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**The effects of ocean acidification, warming
and enhanced nutrients on a Wadden Sea
community - Examined with Mesocosm
experiments**

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Summary

Ocean acidification and global warming are known as two of the most crucial factors impacting marine ecosystems worldwide. While most investigations tested short-term impacts of single environmental drivers (e.g. temperature, salinity) on single species, studies on the combined effect of multiple drivers on a multi-species assemblage in different seasons, which is much more realistic and relevant, are still scarce. Therefore, an experimental mesocosm facility was built to gain information on community changes under the impacts from multiple drivers. In three consecutive experiments, in spring, summer and autumn 2014, compartments of an intertidal macroalgae-mussel community from the Wadden Sea were incubated for 8 to 11 weeks within a large-scale mesocosm facility (Sylt Benthic Mesocosm). In the experiments four different treatments were applied: Ambient, nutrient enrichment (N; doubled natural summer nutrient concentration), warming in combination with acidification (OAW; ambient + 5°C and 1000ppm), and a combination of all three drivers (OAW+N). To find seasonal effects, we compared the responses of (OAW) to that of the ambient treatment in spring, summer and autumn. Carbon flows within the food web of the enclosed species assemblage were analysed by a holistic, static modelling approach (Ecological Network Analysis, ENA).

The combined effects of ocean warming and acidification decreased the biomass of the main grazers and the macrophyte *Fucus vesiculosus*, while epiphytes massively increased due to an altered top-down control during summer. This creates a bottle neck within the energy flow between the first two trophic levels and let less energy pass to higher trophic levels. Enriched nutrients alone did not affect the system substantially, but especially grazers seem to benefit from enriched nutrient concentrations. The effects of climate change on the investigated Wadden Sea community strongly depends on the investigated season. In spring and autumn, OAW affected less or even promoted the system by increased energy flows between the trophic levels. In summer the opposite was found, with decreased energy flow, hampered top-down control and a reduced trophic efficiency, that could propagate through the whole food web and alter the structure and functioning of the investigated community. The Analysis ENA showed a lower relative ascendancy and the trend to an increasing flow diversity, as the result of a high number of multiple pathways between the system components. Theoretically, the resilience of the system shows a tendency to increase and the

Summary

capability of withstanding external disturbances under OAW as compared to an unstressed system.

Zusammenfassung

Die Versauerung der Ozeane und die globale Erwärmung sind zwei der wichtigsten Faktoren, die die Ökosysteme der Meere weltweit beeinflussen. Während die meisten Untersuchungen Kurzzeitauswirkungen von einzelnen Arten und einzelnen Umweltfaktoren testeten, sind Studien zur kombinierten Wirkung mehrerer Faktoren auf eine Gemeinschaft mehrerer Arten in verschiedenen Jahreszeiten, welche realistischer und relevanter sind, noch sehr selten. Aus diesem Grund wurde eine Mesokosmenanlage gebaut, die es ermöglicht, Informationen über Änderungen der Lebensgemeinschaft unter den Auswirkungen mehrerer veränderter Umweltfaktoren zu erhalten. In drei aufeinanderfolgenden Experimenten wurden im Frühjahr, Sommer und Herbst 2014 Teile einer Makroalgen-Muschel-Gemeinschaft aus dem Gezeitenbereich des Wattenmeeres, für 8 bis 11 Wochen in der Sylter Mesokosmenanlage inkubiert. Im Sommerexperiment wurden vier verschiedene Behandlungen angewendet: natürliche Umgebungsbedingungen, Nährstoffanreicherung (N; doppelte sommerliche Nährstoffkonzentration), Erwärmung in Kombination mit Ozeanversauerung (OAW; Umgebungsbedingungen + 5°C und 1000ppm) und die Kombination aller drei Faktoren (OAW+N). Um saisonale Effekte zu ermitteln, haben wir die Einflüsse von OAW mit denen der natürlichen Umgebungsbedingungen im Frühling, Sommer und Herbst verglichen. Die Kohlenstoffflüsse innerhalb des Nahrungsnetzes wurden durch einen ganzheitlichen, statischen Modellierungsansatz (Ecological Network Analysis, ENA) analysiert.

Die kombinierten Effekte von Ozeanerwärmung und -versauerung verringerten die Biomasse der wichtigsten Weidegänger und der Makrophyten *Fucus vesiculosus*, während die Epiphyten aufgrund einer veränderten Top-down-Kontrolle im Sommer massiv zunahm. Dies erzeugt einen Engpass innerhalb des Energieflusses zwischen den ersten beiden trophischen Ebenen und lässt weniger Energie auf höhere Trophiestufen übergehen. Eine Anreicherung mit Nährstoffen hat das System nicht wesentlich beeinflusst. Weidegänger scheinen jedoch von angereicherten Nährstoffkonzentrationen zu profitieren. Wir haben herausgefunden, dass die Auswirkungen des Klimawandels auf die untersuchte Wattenmeergemeinschaft stark von der untersuchten Jahreszeit abhängen. Während im Frühjahr und Herbst OAW das System durch erhöhte Energieflüsse zwischen den trophischen Ebenen weniger negativ beeinflusste oder sogar förderte, wurde im Sommer das Gegenteil festgestellt. Wir fanden einen verringerten Energiefluss, eine veränderten Top-down-Kontrolle und eine verringerte trophische Effizienz, mit dem Potential sich über das gesamte Nahrungsnetz auszubreiten und die Struktur und Funktionsweise der untersuchten

Zusammenfassung

Gemeinschaften zu verändern. Die ENA wies eine verringerte „relative ascendancy“ und den Trend zu einer zunehmenden „flow diversity“ auf, was das Ergebnis einer hohen Anzahl von parallelen Verknüpfungen zwischen den Systemkomponenten ist und theoretisch die Belastbarkeit des Systems vergrößert und die Fähigkeit erhöht, externen Störungen zu widerstehen.

General introduction

Global Climate Change

Increasing human activities during the last 150 years, especially the combustion of fossil fuels and intensified land use, have led to a considerable increase of atmospheric carbon dioxide (Field 2014). While atmospheric CO₂ concentrations have remained between 172 to 300 ppm over the last 800 000 years (Lüthi et al. 2008), CO₂ has increased from about 280 to 400 ppm since the beginning of the industrial age (IPCC 2014).

The Intergovernmental Panel on Climate Change (IPCC) estimates that our global ocean effectively absorbed more than 90% of the atmosphere's excess heat trapped by anthropogenic emissions since 1971 (IPCC 2014). This immense absorption of heat has caused ocean warming and the oceans to expand, leading to sea-level rise threatening currently coastal areas around the world (Clark *et al.* 2016). The projected increase of global mean sea surface temperature by the IPCC by the end of the 21st century is likely to be 4.8 °C in the worst emission scenario (RCP 8.5; Figure I.2; IPCC 2014). Although seawater temperatures and CO₂ concentrations have undergone significant oscillative changes during earth history exceeding current levels (Lüthi et al. 2008; Thornalley et al. 2009), it is the unexampled high rate of changes which are concerning.

The global ocean acts as climate integrator by capturing about 30% of anthropogenic CO₂ emissions since the beginning of the industrial revolution in the second half of the eighteenth century (IPCC 2014). The CO₂ uptake by the oceans contributes essentially towards slowing down the rising atmospheric CO₂ concentrations, dumping the amount of greenhouse gases in the atmosphere and consequently slowing down global warming. But this immense CO₂ absorption by the ocean leads to global changes in seawater chemistry with a reduction in pH, a phenomenon commonly referred to as “ocean acidification” (OA; Figure I.1).

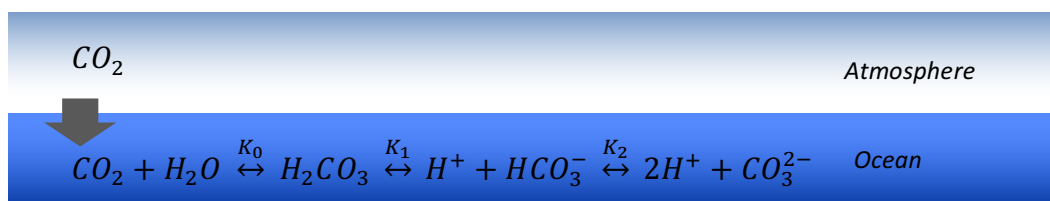


Figure 1: CO₂ dissociation in seawater.

The average pH in surface water of the open ocean has already dropped by 0.1 pH units, compared to pre-industrial values (Feely *et al.* 2009). Based on different CO₂ emission

scenarios called Representative Concentration Pathways (RCPs), the IPCC presented several models for atmospheric pCO₂ increases in the next centuries (IPCC, 2001; IPCC, 2007; IPCC 2014). The highest emission scenario of the last IPCC report (RCP 8.5; IPCC 2014) is based on continuously increasing emissions till the end of the century. The RCP 8.5 scenario predicts an increase to an atmospheric pCO₂ of about 940 ppm by the end of this century (Figure I.2). An atmospheric pCO₂ of around 940 ppm would lead to a decrease of about 0.32 pH units in ocean surface waters. The lower pH is caused by an increase in the concentration of dissolved inorganic carbon (DIC), including HCO₃⁻ and CO₂ (aq), and a decrease in CO₃²⁻ (Doney *et al.* 2009). These changes within the carbonate system of the ocean water are presumed to have consequences for marine organisms, principally on physiological processes such as calcification and photosynthesis.

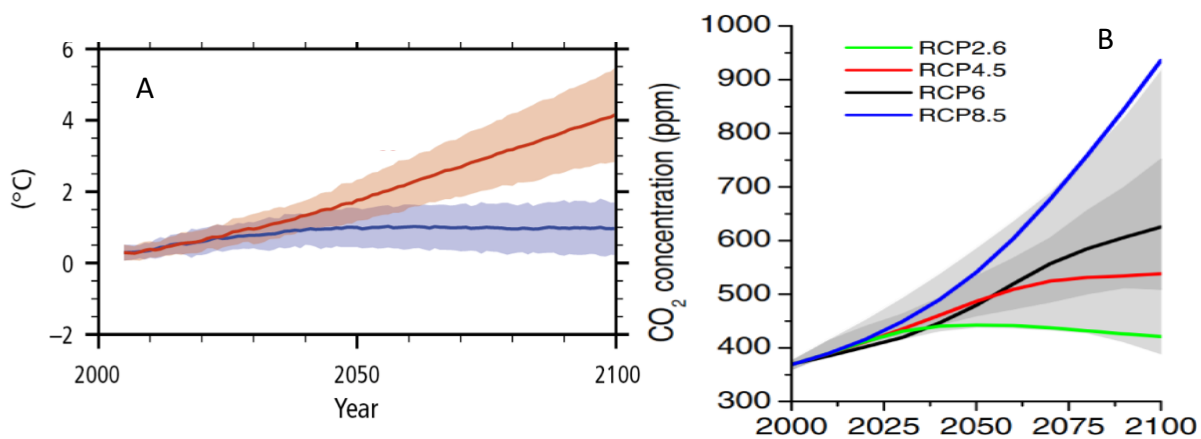


Figure 2: **A:** Average global ocean surface temperature change from 2006 to 2100 for the different RCP scenarios. All changes are relative to 1986–2005. Time series of projections and a measure of uncertainty (shading) are shown for scenarios RCP2.6 (blue) and RCP8.5 (red). Taken from IPCC 2014. **B:** Emissions of CO₂ for the different RCPs (left), and trends in concentrations of carbon dioxide (right). Grey area indicates the 98th and 90th percentiles (light/dark grey) of the values from the literature. Taken from van Vuuren *et al.* (2011).

However, these changes are expected to vary both spatially (e.g. open sea and coast) and temporally (e.g. season). As the carbon chemistry is affected by many processes, coastal waters differ from that of the open ocean in regard to their carbonate chemistry as well as annual and even daily natural fluctuations (Wootton *et al.* 2008; Hofmann *et al.* 2010). In open coasts, fluctuations in the carbon chemistry are usually due to sporadic upwelling events of CO₂-rich deep water (e.g. Feely *et al.* 2008). In estuaries and bights, additional factors, such as the input of nitrogen, phosphorous and sulphur from fossil fuel combustion, agriculture (Doney *et al.* 2007) and input of acidic river water cause a reduced alkalinity of the seawater (Hofmann *et al.* 2010). Furthermore, coastal pH is highly variable due to daily

and seasonal changes in respiration and photosynthesis of the present fauna and flora (Feely *et al.* 2010). Habitats dominated by macroalgae such as *Fucus* spp. with high biological activity show daily fluctuations of even more than 1 pH unit (Saderne *et al.* 2013).

Human-induced nutrient enrichment (eutrophication) is one of the most important anthropogenic pressures affecting estuarine and coastal waters worldwide (e.g. Nixon 2009). There has been a massive global increase in anthropogenic nutrient loading to the sea due to increased population, industrialization and intensification of agriculture with an increased bioavailability of nitrogen and phosphorus. Fertilizers are considered as the main source. High nutrient inputs causing eutrophication events affect especially estuaries and coastal bays with lower water exchange rates. This results in an increased potential for algae blooms with decreasing oxygen levels and possible hypoxic events in the specific system (Anderson *et al.* 2002). In 1988, an agreement on the combat of N problems and reductions in inorganic nitrogen and phosphorus inputs was signed, leading to substantial decreases in waterborne and airborne inputs of nitrogen and phosphorus to many regions (OSPAR 2017). Nevertheless, large parts of the North Sea are still assigned as a problem area with regard to increased nutrient loads including the German Wadden Sea (Figure I.3; OSPAR 2017).

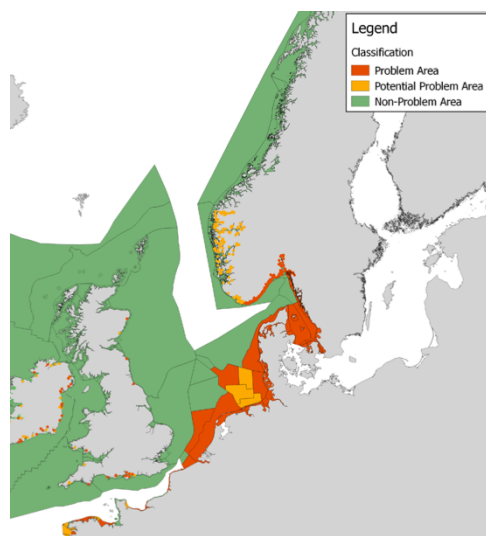


Figure 3: Eutrophication status in areas assessed under the third application of the Common Procedure (2006–2014; OSPAR 2017) in terms of problem areas, potential problem areas and non-problem areas of the greater North Sea.

Biological Impacts of Ocean Acidification, Warming and Enhanced Nutrients

In future oceans, marine organisms will have to cope with a multitude of environmental changes. Rising atmospheric CO₂ concentrations will decrease the ocean pH and the saturation of carbonated minerals, furthermore seawater temperature will increase

(Doney *et al.* 2009; Feely *et al.* 2009). The impacts of global climate change on marine ecosystems were intensively investigated and discussed over the past decades (Doney *et al.* 2012; Koch *et al.* 2013). It is known that ocean acidification (OA) affects various marine autotrophic as well as heterotrophic organisms, leading to changes in their physiology (e.g. Wu *et al.* 2008; Waldbusser *et al.* 2011; Havenhand 2012; Wahl *et al.* 2017), phenology and adaptability (Sunday *et al.* 2011; Calosi *et al.* 2013; Kelly & Hofmann 2013). Ocean acidification also changes the distribution patterns of species (Waldbusser *et al.* 2011). Hence, shifts in biotic interactions are possible (Blois *et al.* 2013), resulting in community restructuring and ecological changes for marine ecosystems (e.g. altered functioning of the ecosystem; Brierley & Kingsford 2009; Doney *et al.* 2009; Doney *et al.* 2012). Considering the high solubility of CaCO_3 in acidified seawater, specifically calcifying organisms should be most susceptible to increases in seawater pCO_2 , but the measured responses were highly variable and depend on species identity, life-history stage, season as well as geographical location and latitude (e.g. Kroeker *et al.* 2013; Wahl *et al.* 2016). However, in some cases calcification was not reduced under acidified conditions, but increased in some organisms instead (Wood *et al.* 2008; Ries *et al.* 2009; Gutowska *et al.* 2010).

However, information on non-calcifying autotrophic organisms and their response to global climate change is still scarce. These organisms may benefit from OA, as enhanced concentrations of CO_2 and HCO_3^- in the seawater could increase photosynthesis and primary production of autotrophic species. Enhanced growth rates have been observed for non-calcifying macroalgae under simulated future CO_2 levels in ocean acidification studies (Giordano *et al.* 2005; Wu *et al.* 2008; Koch *et al.* 2013), with possible ecosystem-wide effects by competition with sensitive calcifying invertebrates (Kroeker *et al.* 2013b). Only very few marine macroalgae such as epiphyte species use dissolved CO_2 as carbon source which enters the cells by passive diffusion, while more complex species possess Carbon Concentration Mechanisms (CCMs; Giordano *et al.* 2005; Hepburn *et al.* 2011; Raven *et al.* 2011). Thus, ocean acidification does not only affect competitive interactions between calcifying invertebrates but likely also the interactions between CCM and non-CCM macrophytes, for example macroalgae vs. epiphytic species, respectively (Hepburn *et al.* 2011; Cornwall *et al.* 2012; Koch *et al.* 2013).

Investigations on naturally acidified ecosystems caused by volcanic vents or coastal upwelling zones, have shown the absence of some, especially calcifying organisms from highly acidified sites near the sources, while macroalgae and seagrass showed an increased

biomass. Furthermore, increased primary production under acidified conditions has been observed for fleshy macroalgae (Kroeker *et al.* 2010; Diaz - Pulido *et al.* 2011; Olischläger & Wiencke 2013) and seagrass (Koch *et al.* 2013).

Multiple drivers can act cumulatively, antagonistically or synergistically, with different impacts on single species and communities (Doney *et al.* 2012). Ocean warming is known to affect the abundance and distribution of different marine species and has the potential to alter species composition and marine ecosystems (Harley *et al.* 2006). Increasing temperatures lead to increasing metabolic rates up to a certain threshold in marine poikilothermic organisms, with altering community composition. It has been suggested, that heterotrophic organisms are more favored by increased temperatures than autotrophic organisms due to an altered duration of development periods, consumption and metabolic rates (Hoppe *et al.* 2008). This could further lead to a reduced primary production due to an increased top-down control within the food web (food web is defined herein as the sum of all intra and interspecific interaction taking place in an ecosystem; O'Connor *et al.* 2009a; Kraufvelin *et al.* 2012). This will influence the ratio between autotrophic and heterotrophic production and hence the trophic balance of an ecosystem. Furthermore, the timing of succession of marine organisms can be expected to change, which may cause drastic alterations in terms of “match or mismatch” in the functioning of ecosystems (Sommer *et al.* 2007).

On the other hand, increased CO₂ from acidification, combined with increased concentrations of limiting nutrients, could act in combination, and stimulate and increase growth rates of primary producers. Nutrient enrichment has been shown to affect marine vegetation by enhanced growth rates of ephemeral algae which alter the turbidity, light, oxygen as well as the nutrient regime. Via these processes ephemeral algae compete with the perennial habitat-forming vegetation (Werner *et al.* 2016a). *Fucus vesiculosus* showed a reduction in growth and cover due to the indirect effects of added nutrients and a subsequently increased growth of epiphytic algae (Berger *et al.* 2004; Werner *et al.* 2016a). Furthermore, environmental changes such as OA will not act alone but in combination with other drivers such as warming or increased nutrients (Harley *et al.* 2006).

When we want to study the effects of climate change, we have to cope with a high number of possible variables influencing the responses of a benthic assemblage to climate change. The responses of the studied system depend on the drivers and their combination, the species composition, different inter- and intraspecific interactions, the species life cycles as

well as the study season. Hence, it is an important task to investigate multiple driver effects on the performance of organisms and on species assemblages in different seasons, in order to reliably predict future ecosystem changes (Doney *et al.* 2012; Kroeker *et al.* 2013a; Wahl *et al.* 2016).

Mesocosms as tool for multiple-driver and multiple-species approaches

It is difficult to predict long-term effects of global climate change on ecosystems structure and functioning, because the majority of experimental climate change research pay particular attention to short-term and small-scale laboratory settings, mainly focusing on single species and single drivers of climate change (Wernberg *et al.* 2012; Kroeker *et al.* 2013a). The advantage of these approaches is the potential for a high number of replications. While field experiments can provide more realistic information (Campbell & Fourqurean 2011), comprising a higher bio-complexity on the community level, a direct and precise control and manipulation of the system is often difficult. They tend to be impractical for experimental handling, with no or a low replication and have hence a low potential for reliably predict climate change responses. Long-term experiments have the advantage to integrate the acclimation and adaptation potential of species to climate change (Eliaison *et al.* 2011; Godbold & Solan 2013). Furthermore, intra- as well as interspecific interactions may change the overall response to climate change. Thus, experiments on single species alone are unlikely to provide sufficient understanding of long-term responses in complex ecological environments, where species also interact. Furthermore, the distinction between direct and indirect effects of climate change drivers on the experimental community is necessary to undertake realistic extrapolations to the natural future scenarios in a changing ocean (Stewart *et al.* 2013). This unavoidably generates a trade-off between space, time and the complexity of different experimental approaches. At best, climate change research should integrate long-term, multiple-driver approaches with high bio-complexity under controlled conditions (Wahl *et al.* 2016; Boyd *et al.* 2018).

Mesocosms provide the potential for multiple-driver and multiple-species approaches including several trophic levels and are recommended as an effective tool for more reliable, near natural climate change experiments (Stewart *et al.* 2013; Wahl *et al.* 2016; Boyd *et al.* 2018). Mesocosms are large enough to enclose a particular assemblage of a natural community with the potential to investigate biotic interactions and biogeochemical processes

under controlled experimental conditions with the advantage of true replication (Pansch *et al.* 2016).

Ecological Network Analysis

Over the past decades, food web models have been increasingly applied and become a useful tool to represent, analyze and compare large-scale systems encompassing complex interactions, with variable responses to environmental changes (Baird *et al.* 1991; Ulanowicz 2004; Leguerrier *et al.* 2007b; Kaufman & Borrett 2010; Fath 2015). The output of these models provides important information on the fundamental functioning of food webs and ecosystems (Baird *et al.* 2004; Fath 2015). The methodology of Ecological Network Analysis (ENA) was evolved to assess complex interactions within a food web. These models can therefore provide a holistic view on food webs and even ecosystems, where large-scale research questions about global climate change can be addressed (Artioli *et al.* 2014). To elucidate the complex structural and functional properties of food webs, ENA is based on input-output analysis, trophic and cycle analysis as well as estimations of system indices, computed with a set of algorithms (Ulanowicz 2004; Kaufman & Borrett 2010; Fath 2015). Detailed theoretical background of ENA and its general applications are described in detail by Ulanowicz (2004) and Wulff *et al.* (2012).

Analyzing the quantitative flows of energy within a food web requires defined compartments (e.g. single species, genus, class or functional group) and the insights into interactions between them (Ulanowicz 2004). Food web analysis is based on empirical data such as biomass, respiration, consumption, egestion of the compartments, the energy flows between them as well as export and import of energy or material between adjoining systems (Fath *et al.* 2007). This creates a static mass-balanced snapshot of the resources within the analyzed system.

Ecological network analysis has been used for ecosystem comparisons (Lacoste *et al.* 2002; Leguerrier *et al.* 2007a), assessing inter-annual variability in food web structures (Baird & Ulanowicz 1989; Borrett *et al.* 2006), as well as for food web comparisons on a global scale (Baird *et al.* 1991). Despite the huge informational output on food web structure and interactions gained by food web models, only few studies used this methodology to analyze the response of marine systems in the frame of climate change experiments (Alsterberg *et al.* 2013; Ullah *et al.* 2018)

Study Area: the Sylt-Rømø Bight

The Sylt-Rømø Bight (Figure I.4) is a semi-enclosed basin within the northern Wadden Sea, situated between the islands of Sylt (Germany) and Rømø (Denmark). Its area covers around 404 km², with one third being intertidal (Baird *et al.* 2004). The tidal range inside the Bight is up to 2 m (Martens & van Beusekom 2008). Each of both islands is connected via a causeway with the mainland and prohibits any water exchange with the adjacent tidal basins via the tidal flat watersheds. The only connection to the North Sea is a 2.8 km wide deep tidal channel (Lister Deep) between these two islands (Figure I.4). Between 2005 and 2013, average salinity ranged between 27.9 ±0.52 in winter and 30.17±0.57 in summer, affected by currents and freshwater inflow. In the same period, average surface seawater temperatures ranged between 2.7±1.2 °C in winter and 17.54±0.52 °C in summer (Rick *et al.* 2017).

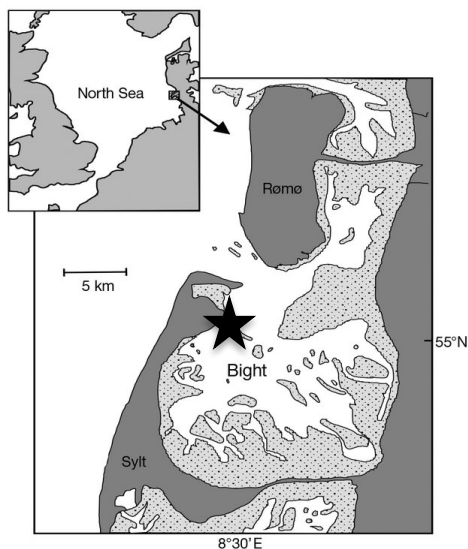


Figure 4: Location and map of the Sylt-Rømø Bight. The sample site is marked with a star and the intertidal areas are indicated in stippled grey. Modified after Baird *et al.* (2012).

The Sylt-Rømø Bight is dominated by sandy sediments with a coverage of 80% of the total area, while mussel-, oyster- and seagrass beds, as well as muddy sediment, cover approximately 20% (Asmus & Asmus 1998). Mussels and oysters form mixed assemblages. The abundance of mussels and oysters within these assemblages are highly variable between years and depend on winter ice coverage and on summer temperatures (Büttger *et al.* 2011), as well as on competition between *Mytilus edulis* and the invasive oyster *Magellana gigas* former known as *Crassostrea gigas* (Büttger *et al.* 2008). Mussel-oyster beds together with seagrass meadows form the only natural epibenthic settling substrates for meroplanktonic larvae within the basin.

Study system: a macroalgae-mussel bed community

The Mussel-oyster beds are dominated by the blue mussel *Mytilus edulis* and the pacific oyster *M. gigas*. The dominant macrophyte in this system is the brown algae *Fucus vesiculosus forma mytili*. It does not possess holdfasts as other *Fucus* forms, such as *Fucus vesiculosus* and cannot attach to hard substrates by itself. Instead *F. vesiculosus forma mytili* is hold to the mussel bed by the byssus threads of *M. edulis*. This macroalgae-mussel bed community provides important nursery ground, shelter, settlement substrate and feeding ground for amphipods (*Gammarus spp.*), shrimps (*Crangon crangon*), winkles (*Littorina littorea* and *L. mariae*), limpets (*Crepidula fornicata*), barnacles (*Balanus crenatus*), crabs (*Carcinus maenas*) and many fish species (Nehls *et al.* 2006; Baird *et al.* 2007; Büttger *et al.* 2008). We investigated a part of this macroalgae-mussel bed community under climate change effects in an experimental, near natural mesocosm approach.

The Sylt-Rømø Bight ecosystem is well investigated and previous studies used network analysis to describe and analyze the structural and functional properties of its food web (Baird *et al.* 2004, 2007, 2008; Baird *et al.* 2011, 2012; de la Vega *et al.* 2018).

Aims and outline

Scope and aims of the thesis

It is known that coastal ecosystems will change under future climate change, but due to the lack of investigations in near natural settings (i.e. multiple stressors, multi-species, multiple seasons), it is unknown in which direction and to which extent they will move. Of capital importance are the consequences of climate change for the structure and functioning of the ecosystems. Hence, the aim of the consortium 2 (Responses of benthic assemblages to interactive stress) within the framework of BIOACID II research project was to advance the understanding for future re-structuring and re-functioning of macrophyte communities in the German Wadden Sea and the western Baltic Sea.

Thus, the aim of this study was to reveal the response of a macroalgae-mussel bed community from the Sylt-Rømø Bight under the interactive effects of climate change. To reach this goal, a species assemblage of this community was tested under effects of ocean acidification, global warming, as well as increased nutrients under near natural conditions within a novel mesocosm facility. In accordance to this scope, I aimed to answer the following questions:

- Is the Sylt benthic mesocosm facility suitable and functional for multiple-stressors, multi-species experiments?
- Which are the quantitative effects of ocean warming, acidification and enriched nutrients on a macroalgae-mussel bed community, regarding growth, survival and reproduction?
- Do ocean warming, acidification and enriched nutrients modify the species interactions within and the functioning of the investigated species assemblage?
- Is seasonality affecting the response of a macroalgae-mussel bed community to ocean warming, acidification and enriched nutrients?

The response of a macroalgae-mussel bed community to global climate change was to our knowledge not investigated before. Since this community provides important functions for the Wadden Sea ecosystem, it is important to enhance the knowledge in regard to its response to climate change and its adaptation potential. This might influence the total Wadden Sea ecosystem and may have consequences on management strategies of the coastal system. The combination of mesocosm experiments and a modelling approach via Ecological Network Analysis is an important step forward to shed light on the response of communities to global climate change.

Outline

The results are presented as 1 publication and 2 manuscripts prepared for submission.

The first chapter outlines a methodology of the newly constructed Sylt benthic mesocosm facility including a detailed description of the function and maintenance as well as information on the need for such a mesocosm approach.

The second chapter focuses on an experimental mesocosm approach, investigating the combined effects of ocean warming and acidification and the interaction with increased nutrients on the macroalgae-mussel bed community. The mesocosm experiments were run in summer and the community responses were analyzed using a holistic food web modeling approach, the Ecological Network Analysis (ENA), assessing the trophic structure and system dynamics.

The third chapter is dedicated to investigate seasonal variations in response of the macroalgae-mussel bed community to climate change. The combined effects of warming and acidification on the investigated species assemblage were tested in spring, summer and autumn 2014. The food web response was also analyzed with a modeling approach by means of ENA.

Chapter I

Tidal benthic mesocosms simulating future climate change scenarios in the field of marine ecology

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Abstract

Increasing human activities cause local to global changes in sea surface temperatures, ocean acidity, eutrophication, and rising sea levels. Many laboratory experiments investigate the effects of these regime shifts on single species and single stressors, showing variable responses within and among species, while different combinations of stressors can have synergistic, additive or antagonistic effects. Large-scale multi-species and multi-stressor experiments can more reliably predict future ecosystem changes. A unique mesocosm facility was developed and set up at the AWI Wadden Sea Station – Sylt, Northern Germany to investigate the particular effects of future climate changes on predominant marine intertidal communities. Each of 12 benthic mesocosms serves as an independent experimental unit with novel techniques of tide and current simulations as well as multi parameter measurement systems to simulate multi-factorial climate change scenarios including the combination of warming, acidification, nutrient enrichment, and sea level rise. Temperature, pH, oxygen, and salinity can be continuously monitored and logged, while discretely collected samples of total alkalinity, light availability, chlorophyll a (Chl a), nutrients and seston supplement these online data- sets. Herein we demonstrate the functionality of the new benthic mesocosm system including first experimental results on the responses of *Fucus vesiculosus* forma *mytili*, and its associated community to the combination of warming, ocean acidification, and increased nutrient enrichment.

Introduction

Due to increasing human activities, especially by burning fossil fuels, our climate is changing at an accelerating rate, leading to a global impact on the abiotic and biotic components of the marine ecosystem (Field 2014). In order to direct political decisions and actions on a local as well as global scale, one of the most important aims of research is to reliably predict future ecosystem changes and its implications (Derous *et al.* 2007). Trustworthy information is, however, not only very urgently needed but also very challenging to obtain due to complex interactions within and between the closely related abiotic and biotic components of a system (Wahl *et al.* 2011).

Many investigations have been undertaken in the field of climate change research, however most of these experiments were run in laboratory settings, mainly with single species and single components of climate change, i.e. single stressors (Harley *et al.* 2006; Walther 2010; Wernberg *et al.* 2012). Their often restricted realism makes extrapolation to the natural situation problematic (Stewart *et al.* 2013). While field observations (Kowalski *et al.* 2009; Winde *et al.* 2014) or field experiments (Campbell & Fourqurean 2011) can reveal important information in a more realistic way and with a higher bio-complexity on the community level, they are more an observational point of view where directed and precise manipulation of the system is difficult. They tend to be impractical for experimental handling, less controllable and have a low potential to reliably predict future consequences. This unavoidably generates a trade-off between space, time and the complexity in the different experimental approaches. Mesocosms have the potential to close the gap between these two approaches and are recommended as an effective tool for more reliable, near natural climate change experiments at the community level (Stewart *et al.* 2013).

Mesocosms are large enough to enclose a particular proportion of a natural community with the ability to investigate biotic interactions and biogeochemical processes under controlled experimental conditions with the advantage of true replication. Moreover, they allow an interdisciplinary way of addressing research questions, bringing together different disciplines within one facility e.g. marine ecology, physiology, biogeochemistry or molecular biology. Only few constraints reveal in mesocosm experiments. Confounding factors such as lower light availability, wall effects, limited space as well as the exclusion of higher trophic levels likely influencing trophic interactions (Mine Berg *et al.* 1999; Petersen & Berg 2009). Another important constraint in many available mesocosm facilities to date is the lack of tidal

and current simulations, with possible non-natural implications for tidal communities such as extended periods of submersion, reduced water mixing and constant grazing pressure. Despite these few constraints, mesocosms remain a valuable tool for climate change experiments at the community level and can contribute important information that cannot be provided by any other approach (Hendriks *et al.* 2010; Yvon-Durocher & Allen 2012; Wahl *et al.* 2015a). Considering the great potential of benthic mesocosm facilities within the field of climate change research, very few facilities exist and even fewer are equipped with continuous monitoring systems.



Figure I-1: Overview of the Sylt benthic mesocosm facility, showing the 12 tanks during the Summer-2014 experiment.

Here, we demonstrate the setup and functioning of the newly developed benthic mesocosm facility at the AWI Wadden Sea Station Sylt, Germany, with a novel integrated tidal and current simulation system and a multi parameter measurement system (MPMS). We present all single hardware compartments, possible manipulation of the different physical parameters as well as physico-chemical data obtained during the first experiments ran in 2013 and 2014. With its new developments, this facility has a great potential to investigate the

effects of multiple stress scenarios at the community level within a near natural setting to predict more reliably future consequences of climate.

Materials and Methods

General set up

The mesocosm facility was set up in August 2013 outdoors on solid ground next to the AWI Wadden Sea Station on the Island of Sylt (55°01'19.2"N, 8°26'17.7"E; Fig. I-1). It consists of 12 round tanks, made of UV stabilized high-density polyethylene (HDPE; Spranger Kunststoffe, Plauen, Germany). Each of 12 tanks serves as independent experimental unit. Hull and bottom of each tank has a double wall construction, insulated with 10 cm of Styrofoam to increase energy efficiency and to be independent from exterior air temperature fluctuations. Each tank has an inner diameter of 170 cm and is 85 cm high, with a net water volume of

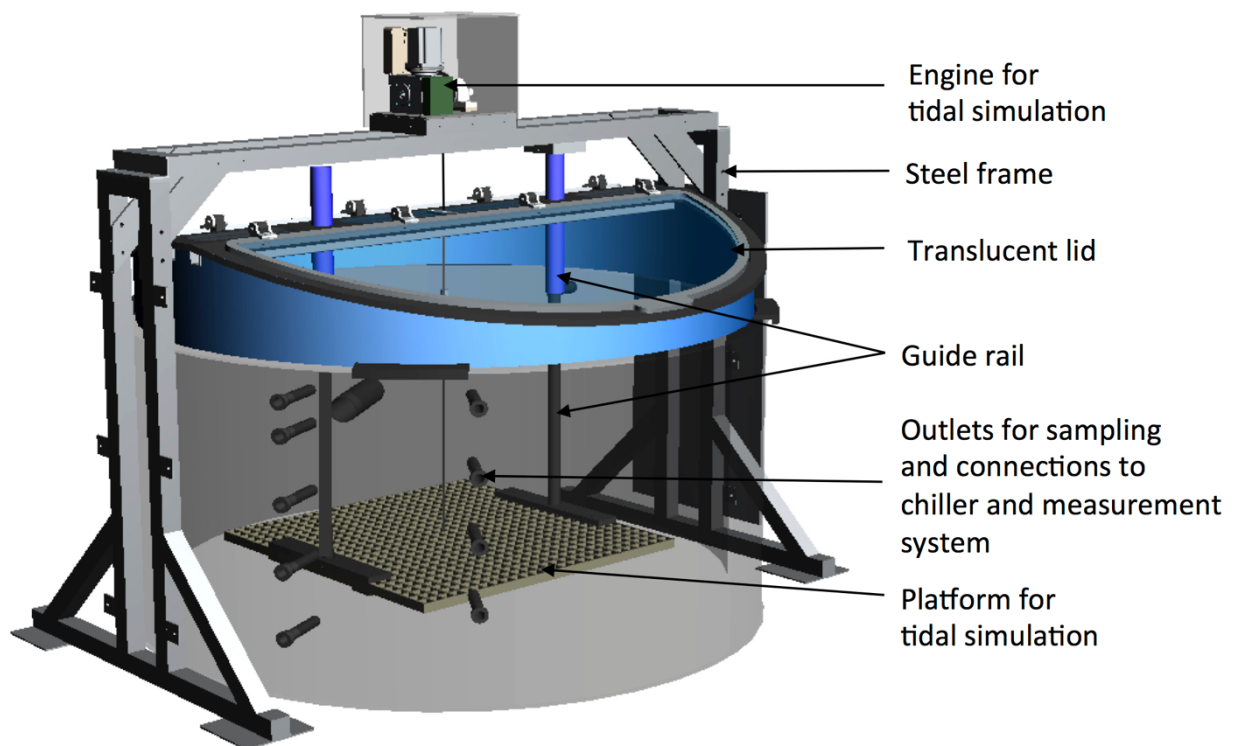


Figure I-2: Schematic of a single mesocosm unit with its components.

The outdoor facility is constantly exposed to ambient light conditions. In order to avoid an introduction of unwanted material such as air-transported dune sand or bird faeces,

and to maintain a constant atmospheric O₂ and CO₂ pressure in the headspace, each mesocosm is covered with a translucent lid (Fig. I-2), allowing 90 % of the photosynthetically active radiation (PAR) to penetrate (LI-250, LI192-SA Quantum Sensor, LI-COR, Lincoln, Nebraska USA). The lid is constructed of HDPE and translucent double wall polycarbonate with a slope of 22 % to avoid accumulation of condensate, rain and dirt.

All tanks are supplied with non-filtered seawater from the AWI Wadden Sea Station facilities (Fig. I-3). Seawater is pumped from 50 m offshore via a storage tank in four subsequent 1000 L tanks, which are located in the second floor of the institute. From here the seawater falls by gravity to the mesocosm facility. The residence time in the storage tanks is around 5 hours and result in reduced sediment loads, preventing an undesired accumulation of sediment in the experimental tanks. The Sylt mesocosms can be run as closed or open systems with an adjustable flow through volume of 1 to 3000 L per day. The wastewater is directed back into the sea. Water samples can be obtained through the sampling outlet in the tank wall of each tank at around 40 cm depth (Fig. I-2).

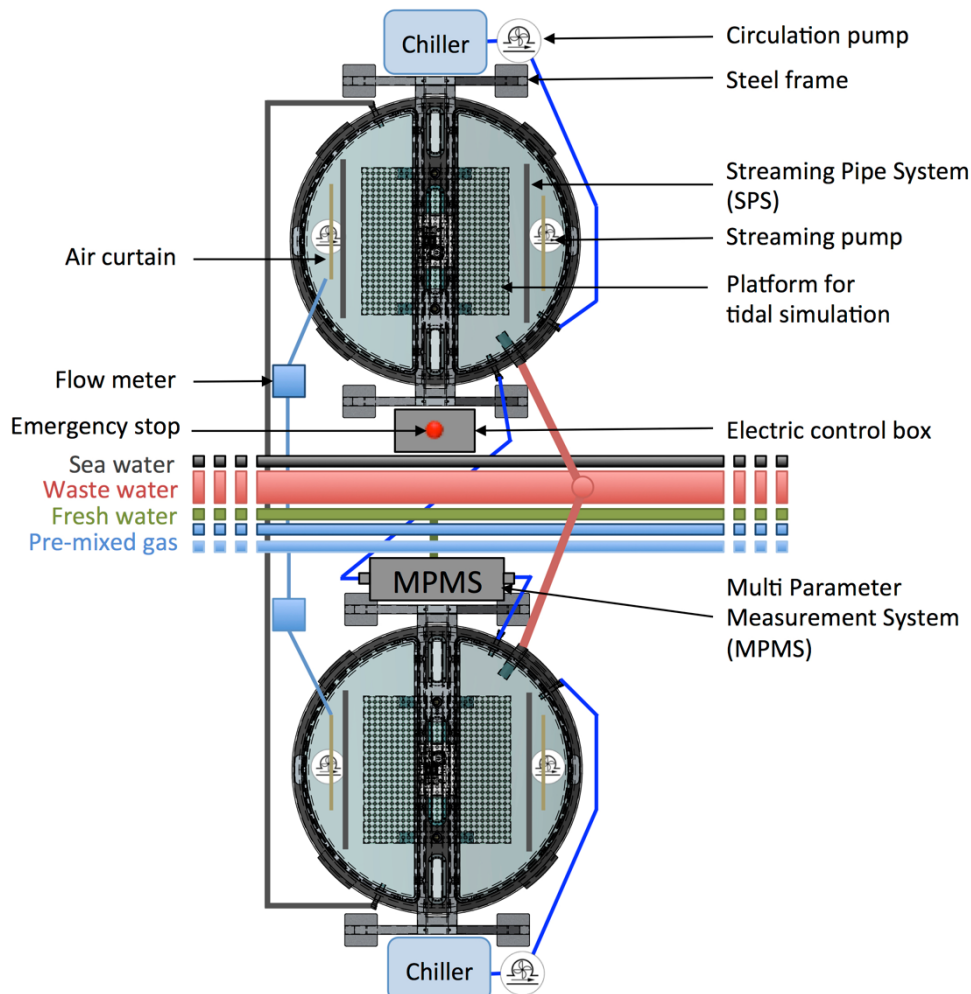


Figure I-3: Schematic overview of one of the six mesocosm tank pairs with their components and connections.

Manipulation and monitoring of environmental parameters is computer controlled. Information technology (IT) facilities are installed in a container next to the mesocosms. A multi parameter measurement system (Fig. I-4a-c) continuously monitors temperature, conductivity, pH and dissolved oxygen. For service works in the tanks during an experiment, all electric loads with 220 Volts can be switched off for each pair of tanks, separately. One of the main components, the tidal simulation system, simulates changing water level and changing current direction simultaneously in each tank (Fig. I-5). A CO₂-mixing facility, heaters and coolers, as well as the tidal simulation system allow the simulation of diverse climate scenarios.

The system is reliably operated in all but the harsh winter months due to the possibility of damages from severe temperatures ($< -5\text{ }^{\circ}\text{C}$). In some parts of the system the water is not continuously flowing as the chance of freezing may negatively affect or even damage the system.

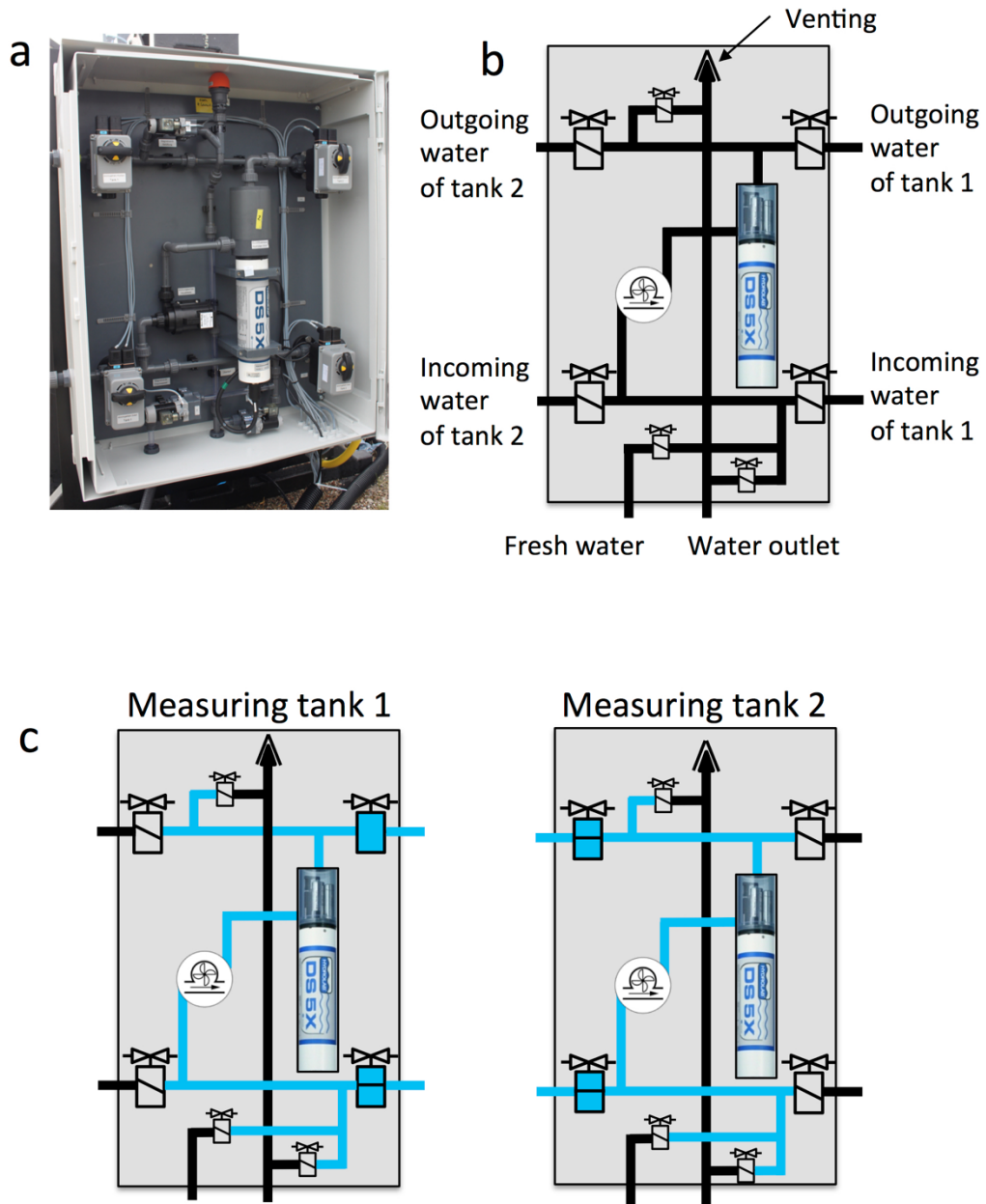


Figure I-4: Foto (a) and diagram (b) of the multi parameter measurement system and (c) an illustration of the alternating pathways for water while parameters of one of the tanks are measured.

Tidal simulation system

The computer-controlled tidal simulation system (Fig. I-5) consists of two parts, the first simulating changing water levels and the second changing the current situation during changes from high to low tide and vice versa. The tidal rhythm can be set as sinus or straight function.

To simulate changing water levels, a 1 m² grating platform (glass-fibre reinforced plastic, GRP), on which the organisms can be planted, is lifted up and down in the water column, with a constant volume of water in the tanks (Fig. I-2). A wire cable and a 220 V engine (Spranger Kunststoffe, Plauen, Germany), attached to a massive steel frame, are constructed to lift the platform with weights up to 250 kg (Fig. I-2), allowing work with sediments, stones or other heavy substrates. A guide rail stabilizes the up and down movements of the platform (Fig. I-2). The maximum tide range can be set from 0 cm (low tide, no water) to 70 cm (high tide) and the platform is moved stepwise (e.g. for a 6 hours tidal period from 0 to 60 cm: 1 cm every 6 minutes). Amplitude, time and type of tide can be regulated with the software without additional mechanical adjustments. Emergency stop buttons are installed between each pair of mesocosms and stop the tide simulation engine immediately in case of problems.

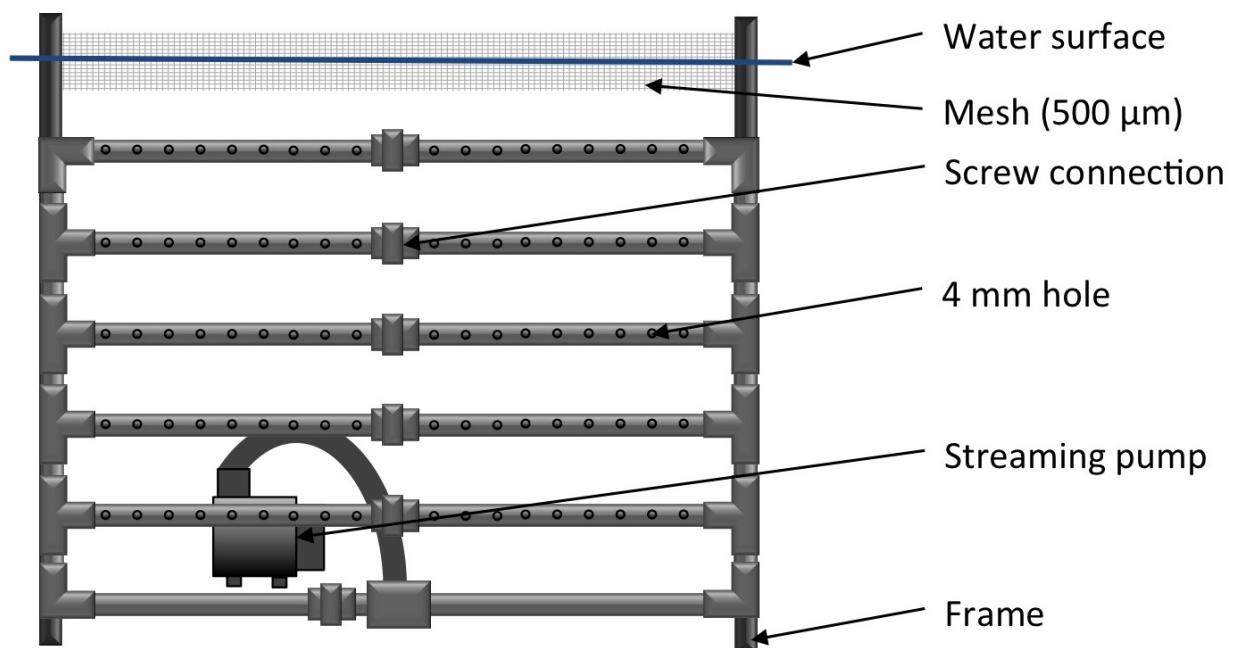


Figure I-5: One of two streaming pipe systems, which are installed opposite to each other in each mesocosm.

The naturally occurring tidal currents are simulated with the second part of the tidal simulation system. To produce a streaming, which is evenly distributed over the whole water body, two streaming pipe systems are opposing each other in the tanks (Fig. I-5). Each system consists of a 60 x 110 cm pipe grid of six horizontal and two vertical PVC-U pipes (20 mm diameter; Georg Fischer Piping Systems, Schaffhausen, Switzerland). Screw connections in

the middle of each pipe allow easy cleaning of the system. Through a total number of 125 holes (4 mm diameter) in the grid, a directed and evenly distributed stream of water (Eco Runner 12000, Aqua medic, Bissendorf, Germany; pumping capacity of 12000 l per hour) is produced for every vertical position of the grating platform in the water column. To achieve this, the pump is connected to the lowest pipe of the streaming pipe systems where the highest ambient water pressure is present. At the bottom of the tank the high pressure of the pump compensates the high ambient water pressure, while near the surface the pressure of the pump, as well as the ambient water pressure is reduced. This creates almost the same current velocity by each single pipe. The current velocity produced is around 10 cm/s, measured in the middle of the tank without any biota (Flo Mate 2000, Hach/Marsh Mc Birney, Loveland, USA). Direction of the current changes with tides and the round shape of the tanks ensure that reflected water runs back along the walls of the tank without interfering with the investigated communities.

Multi Parameter Measurement

Each two tanks share one Multi Parameter Measurement System (MPMS; Fig. I-4a, b). The system consists of a Hydrolab DS5X Probe (OTT Messtechnik GmbH, Kempten Germany), which continuously measures temperature, conductivity, pH and dissolved oxygen. Water is pumped (Eco runner 2700, Aqua Medic, Bissendorf, Germany) from either tank 1 or 2 through the measurement chamber of the Hydrolab DS5X back into the tank. The water gently passes by the sensors in the measurement chamber and a value for each parameter is logged every minute. Electric ball valves and magnetic valves enable the switch between tank 1 and 2 (Fig. I-4c). In order to avoid a mixing of the water between the two tanks and to avoid a fast growing biofilm affecting the sensors, the system is completely emptied and flushed with fresh water between each switching interval, with the use of magnetic valves. Time intervals for measurements in each experimental tank can be adjusted to ensure sensors have stabilized and provide reliable values (30 minutes proved to be optimal for our needs). Verification of drifting or sensors degradation is done by additional manual measurements (pH - pH330i SenTIX81, WTW, Weilheim, Germany; oxygen - Knick Portamess 911 Cond, Berlin, Germany). In case of significant deviations, the sensors are calibrated or replaced and the measured values of the MPMS are corrected by the manual measurements of the hand-held sensors. In addition, to the parameters logged by the MPMS, samples of total alkalinity

(TA), nutrients, chlorophyll a, seston and trace elements (iron, molybdenum and silicate) were performed regularly using the sampling outlets in the wall of the mesocosms.

Software

The software (Labview based, 4H-Jena engineering, Jena, Germany) controls and regulates temperature, tides, currents, energy supply and the MPMS. All logged data from the MPMS are saved automatically as text files and can easily be plotted for a quick check of the measured parameter. Furthermore, a failure notification is integrated, which automatically sends out personalized e-mails in case of system malfunction or if logged parameter deviate from the set thresholds. The system can also be set to different operation modes: SIMULATION mode simulates an experimental run with the predetermined settings of temperature or pH; PLANT mode sets all platforms to the highest position and allows easy access for planting and handling the material on the platforms; SERVICE mode allows for calibration of the MPMS probes; EXPERT mode gives access to more advanced functions and settings e.g. manual control of the MPMS, temperature or tidal system.

Temperature and $p\text{CO}_2$ adjustment

Each tank is equipped with an external cooling unit (Titan 2000 or Titan 4000 Aqua Medic, Bissendorf, Germany) connected via a pump (Eco Runner 2700, Aqua Medic, Bissendorf, Germany). Water runs continuously through the chiller (Eco Runner 2700, Aqua Medic, Bissendorf, Germany), while cooling is automatically switched on or off. Three heaters (Titanium heater 500 W, Aqua Medic, Bissendorf, Germany) are installed directly in each tank. The software regulates cooling devices and heaters in order to achieve the desired temperatures in each tank. Due to the fact that two tanks share one measuring system, it is not possible to simultaneously obtain continuous temperature values in both of these tanks. While the temperature in tank 1 is measured tank 2 is uncoupled from the continuous temperature measurements. By integrating water amount, heat capacity, heat output and difference between measured and desired temperature, the software calculates and regulates the necessary heat amount for the uncoupled period.

In order to manipulate $p\text{CO}_2$ in seawater, each mesocosm is continuously and directly aerated with pre-mixed gas, provided by a central CO_2 -mixing facility from the institute

(GMZ 750, HTK, Hamburg, Germany). Pure CO₂ is mixed with compressed air to the required *p*CO₂ value. A 60 cm air curtain, installed behind each streaming pipe system, aerates the water body continuously with 800 l per hour, controlled with an upstream flow meter (qflow 140, Vögtlin Instruments AG-flow technology, Aesch Bl, Swiss). Direct aerating facilitates the dissolution of CO₂ in seawater allowing a flow through of large amounts of ambient (CO₂ poor) seawater from the facility. A 500-µm, horizontal suspended mesh (Fig. I-5) traps air bubbles that accumulate at the water surface between the streaming pipe system and the tank wall, to avoid an influence on the experimental system. The installed mesh did not affect the light availability in the tanks. The lid ensures similar atmospheric and seawater *p*CO₂ concentrations and reduces the amount of CO₂ required for the experiments. The CO₂-mixing facility is checked and calibrated every year.

2013 – 2014 assessments

The Sylt mesocosms were in operation from October 2013 to December 2014 during four experimental investigations simulating different seasons and different combinations of environmental stressors (Table I-1). Each incubated community consisted of the dominant components of a local Wadden Sea community; the brown alga *Fucus vesiculosus f. mytili*, the pacific oyster *Crassostrea gigas*, the blue mussel *Mytilus edulis*, the periwinkles *Littorina littorea* and *Littorina mariae*, and amphipods of the genus *Gammarus spp.* Algae were bind with wire to the frame, mussels were kept in small baskets on the frame and the rest was free in the tanks. Natural biomass in the field differed between seasons and was adjusted in the mesocosms to mimic the natural population density for the experiments. Within each experiment, the biomass for all species was consistent among the 12 experimental units. The flow through of seawater from the Wadden Sea allowed additional recruitment and settlement of different organism groups such as barnacles, mussels, tunicates, worms and algae.

Table I-1: Periods of investigations of the 4 experiments in 2013 and 2014.

Season	Time	Duration (weeks)	Treatment
Autumn-2013	29 Oct 2013 – 10 Dec 2013	7	Acidification + Warming
Spring-2014	10 Apr 2014 – 25 Jun 2014	11	Acidification + Warming
Summer-2014	17 Jul 2014 – 24 Sep 2014	10	Acidification + Warming + Nutrients
Autumn-2014	16 Oct 2014 – 17 Dec 2014	8	Acidification + Warming + Nutrients

In the Autumn-2013 and Spring-2014 experiments, we applied fully crossed experimental designs with both factors, temperature and $p\text{CO}_2$. To mimic the actual field temperatures, the software calculates a daily and yearly temperature sinus function based on the input of daily and yearly maximum and minimum temperatures (LabVIEW 2013, National Instruments Germany GmbH, München, Germany). The input values of daily temperatures were adjusted at least once a week to actual values measured in the field. Thus, ambient temperatures followed seasonal patterns and warming was achieved by adding a delta value of 5 °C to the ambient temperatures. This led to the following four treatment combinations: (i) ambient, to mimic today's situation, (ii) warm, (ambient temperature +5 °C) as expected for 2100 according to the RCP8.5 temperature scenario of the fifth IPCC report (Field 2014), (iii) acid (ambient temperature and 1000 ppm $p\text{CO}_2$) expected for 2100 according to the RCP8.5 scenario of the fifth IPCC report and (iv) warm+acid (ambient temperature +5 °C and 1000 ppm $p\text{CO}_2$), the combination of increased temperatures and acidification to represent the most realistic future scenario (Field 2014). Each treatment combination was replicated three times.

In the Summer- and Autumn-2014 experiments, we applied partly-crossed designs where the factors temperature, acidification and nutrients were combined in the following four treatment combinations: (i) ambient (ambient temperature, $p\text{CO}_2$ and nutrients), (ii) nutrients (ambient temperature and $p\text{CO}_2$ but doubled concentration of the last 7 year (2006-2013) means of PO_4^{3-} , NO_2^- , NO_3^- , Si), (iii) warm+acid (+5 °C, 1000 ppm $p\text{CO}_2$ but ambient nutrients), and (iv) warm+acid+nutrients (+5 °C, 1000 ppm $p\text{CO}_2$ and doubled nutrients).

Between experiments, all tanks, pipes and hoses were emptied and thoroughly cleaned. Cleaning was done with a high pressure cleaner to remove as much biota as possible, to reduce possible undesired interactions before starting the next experiment. Settled barnacles and tunicates were detached by hand. Except for the Summer-2014 experiment, with very high temperatures (>28 °C) and the addition of nutrients, we did not have many problems with fouling.

During the 2014 experiments, we conducted 24-hour sampling campaigns at the end of each experiment to follow the diurnal, physico-chemical fluctuations in the mesocosms with changing conditions of light, temperature and biological activity. Temperature, pH and salinity were measured and additional samples were taken every other hour from all treatments to determine TA, dissolved inorganic carbon (DIC), nutrients, metals and trace elements.

TA samples were filled bubble-free in 250 ml Winkler bottles and measured immediately after sampling with a titration unit combined with a sample changer (Titroline Alpha Plus, SI Analytics, Mainz, Germany). TA measurements were corrected by using certified seawater standards (Dickson, Scripps Institution of Oceanography, San Diego, USA; Dickson et al. 2003). Chlorophyll *a* was measured weekly with a bbe AlgaeLabAnalyser (bbe Moldaenke, Schwentinal, Germany). Nutrient samples were filtered through 0.45 μm Minisart syringe filter (Sartorius SFCA, Sartorius, Göttingen, Germany); samples were stored at $-20\text{ }^{\circ}\text{C}$ until measurements were performed using a QuAAtro nutrient analyzer (SEAL Analytical GmbH, Norderstedt, Germany; Winde et al. 2014). Accuracy and precision of the nutrient analyses were checked by replicate analyses of standard solution (Winde et al. 2014).

During the Summer-2014 experiment the system was accidentally shut down due to a lightning strike next to the facility. The resulting overvoltage damaged the interface module, connecting pc and mesocosms. Since the temperature regulation and tidal simulation were no longer working, external temperature computers (T-Computer, Aqua Medic, Bissendorf, Germany) were installed on every tank the next morning. The CO_2 mixing facility and water supply work independently from the control system and were not affected by the lightning. An additional lightning protection module was installed with the replacement of the interface module.

This work focuses on the introduction of this new mesocosms facility and the essential abiotic and biotic parameters measured during the experiments in order to demonstrate the functionality of the system. All other data obtained on the community changes under the different climate change scenarios will be presented in detail elsewhere.

Results and Discussion

Chlorophyll a

Chlorophyll *a* values in the mesocosms during the Spring-2014 experiment (Fig. I-6) show the occurrence of the natural algal spring bloom equally in all tanks (peak at the 8th of May), however, with reduced amplitudes compared to field measurements. The maximum concentrations of chlorophyll *a* measured were $15.6\ \mu\text{g l}^{-1}$ and $36.1\ \mu\text{g l}^{-1}$ in the mesocosms and in the field, respectively. Chlorophyll *a* decreased after the spring bloom and varied around $2\ \mu\text{g l}^{-1}$ in all mesocosms until the end of the experiment. A second spring bloom

occurred in the field in end of May 2014 (chlorophyll a of up to $7.05 \mu\text{g l}^{-1}$; Fig. 6) but could not be detected in the incoming water, as well as in the mesocosms. The lower amplitude of the first spring bloom and the absence of the second spring bloom in the mesocosms are likely due to water transport and storage before it is delivered to the mesocosms. Another possible explanation could be the intake of seawater. While field samples of chlorophyll a were taken at the surface, the seawater inlet for the supply to the mesocosms is placed at 2-4 m depths, depending on the tide. Biological activity in the field is, however, mainly concentrated in the surface layers, as is the chlorophyll a concentration (Ryther 1956). However, lots of planktonic species as well as settled barnacles and tunicates, were found during the experiments, suggesting that enough food for the filter feeders was available. In all experiments, we found less than 5% of the dominant filter feeders (*Mytilus edulis* and *Crassostrea gigas*) dead in the ambient treatments. A further explanation of the reduced chlorophyll a concentration could be the reduced light availability in the tanks. Although 90% of the photosynthetically active radiation penetrates the lid, shading effects from the sidewalls of the tanks cannot be avoided. Nevertheless, the successful simulation of the first plankton bloom and apparently abundant phytoplankton and zooplankton species in the tanks, as well as settled barnacles and tunicates at the end of the experiments, demonstrates a strong coupling of the mesocosms and the natural water body of the Wadden Sea. Installing a pump with a direct pipe, connecting the Wadden Sea and the mesocosm facility, should reduce the effects of seawater transport and storage on the chlorophyll a concentration as it is done in the Kiel Outdoor Benthocosm (KOB) facility (Wahl *et al.* 2015). However, without sedimentation tanks the amount of sediment in the Sylt mesocosms would massively increase and unintentional change the biogeochemistry of the water, including the carbonate system.

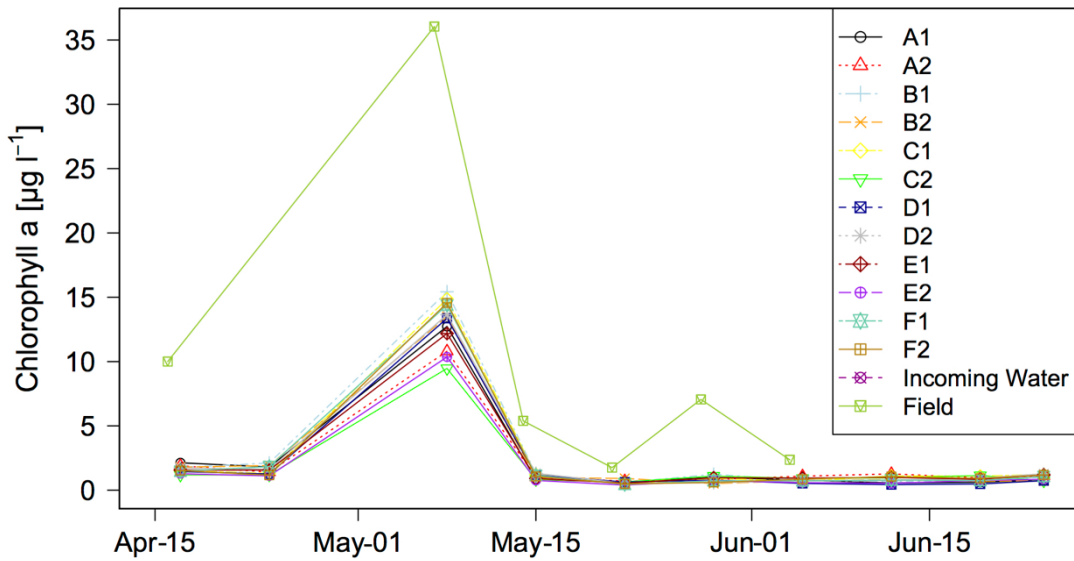


Figure I-6: Chl a concentration during the Spring-2014 experiment for every individual mesocosm, the incoming water and field samples.

Furthermore, we found higher growth rates for *Fucus vesiculosus* in our ambient treatments compared to controls in the field and no signs of light deficiency on the macroalgae during our experiments. While it is still a tank experiment, with some confounding factors, we believe that the lower light availability did not negatively affect the treatments.

Seawater pH

The daily pH fluctuations during the Spring-2014 experiment ranged up to 0.5 pH units per treatment (NBS scale; Fig. I-7a). This was likely due to the natural photosynthetic activity and respiration processes of the biotic components in the tanks (Duarte *et al.* 2013) with minimum pH values in the early morning and maximum pH values in the late afternoon. During the algae bloom of early May, pH reached its highest values of 8.55. Daily pH fluctuations measured during the Autumn-2014 experiment (Fig. I-7b) varied by up to 0.2 units. This much lower variation compared to the Spring-2014 experiment was likely due to reduced temperatures (Fig. I-8a) and less sunlight available due to the lower solar angle during that season.

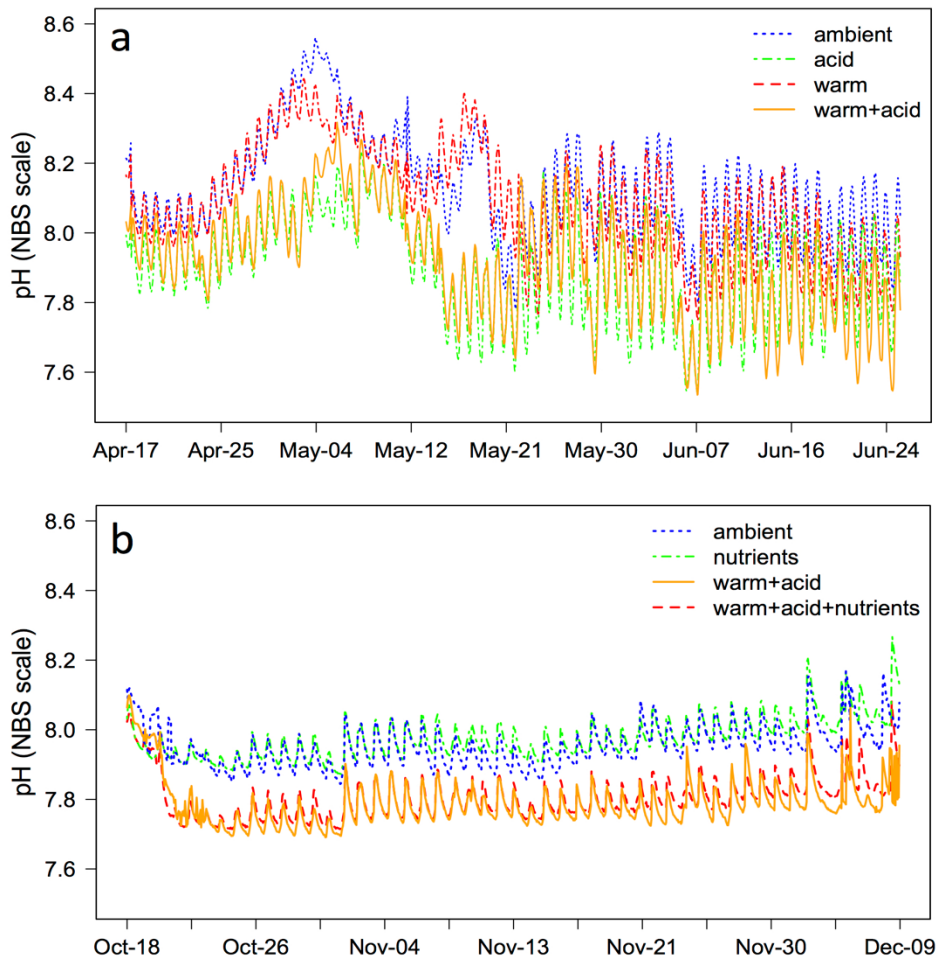


Figure I-7: Daily fluctuations of pH_{NBS} measured by the multi parameter measurement system during the Spring-2014 (a) and the Autumn-2014 (b) experiments. pH values shown are hourly means.

The observed daily fluctuations in seawater pH demonstrate that the naturally occurring variability observed in the field (Saderne *et al.* 2013) can also be simulated in the mesocosms experiments (Fig. I-7a, b), albeit with slightly reduced amplitudes. Nevertheless, the important pH fluctuations could be simulated in our system, which suggests that the amount of incoming, pre-mixed gas and water was not too high to buffer the whole biotic activity within the mesocosms. Furthermore, extremely high fluctuations arising from too high biomass-water-ratios were not observed. The CO₂ mixing facility produced stable treatments during the conducted experiments. The pH within the acid treatments (1000 ppm $p\text{CO}_2$) was consistently 0.2 to 0.3 pH units lower compared to the ambient treatment. This is consistent with the predictions of the fifth IPCC report for the year 2100, where a decreasing pH up to 0.35 units is predicted (IPPC 2013). After the four experiments, single pH sensors

started to drift and had to be replaced. However, as the CO₂ mixing facility acts independently of the pH sensors, the drifting values did not affect the treatments themselves.

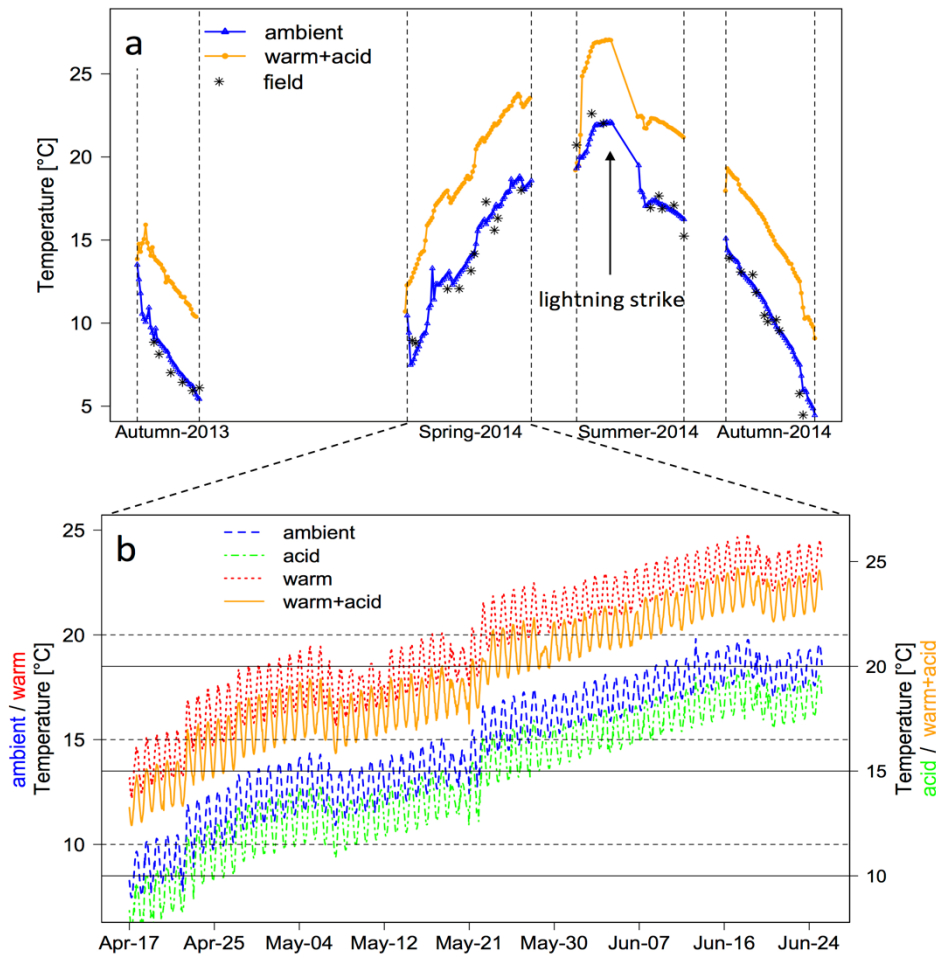


Figure I-8: Daily mean temperatures measured by the multi parameter measurement system during the four experiments in 2013 and 2014 including field temperatures (a) and diel fluctuations of water temperatures (means per hour) during the Spring-2014 experiment (b). The lines of ambient (blue) and acid treatments (green) as well as those of warm (red) and warm+acid (orange) treatments are overlapping due to the same temperature regimes, the right y-axis was therefore shifted downward by 1.58°C (b).

Temperature

Throughout all four experiments in 2013 and 2014, a minimum temperature of 5 °C was only observed at the end of both autumn experiments, in the beginning of December (Fig. I-8a). A maximum temperature of 28 °C was reached at the beginning of August 2014 in the warm treatment. During the Summer-2014 experiment, the values were not continuously logged by

the MPMS between the 12th and 26th of August (Fig. I-8a) due to the lightning strike next to the facility, which damaged the control system.

The yearly (Fig. I-8a) and diurnal variations (Fig. I-8b) in water temperature in the Sylt mesocosms accurately followed the calculation made by the software. A continuous difference of 5 °C between the ambient and warm treatments was maintained with the existing cooling and heating devices at all time during the experiments. Although the temperature simulation is very accurate and reliable, we are aware that even with a lot of adjustments, it only roughly reflects the temperature conditions in the field. All small-scale *in-situ* fluctuations are neglected with this type of simulation.

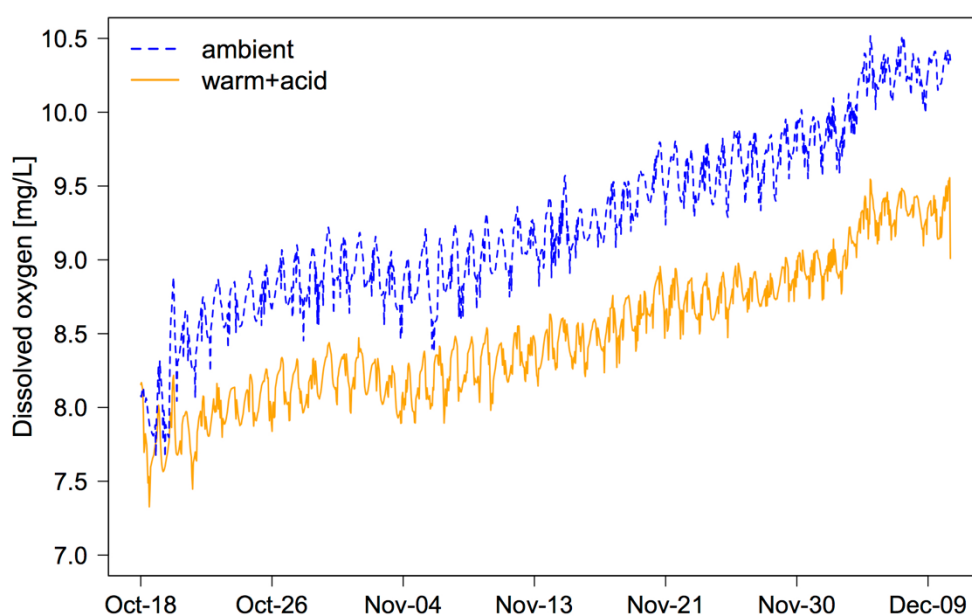


Figure I-9: Daily fluctuations of dissolved oxygen concentrations measured by the multi parameter measurement system during the Autumn-2014 experiment in an ambient and a warm treatment. The oxygen values shown are means per hour.

Oxygen

Oxygen concentrations shown here for the Autumn-2014 experiment (Fig. I-9) constantly increased from 7.5 to 9.25 mg l⁻¹ and from 8.0 to 10.25 mg l⁻¹ in the ambient and the warm+acid treatment, respectively. This is most likely due to decreasing water temperatures during the experiment. The difference between ambient treatment and warm treatments, was consistently highest about 1.0 mg L⁻¹ due to the 5 °C difference in temperature. Biotic

activity, as seen for pH values, induced diurnal variations in oxygen concentrations in the tanks, with an increase of oxygen from dawn to the late afternoon, followed by a decrease until the next morning. We never found any evidence for anoxic conditions in the mesocosms during the conducted experiments.

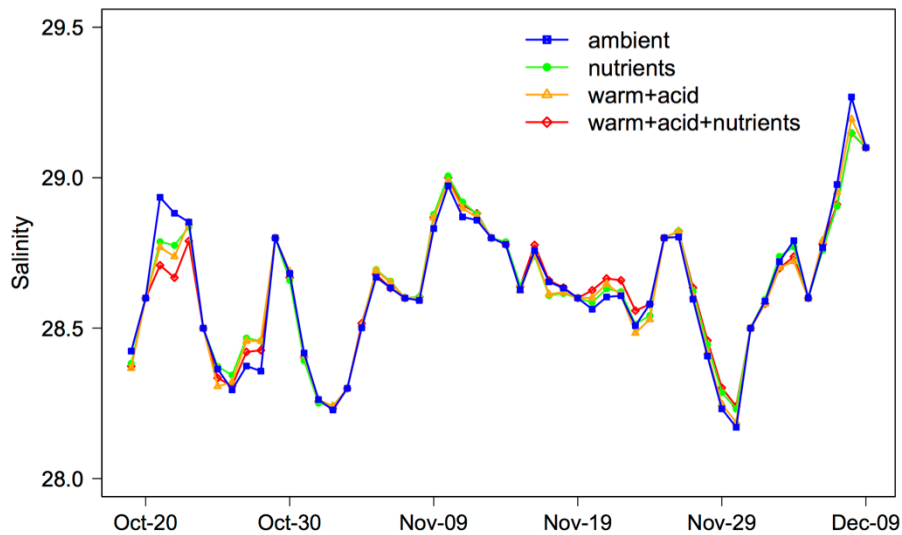


Figure I-10: Fluctuations in salinity during the Autumn-2014 experiment with the four different treatments. Shown are means per day.

Salinity

The salinity, here shown for the Autumn-2014 experiment measured by the MPMS showed only marginal differences between the different treatments (Fig. I-10). The salinity fluctuations within the experiment followed the natural conditions in the field and ranged from 28.2 to 29.3.

Nutrients (PO_4^{3-} , Si and NO_3^-)

During the 24 h sampling campaign of December 2014, we took nutrient samples every 2 hours. Figure I-11 shows the concentration of PO_4^{3-} , Si and NO_3^- during that period. It illustrates the addition of nutrients (red and green lines) at around 3 pm, followed by a strong increase in the concentrations until 5 pm with a gradual subsequent decrease. Part of the added nutrients was likely washed out due to the water flow through while the rest was

reduced by the biota in the tanks. The treatments without the addition of nutrients (orange and blue lines) stayed constant over the 24 hours (Fig. I-11a-c).

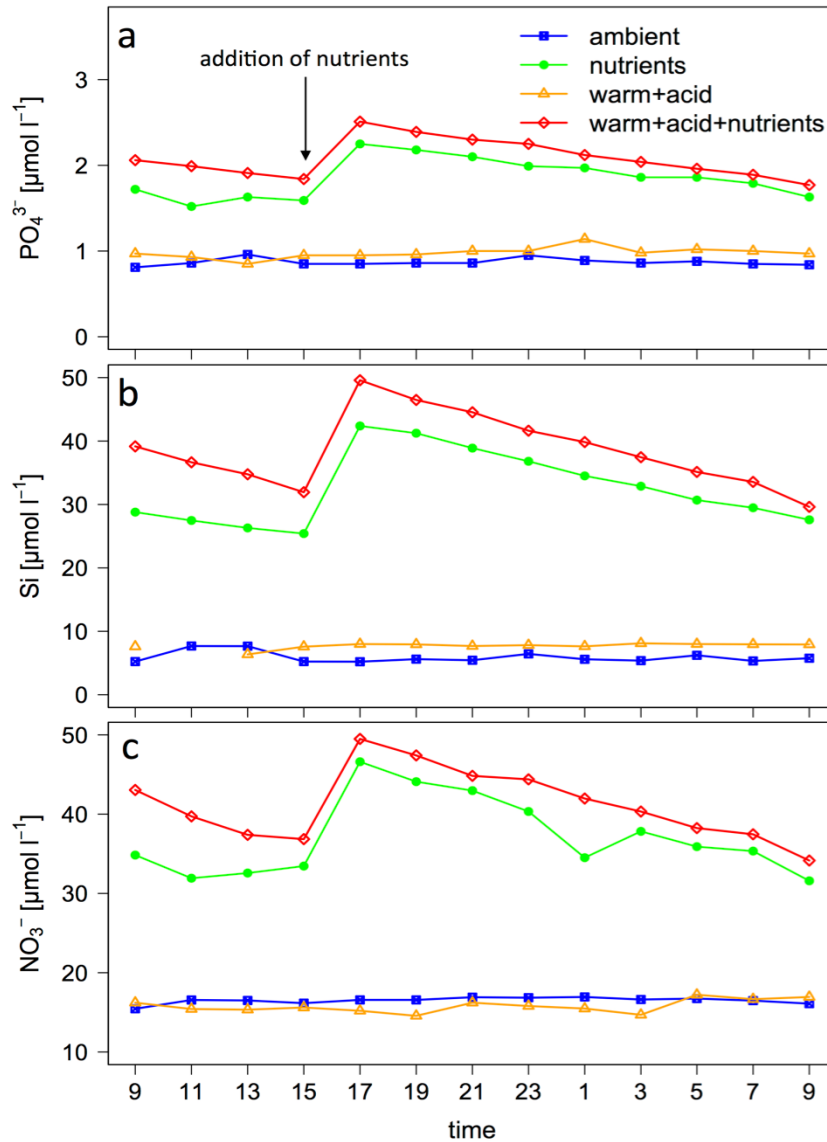


Figure I-11: Nutrient measurements during a 24 h sampling campaign during the Autumn-2014 experiment for (a) PO_4^{3-} , (b) Si and (c) NO_3^-

Conclusions and perspectives

The great potential of mesocosm studies to acquire new knowledge in the context of climate change and its consequences is well known and a reason for the increasing number of new mesocosm facilities worldwide (Stewart *et al.* 2013). Understanding the complex correlations between abiotic factors, biodiversity dynamics, and ecosystem processes is crucial to gain

knowledge on possible shifts in the functioning of ecosystems (Loreau *et al.* 2001). This requires an experimental design, which is controllable, near natural, replicable and feasible at the same time, and based at the community level.

The few transportable pelagic mesocosm facilities that exist allow conducting the same experiments in different aquatic systems around the world (Mostajir *et al.* 2013; Riebesell *et al.* 2013). For benthic systems, however, such transportable facilities do not exist. Nevertheless, in order to reliably predict the consequences of future ecosystem changes, it remains essential to investigate the impact of future changes in the different systems under natural conditions, elucidating local as well as generic patterns in the response to these changes. For the Baltic Sea, a similar system compared to the Sylt mesocosms exists already at the GEOMAR in Kiel (Germany). The KOB system consists of 6 rectangle tanks, with a direct water supply, a hood for each tank and works as well with benthic communities under environmental stress. It did not have a tidal simulation system but a wave generator (Wahl *et al.* 2015a). However, for the Wadden Sea such mesocosm system did not exist before.

The Wadden Sea along the coasts of Germany, Denmark and the Netherlands is one of the most productive marine ecosystems in the world and home to numerous plant and animal species but, at the same time, a heavily used recreational area for humans. It is one of the largest, coherent tidal flat area systems in the world and serves as nursery ground for fish and shrimps as well as important feeding ground for millions of migrating birds (Reise *et al.* 2010). That is why it is important to investigate the response of this exceptional system to future changes and this can be done with this new mesocosm facility.

The mesocosms at the AWI Wadden Sea Station were effectively running during the four experiments with good functionality of all components, it therefore can be recommended as a tool for a variety of experiments in the field of marine biology. Monitoring and controlling the physico-chemical conditions is one of the most important features for climate change experiments. This is done in several comparable, but still different ways (Mostajir *et al.* 2013; Riebesell *et al.* 2013; Leblud *et al.* 2014; Wahl *et al.* 2015a). The MPMS of the Sylt mesocosms is very adaptable, as it can be equipped for different experimental approaches with further sensors (e.g. turbidity, chlorophyll, ammonium, nitrate or chloride). The newly developed tidal simulation system works well and provides new possibilities for mesocosm experiments in coastal systems and tidal areas. It is an important additional feature for near natural mesocosm experiments and not existing in this way in any other mesocosm facility worldwide.

Numerous different types of mesocosm facilities exist, each made with special features for a specific purpose. The targeted purpose of this facility are climate change experiments with benthic communities in tidal areas, but there are far more possibilities for applications. In addition to investigate benthic assemblages, it is possible to investigate pelagic systems with this facility. Although the water depth is only 80 cm, one can test the response of planktonic species to increasing temperatures, acidification or nutrient enrichment as it has been done with comparable mesocosm facilities (Kim *et al.* 2006; Lewandowska *et al.* 2014; Paul *et al.* 2015). The round design of the tanks is favourable for experiments with smaller fish species, juvenile fish, fish larvae or jellyfish, compared to rectangle shaped tanks where it is hard to produce circular flows. Additionally, further stressors or stress combinations can be applied, such as deoxygenation, changes in salinity, changes in turbidity or heat waves. In addition to simulate the tides, it is possible to mimic future sea level rise scenarios with an adjustment of the tidal simulation system. Investigations with non-native species are possible, but they have to be run without a flow-through mode or with an appropriate treatment of the wastewater to avoid an introduction of new species into the Wadden Sea.

So far, the system works with estimated temperatures, calculated by the software, which does not reflect exactly the conditions in the field. To achieve a higher parallelism with field conditions, including natural small-scale fluctuations, which have the potential to modulate environmental stress (Dufault *et al.* 2012; Cornwall *et al.* 2013; Wahl *et al.* 2015a), we suggest installing additional sensors in the field. These would continuously transmit the measured values to the system to improve the simulation of field conditions in the tanks. Direct water supply has great advantages and should be favoured for such experiments (Wahl *et al.* 2015a), but was not possible to build for our system. This adjustment could be considered for future experiments, which focus on small-scale fluctuations of the system. The total costs for the Sylt mesocosms without maintenance and personnel expenses were around 250 K Euro.

The innovations introduced by this facility are the simultaneous control of the important environmental parameters, the manipulation of multiple stressors, their combination with a newly-developed tidal simulation system, and the capability to conduct experiments with inshore benthic communities.

Acknowledgments

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Chapter II

Stressed but robust: a benthic assemblage under the interactive effects of acidification, warming and eutrophication.

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Abstract

Marine ecosystems are expected to respond to global climate change as well as to local anthropogenic drivers with changes in community structure and function. Most investigations to date tested the short-term impacts from particular single drivers on individual species responses. In this study, an experimental mesocosm approach was adopted to gain information on community changes under the cumulative impacts from multiple drivers: nutrient enrichment as regional driver, and ocean acidification and warming as global climate change drivers. Compartments of an intertidal macroalgae-mussel bed community from the Wadden Sea were incubated for 10 weeks in 12 mesocosms at the AWI Wadden Sea laboratory, Germany. Four different treatments were applied, in which single or multiple drivers were manipulated: ‘Ambient’, nutrient enrichment (‘N’), acidification in combination with warming (‘OAW’), and a combination of all three drivers (‘OAW+N’). Nutrient enrichment corresponded to a doubling of natural summer nutrient concentrations. Warming was realized by an increase in temperatures of 5°C and acidification was simulated by 1000 ppm $p\text{CO}_2$ aeration.

OAW reduced biomass of the majority of the investigated species, while N partly mitigated the negative effects of global climate change. Biomass changes were analyzed by a holistic, static modeling approach, the Ecological Network Analysis (ENA), to evaluate energy flows and food web structure of the incubated communities. Shifts in species composition, assemblage activity and connectivity were observed, both under OAW and N, with possible consequences on ecological functioning of the system. Cumulative impacts from all applied factors forced the development of the experimental community towards a stressed and ineffective system. However, the survivors of the persisting species constituted a system with higher activity with enhanced co-operation between compartments and robustness, meaning that these tested systems could better withstand external disturbances. In summary, while certain species suffered from simulated climate change scenarios, ENA revealed that the remaining species created community-level effects, that were mostly beneficial with regard to overall system resilience.

Introduction

Global climatic changes such as ocean acidification, increasing temperatures, and sea level rise, as well as regional anthropogenic drivers such as changing salinities, nutrient regimes and sedimentation loads impact marine ecosystems (Harley *et al.* 2006; Hoegh-Guldberg & Bruno 2010; Doney *et al.* 2012). Surface ocean warming of up to 5°C and rising atmospheric carbon dioxide (CO₂) concentrations of up to 1000 ppm are forecasted for the end of this century (IPCC 2014). Increasing atmospheric CO₂ and its absorption by the oceans leads to increased dissolved CO_{2(aq)} and total inorganic carbon as well as decreased pH, carbonate ion, and calcium carbonate saturation states in seawater (Doney *et al.* 2009). Surface seawater pH already decreased by 0.1 pH units over the past 150 years and future projections predict a further decline by 0.2 to 0.3 pH units until 2100 (Feely *et al.* 2009). At a regional scale, nutrient enrichment (N) is a strong environmental driver for ecosystems changes in numerous coastal seas (Smith 2003; Smith *et al.* 2006; Rabalais *et al.* 2009b). Although in 1988 an agreement on reductions in inorganic nitrogen and phosphorus inputs was signed, a large part of the North Sea is still considered a problem area with regard to increased nutrient loads (Lenhart *et al.* 2010), being true for many more areas all over the world.

The impacts of global climate change on marine ecosystems were intensively investigated and discussed over the past years (Harley *et al.* 2006; Doney *et al.* 2012; Koch *et al.* 2013). Ocean acidification affects various marine organisms, leading to changes in their physiology (e.g. Gazeau *et al.* 2007; Wu *et al.* 2008; Waldbusser *et al.* 2011; Gao *et al.* 2012; Havenhand 2012; Wahl *et al.* 2017), phenology and adaptability (Sunday *et al.* 2011; Calosi *et al.* 2013; Kelly & Hofmann 2013) as well as altered distribution (Waldbusser *et al.* 2011) of species. As different species respond different to ocean acidification, shifts in biotic interactions are likely (Blois *et al.* 2013) and possible result in community restructuring and drastic ecological shifts in marine ecosystems (Brierley & Kingsford 2009; Doney *et al.* 2009; Doney *et al.* 2012). Calcifying organisms were demonstrated to be most susceptible to ocean acidification, but the responses measured were highly variable and depended on species identity, life-history stage of a species, season as well as geographical location and latitude (e.g. Kroeker *et al.* 2013a; Wahl *et al.* 2016).

Photosynthesis of many marine macroalgae is not carbon-saturated at current conditions, and in many macroalgae, energy demanding carbon concentration mechanisms (CCMs) are used to satisfy carbon demands for photosynthesis (Hurd *et al.* 2009; Koch *et al.*

2013). Increased CO₂ concentrations should facilitate access to carbon, reduce the energy costs by down regulating CCMs, and allow plants to allocate more energy to growth, defense or reproduction (Hurd *et al.* 2009).

Ocean acidification will not occur in isolation but will be accompanied by other environmental changes, such as warming, leading to additive, antagonistic, or synergistic effects on marine species and communities (Harvey *et al.* 2013; Sarker *et al.* 2013; Chen *et al.* 2016; Cole *et al.* 2016; Gunderson *et al.* 2016; Boyd *et al.* 2018). Several studies investigating the interactive effects of ocean acidification and warming demonstrated ocean warming to be the main driver of ecosystem changes (Byrne *et al.* 2009; Talmage & Gobler 2011; Paul *et al.* 2015; Horn *et al.* 2016). Seawater temperature is a key factor due to organisms' physiology operating within defined thermal tolerance limits (Helmuth & Hofmann 2001; Pörtner 2008; Paul *et al.* 2015). Various biological responses to temperature changes have been reported, including shifts in species distribution (Parmesan & Yohe 2003; Root *et al.* 2003; Perry *et al.* 2005), decreased body size (Daufresne *et al.* 2009), changes in timing of reproduction and reproductive output (Pankhurst & Munday 2011). Metabolic costs for animals are increased (O'Connor *et al.* 2009a), as also are phenological changes, resulting in predator-prey mismatches (Edwards & Richardson 2004) and increased consumption rates (Sanford 1999), leading to changed food web structures.

In addition, on a more regional scale, intensified land use alters nutrient concentrations of coastal waters. Excessive eutrophication increases the rate of primary production and accumulation of organic matter, with significant impacts on water quality, water chemistry and food web structure (Cloern 2001; Rabalais *et al.* 2009a; Rabalais *et al.* 2014). As relative rates of photosynthetic assimilation and nutrient uptake are considered constant (Sterner & Elser 2002), increased CO₂ conditions from ocean acidification may increase rates of photosynthesis, and enhance primary productivity and nutrient limitation (Pedersen *et al.* 2010). Conversely, primary producers might be limited by present nutrient conditions and enhanced nutrient discharges could increase growth of nutrient-limited primary producers and alter community structure (Lloret *et al.* 2008; Rabalais *et al.* 2009a).

While extensive investigations have been undertaken in the field of marine climate change research, most of these experiments were run in short-term and small-scale settings, mainly using single-driver and single-species assessments (Harley *et al.* 2006; Walther 2010; Wernberg *et al.* 2012). Thus, long-term, multiple-driver approaches with higher bio-complexity are urgently needed (Wahl *et al.* 2016; Boyd *et al.* 2018). Up-to-date mesocosms

provide the potential for multiple driver- and multiple-species approaches including different trophic levels (Stewart *et al.* 2013).

In the present study, replicate subsamples of an intertidal macroalgae-mussel bed community from the Wadden Sea were incubated in a near-natural mesocosm experiment, simulating the single and combined impacts from ocean acidification, warming and nutrient enrichment. We followed the development of species abundance, biomass and network connectivity under the various treatments for 10 weeks during summer 2014. Particular emphasis was given to the question whether the reduction of local drivers (eutrophication) could mitigate the impact of global drivers (acidification and warming) at the community level.

Materials and Methods

Study system

The Sylt-Rømø Bight is a semi-enclosed basin within the North Sea, situated between the islands of Sylt, Germany and Rømø, Denmark. Its area covers around 404 km², with one third being intertidal. The average tidal range in the Sylt-Rømø Bight is 1.8 m. Average salinity related to the time period between 2005 and 2015 ranges between 27.9±0.52 in winter and 30.17±0.57 in summer, depending on currents and freshwater inflow. In the same period, average seawater temperatures range between 2.7±1.2°C in winter and 17.54±0.52°C in summer. The basin is dominated by sandy substrates, while mussel-, oyster- and seagrass beds as well as muddy sediments, together cover approximately 20% of the total area (Asmus & Asmus 1998). The coverage of mussel beds fluctuates strongly between years, depending on factors such as winter ice cover and average summer temperatures (Büttger *et al.* 2011), as well as on competition with the invasive oyster *Magallana gigas* (Büttger *et al.* 2008). Mussel- and oyster beds form the only natural hard substrate, and thus provide important nursery ground, shelter and settlement substrate for hard bottom dwellers.

The investigated system is dominated by the blue mussel *Mytilus edulis* and the pacific oyster *M. gigas*. The dominant macrophyte, *Fucus vesiculosus forma mytili* (hereafter *Fucus*), does not possess a holdfast as other *Fucus* forms do, it is exclusively held to the mussel bed by the byssus threads of *M. edulis*. This macroalgae-mussel community provides settlement substrate, shelter as well as feeding ground for amphipods (*Gammarus spp.*), shrimps (*Crangon crangon*), gastropods (*Littorina littorea* and *L. mariaae*), limpets (*Crepidula*

fornicata), barnacles (*Balanus crenatus*) and many fish species (Nehls *et al.* 2006; Baird *et al.* 2007; Büttger *et al.* 2008).

Mesocosm facility Sylt

The experiment was conducted in the tidal benthic mesocosm facility at the AWI Wadden Sea Station Sylt, described in detail in Pansch *et al.* (2016). In short, the outdoor system consists of 12 x 1.8 m³ circular tanks, exposed to natural light conditions (90% of natural photosynthetic active radiation (PAR) reach the treatment water). In the current study, the tanks were supplied with non-filtered seawater from the AWI Wadden Sea Station, with an exchange rate of approximately one tank volume (1.8 m³) per day. The temperature in each tank was controlled via external cooling units (Titan 2000/4000) and three internal heaters (Titanium heater 500 W), both supplied by Aqua Medic, Bissendorf, Germany.

Software regulated heater and cooler were used to simulated the experimental temperature in the tanks, based on yearly and daily sinusoidal curves, extracted from regular field measurements (unpublished data). In order to manipulate seawater *p*CO₂, each tank was directly aerated via a 60cm air curtain. Pre-mixed gas was supplied by a CO₂-mixing facility (GMZ 750, HTK, Hamburg, Germany), which mixed pure CO₂ and compressed air to the required concentration. To monitor and control the physical parameters, each two tanks shared one Multi Parameter Measurement System (MPMS; 4HJena, Jena, Germany), equipped with a Hydrolab DS5X multi-probe (OTT Messtechnik GmbH, Kempten, Germany). The system continuously logged temperature, conductivity, pH and dissolved oxygen. In addition to the parameters logged by the MPMS, samples for total alkalinity (TA), nutrients, chlorophyll a, and seston were taken manually once per week.

To simulate natural tidal conditions in the field, each tank was equipped with a tidal system, simulating changing water level and currents during tides. A 1m²-grating platform, as basement for the experimental community, was lifted up and down within the water column in line with a simulated tidal rhythm. Changing currents were produced by two opposing streaming pipes per tank, through which the water was pumped alternating with tides. A sinusoidal-based tidal rhythm of 6 hours and 10 minutes with a tidal range of 0 to 70 cm was realized.

During the experiment, the system was temporarily shut down by a lightning, which caused an overvoltage and damage of the interface module, connecting the PC to the

mesocosms (August 11th, 2014). To regulate temperatures during this 2-week period, external temperature computers (T-Computer, Aqua Medic, Bissendorf, Germany) were installed in every tank the following day. As the water supply and the CO₂ mixing facility worked independently from the mesocosm control system, these components were not affected.

Experimental treatments

In order to test for the effects of acidification, warming and nutrient loads, a reduced (partly crossed) experimental design was applied, where the factors acidification, temperature and nutrients were combined in the following four treatment combinations: (1) Ambient (ambient temperature, $p\text{CO}_2$ and nutrients), (2) N (ambient temperature and $p\text{CO}_2$ + doubled nutrients), (3) OAW (ambient nutrients +5°C at 1000ppm $p\text{CO}_2$), and (4) OAW+N (+5°C at 1000 ppm $p\text{CO}_2$ + doubled nutrients). Temperature and CO₂ treatments were chosen as expected for the year 2100 according to the RCP8.5 scenario of the fifth IPCC report (IPCC 2014). Nutrient treatments were chosen after retrieving data from a 7-year dataset (2006-2013) of phosphate (PO_4^{3-}), nitrate (NO_3^-), nitrite (NO_2^-) and silicate (Si) (Rick *et al.* 2015). Mean concentrations for the respective months July, August and September were calculated and doubled in the nutrient enriched treatments (Table S1). Nutrients were added manually daily at 3:00 p.m. (see also Pansch *et al.* 2016 for details). Each of the 4 treatments was replicated three times.

Sampling

A section of a mussel-oyster bed covered by *F. vesiculosus forma mytili* and its associated fauna were collected on July 14th, 2014 from a tidal flat of the Sylt-Rømø Bight (55°01'42.2"N, 8°26'01.1"E). *Fucus* thalli were washed in freshwater to separate animals from the algae. Several thalli were bound to bundles of ~230 g wet weight (Table II-1). Prior to weighting (Sartorius lc 4200s; 0.01 g; Göttingen, Germany), bundles were shaken 5 times in order to remove excess water. After determination of weight and length, 8 of these bundles were fixed to the grating platform of each tank. A selection of the associated fauna (*M. gigas*, *M. edulis*, *Gammarus spp.*, *L. littorea* and *L. mariae*) was collected from the investigated community at the same location and date. After measuring (length and weight) and counting, specimens were distributed at identical proportions to the 12 tanks (Table II-1). Mussels were

put in small nets while oysters were placed in open trays. Amphipods and snails could move freely within the tanks.

Table II-1: Biomass of included species at the start of the experiment (means \pm standard deviation; NA = no data collected).

Species	Individuals per tank	Wet weight \pm SD [g]	Length \pm SD [mm]
<i>Fucus vesiculosus forma mytili</i>	8 bundles of 4-8 thalli	per tank 1868.45 \pm 99.82	44.69 \pm 5.16
<i>Magellana gigas</i>	4	per individual	per individual
S	1	19.00 \pm 5.05	49.26 \pm 4.36
M	1	50.48 \pm 10.84	73.25 \pm 6.70
L	1	88.33 \pm 15.32	97.34 \pm 10.90
XL	1	244.32 \pm 107.20	130.68 \pm 22.04
<i>Mytilus edulis</i>	12	per individual	per individual
S	3	2.05 \pm 0.88	25.22 \pm 4.30
M	4	8.80 \pm 1.81	41.93 \pm 3.11
L	3	16.08 \pm 2.39	51.32 \pm 2.31
XL	2	25.72 \pm 4.10	57.16 \pm 6.32
<i>Gammarus spp.</i>	38	NA	NA
<i>Littorina littorea</i>	85	per tank 237.64 \pm 3.96	NA
<i>Littorina mariae</i>	15	per tank 3.09 \pm 0.44	NA

Besides logged data (MPMS) and regular manual measurements, 24-hour sampling campaigns were conducted at the end of the experiment to follow diurnal, physico-chemical fluctuations with changing conditions of light, temperature and biological activity in the tanks. Here, temperature, pH_{NBS} and salinity (WTW Salinity and pH Meter; WTW pH/Cond 340i, Weilheim, Germany) were measured every other hour. At the same times, water samples were taken to determine total alkalinity (TA), dissolved inorganic carbon (DIC), nutrients, metals and trace elements. More information on sampling and measurements are available in Pansch *et al.* (2016). Means of the data from the regular samplings (temperature, salinity, pH_{NBS} and TA) are provided in Table II-2.

Table II-2: Salinity, temperature ($^{\circ}$ C), pH_{NBS} and TA (μ mol kg⁻¹) means (\pm standard deviation) for each treatment of weekly measurements over the 10-weeks experiment.

	Ambient	N	OAW	OAW+N
Salinity	29.99 \pm 1.00	29.99 \pm 1.00	30.00 \pm 0.98	30.00 \pm 1.00
Temperature	18.60 \pm 2.11	18.59 \pm 2.09	23.40 \pm 2.39	23.44 \pm 2.27
pH_{NBS}	7.950 \pm 0.050	7.960 \pm 0.043	7.744 \pm 0.055	7.742 \pm 0.055
TA	2238.2 \pm 57.1	2242.0 \pm 56.3	2246.2 \pm 54.2	2247.1 \pm 58.4

At the end of the experiment, after 10 weeks (July 17th to September 24th), all organisms were removed from the tanks for further processing, and the tanks were emptied. *Fucus* bundles were weighed (see above) and their length were measured. Epiphytes were gently removed from the algal blades and dried. Settled barnacles, *Balanus crenatus* (entering the tanks as larval stages via unfiltered incoming water) were gently removed and further processed together with all other specimens that were initially introduced to the tanks. Subsamples of *Fucus* from each tank were taken (~100 g fresh weight) to determine the dry weight (DW) and ash-free dry weight (AFDW). All animals were counted and blotted dry before their wet weight was assessed. To determine DW and AFDW of the organisms, all samples were dried for 24 hours at 60°C, and combusted at 550°C for 6 h. The amphipod *Corophium sp.*, which was not part of the starting assemblage, was also counted, dried and combusted to determine DW and AFDW. For the determination of suspended particular organic matter (SPOM), 250 ml water samples were taken from each tank and filtered on pre-combusted (450°C; 4 h) Whatman GF/F glass fibre filters. Conversion factors by Brey *et al.* (2010) were used to calculate the carbon content of the different samples needed for the ENA. Phytoplankton concentrations were estimated by chlorophyll a measurements and converted to carbon content, using a carbon to chlorophyll ratio of 50 (Riemann *et al.* 1989).

Ecological Network Analysis

Ecological network analysis (ENA) is a valuable tool for food web analyses (Baird *et al.* 2004, 2007, 2008). ENA has successfully been applied for ecosystem comparisons (Christian *et al.* 2005; Scharler & Baird 2005; Leguerrier *et al.* 2007b), as well as for assessing inter-annual variability in food web structures (Baird & Ulanowicz 1989; Borrett *et al.* 2006). Furthermore, ENA allows food web comparisons on a global scale (Baird *et al.* 1991). Based on input-output analysis, trophic and cycle analysis as well as estimation of system indices, ENA can elucidate the complex structure of different systems, and systems under climate change stress. The theoretical background of ENA and its general applications are described in detail by Ulanowicz (2004).

For each replicate of the 4 treatments, a food web model was constructed and analyzed by means of ENA as described by Baird *et al.* (2004). Each model consists of 9 living and 3 non-living compartments and is based on the biomass and energy budget for each compartment, as well as on flow of energy and material between the compartments (Figure II-

1). Biomass data were taken at the start and at the end of the experiment. Data on energy budget consisting of production rates, diet composition and energy requirements of the different compartments of the system were obtained from Baird *et al.* (2004). Unit of biomass was mg C per tank. Imports, exports and flows between the compartments are presented in mg C per tank and day.

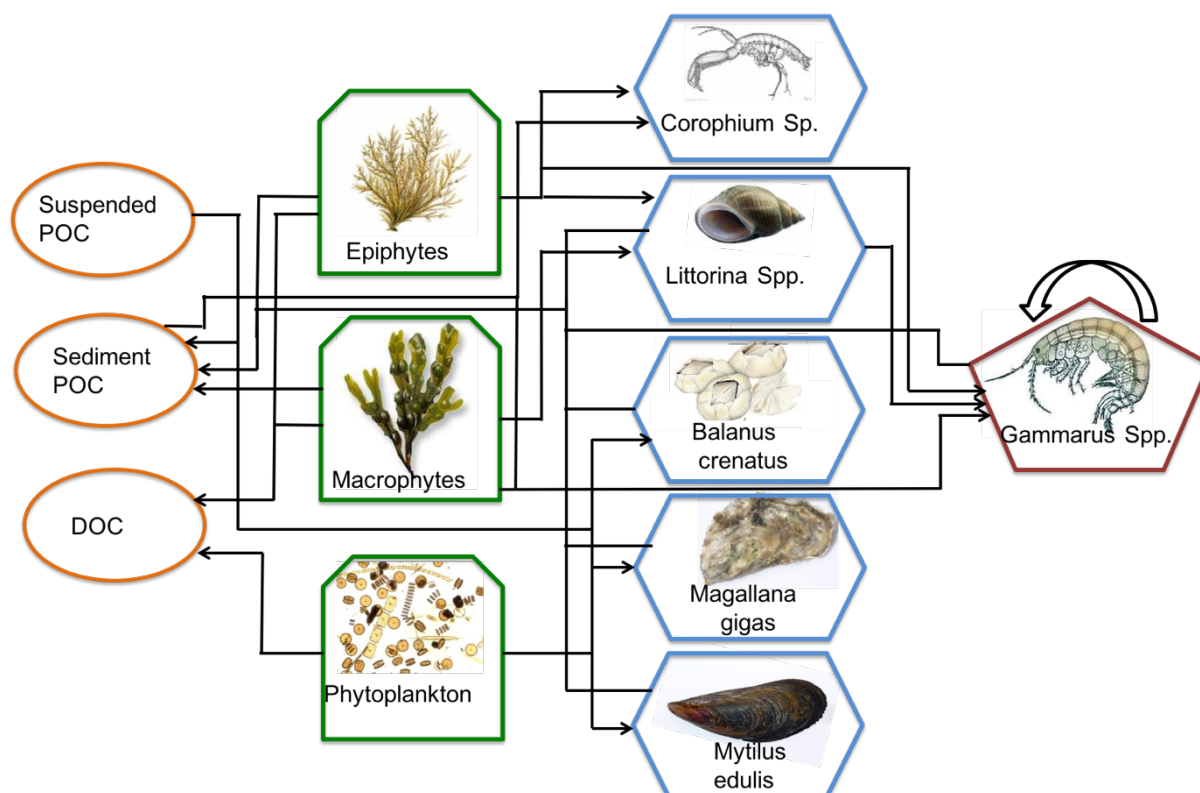


Figure II-1: Food web of the investigated species assemblage. The 12 compartment models are aggregated into various biotic trophic guilds. Non-living compartments are shown as organic carbon (DOC), suspended particulate organic carbon (POC), and sediment POC. Represented are major energy flow pathways (black lines), non-living compartments (orange circles), primary producers (green hexagon shaped boxes), primary consumers (blue hexagons), and secondary consumers (red pentagon; adapted from Baird et al. 2004a).

Each of the living compartments was balanced, regarding energy uptake (consumption), energy loss (respiration), egestion and production. Hence, the inputs of each living compartment of the network were in equilibrium with the corresponding outputs. Regarding the 3 primary-producer compartments phytoplankton, epiphytes and macrophytes, gross primary production (GPP) was assumed equal to the sum of net primary production (NPP) and respiration. The values for GPP were entered as energy input into the subsystems. Exudation by primary producers is considered to be an important source of dissolved organic carbon (DOC) in aquatic ecosystems, in which about 25% of the net phytoplankton

photosynthate and about 2% of macroalgae biomass are assumed to be released as DOC to the water column (Brylinsky 1977; Baird & Ulanowicz 1989; Valiela 2013). Egestion of animals and excess of benthic production was regarded as deposit into sediment in form of particulate organic carbon (POC). To integrate temperature differences between the warm and ambient treatments, we adjusted the respiration and production values considering the multi-parameter artificial neural network model by Brey (2001, 2012). Table S2 provides values of consumed energy, respiration rates, production and egestion by heterotrophs as well as NPP and respiration of autotrophs of all compartments and the 4 different treatments. The diet matrix of predator and prey species is given in Table S3. The network analysis was performed using the R! Software package enaR (Borrett & Lau 2014).

Details about the applied ENA routines are given in Field *et al.* (1989). A review with regard to the underlying quantitative methods was provided by Ulanowicz (2004), who also provided and explained algorithms and their application in ENA, combined with methods for the systematic estimation of ecological flow networks, and calculations of several functional indices and system properties of natural ecosystems. An overview of functional indices and system properties executed by this package is given in Table S4.

The Lindemann trophic analysis converts the complex trophic network into a linear food chain with discrete trophic levels. The generated Lindeman spine defines the carbon amount that is transported from one trophic level to the next higher level, as well the amount leaving each trophic level due to respiration, egestion and export. It further shows the detritus pool, imports of organic matter and energy flow to the different trophic levels. The Lindemann spine enables the quantification of the trophic efficiency for each trophic level, which describes the efficiency of transfer from one level to the next. The trophic efficiency of the entire system is computed as the logarithmic mean of all integer trophic level efficiencies (Baird & Ulanowicz 1989).

Statistical analysis

Log response ratios were calculated of the different treatments (OAW, N, OAW+N) compared to the Ambient treatment, with total as well as single-species biomass.

$$\log (RR) = \ln \left(\frac{\text{Response (Treatment)}}{\text{Response (Ambient)}} \right)$$

The various functional ENA indices obtained from the enaR package, as well as biomass, were analysed using factorial analysis of variance (ANOVA), with the factors climate change (two levels: Ambient and OAW) and nutrients (two levels: Ambient and N). Normality and homogeneity of variances were tested using Shapiro-Wilks and Levenes tests, respectively. Significant ANOVAs were followed by Fischer LSD post-hoc tests. All statistical analyses were carried out using R! Software (R 3.3.2 GUI 1.68).

Results

Water parameters and carbonate chemistry

Salinity, temperature, pH_{NBS} and TA over the 10-weeks experiment are presented in Table II-2 and Figure S1. Salinity followed natural conditions in the field with constantly decreasing values towards the end of summer (end of the experiment). Seawater temperatures followed a similar trend. Temperature differences between the ambient and the warmed treatments were kept at a mean delta of 4.8°C . The mean difference in pH_{NBS} between the ambient CO_2 and increased CO_2 treatments was about 0.2 pH units (Table II-2; Figure S1). Following daily addition of phosphate, nitrate and silicate at 3 p.m. (Table S1), nutrients decreased constantly due to fast uptake by primary producers (Asmus 1986) and due to through-flowing seawater, until the next addition of nutrients the following day (Figure S2b). Furthermore, the nutrient concentration in the sampling area is relatively low during summer and by that the doubled concentrations as well. Thus, overall differences in nutrients between the N and the Ambient treatments were weak but present (Figure S2a).

Experimental community biomass and single compartments

After 10-weeks, climate change (Ambient vs. OAW) had a statistically significant impact on total, heterotrophic and autotrophic biomass, total system throughput (TST), average path length (APL), Finn cycling index (FCI), effective link density (ELD), robustness (R), relative overheads (RelOH), relative ascendancy (RelA), relative internal redundancy and flow diversity (FD; Table S5). Nutrients (Ambient vs. N) had a statistically significant impact on ELD, relative overheads and relative ascendancy only (Table S5).

Total biomass decreased in the OAW and OAW+N treatment compared to the Ambient and N treatment (Table II-3; Figure II-2), representing a decrease of 23%. OAW and

OAW+N showed significantly decreased autotrophic biomass when compared to the Ambient treatment (Table S5; Figure II-2). Heterotrophic biomass in OAW and OAW+N were significantly lower compared to heterotrophic biomass in N (Table II-3; Figure II-2).

Table II-3: General system attributes for the four different treatments (Ambient, OAW (climate change, = ocean acidification and warming, N (nutrient enrichment) and OAW+N (the combination of climate change and nutrient enrichment)). Shown are mean values of three replicates for each treatment. Mussel bed data from the field were retrieved from Baird et al. (2007). Autotrophs comprise phytoplankton, epiphytes and *Fucus*. Heterotrophs comprise *M. gigas*, *M. edulis*, *B. crenatus*, *Gammarus spp.*, *Corophium sp.*, *L. littorea* and *L. mariae*. Filter feeders comprise *M. gigas*, *M. edulis* and *B. crenatus*.

Attribute	Ambient	N	OAW	OAW+N	Mussel bed (literature)
Total biomass (mg C per tank)	241285.8	229697.5	186422.8	192237.9	954798.9
Total biomass autotrophs (mg C per tank)	215145.9	195583.2	169584.4	177357.1	146366.0
Total biomass heterotrophs (mg C per tank)	26139.9	34114.3	16838.4	14880.9	808432.9
Biomass ratio autotrophs to heterotrophs	8.23	5.73	10.07	11.92	0.18
Net primary production (NPP; mg C per tank d ⁻¹)	2905.9	2673.6	3207.6	3402.6	3998.2
Total heterotrophic production (mg C per tank d ⁻¹)	37.1	54.4	31.5	33.5	1097.6
NPP efficiency (%)	8.47	12.80	7.32	5.24	16.90
Production to biomass ratio (P/B; d ⁻¹)	0.012	0.012	0.017	0.018	0.005
Fucus biomass to total biomass ratio (%)	86.4	83.9	79.5	77.6	15.3
Filter feeders/total Biomass (%)	7.3	9.8	5.9	3.9	-
Phytoplankton shortfall (mg C per tank d ⁻¹)	167.13	207.97	149.69	44.96	-

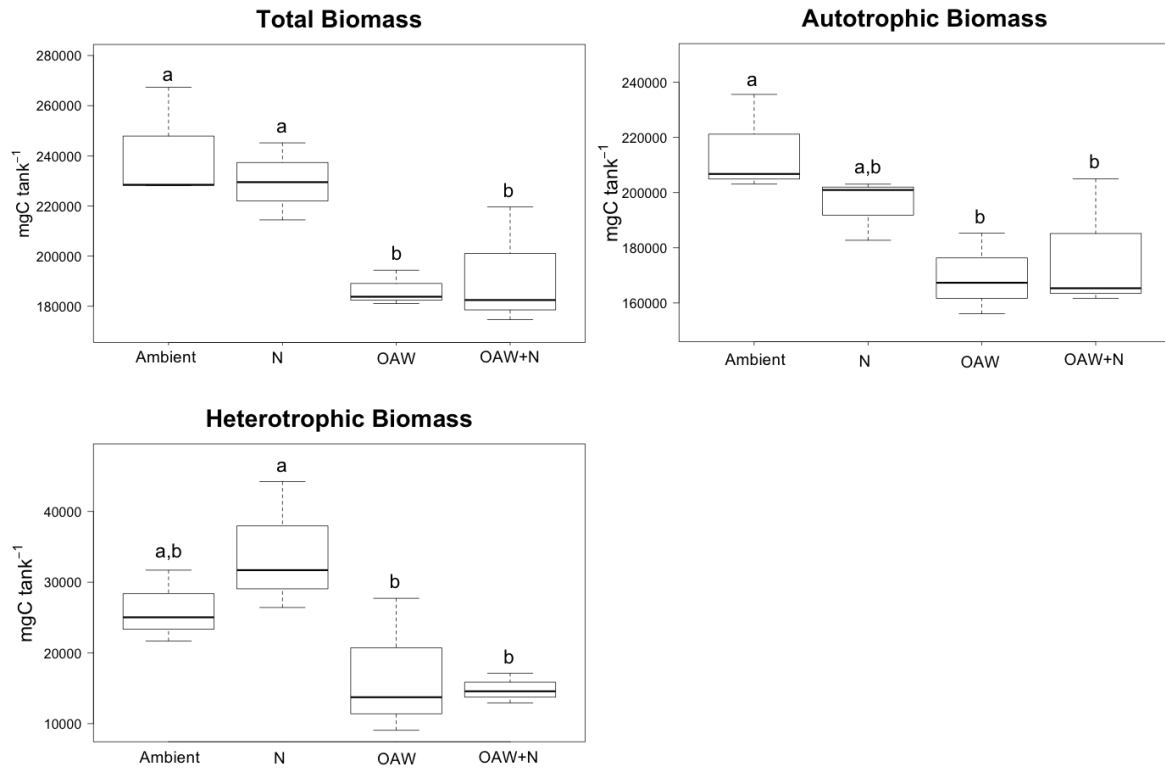


Figure II-2: General system attributes (total, heterotrophic and autotrophic biomass), per treatment (Ambient, OAW (climate change, = ocean acidification and warming, N (nutrient enrichment) and OAW+N (the combination of climate change and nutrient enrichment)), based on the three replicates per treatment. Autotrophs comprise phytoplankton, epiphytes and *Fucus*. Heterotrophs comprise *M. gigas*, *M. edulis*, *B. crenatus*, *Gammarus spp.*, *Corophium sp.*, *L. littorea* and *L. mariae*. The boxplots represent the median, first and third quartile ("box") and 95% confidence interval of the median ("whiskers"). Treatments not sharing the same letter(s) are significantly different (post hoc tests).

Fucus had the highest contribution to the autotrophic biomass in all treatments (Table II-3). The climate change treatments (OAW, OAW+N) significantly decreased *Fucus* biomass, while N showed no effect, when compared to the Ambient treatment (Figure II-3). Filamentous epiphytes were not impacted by the N treatment, but strongly increased in biomass in OAW and OAW+N (Figure II-3). Phytoplankton biomass (measured as chlorophyll a), was impacted by none of the treatments (Figure II-3).

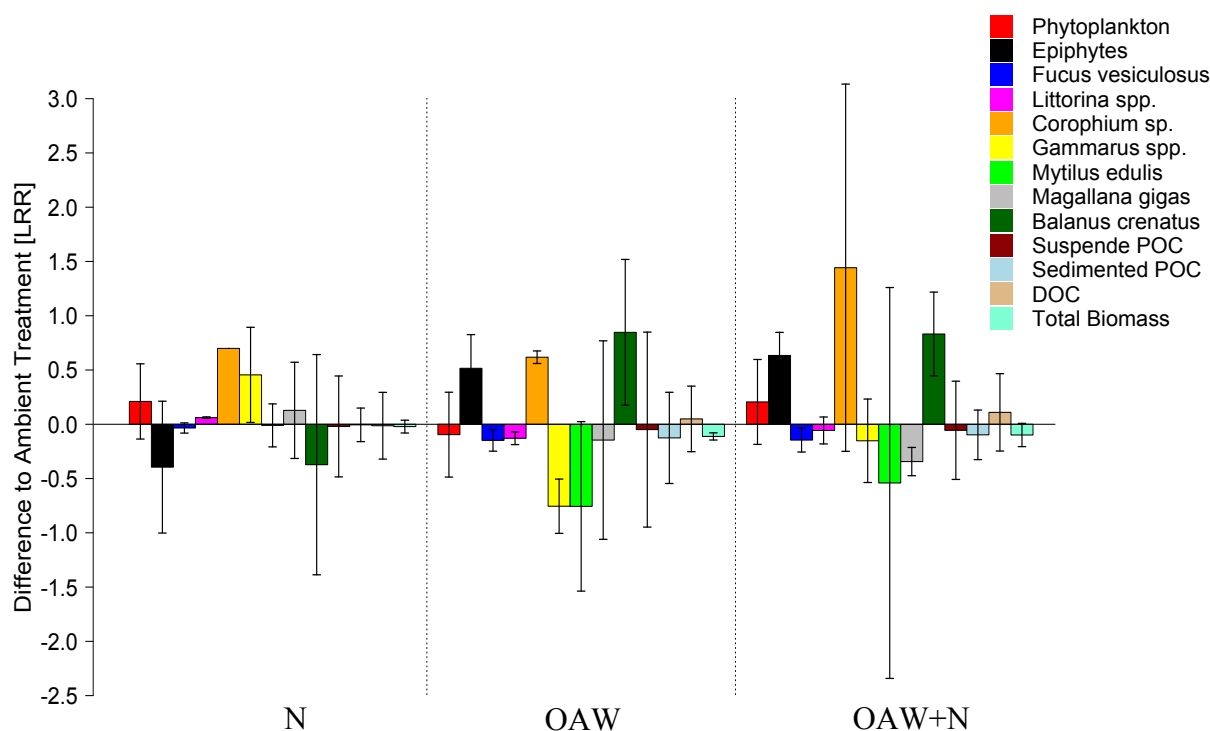


Figure II-3: Log response ratio (LRR) plot of the single compartment biomasses at the end of the experiment (differences to the Ambient treatment; mean \pm 95% CIs). Treatments are shown as differences to the Ambient treatment (OAW (climate change, = ocean acidification and warming, N (nutrient enrichment) and OAW+N (the combination of climate change and nutrient enrichment)).

The largest increase in biomass for single heterotrophic compartments was found for *Corophium sp.*, which showed an increase in all three treatments compared to Ambient, with, however, a strong variability between tanks in OAW+N (Figure II-3). *Gammarus spp.* was positively impacted by the N treatment but suffered in the OAW treatment, leading to an antagonistic effect when both drivers were combined (OAW+N; Figure II-3). Compared to Ambient, *B. crenatus* strongly increased in biomass under both, OAW and OAW+N (Figure II-3). The biomass of the filter feeders *M. gigas*, *M. edulis* and *B. crenatus* accounted for 3.9 to 9.8% of the total biomass (depending on the treatment; Table II-3). While *M. edulis* was not affected by either of the treatments, *M. gigas* showed a decreased biomass under OAW+N, while there were no differences under N and OAW (Figure II-3). The grazer *Littorina spp.* showed a small increase in biomass under N and a decrease imposed from OAW, while OAW+N did not impose an effect (Figure II-3).

Higher autotrophic to heterotrophic biomass ratios were found for OAW+N and OAW, compared to the Ambient and the N treatment, due to a massive reduction of the heterotrophic biomass (Table II-3). The highest mean NPP efficiency was found under N (+51%), while the efficiency was reduced in OAW (-14%) and OAW+N (-38%; Table II-3).

Due to increased production rates under enhanced temperature, the daily P/B ratios were increased by 42% in OAW and by 50% in OAW+N compared to the Ambient treatment (Table II-3).

Possible due to limited water exchange in the system (one tank volume per day), the phytoplankton biomass within the mesocosms were lower compared to natural conditions, thus likely not entirely sustaining the demands for filter feeders (*M. gigas*, *M. edulis* and *B. crenatus*). This resulted in a phytoplankton shortfall of 167, 208, 150 and 45 mg C per tank for Ambient, N, OAW and OAW+N, respectively (Table II-3). The differences in shortfall between the treatments likely derived from enhanced phytoplankton production in the tanks with a simultaneous change in species consumption in OAW+N.

Ecological Network Analysis – Trophic structure

The basic functions of food webs are reflected by average path length (APL) and average residence time (ART). The APLs of the different treatments ranged between 1.50 (OAW+N) and 1.53 (N; Table II-4). This means for all treatments, that a unit of carbon was transferred over very few steps before it exited the system. Climate change (OAW) significantly impacted APL (Table S5). The mean APL of the 4 treatments was 1.514 ($\pm = 0.013$ standard deviation), which is represented between the APL of natural pelagic systems (APL = 1.43) and mussel beds (APL = 1.94) within the Sylt-Rømø Bight (Baird et al. 2004, 2007). Looking at ART, energy and material resided about 11 days (30%) longer in the Ambient and the N treatment when compared to the OAW and OAW+N treatments (Table II-4). The high amount of deposited organic material in Ambient and N coincides with the longer ART values compared to the climate change and combined treatments (OAW and OAW+N), with higher activities and turnover rates (Table II-4). Larger P/B ratios (Table II-3) under OAW and OAW+N are additional indicators for a faster turnover in these systems.

Table II-4: System attributes and indices derived from the ecological network analysis of the four different treatments. Shown are mean values of three replicates for each treatment. Mussel bed data from the field were retrieved from Baird et al. (2007).

System attributes	Ambient	N	OAW	OAW+N	Mussel bed (literature)
Trophic efficiency (TE; %)	3.69	6.40	3.22	3.40	14.92
Detrivory (mgC per tank per day)	37.5	53.7	32.1	24.7	1523.0
Detrivory:herbivory	0.15:1	0.16:1	0.13:1	0.14:1	0.3:1
Finn cycling index (FCI; %)	0.026	0.075	0.006	0.023	2.53
Average path length (APL)	1.52	1.53	1.51	1.50	1.94
Average residence time (ART; d)	36.4	37.1	25.9	25.5	83.7
Total system throughput (TST; mgC m ⁻² d ⁻¹)	16647.9	15657.6	18087.8	18842.8	33571.0
Developmental capacity (DC; mgC tank ⁻¹ d ⁻¹)	44936.1	43855.9	51554.6	55601.4	135620.0
Ascendency (A; mg C m ⁻² d ⁻¹)	26566.2	25227.5	28633.5	29450.5	67521.0
Relative ascendency (RelA; %)	59.1	57.6	55.5	52.9	50
Relative overhead (RelOH; %)	40.9	42.4	44.5	47.1	50
Average mutual information (AMI)	1.60	1.61	1.58	1.56	2.01
Overheads on imports (mgC m ⁻² d ⁻¹)	4980.1	5047.4	7130.6	8258.1	17781
Overheads on exports (mgC m ⁻² d ⁻¹)	483.5	493.1	528.4	624.0	2690
Dissipative overhead (mgC m ⁻² d ⁻¹)	5860.8	6028.6	7175.1	8034.6	23590
Redundancy (R; mgC m ⁻² d ⁻¹)	7045.5	7059.4	8087.0	9234.2	24024
Relative redundancy (RelR; %)	15.71	16.20	15.66	16.63	17.7
Normalized redundancy (R/TST)	0.42	0.45	0.45	0.49	0.72
Internal developmental capacity (DCi; mgC m ⁻² d ⁻¹)	12663.3	12706.3	13938.0	14985.7	54659
Internal ascendency (Ai; mgC m ⁻² d ⁻¹)	5618.0	5646.8	5851.0	5751.7	30624
Relative internal ascendency (Ai/DCi; %)	44.3	44.3	42.0	38.3	56.03
Trophic depth (TD;	3.02	3.05	3.00	2.96	-
Flow diversity (FD; DC/TST; %)	2.70	2.80	2.86	2.96	4.04
Φ (sum of overheads/TST)	1.09	1.17	1.26	1.38	2.03
Effective link density (ELD)	1.47	1.51	1.55	1.62	-
Robustness (R)	0.311	0.318	0.327	0.337	-
Overall connectance	1.33	1.34	1.34	1.36	1.55
Intercompartmental connectance	1.45	1.48	1.51	1.63	1.78
Foodweb connectance (living compartment only, GPP/TST)	1.32	1.38	1.21	1.33	1.29

Simplified Lindeman spines were constructed to visualize the carbon flows within each treatment (Figure II-4). Each box represents an integer trophic level with the associated trophic efficiencies. The first box represents the first trophic level and is divided into primary producers (upper part) and the detritus pool (lower part). The trophic efficiency is calculated as transfer efficiency and considers the first trophic level, the sum of producers and the detritus pool. The total energy passed from the 1st to the 2nd trophic level as recycled material was similar in all treatments (12-14%). The trophic efficiency of the first trophic level was increased in N (6.4%), while it decreased in OAW (3.7%) and OAW+N (2.7%), when compared to the Ambient treatment (4.3%; Figure II-4). From the 1st to the 2nd trophic level, the trophic efficiency increased for N, while it decreased in the OAW and OAW+N treatment (Figure II-4).

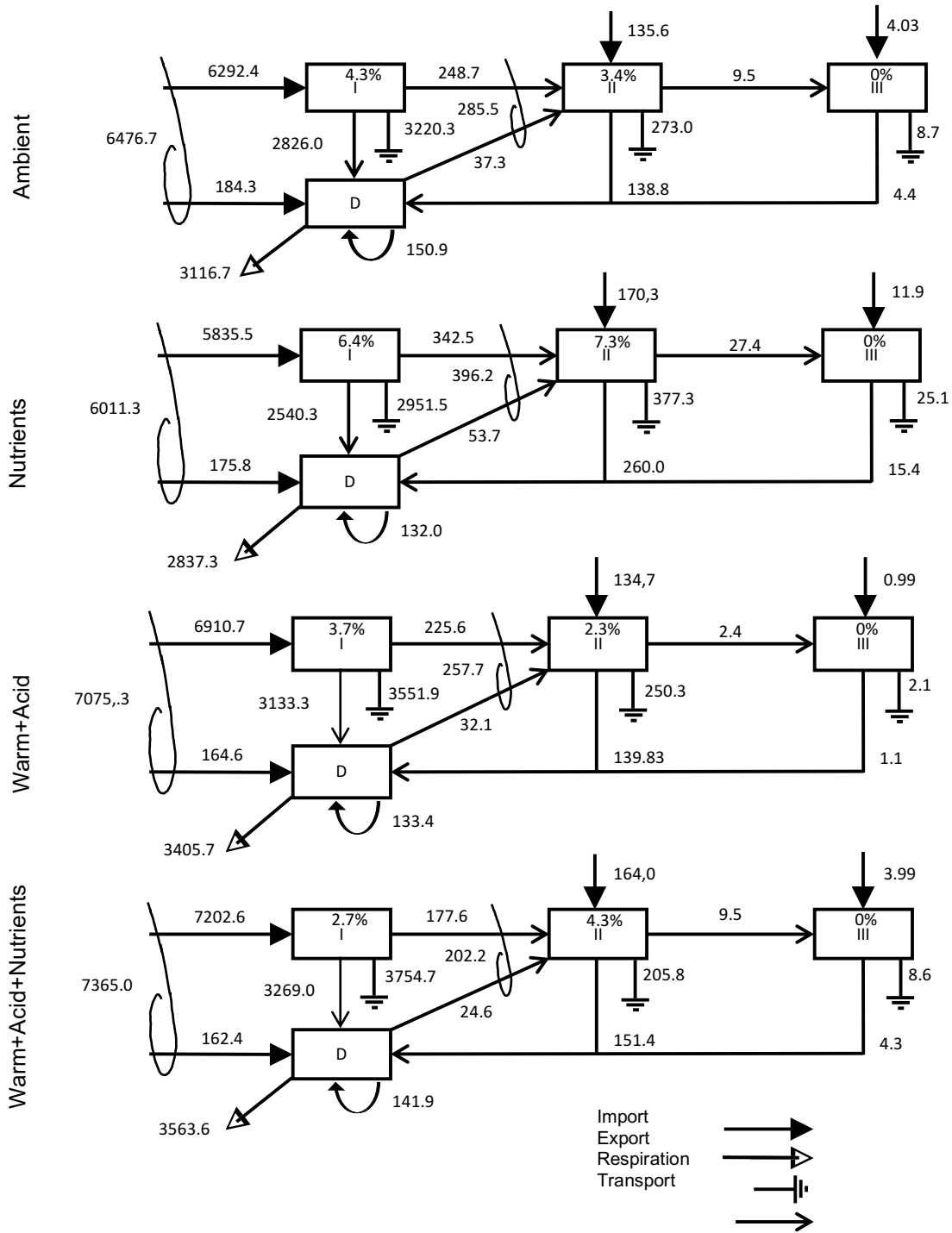


Figure II-4: Trophic aggregation of the investigated networks per treatment (Ambient, OAW (climate change, = ocean acidification and warming, N (nutrient enrichment) and OAW+N (the combination of climate change and nutrient enrichment)), based on three replicates (Lindeman spine). Shown are mean values for each treatment. Roman numbers represent the trophic levels and D the detritus pool. Flow rates are given in mgC per tank per day. The percentage values within the boxes represent trophic efficiencies for each trophic level (N=3).

Mean system trophic efficiency (TE) was lowest for OAW (3.22%), because of low energy transfer between trophic levels (Table 4). The trophic chain under N showed the

highest TE values (6.40%). Separating the detritus pool from the primary producers allows seeing the portions of herbivory and detritivory within the food web. While herbivory exceeded detritivory in all treatments, the relative amounts of herbivory were lower in OAW but enhanced in N and OAW+N (Table II-4).

The trophic depth (TD) was highest under N (3.05 effective trophic levels) and lowest for OAW+N (2.96 effective trophic levels) with intermediate values in the Ambient (3.02 effective trophic levels) and the OAW (3.00 effective trophic levels) treatments (Table II-4).

The main system characteristics of the ENA are given in Table II-4, while the most relevant attributes are presented in Figure II-5.

Ecological Network Analysis – System level properties

The highest total system throughputs (TSTs), representing activity of the system, were found for OAW and OAW+N in comparison to Ambient and N (Table II-4), with however, only significant differences between N and OAW+N (Table S5; Figure II-3). To compare different systems, ratios of relative overhead (RelOH) and relative ascendancy (RelA) represent further system attributes that characterize the degree of organization and specialization. These parameters have been widely used for spatial and temporal system comparisons (Wulff et al. 1989, Baird et al. 1991, Christian et al. 2005). RelA was highest in Ambient and N, indicating better-organized systems compared to OAW+N (Table S5; Figure 5). In contrast, RelOH was highest for OAW+N (47.1%) and lowest in the Ambient treatment (40.9%; Table S5; Figure II-5), indicating a higher capacity for adaptation and development for OAW+N. Considering internal exchanges only, the relative internal ascendancy (A_i/DC_i) was decreased in all 4 treatments compared to the A/DC ratio (Table II-4). Decreasing ratios indicate an increasing dependency of these systems on external exchanges (Baird *et al.* 1991), which indeed reflects the dependence of the “artificial” incubated systems on external imports.

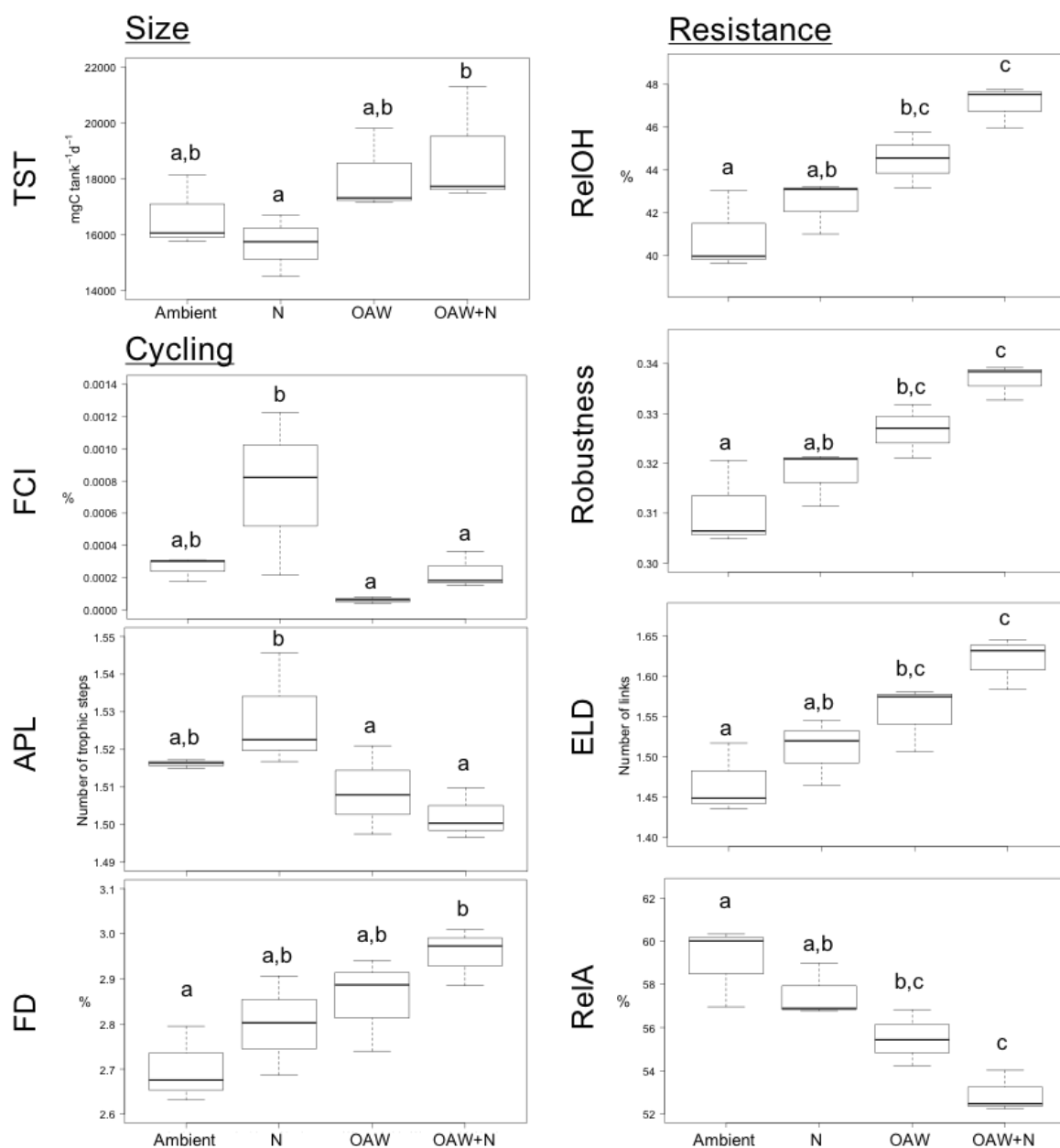


Figure II-5: System indices per treatment (Ambient, OAW (climate change, = ocean acidification and warming, N (nutrient enrichment) and OAW+N (the combination of climate change and nutrient enrichment)), based on three replicates. The boxplots represent the median, first and third quartile ("box") and 95% confidence interval of the median ("whiskers"). Treatments not sharing the same letter(s) are significantly different (post hoc tests). The network indices are shown as TST total system throughput; FCI Finn cycling index; APL average path length; FD flow diversity; RelOH relative overheads; Robustness; ELD effective link density; ReIA relative ascendancy.

Flow diversity (FD), combines the number of interactions and the evenness of flows in the systems, reflecting the diversity of interactions, and thus represents a good indicator of functional diversity within a system. Flow diversity was higher under OAW and OAW+N compared to Ambient conditions and N (Table II-4, S5; Figure II-4a). The same picture is

seen for connectance indices such as overall- and intercompartmental connectance (Table II-4). These indices increased in OAW, and even more so in N when compared to the Ambient treatment, indicating that single compartments of these systems were better connected (Table II-4).

Effective link density (ELD) was highest for OAW+N and lowest in the Ambient treatment, with intermediate values under N and OAW (Table II-4; Figure II-5). Here, a significant effect of climate change was found while no effect was found for nutrients and the interaction of nutrients and climate change (Table S5). Increasing ELD values indicate the better cooperation between the different compartments in a system.

The robustness index (R) ranged from 0.311 (Ambient treatment) to 0.337 (OAW+N; Table II-4) with a significantly increased robustness under OAW+N compared to Ambient and N (Table S5; Figure II-5).

The Finn cycling index (FCI) increased in N when compared to OAW and OAW+N (Table II-4, S5; Figure II-5). Compared to the FCI of the natural mussel bed, analyzed by Baird et al. 2007, much lower values were found in the investigated systems with values of about two magnitudes of order lower than in the field (Table II-4). This indicates that only a small fraction of the material was recycled within this mesocosm study, possibly due to the limited number of species and trophic levels (excluding e.g. fish and birds), resulting in a low number of cycles.

Discussion

We found an overall reduced autotrophic and heterotrophic biomass in treatments with increased CO₂ (reduced pH) and increased temperature, whereas the total system throughput (TST) was enhanced in these treatments, possibly due to an increased production to biomass ratio (P/B) of autotrophs under warming. Thus, in contrast to the overall reduced biomass under the applied climate change scenarios, without or with nutrient addition (OAW and OAW+N), these treatments had a high system activity. This confirms the theory that total biomass is reduced in compartments that survived the OAW showing a higher activity under the new conditions.

With the exception of *Balanus crenatus*, *Corophium sp.* and filamentous epiphytes, biomass of all other species investigated, as well as the total biomass of the system, decreased

under acidification and warming scenarios. The grazing species *Gammarus spp.* and *Littorina spp.*, which are known to mainly consume filamentous epiphytes (Jaschinski & Sommer 2010), were most vulnerable to the combination of acidification and warming. The same treatment massively increased the amount of filamentous epiphytes, whereas biomass of the macroalgae *F. vesiculosus* decreased. Thus, warming possibly decreased the grazer biomass directly, resulting in reduced grazing pressure on epiphytes, and hence a decreased biomass of macroalgae due to competition for nutrients and light with epiphytes (Worm & Sommer 2000; Werner *et al.* 2016b).

With ambient CO₂ and temperature but additional nutrients, epiphytes seemed to be top-down controlled by high numbers of grazers. This top-down control was found by Duffy and Harvilicz (2001) in a similar species assemblage. Filamentous epiphytes, *Corophium sp.* and *B. crenatus* benefitted most from acidification and warming. Increased biomass of *B. crenatus* seemed to be a direct response to warming. As ocean acidification is often negatively associated with the growth of shell forming species such as barnacles (Pansch *et al.* 2014), positive effects from warming must have compensated for the possibly negative effects from acidification. In this study, nutrient enrichment, as a regional driver, seems to mitigate negative impacts from climate change for some groups (*Gammarus*, *Littorina*, *Mytilus*).

Several studies demonstrated that the combination of global climate change (acidification and warming) and local anthropogenic drivers (nutrient enrichment) could have negative impacts on important marine systems (Crain *et al.* 2008; Wahl *et al.* 2011; Ban *et al.* 2014; Strain *et al.* 2015). First studies, however, suggest that the reduction of local drivers can improve the resilience of a system to global stress in the Mediterranean and Baltic Sea system (Strain *et al.* 2015; Werner *et al.* 2016a). For the North Sea, our results indicate that the combination of acidification and warming can threaten parts of the investigated species assemblage and thus, change the structure of the invested community.

Mitigation of climate change drivers by local environmental drivers such as nutrient enrichment could be reversed when these drivers are tested for longer periods or with higher nutrient loads, resulting in additive negative effects of climate change and nutrient enrichment as was shown by Werner *et al.* (2016a) for a Baltic Sea community. Furthermore, this parallel mesocosm experiment, with a *Fucus vesiculosus* community from the Baltic Sea, revealed a collapse of grazers and overgrowth of *F. vesiculosus* by epiphytes under increased CO₂ and temperature (Werner *et al.* 2016b), most likely due to extreme warm temperature during the

peak summer season. The low salinity of the Baltic system may have additionally lowered the general temperature tolerance of marine species. In comparison to Baltic Sea communities, intertidal Wadden Sea communities have to cope with higher temperature variations, and as a consequence, possibly evolved wider temperature tolerance limits (Compton *et al.* 2007). Tide pools, from where the investigated communities originated, can heat up to 28°C and more during summer (pers. observ.). Hence, the experimental community has possibly adapted to higher temperature fluctuations and, thus, was less affected by the applied warming temperature treatments. Thus, in combination with local nutrient enrichment, Wadden Sea communities might be able to better withstand future climate changes, when compared to Baltic Sea communities.

The ecological network analysis (ENA) revealed an increasing robustness under acidified, warmed and eutrophied conditions, i.e. systems that experienced the combination of global and local drivers. These systems appeared to be smaller (smaller ART and APL), but expressed a higher activity (larger TST and NPP). Because only the most adaptable and euryoecious species (= organism able to live under variable conditions) or genotypes survive under the particular treatment, this in addition leads to systems that are less specialized (lower RelA), and show an increasing system resilience under the costs of efficiency (lower TE, NPP efficiency and RelA). This could be understood as selecting process for those compartments in the system, having the highest adaption potential under the combination of global and local drivers.

After the definition by Fath (2015), a system is healthy and sustainable, when its robustness values are between 0.33 and 0.37 (Figure 6) and are thus in the range of the so-called “window of vitality”. This system can, thus, be characterized as a robust system with a strong integrity and a high resistance to external disturbance (Saint-Béat *et al.* 2015). In our study, all treatment combinations as well as the ambient treatment exceeded the upper limit of the window of vitality, i.e. had generally increased efficiency but were also more brittle when compared to field assessments (Baird *et al.* 2007; Baird & Ulanowicz 1989; Scharler & Baird 2005; Chen *et al.* 2011). We believe this finding is due to the simplified and artificial system and the short time period, we used in our study. Interestingly, however, robustness in our experiment increased with an increasing number of drivers/disturbances (nutrient enrichment < acidification and warming < acidification, warming and nutrient enrichment), with, however, a cost of efficiency.

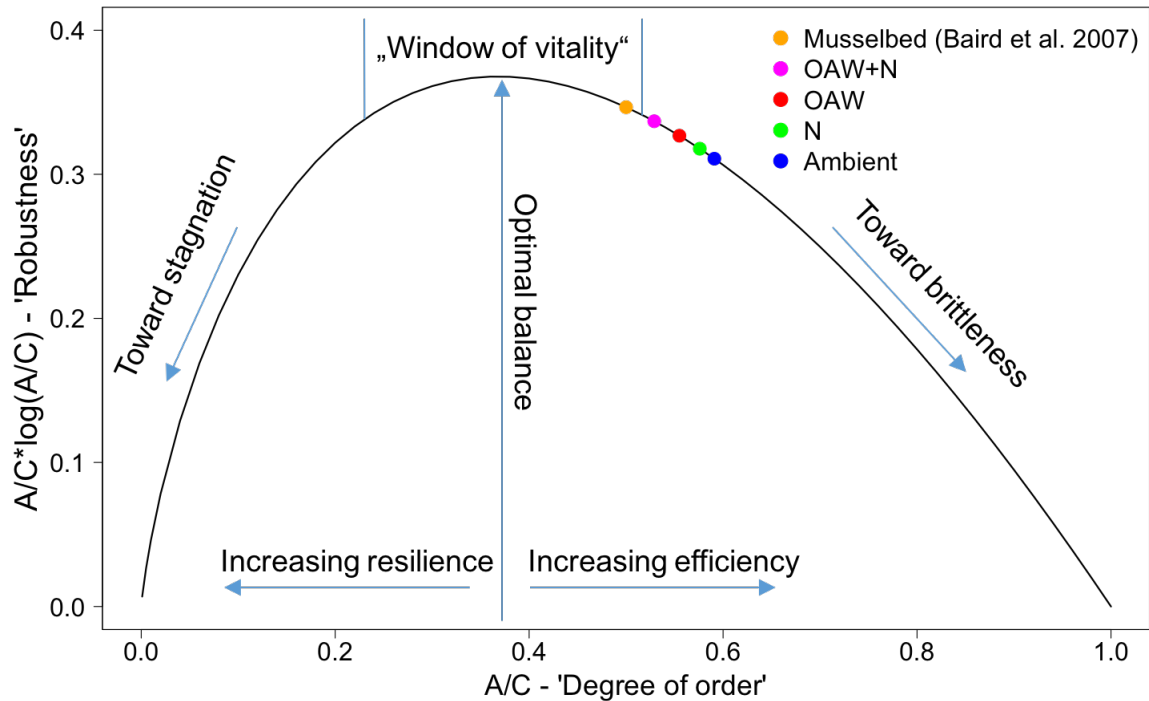


Figure II-6: Window of vitality modified after Fath et al. (2015). Represented are the robustness as a function of efficiency and resilience, shown for the different treatments (Ambient, OAW (climate change, = ocean acidification and warming, N (nutrient enrichment) and OAW+N (the combination of climate change and nutrient enrichment)), and the natural mussel bed.

Higher numbers of interactions, parallel pathways and better connected compartments (indicated by higher ELD and FD in this study), as well as increased relative overhead (RelOH) and decreased relative ascendancy (RelA) in acidified, warmed and eutrophied systems, indicate a higher grade of redundancy with a higher capacity for adaptation, and hence, a higher capability of the system to develop and withstand external disturbances (Heymans *et al.* 2002; Baird *et al.* 2004; Saint-Béat *et al.* 2015). For the Baltic Sea, Ulanowicz and Wulff (1991) concluded that a high Finn cycling index (FCI) and many long cycles (APL) indicate a more developed system for recycling, which might be less vulnerable than systems that recycle carbon over short trophic ways or over short periods. Higher FCI, APL and ART values in systems with ambient CO₂ and temperature indicate a higher magnitude in cycling, and longer cycles compared to acidified and warmed systems. Hence, these systems have a higher efficiency in retaining particulate matter and can be identified as less stressed (Monaco & Ulanowicz 1997; Heymans *et al.* 2002).

Conclusions

Our findings suggest that macroalgae-mussel beds of the temperate Wadden Sea are relative robust to warming, acidification and nutrient enrichment compared to other systems. As an example, open-ocean ecosystems, in which a wide range of global and regional factors, including acidification, warming, overfishing, stratifying surface waters as well as rising ultraviolet radiation, are driving complex changes in food web structure and community dynamics (Rockstrom *et al.* 2009). Another example is that of coral reefs with dramatic changes for the ecosystem functioning as consequence of warming and acidification (Hoegh-Guldberg *et al.* 2007).

Our experimental results of an incubated intertidal macroalgae-mussel assemblage indicate a change in species composition, activity and connectance under future climate change, with possible changes for ecological functions of the system. Although simulated climate change, here applied as the combination of acidification and warming, decreased biomass of the majority of species, as well as overall biomass, the network analysis (ENA) indicates the simulated system, to be capable of withstanding external disturbances but showing a tendency of larger brittleness. While in our example system the potential for resilience seems to increase under the combination of acidification and warming, also other systems may become more resilient and thus may lose organization and specialization when being challenged. As specialization and organization is lost, the potential for a new organization of the community increases and thus the capability for resilience. It is also important to note that the response of an incubated model system to the applied treatments can only be compared to its control but not to the natural system that includes even more compartments and interacting factors at diverse temporal and spatial scales. The potential for resilience and robustness of communities increased under a stress regime, probably due to changes in the structural and functional identity of the particular community after such a stress impact, and the question arises whether this change would have an impact to further entire system elements that were not included in the experiment.

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Supplementary

Table S1: Mean concentrations of nitrite (NO₂⁻), nitrate (NO₃⁻), phosphate (PO₄⁻) and silicate (SiO₄⁻) of the natural mean nutrient concentration over the past 7 years (2006 - 2013) within the Sylt-Rømø Bight (unpublished data), and enriched nutrient concentration calculated for the nutrient treatment over the respective months July, August and September used in the experiment. Nutrient enrichment comprised a doubling of the natural nutrient concentration.

	Nutrient concentrations [$\mu\text{mol L}^{-1}$]					
	July		August		September	
	7y mean	enriched	7y mean	enriched	7y mean	enriched
NO₂⁻	0.08	0.17	0.05	0.09	0.17	0.34
NO₃⁻	0.53	1.06	0.34	0.67	1.69	3.39
PO₄⁻	0.31	0.62	0.39	0.79	0.44	0.88
SiO₄⁻	1.70	3.41	2.01	4.03	3.26	6.53

Table S2: Biomass and energetics of all 12 community compartments (#) in the flow networks per treatment based on three replicates. Biomass and standing stocks in mg C tank⁻¹; GPP, NPP, Consumption (Cons.), Production (Prod.), Respiration (Resp.) and Egestion in mg C tank⁻¹ d⁻¹.

#	Parameter	Ambient			N			OAW			OAW+N			
		R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	
1	Phyto-plankton	Biomass	164.11	214.07	218.64	220.43	275.15	473.52	98.81	141.44	238.76	263.51	206.42	488.61
		GPP	69.012	126.027	180.205	92.695	161.991	390.286	58.170	116.575	275.505	155.135	170.138	563.811
		NPP	39.022	71.260	101.894	52.413	91.595	220.681	32.891	65.916	155.779	87.718	96.201	318.797
		Resp.	30.004	54.791	78.345	40.300	70.427	169.679	25.290	50.682	119.777	67.446	73.968	245.120
2	Epi-phytes	Biomass	10760	6237	2442	2500	4248	1082	30486	17228	16023	27958	34449	21277
		GPP	302.43	245.40	134.54	70.26	167.16	59.63	1199.60	949.05	1235.78	1100.13	1897.76	1640.97
		NPP	143.10	116.12	63.66	33.25	79.09	28.22	567.60	449.05	584.72	520.54	897.95	776.45
		Resp.	159.33	129.29	70.88	37.02	88.06	31.42	632.00	500.00	651.06	579.59	999.81	864.53
3	Macro-phytes	Biomass	192161	229141	204101	200345	196429	181176	136748	167972	139817	133497	170355	143577
		GPP	5401.0	9016.6	11243.8	5631.0	7729.4	9980.9	5381.0	9253.5	10783.4	5253.0	9384.7	11073.4
		NPP	2555.6	4266.3	5320.1	2664.4	3657.2	4722.6	2546.1	4378.4	5102.3	2485.5	4440.5	5239.5
		Resp.	2845.5	4750.3	5923.6	2966.6	4072.1	5258.3	2834.9	4875.1	5681.1	2767.5	4944.2	5833.9
4	Littorina spp.	Biomass	8102.8	7206.1	7347.6	8675.7	8645.9	8775.9	5310.7	6032.3	5489.7	6041.2	6086.6	7739.2
		Prod.	15.150	18.863	26.927	16.221	22.632	32.161	13.901	22.106	28.165	15.813	22.305	39.706
		Resp.	52.118	64.890	92.631	55.803	77.856	110.637	47.822	76.049	96.891	54.400	76.734	136.595
		Egestion	100.11	124.65	177.93	107.19	149.55	212.52	91.86	146.08	186.11	104.50	147.39	262.38
		Cons.	167.51	208.56	297.72	179.35	250.23	355.59	153.70	244.42	311.41	174.84	246.62	439.02
5	Corophium sp.	Biomass	0.6108	0.7635	0.0100	6.8715	0.0100	0.0100	2.6977	3.0031	0.0100	0.7635	3.1558	64.6939
		Prod.	0.0025	0.0044	0.0001	0.0284	0.0001	0.0001	0.0156	0.0243	0.0001	0.0044	0.0256	0.7341
		Resp.	0.0119	0.0208	0.0004	0.1340	0.0003	0.0004	0.0736	0.1148	0.0005	0.0208	0.1206	3.4615
		Egestion	0.0029	0.0050	0.0001	0.0324	0.0001	0.0001	0.0178	0.0277	0.0001	0.0050	0.0292	0.8367
		Cons.	0.0173	0.0303	0.0006	0.1948	0.0004	0.0006	0.1071	0.1669	0.0008	0.0303	0.1753	5.0323
6	Gammarus spp.	Biomass	1107.9	735.7	1151.0	2972.6	1527.0	4041.5	175.2	127.1	224.0	434.7	636.9	1038.4
		Prod.	6.1728	5.7385	12.5689	16.5614	11.9106	44.1327	1.3665	1.3885	3.4239	3.3906	6.9545	15.8744
		Resp.	29.229	27.172	59.514	78.419	56.397	208.970	6.471	6.574	16.212	16.054	32.930	75.166
		Egestion	9.8923	9.1964	20.1425	26.5407	19.0875	70.7256	2.1900	2.2251	5.4870	5.4336	11.1450	25.4398
		Cons.	45.373	42.181	92.387	121.733	87.548	324.395	10.041	10.041	10.041	24.922	51.118	116.684
7	Mytilus edulis	Biomass	2599.8	3196.6	2898.2	2670.7	2275.3	3547.2	966.1	164.1	393.1	85.8	0.1	2417.0
		Prod.	2.5641	4.4137	5.6024	2.6340	3.1416	6.8570	1.3339	0.3173	1.0639	0.1185	0.0002	6.5412
		Resp.	14.103	24.276	30.814	14.487	17.280	37.715	7.337	1.745	5.851	0.652	0.001	35.978
		Egestion	2.3549	4.0536	5.1453	2.4191	2.8853	6.2975	1.2251	0.2914	0.9771	0.1088	0.0002	6.0075
		Cons.	19.022	32.744	41.562	19.540	23.307	50.869	9.896	2.354	7.892	0.879	0.001	48.527
8	Magallana gigas	Biomass	13223	20552	10280	12096	31765	15330	7262	2704	21537	6326	7788	5852
		Prod.	13.050	28.398	19.886	11.939	43.891	29.656	10.035	5.231	58.327	8.741	15.066	15.849
		Resp.	171.90	374.05	261.93	157.25	578.12	390.62	132.17	68.90	768.27	115.13	198.44	208.77
		Egestion	10.542	22.940	16.064	9.644	35.455	23.956	8.106	4.226	47.116	7.061	12.170	12.803
		Cons.	195.49	425.39	297.88	178.84	657.46	444.23	150.31	78.36	873.71	130.94	225.68	237.42
9	Balanus crenatus	Biomass	3.7310	13.3250	0.5330	0.5330	1.5990	10.6600	18.1220	26.6500	78.8840	26.1170	60.7620	32.5130
		Prod.	0.0124	0.0621	0.0035	0.0018	0.0075	0.0696	0.0845	0.1739	0.7208	0.1218	0.3966	0.2971
		Resp.	0.0323	0.1617	0.0091	0.0046	0.0194	0.1811	0.2199	0.4527	1.8760	0.3169	1.0322	0.7732
		Egestion	0.0050	0.0249	0.0014	0.0007	0.0030	0.0279	0.0338	0.0696	0.2885	0.0487	0.1588	0.1189
		Cons.	0.0497	0.2487	0.0139	0.0071	0.0298	0.2785	0.3382	0.6963	2.8854	0.4874	1.5875	1.1892
10	Suspended POC Biomass	185.10	131.54	236.30	150.05	280.02	98.07	57.89	367.06	68.92	267.02	108.70	110.67	
11	Sediment POC Biomass	2121.6	3107.7	1818.7	2651.4	1932.4	1991.0	2486.2	974.2	1819.3	2460.6	1666.1	1506.8	
12	DOC Biomass	63.729	105.463	133.149	67.056	97.626	150.186	70.496	113.028	152.685	82.051	130.819	200.018	

Table S3: Diet matrices of predator and prey species taken from Baird et al. (2007) over different compartments (#). Species and numbers in blue indicate parts, which were not sampled and hence not included in the network analysis, but were treated as system imports.

Prey		Phytoplankton	Zooplankton	Microphytobenthos	Epiphytes	Macrophytes	Bacteria water column	Bacteria Sediment	Meiofauna	Macrofauna	Suspended POC	Sediment POC	DOC
#	Predator	1	-	-	2	3	-	-	-	4 to 7	10	11	12
4	<i>Littorina spp.</i>			0.800	0.100	0.100							
5	<i>Corophium sp.</i>			0.500	0.250			0.125				0.125	
6	<i>Gammarus spp.</i>			0.200	0.100	0.250		0.100		0.250		0.100	
7	<i>Mytilus edulis</i>	0.850					0.010				0.140		
8	<i>Magallana gigas</i>	0.850					0.010				0.140		
9	<i>Balanus crenatus</i>	0.500	0.500										

Table S4: Abbreviations and descriptions of all parameters derived from the ecological network analysis (ENA).

ENA-indices	Description	Reference
A	The ascendancy (A) integrates the size and organization of the system flows and describes its efficiency and performance. High productivity of the system and complex trophic structure increase the system ascendancy. It is defined as the product of TST and average mutual information (AMI)	Ulanowicz 2004
APL	The average path length (APL) is defined as the average number of compartments that a unit of carbon passes through from its entry into the system until its leaving, which is calculated as $(TST-Z)/Z$, where Z is the sum of all exogenous inputs. The APL should increase with increasing flow diversity and cycling within the system.	Baird et al. 1991 Christensen 1995
AMI	The average mutual information (AMI), corresponds to the organization of the flows inherent in a system and is defined as the normalized ascendancy (A/TST).	Ulanowicz 2004
ART	The average residence time (ART) of energy in the system is calculated by the ratio of the total system biomass and the sum of export and respiration. It gives the time, a unit of energy needs from entering until leaving the system.	Christensen 1995
DC	The upper limit of A is defined by the development capacity (DC) and represents the potential of the system to develop. It is defined as the product of TST and the FD, which is quantified as the number of interactions and the evenness of flows in the system.	Christensen 1995
ELD	The effective link density (ELD) represents the average number of links flowing into or out of a compartment.	Ulanowicz 2004
FCI	The Finn cycling index (FCI) is quantifying the importance of cycling within the system and represents the fraction of flow, which is recycled.	Finn 1976
FD	The flow diversity (FD) is quantified as the number of interactions and the evenness of flows in the system.	MacArthur 1955
OH	The difference of $C - A$ is defined as the system overhead (OH), which represents the cost of the system to operate in the way it does.	Ulanowicz 2004
RelA	The relative ascendancy (RelA) is the organized fraction of the DC.	Wulff et al. 1989
RelOH	The relative overhead (RelOH) is the non organized fraction of the DC. High RelOH indicating a high system resilience and a high capacity to adapt to system perturbations.	Heymans et al. 2002, Ulanowicz 2004
Robustness	The Robustness (R) represents the tradeoff between organisation and redundancy of the system and therefore measures and assesses the necessary configuration for sustainability of the system.	Goerner et al. 2009, Fath 2015
TD	The trophic depth (TD) represents the effective number of trophic levels in a system. Systems with a high number of trophic levels is expected to be more specialized.	Ulanowicz 2004
TE	The system trophic efficiency (TE) is defined as the logarithmic mean of the single trophic efficiencies, i.e. the efficiency of transfer from one trophic level to the next.	Wulff et al. 1989, Baird et al. 2004
TST	The total system throughput (TST) represents the sum of all flows through the system and indicates the activity within the system.	Ulanowicz 2004

Table S5: Factorial ANOVA results for biomass and all ENA indices. Significance levels are indicated by *P < 0.05, **P < 0.01, ***P < 0.001, or by ‘.’, when close to significance.

System indices	Treatment	Df	Mean Sq.	F value	P	
Total Biomass	Nutrients	1	25020962	0.0731	0.7938	
	Climate change	1	6392962368	186.71	0.0025	**
	Nutrients * Climate change	1	227088960	0.6632	0.4389	
Biomass Heterotrophs	Nutrients	1	27127229	0.5207	0.4911	
	Climate change	1	610797760	117.23	0.0090	**
	Nutrients * Climate change	1	74022481	14.208	0.2674	
Biomass Autotrophs	Nutrients	1	104253830	0.3372	0.5774	
	Climate change	1	3051643180	98.716	0.0138	*
	Nutrients * Climate change	1	560415804	18.129	0.2151	
TST	Nutrients	1	41531	0.0173	0.8987	
	Climate change	1	16043321	6.6685	0.0325	*
	Nutrients * Climate change	1	2284768	0.9497	0.3583	
APL	Nutrients	1	0.00002357	0.2256	0.6475	
	Climate change	1	0.00084089	8.0503	0.0219	*
	Nutrients * Climate change	1	0.00026084	2.4971	0.1527	
FCI	Nutrients	1	3.3214e-07	4.7862	0.0601	.
	Climate change	1	3.9478e-07	5.6887	0.0442	*
	Nutrients * Climate change	1	7.7640e-08	1.1187	0.3211	
ELD	Nutrients	1	0.0089445	5.6420	0.0449	*
	Climate change	1	0.0290507	18.324	0.0027	**
	Nutrients * Climate change	1	0.0004077	0.2572	0.6258	
Capacity	Nutrients	1	15660202	0.0339	0.8585	
	Climate change	1	594807631	12.875	0.2894	
	Nutrients * Climate change	1	34930145	0.0756	0.7903	
Ascendancy	Nutrients	1	761196	0.0049	0.9457	
	Climate change	1	72536912	0.4712	0.5118	
	Nutrients * Climate change	1	7549408	0.0490	0.8303	
AMI	Nutrients	1	0.00001633	0.0208	0.8888	
	Climate change	1	0.00229633	29.268	0.1255	
	Nutrients * Climate change	1	0.00100833	12.852	0.2898	
Robustness	Nutrients	1	0.00025153	52.431	0.0513	.
	Climate change	1	0.00103528	215.79	0.0017	**
	Nutrients * Climate change	1	0.00000324	0.0676	0.8014	
Overheads	Nutrients	1	23326611	0.2753	0.6140	
	Climate change	1	251914787	29.728	0.1230	
	Nutrients * Climate change	1	10001787	0.1180	0.7400	
TD	Nutrients	1	0.0000806	0.0230	0.8833	
	Climate change	1	0.0099475	28.365	0.1306	
	Nutrients * Climate change	1	0.0044275	12.625	0.2938	
Relative Overheads	Nutrients	1	13.671	57.167	0.0438	*
	Climate change	1	56.044	234.36	0.0013	**
	Nutrients * Climate change	1	0.615	0.2573	0.6257	
Relative Ascendancy	Nutrients	1	13.671	57.167	0.0438	*
	Climate change	1	56.044	234.36	0.0013	**
	Nutrients * Climate change	1	0.615	0.2573	0.6257	
ART	Nutrients	1	0.003	0	0.9948	
	Climate change	1	209	36	0.0937	.
	Nutrients * Climate change	1	0.418	0.0072	0.9342	
Redundancy	Nutrients	1	2611560	0.2680	0.6187	
	Climate change	1	18007255	18.483	0.2111	
	Nutrients * Climate change	1	1838562	0.1887	0.6755	
Relative Redundancy	Nutrients	1	147.741	26.640	0.1413	
	Climate change	1	0.37789	0.6814	0.4330	
	Nutrients * Climate change	1	0.19304	0.3481	0.5715	
Relative internal Redundancy	Nutrients	1	9.889	13.254	0.2829	
	Climate change	1	58.973	79.041	0.0228	*
	Nutrients * Climate change	1	9.853	13.207	0.2837	
TE	Nutrients	1	76.944	34.993	0.0983	.
	Climate change	1	108.254	49.233	0.0573	.
	Nutrients * Climate change	1	34.779	15.817	0.2440	
FD	Nutrients	1	0.030369	29.938	0.1218	
	Climate change	1	0.081777	80.616	0.0218	*
	Nutrients * Climate change	1	0.000013	0.0013	0.9718	

Chapter II

Figure S1: Environmental parameters (salinity (a), pH_{NBS} (b), Temperature (c) and total alkalinity (d)) of weekly measurements over the 10-weeks experiment. Shown are means values (\pm standard deviation) per treatment based on three replicates as well as measurements performed of the inflowing seawater.

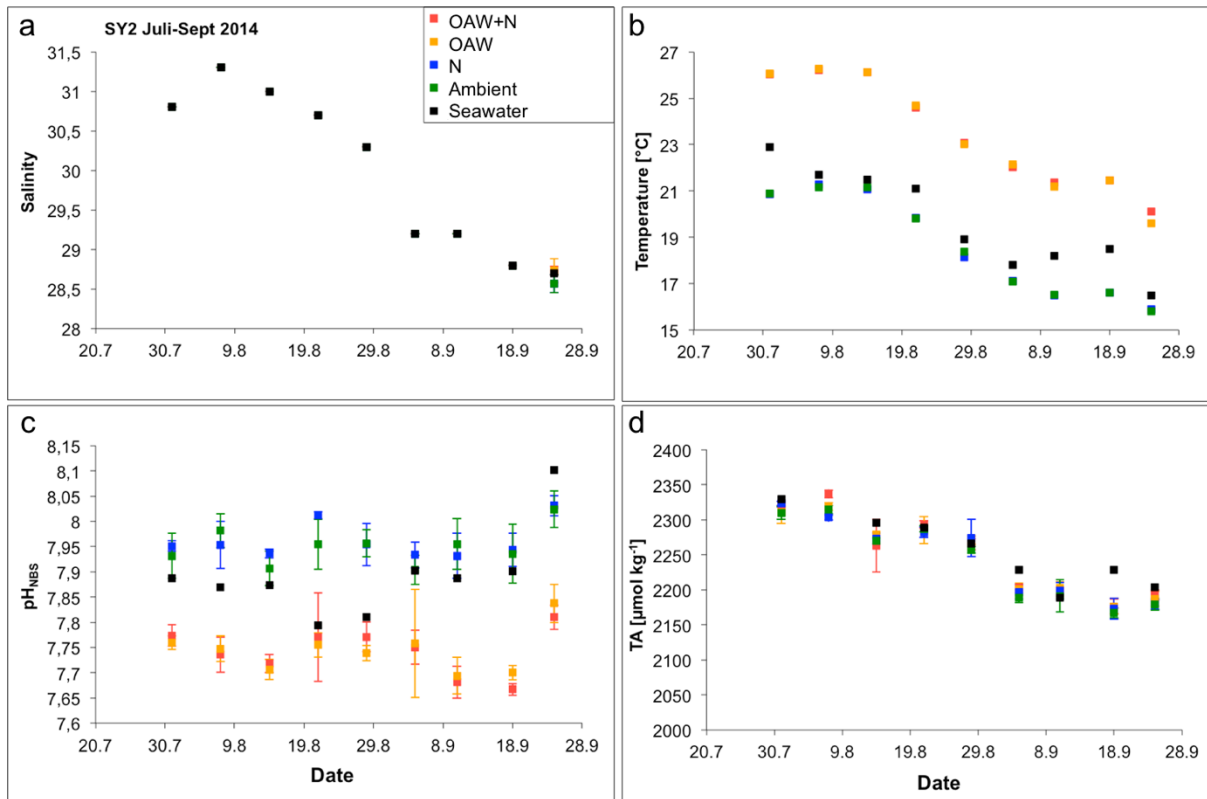
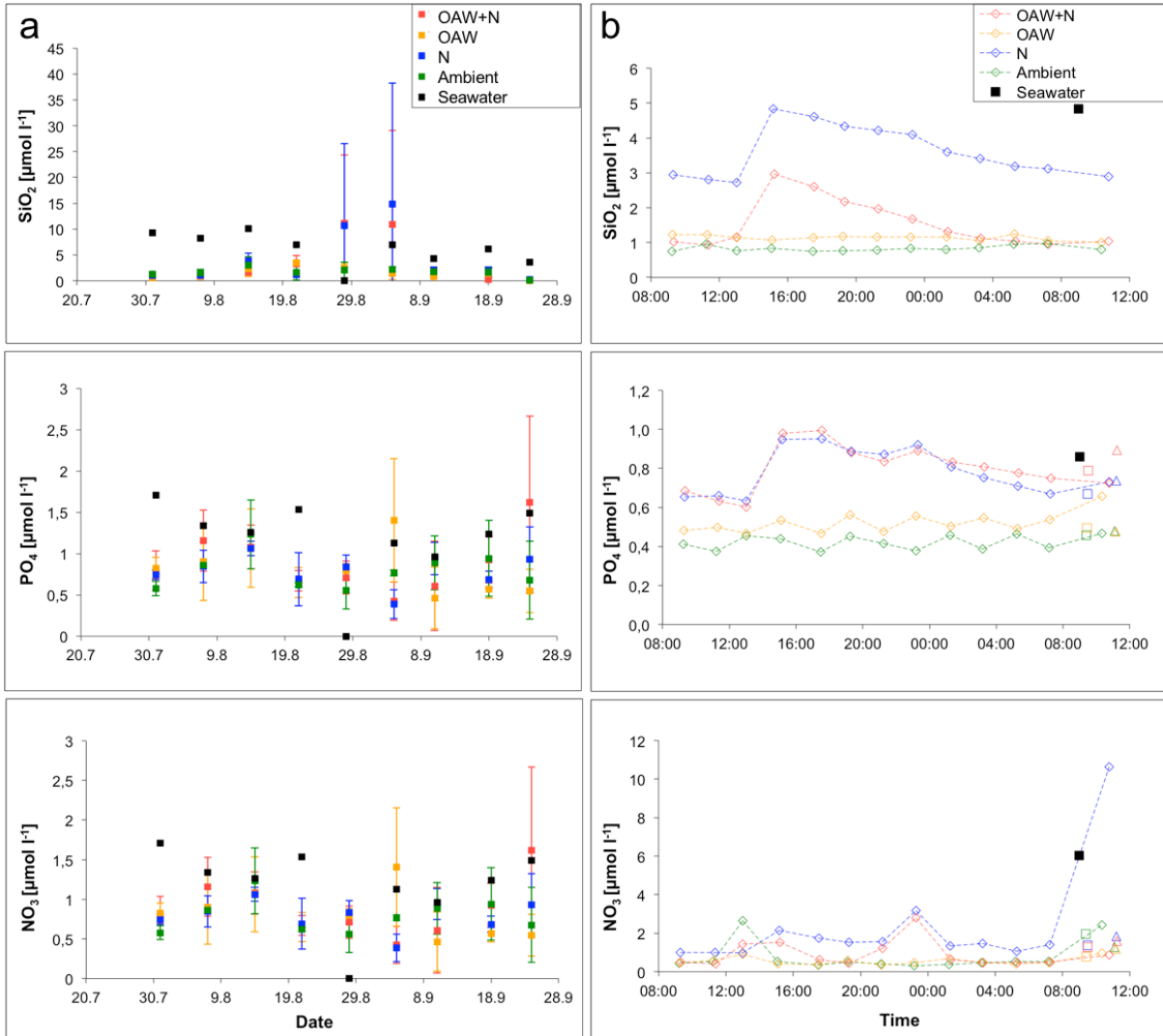


Figure S2: Silicate (SiO_2), phosphate (PO_4) and nitrate (NO_3) concentrations measured weekly at 9 o'clock, after nutrients were manually added a day before at 3:00 p.m., over the 10-weeks experiment (a). Silicate, phosphate and nitrate concentrations measured every other hour during a 24 h sampling campaign at the end of the experiment on the 23rd of September (b). Shown are means values (\pm standard deviation) per treatment based on three replicates as well as measurements of the inflowing seawater.



Chapter III

Strong seasonal differences in the impacts of climate change on a temperate macrophyte-mussel community.

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Abstract

Ocean acidification and global warming are known as two of the most crucial factors impacting marine ecosystems worldwide. Most projections of ecosystem changes are derived from short-term experiments on single species and single drivers, ignoring inter-seasonal differences in the responses. We tested the responses of a temperate intertidal macrophyte-mussel community to the combined effects of elevated seawater temperature and CO₂ concentration. In three consecutive experiments, in spring, summer and autumn, quantitative samples of the epibenthos were incubated for 8 to 11 weeks within a large-scale mesocosm facility (Sylt Benthic Mesocosm). Carbon flows within the food web were analysed through a holistic, static modelling approach (Ecological Network Analysis, ENA). We observed strong interactive effects of ocean warming and acidification on the macroalgae-mussel community, particularly on primary producers, filter feeders and grazers, with a strong dependency on the respective season. Warming and acidification in combination reduced energy flows during summer from the first trophic level to the second and further up the food chain, while this treatment increased energy transfer efficiency during autumn and spring. In summer, warming and acidification reduced biomass of omnivorous *Gammarus spp.* greatly with strong implications for the primary producers, due to a reduced grazing potential and a lowered top down control, resulting in overgrowth of *Fucus vesiculosus forma mytili* by epiphytes. Warming and acidification jointly enhanced primary production in all seasons. In summer, the increased energy was constrained to the food web base. The ENA revealed that warming and acidification jointly reduced the organization within the system during summer, resulting in an increased capacity of the system to develop. In this way, the capability of the system to adapt to environmental changes could be improved. Our experimental results show a strong seasonal variability of transfer efficiency with increased energy transfer efficiency in spring and autumn in comparison to reduced energy transfer efficiency in summer. For systems close to their upper thermal tolerance limits, our experimental results indicate a hampered energy flow to higher trophic levels and altered producer-consumer interactions, potentially resulting in a loss of important key functions of temperate macrophyte-mussel communities.

Introduction

Over the last decade, extensive research has provided valuable information about the impacts of ocean warming and acidification, and there is a clear consensus that a persistent upward trend in these factors will change structure and functioning of ecosystems and entire food webs (Hoegh-Guldberg & Bruno 2010; Walther 2010; Doney *et al.* 2012). Increasing seawater temperatures affect the performance and growth of many species directly through the acceleration of metabolic rates and respiratory demands (Brey 2010), resulting in less energy for important life-supporting activities such as feeding, somatic growth, reproduction, and defence mechanisms (Kroeker *et al.* 2010). Consequently, distribution and abundance of many marine key species will change (Perry *et al.* 2005; Doney *et al.* 2009; Poloczanska *et al.* 2016). Ocean acidification can raise the energetic costs connected with calcification and acid-base regulation (Hannisdal *et al.* 2012), and can harm neural functioning of marine organisms (Briffa *et al.* 2012), leading to altered physiology (e.g. Waldbusser *et al.* 2011; Gao *et al.* 2012; Havenhand 2012; Wahl *et al.* 2017), phenology and adaptability (Calosi *et al.* 2013; Kelly & Hofmann 2013), as well as shifts in distribution (Waldbusser *et al.* 2011).

However, the measured species-specific responses to climate drivers are variable between life-history stages of a species, they depend on the geographical region as well as on the investigated season (e.g. Kroeker *et al.* 2013; Wahl *et al.* 2016). Furthermore, the responses of marine organisms to warming and acidification are linked through biological interactions (e.g., predator-prey relationships, competition, and mutualism), affecting the energy flow between trophic levels (Van der Putten *et al.* 2010). For example, a decreased feeding performance due to climate change can reduce energy flow as well as the energy transfer efficiency between primary producers and consumers with further implication for higher trophic levels (Ullah *et al.* 2018). Possible indirect effects on primary production due to an increased top-down control can impact species persistence and diversity under global change, which are likely to vary with seasons (O'Connor *et al.* 2009a; Walther 2010; Werner *et al.* 2016b). The distinction between direct and indirect effects of warming and acidification on the experimental community is necessary to forecast and manage the effects of global change under future scenarios (Stewart *et al.* 2013).

Ocean warming and acidification will have detrimental effects for many marine organisms and beneficial effects for others. To better predict the responses of marine organisms and ecosystems to global climate change, it is crucial to reduce the uncertainty

around these projections (Boyd *et al.* 2018). It has become more and more evident that factorial manipulations and experiments at the community level are essential to understand how climate drivers such as warming and ocean acidification may impact diversity and functioning of marine ecosystems. Within this framework, however, most studies to date disregard the seasonal variability of global change effects on the investigated species (Wahl *et al.* 2016). Fluctuations of environmental parameters occur on multiple time scales from hours to months to seasons to years (Gunderson *et al.* 2016), with changing temperature, solar radiation and freshwater input. In temperate regions, seasonality might be one of the main covariate for ecosystem responses to environmental changes. The responses to changes in temperature and acidification levels might vary and/or co-vary in strength and direction between seasons, in accordance to growth and reproduction of marine organisms due to their different life-history stages and metabolic rates (Godbold & Solan 2013).

A promising tool for climate change experiments are mesocosms, with the possibility to capture species interactions and indirect effects under near natural, but controlled, conditions (Stewart *et al.* 2013; Pansch *et al.* 2016; Boyd *et al.* 2018). In this study, sections of an intertidal epibenthic macrophyte-mussel bed community from the Wadden Sea, were incubated in a near natural mesocosm experiments (Pansch *et al.* 2016), across three seasons within one year. We tested the combined impacts of ocean warming and acidification, including natural seasonally environmental conditions (temperature and photoperiod), on the diversity, growth, biotic interactions and contribution to ecosystem functioning of this species assemblage. We followed the development of species under the different treatments for 8-11 weeks during spring, summer and autumn in 2014. A holistic ecosystem-modelling approach (Ecological Network Analysis - ENA) was used to analyse quantitative food web structures and pathways of energy flows (Ulanowicz 2004). We hypothesized that the effects of warming and acidification will develop over the experimental time but will vary strongly between the different seasons.

Materials and Methods

Study system and sampling site

For experimental incubations, samples were collected from a Wadden Sea mussel bed community covered with macroalgae from the Sylt-Rømø Bight, representing one of the few dominantly epibenthic communities in this area (Büttger *et al.* 2008). This study system is

situated at the south-eastern shore of the North Sea between the islands of Sylt, Germany and Rømø, Denmark, and the German/Danish mainland. This semi-enclosed basin is dominated by sandy substrates with an area of around 404 km² (Asmus & Asmus 1998). One third of the bight is intertidal with an average tidal range of 1.8 m. The sea surface temperature varies between -1°C in winter and 22°C in summer, and salinities between 28 and 32, depending on freshwater inflow and currents.

Mussel beds have a high ecological importance by providing important nursery ground, shelter, settlement substrate as well as feeding ground for amphipods (*Gammarus spp.*), shrimps (*Crangon crangon*), gastropods (*Littorina littorea* and *L. mariae*), slipper limpets (*Crepidula fornicata*), barnacles (*Balanus crenatus*) and many fish species (Nehls *et al.* 2006; Baird *et al.* 2007; Büttger *et al.* 2008). The blue mussel *Mytilus edulis* and the pacific oyster *Magallana gigas* (formerly known as *Crassostrea gigas*) dominate the heterotrophic biomass of the investigated system. *Fucus vesiculosus forma mytili* (hereafter *Fucus*), as dominant macrophyte, forms a dense cover on the mussels and oysters fixed by byssus threads of *M. edulis* (Büttger *et al.* 2008). The number of incubated specimens for this study were based on field abundance data quantified by counts per square meter, for the respective seasons.

Experimental facility

The experiments were conducted in the Sylt Benthic Mesocosms at the AWI Sylt, an outdoor facility consisting of 12 x 1.8 m³ round tanks, permanently exposed to natural light conditions, described in detail in Pansch *et al.* (2016). In all three experiments, the mesocosms were supplied with non-filtered seawater directly pumped from the Lister Deep, a tidal inlet in the Sylt-Rømø Bight only about 250 m from the AWI Wadden Sea Station, with a constant flow through of approximately 1.8 m³ per tank and day. Temperature in the tanks was simulated based on yearly and daily sinusoidal curves, adjusted by regular field measurements. Temperature simulation was achieved by external cooling units (Titan 2000/4000, Aqua Medic, Bissendorf, Germany) and internal heaters (Titanium heater 500 W, Aqua Medic, Bissendorf, Germany). In order to simulate ocean acidification, each tank was constantly and directly aerated with a pre-mixed gas of 1000 ppm CO₂, through a 60 cm air curtain. In order to control and monitor the physical parameters, each two tanks were equipped with a Multi Parameter Measurement System (MPMS; 4HJena, Jena, Germany).

Each MPMS was equipped with a Hydrolab DS5X multi-probe (OTT Messtechnik GmbH, Kempten, Germany) to log continuously temperature, conductivity, pH and dissolved oxygen. Additionally, samples for total alkalinity (TA), nutrients, chlorophyll a, and seston were taken manually every week. To provide most natural conditions, each tank was further equipped with a tidal simulation system, simulating changing water levels and currents during a tidal rhythm of 6 hours and 10 minutes, and a tidal range of 70 cm.

During the summer experiment, the mesocosm facility was temporarily shut down due to a lightning. Temperature regulation during this time was achieved by external temperature computers (T-Computer, Aqua Medic, Bissendorf, Germany). Water supply and CO₂ mixing facility were not affected as they worked independently from the mesocosm system. All other components were not impaired by the shutdown.

Seasonal mesocosm experiments

In order to determine the seasonal effects of climate change (ocean warming and acidification) on the investigated community, all experiments were performed within one year consecutively, from 10 April to 25 June (spring, 11 weeks), 17 July to 24 September (summer, 10 weeks) and from 16 October to 17 December 2014 (autumn, 8 weeks). A further experiment during wintertime was not possible due to frosty temperatures with possible damages for the system.

A part of a natural mussel-oyster bed covered by *Fucus* and its associated fauna were collected during low tide by hand in the Sylt-Rømø Bight (55°01'42.2"N, 8°26'01.1"E). For all experiments, the organisms were collected 2 days prior to the experimental start and kept in experimental containers (1800 L) at respective field conditions. *Fucus* thalli were rinsed with freshwater to remove motile fauna. Several thalli were bound to bundles, and weighed (Sartorius lc 4200s; 0.01 g; Sartorius, Göttingen, Germany). Afterwards, these bundles were fixed on the grating platform of each tank. The associated fauna (*M. gigas*, *M. edulis*, *Gammarus spp.*, *L. littorea* and *L. mariae*) were collected at the same time and location. After counting and weighing, specimens were distributed evenly to the experimental units (Table III-1). Mussels and oysters were put into small nets and open buckets, respectively, while snails and amphipods could move freely within the mesocosms.

Table III-1: Biomass and abundance of included species at the start of the experiments (means \pm standard deviation; wet weight (g); NA = no data collected).

Species	Spring		Summer		Autumn	
	Individuals	Wet weight	Individuals	Wet weight	Individuals	Wet weight
<i>Fucus vesiculosus</i> <i>forma mytili</i>	bundles	1428.9 \pm 78.9	bundles	1868.5 \pm 99.8	bundles	1568.8 \pm 40.8
<i>Magallana gigas</i>	4	259.3 \pm 59.9	4	416.1 \pm 112.7	4	233.4 \pm 59.7
<i>Mytilus. edulis</i>	16	138.2 \pm 12.6	12	145.2 \pm 8.4	12	114.3 \pm 8.7
<i>Gammarus spp.</i>	12	NA	38	NA	40	NA
<i>Littorina littorea</i>	70	164.2 \pm 3.7	85	238.1 \pm 4.3	70	191.7 \pm 3.9
<i>Littorina mariae</i>	20	4.8 \pm 0.6	15	3.2 \pm 0.5	32	9.8 \pm 0.5

At the end of the respective experiments, all organisms were collected from the tanks, counted and weighed (see above). Species introduced by inflowing seawater such as *Balanus crenatus* (entering as larval stages) and *Corophium sp.* were processed together with all other specimens. *Fucus* subsamples were taken to determine dry weight (DW) and ash-free dry weight (AFDW). Animals were dried for 24 hours at 60°C, and later combusted at 550°C for 6 hours to determine DW and AFDW. In order to determine suspended particular organic matter (SPOM), 250 ml water samples per tank were filtered on pre-combusted (450°C; 4 hours) Whatman GF/F glass fibre filters. To calculate the carbon content for the Ecological Network Analysis (ENA), conversion factors by Brey *et al.* (2010) were used. Concentrations of phytoplankton were determined by chlorophyll *a* measurements and converted to carbon content by using the following equation:

$$\text{mg C}_{\text{Phyto}} \text{ l}^{-1} = \mu\text{g Chl } a \text{ l}^{-1} * 50 * 1000^{-1}.$$

In order to test the effects of climate change and season, we used an ambient treatment (ambient temperature and ambient $p\text{CO}_2$) and a climate change treatment with the combined effects of warming and acidification (+5°C at 1000 ppm $p\text{CO}_2$). Treatments were chosen accordingly to climate change predictions for the year 2100 (Field 2014), with each treatment replicated three times.

Ecological Network Analysis

Community structure of the investigated system was analysed by Ecological Network analysis (ENA). For each replicate of the 2 treatments, a food web model was constructed and analysed by means of ENA as described by Baird *et al.* (2004). Each of the 18 models comprises 9 living and 3 non-living compartments and is based on biomass, energy budgets (production rates, diet composition and energy requirements), as well as on energy flows between the compartments. Biomass data were taken at the end of the experiment and

expressed as mgC per tank. Imports, exports and energy flows are given in mgC per tank and day. Energy budget data were taken from Baird *et al.* (2004). More details on the applied ENA can be obtained from Pansch *et al.* (submitted). The ENA was run with the R! Software package enaR (Borrett & Lau 2014).

Statistical analysis

For comparisons of biomass and ENA-indices, effect sizes of OAW were calculated to compensate for the different initial biomass values between the different seasons. For calculating the effect size, we chose log response ratio (LRR) as index since it quantifies the proportionate change that ensue from experimental manipulation and is often used for conducting meta-analysis of ecological studies (Osenberg *et al.* 1997, Hedges *et al.* 1999). LRR is defined as the dimensionless ratio of the OAW treatment over the ambient response value.

$$\text{LRR} = \text{Ln} \left[\frac{\text{Response (OAW)}}{\text{Response (Ambient)}} \right]$$

When the effect size (LRR) is positive, the response values under OAW increases compared to the ambient treatment, whereas a negative LRR value indicates negative impacts. An effect size with the 95% confidence interval not significantly different from zero (overlapping zero), indicates that OAW had no discernible effect on the specific response variables.

The various functional indices obtained from the enaR package, as well as biomass values, were compared by means of analysis of variance (ANOVA), with the independent variable climate change (warming and acidification). Prior to the analysis, data were tested for normality and homogeneity of variances, using Shapiro-Wilks and Levenes test, respectively, and were transformed if necessary. All statistical analyses were carried out using the R! Software (R 3.3.2 GUI 1.68).

Results

Seasonal variability in water temperature, pH and salinity

The water temperature in the ambient treatment followed the natural seasonal pattern with continuously rising mean temperatures within the spring-experiment (April to June; Table III-

2; Figure III-1). Maximal temperatures around 23°C were reached during the summer-experiment in the beginning of August. Temperature declined during the autumn-experiment to minimum values of around 5°C at the end of December. Mean temperature differences between the ambient and the warmed treatments were constantly at around 4.8°C (Figure III-1). The mean difference in pH_{NBS} between the ambient and the warm+acid treatment ranged from 0.15 to 0.21 pH_{NBS} units (Table III-2), with highest values during spring and lowest values in summer. Salinity followed natural conditions in the field.

Table III-2: Salinity, temperature (°C), pH_{NBS} and TA (μmol kg⁻¹) over the experimental time for each season (means ± standard deviation from three replicate tanks).

	Spring		Summer		Autumn	
	Ambient	Warm+ Acid	Ambient	Warm+ Acid	Ambient	Warm+ Acid
Salinity	28.82 ± 0.54	28.82 ± 0.54	29.99 ± 1.00	30.00 ± 0.98	28.63 ± 0.17	28.79 ± 0.23
Temperature	13.33 ± 3.28	18.33 ± 3.28	18.60 ± 2.11	23.40 ± 2.39	10.18 ± 2.26	14.95 ± 2.34
pH _{NBS}	8.126 ± 0.125	7.971 ± 0.101	7.950 ± 0.050	7.744 ± 0.055	7.961 ± 0.036	7.813 ± 0.044
TA	2306.1 ± 17.1	2301.2 ± 19.0	2238.2 ± 57.1	2246.2 ± 54.2	2281.1 ± 28.7	2282.2 ± 29.3

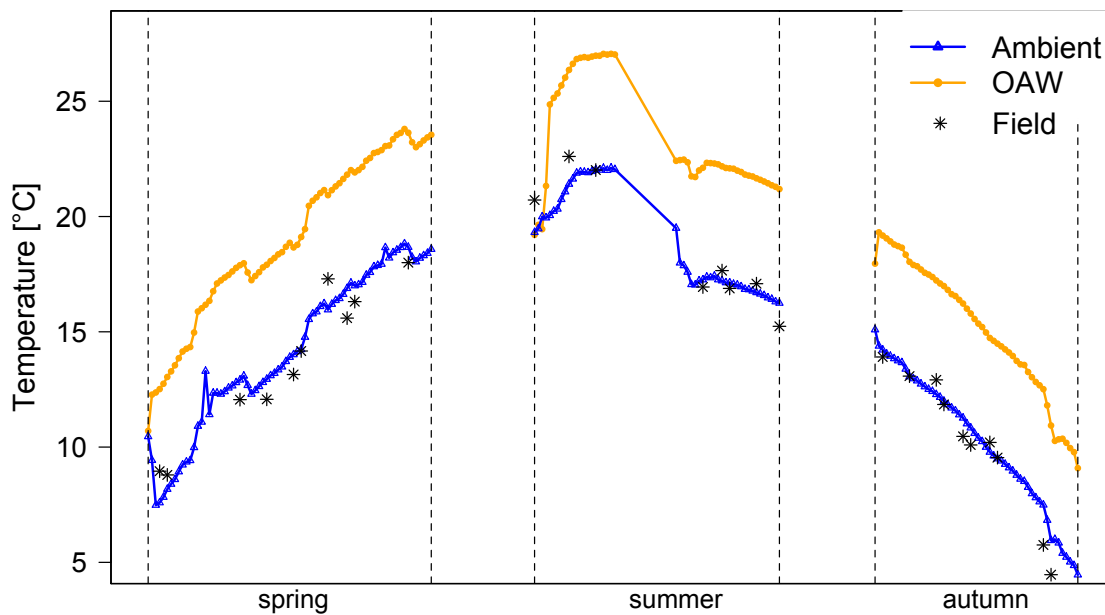


Figure III-1: Daily temperature values logged by the Multi Parameter Measurement System. Shown are temperatures of the Ambient and OAW (ocean acidification and warming) treatments as well as the field measurements.

Biomass of the investigated communities and the single compartments

By the end of the experiments, *Fucus* biomass in the warmed and acidified (OAW) treatment did not differ from control conditions in spring, but in summer and autumn (Figure III-2a).

Here, a decrease in *Fucus* biomass was observed under OAW, with the strongest decrease in summer (Figure III-2a). During the summer experiment, numerous epiphytes settled on *Fucus* and on the grating platform to which *Fucus* was attached, resulting in a strong increase in epiphytic biomass under OAW (Figure III-2a). Filamentous epiphytes were not detectable in spring and autumn, in both treatments. Phytoplankton biomass (measured as chlorophyll a) was not impacted by the treatments (Figure III-2a). Primary consumers responded to OAW depending on the season. During their early settling phase, biomass of the barnacle *Balanus crenatus* decreased under OAW during spring, and increased under OAW during summer (Figure III-2b). Biomass of both *Littorina* species was reduced under OAW during summer but not during spring and autumn, where both species showed a slight but not significant increase in biomass under OAW. While the blue mussel *Mytilus edulis* suffered from OAW in all seasons, the invasive pacific oyster *Magallana gigas* was not affected (Figure III-2b). The secondary consumer *Gammarus spp.* showed a strong seasonal variation with decreasing biomass under OAW during summer and a strong increase in biomass during autumn (Figure III-2c). Suspended POC and DOC increased under OAW during spring, while DOC as well in autumn (Figure III-2d). Sedimented POC showed no significant changes in all seasons (Figure III-2d).

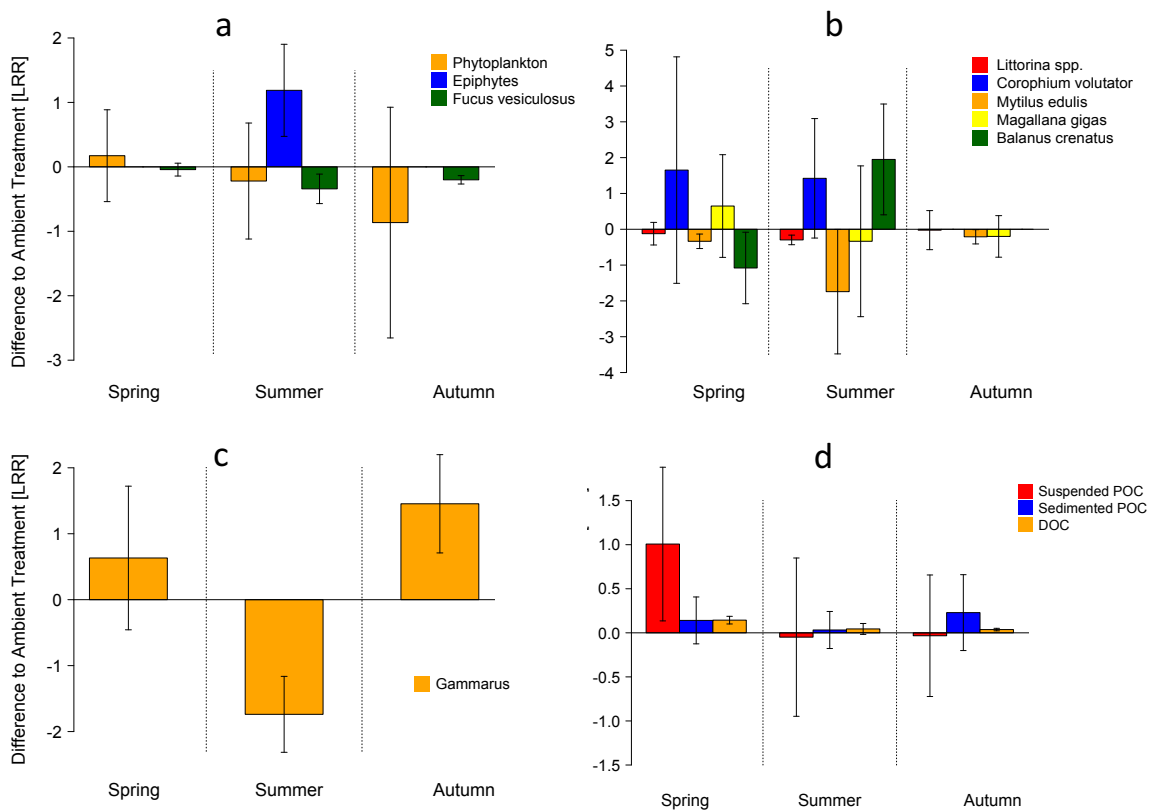


Figure III-2: Log response ratio (LRR) plot of biomass of single compartments at the end of the experiments conducted in spring, summer and autumn (differences of OAW compared to the ambient treatment; mean \pm 95% CIs). Log response ratios are shown across the different seasons for a) primary producers, b) primary consumers, c) secondary consumers and d) detritus. A LRR is statistically significant when the boot-strapped 95% confidence intervals do not overlap the zero line, and is statistically different from other LRR when 95% confidence intervals do not overlap.

Differences between the ambient and OAW treatment of the basic system characteristics are shown by log response ratios (LRR) in Figure III-3, with total biomass, as well as autotrophic and heterotrophic biomass, net primary production (NPP), consumption and daily production to biomass ratio (P/B ratio). In both treatments total biomass, as well as autotrophic biomass decreased at the end of the summer and autumn experiments, while heterotrophic biomass showed no significant differences under the interactive effects of warming and acidification (Figure III-3, Table III-3). Under OAW, NPP was enhanced during spring and autumn when compared to the ambient treatment. The daily P/B ratios increased under OAW in all investigated seasons, indicating a high turnover and activity under warming and enhanced CO_2 concentrations (Figure III-3). Differences in consumption rates due to warming and ocean acidification were masked by a high variability of rates in any season (Figure III-3).

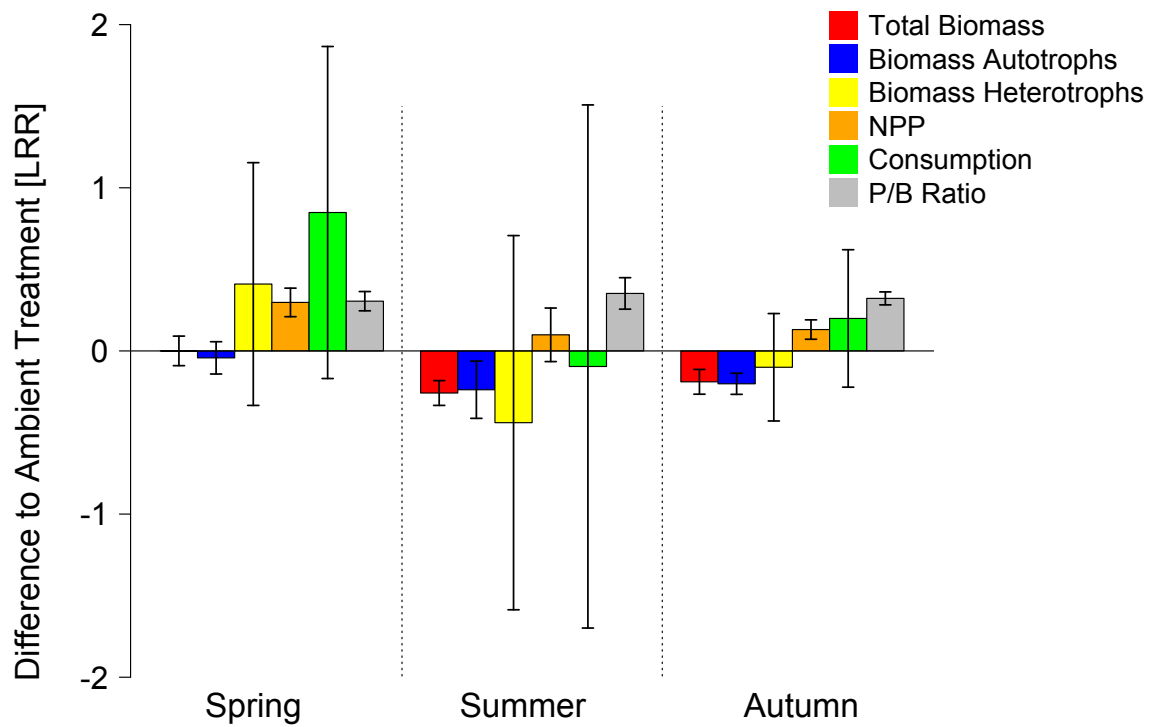


Figure III-3: Log response ratio (LRR) plot of the general system attributes at the end of the experiment showing differences between the climate change treatment (OAW, increased CO₂ and temperature) and the ambient treatment for spring summer and autumn in 2014. Shown are means \pm 95% confidence intervals. A LRR is statistically significant when the boot-strapped 95% confidence intervals do not overlap the zero line, and is statistically different from other LRR when 95% confidence intervals do not overlap.

Table III-3: General system attributes of the four different treatments. Shown are mean values of three replicates for the Ambient and OAW (ocean acidification and warming) treatment. Autotrophic biomass comprises phytoplankton, epiphytes and *F. vesiculosus forma mytili*. Heterotrophic biomass comprises *Magallana gigas*, *M. edulis*, *B. crenatus*, *Gammarus spp.*, *C. volutator*, *L. littorea* and *L. mariae*.

Attribute	Spring		Summer		Autumn	
	Ambient	OAW	Ambient	OAW	Ambient	OAW
Total biomass (mgC per tank)	246173.7	246240.2	241285.8	186422.8	122370.9	101263.3
Total biomass autotrophs (mgC per tank)	227349.8	217877.2	215145.9	169584.4	108430.4	88651.2
Total biomass heterotrophs (mgC per tank)	18823.9	28363.0	26139.9	16838.4	13940.5	12612.1
Biomass ratio autotrophs to heterotrophs	12.08	7.682	8.231	10.07	7.778	7.029
Net primary production (NPP; mgC per tank d ⁻¹)	3067.1	4129.1	2905.9	3207.6	1454.2	1657.7
Total heterotrophic production (mgC per tank d ⁻¹)	35.04	78.57	37.06	31.47	16.74	23.47
NPP efficiency (%)	5.394	9.419	8.471	7.324	9.312	9.999
Production to biomass ratio (P/B; d ⁻¹)	0.013	0.017	0.012	0.017	0.012	0.017
Consumption	165.5	386.5	248.1	225.6	135.8	165.7

Ecological Network Analysis

The trophic functions of a community are reflected by the average path length (APL) and the average residence time (ART; Table III-4; Figure III-4). OAW did not impact the APL in any season. This means for all treatments and seasons, that the energy is used over approximately the same number of steps before leaving the system. The ART strongly decreased under the combination of warming and acidification (Figure III-4). Hence energy and material reside for a shorter period in the OAW treatment. While in the Ambient treatments the mean ART was 35.7 days, the material resided in the OAW treatment only for around 25.4 days (Table III-4). The higher activities and turnover rates under higher temperature and CO₂ resulted in the shorter ART. The potential for recycling indicated by the Finn cycling index (FCI) varied with seasons. While in the summer experiment OAW decreased the FCI, in autumn the FCI was increased (Figure III-4), indicating that only a small fraction of the material is recycled during summer in comparison to spring and autumn.

In spring, the information based indices ascendancy (A), development capacity (DC) and overheads (OH) increases strongly under OAW, while only OH was slightly increased in summer and in autumn (Figure III-4).

Table III-4: System attributes and indices derived from ecological network analysis of the four different treatments. Shown are mean values of three replicates for each treatment. Mussel bed data from the field were retrieved from Baird et al. (2007).

System attributes	Spring		Summer		Autumn	
	Ambient	OAW	Ambient	OAW	Ambient	OAW
Trophic efficiency (TE; %)	5.57	7.38	3.69	1.76	1.25	2.86
Detrivory (mgC per tank per day)	27.0	66.5	37.5	32.1	20.4	24.6
Finn cycling index (FCI; %)	0.060	0.110	0.025	0.006	0.003	0.015
Average path length (APL)	1.50	1.53	1.49	1.51	1.55	1.55
Average residence time (ART; d)	36.5	25.3	36.4	25.9	34.2	25.0
Total system throughput (TST; mgC m ⁻² d ⁻¹)	16858.1	24654.7	16647.9	18087.8	9183.5	10369.4
Developmental capacity (DC; mgC tank ⁻¹ d ⁻¹)	40868.6	67242.1	48929.8	51554.6	24461.3	27998.0
Overheads (OH; mgC m ⁻² d ⁻¹)	14537.2	27818.9	20075.6	22920.2	9356.7	11005.3
Ascendancy (A; mgC m ⁻² d ⁻¹)	26331.4	28633.2	28854.2	28633.5	15104.6	16992.8
Relative ascendancy (RelA; %)	64.4	59.0	58.9	55.5	62.0	60.8
Relative overhead (RelOH; %)	35.6	41.0	41.1	44.5	38.0	39.2
Average mutual information (AMI)	1.56	1.60	1.60	1.58	1.64	1.64
Trophic depth	2.96	3.03	3.00	3.00	3.12	3.11

(TD)						
Flow diversity (FD ; DC/TST; %)	2.42	2.72	2.70	2.86	2.65	2.70
Effective link density (ELD)	1.35	1.48	1.47	1.55	1.42	1.44
Robustness (R)	0.283	0.311	0.311	0.327	0.296	0.303

The highest activity of the system indicated by the total system throughput (TST), was found during spring under the interactive effects of warming and acidification (24654.7 mgC tank⁻¹ d⁻¹; Table III-4, Figure III-4). During spring, OAW strongly increased the TST compared to the ambient treatment while in summer and autumn differences were not obvious (Figure III-4). The flow diversity (FD) reflects the diversity of interactions and is an indicator of functional diversity within a system. Flow diversity was highest during summer under the effects of OAW (2.86) (Table III-4), but OAW affects the FD only slightly compared to the ambient treatment in our experiments. Trophic depth showed no influence of OAW (TD; Figure III-4). The effective link density (ELD) increased in the summer experiment in the warm+acid treatment (Figure III-4). Increasing ELD values indicate the better cooperation between the different compartments.

Relative ascendancy (RelA) and relative overhead (RelOH) have been used for spatial and temporal system comparisons (Wulff et al. 1989, Baird et al. 1991, Christian et al. 2005) indicating the degree of organisation within each system and the system reserves, respectively. While in spring and autumn no changes were found under OAW, in summer OAW decreased the relative ascendancy (relA) and increased the relative overhead and robustness (Figure III-4). This loss of organisation is mainly due to increased respiration and dissipative fluxes under higher temperatures. The robustness index (R) ranged from 0.283 (ambient treatment in spring) to 0.327 (OAW treatment in summer; Table III-4), while OAW significantly increased robustness values under enhanced temperature and CO₂ (Figure III-4), particularly in spring. On system level, increasing robustness under OAW treatment can be interpreted as shifting the community towards a slightly more stabilized status against perturbations, but losing potential for resilience.

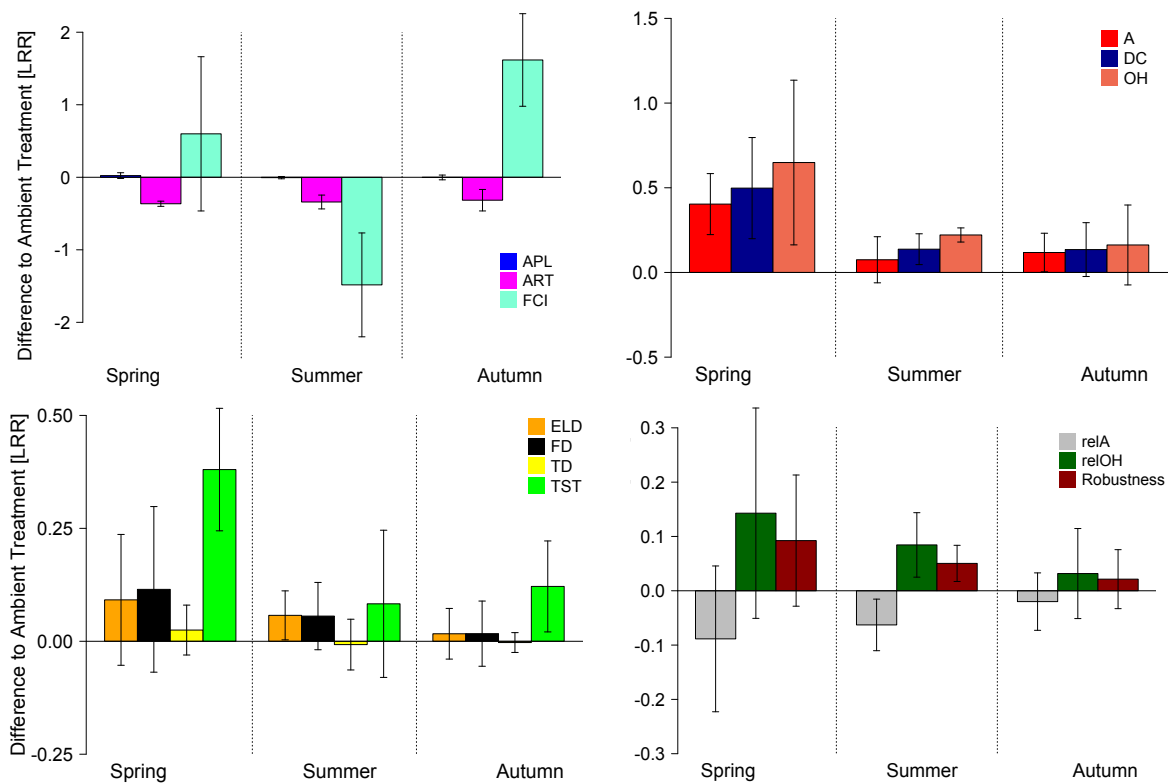


Figure III-4: Log response ratio (LRR) plot of the ENA indices at the end of the experiment showing differences between climate change treatment (OAW, increased CO₂ and temperature) and the ambient treatment for spring, summer and autumn in 2014, with APL (average path length), ART (average residence time), FCI (Finn cycling index), A (ascendancy), DC (development capacity), OH (overheads), ELD (effective link density), FD (flow diversity), TD (trophic depth), TST (total system throughput), reLA (relative ascendancy and ReOH (relative overheads). Shown are means \pm 95% confidence intervals. A LRR is statistically significant when the boot-strapped 95% confidence intervals do not overlap the zero line, and is statistically different from other LRR when 95% confidence intervals do not overlap.

Lindeman spine

To concatenate the complex food web into a food chain and to visualize the differences in carbon flows between ambient and OAW treatment as well as between seasons, simplified Lindeman spines were constructed (Figure III-5). Each box represents an integer trophic level with the associated trophic efficiencies. As we investigated artificial systems, the trophic chains of all treatments and seasons showed the same length of only 3 trophic levels, as the following: primary producers, primary consumers and secondary consumers. The first trophic level is divided into primary producers, in the upper box and the detritus pool in the lower box. Detritivory and herbivory were separated to highlight the differences between them from the first to the second trophic level. Detritivory exceeded herbivory at the end of the spring

experiment under the combined effects of elevated temperature and CO₂ (OAW). Under OAW, gross primary production (GPP) was enhanced from spring to autumn (Figure III-5). While in spring and autumn, OAW increased the energy flows upward the trophic levels as well as the respiration, OAW decreased the flow of energy between the trophic levels and the respiration from the second and third trophic level during summer (Figure III-5).

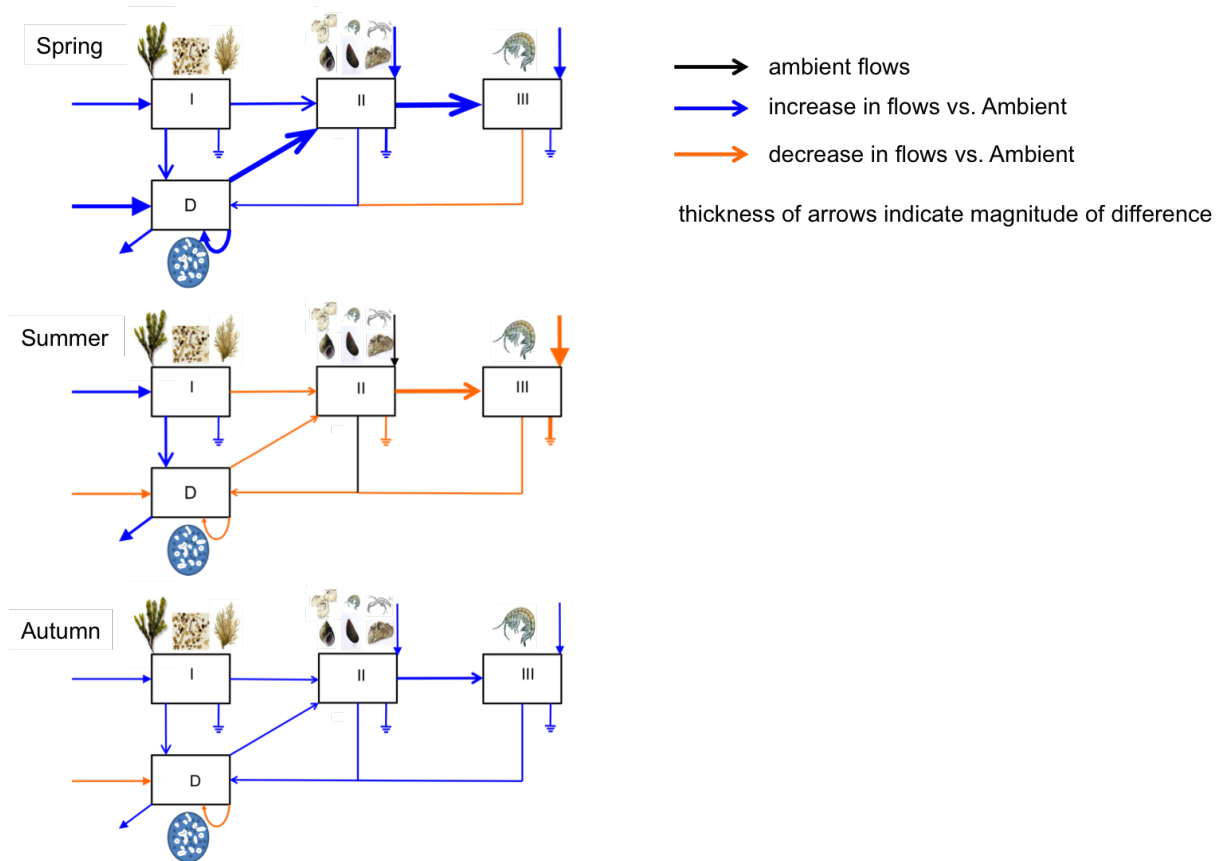


Figure III-5: Lindeman spines showing the differences in energy flows of the investigated networks between the OAW treatment and the ambient treatment per season. Roman numbers represent the trophic levels and D the detritus pool. Colour of arrows indicating the direction and magnitude of changes, with black arrows indicating no changes between ambient and OAW blue arrows an increase in flows an orange arrows decreased flows. The thickness of arrows indicating the magnitude of flows. The thicker the arrows the greater the flows and vice versa.

Hence, the total trophic efficiency (TE) increased during spring and autumn, while it decreased in summer (Figure III-6). Significant changes were seen in summer (ANOVA, $F=26.86$, $p=0.0066$) and autumn (ANOVA, $F=22.58$, $p=0.00896$)

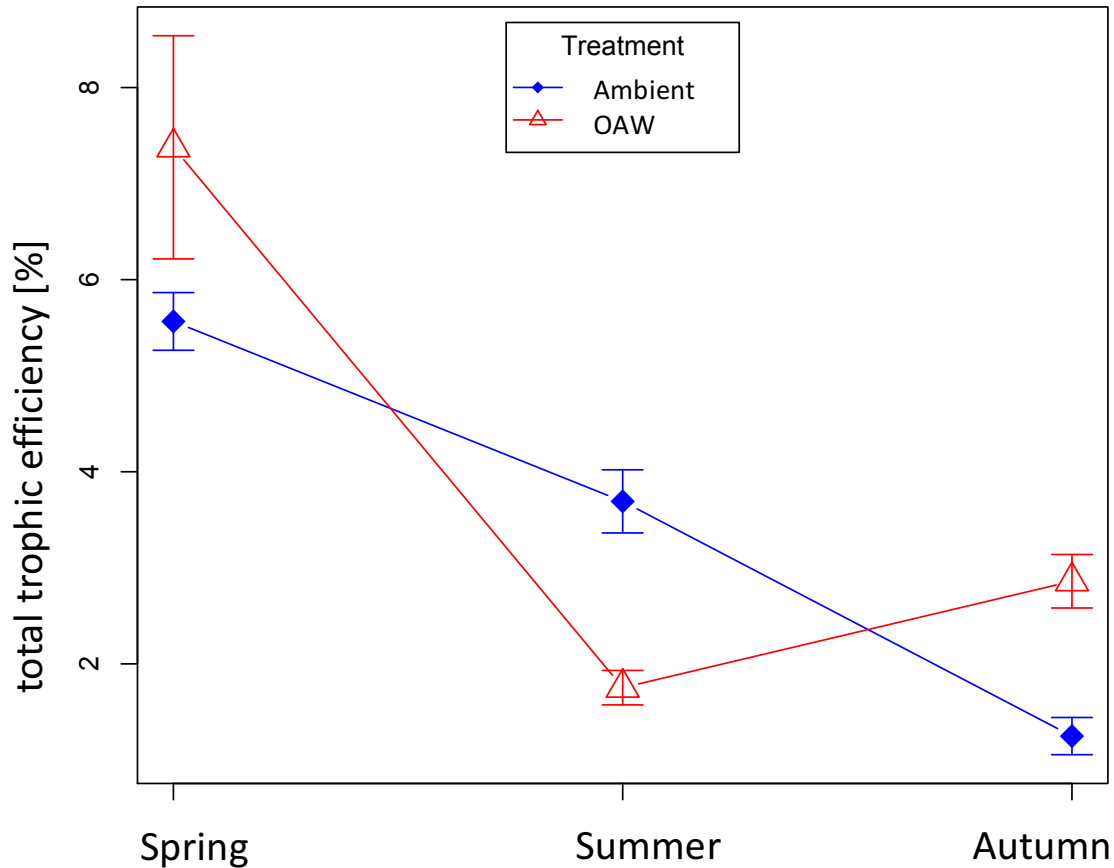


Figure III-6: Total trophic efficiencies for ambient (blue circles) and OAW (orange triangles) treatments across the investigated seasons (spring, summer and autumn). Shown are means \pm SD of 3 replicates.

Discussion

The presented mesocosm experiments on benthic species assemblages demonstrated strong treatment effects by the combined stress of warming and ocean acidification as well as a strong seasonal influence, with changes in species composition, food web functioning as well and altered energy flows between successive trophic levels. While the investigated species assemblages were either not affected or more positively influenced by future treatment conditions (warming and ocean acidification in combination) in spring and autumn, the system responded much stronger and negatively in summer. In spring and autumn, increased temperatures (and possibly ocean acidification) promote overall growth and system activity. In summer, the system is probably already close to its upper temperature limit under ambient conditions, thus higher temperatures under future treatment conditions showed detrimental effects. This summer system had a reduced total as well as autotrophic biomass at future

treatment conditions as a consequence of a reduced *Fucus* biomass, representing the major proportion of the total biomass within the mesocosms. The increment in overall biomass could be caused by the higher temperature as direct effect and additionally by an enormous growth of filamentous epiphytes and thereby an increased competition for nutrients and light (Worm & Sommer 2000). At this time, epiphytes overgrew large parts of the *Fucus* thalli and surrounding mesocosm structures, to which *Fucus* was tied. Additionally, *Gammarus spp.* and *Littorina spp.*, which normally graze on filamentous epiphytes (Jaschinski & Sommer 2010), were strongly negatively impacted by the combination of warming and acidification. Thus, the combination of both stressors possibly affected grazer biomass directly, leading to reduced grazing pressure on the epiphytes, which resulted in an increased epiphytic biomass as was also observed by Worm and Sommer (2000). This is supported by other studies in temperate benthic systems, showing that effects of environmental drivers can be largely mediated by grazers (Alsterberg *et al.* 2013; Werner *et al.* 2016b). Contrarily, in spring and autumn, no measurable amounts of epiphytes were found. Thus, ephemeral epiphytes were possibly able to settle and grow within the mesocosms only during the warmer summer month. Alternatively, an ambient or even higher grazing pressure during spring and autumn could have prevented the colonisation of epiphytes under elevated temperature and CO₂.

The response of the barnacle *B. crenatus* varied strongly with season. While in spring, *B. crenatus* biomass decreased under the future scenario, warming and acidification increased its biomass during summer. In spring, *B. crenatus* seemed to be directly affected by lower pH and higher temperatures, while in summer this negative impact was possibly compensated by a reduced predating pressure and competition for food through a decreased biomass of *Littorina spp.* and *Mytilus edulis*, respectively. A study by Buschbaum (2000) showed a strong negative correlation between the recruitment of barnacles on mussel beds and the abundance of *Littorina littorea*. Furthermore, *Mytilus* was shown to reduce biomass of mesozooplankton, including barnacle larvae by inhaling and ingesting the larvae or expelling them as pseudofaeces (Davenport *et al.* 2000). Additionally, *B. crenatus* and *M. edulis* may compete for larger sized phytoplankton as food (Thomsen *et al.* 2013), particularly so when zooplankton is low. As *Mytilus* biomass decreased under warming and acidification, more phytoplankton was possibly available for barnacles in summer. While *M. edulis* was negatively impacted by warming and acidification in all investigated seasons, biomass of *Magallana gigas* was not affected. *Magallana gigas*, as an invasive species from the Pacific, can tolerate higher summer temperatures than native species in the Wadden Sea. Higher

temperature tolerance could arise from the expression of specific heat shock proteins under temperature stress (Hamdoun *et al.* 2003). While warming and acidification directly affected many species, the results indicate that other species are indirectly affected by OAW due to alterations in top-down control. Hence, future increases in water temperature and CO₂ will impact food web dynamics of marine communities by changing top-down control as suggested by different studies (Jochum *et al.* 2012; Lewandowska *et al.* 2014; Werner *et al.* 2016b). Nevertheless, the outcome of our study shows that the effects of OAW on the investigated assemblage is species specific and vary strongly with season and species identity.

The results of the modelling approach by means of ENA indicate that the interactive effects of warming and acidification have the capacity to alter energy flows between trophic levels. The combination of warming and acidification enhanced the production of primary producers throughout all investigated seasons, but reduced energy flow to herbivores and to the secondary consumer *Gammarus spp.* in summer. Furthermore, OAW decreased the total trophic efficiency between primary producers, primary consumers and secondary consumers in summer, resulting in a reduced standing stock of consumers. Hence, a hampered energy transfer under future climate change from enhanced primary production upward the trophic levels could result in altered producer-consumer interaction within food webs. These findings relate to a study by Ullah *et al.* (2018), where the same picture was seen, while most of the primary production was branched off into blue-green algal biomass and were thus unavailable further up the food chain. In the present summer experiment, primary production could have increased by both, the influence of the physical factor warming (or acidification) or a decreasing grazing pressure by the stressed herbivore assemblage. In contrast to the results from the summer experiment, increased primary production in autumn and winter resulted in an increased transfer of energy and total trophic efficiency of the warmed and acidified systems. Different studies indicate that temperature-driven enhanced basal productivity is likely to propagate through trophic levels, leading to increased heterotrophic to autotrophic biomass ratios (O'Connor 2009; O'Connor *et al.* 2009b; Carr & Bruno 2013). Hence, for systems, which are already close to their upper temperature limits, future climate warming could hamper the energy transfer from enhanced primary production upward the trophic levels resulting in a possibly altered producer-consumer interaction within food webs. Our results indicate a strong seasonal variability with increased energy transfer efficiency in spring and autumn in comparison to a reduced energy transfer in summer.

The ecological network analysis revealed a higher system activity (higher TST and NPP) in spring and autumn under OAW. This is in accordance with a decreased ART, indicating that a unit of energy remains within the system for a shorter period of time. Again, strong seasonal variations are apparent: the system activity was not enhanced in summer but there was a considerable reduction of recycling within the system. Hence, the system had a reduced efficiency in retaining particulate matter, and can therefore be identified as more stressed under warming and acidification in comparison to spring and autumn (Monaco & Ulanowicz 1997; Heymans *et al.* 2002). A decreased relative ascendency (RelA) and increased overhead (OH) in the warmed and acidified system during summer, indicate a highly reduced organization within the system. The resulting increment of the non-organized fraction increases the capacity of the system to develop, and in this way, the capability of the system to adapt to environmental changes (Heymans *et al.* 2002; Baird *et al.* 2004; Saint-Béat *et al.* 2015). This is in line with increasing values of robustness under OAW in summer. Our results do not allow to distinguish between the effects of warming and acidification as we used a treatment with a combination of both drivers only. Some studies showed warming to be the main driver for food web changes (Werner *et al.* 2016b; Ullah *et al.* 2018), while other studies showed ocean acidification to be harmful for different species (Waldbusser & Salisbury 2014). However, ocean warming and acidification will not act separately on marine species or communities as forecasts show that ocean warming and acidification will increase jointly (IPCC 2014) and will affect the marine environment by their combination.

Mesocosm studies, including the complexity of seasonal differences give us important information on food web and community responses to climate change. Investigating the seasons separately, however, is connected with some limitations. As the investigated species assemblages were renewed at the onset of each experiment, we cannot account for natural carry-over effects between seasons. An increased grazer biomass in autumn could have propagated through winter and spring, and could have resulted in an increased grazer biomass in summer, and hence an increased grazing pressure on ephemeral epiphytes (Werner *et al.* 2016b). Additionally, in autumn, a decreased *Fucus* biomass in combination with an increased grazing pressure could have resulted in a reduced fitness at the start of the next growing season, with disadvantages for *Fucus* in competition with fast-growing epiphytes.

The combined impact of ocean warming and acidification on habitats such as the macroalgae-mussel bed communities depends on a certain threshold temperature, resulting from the species-specific tolerance of community constituents, and the consequences for the

interactions within the food web. In temperate areas, this threshold might be reached during summer, whereas in seasons with temperatures far below this threshold an increase in temperature and CO₂ may promote the energy flow through the food web and induce a higher activity at all trophic levels. To investigate these effects on the system level, large scale mesocosms as well as complex analytical tools such as ENA are crucial and will help understanding and predicting complex ecosystem changes within a future ocean.

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Supplementary

Table S1: Biomass and energetics of all compartments in flow networks of the 3 treatments for the Spring experiment. Biomass and standing stocks in mg C tank⁻¹; GPP, NPP, Consumption, Production, Respiration and Egestion in mg C tank⁻¹ d⁻¹.

Spring #	Compartment	Parameter	Ambient Replicate			OAW Replicate		
			1	2	3	1	2	3
1	Phytoplankton	Biomass	213,066	151,686	217,219	327,614	197,586	166,738
		GPP	89,599	63,787	91,345	192,877	116,325	98,163
		NPP	50,662	36,067	51,650	109,059	65,774	55,505
		Respiration	38,954	27,732	39,713	83,854	50,573	42,677
2	Epiphytes	Biomass	0,000	0,000	0,000	0,000	0,000	0,000
		GPP	0,000	0,000	0,000	0,000	0,000	0,000
		NPP	0,000	0,000	0,000	0,000	0,000	0,000
		Respiration	0,000	0,000	0,000	0,000	0,000	0,000
3	Macrophytes	Biomass	223020,1 0	221437,18	237010,05 0	207065,32	228447,23	217427,136
		GPP	6268,373	6223,882	6661,585	8147,910	8989,277	8555,642
		NPP	2965,955	2944,903	3152,008	3855,280	4253,383	4048,203
		Respiration	3302,418	3278,979	3509,577	4292,630	4735,894	4507,439
4	Littorina spp.	Biomass	6268,373	6223,882	6661,585	8147,910	8989,277	8555,642
		Production	11,720	11,637	12,455	21,328	23,530	22,395
		Respiration	40,319	40,033	42,848	73,371	80,948	77,043
		Egestion	77,447	76,897	82,305	140,936	155,490	147,989
		Consumption	129,585	128,665	137,714	235,817	260,168	247,617
5	Corophium sp.	Biomass	36,212	20,903	23,400	364,836	36,863	18,609
		Production	0,150	0,086	0,097	2,112	0,213	0,108
		Respiration	0,706	0,408	0,456	9,960	1,006	0,508
		Egestion	0,171	0,099	0,110	2,407	0,243	0,123
		Consumption	1,027	0,593	0,663	14,479	1,463	0,739
6	Gammarus spp.	Biomass	2152,684	1913,291	2927,048	7203,699	3416,769	2540,733
		Production	11,994	10,660	16,308	56,189	26,651	19,818
		Respiration	56,790	50,474	77,218	266,057	126,193	93,838
		Egestion	19,220	17,083	26,134	90,046	42,710	31,759
		Consumption	88,158	78,354	119,870	413,012	195,895	145,669
7	Mytilus edulis	Biomass	3147,000	2621,440	4203,710	2498,350	2518,870	2110,810
		Production	3,104	2,585	4,146	3,450	3,478	2,915
		Respiration	17,071	14,220	22,803	18,974	19,129	16,030
		Egestion	2,851	2,374	3,808	3,168	3,194	2,677
		Consumption	23,025	19,180	30,757	25,591	25,802	21,622
8	Magallana gigas	Biomass	4940,758	8791,237	6472,669	19420,991	14207,943	5034,754
		Production	4,876	8,677	6,388	26,835	19,632	6,957
		Respiration	64,230	114,286	84,145	353,462	258,585	91,633
		Egestion	3,939	7,009	5,160	21,677	15,858	5,620
		Consumption	73,045	129,972	95,693	401,974	294,075	104,209
9	Balanus crenatus	Biomass	8,048	26,597	32,886	11,886	4,477	6,556
		Production	0,027	0,089	0,110	0,055	0,021	0,031
		Respiration	0,070	0,231	0,285	0,144	0,054	0,080
		Egestion	0,011	0,035	0,044	0,022	0,008	0,012
		Consumption	0,107	0,355	0,438	0,222	0,084	0,122
10	Suspended POC	Biomass	113,639	18,510	16,935	787,286	603,068	126,029
11	Sediment POC	Biomass	121,947	146,752	130,418	248,041	167,053	137,070
12	DOC	Biomass	71,985	67,915	75,953	104,370	101,511	94,840

Table S2: Biomass and energetics of all compartments in flow networks of the 3 treatments for the summer experiment. Biomass and standing stocks in mg C tank⁻¹; GPP, NPP, Consumption, Production, Respiration and Egestion in mg C tank⁻¹ d⁻¹.

Summer			Ambient			OAW		
#	Compartment	Parameter	Replicate			Replicate		
			1	2	3	1	2	3
1	Phytoplankton	Biomass	164,1114	214,0650	218,6370	98,8056	141,4368	238,7574
		GPP	69,0124	126,0265	180,2055	58,1698	116,5754	275,5049
		NPP	39,0219	71,2595	101,8941	32,8911	65,9155	155,7794
		Respiration	30,0035	54,7907	78,3453	25,2897	50,6818	119,7772
2	Epiphytes	Biomass	10760,175	6236,510	2442,156	30485,8	17227,51	16023,07
		GPP	302,4337	245,4034	134,5365	1199,60	949,0508	1235,778
		NPP	143,1001	116,1155	63,6576	567,605	449,0546	584,7232
		Respiration	159,3336	129,2879	70,8790	631,995	499,9962	651,0553
3	Macrophytes	Biomass	192160,54	229140,68	204100,73	136748,	167972,21	139817,30
		GPP	5401,0105	9016,5633	11243,7563	5380,9721	9253,4636	10783,4026
		NPP	2555,5520	4266,2935	5320,1162	2546,0706	4378,3857	5102,2944
		Respiration	2845,4585	4750,2698	5923,6401	2834,9016	4875,0779	5681,1082
4	Littorina spp.	Biomass	8102,7778	7206,0857	7347,6248	5310,6563	6032,3344	5489,6984
		Production	15,1500	18,8628	26,9266	13,9013	22,1065	28,1650
		Respiration	52,1177	64,8902	92,6306	47,8220	76,0489	96,8912
		Egestion	100,1112	124,6453	177,9310	91,8597	146,0798	186,1149
		Consumption	167,5076	208,5586	297,7170	153,7010	244,4230	311,4104
5	Corophium Sp.	Biomass	0,6108	0,7635	0,0100	2,6977	3,0031	0,0100
		Production	0,0025	0,0044	0,0001	0,0156	0,0243	0,0001
		Respiration	0,0119	0,0208	0,0004	0,0736	0,1148	0,0005
		Egestion	0,0029	0,0050	0,0001	0,0178	0,0277	0,0001
		Consumption	0,0173	0,0303	0,0006	0,1071	0,1669	0,0008
6	Gammarus spp.	Biomass	1107,9403	735,7086	1151,0017	175,1978	127,1482	223,9600
		Production	6,1728	5,7385	12,5689	1,3665	1,3885	3,4239
		Respiration	29,2285	27,1722	59,5145	6,4706	6,5744	16,2123
		Egestion	9,8923	9,1964	20,1425	2,1900	2,2251	5,4870
		Consumption	45,3728	42,1806	92,3871	10,0407	10,0407	10,0407
7	Mytilus edulis	Biomass	2599,8100	3196,6100	2898,2100	966,0700	164,1200	393,1047
		Production	2,5641	4,4137	5,6024	1,3339	0,3173	1,0639
		Respiration	14,1029	24,2765	30,8144	7,3368	1,7450	5,8514
		Egestion	2,3549	4,0536	5,1453	1,2251	0,2914	0,9771
		Consumption	19,0219	32,7438	41,5621	9,8957	2,3536	7,8923
8	Magallana gigas	Biomass	13222,850	20552,300	10279,880	7262,310	2704,250	21537,020
		Production	13,0504	28,3980	19,8858	10,0347	5,2312	58,3270
		Respiration	171,8971	374,0519	261,9313	132,1740	68,9043	768,2686
		Egestion	10,5420	22,9397	16,0636	8,1059	4,2257	47,1161
		Consumption	195,4895	425,3896	297,8808	150,3146	78,3612	873,7117
9	Balanus creantus	Biomass	3,7310	13,3250	0,5330	18,1220	26,6500	78,8840
		Production	0,0124	0,0621	0,0035	0,0845	0,1739	0,7208
		Respiration	0,0323	0,1617	0,0091	0,2199	0,4527	1,8760
		Egestion	0,0050	0,0249	0,0014	0,0338	0,0696	0,2885
		Consumption	0,0497	0,2487	0,0139	0,3382	0,6963	2,8854
10	Suspended POC	Biomass	185,1048	131,5426	236,3040	57,8945	367,0589	68,9220
11	Sediment POC	Biomass	2121,5964	3107,6958	1818,6721	2486,1566	974,2126	1819,2522
12	DOC	Biomass	63,7285	105,4631	133,1490	70,4963	113,0277	152,6852

Chapter III

Table S3: Biomass and energetics of all compartments in flow networks of the 3 treatments for the autumn experiment. Biomass and standing stocks in mg C tank⁻¹; GPP, NPP, Consumption, Production, Respiration and Egestion in mg C tank⁻¹ d⁻¹.

Autumn			Ambient			OAW		
#	Compartment	Parameter	Replicate			Replicate		
			1	2	3	1	2	3
1	Phytoplankton	Biomass	91,079	60,884	24,783	36,917	24,783	6,823
		GPP	38,301	25,603	14,591	21,734	14,591	4,017
		NPP	21,657	14,477	8,250	12,289	8,250	2,271
		Respiration	16,652	11,131	6,343	9,449	6,343	1,746
2	Epiphytes	Biomass	0,000	0,000	0,000	0,000	0,000	0,000
		GPP	0,000	0,000	0,000	0,000	0,000	0,000
		NPP	0,000	0,000	0,000	0,000	0,000	0,000
		Respiration	0,000	0,000	0,000	0,000	0,000	0,000
3	Macrophytes	Biomass	106871,89	107613,53	86841,268	87105,242	86841,268	91938,486
		GPP	3003,823	3024,668	3417,157	3427,545	3417,157	3617,730
		NPP	1421,294	1431,157	1616,868	1621,783	1616,868	1711,772
		Respiration	1582,528	1593,511	1800,289	1805,761	1800,289	1905,958
4	Littorina spp.	Biomass	3003,823	3024,668	3417,157	3427,545	3417,157	3617,730
		Production	5,616	5,655	8,945	8,972	8,945	9,470
		Respiration	19,321	19,455	30,771	30,865	30,771	32,577
		Egestion	37,113	37,370	59,107	59,287	59,107	62,577
		Consumption	62,098	62,529	98,899	99,200	98,899	104,704
5	Corophium Sp.	Biomass	0,000	0,000	0,000	0,000	0,000	0,000
		Production	0,000	0,000	0,000	0,000	0,000	0,000
		Respiration	0,000	0,000	0,000	0,000	0,000	0,000
		Egestion	0,000	0,000	0,000	0,000	0,000	0,000
6	Gammarus spp.	Biomass	91,101	36,892	233,651	387,754	233,651	189,842
		Production	0,508	0,206	1,822	3,024	1,822	1,481
		Respiration	2,403	0,973	8,630	14,321	8,630	7,011
		Egestion	0,813	0,329	2,921	4,847	2,921	2,373
		Consumption	3,731	1,511	13,396	22,231	13,396	10,884
7	Mytilus edulis	Biomass	2249,304	2191,555	1810,435	1574,060	1810,435	1902,093
		Production	2,218	2,161	2,500	2,173	2,500	2,626
		Respiration	12,202	11,888	13,749	11,954	13,749	14,445
		Egestion	2,037	1,985	2,296	1,996	2,296	2,412
		Consumption	16,457	16,035	18,545	16,124	18,545	19,484
8	Magallana gigas	Biomass	6943,147	5408,239	8760,048	5030,440	8760,048	7485,536
		Production	6,853	5,338	12,104	6,951	12,104	10,343
		Respiration	90,261	70,307	159,433	91,554	159,433	136,237
		Egestion	5,535	4,312	9,778	5,615	9,778	8,355
		Consumption	102,649	79,957	181,315	104,120	181,315	154,935
9	Balanus crenatus	Biomass	0,000	0,000	0,000	0,000	0,000	0,000
		Production	0,000	0,000	0,000	0,000	0,000	0,000
		Respiration	0,000	0,000	0,000	0,000	0,000	0,000
		Egestion	0,000	0,000	0,000	0,000	0,000	0,000
		Consumption	0,000	0,000	0,000	0,000	0,000	0,000
10	Suspended POC	Biomass	40,861	734,019	251,073	613,898	251,073	128,983
11	Sediment POC	Biomass	418,431	608,317	1432,697	539,530	1432,697	849,990
12	DOC	Biomass	33,840	32,242	34,400	35,508	34,400	34,803

Table S4: Diet matrices predator and prey species taken from Baird et al. (2007). Species and numbers in blue indicate parts, which were not sampled and hence not included in the network analysis, but were treated as system imports.

Prey		Phytoplankton	Zooplankton	Microphytobenthos	Epiphytes	Macrophytes	Bacteria water column	Bacteria Sediment	Meiofauna	Macrofauna	Suspended POC	Sediment POC	DOC
#	Predator	1	-	-	2	3	-	-	-	4 to 7	10	11	12
4	<i>Littorina spp.</i>			0.800	0.100	0.100							
5	<i>Corophium sp.</i>			0.500	0.250			0.125				0.125	
6	<i>Gammarus spp.</i>			0.200	0.100	0.250		0.100		0.250		0.100	
7	<i>Mytilus edulis</i>	0.850					0.010				0.140		
8	<i>Magallana gigas</i>	0.850					0.010				0.140		
9	<i>Balanus crenatus</i>	0.500	0.500										

Table S5: Abbreviations and descriptions of all parameters derived from the ecological network analysis (ENA).

ENA-indices	Description	Reference
A	The ascendancy (A) integrates the size and organization of the system flows and describes its efficiency and performance. High productivity of the system and complex trophic structure increase the system ascendancy. It is defined as the product of TST and average mutual information (AMI)	Ulanowicz 2004
APL	The average path length (APL) is defined as the average number of compartments that a unit of carbon passes through from its entry into the system until its leaving, which is calculated as $(TST-Z)/Z$, where Z is the sum of all exogenous inputs. The APL should increase with increasing flow diversity and cycling within the system.	Baird et al. 1991 Christensen 1995
AMI	The average mutual information (AMI), corresponds to the organization of the flows inherent in a system and is defined as the normalized ascendancy (A/TST).	Ulanowicz 2004
ART	The average residence time (ART) of energy in the system is calculated by the ratio of the total system biomass and the sum of export and respiration. It gives the time, a unit of energy needs from entering until leaving the system.	Christensen 1995
DC	The upper limit of A is defined by the development capacity (DC) and represents the potential of the system to develop. It is defined as the product of TST and the FD, which is quantified as the number of interactions and the evenness of flows in the system.	Christensen 1995
ELD	The effective link density (ELD) represents the average number of links flowing into or out of a compartment.	Ulanowicz 2004
FCI	The Finn cycling index (FCI) is quantifying the importance of cycling within the system and represents the fraction of flow, which is recycled.	Finn 1976
FD	The flow diversity (FD) is quantified as the number of interactions and the evenness of flows in the system.	MacArthur 1955
OH	The difference of $C - A$ is defined as the system overhead (OH), which represents the cost of the system to operate in the way it does.	Ulanowicz 2004
RelA	The relative ascendancy (RelA) is the organized fraction of the DC.	Wulff et al. 1989
RelOH	The relative overhead (RelOH) is the non organized fraction of the DC. High RelOH indicating a high system resilience and a high capacity to adapt to system perturbations.	Heymans et al. 2002, Ulanowicz 2004
Robustness	The Robustness (R) represents the tradeoff between organisation and redundancy of the system and therefore measures and assesses the necessary configuration for sustainability of the system.	Goerner et al. 2009, Fath 2015
TD	The trophic depth (TD) represents the effective number of trophic levels in a system. Systems with a high number of trophic levels is expected to be more specialized.	Ulanowicz 2004
TE	The system trophic efficiency (TE) is defined as the logarithmic mean of the single trophic efficiencies, i.e. the efficiency of transfer from one trophic level to the next.	Wulff et al. 1989, Baird et al. 2004
TST	The total system throughput (TST) represents the sum of all flows through the system and indicates the activity within the system.	Ulanowicz 2004

General discussion

Mesocosm experiments examine the response of a macroalgae-mussel bed community to global climate change

Marine communities will alter under future climate change, but the direction and to which extent they will modify seems to be widely unknown. The responses of a near natural Wadden Sea multi-species assemblage to the combined effects of elevated pCO₂, temperature and enriched nutrients are complex. This thesis used near natural mesocosm experiments in combination with a modeling approach by means of ENA and revealed that global climate change will exert differential effects on growth, reproduction, food web structure and functioning depending on season. Warming in combination with acidification increased the energy flows along trophic levels in spring and autumn, accelerated the total system throughput and enhanced the net primary production. In contrast, in summer, warming and acidification decreased total biomass, abundance of important grazing species as well as energy flows along trophic levels. Surprisingly, the results of ENA indicated a higher robustness of the investigated community in summer that gives theoretically a better capability of the system to adapt to environmental changes. This seeming contradiction was explained with a selection within the community during the experimental treatment allowing only survivors with a low biomass but better adapted to the warmer and more acid conditions to exist and to form a “new” food web. The results of the present study demonstrate that the response of the total assemblage to global climate change tends to sustain community functioning although under stress but is highly species specific and varies with the season. This advances the understanding of future re-structuring and re-functioning and possible consequences of climate change for ecosystems.

Mesocosm experiments as useful tool for climate change research

Throughout the last decade, the need for mesocosm experiments as useful tools for gauging ecological consequences of climate change become more relevant. Thus more mesocosm facilities arose for both marine and freshwater systems (AQUACOSM 2019). All mesocosm facilities are quite different, each with special features for specific purposes. The most similar system to the Sylt benthic mesocosm facility is the GEOMAR Kiel-Outdoor-Benthocosm facility (KOB), that is located at Kiel Fjord in the Baltic Sea of Germany (Wahl *et al.* 2015b).

The targeted purpose of both facilities are climate change experiments with benthic communities and both were used within the BIOACID II project.

The true innovations introduced by the Sylt benthic mesocosm facility are the simultaneous control of important environmental parameters, the manipulation of multiple stressors, their combination with a newly developed tidal simulation system and the capability to conduct near natural experiments with inshore benthic communities. Most importantly, the technology of this facility allows the combination of simulated natural temperature fluctuations with controlled dynamic delta-treatments, the addition of predefined changes (e.g. temperature, CO₂ concentration) to the reference values. In the present study, the Sylt benthic mesocosm facility was used for the first time for assessing partially separate and joint effects of simulated ocean warming, acidification and nutrient enrichment as well as their seasonal variations on a macroalgae-mussel bed community in the northern Wadden Sea. Considering mesocosm experiments within the climate change research is an important step forward, closing the gap between necessary small scale laboratory experiments and large scale field experiments. They can give crucial additional information for a realistic assessment of future consequences to global climate change. Confounding factors of mesocosms are always a limitation for more realistic forecasts. The Sylt benthic mesocosm facility simulated the near natural conditions on a new level by integrating the natural tidal and current rhythm as well as natural temperature fluctuations. This was done for the first time in this way and makes this facility unique. With this study, we demonstrate the setup and functioning of the benthic mesocosm with a great potential to investigate the effects of multiple stress scenarios at the community level within a near natural setting to predict more reliably future consequences of climate change.

The Sylt benthic mesocosms were effectively used in different experiments and can be recommended as a tool for a variety of experiments in the field of climate change. The tidal simulation provides new possibilities for mesocosm experiments in coastal systems and tidal areas and experiments concerning sea level rise scenarios. The combination of changing water level and changing currents is not existing in this way in any other mesocosm facility worldwide. The different existing mesocosms also vary in their general shape from round to rectangular tanks (AQUACOSM 2019). The Sylt mesocosm facility used a round design of the tanks, allowing straight and circular flows within the tanks for several purposes.

Effects of ocean warming, acidification and nutrient enrichment

Coastal ecosystems are impacted by direct physiological effects of acidification, warming and increased nutrients and by indirect effects mediated by changes in interspecific interactions (Harley *et al.* 2006; Harley *et al.* 2012). Hence, shifts in community structure and dynamics are likely to occur, as the phenology, abundance, and species interactions change (Harley *et al.* 2012; Kroeker *et al.* 2013b). Macroalgae compete for nutrients, light and space, and their relative success depends on both, resource availability on the one hand and environmental stress on the other hand (Harley *et al.* 2012). Ocean acidification, warming as well as nutrients can increase resource availability but they can also intensify the environmental stress for primary producers depending on season and functional group (Russell *et al.* 2011; Graiff *et al.* 2015a; Graiff *et al.* 2015b; Mensch *et al.* 2016; Werner *et al.* 2016a). The response of heterotrophs to a changing environment lies mainly in a modification of their behavior (Nagelkerken & Munday 2016). Foraging behavior, consumption rates, egesting rates, predator avoidance, dispersal, migration and reproduction are all directly affected by climate change and can alter species interactions, population dynamics, community structure, biodiversity and finally ecosystem functioning (Edwards & Richardson 2004; Pörtner & Farrell 2008; Briffa *et al.* 2012; Doney *et al.* 2012; Byrne & Przeslawski 2013; Hall-Spencer *et al.* 2015; Moulin *et al.* 2015).

Impacts of ocean acidification and warming as well as the responses of multi-species assemblage were shown by several studies (Alsterberg *et al.* 2013; Eklöf *et al.* 2015; Queirós *et al.* 2015; Werner *et al.* 2016b). Alsterberg *et al.* (2013) showed that acidification in combination with warming had strong, positive direct and indirect effects on different components of a seagrass system in summer. Macroalgae, associated fauna, and *Zostera marina* were positively affected by the combined effects of acidification and warming (OAW). However, this and most other studies did not consider the influence of season, although, it is known that interactions vary strongly between seasons (Godbold & Solan 2013; Strain *et al.* 2014; Graiff *et al.* 2015a; Werner *et al.* 2016b).

Our study showed that OAW decreased the biomass of the majority of species, and hereby the overall biomass, whereas the total system throughput (TST) was enhanced in these treatments. Especially the grazers *Gammarus spp.* and *Littorina spp.*, which are known to dominantly graze on filamentous epiphytes when available (Jaschinski & Sommer 2010), were mostly impacted by OAW, resulting in altered species composition, activity and

connectance of the incubated intertidal macroalgae-mussel bed community under future climate change. Different studies indicate that warming is the main driver of climate change, while ocean acidification showed less impacts (Graiff *et al.* 2015a; Paul *et al.* 2015; Al-Janabi *et al.* 2016; Werner *et al.* 2016b). As we used treatments with combined ocean warming and acidification, we cannot distinguish between these single impacts, but unpublished data from the spring experiment showed that warming had a stronger effect. Warming negatively impacted most species, while acidification had less or even positive effects. It seems, that both drivers acted antagonistically and that acidification hampered the effects of warming when both drivers acted simultaneously (Pansch *et al.* 2019; in preparation).

Enriched nutrient concentrations affected herbivorous species, resulting in a decreased biomass of filamentous epiphytes. Although enriched nutrients should support the growth of epiphytes, increasing biomass of omnivorous *Gammarus* spp. and herbivorous *Littorina littorea* L. possibly enhanced the grazing pressure on epiphytes and reduced their biomass. With increased temperatures and acidification, the system responded differently. Under OAW, both species were negatively impacted, while OAW in combination with enhanced nutrients showed no effects on grazing pressure. Hence, epiphytes developed a greater biomass, presumably as a consequence of a reduced grazing pressure. On the other hand, the increased biomass could be a direct effect of enhanced temperature, CO₂ and nutrients favoring the growth of epiphytes.

Despite the fact, that OAW negatively affected important components of the investigated species assemblage, the applied ecological network analysis (ENA) showed a lower relative Ascendency and the trend to an increasing Flow diversity, as a the result of a high number of multiple pathways between the system components. This indicates that the simulated system is better capable of withstanding external disturbances under OAW as compared with the unstressed system. The treated systems showed higher robustness values and may become more resilient but as their AMI as well as relative ascendency decreases, they lose efficiency, organization and specialization when being challenged by OAW (Fath *et al.* 2019).

Seasonal variations in response to climate change

Environmental factors like temperature, irradiance, photoperiod and nutrient concentrations show a significant seasonal variation within temperate systems. As these factors influence species behavior, performance, food availability growth, reproduction and development, seasonal patterns are the result and should be considered within climate change research (Coma *et al.* 2000).

However, many experiments with multi-species assemblages were done but most of them did not examine the influence of seasons (Alsterberg *et al.* 2013; Queirós *et al.* 2015; Ullah *et al.* 2018). It is known that species responses to climate change vary strongly with seasons (Godbold & Solan 2013; Strain *et al.* 2014). Different studies showed warming worsen the decline of canopy forming algae by enhancing the growth of epiphytes during summer (Andersen *et al.* 2013; Graiff *et al.* 2015a; Werner *et al.* 2016b). This could be a direct effect of promoted epiphytes growth or an indirect effect through a temperature induced lowered grazing pressure on the epiphytes. Effects such as those on epiphytes could be mitigated or reversed during autumn, winter and spring with lower temperatures (Graiff *et al.* 2015a). Werner *et al.* 2016b showed in a similar experiment to ours for the Baltic Sea, that warming during summer induced a collapse of grazers, causing a cascading effect from consumers to primary producers, resulting in overgrowth of *F. vesiculosus* by epiphytes. In the Baltic, the *Fucus* biomass was reduced by up to 70 % under warming in late summer and could not solely be explained by the reduced top down control of epiphytes. Werner *et al.* (2016b) assume that the drop in biomass result from direct heat stress impairing photosynthetic activity, growth and survival of *F. vesiculosus* (Graiff *et al.* 2015b). However, during autumn and winter these epiphyte- grazer interactions were absent and the grazers directly reduced the *Fucus* biomass (Werner *et al.* 2016b). The results of the present thesis show a similar tendency. However, the increased temperatures in summer did neither lead to a collapse of the system nor to such a high reduction of *Fucus* biomass as in the Kiel Bay. Under OAW grazers decreased strongly while epiphytes massively increased and overgrew *Fucus*, resulting in a reduced biomass of *F. vesiculosus*. In summer, the system seems already close to its upper temperature limit under ambient conditions, but showed detrimental effects under the scenario of future conditions. In spring and autumn, increased temperatures and ocean acidification seem to promote the system activity with an increased net primary production. There was no decrease of grazers in spring and even an increased grazer biomass

in autumn under OAW. The observed lowered *Fucus* biomass in autumn resulted possibly from an enhanced grazing pressure or due to shortened day length (Strömberg 1977). Furthermore, OAW reduced the total trophic efficiency between primary producers, primary consumers and secondary consumers in summer. As a result of a reduced standing stock of consumers, most of the primary production was not used further up the food chain. Hence a hampered energy transfer under future climate change could result in altered producer-consumer interaction within food webs. This is in accordance with a study by Ullah *et al.* (2018), where an enhanced primary production due to warming was constrained to the base of the food web. Contrary to the results from our summer experiment, increased primary production in spring and autumn resulted in an enhanced energy transfer and total trophic efficiency under OAW. Other studies point out that enhanced basal productivity due to warming is likely to propagate through higher trophic levels in spring and autumn, resulting in an increased heterotrophic to autotrophic biomass ratio (O'Connor 2009; Carr & Bruno 2013).

The results of the present thesis indicate high seasonal variations in response to global climate change and therefore it is crucial to consider the responses of species and communities to ocean acidification and warming from different geographical areas and over different seasons in order to assess the full ecological implications of future climate change scenarios.

Although the simulated temperature increase was 5 °C for both Baltic Sea and North Sea experiments, the impact of warming seems to be lower for the North Sea organisms, due to higher stress tolerance. This is most probable the result of a better adaptation of the living organisms to higher temperature fluctuations in the intertidal zone, with even extreme temperature fluctuations (Pearson *et al.* 2000). The geographical distribution of species depends on the tolerance limits and optimum temperatures of species as a result of their genotypic adaptation, generating distinct ecotypes with different tolerance limits and optima (Nygård & Dring 2008; Jueterbock *et al.* 2014; Graiff *et al.* 2015b).

Strengths and limitations

Mesocosm experiments provide the possibility to control and monitor environmental conditions and to assess the response of interacting ecosystem components as the conditions come close to ambient. This is why mesocosms close the gap between small scale, constrained but precise laboratory experiments with high replication and on the other hand large scale, natural but mostly observational field investigations mainly with little replication. When planning an experiment, a tradeoff between experimental realism and experimental control and precision is required. The Sylt benthic mesocosm facility showed within the presented experiments its good functionality and usability. However, like other experimental systems, mesocosms have as well some constraints. Confounding factors are mainly lowered light availability, wall effects, limited space as well as the exclusion of influence of organisms such as fish or birds, feeding at higher trophic levels and on larger scales than the size of the mesocosms. This might impact not only single species and influence the trophic interactions, but also total trophic functioning. As a consequence, an extrapolation of experimental results to natural systems should be critically evaluated. For example, due to the wall surface growth of microbiota and filamentous algae might be enhanced. The walls provide additional settlement substrate within the mesocosms, which must be controlled or accounted for. Till now, the Sylt benthic mesocosm facility works with estimated temperatures, to simulate the conditions in the field. This simulation is precise and reliable but still based on calculated temperature values. Installing additional temperature sensors in the field would increase the experimental realism and could include small scale fluctuations, which have the potential to modulate environmental stress (Dufault *et al.* 2012; Cornwall *et al.* 2013; Wahl *et al.* 2015a). A direct water supply could be further considered for future experiments, to focus on small scale fluctuations of the system. Such fluctuations are emphasized by different studies to be very crucial for coastal habitats and should be considered (Duarte *et al.* 2013; Waldbusser & Salisbury 2014).

Another important confounding factor is the exclusion of higher trophic levels in the present study. In order to move forward in the insights that can be generated from mesocosm experiments, the addition of predatory species could be an important consideration. Inclusion of a predator as higher trophic level would change species interactions and trophic structure and allows creating a more comprehensive food web model, resulting in a higher reliability of extrapolations to the natural system. Hence, experimental responses of a model system to the

General discussion

applied treatments can be compared to their control treatments and thus allows to detect development tendencies within the trophic web that could be significant also at the level of the total or natural ecosystem.

Conclusion and Outlook

The Wadden Sea along the coasts of Germany, Denmark and the Netherlands is one of the largest, coherent tidal flat systems in the world. It is highly productive, home for numerous plant and animal species as well as feeding ground for millions of migrating birds. Beyond its outstanding ecological value, it is an important recreational area for humans (Reise et al. 2010). We questioned in this thesis if the newly developed and unique Sylt benthic mesocosm facility is suitable and functional for multiple-stressors, multi-species experiments to investigate the responses of climate change on a Wadden Sea community. After several successful, consecutive running experiments with many useful and reliable results, this facility can be recommended for global climate change experiments on the community level.

The Sylt benthic mesocosm facility was built to investigate the response of a macroalgae-mussel bed community to ocean warming, acidification and enriched nutrients. We showed that the combined effects of warming and acidification decreased the biomass of the main grazing species and the macrophyte *F. vesiculosus* while epiphytes massively increased due to an altered top-down control. Enriched nutrients alone did not affect the system substantially while in combination with warming and acidification the effects of nutrient enrichment become invisible. Especially grazers seem to benefit from enriched nutrient concentrations under OAW. Furthermore, we tried to answer the question, if ocean warming, acidification and enriched nutrients could modify species interactions and functioning of the investigated community. Warming in combination with acidification favored the growth of filamentous epiphytes in combination with or as a result of a reduced grazer biomass. Thus, a bottle neck was created within the energy flow between the first two trophic levels and let less energy passed to higher trophic levels. We tried to answer the question if the response to climate change depends on the investigated season and we found strong indications, that the effects of climate change on the investigated Wadden Sea community strongly varies with on the season. During spring and autumn, the system was less affected or even promoted under OAW with increased energy flows between the trophic levels, while in summer we found the opposite with a decreased energy flow and hampered top-down control. The reduced trophic efficiency could propagate through the whole food web and alter the structure and functioning of the community.

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Curriculum Vitae

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Education

Since 2106 Teacher for biology and mathematics at the Theodor-Fontane-Schule in Menz

Since 2012 PhD thesis, Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Wadden Sea Station Sylt Germany
Thesis: The effects of ocean acidification, warming and enhanced nutrients on a Wadden Sea community - Examined with Mesocosm experiments

2011 Acquisition of the degree: Diplombiologe (diploma biologist)
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2010 - 2011 Diploma thesis: "Tolerance towards abiotic stress in marine benthic species - comparing native and invasive populations of *Magallana gigas*, *Ciona intestinalis* and *Codium fragile*" Within the international research program GAME (Global Approach by Modular Experiments) at the GEOMAR in Kiel, with a six month stay at the Akkeshi Field Science Center for Northern Biosphere, Hokkaido, Japan

2007 - 2010 Continuation of Studies in Biology at the University of Greifswald

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Description of Author contributions

Chapter I: Tidal benthic mesocosms simulating future climate change scenarios in the field of marine ecology

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Author contributions: AP, RA and HA designed the study; AP, and VW collected the data; AP wrote the manuscript with input from all other authors.

Chapter II: Stressed but robust: a benthic assemblage under the interactive effects of acidification, warming and eutrophication.

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Chapter III: Strong seasonal differences in the impacts of climate change on a temperate macrophyte community.

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Eidesstattliche Erklärung

Hiermit erkläre ich, Andreas Pansch, dass die vorgelegte Dissertation:

“The effects of ocean acidification, warming and enhanced nutrients on a Wadden Sea community - Examined with Mesocosm experiments”

von mir, unter Beratung meiner Betreuer, selbständig verfasst wurde, nach Inhalt und Form meine eigene Arbeit ist und keine weiteren Quellen und Hilfsmittel als angegeben verwendet wurden.

Die Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden.

Diese Arbeit wurde an keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt und ist mein erstes und einziges Promotionsverfahren. Kapitel I dieser Arbeit wurde in der wissenschaftlichen Fachzeitschrift *Limnology and Oceanography: Methods* publiziert. Der Anteil der Autoren an den Manuskripten wird im Abschnitt „Description of Author contributions“ erläutert (Seite 147).

Mir wurde noch kein akademischer Grad entzogen.

Stechlin, den 05.08.2019

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Andreas Pansch