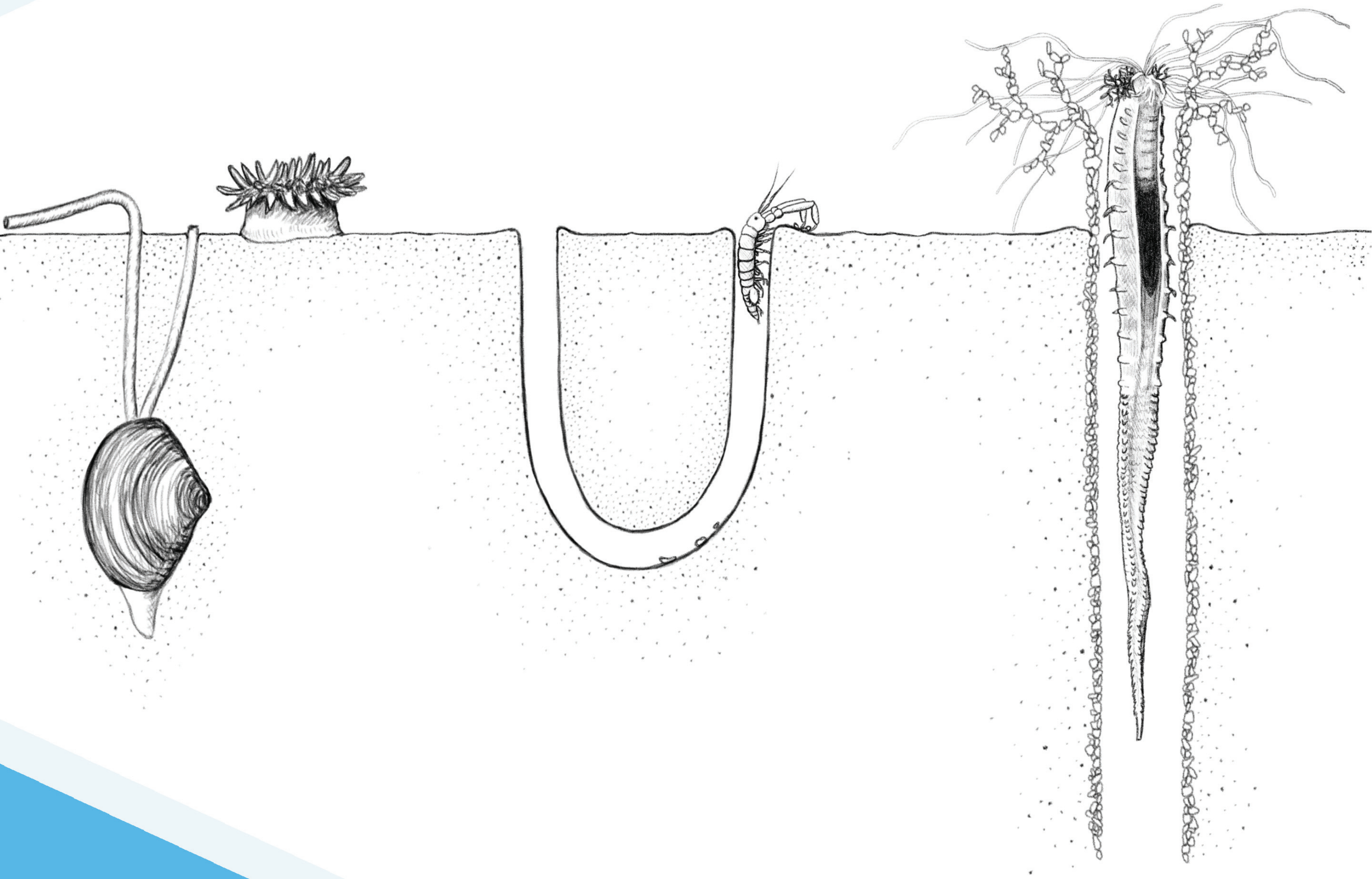
Macrofaunal impact on biogeochemical turnover in German Bight sediments

Alexa Wrede



Universität Bremen

ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR-
UND MEERESFORSCHUNG

Macrofaunal impact on biogeochemical turnover in German Bight sediments

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Dissertation

in fulfilment of the requirements for the

doctoral degree in Natural Sciences (Dr. rer. nat.)

at the Faculty 02 – Biology and Chemistry

of the University of Bremen, Germany

January 2018

Tag des Promotionskolloquiums: 04.04.2018

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Dedicated to my family

Summary

Macrofaunal bioturbation is an important mechanism for the enhancement of remineralization and biogeochemical cycling in marine sediments. Reduction of bioturbation activity may accordingly have far-reaching negative implications for general ecosystem performance. This is especially the case for shallow shelf seas, such as the German Bight, which account for ~ 50% of global benthic remineralization, although they cover only 7% of the total sea surface. Increasing anthropogenic activities (e.g. wind farm construction) in these shallow shelf seas have intensified the need for reliable quantifications and predictions of macrofaunal bioturbation activities and resulting biogeochemical processes. The aim of this thesis was, thus, to develop easily applicable concepts that allow for the quantification of sediment reworking and the prediction of bioirrigation.

In order to simplify the quantification of sediment reworking, which can so far only be assessed experimentally, I compared two of the most commonly applied methods (sediment profile imaging (SPI) and standard slicing technique (ST)). In addition, the time-saving and easily applicable SPI method was tested for its suitability to assess sediment reworking from cylindrical multi-corer samples (*Manuscript I*). The results suggested that SPI is suitable and even more accurate than ST for the investigation of sediment reworking activity. This omits the previously necessary need for time-consuming slicing or the complex transfer into rectangular aquaria. These findings will facilitate studies on spatiotemporal patterns of sediment reworking activity in the German Bight.

Such studies are of special interest as the bioturbation potential (BP_c), which was previously often applied to estimate the potential of communities to rework the sediment, does not correlate with actual sediment reworking rates (*Manuscript II*). Surprisingly BP_c, which includes sediment reworking traits (i.e. mobility and reworking mode) but no specific bioirrigation traits, rather correlated with bioirrigation activity and nutrient fluxes of silicate, ammonium, nitrate, and nitrite (*Manuscript II*).

To overcome ambiguity of BP_c, I developed the irrigation potential (IP_c), as an adaptation from BP_c (*Manuscript III*). By incorporation of bioirrigation effect traits (i.e. burrow type, feeding type, injection pocket depth), IP_c was specifically designed to predict bioirrigation and its influence on biogeochemical processes (*Manuskript III*).

I could demonstrate that, in contrast to BP_c, the modified index provides an accurate quantitative measure of macrofaunal bioirrigation for both single species and entire communities of various infaunal species, if the index is calculated with ash free dry body mass. IP_c provided better estimations of phosphate, silicate, ammonium, nitrate and nitrite fluxes than BP_c (*Manuscript IV*). The estimation of silicate, ammonium, nitrate, and nitrite fluxes may be further increased if IP_c is calculated in wet body mass instead of ash free dry body mass. Wet body mass thereby serves as a proxy of the irrigated sediment volume. In general, IP_c could become a valuable tool to support ecosystem management and future investigations on the effects of anthropogenic activities on biogeochemical turnover in shallow shelf seas.

Findings of *Manuscript IV* however also demonstrated that IP_c is a crucial but insufficient parameter for the modelling of sediment biogeochemical processes because these also dependent on environmental conditions (e.g. temperature, sediment organic matter content, permeability). A newly proposed temperature term (I_{cT}) (*Manuscript III)* may provide a tool to identify spatiotemporal variations in macrofaunal bioirrigation activity. There is however a need to determine further how IP_c or I_{cT} relate to biogeochemical cycling under different environmental conditions as well as how the respective macrofaunal traits are affected by environmental parameters.

Zusammenfassung

Die Bioturbationsaktivitäten der benthischen Macrofauna können die Remineralisierung von organischem Material und Nährstoffflüsse in marinen Sedimenten bedeutend verstärken. Eine Reduzierung von Bioturbationsaktivität kann dementsprechend weitreichende negative Folgen für die generelle Ökosystem Leistung haben. Besonders wenn die Reduzierung von Bioturbationsaktivität flache Schelfmeere betrifft, kann dies erhebliche Konsequenzen haben, denn flache Schelfmeere tragen ~ 50% zur globalen benthischen Remineralisierung bei obwohl sie nur 7% der gesamten Meeresfläche ausmachen. Da jedoch eben jene Schelfmeere besonders von den wachsenden anthropogenen Aktivitäten (z. B. Bau von Offshore-Windparks) betroffen sind, ist es dringend nötig, die Quantifizierung und Vorhersage von Bioturbationsaktivität sowie deren Einfluss auf biogeochemische Prozesse zu erleichtern. Es war daher das Ziel dieser Doktorarbeit einfach anwendbare Konzepte zur Quantifizierung von biogener Sedimentdurchmischung und Vorhersage von Bioirrigationsaktivität zu entwickeln.

Die biogene Sedimentdurchmischung kann bisher nur experimentell gemessen werden. Um ihre Quantifizierung zu vereinfachten habe ich zwei der am häufigsten verwendeten Methoden verglichen (sediment profile imaging (SPI) und slicing technique (ST)) (*Manuskript I*). Des Weiteren habe ich getestet, ob die zeitsparende und leicht einsetzbare SPI-Methode das Potential hat, auch an Multi-Corer-Zylindern die biogene Sedimentdurchmischung per Fotografie zu bestimmen. Die Ergebnisse zeigten, dass eine Messung von biogener Sedimentdurchmischung mit der SPI-Methode an Multi-Corer Zylindern nicht nur möglich ist, sondern dass sich die Genauigkeit aller gemessenen Parameter (mit Ausnahme der maximalen Durchmischungstiefe) gegenüber der ST-Methode erhöhte. Diese Ergebnisse sollten für zukünftige Studien der Sedimentdurchmischungsaktivitäten in der Deutschen Bucht eine nützliche Grundlage darstellen, um zeitliche und räumliche Muster zu untersuchen.

Solche Untersuchungen sind von besonderem Interesse, da das bisher häufig angewandte und für ebensolche Zwecke entwickelte Bioturbationspotential (BP_c) nicht mit gemessenen Sedimentdurchmischungsaktivitäten korreliert (*Manuskript II)*. Stattdessen zeigte sich eine Korrelation mit der Bioirrigationsaktivität und den Nährstoffflüssen von Silikat, Ammonium, Nitrat und Nitrit. Dies war der Fall, obwohl BP_c keine funktionellen Eigenschaften beinhaltet, welche die Bioirrigation beschreiben.

Stattdessen enthält BP_c funktionelle Eigenschaften, welche die Sedimentdurchmischung beschreiben.

Um diese Widersprüchlichkeit im BP_c zu überwinden, wurde das Irrigation Potential (IP_c) als Adaption und Erweiterung des BP_c entwickelt (*Manuskript III*). Durch den Austausch der im Index inkludierten Sedimentdurchmischungseigenschaften durch Merkmale, die die Bioirrigation direkt beschreiben (Art der Wohnröhre, Ernährung Typ, Wasserinjektionstiefe), konnte ich einen Index kreieren, der, wenn er mit aschefreiem Trockengewicht berechnet wird, die quantitative Vorhersage von Bioirrigation in Monokulturen benthischer Tiere sowie in Gemeinschaften ermöglicht. Des Weiteren zeichnete sich IP_c durch eine höhere Vorhersagegenauigkeit von Phosphat-, Silikat-, Ammonium-, Nitrat- und Nitrit-Füssen über die Wasser-Sediment Grenze aus (*Manuskript IV*). Die Vorhersagegenauigkeit von Silikat-, Ammonium-, Nitrat- und Nitrit-Füssen konnte noch weiter erhöht werden, wenn das Körpervolumen der Organismen über ihr Nassgewicht mit in die Berechnung einbezogen wurde. Insgesamt sollte IP_c daher ein wertvolles Werkzeug zur Abschätzung von anthropogenen Effekten auf die Bioirrigation darstellen.

Die Ergebnisse von *Manuskript IV* zeigten jedoch auch, dass IP_c allein keine quantitativen Vorhersagen von biogeochemischen Prozessen erlaubt, da diese von vielen weiteren Umweltbedingungen abhängen (wie z.B. Temperatur, Gehalt an organischem Material im Sediment, Permeabilität). Um zumindest den Einfluss von Temperatur auf die Bioirrigationsaktivität abzuschätzen, wird in *Manuskript III* eine temperaturabhängige Erweiterung (I_{cT}) von IP_c vorgeschlagen. Diese Erweiterung könnte nach einer genauen Validierung zeitliche und räumliche Untersuchungen der Bioirrigationsaktivität unterstützen. Allerdings ist es weiterhin notwendig zu untersuchen, wie IP_c (oder I_{cT}) mit biogeochemischen Prozessen unter unterschiedlichen Umweltbedingungen zusammenhängt und wie die funktionellen Eigenschaften, die IP_c charakterisieren, im Zuge biotischer und abiotischer Effekte variieren.

Content

Abbreviations & Acronyms

AFDM	Ash free dry mass
AIC	Akaike's Information Criterion
AICc	AIC corrected for small sample sized
ANOVA	Analysis of Variance
BP_c	Community bioturbation potential
BP_i	Species bioturbation potential
Br^-	Bromide
D_b	Sediment reworking rate
EEZ	Exclusive Economic Zone
$^{exp}BP_c$	Experimental bioturbation potential
Full-SPI	Sediment profile imaging – full resolution
GLM	Generalized linear model
I_{cT}	Temperature adjusted bioirrigation activity
IP_c	Community irrigation potential
$IP_{c,AFDM}$	IP_c calculated with ash free dry mass
$IP_{c,WM}$	IP_c calculated with wet mass
Lum_{max}	Maximum luminophore burial depth
Lum_{mean}	Mean weighted luminophore burial depth
mIP_c	Modified irrigation potential
MSFD	Marine Strategy Framework Directive
NH_4^+	Ammonium
NLI	Non locality index
NO_2^-	Nitrite
NO_3^-	Nitrate
OM	Organic matter

PO_4^{3-}	Phosphate
SiO_2	Silicate
Reduced-SPI	Sediment profile imaging – reduced resolution
SPI	Sediment profile imaging
ST	Slicing technique
WM	Wet mass

1. Introduction

“It may be doubted whether there are many other animals which have played so important a part in the history of the world, as these lowly organized creatures”

Charles Darwin, 1881

This statement at the end of Charles Darwin’s last book *On the Formation of Vegetable Mounds through the Action of Worms with Observation of their habits* may seem exaggerated. However, it is nowadays common agreement that Darwin, who was the first to realize the profound impact of burrowing macrofaunal organisms on geomorphology and biogeochemical processes of soils, was not too far away from the truth (Meysman et al., 2006a). Many recent studies have demonstrated that key ecosystem services, such as biogeochemical cycling, remineralization, soil formation, soil fertility, oxygen, and water regulation are largely influenced by the bioturbation activities of living organisms (de Bello et al., 2010; Kristensen et al., 2012; Valença et al., 2017; Wilkinson et al., 2009). It is, thus, widely accepted that bioturbation is an archetypal example for ecosystem engineering (Meysman et al., 2006a). Bioturbation directly or indirectly modulates the availability of resources for other organisms as it creates, maintains and modifies habitats. Therefore, it fulfils all criterions of the definition for ecosystem engineering (Jones et al., 1994). Bioturbating organisms can be found in nearly all sediments or soils that cover the surface of the Earth. Accordingly, there are only few other examples of ecosystem engineering that influence as many ecosystems as bioturbation (Darwin, 1881; Meysman et al., 2006a).

1.1 What is bioturbation?

Bioturbation is defined as all transport processes, carried out by animals, that directly affect sediment matrices; including both particle and water transport (Kristensen et al., 2012). Depending on the influenced medium (i.e. particles or water), bioturbation may be subdivided into two categories of activities: (1) the sediment reworking actives which facilitate particle mixing (e.g. construction of tubes and burrows, in- or egestion of particles), and (2) the bioirrigation activities which transport water in and out of the sediment matrix (e.g. ventilation, sub-surface filter-feeding, burrow flushing) (Kristensen et al., 2012). This categorization of activities is necessary in order to assess the effects of macrofaunal behaviors on their surrounding environment. However, the line between sediment reworking and bioirrigation is often unclear.

1.2 Why is bioturbation important for biogeochemical processes?

Sediment reworking activities are one of the most important transport processes for organic matter in sediments (Meysman et al., 2006a; Middelburg et al., 1997). The redistribution of sediment particles by macrofaunal organisms can bury fresh organic matter in deeper anoxic layers or transport buried matter to oxygenized surface sediments (Giangrande et al., 2002; Meysman et al., 2006a). Yet, it is bioirrigation which is in aquatic environments, more important for the enhancement of biogeochemical processes, such as remineralization and nitrification (Braeckman et al., 2014; Mermillod-Blondin et al., 2004). In contrast to terrestrial soils, subtidal aquatic sediments are permanently covered with water. In water, the partial pressure of oxygen is about 30 times lower than in air and oxygen diffusion is about 10 000 times slower. Accordingly, beneath a thin layer, which is oxygenized through diffusion, aquatic sediments are often oxygen depleted (Glud, 2008; Kristensen et al., 2012). Hence, bioirrigation of infaunal organisms is a mechanism that improves oxygen supply to sustain basic metabolic demands (Kristensen et al., 2012).

In the absence of bioirrigation, the bacterial degradation of organic matter causes a vertical depletion of electron acceptors (Bertics et al., 2010; Gilbert et al., 1995; Glud, 2008). The amount of energy, which bacteria can gain from the organic matter degradation with a certain electron acceptor, determines the order in which electron acceptors are depleted (Glud, 2008). As most energy can be gained from oxygen, a stratified sediment is created with a thin oxygenated upper layer of nitrification, followed by anoxic zones of denitrification and sulfate reduction below (Bertics et al., 2010; Gilbert et al., 1995; Glud, 2008). The thickness of the layers may vary strongly with the environmental conditions (i.e. sediment permeability, wave action, oxygen partial pressure in the overlying water column). Bioirrigation disrupts this stratification by introducing oxygenated water into otherwise anoxic layers. A fraction of the oxygenated water is transported from the ventilated burrow into the surrounding sediment by diffusion or advection where it 1) influences biogeochemical processes,

2) provides electron acceptors for remineralization, 3) increases the zone of nitrification, 4) removes metabolites, 5) alters concentration gradients, and 6) transports nutrients and dissolved organic matter (Aller, 1994; Christensen et al., 2000; Koo et al., 2007; Na et al., 2008).

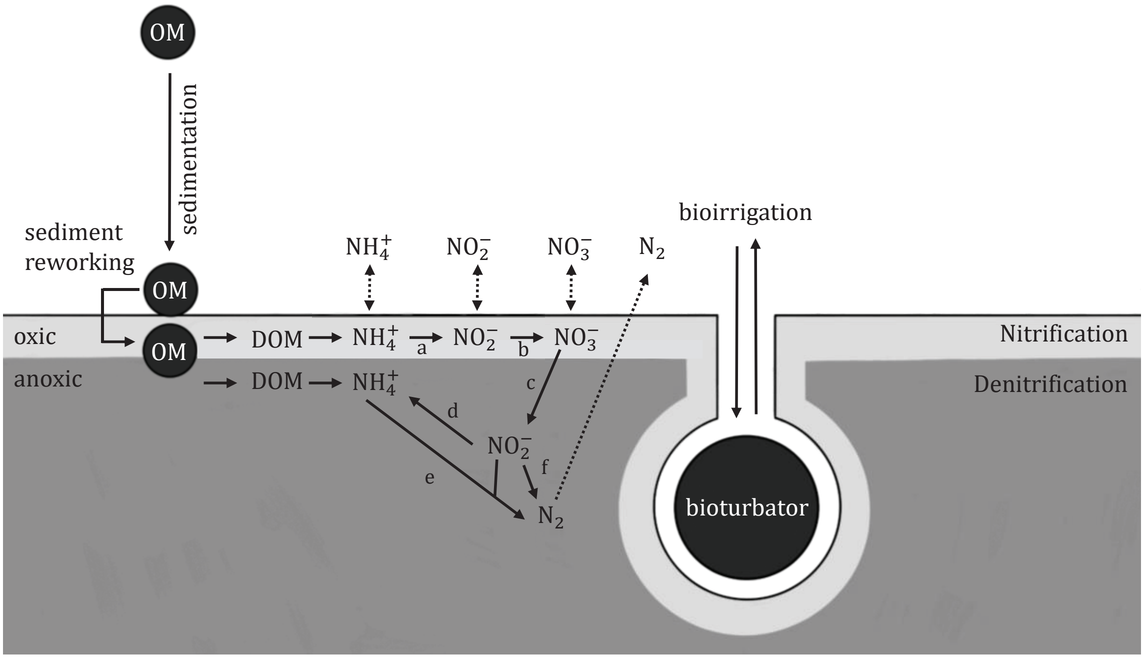

Fig 1. The influence of bioturbating organisms on the nitrogen cycle and the stratification of marine sediments: Nitrification, consisting of ammonia oxidation (a) and nitrite oxidation (b), can only occur in the oxic regions of the sediment, while denitrification (c + f), dissimilatory reduction of nitrite to ammonium (DNRA) (d), and anaerobic ammonium oxidation (anammox) (e) occur only in the absence of oxygen. Bioirrigation introduces oxygenated water and, thus, increases the zone of nitrification, whereas sediment reworking transports organic matter within the sediment. Broken arrows indicate fluxes into or out of the sediment (adapted from Bertics et al. (2010); Glud (2008); Laverock et al. (2011)).

1.3 Why is it important to assess bioturbation in the German Bight?

The loss of macrofaunal activity may have broad ecological and biochemical implications for ecosystem functioning (i.e. remineralization, benthopelagic coupling, primary production) (Lohrer et al., 2004). This is especially the case for coastal shelf seas, such as the German Bight. Due to the shallow water depth in shelf seas, up to 50% of the organic matter produced by pelagic primary production reaches the sea floor, where it is mixed into the sediment and remineralized. In contrast, in the open ocean, the products of primary production are mainly respired in the water column (Jørgensen, 1983; Middelburg et al., 1997; Provoost et al., 2013).

Therefore, coastal shelf sea sediments (0-200m water depth) account for approx. 50% of the global benthic remineralization despite, covering only 7% of the ocean surface (Middelburg et al., 1997). Due to the proximity to land and low water depths, shelf sea systems are exceptionally heavily impacted by increasing human activities (e.g. fisheries, offshore windfarm construction, underwater noise) (Coates et al., 2014; Rijnsdorp et al., 1998). These anthropogenic activities may lead to changes in bioturbation activity. Noise that is emitted by ships or construction activities, may, for example, reduce sediment reworking activities (Solan et al., 2016) while intensive fisheries may directly cause shifts in the composition of communities - from strongly bioturbating species to smaller opportunistic species which tend to have a lower impact on biogeochemical cycling (Hale et al., 2017).

1.4 Are there models to predict bioturbation?

So far, reliable models, that allow for the prediction of broad scale changes in macrofaunal bioturbation activities (caused by shifts in functional community composition and/or species extinction), are lacking. There is no reliable model to extrapolate sediment reworking activities. Estimations of sediment reworking activities, thus, still depend on experimental measurements. However, measurements of subtidal in situ communities are sparse as well, because the common experimental methodology for quantification of in situ sediment reworking is a very time consuming task (Gilbert et al., 2003; Maire et al., 2008; Solan et al., 2004b).

There are some models that describe bioirrigation activities. However, the existing models are either only phenomenological descriptions, that lack a connection to the underlying biology (i.e. diffusive, non-local, and advective one-dimensional models (Meysmann et al. 2006c)), or they rely on uncommon, specific parameters which are known for only few species (e.g. pumping rate, burrow volume, specific burrow morphology) (i.e. see the mechanistic models of Aller (1980) and Meysman et al. (2006b)). In order to provide large scale predictions of bioturbation activities to support management and policy decisions, models should be easily applicable and give realistic predictions for all macrofaunal species and communities.

In an attempt to account for this deficiency, Solan and co-authors (2004a) proposed the trait based non-quantitative concept of community bioturbation potential (BP_c). Traits are defined as functional characteristics of organisms (e.g. size, feeding type, fertility). They can be subdivided into 1) response traits, which determine the ecological niche of organisms as well as their response to disturbances, and 2) effect traits, which describe all characteristics of an organism that influence the functioning of the ecosystem around it (Díaz and Cabido, 2001). Accordingly, effect traits are well suited to estimate the extent of macrofaunal impact on biogeochemical processes. BP_c uses information on sediment reworking mode and mobility of each species together with abundance and biomass data to estimate the potential of the community to rework the sediment (Solan et al., 2004a).

BP_c has been shown to correlate with total organic carbon content, chlorophyll concentrations, oxidation depth, sediment oxygen consumption, ammonium efflux, and denitrification (Birchenough et al., 2012; Braeckman et al., 2014; Solan et al., 2004a). This indicates that BP_c provides an estimate of how macrofaunal organisms modulate biogeochemical cycling and remineralization. Accordingly, many studies consider BP_c as a good tool to support management and policy decisions (Queirós et al., 2013). Unfortunately, BP_c also has limitations; due to its non-quantitative nature, it is neither able to provide definite values of bioturbation activity, nor to assess bioturbation activity true to scale (i.e. double BP_c does not imply double bioturbation activity). Furthermore, BP_c only includes sediment reworking traits and neglects traits that describe bioirrigation activity (Braeckman et al., 2014). However, it is mostly the bioirrigation activities that influence biogeochemical processes (Braeckman et al., 2014; Mermillod-Blondin et al., 2004). Accordingly, an index based on bioirrigation traits may increase the predictability of human-induced macrofaunal impacts on biogeochemical turnover.

2. Aims and Objectives

The aim of this thesis was to facilitate the quantification and prediction of macrofaunal bioturbation activity and its influence on biogeochemical processes in shelf sea areas such as the German Bight. To meet this aim, the four objectives of this thesis were (i) to facilitate the measurement of sediment reworking rates in in situ communities (*Manuscript I*), (ii) to test the applicability of the existing trait-based concept of community bioturbation potential (BP_c) for the identification of bioturbating key species in the German Bight (*Manuscript II*), (iii) to develop a trait-based irrigation model and to relate it to actual irrigation rates (*Manuscript III*), and (iv) to link the irrigation model to biogeochemical cycling in German Bight sediments (*Manuscript IV*).

Manuscript I

Spatial accuracy is crucial in the quantification of bioturbation – a comparison of methods

Sediment reworking is the most important transport process of organic matter in the sedimentary system. Through particle displacement, organisms may burry organic matter or change permeability and erodibility of the sediment. To date, sediment reworking can only be quantified through experiments. Two of the most commonly applied methods use the displacement of luminophore tracers within sediment columns to quantify bioturbation. Tracers may be recovered either by slicing of sediment cores and subsequent quantification of tracers in different horizons or by sediment profile imaging (SPI) of flat surfaced aquaria. In contrast to the SPI method, the slicing technique can be applied in cylindrical in situ cores which are easily taken by a multi-corer. However, the slicing of the cores is time consuming and provides a lower resolution of particle displacements. Accordingly, it was my aim to compare the two methods and improve the applicability of the SPI method by testing whether this method can also be applied to cylindrical multi-corer samples.

Manuscript II

Who really matters: Influence of German Bight key bioturbators on biogeochemical cycling

Biogeochemical cycling in coastal marine sediments depends largely on the bioturbation activities of macrofaunal organisms. The loss of key species (i.e. species that substantially enhance biogeochemical cycling through their specific activities) may consequently affect biogeochemical cycling. Therefore, my major aim was to identify key species in the German Bight and to quantify their impact on biogeochemical cycling. As the impact of the organisms on ecosystem processes is determined by their effect traits, I applied the trait-based concept of community bioturbation potential (BP_c) to identify key species. To test the performance of the BP_c, the trait-based predictions were compared with measured bioturbation rates in laboratory experiments with select species.

Manuscript III

Organism functional traits and ecosystem supporting services – a novel approach to approximate bioirrigation

Marine benthic biogeochemical processes depend on bioirrigation rather than on sediment reworking. However, BP_c includes only sediment reworking traits. Accordingly, it was my aim to develop a trait-based irrigation index which includes effect traits that describe bioirrigation. Based on literature information, I developed the irrigation potential (IP_c) as an adaptation of BP_c. IP_c was validated with data on bioirrigation rates of single species and from multi-species assemblages in a multi-factorial experiment. The organisms were representative faunal elements of three different sediment types of the German Bight.

Manuscript IV

Macrofaunal irrigation traits enhance predictability of biogeochemical cycling

Biogeochemical processes in marine sediments depend largely on the irrigation activity of the inhabiting fauna. However, the impact of the fauna on biogeochemical processes may differ depending on the respective environmental conditions. Accordingly, it was my aim to assess if IP_c can accurately predict the macrofaunal impact across variable environmental conditions. In controlled experiments, I measured the changes in concentration of phosphate, silicate, ammonium, nitrate, and nitrite over eight hours in nine different treatments that differed in community composition, temperature, sediment type, and nutrient gradients across the sediment water inter-face and assessed if IP_c was able to predict nutrient fluxes. Furthermore, I tested if IP_c increased the predictability of these nutrient fluxes compared to BP_c.

3. Manuscript contributions

Manuscript I

Spatial accuracy is crucial in the quantification of bioturbation – a comparison of methods

Alexa Wrede, Jan Holstein, Thomas Brey

AW conceived and designed the study. AW performed the experiment and analyzed the data. JH assisted with the modelling of biodiffusive transport. AW wrote the paper with substantial contributions from TB and JH.

The manuscript was submitted for publication to the *Journal of Experimental Marine Biology and Ecology* in January 2018.

Manuscript II

Who really matters: Influence of German Bight key bioturbators on biogeochemical cycling

Alexa Wrede, Jennifer Dannheim, Lars Gutow, Thomas Brey

The study and experiments were conceived by AW, JD, LG, and TB. AW mapped the bioturbation potential, performed the experiments and analyzed the data. AW wrote the manuscript with support from JD, LG and TB.

The manuscript was published in *Journal of Experimental Marine Biology and Ecology*, DOI 10.1016/j.jembe.2017.01.001; published online 07. January 2017.

Manuscript III

Organism functional traits and ecosystem supporting services – a novel approach to approximate bioirrigation

Alexa Wrede, Jan Beermann, Jennifer Dannheim, Lars Gutow, Thomas Brey

AW, LG and TB developed the irrigation potential. TB developed the temperature dependent expansion of the irrigation potential. The study and experiments were conceived by AW, JD, LG, and TB. AW performed the experiments. AW analyzed the data with the help of JB. AW wrote the manuscript with support from JB, JD, LG and TB.

The manuscript was submitted for publication to *Ecological Indicators* in September 2017 and is currently under review.

Manuscript IV

Macrofaunal irrigation traits enhance predictability of biogeochemical cycling

Alexa Wrede, Ragnhild Asmus, Harald Asmus, Karen Helen Wiltshire, Thomas Brey

The study and experiments were conceived by AW, RD, HG, KHW and TB. AW performed the experiments and analyzed the data. AW wrote the manuscript with support from RD, HG, KHW and TB.

The manuscript is in preparation for submission to *Marine Ecology Progress Series*.

4. Manuscripts

Spatial accuracy is crucial in the quantification of sediment reworking - a comparison of methods

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The manuscript was submitted for publication to the *Journal of Experimental Marine Biology and Ecology* by January 2018 and is currently under review.

Abstract

Macrofaunal sediment reworking activity is a key driver of ecosystem functioning in marine systems. So far it can only be assessed experimentally. This study compares the two most common applications of the luminophore tracer technique: the slicing technique (ST) and the sediment profile imaging approach (SPI). Since its introduction SPI gradually replaced the ST, albeit with two shortcomings: SPI has to date been applied to flat-surfaced (i.e. rectangular) cores only, and a thorough comparison of methods is lacking. Here we apply both methods to cylindrical sediment cores (10 cm diameter), in particular to test the suitability of SPI to sampling devices of the multi-corer type. Cores were taken from mud and fine sand habitats inhabited by *Nucula*- and *Amphiura*-communities in the southern German Bight. We found significant differences between the methods for all investigated sediment reworking parameters: sediment reworking rate, non-locality index, mean weighted luminophore depth, and the maximal luminophore depth. Consequently, the conclusions drawn differ distinctly, too. The ST method failed to show differences between mud and fine sand communities, owing to insufficient spatial resolution and accuracy. The SPI method, on the other hand, did not capture the full extent of maximal sediment reworking depth. We conclude that the ST method is preferable for investigations focusing on the absolute maximal sediment reworking depth, whereas general sediment reworking patterns are better resolved with the SPI method. There was no indication that the curvature of the cylinder walls (diameter 10 cm) caused an underestimation of sediment reworking parameters. Our findings, thus, indicate that SPI is suitable for the investigation of sediment reworking in natural communities by means of multi corer type samples.

Keywords: bioturbation, luminophore tracer technique, sediment profile imaging

1. Introduction

In recent years, there has been increased attention on metazoan impact on the functioning of sedimentary systems. Through burrowing, ventilation, ingestion and defecation activities benthic organisms actively induce a bio-mixing of sediment particles, a transport of particulate organic matter (POM) and an enlargement of the sediment water interface (Kristensen et al., 2012). Accordingly, bioturbators have a strong influence on the functioning of the benthic system and the overlying water body (Lohrer et al., 2004). However, thus far, reliable quantitative models for assessing sediment reworking are lacking (Queirós et al., 2015; Wrede et al., 2017). Thus, the experimental determination of sediment reworking rates remains indispensable when trying to understand biological sediment turnover (Wrede et al., 2017).

There are many different methods to quantify sediment reworking rates in bioturbation research (Maire et al., 2008). A commonly used approach is the luminophore tracer technique by Mahaut and Graf (1987) where fluorescent sand grains applied on a sediment surface are redistributed within the sediment matrix through the sediment reworking activity of the inhabiting fauna (Mahaut and Graf, 1987). The common procedure for assessing the vertical luminophore profile is the slicing of a sediment core into different depth strata (i.e. standard slicing technique (ST)) (Maire et al., 2008). The amount of luminophores per layer is subsequently counted under UV light in the dried and homogenized sediment samples (Mahaut and Graf, 1987; Maire et al., 2008). This technique is very time consuming, has a limited spatial resolution, does not allow for a temporal investigation of sediment reworking patterns, and is error-prone due to the unintentional displacement of luminophores during the slicing process (Gilbert et al., 2003; Maire et al., 2008; Solan et al., 2004).

The sediment profile imaging (SPI) method overcomes these limitations. SPI combines time-laps photography of flat-surfaced aquaria or in situ sediment with automated image analysis (Gilbert et al., 2003; Solan et al., 2004). The drawback of this method, however, is that all information on the 3-D process of sediment reworking is derived from the 2-D profile that presents itself at the aquaria walls. This profile may be misleading due to potential wall effects, i.e., organisms may bioturbate away from the walls. Accordingly, the SPI method has a “blind spot” that is a function of core diameter, photographed surface area and animal behavior. However, for very narrow aquaria (width 1.2 cm) with a small volume to surface ratio, the "blind spot", is very small and a potential distortion of the luminophore distribution through wall-effects was shown to be negligible (Maire et al., 2006). Such narrow aquaria, however, limit the size of the experimental organisms. Consequently, recent studies have used SPI on quadratic aquaria and larger incubation chambers (Murray et al., 2014; Murray et al., 2013; Queirós et al., 2015). So far it has not been tested whether information deduced from SPI is able to reliably represent the biogenic processes in incubation chambers with a larger blind spot (i.e. a higher volume to surface ratio) (Maire et al., 2008).

Another apparent drawback of the SPI method is the need for a rectangular or flat-surfaced design of the incubation chambers, to provide a flat, undistorted photographic plane. In many situations, it is not possible or not desirable to use flat-surfaced aquaria, e.g., in situ community samples are taken by cylindrical cores quite often (multi corer), or cores need to be sealed air tight for oxygen consumption measurements. SPI has not yet been applied in such cases, as the curvature of the cylinders causes a distortion of the objects displayed at the edges of the cylinders. Towards the edges of the cylinder, the distance of objects increases, and, hence their displayed size decreases. This may cause an underestimation of sediment reworking parameters. Consequently, the tedious and error prone core slicing method or a complex transfer of community samples into rectangular aquaria was so far applied in such cases.

This study evaluates (i) whether SPI is also suitable for cylindrical sediment cores and (ii) whether SPI is able to reliably assess macrofaunal sediment reworking activity in cores with a higher volume to (photographed) surface area ratio. We hypothesize that the distortion in the SPI images, from curved cylinder walls (10 cm diameter), is negligible and will not affect the quantification of sediment reworking activity. In case an effect is observed, we hypothesize an underestimation of sediment reworking. Further, we hypothesize that although the SPI method will not be able to capture the full extent of sediment reworking activity due to its larger "blind spot", it will provide a more accurate depiction of small dislocations of particles due to its higher spatial resolution. To assess spatial resolution effects, we treated the SPI data in two different ways (reduced-SPI: reduced spatial resolution identical to ST, full-SPI: full spatial resolution of the SPI data). We thereby created a third method (i.e. reduced-SPI) that we compare to both ST and full-SPI.

2. Material and Methods

2.1 Sediment core sampling for laboratory experiments

In April 2016, Sediment cores (height: 35 cm, inner diameter: 9.0 cm) were sampled by ship from mud and fine sand habitats in the southern German Bight (54°7’21’’N 8°12’96’’E; 54°0’50’’N 7°48’51’’E). The muddy habitat was inhabited by a *Nucua nitidosa*-community (Salzwedel et al., 1985) and the fine sand habitat by an *Amphiura filiformis*-community (Salzwedel et al., 1985). From each of these communities, 10 cylindrical in situ cores (h: 35 cm, d: 9.4 cm) were taken. The cores were carefully extracted from 0.1 m² box cores to maintain an undisturbed sediment column. The cores were then transferred to a climate controlled room (8°C) on board of RV Heincke. The water was exchanged once a day. After 5 days, the cores were transferred to a climate controlled room (8°C) at the Wadden Sea Station of the Alfred Wegener Institute and connected to a continuous flow of filtered seawater (30 µm drumfilter: Spranger; protein skimmer: Sander) from the Sylt-Rømø Bight. Seven days after the sampling, the experiment was launched by pouring a homogenized suspension of luminophores (green color, 80-250 µm, 4 g and pink color, 60 µm, 4 g; Partrac Ltd. U.K.) evenly over the sediment surface. After eight days of incubation, sediment reworking was quantified by sediment profile imaging (SPI) (Gilbert et al., 2003). Images of the sediment column were taken under black light (Phillips, TL-D 18W BLB 1SL) from a defined distance through the glass wall of the cores (Camera: Canon Eos 500D, Canon EF-S 15-85 mm, f/3.5-5.6 IS USM). To test for the variance of this procedure, two pictures were taken per core from the opposite sides of the cores.

Due to time restrains, the slicing technique (ST) had to be conducted one day later. In accordance with Fernandes et al. (2006) and own preliminary experiments (Wrede, 2014) no significant change in the sediment reworking parameters within 24h was assumed. For the ST slicing procedure, the sediment cores were carefully pressed out of the cylinders and sliced into 0.5 cm sections from 0-3 cm depth, 1 cm sections from 4-6 cm depth, 2 cm sections from 6-10 cm depth and 4 cm sections from 10-18 cm depth from which the organisms were manually retrieved. During the slicing process, three cores were lost as they could not be retrieved from their plastic cylinders. Replicate number was thus n = 9 for the *Amphiura*-community and n = 8 for the *Nucula*-community. The sediment of each section was dried for 72 hours at 40°C and homogenized with a mortar and pestle. The homogenized sediment was partitioned into three equal subsamples, which were each spread evenly in a rectangular petri dish (4x4 cm). Digital images of the surface of each subsample were taken (Canon Eos 500D, Sigma 150 mm, F2.8 EX APO DG Makro HSM) under black light (Phillips, TL-D 18W BLB 1SL).

2.2 Image analysis

SPI images were cut to the same size (height: 10 cm, width: 9 cm) with the image analysis software Image J (1.48) (http://imagej.nih.gov/ij/index.html) and scaled. The water column was manually tinted with a preselected uniform marking colour (RGB: 253,003,155) with Adobe Photoshop CS6. The tinted water column was later identified by a custom-made plugin for the software ImageJ. The plugin was used to remove the tinted water column and to smooth the sediment surface so that the sediment-water interface served as the x-axis in a coordinate system at $y = 0$ of an inverse ordinate (i.e. the vertical sediment column) (Wrede et al., 2017). After identifying the luminophores using the threshold function of ImageJ, the image was converted into black and white. The black pixels, representing the luminophores were counted for each pixel row and transferred into a profile of the luminophore distribution.

For the images of the ST samples, luminophorous pixels per area were counted using a custom-made plugin for the image analysis software Image J (1.48) (http://imagej.nih.gov/ij/index.html). For each core, the average number of luminophorous pixels per section was calculated from the three subsamples.

For both methods the luminophorous pixel counts of the core profiles were converted into relative slice-specific concentrations by dividing the number of luminophorous pixels in each slice by the total number of luminophorous pixels in all slices from a core.

2.3 Impact of spatial resolution

Although the ST and SPI method differ in their spatial resolution (mean slice thickness: ST: 0.9 cm; SPI: 0.0064 cm) we chose the lower border of each slice or pixel row as representative depth for the luminophore concentrations for both methods. This means that all data on luminophores within a slice or pixel row was summed up and said to be located at the lower boarder of the slice or pixel row. Thus, all luminophores are treated similarly whether they are at a top of a slice or at its bottom. In the ST, this treatment may, however, result in high luminophore concentrations apparently “moving” over a notable distance (e.g. high luminophore concentration at 6.5 cm will be assigned to a depth of 8 cm). Unfortunately, it does not matter where the representative depth is located in a slice or pixel row, the procedure is always accompanied with an artificial “moving” of particles.

In order to assess whether differences between the two methods were due to this artificial “movement” and therefore due to the spatial resolution of the methods, the SPI profile data was treated in two different ways. For the first treatment (reduced-SPI), the profiles were reduced to the spatial resolution of the slicing technique (ST), whereas for the second treatment (full-SPI), the SPI profile was analyzed in its full resolution. The sediment reworking parameters sediment reworking rate, non-local transport, maximal sediment reworking depth, and mean sediment reworking depth where then determined for all three treatments (i.e. ST, reduced-SPI, full-SPI). A lack of differences between ST and reduced-SPI would provide an indication that a lower spatial resolution drives differences in sediment reworking parameters. If we, however, observe differences between ST and reduced-SPI it would be an indication that not only the lower spatial resolution sets ST and full-SPI apart.

2.4 Sediment reworking rate

The sediment reworking rate D_b was estimated as described in Wrede et al. (2017) using the one-dimensional diffusion model (Crank, 1975) for conservative tracers, such as luminophores, in the absence of sedimentation.

$$C(x,t) = \frac{N}{\sqrt{\pi D_b t}} \, exp\left(\frac{-x^2}{4D_b t}\right) \tag{1}$$

where $C(x,t)$ = the normalized tracer concentration relative to the initial input, t = time (years), x = depth (cm), N = the initial luminophore input and D_b = the biodiffusion coefficient, which is the common measure for the sediment reworking rate. The initial luminophore concentration N was estimated from the normalized profile data as:

$$N = \sum_i n(a_i) * z(a_i) \tag{2}$$

where $n(a_i)$ = the relative concentration of luminophores in the slice a_i and $z(a_i)$ = the thickness of the slice a_i in cm. N was equal to 0.36.

Non-local (i.e. non-diffusive) transport was calculated using the non-locality index (*NLI*) proposed by Fernandes et al. (2006). This requires the calculation of the sediment reworking rate D_b using the actual tracer concentration $C(x,t)$ and of D_b^{log} using the log-transformed tracer concentration: log($C(x,t)$). The log-approach gives more weight to the lower concentrations. The *NLI* is, thus, computed as:

$$NLI = \frac{\left|D_b^{log} - D_b\right|}{\sqrt{D_b^{log} * D_b}} \tag{3}$$

where $NLI = 0$ ($D_b^R = D_b^L$) indicates no non-local transport. The mean luminophore burial depth (Lum_{mean}) was assessed from profiles by weighting each depth by the luminophore concentration in that respective depth. For the SPI images maximum luminophore depth (Lum_{max}) was assessed by measuring the deepest luminophore signal in ImageJ, whereas for the ST slicing method, maximum luminophore depth (Lum_{max}) was assumed to be similar to the deepest slice in which luminophores could still be detected.

2.5 Statistical analysis

An assessment of the differences between the three methods in the response variables of sediment reworking (sediment reworking rate D_b, *NLI*, Lum_{max}, Lum_{mean}) was carried out with linear mixed models. Fixed effects were method (i.e. ST, reduced-SPI, full-SPI) and treatment (i.e. *Amphiura*-community, *Nucula*-community) with no interaction term. Random effects were intercepts for core and sample, which nested in method (i.e. the two samples of the SPI method). Tukey's tests were performed to assess the differences between the methods. The variability of the SPI methods was estimated using a mixed model. The method (i.e. reduced- and full-SPI), treatment, and sample are fixed effects without an interaction term and intercepts for core was the random effect.

In order to decide if differences in sediment reworking parameters were due to the luminophore depth distribution, we compared the luminophore concentrations per slice of the ST and the reduced-SPI method using a nonparametric Mann Whitney t-test (Graph Pad Prism 5, GraphPad Software, Inc.).

To assess whether the application of the different methods would lead to different conclusions about differences in sediment reworking rate D_b, *NLI*, Lum_{max} and Lum_{mean} between the *Amphiura*-community and *Nucula*-community, we applied further generalized linear models. The two samples of both SPI methods were averaged, thus, the replicate number was similar for all three methods.

Visual inspection of residual plots revealed obvious deviation from homoscedasticity and normality for the sediment reworking rate D_b. Accordingly, the log-transformed sediment reworking rates were used for the analysis. P-values of the mixed models were obtained by likelihood ratio tests of the full model including method as fixed effect against the model without method as fixed effect. Analyses were carried out using R v3.1.1 (R Core Team, 2013) and the packages lme4 (Bates et al., 2015), multcomp (Hothorn et al., 2008). Plots were made with the help of ggplot2 (Wickham, 2009).

3. Results

All four sediment reworking parameters showed significant differences between methods, i.e. sediment reworking rate D_b ($\chi^2(1) = 7.96$, $p = 0.019$), non-locality index *NLI* ($\chi^2(1) = 10.86$, $p = 0.004$), mean weighted luminophore depth Lum_{mean} ($\chi^2(1) = 10.72$, $p = 0.005$), and maximum luminophore depth Lum_{max} ($\chi^2(1) = 11.26$, $p = 0.004$).

D_b was significantly higher in the reduced-SPI approach (compared to ST (Tukey: $p = 0.131$) and full-SPI (Tukey: $p < 0.001$)) while no differences were detected between the ST and the full-SPI approach (Fig.1).

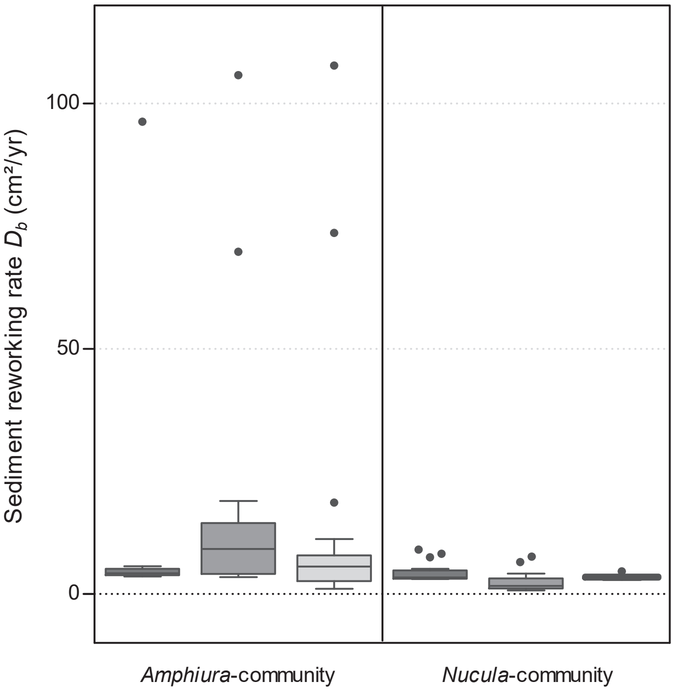

Fig. 1. Sediment reworking D_b (cm²/yr) measured by the different methods (dark grey: ST; grey: reduced-SPI; light grey: full-SPI) for both the *Amphiura*- and the *Nucula*-community.

NLI was significantly lower in the full-SPI approach (compared to ST (Tukey: p < 001) and reduced-SPI (Tukey: p < 0.001)), whereas no differences were observed between the ST and the reduced-SPI approach (Fig.2).

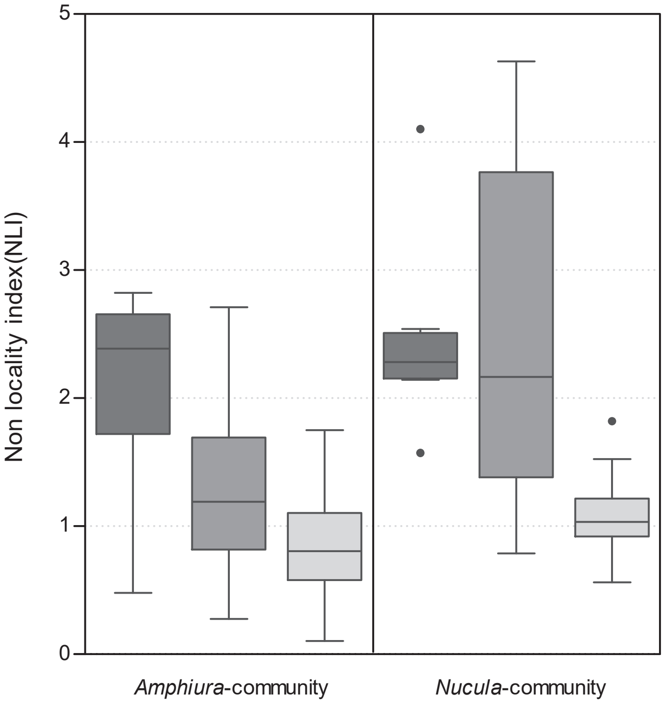

Fig. 2. Dimensionless non-locality index (*NLI*) measured by the different methods (dark grey: ST; grey: reduced-SPI; light grey: full-SPI) for both the *Amphiura*- and the *Nucula*-community. Higher *NLI* denotes higher non-local transport.

Lum_{mean} was significantly deeper in the full-SPI method (compared to ST (Tukey: $p < 0.001$) and reduced-SPI (Tukey: $p < 0.001$)) whereas both low spatial resolution approaches (i.e. ST and the reduced-SPI) showed no difference in Lum_{mean} (Fig.3).

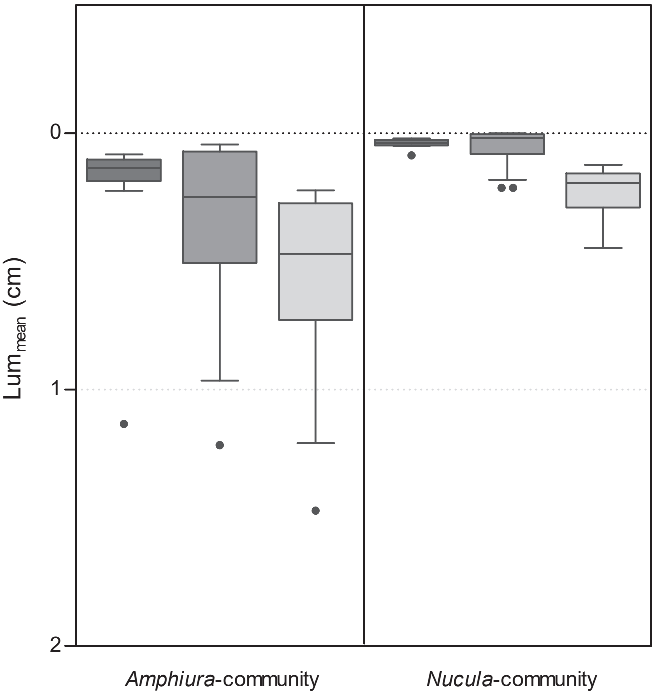

Fig. 3. Mean weighted luminophore depth (cm) (Lum_{mean}) measured by the different methods (dark grey: ST; grey: reduced-SPI; light grey: full-SPI) for both the *Amphiura*- and the *Nucula*-community.

Lum_{max} was significantly deeper in the ST method compared to both SPI approaches (compared to full-SPI (Tukey: $p < 0.001$) and reduced-SPI (Tukey: $p < 0.001$)), which did not differ significantly (Fig.4). Assessment of the variability of the SPI method depending on the sample site yielded only significant differences for Lum_{max} ($\chi^2(1) = 5.79$, $p = 0.016$).

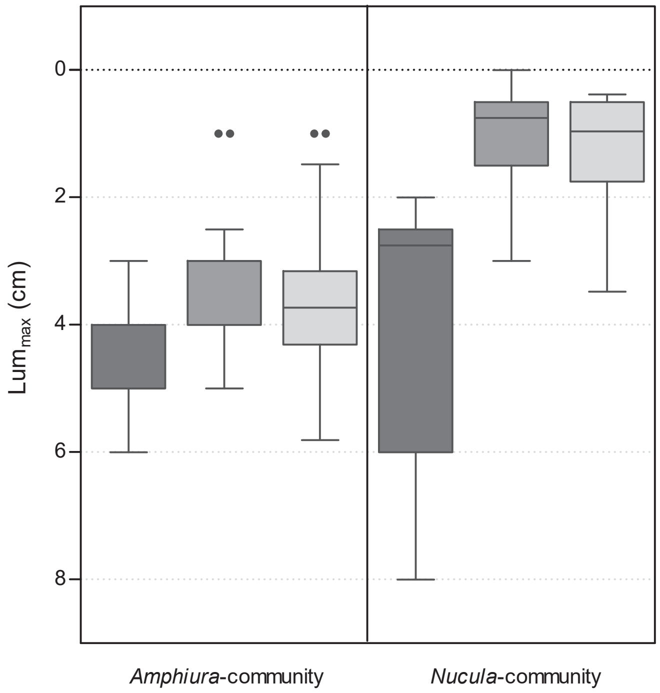

Fig. 4. Maximal luminophore depth (cm) (Lum_{max}) measured by the different methods (dark grey: ST; grey: reduced-SPI; light grey: full-SPI) for both the *Amphiura*- and the *Nucula*-community.

Differences between methods will ultimately lead to different outcomes in community sediment reworking regime comparison. The full-SPI method indicates that there are significant differences in D_b ($F_{1,15} = 5.60$, $p = 0.032$), Lum_{max} ($F_{1,15} = 52.66$, $p < 0.001$), and Lum_{mean} ($F_{1,15} = 7.13$, $p = 0.0175$) between the *Amphiura*- and *Nucula*-community. The reduced-SPI approach indicates significant differences in D_b ($F_{1,15} = 6.00$, $p = 0.027$), *NLI* ($F_{1,15} = 6.89$, $p = 0.019$), Lum_{max} ($F_{1,15} = 47.37$, $p < 0.001$), and Lum_{mean} ($F_{1,15} = 6.48$, $p = 0.022$) between the two communities.

The ST method indicates that there are no significant differences in any of the sediment reworking parameters between the two communities, even though profiles of the wet weight distribution of both communities show that the gross biomass of the *Amphiura*-community is located at three to four cm depth, whereas biomass in the *Nucula*-community is located mainly in the upper two cm (Fig.5).

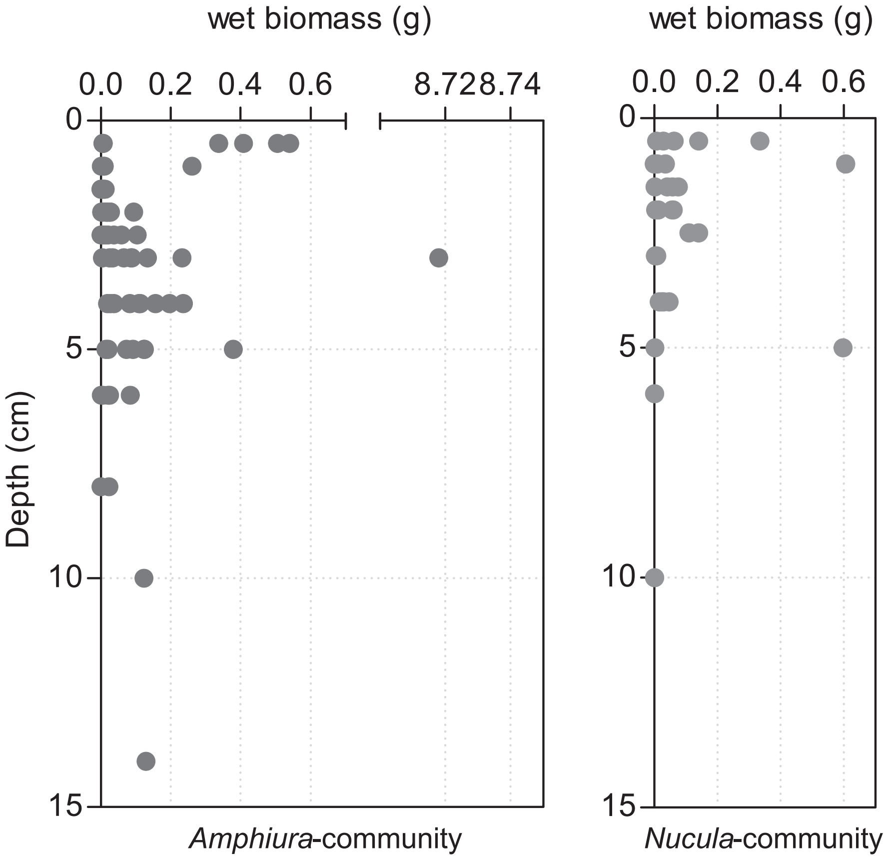

Fig. 5. Distribution of the wet biomass (g) within the communities. Each circle represents the wet biomass (g) found at the respective depth in one of the replicates.

All methods produced similar luminophore depth distributions (Fig.6). However, for two depth strata, the ST and the reduced-SPI method show significant differences in the luminophore depth distribution. The luminophore concentration in the section from 5 - 6 cm (Mann-Whitney $U = 69.00$, $n_1 = n_2 = 17$, p = 0.0086) was significantly higher in the ST approach whereas the reduced-SPI approach found significantly more luminophores in the section from 0.5 - 1 cm depth (Mann-Whitney $U = 83.00$, $n_1 = n_2 = 17$, p = 0.0356).

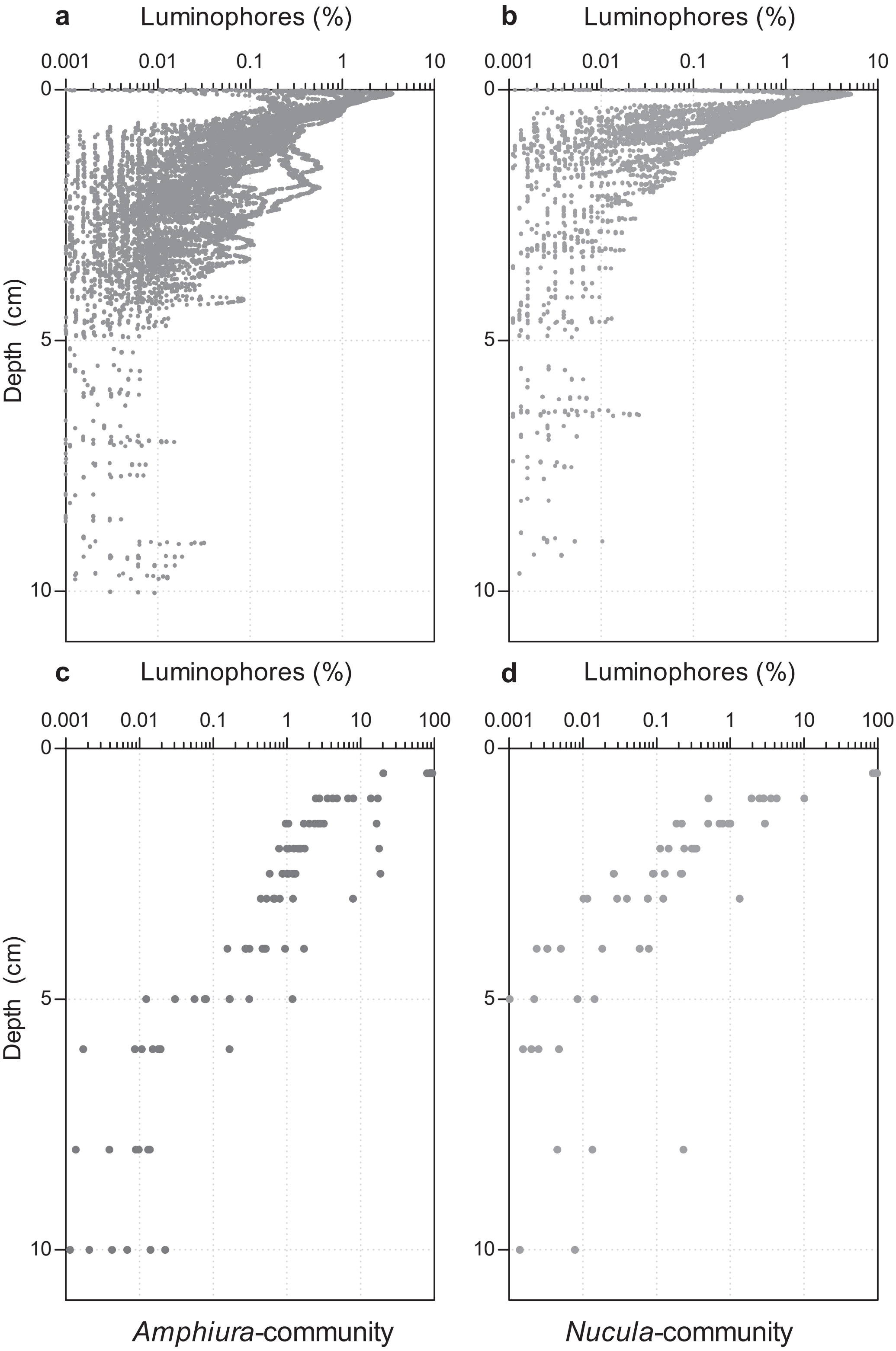

Fig. 6. Distribution of the luminophores (%) within the *Amphiura*-community (a,c) and *Nucula*-community (b,d). Each point present the percentage of luminophores detected by the full-SPI (a,b) and the ST (c,d) method at the respective depth in one of the replicates. Luminophore concentrations smaller that 0.001% are outside the axis limits. Accordingly a lack of points at a certain depth indicates luminophore percentages below 0.001%.

4. Discussion

Our results clearly demonstrate that the slicing technique (ST) approach and the SPI method deliver significantly different results that ultimately may lead to distinctly different interpretations of sediment reworking patterns. This finding not only indicates that data derived by both approaches is difficult to compare, it also raises the question which method produces a more appropriate model of reality. Solan et al. (2004) assumed that the SPI method provides conservative estimates of the sediment reworking rate as particles are likely to be dislocated horizontally away from the glass wall. Yet, in our experiments, the reduced-SPI approach produced the highest sediment reworking rates while no significant differences were detected in sediment reworking rates between the other two methods.

An underestimation of the sediment reworking parameters by the SPI method, as predicted by Solan et al. (2004), could only be observed for the maximum luminophore depth. The ST showed the deepest Lum_{max} which indicates that deep sediment reworking activity was partly not detected by the SPI method as it presumably took place away from any of the cylinder walls. The effect was especially pronounced in the *Nucula*-community. However, from the biomass and luminophore distribution, it has to be concluded that the deep luminophores, responsible for Lum_{max}, are extremes in the *Nucula*-community. Accordingly, SPI functions sufficiently to depict the general picture of sediment reworking activity.

Full-SPI, however, also showed the lowest *NLI*. Yet, this finding does not show that full-SPI underestimates *NLI* but that non-local transport is overestimated by the low spatial resolution of the ST and the reduced-SPI method. Sediment reworking can either be of local nature, where animal activity leads to frequent random dislocations of individual particles over very short distances (Boudreau, 1986; Kristensen et al., 2012), or of a non-local nature, where particles are moved over larger distances in distinct steps (Boudreau and Imboden, 1987; Kristensen et al., 2012; Meysman et al., 2003). This non-local transport may, however, also be caused by the spatial reduction of the luminophore distribution. While the full-SPI approach captures the full extent of the small local random dislocations and depicts them spatially accurate, with both the ST and the reduced-SPI approach, by integrating over larger depth intervals, shifts in depth distribution generally appear as particle dislocation over larger steps.

In the *Nucula*-community, this effect was more pronounced compared to the *Amphiura*-community as the non-local transport was already with the full-SPI approach slightly higher. Yet, compared to the *NLI* described by Fernandes et al. (2006) for *Nereis diversicolor* of 18, neither of our approaches attests one of the communities' high non-local transport.

Differences between the ST and the SPI method are not only caused by spatial resolution. Irregularly sloped sediment surfaces, caused by surface sediment reworking activity, are a hindrance to slicing equally sized surface sections with the ST method. Resulting differences in sediment volume per slice will ultimately skew the luminophore distribution. The effect is most pronounced in the top layers where at the same time the diffusion model is most sensitive to parameter changes, hence, the impact of surface irregularities is correspondingly large. The SPI method compensates for surface bumps by a computer-assisted smoothing and straightening of the sediment surface before biodiffusive transport is modelled. This may explain different conclusions that are drawn about the sediment reworking activity (D_b) in the *Amphiura*- and *Nucula*-community. In contrast to surface bumps, spatial distribution alone does not affect the conclusions for D_b, Lum_{mean} and Lum_{max} of the two communities as is demonstrated by the reduced-SPI method.

Considering all these findings, we can find no evidence that would rebut our first hypothesis, that distortion in the SPI images from the curvature of the cylinders (10 cm diameter) will not affect the sediment reworking parameters. Underestimation in any of the sediment reworking parameters was only observed for Lum_{max}, which can be attributed to the "blind spot" of the SPI method. This supports our second hypothesis that SPI is not able to capture the full extent of sediment reworking activity in a chamber that has a higher volume to surface ratio than the thin aquaria (width 1.2 cm) of Maire et al. (2006).

Likewise, our third hypothesis that SPI will provide a more accurate depiction of small scale particle dislocations due to its higher resolution was confirmed. Additionally, we show that also the spatial accuracy of the SPI method resolved differences in sediment reworking activity better. This is of special interest for the investigation of organisms or communities that have low differences in sediment reworking activity and that require high discrimination power. However, it seems to be rather the norm that communities or species do not differ strongly within their activities.

All parameters, we measured, were well within the common range of sediment reworking activity for macrofauna species measured with either of the techniques (ST (Fernandes et al., 2006; Gilbert et al., 2007; Gilbert et al., 2003); SPI (Queirós et al., 2015; Wrede et al., 2017)).

It may, thus, be assumed that the SPI method functions equivalent to the ST in terms of the general picture of the sediment reworking activity. When it comes to spatial resolution and spatial accuracy, the SPI method was even superior to the ST. Nevertheless, it is crucial to note that the ST method is better suited if the absolute maximum sediment reworking depth is the major focus of an investigation as the "blind spot" of the SPI method may mask some sediment reworking activity. Considering, however, the lower accuracy and the time intensive slicing procedure of the ST method, general sediment reworking patterns are better resolved with the SPI method. However, if SPI is applied, the "blind spot" should be kept as small as possible through a maximization of photographed surface area. We implemented this in our study by taking pictures from the two exact opposite sides of the core. Since significant differences in Lum_{max} between the two sides exist, important information may get lost if the photographed surface area is kept small. In cores with a diameter of 10 cm, it is possible to capture nearly the whole circumference of the core with two pictures. An expansion of this multiple picture technique may be a solution for cylinders with larger diameters. Multiple images can be put together to reduce the distortion to a minimum. Accordingly, SPI is also applicable on the rounded walls of our cylindrical cores as an underestimation of sediment reworking activity by the curvature of the cylinders could not be observed for cores with a 10cm diameter and it can be further minimized. This finding should facilitate the applicability of sediment reworking measurements in natural communities as sediment reworking parameters can be measured directly in multi-corer cores and as the need for time-consuming slicing or the complex transfer into rectangular aquaria is omitted.

Acknowledgements

The authors would like to thank the crew of the RV Heinke as well as J. Meyer, K. Stumpf and P. Kadel for their support during animal collection and laboratory experiments. Further, we would like to thank R. and H. Asmus and K.H. Wiltshire for their support and for providing access to the facilities of the Wadden Sea Station Sylt of the AWI. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. Author contributions: AW conceived and designed the study. AW performed the experiment and analysed the data. JH assisted with the modelling of biodiffusive transport. AW wrote the paper with substantial contributions from TB and JH.

References

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Soft. 67, 1-48.

Boudreau, B.P., 1986. Mathematics of tracer mixing in sediments; II. Nonlocal mixing and biological conveyor-belt phenomena. Am. J. Sci. 286, 199-238.

Boudreau, B.P., Imboden, D.M., 1987. Mathematics of tracer mixing in sediments; III. The theory of nonlocal mixing within sediments. Am. J. Sci. 287, 693-719.

Crank, J., 1975. Mathematics of diffusion. Clarendon Press, Oxford.

Fernandes, S., Meysman, F.J.R., Sobral, P., 2006. The influence of Cu contamination on *Nereis diversicolor* bioturbation. Mar. Chem. 102, 148-158.

Gilbert, F., Hulth, S., Grossi, V., Poggiale, J.-C., Desrosiers, G., Rosenberg, R., Gérino, M., François-Carcaillet, F., Michaud, E., Stora, G., 2007. Sediment reworking by marine benthic species from the Gullmar Fjord (western Sweden): importance of faunal biovolume. J. Exp. Mar. Biol. Ecol. 348, 133-144.

Gilbert, F., Hulth, S., Stromberg, N., Ringdahl, K., Poggiale, J.C., 2003. 2-D optical quantification of particle reworking activities in marine surface sediments. J. Exp. Mar. Biol. Ecol. 285, 251-263.

Hothorn, T., Bretz, F., Westfall, F., 2008. Simultaneous inference in general parametric models. Biometrical J. 50, 346-363.

Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C.O., Banta, G.T., 2012. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Mar. Ecol. Prog. Ser. 446, 285-302.

Lohrer, A.M., Thrush, S.F., Gibbs, M.M., 2004. Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431, 1092-1095.

Mahaut, M., Graf, G., 1987. A luminophore tracer technique for bioturbation studies. Oceanologica acta 10, 323-328.

Maire, O., Duchene, J.C., Rosenberg, R., de Mendonca, J.B., Grémare, A., 2006. Effects of food availability on sediment reworking in *Abra ovata* and *A. nitida*. Mar. Ecol. Prog. Ser. 319, 135.

Maire, O., Lecroart, P., Meysman, F., Rosenberg, R., Duchene, J.C., Gremare, A., 2008. Quantification of sediment reworking rates in bioturbation research: a review. Aquat. Biol. 2, 219-238.

Meysman, F.J., Boudreau, B.P., Middelburg, J.J., 2003. Relations between local, nonlocal, discrete and continuous models of bioturbation. J. Mar. Res. 61, 391-410.

Murray, F., Douglas, A., Solan, M., 2014. Species that share traits do not necessarily form distinct and universally applicable functional effect groups. Mar. Ecol. Prog. Ser. 516, 23-34.

Murray, F., Widdicombe, S., McNeill, C.L., Solan, M., 2013. Consequences of a simulated rapid ocean acidification event for benthic ecosystem processes and functions. Mar. Pollut. Bull. 73, 435-442.

Queirós, A.M., Birchenough, S.N.R., Bremner, J., Godbold, J.A., Parker, R.E., Romero-Ramirez, A., Reiss, H., Solan, M., Somerfield, P.J., Van Colen, C., Van Hoey, G., Widdicombe, S., 2013. A bioturbation classification of European marine infaunal invertebrates. Ecol. Evol. 3, 3958-3985.

Queirós, A.M., Stephens, N., Cook, R., Ravaglioli, C., Nunes, J., Dashfield, S., Harris, C., Tilstone, G.H., Fishwick, J., Braeckman, U., 2015. Can benthic community structure be used to predict the process of bioturbation in real ecosystems? Prog. Oceanogr. 137, 559-569.

R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Salzwedel, H., Rachor, E., Gerdes, D., 1985. Benthic macrofauna communities in the German Bight. Veröff. Instit. Meeresf. Bremerhaven 20, 199-267.

Solan, M., Wigham, B.D., Hudson, I.R., Kennedy, R., Coulon, C.H., Norling, K., Nilsson, H.C., Rosenberg, R., 2004. In situ quantification of bioturbation using time-lapse fluorescent sediment profile imaging (f-SPI), luminophore tracers and model simulation. Mar. Ecol. Prog. Ser. 271, 1-12.

Wickham, H., 2009. ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.

Wrede, A., 2014. Effects of temperatrue on the bioturbation activity of coastal marine benthic species, University of Bremen, p. 53.

Wrede, A., Dannheim, J., Gutow, L., Brey, T., 2017. Who really matters: Influence of German Bight key bioturbators on biogeochemical cycling and sediment turnover. J. Exp. Mar. Biol. Ecol. 488, 92-101.

Who really matters: influence of German Bight key bioturbators on biogeochemical cycling and sediment turnover

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published in *Journal of Experimental Marine Biology and Ecology*,
DOI 10.1016/j.jembe.2017.01.001; published online 07. January 2017.

Abstract

Loss of bioturbating key species from marine sediments has been shown to strongly reduce benthic biogeochemical cycling and ecosystem functioning. It is, thus, of paramount importance to identify key bioturbators and quantify their effect on biogeochemical processes. To do so, trait-based community and species bioturbation potential (BP_c and BP_i) was mapped for 423 North Sea stations in the German Bight. Mapping of BP_c and BP_i identified *Amphiura filiformis*, *Echinocardium cordatum* and *Nucula nitidosa* as major bioturbating species in the German Bight. The effects of these species on benthic nutrient flux (i.e., changing concentrations of silicate $\Delta[SiO_2]$, ammonium $\Delta[NH_4^+]$, nitrate $\Delta[NO_3^-]$ and nitrite $\Delta[NO_2^-]$) were quantified in laboratory experiments together with their bioturbation rate (D_b) and bioirrigation activity. The experiments indicated that mapped species' bioturbation potential (BP_i) may be a poor tool for identifying key bioturbators while calculated experimental BP_i (${}^{exp}BP_i$) was a good indicator for species impact on biogeochemical cycling. Out of the three investigated species only *E. cordatum* significantly influenced biogeochemical cycling, whereas the effect of *A. filiformis* remained inconclusive; potentially because arm damage and regeneration may affect the bioturbation activity of many individuals. The bivalve *N. nitidosa* showed only little impact on biogeochemical cycling, although this species was found to be an active bioturbator. Accordingly, *E. cordatum* may be considered one of the most important contributors to biogeochemical cycling at the sediment-water interface in the German Bight.

Keywords: Bioturbation potential; Sediment reworking; Bioirrigation;
Amphiura filiformis; *Echinocardium cordatum*; *Nucula nitidosa*

1. Introduction

Sediments in aquatic environments are strongly influenced by the inhabiting macrofaunal organisms. Through their burrowing, ventilation, ingestion, and defecation activities, benthic organisms actively mix the sediment (Kristensen et al., 2012), modify the grain size distribution (Giangrande et al., 2002), change structure, porosity and erodibility of the sediment (Grant and Daborn, 1994; Meadows and Meadows, 1991; Rowden et al., 1998; Widdows et al., 2000), and redistribute food resources, eggs, viruses and resting stages (Giangrande et al., 2002; Meysman et al., 2006). Ventilation activities and flushing of burrows redistribute nutrients (Lohrer et al., 2004) and oxygen (Aller, 1982), thereby promoting the remineralization of organic matter in deeper sediment layers (Aller, 1994; Kristensen, 2000). This enhances the exchange of solutes across the sediment-water interface and the nutrient availability for primary production and biogeochemical cycling (Blackburn, 1988; Braeckman et al., 2014). Accordingly, aquatic bioturbators have a strong influence on the functioning of the benthic system and the overlying water body (Jones et al., 1994; Lohrer et al., 2004). Bioturbators are, hence, considered important ecosystem engineers which modify the availability and accessibility of resources for themselves and other organisms (Jones et al., 1994).

Previous studies showed that the effect of a diverse multispecies community on biogeochemical cycling is not just the summation of the single species effects (Hale et al., 2014; Mermillod-Blondin et al., 2005; Waldbusser et al., 2004). The strongest bioturbator often superimposes the effects of the other species while competition for resources, such as food and space, may reduce the overall influence of bioturbation on benthic functioning (Hale et al., 2014). As a consequence, the behavior of the dominant bioturbator is often more important for biogeochemical cycling than species diversity (Lohrer et al., 2004; Mermillod-Blondin et al., 2005; O'Reilly et al., 2006).

The reduction or loss of the bioturbation activity of one keystone species may, thus, have broad ecological and biochemical implications, such as a reduced nutrient exchange across the sediment water interface, with potentially negative implications for primary production (Lohrer et al., 2004). Continuous anthropogenic activities in the North Sea, such as fisheries and offshore wind farming, are expected to induce regional and large scale-changes in the structure of benthic communities (Coates et al., 2014; Rachor, 1990; Tillin et al., 2006). To understand the implications of these changes for ecosystem functioning, it is of utmost importance to identify key bioturbators and quantify their contribution to biogeochemical cycling. So far, there has been no large scale assessment of key bioturbators in the North Sea or any other sea.

The descriptive, non-quantitative concept of community bioturbation potential (BP_c) of Solan et al. (2004) may offer a valuable tool for the identification of important bioturbators. It provides an estimate of relative sediment reworking activity (Queirós et al., 2013) and is correlated with sediment parameters, such as total organic carbon content, chlorophyll concentration, oxygenation depth, sediment oxygen consumption, ammonium efflux, and denitrification (Birchenough et al., 2012; Braeckman et al., 2014; Solan et al., 2004). Due to its non-quantitative nature, BP_c provides neither an absolute value of bioturbation nor a measure of associated biogeochemical cycling. Therefore, these processes have to be quantified experimentally.

This study aims for a first comprehensive spatial assessment of important bioturbators in the German Bight and the identification of species that are essential contributors to biogeochemical cycling in this region. To do so, this study combines computer-based mapping of bioturbation potential with the experimental determination of bioturbation and bioirrigation activity and their influence on nutrient fluxes.

2. Material and Methods

2.1 Identification of key bioturbators

To identify key bioturbators, geo-referenced trait-based bioturbation potential was calculated for 423 North Sea stations in the German Bight. The species and community bioturbation potential ($\mathrm{BP_i}$ and $\mathrm{BP_c}$) was calculated according to Solan et al. (2004) and Queirós et al. (2013):

Species bioturbation potential:

$$\mathrm{BP_i} = \sqrt{B_i / A_i} * A_i * M_i * R_i \tag{1}$$

Community bioturbation potential:

$$\mathrm{BP_c} = \sum_{i=1}^{n} \mathrm{BP_i} \tag{2}$$

where B_i and A_i are biomass and abundance of species (i) at a station, while M_i and R_i are categorical scores describing the mobility and reworking mode of species (i). Categorical scores were adopted from the classifications provided by Queirós et al. (2013). Missing information on mobility and reworking mode was compiled from literature following the rules proposed by Queirós et al. (2013).

Abundance and biomass data were taken from the benthos database for ecological research (BENDER) held by the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (AWI). The subset comprised 423 stations sampled between 1998 and 2011 with van Veen grabs (0.1 m²). All samples were identified to lowest taxonomic level possible. Data from repeatedly sampled stations was averaged.

Key bioturbators were identified by (a) $\mathrm{BP_i}$ contribution to total German Bight $\mathrm{BP_c}$ and to station $\mathrm{BP_c}$, (b) their spatial importance in the German Bight and (c) from literature information. Spatial importance describes the relative frequency (over all stations) at which a species' $\mathrm{BP_i}$ is $\geq 20\%$ of $\mathrm{BP_c}$ at a station. To reduce the effects of heterogeneity in station density, the data were averaged to 5 x 5 km grid cells in ArcGIS (esri). Spatial importance (I) was, thus, calculated for each species (i) separately by:

$$I_i = \frac{sum\ (n_i)}{N} * 100 \tag{3}$$

where *n* is the number of grid cells with $\mathrm{BP_i}/\mathrm{BP_c} \geq 0.2$ while *N* is the number of all grid cells containing at least one station. Literature information on habitat, life style, feeding habit, bioturbation and bioirrigation activity was reviewed for indications that BP_i might be overestimating the potential of a species to bioturbate or to influence the biogeochemical cycling (i.e. reports of low bioturbation activity, restriction to permeable sediments, etc.).

A species was considered a key bioturbator if (i) BP_i contribution to total BP_c and station BP_c was ≥ 1%, (ii) if the spatial importance (*I*) was ≥ 4% and (iii) if there was no evidence from the literature that BP_i overestimated the bioturbation effects. For easier assessment, species were ranked according to these three obligatory criteria. Laboratory experiments were conducted on the identified key bioturbators, the brittle star *Amphiura filiformis* (O.F. Müller, 1776), the sea urchin *Echinocardium cordatum* (Pennant, 1777), and the bivalve *Nucula nitidosa* Winckworth, 1930 to assess actual bioturbation rates and bioirrigation activity and their effects on nutrient fluxes (changing concentrations of silicate $\Delta[SiO_2]$, ammonium $\Delta[NH_4^+]$, nitrate $\Delta[NO_3^-]$ and nitrite $\Delta[NO_2^-]$).

2.2 Sediment and fauna sampling for laboratory experiments

Sediment and benthic organisms for laboratory experiments were collected with a van Veen grab (0.1 m²) in August 2015 at two different stations in the southern German Bight (*Nucula nitidosa*: N 54°07’50’’ E 8°12’90’’; *Amphiura filiformis* and *Echinocardium cordatum*: N 54°0’50’’ E 7°49’00’’) which were representative for the species’ natural habitats. Specimens were carefully isolated from the sediment, separated by species, and directly transferred into seawater aquaria, aerated by air stones and filled with specific sediment (6 cm) from the site were the respective species was sampled. After removal of the organisms, the sediment of each grab was sieved through a 1 mm mesh and left to settle in large tanks. As the sediment characteristics differed between the two stations (Tab.1), one tank was prepared with sediment from the site where *N. nitidosa* was collected and two with sediment from the site where *A. filiformis* and *E. cordatum* were sampled. After 24 hours the overlying water was decanted. The sediment of each tank was homogenized by mixing and poured into rectangular incubation chambers (h: 35 cm, w: 9.4 cm, d: 9.4 cm) (Fig. 1) up to a sediment column height of 18 cm (±1 cm) after settling.

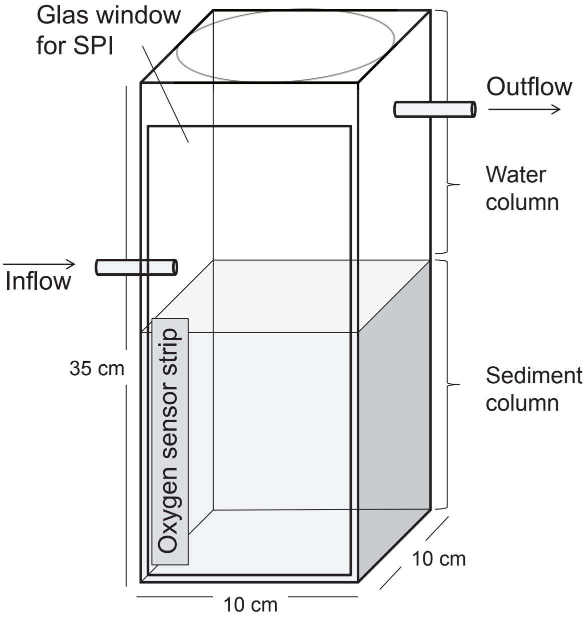

Fig. 1. Set up of the incubation chambers. Bioturbation activity was determined by sediment profile imaging (SPI) through the glass window of the incubation chamber.

For each of the three species, five replicate incubation chambers and five control chambers were filled with the respective sediment (Tab.1). The incubation chambers and aquaria were transferred to a temperature controlled laboratory at the Wadden Sea Station of the AWI at the island of Sylt and connected to a continuous flow of filtered seawater (30 µm drumfilter: Spranger; protein skimmer: Sander) taken directly from the Sylt-Rømø Bight. Each chamber and aquarium was constantly aerated through an air stone.

2.3 Incubation of bioturbators

After two days, the height of the sediment column was controlled and adjusted, if necessary, to a height of 18 cm (±1cm). Temperature, pH, and salinity were controlled every second day. Four days after sampling, individuals of all species where recovered from the aquaria and selected according to number of intact arms ≥ 3 in *A. filiformis* and, for all species, according to observation of activity and size (*A. filiformis* 0.4-0.6 cm disc diameter, *E. cordatum* 3.5-3.8 cm test length, *N. nitidosa* 0.6-0.8 cm max. shell diameter). Selected individuals were placed in the replicate incubation chambers.

The numbers of individuals in each chamber was five for *A. filiformis* and *N. nitidosa*, whereas the incubation chambers for *E. cordatum* were stocked with a single individual each. These densities are within the density range of the species in field populations according to the BENDER data base. The control chambers contained only sediment but no animals. The organisms were allowed to acclimatize to the experimental conditions and to burrow into the sediment for one day. Subsequently, a homogenized suspension of luminophores (green colour: 4 g, 80-250 µm; pink colour: 4 g, 60 µm; Partrac Ltd. U.K.) was poured evenly over the sediment surface. On day 7, the seawater flow was stopped for eight hours to measure bioirrigation and cycling of nitrogen and silicate (see below). Bioturbation rates and oxygen distribution in the sediment were measured on day 8 of the experiment. On day 9, the organisms, that had survived, were recovered, counted and the total wet biomass (g) per incubation chamber was measured (Tab. 1). Based on this abundance and biomass data, the bioturbation potential of the three species ($^{exp}BP_i$) was calculated for each incubation chamber.

Table 1: Average (± 1 SD) mortality (%) and body mass (g) of individuals of *Amphiura filiformis*, *Echinocardium cordatum* and *Nucula nitidosa* in the experimental chambers at the end of the incubation period.

Species	Sediment characteristics* (median grain size (µm)/org. content (%))	Mortality (%)	Mean individual wet weight (g)	Replicates	Controls
Amphiura filiformis	94.67 / 0.81	4.00 (± 8.94)	0.19 (± 0.19)	5	5
Echinocardium cordatum	98.93 / 0.92	40.00 (± 54.77)	22.39 (± 15.05)	3	5
Nucula nitidosa	63.18 / 1.05	0 (± 0)	0.20 (± 0.19)	5	5

*Determined from a pooled sample representative of the sediment characteristics of both replicates and controls

2.4 Bioirrigation and biogeochemical cycling

Bioirrigation activity of the organisms was assessed through changes in the concentration of the inert tracer bromide over eight hours ($\Delta[Br^-] = t_8-t_0$) (Forster et al., 1999; Mermillod-Blondin et al., 2005) where negative $\Delta[Br^-]$ values indicate an increased bioirrigation activity. At the beginning of the measuring period (t_0), a sodium bromide solution (0.668 g NaBr dissolved in seawater aiming at a $[Br^-]$ concentration of 5 mM in the water column above the sediment) was carefully stirred into the water column of each incubation chamber.

After 0, 1, 2, 4 and 8 hours 3 ml samples of the seawater medium were extracted and filtered (0.2 µm surfactant-free cellulose acetate membrane, Minisart Syringe Filter, Satorius) (Forster et al. (1999); Mermillod-Blondin et al. (2005); Murray et al. (2013)). The water samples were stored at 3°C until analysis by ion-chromatography (Metrohm, 930 Compact IC Flex). Additional samples of the seawater medium were taken and filtered after 0 and 8 hours to measure concentrations of silicate (sample volume: 3 ml), ammonium, nitrate and nitrite (sample volume: 5 ml each). Silicate samples were stored at 3°C whereas ammonium and nitrate samples were immediately frozen at -20°C until analysis. Samples were analysed by segmented flow analysis (SEAL AA3 HR Autoanalyzer) and ISO standard procedures (MT 18, MT 19) of the manufacturer (SEAL Analytical). Fluxes of silicate ($\Delta[SiO_2]$), ammonium ($\Delta[NH_4^+]$), nitrate ($\Delta[NO_3^-]$) and nitrite ($\Delta[NO_2^-]$) were determined from the changes in concentration (C) over time and are given as ($\Delta[C] = t_8 - t_0$).

2.5 Bioturbation

Bioturbation was quantified by sediment profile imaging (SPI) (Gilbert et al., 2003). Images of the sediment column were taken under black light (Phillips, TL-D 18W BLB 1SL) from a defined distance through the glass wall of the incubation chambers (Camera: Canon Eos 500D, Canon EF-S 15-85 mm, f/3.5-5.6 IS USM; Fig. 1). All images were cut to the same size with the image analysis software Image J (1.48) (http://imagej.nih.gov/ij/index.html) and scaled. The water column was manually tinted with a preselected uniform marking colour (RGB: 253,003,155) in Adobe Photoshop CS6. The tinted water column was later identified by a custom-made plugin for the software ImageJ. The plugin was used to remove the tinted water column and to smooth the sediment surface so that the sediment-water interface served as the abscissa in a coordinate system at y = 0 of an inverse ordinate (i.e. the vertical sediment column). After identifying the luminophores using the threshold function of ImageJ, the image was converted into black and white. The black pixels representing the luminophores were counted for each pixel row and transferred into a profile of the luminophore distribution.

The bioturbation rate was estimated using a one-dimensional diffusion model, which is applied for conservative tracers, such as luminophores, in the absence of sedimentation. Assuming that initially the luminophores are located in a narrow layer at the sediment-water interface, Crank (1975) gives the solution as:

$$C(x,t) = \frac{N}{\sqrt{\pi D_b t}}\ exp\left(\frac{-x^2}{4D_b t}\right) \tag{4}$$

where $C(x,t)$ = the normalized tracer concentration relative to the initial input, t = time, x = depth, N = the initial luminophore input and D_b = the biodiffusion coefficient, which is the common measure for the bioturbation rate (Crank, 1975; Fernandes et al., 2006; Maire et al., 2008).

The initial luminophore concentration N was estimated from the normalized profile data as:

$$N = \sum_i n(a_i) * z(a_i) \tag{5}$$

where $n(a_i)$ = the relative concentration of luminophores in the slice a_i and $z(a_i)$ = the thickness of the slice a_i in cm. N was equal to 0.36. The biodiffusion coefficient D_b was estimated by matching the model to the observed profiles using non-linear regression with the least sum of squares of the residuals as criterion for the best fit. Non-local (i.e. non-diffusive) transport was calculated using the non-locality index (*NLI*) proposed by Fernandes et al. (2006). This requires the calculation of D_b using the actual tracer concentration $C(x,t)$ and of D_b^{log} using the log-transformed tracer concentration: log($C(x,t)$). The log-approach gives more weight to the lower concentrations. The *NLI* is, thus, computed as:

$$NLI = \frac{\left|D_b^{log} - D_b\right|}{\sqrt{D_b^{log} * D_b}} \tag{6}$$

where $NLI = 0$ ($D_b^R = D_b^L$) indicates no non-local transport. The mean luminophore burial depth (lum_{mean}) was assessed from profiles by weighting each depth by the luminophore concentration in that depth. Maximum luminophore depth (lum_{max}) was assessed from the images by measuring of the deepest luminophore signal in the ImageJ.

2.6 Oxygenation depth

Oxygen profiles were measured using the VisiSens A1 System (PreSense). Planar oxygen sensor foils (height: 15 cm, width: 1 cm; SF-RPSu4, PreSense), glued to the inner glass wall of the incubation chambers (Fig. 1), were photographed with a Detector Unit (DU01, PreSense). Images were analysed in the software VisiSens AnalytiCal 1 (PreSens) which uses pre-calibrated colour values to calculate oxygen concentrations.

Images were converted into black and white with a colour ramp that defined concentrations from 0 to 80% air oxygen concentration. White equalling 80% oxygen, black equaling 0% oxygen, and the continuum in shades of grey between those thresholds defined the oxygen concentrations between 0 and 80%. Thus, each pixel row of the image was related to an oxygen concentration via its grey value (the mean of the single pixels grey values). The images were then scaled and transferred into an oxygen profile by calculating the grey value for each pixel row using ImageJ.

2.7 Statistical analysis

Statistical analyses were performed using the software packages Graph Pad Prism 5 (GraphPad Software, Inc.) and JMP 5.0.1 (SAS Institute GmbH). For each variable describing sediment reworking (D_b, Lum_{mean}, Lum_{max}, NLI), bioirrigation (oxygenation depth, ΔBr^-) and biogeochemical flux ($\Delta[NH_4^+]$, $\Delta[NO_3^-]$, $\Delta[NO_2^-]$, $\Delta[SiO_2]$), comparisons were made for each species separately by contrasting the replicates with animals of that species with the corresponding controls containing the same sediment. Prior to the tests, the data was tested for normality (Kolmogorov-Smirnov test) and variance homogeneity (Levene's test). The data was compared by Welch's t-test (Zimmerman (2004). Data on the species' bioturbation potential under the experimental conditions (${}^{exp}BP_i$) were log-transformed and analyzed by one-way ANOVA with Tukey's multiple comparison test.

3. Results

3.1 Identification of bioturbating key species

15 out of the 373 taxa considered accounted for 75% of the overall bioturbation potential in the German Bight (Tab. 2). Only 6 fulfilled the criterion for spatial importance ($I \geq 4\%$) (Fig. 2 and Fig. 3).

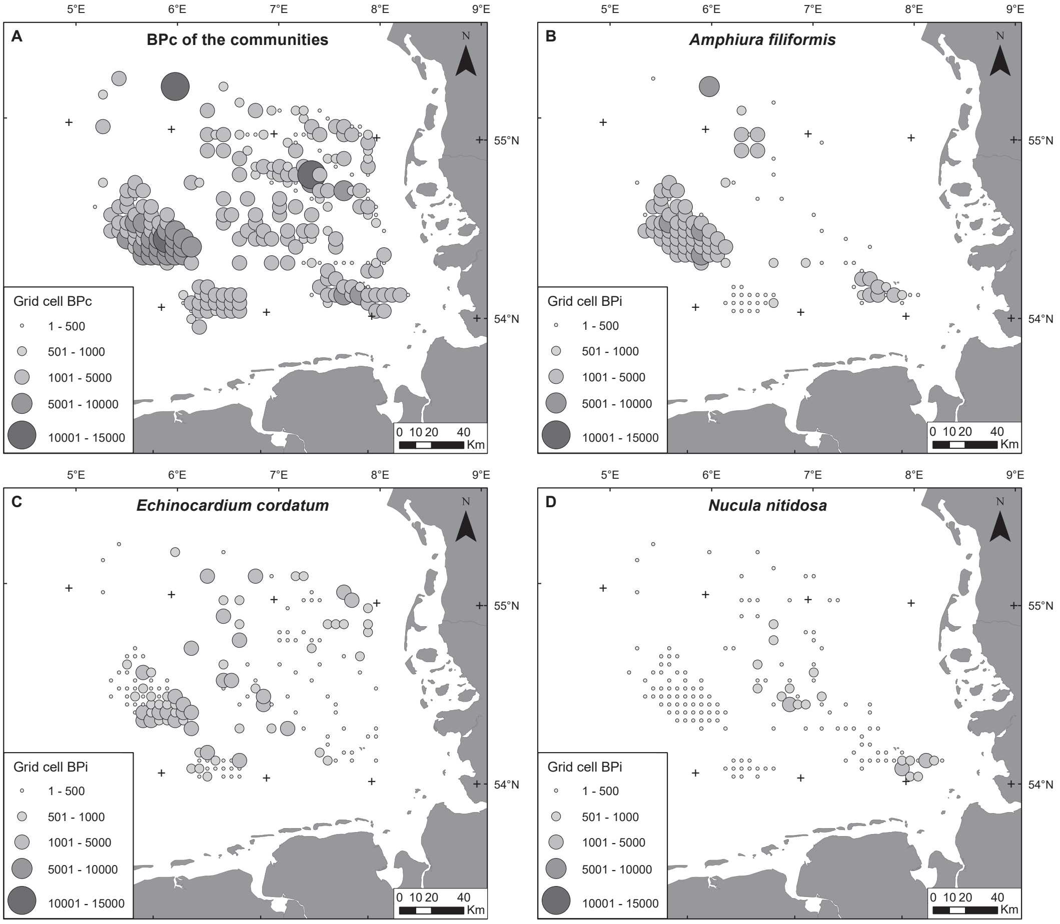

Fig. 2. Bioturbation potential (BP_c and BP_i) of A) the community, B) *Amphiura filiformis*, C) *Echinocardium cordatum* and D) *Nucula nitidosa* at stations sampled from 1998 to 2011 in the German Bight averaged to 5 km grid cell. Size of the circles indicates the relative amount of potential bioturbation activity.

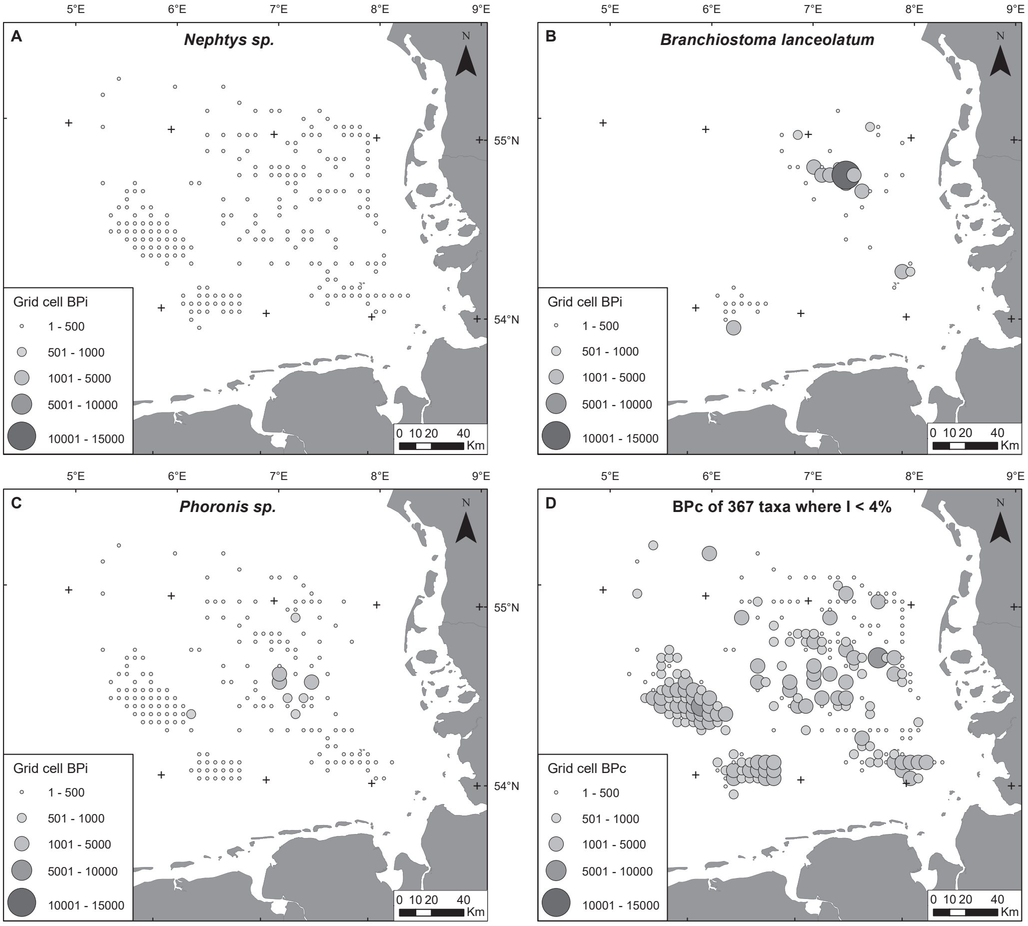

Fig. 3. Bioturbation potential (BP_c and BP_i) of A) *Nephtys* sp., B) *Branchiostoma lanceolatum*, C) *Phoronis* sp. and D) the other 367 taxa where the spatial importance *I* was smaller than 4% at stations sampled from 1998 to 2011 in the German Bight averaged to 5 km grid cell. Size of the circles indicates the relative amount of potential bioturbation activity.

Among these 6 species (Tab. 2), BP_i was likely overestimated in *B. lanceolatum*, *Nephtys* sp. and *Phoronis* sp. (see discussion). Hence, only *Amphiura filiformis, Echinocardium cordatum* and *Nucula nitidosa*, that represent a combined share of 46% of the total BP_c, met all three criteria to be considered a key bioturbator and were, thus, selected for the bioturbation and bioirrigation experiments. The polychaete *Spiophane bombyx* was not considered for the experiments - although equally ranked as *Nucula nitidosa* - due to limited experimental capacity for only 3 species and its low spatial importance (*I*=1.65)

Table 2: Ranking of the 15 species explaining 75% of the overall bioturbation potential BP_c in the German EEZ, contribution (%) to total and station BP_c, and spatial importance (%) of each species (for details see Material and Methods). Asterisks indicate key bioturbators selected for experiments.

Species	Mean Rank	Contribution to total BPc (%)	Mean contribution to station BPc (%)	Spatial importance (%)	Assessment according to literature information
Amphiura filiformis	1.7	25.75	12.17 (± 23.44)	32.64	*
Echinocardium cordatum	1.7	16.95	17.07 (± 20.84)	27.27	*
Nephtys sp.	3.0	6.34	12.38 (± 17.08)	17.77	overestimated
Branchiostoma lanceolatum	4.0	6.86	4.01 (± 15.20)	9.50	overestimated
Spiophanes bombyx	5.3	3.49	4.85 (± 10.82)	1.65	
Nucula nitidosa	5.3	3.29	3.71 (± 10.32)	9.50	*
Phoronis sp.	7.0	2.38	2.41 (± 6.14)	4.55	overestimated
Corbula gibba	9.7	2.25	1.10 (± 3.67)	1.24	
Magelona sp.	8.0	1.53	2.61 (± 4.95)	1.24	
Corystes cassivelaunus	10.3	1.21	1.84 (± 12.69)	0.83	
Callianassa subterranea	11.7	1.47	1.04 (± 3.25)	0.41	
Lanice conchilega	11.7	1.25	1.57 (± 3.81)	0.00	
Tellina fabula	12.0	0.99	1.75 (± 4.87)	0.00	
Ophiura albida	13.3	0.84	0.96 (± 2.48)	0.41	
Urothoe poseidonis	13.3	0.72	1.20 (± 4.46)	0.00	

3.2 Sediment reworking and oxygenation depth

Under the experimental settings, the bioturbation potential ${}^{exp}BP_i$ differed significantly between the three species (one-way ANOVA, $F_{2,10} = 4648$, $p < 0.0001$). The mean ${}^{exp}BP_i$ score of *E. cordatum* was more than seventy times higher than the mean ${}^{exp}BP_i$ scores of *A. filiformis* and *N. nitidosa* (Fig. 4). ${}^{exp}BP_i$ scores were 1.9 times higher for *A. filiformis* than for *N. nitidosa*.

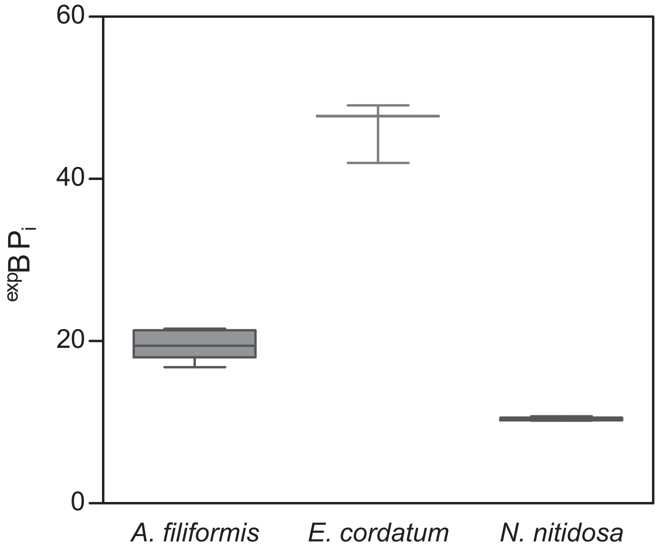

Fig. 4. Bioturbation potential (${}^{exp}BP_i$) of *Amphiura filiformis*, *Echinocardium cordatum* and *Nucula nitidosa* under the experimental conditions. Boxes represent lower and upper quartiles; lines indicate median and whiskers show minima and maxima. ${}^{exp}BP_i$ varied significantly between all species at $p < 0.05$. Logarithmic ordinate was chosen for an optimal representation of the large differences between the ${}^{exp}BP_i$ of the species *A. filiformis* (mean: 20), *E. cordatum* (mean: 1463) and *N. nitidosa* (mean: 10).

Bioturbation rate (D_b; Welch's t-test: $F_{1,8} = 10.75$, $p = 0.029$; Fig. 5a), maximal luminophore distribution (Lum_{max}; Welch's t-test: $F_{1,8} = 33.13$, $p = 0.004$; Fig, 5c) and mean luminophore distribution (Lum_{mean}; Welch's t-test: $F_{1,8} = 35.57$, $p = 0.002$; Fig. 5d) were significantly enhanced by *N. nitidosa* as compared to the controls without animals, whereas *A. filiformis* and *E. cordatum* had no significant influence on the particle distribution (Fig. 5a). Non-local particle transport (NLI) was not enhanced by any of the species (Fig. 5b).

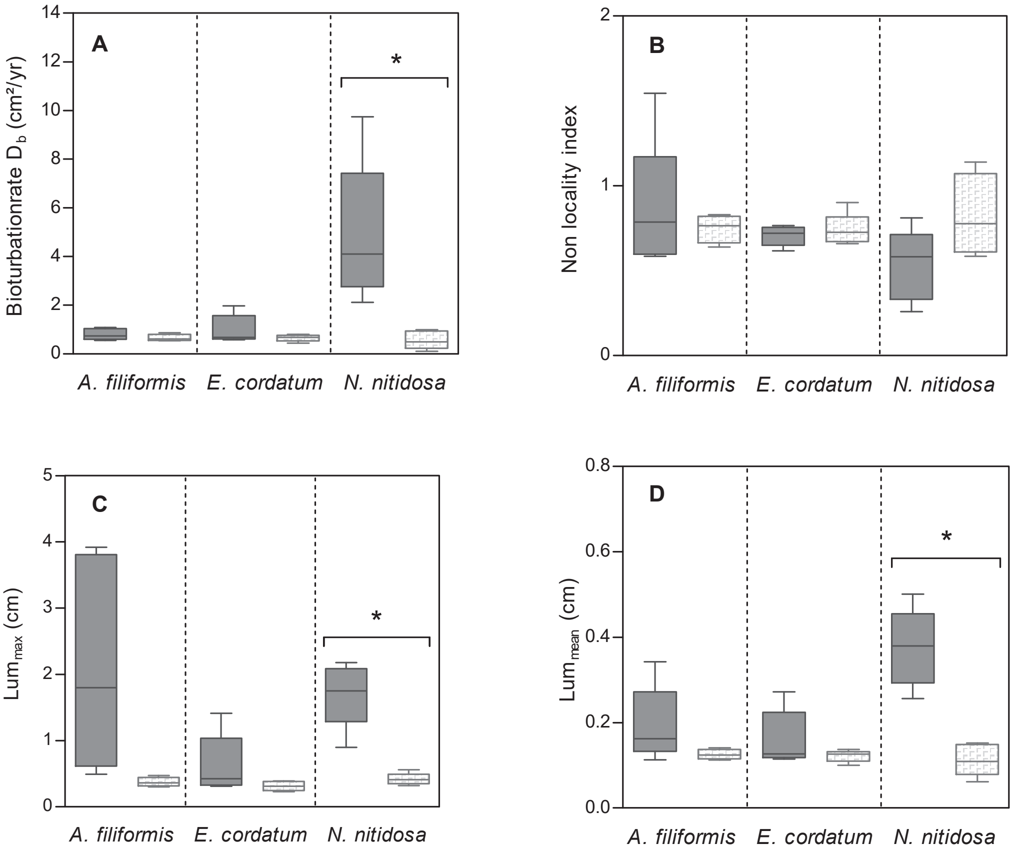

Fig. 5. A) Bioturbation rate (D_b), B) non-locality index (NLI), C) maximal vertical luminophore depth (Lum_{max}) and D) mean vertical luminophore distribution (Lum_{mean}) in *Amphiura filiformis*, *Echinocardium cordatum* and *Nucula nitidosa* experimental cores (dark grey) and in the respective controls without animals (light grey hatched). Boxes represent lower and upper quartiles; lines indicate median and whiskers show minima and maxima. Brackets with asterisks represent significant differences ($p < 0.05$) due to species effects.

3.3 Bioirrigation and nutrient flux

Only *E. cordatum* significantly enhanced the flux of bromide ($\Delta[\mathrm{Br}^-]$) into the sediment (Welch's t-test: $F_{1,6}$ = 19.58, p = 0.006; Fig. 6a). Solely *N. nitidosa* increased the oxygenation depth of the sediment (Welch's t-test: $F_{1,7}$ = 7.71, p = 0.039; Fig. 6b).

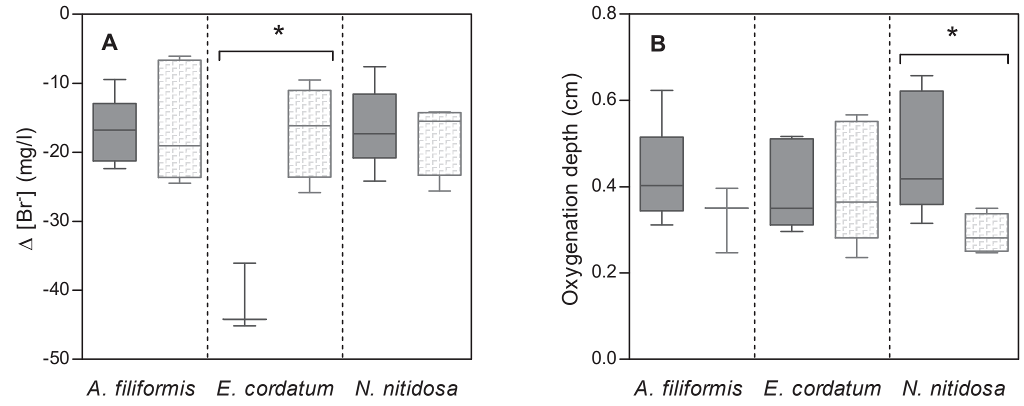

Fig. 6. A) Bioirrigation activity (ΔBr-) and B) sediment oxygenation depth in *Amphiura filiformis*, *Echinocardium cordatum* and *N. nitidosa* experimental cores (dark grey) and in the respective controls without animals (light grey hatched). Boxes represent lower and upper quartiles; lines indicate median and whiskers show minima and maxima. Brackets with asterisks represent significant differences ($p < 0.05$) due to species effects.

Silicate flux was significantly enhanced by all three species (Welch's tests: *A. filiformis*: $F_{1,8} = 205.72$, $p < 0.0001$; *E. cordatum*: $F_{1,6} = 1040.87$, $p < 0.0001$; *N. nitidosa*: $F_{1,8} = 7.16$, $p = 0.041$; Fig. 7a). The mean silicate flux was more than two times higher in the cores containing *E. cordatum* compared to all other treatments. *E. cordatum* also enhanced the flux of ammonium (Welch's test: $F_{1,6} = 41.48$, $p = 0.017$; Fig. 7b), nitrate (Welch's test: $F_{1,6} = 41.09$, $p = 0.002$; Fig. 7c), and nitrite (Welch's test: $F_{1,6} = 65.54$, $p = 0.006$; Fig. 7d) whereas *A. filiformis* and *N. nitidosa* had no effect on the fluxes of these compounds.

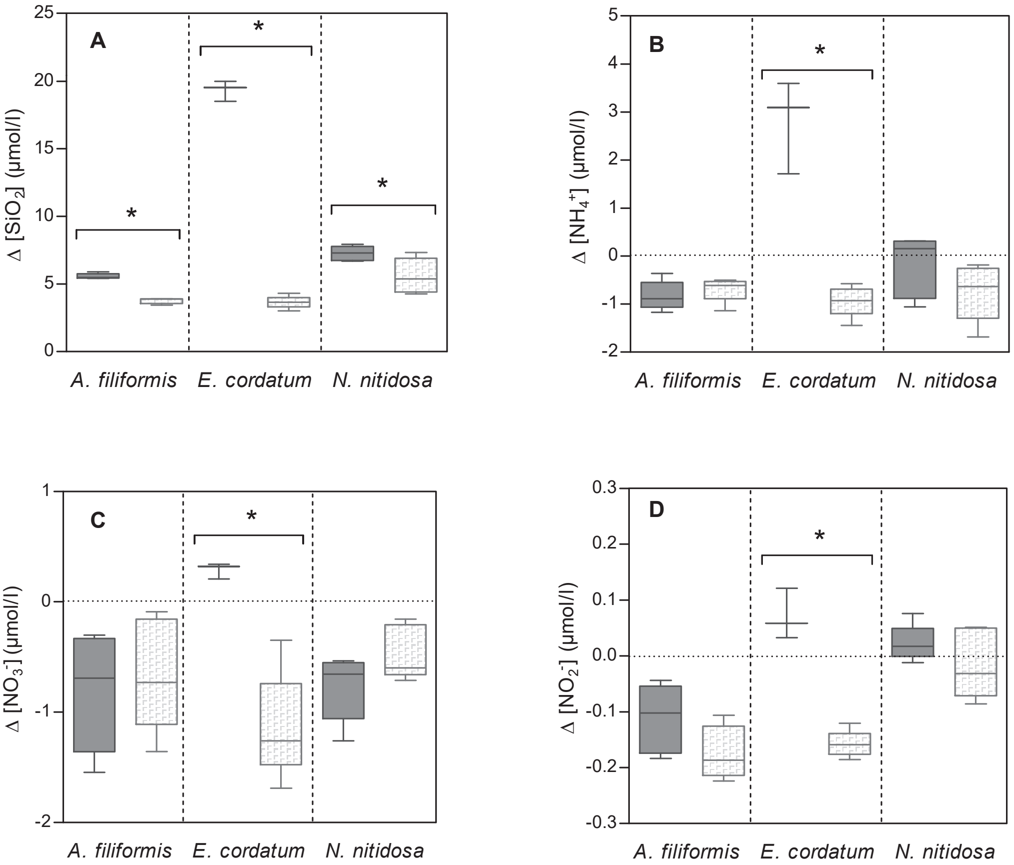

Fig. 7. Fluxes of A) silicate (ΔSi), B) ammonium (ΔNH_4^+), C) nitrate (ΔNO_3^-) and D) nitrite (ΔNO_2^-) in sediments with *Amphiura filiformis*, *Echinocardium cordatum* and *Nucula nitidosa* experimental cores (dark grey) and in the respective control sediments without animals (light grey hatched). Boxes represent lower and upper quartiles; lines indicate median and whiskers show minima and maxima. Brackets with asterisks represent significant differences ($p \geq 0.05$) due to species effects.

4. Discussion

4.1 Key bioturbators in the German Bight

Only a few species in the German Bight account for the bulk potential for bioturbation (BP_c), foremost *Amphiura filiformis* and *Echinocardium cordatum*. Both species occur in various types of sediment across the North Sea (Künitzer et al., 1992) and have been reported to be active bioturbators that sustain important ecosystem functions (Lohrer et al., 2004; Lohrer et al., 2005; Murray et al., 2013; Solan and Kennedy, 2002; Vopel et al., 2003). Apart from *A. filiformis* and *E. cordatum*, only *Nucula nitidosa* was considered a key bioturbator in this study. This bivalve is able to move several centimetres per day beneath the sediment surface thereby efficiently reworking the sediment (Rachor, 1976). Other top ranking species, in particular *Branchiostoma lanceolatum, Nephtys* sp. and *Phoronis* sp., were not considered key bioturbators as literature provided evidence that BP_i overestimated the effects of the species on sediment turnover and biogeochemical cycling. For example, low bioturbation activity was reported for *Phoronis* sp. and *Nephtys* sp. (Braeckman et al., 2010; Emig, 1979; Lindqvist et al., 2009), whereas *B. lanceolatum* is restricted to permeable sediments in which pore water transport overrules the effect of the bioturbating fauna (Braeckman et al., 2014; Courtney and Webb, 1964; Kristensen and Kostka, 2005; Kristensen and Hansen, 1999). Unfortunately, literature information on these species was, apart from the cited studies, scarce. Further important bioturbators might have remained undetected because of the uneven distribution of the sampling stations considered in our analysis. A conspicuous high density cluster of stations in the western part of the German Bight may have led to an overestimation of the spatial importance of common species in the *Amphiura filiformis* benthic association in this region (Salzwedel et al., 1985). However, the good spatial coverage of the southern and central part of the German Bight allows for an accurate estimation of the bioturbation potential and the identification of its main contributors.

4.2 Bioturbation and biogeochemical cycling

Under the settings of this experiment, *N. nitidosa* displayed a higher bioturbation activity than *A. filiformis* and *E. cordatum*. The small bivalve caused a thorough diffusive mixing of the upper 2.5 cm of the sediment, redistributed particles and substantially enhanced the oxygenation depth. The reworking of the sediment was likely a consequence of the foraging activity of *N. nitidosa*. As a highly selective deposit feeder with only little ability for suspension feeding (Davis and Wilson, 1985), *N nitidosa* may creep and dig very actively several centimetres per day beneath the sediment surface in search of food. Thereby it creates trench-like burrows in the upper sediment layers that are oxygenated by the respiratory currents (Rachor, 1976). The bivalve, thus, increases the oxygenation depth but due to its shallow burial depth and irrigation rates too low for detection it does not have a strong impact on biogeochemical cycling ($\Delta[NH_4^+]$, $\Delta[NO_3^-]$, $\Delta[NO_2^-]$, $\Delta[SiO_2]$).

Surprisingly, both *A. filiformis* and *E. cordatum* showed no significant particle reworking activity although both species had higher experimental bioturbation potentials (${}^{exp}BP_i$) than *N. nitidosa* and have repeatedly been reported to be active bioturbators (Lohrer et al., 2004; Lohrer et al., 2005; Murray et al., 2013; O'Reilly et al., 2006; Solan and Kennedy, 2002; Vopel et al., 2003). The sea urchin *E. cordatum* showed a strong bioirrigation activity ($\Delta[Br^-]$) that likely increased the flux of ammonium ($\Delta[NH_4^+]$), nitrate ($\Delta[NO_3^-]$), nitrite ($\Delta[NO_2^-]$) and silicate ($\Delta[SiO_2]$) into the water column, whereas *A. filiformis* caused only a low outflow of silica, indicating only small irrigative activity.

Loss of arms and subsequent regeneration may explain the low bioturbation activity in *A. filiformis*. The brittle star *A. filiformis* readily discards arms when mechanically stimulated, e.g. when facing strong sub-lethal predation (Biressi et al., 2010; Dupont and Thorndyke, 2006; Sköld and Rosenberg, 1996) or rough sampling procedures. Although the specimens of *A. filiformis* were sampled very carefully, it was not possible to obtain individuals without any sign of arm loss and regeneration. In natural populations, 80 to 100 % of the individuals show signs of regeneration (Bowmer and Keegan, 1983; Buchanan, 1964). Bowmer and Keegan (1983) reported an average of 4 regenerating arms out of 5 arms. Only specimens with at least three wholly functional arms were selected for the experiments. Accordingly, the specimens used for this experiment were in a better status than some natural populations.

Bioturbation and irrigation by *A. filiformis* is mainly the result of arm undulation for feeding purposes (Wood et al., 2009). The brittle star, however, retracts damaged arms below the sediment surface (Makra and Keegan, 1999) and invests energy in their regeneration (Dupont and Thorndyke, 2006; Fielman et al., 1991), likely at the expense of bioturbation activity. Accordingly, high sub-lethal predation probably affects bioturbation activity of *A. filiformis* also in natural populations. It is, thus, questionable whether bioturbation by *A. filiformis* plays a key role in the German Bight as suggested by BP_c mapping and whether BP_c is an appropriate tool to quantify the bioturbation activity of all benthic macrofauna.

Queirós et al. (2015) showed that BP_c can only predict bioturbation distance (average distance travelled by sediment particles) but no other attributes of sediment reworking (e.g. bioturbation rate or depth). The index BP_c is, however, correlated with some biogeochemical processes such as ammonium efflux from the sediment and denitrification (Braeckman et al., 2014). Although calculated from traits that describe sediment reworking behaviour, BP_c might, thus, be a better predictor of bioirrigation activity and biogeochemical cycling than of sediment turnover.

This is supported by the high $^{exp}BP_i$ scores of *E. cordatum*, that were in agreement with its strong bioirrigation activity ($\Delta[Br^-]$), that likely increased the flux of ammonium ($\Delta[NH_4^+]$), nitrate ($\Delta[NO_3^-]$) nitrite ($\Delta[NO_2^-]$) and silicate ($\Delta[SiO_2]$) into the water column. In the natural environment, however, also high bioturbation rates - in accordance with the high ^{exp}BPi scores - may be expected of *E. cordatum.* Its bioturbation activity was likely underestimated in this experiment because of spatial restrictions in the small incubation chambers and the surficial luminophore application. Gilbert et al. (2007) showed with a similar experimental set-up, including not only surface but also deep luminophores (i.e. a layer of luminophores placed 3 cm below the sediment surface), that *E. cordatum* readily moves at the surface and transports surface particles into deeper sediment layers. Following these results, only a surficial luminophore application was chosen for this experiment. Surficial luminophore application alone, however, apparently cannot capture the whole scope of *E. cordatum*'s reworking activity as the sea urchin may burrow to a depth of about 20 cm not necessarily mixing the surface layers (Fish and Fish, 1996; Mortensen, 1927). In the experiments of the present study, *E. cordatum* was observed to burrow always beneath the surface at a depth of approx. 5 cm. Burrow depth in *E. cordatum* may change according to environmental factors such as sediment type and temperature but also within a population according to body size or individual preferences (Bergman and Hup, 1992; Beukema, 1985; Buchanan, 1966; Foster-Smith, 1978). Accordingly, the application of both surface and deep luminophores (Gilbert et al. 2007) would be a more appropriate approach to quantify bioturbation in *E. cordatum*.

Nevertheless, the strong effect of *E. cordatum* on nutrient flux suggests that biogeochemical cycling depends more on bioirrigation than on bioturbation (Mermillod-Blondin et al. (2004); Braeckman et al. (2010)). According to Foster-Smith (1978) *E . cordatum* irrigates and oxygenates large volumes of sediment, thereby stimulating microbial mineralization, generation of ammonium and nitrification (Braeckman et al., 2010; Laverock et al., 2011). Excretion by the urchin likely enhances the ammonium efflux from the sediment (Lohrer et al., 2004). Ammonium is the major source of inorganic nitrogen for marine primary producers. Accordingly, irrigation activity by *E. cordatum* likely supports marine primary production (Lohrer et al., 2004).

The strong bioirrigation activity of *E. cordatum* also enhanced the release of silica from the sediment. The oxygenation of the sediment likely promoted the dissolution of amorphous silica (SiO_2) while flushing of the burrow enhanced the flux of silica out of the sediment (Raimonet et al., 2013). The silica efflux created by *E. cordatum* was high in comparison to other species such as the polychaetes *Abrenicola pacifica* and *Eupolymnia heterobranchia* (Marinelli, 1992, 1994; Murray et al., 2013). As *E. cordatum* is widely distributed in the German Bight, the species likely plays an important role in the liberation of amorphous silica from the sediment. Silicate is mainly consumed by diatoms which strongly contribute to primary production in the German Bight (Cadée and Hegeman, 1974; Rousseau et al., 2002).

Nutrient fluxes likely have also been influenced independently of the presence of macrofauna by the different sediment characteristics (e.g. higher $\Delta[NO_2^-]$ values in the control chambers of *N. nitidosa* compared to the control chambers of the other two species). Nevertheless, the very pronounced enhancement of nutrient fluxes by *E. cordatum* demonstrates the importance of this species for biogeochemical cycling.

4.3 Conclusions

The results of this study clearly confirmed that *E. cordatum* plays an important role in the biogeochemical cycling of the German Bight. Given the wide distribution of *E. cordatum* on a great variety of sediments and its strong bioirrigative activity, the species may substantially contribute to benthic biogeochemical cycling, thereby enhancing the availability of nutrients for primary production in the German Bight. Calculation and mapping of bioturbation potential ($^{exp}BP_i$, BP_i and BP_c), however, proofed to be an ambiguous tool in identifying key bioturbators. While it was imprecise in predicting actual bioturbation rates, agreement between $^{exp}BP_i$ and the bioirrigation activity of the three species as well as their influence on the nutrient fluxes suggests that BP_i and BP_c may be useful proxies for biogeochemical cycling. The results of this study clearly show that experiments are indispensable when trying to understand the effects of benthic organisms on sediment turnover and biogeochemical cycling.

Acknowledgements

The authors would like to thank the crews of the RV Mya and RV Uthörn as well as C. Brack, T. Romanova and P. Kadel for their support during animal collection and laboratory experiments. Further, we would like to thank R. and H. Asmus and K.H. Wiltshire for their support and for providing access to the facilities of the Wadden Sea Station Sylt of the AWI. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

References

Aller, R.C., 1982. The effects of macrobenthos on chemical properties of marine sediment and overlying water. In: Mc Call, P.L., Tevesz, M.J.S. (Eds.), Animal-sediment relations. Plenum Press, New York, 53-120.

Aller, R.C., 1994. Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillations. Chem. Geol. 114, 331-345.

Bergman, M., Hup, M., 1992. Direct effects of beamtrawling on macrofauna in a sandy sediment in the southern North Sea. ICES J. Mar. Sci. 49, 5-11.

Beukema, J., 1985. Growth and dynamics in populations of *Echinocardium cordatum* living in the North Sea off the Dutch north coast. Neth. J. Sea Res. 19, 129-134.

Birchenough, S.N.R., Parker, R.E., McManus, E., Barry, J., 2012. Combining bioturbation and redox metrics: potential tools for assessing seabed function. Ecol. Indic. 12, 8-16.

Biressi, A.C.M., Zou, T., Dupont, S., Dahlberg, C., Di Benedetto, C., Bonasoro, F., Thorndyke, M., Carnevali, M.D.C., 2010. Wound healing and arm regeneration in *Ophioderma longicaudum* and *Amphiura filiformis* (Ophiuroidea, Echinodermata): comparative morphogenesis and histogenesis. Zoomorphology 129, 1-19.

Blackburn, T., 1988. Benthic mineralization and bacterial production. In: Blackburn, T.H., Sørensen, J. (Eds.), Nitrogen cycling in coastal marine environments. SCOPE. Wiley and Sons Ltd., Chichester 175-206.

Bowmer, T., Keegan, B., 1983. Field survey of the occurrence and significance of regeneration in *Amphiura filiformis* (Echinodermata: Ophiuroidea) from Galway Bay, west coast of Ireland. Mar. Biol. 74, 65-71.

Braeckman, U., Foshtomi, M.Y., Van Gansbeke, D., Meysman, F., Soetaert, K., Vincx, M., Vanaverbeke, J., 2014. Variable importance of macrofaunal functional biodiversity for biogeochemical cycling in temperate coastal sediments. Ecosystems 17, 720-737.

Braeckman, U., Provoost, P., Gribsholt, B., Van Gansbeke, D., Middelburg, J.J., Soetaert, K., Vincx, M., Vanaverbeke, J., 2010. Role of macrofauna functional traits and density in biogeochemical fluxes and bioturbation. Mar. Ecol. Prog. Ser. 399, 173-186.

Buchanan, J.B., 1964. A comparative study of some features of the biology of *Amphiura filiformis* and *Amphiura chiajei* [Ophiuroidea] considered in relation to their distribution. J. Mar. Biol. Assoc. UK. 44, 565-576.

Buchanan, J.B., 1966. The biology of *Echinocardium cordatum* [Echinodermata: Spatangoidea] from different habitats. J. Mar. Biol. Assoc. UK. 46, 97-114.

Cadée, G., Hegeman, J., 1974. Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea. Neth. J. Sea Res. 8, 260-291.

Coates, D.A., Deschutter, Y., Vincx, M., Vanaverbeke, J., 2014. Enrichment and shifts in macrobenthic assemblages in an offshore wind farm area in the Belgian part of the North Sea. Mar. Environ. Res. 95, 1-12.

Courtney, W.M., Webb, J., 1964. The effects of the cold winter of 1962/63 on the Helgoland population of *Branchiostoma lanceolatum* (Pallas). Helgoland. Wiss. Meer. 10, 301-312.

Crank, J., 1975. Mathematics of diffusion. Clarendon Press, Oxford.

Davis, J., Wilson, J., 1985. The energy budget and population structure of *Nucula turgida* in Dublin Bay. J. Anim. Ecol. 54, 557-571.

Dupont, S., Thorndyke, M.C., 2006. Growth or differentiation? Adaptive regeneration in the brittlestar *Amphiura filiformis*. J. Exp. Biol. 209, 3873-3881.

Emig, C.C., 1979. British and other phoronids. The Linnean Society of London, London.

Fernandes, S., Meysman, F.J.R., Sobral, P., 2006. The influence of Cu contamination on *Nereis diversicolor* bioturbation. Mar. Chem. 102, 148-158.

Fielman, K., Stancyk, S., Dobson, W., Clements, L.J., 1991. Effects of disc and arm loss on regeneration by *Microphiopholis gracillima* (Echinodermata: Ophiuroidea) in nutrient-free seawater. Mar. Biol. 111, 121-127.

Fish, J.D., Fish, S., 1996. A student's guide to the seashore. Cambridge University Press, Cambridge.

Forster, S., Glud, R.N., Gundersen, J., Huettel, M., 1999. In situ study of bromide tracer and oxygen flux in coastal sediments. Estuar. Coast. Shelf S. 49, 813-827.

Foster-Smith, R.L., 1978. An analysis of water flow in tube-living animals. J. Exp. Mar. Biol. Ecol. 34, 73-95.

Giangrande, A., Montresor, M., Cavallo, A., Licciano, M., 2002. Influence of *Naineris laevigata* (Polychaeta: Orbiniidae) on vertical grain size distribution, and dinoflagellate resting stages in the sediment. J. Sea Res. 47, 97-108.

Gilbert, F., Hulth, S., Grossi, V., Poggiale, J.-C., Desrosiers, G., Rosenberg, R., Gérino, M., François-Carcaillet, F., Michaud, E., Stora, G., 2007. Sediment reworking by marine benthic species from the Gullmar Fjord (western Sweden): importance of faunal biovolume. J. Exp. Mar. Biol. Ecol. 348, 133-144.

Gilbert, F., Hulth, S., Stromberg, N., Ringdahl, K., Poggiale, J.C., 2003. 2-D optical quantification of particle reworking activities in marine surface sediments. J. Exp. Mar. Biol. Ecol. 285, 251-263.

Grant, J., Daborn, G., 1994. The effects of bioturbation on sediment transport on an intertidal mudflat. Neth. J. Sea Res. 32, 63-72.

Hale, R., Mavrogordato, M.N., Tolhurst, T.J., Solan, M., 2014. Characterizations of how species mediate ecosystem properties require more comprehensive functional effect descriptors, Sci. Rep. 4, 1-6.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. Oikos 69, 373-386.

Kostka, JE (Eds.), Interactions between macro- and microorganisms in marine sediments. American Geophysical Union, Washington D. C., 125-157.

Kristensen, E., 2000. Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiologia 426, 1-24.

Kristensen, E., Kostka, J.E., 2005. Macrofaunal burrows and irrigation in marine sediment: microbiological and biogeochemical interactions. In: Kristensen, E.H., RR;

Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T. Quintana, C.O., Banta, G.T., 2012.What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Mar. Ecol. Prog. Ser. 446, 285-302.

Kristensen, K., Hansen, K., 1999. Transport of carbon dioxide and ammonium in bioturbated (*Nereis diversicolor*) coastal, marine sediments. Biogeochemistry 45, 147-168.

Künitzer, A., Basford, D., Craeymeersch, J., Dewarumez, J., Dörjes, J., Duineveld, G., Eleftheriou, A., Heip, C., Herman, P., Kingston, P., 1992. The benthic infauna of the North Sea: species distribution and assemblages. ICES J. Mar. Sci. 49, 127-143.

Laverock, B., Gilbert, J., Tait, K., Osborn, A.M., Widdicombe, S., 2011. Bioturbation: impact on the marine nitrogen cycle. Biochem. Soc. T. 39, 315-320.

Lindqvist, S., Norling, K., Hulth, S., 2009. Biogeochemistry in highly reduced mussel farm sediments during macrofaunal recolonization by *Amphiura filiformis* and *Nephtys* sp.. Mar. Environ. Res. 67, 136-145.

Lohrer, A.M., Thrush, S.F., Gibbs, M.M., 2004. Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431, 1092-1095.

Lohrer, A.M., Thrush, S.F., Hunt, L., Hancock, N., Lundquist, C., 2005. Rapid reworking of subtidal sediments by burrowing spatangoid urchins. J. Exp. Mar. Biol. Ecol. 321, 155-169.

Maire, O., Lecroart, P., Meysman, F., Rosenberg, R., Duchene, J.C., Gremare, A., 2008. Quantification of sediment reworking rates in bioturbation research: a review. Aquat. Biol. 2, 219-238.

Makra, A., Keegan, B.F., 1999. Arm regeneration in *Acrocnida brachiata* (Ophiuroidea) at Little Killary, west coast of Ireland. Biol. Environ. 99B, 95-102.

Marinelli, R.L., 1992. Effects of polychaetes on silicate dynamics and fluxes in sediments: importance of species, animal activity and polychaete effects on benthic diatoms. J. Mar. Res. 50, 745-779.

Marinelli, R.L., 1994. Effects of burrow ventilation on activities of a terebellid polychaete and silicate removal from sediment pore waters. Limnol. Oceanogr. 39, 303-317.

Meadows, P.S., Meadows, A., 1991. The geotechnical and geochemical implications of bioturbation in marine sedimentary ecosystems. Sym. Zool. S. of London 63, 157-181.

Mermillod-Blondin, F., Francois-Carcaillet, F., Rosenberg, R., 2005. Biodiversity of benthic invertebrates and organic matter processing in shallow marine sediments: an experimental study. J. Exp. Mar. Biol. Ecol. 315, 187-209.

Mermillod-Blondin, F., Rosenberg, R., François-Carcaillet, F., Norling, K., Maulaire, L., 2004. Influence of bioturbation by three benthic infaunal species on microbial communities and biogeochemical processes in marine sediment. Aquat. Microb. Ecol., 271-284.

Meysman, F.J., Middelburg, J.J., Heip, C.H., 2006. Bioturbation: a fresh look at Darwin's last idea. Trends Ecol. Evol. 21, 688-695.

Mortensen, T., 1927. Handbook of the echinoderms of the British Isles. Oxford University Press, London

Murray, F., Widdicombe, S., McNeill, C.L., Solan, M., 2013. Consequences of a simulated rapid ocean acidification event for benthic ecosystem processes and functions. Mar. Pollut. Bull. 73, 435-442.

O'Reilly, R., Kennedy, R., Patterson, A., 2006. Destruction of conspecific bioturbation structures by *Amphiura filiformis* (Ophiuroida): evidence from luminophore tracers and in situ time-lapse sediment-profile imagery. Mar. Ecol. Prog. Ser. 315, 99-111.

Queirós, A.M., Birchenough, S.N.R., Bremner, J., Godbold, J.A., Parker, R.E., Romero-Ramirez, A., Reiss, H., Solan, M., Somerfield, P.J., Van Colen, C., Van Hoey, G., Widdicombe, S., 2013. A bioturbation classification of European marine infaunal invertebrates. Ecol. Evol. 3, 3958-3985.

Queirós, A.M., Stephens, N., Cook, R., Ravaglioli, C., Nunes, J., Dashfield, S., Harris, C., Tilstone, G.H., Fishwick, J., Braeckman, U., 2015. Can benthic community structure be used to predict the process of bioturbation in real ecosystems? Prog. Oceanogr. 137, 559-569.

Rachor, E., 1976. Structure, dynamics and productivity of a population of *Nucula nitidosa* (Bivalvia; Protobranchiata) in the German Bight. Ber. Deut. Wiss. Komm. 24, 296-331.

Rachor, E., 1990. Changes in sublittoral zoobenthos in the German Bight with regard to eutrophication. Neth. J. Sea Res. 25, 209-214.

Raimonet, M., Ragueneau, O., Andrieux-Loyer, F., Philippon, X., Kérouel, R., Le Goff, M., Mémery, L., 2013. Spatio-temporal variability in benthic silica cycling in two macrotidal estuaries: causes and consequences for local to global studies. Estuar. Coast. Shelf S. 119, 31-43.

Rousseau, V., Leynaert, A., Daoud, N., Lancelot, C., 2002. Diatom succession, silicification and silicic acid availability in Belgian coastal waters (southern North Sea). Mar. Ecol. Prog. Ser. 236, 61-73.

Rowden, A.A., Jones, M.B., Morris, A.W., 1998. The role of *Callianassa subterranea* (Montagu)(Thalassinidea) in sediment resuspension in the North Sea. Cont. Shelf Res. 18, 1365-1380.

Salzwedel, H., Rachor, E., Gerdes, D., 1985. Benthic macrofauna communities in the German Bight. Veröff. Inst. Meeresf. Bremerhaven 20, 199-267.

Sköld, M., Rosenberg, R., 1996. Arm regeneration frequency in eight species of Ophiuroidea (Echinodermata) from European sea areas. J. Sea Res. 35, 353-362.

Solan, M., Cardinale, B.J., Downing, A.L., Engelhardt, K.A.M., Ruesink, J.L., Srivastava, D.S., 2004. Extinction and ecosystem function in the marine benthos. Science 306, 1177-1180.

Solan, M., Kennedy, R., 2002. Observation and quantification of in situ animal-sediment relations using time-lapse sediment profile imagery (t-SPI). Mar. Ecol. Prog. Ser. 228, 179-191.

Tillin, H., Hiddink, J., Jennings, S., Kaiser, M., 2006. Chronic bottom trawling alters the functional composition of benthic invertebrate communities on a sea-basin scale. Mar. Ecol. Prog. Ser. 318, 31-45.

Vopel, K., Thistle, D., Rosenberg, R., 2003. Effect of the brittle star *Amphiura filiformis* (Amphiuridae, Echinodermata) on oxygen flux into the sediment. Limnol. Oceanogr. 48, 2034-2045.

Waldbusser, G.G., Marinelli, R.L., Whitlatch, R.B., Visscher, P.T., 2004. The effects of infaunal biodiversity on biogeochemistry of coastal marine sediments. Limnol. Oceanogr. 49, 1482-1492.

Widdows, J., Brown, S., Brinsley, M.D., Salkeld, P.N., Elliott, M., 2000. Temporal changes in intertidal sediment erodability: influence of biological and climatic factors. Cont. Shelf Res. 20, 1275-1289.

Wood, H.L., Widdicombe, S., Spicer, J.I., 2009. The influence of hypercapnia and the infaunal brittlestar *Amphiura filiformis* on sediment nutrient flux - will ocean acidification affect nutrient exchange? Biogeosciences 6, 2015-2024.

Zimmerman, D.W., 2004. A note on preliminary tests of equality of variances. Brit. J. Math. Stat. Psy. 57, 173-181.

Organism functional traits and ecosystem supporting services - a novel approach to predict bioirrigation

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The manuscript was submitted for publication to *Ecological Indicators* by September 2017 and is currently under review

Abstract

Increasing anthropogenic activities on land and at sea underline the demand for easily applicable indices to effectively predict human mediated changes in ecosystem functioning. Here, we propose a novel bioirrigation index (IP_c) that is based on body mass, abundance, burrow type, feeding type, and injection pocket depth of bottom dwelling animals. Results from both community and single-species experimental incubations indicate that IP_c is able to predict the bioirrigation rate in different sediment types (mud, fine sand, sand). The trait-based index, thus, demonstrates robustness in the prediction of animal-mediated functional processes that support biogeochemical functions under variable abiotic and biotic conditions. Accordingly, we argue that trait-based indices provide a useful tool for the quantitative prediction of ecosystem processes as effect traits provide a direct link to the behavioral mechanisms that drive ecosystem functioning.

Keywords: bioturbation, biogeochemical cycling, bentho-pelagic coupling, bioturbation potential, ecosystem services, Marine Strategy Framework Directive

1. Introduction

The increasing exploitation of natural resources by mankind proposes a growing threat to many ecosystem functions and associated services (Naeem et al., 2012). In marine systems, rising water temperatures, fisheries, construction of offshore installations, and underwater noise progressively change the structure of benthic communities and how benthic organisms influence ecosystem functioning (e.g. biogeochemical cycling, sediment mixing) (Coates et al., 2014; Solan et al., 2016; Tillin et al., 2006). Accordingly, easily applicable descriptors of key ecosystem functions have become of great importance for both policy and science. For example, descriptor 6 (Sea-Floor Integrity) of the EU Marine Strategy Framework Directive (MSFD) specifically calls for multi-metric indices to assess benthic community condition and functionality (European Union 2008) while the Millennium Ecosystem Assessment (Duraiappah, 2005) underlines the urgent need for a broader set of diversity indicators aligned with valued functions. Taxonomic identity, abundance or biomass of species alone have little power in explaining ecosystem processes (e.g. pollination, bioturbation, biocontrol of pests), as these processes are determined by the functional characteristics (i.e. the ecological effect traits) of the organisms involved (Díaz and Cabido, 2001; Gagic et al., 2015). Recently, trait-based models proved their potential to outperform common ecosystem models in the quantitative prediction of ecosystem functioning (Ghimire et al., 2017; Zwart et al., 2015). Indices based on functional traits may, thus, be a promising tool to meet the societal and political demands.

Key services, such as biogeochemical cycling, remineralization, soil formation, soil fertility, oxygen and water regulation, are strongly influenced by the reworking and irrigating (i.e. ventilation and burrow flushing) activities of invertebrates within both terrestrial and aquatic sediment or soil (de Bello et al., 2010; Kristensen et al., 2012; Valença et al., 2017; Wilkinson et al., 2009). Especially in shallow marine areas, the bioirrigation activity of the benthic fauna is of major importance for biogeochemical cycling (Braeckman et al., 2010; Mermillod-Blondin et al., 2004). Bioirrigation promotes the movement of pore water and associated solutes (e.g. O_2, CO_2, dissolved organic matter (DOM), inorganic nutrients) (Kristensen et al., 2012). Accordingly, the oxygenation of deeper, otherwise anoxic layers of the sediment strongly depends on bioirrigation (Aller, 1994; Kristensen, 2000).

Hence, bioirrigation enlarges the sediment volume where aerobic processes can take place (e.g. nitrification) (Na et al., 2008) and, thus, enhances nutrient turnover as well as mineralization (Aller, 1982). As a result, bioirrigation constitutes a significant driver of overall ecosystem functioning (primary production, benthic-pelagic coupling, biogeochemical cycling) (Lohrer et al., 2004). Consequently, the loss of bioirrigation activity may have broad implications for overall ecosystem performance (Lohrer et al., 2004).

So far, there is no reliable index or model to predict broad scale changes in bioirrigation activity that are due to shifts in functional community composition or species extinction. The existing models are either only phenomenological descriptions of the bioirrigation process with a weak connection to the underlying biology (i.e. diffusive, non-local and advective one-dimensional models (Meysman et al., 2006b)) or based on complex microenvironment modelling that is dependent on rarely measured parameters (e.g. pumping rate, burrow volume, specific burrow morphology) (i.e. the mechanistic models of Aller (1980) and Meysman et al. (2006a)).

Recently, the trait-based non-quantitative concept of community bioturbation potential (BP_c) was traded and applied as an approach to estimate the effects of species extinctions on sediment reworking and ecosystem properties (biogenic mixing depth, total organic carbon content, chlorophyll concentrations, oxygenation depth, sediment oxygen consumption, ammonium efflux and denitrification) (Birchenough et al., 2012; Braeckman et al., 2014; Gogina et al., 2017; Solan et al., 2004). Yet, estimations by BP_c may be misleading as they are not quantitatively scaled and based on the assumption that sediment reworking is the main driver of biogeochemical cycling. However, biogeochemical cycling depends more on bioirrigation than on sediment reworking (Braeckman et al., 2010; Mermillod-Blondin et al., 2004; Wrede et al., 2017). Accordingly, BP_c uses effect traits (i.e. body mass, mobility and reworking mode) that are, apart from body mass, poorly suited to describe the major macrofaunal effect on biogeochemical cycling. Especially the mobility trait of BP_c is likely to underestimate the contribution of sessile species, with low mobility but high bioirrigation, to biogeochemical cycling, whereas the contribution of highly mobile species with low bioirrigation is overestimated (Braeckman et al., 2010).

Bioirrigation is mostly driven by ventilation and suspension feeding activities of benthic species (Aller, 1982; Kristensen, 2001; Kristensen et al., 2012). Consequently, it is primarily dependent on body mass and feeding type (Christensen et al., 2000), whereas injection pocket depth and the general burrow morphology have a more indirect effect on bioirrigation and biogeochemical cycling (Kristensen et al., 2012). A deeper injection pocket requires, for a complete exchange of burrow water, the transport of larger volumes of water whereas the burrow morphology essentially determines whether advective transport of water into the surrounding sediment occurs (Kristensen et al., 2012).

Based on these effect traits we propose, in the style of BP_c, the novel irrigation potential IP_c, as a specific estimate of the bioirrigation activity of the benthic fauna. Furthermore, we validated IP_c with a multi-factorial experiment and demonstrate that IP_c will allow for quantitative modelling of macrofaunal bioirrigation activity over large spatial scales. We hypothesize that IP_c will be a better predictor of bioirrigation activity than BP_c.

2. Material and Methods

2.1 Irrigation potential (IP_c)

We adapted the bioturbation potential (BP_c) of Solan et al. (2004) and replaced the traits mobility and reworking mode (defined in more detail by Queirós et al. 2013) by traits that characterize the irrigation behaviour of benthic macrofaunal organisms and its effects on ecosystem functioning: burrow type (BT_i), feeding type (FT_i), injection pocket depth (ID_i). The index now reads as follows:

$$\mathrm{IP_c} = \sum_{i=1}^{n} \left(\frac{B_i}{A_i}\right)^{0.75} * A_i * BT_i * FT_i * ID_i \qquad (1)$$

where B_i is the biomass of species i and A_i its abundance. Following the approach of Solan et al. (2004), the traits are subdivided in categories that describe the species' specific expression of the respective trait (Tab.1). Each category is assigned a descriptive numerical score. A low score indicates low bioirrigation and associated effects, whereas a high value indicates high bioirrigation and associated effects (for example: "feeding type: predator – score: 2, sub surface filter feeder – score: 4"). The body mass term is weighted by an exponent of 0.75 to account for the scaling of metabolic activity with body mass (Brey, 2010; West and Brown, 2005), which we consider to be more appropriate than the square root scaling applied by Solan et al. (2004).

Table 1: Irrigation potential effect traits burrow type, feeding type and injection pocket depth with the respective categories and scores.

Effect trait	Category	Score
burrow type	epifauna, internal irrigation (e.g. siphons)	1
	open irrigation (e.g. U- or Y- shaped burrows)	2
	blind ended irrigation (e.g. blind ended burrows, no burrow systems)	3
feeding type	surface filter feeder	1
	predator	2
	deposit feeder	3
	sub surface filter feeder	4
injection pocket depth	0 - 2 cm	1
	2 - 5 cm	2
	5 - 10 cm	3
	> 10 cm	4

2.2 Experiment

Single and multispecies laboratory experiments were run to test IP_c performance. Sediments and macrofaunal organisms were collected from three different sedimentary habitats within the German Bight (mud, fine sand and sand) (54°7’21’’N 8°12’96’’E; 54°0’50’’N 7°48’51’’E; 55°01’32’’N 8°26’10’’E). The muddy habitat was inhabited by a *Nucula nitidosa*-community (Salzwedel et al., 1985), the fine sand habitat by an *Amphiura filiformis*-community (Salzwedel et al., 1985) and the sand habitat by a *Lanice conchilega*-reef (Rabaut et al., 2008). The *Nucula*- and *Amphiura*- community were sampled in April 2016 by ship while the *Lanice*- reef was sampled in March 2016 by foot during low tide. From each of these communities, 10 cylindrical in situ cores (h: 35 cm, d: 9.4 cm) were taken. In the case of the *Lanice*-reef, cores were randomly sampled from a 12 m² area within the reef. For the other two communities, the cores were carefully extracted from 0.1 m² box cores to maintain an undisturbed sediment column. The cores were then transferred to a climate controlled room (on board of the RV Heincke for the *Nucula*- and *Amphiura*-community and to the Wadden Sea Station of the AWI at the island of Sylt for the *Lanice*-community) where they were constantly aerated through an air stone. Experimental temperature was 8 °C which is about the annual mean bottom temperature in the southern North Sea (Dulvy et al., 2008). The water was exchanged once a day for the ship sampled cores (*Nucula*- and *Amphiura*-community), whereas the cores from the *Lanice*-community were directly connected to a continuous flow of filtered seawater (30 µm drumfilter: Spranger; protein skimmer: Sander) from the Sylt-Rømø Bight. After 5 days, the *Amphiua*- and *Nucula*-community cores were also transferred to the climate controlled room at the Wadden Sea Station of the AWI and connected to the continuous flow system. Seven days after the sampling, the experiment was started.

Parallel to the sampling of in situ cores, two dominant species were chosen from each of the three habitats as model organisms for the single-species treatment. Representative species were: *Nucula nitidosa* Winckworth, 1930 and *Owenia fusiformis* Delle Chiaje, 1844 for the *Nucula*-community, *Amphiura filiformis* (O.F. Müller, 1776) and *Echinocardium coradtum* (Pennant, 1777) for the *Amphiura*-community, *Lanice conchilega* (Pallas, 1766), and *Cerastoderma edule* Linnaeus, 1758 for the *Lanice*-community. Specimens of these six species were sampled simultaneously to the in situ cores. *A. filiformis*, *E. cordatum*, *N. nitidosa* and *O. fusiformis* were sampled with van Veen grabs (0.1 m²) while *L. conchilega* and *C. edule* were dug up directly from the sediment. Specimens of *A. filiformis*, *E. cordaum*, *N. nitidosa*, *O. fusiformis* and *C. edule* were carefully isolated from the sediment, separated by species and directly transferred into seawater aquaria, aerated by air stones and filled with sediment (6 cm). Specimens of *L. conchilega* were extracted and replanted into sweater aquaria filled with sediment (15 cm) as described by Ziegelmeier (1969). Specimens of *O. fusiformis* were carefully replanted with tweezers.

Simultaneous to the sampling of the organisms, the sediment was collected. After the organisms had been carefully removed, the sediment of each sampling site was sieved through a 1 mm mesh and left to settle in large tanks. After 24 hours, the overlying water was decanted. The sediment was homogenized by mixing and poured into rectangular incubation chambers (h: 35 cm, w: 9.4 cm, d: 9.4 cm) up to a sediment column height of 18 cm (±1 cm) after settling. In preparation of the experiments, 25 chambers (20 incubation chambers - 10 for each species and five control chambers) were prepared for each sampling site. The sediment was left to settle for six days.

Identical to the in situ cores, the seawater aquaria and incubation chambers were transferred to a climate controlled room (8°C). The water was exchanged once a day for the seawater aquaria and the respective incubation chambers of *A. filiformis*, *E. cordaum*, *N. nitidosa* and *O. fusiformis* until they were connected on the fifth day after sampling to the continuous flow of filtered seawater at the Wadden Sea Station of the AWI while the aquaria and incubation chambers of *L. conchilega* and *C. edule* were directly connected to the continuous flow system.

Six days after sampling, individuals of all species where recovered from the aquaria and selected according to two size classes per species (Appendix A Table 1), number of intact arms (= 5) in *A. filiformis* and, for all species, according to observation of activity. The selected individuals were placed in the replicate rectangular incubation chambers (n = 5 per size class treatment). Densities were chosen in accordance to natural densities (*A. filiformis*, *N. nitidosa*, *L. conchilega*, and *O. fusiformis* 5 individuals per core, *C. edule* 2 individuals per core, *E. cordatum* 1 individual per core). After one day of acclimatisation within incubation chambers, the experiment was started.

Bioirrigation and biogeochemical cycling was measured on the seventh day after the start of the experiment. For this purpose, the continuous seawater flow was stopped to start the incubation. At the beginning of the measuring period (t_0), a sodium bromide solution (0.668 g NaBr dissolved in seawater aiming at a Br^- concentration of 5 mM in the water column above the sediment) was carefully stirred into the water column of each incubation chamber. Samples of the seawater medium (3ml) were extracted and filtered (0.2 µm surfactant-free cellulose acetate membrane, Minisart Syringe Filter, Satorius) at the start of the incubation and after 1, 2, 4, 8, and 12 hours (Forster et al., 1999). All water samples were stored at 3°C until analysis by ion-chromatography (Metrohm, 930 Compact IC Flex). Additional samples of the seawater medium (sample volume: 8ml) were collected at the start of the incubation and after 8 hours to measure concentrations of nutrients.

The bioirrigation activity of the organisms was assessed as described in De Smet et al. (2016) using the following formula:

$$Q = -\frac{V_{ow}}{C_{T_0} - C_{T_{reference}}} * \frac{dC_{T_0}}{dt} \tag{2}$$

where Q is the bioirrigation activity per core or incubation chamber (l/h), V_{ow} the volume of the water overlying the sediment, C_{T0} the Br^- concentration at T_0, $C_{Treference}$ the ambient Br^- concentration of seawater and C_{T0}/dt the slope of the linear regressions of the Br^- concentration versus time over the 12h of the incubation. The bioirrigation rate in l/m²/h was calculated by dividing Q by the surface area of the respective incubation chambers or cores. In eight cases, the Br^- concentration increased (i.e. negative bioirrigation) which indicates very low or no bioirrigation activity. However, an increase in Br^- concentration is theoretically not possible. Accordingly, these values were considered laboratory artefacts and were excluded from the analysis (Appendix A Table 1). In twelve other cases, the rates were also excluded from the analysis because of accidental loss of the samples (e.g. the breaking of the sample container during freezing) (Appendix A Table 1). In order to solely assess the bioirrigation activity of the organisms and to correct for advective effects caused by water circulation, mean irrigation rates of the controls of each sediment type was subtracted from the irrigation rates for both the single- and community set-ups for each respective sediment type.

Two days after the bioirrigation measurement, the community cores were sliced into different depth strata from which the organisms were manually retrieved. The sediment of the single-species set-ups was extracted from the incubation chambers and sieved through a 1 mm mesh to recover the organisms. All retrieved organisms were fixed in a buffered 5% formaldehyde solution and stored for 3 months. Thereafter, species taxonomy as well as wet weight (WW) (g), dry weight (g), and ash free dry weight (AFDW (g)) per species per core or incubation chamber was determined. Information on species traits were compiled from the literature. This information was translated for each trait into a score according to the predetermined scoring categories (Tab. 1). If no information was available, the scoring was based on taxonomically closely related species.

2.3 Statistical analysis

We applied generalized linear models (GLM) to analyse the relationship between both IP_c and BP_c (calculated for both WM and AFDM) and bioirrigation activity. Grubb's outlier test identified a single outlier that was consequently excluded from the analysis. The GLM included sediment type (mud, fine sand, sand) and the treatment (single-species or community set-up) as independent variables and tested for interaction effects on the bioirrigation activity. The best model was chosen according to Zuur et al. (2007) by comparison of Akaike's information criterion (AICc) where the lowest AICc indicates the best model. Goodness of fit was tested through least squares. All analyses were performed with the software package JMP 13.1.0 (SAS Institute GmbH).

In order to analyse and compare differences between BP_c and IP_c on a spatial scale, values for both indices were mapped in ArcGIS on abundance and biomass data from the German North Sea exclusive economic zone (EEZ), derived from the AWI benthos database for ecological research and from environmental impact assessments of offshore wind farms by the German Federal Maritime and Hydrographic Agency (BSH). The data set covered 2227 stations and 569 species.

The data was taxonomically harmonised and converted from WM (g) to AFDM (g) using the conversion factor data base of Brey (2001). In case of a non-availability of a conversion factor for a taxon, the conversion factor of the next higher taxon level was used. The data was further averaged over 5 km grid cells to prevent species overestimation caused by heterogeneity in station distribution. The species spectrum was taxonomically reduced to cover only those species that accounted for 90% of both total biomass and abundance (83 species in total). IP_c trait scores were compiled from literature information (Appendix C) while scores for BP_c were taken from the classifications of Queirós et al. (2013).

3. Results

In the models, macrofaunal irrigation activity was best described by $IP_{c,AFDM}$ (Prob > $Chi^2 < 0.0001$; $R^2 = 0.6050$; AICc = -391.576). The predicted bioirrigation rates of the community set-ups were higher than the predicted rates of the single-species set-ups, but the slope of the relationship between predicted bioirrigation rate and $IP_{c,AFDM}$ did not differ between the two treatments (Prob > $Chi^2 = 0.0008$) (Fig. 1).

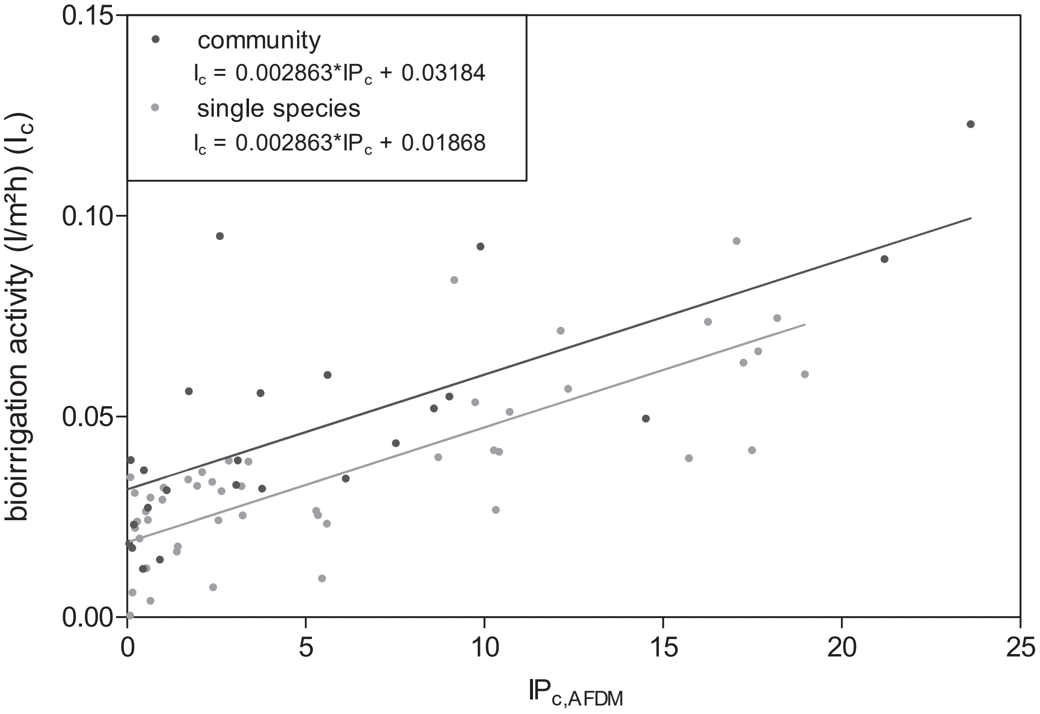

Fig. 1. Relationship between the irrigation potential $IP_{c,AFDM}$ and the bioirrigation activity (l/m²h). The community experiment is highlighted in black, single species experiments in light grey:

Sediment type did not affect the bioirrigation activity of the organisms (Appendix C). $IP_{c,WM}$ (AICc = -390.3283), $BP_{c,WM}$ (AICc = -346.4898) and $BP_{c\ AFDM}$ (AICc = -346.4970) were less good predictors of the bioirrigation activity (Appendix B). Spatial mapping of $IP_{c,AFDM}$ and $BP_{c,AFDM}$ revealed that $BP_{c,AFDM}$ led to an underestimation in the southern, eastern, and central region of the German EEZ, whereas an overestimation was observed along the glacial valley of the river Elbe and in the north-western German Bight (Fig. 2).

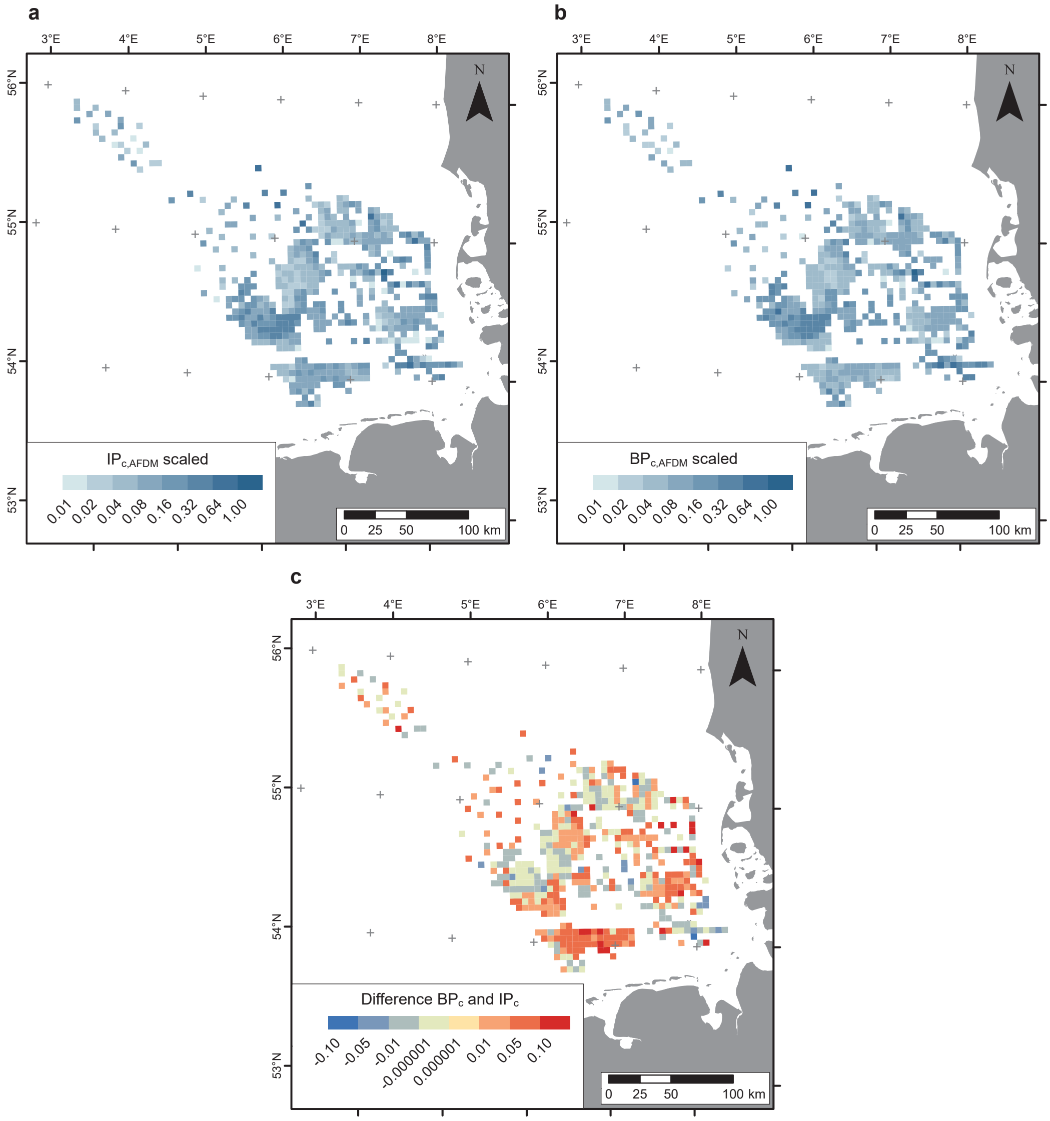

Fig. 2. a) $IP_{c,AFDM}$ and b) $BP_{c,AFDM}$ plotted in the German North Sea EEZ for species contributing 90% of total stations abundance and biomass scaled to 1 for comparability. c) Differences between $IP_{c,AFDM}$ and $BP_{c,AFDM}$: Blue indicates an overestimation and red an underestimation if BP_c would be applied to estimate bioirrigation activity.

4. Discussion

Here we provide the first quantitative model that is able to predict the bioirrigation activity of communities from information on taxonomic identity, abundance and biomass. Compared to one-dimensional bioirrigation models (Meysman et al., 2006b) and to mechanistic models of Aller (1980) and Meysman et al. (2006a), IP_c is, thus, suitable for an upscaling of bioirrigation activity over large spatial scales and for assessing changes due to species extinctions or shifts in species composition. This spatial applicability is supported by the good level of robustness of the of the IP_c against potential trait variation due to interspecific interaction (e.g. competition), regional origin, and sediment type, as neither sediment type nor treatment (single-species or community) affected the relationship between IP_c and the bioirrigation rates. In contrast to IP_c, BP_c was not able to predict bioirrigation activity (Apendix B) and is, therefore, likely to misjudge the effect of macrofaunal organisms on biogeochemical cycling (Mermillod-Blondin et al., 2004).

This is mostly due to the square root scaling of the body mass trait in BP_c. It causes an overestimation of species with low body mass and accordingly, lower respiration and consumption demands. For IP_c we chose a scaling factor of 0.75 which is a better and proven descriptor of energy transfer in marine systems (West and Brown, 2005). IP_c further takes into account the feeding type of organisms, which is essential to assess bioirrigation and biogeochemical cycling, because suspension feeders may cause up to ten times higher irrigation rates and a doubling of biogeochemical cycling as compared to similarly sized deposit feeders (Christensen et al., 2000). In contrast, BP_c focuses on the mobility of organisms (Solan et al., 2004) and is, therefore, likely to underestimate the impact of sessile species with low bioturbation activity such as *Lanice conchilga* and *Chaetopterus variopedatus* (Renier, 1804) which are known for their strong bioirrigation activity and pronounced influence on biogeochemical cycling (Braeckman et al., 2010; Riisgård, 1989).

On a spatial scale, these differences in trait choice become apparent in an underestimation in the eastern and central region of the German EEZ where sessile or semi-sessile species (i.e. *Lanice conchilega* and *Notomasus latericeus* Sars, 1851) were particularly abundant. Similarly, the bioirrigation activity was overestimated by BP_c

along the Elbe glacial valley and in the north-western German Bight in assemblages of species with small AFDM (i.e. *Amphiura filiformis* and *Corbula gibba* (Olivi, 1792)).

In addition to trait choice, trait variation caused by biotic, abiotic and anthropogenic drivers may result in spatial variability in individual trait expressions and, consequently, in bioirrigation activity (Baranov et al., 2016; Murray et al., 2017; Solan et al., 2016; Wohlgemuth et al., 2017). Generally, higher bioirrigation rates in our community experiment than in the single species experiment were most likely related to the differences in core design. Hale et al. (2014a) observed higher bioirrigation rates in circular cores (our community experiment) compared to square-shaped cores (our single-species experiment). Interspecific interactions in the community experiments are unlikely responsible for this difference because species interactions have been shown to reduce rather than increase bioirrigation activity in benthic assemblages (Mermillod-Blondin et al., 2005; Waldbusser et al., 2004).

Bioirrigation rate will, however, certainly be affected by temperature - with higher temperatures, metabolic rates increase and, consequently, the ventilation activity is required to fulfil the higher demands for oxygen and food (Brey, 2010; Grémare, 1990; Kristensen et al., 2012; Ouellette et al., 2004). The effect of temperature on bioirrigation may be accounted for by linking a temperature term to bioirrigation activity (I_c), estimated from IP_c, based on the Q_{10} temperature coefficient (i.e., the rate of change in metabolism as a consequence of increasing the temperature by 10 °C):

$$I_{c\,T} = I_c * Q_{10}^{\left(\frac{T-TQ8}{10}\right)} \tag{3}$$

where T_Q is the reference temperature (i.e., at which rates were determined experimentally, here T_Q = 8°C) and T is ambient temperature in °C. The Q_{10} of macrobenthic metabolism lies around 2 (Davis and McIntire, 1983; Grant, 1986; Provoost et al., 2013), hence, we should expect irrigation activity in the North Sea during summer at temperatures above 16°C to be almost twice the annual mean and in winter, when temperature falls below 2°C, about half the annual mean. These extrapolations have to be verified experimentally.

Already now, IP_c represents, within its validated temperature range, a most promising solution for management purposes that enables us to meet the societal and political demands. On small spatial scales, IP_c constitutes a useful tool to predict human impacts (e.g. offshore installations) on bioirrigation activity and consequently on biogeochemical cycling and related ecosystem services, whereas on broad spatial scales, IP_c may be applied to support descriptor 6 (Sea-Floor Integrity) of the EU Marine Strategy Framework Directive (MSFD) or the costal marine spatial plans in the United States.

Acknowledgements

The authors would like to thank the crews of the RV Heincke as well as H. Pehlke, K. Stieler, B. Ebbe, J. Steger, J. Meyer, K. Stumpf, T. Romanova and P. Kadel for their support during animal collection and laboratory experiments. Further, we would like to thank R. and H. Asmus and K.H. Wiltshire for their support and for providing access to the facilities of the Wadden Sea Station Sylt of the AWI. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Aller, R.C., 1980. Quantifying solute distributions in the bioturbated zone of marine-sediments by defining an average micro-environment. Geochim. Cosmochim. Acta 44, 1955-1965.

Aller, R.C., 1982. The effects of macrobenthos on chemical properties of marine sediment and overlying water, in: Mc Call, P.L., Tevesz, M.J.S. (Eds.), Animal-Sediment Relations. Springer US, pp. 53-102.

Aller, R.C., 1994. Bioturbation and remineralization of sedimentary organic-matter-effects of redox oscillation. Chem. Geol. 114, 331-345.

Baranov, V., Lewandowski, J., Krause, S., 2016. Bioturbation enhances the aerobic respiration of lake sediments in warming lakes. Biol. Letters 12, 20160448.

Birchenough, S.N.R., Parker, R.E., McManus, E., Barry, J., 2012. Combining bioturbation and redox metrics: potential tools for assessing seabed function. Ecol. Indic. 12, 8-16.

Braeckman, U., Foshtomi, M.Y., Van Gansbeke, D., Meysman, F., Soetaert, K., Vincx, M., Vanaverbeke, J., 2014. Variable importance of macrofaunal functional biodiversity for biogeochemical cycling in temperate coastal sediments. Ecosystems 17, 720-737.

Braeckman, U., Provoost, P., Gribsholt, B., Van Gansbeke, D., Middelburg, J.J., Soetaert, K., Vincx, M., Vanaverbeke, J., 2010. Role of macrofauna functional traits and density in biogeochemical fluxes and bioturbation. Mar. Ecol. Prog. Ser. 399, 173-186.

Brey, T., 2001. Population dynamics in benthic invertebrates. A virtual handbook. Version 01.2.

Brey, T., 2010. An empirical model for estimating aquatic invertebrate respiration. Methods Ecol. Evol. 1, 92-101.

Christensen, B., Vedel, A., Kristensen, E., 2000. Carbon and nitrogen fluxes in sediment inhabited by suspension-feeding (*Nereis diversicolor*) and non-suspension-feeding (*N. virens*) polychaetes. Mar. Ecol. Prog. Ser. 192, 203-217.

Coates, D.A., Deschutter, Y., Vincx, M., Vanaverbeke, J., 2014. Enrichment and shifts in macrobenthic assemblages in an offshore wind farm area in the Belgian part of the North Sea. Mar. Environ. Res. 95, 1-12.

Davis, M.W., McIntire, C.D., 1983. Effects of physical gradients on the production dynamics of sediment-associated algae. Mar. Ecol. Prog. Series 13, 103-114.

de Bello, F., Lavorel, S., Díaz, S., Harrington, R., Cornelissen, J.H., Bardgett, R.D., Berg, M.P., Cipriotti, P., Feld, C.K., Hering, D., 2010. Towards an assessment of multiple ecosystem processes and services via functional traits. Biodivers. Conserv. 19, 2873-2893.

de Smet, B., van Oevelen, D., Vincx, M., Vanaverbeke, J., Soetaert, K., 2016. Lanice conchilega structures carbon flows in soft-bottom intertidal areas. Mar. Ecol. Prog. Ser. 552, 47-60.

Díaz, S., Cabido, M., 2001. Vive la difference: plant functional diversity matters to ecosystem processes. Trends Ecol. Evol. 16, 646-655.

Dulvy, N.K., Rogers, S.I., Jennings, S., Stelzenmüller, V., Dye, S.R., Skjoldal, H.R., 2008. Climate change and deepening of the North Sea fish assemblage: a biotic indicator of warming seas. J. Appl. Ecol. 45, 1029-1039.

Duraiappah, A.N., S.; Agardy, T., 2005. Ecosystems and human well-being: biodiversity synthesis; a report of the millennium ecosystem assessment. World Resources Institute: Washington, Washington, USA.

European Union, 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive), Official Journal of the European Union. European Union, pp. 19-40.

Forster, S., Glud, R.N., Gundersen, J., Huettel, M., 1999. In situ study of bromide tracer and oxygen flux in coastal sediments. Estuar. Coast. Shelf S. 49, 813-827.

Gagic, V., Bartomeus, I., Jonsson, T., Taylor, A., Winqvist, C., Fischer, C., Slade, E.M., Steffan-Dewenter, I., Emmerson, M., Potts, S.G., 2015. Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. Proc. R. Soc. Ser. B.-Bio. 282, 20142620.

Ghimire, B., Riley, W.J., Koven, C.D., Kattge, J., Rogers, A., Reich, P.B., Wright, I.J., 2017. A global trait-based approach to estimate leaf nitrogen functional allocation from observations. Ecol. Appl. 27, 1421-1434.

Gogina, M., Morys, C., Forster, S., Gräwe, U., Friedland, R., Zettler, M.L., 2017. Towards benthic ecosystem functioning maps: quantifying bioturbation potential in the German part of the Baltic Sea. Ecol. Indic. 73, 574-588.

Grant, J., 1986. Sensitivity of benthic community respiration and primary production to changes in temperature and light. Mar. Biol. 90, 299-306.

Grémare, A., 1990. Consumption of diatoms and diatom filtrates by the tentaculate deposit-feeder *Eupolymnia nebulosa* (Annelida: Polychaeta). Mar. Biol. 106, 139-143.

Hale, R., Mavrogordato, M., Tolhurst, T., Solan, M., 2014. Characterizations of how species mediate ecosystem properties require more comprehensive functional effect descriptors. Sci. Rep. 4, 2045-2322.

Kristensen, E., 2000. Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiologia 426, 1-24.

Kristensen, E., 2001. Impact of polychaetes (*Nereis* spp. and *Arenicola marina*) on carbon biogeochemistry in coastal marine sediments. Geochem. Trans. 2, 92-103.

Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C.O., Banta, G.T., 2012. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Mar. Ecol. Prog. Ser. 446, 285-302.

Lohrer, A.M., Thrush, S.F., Gibbs, M.M., 2004. Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431, 1092-1095.

Mermillod-Blondin, F., Francois-Carcaillet, F., Rosenberg, R., 2005. Biodiversity of benthic invertebrates and organic matter processing in shallow marine sediments: an experimental study. J. Exp. Mar. Biol. Ecol. 315, 187-209.

Mermillod-Blondin, F., Rosenberg, R., François-Carcaillet, F., Norling, K., Maulaire, L., 2004. Influence of bioturbation by three benthic infaunal species on microbial communities and biogeochemical processes in marine sediment. Aquat. Microb. Ecol. 36, 271-284.

Meysman, F.J.R., Galaktionov, E.S., Gribsholt, B., Middelburg, J.J., 2006a. Bioirrigation in permeable sediments: advective pore-water transport induced by burrow ventilation. Limnol. Oceanogr. 51, 142-156.

Meysman, F.J.R., Galaktionov, O.S., Gribsholt, B., Middelburg, J.J., 2006b. Bio-irrigation in permeable sediments: an assessment of model complexity. J. Mar. Res. 64, 589-627.

Murray, F., Solan, M., Douglas, A., 2017. Effects of algal enrichment and salinity on sediment particle reworking activity and associated nutrient generation mediated by the intertidal polychaete *Hediste diversicolor*. J. Exp. Mar. Biol. Ecol. 495, 75-82.

Na, T., Gribsholt, B., Galaktionov, O.S., Lee, T., Meysman, F.J., 2008. Influence of advective bio-irrigation on carbon and nitrogen cycling in sandy sediments. J. Mar. Res. 66, 691-722.

Naeem, S., Duffy, J.E., Zavaleta, E., 2012. The functions of biological diversity in an age of extinction. Science 336, 1401-1406.

Ouellette, D., Desrosiers, G., Gagne, J.P., Gilbert, F., Poggiale, J.C., Blier, P.U., Stora, G., 2004. Effects of temperature on in vitro sediment reworking processes by a gallery biodiffusor, the polychaete *Neanthes virens*. Mar. Ecol. Prog. Ser. 266, 185-193.

Provoost, P., Braeckman, U., Van Gansbeke, D., Moodley, L., Soetaert, K., Middelburg, J.J., Vanaverbeke, J., 2013. Modelling benthic oxygen consumption and benthic-pelagic coupling at a shallow station in the southern North Sea. Estuar. Coast. Shelf S. 120, 1-11.

Queirós, A.M., Birchenough, S.N.R., Bremner, J., Godbold, J.A., Parker, R.E., Romero-Ramirez, A., Reiss, H., Solan, M., Somerfield, P.J., Van Colen, C., Van Hoey, G., Widdicombe, S., 2013. A bioturbation classification of European marine infaunal invertebrates. Ecol. Evol. 3, 3958-3985.

Rabaut, M., Vincx, M., Degraer, S., 2008. Do Lanice conchilega (sandmason) aggregations classify as reefs? Quantifying habitat modifying effects. Helgoland Mar. Res. 63, 37-46.

Riisgård, H.U., 1989. Properties and energy cost of the muscular piston pump in the suspension feeding polychaete *Chaetopterus variopedatus*. Mar. Ecol. Prog. Ser. 56, 157-168.

Salzwedel, H., Rachor, E., Gerdes, D., 1985. Benthic macrofauna communities in the German Bight. Veröff. Inst. Meeresf. Bremerhaven 20, 199-267.

Solan, M., Cardinale, B.J., Downing, A.L., Engelhardt, K.A.M., Ruesink, J.L., Srivastava, D.S., 2004. Extinction and ecosystem function in the marine benthos. Science 306, 1177-1180.

Solan, M., Hauton, C., Godbold, J.A., Wood, C.L., Leighton, T.G., White, P., 2016. Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rep. 6, 20540.

Tillin, H., Hiddink, J., Jennings, S., Kaiser, M., 2006. Chronic bottom trawling alters the functional composition of benthic invertebrate communities on a sea-basin scale. Mar. Ecol. Prog. Ser. 318, 31-45.

Valença, A.W., Vanek, S.J., Meza, K., Ccanto, R., Olivera, E., Scurrah, M., Lantinga, E.A., Fonte, S.J., 2017. Land use as a driver of soil fertility and biodiversity across an agricultural landscape in the Central Peruvian Andes. Ecol. Appl. 27, 1138-1154.

Waldbusser, G.G., Marinelli, R.L., Whitlatch, R.B., Visscher, P.T., 2004. The effects of infaunal biodiversity on biogeochemistry of coastal marine sediments. Limnol. Oceanogr. 49, 1482-1492.

West, G.B., Brown, J.H., 2005. The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. J. Exp. Biol. 208, 1575-1592.

Wilkinson, M.T., Richards, P.J., Humphreys, G.S., 2009. Breaking ground: pedological, geological, and ecological implications of soil bioturbation. Earth Sci. Rev. 97, 257-272.

Wohlgemuth, D., Solan, M., Godbold, J.A., 2017. Species contributions to ecosystem process and function can be population dependent and modified by biotic and abiotic setting. Proc. R. Soc. Ser. B.-Bio 284, 2016-2805.

Wrede, A., Dannheim, J., Gutow, L., Brey, T., 2017. Who really matters: Influence of German Bight key bioturbators on biogeochemical cycling and sediment turnover. J. Exp. Mar. Biol. Ecol. 488, 92-101.

Ziegelmeier, E., 1969. Neue Untersuchungen über die Wohnröhren-Bauweise von *Lanice conchilega* (Polychaeta, Sedentaria). Helgolander Wiss. Meeresunters 19, 216-229.

Zwart, J.A., Solomon, C.T., Jones, S.E., 2015. Phytoplankton traits predict ecosystem function in a global set of lakes. Ecology 96, 2257-2264.

Zuur, A., Ieno, E.N., Smith, G.M., 2007. Analyzing ecological data. Springer Science & Business Media.

Macrofaunal irrigation traits enhance predictability of biogeochemical cycling

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The manuscript is in preparation for submission to *Marine Ecology Progress Series*.

Abstract

Sediment reworking traits (e.g. as used in the index community bioturbation potential BP_c) are widely used to characterize the impact of macrofauna communities on biogeochemical cycling. However, bioirrigation has a stronger impact on biogeochemical turnover than sediment reworking activities. Thus, we ask the following questions: (i) Are irrigation traits (i.e. as applied in the index community irrigation potential (IP_c)) suitable predictors of biogeochemical cycling (changing concentrations of phosphate $[PO_4^{3-}]$, silicate $[SiO_2]$, ammonium $[NH_4^+]$, nitrate $[NO_3^-]$, and nitrite $[NO_2^-]$) under different environmental conditions (sediment type, gradients across the sediment water column, temperature, faunal inventory), and (ii) do irrigation traits increase the predictability of biogeochemical cycling compared to sediment reworking traits (i.e. BP_c)? To answer these questions, we correlate IP_c and BP_c with experimental nutrient flux data from this, and a previous, experimental study. Generally, IP_c predicts the fluxes of all measured nutrients better than BP_c, i.e., biogeochemical cycling is more closely linked to irrigation traits than to sediment reworking traits. The body volume of the macro-organisms involved plays a significant role for flows of $\Delta[SiO_2]$, $\Delta[NH_4^+]$, $\Delta[NO_3^-]$, and $\Delta[NO_2^-]$ but not for phosphate. Presumably, in our experiments, the release of phosphate into the water column was the result of excretion activity of the animals. Phosphate flow was, thus, linked more closely to metabolic activity than to body volume. We observe a general statistically significant impact of treatment and (except for phosphate) a statistical interaction between IP_c and treatment in our experiments. Hence, the macrofaunal impact, as measured by IP_c is a suitable - albeit not a sufficient - parameter in the modelling of sediment biogeochemical turnover.

Keywords: bioirrigation, bioturbation, functional traits, ecosystem functioning

1. Introduction

Benthic biogeochemical cycling and mineralisation processes are strongly promoted by macrofaunal activities such as sediment reworking (biomixing of sediment particles) and bioirrigation (ventilation and flushing of burrows) (Aller, 1994; Baranov et al., 2016; Kristensen, 2000). The scope of these activities depends largely on the functional characteristics (i.e. traits) of the macrofaunal organisms. Accordingly, many studies have explored the predictability of biogeochemical cycling via macrofaunal traits or functional groups (Braeckman et al., 2009; Murray et al., 2014; Solan et al., 2004). The non-quantitative trait-based index of bioturbation potential (BP_c (Solan et al., 2004)) was widely adopted (Birchenough et al., 2012; Gogina et al., 2017; Queirós et al., 2013). BP_c correlates with biogenic mixing depth, total organic carbon content, chlorophyll concentrations, oxygenation depth, sediment oxygen consumption, ammonium efflux, and denitrification (Birchenough et al., 2012; Braeckman et al., 2014; Gogina et al., 2017).

BP_c, however, may describe macrofaunal impact improperly as it is based on the assumption that sediment reworking is the main driver of sediment biogeochemical cycling. Yet biogeochemical cycling depends more on bioirrigation than on sediment reworking (Braeckman et al., 2014; Mermillod-Blondin et al., 2004; Wrede et al., 2017). To overcome false assumptions of $BP_{c,}$ Wrede et al. (submitted) propose an alternative index, the irrigation potential IP_c. In contrast to BP_c, which incorporates sediment reworking traits (mobility and reworking type), IP_c is based on irrigation traits such as feeding type, burrow type, and injection pocket depth (Solan et al. 2004, Wrede et al. submitted). In contrast to BP_c, IP_c (based on macrobenthic ash free dry mass (AFDM)) shows a consistent correlation with macrofaunal irrigation activity across various sediment types and communities (Wrede et al. submitted). Yet, to date, we lack confirmation that IP_c is as well a suitable predictor of biogeochemical turnover rates, and, thus, may enhance models of sediment biogeochemical cycling.

Additionally, there is a need to resolve the ambiguity in the use of macrofaunal body mass units between IP_c and BP_c. While BP_c is based on wet body mass (WM) in most studies (Braeckman et al., 2014; Gogina et al., 2017; Wrede et al., 2017), Wrede et al (submitted) demonstrated that AFMD based IP_c correlates better with bioirrigation.

Accordingly, this study asks the following questions: (i) can irrigation traits (i.e. IP_c) predict biogeochemical cycling (changing concentrations of phosphate, silicate, ammonium, nitrate, and nitrite under different environmental conditions (sediment type, gradients across the sediment water column, temperature, faunal inventory)), (ii) do irrigation traits (IP_c) increase the predictability of biogeochemical cycling compared to sediment reworking traits (BP_c), (iii) to which extent does it matter which body mass units are used for IP_c or BP_c, and (iv) do the environmental conditions affect the relationships between trait-based indices and biogeochemical cycling?

2. Material and Methods

2.1 Dataset

We used data from this study and a previous study on German Bight sediments (Wrede et al., 2017). The data encompassed biomass (WM and AFDM), abundance, species identity, and nutrient flux measurements ($\Delta[PO_4^{3-}]$, $\Delta[SiO_2]$, $\Delta[NH_4^+]$, $NO_3^-]$ and $\Delta[NO_2^-]$) from laboratory incubations in different seasons (i) of natural communities inhabiting mud, fine sand, and sand sediments (mud: *Nucula nitidosa*-community (Salzwedel et al., 1985), fine sand: *Amphiura filiformis*-community (Salzwedel et al., 1985), sand: *Lanice conchilega*-reef (Rabaut et al., 2008)), and (ii) of artificial mono-cultures (mud: *Nucula nitidosa* Winckworth, 1930 and *Owenia fusiformis* Delle Chiaje, 1844, fine sand: *Amphiura filiformis* (O.F. Müller, 1776) and *Echinocardium coradtum* (Pennant, 1777), sand: *Lanice conchilega* (Pallas, 1766) and *Cerastoderma edule* Linnaeus, 1758). The data of the mono-cultures also included five control cores for every sediment type (cores without macrofaunal organisms).

In total, the data consisted of 140 (110 for NO_3^-) data sets. Regrettably, the heterogeneity of the data did not allow for a full factorial analysis. Due to time and space restraints, the experiments with the different sediment types were conducted in different months (spring: March and April, summer: July and August). Consequently, initial (at start of the experiment) nutrient gradients between sediment and water column differed between experiments with respect to season as well as sediment type (i.e. sand: measured in March and July; fine sand and mud measured in April and August). Further summer experiments (i.e. July and August) were restricted to mono-culture incubations. To be able to test whether different environmental conditions affect the capability of IP_c or BP_c in predicting nutrient fluxes, we split the data into nine sub-groups of homogeneous in-group conditions, i.e. identical initial sediment-water nutrient gradients, sediment type, sediment treatment (sieved homogenised sediment of mono-cultures vs. in situ stratification of communities) and temperature, and established a corresponding categorical variable TREATMENT (Tab.1). Previous studies have shown that these parameters can particularly affect nutrient fluxes (Baranov et al., 2016; Biswas et al., 2017; Mermillod-Blondin and Rosenberg, 2006). The categories contained macrofaunal organisms of the following species or communities: L_{summer} – mono-culture of *L. conchilega*; LC_{spring} – mono-cultures of *L. conchilega* and *C. edule*;

$L_{community}$ - in situ *Lanice*-community; AE_{summer} – mono-cultures of *A. filiformis* and *E. cordatum*; AE_{spring} – mono-cultures of *A. filiformis* and *E. cordatum*; $A_{comunity}$ – in situ *Amphiura*-community; N_{summer} – mono-cultures of *N. nitidosa*; NO_{spring} – mono-cultures of *N. nitidosa* and *O. fusiformis*; $N_{commuity}$ - in situ *Nucula*-community. Sampling of organisms and sediment as well as experiments are described in detail by Wrede et al. (2017) and Wrede et al. (submitted).

2.2 Measurements of biogeochemical cycling

Nutrient fluxes ($\Delta[PO_4^{3-}]$, $\Delta[SiO_2]$, $\Delta[NH_4^+]$, $\Delta[NO_3^-]$ and $\Delta[NO_2^-]$) were measured on the seventh day after the start of the experiments. At the start of the incubation (t_0), water flow was stopped and a sodium bromide solution was cautiously stirred into the water column of each core or incubation chamber for assessment of bioirrigation. Seawater samples of 8ml (summer 2015) or 13ml (spring 2016) were extracted and filtered (0.2 µm surfactant-free cellulose acetate membrane, Minisart Syringe Filter, Sartorius) after 0 and 8 hours (t_0, t_8) for measurement of nutrient concentrations. For bioirrigation measurements, further 3 ml samples were taken at t_0 t_2, t_4 and t_8. Nutrient samples were divided and stored for the measurement of $[SiO_2]$ at 3°C and for $[PO_4^{3-}]$, $[NH_4^+]$, $[NO_3^-]$ and $[NO_2^-]$ at -20°C. Samples were analysed by segmented flow analysis (SEAL AA3 HR Autoanalyzer) and ISO standard procedures (MT 18, MT 19) of the manufacturer (SEAL Analytical). Fluxes of $[PO_4^{3-}]$, $[SiO_2]$, $[NH_4^+]$, $[NO_3^-]$ and $[NO_2^-]$ were determined from the changes in concentration (C) over time and are given as ($\Delta[C] = t_8\text{-}t_0$). $\Delta[PO_4^{3-}]$ was only measured in the spring 2016 treatments. Owing to a malfunction of the Autoanalyser, 33 $\Delta[NO_3^-]$ values had to be excluded from further analysis (Tab.1).

Table 1: Environmental conditions within the nine TREATMENT categories (*tr*): n gives the number of datasets that were allocated to each category of treatment, n_{NO3-} gives the number of datasets which were included in the statistical assessment of $\Delta[NO_3^-]$, *S* the sediment types (sand (s), fine sand (fs) mud (m)), *c* the species composition (community (C), mono-culture (M)) and T the temperature in °C. Ambient nutrient conditions in the Sylt-Rømø Bight at the start of the incubations are given for phosphate, silicate, ammonium, nitrate, and nitrite in µmol/l as a proxy for the nutrient gradients between sediment and water column during the incubation. Blank spaces indicate that no data is available for these treatments (phosphate) or excluded due to errors (nitrate). Asterisks indicate that the data for these categories was taken from Wrede et al. (2017).

tr	*n*	n_{NO3-}	*S*	*c*	*T*	$[PO_4^{3-}]$	$[NO_2^-]$	$[NO_3^-]$	$[NH_4^+]$	$[SiO_2]$
$\mathrm{L}_{\mathrm{community}}$	10	10	s	C	8	0.65 ± 0.017	0.38 ± 0.020	39.25 ± 0.237	1.75 ± 0.450	27.86 ± 0.210
$\mathrm{LC}_{\mathrm{spring}}$	23	22	s	M	8	0.48 ± 0.025	0.28 ± 0.008	38.25 ± 0.351	1.07 ± 0.395	23.08 ± 0.842
$\mathrm{L}_{\mathrm{summer}}$	10	10	s	M	15		0.55 ± 0.027	3.75 ± 0.113	1.65 ± 0.530	6.32 ± 0.0962
$\mathrm{A}_{\mathrm{community}}$	10	10	fs	C	8	0.16 ± 0.005	0.16 ± 0.006	15.28 ± 0.549	1.40 ± 0.221	2.58 ± 0.116
$\mathrm{AE}_{\mathrm{spring}}$	24	-	fs	M	8	0.19 ± 0.005	0.18 ± 0.002		2.05 ± 0.127	2.36 ± 0.116
$\mathrm{AE}_{\mathrm{summer*}}$	18	18	fs	M	19		0.46 ± 0.015	5.36 ± 0.189	1.69 ± 0.319	8.40 ± 0.361
$\mathrm{N}_{\mathrm{community}}$	10	10	m	C	8	0.19 ± 0.013	0.17 ± 0.006	14.02 ± 0.249	2.16 ± 0.284	3.83 ± 0.538
$\mathrm{NO}_{\mathrm{spring}}$	25	20	m	M	8	0.20 ± 0.003	0.19 ± 0.003	12.30 ± 0.141	2.02 ± 0.123	2.30 ± 0.207
$\mathrm{N}_{\mathrm{summer*}}$	10	10	m	M	19		0.43 ± 0.009	5.55 ± 0.088	1.59 ± 0.288	8.33 ± 0.323

Later analysis revealed obvious deviations from the expected values by approximately the same value in the measurements of the nitrate concentrations ($[NO_3^-]$ at t_0 should always be similar within a treatment). As none of the other analyzed nutrients showed similar patterns of deviation, we attributed the deviating values to a malfunction of the Autoanalyser and not the experimental set up. Accordingly, we excluded 33 cores or incubation chambers (from originally 141) for the statistical analysis of $\Delta[NO_3^-]$ (Tab.1).

2.3 Determination of body mass

Organisms that survived the experimental period of 9 days were recovered and fixed in alcohol (summer 2015) or in a buffered 5% formaldehyde solution (spring 2015) for subsequent estimation of macrofaunal body mass. After at least three months of storing, species taxonomy, abundance, and biomass (in wet mass (g WW), dry mass (g DM) and ash free dry mass (g AFDW) of each species, per core or incubation chamber were determined. In accordance with Wetzel et al. (2005), we assumed that there are no significant differences in the body mass measurements between alcohol or formaldehyde fixed samples.

2.4 Calculation of bioturbation and irrigation potential

The irrigation potential (IP) of the community in each core or incubation chamber ($\mathrm{IP_c}$) was calculated according to Wrede et al. (submitted) by:

$$\mathrm{IP_c} = \sum_{i=1}^{n} \left(\frac{B_i}{A_i}\right)^{0.75} * A_i * BT_i * FT_i * ID_i \tag{1}$$

where A_i and B_i are abundance and biomass of species i and BT_i, FT_i and ID_i are categorical scores describing burrow type, feeding type, and injection pocket depth. Categorical scores on irrigation traits were compiled from the classifications provided by Wrede et al. (submitted).

The bioturbation potential (BP) of the community in each core or incubation chamber ($\mathrm{BP_c}$) was calculated according to Solan et al. (2004) and Queirós et al. (2013):

$$\mathrm{BP_c} = \sum_{i=1}^{n} \left(\frac{B_i}{A_i}\right)^{0.5} * A_i * M_i * R_i \tag{2}$$

where A_i and B_i are abundance and biomass of species (i) at a station while M_i and R_i are categorical scores describing the mobility and reworking mode of species (i). Categorical scores were adopted from the classifications provided by Queirós et al. (2013). Missing information on mobility and reworking mode was compiled from literature following the rules proposed by Queirós et al. (2013).

In order to assess whether irrigation traits or the body mass scaling factor are responsible for differences between $\mathrm{IP_c}$ and $\mathrm{BP_c}$, we also calculated a modified $\mathrm{IP_c}$ ($\mathrm{mIP_c}$). We thereby exchanged the $\mathrm{IP_c}$ body mass scaling factor of 0.75 against the $\mathrm{BP_c}$ body mass scaling factor of 0.5. Both $\mathrm{IP_c}$ and $\mathrm{BP_c}$, as well as $\mathrm{mIP_c}$ were calculated based on both WM and AFDM.

2.3 Statistical analysis

We constructed generalized linear models (GLM) of $\Delta[\mathrm{PO_4^{3-}}]$, $\Delta[\mathrm{SiO_2}]$, $\Delta[\mathrm{NH_4^+}]$, $\Delta[\mathrm{NO_3^-}]$ and Δ[NO2-] as a function of either $\mathrm{IP_c}$ or $\mathrm{BP_c}$ (calculated for both in WM and AFDM), the categorical variable TREATMENT ($\mathrm{L_{community}}$, $\mathrm{LC_{sping}}$, $\mathrm{L_{summer}}$, $\mathrm{A_{community}}$, $\mathrm{AE_{spring}}$, $\mathrm{AE_{summer}}$, $\mathrm{N_{community}}$, $\mathrm{NO_{sping}}$, $\mathrm{N_{summer}}$) and the interaction between index and TREATMENT. The best model was identified by backward selection and comparison of Akaike’s information criterion (AIC) where the lowest AIC indicates the best model

(Zuur et al., 2007). Visual inspection of the residual plots of the best models did not indicate deviation from homoscedasticity, but from normality for the GLM's of Δ[PO_4^{3-}], Δ[SiO_2], Δ[NH_4^+] and Δ[NO2-]. Transformation of the data did not achieve normality.

According to Quinn and Keough (2002), normality is an important but not a crucial assumption, whereas Gelman and Hill (2007) do not even recommend the diagnostics of normality for GLM's. Yet, to reduce the likelihood of a type I error due to non-normality of the data, we lowered the significance level to $\alpha < 0.01$.

For the estimation of the effect of the independent variables (index, TREATMENT, interaction between TREATMENT and index) we used the Wald Chi-square test. To test for differences due to the index composition (scaling factor, traits), we constructed further GLM's of the nutrient flux measurements as a function of mIP_c (calculated in both WM and AFDM), the TREATMENT, and the interaction of the index with the TREATMENT. The best model was chosen by comparison of AIC. Subsequently, we compared AIC of the GLM's based on mIP_c against AIC of the GLM's based on IP_c or BP_c. Smaller AIC_{mIPc} indicated for GLM's based on IP_c that nutrient fluxes are better represented with a body mass scaling factor of 0.5, while for GLM's based on BP_c it indicated that irrigation traits represent nutrient flux sediment reworking traits. All analyses were carried out within the R statistical and programming environment (R Core Team, 2013). ANOVA analysis of GLM's was performed with the package car (Fox and Weisberg, 2011).

3. Results

$IP_{c,AFDM}$ correlates with all measured nutrient fluxes significantly ($\alpha < 0.01$; Tab.2). TREATMENT affects the rates of all measured fluxes significantly ($\alpha < 0.01$; Tab.2). A significant interaction of $IP_{c,AFDM}$ and TREATMENT was found for $\Delta[NO_2^-]$, $\Delta[NO_3^-]$ and $\Delta[SiO_2]$. $IP_{c,AFDM}$ is the only index that significantly predicts $\Delta[PO_4^{3-}]$ ($\chi^2(1) = 8{,}51$; $p = 0.0035$).

Table 2: Statistic results (χ^2, *df* and $p > \chi^2$) from the ANOVA analysis of the best (lowest AIC) GLM models of $\Delta[PO4_3^-]$, $\Delta[NO_2^-]$, $\Delta[NO_3^-]$, $\Delta[NH_4^+]$ and $\Delta[SiO_2]$ as a function of the indices (*x*) IP_c and BP_c (both based on WM as well as AFDM), the TREATMENT (*tr*) and the interaction (*in*) between indices and TREATMENT. Asterisks indicate significance $\alpha < 0.01$ in column $p > \chi^2$. In the AIC column, they highlight the lowest AIC (i.e. the model that describes the distribution of the data best). Dashes indicate that backward selection via lowest AIC has either excluded the interaction from the model or, in case of BP_c, the index.

		χ^2			*df*			$p > \chi^2$			
x	nutrient	*x*	*tr*	*in*	*x*	*tr*	*in*	*x*	*tr*	*in*	AIC
$IP_{c,AFDM}$	$\Delta[PO_4^{3-}]$	8.51	48.01	-	1	5	5	*	*	-	-239.27 *
	$\Delta[NO_2^-]$	46.58	166.63	27.76	1	8	8	*	*	*	-231.87
	$\Delta[NO_3^-]$	40.03	104.74	33.68	1	7	7	*	*	*	207.75
	$\Delta[NH_4^+]$	49.99	78.91	-	1	8	8	*	*	-	637.79
	$\Delta[SiO_2]$	20.70	73.43	41.86	1	8	8	*	*	*	762.08
$IP_{c,WM}$	$\Delta[PO_4^{3-}]$	4.12	54.09	10.28	1	5	5		*		-235.64
	$\Delta[NO_2^-]$	33.40	221.11	48.74	1	8	8	*	*	*	-237.34 *
	$\Delta[NO_3^-]$	10.97	137.11	79.75	1	7	7	*	*	*	197.2 *
	$\Delta[NH_4^+]$	65.47	137.98	22.58	1	8	8	*	*	*	623.24 *
	$\Delta[SiO_2]$	58.54	70.16	22.72	1	8	8	*	*	*	748.55 *
$BP_{c,AFDM}$	$\Delta[PO_4^{3-}]$	-	-	-	1	5	5	-	-	-	-232.5
	$\Delta[NO_2^-]$	3.00	161.15	27.75	1	8	8		*	*	-196.67
	$\Delta[NO_3^-]$	28.70	110.79	26.46	1	7	7	*	*	*	220.6
	$\Delta[NH_4^+]$	17.45	95.97	14.78	1	8	8	*	*		666.54
	$\Delta[SiO_2]$	1.96	48.10	-	1	8	8		*	-	801.69
$BP_{c,WM}$	$\Delta[PO_4^{3-}]$	-	-	-	1	5	5	-	-	-	-232.5
	$\Delta[NO_2^-]$	1.30	181.27	40.61	1	8	8		*	*	-206.55
	$\Delta[NO_3^-]$	15.81	115.64	39.27	1	7	7	*	*	*	220.63
	$\Delta[NH_4^+]$	18.98	100.71	-	1	8	8	*	*	-	664.29
	$\Delta[SiO_2]$	6.79	65.27	42.99	1	8	8	*	*	*	772.14

$IP_{c,WM}$ correlates with all measured nutrient fluxes significantly but $\Delta[PO_4^{3-}]$ ($\alpha > 0.01$; Tab.2). TREATMENT affects all measured fluxes significantly ($\alpha < 0.01$; Tab.2). A significant interaction of $IP_{c,WM}$ and TREATMENT was found for $\Delta[NO_2^-]$, $\Delta[NO_3^-]$, $\Delta[NH_4^+]$ and $\Delta[SiO_2]$.

AIC indicates that the GLM of $IP_{c,AFDM}$ and TREATMENT predicts $\Delta[PO_4^{3-}]$ best (Fig.1), while the GLM's of $IP_{c,WM,}$ TREATMENT and the interaction $IP_{c,WM}$ and TREATMENT predict $\Delta[NO_2^-]$, $\Delta[NO_3^-]$, $\Delta[NH_4^+]$ and $\Delta[SiO_2]$ best (Tab.2) (Fig.2, Fig.3, Fig.4, Fig.5).

Fig. 1. Change in phosphate concentration ($\Delta[PO_4^{3-}]$) over eight hours in the overlying water column plotted against $IP_{c,AFDM}$ displayed for each TREATMENT category. Lines indicate the predicted $\Delta[PO_4^{3-}]$ from the GLM that described the change of $\Delta[PO_4^{3-}]$ best (i.e. $\Delta[PO_4^{3-}] = IP_{c,AFDM}$ + TREATMENT). Note that the lines are no indication that the specific treatment significantly affected $\Delta[PO_4^{3-}]$. Full coefficient table is given in the appendix. Note further that the y-axis scaling of $L_{community}$ and the x-axis scaling of NO_{spring} and $N_{community}$ was adjusted for a better representation of the data.

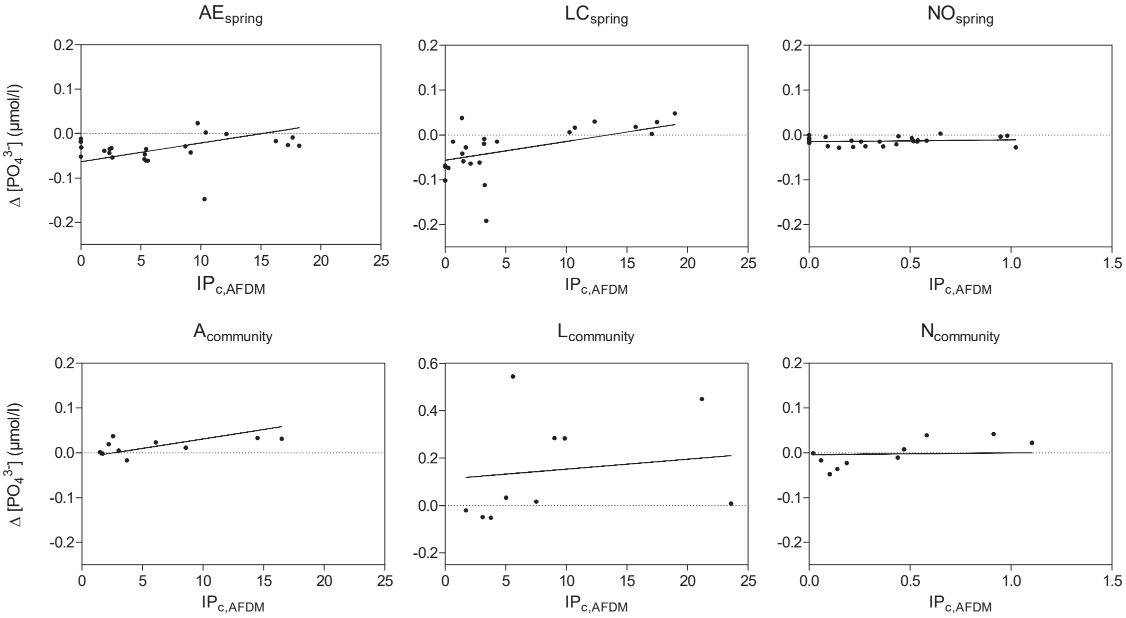


Fig. 2. Change in nitrite concentration ($\Delta[NO_2^-]$) over eight hours in the overlying water column plotted against $IP_{c,WM}$ displayed for each Treatment category. Lines indicate the predicted $\Delta[NO_2^-]$ from the GLM that described the change of $\Delta[NO_2^-]$ best (i.e. $\Delta[NO_2^-]$ = $IP_{c,WM}$ + Treatment + $IP_{c,WM}$*Treatment). Note that the lines are no indication that the specific treatment significantly affected $\Delta[NO_2^-]$. Full coefficient table is given in the appendix. Note further that the y-axis scaling of $L_{community}$ and the x-axis scaling of L_{summer} N_{summer}, NO_{spring} and $N_{community}$ was adjusted for a better representation of the data.

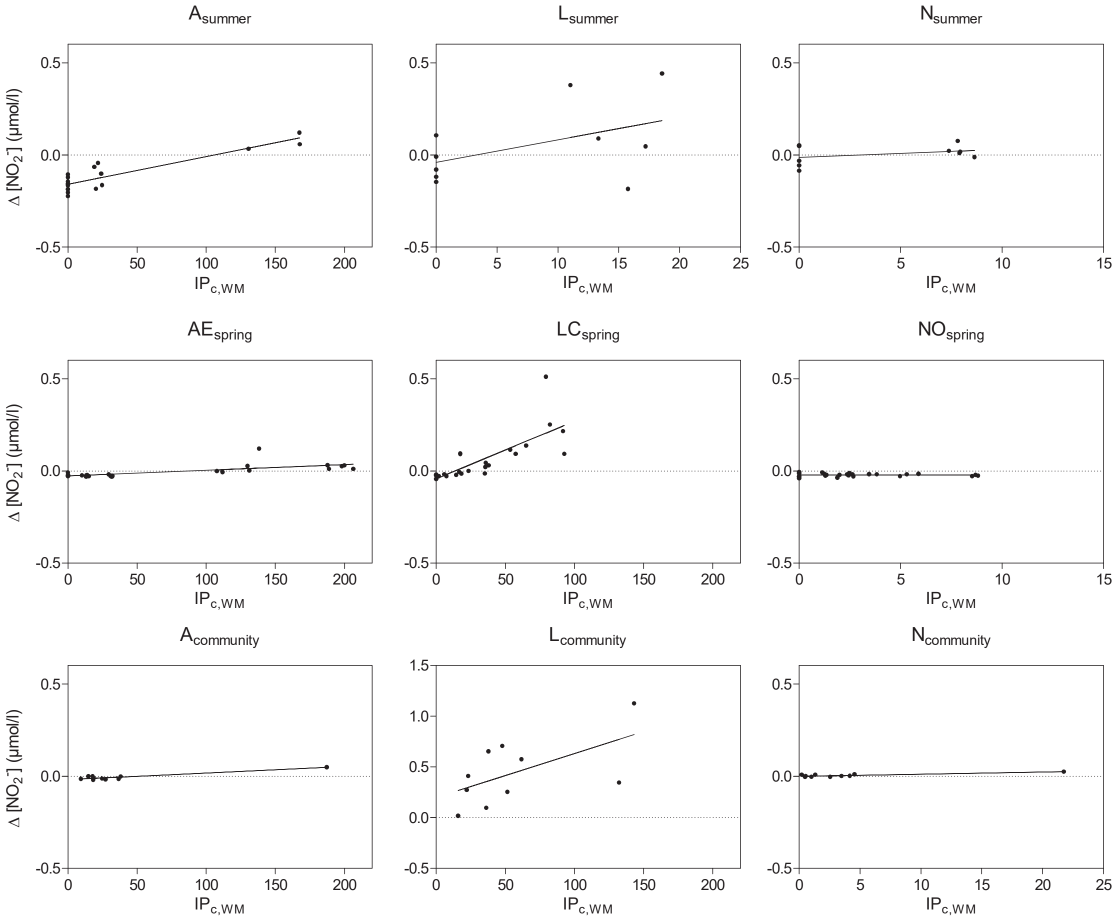


Fig. 3. Change in nitrate concentration ($\Delta[NO_3^-]$) over eight hours in the overlying water column plotted against $IP_{c,WM}$ displayed for each Treatment category. The category AE_{spring} is crossed out as all data had to be removed from the analysis due to malfunction of the Autoanalyzer. Lines indicate the predicted $\Delta[NO_3^-]$ from the GLM that described the change of $\Delta[NO_3^-]$ best (i.e. $\Delta[NO_3^-] = IP_{c,WM}$ + Treatment + $IP_{c,WM}$*Treatment). Note that the lines are no indication, that the specific treatment significantly affected $\Delta[NO_3^-]$. Full coefficient table is given in the appendix. Note further that the y-axis scaling of $L_{community}$ and the x-axis scaling of L_{summer} N_{summer}, NO_{spring} and $N_{community}$ was adjusted for a better representation of the data.

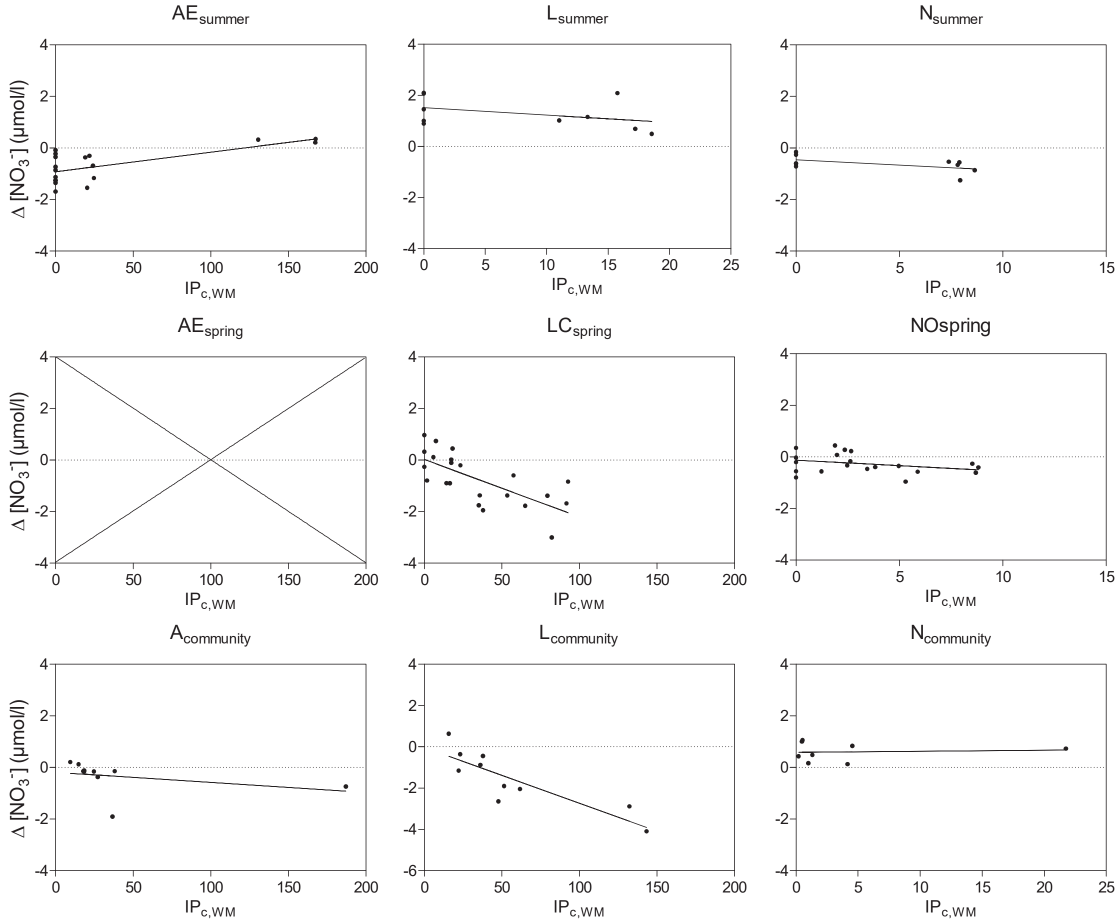


Fig. 4. Change in ammonium concentration ($\Delta[NH_4^+]$) over eight hours in the overlying water column plotted against $IP_{c,WM}$ displayed for each Treatment category. Lines indicate the predicted $\Delta[NH_4^+]$ from the GLM that described the change of $\Delta[NH_4^+]$ best (i.e. $\Delta[NH_4^+]$ = $IP_{c,WM}$ + Treatment + $IP_{c,WM}$*Treatment). Note that the lines are no indication that the specific treatment significantly affected $\Delta[NH_4^+]$. Full coefficient table is given in the appendix. Note further that the y-axis scaling of $L_{community}$ and the x-axis scaling of L_{summer} N_{summer}, NO_{spring} and $N_{community}$ was adjusted for a better representation of the data.

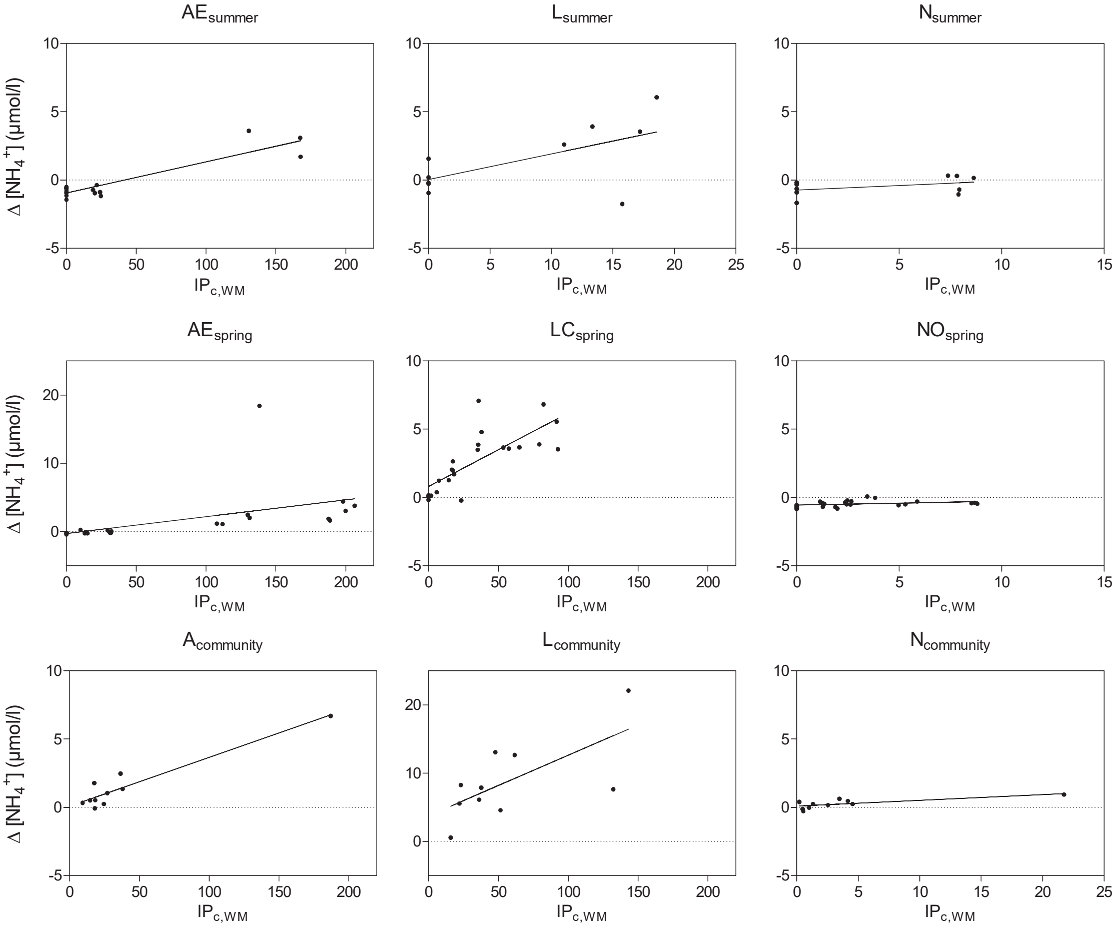
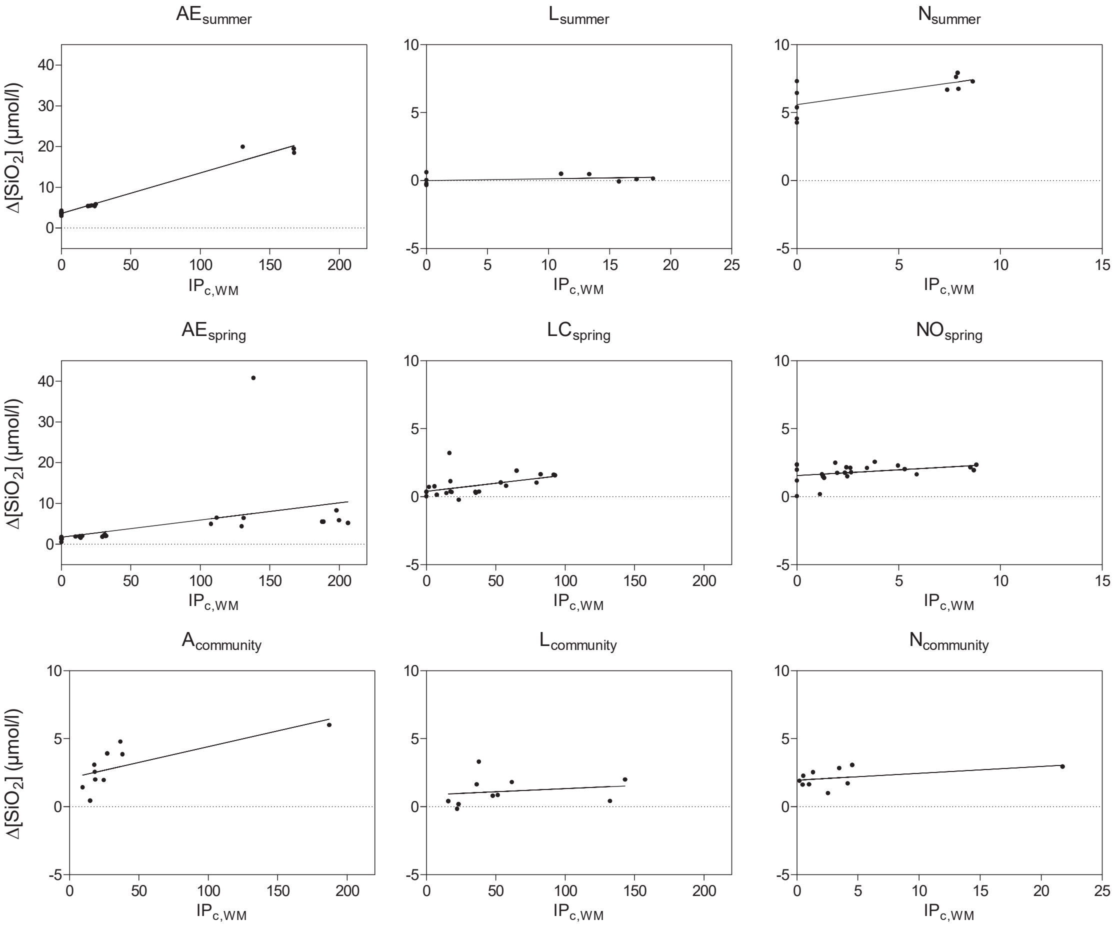


Fig. 5. Change in silicate concentration ($\Delta[SiO_2]$) over eight hours in the overlying water column plotted against $IP_{c,WM}$ displayed for each TREATMENT category. Lines indicate the predicted $\Delta[SiO_2]$ from the GLM that described the change of $\Delta[SiO_2]$ best (i.e. $\Delta[SiO_2] = IP_{c,WM} +$ TREATMENT $+ IP_{c,WM}$*TREATMENT). Note that the lines are no indication that the specific treatment significantly affected $\Delta[SiO_2]$. Full coefficient table is given in the appendix. Note further that the y-axis scaling of AE_{summer} and AE_{spring}, as well as the x-axis scaling of L_{summer} N_{summer}, NO_{spring} and $N_{community}$ was adjusted for a better representation of the data.



All models, based on either $IP_{c,AFDM}$ or $IP_{c,WM}$, produce lower AIC values (i.e. a better representation of the data) compared to the models based on their respective BP_c counterpart.

Both $BP_{c,AFDM}$ and $BP_{c,WM}$ were removed by backward selection from their GLM's of $\Delta[PO_4^{3-}]$, as phosphate flux is better predicted by TREATMENT alone, without either $BP_{c,AFDM}$ or $BP_{c,WM}$ as further fixed effect in the GLM function (Tab.2). $BP_{c,AFDM}$ significantly predicts $\Delta[NO_3^-]$ and $\Delta[NH_4^+]$, whereas $BP_{c,WM}$ predicts $\Delta[NO_3^-]$, $\Delta[NH_4^+]$ and $\Delta[SiO_2]$. The TREATMENT significantly affects the flux of all measured nutrients ($\alpha < 0.01$; Tab.2) in both cases. An interaction of $BP_{c,AFDM}$ with TREATMENT was found for $\Delta[NO_2^-]$ and $\Delta[NO_3^-]$ For $BP_{c,WM}$, a significant interaction with TREATMENT was found for $\Delta[NO_2^-]$, $\Delta[NO_3^-]$ and $\Delta[SiO_2]$.

Comparison of AIC between mIP_c and both IP_c and BP_c shows that AIC is lower compared to IP_c (in both WM and AFDM) for $\Delta[PO_4^{3-}]$. Compared to BP_c, the AIC of mIP_c is lower for all measured nutrient fluxes (Tab.3).

Table 3: AIC of the best GLM models of $\Delta[PO4_3^-]$, $\Delta[NO_2^-]$, $\Delta[NO_3^-]$, $\Delta[NH_4^+]$ and $\Delta[SiO_2]$ as a function of the indices (x) IP_c and BP_c (AIC_x) compared against AIC of the best GLM models of $\Delta[PO4_3^-]$, $\Delta[NO_2^-]$, $\Delta[NO_3^-]$, $\Delta[NH_4^+]$ and $\Delta[SiO_2]$ as a function the index created for comparison of trait effects (AIC_{mIPc}). All indices are calculated both in WM and AFDM. Smaller AIC_{mIPc} (>) indicates for IP_c that nutrient fluxes are better represented with a body mass scaling factor of 0.5 while for BP_c, it indicates that the traits of IP_c represent nutrient flux better than BP_c traits.

		AFDM			WM		
x	nutrient	AIC_x		AIC_{mIPc}	AIC_x		AIC_{mIPc}
IP_c	$\Delta[PO_4^{3-}]$	-239.27	>	-241.56	-235.64	>	-240.96
	$\Delta[NO_2^-]$	-231.87	<	-225.02	-237.34	<	-234.07
	$\Delta[NO_3^-]$	207.75	<	216.64	197.2	<	210.49
	$\Delta[NH_4^+]$	637.79	<	651.36	623.24	<	628.66
	$\Delta[SiO_2]$	762.08	<	783.7	748.55	<	755.16
BP_c	$\Delta[PO_4^{3-}]$	-232.5	>	-241.56	-232.5	>	-240.96
	$\Delta[NO_2^-]$	-196.67	>	-225.02	-206.55	>	-234.07
	$\Delta[NO_3^-]$	220.6	>	216.64	220.63	>	210.49
	$\Delta[NH_4^+]$	666.54	>	651.36	664.29	>	628.66
	$\Delta[SiO_2]$	801.69	>	783.7	772.14	>	755.16

Discussion

Generally, IP_c predicts all nutrient fluxes better than BP_c. The AIC comparison with mIP_c models indicates that the improvement of prediction accuracy is not only caused by the different body mass scaling factor of both models but that it is also a direct result of the description of macrofaunal activity via irrigation traits. This confirms the view that biogeochemical turnover and remineralization is mostly driven by the irrigative introduction of oxygenated water into otherwise anoxic layers of the sediment (Mermillod-Blondin et al., 2004; Na et al., 2008) and the out-flushing of nutrients that do not undergo oxidation or reduction reactions (e.g. silicate) (Marinelli, 1992).

Surprisingly, the use of wet mass (WM) as body mass unit in $IP_{c,WM}$ leads to better predictions of $\Delta[SiO_2]$, $\Delta[NH_4^+]$, $\Delta[NO_3^-]$ and $\Delta[NO_2^-]$ than the use of ash free dry mass (AFDM) in $IP_{c,AFDM}$. This is in contrast to the bioirrigation models of Wrede et al. (submitted), where $IP_{c,AFDM}$ is the superior predictor across all sediment types. AFDM represents the biologically active part of an organism, i.e., it is a reasonable proxy of metabolic activity. WM (including fluid and inorganic parts), on the other hand, may be a better proxy of body volume, i.e. the space an organism occupies in the sediment. While the ratio of AFDM and WM (i.e. AFDM/WM) does not vary dramatically across many taxa, it can be extremely low in taxa with exoskeletons. Some of these species, i.e. the sea urchin *E. cordatum* are highly important for biogeochemical cycling (Lohrer et al., 2004; Wrede et al., 2017). Already Aller (1988) described that burrow radius and, accordingly, burrow volume are crucial determinants of nitrification and denitrification. Hence, besides the absolute flux of water, the volume of sediment, through which this water is flowing, through is a further determinant of nutrient flux (Laverock et al., 2011). This sediment volume is related to the size (volume) of the organism in question.

$\Delta[PO_4^{3-}]$, however, behaves differently, as it is predicted best by $IP_{c,AFDM}$. Under oxic sediment conditions, phosphate is efficiently bound by absorption to ferric oxyhydroxides (Jørgensen, 1983). From these, it may be released during periods of anoxia (Jørgensen, 1983). The irrigative introduction of oxygen into the sediment is, thus, normally counteracting the outflow of phosphate from marine sediments (Clavero et al., 1992; Clavero et al., 1991; Clavero et al., 1994). Yet, phosphate may still be released as a result of excretion or egestion activities of the inhabiting macrofauna (Gardner et al., 1981; Krantzberg, 1985) or by rapid, intensive, advective flushing of anoxic sediment layers (Biswas et al., 2017). As phosphate release scales better with the metabolic activity of the inhabiting fauna, represented by $IP_{c,AFDM}$, than with the flushed volume of sediment, represented by $IP_{c,WM}$, we assume that phosphate release in our experiments was mainly a result of excretion.

Ammonium is also a product of excretion (Henriksen et al., 1983). Yet, $\Delta[NH_4^+]$ was predicted best by $IP_{c,WM}$, indicating that irrigation and metabolic activity alone are not sufficient to describe $\Delta[NH_4^+]$. Next to excretion, ammonium is a product of aerobic and anaerobic remineralisation within natural sediments (Aller, 1988; Kristensen, 1985; Laverock et al., 2011). Irrigation may stimulate ammonium production in the sediment away from the burrow walls by 20 to 30% as it removes metabolites that inhibit ammonification (Aller, 1988). Moreover, irrigation may supply oxidants that increase remineralisation and ammonium production (Aller, 1988). Both processes are critically linked to the physical contact between sediment and irrigation flux which explains why the irrigated volume plays a crucial role in the cycling of ammonium.

Our results indicate that IP_c is a suitable but not sufficient parameter in the modelling of sediment biogeochemical turnover. Biogeochemical cycling is also a function of many other ecosystem characteristics (e.g. total organic content, permeability, wave action, currents, intensity of bacteria mediated processes, temperature, and gradients between the sediment and water column) (Krantzberg, 1985; Mermillod-Blondin and Rosenberg, 2006; Wohlgemuth et al., 2017). Accordingly, the categorical variable TREATMENT, which we introduced to account for the different environmental conditions in our data set, significantly affects all analysed nutrient fluxes and interacts with IP_c for nitrate, nitrite, ammonium, and silicate. This finding may indicate that organisms with similar traits are likely to affect nutrient fluxes differently in different ecosystems, as stated by Mermillod-Blondin and Rosenberg (2006). Quantitative predictions of biogeochemical cycling based on the macrofaunal activity alone will, thus, be difficult to realize. More sophisticated ecosystem models, which incorporate permeability, changing nutrient gradients in the water column, organic matter concentrations, the variable import of organic matter throughout the year and macrofaunal bioirrigation, will be needed.

Acknowledgements

The authors would like to thank the crews of the RV Mya and RV Heincke as well as Constanze Bark, Julia Meyer, Katharina Stumpf, Tatjana Romanova and Petra Kadel for their support during animal collection and laboratory experiments.

References

Aller, R.C., 1988. Benthic fauna and biogeochemical processes in marine sediments: the role of burrow structures, in: Blackburn, T.H., Sørensen, J. (Eds.), Nitrogen cycling in coastal marine environments. John Wiley & Sons, pp. 301-338.

Aller, R.C., 1994. Bioturbation and remineralization of sedimentary organic-matter-effects of redox oscillation. Chem. Geol. 114, 331-345.

Baranov, V., Lewandowski, J., Krause, S., 2016. Bioturbation enhances the aerobic respiration of lake sediments in warming lakes. Biol. Letters 12, 20160448.

Birchenough, S.N.R., Parker, R.E., McManus, E., Barry, J., 2012. Combining bioturbation and redox metrics: potential tools for assessing seabed function. Ecol. Indic. 12, 8-16.

Biswas, J.K., Hazra, S., Majumdar, J., Mandal, S.K., Shaheen, S.M., Sarkar, S.K., Meissner, R., Meers, E., Rinklebe, J., 2017. Impact of raking and bioturbation-mediated ecological manipulation on sediment–water phosphorus diagenesis: a mesocosm study supported with radioactive signature. Environ. Geochem. Hlth., 1-19.

Braeckman, U., Foshtomi, M.Y., Van Gansbeke, D., Meysman, F., Soetaert, K., Vincx, M., Vanaverbeke, J., 2014. Variable importance of macrofaunal functional biodiversity for biogeochemical cycling in temperate coastal sediments. Ecosystems 17, 720-737.

Braeckman, U., Provoost, P., Gribsholt, B., Van Gansbeke, D., Middelburg, J.J., Soetaert, K., Vincx, M., Vanaverbeke, J., 2009. Role of macrofauna functional traits and density in biogeochemical fluxes and bioturbation. Mar. Ecol. Prog. Ser. 399, 173.

Clavero, V., Fernández, J.A., Niell, F.X., 1992. Bioturbation by Nereis sp. and its effects on the phosphate flux across the sediment-water interface in the Palmones River estuary. Hydrobiologia 235, 387-392.

Clavero, V., Niell, F., Fernandez, J., 1991. Effects of Nereis diversicolor OF Muller abundance on the dissolved phosphate exchange between sediment and overlying water in Palmones River Estuary (Southern Spain). Estuar. Coast. Shelf S.33, 193-202.

Clavero, V., Niell, F.X., Fernandez, J., 1994. A laboratory study to quantify the influence of Nereis diversicolor OF Müller in the exchange of phosphate between sediment and water. J. Exp. Mar. Biol. Ecol. 176, 257-267.

Fox, J., Weisberg, S., 2011. An {R} companion to applied regression. Sage, Thousand Oaks CA.

Gardner, W.S., Nalepa, T.F., Quigley, M.A., Malczyk, J.M., 1981. Release of phosphorus by certain benthic invertebrates. Can. J. Fish. Aquat. Sci. 38, 978-981.

Gelman, A., Hill, J., 2007. Data analysis using regression and multilevelhierarchical models. Cambridge University Press New York,, New York, USA.

Gogina, M., Morys, C., Forster, S., Gräwe, U., Friedland, R., Zettler, M.L., 2017. Towards benthic ecosystem functioning maps: quantifying bioturbation potential in the German part of the Baltic Sea. Ecol. Indic. 73, 574-588.

Henriksen, K., Rasmussen, M., Jensen, A., 1983. Effect of bioturbation on microbial nitrogen transformations in the sediment and fluxes of ammonium and nitrate to the overlaying water. Ecol. Bull., 193-205.

Jørgensen, B., 1983. The major biogeochemical cycles and their interactions. John Wiley & Sons.

Krantzberg, G., 1985. The influence of bioturbation on physical, chemical and biological parameters in aquatic environments: a review. Environ. Pollut. A 39, 99-122.

Kristensen, E., 1985. Oxygen and inorganic nitrogen exchange in a Nereis virens(Polychaeta) bioturbated sediment-water system. J. .Coas. Res., 109-116.

Kristensen, E., 2000. Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiologia 426, 1-24.

Laverock, B., Gilbert, J., Tait, K., Osborn, A.M., Widdicombe, S., 2011. Bioturbation: impact on the marine nitrogen cycle. Biochem. Soc. T. 39, 315.

Lohrer, A.M., Thrush, S.F., Gibbs, M.M., 2004. Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431, 1092-1095.

Marinelli, R.L., 1992. Effects of polychaetes on silicate dynamics and fluxes in sediments: importance of species, animal activity and polychaete effects on benthic diatoms. J. Mar. Res. 50, 745-779.

Mermillod-Blondin, F., Rosenberg, R., 2006. Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. Aquat. Sci. 68, 434-442.

Mermillod-Blondin, F., Rosenberg, R., François-Carcaillet, F., Norling, K., Maulaire, L., 2004. Influence of bioturbation by three benthic infaunal species on microbial communities and biogeochemical processes in marine sediment. Aquat. Microb. Ecol. 36, 271-284.

Murray, F., Douglas, A., Solan, M., 2014. Species that share traits do not necessarily form distinct and universally applicable functional effect groups. Marine Ecology Progress Series 516, 23-34.

Na, T., Gribsholt, B., Galaktionov, O.S., Lee, T., Meysman, F.J., 2008. Influence of advective bio-irrigation on carbon and nitrogen cycling in sandy sediments. J. Mar. Res. 66, 691-722.

Queirós, A.M., Birchenough, S.N.R., Bremner, J., Godbold, J.A., Parker, R.E., Romero-Ramirez, A., Reiss, H., Solan, M., Somerfield, P.J., Van Colen, C., Van Hoey, G., Widdicombe, S., 2013. A bioturbation classification of European marine infaunal invertebrates. Ecol. Evol. 3, 3958-3985.

Quinn, G.P., Keough, M.J., 2002. Experimental design and data analysis for biologists. Cambridge University Press.

R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rabaut, M., Vincx, M., Degraer, S., 2008. Do Lanice conchilega (sandmason) aggregations classify as reefs? Quantifying habitat modifying effects. Helgoland Mar. Res. 63, 37-46.

Salzwedel, H., Rachor, E., Gerdes, D., 1985. Benthic macrofauna communities in the German Bight. Veröff. Inst. Meeresf. Bremerhaven 20, 199-267.

Solan, M., Cardinale, B.J., Downing, A.L., Engelhardt, K.A.M., Ruesink, J.L., Srivastava, D.S., 2004. Extinction and ecosystem function in the marine benthos. Science 306, 1177-1180.

Wetzel, M.A., Leuchs, H., Koop, J.H.E., 2005. Preservation effects on wet weight, dry weight, and ash-free dry weight biomass estimates of four common estuarine macro-invertebrates: no difference between ethanol and formalin. Helgoland Mar. Res. 59, 206.

Wohlgemuth, D., Solan, M., Godbold, J.A., 2017. Species contributions to ecosystem process and function can be population dependent and modified by biotic and abiotic setting. Proc. R. Soc. Ser. B.-Bio. 284, 2016-2805.

Wrede, A., Dannheim, J., Gutow, L., Brey, T., 2017. Who really matters: Influence of German Bight key bioturbators on biogeochemical cycling and sediment turnover. J. Exp. Mar. Biol. Ecol. 488, 92-101.

Zuur, A., Ieno, E.N., Smith, G.M., 2007. Analyzing ecological data. Springer Science & Business Media.

5. Synthesis

This thesis addresses the development and application of easily applicable concepts, allowing for the quantification of sediment reworking and the prediction of bioirrigation. This has the potential of facilitating the estimation of macrofaunal influence on biogeochemical processes in shelf sea areas such as the German Bight.

To date, sediment reworking may only be quantified through experimental measurement. Measurements of subtidal *in situ* communities, however, are sparse because the common experimental methodology for quantification of in situ sediment reworking is very time-consuming (Gilbert et al., 2003; Maire et al., 2008; Solan et al., 2004b). This impedes the assessment of sediment reworking over broad spatial and temporal scales (Zhang and Wirtz, 2017). Two of the most commonly used methods (sediment profile imaging (SPI) and standard slicing technique (ST)) were compared and tested for a successful assessment of sediment reworking from cylindrical multi-corer samples (*Manuscript I*). SPI proved to be suitable and more accurate compared to SST for the investigation of general patterns of sediment reworking in natural communities. The need for time-consuming slicing or the complex transfer into rectangular aquaria as previously necessary (Queirós et al., 2015; Solan et al., 2004b), is, thus, omitted. This should increase the feasibility of studies on spatiotemporal patterns of sediment reworking activity. Such studies may support the validation of newly developed mechanistic models (e.g. Zhang and Wirtz, 2017).

Previous efforts to estimate spatiotemporal patterns sediment reworking with indices or mechanistic models have recently been deemed inconsistent (Queirós et al., 2015; Sandnes et al., 2000). Queirós et al. (2015) and findings from *Manuscript II* demonstrated that the bioturbation potential (BP_c), which was specifically created to estimate the potential of communities to rework the sediment, does not correlate with actual sediment reworking rates. BP_c rather correlated with nutrient fluxes of ammonium, nitrate, nitrite, and silicate (*Manuscript II*) which corroborates the findings of previous studies (Birchenough et al., 2012; Braeckman et al., 2014; Solan et al., 2004a). Surprisingly, BP_c was also correlated with bioirrigation activity (*Manuscript II*), although it includes sediment reworking effect traits (i.e. mobility and reworking mode) and no specific bioirrigation effect traits. Bioirrigation is mostly driven by ventilation and filter feeding activities of benthic species (Aller, 1982; Kristensen, 2001; Kristensen et al., 2012), which, in turn, are primarily dependent on body mass and feeding type (Christensen et al., 2000). Next to sediment reworking traits and abundance, BP_c also contains a term that describes the body mass of macrofaunal organisms. This may explain why BP_c correlates with bioirrigation. However, all things considered, BP_c is an ambiguous and inconsistent tool for the prediction of macrofaunal influence on biogeochemical processes.

In order to facilitate reliable estimations of macrofaunal enhancement of biogeochemical processes, a newly developed index is proposed - the Irrigation Potential (IP_c) (*Manuscript III*). The IP_c is an adaptation of the BP_c where the sediment reworking traits of BP_c were replaced with traits related to bioirrigation (i.e. feeding type, burrow type, injection pocket depth). Furthermore, the scaling factor of the body mass term was increased from 0.5 in BP_c to 0.75 in IP_c, because 0.75 is regarded as a better descriptor of energy transfer in marine systems (West and Brown, 2005). When it was calculated from the ash free dry body mass, IP_c quantitatively predicted macrofaunal bioirrigation activity over three different sediment types. No such relationship could be observed between BP_c and the bioirrigation activity (*Manuscript III*). Accordingly, IP_c provides a major improvement for the prediction of macrofaunal bioirrigation activities.

In comparison to sediment reworking, bioirrigation is more important for the enhancement of biogeochemical processes (Braeckman et al., 2014; Mermillod-Blondin et al., 2004). Therefore, it is likely that IP_c may also increase the predictability of biogeochemical cycling. In direct comparison to BP_c, IP_c clearly increased the predictability of macrofaunal effects on a number of nutrient fluxes (phosphate, silicate, ammonium, nitrate, and nitrite) across a multivariate experimental setting (*Manuscript IV)*. Further comparison of IP_c with a modified BP_c-IP_c intermediate (i.e. BP_c - scaling factor, IP_c – traits) suggested that both the irrigation traits and the scaling factor of IP_c increase the predictability of biogeochemical cycling (*Manuscript IV)*. Accordingly, the irrigation traits of the IP_c are linked closer to biogeochemical cycling than the sediment reworking traits of the BP_c. The body volume (approximated through wet body mass) played a significant role for flows of nitrate, nitrite, ammonium, and silicate because fluxes of these nutrients depend on the oxygenized sediment volume (i.e. nitrate and nitrite (Laverock et al., 2011)) or sediment volume that is flushed by irrigative activity (i.e. ammonium and silicate (Aller, 1988; Marinelli, 1992). Although it was demonstrated that IP_c is a necessary parameter to predict the flow of nutrients across the sediment water interface, it should not be regarded as sufficient. The different environmental conditions (i.e. community composition, temperature, sediment type, and nutrient gradients across the sediment water inter face) of the multivariate experiment influenced how macrofaunal activity affected nutrient flux for all nutrients, but phosphate.

5.1 Future challenges

Many parameters, such as total organic matter content, temperature, permeability of the sediment, and intensity of bacteria mediated processes, may determine how biogeochemical processes in the sediment are affected by macrofaunal bioirrigation (Krantzberg, 1985; Mermillod-Blondin and Rosenberg, 2006; Wohlgemuth et al., 2017). Accordingly, there is a need to validate how IP_c or I_{cT} relate to biogeochemical cycling under different environmental conditions. The accuracy of the IP_c may further be restrained by trait variation under differing environmental conditions. Trait variation implies that the expression of particular traits varies within individuals or among populations of the same species due to biotic, abiotic, and anthropogenic drivers (e.g. a species may change from deposit feeding to filter feeding at certain flow velocities) (Bolnick et al., 2011). Known drivers, that affect bioturbation related traits and consequently bioturbation activities, include the temperature, flow velocities, underwater sound, food availability, and even local hereditary population differences (Baranov et al., 2016; Loo et al., 1996; Murray et al., 2017; Solan et al., 2016; Wohlgemuth et al., 2017).

The proposed temperature term (I_{cT}), which approximates the variation in bioirrigation activity from Q_{10} values represents a first step for the assessment of trait variation. If successfully validated, it may provide a tool to identify spatiotemporal variations in macrofaunal bioirrigation activity. A fuzzy coding approach could be a further approximation to account for trait variation caused by factors besides temperature. Fuzzy coding employs numerical scores to describe the affinities of a species to certain trait categories (deposit feeding is for example a trait category of the trait feeding type) (Chevenet et al., 1994). Consequently, a species, which is known for trait variations, could be assigned to several trait categories at the same time according to the likelihood of their expression.

A more promising resolution could be dynamical fuzzy coding linking to the spatiotemporal variations in the environmental factors that drive trait variation. For instance, species that would shift feeding type along a gradient of flow velocities could be assigned to a deposit feeding trait at low flow velocities and simultaneously to sub surface filter feeding at high flow velocities. This approach is, however, limited by the current scarcity of available knowledge about species trait variation that is currently available. Although there could be enough information for some very common and/or dominant species, there is little to no autecological information for the majority of species.

Current attempts aim to determine missing traits and their variability statistically, for example by using the Bayesian extension of probabilistic matrix factorization (Schrodt et al., 2015). These approaches utilize the the phylogenetic trait signal (i.e. the assumption that two closely related species are more similar in their traits), trait-trait correlations, and environmental constraints to fill knowledge gaps. Whether such approaches may significantly increase the resolution and applicability of trait-based indices, such as IP_c, is to date unknown.

References

Aller, R.C., 1980. Quantifying solute distributions in the bioturbated zone of marine-sediments by defining an average micro-environment. Geochim. Cosmochim. Acta 44, 1955-1965.

Aller, R.C., 1982. The effects of macrobenthos on chemical properties of marine sediment and overlying water, in: Mc Call, P.L.a.T., M.J.S. (Ed.), Animal-sediment relations. Plenum Press, New York, pp. 53-120.

Aller, R.C., 1988. Benthic fauna and biogeochemical processes in marine sediments: the role of burrow structures, in: Blackburn, T.H., Sørensen, J. (Eds.), Nitrogen cycling in coastal marine environments. John Wiley & Sons, pp. 301-338.

Aller, R.C., 1994. Bioturbation and remineralization of sedimentary organic-matter-effects of redox oscillation. Chem. Geol. 114, 331-345.

Baranov, V., Lewandowski, J., Krause, S., 2016. Bioturbation enhances the aerobic respiration of lake sediments in warming lakes. Biol. Letters 12, 20160448.

Bertics, V.J., Sohm, J.A., Treude, T., Chow, C.-E.T., Capone, D.G., Fuhrman, J.A., Ziebis, W., 2010. Burrowing deeper into benthic nitrogen cycling: the impact of bioturbation on nitrogen fixation coupled to sulfate reduction. Mar. Ecol. Prog. Ser. 409, 1-15.

Birchenough, S.N.R., Parker, R.E., McManus, E., Barry, J., 2012. Combining bioturbation and redox metrics: potential tools for assessing seabed function. Ecol. Indic. 12, 8-16.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H., Schreiber, S.J., Urban, M.C., Vasseur, D.A., 2011. Why intraspecific trait variation matters in community ecology. Trends Ecol. Evol. 26, 183-192.

Braeckman, U., Foshtomi, M.Y., Van Gansbeke, D., Meysman, F., Soetaert, K., Vincx, M., Vanaverbeke, J., 2014. Variable importance of macrofaunal functional biodiversity for biogeochemical cycling in temperate coastal sediments. Ecosystems 17, 720-737.

Chevenet, F., Doléadec, S., Chessel, D., 1994. A fuzzy coding approach for the analysis of long-term ecological data. Freshwater Biol. 31, 295-309.

Christensen, B., Vedel, A., Kristensen, E., 2000. Carbon and nitrogen fluxes in sediment inhabited by suspension-feeding (Nereis diversicolor) and non-suspension-feeding (N. virens) polychaetes. Mar. Ecol. Prog. Ser. 192, 203-217.

Coates, D.A., Deschutter, Y., Vincx, M., Vanaverbeke, J., 2014. Enrichment and shifts in macrobenthic assemblages in an offshore wind farm area in the Belgian part of the North Sea. Mar. Environ. Res. 95, 1-12.

Darwin, C., 1881. The formation of vegetable mould through the action of worms with observation of their habits. John Murray.

de Bello, F., Lavorel, S., Díaz, S., Harrington, R., Cornelissen, J.H., Bardgett, R.D., Berg, M.P., Cipriotti, P., Feld, C.K., Hering, D., 2010. Towards an assessment of multiple ecosystem processes and services via functional traits. Biodivers. Conserv. 19, 2873-2893.

Díaz, S., Cabido, M., 2001. Vive la difference: plant functional diversity matters to ecosystem processes. Trends Ecol. Evol. 16, 646-655.

Giangrande, A., Montresor, M., Cavallo, A., Licciano, M., 2002. Influence of Naineris laevigata (Polychaeta: Orbiniidae) on vertical grain size distribution, and dinoflagellate resting stages in the sediment. J. Sea Res. 47, 97-108.

Gilbert, F., Bonin, P., Stora, G., 1995. Effect of bioturbation on denitrification in a marine sediment from the West Mediterranean littoral. Hydrobiologia 304, 49-58.

Gilbert, F., Hulth, S., Stromberg, N., Ringdahl, K., Poggiale, J.C., 2003. 2-D optical quantification of particle reworking activities in marine surface sediments. J. Exp. Mar. Biol. Ecol. 285, 251-263.

Glud, R.N., 2008. Oxygen dynamics of marine sediments. Mar. Biol. Res. 4, 243-289.

Hale, R., Godbold, J.A., Sciberras, M., Dwight, J., Wood, C., Hiddink, J.G., Solan, M., 2017. Mediation of macronutrients and carbon by post-disturbance shelf sea sediment communities. Biogeochemistry, 1-13.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers, Ecosystem management. Springer, pp. 130-147.

Jørgensen, B.B., 1983. Processes at the sediment-water interface, in: Bolin, B., Cook, R.B. (Eds.), The Major Biogeochemical Cycles and Their Interactions. John Wiley & Sons Ltd., pp. 477-515.

Koo, B.J., Kwon, K.K., Hyun, J.H., 2007. Effect of environmental conditions on variation in the sediment-water interface created by complex macrofaunal burrows on a tidal flat. J. Sea Res. 58, 302-312.

Krantzberg, G., 1985. The influence of bioturbation on physical, chemical and biological parameters in aquatic environments: a review. Environ. Pollut. A 39, 99-122.

Kristensen, E., 2001. Impact of polychaetes (Nereis spp. and Arenicola marina) on carbon biogeochemistry in coastal marine sediments. Geochem. Trans. 2, 92-103.

Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C.O., Banta, G.T., 2012. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Mar. Ecol. Prog. Ser. 446, 285-302.

Laverock, B., Gilbert, J., Tait, K., Osborn, A.M., Widdicombe, S., 2011. Bioturbation: impact on the marine nitrogen cycle. Biochem. Soc. T. 39, 315.

Lohrer, A.M., Thrush, S.F., Gibbs, M.M., 2004. Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431, 1092-1095.

Loo, L.-O., Jonsson, P.R., Skold, M., Karlsson, Ö., 1996. Passive suspension feeding in Amphiura filiformis (Echinodermata: Ophiuroidea): feeding behaviour in flume flow and potential feeding rate of field populations. Mar. Ecol. Prog. Series 139, 143-155.

Maire, O., Lecroart, P., Meysman, F., Rosenberg, R., Duchene, J.C., Gremare, A., 2008. Quantification of sediment reworking rates in bioturbation research: a review. Aquat. Biol. 2, 219-238.

Marinelli, R.L., 1992. Effects of polychaetes on silicate dynamics and fluxes in sediments: importance of species, animal activity and polychaete effects on benthic diatoms. J. Mar. Res. 50, 745-779.

Mermillod-Blondin, F., Rosenberg, R., 2006. Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. Aquat. Sci. 68, 434-442.

Mermillod-Blondin, F., Rosenberg, R., François-Carcaillet, F., Norling, K., Maulaire, L., 2004. Influence of bioturbation by three benthic infaunal species on microbial communities and biogeochemical processes in marine sediment. Aquat. Microb. Ecol. 36, 271-284.

Meysman, F.J., Middelburg, J.J., Heip, C.H., 2006a. Bioturbation: a fresh look at Darwin's last idea. Trends Ecol. Evol. 21, 688-695.

Meysman, F.J.R., Galaktionov, E.S., Gribsholt, B., Middelburg, J.J., 2006b. Bioirrigation in permeable sediments: advective pore-water transport induced by burrow ventilation. Limnol. Oceanogr. 51, 142-156.

Meysman, F.J.R., Galaktionov, O.S., Gribsholt, B., Middelburg, J.J., 2006c. Bio-irrigation in permeable sediments: an assessment of model complexity. J. Mar. Res. 64, 589-627.

Middelburg, J.J., Soetaert, K., Herman, P.M., 1997. Empirical relationships for use in global diagenetic models. Deep Sea Research Part I: Oceanographic Research Papers 44, 327-344.

Murray, F., Solan, M., Douglas, A., 2017. Effects of algal enrichment and salinity on sediment particle reworking activity and associated nutrient generation mediated by the intertidal polychaete Hediste diversicolor. J. Exp. Mar. Biol. Ecol. 495, 75-82.

Na, T., Gribsholt, B., Galaktionov, O.S., Lee, T., Meysman, F.J., 2008. Influence of advective bio-irrigation on carbon and nitrogen cycling in sandy sediments. J. Mar. Res. 66, 691-722.

Provoost, P., Braeckman, U., Van Gansbeke, D., Moodley, L., Soetaert, K., Middelburg, J.J., Vanaverbeke, J., 2013. Modelling benthic oxygen consumption and benthic-pelagic coupling at a shallow station in the southern North Sea. Estuar. Coast. Shelf S. 120, 1-11.

Queirós, A.M., Birchenough, S.N.R., Bremner, J., Godbold, J.A., Parker, R.E., Romero-Ramirez, A., Reiss, H., Solan, M., Somerfield, P.J., Van Colen, C., Van Hoey, G., Widdicombe, S., 2013. A bioturbation classification of European marine infaunal invertebrates. Ecol. Evol. 3, 3958-3985.

Queirós, A.M., Stephens, N., Cook, R., Ravaglioli, C., Nunes, J., Dashfield, S., Harris, C., Tilstone, G.H., Fishwick, J., Braeckman, U., 2015. Can benthic community structure be used to predict the process of bioturbation in real ecosystems? Prog. Oceanogr. 137, 559-569.

Rijnsdorp, A., Buys, A., Storbeck, F., Visser, E., 1998. Micro-scale distribution of beam trawl effort in the southern North Sea between 1993 and 1996 in relation to the trawling frequency of the sea bed and the impact on benthic organisms. ICES J. Mar. Sci. 55, 403-419.

Sandnes, J., Forbes, T., Hansen, R., Sandnes, B., Rygg, B., 2000. Bioturbation and irrigation in natural sediments, described by animal-community parameters. Mar. Ecol. Prog. Ser. 197, 169-179.

Schrodt, F., Kattge, J., Shan, H., Fazayeli, F., Joswig, J., Banerjee, A., Reichstein, M., Bönisch, G., Díaz, S., Dickie, J., 2015. BHPMF–a hierarchical Bayesian approach to gap-filling and trait prediction for macroecology and functional biogeography. Global Ecol. Biogeogr. 24, 1510-1521.

Solan, M., Cardinale, B.J., Downing, A.L., Engelhardt, K.A.M., Ruesink, J.L., Srivastava, D.S., 2004a. Extinction and ecosystem function in the marine benthos. Science 306, 1177-1180.

Solan, M., Hauton, C., Godbold, J.A., Wood, C.L., Leighton, T.G., White, P., 2016. Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rep. 6, 20540.

Solan, M., Wigham, B.D., Hudson, I.R., Kennedy, R., Coulon, C.H., Norling, K., Nilsson, H.C., Rosenberg, R., 2004b. In situ quantification of bioturbation using time-lapse fluorescent sediment profile imaging (f-SPI), luminophore tracers and model simulation. Mar. Ecol. Prog. Ser. 271, 1-12.

Valença, A.W., Vanek, S.J., Meza, K., Ccanto, R., Olivera, E., Scurrah, M., Lantinga, E.A., Fonte, S.J., 2017. Land use as a driver of soil fertility and biodiversity across an agricultural landscape in the Central Peruvian Andes. Ecol. Appl. 27, 1138-1154.

West, G.B., Brown, J.H., 2005. The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. J. Exp. Biol. 208, 1575-1592.

Wilkinson, M.T., Richards, P.J., Humphreys, G.S., 2009. Breaking ground: pedological, geological, and ecological implications of soil bioturbation. Earth Sci. Rev. 97, 257-272.

Wohlgemuth, D., Solan, M., Godbold, J.A., 2017. Species contributions to ecosystem process and function can be population dependent and modified by biotic and abiotic setting. Proc. R. Soc. Ser. B.-Bio. 284, 2016-2805.

Zhang, W., Wirtz, K., 2017. Mutual dependence between sedimentary organic carbon and infaunal macrobenthos resolved by mechanistic modeling. J. Geophys. Res.-Biogeo. 122, 2509-2526.

Acknowledgements

At this point, I would like to thank all those people without whom this thesis would not have been possible. I am most grateful to all my colleagues and friends who have advised, supported, and accompanied me during the last years.

I like to especially thank my supervisor **Prof. Dr. Thomas Brey**. You not only gave me the opportunity to conduct my thesis in the functional ecology working group at the Alfred Wegener Institute but you always trusted in my abilities, gave me the freedom to independently develop own answers to the scientific questions of my project, and supported me at all times. I am deeply thankful for your support!

Likewise, I am most grateful **to Prof. Dr. Karen Wiltshire**. By funding half of my PhD project you made this project possible in the first place. Further, I would like to thank you very much for the possibility of conducting all my experiments in the laboratories of the AWI Wadden Sea Station on Sylt. The manifold opportunities, which this setting provided, were key to enabling me to conduct my experiments so successfully.

Further, I would like to thank **Prof. Dr. Christian Wild.** You were so kind to review my dissertation and to join my examination committee. I am also very grateful to **Prof. Dr. Wilhelm Hagen** for becoming a member of my examination committee.

Additionally, I would like to thank **Dr. Lars Gutow** for not only being am member of my examination committee but also a constant source of support. You not only brought me in touch with bioturbation research while I was writing my master thesis, but you also supported me throughout this project with your extraordinary ability to always ask the right questions and to nail down a problem in only one sentence.

I, moreover, would like to sincerely thank **Dr. Jennifer Dannheim**. You always took the time to answer my day-to-day questions and no matter how much you had on your own plate, you supported me at all stages of my thesis. Further, you took me along to the BEWG meetings which proved to be a great source of inspiration for me.

Similarly, I would like to thank all the members of the Functional Ecology working group at AWI Bremerhaven and the members of the Coastal Ecology working group at AWI Sylt. In this context, I would like to especially mention **Jan Beermann**, **Henrike Andresen**, **Jan Holstein**, **Manuela Gusky**, **Brigitte Ebbe**, **Hendrik Pehlke, Tatjana**

Romanova, **Petra Kadel**, **Harald Asmus** and **Ragnhild Asmus**. Without your support, I would have never managed to put this thesis together.

Further, my gratitude goes to the silent helpers from the scientific workshops at Bremerhaven and Sylt, **Ehrich Dunker**, **Mattias Lippmann** and **Andreas Kornmann**, who built all my equipment.

Moreover, I like to thank my *HiWi's* and the *FÖJ-lers* of the Functional Ecology working group. **Constanze Bark**, **Julia Meyer**, **Katharina Stumpf** and **Daniel Radin**, without your help during all the experimental work and the laboratory analysis, it would not have been possible to measure so many sediment cores. It would have also been a lot less fun.

Last, but not least, I would like to thank my family and friends. Thank you, **Gerrit G**., **Gerrit B.**, **Flavia**, **Theresa**, **Janina** and **Marieke** for being my friends and constant sources of support during the past three years. I owe you many great experiences and moments that have also carried me through the more challengnging times of writing this thesis. I would also like to thank my boyfriend **Gerrit** for being my connection to the real life at times when the PhD world was threatening to engulf me. And, most of all, I would like to thank my sister **Anne** and **my parents** who supported me at all times and enabled me to write this thesis. Thank you with all my heart.

Appendix Manuscript III

Appendix A

Appendix A Table 1: Treatment, sediment type, mean ash free dry body mass (AFDM) (g) of the single-species biomass treatments, total number of replicates, number of replicates that included in the analysis and number of replicates excluded from the statistical analysis with the respective reasons of exclusion in superscript: 1: replicates were excluded due to increasing Br^- concenrtations (i.e. negative bioirrigation) that indicate very low or no bioirrigation activity but as an increase Br^- concentration is theoretically not possible this cannot be entangled from methodological errors; 2: methodological errors during measuring (e.g. the breaking of the sample container during freezing); 3: outlier. The asterisks for the fine sand and mud controls indicates that the mean bioirrigation was negative thus advective effects in these sediment types were assumend to be non-existent while the superscipt “a” for the sand controls indicates that here mean advective effects could be detected that were subtracted from the treatments, these two replicates are however not part analysis between the indices and the bioirrigation rate.

Treatment	Sediment type	Mean AFDM (g)	Total replicates	Replicates incl. statistical analysis	Excluded replicates + reasons
Lanice conchilega - community	sand	variable	10	8	2^1
Lanice conchilega - high biomass	sand	0,20532	5	5	-
Lanice conchilega - low biomass	sand	0,06136	5	4	1^2
Cerastoderma edule - high biommas	sand	1,19336	5	4	1^2
Cerastoderma edule - low biommas	sand	0,4039	5	3	2^2
sand - control	sand	-	5	2^a	2^2,1^1
Amphiura filiformis - community	fine sand	variable	10	7	3^2
Amphiura filiformis - high biomass	fine sand	0,08048	5	4	1^3
Amphiura filiformis - low biomass	fine sand	0,02732	5	5	-
Echinocardium cordatum - high biommas	fine sand	0,34914	5	5	-
Echinocardium cordatum - low biommas	fine sand	0,17276	5	5	-
fine sand - control	fine sand	-	5	0	*
Nucula nitidosa - community	mud	variable	10	9	1^1
Nucula nitidosa - high biomass	mud	0,0225	5	3	2^2
Nucula nitidosa - low biomass	mud	0,0078	5	5	-
Owenia fusifomis - high biommas	mud	0,01168	5	1	3^1,1^2
Owenia fusiformis - low biommas	mud	0,00402	5	4	1^1
mud - control	mud	-	5	0	*

Appendix B

Model selection

Appendix B Table 1: Backward selection was used to determine the model that best described effects of IP_c (AFDM(g)) on bioirrigation. Other factors in the models were Treatment (community, single-species), sediment type (mud, fine sand, sand) as well as the interactions between all these factors (indicated by asterisk). Crosses denote the variables included in the respective model. The model with lowest AICc was chosen as best (bold font).

	IP_c (AFDM (g))	Treatment	Sediment	IP_c (AFDM (g)) * Treatment	IP_c (AFDM (g)) * Sediment	Treatment * Sediment	AICc
Model 1	x	x	x	x	x	x	-380.2293
Model 2	x	x	x	x	x		-381.8091
Model 3	x	x	x	x			-386.5269
Model 4	x	x	x	x		x	-385.1951
Model 5	x	x	x		x	x	-382.8846
Model 6	x	x	x			x	-387.7424
Model 7	x	x	x		x		-383.4648
Model 8	x	x	x				-387.7555
Model 9	x	x		x			-390.5207
Model 10	x		x		x		-375.1422
Model 11	x	x					**-391.576**
Model 12	x		x				-378.314
Model 13	x						-382.5146
Model 14		x	x				-346.197
Model 15		x	x			x	-345.8122
Model 16		x					-330.4341
Model 17			x				-341.7702

Appendix B Table 2: Backward selection was used to determine the model that best described effects of IP_c (WM(g)) on bioirrigation. Other factors in the models were Treatment (community, single-species), sediment type (mud, fine sand, sand) as well as the interactions between all these factors (indicated by asterisk). Crosses denote the variables included in the respective model. The model with lowest AICc was chosen as best (bold font).

	IP_c (WM (g))	Treatment	Sediment	IP_c (WM (g)) * Treatment	IP_c (WM (g)) * Sediment	Sediment * Treatment	AICc
Model 1	x	x	x	x	x	x	**-390.3283**
Model 2	x	x	x	x	x		-385.8844
Model 3	x	x	x	x		x	-376.7132
Model 4	x	x	x		x	x	-387.2331
Model 5	x	x	x	x			-375.3045
Model 6	x	x	x		x		-387.7262
Model 7	x	x	x			x	-378.5958
Model 8	x	x	x				-377.4348
Model 9	x	x		x			-372.6432
Model 10	x		x		x		-380.0237
Model 11	x		x				-366.4459
Model 12	x	x					-3733.5657
Model 13	x						-363.9739

Appendix B Table 3: Backward selection was used to determine the model that best described effects of BP_c (WM(g)) on bioirrigation. Other factors in the models were Treatment (community, single-species), sediment type (mud, fine sand, sand) as well as the interactions between all these factors (indicated by asterisk). Crosses denote the variables included in the respective model. The model with lowest AICc was chosen as best (bold font).

	BP_c (WM (g))	Treatment	Sediment	BP_c (WM (g)) * Treatment	BP_c (WM (g)) * Sediment	Sediment * Treatment	AICc
Model 1	x	x	x	x	x	x	-342.59
Model 2	x	x	x	x		x	-345.1174
Model 3	x	x	x			x	-343.8318
Model 4	x	x	x	x	x		-343.9632
Model 5	x	x	x		x		-343.5621
Model 6	x	x	x	x			**-346.4898**
Model 7	x	x	x				-343.8339
Model 8	x	x		x			-340.5839
Model 9	x		x				-339.478
Model 10	x	x					-334.0817
Model 11	x						-331.2364
Model 12	x	x	x		x	x	-343.318
Model 13	x		x		x		-341.1074

Appendix B Table 4: Backward selection was used to determine the model that best described effects of BP_c (AFDM(g)) on bioirrigation. Other factors in the models were Treatment (community, single-species), sediment type (mud, fine sand, sand) as well as the interactions between all these factors (indicated by asterisk). Crosses denote the variables included in the respective model. The model with lowest AICc was chosen as best (bold font).

	BP_c (AFDM (g))	Treatment	Sediment	BP_c (AFDM (g)) * Treatment	BP_c (AFDM (g)) * Sediment	Sediment * Treatment	AICc
Model 1	x	x	x	x	x	x	-342.9233
Model 2	x	x	x	x	x		**-346.497**
Model 3	x	x	x	x		x	-344.0918
Model 4	x	x	x		x	x	-339.5091
Model 5	x	x	x	x			-346.3876
Model 6	x	x	x		x		-339.7046
Model 7	x	x	x			x	-344.0101
Model 8	x	x	x				-343.8983
Model 9	x	x		x			-340.9704
Model 10	x		x		x		-334.7526
Model 11	x		x				-339.5433
Model 12	x	x					-337.6159
Model 13	x						-335.1854

Statistical analysis: Models that best described (lowest AIC_c) the relationship between IP_c (AFDM(g)) (Model IP_c (AFDM)), IP_c (WM(g)) (Model IP_c (WM(g))), BP_c (WM(g)) (Model BP_c (WM(g))), BP_c (AFDM(g)) (Model BP_c (AFDM(g))) and irrigation activity.

Model IP_c (AFDM(g))

Generalized Linear Model Fit:

Overdispersion parameter estimated by Maximum Likelihood

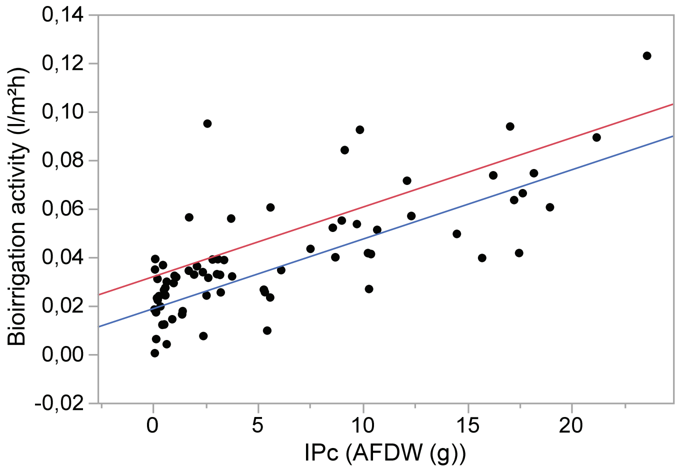
Response: Bioirrigation activity (l/m^{2}h)

Distribution: Normal

Link: Identity

Estimation Method: Maximum Likelihood

Observations (or Sum Wgts) = 72



Appendix B Figure 1: Regression Plot of the relationship between IP_c (AFDM(g)) and the bioirrigation activity. The red line indicates the predicted bioirrigation activity of the community treatment and the blue line the predicted bioirrigation activity of the single-species treatment.

Appendix B Table 5: Whole Model Test of Model IP_c (AFDM(g)). Significance indicated by bold font and asterisk.

Model	-LogLikelihood	L-R ChiSquare	DF	Prob>ChiSq
Difference	33.4413021	66.8826	2	**<.0001***
Full	-200.08651			
Reduced	-166.6452			

Appendix B Table 6: Goodness of Fit Statistics of Model IP$_c$ (AFDM(g)).

Goodness of Fit Statistic	ChiSquare	DF	Prob>ChiSq	Overdispersion
Pearson	0.0163	69	1.0000	0.0002
Deviance	0.0163	69	1.0000	

AICc

-391.5760*

Appendix B Table 7: Effect Summary of Model IP$_c$ (AFDM(g)).

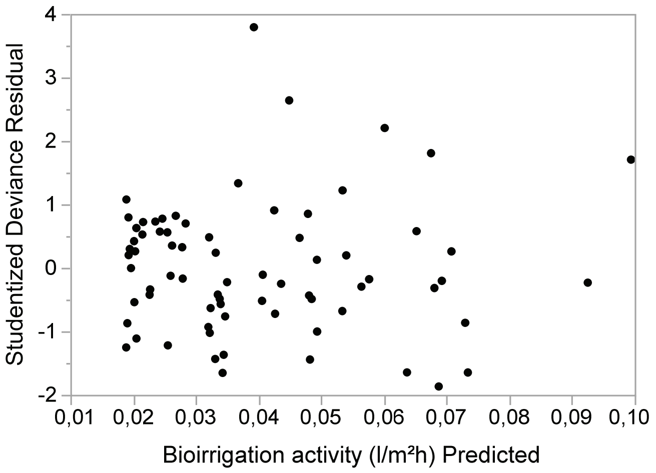
Source	LogWorth	PValue
IPc (AFDM (g))	14.770	0.00000
Treatment	3.112	0.00077

Appendix B Table 8: Effect Tests of Model IP$_c$ (AFDM(g)). Significance indicated by bold font and asterisk.

Source	DF	L-R ChiSquare	Prob>ChiSq
IPc (AFDM (g))	1	63.385987	**<.0001***
Treatment	1	11.305484	**0.0008***

Appendix B Table 9: Parameter Estimates of Model IP$_c$ (AFDM(g)). Significance indicated by bold font and asterisk.

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
Intercept	0.0252575	0.0024803	64.229911	**<.0001***	0.0203306	0.0301845
IPc (AFDM (g))	0.0028627	0.0002839	63.385987	**<.0001***	0.0022987	0.0034268
Treatment	0.0065822	0.0018813	11.305484	**0.0008***	0.0028452	0.0103192



Appendix B Figure 2: Studentized Deviance Residual by Predicted plot of Model IP$_c$ (AFDM(g)).

Model IP_c (WM(g))

Generalized Linear Model Fit

Overdispersion parameter estimated by Maximum Likelihood

Response: Bioirrigation activity (l/m²h)

Distribution: Normal

Link: Identity

Estimation Method: Maximum Likelihood

Observations (or Sum Wgts) = 72

Appendix B Table 10: Whole Model Test of Model IP_c (WM(g)). Significance indicated by bold font and asterisk.

Model	-LogLikelihood	L-R ChiSquare	DF	Prob>ChiSq
Difference	41.7189679	83.4379	9	**<.0001***
Full	-208.36417			
Reduced	-166.6452			

Appendix B Table 11: Goodness of Fit Statistics of Model IP_c (WM(g)).

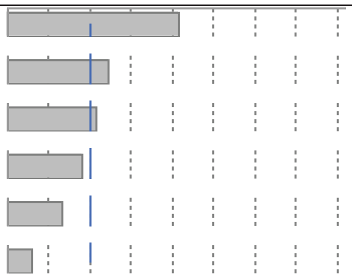
Goodness Of Fit Statistic	ChiSquare	DF	Prob>ChiSq	Overdispersion
Pearson	0.0129	62	1.0000	0.0002
Deviance	0.0129	62	1.0000	

AICc

-390.3283*

Appendix B Table 12: Effect Summary of Model IP_c (WM(g)). Caret (^) indicates that the containing higher-order effect is more significant than the lower-order effect.

Source	LogWorth	PValue
IPc (WM (g))*Sediment	4.150	0.00007
Treatment	2.462	0.00345
Sediment*Treatment	2.159	0.00694
Treatment*IPc (WM (g))	1.817	0.01524
IPc (WM (g))	1.351	0.04452 ^
Sediment	0.629	0.23510 ^

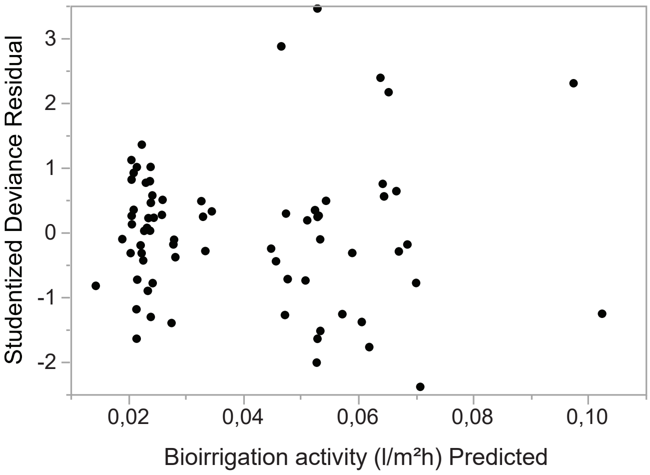


Appendix B Table 13: Effect Tests of Model IP_c (WM(g)). Significance indicated by bold font and asterisk.

Source	DF	L-R ChiSquare	Prob>ChiSq
IPc (WM (g))	1	4.0366998	**0.0445***
Sediment	2	2.8955207	0.2351
Treatment	1	8.5501847	**0.0035***
IPc (WM (g))*Sediment	2	19.111896	**<.0001***
Treatment*IPc (WM (g))	1	5.8886662	**0.0152***
Sediment*Treatment	2	9.9407303	**0.0069***

Appendix B Table 14: Parameter Estimates of Model IP_c (WM(g)). Significance indicated by bold font and asterisk.

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
Intercept	0.0269661	0.0025796	66.482108	**<.0001***	0.021842	0.0320903
IPc (WM (g))	0.0004369	0.0002144	4.0366998	**0.0445***	1.0987e-5	0.0008628
Sediment[fine sand]	-0.00518	0.009699	0.2846336	0.5937	-0.024446	0.0140864
Sediment[mud]	0.0029447	0.0188674	0.0243545	0.8760	-0.034534	0.0404229
Treatment[community]	0.0053559	0.0017775	8.5501847	**0.0035***	0.0018249	0.0088868
(IPc (WM (g))-48.1353)* Sediment[fine sand]	-0.000308	0.0002172	1.9840235	0.1590	-0.00074	0.0001234
(IPc (WM (g))-48.1353)* Sediment[mud]	0.0001866	0.0004266	0.1911629	0.6620	-0.000661	0.0010339
Treatment[community]* (IPc (WM (g))-48.1353)	-0.000103	4.163e-5	5.8886662	**0.0152***	-0.000186	-2.043e-5
Sediment[fine sand]* Treatment[community]	0.0055478	0.0025995	4.4163815	**0.0356***	0.0003841	0.0107114
Sediment[mud]* Treatment[community]	-0.009106	0.002808	9.8165778	**0.0017***	-0.014684	-0.003529



Appendix B Figure 3: Studentized Deviance Residual by Predicted plot of Model IP_c (WW(g)).

Model BP_c (WM(g))

Generalized Linear Model Fit:

Overdispersion parameter estimated by Maximum Likelihood

Response: Bioirrigation activity (l/m²h)

Distribution: Normal

Link: Identity

Estimation Method: Maximum Likelihood

Observations (or Sum Wgts) = 72

Appendix B Table 15: Whole Model Test of Model BP_c (WM(g)). Significance indicated by bold font and asterisk.

Model	-LogLikelihood	L-R ChiSquare	DF	Prob>ChiSq
Difference	14.474683	28.9494	5	**<.0001***
Full	-181.11989			
Reduced	-166.6452			

Appendix B Table 16: Goodness of Fit Statistics of Model BP_c (WM(g)).

Goodness Of Fit Statistic	ChiSquare	DF	Prob>ChiSq	Overdispersion
Pearson	0.0275	66	1.0000	0.0004
Deviance	0.0275	66	1.0000	

AICc

-346.4898*

Appendix B Table 17: Effect Summary of Model BP_c (WM(g)). Caret (^) indicates that the containing higher-order effect is more significant than the lower-order effect.

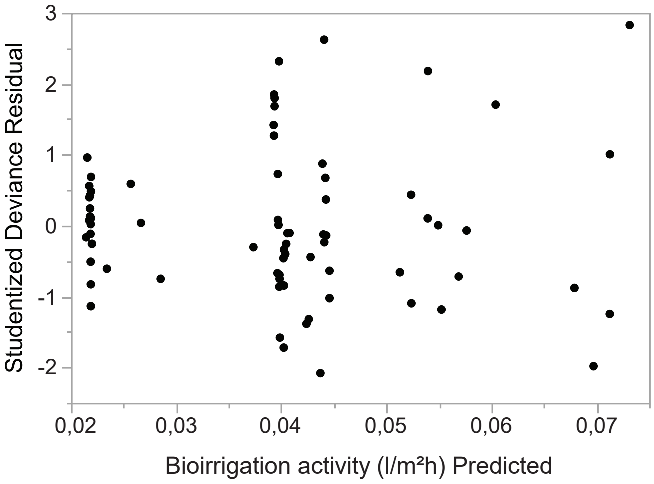
Source	LogWorth		PValue
Treatment	2.675		0.00211
Sediment	2.334		0.00464
Treatment*BPc (WM (g))	1.625		0.02374
BPc (WM (g))	1.144		0.07176 ^

Appendix B Table 18: Effect Tests of Model BP_c (WM(g)). Significance indicated by bold font and asterisk.

Source	DF	L-R ChiSquare	Prob>ChiSq
Treatment	1	9.4488657	**0.0021***
BPc (WM (g))	1	3.2422084	0.0718
Sediment	2	10.746786	**0.0046***
Treatment*BPc (WM (g))	1	5.1135746	**0.0237***

Appendix B Table 19: Parameter Estimates of Model BP_c (WM(g)).

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
Intercept	0.0354026	0.004314	47.541282	**<.0001***	0.0268333	0.0439719
Treatment[community]	0.0080523	0.0025341	9.4488657	**0.0021***	0.0030185	0.0130861
BPc (WM (g))	0.0002527	0.0001387	3.2422084	0.0718	-0.000023	0.0005283
Sediment[fine sand]	0.0049294	0.0034266	2.0402476	0.1532	-0.001877	0.0117361
Sediment[mud]	-0.013926	0.0041377	10.520164	**0.0012***	-0.022145	-0.005707
Treatment[community]*(BPc (WM (g))-30.1892)	0.0002826	0.0001228	5.1135746	**0.0237***	3.8754e-5	0.0005265



Appendix B Figure 4: Studentized Deviance Residual by Predicted plot of Model BP$_c$ (WM(g)).

Model BP$_c$ (AFDM(g))

Generalized Linear Model Fit

Overdispersion parameter estimated by Maximum Likelihood

Response: Bioirrigation activity (l/m²h)

Distribution: Normal

Link: Identity

Estimation Method: Maximum Likelihood

Observations (or Sum Wgts) = 72

Appendix B Table 20: Whole Model Test of Model BP$_c$ (AFDM(g)). Significance indicated by bold font and asterisk.

Model	-LogLikelihood	L-R ChiSquare	DF	Prob>ChiSq
Difference	17.0549271	34.1099	7	**<.0001***
Full	-183.70013			
Reduced	-166.6452			

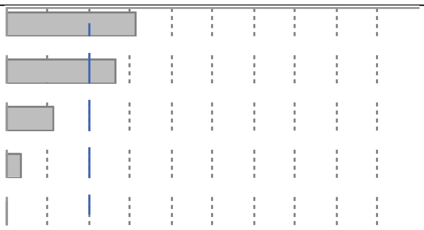
Appendix B Table 21: Effect Tests of Model BP$_c$ (AFDM(g)).

Goodness Of Fit Statistic	ChiSquare	DF	Prob>ChiSq	Overdispersion
Pearson	0.0256	64	1.0000	0.0004
Deviance	0.0256	64	1.0000	

AICc

-346.4970*

Appendix B Table 22: Effect Summary of Model BP_c (AFDM(g)). Caret (^) indicates that the containing higher-order effect is more significant than the lower-order effect.

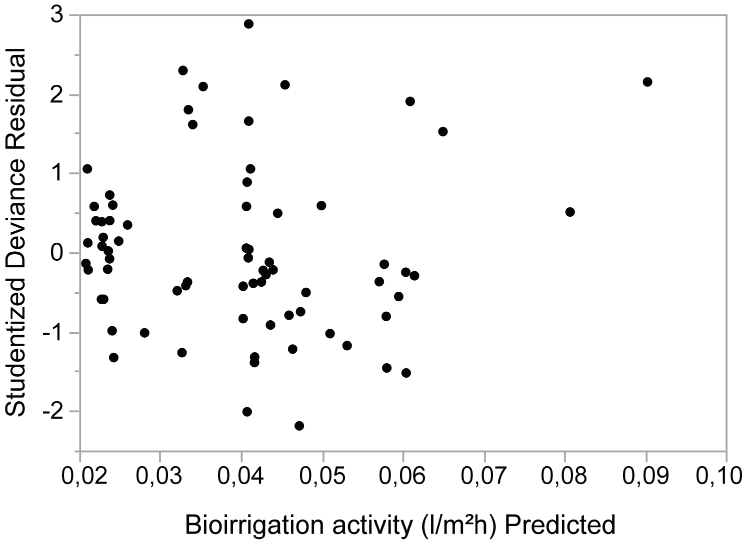
Source	LogWorth		PValue
Treatment	3.129		0.00074
BPc (AFDM (g))*Treatment	2.666		0.00216
BPc (AFDM (g))*Sediment	1.143		0.07198
Sediment	0.383		0.41414 ^
BPc (AFDM (g))	0.020		0.95598 ^

Appendix B Table 23: Effect Tests of Model BP_c (AFDM(g)). Significance indicated by bold font and asterisk.

Source	DF	L-R ChiSquare	Prob>ChiSq
BPc (AFDM (g))	1	0.003047	0.9560
Sediment	2	1.7631088	0.4141
Treatment	1	11.380251	**0.0007***
BPc (AFDM (g))*Sediment	2	5.2626485	0.0720
BPc (AFDM (g))*Treatment	1	9.4099881	**0.0022***

Appendix B Table 24: Parameter Estimates of Model BP_c (AFDM(g)). Significance indicated by bold font and asterisk.

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
Intercept	0.0418734	0.0049853	49.177392	**<.0001***	0.0319706	0.0517763
BPc (AFDM (g))	-6.965e-5	0.0012618	0.003047	0.9560	-0.002576	0.0024367
Sediment[fine sand]	0.0104248	0.0079438	1.7019111	0.1920	-0.005355	0.0262043
Sediment[mud]	-0.018915	0.0144657	1.6897003	0.1936	-0.047649	0.0098201
Treatment	0.009112	0.0025951	11.380251	**0.0007***	0.0039571	0.0142668
(BPc (AFDM (g))-7.24768)* Sediment[fine sand]	-0.001774	0.0014604	1.4608575	0.2268	-0.004675	0.0011268
(BPc (AFDM (g))-7.24768)* Sediment[mud]	5.0632e-5	0.0023655	0.0004581	0.9829	-0.004648	0.0047495
(BPc (AFDM (g))-7.24768)* Treatment[community]	0.0015428	0.0004866	9.4099881	**0.0022***	0.0005762	0.0025093



Appendix B Figure 5: Studentized Deviance Residual by Predicted plot of Model BP_c (AFDM(g)).

Appendix C

Appendix C Table 1: Trait scores for species found in the community experiments and species contributing to 90% of total abundance and biomass (target species*) at stations (Fig. 2 in the manuscript)

Species	Burrow type	Feeding behaviour	Injection pocket depth	target species
Abludomelita obtusata	1	3	1	*
Abra alba	1	1	3	*
Abra nitida	1	1	2	*
Acrocnida brachiata	2	4	3	*
Actinaria sp.	1	2	1	
Ampelisca brevicornis	1	1	1	*
Ampharete sp.	3	1	1	
Ampharete lindstroemii	3	1	1	
Aphelochaeta sp.	3	3	2	
Amphictene auricoma	3	3	2	*
Amphiura filiformis	2	4	3	*
Anthozoa spp.	1	1	1	*
Aonides paucibranchiata	1	3	1	*
Aora gracilis	1	1	1	*
Aphrodita aculeata	1	2	1	*
Arctica islandica	1	1	3	*
Arenicola marina	3	3	4	
Ascidiella aspersa	1	1	1	*
Asterias rubens	1	2	1	*
Astropecten irregularis	1	2	1	*
Balanus crenatus	1	1	2	
Bathyporeia	1	3	1	*
Branchiostoma lanceolatum	3	4	2	*
Callianassa subterranea	2	3	3	*
Capitella capitata	3	3	4	
Carcinus maenas	1	2	1	
Cerastoderma edule	1	1	2	
Chaetopterus variopedatus	2	4	4	*
Chaetozone	3	3	2	*
Chamelea gallina	1	1	2	*
Corbula gibba	1	1	1	*
Corystes cassivelaunus	3	2	3	*
Cylichna cylindracea	3	2	2	*
Diastylis bradyi	3	2	1	*
Dosinia exoleta	1	1	3	*
Dosinia lupinus	1	1	3	*
Echinocardium cordatum	3	3	4	*
Echinocyamus pusillus	3	3	1	*

Species	Burrow type	Feeding behaviour	Injection pocket depth	target species
Echiurus echiurus	2	3	4	*
Edwardsia	1	1	1	*
Ensis directus	1	1	4	*
Ensis ensis	1	1	4	*
Ensis magnus	1	1	4	*
Ensis siliqua	1	1	4	*
Eteone longa	1	2	1	*
Eumida	1	2	1	*
Euspira nitida	1	2	1	*
Exogone naidina	1	3	1	
Galathowenia oculata	3	3	1	*
Gattyana cirrhosa	3	2	2	*
Glycera lapidum	2	2	2	*
Glycinde nordmanni	3	2	3	*
Goniada maculata	3	2	3	*
Harpinia antennaria	3	3	3	*
Hyala vitrea	1	3	1	*
Iphinoe trispinosa	1	3	1	*
Kurtiella bidentata	2	4	3	*
Lagis koreni	3	3	2	*
Lanice conchilega	3	3	4	*
Leptopentacta elongata	1	1	1	*
Lutraria lutraria	1	1	4	*
Macoma balthica	1	3	3	
Mactra stultorum	1	1	2	*
Magelona	3	3	4	*
Malacoceros fulginosus	2	3	1	
Malmgreniella	1	2	4	*
Microphtalmus sczellcowii	3	3	4	
Mya	1	1	4	*
Mytilus edulis	1	1	1	
Nemertea	2	2	2	*
Nephtys assimilis	3	2	1	*
Nephtys caeca	3	2	1	*
Nephtys cirrosa	3	2	1	*
Nephtys hombergii	3	2	1	*
Notomastus latericeus	3	3	4	*
Nucula nitidosa	3	3	1	*
Ophelia limacina	3	3	2	*
Ophiura albida	1	3	1	*
Ophiura ophiura	1	3	1	
Owenia fusiformis	3	3	1	*

Species	Burrow type	Feeding behaviour	Injection pocket depth	target species
Paramphinome jeffreysii	3	2	1	*
Pariambus typicus	1	1	1	*
Phaxas pellucidus	3	1	1	*
Pholoe baltica	1	2	1	*
Phoronida	3	1	1	*
Phyllodoce rosea	1	2	1	*
Phyllodoce maculata	1	2	1	
Pisione remota	1	2	3	*
Podarkeopsis helgolandica	1	1	1	
Poecilochaetus serpens	2	3	1	*
Polydora cilliata	1	1	2	
Polygordius	3	2	1	*
Processa	1	2	1	*
Protodorvillea kefersteini	1	2	1	*
Scalibregma inflatum	2	3	3	*
Scolelepis bonnieri	3	3	3	*
Scoloplos (Scoloplos) armiger	3	3	4	*
Semibalanus balanoides	1	1	2	
Spatangus purpureus	2	3	3	*
Spio	3	3	1	*
Spiophanes bombyx	3	3	4	*
Spisula solida	1	1	2	*
Sthenelais limicola	1	2	1	*
Tellimya ferruginosa	3	4	4	*
Tellina fabula	1	3	3	*
Thracia phaseolina	1	1	1	*
Thyasira flexuosa	3	3	3	*
Tryphosa nana	1	2	1	*
Turritella communis	1	1	1	*
Upogebia deltaura	2	4	4	*
Urothoe poseidonis	3	3	3	*

Appendix C References:

Note that references are in the style of the Journal Manuscript III was submitted to (i.e. Ecological Indicators)

Alexander, R. R., R. J. Stanton Jr, and J. R. Dodd. 1993. Influence of sediment grain size on the burrowing of bivalves: correlation with distribution and stratigraphic persistence of selected neogene clams. Palaios **8**:289-303.

Allen, P. 1983. Feeding behaviour of *Asterias rubens* (L.) on soft bottom bivalves: a study in selective predation. Journal of Experimental Marine Biology and Ecology **70**:79-90.

Ansell, A. D. 1961. The functional morphology of the British species of Veneracea (Eulamellibranchia). Journal of the Marine Biological Association of the United Kingdom **41**:489-517.

Ansell, A. D. 1969. Leaping movements in the Bivalvia. Journal of Molluscan Studies **38**:387-399.

Ball, B., G. Fox, and B. Munday. 2000. Long-and short-term consequences of a *Nephrops* trawl fishery on the benthos and environment of the Irish Sea. ICES Journal of Marine Science **57**:1315-1320.

Berelson, W., T. Townsend, D. Heggie, P. Ford, A. Longmore, G. Skyring, T. Kilgore, and G. Nicholson. 1999. Modelling bio-irrigation rates in the sediments of Port Phillip Bay. Marine and freshwater research **50**:573-579.

Beukema, J. 1985. Growth and dynamics in populations of *Echinocardium cordatum* living in the North Sea off the Dutch north coast. Netherlands Journal of Sea Research **19**:129-134.

Boos, K., L. Gutow, R. Mundry, and H.-D. Franke. 2010. Sediment preference and burrowing behaviour in the sympatric brittlestars *Ophiura albida* Forbes, 1839 and *Ophiura ophiura* (Linnaeus, 1758)(Ophiuroidea, Echinodermata). Journal of Experimental Marine Biology and Ecology **393**:176-181.

Borsje, B. W., M. B. de Vries, T. J. Bouma, G. Besio, S. Hulscher, and P. M. J. Herman. 2009a. Modeling bio-geomorphological influences for offshore sandwaves. Continental Shelf Research **29**:1289-1301.

Borsje, B. W., S. Hulscher, P. M. J. Herman, and M. B. de Vries. 2009b. On the parameterization of biological influences on offshore sand wave dynamics. Ocean Dynamics **59**:659-670.

Bourgoin, A., and M. Guillou. 1994. Arm regeneration in two populations of *Acrocnida brachiata* (Montagu)(Echinodermata: Ophiuroidea) in Douarnenez Bay,(Brittany: France): an ecological significance. Journal of Experimental Marine Biology and Ecology **184**:123-139.

Braeckman, U., P. Provoost, B. Gribsholt, D. Van Gansbeke, J. J. Middelburg, K. Soetaert, M. Vincx, and J. Vanaverbeke. 2010. Role of macrofauna functional traits and density in biogeochemical fluxes and bioturbation. Marine Ecology Progress Series **399**:173-186.

Brey, T. 1991. The relative significance of biological and physical disturbance: an example from intertidal and subtidal sandy bottom communities. Estuarine, Coastal and Shelf Science **33**:339-360.

Buchanan, J. B. 1964. A comparative study of some features of the biology of *Amphiura filiformis* and *Amphiura chiajei* [Ophiuroidea] considered in relation to their distribution. Journal of the Marine Biological Association of the United Kingdom **44**:565-576.

Buhr, K.-J. 1976. Suspension-feeding and assimilation efficiency in *Lanice conchilega* (Polychaeta). Marine Biology **38**:373-383.

Buhr, K., and J. Winter. 1976. Distribution and maintenance of a *Lanice conchilega* association in the Weser estuary (FRG), with special reference to the suspension-feeding behaviour of *Lanice conchilega*. Pages 5-11 *in* Proceedings of the Eleventh European Symposium of Marine Biology, University College, Galway.

Callaway, R. 2006. Tube worms promote community change. Marine Ecology Progress Series **308**:49-60.

Clark, R. B. 1962. Observations on the food of *Nephtys*. Limnology and Oceanography **7**:380-385.

Coonfield, B. R. 1931. The cilia of *Nephthys bucera*. Procedings of the National Academy of Science **17**:416.

Courtney, W. A. M., and J. E. Webb. 1964. The effects of the cold winter of 1962/63 on the Helgoland population of *Branchiostoma lanceolatum* (Pallas). Helgoländer wissenschaftliche Meeresuntersuchungen **10**:301-312.

Creutzberg, F., P. Wapenaar, G. Duineveld, and N. L. Lopez. 1984. Distribution and density of the benthic fauna in the southern North Sea in relation to bottom characteristics and hydrographic conditions. Rapports et procès-verbaux des réunions / Conseil permanent international pour l'exploration de la mer **183**:101-110.

Dales, R. P. 1957. The feeding mechanism and structure of the gut of *Owenia fusiformis* Delle Chiaje. Journal of the Marine Biological Association of the United Kingdom **36**:81-89.

Dando, P., and A. Southward. 1986. Chemoautotrophy in bivalve molluscs of the genus *Thyasira*. Journal of the Marine Biological Association of the United Kingdom **66**:915-929.

Dando, P., A. Southward, and E. Southward. 2004. Rates of sediment sulphide oxidation by the bivalve mollusc *Thyasira sarsi*. Marine Ecology Progress Series **280**:181-187.

Dauer, D. M., C. A. Maybury, and R. M. Ewing. 1981. Feeding behavior and general ecology of several spionid polychaetes from the Chesapeake Bay. Journal of Experimental Marine Biology and Ecology **54**:21-38.

Dauwe, B., P. M. Herman, and C. Heip. 1998. Community structure and bioturbation potential of macrofauna at four North Sea stations with contrasting food supply. Marine Ecology Progress Series **173**.

Davis, J., and J. Wilson. 1985. The energy budget and population structure of *Nucula turgida* in Dublin Bay. The Journal of Animal Ecology:557-571.

De Ridder, C., M. Jangoux, and L. De Vos. 1987. Frontal ambulacral and peribuccal areas of the spatangoid echinoid *Echinocardium cordatum* (Echinodermata): a functional entity in feeding mechanism. Marine Biology **94**:613-624.

Dekker, R. 1989. The macrozoobenthos of the subtidal western Dutch Wadden Sea. I. Biomass and species richness. Netherlands Journal of Sea Research **23**:57-68.

Drew, G. A. 1907. The habits and movements of the razor-shell clam, *Ensis directus*, Con. The Biological Bulletin **12**:127-140.

Ellis, J. I., and D. C. Schneider. 1997. Evaluation of a gradient sampling design for environmental impact assessment. Environmental Monitoring and Assessment **48**:157-172.

Emig, C. C. 1979. British and other phoronids. The Linnean Society of London, London.

Farke, H. 1979. Population dynamics, reproduction and early development of *Tharyx marioni* (Polychaeta, Cirratulidae) on tidal flats of the German Bight. Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven **18**:69-99.

Fauchald, K., and P. A. Jumars. 1979. The diet of worms: a study of polychaete feeding guilds. Aberdeen University Press.

Fish, J. D. 1967. The biology of *Cucumaria elongata* (Echinodermata: Holothuroidea). Journal of the Marine Biological Association of the United Kingdom **47**:129-144.

Fish, J. D., and S. Fish. 1996. A student's guide to the seashore. Cambridge University Press, Cambridge.

Forster, S., and G. Graf. 1995. Impact of irrigation on oxygen flux into the sediment: intermittent pumping by *Callianassa subterranea* and "piston-pumping" by *Lanice conchilega*. Marine Biology **123**:335-346.

Forster, S., and M. Zettler. 2004. The capacity of the filter-feeding bivalve *Mya arenaria* L. to affect water transport in sandy beds. Marine Biology **144**:1183-1189.

Foster-Smith, R. L. 1978. An analysis of water flow in tube-living animals. Journal of Experimental Marine Biology and Ecology **34**:73-95.

Gage, J. 1966. Observations on the bivalves *Montacuta substriata* and *M. ferruginosa*,'commensals' with spatangoids. Journal of the Marine Biological Association of the United Kingdom **46**:49-70.

Ghiold, J. 1982. Observations on the clypeasteroid Echinocyamus pusillus (O. F. Müller). Journal of Experimental Marine Biology and Ecology **61**:57-74.

Gilbert, F., S. Hulth, V. Grossi, J.-C. Poggiale, G. Desrosiers, R. Rosenberg, M. Gérino, F. François-Carcaillet, E. Michaud, and G. Stora. 2007. Sediment reworking by marine benthic species from the Gullmar Fjord (western Sweden): importance of faunal biovolume. Journal of Experimental Marine Biology and Ecology **348**:133-144.

Gilbert, M. A. 1973. Growth rate, longevity and maximum size of *Macoma balthica* (L.). The Biological Bulletin **145**:119-126.

Gillan, D. C., J. Ribesse, and C. De Ridder. 2004. The iron-encrusted microbial community of *Urothoe poseidonis* (Crustacea, Amphipoda). Journal of Sea Research **52**:21-32.

Hagmeier, A. H., J. 1931. Bemerkungen über die Ökologie von *Branchiostoma lanceolatum* (Pallas) und das Sediment seines Wohnortes. Senckenbergia **13**:259-285.

Haines, J., and D. Maurer. 1980. Quantitative faunal associates of the serpulid polychaete *Hydroides dianthus*. Marine Biology **56**:43-47.

Hansen, L. 1998. The intertidal macrofauna and macroalgae at five Arctic localities (Disko, West Greenland). Alfred Wegener Institute for Polar and Marine Research, Bremerhaven.

Hartmann-Schröder, G., F. Dahl, and H. Schumann. 1996. Annelida, Borstenwürmer, Polychaeta. Gustav Fischer Jena.

Hartnoll, R. 1972. The biology of the burrowing crab, *Corystes cassivelaunus*. Bijdragen tot de Dierkunde **42**:139-155.

Hayward, P. J., and J. S. Ryland. 2012. Handbook of the marine fauna of North-West Europe. Oxford University Press.

Hollertz, K., M. Sköld, and R. Rosenberg. 1998. Interactions between two deposit feeding echinoderms: the spatangoid *Brissopsis lyrifera* (Forbes) and the ophiuroid *Amphiura chiajei* Forbes. Hydrobiologia **375**:287-295.

Holme, N. 1950. Population-dispersion in *Tellina tenuis* da Costa. Journal of the Marine Biological Association of the United Kingdom **29**:267-280.

Holme, N. 1961. Notes on the mode of life of the Tellinidae (Lamellibranchia). Journal of the Marine Biological Association of the United Kingdom **41**:699-703.

Horton, T., A. Kroh, N. Bailly, N. Boury-Esnault, S. N. Brandão, M. J. Costello, S. Gofas, F. Hernandez, J. Mees, G. Paulay, G. Poore, G. Rosenberg, S. Stöhr, W. Decock, S. Dekeyzer, L. Vandepitte, B. Vanhoorne, K. Verfaille, S. Vranken, M. J. Adams, R. Adlard, P. Adriaens, S. Agatha, K. J. Ahn, S. Ahyong, N. Akkari, B. Alvarez, G. Anderson, M. Angel, C. Arango, T. Artois, S. Atkinson, R. Bank, A. Barber, J. P. Barbosa, I. Bartsch, D. Bellan-Santini, J. Bernot, A. Berta, R. Bieler, S. Blanco, I. Blasco-Costa, M. Błażewicz, P. Bock, R. Böttger-Schnack, P. Bouchet, C. B. Boyko, R. Bray, N. L. Bruce, S. Cairns, T. N. Campinas Bezerra, P. Cárdenas, E. Carstens, B. K. Chan, T. Y. Chan, L. Cheng, M. Churchill, C. O. Coleman, A. G. Collins, R. Cordeiro, A. Cornils, M. Coste, K. A. Crandall, T. Cribb, S. Cutmore, F. Dahdouh-Guebas, M. Daly, M. Daneliya, J. C. Dauvin, P. Davie, S. De Grave, V. de Mazancourt, P. Decker, D. Defaye, J. L. d'Hondt, H. Dijkstra, M. Dohrmann, J. Dolan, D. Domning, R. Downey, I. Drapun, L. Ector, U. Eisendle-Flöckner, M. Eitel, S. C. d. Encarnação, H. Enghoff, J. Epler, C. Ewers-Saucedo, M. Faber, S. Feist, D. Figueroa, J. Finn, C. Fišer, G. Fonseca, E. Fordyce, W. Foster, J. H. Frank, C.

Fransen, H. Furuya, H. Galea, O. Garcia-Alvarez, R. Garic, R. Gasca, S. Gaviria-Melo, S. Gerken, H. Gheerardyn, D. Gibson, J. Gil, A. Gittenberger, C. Glasby, A. Glover, S. E. Gómez-Noguera, D. González-Solís, D. Gordon, M. Grabowski, C. Gravili, J. M. Guerra-García, R. Guidetti, K. Guilini, M. D. Guiry, K. A. Hadfield, E. Hajdu, J. Hallermann, B. Hayward, E. Hendrycks, A. Herrera Bachiller, J. s. Ho, J. Høeg, O. Holovachov, J. Hooper, R. Houart, L. Hughes, W. Hummon, M. Hyžný, L. F. M. Iniesta, T. Iseto, S. Ivanenko, M. Iwataki, G. Jarms, D. Jaume, K. Jazdzewski, I. Karanovic, B. Karthick, Y. H. Kim, R. King, P. M. Kirk, J. P. Kociolek, J. Kolb, A. Kotov, T. Krapp-Schickel, A. Kremenetskaia, R. Kristensen, M. Kulikovskiy, S. Kullander, R. La Perna, G. Lambert, D. Lazarus, F. Le Coze, S. LeCroy, D. Leduc, E. J. Lefkowitz, R. Lemaitre, Y. Liu, A. N. Lörz, J. Lowry, T. Ludwig, N. Lundholm, E. Macpherson, L. Madin, C. Mah, T. Mamos, R. Manconi, G. Mapstone, P. E. Marek, B. Marshall, D. J. Marshall, P. Martin, S. McInnes, T. Meidla, K. Meland, K. Merrin, R. Mesibov, C. Messing, D. Miljutin, C. Mills, Ø. Moestrup, V. Mokievsky, T. Molodtsova, F. Monniot, R. Mooi, A. C. Morandini, R. Moreira da Rocha, F. Moretzsohn, J. Mortelmans, J. Mortimer, L. Musco, T. A. Neubauer, E. Neubert, B. Neuhaus, P. Ng, A. D. Nguyen, C. Nielsen, T. Nishikawa, J. Norenburg, T. O'Hara, H. Okahashi, D. Opresko, M. Osawa, Y. Ota, D. Patterson, H. Paxton, V. Perrier, W. Perrin, I. Petrescu, B. Picton, J. F. Pilger, A. Pisera, D. Polhemus, M. Potapova, P. Pugh, J. D. Reimer, H. Reip, M. Reuscher, J. W. Reynolds, F. Rimet, P. Rios Lopez, M. Rius, K. Rützler, A. Rzhavsky, K. Sabbe, J. Saiz-Salinas, S. Sala, S. Santos, E. Sar, A. F. Sartori, A. Satoh, H. Schatz, B. Schierwater, A. Schmidt-Rhaesa, S. Schneider, C. Schönberg, P. Schuchert, A. R. Senna, C. Serejo, S. Shamsi, J. Sharma, W. A. Shear, N. Shenkar, A. Shinn, M. Short, J. Sicinski, V. Siegel, P. Sierwald, E. Simmons, F. Sinniger, D. Sivell, B. Sket, H. Smit, N. Smit, N. Smol, J. F. Souza-Filho, J. Spelda, S. N. Stampar, W. Sterrer, E. Stienen, P. Stoev, M. Strand, E. Suárez-Morales, M. Summers, C. Suttle, B. J. Swalla, S. Taiti, M. Tanaka, A. H. Tandberg, D. Tang, M. Tasker, J. Taylor, J. Taylor, A. Tchesunov, H. ten Hove, J. J. ter Poorten, J. Thomas, E. V. Thuesen, M. Thurston, B. Thuy, J. T. Timi, T. Timm, A. Todaro, X. Turon, S. Tyler, P. Uetz, S. Utevsky, J. Vacelet, W. Vader, R. Väinölä, B. Van de Vijver, S. E. van der Meij, T. van Haaren, R. van Soest, R. Van Syoc, V. Venekey, R. Vonk, C. Vos,

G. Walker-Smith, T. C. Walter, L. Watling, M. Wayland, T. Wesener, C. Wetzel, C. Whipps, K. White, D. Williams, G. Williams, R. Wilson, A. Witkowski, J. Witkowski, N. Wyatt, C. Wylezich, M. Yasuhara, J. Zanol, and W. Zeidler. 2017. World Register of Marine Species (WoRMS). WoRMS Editorial Board.

Howe, R. L., A. P. Rees, and S. Widdicombe. 2004. The impact of two species of bioturbating shrimp (*Callianassa subterranea* and *Upogebia deltaura*) on sediment denitrification. Journal of the Marine Biological Association of the United Kingdom **84**:629-632.

Hummel, H. 1985. Food intake of *Macoma balthica* (Mollusca) in relation to seasonal changes in its potential food on a tidal flat in the Dutch Wadden Sea. Netherlands Journal of Sea Research **19**:52-76.

IfAÖ, and AWI. 2008. Autökologischer Atlas benthischer wirbelloser Tiere in der Deutschen Nord- und Ostsee, Version 1.2. Institut für Angewandte Ökologie und Alfred-Wegener-Institut für Polar und Meeresforschung.

Janke, K. K., Bruno, P. 2010. Düne, Strand und Wattenmeer - Tiere ubd Pflanzen unserer Küsten. Franckh-Kosmos Verlag GmbH & Co. KG, Stuttgard.

Janson, A.-L., L. Denis, M. Rauch, and N. Desroy. 2012. Macrobenthic biodiversity and oxygen uptake in estuarine systems: the example of the Seine estuary. Journal of soils and sediments **12**:1568-1580.

Jenner, H. A., and T. Bowmer. 1990. The accumulation of metals and their toxicity in the marine intertidal invertebrates *Cerastoderma edule*, *Macoma balthica*, *Arenicola marina* exposed to pulverised fuel ash in mesocosms. Environmental Pollution **66**:139-156.

Jones, J. 1955. Observations on the respiratory physiology and on the haemoglobin of the polychaete genus *Nephthys*, with special reference to *N. hombergii* (Aud. et M.-Edw.). Journal of experimental biology **32**:110-125.

Jones, N. S. 1948. The ecology of the Amphipoda of the south of the Isle of Man. Journal of the Marine Biological Association of the United Kingdom **27**:400-439.

Kamermans, P., E. Brummelhuis, and M. Dedert. 2013. Effect of algae-and silt concentration on clearance-and growth rate of the razor clam *Ensis directus*, Conrad. Journal of Experimental Marine Biology and Ecology **446**:102-109.

Kikuchi, E. 1987. Effects of the brackish deposit-feeding polychaetes *Notomastus* sp. (Capitellidae) and *Neanthes japonica* (Izuka)(Nereidae) on sedimentary O_2 consumption and CO_2 production rates. Journal of Experimental Marine Biology and Ecology **114**:15-25.

Kinner, P., and D. Maurer. 1978. Polychaetous annelids of the Delaware Bay region. Fishery Bulletin **76**:209-224.

Lackschewitz, D., and K. Reise. 1998. Macrofauna on flood delta shoals in the Wadden Sea with an underground association between the lugworm *Arenicola marina* and the amphipod *Urothoe poseidonis*. Helgoländer Meeresuntersuchungen **52**:147-158.

Lee, C.-G., M. Huettel, J.-S. Hong, and K. Reise. 2004. Carrion-feeding on the sediment surface at nocturnal low tides by the polychaete *Phyllodoce mucosa*. Marine Biology **145**:575-583.

Li, J., D. Ó. Foighil, and P. Middelfart. 2012. The evolutionary ecology of biotic association in a megadiverse bivalve superfamily: sponsorship required for permanent residency in sediment. Plos One **7**:e42121.

Lindqvist, S., K. Norling, and S. Hulth. 2009. Biogeochemistry in highly reduced mussel farm sediments during macrofaunal recolonization by *Amphiura filiformis* and *Nephtys* sp. Marine Environmental Research **67**:136-145.

Lindroth, A. 1941. Atmungsventilation der Polychäten. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology **28**:485-532.

Loo, L.-O., P. R. Jonsson, M. Skold, and Ö. Karlsson. 1996. Passive suspension feeding in *Amphiura filiformis* (Echinodermata: Ophiuroidea): feeding behaviour in flume flow and potential feeding rate of field populations. Marine Ecology Progress Series **139**:143-155.

Maire, O., J. C. Duchene, R. Rosenberg, J. B. de Mendonca, and A. Grémare. 2006. Effects of food availability on sediment reworking in *Abra ovata* and *A. nitida*. Marine Ecology Progress Series **319**:135.

Makra, A., and B. F. Keegan. 1999. Arm regeneration in *Acrocnida brachiata* (Ophiuroidea) at Little Killary, west coast of Ireland. Pages 95-102 *in* Biology and Environment: Proceedings of the Royal Irish Academy. JSTOR.

MarLIN. 2006. BIOTIC - Biological traits information catalogue.*in* M. L. I. Network, editor. Marine Biological Association of the United Kingdom, Plymouth.

Martin, W., and G. Banta. 1992. The measurement of sediment irrigation rates: a comparison of the Br− tracer and 222Rn/226Ra disequilibrium techniques. Journal of Marine Research **50**:125-154.

Mattson, S. 1981. Burrowing and feeding of *Goniada maculata* Ørsted (Polychaeta). Sarsia **66**:49-51.

Meixner, R. 1994. Über die Trogmuschel und deren Nutzung in der Deutschen Bucht. Informationen für die Fischwirtschaft aus der Fischereiforschung **41**:166-170.

Michaelis, H., and L. Vennemann. 2005. The "piece-by-piece predation" of *Eteone longa* on *Scolelepis squamata* (Polychaetes)—traces on the sediment documenting chase, defence and mutilation. Marine Biology **147**:719-724.

Miller, D. C., M. J. Bock, and E. J. Turner. 1992. Deposit and suspension feeding in oscillatory flows and sediment fluxes. Journal of Marine Research **50**:489-520.

Moksnes, P.-O. 2002. The relative importance of habitat-specific settlement, predation and juvenile dispersal for distribution and abundance of young juvenile shore crabs *Carcinus maenas* L. Journal of Experimental Marine Biology and Ecology **271**:41-73.

Moore, P., and K. Cameron. 1999. A note on a hitherto unreported association between *Photis longicaudata* (Crustacea: Amphipoda) and *Cerianthus lloydii* (Anthozoa: Hexacorallia). Journal of the Marine Biological Association of the United Kingdom **79**:369-370.

Mortensen, T. 1927. Handbook of the echinoderms of the British isles. Oxford University Press, London

Mortimer, K., and A. S. Y. Mackie. 2014. Morphology, feeding and behaviour of British *Magelona* (Annelida: Magelonidae), with discussions on the form and function of abdominal lateral pouches. Memoirs of Museum Victoria **71**:177-201.

Nair, N. B., and A. D. Ansell. 1968. Characteristics of penetration of the substratum by some marine bivalve molluscs. Journal of Molluscan Studies **38**:179-197.

Nichols, D. 1959a. Changes in the chalk heart-urchin *Micraster* interpreted in relation to living forms. Philosophical Transactions of the Royal Society of London B: Biological Sciences **242**:347-437.

Nichols, D. 1959b. The histology of the tube-feet and clavulae of *Echinocardium cordatum*. Quarterly Journal of Microscopical Science **3**:73-87.

Norling, K., R. Rosenberg, S. Hulth, A. Grémare, and E. Bonsdorff. 2007. Importance of functional biodiversity and species-specific traits of benthic fauna for ecosystem functions in marine sediment. Marine Ecology Progress Series **332**:11-23.

O'Connor, B., T. Bowmer, and A. Grehan. 1983. Long-term assessment of the population dynamics of *Amphiura filiformis* (Echinodermata: Ophiuroidea) in Galway Bay (west coast of Ireland). Marine Biology **75**:279-286.

O'Connor, B., T. Bowmer, D. McGrath, and R. Raine. 1986. Energy flow through an *Amphiura filiformis* (Ophiuroidea: Echinodermata) population in Galway Bay, west coast of Ireland: a preliminary investigation. Ophelia **26**:351-357.

Ockelmann, K. W., and K. Muus. 1978. The biology, ecology and behaviour of the bivalve *Mysella bidentata* (Montagu). Ophelia **17**:1-93.

Olafsson, E. 1986. Density dependence in suspension-feeding and deposit-feeding populations of the bivalve *Macoma balthica*: a field experiment. The Journal of Animal Ecology:517-526.

Oldfield, E. 1961. The functional morphology of *Kellia suborbicularis* (Montagu), *Montacuta ferruginosa* (Montagu) and *M. substriata* (Montagu),(Mollusca, Lamellibranchiata). Journal of Molluscan Studies **34**:255-295.

Pohlo, R. 1969. Confusion concerning deposit feeding in the Tellinacea. Journal of Molluscan Studies **38**:361-364.

Queirós, A. M., S. N. R. Birchenough, J. Bremner, J. A. Godbold, R. E. Parker, A. Romero-Ramirez, H. Reiss, M. Solan, P. J. Somerfield, C. Van Colen, G. Van Hoey, and S. Widdicombe. 2013. A bioturbation classification of European marine infaunal invertebrates. Ecology and evolution **3**:3958-3985.

Rachor, E. 1976. Structure, dynamics and productivity of a population of *Nucula nitidosa* (Bivalvia; Protobranchiata) in the German Bight. Berichte der Deutschen Wissenschaftlichen Kommision für Meeresforschung **24**:296-331.

Rachor, E., and S. Bartel. 1981. Occurrence and ecological significance of the spoon-worm *Echiurus echiurus* in the German Bight. Veröffentlichungen Inst. Meeresforschung Bremerhaven **19**:71-88.

Redmond, M. S., and K. J. Scott. 1989. Amphipod predation by the infaunal polychaete, *Nephtys incisa*. Estuaries **12**:205-207.

Rees, H., and P. J. Dare. 1993. Sources of mortality and associated life-cycle traits of selected benthic species: a review. Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research.

Riedl, R. 1983. Fauna und Flora der Mittelmeeres. P. Parey.

Riisgård, H. 2002. Methods of ciliary filter feeding in adult *Phoronis muelleri* (phylum Phoronida) and in its free-swimming actinotroch larva. Marine Biology **141**:75-87.

Riisgård, H. U., and I. Svane. 1999. Filter feeding in lancelets (amphioxus), *Branchiostoma lanceolatum*. Invertebrate Biology:423-432.

Rouse, G. W., and F. Pleijel. Polychaetes. Oxford University Press, New York.

Schaffner, L. C. 1990. Small-scale organism distributions and patterns of species diversity: evidence for positive interactions in an estuarine benthic community. Marine Ecology Progress Series:107-117.

Schöttler, U. 1982. An investigation on the anaerobic metabolism of *Nephtys hombergii* (Annelida: Polychaeta). Marine Biology **71**:265-269.

Schöttler, U., and M. Grieshaber. 1988. Adaptation of the polychaete worm *Scoloplos armiger* to hypoxic conditions. Marine Biology **99**:215-222.

Solan, M., and R. Kennedy. 2002. Observation and quantification of in situ animal-sediment relations using time-lapse sediment profile imagery (t-SPI). Marine Ecology Progress Series **228**:179-191.

Srikrishnadhas, B., and K. Ramamoorthi. 1981. Studies on the life-history of *Spio filicornis* (Müller 1776). Mahasagar-Bulletin of the National Institute of Oceanography **14**:303-307.

Stamhuis, E., T. Reede-Dekker, Y. Van Etten, J. De Wiljes, and J. Videler. 1996. Behaviour and time allocation of the burrowing shrimp *Callianassa subterranea* (Decapoda, Thalassinidea). Journal of Experimental Marine Biology and Ecology **204**:225-239.

Swennen, C., M. Leopold, and M. Stock. 1985. Notes on growth and behaviour of the American razor clam *Ensis directus* in the Wadden Sea and the predation on it by birds. Helgoländer Meeresuntersuchungen **39**:255-261.

Taylor, A. C. 1976. Burrowing behaviour and anaerobiosis in the bivalve *Arctica islandica* (L.). Journal of the Marine Biological Association of the United Kingdom **56**:95-109.

Tebble, N. 1966. British bivalve seashells: a handbook for identification. British Museum (Natural History) London.

Telford, M., A. S. Harold, and R. Mooi. 1983. Feeding structures, behavior, and microhabitat of *Echinocyamus pusillus* (Echinoidea: Clypeasteroida). The Biological Bulletin **165**:745-757.

Trevallion, A. 1971. Studies on *Tellina tenuis* da Costa. III. Aspects of general biology and energy flow. Journal of Experimental Marine Biology and Ecology **7**:95-122.

Trevor, J. 1977. The burrowing of *Nereis diversicolor* O. F. Müller, together with some observations on *Arenicola marina* (L.)(Annelida: Polychaeta). Journal of Experimental Marine Biology and Ecology **30**:129-145.

Tunberg, B. 1984. Population ecology of *Dosinia exoleta* (L.)(Bivalvia) in Raunefjorden, western Norway. Sarsia **69**:159-168.

Tyler, P., and S. Shackley. 1978. Comparative efficiency of the Day and Smith-McIntyre grabs. Estuarine and Coastal Marine Science **6**:439-445.

Vader, W. 1964. A preliminary investigation into the reactions of the infauna of the tidal flats to tidal fluctuations in water level. Netherlands Journal of Sea Research **2**:189-222.

Van Dam, L. 1940. On the mechanism of ventilation in *Aphrodite aculeata*. Journal of experimental biology **17**:1-7.

van der Loeff, M. M., L. G. Anderson, P. O. Hall, Å. Iverfeldt, A. B. Josefson, B. Sundby, and S. F. Westerlund. 1984. The asphyxiation technique: an approach to distinguishing between molecular diffusion and biologically mediated transport at the sediment-water interface. Limnology and Oceanography **29**:675-686.

Vittor, B. A. 1999. Benthic macroinfauna of the northeastern Golf of Mexico OCS, near de Soto Canyon. Page 73 *in* Physical/Biological Oceanographic Integration Workshop for the.

Vopel, K., D. Thistle, and R. Rosenberg. 2003. Effect of the brittle star *Amphiura filiformis* (Amphiuridae, Echinodermata) on oxygen flux into the sediment. Limnology and Oceanography **48**:2034-2045.

Webb, J. 1976. A review of swimming in amphioxus. Pages 447-454 *in* P. Davis, editor. Perspectives in Experimental Biology. Pergamon Press, Oxford.

Wells, G., and R. P. Dales. 1951. Spontaneous activity patterns in animal behaviour: the irrigation of the burrow in the polychaetes *Chaetopterus variopedatus* Renier and *Nereis diversicolor* OF Müller. Journal of the Marine Biological Association of the United Kingdom **29**:661-680.

Westheide, W. 1967. Monographic der Gattungen *Hesionides* Friedrich und *Microphthalmus* Mecznikow (Polychaeta, Hesionidae). Zeitschrift für Morphologie der Tiere **61**:1-159.

Widdicombe, S., M. C. Austen, M. A. Kendall, F. Olsgard, M. T. Schaanning, S. L. Dashfield, and H. R. Needham. 2004. Importance of bioturbators for biodiversity maintenance: indirect effects of fishing disturbance. Marine Ecology Progress Series **275**:1-10.

Wieking, G., and I. Kröncke. 2003. Macrofauna communities of the Dogger Bank (central North Sea) in the late 1990s: spatial distribution, species composition and trophic structure. Helgoland Marine Research **57**:34-46.

Wilson, J. 1990. Gill and palp morphology of *Tellina tenuis* and *Tellina fabula* in relation to feeding. The Bivalvia.

Wilson, J. G., and J. P. Davis. 1984. The effect of environmental variables on the oxygen consumption of the protobranch bivalve *Nucula turgida* (Leckenby and Marshall). Journal of Molluscan Studies **50**:73-77.

Winter, A. G., R. L. Deits, and A. E. Hosoi. 2012. Localized fluidization burrowing mechanics of *Ensis directus*. The Journal of experimental biology **215**:2072-2080.

Wlodarska-Kowalczuk, M., and T. H. Pearson. 2004. Soft-bottom macrobenthic faunal associations and factors affecting species distributions in an Arctic glacial fjord (Kongsfjord, Spitsbergen). Polar Biology **27**:155-167.

Wolff, W. J. 1973. The estuary as a habitat: an analysis of data on the soft-bottom macrofauna of the estuarine area of the rivers Rhine, Meuse and Scheldt. Brill.

Wood, H. L., S. Widdicombe, and J. I. Spicer. 2009. The influence of hypercapnia and the infaunal brittlestar *Amphiura filiformis* on sediment nutrient flux - will ocean acidification affect nutrient exchange? Biogeosciences **6**:2015-2024.

Woodley, J. 1975. The behaviour of some amphiurid brittle-stars. Journal of Experimental Marine Biology and Ecology **18**:29-46.

Word, J. Q. 1979. Classification of benthic invertebrates into infaunal trophic index feeding groups.

Yonge, C. 1946. On the habits and adaptations of *Aloidis* (*Corbula*) *gibba*. Journal of the Marine Biological Association of the United Kingdom **26**:358-376.

Yonge, C. M. 1948. Cleansing mechanisms and the function of the fourth pallial aperture in *Spisula subtruncata* (da Costa) and *Lutraria lutraria* (L.). Journal of the Marine Biological Association of the United Kingdom **27**:585-596.

Ziegelmeier, E. 1952. Beobachtungen über den Röhrenbau von *Lanice conchilega* (Pallas) im Experiment und am natürlichen Standort. Helgoländer wissenschaftliche Meeresuntersuchungen **4**:107.

Ziegelmeier, E. 1957. Die Muscheln (Bivalvia) der deutschen Meeresgebiete. Helgoland Marine Research **6**:1-51.

Ziegelmeier, E. 1969. Neue Untersuchungen über die Wohnröhren-Bauweise von *Lanice conchilega* (Polychaeta, Sedentaria). Helgoländer wissenschaftliche Meeresuntersuchungen **19**:216-229.

Zimmermann, K. E. 1912. Notes on the respiratory mechanism of *Corystes cassivelaunus*. Journal of the Marine Biological Association of the United Kingdom **9**:288-291.

Appendix Manuscript IV

Statistical analysis:

Models that best described (lowest AIC_c) the nutrient flow (i.e. $\Delta[PO_4^{3-}]$, $\Delta[NO_2^-]$, $\Delta[NO_3^-]$, $\Delta[NH_4^+]$, $[NO_3^-]$ and $\Delta[SiO_2]$)

Best model of $\Delta[PO_4^{3-}]$

GLM formula: $\Delta[PO_4^{3-}] = IP_{c,AFDM} + \text{TREATMENT}$)

Table 1. Deviance residuals of GLM formula: $\Delta[PO_4^{3-}] = IP_{c,AFDM} + \text{TREATMENT}$

Min	1Q	Median	3Q	Max
-0.20260	-0.01674	0.00112	0.01885	0.40905

Table 2. Coefficients of GLM formula: $\Delta[PO_4^{3-}] = IP_{c,AFDM} + \text{TREATMENT}$

	Estimate	Std. Error	t value	Pr (> |t|)
Intercept	-0.063400	0.0174779	-3627	0.000461
$IP_{c,AFDM}$	0.004203	0.001441	2.918	0.004391
$A_{community}$	0.052031	0.027180	1.914	0.058554
$L_{community}$	0.174782	0.027360	6.388	6e-09
$N_{community}$	0.059211	0.028662	2.066	0.041541
LC_{spring}	0.006836	0.021025	0.325	0.745800
NO_{spring}	0.048019	0.022501	2.134	0.035384

(Dispersion parameter for gaussian family taken to be 0.005269455)

Null deviance: 0.83441 on 102 degrees of freedom

Residual deviance: 0.50587 on 96 degrees of freedom

AIC: -239.27

Number of Fisher Scoring iterations: 2

Best model of $\Delta[NO_2^-]$

GLM formula: $\Delta[NO_2^-] = IP_{c,WM} + \text{Treatment} + IP_{c,WM} {}^{*} \text{Treatment}$

Table 3: Deviance residuals of GLM formula: $\Delta[NO_2^-] = IP_{c,WM} + \text{Treatment} + IP_{c,WM} * \text{Treatment}$

Min	1Q	Median	3Q	Max
-0.42751	-0.01786	-0.00280	0.00781	0.30818

Table 4: Coefficients of GLM formula: $\Delta[NO_2^-] = IP_{c,WM\,+}\,\text{Treatment} + IP_{c,WM} * \text{Treatment}$

	Estimate	Std. Error	t value	Pr (> |t|)
Intercept	-2.762e-02	2.711e-02	-1.019	0.310243
$IP_{c,WM}$	3.098e-04	2.581e-04	1.200	0.232326
AE_{summer}	-1.316e-01	3.787e-02	-3.476	0.000705
$A_{community}$	9.436e-03	4.770e-02	0.198	0.843498
$L_{community}$	2.261e-01	5.837e-02	3.874	0.000173
$N_{community}$	2.748e-02	4.579e-02	0.600	0.549527
LC_{spring}	-1.509e-02	4.113e-02	-0.367	0.714437
L_{summer}	-1.285e-02	5.082e-02	-0.253	0.800834
NO_{spring}	5.805e-03	3.950e-02	0.147	0.883410
N_{summer}	1.348e-02	5.133e-02	0.263	0.793323
$IP_{c,WM}*AE_{summer}$	1.191e-03	4.829e-04	2.468	0.014980
$IP_{c,WM}*A_{community}$	4.085e-05	6.686e-04	0.061	0.951381
$IP_{c,WM}*L_{community}$	4.028e-03	7.699e-04	5.232	6.98e-07
$IP_{c,WM}*N_{community}$	8.238e-04	5.059e-03	0.163	0.870916
$IP_{c,WM}*LC_{spring}$	2.808e-03	7.262e-04	3.867	0.000178
$IP_{c,WM}*L_{summer}$	1.190e-02	3.951e-03	3.012	0.003155
$IP_{c,WM}*NO_{spring}$	-4.154e-04	7.303e-03	-0.057	0.954735
$IP_{c,WM}*N_{summer}$	4.211e-03	7.758e-03	0.543	0.588304

(Dispersion parameter for gaussian family taken to be 0.009522958)

Null deviance: 4.2021 on 140 degrees of freedom

Residual deviance: 1.1713 on 123 degrees of freedom

AIC: -237.34

Number of Fisher Scoring iterations: 2

Best model of $\Delta[NO_3^-]$

GLM formula: $\Delta[NO_3^-] = IP_{c,WM} + \mathrm{TREATMENT} + IP_{c,WM} * \mathrm{TREATMENT}$

Table 5: Deviance residuals of GLM formula: $\Delta[NO_3^-] = IP_{c,WM} + \mathrm{TREATMENT} + IP_{c,WM} * \mathrm{TREATMENT}$

Min	1Q	Median	3Q	Max
-1.57883	-0.34424	0.04706	0.31237	1.20382

Table 6: Coefficients of GLM formula: $\Delta[NO_3^-] = IP_{c,WM} + \mathrm{TREATMENT} + IP_{c,WM} * \mathrm{TREATMENT}$

	Estimate	Std. Error	t value	Pr (> |t|)
Intercept	-0.916143	0.151255	-6.057	2.99e-08
$IP_{c,WM}$	0.007554	0.002334	3.237	0.001682
$A_{community}$	0.729075	0.270649	2.694	0.008396
$L_{community}$	0.887773	0.332076	2.673	0.008884
$N_{community}$	1.502703	0.277569	5.414	4.89e-07
LC_{spring}	0.938283	0.233973	4.010	0.000123
L_{summer}	2.432751	0.288647	8.428	4.60e-13
NO_{spring}	0.789436	0.241184	3.273	0.001499
N_{summer}	0.455180	0.291568	1.561	0.121923
$IP_{c,WM}*A_{community}$	-0.011472	0.004229	-2.713	0.007969
$IP_{c,WM}*L_{community}$	-0.034652	0.004760	-7.280	1.10e-10
$IP_{c,WM}*N_{community}$	-0.003278	0.029103	-0.113	0.910554
$IP_{c,WM}*LC_{spring}$	-0.029816	0.004530	-6.583	2.80e-09
$IP_{c,WM}*L_{summer}$	-0.036576	0.022666	-1.614	0.110021
$IP_{c,WM}*NO_{spring}$	-0.049964	0.043438	-1.150	0.253033
$IP_{c,WM}*N_{summer}$	-0.047570	0.044408	-1.071	0.286883

(Dispersion parameter for gaussian family taken to be 0.3114795)

Null deviance: 112.398 on 107 degrees of freedom

Residual deviance: 28.656 on 92 degrees of freedom

AIC: 197.2

Number of Fisher Scoring iterations: 2

Best model of $\Delta[NH_4^+]$

GLM formula: $\Delta[NH_4^+] = IP_{c,WM} + \text{TREATMENT} + IP_{c,WM} * \text{TREATMENT}$

Table 7: Deviance residuals of GLM formula: $\Delta[NH_4^+] = IP_{c,WM} + \text{TREATMENT} + IP_{c,WM} * \text{TREATMENT}$

Min	1Q	Median	3Q	Max
-7.8547	-0.4858	-0.0875	0.2704	15.3153

Table 8: Coefficients of GLM formula: $\Delta[NH_4^+] = IP_{c,WM} + \text{TREATMENT} + IP_{c,WM} * \text{TREATMENT}$

	Estimate	Std. Error	t value	Pr (> |t|)
Intercept	-0.283185	0.573339	-0.494	0.622242
$IP_{c,WM}$	0.024690	0.005459	4.523	1.42e-05
AE_{summer}	-0.664410	0.801015	-0.829	0.408451
$A_{community}$	0.354903	1.008797	0.352	0.725585
$L_{community}$	4.040637	1.234525	3.273	0.001382
$N_{community}$	0.372608	0.968481	0.385	0.701099
LC_{spring}	1.089506	0.870029	1.252	0.212850
L_{summer}	0.322696	1.074871	0.300	0.764518
NO_{spring}	-0.276487	0.835523	-0.331	0.741271
N_{summer}	-0.462317	1.085600	-0.426	0.670952
$IP_{c,WM}*AE_{summer}$	-0.001849	0.010213	-0.181	0.856619
$IP_{c,WM}*A_{community}$	0.011127	0.014141	0.787	0.432871
$IP_{c,WM}*L_{community}$	0.064080	0.016284	3.935	0.000138
$IP_{c,WM}*N_{community}$	0.017271	0.107002	0.161	0.872040
$IP_{c,WM}*LC_{spring}$	0.029263	0.015359	1.905	0.059081
$IP_{c,WM}*L_{summer}$	0.163268	0.083559	1.954	0.052980
$IP_{c,WM}*NO_{spring}$	0.004584	0.154464	0.030	0.976371
$IP_{c,WM}*N_{summer}$	0.043634	0.164096	0.266	0.790759

(Dispersion parameter for gaussian family taken to be 4.260144)

Null deviance: 1671.6 on 140 degrees of freedom

Residual deviance: 524.0 on 123 degrees of freedom

AIC: 623.24

Number of Fisher Scoring iterations: 2

Best model of $\Delta[SiO_2]$

GLM formula: $\Delta[SiO_2] = IP_{c,WM} + \textsc{Treatment} + IP_{c,WM} * \textsc{Treatment}$

Table 9: Deviance residuals of GLM formula: $\Delta[SiO_2] = IP_{c,WM} + \textsc{Treatment} + IP_{c,WM} * \textsc{Treatment}$

Min	1Q	Median	3Q	Max
-5.218	-0.506	-0.211	0.271	33.264

Table 10: Coefficients of GLM formula: $\Delta[SiO_2] = IP_{c,WM} + \textsc{Treatment} + IP_{c,WM} * \textsc{Treatment}$

	Estimate	Std. Error	t value	Pr (> |t|)
Intercept	1.728539	0.894123	1.933	0.055507
$IP_{c,WM}$	0.042194	0.008513	4.956	2.32e-06
AE_{summer}	1.933318	1.249184	1.548	0.124272
$A_{community}$	0.367231	1.573220	0.233	0.815818
$L_{community}$	-0.858390	1.925242	-0.446	0.656482
$N_{community}$	0.230957	1.510347	0.153	0.878715
LC_{spring}	-1.333325	1.356811	-0.983	0.327689
L_{summer}	-1.720211	1.676262	-1.026	0.306803
NO_{spring}	-0.169888	1.302999	-0.130	0.896477
N_{summer}	3.867869	1.692994	2.285	0.024048
$IP_{c,WM}*AE_{summer}$	0.056870	0.015927	3.571	0.000509
$IP_{c,WM}*A_{community}$	-0.018955	0.022052	-0.860	0.391713
$IP_{c,WM}*L_{community}$	-0.037598	0.025395	-1.481	0.141287
$IP_{c,WM}*N_{community}$	0.008162	0.166870	0.049	0.961068
$IP_{c,WM}*LC_{spring}$	-0.030228	0.023952	-1.262	0.209336
$IP_{c,WM}*L_{summer}$	-0.029485	0.130311	-0.226	0.821369
$IP_{c,WM}*NO_{spring}$	0.042048	0.240887	0.175	0.861715
$IP_{c,WM}*N_{summer}$	0.168292	0.255908	0.658	0.512007

(Dispersion parameter for gaussian family taken to be 10.36085)

Null deviance: 2850.8 on 140 degrees of freedom

Residual deviance: 1274.4 on 123 degrees of freedom

AIC: 748.55

Number of Fisher Scoring iterations: 2

Eidesstattliche Erklärung

Hiermit erkläre ich,

Alexa Wrede

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dass ich die Doktorarbeit mit dem Titel:

Macrofaunal impact on biogeochemical turnover in German Bight sediments

selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

Bremen, 30. Januar 2018

Alexa Wrede, Msc