Bioturbation of *Marenzelleria* spp. in Baltic Sea sediments

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‘All animals are equal –

but some animals are more equal than others.’

G. Orwell

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List of abbreviations

BC	bioturbation experiment - control
Biex	bioturation experiment
BMA	bioturbation experiment - *M. arctia* treatment
BMN	bioturbation experiment - *M. neglecta* treatment
BMV	bioturbation experiment - *M. viridis* treatment
Br^-	bromide
CT	micro-computed tomography
d_x	day x of the experiment
DNRA	dissimilatory nitrate reduction to ammonium
FEMME	flexible environment for mathematically modeling the environment
GF/F	glass microfiber filters
ind.	individuals
n	number of individuals
N	nitrogen
N_2	dinitrogen
NaCl	sodium chloride
NC	nutrient experiment - control
Näex	nutrient experiment
NH_4^+	ammonium
NMA	nutrient experiment - *M. arctia* treatment
NMN	nutrient experiment - *M. neglecta* treatment
NMV	nutrient experiment - *M. viridis* treatment
NO_2^-	nitrite
NO_3^-	nitrate
O_2	oxygen
p	significance level
P	phosphorous
PO_4^3	phosphate
PVC	poly vinyl chloride
SO_4^{2-}	sulfate
S^{2-}	sulfide
TOU	total oxygen uptake
UV	ultra violet

List of formula symbols

A	(m^2)	surface area of cores
α	(yr^{-1})	non-local exchange coefficient
C_{olw}	(mmol L^{-1})	concentration overlying water
C_{add}	(mmol L^{-1})	added tracer concentration
C_{ini}	(μmol L^{-1})	external (initial) tracer concentration
δ		delta
Db	(cm^2 yr^{-1})	bioturbation coefficient
D_s	(cm^2 s^{-1})	diffusion coefficient of solutes
ε		enhanced diffusion coefficient
$F_{biodiff}$	(mmol m^{-2} h^{-1})	(bio)-diffusion-flux
$F_{non\text{-}local}$	(mmol m^{-2} h^{-1})	non-local exchange-flux
F_{olw}	(mmol m^{-2} d^{-1})	flux overlying water
h	(m)	height of water inside the cores
J	(mmol m^{-2} h^{-1})	flux across the sediment-water interface
$k_{\varepsilon,\alpha}$	(cm)	enhancement attenuation
k^{Φ}		porosity attenuation
L_b	(cm)	depth of bioturbated layer
m		tortuosity power
Φs		porosity at surface
Φ_∞		porosity at depth
r	(yr^{-1})	non-local transport coefficient
T	(°C)	temperature

Explanation

Large parts of the present thesis are already published or in press in two scientific journals cited below. In both these publications I am the first and responsible author having done all the data compilation, most of the discussion and writing of the draft for publications.

The concept and experimental design of this PhD thesis was developed together with Stefan Forster, the implementation of experiments as well as the analysis and modeling of data has been completed independently. Molecular genetic determination of the studied polychaetes was conducted by Ralf Bastrop. Additional data compilation, calculations and discussion only available in this PhD thesis were primarily my own work, however with the aid of Stefan Forster. This PhD thesis has been written and arranged without additional external assistance.

Contents referring to the investigation of particle and solute transport, modeling, as well as the micro-computed scan of the burrow structure of *M. arctia* are published in Limnology & Oceanography:

Renz, J. and Forster, S. (2013) Are similar worms different? A comparative tracer study on bioturbation in the three sibling species *Marenzelleria arctia*, *M. viridis*, and *M. neglecta* from the Baltic Sea. Limnology and Oceanography 58(6), 2046–2058.

Contents referring to nutrient release and total oxygen uptake are submitted in Marine Ecology Progress Series and in press:

Renz, J. R. and Forster, S. (in press) Effects of bioirrigation by the three sibling species of *Marenzelleria* spp. on solute fluxes and pore water nutrient profiles. Marine Ecology Progress Series. doi: 10.3354/meps10756

Summary

The invasive spionid polychaetes of the genus *Marenzelleria* spp., consisting of the three sibling species *M. neglecta*, *M. viridis,* and *M. arctia* have been found in the Baltic Sea since the 1980s. Because of difficulties in species identification, little is known about their species dependent sediment reworking and solute transport pattern as well as their various effects on the biogeochemistry of bioturbated sediments. The closely related species are apparently similar in feeding and sediment dwelling behavior, but size and burrowing depth indicate differences in bioturbation. To investigate these potential differences with regard to their bioturbation and bioirrigation pattern, a tracer experiment with artificial particles (luminophores) and solute tracer (bromide) was conducted. The influence on benthic nutrient fluxes (NH_4^+, NO_3^- PO_4^{3-}) and oxygen uptake was examined in a further laboratory experiment. Polychaetes were identified to species level using a molecular genetic key.

Modeled results show that all three species display markedly low particle reworking rates with biodiffusion coefficients (*Db*) of 1.76 cm^2 yr^{-1} (*M. viridis*) and 0.07 cm^2 yr^{-1} (*M. neglecta*) at an abundance of 1273 individuals m^{-2}, and 0.44 cm^2 yr^{-1} (*M. arctia*) at twice that abundance. Non-local transport coefficients are negligible in all cases. Solute transport by *M. neglecta* and *M. viridis* are more similar to one another than to *M. arctia* whose solute transport mode is much more diffusive in character (10.9 fold enhanced diffusivity with non-local irrigation coefficients (α) of 55.3 yr^{-1}) than that of the two other species which affect tracer distributions in the sediment predominantly through a non-local, advective transport mode (α of 108.9 yr^{-1} *M. viridis* and 130.9 yr^{-1} *M. neglecta*) These general bioirrigation patterns were also confirmed by the results of the second experiment, where solute transport modes were investigated in analogy to the first experiment. Thus, a functional grouping of the sibling species in terms of bioirrigation is not recommended.

While the release of ammonium and phosphate, as well as the total oxygen uptake of the sediment, was stimulated by the presence of all polychaetes, the fluxes (NH_4^+, PO_4^{3-}, TOU) in cores colonized with *M. viridis* and *M. neglecta* were substantially higher than those of *M. arctia*. The presence of *M. arctia* had only slight stimulatory effects on nitrification, whereas *M. neglecta* and *M. viridis* enhanced nitrification. This suggests negligible stimulation of denitrification for *M. arctia* and leaves the source of ammonium in *M. neglecta* and *M. viridis* unresolved.

Consequently *M. viridis* and *M. neglecta* may affect eutrophication-related benthic fluxes considerably more than *M. arctia*. Furthermore, the ecological consequences of a potential spread of *M. arctia* into the southern areas of the Baltic Sea are assumed to be less dramatic than a comparable spread of *M. viridis*.

Zusammenfassung

Die invasiven spioniden Polychaeten der Gattung *Marenzelleria* spp., die unter anderem die drei Schwesterarten *M. neglecta*, *M. viridis* und *M. arctia* umfasst, wurden seit den frühen 1980'er Jahren in der Ostsee nachgewiesen. Da sich die morphologische Bestimmung der drei Geschwisterarten aufgrund der großen Ähnlichkeit der Tiere sehr schwierig gestaltet, ist jedoch nur wenig über den artspezifischen Partikel- und Flüssigkeitstransport sowie die daraus folgenden zahlreichen und komplexen Auswirkungen auf Sedimente bekannt. Die eng miteinander verwandten Arten verfügen über ein ähnliches Fraß- und Grabverhalten, dennoch verweisen Größe und Eingrabtiefe der Organismen auf substantielle Unterschiede hinsichtlich ihrer Bioturbation.

Um diese potentiellen Unterschiede der Schwesterarten in Bezug auf Bioturbations- und Bioirrigationsmuster zu untersuchen, wurde ein Tracer-Experiment mit künstlich hergestellten Partikeln (Luminophoren) und einem Flüssigkeitstracer (Bromid) durchgeführt. Ferner wurden der Einfluss der Polychaeten auf benthische Nährstoffflüsse (NH_4^+, NO_3^- PO_4^{3-}) und die Aufnahme von Sauerstoff in einem zweiten Laborexperiment ermittelt. Die Bestimmung der verwendeten Tiere erfolgte mit Hilfe eines molekulargenetischen Schlüssels auf Artniveau.

Die modellierten Ergebnisse zeigen, dass alle drei Arten ausgesprochen geringe Partikeltransportraten mit Biodiffusionskoeffizienten (*Db*) im Bereich von 1.76 $cm^2\ yr^{-1}$ (*M. viridis*) und 0.07 $cm^2\ yr^{-1}$ (*M. neglecta*) bei einer Abundanz von 1273 Individuen m^{-2} und 0.44 $cm^2\ yr^{-1}$ (*M. arctia*) bei doppelter Abundanz, aufweisen. In allen Fällen sind die ‚non-local' Transportkoeffizienten vernachlässigbar klein.

Bezüglich des untersuchten Flüssigkeitstransports sind sich *M. neglecta* und *M. viridis* untereinander sehr ähnlich und unterscheiden sich deutlich von *M. arctia*, deren Flüssigkeitstransportmodus durch einen ausgeprägten diffusiven Charakter geprägt wird (10.9-fach erhöhte Diffusion mit einem ‚non-local' Irrigationskoeffizienten (α) von 55.3 yr^{-1}). Im Gegensatz dazu beeinflussen *M. neglecta* und *M. viridis* die Tracerverteilung im Sediment hauptsächlich durch einen ‚non-local', advektiven Transportmodus (α), der im Bereich von 108.9 yr^{-1} (*M. viridis*) und 130.9 yr^{-1} (*M. neglecta*) liegt. Diese grundlegenden Bioirrigationsmuster wurden auch durch die Ergebnisse eines zweiten Experiments, in welchem die Art und Weise des Flüssigkeitstransports in Analogie zum ersten Experiment untersucht wurde, bestätigt. Aufgrund dieser Resultate ist eine einheitliche funktionelle Gruppierung der drei Schwesterarten in Bezug auf ihre Bioirrigationsleistung nicht zu empfehlen.

Während die Freisetzung von Ammonium und Phosphat, ebenso wie die Sauerstoffaufnahme des Sediments durch die Anwesenheit aller untersuchter Polychaeten

stimuliert wurde, konnte nachgewiesen werden, dass die durch *M. viridis* und *M. neglecta* beeinflussten Flüsse im Vergleich zu *M. arctia* deutlich erhöht wurden. Die Anwesenheit von *M. arctia* hatte lediglich einen geringen stimulierenden Effekt auf die Nitrifikation, wohingegen *M. neglecta* und *M. viridis* die Nitrifikation erhöhten. Dies lässt im Falle von *M. arctia* eine vernachlässigbare Stimulation der Denitrifikation vermuten. Die Ursache für eine Ammonium-Quelle im Falle von *M. neglecta* und *M. viridis* bleibt jedoch ungelöst.

Infolgedessen weisen *M. neglecta* und *M. viridis* einen deutlich größeren Einfluss auf benthische Flüsse, die auch in enger Beziehung zur Eutrophierung stehen, auf als *M. arctia*. Darüber hinaus kann angenommen werden, dass die potentielle Ausbreitung von *M. arctia* in südlichere Regionen der Ostsee weniger dramatische Auswirkungen besitzt, als beispielsweise eine vergleichbare Ausbreitung von *M. viridis*.

1. Introduction

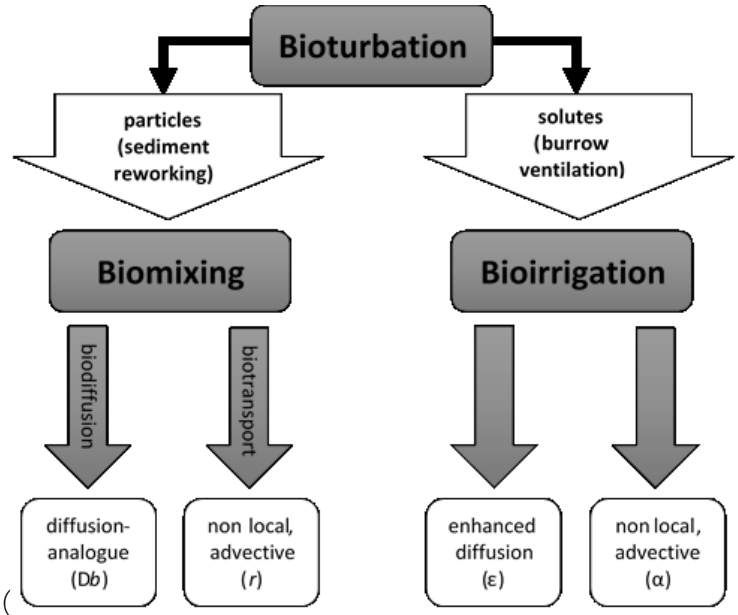
The concept of bioturbation defines all animal induced transport processes that directly or indirectly affect the sediment in aquatic environments and comprises the transport of particles (biomixing) (

Figure 1) as well as the transport of solutes (bioirrigation) (Kristensen et al. 2012). In particular, marine and estuarine environments are strongly affected by benthic infauna (Boudreau 1998; Duport et al. 2006), causing spatial and temporal heterogeneity of fluxes across the sediment-water interface as well as of reactions within the sediment. The translocation of particles due to sediment reworking e.g., burrowing or construction activities, feeding and defecation, as well as the pore water irrigation as a consequence of burrow ventilation, alters the biogeochemical sediment properties and plays an essential role in ecosystem functioning (Kristensen 1988). Beside the abundance and the burrowing depth (Aller 1982), the morphology of burrows (i.e., open- or blind-ended), the type of sediment, and the ventilation rate are constitutive characteristics, determining the effects of bioirrigation on pore water transport (Kristensen et al. 2012).

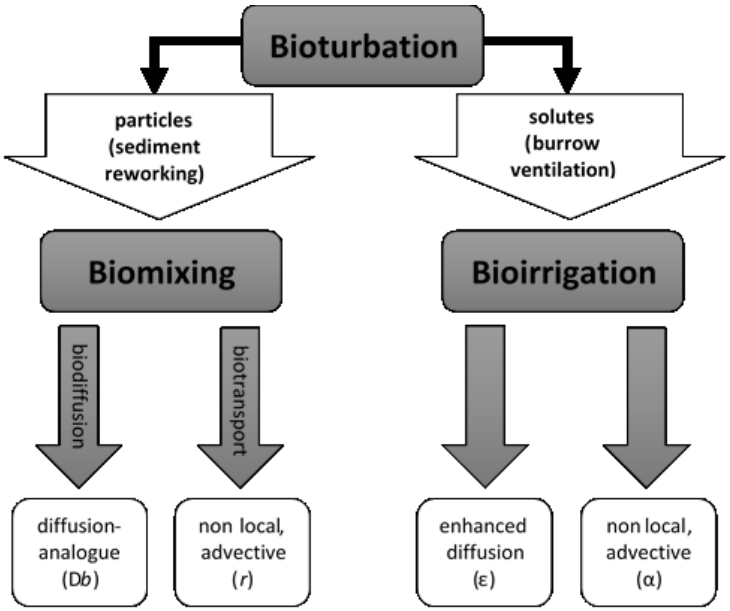


Figure 1: The concept of bioturbation inspired by Kristensen et al. (2012).

Quantification of bioturbation processes is useful for comparison of varying effects on ecosystems caused by the particle and solute transport of benthic organisms (François et al. 2002). These relate to for example, benthic pelagic coupling (Graf 1992), altered oxygen supply and microbial degradation rates in the sediment and contaminant transport (Granberg et al. 2008; Josefsson et al. 2011; Timmermann et al. 2011), especially in the Baltic where indigenous soft bottom fauna does not burrow very deep.

Because bioturbation includes many complex processes, particle and solute transport are often investigated separately (Martin and Banta 1992) even though the transport mechanisms influence each other (Berg et al. 2001; Kristensen et al. 2012). Sediment reworking is often examined by determining the vertical distribution of inert particle tracers (e.g., luminophores) (Mahaut and Graf 1987; Maire et al. 2008) and reactive tracers (Gérino et al. 1998) within the sediment. Subsequently the sediment reworking is studied in terms of a diffusion analogue (*Db*) and a non-local, advective mode (*r*) of particle translocation. Inert solute tracers such as bromide are used to investigate solute transport across the sediment-water interface due to ventilation and also in association with particle translocation. These transport mechanisms are specified either as a factor of enhanced diffusion (ε), a non-local, advective exchange coefficient (α), or a combination of both. To quantify particle or solute transport, mathematic models are fitted to the tracer profiles (Boudreau 1987; Wheatcroft et al. 1990; François et al. 1997).

The bioturbation activities of benthic macrofauna, dependent on biomass, density, and species composition, have profound influence on sediment biogeochemistry (Welsh 2003).

Beside abundance and burrowing depth (Aller 1982), the morphology of burrows (i.e. open- or blind-ended), and the ventilation rate are constitutive characteristics, determining the effects of bioirrigation on pore water transport (Kristensen et al. 2012). Bioturbating macrofauna determines the input and vertical depth distribution of organic material in the sediment and its mineralization (Shull 2009). In the absence of bioturbation, the mineralization of organic material mainly occurs near the sediment surface and associated the steep concentration gradients of metabolites between pore water and overlying water is the driving force for upward diffusive transport to the water column (Blackburn and Blackburn 1993). While the construction of burrows and feeding activities increase the sediment-water interface and, therefore, the effective area for any solute exchange and for biogeochemical reactions (Forster and Graf 1992), pore water irrigation as a consequence of burrow ventilation leads to enhanced water exchange between overlying water and pore water (Graf and Rosenberg 1997). Thus, bioturbation not only affects the input of organic matter, but also stimulates microbial activities by providing additional electron acceptors (e.g., O_2, NO_3^- and SO_4^{2-}) for benthic mineralization (Andersen and Kristensen 1988) and by removing toxic metabolites (e.g., NH_4^+ and S^{2-}) (Quintana et al. 2013).

Nutrient recycling in shallow coastal sediments is assumed to provide between 20 and 100% of the annual nitrogen demand of the phytoplankton production (Kristensen 1988), therefore, the primary production in the overlying water is controlled by the rates of nutrient regeneration (Welsh 2003). The balance between different nitrogen species is influenced by the presence of benthic macrofauna (Blackburn and Blackburn 1993). Due to the combined effects of bioturbation, microbial ammonification is stimulated, leading to an increased ammonium level in oxic sediments (Karlson et al. 2007). Excretion by benthic organisms contributes considerably to the enhanced ammonium pool (Blackburn and Henriksen 1983; Kristensen 1988). The produced ammonium will either be transported to the water column by the ventilation current or be nitrified by ammonium- and nitrite-oxidizing bacteria. Nitrification is restricted to oxic surface sediments and the nitrate produced will either be transported to the water column or be transported to anoxic sediment layers and converted into dinitrogen (N_2) by denitrification. Sedimentary nitrification and denitrification, as well as the coupling of both processes, are stimulated by benthic macrofauna (Kristensen et al. 1991; Henriksen et al. 1993; Pelegri and Blackburn 1994; Karlson et al. 2007). However, due to an intensive irrigation and, therefore, oxygenation of the sediment, the denitrification rates may decline (Gilbert et al. 2003). Besides denitrification, nitrate can be used as substrate for anaerobic ammonium oxidation (anammox), or can be reduced to ammonium by a dissimilatory nitrate reduction (DNRA). The role of the sediment acting as sink or net source

for recycled nitrogen is thereby affected by the availability of oxygen and organic material as well as by the reactivity of the organic matter (Dale et al. 2011).

Release of dissolved phosphate to the pore water through microbial mineralization and macrofaunal excretion may be transported to the water column or adsorbed to sediment particles, depending on the prevailing redox conditions (Anschutz et al. 1998). Adsorption to Fe-oxides retains substantial amounts of phosphate in oxidized sediment layers (Sundby et al. 1993). Therefore, the retention and release of phosphate can be directly linked to the bioturbation activity of benthic macrofauna. Sediment oxidation by ventilation and irrigation of burrows favors the adsorption of phosphate and limits its diffusion to the water column (Davenport et al. 2012). Irrigation of macrofaunal burrows may instead result in non-local transport of dissolved phosphate from reduced sediment layers to the water column (Kristensen 1988; Aller 1994).

The spionid polychaetes of the genus *Marenzelleria* consist of five species (Sikorski and Bick 2004) with the three sibling species *M. neglecta*, *M. viridis*, and *M. arctia* present in the Baltic Sea since the 1980s (Bick and Burckhardt 1989; Bastrop 1997; Blank et al. 2008). Sibling species are two or more related species that are morphologically difficult or impossible to distinguish (Mayr and Ashlock 1991). Therefore, their identification is primarily based on molecular genetic and biochemical factors but a precise characterization of the biology and ecology of the sibling species is essential to assess their environmental effects (Knowlton 1993). Considerable numbers of investigations with *Marenzelleria* spp. have been done so far but Blank et al. (2008) found incorrect species nominations in astonishingly 58% of all considered papers as a result of the difficulties in species discrimination. Due to the similarity in morphological characteristics, the sibling species are thought to have only minor, if any, functional differences (Hietanen et al. 2007; Norkko et al. 2012). Hence little is known about species dependent sediment reworking and solute transport, although size and burrowing depth of the organisms indicate differences.

Feeding modes of *Marenzelleria* spp. include facultative deposit-feeding and suspension-feeding (Fauchald and Jumars 1979; Dauer et al. 1981; Sikorski and Bick 2004), although for *M. arctia* no information is available. *M. neglecta* (Zettler et al. 1994) and *M. viridis* (Essink and Kleef 1988; Quintana et al. 2011) are known to construct L-, J-, or I-shaped, unbranched mucus-lined burrows to a sediment depth of 25 to 35 cm with a mean diameter of 2 mm. In contrast, *M. arctia* creates J-, Y-, or U-shaped burrows with a thin transparent membrane lining to maximum depths of 6 - 8 cm (Hietanen et al. 2007) (and own observation).

Knowledge on the distribution of the three sibling species in the Baltic assumes that *M. neglecta* is established over the whole Baltic Sea, whereas *M. viridis* and *M. arctia* are still spreading (Blank et al. 2008). *M. viridis* and *M. neglecta* originate from North America (Leppäkoski and Olenin 2000) in contrast to *M. arctia*, which was restricted originally to the European Arctic (Jørgensen et al. 1999). Range expansion from the North Sea in case of *M. viridis* and displacement via ship ballast water for all of the three sibling species are the most likely reasons for facilitated invasion to the Baltic Sea (Blank et al. 2008). *M. viridis* and *M. neglecta* inhabit estuarine sandy and muddy sediments in abundances of several thousand individuals (ind.) m^{-2} (Kube et al. 1996; Delefosse 2012) and can occur sympatrically in the eastern Baltic Sea. *M. arctia* mainly occurs in various sediments in deeper, cooler habitats in the northern Baltic region, at reported abundances of 4000 ind. m^{-2} (Hietanen et al. 2007) and can be found there sympatrically with the other two species (Blank et al. 2008).

The influence of *Marenzelleria* spp. on sediment nutrient dynamics is poorly understood, and, though a few studies have investigated the effects of *M. viridis* (Karlson et al. 2005; Kristensen et al. 2011; Quintana et al. 2013) and *M. arctia* (Hietanen et al. 2007; Viitasalo-Frösen et al. 2009), no information on the biogeochemical influence of *M. neglecta* is available. The missing taxonomic identification of species in several recent studies (Bonaglia et al. 2013; Urban-Malinga et al. 2013) renders the interpretation of some of their results difficult. Recent studies have shown that the mineralization of organic material is increased and sulfate reduction is stimulated by the presence of *M. viridis* (Kristensen et al. 2011; Quintana et al. 2013). On the other hand, the influence of *M. arctia* and *M. viridis* on denitrification is minor despite increased ammonium fluxes from the sediment (Hietanen et al. 2007; Kristensen et al. 2011). The high fluxes of nitrate into the sediment apparently stimulate dissimilatory reduction of nitrate to ammonium (DNRA) more than denitrification (Karlson et al. 2005). The efflux of phosphate from the sediment is increased by the presence of *M. arctia* (Hietanen et al. 2007; Viitasalo-Frösen et al. 2009), but Hietanen et al. (2007) speculated that the oxygenation of the sediment surface may lead to improved long-term retention of P and, therefore, enhance the amount of Fe-bound P in sediments (Norkko et al. 2012).

The concept of grouping benthic animals by ecological equivalency into functional groups, sharing common biogeochemical and interspecific attributes (Gérino et al. 2003), has been used to link bioturbation aspects with ecosystem functions in a conceptual framework (Mermillod-Blondin and Rosenberg 2006). Ecosystem engineering comprises direct or indirect modifications by organisms that modulate the physical environment (Jones et al. 1994) and, therefore, cause qualitative and quantitative changes in the distribution and availability of resources for other species (Kristensen et al. 2012). Especially invasive species have been

identified to cause extensive changes in the function of ecosystems (Leppäkoski and Olenin 2000). In association with the spreading of the three sibling species of *Marenzelleria* spp. in the Baltic Sea, complex effects on e.g., nutrient dynamics (Hietanen et al. 2007; Norkko et al. 2012; Quintana et al. 2013) and eutrophication (Karlson et al. 2007; Viitasalo-Frösen et al. 2009) have to be discussed. In the Baltic Sea, where deep burrowing species are naturally absent, the burrowing activity of *Marenzelleria* spp. also contributes to a significant remobilization of buried contaminants (Granberg et al. 2008; Josefsson et al. 2011).

Aim of the present study was to assess the effects of the introduction of the three species of *Marenzelleria* spp. into the Baltic Sea and to examine the potential differences among closely related species. Due to the similarity in morphological characteristics, these species are thought to have only minor, if any, functional differences. Therefore, the basic bioturbation characteristics e.g., the species specific transport mechanisms of particles and solutes of the three sibling species *M. viridis*, *M. neglecta,* and *M. arctia* (the denomination in the present study follows Sikorski and Bick (2004) and Bick (2005)) were investigated in a first microcosm tracer experiment (Biex). This study raises the question if differences in transport rates and mode are related to burrowing depth and if the three species are indeed alike. The hypothesis is that, based on the few observations available, *M. arctia* will differ from the other two species in its transport characteristics. The present investigation also inquires whether similar types of organisms can be viewed as one functional group with respect to their function, in this case bioturbation (Pearson 2001; Gérino et al. 2003). Here the pronounced morphological similarity of the worms may suggest *one* functional group. However, since biogenic solute transport in soft sediments depends predominantly on burrow geometry the concept of a functional grouping in relation to solute transport may fail in this case. Furthermore the effects of the three closely related polychaetes on solute fluxes (bromide), nutrient fluxes (NH_4^+, NO_3^-, PO_4^{3-}) and oxygen uptake were examined to emphasize potential differences among them. This second experiment (Näex) raises the question if differences in nutrient release and total oxygen uptake are driven by differences in size and burrowing depth of the species. We predicted that the morphologically similar organisms would differ in their direct (solute transport) and indirect functions (biogeochemical effects) and, that the three sibling species therefore, cannot be categorized as a single functional group.

2. Methods

2.1 Experimental setup

2.1.1. Sediment and polychaete collection

Sediment was collected in July 2008 in a shallow brackish water bay (Schnatermann), located at the Warnow River near Rostock, Germany. The bay is characterized by well sorted fine-sand mixed with shell debris and a high organic content. Water temperature varies seasonally between 0°C and 20°C and salinity alternates between 3 and 13. Sediment was sieved through 1000 and 500 μm mesh to remove macrofauna and larger particles (e.g., shells) and homogenized before use to obtain equal starting conditions. Organic matter content, determined as loss on ignition at 500°C for 12 h, and grain size of the used sediment were determined before establishing the sediment cores (Bale and Kenny 2005). Polychaetes were collected in September 2008 at three different sampling sites in the Baltic Sea (

Figure 2) and were kept in aquaria filled with 3 cm sediment for approximately two weeks under constant conditions (14°C, salinity 10) for acclimation. Further information characterizing the different sampling sites is specified in Table 1.

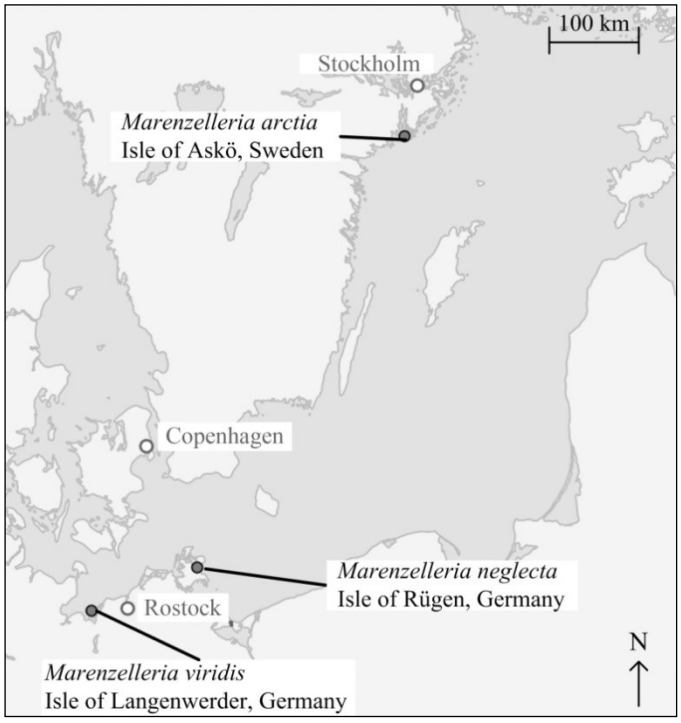


Figure 2: Samplings sites of the three sibling species of *Marenzelleria* spp. in the Baltic Sea.

Table 1: *Marenzelleria* spp. sampling sites and abiotic parameters.

	Species	Position	Water temperature (°C)	Depth (m)	Salinity	Sediment type
Askö (Sweden)	*M. arctia*	58° 50' N 17° 32' E	15.3*	30	6.1	mud
Langenwerder, Poel (Germany)	*M. viridis*	54° 03' N 11° 49' E	17.0	1	14.0	sand
Banzelwitz Rügen (Germany)	*M. neglecta*	54° 52' N 13° 41'E	15.6	1.2	8.3	sand

*surface temperature

2.1.2 Preparation of sediment cores

Transparent 55 cm long polyvinyl chloride (PVC) cores with an internal diameter of 10 cm were filled with 40 cm homogenized sediment overlaid by 1 L seawater (salinity 10). Cores were sealed with plastic wrap and pre-incubated for five weeks with air-saturated water in a dark, temperature controlled (14°C) container to restore near steady conditions after sediment homogenization. Aeration of the water column in each core provided convection and thus a complete mixing of the overlying water, while care was taken not to resuspend the sediment. Hansen and Kristensen (1998) suggested a stabilization period of 15 days for solute fluxes across the sediment-water interface and a period of 30 days for pore water profiles after sediment homogenization for comparable sediments. Until the beginning of the bromide experiment, the overlying water was replaced once per week and height of overlying water and salinity were controlled daily and corrected appropriately all the way through the experiment.

2.1.3 Particle and solute transport

The experimental set-up of the bioturbation experiment (Biex; B) consisted of 4 replicate cores within four treatments, including the azoic controls (BC) and cores colonized with *M. arctia* (BMA), *M. neglecta* (BMN), and *M. viridis* (BMV). Given the much smaller size of *M. arctia*, the number of individuals per core differed within the treatments (BMA n = 20 ind. per core, equating to 2546 ind. m^{-2}; BMV and BMN n = 10 ind. per core equating to 1273 ind. m^{-2}). Total biomass, measured as fresh weight of polychaetes per core (BMA 0.43 ± 0.04 g, BMN 1.86 ± 0.11 g and BMV 2.16 ± 0.02 g) was determined before introducing the animals

to the sediment cores on day 0 (d_0) of the experiment. After animals were added to the cores, they burrowed in immediately except for one individual of *M. arctia*, which was replaced after 24 hours. Sediment cores were kept under constant conditions (see above) for another 40 days after introduction of polychaetes (Figure 3).

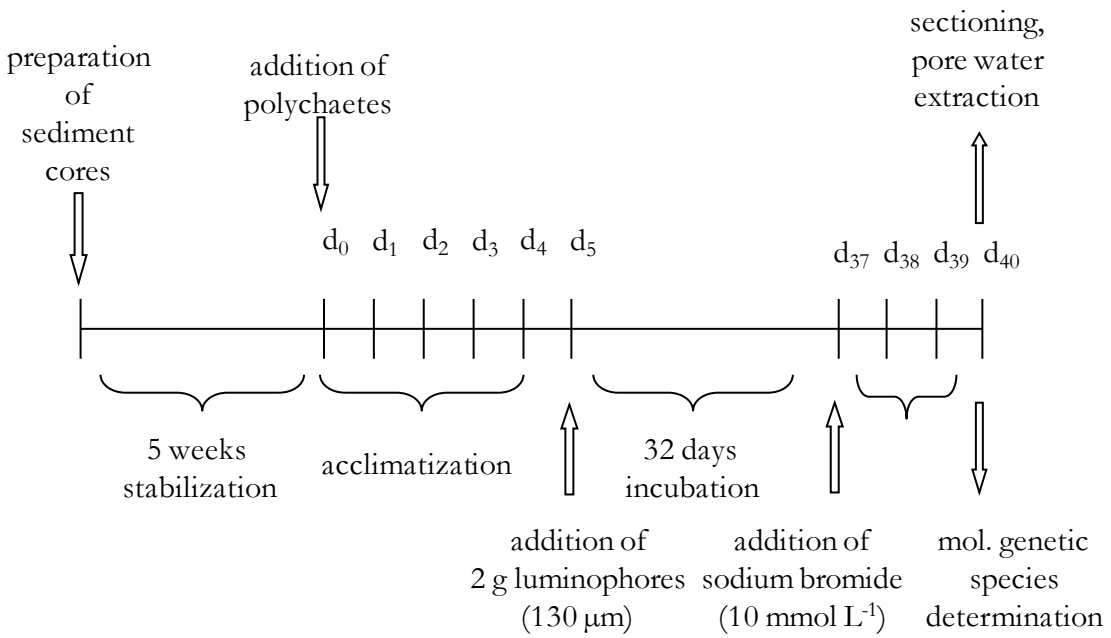


Figure 3: Timeline of the bioturbation experiment (Biex). Total duration of the investigation was 40 days (d), bromide was added on d_{37} of the experiment.

Sediment particle mixing was quantified by luminophores, which are natural sediment particles, covered with fluorescent dye (Mahaut and Graf 1987; Gérino et al. 1998). On d_5, a suspension of 2 g luminophores (mean diameter 130 μm) and approximately 5 mL seawater was added with a syringe to the sediment surface of each core and cores were incubated for 32 days as described above.

The influence of pore water irrigation was quantified after the addition of sodium bromide in a final concentration of 10 mmol L^{-1} to the overlying water on d_{37} (Martin and Banta 1992; Forster et al. 1999). After an initial incubation time of 15 minutes, 2 mL water samples were taken after 0.25, 2, 5, 9, 23, 32, 49, 57, and 72.5 hours. The samples were stores cold (5°C) and dark until analysis. Additionally, height of the water column for each sampling was noted.

Before sectioning the cores at the end of the experiment on d_{40}, overlying water was removed carefully to avoid resuspension. Sediment cores were sliced into 0.5 cm layers to the depth of 2 cm, followed by 1 cm layers to the depth of 10 cm (BC and BMA) and 30 cm

(BMN and BMV), respectively. Additional layers were taken at 16 and 20 cm (BC), at 12, 14, 16, 18 and 20 cm (BMA) and at 35 cm (BMN and BMV). The residence depth of polychaetes was noted during the sectioning and collected worms were preserved in 80% ethanol for identification according to Blank and Bastrop (2009). Sediment layers were weighed and pore water was extracted by centrifuging for 1 minute at 500 rpm in cups with GF/F filters as described by Saager et al. (1990). The remaining sediment was freeze dried, weighed, homogenized and stored dark until analysis. For luminophore profiles, three sub samples (0.25 g) of each sample were removed and photographed under ultraviolet (UV) light. An image analysis was conducted with IMAQ^{TM} Vision builder (Figure 4) and fluorescent pixels were counted as described in Rau (2007).

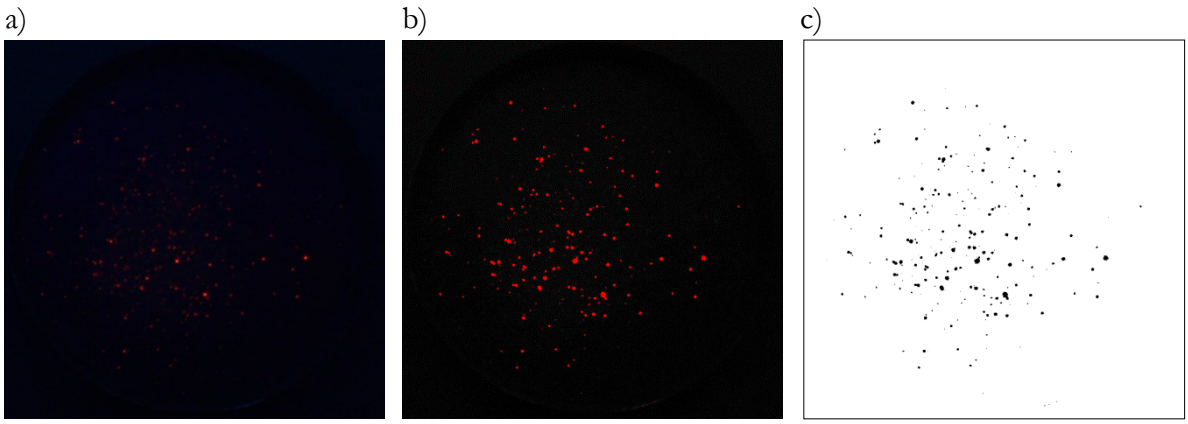


Figure 4: Fluorescent luminophores under UV-light; a) raw data; b) processed image; c) mask of analyzed particles

Of the pore water retrieved, ~ 2 mL were collected and stored dark and cold (5°C). Bromide was analyzed by anion exchange chromatography (Sykam, IBJ A06 column, Merck-Hitachi L-4200 UV-VIS detector) with a 25 mmol L^{-1} NaCl eluent and a constant flow rate of 1 mL min^{-1}. Analytical precision was ± 0.1 mmol L^{-1} and the detection limit was 0.4 mmol L^{-1} Br^-.

The porosity Φ was calculated for each depth interval from the weight difference before and after drying the sediment samples.

2.1.4 Nutrient fluxes

The experimental set-up of the nutrient experiment (Näex; N) consisted of four treatments with 3 replicate cores each, comprising azoic controls (NC) and cores colonized with *M. arctia* (NMA), *M. neglecta* (NMN) and *M. viridis* (NMV), taking into account the natural densities of the polychaetes. Individuals were of typical size for adults of the respective species

from the Baltic Sea (Sikorski and Bick 2004). Given the small size of *M. arctia*, the number of individuals per core differed among the treatments (NMA: n = 20 ind. per core, equating to 2546 ind. m^{-2}; NMV and NMN: n = 10 ind. per core equating to 1273 ind. m^{-2}). Total fresh weight of polychaetes per core (NMA: 0.5 ± 0.1 g, NMN: 1.0 ± 0.1 g and NMV: 1.7 ± 0.2 g) was determined before introducing the animals to the randomly chosen sediment cores on d_1 (Figure 5). All animals buried immediately into the sediment of the microcosms.

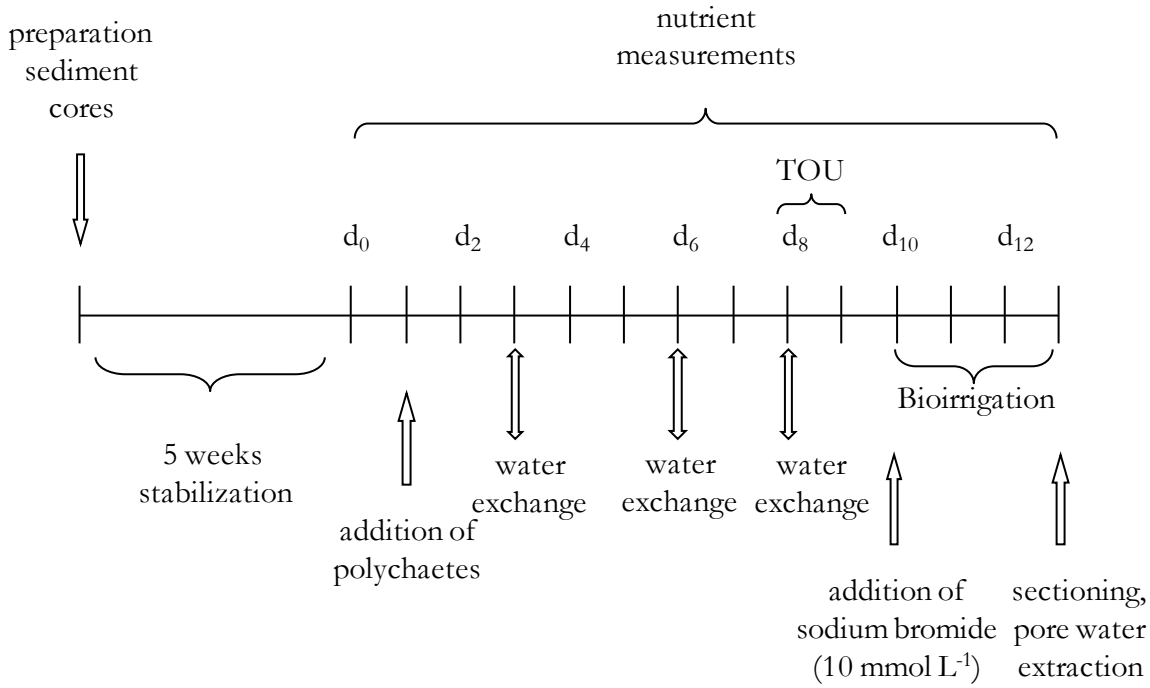


Figure 5: Timeline of the laboratory experiment investigating effects of *Marenzelleria* spp. on benthic nutrient fluxes and total oxygen uptake.

Samples of 10 mL water for N and P nutrient analysis were taken from the overlying water of each core before animals were introduced on d_0, to control variability among cores. Similar samples were taken daily from d_2 to d_{13} to determine nutrient fluxes from concentration differences in the overlying water against time. The overlying water was replaced three times (d_3, d_6 and d_8) with sea water stored in a reservoir, to avoid exceedingly high nutrient levels, resulting in three sequential incubations. The nutrient concentrations of the reservoir (NH_4^+ 3.4 ± 0.9 µmol L^{-1}, NO_2^- 1.8 ± 0.6 µmol L^{-1}, NO_3^- 20.0 ± 1.3 µmol L^{-1}, PO_4^{3-} 0.17 ± 0.18 µmol L^{-1}) were monitored daily during the experiment to assure that no changes occurred.

Pore water irrigation was quantified from vertical sediment profiles after the addition of sodium bromide to a final concentration of 10 mmol L^{-1} to the overlying water on d_{10} of the experiment (Martin and Banta 1992; Forster et al. 1999). After an initial incubation time of

15 minutes, 2 mL water samples were taken sequentially after 0.25, 2, 5, 9, 24, 32, 50, 69 and 70.5 hours. The samples were stored cold (5°C) in the dark until analysis.

The microcosms were sectioned at the end of the experiment on d_{13}. The overlying water was first removed and, 1 cm slices were taken from the sediment cores at variable depths depending on treatment (NC: 1-10, 15 and 20 cm; NMA 1-10, 12, 14, 16, 18, 20 cm; NMV and NMN: 1-20, 22, 24, 26, 28, 30, 35 cm). The remaining layers were discarded. Residence depth of polychaetes was noted during sectioning, and collected worms were preserved in 80 % ethanol for molecular identification. Sediment layers were weighed fresh and dry (60°C) for porosity determination. Pore water was extracted from a subsample of each slice by centrifuging (1 min, 500 rpm) through GF/F filters as described by Saager et al. (1990). Of the pore water retrieved, ~ 5 mL were collected and frozen (-18°C) for nutrient analysis and 2 mL was stored dark and cold (5°C) for bromide measurements.

Bromide was analyzed by anion exchange chromatography as described above for the bioturbation experiment. Ammonium and phosphate were analyzed spectrophotometrically immediately after sampling (Parsons et al. 1984). Samples for nitrate and nitrite were preserved (-18°C) and measured using the spongy cadmium technique (Jones 1984). Methods were modified for small sample volumes of 1 mL for all nutrient analysis.

Total nutrient fluxes were calculated for each stage from concentration changes in the overlying water (F_{olw}) using a linear regression against time.

2.1.5 Total oxygen uptake

To assess benthic oxygen uptake, the ventilation of all cores of the nutrient experiment (Näex) was stopped on d_7 and the cores were sealed with tight lids. Magnetic stirrers driven by an external rotating magnet ensured mixing of the water column during the respiration measurements (stirring speed of approximately 60 rotations per minute). Oxygen concentrations were measured with two optodes integrated into the lids (oxygen sensor spots SP-PSt3-PSUP-YOP-D5 PreSens), and the flux rates were calculated from changes in O_2 concentrations over time for all cores. The measurements were stopped when oxygen depletion reached 80 % saturation. The optodes were calibrated in distilled water (Arain et al. 2005), and, O_2 concentrations were analyzed using the Winkler titration technique (Grasshoff et al. 1983).

2.2 Modeling

2.2.1 Gallery-diffusion model

To describe the macrofauna induced luminophore distribution within the sediment, the mechanistic one-dimensional gallery-diffusion model (François et al. 1997; François et al. 2002; Duport et al. 2007), was used. The model is based on the general diagenetic equation (Berner 1980) and applies two different transport coefficients to quantify the sediment reworking:

$$\frac{\delta C}{\delta t} = Db\frac{\delta^2 C}{\delta x^2} + R(C(x,t)) \qquad (1)$$

The biodiffusion coefficient (*Db*) describes the movement of a quantity of tracer *C*(*x,t*) at a depth *x* and a time *t*, in a random, diffusion analogue manner over short distances in the region of intense burrowing activity (Figure 6).

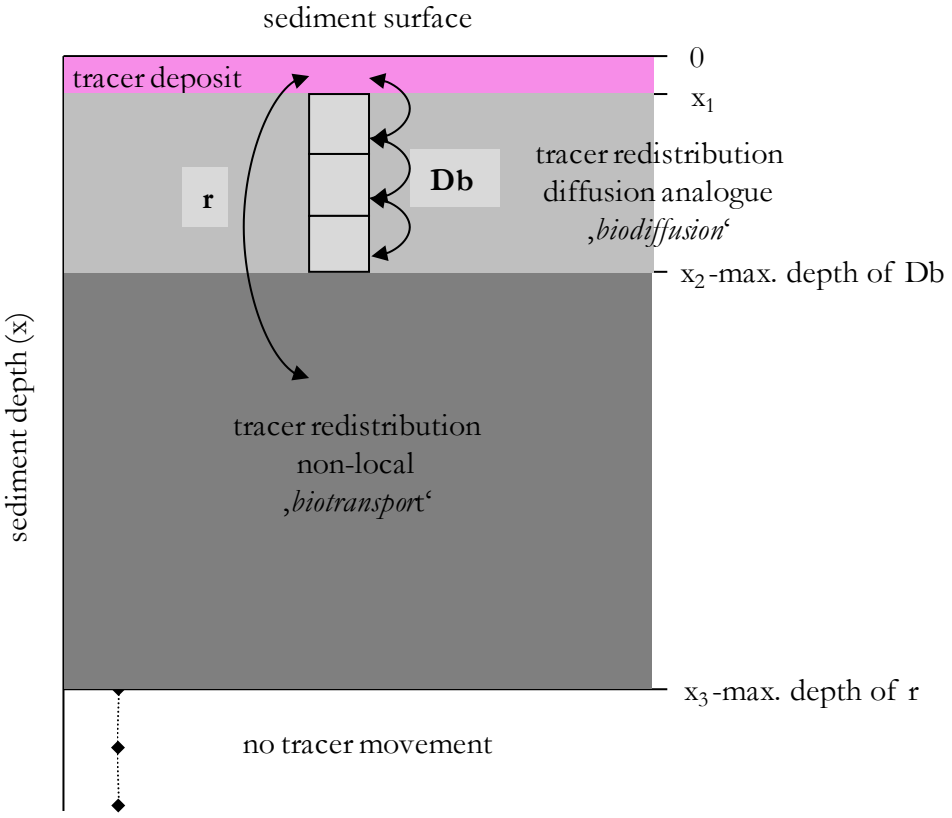


Figure 6: Schematic drawing of the gallery-diffusion-model.

The rapid vertical discontinuous transport of particles $R(C(x,t))$ from upper to lower sediment layers is simulated by applying a non-local transport coefficient (r) and is defined by:

$$R\left(C\left(x,t\right)\right)=\begin{cases}-rC(x,t)\text{ if }x\in\left[0;x_1\right]\\ \dfrac{r}{x_3-x_2}\int_0^{x_1}C\left(x,t\right)dx\text{ if }x\in\left[x_2;x_3\right]\\ 0\text{ if }x>x_3\end{cases} \quad (2)$$

where x_2 and x_3 define the upper and lower border of the tracer redistribution and x is a depth variable. r is defined as the percentage of tracer that left the [0, x_1] deposit and was redistributed in the [x_2; x_3] layer. In eq. (2) the redistribution of tracer between x_2 and x_3, the disappearance of tracer from the $0 - x_1$ layer and the inexistent tracer movement below x_3 are defined. This displacement term was originally exemplified in a model describing gallery-diffusion of macrofaunal reworking (François et al. 2002).
The initial conditions are described as:

$$C(x,0)=\begin{cases}C_0\text{ if}x\in[0;x_1]\\ 0\quad\text{else}\end{cases} \quad (3)$$

At time $t = 0$, all tracer is uniformly spread within the tracer deposit layer [0; x_1]. The zero-flux Neuman boundary condition was considered:

$$\frac{\delta C}{\delta x}(0,t)=\lim_{x\to+\infty}\frac{\delta C}{\delta x}(x,t)=0 \quad (4)$$

Both particle mixing coefficients were obtained by comparing the experimental and simulated luminophore profiles using the least square criterion (François et al. 2002).

2.2.2 Bioirrigation model

Tracer results, which refer to the influence on solute transport of the three polychaetes, were interpreted with a numerical model, described in detail in Andersson et al. (2006) within the flexible environment for mathematically modeling the environment (FEMME) (Soetaert et al. 2002). The irrigation model is based on the distribution of an inert tracer inside a one-dimensional sediment column, covered with a fully mixed water column. The solute transport is specified with either a factor of enhanced diffusion (ε), a non-local exchange coefficient (α), or as a combination of both. The tracer concentration C in the sediment is described by,

$$\phi \frac{\delta C}{\delta t} = \frac{\delta}{\delta x}\left(\varepsilon \cdot D_s \cdot \Phi \frac{\delta C}{\delta x} \right) + \alpha \cdot \Phi (C_{\text{olw}} - C) \qquad (5)$$

where Φ is the porosity, that enters the model as a function of porosity vs. depth, t is the time after tracer addition and D_s is the diffusion coefficient in pore water corrected for tortuosity effects according to Archie's Law with m = 2 (Ullman and Aller 1982), x is the sediment depth, ε is a factor of enhancement over molecular diffusion and α is a non-local exchange coefficient, defined as volume fraction per unit time that is exchanged between pore water and overlying water (C_{olw}). Thereby, the tracer concentration in the water column (C_{olw}) determines the upper boundary of the model. The flux (J) across the sediment-water interface consists of both, diffusion at the sediment surface and the integrated mass exchange by burrow irrigation.

$$J = -\varepsilon \cdot D_s \cdot \Phi \left. \frac{\delta C}{\delta x} \right|_{x=0} + \int_0^{\infty} \alpha \cdot \Phi (C_{\text{olw}} - C) dx \qquad (6)$$

By taking into account the height of water inside the chamber (h), the concentration in the water column is altered by the flux (J).

$$\frac{\delta C_{olw}}{\delta t} = -\frac{J}{h} \qquad (7)$$

ε (and in analogy α) are assumed to be constant down to a bioturbated layer L_b and decrease exponentially with an e-folding depth ($k_{\varepsilon,a}$) below L_b:

$$\varepsilon(x) = \begin{cases} \varepsilon, & \text{if } x \leq L_b \\ \varepsilon \cdot e^{-(x-L_b)/k_{\varepsilon,\alpha}}, & \text{if } x > L_b \end{cases} \quad (8)$$

The parameters used for the irrigation model are described in Table 2. A calibration analysis (Soetaert et al. 2002), using the Levenberg-Marquardt algorithm, was used to fit the model to the observed data and to obtain the solute transport parameters. Each sediment core was modeled separately.

Table 2: Parameters used for the bioirrigation model. Parameters termed 'variable' are assigned a value through the software routine itself.

Parameter	Biex	Näex	Unit	Description
ε	variable	variable		enhanced diffusion coefficient
a	variable	variable	(yr^{-1})	non-local exchange coefficient
L_b	variable	variable	(cm)	depth of bioturbated layer
$k_{\varepsilon,a}$	variable	variable	(cm)	enhancement attenuation
h	0.1274	0.1274	(m)	height of water inside the cores
A	0.00785	0.00785	(m^2)	surface area of cores
Φ_s	0.42	0.51		porosity at surface
Φ_∞	0.36	0.38		porosity at depth
k^Φ	0.36	1.23		porosity attenuation
T	14	14	(°C)	temperature
C_{add}	10	10	(mmol L^{-1})	final tracer concentration in the cores
C_{ini}	279 ± 29	374±111	(μmol L^{-1})	external tracer concentration
m	2	2		tortuosity power

2.2.3 Relating solute transport coefficients

To directly compare the proportion of mass transported by the two solute transport mechanisms (enhanced diffusion (ε) and non-local advective transport (α)), the bio-irrigation-flux (mmol m^{-2} h^{-1}) of each core was calculated from modeled bromide pore water profiles at the end of the experiment after 72.5 hours. The (bio)-diffusion-flux ($F_{biodiff}$), resulting from both molecular and bio-enhanced diffusion, was calculated for the steepest concentration gradient within the first sediment layers via:

$$F_{\text{biodiff}} = \Phi \cdot \varepsilon \cdot D_s \left(\frac{\delta C}{\delta x} \right) \qquad (9)$$

with a mean porosity value Φ of 0.42, the modeled factor of enhanced diffusion ε, the corrected molecular diffusion coefficient of bromide D_s and the bromide concentration difference δC between the sediment surface and a depth (x) of 1.5 cm.
The non-local exchange-flux ($F_{\text{non-local}}$) was calculated for each sediment layer via

$$F_{\text{non–local}} = x \cdot \Phi \cdot \delta C \cdot \alpha \qquad (10)$$

with the thickness x (cm) of the sediment layers, the mean porosity value Φ, the concentration difference δC between bromide concentration in overlying water at the end of the experiment and the concentration C in the respective sediment layer and the modeled non-local transport coefficient α. The calculated fluxes to each sediment layer were summed down to the bioturbated depth (L_b), obtained from modeling. Below this depth L_b only molecular diffusion occurs.

2.3 Burrow structures: Micro-computed tomography

A micro-computed tomography scan (phoenix nanotom® s, micro x-ray) was conducted to visualize burrow structure of *M. arctia*. Five individuals of *M. arctia* were added to a 15 cm long PVC tube with an inner diameter of 36 mm, filled to a high of 6 cm with the same sediment used for the tracer studies. After 5 days of burrow establishing (10°C and salinity 10), the microcosm was scanned and raw data were visualized with the software Imaris. Unfortunately, a micro-computed tomography scan of *M. neglecta* and *M. viridis* burrows was not practicable due to the vertical extension of their burrows.

2.4 Statistical analyses

Differences in bioturbation coefficients between control cores and inhabited sediments were tested using the Kolmogorov-Smirnov test to ensure normal distribution of results. When no normal distribution of data existed the non-parametric tests after Whitney and Mann (*U*-test) or Kruskal and Wallis (*H*-test) were used on raw data. With these tests, all treatments during each incubation period were tested pair wise at a 95% level. A linear regression analysis ($p < 0.01$) was conducted to test the correlation between ammonium fluxes and the bromide-affected sediment depth.

A nonparametric multivariate analysis (cluster and SIMPER) on modeled co-dependent solute transport parameters (PRIMER 6.0) was conducted to show resemblance between the treatments and to test for significant differences (SIMPROF, $p < 0.05$)(Clarke and Warwick 2001; Clarke et al. 2008). Raw data were fourth-root transformed, and for the resemblance Bray-Curtis similarity was used (stress: 0.01). A linear regression analysis ($p < 0.01$) was conducted to test the correlation between ammonium fluxes, biomass of the polychaetes and the bromide-affected sediment depth.

3. Results

3.1 Particle- and solute transport

3.1.1 Visual observation

The general appearance of control cores and those inhabited by the polychaetes was equal in both experiments, in the bioturbation experiment (Biex) as well as in the nutrient experiment (Näex). Therefore, results of both experiments were summarized in this section and data of the nutrient experiment were enclosed in parentheses.

At the end of the incubation, sediment of control cores (Figure 7a) was distinctly layered, with an organic-rich, brown-green fluff layer on top of the sediment surface covered with luminophores and followed by an approximately 0.5 cm thin bright oxidized layer. Deeper down sediment in the control cores was uniform and grey to grayish-black in color. Patches of gas bubbles and a few small burrows of juvenile polychaetes were noted, indicating that even 500 μm mesh sieving was not fully sufficient to remove macrofauna. In populated cores the fluff layer appeared less distinct due to surface deposit feeding and the bulk of luminophores in the bioturbation experiment seemed to disappear from the sediment surface. Burrow openings and characteristic string-like fecal pellets (Figure 7b, c), partially permeated with luminophores on the sediment surface, appearing a few days after introduction of worms, indicated well acclimatized polychaetes in all treatments. One core (BMN18) was excluded from further considerations, because no vital signs of the animals could be observed. In cores BMA5, BMA8, BMV9, BMV11, and BMV12 of the bioturbation experiment small burrows of juvenile *Hediste diversicolor* were noted, but individuals were small and effect on solute and particle transport compared with *Marenzelleria* spp. is assumed to be negligible.

A multiplicity of L- and U-shaped permanent burrows reached a depth of 6 cm in the *M. arctia* treatments (Figure 7d) and indicate the depth affected by bioturbation. Burrows of both *M. neglecta* and *M. viridis* appeared L-shaped (Figure 7e, f) and the oxygenated halo around the burrows was distinct and visibly developed in *M. viridis* and *M. neglecta* treatments. In a few cores, sporadic white *Beggiatoa* spp.-patches (1 cm diameter) could be noted at the sediment surface.

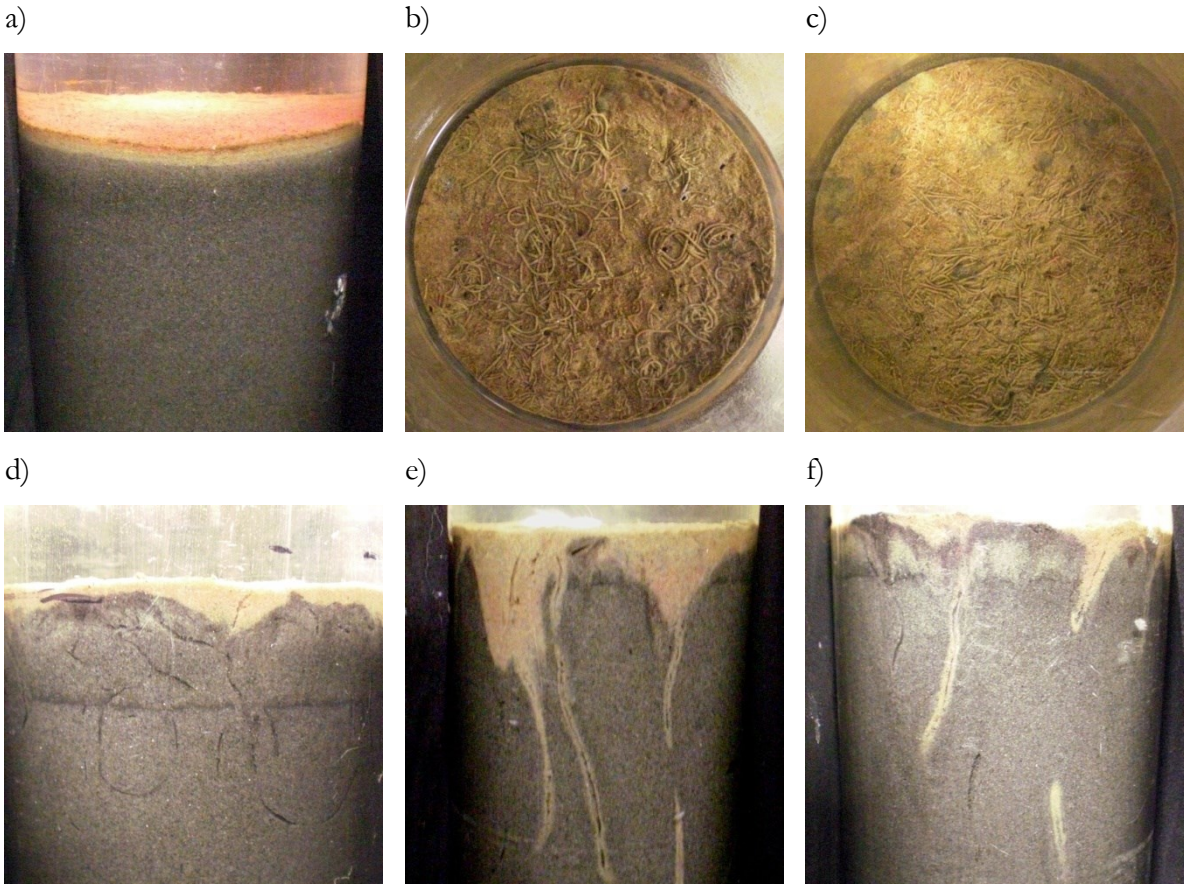


Figure 7: Impressions of sediment cores; a) Control with luminophores, b) fecal pellets of *M. viridis*; c) fecal pellets of *M. neglecta*, d) burrow structure of *M. arctia*, e) burrow structure of *M. viridis*; f) burrow structure of *M. neglecta.*

3.1.2 Recovery and molecular identification

In our experiments recovery of polychaetes differed between the treatments and varied between 26.3 % BMA, 33.3 % BMN and 47.5 % BMV (18.3% NMA, 46.7% NMN and 50% NMV). Overall 36% (33%) of all introduced individuals were found. Recovery of polychaetes during sectioning of sediment cores and subsequent pore water extraction is generally difficult. Mortality of polychaetes can easily be judged when dead or injured individuals are found on the sediment surface. When relying on recovery for mortality it therefore, remains unclear whether animals were truly dead or not found during handling. In some cases worms had been cut into pieces, but only head pieces were counted and used for identification. The molecular species identification (Blank and Bastrop 2009), that was done with recovered polychaetes and reference animals from the sampling sites (data not shown), confirmed the successful grouping of polychaete sibling species in separate experimental treatments as originally intended.

3.1.3 Sediment properties

The experimentally modified sediment of both experiments was classified as fine to very fine sand with a median particle size of 178 μm (sorting 0.31 (Folk and Ward 1957), with a mean organic content of 0.47 ± 0.018 % dry weight (n = 3). Mean sediment porosity near the sediment surface was 0.43 ± 0.07 (0.51 ± 0.08) and decreased with increasing sediment depth to a mean value of 0.37 ± 0.01 (0.38 ± 0.01).

3.1.4 Scan of M. arctia burrow

The burrows of *M. arctia* (

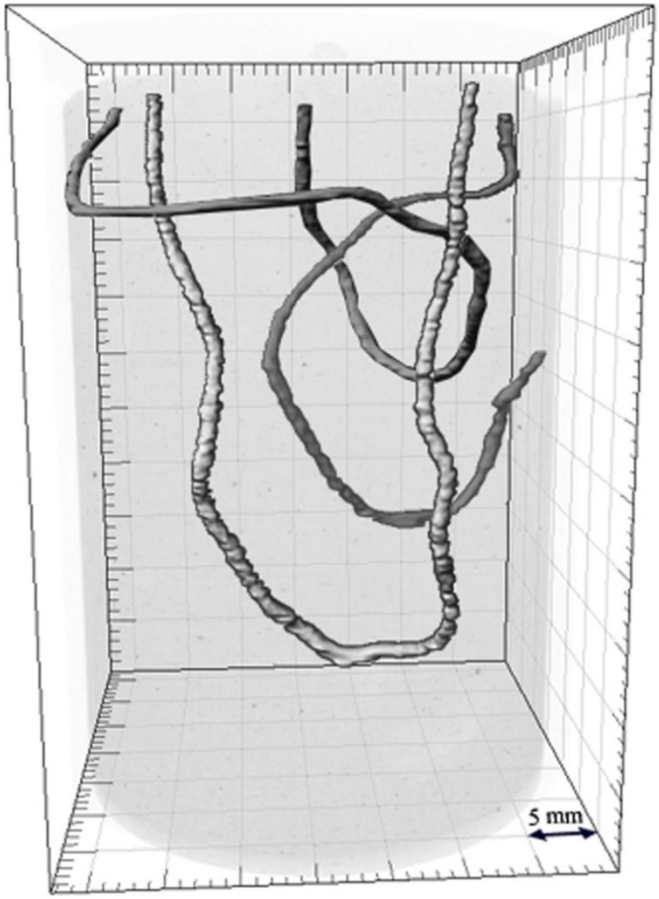


Figure 8: Micro-computed tomography scan of a sediment microcosm colonized with *M. arctia*. The total width is 37 mm.

) reached a length between 7 and 11 cm with an inner diameter of 0.5 mm. They were mainly U-shaped and appeared somehow twisted. These burrows were constructed to depths similar to those observed in the cores during the experiments. We conclude that the burrow shape typical for *M. arctia* in this sediment is a U-shaped, relatively shallow construction.

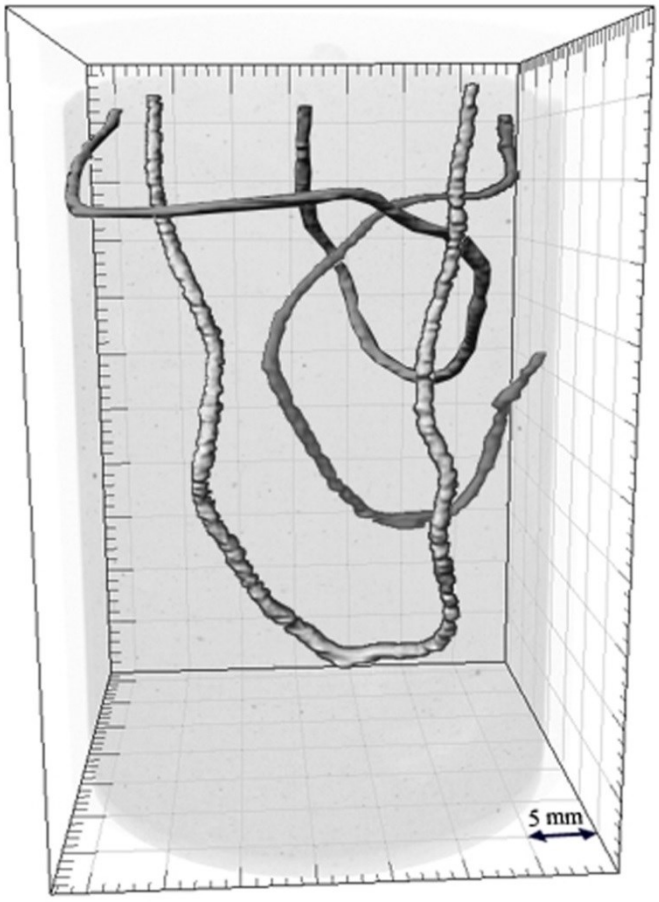


Figure 8: Micro-computed tomography scan of a sediment microcosm colonized with *M. arctia*. The total width is 37 mm.

3.1.5 Particle transport

Slight subsurface peaks in luminophore concentration at a sediment depth of 1.5 to 2.5 cm occurred in all four replicates of the control treatments (Figure 9). An amount of 88.1 ± 1.2 % of the total luminophores added to the sediment surface remained in the 0.5 cm surface layer. The maximum penetration depth of luminophores, defined as depth where 99 % of tracer were recovered (Duport et al. 2006) was 3.2 ± 0.6 cm in control cores.

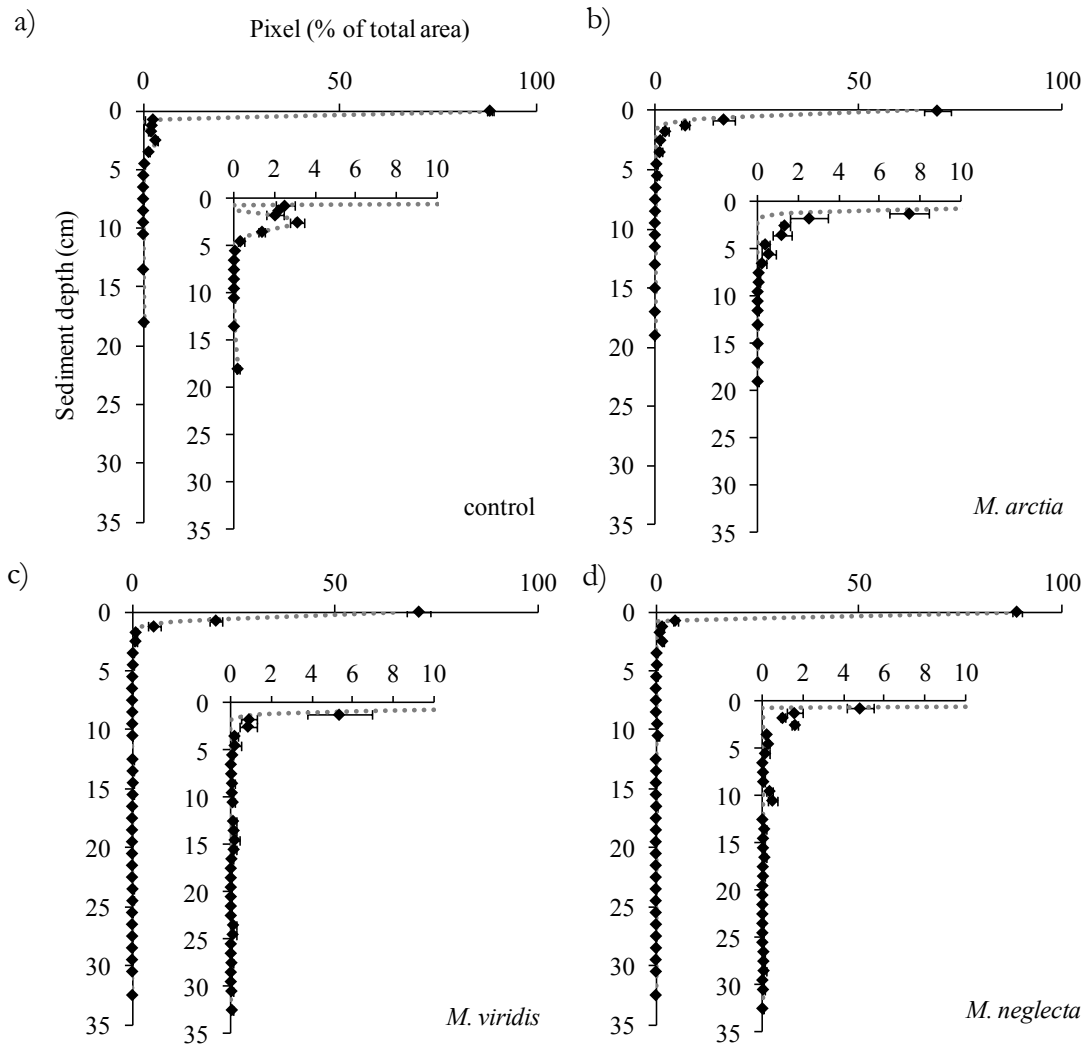


Figure 9: Example of luminophore profiles a) BC3, b) BMA6, c) BMV12 and d) BMN17 after 40 days incubation time. Dotted lines indicate model fit obtained to the concentration depth profiles. Inserts depict low concentration range.

Similar small subsurface peaks within the first 3 cm emerged in almost all polychaete treatments except *M. arctia* cores. Here peaks were possibly masked by sediment reworking activity of the worms. The tracer distribution in *M. arctia* treatments decreased almost

exponentially with increasing sediment depth and profiles showed only a slight vertical particle displacement. An amount of 85.7 ± 11.2 % of total luminophores remained in the 0.5 cm surface layer and the maximum penetration depth of luminophores was 2.6 ± 1.7 cm. In *M. viridis* inhabited sediment, maximum penetration of luminophores (20.0 ± 4.8 cm) was highest among the sibling species. Accordingly only 67.1 ± 4.9 % of total luminophores were recovered in the 0.5 cm surface layer. At a sediment depth of 8-30 cm slight accumulations of luminophores occurred. The luminophore profiles of *M. neglecta* treatments do not considerably differ from controls, because 88.6 ± 4.2 % of luminophores remained in the surface layer and maximum penetration of luminophores (12.2 ± 3.8 cm) was shallow compared to *M. viridis*.

3.1.6 Modeled particle transport

In the azoic control cores no transport of luminophores into deeper sediment layers should occur. By applying the gallery-diffuser model (Figure 9), the best fit of sediment reworking coefficients to the tracer distributions in control experiments (Table 3) was achieved by using both transport mechanisms, the diffusion analogue and the non-local particle mixing.

Table 3: Modeled sediment reworking coefficients (gallery-diffusion model). Significant differences of polychaete treatments compared to control cores (Mann-Whitney-*U*-test) are assigned with an asterisk (*$p < 0.05$). Standard deviation obtained from 4 replicates per treatment.

treatment	*Db* ($cm^2\ yr^{-1}$)	*r* (yr^{-1})
control	0.03 ± 0.00	0.68 ±0.18
M. arctia	0.44 ± 0.80	0.15 ± 0.15*
M. viridis	1.76 ± 0.30*	0.00 ± 0.00*
M. neglecta	0.07 ± 0.05*	0.02 ± 0.04 *

The modeled luminophore distributions of *M. arctia* treatments showed a high variability of biodiffusion coefficients 0.44 ± 0.80 $cm^2\ yr^{-1}$ (*Db*) and a slight non-local transport of 0.15 ± 0.15 yr^{-1}, which was significantly different to control cores. *M. viridis* inhabited cores yielded the highest biodiffusion coefficient (*Db*) 1.76 ± 0.30 $cm^2\ yr^{-1}$ and no non-local transport coefficient despite detectable deep subsurface concentration of luminophores. All sediment reworking coefficients of *M. viridis* and *M. neglecta* were significantly different ($p < 0.05$) from control cores. Particle transport of *M. neglecta* was

minute and the diffusion coefficients differed significantly from *M. viridis*. Sediment reworking coefficients of *M. neglecta* and *M. arctia* did not differ significantly.

3.1.7 Solute transport

The overall solute transport pattern investigated with bromide were comparable in both experiments rendering a collective description of all results possible. Below the results concerning the nutrient experiment (Näex) are parenthesized. Mean natural background concentration of bromide was 0.28 ± 0.03 mmol L^{-1} (0.37 ± 0.11 mmol L^{-1}), indicating a uniform distribution within the cores at the beginning of the experiment. Initial concentration in overlying water consisted of added bromide (C_{add}) and background tracer (C_{ini}) and was approximately 10 ± 0.56 mmol L^{-1} (9.8 ± 0.6 mmol L^{-1}). In the overlying water, the dynamics of bromide concentration declined with time was distinctly different between treatments (

a) b)

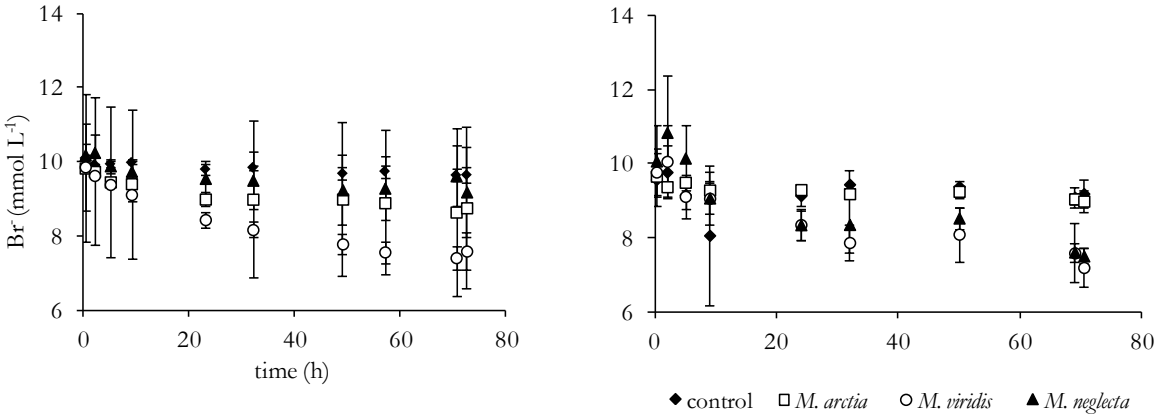


Figure 10). In the nutrient experiment concentrations during the first samples increased, ascribing an insufficient mixing of the added bromide within the overlying water.

a) b)

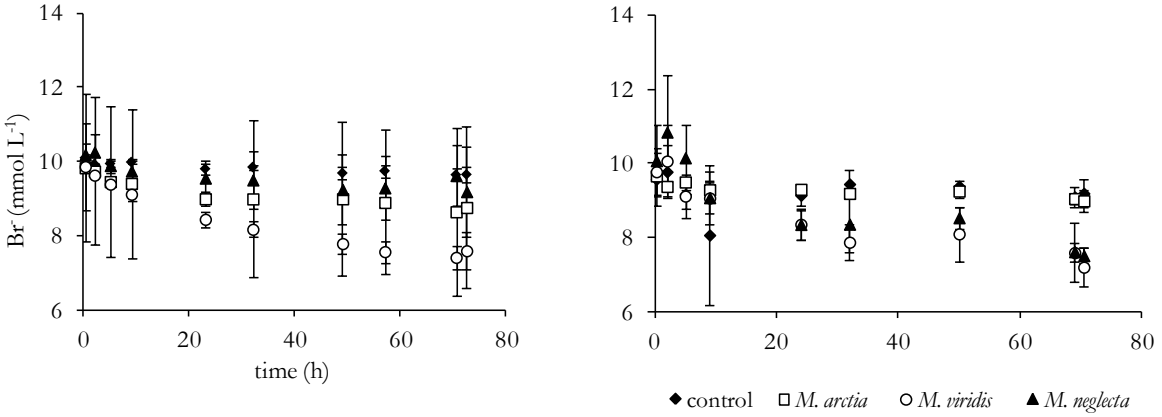


Figure 10: Bromide concentrations with time in the overlying water of a) the bioturbation experiment and b) of the nutrient experiment. Standard deviation obtained from 4 (Biex), and 3 (Näex) replicates per treatment.

The concentration differences calculated from bromide concentrations in overlying water from the beginning to the end of incubation time of *M. viridis* were 6.4-fold (4.7-fold), *M. neglecta* 4.3-fold (4.4-fold), and *M. arctia* 2.4-fold (1.2-fold) higher than in control cores with 30.25 ± 6.60 mmol L^{-1} (38.0 ± 55.7 mmol L^{-1}).

In both experiments pore water tracer profiles (

a) b)

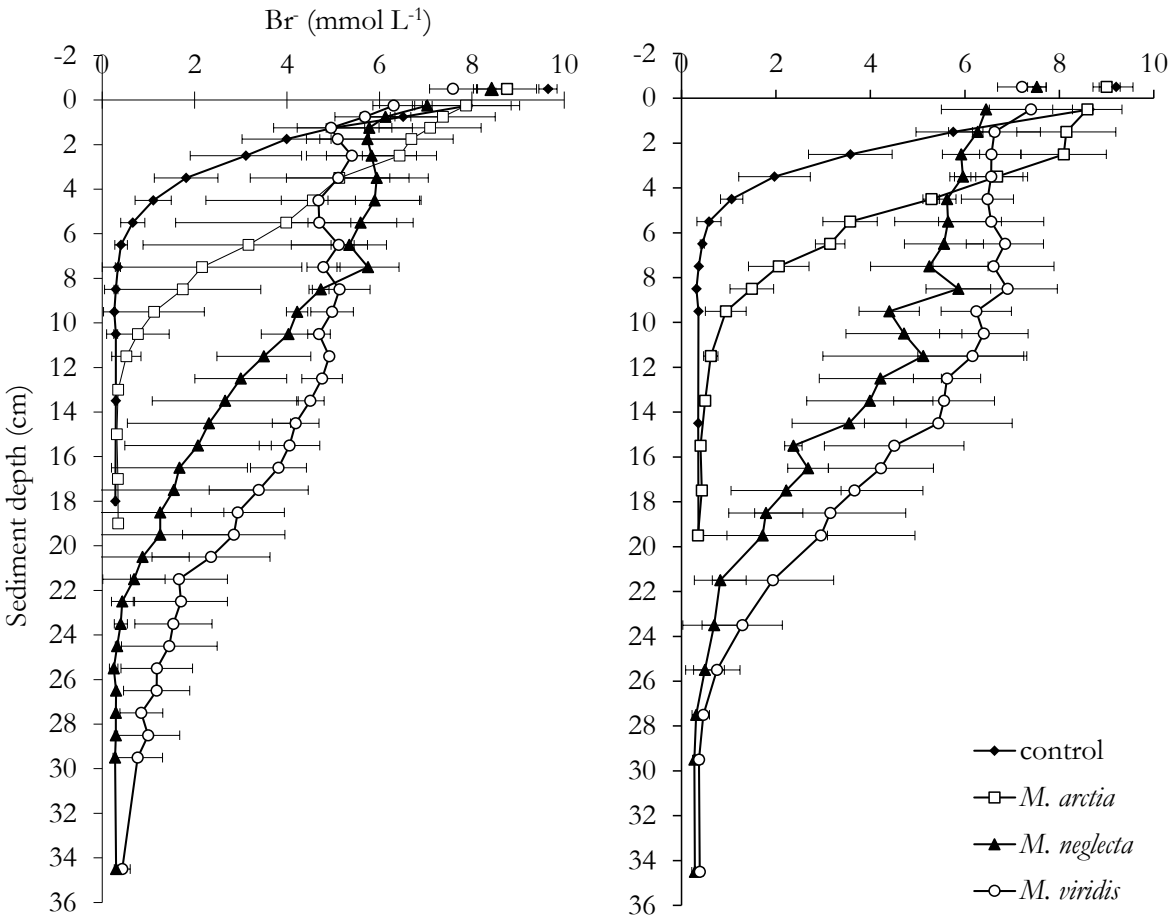


Figure 11) of control cores exhibited a highly reproducible and steep decrease within all replicates, basically driven by the vertical molecular diffusion. Bromide background concentration was reached at a depth of 8.0 ± 1.0 cm (6.8 ± 1.1 cm). In *M. arctia* treatments, the near surface decrease was less steep and concentrations were significantly higher compared to control cores from 3 - 13 cm depth. Background level was reached at a depth of 12.8 ± 1.7 cm (14.1 ± 1.2 cm). In the bioturbation experiment, the burrowing depth of *M. viridis* reached the maximum of 35 cm dictated by the experimental design in two of four replicates, thus the determination of L_b is questionable. Pore water profiles suggest that *M. viridis* achieved the highest irrigation effect below a depth of 8 cm, where bromide concentration was significantly higher than in all other treatments.

a) b)

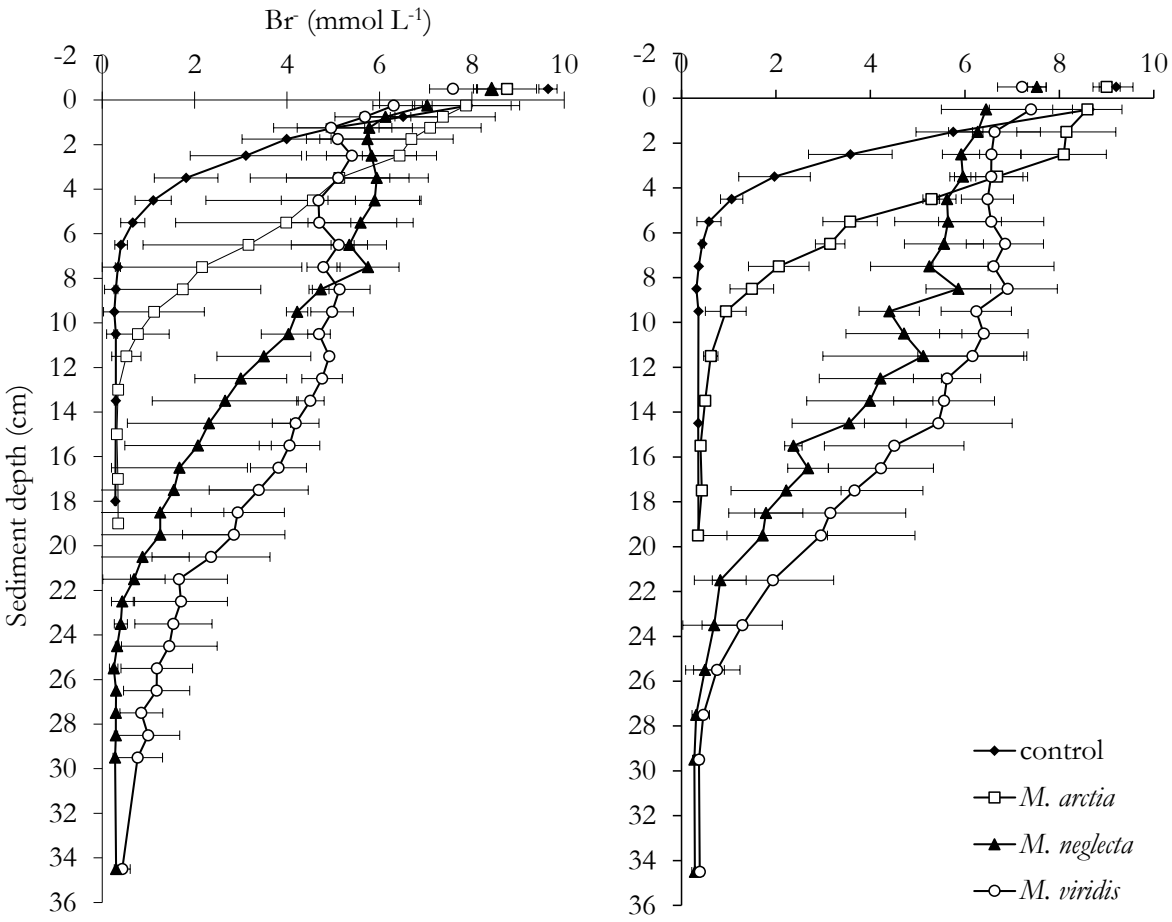


Figure 11: Average pore water bromide concentrations of a) the bioturbation experiment (Biex) after an incubation time of 72.5 h and b) the nutrient experiment (Näex) after an incubation time of 70.5 h. Single symbols displayed above the sediment surface represent mean bromide concentration in overlying water (C_{olw}) at the end of the experiment (point of sectioning). Standard deviation obtained from 4 (Biex) and 3 (Näex) replicates per treatment.

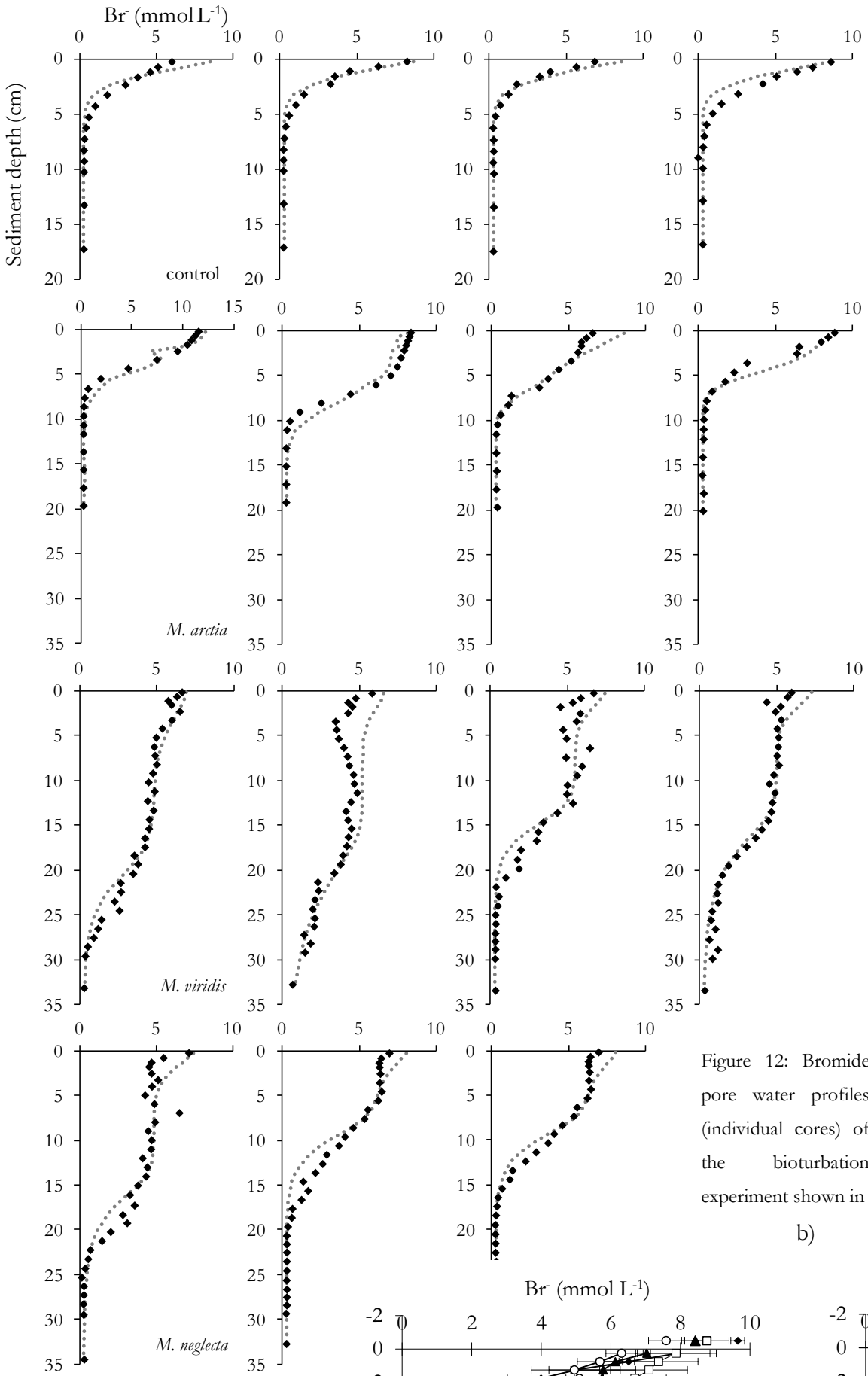
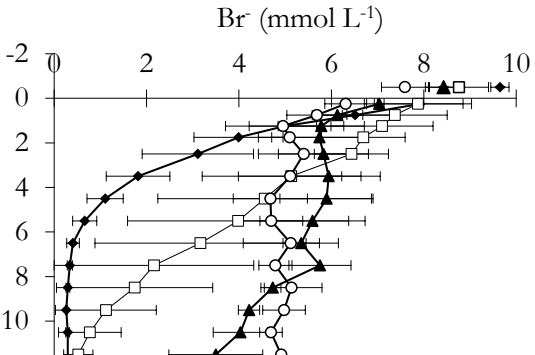


Figure 12: Bromide pore water profiles (individual cores) of the bioturbation experiment shown in

b)



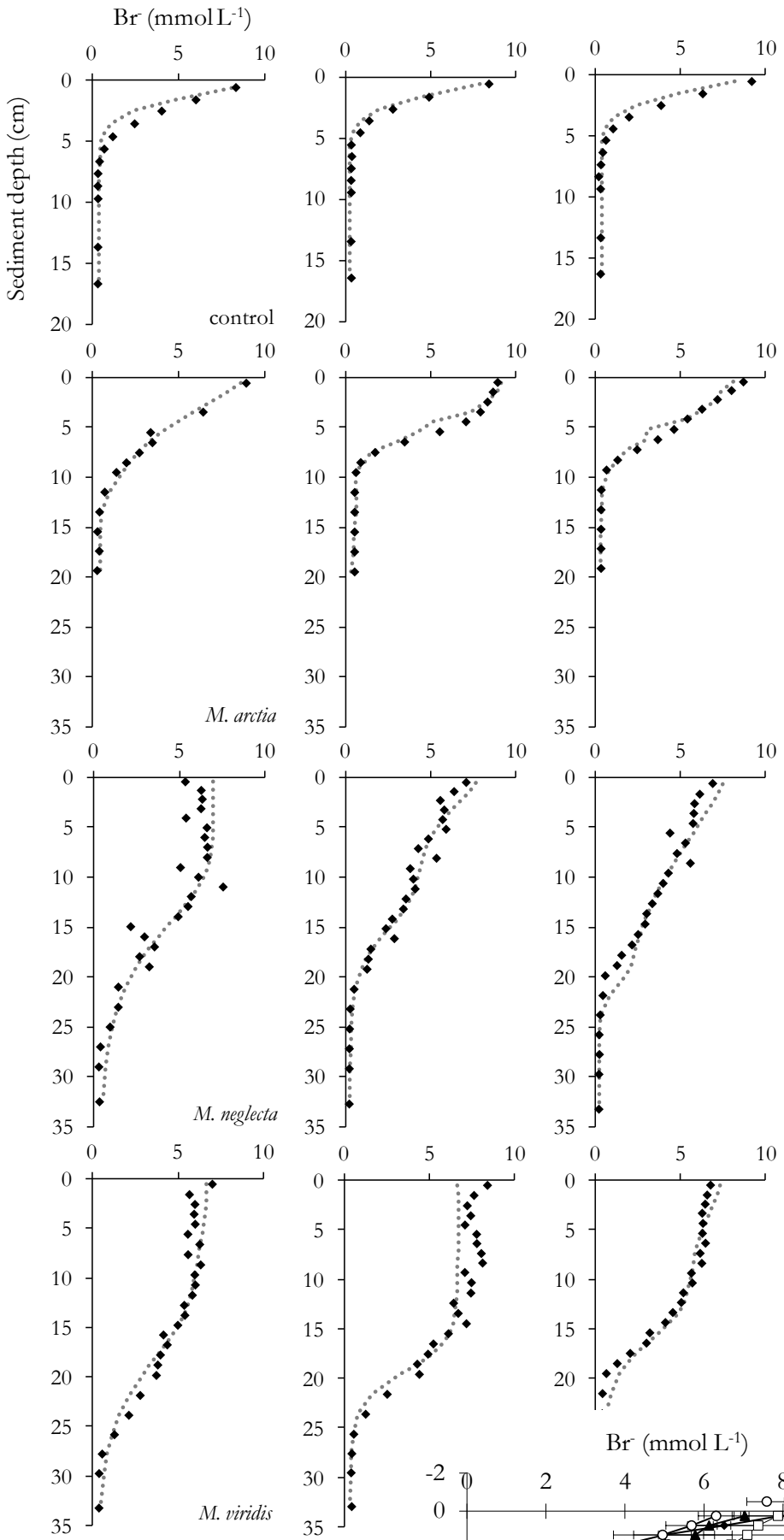
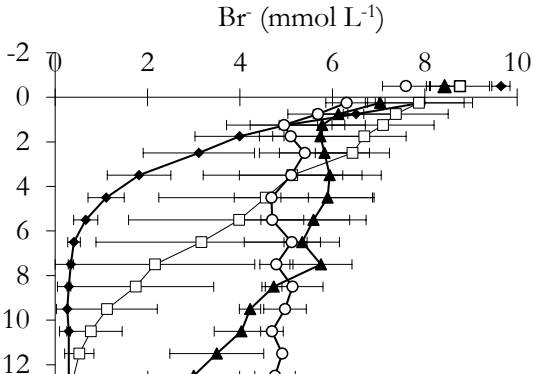


Figure 13: Bromide pore water profiles (individual cores) of the nutrient experiment shown in b)



The volume of overlying water transported into the sediment was calculated from bromide pore water inventory divided by the overlying water concentration as mean values of both experiments (Figure 14). For *M. arctia* it amounts to 1.2 mL ind.$^{-1}$ d^{-1}, for *M. viridis* to 12.2 mL ind.$^{-1}$ d^{-1} and for *M. neglecta* to 8.6 mL ind.$^{-1}$ d^{-1}. Related to one gram fresh weight (fresh wt) of the polychaetes a similar volume of overlying water was transported into the sediment. To assess the impact of the reduced biomass (recovery), reduced abundance for each treatment was also considered to calculate the volume of overlying water transported into the sediment, accentuating the calculated individual effects of the different species.

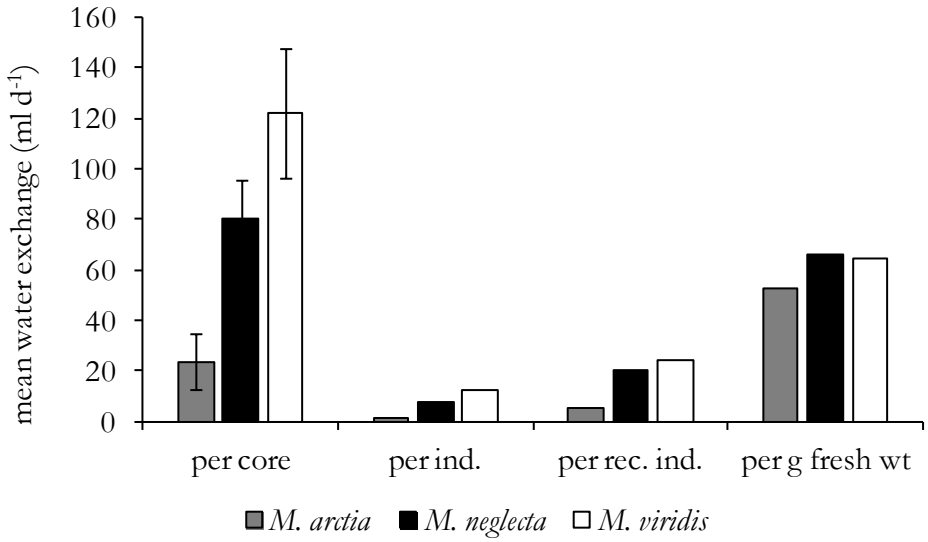


Figure 14: Volume of overlying water transported into the sediment calculated as mean values from the bromide inventory per core of both experiments, per individual (per ind.), per recovered individual (per rec. ind.) and per gram fresh weight (per g fresh wt) at the end of the incubation. Standard deviation obtained from a total of 7 replicates.

3.1.8 Modeled solute transport

In control cores, where molecular diffusion is the determining mechanism in the top centimeters of the sediment core, the solute tracer model and its routine for assigning parameters yielded a good fit to the observed data (Table 4). In one control of the bioturbation experiment and one control core of the nutrient experiment, the decrease of measured bromide concentrations below a depth of 4 cm was less than in modeled data. Here an enhanced diffusion coefficient (ε), 2.2 times (3.0 times) higher than molecular diffusion, resulted in the best fit.

Table 4: With FEMME modeled solute transport (bioirrigation). Parameters ε, α, $k_{\varepsilon,a}$, and L_b were obtained from modeling; Significant differences (SIMPROF, $p < 0.05$) from multivariate analysis between the treatments are labeled with an asterisk (* polychaete treatments compared to control cores). Standard deviation obtained from 4 (Biex) and 3 (Näex) replicates per treatment.

	Treatment	ε	α (yr^{-1})	$k_{\varepsilon,a}$ (cm)	L_b (cm)
Biex	Control	1.3 ± 0.6	0.0 ±0.0	0.0 ± 0.0	4.0 ± 0.0
	*M. arctia**	10.9 ±11.5	55.3 ± 32.6	2.1 ±1.4	2.8 ±2.9
	*M. viridis**	1.9 ±1.8	108.9 ± 13.3	5.4 ±2.0	15.3 ±2.5
	*M. neglecta**	1.0 ±0	130.9 ± 26.5	3.7 ±0.6	9.5 ±4.2
Näex	Control	1.7 ± 1.1	0.0 ±0.0	0.0 ± 0.0	0.7 ± 0.0
	*M. arctia**	11.8 ± 4.2	4.3 ± 3.9	3.3 ± 2.9	3.4 ± 4.8
	*M. viridis**	6.3 ± 3.3	277.1 ± 336.6	4.4 ± 0.7	9.4 ±4.0
	*M. neglecta**	4.9 ± 4.6	284.1 ±184.6	3.7 ± 2.1	13.1 ± 1.4

Multivariate analysis of both experiments resulted in three clusters (Figure 15 and 16) and showed significant differences (SIMPROF, $p > 0.05$) between polychaete treatments and control cores (Table 4). The SIMPER analysis (Clarke and Warwick 2001) thereby confirmed the species-specific differences described as similarities or dissimilarities with regard to the different transport parameters (Table 5). The modeled solute transport coefficients for *M. arctia* yielded a combination of 10.9 ± 11.5-fold enhanced molecular diffusion with a similarity of 25.7 % (11.8 ± 4.2-fold enhanced, similarity of 39.1 %) and a non-local transport coefficient α, with mean values of 55.3 ± 32.6 yr^{-1} and a similarity of 42.9 % (4.3 ± 3.9 y^{-1}, similarity of 18.8 %). The non-local transport α was the dominant mechanism influencing solute transport patterns in *M. neglecta* and *M. viridis* cores with a similarity of 43.5 (43.2 %), and the enhanced diffusion was seemingly negligible.

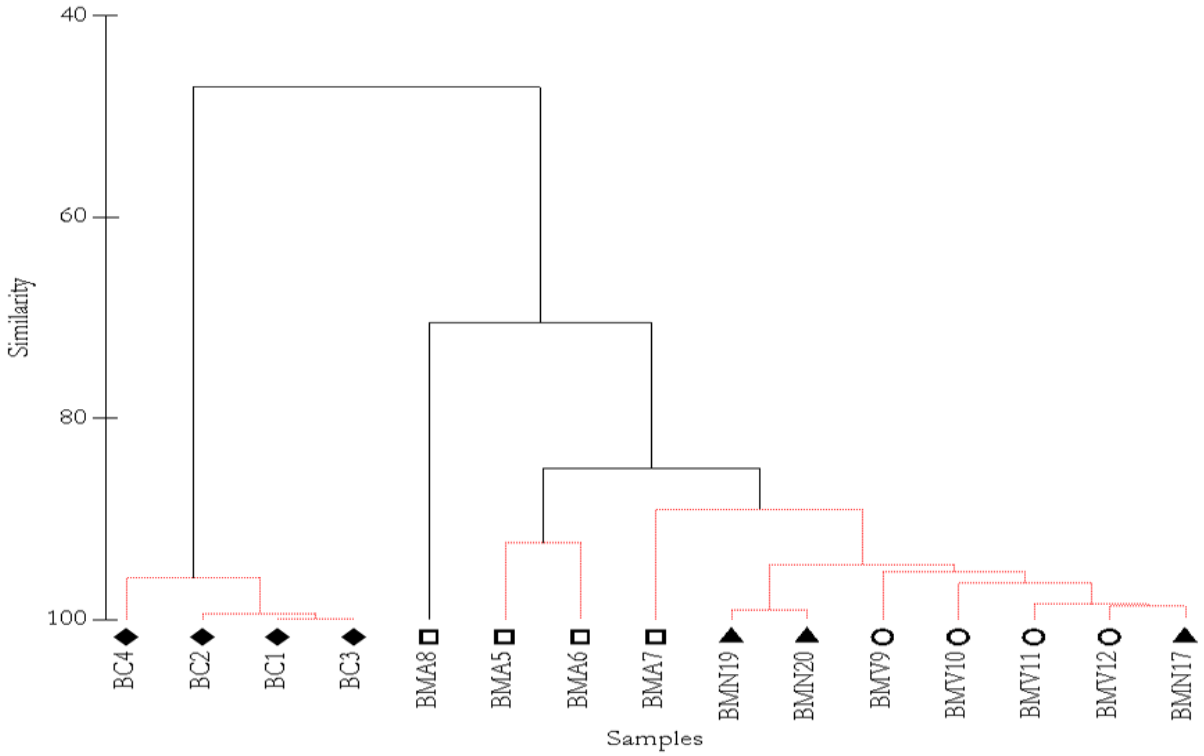


Figure 15: Dendrogram of modeled solute transport parameters of the treatments of the bioturbation experiment. Raw data were fourth-root transformed and for the resemblance Bray Curtis similarity was used (stress 0.01).

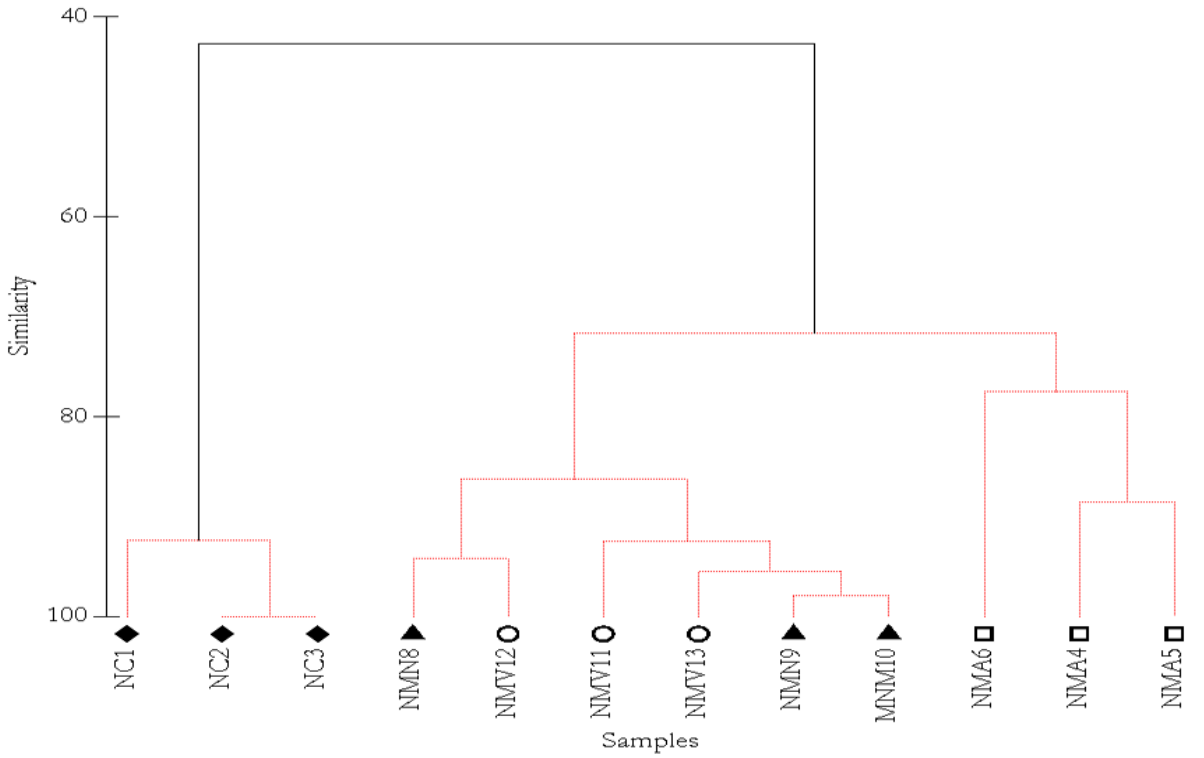


Figure 16: Dendrogram of modeled solute transport parameters of the treatments of the nutrient experiment. Raw data were fourth-root transformed and for the resemblance Bray Curtis similarity was used (stress 0.01).

Table 5: Similarity and dissimilarity (SIMPER analysis) of modeled solute transport parameters of a) the bioturbation experiment (Biex) and b) the nutrient experiment (Näex). Average similarity or dissimilarity (in parentheses) within or between the groups obtained from 4 (Biex) and 3 (Näex) replicates per treatment.

a)	Similarity (%)	BC	BMA	BMV + BMN
	within group	(97.76)	(80.48)	(95.80)
	ε	41.53	25.73	13.59
	L_b	58.47	13.25	24.04
	a	0.00	42.92	43.49
	$k_{\varepsilon,a}$	0.00	18.10	18.88
	Dissimilarity (%)	BC – BMA	BC - BMV+BMN	BMA - BMV+BMN
	between groups	(53.91)	(52.28)	(17.92)
	$k_{\varepsilon,a}$	23.68	27.40	13.59
	a	53.47	62.00	29.44
	ε	12.57	2.01	23.95
	L_b	10.28	8.59	33.02
b)	Similarity (%)	NC	NMA	NMV + NMN
	within group	(94.91)	(81.20)	(90.04)
	ε	52.30	39.07	16.85
	L_b	47.70	17.92	22.67
	a	0.00	18.78	43.20
	$k_{\varepsilon,a}$	0.00	24.23	17.28
	Dissimilarity (%)	NC – NMA	NC - NMV+NMN	NMA - NMV+NMN
	between groups	(49.03)	(61.95)	(28.36)
	$k_{\varepsilon,a}$	36.23	21.63	6.61
	a	33.35	58.38	65.56
	ε	20.42	5.99	9.80
	L_b	10.00	14.00	18.03

For a better comparison of the effect of the two different transport mechanisms the masses of bromide transported via enhanced (bio-)-diffusion as opposed to non-local exchange were calculated and related to the total bromide flux. In *M. arctia* treatments the flux attributed to enhanced (bio-) diffusion dominates with at least 73% of total bromide flux (

Figure 17). This proportion is very different in the other two siblings with tracer flux due to non-local exchange representing more than 95% of total bromide flux.

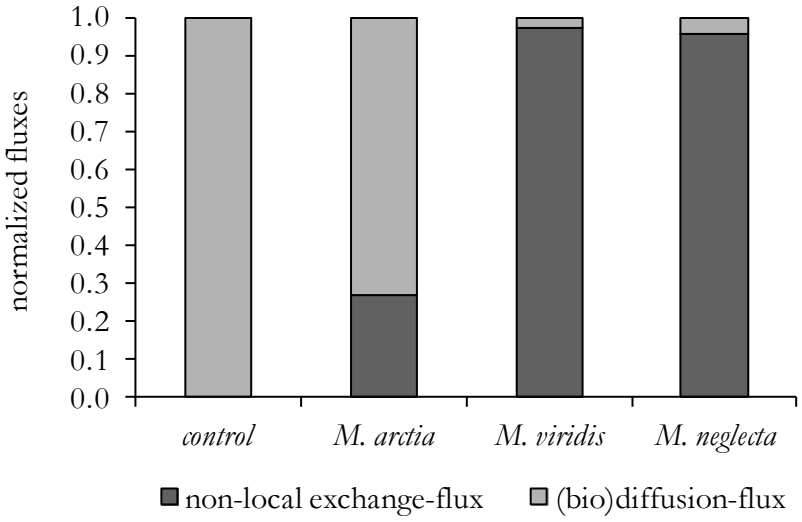


Figure 17: Normalized fluxes. Masses of bromide transported via enhanced diffusion as opposed to non-local exchange were related to the total bromide flux.

3.2 Benthic nutrient fluxes and oxygen uptake

3.2.1 Nutrient fluxes

At the beginning of the experiment (d_0), before animals were introduced, concentrations in the water column of ammonium (4.3 ± 8.2 µmol L^{-1}), nitrite (3.5 ± 1.5 µmol L^{-1}) and phosphate (2.9 ± 2.2 µmol L^{-1}) were similar and low in all 12 replicates. In contrast, nitrate concentrations varied considerably (56.7 ± 35.2 µmol L^{-1}), indicating non-uniform starting conditions. Ammonium and phosphate concentrations in the water column stayed on a steady low level in control cores, while these nutrients increased with time in almost all polychaete treatments (Figure 18, left panel). Only phosphate concentrations decreased from d_7 to d_8 in NC, NMA and NMN treatments. NH_4^+ concentrations on d_{13} were excluded from calculations due to analytical problems. Microcosms colonized by *M. viridis* and *M. neglecta* displayed considerably higher increases in ammonium and phosphate concentrations than those with *M. arctia*. In contrast to increasing nitrate concentrations with time in azoic controls and cores colonized with *M. arctia*, NO_3^- concentrations in *M. neglecta* and *M. viridis* microcosms were significantly lower and tended to decrease during the experiment.

Ammonium fluxes derived from the concentration changes in the overlying water in each incubation period (Figure 18, right panel) were directed out of the sediment and increased with time, except for control cores on d_4 to d_6. The efflux of NH_4^+ in all polychaete cores was significantly enhanced compared to the control cores (*U*-test, $p < 0.05$). The highest effluxes in *M. viridis* cores did not differ significantly from cores colonized with *M. neglecta*, except on d_9 to d_{10}. The fluxes in both these treatments were significantly higher than fluxes in *M. arctia* inhabited cores except on d_6 to d_7.

Nitrate fluxes of control and *M. arctia* cores were directed out of the sediment and increased with time, except from d_9 to d_{13}. The NO_3^- fluxes of cores colonized with *M. viridis* and *M. neglecta* were directed into the sediment, except for *M. neglecta* on d_7 to d_8 and differed significantly from control and *M. arctia* cores, respectively, except on d_6 to d_7 (*U*-test, $p < 0.05$). No statistical differences were found between NO_3^- fluxes in *M. viridis* and *M. neglecta* cores, except on d_9 to d_{13}.

Phosphate fluxes were highly variable and in the majority of cases directed out of the sediment, except for d_7 to d_8 when fluxes in almost all treatments were low and directed into the sediment. Beside the significantly higher fluxes of inhabited cores compared to control cores, only marginal statistical differences were found for phosphate fluxes (*U*-test, $p < 0.05$).

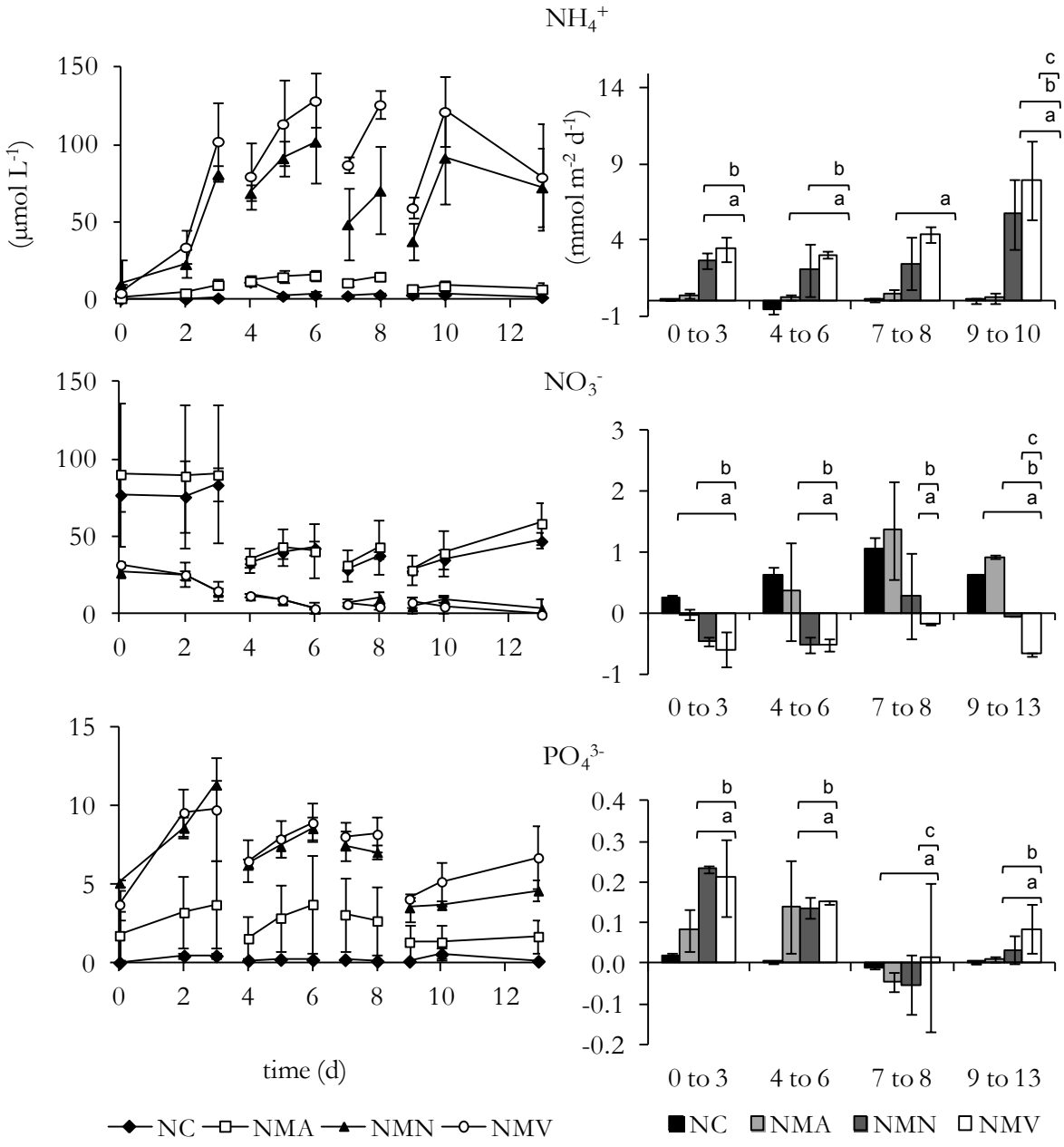


Figure 18: Nutrient concentrations (left panel) and fluxes calculated from concentration gradients (right panel) in the water column. Standard deviation obtained from 3 replicates per treatment. Significant differences (*U*-test, $p < 0.05$) of fluxes during each incubation period were tested pair wise, differences of the respective treatment are labeled with uppercase letters (e.g., (a) treatments compared to control cores NC, (b) NMN or NMV compared to NMA, (c) NMN compared to NMV).

3.2.2 Nutrient pore water profiles

Ammonium pore water profiles (Figure 19) of control cores were characterized by increasing concentrations within the first sediment layers, a typical subsurface maximum at a sediment depth of 3.5 cm (205.5 ± 10.6 μmol L^{-1}), and a subsequent slight decrease in concentrations. The pore water NH_4^+-concentration gradient and subsurface maximum in *M. arctia* cores (189.2 ± 34.9 μmol L^{-1}) were smaller than in control cores. NH_4^+-concentrations in NMN and NMV cores increased within the first sediment layers, followed by fluctuation at approximately 160 and 145 μmol L^{-1}, respectively.

The nitrate pore water profiles of control and *M. arctia* cores were characterized by highest concentrations within the first sediment layer (NC 20.8 ± 18.8 μmol L-1; NMA 12.0 ± 5.3 μmol L^{-1}) followed by a continuous decrease with depth to a mean value of 4.3 ± 0.6 and 0.7 ± 0.3 μmol L^{-1}, respectively. Microcosms colonized by *M. neglecta* likewise displayed highest nitrate values within the first sediment layer (4.9 ± 2.0 μmol L^{-1}), followed by decreasing levels to a depth of 2.5 cm, and subsequent fluctuating concentrations around a mean value of 2.2 ± 0.7 μmol L^{-1}. In cores inhabited by *M. viridis* almost no nitrate was detected in the surface layer (1.2 ± 0.3 μmol L^{-1}), and below a subsurface maximum at a sediment depth of 2.5 cm concentrations decreased.

The control pore water profiles of phosphate were characterized by a typical subsurface maximum (2.0 ±1.9 μmol L^{-1}) at a sediment depth of 6 to 9 cm. The concentrations in *M. arctia* inhabited cores did not change considerably from the sediment surface to a sediment depth of approximately 4 cm. Below this depth, concentrations increased to approximately 9.9 ± 2.7 μmol L^{-1} at a depth of 15 cm, followed by a decrease. In contrast, the maximum concentrations of phosphate in the pore water profiles of *M. neglecta* (4.9 ± 4.3 μmol L^{-1}) and *M. viridis* (11.4 ± 1.9 μmol L^{-1}) were observed within the first sediment layer. Subsequently, decreasing concentrations to a sediment depth of 3 cm were followed by pore water distribution patterns that were inconsistent in shape and fluctuating in concentrations.

3.2.3 Total oxygen uptake

Compared to control cores with a mean total oxygen uptake (TOU) of 8.5 ± 1.3 mmol m^{-2} d^{-1} (Table 6), the presence of polychaetes increased TOU significantly (*U*-test, $p < 0.05$): 2.8-fold (*M. arctia*), 8.9-fold (*M. neglecta*), and 10.8-fold (*M. viridis*). While the effects of *M. viridis* (91.5 ± 13.1 mmol m^{-2} d^{-1}) and *M. neglecta* (75.6 ± 7.8 mmol m^{-2} d^{-1}) did not differ significantly,

TOU in *M. arctia* cores (23.9 ± 6.3 mmol m^{-2} d^{-1}) was significantly lower than in the former species.

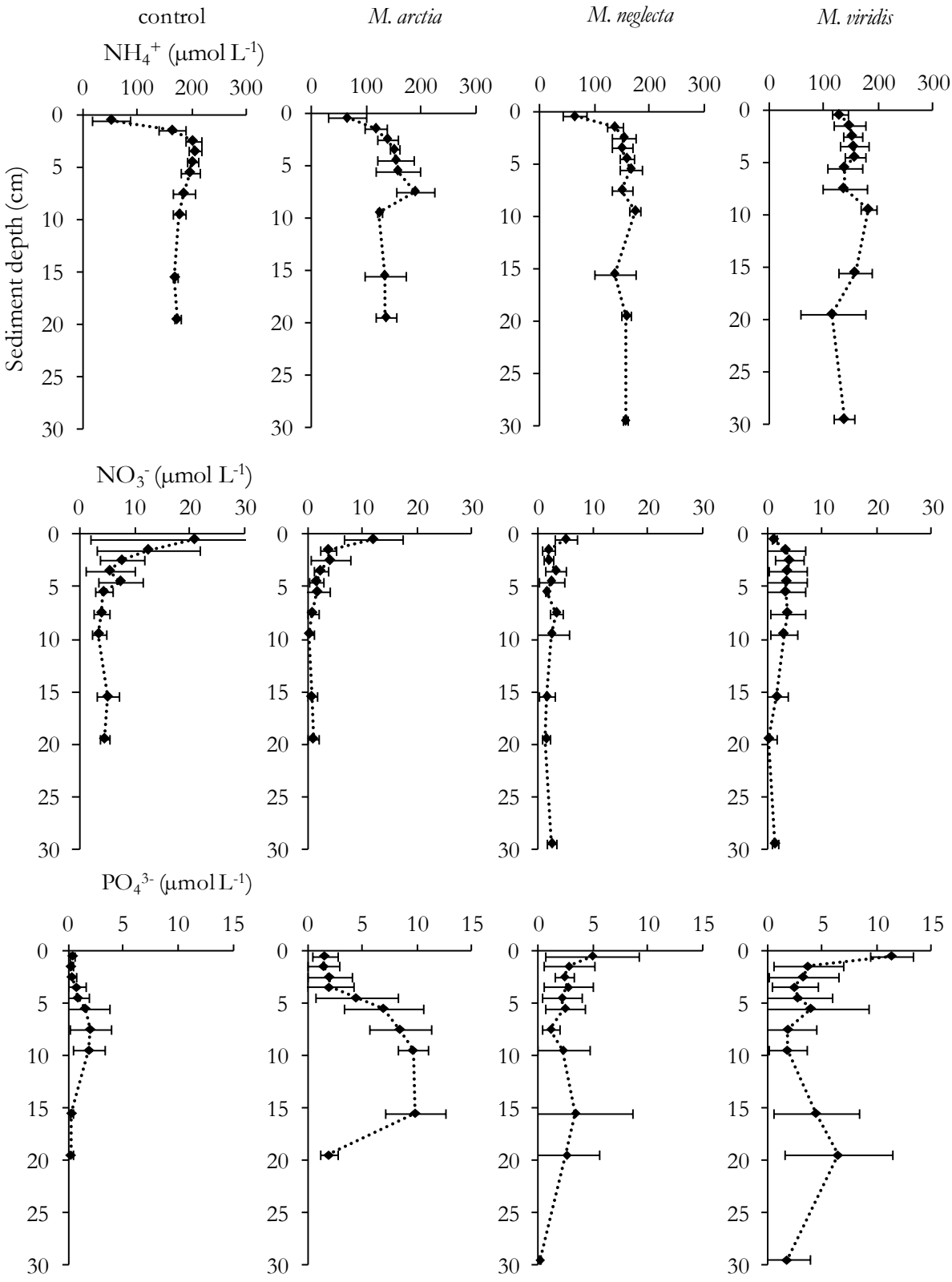


Figure 19: Nutrient pore water profiles at the end of the nutrient experiment. Standard deviation obtained from 3 replicates per treatment.

4. Discussion

4.1 Burrowing depth and shape of burrows

The differing shape of burrows is a constitutive factor concerning the bioturbation of the three sibling species. *M. arctia* dwells in a restricted depth interval in the top 6 - 11 cm of the sediment with burrows seemingly U, J, or L shaped if viewed from outside the core. These burrows appear to create a 'dense network' close to the interface. This observation coincides with findings of Hietanen et al. (2007) and Viitasalo-Frösen et al. (2009). The CT scan confirmed that the apparent dense network resulted from the multiplicity of burrows and that *M. arctia* likely constructs individual, unbranched and twisted burrows of ~0.5 mm diameter, which are U-shaped. In general, shape and depth of burrows of both *M. viridis* (Essink and Kleef 1988; Quintana et al. 2011) and *M. neglecta* (Zettler et al. 1994) were similar, building L, J or I shaped burrows to a sediment depth of 35 cm with, a mean diameter of 2 mm and clearly differed from those of *M. arctia*. Thus these sibling species can be distinguished from *M. arctia* according to their burrows and the predicate 'deep burrowing' species may only be applicable for *M. viridis* and *M. neglecta*. Burrowing depth of *M. neglecta* in the present investigation was somewhat shallower (26 cm) compared to *M. viridis*, reaching more than 35 cm deep into the sediment.

Burrow geometry, predominantly abundance and less size of burrows, is the one governing variable affecting solute exchange in soft bottom sediments (Aller 1982). This may also hold for particle reworking though to a lesser degree. In this study twice the abundance of *M. arctia* compared to the other siblings was employed. Moreover, one individual of *M. arctia* with its U-shaped burrow always constructed two burrow branches pervading the bioturbated surface sediment, while in the other species only one or two branches per individual were constructed. As a result of the established burrow structures, the interface between sediment and water, and, therefore, the effective area of exchange, increases. Based on the scan results, *M. arctia* (assumed abundance of 1273 individuals m^{-2}) creates 0.4 m^2 of burrow surface below each square meter of sediment surface. In contrast, the deep burrowing polychaetes enhanced the interface by 2.8 m^2 (*M. viridis*) and 2.1 m^2 (*M. neglecta*), when assuming the above mentioned burrow depth and diameter. The combined effect of both higher individual numbers and different burrow geometry in *M. arctia* treatments, with at least twice as many burrow branches transgressing the surficial sediment, has to be kept in mind in the following discussion.

4.2 Particle reworking

Macrofaunal induced sediment reworking influences the sediment structure depending on feeding modes, mobility and burrowing activities of the organisms involved (Aller 1982; Kristensen 1988). Feeding mode of *Marenzelleria* spp. in general includes surface and subsurface deposit feeding, i.e. ingestion and egestion of faecal pellets and suspension feeding (Aller 1982; Zettler 1995; Granberg et al. 2008) although no specific information for *M. arctia* is available. The active searching behavior around the burrow openings on the sediment surface or within burrows (Dauer 1997) in combination with the particle transport due to moving in and out the sediment are likely the major mechanisms that influence sediment reworking (Aller 1982) leading to a diffusion-analogue transport of particles within the first centimeters of the sediment. In deeper sediment layers non-local vertical transport and passive falling of particles inside the burrow (Powilleit et al. 1994) may influence the transport process. The applied gallery-diffusion model (François et al. 2002) provided quantitative estimates of both particle transport mechanisms either as separate transport processes or as a combination of both. The slight non-local transport of particles that was apparent in the azoic control cores likely indicates bioturbation by meiofauna (Aller and Aller 1992; Berg et al. 2001) or a mechanical artifact during sediment handling. However values are minor and were masked by higher transport coefficients in all polychaete treatments. In this study *M. viridis* achieved the highest biodiffusion coefficients among the sibling species (4 fold higher than *M. arctia* and 25 fold higher than *M. neglecta*). The non-local transport of all three siblings was quantitatively negligible. The mixing intensity of *M. arctia* remains restricted to a shallow sediment region within the first 11 centimeters and reflects the multiplicity of burrows in vertical and horizontal directions (Hietanen et al. 2007), despite the fact that *M. arctia* were roughly 5-fold lighter in fresh weight that *M. viridis*. The high variability in reworking coefficients of *M. arctia* was caused by one replicate with a surprisingly high diffusion analogue transport and no non-local transport at all. Thus, relative to biomass as a measure, *M. arctia* and *M. viridis* reworked sediments with similar intensity. Sediment reworking of *M. neglecta* was negligible compared to the other siblings and does not reflect expectations drawn from burrow geometry. The divergence of maximum penetration depth of luminophores and the maximum burrowing depth of polychaetes suggests that sediment was displaced basically horizontally during burrowing. In such a case luminophores applied at the sediment surface could not trace the presumed small horizontal displacement of sediment particles during burrowing. Though the restricted vertical particle tracer distribution remains remarkable, the data possibly indicate a different, yet unknown, mode of reworking. They also display classical

reworking and non-local transport coefficients that are close to zero and the lowest among the siblings.

To compare the impact on sediment reworking of the three sibling species in the upper part of the sediment, bioturbation of averaged sediment profiles was modeled to a depth of 10 cm in exclusive consideration of diffusion-analogue transport mechanisms. Diffusion coefficients of *M. neglecta* were marginal with 0.06 $cm^2\ y^{-1}$ followed by 0.11 $cm^2\ y^{-1}$ for *M. arctia* and 1.75 $cm^2\ y^{-1}$ for *M. viridis* but are not exceptional and integrate well into a global plot of mixing coefficients obtained by different tracers and methods (Boudreau 1987). The calculations allow a direct comparison to a few studies where particle reworking coefficients were modeled via diffusion analogue transport exclusively without regarding any non-local transport mechanisms. In a study of Hedman et al. (2011) sediment reworking of *M. neglecta* was investigated under similar experimental conditions (sediment type, organic content, type of tracer) although species identity was merely through the sampling location and not confirmed by taxonomical or molecular means. The quantification of particle transport was done via the same model (François et al. 2002) including a diffusion coefficient (Db) and non-local transport (r). With a Db of 0.88 $cm^2\ y^{-1}$ the diffusion coefficient is higher compared to the results of the present study. As higher abundance in e.g., *H. diversicolor* has been shown to result in an increased biodiffusive mixing (Duport et al. 2006), the comparison may reflect in part a 2.5 fold higher abundance (3185 ind. m^{-2}) used by Hedman et al. (2011). In analogy to the present investigation the non-local transport was negligible. However, the possibility that measurable Db and no r in the study of Hedman et al. (2011) describe a species other than *M. neglecta* can presently not be ruled out either. Sediment reworking of *M. viridis* was investigated in a study of Quintana et al. (2007) under similar experimental conditions but a 2 fold higher abundance (2548 ind. m^{-2}) was chosen. Quantification was done with a 1D diffusion model (standard diagenetic transient transport-reaction model (Martin and Banta 1992) where luminophore transport is described as simple diffusion analogue transport. The obtained diffusion coefficients are in a range of 1.2 – 1.5 $cm^2\ y^{-1}$ and biodiffusion increased with increasing incubation time. Compared to the coefficients of the present investigation when calculated in exclusive consideration of diffusion-analogue transport mechanisms the diffusion coefficients are slightly lower despite the higher abundances. These differences could in part be due to the choice of models, fitness of organisms or sediment characteristics not measured here. A Db on the order of 1 to 1.7 $cm^2\ y^{-1}$ for *M. viridis* can be confirmed, however. The particle transport of *M. arctia* was investigated using two different abundances (390 and 1950 ind. m^{-2}) in a study of Viitasalo-Frösen et al. (2009). They used an organic rich sediment and fluorescent microspheres with a diameter of 1 μm. Sediment reworking was quantified via the

biodiffusion-model (François et al. 1997), where particle transport is described exclusively as diffusion analogue transport. The obtained diffusion coefficients are in a range of 3.76 to 5.11 $cm^2\ y^{-1}$. The surprisingly high values originate most likely from the initial burrowing effect included in the investigation, because tracer were added before the introduction of polychaetes to the mesocosms, while the different tracer used may also influence the results.

Differing incubation times may also influence the magnitude of the transport coefficients. Compared to 10 days in Hedman et al. (2011) the incubation time in Viitasalo-Frösen et al. (2009) was 2 fold higher, it was 4 fold higher in the present investigation and 5 fold higher in Quintana et al. (2007). These relations support a close agreement of reworking coefficients for *M. viridis* with literature values. They further suggest that *M. arctia* reworks sediments less and *M. neglecta* does so to the least extent.

The complexity of bioturbation processes implies that particle and solute transport are often investigated separately; however a transport of solutes influences particle translocation and vice versa. During the ventilation of burrows for example substantial amounts of particles can be transferred into burrows (Riisgård 1991). Conversely pore water is transported during sediment reworking in relation to sediment porosity (Aller 1982; Berg et al. 2001; Kristensen 2001). Compared to the ventilation of burrows, however, solute transport generated by particle movement plays a minor role.

4.3 Solute transport

Molecular diffusion, advection of fluid and macrofauna induced mixing processes, i.e., construction and ventilation of burrows are the main mechanisms that influence solute transport in sediments (Aller 1982). Aller and Aller (1992) demonstrated that meiofauna can double molecular solute transport rates of bromide compared to meiofauna-free sediments. However, with regard to the polychaete treatments in this study, the effect of meiofauna is negligible (Forster et al. 2003).

In general, the species specific differences in solute transport patterns reflect the mode of ventilation and the geometry of burrows constructed by the polychaetes. In the current literature only limited information about the burrow ventilation of *Marenzelleria* spp. is available. The ventilation mechanism of *M. viridis* is described as a combination of ciliar and muscular pumping of water into and out of the blind-ending burrows (Quintana et al. 2011). The more distinctive ciliar ventilation is thereby directed into the burrow lines and forces an advective transport of water across the burrow walls into the surrounding sediment. This leads to a subsequent percolation of pore water through the bulk sediment to the overlying water (Quintana et al. 2011). There is no information concerning the ventilation behavior of *M.*

neglecta and *M. arctia* so far either, but based on the similar results obtained for *M. neglecta* and *M. viridis*, comparable ventilation patterns may be assumed for these species. The higher number of burrows and branches close to the interface in sediment inhabited by *M. arctia* generates a bromide ion distribution that resembles a diffusion of tracer into the sediment. In contrast, the U-shaped burrows of *M. arctia* allow unidirectional ventilation of burrows, with probably less energy demand. The enhanced diffusive pore water transport originating from open-ended burrows, however, is thought to be less intensive and deep compared to the advective irrigation of pore water from blind-ended burrows (Kristensen et al. 2012). While details of irrigation patterns remain unknown, some studies suggest that continuous ventilation in open-ended burrows creates a more stable environment compared to oscillating conditions (Forster and Graf 1992; Kristensen and Kostka 2005). This may add to the differences observed here between the species.

With respect to their solute transport patterns *M. neglecta* and *M. viridis* are more similar to one another than to *M. arctia*. While the modeled solute transport mode of *M. arctia* is largely determined by enhanced diffusivity, the deep-burrowing species *M. viridis* and *M. neglecta* affect tracer distributions in the sediment predominantly through a non-local, advective transport mode. The solute transport coefficients obtained for *M. viridis* in the present investigation are comparable to the findings of Quintana et al. (2007), where the diffusion coefficient ranged from 1.3 to 2.2 cm d^{-1}, corresponding to a diffusion enhanced by a factor 3 to 7. The non-local parameter was in a range of 0.18 to 0.28 d^{-1} and in good agreement with our results. The high solute transport coefficients (11 to 32 cm d^{-1}, corresponding to an approximately 30 to 80 fold enhanced diffusion) in a study of Hedman et al. (2011) can be explained as a result of the absence of non-local transport, which is thought to be the main driving force of solute transport in the present investigation. No quantification of the solute transport of *M. arctia* other than the present study is known from the current literature. The comparison of solute transport coefficients in general has to be made with care, because study specific variables such as incubation time, abundance and different models used clearly affect the magnitude of coefficients.

The polychaete induced volume of overlying water transported into the sediment (water exchange) per individual calculated from the bromide pore water inventory over time is therefore, a meaningful parameter to compare the solute transport of *Marenzelleria* spp. between different investigations with varying settings. Related to one individual at the end of the incubation period and as a mean of both experiments, the water exchange was 5.1-fold and 3.3-fold higher for *M. viridis* or *M. neglecta* compared to *M. arctia*. This contrasts the volume transport relative to fresh weight of the polychaetes. Here, *M. arctia* seems to be just as

effective as the deep burrowing species *M. viridis* and *M. neglecta*. Calculated with the reduced abundance for each treatment, the volume of overlying water transported into the sediment per recovered individual merely accentuated the individual effects of the different species. The calculated water exchange of *M. viridis* with 12 mL d^{-1} $ind.^{-1}$ (Quintana et al. 2011) and of *M. neglecta* with 6.6 mL d^{-1} $ind.^{-1}$ (Hedman et al. 2011) corresponds to the findings of the present investigation. In case of *M. arctia* no information about the water exchange are available. In all these studies, including the present one, mortality poses a problem (see discussion below). Still, all results deviate by less than 30% from one another indicating reproducibility in the incubation methods used.

The high volume of water exchange per biomass caused by *M. arctia* may be related to the distinctly different geometry of its burrows. The radial diffusive pore water transport in open-ended burrows is likely more intense than in blind-ended burrows. However, given a certain permeability of the surrounding sediment, percolation into the pore water from blind-ended burrows will exceed the percolation from U-shaped burrows (Kristensen et al. 2012). Unfortunately no permeability data are available for this experimental setup.

4.4 Modeling solute transport

The addition of an inert tracer to the water column of sediment cores, subsequently analyzed in the overlying water as time series of concentrations or from pore water tracer profiles at the end of the incubation is a helpful tool to quantify different aspects of bioirrigation. An identifiability analysis in Andersson et al. (2006) showed that the sole use of tracer data from overlying water during the incubation is insufficient to describe the mode of solute transport on the basis of different transport processes. Because only pore water tracer profiles were used for the modeling, the increasing concentrations of bromide in the overlying water at the beginning of the nutrient experiment, arising from insufficient mixing after the tracer was added, remain without consequences for the modeling.

Fitting experimentally investigated pore water profiles with a theoretical model enables an interpretation of bioirrigation on known parameters as enhanced molecular diffusion (ε), non-local exchange (α), depth of the bioturbated layer (L_b) attenuation coefficients ($k_{\varepsilon,\alpha}$) and porosity gradients. But especially with regard to their impact on solute transport processes the parameters enhanced diffusion and non-local exchange interfere in their effects. Contrasting the dominant influence of the enhanced molecular diffusion at the sediment-water interface, where the gradients are steep, the non-local exchange has the greatest effects in deeper sediment regions because of wide concentration differences between overlying water and the local concentration (Boudreau 1987). The different quality of the two irrigation mechanisms

complicates their quantitative comparison. A simplified approach was used in the bioturbation experiment to relate the proportions of matter flux by enhanced diffusion, acting close to the sediment surface, and non-local transport across the burrow walls at greater sediment depth driven by ventilation. The bio-irrigation-flux caused by each mechanism was calculated from modeled bromide pore water profiles at the end of the experiment. In this simple approach, we assumed a constant porosity and constant solute transport coefficients, even though the concentration differences across the sediment-water interface as well as between overlying water and pore water at depth were higher at the beginning and decreased with time. Thus the calculated fluxes underestimate the actual fluxes, but their proportion reflects a feature of the solute exchange in each species otherwise not visible. In *M. viridis* and *M. neglecta* treatments, the tracer exchange is completely dominated by non-local processes. This may be due to the diffusion and advection of solutes through the burrow walls and occurs at considerable depths. On the other hand, solute transport in *M. arctia* inhabited cores is dominated by the enhanced diffusion mechanism within the upper sediment layers only.

Furthermore the co-dependence of the modeled parameters (porosity, ε, α, L_b, $k_{\varepsilon,a}$) in the present study renders a comparison with literature difficult. One solution at the expense of accuracy might therefore, be the predetermination or estimation and fixation of parameters, such as sediment porosity, depth of bioturbated layer (L_b) or the attenuation coefficient ($k_{\varepsilon,a}$) (Andersson et al. 2006). Because the sensitivity analysis in Andersson et al. (2006) identified sediment porosity as most sensitive parameter of the solute transport model, porosity gradient of each core was derived from profiles and integrated into the model as fixed mean values as a function of porosity vs. depth.

At first sight, bioirrigation coefficients of *M. viridis* and *M. neglecta* are comparably high, but with respect to the maximum irrigation depth (L_b), some differences become obvious. In reality, the different depths of individual burrows cause a gradual attenuation of solute transport in deeper sediment layers (Martin and Banta 1992) down to molecular diffusion below a depth L_b. The modeling routine of the irrigation model used facilitated a smooth transition between these two different solute transport mechanisms by the use of a factor describing the 'enhancement attenuation'. The maximum irrigation depth (L_b) assigned by the modeling routine, i.e., the upper boundary of the transition from non-local to molecular diffusive transport, was 1.6 fold deeper in *M. viridis* (15.3 ± 2.5 cm) treatments than in *M. neglecta* (9.5 ± 4.2 cm). Thus the vertical distance in which irrigation occurred was greater in *M. viridis* and consequently, the overall effect through radial diffusion and advection more distinct.

Based on the modeled co-dependent solute transport parameters the conducted nonparametric multivariate analysis (cluster and SIMPER) (Clarke and Warwick 2001; Clarke et al. 2008) seems to be an assistant tool to assess the resemblance between the treatments. The SIMPER analysis confirmed the species specific trends by the use of similarities or dissimilarities. Emphasizing the meaning of molecular diffusion down to a bromide influenced layer (L_b) in the control cores, solute transport pattern in *M. arctia* cores are dominated by a combination of enhanced molecular diffusion and a non-local transport, whereas the non-local transport in combination with the depth of bioturbated layer (L_b) characterizes the deep burrowing polychaetes *M. neglecta* and *M. viridis*. In correspondence with the sensitivity analysis of Andersson et al. (2006), the attenuation coefficient ($k_{\varepsilon,a}$) was of minor importance in the present investigation.

4.5 Benthic nutrient fluxes

To compare the biogeochemical impact of the three sibling species, natural, sieved sediment was used in laboratory experiments. While the use of this kind of sediments is a prerequisite for such a comparison, it does not represent the sediment that the polychaete individuals originated from. At the polychaete sampling sites abiotic parameters and sediment characteristics varied considerably. Consequently, unknown ecological constraints to the behavior of the polychaetes may emerge that cannot be addressed here. Variations of the patterns observed in this study due to e.g., porosity and permeability differences cannot be excluded. The use of homogenized sediment in artificial cores requires that sufficient time is given to facilitate steady-state conditions with regard to pore water profiles and solute fluxes across the sediment-water interface (Hansen and Kristensen 1998). Despite a stabilization period of five weeks, the initial starting conditions within the replicates varied, reflecting the individual growth and evolution of microorganisms in each sediment core. To reduce effects related to these differences and to facilitate comparable starting conditions, the choice of cores during the colonization with polychaetes was random. Nevertheless, starting conditions in NO_3^- concentrations incidentally fell into distinct groups (NC and NMA vs. NMN and NMV), reflecting for example the slow growth of nitrifying bacteria compared to denitrifying bacteria (Welsh 2003).

Shortly after their introduction to sediment cores, the polychaetes affected the nutrient fluxes. Overall, concentrations of ammonium were higher in sediments than in overlying water, driving a constant nutrient release from the sediment. Compared to azoic control cores, the presence of burrowing polychaetes enhanced the effluxes of ammonium, thus reflecting the grouping of the sibling species, as also shown in solute-transport and oxygen-uptake

patterns, with the strongest impact of 'deep-burrowing' *M. neglecta* and *M. viridis* compared to that of *M. arctia*.

The burrowing and ventilating polychaetes influence the magnitude of nutrient fluxes in several ways. As a result of the established burrow structures, the additional sediment-water interface and, therefore, the effective area of exchange for any solutes increases. Since biomass, size, and abundance of macrofauna, as well as the specific burrowing depth, have a major impact on the geometry of diffusion and the vertical solute concentration gradient (Aller 1982), deeper irrigation will increase pore water solute exchange most. To assess the influence of burrowing depth and the biomass of polychaetes on nutrient dynamics, the ammonium efflux determined at the end of the experiment was correlated with the simultaneously measured bromide-affected sediment depth and with the biomass used in the different treatments (Figure 20a and b). Thereby, ammonium fluxes significantly correlate with both, biomass ($n = 12$, $p < 0.01$, $R^2 = 0.85$) and sediment irrigation depth ($n = 12$, $p < 0.01$, $R^2 = 0.84$). Thus, the magnitude of ammonium fluxes of *M. viridis* and *M. neglecta* compared to *M. arctia* can be directly linked to the differences in biomass and irrigation depth, but no evidence can be gleaned on the causality of parameters (e.g. size-dependent burrowing depth) influencing the magnitude of fluxes. In addition, as drawn from the calculated water exchange and from solute transport coefficients, the ventilation patterns of the three sibling species are different in magnitude and quality, which, consequently, affect the stimulation of microbial nutrient mineralization differently.

A part of the produced ammonium is assumed to be transported to the water column by the ventilation current, as is visible in the decreasing ammonium pore water inventories in the polychaete treatments compared to control cores. In the water column and oxidized surface sediments, ammonium can be directly nitrified to nitrite and nitrate. In control and *M. arctia* cores, the increasing nitrate concentrations in overlying water (e.g. between d_9 and d_{13}), in combination with the pore water nitrate distributions, suggest high nitrification rates within the water column. The produced nitrate will be transported into the sediment and possibly fuel benthic denitrification. However, compared to control cores, *M. arctia* had no significant stimulatory effect on nitrification: hence, the stimulatory effect on denitrification is assumed to be minor.

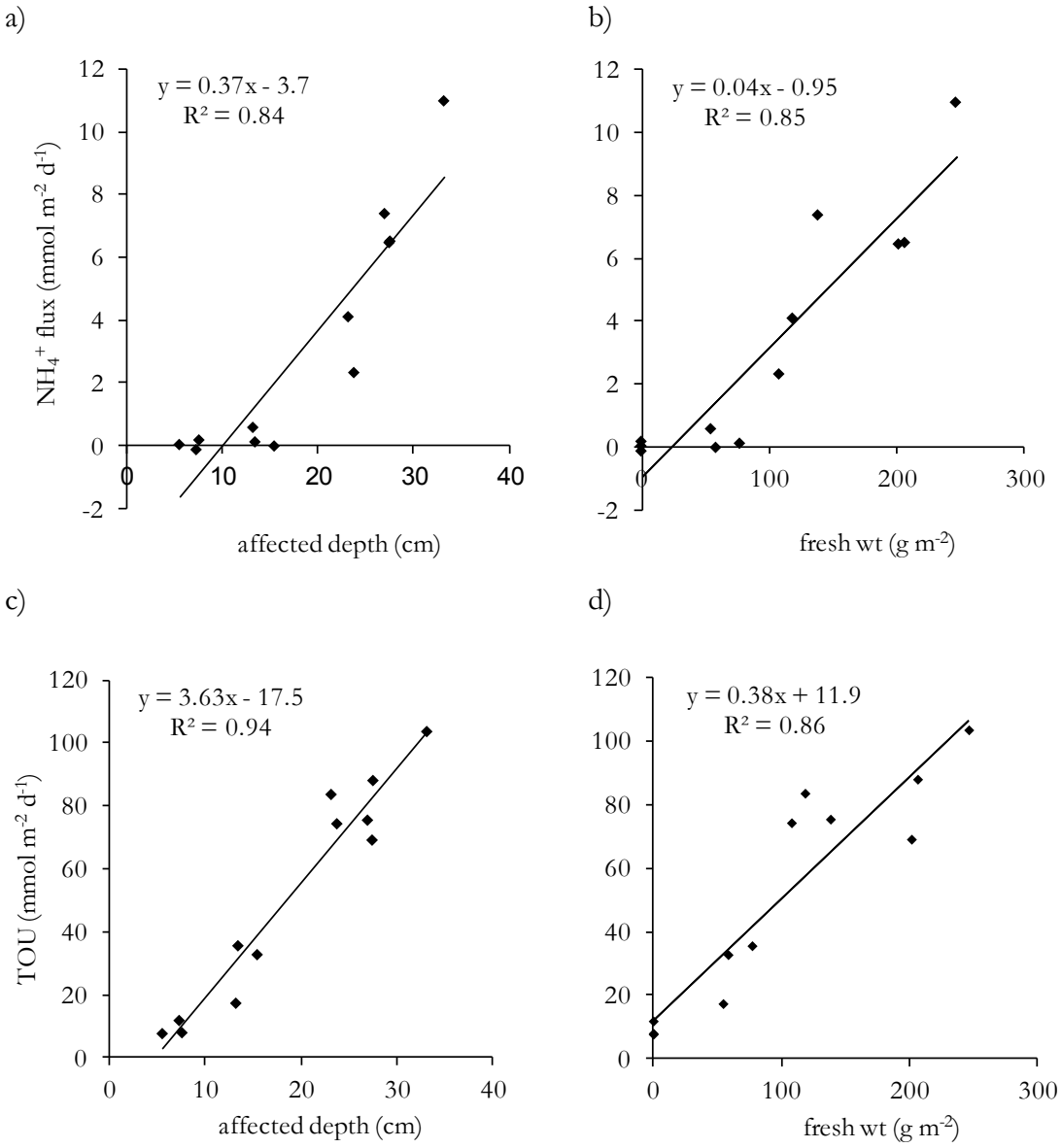


Figure 20: Linear regression of ammonium fluxes and total oxygen uptake (TOU) with a) and c) the bromide affected sediment depth and b) and d) with fresh weight of the polychaetes. Diamonds represent the individual cores.

In sharp contrast to the control and *M. arctia* cores, nitrate concentrations in the overlying water of *M. neglecta* and *M. viridis* cores continuously decreased during the experiment. This decrease is even higher, taking into account that an additional amount of nitrate (20 µmol L^{-1}) was added with each water exchange. If rates of nitrification and denitrification match, a steady-state condition exists, as no net nitrate fluxes across the sediment-water interface are evident (Hietanen et al. 2007). Nitrate pore water concentrations in the present investigation remain low, suggesting a little transport of nitrate across the sediment-water boundary. Consequently, no considerable accumulations of nitrate occur

within the sediment, and any nitrate produced will either be denitrified or used as substrate for DNRA. Ammonium fluxes from the sediment and small nitrate fluxes into the sediment agree with results of other studies of *M. arctia* (Hietanen et al. 2007; Viitasalo-Frösen et al. 2009) and *M. viridis* (Karlson et al. 2005; Kristensen et al. 2011). Hietanen et al. (2007) found the influence of *M. arctia* on denitrification to be minor. Similarly, in a study by Karlson et al. (2005) the effects of *M. viridis* on denitrification were low and DNRA was thought to be the major pathway of nitrate removal in reduced sediments. No information on the influence of the nutrient dynamics of *M. neglecta* is currently available from the literature. While the pathway of nitrate removal cannot be deduced from this study, the literature suggests DNRA as the likely mechanism.

The phosphate pore water profiles of *M. arctia* inhabited cores are typically S-shaped (Slomp et al. 1998) and characterized by a sharp increase in phosphate concentrations below the oxidized surface sediment. Below this subsurface maximum the declining concentrations are caused by a general decrease in metabolic activity with depth. The similar shape but generally lower concentrations, of profiles in control cores indicate a stimulation of the microbial production of phosphate, as well as enhanced desorption in the presence of *M. arctia*. Consequently, the produced phosphate will be transported upward by diffusion to the more oxidized sediment layers at the sediment surface, where an adsorption on Fe-oxides is likely. The potential irrigation effect of *M. arctia*, caused by non-local transport of dissolved phosphate from below the oxidized sediment layers, is minor, as indicated by only slowly increasing phosphate concentrations in the water column. Therefore, the phosphate-buffering capacity of the sediment will not be exploited. Similar effects of *M. arctia* on the remobilization of phosphate have been reported by Hietanen et al. (2007) and Viitasalo-Frösen et al. (2009).

The pore water phosphate profiles of *M. neglecta* and *M. viridis* are completely different from those of the control and *M. arctia* cores. Highest phosphate concentrations occur within the surface layer, followed by decreasing concentrations with depth. Below a sediment depth of 10 cm, the higher phosphate concentrations indicate a stimulated release in both treatments, resulting from enhanced microbial production, as well as from enhanced desorption. The produced phosphate will enter the surrounding sediment via diffusion along a concentration gradient and is transported via non-local transport to the water column. The latter process may partly explain the unusually high phosphate concentrations near the sediment surface. However, the bulk may result from a shift to a more intense anaerobic metabolism, also consistent with the appearance of *Beggiatoa* spp. in these cores. As a consequence of increased sulfate reduction in the upper sediment layers, the produced sulfide causes desorption of phosphate, which diffuses into the water column (Heijs 2000). The

phosphate release caused by *M. viridis* is comparable to that found in a study by Karlson et al. (2005), with a benthic flux of approximately 0.1 mmol m^{-2} d^{-1} and a measured flux of 0.09 to 0.12 mmol m^{-2} d^{-1} in a study by Urban-Malinga et al. (2013), most likely caused by *M. neglecta* (pers. Comm.); these data are consistent with the results of the present investigation.

4.6 Total oxygen uptake

Benthic oxygen uptake reflects the oxygen demand of the heterotrophic activity of fauna and bacteria, as well as the reoxidation of reduced inorganic products that are released during the anaerobic heterotrophic degradation (Glud 2008). The increased TOU in sediments colonized with polychaetes is caused by effects such as stimulation of microbial degradation, as well as directly by the metabolism of macrofauna. There might be additional oxygen consumption associated with decomposing dead individuals if polychaetes not recovered are assumed to have died.

The impact of animal respiration is still controversial, with ranges cited from 10 to 41 % of the total benthic metabolism in the case of *Marenzelleria* spp. (Quintana et al. 2007; Bonaglia et al. 2013; Quintana et al. 2013; Urban-Malinga et al. 2013). Since no information on the respiration rates of *M. neglecta* and *M. arctia* are available from the literature, the respiration rates (R_r) based on biomass (Mahaut et al. 1995) of all three polychaetes according to Urban-Malinga et al. (2013) were roughly estimated, using the relation $R_r = 0.0174\ W^{0.844}$ (mg C d^{-1}) (Braeckman et al. 2010). To assess the impact of reduced biomass (recovery) on the oxygen budgets, respiration rates were subsequently calculated considering the reduced abundance for each treatment (Table 6). With 0.4 to 2.1 mmol m^{-2} d^{-1} the respiration rate of *M. arctia* was slightly lower than the 1.6 to 3.3 mmol m^{-2} d^{-1} and 2.7 to 5.4 mmol m^{-2} d^{-1} for *M. neglecta* and *M. viridis*, respectively. Subsequently, oxygen uptake at the burrow walls (OU_{bw}), estimated from $OU_{bw} = TOU - TOU_{control} - R_r$ was comparable in *M. neglecta* and *M. viridis* cores (84.5 to 86.6% of TOU and 84.9 to 87.8% of TOU) and differed considerably from that at *M. arctia* burrow walls (55.7 to 62.8% of TOU). This indicates that sediment-water interfaces are the most intense sinks for oxygen. Changing respiration rates of the estimated magnitude would not affect this conclusion in either case. In the case of mortality, reduced burrow wall surface would also affect the above calculations, increasing the importance of oxygen removal at these sediment-water interfaces. Therefore, absolute numbers should be interpreted with caution.

Table 6: Total oxygen uptake (TOU), estimated respiration rates (R_r) of the polychaetes, and calculated oxygen uptake of the burrow walls (OU_{bw}). Values in parentheses are related to the total oxygen uptake (% of TOU). R_r and OU_{bw} were calculated (subscripted I and rec, respectively).

	control	*M. arctia*	*M. neglecta*	*M. viridis*
TOU (mmol m^{-2} d^{-1})	8.5 ± 1.3	23.9 ± 6.3	75.6 ± 7.8	91.5 ± 13.1
TOU_{contro} (% of TOU)		8.5 (35.4%)	8.5 (11.2%)	8.5 (9.2%)
R_r (mmol m^{-2} d^{-1})		0.4_{rec} -2.1_i	1.6_{rec} -3.3_i	2.7_{rec} - 5.4_i
		(1.8_{rec} - 8.9_i%)	(2.2_{rec} -4.4_i%)	(2.9_{rec} - 5.9_i%)
OU_{bw} (mmol m^{-2} d^{-1})		13.3_i – 15.0_{rec}	63.9_i – 65.5_{rec}	77.6_i - 80.3_{rec}
		(55.7_i -62.8_{rec}%)	(84.5_i – 86.6_{rec}%)	(84.9_i – 87.8_{rec}%)

The additional sediment-water interface resulting from the established burrow structures increases the effective area of exchange for any solutes. Due to the major effects of biomass and size as well as the specific burrowing depth of the polychaetes, deeper burrows increase the magnitude of total oxygen uptake in the sediment cores. In analogy to the linear regressions of the ammonium fluxes with the bromide affected sediment depth and with the biomass used in the different treatments, TOU was correlated with the same parameters (Figure 20c and d). Total oxygen uptake thereby significantly correlated with both, biomass (n = 12, $p < 0.01$, $R^2 = 0.86$) as well as sediment irrigation depth (n = 12, $p < 0.01$, $R^2 = 0.94$), confirming the hypothesis that differences in total oxygen uptake are driven by differences in biomass and burrowing depth of the species.

The high TOU, especially in cores colonized with *M. neglecta* and *M. viridis*, indicate stimulation of the anaerobic bacterial processes around the burrows. Therefore, a considerable amount of the oxygen uptake in the sediment cores was likely due to the reoxygenation of reduced components. *M. viridis* is known to increase sulfate reduction (Kristensen et al. 2011; Quintana et al. 2013), and the appearance of *Beggiatoa* spp. in *M. neglecta* and *M. viridis* cores at the sediment surface indicates reduced conditions near the sediment surface by reason of upward drifting sulfide.

4.7 Functional diversity

The concept of grouping benthic animals by ecological equivalency into functional groups is a helpful tool for understanding the complexity of ecological systems. Within a functional group species share common biogeochemical and interspecific attributes (Gérino et al. 2003) as feeding and mode of locomotion (Fauchald and Jumars 1979; Diaz and Schaffner 1990; Dauer 1997) or sediment mixing (François et al. 2002). The feeding types of *Marenzelleria*

spp. are assumed to be consistent and comprise surface deposit and suspension feeding (Fauchald and Jumars 1979; Dauer et al. 1981; Sikorski and Bick 2004), although for *M. arctia* no information is available. Based on the close taxonomical relation and their similarity in morphological characteristics, the functional differences of the three sibling species are frequently neglected (Hietanen et al. 2007; Norkko et al. 2012).

The classification of organisms to functional groups on the basis of different mixing modes helps to systemize the various effects of bioturbation on ecosystem processes (François et al. 2002).With regard to sediment reworking and from a model point of view all three sibling species of *Marenzelleria* spp. are assigned to the same functional group of the 'biodiffuser', although a slight but quantitatively negligible non-local transport of sediment particles into deeper sediment layers occurred. Organisms within this group cause a vertical transport of particles by a random particle translocation over short spatial and time distances (François et al. 1997; François et al. 2002). Referring to solute transport a bisection of the sibling species is apparent into *M. arctia* on one hand, with mainly open-ended burrows and a solute transport mechanism mostly driven by enhanced molecular diffusion, and the group of *M. viridis* and *M. neglecta* on the other hand, characterized by blind-ending burrows and a non-local solute transport. Thus, grouping the morphologically similar species in relation to their solute transport will be inappropriate in this case.

The indirect functions measured as changes in nutrient dynamics and oxygen uptake in the present investigation emphasize the complexity of macrofaunal bioturbation activities and their effects on microbial communities (Meysman et al. 2006). Thereby, the three closely related polychaetes emerged to have different activity patterns that are related to their individual species traits. With regard to their oxygen consumption as a key ecosystem function (Norling et al. 2007), the periodic irrigation of the blind-ended burrows of *M. viridis* and *M. neglecta* creates oscillating redox conditions in the surrounding sediment, consequently stimulating the rates of mineralization of organic material (Forster and Graf 1992; Aller 1994; Kristensen and Kostka 2005). Due to the different individual species traits (e.g. burrow structure and irrigation patterns) a similar magnitude of stimulation may not occur in the presence of *M. arctia*. Assessing the relevance of the three morphologically similar species related to all these indirect functions, the deep-burrowing species *M. viridis* and *M. neglecta* may, cause profound changes in resources, acting as important ecosystem engineers. They should, therefore, belong to one functional group, distinctly different from that of *M. arctia*.

5. Synthesis: Ecological consequences of the invasion

In a constrained and reduced definition which is commonly used for public purposes, 'invasive species' cause negative environmental or economic impact or harm to human health (Convention of Biological Diversity). In contrast the broad definition that comprises any species that is not native to a certain ecosystem renders an unprejudiced assessment of the effects caused by the introduction possible. The synonyms most commonly used for 'invasive species' are 'alien' 'foreign', 'non-indigenous', 'exotic', 'introduced', 'naturalized' or even 'pest species', (e.g., Bax et al. 2003), some reflecting the prejudiced public perception of this subject. Introduction mechanisms are basically a result of human activities, but natural range expansion of species is also a possible way to spread. Because invasive species are known to interact with and replace native species, to alter community structures and to cause significant changes in the ecosystem function (Leppäkoski and Olenin 2000), they frequently are assessed as major threat to marine biodiversity and a contributor to environmental change. In case of the spionid polychaetes the spreading of *M. viridis* might e.g., be accompanied by decreasing populations of *H. diversicolor* (Delefosse 2012). *M. neglecta* is assumed to competitively interact with *M. affinis* (Kotta et al. 2006). In contrast no considerable changes in benthic fauna communities could be related with the spreading of *M. neglecta* in the Gulf of Finland (Maximov 2011). Hence the magnitude of interactions between *Marenzelleria* spp. and native fauna in the Baltic Sea still remains unresolved (Villnäs and Norkko 2011).

Besides various influences on nutrient dynamics (Hietanen et al. 2007; Norkko et al. 2012; Quintana et al. 2013), eutrophication (Karlson et al. 2007; Viitasalo-Frösen et al. 2009) and remobilization of buried contaminants (Granberg et al. 2008; Josefsson et al. 2011), bioturbation activities of *Marenzelleria* spp. may also have positive effects on ecosystems, providing important ecosystem-services. A model approach of Norkko et al. (2012), assessing the long term bioturbation effects of the polychaete indicates the positive potential of *Marenzelleria* spp. to reduce sediment induced eutrophication by oxygenating the sediment, consequently retaining phosphate. However, assessing the ecological effects on species level of all three polychaetes, our results clearly indicate species specific differences, basically referring to burrow morphology, burrowing depth and bioturbation pattern. Thereby the present investigation suggests that the effects of *M. arctia* on the ecosystem will be less dramatic and different in basic pattern from the deep burrowing *M. neglecta* and *M. viridis,* that are similar in many bioturbation characteristics. For instance, nutrient release from the sediments may be less in *M. arctia* and their physical effects through bioturbation will not reach as deep. Since e.g., depth of bioturbation and bioirrigation of *Marenzelleria* spp. in Norkko et al. (2012) was

assumed to merely extend a sediment depth of 7 cm, a sediment depth that seems to be characteristic for burrowing activities of *M. arctia*, the investigated potential to enhance phosphorus retention in the sediment may be constrained to this single species. Effects possibly arising from the deeper burrowing *M. neglecta* and *M. virids* are not considered within that modeling approach. Thus, given my information on species specific effects, this topic needs further modeling to arrive at more precise views.

In a proposal of 2013 the European Commission tries to find a common regulation on the prevention and the management of invasive species (Ec 2013). Considering prevention, early warning and rapid response as well as the management of those species the ecological (e.g., effects on native biodiversity and ecosystem services), social and economic influences caused by spreading species should be minimized. In this context constitutive information on invasive species in Europe is provided by different databases such as NOBANIS (European Network on Invasive Alien Species) and DAISIE (Delivering Alien Invasive Species Inventories for Europe) compiling information on risk assessing and scientific evidence. With regard to provided information on *Marenzelleria* spp. both databases seem to be obsolete and rather unsophisticated approaches to compile information. Since the accessible data are used for actual political decisions and the fact sheets on *M. neglecta* date from 2006, more frequent updates than within 8 years are required for both databases. The fact sheet '*Marenzelleria* spp.' provided on DAISIE (Olenin 2006) contains no information on the three sibling species in the Baltic and the species *M. viridis* is simply used as a synonym for *M. neglecta*. Besides being a numerically dominant organism competing for food and space with native benthic macrofauna, the authors address the high effects of *M. neglecta* on solute transport as well as the improved conditions for degradation of organic material caused by their bioturbation activity as possible impact. Apart from these appraisements, the species seems to have no health and social effects and the economic impact is largely unknown. Coexistent on the database *M. neglecta* also emerges within a supplied list termed '100 of the worst', emphasizing a rather undifferentiated way that easily may be misused to prejudice perceptions of the general public, since no criteria on compilation of the list are given. In contrast the prevailing HELCOM Baltic Sea Environment Fact Sheet on *Marenzelleria* spp. (Michalek and Werner 2013) provides more detailed and updated information on abundance and distribution of all three sibling species in the Baltic, more convenient for the use as base for political decisions.

Management approaches associated with the handling of *Marenzelleria* spp. are limited, reflecting the problem of invasive species entering a marine habitat as well as the insufficient knowledge on ecological effects and interactions of these invasive species due to the lack of consistent monitoring programs. Besides removing planctic larvae from ships ballast water,

prevention methods for *Marenzelleria* spp. are unknown and the elimination of marine species is nearly impossible because interception and removal are unsuitable for marine environments. Furthermore the classical scientific research on invasive species, basically focusing on ecological components, seems to be not sufficient to describe the whole extent of the complex topic (Molnar et al. 2008). Since natural scientists are not able to assess any economical or social impact of invasive species in the majority of cases a broad range of experts (e.g., economists and public-health experts) would be necessary to decide on general impacts (Lodge et al. 2006). Therefore, any risk assessment, policy recommendations and management approaches should be implemented locally and globally taking into account the different components of this complex issue.

Increased abundances of *Marenzelleria* spp. have been reported in the Baltic since 2004, especially in deeper areas (> 30 m water depth) of the open sea below the summer thermocline (Eriksson Wiklund 2009), most likely caused by the successful spreading of *M. arctia* in these areas. Until 2008 *M. arctia* was recorded at five different sampling sites in the northern and eastern Baltic Sea (Blank et al. 2008) with increasing densities up to several thousand individuals per m^2 (Hietanen et al. 2007). Since the population development of invasive species is separated into different phases (initial increase, periods of stabilization and decline) (Essink and Dekker 2000; Essink and Dekker 2002) the assessment of abundance and biomass of the three sibling species is difficult and needs to take the population dynamics of invasions into account. Considering the actual distribution of the three sibling species, proceeding on the assumption, that *M. neglecta* and *M. viridis* can be found in the whole Baltic Sea, a further spreading of *M. arctia* into southern regions of the Baltic Sea is likely, depending on the parameters described in the following:

1) All three sibling species are equally tolerant towards lower water temperatures (0°C), but because *M. arctia* is the most temperature sensitive species (Blank et al. 2006), increasing water temperatures will restrict the geographical range of this species to regions with a maximum water temperature of approximately 12°C . In the Yenisei estuary of the Kara Sea, region of origin of *M. arctia*, the water temperature varies between 0 - 12°C (Jørgensen et al. 1999), deducing potential areas in the Baltic for a successful spreading. This assumption is also supported by the small scale distribution pattern of all three sibling species around the Hanko peninsula in southern Finland, where recently the sympatric occurrence of all three sibling species has been verified and basically *M. neglecta* and *M. viridis* were found in near-shore, more sandy bottoms, whereas *M. arctia* was present as single species at water depths below 5 m (Kauppi pers. communication).

2) Due to a high tolerance towards salinity variations (0 to 32, Yenisei estuary of the Kara Sea (Jørgensen et al. 1999)) this factor is assumed to not limit a further spreading into southern Baltic regions of *M. arctia*.

3) Grain size and water content of the substratum determine the successful settlement and development of juvenile burrowing polychaetes. In a study of Kube et al. (1996) no preferred grain size range between a median of 0.15 and 0.35 mm could be substantiated for *M. neglecta*. Because higher contents of water or silt are thought to be unfavorable for burrow stability, adult *M. neglecta* were assumed to be restricted to sediments with low organic or silt contents of 5 to 10% (Kube et al. 1996). In case of *M. arctia* in the Baltic Sea no information on sediment preferences is available, but the different shape of burrows demonstrated in the present study may reflect the adoption of *M. arctia* to muddy sediments. Furthermore, sediment in the Yenisei estuary consists of 100% mud (Jørgensen et al. 1999).

4) Hypoxia, a low bottom water oxygen content of < 2 mg L^{-1}, is a widespread and well described phenomenon in the Baltic Sea and large areas especially in the offshore-deep waters are periodically affected by hypoxia (Conley et al. 2009). In a recent study of Conley et al. (2011) hypoxia and its consequences (e.g., effects on benthic communities, biogeochemical cycles and eutrophication) were also attested to coastal areas of the Baltic Sea. Due to the ability to cope with low oxygen concentrations by falling back on an alternative anaerobic metabolic pathway and the creation of sulphide detoxification products (Bochert et al. 1997; Schiedek 1997) *M. neglecta* and *M. viridis* are equipped with basic requirements facilitating a successful invasion in areas affected by hypoxia or even anoxia. Although there is no information on corresponding pathways of *M. arctia* in literature, the already existing successful spreading of this species within deeper areas, periodically affected by hypoxia, indicates similar abilities in this species.

Habitats that are successfully colonized by invasive species are often characterized as low diversity systems (Leppäkoski and Olenin 2000) with an early successional stage of native species or as disturbed habitats (Essink and Dekker 2002), attributes that all may be dedicated to the deeper areas of the Baltic Sea. In particular the low diversity in the deeper parts of the Baltic Sea implicates potential areas that may be colonized with invasive species (Norkko et al. 2012). As a consequence of the successful spreading of *M. arctia* in southern regions of the Baltic Sea, there will be a considerable increase in biomass in these colonized areas. Due to their adaption to low oxygen conditions (Hahlbeck et al. 2000), *M. arctia* has a great potential to successfully fill that available niche (Villnäs and Norkko 2011). In a study of Maximov (2011) the effects of *M. arctia* due to their spreading into the deeper parts of the eastern Gulf of Finland were especially notable in regions formerly affected by hypoxia.

Consequently, the spreading of *M. arctia* over the next few years will introduce the functional group of the 'biodiffuser' into the deep areas of the Baltic Sea. But due to the minor ecological effects of *M. arctia* compared to the deep burrowing and intensively irrigating species *M. neglecta* and *M. viridis*, substantiated in the present study, a "*cardinal restructuring of the entire ecosystem in the eastern part of the Gulf of Finland as a result of considerable changes in biogeochemical processes and trophic relations*" (Maximov 2011, p. 17) or even the "*changing state of the ecosystem of the entire Baltic Sea*" (Maximov 2011, p. 16) may be contested. Since the ecological consequences of a further spreading of *M. arctia* into the southern areas of the Baltic Sea are assumed to be not as dramatic as a comparable spreading of *M. viridis* in coastal areas, there is an obvious need for further research and consequent monitoring of the ecological effects of *Marenzelleria* spp., especially highlighting potential differences on species level.

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Declaration / Selbständigkeitserklärung

I hereby declare that I have completed the work submitted here independently and have composed it without outside assistance. Furthermore, I have not used anything other than the resources and sources stated and where I have taken sections from these works in terms of content or text, I have identified this appropriately.

Ich versichere hiermit an Eides statt, dass ich die vorliegende Arbeit selbstständig angefertigt und ohne fremde Hilfe verfasst habe. Dazu habe ich keine außer den von mir angegebenen Hilfsmitteln und Quellen verwendet und die den benutzten Werken inhaltlich und wörtlich entnommenen Stellen habe ich als solche kenntlich gemacht.

Rostock, den 22.05.2014