Denitrification in estuarine sediment stimulated by the irrigation activity of the amphipod *Corophium volutator*

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ABSTRACT: Sediment with different densities of *Corophium volutator* (Pallas), ranging from 0 to 19 800 ind. m$^{-2}$, were incubated in laboratory microcosms, and rates of oxygen uptake, denitrification and nitrate ammonification were determined from sediment-water fluxes. The measured processes were stimulated differently by *C. volutator*: oxygen uptake, denitrification of NO$_3^-$ from nitrification within the sediment, and denitrification of NO$_3^-$ from the overlying water were enhanced 2-, 3- and 5-fold respectively in the presence of 19 800 ind. m$^{-2}$. This differential stimulation was explained by the different characteristics of diffusional solute transport at the sediment-water interface and mass transfer of water into the burrows where O$_2$ and NO$_3^-$ was depleted. Denitrification rates were calculated by using the $^{15}$N isotope pairing technique. The applicability of the $^{15}$N isotope pairing technique for measuring coupled nitrification-denitrification in bioturbated sediment was confirmed in a test incubation with different levels of $^{15}$NO$_3^-$ added to microcosms with 12 000 *C. volutator* ind. m$^{-2}$

KEY WORDS: Denitrification · Bioturbation · Estuarine sediment

INTRODUCTION

Nitrogen has been identified as the most limiting nutrient for phytoplankton production in coastal seas (e.g. Ryther & Dunstan 1971) and denitrification may therefore control eutrophication by eliminating available nitrogen through NO$_3^-$ reduction to N$_2$O and N$_2$ (e.g. Nixon et al. 1976, Seitzinger 1980). Bottom-dwelling animals affect both physical and chemical processes taking place in the sediment through burrow construction, bioturbation and irrigation (Rhoads 1974, Aller 1988, Kristensen 1988). The objective of the present study was to measure the effect of bioturbation by the amphipod *Corophium volutator* (Pallas) on denitrification in estuarine sediments.

*Corophium volutator* lives in 'U'-shaped burrows 2 to 6 cm deep that are continuously irrigated. The burrow represents an extension of the sediment surface, and thus of all processes taking place at the surface (Hylleberg & Henriksen 1980, Kristensen 1984). The burrow wall is often a site of high bacterial numbers and metabolic activity compared to bulk sediment (Aller & Yingst 1978, Henriksen et al. 1983, Koike & Mukai 1983, Kristensen et al. 1985, 1991, Kristensen & Blackburn 1987). Higher potential nitrification rates have been reported in *C. volutator*'s burrow wall relative to surface sediment, suggesting better conditions for nitrifiers (Henriksen et al. 1983). The same authors indicated that *C. volutator*'s excretion rates could account for 80% of the net NH$_4^+$ flux from the sediment and eventually constitute an important pool of NH$_4^+$ for nitrifying bacteria. Irrigation of the burrow increases NO$_3^-$ transport from the water column into the sediment and thus denitrification of NO$_3^-$ coming from the overlying water (Aller 1988, Kristensen 1988), while O$_2$ transport to the burrow lining may increase nitrification (Aller 1988, Kristensen 1988) and thus denitrification of NO$_3^-$ generated within the sediment. Henriksen et al. (1980) reported, from indirect calculations, higher nitrification and denitrification rates in sediment bioturbated by *C. volutator* (6000 ind. m$^{-2}$) relative to non-bioturbated sediment.

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The isotope pairing technique (Nielsen 1992), involving $^{15}$NO$_3^-$, makes possible the measurement of denitrification of NO$_3^-$ coming from both sources: the overlying water ($d_{w}$) and nitrification within the sediment ($d_{n}$). Oxygen consumption, $d_{w}$ and $d_{n}$ rates were measured and compared in sediment microcosms containing different amphipod densities and with different NO$_3^-$ concentrations in the overlying water.

**MATERIAL AND METHODS**

**Test incubation.** A small incubation series was set up to test the applicability of the denitrification assay in sediment bioturbated by Corophium volutator. Specimens approximately 3 to 5 mm in length were collected in a shallow mesohaline estuary (Norsminde Fjord, Denmark). The amphipods were placed in cores (13 cm long and 3.4 cm inner diameter, containing 6 cm intact sandy sediment), at a density of 12000 ind. $m^{-2}$. The amphipods dug immediately into the sediment. The microcosms were covered with a 340 µm net to prevent the specimens from escaping, were placed in a reservoir containing aerated in situ water (24% salinity), and kept in darkness at 13°C. After 1 d, $^{15}$NO$_3^-$ was added to each core to give concentrations ranging from 15 to 330 µM NO$_3^-$ and the cores were stoppered. Incubation time was around 80 min for all the cores, ensuring a maximum oxygen depletion of 20%. Stoppers were removed at the end of the incubation and ZnCl$_2$ (240 µl 50% solution) was added to stop microbial activity. Water column and porewater was gently mixed with a stick, and samples of the slurry were stored in 12 ml glass tubes containing 2% ZnCl$_2$ closed with gastight caps. Two days later, N$_2$ gas was extracted from these water samples with 1 ml argon before analysis.

**Main incubation.** Corophium volutator was distributed in artificial microcosms containing homogenized and sieved (500 µm mesh) silty sediment. Three different sets of microcosms were used: 0, 6600 (normal density) and 19800 (high density) ind. $C. volutator$ $m^{-2}$. The experiment was run at 11°C. Oxygen fluxes were measured regularly to ensure steady state conditions before the experiment was conducted (results not shown). Twelve days after setup, $^{15}$NO$_3^-$ was added to the reservoir to give $72 \pm 2$ µM NO$_3^-$ (80 ± 1% $^{13}$NO$_3^-$). One day later an incubation was carried out. The cores were stoppered during a period ranging from 0.5 to 4 h (to assure maximum 20% oxygen depletion). At the end of the incubation the stoppers were removed and 30 ml water samples were taken with a 50 ml plastic syringe. Dissolved N$_2$ was extracted with 5 ml argon and collected in a pre-evacuated 3.4 ml Venoject blood collection tube (Risgaard-Petersen et al. 1993). Water samples for analyzing NO$_3^-$, %$^{15}$NO$_3^-$, and $^{15}$NH$_4^+$ were collected and frozen. The water in the reservoir was subsequently exchanged with water containing 152 ± 7 µM NO$_3^-$ (90% $^{15}$NO$_3^-$) and the microcosms were incubated again following the same steps as described above. Mortality at the end of the experiment (17 d after setup) was around 20% of the initial number of amphipods introduced. Dead amphipods were removed each day from the microcosms.

**Analysis.** Oxygen was measured directly in the microcosms with an oxygen microsensor provided with a guard cathode (Revshbech 1989) and NO$_3^-$ plus NO$_2^-$ by standard methods (Grasshoff et al. 1983) in a flow injection analyzer (Tecator, Höganas, Sweden). The formation of $^{15}$N-labelled dinitrogen pairs ($^{14}$N$^{15}$N and $^{15}$N$^{15}$N) by denitrification was measured on an isotope ratio mass spectrometer as described by Nielsen (1992). The %$^{15}$NO$_3^-$ enrichment in the water phase was analyzed on a mass spectrometer after using denitrifying bacteria cultures to transform NO$_3^-$ to gaseous N$_2$ (Risgaard-Petersen et al. 1993). Accumulation of $^{15}$NH$_4^+$ in the water phase was analyzed by mass spectrometry after NH$_3$ in the samples was liberated as NH$_3$ into a headspace (after addition of a strong base), captured in a capsule containing acidified Al$_2$O$_3$, and converted to dinitrogen by heating the sample (Blackburn 1993). Due to the small concentrations, the water samples were enriched with 3 µmol unlabelled NH$_4^+$ to obtain sufficient N for analysis on the mass spectrometer.

**Calculations.** Denitrification rates were calculated by using the $^{15}$N isotope pairing technique (Nielsen 1992), which assumes that addition of $^{15}$NO$_3^-$ does not influence denitrification of the natural $^{14}$NO$_3^-$ and that the NO$_3^-$ species are uniformly mixed in the denitrification zone. The formation rates of single-labelled ($^{14}$N$^{15}$N) and double-labelled ($^{15}$N$^{15}$N) dinitrogen pairs were used to calculate $d_w$ and $d_n$:

$$d_{15} = (14N^{15}N) + 2(15N^{15}N) \quad (Koike \& Hatton 1978);$$

$$d_{14} = d_{15} \frac{(14N^{15}N)}{2(15N^{15}N)};$$

$$dw = d_{15} \frac{100}{e_{15}};$$

$$dn = d_{14} + d_{15} - dw \quad (Nielsen 1992)$$

where $e_{15}$ is the %$^{15}$NO$_3^-$ enrichment of the reservoir water.

An analysis of variance (ANOVA) was carried out to test the significance of the differences between treatments.
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RESULTS

Test incubation

Fig. 1A, B shows the calculated rates of denitrification of NO3\(^-\) coming from the overlying water (dw) and NO3\(^-\) generated within the sediment (dn). Denitrification of NO3\(^-\) coming from the overlying water was linearly correlated with NO3\(^-\) concentration in the overlying water (r = 0.88) in the test incubation with 12,000 ind. Corophium volutator m\(^{-2}\) (Fig. 1A). The calculated rates of dn were independent of the concentration of NO3\(^-\) present in the overlying water at concentrations above 40 \(\mu\)M (slope not significantly different from zero, p = 0.05) (Fig. 1B). Oxygen consumption rates (not shown) were 1200 ± 200 \(\mu\)mol O\(_2\) m\(^{-2}\) h\(^{-1}\).

Main incubation

Oxygen consumption rates and denitrification rates were higher in bioturbated than in non-bioturbated sediments (Figs. 2, 3 & 4). Both oxygen uptake and dn rates were significantly higher in microcosms containing 19,800 ind. m\(^{-2}\) than in microcosms without amphipods (p < 0.001), while no statistically significant difference was found between the rates measured in microcosms containing 0 and 6600 ind. m\(^{-2}\) (p = 0.1) (Figs. 2 & 4). Oxygen consumption rates by Corophium volutator, calculated from the literature (McLusky 1969, Birklund 1977), indicated that the amphipod metabolism accounted for less than 2% of the total sediment oxygen uptake in our bioturbated microcosms. Denitrification of NO3\(^-\) coming from the overlying water also increased with increasing NO3\(^-\) concentration in the overlying water (from 0 to 152 \(\mu\)M) (Fig. 3). Apparent rates of denitrification of NO3\(^-\) generated within the sediment were not affected by the NO3\(^-\) concentration present in the overlying water (p = 0.05) but increased with amphipod density.

Fig. 1. Corophium volutator. Test incubation. Denitrification rates in intact sediment cores containing 12,000 ind. m\(^{-2}\), versus NO3\(^-\) concentration in the overlying water. Denitrification of NO3\(^-\) (A) coming from the overlying water (dw) and (B) produced within the sediment by nitrification (dn). Each data point represents 1 core.
Nitrate ammonification (Fig. 5), only measured at the highest NO$_3^-$ concentration, was also higher at greater C. volutator densities. Less than 5% of the $^{15}$NO$_3^-$ reduced was recovered as $^{15}$NH$_4^+$. Denitrification was shown to be the only significant NO$_3^-$ reducing pathway in these sediments.

Oxygen consumption, dn and dw were stimulated 2-, 3- and 5-fold in sediment inhabited by Corophium volutator (19 800 ind. m$^{-2}$) when compared with non-bioturbated sediment (Fig. 6).

**DISCUSSION**

Calculation of coupled nitrification-denitrification rates by the $^{15}$N isotope pairing technique assumes uniform mixing of both NO$_3^-$ species ($^{15}$NO$_3^-$ and $^{14}$NO$_3^-$) in the denitrification zone (Nielsen 1992). This is because production of unlabelled N$_2$ ($^{14}$N$^{14}$N) by denitrification is not measured directly but estimated from the production of single-labelled N$_2$ ($^{15}$N$^{14}$N) relative to double-labelled N$_2$ ($^{15}$N$^{15}$N). Bioturbation may create local or temporal variations in the ratio of dw to dn, thus questioning the assumption of the technique. The possible underestimation of dn can be tested in incubations with different concentrations of $^{15}$NO$_3^-$ (Nielsen 1992): at higher $^{15}$NO$_3^-$ levels, more $^{14}$NO$_3^-$ will be paired with $^{15}$NO$_3^-$ to form measurable $^{15}$N$^{14}$N and less of the immeasurable $^{14}$N$^{14}$N is formed. The degree of underestimation will therefore diminish at higher $^{15}$NO$_3^-$ levels. Too high $^{15}$NO$_3^-$ concentrations, however, will affect the assumed first-order kinetics of denitrification, therefore the optimal $^{15}$NO$_3^-$ concentration range has to be tested. The test of the present sediment with Corophium volutator showed that $^{15}$NO$_3^-$ concentrations above 40 $\mu$M did not affect the estimate of dn, an indication that the heterogeneity effect was eliminated and the estimates represented true dn rates (Fig. 1B). First-order kinetics were confirmed by the linear correlation between dw and NO$_3^-$ concentration up to 330 $\mu$M NO$_3^-$ (Fig. 1A). The NO$_3^-$ concentrations of 72 and 152 $\mu$M applied in the main experiments were consequently within the optimal range of 40 to 330 $\mu$M.

Oxygen uptake, dn and dw were enhanced 2-, 3- and 5-fold in the presence of 19 800 ind. m$^{-2}$ (Fig. 6). This differential stimulation showed that the amphipod burrows could not be considered as a simple extension of the sediment surface with associated processes. The
observed pattern could be explained by differences between diffusional transport of \( O_2 \) and \( NO_3^- \) in the sediment surface and mass transport of \( O_2 \) and \( NO_3^- \) with water pumped through amphipod burrows: diffusion of \( NO_3^- \) at the sediment surface from the water column to the anoxic zone of denitrification is impeded by the presence of the oxic layer acting as a diffusion barrier. Model calculations confirmed by field studies in stream sediment have shown that the uptake of \( NO_3^- \) relative to the \( NO_3^- \) concentration therefore should be about one-third of the \( O_2 \) uptake relative to the \( O_2 \) concentration (Christensen et al. 1990). In the amphipod burrows, however, \( NO_3^- \) and \( O_2 \) should be consumed with the same efficiency provided that both \( NO_3^- \) and \( O_2 \) are depleted from burrow water as it passes through the sediment. Bioturbation by \textit{Corophium volutator} should therefore stimulate denitrification of \( NO_3^- \) from the water 3 times more than \( O_2 \) consumption, reasonably consistent with the actually observed 4 times higher stimulation (Fig. 6). Coupled nitrification-denitrification depends on the transport of \( NO_3^- \) from the oxic nitrification zone to the anoxic denitrification zone. Microsensor studies and flux measurements have indicated that nitrification activity usually is evenly distributed in the oxic surface zone of sediment, and about half the \( NO_3^- \)-produced diffuses down and is denitrified and half diffuses up into the overlying water column (Blackburn & Henriksen 1983, Jensen et al. 1993). In the burrows, \( NO_3^- \) diffusing out from the oxic nitrification zone of the walls is transported further through the burrow and is eventually denitrified; i.e. the coupling of nitrification and denitrification will be 100%. Assumed that nitrification constitutes a constant fraction of sediment oxygen consumption, bioturbation should thus stimulate nitrification and oxygen consumption to the same extent, while coupled nitrification-denitrification should increase twice as much. The measurements indeed showed 2 times more stimulation of coupled nitrification-denitrification than of oxygen consumption in the presence of 19 800 ind. m\(^{-2}\) (Fig. 6).

This study has shown that \textit{Corophium volutator} increases not only the absolute rate of denitrification but also denitrification relative to oxygen consumption, particularly when \( NO_3^- \) from the overlying water is the prime source. \textit{C. volutator} is one of the dominating species in the benthic fauna of Norsminde Fjord, with common densities of 6000 ind. m\(^{-2}\) and high densities up to 65 000 ind. m\(^{-2}\) (Thorson 1975), and because of riverine discharge, the water phase concentration of \( NO_3^- \) is often high (up to 600 \( \mu M \)) (Binnerup et al. 1992). This will indicate that different abundances of the amphipod through the same and successive years could have an important effect on nitrogen retention in the estuary.

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LITERATURE CITED


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