Seasonal variation in nitrification and denitrification in estuarine sediment colonized by benthic microalgae and bioturbating infauna

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ABSTRACT: Measurements of seasonal variation in oxygen fluxes, nutrient fluxes, and denitrification were obtained in an estuarine sediment inhabited by benthic microalgae and bioturbating infauna. Oxygen dynamics in the upper sediment strata were found to be controlled by the microalgae and there was a net flux of O₂ out of the sediment during spring and autumn. High assimilation by the microalgae reduced the efflux of NH₄⁺ and PO₄³⁻ from the sediment to the water column during daytime. Denitrification based on NO₃⁻ from the water column (Dₙ) only occurred in winter and spring, when NO₃⁻ was present in the water column, and activity was proportional to the water column NO₃⁻ concentration. The rate of Dₙ was reduced during daytime when the upper oxic zone of the sediment increased due to O₂ production by benthic microalgae. Coupled nitrification-denitrification (Dₙ) in the sediment was stimulated by the O₂ production during winter and spring, at which times NO₃⁻ and NH₄⁺ were present in the water column and high concentrations. In contrast, during summer, when the concentration of NO₃⁻ and NH₄⁺ in the water column was low, benthic microalgae inhibited Dₙ by competing with nitrifying bacteria for NH₄⁺. Dₙ accounted for 80% of the total denitrification during winter, while on an annual basis, Dₙ and Dₚ each accounted for 50% of the total denitrification activity. Benthic infauna, such as Corophium spp., Hydrobia spp., and Nereis spp., occurred in densities of up to several thousand ind. m⁻² from May to October. Oxygen consumption, Dₙ, and Dₚ were linearly correlated with the density of the amphipod Corophium spp., all the processes studied being stimulated by the pumping of O₂- and NO₃⁻-rich water through the burrows in the upper 2 to 6 cm of the sediment. During summer, the Dₚ activity was, therefore, the net result of the inhibitory effect by benthic microalgae and the stimulatory effect of the benthic infauna. However, as the concentration of inorganic nitrogen in the overlying water and the sediment nitrification potential are both low in shallow coastal waters during summer, when benthic infauna density is high, we conclude that the stimulatory effect of bioturbating infauna on both Dₙ and Dₚ is of minor importance to the annual denitrification budget.

KEY WORDS: Denitrification, Nitrification, Microalgae, Bioturbation, ISN regulation

INTRODUCTION

Denitrification provides a sink in the global nitrogen budget and thereby plays an important part in controlling the degree of eutrophication in waters subjected to substantial anthropogenic input of nutrients. Denitrification in estuarine sediments thus decreases the transport of nitrogen from land to the open sea (Seitzinger 1988). The process may be supported either by NO₃⁻ diffusing from the overlying water into the sediment or by NO₃⁻ being produced within the sediment by nitrification (Vanderborght & Billen 1975, Nishio et al. 1983, Jenkins & Kemp 1984). Diffusion of NO₃⁻ from overlying water is mainly controlled by a concentration gradient determined by the water NO₃⁻ concentration and the length of the diffusion path through the oxygen zone (Christensen et al. 1990). Nitrifi-
cation activity in sediments is mainly controlled by the availability of NH$_4^+$ and O$_2$, as well as by population dynamics of nitrifying bacteria (Hansen et al. 1981, Henriksen et al. 1981).

Major seasonal and diurnal variation in nitrification and denitrification in shallow water sediments is explicable by changes in oxygen penetration depth caused by benthic microagal growth and mineralization (Christensen et al. 1990, Risgaard-Petersen et al. 1994). Nitrification is preferentially stimulated in the daytime due to photosynthetic production of oxygen and deeper oxygen penetration, while the diffusion of nitrate from the water column is stimulated during night due to high oxygen demand for mineralization and low oxygen penetration. Benthic microalgae also assimilate nitrogen and may successfully compete with nitrifiers and denitrifiers for NH$_4^+$ and NO$_3^-$ (Sundbäck & Graneli 1988, Nielsen et al. 1990, Nielsen & Sloth 1994). In addition, nitrifying bacteria in sediment with active phototrophs may become inhibited by high pH, high O$_2$ concentrations, CO$_2$ limitation, and toxic organic products (Henriksen & Kemp 1988).

Benthic infaunal activity also affects the physical and chemical processes within sediments, e.g. through burrow building, bioturbation and irrigation (Rhoads 1974, Kristensen 1984, Aller 1988). Recently, it has been shown that the amphipod Corophium volutator stimulates oxygen uptake, denitrification of water phase NO$_3^-$ and coupled nitrification-denitrification by mass transport of water into its burrows (Pelegri et al. 1994).

The purpose of the present study was to measure and explain the diurnal and seasonal variation in O$_2$ consumption, nutrient fluxes, nitrification and denitrification in a shallow estuarine sediment colonized by benthic microalgae and bioturbating infauna.

**MATERIALS AND METHODS**

**Study site.** The study was carried out in Kertinge Nor, a small shallow estuary located on the east coast of the island of Funen, Denmark (Fig. 1). Kertinge Nor is connected to the sea through a narrow entrance to the east. The system receives only minor amounts of freshwater, mostly from small streams and precipitation, and the water residence time at the sampling locality is ca 4 to 6 wk (Christensen 1994). Salinity fluctuates on an annual basis but is generally around 17%. During the study year, temperature varied from 2 to 4°C during winter up to 20 to 24°C during summer. The sampling site was located in shallow water (0.5 m depth) where the sediment was sandy. In early spring and late autumn, a dark-brown layer of benthic microalgae was observed on the sediment surface. Amphipods (Corophium spp.) dominated the benthic fauna from May to August at densities of up to 20,000 ind. m$^{-2}$. Later in summer, a mixture of polychaetes (Nereis spp.), oligochaetes, mud snails (Hydrobia spp.) and amphipods (Corophium spp.) were present in the sediment at a total density of up to 50,000 ind. m$^{-2}$.

**Sampling.** The sediment was sampled on 8 different dates during 1992. On each sampling date, 16 intact sediment cores were sampled by hand in 30 cm long and 52 mm wide Plexiglas tubes and brought to the laboratory within 4 h. In the laboratory the sediment cores were adjusted to give a sediment depth of ca 11 cm and a water column of ca 20 cm. The water column was stirred by a 2.5 cm teflon-coated magnet positioned 5 cm above the sediment surface the magnets receiving momentum from an external rotating magnet (60 rpm). The adjusted cores were left uncapped at the in situ temperature in a water bath containing 10 l of water from the locality. Five cores were incubated in the dark and five at the in situ light conditions. During winter, spring and autumn the sediment surfaces were illuminated with ca 70 µmol photons m$^{-2}$ s$^{-1}$, and during summer ca 120 µmol photons m$^{-2}$ s$^{-1}$.

**Flux measurements.** Net fluxes of oxygen, inorganic nitrogen and inorganic phosphorus were measured by closing the cores with a rubber stopper and incubating for 1 to 12 h depending on the season. The incubation time was adjusted to ensure that the oxygen consumption in the cores never reduced the initial O$_2$ concentration by more than 20%. Water samples were collected just before closing the cores and after the incubation. Parallel incubated cores containing only water were used to correct for water column activity. Water samples were analyzed for O$_2$ by Winkler titration within a few hours of sampling and GF/C filtered samples for NO$_3^-$, NH$_4^+$ and PO$_4^{3-}$ were immediately...
frozen for later analysis. *In situ* fluxes of oxygen and inorganic nitrogen and phosphorus were calculated for each sediment core from the change in concentration during incubation and expressed as the rate per square meter. On several sampling dates, time series were made to ensure that the changes in oxygen and nutrient concentrations were linear over the incubation times used.

**Denitrification measurements.** Following measurement of the oxygen and nutrient fluxes, denitrification activity was determined on the same sediment cores by means of the isotope pairing technique (Nielsen 1992). $^{15}$NO$_3^-$ (20 to 60 μM) was added to the water column. However, in winter when high *in situ* NO$_3^-$ concentrations were present, 250 μM $^{15}$NO$_3^-$ was added in order to obtain a uniform mixing of the isotopes. The added $^{15}$NO$_3^-$ was allowed to equilibrate with sediment porewater NO$_3^-$ before the cores were closed with rubber stoppers. The cores were dark or light incubated for the same period as used for the flux measurements.

After incubation, samples of the water column and sediment porewater were collected for analysis of the $^{15}$N labelling of N$_2$, NH$_4^+$ and NO$_3^-$. The water column was sampled immediately upon removal of the stopper. 250 μl ZnCl$_2$ solution (50% w/w) was then added to the sediment surface to stop all bacterial activity and the sediment porewater and water column were carefully mixed with a Plexiglas rod. A sample of the resultant sediment slurry was taken by syringe. All samples for $^{15}$N isotope analysis were preserved in gastight containers (Exetainers, Labco, High Wycombe, UK) with 2% (vol) of the ZnCl$_2$ solution. Finally, the sediment cores were sieved through a 1 mm sieve to recover the benthic infauna. Sediment porosity was measured at each sampling date in separate cores.

**Test incubation.** A test incubation was performed in order to find the optimal $^{15}$NO$_3^-$ concentration range for the denitrification measurements (Nielsen 1992). Four different concentrations of $^{15}$NO$_3^-$ in the overlying water (20, 40, 50 and 80 μM) were selected, and for each concentration, 4 intact sediment cores (22 cm$^2$, 11 cm sediment and 20 cm water) were incubated as described above.

**Analysis and calculations.** The concentration of NO$_3^-$ + NO$_2^-$ was determined on a flow injection analyzer (Tecator, Höganas, Sweden) using the method described by Grasshoff et al. (1983). NH$_4^+$ concentration was determined manually using the method of Bower & Hansen (1980), and PO$_4^{3-}$ was determined by a standard colorimetric method described by Grasshoff et al. (1983). The $^{15}$N$_2$ (14N$^{15}$N and 14N$^{15}$N) in the water and slurry samples was extracted into a helium headspace introduced in the Exetainers. After 5 min of vigorously shaking most of the N$_2$ is found in the headspace, less than 2% of the N$_2$ gas being dissolved in the water at equilibrium. The gas in the headspace was then injected into a gas chromatograph coupled to a triple-collector isotopic ratio mass spectrometer (Robo-Prep-G+ in line with TracerMass, Europa Scientific, Crewe, UK) and the abundance and concentrations of $^{15}$N$^{15}$N and $^{15}$N$^{14}$N analyzed.

The production rate of the isotopes [p*(14N$^{15}$N and 15N$^{15}$N)] was calculated as follows:

$$p_{14N^{15}N} + p_{^{15}N^{15}N} = \frac{[V_t(C_{water} - C_{init}) + (C_{slurry} - C_{init})V_2]}{At}$$

(1)

where $C_{water}$ and $C_{slurry}$ are the concentrations of the isotope in the water column and the sediment slurry, respectively, $C_{init}$ is the initial concentration of the isotope, $V_t$ is the volume of the sampled water, $V_2$ is the volume of porewater plus the remaining water column after the initial sampling, $A$ is the area, and $t$ is the incubation time.

Denitrification rates were estimated from the production of $^{15}$N isotopes (Nielsen 1992):

$$D_{15} = p_{14N^{15}N} + 2p_{^{15}N^{15}N}$$

(2)

$$D_{14} = \frac{p_{14N^{15}N}}{2p_{^{15}N^{15}N}} D_{15}$$

(3)

where $D_{15}$ and $D_{14}$ are the rates of denitrification based on $^{15}$NO$_3^-$ and $^{14}$NO$_3^-$, respectively, and $p_{14N^{15}N}$ and $p_{^{15}N^{15}N}$ are the rates of production of the 2 labelled N$_2$ species ($^{14}$N$^{15}$N and $^{15}$N$^{15}$N, respectively). While $D_{15}$ expresses denitrification activity of added $^{15}$NO$_3^-$, $D_{14}$ expresses the total *in situ* denitrification activity.

The proportion of $D_{14}$ that is based on NO$_3^-$ from the water phase ($D_w$) was calculated from $D_{15}$ and the $^{14}$N:$^{15}$N ratio of water column NO$_3^-$:

$$D_w = D_{15} \frac{[^{14}NO_3^-]_{w}}{[^{15}NO_3^-]_{w}}$$

(4)

where $[^{14}NO_3^-]_{w}$ is the concentration of unlabelled NO$_3^-$ and $[^{15}NO_3^-]_{w}$ the concentration of labelled NO$_3^-$ in the water column. Finally, *in situ* denitrification of NO$_3^-$ produced by nitrification ($D_a$) was calculated as:

$$D_a = D_{14} - D_w$$

(5)

To estimate $D_a$ it was, as indicated above, necessary to measure the $^{15}$N labelling of the water column NO$_3^-$. A pure culture of denitrifying *Pseudomonas nauticus* was used to convert $^{15}$NO$_3^-$ and $^{15}$NO$_3^-$ into N$_2$ gas composed of $^{28}$N$_2$, $^{29}$N$_2$ and $^{30}$N$_2$, which was subsequently analyzed by mass spectrometry (Risgaard-Petersen et al. 1993). Labelling of NO$_3^-$ in the water column of the sediment cores was then calculated from the $^{28}$N$_2$:$^{30}$N$_2$ ratio in the analyzed gas.
RESULTS

Oxygen and nutrient fluxes

Marked seasonal variation was observed in the water column concentration and sediment-water flux of oxygen, inorganic nitrogen and phosphorus (Fig. 2). The benthic microalgae at the sediment surface produced O$_2$ throughout the year, as indicated by the net efflux or reduced net uptake of O$_2$ in the light-incubated sediment cores (Fig. 2A, B). Oxygen uptake in the dark-incubated cores, which represents the total sediment oxygen consumption, was significantly higher during the summer months of May until October (Fig. 2B). During summer, the water phase O$_2$ concentration exceeded 100% atmospheric saturation due to O$_2$ production by phytoplankton.

The NO$_3^-$ concentration in the water column of Kertinge Nor displayed distinct seasonal fluctuation, the concentrations being high in winter and spring but negligible (<0.2 µM) throughout the summer. NO$_3^-$ uptake by the sediment correlated to the water column NO$_3^-$ concentration in both light- and dark-incubated sediment (Fig. 2C, D), as a result uptake was high in spring and winter but negligible during the summer period. The water column NH$_4^+$ concentration followed that of NO$_3^-$, although at a lower level. NH$_4^+$ efflux from the sediment was highest during the summer, with the rate being maximal in August (Fig. 2E, F). Throughout the growth season, NH$_4^+$ efflux was significantly lower in the light- than in the dark-incubated cores. The PO$_4^{3-}$ flux changed during the year from sediment uptake in winter to efflux during summer. Moreover, PO$_4^{3-}$ release from the sediment was higher in the dark-incubated sediment (Fig. 2H) than in the light-incubated sediment (Fig. 2G).

Fig. 2. Seasonal variation (1992) in O$_2$, NO$_3^-$, NH$_4^+$, and PO$_4^{3-}$ fluxes in light (open bars) and dark (solid bars) incubated sediment shown together with the respective in situ concentration. Error bars on the flux measurements indicate SE (n = 5).
Optimization of the $^{15}$NO$_3^-$ concentration for the denitrification assay

Denitrification of $^{15}$NO$_3^-$ ($D_{v(15)}$) was proportional to the $^{15}$NO$_3^-$ concentration over the range 20 to 80 $\mu$M (Fig. 3A). Further, coupled nitrification-denitrification activity ($D_n$) was independent of the water column $^{15}$NO$_3^-$ concentration (Fig. 3A), thus verifying that incomplete isotope mixing due to heterogeneity was not a problem (Nielsen 1992, Pelegri et al. 1994, Rysgaard et al. 1994). On this basis we concluded that addition of 20 to 80 $\mu$M $^{15}$NO$_3^-$ would give real values of $D_n$. In February, however, when the in situ NO$_3^-$ concentration was higher than 1000 $\mu$M, $^{15}$NO$_3^-$ was added to a final concentration of 250 $\mu$M.

The concentrations of both $^{14}$N$^{15}$N and $^{15}$N$^{15}$N increased linearly with incubation time (Fig. 3B). The intercept with the x-axis ($y = 0$) represents the time necessary for the NO$_3^-$ profile to stabilize within the surface sediment following addition of the $^{15}$NO$_3^-$. In situ denitrification activity

Seasonal variation in in situ denitrification is shown both as denitrification based on water phase NO$_3^-$ ($D_w$: Fig. 4A) and denitrification based on NO$_3^-$ from nitrification ($D_n$: Fig. 4B). In general, the rate of total denitrification ($D_{w+n}$) was highest in late winter and spring in both light- and dark-incubated sediment (Fig. 4). Denitrification based on water phase NO$_3^-$ correlated with the NO$_3^-$ concentration in the overlying water; it decreased from ca 60 $\mu$mol N m$^{-2}$ h$^{-1}$ in February to <1 $\mu$mol N m$^{-2}$ h$^{-1}$ in May and remained low throughout the summer period until the NO$_3^-$ concentration increased in the autumn (Fig. 4A). Denitrification of water phase NO$_3^-$ was always slightly higher in dark-incubated cores than in light-incubated cores.

Denitrification of NO$_3^-$ produced by nitrification within the sediment was almost constant from February to June, but was lower and more variable during the rest of the year (Fig. 4B). Coupled nitrification-denitrification activity was significantly higher in light- than in dark-incubated cores during winter and spring, with the pattern being opposite in the summer period, when the concentration of inorganic nitrogen in the overlying water was very low. Coupled nitrification-denitrification accounted for ca 50% of total denitrification on an annual basis.

The presence of benthic infauna had a marked effect on oxygen consumption and denitrification rates, sediment oxygen consumption, $D_w$ and $D_n$ all being stimulated at increasing amphipod density (Corophium spp.) under both light and dark conditions (Fig. 5).

DISCUSSION

Use of the isotope pairing technique for measuring denitrification in estuarine sediments

Correct determination of actual in situ denitrification using the isotope pairing technique requires that 3 important assumptions are fulfilled (Nielsen 1992).
Corophium density (ind. m$^{-2}$)

(1) Addition of $^{15}$NO$_3^-$ must not alter the rate of denitrification based on in situ NO$_3^-$. Microsensor and modelling studies (Christensen et al. 1989, 1990, Nielsen et al. 1990) have demonstrated a 1st order kinetic relationship between denitrification based on water phase NO$_3^-$ and the NO$_3^-$ concentration in the overlying water, denitrification of water phase NO$_3^-$ primarily being determined by the NO$_3^-$ concentration gradient within the upper oxic surface layer of the sediment. Since $D_{15}$ of the Kertinge Nor sediment was linearly correlated to the water phase NO$_3^-$ concentration (Fig. 3A), the first assumption was therefore fulfilled.

(2) The added $^{15}$NO$_3^-$ must mix uniformly with the NO$_3^-$ already present in water column and in the sediment. Heterogeneous topography, bioturbation, inhomogenous nitrification activity, etc., may cause local variations in the transport of $^{15}$NO$_3^-$ and $^{15}$NO$_2^-$ to the anoxic denitrification zone, and hence underestimate in situ denitrification activity ($D_{15}$) since $^{15}$N production would then be less than that predicted on the assumptions of homogeneity (Broast et al. 1988). As demonstrated by several authors (Nielsen 1992, Pellegri et al. 1994, Rysgaard et al. 1994) this possible underestimation can be analyzed by incubating the sediment cores at different $^{15}$NO$_3^-$ concentrations. At increasing $^{15}$NO$_3^-$ concentration, an increased denitrification of $^{15}$NO$_3^-$ will be detected directly as $^{15}$N on the mass spectrometer, thereby minimizing the possible underestimation of $D_{15}$. As demonstrated in the optimization experiment, coupled nitrification-denitrification was independent of the water phase NO$_3^-$ concentration at concentrations greater than 20 µM, thus indicating uniform mixing of the added $^{15}$NO$_3^-$ (Fig. 3A). We always used a higher $^{15}$NO$_3^-$ concentration, thereby ensuring correct determination of both coupled denitrification ($D_n$) and total denitrification ($D_{15}$).

(3) A stable NO$_3^-$ concentration gradient must be established in the surface layer of the sediment within a short time of $^{15}$NO$_3^-$ addition relative to the duration of incubation. If this is not the case, denitrification activity will be underestimated since the added $^{15}$NO$_3^-$ will not be immediately available to the denitrifying bacteria in the anoxic zone of the sediment. The time needed to establish a stable NO$_3^-$ gradient depends on the O$_2$ penetration depth. During summer, when oxygen typically penetrates 1 mm down into shallow sediments, the 90% equilibration time is ca 5 min (Nielsen 1992). During winter, when the O$_2$ penetration is deeper, the establishment of a new NO$_3^-$ gradient takes longer. Nevertheless, as the optimization experiment shows that production of $^{15}$N-dinitrogen was linear after 30 min in March (Fig. 3B), the establishment of a stable NO$_3^-$ profile took less than 30 min, this being a short period compared to the total incubation time of up to 12 h during winter. The third assumption was therefore also fulfilled at the Kertinge Nor sediment.

**Effect of benthic microalgae and infauna on oxygen and nutrient dynamics**

When benthic microalgae colonize the sediment surface of shallow waters, they may greatly influence oxygen and nutrient dynamics at the sediment-water interface. Oxygen production by benthic microalgae...
may increase the oxygen penetration into the sediment by several mm (Revsbech & Jørgensen 1983) and thereby influence sediment metabolism as well as the turnover and flux of nutrients on both a diurnal and seasonal basis (Sundbäck 1986, Sundbäck & Graneli 1988, Rysgaard-Petersen et al. 1994). Even though $\text{NH}_4^+$ was present at a high concentration in the sediment porewater, the benthic microalgae reduced the $\text{NH}_4^+$ flux to the water column significantly as a result of nitrogen assimilation as demonstrated by Rysgaard et al. (1993). Efflux of $\text{NH}_4^+$ was not measurable when photosynthesis was taking place, except in August (Fig. 2E). The microalgae therefore acted as an efficient filter, adsorbing the flux of ammonium from the deeper, anoxic sediment layers. Henriksen et al. (1980) and Sundbäck (1986) have also reported that a thin layer of benthic microalgae is able to control the flux of inorganic nitrogen from sediment to the overlying water. When $\text{NO}_3^-$ was present in the water column in spring and early winter, the flux of $\text{NO}_3^-$ was generally directed into the sediment, this being attributable to benthic assimilation in the surface layers and denitrification in the deeper sediment layers. Assimilation of inorganic nutrients by the benthic microalgae also influenced the $\text{PO}_4^{3-}$ flux, a lower efflux being observed in light- than in dark-incubated sediment. The $\text{PO}_4^{3-}$ flux into the sediment during winter was most likely due to binding of phosphate to oxidized iron, which is more abundant within the sediment during the cold season, when oxygen demand is lower and oxygen penetration into the sediment therefore deeper (Rasmussen & Jørgensen 1992).

Benthic oxygen production may also greatly influence sediment nitrification and denitrification. In the sediment from Kertinge Nor, we found that $D_o$ was slightly less in the light than in the dark (Fig. 4A). However, it has recently been demonstrated that photosynthesis by benthic microalgae reduced denitrification based on water phase $\text{NO}_3^-$ ($D_{w}$) by ca 50% during the day as a result of deeper $O_2$ penetration into the sediment when photosynthesis was taking place (Rysgaard-Petersen et al. 1994). This deeper oxygen penetration enhances the diffusion path from the water column to the denitrifying zone, thereby reducing the $\text{NO}_3^-$ supply for denitrification (Christensen et al. 1989, Nielsen et al. 1990). The difference in inhibition of denitrification in the 2 studies can be ascribed to the relatively higher activity (as judged from the much higher $O_2$ production) of benthic microalgae in the laboratory experiment by Rysgaard-Petersen et al. (1994) as compared to our measurements of in situ activity in Kertinge Nor.

During winter and early spring when the availability of inorganic nitrogen was high, benthic photosynthesis stimulated coupled nitrification-denitrification (Fig. 4B), this probably being due to oxygen stimulation of nitrification. This is in agreement with the study of Rysgaard-Petersen et al. (1994), who found that benthic microalgae stimulated $D_o$ during the day as a result of their $O_2$ excretion. However, both findings are in conflict with the hypothesis of Henriksen & Kemp (1988) that photosynthesis by benthic diatoms reduces nitrification due to a combination of a high competition for $\text{NH}_4^+$, high pH and high $O_2$ concentration in the upper sediment layers, all of which inhibit the nitrification process, and thereby also the coupling between nitrification and denitrification. The competition between nitrifiers and benthic microalgae for inorganic nitrogen is particularly intense during periods of illumination; moreover, the concentration of available inorganic nitrogen in the overlying water and within the sediment is of major importance when evaluating the effect of benthic photosynthesis on nitrogen processes. Thus, during the summer period from May until November, when both $\text{NO}_3^-$ and $\text{NH}_4^+$ was almost absent in the water column of Kertinge Nor, the photosynthetic activity of benthic microalgae actually reduced the activity of coupled nitrification-denitrification (Fig. 4B). Further, $D_o$ reflected the $\text{NH}_4^+$ efflux from the sediment (Fig. 2F), thus indicating strong competition for $\text{NH}_4^+$ between nitrifiers and benthic microalgae in the surface layers of the sediment during the summer period, as has been suggested by Henriksen & Kemp (1988). Since benthic microalgae can assimilate $\text{NO}_3^-$ and $\text{NH}_4^+$ at high rates for up to 60 h after sediment has been darkened, they therefore represent an efficient competitor for nitrifying bacteria at low nitrogen concentrations (Rysgaard et al. 1993). However, when $\text{NO}_3^-$ or $\text{NH}_4^+$ are present at high concentrations in the overlying water column they can act as a nitrogen source for the benthic assimilatory demand, and thereby reduce the competition with nitrifiers for porewater $\text{NH}_4^+$. This was the situation in Kertinge Nor during winter and spring as well as in the laboratory experiments of Rysgaard-Petersen et al. (1994), where benthic primary production stimulated the coupled nitrification-denitrification. Thus, as indicated by the present study, there may be both diurnal and seasonal variation in the effect of benthic microalgae on the coupling between nitrification and denitrification in estuarine sediments, i.e. microalgal photosynthesis may stimulate denitrification during the cold season, when nitrogen availability is high, and inhibit denitrification during summer, when nitrogen availability is low.

Oxygen and nitrogen dynamics within the sediment may also be affected by bioturbating infauna (Henriksen et al. 1980, Aller 1982, Kristensen et al. 1991). In the sandy sediment of Kertinge Nor, sediment oxygen respiration and denitrification was significantly enhanced at increasing densities of the amphipod Coro-
phium spp. (Fig. 5). Stimulation of sediment oxygen consumption, \( D_n \) and \( D_w \), in the presence of Corophium spp. can be explained by mass transport of \( O_2 \)- and \( NO_3^- \)-rich water within the U-shaped amphipod burrows, which may penetrate as much as 2 to 6 cm into the sediment (Pelegri et al. 1994). Infauna density was particularly high from May until November (Fig. 6). During this period, the water column \( NO_3^- \) concentration was very low (Fig. 2) and the effect of bioturbating fauna on \( D_n \) was consequently negligible; the high infauna density primarily stimulated \( D_w \) activity (Fig. 5). The stimulation of coupled nitrification-denitrification activity was higher in light, probably because the infauna were more active during the day and therefore able to pump more \( O_2 \)-rich water into the sediment. Coupled nitrification-denitrification activity in May ranged from 8 \( \mu \)mol N m\(^{-2} \) h\(^{-1} \) in the dark to 13 \( \mu \)mol N m\(^{-2} \) h\(^{-1} \) in the light (Fig. 4B). From the data presented in Fig. 5, the activity in sediments devoid of Corophium spp. can be extrapolated to be less than 3 \( \mu \)mol N m\(^{-2} \) h\(^{-1} \). It is therefore obvious that the presence of Corophium spp. had a marked influence on coupled nitrification-denitrification activity in Kertinge Nor during the summer period. However, total nitrification activity is generally reduced in shallow coastal waters during summer since the population of nitrifiers in the sediment is reduced due to lower \( O_2 \) availability and higher \( NH_4^+ \) competition (Hansen et al. 1981). As a consequence, coupled nitrification-denitrification activity was low during summer in Kertinge Nor compared to that obtained during winter (Fig. 4B). The stimulatory effect of the bioturbating infauna (mainly present during summer) on \( D_w \) and \( D_n \) was therefore limited by the predominantly low water column \( NO_3^- \) concentration and the reduced nitrifying population in the sediment, respectively.

Stimulation of coupled nitrification-denitrification with increasing infauna density was 2-fold greater than that of \( O_2 \) consumption rate (Fig. 7), a finding consistent with the observations of Pelegri et al. (1994). Nitrate produced by nitrification in a sediment devoid of bioturbating animals will diffuse both upwards to the water column and downwards to the anoxic denitrification zone; assuming homogeneous distribution of nitrifiers throughout the oxic surface layers of the sediment, approximately half of the \( NO_3^- \) will diffuse in each direction (Nielsen et al. 1990, Pelegri et al. 1994). Nitrate diffusing out of the oxic layer in an amphipod burrow can be denitrified further down the burrow, however, and coupling between nitrification and denitrification will therefore be much closer in bioturbated sediment. Thus, as a general rule, bioturbation should stimulate nitrification and sediment oxygen uptake to an equivalent extent, while coupled nitrification-denitrification should be stimulated twice as much as oxygen consumption when coupling of nitrification and denitrification is almost 100\% (Pelegri et al. 1994).

**Control and relative importance of \( D_w \) and \( D_n \)**

Denitrification based on water phase \( NO_3^- \) (\( D_w \)) was highly correlated to the \( NO_3^- \) concentration in the water, activity being high during winter and spring and almost negligible throughout summer (Fig. 4A). A model relating \( D_w \) to \( O_2 \) uptake and the water column concentrations of \( O_2 \) and \( NO_3^- \) (Christensen et al. 1990) was tested on the present data set. The model is based on the fact that \( O_2 \) penetration within the sediment is a function of \( O_2 \) concentration and \( O_2 \) uptake. The \( O_2 \) penetration depth represents the diffusional path for \( NO_3^- \) to the underlying, anaerobic denitrification zone.
and the concentration of $\text{NO}_3^-$ in the water column divided by the diffusional path of $\text{NO}_3^-$ thus represents the concentration gradient of $\text{NO}_3^-$. The flux of $\text{NO}_3^-$ to the denitrification zone can therefore be calculated by multiplying this concentration gradient with the diffusion coefficient of $\text{NO}_3^-$ in the sediment:

$$D_w = F_O \times \alpha \times \left[\frac{D_{\text{NO}_3^-}}{D_O} \times \frac{C_{\text{NO}_3^-}}{C_O} \times \frac{1}{\alpha} - 1\right]$$

where $F_O$ is the sediment oxygen consumption, $C_O$ and $C_{\text{NO}_3^-}$ are the respective concentrations in the water column, $D_O$ and $D_{\text{NO}_3^-}$ are the respective coefficients of diffusion, and $\alpha$ is the ratio between the volume specific denitrification and oxygen respiration rates. The model is based on the fact that $D_w$ depends on the water column $\text{NO}_3^-$ concentration, the volume specific denitrification rate in the anoxic zone, and the length of the diffusion path through the oxic zone. The thickness of the oxic zone, in turn, is a function of $\text{O}_2$ uptake and $\text{O}_2$ concentration in the water column, assuming the same volume specific $\text{O}_2$ consumption rate throughout the oxic zone. The diffusion coefficient $D_{\text{NO}_3^-}$ and $D_O$ need not to be measured since the ratio between them is invariably 0.8 in an aquatic medium. The volume specific denitrification and oxygen respiration rates are not determined either, but the ratio between them ($\alpha$) is set to 0.8 on the basis of bacterial kinetics and microsensor studies (Christensen et al. 1989). As demonstrated in Fig. 8, the measured rates of $D_w$ corresponded very well with the rates predicted by the model. Denitrification based on $\text{NO}_3^-$ may therefore be estimated by this model using easily obtainable parameters such as $\text{O}_2$ consumption and water column $\text{O}_2$ and $\text{NO}_3^-$ concentrations.

$D_o$ activity is related to the nitrification rate and the efficiency with which the nitrification process is coupled to denitrification. In the sediment from Kertinge Nor, the highest rates of coupled nitrification-denitrification were observed during the cold months, when the temperature was below 4°C (Fig. 4B). Total microbial respiration in the sediment is reduced by the cold temperatures, thereby reducing total sediment $\text{O}_2$ consumption. Oxygen penetration within the sediment is therefore enhanced during winter (Rasmussen & Jorgensen 1992), which may activate nitrifying bacteria located deeper in the sediment, and thereby enhance total nitrification activity. A seasonal horizontal shift that ensures maximal nitrifying activity in the deeper sediment layers during winter will result in tight coupling between nitrification and denitrification in this part of the year, as demonstrated by Rysgaard et al. (1994) and Jensen et al. (1994).

Denitrification based on water phase $\text{NO}_3^-$ and denitrification coupled to nitrification within the sediment both played an important role in nitrogen removal from Kertinge Nor estuary. At the shallow, sandy sampling site, $D_w$ accounted for ca 50% of total annual denitrification. It has previously been stated that coupled nitrification-denitrification is the most important denitrification process in aquatic sediments (Seitzinger 1988). However, the present study indicates that $D_w$ may be important in shallow coastal systems that receive significant nutrient input. Moreover, as the study also demonstrates, the relative importance of the 2 processes may change dramatically throughout the year. In February, when $\text{NO}_3^-$ was present in high concentrations, $D_w$ accounted for more than 80% of the total denitrification, while in the summer months, almost all denitrification was due to coupled nitrification-denitrification (Fig. 4).

We conclude that benthic microalgae may have a strong regulating effect on the efflux of nutrients from the sediment surface to the overlying water in shallow estuarine waters. Due to their assimilatory demand, they may efficiently reduce the flux of inorganic nutrients during the day and may also cause significant uptake of, for example, $\text{NO}_3^-$ from the water column. From this and previous studies it is evident that the presence of benthic microalgae also has a marked influence on the diurnal and seasonal variation in sediment denitrification. The rather complex means whereby they regulate these processes can be summarized as follows. (1) Oxygen production by benthic microalgae increases the oxic zone within the sediment, thereby lengthening the diffusional path for $\text{NO}_3^-$ from the water column to the anoxic denitrification zone and hence reducing denitrification activity based on water phase $\text{NO}_3^-$ ($D_o$). (2) When inorganic nitrogen is present in excess, benthic oxygen production stimulates nitrification and the increased oxic surface layers caused by benthic primary production may stimulate coupled nitrification-denitrification ($D_o$).
(3) During summer, when the water column concentration of NO$_3^-$ and NH$_4^+$ is low, there is strong competition for inorganic nitrogen between benthic microalgae, nitrifiers and denitrifiers. In this situation benthic primary production will reduce nitrification activity and thereby $D_n$ and nitrogen assimilation can decrease the activity of $D_n$ as well.

A further conclusion to be drawn from this study is that bioturbation by benthic infauna can significantly stimulate sediment oxygen consumption, $D_n$ and $D_o$ within the sediment as long as excess inorganic nitrogen is present in the water column. However, since benthic infauna density is generally highest during summer, when the NO$_3^-$ concentration is low, their effect on $D_n$ will be of minor importance on an annual basis. Moreover, as the nitrification potential of sediment in shallow coastal waters is reduced during summer, the stimulatory effect on $D_n$ of bioturbation by benthic infauna will also be of minor importance on an annual basis.

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