Denitrification by benthic foraminifera and their contribution to N-loss from a fjord environment

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Abstract.

Oxygen and nitrate availabilities impact the marine nitrogen cycle at a range of spatial and temporal scales. Here, we demonstrate the impact of denitrifying foraminifera on the nitrogen cycle at two oxygen and nitrate contrasting stations in a fjord environment (Gullmar Fjord, Sweden). Denitrification by benthic foraminifera was determined through the combination of specific density countings per microhabitat and specific nitrate respiration rates obtained through incubation experiments using N_2O microsensors. Benthic nitrate removal was calculated from submillimeter chemical gradients extracted from 2D porewater images of the porewater nitrate concentration. These were acquired combining the DET technique (Diffusive Equilibrium in Thin film) with chemical colorimetry and hyperspectral imagery. Sediments with high nitrate concentrations in the porewater and oxygenated overlying water were dominated by the non-indigenous species (NIS) *Nonionella* sp. T1. Denitrification by this species could account for 50-100 % of the nitrate loss estimated from the nitrate gradients. In contrast sediments below hypoxic bottom waters had low inventories of porewater nitrate, and denitrifying foraminifera were rare. Their contribution to benthic nitrate removal was negligible (<5%). Our study showed that benthic foraminifera can be a major contributor of nitrogen mitigation in oxic coastal ecosystems and should be included in ecological and diagenetic models aiming to understand biogeochemical cycles coupled to nitrogen.

1 Introduction

Hypoxic water (i.e. $[O_2] < 63$ µmol L^{-1} Diaz et al., 2008; Breitburg et al., 2018) occurs frequently in bottom-waters of shallow coastal seas, due to remineralization of organic matter and water stratification. Hypoxia may have large ecological effects (Levin et al., 2009; Rabalais et al., 2010; Zhang et al., 2010), such as an increase of fauna mortality (Stachowitsch et al., 1984; Diaz et al., 2001). However, certain microorganisms, e.g. bacteria and foraminifera, can perform denitrification by respiring nitrate (Risgaard-Petersen et al., 2006) and thereby survive in depleted oxygen environments.

The effects of decreasing dissolved oxygen availability at spatial and temporal scales will impact biogeochemical cycles such as the nitrogen cycle (Childs et al., 2002; Kemp et al., 2005; Conley et al., 2007; Diaz et al., 2008; Neubacher et al., 2013; Breitburg et al., 2018). The nitrogen cycle in marine sediments is a perpetual balance between nitrogen inputs (e.g. terrestrial runoff, atmospheric precipitations) and outputs (e.g. denitrification from sediment and water column) (Galloway et al., 2004; Sigman et al., 2009). In most semi-enclosed marine environments as the Baltic Sea, the nitrogen loss through benthic denitrification exceeds the inputs of nitrogen through nitrogen fixation. These nitrogen sink regions of the ocean are mostly associated with anoxic regions (Gruber and Sarmiento 1997).

At oxic bottom water conditions (Fig. 1a), ammonium (NH_4^+) produced from remineralization of particulate organic nitrogen (PON) in sediments, diffuses toward the oxic sediment-superficial layer and through the sediment-water interface (SWI). Nitrification is an aerobic process which converts NH_4^+ to nitrate (NO_3^-) in the oxic sediment and in the oxic water column (Rysgaard et al., 1994; Thamdrup and Dalsgaard, 2008). Total denitrification, the sum of "canonical denitrification" ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) and anammox is an anaerobic process, that converts NO_2^- or NO_3^- to N gasses, such as e.g. N_2 (Brandes et al., 2007 and references within) generating N removal from the environment. The process typically occurs in sediment layers where oxygen is scarce (i.e. < 5 µmol L^{-1}, Devol et al., 2008) and is the dominant process of nitrate reduction in coastal marine sediments (Thamdrup and Dalsgaard, 2008; Herbert, 1999). Denitrification depends on the nitrate transported from the water column and adjacent sedimentary nitrification zones. Nitrification and denitrification are thereby strongly coupled (Kemp et al., 1990; Cornwell et al., 1999). This dependency on nitrification can imply a reduction of denitrification rates as bottom water turns hypoxic, (Fig. 1 b) since nitrification rates are reduced as nitrification cannot proceed under low oxygen concentrations (~ 0 µmol L^{-1}; Rysgaard et al., 1994; Mortimer et al., 2004). The exception however is anoxic nitrification occurring through secondary reactions with NH_4^+ oxidation by Mn and Fe oxides (Luther et al., 1997; Mortimer et al., 2004). In reduced sediment, dissimilatory nitrate reduction to ammonium (DNRA) can also contribute to nitrate depletion leading to NO_3^- conversion into NH_4^+ instead of nitrogen (N_2) (Christensen et al., 2000) and compete denitrification.

Benthic foraminifera were the first marine eukaryotes found to perform complete denitrification (Risgaard-Petersen et al., 2006), but not all foraminifera species can denitrify (Piña-Ochoa et al., 2010). Denitrifying foraminifera species are defined in our study as species able to perform denitrification proved by denitrification rate measurements. The denitrifying

species have a facultative anaerobic metabolism and store nitrate in their cells, which can be used for denitrification. *Nonionella* cf. *stella* (Charrieau et al., 2019 and references therein) and *Globobulimina turgida* were identified as the first denitrifying foraminifera species (Risgaard-Petersen et al., 2006) but currently, nineteen denitrifying species within 9 genera are known (Glock et al., 2019). Their cell specific rate range from 7 ± 1 pmol N $indiv^{-1}$ d^{-1} to 2241 ± 1825 pmol N $indiv^{-1}$ d^{-1} (Glock et al., 2019), and the contribution of benthic foraminiferal communities to benthic denitrification lies in the range from 1 to 90 % (Kamp et al., 2015, Dale et al., 2016; Xu et al., 2017).

Recently, a non-indigenous and suspected invasive *Nonionella stella* morphotype: *Nonionella* sp. T1 was described in the North Sea region (Deldicq et al., 2019) and also reported from the Gullmar Fjord (Sweden) (< 5 %, Polovodova Asteman and Schönfeld, 2015). The genus *Nonionella* is potentially capable to denitrify as demonstrated for *Nonionella* cf. *stella* by Risgaard-Petersen et al. (2006). However, the NIS *Nonionella* sp. T1 morphotype, differs both morphologically and genetically from *Nonionella stella* specimens sampled previously at other localities, such as the Santa Barbara Basin (California USA) (Charrieau et al., 2018), the Kattegat and Oslo Fjord (Norway) (Deldicq et al., 2019). As a consequence, the denitrification capacity of the NIS *Nonionella* sp. T1 is unclear.

In the present study, we investigate if the suspected invasion of the NIS *Nonionella* sp. T1 has any implication for the nitrogen cycle in sections of the Gullmar Fjord (Sweden) that is subjected to hypoxic events. Several denitrifying foraminifera species are present in the Gullmar Fjord sediments: *Globobulimina turgida* (Risgaard-Petersen et al., 2006), *Globobulimina auriculata* (Woehle et al., 2018), *Stainforthia fusiformis* and *Bolivina pseudopunctata* (Gustafsson and Nordberg, 2001; Filipsson and Nordberg, 2004). The denitrification capacity of the latter two species in the Gullmar Fjord is indicative from direct measurement on affiliated species sampled at the coast of Peru, Bay of Biscay (France) and Santa Barbara Basin (Glock et al. (2019); Piña-Ochoa et al. (2010) and Bernhard et al. (2012). Several species, which apparently lack the ability to denitrify, but are able to survive anoxia, are, however, also present in the sediments of the fjord. These include *Bulimina marginata*, *Cassidulina laevigata*, *Hyalinea balthica*, *Leptohalysis scotti*, *Liebusella goesi*, *Nonionellina labradorica* and *Textularia earlandi*. In the context of ecosystem function and service, it is therefore of interest if the NIS *Nonionella* sp. T1 can denitrify and thereby if its invasion into the Gullmar Fjord maintains (or elevates) the denitrification capacity of the overall foraminifera community and thus the sediment or, alternatively, if the organism share a metabolism similar to the non-denitrifying

specimens above, with the possible consequence that the suspected invasion of NIS *Nonionella* sp. T1 implies reduced contribution of foraminifera based denitrification to the loss of N from the fjord.

Estimates of foraminifera contribution to benthic denitrification are limited by the high spatial and temporal variability of sediment geochemistry and distribution of denitrifying foraminifera. Marine sediments often include chemical micro-heterogeneities (Aller et al., 1998; Stockdale et al., 2009), which can be averaged out within the volume of a sediment slice. Moreover, sediment core slicing or centrifugation can induce cell lysis, which can lead to a bias in porewater nitrate concentrations (Risgaard-Petersen et al., 2006). To obtain better estimates of the chemical microenvironments at relevant submillimeter/ millimeter scales, new approaches have to be used. Recently, a 2D-DET (two Dimensions Diffusive Equilibrium in Thin-film) technique combined with colorimetry and hyperspectral imagery was developed to obtain the distribution of nitrite and nitrate in sediment porewater at millimeter resolution in two dimensions (Metzger et al., 2016). This method avoids mixing of intracellular nitrate and the nitrate contained in the sediment porewater. We will apply this technique here to get information about the distribution and concentration of nitrate at a scale relevant for modeling denitrification rates.

The general objectives of the study are (1) to characterize the density of the living benthic foraminifera at two contrasting stations in the Gullmar fjord: one with oxic bottom water and one with hypoxic bottom water. We will in particular focus on the relative abundance of the NIS *Nonionella* sp. T1 (2) to investigate if this NIS *Nonionella* sp. T1 can denitrify and (3) quantify its eventual contributions to benthic denitrification in the sediments. On the basis of the results we will discuss the probable future impact of the NIS *Nonionella* sp. T1 on the foraminifera fauna and the nitrogen cycle in the Gullmar Fjord.

2 Material and Methods

2.1 Site description and sampling conditions

The Gullmar Fjord is 28 km long, 1-2 km wide and located on the Swedish West coast (Fig. 2). The fjord undergoes fluctuations between cold and temperate climates (Svansson, 1975; Nordberg, 1991; Polovodova Asteman and Nordberg, 2013; Polovodova Asteman et al., 2018). The fjord is stratified (Fig. 2 d) in four water masses (Svansson, 1984; Arneborg, 2004). Hypoxia events in the fjord have been linked to the influence of the North Atlantic Oscillation (NAO) (Nordberg et al., 2000; Björk and Nordberg, 2003; Filipsson and Nordberg, 2004). Several monitoring stations are located in the fjord: Släggö

(65 m water depth), Björkholmen (70 m water depth) and Alsbäck (117 m water depth), the hydrographic and nutrient data were obtained from the Swedish Meteorological and Hydrological Institute's (SMHI) publicly available data-base SHARK (SMHI, 2020). Since 2010, the threshold of hypoxia ($[O_2] < 2$ mg L^{-1}, i.e. 63 µmol L^{-1}) in Alsbäck station (red squares, Fig. 3) is reached typically in late autumn and winter. Deep-water exchanges usually occur in late water-early spring. However, the duration of hypoxia varies between years and hypoxia events occurred in the summer 2014 and 2015, due to lack of deep-water exchange. The frequency of hypoxic events has increased in the fjord (Nordberg et al., 2000; Filipsson and Nordberg, 2004).

Two sampling cruises were conducted in the Gullmar Fjord on board R/V *Skagerak* and *Oscar von Sydow*, respectively. The 2017 cruise (GF17) took place between 14th and 15th November 2017 and two stations were sampled (GF17-3 and GF17-1, Fig. 2 c and d) to define the living foraminifera fauna and the sediment geochemistry at two contrasted stations. The 2018 cruise (GF18) took place on the 5th September 2018 with the focus to collect living *Nonionella* sp. T1 for O_2 respiration and denitrification rates measurements. Only one station (at the same position as GF17-3) was sampled.

GF17-3 (50 m water depth) is located closest to the mouth of the fjord (58°16'50.94"N/ 11°30'30.96"E) with bottom waters from Skagerrak (blue diamond, Fig. 3) and GF17-1 (117 m water depth) close to the deepest part of the fjord (58°19'41.40"N/11°33'8.40"E) near Alsbäck monitoring station in the middle of the stagnant basin (red square, Fig. 3). In November 2017, CTD profiles indicated the water mass structures at both stations (Fig. S1). Bottom water at GF17-3 station was oxic with a dissolved oxygen content of 234 µmol L^{-1}. The dissolved oxygen content decreased strongly with depth at the GF17-1 station reaching 9 µmol L^{-1} at the seafloor, which is below the severe hypoxia threshold.

2.2 Foraminifera sampling and processing

During the 2017 cruise, two sediment cores per station (1A, 1C and 3A, 3C for GF17-1 and GF17-3 stations respectively) were immediately subsampled with a smaller cylindrical core (Ø 8.2 cm) and sliced every 2 mm from the sediment surface to 2 cm depth and every 5 mm from 2 cm to 5 cm depth to study living foraminifera distribution. The samples were incubated without light for 10–19 hours in ambient seawater with Cell Tracker Green (CMFDA, 1 mM final concentration) at *in situ* temperature (Bernhard et al., 2006) and then fixed with ethanol 96°. Fixed samples were sieved (> 355, 150, 125 and 100 µm)

and the > 100 µm fraction, the most commonly fraction used for foraminiferal analyses in the Gullmar Fjord (see Charrieau et al., 2018 and references therein) was examined using an epifluorescence microscope equipped for fluorescein detection (i.e., 470 nm excitation; Olympus SZX13). In the present study, the foraminifera distribution will be described highlighting the NIS *Nonionella* sp. T1.

2.3 Geochemical sampling and processing

One core from the shallow GF17-3 station was reserved for O_2 microelectrode profiling. Oxygen concentration was measured in the dark with a Clark electrode (50 µm tip diameter, Unisense ®, Denmark) within the first 5 mm depth at a 100 µm vertical resolution. Due to technical problems, no oxygen profiling was done at the GF17-1 station.

One core per station was dedicated for geochemical analyses; they were carefully brought to Lund University (Sweden) and stored at *in situ* temperature (10°C) until further analysis the next day. Overlaying water of the GF17-3 core was gently air bubbled to maintain the oxygenated conditions recorded at this station. Hypoxia in the overlaying water of the GF17-1 core was maintained by bubbling with N_2 gas passed through a solution of carbonate/bicarbonate to avoid pH rise due to degassing of CO_2.

Nitrite/Nitrate were analyzed using the 2D-DET method from Metzger et al. (2016). In brief, for each core, a DET (Diffusive Equilibrium in Thin films) gel probe (16 cm x 6.5 cm and 0.1 cm thickness) was hand-made prepared. The gel probe was inserted into the sediment and left for 5 hours to allow diffusive equilibration between the gel and porewaters. After equilibration, the gel was removed of the core and laid on a first NO_2^- reagent gel. After 15 minutes at ambient temperature a pink coloration must appear where nitrite is detected. A reflectance image of the nitrite gels was taken with a hyperspectral camera (HySpex VNIR 1600). The next step was to convert existing nitrate into nitrite with the addition of a reagent gel of vanadium chloride (VCl_3). After 20 minutes at 50°C, additional pink coloration is interpreted as porewater nitrate concentration. Followed by the acquisition of another hyperspectral image and the conversion into false colors through a calibrated scale of concentrations, the final gel images were cropped to avoid border effects. Each pixel (190 µm x 190 µm) was decomposed as a linear combination of the logarithm of the different end-member spectra using ENVI software (unmixing

function) (Cesbron et al., 2014; Metzger et al., 2016). Nitrite and nitrate detection limits are 1.7 µmol L^{-1} (Metzger et al., 2016).

2.4 Oxygen and nitrate respiration rate measurements of the NIS *Nonionella* sp. T1

The two cores sampled during the 2018 cruise (GF18) at the shallower GF17-3 station were carefully transported and stored at *in situ* temperature (8 °C) for three days at the Department of Geosciences, Aarhus University (Denmark). *Nonionella* sp. T1 specimens were picked at *in situ* temperature and collected in a Petri dish, containing a thin layer of sediment (32 µm) to check their vitality. Only living, active *Nonionella* sp. T1 specimens were picked using a brush and cleaned several times with micro-filtered, nitrate-free artificial seawater.

Oxygen respiration rates were measured, following the method developed by Høgslund et al. (2008) using a Clark type oxygen microsensors (50 µm tip diameter, Unisense ®, Denmark) (Revsbech, 1989). The O_2 sensor was calibrated at *in situ* temperature (8 °C) in 0.7 M alkaline ascorbate solution (zero O_2) and air-saturated sea water. Then, a pool of five living *Nonionella* sp. T1 was transferred into a glass microtube (inner diameter 0.5 mm, height 7.5 mm), that was fixed inside a 20 ml test tube mounted in a glass-cooling bath (8 °C). A motorized micromanipulator was used to measure O_2 concentration profiles along a distance gradient that ranged from 200 µm of the foraminifera to 1200 µm using 100 µm steps. Seven O_2 concentration profiles were generated with one incubation containing the pool of *Nonionella* sp. T1. Negative controls were done by measuring O_2 rates from microtube with empty foraminifera shells and blanks with empty microtube. Oxygen respiration rates were calculated with Fick's first law of diffusion, J = -D * dC/dx, where J is the flux, dC/dx is the concentration gradient obtained by profiles and D is the free diffusion coefficient of oxygen at 8 °C for a salinity of 34 (1.382×10^{-5} cm^{-2} s^{-1}, Ramsing and Gundersen, 1994). The seven O_2 respiration rates were calculated as the product of the flux by the cross section area of the microtube (0.196 mm^2). Then, the average O_2 respiration rate was divided by number (n=5) of *Nonionella* sp. T1 present in the microtubes to obtain the respiration rate per individual.

The same pool of *Nonionella* sp. T1 specimens as for the O_2 respiration measurements was used for denitrification measurements. These measurements were performed in the microtubes as described in Høgslund et al. (2017). A N_2O microprobe (Andersen et al., 2001) with a 50 µm tip diameter was used to measure the N_2O concentration profile, that

developed in the chamber after acetylene inhibition of the final step in the denitrification process ($N_2O \rightarrow N_2$). Calibration of the sensor was performed using the standard addition method by successive injections of a N_2O saturated solution in order to have 14 µM steps of final concentration. The cell specific N_2O production rate was calculated from the N_2O flux (estimated from the concentration gradient and Fick's first law), the surface area of the microtube (0.25 mm^2) and the number of *Nonionella* sp. T1 in the tubes (n=5) as described above. Rates are reported with the unit pmol N $indiv^{-1}$ d^{-1}.

Since O_2 respiration and denitrification rates are linked to cytoplasmic volumes or biovolumes (BV) (Geslin et al., 2011; Glock et al., 2019), the specimens from the pool of *Nonionella* sp. T1 were measured (width (a) and length (b), Fig. 4) using a micrometer mounted on a Leica stereomicroscope (MZ 12.5) to estimate the average BV. The volume of each shell was estimated by using the best resembling geometric shape, a spheroid prolate ($V = \frac{4}{3}\pi\left(\frac{a}{2}\right)^2\left(\frac{b}{2}\right)$). Then, according to Hannah et al. (1994), 75 % of the measured entire volume of the shell was used as the estimated cytoplasmic volume. Five *Nonionella* sp. T1 specimens sampled during the 2017 cruise (GF17, study of the fauna) were also measured to compare their average size with the size of the specimens sampled during the 2018 cruise (GF18, denitrification rate measurements).

2.5 Contributions of the NIS *Nonionella* sp. T1 to benthic denitrification.

Benthic denitrification was estimated using the 2D nitrate concentrations obtained with the DET technique. An average 1D nitrate profile was obtained by calculating the mean of 290 vertical profiles ((5.5 cm width x 1 pixel) / 0.019 cm for 1-pixel size) extracted from the 2D concentration image. Then, nitrate production and consumption zones were calculated with PROFILE software (Berg et al., 1998). With the assumption that nitrate consumption was equivalent to denitrification, the benthic denitrification rate was calculated by integrating nitrate consumption over the depth.

The denitrification activity of the NIS *Nonionella* sp. T1 population was calculated using the specimen abundances in the nitrate consumption zones and their cell specific activity. The size of the *Nonionella* sp. T1 specimens sampled during the two cruises, however, differed markedly (Table 1). The cell specific denitrification rate of denitrifying foraminifera is correlated with their size according to the model: ln (y) = 0.68 ln (x) – 5.57, where y is the denitrification rate (pmol ind^{-1} d^{-1}) and x is the shell BV (μm^3) (Geslin et al., 2011; Glock et al., 2019; Equation S1) and we therefore used this model to correct the denitrification estimates for size specific variations.

A maximum estimate of the contribution of the NIS *Nonionella* sp. T1 population to benthic denitrification was obtained from the ratio of the denitrification activity of *Nonionella* sp. T1 population and the benthic denitrification rate estimated from the porewater nitrate concentration profiles. This presumes that *Nonionella* sp. T1 exclusively use nitrate dissolved in the sediment porewater as source for denitrification (calculation approach A). A minimum estimate of the contribution of *Nonionella* sp. T1 population to benthic denitrification was obtained from the ratio between the denitrification activity of *Nonionella* sp. T1 population and the benthic denitrification rate estimated from porewater nitrate concentration profiles plus the denitrification activity of *Nonionella* sp. T1 population. This presumes that *Nonionella* sp. T1 exclusively use intracellular nitrate as source for denitrification (calculation approach B).

3 Results

3.1 The NIS *Nonionella sp.* T1 oxygen and nitrate respiration rates in the Gullmar Fjord

The O_2 respiration rate measured from the pool of *Nonionella* sp. T1 specimens collected during the 2018 cruise (GF18) was 169 ± 11 pmol O_2 indiv.$^{-1}$ d^{-1} with an average BV of 1.3 ± 0.7 10^{+06} µm^3 (BV details, Table 1). The denitrification rate measured from the same pool of specimens was 21 ± 9 pmol N indiv^{-1} d^{-1}.

The *Nonionella* sp. T1 average BV of the specimens collected during the 2017 cruise (GF17-3) was 4.0 ± 0.6 10^{+06} µm^3, i.e. more than three times the *Nonionella* sp. T1 average BV of the 2018 samples (1.3 ± 0.7 10^{+06} µm^3). As denitrification rates and foraminifera BV are related (see method), the measured denitrification rate was corrected using the BV of *Nonionella* sp. T1 from the 2017 cruise. Hence, the *Nonionella* sp. T1 corrected denitrification rate was 38 ± 8 pmol N indiv.$^{-1}$ d^{-1} (Equation S1).

3.2 The NIS *Nonionella* sp. T1 and foraminifera fauna regarding porewater nitrate micro-distribution

The bottom water at GF17-3 station was oxic (Fig. S1, [O_2] = 234 µmol L^{-1}) and the measured oxygen penetration depth (OPD) in the sediment was 4.7 ± 0.2 mm (n = 3). No nitrite was revealed on the gel (< 1.7 µmol L^{-1}), only nitrate was detected. Bottom water average NO_3^- concentration was 14.6 ± 2.3 µmol L^{-1} and nitrate concentration decreased with depth in the

sediment (Fig. 5 c, d). Nitrate concentrations ranged between 13.1 ± 3.2 to 11.7 ± 3.4 µmol L^{-1}, from the SWI to the OPD. Nitrate concentrations decreased strongly under the OPD from 11.7 ± 3.4 to 2.8 ± 0.9 µmol L^{-1} at 4.0 cm depth. From 4.0 to 5.0 cm depth, NO_3^- concentration was very low with an average value of 2.7 ± 0.9 µmol L^{-1} (Fig. 5 c, d). The PROFILE parameters (Berg et al., 1998) used on laterally averaged nitrate porewater vertical distribution of both stations are available in Table S1. Thus, the PROFILE modelling of the averaged nitrate porewater profile revealed one nitrification zone from 0 to 1.2 cm depth and two denitrifying zones (red line, Fig. 5 d). The first denitrification zone occurred between 1.2 to 3.5 cm depth with a nitrate consumption of 3.92 E^{-05} nmol cm^{-3} s^{-1} and the second smaller consumption zone was from 3.5 to 5 cm depth (1.53 E^{-06} nmol cm^{-3} s^{-1}). The total denitrification rate from 1.2 to 5 cm depth was 4.07 E^{-05} nmol cm^{-3} s^{-1} (Fig. 5 d).

The total densities of living foraminifera were similar between the cores GF17-3A and 3C (Ø 8.2 cm, 5 cm depth) with 1256 individuals and 1428 individuals, respectively (Fig. 5 a and b; Table S2, GF17-3A and 3C). *Nonionella* sp. T1 was the main denitrifying species, accounting for 34 % of the total living fauna in the core GF17-3A and 74 % in GF17-3C (Fig. 5 a, b; Table S3). One other candidate for denitrification, *Stainforthia fusiformis*, was in minority: 1 % of the total fauna in both cores (Fig. 5 a, b; Table S3, GF17-3A and 3C). The other known denitrifying species previously reported in the Gullmar Fjord, *Globobulimina turgida* (Risgaard-Petersen et al., 2006) and *Globobulimina auriculata* (Whoele et al., 2018) were absent. Three non-denitrifying species (Piña-Ochoa et al., 2010; Xu et al., 2017; Glock et al., 2019) were dominant in the cores GF17-3A and 3C: *Bulimina marginata* (37 and 5 %, respectively), *Cassidulina laevigata* (9 and 5 %) and *Leptohalysis scotti* (11 and 9 %).

The density and the micro-distribution of *Nonionella* sp. T1 differed between the two cores (Fig. 5 a and b; Table S2, GF17-3A and 3C)..*Nonionella* sp. T1 relative abundance accounted for 18 % and 50 % of the fauna in the nitrification zone (from the SWI to 1.2 cm depth) for the cores GF17-3A and 3C respectively (Table S3). In the main denitrifying zone (from 1.2 cm to 3.5 cm), the *Nonionella* sp. T1 relative abundance represented 27 % of the fauna for the core GF17-3A and 78 % for the core GF17-3C. In the second denitrifying zone, the *Nonionella* sp. T1 relative abundance increased from 3.5 to 5 cm depth and dominated the fauna with relative abundances of 60 % and 98% (GF17-3A and 3C respectively). The relative abundance of the denitrifying candidate, *Stainforthia fusiformis*, was a minor component in each zones of both cores and did not exceed 2 % (Table S3, GF17-3A and 3C). The three non-denitrifying species (e.g. *B. marginata*, *C. laevigata* and *L. scotti*) also

dominated the fauna of both cores (Table S2 and S4). From the SWI to 1.2 cm depth, *B. marginata* accounted for 42 % and 12 %, *C. laevigata* 16 % and 13 % and *L. scotti* 6 % and 11 %, for the cores GF17-3A and 3C respectively. In the first denitrifying zone (1.2-3.5 cm depth), *B. marginata* accounted for 34 % and 2 %, *C. laevigata* for 7 % and 2% and *L. scotti* for 25 % and 13 % (GF17-3A and 3C respectively). In the second denitrifying zone (3.5-5 cm depth), *B. marginata* accounted for 34 % and 0 %, *C. laevigata* was absent and *L. scotti* 5 % and 1 % (GF17-3A and 3C respectively).

Due to severe hypoxia at the GF17-1 station, oxygen was assumed to be below detection limit within the sediment. No nitrite was detected at this station (< 1.7 µmol L^{-1}). Average NO_3^- concentration in the bottom water reached 5.7 ± 1.0 µmol L^{-1} (Fig. 5 g and h). Nitrate concentrations decreased from the SWI (4.2 ± 1.0 µmol L^{-1}) to 1.6 cm depth (1.8 ± 0.6 µmol L^{-1}) and then average nitrate concentration remained below the detection limit (1.7 µmol L^{-1}). However, a micro-environment with higher nitrate concentration was visible on the left part of the gel between 2.0 and 3.0 cm depth. A 1D vertical profile passing through this micro-environment (white line, Fig. 5 g) was extracted from the 2D image and the maximal nitrate concentration of this patch was above the detection limit with a value of 6.5 µmol L^{-1} at 2.3 cm depth (blue square profile, Fig. 5 h). The PROFILE modelling (Table S1) of the laterally averaged nitrate vertical distribution revealed at the sampling time one denitrifying zone from the SWI to 1.6 cm depth with a nitrate consumption of 2.71 E^{-05} nmol cm^{-3} s^{-1} (red line, Fig. 5 h). No PROFILE modelling was done under 1.6 cm depth, because nitrate concentration was below the detection limit (hatched grey zone, Fig. 5 h).

Living foraminifera showed a large difference in both species distribution and total densities between the two cores GF17-1A and 1C (Fig. 5 e, f; Table S2) with 1457 individuals and 786 individuals respectively (Ø 8.2, 5 cm depth). *Nonionella* sp. T1 represented a low relative abundance of the total fauna with 5 % for the core GF17-1A and was almost absent (1 %) for the core GF17-1 C (Table S3). The known denitrifying *G. auriculata* was minor in the fauna with relative abundances of 1 % and 2% (GF17-1A and 1C respectively). The denitrifying candidate *S. fusiformis* was also found in both cores reaching only 3% of the total fauna (Figure 5 e, f; Table S3). The other denitrifying candidate *B. pseudopunctata*, was almost absent of the total fauna with relative abundances of 0 % and 2 % for the cores GF17-1A and GF17-1C respectively (Table S3). The same three non-denitrifying species observed in oxic station were also dominant for both cores GF17-1A and 1C: *B. marginata* (64 and 30 %), *C. laevigata* (16 and 15 %) and *L. scotti* (4 and 36 %).

In the denitrifying zone (0-1.6 cm), *Nonionella* sp. T1 relative abundance was rare (2 %) for the core GF17-1A) and almost absent from the fauna for the core GF17-1C. For the core GF17-1A, *Nonionella* sp. T1 relative abundance reached 26 % of the fauna between 1.6 and 2.5 cm depth (Fig. 5 e, GF17-1A), whereas it was almost absent from the rest of the core GF17-1A and it was absent from the core GF17-1C (Table S3). For the cores GF17-1A and 1C, *S. fusiformis* reached respectively 2 % and 3 % in the denitrifying zone (0-1.6 cm). Under the denitrifying zone (1.6-5 cm), *S. fusiformis* represented 4 and 1 % of the fauna (GF17-1A and 1C respectively). The three other non-denitrifying species dominated both cores in the denitrifying zone (0-1.6 cm): *B. marginata* accounted for 66 % and 35 %, *C. laevigata* 19 % and 19 % and *L. scotti* 4 % and 24 % for the cores GF17-1A and 1C respectively. From 1.6 to 5 cm depth, *B. marginata* accounted for 61 % and 11 %, *C. laevigata*for 5 % and 2 % and *L. scotti* for 6 % and 75 % (GF17-1A and 1C respectively).

4 Discussion

4.1 The NIS *Nonionella* sp. T1 density in comparison with other species from the Gullmar Fjord

The presence and relative abundance of the NIS *Nonionella* sp. T1 in the Gullmar Fjord and in the Skagerrak-Kattegat strait have been documented during the last decades. The earliest SEM observations of specimens resembling *Nonionella* sp. T1 morphotype in the deepest part of the fjord date back to summer 1993 (identified as *Nonionella turgida*, Gustafsson and Nordberg, 2001). The invasive characteristics of *Nonionella stella* were firstly revealed by Polovodova Asteman and Schönfeld, (2015). Then, *Nonionella stella* was identified as *Nonionella* sp. T1 morphotype also described as a NIS and potentially invasive species in the Oslofjord by Deldicq et al. (2019). The estimated introduction date of *Nonionella* sp. T1 into the deepest part of the Gullmar Fjord is 1985 according to Polovodova Asteman and Schönfeld, (2015). The relative abundance of *Nonionella* sp. T1 in the deepest fjord station was less than 5 % between 1985 and 2007 (Polovodova Asteman and Schönfeld, 2015 and references within). At the GF17-1 hypoxic station, the *Nonionella* sp. T1 relative abundance was between 1-5 % (Table S3, GF17-1A and 1C). Thus, the *Nonionella* sp. T1 relative abundance in the deepest part of the fjord seems to remain stable. In contrast to GF17-1 station, the GF17-3 oxic station was sampled for the first time in this study. In this station closer to the mouth of the fjord than GF17-1, the relative abundance of *Nonionella* sp. T1 varied between 34 and 74 % (Table S3, GF17-3A and 3C). Previous studies showed an increase in the relative abundance of *Nonionella* sp. T1

morphotype in the Skagerrak-Kattegat region (near the entrance of the Gullmar Fjord). The *Nonionella* sp. T1 represented 10 % of the fauna in June 2013 (Polovodova Asteman and Schönfeld, 2015). The Öresund strait linking the North Skagerrak, the Kattegat and the Baltic Sea showed an increase in *Nonionella* sp. T1 relative abundance from 1 % to 14 % observed between 1998 and 2009 (Charrieau et al., 2019). The foraminifera fauna in the Gullmar Fjord changed over the last decennium and *Nonionella* sp. T1 seems to become an invasive species in the Gullmar Fjord oxic shallow water area.

The foraminifera fauna found at the GF17-1 station in the deepest part of the fjord differed from previous studies (Nordberg et al., 2000; Filipsson and Nordberg, 2004; Risgaard-Petersen et al., 2006; Polovodova Asteman and Nordberg, 2013; Polovodova Asteman and Schönfeld, 2015). Indeed, until the early 1980s, the foraminifera fauna in the deepest part of the fjord was dominated by a typical Skagerrak – Kattegat fauna (*Bulimina marginata, Cassidulina laevigata, Hyalinea balthica, Liebusella goësi*, *Nonionellina labradorica* and *Textularia earlandi*) (Nordberg et al., 2000). However, the fauna changed. *S. fusiformis* and *B. pseudopunctata* became the major species (Nordberg et al., 2000; Filipsson and Nordberg, 2004). Further studies by Polovodova Asteman and Nordberg (2013) demonstrated that at least until 2011 *S. fusiformis*, *B. pseudopunctata* and *T. earlandi* dominated the fauna. Foraminifera fauna described in the present study differs. In November 2017 *S. fusiformis* did not exceed 3 % of the fauna (Table S3, GF17-1A and 1C), *B. pseudopunctata* reached only 2 % in the core GF17-1C (Table S3, GF17-1C) and *T. earlandi* was a minor species < 1 %. Then, in November 2017, *B. marginata, C. laevigata* and *L. scotti* were the dominant species in the fjord. The *Elphidium clavatum-selseyensis* species complex (following the definition from Charrieau et al., 2018), *H. baltica*, *N. labradorica,* and *T. earlandi* were present with a low relative abundance (< 5 %, Table S3). Namely, *G. turgida* reached 37 % of the foraminifera fauna in August 2005 at the deepest station (Risgaard-Petersen et al., 2006); whereas in November 2017 this species relative abundance decreased to become a minor species of the assemblage. However, such trend for *S. fusiformis* and *B. pseudopunctata* must be interpreted with caution since our study used the > 100 µm fraction whereas some of the previous studies used the > 63 µm fraction. We also wet picked the specimens and used Cell Tracker Green to identify living foraminifera, which might affect the results compared to Rose Bengal studies of dry sediment residuals.

The relative abundance of the potential invasive *Nonionella* sp. T1 in 2017increased compared to the study of Polovodova Asteman and Schönfeld (2015) in the oxic part of the fjord. It is also noteworthy that the two non-denitrifying species *B.*

marginata and *C. laevigata* described as typical species of the Skagerrak-Kattegat fauna (Filipsson and Nordberg, 2004) increased markedly in the fjord as well. It is evident that the foraminifera fauna in the Gullmar Fjord is presently very dynamic with considerable species composition shifts probably following seasonal water body stratification and consecutive oxygen depletion occurring in the fjord (Fig. 3).

4.2 Foraminifera ecology considering porewater nitrate micro-distribution

For the first time a core sampled in the Gullmar fjord shows *Nonionella* sp. T1 as a dominant species. This observation was made under oxic conditions at GF17-3 station (50 m depth) during November 2017 (Fig. 5 a, b; Table S2; S4). *Nonionella* sp. T1 density increased with sediment depth below the sedimentary oxic zone (Fig. 5 a – d; Table S2), which could be explained by its preference to respire nitrate rather than oxygen. This would be in agreement with the hypothesis of using nitrate as the preferred electron acceptor suggested by Glock et al. (2019). *Nonionella* sp. T1 distribution could be explained by its capacity to store nitrate intracellularly before porewater nitrate is denitrified by other organisms such as bacteria. In detail, in the upper part of the sediment, within the oxic zone, *Nonionella* sp. T1 would respire oxygen at the rate of 169 ± 11 pmol O_2 $indiv^{-1}$ d^{-1}(Fig. 5 c and d). Below the oxygen penetration depth (from 4.7 ± 0.2 mm to 3.5 cm), *Nonionella* sp. T1 could store and respire the ambient nitrate at the rate of 38 ± 8 pmol N $indiv^{-1}$ d^{-1}. Further down, where the nitrate porewater is depleted (Fig. 5 c, d; from 3.5 to 5 cm depth), *Nonionella* sp. T1 would respire on its intracellular nitrate reserves to survive (Fig. 5 a, b; from 3.5 to 5 cm depth). When the intracellular nitrate reserve runs out, *Nonionella* sp. T1 would be able to migrate to an upper zone where nitrate is still present in the sediment to regenerate its intracellular nitrate reserve (Fig. 5 a, b; from 1.2 to 3.5 cm depth).

Hypoxia occurred approximately at least one month before the sampling cruise in the deepest part of the fjord (Fig. 3). When hypoxia is extended to the water column, nitrification both in the water column and the sediment is reduced or even stopped, as oxygen is almost absent (Fig. 1 b; Childs et al., 2002; Kemp et al., 2005; Conley et al., 2007; Jäntti and Hietanen, 2012). Under this condition, the coupled nitrification-denitrification processes are strongly reduced (Kemp et al., 1990). At the GF17-1 station, no nitrification in superficial sediment was showed by our data and nitrate was low but still detectable in the

bottom water. Nitrate can diffuse from the water column into the sediment, and thereby generate the denitrification zone as modelled by PROFILE between the SWI and 1.6 cm depth (Fig. 5 h).

The rare presence of the NIS *Nonionella* sp. T1 and other denitrifying species as *Globobulimina auriculata, Bolivina pseudopunctata* and *Stainforthia fusiformis* in the hypoxic station indicates that sediment chemical conditions turned unfavorable towards denitrification during prolonged hypoxia. Instead, the non-denitrifying species *Bulimina marginata*, *Cassidulina laevigata,* and *Leptohalysis scotti* dominated in this hypoxic environment. Their survival could be due to seasonal dormancy (Ross and Hallock, 2016; LeKieffre et al., 2017) and their ability to release propagules which can disperse and grow when environmental conditions turn favorable again (Alve and Goldstein, 2003). The suspected deep nitrification zone (blue square profile, Fig. 5 h) could indicate the presence of nitrate micro-niches deeper in the sediment and might explain the patchy distribution of *Nonionella* sp. T1 also at the hypoxic site (see Fig. 5 e; Table S2, GF17-1A). Therefore, deep nitrate production in these micro-environments could favor the presence of *Nonionella* sp. T1, which can be attracted by this nitrate source of electron acceptor to respire (Nomaki et al., 2015; Koho et al., 2011). This deep nitrification zone could be the result of macrofaunal activity (burrowing activity) that introduce some oxygen deeper into anoxic sediment (Aller, 1982; Karlson et al., 2007; Nizzoli et al., 2007; Stief, 2013; Maire et al., 2016). This nitrification zone could also be due to an anaerobic process. The Gullmar Fjord is Mn-rich (Goldberg et al., 2012) and metal-rich particles can be bio-transported into the anoxic sediment, thus allowing ammonium oxidation into NO_3^- by Mn and Fe-oxides in the absence of oxygen deeper in the sediment (Aller, 1994; Luther et al., 1997).

4.3 Contributions and potential impacts of the NIS *Nonionella* sp. T1 to benthic denitrification in the Gullmar Fjord

Considering that *Nonionella* sp. T1 is denitrifying the nitrate from sediment porewater (approach A, Table 2; see method 2.5), its contribution to benthic denitrification in the oxic station would be 47 % in the core GF17-3A and would reach 100 % in the core GF17-3C. If we consider that *Nonionella* sp. T1 uses its intracellular nitrate pool for denitrification (approach B), its contribution to benthic denitrification would be 32 % in the core GF17-3A and would reach 50 % in the core GF17-3C (Table 2). These two calculation approaches highlight the difficulties and the importance of knowing the concentration of environmental nitrate and foraminifera intracellular nitrate at the same time to estimate the contributions of foraminifera to

benthic denitrification. Moreover, in this study there is no data on anammox process which contributes also to the total denitrification (Brandes et al., 2007). The results reported in previous studies as Engström et al. (2005) do not allow us to extrapolate their data at our oxic station, located at the entrance of the fjord. Thus, we assume that our estimate of denitrification is conservative since the possible contribution of anammox is not included in the calculation. However, despite these uncertainties *Nonionella* sp. T1 contribution to benthic denitrification supports the hypothesis that this non-indigenous denitrifying foraminifer plays a major role in the benthic nitrogen cycle.

At the hypoxic station, the opposite was shown where the estimated contribution of *Nonionella* sp. T1 to benthic denitrification was below 1 % whatever the calculation approach. The estimated contributions of the other denitrifying foraminifera found in this station were low. Foraminifera contributed to almost 5 % of benthic denitrification. Compared to the oxic station, the NIS *Nonionella* sp. T1 and the other denitrifying species contributions to benthic denitrification were weak in a prolonged hypoxic station of the Gullmar Fjord.

Overall, the Gullmar Fjord is well oxygenated except for the deepest basin where oxygen goes down when there is no deep water exchange (Fig. 3 c). Therefore, the GF17-3 oxic station could be considered representative of the Gullmar Fjord benthic ecosystem. *Nonionella* sp. T1 is not the most efficient denitrifying species compared to *Globobulimina turgida* (42 pmol N ind^{-1} d^{-1}, with BV = 1.3 10^{+06} μm^3) and also less efficient than *Nonionella* cf. *stella* from Perú. However, *Nonionella* sp. T1 high density could accelerate sediment denitrification and participate to increase the contrast between the two hydrographic conditions. Indeed, the increasing discrepancy of bottom water oxygenation between stations induces a gap in the availability of nitrate for anaerobic facultative metabolisms in the sediment. In the oxygenated part of the fjord, high contribution to benthic denitrification (estimated between 50 and 100%) by *Nonionella* sp. T1 could take part to a potential de-eutrophication of the system by increasing the nitrogen loss. Primary production (PP) in the Gullmar Fjord is dominated by diatom blooms in spring and autumn (Lindahl and Hernroth, 1983). Since the 1990s, Lindahl et al. (2003) observed an increase in PP in the Gullmar Fjord, potentially changing its trophic status towards eutrophic. This increase in PP also shown in the adjacent Kattegat could be related to the nitrogen input loading from the land and atmosphere (Carstensen et al., 2003). Lindahl et al. (2003) argued that the PP in the Gullmar Fjord was due to climatic forces resulting from a strong positive North Atlantic Oscillation (NAO) index, which increased the availability of deep-water nutrients (Kattegat nitrate-rich) through changes in

the thermocline. The benthic denitrification of the Gullmar Fjord produces nitrogen unassimilable by primary producers. Moreover, foraminiferal nitrate uptake and intracellular storage act as an additional sink through bio-transportation and permanent sequestration in sediments (Glock et al., 2013; Prokopenko et al., 2011). Thus, denitrifying foraminifera including *Nonionella* sp. T1 could help counterbalance a potential eutrophication of the system via nitrogen loss (Seitzinger, 1988).

Contrastingly, in the hypoxic part of the fjord, nitrate and nitrite rapidly exhausted become scarce, resulting in a decrease in benthic denitrification including foraminifera contribution. As a consequence of oxygen and nitrate scarcity, nitrification, denitrification and anammox processes are less intense resulting in a decrease of nitrogen mitigation and accumulation of ammonium in the deeper part of the fjord subjected to prolonged severe hypoxia (Fig. 1). Moreover, the low availability of nitrate in the sediment would possibly increase the benthic transfer towards the water column of reduced compounds such as manganese and iron produced deeper in the sedimentary column by other anaerobic metabolisms (Hulth et al., 1999). These new results demonstrate that the role of denitrifying foraminifera is underestimated in the nitrogen cycle and that overlooking this part of the meiofauna may lead to a misunderstanding of environments subject to hydrographic changes.

5 Conclusion

This study revealed a drastic change in living foraminifera fauna due to several hypoxic events that occurred in the last decennium in the Gullmar Fjord. For the first time, the non-indigenous species (NIS) *Nonionella* sp. T1 dominated up to 74 % the foraminifera fauna at a station with oxygenated bottom waters and high nitrate content in sediment porewater. This NIS can denitrify up to 50-100 % of the nitrate porewater sediment under oxic conditions in the fjord. Whereas, under prolonged hypoxia, nitrate depletion turns environmental conditions unfavorable for foraminifera denitrification, resulting in a low density of *Nonionella* sp. T1 and other denitrifying species. Foraminifera contribution to benthic denitrification was negligible (~ 5 %) during prolonged seasonal hypoxia in the fjord. Moreover, the potential invasive denitrifying *Nonionella* sp. T1 could impact the nitrogen cycle under oxic conditions by increasing the sediment denitrification and could counterbalance potential eutrophication of the Gullmar Fjord. Our study demonstrated that the role of denitrifying foraminifera is underestimated in the nitrogen cycle especially in oxic environments.

Figure list

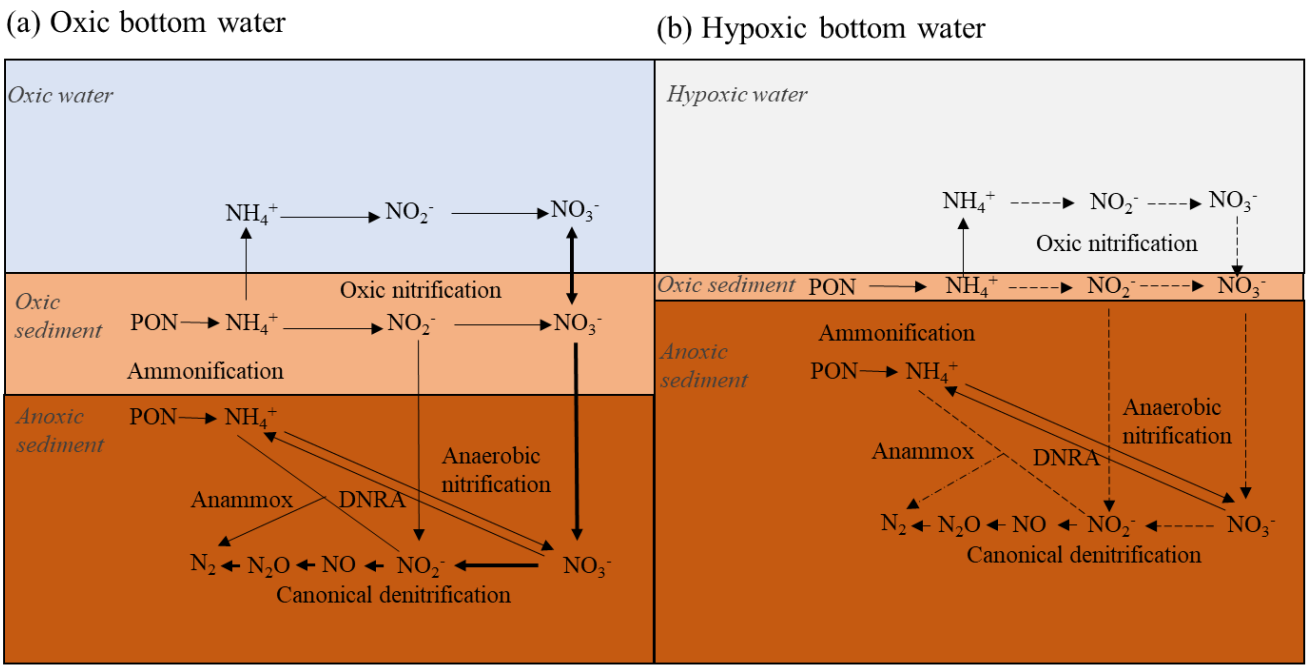
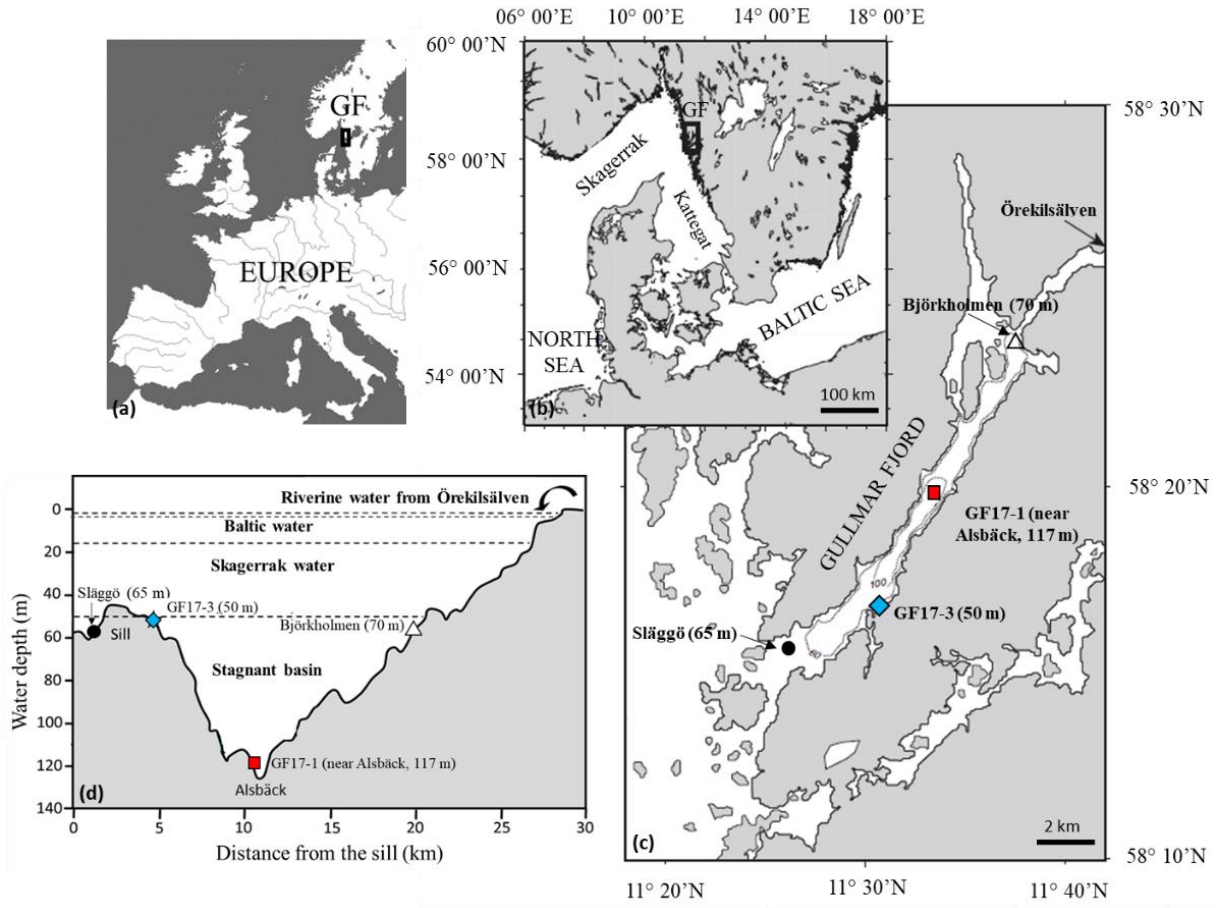


Figure 1. Simplified nitrogen cycling in marine sediments when the bottom water is oxic (a) and hypoxic (b). Chemical formulae: PON (particulate organic nitrogen), NH_4^+ (ammonium), NO_3^- (nitrate), NO_2^- (nitrite), NO (nitrogen oxide), N_2O (nitrous oxide), N_2 (nitrogen). The bold/dotted arrows indicate reactions advantaged/reduced by oxygen and nitrate presence/depletion. See text for more details. Modified from Jantti and Hietanen, (2012).



445 **Figure 2. (a-c) Location of studied stations in the Gullmar Fjord (Sweden); blue diamond: GF17-3 oxic station (50 m water depth); red square: GF17-1 hypoxic station (117 m water depth); dark circles: monitoring stations Släggö (65 m water depth) and Björkholmen (70 m water depth). (d) Transect from the sill with the four Gullmar Fjord water masses and the studied stations (modified from Arneborg et al., 2004).**

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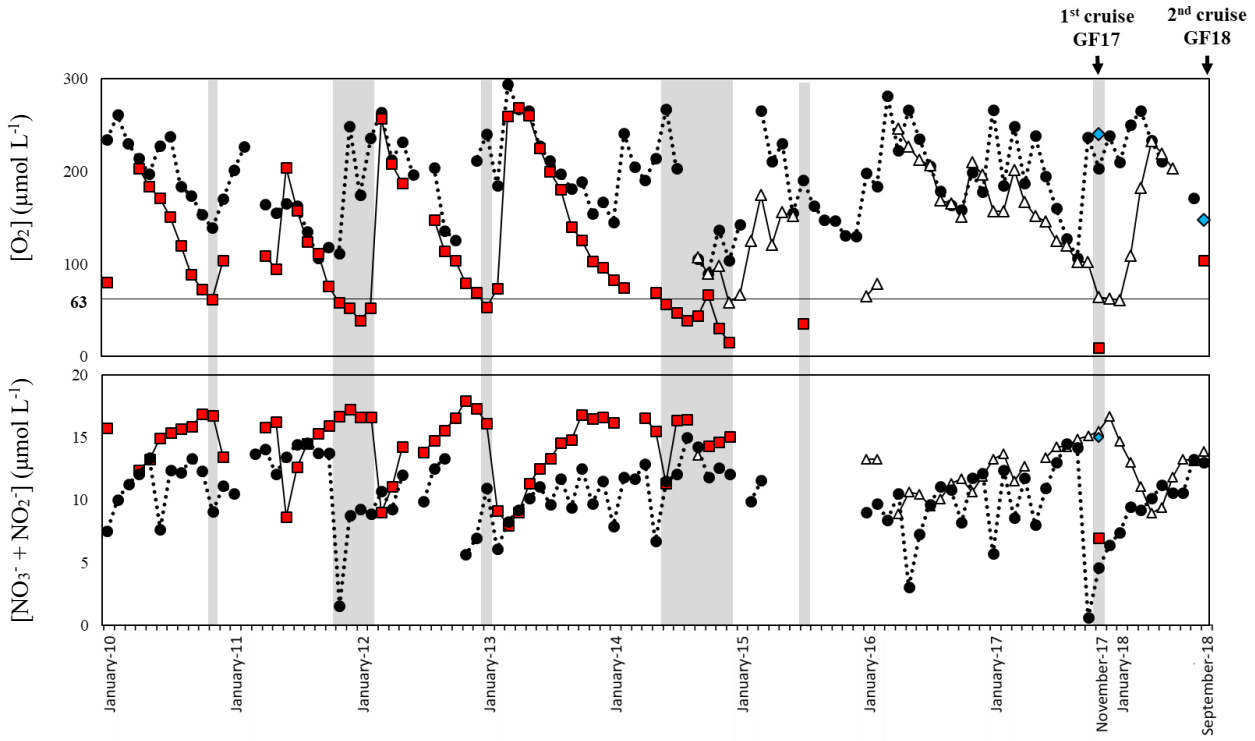

Figure 3. Record from January 2010 to September 2018 of bottom water oxygen ($[O_2]$) and nitrite + nitrate ($[NO_3^- + NO_2^-]$) measurements from the monitoring stations Släggö (65 m water depth; black dot), Björkholmen (70 m water depth; white triangle) and the sampling stations GF17-1 (Alsbäck, 117 m water depth; red square) and GF17-3 (50 m water depth; blue diamond). The arrows indicate the date of the two sampling cruises: the 2017 cruise (14th, 15th November 2017) and the 2018 cruise (5th September 2018). The grey zones indicate hypoxic periods with a threshold of $[O_2] < 63$ µmol L^{-1}.

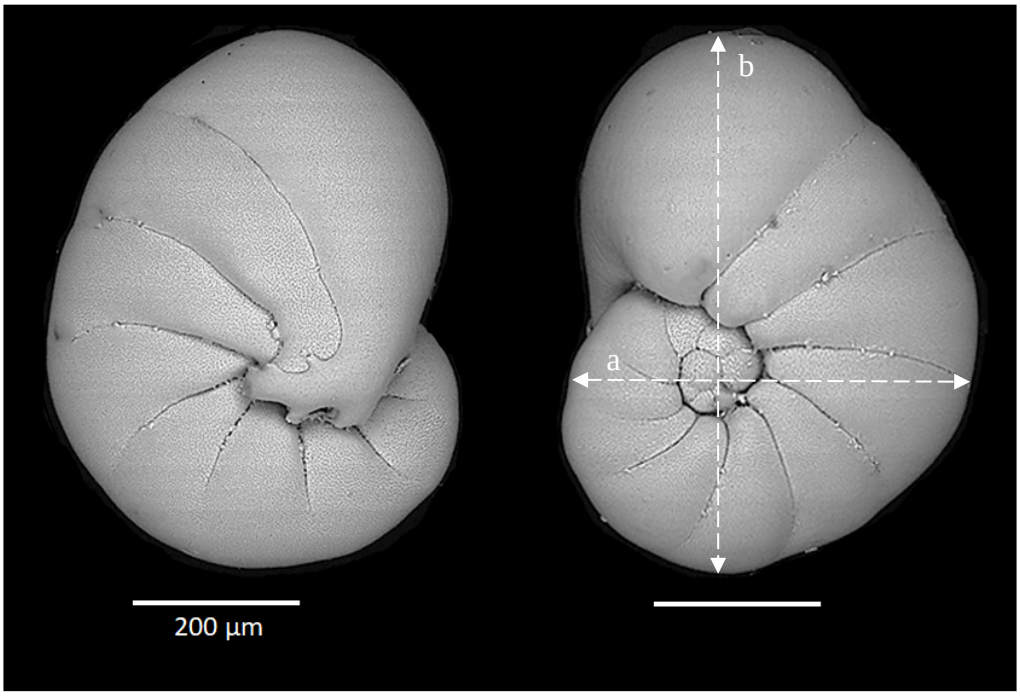

Figure 4. Scanning Electronic Microscope images of a *Nonionella* sp. T1 from the GF17-3 oxic station in the Gullmar Fjord. White lines (a, b) correspond to measured distances serving for a spheroid prolate volume model.

Table 1. Total shell volume (µm^3) and the biovolume (BV, µm^3) corresponding to 75 % of the total shell volume measured on the pool of five *Nonionella* sp. T1 from the 2017 and the 2018 cruises in the Gullmar Fjord. Abbreviations: sd (standard deviation), ind. (individual).

Nonionella sp. T1	1st cruise total shell volume	1st cruise BV	2nd cruise total shell volume	2nd cruise BV
ind. 1	6.7 10 $^{+06}$	5.0 10 $^{+06}$	3.1 10 $^{+06}$	2.3 10 $^{+06}$
ind. 2	4.5 10 $^{+06}$	3.4 10 $^{+06}$	2.4 10 $^{+06}$	1.8 10 $^{+06}$
ind. 3	5.1 10 $^{+06}$	3.8 10 $^{+06}$	1.4 10 $^{+06}$	1.0 10 $^{+06}$
ind. 4	4.9 10 $^{+06}$	3.7 10 $^{+06}$	9.2 10 $^{+05}$	6.9 10 $^{+05}$
ind. 5	5.8 10 $^{+06}$	4.4 10 $^{+06}$	6.2 10 $^{+05}$	4.7 10 $^{+05}$
Average (µm^3)	5.4 10 $^{+06}$	4.0 10 $^{+06}$	1.7 10 $^{+06}$	1.3 10 $^{+06}$
sd (µm^3)	0.8 10 $^{+06}$	0.6 10 $^{+06}$	1.0 10 $^{+06}$	0.7 10 $^{+06}$

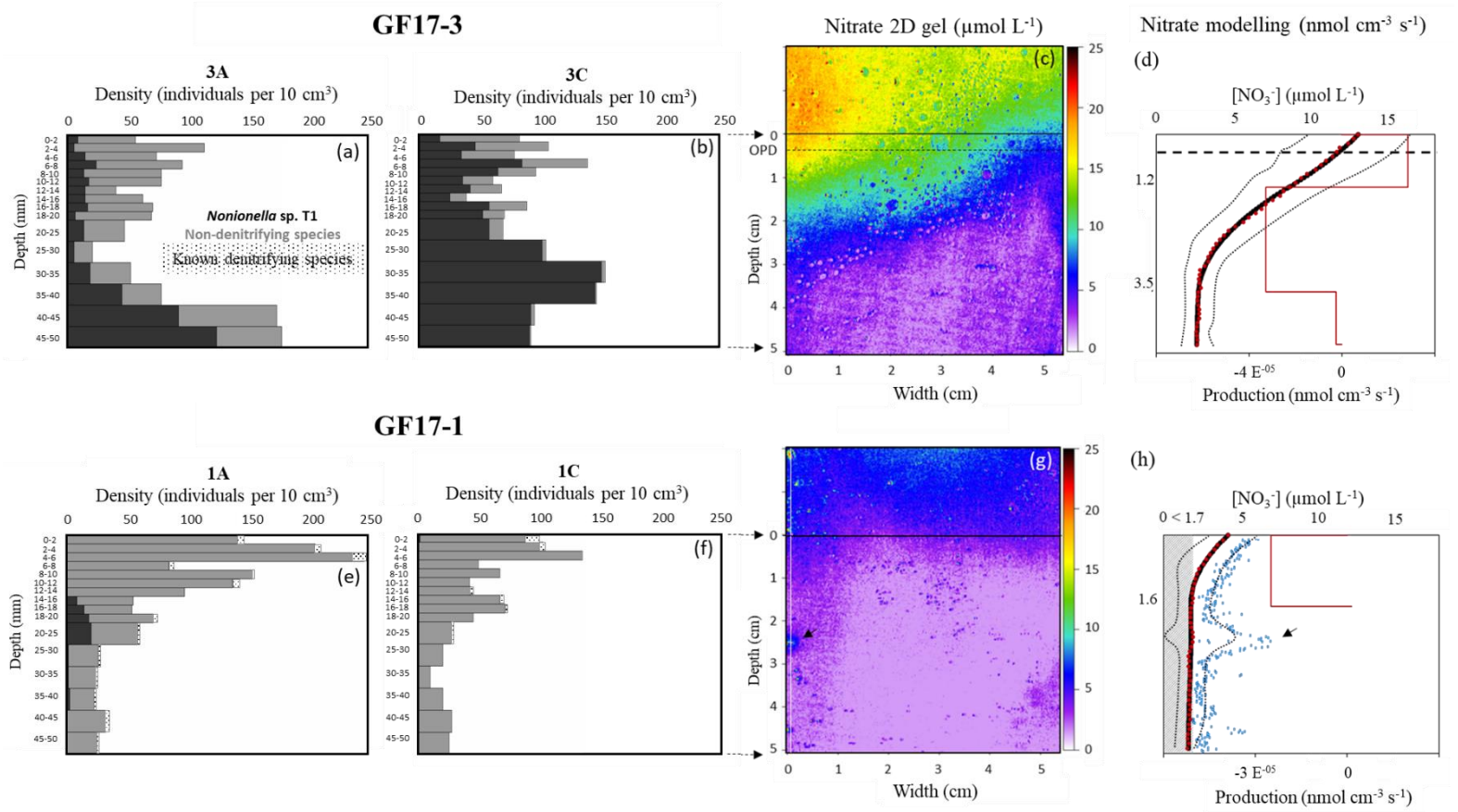

Figure 5. Micro-distributions of living foraminifera densities in GF17-3 oxic station (a, b) and in GF17-1 hypoxic station (e, f). *Nonionella* sp. T1 specimens are in black, the sum of the non-denitrifying species in grey and the small dots (e, f) show the other denitrifying species (known and potential candidates). The maps of porewater nitrate 2D gels are presented for the stations GF17-3 (c) and GF17-1 (g). The SWI is represented by a black line at 0 cm depth (c, g) and the OPD is represented by the dashed line in bold at 4.7 ± 0.2 mm depth (c). Nitrate 1D profiles (d and h, black dots) are calculated using the average value of each pixel line of the nitrate distribution image (290 pixels wide), the standard deviation is represented by two fine dotted lines. The corresponding best-fitting concentration profiles (red dots, d and h) and the production zones (red line) are modelled with PROFILE software. The 1D profile corresponding to x = 1 mm (white line, g) is represented with a blue square profile (h) and the deep nitrate spot is indicated by a black arrow. The hatched grey zone (h) represents the detection limit of the nitrate 2D gel (<1.7 µmol L^{-1}).

Table 2. Summary of the NIS *Nonionella* sp. T1 contributions to benthic denitrification in the Gullmar Fjord. The porewater denitrification zones come from PROFILE modelling (Fig. 5 d, h). To estimate the contributions of *Nonionella* sp. T1 the number of counted specimens per zones was used. Two different approaches were used to estimate the contribution of *Nonionella* sp. T1: (A) *Nonionella* sp. T1 denitrification rate divided by nitrate porewater denitrification rate; (B) *Nonionella* sp. T1 denitrification rate divided by nitrate porewater denitrification rate plus *Nonionella* sp. T1 denitrification rate. The calculations are detailed in Equation S2.

Stations	Sediment depth interval of denitrification (cm)	*Nonionella* sp. T1 (counted specimens per zone)	Nitrate porewater denitrification rates (nmol cm^{-3} s^{-1})	*Nonionella* sp. T1 denitrification rates (nmol cm^{-3} s^{-1})	*Nonionella* sp. T1 contribution (%), approach A	*Nonionella* sp. T1 contribution (%), approach B
GF17-3A	1.2 to 5	841	4.07 E^{-07}	1.90 E^{-05}	47	32
GF17-3C	1.2 to 5	1807	4.07 E^{-07}	4.06 E^{-05}	100	50
GF17-1A	0 to 1.6	3	2.71 E^{-05}	6.72 E^{-08}	0	0
GF17-1C	0 to 1.6	12	2.71 E^{-05}	2.69 E^{-07}	1	0

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C.C. participated in the sampling cruise, did the foraminifera taxonomy, contributed to 2D gel experiments and analyses by hyperspectral camera. C.C. did the nitrate and oxygen respiration measurements and wrote the present manuscript. E.G. participated in the sampling cruise, contributed to foraminifera analysis, scientific discussions. E.M. participated in the sampling cruise, managed with A.M. the 2D gels experiments, and contributed to hyperspectral camera treatments and scientific discussions and manuscript rewriting. H.L.F managed with A.M the sampling cruise and contributed to foraminifera taxonomy and scientific discussions and manuscript rewriting. N.R.P. managed the oxygen and nitrate respiration measurements and contributed to the scientific discussions. P.L. managed hyperspectral treatments for 2D gels and contributed

to scientific discussion. M.G. participated in the 2D gel lab experiments and hyperspectral treatments. T.J. participated to the sampling cruise, contributed to 2D gels experiments and scientific discussions and manuscript rewriting. B.J. contributed to scientific discussion and manuscript rewriting. A.M. managed the sampling cruise and 2D gels experiments and contributed to hyperspectral camera treatments and scientific discussions and manuscript rewriting.

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Competing interests

565 The authors declare no competing interest.

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