Meiofauna increases bacterial denitrification in marine sediments

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Denitrification is a critical process that can alleviate the effects of excessive nitrogen availability in aquatic ecosystems subject to eutrophication. An important part of denitrification occurs in benthic systems where bioturbation by meiofauna (invertebrates <1 mm) and its effect on element cycling are still not well understood. Here we study the quantitative impact of meiofauna populations of different abundance and diversity, in the presence and absence of macrofauna, on nitrate reduction, carbon mineralization and methane fluxes. In sediments with abundant and diverse meiofauna, denitrification is double that in sediments with low meiofauna, suggesting that meiofauna bioturbation has a stimulating effect on nitrifying and denitrifying bacteria. However, high meiofauna densities in the presence of bivalves do not stimulate denitrification, while dissimilatory nitrate reduction to ammonium rate and methane efflux are significantly enhanced. We demonstrate that the ecological interactions between meio-, macrofauna and bacteria are important in regulating nitrogen cycling in soft-sediment ecosystems.

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Markedly increased nitrogen (N) loading of many coastal aquatic environments worldwide has had negative global ecological and economical consequences for biodiversity and water quality. Denitrification is a potentially important ecosystem process in coastal sediments that experience high anthropogenic N loads, because it is estimated to remove globally ~24 Tg of fixed N from the system per year. Although denitrification occurs globally, aquatic environments are far more important N sinks than terrestrial ones. Sediments are preferential places for denitrification, because they are often characterized by low oxygen (O₂) concentrations, sharp oxic/anoxic interfaces and high rates of nitrate (NO₃⁻) and organic matter supply.

Besides denitrification, two other pathways of nitrate reduction have been shown to play a role in marine sediments: anammox—the anaerobic oxidation of ammonium (NH₄⁺) to dinitrogen (N₂) by reduction of nitrite (NO₂⁻), and the dissimilatory nitrate reduction to ammonium (DNRA). Generally, anammox accounts for less than 20–30% of the total benthic N₂ production in shallow coastal environments, while DNRA can be more important than denitrification in coastal and estuarine areas. DNRA retains fixed nitrogen in the environment and further enhances eutrophication when denitrification is outcompeted. Given the magnitude of the ecological and economic problems caused by eutrophication, understanding the mechanisms that control nitrogen removal from aquatic ecosystems remains a central point in ecology, with clear environmental policy and management implications. However, the complexity of benthic N cycling, in particular of the multiple nitrate reduction pathways, is hard to tackle in field studies, and experimental investigations of feedbacks between trophic levels and their effects on ecosystem processes are needed.

Benthic macrofauna (invertebrates >1 mm) is widely recognized to play an important role in the regulation of carbon (C) mineralization, nutrient regeneration and coupled nitrification/denitrification. Macrofaunal activity is generally known to enhance denitrification due to particle reworking and burrowing, ventilation and bioirrigation. Most studies dealing with the effects of fauna on benthic biogeochemistry have considered large animals because they are easy to manipulate in the laboratory and are expected to physically alter microbial pathways and process rates limited by diffusive supply or other constraints (for example, sediment aging, burial to strictly anoxic zones or exhaustion of energy-yielding electron acceptors). While there is a vast body of literature dealing with macrofauna and its effect on sediment biogeochemistry, only a few papers deal with the role of other potentially important benthic organisms such as meiofauna (benthic animals between 0.04 and 1 mm) on benthic ecosystem services. Meiofauna is the most abundant and diverse metazoan group in aquatic sediments and corresponds to ~60% of total metazoan abundance on Earth.

Moreover, in benthic environments with low input of organic matter where endobenthic macrofauna abundance is low, meiofauna is often not only the most abundant but also the faunal group with the highest biomass. Interactions between meiofauna and sediment prokaryotes have recently been shown to have an important effect on benthic ecosystem processes such as organic matter mineralization or degradation of organic pollutants and only a few authors have acknowledged the importance of meiofauna for solute transport and oxygen and nutrient cycling. Recently, it has also been shown that certain meiofaunal groups (foraminifera) are even capable of complete denitrification, but the general role of meiofauna for benthic nitrogen cycling remains poorly understood.

Here, we test whether microbenthos and meiofauna–macrofauna interactions have a significant effect on (1) pathways of relevant electron acceptors (oxygen and nitrate) in surface sediments and on (2) fluxes of end products of anaerobic metabolism, such as methane. We show that meiofauna positively affects nitrification and denitrification, thus enhancing sedimentary nitrogen loss. On the other hand, macrobenthic bivalves increase N recycling by stimulating DNRA and the efflux of methane. Biological interactions between meio-, macrofauna and bacteria are therefore important factors that regulate essential benthic biogeochemical processes such as nitrogen loss and methane release.

**Results**

**Infauna abundances and community structure.** This experiment had four main treatments with changing infaunal composition. It included two treatments with high meiofauna abundance and diversity: HM, high meiofauna and no macrofauna, and HMM, high meiofauna + the macrofaunal bivalve Macoma balthica; plus two treatments with low meiofauna abundance and diversity: LM, low meiofauna and no macrofauna, and LMM, low meiofauna + M. balthica. In addition, we also included a treatment of undisturbed and unmanipulated sediment cores (CTRL) with the natural infaunal community composition and structure.

The extraction methodology established a large difference in meiofauna abundances between the treatments with low meiofauna (LM and LMM) and the two treatments with high meiofauna (HM and HMM). The meiofauna organisms remaining in the LM and LMM treatments were predominantly small nematodes (on average 86 and 74 ind. 10⁻³ m⁻², respectively) and ostracods (15 and 16 ind. 10⁻² m⁻², respectively; Table 1). The HM and HMM treatments contained a more diverse meiofauna community with high abundances of nematodes (718 and 680 ind. 10⁻³ m⁻², respectively) and ostracods (44 and 38 ind. 10⁻³ m⁻², respectively) together with copepods, kinorhynchs and low abundances of oligochaetes, which resulted in abundances on average seven times higher than in the LM and LMM treatments (Table 1). When compared with the control treatment, meiofauna abundance was significantly higher in the HM and HMM and lower in the LM and LMM treatment (analysis of variance (ANOVA), P<0.0001), and this was mainly due to differences in nematode abundances (Table 1). However, the meiofauna abundances in the HM, HMM and unmanipulated control treatment were within the same order of magnitude reported for the field area where the sediment was collected.

All macrofauna specimens that we added to the LMM and HMM treatments were recovered alive at the end of the experiment, that is, two individuals of M. balthica in each sediment core, corresponding to ~2,000 ind. m⁻² and a biomass of 1.92 g C m⁻². The unmanipulated control treatment was found to contain individuals of both Marenzelleria spp. (Polychaeta) and M. balthica, with Marenzelleria reaching higher densities than the bivalve (~1,400 and ~400 ind. m⁻², respectively) as well as higher biomasses (0.97 versus 0.38 g C m⁻², respectively).

**Concentration gradients and gas fluxes.** The average O₂ penetration depth and concentration profiles of O₂ in surface sediments and on (2) fluxes of end products of anaerobic metabolism, such as methane. We show that meiofauna positively affects nitrification and denitrification, thus enhancing sedimentary nitrogen loss. On the other hand, macrobenthic bivalves increase N recycling by stimulating DNRA and the efflux of methane. Biological interactions between meio-, macrofauna and bacteria are therefore important factors that regulate essential benthic biogeochemical processes such as nitrogen loss and methane release.
abundance did not significantly enhance O₂ penetration compared with low meiofauna abundance, whereas the presence of *M. balthica* increased O₂ penetration depth (LMM, HMM, CTRL; Table 3 and Fig. 1).

The theoretical molecular diffusive O₂ flux (*Jₐ₈*), calculated without taking into consideration biodiffusivity, decreased in the order HMM < LMM < HM < LM (Table 2). The CTRL treatment was included to determine how the processes measured in our study occur in unmanipulated intact sediment. As the remaining treatments intended to vary meio- and macrofauna abundances, they are not directly comparable to the CTRL treatment. Molecular diffusivity (*Dₘ*) was very similar among treatments (range 9.9 to 10.7 × 10⁻⁶ cm² s⁻¹) and reflected the narrow range of porosities in the different treatments (Table 2). The total oxygen flux (*Jₜ₇*), measured by whole-core incubation and representing the sum of the O₂ consumption due to infauna activity and *Jₐ₈* was significantly different among treatments (ANOVA, *P* = 0.003, Table 3) and decreased in the order LM < LMM < HM < HMM (Table 2). Biodiffusivity (*Dₜ₇*) was dependent on the biomass of the animals in each treatment and followed the same trend as the total oxygen flux, with the lowest *Dₜ₇* recorded in LM (2.4 ± 10⁻⁶ cm² s⁻¹) and the highest in HMM (27.2 ± 10⁻⁶ cm² s⁻¹) (Table 2).

Methane (CH₄) efflux to the water column was significantly different among the treatments (Kruskal–Wallis, *P* < 0.001, Table 3), with significantly higher emissions associated with sediments inhabited by bivalves when compared with those with only meiofauna, irrespective of whether they had high or low abundances and diversity (Table 3 and Fig. 2). The control treatment showed an intermediate situation, with CH₄ fluxes ranging between HM and LMM. HMM showed a CH₄ efflux 10 times higher than HM, whereas in LMM the CH₄ efflux was seven times higher than in LM, suggesting that bivalve activity coupled to high meiofauna abundance and diversity either stimulated methanogenesis or methane transport. The higher effluxes of CH₄ in HMM and LMM when compared with CTRL, which was dominated by *Marenzelleria* spp., suggest that *M. balthica* stimulated CH₄ emission over *Marenzelleria* spp.

**Table 1 | Abundances and biomass of meiofauna.**

| Treatment | Nematoda | Ostracoda | Harpacticoida | Kinorhyncha | Oligochaeta | Total |
|-----------|----------|-----------|---------------|-------------|-------------|-------|
| LM        | 2.4 ± 11 | 2.4 ± 7.3 | 1.5 ± 4.3     | 0.0 ± 0     | 0.0 ± 0     | 3.1 ± 2 |
| HM        | 1.2 ± 17 | 1.7 ± 2.7 | 1.5 ± 4.3     | 0.0 ± 0     | 0.0 ± 0     | 3.3 ± 2 |
| LMM       | 4.3 ± 16 | 4.8 ± 1.6 | 4.8 ± 1.6     | 0.0 ± 0     | 0.0 ± 0     | 4.3 ± 2 |
| Control   | 4.7 ± 16 | 4.7 ± 1.6 | 4.7 ± 1.6     | 0.0 ± 0     | 0.0 ± 0     | 4.7 ± 2 |

CTRL, unmanipulated sediment cores; HM, high meiofauna; HMM, high meiofauna + macrofauna; LM, low meiofauna; LMM, low meiofauna + macrofauna. Meiofaunal densities (ind 10⁻³ m⁻²) are in top rows in roman style and biomasses (µg C 10⁻³ m⁻²) are in second rows in italic style. Values represent average ± s.d. (n = 8 per treatment). 

**Table 2 | Oxygen penetration, oxygen fluxes and diffusivity among treatments.**

| Treatment | OPD (mm) | *Jₜ₇* (µmol m⁻² h⁻¹) | *Jₐ₈* (µmol m⁻² h⁻¹) | *Dₘ* (cm² s⁻¹) | *Dₜ₇* (cm² s⁻¹) |
|-----------|----------|----------------------|----------------------|----------------|-----------------|
| LM        | 3.3 ± 0.3 | -1.098 ± 34          | -882 ± 58            | 9.9 × 10⁻⁶     | 2.4 × 10⁻⁶      |
| HM        | 3.7 ± 0.2 | -1.321 ± 69          | -695 ± 51            | 10.1 × 10⁻⁶    | 9.0 × 10⁻⁶      |
| LMM       | 4.2 ± 0.1 | -1.211 ± 50          | -510 ± 32            | 10.5 × 10⁻⁶    | 14.4 × 10⁻⁶     |
| HMM       | 4.7 ± 0.2 | -1.412 ± 64          | -399 ± 35            | 10.7 × 10⁻⁶    | 27.2 × 10⁻⁶     |
| Control   | 5.1 ± 0.1 | -1.240 ± 113         | -540 ± 68            | 10.3 × 10⁻⁶    | 13.3 × 10⁻⁶     |

CTRL, unmanipulated sediment cores; HM, high meiofauna; HMM, high meiofauna + macrofauna; LM, low meiofauna; LMM, low meiofauna + macrofauna; OPD, *Dₜ₇* penetration depth. Average OPD, total benthic O₂ flux (*Jₜ₇*), and molecular diffusive O₂ flux (*Jₐ₈* ± s.d. (n = 8 per treatment). *Dₘ* represents molecular diffusivity and *Dₜ₇* represents biodiffusivity in the top sediment layer. *Dₜ₇* was calculated from the previous three parameters (see Methods).
and was highest in HMM where DNRA accounted for ~19% of total NO$_3^-$ reduction, followed by LMM (~11%) and the other treatments (~5%). DNRA rates followed the same trend as the CH$_4$ fluxes and were significantly higher in sediments inhabited by bivalves (LMM and HMM) compared with those with only meiofauna (LM and HM), irrespective of whether they had a high or low meiofauna abundance (Kruskal–Wallis, $P = 0.004$, Table 3). Once again, in the control treatment, DNRA was intermediate between the rates in HM and LMM.

Discussion

Our results show that high meiofauna bioturbation (Fig. 4) enhances the sedimentary production of dinitrogen gas. This enhanced dinitrogen production is not due to direct respiration of nitrate by the meiofauna, which has been shown for some species of foraminifera, a common unicellular meiofaunal group$^{28,29}$. In line with previous studies that found foraminifera to be generally present in low abundances in the Baltic Sea$^{32}$, our experimental sediments did not contain significant numbers of individuals of this meiofaunal group. In addition, our incubations

### Table 3 | Summary of statistical test results.

| Parameter                              | Analysis  | $P$ value | Differences among treatments |
|----------------------------------------|-----------|-----------|------------------------------|
|                                        | $H_{3,19} = 14.450$ | 0.002     | a, b                         |
| Meiofauna abundances                   |           |           | a, b                         |
| O$_2$ penetration depth                 | $F_{3,23} = 10.362$ | <0.001    | a, ab, bc                    |
| Molecular diffusive O$_2$ flux ($J_{dif}$) | $F_{3,23} = 22.814$ | <0.001    | a, b, c                      |
| Total O$_2$ flux ($J_{tot}$)            | $F_{3,31} = 5.901$ | 0.003     | a, b, ab                     |
| Total denitrification rate ($D_{tot}$)  | $F_{3,27} = 11.371$ | <0.001    | a, b, a                      |
| Denitrification from water NO$_3^-$ ($D_w$) | $F_{3,27} = 5.707$ | 0.004     | a, a, b                      |
| Coupled nitrification–denitrification ($D_n$) | $F_{3,27} = 11.425$ | <0.001    | a, a, a, b                   |
| DNRA rate                              | $H_{3,27} = 13.381$ | 0.004     | a, a, b                      |
| CH$_4$ flux                            | $H_{3,31} = 24.662$ | <0.001    | a, a, b                      |

DNRA, dissimilatory nitrate reduction to ammonium; HM, high meiofauna; HMM, high meiofauna + macrofauna; LM, low meiofauna; LMM, low meiofauna + macrofauna.

One-way parametric ($F$ values) and non-parametric Kruskal–Wallis analysis of variance ($H$ values) among the different treatments. Pairwise comparison was performed by means of Tukey test. Different letters represent significant differences ($P < 0.05$), while the same letter represents no significant differences ($P > 0.05$) among treatments.

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In estuarine and coastal marine sediments, nitrification is often the electron donor (for example, organic matter, sulfide and so on). Capable of nitrate respiration, prokaryotes and especially denitrification. Although certain protists and foraminifera are denitrifying bacterial activity rather than direct eukaryotic stimulation of nitrification–denitrification as increased availability of ammonium would have stimulated nitrifiers, and increased availability of nitrate and labile organic compounds would have specifically stimulated heterotrophic denitrification. Moreover, in sediments with more active irrigation by meiothemos transport of solutes like oxygen, ammonium and nitrate was low, could have enhanced growth of aerobic bacteria and protozoans. It is also possible that less competition for oxygen penetration significantly. This indicates that meiofauna was mainly active in the uppermost millimeters, as supported by visual inspection (Fig. 4). The oxygen consumption rate calculated from the concentration profiles in the uppermost sediment layer (0–2 mm depth) was consistently lower in HMM and HM than in LMM and LM (Fig. 1). Even when considering biodiffusivity in our numerical interpretations, the O2 consumption in this top layer was greater in LMM and LM than in HMM and HM. This may suggest that aerobic microorganisms were more active and abundant in close proximity to the sediment–water interface in low meiofauna than in high meiofauna conditions. Lower predation pressure, when meiofauna density was low, could have enhanced growth of aerobic bacteria and protozoans. It is also possible that less competition for...
oxygen in the low meiofauna treatments could have favored aerobic microorganisms. Nonetheless, a recent experiment using soft Baltic Sea sediments mobilized at greater depth by macrofaunal bioturbation16. This is a fundamental limitation of the IPT, which heterogeneity of the sediment due to large polychaete burrow structures52. This is a fundamental limitation of the IPT, which due to interference competition for freshly deposited organic matter48, while no obvious effect on bacterial abundances has been reported49. Thus, it appears that M. balthica counteracted the stimulating effect for the nitrifying and denitrifying microbial communities by meiofauna as suggested above. In addition, in sediments with bivalves, nitrification could have been partly inhibited by the presence of sulfides49, which could have been mobilized at greater depth by macrofaunal bioturbation16. This is supported by the fact that in LMM and HMM we measured the highest rates of DNRA, a process that is tightly coupled to the oxidation of sulfides in sediments51. It is likely that all these factors contributed to the decrease in coupled nitrification–denitrification we observed in HMM compared with HM. The treatments with macrofauna had higher Dn compared with the treatments where M. balthica was absent, which agrees with previous studies17,18.

Macrofaunal bioturbation has previously been shown to restrain the uniform mixing of endogenous 14NO3 and added 15NO3 in the denitrification zone because of increased heterogeneity of the sediment due to large polychaete burrow structures52. This is a fundamental limitation of the IPT, which may lead to the underestimation of total denitrification activity31. Nonetheless, a recent experiment using soft Baltic Sea sediments bioturbated by Marenzelleria spp, proved that at high polychaete biomass, the two nitrate isotopes were homogeneously mixed16, that is, the significant positive correlation between polychaete biomass and degree of denitrification underestimation32 was not observed. In our study, meiofauna likely exerts an opposite effect compared with large polychaetes: the former creates a more homogeneous sediment texture because it freely moves in the interstices53 and enhances solute and particle transport within the oxic zone24,26,27. Moreover, since the siphons of M. balthica do not secrete mucus and are mostly active in the nitrification zone54, its bioturbation may have even helped nitrate mixing.

Anammox is a factor that may lead to the overestimation of total N2 production in marine sediments incubated using the IPT55. Our tests with anoxic sediment slurries collected from the same geographical area as our experimental sediments and amended with 15NH4 and 14NO3 did not result in any significant production of labelled N2, suggesting that anammox bacteria were either not active or not present in this shallow coastal sediment. So far, no studies have examined the effect of macro- and microbioturbation on the anammox process. We therefore recommend that future experiments should investigate the effect of faunal bioturbation on anammox in sediments where anammox contributes significantly to N2 production rates6.

Methane effluxes and DNRA rates showed the same trend among different treatments, with a significantly higher flux/rate in sediments inhabited by bivalves. It is not clear if the increase in methane and ammonium release was due to the presence of symbionts living in the gut of M. balthica or to the bioturbation activity by the bivalve. Macrofauna can have anoxic niches inside its gut, which allow the colonization and metabolic activity of anaerobic bacteria88. In particular, bivalves can host symbionts in every region of their gut, where fermenting bacteria have been documented76. The fact that the bivalves were not found deeper than 1.5–2 cm in our sediments further indicates that there were anoxic microenvironments inside M. balthica colonized by nitrate reducers and methanogens, as it has been suggested for other metazoans58,59. Further investigations are required to examine if bivalves are not only able to consume methane thanks to their symbionts60, but also capable of methane production.

The enhancement of nitrification and denitrification by meiofauna suggests that this faunal group can mediate nitrogen cycling in sediments with little or no macrofauna. Indeed, Danovaro et al.41 has found deep-sea ecosystem functioning and efficiency to be linked to high meiofauna diversity. Meiofauna dominates over macrofauna in terms of biomass, abundance and diversity in low-energy benthic systems51, and in systems affected by oxygen depletion32. The results presented here show that denitrification is even higher when macrofauna abundance is reduced.

Denitrification by foraminifera can make important contributions to total N2 production especially in continental shelves28 and deep see sediments61. Generally, rates of foraminiferal denitrification may vary between 2.1 and 7.2 μmol N m⁻² h⁻¹ (refs 28,61). The increase in denitrification rate by meiofauna bioturbation measured in our experiments (5.1 μmol N m⁻² h⁻¹) is even higher than the average denitrification rate by foraminifera suggesting that stimulation of bacterial denitrification by meiofauna activity may be as important as the direct meiofaunal denitrification itself. This is important in light of the strong anthropogenic pressure on benthic ecosystems and their vulnerability to biodiversity loss62. Meiofauna has shorter generation times and faster turnover rates than macrofauna21, and recovers generally faster from perturbation events related to eutrophication like anoxia or hypoxia53. Our findings suggest that meiofauna community recovery could stimulate benthic microbial processes by enhancing biodiffusivity, even if the macrofauna population fails to recover or does so later in time.

Our results demonstrate that meiofauna activity increases the removal of fixed nitrogen from aquatic ecosystems by stimulating nitrification and denitrification in the oxic–anoxic transition zone of the marine sediment. Bivalves stimulate microbial processes as DNRA and net methane release, and this stimulation is also affected by meiofauna bioturbation. By enhancing DNRA, bivalves can to a certain extent counteract the beneficial effects of meiofauna on total N loss. Effects of macrofauna–meiofauna–bacteria interactions on nitrogen transformation processes have been largely unexplored. This study provides important new information on how benthic meiofauna can mitigate environmental problems caused by excessive nitrogen loads in aquatic ecosystems (that is, eutrophication). Further understanding of the mechanisms regulating benthic ecosystem processes such as denitrification requires studies that take into account how ecological interactions between macro-, meio- and microbiological communities of the benthos impact such processes. In particular, it is important to consider other potentially important faunal groups, such as protozoans, in regulating bacteria-mediated processes with important ecosystem function.

**Methods**

**Sediment sampling.** Sediment cores were collected in July 2012 with a multicorer in Hållsviken (Stockholm Archipelago, Baltic Sea: 58°50'N, 17°32'E) at 28 m depth. Sampling with a multicorer minimizes the 'bow-wave' effect on the sediment surface, which can reduce the abundance of epibenthic fauna in the water overlying the sediment. A modified Niskin sampler allowed the collection of bottom water, and a digital multimeter was used to measure temperature (9.4 °C), salinity (6.5), and dissolved oxygen (307 μmol l⁻¹). Multicorer sediment liners (n = 16, i.d. 9 cm, height 60 cm) were subsampled onboard with smaller liners (n = 45, i.d. 3.6 cm, height 25 cm) to have 12 cm height of sediment and 10 cm of overlying water. The cores were capped with rubber stoppers and transported within 30 min to the Åskö Laboratory, Stockholm University Marine Research Center, where they were stored in a cold room and constantly stirred with magnetic bars at in situ temperature.

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Meiofauna extractions and experimental setup. Meiofauna extractions were carried out the day after sampling using the procedure described in Næslund et al. Briefly, the upper 4 cm of each core were sliced and sieved through a 1,000 and 40-μm sieve. The animals retained in the 1,000-μm sieve were removed as macrofauna, while meiofauna and sediment in the 40-μm sieve were submersed in a MgCl₂ solution (740 mmol l⁻¹) for 5 min to anaesthetize the animals. Meiofauna was separated from the sediment by density extraction using a Levasil colloidal solution (H.C. Starck) with a density of 1.3 kg m⁻³. The extractions were made by shaking an Erlenmeyer flask with sediment and Levasil and let it stand for 5 min while the sediment settled and the animals floated up (settling time). The top part of the solution containing the animals was decanted and washed with seawater (salinity 6.5). This extraction procedure was repeated twice (a second extraction with 5 min of settling time, followed by a third and final extraction with 30 min of settling time). After the last extraction, the sediment retained in the 40-μm sieve was washed thoroughly with seawater to remove the Levasil and reintroduced to the sliced core. The meiofauna individuals extracted from two cores were pooled and added to one of the two experimental units, creating a high meiofauna abundance/diversity. The second experimental unit was left with only extracted sediment and low meiofauna abundance/diversity, that is, the meiofauna that could not be re-introduced. After this procedure, we added this pre-extracted sediment particles that passed through the 40-μm sieve to each experimental unit to reconstitute the finer sediment particle fraction of the original sediments. A total of 45 microcosms were setup, with five different treatments (n = 9 per treatment): (1) high meiofauna (HM), microcosms with a high abundance and diversity of extracted meiofauna; (2) high meiofauna and sediment with macrofauna (HMM), identical to HM treatment but with two individuals of Macoma balthica; (3) low meiofauna (LM) microcosms with the extracted sediment particles only and no meiofauna addition; (4) low meiofauna with macrofauna (LMM), identical to the LM treatment but with two individuals of M. balthica and (5) a control (CTRL) consisting of unmanipulated intact sediment cores. The CTRL treatment was not directly comparable to the other manipulated treatments (HM, HMM, LM and LMM). Its main function was to provide an experimental control, and enable a comparison to field-like conditions.

The abundance of M. balthica used in the experimental units of the HM and LMM treatments corresponds to 1–4.5 days of peak sedimentation of phytoplankton material (see below for details). In the numerical procedure used to calculate sediment core incubation (see below for details). In the numerical procedure used to calculate O₂ concentration at the bottom and the flux at the bottom of the profile were selected as boundary conditions.

**Sediment core incubations.** A total of 40 sediment cores (n = 8 per treatment) with about 100 ml water on top of the sediment were capped with rubber stoppers without headspace and stirred with small magnetic stirrers. Incubations were limited to 6 h to ensure that the O₂ concentration did not decrease below 20% of the initial value. Water samples for CH₄ were taken at the beginning and the end of the incubation, transferred to 12 ml Exetainer vials (Labco Scientific) and poisoned with 100 μl ZnCl₂ (7 mol l⁻¹). O₂ concentrations were measured before and after the incubation using a specialised mid-infrared spectrophotometer (OX 500, Unisense).

After the flux incubation, a second incubation was carried out to determine denitrisfication and N₂O release according to the IPT₊⁻. Each sediment core was treated with the same concentration of ¹⁵NO₃⁻ to avoid pseudoreplication. Briefly, the water on top of each sediment core was amended with a 20 mmol l⁻¹ Na₂NO₃ solution (6.3 atom %) to a final concentration of 50 μmol l⁻¹ ¹⁵NO₃⁻. Samples for N₂O analysis were taken before and after ¹⁵NO₃⁻ addition to determine the labelling percentage of the N₂O pool. The water samples were filtered through 0.45 μm filters disposable and frozen for later analysis. The cores left uncapped and preincubated for 1.5 h to establish a linear production of ¹⁵N₂ and ¹⁵N₂O and to simulate laboratory conditions. One core after treatment was sacrificed thereafter to measure the background concentration of ²⁹N₂ and ³⁰N₂. The other cores (n = 35) were capped without headspace and incubated for 6 h while stirring. The incubation was terminated by gently mixing water and sediment in each core to thoroughly mix. Approximately, 20 ml slurry were sampled with a plastic syringe and a Viton tubing equipped with a plastic net, placed in a 12-ml Exetainer, and immediately poisoned with 100 μl ZnCl₂ (7 mol l⁻¹) for later analysis of ²⁹N₂ and ³⁰N₂. An additional poisoned slurry sample (∼10 ml) was taken, treated with KCl (2 mol l⁻¹), centrifuged, filtered and frozen at −20 °C for later analysis of ¹⁵NH₄⁺ fraction in the ammonium pool.

**Anoxic slurry incubation for anammox activity.** The presence/absence of sedimentary anammox activity was tested by means of anoxic slurry incubations performed in 12 ml Exetainers. Briefly, the topmost 1.5 cm sediment was collected from a multicorer sediment liner (d = 9 cm, height 60 cm) and homogenized in a glass beaker. Then, 2 ml of this sediment was transferred to each of 18 Exetainers, which were filled with anoxic bottom water. Each vial also received a 4-mm glass bead and was immediately sealed. The slurries were kept homogenously on a rotating stirrer and preincubated for 12 h to consume all residual O₂ and N₂O.

Following preincubation, a degassed 10 mmol l⁻¹ stock solution of ¹⁴NO₃⁻ and ¹⁵NH₄⁺ (99.1 atom %) was injected through the vial septa to reach a final concentration of 150 μmol l⁻¹⁻¹. Nine vials received the ¹⁵N solution, while nine were left unamended to act as a control. Biological activity was stopped in triplicate samples directly after the addition of substrates by injecting 100 μl ZnCl₂ (7 mol l⁻¹) to each Exetainer. In the same way, triplicate samples were taken after 6 and 12 h incubation at in situ temperature (9.5 °C) on the rotating stirrer. The poisoned slurry samples were subsequently analysed for concentrations of ²⁹N₂.

**Infauna quantification and porosity determination.** After incubation, the surface sediment layer (~ 2 cm) of each core was sliced off and sieved sequentially through 1,000 and 40-μm sieves to retrieve samples of macro- and meiofauna. The remaining sediment in each sediment core was sieved through a 1-mm sieve to retrieve deeper burrowing macrofauna individuals. The 40-μm fraction of the sediment from each core was preserved in 4% buffered formalin before extracting the meiofauna, using the method described above. Meiofauna was sorted and counted under a × 30 binocular stereomicroscope. Meiobenthos biomass was calculated according to Olabsson et al. Macrofaunal biomass was determined from the animal dry weight and the carbon content was assumed to be 40% (ref. 69).

One core per treatment was sacrificed for porosity determination. The sediment core was bisected at 1 cm intervals and porosity was determined from water content and assuming a dry sediment density of 2.6 g cm⁻³. Water content was measured as water loss after drying at 105 °C until constant weight. To use
dynamic porosity values for interpretations of O₂ concentration profiles, the porosity profiles were fitted with polynomial functions.26

Nematode incubations. Exeterior vials were incubated with anoxic water amended with 15NO₃⁻ and increasing numbers of nematodes to test if the animals and/or their symbionts were actively denitrifying. Briefly, 400 ml in situ waters were filtered and amended with 1 ml of a Na₂SO₄ solution (20 mmol l⁻¹ 15NO₃⁻) to reach a concentration of ~50μmol l⁻¹ 15NO₃⁻. The water was degassed for 20 min with Helium 5.0 in a glass bottle equipped with a gas-tight inlet and outlet. One ml of this solution was transferred to each Exeterior (5.9 ml volume) and increasing numbers (5 to 35, in intervals of 5 so that n = 7) of nematodes were placed in each vial; three additional Exeteriors were used as controls with only filtered water and no animals. The nematodes were collected from depths between 1 and 3 cm and extracted alive with the method described above. The Exeteriors received additional 3.5 ml of degassed and 15NO₃⁻-amended water and were capped right away. The vials were additionally degassed with Helium for 5 min so that the O₂ concentration at the beginning of the incubation (tested with a precalibrated microelectrode) was always <1 μmol l⁻¹. After 8 h at 9.5 °C, the incubation was terminated by adding 100 μl ZnCl₂ (7 mol l⁻¹) to each of the vials, which were subsequently analysed for concentrations of 28N₂ and 30N₂. Stereomicroscope observations showed that the nematodes were killed immediately after the concentrated ZnCl₂ solution was added.

Analyses and calculation. Nitrate and ammonium were determined on a seg-
mented flow autoanalyzer (ALPKEM, Flow Solution IV). Precision was ±0.036 micromol l⁻¹ for NH₄⁺ and ±0.021 micromol l⁻¹ for NO₃⁻. Methane was ana-
ysed by headspace analysis on a gas chromatograph equipped with a FID (SRI 8610C). Precision was ±1 mmol l⁻¹. Net fluxes of O₂ and CH₄ across the sediment–water interface were calculated from the difference in concentration in the water column at the beginning and end of the incubation period. The concentrations of 28N₂ and 30N₂ in the Exeteriors were determined by means of headspace analysis on a gas chromatograph-isotope ratio mass spectrometer. Excess 28N₂ and 30N₂ were used to calculate the N₂ production over time, 31, and the denitrification rate (D₄) was calculated from the relation:

\[ D₄ = D₁₅O \times \left( \frac{2p^{28}N₂}{2p^{30}N₂} \right) \]  

where \( p^{28}N₂ \) and \( p^{30}N₂ \) are the production rates of 28N₂ and 30N₂, respectively, and D₁₅O is calculated as follows:

\[ D₁₅O = D₁₅N × r_NH₄ \]  

where r_NH₄ is the ratio between the concentrations of 15NO₃⁻ and 15NO₂⁻ in the water column;

\[ D₁₅N = D₄₁ × D₄₂ \]  

The isotopic composition of NH₄⁺ was analysed after the conversion of NH₄⁺ to N₂ with hypobromite.9 Analytical precision was 0.5 nmol l⁻¹. Dnra rate was calculated as follows:

\[ DNRA = p^{15}NH₄⁺ \times \frac{D₄₁}{D₄₂} \]  

where \( p^{15}NH₄⁺ \) is the production rate of 15N-labelled ammonium during incubation.

Statistical analyses. Differences between flux/rate calculated for each treatment (LM, HM, LMM, HMM) were tested using parametric (ANOVA) and non-para-
metric (Kruskal–Wallis) one-way analysis of variance. The CTRL treatment was among treatments were carried out with the Tukey test. Statistical analyses were performed with Statistica 9.0 (StatSoft). Average values (μmol m⁻² h⁻¹) are reported with associated s.e.m., unless otherwise noted.

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We are grateful to Dr. Barbara Deutsch and the staff of the Askö laboratory for support during sampling. We thank the chemical laboratory at Department of Ecology, Environment and Plant Sciences (Stockholm University) for nutrient analysis; Silvia Fedruzzi for practical help during the experiment phase; and Dr Johan Nilslund for the sediment pictures. This study was supported by the Swedish Research Council Formas (Grant no. 215-2009-813 to V.B.), by Baltic Ecosystem Adaptive Management (BEAM) to V.B. and by a grant from Stockholm University’s Baltic Sea Centre to S.B. and I.K.

Author contributions

S.B. and F.J.A.N. performed the sampling; S.B. and I.K. carried out the experiments; S.B. and M.B. carried out the isotope and gas analysis; F.J.A.N. performed the meiofauna identification. The research was designed by S.B., F.J.A.N., M.B. and V.B. All authors contributed to interpreting the data and writing the paper.

Additional information

Competing financial interests: The authors declare no competing financial interests.

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How to cite this article: Bonaglia S. et al. Meiofauna increases bacterial denitrification in marine sediments. Nat. Commun. 5:5133 doi: 10.1038/ncomms6133 (2014).

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