

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

3
4 Thomas Valdemarsen^{1*}, Cintia O. Quintana^{1,2}, Mogens R. Flindt¹ and Erik Kristensen¹

5
6
7 *Corresponding Author: valdemarsen@biology.sdu.dk

8 ¹⁾ Institute of Biology, University of Southern Denmark, Denmark

9 ²⁾ Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brazil

10
11
12 **Key words:** nutrient release, fluxes, reactivity, ammonium production, phosphate production,
13 mineralization, oligotrophication, recovery, adsorption

14 **Abstract**

15 Nutrient release from the sediments in shallow eutrophic estuaries may counteract reductions of the
16 external nutrient load and prevent or prolong ecosystem recovery. The magnitude and temporal
17 dynamics of this potential source, termed internal nutrient loading, is poorly understood. We
18 quantified the internal nutrient loading driven by microbial mineralization of accumulated organic
19 N (ON) and P (OP) in sediments from a shallow eutrophic estuary (Odense Fjord, Denmark).
20 Sediments were collected from 8 stations within the system and nutrient production and effluxes
21 were measured over a period of ~2 years. DIN effluxes were high initially but quickly faded to low
22 and stable levels after 50-200 d, whereas PO_4^{3-} effluxes were highly variable in the different
23 sediments. Mineralization patterns suggested that internal N-loading would quickly (<200 days)
24 fade to insignificant levels whereas internal PO_4^{3-} loading could be sustained for extended time
25 (years). When results from all stations were combined, internal N-loading and P-loading from the
26 fjord bottom was up to $121 \cdot 10^3 \text{ kg N yr}^{-1}$ ($20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) and $22 \cdot 10^3 \text{ kg P yr}^{-1}$ ($3.6 \text{ kg P ha}^{-1} \text{ yr}^{-1}$)
27 ¹) corresponding to 6% (N) and 36% (P) of the external nutrient loading to the system. We conclude
28 that the internal N-loading resulting from degradation of accumulated ON is low in shallow
29 eutrophic estuaries, whereas microbial mineralization of accumulated OP is a potential source of P.
30 Overall it appears that in N-limited eutrophic systems, internal nutrient resulting from
31 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.

56 2005). This may be caused by substantial release of nutrients, which have accumulated to high
57 concentrations over time in the sediments exposed to eutrophication (Pitkanen et al. 2001;
58 Carstensen et al. 2006). Such delayed nutrient release is thought to counteract reductions in the
59 external nutrient load and cause delayed recovery.

60 Determining the magnitude and temporal dynamics of the internal nutrient loading
61 originating from ON and OP accumulated in sediments requires detailed biogeochemical studies.
62 Organic matter degradation in sediments follow exponential decay kinetics (Westrich & Berner,
63 1984; Burdige 1991; Valdemarsen et al. 2014) and inorganic nutrient production from ON and OP
64 is therefore expected to decrease exponentially with time. Not all produced inorganic nutrients will
65 result in internal nutrient loading, however, since chemical and biological processes within
66 sediments lead to nutrient retention or transformation before efflux to the overlying water. NH_4^+ , for
67 instance, can be adsorbed to the sediment matrix (Mackin and Aller 1984), assimilated by microbes
68 or benthic microalgae or microbially transformed to other nitrogenous compounds (Christensen et
69 al. 2000; Tyler & McGlathery 2003; Hulth et al. 2005). Coupled nitrification-denitrification in the
70 oxic-anoxic transition of surface sediments, whereby NH_4^+ is converted to inert N_2 -gas, is for
71 instance an ecologically important process which reduces the amount of bioavailable N (Seitzinger
72 1988; Burgin & Hamilton 2007). Due to adsorption and denitrification, the efflux of dissolved
73 inorganic nitrogen ($\text{DIN} = \text{NH}_4^+ + \text{NO}_3^- + \text{NO}_2^-$) is generally much lower than anticipated from total
74 ON mineralization in the sediment (Mackin and Swider 1989). As for NH_4^+ , PO_4^{3-} may adsorb to
75 the sediment matrix; mainly to Fe-minerals in oxidized surface sediment (Sundby et al. 1992). PO_4^{3-}
76 efflux is therefore generally low in marine sediments lined with an oxic surface layer (Sundby et al.
77 1992; Jensen et al. 1995; Viktorsson et al. 2013).

78 In this study an experimental approach was used to determine the internal nutrient
79 loading resulting from long-term mineralization of accumulated ON and OP in various sediment

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

91

92 **2. Materials and methods**

93 *2.1 Study area*

94 Odense Fjord is a shallow eutrophic estuary located on the island of Fyn, Denmark. It is divided
95 into a 16 km² shallow inner basin and a 45 km² deeper outer basin, with average depths of 0.8 and
96 2.7 m, respectively (Fig. 1). The fjord is connected to Kattegat through a narrow opening in the
97 northeast. The main external nutrient source to Odense Fjord is Odense River, which has a
98 catchment area of 1095 km², consisting mainly of farmland and urban areas (Petersen et al. 2009).
99 Odense Fjord was critically eutrophic in the past due to high external nutrient loading exceeding
100 $3000 \cdot 10^3$ kg N y⁻¹ and $300 \cdot 10^3$ kg P y⁻¹ before 1990 (Petersen et al. 2009). The massive nutrient
101 loading caused extensive problems with high pelagic primary production, low water transparency,
102 hypoxic events and blooms of opportunistic macroalgae. Implementation of several water action
103 plans has reduced the external nutrient loading considerably to current levels of about $2000 \cdot 10^3$ kg

104 N y⁻¹ and 60*10³ kg P y⁻¹. This has improved the ecological quality of the system, since hypoxia is
105 now rare and levels of opportunistic macroalgae have decreased. Nonetheless, excessive nutrient
106 levels and high primary production are still a problem in Odense Fjord, which may be due to high
107 and sustaining internal nutrient loading.

108

109 *2.2 Sampling of sediment and water*

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

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

126 *2.3 Experimental setup*

127 Sediment cores were pre-treated before the experiment to assure that they had equal sediment height
128 and were free of macrofauna. The sediment cores were adjusted to 20 cm depth by removing the
129 bottom stopper and carefully removing excess sediment from below. After reinserting the bottom
130 stopper, the overlying water was purged with N₂ for 30 min to induce anoxia and the top stopper
131 was reinserted. Asphyxiated macrofauna was removed from the sediment surface after ~48 h in
132 darkness.

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

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

149

150 *2.4 Flux measurements*

151 The net exchange of nutrients (DIN and PO_4^{3-}) between sediment and water was determined in flux
152 experiments with 3 random sediment cores from each station. Flux experiments were conducted
153 weekly during the first 30 days, monthly until day 180 and every 2-3 months to the end. One day
154 prior to flux measurements, the inside headspace wall of the cores designated for flux
155 measurements were cleaned with a Q-tip to avoid biased flux measurements resulting from bacterial
156 biofilms on the inner surface of core liners (Valdemarsen and Kristensen 2005). These cores were
157 removed from the incubation tanks the next day, equipped with 4 cm long magnetic stirring bars a
158 few cm above the sediment surface and placed around a central magnet rotating at 60 rpm. Initial
159 water samples were taken from all cores, before they were closed with rubber stoppers. The cores
160 were incubated in darkness for 4 hours initially and up to 24 hours at the end of the experiment,
161 before the rubber stoppers were removed and final water samples were taken. Nutrient samples
162 were stored frozen (-20°C) until analyzed for NH_4^+ , NO_x^- ($\text{NO}_3^- + \text{NO}_2^-$) and PO_4^{3-} on a Lachat
163 Quickchem 8500 Flow injection Analyzer.

164

165 *2.5 Core sectioning*

166 Three sediment cores from each station were sectioned into 2 cm intervals to 16 cm depth at various
167 times (after 1 day and 1, 7-8, 16-17 and 20-21 months). Core sectioning and subsequent sediment
168 and porewater handling was done inside a N_2 -filled glovebag. Individual sediment slices were
169 homogenized and porewater for nutrient analysis was obtained after centrifugation of sediment
170 subsamples in double centrifuge tubes (10 min, ~ 500 g) and GF/C-filtration. Samples for NH_4^+ and
171 PO_4^{3-} were stored frozen (-20°C) until analysis as described above.

172 Sediment characteristics were determined on subsamples from every depth interval
173 during the core sectioning on day 1. Grain size composition, loss on ignition (LOI), total organic C
174 (TOC) content, density and porosity was determined as described in Valdemarsen et al. (2014).

175 Total N (TN) was measured by elemental analysis on dried sediment subsamples on a Carlo Erba
176 CHN EA1108 Elemental Analyzer. Total P (TP) was extracted by boiling combusted sediment
177 subsamples for 1 h in 1 M HCl. After centrifugation (10 min, 500 g) the supernatants were stored
178 until analyzed for PO_4^{3-} by colorimetric analysis (Koroleff 1983).

179 During initial and final core sectionings, reactive Fe was extracted from ~0.2 g
180 sediment subsamples with 0.5 M HCl. After 30 min extraction on a shaking table and centrifugation
181 (10 min, 500 g) the supernatants were stored in 4 mL plastic vials at room temperature until
182 analysis. Supernatants were analysed for reduced Fe (FeII) and total Fe by the ferrozine method
183 before and after reduction with hydroxylamine (Stookey 1970; Lovley and Phillips 1987). Oxidized
184 iron (FeIII) was determined as the difference between Total Fe and FeII.

185 Linear dimensionless NH_4^+ adsorption coefficients were determined during the initial
186 core sectioning on wet sediment subsamples from 0-2, 4-6 and 8-10 cm depth intervals in NH_4^+ -
187 adsorption experiments as described in Holmboe and Kristensen (2002). Sediment subsamples were
188 incubated for 2 d in slurries with different NH_4^+ -concentrations (0, 1, 2 and 3 mM) and 10 mg/L
189 allylthiourea to inhibit nitrification. After centrifugation (10 min, 500 g) the supernatant was
190 decanted and adsorbed NH_4^+ was extracted from the sediment pellet in 2 M KCl (Mackin and Aller,
191 1984). Supernatants from slurries and KCl-extractions was stored frozen (-20°C) and analyzed for
192 NH_4^+ by the salicylate-hypochlorite method (Bower and Holm-Hansen 1980).

193

194 *2.6 Jar experiments*

195 Closed anoxic sediment incubations ('jar experiments') were performed with sediment from
196 different depths (0-2, 4-6 and 8-10 cm) right after core sectionings. Jar experiments measure the
197 total anaerobic mineralization rates of ON and OP from temporal accumulation of metabolic end-
198 products (NH_4^+ and PO_4^{3-}) in the porewater and yields solid results under a wide range of

199 environmental and experimental conditions (Kristensen and Hansen 1995; Kristensen et al. 2011;
200 Valdemarsen et al. 2012; Quintana et al. 2013). Sediment from different depths was homogenized
201 and fully packed into 6-8 glass scintillation vials ('jars'), leaving no headspace. The jars were
202 closed with screw caps and buried in anoxic sediment at 15°C. Two jars were sacrificed at 3-5 day
203 intervals for porewater extraction by centrifugation. The jars were fitted with a perforated lid
204 containing a GF/C-filter inside before centrifugation and were then centrifuged head-down in a
205 centrifuge tube (10 min, ~500 g). Extracted porewater was stored frozen (-20°C) and analyzed for
206 NH_4^+ and PO_4^{3-} by colorimetric analysis as described above.

207

208 *2.7 Calculations and statistics*

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

215 NH_4^+ adsorption coefficients (K_{NH}) in individual sediment layers were determined
216 based on NH_4^+ -adsorption experiments. Extracted NH_4^+ ($\mu\text{mol g dw sediment}$) was plotted against
217 NH_4^+ -concentration ($\mu\text{mol cm}^{-3}$) and the linear slope, K' , was determined by least squares
218 regression. K_{NH} could hereafter be determined from the relationship $K_{\text{NH}} = ((1-\phi)/\phi) * \rho_{\text{ds}} * K'$, where
219 ϕ is sediment porosity and ρ_{ds} is dry sediment density (Holmboe and Kristensen 2002).

220 Rates of microbial ON and OP mineralization in discrete depth intervals (0-2, 4-6 and
221 8-10 cm) were obtained from jar experiments by fitting the time dependent linear concentration
222 change of NH_4^+ and PO_4^{3-} by least-squares regression (Aller and Yingst 1980). When slopes were

223 significant ($p < 0.05$) the volume specific reaction rates ($\text{nmol cm}^{-3} \text{ d}^{-1}$) in individual depth layers
224 were calculated from the slopes and corrected for sediment porosity and adsorption (Kristensen and
225 Hansen 1995). The mineralization rates at 10-20 cm depth were calculated from exponential
226 regressions based on ON and OP mineralization rates in the top 10 cm. Total area specific ON and
227 OP mineralization were calculated by depth integration (0-20 cm) of measured NH_4^+ and PO_4^{3-}
228 production at different depths. The temporal patterns of total area specific ON and OP
229 mineralization were fitted to a double exponential decay regression model of the form $y = C_L \cdot \exp(-$
230 $k_L \cdot t) + C_R \cdot \exp(-k_R \cdot t)$, where t is time, C_L and C_R are constants and k_L and k_R denote the first order
231 decay constants for labile and refractory ON and OP, respectively. We hereby assume that
232 considerations based on organic C degradation kinetics (Westrich and Berner 1984) are also valid
233 for ON and OP mineralization. Half lives of labile and refractory ON and OP could hereafter be
234 calculated from the formula $T_{0.5} = \ln(2)/k'$, where k' denote k_L and k_R .

235

236 **3. Results**

237 *3.1 Sediment characteristics*

238 Detailed sediment characteristics of the 8 stations in Odense Fjord were previously described in
239 Valdemarsen et al. (2014) and only a brief summary is given here. The sediments from all stations
240 had high sand content and variable silt-clay content with wet densities ranging from $1.2\text{-}1.8 \text{ g cm}^{-3}$
241 and porosities of 0.3-0.8. The medium grain size varied from 87 to 397 μm among stations. The
242 sediments from the innermost stations (St 1-3) and most of the stations in the outer basin (St 4 and
243 6-8) contained a high proportion of silt-clay particles (13-63%). Furthermore, the stations rich in
244 silt-clay particles were organic rich with 0.6-5.2% POC compared to the more sandy St 5 (0.1-0.2%
245 POC).

246 NH_4^+ -adsorption coefficients varied erratically among stations and sediment depths
247 (Table 1). K_{NH} ranged from 0.14 in the 8-10 cm deep sediment on St 7 to 1.06 in the surface
248 sediment on St 2.

249 St 1 and St 3 from the inner basin had similar TN content ranging between 57-156
250 $\mu\text{mol cm}^{-3}$ (Fig. 2). St 2 had slightly higher TN (103-227 $\mu\text{mol cm}^{-3}$) with a pronounced subsurface
251 peak occurring at 3 cm depth. In the outer basin the shallow and deep silty stations (St 4 and 6-8)
252 had similar TN-content (92-154 $\mu\text{mol cm}^{-3}$), except at the surface where TN was lower at St 4 (38-
253 60 $\mu\text{mol cm}^{-3}$). The sandy St 5 contained exceptionally low TN (8-16 $\mu\text{mol cm}^{-3}$). Depth integrated
254 TN was therefore lowest on St 5 ($4.5 \pm 0.1 \text{ mol N m}^{-2}$), intermediate at St 1 ($13.5 \pm 0.4 \text{ mol N m}^{-2}$)
255 and similarly high on the remaining stations (16.0 to 21.4 mol N m^{-2} , Table 2).

256 Two of the stations in the inner basin (St 1 and 2) had similar TP profiles, with 10-11
257 $\mu\text{mol cm}^{-3}$ at the sediment surface and a gradual decrease to 5.1-5.8 $\mu\text{mol cm}^{-3}$ at 15 cm depth (Fig.
258 2). St 3 had the lowest TP content of the stations in the inner basin. The shallow silty sediments in
259 the outer basin (St 4) were similar to St 1-2 with respect to TP, whereas the shallow sandy sediment
260 (St 5) was similar to St 3. The deep silty sediments in the outer basin (St 6-8) were characterized by
261 constant TP with depth (9.6-13.5 $\mu\text{mol cm}^{-3}$). Depth integration showed that the highest area
262 specific TP content was found on the deep outer fjord stations (1.8-1.9 mol P m^{-2}), whereas shallow
263 silty sediments in the inner and outer fjord contained intermediate TP content (1.2-1.3 mol P m^{-2} ; St
264 1, 2 and 4; Table 2). The lowest TP content ($\sim 0.7 \text{ mol P m}^{-2}$) was found on the silty St 3 and sandy
265 St 5 in inner and outer fjord, respectively.

266 Initial FeIII pools varied 30-fold between stations (6-243 mmol m^{-2} ; Table 3), with the
267 lowest FeIII content found in shallow sandy sediment from the outer basin (St 5). FeIII only
268 constituted a minor fraction (2-10%) of total Fe on all stations. No statistically significant

269 differences were detected between initial and final FeIII-pools ($p > 0.17$), but there were trends
270 towards higher final FeIII content, except on St 1 and 5.

271

272 3.2 ON and OP mineralization

273 Mineralization rates obtained in the fully anoxic jar experiments might have underestimated
274 mineralization rates at the sediment surface, where O_2 can stimulate mineralization of O_2 -sensitive
275 organic matter (Hulthe et al. 1998). In coastal and estuarine sediments O_2 only penetrates to 1-3 mm
276 depth, suggesting a minor importance of this artefact at the beginning of the experiment.
277 Surprisingly the sediments did not become significantly more oxidized during the long term
278 incubations as indicated by a modest build-up of oxidized FeIII and continuous presence of
279 hydrogen sulfide in the porewater of surface sediment from all stations (data not shown). Hence we
280 assume that mineralization rates in the sediment cores underlying an oxic water phase were closely
281 approximated by the rates obtained in jar experiments.

282 NH_4^+ production in jar experiments was significant throughout the experiment, except
283 for St 1, 8-10 cm depth after 607 d. Initially NH_4^+ production was highest in the surface 0-2 cm
284 sediment from the silty St 1-2 in the inner fjord and the sandy St 5 in the outer fjord (159-338 nmol
285 $cm^{-3} d^{-1}$) and was similar on remaining stations (63-101 nmol $cm^{-3} d^{-1}$; Fig. 3). Surface NH_4^+
286 production decreased rapidly over time in sediments from shallow locations in the inner and outer
287 fjord, by 96% of initial rates on St 1 and by 61–82% on St 2–5. The surface NH_4^+ production in the
288 sediments sampled in the deep outer basin (St 6–8) decreased by 8–67% during the experiment.
289 NH_4^+ production at 4–6 cm depth was initially 18–60 nmol $cm^{-3} d^{-1}$ on all stations and temporal
290 changes were also observed in this layer, especially in shallow silty sediments from the inner basin
291 where NH_4^+ production decreased by 75–96% to 1.4–12 nmol $cm^{-3} d^{-1}$ by the end (Fig. 3). In
292 sediments from the outer basin NH_4^+ production at 4-6 cm depth only decreased by 19-58%. At 8-

293 10 cm depth NH_4^+ production at all stations occurred at similar rates and showed similar temporal
294 trends as observed at 4-6 cm depth (Fig. 3).

295 Significant PO_4^{3-} production was measured in the surface sediment from all stations
296 throughout the experiment (Fig. 4). Initial rates were highest ($30\text{-}35 \text{ nmol cm}^{-3} \text{ d}^{-1}$) on
297 St 1 and 2 from the shallow inner basin and considerably lower ($7\text{-}18 \text{ nmol cm}^{-3} \text{ d}^{-1}$) on the
298 remaining stations. PO_4^{3-} production initially decreased rapidly in the surface sediment from St 1
299 and 2 and stabilized at relatively low and stable levels after $\sim 200 \text{ d}$ ($0.7\text{-}6.0 \text{ nmol cm}^{-3} \text{ d}^{-1}$). Surface
300 PO_4^{3-} production also decreased over time on the other stations, but temporal trends were more
301 erratic. PO_4^{3-} production in deeper sediment was generally lower than at the surface, and with less
302 variability among stations (Fig. 4). PO_4^{3-} production at 4-6 cm depth was $0\text{-}6 \text{ nmol cm}^{-3} \text{ d}^{-1}$ and
303 remained quite stable throughout the experiment on all stations. The only significant decrease ($p =$
304 $0.01\text{-}0.03$) occurred in silty sediments from the inner basin (St 1-3) and St 6 and 8 from the deep
305 outer basin. PO_4^{3-} production varied between $0\text{-}5 \text{ nmol cm}^{-3} \text{ d}^{-1}$ at 8-10 cm depth and was stable
306 throughout the experiment.

307 Area-specific ON mineralization was calculated by depth integration of NH_4^+
308 production rates (Fig. 3). The sediments from the inner basin (St 1-3) showed high initial ON
309 mineralization ($6\text{-}11 \text{ mmol m}^{-2} \text{ d}^{-1}$) in the same range as the shallow silty and sandy sediments from
310 the outer basin (6 and $10 \text{ mmol m}^{-2} \text{ d}^{-1}$ on St 4 and 5, respectively). The deep silty sediments from
311 the outer basin showed the lowest initial ON mineralization (St 6-8; $3\text{-}5 \text{ mmol m}^{-2} \text{ d}^{-1}$). Area
312 specific ON mineralization decreased during the experiment on all stations, by 82-93% for the silty
313 inner fjord and 34-71% on remaining stations. The temporal decrease was mainly driven by
314 successively lower ON mineralization in surface sediment during the first $\sim 200 \text{ d}$ and area-specific
315 ON mineralization was fairly constant hereafter. Initial area-specific OP mineralization was $0.2\text{-}1.0$
316 $\text{mmol m}^{-2} \text{ d}^{-1}$ (Fig. 4) and decreased (59-70%) over time on several of the stations (St 1-3 and St 6).

317 As for ON mineralization, the successively lower OP mineralization was mainly due to decreased
318 OP mineralization in surface sediment. On the other stations area-specific OP mineralization
319 remained relatively high and did not show clear temporal trends.

320 Double exponential decay models fitted the ON mineralization kinetics on St 1-6 and
321 the OP mineralization kinetics on St 1-3 and 6. Erratic mineralization patterns prevented the use of
322 exponential decay models on remaining stations (see Fig. 3-4). Decay constants for labile and
323 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
324 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
325 lives for ON and OP were in the range of 0.01-0.11 and 1.3-6.3 years for labile and refractory
326 fractions, respectively.

327

328 *3.3 DIN- and DIP-fluxes*

329 DIN fluxes followed a similar exponentially decreasing pattern for all stations (Fig. 5), and ranged
330 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
331 of DIN released initially was NH_4^+ , which contributed 59-100% of DIN-release. Subsequently the
332 NH_4^+ efflux decreased while NO_x^- switched from uptake to release and after 0.5-1 y to the end of
333 the experiment, 68-100% of the DIN was released as NO_x^- .

334 The 8 stations showed different patterns of PO_4^{3-} fluxes. The stations from the shallow
335 inner basin, St 1-3, showed exponentially decreasing PO_4^{3-} fluxes over time (initial fluxes of 0.1-0.2
336 $mmol\ m^{-2}\ d^{-1}$ decreasing to 0.01-0.05 $mmol\ m^{-2}\ d^{-1}$ by the end; Fig. 5). Initial (day 0-90) PO_4^{3-}
337 fluxes on the shallow silty St 4 was around zero, but increased to 0.07-0.14 $mmol\ m^{-2}\ d^{-1}$ during d
338 90-360 of the experiment. The highest PO_4^{3-} fluxes (0.07-0.21 $mmol\ m^{-2}\ d^{-1}$) were observed on the
339 TP-poor sandy St 5, particularly towards the end of the experiment, while the TP-rich outer fjord

340 stations 6-8 had the lowest and most irregular PO_4^{3-} fluxes ranging from slightly negative to 0.1
341 $\text{mmol m}^{-2} \text{d}^{-1}$.

342

343 *3.4 PO_4^{3-} and NH_4^+ in porewater*

344 Porewater nutrient concentrations increased gradually at all depths during the experiment (data not
345 shown). NH_4^+ and PO_4^{3-} only increased moderately in the upper 2 cm, but accumulated to high
346 levels in the deeper diffusion limited sediment. Depth-averaged initial porewater NH_4^+
347 concentration varied between 171-407 μM on the stations. The sandy St 5 showed the highest NH_4^+
348 accumulation over time with a depth-average of 1473 μM in porewater by the end. On the
349 remaining stations, NH_4^+ only accumulated to 259-587 μM . Depth-averaged PO_4^{3-} concentrations at
350 the beginning varied between 17-71 μM depending on station. As for NH_4^+ , the nutrient-poor sandy
351 St 5 showed the highest PO_4^{3-} accumulation to 368 μM compared with 43-170 μM on the other
352 stations.

353

354 *3.5 N- and P-budgets*

355 Area-specific nutrient mineralization obtained in jar-experiments was used to calculate total ON and
356 OP mineralization during the experiment. ON mineralization was fairly constant for all stations
357 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
358 hand, had 3-fold higher ON mineralization that accounted for 80% of the initial ON. A 3-fold range
359 among stations was also evident for OP mineralization, but with lowest rates of 0.12-0.18 mol m^{-2} at
360 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
361 was no apparent relationship between sediment TN and TP content and mineralization activity as
362 some of the highest N- and P-mineralization rates were observed on the organic-poor St 5 (Table 4).
363 DIN-effluxes, porewater accumulation and adsorption only accounted for 18-32% of total ON

364 mineralization, indicating that most of the generated NH_4^+ was not accounted for by our
365 measurements. For P, the sum of PO_4^{3-} efflux and porewater accumulation only accounted for 10-
366 48% of total OP mineralization.

367

368 **4. Discussion**

369 *4.1 Sediment nutrient content*

370 TN and TP in sediments from Odense Fjord were in the same range or higher than reported for
371 other eutrophic systems (e.g. Boynton and Kemp 1985; Cowan & Boynton 1996; Lomstein et al.
372 1998; Coelho et al. 2004; Viktorsson et al. 2013) emphasizing the history of intense eutrophication
373 in Odense Fjord. TN and TP in the silty sediments of Odense Fjord (all stations except St 5) were
374 remarkably similar and only varied ~1.5 (TN) and ~3 (TP) times among stations. Despite these
375 overall similarities, the silty sediments from the shallow inner basin showed higher initial ON- and
376 OP-mineralization and nutrient effluxes than silty sediments from the outer fjord. This could be due
377 to higher availability of labile ON and OP in the sediments from the inner basin, reflecting the
378 nutrient rich conditions in the inner compared to the outer basin (Petersen et al.2009).

379 The sandy St 5 was markedly different from the other stations. It had the lowest total
380 nutrient content and yet exhibited some of the highest rates of ON and OP mineralization. The
381 frequent erosion by wind driven waves in this area (Valdemarsen et al. 2010) and deep (>20 cm)
382 reworking by lugworms (*Arenicola marina*) (Riisgaard & Banta 1998; Valdemarsen et al. 2011)
383 may remove fine particles and refractory organic matter from St 5 sediments (Wendelboe et al.
384 2012) and prevent organic matter accumulation, hence explaining the low organic content on this
385 station. On the other hand, intense growth and burial of microphytobenthos and other reactive
386 detritus by the strong physical disturbance and vertical mixing, can explain the unexpected high TN
387 and TP reactivity of St 5 sediment.

388 A rough areal estimate based on the measured TN and TP content on the examined
389 stations (Table 2) suggest that $12.6 \cdot 10^6$ kg N and $3.7 \cdot 10^6$ kg P are stored in the upper 20 cm of
390 Odense Fjord sediments, corresponding to ~6 (N) and ~62 (P) years of the current annual external
391 nutrient loading to the system.

392

393 *4.2 Organic N and P mineralization*

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

409 Total jar-based microbial ON and OP mineralization over the ~2 years experimental
410 period (Table 5) only accounted for a minor fraction of initial TN and TP in sediments from Odense
411 Fjord suggesting that the standing stock of organic N and P will be a source of nutrients for

412 extended time. Decay constants from the exponential decay model suggested that labile ON and OP
413 was rapidly degraded on all stations within 10-240 d, whereas depletion of more refractory ON and
414 OP will only occur on decadal time-scales (8-40 years), indicating that depletion of buried and
415 degradable ON and OP in eutrophic ecosystems will take considerable time.

416

417 *4.3 Fate of inorganic nutrients*

418 NH_4^+ and PO_4^{3-} produced by microbial mineralization accumulated in porewater of all sediments
419 within the first 1-6 months and only changed slightly hereafter. However, over the whole
420 experiment, porewater accumulation explained only a minor fraction of the jar-based total ON and
421 OP mineralization (0.8-8.1%). It was also investigated if NH_4^+ adsorption to mineral surfaces was
422 an important N sink. Despite the large spatial heterogeneity of NH_4^+ adsorption, this process never
423 accounted for more than 1% of the total produced NH_4^+ over the whole experiment and was
424 therefore not quantitatively important.

425 Nutrient release to the overlying water was the most important route for inorganic
426 nutrients produced by microbial mineralization. We could not account for all the produced
427 nutrients, since nutrient mineralization in jar experiments exceeded DIN and PO_4^{3-} effluxes by 70-
428 84% and 62-93%, respectively. The missing NH_4^+ may have been lost through coupled nitrification-
429 denitrification (e.g. Mackin and Swider 1989; Quintana et al. 2013). The conspicuous shift from
430 NH_4^+ to NO_3^- release indicated that nitrification was an active process in all sediment types, and
431 denitrifying bacteria probably proliferated in the NO_3^- -rich surface sediment. In the present case,
432 coupled nitrification-denitrification rates of 1-2 $\text{mmol m}^{-2} \text{d}^{-1}$ are required to account for the missing
433 NH_4^+ , which is within the range reported in previous studies (e.g. Nielsen et al. 1995; Christensen et
434 al. 2000; Tobias et al. 2003). On the other hand, the missing PO_4^{3-} must have been retained within
435 the sediments. Several studies suggest almost complete PO_4^{3-} retention in marine sediments with an

436 oxic sediment surface (Rozaan et al. 2002; Viktorsson et al. 2013) where PO_4^{3-} adsorbs to oxidized
437 FeIII-minerals preventing PO_4^{3-} efflux (Sundby et al. 1992). Experimental studies suggest that every
438 FeIII molecule can retain more than 0.5 PO_4^{3-} molecules (Gunnars & Blomqvist, 1997; Rozaan et al.
439 2002). Hence the FeIII levels on all the silty stations were sufficient to retain the missing PO_4^{3-} ,
440 especially when considering that 0.5 M HCl extractions only extract a fraction of the available
441 FeIII. On the sandy St 5 the FeIII levels were too low to account for the missing PO_4^{3-} , indicating
442 that there were other PO_4^{3-} sinks. PO_4^{3-} adsorption in the anoxic sediment (Krom & Berner, 1980)
443 or precipitation of PO_4^{3-} - CaCO_3 complexes (Coelho et al. 2004) are possible sinks that were not
444 quantified in this experiment.

445

446 *4.4 Internal nutrient loading*

447 We calculated the potential internal nutrient loading in Odense Fjord resulting from microbial
448 mineralization of ON and OP for a 2 y period based on the measured nutrient effluxes. Average
449 nutrient fluxes were calculated for each sediment type, i.e. shallow inner fjord (St 1-3), shallow silty
450 outer fjord (St 4), sandy outer fjord (St 5) and deep outer fjord (St 6-8). The monthly time-weighted
451 DIN and PO_4^{3-} fluxes and the total areal distribution of the different sediment types in Odense Fjord
452 were then used to calculate the total internal nutrient loading (10^3 kg N and P mo^{-1}) for each
453 sediment type and for the whole ecosystem. Evidently these calculations do not represent the *in situ*
454 internal nutrient loading, since effects of the otherwise continuous deposition of organic matter
455 were omitted by the experimental setup. It can also be debated if all the released nutrients can be
456 considered internal nutrient loading, since the mineralization of recently deposited organic matter in
457 surface sediments drove the majority of nutrient release during the first ~200 d. This nutrient release
458 is largely determined by the ecosystem primary productivity extending only a few years back, and is
459 therefore closely coupled to the recent levels of external nutrient loading. In any case the

460 calculations represent the nutrient release resulting from the mineralization of slowly reacting ON
461 and OP, which have accumulated in the sediments.

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

474 The internal P-loading showed different temporal dynamics than internal N-loading.
475 Total P-release from the whole fjord bottom was stable over time at rates of $21\text{-}22 \cdot 10^3 \text{ kg P y}^{-1}$
476 ($\sim 3.4\text{-}3.6 \text{ kg P ha}^{-1} \text{ y}^{-1}$; Fig. 6) while internal N-loading decreased exponentially. The stability was
477 driven by the increasing P release in shallow sandy outer fjord sediment and constant P release in
478 deep outer fjord sediment. As for N, the shallow sandy sediments in the outer fjord was most
479 important for total internal P-loading (57%) and the remaining 3 sediment types contributed equally
480 (14-15%). The internal P-loading corresponded to 35-36% (P) of the current external P-loading to
481 Odense Fjord ($60 \cdot 10^3 \text{ kg P y}^{-1}$; Petersen et al. 2009) and thus potentially constitutes a stable and
482 significant P-source in the system. However, since Odense Fjord and most other temperate coastal

483 ecosystems are mostly N-limited (Howarth et al. 2011) it is uncertain to which degree this excess P
484 will affect ecosystem productivity.

485

486 *4.5 Ecological implications*

487 In many shallow eutrophic estuaries the external nutrient loading has been reduced to induce
488 oligotrophication, but lower nutrient concentrations in the recipient estuary often occur after
489 considerable delay and rarely correspond proportionally to the reductions (Kronvang et al. 2005;
490 Carstensen 2006). This indicates that a transient phase occurs, where accumulated nutrients are
491 being released from the soils and sediments in the water shed and receiving estuary, respectively,
492 while the system equilibrates to a new level of external nutrient loading. Our study shows the
493 magnitude and temporal dynamics of the internal nutrient loading that can be expected in shallow
494 estuaries recovering from eutrophication. It appears that internal N-loading will be insignificant
495 during recovery since it only corresponded to 2-6% of the external N-loading in our example and
496 decreased rapidly. Internal N-loading will therefore only lead to marginally elevated N-availability
497 and have minor effects on primary productivity and eutrophication status. The results are different
498 with respect to PO_4^{3-} , since the internal P-loading was stable and corresponded to $>1/3$ of the
499 external P-loading. Internal P-loading may therefore be a significant source of dissolved PO_4^{3-} for
500 extended time in shallow eutrophic estuaries, and at a sufficiently high level to counteract
501 reductions in the external P-loading. Most shallow estuaries are N-limited (Conley et al. 2000;
502 Howarth & Marinho 2006; Howarth et al. 2011) so a high internal P-loading might only exacerbate
503 N-limitation while having no further consequences for ecological quality. Decreasing internal N-
504 loading and stable internal P-loading could also lead to increased dominance of cyanobacteria,
505 which have low requirements for dissolved N. However, major shifts in phytoplankton communities
506 would only occur in systems where decreased internal nutrient loading results in markedly lower

507 DIN-concentrations in the water phase, i.e. in systems where N-loading is low and internal nutrient
508 sources dominate.

509 The estimates of internal nutrient loading presented here provide an illustrative
510 example, but the exact values are only valid for the experimental conditions and must be
511 extrapolated with caution. Microbial reaction rates and DIN and PO_4^{3-} release from sediments are
512 strongly influenced by ambient conditions. For instance, sediment macrofauna may stimulate the
513 rates of organic matter degradation and sediment nutrient release through bioturbation (e.g.
514 Kristensen et al. 2012; 2014) leading to higher internal nutrient loading than estimated from
515 defaunated sediment cores in this experiment. Similarly microbial mineralization processes and
516 hence sediment DIN and PO_4^{3-} release are strongly temperature dependent (Westrich and Berner
517 1988; Sanz-Lazaro et al. 2011) and the magnitude of internal nutrient loading will therefore vary
518 seasonally compared to our estimates based on a constant temperature experiment. Finally, in our
519 experimental setup we also omitted hydrodynamics and porewater advection which are known to
520 stimulate nutrient cycling in shallow permeable sediments (Cook et al. 2007; Huettel et al. 2014).
521 This will especially affect the estimated nutrient release from the sandy sediments from this study.
522 Given the multitude of factors influencing nutrient mineralization rates, the actual magnitude of
523 internal nutrient loading and related consequences for primary productivity will therefore follow a
524 seasonal pattern driven by e.g. temperature, hydrodynamics and composition and activity of benthic
525 fauna. Other environmental variables such as hypoxia in the water column may also influence the
526 magnitude of internal nutrient loading, since it hampers PO_4^{3-} retention by Fe-oxides (Azzoni et al.
527 2005; Mort et al. 2010; Viktorsson et al. 2013) and limits coupled nitrification-denitrification while
528 stimulating dissimilatory nitrate reduction to NH_4^+ (Christensen et al. 2000; Jäntti & Hietanen
529 2012). Ecosystems suffering from hypoxia may therefore experience a much higher internal nutrient
530 loading than measured in this experiment. A comparison between total ON and OP mineralization

531 and effluxes from this experiment, suggests that nutrient effluxes could potentially increase 3-6
532 (DIN) and 2-10 (PO_4^{3-}) times if there are no mechanisms to transform or retain inorganic nutrients
533 at the sediment surface.

534

535 *4.6 Conclusions*

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

548

549 **5. Acknowledgements**

550 The authors thank the people who have helped with sampling, experimental work or analysis during
551 this experiment (the crew aboard Liv II, Birthe Christensen, Rikke Orloff Holm, Maria Del Mar
552 Sánchez Huertas and Maria Jensen). This project was funded by the Danish Strategic Science
553 Foundation through grant 09-063190/DSF. C.O. Quintana was funded by FAPESP (Sao Paulo
554 Research Support Foundation) grant # 2012/06121-1.

555

556 **6. References**

- 557 Aller RC, Yingst JY (1980) Relationships between microbial distributions and the anaerobic
558 decomposition of organic-matter in surface sediments of Long-Island Sound, USA. *Marine*
559 *Biology* 56:29-42
- 560 Azzoni R, Gianmarco G, Viaroli P (2005) Iron-sulphur-phosphorous interactions: implications for
561 sediment buffering capacity in a mediterranean eutrophic lagoon. *Hydrobiologia* 550:131-148
- 562 Bower CE, Holm-Hansen T (1980) A salicylate-hypochlorite method for determining ammonia in
563 seawater. *Canadian Journal of Fisheries and Aquatic Sciences* 37:794-798
- 564 Boynton WR, Kemp WM (1985) Nutrient regeneration and oxygen-consumption by sediments
565 along an estuarine gradient. *Marine Ecology Progress Series* 23:45-55
- 566 Bukaveckas PA, Isenberg WN (2013) Loading, Transformation, and Retention of Nitrogen and
567 Phosphorus in the Tidal Freshwater James River (Virginia). *Estuaries and Coasts* 36:1219-
568 1236
- 569 Burdige DJ (1991) The kinetics of organic-matter mineralization in anoxic marine sediments
570 *Journal of Marine Research* 49:727-761
- 571 Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic
572 ecosystems? A review of nitrate removal pathways. *Frontiers in Ecology and Environment*
573 5:89-96
- 574 Carstensen J, Conley DJ, Andersen JH, Aertebjerg G (2006) Coastal eutrophication and trend
575 reversal: A Danish case study. *Limnology and Oceanography* 51:398-408
- 576 Christensen PB, Rysgaard S, Sloth NP, Dalsgaard T, Schwærter S (2000) Sediment mineralization,
577 nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an
578 estuarine fjord with sea cage trout farms. *Aquatic Microbial Ecology* 21:73-84

579 Coelho JP, Flindt MR, Jensen HS, Lillebo AI, Pardal MA (2004) Phosphorus speciation and
580 availability in intertidal sediments of a temperate estuary: relation to eutrophication and
581 annual P-fluxes. *Estuarine Coastal and Shelf Science* 61:583-590

582 Conley DJ, Markager S, Andersen J, Ellermann T, Svendsen LM (2002) Coastal eutrophication and
583 the Danish National Aquatic Monitoring and Assessment Program. *Estuaries* 25:848-861

584 Cook PLM, Wenzhofer F, Glud RN, Janssen F, Huettel M (2007) Benthic solute exchange and
585 carbon mineralization in two shallow subtidal sandy sediments: Effect of advective pore-
586 water exchange. *Limnology and Oceanography* 52:1943-1963

587 Cowan JLW, Boynton WR (1996) Sediment-water oxygen and nutrient exchanges along the
588 longitudinal axis of Chesapeake Bay: Seasonal patterns, controlling factors and ecological
589 significance. *Estuaries* 19:562-580

590 Fulweiler RW, Nixon SW, Buckley BA (2010) Spatial and Temporal Variability of Benthic Oxygen
591 Demand and Nutrient Regeneration in an Anthropogenically Impacted New England Estuary.
592 *Estuaries and Coasts* 33:1377-1390

593 Fyns Amt (2006) Miljøfarlige stoffer og ålegræs i Odense Fjord. Fyns Amt, Natur- og
594 Vandmiljøafdelingen, Odense

595 Gunnars A, Blomqvist S (1997) Phosphate exchange across the sediment-water interface when
596 shifting from anoxic to oxic conditions - an experimental comparison of freshwater and
597 brackish-marine systems. *Biogeochemistry* 37:203-226

598 Holmboe N, Kristensen E (2002) Ammonium adsorption in sediments of a tropical mangrove forest
599 (Thailand) and a temperate Wadden Sea area (Denmark). *Wetlands Ecology and Management*
600 10:453-460

601 Howarth RW, Marino R (2006) Nitrogen as the limiting nutrient for eutrophication in coastal
602 marine ecosystems: Evolving views over three decades. *Limnology and Oceanography*
603 51:364-376

604 Howarth R, Chan F, Conley DJ, Garnier J, Doney SC, Marino R, Billen G (2011) Coupled
605 biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine
606 ecosystems. *Frontiers in Ecology and the Environment* 9:18-26

607 Huettel M, Berg P, Kostka JE (2014) Benthic Exchange and Biogeochemical Cycling in Permeable
608 Sediments. *Annual Review of Marine Science* 6:23-51

609 Hulth S, Aller RC, Canfield DE, Dalsgaard T, Engström P, Gilbert F, Sundbäck K, Thamdrup B
610 (2005) Nitrogen removal in marine environments: recent findings and future research
611 challenges. *Marine Chemistry* 94:125-145

612 Hulthe G, Hulth S, Hall POJ (1998) Effect of oxygen on degradation rate of refractory and labile
613 organic matter in continental margin sediments. *Geochimica et Cosmochimica Acta* 62:1319-
614 1328

615 Jantti H, Hietanen S (2012) The Effects of Hypoxia on Sediment Nitrogen Cycling in the Baltic
616 Sea. *Ambio* 41:161-169

617 Jensen HS, Mortensen PB, Andersen FO, Rasmussen E, Jensen A (1995) Phosphorous cycling in a
618 coastal marine sediment, Aarhus Bay, Denmark. *Limnology and Oceanography* 40:908-917

619 Kelly JR, Nixon SW (1984) Experimental studies of the effect of organic deposition on the
620 metabolism of a coastal marine bottom community. *Marine Ecology Progress Series* 17:157-
621 169

622 Koroleff F (1983) Determination of phosphorus. In: Grashof K, Erhardt M, Kremling K (eds)
623 Method of seawater analysis. Verlag Chemie, Weinheim

624 Kristensen E, Hansen K (1995) Decay of plant detritus in organic-poor marine sediment: Production
625 rates and stoichiometry of dissolved C and N compounds. *Journal of Marine Research*
626 53:675-702

627 Kristensen E, Mangion P, Tang M, Flindt MR, Holmer M, Ulomi S (2011) Microbial carbon
628 oxidation rates and pathways in sediments of two Tanzanian mangrove forests.
629 *Biogeochemistry* 103:143-158

630 Kristensen E, Penha-Lopes G, Delefosse M, Valdemarsen T, Quintana C, Banta G (2012) What is
631 bioturbation? The need for a precise definition for fauna in aquatic sciences. *Marine Ecology*
632 *Progress Series* 446:285-302

633 Kristensen E, Delefosse M, Quintana CO, Flindt MR and Valdemarsen T (2014) Influence of
634 benthic macrofauna on ecosystem functioning in a shallow Danish estuary. *Frontiers in*
635 *Marine Science* 1, Article 41

636 Krom MD, Berner RA (1980) Adsorption of phosphate in anoxic marine sediments. *Limnology and*
637 *Oceanography* 25:797-806

638 Kronvang B, Jeppesen E, Conley DJ, Sondergaard M, Larsen SE, Ovesen NB, Carstensen J (2005)
639 Nutrient pressures and ecological responses to nutrient loading reductions in Danish streams,
640 lakes and coastal waters. *Journal of Hydrology* 304:274-288

641 Lomstein BA, Jensen AGU, Hansen JW, Andreasen JB, Hansen LS, Berntsen J, Kunzendorf H
642 (1998) Budgets of sediment nitrogen and carbon cycling in the shallow water of Knebel Vig,
643 Denmark. *Aquatic Microbial Ecology* 14:69-80

644 Lovley DR, Phillips EJP (1987) Rapid assay for microbially reducible ferric iron in aquatic
645 sediments. *Applied and Environmental Microbiology* 53:1536-1540

646 Mackin JE, Aller RC (1984) Ammonium adsorption in marine sediments. *Limnology and*
647 *Oceanography* 29:250-257

- 648 Mackin JE, Swider KT (1989) Organic matter decomposition pathways and oxygen consumption in
649 coastal marine sediments. *Journal of Marine Research* 47:681-716
- 650 Mort HP, Slomp CP, Gustafsson BG, Andersen TJ (2010) Phosphorus recycling and burial in Baltic
651 Sea sediments with contrasting redox conditions. *Geochimica et Cosmochimica Acta*
652 74:1350-1362
- 653 Mortazavi B, Riggs AA, Caffrey JM, Genet H, Phipps SW (2012) The Contribution of Benthic
654 Nutrient Regeneration to Primary Production in a Shallow Eutrophic Estuary, Weeks Bay,
655 Alabama. *Estuaries and Coasts* 35:862-877
- 656 Nielsen, K., P. L, Rasmussen, P. (1995) Estuarine nitrogen retention independently estimated by the
657 denitrification rate and mass balance methods: a study of Norsminde Fjord, Denmark. *Marine*
658 *Ecology Progress Series* 119:275-283
- 659 Petersen JD, Rask N, Madsen HB, Jorgensen OT, Petersen SE, Nielsen SVK, Pedersen CB, Jensen
660 MH (2009) Odense Pilot River Basin: implementation of the EU Water Framework Directive
661 in a shallow eutrophic estuary (Odense Fjord, Denmark) and its upstream catchment.
662 *Hydrobiologia* 629:71-89
- 663 Pitkanen H, Lehtoranta J, Raike A (2001) Internal nutrient fluxes counteract decreases in external
664 load: The case of the estuarial eastern Gulf of Finland, Baltic Sea. *Ambio* 30:195-201
- 665 Quintana C, Kristensen E, Valdemarsen T (2013) Impact of the invasive polychaete *Marenzelleria*
666 *viridis* on the biogeochemistry of sandy marine sediments. *Biogeochemistry* 115:95-109
- 667 Riisgard HU, Banta GT (1998) Irrigation and deposit feeding by the lugworm *Arenicola marina*,
668 characteristics and secondary effects on the environment. A review of current knowledge. *Vie*
669 *Milieu* 48:243-257
- 670 Rozan TF, Taillefert M, Trouwborst RE, Glazer BT, Ma SF, Herszage J, Valdes LM, Price KS,
671 Luther GW (2002) Iron-sulfur-phosphorus cycling in the sediments of a shallow coastal bay:

672 Implications for sediment nutrient release and benthic macroalgal blooms. *Limnology and*
673 *Oceanography* 47:1346-1354

674 Sanz-Lazaro C, Valdemarsen T, Marin A, Holmer M (2011) Effect of temperature on
675 biogeochemistry of marine organic-enriched systems: implications in a global warming
676 scenario. *Ecological Applications* 21:2664-2677

677 Seitzinger SP (1988) Denitrification in fresh-water *and* coastal marine ecosystems - ecological and
678 geochemical significance. *Limnology and Oceanography* 33:702-724

679 Stookey LL (1970) Ferrozine - a New Spectrophotometric Reagent for Iron. *Analytical Chemistry*
680 42:779-781

681 Sundby B, Gobeil C, Silverberg N, Mucci A (1992) The phosphorous cycle in coastal marine
682 sediments. *Limnology and Oceanography* 37:1129-1145

683 Tobias C, Giblin A, McClelland J, Tucker J, Peterson B (2003) Sediment DIN fluxes and
684 preferential recycling of benthic microalgal nitrogen in a shallow macrotidal estuary. *Marine*
685 *Ecology Progress Series* 257:25-36

686 Tyler AC, McGlathery KJ, Anderson IC (2003) Benthic algae control sediment-water column
687 fluxes of organic and inorganic nitrogen compounds in a temperate lagoon. *Limnology and*
688 *Oceanography* 48:2125-2137

689 Valdemarsen T, Kristensen E (2005) Diffusion scale dependent change in anaerobic carbon and
690 nitrogen mineralization: True effect or experimental artifact? *Journal of Marine Research*
691 63:645-669

692 Valdemarsen T, Kristensen E, Holmer M (2009) Metabolic threshold and sulfide-buffering in
693 diffusion controlled marine sediments impacted by continuous organic enrichment.
694 *Biogeochemistry* 95:335-353

- 695 Valdemarsen T, Canal-Verges P, Kristensen E, Holmer M, Kristiansen MD, Flindt MR (2010)
696 Vulnerability of *Zostera marina* seedlings to physical stress. *Marine Ecology Progress Series*
697 418:119-130
- 698 Valdemarsen T, Wendelboe K, Egelund JT, Kristensen E, Flindt MR (2011) Burial of seeds and
699 seedlings by the lugworm *Arenicola marina* hampers eelgrass (*Zostera marina*) recovery.
700 *Journal of Experimental Marine Biology and Ecology* 410:45-52
- 701 Valdemarsen T, Bannister RJ, Hansen PK, Holmer M, Ervik A (2012) Biogeochemical
702 malfunctioning in sediments beneath a deep-water fish farm. *Environmental Pollution* 170:15-
703 25
- 704 Valdemarsen T, Quintana CO, Kristensen E, Flindt MR (2014) Recovery of organic-enriched
705 sediments through microbial degradation - implications for eutrophic estuaries. *Marine*
706 *Ecology Progress Series* 503:41-58
- 707 Viktorsson L, Ekeröth N, Nilsson M, Kononets M, Hall POJ (2013) Phosphorus recycling in
708 sediments of the central Baltic Sea. *Biogeosciences* 10:3901-3916
- 709 Wendelboe K, Egelund JT, Flindt MR, Valdemarsen T (2013) Impact of lugworms (*Arenicola*
710 *marina*) on mobilization and transport of fine particles and organic matter in marine
711 sediments. *Journal of Sea Research* 76:31-38
- 712 Westrich JT, Berner RA (1984) The role of sedimentary organic matter in bacterial sulfate
713 reduction: The G-model tested. *Limnology and Oceanography* 29:236-249
- 714 Westrich JT, Berner RA (1988) The effect of temperature on rates of sulfate reduction in marine
715 sediments. *Geomicrobiology Journal* 6:99-117

716 **Tables**

717 Table 1. Dimensionless linear NH_4^+ adsorption coefficients, K_{NH} , for different sediment depths at St
718 1-8.

	St 1	St 2	St 3	St 4	St 5	St 6	St 7	St 8
0-2 cm	0.26	1.06	0.33	0.46	0.64	0.31	0.57	0.48
6-8 cm	0.52	0.76	0.49	0.45	0.82	0.51	0.62	0.36
8-10 cm	0.40	0.82	0.20	0.79	0.55	0.66	0.14	0.45

719

720 Table 2. Depth integrated (0-16 cm) area specific TN and TP content \pm SE (n=3) on St 1-8.

721 Superscript capital letters indicate the grouping of data obtained by ANOVA and subsequent post

722 hoc analysis. Average TN:TP ratios are also shown.

	TN	TP	TN:TP
	(mol m ⁻²)	(mol m ⁻²)	-
St 1	13.5 \pm 0.4 ^A	1.34 \pm 0.04 ^A	10.1
St 2	21.5 \pm 0.5 ^B	1.31 \pm 0.02 ^A	16.4
St 3	16.0 \pm 0.2 ^B	0.70 \pm 0.06 ^B	22.9
St 4	16.6 \pm 1.1 ^B	1.18 \pm 0.06 ^A	14.1
St 5	4.5 \pm 0.1 ^C	0.73 \pm 0.04 ^B	6.2
St 6	17.1 \pm 0.1 ^B	1.94 \pm 0.03 ^C	8.8
St 7	18.1 \pm 0.0 ^B	1.86 \pm 0.05 ^C	9.7
St 8	19.5 \pm 0.2 ^B	1.83 \pm 0.03 ^C	10.7

723

724

725 Table 3. Initial and final depth integrated pools (0-20 cm) of FeIII \pm SE (n=3) on St 1-8. t-tests
 726 showed no significant difference between initial and final FeIII pools on any station.

	Initial		Final	
	FeII (mmol m ⁻²)	FeIII (mmol m ⁻²)	FeII (mmol m ⁻²)	FeIII (mmol m ⁻²)
St 1	2390 \pm 34	243 \pm 24	2294 \pm 153	92 \pm 22
St 2	2302 \pm 160	157 \pm 32	2399 \pm 189	271 \pm 161
St 3	1356 \pm 155	62 \pm 25	1358 \pm 154	109 \pm 40
St 4	1054 \pm 86	28 \pm 20	996 \pm 23	97 \pm 37
St 5	258 \pm 2	6.3 \pm 1.0	274 \pm 39	6.4 \pm 1.2
St 6	1887 \pm 37	75 \pm 12	1813 \pm 43	141 \pm 40
St 7	2464 \pm 105	52 \pm 2.0	2142 \pm 60	137 \pm 48
St 8	1697 \pm 63	156 \pm 8.0	1813 \pm 43	210 \pm 89

727

728

729 Table 4. Double exponential regression statistics for the temporal trends of total ON and OP
 730 degradation in jar experiments. Total organic N (ON) and P (OP) degradation were fitted to the
 731 exponential decay function $y = C_L \cdot \exp(-k_L \cdot x) + C_R \cdot \exp(-k_R \cdot x)$, where C_L and C_R denote constants
 732 and k_L and k_R denote decay constants for labile and refractory organic ON and OP, respectively.
 733 Statistics were not calculated for St 7-8 (ON) and for St 4-5 and 7-8 (OP), since the temporal
 734 degradation patterns did not fit the double exponential decay model. $T_{L,0.5}$, L and $T_{R,0.5}$, R denote
 735 the half life (y) of labile and refractory ON and OP, respectively.

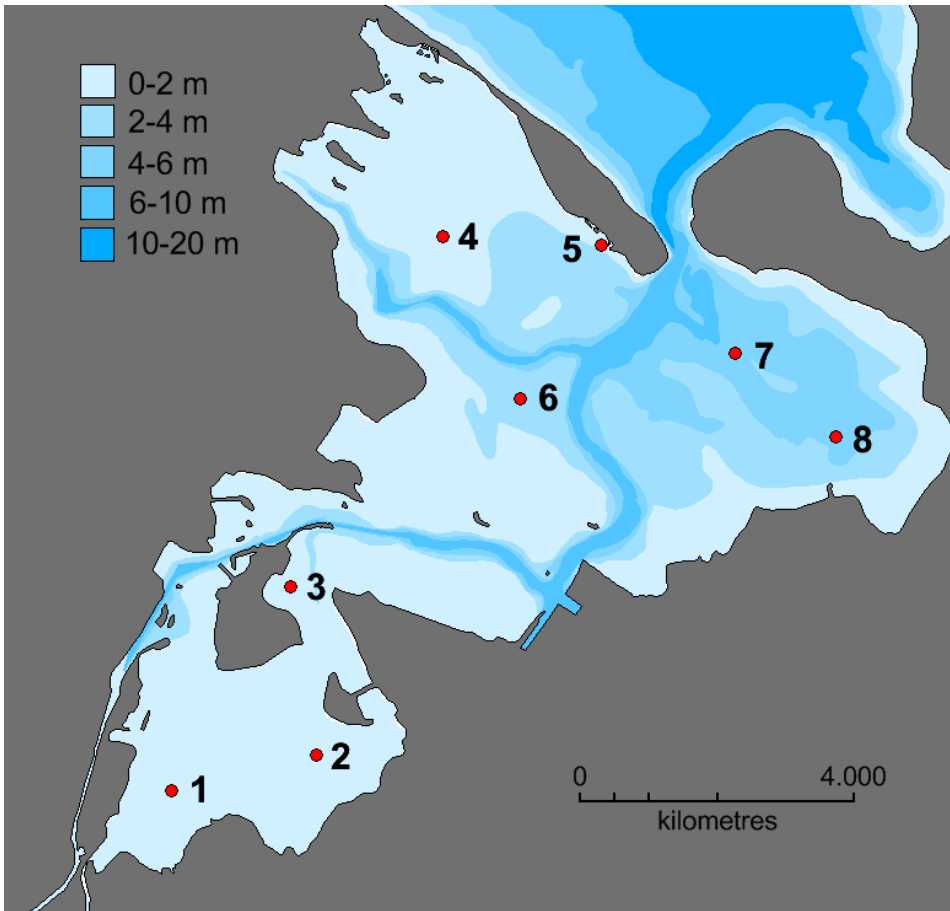
ON						
	k_L	k_R	C_L	C_R	$T_{L,0.5}$	$T_{R,0.5}$
St 1	$4.6 \cdot 10^{-2}$	$1.1 \cdot 10^{-3}$	7.7	2.4	0.04	1.73
St 2	$2.3 \cdot 10^{-2}$	$1.0 \cdot 10^{-3}$	3.1	2.9	0.08	1.90
St 3	$5.3 \cdot 10^{-2}$	$1.1 \cdot 10^{-3}$	8.6	2.8	0.04	1.73
St 4	$4.3 \cdot 10^{-2}$	$0.4 \cdot 10^{-3}$	4.0	1.8	0.04	4.75
St 5	$5.7 \cdot 10^{-2}$	$0.6 \cdot 10^{-3}$	2.7	7.2	0.03	3.17
St 6	$52.4 \cdot 10^{-2}$	$0.3 \cdot 10^{-3}$	3.2	2.9	0.01	6.33
St 7	-	-	-	-	-	-
St 8	-	-	-	-	-	-
OP						
	k_L	k_R	C_L	C_R	$T_{L,0.5}$	$T_{R,0.5}$
St 1	$3.9 \cdot 10^{-2}$	$0.4 \cdot 10^{-3}$	0.6	0.3	0.05	4.75
St 2	$56.0 \cdot 10^{-2}$	$1.5 \cdot 10^{-3}$	1.1	0.3	0.01	1.27
St 3	$2.2 \cdot 10^{-2}$	$1.3 \cdot 10^{-3}$	0.1	0.3	0.08	1.46
St 4	-	-	-	-	-	-
St 5	-	-	-	-	-	-
St 6	$1.7 \cdot 10^{-2}$	$0.9 \cdot 10^{-3}$	0.4	0.5	0.11	2.11
St 7	-	-	-	-	-	-
St 8	-	-	-	-	-	-

736

737 Table 5. N and P budgets for the experiment. Initial TN and TP are the depth integrated values
738 based on initial measurements. ON and OP degradation were calculated based on area specific rates
739 obtained from jar experiments. Total NH_4^+ -, NO_x^- - and PO_4^{3-} -effluxes were calculated by time
740 integration of effluxes over the entire experimental period. NH_4^+ - and PO_4^{3-} -accumulation in
741 porewater (pw) was calculated from the difference between initial and final pw profiles. NH_4^+
742 adsorption was calculated from initial and final pw-inventories of NH_4^+ and the average NH_4^+ -
743 adsorption coefficient for each station. Values in parentheses marked with * or ** represent
744 percentage relative to initial TN and TP or total N and P mineralization, respectively.

<i>N-mineralization</i>	St 1	St 2	St 3	St 4	St 5	St 6	St 7	St 8
Initial TN (mol m^{-2})	13.5	21.5	16.0	16.6	4.5	17.1	18.1	19.5
ON degradation, jars (mol m^{-2})*	1.38 (10.2)	1.62 (7.5)	1.56 (9.8)	1.44 (8.6)	3.62 (80.1)	1.77 (10.1)	1.61 (8.9)	1.86 (9.6)
NH_4^+ efflux (mol m^{-2})**	0.26 (19.1)	0.10 (6.2)	0.23 (14.6)	0.15 (10.7)	0.38 (10.6)	0.09 (5.1)	0.27 (16.6)	0.12 (6.2)
NO_x^- efflux (mol m^{-2})**	0.15 (11.2)	0.21 (13.0)	0.19 (12.1)	0.11 (7.3)	0.18 (5.0)	0.22 (12.9)	0.17 (10.8)	0.20 (10.9)
NH_4^+ accumulation, pw (mol m^{-2})**	0.02 (1.6)	0.01 (0.7)	0.00 (0.0)	0.08 (5.9)	0.06 (1.6)	0.03 (1.8)	0.01 (0.7)	0.02 (1.3)
NH_4^+ adsorption (mol m^{-2})**	0.01 (0.7)	0.01 (0.6)	0.00 (0.0)	0.01 (0.4)	0.04 (1.0)	0.02 (1.0)	0.00 (0.2)	0.01 (0.6)
<i>P-mineralization</i>	St 1	St 2	St 3	St 4	St 5	St 6	St 7	St 8
Initial TP (mol m^{-2})	1.34	1.31	0.70	1.18	0.73	1.94	1.86	1.83
OP degradation, jars (mol m^{-2})*	0.16 (12.6)	0.12 (7.9)	0.18 (19.6)	0.13 (11.2)	0.29 (47.7)	0.28 (14.5)	0.22 (11.5)	0.33 (17.4)
PO_4^{3-} efflux (mol m^{-2})**	0.02 (12.6)	0.04 (38.0)	0.02 (16.8)	0.02 (15.8)	0.10 (27.8)	0.02 (7.4)	0.03 (12.8)	0.02 (6.5)
PO_4^{3-} accumulation, pw (mol m^{-2})**	0.01 (4.8)	0.01 (9.6)	0.00 (1.6)	0.00 (0.8)	0.02 (5.1)	0.01 (3.0)	0.00 (1.7)	0.01 (3.1)

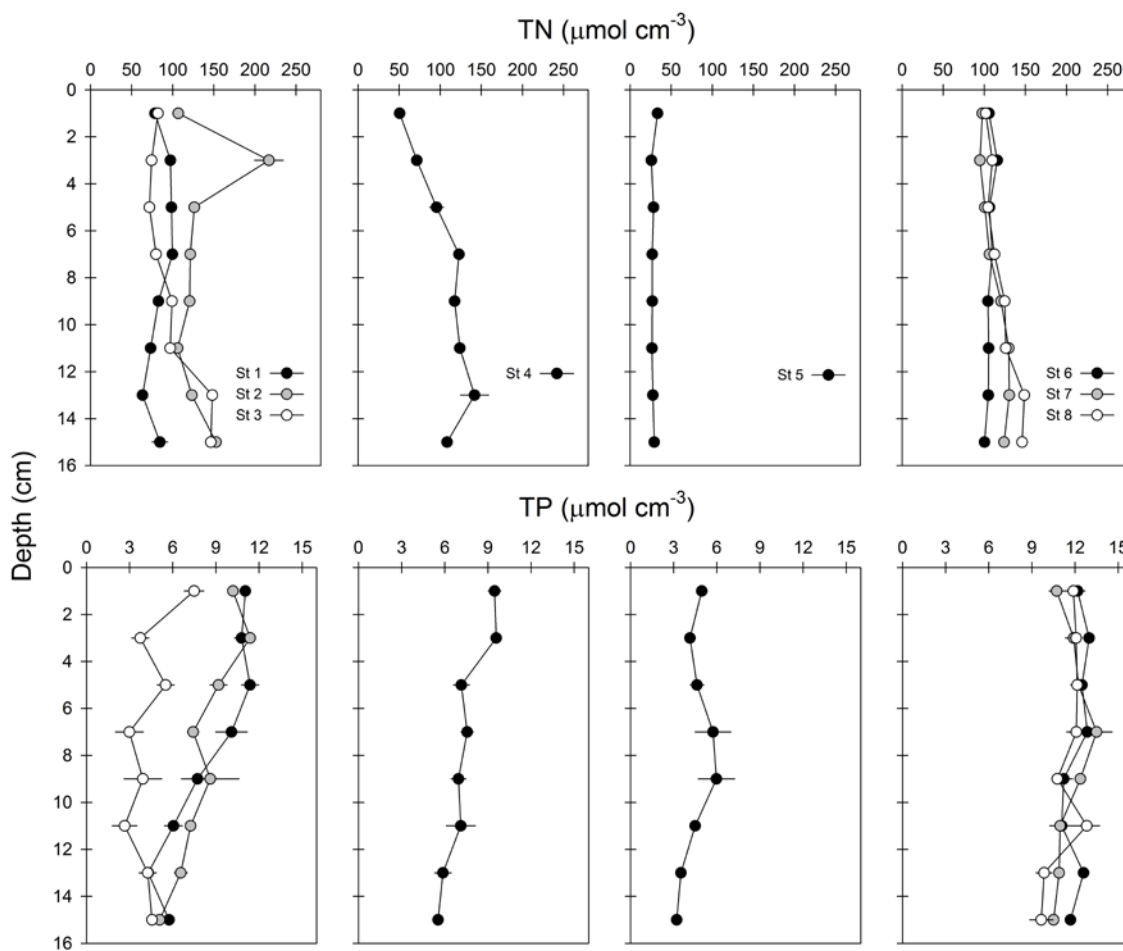
745



747

748 Figure 1. Map of Odense Fjord (55°29'15" N; 10°31'09") showing the 8 stations, where sediments
749 were sampled for the long term degradation experiment. Gray color indicates land and different
750 shades of blue indicate water depth.

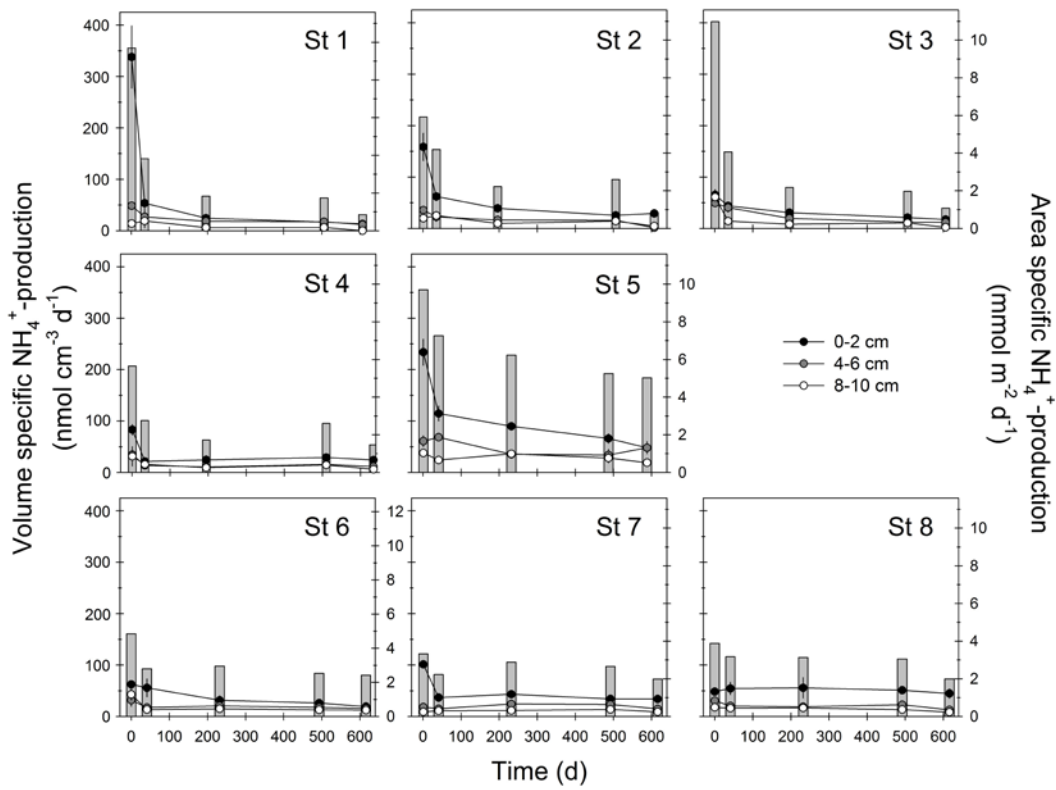
751



752

753 Figure 2. Total nitrogen (TN) and total phosphorous (TP) in sediments from Odense Fjord. Left
 754 panels show stations from the shallow inner fjord (St 1, 2 and 3), middle panels show shallow silty
 755 and sandy sediments in the outer fjord (St 4 and 5, respectively) and right panels show deep silty
 756 sediments in the outer fjord (St 6, 7 and 8). Error bars indicate standard error ($n = 3$).

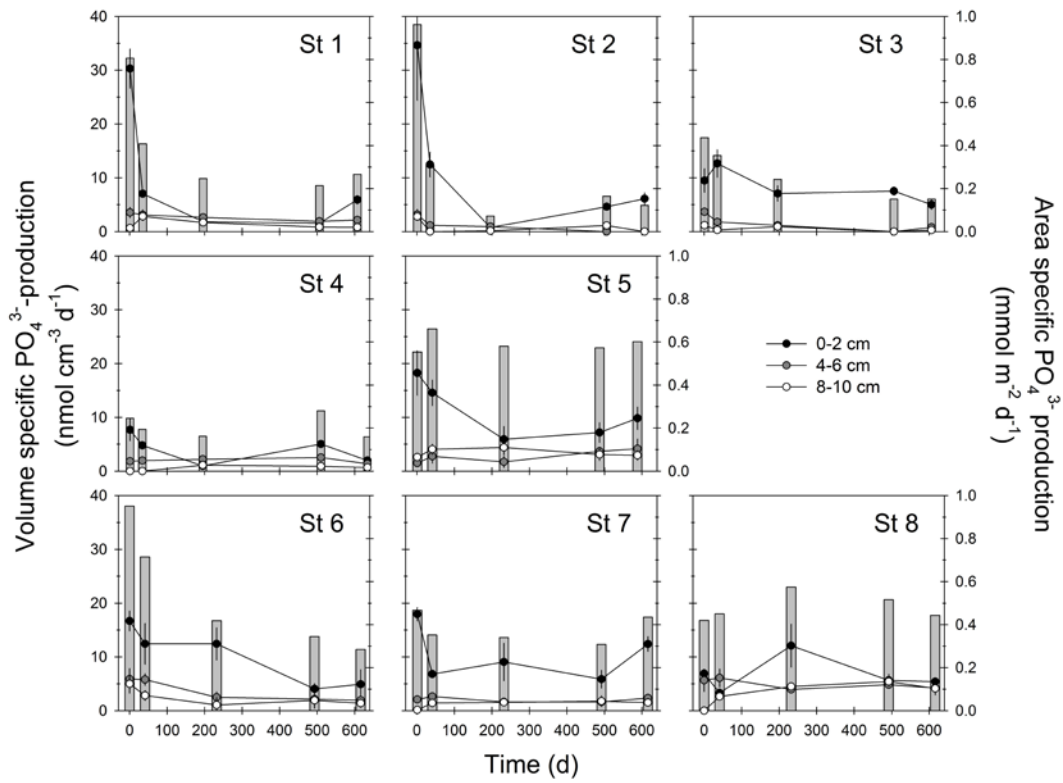
757



758

759 Figure 3. NH_4^+ production measured in jar experiments with sediment from shallow inner basin
 760 (upper panels), shallow silty and sandy outer basin (middle panels) and deep silty outer basin (lower
 761 panels). Black, gray and white symbols indicate volume specific NH_4^+ production in sediment from
 762 0-2, 4-6 and 8-10 cm depth, respectively (left y-axis). Bars indicate depth integrated (0-20 cm)
 763 NH_4^+ production based on volume specific production rates (right y-axis).

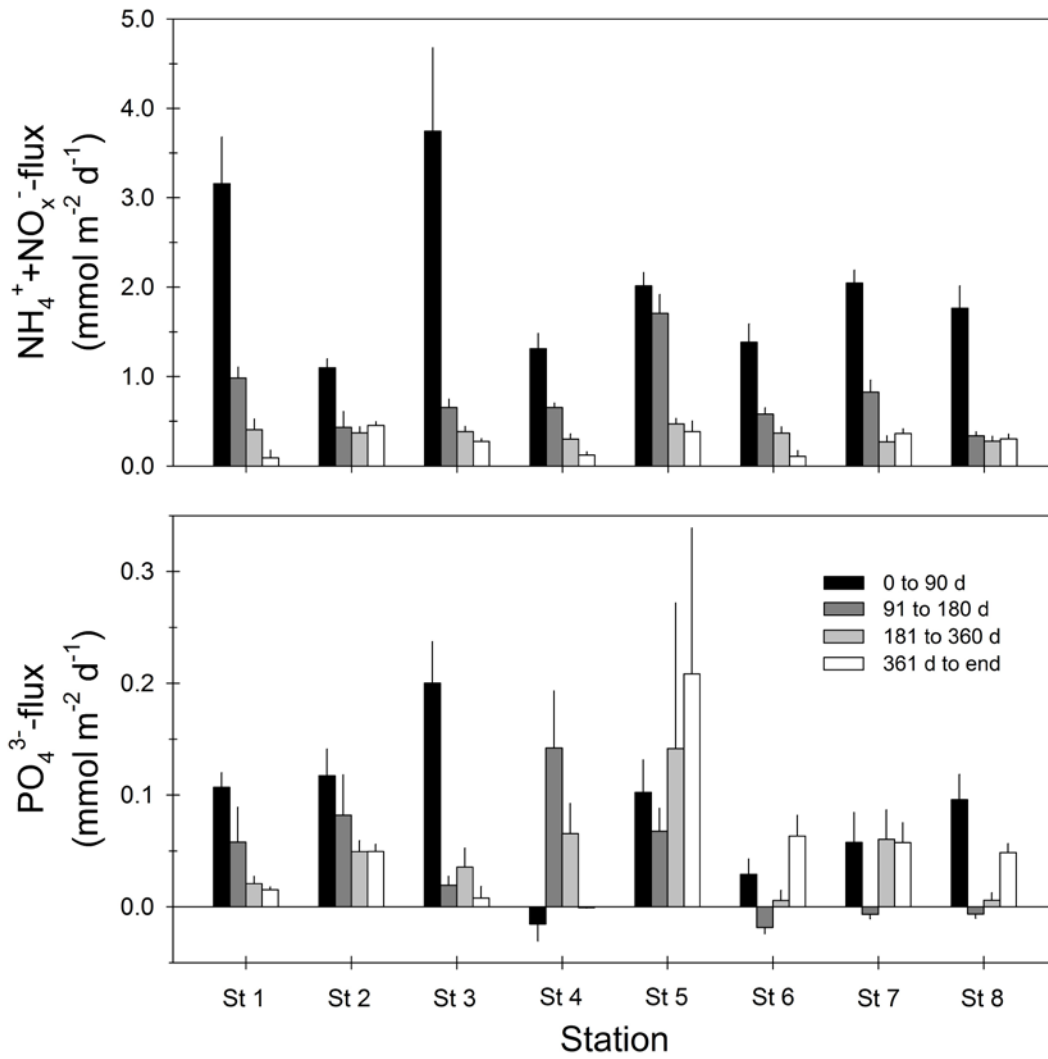
764



765

766 Figure 4. PO_4^{3-} production measured in jar experiments performed with sediment from shallow
 767 inner fjord (upper panels), shallow silty and sandy outer fjord (middle panels) and deep silty outer
 768 fjord (lower panels). Black, gray and white symbols indicate volume specific PO_4^{3-} production in
 769 sediment from 0-2, 4-6 and 8-10 cm depth, respectively (left y-axis). Bars indicate depth integrated
 770 (0-20 cm) PO_3^{3-} production based on volume specific production rates (right y-axis).

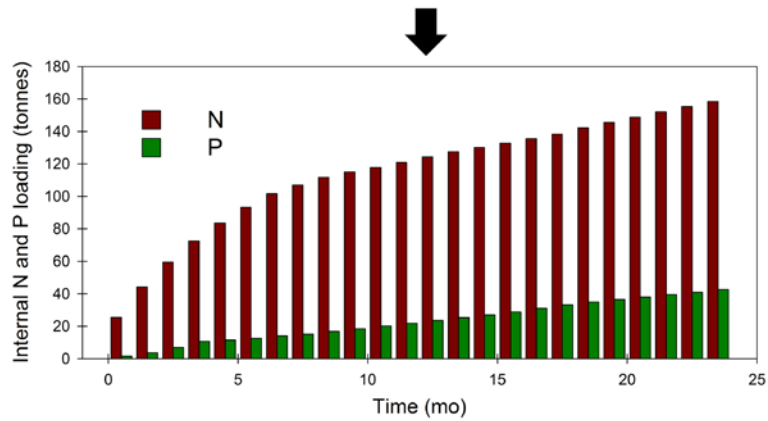
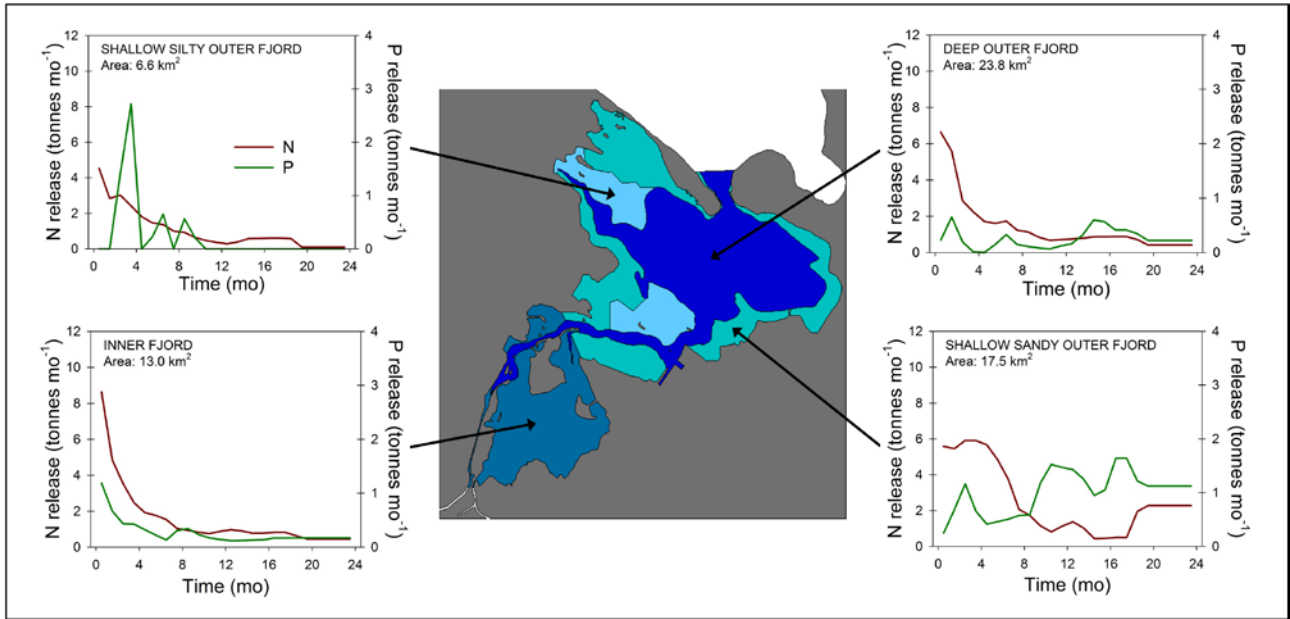
771



772

773 Figure 5. Fluxes of dissolved inorganic nitrogen ($\text{DIN} = \text{NH}_4^+ + \text{NO}_x^-$) and PO_4^{3-} at various times
 774 during the experiment. Error bars represent standard error ($n = 6-24$).

775



776

777 Figure 6. Estimated internal nutrient loading in Odense Fjord. The upper figure shows a schematic
 778 overview of Odense Fjord with the distribution of sediment types included in this study and their
 779 nutrient release over a 24 month period. The lower figure shows the cumulated nutrient release from
 780 the entire fjord bottom.