Journal of Foraminiferal Research, v. 45, no. 3, p. 220–234, July 2015

DIFFERENTIAL RESPONSE OF INTERTIDAL FORAMINIFERA TO COMMUNITY RECOVERY FOLLOWING EXPERIMENTALLY INDUCED HYPOXIA

MARGREET G. M. BROUWER[1,7], MARIËTTE WOLTHERS[2,1], JOHAN H. HAZELEGER[1], FRANCESCA ROSSI[3,4], LUCAS J. LOURENS[1], JACK J. MIDDELBURG[1,4] AND IVO A. P. DUIJNSTEE[1,5,6]

ABSTRACT

Conditions of low oxygen, as well as strong fluctuations in dissolved oxygen concentrations, can substantially affect marine benthic communities. An early assessment of the status of a community after such an event is of fundamental ecological importance and may help to inform management measures. This paper investigates the response of a foraminiferal assemblage, and its relationship with sediment biogeochemistry, micro-organisms and other fauna, following experimentally induced hypoxia on an intertidal flat in the Scheldt Estuary, The Netherlands. Sediment hypoxia was induced during one experiment in winter and a second in late spring. The foraminiferal assemblages in the upper 0–1 cm of sediment were sampled at two and five months post-hypoxia. Changes in foraminiferal abundance and biovolume were compared to responses of microphytobenthos, bacteria, meiofauna and macrofauna, which have been reported in separate papers. The foraminiferal assemblage comprised three species, *Haynesina germanica*, *Ammonia beccarii* and *Elphidium excavatum*. Their species-specific abundance and estimated biovolume varied with timing of disturbance (winter vs. spring hypoxia) and the duration of recovery (2 vs. 5 months). Although all foraminiferal species were expected to benefit from recolonization of macrofauna, *H. germanica* was negatively correlated to the abundance of macrofaunal bioturbators during recovery. The abundance of *A. beccarii* was positively correlated with microalgal biomass. These findings revealed species-specific responses by foraminifera after hypoxia and the concomitant recovery of other biota, further demonstrating the usefulness of foraminifera as ecological indicators.

INTRODUCTION

Bottom-water hypoxia (oxygen concentrations <2–3 ppm) events in coastal areas have become more frequent, more intense, more extensive, and longer in duration since the 1960s (Diaz & Rosenberg, 2008). Hypoxia occurs naturally in some environments due to limited bottom-water renewal and circulation, water-column stratification and high organic matter input. Nevertheless, there is strong evidence that the global increase of coastal hypoxia is associated with human-induced eutrophication (Diaz & Rosenberg, 2008).

Low oxygen conditions and strong fluctuations in the concentration of dissolved oxygen can affect the benthic community in multiple ways. Seasonal depletion of oxygen can lead to behavioral changes, a reduction in diversity, and mortality of sensitive species (e.g., Gray et al., 2002; Diaz et al., 2009; Zhang et al., 2009). A sudden lack of oxygen is often associated with the loss of standing biomass and diversity, while continued hypoxia results in a collapse of communities and their functioning (Conley et al., 2007; Diaz et al., 2009). As hypoxia-induced disturbances are increasing, a better understanding of the effects of such disturbances on the ecological resilience of coastal communities is essential for their management.

The recently published recommendations on standardizing methodologies for foraminiferal bio-monitoring (Bouchet et al., 2012; Schönfeld et al., 2012) may contribute to a more central role for foraminifera to describe the state of marine ecosystems. Foraminifera possess several characteristics that enhance their potential utility as bioindicators. Besides their small size, short life cycle, high abundance and high fossilization potential, which all facilitate collecting data covering longer time scales, foraminifera are highly resistant to local extinction associated with environmental perturbations (Josefson & Widbom, 1988; Bernhard, 1993; Moodley et al., 1997; Pucci et al., 2009 and references therein). Foraminifera are often among the most numerous organisms within the benthic fauna of marine ecosystems. Species of these unicellular organisms thrive in a wide range of environmental conditions and can be found in virtually all marine habitats.

Some foraminiferal genera seem to profit from the conditions of low bottom-water oxygenation, suggesting that hypoxia may increase their survival rates through decreased biological interactions such as sediment reworking or predation by macrofauna, and interspecific competition for food. Moreover, some taxa can survive H_2S-rich conditions for weeks (Moodley et al., 1998). Recently, the capacity of several foraminiferal species to use nitrate instead of oxygen as an alternative electron acceptor for respiration has been described (Risgaard-Petersen et al., 2006; Piña-Ochoa et al., 2010a, b). Additionally, changes in their vertical-migratory behavior (Duijnstee et al., 2003) and life history (e.g., reduced growth and enhanced fecundity, Duijnstee et al., 2005) may contribute to their ability to tolerate low-oxygen concentrations.

Foraminifera rapidly respond to environmental changes by modifications in populations and assemblages (i.e., changes in diversity, abundance and biomass). The distribution of living foraminifera is mainly governed by the interplay of their species-specific tolerance for, or

[1] Utrecht University, Faculty of Geosciences, Department of Earth Sciences, P.O. Box 80.021, 3584 TA Utrecht, The Netherlands
[2] University College London, Department of Chemistry, 20 Gordon Street, London, WC1H 0AJ, United Kingdom
[3] UMR 5119-Ecosym, CNRS-IRD-IFREMER-Université Montpellier 2, 34 095 Montpellier Cedex 5, France
[4] Department of Ecosystem Studies, Royal Netherlands Institute for Sea Research, Yerseke, The Netherlands
[5] University of California, Berkeley, Department of Integrative Biology, Berkeley, CA 94720, USA
[6] University of California Museum of Paleontology, Berkeley, CA 94720, USA
[7] Correspondence author. E-mail: brouwer.margreet@gmail.com

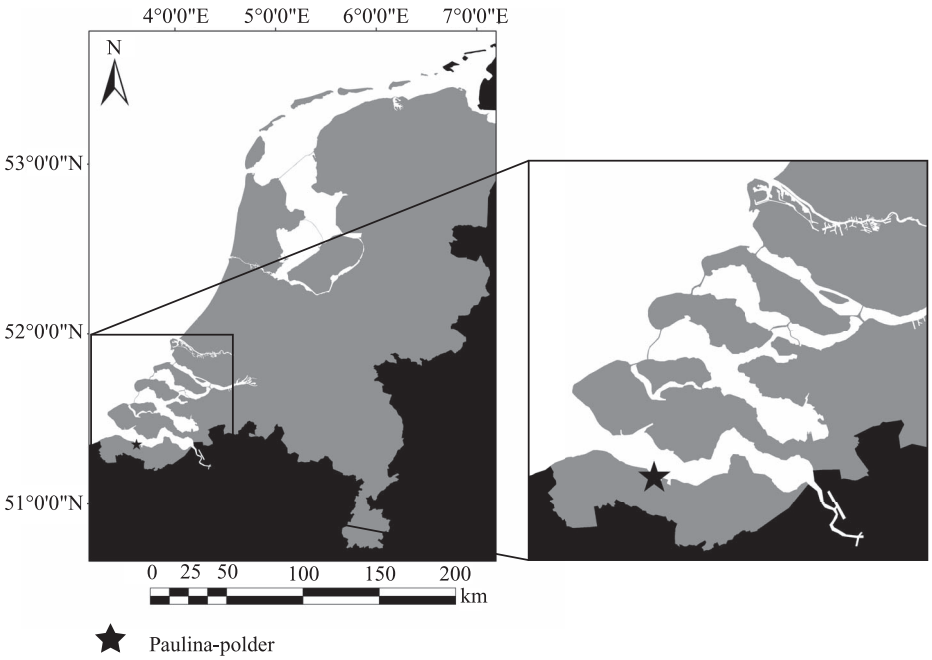

FIGURE 1. Map of the Scheldt Estuary.

dependence on, certain levels of oxygen, nitrate and required food sources (e.g., Jorissen et al., 1992, 1995; Linke & Lutze, 1993; Van der Zwaan et al., 1999; Gross, 2000). Geochemical properties of the sediment are closely coupled to activities of benthic organisms; bioturbation, bioirrigation and feeding strategies significantly influence seafloor geochemistry and sediment stability (Lohrer et al., 2004; Meysman et al., 2006; Erwin, 2008; Montserrat et al., 2008; Passarelli et al., 2012). Infaunal activity creates heterogeneity in biogeochemical properties of sediments and extends the sediment-water interface, resulting in an increase in micro-niches suitable for micro- and meiobenthic organisms (Aller, 1983; Ray & Aller, 1985; Fenchel, 1996; Zorn et al., 2006; Bouchet et al., 2009).

Many experimental studies on the importance of oxygen and food availability for the distribution of foraminifera have been carried out under controlled laboratory conditions (e.g., Bernhard, 1993; Moodley et al., 1997; Duijnstee et al., 2003). Although fundamental insights into the response of foraminifera to different environmental factors were gained, the full complexity of natural conditions cannot be taken into account in such studies and more in situ studies are needed to account for environmental complexity (e.g., Jorissen et al., 1992; Moodley & Hess, 1992; Gooday et al., 2000).

Our study documents the response of benthic foraminifera following experimentally-induced hypoxia in an in situ field experiment. Specifically, we analyzed changes in the abundance and biovolume of foraminiferal species during recovery of the benthic community, and compared these with observations from undisturbed (control) sediments where no hypoxia occurred. This approach provided the opportunity to distinguish between foraminiferal assemblage responses to natural (seasonal) fluctuations in environmental properties (e.g., variation in temperature, length of daylight, food availability) and responses during recovery of the community (e.g., macrofaunal recovery, microphytobenthos bloom) after experimentally-induced hypoxia. We tested whether the assemblage and the abundances of foraminiferal species varied according to the duration of recovery (2 vs. 5 months) and whether these changes were affected by the timing of the disturbance (winter vs. spring). The results are presented in the context of parallel studies that focused on the recovery and development of macrofauna, nematodes, microphytobenthos and bacteria after induced hypoxia (Montserrat et al., 2008, 2009; Rossi et al., 2008, 2009; Van Colen et al., 2008, 2009, 2010a, 2010b, 2012; Rossi & Middelburg, 2011). This strategy allowed us to examine the foraminiferal assemblage alongside the recovery of other faunal assemblages and changes in food availability. Our goal was to improve the applicability of foraminifera as ecological indicators of the state and functioning of marine communities recovering from hypoxia.

MATERIALS AND METHODS

SITE DESCRIPTION

The site of the field experiment is in the Paulinapolder, an intertidal flat in the Scheldt Estuary, along the southwestern coast of The Netherlands (at 51°21'24''N, 3°42'51''E, Fig. 1). The sediment contains an average mud content (i.e., % of particles <63 μm) of 50% (Rossi et al., 2008). This intertidal flat covers an area of approximately 1 km^2 and has a mean tidal range of 3.9 m, with a semidiurnal regime and a yearly average salinity of 24 (Van Colen et al., 2012). The assemblage of hard-shelled foraminifera in the Paulinapolder consists of species from the genera *Ammonia*, *Haynesina* and *Elphidium* (Moodley et al., 2000). This tidal flat has a rich assemblage of macrobenthos, dominated by polychaetes (*Heteromastus filiformis, Arenicola marina,*

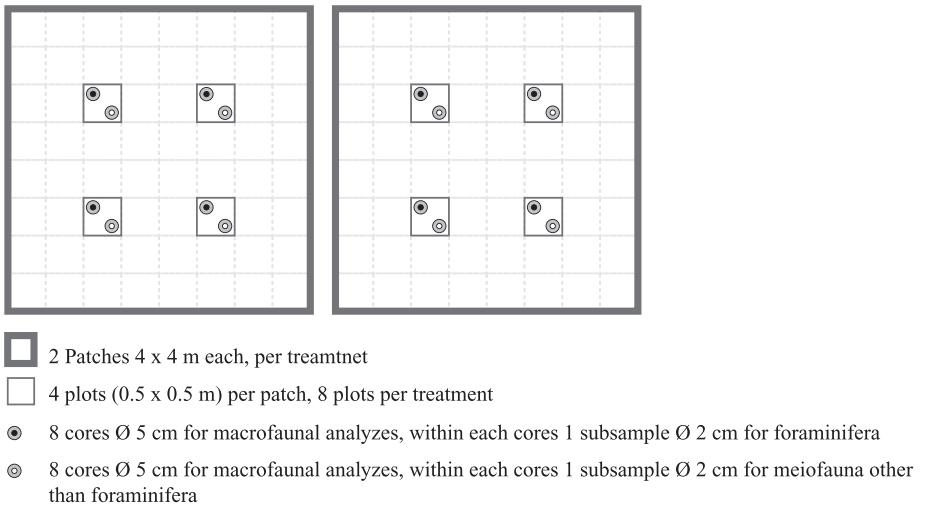

FIGURE 2. Schematic overview of sampling design.

Pygospio elegans) and mollusks [*Macoma balthica, Cerastoderma edule, Hydrobia ulvae* (Rossi et al., 2007)]. The main nematode species include *Daptonema* spp., *Chromadora* spp., *Anoplostoma viviparum*, *Oncholaimellus* sp.1., *Viscosia* spp. and *Ptycholaimellus ponticus* (Van Colen et al., 2009).

Experimental Set-up and Sampling

Hypoxic conditions, two months in duration, were induced in two separate experiments, one in winter and the second beginning in late spring. During both experiments, two large sediment patches (4 x 4 m), 5 to 10 m apart within a 50 x 50 m location (51°21'23''N, 3°42'49''E), were covered with black waterproof polyethylene sheets (Fig. 2). These sheets sharply decreased the replenishment of oxygen from the water column into the sediment and strongly reduced the penetration of sunlight (Van Colen et al., 2012). The winter experiment began on January 30 and continued until March 30, 2005, whereas the spring experiment began on May 9 and ended on July 6, 2005 (Fig. 3). Two untreated sediment patches (4 x 4 m) were used as controls.

In April, 12 days after removal of the plastic sheets that had induced the winter hypoxia, sediment was collected in the hypoxia-treated patches for a field observation on foraminiferal abundances. Subsequently, the sediment patches were left to recover for nearly two months.

Samples were taken on June 10 (winter treatment–2J) and September 9 (late spring treatment–2S). In addition, winter treatment sites were again sampled in September (hereafter 5S), after five months of recovery (Rossi et al., 2008, 2009; Table 1, Fig. 3). The control patches were sampled both in June (hereafter CJ) and September (CS). In each patch, four subplots of 50 x 50 cm were randomly chosen during the field observation and each sampling occasion. Per subplot, two cores (5 cm internal diameter) were collected to 8 cm sediment depth (Rossi et al., 2009). In the laboratory, eight cores per treatment were subsampled for foraminifers and eight for other meiofauna, using a cut syringe with an internal diameter of 2 cm. In accordance with proposed standardized biomonitoring methods (Schönfeld et al., 2012; Bouchet et al., 2012), the top centimeter of each core was used for foraminiferal analyses (analyzed volume 3.14 cm^3 per sample). Per sampling occasion and treatment,

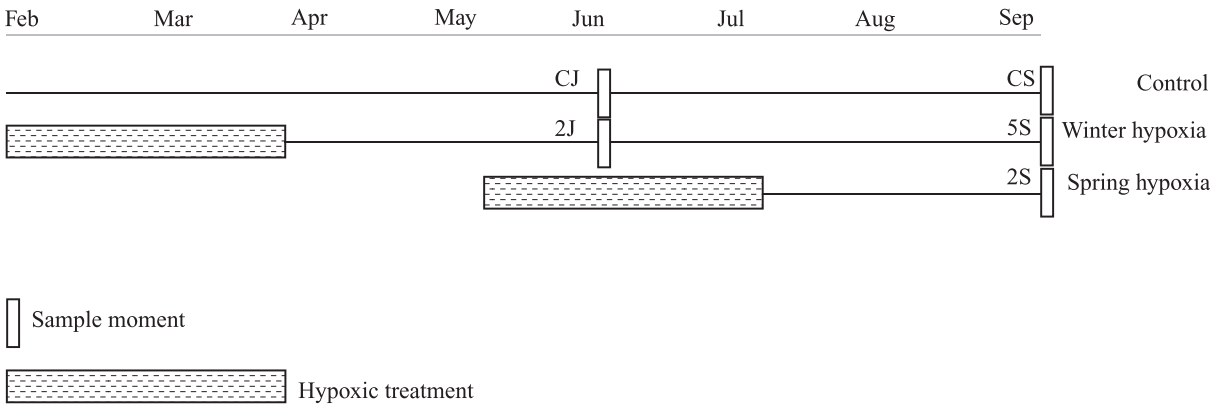

FIGURE 3. Experimental time scale and sample set-up. Hypoxia was induced in winter from January 30 until March 30, 2005, and in late spring from May 9 until July 6, 2005. Samples were taken on June 10 and September 9, 2005.

TABLE 1. Sampling schedule

Sampling June	
Control patches	CJ
Winter hypoxia patches	2J
Spring hypoxia patches	
Sampling September	
Control patches	CS
Winter hypoxia patches	5S
Spring hypoxia patches	2S

four replicate cores were analyzed, except for the control patches in September, for which five replicate samples were analyzed. The sediment samples were stored in 4% buffered formalin with rose Bengal staining (1 g/l).

Sample Processing

Foraminiferal samples were sieved over a 63 μm mesh-size sieve. Well-stained specimens were picked from wet residues and enumerated at the species level. Only individuals fully filled with pink-stained cytoplasm (except for the ultimate chamber) were considered alive at or shortly before the time of sampling (for a discussion on the method of staining see 'experimental limitations'). For a small number of foraminifer-rich samples, a split was used; the foraminiferal counts of these samples were recalculated to estimate the whole sample abundances and biovolumes. After picking, photos were taken using a camera mounted on a microscope with a calibrated internal scale to measure the maximum test diameter (L) of each specimen. Then a crude estimate of protoplasm biovolume (BV) of each specimen was calculated, based on the maximum test-size dimension, using the equation,

$$BV = \pi L^3/16$$

where BV is the volume of a disk with diameter L and a height of 0.25L. The three foraminiferal species, *Ammonia beccarii* (Linnaeus, 1758), *Haynesina germanica* (Ehrenberg, 1840) and *Elphidium excavatum* (Terquem, 1875), have planspiral or very low trochospiral chamber arrangements and are thus approximately cylinder-shaped.

Statistical Analysis

Statistical tests were performed in PAST (PAleontological STatistics; Hammer et al., 2001). First, the SIMPER (Similarity Percentage) test was used to identify the dissimilarity among assemblages and individual species contributions to these differences among assemblages following recovery from hypoxia (Appendix 2). A non-parametric Kruskal-Wallis test was used to test for pairwise differences in the abundance and biovolume of the dominant species (Appendix 3). The Kruskal-Wallis test is a non-parametric ANOVA and was used because it does not require a normal distribution. The p-values were Bonferroni corrected to conservatively correct for multiple testing. A Two-Way ANOSIM (using Bonferroni-corrected p-values) tested for differences between the composition of the foraminiferal assemblages due to timing of disturbance (CJ, CS, 2J, 2S) and recovery development (CJ, CS, 2J, 5S). To assess differences in the foraminiferal assemblage on account of dissimilar recovery stages (CS, 5S, 2S), a One-Way ANOSIM test was used.

To explore the possible influence of microphytobenthos, bacteria and macrofauna on foraminiferal populations, additional data (as published by Rossi et al., 2009) were used in a multivariate analysis using the software package CANOCO (Ter Braak and Šmilauer, 1998). Redundancy Analysis (RDA, see Rao, 1964) was employed to explore relationships between the assemblages of foraminifera and other biota during community recovery. Principal response curves (PRC, see Van den Brink & Ter Braak, 1998) were used to summarize time-dependent treatment effects on the community of microphytobenthos, bacteria, macrofauna and foraminifera. This technique is based on a reduced-rank regression that is adjusted for temporal changes in the control treatment (Van den Brink & Ter Braak, 1998), thus allowing focus on the time-dependent treatment (induced winter hypoxia versus induced spring hypoxia versus undisturbed control) effects. The PRCs on the first axis are plotted against time in a PRC diagram. We used centered and standardized species abundances (mean subtracted, and divided by the standard deviation) to adjust for the various ways in which macrofauna, foraminifera, bacteria and microphytobenthos abundances are expressed. See Ter Braak & Šmilauer (1998) for a detailed description.

To test the explanatory potential of the co-occurring macrobenthos, bacteria and microphytobenthos as possible driving factors in the foraminiferal distributions, we used partial Redundancy Analyses (RDA) in addition to the PRC analysis. Standardized and centered abundances of the former three served as explanatory variables and foraminiferal assemblages were introduced as species data. Redundancy Analysis is the canonical version of Principal Component Analysis, in which the sample scores on the axes are constrained to being linear combinations of environmental variables (in this case, abundances of bacteria, microphytobenthos and macrobenthic taxa) that best separate species' responses along those axes. The potential of individual explanatory variables was first tested separately (marginal effects). A second analysis tested for conditional effects: a set of variables was chosen, starting with the one with the highest marginal effect, the second variable was subsequently added that had the highest explanatory potential in addition to the first; then a third with the highest explanatory value in addition to the first two, and so on. Direction and length of the vectors indicate the rate of change among foraminifera along the first two RDA axes and the contribution of the other benthos to the axes. It offers a graphic summary of the degree of (anti)correlation between them. The analyses were carried out using foraminiferal abundances and foraminiferal biovolume estimates.

Experimental Limitations

Before exploring the complex set of relationships and their potential influence on foraminifera at different stages of recovery, we will discuss the limitations of the

experiment. To distinguish recently living foraminifera from dead specimens, their protoplasm was stained with rose Bengal. Bearing in mind the drawbacks of this staining method (rose Bengal will stain dead but not yet decomposed foraminiferal protoplasm and may result in a overestimation of the number of living foraminifera; Murray & Bowser, 2000; Figueri et al., 2012), the well-stained specimens were assumed to represent foraminifera that survived and/or proliferated during or after the period of hypoxia. Oxygen concentrations in the top layer of the sediment re-established within a few days after removal of the plastic sheets (Van Colen et al., 2012). Hence, all sample moments (including field observations that took place 12 days after removal of the plastic sheets used for the winter hypoxia treatment) were taken from re-oxygenated sediments that, in general, have high decomposition rates for labile organic matter (such as foraminiferal protoplasm). By selecting only specimens fully filled with brightly stained cytoplasm except for the ultimate chamber, the chance of false positive vital staining was further reduced.

The experimental set-up had a limited number of sampling occasions, chosen at the scale of months because the original purpose of the experiment was designed to follow macrofaunal recovery and the relationship between carbon cycling and macrofaunal diversity (Rossi et al., 2009; Van Colen et al., 2012). Foraminifera can respond more quickly than macrofauna to some types of environmental change, often at a scale of days or weeks (Alve, 1999 and references therein). Hence, the time scale of sampling was not optimal for foraminiferal studies. Notwithstanding, the data presented compare the development within the foraminiferal assemblage to dynamics of other biota during recovery after hypoxia.

The large size of the patches (16 m^2) permitted multiple within-patch subsamples taken in plots of 0.25 m^2. This enabled evaluation of foraminiferal variability at small scales, which is important for environmental studies. Schönfeld et al. (2012) recommended the use of at least three replicate samples for foraminiferal bio-monitoring studies to obtain a good estimate of population distributions. We used four replicates.

In accordance with the recommendations by Schönfeld et al. (2012), we studied samples derived from the first centimeter below the sediment-water interface. Unfortunately, this method does not allow studying vertical migration of foraminifera through the sediment. Consequently, we can only speculate about the contribution of vertical migration to the observed foraminiferal dynamics in the experiment.

Taxonomic Remarks

The second most abundant foraminiferal species in the assemblage was *Ammonia* cf. molecular type T6 in Hayward et al. (2004), referred to as *Ammonia tepida* in De Nooijer et al. (2006, 2008 and 2009) for the Dutch Wadden Sea, and referred to as *Ammonia beccarii* by Moodley & Hess (1992) and Moodley & Van Weering (1993) for specimens occurring in the Dutch Scheldt Estuary. Hayward et al. (2004) made a strong case that both *Ammonia* species names should be abandoned for these specimens, but since we lack a taxonomic revision of the genus *Ammonia*, we use *A. beccarii* in accordance with historical local terminology.

RESULTS

In both experiments, the hypoxia resulted in mortality of all macrobenthic species (Van Colen et al., 2008; Rossi et al., 2009; Van Colen et al., 2010a, b) and a sharp decline in the abundance of (meiofaunal) nematodes (Van Colen et al., 2009), but did not affect bulk organic carbon concentration or mineralization rates (Rossi et al., 2009; Van Colen et al., 2012). Oxygen concentrations in the top layer of the sediment re-established to pre-hypoxia levels within a few days after removal of the plastic sheets (Van Colen et al., 2012). Twelve days after the winter hypoxia treatment ended, a field observation at the treatment sites revealed that the foraminifera were abundant (foraminiferal density of *H. germanica* was 394 ± 104, *A. beccarii* 39.8 ± 9.0 and *E. excavatum* 47.5 ± 2.6 specimen per 3.14 cm^3).

The variation in abundance and total biovolume of each of the three foraminiferal species did not differ significantly between samples taken in June and September (CJ and CS) in the natural, undisturbed sediments (Fig. 4A, B, C; Appendix 3). However, the recolonization stage (2 or 5 months after the winter hypoxia) and timing of disturbance (winter or late spring) did produce dissimilarities in assemblage composition and in the abundance and biovolume of the three foraminiferal species in the assemblage. These differences are described below.

The foraminiferal assemblage comprised three species *H. germanica*, *A. beccarii* and *E. excavatum* (Table 2, Appendix 1). The most abundant species was *H. germanica,* which was responsible for 57–84% of the observed differences among assemblages sampled in control and hypoxia-treated patches at different stages of recovery (Table 3 and Appendices 2, 3). Specimens of *A. beccarii* had relatively large test sizes, resulting in a high average biovolume per individual.

Timing: Winter Hypoxia versus Spring Hypoxia (CJ-CS-2J-2S)

Differences in the foraminiferal assemblages were observed between the patches that recovered for two months after hypoxia induced in different seasons. The overall dissimilarity due to timing of disturbance was 27%. The dissimilarity between control and experimental assemblages was larger two months after the winter hypoxia (CJ vs. 2J, 33%), than after the spring hypoxia (CS vs. 2S, 22%; Appendix 2). In the case of the individual species, only *E. excavatum* was significantly more abundant two months after the winter hypoxia than two months after the spring hypoxia (2J > 2S, Fig. 4C, Appendix 3, $p=0.03$). *Haynesina germanica* contributed more to the observed differences between winter and spring treatments and their corresponding control patches than *A. beccarii* and *E. excavatum* and in the opposite direction (Fig. 4A, B, C, Appendix 2). *Haynesina germanica* was more abundant two months after the spring than after the winter hypoxia (2J < 2S, $p=0.19$)

TABLE 2. Foraminiferal abundance and biovolume after hypoxia. (a) Mean abundance (± 1SE) of foraminifera in the top 1 cm of the sediment surface (no x 3.14 cm^{-3}), (b) average biovolume per specimen in the top 1 cm of the sediment surface (mm^3), (c) mean total foraminiferal biovolume in the top 1 cm of the sediment surface (mm^3).

	Winter hypoxia patches				Spring hypoxia patches	
	2J	SE	5S	SE	2S	SE
(a)						
Haynesina germanica	1.17E+02	± 1.11E+01	1.55E+02	± 1.68E+01	1.92E+02	± 3.07E+01
Ammonia beccarii	3.54E+01	± 4.97E+00	3.61E+01	± 1.15E+01	2.58E+01	± 7.56E+00
Elphidium excavatum	1.90E+01	± 3.24E+00	0.00E+00	± 0.00E+00	2.00E+00	± 1.41E+00
(b)						
Haynesina germanica	1.27E-03	± 1.31E-04	2.43E-03	± 1.58E-04	2.78E-03	± 8.86E-05
Ammonia beccarii	9.98E-03	± 1.33E-03	4.19E-03	± 6.70E-04	5.34E-03	± 6.35E-04
Elphidium excavatum	4.37E-03	± 6.84E-04			3.85E-03	± 9.04E-04
(c)						
Haynesina germanica	1.50E-01	± 2.46E-02	3.77E-01	± 4.91E-02	5.29E-01	± 7.65E-02
Ammonia beccarii	3.71E-01	± 1.02E-01	1.40E-01	± 4.06E-02	1.36E-01	± 3.76E-02
Elphidium excavatum	8.62E-02	± 2.66E-02			1.72E-02	± 1.13E-02
	Control patches					
	CJ	SE	CS	SE		
(a)						
Haynesina germanica	1.56E+02	± 3.32E+01	1.01E+02	± 1.93E+01		
Ammonia beccarii	1.98E+01	± 3.52E+00	2.20E+01	± 5.00E+00		
Elphidium excavatum	2.47E+01	± 6.48E+00	8.47E+00	± 3.20E+00		
(b)						
Haynesina germanica	1.70E-03	± 1.66E-04	1.84E-03	± 1.08E-04		
Ammonia beccarii	5.24E-03	± 9.89E-04	3.24E-03	± 2.88E-04		
Elphidium excavatum	4.47E-03	± 3.39E-04	4.71E-03	± 8.25E-04		
(c)						
Haynesina germanica	2.49E-01	± 3.33E-02	1.89E-01	± 4.62E-02		
Ammonia beccarii	1.04E-01	± 2.36E-02	7.27E-02	± 2.13E-02		
Elphidium excavatum	1.09E-01	± 2.74E-02	3.31E-02	± 1.06E-02		

and also the mean total biovolume of this species was more than three times higher two months after the spring hypoxia than two months after the winter hypoxia (2J < 2S, Appendix 3, p=0.03). Although the effect of hypoxia timing on the mean total biovolume of *A. beccarii* was not significant (Appendix 3, p=0.19), the trend for total biovolume of this species indicated higher abundances and per-individual biovolumes two months after the winter hypoxia than two months after the spring hypoxia, in contrast with *H. germanica* (Fig. 4G, H).

TABLE 3. Statistical analyses of the foraminiferal abundance of the assemblage (ANOSIM). The Two-Way ANOSIM tests for the combined effect of time and treatment on the foraminiferal assemblage due to timing of disturbance and recovery development (taxa in). The One-Way ANOSIM tests for the effect of recovery stages on the foraminiferal assemblages (taxa in). Bold numbers represent significant differences.

	R	P
Two-Way ANOSIM		
2J - CJ - 2S - CS		
Time	0.34	**0.03**
Treatment	0.20	0.07
2J - CJ - 5S - CS		
Time	0.27	**0.04**
Treatment	0.17	0.10
One-Way ANOSIM		
2S - 5S - CS	0.22	0.08

COMMUNITY RECOVERY AFTER WINTER HYPOXIA (CJ-CS-2J-5S)

The dissimilarity between foraminiferal assemblages in the patches exposed to the winter hypoxia and the concurrent control patches was higher in September after five months of recovery compared to June after two months of recovery (CJ vs. 2J and CS vs. 5S, Appendix 2). At species level, the abundance and mean total biovolume of *H. germanica* increased in the recovery patches whereas they decreased in the control patches (Fig. 4A, D, and G). *Ammonia beccarii* had a significantly higher biovolume in the recovery patches in June (2J vs. CJ and 5S vs. CS, Appendix 3, p=0.03 and p=0.11). Although the estimated per-individual biovolume of *A. beccarii* in the recovery patches reduced by more than half from June to September (2J > 5S), the total biovolumes of *A. beccarii* did not differ significantly between these recovery stages (Fig. 4H, Appendix 3, p=0.11).

COMMUNITY RECOVERY IN SEPTEMBER (CS-5S-2S)

In September, the dissimilarity between the patches treated with hypoxia and their concurrent control patches (CS vs. 2S and CS vs. 5S) was greater than the dissimilarity between the winter and spring hypoxia treatments (2S vs. 5S, Appendix 2). *Haynesina germanica* contributed most to these observed dissimilarities. The highest September density of *H. germanica* was observed in the patches that had recovered for two months after the spring hypoxia (2S

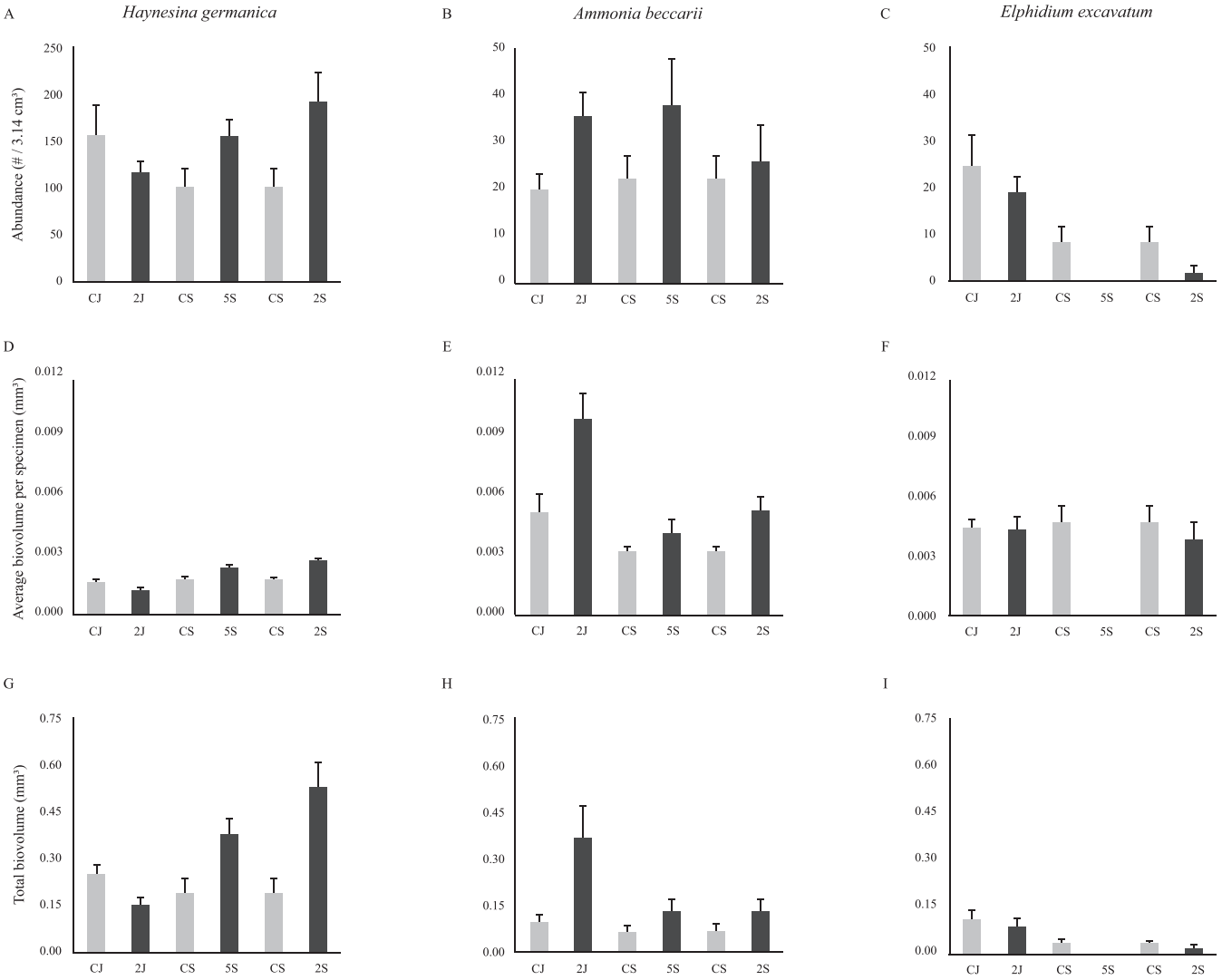

FIGURE 4. (A, B, C) Abundances of the three most prominent species *Haynesina germanica*, *Ammonia beccarii* and *Elphidium excavatum*. (D, E, F) Average biovolumes (mm³) of *H. germanica*, *A. beccarii* and *E.excavatum*. (G, H, I) Total biovolumes (mm³) of *H. germanica*, *A. beccarii and E. excavatum*.

> 5S > CS, Fig. 4A), while the abundance of *A. beccarii* showed smaller and non-significant differences (5S > 2S > CS, Fig. 4B, Appendix 3). *Elphidium excavatum* abundances in September were very low (<10 individuals) in the control (CS) and the two-month recovery patches (2S). *Elphidium excavatum* was absent in the patches that recovered for five months after the winter hypoxia (Fig. 4C). After the spring hypoxia, the total biovolume of *H. germanica* was significantly higher in the experimental patches than in the control (2S > CS, Appendix 3, p=0.04). The total biovolumes of *A. beccarii* did not differ significantly between these plots in September (Fig. 4H, Appendix 3, p=0.11).

Correlations With Macrofauna, Microphytobenthos and Bacteria

The main changes in abundance within the benthic community (i.e., foraminifera, macrofauna, bacteria and microphytobenthos) are summarized in principal response curves (PRC, Fig. 5). The PRCs illustrate the differential community response to the induced hypoxia, relative to changes in the control samples. The main type of community change (after temporal variability in control samples was filtered out), as highlighted by the winter hypoxia PRC, remains consistent 2 and 5 months after the hypoxia. The initial response to the spring hypoxia is similar to winter hypoxia response, but deviates more from the control (Fig. 5). The species weight for the two most dominant foraminiferal species, *H. germanica* and *A. beccarii,* point in the opposite direction of most of the community. The abundances of the macrofaunal species *Eteone longa* (Polychaeta), and especially *Heteromastus filiformis* (Polychaeta) and large (>5 mm) *Macoma balthica* (Bivalvia), have the highest positive species weights, indicating that their reduced abundance contributed strongly to the negative values of the presented PRC. If we consider macrofauna, bacteria and microphytobenthos as the main drivers of foraminiferal dynamics, their abundances could account for a maximum of 34% of

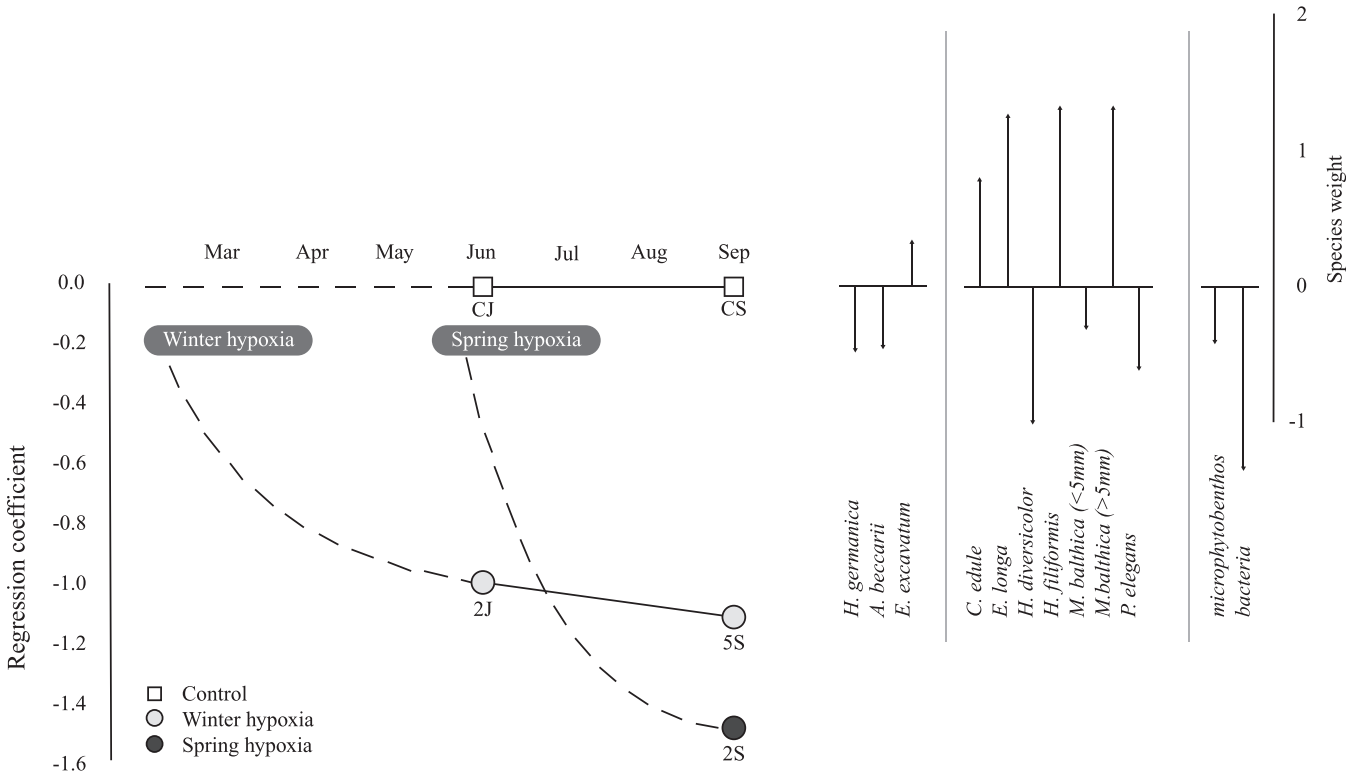

FIGURE 5. Principle Response Curve (PRC) of Scheldt Estuary biota. Regression coefficients on the first PRC summarize the response of the benthic community to the experimental treatments across time. Species weights can be interpreted as the species' affinities with the PRC; see Methods (Statistical Analysis) for further explanation. The percentage of total variance accounted for by "time" was 14% and by "time treatment" was 57%; 82% of the variance explained by the treatment regime was captured by the first PRC axis.

variance in the foraminiferal abundance data (Fig. 6A), and 49% of variance within the foraminiferal biovolume data (Fig. 6B). Figure 6A suggests that the explanatory variables (i.e., macrofaunal abundances, bacteria and microphytobenthos) may have contributed more to the abundances of *H. germanica* than to *A. beccarii and E. excavatum*. The ordination diagrams (Fig. 6A, B) indicate an inverse correlation between abundances and biovolumes of *H. germanica*, and the abundances of most macrofaunal species. Notably, the analyses indicate that, in their potential role as explanatory variables, the non-foraminiferal community members may have had a stronger impact on biovolumes of *A. beccarii* (Fig. 6B), than on the abundance of this species (Fig. 6A). The second axis in the abundance-based RDA is of little relevance, explaining only 3% of total variance in species data.

DISCUSSION

Hypoxia can have a major impact on benthic communities, including foraminifera (Gooday et al., 2009; Levin et al., 2009). Nonetheless, our findings show that the same species, *Haynesina germanica*, *Ammonia beccarii* and *Elphidium excavatum*, dominated the foraminiferal assemblage throughout community recovery after experimentally-induced hypoxia as in the surrounding (control) sediment. There were, however, changes in their abundances and biovolumes depending on timing of disturbance (winter hypoxia vs. spring hypoxia) and recovery stage (2 or 5 months after hypoxia). Various factors, including sulfide toxicity, oxygen concentration, food availability, predation and macrofaunal bioturbation, probably played a role over the entire recovery period, but their relative contributions to the dynamics of the foraminiferal assemblage seemed to vary with time and between control and experimental patches.

COMMUNITY RESPONSE TO HYPOXIA

At the end of hypoxia, directly after removing sheets used to induce hypoxia, the sediment was black and anoxic (see Montserrat et al., 2008). The surrounding sediment was well-oxygenated. Interestingly, we observed numerous foraminifera, especially *H. germanica*, in samples taken during the field observation in April, less than two weeks after hypoxia. The total foraminiferal abundance in the experimental patches was almost three times higher compared to two months later in June.

Nematodes, just like their foraminiferal companions in the meiobenthic community, were expected to better tolerate hypoxic conditions (Moodley et al., 1997; Duijnstee et al., 2003, 2005) than macrofauna, which were killed by the induced hypoxia (Van Colen et al., 2008 and references therein; Rossi et al., 2009). Although the winter hypoxia did not result in complete mortality of the nematode assemblage, their abundance was reduced by roughly 70% directly after the hypoxic period in the treated patches (Van Colen et al., 2009), in contrast to the foraminifera.

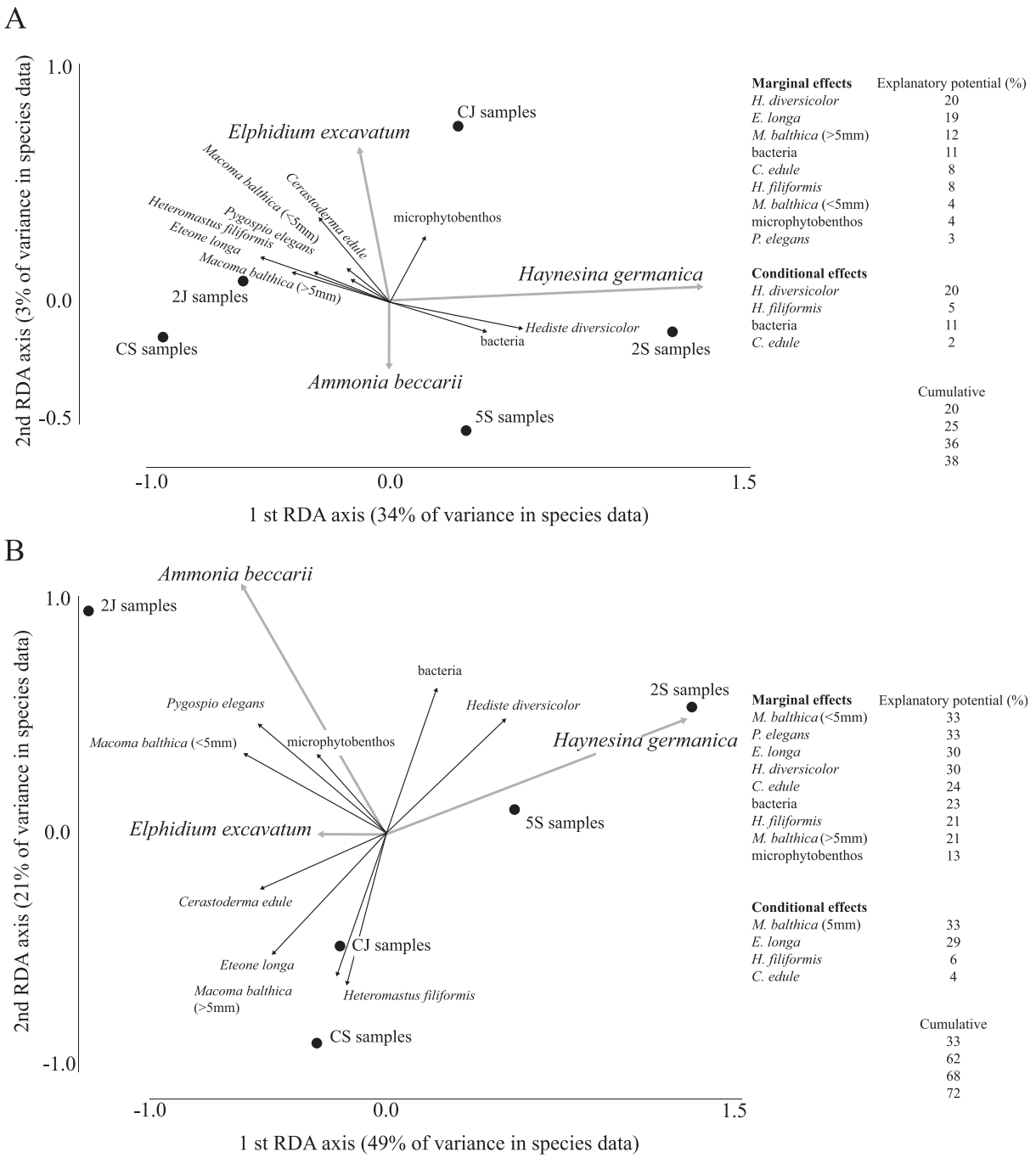

FIGURE 6. Redundancy Analyses (RDA) of foraminiferal species (wide, grey arrows) and the remaining benthos (narrow, black arrows). Foraminiferal abundances were used in (A); foraminiferal biovolumes were used in (B). Macrofaunal, bacterial and microphytobenthos abundances were used as explanatory variables and the RDA axes are linear combinations of these abundances. First and second RDA axes are shown, as well as marginal and conditional effects; see Methods (Statistical Analysis) for further explanation.

COMMUNITY RECOVERY: FIRST STAGE

Field observations made 12 days after the hypoxia-inducing black sheets were removed on March 30, 2005, indicated re-oxygenated surficial sediments and extremely abundant *H. germanica,* which are small, opportunistic foraminifera known to feed on bacteria. *Elphidium excavatum* also apparently thrived in this microbial bloom, as the highest densities reported for this species were recorded during the preliminary observations. Samples taken on June 10 revealed abundant *A. beccarii* and nematodes, thriving on the bloom of microphytobenthos observed beginning in April (Van Colen et al., 2008, 2012) and continuing into June (Montserrat et al., 2008). The average individual biovolume of *A. becarrii* was also more than double that seen in any other set of samples. In contrast, by June the abundance of *H. germanica* dropped to roughly one quarter and *E. excavatum* to about half that seen in the April field

observations. Further details regarding this succession and supporting observations of feeding strategies of these foraminiferal species are discussed below.

Bacteria are widely recognized as food sources for foraminifera (e.g., Bernhard & Bowser 1992; Langezaal et al., 2005). Muller & Lee (1969) even suggested that bacterial feeding is a prerequisite for sustained reproduction. Consistently, the RDA analysis indicates a positive correlation of *H. germanica* (abundances and total biovolumes) with bacterial biomass. In addition, field observations within 12 days after the winter treatment ended indicated extremely abundant *H. germanica*.

The next apparent development with the onset of recovery from hypoxia was a microphytobenthos bloom within the first month (Van Colen et al., 2008, 2012). After abundance peaked at day 28, it decreased, but remained higher than control patches for about three months. The abundance of nematodes recovered to near control values within eight weeks (Van Colen et al., 2009), primarily near the end of the early stage of recovery. The early stage of macrofaunal recovery was characterized first by lateral colonization by, most notably, the gastropod *Hydrobia ulvae* and subsequently (starting at 28 days following the end of the hypoxia) by an increase of the small tube-building polychaete *Pygospio elegans* (Van Colen et al., 2008, 2012; Rossi et al., 2009).

The observed species-specific foraminiferal response to hypoxia is similar to that reported by Bouchet et al. (2007). They investigated the response of foraminiferal assemblages to short-term biogeochemical disturbances in marine sediments of the Marennes-Oléron Bay (SW France), where hypoxia may be involved in summer oyster mortality. Species-specific responses were observed, with *A. tepida* relatively tolerant of elevated temperature and hypoxic conditions, and *H. germanica* more sensitive to organic degradation and hypoxia.

As reflected by the strong increase in biovolume in June, *A. beccarii* thrived in the benthic algal blooms in the treatment patches (Fig. 4E). The PRC based on foraminiferal abundance (Fig. 5) and the RDA analysis based on foraminiferal biovolumes (Fig. 6B) respectively explored relationships between responses of foraminifera and other faunal and microfloral elements. The PRC and RDA revealed that the abundance and biovolumes of *A. beccarii* correlated positively with microphytobenthos, consistent with results from several previous studies. Bacteria (Langezaal et al., 2005; Pascal et al., 2008), as well as algal material (Moodley et al., 2000), were shown to be ingested by *A. beccarii*; their microphytobenthos uptake resembled the pattern of uptake found for nematodes (Middelburg et al., 2000). Previous studies have reported high abundances of *Ammonia* spp. at sites around sewage outfalls (e.g., Thomas et al., 2000) from Long Island Sound.

As noted above, the microphytobenthos bloom also positively influenced the nematode assemblage (Van Colen et al., 2009). If competition for food between *A. beccarii* and nematodes plays a role in their population dynamics, one could argue that food was not limiting during the recovery period. Moreover, a recent experimental study by Dupuy et al. (2010) revealed predation on live nematodes, copepods and gastropod larvae by *Ammonia* individuals identified as *Ammonia tepida* (likely the same species as our *A. beccarii*; see 'taxonomic remarks' in 'Material and Methods' section). If nematodes were preyed upon by *A. beccarii*, the microphytobenthos bloom provided both direct and indirect food sources for *A. beccarii*.

Our results, showing a decline in abundances of the other two foraminiferal taxa as microphytobenthos abundance increased, are consistent with observations of Moodley et al. (2000) indicating lack of algal uptake by *H. germanica* and *E. excavatum*. However, Ward et al. (2003) demonstrated that *H. germanica* can actively feed on pennate diatoms, but not on sewage-derived degrading organic matter. Clearly more studies of food requirements of these foraminifera would aid interpretation of succession studies such as ours.

Besides a response to fluctuations in microphytobenthos and bacterial densities, additional mechanisms might elucidate the sharp decrease in the densities of *H. germanica* and *E. excavatum* in the uppermost centimeter of the sediment during the early stage of recovery. Although we cannot verify this with the current data, foraminifera might have migrated downwards following the re-oxygenation of the sediment. Vertical foraminiferal migration in response to fluctuations in oxygen concentrations has previously been reported. Geslin et al. (2004), for instance, showed that foraminifera can actively move to preferred habitats, and oxygen was suggested to be the main driver for this foraminiferal migration. Alve & Bernhard (1995) indicated that foraminifera actively migrated downwards to their original depth distributions after re-oxygenation of sediments that had experienced a period of anoxia. And although their results were not based on material from intertidal muds, which are characterized by steep geochemical gradients and limited vertical distribution, we cannot rule out some migratory effect on our observed distributions. Moreover, part of a decline in the abundance of the foraminifera in the top centimeter of the sediment may be ascribed to passive transport (as previously reported by Moodley, 1990). Although the total macrofaunal biomass was very low in the recovering patches in June (10 times lower compared to the controls), the abundance of macrofauna was actually higher in the recovery patches compared to the controls (Rossi et al., 2009). This high abundance of tube-building polychaete species (Van Colen et al., 2008) may have resulted in stronger reworking of the sediment surface, transporting foraminifera to layers below the top centimeter of the sediment.

Community Recovery (June to September): Second Stage

Similar to the first stage of recovery, an inverse correlation between macrofaunal density and the abundance of *Haynesina germanica* is observed during prolonged recovery (Fig. 4A, D, G and Fig. 6A, B). Contrastingly, in the undisturbed control patches both macrofaunal abundances and the density of *H. germanica* decreased, indicating a positive correlation which suggests that the observed inverse correlation in the treated patches does not imply a linear causal relationship between the abundances of macrofauna and *H. germanica*. The dynamics in

abundance of *Ammonia beccarii* observed in the recovery patches do not seem to be strongly affected by macrofaunal abundances (Fig. 6).

Given the widely accepted concept that bioturbation and bioirrigation promote the occurrence of oxygen-dependent meiobenthos (Aller, 1983; Ray & Aller, 1985; Fenchel, 1996; Zorn et al., 2006), we expected that high abundances of foraminifera would indicate high densities of macrofauna and *vice versa*. In the second stage of community recovery, from two to five months after hypoxia, macrofauna assemblages became dominated by larger species, such as the polychaete *Heteromastus filiformis*, inhabiting deeper sediment layers (Rossi et al., 2009). While the total macrofaunal biomass tripled, its abundance decreased (~60%) in the disturbed patches. Simultaneously, the control patches revealed a small increase in macrofaunal biomass (approximately 6%) and a decrease in abundance of ~30% (Rossi et al., 2009). In the patches disturbed by winter hypoxia, the replacement of numerous small tube-building polychaetes by fewer large macrofaunal elements such as the polychaete *Heteromastus filiformis* co-occurred with an increase in abundance of *H. germanica*.

The contradiction between the expected and observed relationship between macrofaunal abundance and foraminiferal densities (of especially *H. germanica* in the recovery patches) may be attributable to several factors. First, the expected increase in micro-niches, mediated through macrofaunal bioturbation, may have predominantly affected the deeper sediment layers. As mentioned before, a higher availability of foraminiferal niches below the top centimeter of the sediment may lead to lower foraminiferal densities in the top layer by stimulating downwards migration of foraminifera. Analyzing the top centimeter of the sediment, in correspondence with the recommendations for foraminiferal bio-monitoring studies (Bouchet et al., 2012; Schönfeld et al., 2012), may lead to an unexpected outcome if processes in deeper sediment layers become increasingly important for foraminiferal dynamics. Hence, in the hypoxia-exposed patches, an increased physical disturbance and predation pressure due to increased macrofaunal density may have counteracted and outweighed the expected positive effects of bioturbation as a community-engineering force for the establishment of additional micro-niches. Moreover, sediment reworking activities by macrofauna may injure foraminifera. For locomotion and feeding, foraminifera use reticulopodia, a network of fine extensions of the cytoplasm, emerging from the test's aperture(s). This network ventures out into the surrounding sediment in a sphere with a radius many times the test diameter. Higher abundances of macrofauna amplify the amount of sediment reworking and, consequentially might augment the disturbance by severing reticulopodia, which requires very little physical disturbance (Duijnstee, personal communication 2010). In addition, macrofaunal predation pressure may negatively affect foraminiferal densities. Buzas (1978, 1982) reported that foraminifera are a food source for macrofauna. He demonstrated that predation on foraminifera plays a regulatory role in foraminiferal densities.

In summary, an unexpected inverse relation between foraminiferal and macrofaunal abundances in the recovery patches may be attributable to direct biotic interactions, as well as environmental factors. Hypoxia disturbed the interplay of direct (e.g., predation, availability of niches) and indirect (e.g., food availability) interactions among macrofauna and foraminifera (Fig. 5), and this may have resulted in the observed differences among the foraminiferal species and macrofauna in the disturbed versus control patches.

Effects of Winter versus Spring Hypoxia

The first PRC for the benthic community (species-specific macrofaunal and foraminiferal abundances, bacteria and microphytobenthos, Fig. 5), shows a relatively consistent degree of deviation from the control patches, regardless of timing of treatment and recovery. This pattern, however, is strongly dominated by the almost complete loss and slow recovery of the larger macrofauna (i.e., *Heteromastus filiformis* and *Macoma balthica* > 5mm) associated with the hypoxia, and, to a lesser extent, with the foraminiferal response or the recovery dynamics of the entire benthic community.

When individual taxa are followed throughout the experiments, a more differentiated pattern emerges. The early stages of succession of the macrofaunal community, within 2 months after hypoxic conditions eliminated most macrofauna, differed in terms of species richness and abundance depending on whether hypoxia took place in winter or late spring (Rossi et al., 2009). The recovering macrofaunal community comprised more individuals and species two months after the winter hypoxia (2J) compared to two months after the spring hypoxia (2S; Rossi et al., 2009). The differences found between the macrofaunal communities were ascribed to seasonal differences in the availability of larvae and juveniles for recruitment (Rossi et al., 2009).

De Nooijer et al. (2007) observed that, on intertidal flats in the Dutch Wadden Sea, abundances of *Haynesina germanica* peaked in spring, whereas those of *Ammonia* (*tepida*) had their optimum in summer. Foraminiferal abundances in our control patches (Fig. 4A and B) seem consistent with the findings of De Nooijer et al. (2007). For our recovering patches two months after winter and spring hypoxia, the patterns point another direction: the timing of the hypoxic treatment resulted in higher abundances of *H. germanica* and higher per-individual and total biovolumes (Fig. 4A, D, G) two months after the spring hypoxia compared to two months after the winter hypoxia. The opposite was found for *Ammonia beccarii,* which was more abundant and had higher per-individual and total biovolumes two months after the winter hypoxia.

In summary, a species-specific foraminiferal response was observed for the timing of hypoxia. Altogether, the timing of hypoxia, the interplay of direct and indirect interactions within the community, and the dissimilarities in the lateral availability of larvae and juveniles for repopulation, may have led to the species-specific foraminiferal responses. Similarly, as suggested for the macrofauna, timing differences among the foraminiferal species reproduction may have contributed to the foraminiferal species-specific responses to the timing of hypoxia.

CONCLUSIONS

The variation in abundance and total biovolume of each of the three foraminiferal species, *Haynesina germanica*, *Ammonia beccarii* and *Elphidium excavatum*, did not differ significantly between samples taken in June and September in the natural, undisturbed sediments. Differences in the foraminiferal assemblages were observed between the patches that recovered for two months after hypoxia in winter or late spring. Furthermore, the dissimilarity was even higher between the foraminiferal assemblage in the patches exposed to the winter hypoxia in September, after five months of recovery, and the concurrent control patches. In September, the dissimilarity between the patches treated with hypoxia and their concurrent control patches was greater than the dissimilarity between the winter and spring hypoxia. In the patches exposed to experimentally-induced hypoxia, the benthic foraminifera showed species-specific and complex patterns of development. During community recovery, the expected positive impact of macrofauna on foraminiferal abundances through bioturbation and bioirrigation was not observed. In the patches that had been exposed to hypoxia, *H. germanica* decreased with increasing macrofaunal abundances during macrofaunal recovery. Contrasting results were found among the undisturbed control patches. The abundance of *H. germanica* in the upper centimeter of the sediment in the disturbed patches was inversely related to macrofaunal abundance whereas it was positively related in control patches. *Ammonia beccarii* instead seemed to be driven by the microphytobenthos surplus due to the lack of grazing after hypoxia-induced mortality of the macrofauna. Our study indicates species-specific dynamics in the abundance and biovolume of foraminifera in response to fluctuations in environmental factors.

ACKNOWLEDGMENTS

This is publication number DW-2012-1009 of the Darwin Center for Biogeosciences, which partially funded this project. Financial support to M.W. is acknowledged from the Netherlands Organization for Scientific Research (NWO, fellowship #863.06.006) and the U.K.'s Natural Environment Research Council (fellowship #NE/J018856/1).

REFERENCES

Aller, R. C., 1983, The importance of the diffusive permeability of animal burrow linings in determining marine sediment chemistry: Journal of Marine Research, v. 41, p. 299–322.

Alve, E., 1999, Colonization of new habitats by benthic foraminifera: a review: Earth-Science Reviews, v. 46, p. 167–185.

Alve, E., and Bernhard, J. M., 1995, Vertical migratory response of benthic foraminifera to controlled oxygen concentrations in an experimental mesocosm: Marine Ecology Progress Series, v. 116, p. 137–151.

Bernhard, J. M., 1993, Experimental and field evidence of Antarctic foraminiferal tolerance to anoxia and hydrogen-sulfide: Marine Micropaleontology, v. 20, p. 203–213.

Bernhard, J. M., and Bowser, S. S., 1992, Bacterial biofilms as a trophic resource for certain benthic foraminifera: Marine Ecology Progress Series, v. 83, p. 263–272.

Bouchet, V. M. P., Debenay, J., Sauriua, P., Radford-Knoeryd, J., and Soletchnike, P., 2007, Effects of short-term environmental disturbances on living benthic foraminifera during the Pacific oyster summer mortality in the Marennes-Oléron Bay (France): Marine Environmental Research, v. 64, p. 358–383.

Bouchet, V. M. P., Sauriau, P., Debenay, J., Mermillod-Blondin, F., Schmidt, S., Amiard. J., and Dupas, B., 2009, Influence of the mode of macrofauna-mediated bioturbation on the vertical distribution of living benthic foraminifera: First insight from axial tomodensitometry: Journal of Experimental Marine Biology and Ecology, v. 371, p. 20–33.

Bouchet, V. M. P., Alve, E., Rygg, B., and Telford, R. J., 2012, Benthic foraminifera provide a promising tool for ecological quality assessment of marine waters: Ecological Indicators, v. 23, p. 66–75.

Buzas, M. A., 1978, Foraminifera as prey for benthic deposit feeders—results of predator exclusion experiments: Journal of Marine Research, v. 36, p. 617–625.

Buzas, M. A., 1982, Regulation of foraminiferal densities by predation in the Indian river, Florida: Journal of Foraminiferal Research, v. 12, p. 66–71.

Conley, D. J., Carstensen, J., Ærtebjerg, G., Christensen, P. B., Dalsgaard, T., Hansen, J. L. S., and Josefson. A. B., 2007, Long-term changes and impacts of hypoxia in Danish coastal waters: Ecological Applications, v. 17, p. 165–184.

De Nooijer, L. J., Duijnstee, I. A. P., and Van der Zwaan, G. J., 2006, Novel application of MTT reduction: A viability assay for temperate shallow-water benthic foraminifera: Journal of Foraminiferal Research, v. 36, p. 195–200.

De Nooijer, L. J., Reichart, G. J., Duenas-Bohorquez, A., Wolthers, M., Ernst, S. R., Mason, P. R. D., and Van der Zwaan, G. J., 2007, Copper incorporation in foraminiferal calcite: Results from culturing experiments: Biogeosciences, v. 4, p. 493–504.

De Nooijer, L. J., Duijnstee, I. A. P., Bergman, M. J. N., and Van der Zwaan, G. J., 2008, The ecology of benthic foraminifera across the Frisian front, southern North Sea: Estuarine, Coastal and Shelf Science, v. 78, p. 715–726.

De Nooijer, L. J., Langer, G., Nehrke, G., and Bijma, J., 2009, Physiological controls on seawater uptake and calcification in the benthic foraminifer *Ammonia tepida*: Biogeosciences, v. 6, p. 2669–2675.

Diaz, R. J., and Rosenberg, R., 2008, Spreading dead zones and consequences for marine ecosystems: Science, v. 321, p. 926–929.

Diaz, R. J., Rosenberg, R., Rabalais, N. N., and Levin, L. A., 2009, Dead zone dilemma: Marine Pollution Bulletin, v. 58, p. 1767–1768.

Duijnstee, I. A. P., Ernst, S. R., and Van der Zwaan, G. J., 2003, Effect of anoxia on the vertical migration of benthic foraminifera: Marine Ecology Progress Series, v. 246, p. 85–94.

Duijnstee, I. A. P., De Nooijer, L. J., Ernst, S. R., and Van der Zwaan, G. J., 2005, Population dynamics of benthic shallow-water foraminifera: Effects of a simulated marine snow event: Marine Ecology Progress Series, v. 285, p. 29–42.

Dupuy, C., Rossignol, L., Geslin, E., and Pascal, P., 2010, Predation of mudflat meio-macrofaunal metazoans by a calcareous foraminifer, *Ammonia tepida* (Cushman, 1926): Journal of Foraminiferal Research, v. 40, p. 305–312.

Ehrenberg, G. C., 1840, Das grössere Infusorienwerke: Königliche Preussische Akademie der Wissenschaften, Abhandlungen, Berlin, p. 198–219.

Erwin, D. H., 2008, Macroevolution of ecosystem engineering, niche construction and diversity: Trends in Ecology and Evolution, v. 23, p. 304–310.

Fenchel, T., 1996, Worm burrows and oxic microniches in marine sediments. 1. Spatial and temporal scales: Marine Biology, v. 127, p. 289–295.

Figueri, B. O., Grenfell, H. G., Hayward, B. W., and Alfaro, A. C., 2012, Comparison of rose Bengal and CellTracker green staining for identification of live salt-marsh foraminifera: Journal of Foraminiferal Research, v. 42, p. 206–215.

Geslin, E., Heinz, P., Jorissen, F., and Hemleben, C., 2004, Migratory responses of deep-sea benthic foraminifera to variable oxygen conditions: laboratory investigations: Marine Micropaleontology, v. 53, p. 227–243.

Gooday, A. J., Bernhard, J. M., Levin, L. A., and Suhr, S. B., 2000, Foraminifera in the Arabian Sea oxygen minimum zone and other

oxygen-deficient settings: Taxonomic composition, diversity, and relation to metazoan faunas: Deep Sea Research Part II, v. 47, p. 25–54.

Gooday, A. J., Jorissen, F. J., Levin, L. A., Middelburg, J. J., Naqvi, S. W. A., Rabalais, N. N., Scranton, M., and Zhang, J., 2009, Historical records of coastal eutrophication-induced hypoxia: Biogeosciences, v. 6, p. 1707–1745.

Gray, J. S., Wu, R. S. S., and Or, Y. Y., 2002, Effects of hypoxia and organic enrichment on the coastal marine environment: Marine Ecology Progress Series, v. 238, p. 249–279.

Gross, O., 2000, Influence of temperature, oxygen and food availability on the migrational activity of bathyal benthic foraminifera: Evidence by microcosm experiments: Hydrobiologia, v. 426, p. 123–137.

Hayward, B. W., Holzmann, M., Grenfell, H. R., Pawlowski, J., and Triggs, C. M., 2004, Morphological distinction of molecular types in *Ammonia* — towards a taxonomic revision of the world's most commonly misidentified foraminifera: Marine Micropaleontology, v. 50, p. 237–271.

Hammer, Ø., Harper, D. A. T., and Ryan, P. D., 2001, PAST: Paleontological Statistics Software Package for education and data analysis: Palaeontologica Electronica 4.1.

Jorissen, F. J., Barmawidjaja, D. M., Puskaric, S., and Van der Zwaan, G. J., 1992, Vertical distribution of benthic foraminifera in the northern Adriatic Sea – the relation with the organic flux: Marine Micropaleontology, v. 19, p. 131–146.

Jorissen, F. J., de Stigter, H. C., and Widmark, J. G. V., 1995, A conceptual model explaining benthic foraminiferal microhabitats: Marine Micropaleontology, v. 26, p. 3–15.

Josefson, A. B., and Widbom, B., 1988, Differential response of benthic macrofauna and meiofauna to hypoxia in the Gullmar fjord basin: Marine Biology, v. 100, p. 31–40.

Langezaal, A. M., Jannink, N. T., Pierson, E. S., and Van der Zwaan, G. J., 2005, Foraminiferal selectivity towards bacteria: An experimental approach using a cell-permeant stain: Journal of Sea Research, v. 54, p. 256–275.

Levin, L. A., Ekau, W., Gooday, A. J., Jorissen, F. J., Middelburg, J. J., Naqvi, S. W. A., Neira, C., Rabalais, N. N., and Zhang, J., 2009, Effects of natural and human-induced hypoxia on coastal benthos: Biogeosciences, v. 6, p. 2063–2098.

Linke, P., and Lutze, G. F., 1993, Microhabitat preferences of benthic foraminifera – a static concept or a dynamic adaptation to optimize food acquisition: Marine Micropaleontology, v. 20, p. 215–234.

Linné, C., 1758, Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis: G. Engelmann (Lipsiae), v. 10, p. 1–824.

Lohrer, A. M., Thrush, S. F., and Gibbs, M. M., 2004, Bioturbators enhance ecosystem function through complex biogeochemical interactions: Nature, v. 431, p. 1092–1095.

Meysman, F. J. R., Galaktionov, E. S., Gribsholt, B., and Middelburg, J. J., 2006, Bioirrigation in permeable sediments: Advective pore-water transport induced by burrow ventilation: Limnology and Oceanography, v. 51, p. 142–156.

Middelburg, J. J., Barranguet, C., Boschker, H. T. S., Herman, P. M. J., Moens, T., and Heip, C. H. R., 2000, The fate of intertidal microphytobenthos carbon: An in situ C-13-labeling study: Limnology and Oceanography, v. 45, p. 1224–1234.

Montserrat, F., Van Colen, C., Degraer, S., Ysebaert, T., and Herman, P. M. J., 2008, Benthic community-mediated sediment dynamics: Marine Ecology Progress Series, v. 372, p. 43–59.

Montserrat, F., Van Colen, C., Provoost, P., Milla, M., Ponti, M., Van den Meersche, K., Ysebaert, T., and Herman, P. M. J., 2009, Sediment segregation by biodiffusing bivalves: Estuarine, Coastal and Shelf Science, v. 83, p. 379–391.

Moodley, L., 1990, Southern North Seafloor and subsurface distribution of living benthic foraminifera: Netherlands Journal of Sea Research, v. 27, p. 57–71.

Moodley, L., and Hess, C., 1992, Tolerance of infaunal benthic foraminifera for low and high oxygen concentrations: The Biological Bulletin, v. 183, p. 94–98.

Moodley, L., and Van Weering, T. C. E., 1993, Foraminiferal record of the Holocene development of the marine environment in the southern North Sea: Netherlands Journal of Sea Research, v. 31, p. 43–52.

Moodley, L., Van der Zwaan, G. J., Herman, P. M. J., Kempers, L., and Van Breugel, P., 1997, Differential response of benthic meiofauna to anoxia with special reference to foraminifera (Protista: Sarcodina): Marine Ecology Progress Series, v. 158, p. 151–163.

Moodley, L., Schaub, B. E. M., Van der Zwaan, G. J., and Herman, P. J. M., 1998, Tolerance of benthic foraminifera (Protista: Sarcodina) to hydrogen sulphide: Marine Ecology Progress Series, v. 169, p. 77–86.

Moodley, L., Boschker, H. T. S., Middelburg, J. J., Pel, R., Herman, P. M. J., De Deckere, E., and Heip, C. H. R., 2000, Ecological significance of benthic foraminifera: ^{13}C labelling experiments: Marine Ecology Progress Series, v. 202, p. 289–295.

Muller, W. A., and Lee, J. J., 1969, Apparent indispensability of bacteria in foraminiferan nutrition: Journal of Protozoology, v. 16, p. 471–478.

Murray, J. W., and Bowser, S. S., 2000, Mortality, protoplasm decay rate, and reliability of staining techniques to recognize 'living' foraminifera: A review: Journal of Foraminiferal Research, v. 30, p. 66–70.

Pascal, P., Dupuy, C., Richard, P., and Niquil, N., 2008, Bacterivory in the common foraminifer *Ammonia tepida*: Isotope tracer experiment and the controlling factors: Journal of Experimental Marine Biology and Ecology, v. 359, p. 55–61.

Passarelli, C., Olivier, F., Paterson, D. M., and Hubas, C., 2012, Impacts of biogenic structures on benthic assemblages: microbes, meiofauna, macrofauna and related ecosystem functions: Marine Ecology Progress Series, v. 465, p. 85–97.

Piña-Ochoa, E., Høgslund, S., Geslin, E., Cedhagen, T., Revsbech, N. P., Nielsen, L. P., Schweizer, M., Jorissen, J. F., Rysgaard, S., and Risgaard-Petersen, N., 2010a, Widespread occurrence of nitrate storage and denitrification among Foraminifera and Gromiida: Proceedings of the National Academy of Sciences of the United States of America, v. 107, p. 1148–1153.

Piña-Ochoa, E., Koho, K. A., Geslin, E., and Risgaard-Petersen, N., 2010b, Survival and life strategy of the foraminiferan *Globobulimina turgida* through nitrate storage and denitrification: Marine Ecology Progress Series, v. 417, p. 39–49.

Pucci, F., Geslin, E., Barras, C., Morigi, C., Sabbatini, A., Negri, A., and Jorissen, F. J., 2009, Survival of benthic foraminifera under hypoxic conditions: Results of an experimental study using the CellTracker green method: Marine Pollution Bulletin, v. 59, p. 336–351.

Rao, C. D., 1964, The use and interpretation of principal components analysis in applied research: Sankhyā: The Indian Journal of Statistics, Series A, v. 26, p. 329–358.

Ray, A. J., and Aller, R. C., 1985, Physical irrigation of relict burrows – implications for sediment chemistry: Marine Geology, v. 62, p. 371–379.

Risgaard-Petersen, N., Langezaal, A. M., Ingvardsen, S., Schmid, M. C., Jetten, M. S. M., Op den Camp, H. J. M., Derksen, J. W. M., Piña-Ochoa, E., Eriksson, S. P., and Nielsen, L. P., 2006, Evidence for complete denitrification in a benthic foraminifer: Nature, v. 443, p. 93–96.

Rossi, F., and Middelburg, J. J., 2011, Intraspecific diet shift of *Macoma balthica* during community reassembly in an estuarine intertidal flat: Estuarine, Coastal and Shelf Science, v. 92, p. 496–501.

Rossi, F., Forster, R. M., Montserrat, F., Ponti, M., Terlizzi, A., Ysebaert, T., and Middelburg, J. J., 2007, Human trampling as short-term disturbance on intertidal mudflats: Effects on macrofauna biodiversity and population dynamics of bivalves: Marine Biology, v. 151, p. 2077–2090.

Rossi, F., Gribsholt, B., Middelburg, J. J., and Heip, C., 2008, Context-dependent effects of suspension feeding on intertidal ecosystem functioning: Marine Ecology Progress Series, v. 354, p. 47–57.

Rossi, F., Vos, M., and Middelburg, J. J., 2009, Species identity, diversity and microbial carbon flow in reassembling macrobenthic communities: Oikos, v. 118, p. 503–512.

Schönfeld, J., Alve, E., Geslin, E., Jorissen, F. J., Korsun, S., and Spezzaferri, S., 2012, The FOBIMO (FOraminiferal BIo-MOnitoring) initiative—Towards a standardised protocol for

soft-bottom benthic foraminiferal monitoring studies: Marine Micropaleontology, v. 94, p. 1–13.

Ter Braak, C. J. F., and Šmilauer, P., 1998, CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4): Micro Computer Power, Ithaca, New York.

Terquem, O., 1875, Essai sur le classement des animaux qui vivent sur la plage et dans les environs de Dunkirque, v. 1, p. 1–54.

Thomas, E., Gapotchenko, T., Varekamp, J. C., Mecray, E. L., and Ten Brink, M. R. B., 2000, Benthic foraminifera and environmental changes in Long Island Sound: Journal of Coastal Research, v. 16, p. 641–655.

Van Colen, C., Montserrat, F., Vincx, M., Herman, P. M. J., Ysebaert, T., and Degraer, S., 2008, Macrobenthic recovery from hypoxia in an estuarine tidal mudflat: Marine Ecology Progress Series, v. 372, p. 31–42.

Van Colen, C., Montserrat, F., Verbist, K., Vincx, M., Steyaert, M., Vanaverbeke, J., Herman, P. M. J., Degraer, S., and Ysebaert, T., 2009, Tidal flat nematode responses to hypoxia and subsequent macrofauna-mediated alterations of sediment properties: Marine Ecology Progress Series, v. 381, p. 189–197.

Van Colen, C., Montserrat, F., Vincx, M., Herman, P. M. J., Ysebaert, T., and Degraer, S., 2010a, Long-term divergent tidal flat benthic community recovery following hypoxia-induced mortality: Marine Pollution Bulletin, v. 60, p. 178–186.

Van Colen, C., Montserrat, F., Vincx, M., Herman, P. M. J., Ysebaert, T., and Degraer, S., 2010b, Macrobenthos recruitment success in a tidal flat: Feeding trait dependent effects of disturbance history: Journal of Experimental Marine Biology and Ecology, v. 385, p. 79–84.

Van Colen, C., Rossi, F., Montserrat, F., Andersson, M. G. I., and Gribsholt, B., 2012, Organism-sediment interactions govern post-hypoxia recovery of ecosystem functioning: Plos One, v. 7(11), p. e49795.

Van den Brink, P. J., and Ter Braak, C. J. F., 1998, Multivariate analysis of stress in experimental ecosystems by Principal Response Curves and similarity analysis: Aquatic Ecology, v. 32, p. 163–178.

Van der Zwaan, G. J., Duijnstee, I. A. P., den Dulk, M., Ernst, S. R., Jannik, N. T., and Kouwenhoven, T. J., 1999, Benthic foraminifers: Proxies or problems? A review of paleoecological concepts: Earth-Science Reviews, v. 46, p. 213–236.

Ward, J. N., Pond, D. W., and Murray, J. W., 2003, Feeding of benthic foraminifera on diatoms and sewage-derived organic matter: An experimental application of lipid biomarker techniques: Marine Environmental Research, v. 56, p. 515–530.

Zhang, H., Ludsin, S. A., Mason, D. M., Adamack, A. T., Brandt, S. B., Zhang, X., Kimmel, D. G., Roman, M. R., and Boicourt, W. C., 2009, Hypoxia-driven changes in the behavior and spatial distribution of pelagic fish and mesozooplankton in the northern Gulf of Mexico: Journal of Experimental Marine Biology and Ecology, v. 381, p. S80–S91.

Zorn, M. E., Lalonde, S. V., Gingras, M. K., Pemberton, S. G., and Konhauser, K. O., 2006, Microscale oxygen distribution in various invertebrate burrow walls: Geobiology, v. 4, p. 137–145.

Received 16 October 2013
Accepted 15 November 2014

APPENDIX 1. Foraminiferal abundance per sample (no x 3.14 cm^{-3}).

	Foraminiferal abundances per sample (3.14 cm^3)		
Sample name (split)	*Haynesina germanica*	*Ammonia beccarii*	*Elphidium excavatum*
2J (1)	123	50	21
2J (3/4)	133	31	16
2J (1)	126	33	27
2J (1)	84	28	12
CJ (1/4)	80	28	28
CJ (3/4)	176	19	17
CJ (3/4)	236	21	41
CJ (1)	130	11	12
2S (1)	116	14	0
2S (3/4)	207	21	0
2S (1)	181	20	2
2S (1/2)	264	48	6
5S (1)	109	30	0
5S (1)	151	29	0
5S (3/4)	177	16	0
5S (3/4)	183	69	0
CS (1)	62	22	6
CS (1)	109	13	5
CS (1)	83	19	10
CS (3/4)	79	15	1
CS (3/4)	172	41	20

APPENDIX 2. SIMPER analyses of the contribution per taxa to observed differences between foraminiferal assemblages.

SIMPER - foraminiferal abundances				
Time and treatment	dissimilarity %	*H. germanica* %	*A. beccarii* %	*E. excavatum* %
CJ - CS - 2J - 5S	25.0	64.4	18.3	17.3
CJ - CS - 2J - 2S	26.8	70.8	14.2	15.0
CS - 5S - 2S	27.2	76.9	16.8	6.3
Time or treatment	%	%	%	%
CJ - CS	28.8	71.8	9.7	18.5
2J - CJ	21.7	67.4	19.6	13.0
5S - CS	28.6	70.8	20.5	8.7
2S - CS	32.5	84.1	9.7	6.1
2J - 5S	22.0	56.7	19.5	23.8
2J - 2S	25.5	68.6	15.4	16.0
2S - 5S	19.0	72.6	25.1	2.2

APPENDIX 3. Statistical analyses of the foraminiferal abundance per species (Kruskal-Wallis). Statistical analyses of the total biovolumes of *Haynesina germanica* and *Ammonia beccarii*. All p-values are Bonferroni corrected. Bold numbers represent significant differences.

Kruskal-Wallis					
	Abundance			Total biovolumes	
Time* Treatment	*H. germanica* p	*A. beccarii* p	*E. excavatum* p	*H. germanica* p	*A. beccarii* p
CJ - CS	0.18	1.00	0.07	0.18	0.71
2J - CJ	0.47	**0.04**	0.56	0.06	**0.03**
5S - CS	0.09	0.27	0.02	0.07	0.11
2S - CS	**0.04**	0.71	0.14	**0.04**	0.11
2J - 5S	0.19	0.67	**0.02**	**0.03**	0.11
2J - 2S	0.19	0.19	**0.03**	**0.03**	0.11
2S - 5S	0.31	0.47	0.19	0.19	0.67