* was greater within mussel beds than in mud-flats and in plots with 100 % than in those with 0, 5 or 50 % cover of oysters (Table 10, Figure 12a, SNK procedure). The density of *E. modestus* was greater in plots with 100 % than in plots with 0 or 5 % cover of oysters on mussel beds and was greater in plots with 100 % than those with 0, 5 or 50 % cover of oysters in mud-flat habitats (Table 7, Figure 12c, SNK

procedure). The density of *C. maenas* was greater in mussel-bed than in mud-flat habitats (Figure 11e, SNK procedure). The density of polychaetes was greater in plots with 5, 50 or 100 % cover of oysters than in plots with no oysters (Figure 12g, SNK procedure).

3.3.8 Effects on individual taxa after 15 months

At Lough Foyle the density of *L. littorea* and *E. modesutus* greater in mussel-bed than in mud-flat habitats and greater in plots with 100 % than those with 0 or 5 % cover of oysters (Table 10, Figure 12b and d, SNK procedure). The density of *C. maenas* was greater on mussel-bed than mud-flat habitats (Table 10, Figure 12f, SNK procedure). The density of polychaetes was greater in mussel-bed than in mud-flat habitats (Table 10, Figure 12h, SNK procedure). Although not significant, the density of polychaetes in mud-flat habitats increased with increasing cover of oysters.

At Lough Swilly the density of *L. littorea* was greater in plots with 50 or 100 % than those with 0 or 5 % cover of oysters within mud-flat habitats, and no differences were found in mussel-bed habitats (Table 10, Figure 12b, SNK procedure). The density of *E. modestus* was greater in plots with 50 or 100 % than those with 0 or 5 % cover of oysters (Table 9, Figure 12d, SNK procedure). The density of *C. maenas* was greater in mussel-bed than in mud-flat habitats (Table 10, Figure 12f, SNK procedure). The density of polychaetes was greater in mussel-bed than in mud-flat habitats (Table 10, Figure 12h, SNK procedure). Although not significant, the density of polychaetes in mussel-bed habitats was greater in plots with 0, 5 or 50 % cover of oysters than in those with 100 % (Table 9).

Table 4. ANOVA for the total number of individuals, species richness and Shannon-Weiner diversity on plots with increasing cover of oysters on mussel-beds and mud-flats after 4 and 15 months. Significant results are indicated, * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Source of variation	4mths	L. Foyle			4mths	L. Swilly			15mths	L. Foyle			15mths	L. Swilly		
Number of individuals	*d.f.*	MS	F		*d.f.*	MS	F		*d.f.*	MS	F		*d.f.*	MS	F	
Habitat (=H)	1	3.21	61.42	***	1	1997.68	22.83	***	1	8953.59	16.54	***	1	545.72	8.96	**
Cover of oysters (=C)	3	0.55	10.60	***	3	203.71	2.33		3	492.65	0.91		3	748.39	12.28	***
H x C	3	0.41	7.80	***	3	695.99	7.95	***	3	1027.85	1.90		3	446.68	7.33	**
Residual	24	0.50			24	87.51			16	541.28			24	60.93		
Species richness																
Habitat (=H)	1	105.12	20.85	***	1	24.50	4.45	*	1	8.17	1.75		1	60.50	8.02	**
Cover of oysters (=C)	3	11.58	2.30		3	31.42	5.71	**	3	17.00	3.64	*	3	53.42	53.42	**
H x C	3	15.71	3.12	*	3	22.42	4.08	*	3	7.17	1.54		3	90.08	11.94	***
Residual	24	5.04			24	5.50			16	4.67			24	7.54		
Shannon-Weiner (H')																
Habitat (=H)	1	1.51	32.81	***	1	0.04	0.73		1	0.08	0.79		1	0.21	4.98	*
Cover of oysters (=C)	3	0.14	3.09	*	3	0.33	6.54	**	3	0.34	3.53	*	3	0.32	7.52	**
H x C	3	0.22	4.80	**	3	0.27	5.40	**	3	0.04	0.45		3	0.57	13.22	***
Residual	24	0.05			24	0.05			16	0.10			24	0.04		

Table 5. PERMANOVA of assemblage structure on square root transformed data of plots with increasing cover of oysters on mussel-beds and mud-flats at Lough Foyle and Lough Swilly after 4 and 15 months with 9999 permutations under the reduced model.

Source of variation	4 mths				15 mths			
Lough Foyle	*d.f.*	MS	Pseudo F		*d.f.*	MS	Pseudo F	
Habitat (=H)	1	12326	22.94	**	1	12620	13.18	**
Cover of oysters (=C)	3	1924	3.58	**	3	1798	1.88	*
H x C	3	2438	4.54	**	3	2238	2.34	**
Residual	24	537			16	957		
Lough Swilly								
Habitat (=H)	1	7075	14.49	**	1	6978	7.81	**
Cover of oysters (=C)	3	1608	3.29	**	3	2771	3.10	**
H x C	3	2024	4.15	**	3	1776	1.98	**
Residual	24	488			24	893		

Significant results are indicated, * = P < 0.05, ** = P < 0.001

Table 6. SIMPER analyses based on square-root transformed data corresponding to significant PERMANOVA results at Lough Foyle after 4 months (Table 2). Listed are the 5 taxa that contributed most to the dissimilarity between pair-wise comparisons of different covers of oysters.

Mud-flat	Average density					
Taxon	0%	5%	Av.Diss	Diss/SD	Contrib%	Cum.%
E. modestus	4.60	0.00	12.26	1.35	25.59	25.59
C. seurati	4.01	0.00	9.23	0.80	19.26	44.84
Spionidae	4.14	1.38	7.75	1.62	16.17	61.02
M. palmata	1.57	1.36	2.69	1.13	5.62	66.64
Nematoda	7.78	7.10	2.68	1.33	5.59	72.23
	0%	50%				
C. seurati	0.00	11.00	22.42	5.08	38.45	38.45
Spionidae	1.38	4.42	6.56	1.74	11.25	49.70
L. littorea	0.00	2.47	4.89	5.99	8.39	58.09
E. modestus	0.00	2.18	3.90	0.72	6.68	64.77
Oligochaete	0.35	1.99	3.42	1.94	5.87	70.64
	0%	100%				
C. seurati	0.00	11.40	18.61	3.87	25.69	25.69
E. modestus	0.00	10.80	17.67	5.94	24.39	50.09
Spionidae	1.38	5.89	7.24	1.97	10.00	60.08
L. littorea	0.00	3.09	5.20	2.45	7.18	67.27
Nematoda	7.10	5.47	4.57	0.83	6.31	73.57
	5%	100%				
C. seurati	4.01	11.40	11.51	1.76	25.44	25.44
E. modestus	4.62	10.80	8.33	1.89	18.40	43.84
L. littorea	0.00	3.09	4.32	2.49	9.54	53.37
Nematoda	7.78	5.47	4.30	0.90	9.50	62.87
Spionidae	4.14	5.89	3.67	1.60	8.12	70.99
	50%	100%				
E. modestus	2.18	10.80	10.63	2.15	29.56	29.56
C. seurati	11.16	11.40	4.42	1.44	12.29	41.85
Nematoda	8.49	5.47	4.33	0.98	12.06	53.91
Spionidae	4.42	5.89	2.98	1.31	8.29	62.20
Copepoda	1.30	1.54	1.75	1.32	4.88	67.08

Table 7. SIMPER analyses based on square-root transformed data corresponding to significant PERMANOVA results at Lough Swilly after 4 months (Table 2). Listed are the 5 taxa that contributed most to the dissimilarity between pair-wise comparisons of different covers of oysters.

Mud-flat	Average density					
Taxon	0%	5%	Av.Diss	Diss/SD	Contrib%	Cum.%
E. modestus	0.00	6.34	9.74	3.89	18.71	18.71
C. seurati	0.00	3.64	4.98	0.73	9.56	28.27
Nematoda	9.00	9.18	3.51	1.27	6.74	35.02
Spionidae	1.24	3.23	3.13	1.69	6.02	41.04
Chiton	0.00	1.98	3.02	8.29	5.80	46.84
	0%	50%				
C. seurati	0.00	12.08	17.19	3.88	29.80	29.80
E. modestus	0.00	5.53	7.88	4.84	13.66	43.46
Spionidae	1.24	3.57	3.32	1.38	5.76	49.22
Nematoda	9.00	7.86	3.16	2.91	5.47	54.69
Copepoda	2.75	4.71	3.07	1.16	5.32	60.00
	0%	100%				
E. modestus	0.00	9.80	14.38	7.55	23.76	23.76
C. seurati	0.00	6.66	9.64	1.50	15.93	39.69
Nematoda	9.00	7.00	6.03	1.09	9.96	49.65
L. littorea	1.37	4.12	4.07	5.42	6.73	56.38
Copepoda	2.75	5.17	3.62	1.84	5.98	62.36
	50%	100%				
C. seurati	12.08	6.66	6.31	1.31	18.68	18.68
E. modestus	5.53	9.80	4.30	2.46	12.72	31.40
Nematoda	7.86	7.00	3.78	1.23	11.19	42.59
Copepoda	4.71	5.17	2.12	1.33	6.27	48.86
M. palmata	2.10	2.41	1.41	1.73	4.17	53.03

Table 8. SIMPER analyses based on square-root transformed data corresponding to significant PERMANOVA results at Lough Foyle after 15 months (Table 2). Listed are the 5 taxa that contributed most to the dissimilarity between pair-wise comparisons of different covers of oysters.

Mud-flat	Average density					
Taxon	0%	50%	Av.Diss	Diss/SD	Contrib%	Cum.%
Copepod	17.19	3.41	18.10	1.19	29.90	29.90
E. modestus	0.00	5.67	8.90	2.67	14.70	44.60
C. seurati	0.00	5.89	8.82	0.65	14.57	59.17
L. littorea	0.00	5.38	8.40	5.40	13.87	73.04
Oligochaete	2.88	1.63	1.90	1.07	3.14	76.19
	0%	100%				
C. seurati	0.00	19.45	22.79	1.86	34.67	34.67
Copepoda	17.19	4.80	12.91	0.94	19.63	54.30
L. littorea	0.00	6.01	7.51	3.52	11.43	65.73
E. modestus	0.00	4.68	6.04	1.16	9.19	74.92
Spionidae	5.88	7.22	2.32	0.94	3.53	78.45

Table 9. SIMPER analyses based on square-root transformed data corresponding to significant PERMANOVA results at Lough Swilly after 15 months (Table 2). Listed are the 5 taxa that contributed most to the dissimilarity between pair-wise comparisons of different covers of oysters.

Mud-flat	Average density					
Taxon	50%	0%	Av.Diss	Diss/SD	Contrib%	Cum.%
F. vesiculosus	0.00	5.31	8.22	4.02	12.04	12.04
L. littorea	0.56	5.48	8.12	2.69	11.90	23.94
Copepoda	5.09	3.58	5.38	1.20	7.88	31.82
E. modestus	0.00	2.76	4.31	1.16	6.32	38.14
Phyllodocidae	0.25	2.67	3.86	1.69	5.66	43.80
	0%	100%				
E. modestus	0.00	6.46	9.37	4.59	14.35	14.35
F. vesiculosus	0.00	5.99	8.65	7.72	13.25	27.60
L. littorea	0.56	6.34	8.27	3.93	12.66	40.27
Phyllodocidae	0.25	3.70	5.01	3.94	7.67	47.94
Copepoda	5.09	4.77	3.51	1.76	5.38	53.31
	5%	100%				
F. vesiculosus	1.12	5.99	6.45	2.10	12.41	12.41
E. modestus	2.35	6.46	5.42	1.48	10.43	22.84
L. littorea	3.19	6.34	4.22	1.79	8.11	30.95
Copepoda	2.98	4.77	3.47	2.28	6.67	37.62
Oligochaete	2.27	3.90	2.99	1.27	5.76	43.38
Mussel-bed	0%	100%				
E. modestus	1.74	7.10	7.14	2.23	16.48	16.48
F. vesiculosus	1.12	4.68	5.30	1.85	12.22	28.69
Oligochaete	6.72	6.79	2.97	1.51	6.85	35.54
Phyllodocidae	1.96	0.35	2.23	1.97	5.15	40.69
Copepoda	1.93	0.87	2.01	1.28	4.63	45.32

Table 10. Results of analyses of densities (No. per m^2) of *L. littorea*, *E. modestus*, *C. maenas* and Polychaetes on plots with increasing cover of oysters on mussel-beds and mud-flats at Lough Foyle and Lough Swilly after 4 and 15 months.

Source of variation	4mths	L. Foyle			4mths	L. Swilly			15mths	L. Foyle			15mths	L. Swilly		
L. littorea	*d.f.*	MS	F		*d.f.*	MS	F		*d.f.*	MS	F		*d.f.*	MS	F	
Habitat (=H)	1	5151.13	49.67	***	1	693.78	40.39	***	1	7704.17	37.34	***	1	5151.13	42.84	***
Cover of oysters (=C)	3	311.46	3.00	*	3	215.53	12.55	***	3	1079.00	5.23	*	3	1188.54	9.89	***
H x C	3	49.13	0.70		3	9.61	0.65		3	237.17	1.15		3	373.88	3.11	*
Residual	24	103.71			24	17.18			16	206.33			24	120.23		
E. modestus																
Habitat (=H)	1	478.83	50.99	***	1	49.45	21.81	***	1	357.46	74.86	***	1	693.78	1.94	
Cover of oysters (=C)	3	88.45	9.42	***	3	49.44	21.81	***	3	32.49	6.80	**	3	3328.03	9.33	***
H x C	3	56.58	6.02	**	3	13.92	6.14	**	3	5.24	1.10		3	340.36	0.95	
Residual	24	9.39			24	2.27			16	4.78			24	356.84		
C. maenas																
Habitat (=H)	1	31.08	41.02	***	1	800.00	26.37	***	1	3.43	10.44	**	1	28.13	21.43	***
Cover of oysters (=C)	3	5.85	7.71	***	3	12.00	0.40		3	0.75	2.29		3	3.08	2.35	
H x C	3	2.92	3.86	*	3	12.00	0.40		3	0.32	0.99		3	0.38	0.29	
Residual	24	0.76			24	30.33			16	0.32			24	1.31		
Polychaetes																
Habitat (=H)	1	133.40	0.46		1	0.81	2.15		1	8523.47	14.35	**	1	5.00	16.05	***
Cover of oysters (=C)	3	231.33	0.80		3	1.14	3.04	*	3	210.94	0.36		3	0.34	1.10	
H x C	3	601.94	2.08		3	1.50	4.00	*	3	159.15	0.27		3	0.59	1.88	
Residual	24	289.32			24	0.38			16	594.07			24	0.31		

Significant results are indicated, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

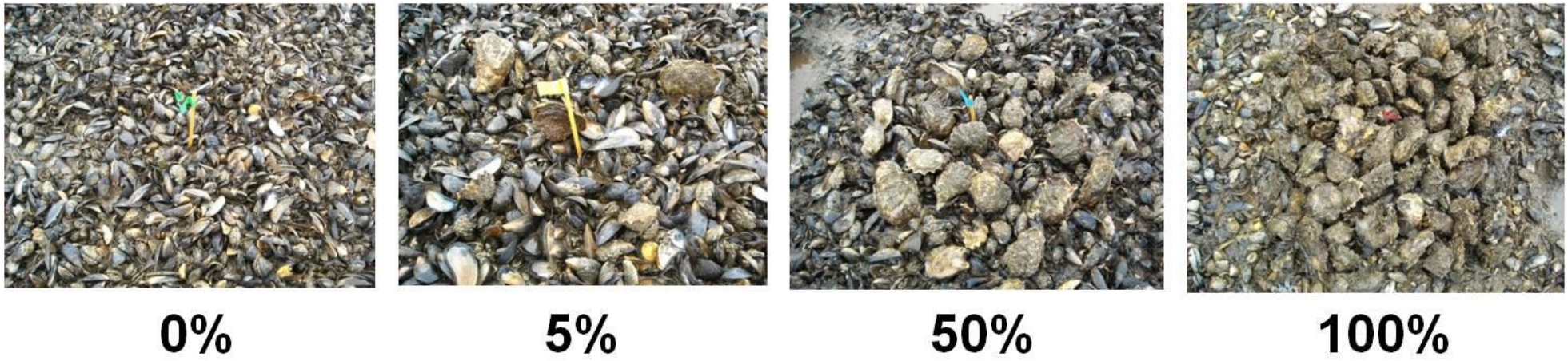

Figure 9. Experimental plots measuring 50 x 50 cm with increasing cover of *C. gigas* on mussel-beds

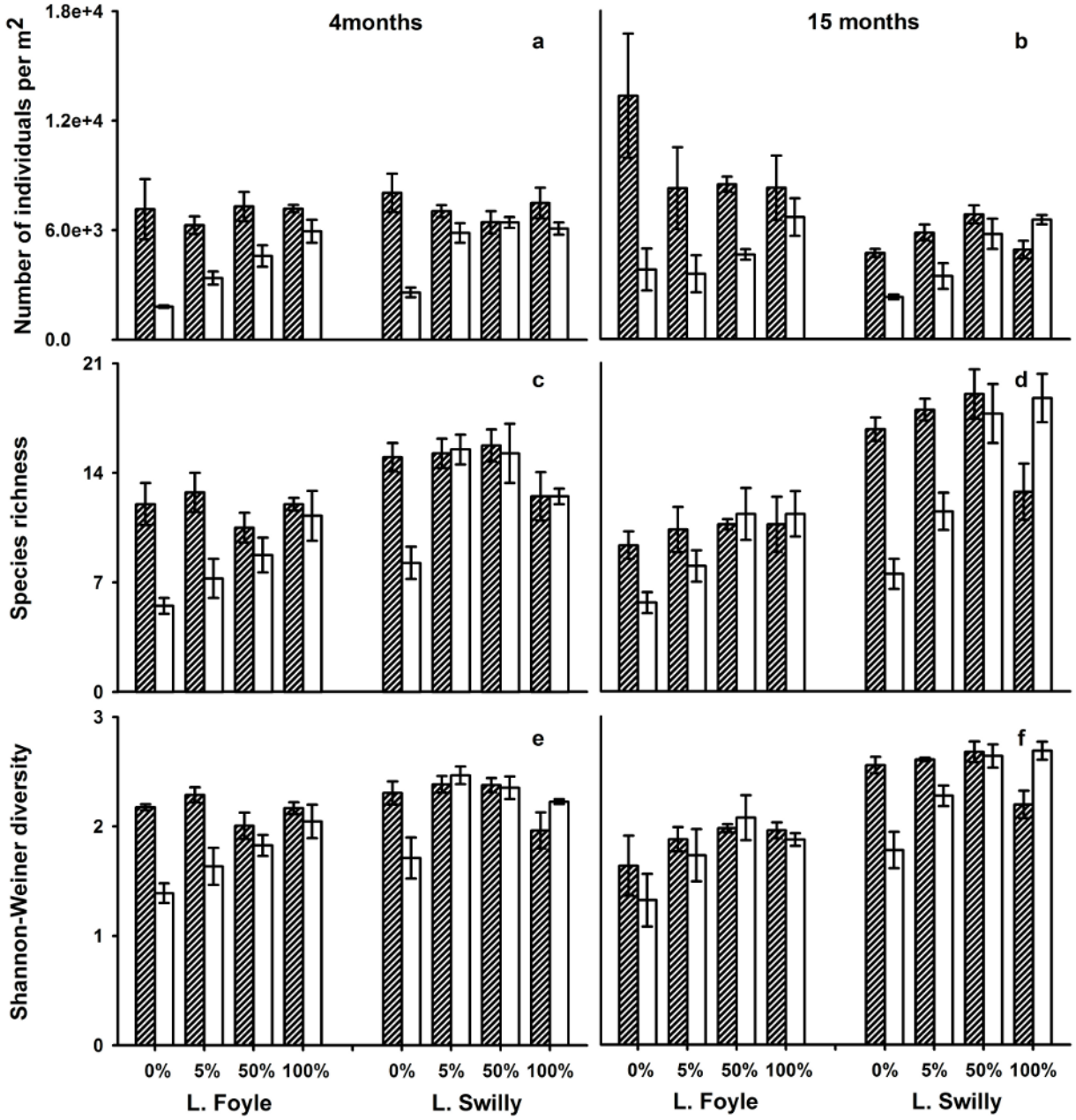

Figure 10. Mean (+/- S.E.) numbers of individuals per m^2 after (a) 4 and (b) 15 months, species richness after (c) 4 and (d) 15 months and Shannon-Wiener diversity after (e) 4 and (f) 15 months in mussel-beds (hatched bars) and mud-flats (white bars) with increasing cover of *C. gigas* at Lough Foyle and Lough Swilly. Note that at Lough Foyle by 15 months the 100 % cover plots had decreased to approximately 75 %.

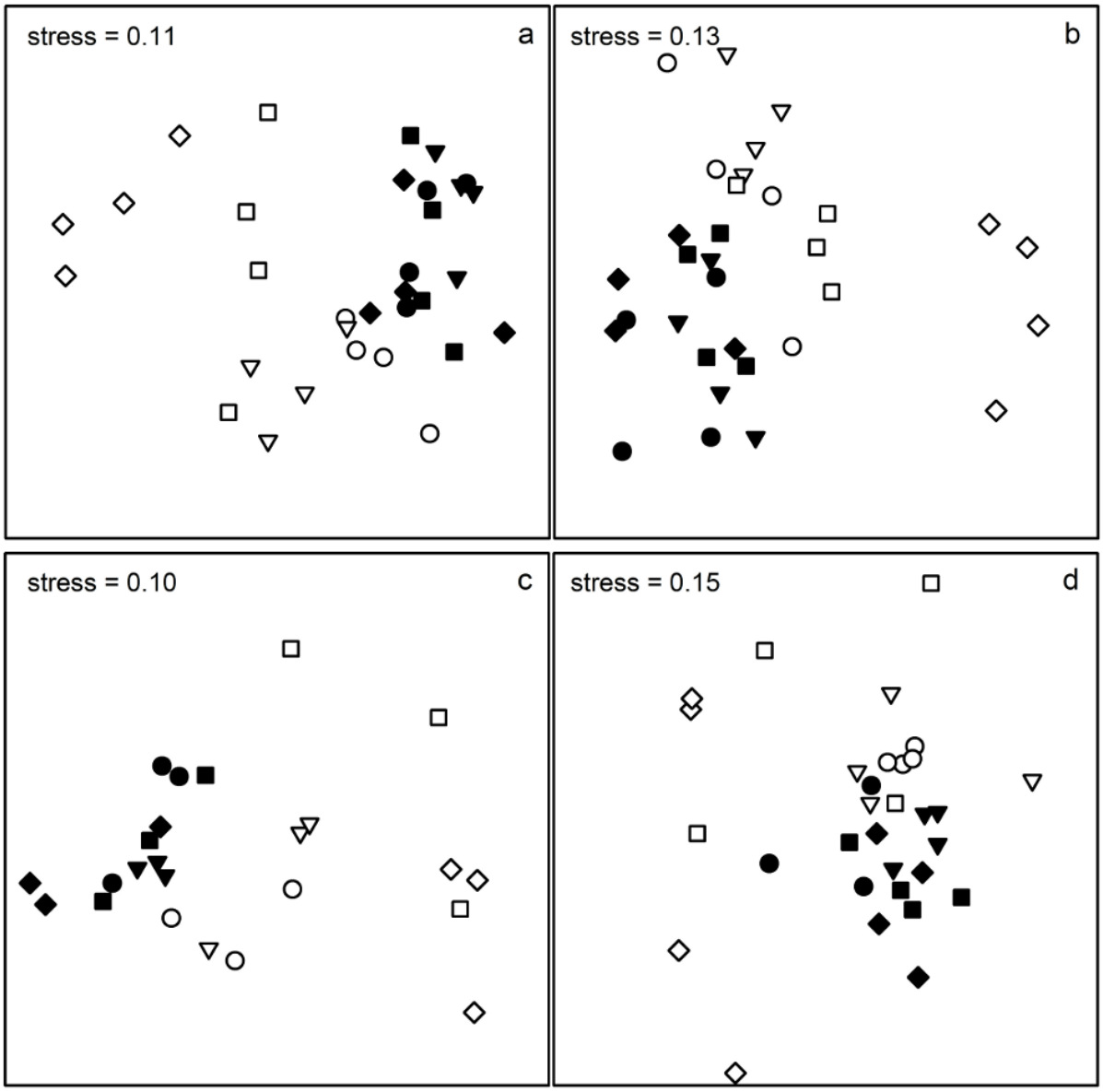

Figure 11. nMDS plots with stress values on square root transformed data of assemblages on mussel-beds (black) and mud-flats (white) with 0 (♦), 5 (■), 50 (▼) or 100 (●) % cover of *C. gigas* at Lough Foyle after 3 (a), and 15 (c) months and Lough Swilly after 4 (b) and 15 (d) months.

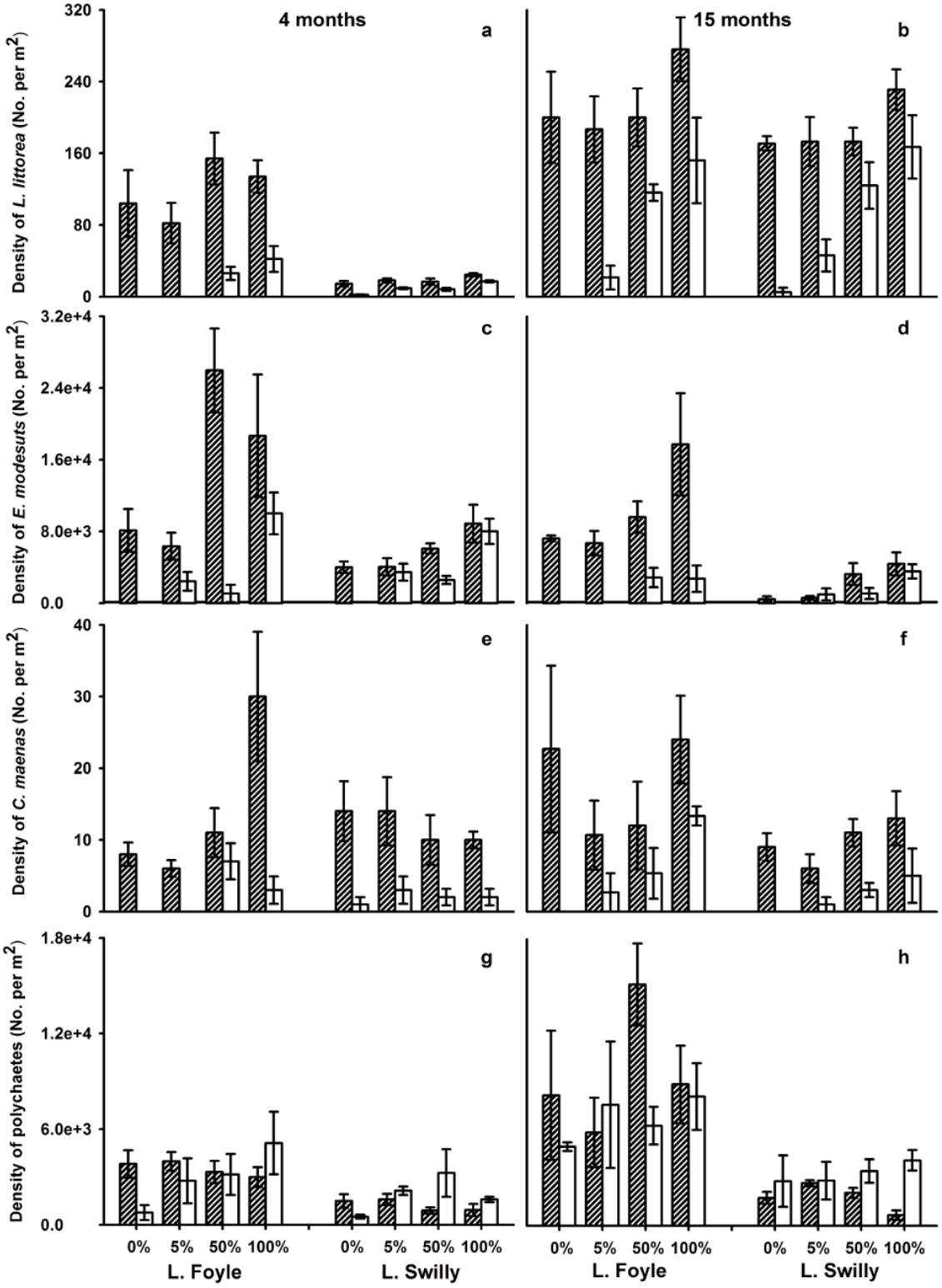

Figure 12. Mean (+/- S.E.) density (No. per m^2) of *L. littorea* after (a) 4 and (b) 15 months, *E. modestus* after (c) 4 and (d) 15 months, *C. maenas* after (e) 4 and (f) 15 months and polychaetes after (g) 4 and (h) 15 months in mussel-beds and mud-flats with increasing cover of *C. gigas* at Lough Foyle and Lough Swilly.

3.4 Discussion

C. gigas affected biodiversity, assemblage structure and the abundance of organisms, and the nature of these effects depended both on the environmental context (i.e. the location, habitat and time since establishment) and on the cover of *C. gigas*.

Overall effects on biota were more consistent in mud-flat than in mussel-bed habitats. This is not surprising since the addition of *C. gigas* to mud-flat habitats provides hard substratum where it was formerly rare, thus completely changing the habitat from soft unstructured mud-flat to one with hard biogenic reef (Lejart and Hily, 2011). This is in contrast to mussel-bed habitats, where *C. gigas* are overgrowing an existing hard biogenic habitat. Regardless, the formation of oyster reefs within either habitat results in an increase of habitat complexity and heterogeneity (McCoy and Bell, 1991), and although not always consistent, there were also strong effects of *C. gigas* within mussel-bed habitats. In mud-flats the total number of individuals, species richness and Shannon-Wiener diversity all increased with increasing cover of *C. gigas*. The importance of availability of refuge and structural complexity of habitat on intertidal assemblage structure has long been recognised (Barshaw and Lavalli, 1988; Gee and Warwick, 1994) and the increase in the total number of individuals with increasing cover of oysters may be a result of reduced mortality or increased recruitment (Crooks and Khim, 1999). Effects on assemblage structure within mud-flat habitats were also consistent across locations and time, with plots with the greatest cover differing from those with less or no cover of *C. gigas*. These changes were mostly underpinned by increases of organisms which require a hard surface for colonisation, such as barnacles, bryozoans and fucoid algae, with the oysters' shell allowing these organisms to exist in a habitat where they previously could not. Assemblage structure in mussel-bed habitats also differed at Lough Swilly after 15 months, with plots with the greatest cover differing from those with no cover of *C. gigas*. In both habitats differences in assemblage structure could be due to an increase in habitat

complexity, since *C. gigas* has overlapping ridges and layers on its shell and is more complex in structure than *M. edulis* (Lejart and Hily, 2011).

Because *C. gigas* enhances habitat heterogeneity with its shell and forms complex reefs, it is expected to increase biodiversity (McCoy and Bell, 1991). Previous studies have confirmed this hypothesis, finding *C. gigas* to increase biodiversity and the abundance of taxa compared to adjacent habitats (Dubois et al., 2006a; Markert et al., 2010; Troost, 2010; Lejart and Hily, 2011). As in the current study, others have also found increases in the number of individuals and species richness associated with *C. gigas* in mud-flat habitats (Hosack, 2003; Kelly et al., 2008; Lejart and Hily, 2011). Although another study done in a similar mud-flat habitat found no effect of *C. gigas* on native assemblages (Nicastro et al., 2009). Lang and Buschbaum (2010) found an increase in algal diversity associated with *C. gigas*, while others have found algal diversity and/or survival reduced by *C. gigas* (Kelly and Volpe, 2007; Kelly et al., 2008). Previous studies within mussel-bed habitats similar to those in the present study, found that *C. gigas* reefs had greater biodiversity than adjacent mussel-beds (Markert et al., 2010) or a greater abundance of organisms (Kochmann et al., 2008). The current study is the first to find a decrease in macro-invertebrate biodiversity caused by *C. gigas*. Species richness and Shannon-Wiener diversity decreased at the greatest cover of *C. gigas* on mussel-beds at Lough Swilly at both times, and reached a maximum and asymptoted with the lowest cover of *C. gigas* on mussel-beds at Lough Foyle. The high cover plots decreased in cover at Lough Foyle which may explain why there was no decrease in diversity in mussel-beds here, since high cover plots actually represented 75 % cover by 15 months. Also at Lough Swilly after 15 months, β-diversity (multivariate dispersion indices) was reduced with the greatest cover of *C. gigas* in mud-flat habitats. Tests for homogeneity of multivariate dispersions have been identified as a way of measuring β-diversity (Anderson et al., 2006). The exact meaning of this measure, however, is largely dependent on the dissimilarity measure and

any transformations used and therefore should be carefully interpreted (Anderson et al., 2011). β-diversity, as measured here, based on Bray-Curtis, square-root data measures variation in assemblage structure with emphasis on both species composition and relative abundance (Anderson et al., 2011). Therefore, the reduction in β-diversity with increasing cover of *C. gigas* means that assemblages were more homogeneous in terms of their composition (the types of taxa present) and the relative abundance of taxa. This corroborates another study comparing assemblages associated with bare mud to those underneath oysters, which found more uniform assemblages associated with *C. gigas* (Lejart and Hily, 2011). The issue of homogenisation may become magnified if invasive species also cause homogenisation of the habitat and / or cause endemic species to become extinct (Rahel, 2002). Although in the current study there was little evidence of the latter, *C. gigas* is an ecosystem engineer which can dominate large areas (Diederich, 2005) thereby causing extensive habitat homogenisation. The potential for invasive species to cause homogenisation (Olden and Rooney, 2006), in terms of a decrease in β-diversity (McKinney and Lockwood, 1999; Olden et al., 2008) is an important, but often overlooked, consequence of invasive species (Wright, 2011).

The reductions in biodiversity found in the current study may not have been uncovered by an experiment which did not include treatments of different covers of *C. gigas* or which was sampled only after a shorter duration. Previously 9 months was the longest experimental manipulation of *C. gigas* (Kochmann et al., 2008), as opposed to 15 months in the current study.

It is not uncommon for invasive species to have “positive” effects, for example by increasing biodiversity and the abundance of native organisms (Simberloff and Von Holle, 1999; Altieri et al., 2010). In fact, some previous studies have found positive effects to increase with invader density (Thomsen, 2010), but it is possible that there is a density

threshold whereupon the positive effects on an invasive species become negative, or the negative effects increase, after a critical invader density is reached (Foxcroft and Downey, 2008). Despite this, very few studies have explicitly tested the effects of invasive species at a range of densities (Thomsen et al., 2011a), but this is paramount if we are to avoid the detrimental consequences of under-estimating their effects at greater densities (Yokomizo et al., 2009). For example in the current study, at lower densities, *C. gigas* might be interpreted as having positive effects by increasing biodiversity. At greater densities, however, there was evidence of a threshold being reached, and in mussel beds, the effects on biodiversity even became negative.

While the effects of *C. gigas* on biodiversity can be difficult to predict, some single species, such as *L. littorea* and *E. modesutus*, exhibited very consistent responses, increasing with increasing cover of *C. gigas* within both habitats, locations and at both times. Similar results have been found by others for both of these species (Kochmann et al., 2008; Markert, 2010) and are probably due to an increase in habitat complexity provided by oyster shells providing more refuges from desiccation or predation, or in the case of the periwinkles, an increase in available microalgae for grazing. *C. maenas*, although initially increased at Lough Foyle with increasing cover of oysters, was mostly affected by the type of habitat, occurring in greater density in mussel bed than in mud-flat habitats. This was in contrast to past studies which have found *C. maenas* to increase in association with *C. gigas* (Kochmann et al., 2008; Markert, 2010). Although the density of polychaetes was mostly affected by the type of habitat, in mud-flat habitats it generally increased with increasing cover of *C. gigas* whereas it was decreased at the greatest cover of *C. gigas* in mussel-bed habitats.

Declines in polychaetes and biodiversity at the greatest cover of oysters may have been caused by an increase in the concentration of nutrients in the pore-water, such as

ammonium, resulting either directly from oyster excretion (Dame et al., 1984 and 1985) or indirectly due to the breakdown of organic matter in oyster “biodeposits” which may be composed of faeces or pseudofaeces. Oysters produce large quantities of biodeposits (Hayakawa et al., 2001) which organically enrich sediments and supply food for other organisms (Castel et al., 1989). But the decomposition of biodeposits increases the uptake of oxygen in the sediment (Christensen et al., 2003) and if the rate of biodeposition is great, this can lead to sediment anoxia, thus making the habitat unsuitable, and decreasing the diversity and abundance of infaunal organisms (Callier et al., 2009). Alternatively an increase in hydrogen sulphide may be responsible for the decrease in diversity, as toxic levels have been reached before with regards to the effects of *C. gigas* on eelgrass (Kelly and Volpe, 2007; Kelly, 2008). Some of the differences in assemblage structure or biodiversity could also be due to structural effects, such as alterations to hydrodynamics, caused by the oysters’ shells (Moulin, 2007). An experiment by Lenihan (1999) in which oyster reefs were artificially created using a similar species of oyster, *Crassostrea virginica* (Gmelin, 1791), found that flow speed was altered, increasing with increasing reef height. Flow, in turn, controls the rate of sedimentation (Lenihan, 1999) which exerts strong influence on the growth and survival of benthic organisms (Muschenheim, 1987). Of course, in order to understand the mechanisms underpinning these changes, it would be necessary to separate the effects of the physical structure and the biological activities of *C. gigas*. This was not explored in the current study, but in Chapter II, it was found that structure was more important than biological activity in determining patterns of difference associated with increasing cover of *C. gigas* in boulder-fields.

It is also possible that indirect effects of organisms that were facilitated by increasing cover of *C. gigas* may have excluded the establishment of others, thereby decreasing biodiversity. For example, some species which consistently increased in density with

increasing cover of *C. gigas* are known to be able to reduce the establishment of other invertebrates and algae, for example, *E. modestus* through competition for space and food (Little et al., 1992), *L. littorea* via bull-dozing effects from grazing (Buschbaum, 2000) and *F. vesiculosus* via mechanical abrasion (Grant, 1977; Kiirikki, 1996). The ecological importance of facilitation of native organisms by invaders, and the cascading indirect effects on biodiversity, have recently been highlighted (Rodriguez, 2006; Pope et al., 2008) and warrants further investigation.

These findings emphasise how difficult it is to predict the effect of invasive species on biodiversity, since the outcome depends not only on the type of substratum but also on the physical and chemical properties of the receiving environment and on the types of species already present (Padilla, 2010; Queiros et al., 2011). Estuarine habitats such as those examined here are inherently patchy (Morrisey et al., 1992) characterised by a high level of spatial and temporal variability not only in the abundance and distribution of invertebrates (Underwood and Chapman, 1996) but also geochemical properties of sediments (Tolhurst and Chapman, 2007). The current study found that some impacts of *C. gigas* on individual taxa were consistent across habitats, locations and time, but the majority were context dependent, especially with regards to the type of habitat being invaded. In addition, these effects varied in direction and magnitude depending on the density of *C. gigas*. This confirms the recommendations of others (Sousa et al., 2009; Padilla, 2010; Thomsen et al., 2011a) that more experimental studies spanning a range of habitats and a range of invader abundances are needed in order to assess the context-dependency of invasive species.

Chapter IV - Effects of non-indigenous oysters on ecosystem processes of estuarine habitats

4.1 Introduction

Non-indigenous invasive species can profoundly affect ecosystems and economies worldwide (Chapin et al., 2000; Mack et al., 2000), thus necessitating effective management procedures (Keller et al., 2008). Characterising the costs of invasive species to society is difficult, but important if decisions are to be made involving tradeoffs between investing in the control of invasive species or control of other pressures on ecosystems (Oreska and Aldridge, 2011).

Invasive species can alter native biodiversity (Bax et al., 2003; Molnar et al., 2008) and ecosystem functioning (Ehrenfeld, 2010) and, as such, affect the provision of ecosystem services (Pejchar and Mooney, 2009; Vilá et al., 2010). Although the effects of invasive species on biodiversity have been well documented, experimental tests of their effects on ecosystem functioning are rare (Ehrenfeld, 2010; Sousa et al., 2011). Alterations to biodiversity may alter the magnitude and stability of ecosystem processes, and therefore may exacerbate effects on ecosystem functioning (Naeem, 1999; Gamfeldt and Hillebrand, 2008). Similarly, alterations to ecosystem processes, arising either directly from the activities of invasive species or indirectly via consequent changes to native biodiversity, may generate feedbacks that further exacerbate changes to biodiversity (Duke and Mooney, 2004; Gomez-Aparico and Canham, 2008). Combinations of these mechanisms may operate simultaneously, depending on the species and/or processes being affected. Invasive species can affect the abundance and distribution of other organisms through interactions (such as predation or competition) or alterations to habitats and they may alter processes important for the functioning of ecosystems, such as biogeochemical cycling rates (Kurten et al., 2008), nutrient availability (Gomez-Aparico and Canham, 2008),

productivity (Sousa et al., 2008), rates of decomposition (van der Putten et al., 2007) and community respiration (Martin et al., 2007), again either directly or indirectly via changes to biodiversity.

The properties of the receiving environment can strongly influence the effects of invasive species (Padilla, 2010; Queiros et al., 2011). Hence there is increasing recognition of the need to explore the effects of invasive species in different types of habitats (Sousa et al., 2009; Thomsen et al., 2011a). The nature and magnitude of effects may also change depending on the abundance or density of the invading organism, which will vary in different places and also at different stages of the establishment of the species in non-native habitats and its subsequent invasion (Sousa et al., 2009). Indeed variation in impacts with abundance or density has been identified as a key gap in empirical knowledge of biological invasions (Thomsen et al., 2011a), particularly concerning effects on ecosystem functioning (Ehrenfeld, 2010).

Invasive 'ecosystem engineers' (Jones et al., 1994) cause some of the most significant changes to the physical and / or chemical properties of the native ecosystems (Cuddington and Hastings, 2004). Many bivalves are ecosystem engineers, often creating complex habitats that persist in the environment for a long time (Sousa et al., 2009). Several species can form dense monocultures, profoundly disrupting ecosystem processes (Hall et al., 2006; Queiros et al., 2011).

The Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), one of the best known invasive marine species, is an ecosystem engineer which can construct very dense reefs (over 400 individuals per m^2; Markert et al., 2010). Invasive populations of *C. gigas* have been found throughout much of the world and their effects on biodiversity have been extensively documented (Troost, 2010). In several cases increases in biodiversity have been attributed

to the presence of *C. gigas* (Gutierrez et al., 2003; Peterson et al., 2003; Dubois et al., 2006a; Markert et al., 2010; Lejart and Hily, 2011). The effects of invasive *C. gigas* on ecosystem functioning, however, remain mostly unexplored.

Oysters can alter the physical and chemical environment through their physical structure and biological activities. The shells of oysters can be particularly complex, increasing the heterogeneity of the receiving habitat and possibly facilitating the establishment of other organisms (Lejart and Hily, 2011). Oyster shells can also alter local hydrodynamic patterns, thereby altering the flow of nutrients and sedimentation rates (Lenihan, 1999). Oysters filter-feed, removing suspended phytoplankton and inorganic particulates from the water column, depositing faeces or pseudofaeces (collectively termed “biodeposits”) onto the benthos (Haven and Morales-Alamo, 1966) and modifying nutrient cycling through benthic-pelagic coupling (Arnott and Vanni, 1996; Norkko et al., 2001). Increased sedimentation of organic matter in the form of biodeposits may lead to carbon and nitrogen accumulation, followed by increased oxygen consumption resulting in anoxic conditions (Kaspar et al., 1985). High mineralisation rates of biodeposits can increase nutrient turnover at the sediment-water interface and may increase the release of ammonium and silicate into the water column (Christensen et al., 2003). As a result of these biogeochemical changes, infaunal biodiversity and community structure may be affected (Tenore, 1982). Different species of oysters can differ in their clearance rates (Haure et al., 2003), tolerance to environmental stress (Piano et al., 2002; Brownlee et al., 2008) and biodeposition rates (Hayakawa et al., 2001), therefore their effects may change as they become established in habitats outside of their native range.

Understanding the effects of invasive oysters on ecosystem processes is essential for predicting their impact on provisioning ecosystem services, such as aquaculture. The development of sustainable aquaculture requires determination of the relative roles of wild,

feral and cultivated populations, on the partitioning of resources and the incorporation of these estimates into existing models of carrying capacities (Sequeira et al., 2008; Cugier et al., 2010). The extent to which ecosystem processes and services are altered, however, is also likely to depend on the cover of *C. gigas* that is established and on the nature of the habitat being invaded. For example, the effects of *C. gigas* becoming established in habitats already dominated by biogenic reef forming organisms, such as mussel-beds, would be expected to differ from those caused by its establishment in habitats without biogenic reef, such as mud-flats.

In the current study, we experimentally manipulated the cover of *C. gigas* in the field to test the following hypotheses 1.) *C. gigas* will affect ecosystem functioning in invaded habitats, 2.) The effects of *C. gigas* will differ between different mussel-bed and mud-flat habitats 3.) These effects will change with increasing cover of *C. gigas* and finally 4.) That the effects on biodiversity and ecosystem functioning will co-vary (using data on biodiversity reported in Chapter III).

4.2 Materials and methods

4.2.1 Study site

This experiment was done at Lough Swilly (Ballylin Point, County Donegal, Ireland: 55° 2' 36.12", -7° 33' 36.09") on lower intertidal mussel-bed and mud-flat habitats. Mussel-bed habitats consisted of dense populations of the blue mussel, *Mytilus edulis* (Linneaus, 1758), while mud-flat habitats were patches of mud interspersed between the mussel beds which were not dominated by mussels or any other habitat-forming species. Sediment within mussel-beds was a mixture of fine sand and silt with large shell fragments throughout. Sediment within mud-flats was a mixture of very fine sand, silt and clay.

4.2.2 Experimental design

The experiment was set-up during late April 2009, with 2 orthogonal factors; habitat (2 levels, mussel-beds and mud-flats) and cover (4 levels, zero, (0 %), low (5 %), medium (50 %) or high (100 %) cover of *C. gigas*). These four levels of cover were applied to 50 x 50cm plots in each habitat and equated to densities of 0, 16 +/- 0.5, 120 +/- 8 and 240 +/- 12 individuals per m^2 and biomasses of 0, 26.06 +/- 2.13, 390.88 +/- 31.95 and 781.77 +/- 63.90 g per m^2 ash free dry weight. All oysters used in this experiment were found *in situ* and were not moved from other locations. Oysters were rinsed with seawater and cleaned of any epibionts prior to use and were then simply inserted upright into the mud-flat and mussel-bed habitats to simulate how they are typically found in natural populations. Four replicate plots were randomly allocated to each treatment and were sampled after 15 months (July 2010).

4.2.3 Organic matter (OM) content and C/N ratios

Sediment was collected from three depths (0 - 2, 2 - 4 and 4 – 6 cm) using a mini-corer adapted from a 60 ml syringe. At each plot 5 sediment samples were taken from each depth and pooled together in order to minimise variation among plots due to spatial heterogeneity within plots. OM content was determined by loss on ignition (Eleftheriou and McIntyre, 2005) as a percentage of ash-free dry weight. In addition, total organic carbon (TOC %) and nitrogen (TON %) was analysed from oven dried (80°C) samples of the surface layer (0 – 2 cm). For this, 50 mg of pulverised sediment was weighed into silver capsules and pre-treated with HCl to remove carbonates (Hedges and Stern, 1984). Total C and N were determined using the Dumas principle of complete and instantaneous oxidation of the sediment through combustion at 950°C with oxygen injection on an Elementar vario EL cube.

4.2.4 Pore-water nutrient concentrations

Pore-water samples were collected using purpose-built Rhizon *in situ* profilers modified from the design of Seeberg-Elverfeldt et al., (2005). These consisted of perspex sheets into which grooves were cut at certain intervals to allow the attachment of Rhizon soil moisture samplers (Figure 13). Rhizons were 10 cm long filters made of a hydrophilic porous polymer with 0.1 µm pore size (PES, Polyester Sulphone membranes) designed to extract water using a vacuum. Rhizons were placed onto the profilers at 0 (sediment-water interface), 1, 2, 3, 4, 5, 8 and 10cm depths and were carefully inserted into the sediment and left for 24 hours prior to sampling in order to reduce the effects of disturbance. Over-lying surface water (approximately 2 - 4 mm above the sediment-water interface) was collected from plots using separate Rhizons. This method allows pore-water profiles to be sampled with minimum disturbance to a vertical resolution of 1cm (Seeberg-Elverfeldt et al., 2005; Rocha et al., 2009). All water samples were stored in vacuum tubes and were analysed for ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and silicate ($Si(OH)_4$) using a Lachat Quick-Chem 8000 flow injection autoanalyser using Lachat methods 31-107-06-1-B (ammonia), 31-107-04-1-A (nitrite and nitrate) and 31-114-27-1-A (silicate). Concentrations of NO_2^- and NO_3^- were always below the detection limit and were omitted from further analyses.

Pore-water nutrient concentrations were corrected for porosity (ϕ) using the following equation:

i) $$\phi = V_p / V_b$$

Where V_p is the volume of pores in each 2 cm layer of sediment (weight of sediment dried to a constant weight) and V_b is the bulk volume of the sediment (weight of wet sediment). The concentrations were standardised to dry bulk density (BD_d) (Eleftheriou and McIntyre, 2005) using the following equation:

ii) $$BD_d = M_s / V_t$$

Where M_S is the mass of dry solids and V_t is the volume of the original sample.

4.2.5 Flux across the sediment-water interface (SWI)

Diffusive fluxes (J_D) of NH_4^+ and $Si(OH)_4$ across the sediment-water interface were calculated from vertical pore water concentration gradients according to Fick's first law of diffusion. Concentration gradients ($\partial C/\partial z$) were determined from the sediment pore-water data using simple linear regression (Moore et al., 1991). These gradients were then used to estimate the diffusive nutrient flux (J_D). Calculations were based on the linear portion of the nutrient profiles at the sediment-water interface (from surface water to 1 cm depth).

i) $$J_D = - \phi_0 D_s (\partial C/\partial z)$$

Where J_D is the diffusive flux (µmol m^{-2} d^{-1}),

ϕ_0 is the porosity in the top 2 cm of the sediment,

$\partial C/\partial z$ is the concentration gradient of the dissolved nutrient in the interstitial water (µmol cm^{-3}),

C is the concentration of the dissolved nutrient,

z is the depth in cm (Berner, 1980),

D_S is the whole sediment diffusion coefficient (cm^2 h^{-1}). Values of 0.0637 for ammonium and 0.0243 for silicate (Schulz and Zabel, 2000) were used in the calculations.

Inventories for NH_4^+ and $Si(OH)_4$ were calculated down to 10 cm and were determined using pore-water profiles, corrected for porosity, by depth using trapezoidal integration of concentrations between sediment layers. Residence times for NH_4^+ and $Si(OH)_4$ were calculated as the ratio of inventory to diffusive flux rates.

4.2.6 Community respiration

Gas samples were obtained using the closed chamber technique (Hutchinson and Mosier, 1981) with chambers with a volume of 8L fitted with airtight rubber septums. Cuvettes were

painted black to eliminate light and measure community respiration (Figure 14). Samples were taken at time zero and at hourly intervals for 3 hours with 60 ml syringes closed with a 3-way stopcock. The air was mixed by gently pumping the syringe 3 times before each sample was taken. Temperature was measured inside the cuvettes using a thermometer and an estimation of atmospheric pressure was taken from the Met Éireann website. Analysis for CO_2, N_2O and CH_4 was done using a gas chromatographer (Shimadzu GC-2024) with an automated injection system (Loftfield et al., 1997). The flux rates were calculated by using the ideal gas law and linear regression with chamber temperature and average air pressure during cover period. An exponential equation (Hutchinson and Mosier, 1981) was used if R-squared was greater than 0.985 but less than 1.

4.2.7 Biological assemblages

Easily identifiable epiflora and fauna was counted or recorded as percentage cover using a 25 x 25 cm quadrat which was placed randomly onto plots. A sediment core with a 10 cm cross-section was also taken down to 10 cm depth in each plot. This was processed to extract epifauna and infauna according to protocols described in detail in Chapter III.

4.2.8 Statistical analysis

Differences in OM %, diffusive fluxes of NH_4^+ and $Si(OH)_4$ and measured gas fluxes of CO_2, N_2O and CH_4 were all evaluated using 2-factor analyses of variance (ANOVAs) based on the design described in Section 2.2. The factors ‘habitat’ and ‘cover’ were both treated as fixed. Homogeneity of univariate variance was tested using Cochran’s C-test and corrected for by the same method as detailed in Chapter II. When significant differences were detected by ANOVA ($P < 0.05$), Student-Newman Keuls (SNK) tests were done to identify patterns of difference. All calculations were done using Win-GMAV (Underwood and Chapman, 1998).

A distance-based linear model (DISTLM) procedure was used to perform a permutational test of the null hypothesis of no relationship between biological assemblages and functional variables (Legendre and Anderson, 1999; McArdle and Anderson, 2001) calculated on Bray-Curtis similarity measures (Bray and Curtis, 1957) and analysed using the PRIMER package (PRIMER-e, 2009). Functional variables included in the analyses were fluxes of NH_4^+, $Si(OH)_4$, CO_2, N_2O and CH_4, mean pools and residence times of NH_4^+ and $Si(OH)_4$ and OM, TOC and TON %. Marginal tests (i.e. fitting of each variable individually, ignoring other variables) were followed by the *all specified* selection procedure with the *adjusted* R^2 selection criterion. The significance of the marginal tests was determined based on 9999 permutations of raw data (Anderson, 2003). Distance-based redundancy analysis (dbRDA) was used to visualise the fitted DISTLM model (Legendre and Anderson, 1999). Models were fitted using 9999 unrestricted permutations of raw data. Analyses were done on square-root transformed assemblage data in order to reduce the contribution of highly abundant taxa (Clarke and Warwick, 2001). Functional variables were automatically normalised as part of the DISTLM procedure.

4.3 Results

4.3.1 Organic matter (OM) content and C/N ratio

OM % only differed significantly in the 2 – 4 cm depth within mussel-beds, where plots with 50 % cover had greater OM % than those with 0, 5 or 100 % cover of *C. gigas* (F = 6.29, MS = 0.22, *d.f.* = 3, $P < 0.01$). Within mud-flats, there were no significant differences in OM % at any depth. Although not significantly different, the OM % in the 0 – 2 cm depth tended to increase with increasing cover of *C. gigas* within mussel-beds (Table 11). TOC %, TON % and C/N ratios did not significantly differ between habitats or among different cover of *C. gigas* (Table 12). Although not significantly different, TOC and TON % in mussel-beds were greatest in plots with 50 % cover of *C. gigas* and in mud-flats TOC and TON % tended to

increase with increasing cover of *C. gigas* (Table 13).

4.3.2 Pore-water nutrient concentrations

There was greater NH_4^+ concentration in plots with 50 or 100 % cover than in plots with 5 or 0 % cover of *C. gigas* in mussel-beds and no differences in mud-flats ($F = 3.07$, $MS = 1.13$, *d.f.* = 3, $P < 0.05$). In the shallow layers (0 to 3 cm depth) within mussel-beds NH_4^+ concentration was greatest in plots with 50 % cover of *C. gigas*, but in deeper layers (from 3 to 10 cm depth) NH_4^+ concentration was greatest in plots with 100 % cover of *C. gigas* (Figure 15). Within sediment habitats, NH_4^+ concentration was greatest within plots with 50 % cover of *C. gigas* at each depth (Figure 15). In the deeper layers (from 5 to 10 cm depth) in mussel-beds, NH_4^+ concentration increased with increasing cover of *C. gigas*. $Si(OH)_4$ concentration was significantly greater in mussel-beds than in mud-flats ($F = 49.50$, $MS = 168.76$, *d.f.* = 3, $P < 0.0001$) and within plots with 100, 50 or 0 % cover than in plots with 5 % cover of *C. gigas* ($F = 5.45$, $MS = 18.59$, *d.f.* = 3, $P < 0.01$). Within the shallow layers (0 to 3 cm depth) of mussel-beds, plots with 50 % cover of *C. gigas* have the greatest $Si(OH)_4$ concentration (Figure 16). Within the shallowest layers (from 0 to 1cm depth) of mud-flats, plots with 100 % cover of *C. gigas* have the greatest $Si(OH)_4$ concentration (Figure 16).

4.3.3 Diffusive nutrient fluxes

The average nutrient fluxes of NH_4^+ and $Si(OH)_4$ were unidirectional, from the sediment pore-water into the overlying bottom water. Fluxes of NH_4^+ differed between habitats and among different covers of *C. gigas* (Table 14). In mussel-beds, fluxes of NH_4^+ were greater from plots with 50 % cover than from those with 100, 5 or 0 % cover of *C. gigas* (Figure 17a, Table 14, SNK procedure). Although there were no significant differences due to oyster cover in mud-flats, the average flux of NH_4^+ from plots with 50 % cover of *C. gigas*

was more than twice that from plots with 100, 5 or 0 % cover of *C. gigas* (Figure 17a). The flux of $Si(OH)_4$ was not significantly affected by the type of habitat or the cover of *C. gigas* (Table 14), but within mussel-beds was reduced in plots with 100 % cover of *C. gigas* compared to those with 50, 5 or 0 % cover of *C. gigas* (Figure 17b). Within sediment habitats, plots with 50 or 100 % cover had greater average fluxes than those with 5 or 0 % cover of *C. gigas* (Figure 17b).

4.3.4 Inventory and residence time of nutrients

The benthic pools of NH_4^+ and $Si(OH)_4$ were greater in mussel-beds than in mud-flats (Table 14, Figure 18). Overall, the NH_4^+ benthic pools were greater in plots with 50 or 100 % cover than in plots with 5 or 0 % cover of *C. gigas* (Table 14, Figure 18a, SNK procedure). The residence time of NH_4^+ in mussel-beds was significantly longer in plots with 100 % cover than in plots with 50 or 0 % cover of *C. gigas*, but in mud-flats it was significantly shorter in plots with 50 % cover than in plots with 5 % cover of *C. gigas* (Table 14, Figure 19a, SNK procedure). The residence time of $Si(OH)_4$ in mussel-beds was significantly longer in plots with 100 % cover than in plots with 0, 5 or 50 % cover of *C. gigas* (Table 14, Figure 19b, SNK procedure) and although not significant, it was longer in plots with 0, 5 or 50 % cover than in plots with 100 % cover of *C. gigas* in mud-flats.

4.3.5 Community respiration

The CO_2 flux from the sediment was significantly affected by the cover of oysters alone, with greater fluxes in plots with 100 % cover than in those with 50, 5 or 0 % cover of *C. gigas* regardless of the type of habitat (Table 15, Figure 20a, SNK procedure). The fluxes of CH_4 and N_2O did not differ significantly between habitats or among different covers of oysters (Table 15, Figure 20b and c), however, CH_4 flux followed a similar pattern to that of CO_2 and was increased by 100 % cover of oysters.

4.3.6 Relationship between changes in assemblage structure and functional variables

The functional variables together were associated with 33.2 % of the total variation in assemblage structure (Figure 21). Marginal tests of variables indicated that changes in overall assemblage structure related most strongly to changes in NH_4^+ and $Si(OH)_4$ pools and NH_4^+ flux which was associated with 18, 10 and 9 % of the variation in assemblage data respectively (Table 16).

Table 11. Average (+/- S.E.) percentage of organic matter from loss on ignition (OM %) at 0 – 2, 2 – 4 and 4 – 6 cm depth in plots with increasing cover of oysters in mussel-beds and mud-flats.

	0 – 2 cm	2 – 4 cm	4 – 6 cm
Mussel-bed			
0 %	0.91 +/- 0.13	0.63 +/- 0.02	0.93 +/- 0.15
5 %	0.90 +/- 0.09	0.82 +/- 0.13	0.84 +/- 0.15
50 %	1.10 +/- 0.16	1.16 +/- 0.16	0.95 +/- 0.04
100 %	1.27 +/- 0.15	0.87 +/- 0.05	1.01 +/- 0.14
Mud-flat			
0 %	0.89 +/- 0.13	0.86 +/- 0.06	0.85 +/- 0.14
5 %	1.28 +/- 0.26	0.90 +/- 0.11	0.96 +/- 0.15
50 %	1.02 +/- 0.03	0.63 +/- 0.03	0.94 +/- 0.21
100 %	1.19 +/- 0.09	0.93 +/- 0.09	0.98 +/- 0.10

Table 12. ANOVA for total organic carbon (TOC %) and nitrogen (TON %) and C/N ratios from surface sediment (0 – 2 cm) in plots with increasing cover of oysters in mussel-beds and mud-flats.

Source		TOC %		TON %		C/N	
	d.f.	MS	F	MS	F	MS	F
Habitat (=H)	1	0.05	0.79	0.00	0.84	0.07	0.08
Cover of oysters (=C)	3	0.14	2.05	0.00	2.05	0.87	1.10
H x C	3	0.04	0.59	0.00	0.67	0.22	0.28
Res	16	0.07		0.00		0.79	

Significant results are indicated, * = $P < 0.05$, ** = $P < 0.01$.

Table 13. Average (+/- S.E.) TON %, TOC % and C/N ratios from surface sediments (0 – 2 cm) of plots with increasing cover of oysters in mussel-beds or mud-flats.

	TON %	TOC %	C/N
Mussel-bed			
0 %	0.14 +/- 0.03	1.01 +/- 0.18	7.33 +/- 0.35
5 %	0.14 +/- 0.01	1.00 +/- 0.03	7.16 +/- 0.57
50 %	0.16 +/- 0.02	1.16 +/- 0.04	7.39 +/- 0.70
100 %	0.16 +/- 0.01	1.16 +/- 0.11	7.39 +/- 0.59
Mud-flat			
0 %	0.10 +/- 0.03	0.77 +/- 0.22	7.49 +/- 0.27
5 %	0.12 +/- 0.02	0.86 +/- 0.14	7.24 +/- 0.10
50 %	0.13 +/- 0.02	0.95 +/- 0.08	7.46 +/- 0.82
100 %	0.14 +/- 0.02	1.02 +/- 0.24	7.44 +/- 0.33

Table 14. ANOVA for diffusive fluxes, inventories and residence times of NH_4^+ and $Si(OH)_4$ in plots with increasing cover of oysters in mussel-beds and mud-flats.

Source		NH_4^+			$Si(OH)_4$		
Flux	*d.f.*	MS	F		MS	F	
Habitat (=H)	1	0.73	11.68	**	0.01	0.94	
Cover of oysters (=C)	3	0.09	1.53		0.01	1.47	
H x C	3	0.21	3.36	*	0.01	1.37	
Res	16	0.06			0.01		
Inventory							
Habitat (=H)	1	13.35	58.78	***	0.00	23.50	***
Cover of oysters (=C)	3	0.97	4.31	*	0.00	2.12	
H x C	3	0.57	2.53		0.00	0.19	
Res	16	0.23			0.00		
Residence time							
Habitat (=H)	1	0.01	0.05		2.77	2.68	
Cover of oysters (=C)	3	0.24	1.25		2.49	2.41	
H x C	3	0.76	3.90	*	3.95	3.83	*
Res	16	0.19			1.03		

Significant results are indicated, * = $P < 0.05$, ** = $P < 0.01$.

Table 15. ANOVA for gas flux of CO_2, CH_4 and N_2O on plots with increasing cover of oysters in mussel-beds and mud-flats.

Source		CO_2			CH_4		N_2O	
	d.f.	MS	F		MS	F	MS	F
Habitat (=H)	1	20.01	0.16		187.82	0.22	6.35	0.27
Cover of oysters (=C)	3	4525.33	5.87	**	1511.62	1.74	8.77	0.19
H x C	3	229.76	0.30		289.37	0.33	1.89	0.76
Res	16	770.54			868.99		4.87	

Significant results are indicated, * = P < 0.05, ** = P < 0.01.

Table 16. Marginal tests of distance-based linear model (DISTLM) analyses on relationships between assemblage structure and individual functional variables in plots with increasing cover of oysters in mussel-beds and mud-flats. Prop is the proportion of variance in the assemblage structure that is explained by the functional variable.

Variable	Pseudo-F	Prop.	
NH_4^+ pool	4.68	0.18	***
$Si(OH)_4$ pool	2.34	0.10	*
NH_4^+ flux	2.06	0.09	*
TOC%	2.03	0.08	
TON%	1.66	0.07	
$Si(OH)_4$ residence time	0.91	0.04	
CH_4 flux	0.91	0.04	
NH_4^+ Residence time	0.75	0.03	
CO_2 flux	0.72	0.03	
$Si(OH)_4$ flux	0.67	0.03	
Mean OM%	0.66	0.03	
N_2O flux	0.46	0.02	

Significant results are indicated: * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

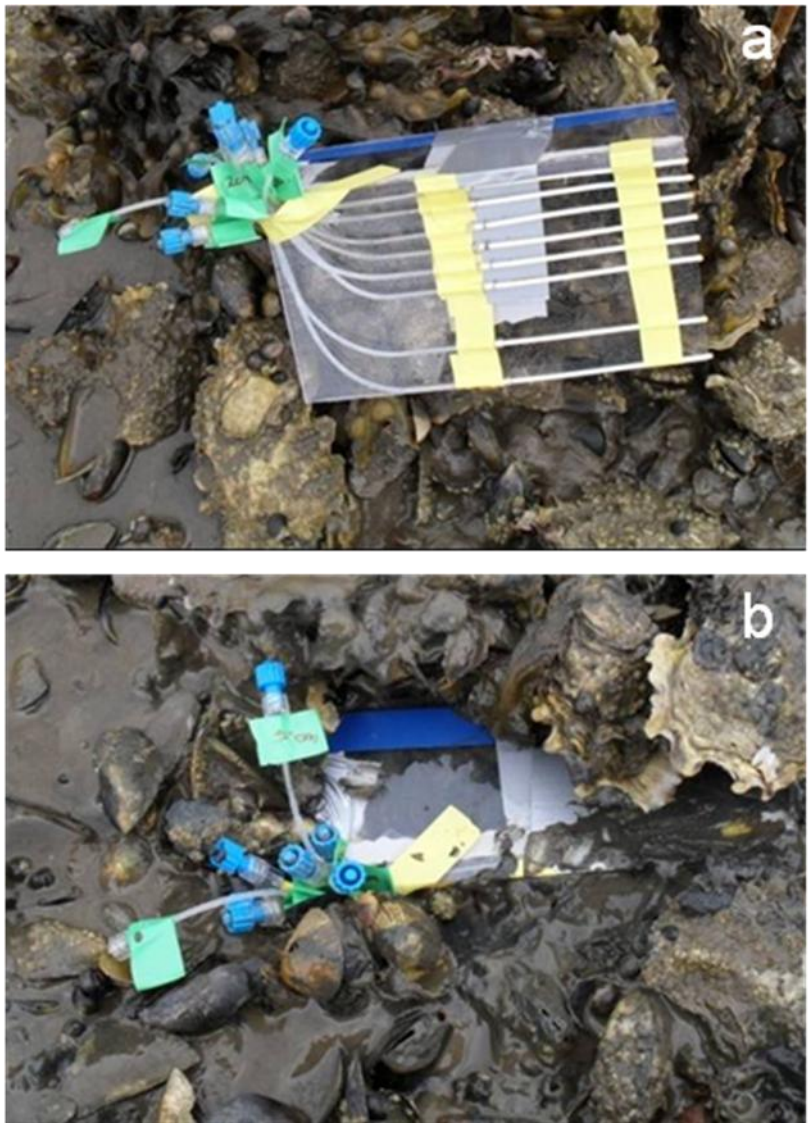

Figure 13. (a) Pore-water profiler with Rhizon^{TM} soil moisture samplers placed at 0, 1, 2, 3, 4, 5, 8 and 10 cm intervals. (b) Pore-water profiler inserted into a mussel-bed.

Figure 14. Sample being extracted from a gas collection chamber situated on a mussel-bed.

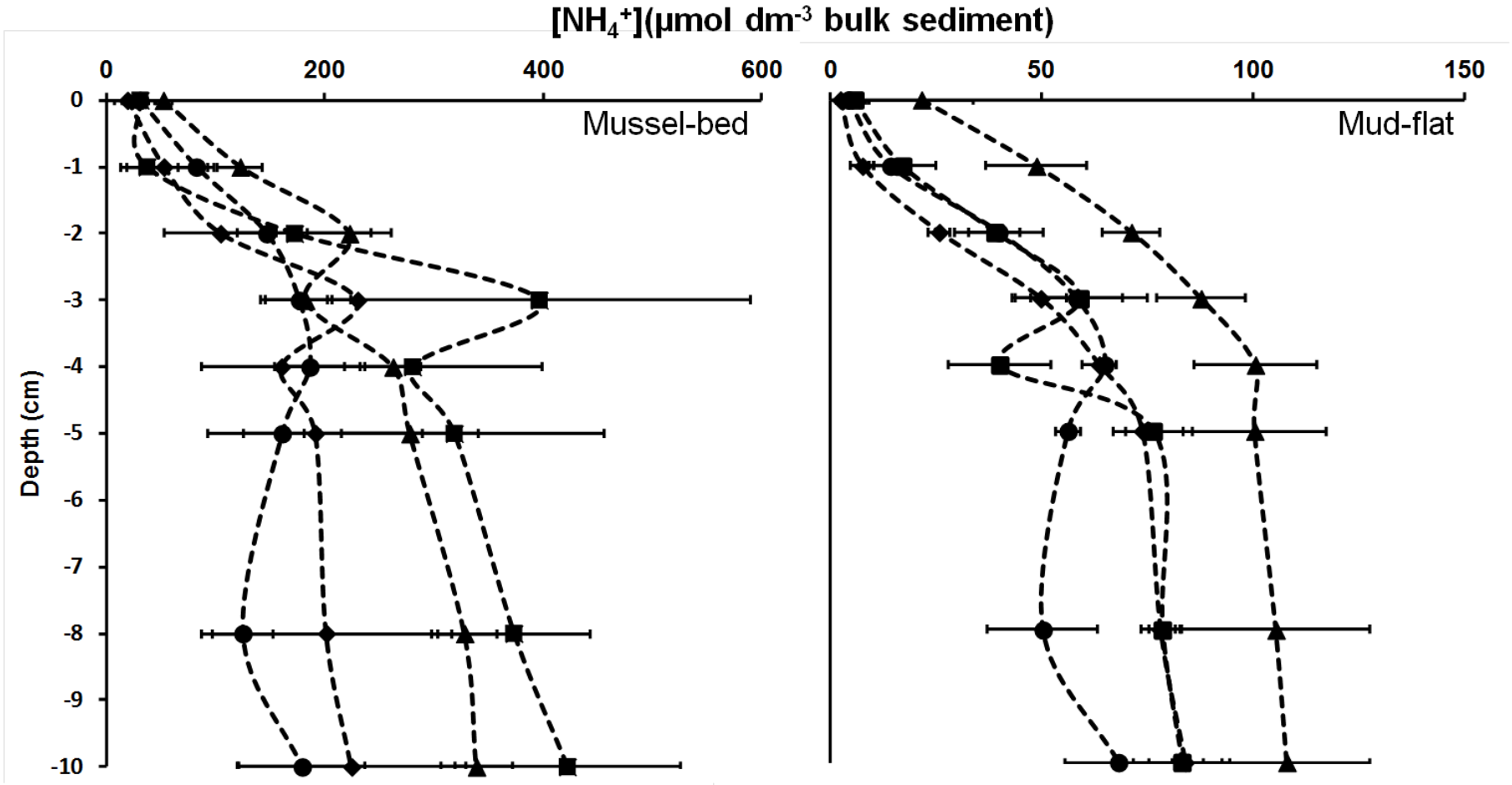

Figure 15. Concentration of ammonium (NH_4^+) in pore water from the sediment-water interface (0 cm) down to 10 cm depth in experimental plots with 0 (●), 5 (♦), 50 (▲) or 100 (■) % cover of oysters in mussel beds or mud-flats.

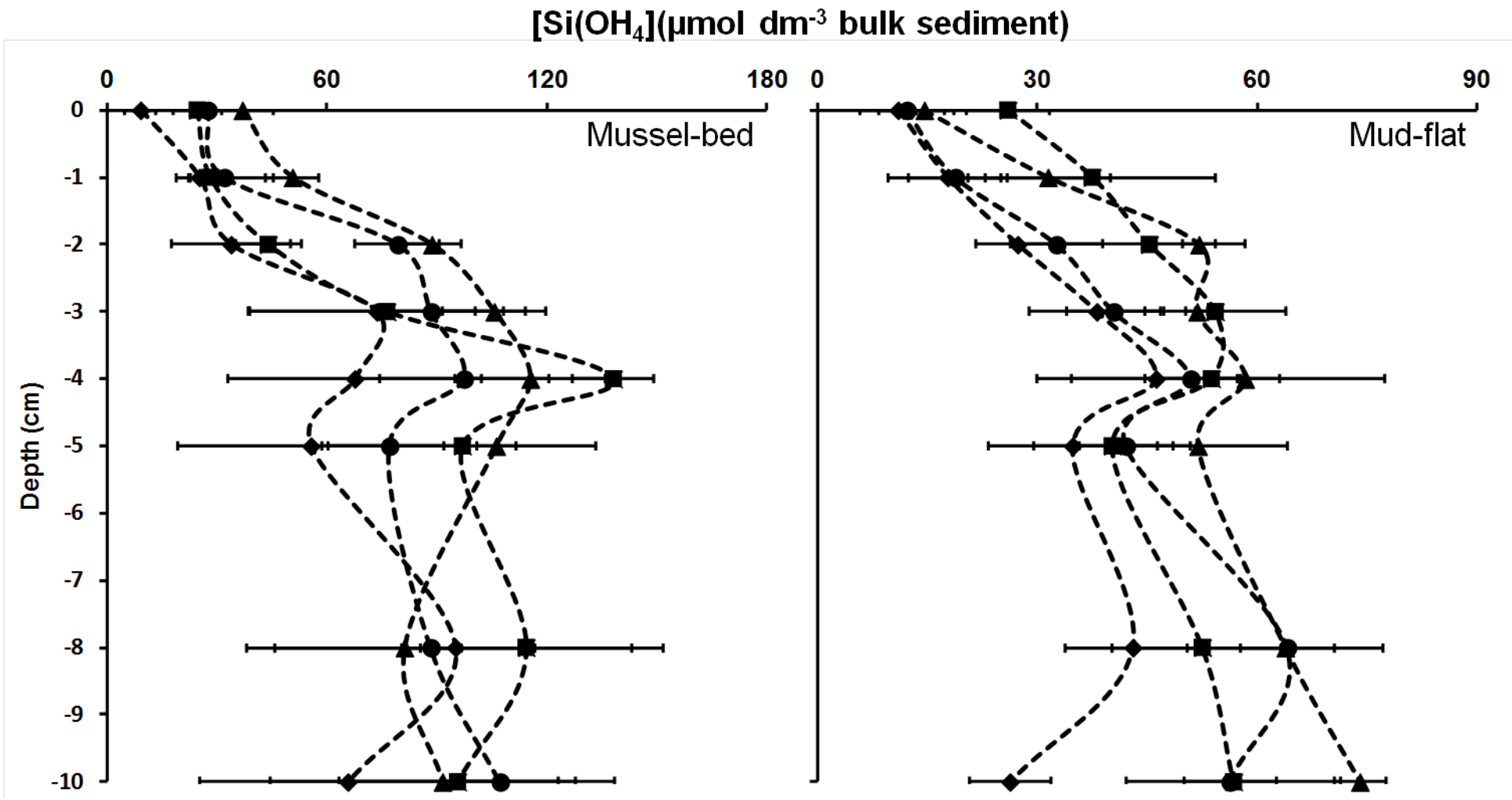

Figure 16. Concentration of silicate ($Si(OH)_4$) in pore water from the sediment-water interface (0 cm) down to 10 cm depth in experimental plots with 0 (●), 5 (♦), 50 (▲) or 100 (■) % cover of oysters in mussel beds or mud-flats.

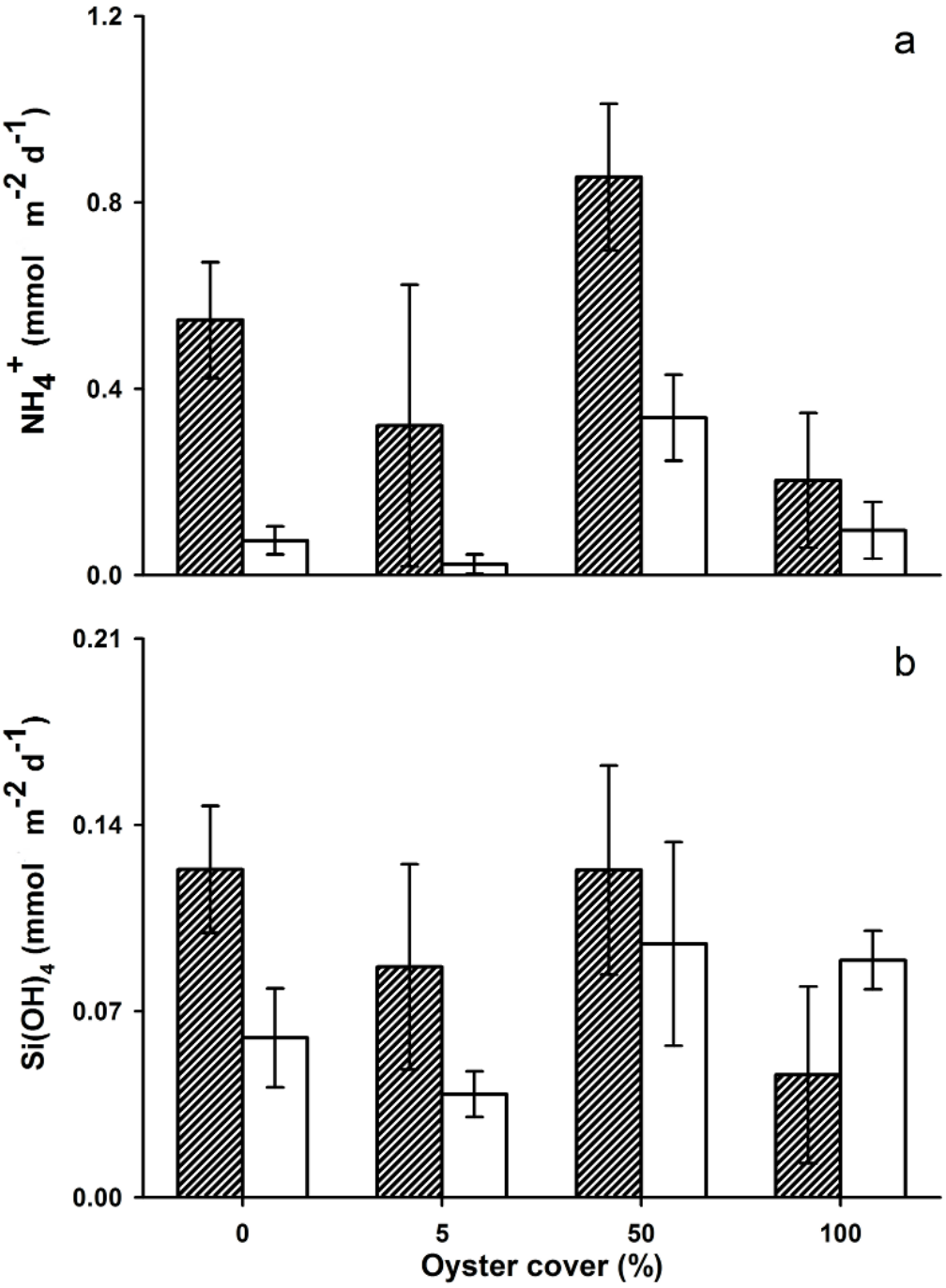

Figure 17. Modelled daily diffusive fluxes of NH_4^+ (a) and $Si(OH)_4$ (b) across the sediment-water interface in experimental plots with 0, 5, 50 or 100 % cover of oysters in mussel beds (shaded bars) or mud-flats (clear bars).

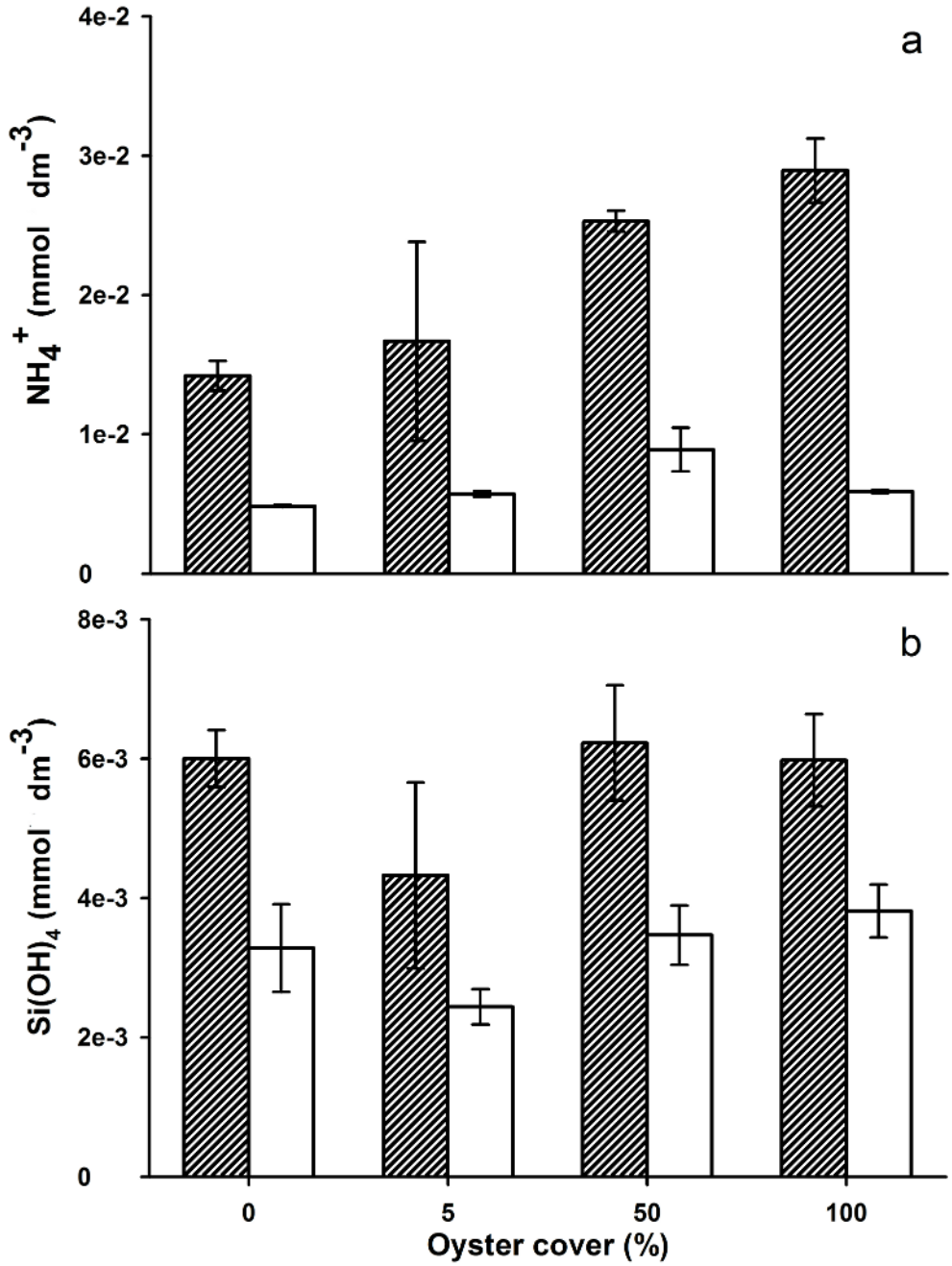

Figure 18. Inventory of pore-water NH_4^+ (a) and $Si(OH)_4$ (b) in experimental plots with 0, 5, 50 or 100 % cover of oysters in mussel beds (shaded bars) or mud-flats (clear bars).

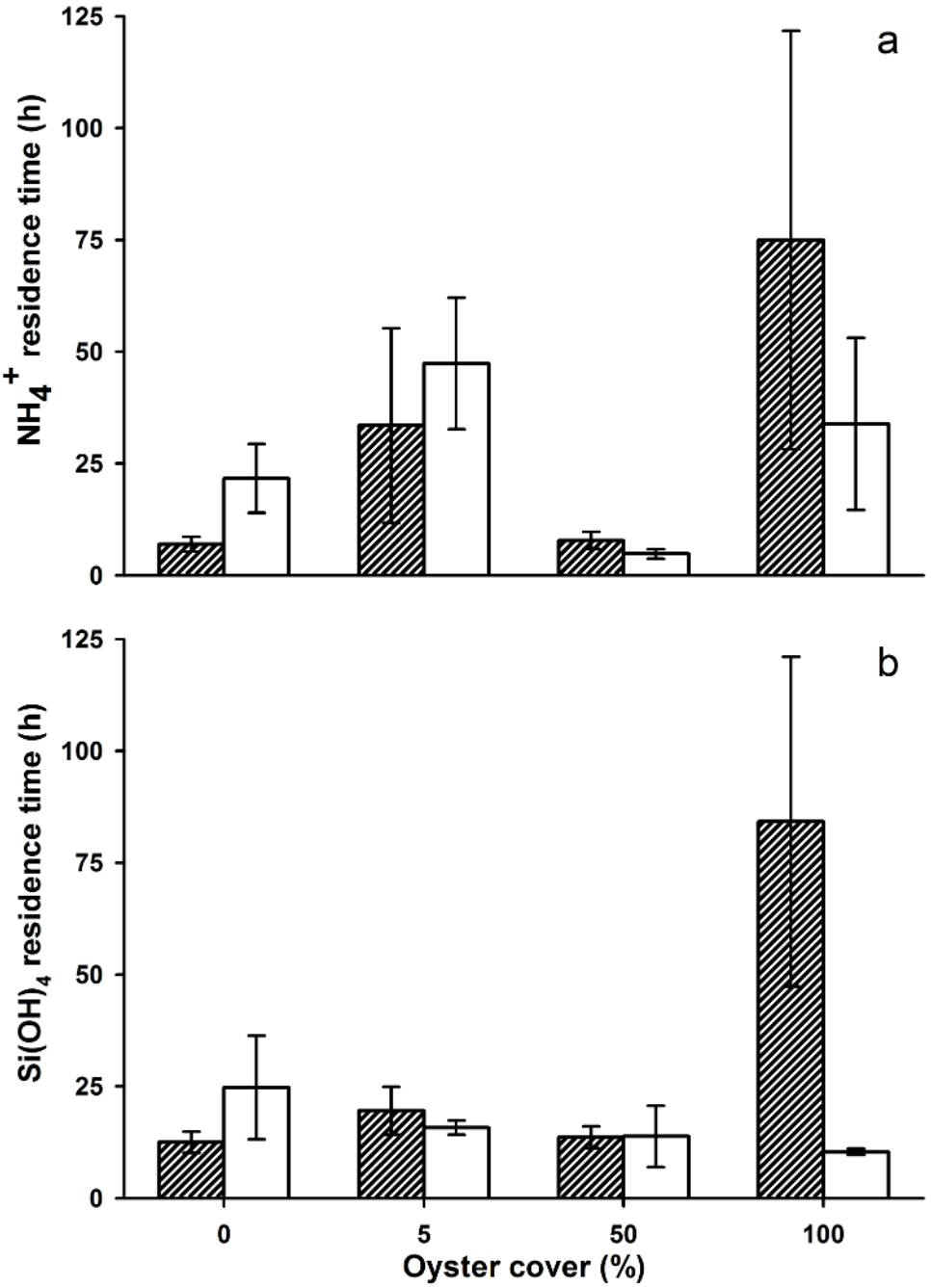

Figure 19. Mean residence times for benthic pools of (a) NH_4^+ and (b) $Si(OH)_4$ in experimental plots with 0, 5, 50 or 100 % cover of oysters in mussel beds (shaded bars) or mud-flats (clear bars).

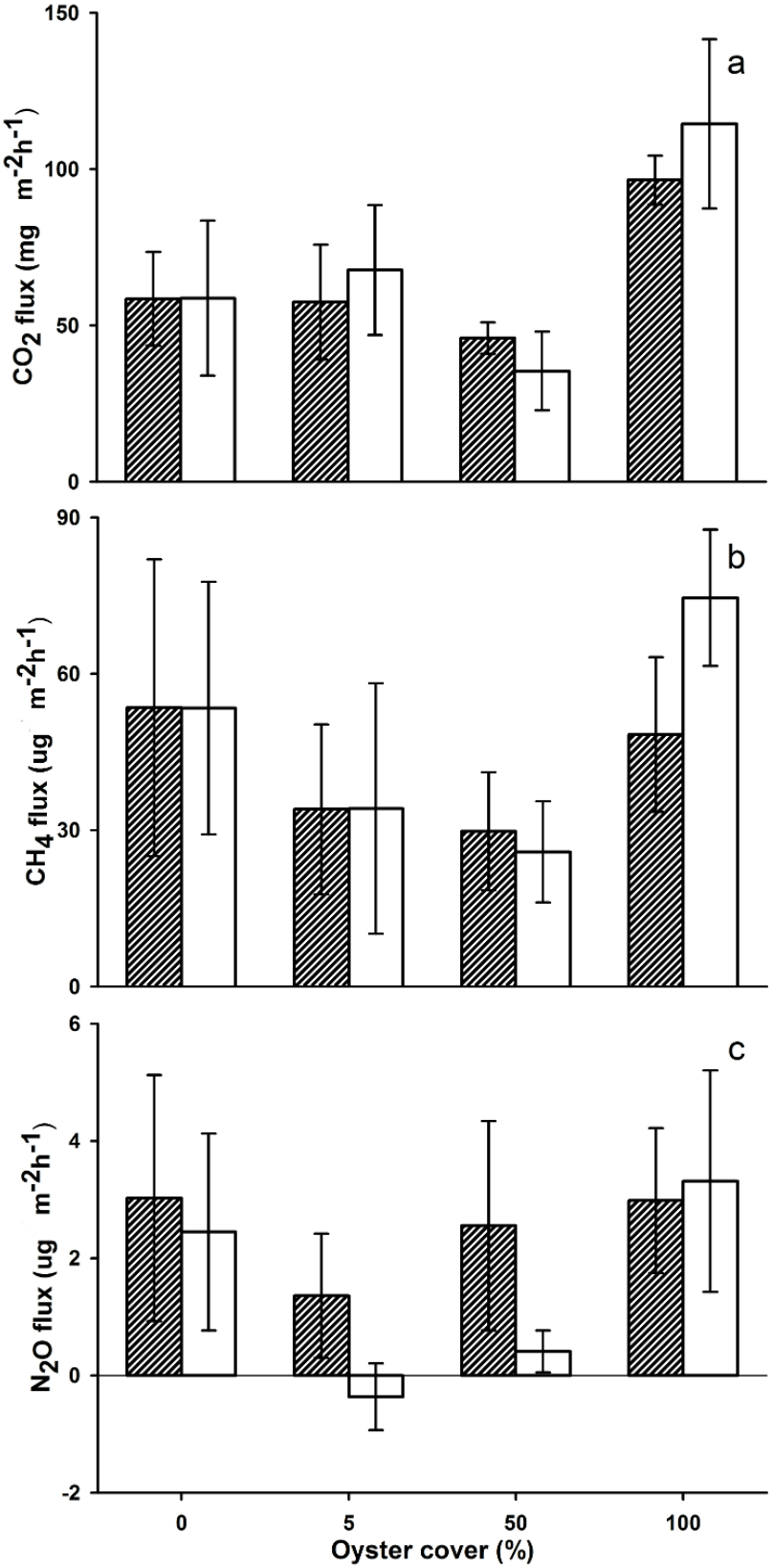

Figure 20. Hourly fluxes of CO_2 (a), CH_4 (b) or N_2O (c) in experimental plots with 0, 5, 50 or 100 % cover of oysters in mussel beds (shaded bars) or mud-flats (clear bars).

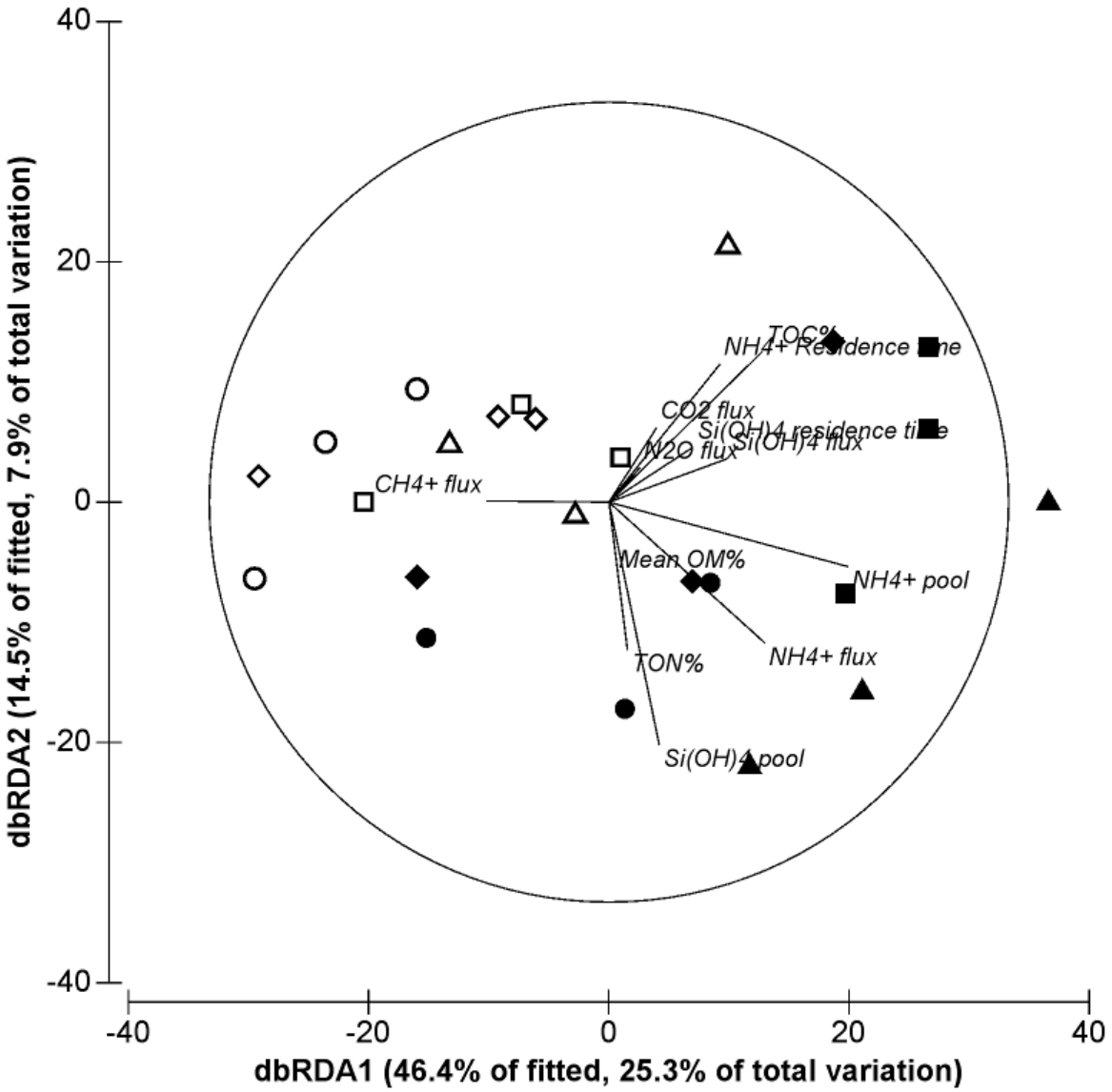

Figure 21. Distance-based redundancy analysis (dbRDA) of square-root transformed assemblages in experimental plots with 0 (●), 5 (♦), 50 (▲) or 100 (■) % cover of *C. gigas* in mussel-beds and 0 (○), 5 (◊), 50 (Δ) or 100 (□) % cover of *C. gigas* in mud-flats. The functional variables used to generate the dbRDA were NH_4^+ inventory, fluxes of pore-water NH_4^+ and $Si(OH)_4$, gas fluxes of CO_2, CH_4 and N_2O, average OM %, TOC and TON %.

4.4 Discussion

C. gigas altered key processes involved in nutrient cycling, vital for the functioning of estuarine ecosystems, but the nature and magnitude of some of these alterations depended on the habitat and on the cover of *C. gigas*.

Although not significantly different, TOC and TON % increased slightly with increasing cover of *C. gigas* in each habitat, indicating increased biodeposition. C/N ratios did not differ between habitats or among different levels of cover of *C. gigas* and fell into the range of previously measured C/N ratios of biodeposits from other suspension-feeding bivalves (Miller et al., 2002; Giles and Pilditch, 2004), with values between 4 and 8, indicating a composition of phytoplankton and faecal material (Kautsky and Evans, 1987).

Gas fluxes were similarly affected by the cover of *C. gigas* in each habitat. The flux of N_2O, a product of denitrification, was not affected by habitats or cover of *C. gigas* and the measured values were low (less than 5µg m^{-2} h^{-1}). This is not surprising since in marine ecosystems the flux of N_2O only represents about 5 % of the N_2 flux (Seitzinger and Nixon, 1985). Also denitrification relies on the respiration of nitrate and nitrite, both of which were found in negligible concentrations in this study. CO_2 fluxes, however, increased at the highest cover of *C. gigas* in each habitat, likely due to an increase in microbial activity from the decomposition of OM supplied in biodeposits of *C. gigas* (Newell et al., 2005). Although not significant, the flux of CH_4 followed a similar pattern to that of CO_2, indicating that anoxic decomposition of OM (through methanogenesis) also increased at high cover of *C. gigas*. This could be due to an increase in the amount of buried OM available for anaerobic decomposition (Newell, 2004). In situations with high densities of bivalves, biodeposition can stimulate microbial metabolism sufficiently to cause sediments to become anoxic (Tenore et al., 1982). In these situations organic nitrogen is primarily

regenerated as NH_4^+ (Newell et al., 2005), however, mineralisation of OM may be less effective in anoxic conditions and burial of OM can increase (Hansen and Blackburn, 1991). Alternatively, the increase in CO_2 and CH_4 fluxes could be due to the "priming effect" (Löhnis, 1926), whereby the addition of fresh labile OM stimulates microbial OM decomposition, including that of older, buried, recalcitrant OM (Guenet et al., 2010). The labile OM necessary to stimulate the priming effect in aquatic ecosystems is present in phytoplankton (McKinley and Vestal, 1992) and could have been present in, or enhanced by, oyster biodeposits. Regardless of the mechanisms, the combination of CO_2 and CH_4 fluxes are an indicator of the total carbon catabolism in the sediment (Griffiths et al., 1983), meaning that the total decomposition of organic carbon increased at high cover of *C. gigas* in both habitats. Of course, one cannot ignore the possibility that the increase in community respiration was due to respiration of the oysters themselves, or that of the associated macrofauna, or that it was simply due to a decrease in air volume due to the oysters occupying space in the gas collection chambers. Although the current experiment cannot separate between these models, tests were made in a subsequent experiment and changes were found to be mostly attributable to fluxes from the sediment (Chapter V).

NH_4^+ can be directly excreted by *C. gigas* (Dame et al., 1984 and 1985) and associated fauna which were facilitated by *C. gigas*, but the main origin is likely from microbially mediated mineralisation of OM from biodeposits (Newell et al., 2005) which increased in supply with increasing cover of *C. gigas*. In both habitats, the pools and fluxes of NH_4^+ were greatest, and the residence times were shortest, in plots with medium cover of *C. gigas*, rather than in plots with high cover as might be expected. NH_4^+ may have been depleted from the plots with high cover of *C. gigas*, for example, actively growing algae can intercept and assimilate NH_4^+ in the surface layers of sediment, thereby limiting its release to the water column (Rysgaard et al., 1995; Newell et al., 2002; Newell, 2004). It is common for the growth and production of algae to be enhanced by bivalves (Newell et al.,

2005) and an increase in algae could account for the decrease in NH_4^+ with high cover of *C. gigas*. In fact, there was a significant increase in the cover of brown macroalgae, *Fucus vesiculosus* (Linnaeus, 1753), within plots with high cover of *C. gigas* in both habitats (Chapter III) and despite slow growth, *F. vesiculosus* can utilize NH_4^+ very efficiently (Pedersen and Borum, 1997).

$Si(OH)_4$ fluxes decreased within plots with high cover of *C. gigas* in mussel-beds but increased with high and medium cover of *C. gigas* within mud-flats. Dissolved silica plays a major role in the functioning of marine ecosystems (Ragueneau et al., 2002) as an essential nutrient for the growth of diatoms (a vital component of marine food webs) and so its depletion and cycling is closely tied to diatom productivity (Cohen, 2003). Diatoms taken up by filter-feeders are regenerated as dissolved silica ($Si(OH)_4$) from the dissolution of biodeposits in the sediments (Ittekkot et al., 2006). Filter-feeders can produce such high quantities of biodeposits that the subsequent dissolution of biogenic silica allows for high flux rates of $Si(OH)_4$ and facilitates diatom dominance (Ragueneau et al., 2002). This may be the case within mud-flats, where the benthic turnover time of $Si(OH)_4$ was slightly accelerated with increasing cover of *C. gigas*, meaning that these sediments may act as an important source of $Si(OH)_4$ to the water column. In mussel-beds, however, the turnover time was greatly decelerated with the highest cover of *C. gigas*, possibly converting sediments into a sink for $Si(OH)_4$, limiting release into the water column. The retention of $Si(OH)_4$ in sediments due to increased biodeposition has recently become a concerning consequence of invasive filter-feeding organisms (Ragueneau et al., 2005). There are two groups of phytoplankton, those dominated by diatoms and those not (Officer and Ryther, 1980). When silicate is absent, diatoms become replaced by other phytoplankton groups that do not have any requirement for this nutrient, such as dinoflagellates, which can form harmful algal blooms (Smayda, 1997). These can have deleterious effects on human health (Officer and Ryther, 1980) and support food-webs that are not economically

desirable (Chorus and Bartram, 1999). Shifts of ecosystems from siliceous-based to non-siliceous-based phytoplankton communities have typically been attributed to anthropogenically induced enrichment of nitrogen and phosphorus (Rocha et al., 2002). These effects, coupled with those of invasive bivalves, could lead to long-term deterioration of the water quality and economical value of this estuary.

It is important to note that diffusive fluxes calculated using Fick's Law only represent diffusive mechanisms of nutrient transport and do not include fluxes caused by advective pore-water exchange or bioturbation (Clavero et al., 2000). Advective pore-water exchange processes in sediments are altered by changes to local pressure gradients which may be caused by protruding structures, such as shells (Huettel and Gust, 1992). For example, during inundation, a single shell protruding from the sediment surface can force water from the water column into the interstices of the sediment and simultaneously draw pore-water out, thus increasing the flux of inorganic nutrients into the water column and decreasing the concentration in the surface layers of sediment (Huettel et al., 1998). Oscillation of the redox boundary caused by advection can promote the degradation of OM by controlling microbial diversity and process rates (Rocha, 2008). The structure of oysters may, therefore, cause differences in pore-water nutrient concentrations by altering advective pore-water exchange. Pore-water exchange in muddy sediments (such as in this study) are, however, more likely to be dominated by diffusion and bioturbation, rather than advection (Mermillod-Blondin, 2011). Bioturbation is an important ecosystem process influencing the distribution of nutrients in sedimentary habitats (Rosenberg, 2001). Bioturbating polychaetes enhance the exchange of inorganic nutrients from sediment pore-water into the overlying water (Henriksen et al., 1983; Banta et al., 1999). There was a decrease in the abundance of infaunal polychaetes in plots with the highest cover of *C. gigas* in mussel-beds (Chapter III), which may have reduced bioturbation in these plots and contributed to the reduction in $Si(OH)_4$ and NH_4^+ fluxes. This same model cannot,

however, explain the decrease in NH_4^+ flux in plots with high cover of oysters in mud-flats where the abundance of polychaetes was unaffected by *C. gigas* (Chapter III). Community respiration can indicate microbial activity (Raina et al., 2009), and was similarly affected by *C. gigas* in both habitats, suggesting that microbially mediated processes may have played a substantial role in determining the differences in nitrogen cycling.

The relationship between biodiversity and ecosystem functioning has received a lot of attention in recent years (Gamfeldt and Hillebrand, 2008), but the effect of invasive species on this interaction has not (Sousa et al., 2011). Ecosystem functioning may be affected by *C. gigas* either directly due to their biological activities, or indirectly as a result of consequent changes to biodiversity (Chapin et al., 2000). Or indeed, biodiversity may be altered as a result of changes to ecosystem processes resulting directly or indirectly from *C. gigas*. The current study cannot separate these models, but we can comment on covariance between biodiversity and the functional properties of the ecosystems studied. Differences in pools of NH_4^+ and $Si(OH)_4$ and fluxes of NH_4^+ co-varied with the differences in assemblage structure of plots. Biodiversity (measured as species richness and Shannon-Weiner diversity) in mussel beds followed a similar pattern to NH_4^+ and $Si(OH)_4$ fluxes, increasing up until medium cover of *C. gigas* but significantly decreasing at the highest cover (Chapter III). Differences in pore-water nutrient fluxes, therefore, may have resulted from the differences in associated macrofaunal biodiversity (Loreau et al., 2001; Cardinale et al., 2006). Alternatively, biodiversity may have responded to changes in the physical or chemical environment due to *C. gigas*. Biodiversity is often increased by the shells of bivalves which provide habitat and increase habitat complexity (McCoy and Bell, 1991), but high rates of biodeposition associated with high densities of filter-feeding bivalves can decrease macrofaunal diversity by reducing O_2 availability at the water-sediment interface (Commito and Boncavage, 1989). There was very little mortality of mussels or oysters in the experimental plots (personal observation), so there would have

been a great amount of biodeposition in plots with high cover of *C. gigas*, arising from both oysters and mussels. None of these models can explain the decrease in NH_4^+ flux at the highest cover of *C. gigas* in mud-flats since here biodiversity was increased by *C. gigas*. This is not surprising since at lower densities of bivalves, such as on mud-flats, biodeposits provide an important resource for benthic species without producing unfavourable anoxic conditions and so often increase macrofaunal diversity (Norkko et al., 2001). Differences between habitats likely occurred because of pre-existing differences in the processes determining OM deposition. For example, mussel-beds are dominated by filter-feeders and so already have strong benthic-pelagic coupling and a plentiful supply of hard substrata, whereas mud-flats are dominated by passive settlement of particulate OM and soft sediment. It is possible that different mechanisms may have operated within the different habitats, despite causing similar patterns.

The same densities or cover, however, may have different effects depending on the characteristics of the receiving environment. For example, although nitrogen cycling and community respiration were similarly affected in both habitats, effects on biodiversity and silicate cycling differed. Our results confirm the recommendations of Sousa et al. (2009) and Padilla (2010) that more experimental studies spanning a range of habitats are needed in order to assess the context-dependency of invasive species and to avoid detrimental economic consequences (Yokomizo et al., 2009).

Through their biological activities, filter-feeding bivalves strongly affect physical, chemical and biological properties of sediments, typically resulting in local deposition rates exceeding those of passive physical sedimentation (Dobson and Mackie, 1998). The increased deposition of OM from biodeposits (Kautsky and Evans, 1987; Chamberlain et al., 2001) can increase carbon and nitrogen burial in sediments, oxygen consumption, anoxia and denitrification rates (Kaspar et al., 1985). In this way, they can strongly

influence the cycling of important biogenic elements such as carbon (Doering et al., 1986; Chauvaud et al., 2003), nitrogen (Dame et al., 1985) and silica (Ragueneau et al., 2002). Because of the intensity of benthic-pelagic coupling in coastal waters, they play an essential role in the functioning of coastal ecosystems (Wildish and Kristmanson, 1984; Alpine and Cloern, 1992; Dame, 1996). Our results show that this role, when played by an invasive bivalve, can alter the rates of OM decomposition and nutrient regeneration to varying extents depending on the receiving habitat and on the cover of bivalves. At the highest cover of *C. gigas* in mussel-beds the benthic turnover times of NH_4^+ and $Si(OH)_4$ were decelerated, leading to increased retention of these nutrients in the sediment, possibly limiting productivity and causing changes in the phytoplankton community (see above). In mud-flats, the same pattern occurred with regards to NH_4^+, but not for $Si(OH)_4$. Benthic nutrient pools were lower in mud-flats than in mussel-beds, so the magnitude of the effects are greater and mitigation of the retention of $Si(OH)_4$ observed in mussel-beds is unlikely.

Our results show that when present at high cover, especially in mussel-beds, *C. gigas* can decrease biodiversity and reduce the efficiency of ecosystem processes such as benthic nutrient cycling, thus potentially altering ecosystem services. The importance of the effects of invasive species on ecosystem functioning has only recently being recognised (Ehrenfeld, 2010), but the link between alterations to ecosystem processes and affects on ecosystem services is often overlooked (Charles and Dukes, 2007). Alterations to ecosystem processes directly affect supporting ecosystem services (Charles and Dukes, 2007) which are vital for the maintenance of other ecosystem services that are of benefit to humans (Daily, 1997). For example, if *C. gigas* becomes a dominant species in Lough Swilly, nutrient cycling may be altered to the extent that greater burial of OM and reduced regeneration of limiting biogenic elements, reduces the carrying capacity of the estuary (Cugier et al., 2010), leading to reductions in a provisioning ecosystem service,

commercial shellfish production. In addition, invasive species which cause reductions to biodiversity may threaten the delivery and quality of ecosystem services by altering ecosystem functioning (Charles and Dukes, 2007).

Chapter V - Effects of non-indigenous oysters on the diversity and functioning of microbial assemblages

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5.1 Introduction

Biological invasions of non-indigenous species are considered among the most serious global threats to biodiversity, ecosystem functioning and the provision of ecosystem services in terrestrial (Pejchar and Mooney, 2009), freshwater (Simon and Townsend, 2003) and marine (Molnar et al., 2008) environments. The nature and magnitude of the effects of invasive species on the receiving environment, however, may vary depending on the density of the invading organism (Sousa et al., 2009). Many invasive species can form dense populations, dominating habitats after successful establishment (Crooks and Soulè, 1999).

Although the majority of studies have focused on immediately apparent impacts of invasive species, such as changes to macrofaunal biodiversity, there is growing awareness that impacts on the less obvious microbial communities, and on the processes that they drive, are paramount to the functioning of ecosystems (Van der Putten et al., 2007). Microbial communities and the processes they perform are extremely important for the functioning of all ecosystems (Ortega-Morales et al., 2010), yet we know relatively little about external environmental controls on microbial community composition. Research has typically focused on either processes (e.g. biogeochemical fluxes) or, less often, on microbial

community characterisation. To gain a true mechanistic understanding of how ecosystem functioning can be affected by invasive species, research that couples studies of biogeochemical process with characterisation of microbial communities is required (Gutknecht et al., 2006; Oremland et al., 2005).

Invasive species have the potential to modify diversity or community composition, activity levels or abundance or biomass of recipient microbial communities leading to changes in ecosystem processes and functioning (Windham, 2001). This can be facilitated by an increased supply of organic matter, or the provision of organic substrates with a different chemical composition from that of pre-invasion conditions. Subsequent changes to microbial communities may be manifested as changes in the rates of decomposition and nutrient cycling (Pedersen et al., 1999; Naeem et al., 2000). Many researchers have studied the effects of invasive species on microbial communities and processes in terrestrial systems (Ehrenfeld, 2003; Mitchell, 2006), however, with few exceptions (Moseman et al., 2009), there is still a dearth of information concerning these effects in marine ecosystems.

Marine coastal ecosystems contribute substantially to global ecosystem services (Martinez et al., 2007), such as animal nutrition, organic matter (OM) decomposition, nutrient regeneration and stabilisation of pollutants (Ortega-Morales et al., 2010). Intertidal habitats contribute the majority of coastal ecosystem productivity (Bertness et al., 2001), with intertidal mud-flats having disproportionally high productivity, compared to similar subtidal habitats (Elliott and Taylor, 1989). Of particular importance is the role microbes play in nitrogen (Francis et al., 2005) and carbon (Kristensen et al., 1995) cycling. In the sediment microbes are involved in a myriad of processes resulting in the production of pore-water ammonium, nitrite and nitrate, and also important greenhouse gases (IPCC, 2011) carbon dioxide (CO_2) and methane (CH_4). These microbially mediated processes depend on the

redox condition within the sediment (Brune et al., 2000). Many reducing processes, such as the production of CH_4 by methanogens, occur in the anoxic layer of sediments. Methanogens are strictly anaerobic members of the Archaea, but they can survive momentarily under oxic conditions (Peters and Conrad, 1995). Methanogenesis is an important part of the global carbon cycle (Edwards et al., 1998). CO_2 generated as a by-product during anaerobic decomposition of organic matter can contribute to the formation of methane (Madigan and Martinko, 2006) since some methanogens convert CO_2 to methane (Zinder, 1993), including those in wetlands (Whalen, 2005). Methane is consumed via methanotrophy by methane-oxidising microbes which utilise methane instead of CO_2 (Hanson and Hanson, 1996). The oxidation of methane is greatest at the oxic-anoxic interface (Dedysh, 2002), but it has been found that methane can be oxidised anaerobically within the anoxic sediment by a complex interplay between several methane-oxidising archaea and sulphate-reducing bacteria (Holler et al., 2011). As part of the carbon cycle it is important to understand what role methane-oxidising microbes have in sediments as the balance between methane producers and methane consumers may be tipped when the supply of organic matter is changed (Oremland and Culbertson, 1992).

Furthermore, of great importance for ecosystem functioning is the mineralisation and recycling of nitrogen which is carried out by highly diverse assemblages of aerobic and anaerobic microbes (Hayatsu et al., 2008), including ammonia-oxidisers (Francis et al., 2007). Ammonia-oxidisers obtain energy from the oxidation of ammonia to nitrite, while assimilating CO_2 as the major carbon source.

Modern genetic techniques enable assessment of the community diversity and composition of functionally important microbial groups by targeting genes involved in specific processes (Monsen-Collar and Dolcemascolo, 2010). For example, the coenzyme “methyl coenzyme reductase A” plays an important role in the final steps of the formation

of CH_4 and its coding gene (mcrA) can be targeted to assess methanogen assemblages (Luton et al, 2002). Similarly, the “methanol dehydrogenase structural gene” (mxaF), encodes part of the enzyme involved in the oxidation of methane (McDonald and Murrell, 1997) and so can be used to assess methane-oxidising assemblages. Ammonia-oxidiser assemblages can be targeted using the “ammonia monooxygenase structural gene A” (amoA) which encodes for an enzyme involved in the oxidation of ammonia to nitrite (Rotthauwe et al., 1997). Bacterial assemblages in general can be measured by targeting the small sub-unit of ribosomal RNA (16S) gene since it is a component of the ribosome, important in the synthesis of proteins in most prokaryotic cells (Woese, 1987).

The Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), one of the most successful invasive marine species, has spread throughout much of the world can construct very dense reefs (Markert et al., 2010) covering large areas of shores (Diederich et al., 2005). Similar to other bivalves, *C. gigas* may alter the physical and chemical environment through their physical structure and their biological activities. Their shells provide complex habitat (Lejart and Hily, 2011) and can alter hydrodynamics, thereby altering the flow of nutrients and sedimentation rates (Lenihan, 1999). Through their biological activity, they filter-feed, removing small suspended phytoplankton and inorganic particulates from the water column and depositing them as larger particles, either faeces or pseudofaeces and hence play an important role in nutrient cycling and benthic-pelagic coupling (Norkko et al., 2001). These changes have strong potential to influence and be mediated by microbes, but the nature of changes to microbial assemblages and their activity in response to *C. gigas* has not previously been characterised.

The aims of this study were to test the following hypotheses (i) *C. gigas* will alter the diversity and composition of assemblages of microbes in general and of several functional groups important in nutrient cycling in the oxic and anoxic layers of sediment (ii) *C. gigas*

will alter microbial activity (iii) *C. gigas* will alter the biomass of primary producers and (iv) *C. gigas* will alter processes important in carbon and nitrogen cycling and finally (v) These effects will differ depending on the cover of *C. gigas*.

5.2 Methods

5.2.1 Study site and experimental design

The experiment was set-up on extensive lower intertidal mud-flat at Ballylin Point, Lough Swilly in County Donegal, Ireland (55° 2' 36.12", -7° 33' 36.09") adjacent to mussel-beds consisting of blue mussels, *Mytilus edulis* (Linnaeus, 1758). The mud-flats were interspersed between patches of mussel-bed and were not dominated by any other biogenic habitat forming organism or hard substratum. Four levels of cover including "Zero", "Low", "Medium" and "High" equating to approximately 0, 5, 40 and 80 % cover of *C. gigas* respectively were set-up (Figure 22). The high level of cover was set at 80 % in this experiment as opposed to 100 % as was used in Chapters III and IV, because this was the maximum level of cover possible which still allowed undisturbed sampling of the sediment between oysters. Oysters in the range 40 – 100 mm maximal length were randomised among plots. There were seven replicate plots, each measuring 50 x 50 cm, for each cover treatment. Oysters were simply inserted upright into the sediment to simulate the way they are found at the site. All oysters used in this experiment were taken from the area surrounding the plots and were rinsed with seawater to remove attached sediment and flora and fauna. After three months (June 2011) all plots were sampled.

5.3 Sampling techniques for ecosystem processes

5.3.1 Gas flux of CO_2 and CH_4

Gas samples were obtained using a closed chamber technique based on Hutchinson and

Mosier (1981). This involved using 6 L airtight, tinfoil covered chambers fitted with rubber septums to allow sampling the volume. Samples were taken at 0, 45 and 90 minutes intervals using 60 ml syringes fitted with an airtight, three-way stopcock. The air within the chambers was homogenised by gently pumping the syringe three times immediately before each sample was taken. Air temperature was measured inside the chambers and the atmospheric pressure was estimated from data on the national meteorological website (www.meteireann.ie). Concentrations of CO_2 and CH_4 were measured using a gas chromatograph (Shimadzu GC-2024) with an automated injection system (Loftfield et al., 1997). The flux rates were calculated by assuming the ideal gas law coupled with linear regression, correcting for differences in chamber temperature and average air pressure during the sampling period. An exponential equation was used if R^2 was greater than 0.985 but less than 1 (Hutchinson and Mosier, 1981).

5.3.2 Procedural controls for gas fluxes

Three procedural controls were used to quantify effects on community respiration of the volume in the chamber taken up by *C. gigas* themselves, their respiration and the respiration of macro-organisms attached to their shells. To account for space occupied by *C. gigas*, empty *C. gigas* shells which displaced similar volumes of air as the live *C. gigas* in the high density treatment were used. Additionally, cleaned (to remove flora and fauna) or uncleaned live *C. gigas* oysters were used to determine direct gas emission from *C. gigas* and the associated flora and fauna respectively. Volume, oyster and macro-organism controls were set-up on an airtight impermeable barrier which was placed over the sediment to eliminate respiration from the sediment itself. These controls were set-up simultaneously with the experimental plots and were interspersed randomly with them.

5.3.3 Pore-water nutrients

Pore-water was sampled from the sediment-water interface (0 cm), 1 and 4 cm depth

using modified Rhizon^{TM} in-situ profilers (Seeberg-Elverfeldt et al., 2005). These consisted of Perspex sheets into which grooves were cut at intervals to allow the insertion of Rhizon^{TM} (Rhizosphere, Wageningen, the Netherlands) soil moisture samplers, consisting of 10 cm long filters made of a hydrophilic porous polymer with 0.1 ìm pore size. The profilers were carefully inserted into the sediment and left for 24 hours prior to sampling in order for the sediment to reach equilibrium. Over-lying surface water (approximately 2 - 4 mm above the sediment-water interface) was also collected from each plot. This method allows pore-water profiles to be sampled with minimum disturbance to a vertical resolution of 1 cm (Seeberg-Elverfeldt et al., 2005). Water samples were transported and stored in separate vacuum tubes. Ammonium (NH_4^+) and total oxidised nitrogen (TOxN), which is NO_3^- plus NO_2^-, concentrations were determined using a Lachat Quikchem 8000 (Hach Company, Colorado USA) flow injection autoanalyser. Pore-water nutrient concentrations were corrected for porosity, standardised to dry bulk density and used to calculated diffusive fluxes according to the equations presented in Chapter IV.

5.3.4 Chlorophyll content of the oxic layer of sediment

Sediment was collected from the oxic surface layer (approximately the top 2 mm) using sterile spatulas, immediately wrapped in foil and stored in a refrigerator. Within 24 hours of collection 10 ml of 90 % acetone was added, centrifuged at 3000g for 3 min and chlorophyll a, b and c was measured from the supernatant using a spectrophotometer (λ = 430 and 664, 460 and 647, 630nm respectively as described by Lorenzen, 1967). Concentrations of chlorophyll were calculated according to equations by Jeffrey and Humphrey (1975).

5.3.5 Total organic carbon and total nitrogen in the oxic and anoxic sediment

Total organic carbon (TOC) and total nitrogen (TN) were measured on 50 mg of pulverised

oven dried (80°C for 24 h) sediment after being fumigated with HCl vapour to remove carbonates (Hedges and Stern, 1984). C and N content was determined on a vario EL cube (Elementar, Germany) and expressed as percentages.

5.3.6 Dehydrogenase enzyme activity as an estimate of microbial activity

Dehydrogenase enzyme activity can be used as a measure for microbial activity and was done for both the oxic (top 2 mm) and anoxic (4 cm deep) sediment using a modified triphenyltetrazolium chloride (TTC) method as described by Alef and Nannipieri (1995). This method utilises TTC as an electron acceptor for enzymes associated with microbial oxidation of organic substrates under aerobic conditions (Lenhard 1956). The reaction has triphenyl formazan (TPF) as an end-product which can be estimated colorimetrically at λ=546 nm. Dehydrogenase activity is reported as µgTPF g^{-1} dry sediment 24 h^{-1}. Since the reaction involves aerobic oxidation of organic substrates additional measurements were done on anoxic sediment samples which were sterilised by autoclaving (121°C, 20 min). Microbial activity was estimated by subtracting results obtained from sterilised from non-sterilised anoxic sediments.

5.4 Microbial diversity

5.4.1 DNA extraction from the oxic and anoxic sediment

DNA was extracted from 0.5 g of field-moist, homogenised sediment using the method described by Griffiths et al. (2000). Samples were added to sterile 2 ml polyethylene screw-capped centrifuge tubes, containing 0.5 g of 0.1 mm sterile glass beads (Thistle Scientific), 0.5 g of 0.5 mm sterile zirconium beads (Thistle Scientific) and 0.5 ml modified hexadecyltrimethylammonium bromide (CTAB) extraction buffer (equal volumes of 10 % CTAB in 0.7 M NaCl with 240 mM potassium phosphate buffer at *p*H 8.0). After 10 min incubation at 70ºC, 0.5 ml of phenol:chloroform:iso-amylalcohol (25:24:1) was added.

Physical lysis of cells was carried out by bead beating using the glass and zirconium beads with a Hybraid Ribolyser at 5.5 m s^{-1} for 30 s. Supernatant was collected after centrifugation at 14000 g at 4ºC for 5 min and cleaned twice with chloroform:isoamylalcohol (24:1) to remove impurities. DNA yield was improved by ethanol-precipitation. The resulting DNA was further purified with a PCR product purification kit (Roche) according to manufacturer's protocol. Presence of DNA was confirmed by electrophoreses on a 1.2 % agarose (Roche) gel made with 1x TAE (Tris-Acetate-EDTA, Sigma) buffer. DNA concentrations were quantified on a UV spectrophotometer (Nanodrop) and standardised to ~30 ng μl^{-1} for downstream analyses. Each sample was extracted in triplicate.

5.4.2 Polymerase chain reactions

All forward primers were fluorescently labelled for the generation of PCR amplicons, whereas all reverse primers were unlabelled. The primers used are shown in Table 17. For all reactions, PCR was carried out in 50 µl volumes, containing 10 µl of 10X PCR buffer (Promega), 5 µl each of 0.3 µM forward and reverse primers, 1.25 µl of 10 mg ml^{-1} BSA (New England Biolabs Inc.), 1 µl of each dNTP (10 mM each, Sigma), 2.5 µl of ultra clean H_2O (Fluka) and 0.25 µl (2.5 U) of *Taq* DNA polymerase (Promega). 1 µl of template DNA was added to 25 µl of ultra clean H_2O prior to adding the PCR mix. Thermocycler (PX2 ThermoHybaid) PCR conditions for each primer pair are shown in Table 18. Ultra clean H_2O (Sigma) served as a negative control for all reactions. PCR products were confirmed on a 1 % agarose gel and subsequently purified using a high pure PCR product cleanup kit (Roche) as per manufacturer's instructions. Approximately 50 ng of each PCR product was digested using different restriction endonucleases in final volumes of 20 µl. 16S rRNA and mcrA fragments were digested at 37º C for 4 h in 2 µl of 10X NEBuffer4 together with 20 U of MspI restriction endonuclease (New England Biolabs Inc.). Similarly, approximately 50

ng of mxaF was digested at 37ºC for 4 h in 2 µl of 10X NEBuffer4 together with 20 U of HhaI restriction endonuclease (New England Biolabs Inc.) and amoA PCR product was digested at 65º C for 4 h in 2 µl of 10X NEBuffer4 together with 20 U of TaqI restriction endonuclease (New England Biolabs Inc.). The digested products were desalted and cleaned in ethanol.

5.4.3 Terminal restriction fragment length polymorphism and fragment analysis

The differently labelled TRFLP digests were pooled for each sample, and 2 µl of this was mixed with 0.5 µl 600LIZ size standard (GenStat, Applied Biosystems) and 9.0 µl formamide loading solution (Applied Biosystems) in 96 well analytical loading plates (Applied Biosystems). 600LIZ size standard contains fluorescently labelled fragment sizes of known base pair numbers, ranging from 20bp to 600bp. Terminal restriction lengths were analysed by electrophoresis using a 36 cm capillary for 30 min at 8 kV on a 3031 ABI Genetic Sequencer (Applied Biosystems).

Fragment analysis was performed using software supplied by the manufacturer of the genetic sequencer used (Genemapper, Applied Biosystems). Threshold levels for peak detection were set at 20 rfu, and peaks were called using a quartic polynomial model. Microbial assemblage profiles obtained were sorted and aligned using RiboSort in R (Scallan et al., 2008). This program automatically classifies microbial fingerprints by assigning fragments and their respective relative abundances to appropriate ribotype numbers expressed in base pairs. Fragment sizes were rounded up or down at 0.5 bp and samples that have been processed in triplicate were merged. Only fragments that contributed more than 1 % of the total abundance, relative fluorescent units, (rfu) were considered true fragments.

5.5 Statistical analyses

5.5.1 Univariate data analyses

Differences in gas fluxes of CO_2 and CH_4, diffusive flux of NH_4^+, concentrations of chlorophyll a, b and c, TOC %, TN %, C/N ratios, microbial activity and species richness (using number of gene fragments as a proxy) among different levels of oyster cover were evaluated using 1-factor ANOVA with one fixed factor “cover” with four levels (zero, low, medium and high) using Win-GMAV (Underwood and Chapman, 1998). Procedural controls for gas flux were evaluated using 1-Factor ANOVA with four levels (Volume, oyster and macro-organism controls and high cover plots of oysters). Pore-water concentrations of NH_4^+ and TOxN were evaluated using a 2-factor ANOVA with cover of oysters with four levels (zero, low, medium and high) and depth of water with four levels (surface, 0, 1 and 4 cm depth) as fixed, orthogonal factors. Homogeneity of univariate variance was tested using Cochran’s C-test and corrected for by the same method as detailed in Chapter II. When significant differences were detected by ANOVA (at $\alpha=0.05$), Student-Newman Keuls (SNK) tests were done to compare means and identify patterns of difference.

5.5.2 Multivariate data analyses

Differences in assemblage structure among different covers of oysters in the oxic and anoxic sediment was assessed using 1-factor PERMANOVA (Anderson, 2001) on Bray-Curtis dissimilarities (Bray and Curtis, 1957) of 4th root transformed data with 9999 permutations of the raw data. Assemblage data were ordinated on a 2-dimensional non-metric multidimensional scaling (nMDS) plot, with stress values representing the level of distortion of the actual rank order of distance among samples (Clarke, 1993). All multivariate analyses were computed using the PRIMER and PERMANOVA package (PRIMER-e, Plymouth, UK).

5.6 Results

5.6.1 Gas fluxes and procedural controls

CO_2 flux was greater from plots with high or medium cover than from plots with zero cover of *C. gigas* (Figure 23a, Table 19, SNK procedure). CH_4 flux was greater from plots with high than with zero cover of *C. gigas* (Figure 23b, Table 19, SNK procedure). Differences in gas fluxes between plots with high cover of C. gigas and controls could not be explained by the volume of *C. gigas* shells, the respiration of *C. gigas* themselves nor the respiration of the macro-organisms on the shells of *C. gigas*, since fluxes of CO_2 and CH_4 from plots with high cover of *C. gigas* were greater than from volume, oyster or macro-organism controls (ANOVA: F = 6.27, *d.f.* = 3, P = 0.0084 and F = 6.98, *d.f.* = 3, P = 0.0057, respectively) (SNK procedure). Changes in sedimentary processes must therefore have contributed substantially to the differences induced by the increased cover of *C. gigas*.

5.6.2 Pore-water nutrients

The concentration of NH_4^+ differed among depths and among covers of *C. gigas*, being greater at 4 cm than at surface, 0 or 1 cm depth and greater within plots with high, medium or low cover than within plots with zero cover of oysters (Figure 24a, Table 19, SNK procedure). The concentration of TOxN was greater within plots with low cover than within plots with high, medium or zero cover of *C. gigas* (Figure 24b, Table 19) but did not differ among different depths. Diffusive fluxes of NH_4^+ were greater in plots with medium than within plots with zero cover of *C. gigas* (Figure 25, Table 19, SNK procedure).

5.6.3 Chlorophyll a, b and c concentration

The concentrations of Chlorophyll a and c were greater within plots with high cover than within plots with medium, low or zero cover of *C. gigas* (Figure 26, Table 19, SNK procedure), but chlorophyll b did not differ significantly among different covers of *C. gigas*.

5.6.4 Sediment carbon and nitrogen content

C/N ratios and the concentration of TOC and TN did not differ among covers of *C. gigas* within the oxic or anoxic sediment (Tables 19 and 20). Within the oxic sediment, the concentration of TOC and TN tended to increase with increasing cover of *C. gigas*, but not significantly so (Figure 27).

5.6.5 Microbial activity

Microbial activity was greater in plots with high cover than in plots with medium, low or zero cover of *C. gigas* in the oxic sediment (Figure 28, Table 19, SNK procedure), but did not differ among covers of *C. gigas* in the anoxic sediment (Figure 28, Table 20).

5.6.6 Microbial diversity and assemblage structure

The number of gene fragments (as a proxy for species richness) did not differ among different covers of *C. gigas* for mcrA or mxaF assemblages (Tables 19 and 20). In anoxic sediment mcrA assemblages within plots with low cover differed from those within plots with high, medium or zero cover of *C. gigas* (Table 22, Figure 29f, pair-wise comparisons). Assemblages of 16S or mxaF did not differ among different covers of *C. gigas* in the oxic or anoxic sediment (Table 22, Figure 29a, b, g and h). The number of gene fragments of amoA assemblages in the oxic sediment was greater within plots with high cover than within plots with zero, low or medium cover of *C. gigas* (Table 19, SNK procedure) and amoA assemblages within plots with high cover were different from those with medium, low or zero cover of *C. gigas* (Table 22, Figure 29c, pair-wise comparisons). In the anoxic sediment amoA assemblages in plots with low cover differed from those within plots with high or medium cover of *C. gigas* (Table 22, Figure 29d, pair-wise comparisons).

Table 17. Oligonucleotides (forward and reverse primers) used to assess total bacterial and functional gene assemblages.

Target	Name	Primer sequence '5-'3	Label*	Reference
16S rRNA (bacteria)	F27	AGAGTTTGATC(C/A)TGGCTCAG	NED	Lane et al. 1991
	R1469	ACGG(C/T)TACCTTGTTACGACT		
amoA (ammonia-oxidisers)	amoa1-F	GGGGTTTCTACTGGTGGT	6FAM	Rotthauwe et al. 1997
	amoaR	CCCCTCKGSAAAGCCTTCTTC		
mxaF (methane-oxidisers)	mxa-f1003	GCGGCACCAACTGGGGCTGGT	PET	McDonald and Murrell 1997
	mxa-r1561	GGGCAGCATGAAGGGCTCCC		
mcrA (methanogens)	mlf	GGTGGTGTMGGATTCACACARTAYGCWACAGC	VIC	Luton et al. 2002
	mlr	TTCATTGCRTAGTTWGGRTAGTT		

* Fluorescent labels were attached to the '5 end of the forward primer

Table 18. PCR conditions used during amplification of bacterial and functional genes.

Target	Hotstart	Denaturing	Annealing	Extension	Cycles	Final
16S rRNA	95°C - 2 min	95°C - 30 sec	55°C - 90 sec	72°C - 90 sec	26	72°C - 5 min
amo*A*	95°C - 2 min	95°C - 30 sec	45°C - 45 sec	72°C - 30 sec	35	72°C - 5 min
mxa*F*	95°C - 2 min	95°C - 60 sec	55°C - 60 sec	72°C - 60 sec	30	72°C - 5 min
mcr*A**	95°C - 2 min	95°C - 30 sec	55°C - 30 sec	72°C - 90 sec	30	72°C - 5 min

*mcrA primers (mlf-mlr) were degenerate, therefore following the hotstart, DNA was amplified with an initial 5 cycles of 95°C for 30 sec followed by 45°C for 30 sec with an increasing 0.1°C/sec to 72°C for 90 sec to allow for mismatches (Compton, 1990) prior continuing the program in the table.

Table 19. ANOVA for functional variables and microbial diversity in the oxic layer of sediment, pore-water concentrations and NH_4^+ flux in plots with zero, low, medium and high cover of oysters.

Source	d.f.	M.S.	F		M.S.	F		M.S.	F		M.S.	F
Gas flux		CO_2 flux			CH_4							
Cover	3	10540.24	3.75	*	6443.59	4.02	*					
Residual	24	2812.88			1602.36							
N concentration		$[NH_4^+]$			[TOxN]							
Cover (C)	3	0.80	3.33	*	0.00	3.21	*					
Depth (dp)	3	8.59	35.60	***	0.00	0.60						
C x dp	9	0.38	1.56		0.00	0.94						
Residual	64	0.24			0.00							
N flux		NH_4^+ flux										
Cover	3	0.09	4.16	*								
Residual	16	0.02										
Chlorophyll		Chl a			Chl b			Chl c				
Cover	3	2.88	7.53	**	0.06	0.83		1.00	6.98	**		
Residual	24	0.38			0.07			0.14				
Total C and N		TC %			TN %			C/N				
Cover	3	0.11	2.14		0.00	2.46		0.58	1.07			
Residual	24	0.05			0.00			0.54				
Microbial		Activity										
Cover	3	1.16	13.70	***								
Residual	24	0.08										
Microbial		16S			amoA			mxaF			mcrA	
Cover	3	11.90	0.51		69.65	3.45	*	0.42	0.22		0.04	0.01
Residual	24	23.49			20.18			1.87			2.45	

Significant results are indicated: * = P < 0.05, ** = P < 0.01, *** P < 0.001.

Table 20. ANOVA for functional variables and microbial diversity in the anoxic layer of sediment in plots with zero, low, medium and high cover of oysters.

	d.f.	M.S.	F	M.S.	F	M.S.	F	M.S.	F
Source		TC %		TN %		C/N			
Cover	3	0.00	1.66	0.01	0.23	0.85	1.30		
Residual	24	0.00		0.03		0.64			
Source		Activity							
Cover	3	43589.91	0.93						
Residual	24	47052.24							
Source		16S		amoA		mxaF		mcrA	
Cover	3	4.24	0.08	6.70	0.36	6.89	1.10	4.48	0.67
Residual	24	50.65		18.77		6.25		6.68	

Significant results are indicated: * = P < 0.05, ** = P < 0.01, *** P < 0.001.

Table 21. Permutational multivariate analysis of variance (PERMANOVA) of assemblages of bacteria in general (16S), ammonia-oxidisers (amoA), methanogens (mcrA) and methane-oxdisers (mxaF) in oxic or anoxic sediment in plots with increasing density of *C. gigas*.

Source		Oxic			Anoxic		
16 S	*d.f.*	MS	Pseudo-F		MS	Pseudo-F	
Cover	3	807.93	0.93		1480.10	1.26	
Res	24	866.66			1171.70		
amoA							
Cover	3	5170.40	2.63	***	1680.90	1.67	*
Res	24	1969.60			1005.50		
mcrA							
Cover	3	500.51	0.47		3304.10	2.81	**
Res	24	1057.40			1175.90		
mxaF							
Cover	3	400.97	0.51		1037.20	1.24	
Res	24	789.03			836.02		

Significance is indicated, * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$).

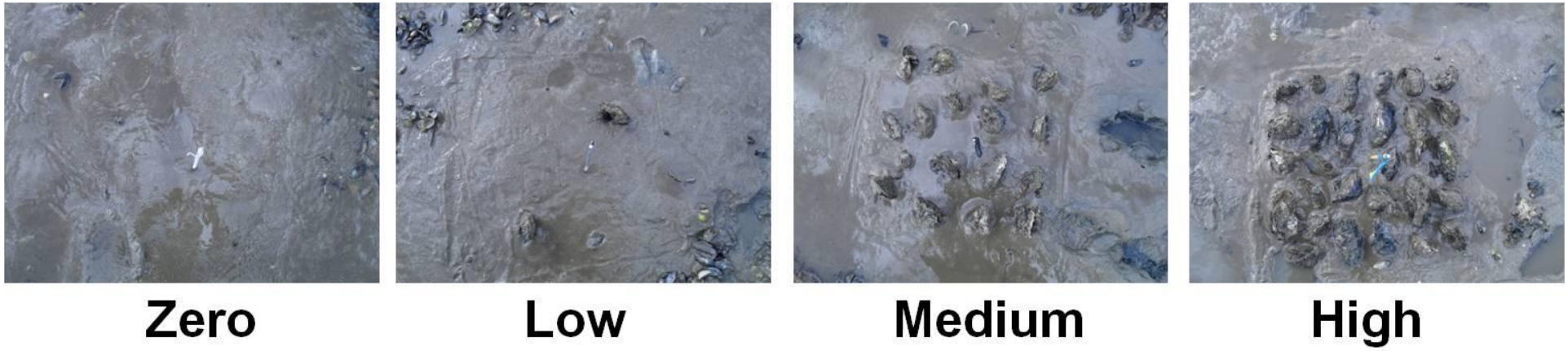

Figure 22. Experimental plots of increasing cover of *C. gigas* on mud-flats including zero (0%), low (5%), medium (40%) and high (80%) cover.

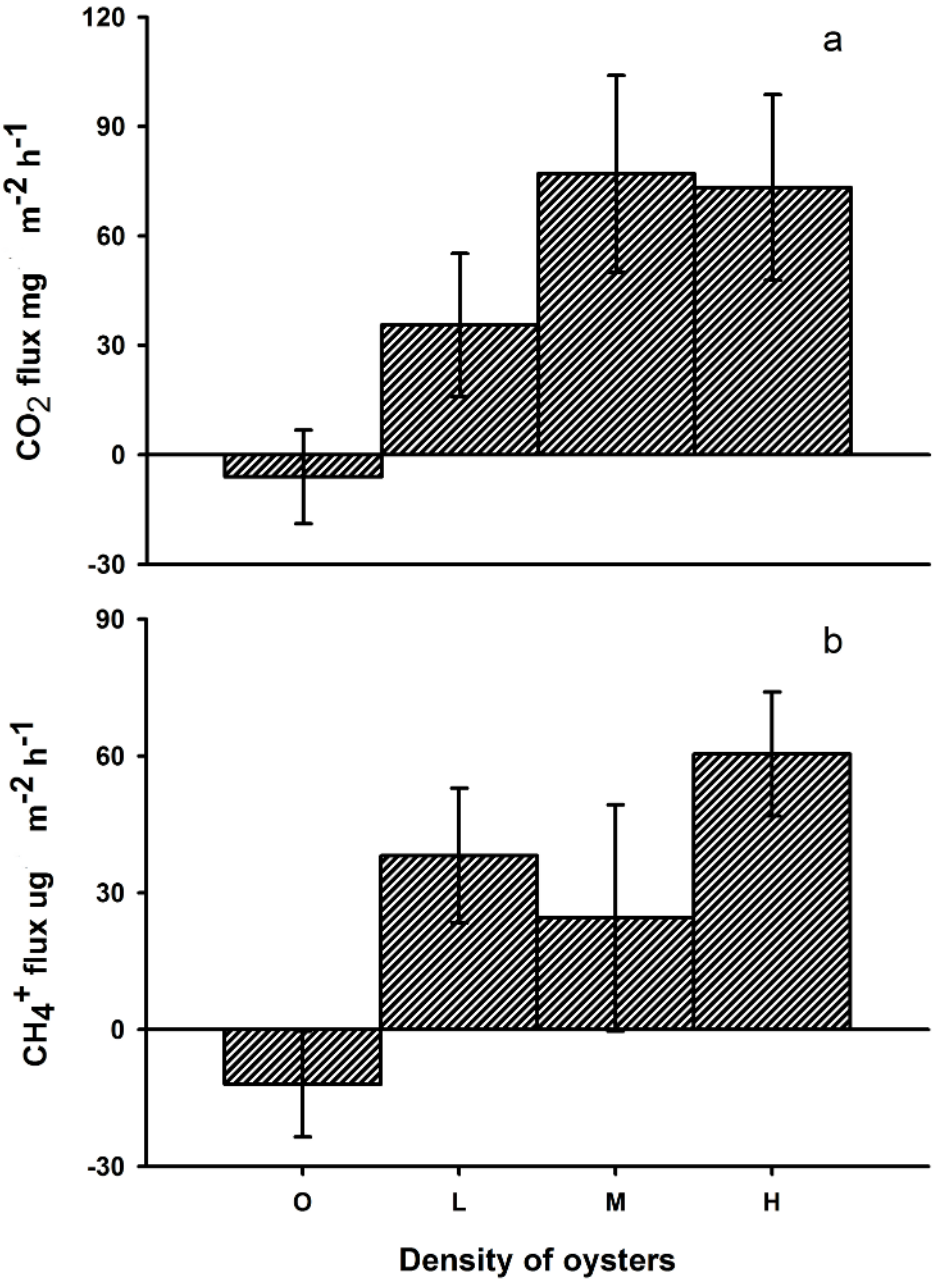

Figure 23. Mean hourly flux (± S.E.) of (a) CO_2 and (b) CH_4 from experimental plots with zero (O), low (L), medium (M) and high (H) cover of oysters.

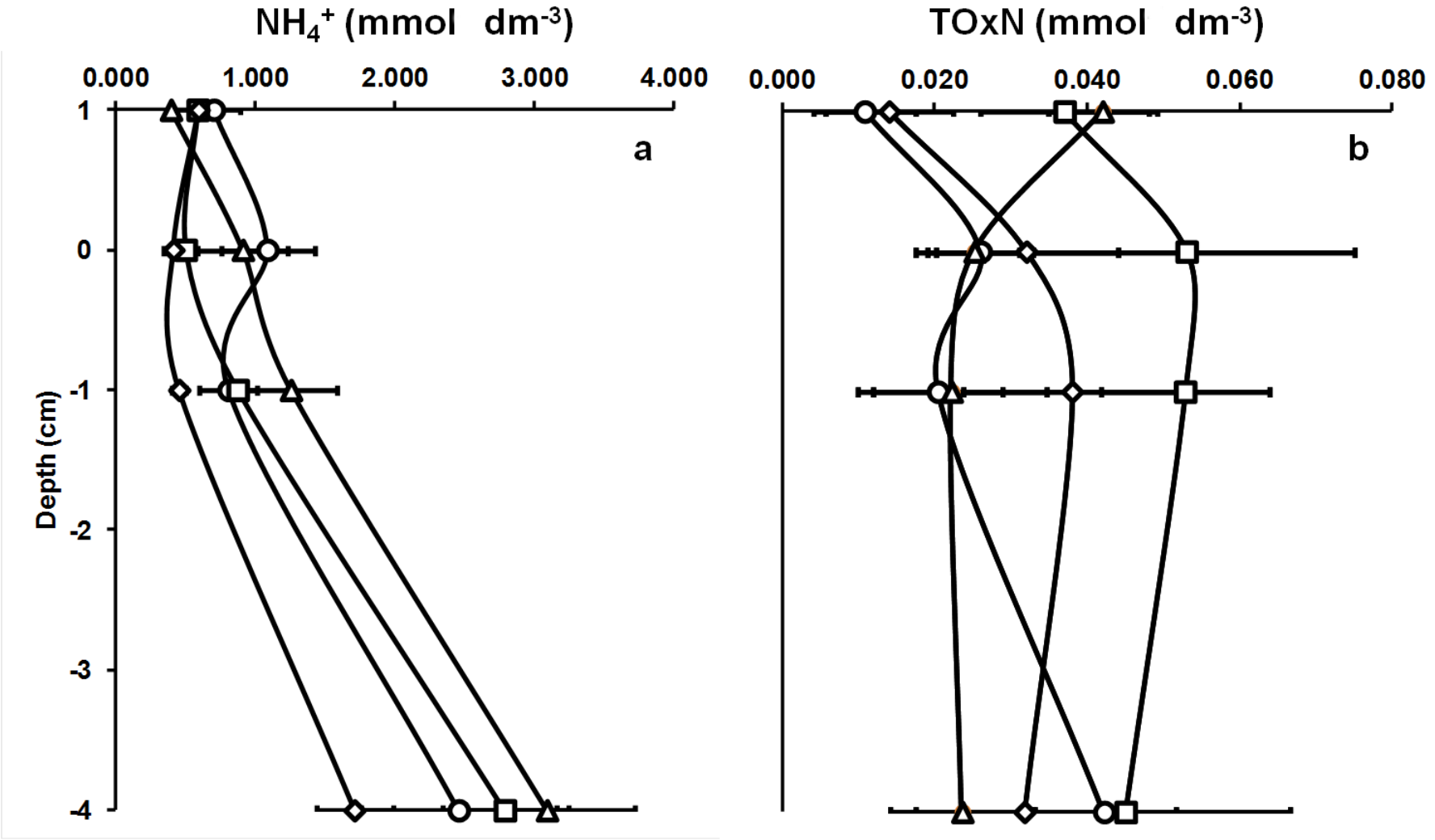

Figure 24. Mean concentration of (a) NH_4^+ and (b) TOxN from surface water (1 cm) down to 4 cm depth in the sediment in experimental plots with zero (◊), low (□), medium (Δ) and high (○) cover of oysters.

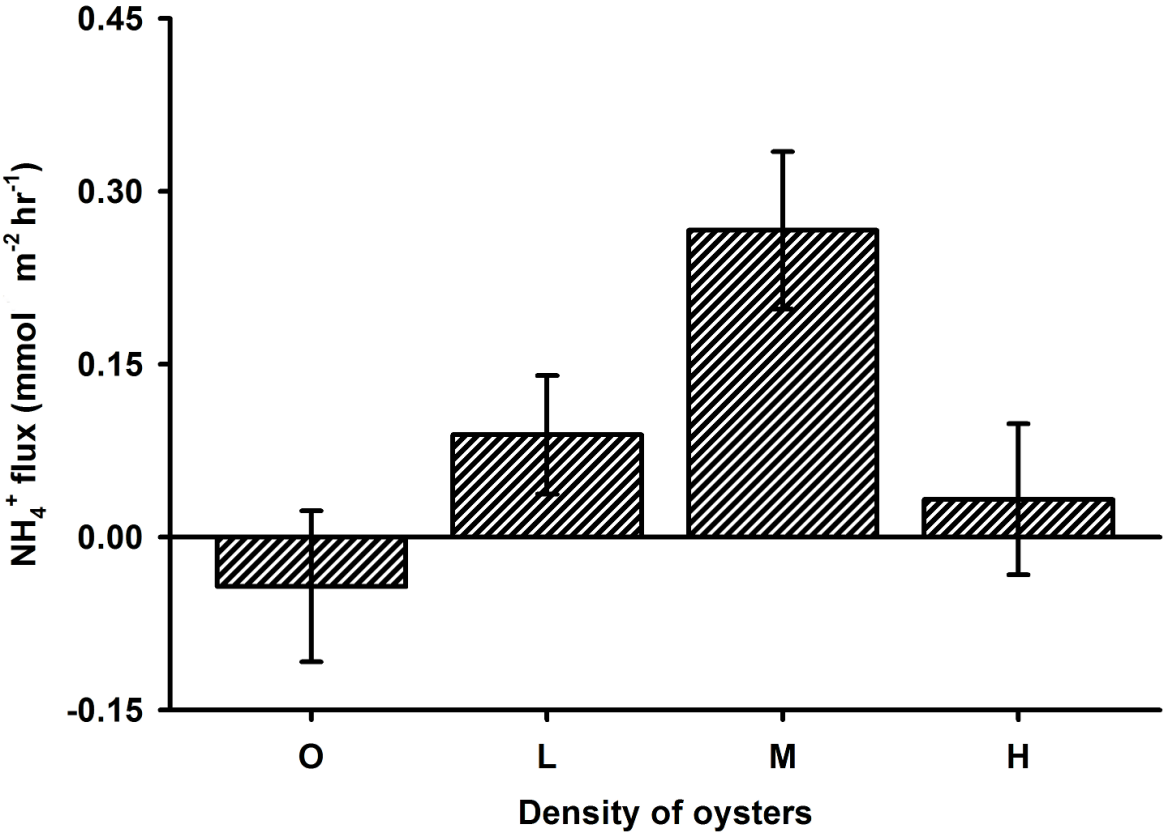

Figure 25. Mean diffusive flux of NH_4^+ from experimental plots with zero (O), low (L), medium (M) and high (H) cover of oysters.

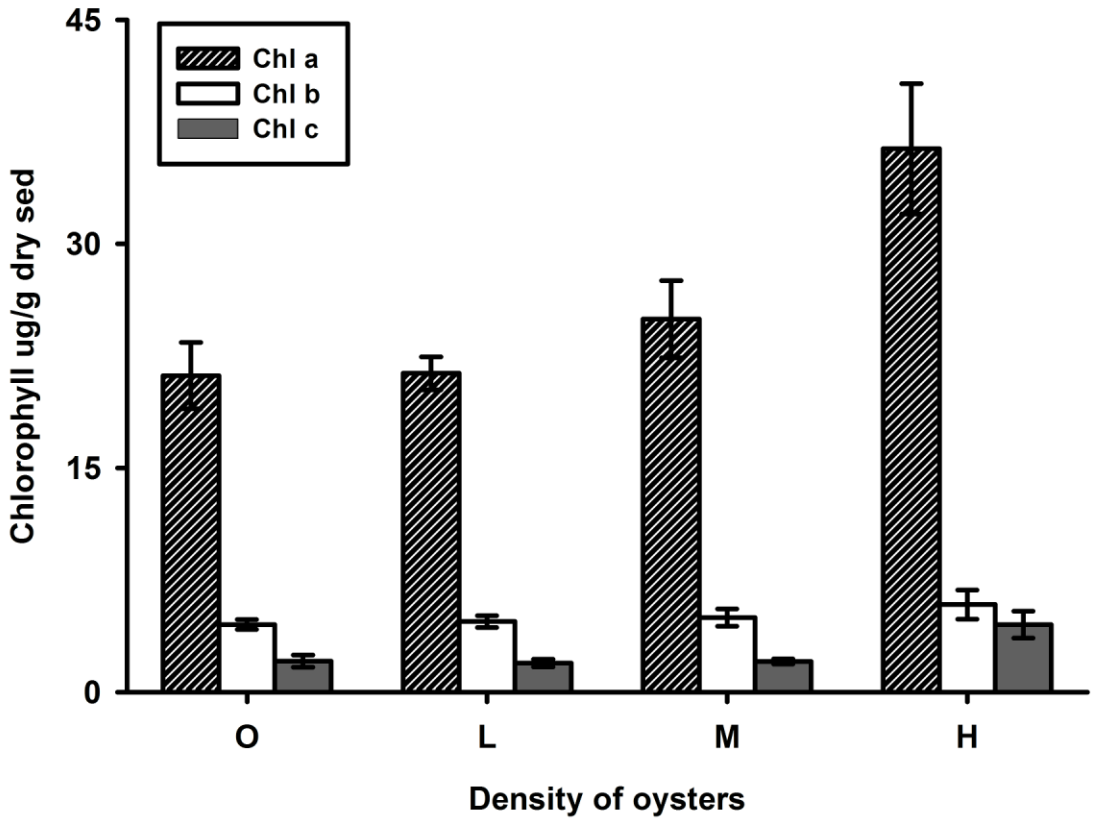

Figure 26. Mean (± S.E.) concentration of chlorophyll a (hashed), b (clear) and c (grey) per gram of dry surface sediment from experimental plots with zero (O), low (L), medium (M) and high (H) cover of oysters.

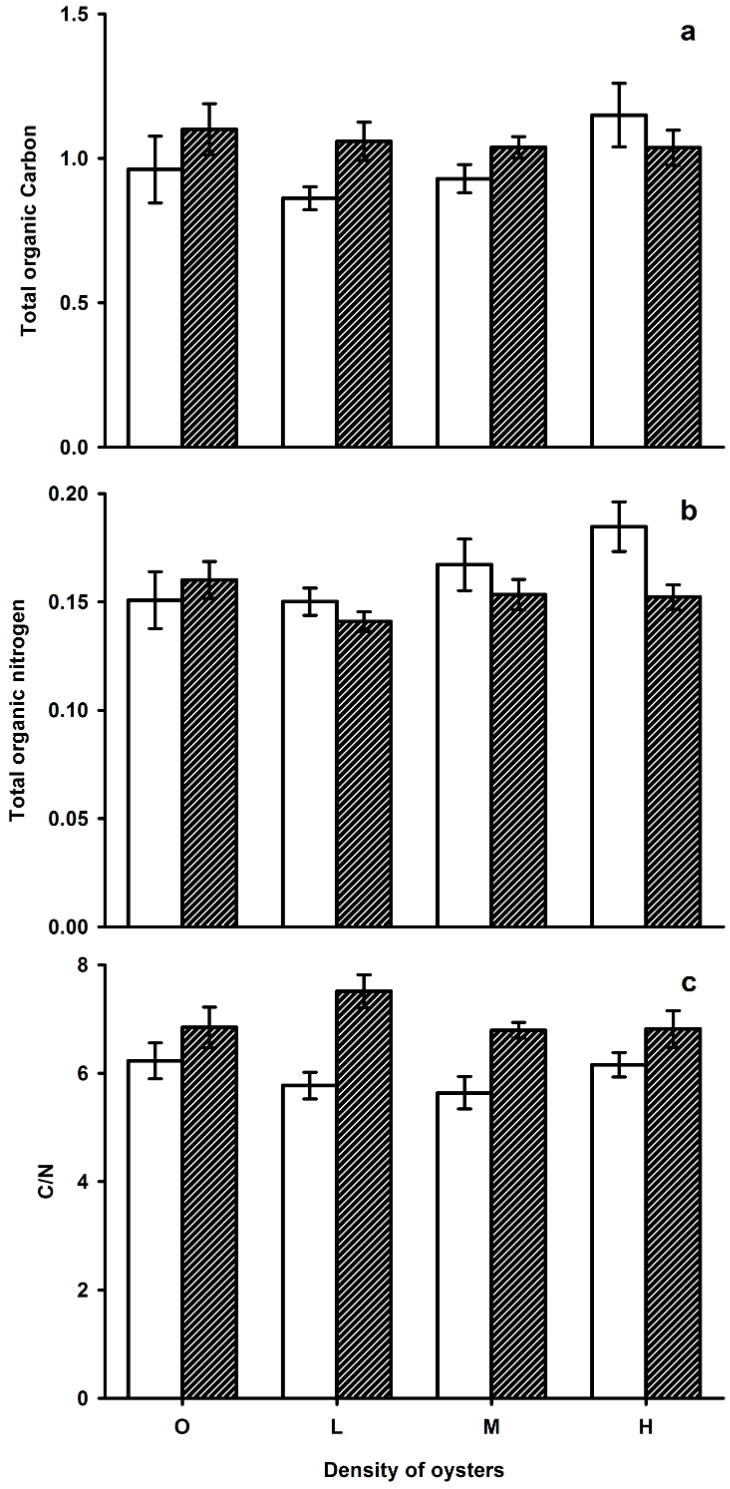

Figure 27. Mean (a) total organic carbon (%) and (b) nitrogen (%) and (c) C/N ratio from oxic and anoxic sediment from experimental plots with zero (O), low (L), medium (M) and high (H) cover of oysters.

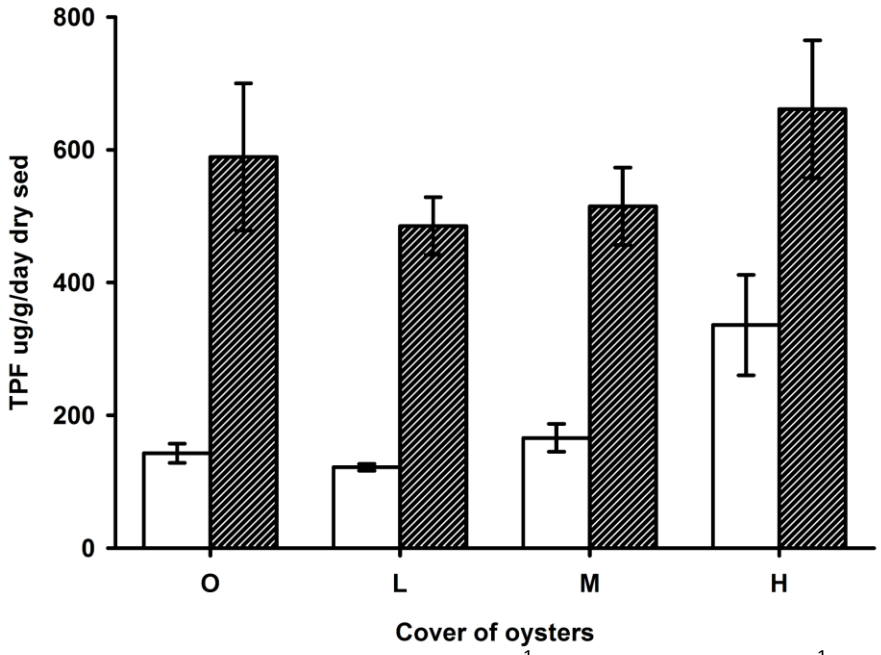

Figure 28. Mean (± S.E.) flux of TPF g^{-1} of dry sediment 24 h^{-1} for oxic (white) and anoxic (hashed) sediments from experimental plots with zero (O), low (L), medium (M) and high (H) cover of oysters.

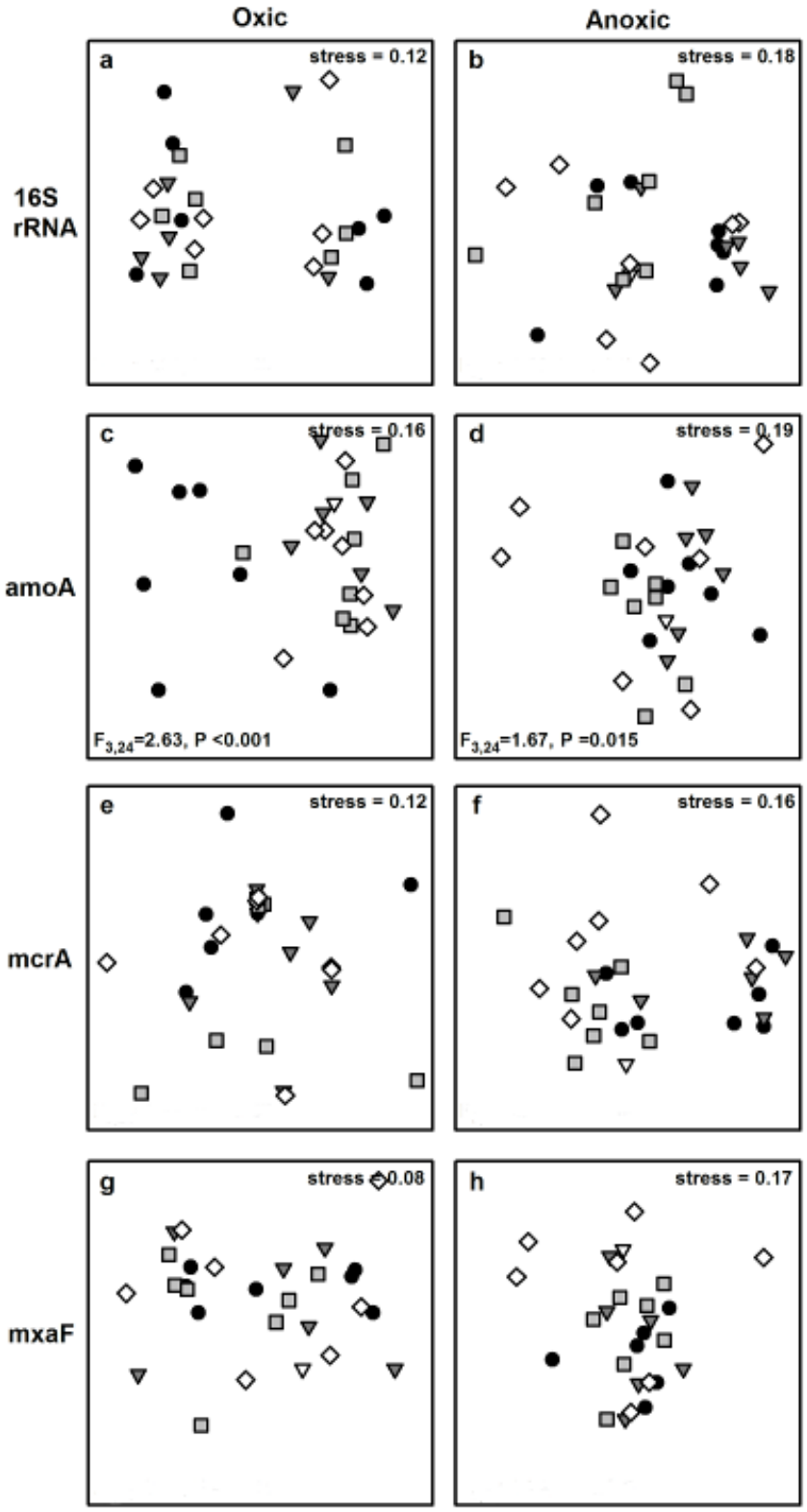

Figure 29. nMDS plots of assemblage structure of total bacteria (16S), ammonia oxidisers (amoA), methanogens (mcrA) and methane oxidisers (mxaF) in oxic and anoxic sediment from experimental plots with zero (◊), low (■), medium (▼) and high (●) cover of oysters.

5.7 Discussion

5.7.1 Effects of *C. gigas* on carbon cycling

This is the first study linking invasive species to changes in microbial processes, diversity, gas fluxes and nutrient cycling in the marine environment. In this study, at the highest cover of *C. gigas*, emissions of CO_2 and CH_4 were 13 and 6 fold greater compared to mud-flat without *C. gigas*. Analysis of procedural controls indicated that differences in CO_2 and CH_4 fluxes between control treatments and those with high cover of *C. gigas* could not be explained by the volume occupied by the oysters in the chamber, nor their own respiration or the respiration of macro-organisms attached to their shells. This means that the differences in CO_2 and CH_4 fluxes resulted substantially from the effects of *C. gigas* on the sediment. Infauna in the sediment has been found to increase with increasing density of *C. gigas* in mud-flats (Chapter III), and may have contributed to carbon fluxes. On average, the contribution of meiofauna is negligible, and the contribution by macrofauna is usually between 10 and 30 % (Hopkinson and smith, 2004). The majority of the carbon flux, therefore, cannot be explained by an increase in macrofauna, but rather can be attributed to microbially mediated processes in the sediment (Hopkinson and smith, 2004).

Oysters can increase organic matter content locally by increased deposition either directly due to biodeposits (Newell et al., 2005), but also indirectly, as a result of shell structures enhancing sedimentation rates of particulate organic matter (Lenihan, 1999). There was, however, only slight evidence for enrichment of total organic carbon in the sediment. This suggests that rather than being sequestered, the majority of the additional organic carbon was rapidly decomposed, as was evident from increased sediment CO_2 and CH_4 emission. There was no effect of *C. gigas* on the diversity or structure of the total bacterial assemblage, indicating that altered microbial diversity or structure did not account for the greater release of CO_2. This may mean that the bacteria are generally resistant or resilient

to potential disturbance from *C. gigas* (Allison and Martiny, 2008). It is possible that greater decomposition rates at the highest cover of *C. gigas*, as may be indicated by greater levels of microbial activity at this cover, account for the greater release of CO_2. Indeed, microbes can display greater activity with higher levels of labile carbon resulting in increased respiration in a range of ecosystems (Mallik and Hu, 1997; Hopkinson and smith, 2004; Plaza et al., 2004).

Algal respiration may have also contributed to the greater CO_2 flux (Hansen et al., 2000), since at the highest cover of *C. gigas*, there was also an increase in chlorophyll a and c, indicating an increase in the biomass of microphytobenthos (MPB) (Aminot and Rey, 2000). However, MPB only increased at the highest cover of *C. gigas*, which was not accompanied by a further increase in CO_2 flux compared to medium cover. Plots with the highest cover of *C. gigas*, did however, exhibit greater emission of CH_4 than plots with medium cover. An increase in anaerobic reduction of CO_2 by methanogenesis may explain the lack of increase in CO_2, since about one third of CH_4 is produced this way (Ferry and Lessner, 2008). The greater CH_4 flux indicates more anaerobic decomposition of OM resulting in more methanogenesis (Sowers and Ferry, 1983), due to a temporary increase in available buried OM (Newell, 2004). Alternatively, the increase in CO_2 and CH_4 emission can be stimulated by the “priming effect” (Löhnis, 1926), whereby the addition of fresh labile OM (such as from biodeposits) temporarily stimulates microbial decomposition of OM, including that of older, buried, recalcitrant OM (Guenet et al., 2010). The labile OM necessary to cause a priming effect in aquatic ecosystems is present in MPB (McKinley and Vestal, 1992) which are commonly present in, or enhanced by, oyster biodeposits.

Methanogen assemblages in anoxic sediments were significantly different in the presence of low cover of *C. gigas*, compared to those occurring with zero, medium or high covers of *C. gigas*. Similar to several previous studies, we found no relationship between differences

in methane emissions and methanogen assemblage structure (Lueders and Friedrich, 2000; Hoj et al., 2005; Kniffin et al., 2010), suggesting that microbially mediated methane emissions are, in fact, independent of methanogenic community structure. This implies that methanogenic communities may include many functionally redundant members, meaning that even if the composition changes, functionality with regards to CH_4 production will be retained (Allison and Martiny, 2008).

CH_4 is used as an electron donor by many methane-oxidising bacteria (Khalil et al., 1993, Quay et al., 1999) which typically occur in oxic sediment and regulate the flux of CH_4 to the atmosphere or water column (Reeburgh et al., 1993; Reeburgh, 1996). This process is however, dependent on gradients of CH_4 concentration in the sediment (Sotomayor et al., 1994), the quality of organic matter (Lojen et al., 1999) and the position of the oxic-anoxic interface (Ogrinc et al., 1997). Neither diversity nor assemblage structure of methylotrophs was altered by *C. gigas*. Methanogens in anaerobic marine sediments can be out-competed for labile OM by sulfate-reducing bacteria (Oremland and Polcin, 1982), but the addition of nitrogen can alleviate this by reducing competition for carbon (Oremland and Polcin, 1982). Alternatively increased nitrogen can suppress methane-oxidisers (Schimel and Gulledge, 1998), thereby reducing the loss of methane. A recent study stimulating multiple levels of nitrogen addition to marine sediments, found that methane production increased with increasing nitrogen, but there were no effects on the community structure, diversity or activity of methanotrophs (Irvine et al., 2012). Oyster biodeposits can enrich the sediment in nitrogen (Newell et al., 2005) altering processes such as methanogenesis or methanotrophy and thereby alter carbon cycling.

5.7.2 Effects of *C. gigas* on nitrogen cycling

In coastal sediments, a complex interplay between nitrification, denitrification, and ammonification occurs at the oxic-anoxic interface, driving rapid nitrogen transformations

which result in the loss of nitrogen to the water column and atmosphere (Sloth et al., 1995). Nitrogen cycling was altered by *C. gigas* in the current study, although there was only a slight increase in total nitrogen with increasing cover of *C. gigas*, the concentration of pore-water NH_4^+ was greater. Mirroring findings from the carbon cycle, this indicates an increase in the remineralisation of OM (Pepper et al, 2001), probably due to increased biodeposition. Diffusive fluxes of NH_4^+ changed from negative to positive with low and medium covers of *C. gigas*, compared to plots without *C. gigas*. Fluxes were, however, greatest at medium cover, rather than at high cover, as might be expected. This may be due to the increase in MPB found in high cover plots, since actively growing MPB can intercept and absorb and assimilate NH_4^+ from the surface layers of sediment, thereby limiting its release to the water column (Rysgaard et al., 1995; Hansen et al., 2000; Newell et al., 2002; Newell, 2004). Ammonia-oxidising assemblages in oxic sediments at high cover of *C. gigas* were more diverse and their structure differed from those in zero, low or medium cover plots. Increased diversity of ammonia-oxidisers has been suggested to increase the stability of nitrification in waste water treatment plants leading to enhanced removal of NH_4^+ (Daims et al., 2001; Rowan et al., 2003), but we cannot determine whether this was the case in the high cover sediment. Alternatively, others have found no correlation between nitrification and community composition of ammonia-oxidisers (Hallin et al., 2009). Coastal sediments are critical areas for global nitrogen cycling, and ammonia-oxidising microbes (amoA) play a significant role in nitrification (Francis et al., 2005). Interestingly, the concentration of TOxN was greater at low than at zero, medium or high covers of *C. gigas*. This corresponds to a difference in assemblage structure of ammonia oxidisers in anoxic sediments in low cover plots compared to those with medium or high covers of *C. gigas*. There may have been greater nitrification efficiency within these plots resulting in increased TOxN, but we cannot determine this directly. Nevertheless, there is growing evidence suggesting that many microbial communities are not functionally redundant and different communities are not functionally similar (Allison and Martiny,

2008).

5.7.3 Links between invasion and functioning mediated by microbial assemblages

The addition of biodeposits is likely to explain many of the changes to microbial community structure observed in our study. The concentration and composition of OM can determine microbial growth, activity rates and community structure (Blum et al., 2004; Koster et al., 2005). Biodeposits of oysters are more enriched in carbon and nitrogen than OM from passive settlement (Newell et al., 2005). Causal links between changes to the diversity or structure of microbial assemblages and alterations to ecosystem process rates are possible, and even probable (Loreau et al., 2001; Van der Putten et al., 2007), but require further experimentation.

Many processes involved in OM mineralisation occur at the oxic-anoxic interface in sediments (Kristensen et al., 1995). The depth of the oxic layer depends greatly on the input of OM to the sediment and is usually limited to a few millimetres in productive systems, such as estuaries (Brune et al., 2000). Processes occurring at the oxic-anoxic interface are controlled by temperature, light penetration, water currents, organic matter supply and bioturbation (Kristensen et al., 2005). In a similar study (Chapter III) the density of polychaetes increased with increasing densities of *C. gigas* in mud-flats. Sediment-inhabiting organisms, especially macrofauna, can have extensive effects on the position of the oxic-anoxic interface and hence on the microbial assemblages and nutrient fluxes of sediments (Banta et al., 1999) through bioturbation (sediment reworking) and irrigation (enhanced water exchange between sediment and overlying water). It is quite likely that there was some influence of macrofauna, facilitated by *C. gigas*, on the flux of nutrients in the current study.

The effects of invasive species on the structure and functioning of microbial decomposer

communities has received much attention in terrestrial habitats (van der Putten, 2007), for example, changes to microbial community structure induced by invasive grasses have been suggested as a causal mechanism for co-occurring changes to decomposition rates (Holly et al., 2009). Similarly, in freshwater habitats, invasive plants have been found to alter microbial biomass, activity or nitrogen cycling (Otto et al., 1999) or to alter microbial community composition and increase nitrogen storage in the sediment (Angeloni et al., 2006). In addition, invasive zebra mussels were found to increase NH_4^+ flux to the water column and to change microbial community composition (Lavrentyev et al., 2000). There are very few studies addressing changes to microbial community structure in marine ecosystems, but Hahn (2003) found invasive algae altered microbial decomposition rates and decomposer community structure in sediments.

5.7.4 Density dependent effects of invaders

After successful establishment, invasive species increase in abundance, density or percentage cover, either suddenly or after a lag period (Crooks and Soulè, 1999). Despite this, knowledge of how the effects of invasive species change with invader density is lacking (Thomsen et al., 2011a). Experiments with a range of invader densities, however, may help identify critical thresholds, beyond which an ecosystem dramatically changes its functioning. Quantitative results obtained from studies examining multiple density levels can be incorporated into ecosystem models to predict possible ecosystem responses of invasion and avoid detrimental ecological or economic consequences of underestimation (Yokomizo, 2009). Many of the effects of *C. gigas* did not change linearly with their abundance, thus making predictions a challenge. For example, as discussed in Section 4.2, NH_4^+ flux was greater at medium cover compared to plots without *C. gigas*, but was unchanged at high cover, possibly due to mediation from increased MPB. Several effects, such as increased ammonia-oxidiser diversity, primary productivity and microbial activity, were only evident at high cover of *C. gigas*. Whilst other responses were only evident at

low cover of *C. gigas*, such as altered assemblage structure of anoxic ammonia oxidisers and methanogens and increased concentration of TOxN. The explanation for this is not simple and can only be speculated upon, emphasising that the complexity of microbial interactions and biogeochemical processes occurring in sediments, in many respects, is still a “black box” (Kristensen et al., 2005).

5.7.5 Broader scale implications – climatic change and nutrient cycling

Total carbon flux (CO_2 and CH_4) differed from being a sink in areas of mud-flat without *C. gigas*, to a source when *C. gigas* was present. It is not uncommon for *C. gigas* to dominate large areas of shores (Markert et al., 2010). Recently, Lejart et al. (2012) measured the aerial and underwater respiration (CO_2) of *C. gigas* in laboratory conditions in order to estimate their direct contribution to CO_2 emissions from the Bay of Brest during the whole tidal cycle. They found that the underwater CO_2 emissions due to respiration and calcification of *C. gigas* were greater, per unit of area, than the estimated CO_2 production for the entire bay, but that aerial respiration was negligible (Lejart et al., 2012). They did not, however, take into account the emission of CO_2 resulting indirectly as a result of the influence of *C. gigas* on the sediment. The rate of increase and the potential effects of rising CO_2 levels have received much attention (Raich and Schlesinger, 1992; Sala et al., 2000), but recently researchers have focused on the potential effects of increased CH_4 emissions (Wuebbles and Hayhoe, 2002) and this is the first study to estimate the effect of an invasive bivalve on CH_4 emissions. Since the industrial revolution, atmospheric CH_4 concentration has been steadily increasing by 1% yr^{-1} and its relative increase since pre-industrial times is about 150 % compared to 35 % for CO_2 (Upton et al., 2000). Methane is the second most abundant carbon based atmospheric gas (Galand, 2004), and is considered to be 20 times more effective as a greenhouse gas than CO_2 (IPCC, 2007). At larger scales, greater emissions of greenhouse gases arising indirectly due to *C. gigas* and

other species with similar impacts, could contribute to global warming (Houghton, 2001; Karl and Trenberth, 2003) and should be taken into account for climatic change models.

Even at low levels of cover *C. gigas* can alter ecosystem level processes such as decomposition and nutrient cycling. Rapid decomposition could lead to more rapid nutrient cycling, which could facilitate higher levels of primary production within the estuary, as was seen at the highest cover of *C. gigas*. The assemblage structure and diversity of microbes involved in decomposition were also altered by *C. gigas*, possibly leading to further changes in decomposition, nutrient cycling and nutrient retention within the system (Pedersen et al., 1999; Naeem et al., 2000). Further research is required to assess to what extent changes in microbial assemblages affect ecosystem processes. Now that it is evident that there are effects of *C. gigas* on microbial assemblages, the use of more detailed techniques is warranted. For example, approaches focusing on functional genomics , such as metatranscriptomics on mRNA-based massive parallel sequencing, may provide detailed information on microbial processes involved within the sediment. Investigation of microbial responses to invasions can illuminate the mechanisms underpinning functional changes induced by biological invasions. This can be aided by experiments manipulating a range of invader percentage covers or densities, helping to develop quantitative estimates of future effects and therefore aid in making decisions about appropriate management or mitigation actions. This research was achieved by interdisciplinary collaborations involving ecologists, biogeochemists and microbiologists. Such an approach is necessary to advance knowledge of the important functional roles played by microbes in biogeochemical processes, and to understand how they may be impacted directly and indirectly by other anthropogenic stressors.

Chapter VI - General Discussion

Three field experiments were done in which the percentage cover of *Crassostrea gigas* (Thunberg, 1793) was manipulated to test effects on biodiversity and ecosystem functioning in different habitats. The nature and magnitude of many of these effects were dependent on the environmental context, including the type of habitat and the abundance of *C. gigas*. In boulder-fields, *C. gigas* inhibited the establishment of *Sabellaria alveolata* (Linnaeus, 1767), thereby threatening the establishment of a protected biogenic habitat (Chapter II). Several organisms, such as *Fucus vesiculosus* (Linnaeus, 1767), *Littorina littorea* (Linnaeus, 1758) and *Elminius modestus* (Darwin, 1854) were consistently facilitated by *C. gigas* regardless of the type of habitat. In boulder-fields, most of these effects were due to the physical structure of *C. gigas* rather than its biological activities. Although generally *C. gigas* increased biodiversity in all three habitats examined, biodiversity decreased at the highest cover of *C. gigas* in mussel beds at Lough Swilly (Chapter III). Using an interdisciplinary approach, this research was the first to experimentally assess the effects of *C. gigas* on ecosystem functioning. Similar to the effects on biodiversity, the turnover rate of some important nutrients also decreased at high levels of cover of *C. gigas*, whilst community respiration increased (Chapters IV and V). Potential mechanisms for this were assessed using a novel approach in which the link between the effects of *C. gigas* on microbial assemblages and ecosystem functioning were explored (Chapter V). The increase in community respiration was mostly attributable to an increase in microbial activity with high cover of *C. gigas*. Figure 30 shows a summary of the potential ways in which the addition of a high cover of *C. gigas* altered ecosystem processes involved with the decomposition of organic matter. The assemblage structure and composition of some functional groups of microbes were also altered by *C. gigas* but this could not account for any of the differences in process rates.

6.1 Context-dependency of impacts of invasions

It is a common assertion that the impacts of invasive species strongly depend on the spatial and temporal scales of analysis, the type of habitat and on the abundance and size of the invader (Carlsson and Bronmark, 2006; Padilla, 2010; Thomsen et al., 2011b). For instance, the nature and magnitude of their effects can vary at small (Ceccherelli and Campo, 2002) and large (Bulleri et al., 2010) spatial scales, between seasons (Heiman and Micheli, 2010) and over years (Phillips and Shine, 2005). Space and time have no inherent causality in themselves, rather, it is the biotic and abiotic conditions of the environment that determines the nature and magnitude of impacts at any place or time (Levine et al., 2003; Catford et al., 2009; Thomsen et al., 2011b). The characteristics of invaded systems, including the composition of native communities and the physical and chemical composition of the habitat, can regulate the impacts of invasive ecosystem engineering on biodiversity and ecosystem functioning (Queiros et al., 2011). Moreover, effects may be more pervasive if the invasive species provides novel habitat, since native species would share little to no evolutionary history with the species providing the habitat (Byers et al., 2010). Therefore, the impact of an invasive ecosystem engineer depends on the existence or otherwise of native ecosystem engineers and on their function (Padilla, 2010). For example, if a native ecosystem engineer is present and performs similar engineering functions as the invader, then the impacts are likely to involve a change in the magnitude or character of these effects, but may not be substantial. Alternatively, if a native ecosystem engineer is absent or performs different engineering functions from the invader, then the impacts of the invader are likely to be more fundamental. In the current research, the effects of *C. gigas* were assessed in three different types of habitat: boulder-fields, mud-flats and mussel-beds, representing a fairly comprehensive range of their potential intertidal inhabitance (Padilla, 2010). Some of the effects on biodiversity depended on the type of habitat, while others did not. Although biodiversity was increased by the presence of *C. gigas* in all three habitats, the magnitude of increase was greater for

mud-flats than for boulder-fields and mussel-beds (Chapters II and III). This could be due to the lack of hard substrata and typically lower habitat complexity in mud-flats, compared to rocky shores or mussel-beds, which is increased by the arrival of *C. gigas*. Indeed, similar to findings in this thesis, the effects of *C. gigas* have previously been identified as context-dependent, varying with the type of substratum and the presence of native ecosystem engineers (Padilla, 2010). For example, the effects of *C. gigas* on $Si(OH)_4$ cycling differed in direction between the habitats. The highest covers of *C. gigas* caused greater retention of $Si(OH)_4$ in mussel-beds but the opposite occurred in mud-flats (Chapter IV). This is probably due to the greater abundance of filter-feeders (mussels and *C. gigas*) in mussel-beds which deposit $Si(OH)_4$ bound in biodeposits. There were also some differences between locations. For example biodiversity decreased with the highest cover of *C. gigas* in mussel-beds at Lough Swilly, but was unchanged at Lough Foyle (Chapter III). This could be due to differences in numerous environmental variables, such as the age and size of mussel-beds (O'Connor and Crowe, 2007), nutrient loading (Korpinen et al., 2007) wave exposure (Tuya and Haroun, 2006) or tidal regime (McQuaid and Lindsay, 2005) all which can alter the abundance and distribution of species.

Along with spatial and temporal variability, non-linear dynamics contribute substantially to the complexity of ecological systems (Koch et al., 2009). It is often assumed that ecological properties, interactions or processes are provided linearly (unvaryingly, at a steady rate), but many are non-linear, characterised by erratic shifts and asymptotes (Nicolis and Prigogine, 1989). This can greatly complicate the estimation of the current impacts of invasive species, let alone the extrapolation of future impacts (Parker et al., 1999). The effects of different levels of cover of *C. gigas* on biodiversity and ecosystem processes in the current research were often non-linear, for example, biodiversity and the concentration of TOxN were greatest at low levels of invasion with no clearly defined pattern at greater levels (Chapters III and V).

The impacts of other invasive species, such as earthworms (Straube et al., 2009), shrubs (Elgersma and Ehrenfeld, 2011) and aquatic algae (White and Shurin, 2011) on biotic and abiotic properties of ecosystems can also be non-linear with respect to invader abundance. Nevertheless, there are few studies which have explicitly examined the effects of invasive species across a gradient of invasion (Thomsen et al., 2011a), as most studies tend to examine “worst case scenarios”, contrasting heavily invaded with uninvaded sites. This leaves the question of how ecosystems respond to low levels of invasion unanswered, and in fact, very little is known about the impact of invaders at low abundances (Elgersma and Ehrenfeld, 2011).

Yokomizo et al. (2009) demonstrated that the cost of falsely assuming a linear abundance-impact relationship can be substantial, especially when the true abundance-impact relationship exhibits an ecological threshold effect. Ecological thresholds are critical values of independent variables (such as invader abundance) after which there are abrupt changes to an ecosystem process or property (Groffman et al., 2006). For example, ecological thresholds of 75 % cover have been identified for invasive plants due to rapid declines in species richness above this cover (Gooden et al., 2009). In the current research, some responses appeared to reach a threshold at medium levels of cover. For example, there was a marked increase in the flux and turnover rate of NH_4^+ with medium cover of *C. gigas* in mussel-beds and mud-flats (Chapter IV). Marinelli and Williams (2003) also found the greatest flux of NH_4^+ with medium densities of another bivalve, *Macoma balthica* (Linnaeus, 1758), and could not find a satisfactory explanation for this. Similarly, Callier et al. (2009) found non-linear and ecological threshold effects on NH_4^+ flux with different densities of mussels, and were also unable to resolve the mechanisms for this. Environmental managers have a pressing need for information about ecological thresholds because of the potentially negative consequences of exceeding them, which in some circumstances can be irreversible (Groffman et al., 2006). Consequently, there is a need

for empirical information that will help assess the position of ecological thresholds and to aid in the development of predictive tools (Muradian, 2001). If ecological thresholds exist, then such information would be useful for prioritising management intervention at sites where negative impacts are likely to be greatest.

The physical conditions of the environment can also alter the physical or biological characteristic of organisms (Denny, 1999) and thereby may alter their impacts (Parker et al., 1999). This was the first research to find a decrease in macrofaunal diversity associated with *C. gigas*. This occurred at the highest cover (100 %) in mussel beds, which represented a density of 240 individuals per m^2, which is similar to densities of invasive *C. gigas* found elsewhere (Wrange et al., 2010) and could realistically become established in Ireland. In fact, this density has been greatly surpassed in some other parts of the world such as the Wadden Sea in The Netherlands (Markert et al., 2010), but with no associated decrease in biodiversity. This may be due to differences in body size and / or shape. In Markert et al. (2010), the biomass of oysters per m^2 was similar to that of the current study, suggesting that the oysters used in our study had a larger average biomass. The shape and size of oysters can vary considerably depending on the type of substratum they are on and on their density (Seed, 1968; Quayle, 1988) and may alter their effect on the receiving environment. Evidence from the current study suggests that, on rocky shores at least, the effects of *C. gigas* on biodiversity may be due to its physical structure rather than the biological activities. Further research is needed, however, to determine whether the physical condition, in terms of body size, shape and packing arrangements of *C. gigas* alters their impact (Carlsson and Bronmark, 2006) and how these effects differ may vary in different habitats.

6.2 Effects of invasive species on biogeochemical pathways and processes

Earth is essentially a biogeochemically closed system (with the exception of radiation from

the sun and the introduction of materials by meteorites) and most elements processed by living organisms are recycled through the processes of birth, growth, death and decay (Ogunseitan, 2005). Biogeochemical cycling is a complex and fundamentally important ecological process which is largely driven by microbes. Microbes contribute disproportionately to the stimulation and continuation of important biogeochemical processes and are termed the “biological catalysts” of element cycling, accelerating some processes up to 10^{20}-fold relative to non-biological reaction rates (Jørgensen, 2000). The effects of invasive species on microbial assemblage structure and processes is, therefore, of fundamental importance to ecosystem functioning and has been investigated in terrestrial (van der Putten et al., 2007) and freshwater habitats (Angeloni et al, 2006), but has received less attention in marine habitats.

Microbes are difficult to characterise and the majority of them cannot be cultured (Torsvik et al., 1998; McCaig et al., 2001), therefore there may be a bias if assessments of microbial diversity are based only on cultured microbes (Tiedje et al., 1999). Molecular techniques, such as PCR of evolutionary conserved genes (for example, 16S rRNA) combined with terminal restriction fragment length polymorphism, are faster and provide better information on microbial assemblages and diversity than culture-based approaches (Øvreås and Torsvik, 1998). In addition, the ability of PCR-based techniques to target specific genes that play specific roles in an ecosystem process (such as the gene that encodes for an enzyme resulting in the production of methane) allows insight into the functional diversity of microbes. It is important to remember, however, that fragment lengths obtained from terminal restriction fragment length polymorphism are only indicative of a species, as different species may have closely related sequences or some species may have several different rDNA sequences (Ranjard et al., 2001). Therefore, conclusive species names cannot be assigned and the data are regarded as a “fingerprint” of the

microbial community. Fingerprinting approaches can be made more sensitive by applying analysis of RNA rather than DNA. RNA, especially messenger RNA (mRNA), is considered a better indicator of metabolically active microbes than DNA. DNA can persist in the sediment after the death of an organism (Keer and Birch, 2003). RNA-based approaches have become more routine, however, extraction of mRNA from complex systems such as marine sediments may be difficult. Nevertheless, the validity of DNA-based approaches is still accepted, and fragment length profiles are valuable ways to track changes in microbial assemblages. Alternatively, second generation sequencing (e.g. pyrosequencing (Ronaghi and Elahi 2002; Hall 2007) is increasingly being used. This typically can lead to extremely complex and extensive datasets to analyse, with equally demanding computation power and skills (Hall 2007). Furthermore, these types of analyses currently are very costly, which would restrict the ability to have enough replication in experiments. In the following decades, however, the costs of second generation sequencing are expected to decrease substantially, similar to the trend that happened to terminal restriction length polymorphism during the last decades (Stres, 2006).

Ascertaining the effects of invasive species on biogeochemical processes and properties is further complicated by the fact that, even for very well studied systems, the mechanistic interactions that lead to changes in nutrient pools are not fully understood (Hansen and Kristensen 1998, Kristensen and Kostka 2005). This is partly because the information on the role of different microbes is lacking. Improved knowledge of population, functional diversity and environmental controls of microbes could help resolve this issue (Ortega-Morales et al., 2010). It is known, however, that microbial communities are affected by the availability and quality of carbon sources (Wilms et al., 2006). Biodeposits produced by bivalves are rapidly colonised by microbes and are sites of great microbial activity (Yingst and Rhoads, 1980) requiring a high rate of oxygen consumption to decompose, and thus reducing the oxygen concentration in surface sediments (Bianchi, 2007). This, in turn,

could promote anaerobic processes, such as denitrification and ammonification, increasing the total removal of nitrogen from the system (Kristensen, 1988). Although there was no evidence of an increase in denitrification in the current research (Chapter IV), there was an increase in the concentration of TOxN in plots with the lowest cover of *C. gigas*, possibly indicating an increase in nitrification efficiency. This corresponded with a change in the assemblage structure of ammonia-oxidising microbes, however, whether or not this is a causal mechanism for the increase in TOxN is uncertain. Rodriguez-Caballero et al. (2012) explored whether differences in the community composition of ammonia-oxidisers corresponded to differences in the efficiency of ammonium removal and nitrification. They found that efficiency of processes was related to a shift in community composition related to organic matter loading. As in the current study, these mechanisms underlying these links could not be determined. It is possible that the addition of biodeposits from *C. gigas* may have altered the nature or strength of interactions between functional groups of microbes. A change in the supply rate or quality of organic sources can alter ecological interactions among different groups of microbes resulting in a change to their process rates (Okabe et al., 2005). Understanding of microbial community structure and composition continues to advance rapidly owing to the ongoing development and application of molecular methods (Wu et al., 2001). These techniques, coupled with appropriately designed experiments, will help to identify the links between microbial community composition, function and the stability of processes.

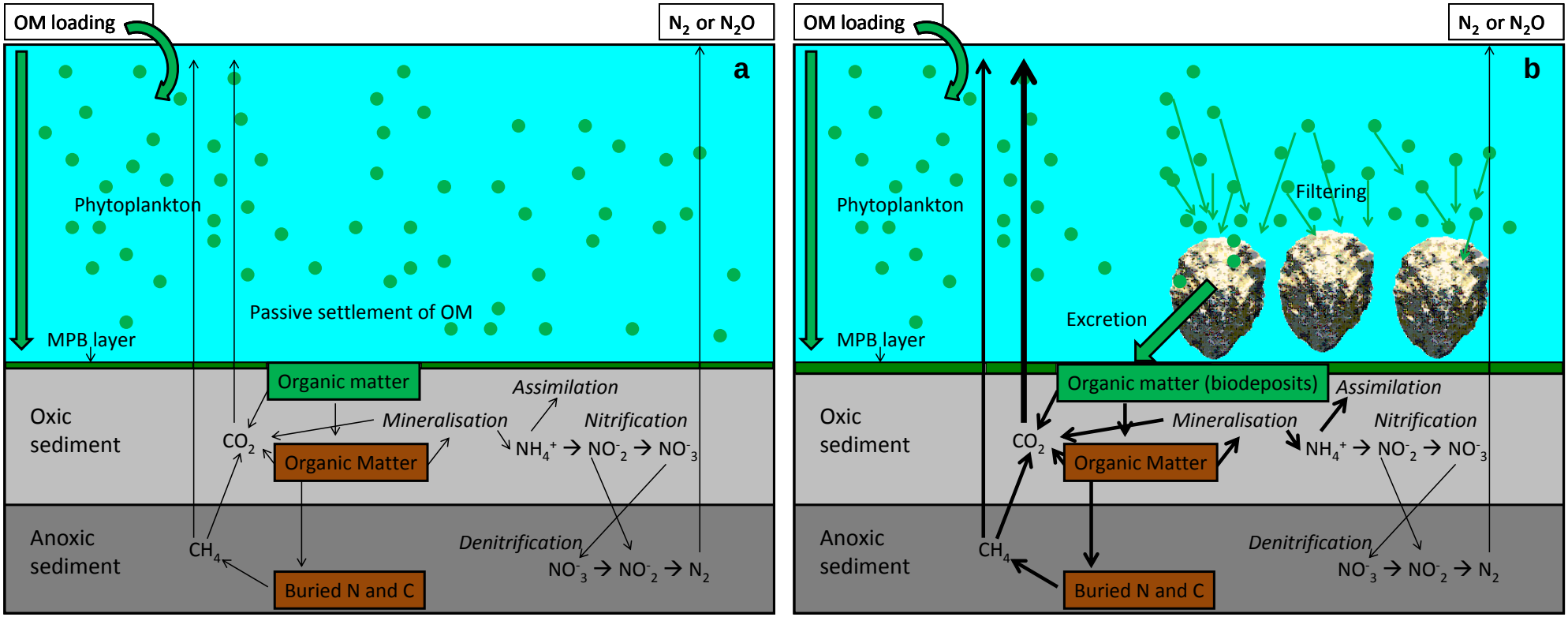

Figure 30. Conceptual model exemplifying some of the effects of *C. gigas* on biogeochemical processes in the oxic and anoxic layers of sediment. Part (a) represents organic matter cycling in a situation without *C. gigas* or any other dominant epifauna where organic matter settles passively. Part (b) represents organic matter cycling after the addition of high abundances of *C. gigas* where oysters actively filter phytoplankton from the water column and deposit it as biodeposits onto the sediment. The biomass of microphytobenthos (MPB) is enhanced by oysters and assimilates excess NH_4^+. The decomposition of organic matter is increased with oysters leading to an increase in gaseous carbon emissions. (The thickness of the arrows represents the magnitude of the flux).

6.3 Links between microbial and macrobiotic biodiversity and ecosystem functioning

The current research also found several alterations to biodiversity and assemblage composition (Chapters I, II, III and V), which may result in further alterations to ecosystem services (Hooper et al., 2005; Worm et al., 2006). The functional consequences of some aspects of biodiversity, however, are still little understood. The influence of the biodiversity of macro-biota in mediating ecosystem functions, such as nutrient cycling, has been well established (Emmerson et al., 2001; Mermillod-Blondin et al., 2005). On the contrary, the interaction between microbial biodiversity and ecosystem functioning is largely unexplored (Prosser et al. 2007). For example, *C. gigas* altered the assemblage composition of methanogens, but it is not known how this might alter their ability to produce methane. Indeed the effects of alterations to community composition of microbes in general are little understood (Allison and Martiny, 2008). Investigation of specific microbial functional groups coupled with measurements of ecosystem processes, as undertaken in the current research, is fundamental in establishing a mechanistic understanding of how the effect of invaders may indirectly alter nutrient cycling through their effects on microbial assemblages. However, the current research cannot separate the direct effects of *C. gigas* on microbes from the indirect effects from changes to macro-biota biodiversity induced by *C. gigas*. For instance, in Chapter III, the biodiversity and abundance of macro-biota was changed by *C. gigas*. Macro-biota in sediments, particularly those which redistribute particles or fluids through bioturbation or bioirrigation, can alter the availability of organic matter (Levin et al., 1997), the position of the oxic-anoxic interface (Forster and Graf, 1992) and the distribution of metabolic electron acceptors (Fanjul et al., 2007), thereby strongly influencing microbial communities and nutrient cycling (Chapter I). The current research confirmed that some of the functional changes to ecosystems caused by biological invasions are associated with the responses of microbes to invaders. For example, an increase in microbial activity or biomass was linked with an increase in

community respiration (Chapter V). Whether this occurred directly from the influence of biodeposits, or indirectly via changes to macro-biota induced by *C. gigas* cannot be determined in the current work. Indeed, there is a critical knowledge gap regarding how the interactions between multiple abiotic and multi-trophic biotic factors (both in terms of microbes and macro-biota) vary under the influence of anthropogenic stressors, such as invasive species, and link to observed levels of ecosystem functioning (Gilbertson et al., 2012).

There is also a lack of information regarding the functional consequences of a loss of β-diversity, which decreased with the highest cover of *C. gigas* (Chapter III). Despite increasing awareness that β-diversity quantifies fundamental aspects of spatial biodiversity, it has received comparatively limited attention, particularly in marine environments (Gray, 2000). As with alpha diversity, loss of β-diversity may also imply loss of function (Chapin et al. 2000) and, as such, β-diversity is thought to be important in sustaining ecosystem services (Sekercioglu, 2010). Its functional consequences are largely unknown, but limited research indicates that greater β-diversity may increase the stability of ecosystems (France and Duffy, 2006). Although a reduction in β-diversity with the highest cover of *C. gigas* was correlated with a change in ecosystem processes, the current work cannot separate the effects of α-diversity (which also declined) from β-diversity, nor can it unravel whether changes to biodiversity altered ecosystem functioning or the other way around (Chapter IV).

The effects of invasive species on biodiversity and ecosystem functioning can be caused directly by the invader or indirectly, mediated through other organisms with cascading effects on ecosystem processes. Some of the alterations to ecosystem processes observed in Chapter V, likely resulted indirectly from changes to microbial processes which were altered by increased biodeposition by *C. gigas*. This has also been proposed as the

mechanism by which invasive Zebra mussels, *Dreissena polymorpha* (Pallas, 1771), alter freshwater ecosystem functioning (Gergs et al., 2009). Moreover, facilitation of algae at high levels of cover of *C. gigas* is proposed to have reduced sediment NH_4^+ fluxes in Chapters IV and V. Indeed, the indirect effects of invaders are likely to contribute substantially to their overall effects on ecosystems, but are understudied (White et al., 2006). The effects of invaders can be influenced by complex feedbacks between invaders, their environment and the species present in the receiving environment (Wootton, 1994; Elgersma and Ehrenfeld, 2011). Further experimental research is required to disentangle these feedbacks.

6.4 Potential effects of C. gigas *on ecosystem services*

Coastal ecosystems are highly productive and have been a main focal point of human settlement and exploitation because they comprise a rich array of social, economic and environmental resources supplying many ecosystem services (Gray, 1997; Lotze et al., 2006). The current research found evidence for the alteration of several supporting services, including decomposition, nutrient cycling and primary productivity, with potential cascading effects on provisioning services (Chapters IV and V). For example, changes to NH_4^+ and $Si(OH)_4$ fluxes, observed in Chapters IV and V, on a larger scale may affect the abundance, biomass and specific composition of primary producers thereby altering the estuarine carrying capacity (Baudinet et al., 1990; Ragueneau et al., 2002; Rocha et al., 2002). Similar responses have been hypothesised in relation to farmed bivalves (Christensen et al., 2003; Richard et al., 2007; Callier et al., 2009). With the rapid expansion of aquaculture, a provisioning ecosystem service, in recent decades (Naylor et al., 2000), assessing the carrying capacities of bays, loughs or estuaries has become an important task.

Ecosystem models have become a widely used tool to describe the interactions between

the ecosystem and fish or shellfish farms (McKindsey et al., 2006). These models seek to answer the following question: what is the maximum standing stock the ecosystem can support without threatening the sustainability of human activities? Some specifically couple complex ecological models with physiological parameters for filter feeders (Ferreira et al., 2008), while others focus on trophic networks and the mass balance (Leloup et al., 2008). Recently, the inclusion of wild populations of native and invasive filter-feeders, and their contribution to benthic nutrient fluxes, has vastly improved carrying capacity estimates (Sequeira et al., 2008; Cugier et al., 2010).

Benthic nutrient (re)mineralisation can provide up to 80 % of the nutrients required for primary production in coastal ecosystems (Jensen et al., 1990; Giles et al., 2006). A change in the rate of supply of organic matter, such as an increase in biodeposition, can alter nutrient cycling at the sediment-water interface with cascading effects for the pelagic environment. Dense populations of bivalves, particularly oysters, can control and limit the standing stock of phytoplankton in the water column (Gibbs et al., 2005). This has the potential to affect not only aquaculture production, but also natural assemblages of filter feeders through food depletion and through alteration of the average size of inorganic particulate matter and species of phytoplankton and zooplankton available for other consumers (Pietros and Rice, 2003). It is also important to note that although oysters deplete phytoplankton, this loss may be offset by the release of dissolved nutrients from the (re)mineralisation of their biodeposits, which can stimulate phytoplankton production (Pietros and Rice, 2003). The current research suggests, however, that high abundances of *C. gigas* may hamper the ability of sediments to mediate their effects in this way, since it is likely that in these situations the nutrients are either buried or become assimilated into benthic primary production rather than pelagic (Chapter IV). The magnitude of effects on carrying capacity depends on several factors, including the total extent and identity of bivalves, the intensity of aquaculture, seasonal variability of phytoplankton, topography,

local hydrodynamics and flushing characteristics in an estuary (Forrest, 1991; Gibbs et al., 2005).

6.5 Future directions and recommendations

The current research indicates that at low abundances, *C. gigas* can increase biodiversity with minimal impacts on ecosystem functioning. Beyond low abundances, however, *C. gigas* can pose a considerable threat to native biodiversity and ecosystem functioning. In the current study, they have been shown to negatively impact the establishment of a protected biogenic habitat (*S. alveolata* reefs). At its highest cover, *C. gigas* can decrease biodiversity, increase the homogenisation of habitats, increase the emission of gaseous carbon and decrease the turnover rate of important limiting nutrients, possibly leading to a reduction in provisioning services, such as aquaculture production. Action should be taken at an early stage to restrict the spread of *C. gigas* in Ireland before dense reefs are formed. The task would already be very challenging, but if large populations become established, the challenge would be far greater. At present, feral populations are being harvested (F. O'Beirn, pers. comm.), which will contribute considerably to their control and should be encouraged. However, this would cease to be done if populations become too dense: once they have formed dense reefs, they are not harvested commercially because individuals with distorted shells have limited value. Furthermore, *C. gigas* impacted biodiversity and *S. alveolata* even when dead, albeit to a lesser extent, so management action should include removal of dead shells where feasible.

This research assessed the effects of an invasive species on six ecosystem processes and has simultaneously assessed the changes to biodiversity and assemblage structure and composition of macro-biota and microbes. By measuring multiple, rather than single, ecosystem processes in order to estimate the effects on ecosystem functioning, the

current research (Chapters IV and V) complies with recent recommendations in biodiversity and ecosystem functioning research (Gamfeldt, 2008; Naeem et al., 2009). The use of single processes to assess ecosystem functioning may ignore other important ecosystem processes (Rosenfeld, 2002). Therefore, equating single processes with overall functioning can be misleading, especially if research ultimately aims to inform management and conservation. The overall effect for society cannot be adequately estimated without considering a range of ecosystem services. In addition, different elements of biodiversity may contribute to different processes (Gamfeldt, 2008) so it is important to measure different species, functional groups or genotypes of micro and macro organisms (Chapin et al., 1997).

This research was facilitated by taking an interdisciplinary approach involving ecology, biogeochemistry and molecular biology. It stands to reason that complex environmental issues involving interactions and interfaces with multiple disciplines require multidisciplinary research for their solution. Recognition of the need to pursue interdisciplinary approaches in order to manage and conserve natural habitats is not new (Hilborn and Ludwig, 1993), but in many cases, it is still lacking (Sievanen et al., 2011). Future studies on the impacts of invasive species, or indeed any environmental stressor, would benefit from such an approach. This research cannot provide generality beyond *C. gigas*, because it did not examine a suite of invasive species across a suite of locations and times. It does suggest, however, that the degree of impact and the abundance of the invader do not always correspond to one another, highlighting the need to quantify the relationship between abundance and impact in other invasive species. This work also identified ecological thresholds in response to invasion by *C. gigas*. Ecological thresholds do not exist in all systems and even when they do, it remains very difficult to incorporate their, often non-linear, dynamics into predictive models (Scheffer and Carpenter, 2003). Thus, future work involving ecologists, economists and ecosystem modellers is required in

order to improve understanding and knowledge of these complex effects.

The effects of invasive species will also depend on how the invader becomes established, for example, some species establish gradually whilst others establish high densities initially and then decline and stabilise over time, otherwise known as a boom and bust scenario (Parker et al., 1999). This matters because it is possible that the system being invaded will adjust to the invaders' impacts over time. Therefore, future research which experimentally increases abundances of invaders either gradually, or in a fluctuating manner to simulate natural situations, will expose the system to a more realistic invasion process and may yield more realistic results.

6.6 Concluding remarks

These experiments have provided important insight into the potential impacts of invasive species on biodiversity and ecosystem functioning using a novel interdisciplinary approach. It was demonstrated that *C. gigas* can threaten the establishment of a protected biogenic habitat, change biodiversity and alter important ecosystem processes. At its highest cover *C. gigas* can decrease biodiversity, increase the homogenisation of habitats, increase the emission of gaseous carbon and decrease the turnover rate of important limiting nutrients, possibly leading to a reduction in provisioning services, such as aquaculture production.

Invasive species are continuing to proliferate due to human activities (Vitousek et al., 1997) and their impacts on ecosystems are increasingly altering biodiversity and ecosystem functioning on a global scale (Grosholz, 2005; MEA, 2005). It is important to remember that the effects of invasive species depend on their density and the nature of their receiving environment (Thomsen et al., 2011b) and that not all invasive species cause negative effects (Colautti and MacIsaac, 2004). In addition, the costs of mitigation and

management are substantial (Olson, 2006). It is, therefore, vital to carefully ascertain the nature and magnitude of the potential effects of invaders, across a range of different environmental contexts, in order to justify the monetary costs of control. Of course, the costs of failing to mitigate invaders can also be great, especially if they result in the deterioration of ecosystem services (Pejchar and Mooney, 2009; Vilá et al., 2010). If the loss to biodiversity and ecosystem services due to invasive species is to be mitigated or prevented, more interdisciplinary research is required to aid in development of appropriate management strategies.

Appendices

Appendix 1. Species list detailing taxa found on experimental boulders with control (a) and different covers of living (b, c, d = 5, 50 and 100 % cover) and dead (e, f, g, = 5, 50 and 100% cover) oysters at Lough Swilly for combined sampling times and treatments (Chapter II), all = where taxa occurred in all treatments.

Kingdom	Phylum	Class	Order	Family	Genus	Species	Occurence
Plantae	Heterokontophyt	Phaeophyceae	Fucales	Fucaceae	*Fucus*	*vesiculosus*	all
	Rhodophyta	Rhodophyceae	Ceramiales	Rhodomelaceae	*Laurencia*		a
Animalia	Annelida	Polychaeta	Canalipalpata	Serpulidae	*Pomatoceros*	*triqueter*	all
			Phyllodocida	Nephtyidae	*Nephtys*		a
				Phyllodocidae	*Phyllodoce*	*lamelligera*	f
				Polynoidae			all
			Sabellida	Serpulidae			bdg
				Spirorbidae	*Spirorbis*	*spirorbis*	all
			Terebellida	Sabellariidae	*Sabellaria*	*alveolata*	all
	Bryozoa	Gymnolaemata	Cheilostomata	Membraniporida	*Conopeum*	*seurati*	all
	Chordata	Ascidiacea	Enterogona	Ascidiidae	*Ascidia*	*conchilega*	all
					Ascidia	*mentula*	abcfg
				Cionidae	*Ciona*	*intestinalis*	all
				Styelidae	*Botryllus*	*schlosseri*	abcdeg
	Cnidaria	Anthozoa	Actiniaria	Actiniidae	*Actinia*	*equina*	all
					Anemonia	*viridis*	cg
	Crustacea	Cirripedia	Sessilia	Balanidae	*Elminius*	*modestus*	all
		Malacostraca	Decapoda	Paguridae	*Pagurus*	*bernhurdas*	g
				Portunidae	*Carcinus*	*maenas*	abcdfg
			Isopoda	Janiridae			abcdf
	Echinodermata	Asteroidea	Forcipulatida	Asteriidae	*Asterias*	*rubens*	a
		Echinoidea					be
		Ophiuroidea					adg
	Mollusca	Bivalvia	Mytiloida	Mytilidae	*Mytilus*	*edulis*	a
			Ostreoida	Ostreidae	*Crassostrea*	*gigas*	all
				Pectinidae	*Chlamys*	*varia*	all
	Mollusca	Gastropoda	Caenogastropod	Cerithiidae	*Bittium*	*reticulatum*	all
			Hypsogastropoda	Turritellidae	*Turritella*		f
			Opistobranchia				a
				Zephyrinidae	*Janolus*	*cristatus*	a
				Littorinidae	*Littorina*	*littorea*	all

				Littorina	*obtusata*	abcg
			Muricidae	*Nucella*	*lapillus*	all
			Patellidae	*Patella*	*vulgata*	all
				Patella	*depressa*	all
			Trochidae	*Gibbula*	*umbilicalis*	all
	Mollusca	Polyplacophor	Chitonida	Lepidochitonidae	*Lepidochiton*	*cinerea*	all
	Porifera	Demospongiae	Halichondrida	Halichondriidae	*Halichondria*	*panicea*	ag

Appendix 2. Species list detailing taxa found in experimental plots of different % cover at Lough Foyle in mussel-beds (a, b, c, d = 0, 5, 50 and 100 % cover) and mud-flats (e, f, g, h = 0, 5, 50 and 100 % cover) and at Lough Swilly mussel-beds (i, j, k, l = 0, 5, 50 and 100 % cover) and mud-flats (m, n, o, p = 0, 5, 50 and 100 % cover) for combined times and treatments (Chapter III), all = where taxa occurred in all treatments.

Kingdom	Phylum	Class	Order	Family	Genus	Species	Occurrence
Plantae	Heterokontophyta	Phaeophyceae	Fucales	Fucaceae	*Fucus*	*vesiculosus*	ijklnop
	Rhodophyta	Florideophyceae	Ceramiales	Ceramiaceae	*Ceranium*	*rubrum*	bcn
		Rhodophyceae	Ceramiales	Rhodomelaceae	*Laurencia*		a
Animalia	Annelida	Hirudinea					a
		Oligochaeta					all
			Haplotaxida	Tubificidae	*Tubificoides*	*benedii*	abcdefgijklnop
		Polychaeta	Aciculata	Amphinomidae			ijklmp
				Hesionidae			efghijklmnop
				Nereidae	*Nereis*		defghijklmnop
					Nereis	*diversicolor*	all
			Canalipalpata	Ampharetidae			ijklmp
				Serpulidae	*Pomatoceros*	*triqueter*	bhijklm
			Eunicida	Eunicidae			go
			Phyllodocida	Aphroditidae			g
				Glyceridae	*Glycera*		bdefghijlmop
					Glycera	*tridactyla*	jkl
				Nephtyidae	*Nephtys*		befghm
				Phyllodocidae			bcdefghijklmnop
					Phyllodoce	*lamelligera*	bginop
				Polynoidae			adfghjknop
				Syllidae			eghn
			Spionida	Spionidae			all
					Polydora	*ciliata*	dgo
					Prionospio	*fallax*	abehijklmnp
			Terebellida	Cirratulidae			bdefghjklno
				Capitellidae	*Capitella*	*capitata*	abcdefghijklnop
				Orbiniidae			all
					Scoloplos	*armiger*	all
	Bryozoa	Gymnolaemata	Cheilostomata	Membraniporidae	*Conopeum*	*seurati*	abcdfghijklnop
	Chordata	Thaliacea					f
	Crustacea	Cirripedia	Sessilia	Balanidae	*Elminius*	*modestus*	ei
		Copepoda	Harpacticoida				all

	Eumalacostraca	Amphipoda	Corophiidae	*Corophium*	*volutator*	abcdeghijklmp
			Ischyroceridae	*Siphonoecetes*	*striatus*	abcdefghjkmn
			Melitidae	*Melita*	*palmata*	acdefghijlmnop
	Malacostraca	Amphipoda	Caprelloidea	*Pariambus*	*typicus*	bdfh
		Cumacea				mo
		Decapoda	Crangonidae			e
			Paguridae	*Pagurus*	*bernhurdas*	ah
			Portunidae	*Carcinus*	*maenas*	all
		Isopoda	Janiridae			abcdefghijklnop
Echinodermata	Holothuroidea					ijko
Mollusca	Bivalvia	Myoida	Myidae	*Mya*	*arenaria*	ijklmnop
		Mytiloida	Mytilidae	*Mytilus*	*edulis*	abcdhijklmnop
		Veneroida	Cardiidae	*Cerastoderma*	*edule*	abijkmno
			Pectinidae	*Chlamys*	*varia*	p
			Tellinidae	*Macoma*	*balthica*	abcdefghijklmo
			Veneridae	*Tapes*	*rhomboides*	coikmno
	Gastropoda	Caenogastropoda	Cerithiidae	*Bittium*	*reticulatum*	ijklmnop
			Hydrobiidae	*Hydrobia*	*ulvae*	ek
			Littorinidae	*Littorina*	*littorea*	abcdfghijklmnop
				Littorina	*obtusata*	jko
				Littorina	*saxatalis*	ijklnop
			Muricidae	*Nucella*	*lapillus*	bcijklmnop
			Patellidae	*Patella*	*vulgata*	ijklnop
			Trochidae	*Gibbula*	*cineraria*	knop
				Gibbula	*umbilicalis*	dklop
	Polyplacophora	Chitonida	Lepidochitonidae	*Lepidochitona*	*cinerea*	ijklmnop
Nematoda						all
Porifera	Demospongiae	Halichondrida	Halichondriidae	*Halichondria*	*panicea*	ko

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