Cover letter regarding manuscript Bg-2014-411 “Organic N and P in eutrophic fjord sediments - rates of mineralization and consequences for internal nutrient loading”

Dear editor,

We have carefully considered the comments provided by the two reviewers and have edited the manuscript where appropriate. In the text below we have written our response to the individual reviewer comments. By the end of this document we have attached the revised manuscript with changes highlighted by track changes – here it is indicated which changes were made in response to specific comments.

We hope that with these changes, the revised version of the manuscript can be accepted for publication in Biogeosciences.

Sincerely,

Thomas Valdemarsen

RESPONSE TO REVIEWER#1, Susanna Hietanen

The authors thank Susanna Hietanen (Reviewer#1) for her constructive criticism of the manuscript. In the text below, the Reviewers original comment is indicated by the headline “REVIEWER COMMENT” and author´s responses are indicated by the headlines “REPLY” and “ACTION”

REVIEWER#1, COMMENT 1:
This MS discusses a topical, important and controversial aspect in aquatic ecosystem protection and restoration – the effect internal, independently of external, nutrient loading has on the water quality. While internal nutrient loading has traditionally been seen only as phosphorus release from iron hydroxides under oxygen stress, these authors define it wider, as the release of inorganic nutrients by mineralization of organic matter. To quantify such a release they performed a two-year experiment in which they incubated different types of sediments from different areas of a eutrophied fjord in both oxic and anoxic conditions and followed the fluxes of inorganic nutrients in and out of the sediments, production of ammonium and phosphate in anoxic conditions and the concentrations of total nutrients in the sediments. The same authors have recently published the results of organic carbon mineralization in this same experiment in Marine Ecology Progress Series (503: 41-58; 2014), stating that organic carbon accumulates in sediments and it’s degradation is a slow process that delays the recovery of the water ecosystem. In this discussion paper the authors present data on nitrogen and phosphorus mineralization, concluding that internal nitrogen loading ceases much faster than that of phosphorus, which can be seen as good news for nitrogen limited

systems (although the authors fail to mention e.g. that low N:P ratio favors the growth of cyanobacteria that, in turn, may release plenty of freshly fixed nitrogen to the system).

REPLY:
The same point was addressed by reviewer#2. The reviewers are right that we did not mention that decreasing internal nitrogen loading could lead to stimulation of cyanobacteria, which could potentially buffer the decreasing internal nitrogen loading through N-fixation. This was omitted because we thought it was too speculative. In our experiment we only looked at N coming from the sediment and neglected the external N-loading, which is still the dominating source of N in the studied system. Decreasing internal nutrient loading may therefore not necessarily lead to reduced DIN concentrations as long as external N-loading remains high. Therefore major changes in phytoplankton composition towards increased dominance of cyanobacteria will probably not occur.

ACTION:
In the revised manuscript we mention that the decreasing internal N-loading and stable internal P-loading could lead to increased dominance of cyanobacteria. However, a major shift in phytoplankton community can only occur in systems where decreased internal nutrient loading results in markedly lower DIN-concentrations, i.e. in systems where (1) internal nutrient sources dominate, or where (2) external N-loading is significantly reduced.

REVIEWER#1, COMMENT 2:
The experiment has been carefully conducted and the paper is well written, with good quality tables and figures. The authors show that they are quite well aware of the shortcomings of the experimental setup used and discuss the results mainly accordingly. While the paper does not present especially novel ideas, it strengthens theories on shallow-water ecosystem recovery processes. I have very few specific comments: Page 15114 row 5: The selected habitat types are said to have covered the whole fjord (100%) – still, for example the highest porosity found in these samples was 0.8 which is surprisingly low – are there no high-organic muddy areas in this estuary, with porosities well over 0.9?

REPLY:
The 3 stations located in the outer fjord (St 6-8) were chosen to represent the most organic rich, muddy areas in the fjord. A previous survey of sediment characteristics in Odense fjord covering >100 stations, showed that the most fine grained sediments had porosities around 0.8. The absence of extremely muddy sites in Odense Fjord is probably due to wind driven resuspension events, which may affect even the deep parts of this shallow system (down to 10 m depth) and prevent accumulation of the finest sediment fractions.

ACTION:
This comment will not lead to changes in the manuscript.

REVIEWER#1, COMMENT2b:
Page 15114 row 20 on; removing macrofauna from naturally permanently oxic system makes the interpretation and generalization of the results dubious. The authors mention this shortcoming briefly in discussion as a possible source of error, and probably this is the only way to study slow processes in laboratory conditions. However, the role of macrofauna in benthic mineralisation in shallow, oxic waterbodies is very large and excluding them from the experimental setup a very drastic manipulation of the system. This might merit a bit longer discussion about the reliability of the results from this point of view.

REPLY:
A similar point was raised by Reviewer#2. We agree with the reviewers that removing macrofauna was a major manipulation, which undoubtedly changed/stimulated mineralization rates and temporal patterns. However, the main goal of the experiment was to assess how much nutrients could be released from the organic matter that had accumulated in the sediments during eutrophication, and this could not have been accomplished if macrofauna had been included in the study. To keep macrofauna alive in a 2 year laboratory experiment is virtually impossible. Macrofauna would need a stable food source (addition of some source of organic matter, including organic N and P), and then we would not know how much of the generated nutrients were coming from the sediment and added organic matter, respectively. Furthermore, most benthic infauna has a life span of less than 2 years, meaning that they would eventually die out in the lab experiment since there was no recruitment. Finally, the infauna composition is highly variable at the different stations (i.e. some stations have large bioturbators and others not), and this variability would have disturbed the major patterns in sediment nutrient generation at the different stations.

ACTION:
In the revised manuscript we will strengthen our discussion of the consequences of removing infauna at the beginning of the experiment. We will emphasize that our estimates of nutrient regeneration are probably conservative, since macrofauna would have stimulated the remineralization of organic matter and nutrients.

REVIEWER#1, COMMENT 3:
Similarly, some mention could be made about the annual oxygen and temperature conditions these sediments might be exposed to in contrast to fully oxic, 15 degree incubation used in the experiment.

REPLY:
A similar comment was made by Reviewer#2. The incubation temperature of 15˚C was chosen to reflect the average annual temperature in Odense Fjord. Hypoxia/anoxia is no longer occurring in Odense Fjord, so the incubation condition of fully oxygenated water is fully representative for Odense Fjord. It is true that annual temperature variations occur in the system and that this will influence microbial reaction rates. However, the main goal of the experiment was to provide an estimate of the total nutrient release from Odense Fjord sediments and not to simulate seasonal changes in microbial reaction rates. We therefore

choose to omit any temperature driven variation in microbial reaction rates, which would complicate the interpretation of results.

ACTION:
The influence of temperature variations were briefly mentioned in the original manuscript (Page 15129, Line 23). In the revised manuscript we have strengthened the discussion of consequences of constant contra variable temperature for internal nutrient loading.

REVIEWER#1, COMMENT 4:
Page 15117 Jar experiments. These experiments were run fully anoxic to prevent oxidation of end products, and homogenized probably for minimizing variation between samples. Both cutting the contact with oxygen for the surface sediments and homogenizing across redox zones heavily changes conditions compared to core incubations.These effects are not discussed at all. Was oxygen penetration to the sediments so shallow that it merited the anoxic incubation in the top layer?

REPLY:
It is true that the jar technique may underestimate the actual mineralization rates in oxidized sediments where O_2 sensitive organic matter is degraded. However, most coastal and estuarine sediments are predominantly anoxic beneath a shallow (1-3 mm deep) oxidized sediment layer, i.e. conditions similar to the beginning of this experiment. We (and probably the Reviewer) expected the sediments to become more and more oxic as the experiment progressed and labile organic matter was depleted, but this did not occur. This was for instance shown by hydrogen sulfide (data not shown), which was present at all sediment depths at all stations throughout the experiment, suggesting that sediments remained predominantly anoxic except for the upper few mm. Anoxic sediment incubations was therefore an appropriate technique to measure microbial reaction rates throughout the experiment.

ACTION:
A paragraph has been added where it is mentioned that the sediments remained mostly anoxic throughout the experiment and that closed anoxic sediment incubations therefore was an appropriate technique to measure microbial reaction rates throughout the experiment.

REVIEWER#1, COMMENT 5:
Page 15117 row 25; you probably mean that the concentrations of Fe (III) were compared at the beginning (initial) and end (final) of the experiments using pairwise t-tests?
the results are really surprising – according to table 3 there are no significant differences, despite increase or decrease by 2.5-3.4 times.

REPLY:
True – we compared total pools of Fe(III) at the beginning and end by pairwise t-tests. We were also very surprised that 2 year incubation with no addition of new organic matter did

not lead to significant changes in Fe(III)-pools, owing to large spatial heterogeneity between sediment cores. Unfortunately, intercore variability is one of the drawbacks of using non-manipulated sediment cores.

ACTION:
We rephrased the lines (Page 15117 row 25-26) according to the reviewer´s suggestion.

REVIEWER#1, COMMENT 6:
Page 15120 rows 18-25; there seems to be some words missing and some in excess in the text (check grammar).

ACTION:
The indicated section was rephrased to: "Surface 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 NH_4^+ production in the sediments sampled in the deep outer basin (St 6–8) decreased by 8–67% during the experiment. 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 NH_4^+ production decreased by 75–96% to 1.4–12 nmol cm^{-3} d^{-1} by the end (Fig. 3)."

REVIEWER#1, COMMENT 7:
Page 15123 general comment on all the discussion about the sandy site: Sandy sediments are usually permeable, which means they operate by advection, not by diffusion. Enclosing sand in a core, out of reach of advective flow, changes conditions in the porewater dramatically. Recent research has indicated sandy sediments as areas of extremely high mineralization despite the low organic content. The authors mention that the sandy sediment came from area affected by with wind driven waves, deeply burying macrofauna and intense microphytobenthic production. None of this could be reproduced in the experimental setup, which questions the interpretation of the results on this site.

REPLY:
A similar comment was made by reviewer#2.We are aware that shallow sandy sediments may be highly reactive due to a very dynamic environment. As mentioned in previous comments the main purpose of the experiment was not to simulate in situ degradation rates, but to measure how much nutrients could actually be made available by degradation and efflux to the overlying water column in different sediment types. In this respect the omission of waves, light and microphytobenthos is critical to be able to compare the reactivity of sedimentary organic matter and nutrients at different sites.

ACTION:

In the revised manuscript we will underline that shallow sandy sediments are highly dynamic environments supporting a rapid cycling of nutrients and organic matter. We will also emphasize that this might have lead to a bias in our estimates of nutrient release.

REVIEWER#1, COMMENT 8:
Page 15124 row 25 typos- subscripts instead of superscripts in Mkg.

ACTION:
This was corrected.

REVIEWER#1, COMMENT 9:
Page 15128 Ecological implications – the first half of the chapter is very repetitive and could be pruned heavily.

ACTION:
We will shorten this paragraph.

REVIEWER#1, COMMENT 10:
Page 15138 Table 2 should show “average TN:TP ratios” but it does not.

ACTION:
TN:TP ratios were added to Table 2.

REVIEWER#1, COMMENT 11:
Page 15146 Figure 5 DIN= NH4+ + NOx- in figure but DIN= NH4+ + NO3- in legend.

ACTION:
The text in the figure legend was changed to “DIN = NH_4^+ + NO_x^-“

REVIEWER#1, COMMENT 12:
Page 15147 Figure 6 I am not sure this figure is really needed, although it is nice.

REPLY:
Reviewer#2 had the same comment. The temporal trends in NH_4^+ and NO_x^- are described sufficiently in the text.

ACTION:

The figure was deleted.

RESPONSE TO REVIEWER#2, Anonymous

The authors thank the reviewer for his/her constructive criticism of the manuscript. In the text below, the Reviewers original comments are indicated by the headline "REVIEWER COMMENT" and author responses are indicated by the headlines "REPLY" and "ACTION"

REVIEWER#2, COMMENT 1:
The manuscript discusses the importance of internal nutrient loading in the recovery of eutrophic estuaries by examining the mineralisation of organic nitrogen (ON) and phosphorus (OP) buried in the sediment. The magnitude of the internal loading of ON and OP was investigated in a two year experiment spanning a number of different sediment types found within the Odense Fjord. Cores were kept oxic throughout the experimental phase in which sediment porewater profiles, dissolved nutrient fluxes and anaerobic jar experiments (anaerobic mineralisation rates) were periodically measured over the two year period. The authors concluded that internal nitrogen loading is of minor importance (6% external N) and DIN fluxes stabilised after 50-200 days, alternatively internal phosphorus loading is potentially a source of P (36% external P) and PO43- fluxes throughout the two year period were variable and P efflux could be sustained over years which may be important for the management of P.
The principles within the manuscript are not necessarily novel, however, the authors do provide further understanding of internal nutrient loading which is an important measure in the management of these systems and the potential for a delayed response after reducing external inputs. The manuscript is generally well written some areas could be shortened. There are, however, some limitations in the experimental design, possibly unavoidable due to the required length of the experiment but they do raise the question as to how relevant long term laboratory experiments are to the natural environment. Some of these limitations have been discussed briefly in the discussion and more thoroughly in the partner paper on carbon mineralisation published in the Marine Ecology Progress Series (Vol. 503: 41–58, 2014) however these need to be addressed more thoroughly.

REPLY:
Both reviewers indicate that sources of errors and uncertainties regarding experimental setup and estimates of internal nutrient loading should be emphasized more clearly in the text. We agree with both reviewers that variation in temperature, macrofauna and hydrodynamics/advection will impact nutrient regeneration and hence the magnitude of internal nutrient loading compared to our estimates.

ACTION:
Where appropriate we will emphasize sources of errors and consequences of omitting different environmental variables in the experiment set-up. See our reply to specific comments for more details.

REVIEWER#2, COMMENT 2:
Page 15114 Line 1 – Station 5 represents sandy sediment which has been collected in a closed core. Measuring fluxes in sediment within closed cores is based on diffusion principles, however in the natural environment processes within sandy sediments are not only governed by diffusion but also advective flow. This will likely alter the estimate of nutrient effluxes from the sediment depending on residence time of the porewaters etc. The difference of this site to the others is briefly touched on in the discussion (15124 – line 14) however it is not discussed in terms of an experimental limitation and how this influences the interpretation of the results at this site and requires further comment.

REPLY:
A similar comment was made by reviewer#1.We are aware that shallow sandy sediments may be highly reactive due to a very dynamic environment. However, the main purpose of the experiment was not to simulate in situ degradation rates, but to measure how much nutrients could actually be made available by degradation and efflux to the overlying water column in different sediment types. In this respect the omission of waves, light and microphytobenthos is critical to be able to compare the reactivity of sedimentary organic matter and nutrients at different sites.

ACTION:
In the revised manuscript we will underline that shallow sandy sediments are highly dynamic environments supporting a rapid cycling of nutrients and organic matter. We will also emphasize that this might have lead to a bias in our estimates of nutrient release.

REVIEWER#2, COMMENT 3:
Page 15114 Line 20 – What was the density of the macrofauna at each of the sites? In the literature macrofauna play an important role in nutrient cycling in the Odense Fjord turning over large volumes of sediment annually if this also applies to these sites excluding them from your experiments (even though it would be challenging to include them in a long term experiment such as this) could drastically change your estimates ON and OP released from the sediment. This also changes the redox conditions at the sediment surface where burrowing macrofauna can increase the depth of oxygen penetration and nutrient transport into the sediment and in inturn potentially influence your mineralisation rates. I am uncertain here how relevant to natural conditions your estimates of internal nutrient loadings are having excluded all macrofauna from your experiments. This requires further discussion.

REPLY:
A similar point was raised by Reviewer#1. We agree with the reviewers that removing macrofauna was a major manipulation, which undoubtedly changed mineralization rates and temporal patterns. However, the main goal of the experiment was to assess how much nutrients could be released from the organic matter that had accumulated in the sediments during eutrophication, and this could not have been accomplished if macrofauna had been included in the study. To keep macrofauna alive in a 2 year laboratory experiment is virtually impossible. Macrofauna would need a stable food source (addition of some source of organic matter, including organic N and P), and then we would

not know how much of the generated nutrients were coming from the sediment and added organic matter, respectively. Furthermore, most benthic infauna has a life span of less than 2 years, meaning that they would eventually die out in the lab experiment since there was no recruitment. Finally, the infauna composition is highly variable at the different stations (i.e. some stations have large bioturbators and others not), and this variability would have disturbed the major patterns in sediment nutrient generation at the different stations.

ACTION:
In the revised manuscript we will strengthen our discussion of the consequences of removing infauna at the beginning of the experiment. We will emphasize that our estimates of nutrient regeneration are probably conservative, since macrofauna would have stimulated the remineralization of organic matter and nutrients.

REVIEWER#2, COMMENT 4:
Page 15114 Line 11/25 - The authors state that at the time of sampling the in situ temperature was 10 – 12oC, however the experimental conditions were set to 15oC I am uncertain why this temperature was chosen. Furthermore the authors then extrapolate annual ON and OP release under these conditions where on an annual cycle temperatures in the Odense Fjord range from _3 - 19oC. Bacterial populations could also vary throughout this time based on temperature differences. The influence of temperature on mineralisation rates should be something that is considered and discussed further particularly when temperature regulation is quite easy to manipulate in an experimental setup.

REPLY:
A similar comment was made by Reviewer#1. The incubation temperature of 15˚C was chosen to reflect the average annual temperature in Odense Fjord. It is true that annual temperature variations occur in the system and that this will influence microbial reaction rates. However, the main goal of the experiment was to provide an estimate of the total potential nutrient release from Odense Fjord sediments and not to simulate seasonal changes in microbial reaction rates. We therefore choose to omit any temperature driven variation in microbial reaction rates, which would complicate the interpretation of results.

ACTION:
The influence of temperature variations were briefly mentioned in the original manuscript (Page 15129, Line 23). In the revised manuscript we have strengthened the discussion of consequences of constant contra variable temperature for internal nutrient loading.

REVIEWER#2, COMMENT 5:
Page 15117 Line 6 – Was there a control to compare the rate of aerobic and anaerobic mineralisation to confirm that anaerobic mineralisation measurements were representative of all mineralisation within the fjord? This would not be an issue for deep anaerobic sediments but in aerobic surface sediments an aerobic mineralisation measurement

should have been considered.

REPLY:
We have evidence (hydrogen sulfide concentrations in porewater) suggesting that sediments from all stations were predominantly anoxic throughout the experiment, indicating that anaerobic degradation was most important for organic matter degradation in this experiment. Furthermore, results obtained in anoxic jar experiments are evaluated against flux measurements, which include both anaerobic and aerobic processes. Hence both anaerobic and aerobic processes are actually accounted for in the manuscript.

ACTION:
A paragraph was added where it is mentioned that sediments remained mostly anoxic throughout the experiment and that anaerobic degradation processes were most important in the present experiment.

REVIEWER#2, COMMENT 6:
Page 15126 Line 15 – The authors mention that the missing NH4+ could be lost due to nitrification-denitrification coupling. I do agree the missing NH4+ in the oxic fluxes is most likely accounted for by nitrification however in Figure 6 it does not appear that nitrification and denitrification are strongly coupled with most of the NO3- released from the sediment after _20 days. Possibly revise this statement.

REPLY:
We are not sure we understand the reviewer´s argument. We measure much higher NH_4^+ production in the sediments than is released as DIN-efflux or accumulates as NH_4^+ in porewater. We argue that the deficit between NH_4^+ production and DIN efflux must be caused by closely coupled nitrification-denitrification (i.e. we do not see the produced NO_3^- as it is consumed as fast as it is produced). This is the only explanation for the observed deficit and we see no reason to revise this statement.

ACTION:
This comment will not lead to major changes in the text. However, Fig. 6 will be deleted from the revised manuscript as requested by both reviewers.

REVIEWER#2, COMMENT 7:
Page 15128 Line 3 – The authors state in the discussion that the sandy sediments are the most important for the total fjord release of N (39%) I would note that this estimate probably has the most uncertainty out of all sites due to the absence of advective flow in these estimates. This statement is also made about P release (Line 16).

ACTION:
As also mentioned for other comments, we will emphasize in the revised manuscript that our estimates of nutrient release should be extrapolated with caution because we have omitted important factors in our experimental setup (temperature variation, macrofauna

and hydrodynamics/advection). The error by omitting these factors is probably largest for shallow sandy sediments, which are impacted by e.g. the largest temperature fluctuations and more intense hydrodynamics compared to the other environments.

REVIEWER#2, COMMENT 8:
Page 15129 Line 17 – As mentioned by the previous referee (S. Hietanen) – the authors need to explore further how a change in the N:P ratio from high internal P loading could potentially result in N2 fixers dominating the system and be a long term management issue.

REPLY:
The same point was addressed by reviewer#1. The reviewers are right that we did not mention that decreasing internal nitrogen loading could lead to stimulation of cyanobacteria, which could potentially buffer the decreasing internal nitrogen loading through N-fixation. This was omitted because we thought it was too speculative. In our experiment we only looked at N coming from the sediment and neglected the external N-loading, which is still the dominating source of N in the studied system. Decreasing internal nutrient loading may therefore not necessarily lead to reduced DIN concentrations as long as external N-loading remains high. Therefore major changes in phytoplankton composition towards increased dominance of cyanobacteria will probably not occur.

ACTION:
In the revised manuscript we mention that the decreasing internal N-loading and stable internal P-loading could lead to increased dominance of cyanobacteria. However, a major shift in phytoplankton community can only occur in systems where decreased internal nutrient loading results in markedly lower DIN-concentrations, i.e. in systems where (1) internal nutrient sources dominate, or where (2) external N-loading is significantly reduced.

REVIEWER#2, COMMENT 9:
Page 15120 Line 6 – This line is not essential. Why Fe(III) was measured is already mentioned in the introduction.

ACTION:
Line was deleted.

REVIEWER#2, COMMENT 10:
Page 15124 Line 25 subscript on numbers instead of superscript

ACTION:
This was corrected.

REVIEWER#2, COMMENT 11:
Page 15138 - Table 2 is missing the average TP:TN ratios

ACTION:
TN:TP ratios were added to Table 2.

REVIEWER#2, COMMENT 12:
Page 15147 - Figure 6 is not essential it is explained well in results.

REPLY:
Reviewer#2 had the same comment. The temporal trends in NH_4^+ and NO_x^- are described sufficiently in the text.

ACTION:
The figure was deleted.

REVIEWER#2, COMMENT 13:
Page 15125 Lines 1-2 are not necessary this is clear in the previous sentence and was set out in the introduction

ACTION:
The indicated section was deleted.

REVIEWER#2, COMMENT 14:
Page 15141 - Table 5 should ON and OP degradation or any of the other measurements here be time integrated? the units are in mmol m-2

REPLY:
Values in table 5 are time integrated – hence the unit mol m^{-2}. This is clearly indicated in the table legend: "Total NH_4^+, NO_x^- and PO_4^{3-} effluxes were calculated by time integration of effluxes over the entire experimental period."

REVIEWER#2, COMMENT 15:
Page 15127 Line 13 – Normal text for N-1 should be superscript

ACTION:
This was corrected.

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

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Feltkode ændret

Key words: nutrient release, fluxes, reactivity, ammonium production, phosphate production, mineralization, oligotrophication, recovery, adsorbtion

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 al. 2000; Tyler & McGlathery 2003; Hulth et al. 2005). Coupled nitrification-denitrification in the 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 adsorbtion 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. 1992; Jensen et al. 1995; Viktorsson et al. 2013).

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 km^2 shallow inner basin and a 45 km^2 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 km^2, 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 $3000*10^3$ kg N y^{-1} and $300*10^3$ 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 $2000*10^3$ kg

104 N y^{-1} and $60*10^3$ 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*

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 ~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 ~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.

Kommentar [TBV1]: Line was added in response to REVIEWER#2, COMMENT 4

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

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^- ($NO_3^- + NO_2^-$) 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^+ adsorbtion 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 adsorbtion 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

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^+-adsorbtion 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 ϕ is sediment porosity and ρ_{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

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Slettet: temporal changes in area specific FeIII pools over the whole experimental period were detected by

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 adsorbtion (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 $y = C_L*\exp(-k_L*t) + C_R*\exp(-k_R*t)$, 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

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Slettet: Oxidized FeIII binds PO_4^{3-} in marine sediments (Sundby et al. 1992) and it was therefore essential to know the temporal behavior of FeIII in the studied sediments.

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 O_2 can stimulate mineralization of O_2-sensitive organic matter (Hulthe et al. 1998). In coastal and estuarine sediments O_2 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.

NH_4^+ production in jar experiments was significant throughout the experiment, except for St 1, 8-10 cm depth after 607 d. Initially 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 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 NH_4^+ production in the sediments sampled in the deep outer basin (St 6–8) decreased by 8–67% during the experiment. 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 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 NH_4^+ production at 4-6 cm depth only decreased by 19-58%. At 8-

Kommentar [TBV4]: This paragraph was added in response to REVIEWER#1, COMMENT 4 and REVIEWER#2, COMMENT 5

Kommentar [TBV5]: Section was edited in response to REVIEWER#1, COMMENT 6

Slettet: Surface NH_4^+ production decreased rapidly over time in sediments sampled from shallow locations in the inner and outer, by 96% on St 1 and by 61-82 on St 2-5 of initial rates. The surface NH_4^+ production in the sediments sampled on deep locations (St 6-8) in the outer basin only decreased by 8-67% during the experiment. 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 NH_4^+ production had decreased by 75-96% to 1.4-12 nmol cm^{-3} d^{-1} by the end (Fig. 3).

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^-.

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Slettet: (Fig. 6)

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

366 stations 6-8 had the lowest and most irregular PO_4^{3-} fluxes ranging from slightly negative to 0.1
367 mmol m^{-2} d^{-1}.
368
369 *3.4 PO_4^{3-} and NH_4^+ in porewater*
370 Porewater nutrient concentrations increased gradually at all depths during the experiment (data not
371 shown). NH_4^+ and PO_4^{3-} only increased moderately in the upper 2 cm, but accumulated to high
372 levels in the deeper diffusion limited sediment. Depth-averaged initial porewater NH_4^+
373 concentration varied between 171-407 μM on the stations. The sandy St 5 showed the highest NH_4^+
374 accumulation over time with a depth-average of 1473 μM in porewater by the end. On the
375 remaining stations, NH_4^+ only accumulated to 259-587 μM. Depth-averaged PO_4^{3-} concentrations at
376 the beginning varied between 17-71 μM depending on station. As for NH_4^+, the nutrient-poor sandy
377 St 5 showed the highest PO_4^{3-} accumulation to 368 μM compared with 43-170 μM on the other
378 stations.
379
380 *3.5 N- and P-budgets*
381 Area-specific nutrient mineralization obtained in jar-experiments was used to calculate total ON and
382 OP mineralization during the experiment. ON mineralization was fairly constant for all stations
383 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
384 hand, had 3-fold higher ON mineralization that accounted for 80% of the initial ON. A 3-fold range
385 among stations was also evident for OP mineralization, but with lowest rates of 0.12-0.l8 mol m^{-2} at
386 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
387 was no apparent relationship between sediment TN and TP content and mineralization activity as
388 some of the highest N- and P-mineralization rates were observed on the organic-poor St 5 (Table 4).
389 DIN-effluxes, porewater accumulation and adsorbtion only accounted for 18-32% of total ON

390 mineralization, indicating that most of the generated NH_4^+ was not accounted for by our
391 measurements. For P, the sum of PO_4^{3-} efflux and porewater accumulation only accounted for 10-
392 48% of total OP mineralization.
393
394 **4. Discussion**
395 *4.1 Sediment nutrient content*
396 TN and TP in sediments from Odense Fjord were in the same range or higher than reported for
397 other eutrophic systems (e.g. Boynton and Kemp 1985; Cowan & Boynton 1996; Lomstein et al.
398 1998; Coelho et al. 2004; Viktorsson et al. 2013) emphasizing the history of intense eutrophication
399 in Odense Fjord. TN and TP in the silty sediments of Odense Fjord (all stations except St 5) were
400 remarkably similar and only varied ~1.5 (TN) and ~3 (TP) times among stations. Despite these
401 overall similarities, the silty sediments from the shallow inner basin showed higher initial ON- and
402 OP-mineralization and nutrient effluxes than silty sediments from the outer fjord. This could be due
403 to higher availability of labile ON and OP in the sediments from the inner basin, reflecting the
404 nutrient rich conditions in the inner compared to the outer basin (Petersen et al.2009).
405 The sandy St 5 was markedly different from the other stations. It had the lowest total
406 nutrient content and yet exhibited some of the highest rates of ON and OP mineralization. The
407 frequent erosion by wind driven waves in this area (Valdemarsen et al. 2010) and deep (>20 cm)
408 reworking by lugworms (*Arenicola marina*) (Riisgaard & Banta 1998; Valdemarsen et al. 2011)
409 may remove fine particles and refractory organic matter from St 5 sediments (Wendelboe et al.
410 2012) and prevent organic matter accumulation, hence explaining the low organic content on this
411 station. On the other hand, intense growth and burial of microphytobenthos and other reactive
412 detritus by the strong physical disturbance and vertical mixing, can explain the unexpected high TN
413 and TP reactivity of St 5 sediment.

414 A rough areal estimate based on the measured TN and TP content on the examined
415 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
416 Odense Fjord sediments, corresponding to ~6 (N) and ~62 (P) years of the current annual external
417 nutrient loading to the system.
418
419 *4.2 Organic N and P mineralization*
420 Microbial mineralization of ON and OP in Odense Fjord sediments led to marked release of
421 inorganic nutrients, especially in the initial phase of the experiment. Initially there were strong
422 vertical gradients of ON and OP mineralization in silty and sandy sediments from shallow
423 environments, indicating that newly deposited and relatively labile organic matter was being
424 degraded near the sediment surface, with the depth gradient reflecting a gradual and time-dependent
425 depletion of labile ON and OP (Westrich & Berner 1984; Mackin and Swider 1989; Valdemarsen et
426 al. 2014). It was expected that ON and OP mineralization would decrease with time at all depths
427 due to diminishing reactivity of the organic pools. However, significant temporal decreases were
428 only observed in surface sediments from shallow locations, whereas mineralization rates were
429 surprisingly stable in the underlying sediment and the entire sediment column in the deep outer
430 fjord. Assuming that organic matter degradation follows an exponential decay pattern, the lack of a
431 detectable attenuation in mineralization rates over a ~2 yr period indicates very low initial reactivity
432 of ON and OP in the deeper layers (Westrich & Berner 1984). Nevertheless, since ON and OP of
433 low reactivity was present at high concentrations, it remained a significant source for inorganic
434 nutrients.
435 Total jar-based microbial ON and OP mineralization over the ~2 years experimental
436 period (Table 5) only accounted for a minor fraction of initial TN and TP in sediments from Odense
437 Fjord suggesting that the standing stock of organic N and P will be a source of nutrients for

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Kommentar [TBV8]: Line deleted in response to REVIEWER#2, COMMENT 13
Slettet: The main goal of this experiment was to evaluate the extent to which these accumulated nutrients can be recycled to the overlying water as internal loading.

446 extended time. Decay constants from the exponential decay model suggested that labile ON and OP
447 was rapidly degraded on all stations within 10-240 d, whereas depletion of more refractory ON and
448 OP will only occur on decadal time-scales (8-40 years), indicating that depletion of buried and
449 degradable ON and OP in eutrophic ecosystems will take considerable time.
450
451 *4.3 Fate of inorganic nutrients*
452 NH_4^+ and PO_4^{3-} produced by microbial mineralization accumulated in porewater of all sediments
453 within the first 1-6 months and only changed slightly hereafter. However, over the whole
454 experiment, porewater accumulation explained only a minor fraction of the jar-based total ON and
455 OP mineralization (0.8-8.1%). It was also investigated if NH_4^+ adsorbtion to mineral surfaces was
456 an important N sink. Despite the large spatial heterogeneity of NH_4^+ adsorbtion, this process never
457 accounted for more than 1% of the total produced NH_4^+ over the whole experiment and was
458 therefore not quantitatively important.
459 Nutrient release to the overlying water was the most important route for inorganic
460 nutrients produced by microbial mineralization. We could not account for all the produced
461 nutrients, since nutrient mineralization in jar experiments exceeded DIN and PO_4^{3-} effluxes by 70-
462 84% and 62-93%, respectively. The missing NH_4^+ may have been lost through coupled nitrification-
463 denitrification (e.g. Mackin and Swider 1989; Quintana et al. 2013). The conspicuous shift from
464 NH_4^+ to NO_3^- release indicated that nitrification was an active process in all sediment types, and
465 denitrifying bacteria probably proliferated in the NO_3^--rich surface sediment. In the present case,
466 coupled nitrification-denitrification rates of 1-2 mmol m^{-2} d^{-1} are required to account for the missing
467 NH_4^+, which is within the range reported in previous studies (e.g. Nielsen et al. 1995; Christensen et
468 al. 2000; Tobias et al. 2003). On the other hand, the missing PO_4^{3-} must have been retained within
469 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-} adsorbtion 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

Kommentar [TBV9]: Change in response to REVIEWER#2, COMMENT 15

Slettet: t

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.

Kommentar [TBV10]: The original Fig. 7 is now Fig. 6

Slettet: 7

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

Kommentar [TBV11]: The original Fig. 7 is now Fig. 6

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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

Slettet: management efforts have been implemented to reduce

Slettet: and

Slettet: (e.g Carstensen et al. 2006). This generally results in

Slettet: , but

Slettet: in the external nutrient loading

Slettet: during recovery from eutrophication

Kommentar [TBV12]: The first half of this paragraph was shortened in response to REVIEWER#1, COMMENT 9

Slettet: in our example

Slettet: in all sediments types

Slettet: in eutrophic estuaries

Slettet: However, since m

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

Kommentar [TBV13]: This section was edited according to REVIEWER#1, COMMENT 1 and REVIEWER#2, COMMENT 8

Slettet: a high internal P-loading will probably only exacerbate N-limitation while having no further consequences for ecological quality.

Kommentar [TBV14]: This line was edited in response to REVIEWER#2, COMMENT 7

Kommentar [TBV15]: This line was edited in response to REVIEWER#1, COMMENT2b and REVIEWER#2, COMMENT 3

Kommentar [TBV16]: This line was edited in response to REVIEWER#1, COMMENT 3 and REVIEWER#2, COMMENT 4

Kommentar [TBV17]: This line was added in response to REVIEWER#1, COMMENT 7, REVIEWER#2, COMMENT 2, REVIEWER#2, COMMENT 7

Slettet: and temperature changes can lead to several fold variation in DIN and PO_4^{3-} release from sediments (Sanz-Lazaro et al. 2011). T

Slettet: ,

Slettet: and benthic primary production

Slettet: During warm summer periods, for instance, where nutrient concentrations in the water column are kept low by primary producers, even moderate nutrient release from sediments can have a high impact on ecosystem functioning and may even shift the balance between limiting nutrients (Kristensen et al. *submitted*).

599 and effluxes from this experiment, suggests that nutrient effluxes could potentially increase 3-6
600 (DIN) and 2-10 (PO_4^{3-}) times if there are no mechanisms to transform or retain inorganic nutrients
601 at the sediment surface.
602
603 *4.6 Conclusions*
604 In this study we investigated the mineralization of organic N and P buried in the sediments from a
605 shallow eutrophic estuary and obtained estimates of the magnitude and temporal dynamics of
606 internal nutrient loading. Total internal N-loading, which attenuated rapidly, corresponded to only a
607 minor fraction of the external N-loading and was therefore not important for the ecological state in
608 the studied ecosystem. Total internal P-loading showed no temporal attenuation and was
609 quantitatively more important as it corresponded to >1/3 of the external P-loading. However, the
610 studied ecosystem was N-limited, and it is therefore uncertain if high internal P-loading will result
611 in negative ecological effects. This study indicates that internal nutrient loading, and especially
612 internal N-loading, is a transient phenomena that can only temporarily influence the recovery
613 trajectory of ecosystems recovering from eutrophication. In turn, internal nutrient loading driven by
614 mineralization of organic N and P in sediments, cannot explain the lack of recovery in shallow
615 estuaries where external nutrient loading has been reduced.
616
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622

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785 **Tables**

786 Table 1. Dimensionless linear NH_4^+ adsorbtion coefficients, K_{NH}, for different sediment depths at St

787 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

788

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.

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

Kommentar [TBV18]: TN:TP ratios were added to the table in response to REVIEWER#1, COMMENT 10 and REVIEWER#2, COMMENT 11

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

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

796

797

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 $y = C_L*\exp(-k_{L*}x) + C_R*\exp(-k_R*x)$, 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.

ON

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

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_x^-- 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^+ adsorbtion was calculated from initial and final pw-inventories of NH_4^+ and the average NH_4^+-adsorbtion 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.

N-mineralization	**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^+ adsorbtion (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)
P-mineralization	**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)

Figures

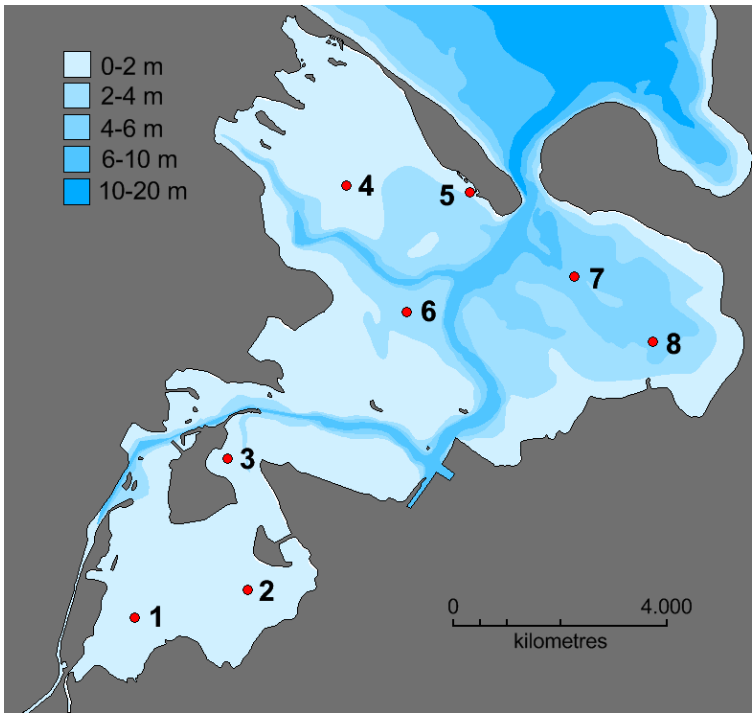

Figure 1. Map of Odense Fjord (55°29´15” N; 10°31´09”) showing the 8 stations, where sediments were sampled for the long term degradation experiment. Gray color indicates land and different shades of blue indicate water depth.

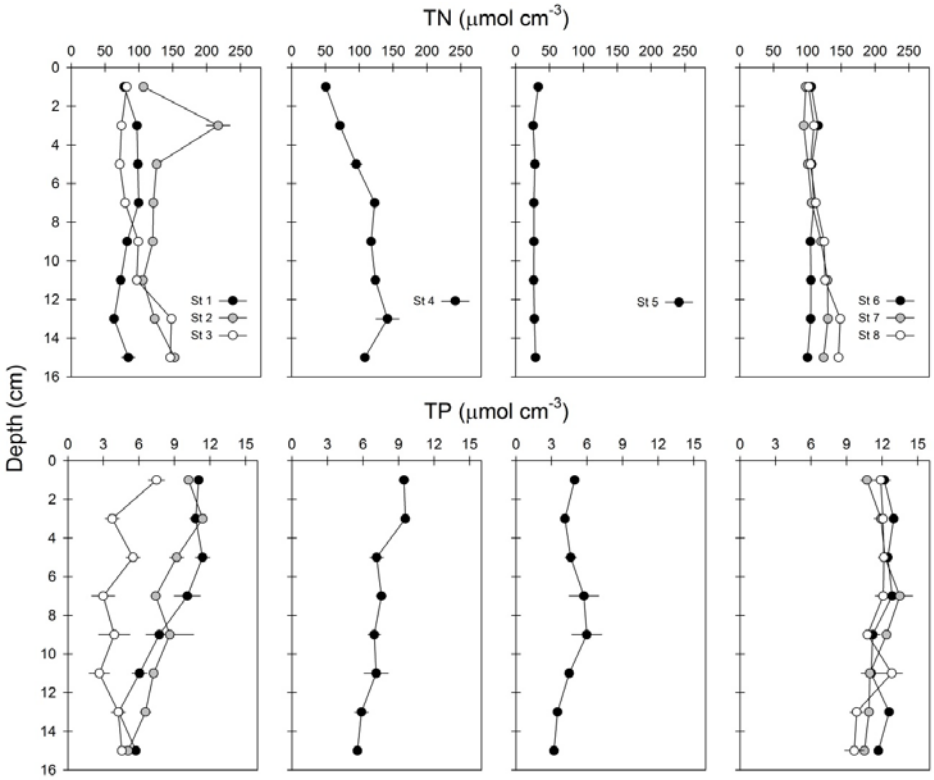

Figure 2. Total nitrogen (TN) and total phosphorus (TP) in sediments from Odense Fjord. Left panels show stations from the shallow inner fjord (St 1, 2 and 3), middle panels show shallow silty and sandy sediments in the outer fjord (St 4 and 5, respectively) and right panels show deep silty sediments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

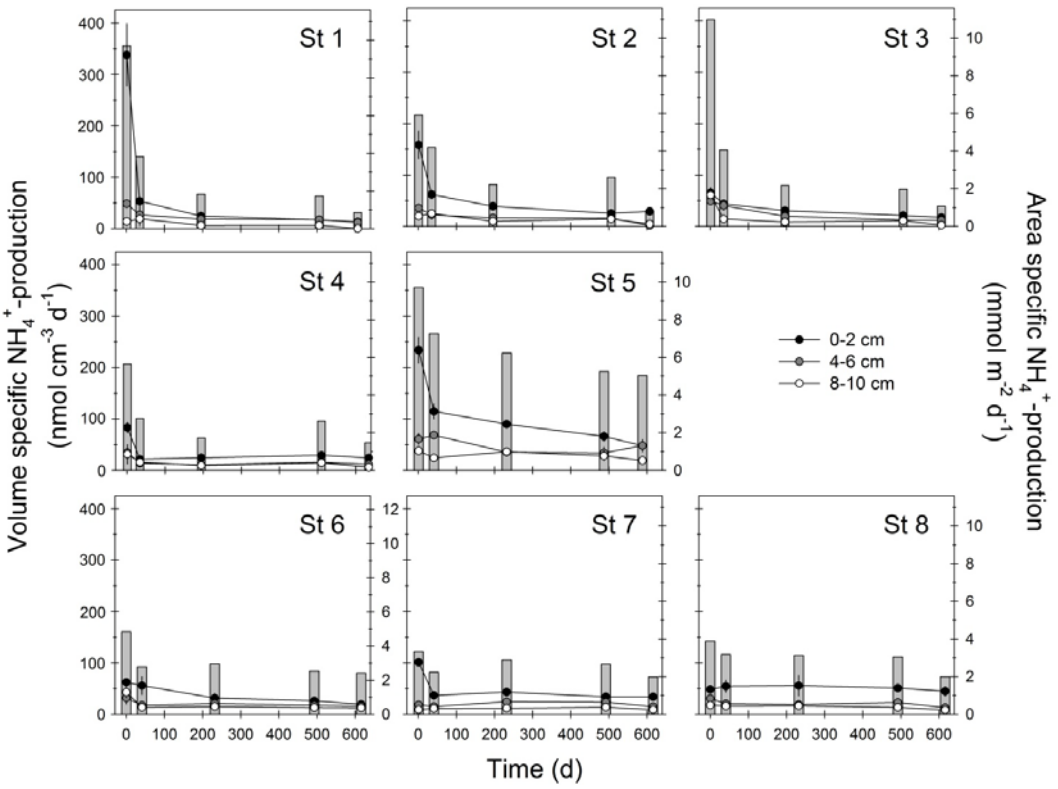

Figure 3. NH_4^+ production measured in jar experiments with sediment from shallow inner basin (upper panels), shallow silty and sandy outer basin (middle panels) and deep silty outer basin (lower panels). Black, gray and white symbols indicate volume specific NH_4^+ production in sediment from 0-2,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

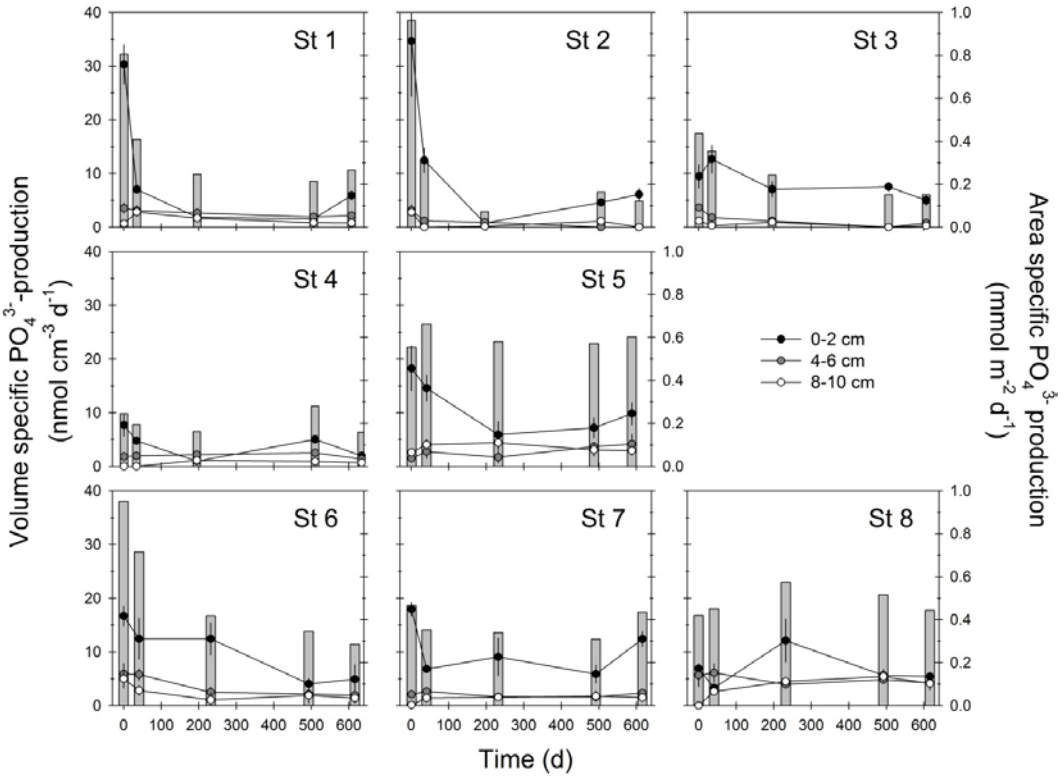

Figure 4. PO_4^{3-} production measured in jar experiments performed with sediment from shallow inner fjord (upper panels), shallow silty and sandy outer fjord (middle panels) and deep silty outer fjord (lower panels). Black, gray and white symbols indicate volume specific PO_4^{3-} production in sediment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

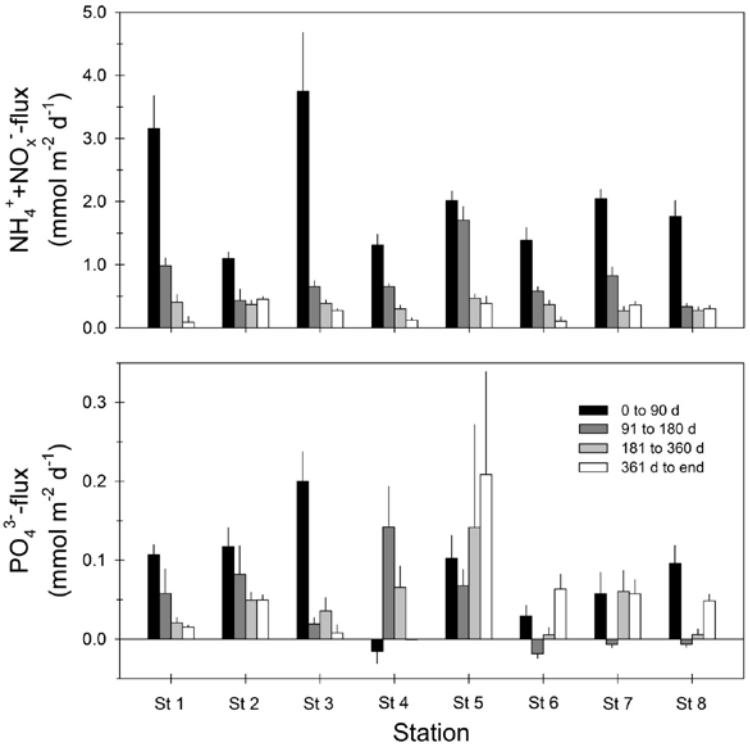

Figure 5. Fluxes of dissolved inorganic nitrogen (DIN = NH_4^+ + NO_X^-) and PO_4^{3-} at various times during the experiment. Error bars represent standard error (n = 6-24).

Kommentar [TBV19]: Change in response to REVIEWER#1, COMMENT 11

Slettet: NO_3

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Kommentar [TBV20]: Figure was deleted in response to REVIEWER#1, COMMENT 12 and REVIEWER#2, COMMENT 12

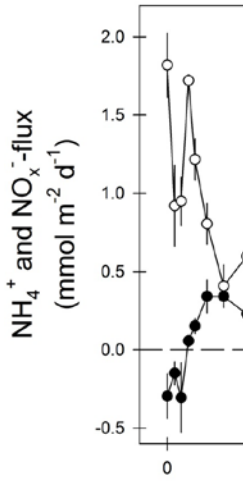

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Figure 6. Example of changes in NH_4^+ and NO_x^- fluxes measured during 600 d on sediment from the silty inner fjord (St 2). NH_4^+ was the main form of N released in the beginning of the experiment and NO_x^- was the main form released at the end. A similar pattern was observed on all stations. Error bars indicate standard error (n=3) and the dashed horizontal line indicate y=0

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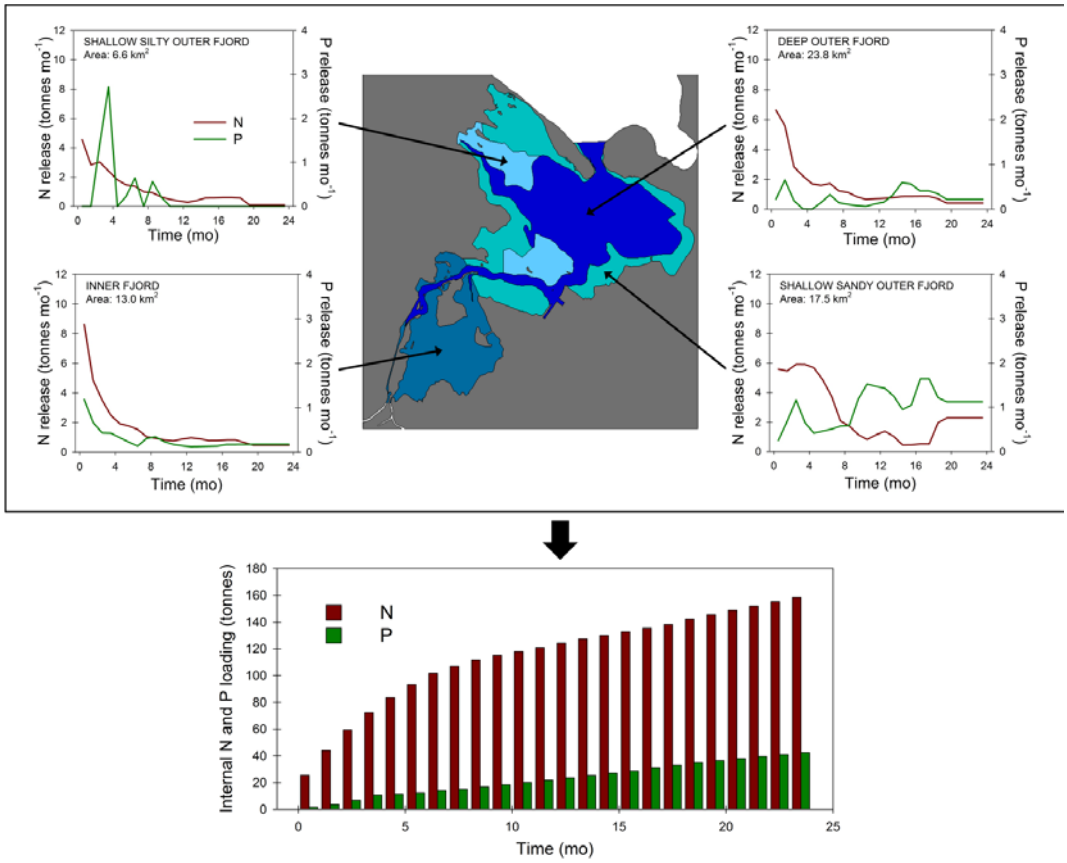

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856 Figure 6. Estimated internal nutrient loading in Odense Fjord. The upper figure shows a schematic

857 overview of Odense Fjord with the distribution of sediment types included in this study and their

858 nutrient release over a 24 month period. The lower figure shows the cumulated nutrient release from

859 the entire fjord bottom.

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