Organic N and P in eutrophic fjord sediments - rates of mineralization and consequences for internal nutrient loading

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Abstract

Nutrient release from the sediments in shallow eutrophic estuaries may counteract reductions of the external nutrient load and prevent or prolong ecosystem recovery. The magnitude and temporal dynamics of this potential source, termed internal nutrient loading, is poorly understood. We quantified the internal nutrient loading driven by microbial mineralization of accumulated organic N (ON) and P (OP) in sediments from a shallow eutrophic estuary (Odense Fjord, Denmark).

Sediments were collected from 8 stations within the system and nutrient production and effluxes were measured over a period of ~2 years. DIN effluxes were high initially but quickly faded to low and stable levels after 50-200 d, whereas PO$_4^{3-}$ effluxes were highly variable in the different sediments. Mineralization patterns suggested that internal N-loading would quickly (<200 days) fade to insignificant levels whereas internal PO$_4^{3-}$ loading could be sustained for extended time (years). When results from all stations were combined, internal N-loading and P-loading from the fjord bottom was up to 121*10$^3$ kg N yr$^{-1}$ (20 kg N ha$^{-1}$ yr$^{-1}$) and 22*10$^3$ kg P yr$^{-1}$ (3.6 kg P ha$^{-1}$ yr$^{-1}$) corresponding to 6% (N) and 36% (P) of the external nutrient loading to the system. We conclude that the internal N-loading resulting from degradation of accumulated ON is low in shallow eutrophic estuaries, whereas microbial mineralization of accumulated OP is a potential source of P.

Overall it appears that in N-limited eutrophic systems, internal nutrient resulting from mineralization of ON and OP in sediments is of minor importance.
1. Introduction

The nutrient loading of coastal ecosystems is often divided into internal and external sources, i.e. release from sediments resulting from organic N (ON) and P (OP) mineralization, and natural and anthropogenic supplies via the water shed and atmospheric deposition, respectively. The external nutrient loading can be quantified by summing up the external sources (e.g. Petersen et al. 2009). It is difficult, however, to use a mass balance approach to obtain reliable estimates of internal nutrient loading, since release from sediments and export to adjacent water bodies are difficult to quantify with sufficient temporal and spatial precision in large and dynamic estuaries with extensive spatial variability and open boundaries.

To complicate matters more, the internal nutrient loading can be divided into two fractions with different temporal dynamics. The first is rapid nutrient release from mineralization of fresh and newly deposited labile organic material, and the second is slow and continued nutrient release from mineralization of buried organic material with lower reactivity. High turnover of labile ON and OP deposited at the sediment-water interface ensures a rapid recycling of inorganic nutrients to the water column (Kelly & Nixon 1984; Valdemarsen et al. 2009). The primary productivity in many shallow estuaries is therefore partially controlled by nutrients released from the sediments (Cowan & Boynton 1996; Fullweiler et al. 2010; Mortazavi et al. 2012; Bukaveckas & Isenberg 2013). The contribution from mineralization of low reactivity and often deeply buried ON and OP to total sediment nutrient release, however, remains largely unknown. Nutrient release reported in most published studies is dominated by the nutrients generated by labile ON and OP mineralization due to the short time-scale applied for measurements. It is nonetheless important to obtain reliable estimates of the nutrient generation and efflux resulting from mineralization of low reactivity ON and OP. In many instances the recovery of eutrophic ecosystems after reductions of the external nutrient loading does not occur or only occurs after considerable delay (Kronvang et al.
2005). This may be caused by substantial release of nutrients, which have accumulated to high
concentrations over time in the sediments exposed to eutrophication (Pitkanen et al. 2001;
Carstensen et al. 2006). Such delayed nutrient release is thought to counteract reductions in the
external nutrient load and cause delayed recovery.

Determining the magnitude and temporal dynamics of the internal nutrient loading
originating from ON and OP accumulated in sediments requires detailed biogeochemical studies.
Organic matter degradation in sediments follow exponential decay kinetics (Westrich & Berner,
1984; Burdige 1991; Valdemarsen et al. 2014) and inorganic nutrient production from ON and OP
is therefore expected to decrease exponentially with time. Not all produced inorganic nutrients will
result in internal nutrient loading, however, since chemical and biological processes within
sediments lead to nutrient retention or transformation before efflux to the overlying water. NH$_4^+$, for
instance, can be adsorbed to the sediment matrix (Mackin and Aller 1984), assimilated by microbes
or benthic microalgae or microbially transformed to other nitrogeneous compounds (Christensen et
oxic-anoxic transition of surface sediments, whereby NH$_4^+$ is converted to inert N$_2$-gas, is for
instance an ecologically important process which reduces the amount of bioavailable N (Seitzinger
1988; Burgin & Hamilton 2007). Due to adsorption and denitrification, the efflux of dissolved
inorganic nitrogen (DIN = NH$_4^+$ + NO$_3^-$ + NO$_2^-$) is generally much lower than anticipated from total
ON mineralization in the sediment (Mackin and Swider 1989). As for NH$_4^+$, PO$_4^{3-}$ may adsorb to
the sediment matrix; mainly to Fe-minerals in oxidized surface sediment (Sundby et al. 1992). PO$_4^{3-}$
efflux is therefore generally low in marine sediments lined with an oxic surface layer (Sundby et al.

In this study an experimental approach was used to determine the internal nutrient
loading resulting from long-term mineralization of accumulated ON and OP in various sediment
types of a large shallow, eutrophic estuary (Odense Fjord, Denmark). The goals of the study were
two-fold; (1) to quantify the magnitude and temporal dynamics of internal nutrient loading resulting
from mineralization of ON and OP accumulated in sediments and (2) to evaluate the role of internal
nutrient loading for the recovery of eutrophic ecosystems. Sediment cores were collected from
various locations representing the dominating sediment types and environments in the estuary.
These were maintained in experiments lasting ~2 years, during which the mineralization of ON and
OP and resulting effluxes of inorganic nutrients were measured with high spatial and temporal
resolution. By comparing total inorganic nutrient production to effluxes, the fate of inorganic
nutrients was elucidated. The total internal nutrient loading of the entire system was estimated
based on the measured nutrient effluxes and the areal distribution of dominating sediment types.
Finally, the importance of internal nutrient loading in shallow eutrophic ecosystems is evaluated.

2. Materials and methods

2.1 Study area

Odense Fjord is a shallow eutrophic estuary located on the island of Fyn, Denmark. It is divided
into a 16 km² shallow inner basin and a 45 km² deeper outer basin, with average depths of 0.8 and
2.7 m, respectively (Fig. 1). The fjord is connected to Kattegat through a narrow opening in the
northeast. The main external nutrient source to Odense Fjord is Odense River, which has a
catchment area of 1095 km², consisting mainly of farmland and urban areas (Petersen et al. 2009).
Odense Fjord was critically eutrophic in the past due to high external nutrient loading exceeding
300*10³ kg N y⁻¹ and 300*10³ kg P y⁻¹ before 1990 (Petersen et al. 2009). The massive nutrient
loading caused extensive problems with high pelagic primary production, low water transparency,
hypoxic events and blooms of opportunistic macroalgae. Implementation of several water action
plans has reduced the external nutrient loading considerably to current levels of about 2000*10³ kg
N \, y^{-1} and 60*10^3 \, kg \, P \, y^{-1}. This has improved the ecological quality of the system, since hypoxia is now rare and levels of opportunistic macroalgae have decreased. Nonetheless, excessive nutrient levels and high primary production are still a problem in Odense Fjord, which may be due to high and sustaining internal nutrient loading.

2.2 Sampling of sediment and water

Intact sediment cores were collected on 8 stations from 4 habitat types in Odense Fjord during October and November 2009 (Fig. 1). The stations were chosen to cover all major sediment types in the fjord; 3 stations (St 1-3) represented shallow silty sediments in the inner fjord, St 4 and 5 represented shallow (< 1 m) silty and sandy sediments in the outer fjord, respectively, and finally, 3 stations (St 6-8) represented deep (2-6 m) silty sediments in the outer fjord. A detailed survey of sediment characteristics conducted in 2009 (partially presented in Valdemarsen et al. 2014) revealed that the four selected habitat types (shallow silty inner fjord, shallow silty outer fjord, shallow sandy outer fjord and deep silty outer fjord) represented 21, 11, 29 and 39% of the fjord area, respectively. Fifteen sediment cores were sampled from each station with 30 cm long, 8 cm internal diameter Plexiglas core liners. The shallow stations (St 1-5) were sampled from a dinghy using a hand operated coring device. Cores from the deeper stations (St 6-8) were subsampled from a ‘HAPS’ box corer on board a larger vessel (“Liv II”, Danish Nature Agency). Water temperatures were 10-12°C at the time of sampling.

Seawater used for the experiment was collected at Kerteminde Harbor at various times during 2009-2011. The seawater was GF/C-filtered and adjusted to the appropriate salinity (10 or 20) before it was used for experiments.

2.3 Experimental setup
Sediment cores were pre-treated before the experiment to assure that they had equal sediment height and were free of macrofauna. The sediment cores were adjusted to 20 cm depth by removing the bottom stopper and carefully removing excess sediment from below. After reinserting the bottom stopper, the overlying water was purged with $N_2$ for 30 min to induce anoxia and the top stopper was reinserted. Asphyxiated macrofauna was removed from the sediment surface after $\sim$48 h in darkness.

The pre-treatment was completed 2-4 days after sampling and sediment cores were then transferred to the experimental setup consisting of eight $\sim$70 L water tanks located in a temperature controlled room at 15°C. The incubation temperature of 15°C approximately corresponds to the average annual water temperature in Odense Fjord. Each tank contained all sediment cores from one station, and was filled with filtered seawater with salinity 10 for St1-3 and salinity 20 for St 4-8, corresponding to the average salinity in the inner and outer basins of Odense Fjord (Fyns Amt, 2006). The water reservoir in each tank was vigorously mixed and aerated by air pumps, and kept at a level 0.5 cm above the upper rim of the open core liners to assure mixing of the headspace. The tanks were kept in darkness and about 1/3 of the water was renewed with fresh seawater every 2 weeks.

The sediment cores were maintained in this setup for the entire experiment, which lasted 589-635 days, depending on station. The time when cores were first transferred to the incubation tanks is referred to as $t = 0$. At selected times, 3 random sediment cores from each station were temporarily removed for flux measurements, and at other times 3 sediment cores were removed permanently for porewater and solid phase analysis as well as anoxic sediment incubations (see detailed sections below).

2.4 Flux measurements
The net exchange of nutrients (DIN and PO$_4^{3-}$) between sediment and water was determined in flux experiments with 3 random sediment cores from each station. Flux experiments were conducted weekly during the first 30 days, monthly until day 180 and every 2-3 months to the end. One day prior to flux measurements, the inside headspace wall of the cores designated for flux measurements were cleaned with a Q-tip to avoid biased flux measurements resulting from bacterial biofilms on the inner surface of core liners (Valdemarsen and Kristensen 2005). These cores were removed from the incubation tanks the next day, equipped with 4 cm long magnetic stirring bars a few cm above the sediment surface and placed around a central magnet rotating at 60 rpm. Initial water samples were taken from all cores, before they were closed with rubber stoppers. The cores were incubated in darkness for 4 hours initially and up to 24 hours at the end of the experiment, before the rubber stoppers were removed and final water samples were taken. Nutrient samples were stored frozen (-20°C) until analyzed for NH$_4^+$, NO$_x^-$(NO$_3^- + NO_2^-$) and PO$_4^{3-}$ on a Lachat Quickchem 8500 Flow injection Analyzer.

2.5 Core sectioning

Three sediment cores from each station were sectioned into 2 cm intervals to 16 cm depth at various times (after 1 day and 1, 7-8, 16-17 and 20-21 months). Core sectioning and subsequent sediment and porewater handling was done inside a N$_2$-filled glovebag. Individual sediment slices were homogenized and porewater for nutrient analysis was obtained after centrifugation of sediment subsamples in double centrifuge tubes (10 min, ~500 g) and GF/C-filtration. Samples for NH$_4^+$ and PO$_4^{3-}$ were stored frozen (-20°C) until analysis as described above.

Sediment characteristics were determined on subsamples from every depth interval during the core sectioning on day 1. Grain size composition, loss on ignition (LOI), total organic C (TOC) content, density and porosity was determined as described in Valdemarsen et al. (2014).
Total N (TN) was measured by elemental analysis on dried sediment subsamples on a Carlo Erba CHN EA1108 Elemental Analyzer. Total P (TP) was extracted by boiling combusted sediment subsamples for 1 h in 1 M HCl. After centrifugation (10 min, 500 g) the supernatants were stored until analyzed for PO$_4^{3-}$ by colorimetric analysis (Koroleff 1983).

During initial and final core sectionings, reactive Fe was extracted from ~0.2 g sediment subsamples with 0.5 M HCl. After 30 min extraction on a shaking table and centrifugation (10 min, 500 g) the supernatants were stored in 4 mL plastic vials at room temperature until analysis. Supernatants were analysed for reduced Fe (FeII) and total Fe by the ferrozine method before and after reduction with hydroxylamine (Stookey 1970; Lovley and Phillips 1987). Oxidized iron (FeIII) was determined as the difference between Total Fe and FeII.

Linear dimensionless NH$_4^+$ adsorption coefficients were determined during the initial core sectioning on wet sediment subsamples from 0-2, 4-6 and 8-10 cm depth intervals in NH$_4^+$-adsorption experiments as described in Holmboe and Kristensen (2002). Sediment subsamples were incubated for 2 d in slurries with different NH$_4^+$-concentrations (0, 1, 2 and 3 mM) and 10 mg/L allylthiourea to inhibit nitrification. After centrifugation (10 min, 500 g) the supernatant was decanted and adsorbed NH$_4^+$ was extracted from the sediment pellet in 2 M KCl (Mackin and Aller, 1984). Supernatants from slurries and KCl-extractions was stored frozen (-20°C) and analyzed for NH$_4^+$ by the salicylate-hypochlorite method (Bower and Holm-Hansen 1980).

2.6 Jar experiments

Closed anoxic sediment incubations (‘jar experiments’) were performed with sediment from different depths (0-2, 4-6 and 8-10 cm) right after core sectionings. Jar experiments measure the total anaerobic mineralization rates of ON and OP from temporal accumulation of metabolic end-products (NH$_4^+$ and PO$_4^{3-}$) in the porewater and yields solid results under a wide range of
environmental and experimental conditions (Kristensen and Hansen 1995; Kristensen et al. 2011; Valdemarsen et al. 2012; Quintana et al. 2013). Sediment from different depths was homogenized and fully packed into 6-8 glass scintillation vials (‘jars’), leaving no headspace. The jars were closed with screw caps and buried in anoxic sediment at 15°C. Two jars were sacrificed at 3-5 day intervals for porewater extraction by centrifugation. The jars were fitted with a perforated lid containing a GF/C-filter inside before centrifugation and were then centrifuged head-down in a centrifuge tube (10 min, ~500 g). Extracted porewater was stored frozen (-20°C) and analyzed for NH$_4^+$ and PO$_4^{3-}$ by colorimetric analysis as described above.

2.7 Calculations and statistics

Initial area specific pools of TN and TP were calculated by depth integration (0-20 cm) of TN and TP content in individual sediment layers. Differences in area specific pools of TN and TP between stations were detected by one-way ANOVA followed by Tuckey’s post hoc test. Data were log-transformed before statistical analysis when assumptions of homoscedasticity were not met (only TN). Area specific pools of FeIII were calculated by depth integration at the beginning (initial) and end (final) and compared by pairwise t-tests.

NH$_4^+$ adsorption coefficients (K$_{NH}$) in individual sediment layers were determined based on NH$_4^+$-adsorption experiments. Extracted NH$_4^+$ (μmol g dw sediment) was plotted against NH$_4^+$-concentration (μmol cm$^{-3}$) and the linear slope, K’, was determined by least squares regression. K$_{NH}$ could hereafter be determined from the relationship K$_{NH} = ((1 - \phi)/\phi) \rho_{ds} * K'$, where $\phi$ is sediment porosity and $\rho_{ds}$ is dry sediment density (Holmboe and Kristensen 2002).

Rates of microbial ON and OP mineralization in discrete depth intervals (0-2, 4-6 and 8-10 cm) were obtained from jar experiments by fitting the time dependent linear concentration change of NH$_4^+$ and PO$_4^{3-}$ by least-squares regression (Aller and Yingst 1980). When slopes were
significant (p < 0.05) the volume specific reaction rates (nmol cm$^{-3}$ d$^{-1}$) in individual depth layers were calculated from the slopes and corrected for sediment porosity and adsorption (Kristensen and Hansen 1995). The mineralization rates at 10-20 cm depth were calculated from exponential regressions based on ON and OP mineralization rates in the top 10 cm. Total area specific ON and OP mineralization were calculated by depth integration (0-20 cm) of measured NH$_4^+$ and PO$_4^{3-}$ production at different depths. The temporal patterns of total area specific ON and OP mineralization were fitted to a double exponential decay regression model of the form $y = C_L \times \exp(-k_L \times t) + C_R \times \exp(-k_R \times t)$, where $t$ is time, $C_L$ and $C_R$ are constants and $k_L$ and $k_R$ denote the first order decay constants for labile and refractory ON and OP, respectively. We hereby assume that considerations based on organic C degradation kinetics (Westrich and Berner 1984) are also valid for ON and OP mineralization. Half lives of labile and refractory ON and OP could hereafter be calculated from the formula $T_{0.5} = \ln (2)/k'$, where $k'$ denote $k_L$ and $k_R$.

3. Results

3.1 Sediment characteristics

Detailed sediment characteristics of the 8 stations in Odense Fjord were previously described in Valdemarsen et al. (2014) and only a brief summary is given here. The sediments from all stations had high sand content and variable silt-clay content with wet densities ranging from 1.2-1.8 g cm$^{-3}$ and porosities of 0.3-0.8. The medium grain size varied from 87 to 397 μm among stations. The sediments from the innermost stations (St 1-3) and most of the stations in the outer basin (St 4 and 6-8) contained a high proportion of silt-clay particles (13-63%). Furthermore, the stations rich in silt-clay particles were organic rich with 0.6-5.2% POC compared to the more sandy St 5 (0.1-0.2% POC).
NH$_4^+$-adsorbtion coefficients varied erratically among stations and sediment depths (Table 1). $K_{NH}$ ranged from 0.14 in the 8-10 cm deep sediment on St 7 to 1.06 in the surface sediment on St 2.

St 1 and St 3 from the inner basin had similar TN content ranging between 57-156 μmol cm$^{-3}$ (Fig. 2). St 2 had slightly higher TN (103-227 μmol cm$^{-3}$) with a pronounced subsurface peak occurring at 3 cm depth. In the outer basin the shallow and deep silty stations (St 4 and 6-8) had similar TN-content (92-154 μmol cm$^{-3}$), except at the surface where TN was lower at St 4 (38-60 μmol cm$^{-3}$). The sandy St 5 contained exceptionally low TN (8-16 μmol cm$^{-3}$). Depth integrated TN was therefore lowest on St 5 (4.5 ± 0.1 mol N m$^{-2}$), intermediate at St 1 (13.5 ± 0.4 mol N m$^{-2}$) and similarly high on the remaining stations (16.0 to 21.4 mol N m$^{-2}$, Table 2).

Two of the stations in the inner basin (St 1 and 2) had similar TP profiles, with 10-11 μmol cm$^{-3}$ at the sediment surface and a gradual decrease to 5.1-5.8 μmol cm$^{-3}$ at 15 cm depth (Fig. 2). St 3 had the lowest TP content of the stations in the inner basin. The shallow silty sediments in the outer basin (St 4) were similar to St 1-2 with respect to TP, whereas the shallow sandy sediment (St 5) was similar to St 3. The deep silty sediments in the outer basin (St 6-8) were characterized by constant TP with depth (9.6-13.5 μmol cm$^{-3}$). Depth integration showed that the highest area specific TP content was found on the deep outer fjord stations (1.8-1.9 mol P m$^{-2}$), whereas shallow silty sediments in the inner and outer fjord contained intermediate TP content (1.2-1.3 mol P m$^{-2}$; St 1, 2 and 4; Table 2). The lowest TP content (~0.7 mol P m$^{-2}$) was found on the silty St 3 and sandy St 5 in inner and outer fjord, respectively.

Initial FeIII pools varied 30-fold between stations (6-243 mmol m$^{-2}$; Table 3), with the lowest FeIII content found in shallow sandy sediment from the outer basin (St 5). FeIII only constituted a minor fraction (2-10%) of total Fe on all stations. No statistically significant
differences were detected between initial and final FeIII-pools (p > 0.17), but there were trends towards higher final FeIII content, except on St 1 and 5.

3.2 ON and OP mineralization

Mineralization rates obtained in the fully anoxic jar experiments might have underestimated mineralization rates at the sediment surface, where O₂ can stimulate mineralization of O₂-sensitive organic matter (Hulthe et al. 1998). In coastal and estuarine sediments O₂ only penetrates to 1-3 mm depth, suggesting a minor importance of this artefact at the beginning of the experiment.

Surprisingly the sediments did not become significantly more oxidized during the long term incubations as indicated by a modest build-up of oxidized FeIII and continuous presence of hydrogen sulfide in the porewater of surface sediment from all stations (data not shown). Hence we assume that mineralization rates in the sediment cores underlying an oxic water phase were closely approximated by the rates obtained in jar experiments.

\[ \text{NH}_4^+ \] production in jar experiments was significant throughout the experiment, except for St 1, 8-10 cm depth after 607 d. Initially \[ \text{NH}_4^+ \] production was highest in the surface 0-2 cm sediment from the silty St 1-2 in the inner fjord and the sandy St 5 in the outer fjord (159-338 nmol cm\(^{-3}\) d\(^{-1}\)) and was similar on remaining stations (63-101 nmol cm\(^{-3}\) d\(^{-1}\); Fig. 3). Surface \[ \text{NH}_4^+ \] production decreased rapidly over time in sediments from shallow locations in the inner and outer fjord, by 96% of initial rates on St 1 and by 61–82% on St 2–5. The surface \[ \text{NH}_4^+ \] production in the sediments sampled in the deep outer basin (St 6–8) decreased by 8–67% during the experiment. \[ \text{NH}_4^+ \] production at 4–6 cm depth was initially 18–60 nmol cm\(^{-3}\) d\(^{-1}\) on all stations and temporal changes were also observed in this layer, especially in shallow silty sediments from the inner basin where \[ \text{NH}_4^+ \] production decreased by 75–96% to 1.4–12 nmol cm\(^{-3}\) d\(^{-1}\) by the end (Fig. 3). In sediments from the outer basin \[ \text{NH}_4^+ \] production at 4-6 cm depth only decreased by 19-58%. At 8-
10 cm depth NH$_4^+$ production at all stations occurred at similar rates and showed similar temporal trends as observed at 4-6 cm depth (Fig. 3).

Significant PO$_4^{3-}$ production was measured in the surface sediment from all stations throughout the experiment (Fig. 4). Initial rates were highest (30-35 nmol cm$^{-3}$ d$^{-1}$) on St 1 and 2 from the shallow inner basin and considerably lower (7-18 nmol cm$^{-3}$ d$^{-1}$) on the remaining stations. PO$_4^{3-}$ production initially decreased rapidly in the surface sediment from St 1 and 2 and stabilized at relatively low and stable levels after ~200 d (0.7-6.0 nmol cm$^{-3}$ d$^{-1}$). Surface PO$_4^{3-}$ production also decreased over time on the other stations, but temporal trends were more erratic. PO$_4^{3-}$ production in deeper sediment was generally lower than at the surface, and with less variability among stations (Fig. 4). PO$_4^{3-}$ production at 4-6 cm depth was 0-6 nmol cm$^{-3}$ d$^{-1}$ and remained quite stable throughout the experiment on all stations. The only significant decrease (p = 0.01-0.03) occurred in silty sediments from the inner basin (St 1-3) and St 6 and 8 from the deep outer basin. PO$_4^{3-}$ production varied between 0-5 nmol cm$^{-3}$ d$^{-1}$ at 8-10 cm depth and was stable throughout the experiment.

Area-specific ON mineralization was calculated by depth integration of NH$_4^+$ production rates (Fig. 3). The sediments from the inner basin (St 1-3) showed high initial ON mineralization (6-11 mmol m$^{-2}$ d$^{-1}$) in the same range as the shallow silty and sandy sediments from the outer basin (6 and 10 mmol m$^{-2}$ d$^{-1}$ on St 4 and 5, respectively). The deep silty sediments from the outer basin showed the lowest initial ON mineralization (St 6-8; 3-5 mmol m$^{-2}$ d$^{-1}$). Area-specific ON mineralization decreased during the experiment on all stations, by 82-93% for the silty inner fjord and 34-71% on remaining stations. The temporal decrease was mainly driven by successively lower ON mineralization in surface sediment during the first ~200 d and area-specific ON mineralization was fairly constant hereafter. Initial area-specific OP mineralization was 0.2-1.0 mmol m$^{-2}$ d$^{-1}$ (Fig. 4) and decreased (59-70%) over time on several of the stations (St 1-3 and St 6).
As for ON mineralization, the successively lower OP mineralization was mainly due to decreased OP mineralization in surface sediment. On the other stations area-specific OP mineralization remained relatively high and did not show clear temporal trends.

Double exponential decay models fitted the ON mineralization kinetics on St 1-6 and the OP mineralization kinetics on St 1-3 and 6. Erratic mineralization patterns prevented the use of exponential decay models on remaining stations (see Fig. 3-4). Decay constants for labile and refractory ON and OP in were fairly similar at all stations, with $k_L$'s of 0.02-0.06 d$^{-1}$ (except for 10 times higher values for ON at St 6 and for OP at St 2) and $k_R$'s of 0.0003-0.0015 (Table 4). The half lives for ON and OP were in the range of 0.01-0.11 and 1.3-6.3 years for labile and refractory fractions, respectively.

### 3.3 DIN- and DIP-fluxes

DIN fluxes followed a similar exponentially decreasing pattern for all stations (Fig. 5), and ranged from 1.1-3.7 mmol m$^{-2}$ d$^{-1}$ initially (t =0-90 d) to 0.09-0.5 mmol m$^{-2}$ d$^{-1}$ by the end. The main form of DIN released initially was NH$_4^+$, which contributed 59-100% of DIN-release. Subsequently the NH$_4^+$ efflux decreased while NO$_x^-$ switched from uptake to release and after 0.5-1 y to the end of the experiment, 68-100% of the DIN was released as NO$_x^-$. The 8 stations showed different patterns of PO$_4^{3-}$ fluxes. The stations from the shallow inner basin, St 1-3, showed exponentially decreasing PO$_4^{3-}$ fluxes over time (initial fluxes of 0.1-0.2 mmol m$^{-2}$ d$^{-1}$ decreasing to 0.01-0.05 mmol m$^{-2}$ d$^{-1}$ by the end; Fig. 5). Initial (day 0-90) PO$_4^{3-}$ fluxes on the shallow silty St 4 was around zero, but increased to 0.07-0.14 mmol m$^{-2}$ d$^{-1}$ during d 90-360 of the experiment. The highest PO$_4^{3-}$ fluxes (0.07-0.21 mmol m$^{-2}$ d$^{-1}$) were observed on the TP-poor sandy St 5, particularly towards the end of the experiment, while the TP-rich outer fjord
stations 6-8 had the lowest and most irregular \( \text{PO}_4^{3-} \) fluxes ranging from slightly negative to 0.1 mmol m\(^{-2}\) d\(^{-1}\).

3.4 \( \text{PO}_4^{3-} \) and \( \text{NH}_4^+ \) in porewater

Porewater nutrient concentrations increased gradually at all depths during the experiment (data not shown). \( \text{NH}_4^+ \) and \( \text{PO}_4^{3-} \) only increased moderately in the upper 2 cm, but accumulated to high levels in the deeper diffusion limited sediment. Depth-averaged initial porewater \( \text{NH}_4^+ \) concentration varied between 171-407 μM on the stations. The sandy St 5 showed the highest \( \text{NH}_4^+ \) accumulation over time with a depth-average of 1473 μM in porewater by the end. On the remaining stations, \( \text{NH}_4^+ \) only accumulated to 259-587 μM. Depth-averaged \( \text{PO}_4^{3-} \) concentrations at the beginning varied between 17-71 μM depending on station. As for \( \text{NH}_4^+ \), the nutrient-poor sandy St 5 showed the highest \( \text{PO}_4^{3-} \) accumulation to 368 μM compared with 43-170 μM on the other stations.

3.5 N- and P-budgets

Area-specific nutrient mineralization obtained in jar-experiments was used to calculate total ON and OP mineralization during the experiment. ON mineralization was fairly constant for all stations except St 5 (1.4 to 1.9 mol m\(^{-2}\)) corresponding to 8-10% of initial TN (Table 5). St 5, on the other hand, had 3-fold higher ON mineralization that accounted for 80% of the initial ON. A 3-fold range among stations was also evident for OP mineralization, but with lowest rates of 0.12-0.18 mol m\(^{-2}\) at St 1-4 and the highest rates of 0.22-0.33 mol m\(^{-2}\) at St 5-8 (8-48% of initial TP). Interestingly, there was no apparent relationship between sediment TN and TP content and mineralization activity as some of the highest N- and P-mineralization rates were observed on the organic-poor St 5 (Table 4). DIN-effluxes, porewater accumulation and adsorption only accounted for 18-32% of total ON.
mineralization, indicating that most of the generated NH$_4^+$ was not accounted for by our measurements. For P, the sum of PO$_4^{3-}$ efflux and porewater accumulation only accounted for 10-48% of total OP mineralization.

4. Discussion

4.1 Sediment nutrient content

TN and TP in sediments from Odense Fjord were in the same range or higher than reported for other eutrophic systems (e.g. Boynton and Kemp 1985; Cowan & Boynton 1996; Lomstein et al. 1998; Coelho et al. 2004; Viktorsson et al. 2013) emphasizing the history of intense eutrophication in Odense Fjord. TN and TP in the silty sediments of Odense Fjord (all stations except St 5) were remarkably similar and only varied ~1.5 (TN) and ~3 (TP) times among stations. Despite these overall similarities, the silty sediments from the shallow inner basin showed higher initial ON- and OP-mineralization and nutrient effluxes than silty sediments from the outer fjord. This could be due to higher availability of labile ON and OP in the sediments from the inner basin, reflecting the nutrient rich conditions in the inner compared to the outer basin (Petersen et al. 2009).

The sandy St 5 was markedly different from the other stations. It had the lowest total nutrient content and yet exhibited some of the highest rates of ON and OP mineralization. The frequent erosion by wind driven waves in this area (Valdemarsen et al. 2010) and deep (>20 cm) reworking by lugworms (* Arenicola marina*) (Riisgaard & Banta 1998; Valdemarsen et al. 2011) may remove fine particles and refractory organic matter from St 5 sediments (Wendelboe et al. 2012) and prevent organic matter accumulation, hence explaining the low organic content on this station. On the other hand, intense growth and burial of microphytobenthos and other reactive detritus by the strong physical disturbance and vertical mixing, can explain the unexpected high TN and TP reactivity of St 5 sediment.
A rough areal estimate based on the measured TN and TP content on the examined stations (Table 2) suggest that 12.6*10^6 kg N and 3.7*10^6 kg P are stored in the upper 20 cm of Odense Fjord sediments, corresponding to ~6 (N) and ~62 (P) years of the current annual external nutrient loading to the system.

4.2 Organic N and P mineralization

Microbial mineralization of ON and OP in Odense Fjord sediments led to marked release of inorganic nutrients, especially in the initial phase of the experiment. Initially there were strong vertical gradients of ON and OP mineralization in silty and sandy sediments from shallow environments, indicating that newly deposited and relatively labile organic matter was being degraded near the sediment surface, with the depth gradient reflecting a gradual and time-dependent depletion of labile ON and OP (Westrich & Berner 1984; Mackin and Swider 1989; Valdemarsen et al. 2014). It was expected that ON and OP mineralization would decrease with time at all depths due to diminishing reactivity of the organic pools. However, significant temporal decreases were only observed in surface sediments from shallow locations, whereas mineralization rates were surprisingly stable in the underlying sediment and the entire sediment column in the deep outer fjord. Assuming that organic matter degradation follows an exponential decay pattern, the lack of a detectable attenuation in mineralization rates over a ~2 yr period indicates very low initial reactivity of ON and OP in the deeper layers (Westrich & Berner 1984). Nevertheless, since ON and OP of low reactivity was present at high concentrations, it remained a significant source for inorganic nutrients.

Total jar-based microbial ON and OP mineralization over the ~2 years experimental period (Table 5) only accounted for a minor fraction of initial TN and TP in sediments from Odense Fjord suggesting that the standing stock of organic N and P will be a source of nutrients for
extended time. Decay constants from the exponential decay model suggested that labile ON and OP was rapidly degraded on all stations within 10-240 d, whereas depletion of more refractory ON and OP will only occur on decadal time-scales (8-40 years), indicating that depletion of buried and degradable ON and OP in eutrophic ecosystems will take considerable time.

4.3 Fate of inorganic nutrients

NH$_4^+$ and PO$_4^{3-}$ produced by microbial mineralization accumulated in porewater of all sediments within the first 1-6 months and only changed slightly hereafter. However, over the whole experiment, porewater accumulation explained only a minor fraction of the jar-based total ON and OP mineralization (0.8-8.1%). It was also investigated if NH$_4^+$ adsorption to mineral surfaces was an important N sink. Despite the large spatial heterogeneity of NH$_4^+$ adsorption, this process never accounted for more than 1% of the total produced NH$_4^+$ over the whole experiment and was therefore not quantitatively important.

Nutrient release to the overlying water was the most important route for inorganic nutrients produced by microbial mineralization. We could not account for all the produced nutrients, since nutrient mineralization in jar experiments exceeded DIN and PO$_4^{3-}$ effluxes by 70-84% and 62-93%, respectively. The missing NH$_4^+$ may have been lost through coupled nitrification-denitrification (e.g. Mackin and Swider 1989; Quintana et al. 2013). The conspicuous shift from NH$_4^+$ to NO$_3^-$ release indicated that nitrification was an active process in all sediment types, and denitrifying bacteria probably proliferated in the NO$_3^-$-rich surface sediment. In the present case, coupled nitrification-denitrification rates of 1-2 mmol m$^{-2}$ d$^{-1}$ are required to account for the missing NH$_4^+$, which is within the range reported in previous studies (e.g. Nielsen et al. 1995; Christensen et al. 2000; Tobias et al. 2003). On the other hand, the missing PO$_4^{3-}$ must have been retained within the sediments. Several studies suggest almost complete PO$_4^{3-}$ retention in marine sediments with an
oxic sediment surface (Rozan et al. 2002; Viktorsson et al. 2013) where PO$_4^{3-}$ adsorbs to oxidized FeIII-minerals preventing PO$_4^{3-}$ efflux (Sundby et al. 1992). Experimental studies suggest that every FeIII molecule can retain more than 0.5 PO$_4^{3-}$ molecules (Gunners & Blomqvist, 1997; Rozan et al. 2002). Hence the FeIII levels on all the silty stations were sufficient to retain the missing PO$_4^{3-}$, especially when considering that 0.5 M HCl extractions only extracts a fraction of the available FeIII. On the sandy St 5 the FeIII levels were too low to account for the missing PO$_4^{3-}$, indicating that there were other PO$_4^{3-}$ sinks. PO$_4^{3-}$ adsorption in the anoxic sediment (Krom & Berner, 1980) or precipitation of PO$_4^{3-}$-CaCO$_3$ complexes (Coelho et al. 2004) are possible sinks that were not quantified in this experiment.

4.4 Internal nutrient loading
We calculated the potential internal nutrient loading in Odense Fjord resulting from microbial mineralization of ON and OP for a 2 y period based on the measured nutrient effluxes. Average nutrient fluxes were calculated for each sediment type, i.e. shallow inner fjord (St 1-3), shallow silty outer fjord (St 4), sandy outer fjord (St 5) and deep outer fjord (St 6-8). The monthly time-weighted DIN and PO$_4^{3-}$ fluxes and the total areal distribution of the different sediment types in Odense Fjord were then used to calculate the total internal nutrient loading (10$^3$ kg N and P mo$^{-1}$) for each sediment type and for the whole ecosystem. Evidently these calculations do not represent the *in situ* internal nutrient loading, since effects of the otherwise continuous deposition of organic matter were omitted by the experimental setup. It can also be debated if all the released nutrients can be considered internal nutrient loading, since the mineralization of recently deposited organic matter in surface sediments drove the majority of nutrient release during the first ~200 d. This nutrient release is largely determined by the ecosystem primary productivity extending only a few years back, and is therefore closely coupled to the recent levels of external nutrient loading. In any case the
calculations represent the nutrient release resulting from the mineralization of slowly reacting ON and OP, which have accumulated in the sediments.

The calculations show the magnitude of nutrient release driven by microbial mineralization of sediment-bound ON and OP in eutrophic ecosystems (Fig. 6). Total DIN release from the whole fjord bottom is equivalent to 121*10^3 kg N y^{-1} (~20 kg N ha^{-1} y^{-1}) the first year after sedimentation of new organic matter has ceased, but only 38*10^3 kg N y^{-1} (~6.2 kg N ha^{-1} y^{-1}) the second year, since ON effluxes decreased exponentially on all stations. The shallow sandy sediments in the outer fjord were most important for the total fjord-wide N-release (39%), whereas the remaining 3 sediment types contributed equally (16-23%). The numbers for internal N-loading are impressive at first, but only correspond to maximum 2-6% (N) of the current external N-loading to Odense Fjord (about 2000*10^3 kg N y^{-1}; Petersen et al. 2009). In the shallow N-limited Odense Fjord the internal N-loading can therefore only have minor effects for overall ecosystem productivity. In any case the external N-loading is far more important for the overall primary productivity and ecological status.

The internal P-loading showed different temporal dynamics than internal N-loading. Total P-release from the whole fjord bottom was stable over time at rates of 21-22*10^3 kg P y^{-1} (~3.4-3.6 kg P ha^{-1} y^{-1}; Fig. 6) while internal N-loading decreased exponentially. The stability was driven by the increasing P release in shallow sandy outer fjord sediment and constant P release in deep outer fjord sediment. As for N, the shallow sandy sediments in the outer fjord was most important for total internal P-loading (57%) and the remaining 3 sediment types contributed equally (14-15%). The internal P-loading corresponded to 35-36% (P) of the current external P-loading to Odense Fjord (60*10^3 kg P y^{-1}; Petersen et al. 2009) and thus potentially constitutes a stable and significant P-source in the system. However, since Odense Fjord and most other temperate coastal
ecosystems are mostly N-limited (Howarth et al. 2011) it is uncertain to which degree this excess P will affect ecosystem productivity.

4.5 Ecological implications

In many shallow eutrophic estuaries the external nutrient loading has been reduced to induce oligotrophication, but lower nutrient concentrations in the recipient estuary often occurs after considerable delay and rarely corresponds proportionally to the reductions (Kronvang et al. 2005; Carstensen 2006). This indicates that a transient phase occurs, where accumulated nutrients are being released from the soils and sediments in the water shed and receiving estuary, respectively, while the system equilibrates to a new level of external nutrient loading. Our study shows the magnitude and temporal dynamics of the internal nutrient loading that can be expected in shallow estuaries recovering from eutrophication. It appears that internal N-loading will be insignificant during recovery since it only corresponded to 2-6% of the external N-loading in our example and decreased rapidly. Internal N-loading will therefore only lead to marginally elevated N-availability and have minor effects on primary productivity and eutrophication status. The results are different with respect to PO$_4^{3-}$, since the internal P-loading was stable and corresponded to >1/3 of the external P-loading. Internal P-loading may therefore be a significant source of dissolved PO$_4^{3-}$ for extended time in shallow eutrophic estuaries, and at a sufficiently high level to counteract reductions in the external P-loading. Most shallow estuaries are N-limited (Conley et al. 2000; Howarth & Marinho 2006; Howarth et al. 2011) so a high internal P-loading might only exacerbate N-limitation while having no further consequences for ecological quality. Decreasing internal N-loading and stable internal P-loading could also lead to increased dominance of cyanobacteria, which have low requirements for dissolved N. However, major shifts in phytoplankton communities would only occur in systems where decreased internal nutrient loading results in markedly lower
DIN-concentrations in the water phase, i.e. in systems where N-loading is low and internal nutrient sources dominate.

The estimates of internal nutrient loading presented here provide an illustrative example, but the exact values are only valid for the experimental conditions and must be extrapolated with caution. Microbial reaction rates and DIN and PO$_4^{3-}$ release from sediments are strongly influenced by ambient conditions. For instance, sediment macrofauna may stimulate the rates of organic matter degradation and sediment nutrient release through bioturbation (e.g. Kristensen et al. 2012; 2014) leading to higher internal nutrient loading than estimated from defaunated sediment cores in this experiment. Similarly microbial mineralization processes and hence sediment DIN and PO$_4^{3-}$ release are strongly temperature dependent (Westrich and Berner 1988; Sanz-Lazaro et al. 2011) and the magnitude of internal nutrient loading will therefore vary seasonally compared to our estimates based on a constant temperature experiment. Finally, in our experimental setup we also omitted hydrodynamics and porewater advection which are known to stimulate nutrient cycling in shallow permeable sediments (Cook et al. 2007; Huettel et al. 2014). This will especially affect the estimated nutrient release from the sandy sediments from this study.

Given the multitude of factors influencing nutrient mineralization rates, the actual magnitude of internal nutrient loading and related consequences for primary productivity will therefore follow a seasonal pattern driven by e.g. temperature, hydrodynamics and composition and activity of benthic fauna. Other environmental variables such as hypoxia in the water column may also influence the magnitude of internal nutrient loading, since it hampers PO$_4^{3-}$ retention by Fe-oxides (Azzoni et al. 2005; Mort et al. 2010; Viktorsson et al. 2013) and limits coupled nitrification-denitrification while stimulating dissimilatory nitrate reduction to NH$_4^+$ (Christensen et al. 2000; Jäntti & Hietanen 2012). Ecosystems suffering from hypoxia may therefore experience a much higher internal nutrient loading than measured in this experiment. A comparison between total ON and OP mineralization...
and effluxes from this experiment, suggests that nutrient effluxes could potentially increase 3-6 (DIN) and 2-10 (PO$_4^{3-}$) times if there are no mechanisms to transform or retain inorganic nutrients at the sediment surface.

4.6 Conclusions

In this study we investigated the mineralization of organic N and P buried in the sediments from a shallow eutrophic estuary and obtained estimates of the magnitude and temporal dynamics of internal nutrient loading. Total internal N-loading, which attenuated rapidly, corresponded to only a minor fraction of the external N-loading and was therefore not important for the ecological state in the studied ecosystem. Total internal P-loading showed no temporal attenuation and was quantitatively more important as it corresponded to >1/3 of the external P-loading. However, the studied ecosystem was N-limited, and it is therefore uncertain if high internal P-loading will result in negative ecological effects. This study indicates that internal nutrient loading, and especially internal N-loading, is a transient phenomena that can only temporarily influence the recovery trajectory of ecosystems recovering from eutrophication. In turn, internal nutrient loading driven by mineralization of organic N and P in sediments, cannot explain the lack of recovery in shallow estuaries where external nutrient loading has been reduced.

5. Acknowledgements

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Table 1. Dimensionless linear NH$_4^+$ adsorption coefficients, K$_{NH}$, for different sediment depths at St 1-8.

<table>
<thead>
<tr>
<th>Depth</th>
<th>St 1</th>
<th>St 2</th>
<th>St 3</th>
<th>St 4</th>
<th>St 5</th>
<th>St 6</th>
<th>St 7</th>
<th>St 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2 cm</td>
<td>0.26</td>
<td>1.06</td>
<td>0.33</td>
<td>0.46</td>
<td>0.64</td>
<td>0.31</td>
<td>0.57</td>
<td>0.48</td>
</tr>
<tr>
<td>6-8 cm</td>
<td>0.52</td>
<td>0.76</td>
<td>0.49</td>
<td>0.45</td>
<td>0.82</td>
<td>0.51</td>
<td>0.62</td>
<td>0.36</td>
</tr>
<tr>
<td>8-10 cm</td>
<td>0.40</td>
<td>0.82</td>
<td>0.20</td>
<td>0.79</td>
<td>0.55</td>
<td>0.66</td>
<td>0.14</td>
<td>0.45</td>
</tr>
</tbody>
</table>
Table 2. Depth integrated (0-16 cm) area specific TN and TP content ± SE (n=3) on St 1-8.

Superscript capital letters indicate the grouping of data obtained by ANOVA and subsequent post hoc analysis. Average TN:TP ratios are also shown.

<table>
<thead>
<tr>
<th></th>
<th>TN (mol m⁻²)</th>
<th>TP (mol m⁻²)</th>
<th>TN:TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>St 1</td>
<td>13.5 ± 0.4</td>
<td>1.34 ± 0.04</td>
<td>10.1</td>
</tr>
<tr>
<td>St 2</td>
<td>21.5 ± 0.5</td>
<td>1.31 ± 0.02</td>
<td>16.4</td>
</tr>
<tr>
<td>St 3</td>
<td>16.0 ± 0.2</td>
<td>0.70 ± 0.06</td>
<td>22.9</td>
</tr>
<tr>
<td>St 4</td>
<td>16.6 ± 1.1</td>
<td>1.18 ± 0.06</td>
<td>14.1</td>
</tr>
<tr>
<td>St 5</td>
<td>4.5 ± 0.1</td>
<td>0.73 ± 0.04</td>
<td>6.2</td>
</tr>
<tr>
<td>St 6</td>
<td>17.1 ± 0.1</td>
<td>1.94 ± 0.03</td>
<td>8.8</td>
</tr>
<tr>
<td>St 7</td>
<td>18.1 ± 0.0</td>
<td>1.86 ± 0.05</td>
<td>9.7</td>
</tr>
<tr>
<td>St 8</td>
<td>19.5 ± 0.2</td>
<td>1.83 ± 0.03</td>
<td>10.7</td>
</tr>
</tbody>
</table>
Table 3. Initial and final depth integrated pools (0-20 cm) of FeIII ± SE (n=3) on St 1-8. t-tests showed no significant difference between initial and final FeIII pools on any station.

<table>
<thead>
<tr>
<th></th>
<th>Initial FeII (mmol m⁻²)</th>
<th>Initial FeIII (mmol m⁻²)</th>
<th>Final FeII (mmol m⁻²)</th>
<th>Final FeIII (mmol m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St 1</td>
<td>2390 ± 34</td>
<td>243 ± 24</td>
<td>2294 ± 153</td>
<td>92 ± 22</td>
</tr>
<tr>
<td>St 2</td>
<td>2302 ± 160</td>
<td>157 ± 32</td>
<td>2399 ± 189</td>
<td>271 ± 161</td>
</tr>
<tr>
<td>St 3</td>
<td>1356 ± 155</td>
<td>62 ± 25</td>
<td>1358 ± 154</td>
<td>109 ± 40</td>
</tr>
<tr>
<td>St 4</td>
<td>1054 ± 86</td>
<td>28 ± 20</td>
<td>996 ± 23</td>
<td>97 ± 37</td>
</tr>
<tr>
<td>St 5</td>
<td>258 ± 2</td>
<td>6.3 ± 1.0</td>
<td>274 ± 39</td>
<td>6.4 ± 1.2</td>
</tr>
<tr>
<td>St 6</td>
<td>1887 ± 37</td>
<td>75 ± 12</td>
<td>1813 ± 43</td>
<td>141 ± 40</td>
</tr>
<tr>
<td>St 7</td>
<td>2464 ± 105</td>
<td>52 ± 2.0</td>
<td>2142 ± 60</td>
<td>137 ± 48</td>
</tr>
<tr>
<td>St 8</td>
<td>1697 ± 63</td>
<td>156 ± 8.0</td>
<td>1813 ± 43</td>
<td>210 ± 89</td>
</tr>
</tbody>
</table>
Table 4. Double exponential regression statistics for the temporal trends of total ON and OP degradation in jar experiments. Total organic N (ON) and P (OP) degradation were fitted to the exponential decay function \( y = C_L \exp(-k_L \cdot x) + C_R \exp(-k_R \cdot x) \), where \( C_L \) and \( C_R \) denote constants and \( k_L \) and \( k_R \) denote decay constants for labile and refractory organic ON and OP, respectively. Statistics were not calculated for St 7-8 (ON) and for St 4-5 and 7-8 (OP), since the temporal degradation patterns did not fit the double exponential decay model. \( T_{L,0.5} \), \( L \) and \( T_{R,0.5} \), \( R \) denote the half life (y) of labile and refractory ON and OP, respectively.

<table>
<thead>
<tr>
<th></th>
<th>( k_L )</th>
<th>( k_R )</th>
<th>( C_L )</th>
<th>( C_R )</th>
<th>( T_{L,0.5} )</th>
<th>( T_{R,0.5} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St 1</td>
<td>4.6*10^{-2}</td>
<td>1.1*10^{-3}</td>
<td>7.7</td>
<td>2.4</td>
<td>0.04</td>
<td>1.73</td>
</tr>
<tr>
<td>St 2</td>
<td>2.3*10^{-2}</td>
<td>1.0*10^{-3}</td>
<td>3.1</td>
<td>2.9</td>
<td>0.08</td>
<td>1.90</td>
</tr>
<tr>
<td>St 3</td>
<td>5.3*10^{-2}</td>
<td>1.1*10^{-3}</td>
<td>8.6</td>
<td>2.8</td>
<td>0.04</td>
<td>1.73</td>
</tr>
<tr>
<td>St 4</td>
<td>4.3*10^{-2}</td>
<td>0.4*10^{-3}</td>
<td>4.0</td>
<td>1.8</td>
<td>0.04</td>
<td>4.75</td>
</tr>
<tr>
<td>St 5</td>
<td>5.7*10^{-2}</td>
<td>0.6*10^{-3}</td>
<td>2.7</td>
<td>7.2</td>
<td>0.03</td>
<td>3.17</td>
</tr>
<tr>
<td>St 6</td>
<td>52.4*10^{-2}</td>
<td>0.3*10^{-3}</td>
<td>3.2</td>
<td>2.9</td>
<td>0.01</td>
<td>6.33</td>
</tr>
<tr>
<td>St 7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>St 8</td>
<td>-</td>
<td>-</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>( k_L )</th>
<th>( k_R )</th>
<th>( C_L )</th>
<th>( C_R )</th>
<th>( T_{L,0.5} )</th>
<th>( T_{R,0.5} )</th>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St 1</td>
<td>3.9*10^{-2}</td>
<td>0.4*10^{-3}</td>
<td>0.6</td>
<td>0.3</td>
<td>0.05</td>
<td>4.75</td>
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<tr>
<td>St 2</td>
<td>56.0*10^{-2}</td>
<td>1.5*10^{-3}</td>
<td>1.1</td>
<td>0.3</td>
<td>0.01</td>
<td>1.27</td>
</tr>
<tr>
<td>St 3</td>
<td>2.2*10^{-2}</td>
<td>1.3*10^{-3}</td>
<td>0.1</td>
<td>0.3</td>
<td>0.08</td>
<td>1.46</td>
</tr>
<tr>
<td>St 4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>St 6</td>
<td>1.7*10^{-2}</td>
<td>0.9*10^{-3}</td>
<td>0.4</td>
<td>0.5</td>
<td>0.11</td>
<td>2.11</td>
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<tr>
<td>St 7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>St 8</td>
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</tbody>
</table>
Table 5. N and P budgets for the experiment. Initial TN and TP are the depth integrated values based on initial measurements. ON and OP degradation were calculated based on area specific rates obtained from jar experiments. Total NH$_4^+$-, NO$_3^-$- and PO$_4^{3-}$-effluxes were calculated by time integration of effluxes over the entire experimental period. NH$_4^+$- and PO$_4^{3-}$-accumulation in porewater (pw) was calculated from the difference between initial and final pw profiles. NH$_4^+$ adsorption was calculated from initial and final pw-inventories of NH$_4^+$ and the average NH$_4^+$-adsorption coefficient for each station. Values in parentheses marked with * or ** represent percentage relative to initial TN and TP or total N and P mineralization, respectively.

<table>
<thead>
<tr>
<th>N-mineralization</th>
<th>St 1</th>
<th>St 2</th>
<th>St 3</th>
<th>St 4</th>
<th>St 5</th>
<th>St 6</th>
<th>St 7</th>
<th>St 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial TN (mol m$^{-2}$)</td>
<td>13.5</td>
<td>21.5</td>
<td>16.0</td>
<td>16.6</td>
<td>4.5</td>
<td>17.1</td>
<td>18.1</td>
<td>19.5</td>
</tr>
<tr>
<td>ON degradation, jars (mol m$^{-2}$)$^*$</td>
<td>(10.2)</td>
<td>(7.5)</td>
<td>(9.8)</td>
<td>(8.6)</td>
<td>(80.1)</td>
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<td>0.26</td>
<td>0.10</td>
<td>0.23</td>
<td>0.15</td>
<td>0.38</td>
<td>0.09</td>
<td>0.27</td>
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<td>(19.1)</td>
<td>(6.2)</td>
<td>(14.6)</td>
<td>(10.7)</td>
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<td>(5.1)</td>
<td>(16.6)</td>
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<td>0.21</td>
<td>0.19</td>
<td>0.11</td>
<td>0.18</td>
<td>0.22</td>
<td>0.17</td>
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<tr>
<td>(11.2)</td>
<td>(13.0)</td>
<td>(12.1)</td>
<td>(7.3)</td>
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<td>(10.8)</td>
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<td>NH$_4^+$ accumulation, pw (mol m$^{-2}$)$^{**}$</td>
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<td>0.01</td>
<td>0.00</td>
<td>0.08</td>
<td>0.06</td>
<td>0.03</td>
<td>0.01</td>
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<td>(1.6)</td>
<td>(0.7)</td>
<td>(0.0)</td>
<td>(5.9)</td>
<td>(1.6)</td>
<td>(1.8)</td>
<td>(0.7)</td>
<td>(1.3)</td>
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<tr>
<td>NH$_4^+$ adsorption (mol m$^{-2}$)$^{**}$</td>
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<td>0.01</td>
<td>0.00</td>
<td>0.01</td>
<td>0.04</td>
<td>0.02</td>
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<td>(0.7)</td>
<td>(0.6)</td>
<td>(0.0)</td>
<td>(0.4)</td>
<td>(1.0)</td>
<td>(1.0)</td>
<td>(0.2)</td>
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<td>0.02</td>
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<td>0.02</td>
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<tr>
<td>(12.6)</td>
<td>(38.0)</td>
<td>(16.8)</td>
<td>(15.8)</td>
<td>(27.8)</td>
<td>(7.4)</td>
<td>(12.8)</td>
<td>(6.5)</td>
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<tr>
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<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.01</td>
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<tr>
<td>(4.8)</td>
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<td>(0.8)</td>
<td>(5.1)</td>
<td>(3.0)</td>
<td>(1.7)</td>
<td>(3.1)</td>
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Figure 1. Map of Odense Fjord ($55^\circ 29' 15''$ N; $10^\circ 31' 09''$) showing the 8 stations, where sediments were sampled for the long term degradation experiment. Gray color indicates land and different shades of blue indicate water depth.
Figure 2. Total nitrogen (TN) and total phosphorus (TP) in sediments from Odense Fjord. Left panels show stations from the shallow inner fjord (St 1, 2 and 3), middle panels show shallow silty and sandy sediments in the outer fjord (St 4 and 5, respectively) and right panels show deep silty sediments in the outer fjord (St 6, 7 and 8). Error bars indicate standard error (n = 3).
Figure 3. \(\text{NH}_4^+\) production measured in jar experiments with sediment from shallow inner basin (upper panels), shallow silty and sandy outer basin (middle panels) and deep silty outer basin (lower panels). Black, gray and white symbols indicate volume specific \(\text{NH}_4^+\) production in sediment from 0-2, 4-6 and 8-10 cm depth, respectively (left y-axis). Bars indicate depth integrated (0-20 cm) \(\text{NH}_4^+\) production based on volume specific production rates (right y-axis).
Figure 4. PO₄³⁻ production measured in jar experiments performed with sediment from shallow inner fjord (upper panels), shallow silty and sandy outer fjord (middle panels) and deep silty outer fjord (lower panels). Black, gray and white symbols indicate volume specific PO₄³⁻ production in sediment from 0-2, 4-6 and 8-10 cm depth, respectively (left y-axis). Bars indicate depth integrated (0-20 cm) PO₃⁻ production based on volume specific production rates (right y-axis).
Figure 5. Fluxes of dissolved inorganic nitrogen (DIN = NH$_4^+$ + NO$_x^-$) and PO$_4^{3-}$ at various times during the experiment. Error bars represent standard error (n = 6-24).
Figure 6. Estimated internal nutrient loading in Odense Fjord. The upper figure shows a schematic overview of Odense Fjord with the distribution of sediment types included in this study and their nutrient release over a 24 month period. The lower figure shows the cumulated nutrient release from the entire fjord bottom.